Université de Lille (ULille)

Ecole Doctorale Biologie-Santé (EDBSL)
CANTHER – Hétérogénéité, Plasticité et Résistance aux Thérapies des Cancers
Univ. Lille, Institut Pasteur de Lille, CHU de Lille, CNRS, Inserm –
UMR9020, U1277
Equipe SENFIB : Sénescence, fibrose et cancer

Université de Namur (UNamur)

Faculté des sciences
Département de Biologie Namur
NARILIS - Research Institute for Life Sciences
Unité de Recherche en Biochimie et Biologie Cellulaire (URBC)

THESE DE DOCTORAT

Dissertation originale présentée par **Joëlle Giroud**

En vue de l'obtention du grade de docteur en Sciences de l'Université de Lille et de l'Université de Namur

Vendredi 5 juillet 2024

Impact of the UPR pathway on the establishment of the senescent phenotype induced by UVB

Composition du jury :

Dr. Florence Debacq-Chainiaux
(**Directeur de thèse**)
URBC- Stress and ageing
NARILIS, UNamur,Namur

Dr. Olivier Pluquet
(**Directeur de thèse**)
UMR9020-U1277 SENFIB
CANTHER, ULille, Lille

Prof. Yves Poumay
(**Président**)
URPHYM – LabCeTi
NARILIS, UNamur,Namur

Prof. Jerôme Lamartine
(**Rapporteur**)
UMR5305 – SKIN
LBTI, Université Claude Bernard Lyon 1, Lyon

Prof. Isabelle Petropoulos
(**Rapportrice**)
UMR8256- Vieillissement intégré et inflammation
Adaptation Biologique et Vieillissement, Sorbonne Université, Paris

Dr. Fabienne Foufelle
(**Examinatrice**)
U1138 - Metabolic diseases, diabetes and co-morbidities
Centre de Recherche des Cordeliers, Paris

Acknowledgments

Tout d'abord, je tiens à exprimer ma profonde gratitude envers l'ensemble des membres de mon jury de thèse, pour avoir accepté d'évaluer ce travail.
Merci au Professeur Yves Poumay d'avoir accepté sans hésitation de présider ce jury et merci de m'avoir ouvert les portes de votre laboratoire, le LabCeti, quand j'en ai eu besoin. Merci au Professeur Jérôme Lamartine et au Docteur Fabienne Foufelle qui ont respectivement pris les rôles de rapporteur et examinatrice de cette thèse. Enfin, merci au Professeur Isabelle Petropoulos d'être également rapportrice de ce travail, votre présence ainsi que votre soutien depuis mon master 2 me touchent énormément.

Je souhaite particulièrement remercier mes directeurs de thèse, les Docteurs Florence Debacq-Chainiaux et Olivier Pluquet. Merci de m'avoir fait confiance tout au long de ce projet. Cette cotutelle aura été pour moi, une grande aventure professionnelle et personnelle. Sans votre bonne entente et votre complémentarité, elle n'aurait pas été aussi belle. Florence, merci de votre bienveillance, du suivi presque quotidien que vous avez accordé à ce projet, de votre investissement dans tous les aspects de la thèse et de votre soutien indéfectible à chaque nouvelle étape que je devais franchir. Je vous souhaite le meilleure dans votre nouvelle vie. Olivier, merci pour toutes ces discussions scientifiques enrichissantes (voire parfois philosophiques !), merci d'avoir su me rassurer quand j'en ai eu besoin. Votre optimisme à toute épreuve m'aura appris que n'importe quelle situation peut toujours se régler dans le calme et l'intelligence. Henry Ford a dit un jour : « Se réunir est un début, rester ensemble est un progrès, travailler ensemble est la réussite », il avait raison et je suis très reconnaissante d'avoir pu le faire avec vous deux.

Ayant commencé ma thèse en Belgique je souhaite commencer par remercier les membres de l'**UNamur** :

Merci à Jean-Paul Leonis et Florence Collot, lesquels, dans le cadre de la pandémie COVID-19, ont largement facilité mon arrivée à Namur. Votre souci du bien-être des étudiants étrangers est une grande chance pour l'Université.

Je souhaite également remercier tous les autres PIs de l'URBC pour leurs conseils lors de nos réunions de laboratoire.

Merci à Marc qui, dans toutes nos galères avec le sécrétome, a toujours fait de son mieux pour améliorer les résultats. Ton humour légendaire aura rendu cette période plus légère. Merci à Benjamin d'avoir pris sur son temps pour me former aux nouveaux microscopes. Merci à Catherine et Antoine de faciliter notre quotidien au labo et merci beaucoup à Sophie de m'avoir

aidée dans la préparation des échantillons de spectrométrie de masse, promis les centris c'est terminé !

Merci à tous les SAGE d'avoir suivi l'évolution de ce projet depuis bientôt 4 ans, je vous souhaite à toutes (girl power) une très belle thèse ! Inès, la suite de ton parcours sera sans aucun doute à ton image : brillant ! Je te souhaite bon courage dans la continuité de ton projet.

Que serait la thèse en Belgique sans les fameux « mémos » ? Florent, merci pour tout le travail que tu as réalisé avant ce projet de thèse et merci beaucoup de m'avoir si vite intégrée à l'équipe, grâce à toi j'ai pu découvrir les coutumes Belges mais je dois te l'avouer m'fi, mon Wallon est toujours très mauvais et je n'aime toujours pas la bière ! Ma petite Pauline, merci beaucoup de ton aide lors de ma deuxième année. Ton travail sans relâche aura rendu ce projet meilleur et ta personnalité pétillante aura égayé toutes nos journées de travail, même les plus difficiles. Je suis ravie de savoir que tu t'épanouies dans ta nouvelle vie scientifique ! Merci également à mes stagiaires, Fiona et Loic, je vous souhaite à tous les deux un beau parcours. Enfin, bon courage à la cuvée mémo 2024 et particulièrement à Laura.

L'URBC est avant tout une grande famille et je veux ici remercier chaque doctorant du labo de participer à cette bonne ambiance, nos Beer hours sont gravées pour toujours dans ma mémoire, en particulier celles qui se sont terminées en cache-cache ou en karaoké. J'aimerais remercier plus particulièrement Jia-Wei et Jérémy pour m'avoir recueillie au 5711. Partager ce bureau avec vous m'aura fait prendre quelques kilos, mais finalement vous aviez raison « manger c'est la vie » ! Merci à Natacha pour ses conseils, ses invitations et sa gentillesse. Je voudrais souhaiter bon courage à Louise P. et Shiqiang, vous êtes dans la dernière ligne droite aussi ! Enfin, j'aimerais particulièrement remercier mes deux nouveaux amis, Emilie et Andréa. « Madame Emilie », merci pour tous ces moments passés ensemble à débriefer, râler, pleurer, rigoler, (manger)… Tu auras été une véritable confidente pour moi et je suis ravie que tu fasses partie de ma vie (et Tom aussi !). « Pancetta », do I really have to write this in English? Ok…Thank you for being you; a true friend, a clown, and a pretty good chef. I know that I can always count on you. Love you guys and good luck with the lab! I know that your theses will be perfect, just like you.

Ayant passé quelques semaines au **LabCeti** j'aimerais tout particulièrement remercier Valérie sans qui toute la partie des RHE n'aurait pas été possible. Merci de ta patience, de t'être toujours rendue disponible pour moi et de ton aide. Merci également aux doctorants du labo qui m'ont toujours apporté de l'aide (ainsi que des pipettes et des tips) quand j'en avais besoin. Enfin, merci au duo Nicolas et Valéry, vos blagues vont me manquer.

Après un petit peu plus d'un an passé à Lille, j'aimerais remercier les membres de l'équipe **SENFIB** avec qui j'ai pu terminer cette thèse :

Tout d'abord, j'aimerais remercier tous les chercheurs de l'équipe pour leurs retours sur mon projet. Merci au Professeure Corinne Abbadie de m'avoir accueillie dans l'équipe et merci au Docteur Albin Pourtier pour son aide dans la rédaction de notre review.

Je souhaite particulièrement remercier Nathalie, sans qui je n'aurais jamais trouvé mes marques à mon arrivée. Ta gestion parfaite du labo, ta mémoire sans faille et ton amour de la science auront sans aucun doute contribué à bien terminer ce projet. Merci également à Clémentine pour son aide sur Skinage et pour sa bonne humeur tous les jours de la semaine. Je garderai la photo du westernblot d'ATF6α près de moi pour toujours ! Merci à Nihad qui avec seulement un « bonjour » arrive à donner toute l'énergie qu'il faut pour une journée. Merci à Ingrid de nous chouchouter à chaque fête et de toujours avoir un gentil mot à partager et évidemment, merci de ton aide avec (le capricieux) Incucyte.

Merci à Matthieu, mon « mémo-stagiaire ». Je suis ravie d'avoir fait mes débuts à Lille en ta compagnie. Tes petits mots m'ont manqué à ton départ, mais je suis ravie de savoir que tu as trouvé ta voie.

Merci aux doctorants de l'équipe pour nos partages d'expériences. Henry, je te souhaite bon courage pour tes manips, tu verras, les kéras c'est aussi gentil que les patients ! Merci à Marine et Elodie pour nos discussions tout au long de l'année et bon courage pour votre rédaction. Quentin et Emilie, la thèse est un long parcours mais vous vous en sortirez très bien, je vous souhaite le meilleur.

Enfin, j'aimerais remercier **mes amis de toujours et ma famille** :

Maman et Pady, je ne saurais pas suffisamment vous remercier pour tout ce que vous avez fait et continuez de faire pour mon bonheur. Merci de m'avoir enseigné le courage et l'amour du travail, de m'avoir guidée dans tous mes choix et d'avoir cru en moi depuis toujours et pour toujours, vous êtes mes piliers.

Papa, merci d'avoir suivi mes avancées dans cette thèse et de t'être intéressé à mon projet depuis le début. Tes conseils du dimanche soir durant ces 4 ans m'auront beaucoup aidé.

J'aimerais également remercier ma belle-famille et particulièrement Brigitte et Pierrette, lesquelles, depuis mon arrivée chez elles se sont toujours souciées de mes besoins et de mon bien-être avec beaucoup de tendresse. Merci également à François de toujours avoir eu des

mots encourageants envers moi. Ma vie en Belgique m'aura apporté bien plus que ce que je n'aurais pu imaginer.

Merci à mes amis de toujours de m'avoir supportée dans tous les sens du terme. Merci à Rémy et Jordan pour vos encouragements de groupe, on y sera tous les trois arrivés ! Merci à Florian d'avoir compris tous mes choix, je suis heureuse de savoir que tu arrives aussi bientôt à la fin de ta thèse, tu mérites ce beau parcours. Merci à Samir pour son aide et sa présence. Merci à Gilou, promis le traitement antivieillissement c'est pour bientôt ! Merci à Yasmine, Maxou, Lucas, et Loumie d'avoir essayé de comprendre ce que je faisais, vos résumés de ma recherche me font toujours autant rire. Merci à Antoinette et Jeanne d'être venues me voir en Belgique quand j'en avais besoin, je vais bientôt pouvoir vous rendre la pareille. Merci à Lélé qui fait toujours son maximum pour me rendre heureuse, ta présence quotidienne me rappelle tous les jours la chance que j'ai de t'avoir comme amie, merci également à ta famille et aux cats pour leurs encouragements et autres douceurs.

Enfin, je terminerai en remerciant Sébastien sans qui ces dernières années n'auraient pas été pareilles. Merci d'être rentré dans ma vie, de m'aider chaque jour à prendre un peu plus confiance en moi, d'agir alors même que je n'ai rien dit et de faire de nous une priorité. Nos chemins ne se sont pas croisés par hasard et je nous souhaite d'en faire une belle route, ensemble.

Résumé: Impact de la voie UPR sur l'établissement du phénotype sénescent induit par les UVB

Le vieillissement cutané, influencé par une combinaison de facteurs intrinsèques et extrinsèques, entraine des dommages capables d'altérer les fonctions cutanées. Parmi les facteurs extrinsèques, les rayonnements ultraviolets (UV) sont responsables du photo-vieillissement de la peau. Ces éléments conduisent notamment à une accumulation de cellules sénescentes capables de contribuer au développement de pathologies liées à l'âge, telles que les cancers cutanés. En effet, la sénescence s'accompagne de profonds changements morphologiques et moléculaires au sein de la cellule. Cela inclut notamment une modification de son sécrétome, qui s'enrichit en cytokines pro-inflammatoires, en facteurs de croissance et en enzymes remodelant la matrice extracellulaire, altérants les caractéristiques des tissus lors du vieillissement. Néanmoins, les mécanismes précis qui aboutissent au phénotype sénescent induit par les UVB restent largement inconnus.

Dans ce contexte, l'objectif principal de ce travail a été d'identifier des mécanismes moléculaires sous-jacents à l'établissement de la sénescence induite par les UVB dans des fibroblastes de derme humains normaux (NHDFs), mécanismes qui pourraient contribuer au vieillissement cutané.

In vitro, nous avons confirmé que des expositions répétées aux UVB induisent la sénescence prématurée des NHDFs et que cet état est associé à l'activation des trois branches de la voie UPR (Unfolded Protein Response) responsables du maintien de l'homéostasie du réticulum endoplasmique (RE), le premier compartiment de sécrétion. Ces observations ont été supportées par une analyse transcriptomique, révélant des éléments de régulation liés aux grandes voies de sénescence et aux fonctions du RE dans les NHDFs exposés aux UVB. Par la suite, nous avons montré que la branche ATF6α joue un rôle central dans la survenue des biomarqueurs du phénotype sénescent induit par les UVB. En effet, l'invalidation d'ATF6α protège non seulement des changements morphologiques induits par les UVB, mais réduit le pourcentage de cellules positives pour la SA-βgalactosidase (SA-βgal), prévient la persistance des dommages à l'ADN, et modifie l'expression de facteurs majeurs du phénotype sécrétoire associé à la sénescence (SASP).

Le SASP exerçant entre autres une action pro-tumorale, nous avons cherché à évaluer si le milieu conditionné (MC) des fibroblastes exposés aux UVB et invalidé pour ATF6α

pouvait impacter le potentiel de migration et d'invasion de cellules issues de mélanomes. Cependant, nous n'avons pas observé d'effets pro-migratoires ou pro-invasifs dépendants d'ATF6α.

Afin de mettre en évidence un potentiel rôle d'ATF6α dans un autre processus biologique, nous avons exploité nos analyses transcriptomiques et sécrétomiques et avons identifié un possible effet d'ATF6α sur le contrôle paracrine de l'environnement cutané. Pour explorer cela, nous nous sommes concentrés sur les facteurs du SASP (cytokines et métalloprotéases) régulés par ATF6α et dont l'impact sur l'environnement tissulaire était connu. Ensuite, nous avons traité un modèle d'épiderme humain reconstruit (RHE) avec du MC issu de NHDFs exposés aux UVB ou non, et invalidés ou non pour ATF6α. Etonnement, nous avons observé que le MC des NHDFs exposés aux UVB augmente l'épaisseur du RHE ainsi que la prolifération des kératinocytes basaux, via un mécanisme dépendant d'ATF6α. Enfin, nous avons identifié l'IL-8 comme un facteur paracrine majeur impliqué dans ce processus, puisque le blocage d'IL-8 par des anticorps neutralisants prévient la prolifération excessive des kératinocytes.

En conclusion, nous rapportons le rôle d'ATF6α dans la sénescence induite par les UVB ainsi que son impact sur la préservation de l'homéostasie cutanée en condition de stress, notamment par la régulation de l'expression des composants du SASP. Cela suggère qu'ATF6α et ses effecteurs pourraient être des cibles prometteuses contrôlant les effets du vieillissement cutané.

Abstract: Impact of the UPR pathway on the establishment of the senescent phenotype induced by UVB

Skin ageing, influenced by a combination of intrinsic and extrinsic factors, can result in damage that has the potential to alter skin functions. Among extrinsic factors, ultraviolet (UV) radiation is responsible for skin photoageing. These factors notably contribute to the accumulation of senescent cells which in turn can contribute to the development of age-related pathologies, including skin cancers. Indeed, senescence is characterized by profound morphological and molecular changes within the cell. This includes a modification of its secretome, which becomes enriched in pro-inflammatory cytokines, growth factors, and matrix-remodelling enzymes, altering tissue characteristics during ageing. However, the exact mechanisms driving the senescent phenotype induced by UVB remain largely unknown.

In this context, the main objective of this work was to identify the underlying molecular mechanisms responsible for the establishment of UVB-induced senescence in normal human dermal fibroblasts (NHDFs), mechanisms that may play a role in skin ageing.

In vitro, we confirmed that repeated exposures to UVB induce premature senescence of NHDFs and that this state is associated with the activation of the three branches of the Unfolded Protein Response (UPR), which are responsible for maintaining endoplasmic reticulum (ER) homeostasis, the primary cellular secretion compartment. These observations were supported by transcriptomic analysis, revealing regulatory elements related to major senescence pathways and ER functions in UVB-exposed NHDFs. Subsequently, we demonstrated that the ATF6α branch plays a central role in the development of the UVB-induced senescent phenotype. Indeed, the silencing of ATF6α not only protects against morphological changes induced by UVB, but also reduces the percentage of senescence-associated β-galactosidase (SA-βgal) positive cells, prevents the persistence of DNA damage, and alters the expression of major factors associated with the senescence-associated secretory phenotype (SASP).

The SASP, exerting a pro-tumoral action, led us to assess whether the conditioned medium (CM) from UVB-exposed fibroblasts invalidated for ATF6α could impact the migration and invasion potential of melanoma cells. However, we did not observe any ATF6α-dependent pro-migratory or pro-invasive effects.

To highlight a potential role of ATF6α in another biological process, we further analyzed our transcriptomic and secretomic analyses and identified a possible effect of ATF6α on the paracrine control of the skin environment. To explore this, we focused on SASP factors (cytokines and metalloproteinases) regulated by ATF6α and whose impact on tissue environment was known. Subsequently, we treated a reconstructed human epidermis (RHE) model with CM from NHDFs exposed or not to UVB and invalidated or not for ATF6α.

Surprisingly, we observed that the CM from UVB-exposed NHDFs increased the thickness of the RHE as well as the proliferation of basal keratinocytes, via an ATF6α-dependent mechanism. Finally, we identified IL-8 as a major paracrine factor involved in this process, as blocking IL-8 with neutralizing antibodies prevented excessive proliferation of keratinocytes. In conclusion, we report the role of ATF6α in UVB-induced senescence and its impact on the preservation of skin homeostasis under stress conditions, particularly through the regulation of the expression of SASP components. This suggests that ATF6α and its effectors could be promising targets for controlling the effects of skin ageing.

Table of contents

Abbreviations

3D	Three-dimensional
53BP1	P53 Binding Protein 1
ACAN	Aggrecan core protein
ADP	Adenosine diphosphate
AGE	Advanced glycation end product
AKT	Protein kinase B
AMPK	AMP-activated protein kinase
AMPs	Antimicrobial peptides and/ or proteins
AP-1	Jun Proto-Oncogene, AP-1 Transcription Factor Subunit
Asf1	Anti-Silencing Factor 1
ASK1	Apoptosis signal-regulating kinase 1
Asn	Asparagine
ATF4	Activating transcription factor 4
ATF6α	Activating transcription factor 6α
ATM	Ataxia Telangiectasia mutated
ATP	Adenosine triphosphate
ATP2A2	ATPase Sarcoplasmic/Endoplasmic Reticulum Ca2+ Transporting 2
ATR	Ataxia telangiectasia- and Rad3-related
ATRIP	ATR-interacting protein
ATV	Atazanavir
BAK	BCL2 Antagonist/Killer
BAX	BCL2 Associated X, Apoptosis Regulator
BCL-2	B-cell leukemia/lymphoma 2 protein
BDNF	Brain-derived neurotrophic factor
BiP	Binding immunoglobulin protein
BME	Basal Medium Eagle
BP	Biological Process
BRCA-1	Breast cancer type 1 susceptibility protein
C/EBPβ	CCAAT/enhancer binding protein beta
C12FDG	5-Dodecanoylaminofluorescein di-β-D-Galactopyranoside
C1R	Complement C1r subcomponent
C1RL	Complement C1r subcomponent-like protein
C1S	Complement C1s subcomponent
CA7	Ceapin A7
CAFs	Carcinoma-Associated Fibroblasts
CAR-T cells	Chimeric antigen receptor T cells
CCFs	Cytosolic chromatin fragments
CCL	Chemokine (C-C motif) ligand
CDC25	Cell Division Cycle 25
CDK	Cyclin-dependent kinase
CDKI	Cyclin dependent kinase inhibitor
CDKN2A	Cyclin Dependent Kinase Inhibitor 2A
CDKN2B	Cyclin Dependent Kinase Inhibitor 2B
cGAS-STING	cyclic GMP-AMP synthase linked to a stimulator of interferon genes
CHK1	Checkpoint Kinase 1
CHK2	Checkpoint Kinase 2
CHOP	CCAAT/enhancer-binding protein homologous protein

CM	Conditioned media
CME	Clathrin-mediated endocytocis
COL	Collagen
COVID-19	coronavirus disease 2019
COX2	Cyclooxygenase 2
CPS	conventional protein secretion
CTL	Control
$CuSO_4$	Copper sulfate
CXCL	C-X-C Motif Chemokine Ligand
DAMPs	Damage-associated molecular patterns
DCR	Decoy receptor
DDA	Data-independent acquisition
DDR	DNA damage response
DEJ	Dermoepidermal junction
DISC	Death-inducing signalling complex
DNA	deoxyribonucleic acid
DNA-SCARS	DNA segments with chromatin alterations reinforcing senescence
DPP4	Dipeptidyl peptidase 4
DREAM	Dimerization partner Rb-like E2F multi-vuval class B
DRs	Death receptors
DSB	Double-strand beak
dWAT	Dermal white adipose tissue
E2F	E2F transcripton factor
ECFC	Endothelial colony-forming cells
ECM	Extracellular matrix
EDEM1	ER degradation-enhancing alpha-mannosidase-like protein 1
eiF2α	Eukaryotic translation initiator factor-2
EMT	Epithelial–mesenchymal transition
EP4	E-type prostanoid receptor 4
ER	Endoplasmic reticulum
ERAD	Endoplasmic reticulum-associated protein degradation
ERES	ER exit sites
Erp57	ER-resident protein 57
ERQC	ER quality control
ERSE	ER-stress response element
EV	Extracellular vesicle
FA	Fatty acids
FAM134B	Reticulophagy Regulator 1
FBLN1	Fibulin-1
FGF2	Fibroblast growth factor 2
Fis1	Mitochondrial fission 1 protein
FN1	Fibronectin-1
FSTL1	Follistatin-related protein 1
G1	Glucosidases I
G2	Glucosidases II
GADD34	Growth arrest and DNA damage-inducible protein 34
GATA4	GATA Binding Protein 4
GDF15	Growth Differentiation Factor 15
GDP	Guanosine diphosphate

GLB1	Galactosidase Beta 1
Glc	Glucose
GlcNAc	N-acetylglucosamine
GLS	Golgi localization site
GO	Gene ontology
GPC6	Glypican-6
GRASPs	Golgi reassemble stacking proteins
GTP	Guanosine triphosphate
H+	proton
H_2O_2	Hydrogen peroxide
HDAC	Histone deacetylase
HDFs	Human dermal fibroblasts
HERPUD1	Homocysteine-responsive endoplasmic reticulum-resident ubiquitin-like domain member 1 protein
HGPS	Hutchinson-Gilford progeria syndrome
HIRA	histone cell cycle regulator
HMGB	High Mobility Group Box
HP1	Heterochromatin protein 1
HSPs	Heat shock proteins
IL	Interleukin
IRE1α	Inositol-requiring enzyme 1α
IRIS	Irradiation-induced senescence
JAK/STAT	Janus kinase/signal transducers and activators of transcription
JNK	c-Jun N-terminal kinase
KEAP1	Kelch-like ECH-associated protein 1
KLF4	Kruppel-like factor 4
KRT	Cytokeratin
LAM	Laminin
LIR	LC3 interacting region
LSECs	Liver Sinusoïd Endothelial Cells
LUM	Lumican
MAGS	Melanoma aggressiveness score
MAMs	Mitochondria-associated membranes
Man	Mannose
MAPK	Mitogen-activated protein kinase
MAPKAPK2	MAP kinase-activated protein kinase 2
MCSs	Membrane contact sites
MDC1	DNA-damage checkpoint 1
MDM2	MDM2 Proto-Oncogene
MEGF6	Multiple epidermal growth factor-like domains protein 6
MF	Molecular Function
MiDAS	Mitochondrial dysfunction associated senescence
MMP	Matrix metalloproteinase
MOMP	Mitochondrial outer membrane permeabilization
MRE11	Mre11 homolog double strand beak repair nuclease
MRN	MRE11-RAD50-NSB1 complex
MS	mass spectrometry
mtDNA	Mitochondrial DNA
mTOR	Mamalian Target Of Rapamycin

mTORC	Mammalian Target Of Rapamycin
NAD	Nicotinamide adenine dinucleotide
NBS1	Nibrin
NCT	Nucleocytoplasmic trafficking
NEF	Nucleotide exchange factors
NF1	Neurofibromin 1
NF-κB	Nuclear Factor κB
NHDFs	Normal human dermal fibroblasts
NHEK	Normal human epidermal kertainocytes
NID1	Nidogen-1
NID2	Nidogen-2
NK	Natural killer
NLRP3	Nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3
NMFs	Natural moisturizing factors
Nrf2	Nuclear factor erythroid 2-related factor 2
OA	Osteoarthritis
OIIS	Oncogene inactivation induced senescence
OIS	Oncogene-induced senescence
OST	Oligosaccharyltransferase complex
OXPHOS	Oxidative phosphorylation
p53	Tumor protein 53
PAMPs	Pathogen-associated molecular patterns
PAR-1	Protease-activated receptor 1
PDGF-AA	Platelet-derived growth factor AA
PDIs	Protein disulfide isomerases
PER1	Period Circadian Regulator 1
PERK	PKR-like ER kinase
PGC1-β	Peroxisome proliferator-activated receptor gamma coactivator 1-beta
PGE2	Prostaglandin E_2
pH	potential of Hydrogen
PINK1	PTEN-induced putative protein kinase 1
PML bodies	Promyelocytic leukemia nuclear bodies
PP1	Protein phosphatase 1
PPI	Peptidyl-prolyl isomerases
PRAK	p38-regulated/activated protein kinase
PSNE	post-senescence neoplastic emergence
PTEN	Phosphatase and tensin homolog
Rad17	Rad17 checkpoint clamp loader component
RAF	Rapidly accelerated fibrosarcoma
RAS	Rat sarcoma
Rb	Retinoblastoma protein
RHE	Reconstructed human epidermis
RHS	Reconstructed human skin
RIDD	IRE1α -dependent mRNA decay
RNA	Ribonucleic acid
RNC	Ribosome-nascent chain complex
ROS	Reactive oxygen species
RS	Replicative senesence

RTCB	RNA 2',3'-Cyclic Phosphate And 5'-OH Ligase
S1P	Site-1 Protease
S2P	Site-2 Protease
SAHF	Senescence-associated heterochromatic foci
SAMPs	Senescence-associated morphological profiles
SAPA	Senescence-associated proliferation arrest
SASP	Senescence-associated secretory phenotype
SA-βgal	Senescence-associtaed beta-galactosidase
SCAFs	Senescent-cell adhesion fragments
SCAPs	Senescent cell anti-apoptotic pathways
SCC	Cutaneaous squamous-cell carcinoma
Ser	Sérine
SERCA	Sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase
SERPINE1/PAI-1	Serpin Family E Member 1
SERPINH1	Serpin H1
SIPS	Stress-induced premature senescence
SIRT	Sirtuin
SMS	Senescence-messaging secretome
SOD3	Extracellular superoxide dismutase [Cu-Zn]
SP	Signal peptidase
SR	SRP receptor
SRF	serum response factor
SRP	Signal recognition particle
SS	Signal sequence
SSB	Single-strand break
STC1	Stanniocalcin 1
sWAT	Subcutaneous white adipose tissue
TA	Tail-anchored
t-BHP	Tert-butylhydroperoxide
TCA	Tricarboxylic acid cycle
TCD	N-deacetyl-N-(chromone-2-carbonyl)-thiocolchicine
TEAD1	TEA Domain Transcription Factor 1
TERC	Telomerase RNA component
TERS	Transmissible ER stress
TERT	Telomerase reverse transcriptase
TEWL	Transepidermal water loss
TFEB	Transcription Factor EB
TG	Tryglyceride
TGF-β	Transforming growth factor-beta
THBS1	Thrombospondin-1
Thr	Thréonine
TIF	Telomere dysfunction-induced focus
TIMP1	Metalloproteinase inhibitor 1
TIS	Therapy-induced senescence
TMD	Transmembrane domain
TNF	Tumor Necrosis Factor
TOPBP1	Topoisomerase-binding protein 1
TRAF2	tumor necrosis factor receptor- associated factor 2
TRAIL	TNF related apoptosis inducing ligand

TRC40/GET3	Guided Entry Of Tail-Anchored Proteins Factor 3
TREX	Transcription-export
TrkB	Tropomyosin receptor kinase B
TXNIP	Thioredoxin-interacting protein
UGGT	UDP-glucose glycoprotein glucosyltransferase
UHDR	Ultra-high dose rate
UN	Untransfected
uORFs	Upstream open reading frames
uPAR	Urokinase-type plasminogen activator receptor
UPR	Unfolded Protein Response
UPRE	Unfolded protein response element
UPS	Unconventional protein secretion
UV	Ultraviolet rays
XBP1	X-box Binding Protein 1
x-gal	5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside
ZFP36L1	mRNA decay activator protein ZFP36L1
α-Fuc	α-L-fucosidase
γ-H2AX	Gamma-H2A histone family member X

Foreword

According to the United Nations, old age is considered to begin at 60 years old (Scherbov and Sanderson 2020). By the year 2050, it is projected that 25% of the population in Europe and Northern America will be aged 65 or older. Worldwide, life expectancy at birth has increased since 1950 (**Figure 1**). This is mainly related to an overall drop in early and mid-life mortality, coupled with improvements in hygiene, healthcare, lifestyle, and medicine.

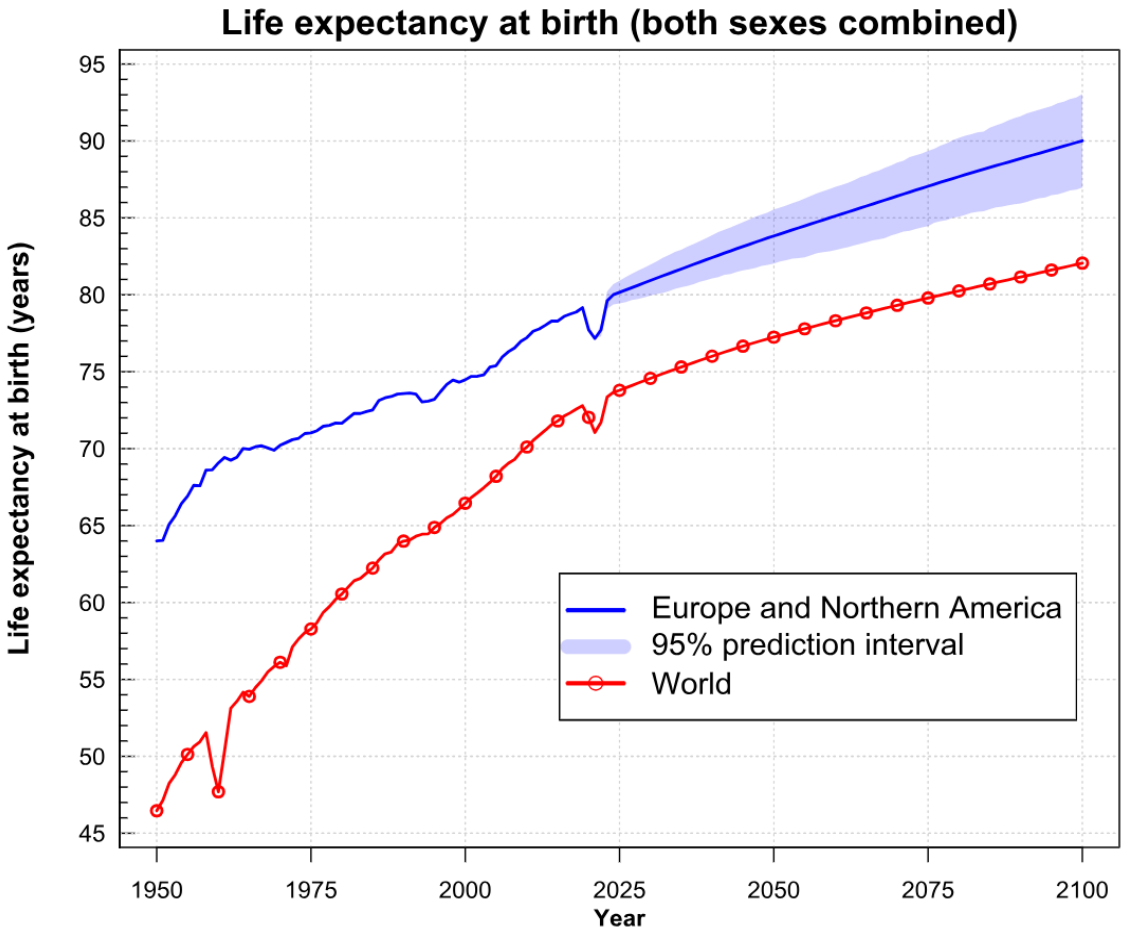


Figure 1. Graphical representation of life expectancy at birth for both sexes combined. Life expectancy at birth is the average number of years a newborn is projected to live if current mortality patterns of the year persist. *From © 2022 United Nations, DESA, Population Division. Licensed under Creative Commons license CC BY 3.0 IGO. United Nations, DESA, Population Division. World Population Prospects 2022.* **http://population.un.org/wpp/**

However, fluctuations in life expectancy are not uncommon and are often attributed to excess mortality caused by war-related shocks, famines, or epidemics, such as the COVID-19 pandemic in 2020 (Schöley et al. 2022).

In January 2023, INSEE published that the life expectancy for French people born in 2022 is

85.2 years for women and 79.3 years for men. Similarly, they addressed the decline in natality in France, which is 20% less than in 2010, raising concerns about the challenge of a global ageing population *(https://www.insee.fr/en/statistiques/7757334)*. For instance, this ageing population presents several socioeconomic challenges, such as increased healthcare costs, pension and social security burdens, and the need for adjustments in housing and infrastructure.

However, these demographic shifts are foreseeable, allowing us to proactively respond and devise appropriate policies to tackle these issues (Banks 2020). Hence, addressing population ageing, especially promoting healthier ageing, is gaining recognition as a critical societal challenge. Consequently, there is a significant need for robust research to enhance our understanding of ageing ("Enhancing the Impact of Aging Research for Its Intended Beneficiaries" 2021).

Among the initial signs of ageing, the appearance of wrinkles in the skin is likely one of the earliest and most noticeable traits, often preceding the onset of other age-related changes. According to IFOP (Institut français d'opinion publique), 61% of women over 50 years old are self-conscious about skin ageing (Ifop and Septembre 2023). Moreover, in everyday unconsciousness, we often associate the appearance of the skin with health; you look good, or you look healthy…

As the first barrier between the body and the environment, the skin represents a great model for studying mechanisms underlying ageing. Beyond what may seem superficial, investigating skin ageing can substantially enhance our understanding of broader mechanisms associated with ageing.

Introduction

The introduction of this thesis manuscript draws partially from our review article published in the International Journal of Molecular Sciences, titled: Exploring the Communication of the SASP: Dynamic, Interactive, and Adaptive Effects on the Microenvironment (Giroud et al. 2023).

1. The dynamics of ageing

A. Cellular senescence: An adaptative response to stress influencing the ageing process

A.1 Healthy versus pathological ageing

From a biomedical perspective, ageing can be considered pathological, normal, or successful (reviewed in; Gangbè and Ducharme 2006). Successful ageing refers to the process by which individuals maintain a high level of physical and cognitive function while presenting low-risk factors for diseases. For instance, aged people with normal blood pressure and low-density lipoprotein cholesterol are less susceptible to developing cardiovascular diseases than those with high blood pressure and high-low-density lipoprotein cholesterol (Rowe and Kahn 1987). In addition, an important determinant of successful longevity seems to be the environment and lifestyle. In the world, five areas, referred to as the “blue zones” (Loma Linda (USA), Nicoya (Costa Rica), Sardina (Italy), Ikaria (Greece), and Okinawa (Japan)) have been identified as unexcepted regions where people are known to live significantly longer and healthier, partly due to extrinsic factors playing a positive role in their ageing (Buettner and Skemp 2016). Particularly, in Okinawa, one identified habit that significantly increases lifespan is a diet rich in seafood and plants. Apart from genetic factors, the distinction between successful and normal ageing seems to precisely depend on extrinsic factors (Rowe and Kahn 1987).
However, more generally, living longer has led to an increased prevalence of age-related diseases, disability, and dementia (reviewed in; Redmond 2022). Moreover, as individuals age, they often develop multiple comorbidities, and managing these conditions necessitates a combination of treatments. Nevertheless, it is worth noting that the available therapeutic interventions for various age-related disorders may interact with each other (reviewed in; Guo et al. 2022). As a consequence, current research on ageing aims to improve late-life health and promote healthy ageing by preventing the progression of age-related issues (reviewed in; Partridge, Deelen, and Slagboom 2018) and increasing the autonomy of elderly people. This underscores the need for a better understanding of the mechanisms involved in the ageing

process. Additionally, rare “premature ageing” syndromes, such as the Hutchinson-Gilford progeria syndrome (HGPS), are conditions that require comprehensive understanding at both the cellular and organismal levels to better assist the affected children.

Interestingly, at the cellular level, HGPS cells exhibit similar features to those observed in aged cells, including reduced telomere length, premature cellular senescence (Benson, Lee, and Aaronson 2010), and an inability to produce a functional extracellular matrix (Hernandez et al. 2010).

Worldwide, it is estimated that approximately 350-400 children are living with progeria, a rare laminopathy caused by a mutation in the *LMNA* gene (https://www.progeriaresearch.org/). This mutation leads to the production of a truncated lamin A scaffolding protein called progerin (a mutant permanently farnesylated and carboxymethylated), which results in nuclear instability, increased DNA damage, and premature ageing. Clinically, children with HGPS have severe physical manifestations in many, but not all, organs. At the level of the skin, it appears thin, dry, scaly, and wrinkled with scleroderma-like changes. They often develop areas of hyperpigmentation and have reduced wound healing capacities, with more visible veins and diminished subcutaneous fat. The most significant problems that arise include low bone mineral density, osteolysis, atherosclerosis, narrow coronary arteries, and hypertension, which can ultimately lead to cardiovascular complications and early death (Kreienkamp and Gonzalo 2020). To date, clinical trials using lonafarnib, a farnesyltransferase inhibitor, have demonstrated beneficial effects in improving vascular stiffness and bone structure in children born with progeria. However, this treatment remains the only one available and does not offer a definitive cure. Instead, it can slow the onset of certain HPGS consequences and extend the lifespan of children by a few years (Murtada et al. 2023).

Therefore, research on aging seems to be a priority to help these children and to improve the quality of life for millions of people in their later years.

A.2 Hallmarks of ageing

Research on ageing has seen significant progress in recent years, particularly with the identification of well-conserved genetic pathways capable of modulating the rate of ageing. In 2013, López-Otín *et al.* proposed nine potential ageing hallmarks, referencing biological ageing processes shared across various organisms (reviewed in; López-Otín et al. 2013). Last year, they edited this list at twelve ageing hallmarks (**Figure 2**).

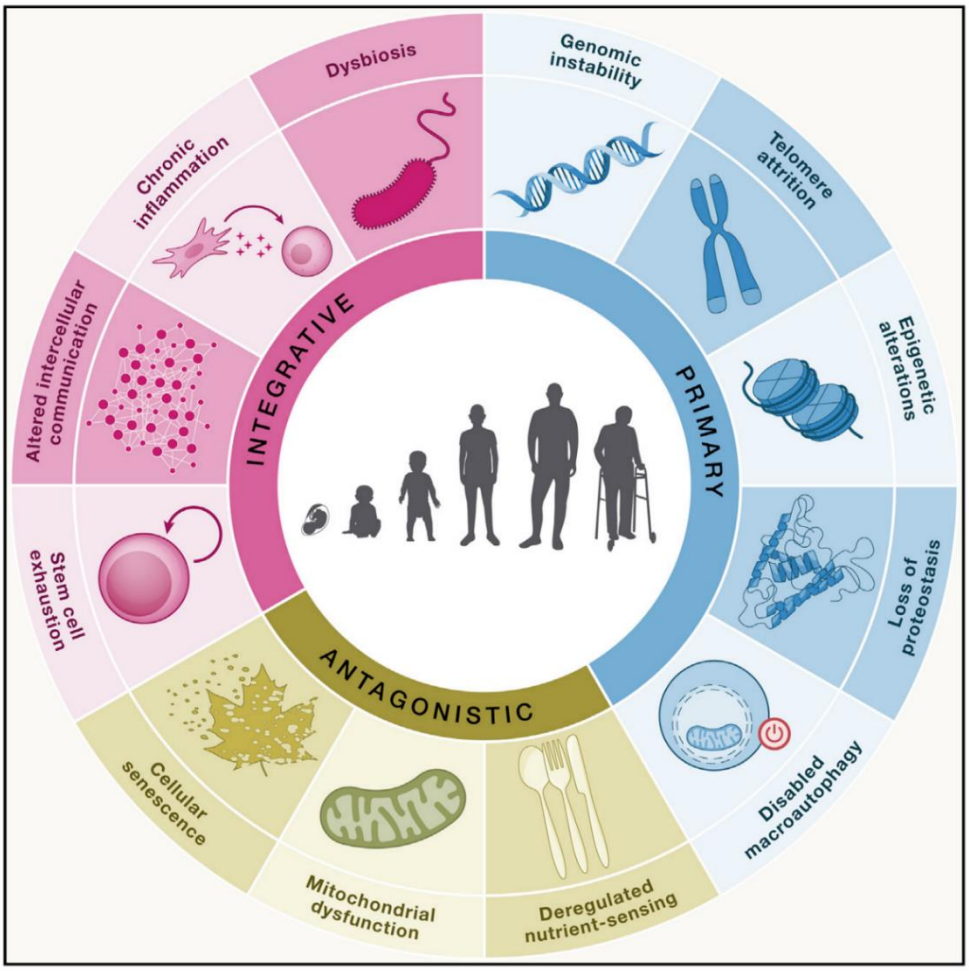


***Figure 2*. The twelve updated hallmarks of ageing proposed in 2023.** Hallmarks are grouped into three categories: Primary hallmarks, including genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, and disabled macroautophagy; antagonistic hallmarks, including deregulated nutrient-sensing, mitochondrial dysfunction, and cellular senescence; and integrative hallmarks, including stem cell exhaustion, altered communication, chronic inflammation, and dysbiosis. From López-Otín *et al.* 2013.

Notably, they proposed a new classification based on the hierarchy among the hallmarks (reviewed in; López-Otín et al. 2023). They defined primary hallmarks as processes responsible for causing cellular damage, including genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, and disabled macroautophagy. Antagonist hallmarks depict processes responsible for the damage response, including deregulated nutrient-sensing, mitochondrial dysfunction, and cellular senescence. Integrative hallmarks define the processes involved in the ageing phenotype, including stem cell exhaustion, altered intercellular communication, chronic inflammation, and dysbiosis. All these hallmarks conform to three conditions: they develop with age, their worsening accelerates ageing, and targeting them can delay, halt, or reverse the ageing process. While it is possible to address each hallmark individually, they seem strongly interconnected. Besides, anti-ageing compounds

often address multiple hallmarks, as seen with Metformin, one of the most widely studied drugs in clinical trials, exhibiting pleiotropic beneficial effects in attenuating several previously mentioned hallmarks of ageing, such as delaying cellular senescence (Kulkarni, Gubbi, and Barzilai 2020).

A.3 Discovery of cellular senescence

Until the second half of the 20th century, normal cultured cells were considered immortal. This belief was partly reinforced by Alexis Carrel, whose research demonstrated that cell cultures derived from chicken heart tissue could be maintained indefinitely *in vitro*. Therefore, when Leonard Hayflick and Paul Moorhead published their observations, revealing that normal human fibroblasts grown in optimal culture conditions have a limited proliferative capacity, they faced significant challenges in convincing the scientific community (reviewed in; Shay and Wright 2000). In their first research article in 1961, they described the culture of normal fibroblasts in three phases: Phase I is the primary culture, an adaptation phase for cells isolated from biopsies to culture condition. Phase II is the exponential growth phase of cultured cells, and phase III is the phase of progressive cell replication arrest, named the "Hayflick limit" (Hayflick and Moorhead 1961). Then, in 1965, Leonard Hayflick hypothesized that the limited proliferative capacity of normal human cells is probably linked to the doubling potential of cells. He suggested that the accumulation of non-dividing cells may result from accumulated damage in addition to chromosome abnormalities he observed in phase III (Hayflick 1965). At this time, he named this process "replicometer" until 1990 when Calvin Harley observed that telomeres (long repeated DNA segments (in humans; 5'TTAGGG-3') at the end of chromosomes) shorten as cultured normal cells approach the Hayflick limit (Harley, Futcher, and Greider 1990). Nowadays, this phenomenon is recognized as replicative senescence (RS), and the definition of cellular senescence persists as a stable and strong cell cycle arrest in which cells remain metabolically active.

A.4 Types of cellular senescence and initiating stimuli

Since this discovery, our understanding of cellular senescence has evolved. It is now recognized as a complex response to the cell proliferative exhaustion or stressors, mainly induced through the DNA Damage Response (DDR) triggered by events like single-strand breaks (SSB) or double-strand breaks (DSB), with specific exceptions noted. In addition, cellular senescence can be induced in both normal cells but also in immortalized and cancer cells. In this section, the origin of cells will be discussed to provide a better understanding of the mechanism leading to senescence depending on the context.

A.4.1 Replicative senescence (RS)

Mammalian telomeres comprise kilobases of G-rich repetitive DNA sequences and protein complexes located at the ends of linear chromosomes, preserving the genetic information (O'Sullivan and Karlseder 2010). Telomeric DNA forms protective loops, known as T-loops, which consist of double-strand DNA ending with a single-strand 3' tail (referred to as the G-overhang) that can fold into double-strand DNA sequences, forming the D-loop (**Figure 3**).

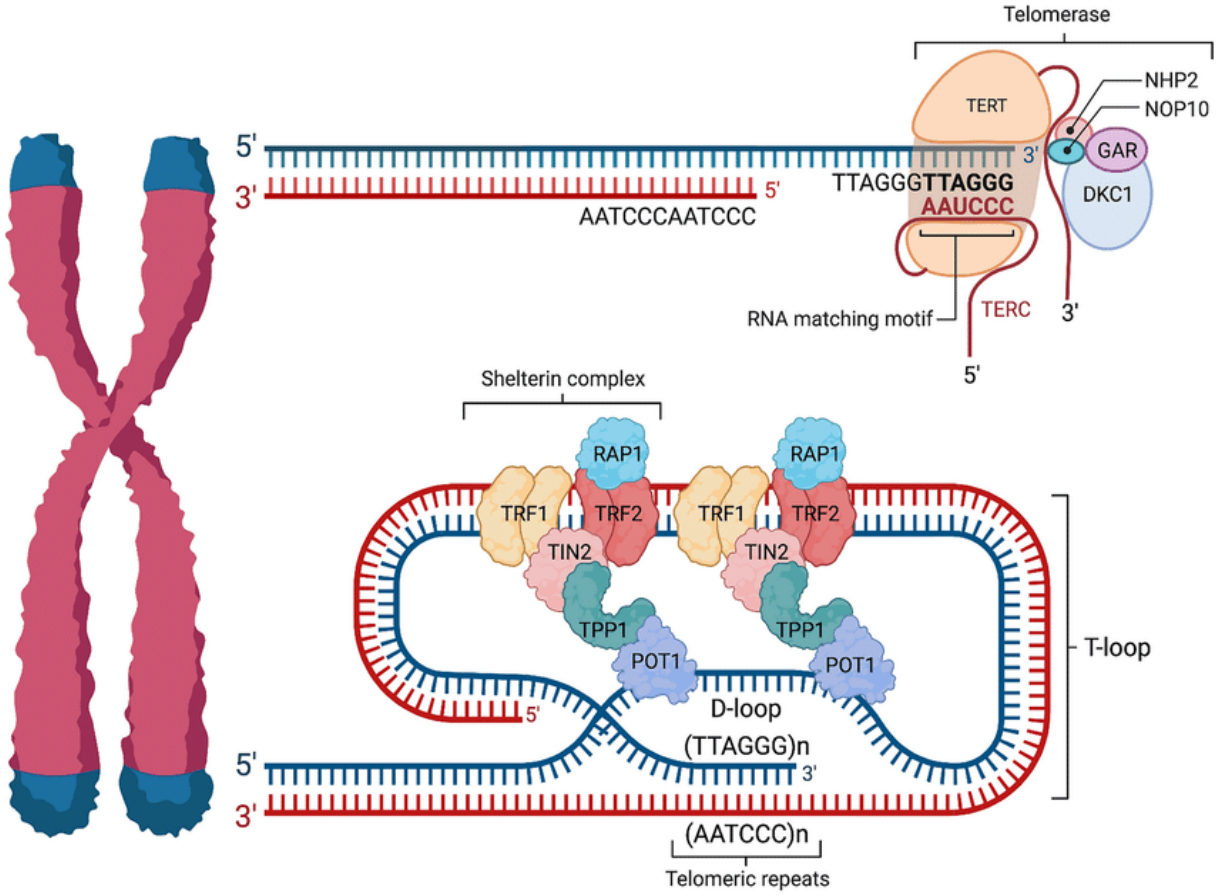


Figure 3. **The telomere structure and maintenance.** Telomeres are made up of 5'-TTAGGG-3' telomeric repeats organized in large loop structures protected by shelterin complexes (TRF1, TRF2, TIN2, TPP1, POT1, and RAP1). Complete telomere replication is performed by telomerase. From Muoio *et al.* 2022.

Regulation of this circular structure is managed by shelterin complexes, formed by six telomere-specific proteins (reviewed in; Maestroni, Matmati, and Coulon 2017).

During replication, DNA polymerase synthesizes the DNA strand in the 5' to 3' direction, allowing continuous replication of the leading strand. However, lagging strand replication is discontinuous and requires RNA for DNA synthesis. Upon the removal of RNA primers, the last primer at the end of the 3' tail results in a gap where DNA is not fully synthesized, an issue known as "the end-replication problem" (Wynford-Thomas and Kipling 1997). This process

causes telomeres to shorten with each cellular division until they reach a critical length that renders them unable to form the T-loop. Chromosomal DNA ends are then recognized as DSBs, triggering the DDR pathway and subsequent cell cycle arrest through p53/p21^{CIP1} (D'Adda Di Fagagna et al. 2003; Herbig et al. 2004). These DSBs lead to the specific accumulation of DNA damage response proteins, such as 53BP1, γ-H2AX, Rad17, ATM, and MRE11 creating domains named telomere-dysfunction-induced foci (TIF) (Takai, Smogorzewska, and de Lange 2003). However, a new hypothesis suggests a multistep model for the natural entry into replicative cellular senescence. Indeed, Ghadaouia *et al.* propose that TIFs alone should only induce a mild DDR and an unstable cell cycle arrest. However, they demonstrate that telomere uncapping is prone to homologous recombination-mediated sister chromatid fusion, generating abnormal chromosomal segregation, irreversible genome lesions, and instability that ensures persistent proliferative arrest (Ghadaouia et al. 2021). Regarding signalling pathways leading to cell cycle arrest, they will be detailed in section A.5. Nevertheless, it is relevant to mention that the two main pathways, p53/p21^{CIP1} and p16^{INK4}/Rb, which often drive cell cycle arrest, are both necessary for cell cycle arrest in normal human cells during RS. Moreover, it appears that p21^{CIP1} plays a role in the initiation of senescence, whereas p16^{INK4} seems to be induced later and plays a role in the maintenance of a stable and strong cell cycle arrest (Alcorta et al. 1996; Stein et al. 1999).

Conversely, the end-replication problem could be counteracted by the telomerase, a ribonucleoprotein that adds telomeric DNA sequences to the 3' tail to prevent the shortening of telomeres (Greider and Blackburn 1985). Telomerase is composed of two subunits: the telomerase RNA component (TERC), which acts as a template for the enzymatic subunit telomerase reverse transcriptase (TERT). However, in normal human cells, telomerase is expressed only during embryonic differentiation, and then in adulthood, its expression is limited in germ cells, activated lymphocytes, and some human stem cells (Cong, Wright, and Shay 2002; Hiyama and Hiyama 2007). In addition, the reactivation of telomerase activity in cancer cells occurs in up to 90% of cases, offering high proliferative potential to these cells and representing an interesting target for anticancer therapeutics (Jafri et al. 2016). Nowadays, exogenous hTERT is used to immortalize primary human cells (K. M. Lee, Choi, and Ouellette 2004).

A.4.2 Oncogene-Induced Senescence (OIS)

According to the cellular microenvironment, abnormal proliferative signals from oncogenes (mutated versions of normal genes with the potential to induce carcinogenesis) can be a source of stress for cells (reviewed in; V. G. Gorgoulis and Halazonetis 2010). Although the activation of the Ras pathway is common in human cancers, it is noteworthy that in benign tumors,

oncogenic signalling has the potential to induce cellular senescence. Indeed, the expression of the oncogenic form of *RAS (H-RAS^{G12V})* in normal human and murine cells results in permanent G1 arrest, accumulation of p53 and p16^{INK4}, and a premature senescent phenotype (Serrano et al. 1997). This process, referred to as "oncogene-induced senescence" (OIS) has now been described in various *in vivo* models, including the activation of other oncogenes like *K-RAS^{G12V}*, *N-RAS^{G12D}* and *B-RAF^{V600E}* (Collado et al. 2005; Braig et al. 2005; Dhomen et al. 2009). Often, OIS initiates a hyper-replicative phase, causing disruptions in the DNA replication fork progression and thus DDR activation (Di Micco et al. 2006). Another hypothesis is that DNA damage can also be induced due to the accumulation of reactive oxygen species (ROS) induced by oncogenes (A. C. Lee et al. 1999), leading to the activation of p38^{MAPK} and its downstream effector PRAK (Sun et al. 2007). At least, these two models trigger the activation of p53 and thus senescence. Additionally, OIS could also be reinforced due to the accumulation of senescence-associated heterochromatic foci (SAHFs) (Narita et al. 2003).

A.4.3 Oncogene inactivation-induced senescence (OIIS)

Additionally, the inactivation of tumor suppressor genes like *PTEN* or *Rb* can also trigger senescence *in vivo* (Z. Chen et al. 2005; Shamma et al. 2009). This process, known as oncogene inactivation-induced senescence (OIIS), can lead to different cell fates depending on the cell's origin. For instance, invalidation of *NF1* (a tumor suppressor) in mouse embryonic fibroblasts promotes RAS and AKT activation, leading to the immortalization of these cells, whereas, in normal human diploid fibroblasts, it induces a transient activation of RAS, resulting in premature senescence (Stéphanie Courtois-Cox et al. 2006). Wu *et al.* demonstrated that the inactivation of c-MYC in diverse tumor types including lymphoma, osteosarcoma, and hepatocellular carcinoma induces senescence with distinct consequences depending on the tumor type (C.-H. Wu et al. 2007).

Therefore, it appears that the cell sensitivity to mitogenic stress is an important parameter in the establishment of OIS and OIIS and that different cell types may exhibit different responses to oncogenic stress (S. Courtois-Cox, Jones, and Cichowski 2008).

A.4.4 Stress-induced premature senescence (SIPS)

It is now recognized that senescence can be prematurely induced by intrinsic and/or extrinsic sublethal stressors. This phenomenon has been referred to as stress-induced premature senescence (SIPS) and has been explained by thermodynamic arguments, suggesting that stresses could result in distinct cell fates, including one characterized by higher levels of damage and lower global metabolic activity (Toussaint et al. 2000). In response to DNA damage, cells enter transient cell cycle arrest to facilitate repair. However, depending on the

stress origin (e.g. oxidative stress, genotoxic stress…), intensity, and duration, cells can undergo three different fates (**Figure 4**).

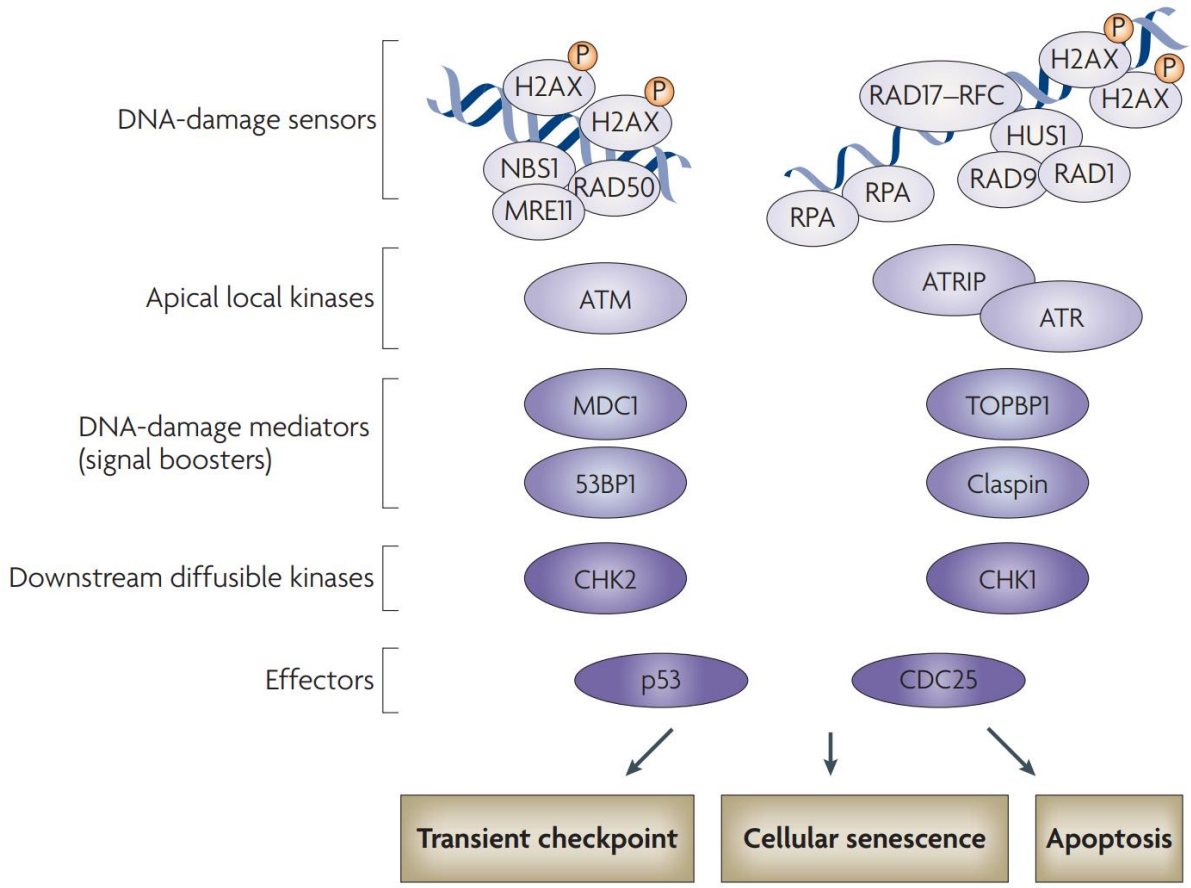


***Figure 4*. Fate of cells encompassing DNA damage lesions.** DDR signalling involves two DNA damage sensors: the MRE11-RAD50-NBS1 (MRN) complex for DSBs, and the RAD9-RAD1-HUS1 for SSBs. Upon detection, DSBs recruit and activate the apical kinase ataxia Telangiectasia mutated (ATM), leading to CHK2 activation through DNA-damage mediators like p53-binding protein 1 (53BP1) or DNA-damage checkpoint 1 (MDC1). Conversely, SSBs recruit and activate ataxia telangiectasia- and Rad3-related (ATR) associated with ATR-interacting protein (ATRIP), activating CHK1 through topoisomerase-binding protein 1 (TOPBP1) and Claspin. Ultimately, p53 and CDC25 phosphatases serve as the terminal components of the DDR signalling cascade, culminating in cell-cycle arrest. In addition, the recruitment of ATM and ATR mediate the phosphorylation of the histone H2AX at the chromatin (γH2AX). From d'Adda Di Fagagna *et al.* 2008.

Mild DNA damage induces a transient DDR-mediated cell cycle arrest, which allows proper repair and the resumption of normal proliferation. Conversely, chronic or acute subcytotoxic stresses lead to a permanent cell-cycle arrest mediated by the robust activation of the DDR pathway, resulting in a senescent phenotype. In cases of more severe DNA damage, cells may undergo apoptosis or necrosis, which are programmed cell death mechanisms designed to eliminate damaged cells from the microenvironment (D'Adda Di Fagagna 2008; Sulli, Di Micco, and Di Fagagna 2012). SIPS can result from direct DNA damage caused by ionizing radiation

or by cytotoxic drugs, often utilized in cancer treatments, as further detailed in section A.4.4.

Other natural sources of radiation include ultraviolet rays (UV). Various models of UV-induced premature senescence have been developed in different cell types, often focusing on skin cells. These models include among others, fibroblasts (UVB-induced: Chainiaux et al. 2002; Debacq-Chainiaux et al. 2005; UVA-induced: Yi et al. 2018 ; Berneburg et al. 1999) keratinocytes (UVB-induced: Bertrand-Vallery et al. 2010; Bauwens et al. 2023; UVA-induced: Valerio et al. 2021) and melanocytes (UVB-induced: Medrano et al. 1995; Martic et al. 2020). The biological damage induced by UV will be described later in part 3.
In addition to direct DNA damage, SIPS can also be induced because of oxidative stress. Mitochondria are considered the major source of ROS and mitochondrial dysfunction-associated senescence (MiDAS) induces a specific senescent phenotype described in A.4.5. Besides, normal human dermal fibroblasts treated with hydrogen peroxide (H_2O_2), *tert*-butylhydroperoxide (*t*-BHP), or exposed to hyperoxia enter prematurely into senescence (Toussaint et al. 2001). Additionally, fibroblasts exposed to sublethal concentrations of copper sulfate ($CuSO_4$) exhibit increased levels of ROS and undergo premature senescence through a $p38^{MAPK}$-dependent signalling pathway (Boilan et al. 2013). This pathway has been described, among others, as a crucial signalling pathway to stress-induced senescence (Debacq-Chainiaux et al. 2010). It is noteworthy that, in addition to normal cells, immortalized cells can also enter into senescence after stress exposures (De Magalhães et al. 2002). Indeed, while some DNA damage may occur in telomere regions, the establishment of the senescent phenotype occurs independently of telomere length (de Magalhães and Passos 2018; Hewitt et al. 2012). Besides, the designation of the senescent-induced phenotype is intrinsically linked to the stress inducer. Finally, the signalling pathways responsible for cell cycle arrest in SIPS often rely on $p21^{CIP1}$ and $p16^{INK4}$. However, depending on the stress inducer, it may initially induce another cyclin-dependent kinase inhibitor specific to that stress. Examples will be provided in section A.5.

A.4.5 Therapy-Induced Senescence (TIS)

Nowadays, conventional treatments for cancers including radiotherapy and/or chemotherapy have been linked to the onset of cellular senescence in tumors but also in non-tumor cells and tissues (reviewed in; Wang, Kohli, and Demaria 2020). Briefly, therapy effectors inducing senescence can be labeled based on the nature of the inducer: Irradiation-induced senescence (IRIS) is based on the use of ionizing radiation, like X-rays, and conventional or ultra-high dose rate (UHDR) proton irradiation (Liao et al. 2014; Răileanu et al. 2022; Buonanno, Grilj, and Brenner 2019). Chemotherapy-induced senescence depends on the nature of the cytotoxic drugs used, such as topoisomerase inhibitors like Doxorubicin, and

alkylating agents like Cisplatin (reviewed in; Chang et al. 1999). Additionally, CDK4/6 inhibitors, epigenetic modulators, and immunotherapeutic drugs have the potential to induce senescence (reviewed in; Wang, Kohli, and Demaria 2020). One common feature of these different senescence inducers is the generation of irreparable DNA lesions that cause sustained cell cycle arrest in cancer and non-cancer cells.

A.4.6 Mitochondrial Dysfunction Associated Senescence (MiDAS)

Accumulating evidence indicates a dynamic connection between dysfunctional mitochondria and senescence. For instance, a causative link between mtDNA mutations and premature ageing phenotype has been described in mice (Trifunovic et al. 2004). In addition, mitochondrial dysfunction and mitochondrial ROS production (i.e. superoxide) are factors influencing replicative senescence as well as oncogene-induced senescence (Passos et al. 2007; Moiseeva et al. 2009). Indeed, mitochondrial ROS have the potential to induce DNA damage and DDR, leading to the induction of senescence. It has also been described that direct mitochondrial dysfunction, like SIRT3 and SIRT5 depletion, electron transport chain inhibition, mtDNA depletion, or mitochondrial HSPA9 depletion, trigger a senescence phenotype characterized by a cell cycle arrest and a distinctive SASP (Wiley et al. 2016). This senescence, known as MiDAS, occurs independently of DNA damage. The MiDAS signalling pathway is initiated by a decreased ratio of NAD+/NADH, causing AMPK-mediated p53 activation and subsequent NF-κB inhibition. Depleting mitochondria has been shown to reduce pro-inflammatory characteristics in cellular senescence. Moreover, *in vivo*, the reduction of mitochondrial content, achieved by inhibiting the mTORC1/PGC1-β axis, affects the persistence of DDR and thus prevents the appearance of certain senescence features (Correia-Melo et al. 2016).

A.5 Biomarkers and features of senescent cells

The scientific community has faced challenges in identifying robust and specific markers that characterize the senescent state, likely due to the heterogeneity of cellular senescence (reviewed in; Cohn et al. 2023). While growth arrest is the main characteristic of senescent cells, it is not sufficient to distinguish them from other non-proliferative cell states, such as quiescence or terminal differentiation (V. Gorgoulis et al. 2019). For these reasons, a combination of specific cellular and molecular markers and phenotypic features is necessary to identify senescent cells (**Figure 5**). However, there is no consensus on the number and type of markers required to identify senescent cells (Deruy et al. 2014), and the detection of primary biomarkers is needed before validating the senescent phenotype (reviewed in; Gil 2023).

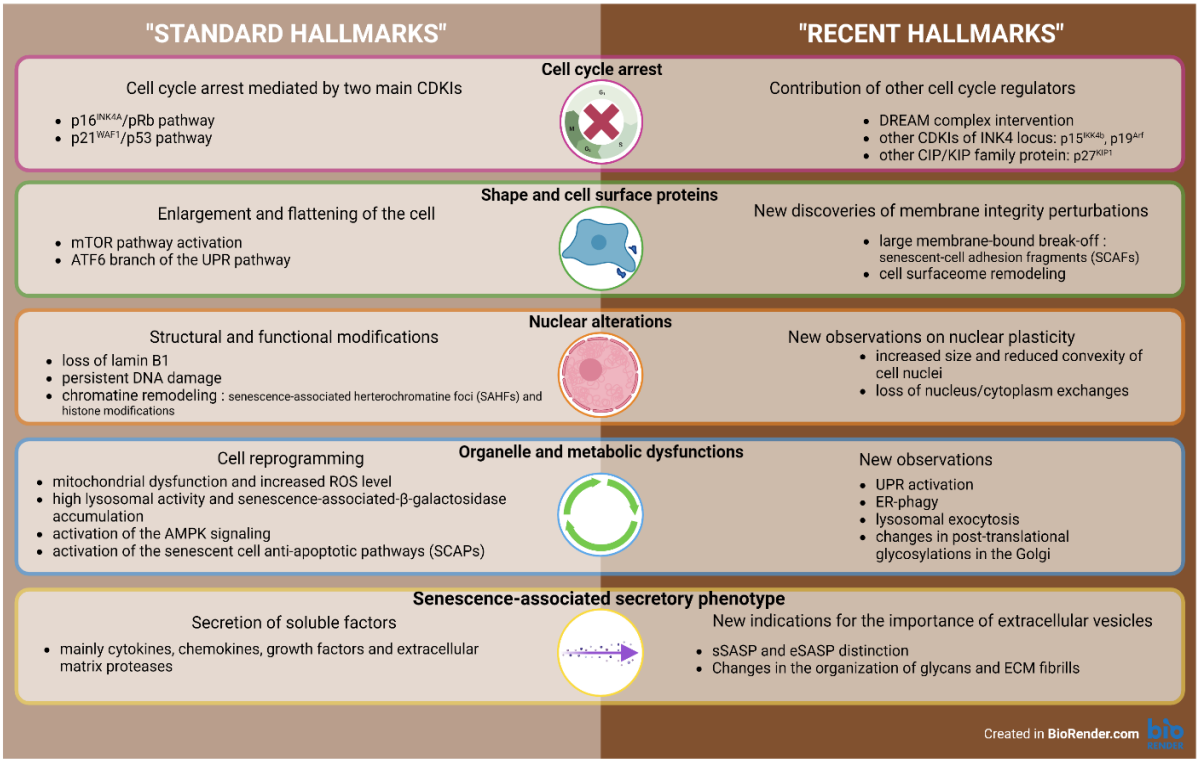


***Figure 5*. The updating hallmarks of senescence.** The scheme compiles the major hallmarks of senescent cells, which are classified into five characteristic groups: cell cycle arrest, shape and cell surface proteins, nuclear alterations, organelle and metabolic dysfunctions, and senescence-associated secretory phenotype. The light and dark brown portions indicate the well-described standard hallmarks and the more recent ones, respectively. From Giroud *et al.* 2023.

A.5.1 Cell cycle arrest

Cellular senescence is first characterized by a stable and strong cell cycle arrest. In recent years, the idea that this cell cycle arrest could be irreversible has been debated, as some studies demonstrated that specific senescent cells can escape it, leading to new clones associated with hallmarks of transformed cells (Nassour et al. 2016; Guillon et al. 2019; Martin et al. 2014). In their study, Gosselin *et al.* used normal human epidermal keratinocytes (NHEKs) and observed that after 3 weeks of culture, cells became senescent. Surprisingly, they demonstrated that some of these cells spontaneously emerge from senescent culture (from 10^{-5} to 10^{-2}) before reaching a second senescent plateau and undergoing a second emergence process (from 10^{-5} to 10^{-4}) (Gosselin et al. 2009). Later, they showed that the extent of oxidative damage and subsequent macroautophagic activity may serve as primary factors influencing the initial stages of neoplastic transformation through evasion of senescence (Deruy et al. 2014).

Hence, as emergence remains of rare occurrence, cell cycle arrest continues to be a widely

used biomarker for assessing senescence within a cell population. Indeed, senescent signals induce the expression of specific members of the INK4/ARF or CIP/KIP families of cyclin-dependent kinase inhibitors (CDKIs). The senescent cell cycle arrest occurs frequently in the G1/S transition and often depends on the activation of two main pathways: p53/p21^{CIP1} and p16^{INK4}/Rb (**Figure 6**). Both are interconnected and act in a complementary manner (reviewed in; Campisi and D'Adda Di Fagagna 2007).

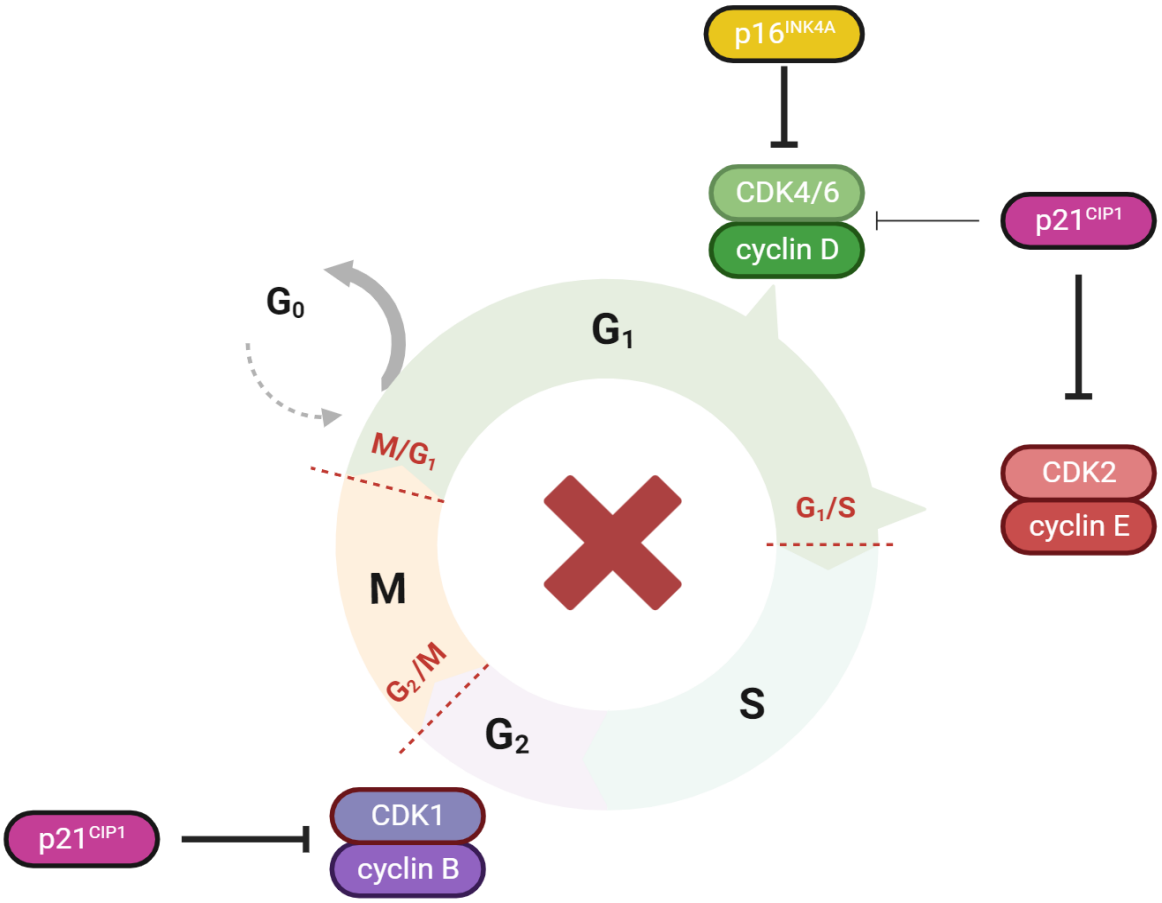


Figure 6. The main pathways leading to cell cycle arrest during senescence. Cell cycle progression is mediated by cyclin-dependent kinases (CDKs) and requires checkpoints to ensure proper timing of the different cell cycle phases. However, during DNA damage, inhibitors of CDKs (CDKIs) can induce a halt in different phases. During senescence, two main CDKIs result in cell cycle arrest; p16^{INK4} can induce a cell cycle arrest in G1/S by inhibiting the cyclin D/CDK4-6 complex, while p21^{CIP1} can additionally inhibit the cyclin E/CDK2 complex and the cyclin B/CDK1 complex involved in cell cycle arrest at G2/M. Figure created using Biorender.

After DNA damage, especially double-strand breaks, replication forks stalling, or telomere shortening, the DDR is activated. This activation involves the phosphorylation and activation of the transcription factor p53. The DDR acts through ATM and the ataxia telangiectasia RAD3-related protein (ATR) to recruit DNA damage repair proteins and downstream checkpoints kinase such as CHK2 and CHK1 respectively. The ATR/CHK1 pathway rapidly inhibits CDC25,

a positive regulator of the cyclin B/CDK1 complex (involved in the G2/M transition). Simultaneously, the ATM/CHK2 pathway activation leads to the phosphorylation of p53 and allows its dissociation from the ubiquitin ligase MDM2 (responsible for the constitutive degradation of p53). This activation of p53 results in the expression of *p21*^{CIP1}, coding for a CDKI responsible for inhibiting the cyclin E/CDK2 complex (G1/S). This inhibition prevents the hyperphosphorylation of the retinoblastoma protein (Rb) and subsequently allows the sequestration of the E2F transcription factor thanks to the recruitment of histone deacetylase (HDAC)-repressor complex (Sulli, Di Micco, and Di Fagagna 2012; Kumari and Jat 2021). Among the genes regulated by E2F are cyclins (e.g. cyclin E), cyclin-dependent kinases (e.g. CDK2), DNA replication factors, and regulators of apoptosis (DeGregori et al. 1997). Moreover, p21^{CIP1} is also able to inhibit the cyclin D/CDK4-6 complex (G1/S) which also results in E2F inhibition. Regarding the growth arrest in G2/M, the involvement of p21^{CIP1} in inhibiting the cyclin B/CDK1 complex is noteworthy (Gire and Dulic 2015). Interestingly, the p53/p21^{CIP1} pathway is also involved in assembling the repressive dimerization partner Rb-like E2F multi-vulval class B (DREAM) complex (reviewed in; Quaas, Müller, and Engeland 2012; Kumari and Jat 2021).

During senescence, the activation of p53/p21^{CIP1} is often transient and gives way to another CDKI, p16^{INK4} which is responsible for maintaining the cell cycle arrest (Stein et al. 1999). During DNA damage, replicative senescence, or OIS, the INK4/ARF locus is activated. This locus encodes two genes, *CDKN2A* and *CDKN2B*, allowing the expression of three proteins, p16^{INK4} and p14 ^{ARF} for *CDKN2A*, and p15^{INK4B} for *CDKN2B.* The INK4/ARF activation leads to two main signalling cascades responsible for cell cycle arrest. On one hand, p14^{ARF} can control the p53-MDM2 pathway responsible for the G1/S arrest. On the other hand, the Rb pathway is regulated by p16^{INK4} and p15^{INK4B}, which are CDKIs inhibiting cyclin D/CDK4-6 complex (reviewed in; W. Y. Kim and Sharpless 2006).

As mentioned, other cell cycle regulators are also involved in senescence-associated cell cycle arrest. Additionally, the actors of the cell cycle arrest can vary over time, with some being expressed at the onset of senescence and others during its late phases. As an example, in glyoxal-induced senescent keratinocytes, the cell cycle arrest is first mediated by the protein kinase B-FOXO3a-p27^{KIP1} pathway but is sustained over time by the p16^{INK4}/Rb pathway (Halkoum et al. 2022). Furthermore, in therapy-induced senescent breast cancer cell lines, the tyrosine kinase inhibitor Lapatinib can induce senescence by increasing the expression of p27^{KIP1} and p15^{INK4B} (McDermott et al. 2019), and in prostate cancer cells, supraphysiological androgen levels regulate the establishment of senescence through p15^{INK4B} (Mirzakhani et al. 2021).

A.5.2 Morphological changes and cell surface proteins

Experimental descriptions of senescent cells are consistently characterized by changes in cell size, as they are observed to be enlarged and flattened compared with proliferative cells (Greenberg, Grove, and Cristofalo 1977). Furthermore, it has been demonstrated that senescence-associated beta-galactosidase (SA-βgal) positive cells (another biomarker of senescent cells) from old mice have a cell area significantly increased compared to SA-βgal negative cells *in vivo* (Biran et al. 2017). The reason behind this increase in size is still unclear, although excessive cell size growth is known to contribute to cell signalling impairments, probably due to a decrease in the DNA: cytoplasm ratio that leads to altered cellular processes (Neurohr et al. 2019).

Interestingly, senescent cells exhibit cytoskeleton rearrangements. For instance, senescent fibroblasts manifest overproduction of vimentin, resulting in large bundles of long vimentin filaments (Nishio et al. 2001), and senescent epithelial kidney cells display reorganization of F-actin filaments and stabilization of microtubules (Moujaber et al. 2019). Additionally, H_2O_2-induced senescent fibroblasts showed a redistribution of focal adhesion proteins like vinculin or paxillin (Q. M. Chen et al. 2000).

Interestingly, a recent study demonstrates that senescent cells can release large membrane-bound fragments of themselves through cell-to-cell adhesion, a process named senescent-cell adhesion fragments (SCAFs) (Durik et al. 2023).

Mechanistically, morphological changes associated with senescence appear multifactorial, likely dependent on the cell type and the senescence inducer. In mammals, cell size has been described as an mTOR-dependent signal cascade (Fingar et al. 2002). In senescent fibroblasts, caveolin-1 seems to play a role in the morphological changes associated with senescence by controlling the activity of focal adhesion kinase and the formation of actin stress fibres (K. A. Cho et al. 2004). Conversely, Druelle *et al.* demonstrated that the activating transcription factor 6 (ATF6α) branch of the Unfolded Protein Response (UPR) controls the enlarged morphology in replicative senescent dermal fibroblasts (Druelle et al. 2016). The biological interconnection between UPR and senescence will be described later in section D.2.

Moreover, senescent cells undergo remodelling of their surface proteome (surfaceome) (Althubiti et al. 2014). Indeed, the proteins at the surface of senescent cells are for example enriched in dipeptidyl peptidase 4 (DPP4) (K. M. Kim et al. 2018) and in urokinase-type plasminogen activator receptor uPAR (Amor et al. 2020). In addition, the lipid composition of the plasma membrane is also modified during senescence. Mound *et al.* have demonstrated the gradual disappearance of sphingomyelin-rich domains during senescence in keratinocytes, impacting their migration ability (Mound et al. 2017).

A.5.3 Nuclear alterations

Senescence entails structural and functional modifications of the nucleus, including alterations in heterochromatin structure that form specialized domains known as SAHF (Senescence Associated Heterochromatin Foci) (Narita et al. 2003). These domains, described as transcriptionally inactive, harbor heterochromatin protein 1 (HP1), methylated histones like K9M-H3, and the variant of histone H2A macroH2A. The incorporation of macroH2A into SAHF is facilitated by two histone chaperones, HIRA and Asf1 (R. Zhang et al. 2005). It's worth noting that promyelocytic leukemia nuclear bodies (PML bodies), potential sites of transcription, may also contribute to SAHF formation, although the mechanism is not well understood (**Figure 7**).

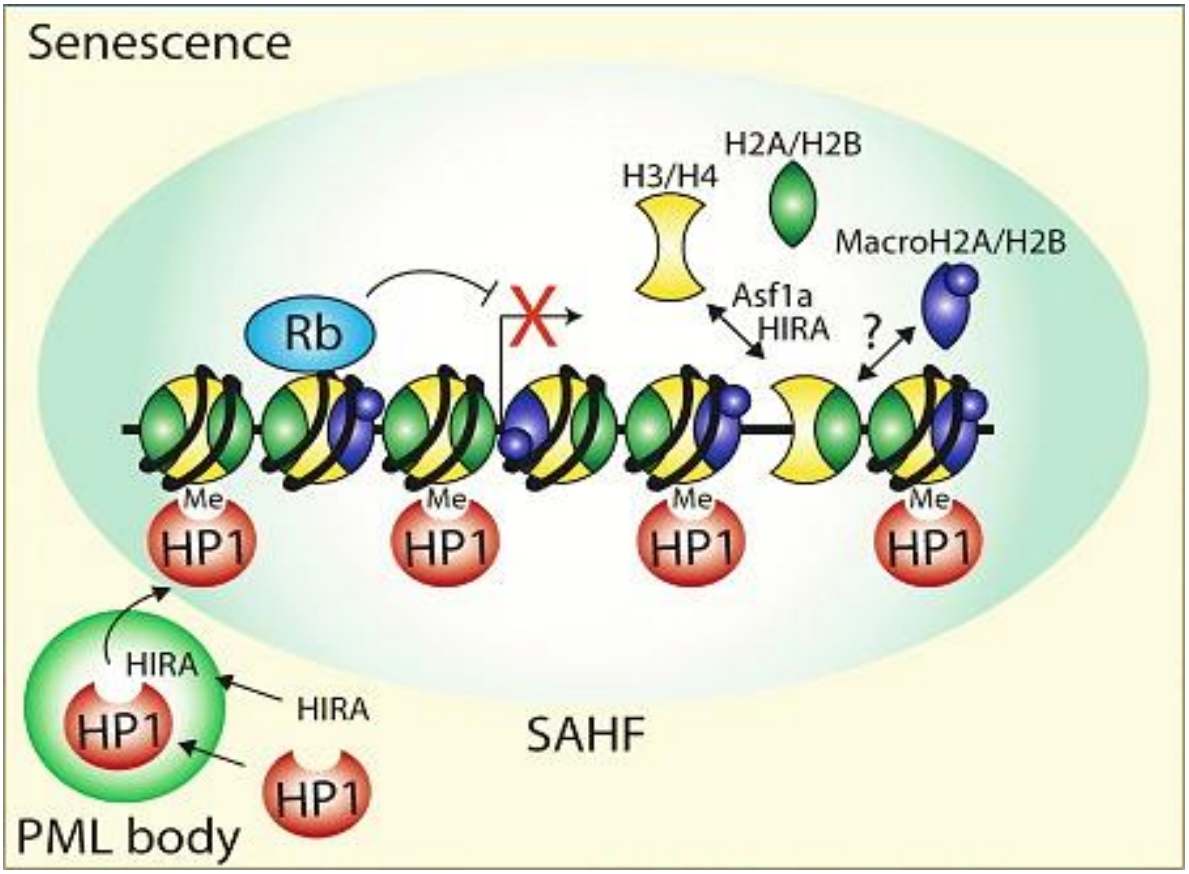


Figure 7. **Model for SAHFs formation**. Senescence-inducing factors trigger the repression of proliferating genes, recruiting the retinoblastoma protein (Rb). Senescence-associated heterochromatic foci are formed by various chromatin proteins, such as HP1, methylated histone, macroH2A, and histone chaperones like HIRA and Asf1. HP1 and HIRA transiently localize to PML bodies. From Schulz *et al.* 2005.

SAHF formation has been linked to the recruitment of Rb and appears to play a role in silencing E2F proliferative target genes, indicating that nuclear rearrangement correlates with gene

expression changes. The *nuclear lamina* (i.e. a fibrillar network located on the inner surface of the nuclear envelope which provides structural support to the nucleus and maintains its shape) is known to play a crucial role in regulating gene expression (Reddy et al. 2008). However, during senescence, a decline in mRNA and protein abundance of lamin B1, a structural component of the *nuclear lamina* that allows its stability, has been observed (Freund et al. 2012). The compromised integrity of the nuclear envelope in senescent cells results in the release of nuclear DNA fragments into the cytosol, known as cytosolic chromatin fragments (CCFs) (Adams et al. 2013). These CCFs are recognized by the cytosolic DNA sensor cyclic GMP-AMP synthase linked to a stimulator of interferon genes (cGAS-STING). Activation of the intracellular immune cGAS-STING pathway leads to an increased expression of pro-inflammatory cytokines through activation of NF-κB (Dou et al. 2017).

Additionally, senescent cells exhibit persistent DNA damage, collectively termed DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS) (Rodier et al. 2011). These persistent DNA damage aggregates in foci and primarily involve the accumulation of DDR-associated proteins, implicated both in the establishment of senescence-associated proliferation arrest (SAPA) and the late SASP (Rodier et al. 2009). The link between the DDR and the SASP will be further described in section B.2 of part 1.

Furthermore, new observations focused on nuclear plasticity during senescence, including alterations of the nuclear matrix, nucleolus, heterochromatin, and even nuclear shape and size (Pathak, Soujanya, and Mishra 2021). In particular, the increased nuclear shape is a promising biomarker to predict the senescent state (Heckenbach et al. 2022). Moreover, the nucleus–cytoplasm exchanges are altered in senescence, as the transcription-export (TREX) machinery and the nucleocytoplasmic trafficking (NCT) are downregulated (S. Y. Kim et al. 2020; S. S. Park et al. 2021). Interference in exchanges between the nucleus and the cytoplasm of senescent cells leads to a reduction in the transmission of extrinsic signals toward the nucleus and alters the nucleus-to-cytoplasm protein–RNA transport, resulting in the establishment of a “nuclear barrier” (S. Y. Kim et al. 2020; S. S. Park et al. 2021).

A.5.4 Organelles and biological process dysfunctions

Entering senescence impacts the cell’s organelles. Various studies describe morphological organelles alterations during senescence, such as an increase in the number and size of lysosomes, an increase in mitochondrial mass, expansion of the endoplasmic reticulum (ER), and altered structure of Golgi apparatus (Kurz et al. 2000; Correia-Melo and Passos 2015; Druelle et al. 2016; Despres et al. 2019).

A.5.4.1 Mitochondrial anomalies and increased ROS level

At the level of mitochondria, many changes are detected in their structure, dynamics, and function (**Figure 8**).

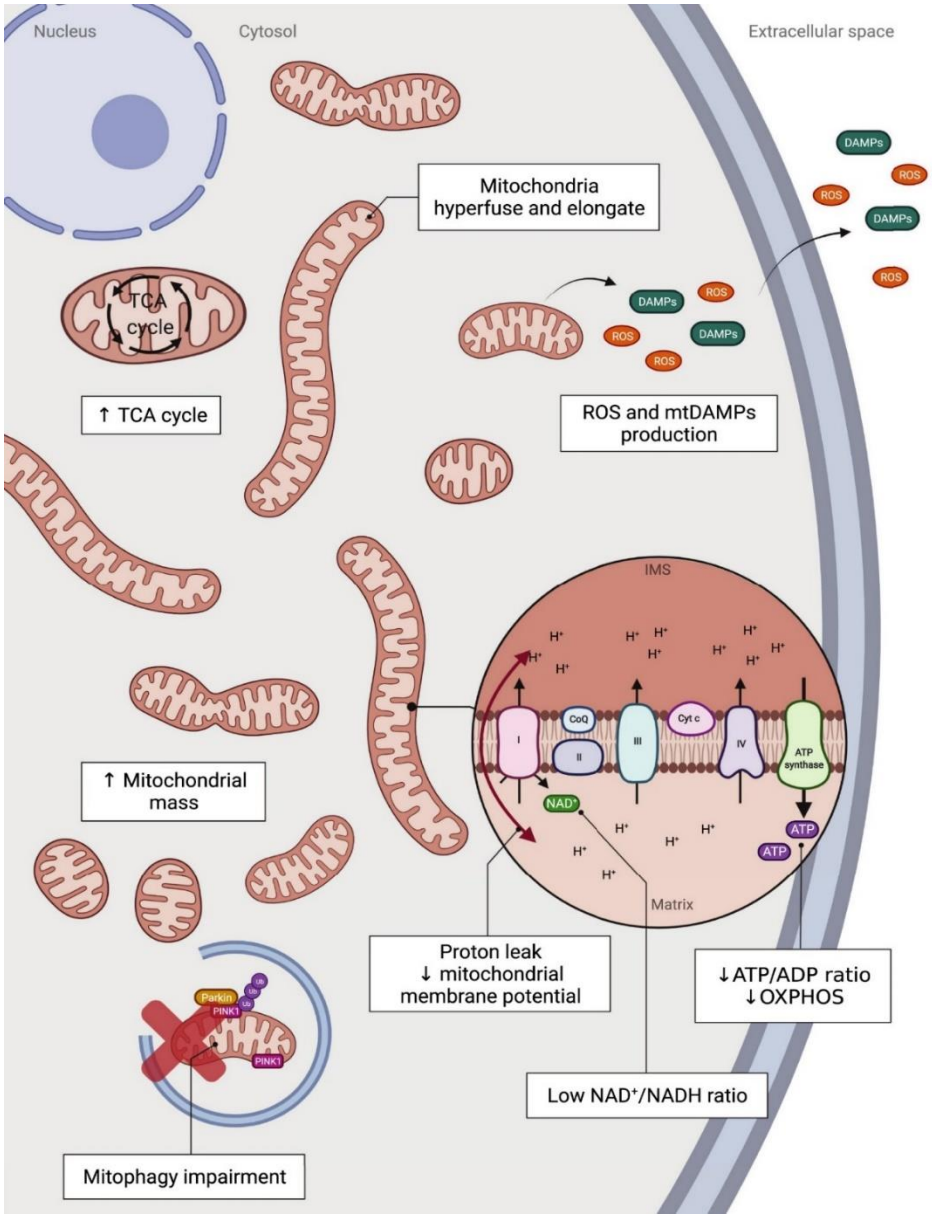


Figure 8. **Characterization of mitochondria in senescent cells.** In senescent cells, mitochondria undergo structural (i.e. hyperfused and elongated morphology), dynamic (i.e. elevated mitochondrial mass), and functional (i.e. enhanced TCA cycle, increased ROS and mtDAMPs, decreased membrane potential, decreased NAD+/NADH ratio and ATP/ADP ratio as well as OXHOPS) alteration. From Martini *et al.* 2023.

In senescent cells, mitochondria are elongated, and the mitochondrial network looks fused. This is mainly due to a decreased abundance of the mitochondrial fission protein Fis1, leading to an important fusion of mitochondria (Yoon et al. 2006). In various cell types, the

mitochondrial mass is also increased, such as in X-ray-induced senescent hepatocytes (Correia-Melo et al. 2016).

Several studies pointed out the deregulation of the tricarboxylic acid (TCA) cycle which is tightly coordinated with the oxidative phosphorylation (OXPHOS) and is characterized by a reduced efficiency during senescence. Moreover, those mitochondria often exhibit a loss of membrane potential (Passos et al. 2007). The decrease in proton (H+) in the intermembrane space leads to a less efficient production of ATP. All these perturbations result in a bioenergetic imbalance characterized by a decreased ATP/ADP and NAD^+/NADH ratios (reviewed in; Martini and Passos 2023) and increased AMP/ATP ratio. AMPK is one of the master regulators of metabolism, and its activation *in vitro* leads to increased biomarkers of senescence in fibroblasts (W. Wang et al. 2003).

Additionally, the autophagic degradation of damaged mitochondria is perturbed due to a decrease in mitophagy, primarily controlled by the PTEN-induced putative protein kinase 1 (PINK1) – Parkin pathway. This leads to the accumulation of dysfunctional mitochondria in senescent cells (Korolchuk et al. 2017).

More than being consequences of the senescent cellular state, some mitochondrial dysfunctions mentioned above can causally contribute to the induction of senescence, notably through the activation of AMPK signalling, as explained in section A.4.5 of part 1. More particularly, studies from the team of J.F. Passos demonstrated that the establishment of certain characteristics of the senescent phenotype, such as the SASP, is partly dependent on mitochondria. By removing mitochondria from the cells through a forced increase of mitophagy, they succeeded in attenuating the pro-inflammatory phenotype of SASP (Correia-Melo et al. 2016).

Mitochondria are an important source of ROS, but during senescence, their production is excessively increased (Passos et al. 2010). On the one hand, ROS can cause reversible or irreversible protein modifications, such as disulfides or protein carbonyls respectively, and on the other hand, they can induce lipid peroxidation and DNA damage, including mitochondrial DNA (mtDNA) damage (reviewed in; Avery 2011).

Furthermore, oxidized proteins accumulate in senescent cells due to a decline in protein degradation and endogenous antioxidants, such as a decrease in detoxifying enzymes system (e.g. superoxide dismutases, glutathione reductases, thioredoxin reductases or methionine sulfoxide reductases) (Lourenço Dos Santos, Petropoulos, and Friguet 2018). For example, under oxidative stress and increased ROS levels, lipofuscin, which is a covalently cross-linked brown-yellow aggregate of oxidized proteins, lipids, and metal, accumulates in lysosomes while it cannot be degraded by lysosomal enzymes or proteasomal system, and is therefore named “age-pigment” (Jung, Bader, and Grune 2007). Since this description, protocols for

efficiently detecting lipofuscin in senescent cells have emerged and are used in combination with other senescence biomarkers (Evangelou et al. 2017; Magkouta et al. 2024).

A.5.4.2 Activation of the senescent cell anti-apoptotic pathways (SCAPs)

Strikingly, senescent cells are resistant to apoptosis (E. Wang 1995). Senescent cell anti-apoptotic pathways (SCAPs) involve intrinsic and extrinsic pathways. The intrinsic pathway is a mitochondrial-mediated pathway involving the BAX/BAK pro-apoptotic proteins family. Upon an apoptotic signal, BAX/BAK proteins oligomerize in the outer membrane of mitochondria, forming a permeabilizing pore that provokes the mitochondrial outer membrane permeabilization (MOMP), the release of cytochrome c, and the subsequent formation of the apoptosome and activation of caspases (reviewed in; Jan and Chaudhry 2019). Under physiological conditions, the stoichiometric balance between BAX/BAK proteins and antiapoptotic proteins of the BCL-2 family regulates the permeabilization of mitochondria. Indeed, BCL-2 can heterodimerize with BAX/BAK proteins, preventing the pore formation. During senescence, BCL-2 family proteins are overexpressed, promoting resistance to apoptosis (E. Wang 1995; Yosef et al. 2016). The extrinsic pathways involve death receptors (DRs) activated by external stimuli or ligand molecules, resulting in a death-inducing signalling complex (DISC), which activates a cascade of caspase activation and apoptotic signalling. Resistance may occur because of an increased expression of Decoy receptors DCR1 and DCR2. These receptors can structurally bind ligands such as TNF-related apoptosis-inducing ligands (TRAIL) but are incapable of signalling, and it has been shown that senescent fibroblasts exhibit an increase in the expression of DCR1 and DCR2 receptors (reviewed in; Soto-Gamez, Quax, and Demaria 2019).

A.5.4.3. Alterations to the Endoplasmic reticulum and Golgi apparatus

During senescence, the ER homeostasis is perturbed and thus activates multiple signalling networks, such as the unfolded protein response (UPR) (Kaufman 1999). The link between UPR and senescence is described in part 2. However, the other ER quality control systems referred to as endoplasmic reticulum-associated protein degradation (ERAD) and ER-phagy, are poorly understood in the senescence context. To date, only one study has reported a relationship between ER-phagy and senescence. The authors demonstrated activation of the ER-phagy mediated by FAM134B, an ER-phagy receptor, upon advanced glycation end products (AGEs) stress-induced senescence and showed that enhancement of ER-phagy reduces the percentage of SA-βgal positive cells (Luo et al. 2021).

The Golgi structure is also altered in senescent cells (Despres et al. 2019). These alterations can not only be mediated by the translocation of a G protein γ subunit from the plasma membrane to the Golgi (J. H. Cho et al. 2011) but also by the impaired expression of the vacuolar ATPase ATP6V0A2, which acidifies organelles such as Golgi, endosomes, or lysosomes (Udono et al. 2015). This, results in deep changes in post-translational glycosylation in the Golgi, impacting SASP compounds.

A.5.4.4 High lysosomal activity and senescence-associated β-galactosidase

β-galactosidase is an enzyme located in lysosomes, catalyzing the hydrolysis of terminal β-D-galactose residues, with optimal activity observed at pH 4 in proliferative cells. However, in 1995, Dimri *et al.* published that upon senescence induction, the cytochemical detection of β-galactosidase is noticeable at a suboptimal pH of 6,0 in cultured human senescent cells. Their study also demonstrated that this marker, referred to as senescence-associated β-galactosidase (SA-βgal), is detected and accumulates in an age-dependent manner in human skin samples (Dimri et al. 1995). Since this observation, the origin of SA-βgal has been linked to several factors. Firstly, to confirm the hypothesis of a residual lysosomal activity at a suboptimal pH and avoid the possibility of an extra lysosomal pH 6,0 β-galactosidase in senescent cells, Kurz *et al.* used flow cytometric assays to assess the involvement of the lysosome in the detected activity at pH 6,0. Not only did they show the lysosomal origin of SA-βgal, but they also correlated its activity to an increase in lysosomal content and its related increased protein abundance in senescent endothelial cells (Kurz et al. 2000). These observations are in line with previous studies showing an increase in the number and size of residual bodies at late passages (Brunk et al. 1973). Then, it has been demonstrated in senescent fibroblasts, that the activity of SA-βgal was concentrated in autophagic vacuoles, indicating an accumulation of degradative autolysosomes (Gerland et al. 2003). Finally, the gene encoding the classic lysosomal enzyme named *GLB1* (galactosidase beta 1) was found to be overexpressed in senescent cells (B. Y. Lee et al. 2006). The mechanisms and regulators of lysosomal biogenesis remain not completely understood. Interestingly, mTOR can control the TFEB transcription factor which, in turn, regulates an important network of lysosomal genes (Sardiello et al. 2009; Roczniak-Ferguson et al. 2012). However, not all lysosomal enzymes exhibit a TFEB-binding motif on the promoter of their coding gene, such as for the α-L-fucosidase (α-Fuc), a member of the glycoside hydrolases, whose increased activity at senescence is higher than the one of SA-βgal (Hildebrand et al. 2013). Nonetheless, the detection of SA-βgal is still one of the most used biomarkers to assess cellular senescence in cultured cells or tissues. Indeed, several techniques are used to identify SA-βgal-positive cells,

such as cytochemical detection based on the cleavage of a chromogenic substrate of β-galactosidase named 5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside (x-gal), or fluorescent detection using another substrate named C_{12}FDG, which becomes fluorescent after cleavage by β-gal (Debacq-Chainiaux et al. 2009). While this senescent biomarker is widely used, some studies pointed out its lack of specificity. As an example, one of them demonstrated that SA-βgal is detectable in confluent quiescent cells or under serum starvation (Yang and Hu 2005). In addition, fibroblasts derived from patients suffering from a genetic disorder characterized by defective lysosomal β-galactosidase, become senescent without expressing SA-βgal (B. Y. Lee et al. 2006). Although SA-βgal is widely used as a biomarker of senescent cells, its detection alone is insufficient to characterize the senescent phenotype. It must therefore be used in combination with other senescence biomarkers.

A.5.4.5 Lipids modifications

Lipids and lipid metabolism are central to cellular signalling. However, in the context of cellular senescence, particularly regarding the composition and effects of the SASP, lipid components are understudied compared to secreted proteins. It is now known that senescent cells generate a variety of oxylipins, which are biologically active lipids deriving from the oxygenation of polyunsaturated fatty acids (PUFAs), that can contribute to inflammation and fibrosis (Wiley et al. 2021). In addition, the cyclooxygenase 2 (COX-2) has been described as a potent inducer of senescence in human fibroblasts, primarily through the secretion of prostaglandin E2 (PGE2) (Martien et al. 2013).

As lipids are classified into three groups: triglycerides, which are used for energy; steroids, including cholesterol and hormones; and phospholipids, which are essential for membrane structures, it's clear that lipid metabolism plays a crucial role in the senescent phenotype. In their review, Hamsanathan et *al.*, summarized the changes in the composition of these different class of lipids during senescence and raises the importance of using lipidomics and phospholipidomics mass spectrometry analyses to better characterized the lipid composition of senescent cells and to identified new targets (Hamsanathan and Gurkar 2022). For instance, quantitative mass spectrometry analyses of TIS cells demonstrated an enrichment of lipid droplets, an enhanced lipid metabolism and lipid peroxidation when compared to proliferative or quiescent cells (Flor et al. 2017). In addition, an encouraging result has recently demonstrated that CD36, a membrane receptor involved in the uptake of long-chain fatty acids, may play a role in the accumulation of triacylglycerols during the replicative senescence of fibroblasts, emphasizing the importance of studying the regulation of lipids in the context of cellular senescence in mammals (Lizardo et al. 2017). Furthermore, CD36 seems to be implicated in membrane remodeling in replicative senescent cells as its overexpression

induces a senescence-like phenotype in proliferative cells and may contribute to the production of an active SASP (Saitou et al. 2018).

B. SASP communication and its impact on the microenvironment

B.1 Characteristics of SASP

The secretory profile of a cell is shaped by intrinsic features, dictated by its differentiation stage, as well as extrinsic factors like changes in the cellular environment. Notably, cells undergoing senescence exhibit a distinct remodelling of their secretory profile known as SASP (reviewed in; Tan et al. 2021). Examining the secretome of senescent cells reveals modifications in the levels of secreted and extracellular vesicle (EV)-related components. Senescence establishment can cause these components to be either exacerbated or partially depleted, and can also lead to the secretion of new components when compared to proliferative cells (Matos, Gouveia, and Almeida 2015).

B.1.1 Reported SASP factors

SASP is complex and is composed of hundreds of different proteins and non-protein signalling molecules (Basisty et al. 2020; Wiley et al. 2019). Despite the diversity of the factors secreted, a core protein secretome, known as the sSASP is mainly composed of pro-inflammatory cytokines, chemokines, growth factors, and matrix metalloproteinases (MMPs) (**Table 1**).

Table 1. Shared SASP proteins between different cell types under different senescent inducers. From Giroud *et al.* 2023.

SASP factor	Cell type	Inducer	Detection	References
IL-6	Human fibroblasts (WI-38, IM90, BJ) Prostate epithelial cells (PrECs) Human keratinocytes (NHEK)	Irradiation-induced senescence Replicative Senescence Ras overexpression UVB-induced senescence	Antibodies array ELISA	(Rodier et al. 2009; Coppé et al. 2008; Bauwens et al. 2022)
CXCL1-2-3	Human fibroblasts (IMR90, HCA2) Prostate epithelial cells (PrECs)	Irradiation-induced senescence Replicative Senescence	Antibodies array	(Rodier et al. 2009; Coppé et al. 2008)
IL-8	Human fibroblasts (WI-38, IM90, BJ), Prostate epithelial cells (PrECs) Renal epithelial cells (ATCC) Human keratinocytes (NHEK)	Irradiation-induced senescence Replicative Senescence UVB-induced senescence Glyoxal-induced senescence	Antibodies array Mass spectrometry ELISA	(Rodier et al. 2009; Coppé et al. 2008; Basisty et al. 2020; Bauwens et al. 2022; Halkoum et al. 2022)
IGFBP-2	Human fibroblasts (IMR90, WI-38, HCA-2, BJ) Renal epithelial cells (ATCC)	Irradiation-induced senescence	Antibodies array Mass spectrometry	(Rodier et al. 2009; Basisty et al. 2020)
IL-7	Human fibroblasts (WI-38, HCA-2, BJ) Prostate epithelial cells (PrECs)	Irradiation-induced senescence	Antibodies array	(Coppé et al. 2008)

GDF15	Human fibroblasts (IMR-90) Epithelial renal cells (ATCC)	Irradiation-induced senescence Replicative Senescence	Mass spectrometry	(Basisty et al. 2020)
Macrophage migration inhibitory factor (MIF)	Human fibroblasts (IMR-90, WI-38) Epithelial renal cells (ATCC) Human keratinocytes (NHEK) Prostate epithelial cells (PrECs) Bone marrow MSC	Irradiation-induced senescence UVA-induced senescence RAS overexpression Chemical-induced senescence (ATZ) H_2O_2-induced senescence	Mass spectrometry Antibodies array	(Basisty et al. 2020; Valerio et al. 2021; Coppé et al. 2008; Özcan et al. 2016)
Filamin B	Human fibroblasts (IMR-90) Epithelial renal cells (ATCC) Human keratinocytes (NHEK) Prostate epithelial cells (PrECs) Bone marrow MSC	Irradiation-induced senescence UVA-induced senescence RAS overexpression Chemical-induced senescence (ATZ) H_2O_2-induced senescence	Mass spectrometry	(Basisty et al. 2020; Valerio et al. 2021; Coppé et al. 2008; Özcan et al. 2016)
Cathepsin D	Mesenchymal stem cells	Replicative Senescence Chemical-induced senescence (doxorubicin) H_2O_2-induced senescence Irradiation-induced senescence	Mass spectrometry	(Özcan et al. 2016)

Additionally, non-protein signalling molecules, including various bioactive oxidized lipid metabolites, prostaglandins, and nitric oxide, are enriched in the sSASP of senescent cells (Ni et al. 2016; Narzt et al. 2021; Wiley et al. 2021b; Hattori et al. 2021). While prior research has predominantly focused on secreted factors, emerging studies indicate that EVs containing proteins, lipids, and nucleic acids, also contribute to the SASP (reviewed in; Takasugi 2018).

B.1.2 SASP Heterogeneity and Plasticity

Coppé and colleagues demonstrated initially that only a subset of SASP proteins was shared between fibroblasts and prostate epithelial cells upon IRIS (Coppé et al. 2008). A subsequent large-scale proteomic analysis of SASP then revealed only 48 shared SASP factors between fibroblasts and renal epithelial cells in IRIS (Basisty et al. 2020). When considering other proteomic studies on various cell types and senescence inducers, such as UVA-induced senescent keratinocytes and IRIS mesenchymal stem cells (Valerio et al. 2021; Özcan et al. 2016), the number of shared SASP factors drops to 19, suggesting that only a handful of proteins are commonly secreted across all types of senescent cells.

Moreover, SASP factors vary over time, exhibiting cell-type-specific patterns. For instance, in IRIS, the *IL-1β* gene is upregulated on days 10 and 20 in fibroblasts, but only on day 10 in keratinocytes, and on day 20 in melanocytes (Hernandez-Segura et al. 2017). The secretion of IL-6 and IL-8 in UVB-induced senescent keratinocytes is overexpressed on day 3 but diminishes by day 7 following senescence induction (Bauwens et al. 2023). In addition, HDFs in replicative senescence also exhibit two distinct phases of SASP-related genes expression.

The first phase is often characterized by an overexpression of SASP genes related to inflammation, such as *IL-6* and *IL-8*, while the second phase is more characterized by changes in the expression of genes associated with extracellular matrix modulation, such as *MMPs* (Y. M. Kim et al. 2013).

SASP composition is also influenced by the senescence inducers. Senescent IMR-90 fibroblasts present distinct secretome profiles depending on whether the senescence was induced by X-rays, atazanavir (ATV), or RAS overexpression (Basisty et al. 2020). Similar findings were noted in mesenchymal stem cells in senescence induced by oxidative stress, doxorubicin treatment, X-ray irradiation, or replicative exhaustion (Özcan et al. 2016).

Finally, the matrix and cellular microenvironment of senescent cells can impact their secretome composition. The substrate stiffness impacts the NF-κB phosphorylation status in UV-induced senescence in fibroblasts (Yao et al. 2022), suggesting that the extracellular matrix (ECM) composition could have an impact on SASP composition. Moreover, it has been demonstrated that co-cultures of squamous cell carcinoma and RS fibroblasts exacerbate some SASP gene expressions (Toutfaire et al. 2018).

B.2 Regulation of SASP

The regulation of SASP involves transcriptional, post-transcriptional, epigenetic, and translational mechanisms. In addition, the secretion of SASP components is regulated through intracellular trafficking, and many compartments of secretion are altered during senescence. These alterations could potentially affect the dynamic and create a heterogeneous composition of SASP.

B.2.1 Transcriptional, post-transcriptional, and epigenetic regulation

Multiple signalling pathways have been identified to activate transcription factors that play a crucial role in regulating the expression of inflammatory cytokines.

First, there is a clear link between the expression of SASP and the DDR pathway, as several DDR proteins (ATM, CHK2, and NBS1, i.e a protein of the MRN complex) are necessary for the initiation and maintenance of the cytokine response in IRIS fibroblasts (Rodier et al. 2009). It has recently been described that in the absence of DNA damage, such as after sodium butyrate treatment, the SASP of fibroblasts still relies on the non-canonical activation of DDR and the accumulation of ATM, MRE11, and NF-κB on chromatin (Malaquin et al. 2020). Then, the NF-κB and the CCAAT/enhancer binding protein beta (C/EBPβ) transcription factors were identified to be involved in the regulation of CXCR2 ligands expression, including IL-8 in fibroblasts in OIS (Juan C. Acosta et al. 2008).

Regarding the regulation of inflammatory cytokine expression, senescent preadipocytes

exhibit activation of the JAK/STAT signalling pathway, a pathway associated with inflammation. Notably, the application of a JAK inhibitor on IRIS preadipocytes effectively suppresses crucial components of SASP (Xu et al. 2015).
Finally, the cGAS/STING pathway has been implicated in the control of inflammatory SASP factors, specifically, IL-6 and CXCL-10 secretion, through NF-κB activation *in vitro* and *in vivo* (Glück et al. 2017).
It has recently been shown that COX2 plays a crucial role in regulating the expression of various inflammatory SASP components in OIS through an autocrine feedback loop involving prostaglandin E2 (PGE2) binding to the E-type prostanoid receptor 4 (EP4). However, the downstream pathways of PGE2 and EP4 remain unknown. Nonetheless, the COX2 pathway is thought to be able to activate major SASP transcriptional regulators, such as NF-κB, C/EBPβ, and GATA4 (Gonçalves et al. 2021).

While early SASP is mainly regulated at the transcriptional level, its long-term SASP expression is mainly driven by post-transcriptional mechanisms. This has been demonstrated by the lack of impact of actinomycin D treatment, an inhibitor of transcription, on the expression of several SASP factors (Alspach et al. 2014).
$P38^{MAPK}$ seems to play a role in timely regulating SASP. Indeed, its early activation during X-ray-induced senescence or RAS-induced senescence facilitates the expression of SASP factors, such as IL-6 and IL-8, through NF-κB activation (Freund, Patil, and Campisi 2011).

The mTOR pathway is also involved in the post-transcriptional regulation of SASP. Specifically, mTOR activates the translation of MK2 (or MAPKAPK2), which can phosphorylate and inhibit the RNA-binding protein ZFP36L1, also involved in the destabilization of several SASP mRNAs (Herranz et al. 2015). The mTORC1 kinase has also been shown to modulate senescence-induced inflammation and SASP (Machado-Oliveira et al. 2020).
Previous research on SASP has mainly concentrated on the transcriptional and post-transcriptional control of inflammatory cytokines. Yet, the regulatory mechanisms for other SASP factors like growth factors and proteases remain poorly understood. A recent study on fibroblasts has described that E2F4, TEAD1, and AP-1 transcription factors are major regulators of SASP in RS (Y. Wang et al. 2022). Moreover, AP-1 is involved in the expression of IL-6, IL-1β, and MMP-10, as their expression is abrogated when expressing a dominant-negative isoform of c-Jun, one of the subunits of AP-1, during OIS in fibroblasts (Martínez-Zamudio et al. 2020).

The physical clustering of SASP genes suggests that the regulation of their expression may depend, at least in part, on broader changes in chromatin conformation (Coppé et al. 2010). Indeed, several histone variants can influence the expression of SASP genes. For example,

the relocation of the macroH2A1 histone variant away from SASP genes in OIS-induced senescent fibroblasts is involved in the maintenance of SASP gene expression (H. Chen et al. 2015).

Moreover, the increased expression of histone variant H2A.J in fibroblasts undergoing etoposide-induced senescence enhances the expression of multiple genes associated with inflammation and immune response (Contrepois et al. 2017).

In addition, nuclear HMGBs bind to DNA, facilitating the access of transcription factors to promoter regions. In fibroblasts in RS or IRIS, HMGB1 can be released into the extracellular space and act as an alarmin to activate NF-κB, which subsequently upregulates the expression of pro-inflammatory target genes (Davalos et al. 2013). Furthermore, HMGB2 preferentially localizes to SASP gene regions during OIS in fibroblasts, protecting them from being incorporated in transcriptionally repressed SAHF regions (Aird et al. 2016).

The SASP emerges as a hallmark of senescence common to all cell types. However, its composition varies depending on the cell type and the senescence inducer. Furthermore, while the sharing of signalling pathways governing the SASP is being studied in different models of senescence, understanding the extent of pathway sharing among different models and their respective contributions to the phenotype remains limited.

B.2.2 Secretory control

The ER is the site of membrane biosynthesis used in secretory and excretory pathways. It is responsible for folding and maturating secreted proteins, making it the first compartment of secretion. Recently, it has been proposed that ER stress and the subsequent activation of the UPR upon senescence could contribute to the modified secretome of senescent cells, as further detailed in part 2.

Lysosomes are at the crossroads of endocytic and exocytic pathways, and their increased abundance in senescent cells may be associated with the exacerbation or deregulation of these pathways. Besides their partnership with the Golgi apparatus and the endosomal compartments, lysosomes are also important for the clearance of cytoplasmic chromatin fragments (CCFs). CCFs may leak from the nucleus in the cytoplasm of senescent cells and induce SASP; both CCFs and SASP inductions would be related to a retrograde mitochondrial–nucleus signalling pathway associated with the mitochondrial increase in ROS species (reviewed in; Machado-Oliveira et al. 2020). In melanoma cells, the lysosomal exocytosis mediated by the small GTPase RAB27A has also been shown to be upregulated in TIS and to participate in SASP factors secretion, including the chemokines CCL-2 and CXCL-12 (Rovira et al. 2022). Along with this enhanced lysosomal secretion, senescent cells exhibit a

remodelling of their lysosomal proteome with selective enrichment in some lysosomal resident proteins such as those implicated in vesicular transport and fusion (Rovira et al. 2022).

Small EV and exosome secretions are now part of the specific secretory phenotype. The release of senescence-associated exosomes is linked to RAB27A expression, as silencing of RAB27A leads to decreased exosome secretion in fibroblasts undergoing RS or OIS (Takahashi et al. 2017). Rab27 GTPases are associated with the connection of multivesicular endosomes and the secretion of exosomes (Ostrowski et al. 2010). The enhanced biogenesis of EVs and their release by senescent cells have been demonstrated to be associated with the extent of DNA damage generated by the senescence inducer, as well as the activation of the ceramide synthetic pathway (Hitomi et al. 2020). EVs and exosomes also contribute to SASP and its paracrine impact. For example, EVs from senescent stromal cells can enhance the proliferation of cancer cells by promoting the activation of the ephrin-A2 tyrosine kinase receptor, which interacts with overexpressed ephrin-A1 on the surface of the cancer cells, thereby boosting an Erk-dependent proliferation pathway (Takahashi et al. 2017; Misawa et al. 2020). In addition to being components of SASP, the release of senescence-associated EVs seems to be a mechanism used by senescent cells to discard cytoplasmic chromatin DNA fragments, thus limiting DNA damage accumulation caused by major stress exposure and potentially modulating SASP (Takahashi et al. 2017; Hitomi et al. 2020).
Finally, a causal relationship has been established between disrupted endocytosis, particularly clathrin-mediated endocytosis (CME), and cellular senescence (reviewed in; Shin et al. 2021). For example, Cao *et al.* demonstrated that the loss of the myosin light chain 3 (i.e. a specific binding partner of clathrin) in chondrocytes leads to increased CME, activation of Notch signalling, induction of senescence, and promotion of osteoarthritis (OA) in mice (Cao et al. 2023).

B.3 Cellular senescence and SASP *in vivo*

Senescent cells accumulate in tissues with age (Herbig et al. 2006; Jeyapalan et al. 2007; Kavanagh et al. 2021). A meta-analysis showed that even if the proportion of senescent cells in 14 different human tissues is correlated with chronological age, it varies depending on the tissue type and the senescence marker used (reviewed in; Tuttle et al. 2020). Therefore, a broad range of senescent cells exists throughout the body. Basisty and colleagues defined in senescent culture cells a core SASP including GDF15, STC1, SERPINE1/PAI-1, and MMP-1, which are also reported to be significantly increased among the plasma markers of ageing in humans (Basisty et al. 2020). Another study showed that doxorubicin-induced senescence enriched the SERPINE1/PAI-1 SASP factor in plasma *in vivo* (Wiley et al. 2019). However, these markers can also serve as diagnostic tools for various diseases, including

cardiovascular, metabolic, neurodegenerative, and malignant diseases, regardless of age. This makes them indicators of a “state of ageing” rather than a chronological accumulation of senescent cells.

Moreover, the accumulation of senescent cells is also detected at pathological sites due to various stress signals regardless of age (reviewed in; Song et al. 2020). Various studies have indicated that eliminating senescent cells, achieved through transgenic mice like INK-ATTAC and p16-3MR models targeting p16-positive cells (Demaria et al. 2014; Baker et al. 2011; 2016), or using small pharmacological compounds (J. Chang et al. 2016; Baar et al. 2017), results in enhanced healthspan, alleviates several age-associated conditions, and delays tumor formation. Thus, these findings highlight senescence as a significant contributor to age-related pathology.

B.4 Pleiotropic roles of SASP

Given its diverse composition, SASP can exert pleiotropic effects on the cellular environment, which may manifest as either beneficial or deleterious outcomes (reviewed in; Birch and Gil 2020; Hoare and Narita 2018). The most described effects of SASP are presented in **Figure 9**, but only some of these roles will be described more precisely in the following part. However, it should be noted that two important roles of SASP are missing in the figure. Indeed, it has been demonstrated that OIS-induced cells can transmit paracrine senescence to neighboring cells, notably through the inflammasome and IL-1 signalling (Juan Carlos Acosta et al. 2013), and that the SASP could also have autocrine cell-autonomous functions, including reinforcement of proliferation arrest (Juan C. Acosta et al. 2008).

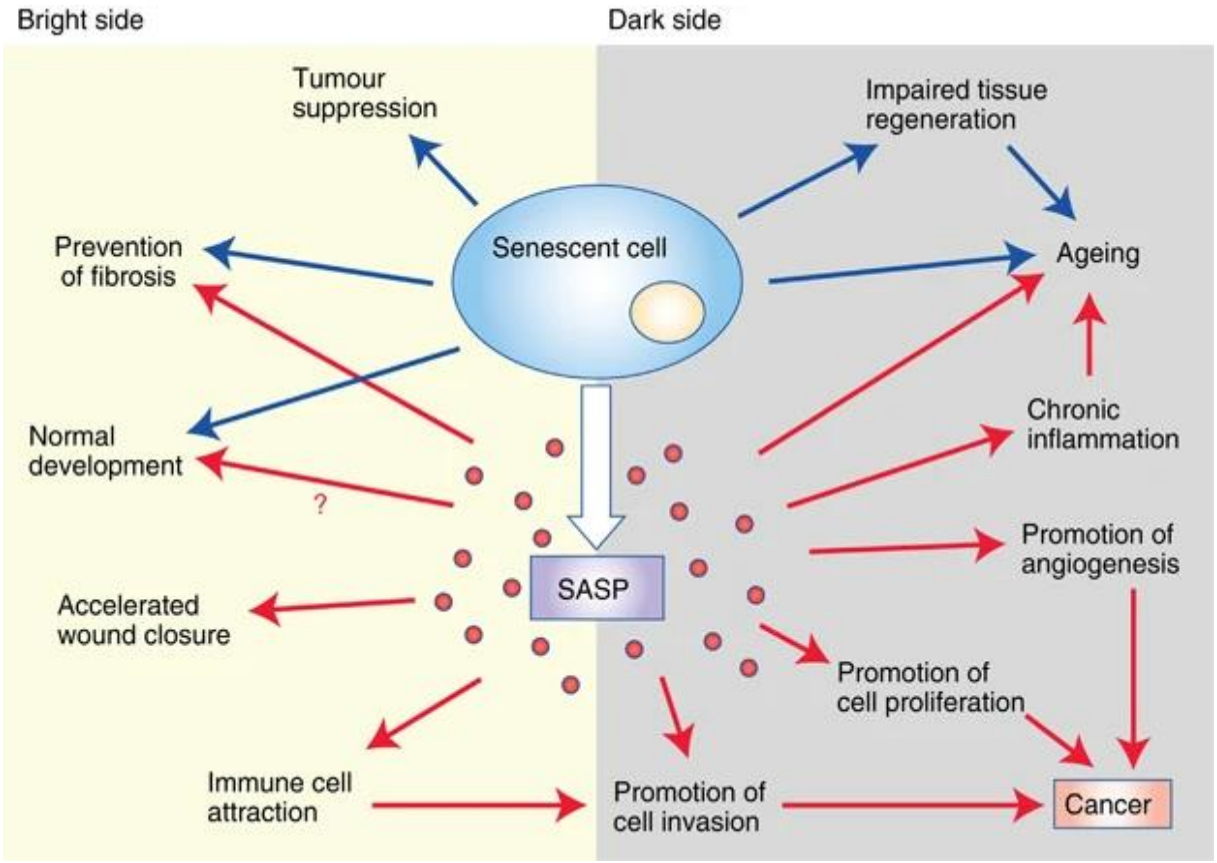


Figure 9. The effects of SASP on the microenvironment. The SASP can exert pleiotropic effects on the microenvironment, often categorized as beneficial and deleterious. On one hand, positive effects of the SASP may include tumor suppression, prevention of fibrosis, normal development, accelerated wound closure, and attraction of immune cells. On the other hand, negative effects may include impairment in tissue regeneration, aging, chronic inflammation, promotion of angiogenesis, cell proliferation, and invasion, which are major components responsible for cancer development. From Lecot *et al.* 2016.

B.4.1 The protective role of SASP on neighboring cells in cancer-prone contexts

Senescent cells have been detected in benign tumor sites like prostatic hyperplasia and melanocytic naevi (Castro et al. 2003; Michaloglou et al. 2005). However, they are absent in malignant stages as observed in melanoma (Gray-Schopfer et al. 2006), strongly suggesting that cellular senescence may act as a barrier against tumorigenesis.

In addition to intrinsically inhibiting the growth of cells exposed to various potentially oncogenic stimuli, senescent cells recruit innate immune cells (i.e; macrophages, natural killer (NK), neutrophils, and T lymphocytes) for their elimination through the secretion of pro-inflammatory cytokines (reviewed in; Kale et al. 2020). This process is a vital anti-tumoral mechanism, as observed in mice, where a deficit in immune surveillance of pre-malignant senescent hepatocytes leads to the development of hepatocarcinoma (T.-W. Kang et al. 2011).

Moreover, senescent cells have been suggested to trigger cell cycle arrest in neighboring cells,

promoting the spread of the anti-tumorigenic response, and ensuring that cells in an unfavorable environment stop dividing. SASP factors such as TGF-β family ligands, VEGF, and chemokines such as CCL-2 and CCL-20 play an important role in inducing paracrine senescence in neighboring cells (Acosta et al. 2013). Additionally, the intensity of SASP can impact local homeostasis paracrine through signals that propagate the senescent state, exacerbating local stress, and inducing ROS-mediated damage in neighboring cells. This is the so-called senescence-messaging secretome (SMS) effect of SASP. Hence, conditioned media (CM) of cells exposed to UV radiations initiate bystander DNA damage in non-exposed neighboring cells (Dickey et al. 2009). Moreover, new studies have shown the important contribution of microvesicles in the propagation of the senescent phenotype, for example, via the transfer of interferon or miRNA cargo factors (Borghesan et al. 2019; Fulzele et al. 2019). For instance, Nedachi *et al.* demonstrated that aged keratinocytes release more extracellular vesicles compared to young keratinocytes.

B.4.2 Tumor promotion effects of some SASP components

It is now clear that factors secreted by senescent cells impact cancer cell's behavior.
The first studies pointing out the role of the cellular microenvironment in the promotion of cancer progression highlighted the role of Carcinoma-Associated Fibroblasts (CAFs) in prostate cancer progression (Olumi et al. 1999). It has been subsequently reported that senescent fibroblasts share many features with CAFs, and can have a similar impact on tumor growth (Trylcova et al. 2015).
Moreover, CM from replicative senescent fibroblasts enhances cutaneous squamous-cell carcinoma (SCC) cell migration and invasion *in vitro* through their secretion of chemerin (Farsam et al. 2016).
Co-culture systems and xenograft models have shown that SASP from senescent fibroblasts promotes the tumorigenesis of premalignant epithelial cells, induces epithelial-mesenchymal transition (EMT), and increases tumor vascularization, suggesting its pro-tumorigenic properties (Pluquet and Abbadie 2021; Parrinello et al. 2005).

In addition, SASP can also enhance the transition from normal epithelial cells into cells with mesenchymal properties. Indeed, senescent dermal fibroblasts can promote neoplastic escape from normal human keratinocytes and increase markers of EMT as well as the migration of emerging cells. This was attributed to the activation of the membrane PAR-1/Thrombin receptor by MMPs among SASP of senescent fibroblasts (Malaquin et al. 2013). Furthermore, a recent study has identified a BDNF-TrkB axis as being associated with the role of SASP of aged fibroblasts in promoting EMT initiation in primary keratinocytes from aged donors (Tinaburri et al. 2021). Another point is that the alteration in the secretion of ECM

components and regulators by senescent prostate cells generates a favorable environment for tumor development (reviewed in; Sprenger, Plymate, and Reed 2010). UVB-induced senescent fibroblasts were shown to produce an ECM that promotes proliferative signalling pathways of preneoplastic HaCaT epidermal keratinocytes (J. Kang et al. 2008).

B.4.3 Extracellular Matrix Remodelling

Another impact of the SASP that is much less studied and underrepresented in the literature is the effect of SASP-related changes in ECM components that could have a notable effect on cell functions and fates.

For instance, the transient secretion of PDGF-AA (Platelet-derived growth factor AA) from senescent fibroblasts is necessary for effective healing following skin injury (Demaria et al. 2014). Recently, efforts have been made to better characterize the changes in the matrisome of senescent cells and their effects on the environment. Hierbert and colleagues described that the activation of Nrf2 in fibroblasts triggers the production of a senescence-promoting extracellular matrix (ECM) via the expression and secretion of certain ECM proteins, such as PAI-1 (Hiebert et al. 2018). This can accelerate wound closure and promote re-epithelization *in vivo*. In addition, Nrf2 inhibition in fibroblasts reduces the production of collagen I and alters ECM deposition (Salamito et al. 2023).

In addition, the SASP of senescent cells is intricately linked with fibrosis. When muscle stem cells are seeded onto decellularized ECM maintained by senescent fibroblasts, their responses and functions are affected, resulting in enhanced expression of fibrogenic markers (Stearns-Reider et al. 2017). Moreover, the elimination of senescent p16High LSECs (Liver Sinusoïd Endothelial Cells) in mice induces fibrosis (Grosse et al. 2020).

Finally, a recent *in vivo* study on mice shows that the susceptibility of various body regions to tumor initiation and invasion is regulated by the composition of the extracellular matrix (Bansaccal et al. 2023). This latest study may lead to the reflection that changes induced by the SASP in the composition of the ECM during ageing could hypothetically promote or inhibit the initiation of certain cancers.

C. Strategies to eliminate senescent cells

Considering the crucial role of senescence in physiological processes, there is likely a threshold where the accumulation of senescent cells creates a microenvironment favorable to the onset of pathologies through SASP (**Figure 10**).

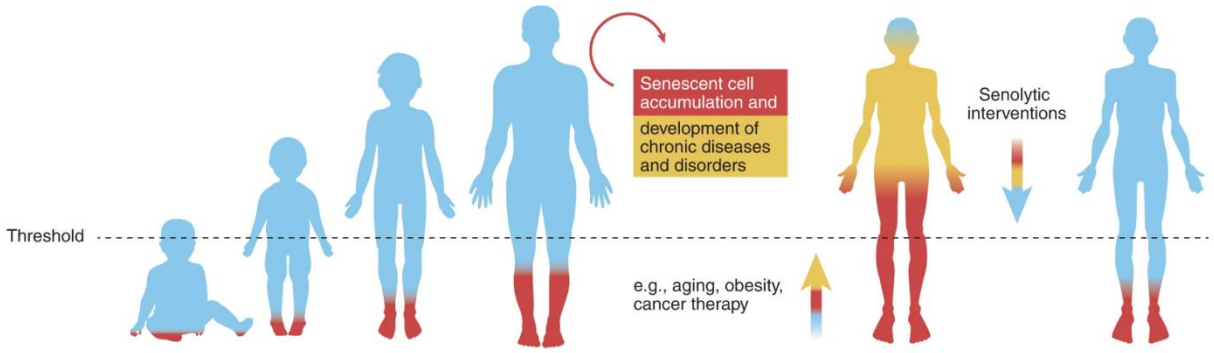


Figure 10. **Senescent cells build-up during life results in the development of chronic diseases and disorders.** An established hypothesis suggests that when the burden of senescent cells surpasses a threshold, the self-amplifying spread of senescence through SASP could exceed immune system clearance and lead to detrimental effects such as ageing, obesity, etc… From Chaib *et al.* 2022.

To address this, there should be an open framework to eliminate these senescent cells, preventing their detrimental impact on the microenvironment. Indeed, the elimination of senescent cells demonstrated their contributive role in ageing and age-related diseases and paved the way for the development of senotherapeutic approaches (reviewed in; Zhu et al. 2015). Over the past five years, senotherapeutic research has emerged to slow down the ageing phenotypes. Current senotherapeutic strategies targeting senescent cells are mainly based on drugs that specifically kill senescent cells (senolytics) and components that suppress the detrimental effects of SASP without inducing senescent cell death (senomorphics) (reviewed in; E. C. Kim and Kim 2019).

C.1 Senolytics approaches

As previously mentioned, a characteristic of senescent cells is their resistance to apoptosis. Several SCAPs have been identified, and specific compounds can target them for the elimination of senescent cells through apoptosis (reviewed in; Zhu et al. 2015) (**Figure 11**).

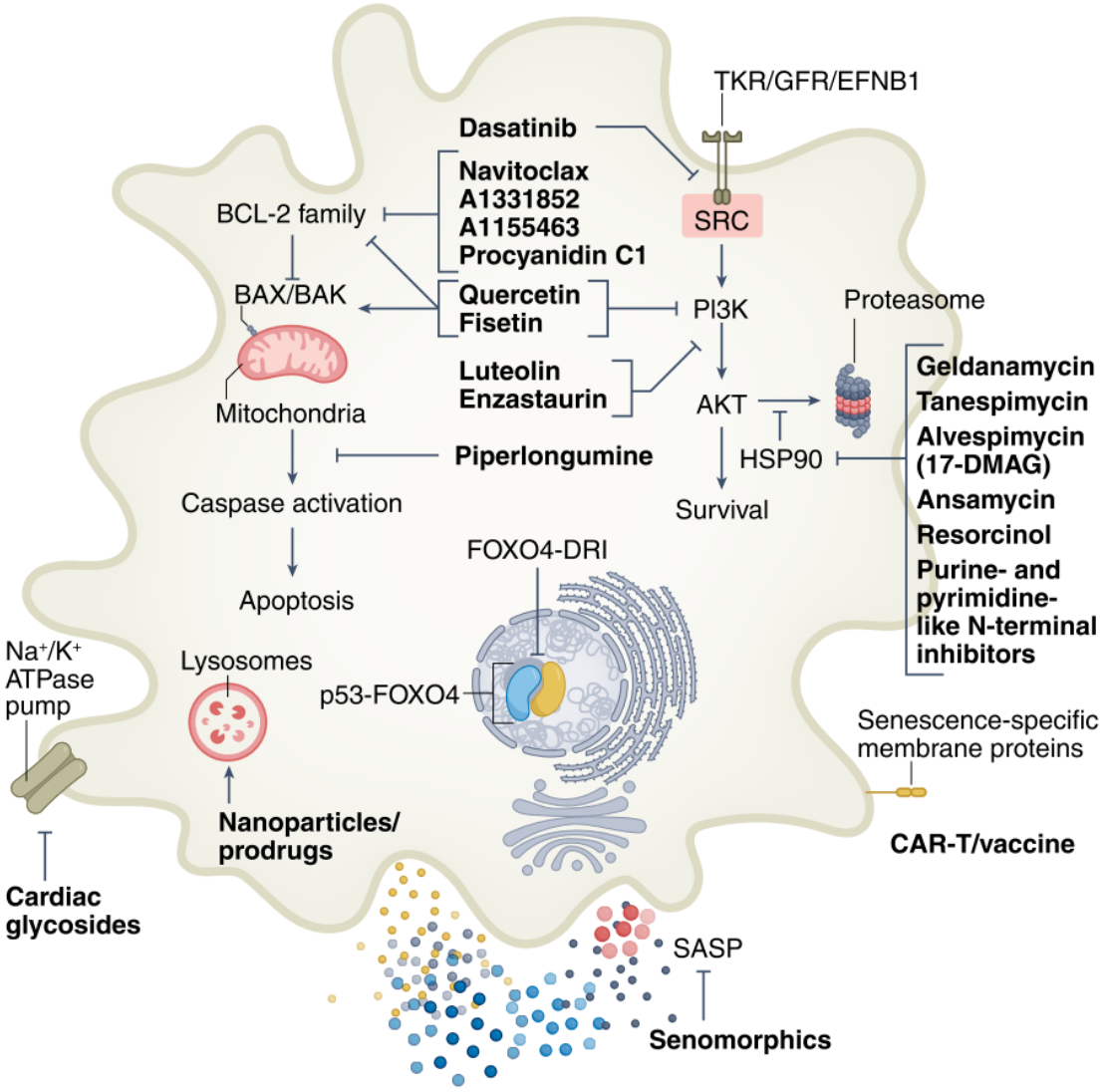


Figure 11. The main senolytics compounds and their modes of action. Senolytics target various senescent cell anti-apoptotic pathways (SCAPs), including SRC kinases, PI3K–AKT signalling pathway, HSP90, BCL-2 family members, and caspase inhibition. Additionally, new generation senolytic strategies focus on lysosomal and SA-β-gal-activated prodrugs and nanoparticles, sodium–potassium pump (Na+/K+-ATPase)-dependent apoptosis, SASP inhibition, and immune-mediated clearance by CAR T cells, antibody-drug conjugates, or vaccines. From Chaib *et al.* 2022.

First-generation senolytics, such as Dasatinib (an SRC/tyrosine kinase inhibitor), Quercetin and Fisetin (PI3K/AKT pathway inhibitors as well as BCL-2 pathway inhibitor), or Navitoclax (a BCL-2 pathway inhibitor), have safety profiles that allow them to effectively target SCAPs (reviewed in; Chaib, Tchkonia, and Kirkland 2022). However, senotherapeutic often combine two agents targeting different SCAPs, such as the most used Dasatanib and Quercetin combination (Hickson et al. 2019).

Second-generation senolytics aim to target other specificity than apoptosis resistance. As an example, eliminating senescent cells using chimeric antigen receptor (CAR) T cells that specifically target senescence-specific surface antigens, such as uPAR, improves the survival of mice with lung adenocarcinoma and restores tissue homeostasis in a chemical-induced liver fibrosis mouse model (Amor et al. 2020).

Finally, emerging preclinical evidence has highlighted the significant potential of these approaches (reviewed in; Raffaele and Vinciguerra 2022). However, further analyses are necessary to rule out the potential adverse effects of long-term administration. Additionally, there are ongoing efforts to evaluate combinations of senotherapies in individuals with multiple age-related diseases (Wissler Gerdes et al. 2021).

C.2 Senomorphics approaches

Senomorphics are small molecules designed to inhibit all, or, at least, multiple characteristics of senescent cells by blocking SASP without killing the cell. To do so, various strategies have been developed. The first therapy is based on the use of compounds able to target pathways related to SASP expression like NF-κB, C/EBPβ, $p38^{MAPK}$, or JAK/STAT pathways. The second one is based on the neutralization of specific SASP factors' activity and function using for example neutralizing antibodies. In a model of bleomycin-induced senescence, the secretion of certain SASP factors (including IL-6 and IL-8) can be directly inhibited with neutralizing antibodies such as those against the membrane-bound IL-1α (Orjalo et al. 2009). Additionally, neutralizing antibodies against specific surface proteins upregulated at senescence can also regulate the SASP. Secretion of IL-6 has been decreased in senescent HUVECs and fibroblasts treated with anti-TNFα or anti-ephrin B2 antibodies, respectively (Prattichizzo et al. 2016; Lister, Chrysovergi, and Lagares 2022). Numerous senomorphics are polyphenols, like flavonoids, phenolic acids, lignans, and stilbenes, known for their antioxidant properties. However, their mechanisms of action remain not fully elucidated. Overall, most senomorphics modulate the senescent phenotypes by disrupting the proinflammatory nature of senescent cells (**Table 2**).

Table 2. Senomorphics that block SASP components at the secreted level only. From Giroud *et al.* 2023.

Compound	Function	Cell type	Inducer	Effect on SASP factors	References
Adalimumab (monoclonal antibody)	TNFα inhibitor	HUVECs	Replicative senescence	IL-6 ↓	(Prattichizzo et al. 2016)
Anti-ephrin B2 antibody (clone B11)	Ephrin B2 inhibitor	Human fibroblasts	Chemical-induced senescence Irradiation-induced senescence	IL-6 ↓	(Lister, Chrysovergi, and Lagares 2022)

Apigenin (flavonoid)	NF-κB inhibitor	BJ fibroblasts	Bleomycin-induced senescence	IL-6 ; IL-8 ; IL-1β ↓	(H. Lim, Park, and Kim 2015)
Avenanthramicine C	AMPK activator p38/NF-kB inhibitor	Human fibroblasts (HDFs)	Replicative Senescence	IL-6 ; IL-8 ; TGF-β ↓	(J. S. Lim et al. 2020)
BIRB796	p38 inhibitor	Human fibroblasts (NHDFs)	Replicative Senescence	IL-6 ↓	(Alimbetov et al. 2016)
Hydroxytyrosol (olive phenolic compound)	NF-kB inhibitor	Human fibroblasts (NHDFs ,MRC5)	Replicative senescence	IL-6 ; MMP-2 ; MMP-9 ↓	(Menicacci et al. 2017)
IPI-504	HSP90 inhibitor	ARPE-19	H_2O_2-induced senescence	IL1-b ; IL-8 ↓	(D. D. Chen et al. 2021)
Isatis tinctoria L. Leaf extract (ITE)	mTOR/ MAPK/ NF-kB inhibitor	Human fibroblasts (HDFs)	Replicative Senescence	IL-6 ; IL1-b ; IL-8 ↓	(Signalling et al. 2022)
Kaempferol (flavonoid)	NF-κB inhibitor	BJ fibroblasts	Bleomycin-induced senescence	IL-6 ; IL-8 ; IL-1β ↓	(H. Lim, Park, and Kim 2015)
Lamivudine	Nucleoside reverse transcriptase inhibitor	Human fibroblasts	Replicative Senescence	IFN-1 ↓	(De Cecco et al. 2019)
Metformin	Several pathways	Human HNSCC cell line Cal27	LY2835219 (CDK4/6 inhibitor)-induced senescence	NT3 ; MCP-1 ; IL-6 ; IL-8 ; GRO ; IGFBP1 ; BMP4 ; BLC ↓	(Hu et al. 2020)
Metformin	Several pathways	Primary VSMCs from the aortas of elderly patients	Ang II-induced premature senescence	MMP-2 ; IL-6 ; TGFβ ↓	(Tai et al. 2022)
Mix of bioCurcumin, Polydatin and liposomal-b-caryophyllene	Several pathways	HUVECs	Replicative Senescence Doxorubine-induced senescence	IL-6 ; IL-1β ↓	(Matacchione et al. 2021)
MK2.III	MK2 kinase inh	Human fibroblasts (NHDFs)	Replicative Senescence	IL-6 ↓	(Alimbetov et al. 2016)
Oleuropein (olive phenolic compound)	NF-kB inhibitor	Human fibroblasts (NHDFs ,MRC5)	Replicative senescence Irradiation induced senescence	IL-6 ; MMP-2 ; MMP-9↓ IL-6 ; IL-8 ; MCP-1 ; RANTES ↓	(Frediani et al. 2022; Menicacci et al. 2017)
Simvastatin	HMG-CoA reductase inhibitor	Normal Human Fibroblasts (HCA2)	Irradiation-induced senescence	IL-6 ↓	(S. Liu et al. 2015)
Rapamycin	mTOR inhibitor	Normal Human Fibroblasts (HCA2)	Irradiation-induced senescence	IL-6 ↓ IL-6 ; CSF2 ; CCL7 ; CCL8 ; IGF1 ; TGFB3 ; IL-8 ; BMP4 ; IL-10	(R. M. Laberge et al. 2015)
Rapamycin	mTOR inhibitor	Murine MEFs	H_2O_2-induced senescence	TNFα ; LIX ; Leptin R ; MIP-1a	(R. Wang et al. 2017)
Resveratrol	SIRT1 activator NF-kB inhibitor NRF2 activator	Arterial VSMCs derived from aged rhesus monkeys	Chronological age	MCP-1; TNFα, VEGF	(Csiszar et al. 2012)

Ruxolitinib	JAK1/2 inh	Preadipocytes from healthy human kidney transplant donors	Irradiation-induced senescence Replicative senescence	IL-6 ; GM-CSF ; G-CSF ; IL-10 ; CXCL-1 ; MIP-1a ; IL-8, MCP-1 ; RANTES, MCP-3 ; PAI-1 ; MIP-1β ; TNFα ; IFN-α2 ; IL-1α ; VEGF ; CCL-11 ; PDGF-AA IL-6 ; IL-8 ; MCP-1 ; PAI-1 ↓	(Xu et al. 2015)
SB203580	p38 inhibitor	Human fibroblasts (NHDFs) Normal Human Fibroblasts (HCA2) Normal Human Fibroblasts (HCA2)	Replicative Senescence Irradiation-induced senescence Ras-induced senescence	IL-6 ↓ IL-6 ; IL-8 ; GM-CSF ↓ GRO ; IL-6 ; IL-8 ; MCP-2 ; MCP-1 ; GCP-2 ; GM-CSF ; IL-10 ; GDNF ; IGFBP4, CNTF ; GROα ; TGF-β1, Angiogenin ; IL-2 ; Eotaxin ; IL-7 ; MIG ; IL-1α ; TNFα ; IL-5 ; TNFβ ; Sgp130 ; Osteoprotegerin IL-6 ; IL-8 ; GM-CSF ↓ GM-CSF ; IL-6 ; GRO ; MIP-1α ; IL-1β ; ENA78 ; GROα ; IL-8 ; MCP-3 ; HGF ; ICAM3 ; MIP-1β ; uPAR ; Dtk ; IGF-1SR ; IL-1α ; Sgp130 ; IL-12 p40 ; IL-4 ; TIMP1 ; IL-11 ; PIGF ; IL-15 ; IL-2 ; RANTES ; IL-2 Rα ; Oncostatin M ; GDNF ; MIP-3α ; IL-12 p70 ; Thrombopoietin	(Alimbetov et al. 2016; Freund, Patil, and Campisi 2011)
Silybum marianum flower extract (SMFE)	-	Human fibroblasts (HDFs)	Replicative Senescence	IL-6 ; MMP-1 ↓	(Woo et al. 2021)
SR9009	Reduces ROS level via the activation of the NRF2 pathway	Human fibroblasts (HDFs)	Doxorubicin-induced senescence	IL-1α ; IL-1β ↓	(Gao et al. 2021)
UR-13756	p38 inhibitor	Human fibroblasts (NHDFs)	Replicative Senescence	IL-6 ↓	(Alimbetov et al. 2016)
Wogonin (flavonoid)	NF-κB inhibitor	BJ fibroblasts	Bleomycin-induced senescence	IL-6 ; IL-8 ; IL-1β ↓	(H. Lim, Park, and Kim 2015)
Zileuton	5-LO inhibitor	Human fibroblasts (HDFs)	Irradiation-induced senescence	IL-6 ↓	(M. Park et al. 2022)

To conclude, senotherapeutics have exhibited encouraging outcomes in eliminating senescent cells and mitigating diverse diseases in animal models. Nonetheless, there is a lack of data on the effectiveness and safety of these strategies in humans and it's probable that the optimal senotherapeutic treatment for age-related diseases has yet to be identified (discussed in; Raffaele and Vinciguerra 2022).

2. The endoplasmic reticulum stress, the unfolded protein response, and their impact on cell fate

A. Functional role of the endoplasmic reticulum (ER)

A.1 Multifaceted functions of the ER

The ER is a large and dynamic membranous intracellular organelle considered as the first compartment of the secretory pathway (Pelham 1988). One-third of cellular proteins, including resident proteins of the secretory organelles, plasma membrane proteins, and secreted proteins are produced and folded in the ER. Therefore, the ER plays a major role in maintaining cellular homeostasis and acts as an alarm for the cell by being able to activate stress signalling pathways to maintain the balance between health and disease (reviewed in; Yoshida 2007). In addition, the ER serves many other roles in the cell, including calcium storage (Bygrave and Benedetti 1996) and lipid metabolism (reviewed in; Jacquemyn, Cascalho, and Goodchild 2017), which will not be detailed in this introduction. In addition, the ER makes several membrane contact sites (MCSs) to facilitate the exchanges and communication with other organelles such as mitochondria (MAMs for mitochondria-associated membranes), Golgi apparatus, lysosomes, endosomes and plasma membrane (reviewed in; Phillips and Voeltz 2016). To ensure all these different functions and to especially regulate protein processing and cell fate, the metabolic pathways are compartmentalized in the ER, including the rough ER where proteins are translated and glycosylated, and the smooth ER where are located the ER quality control (ERQC), the ER exit sites (ERES) and the ER-associated protein degradation (ERAD). Additionally, to fulfill all these functions, the ER uses a diverse array of resident proteins, spanning multiple families such as heat shock proteins (HSPs), lectins, and protein disulfide isomerases (PDIs).

A.2 Import of proteins targeted into the lumen of the ER

The location where a protein is synthesized is often different from its site of action, and the proper transport of proteins to their destinations is crucial for maintaining order and organization within all cells. In mammalian cells, for proteins requiring transit through the ER or for resident ER proteins, nascent polypeptides from cytosolic ribosomes are predominantly imported co-translationally (Corsi and Schekmant 1996). Proteins displaying a signal sequence (SS) (i.e. a short peptide at the N-terminal emerging from the ribosome) enable the ribosome-nascent chain complex (RNC) to interact with the 54 kDa subunit of the signal recognition particle (SRP) complex (SRP54) (Tajima et al. 1986). Meanwhile, other SRP polypeptides and the ARN 7S cause a temporary halt in the elongation of the polypeptide chain

(Egea, Stroud, and Walter 2005). Subsequently, the RNC-SRP complex binds to the SRP receptor (SR) anchored to the ER membrane, mediated by a GTP-dependent interaction between SRP and SR (Connolly and Gilmore 1993). The ribosome, while still associated with the SRP and the nascent polypeptide chain, docks onto the translocon complex (Johnson and van Waes 1999), composed of the Sec61αβγ complex embedded in the ER membrane (Greenfield and High 1999). Successively, GTP hydrolysis occurs, resulting in the release of SRP. Concurrently, the SS is cleaved by signal peptidase (SP) as the polypeptide continues to elongate (**Figure 12**).

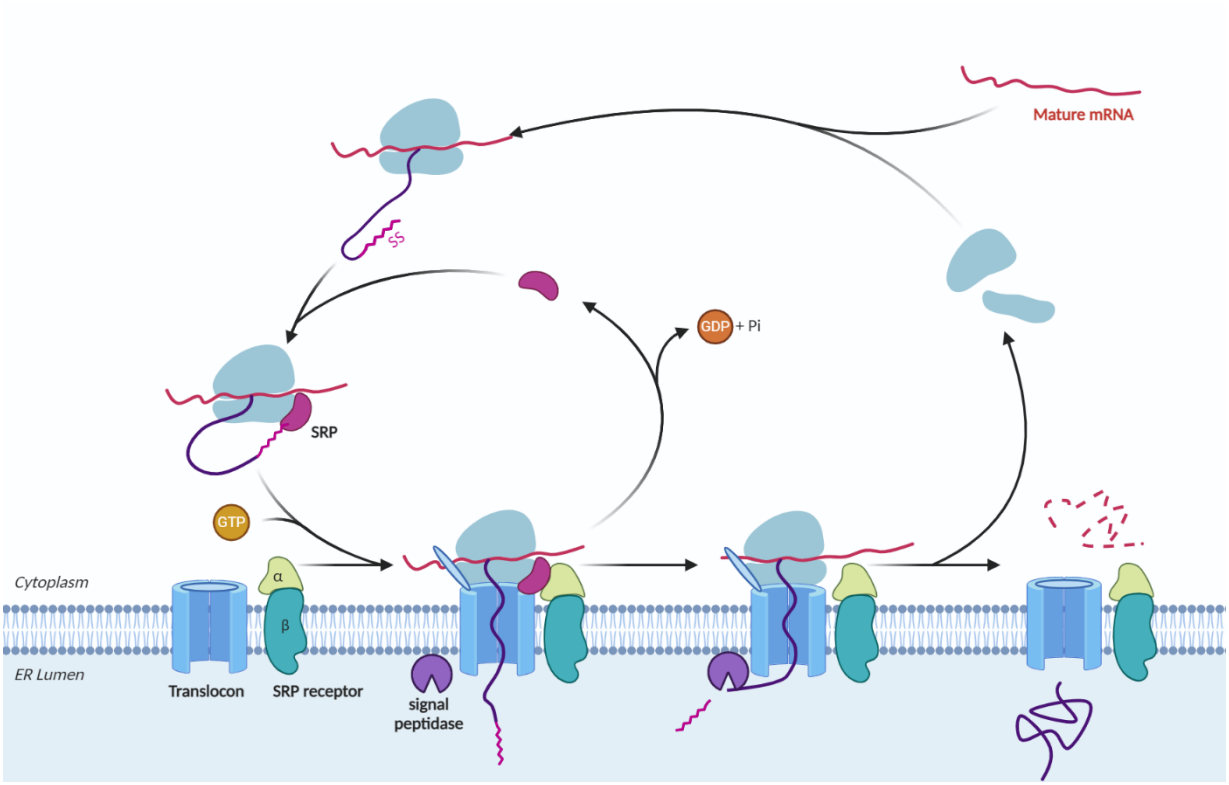


Figure 12. Co-translational import of proteins to the ER. The ER sequence signal (SS) of a newly synthesized polypeptide is recognized by the signal recognition particle (SRP) complex, which then directs it to the translocon complex via an ER membrane-bound SRP receptor. Then the SRP dissociates from the complex through a GTP-depend mechanism, allowing the polypeptide to restart translocation. Following this, the signal sequence is cleaved by a signal peptidase (SP), and the translation completes in the ER. From a Biorender template created by Luozheng Kong (Creator) and Nima Vaezzadeh.

Regarding the post-translationally mechanism (known as tail-anchored (TA)), it implicates that small proteins are translated in the cytosol. Their transmembrane domain (TMD) is recognized by a cytoplasmic complex composed of TRC40/GET3 to facilitate their insertion into the ER membrane in a Sec61-independent manner (reviewed in; Araki and Nagata 2012).

A.2.1 Protein Folding and post-translational modifications

Then, both co- and post-translationally translocated preproteins are recognized by the oligosaccharyltransferase complex (OST) (Shibatani et al. 2005).
This complex, associated with the translocon complex, can recognize the Asn-X-Ser/Thr consensus sequence on polypeptide chains and catalyzes the formation of the N-glycosidic linkage between the asparagine side chain and the oligosaccharide $Glc_3Man_9GlcNAc_2$ (Glc: glucose, Man: mannose, GlcNAc: N-acetylglucosamine) (reviewed in; Breitling and Aebi 2013). Following the addition of the N-glycan, the two terminal glucose residues are sequentially removed by glucosidases I (G1) and II (G2), resulting in monoglucosylated oligosaccharides ($GlcMan_9GlcNAc_2$) which can be recognized by calnexin and calreticulin, two lectins-based chaperones that ensure the quality control of newly synthesized glycoproteins, along with the ER-resident co-chaperones Erp57. Subsequently, G2 removes the innermost glucose residue ($Man_9GlcNAc_2$), leading to the release of calnexin and calreticulin.

Finally, if the glycoprotein is correctly folded, it can exit from the ER and be transferred to the Golgi by membrane clusters that are coated with COPII coatomer proteins before being addressed to their final destination (Vassilakos et al. 1998; Kuehn, Herrmann, and Schekman 1998). However, resident proteins of the ER contain a specific motif known as the KDEL motif to prevent their secretion by interacting with KDEL receptors found in the intermediate compartment and Golgi apparatus. This interaction also prompts retrieval back to the ER through a COP1 dependent pathway (Raykhel et al. 2007).

However, if the glycoprotein is incompletely folded, the UDP-glucose glycoprotein glucosyltransferase (UGGT) senses it and adds back a glucose residue to the N-glycan allowing its re-association with calnexin and calreticulin for an additional cycle of chaperone-mediated folding (Caramelo et al. 2003) (**Figure 13**). In addition, other important ER-resident chaperones play a role in the protein folding, such as glucose-related proteins GRP78/BiP/HSPA5 (for which the abbreviation BiP will be used throughout this chapter) and its associated DnaJ/HSP40 co-chaperone, and GRP94/Endoplasmin/HSP90B1. Finally, different foldases act in the ER, such as protein disulfide isomerases (PDIs) which catalyze disulfide bond formation, and peptidyl-prolyl isomerases (PPIs), that both lead to covalent modifications stabilizing the folding intermediate (Jansen et al. 2012).

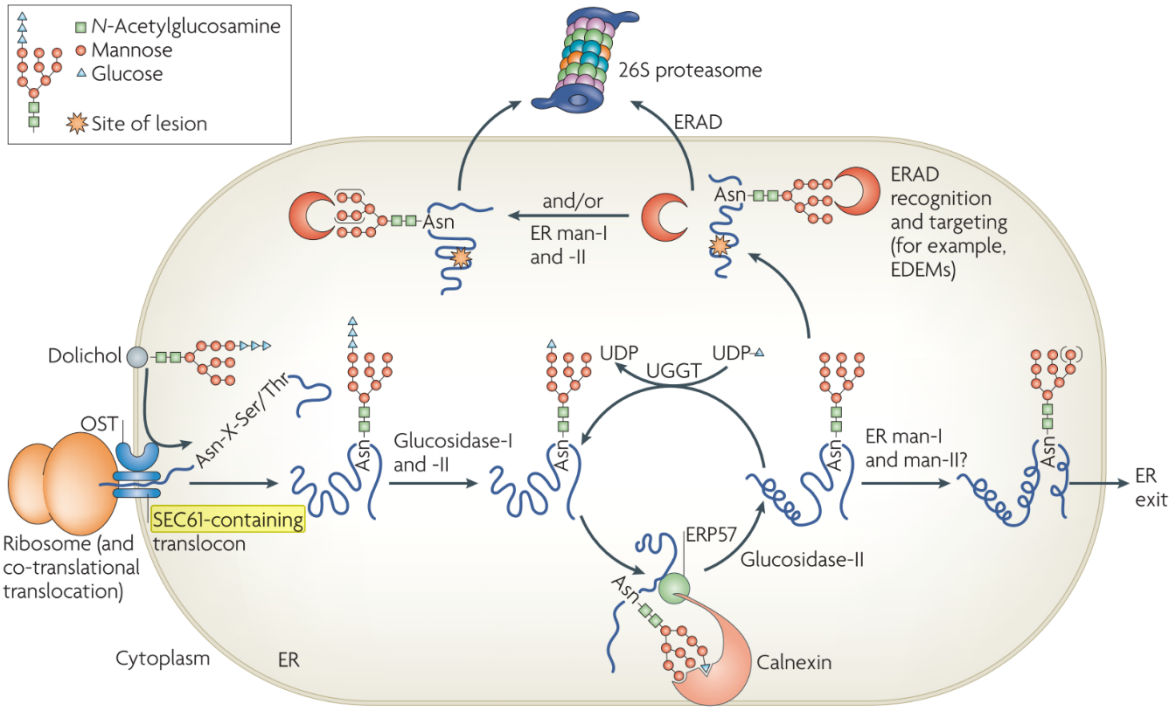


Figure 13. N-linked glycosylation and glycosylated proteins degradation. Proteins entering the ER are N-glycosylated. This process involves the translocon-associated oligosaccharyl transferase (OST) complex, which transfers the glycan to the substrate proteins during translation. Subsequent activity of glucosidase-I and glucosidase-II generates monoglucosylated substrates which is recognized by calnexin and calreticulin to facilitate its folding. If glycoproteins have adopted their native conformations, they undergo demannosylation by ER mannosidases I and II and exit the ER via coatomer protein complex-II vesicles. However, glycoproteins that have undergone multiple folding cycles through reglucosylation and still remain misfolded are targeted for proteasomal degradation. From Vembar and Brodsky, 2008.

A.3 Misfolded protein degradation

In some cases, despite multiple cycles involving calreticulin and calnexin, the glycoprotein may still be misfolded or unfolded. In such cases, it will be directed towards degradation.

Indeed, extended ER retention could result in the cleavage of the terminal mannoses (from $Man_9GlcNAc_2$ to $Man_8GlcNAc_2$ and $Man_7GlcNAc_2$) by ER-mannosidase I (Tremblay and Herscovics 1999) and mannosidase-like proteins named EDEM (EDEM1-3) (Olivari and Molinari 2007) (**Figure 13**). This mechanism seems to act as a quality control timer for misfolded glycoproteins. Specifically, EDEM proteins appear to impair UGGT efficacy, decrease the probability of transport to the Golgi apparatus, and enhance the recognition by ERAD factors such as OS9 and XTP3-B (Groisman et al. 2011). Indeed, the absence of proteases in the ER necessitates that proteins to be degraded are extracted from the ER and directed either to the proteasome or to lysosomes. Consequently, multiple degradation mechanisms exist in the ER.

The majority of misfolded or unfolded proteins are degraded by the ERAD quality control pathway. Once recognized, ERAD substrates are retrotranslocated from the ER to the cytoplasm (McCracken and Brodsky 1996). In mammals, several complexes have been proposed to serve as retrotranslocation channels, such as Sec61, Derlin family proteins, and HRD1 (reviewed in; Römisch 2017). Once in the cytosol, the substrate is ubiquitinated by ER-associated ubiquitin ligases and subsequently extracted by the ATPase p97/VCP complex (Meyer, Bug, and Bremer 2012). Finally, ubiquitinated substrates are transferred to the proteasome by shuttle proteins for degradation.

In addition, ER displays additional degradation pathways, such as ER-phagy and EDEMosomes. ER-phagy is a selective form of autophagy that consists of the selective sequestration of portions of the ER into autophagosomes before being degraded into lysosomes or vacuoles (Bernales, Schuck, and Walter 2007). Briefly, this process relies on ER-phagy receptors, such as members of the FAM134 family (i.e. FAM134B, FAMS134A, FAM134C) that exhibit an LC3-interacting (LIR) domain that can bind to LC3 at the membrane of autophagosomes. During this step, portions of the ER are sequestered and closed inside the autophagosome. Then, their transport occurs through various mechanisms, including macro-ER-phagy, where ER fragments are enclosed by double-membrane autophagosomes that later merge with lysosomes/vacuoles; micro-ER-phagy, where ER fragments are directly engulfed by endosomes/lysosomes/vacuoles; or through the direct fusion of ER-derived vesicles with lysosomes/vacuoles (reviewed in; Reggiori and Molinari 2022).
EDEMosomes are COPII-independent, and they seem to be able to degrade ERAD components including EDEM1 and OS9 in addition to misfolded proteins probably by fusion with lysosomes (Calì et al. 2008; Zuber et al. 2007). All of these quality control mechanisms are crucial for the cell to maintain ER homeostasis, particularly because protein folding, unlike transcription or translation mechanisms, is a highly error-prone process (Guerriero and Brodsky 2012).

A.4 Unconventional protein secretion

The previous description of protein secretion between the ER and the Golgi (anterograde transport from the ER to the Golgi and retrograde transport from the Golgi to the ER) is referred to as conventional protein secretion (CPS) (Marcus C.S. Lee et al. 2004). However, it has been shown that under stress conditions, proteins can use unconventional protein secretion (UPS) (reviewed in; Nickel and Rabouille 2009). There are two classes of proteins that have been identified as secreted via unconventional pathways: cytoplasmic “leaderless” proteins that do not enter the ER and proteins that display a signal peptide or a transmembrane domain that enter the ER but bypass the Golgi. Regarding proteins lacking a signal peptide, such as

fibroblast growth factor 2 (FGF2) or interleukin-1 beta (IL-1β), they can translocate through the plasma membrane thanks to the formation of self-sustained plasma pores (La Venuta et al. 2015) or mediated caspase-1/gadersmin-D plasma pores (Heilig et al. 2018). In addition, this kind of proteins can also be secreted in the extracellular space using ABC-transporters or organelles including autophagosomes, endosomes, and lysosomes. Surprisingly, certain proteins transiting by the ER can bypass the Golgi to reach the plasma membrane. For now, the most described sorting machinery is Golgi reassemble stacking proteins (GRASPs) that allow the delivery of cargo to the plasma membrane without passing through the Golgi (Gee et al. 2011). Interestingly, as numerous SASP factors lack signal peptides, UPS pathways could play an important role in SASP. Investigating the secretory route of SASP factors and uncovering their regulatory elements will be crucial for future ageing research (J. Kim, Gee, and Lee 2018).

B. Endoplasmic Reticulum (ER) stress

B.1 ER Stress detection sensors

To maintain protein-folding balance in the ER, the cell must regulate the ER protein folding load by ensuring a sufficient abundance of ER protein folding machinery, notably chaperones like BiP (Pobre, Poet, and Hendershot 2019).
However, when conditions are unfavorable for proper protein folding, proteins start to accumulate in the ER. If this accumulation persists over time, it leads to a cellular condition referred to as “ER stress”, which can adversely affect the physiological function of cells.

ER stress can be induced by various factors such as heat shock (Liu et al. 2012), viral infection (reviewed in; He 2006), hypoxia (Koumenis 2006), ultraviolet radiation (Komori et al. 2012), and oxidative stress (Ong and Logue 2023). In the last study, the authors used UVA and demonstrated that it triggers the mammalian ER stress response in NHDFs. Similar results were observed in another study using UVC on the human breast adenocarcinoma cell line MCF-7 (H. S. Kim et al. 2019). Indeed, ultraviolet radiation is an external harmful stimulus known to induce oxidative stress, as well as damage to DNA, lipids, and proteins. For example, Chainiaux et *al.*, demonstrated that human dermal fibroblasts exposed to UVB exhibited increased levels of oxidized proteins and modifications in oxidation patterns (Debacq-Chainiaux et al. 2005). Additionally, UVA and UVB can impair the proteasome function of human keratinocytes by reducing the activity of proteasome peptidase, increasing levels of oxidized an ubiquitinated proteins, as well as lipid peroxidation (Bulteau et al. 2002). In addition, ER stress can be provoked by several chemical molecules. For instance, tunicamycin blocks the initial stage of N-linked glycan biosynthesis in proteins (Olden et al. 1979),

dithiothreitol inhibits protein disulfide bond formation (Cleland 1964) and thapsigargin reduces the ER Ca^{2+} concentration by disrupting the Sarco/ER Ca^{2+}-ATPase (SERCA) (Thastrup et al. 1990). The nature and severity of the stressor or condition determine the intensity of the ER stress response (Hetz 2012).

Additionally, It has been demonstrated that ER stress is triggered in physiological conditions, such as in cells with high secretory capacity, like plasma cells (Y. Ma et al. 2010), and in various tissues under pathological conditions associated with obesity, type 2 diabetes (reviewed in; Cnop, Foufelle, and Velloso 2012), and cancer (reviewed in; Oakes 2020). Moreover, as mentioned at the beginning of this introduction, physiological ageing is accompanied by a loss of proteostasis (López-Otín et al. 2023) which is correlated with alterations in the expression of ER chaperones (Macario and Conway de Macario 2002). In addition, the gradual breakdown of chaperone systems can lead to the conversion of soluble proteins or protein fragments into insoluble fibrils or plaques. This process may serve as the initial stages of age-related disorders such as Alzheimer's or Parkinson's diseases (reviewed in; Brown and Naidoo 2012), making ER stress as one of the regulators influencing the balance between healthy and pathological ageing.

However, these observations could also lead us to believe that, during the ageing process, chronic low-grade ER stress might need to be established to compensate for these effects and activate the necessary signalling networks if needed. Indeed, to cope with ER stress and restore proteostasis, cells initiate a variety of adaptative responses, some of which are described above.

One of these is known as the unfolded protein response (UPR). Actually, if ERAD is unable to eliminate misfolded proteins, these proteins may aggregate, becoming substrates for ER-phagy. If not effectively cleared, misfolded proteins can trigger the UPR, aiming to decrease the accumulation of misfolded proteins and prevent abnormal protein accumulation.

It is important to note that this thesis focused solely on the UPR^{ER}, while a similar mechanism exists in mitochondria, and is known as UPR^{mt}. In response to impaired mitochondrial proteostasis, the UPR^{mt} system, comprising its own chaperones and proteases, communicates with the nucleus to restore mitochondrial homeostasis. In mammals, several UPR^{mt} axes have been identified: The canonical UPR^{mt} response involves transcription factors CHOP, ATF4, and ATF5, which enhance mitochondrial folding capacity. The UPR^{mt} sirtuin axis increases antioxidant capacity through the transcription of SOD2 and catalase. The UPR^{mt} translation axis reduces the folding load by regulating pre-RNA processing and translation, and finally the The UPR^{mt}/ER axis improves protein quality control mechanisms (Münch 2018). Interestingly, it has long been believed that UPR^{mt} activation is associated with lifespan extension in C.

elegans. In 2022, Xin et *al.*, demonstrated that the import machinery is necessary for UPR-induced lifespan extension (Xin et al. 2022).

B.1.1 Unfolded protein response (UPR) activation in the endoplasmic reticulum

ER stress is sensed by the luminal domain of three transmembrane proteins of the ER: Inositol-requiring enzyme 1α (IRE1α), PKR-like ER kinase (PERK), and activating transcription factor 6α (ATF6α) (reviewed in; Foufelle and Ferré 2007). To date, two main stress-sensing mechanisms have been observed: the first involves an indirect mechanism where the ER sensing process relies on the co-chaperone BiP, while the second entails activation of the three sensors by direct sensing of unfolded proteins.

In the first model, it was initially proposed that PERK and IRE1α remain inactive under resting conditions due to their physical interaction with the ER chaperone BiP, and that disruption of protein folding leads to the reversible dissociation of BiP from the luminal domains of these two sensors and thus to their activation (Bertolotti et al. 2000). Two years later, a similar mechanism was described for the constitutive activation of the ATF6 branch (Shen et al. 2002). BiP functions as a typical HSP70 chaperone, cycling between an open ATP-bound state and a closed ADP-bound state, facilitated by co-chaperones (Blond-Elguindi et al. 1993). Misfolded proteins are recruited to BiP substrate-binding domain by co-chaperones when BiP is in the open ATP-bound state. This association stimulates BiP ATPase activity, causing it to convert to a closed ADP-bound state, trapping the misfolded protein substrate. Nucleotide exchange factors (NEF) promote the exchange of ADP to ATP, causing BiP to revert to the open ATP form and release the bound substrate (reviewed in; Kampinga and Craig 2010).

Additionally, other BiP-independent mechanisms have been described, particularly in yeast, where Ire1p activity, the unique ER stress-sensory system, does not primarily depend on BiP (Kimata et al. 2006). Although a similar mechanism remains debated for the ER stress sensors of mammals it has been demonstrated that IRE1α and ATF6α can detect changes in the ER membrane lipid bilayer independently of the ER luminal domain for signalling (Halbleib et al. 2017; Tam et al. 2018). Finally, studies have also reported the possibility of a transmissible ER stress (TERS). Firstly,it has been demonstrated that the vascular endothelial growth factor (VEGF) could lead to a rapid activation of the three UPR sensors in HUVEC, without accumulation of misfolded proteins in the ER (Karali et al. 2014). Secondly, TERS has been demonstrated between two different cell types (e.g. cancer cells and immune cells), suggesting that it may facilitate tumor progression (Mahadevan et al. 2011; Rodvold et al. 2017).

B.2 UPR signalling pathways

Activation of the three UPR branches usually enhances the expression of ER chaperones, improves the degradation of misfolded proteins, enhances quality control mechanisms, and reduces protein translation. Some of these effects are synergistic across all three branches, while others depend on a specific signalling cascade activated by one sensor following ER stress sensing. In this section, each branch will be described individually (**Figure 14**).

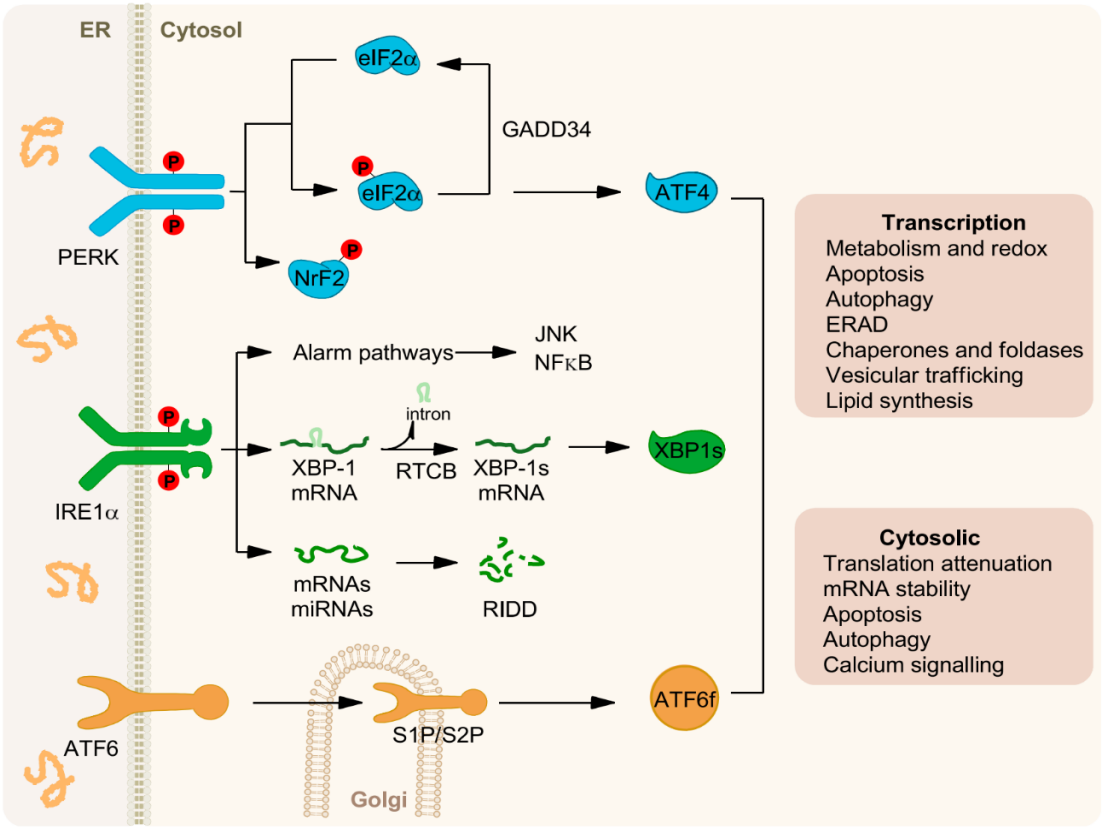


Figure 14. The signalling of the three branches of the UPR. During ER stress, the accumulation of misfolded proteins triggers the dissociation of the chaperone BiP from the three UPR sensors: PERK, IRE1α, and ATF6α. This initiates a cascade of events: PERK activates NrF2 and eIF2α. The latter inhibits global translation while selectively enhancing ATF4 translation; IRE1α splices XBP1 mRNA to produce the active transcription factor XBP1s and mediates mRNA degradation via the RIDD pathway. In addition, IRE1α can induce alarm pathways such as JNK or NFκB during ER chronic stress; ATF6 migrates to the Golgi apparatus, undergoing cleavage by the proteases S1P and S2P, which releases its cytosolic domain (ATF6f). Together, these UPR effectors activate a set of genes to cope with ER stress. From Hetz, Papa. 2018.

B.2.1 IRE1α arm

In mammals two IRE1 genes exist: IRE1α and IRE1β. While IRE1α is ubiquitously expressed in all cells and tissues, IRE1β expression is confined to intestinal epithelial cells (Tirasophon et al. 2000). Among mammalian UPR sensors, IRE1α is considered the most evolutionarily conserved, as it was first identified in the yeast *Saccharomyces cerevisiae* before its mammalian homolog in 1998 (Tirasophon, Welihinda, and Kaufman 1998). Since then, IRE1α has been recognized as a type 1 transmembrane protein with both serine/threonine kinase and endoribonuclease (RNase) domains located in its cytosolic region. Upon ER stress, IRE1α undergoes dimerization and trans-autophosphorylation, triggering a conformational change that activates its RNase domain (Zhou et al. 2006).

This activation, in combination with the tRNA ligase RTCB, catalyzes the excision of a 26-nucleotide intron within the (X-box Binding Protein 1) *XBP1* mRNA into an active transcription factor termed *XBP1s* for the spliced (H Yoshida et al. 2001; Calfon et al. 2002). XBP1s translocates to the nucleus, where it stimulates the transcription of UPR-associated genes by binding to the ER-stress response element (ERSE) and unfolded protein response element (UPRE), thereby regulating genes involved in folding, quality control, and lipid synthesis (reviewed in; S. M. Park, Kang, and So 2021).

In addition to *XBP1* mRNA, IRE1α degrades a set of ER-associated mRNAs or microRNA precursors, which may contribute to reducing the protein folding load. This activity of IRE1α, called IRE1α-dependent mRNA decay (RIDD), is independent of *XBP1* mRNA splicing (Hollien and Weissman 2006; Upton et al. 2012). For instance, the circadian clock *PER1* mRNA is a substrate of RIDD (Pluquet et al. 2013).

The IRE1α cytosolic domain may also serve as a scaffold to recruit adaptor proteins which can trigger the activation of additional signalling pathways, including the Mitogen-activated protein kinase (MAPK) pathways (reviewed in; Darling and Cook 2014). This additional role of IRE1α will be described later in section B.3.

B.2.2 PERK arm

Under ER stress conditions, PERK, a type 1 transmembrane kinase, undergoes oligomerization and trans-autophosphorylation. This process leads to the inhibition of general protein translation by phosphorylating eukaryotic translation initiator factor-2 (eIF2α) at serine 51 (Harding, Zhang, and Ron 1999). Nonetheless, a select group of mRNAs containing specific upstream open reading frames (uORFs) in their 5' end can counteract this inhibition and undergo more efficient translation when P-eIF2α levels are elevated, such as activating transcription factor 4 (ATF4) (Harding et al. 2000; P. D. Lu, Harding, and Ron 2004). ATF4

triggers the transcription of UPR target genes responsible for various cellular processes, including amino acid biosynthesis, antioxidative response, autophagy (to remove aggregated proteins), and apoptosis (Harding et al. 2003).
In addition, Cullinan *et al.* have demonstrated that PERK is also able to phosphorylate Nrf2 which promotes its dissociation from Keap1 and its nuclear localization to regulate genes involved in cellular redox homeostasis (Cullinan et al. 2003).

B.2.3 ATF6α arm

The gene sequence of *ATF6* was discovered in 1989 (Hai et al. 1989), and it was first shown that ATF6 can interact with the serum response factor (SRF) transcription factor (C. Zhu, Johansen, and Prywes 1997).
Nowadays, ATF6 is known as a type 2 transmembrane protein of 90kDa located in the ER. The cytoplasmic segment of ATF6 comprises a basic region/leucine zipper motif (bZIP domain) responsible for DNA binding and a transactivation domain (Haze et al. 1999) whereas its carboxy-terminal luminal region comprises the ER stress-sensing domain (X. Chen, Shen, and Prywes 2002).
In mammals, two homologous ATF6 proteins are expressed: ATF6α and ATF6β that are both functional and respond to ER stress (H Yoshida et al. 1998; Haze et al. 2001). Surprisingly, the double ATF6α and ATF6β-knockout in mice resulted in embryonic lethality (Yamamoto et al. 2007). However, the N-terminal region of ATF6α contains an 8-amino acid sequence known as VN8, which exhibits high transcriptional activity compared to ATF6β (Thuerauf et al. 2002). Additionally, ATF6β may inhibit ATF6α and regulate the duration of ATF6α signalling (Forouhan, Mori, and Boot-Handford 2018). As a result, studies often prioritize exploring the function of ATF6α.

In their study, Nadanaka *et al.* highlighted clear distinctions in the activation and mechanisms of IRE1α and PERK compared to ATF6α. Particularly, they showed that in the absence of ER stress, ATF6α exists in multiple forms (monomer, dimer, and oligomer) due to the formation of inter- and intramolecular disulfide bridges in its luminal domain. Whereas, upon ER stress, the full-length ATF6α (ATF6αp90) is deoligomerized because of the reduction of disulfide bonds, notably via the action of PDIA5 (Higa et al. 2014). Simultaneously, the release of BiP and subsequent unmasking Golgi localization site (GLS), leads to the relocation of the reduced monomeric form to the Golgi apparatus (Shen et al. 2002), where it undergoes proteolytic cleavage by site-1 and site-2 proteases (S1P and S2P) (Ye et al. 2000). This process releases an active cytosolic fragment containing the bZIP domain known as ATF6αp50, which acts as a transcription factor by binding to specific ER stress response elements (Kokame, Kato, and

Miyata 2001).

This fragment then relocates to the nucleus to initiate a subset of UPR target genes. Activation of ATF6α increases the protein folding capacity within the ER, as its targets include key proteins located in the ER involved in protein folding, such as BiP and GRP94/Endoplasmin/HSP90B1, to maintain ER function (J. Wu et al. 2007). In addition, ATF6α induces the transduction of ER-associated degradation (ERAD) components and modulates XBP1 mRNA levels (J. Wu et al. 2007; H Yoshida et al. 2001). Interestingly, Adachi *et al.* conducted a genome-wide search for ATF6α target genes using microarray analysis of mouse embryonic fibroblasts deficient in ATF6α, revealing the identification of 30 genes as ATF6α target genes. Among this list, 7 genes were ER chaperones, 5 were ERAD components and 6 were ER proteins (Adachi et al. 2008). Finally, ATF6α can also drive the expansion of the ER (Bommiasamy et al. 2009).

B.3 From ER stress recovery to chronic ER stress

In general, the activation of the UPR response *in vivo* remains somewhat unclear. While its regulation may depend on factors such as the type, intensity, and duration of stress, several questions persist: Do all three pathways always contribute together to the response? Do they share the same activation kinetics?

Therefore, it would be more prudent to consider the UPR response as a rheostat, capable of adjusting the intensity of its response based on varying conditions.

In cases of the adaptive responses provided by the UPR signalling are unable to restore protein-folding balance, the persistence of the response could lead to alternative signalling pathways known as the "terminal UPR" (reviewed in; Hetz, Chevet, and Oakes 2015). This dynamic response is primarily driven by IRE1α and PERK and consists of two main phases; firstly a transition phase, and an end-stage that upregulates specific pro-apoptotic genes (reviewed in; Woehlbier and Hetz 2011; Hetz and Papa 2018).

B.3.1 Apoptosis signalling

These phases leading to pro-survival or pro-apoptotic cell fate, are primarily regulated by PERK through ATF4 which induces the proapoptotic factor CCAAT/enhancer-binding protein homologous protein (CHOP) and GADD34. On the one hand, CHOP promotes apoptosis by inhibiting the expression of anti-apoptotic BCL-2 and activating pro-apoptotic members such as BIM, as well as death receptor DR5 via the caspase-8 (McCullough et al. 2001; Puthalakath et al. 2007; M. Lu et al. 2014). On the other hand, GADD34 facilitates the dephosphorylation of eIF2α by targeting protein phosphatase 1 (PP1) to restore mRNA translation (Novoa et al. 2001). As mentioned above IRE1α's RNase activity decreases specific microRNA levels which

can inhibit pro-apoptotic targets, such as the pro-oxidant protein TXNIP (thioredoxin-interacting protein). Elevated TXNIP protein levels activate the NLRP3 inflammasome and its caspase-1-dependent pro-death pathway (Lerner et al. 2012). In addition, chronic ER stress results in the recruitment of adaptor proteins to IRE1α. For instance, tumor necrosis factor receptor-associated factor 2 (TRAF2) elicits the activation of apoptosis signal-regulating kinase 1 (ASK1) and subsequently activates its downstream targets, notably c-Jun N-terminal kinase (JNK) which could induce apoptosis (Urano et al. 2000). In summary, the control of ER stress-induced apoptosis involves a complex interplay of multiple mechanisms and only some of them are described in this section.

B.3.2 UPRosome concept

The transition from an adaptive to a terminal pro-apoptotic UPR is not fully understood, but models are proposed to elucidate how the intensity and duration of stress stimuli are integrated. In the case of IRE1α signalling, it has been suggested that the amplitude and kinetics of its signal could be regulated by its interactome (protein-protein interaction) termed the “UPRosome” in which positive and negative co-factors act as a checkpoint to determine downstream signalling responses as well as the duration of the response (Hetz and Glimcher 2009). Hence, questions arise: What is the threshold at which mild ER stress induced homeostatic UPR transitions to a chronic and terminal UPR state promoting apoptosis and inflammation? Is it possible to quantify the intensity of this response in cellular or animal models? Can we mitigate the adverse effects of prolonged ER stress on cellular fate?

C. Implication of Endoplasmic reticulum stress in diseases

C.1 Role of ER stress in skin physiology and related disorders

ER stress arises in cells due to various internal and external stresses. Chronic ER stress is implicated in numerous human diseases and has been extensively investigated in conditions such as type 2 diabetes, neurodegenerative disorders, atherosclerosis, liver diseases, and cancers (reviewed in; Ozcan and Tabas 2012). However, this section will delve into the lesser-explored role of ER stress in the physiology and pathophysiology of the skin. While subtoxic levels of ER stress-induced UPR are essential for normal cellular functions, including differentiation, recent findings suggest that prolonged UPR activation contributes to the onset of certain skin conditions.

C.1.1 ER stress in the physiology of the skin

In the skin, disruptions in the epidermal barrier and exposure to external stressors like UV radiation can induce ER stress (Celli et al. 2011; Anand et al. 2005). Surprisingly, the UPR is activated during normal epidermal keratinocyte differentiation and induces the expression of genes essential for this process (Sugiura et al. 2009). Among the regulators of keratinocyte differentiation *in vitro* and *in vivo*, the sequential differentiation of keratinocytes is facilitated by a calcium gradient that increases from the basal layer until the most superficial layer of the skin (Hennings et al. 1980; Bikle et al. 2001). As the largest calcium store in the cell, the ER controls the balance between CA^{2+} release and uptake. Interestingly, Celli *et al.* showed that ER CA^{2+} depletion using thapsigargin in human keratinocytes and mice triggered the activation of XBP1 and controls terminal differentiation (Celli et al. 2011).

C.1.2 Chronic ER stress is associated with skin diseases

In vivo, deregulation of ER calcium balance can lead to numerous diseases (reviewed in; (Mekahli et al. 2011).

For instance, mutations in the *ATP2A2* gene, which encodes the sarco/endoplasmic reticulum CA^{2+}-ATPase 2 (SERCA2) pump, result in dysfunctions in CA^{2+} exchanges from the cytosol to the ER, consequently inducing chronic ER stress in keratinocytes. This defective ER calcium homeostasis in keratinocytes, observed in the rare genetic Darier's skin disease, leads to loss of adhesion due to impaired trafficking of junctional components, such as desmosomal cadherins causing acantholysis, and to UPR-induced apoptosis in keratinocytes, resulting in dyskeratosis (Savignac et al. 2014; Hovnanian 2004). Clinically, individuals suffering from Darier's disease exhibit fragile and often malformed fingernails, along with multiple red papules and plaques characterized by hyperkeratosis that can affect large areas of the body, typically appearing on the upper trunk or neck during adolescence and worsening throughout life, notably with sun exposure. This skin condition can be highly painful, particularly when fissures develop or when the lesions become infected, and Darier patient have an increased risk of neuropsychiatric disorders. Currently, there is no cure for Darier's disease, but symptoms can be managed through lifestyle adjustments, topical therapies such as retinoids, and surgical intervention if needed (Bernard et al. 2024).

As explained above, mild ER stress is implicated in keratinocyte differentiation suggesting that chronic ER stress can contribute to skin diseases associated with abnormal differentiation (reviewed in; K. Park et al. 2019).

Additionally, even if the precise mechanisms have not been already understood, increased ER stress has been described in common inflammatory skin diseases like psoriasis and rosacea (Zhao et al. 2020; Melnik 2014).

Moreover, keratinocytes are not the only type of epidermal cells implicated in skin diseases. As an example, Vitiligo is a condition characterized by depigmentation in specific areas, affecting melanocytes and resulting in loss of pigment in the skin, mucous membranes, and hair. Remarkably, the study of Birlea *et al.* established a genetic association between XBP1 and vitiligo using a genome-wide dataset of human patients (Birlea et al. 2011).

Finally, studies reported that ER stress in melanoma, even at early stages, exhibit a high expression of BiP that was associated with poor survival prediction against malignant melanoma (Hersey and Zhang 2008; Shimizu et al. 2017). Conversely, mild ER stress in melanoma cells could sustain autophagy in resistant cells notably through PERK activation and autophagy-related genes transcription particularly, via ATF4 and CHOP (Ohoka et al. 2005; B'chir et al. 2013).

C.2 UPR during ageing

For many years, the ability of organisms to handle stress by activating cellular stress responses has been linked to longevity. Studies indicate that, as individuals age, their ability to initiate these stress responses diminishes. However, external activation of these responses and prevention of this decline have been associated with improvements in stress resistance and a delay in the ageing process. (reviewed in; Haigis and Yankner 2010).

With age, ER chaperones and folding enzymes exhibit reduced expression and impaired capacity in murine livers due to the progressive accumulation of oxidative stress, leading to dysfunctional ER. This may consequently diminish the UPR's ability to regulate proteostasis effectively (Rabek, Boylston, and Papaconstantinou 2003; Nuss et al. 2008). In addition, studies on various animal models demonstrated that this long-term dysfunction ER stress response is associated with robust pro-apoptotic signalling (reviewed in; Brown and Naidoo 2012). For instance, Hussain *et al.* demonstrated that although the absolute levels of UPR sensors, such as PERK, may not decrease with age, their activity is reduced, resulting in a decrease in overall protein translation and diminished phosphorylation of eIF2α. This leads to a switch from the synthesis of pro-survival to pro-apoptotic proteins, rendering cells more susceptible to stress-induced death pathways (Hussain and Ramaiah 2007). Interestingly, a study on human subjects revealed that the activation of the UPR in the muscles of older individuals in response to exercise is attenuated compared to young adults (Hart et al. 2019).

Together, these results suggest that modulation of the UPR pathways with age could either extend or reduce longevity. Actually, it has been proved in *C. elegans* that extending longevity could potentially be achieved by circumventing loss of stress response activation, including UPR, and particularly the IRE1α branch (Henis-Korenblit et al. 2010; Matai et al. 2019).

However, some studies suggest a more complex relationship regarding the role of the UPR in ageing. For example, Kristensen *et al.* reported an increased activity of the ATF6α pathway in the liver with ageing (Kristensen et al. 2017). Moreover, another study revealed an age-related increase of components of the ATF6α and IRE1α branches in aged β-cells of monkeys (Li et al. 2021). These recent studies suggest that, in more complex models, there might be a more nuanced regulation of the UPR with age, potentially specific to tissues and contexts.

D. Evidence of an interconnection between UPR and cellular senescence

D.1 Alteration of ER homeostasis during senescence

Various cell types experiencing senescence in response to diverse stressors exhibit structural changes in the ER and trigger UPR activation (reviewed in; Abbadie and Pluquet 2020). However, depending on the cell type and the senescence inducer, it appears that not all three branches are activated.

For instance, HRAS-driven melanocyte senescence displays vacuolization and expansion of the ER as well as activation of the three branches of the UPR (Denoyelle et al. 2006) whereas in HRAS-induced senescent epidermal keratinocytes only IRE1α, is activated (Blazanin et al. 2017). Moreover, in senescent lymphoma cells induced by TIS, Dörr *et al.* observed ultrastructure ER stress alterations and activation of IRE1α/XBP1 and PERK/ATF4 (Dörr, Yu, Milanovic, Beuster, Zasada, Däbritz, et al. 2013). In addition, in replicative senescent NHDFs and UVC-induced senescent human breast adenocarcinoma cells, all the three arms of the UPR have been showed to be increased (Druelle et al. 2016; H. S. Kim et al. 2019). Finally, as a proof of concept demonstrating the interconnection between ER stress and senescence, these two studies have shown that the application of chemical ER stress inducers on proliferating cells was sufficient to induce major hallmarks of senescence in these cells (Druelle et al. 2016; H. S. Kim et al. 2019).

D.2 Impact of UPR modulation on senescence biomarkers

As the first secretion compartment of cells, the ER plays a crucial role in generating, processing, and releasing SASP factors (Machado-Oliveira et al. 2020).

Recently, it has been proposed that ER stress and the subsequent activation of the UPR upon senescence could contribute to the modified secretome of senescent cells (Pluquet and Abbadie 2021). While there are multiple connections between the UPR and inflammation (Dandekar, Mendez, and Zhang 2015; Schmitz et al. 2018), the UPR and normal or tumoral secretome (Smith et al. 2020; Logue et al. 2018; Rubio-Patiño et al. 2018) the data directly linking ER stress with SASP are scarce. Our group demonstrated that knocking-down ATF6α in replicative fibroblasts decreased *IL-6* mRNA levels (Druelle et al. 2016). Another group

proposed that UPR induction in RAS-mediated senescence promotes macroH2A1 expression, subsequently leading to the expression of various SASP-associated genes in fibroblasts (H. Chen et al. 2015). Moreover, a third group suggested that OIS and TIS induce proteotoxic stress and UPR activation to ensure SASP production (Dörr, Yu, Milanovic, Beuster, Zasada, Däbritz, et al. 2013).

Furthermore, among the senescence biomarkers typically overlooked in studies, the literature indicates a complex interplay between the modulation of UPR branches and the senescent phenotype (reviewed in; Pluquet, Pourtier, and Abbadie 2015) (**Table 3**). Indeed, it appears that depending on the cell type and the senescent inducer, modulation of one or more UPR branches could either diminish or enhance senescence. These observations are particularly true for the SA-βgal and cell cycle arrest (reviewed in; Abbadie and Pluquet 2020). Additionally, as the cell cycle arrest observed in senescence primarily arises from the accumulation of unresolved DNA damage, the relationship between DNA damage/repair mechanisms and ER stress/UPR during senescence needs to be more investigated. Indeed, the interconnection between UPR and DDR is still poorly understood. To date, only a limited number of studies have explored this connection, with a predominant focus on understanding how these two signalling pathways are associated with the development and progression of diseases, particularly cancers. These studies have revealed a particularly significant role of PERK and IRE1α at the intersection of this process whereas the role of ATF6α remains to be clarified (reviewed in; González-Quiroz et al. 2020).

The literature on the effects of UPR modulation on the establishment of senescence biomarkers is limited, and the details of certain studies mentioned above will be further discussed in the conclusion section of the manuscript to link the results obtained in our project with these studies.

Table 3. The effects of UPR modulation on senescent biomarkers in different models. *Adapted from Pluquet et al. 2015.*

Model	ER stress modulator	Senescence characteristic	Reference
HRas-driven senescence in melanocytes	ATF4 siRNA DNA-ATF6α XBP1 siRNA DN-IRE1α	Reduced the % of SA-βgal-positive cells	(Denoyelle et al. 2006)
Primary embryo fibroblasts HRAS and SV40 large T antigen	$ATF4^{-/-}$	Triggered senescence by expressing constitutively p16INK4 and p19ARF	(Horiguchi et al. 2012)
HRas-driven senescence in primary murine keratinocytes	ATF4 siRNA XBP1 siRNA	Increased the % of SA-βgal-positive cells Reduced p21 protein expression	(B. Zhu et al. 2014)

HT1080 human fibrosarcoma	E235: activator of ATF4	Increase in perinuclear SA-βgal-staining Increase in cell size Increase in p21 protein expression	(Sayers et al. 2013)
H2O2-induced senescence in WI38 cells	Chemical inhibitor GSK2606414 Chemical inhibitor 4μ8c	Increase in p21 protein expression Reduced the % of SA-βgal positive cells Reduced the % of SA-βgal positive cells	(Matos, Gouveia, and Almeida 2015)
Human endometrial stromal cells (ESCs)	Calreticulin siRNA	Increased the % of SA-βgal positive cells	(Kusama et al. 2014)
Primary MEFs Doxorubicin-induced senescence in HT1080 cells and tumor xenograft assays HRas-driven	$eIF2\alpha^{A/A}$ $PERK^{-/-}$ eIF2α siRNA	Increased the % of SA-βgal positive cells	(Rajesh et al. 2013)
B16F10 mouse melanoma HT1080 MCF7 Human	PERK siRNA	Increased the % of SA-βgal positive cells Increase in p21 protein expression	(H. D. Kim et al. 2012)
RS Normal human dermal fibroblasts NHDFs	ATF6α siRNA IRE1α siRNA	Reduced the % of SA-βgal positive cells	(Druelle et al. 2016)
MCF7 Human + siRNA NBR1	ATF6α siRNA PERK siRNA IRE1α siRNA	Reduced the % of SA-βgal positive cells Increased the % of SA-βgal positive cells	(H. S. Kim et al. 2019)

3. The skin: A flexible model for investigating adaptative stress responses

A. Physiology of the skin

A.1 Skin structure and cellular composition

The human skin is a dynamic and complex organ composed of three overlaying layers, arranged from the outermost to the deepest: the epidermis, a pluristratified epithelium; the dermis, intricately connected to the epidermis through the dermo-epidermal junction; and the hypodermis. Each layer displays notable variations in both structure and cellular composition.

A.1.1 Epidermis

The epidermis derives from the non-neural ectoderm, and its stratification during embryogenesis is orchestrated by basal epidermal cells, referred to as basal keratinocytes. Epidermal stem cells are found in the epidermal basal layer and hair follicle niches (Blanpain and Fuchs 2006). This cell population constitutes a pool of mitotically active progenitors that undergo asymmetric division to drive the differentiation and stratification of the epidermis (Lechler and Fuchs 2005).

In adulthood, epidermal homeostasis is also sustained by the delamination of basal keratinocytes, leading to their detachment from the cutaneous basement membrane and initiating their differentiation process along the suprabasal layers (Moreci and Lechler 2020). In addition, many signalling pathways drive epidermal differentiation, such as Notch, the transcriptional regulator p63, the C/EBPβ transcriptional regulators, and the Kruppel-like factor 4 (KLF4) (Blanpain and Fuchs 2009).

In the healthy epidermis, the basal layer consists of a monolayer predominantly composed of cuboid keratinocytes, along with a small number of melanocytes and Merkel cells, whereas Langerhans cells are located in suprabasal layers (**Figure 15**).

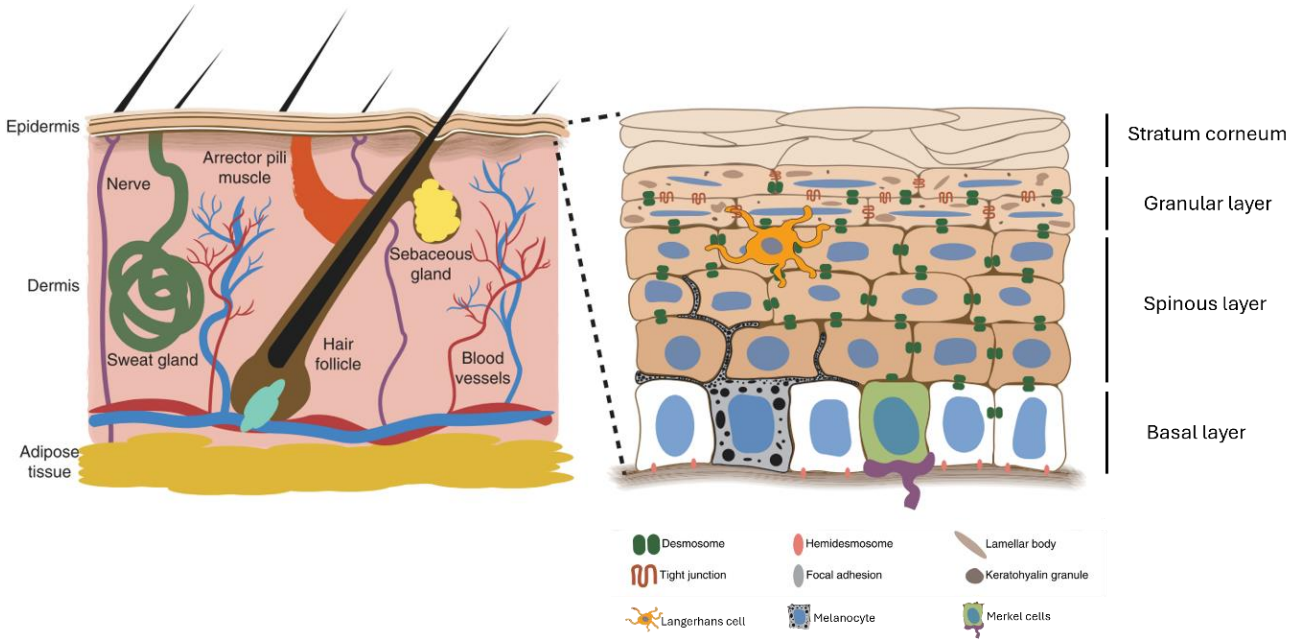


***Figure 15. Schematic illustration of the skin structure and epidermal layers*.** The skin consists of three major layers: the epidermis, with detailed views of its structure and cell composition shown on the right part of the scheme; the dermis, with the representation of all cutaneous appendages; the hypodermis, characterized as adipose tissue. Adapted from R. Moreci, T. Lechler, 2020.

Melanocytes play a crucial role in skin pigmentation and protection against ultraviolet (UV) which will be further explained (Cichorek et al. 2013). Merkel cells interact with sensory nerve endings in the skin and play a specific role in mechanotransduction (Maksimovic et al. 2014). All the basal cells adhere together by cell junctions. While adherent junctions allow the cadherin-actin cytoskeleton connections, desmosomes join the keratin intermediate filaments of neighboring keratinocytes (Rübsam et al. 2018). The proliferating keratinocytes from the basal layer express cytokeratin 5 (KRT5) and cytokeratin 14 (KRT14). Conversely, the transition to the spinous layer leads to a switch in the expression of cytokeratin, favoring the expression of cytokeratin 1 (KRT1) and cytokeratin 10 (KRT10) in the early differentiating keratinocytes (Moll, Divo, and Langbein 2008).

The spinous layer is composed of multiple layers of polyhedral-shaped keratinocytes and constitutes the initial stage of differentiation. During this phase, keratinocytes lose their proliferative capacity and establish robust adhesion through desmosomes.

Then, in the granular layer, the keratinocyte's shape, organization, and adhesion are further modified. At this stage, keratinocytes adopt a squamous shape, get bigger and flattened, express loricrin and filaggrin, undergo changes in nuclear morphology, and develop tight junctions. Granular keratinocytes exhibit keratohyalin granules containing loricrin and profilaggrin and produce lamellar bodies (Kanitakis 2002; Moreci and Lechler 2020). Moreover, the gradual gradient of calcium from the basal to the granular layer coordinates the differentiation process with vitamin D by sequentially activating and inhibiting the genes involved in the differentiation stage (Bikle et al. 2001).

Between the spinous and the granular layers, Langerhans cells, distinct epidermis-resident macrophages with dendritic cell functionality, act as antigen-presenting cells during immune response (West and Bennett 2018).

Finally, in the late differentiation stage, the cornified layer (i.e. *stratum corneum*), consists of thickened, anucleate, and dead keratinocytes named corneocytes. In this layer, profillagrin from keratohyalin granules mature into fillagrin, promoting cell keratinization. Structurally, proteins like loricrin and involucrin replace the cell's membrane, and lamellar bodies secret their contents into extracellular space, forming the cornified envelope (Eckhart et al. 2013).

The basement membrane, named the dermo-epidermal junction (DEJ) separates the basal epidermal layer from the dermis. It consists of the *lamina lucida* and the *lamina densa,* which allow adhesion between basal keratinocytes and the underlying extracellular matrix (ECM). This mechanism is facilitated by a structural network composed of hemidesmosomes and anchoring filaments rich in laminin, including laminin 332, and type IV collagen. Additionally, the third layer, named the fibrillar zone, enables adhesion between the *lamina densa* and the anchoring plaques of the papillary dermis (Breitkreutz, Mirancea, and Nischt 2009).

A.1.2 Dermis

The dermis is a connective tissue in contact with basal keratinocytes through the DEJ. In contrast to the epidermis, the dermis has a low cell density and is predominantly constituted by an extracellular fibrous matrix (Stojic et al. 2019).

The dermal structure consists of two distinct layers: the upper *papillary dermis* and the lower *reticular dermis* both exhibiting differences in their composition and organization (**Figure 16**).

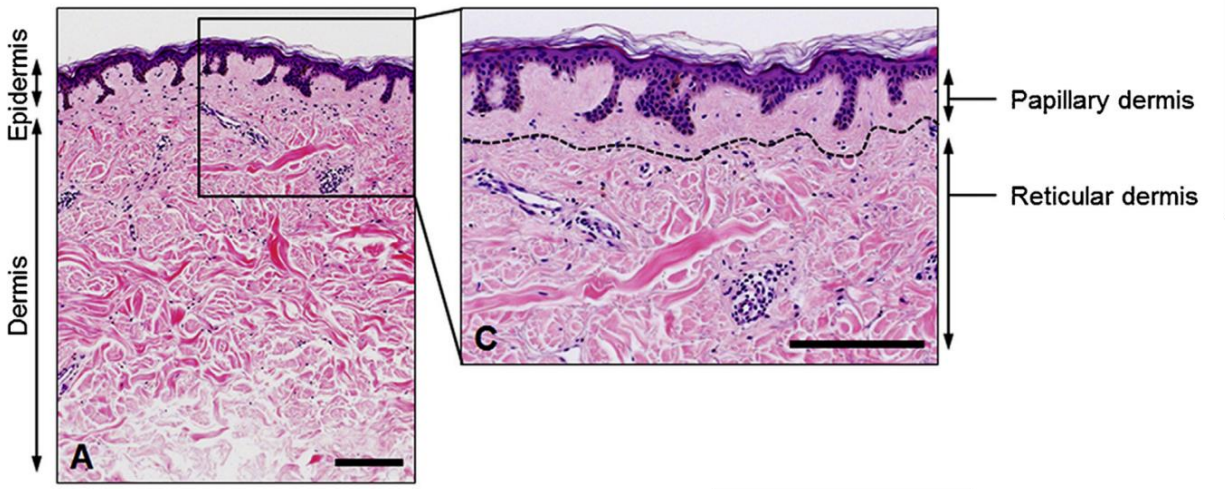


***Figure 16*. Histological cross-section of human skin.** The dermis is composed of two distinct layers: the upper papillary dermis and the lower reticular dermis. The line shows the separation between the two parts. Adapted from G. Sriram *et al.* 2015.

The *papillary dermis* includes the rete ridges named dermal papillae, which are projections of the epidermis facilitating interactions between the epidermis and dermis. It is the most vascular and innervated region of the dermis, composed of thin and poorly organized collagen fibre bundles (majority of type I and type III collagens) and contains mast cells, leukocytes, and macrophages.

The *reticular dermis* is thicker and provides secure anchorage for skin appendages like sweat glands and pilosebaceous apparatus composed of hair follicles, sebaceous glands, and arrector pili muscle, and contains blood vessels and nerves. The reticular dermis acts as the interface separating the dermis and hypodermis and is a well-organized structure (Sriram, Bigliardi, and Bigliardi-Qi 2015) that contains fewer cells than the papillary dermis.

The dermis primarily consists of an extracellular matrix (ECM) secreted by fibroblasts, the predominant cell type in the dermis. The ECM is mainly composed of collagens, which provide tensile strength, elastic fibres, in particular elastin and microfibrils, glycoproteins such as fibronectin, matricellular proteins, proteoglycans and glycosaminoglycans including hyaluronic acid (Pfisterer et al. 2021). It creates a “gel” that provides the skin resistance to compression

forces and ensures hydration retention. This gel undergoes continuous remodelling to maintain tissue homeostasis. Indeed, depending on their localization within the dermis, fibroblasts may exhibit distinct matrix secretion profiles. For instance, they demonstrated that the ECM produced from papillary fibroblasts or reticular fibroblasts exhibits differences in their structures, due to differences in fibres morphology and matrix compositions. Particularly, mass spectrometry revealed that ECM from papillary fibroblasts lacks COL1A2, THBS1, and FN1 compared to ECM from reticular fibroblasts (Ghetti et al. 2018). Additionally, fibroblasts are also responsible for synthesizing a wide range of enzymes that modify the matrix, along with their regulators, including matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) (reviewed in; Bonnans, Chou, and Werb 2014). Surprisingly, Haydont *et al.* identified a specific transcriptomic signature of dermo-hypodermal junction fibroblasts regarding genes involved in ECM synthesis-processing compared to papillary fibroblasts or reticular fibroblasts, resulting in architectural differences in human skin (Haydont et al. 2020).

A.1.3 Hypodermis

The hypodermis, also known as the subcutaneous layer, constitutes the deepest and thickest layer of the skin. It serves to anchor the skin to the fibrous tissue enveloping the bones and muscles. This layer is highly vascularized and innervated, and consists of sparse connective tissue containing adipocytes (reviewed in; Arda, Göksügür, and Tüzün 2014). Adipocytes are multifunctional cells residing in the hypodermis, where they form the subcutaneous white adipose tissue (sWAT), as well as in the reticular dermis (dermal white adipose tissue, dWAT) (reviewed in; Guan et al. 2023). Their first role in the skin is to store excessive energy substrates in the form of intracellular triglycerides (TG) deposits. The release of stored fat is facilitated by lipolytic enzymes, which break down triglycerides stored in adipose tissue and release nonesterified fatty acids (FAs) into the bloodstream (Zimmermann et al. 2004). Additionally, skin-associated adipocytes have been implicated in thermal insulation, mechanical support, and in the modulation of immune and infection responses through the secretion of cytokines, adipokines, and antimicrobial peptides (reviewed in; S. X. Chen, Zhang, and Gallo 2019).

A.2 Skin barrier functions

The skin has several protective functions, including protection against external stressors, regulation of body temperature, hydration, and detection of stimuli like heat, cold, touch, and pain. These functions are maintained by multiple and complex barriers, ensuring the body's integrity (reviewed in; Lefèvre-Utile et al. 2021) (**Figure 17**).

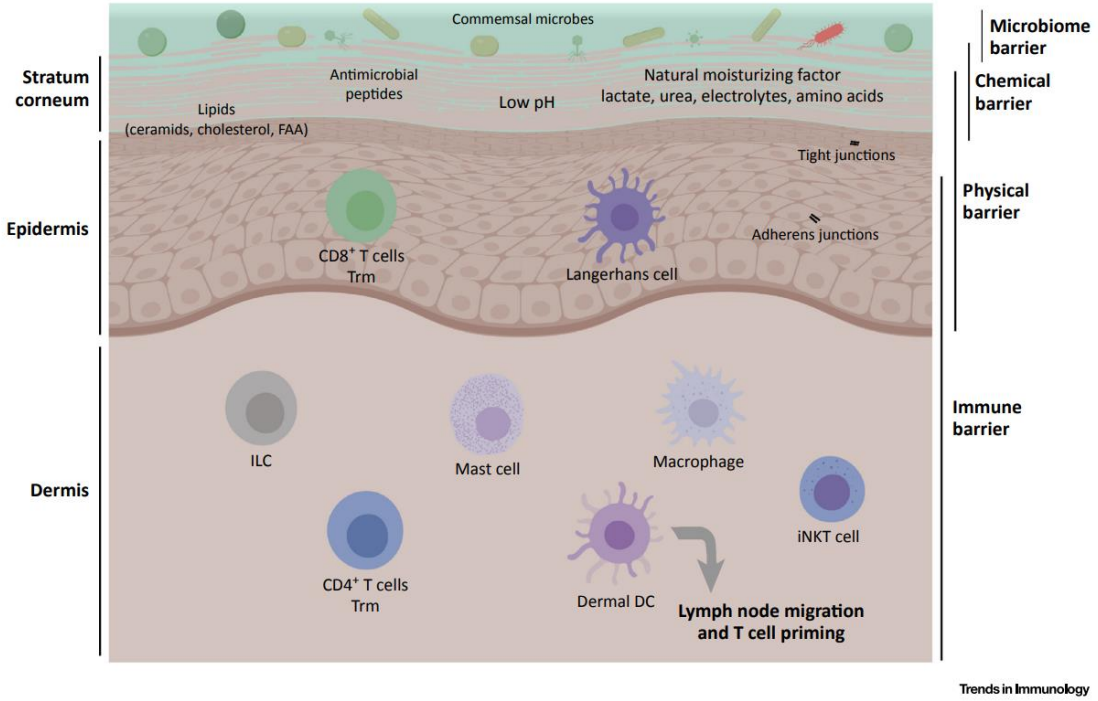


Figure 17. The components responsible for the skin barrier's function. Among the layers of the skin, various barrier functions are provided, notably including the microbiome, chemical, physical, and immune barriers, all designed to prevent skin aggressions. From Eyerich *et al.* 2018.

For instance, the microbiome barrier, spanning all surface areas of the skin, is composed of bacteria, fungi, and viruses and constitutes the first cutaneous barrier known as the microbiome barrier (reviewed in; Byrd, Belkaid, and Segre 2018).

Then, the stratum corneum acts as a chemical barrier, preventing exogenous agents from penetrating thanks to a series of protective molecules produced such as antimicrobial peptides and/ or proteins (AMPs) and epidermal lipids. In addition, the production of natural moisturizing factors (NMFs) and the acidic pH of the surface also contribute to the fight against pathogens (reviewed in; Eyerich et al. 2018).

The physical barrier is closely linked to the chemical barrier. At the top of the stratum corneum, corneocytes are connected by corneodesmosomes and surrounded by a lipid-enriched extracellular matrix. This barrier maintains the integrity of the skin and prevents the loss of water referred to as transepidermal water loss (TEWL). Below the corneocytes, desmosomes, adherents junction, tight junctions and keratins provide a mechanical cohesion between keratinocytes, forming a protective layer (reviewed in; Proksch, Brandner, and Jensen 2008; Matsui and Amagai 2015).

In addition, the skin offers protection from UV rays thanks to melanocytes. Indeed, melanocytes are specialized cells containing lysosome-related organelles called melanosomes, where melanin is synthesized and stored. Melanin exists in two forms in the

skin: eumelanin, a brown to dark pigment, and pheomelanin, a yellow-reddish pigment (reviewed in; Solano 2020). Research has shown that eumelanin offers significantly greater protection against UV radiation than pheomelanin, which can act as a photosensitizing agent, leading to lipid peroxidation and the generation of reactive oxygen species (ROS) (Mariano et al. 2021). Once mature, melanosomes are transferred to the keratinocytes, likely through various mechanisms (reviewed in; Bento-Lopes et al. 2023). Upon internalization by keratinocytes, melanin undergoes processing and contributes to the protection of keratinocyte DNA from UV damage (Byers et al. 2003).

Additionally, skin has also developed an immune barrier performed by a variety of immune cells that are present in normal skin such as dendritic cells, T cells, mast cells, and macrophages. These immune effectors efficiently detect danger signals through pathogen and damage-associated molecular patterns (PAMPs and DAMPs) triggering an appropriate immune response. Additionally, resident immune cells contribute to barrier repair and homeostasis maintenance (reviewed in; Eyerich et al. 2018).

A.3 Communication crosstalk between skin cell populations

Considering the skin's organization, communication between the cells within each layer is vital for maintaining skin homeostasis. As the major representatives of the skin cell population within the epidermis and the dermis, the communication between keratinocytes and fibroblasts has gained attention.

In their review, *Russo et al.* investigated 73 published papers to report the effects of keratinocytes on fibroblasts and vice versa (Russo, Brembilla, and Chizzolini 2020). In summary, among the major effects, they identified that keratinocytes impact fibroblast secretion by increasing the release of soluble factors such as interleukins, notably IL-1β, IL-6, and IL-8, as well as growth factors like fibroblast growth factor 7. Additionally, they influence the secretion of extracellular matrix components, including fibronectin, and matrix metalloproteinases (MMP-1, MMP-2, MMP-3, MMP-8, and MMP-12). Conversely, they observed that in most cases fibroblasts enhanced the proliferation and differentiation of keratinocytes.

Finally, they emphasize the various models that exist to assess the crosstalk between keratinocytes and fibroblasts, ranging from the use of conditioned media in monolayer cell culture to co-culture systems, transwell co-culture assays, skin equivalents, and skin explants.

A.4 *In-vitro* reconstructed skin models

The limitations of two-dimensional cell culture systems to accurately represent the structure and function of complex organs, such as the skin, highlight the need to develop three-

dimensional (3D) skin models for research, drug testing, and preclinical studies (Hennies and Poumay 2021).

In 1981, Bell *et al.* pioneered the creation of a tissue-engineered 3D human skin equivalent, comprising both dermal and epidermal layers (Bell et al. 1981). To date, studies aiming to investigate the complex properties of the skin often use reconstructed human epidermis (RHE), composed of keratinocytes, or reconstructed human skin (RHS), which incorporates additional fibroblasts and extracellular matrix components. Both models can be improved by adding, for example, melanocytes, Langerhans cells, or even cancer cells to increase their complexity (Hall et al. 2022; Bock et al. 2018; Michielon et al. 2020). Various strategies are used to create these 3D models, but today, most studies use scaffold-based 3D models where cells are grown on a support (e.g. that could be either natural or synthetic polymers), or use bioprinting (reviewed in; Choudhury and Das 2021). However, these strategies are experimental models that enhance our understanding of skin biology, but they require further improvement to achieve fully functional skin suitable for clinical applications (discussed in; Randall et al. 2018).

In addition, it should be noted that reconstructed human epidermis and skin equivalents can be used as good models for studying skin ageing. Various strategies have been developed to address the consequences of ageing on reconstructed skin model. One such strategy involves adding senescent cells at the beginning of the reconstruction process. For instance, the inclusion of senescent fibroblasts in skin equivalents leads to the development of ageing hallmarks, such as filaggrin expression reduction, increased water loss, or reduced collagen abundance, effectively mimicking skin ageing *in vitro* (Diekmann et al. 2016; Janson et al. 2013). Moreover, long-term culture (120 days) can be used to study chronological skin aging, with notably an increased number of p16-positive cells in both the epidermis and dermis from day 60, peaking at day 120 (Dos Santos et al. 2015).Surprisingly, a study investigating the impact of the extracellular matrix demonstrated that the longevity of keratinocytes increased when grown on a matrix derived from papillary fibroblasts, compared to a matrix derived from reticular fibroblasts (Janson et al. 2017). However, 3D skin models can also be directly treated with agents known to contribute to aging, such as ultraviolet radiation (Martin et al. 2008). Additionally, numerous studies have explored the role of advanced glycation end products in inducing skin aging. For instance, some have created modified models by glycation of collagen (Pageon et al. 2014). In conclusion, these models offer a degree of flexibility in studying ageing.

B. Skin ageing

As previously mentioned, the skin is a dynamic organ that contributes to the maintenance of body homeostasis. However, with age, the skin undergoes continuous degenerative changes. Indeed, skin ageing results from a combination of genetically programmed and irreversible

factors, along with external stressors dependent on the environment (reviewed in; Farage et al. 2008). These external factors overlap with intrinsic parameters contributing to the ageing process.

B.1 Intrinsic skin ageing

Chronological ageing refers to the internal process of ageing due to intrinsic factors, such as genetics. It leads to gradual changes in the whole body as individuals age.
Intrinsic ageing is mainly due to the dysfunctions of biological processes or mechanisms described in Figure 2 (López-Otín et al. 2023).
Surprisingly, some of these hallmarks of ageing have led to the search for an "ageing clock," aiming to measure the biological age of individuals. Unlike chronological age, which depends solely on our date of birth, biological age considers all external factors, apart from time, and the pathological context that can influence our organism. Thus, ageing clock can reflect the overall functionality of an individual's body and can indicate the susceptibility to age-related diseases and mortality risk. Indeed, numerous studies have examined various indicators, including epigenetic clocks based on genome-wide methylation profiles, telomere length analysis, cell-surface glycome, exosomal analysis, and blood-based biomarkers (reviewed in; Palmer 2022). This research area has gained attention in the field of skin biology (reviewed in; Bienkowska et al. 2023), and new markers have emerged, such as age-dependent specific microRNAs in keratinocytes, particularly miR-30a (Muther et al. 2017).

In the skin, many changes occur in both structure and function as individuals age. For instance, a meta-analysis investigated the proportion of senescent cells across various human tissues, revealing a positive correlation between chronological age and increased levels of senescent cells in the skin (Tuttle et al. 2020). Furthermore, *Ogata et al.* demonstrated a significant increase of $p16^{INK4}$ senescent cells with donor age in both the epidermis and dermis (Ogata et al. 2023). In addition, with age, the epidermal thickness diminishes, and the dermo-epidermal junction becomes finer, resulting in a gradual loss of the barrier function leading to skin dehydration and increased susceptibility to infections. Moreover, in the dermis, the dense network of collagen fibres becomes disorganized mainly due to an imbalance between synthesis and degradation. The number of elastin fibres decreases, resulting in a looser matrix network and the appearance of wrinkles. The cutaneous microcirculation is affected by a reduction in the size and number of blood vessels, leading to various side effects such as paleness or reduced vascular reactivity (reviewed in; Rorteau et al. 2020).
Finally, the pace of skin ageing depends on factors such as ethnic origin, the specific region of the body due to variations in skin thickness, and hormonal fluctuations (reviewed in; Farage et al. 2008).

B.2 Extrinsic skin ageing

In addition, skin ageing is influenced by many non-genetic factors that act in tandem with chronological ageing. The set of factors that can accelerate the rate of normal aging is referred to as the exposome (e.g., the totality of exposures an individual faces from birth to death). In the initial definition provided by Krutmann *et al.* the major exposomal factors influencing skin ageing are sun radiation, tobacco, temperature, nutrition, stress, lack of sleep, and pollution (reviewed in; Krutmann et al. 2017). Although these factors are often studied separately, their synergistic effects can lead to significant biological consequences on skin physiology. Thus, both intrinsic and extrinsic skin aging together lead to inevitable morphological alterations and, more significantly, integrated functional changes and increased skin fragility (**Table 4**).

Table 4: Morphological alterations and integrated function modifications of the skin during ageing. *Based on Farage et al., 2008 and Makrantonaki et al., 2008.*

Skin compartment and appendix	Morphological alterations		Integrated functional modifications
	Intrinsic ageing	**Photoageing**	
Epidermis	Skin Color changes appearance		Altered protection against sun radiation
	Pale (fewer melanocytes)	Irregular pigmentation Lentigines (melanocytic hyperplasia)	
	Epidermal thickness modification		Increased vulnerability to exogenous agents
	Thinning	Thickening	
	Keratinocytes proliferation alteration		
	Decreased mitotic activity Reduction of the stratum spinosum	Impaired proliferation (Actinic keratosis) Impaired differentitation (Dyskeratosis)	Loss of barrier function and skin integrity
	Decrease of epidermal stem cells pool		Impaired wound healing
Dermo-epidermal junction	Flattening of the DEJ		Fragility
Dermis	Altered network of collagen fibers and eslatin matrix		Reduced strengt Heightened sensitivity to deformational forces and fine wrinkle formation Reduced contractility
	Reduction in elastic fibers, collagen fibers, and crossliking enzyme	Increase collagen degradation Increase in abnormal elastic fibers (Solar elastosis) Sparse distribution	
Vascular	Decrease in vascular responsiveness		Alteration of thermoregulation function
	Microvessels decrease	Vessels dilatation Small blood vessels disorganisation	
Nerve endings	Reduction of nerve endings		Disturbed sensory function

B.2.1 External stressors impacting the skin

It's evident that the exposome is closely intertwined with the individual's lifestyle. For example, a survey conducted on monozygotic twins investigated the influence of non-genetic factors on facial skin ageing, such as sun exposure (Guyuron et al. 2009) (**Figure 18**).

Figure 18. **Effects of sun exposure on skin ageing of homozygote twin.** These two women are 61 years old. However, the twin on the right in the picture has been significantly more exposed to the sun during her life compared to her sister on the left. *From Guyuron et al.2019.*

The findings revealed that sun exposure and smoking are significant factors that accelerate the ageing process and contribute to an increase in perceived age. Indeed, the skin of smokers exhibits premature facial wrinkles and loss of elasticity (Koh et al. 2002).

Additionally, good nutrition (e.g. high intake of vegetables and legumes and low intake of meat and sugar), alcohol avoidance, and sufficient hours of sleep contribute to improving facial ageing. Another exposomal factor that is more challenging to quantify but still significantly impacts skin ageing is air pollution (reviewed in; Krutmann et al. 2017). It comprises two main types of pollutants: fine particles and gases, including CO_2 and NO_2 which are global health

concerns. In his study, *Hüls et al.* demonstrated that the interaction between air pollution and sun exposure could result in skin irreversible alterations, such as lentigines (Hüls et al. 2019).

B.2.2 Photo-ageing

Sun exposure is perhaps the most significant factor contributing to extrinsic skin ageing. In 1969, Kligman was the first to report that sunlight can lead to early destructive effects on human skin (Kligman 1969). While photo-ageing primarily refers to exposure to sunlight, the effects on the skin are mainly attributed to ultraviolet radiation (reviewed in; Gromkowska-Kępka et al. 2021). Further details on the spectral composition and effects of each type of sun radiation will be described in the next section.
Since then, the biological mechanisms and clinical consequences of UV on the skin have been well understood. For instance, a study on 298 Caucasian women demonstrated that UV exposure should be responsible for 80% of visible facial ageing signs (Flament et al. 2013). Histologically, photoaged skin is characterized by an epidermal thickening, irregular pigmentation, roughness appearance, and deep wrinkles (reviewed in; Farage et al. 2008).

In addition, recent findings have demonstrated that dermal fibroblasts contribute significantly to the photodamaged phenotype. Unlike keratinocytes, which can eliminate intracellular material during differentiation, fibroblasts must rely on adaptation pathways and damage repair mechanisms to maintain homeostasis (reviewed in; Krutmann et al. 2021). Dermal alterations caused by UV radiation led to disorganized and partially degraded collagen fibres, contributing to skin wrinkling. Additionally, the disintegration of elastic fibres and the formation of abnormal elastic tissue are characteristic features of a process known as solar elastosis (El-Domyati et al. 2002). The study by Berneburg *et al.* also showed a correlation between the induction of photoageing in human skin and mutations in mitochondrial DNA, notably the 4977 base-pair common deletion. This mutation was particularly evident in dermal fibroblasts, serving as long-term biomarkers for skin damage in humans (Mark Berneburg et al. 2004). Moreover, literature revealed that photoageing is associated with a significantly decreased content of antioxidant enzymes, including copper-zinc superoxide dismutase (CuZnSOD), manganese superoxide dismutase (MnSOD), and catalase (CAT), and with a significant accumulation of protein oxidation and carbonyls (Sander et al. 2002).

Hence, even if sunlight has been crucial for life's evolution on Earth, chronic sun exposure can induce a broad range of biological damage to the skin ranking from immediate reactions like sunburn, to chronic conditions like photo-ageing and even life-threatening issues including skin cancer (reviewed in; Polefka et al. 2012).

C. Solar radiation and biological damage to the skin

Solar radiation is electromagnetic radiation (i.e. a form of energy ranking from 10^{3} to 10^{-12} m that includes radio waves, microwaves, infrared light, visible light, ultraviolet light, X-rays, and gamma rays). However, the solar radiation that reaches the Earth's surface only contains infrared (~55%), visible light (~45%), and ultraviolet (~5%), as much of the harmful radiation is intercepted by the Earth's magnetic field and upper atmosphere before reaching the ground (**Figure 19**). The quantity of solar radiation reaching the Earth fluctuates due to changes in the distance between the Earth and the sun and, to a lesser degree, day-to-day shifts in the spectrum caused by solar activity.

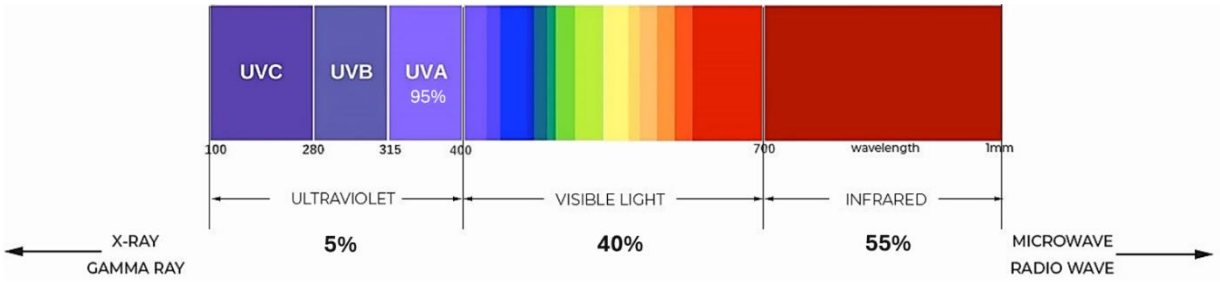


Figure 19. **Solar radiation spectrum.** The sun emits a broad range of wavelengths, ranging from X-ray or gamma ray to microwaves and radio waves. However, only regions from 280 to 315 nm (UVB), 315 to 400 nm (UVA), 400 to 700 nm (visible light), and 700 to 1000 nm (infrared) reach the Earth's surface, with the others being absorbed by the earth's atmosphere.

C.1 Infrared radiation and visible light

For a long time, visible light (400-700 nm) and infrared (> 700 nm) were believed to have little effect on the skin. However, evidence in the literature demonstrated their contribution to skin photo-ageing. Indeed, both wavelengths can penetrate the epidermis and dermis and reach the hypodermis, creating damage to skin cells.

For instance, exposure of fibroblasts and keratinocytes to high-energy visible light (~400 nm) can induce ROS formation, as well as DNA damage in keratinocytes (Mann et al. 2020; Lawrence et al. 2018).

In addition, short infrared wavelengths can significantly increase the protein level of MMP-1 in fibroblasts (Schieke et al. 2002). Moreover, Kim *et al.* demonstrated that a single exposure to infrared radiation did not change the protein expression of MMP-1 whereas repeated exposures did (M.-S. Kim et al. 2006). However, when looking at the combination of both visible light and infrared on human skin, an increased expression of MMP-1 and MMP-9 and a decreased expression of type 1 procollagen was observed 24 hours after the irradiation (S. Cho et al. 2008). Nonetheless, some specific wavelengths in visible light and infrared are used

in dermatology practice because they showed significant benefits for skin rejuvenation and healing (Lask et al. 2005) demonstrated that the time of exposure and the band determine the effects on the skin (van Breugel and Bär 1992).

C.2 Ultraviolet rays

The spectral composition of total solar irradiance fluctuates over time. The visible and infrared lights are relatively stable, whereas the ultraviolet (UV) component exhibits significant variability. The UV region spans wavelengths ranging from 100 to 400 nm and is divided into three bands: UVC (100-280 nm), UVB (280-315 nm), and UVA (315-400 nm). The UV portion of sunlight that arises on Earth consists mainly of approximately 95% UVA and 5% UVB radiation. Indeed, UVC and almost 90% of the UVB radiations are absorbed by the Earth's atmosphere.

The Global Solar UV Index (UVI) measures the intensity of solar UV radiation reaching the Earth's surface, serving to alert the public to heightened risks of skin and eye damage during periods of high UV levels (Health World Organization 2002). The UVI is based on the action spectrum for UV-induced erythema on human skin, its equation is:

$$I_{UV} = k_{er} \cdot \int_{250\ nm}^{400\ nm} E_{\lambda} \cdot s_{er}(\lambda) d\lambda$$

where I_{UV} is the UV index; k_{er} is a constant equal to 40 m²/W; E_{λ} the solar irradiance at wavelength λ; dλ the wavelength interval used in summation; $s_{er}(\lambda)$ the erythema reference action spectrum.

Overall, determining the precise UV dose that human skin receives per day is challenging and highly variable around the world. However, a hypothetical estimation for Europeans falls between 10,000 and 20,000 J/m² per year. This estimate excludes holidays, which may elevate the dosage by 30% or higher (Godar 2005).

C.2.1 UV penetration and energy

UV rays penetrate human skin depending on their wavelength (Meinhardt et al. 2008). Longer wavelengths can penetrate deeply into the skin, with UVA observed to penetrate both the epidermis and dermis. In contrast, UVB has limited penetration, with most of its effects observed in the epidermis, although it can reach the upper dermis where most fibroblasts are located (reviewed in; D'Orazio et al. 2013).

Conversely, longer wavelengths are less energetic than shorter wavelengths, as demonstrated by the Planck relation:

$$E = \frac{hc}{\lambda}$$

where E is the energy; h the Planck's constant (6,63.10^{-34} J/s); c the speed of light (~ 3.10^{8} m/s); λ the wavelength.

This physical relation means that shorter wavelengths have a higher potential to damage biological tissues.

C.2.2 UV-induced DNA damage

DNA damage from UV radiation can be either direct or indirect, depending on the energy level. However, as none of these damages were studied in this work, the UV damage signature and the main repair systems will be briefly explained.

C.2.2.1 Direct DNA damage

Only high-energy wavelengths can cause direct DNA damage. Consequently, since UVC radiation is not expected to reach human skin, only UVB radiation should be directly absorbed by DNA *in vivo*. As a result, UVC rays induce the formation of pyrimidine dimers, preferentially between adjacent thymine (T) bases or to a lesser extent cytosine (C) and 5-methylcytosine (mC). The two major photoproducts induced by UVB are cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts (6-4PPs) (reviewed in; Cadet, Sage, and Douki 2005) (**Figure 20**). CPDs result from a cycloaddition reaction involving the C5=C6 double bonds of neighboring pyrimidines, with a higher proportion of T=T and T=C than C=C. The formation of CPDs is an extremely rapid process where two adjacent thymines can dimerize in a picosecond (Schreier et al. 2009).

6-4PPs are formed by the single covalent bond between the C6 of one pyrimidine and the C-4 of the adjoining base's ring. Additionally, 6-4PPs by absorption of UVB or UVA can give rise to a third type of photoproduct known as the Dewar valence isomers (reviewed in; Cadet, Grand, and Douki 2015; Douki and Sage 2016).

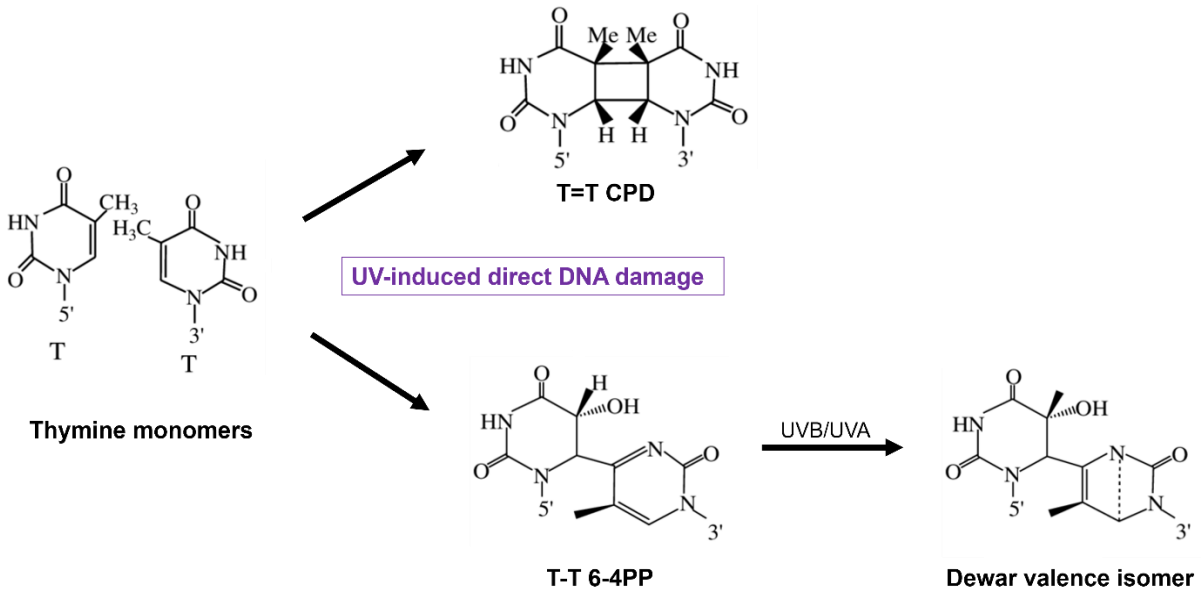


Figure 20. **Major direct DNA damage that could be induced by UV irradiation.** This example shows the formation of T=T CPD and T-T 6-4PP. The latter could undergo photoconversion into Dewar valence isomers via UVB or UVA absorption.

The formation of direct DNA damage has long been considered specific to UVB and UVC radiation. However, several studies have demonstrated that UVA can also cause direct DNA damage, notably CPDs (Thierry Douki et al. 2003; Rochette et al. 2003). Finally, if not correctly repaired, these damage induce structural distortion in the DNA, disrupting DNA replication, and could result in DSBs, particularly at the locations where replication forks with CPDs-containing DNA have collapsed (reviewed in; Rastogi et al. 2010). The physiological and pathological cutaneous reactions to UV will be further detailed in section C. 2.3.

C.2.2.2 Indirect DNA damage

Absorption of UVB and UVA photons can additionally trigger the creation of photoexcited states in skin photosensitizers, leading to the production of reactive oxygen species (ROS) resulting in DNA base modifications, single- and double-strand breaks and oxidative damage to pyrimidine and purine bases (reviewed in; Rastogi et al. 2010).

The skin contains endogenous photosensitizers, such as urocanic acid or flavins, that can be excited by UV radiation. Once excited they can react directly with DNA by one electron transfer (type I reaction) or indirectly by transferring the energy to a molecule of oxygen (type II reaction). Type II photosensitization can lead to the formation of either singlet oxygen (O_2) or superoxide anion ($O_2^{\cdot-}$). $O_2^{\cdot-}$ is typically converted to hydrogen peroxide (H_2O_2) (i.e. Fenton's reaction) that can then be converted to hydroxyl radical (•OH). Singlet oxygen and other ROS specifically target guanine residues which are the most readily oxidized DNA bases, resulting

in the preferentially formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) (reviewed in; Wondrak, Jacobson, and Jacobson 2006). Additionally, ROS can attack other macromolecules such as proteins and lipids leading to protein carbonylation and lipid peroxidation (reviewed in; Andrés Juan et al. 2021).

C.2.2.3 UV-induced DNA repair

To maintain genome integrity, a variety of cellular mechanisms are in place to repair DNA damage (reviewed in; Sancar et al. 2004).

CPDs and 6-4PPs are preferentially repaired through the nucleotide excision repair (NER) pathway, which consists of the removal of bulky DNA damage.

NER consists of two sub-pathways: transcription-coupled NER (TC-NER), which repairs damage from the transcribed strand of active genes, and global genome NER (GG-NER), which can occur anywhere in the genome. These two pathways are initiated by different factors, but after DNA damage recognition, both pathways converge for additional steps, including damage demarcation, incision of the damaged strand, gap filling, and ligation (reviewed in; Marteijn et al. 2014). Mutations in proteins involved in the NER pathway can lead to diseases such as Xeroderma Pigmentosum, a rare genetic disorder (Coin et al. 1999). Additionally, the photoreactivation of CPDs involves the cleavage of the bond between the pyrimidine dimer, leading to the reversal of DNA damage through an enzyme called photolyase (Mees et al. 2004).

Finally, the oxidative DNA damage is repaired through the base excision repair (BER) pathway. BER begins with the recognition and the removal of damaged bases by DNA glycosylases. Next, AP-endonuclease (APE) creates a 3'OH terminus at the damaged site, followed by repair synthesis with a DNA polymerase and DNA ligase. At this stage, it's worth noting that two pathways exist; the short-patch and long-patch BER, which depend on the number of nucleotides to be added. These pathways involve different factors to maintain DNA repair and stability (reviewed in; Hindi, Elsakrmy, and Ramotar 2021).

C.2.3 Pathological and physiological cutaneous reactions to UV

UV irradiation is responsible for various types of DNA damage, some of which can result in specific mutations. For example, mutations identified in the p53 gene of certain human skin cancers exhibit CC → TT tandem base substitutions, which are specific to the UV signature (Brash et al. 1991).

The incidence of skin cancer is steadily rising, notably due to a combination of overexposure to UV, increased outdoor activities, increased longevity and better medical monitoring and earlier diagnosis (reviewed in; Leiter, Eigentler, and Garbe 2014). There are three major types

of skin cancers: basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma. In 2019, ~ 60% of reported cancers were BCC, 35% SCC, and 5% melanoma (W. Zhang et al. 2021).

Exposure and high susceptibility to UV are major risk factors for BCC, which originate from basal cells of the epidermis. It is typically a slow-growing tumor, and metastases are rare. BCCs often present a diagnostic delay of many years from the initial UV damage to clinical manifestation. The primary treatment is usually surgery; however, for "difficult-to-treat" BCCs, additional treatments such as Hedgehog inhibitors, radiotherapy, and more recently, immunotherapy, may be considered (Peris et al. 2023).

SCC originates from malignant proliferation of epidermal keratinocytes known as actinic keratoses. UV overexposure is the most important environmental risk factor as most of the lesions developed are located in sun-exposed skin areas. SCC tumor can be locally invasive, and metastases can be formed in rare cases. Treatment for SCC is first based on surgery and different clinical trials are ongoing to approve drugs for the treatment of metastatic SCC such as PD-L1 inhibitors (Corchado-Cobos et al. 2023).

Melanoma arises from the malignant transformation of melanocytes. The primary etiological factors include intermittent UV exposure and a history of sunburn. A family history of melanoma (*CDKN2a* mutation) is also a contributing factor. Melanomas may arise within or near pre-existing lesions such as dysplastic nevi or in healthy skin. Melanomas are significantly more aggressive than carcinomas. Despite constituting only a minor fraction of skin cancers, they are responsible for most deaths associated with this disease. Surgical resection is the treatment of choice in melanoma cases. However, metastatic melanomas present significant challenges in treatment and are associated with a poor prognosis. Hopefully, recent advances in targeted therapy and immunotherapy have enhanced patient prognosis, notably using kinase inhibitors targeting BRAF, an oncogene frequently mutated in melanoma ($BRAF^{V600E}$) (Castellani et al. 2023).

Furthermore, other skin cell types such as Merkel cells or fibroblasts can be at the origin of rare skin cancers.

Conversely, UV irradiation can also have beneficial effects. Firstly, UVB is necessary for converting 7-dehydrocholesterol into previtamin D3 in the epidermis, which is then isomerized to vitamin D3 (reviewed in; Webb 2006). Additionally, sunlight has a significant positive impact on mood and cosmetic tanning (reviewed in; Juzeniene and Moan 2012), although excessive exposure can lead to sunburns, especially due to UVB. Finally, phototherapy is a highly effective treatment modality in dermatology and has improved the management of various skin conditions. For example, PUVA phototherapy utilizes psoralens, a photosensitizing drug, and

UVA light to treat inflammatory skin diseases like psoriasis (reviewed in; Branisteanu et al. 2022).

D. Mosaic of skin senescent cells

As mentioned previously, there is growing evidence of senescent cells accumulating in various compartments of the skin. However, identifying senescent cells *in vivo* in humans remains challenging. This obstacle, rather than a hypothetical technical issue, could be due to the heterogeneity of senescent cells within the organism. It is conceivable that *in vivo*, the appearance of senescent cells is not synchronized as observed in the *in vitro* models commonly used in labs. Consequently, neighboring cells may become senescent due to different inducers or combinations of them. As a result of this mosaic of senescent cells, a multitude of senescent phenotypes could coexist, particularly in the skin, which is one of the body' organs most exposed to numerous extrinsic stressors (reviewed in; Toutfaire, Bauwens, and Debacq-Chainiaux 2017).

D.1 Similarities and differences among senescent skin cells

Interestingly, while *in vitro* studies on senescence often focus on fibroblasts, their implication in ageing using *ex vivo* skin samples seem to be underrepresented compared to epidermal cells. While some markers of senescence, such as SA-βgal and lamin B1, have been identified in both the epidermis and dermis (Dimri et al. 1995; Dreesen et al. 2013), the common expression of other previously established biomarkers of senescence appears to be less clear.

For instance, Nassour *et al.* demonstrated by immunohistodetection in human-aged skin samples, that epidermal cells display XRCC1 foci accumulation, which correlates with a decrease in PARP1 (Nassour et al. 2016). This observation notably reinforces one of the *in vitro* hypotheses regarding keratinocyte senescence establishment, which suggests that the accumulation of oxidative stress and decreased PARP1 activity lead to persistent SSB and thus to senescence via the $p38^{MAPK}$-$p16^{INK4}$-Rb axis, while replicative senescence of fibroblasts seems to rely on persistent DDR activation and the p53-$p21^{CIP1}$ pathway (reviewed in; Nassour and Abbadie 2016).

However, the distribution of $p16^{INK4}$ and $p21^{CIP1}$-positive cells across the skin, and their biological consequences, remain challenging questions. In a recent preprint study that utilized scRNA-seq data of human eyelid skin from donners of different ages (Zou et al. 2021), it has been further revealed that three distinct non-proliferative cell populations co-exist in human skin with age: a $p21^{CIP1}$-only positive cell population, a $p16^{INK4}$-only positive cell population, and a population of cells co-expressing $p21^{CIP1}$/$p16^{INK4}$, and that the two first categories exhibit

distinct SASP compositions, suggesting that both can drive different biological consequences (Saul et al. 2023). In addition, $p16^{INK4}$-positive cells have been observed significantly increased in both the dermis and the epidermis with age, although in the dermis, a majority of the $p16^{INK4}$-positive fibroblasts was also positive for $p21^{CIP1}$ (Hasegawa et al. 2023). Finally, Victorelli *et al.* revealed that in aged epidermis, most of the $p16^{INK4}$-positive cells in the skin were not keratinocytes but melanocytes. Moreover, they demonstrated that melanocytes can induce paracrine telomere damage to neighboring keratinocytes, leading to a change in their behavior without strictly inducing senescence, but rather a “senescence-like” process. (Victorelli et al. 2019).

Overall, these few examples illustrated well the important heterogeneity of skin senescent cells across the skin.

D.2 From high expectations to limited results: the flip side of killing skin senescent cells

One evidence of this heterogeneity could be the difficulty of demonstrating the efficacy of senolytics *in vivo*. Indeed, preliminary *in vitro* studies that identify the potential effects of senolytic drugs have often focused on relatively homogeneous populations of a single type of senescent cells (Yi Zhu et al. 2016). However, in more complex tissues like the skin, not all the populations of senescent cells respond to senolytics, which limits their potential beneficial effects on the ageing phenotype in humans (reviewed in; Zhu et al. 2015). To date, few studies have reported the potential reversing effects of eliminating senescent cells on the skin ageing phenotype. For instance, only one study showed that daily topical application of rapamycin on one hand of aged individuals for 8 months significantly decreased fine wrinkles, and increased dermal volume, notably through increased collagen VII in the skin (Chung et al. 2019).

Additionally, the use of senolytics compounds to kill senescent cells involves inducing apoptosis in these cells. In their review, McHugh and Gil wondered about the consequences of possible chronic inflammation if these apoptotic cells are not properly cleared by the immune system (McHugh and Gil 2018). Furthermore, predictive hypothetical models suggest that treatments with senolytics could yield benefits in the short to middle term, but may also pose potential negative effects in the long term. These theories are based on two hypotheses: firstly, the lack of specificity of senolytics and the potential negative effects of the chemical compounds on tissue function over the long term (Kobbe 2019). Secondly, the idea that the neighboring cells may begin to accelerate their own division to compensate for the elimination of senescent cells, ultimately leading to an increase in their senescence (Fossel 2019). However, these theories remain hypothetical, and only long-term human studies will be able to validate or refute them. As a result, new therapies were developed shortly afterward,

including the use of senomorphics, which today represent an equally promising avenue as senolytics.

Objectives

As discussed in the introduction, cellular senescence can be viewed as an adaptative response to multiple stresses. In the context of the skin, determining the origin of senescent cells *in vivo* remains challenging. Therefore, efforts are needed to identify common signalling pathways to uncover features shared between all senescent cells, regardless of their original senescence inducer.

In light of this context, the project was initially established through a collaboration between Dr. Olivier Pluquet (University of Lille, UMR9020-U1277 CANTHER) and Dr. Florence Debacq-Chainiaux (University of Namur, NARILIS, URBC). The main goal was to identify molecular mechanisms involved in both replicative and UV-induced senescence of dermal fibroblasts and to determine common effectors that can be targeted in the physiological context of skin ageing.

Indeed, previous work in Dr. Olivier Pluquet's team demonstrated the central role of ATF6α in establishing specific biomarkers of replicative senescence in normal human primary fibroblasts, particularly regarding the morphological changes. However, it is evident from the literature that UPR activity and the regulation of one or more branches on the senescent phenotype vary depending on the cell type and the inducer of senescence being studied. In addition, in models of UV-induced senescence, little information is available regarding the contribution of UPR in the establishment of the senescent phenotype. Therefore, using a physiological model of UVB-induced senescence developed by Dr. Florence Debacq-Chainiaux, we investigated the hypothesis that ATF6α could potentially exert similar effects on the phenotype of UVB-induced senescent fibroblast. Supposing common effects of this pathway in two relevant models of senescence, which are assumed to potentially contribute to skin ageing, could be an interesting approach to delay skin-ageing-related alterations. To reach this purpose several objectives have been established:

- The first objective was to ascertain whether UVB-induced senescent normal human dermal fibroblasts were associated with ER stress and UPR activation or not.

- If such associations were identified, the second objective was to decipher whether disrupting one branch of the UPR would affect the onset of the UVB-induced senescent phenotype with specific attention given to the characterization of the SASP.

- Given that the ER is the first cellular compartment of secretion, a key aim was to focus on the UPR branch that prevents the establishment of most senescent biomarkers, and

to establish the complete secretomic and transcriptomic profile of NHDFs exposed or not to UVB and invalidated for this branch of interest.

- Then, based on the results obtained, the third objective was to study the biological effects of this modified SASP on the skin environment using either skin cancer cells or a more physiological model using keratinocytes embedded in a reconstructed human epidermis.

- Finally, the ultimate aim was to identify paracrine factors regulated by the UPR branch of interest, which could be safely targeted, and to understand their mode of action on the behavior of neighboring cells.

Results

1. Research Article:

Most of the results of this thesis are formatted as a scientific research article.

Through this initial study:

- We investigated the expression of ER stress and UPR markers in skin samples from donors of various ages and degrees of sun exposure.

- We have perfected a model of UVB-induced senescence in normal human dermal fibroblasts (NHDFs), subjecting them to two exposures per day for five days at 500mJ/cm² of NB-UVB (312 nm), and we established their complete transcriptomic signature.

- We assessed the impact of knocking down or chemically inhibiting each UPR sensor on the development of the senescent phenotype, including the SASP.

- Utilizing conditioned media, we evaluated the physiological significance of our findings using reconstructed human epidermis and cultured keratinocytes.

Targeting ATF6α attenuates UVB-induced senescence and improves skin homeostasis by controlling IL8

Joëlle Giroud[1,2], Pauline Delvaux[1], Clémentine De Schutter[2], Nathalie Martin[2], Raphaël Rouget[4], Ayeh Boulouki[1], Valérie De Glas[3], Florent Bourdoux[1], Sophie Burteau[1], Julien Théry[5], Gauthier Decanter[5], Nicolas Penel[5], Corinne Abbadie[2], Yves Poumay[3], Olivier Pluquet[2, #, *] and Florence Debacq-Chainiaux[1,#,*].

[1]Laboratory of Biochemistry and Cell Biology (URBC), Namur Research Institute for Life Sciences (NARILIS), University of Namur, 5000 Namur, Belgium.

[2]University of Lille, CNRS, Inserm, Pasteur Institute of Lille, UMR9020-U1277-CANTHER-Cancer Heterogeneity Plasticity and Resistance to Therapies, 59000 Lille, France.

[3]Research Unit of Molecular Physiology (URPhyM), Namur Research Institute for Life Sciences (NARILIS), University of Namur, 5000 Namur, Belgium.

[4]University of Lorraine, CNRS, CRAN-UMR7039, 54506 Vandoeuvre-lès-Nancy, France.

[5]Direction of Clinical Research and Innovation, Oscar Lambret Center, 59000 Lille, France.

[#]Co-senior authors

[*]Corresponding author. Tel: +33 320965276; E-mail: olivier.pluquet@ibl.cnrs.fr

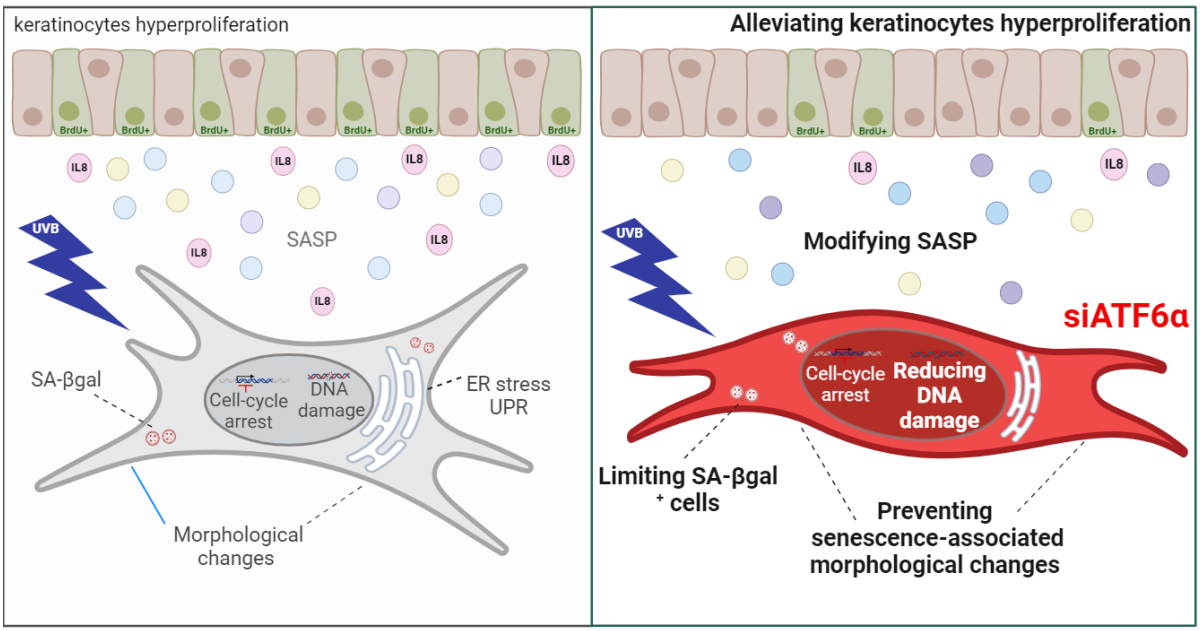
[*]Corresponding author. Tel: +32 81 72 4457; E-mail: florence.chainiaux@unamur.be

Abstract

Skin aging is influenced by both intrinsic and extrinsic factors, particularly UV radiation, and is characterized by an accumulation of senescent cells. Remarkably, exposure to UV can trigger senescence in various skin cells, including dermal fibroblasts. However, the molecular mechanisms underlying UV-induced senescence and the impact of the related senescence-associated secretory phenotype (SASP) on the homeostasis of surrounding skin cells remain poorly understood. Here, we identified that both chronological aging and photoaging induce the unfolded protein response (UPR) in human dermal samples. We demonstrated that silencing ATF6α disrupts the establishment of the UVB-induced senescent phenotype by preventing the onset of several senescent biomarkers and alters the composition of the SASP, consequently affecting its impact on the increased proliferation of keratinocytes embedded in reconstructed human epidermis. Moreover, we found that ATF6α partially controls IL8 expression involved in the hyperproliferation of cultured keratinocytes. Together, our findings highlight the importance of the ATF6α/IL8 axis in regulating the homeostasis of neighboring cells during skin photoaging, thus suggesting ATF6α as a potentially promising target for senotherapeutic interventions.

Keywords

ATF6α / normal human dermal fibroblasts / skin / UPR / UVB-induced senescence



Synopsis

Senescent cells display alterations of Endoplasmic Reticulum (ER) homeostasis and elicit Unfolded Protein Response (UPR). This study examines the impact of ATF6α invalidation, one UPR branch, on the establishment of the UVB-induced senescent phenotype in human dermal fibroblasts, and its implications for the skin microenvironment.

- UPR is increased during human dermal aging and is intensified by chronic sun exposure.
- Transcriptomic analyses of UVB-induced senescent human dermal fibroblasts reveal an enrichment in protein processing in ER.
- UVB-induced senescent fibroblasts exhibit activation of all three branches of the UPR (ATF6α, PERK, and IRE1α).
- Targeting ATF6α in human dermal fibroblasts prevents the establishment of UVB-induced SA-βgalactosidase, persistent DNA damage, morphological changes, and modifies the SASP composition.
- The SASP of UVB-exposed human dermal fibroblasts influences the thickness of reconstructed human epidermis and keratinocyte proliferation through an ATF6α/IL8-dependent mechanism.

The paper explained

Problem

Aging is a key risk factor for age-related diseases, and the associated biological processes are commonly referred to as “hallmarks of aging”. Among them, cellular senescence is an adaptative response to damage. In the skin, evidence indicates the accumulation of senescent cells. As a result, new strategies seek to uncover the underlying mechanisms behind the development of the senescent phenotype to attenuate the adverse effects of senescent cells on skin health. This is particularly relevant in the context of age-related skin disorders, pre-cancerous lesions, and skin cancers.

Results

Our study demonstrates an elevated level of endoplasmic reticulum (ER) stress and unfolded protein response (UPR) in aged and chronically sun-exposed human dermal samples. In UVB-induced senescent fibroblasts, we observed activation of all three branches (ATF6α, PERK, and IRE1α) of the UPR pathway. Invalidation or inhibition of ATF6α prevented the establishment of certain UVB-induced senescent biomarkers. Importantly, in a model of reconstructed human epidermis (RHE), we showed that secreted factors from UVB-induced

senescent fibroblasts not only enhance the proliferation of keratinocytes but also contribute to the thickening of the RHE. Finally, we identified the interleukin-8 (IL8) as a target of ATF6α responsible for mediating this effect on 2D cultured keratinocytes.

Impact

Our study highlights ATF6α's role in both establishing the UVB-induced senescence phenotype and maintaining skin homeostasis under stress through the modified expression of major SASP components, suggesting a potential new senotherapeutic target to investigate.

Introduction

Aging is defined as a gradual and cumulative decline of physiological functions and is associated with an increased risk of developing age-related diseases (Childs et al. 2017). Over the years, the skin undergoes structural and morphological changes, leading to the deterioration of its functions (Russell-Goldman and Murphy 2020). Skin aging is associated with both intrinsic and extrinsic factors including sun exposure, a phenomenon known as photoaging, primarily attributable to ultraviolet (UV) rays (Kammeyer and Luiten 2015; Krutmann et al. 2017). The main effects of UV exposure on skin aging include epidermal thickening, wrinkles, solar elastosis, a decreased amount of extracellular matrix (ECM) proteins, and an increased collagen fragmentation due to elevated activity of matrix metalloproteinases (MMPs) (Wlaschek et al. 2001). Furthermore, UV radiation is a major risk factor for the development of precancerous skin lesions (actinic keratosis) and skin cancers (Narayanan, Saladi, and Fox 2010), including melanoma and non-melanoma skin cancers, which represent the human cancers with the highest incidence (D'Orazio et al. 2013).

Cellular senescence, a hallmark of aging, was initially associated with replicative lifespan exhaustion and later linked to telomere shortening, a phenomenon referred to as replicative senescence (Hayflick and Moorhead 1961; Zhu et al. 2019; Di Micco et al. 2021). Additionally, as an adaptative stress response, senescence can also be triggered following telomere-independent mechanisms including oncogene expression, mitochondrial dysfunction, or exposure to various stresses like ultraviolet radiation (Gorgoulis et al. 2019; Wiley et al. 2016; Di Micco et al. 2020). Despite their likely differing origins, senescent cells share common features, such as a stable and strong cell cycle arrest that mainly depends on the activation of p53/$p21^{WAF1}$ and/or $p16^{INK4}$/pRB pathways in an interconnected or independent manner. Additionally, they exhibit senescence-associated β-galactosidase activity (SA-βgal), enlarged morphology, persistent DNA damage, and a specific inflammatory secretome referred to as the senescence-associated secretory phenotype or SASP (Hernandez-Segura, Nehme, and

Demaria 2018). The composition of SASP can vary depending on the context, although some secreted factors such as interleukins and cytokines (e.g. IL6, IL8, CXCL1, CXCL2, and CXCL3) are frequently present (Giroud et al. 2023; Gorgoulis et al. 2019).

Repeated UVB exposures can induce senescence in dermal fibroblasts, melanocytes, and keratinocytes (Debacq-Chainiaux et al. 2005; Martic et al. 2020; Bauwens et al. 2023). *In vivo*, the build-up of senescent cells is detected in the epidermis and dermis with age (Dimri et al. 1995; A. S. Wang et al. 2017; Tuttle et al. 2020; Ogata et al. 2023). Additionally, the accumulation of senescent cells in the skin has been associated with skin aging characteristics (Waaijer et al. 2016). The detrimental effects of senescent cells can be mainly attributed to their secretome (Coppé et al. 2010), notably because of their potential to influence their microenvironment (Acosta et al. 2013; Ogata et al. 2021). For example, in the skin, senescent melanocytes have been shown to affect keratinocytes proliferation (Victorelli et al. 2019). Besides, keratinocytes exposed to conditioned media from oncogene-induced senescent keratinocytes (OIS-CM) exhibited increased clonogenic capacity, as well as *in vivo* regenerative capacity (Ritschka et al. 2017).

The Endoplasmic Reticulum (ER) is the first compartment of secretion, responsible for the folding and maturation of secreted proteins. Despite profound changes in the composition of the secretome in senescent cells, there is limited understanding of how the ER adapts and copes with these changes. Interestingly, there is evidence that different cell types, when undergoing senescence, display unfolded protein response (UPR) activation (Denoyelle et al. 2006; Dörr, Yu, Milanovic, Beuster, Zasada, Henry, et al. 2013; Blazanin et al. 2017; Kim et al. 2019; Sabath et al. 2020). The UPR pathway is initiated by three transmembrane sensors, the activating transcription factor 6 (ATF6α), the PKR-like ER kinase (PERK), and the Inositol Requiring Enzyme 1 (IRE1α). However, the three branches are not always activated together at senescence and the specific activation of one pathway varies depending on the cell type and the senescence inducer (Matos, Gouveia, and Almeida 2015; Abbadie and Pluquet 2020). We and others have demonstrated that in replicative senescence, UVC-induced senescence, and HRas-induced senescence, the disruption of ATF6α alters the senescent phenotype in different skin cell types (Druelle et al. 2016; Drullion et al. 2018; Kim et al. 2019; Denoyelle et al. 2006). Indeed, ATF6α invalidation in replicative senescent normal human dermal fibroblasts (NHDFs) causes the reversal of some characteristics of the senescence phenotype, such as SA-βgal, morphology, and SASP composition (Druelle et al. 2016). Nonetheless, the significance of ATF6α in driving UVB-induced senescence in NHDFs, as well as its role in determining SASP composition and the associated paracrine effects, remain poorly understood.

Therefore, we investigated whether the ATF6α branch of the UPR plays a role in the UVB-induced senescent phenotype of NHDFs and examined whether the ATF6α -dependent SASP influences the homeostasis of skin cells. Our findings suggest that ATF6α controls the UVB-induced senescent phenotype and partly drives the SASP. We showed that ATF6α is required for IL8 secretion, and that the paracrine effect of IL8 impacts the proliferation of surrounding keratinocytes. Interestingly, invalidation of ATF6α or IL8 inhibition was sufficient to alleviate hyperproliferation of keratinocytes. Overall, our data unveiled a novel role for ATF6α in UVB-induced senescence regulation and skin homeostasis by influencing the behavior of neighboring cells through the paracrine control of SASP factors such as IL8.

Results

UPR is triggered during normal dermal aging and is enhanced by chronic sun exposure

To study UPR activation *in vivo*, we established a collection of skin biopsies obtained from both non-sun-exposed or sun-exposed human dermal samples, encompassing both young and elderly donors (Table 1 and Figure 1A). To visualize the accumulation of senescent cells in the aged dermis, we performed immunostaining against the DNA damage protein 53BP1. Indeed, 53BP1 foci have been reported as a reliable *in vivo* biomarker of senescence in the skin (Nassour et al. 2016; Bauwens et al. 2023). In addition, vimentin staining was used to identify dermal fibroblasts. Expectedly, the percentage of 53BP1 positive cells significantly increased with age, supporting our previous observations (Figure 1B) (Nassour et al. 2016; Bauwens et al. 2023). Interestingly, less than 5 % of dermal fibroblasts in young non-sun-exposed dermis displayed 53BP1 foci, while 12 % of young sun-exposed dermis did. Moreover, sun exposure significantly increased the percentage of 53BP1 foci from 15 % to 24 % in fibroblasts from non-sun-exposed compared to sun-exposed sections, in elderly donors (Figure 1B). To investigate whether UPR activation is modulated by age and sun exposure, we analyzed the percentage of positive cells for proteins involved in ER stress (PDI) and UPR (HERPUD1, XBP1s) in dermal sections. Analyses of the UPR-induced proteins HERPUD1 and XBP1s showed a significantly increased abundance in dermal fibroblasts from aged non-sun-exposed donors compared to young donors, whereas no obvious difference for the protein PDI was observed (Figure 1B). Moreover, the proportion of HERPUD1-, PDI-, and XBP1s-positive fibroblasts in aged sun-exposed dermis compared to young sun-exposed counterparts was increased (Figure 1A and 1B). Additionally, sun exposure significantly increased HERPUD1-positive fibroblasts in skin samples from young donors.

Together, these results suggest that dermal (photo)aging may involve the activation of UPR-induced proteins.

Conditions	Y_NSE	Y_SE	A_NSE	A_SE
Donors age	28.3 ± 10.1	31.7 ± 1.5	79.7 ± 2.1	66.7 ± 11
Sample localization	Right thigh; Left wrist; Right thigh	Right forearm; Left elbow; Right forearm	Left thigh; Right shoulder; Right buttock	Right arm; Neck; Neck
Fitpatrick type	III; III; III	IV; II; III	I;III;V	IV; II; III

Table 1. Characterisitics of skin samples donors
Abbreviations : Y, young; A, aged; NSE, non-sun-exposed ; SE, sun-exposed.

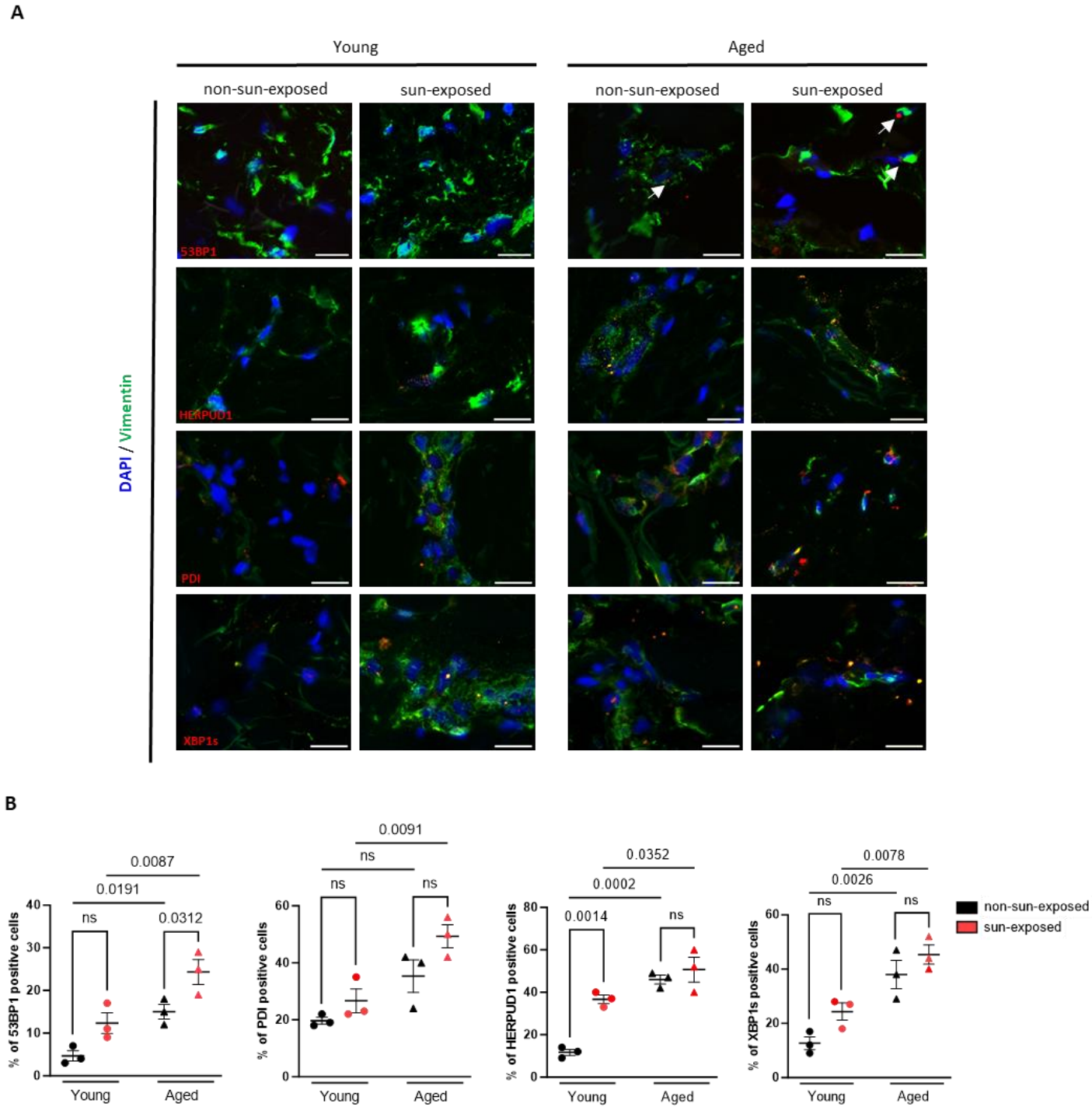


Figure 1. Aging and sun-exposition induce the expression of UPR markers in human dermis

A. Histological imaging of the dermis from both young (19-39 years old) and aged donors (58-82 years old) non-sun-exposed or sun-exposed. Samples were stained using immunofluorescence targeting 53BP1, HERPUD1, PDI, or XBP1s (red). Vimentin (green) was used to delineate the dermal regions and DAPI (blue) for nuclei. Scale bar indicates 20 µm.
B. Quantifications of the percentage of positive fibroblasts for 53BP1, HERPUD1, PDI, and XBP1s, determined by counting at least 100 cells per conditions, (*n*=3).

Data information : Data in **(B)** are presented as means ± SEM of three donors per group. Statistical comparisons were performed using ANOVA2 followed by Šídák's multiple comparaison tests. *p*-values shown represent statistical differences between non-sun-exposed (NSE) and sun-exposed (SE) dermis samples, and differences between young and aged group under the same sun exposure.

Repeated UVB exposures induce premature senescence in normal human dermal fibroblasts

Repeated UVB exposures have been established as a robust experimental model for inducing premature senescence in various skin cell types (Debacq-Chainiaux et al. 2012), and can be considered as an *in vitro* simplified model for dermal photoaging. Consistent with these findings, we generated an experimental approach in which normal human dermal fibroblasts (NHDFs) were exposed to narrowband (NB)-UVB irradiation (500 mJ/cm²) twice a day for five consecutive days and confirmed that these repeated UVB doses led to stress-induced premature senescence (UVB-SIPS). To do so, biomarkers of senescence were all studied three days after the last UVB exposure. Phalloïdin staining indicated that UVB-exposed NHDFs underwent a drastic change in morphology, adopting a star-shaped, enlarged, and irregular morphology (Figure 2A). A minimal proportion (25 %) of SA-βgal-positive NHDFs was observed in proliferative controls, whereas it increases to 51 % in UVB-exposed fibroblasts (Figure 2B). As expected, repeated UVB exposures led to a decrease in proliferation, as evidenced by a lower percentage of EdU-positive cells (Figure 2C), and an increased abundance of $p21^{WAF1}$ (Figure 2D). Additionally, UVB-exposed NHDFs displayed an increased proportion of nuclei with at least four 53BP1 foci (Figure 2E). Repeated UVB exposures also increased the expression of SASP-associated genes, such as *IL1β*, *IL8*, *CCL2, CXCL1, MMP1,* and *MMP3* (Figure 2F), and increased IL8 and TGFβ1 secretion (Figure 2G). This confirms that UVB-exposed NHDFs exhibit the standard hallmarks of senescence.

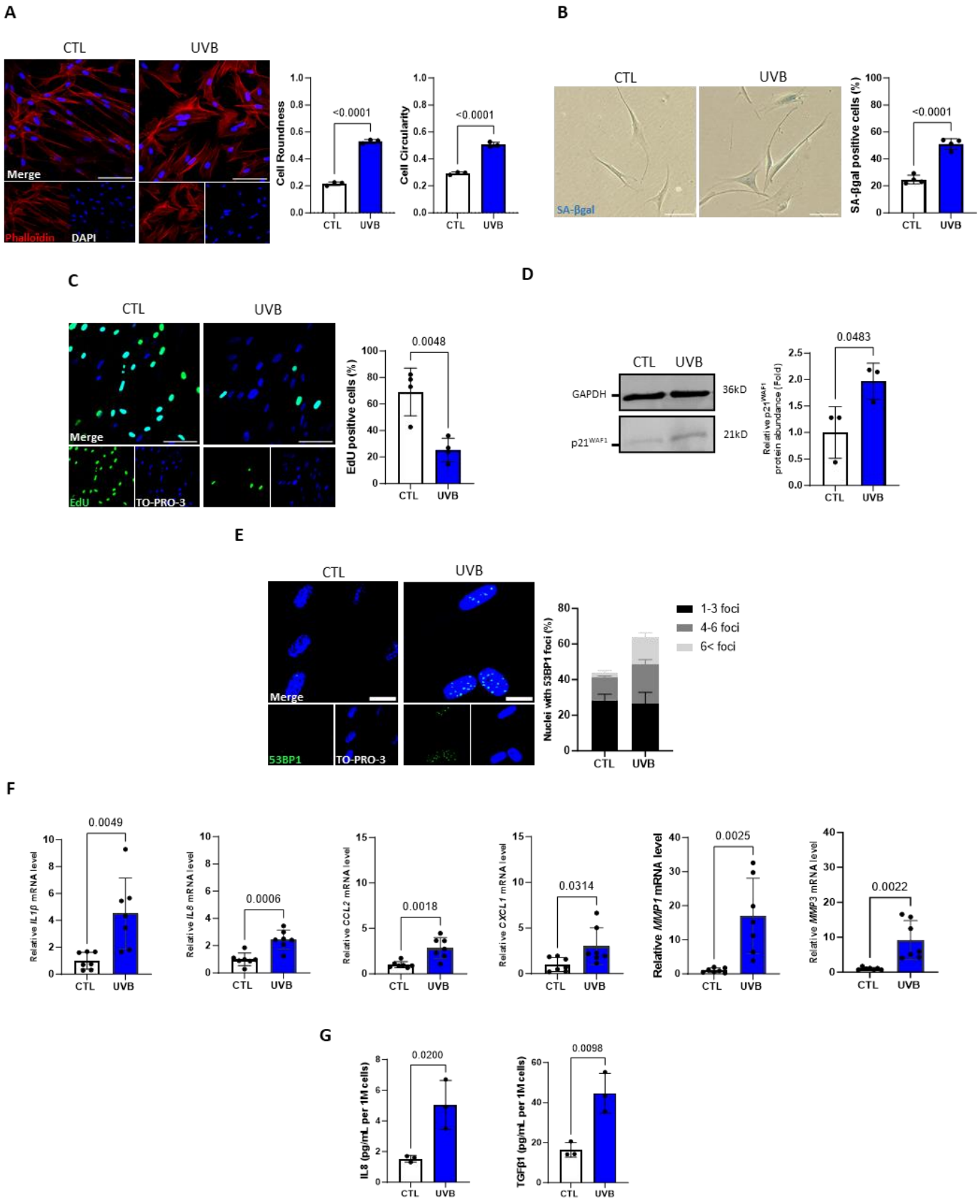


Figure 2. Repeated UVB exposures at 500 mJ/cm² induce premature senescence in NHDFs

NHDFs were exposed (UVB) or not (CTL) to 500 mJ/cm² UVB twice a day for 5 days. At three days after the last UVB exposure, the biomarkers of senescence were studied.

A. (Left panel) Representative micrographies of phalloïdin (red) and TO-PRO-3 (blue). Scale bar indicates 100 µm. (Right panel) Quantification of cell roundness ($4*area/(\pi*major\_axis^2)$) and cell circularity ($4\pi*area/perimeter^2$). Circularity measures the deviation from a perfect circle (Cl=1) whereas roundness approximates a "best-fit" to an idealized ellipse (Ro=1). For each conditions 50 cells have been counted, (*n*=3).

B. (Left panel) Representative micrographies of SA-βgal staining (blue). Scale bar indicates 100 µm. (Right panel) Quantification of SA-βgal positive cells determined by counting 300 cells per conditions, (*n*=4).

C. (Left panel) Representative micrographies of EdU staining (green) and TO-PRO-3 (blue). Scale bar indicates 100 µm. (Right panel) Quantification of EdU positive cells determined by counting 200 cells per conditions, (*n*=4).

D. (Left panel) Representative western blot for p21. GAPDH was used as a loading control. (Right panel) Western blots quantifications, (*n*=3).

E. (Left panel) Representative micrographies of 53BP1 staining (green) and TO-PRO-3 (blue). Scale bar indicates 20 µm. (Right panel) Quantification of the percentage of cells harboring respectively one to three, or four to six, or more than six 53BP1 foci per nucleus determined by counting 200 cells per conditions, (*n*=3).

F. Relative mRNA level of *IL1β*, *IL8*, *CCL2*, *CXCL1*, *MMP1* and *MMP3* were quantified by RT-qPCR and normalized to *RPL13A*. Results are expressed as ratio related to CTL cells, (*n*=7).

G. Quantifications of the level of secreted IL8 and TGF-β1 by ELISA assay. The level of secreted proteins is expressed as pg/mL normalized by the total number of cells per conditions and is represented as IL8 and TGF-β1 concentration per 1 million NHDFs, (*n*=3).

Data information : Data in (**A-G**) are presented as means ± SD. Statistical comparison was performed by unpaired *t*-test. *p*-value shown represents difference between unexposed (CTL) and exposed (UVB) cells.

Transcriptomic signature of UVB-induced premature senescence revealed regulatory elements associated with key pathways in senescence and endoplasmic reticulum functions

To further characterize the UVB-induced senescence signature in NHDFs, we conducted an RNA sequencing (RNA-seq) analysis. For this purpose, RNA was extracted 3 days after the last UVB exposure. Interestingly, we observed significant changes in the global gene expression profiles when compared to young proliferative fibroblasts (CTL). Principal-component analysis (PCA) illustrated a clear distinction between the transcriptional profiles of control and UVB-induced senescent fibroblasts (UVB), with the first principal component accounting for 85% of the variation (Figure 3A). Then, using EdegR, the differentially expressed genes (DEGs) between UVB vs. CTL were performed on three independent biological replicates (Figure 3B and 3C). The number of up and down-regulated genes was 430 and 216 respectively. The relevance of up and down-regulated genes was first compared and examined with gene sets or lists from publications or publicly accessible databases such as, the replicative senescence signature (LIST 1) (Druelle et al. 2016), GeneCards based ontology “UVB-senescence” (LIST 2), “ER stress” (LIST 3), KEGG-ko04141 Protein processing in ER (LIST 4) and KEGG-ko04062 Chemokine Signalling Pathway (LIST 5). Comparative analysis revealed that our lists of differentially expressed genes overlap with genes belonging to one or more gene sets of the aforementioned signatures (Figure 3B and 3C). Subsequently, we searched for processes that could be linked with UVB-induced senescence. To do so, enrichment analysis was performed on up- and downregulated genes from the UVB vs. CTL comparison using the over-representation analysis (ORA) method with the Gene Ontology (GO) database focusing on Biological Processes (BP) (Figure 3D). This analysis revealed that, among the significantly enriched GO terms, the “aging” and “skin development” terms showed up (Figure 3D). Additionally, the sub-ontologies Molecular Function (MF) and Cellular Component (CC) revealed GO terms consistent with the literature such as “growth factor binding” and “extracellular matrix” terms (Saul et al. 2022) (Figure EV1A and EV1B). Interestingly, we observed that “endoplasmic reticulum lumen” GO terms also showed up in CC sub-ontologies. Importantly, Gene set enrichment analysis (GSEA) using the KEGG database predicted highly significant enrichment of “protein processing in ER” term (Figure 3E). Next, using the Reactome pathway database, “cell-cycle checkpoints” term (Figure 3F) and other cell-cycle gene signatures (Figure EV1C) were also significantly enriched. Finally, GSEA unveiled an almost significant enrichment of the publicly available universal senescence signature with the whole transcriptome of UVB vs control comparison (Hernandez-Segura et al. 2017) (Figure EV1D). Our data are in accordance with the literature and show that the implemented senescence program strongly depends on the nature of the

stressor. Based on these results and the knowledge that endoplasmic reticulum stress and UPR activation have been shown to occur in response to various senescence inducers (Denoyelle et al. 2006; Dörr, Yu, Milanovic, Beuster, Zasada, Däbritz, et al. 2013; Blazanin et al. 2017), we chose to monitor the UPR activation status in UVB-induced premature senescent fibroblasts. In addition to the detection of senescence biomarkers, the expression of a panel of UPR target genes downstream of ATF6α (*HSPA5*/*Grp78*, *P4HB*, *XPB1*), PERK (*CHOP*, *PP1R15A*/*GADD34*) and IRE1α (*ERO1β*, *SEL1*, *PER1*) was assessed by RT-qPCR.

Interestingly, mRNA levels of all tested genes except *CHOP*, *SEL1*, and *PER1* were significantly upregulated three days after the last UVB stress (Figure 3G). Moreover, using immunoblotting, we showed that GRP78/BIP level was increased in UVB-induced senescent NHDFs (Figure 3H).

Collectively, these data revealed that UVB-induced premature senescence transcriptomic signature displays common senescence-associated genes and is associated with aging, skin development, and ER stress.

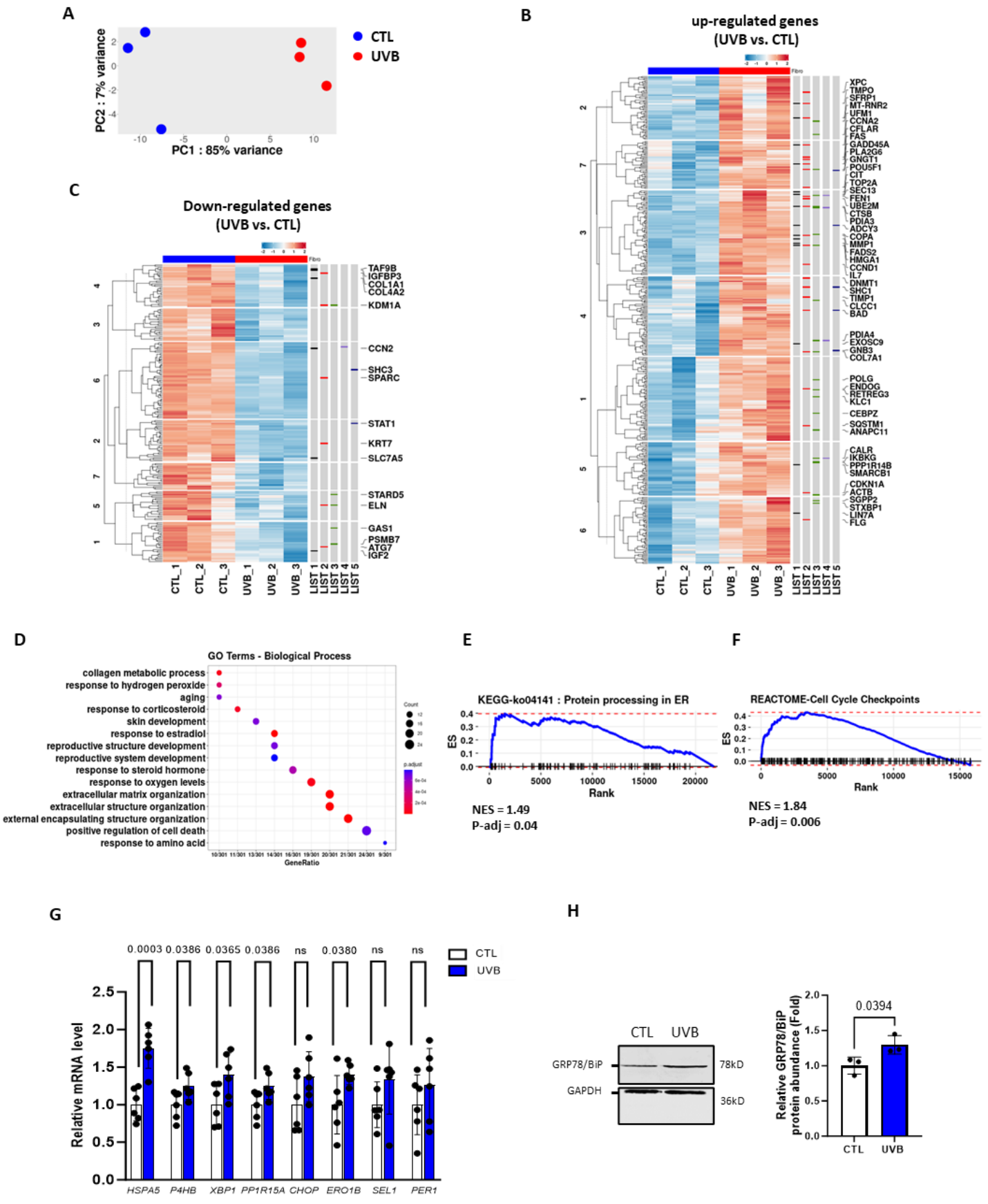


Figure 3. UVB-induced premature senescent NHDFs are associated with ER stress and UPR activation

NHDFs were exposed (UVB) or not (CTL) to 500 mJ/cm² UVB twice a day for 5 days. At three days after the last UVB exposure, mRNA was extracted.
A. Principal Component Analysis (PCA) of transcriptional profiles obtained via RNA-seq for three independent experiments corresponding to the samples from unexposed (CTL) and exposed (UVB) NHDFs represented by blue or red dots respectively. The two-dimensional scatter plot shows the first two principal components of the analysis of all genes, (*n*=3).
B. Heatmap of the differentially up-regulated genes in UVB induced senescent NHDFs. (FDR < 0.05 , log2(FC)+/-1.5) from UVB vs. CTL dataset.
C. Heatmap of the differentially down-regulated genes in UVB induced senescent NHDFs. (FDR < 0.05 , log2(FC)+/-1.5) from UVB vs. CTL dataset. Both up- and downregulated gene lists were compared to gene sets published or deposited into free access databases. LIST 1: Replicative senescence signature (Druelle et al. 2016); LIST 2: GeneCards base dontology « UVB-senescence »; LIST 3: GeneCards based ontology « ER stress »; LIST4: KEGG Protein processing in ER (ko04141); LIST 5: KEGG Chemokine Signaling Pathway (ko04062).
D. ORA (Over-Representation Analysis) of Biological Processes (BP) Gene Ontology (GO) terms of differentially expressed genes in UVB vs. CTL NHDFs. Gene ratio provides ratio of input DEGs that are annoted in BP terms.
E. GSEA enrichment plot of one of the most positively enriched pathway associated with the whole UVB-induced senescent NHDFs using KEGG- ko04141 database. (NES, normalised enrichment score).
F. GSEA enrichment plot of one of the most positively enriched pathway associated with the whole UVB-induced senescent NHDFs using reactome database. (NES, normalised enrichment score).
G. Relative mRNA level of *HSPA5*, *P4HB*, *XBP1*, *PP1R15A*, *CHOP*, *ERO1β*, *SEL1* and *PER1* were quantified using RT-qPCR and were normalized to *RPL13A*. Results are expressed as ratio related to CTL cells, (*n*=6).
H. (Left panel) Representative western blot for GRP78. GAPDH was used as a loading control. (Right panel) Western blots quantifications, (*n*=3).

Data information : Datas in (**G-H**) are presented as means ± SD. Statistical comparison was performed using unpaired *t*-test. *p*-value shown represents difference between unexposed (CTL) and exposed (UVB) cells.

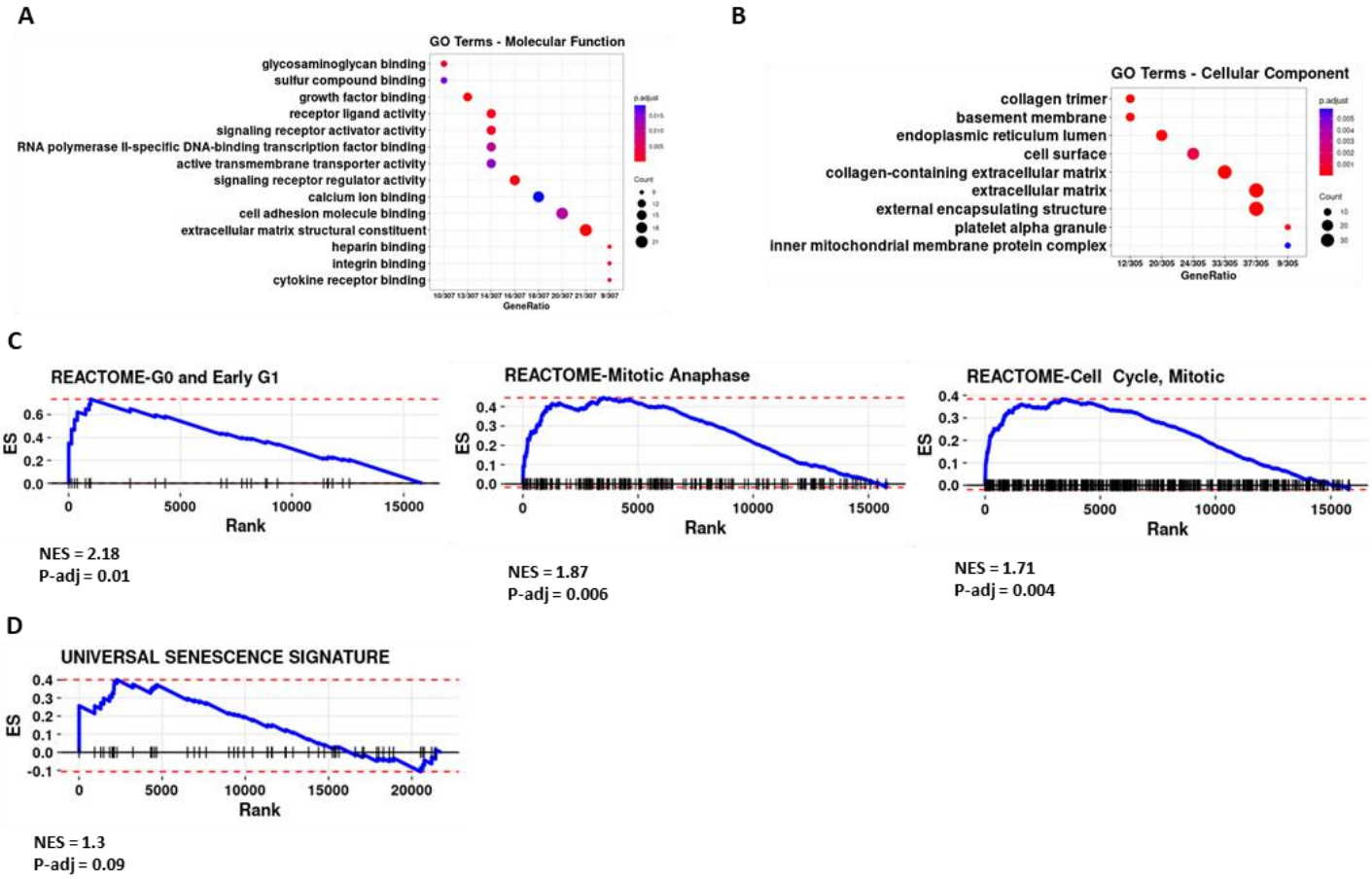


Figure EV1. DEGs and transcriptome from UVB-induced premature senescence display common senescence-related patterns.

NHDFs were exposed (UVB) or not (CTL) to 500 mJ/cm^2 UVB twice a day for 5 days. At three days after the last UVB exposure, mRNA was extracted.
A. ORA of Molecular Function (MF) GO terms of differentially expressed genes in UVB vs. CTL NHDFs. Gene ratio provides ratio of input DEGs that are annoted in MF terms.
B. ORA of Cellular component (CC) GO terms of differentially expressed genes in UVB vs. CTL NHDFs. Gene ratio provides ratio of input DEGs that are annoted in CC terms.
C. GSEA enrichment plot of the most positively enriched pathway associated with the whole UVB-induced senescent NHDFs using reactome database. (NES, normalised enrichment score).
D. GSEA enrichment plot of the most positively and negatively enriched pathway associated with the whole UVB-induced senescent NHDFs using Universal senescence signature published in *Hernandez-Segura et al.* 2017. (NES, normalised enrichment score).

Repeated UVB exposures initiate premature senescence in an ATF6α-dependent manner

To investigate whether a functional link exists between ER stress and the onset of UVB-induced premature senescence in NHDFs, we individually silenced the expression of each UPR sensor (ATF6α, PERK, and IRE1α) using RNA interference (Figure EV2A). The knockdown efficiency of each siRNA was validated at the protein level until eight days following siRNA transfection (Figure EV2B). Subsequently, transfected fibroblasts were exposed to UVB for five days. The specificity of the RNA invalidation was examined by RT-qPCR, demonstrating that each siRNA specifically and efficiently invalidated the targeted UPR sensor three days after the last UVB exposure. It is worth noting that the expression of all three sensors was upregulated upon UVB exposures (Figure EV2C). Next, we assessed whether invalidating the expression of ATF6α, PERK, and IRE1α prior to repeated UVB exposures could impact the establishment of senescence biomarkers. Since ATF6α is known to mediate cytoskeleton morphological changes in replicative senescent NHDFs (Druelle et al. 2016), we first examined the cell morphology. Immunostaining of F-actin demonstrated that upon ATF6α invalidation, UVB-exposed fibroblasts retained a spindle-shape morphology and exhibited cell roundness and circularity similar to that of unexposed NHDFs (Figure 4A and EV3A). By contrast, neither PERK silencing nor IRE1α impacted the morphology of UVB-induced senescent fibroblasts (Figure 4A). Interestingly, ATF6α and PERK silencing prevented the increase of SA-βgal-positive cells after UVB exposures. However, only ATF6α invalidation significantly limited the percentage of SA-βgal-positive NHDFs compared to UVB-exposed fibroblasts transfected with a non-targeting control siRNA (Figure 4B). In addition, ATF6α invalidation, but not IRE1α or PERK, in UVB-exposed fibroblasts, resulted in a non-significant decrease in EdU incorporation compared to control NHDFs (Figure 4C). Surprisingly, the increase in $p16^{INK4A}$ expression after UVB was limited in fibroblasts knocked down for PERK and IRE1α, but not for ATF6α, even though no significant difference between proliferative and UVB-exposed NHDFs is observable following invalidation of either branch (Figure EV3B). The same tendency was noted for $p21^{WAF1}$ (Figure EV3B) suggesting that the potential influence of ATF6α on the cell cycle regulation may not directly control classical cell cycle mediators. Interestingly, an assessment of 53BP1 foci showed that only the silencing of ATF6α reduced the percentage of cells with at least six 53BP1 foci within the nuclei of UVB-induced senescent NHDFs (Figure 4D and EV3C). These findings suggest that ATF6α could potentially protect against the persistence of DNA damage.

Altogether these data suggest that the UVB-induced premature senescent phenotype is partially controlled by ATF6α. In line with this hypothesis, we investigated to what extent the addition of chemical inhibitors for each UPR branch could impair the establishment of UVB-

induced phenotype. In this context, CA7 (Ceapin A7, ATF6α inhibitor), GSK2606414 (PERK inhibitor), or 4μ8c (IRE1α inhibitor) were added directly after the last UVB exposure (Figure EV4A). The results showed similar trends with a protective role of ATF6α inhibition using Ceapin A7 on the increased number of SA-βgal-positive cells and persistent DNA damage caused by UVB exposures (Figure EV4B and EV4D). However, none of the chemical inhibitors had any effect on the UV-induced cell cycle arrest (Figure EV4C). Nevertheless, it seems that targeting ATF6α prevents the appearance of some biomarkers of senescence induced by UVB exposures.

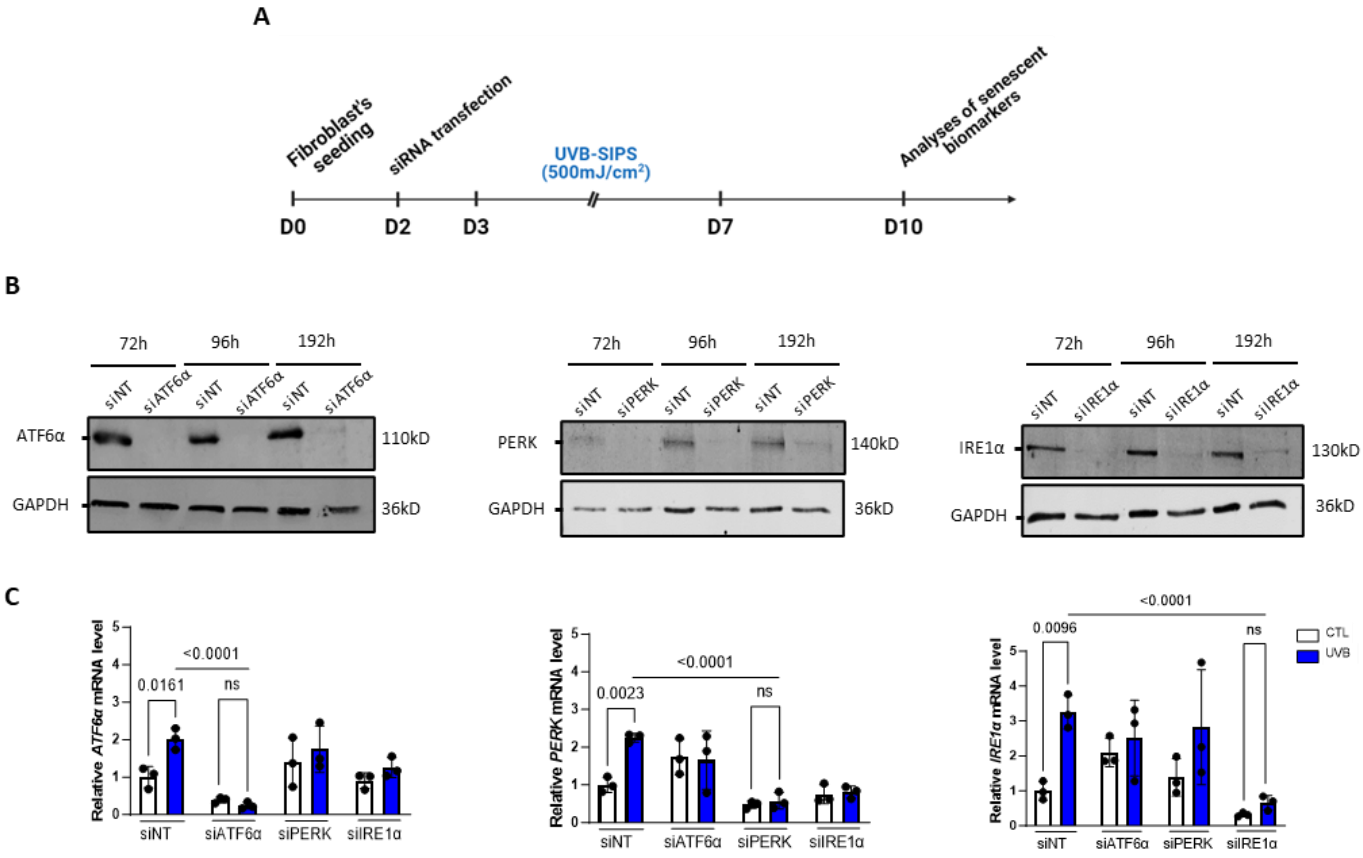


Figure EV2. Validation of ATF6α, PERK, and IRE1α knockdown efficiency in NHDFs

A. Schematic experimental RNA interference strategy for *in vitro* knockdown of the three UPR arms (ATF6α, PERK and IRE1α) in UVB-SIPS model. Details of the model are provided in Materiel and Methods.
B. NHDFs were transfected with non-targeting control siRNA (siNT) or with siATF6α, siPERK or siIRE1α. Representative Western Blots for ATF6α, PERK, and IRE1α at 72h, 96h, and 192h post transfection. GAPDH was used as loading control, (*n*=1).
C. NHDFs were transfected with non-targeting control siRNA (siNT) or with siATF6α, siPERK or siIRE1α 16 hours before repeated UVB exposures at 500 mJ/cm² twice a day for five consecutive days. Three days after the last UVB stress, mRNA was extracted, (*n*=3). Relative mRNA level of *ATF6α*, *PERK*, and *IRE1α* were quantified using RT-qPCR and were normalized to *RPL13A*. Results are expressed as ratio related to CTL siNT cells.

Data information : Data in (**C**) are presented as means ± SD. Statistical comparison was performed using ANOVA2 followed by Šídák's multiple comparaison tests. *p*-value shown represents differences between unexposed (CTL) and exposed (UVB) cells and differences between non-targeting control siRNA (siNT) and siATF6α, or siPERK, and siIRE1α.

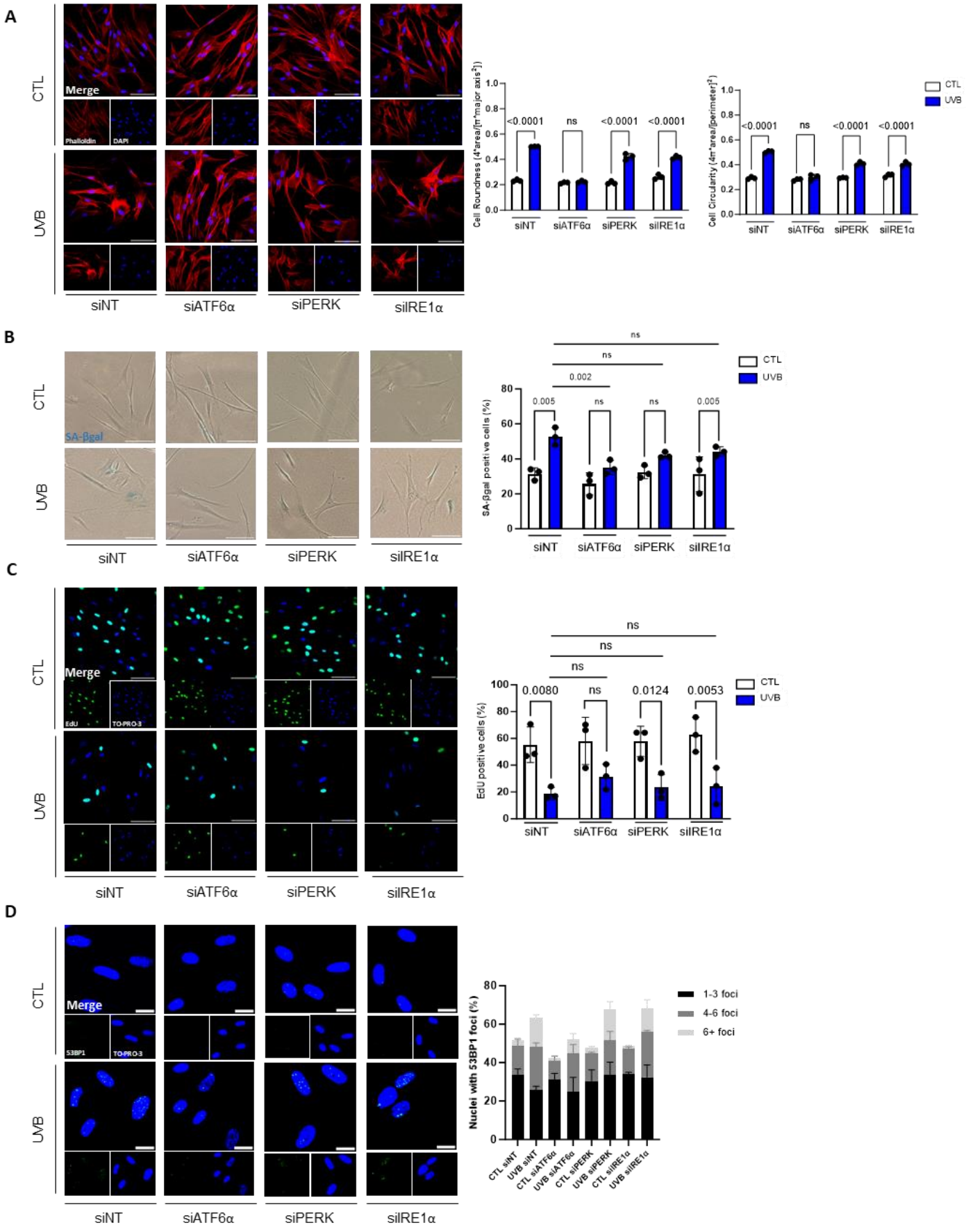


Figure 4. Knockdown of ATF6α prevents the complete establishment of UVB-induce senescent phenotype

NHDFs were transfected with non-targeting control siRNA (siNT) or with siATF6α, siPERK or siIRE1α 16 hours before repetitive UVB exposures at 500 mJ/cm² twice a day for five consecutive days. The biomarkers of senescence were studied three days after the last UVB exposure.

A. (Left panel) Representative micrographies of phalloïdin (red) and DAPI (blue). Scale bar indicates 100 µm. (Right panel) Quantification of the cell circularity and the cell roundness. Circularity measures the deviation from a perfect circle (Cl=1) whereas roundness approximates a "best-fit" to an idealized ellipse (Ro=1). For each conditions 50 cells have been counted, (*n*=3).

B. (Left panel) Representative micrographies of SA-βgal staining (blue). Scale bar indicates 100 µm. (Right panel) Quantification of SA-βgal positive cells determined by counting 300 cells per conditions, (*n*=3).

C. (Left panel) Representative micrographies of EdU staining (green) and TO-PRO-3 (blue). Scale bar indicates 100 µm. (Right panel) Quantification of EdU positive cells determined by counting 200 cells per conditions, (*n*=3).

D. (Left panel) Representative micrographies of 53BP1 staining (green) and TO-PRO-3 (blue). Scale bar indicates 20 µm. (Right panel) Quantification of the percentage of cells harboring respectively one to three, or four to six, or more than six 53BP1 foci determined by counting 200 cells per conditions, (*n*=3).

Data information : Data in (**A-D**) are presented as means ± SD. Statistical comparison was performed using ANOVA2 followed by Šídák's multiple comparaison tests. *p*-value shown represents differences between unexposed (CTL) and exposed (UVB) cells and differences between non-targeting control siRNA (siNT) and siATF6α, or siPERK, and siIRE1α.

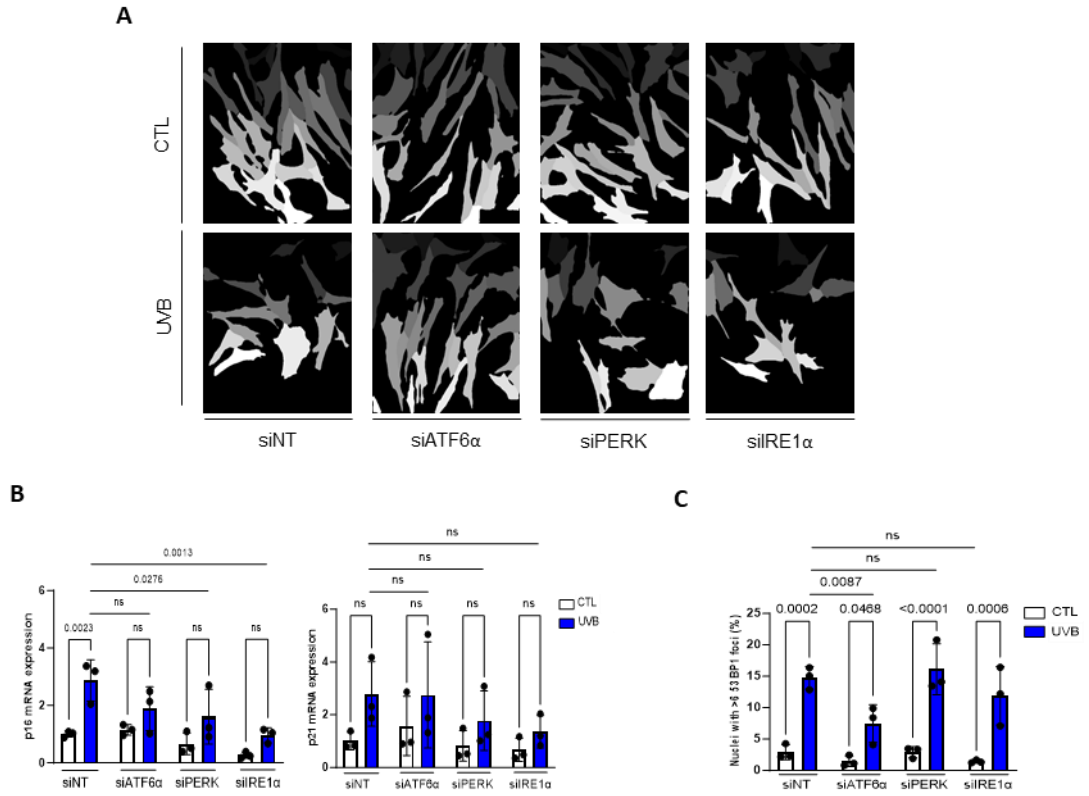


Figure EV3. Impact of UPR invalidation before repeated UVB stresses on the senescent hallmarks

NHDFs were transfected with non-targeting control siRNA (siNT) or with siATF6α, siPERK or siIRE1α 16 hours before repetitive UVB exposures at 500 mJ/cm² twice a day for five consecutive days.

A. Predicted mask of F-actin micrographies from the generalist algorithm for cellular segmentation, Cellpose. Masks have been generated to use shape description tools with Fiji.

B. Three days after the last UVB stress, mRNA was extracted, (*n*=3). Relative mRNA level of *p16*^{INK4A} *and p21*^{WAF1} were quantified using RT-qPCR and were normalized to *RPL13A*. Results are expressed as ratio related to CTL siNT cells.

C. Percentage of NHDFs harboring respectively six or more 53BP1 foci determined by counting 200 cells per conditions, (*n*=3).

Data information : Data in (**B-C**) are presented as means ± SD. Statistical comparison was performed using ANOVA2 followed by Šídák's multiple comparaison tests. *p*-value shown represents differences between unexposed (CTL) and exposed (UVB) cells and differences between non-targeting control siRNA (siNT) and siATF6α , or siPERK, and siIRE1α.

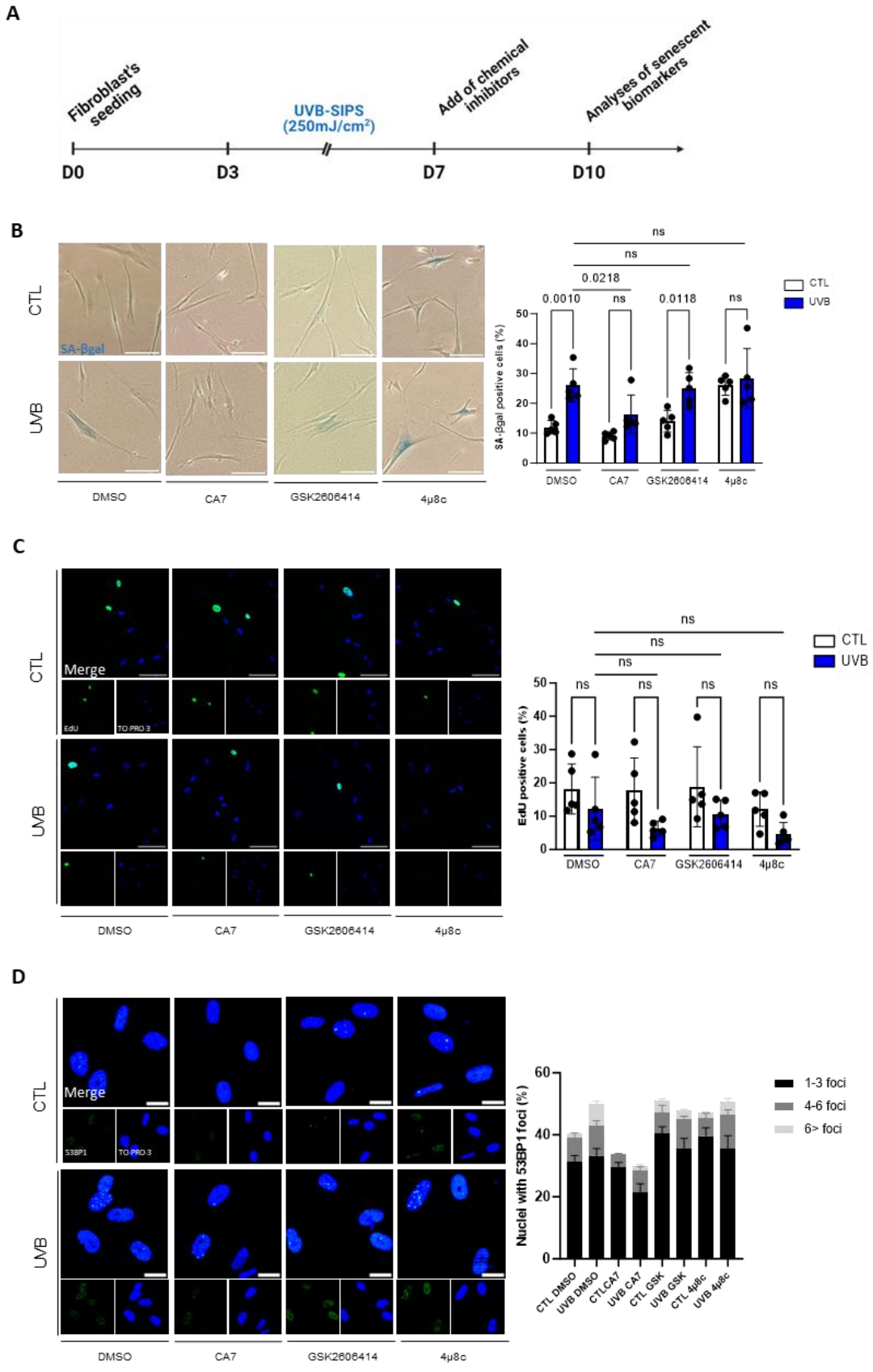


Figure EV4. Inhibition of ATF6α after UVB-exposures prevents the complete establishment of the senescent phenotype

AG04431 were repetitively exposed to 250 mJ/cm² of UVB, twice a day for five consecutive days. After the last UVB stress, chemical inhibitors of the ATF6α arm (CA7), PERK (GSK2606414) or IRE1α (4μ8c) were added. The biomarkers of senescence were studied three days after the last UVB exposure.
A. Schematic experimental strategy for *in vitro* inhibition of the three UPR arms in UVB-SIPS model. Details of the model are provided in Materiel and Methods.
B. (Left panel) Representative micrographies of SA-βgal staining (blue). Scale bar indicates 100 μm. (Right panel) Quantification of SA-βgal positive cells determined by counting 300 cells per conditions, (*n*=5).
C. (Left panel) Representative micrographies of EdU staining (green) and TO-PRO-3 (blue). Scale bar indicates 100 μm. (Right panel) Quantification of EdU positive cells determined by counting 200 cells per conditions, (*n*=5).
D. (Left panel) Representative micrographies of 53BP1 staining (green) and TO-PRO-3 (blue). Scale bar indicates 20 μm. (Right panel) Quantification of the percentage of cells harboring respectively one to three, or four to six, or more than six 53BP1 foci determined by counting 200 cells per conditions, (*n*=3).

Data information : Data in (**B-D**) are presented as means ± SD. Statistical comparison was performed using ANOVA2 followed by Šídák's multiple comparaison tests. *p*-value shown represents differences between unexposed (CTL) and exposed (UVB) cells and differences between control DMSO and CA7, or GSK2606414, and 4μ8c.

ATF6α silencing impairs the expression of major SASP factors after UVB exposures

To further investigate whether the UPR disruption could impact the establishment of UVB-induced senescence, we analyzed the mRNA levels of genes encoding key secreted proteins associated with the Senescence-Associated Secretory Phenotype (SASP). This includes pro-inflammatory cytokines, as well as matrix metalloproteases (Figure 5). It is well-described that the inflammatory SASP of senescent cells is mainly regulated at the transcriptomic level (Acosta et al. 2008; Kuilman et al. 2008; Rodier et al. 2009). We noted that the overexpression of *IL1β, IL8*, *MMP1,* and *MMP3* following repeated UVB exposures was no longer detectable upon ATF6α invalidation (Figure 5A, 5B, 5C, and 5D). It is noteworthy that IRE1α knockdown showed similar effects for *IL1β* and *MMP3*, and PERK silencing for *MMP1* (Figure 5B, 5D, and 5C). This suggests that ATF6α invalidation modifies the secretory phenotype in UVB-induced senescence by altering the expression of major inflammatory cytokines and remodelling enzymes, which can alter the tissue microenvironment.

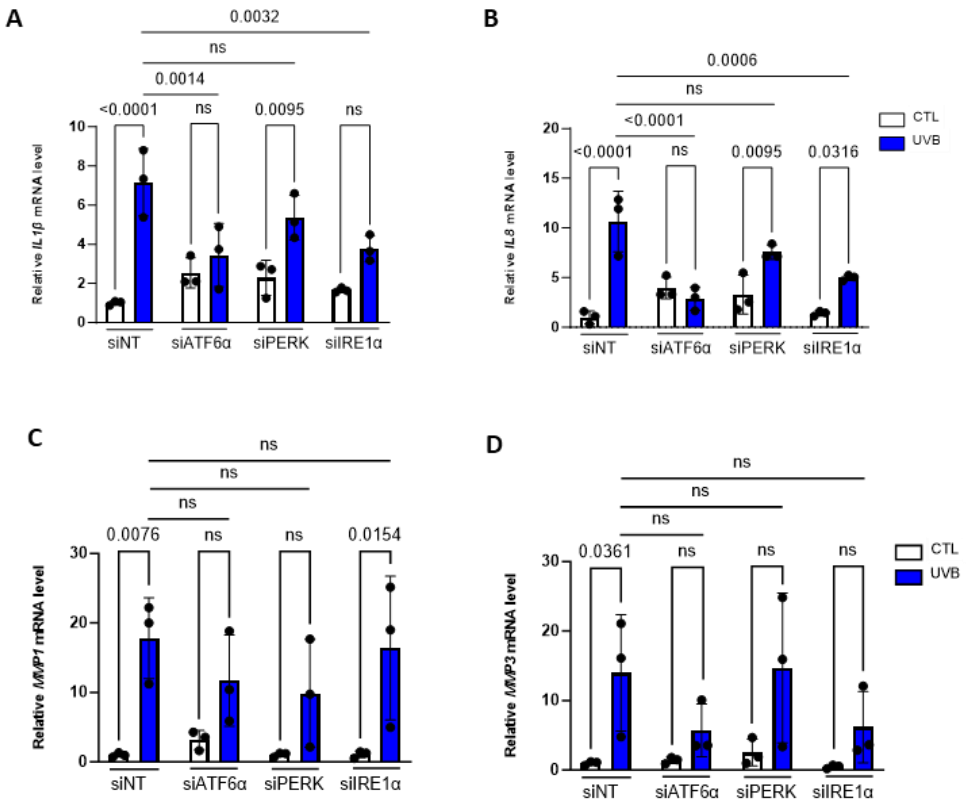


Figure 5. UPR disruption alters the SASP of UVB-induced senescent fibroblasts

NHDFs were transfected with non-targeting control siRNA (siNT) or with siATF6α, siPERK or siIRE1α 16 hours before repetitive UVB exposure at 500mJ/cm² twice a day for five consecutive days. Three days after the last UVB stress, mRNA was extracted.
A-D. Relative mRNA level of *IL1β* (A), *IL8* (B), *MMP1* (C), and *MMP3* (D) were quantified using RT-qPCR and were normalized to *RPL13A*. Results are expressed as ratio related to CTL siNT cells, (*n*=3).

Data information : Data in (**A-D**) are presented as means ± SD. Statistical comparison was performed using ANOVA2 followed by Šídák's multiple comparaison tests. *p*-value shown represents differences between unexposed (CTL) and exposed (UVB) cells and differences between non-targeting control siRNA (siNT) and siATF6α , or siPERK, and siIRE1α.

Conditioned media from UVB-senescent fibroblasts induce ATF6α-dependent paracrine keratinocyte proliferation in RHE

The SASP of senescent cells can exert pleiotropic paracrine effects on the cellular environment (Birch and Gil 2020). While numerous studies have demonstrated the pro-tumorigenic effects of SASP from senescent fibroblasts (Farsam et al. 2016; Krtolica et al. 2001; Laberge et al. 2012), few data showed its impact in a physiological context. To investigate this, we hypothesized that the alteration of SASP composition by ATF6α invalidation in NHDFs could lead to changes in its impact on neighboring cells. Given that our previous RNA-seq data associated the transcriptomic signature of UVB-induced senescent fibroblasts with skin development, we leveraged an established model of reconstructed human epidermis (RHE) utilizing keratinocytes (Aurélie Frankart et al. 2012). Additionally, we devised a novel method of reconstruction involving the contact of keratinocytes with conditioned media (CM) from fibroblasts exposed to UVB, with or without ATF6α invalidation, to decipher the impact of secreted components on epidermal homeostasis. Over 11 days, keratinocytes develop into multi-layered differentiated RHE under the influence of fibroblasts conditioned media, added from day 4 to day 11 of the reconstruction. The day before the end of the reconstruction, BrdU was added for 24h (Figure 6A). H&E staining of RHE section grown in contact with CM of UVB-induced premature senescent fibroblasts displayed the same histological structure and layer organization as those grown in contact with CM from proliferative control fibroblasts (Figure 6B). In addition, no differences in the RHE reconstruction or the staining of early (K10), intermediated (involucrin), and late (loricrin) differentiation markers were observed in the various epidermal layers (Frankart et al. 2012) (Figure EV5A, EV5B and EV5C). However, CM from UVB-exposed fibroblasts appeared to slightly increase the epidermal thickness of RHE, and surprisingly, this effect was no longer observable in RHE grown with CM from fibroblasts invalidated for ATF6α (Figure 6C). Further analysis revealed that UVB-conditioned media significantly increased the number of proliferative keratinocytes from 13,5% to 24% compared to the total number of cells. Interestingly, the knockdown of ATF6α was sufficient to prevent this effect (Figure 6D). These findings provide the first evidence that some secreted components from UVB-induced senescent fibroblasts' secretome can induce keratinocytes hyperproliferation and epidermal thickening in an ATF6α dependent manner, without altering epidermal differentiation.

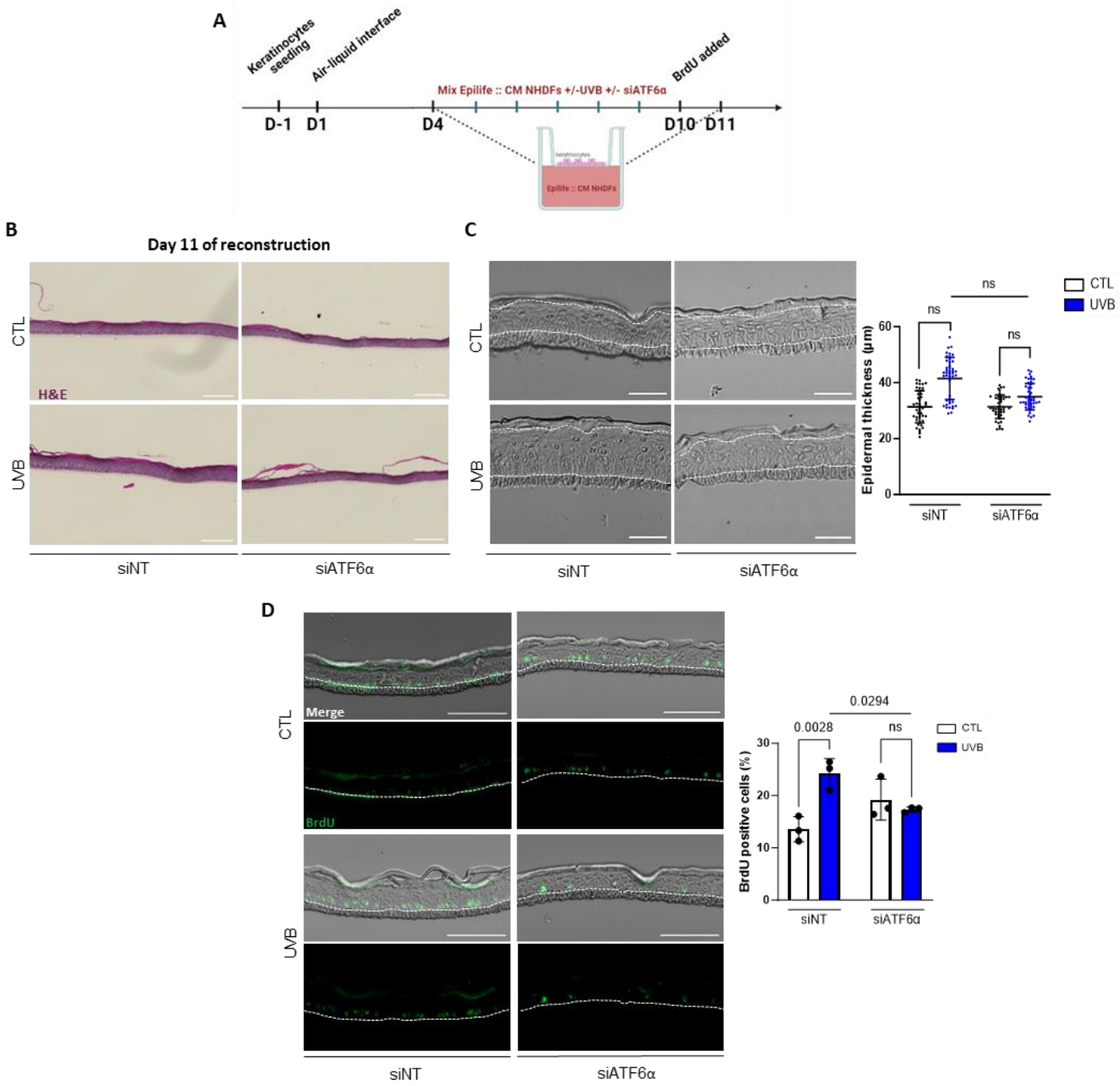


Figure 6. Proliferation of keratinocytes in RHEs is increased by the secretome of UVB-senescent fibroblasts through an ATF6α-dependent mechanism

Reconstructed human epidermises (RHEs) were grown using conditioned media from NHDFs exposed or not (CTL) to UVB (UVB) and transfected or not (siNT) with an siATF6α for 8 consecutive days.

A. Schematic experimental model used for the *in vitro* production of RHE. Epidermises were reconstructed in conditioned media derived from fibroblasts, with BrdU incorporation occurring the day before the completion of the reconstruction.

B. Representative hematoxylin-eosin (H&E) staining on histological sections prepared from RHE. Scale bar indicates 100 µm.

C. (Left panel) Representative brightfield micrographies of RHE thickness. Scale bar indicates 50 µm. (Right panel) Quantification of the epidermal thickness. For each RHE, fifteen measurements have been realised across the entire RHE section, (n=3).

D. (Left panel) Representative micrographies of BrdU staining (green) and brightfield. Scale bar represents 100 µm. (Right panel). Quantification of BrdU positive cells across the entire RHE section, (n=3).

Data information : Data in (**C-D**) are presented as means ± SD. Statistical comparison was performed using ANOVA2 followed by Šídák's multiple comparaison tests. *p*-value shown represents differences between RHE grown in CM from NHDFs unexposed (CTL) and exposed (UVB) and differences between non-targeting control siRNA (siNT) and siATF6α.

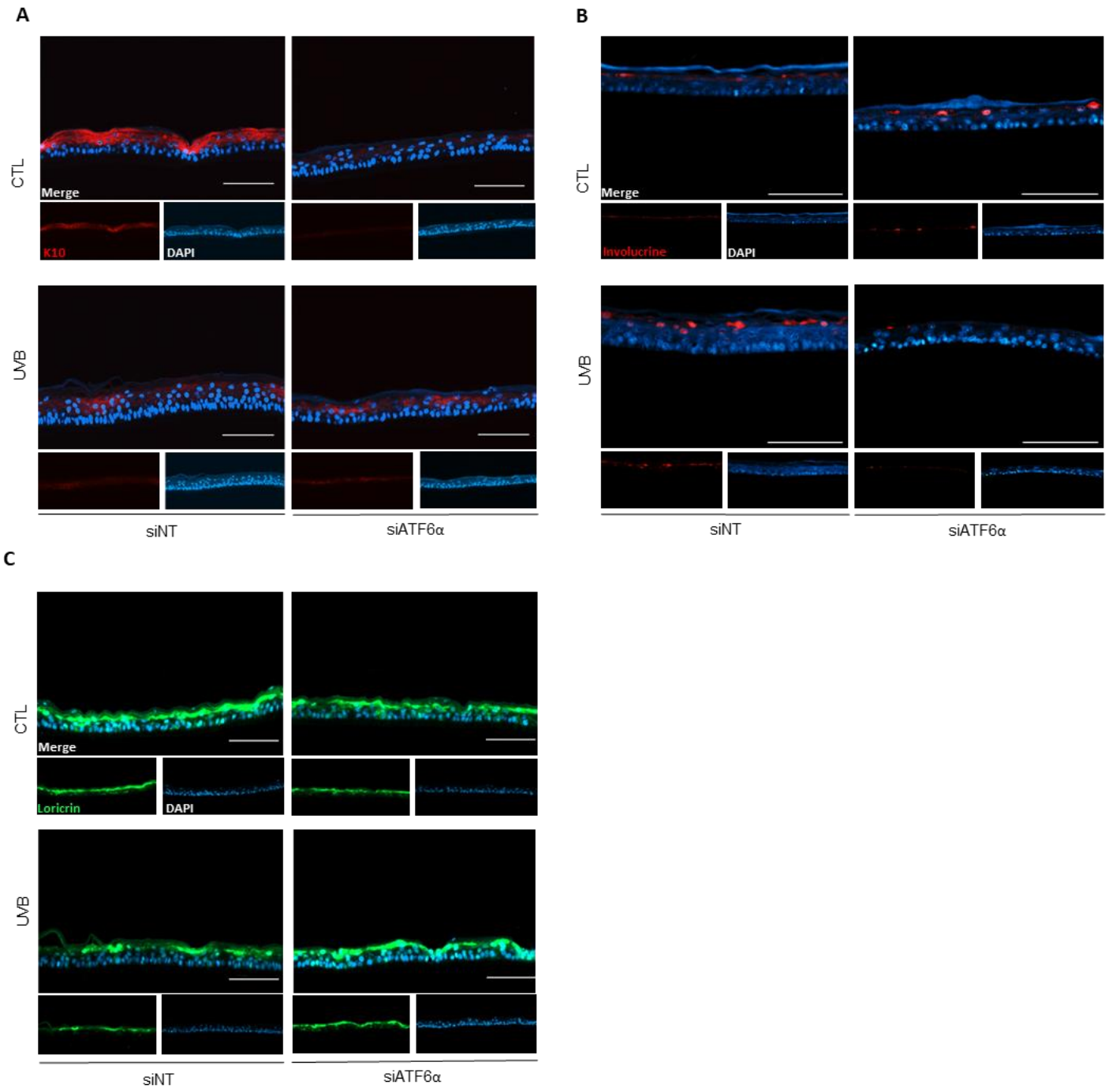


Figure EV5. Conditioned media from fibroblasts do not impact keratinocytes differentiation in RHE.

RHE were grown using CM from NHDFs exposed or not (CTL) to UVB and transfected or not (siNT) with a siATF6α for 8 consecutive days.
A-C. RHE were stained using immunofluorescence targeting K10(red) (**A**), involucrin (red) (**B**), or loricrin (green) (**C**). DAPI (blue) was used to stain nuclei. Scale bar indicates 100 µm.

Increased proliferation of keratinocytes is mediated by an ATF6α/IL8 axis

To further describe the pro-proliferative effect of CM from UVB-induced fibroblasts on keratinocytes, we took advantage of the ability of keratinocytes to form colonies and conducted a clonogenic assay to assess their proliferation (Rafehi et al. 2011). To achieve this, keratinocytes were seeded at low density and incubated with conditioned media from NHDFs exposed or not to UVB and invalidated or not for ATF6α for three days. Colonies were fixed 48 hours after the last media change, and only colonies with at least 20 cells were counted (Figure 7A). We observed a significant increase in the number of colonies when keratinocytes were grown with CM from UVB-exposed fibroblasts compared to CM from proliferative control fibroblasts (Figure 7B). Interestingly, focusing on CM from UVB-exposed fibroblasts we showed that ATF6α invalidation allowed to significantly reduce the number of colonies formed (Figure 7C). To go further into our understanding of ATF6α-dependent effectors involved in keratinocyte proliferation, we took advantage of our findings concerning the expression of SASP factors as depicted in Figure 5. Among the components under the control of ATF6α and based on the literature, we identified IL8 as a potent candidate. Although little is known about the biological effects of IL8 on epidermal cells, a study showed that the addition of exogenous IL8 in a culture medium stimulates keratinocyte proliferation (Steude, Kulke, and Christophers 2002). To delve deeper into this hypothesis, the secreted level of IL8 was quantified by ELISA, revealing that ATF6α invalidation significantly reduces the quantity of IL8 in UVB-induced senescent fibroblasts from 14.8 to 4.9 [pg/mL] per one million fibroblasts (Figure 7D). To determine whether IL8 was necessary for the hyperproliferation of keratinocytes induced by CM from UVB-exposed fibroblasts, a neutralizing antibody against IL8 was freshly added to the CM daily. Results showed a more pronounced decrease in colony formation with IL8 inhibition (Figure 7E), supporting our hypothesis regarding the paracrine effects of UVB-induced senescent fibroblasts on keratinocytes proliferation, possibly mediated by an ATF6α/IL8 axis. This demonstrates that ATF6α can control IL8 production and promote epidermal hypertrophy by impacting keratinocyte proliferation.

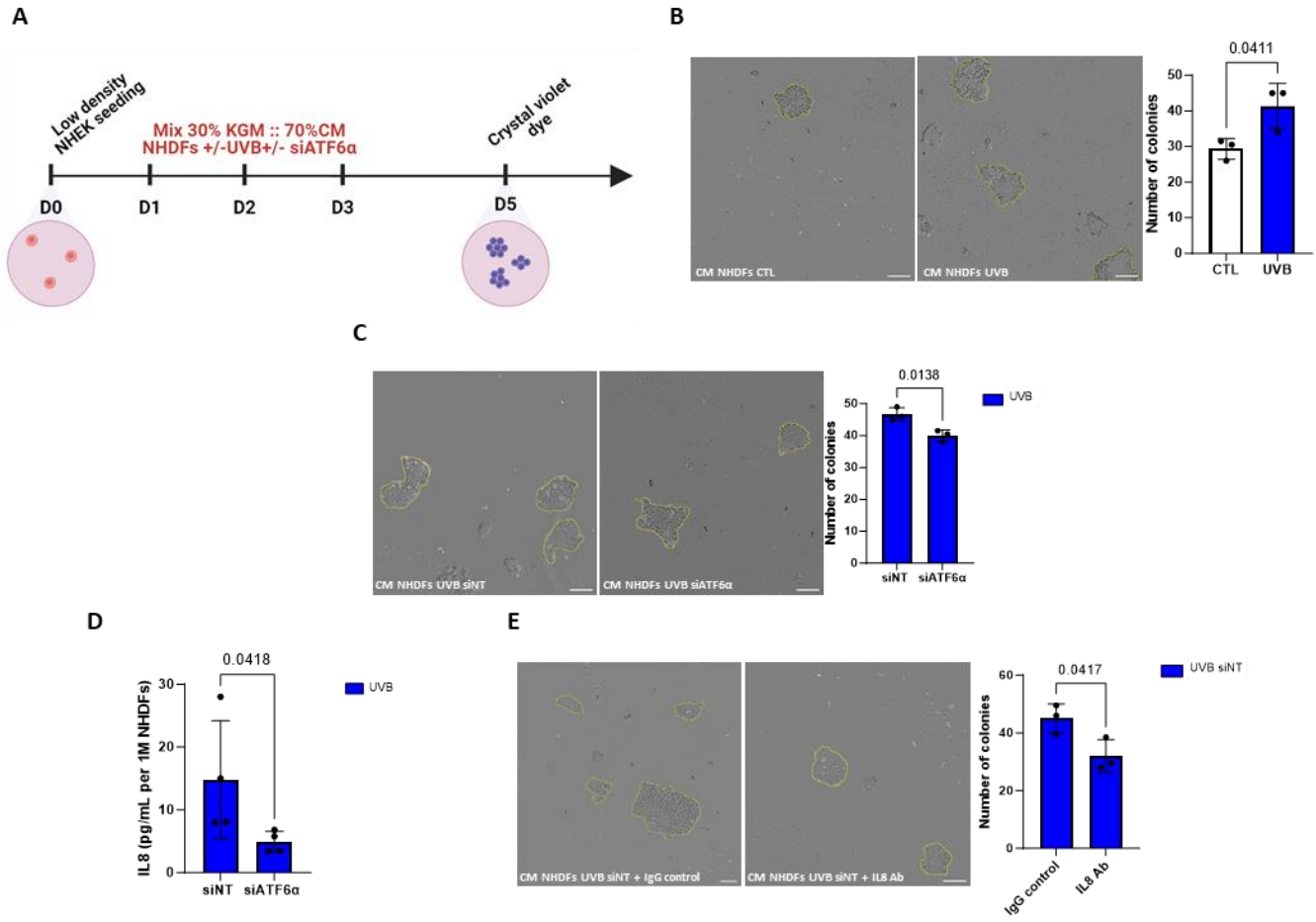


Figure 7. Inhibition of secreted IL8 replicates ATF6α invalidation effect on keratinocyte proliferation.

NHEKs were grown in 24-wells plate using conditioned media from NHDFs exposed to UVB (UVB) and invalidated or not (siNT) for ATF6α (siATF6α) for five consecutive days.

A. Schematic experimental model used to evaluate keratinocytes proliferation. NHEKs were seeded at a low density and then cultured in a mixed media containing 70% conditioned media derived from fibroblasts. The media were changed every day for three days, and the ability of keratinocytes to form colonies was evaluated after staining with 0.025% crystal violet. Then, gray scale pictures of each well were acquired using Incucyte SX3.

B-C. (Left panel) Representative pictures of NHEKs forming colonies at day 5. Scale bar represents 200 µm. (Right panel) Quantification of colonies formed after 5 days (*n*=3).

D. Quantification of the level of secreted IL8 in UVB-induced senescent fibroblasts invalidated or not (siNT) for ATF6α (siATF6α) by ELISA assay. The level of secreted proteins is expressed as pg/mL normalized by the total number of cells per conditions and is represented as IL8 concentration per 1 million NHDFs, (*n*=4).

E. An IL8-neutralizing Ab or a control IgG was added in the conditioned media from UVB-induced fibroblasts transfected with non-targeting control siRNA (siNT).

Data information : Datas in (**B-E**) are presented as means ± SD. Statistical comparison was performed by unpaired t-test. *p*-value shown represents difference between CTL and UVB, or UVB siNT and UVB siATF6α, or UVB IgG control and UVB IL8 Ab.

Discussion

To date, studies have established the involvement of UPR in various models of senescence, with specific branches being activated depending on the cell type and the senescence inducer (reviewed in; Abbadie and Pluquet 2020). We first highlighted in *ex vivo* samples that UPR-related proteins are overexpressed in dermal (photo)aging. More specifically, through a combination of *in vitro* studies on skin cells and an experimental model of reconstructed human epidermis, we have uncovered the regulatory role of the ATF6α branch in the onset of UVB-induced senescence and highlighted its potential effect in maintaining epidermal homeostasis under stress. The silencing of ATF6α prevented a complete establishment of UVB-induced senescent phenotype in NHDFs, evidenced by reduced persistent DNA damage, prevention of increased SA-βgal positive cells, morphological changes, and selective alteration of the SASP. Interestingly, several studies have shown that the SASP can spread to neighboring normal cells and impair their function (reviewed in; Gonzalez-Meljem et al. 2018). However, these studies often focused on the effect of senescent cells in the tumor microenvironment, promoting the growth and proliferation of tumor cells, but did not address the intercommunication of senescent cells in a physiological context. Here, we identified an unexpected consequence of conditioned media from UVB-induced senescent fibroblasts in causing keratinocyte hyperproliferation in an ATF6α/IL8-dependent mechanism. However, we cannot exclude the potential participation of other SASP factors regulated by ATF6α in these processes, which remains to be investigated.

The buildup of p16-positive senescent cells in the skin has been associated with donor age (Ogata et al. 2023). Nonetheless, the exact origin of cellular senescence in the skin remains elusive, with multiple potential sources being considered, such as sun exposure (Toutfaire, Bauwens, and Debacq-Chainiaux 2017). Photoaging denotes skin aging induced by ultraviolet radiation, recognized as the most extensively researched type of extrinsic skin aging (Krutmann et al. 2021). *In vitro*, cell exposure to UV radiation has been linked to the activation of adaptative stress responses, with cellular senescence being among the most extensively studied (Moon et al. 2019). Additionally, chronic UV exposure can induce other biological responses such as endoplasmic reticulum (ER) stress and activation of the unfolded protein response (UPR) (Komori et al. 2012; Karagöz, Acosta-Alvear, and Walter 2019). However, the *in vivo* contribution of the UPR in skin aging remains poorly studied. We found that UPR markers including HERPUD1 and XBP1s are detected in the dermis and increased with age. Similar results were observed in aged sun-exposed dermis compared to young sun-exposed counterparts. This indicates that dermal fibroblasts elicit UPR during skin aging, but this seems to be tissue-specific, since a decrease in UPR activation has also been reported in other models of aging (Chalil et al. 2015). This observation prompted us to investigate the role of

UPR branches in UVB-induced senescence in dermal fibroblasts and the molecular mechanisms contributing to age-related changes in the skin. Previous studies have demonstrated that UV-induced senescent human dermal fibroblasts display several common biomarkers of senescence (Mark Berneburg et al. 2004; Herrmann et al. 1998; Debacq-Chainiaux et al. 2005). In our study, we used primary dermal fibroblasts, necessitating adjustments in the UVB dose compared to the original model related to normal human fetal dermal fibroblasts. This adaptation enabled the induction of a senescent phenotype, characterized by SA-βgal positive cells, enlarged morphology, cell cycle arrest, persistent DNA damage, and establishment of the SASP in NHDFs. Interestingly, ER stress is now admitted as one of the signalling pathways connected to senescence (reviewed in; Pluquet, Pourtier, and Abbadie 2015; Hernandez-Segura, Nehme, and Demaria 2018). Indeed, we and others have shown the involvement of ER stress and UPR activation in replicative and OIS-induced senescence models (Druelle et al. 2016; Drullion et al. 2018; Blazanin et al. 2017). In this study, we clearly showed that UVB-induced senescent fibroblasts are associated with ER stress and UPR activation. Additionally, we demonstrated that silencing the UPR before repeated exposures to UVB altered the complete establishment of the senescent phenotype. We found that ATF6α invalidation, more than PERK and IRE1α invalidation, can prevent the establishment of most UVB-induced senescent biomarkers. This observation reinforces the suggested role of ATF6α as a key player in various types of senescence (Druelle et al. 2016; Kim et al. 2019). However, the importance of PERK and IRE1α should not be overlooked, as we observed that invalidation of PERK prevents the increased proportion of SA-βgal positive cells, while invalidation of IRE1α prevents the overexpression of *IL1β*, *IL8*, and *MMP3*. In comparison, we showed that ATF6α silencing impacts SA-βgal, morphology, SASP composition, and persistent DNA damage. Interestingly, it has been recently demonstrated in cancer cells that ATF6α can prevent DNA damage in response to the cytotoxic effects of ER stressors (Benedetti et al. 2022). This effect involves the stabilization of BRCA-1 through the activation of mTOR signalling by ATF6α. Several groups already reported a link between ATF6α and activation of mTOR signalling in different biological contexts (Blackwood et al. 2019; Allen and Seo 2018a; Schewe and Aguirre-Ghiso 2008), and others described mTOR as necessary for SASP control and particularly for IL6 and IL8 expression (R. M. Laberge et al. 2015; Herranz et al. 2015a). These results supported the possibility that in our model the ATF6α-dependent SASP composition may involve mTOR. However, we cannot rule out the implication of other pathways involved in the regulation of inflammatory SASP factors, such as the DDR or cGAS/STING, as none of these hypotheses have been investigated in this study (Bolland et al. 2021; Rösing et al. 2024).

To study the functional consequences of paracrine factors regulated by ATF6α, we used reconstructed human epidermis composed of normal human keratinocytes to partly mimic the human epidermal environment (Aurélie Frankart et al. 2012). We observed that conditioned media from UVB-induced fibroblasts thickened the epidermis without altering the expression of differentiation markers in RHE. Interestingly, this thickening resembles the phenotype induced directly by keratinocytes injury after UVB exposure (Scott et al. 2012), suggesting that paracrine factors released by UVB-induced fibroblasts may partially overlap some effects of direct UVB exposure on keratinocytes. Surprisingly, we highlighted that this process involves paracrine factors under the control of ATF6α. We identified IL8 as a factor influencing keratinocyte proliferation. Remarkably, IL8-neutralizing antibody added to conditioned media from UVB-induced fibroblasts was able to prevent the increased number of keratinocyte clones. This experiment revealed that IL8 selectively promotes keratinocyte proliferation, in addition to its known chemotactic properties. Furthermore, the addition of exogenous IL8 in an organotypic culture of keratinocytes has been shown to result in marked hyperproliferation (Steude, Kulke, and Christophers 2002). Thus, these results connect the ATF6α/IL8 axis to pro-proliferative phenotypes, shedding light on ATF6α's role as a potential regulator of a key SASP component. Nevertheless, the mechanistically regulatory relationship between ATF6α and IL8 remains unclear. The SASP is known to be dependent on the senescence inducer and the cell type (Basisty et al. 2020; Ito, Hoare, and Narita 2017), and varies over time (Schmitt 2016). Therefore, SASP can modulate the fate of normal neighboring cells in several ways. For example, the early secretion of PDGF-AA SASP factor by senescent cells affects myofibroblasts differentiation (Demaria et al. 2014) whereas long-term exposure of primary keratinocytes to SASP of senescent cells promotes senescence and reduces regenerative capacities (Ritschka et al. 2017). This aligns well with our findings, suggesting that the transient presence of SASP from senescent fibroblasts may initially stimulate processes such as keratinocyte proliferation, before potentially leading to the establishment of a chronic inflammatory environment. Indeed, several skin-related diseases have been linked to keratinocytes hyperproliferation, such as psoriasis, atopic dermatitis (Niehues et al. 2022), and in severe cases, actinic keratoses (AK). Actinic keratoses are precancerous skin lesions arising from the excessive proliferation of transformed neoplasic keratinocytes in the epidermis, caused by solar UV radiation. Common among the elderly population, actinic keratoses present an elevated risk of developing squamous cell carcinoma (SCC) (Berman, Cockerell, and Zografos 2013). In our model, normal keratinocytes were not directly exposed to UVB, but were grown with conditioned media of UVB-induced senescent fibroblasts. This suggests that, aside from direct acute physical stressors, long-term alterations in the cellular microenvironment could also have additional effects on tissue homeostasis.

Targeting senescent cells holds great promise. Indeed, emerging senotherapeutic strategies fall into two categories: senolytics, which specifically kill senescent cells, and senomorphics, which suppress certain properties of senescent cells, primarily SASP factors. Several studies reported senomorphic properties of plant extracts exerting senomorphic properties on senescent dermal fibroblasts, epidermal keratinocytes, and melanocytes (reviewed in; Csekes and Račková 2021). Nevertheless, very few studies investigated the senotherapeutic approach in skin aging and age-related disorders. Azameh *et al*., showed that a prolonged expression of $p16^{INK4A}$ in mouse epidermis leads to hyperplasia and dysplasia, and that senolytic elimination of p16-positive cells suppresses hyperplasia (Azazmeh et al. 2020). In this respect, further experiments are required to determine whether senotherapy could revert skin aging. In conclusion, this work has identified the ATF6α/IL8 axis in UVB-induced senescent fibroblasts as a possible mechanism controlling paracrine proliferation in surrounding normal keratinocytes. Understanding the regulation of SASP and developing strategies to counteract its effects on skin aging and age-related skin disorders is imperative for future research. Finally, manipulating the ATF6α pathway may offer potential therapeutic opportunities to target senescent cells by modulating SASP composition.

Materials and methods

Immunofluorescence on human dermis samples

Human skin samples were obtained from the SkinAge Project (SKINAGE: NCT02553954) at Oscar Lambret Centre (Lille, France), Department of Anatomopathology, in compliance with French regulations. Skin punch biopsies of 5 mm were collected from both healthy young (aged 19-39 years) and older (aged 58-82 years) volunteers. The study was approved by local (Le Comité de Protection des Personnes Nord-Ouest) and national (Agence nationale de sécurité du médicament et des produits de santé) ethics committees. Participants provided informed written consent prior to inclusion in this study. Biopsies were embedded with optimal-cutting temperature into plastic cryomolds before freezing. After frozen sectioning (6 µm thick) on a microtome-cryostat, sections were mounted onto slides for analysis. Sections were fixed in paraformaldehyde 4% for 10 minutes and washed in PBS. Nonspecific binding was blocked by incubation in 5% BSA/PBS. The primary antibody was incubated overnight at 4 °C. The used antibodies were anti-vimentin (AF2105, 1:100, R&D Systems, Minneapolis, MN), and anti-XBP1s (2G4-3 E11-3 E9, 1:50) kindly provided by Eric Chevet (Inserm U1242, University of Rennes, France), or anti-PDI (Ab2792, 1:100, Abcam, Cambridge, United Kingdom), or anti-herpud1 (H00009709-M04, 1:300, Abnova, Taipei, Taiwan), or anti-53BP1 (NB100-304, 1:400, Novus Biologicals, Englewood, CO, USA). After washes in PBS, tissue sections were

incubated with Alexa Fluor 555 anti-IgG Mouse (A21422, 1:1000 Invitrogen, Waltham, MA), or Alexa Fluor 568 anti-IgG Rabbit (A10042, 1:1000, Invitrogen), or Alexa Fluor 488 anti-IgG Goat (A11055, 1:1000, Invitrogen) for 1 hour at room temperature. For double immunofluorescence, the two primary and two secondary antibodies were co-incubated. Finally, tissue sections were washed in PBS, and nuclei were stained for 5 minutes with DAPI (D9542, Sigma-Aldrich, St Louis, MO, USA) at 2 µg/ml and mounted using Dako Glycergel Mounting Medium (C0563, Dako, Santa Clara, CA, USA). Images were acquired on confocal Zeiss (LSM880 Airyscan, Oberkochen, Germany).

Isolation of Primary Dermal Fibroblasts

Normal human dermal fibroblasts (NHDFs) were isolated from the foreskins of young and healthy donors (aged < 10 years) as previously described (Tigges et al. 2013). Skin samples were obtained from Clinique St-Luc (Dr. L. de Visscher, Bouge, Belgium) following approval of the Medical Ethical Committee of the Clinique St-Luc and according to the principles set out in the Declaration of Helsinki.

Cell Cultures

NHDFs and normal human fetal dermal fibroblasts AG04431 (Coriell Institute for Medical Research) were cultured in Basal Medium Eagle (BME, 41010026, Gibco) supplemented with 10% Fetal Bovine Serum (FBS, Corning), 2 mM L-glutamine (Thermo Fisher Scientific, Waltham, MA, USA), and penicillin-streptomycin (50 U/mL).

Human Epidermal Keratinocytes neonatal, HEKn (Thermo Fisher Scientific, C0015C) used for reconstructed human epidermises were grown in a specific keratinocyte serum-free medium, completed with bovine pituitary extract, epidermal growth factor, insulin, hydrocortisone, transferrin, calcium, penicillin and streptomycin (KGM-2 BulletKit, CC-3107, Lonza, Basel, Switzerland).
Normal human epidermal keratinocytes (NHEKs, 00192627, Lonza) used for clonogenic assays were grown in KGM™-Gold Keratinocyte Basal Medium (KGM™-Gold Keratinocyte Basal Medium BulletKit™, 192060, containing basal medium and supplements)

UVB-induced senescence, RNA interference and UPR inhibition

NHDFs at their exponential growth phase were seeded at 4,000 cells/cm^2 (CTL) or 8,000 cells/cm^2 (UVB) (or at 8,000 cells/cm^2 for AG004431) in BME with 1 % FBS, 2 mM L-glutamine, and penicillin-streptomycin (50 U/mL). At day three after seeding, cells were exposed to NB-UVB (312 nm) (TL20W/01, Philips) at 500 mJ/cm^2 two times a day for five days (or at 250 mJ/cm^2 for AG004431 cells, as previously described in Debacq-Chainiaux 2005).

For ATF6α, PERK, or IRE1α knockdown, NHDFs were transfected at 48 hours after seeding with specific siGENOME SMARTpool (Dharmacon, Lafayette, CO), siATF6α (GAACAGGGCUCAAAUUCUC, AACCAAAUCUGUACAGUUA, UCACACAGCUCCCUAAUCA, GAACAGGAUUCCAGGAGAA), siPERK (CCAAGAUGCUGGAGAGAUU, GGAAGUACCAGCACAGUGA, AGAACAAGCUCAACUACUU, CCCAAAAGCCUUACGGUCA), or siIRE1α (CCAAGAUGCUGGAGAGAUU, GGAAGUACCAGCACAGUGA, AGAACAAGCUCAACUACUU, CCCAAAAGCCUUACGGUCA) at 25 nM using DharmaFECT 1 (Dharmacon) transfection reagent according to the manufacturer's instructions. A siGENOME non-targeting control siRNA pool (D-001206-13, Dharmacon), siNT (UAAGGCUAUGAAGAGAUAC, AUGUAUUGGCCUGUAUUAG, AUGAACGUGAAUUGCUCAA, UGGUUUACAUGUCGACUAA) was used as control. The following day, NHDFs were exposed to UVB as described above.

For ATF6α, PERK, or IRE1α inhibition, AG04431 cells were treated directly after the last UVB exposure for up to 72 hours with respectively Ceapin-A7 (CA7, 10 µM, SML2330, Sigma-Aldrich), GSK2606414 (GSK, 10 µM, 516535, Sigma-Aldrich), 4µ8c (4µ8c, 100 µM, SML0949, Sigma-Aldrich), or with DMSO (A994.1, Carl Roth, Karlsruhe, Germany) as control.

SA-βgal staining

Three days after the last UVB exposure, cells were detached and seeded at a density of 15,000 cells per well in technical triplicates within 6-well plates. The next day, cytochemical detection of SA-βgal activity was carried out as previously described (Debacq-Chainiaux et al. 2009).

EdU staining

Three days after the last UVB exposure, cells were detached and seeded onto glass coverslips. The next day, cells were incubated with 10 µM EdU for 16 hours before being fixed with 4% paraformaldehyde (Merck, Rahway, NJ). EdU detection was performed using the Click-iT EdU Cell Proliferation Kit (BCK-EDU488, Sigma-Aldrich) according to the manufacturer's instructions. Cell nuclei were counterstained with TO-PRO-3 (T3605, 1:80 in RNAse 2 mg/mL, Thermo Fisher Scientific). Images were acquired on Leica Microsystems (TCS SP5, Leica, Wetzlar, Germany).

Immunofluorescence of cultured cells

Three days after the last UVB exposure, cells were detached and seeded onto glass coverslips. The next day, cells were fixed with 4 % paraformaldehyde (Merck) and permeabilized using 1 % Triton X-100 in PBS (Sigma-Aldrich).

For the detection of 53BP1, the primary antibody anti-53BP1 (N100-305, 1:2000, Novus) was incubated overnight at 4 °C using 3 % BSA in PBS, followed by secondary antibody Alexa Fluor 488 anti-IgG Rabbit (A11011, Invitrogen) for 1 hour at room temperature, then nuclei were counterstained with TO-PRO-3 (T3605, 1:80 in PBS-RNAse 2 mg/mL, Thermo Fisher Scientific).
For the detection of actin, the phalloidin probe (A12380, 1:100, Thermo Fisher Scientific) was incubated overnight at 4 °C using 3 % BSA in PBS, followed by nuclei counterstaining with DAPI (D9542, in PBS 1µg/mL, Sigma Aldrich). Images were acquired with a Broadband Confocal TCS SP5 (Leica).

RNA isolation and RT-qPCR

Total RNA was isolated from cultured cells using the ReliaPrep™ RNA Tissue Miniprep System (Z6111, Promega) and reverse transcribed using GoScript Reverse Transcriptase Kit (A2791, Promega) in a final volume of 20 µL following to manufacturer's instructions. Quantitative real-time polymerase chain reaction (PCR) was performed using GoTaq qPCR Mix (Promega), primers (sequences are provided in Table 1), and the Viaa7™ instrument (Applied Biosystems). Relative mRNA level was determined using the ΔΔCt method normalized to the mRNA abundance of *RPL13A* and expressed relative to the stated control (Schmittgen and Livak 2008).

Protein extraction and western blot

Total cell protein extracts were harvested using homemade lysis buffer (40 mM Tris; pH 7.5, 150 mM KCl, 1 mM EDTA, and 1% Triton X-100) supplemented with a protease inhibitor cocktail (Roche). Protein extracts were then incubated on a rotating wheel for 30 minutes at 4°C before centrifugation for 10 minutes at 16,000 *g*, at 4°C, to collect supernatants. Protein concentration was determined with Pierce 660 nm Protein Assay Reagent (22660, Thermo Fisher Scientific), complemented with Ionic Detergent Compatibility Reagent (IDC) according to the manufacturer's recommendations. For GRP78/BiP, PERK, and IRE1α blots, equal protein concentration (5-10 µg) was loaded onto SDS-PAGE homemade polyacrylamide gels and then transferred to PolyVinyliDene Fluoride (PVDF) membrane (Immobilon-P, Merck Millipore, Burlington, MA, USA). Membranes were blocked for 1 hour at room temperature in blocking solution Intercept Blocking Buffer (TBS-T, LI-COR Biosciences, Lincoln, NE, USA) and then incubated with primary antibodies overnight at 4°C diluted in TBS supplemented with 0,1% Tween-20 (Carl Roth). The used antibodies were, anti-PERK (3192, 1:1000, Cell Signalling, Danvers, MA, USA), anti-IRE1α (3294, 1:1000, Cell signalling), anti-GRP78 (3177, 1:1000, Cell Signalling), anti-GAPDH (G8795, 1:1000, Sigma-Aldrich or Ab128915, 1:1000, Abcam). After washes in homemade TBS, membranes were incubated with anti-IgG rabbit

goat polyclonal antibody coupled with IRDye 800CW (926-322111, LI-COR Biosciences) or with anti-IgG mouse goat polyclonal antibody coupled with IRDye 680RD (926-68070, LI-COR Biosciences) 1 hour at room temperature in TBS-T supplemented with 0,1% Tween-20 (9127.1, Carl Roth) and 0,01% SDS (A3942, Carl Roth). Membranes were finally dried for 1 hour at 37°C and scanned using Amersham Typhoon™.

For ATF6α blot, 4 µg of proteins were loaded onto SDS-PAGE homemade polyacrylamide gels and then transferred to nitrocellulose membrane (88018, Thermo Fisher Scientific). The membrane was blocked for 90 min using 5% non-fat milk in PBS and then incubated with anti-ATF6α (Ab122897, 1:1000, Abcam) overnight at 4°C in the blocking solution. After washes in PBS supplemented with 0,1 % Tween-20 (Carl Roth), the membrane was incubated with anti-IgG mouse goat polyclonal antibody coupled with HRP (115-035-146, 1:10,000, Jackson ImmunoResearch, West Grove, PA, USA) during 45 minutes at room temperature in the blocking solution. ATF6α blot was revealed using the SuperSignal™ West Femto Maximum Sensitivity Substrate (34096, Thermo Fisher Scientific) and scanned using Bio-Rad ChemiDoc™ XRS+ (Bio-Rad, Hercules, CA, USA). GAPDH was used as loading control (Ab128915, 1:1000, Abcam) and revealed using Amersham Typhoon™.

Conditioned media (CM)

At 48 hours after the last UVB exposure, NHDFs were washed three times with PBS and the medium was changed to serum-free medium. Cells were cultured for an additional 16 hours, and conditioned media were centrifuged at 200 *g*, at 4°C for 5 minutes to remove cellular debris, then, media supernatant was filtered using a 0.2 µm filter. Conditioned media were then aliquoted and frozen at -20°C until use.

Reconstructed Human Epidermis (RHE)

RHE were produced following the protocol described by (Aurélie Frankart et al. 2012). Briefly, HEKs at early passage were thawed in complete KGM-2 medium. The following day, the medium was replaced by complete Epilife medium (Gibco, MEPI500CA). When keratinocytes reached 70-80% of confluency, HEKs were seeded on a polycarbonate filter (Millipore, PIHP01250) in complete EpiLife medium supplemented with 1.5 mM Ca^{2+}, at a density of 250,000 cells/cm^2. The next day, keratinocytes were exposed to the air-liquid interface for 11 days, and the medium under the polycarbonate filter was replaced by complete EpiLife medium supplemented with 1.5 mM Ca^{2+}, 10 ng/mL keratinocyte growth factors (KGF, R&D systems), and 50 µg/mL vitamin C.

At 4 days after exposure to the air-liquid interface, the complete Epilife medium under the polycarbonate filter was mixed with CM from NHDFs exposed or not (CTL) to UVB and

transfected with siNT or siATF6α. CM was normalized to 95,000 cells/mL using serum-free BME and then diluted to a 50%-50% ratio with complete Epilife medium supplemented with 1.5 mM Ca^{2+}, 10 ng/mL keratinocyte growth factors (KGF, R&D systems), and 50 µg/mL vitamin C. The medium was changed every two days. At day 10, bromodeoxyuridine (BrdU, 10 µM, b9285, Sigma) was added to the mixed medium. The reconstruction was stopped on day 11.

RHE were fixed after 11 days of reconstruction in 4% formaldehyde for at least 24 hours. The following day, they were dehydrated in methanol and incubated in toluene to facilitate the detachment of the polycarbonate filter from the insert before embedding in paraffin. Tissue sections (6 µm thick) were prepared perpendicular to the filter using a microtome and mounted onto slides for analysis. Paraffin-embedded tissues were stained using hematoxylin-eosin.
For immunofluorescence, non-specific binding was blocked by incubating slides in PBS containing 0.2% BSA and 0.02% Triton X-100 for 1 hour followed by incubation for 1 hour at room temperature with the primary antibody. The used antibodies were anti-keratin 10 (Ab9026, 1:100, Abcam), anti-involucrin (I9018, 1:200, Sigma-Aldrich), or anti-loricrin (Ab176322, 1:100, Abcam). After washes in blocking solution, tissue sections were incubated with Alexa Fluor 568 anti-IgG mouse (A10037, 1:1000, Cell signalling), or Alexa Fluor 488 anti-IgG rabbit (A11001, 1:200, Invitrogen) for 1 hour at room temperature, then nuclei were counterstained with DAPI for 10 minutes (D9542, Sigma) at 2 µg/ml.
Visualization of $BrdU^+$ cells was performed on tissue sections deparaffinized and incubated for 30 minutes in 10 mM citrate buffer pH 6.0 at 95°C. DNA was denatured by incubation of section for 30 minutes with 2N HCl at 37°C and then neutralized using 0,1 M borax pH 8.5. Anti-BrdU (347580, 1:50, BD Biosciences, Franklin Lakes, NJ, USA) was incubated in PBS containing 0.2% BSA for 1 hour at room temperature. After washes in PBS, sections were incubated with Alexa Fluor 488 anti-IgG mouse (A11001, 1:1000, Invitrogen) for 1 hour at room temperature. All sections were mounted with coverslips using Dako Glycergel Mounting Medium (C0563, Dako) and images were acquired on a Celldiscoverer 7 microscope (Zeiss).

Clonogenic assay and treatment with neutralizing antibody

NHEKs at early passage were seeded at a density of 400 cells/cm² per well in 24-well plates. The next day, culture media was replaced by mixed with CM from NHDFs exposed or not (CTL) to UVB and transfected or not (UN) with siNT or siATF6α. CM was normalized to 5,000 cells/mL using serum-free BME and then diluted to a 70%-30% ratio with complete KGM-2 medium. For neutralization experiments, 0.1 µg/mL of anti-IL8 (AF-208, R&D Sytems) or control IgG (31245, Invitrogen) at the same concentration was added as the instructor suggested a neutralization dose (ND50) of 0.1-0.5 µg/mL in the presence of 20 ng/mL

recombinant human IL8. The media was changed daily for three days. 48 hours after the last change media colonies were fixed and colored with crystal violet. Images of each well were acquired using the IncuCyte® S3 Live-Cell Analysis System (Sartorius, Göttingen, Germany).

Transcriptomic analysis of fibroblasts

Total RNA was isolated from NHDFs exposed or not (CTL) to UVB (n=3) using the same procedure as above. RNA quality was assessed using a NanoDrop 2000 (Thermo Fisher Scientific). RNA integrity (RIN) was evaluated with an Agilent 2100 Bioanalyzer (G2938B, Agilent, Santa Clara, CA, USA). RNA-seq was performed by the Giga's Genomics platform (GIGA Genomics, Liège, Belgium) using the Illumina RNA-Seq workflow. In brief, mRNA sequencing libraries were generated using the Illumina mRNA Stranded Ligation Kit (Illumina, San Diego, CA), following the manufacturer's instructions. A NovaSeq6000 sequencer was used to generate 40 million PE reads of 2X150 base pair per sample. Differential Expression Analysis of the UVB vs. CTL dataset was performed using EdgeR R-package (Robinson, McCarthy, and Smyth 2010) with applied statistical cutoffs (FDR<0.05, log2(FoldChange)±log2(1.5)). Gene Ontology analysis, based on the DEGs list, was first conducted with a published gene set or retrieved gene lists from publicly available databases, such as MSigDB (Liberzon et al. 2011) and GeneCards (Stelzer et al. 2016) (www.genecards.org), respectively. Further Gene Set Enrichment Analysis (GSEA) and Over-representation analyses (ORA) were achieved with "fgsea" (Korotkevich et al. 2021) and "ClusterProfiler" (T. Wu et al. 2021) R-packages, by querying Reactome Pathway (Fabregat et al. 2018), MSigDB, KEGG, and DOSE (Yu et al. 2015) databases. In our analysis, a very small gene set (n<9) has not been considered. The significance of GO-terms or enriched pathways was determined according to the statistical cut-offs from p-adjusted value (padj<0.05) and Normalized Enrichment Score (NES±1.5) and then plotted using R.

Quantifications

All images were processed using Fiji software (ImajeJ2).

For the quantification of the proportion of SA-βgal$^+$ cells, it was determined by counting a total of at least 300 cells per condition.

For the quantification of the proportion of EdU$^+$ cells, it was determined by counting a total of at least 200 cells per condition.

For the quantification of actin, micrographies were processed using a generalist algorithm for cellular segmentation named Cellpose. Predictive masks were further analyzed using Fiji to assess the morphology of fibroblasts, employing Fiji shape descriptors such as cell circularity (4π*area/perimeter^2), or cell roundness (4*area/(π*major_axis^2).

For quantification on RHE, the epidermal thickness was measured in BrightField micrographies, by determining the distance between the top of the polycarbonate filter and the bottom of the cornified layer. Fifteen measurements were performed throughout the tissue section for each RHE. $BrdU^+$ keratinocytes in RHE were calculated as (number of $BrdU^+$ cells/ total number of cells in basal layer) *100 for each condition, as described in (Aurélie Frankart et al. 2012).
For quantification of colony formation, pictures of each well were obtained using the Incucyte imaging system. The number of colonies (containing more than 20 cells) was counted.

Data plotting and statistics

Statistical analyses were conducted using GraphPad Prism (GraphPad Software, La Jolla, CA, USA). Data used for statistics were obtained from at least three independent biological experiments. Results obtained from skin biopsies samples are expressed as mean ± SEM, while results obtained from cells and RHE are expressed as mean ± SD. All the statistical tests applied are specified in the figures' legend. Differences with a significance level of at least $P<0.05$ were considered statistically significant.

Author contributions

Joëlle Giroud: Conceptualization; Methodology; Validation; Formal analysis; Investigation; Writing – Original Draft; Writing- Review & Editing; Visualization. **Pauline Delvaux:** Validation; Investigation. **Clémentine De Schutter:** Investigation. **Nathalie Martin:** Methodology; Investigation; Writing- Review & Editing. **Raphaël Rouget:** Software; Data Curation; Visualization; Writing- Review & Editing. **Ayeh Boulouki:** Software; Data Curation; Resources; Writing- Review & Editing. **Valérie De Glas**: Investigation; **Florent Bourdoux**: Investigation. **Sophie Burteau:** Validation. **Julien Théry**: Resources. **Gauthier Decanter:** Resources. **Nicolas Penel:** Resources. **Corinne Abbadie:** Writing- Review & Editing. **Yves Poumay:** Resources; Writing- Review & Editing. **Olivier Pluquet:** Conceptualization; Methodology; Writing – Original Draft; Writing- Review & Editing; Visualization; Supervision; Project administration; Funding acquisition. **Florence Debacq-Chainiaux:** Conceptualization; Methodology; Resources; Writing – Original Draft; Writing- Review & Editing; Visualization; Supervision; Project administration; Funding acquisition.

Disclosure and competing interest statement

The authors declare that they have no conflict of interest.

Acknowledgments

The authors thank Dr. Luc de Visscher for providing skin samples (Clinique Saint Luc Bouge, Bouge, Belgium), Wouter Coppieters and the Giga-Genomics platform for their assistance with the RNA-sequencing data, and the light microscopy team from MORPH-IM platform (UNamur). This work was supported by the Centre National de la Recherche Scientifique, the Institut National de la Santé et de la Recherche Médicale, the Université de Lille, the Ligue contre le Cancer (Comité du Nord), the Institut Pasteur de Lille, the Contrat Plan État Région—CTRL (Centre Transdisciplinaire de Recherche sur la Longévité) and the Contrat de Plan Etat Région CPER Cancer 2015–2020 to Olivier Pluquet.

Joëlle Giroud is supported by a co-funded FSR fellowship (UNamur) and doctoral fellowship (Univ Lille).

Florence Debacq-Chainiaux is a research associate at Fonds de la Recherche Scientifique-FNRS

References

Abbadie, Corinne, and Olivier Pluquet. 2020. “Unfolded Protein Response (UPR) Controls Major Senescence Hallmarks.” Trends in Biochemical Sciences 45 (5): 371–74. https://doi.org/10.1016/J.TIBS.2020.02.005.

Acosta, Juan C., Ana O’Loghlen, Ana Banito, Maria V. Guijarro, Arnaud Augert, Selina Raguz, Marzia Fumagalli, et al. 2008. “Chemokine Signalling via the CXCR2 Receptor Reinforces Senescence.” Cell 133 (6): 1006–18. https://doi.org/10.1016/J.CELL.2008.03.038.

Acosta, Juan Carlos, Ana Banito, Torsten Wuestefeld, Athena Georgilis, Peggy Janich, Jennifer P. Morton, Dimitris Athineos, et al. 2013. “A Complex Secretory Program Orchestrated by the Inflammasome Controls Paracrine Senescence.” Nature Cell Biology 15 (8): 978. https://doi.org/10.1038/NCB2784.

Allen, Dylan, and Jin Seo. 2018. “ER Stress Activates the TOR Pathway through Atf6.” Journal of Molecular Signalling 13: 1–9. https://doi.org/10.5334/1750-2187-13-1.

Azazmeh, Narmen, Benjamin Assouline, Eitan Winter, Shmuel Ruppo, Yuval Nevo, Alexander Maly, Karen Meir, et al. 2020. “Chronic Expression of P16INK4a in the Epidermis Induces Wnt-Mediated Hyperplasia and Promotes Tumor Initiation.” Nature Communications 11 (1): 1–13. https://doi.org/10.1038/s41467-020-16475-3.

Basisty, Nathan, Abhijit Kale, Ok Hee Jeon, Chisaka Kuehnemann, Therese Payne, Chirag Rao, Anja Holtz, et al. 2020. “A Proteomic Atlas of Senescence-Associated Secretomes for Aging Biomarker Development.” PLoS Biology 18 (1): e3000599. https://doi.org/10.1371/JOURNAL.PBIO.3000599.

Bauwens, Emilie, Tom Parée, Sébastien Meurant, Inès Bouriez, Clotilde Hannart, Anne-Catherine Wéra, Alexis Khelfi, et al. 2023. “Senescence Induced by UVB in Keratinocytes Impairs Amino Acids Balance.” The Journal of Investigative Dermatology 143 (4): 554-565.e9. https://doi.org/10.1016/j.jid.2022.11.017.

Benedetti, Rossella, Maria Anele Romeo, Andrea Arena, Maria Saveria, Gilardini Montani, Livia Di Renzo, Gabriella D'orazi, and Mara Cirone. 2022. "ATF6 Prevents DNA Damage and Cell Death in Colon Cancer Cells Undergoing ER Stress." Cell Death Discovery 2022 8:1 8 (1): 1–12. https://doi.org/10.1038/s41420-022-01085-3.

Berman, Brian, Clay J. Cockerell, and Panagiotis Zografos. 2013. "Pathobiology of Actinic Keratosis: Ultraviolet-Dependent Keratinocyte Proliferation." Journal of the American Academy of Dermatology 68 (1 SUPPL.1): S10–19. https://doi.org/10.1016/j.jaad.2012.09.053.

Berneburg, Mark, Heidi Plettenberg, Kathrin Medve-König, Annette Pfahlberg, H Gers-Barlag, Olaf Gefeller, and Jean Krutmann. 2004. "Induction of the Photoaging-Associated Mitochondrial Common Deletion in Vivo in Normal Human Skin." The Journal of Investigative Dermatology 122 (5): 1277–83. https://doi.org/10.1111/j.0022-202X.2004.22502.x.

Blackwood, Erik A., Khalid Azizi, Donna J. Thuerauf, Ryan J. Paxman, Lars Plate, Jeffery W. Kelly, R. Luke Wiseman, and Christopher C. Glembotski. 2019. "Pharmacologic ATF6 Activation Confers Global Protection in Widespread Disease Models by Reprograming Cellular Proteostasis." Nature Communications 10 (1): 1–16. https://doi.org/10.1038/s41467-018-08129-2.

Blazanin, Nicholas, Jeongin Son, Alayna B. Craig-Lucas, Christian L. John, Kyle J. Breech, Michael A. Podolsky, and Adam B. Glick. 2017. "ER Stress and Distinct Outputs of the IRE1α RNase Control Proliferation and Senescence in Response to Oncogenic Ras." Proceedings of the National Academy of Sciences 114 (37): 9900–9905. https://doi.org/10.1073/PNAS.1701757114.

Bolland, Hannah, Tiffany S. Ma, Syafiq Ramlee, Kristijan Ramadan, and Ester M. Hammond. 2021. "Links between the Unfolded Protein Response and the DNA Damage Response in Hypoxia: A Systematic Review." Biochemical Society Transactions 49 (3): 1251–63. https://doi.org/10.1042/BST20200861.

Chalil, Sreeda, Nicolas Pierre, Astrid D. Bakker, Ralph J. Manders, Annelies Pletsers, Marc Francaux, Jenneke Klein-Nulend, Richard T. Jaspers, and Louise Deldicque. 2015. "Aging Related ER Stress Is Not Responsible for Anabolic Resistance in Mouse Skeletal Muscle." Biochemical and Biophysical Research Communications 468 (4): 702–7. https://doi.org/10.1016/j.bbrc.2015.11.019.

Childs, Bennett G., Martina Gluscevic, Darren J. Baker, Remi Martin Laberge, Dan Marquess, Jamie Dananberg, and Jan M. Van Deursen. 2017. "Senescent Cells: An Emerging Target for Diseases of Ageing." Nature Reviews Drug Discovery 2017 16:10 16 (10): 718–35. https://doi.org/10.1038/nrd.2017.116.

Coppé, Jean Philippe, Pierre Yves Desprez, Ana Krtolica, and Judith Campisi. 2010. "The Senescence-Associated Secretory Phenotype: The Dark Side of Tumor Suppression." Annual Review of Pathology 5 (February): 99. https://doi.org/10.1146/ANNUREV-PATHOL-121808-102144.

Csekes, Erika, and Lucia Račková. 2021. "Csekes, E.; Racková, L. Skin Aging, Cellular Senescence and Natural Polyphenols. Int. J. Mol. Sci. 2021, 22, 12641. Https://Doi.Org/ 10.3390/Ijms222312641." International Journal of Molecular Sciences 22 (23).

D'Orazio, John, Stuart Jarrett, Alexandra Amaro-Ortiz, and Timothy Scott. 2013. "UV Radiation and the Skin." International Journal of Molecular Sciences 14 (6): 12222–48. https://doi.org/10.3390/ijms140612222.

Debacq-Chainiaux, Florence, Céline Borlon, Thierry Pascal, Véronique Royer, François Eliaers, Noëlle Ninane, Géraldine Carrard, et al. 2005. "Repeated Exposure of Human Skin Fibroblasts to UVB at Subcytotoxic Level Triggers Premature Senescence through the TGF-B1 Signalling Pathway." Journal of Cell Science 118 (4): 743–58. https://doi.org/10.1242/JCS.01651.

Debacq-Chainiaux, Florence, Jorge D Erusalimsky, Judith Campisi, and Olivier Toussaint. 2009. “Protocols to Detect Senescence-Associated Beta-Galactosidase (SA-Bgal) Activity, a Biomarker of Senescent Cells in Culture and in Vivo.” Nature Protocols 2009 4:12 4 (12): 1798–1806. https://doi.org/10.1038/nprot.2009.191.

Debacq-Chainiaux, Florence, Cedric Leduc, Alix Verbeke, and Olivier Toussaint. 2012. “UV, Stress and Aging.”
Http://Www.Tandfonline.Com/Action/AuthorSubmission?JournalCode=kder20&page=instructions 4 (3): 236–40. https://doi.org/10.4161/DERM.23652.

Demaria, Marco, Naoko Ohtani, Sameh A. Youssef, Francis Rodier, Wendy Toussaint, James R. Mitchell, Remi Martin Laberge, et al. 2014. “An Essential Role for Senescent Cells in Optimal Wound Healing through Secretion of PDGF-AA.” Developmental Cell 31 (6): 722–33. https://doi.org/10.1016/j.devcel.2014.11.012.

Denoyelle, Christophe, George Abou-Rjaily, Vladimir Bezrookove, Monique Verhaegen, Timothy M. Johnson, Douglas R. Fullen, Jenny N. Pointer, et al. 2006. “Anti-Oncogenic Role of the Endoplasmic Reticulum Differentially Activated by Mutations in the MAPK Pathway.” Nature Cell Biology 8 (10): 1053–63. https://doi.org/10.1038/ncb1471.

Dimri, Goberdhan P, Xinhua Leet, George Basile, Meileen Acosta, Glynis Scorrt, Calvin Roskelley, Estela E Medrano, et al. 1995. “A Biomarker That Identifies Senescent Human Cells in Culture and in Aging Skin in Vivo (Replicative Senescence/Tumor Suppression/18-Galactosidase) Communicated by Arthur.” Cell Bioiogy 92: 9363–67.

Dörr, Jan R., Yong Yu, Maja Milanovic, Gregor Beuster, Christin Zasada, J. Henry M. Däbritz, Jan Lisec, et al. 2013. “Synthetic Lethal Metabolic Targeting of Cellular Senescence in Cancer Therapy.” Nature 2013 501:7467 501 (7467): 421–25. https://doi.org/10.1038/nature12437.

Druelle, Clémentine, Claire Drullion, Julie Deslé, Nathalie Martin, Laure Saas, Johanna Cormenier, Nicolas Malaquin, et al. 2016. “ATF6α Regulates Morphological Changes Associated with Senescence in Human Fibroblasts.” Oncotarget 7 (42): 67699. https://doi.org/10.18632/ONCOTARGET.11505.

Drullion, Claire, Guillemette Marot, Nathalie Martin, Julie Desle, Laure Saas, Clara Salazar-Cardozo, Fatima Bouali, Albin Pourtier, Corinne Abbadie, and Olivier Pluquet. 2018. “Pre-Malignant Transformation by Senescence Evasion Is Prevented by the PERK and ATF6alpha Branches of the Unfolded Protein Response.” Cancer Letters 438 (September): 187–96. https://doi.org/10.1016/j.canlet.2018.09.008.

Fabregat, Antonio, Steven Jupe, Lisa Matthews, Konstantinos Sidiropoulos, Marc Gillespie, Phani Garapati, Robin Haw, et al. 2018. “The Reactome Pathway Knowledgebase.” Nucleic Acids Research 46 (D1): D649–55. https://doi.org/10.1093/nar/gkx1132.

Frankart, Aurélie, Jérémy Malaisse, Evelyne De Vuyst, Frédéric Minner, Catherine Lambert de Rouvroit, and Yves Poumay. 2012. “Epidermal Morphogenesis during Progressive in Vitro 3D Reconstruction at the Air–Liquid Interface.” Experimental Dermatology 21 (11): 871–75. https://doi.org/https://doi.org/10.1111/exd.12020.

Giroud, Joëlle, Inès Bouriez, Hugo Paulus, Albin Pourtier, Florence Debacq-Chainiaux, and Olivier Pluquet. 2023. “Exploring the Communication of the SASP: Dynamic, Interactive, and Adaptive Effects on the Microenvironment.” International Journal of Molecular Sciences 24 (13): 10788. https://doi.org/10.3390/ijms241310788.

Gonzalez-Meljem, Jose Mario, John Richard Apps, Helen Christina Fraser, and Juan Pedro Martinez-Barbera. 2018. “Paracrine Roles of Cellular Senescence in Promoting Tumourigenesis.” British Journal of Cancer 118 (10): 1283–88. https://doi.org/10.1038/s41416-018-0066-1.

Gorgoulis, Vassilis, Peter D. Adams, Andrea Alimonti, Dorothy C. Bennett, Oliver Bischof, Cleo Bishop, Judith Campisi, et al. 2019. “Cellular Senescence: Defining a Path Forward.” Cell 179 (4): 813–27. https://doi.org/10.1016/j.cell.2019.10.005.

Hayflick, L., and P. S. Moorhead. 1961. “The Serial Cultivation of Human Diploid Cell Strains.” Experimental Cell Research 25 (3): 585–621. https://doi.org/10.1016/0014-4827(61)90192-6.

Hernandez-Segura, Alejandra, Tristan V. de Jong, Simon Melov, Victor Guryev, Judith Campisi, and Marco Demaria. 2017. “Unmasking Transcriptional Heterogeneity in Senescent Cells.” Current Biology : CB 27 (17): 2652. https://doi.org/10.1016/J.CUB.2017.07.033.

Hernandez-Segura, Alejandra, Jamil Nehme, and Marco Demaria. 2018. “Hallmarks of Cellular Senescence.” https://doi.org/10.1016/j.tcb.2018.02.001.

Herranz, Nicolás, Suchira Gallage, Massimiliano Mellone, Torsten Wuestefeld, Sabrina Klotz, Christopher J. Hanley, Selina Raguz, et al. 2015. “MTOR Regulates MAPKAPK2 Translation to Control the Senescence-Associated Secretory Phenotype.” Nature Cell Biology 17 (9): 1205–17. https://doi.org/10.1038/ncb3225.

Herrmann, Gernot, Peter Brenneisen, Meinhard Wlaschek, Jutta Wenk, Katrin Faisst, Gudrun Quel, Christina Hommel, et al. 1998. “Psoralen Photoactivation Promotes Morphological and Functional Changes in Fibroblasts in Vitro Reminiscent of Cellular Senescence.” Journal of Cell Science 111 (6): 759–67. https://doi.org/10.1242/jcs.111.6.759.

Ito, Yoko, Matthew Hoare, and Masashi Narita. 2017. “Spatial and Temporal Control of Senescence.” Trends in Cell Biology 27 (11): 820–32. https://doi.org/10.1016/j.tcb.2017.07.004.

Kammeyer, A., and R. M. Luiten. 2015. “Oxidation Events and Skin Aging.” Ageing Research Reviews 21: 16–29. https://doi.org/10.1016/j.arr.2015.01.001.

Karagöz, G. Elif, Diego Acosta-Alvear, and Peter Walter. 2019. “The Unfolded Protein Response: Detecting and Responding to Fluctuations in the Protein-Folding Capacity of the Endoplasmic Reticulum.” Cold Spring Harbor Perspectives in Biology 11 (9): 1–18. https://doi.org/10.1101/cshperspect.a033886.

Kim, Hee Suk, Yongjin Kim, Min Jae Lim, Yun-Gyu Park, Serk In Park, and Jeongwon Sohn. 2019. “The P38-Activated ER Stress-ATF6α Axis Mediates Cellular Senescence.” The FASEB Journal 33 (2): 2422–34. https://doi.org/10.1096/FJ.201800836R.

Komori, Ryota, Mai Taniguchi, Yoshiaki Ichikawa, Aya Uemura, Masaya Oku, Sadao Wakabayashi, Kazuhiko Higuchi, and Hiderou Yoshida. 2012. “Ultraviolet a Induces Endoplasmic Reticulum Stress Response in Human Dermal Fibroblasts.” Cell Structure and Function 37 (1): 49–53. http://www.ncbi.nlm.nih.gov/pubmed/22251794.

Korotkevich, Gennady, Vladimir Sukhov, Nikolay Budin, Maxim N. Atryomov, and Alexey Sergushichev. 2021. “Fast Gene Set Enrichment Analysis. BioRxiv.” BioRxiv, 1–29.

Krutmann, Jean, Anne Bouloc, Gabrielle Sore, Bruno A. Bernard, and Thierry Passeron. 2017. “The Skin Aging Exposome.” Journal of Dermatological Science 85 (3): 152–61. https://doi.org/10.1016/J.JDERMSCI.2016.09.015.

Krutmann, Jean, Tamara Schikowski, Akimichi Morita, and Mark Berneburg. 2021. “Environmentally-Induced (Extrinsic) Skin Aging: Exposomal Factors and Underlying Mechanisms.” Journal of Investigative Dermatology 141 (4): 1096–1103. https://doi.org/10.1016/j.jid.2020.12.011.

Kuilman, Thomas, Chrysiis Michaloglou, Liesbeth C.W. Vredeveld, Sirith Douma, Remco van Doorn, Christophe J. Desmet, Lucien A. Aarden, Wolter J. Mooi, and Daniel S. Peeper. 2008. “Oncogene-Induced Senescence Relayed by an Interleukin-Dependent Inflammatory Network.” Cell 133 (6): 1019–31. https://doi.org/10.1016/J.CELL.2008.03.039.

Laberge, Remi Martin, Yu Sun, Arturo V. Orjalo, Christopher K. Patil, Adam Freund, Lili Zhou, Samuel C. Curran, et al. 2015. “MTOR Regulates the Pro-Tumorigenic Senescence-Associated Secretory Phenotype by Promoting IL1A Translation.” Nature Cell Biology 17 (8): 1049. https://doi.org/10.1038/NCB3195.

Liberzon, Arthur, Aravind Subramanian, Reid Pinchback, Helga Thorvaldsdóttir, Pablo Tamayo, and Jill P Mesirov. 2011. “Molecular Signatures Database (MSigDB) 3.0.” Bioinformatics (Oxford, England) 27 (12): 1739–40. https://doi.org/10.1093/bioinformatics/btr260.

Martic, Ines, Sophia Wedel, Pidder Jansen-Dürr, and Maria Cavinato. 2020. “A New Model to Investigate UVB-Induced Cellular Senescence and Pigmentation in Melanocytes.” Mechanisms of Ageing and Development 190 (September): 111322. https://doi.org/10.1016/J.MAD.2020.111322.

Matos, Liliana, Alexandra Monteiro Gouveia, and Henrique Almeida. 2015. “ER Stress Response in Human Cellular Models of Senescence.” The Journals of Gerontology: Series A 70 (8): 924–35. https://doi.org/10.1093/GERONA/GLU129.

Micco, Raffaella Di, Valery Krizhanovsky, Darren Baker, and Fabrizio d’Adda di Fagagna. 2020. “Cellular Senescence in Ageing: From Mechanisms to Therapeutic Opportunities.” Nature Reviews Molecular Cell Biology 2020 22:2 22 (2): 75–95. https://doi.org/10.1038/s41580-020-00314-w.

Moon, Kyung-Chul, Jong-Phil Yang, Jae-Sun Lee, Seong-Ho Jeong, Eun-Sang Dhong, and Seung-Kyu Han. 2019. “Effects of Ultraviolet Irradiation on Cellular Senescence in Keratinocytes Versus Fibroblasts.” The Journal of Craniofacial Surgery 30 (1): 270–75. https://doi.org/10.1097/SCS.0000000000004904.

Narayanan, Deevya L., Rao N. Saladi, and Joshua L. Fox. 2010. “Ultraviolet Radiation and Skin Cancer.” International Journal of Dermatology 49 (9): 978–86. https://doi.org/10.1111/j.1365-4632.2010.04474.x.

Nassour, Joe, Sébastien Martien, Nathalie Martin, Emeric Deruy, Elisa Tomellini, Nicolas Malaquin, Fatima Bouali, et al. 2016. “Defective DNA Single-Strand Break Repair Is Responsible for Senescence and Neoplastic Escape of Epithelial Cells.” Nature Communications 7. https://doi.org/10.1038/ncomms10399.

Niehues, Hanna, Gijs Rikken, Ivonne M.J.J. van Vlijmen-Willems, Diana Rodijk-Olthuis, Piet E.J. van Erp, Patrick L.J.M. Zeeuwen, Joost Schalkwijk, and Ellen H. van den Bogaard. 2022. “Identification of Keratinocyte Mitogens: Implications for Hyperproliferation in Psoriasis and Atopic Dermatitis.” JID Innovations 2 (1): 100066. https://doi.org/10.1016/j.xjidi.2021.100066.

Ogata, Yuichiro, Takaaki Yamada, Seiji Hasegawa, Ayumi Sanada, Yohei Iwata, Masaru Arima, Satoru Nakata, Kazumitsu Sugiura, and Hirohiko Akamatsu. 2021. “SASP-Induced Macrophage Dysfunction May Contribute to Accelerated Senescent Fibroblast Accumulation in the Dermis.” Experimental Dermatology 30 (1): 84–91. https://doi.org/10.1111/exd.14205.

Pluquet, Olivier, Albin Pourtier, and Corinne Abbadie. 2015. “A Review in the Theme: Cellular Mechanisms of Endoplasmic Reticulum Stress Signalling in Health and Disease.” Am J Physiol Cell Physiol 308: 415–25. https://doi.org/10.1152/ajpcell.00334.2014.

Rafehi, Haloom, Christian Orlowski, George T Georgiadis, Katherine Ververis, Assam El-Osta, and Tom C Karagiannis. 2011. “Clonogenic Assay: Adherent Cells.” Journal of Visualized Experiments : JoVE, no. 49 (March). https://doi.org/10.3791/2573.

Ritschka, Birgit, Mekayla Storer, Alba Mas, Florian Heinzmann, Mari Carmen Ortells, Jennifer P. Morton, Owen J. Sansom, Lars Zender, and William M. Keyes. 2017. “The Senescence-Associated Secretory Phenotype Induces Cellular Plasticity and Tissue Regeneration.” Genes and Development 31 (2): 172–83. https://doi.org/10.1101/GAD.290635.116/-/DC1.

Robinson, Mark D, Davis J McCarthy, and Gordon K Smyth. 2010. “EdgeR: A Bioconductor Package for Differential Expression Analysis of Digital Gene Expression Data.” Bioinformatics (Oxford, England) 26 (1): 139–40. https://doi.org/10.1093/bioinformatics/btp616.

Rodier, Francis, Jean Philippe Coppé, Christopher K. Patil, Wieteke A.M. Hoeijmakers, Denise P. Muñoz, Saba R. Raza, Adam Freund, Eric Campeau, Albert R. Davalos, and Judith Campisi. 2009. “Persistent DNA Damage Signalling Triggers Senescence-Associated Inflammatory Cytokine Secretion.” Nature Cell Biology 11 (8): 973. https://doi.org/10.1038/NCB1909.

Rösing, Sarah, Fabian Ullrich, Susann Meisterfeld, Franziska Schmidt, Laura Mlitzko, Marijana Croon, Ryan G Nattrass, et al. 2024. “Chronic Endoplasmic Reticulum Stress in Myotonic Dystrophy Type 2 Promotes Autoimmunity via Mitochondrial DNA Release.” Nature Communications 15 (1): 1534. https://doi.org/10.1038/s41467-024-45535-1.

Russell-Goldman, Eleanor, and George F. Murphy. 2020. “The Pathobiology of Skin Aging: New Insights into an Old Dilemma.” American Journal of Pathology 190 (7): 1356–69. https://doi.org/10.1016/j.ajpath.2020.03.007.

Sabath, Niv, Flonia Levy-Adam, Amal Younis, Kinneret Rozales, Anatoly Meller, Shani Hadar, Sharon Soueid-Baumgarten, and Reut Shalgi. 2020. “Cellular Proteostasis Decline in Human Senescence.” Proceedings of the National Academy of Sciences of the United States of America 117 (50): 31902–13. https://doi.org/10.1073/pnas.2018138117.

Saul, Dominik, Robyn Laura Kosinsky, Elizabeth J. Atkinson, Madison L. Doolittle, Xu Zhang, Nathan K. LeBrasseur, Robert J. Pignolo, et al. 2022. “A New Gene Set Identifies Senescent Cells and Predicts Senescence-Associated Pathways across Tissues.” Nature Communications 2022 13:1 13 (1): 1–15. https://doi.org/10.1038/s41467-022-32552-1.

Schewe, Denis M., and Julio A. Aguirre-Ghiso. 2008. “ATF6α-Rheb-MTOR Signalling Promotes Survival of Dormant Tumor Cells in Vivo.” Proceedings of the National Academy of Sciences of the United States of America 105 (30): 10519–24. https://doi.org/10.1073/pnas.0800939105.

Schmitt, Clemens A. 2016. “The Persistent Dynamic Secrets of Senescence.” Nature Cell Biology 18 (9): 913–15. https://doi.org/10.1038/ncb3403.

Schmittgen, Thomas D, and Kenneth J Livak. 2008. “Analyzing Real-Time PCR Data by the Comparative CT Method.” Nature Protocols 3 (6): 1101–8. https://doi.org/10.1038/nprot.2008.73.

Scott, Timothy L., Perry A. Christian, Melissa V. Kesler, Kevin M. Donohue, Brent Shelton, Kazumasa Wakamatsu, Ito Shosuke, and John D’Orazio. 2012. “Pigment-Independent CAMP-Mediated Epidermal Thickening Protects against Cutaneous UV Injury by Keratinocyte Proliferation.” Experimental Dermatology 21 (10): 771–77. https://doi.org/10.1111/exd.12012.

Stelzer, Gil, Naomi Rosen, Inbar Plaschkes, Shahar Zimmerman, Michal Twik, Simon Fishilevich, Tsippi Iny Stein, et al. 2016. “The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses.” Current Protocols in Bioinformatics 54 (June): 1.30.1-1.30.33. https://doi.org/10.1002/cpbi.5.

Steude, Judith, Reinhard Kulke, and Enno Christophers. 2002. “Interleukin-1-Stimulated Secretion of Interleukin-8 and Growth-Related Oncogene-α Demonstrates Greatly Enhanced Keratinocyte Growth in Human Raft Cultured Epidermis.” Journal of Investigative Dermatology 119 (6): 1254–60. https://doi.org/10.1046/j.1523-1747.2002.19616.x.

Tigges, Julia, Heike Weighardt, Sandra Wolff, Christine Götz, Irmgard Förster, Zippora Kohne, Ulrike Huebenthal, et al. 2013. “Aryl Hydrocarbon Receptor Repressor (AhRR) Function Revisited: Repression of CYP1 Activity in Human Skin Fibroblasts Is Not Related to AhRR Expression.” Journal of Investigative Dermatology 133 (1): 87–96. https://doi.org/10.1038/jid.2012.259.

Toutfaire, Marie, Emilie Bauwens, and Florence Debacq-Chainiaux. 2017. “The Impact of Cellular Senescence in Skin Ageing: A Notion of Mosaic and Therapeutic Strategies.” Biochemical Pharmacology 142: 1–12. https://doi.org/10.1016/j.bcp.2017.04.011.

Tuttle, Camilla S.L., Mariette E.C. Waaijer, Monique S. Slee-Valentijn, Theo Stijnen, Rudi Westendorp, and Andrea B. Maier. 2020. “Cellular Senescence and Chronological Age in Various Human Tissues: A Systematic Review and Meta-Analysis.” Aging Cell. https://doi.org/10.1111/acel.13083.

Victorelli, Stella, Anthony Lagnado, Jessica Halim, Will Moore, Duncan Talbot, Karen Barrett, James Chapman, et al. 2019. “Senescent Human Melanocytes Drive Skin Ageing via Paracrine Telomere Dysfunction.” The EMBO Journal 38 (23). https://doi.org/10.15252/EMBJ.2019101982.

Waaijer, Mariëtte E.C., David A. Gunn, Peter D. Adams, Jeff S. Pawlikowski, Christopher E.M. Griffiths, Diana Van Heemst, P. Eline Slagboom, Rudi G.J. Westendorp, and Andrea B. Maier. 2016. “P16INK4a Positive Cells in Human Skin Are Indicative of Local Elastic Fiber Morphology, Facial Wrinkling, and Perceived Age.” Journals of Gerontology - Series A Biological Sciences and Medical Sciences 71 (8): 1022–28. https://doi.org/10.1093/gerona/glv114.

Wang, Audrey Shimei, Peh Fern Ong, Alexandre Chojnowski, Carlos Clavel, and Oliver Dreesen. 2017. “Loss of Lamin B1 Is a Biomarker to Quantify Cellular Senescence in Photoaged Skin.” Scientific Reports 7 (1): 1–8. https://doi.org/10.1038/s41598-017-15901-9.

Wiley, Christopher D., Michael C. Velarde, Pacome Lecot, Su Liu, Ethan A. Sarnoski, Adam Freund, Kotaro Shirakawa, et al. 2016. “Mitochondrial Dysfunction Induces Senescence with a Distinct Secretory Phenotype.” Cell Metabolism 23 (2): 303. https://doi.org/10.1016/J.CMET.2015.11.011.

Wlaschek, Meinhard, Iliana Tantcheva-Poór, Lale Naderi, Wenjian Ma, Lars Alexander Schneider, Ziba Razi-Wolf, Jutta Schüller, and Karin Scharffetter-Kochanek. 2001. “Solar UV Irradiation and Dermal Photoaging.” Journal of Photochemistry and Photobiology B: Biology 63 (1): 41–51. https://doi.org/https://doi.org/10.1016/S1011-1344(01)00201-9.

Wu, Tianzhi, Erqiang Hu, Shuangbin Xu, Meijun Chen, Pingfan Guo, Zehan Dai, Tingze Feng, et al. 2021. “ClusterProfiler 4.0: A Universal Enrichment Tool for Interpreting Omics Data.” Innovation (Cambridge (Mass.)) 2 (3): 100141. https://doi.org/10.1016/j.xinn.2021.100141.

Yu, Guangchuang, Li-Gen Wang, Guang-Rong Yan, and Qing-Yu He. 2015. “DOSE: An R/Bioconductor Package for Disease Ontology Semantic and Enrichment Analysis.” Bioinformatics (Oxford, England) 31 (4): 608–9. https://doi.org/10.1093/bioinformatics/btu684.

Zhu, Yukun, Xuewen Liu, Xuelu Ding, Fei Wang, and Xin Geng. 2019. “Telomere and Its Role in the Aging Pathways: Telomere Shortening, Cell Senescence and Mitochondria Dysfunction.” Biogerontology 20 (1): 1–16. https://doi.org/10.1007/s10522-018-9769-1.

List of primers used.

Target	Forward	Reverse
p16	GCCCAACGCACCGAATAGT	CGCTGCCCATCATCATGAC
p21	CTGGAGACTCTCAGGGTCGAA	CCAGGACTGCAGGCTTCCT
HSPA5	GGTGTGCTCTCTGGTGATCAAG	ATGACACCTCCCACAGTTTCAAT
P4HB	AGTGACGTGTTCTCCAAATACC	AAAGTTGTTCCGGCCTTCAT
XBP1	TGCCAGAGATCGAAAGAAGG	CCTGGTTCTCAACTACAAGGC
PP1R15A	GGACACTGCAAGGTTCTGAT	CCGGTGTGATGGTGGATAAG
CHOP	GCAAGAGGTCCTGTCTTCAGATG	CTCAGTCAGCCAAGCCAGAGA
ERO1B	GAGCCCTGTCCAGAGAGTAA	GCTCCCAGTTTATTAGCTTGC
SEL1	GCAGCGAGAGAGATGTTTGA	CACCAAGTCCAGAGGCATAC
PER1	GCGTGCGGAGGACACT	GAGGGAGTGAGGTGGAAGAT
ATF6α	GATGATGAAAAATGGAGCAGCTT	AGACTGAAGAGCAGGTGAGCAAA
PERK	ATGCTTTCACGGTCTTGGTC	TCATCCAGCCTTAGCAAACC
IRE1α	CGAAACTTCCTTTTACCATCCC	CGATGACAAAGTCTGCTGCTT
IL1β	GCCCTAAACAGATGAAGTGCTC	GAGATTCGTAGCTGGATGCC
IL8	CTGGCCGTGGCTCTCTTG	GGGTGGAAAGGTTTGGAGTATG
MMP1	TCGGGGAGAAGTGATGTTCT	GTCGGCAAATTCGTAAGCAG
MMP3	CCCTTTTGATGGACCTGGAA	CATGAGCAGCAACGAGAAATAA
RPL13A	GCCTACAAGAAAGTTTGCCTATCTG	TGAGCTGTTTCTTCTTCCGGTAGT

Research article annex for the PhD thesis manuscript

In Figure 7 of our research article, we investigated the effect of conditioned medium (CM) from UVB-induced fibroblasts on keratinocytes, focusing on their colony-forming ability. More than a proliferation assay, we wanted to evaluate the potential of keratinocytes cultured with CM from fibroblasts to survive over time, capacity to proliferate into clonal populations under our experimental conditions. To do so, we conducted a clonogenic assay to assess keratinocyte proliferation. To quantify the results, we focused on counting the number of colonies under our different conditions of interest. However, other parameters, such as colonies area and circularity, could provide valuable information on the behavior of keratinocytes when exposed to CM from UVB-induced fibroblasts. Indeed, when primary cultures of normal cells like primary keratinocytes are cloned, three types of colonies grow known as holoclones, meroclones, and paraclones, each displaying distinct criteria of shape and size (Barrandon and Green 1987).

Firstly, when we compared the colony area between keratinocytes exposed to CM from UVB-exposed fibroblasts and CM from proliferative control fibroblasts, we observed that the colony area was similar between these two conditions (**Figure 21A**). Additionally, neither the CM from UVB-exposed fibroblasts invalidated for ATF6α nor the addition of a neutralizing antibody against IL-8 affected the colony area (**Figure 21A**). Additionally, same conclusions were drawn regarding colonies circularity, even if a slight non-significant decrease were observed with the CM from from UVB-exposed fibroblasts invalidated for ATF6α (**Figure 21B**).

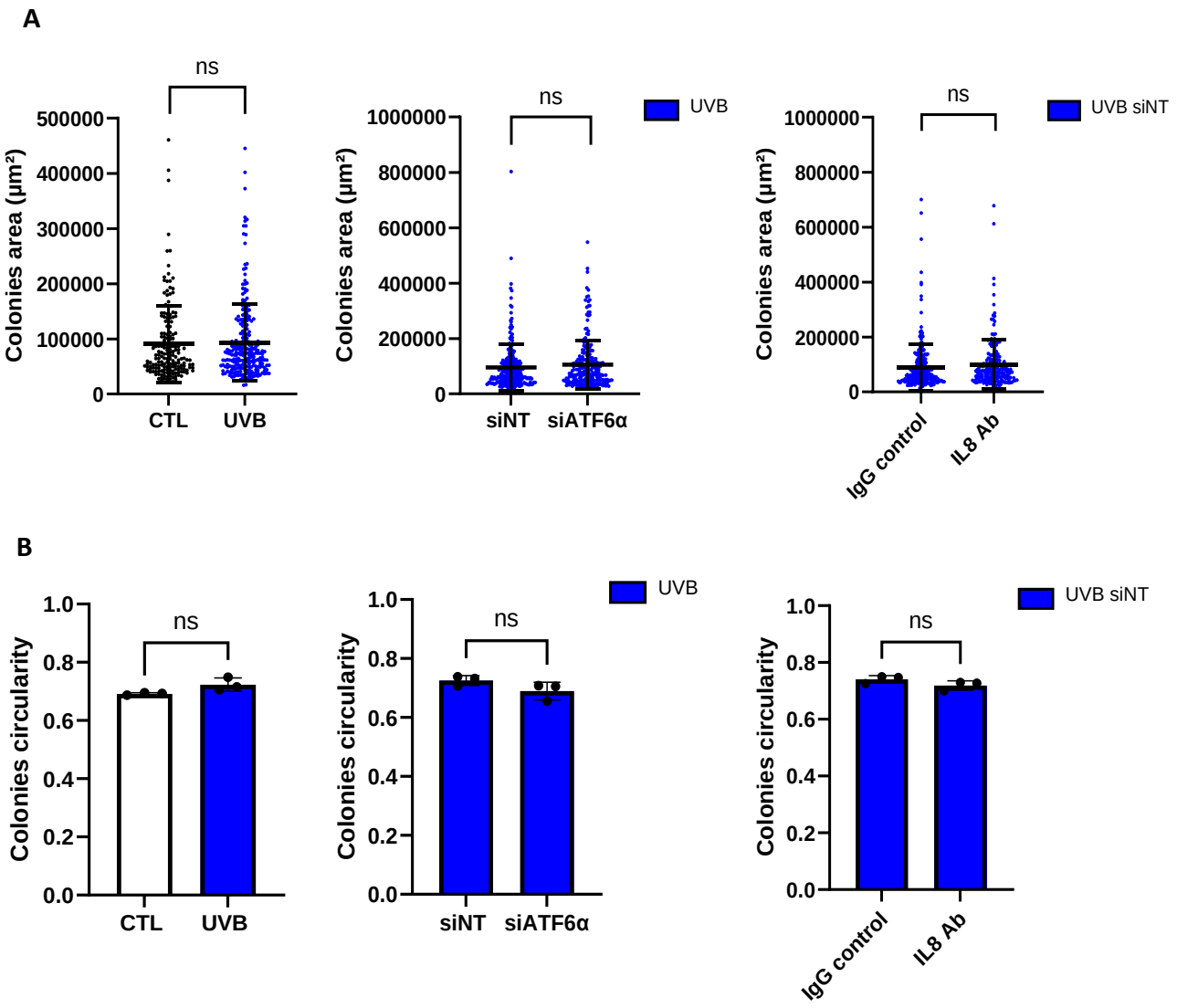


Figure 21. **Conditioned media from UVB-induced senescent fibroblasts do not impact keratinocyte colonies area and circularity.**

NHEKs were grown in 24-wells plate using conditioned media from NHDFs exposed to UVB (UVB) and invalidated or not (siNT) for ATF6α (siATF6α) for five consecutive days.
A. Quantification of NHEK colonies area formed after 5 days (*n*=3).
B. Quantification of NHEK colonies circularity formed after 5 days (n=3).

Data information: Datas in (**A-B**) are presented as means ± SD. Statistical comparison was performed by unpaired t-test. *p*-value shown represents difference between CTL and UVB, or UVB siNT and UVB siATF6α, or UVB IgG control and UVB IL8 Ab.

2. Complementary results

A. Study of the potential pro-tumorigenic effects of the altered SASP of UVB-induced senescent NHDFs invalidated or not for ATF6α

A.1 Context and objectives

As mentioned, the SASP from senescent cells has been described both in cell culture and *in vivo* animal models as capable of promoting tumor development or enhancing it (reviewed in; Schmitt, Wang, and Demaria 2022). However, little is known about the potential pro-tumorigenic effects of UVB-induced senescent SASP of NHDFs on skin cancer cells.

Previous results obtained in our lab showed that the conditioned media from UVB-induced senescent keratinocytes and replicative senescent fibroblasts increase the migration of A431 cutaneous squamous cell carcinoma cells (Bauwens et al. 2023; Toutfaire et al. 2018). Moreover, Menicacci *et al.* demonstrated that treating pre-replicative senescent fibroblasts with resveratrol counteracted the pro-proliferative and pro-invasive effects of the conditioned media harvested from replicative senescent fibroblasts on A375 melanoma cells (Menicacci et al. 2019). In addition, a study identifying IL-8 as an essential autocrine growth factor for melanoma cell proliferation demonstrated that blocking IL-8 was sufficient to inhibit melanoma cell proliferation. Conversely, this effect was reversed when exogenous IL-8 was reintroduced into the culture media (Schadendorf et al. 1993).

Thus, knowing that in our conditions IL-8 secretion increases after UVB exposures and that its expression is potentially regulated by ATF6α, we have established two main hypotheses: Firstly, the CM from UVB-exposed NHDFs could enhance the migration and/or invasion of skin melanoma cells. Secondly, the modifications induced by ATF6α silencing in NHDFs could modify the behavior of skin cancer cells.

Consequently, our first aim was to investigate the impact of conditioned media harvested from UVB-induced senescent NHDFs on several skin melanoma cancer cell lines with different degrees of aggressiveness. Therefore we used three different cell lines ranked from *in vitro* high- to low- melanoma aggressiveness score (MAGS = (growth x migration x invasion) / doubling time): A375, SK-MEL-2, and SK-MEL-28 (Rossi et al. 2018). Secondly, we sought to assess the influence of ATF6α invalidation on the potential observed pro-tumoral characteristics of UVB-induced conditioned media in these three cell lines. To this end, we performed migration and invasion assays using two distinct techniques: a precise approach enabling live-cell analysis through Incucyte technology, and an established method employing Boyden-chambers.

A.2 Material and methods

Cells culture

Human melanoma cell lines A375, SK-MEL-2, and SK-MEL-28 were kindly provided by Prof. Jean-Pierre Gilet who received them from the Center for Cancer Research, NCI-NIH, Laboratory of Cell Biology, Bethesda USA. The three cell lines were maintained in Dulbecco's Modified Eagle Medium - High Glucose (DMEM, 41966, Gibco) supplemented with 10% FBS (Corning), 1 mM pyruvate (Gibco), 2 mM GlutaMAX^{TM}, and penicillin-streptomycin (50 U/mL).

NHDFs were cultured in Basal Medium Eagle (BME, 41010026, Gibco) supplemented with 10% Fetal Bovine Serum (FBS, Corning), 2 mM L-glutamine (Thermo Fisher Scientific, Waltham, MA, USA), and penicillin-streptomycin (50 U/mL).

UVB-induced senescence

NHDFs at their exponential growth phase were exposed to NB-UVB (TL20W/01, Philips) at 500 mJ/cm^2 twice a day for five days and maintained in BME with 1% FBS, 2 mM L-glutamine, and penicillin-streptomycin (50 U/mL) between each UVB exposure.

Conditioned media from NHDFs

48 hours after the last UVB exposure, NHDFs were washed three times with PBS and the medium was changed to serum-free medium. Cells were cultured for an additional 16 hours, and conditioned media were centrifuged at 200 *g*, at 4°C for 5 minutes to remove cellular debris and filtered using a 0.2 µm filter. Conditioned media were then aliquoted and frozen at -20°C until use.

Scratch wound assay

Melanoma cell lines A375, SK-MEL-2, and SKMEL-28 were respectively plated at 50,000 cells/well, 45,000 cells/well, and 40,000 cells/well on Incucyte® Imagelock 96-well plate (Sartorius, BA-04856, Göttingen Germany) to obtain a confluent cell monolayer. At 24 hours after plating, cells were treated for 1 hour with 10 µg/mL of mitomycin C (M4297, Sigma-Aldrich), and scratch wounds (700-800 microns wide) were made using the Incucyte® Cell Migration Kit (BA-04858, Sartorius). Cells were then washed with PBS and replaced with 200 µL of fresh mix media. Conditioned media were normalized to 43,000 cells/mL using serum-free fibroblast media and were then diluted to a 75%-25% ratio with complete DMEM, corresponding to 2,5% serum final concentration in the wells. Cells were monitored every 2 hours for 48 hours using Incucyte® S3 Live-Cell Analysis System (Sartorius) at 10x magnification.

Cultrex cell migration and invasion assays

Cellular migration and invasion were carried out using the Cultrex™ cell migration or invasion assay 96-well kits (3465-096-K or 3455-096-K, R&D Systems). Briefly, these kits utilize a simplified Boyden chamber containing a polyethylene terephthalate (PET) membrane with pores of 8µm to facilitate the migration of cells from the upper chamber to the bottom chamber. For invasion assays, the chambers were coated with 0.5x basement membrane matrix the day before the seeding. Melanoma cell lines SK-MEL-2 and SK-MEL-28 were serum-deprived for 18 h before being seeded at 50,000 cells/well in the upper chamber, while 150 µL of CM from NHDFs exposed or not (CTL) to UVB and transfected or not (siNT) with siATF6α were added to the bottom chambers. CM was normalized to 75,000 cells/mL using serum-free BME but was not mixed with complete DMEM, allowing for the exclusive evaluation of the impact of the conditioned media for 24 h. The next day, the upper and bottom chambers were aspirated and washed. Cells attached to the bottom filter were dissociated using a dissolution solution with fluorescent Calcein-AM for 1 h. Subsequently, the upper chambers were removed, and the optical density (OD) of each bottom well was read at 485 nm. The number of migrating or invading cells was calculated from a standard curve following the manufacturer's instructions.

Quantifications

For quantifications of scratch wound assays, pictures of each were analyzed using the Incucyte imaging system. The selected metric used to compare the wound closure between each condition was the relative wound density (RWD). RWD relies on comparing the spatial cell density within the wound area to the spatial cell density outside the wound area at each time point. It is calibrated to be zero at t=0 and reaches 100% when the cell density inside the wound matches the cell density outside the initial wound. This tool is consistent across various cell types and does not depend on identifying cell boundaries (Roddy et al. 2017).

For quantifying migration and invasion using Cultrex assays, the optical density (OD) values were converted into the number of migrated/invaded cells using a standard curve generated for each experiment.

Statistical analyses were conducted using GraphPad Prism (GraphPad Software, La Jolla, CA, USA). Data used for statistics were obtained from at least three independent biological experiments. All the statistical tests applied are specified in the figures' legend. Differences with a significance level of at least $P<0.05$ were considered statistically significant.

A.3 Results

To investigate other functional consequences of the altered SASP of UVB-induced senescent NHDFs silenced for ATF6α, we conducted experiments using skin melanoma cancer cells. This model could be physiologically relevant as melanoma cells (in their growth phase) can invade the dermis *in vivo*.

We selected three different cell lines with known differences in migratory capacities and seeded them at high density. The following day, scratch wounds were performed. Conditioned media (CM) from UVB-induced senescent NHDFs or young and proliferative fibroblasts (CTL) were added, and the relative wound density was recorded over two days for each cell line. Unexpectedly, we did not observe an increased migratory capacity in either the A375 or SK-MEL-2 and SK-MEL-28 cell lines, when incubated with CM from UVB-exposed fibroblasts (**Figure 22A-C**).

Surprisingly, we have noticed that for SK-MEL-2, the cell density in the wound area expressed relative to the cell density outside of the wound area was lower with CM from UVB-exposed fibroblasts transfected with a siNT compared to CTL siNT conditioned media (**Figure 23B**). In addition, due to the high variability observed in live cell imaging, we encountered difficulties in interpreting the potential effects of CM from UVB-induced senescent NHDFs silenced for ATF6α **(Figure 23A-C).**

To overcome this technical bias, we used another technique to assess cell migration and invasion, based on Boyden chambers (**Figure 24A and 24D**). To do so, we seeded SK-MEL-2 and SK-MEL-28 in the upper chamber of each Cultrex transwell migration assay. The following day, CM was added at the bottom of the chambers and the capability of each cell line to migrate was measured. Curiously, when investigating whether the CM of NHDFs would attract melanoma cells, we found that the attraction potential of CM for each condition closely resembled that of the negative control, which consists of culture media used for fibroblasts depleted in serum (**Figure 24B-C**). In addition, when compared to the potential of cancer cell media supplemented with 10% FBS, the latter exhibited six times more efficiency than the conditioned media in attracting skin cancer cells (**Figure 24B-C**). Besides, upon rationalizing the number of migrated cells to the total number of seeded cells, we observed minimal migration in our conditions of interest. Similarly, we obtained consistent results using the Cultrex transwell invasion assay (**Figure 24E-F**).

Collectively, these data underscore two key concepts: Firstly, CM derived from UVB-induced senescent NHDFs does not enhance the migration or invasion of melanoma cancer cells compared to CM from CTL NHDFs. Secondly, for cells invalidated for ATF6α, the effect of the

CM on the behavior of melanoma cancer cells remained the same. This second result is particularly encouraging, as the silencing of ATF6α did not enhance the pro-tumorigenic potential of the melanoma cells, further emphasizing the significance of this target for the development of a safe senomorphic strategy.

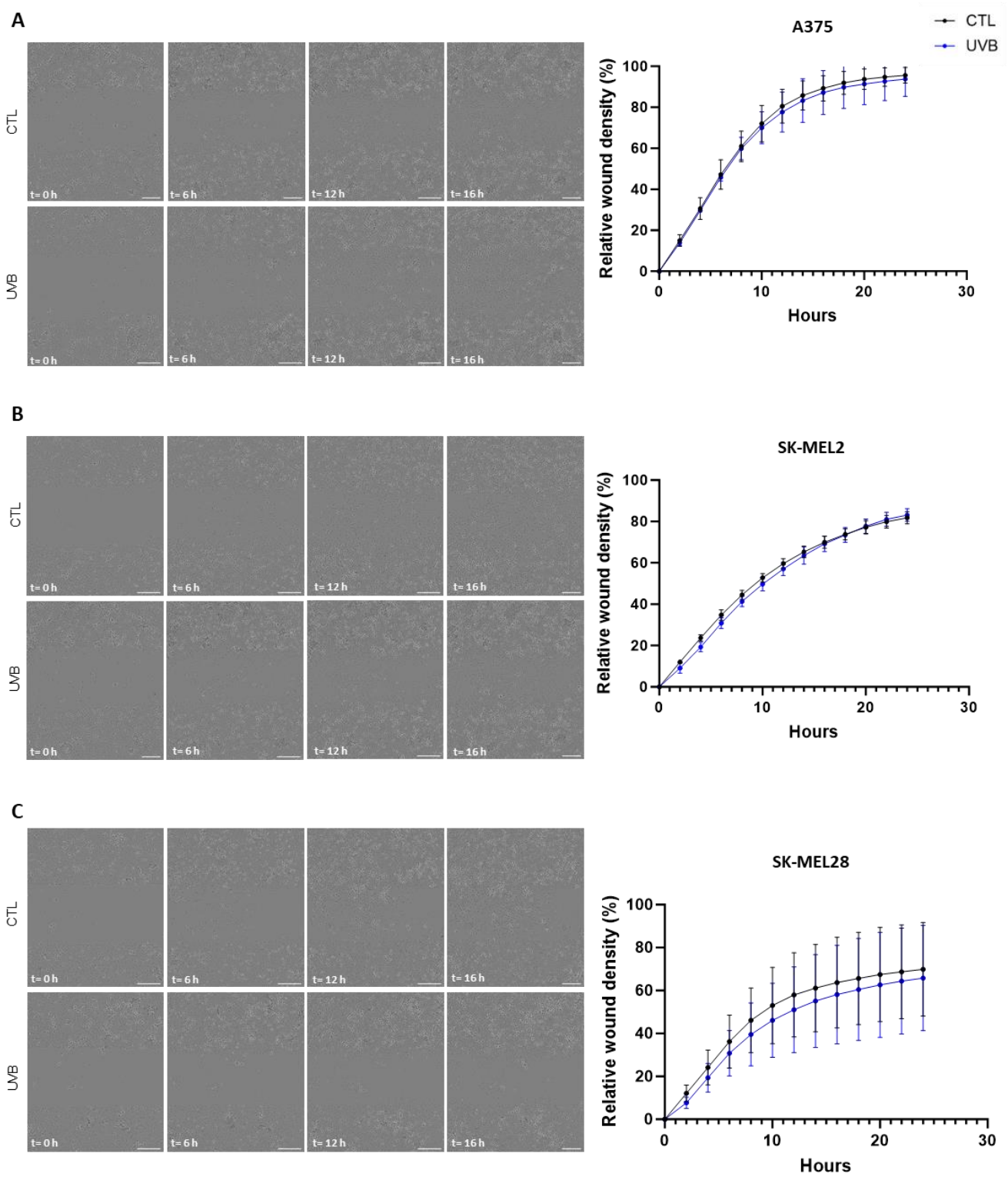


Figure 22. **Conditioned media from UVB-induced senescent fibroblasts do not increase the migration of skin melanoma cancer cells.** CM was harvested from NHDFs exposed or not (CTL) to UVB, and then adjusted with serum -free BME to normalize the number of secreted cells to 43,000 cells/mL before being mixed with skin cancer cells media to a final ratio of 75:25. Migration of skin cancer cells was measured by evaluating the relative wound density.

A-C (Left panel) Representative images of scratch wounds at 0, 6, 12 and 18 hours for A375 (A), SK-MEL-2 (B), and SK-MEL-28 (C) measured by wound healing assay using Incucyte Sartorius. Scale bar indicates 20 µm. (Right panel) Quantification of the relative wound density calculated (*n*=3).

Data information: Each point represents the mean ± SD

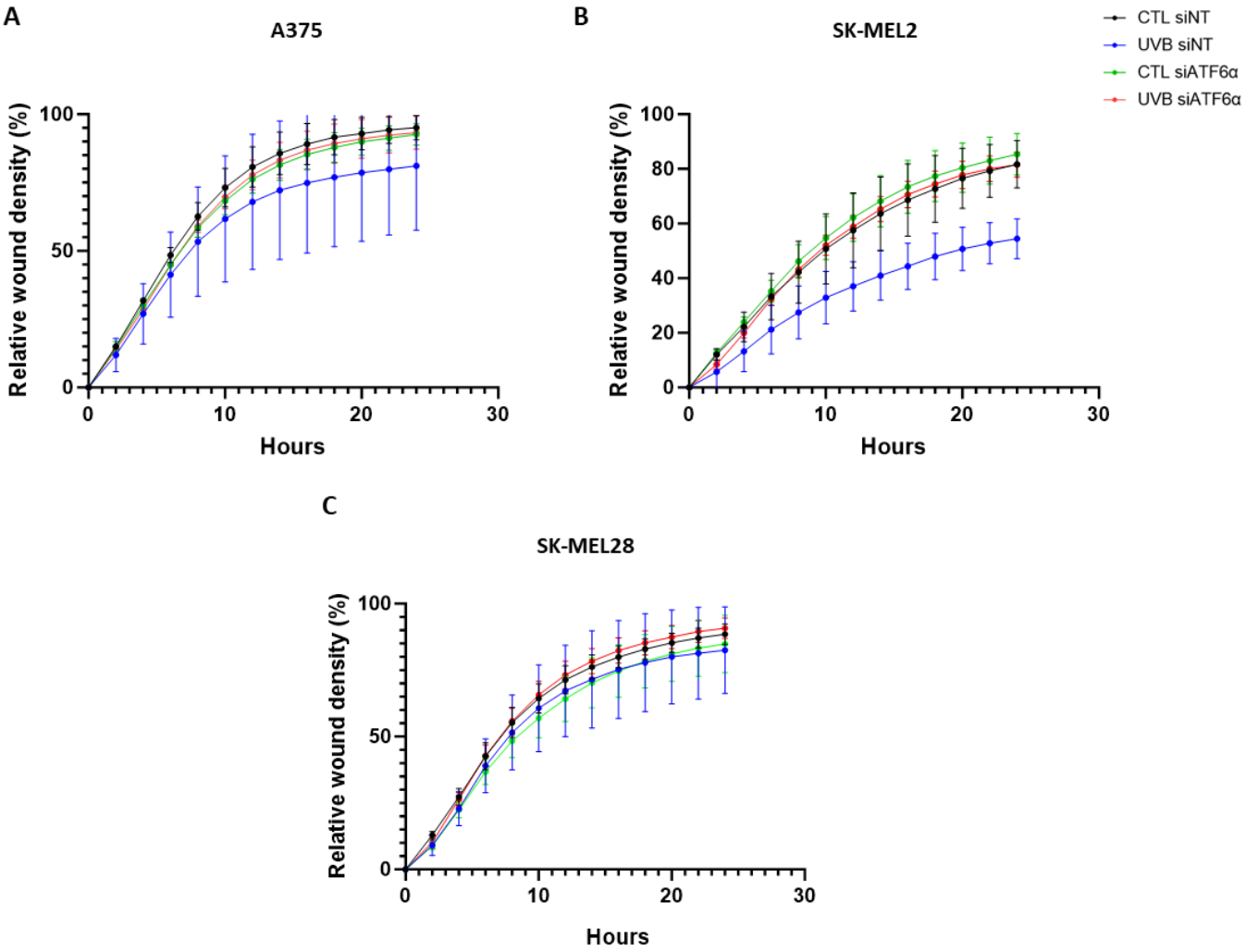


Figure 23. **Conditioned media from UVB-induced senescent fibroblasts invalidated for ATF6α do not impact the migration melanoma cancer cells.** CM was harvested from NHDFs exposed or not (CTL) to UVB, silenced for ATF6α or not (siNT), and then adjusted with serum-free BME to normalize the number of secreted cells to 43,000 cells/mL before being mixed with skin cancer cells media to a final ratio of 75:25. Migration of skin cancer cells was measured by evaluating the relative wound density.

A-C Quantification of the relative wound density of A375 (A), SK-MEL-2 (B) and SK-MEL-28 (C), (*n*=3).

Data information: Each point represents the mean ± SD.

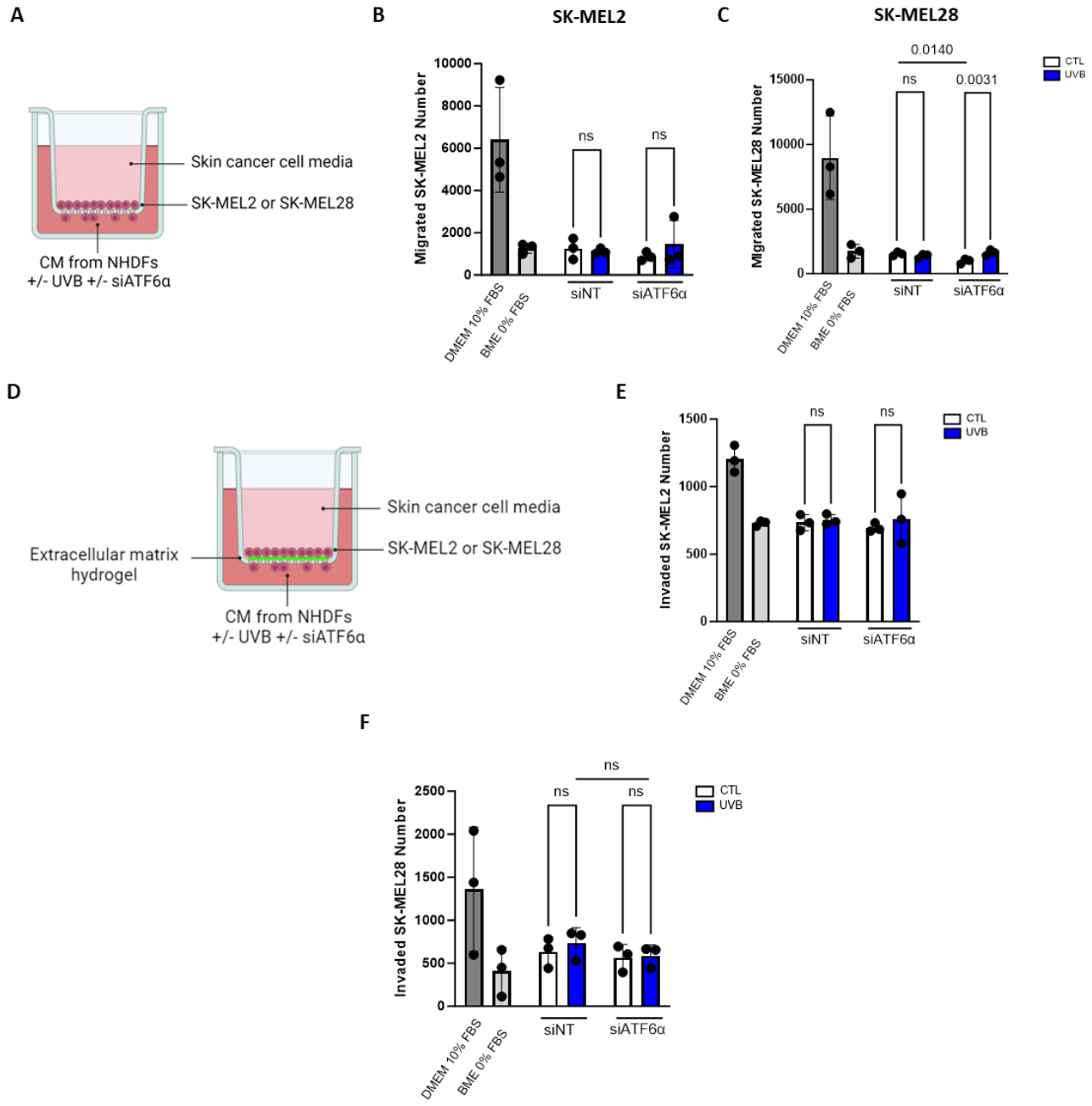


Figure 24. **Conditioned media from UVB-induced senescent fibroblasts invalidated for ATF6α do not impact the migration and invasion potential of melanoma cancer cells.** CM was harvested from NHDFs exposed or not (CTL) to UVB, and then adjusted with serum-free BME to normalize the number of secreted cells to 75,000 cells/mL. Cells migrate from the upper chamber through the filter pores, previously coated with basement membrane extract or not, to the bottom chamber. After 24 hours, a dissociation solution was added in the bottom chamber to allow the complete dissociation of skin cancer cells from the filter. Detection of migrating cells was quantified using Calcein AM.

A. Schematic experimental model used for the in vitro cell migration assay using the Cultrex cell migration kit. SK-MEL2 and SK-MEL 28 were seeded on the upper chamber and incubated for 24 hours in the presence of conditioned media in the bottom chamber.
B-C. Quantification of the number of migrating SK-MEL-2 (B) and SK-MEL-28 (C), (*n*=3).
D. Schematic experimental model used for the in vitro cell invasion assay. Extracellular matrix hydrogel is used to measure the ability of cells to degrade the basement membrane.
E-F. Quantification of the number of invading SK-MEL-2 (E) and SK-MEL-28 (F), (*n*=3).

Data information: Data in (**B,C,E,F**) are presented as means ± SD. Statistical comparison was performed using ANOVA2 followed by Šídák's multiple comparison tests. p-value shown represents differences between unexposed (CTL) and exposed (UVB) cells and differences between non-targeting control siRNA (siNT) and siATF6α.

B. Study of the whole secretome of UVB-induced senescent NHDFs invalidated or not for ATF6α by mass spectrometry analysis

B.1 Context and objectives

As mentioned earlier, the complexity and variety of SASP arise from both the cell type and the senescence inducer, resulting in a diverse array of secretomes. Therefore, to address the challenge of characterizing senescent phenotypes, “omics” approaches are increasingly utilized. Particularly, the use of mass spectrometry (MS) for SASP characterization enables detailed exploration of the secretome's composition, delving into finer details than other techniques, and allowing its monitoring over time.

Secretome analyses of the SASP using MS have attracted significant attention, particularly since the publication of Basisty *et al.* (2020). In their study, they not only demonstrated that the composition of the soluble SASP (sSASP) and the extracellular vesicles SASP (eSASP) differed significantly among senescent inducers and cell types, but they also identified a “core” SASP consisting of a reduced fraction of few secreted proteins shared among all the models used (Basisty et al. 2020). This work not only provided insights into the composition of the SASP across different cell types and stressors but also created a valuable resource for secretomic analysis known as the “SASP Atlas” (http://www.saspatlas.com/). This freely accessible database containing a list of positively or negatively enriched secreted proteins and exosomal cargo components from various models of senescence (e.g. different cell types and senescence inducers) is a highly relevant tool allowing the comparison of secretomes between several senescence studies.

However, it is important to clarify two points here: Firstly, certain secreted proteins with small molecular weights, such as interleukins, are typically not detected by MS. This is not a bias, but a technical limitation that must be taken into account directly. Secondly, the heterogeneity of SASP is such that we do not yet understand which type of composition is associated with specific biological effects, and it’s probably not the small common fraction that plays a major role.

Within this context, we took advantage of the powerful utility of MS to uncover new effects of UVB-induced SASP of NHDFs on biological processes, and to investigate the impact of ATF6α silencing on them. Previously, we observed that these SASPs did not contribute to pro-migratory or pro-invasive effects, but for the moment we only have limited information on the SASP from UVB-induced senescent cells, and its impact on the cellular environment. Thus, utilizing omics data would enable us to predict these potential effects more accurately.

Our primary objective was to exclude microvesicles and exosomes from conditioned media, focusing solely on studying the class of proteins whose secretion would be modified in UVB-induced senescent NHDFs compared to control cells.

Then, our second aim was to analyze the impact of ATF6α invalidation on the secretome profile. As demonstrated in the first part of the results, we observed a modified gene expression of some of the main SASP factors by ATF6α. However, at this stage of the study, we did not have an idea of the effects of ATF6α at the protein level, except for IL-8. Therefore, we aimed to determine globally what kind of changes the knockdown of ATF6α led to and to answer these questions: Does siATF6α alter a specific class of secreted proteins preferentially, and if yes, what biological processes or molecular functions are they associated with?

Indeed, the aim of this section was not to directly identify secreted proteins as potent candidates for further validation, but rather to gain an overview of the modifications induced in the SASP by UVB exposure and siATF6α.

Additionally, it is worth mentioning that data on replicative senescent NHDFs, with or without ATF6α inhibition using CA7, have already been generated in Dr. Olivier Pluquet's team (unpublished). Ultimately, comparing these two datasets – replicative and UVB-exposed senescence with or without ATF6α – would be valuable to identify common pathways or components under the control of ATF6α in two different models of senescence, thereby strengthening the potential of the results.

B.2 Material and methods

Cells culture

NHDFs were cultured in Basal Medium Eagle (BME, 41010026, Gibco) supplemented with 10% Fetal Bovine Serum (FBS, Corning), 2 mM L-glutamine (Thermo Fisher Scientific, Waltham, MA, USA), and penicillin-streptomycin (50 U/mL). 48 hours after the seeding, NHDFs were transfected with either a siGENOME non-targeting control siRNA pool (D-001206-13, Dharmacon) referred to as siNT, or a siGENOME SMARTpool (Dharmacon, Lafayette, CO), siATF6α, or were left untransfected (UN). The day before, cells were exposed to NB-UVB (TL20W/01, Philips) at 500 mJ/cm^2 twice a day for five days and maintained in BME with 1% FBS, 2 mM L-glutamine, and penicillin-streptomycin (50 U/mL) between each UVB exposure.

Conditioned media harvesting

48 hours after the last UVB exposure, NHDFs were washed three times with PBS and the medium was changed to serum-free medium. Cells were cultured for an additional 16 hours, before the harvesting of the conditioned medium and centrifugating it at 200 g, at 4°C for 5 minutes to remove cellular debris.

Exclusion of microvesicles and exosomes from the secreted soluble fraction

The day after harvesting the CM, the supernatant was centrifuged at 2,200 *g* for 10 minutes at 4°C to remove dead cells. Next, the supernatant was further centrifuged at 10,000 g for 30 minutes to eliminate cellular debris, followed by centrifugation at 20,000 g for 70 minutes to remove microvesicles. Subsequently, the supernatant underwent ultracentrifugation at 100,000 g for 70 minutes to eliminate exosomes. All these steps were carried out at 4°C, using a Beckman Optima LE-80K Ultracentrifuge equipped with a swinging bucket rotor (SW-41) in 13.2 mL polypropylene tubes (50 Pk) (331372, Beckman Coulter).

Protein concentration and quantification

Soluble secreted proteins were concentrated using Amicon Ultra Centrifugal Filter Units (3 kDa cutoff) (UFC9003, Sigma-Aldrich). Prior to adding the supernatant, the Amicon filters were rehydrated with PBS and centrifuged at 2,200 g for 40 minutes at 4°C. Excess PBS was removed, and then the supernatant was centrifuged at 2,200 *g* for 60 to 90 minutes at 4°C. Once most of the supernatant had passed through the collection tube, leaving approximately 500 µL on top of the filter device, 2 mL of 8 M urea buffer (8 M urea in 0.1 M Tris buffer at pH 8.5) was added. The secreted proteins mixed with the buffer were then centrifuged at 2,200 g for approximately 60 minutes at 4°C until less than 300 µL of supernatant remained on top of the filter device. The filters were scraped to recover as much protein as possible, and the concentration of each sample was quantified using the Pierce 660 assay.

Protein digestion

The samples were then treated using Filter-aided sample preparation (FASP).

Filters of Millipore Microcon 30 (MRCFOR030 Ultracel PL-30) were first washed using 100 µL of formic acid 1 %, before being centrifugated at 14,500 rpm for 15 minutes.
For each sample, 10 µg of protein adjusted in urea buffer 8 M (urea 8 M in buffer Tris 0.1 M at pH 8,5) to obtain a final volume of 325 µL were placed individually in a column and centrifuged at 14,500 rpm for 15 minutes.
The filtrate was discarded, and the columns were washed three times by adding 200 µL of urea buffer before being centrifugated at 14,500 rpm for 15 minutes.

For the reduction step, 100 µL of dithiothreitol (DTT) was added and mixed for 1 minute at 400 rpm with a thermomixer before an incubation of 15 minutes at 24 °C. Samples were then centrifugated at 14,500 rpm for 15 minutes, the filtrate was discarded, and the filter was washed by adding 100 µL of urea buffer before another centrifugation step at 14,500 rpm for 15 minutes. An alkylation step was performed by adding 100 µL of iodoacetamide (IAA), in urea buffer, in the column and mixing at 400 rpm for 1 minute in the dark before an incubation of 20 minutes in the dark and a centrifugation at 14,500 rpm for 10 minutes. To remove the excess of IAA, 100 µL of urea buffer was added and the samples were centrifugated at 14,500 rpm for 15 minutes.

To quench the rest of IAA, 100 µL of DTT were placed on the column, mixed for 1 minute at 400 rpm, and incubated for 15 minutes at 24°C before centrifugation at 14,500 rpm for 10 minutes.

To remove the excess of DTT, 100 µL of urea buffer was placed on the column and centrifuged at 14,500 rpm for 15 minutes.

The filtrate was discarded, and the column was washed three times by adding 100 µL of sodium bicarbonate buffer 50 mM ((ABC), in ultrapure water) followed by a centrifugation at 14,500 rpm for 10 minutes. The last 100 µL were kept at the bottom of the column to avoid any evaporation in the column.

The digestion process was performed by adding mass spectrometry grade trypsin (1/50 in ABC buffer) in the column and mixed at 400 rpm for 1 minute before being incubated overnight at 24°C in a water-saturated environment.

The next day, Microcon columns were placed on a LoBind tube of 1.5 ml and centrifuged at 14,500 rpm for 10 minutes. 40 µL of ABC buffer was placed on the column before centrifugation at 14,500 rpm for 10 minutes. Trifluoroacetic acid (TFA) 10 % in ultrapure water was added to the contain of the LoBind Tube to obtain 0.2 % TFA.

Finally, samples were dried in a SpeedVac up to 20 µL and transferred in an injection vial.

Mass Spectrometry

The digested proteins were analyzed using nano-LC-ESI-MS/MS tims TOF Pro (Bruker, Billerica, MA, USA) coupled with a UHPLC nanoElute (Bruker). Peptides were separated by nanoUHPLC (nanoElute, Bruker) on a 75 µm ID, 25 cm C18 column with integrated CaptiveSpray insert (Aurora, ionopticks, Melbourne) at a flow rate of 200 nl/min, at 50°C. LC mobile phases A was water with 0.1% formic acid (v/v) and B ACN with formic acid 0.1% (v/v). Samples were loaded directly on the analytical column at a constant pressure of 800 bar. The digest (1 µl) was injected, and the organic content of the mobile phase was increased linearly from 2% B to 15 % in 22 min, from 15 % B to 35% in 38 min, from 35% B to 85% in 3 min. Data acquisition on the tims TOF Pro was performed using Hystar 6.1 and tims Control 2.0.

Tims TOF Pro data were acquired using 160 ms TIMS accumulation time, mobility (1/K0) range from 0.75 to 1.42 Vs/cm². Mass-spectrometric analysis was carried out using the parallel accumulation serial fragmentation (PASEF) acquisition method (Meier et al. 2018). One MS spectra followed by six PASEF MSMS spectra per total cycle of 1.16 s.

Scaffold analyses

Data analysis was performed using Mascot 2.8.1 (Matrix Science). Tandem mass spectra were extracted, charge state deconvoluted and deisotoped by Data analysis (Bruker) version 5.3. All MS/MS samples were analyzed using Mascot (Matrix Science, London, UK; version 2.8.1). Mascot was set up to search the Human Proteome from UniProt (220705-79687 entries) assuming the digestion enzyme trypsin. Mascot was searched with a fragment ion mass tolerance of 0.050 Da and a parent ion tolerance of 15 PPM. Carbamidomethyl of cysteine was specified in Mascot as fixed modifications. Oxidation of methionine and acetyl of the N-terminus were specified in Mascot as variable modifications.

Scaffold (version Scaffold_5.0.0, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 96.0% probability to achieve an FDR less than 1.0% by the Scaffold Local FDR algorithm. Protein identifications were accepted if they could be established at greater than 5.0% probability to achieve an FDR less than 1.0% and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm (Nesvizhskii et al. 2003). Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony. Proteins sharing significant peptide evidence were grouped into clusters.

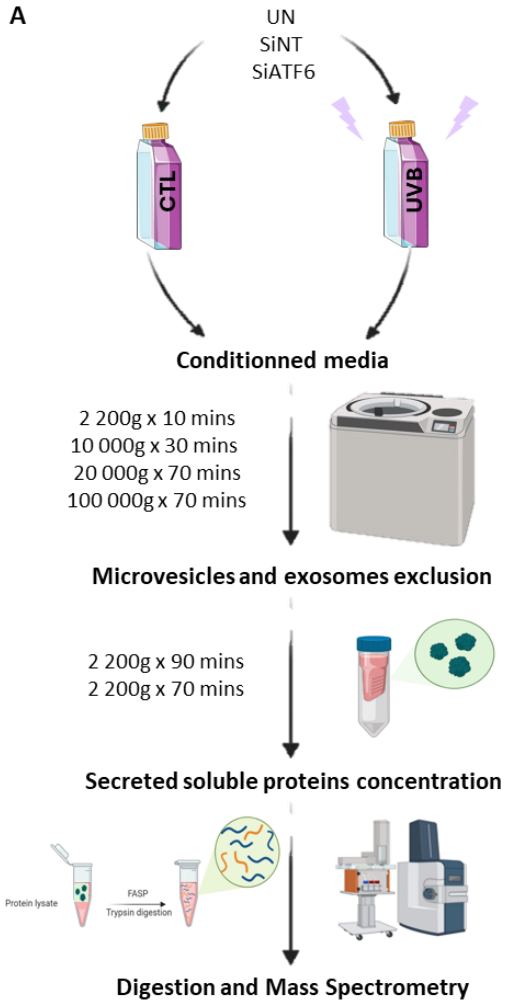
B.3 Results

Before harvesting the conditioned media (CM), we employed the same transfection model and UVB exposures as previously described. At three days after the last UVB exposure, the conditioned media was collected from 1 T75 flask per condition. Simultaneously, biomarkers of senescence, such as $p16^{INK4}$, $p21^{CIP1}$, SASP factors, and *ATF6α* invalidation were confirmed at the mRNA levels to ensure that UVB-induced senescence was correctly established.

After these validations, the CM was processed freshly. As outlined in the introduction, our first objective was to remove microvesicles and exosomes from the secreted proteins of the SASP. To achieve this, we devised an ultracentrifugation protocol based on literature and adapted it to our equipment (Théry et al. 2006; Basisty et al. 2020). In brief, the conditioned media underwent multiple centrifugation steps (2,200g for 10 minutes, 10,000g for 30 minutes, 20,000g for 30 minutes, and ultracentrifugation at 100,000g for 70 minutes) allowing the removal of dead cells, cellular debris, microvesicles and exosomes (**Figure 25A**). At the final stage of this protocol, a pellet containing exosomes should have been formed at the bottom of the ultracentrifuge tubes. Subsequently, the soluble fraction was concentrated using Amicon Ultra-15 centrifugal filters, and the concentration of secreted proteins was quantified. Finally, the secreted proteins were digested and analysed by mass spectrometry.

Before starting the secretomic analyses, we first ensured the efficient removal of microvesicles and exosomes (MVs/EVs) from the soluble fraction. To do so, we examined 100 MVs/EVs markers referenced in a database called ExoCarta (http://www.exocarta.org/), and we compared the number of identified spectral counts of these biomarkers in conditioned media (CM) that underwent ultracentrifugation steps. Among the 100 major markers identified from published proteomic exosomal studies of several contexts, we detected 27 of them in our experiments. Given the particular context of senescence, and the variation in exosomal content which are not taken into account in the database, this ratio appears to be quite promising. Additionally, we observed a reduction of more than half of these markers after the ultracentrifugation steps. To illustrate this, we have provided a table representing four of the most found markers (TSPAN4, CD9, ANXA1, and HSP90AA1) in the literature, along with the number of spectral counts identified in CMs with or without ultracentrifugation steps (**Figure 25B**). These results indicate that our subsequent analysis will indeed mostly focus on directly secreted proteins.

A



B

	# spectral counts	
MVs/Evs markers	**CM - ultracentrifugations steps**	**Mean CM + ultracentrifugations steps**
TSPAN4	6	5,3
CD9	17	1,3
ANXA1	102	1,7
HSP90AA1	561	154,5

Figure 25. **Proteomic workflow for microvesicles and exosomes exclusion and secreted soluble proteins concentration.**

A. NHDFs were transfected with non-targeting control siRNA (siNT), siATF6α, or left untransfected. The following day, cells were either exposed or not (CTL) to UVB twice daily for five days. Three days after the last UVB exposure, conditioned media (CM) from one T75 flask was harvested. Microvesicles and exosomes were removed from the secreted soluble proteins using centrifugation and ultracentrifugation steps. Only the fraction containing secreted soluble proteins was processed and concentrated using Amicon centrifugal filter devices. Finally, the samples were digested and subjected to mass spectrometry (data-dependent acquisition (DDA) - label free).

B. Table of four microvesicle and exosome-specific markers in conditioned media (CM), with or without ultracentrifugation steps, identified by mass spectrometry. Markers were selected based on literature and ExoCarta database's top 100 published markers. Data for CM without ultracentrifugation steps was obtained from young proliferative fibroblasts (*n*=1), while data for CM with ultracentrifugation steps represent the mean of each of our six conditions of interest across three independent replicates.

To begin with, we examined untransfected cells (UN) and compared the secretome of UVB-exposed cells (UVB) with that of respective control (CTL) cells.
Quantitative profiling of protein clusters between these two conditions revealed that 31 secreted proteins were negatively enriched, while 113 were positively enriched in UVB compared to CTL. Among these, 24 and 6, respectively, were identified as secreted proteins in the UniProt database (**Figure 26A**).

Using the STRING database, we constructed an interaction network based on these 30 differentially secreted proteins. This representation not only reveals the extent of interactions among the proteins, drawn from verified experimental data or predictions but also emphasizes the nodes of interaction between them (**Figure 26B**). Among the differentially enriched secreted proteins in UVB, we observed an increase of the metalloproteinase inhibitor 1 (TIMP1) and a decrease in various collagens, notably COL1A1, COL2A1, COL5A1, COL5A2, decorin (DCR), fibulin-1 (FBLN1), fibronectin (FN1) (**Figure 26C-D**). Interestingly, the STRING interactions predicted the strongest connection for them.

Subsequently, we conducted enrichment analysis using the STRING method with the Gene Ontology (GO) database, focusing on Biological Processes, with the lists of differentially secreted proteins in UVB UN vs. CTL UN. This analysis revealed that among the significant GO terms, those associated with “adhesion” and “ECM/collagen/fibres organization” showed up (**Figure 26E**).

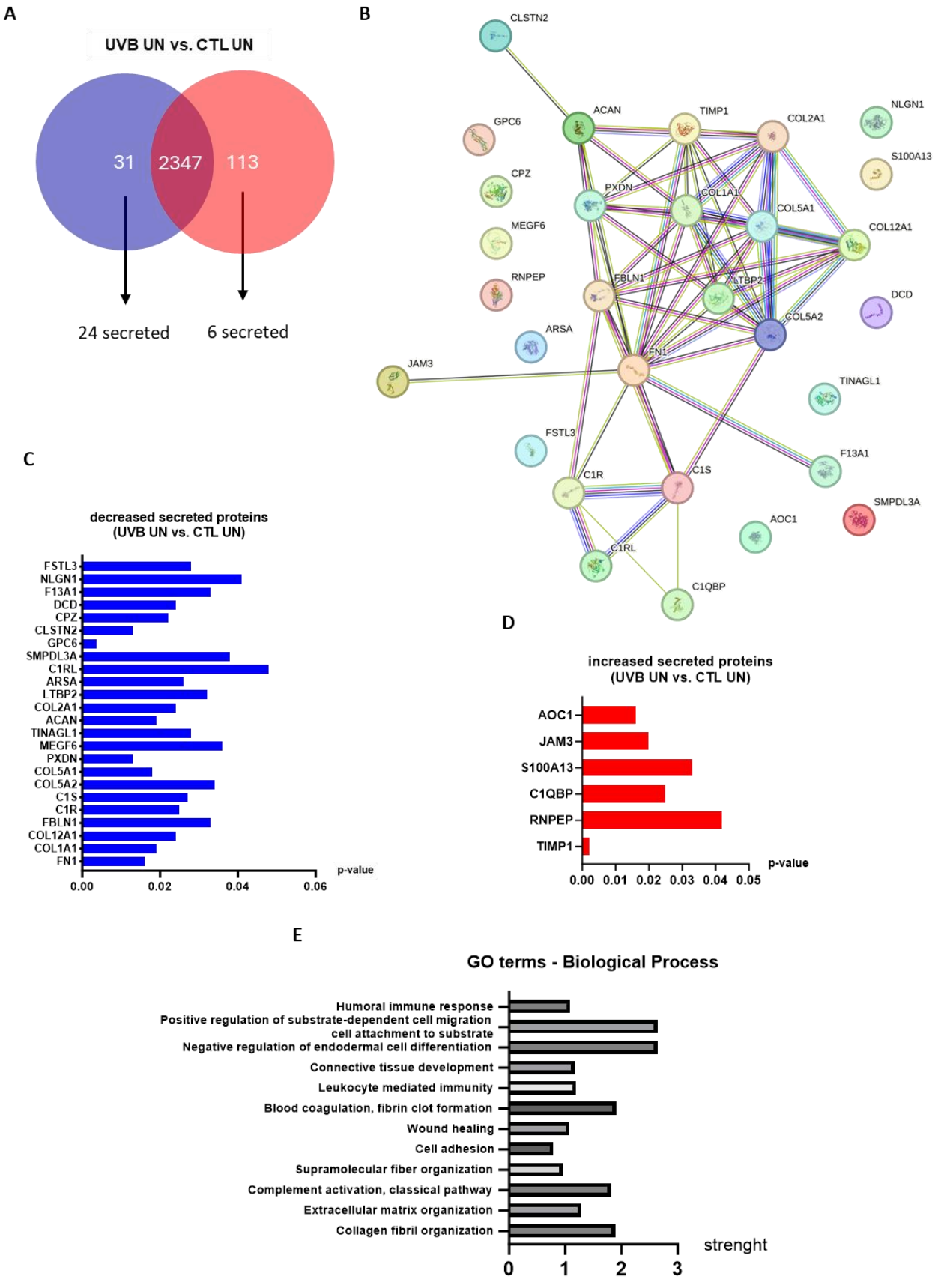


Figure 26. **Only few secreted proteins implicated in cell adhesion, ECM/collagen/fiber organization are differentially enriched in UVB-induced senescent NHDFs compared to CTL.**

A. Venn Diagram of protein clusters showing the quantitative profile between UVB UN and CTL UN identified proteins using Scaffold 5.0. Among them, 2347 proteins showed statistically insignificant changes across UVB and CTL conditions, whereas 113 were significantly increased only in UVB and 31 were significantly decreased. Among the latter, only 6 and 24 proteins, respectively, were identified as secreted proteins in the UniProt database.

B. Protein-protein interactions network of the 30 and down secreted proteins using STRING database. Green represents neighborhood evidence, blue cooccurrence evidence, purple experimental evidence, light blue database evidence, and black line coexpression.

C. Barplot of the differentially 24 decreased secreted proteins in untransfected UVB-induced senescent NHDFs.

D. Barplot of the differentially 6 increased secreted proteins in untransfected UVB-induced senescent NHDFs.

E. STRING analysis of Biological Processes (BP) Gene Ontology (GO) terms of differentially enriched secreted proteins in UVB vs. CTL NHDFs. Strength provides the protein-protein interaction score. Only terms with FDR < 0.05) are shown.

Data information: The Scaffold 5.0 quantitative profile was done using a *t*-test without corrections and a p-value significance threshold of 0.05**.**

When comparing UVB siNT vs. CTL siNT, we identified 10 common differentially enriched proteins (GPC6, COL2A1, C1RL, ACAN, MEGF6, COL5A1, COL5A2, C1S, C1R, and COL1A1) also found in the comparison of UVB UN vs. CTL UN.

In fact, we observed 26 negatively and 7 positively enriched secreted proteins in UVB siNT compared to CTL siNT (**Figure 27A and 27C-D**). Moreover, the STRING database once again established strong protein-protein interactions between most of these proteins, particularly for collagens (COL4A5, COL4A1, COL2A1, COL1A1, COL5A1, COL6A1) and Follistatin-related protein 1 (FSTL1) (**Figure 27B**) that are all decreased in UVB siNT compared to CTL siNT. Although 33 proteins appeared to be differentially secreted in UVB siNT vs. CTL siNT, the enrichment analysis using the STRING method with the GO database for the BP sub-family shared common terms with the comparison of UVB UN vs. CTL UN, such as “collagen fibril organization” and “negative endodermal cell differentiation” (**Figure 27E**).

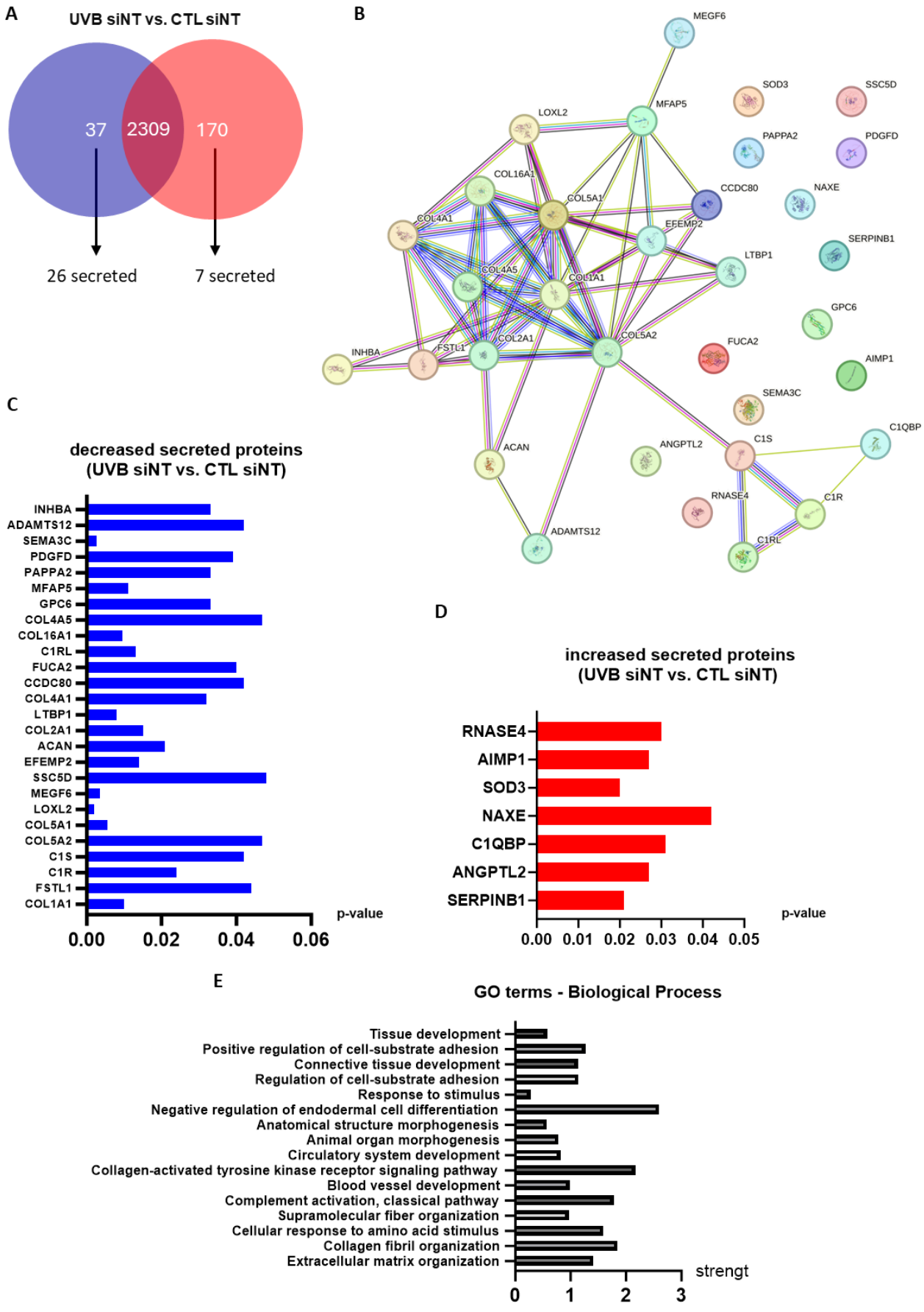


Figure 27. **Transfection with a siRNA seems to slightly modify the secretomic profile.**

A. Venn Diagram of protein clusters showing the quantitative profile between UVB siNT and CTL siNT identified proteins using Scaffold 5.0. Among them, 2309 proteins showed statistically insignificant changes across UVB and CTL conditions, whereas 170 were significantly increased only in UVB and 37 were significantly decreased. Among the latter, only 7 and 26 proteins, respectively, were identified as secreted proteins in the UniProt database.

B. Protein-protein interaction network of the 33 up and down secreted proteins using STRING database. Green represents neighborhood evidence, blue cooccurrence evidence, purple experimental evidence, light blue database evidence and black line coexpression.

C. Barplot of the differentially 26 decreased secreted proteins in UVB-induced senescent NHDFs transfected with a siNT.

D. Barplot of the differentially 7 increased secreted proteins in UVB-induced senescent NHDFs transfected with a siNT.

E. STRING analysis of Biological Processes (BP) Gene Ontology (GO) terms of differentially enriched secreted proteins in UVB vs. CTL NHDFs. Strength provides the protein-protein interaction score. Only terms with FDR < 0.05) are shown.

Data information: The Scaffold 5.0 quantitative profile was done using a *t*-test without corrections and a p-value significance threshold of 0.05**.**

The last comparison we examined was the difference between the secreted proteins from UVB-exposed NHDFs transfected with siATF6α and those from UVB-exposed NHDFs transfected with siNT (UVB siATF6α vs. UVB siNT).

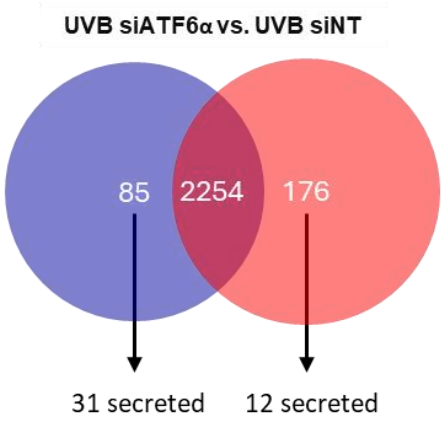
Protein clusters analysis revealed that, on the one hand, 2254 protein clusters were shared between UVB siATF6α and UVB siNT NHDFs, and on the other hand, 12 and 31 secreted proteins, respectively, were positively and negatively enriched in UVB siATF6α compared to UVB siNT NHDFs (**Figure 28A**). Surprisingly, more proteins appeared to be differentially enriched after the invalidation of ATF6α, and their interactions together seem to be stronger than in the two other comparisons (**Figure 28B**). This was particularly true for the extracellular matrix protein lumican (LUM), laminins, and laminin-associated proteins (LAMB1, LAM4, NID1, NID2).

Interestingly, when investigating more in detail the list of positively- and negatively enriched proteins, we identified one protein known as extracellular superoxide dismutase [Cu-Zn] (SOD3) whose secretion seems to increase in UVB siNT compared to CTL siNT and decreased in the comparison UVB siATF6α vs. UVB siNT (**Figure 27D and 29**). Moreover, SERPINH1 and CCL2 were significantly increased (**Figure 29B**).

Finally, on a more general note, the STRING method using the GO database for the BP sub-family on UVB siATF6α vs. UVB siNT revealed a significant enrichment of 45 terms. Many term descriptions were linked to “adhesion” and “ECM assembly/organization” as well as “morphogenesis/development”, “differentiation” and “migration” (**Figure 30**). Moreover, Molecular Function (MF) sub-family predicted significant enrichment in secreted proteins associated with for instance, “ECM constituent” and “laminin binding” (**Figure 31**).

Overall, although these results may not entirely reflect biological reality, they could lead to reflexion, especially if biological processes or pathways appear to be common with the already performed secretome analysis of NHDFs undergoing replicative senescence, inhibited or not for ATF6α. At least, our results may encourage the exploration of interrelated functions.

A



B

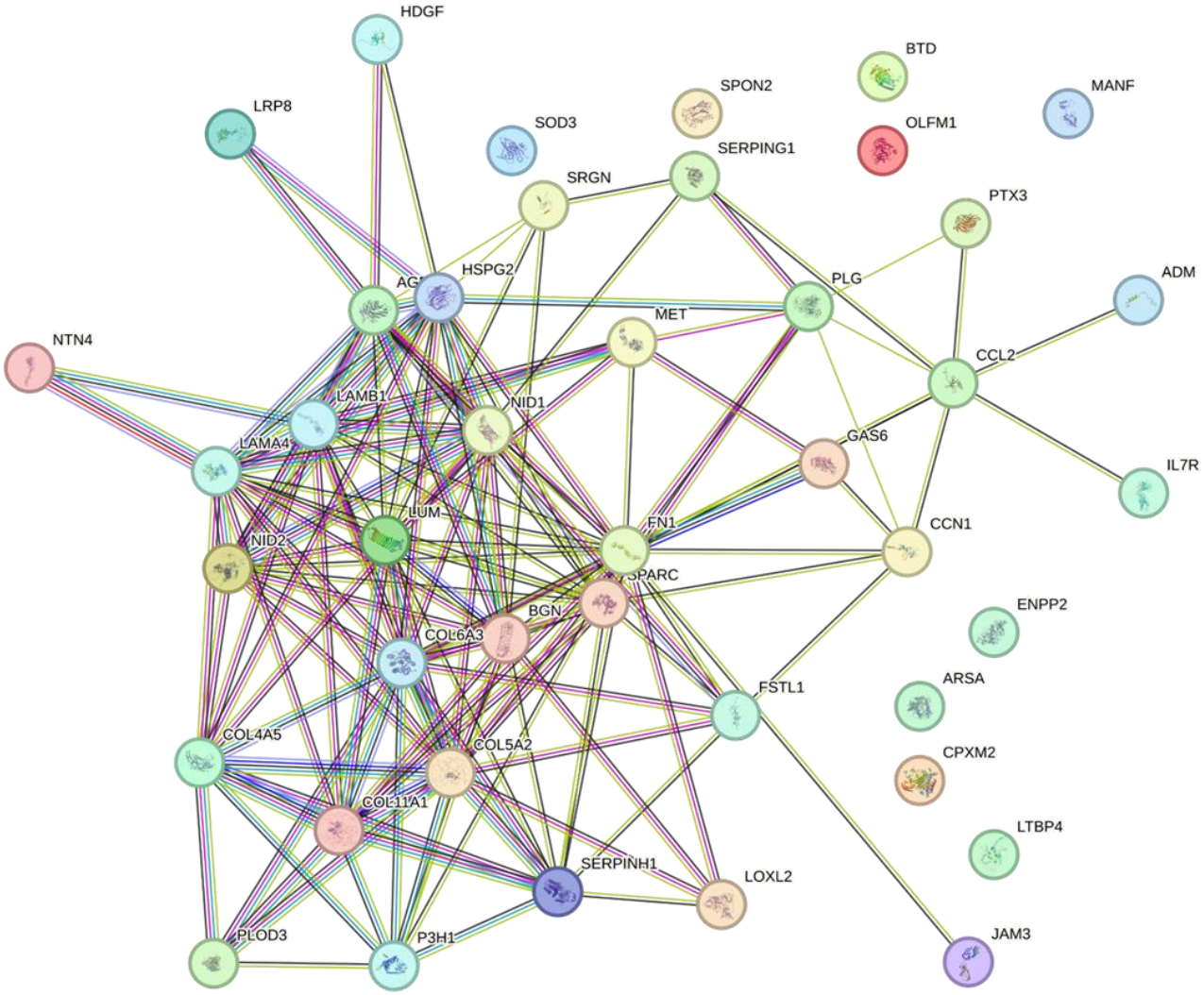


Figure 28. Invalidation of ATF6α in UVB-induced senescent NHDFs leads to a secretome profile containing more positively- and negatively-enriched secreted proteins.

A. Venn Diagram of protein clusters showing the quantitative profile between UVB siNT and UVB siATF6α identified proteins using Scaffold 5.0. Among them, 2254 proteins showed statistically insignificant changes across UVB and CTL conditions, whereas 176 were significantly increased only in UVB and 86 were significantly decreased. Among the latter, only 12 and 31 proteins, respectively, were identified as secreted proteins in the UniProt database.

B. Protein-protein interaction network of the 43 up and down secreted proteins using STRING database. Green represents neighborhood evidence, blue cooccurrence evidence, purple experimental evidence, light blue database evidence and black line coexpression.

Data information: The scaffold 5.0 quantitative profile was done using a *t*-test without corrections and a p-value significance threshold of 0.05.

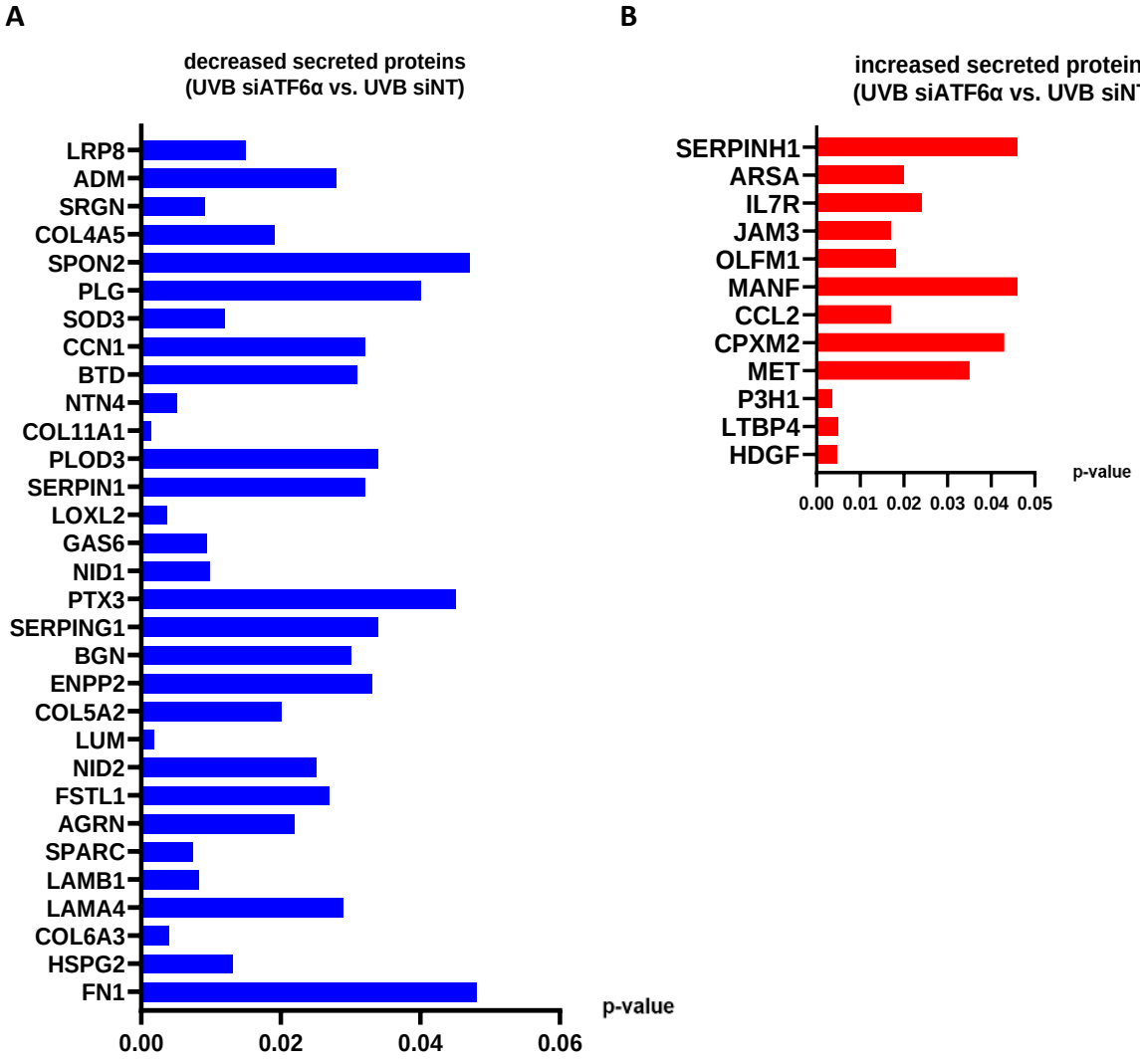


***Figure 29*. Invalidation of ATF6α in UVB-exposed NHDFs significantly modify the secretion of 43 proteins.**

A. Barplot of the differentially 31 decreased secreted proteins in UVB-induced senescent NHDFs transfected with a siATF6α.

B. Barplot of the differentially 12 increased secreted proteins in UVB-induced senescent NHDFs transfected with a siATF6α.

GO- Biological Process

#term ID	term description	strength
GO:0097241	Hematopoietic stem cell migration to bone marrow	2.27
GO:0070831	Basement membrane assembly	2.15
GO:0071711	Basement membrane organization	1.89
GO:0085029	Extracellular matrix assembly	1.83
GO:0030199	Collagen fibril organization	1.68
GO:0035987	Endodermal cell differentiation	1.5
GO:0008347	Glial cell migration	1.49
GO:0060711	Labyrinthine layer development	1.48
GO:0030198	Extracellular matrix organization	1.43
GO:0031589	Cell-substrate adhesion	1.23
GO:0001525	Angiogenesis	0.94
GO:0001568	Blood vessel development	0.92
GO:0030335	Positive regulation of cell migration	0.9
GO:0048514	Blood vessel morphogenesis	0.89
GO:0050878	Regulation of body fluid levels	0.88
GO:0007155	Cell adhesion	0.8
GO:0016477	Cell migration	0.79
GO:0048870	Cell motility	0.76
GO:0072359	Circulatory system development	0.76
GO:0009887	Animal organ morphogenesis	0.75
GO:0030334	Regulation of cell migration	0.75
GO:0035239	Tube morphogenesis	0.75
GO:0048646	Anatomical structure formation involved in morphogenesis	0.74
GO:0045597	Positive regulation of cell differentiation	0.73
GO:0030155	Regulation of cell adhesion	0.73
GO:0035295	Tube development	0.68
GO:0051336	Regulation of hydrolase activity	0.67
GO:0051241	Negative regulation of multicellular organismal process	0.66
GO:0009888	Tissue development	0.64
GO:2000026	Regulation of multicellular organismal development	0.64
GO:0051094	Positive regulation of developmental process	0.63
GO:0009653	Anatomical structure morphogenesis	0.58
GO:0048513	Animal organ development	0.57
GO:0051240	Positive regulation of multicellular organismal process	0.57
GO:0050793	Regulation of developmental process	0.55
GO:0048468	Cell development	0.55
GO:0007399	Nervous system development	0.54
GO:0048731	System development	0.53
GO:0051239	Regulation of multicellular organismal process	0.53
GO:0030154	Cell differentiation	0.49
GO:0048856	Anatomical structure development	0.47
GO:0032502	Developmental process	0.44
GO:0032501	Multicellular organismal process	0.36
GO:0016043	Cellular component organization	0.35
GO:0048519	Negative regulation of biological process	0.31

Figure 30. **Biological Processes enriched in UVB siATF6α vs. UVB siNT based on the list of the 43 secreted proteins.**

STRING analysis of Biological Processes (BPs) Gene Ontology (GO) terms of differentially enriched secreted proteins in UVB siATF6α vs. UVB siNT. Strength provides the protein-protein interaction score. Only terms with FDR < 0.05) are shown.

GO- Molecular function

#term ID	term description	strength
GO:0043237	laminin-1 binding	2.13
GO:0030021	Extracellular matrix structural constituent conferring compression resistance	1.97
GO:0050840	Extracellular matrix binding	1.77
GO:0030020	Extracellular matrix structural constituent conferring tensile strength	1.7
GO:0043236	Laminin binding	1.7
GO:0005201	Extracellular matrix structural constituent	1.67
GO:0005518	Collagen binding	1.63
GO:0043394	Proteoglycan binding	1.58
GO:0005178	Integrin binding	1.25
GO:0005539	Glycosaminoglycan binding	1.24
GO:0008201	Heparin binding	1.21
GO:1901681	Sulfur compound binding	1.08
GO:0061134	Peptidase regulator activity	1.01
GO:0005198	Structural molecule activity	0.93
GO:0005509	Calcium ion binding	0.89
GO:0044877	Protein-containing complex binding	0.68
GO:0005102	Signaling receptor binding	0.61

Figure 31. **Molecular functions enriched in UVB siATF6α vs. UVB siNT based on the list of the 43 secreted proteins.**

STRING analysis of Molecular Functions (MFs) Gene Ontology (GO) terms of differentially enriched secreted proteins in UVB siATF6α vs. UVB siNT. Strength provides the protein-protein interaction score. Only terms with FDR < 0.05) are shown.

Discussion and perspectives

Throughout this work, cellular senescence has been described as an adaptive response triggered by several stresses. Among physiological stressors, ultraviolet can induce premature senescence in the major skin cell types *in vitro*. However, the mechanisms underlying the establishment of UV-induced senescence remain to be understood.

In vivo, chronic exposure to UV rays is known to induce photoageing. This, results in specific histological consequences in the skin, overlapping with those of chronological ageing, which accelerates the appearance of age-related signs, such as wrinkles. Although cells expressing senescence markers accumulate with age in the different compartments of the skin, their contribution in skin ageing and particularly skin photoageing is still being discussed. Nonetheless, the chronic effect of their SASP seems to be implicated in the onset of age-related disorders and diseases.

Since its discovery, studies on cellular senescence have focused mainly on understanding the signalling pathways involved in the development of the phenotype and their role in its maintenance. For decades, the main goal in the field has been to identify key players, sometimes common to several inducers and cell types, with the aim of modulating the effects of senescent cells depending on the context.

Since then, several molecular regulators of the senescent phenotype have been identified. However, most of them are mainly involved in the development of the SASP and do not necessarily appear to be involved in the establishment of other biomarkers. Conversely, for a few years, endoplasmic reticulum (ER) stress and unfolded protein response (UPR) have been described as a signalling platform that seems to be associated with the major senescence hallmarks.

In this context, we investigated whether the UPR pathway could also be involved in the appearance of the senescent phenotype following repeated UVB exposures in dermal fibroblasts, with a particular interest in the composition and impact of the SASP.

A. Is the senescent phenotype induced by UVB exposures dependent on the UPR pathway?

Models of UV-induced senescence have been developed to mimic and study skin photoageing. These useful models have been established for all the wavelengths of ultraviolet light and are sometimes used in combination with drugs such as the combination of UVA with psoralen (W. Ma et al. 2002). In this study, we focused on UVB rays because they are the most energetic fraction of the sunlight encountered by the skin, and thus are the most damaging for the skin as they can penetrate until the upper dermis.

Among the models of stress-induced premature senescence (SIPS), the primary technical challenge lies in determining a dose that adequately induces senescence without causing toxicity, a constraint common to all stress agents. In UV-induced senescent models, the dose and number of exposures vary depending on the irradiation system and UV band utilized, as well as the cell type and origin (Chainiaux et al. 2002; Greussing et al. 2013; Zeng et al. 2014). In this work, we used dermal fibroblasts from two different origins: human dermal fibroblasts (HDFs or AG04431 cells, Coriell Institute for Medical Research, USA) and human primary dermal fibroblasts (NHDFs), isolated in our lab from foreskins of young donors. These two populations of fibroblasts exhibit different proliferative lifespans *in vitro*. Embryonic fibroblasts typically enter replicative senescence after 35-40 passages, whereas primary fibroblasts can continue to proliferate for over 80 passages. Consequently, although the UVB-induced senescent model for AG04431 consisting of UVB exposures at 250 mJ/cm² twice a day for five days has already been published (Debacq-Chainiaux et al. 2005), the UVB-induced senescent model using NHDFs needed optimisation. Therefore, the UVB dose was readjusted, and we set up a new model using sub-cytotoxic UVB exposures at 500 mJ/cm² twice a day for five days.

We then assessed in our new model using NHDFs whether the senescent phenotype was effectively induced after UVB exposures. We confirmed this through several observations: an increase in the size of fibroblasts exposed to UVB, as indicated by a significant increase in cell circularity and roundness index, enrichment in SA-βgal-positive cells, cell cycle arrest identified by a decrease in EdU incorporation and an

increased abundance of $p21^{CIP1}$, persistent DNA damage evidenced by the accumulation of 53BP1 foci accumulation in the nuclei, and finally, a significant increase in the expression of major SASP factors.

An important point to note about SIPS models is that it allows the study of a cellular population enriched in senescent cells, but not exclusively composed of senescent cells, such as in models of replicative senescence. In our model, if we consider the number of SA-βgal-positive NHDFs after UVB exposure, 51% of the cells appear positive, which means that 49% of cells are, either not senescent or are in an early phase of senescence induction, for which SA-βgal activity may not yet be evident (Halkoum et al. 2022). A possible technical improvement of this kind of senescence model would be to selectively sort out these well-established senescent cells and work only on them. To do so, cells can be sorted by flow cytometry according to SA-βgal activity, cell size and granularity (Goy et al. 2023). However, this protocol requires specific equipment with nozzles of an unusually large diameter, which was not available during this project.

Furthermore, the choice of a three-day timeframe to study the senescent phenotype was based not only on the enrichment of senescent cells in the population but also on the expression of UPR markers in this population. Indeed, our initial question was; Are UVB-induced senescent NHDFs associated with ER stress and UPR activation? We monitored the expression of several UPR-associated genes via RT-qPCR at one, two, three, and seven days after the last UVB exposure in HDFs and NHDFs. We observed that the UPR was indeed activated after UVB exposures on day one and that the expression of these markers was maximal three days after the last UVB exposure in both models (preliminary results).

Several studies have reported that UV exposures can induce ER stress, however, most of them often used higher doses of UV to rapidly induce important damage in cells and studied the link between, for example, UV, UPR, and autophagy (reviewed in; Bahamondes Lorca and Wu 2023). To date, only another study has demonstrated that UV-induced senescent cells are associated with ER stress and UPR but this model used UVC at 5 J/m² on human breast adenocarcinoma cell line MCF-7 (H. S. Kim et al. 2019). As UVC rays cannot penetrate the ozone layer, this model seems less relevant from a physiological point of view. However, interestingly, this study observed

that invalidation of ATF6α before UVC exposure prevents the increase of SA-βgal positive cells, but this senescence biomarker was the only one they investigated (H. S. Kim et al. 2019).

Other studies have explored the link between UPR and senescence, however no general conclusion can be drawn from them. Indeed, depending on the senescence inducer and the cell type, the involvement of one or several UPR branches in the appearance and maintenance of the senescent phenotype can vary. For instance, while the knockdown of ATF4 allows a significant reduction of SA-βgal positive cells in human melanocytes induced in senescence by H-RAS (Denoyelle et al. 2006), its invalidation in H-RAS-induced senescent primary murine keratinocytes increased SA-βgal positive cells (B. Zhu et al. 2014). Concerning the role of ATF6α in senescent cells, it appears that its invalidation in replicative senescent NHEKs and NHDFs, or the expression of its dominant negative form in H-RAS melanocytes resulted in a decreased percentage of SA-βgal positive cells (Drullion et al. 2018; Druelle et al. 2016; Cormenier et al. 2018; Denoyelle et al. 2006)

In our study, we demonstrated that silencing ATF6α using siRNA in NHDFs before the first UVB exposure, not only prevents the morphological changes but also reduces the increased percentage of SA-βgal positive cells and 53BP1 foci, as well as the overexpression of several SASP factors in UVB-induced senescent cells. In line with these observations, we obtained similar results using ATF6α chemical inhibitor ceapin A7 in HDFs, following the last UVB stress.

Interestingly, some of these effects were consistent between our model and replicative senescent NHDFs (Druelle et al. 2016). However, in their case, the invalidation of ATF6α led to a reversion of the replicative senescent phenotype previously established, whereas in our UVB-induced senescent model, we prevented its establishment.

For instance, they also observed a significant impact of ATF6α invalidation on the shape and size of senescent cells, as well as an expansion of the ER that we did not check in our model. Similar to the study of Cormenier *et al.,* they both examined the impact of ATF6α invalidation on the network of intermediate filaments by staining vimentin (Druelle et al. 2016; Cormenier et al. 2018), whereas in our model, we

investigated microfilaments. Considering that the cytoskeleton is made of both these fibres and microtubules, and that a microtubule-disrupting agent, TCD, induced ATF6α activation (Ho et al. 2015), this would suggest a larger role of ATF6α on the cytoskeleton of senescent cells. Furthermore, as the ER expansion and cell morphology changes have been described as being mediated through an ATF6α/COX2/PGE_2 in replicative senescent cells, it could be of interest to examine its potential implication in morphological changes in UVB-induced senescent NHDFs, starting by finding out whether COX2 is activated after UVB exposure.

Surprisingly, in most studies investigating the impact of ATF6α knockdown or inhibition on the senescent phenotype, little or no information is available on its effect on the cell cycle arrest. While in RS-NHDFs ATF6α knockdown did not impact the cell growth due to the strong telomere attrition that occurs within senescent fibroblasts (Druelle et al. 2016), in senescent NHEKs its invalidation and the one of PERK led to an increased post-senescence neoplastic emergence (PSNE) (Drullion et al. 2018). In our model, the silencing of ATF6α prior to UVB exposures seems to slightly increase the number of EdU-positive cells after UVB exposure. However, more generally, it seems difficult to draw a global conclusion on the impact of UPR on cell cycle arrest.

At present, linking the effects of ATF6α invalidation on these various biomarkers remains challenging. One of the logical outcomes of this project would be to identify a target upstream of ATF6α that could be the missing link and that could be easily targeted to prevent their appearance.

Our first hypothesis regarding the effect on the persistence of DNA damage and the regulation of SASP, particularly cytokines, suggested a potential link between ATF6α and the DDR. Indeed, there is a known association between SASP expression and some DDR proteins (Malaquin et al. 2020), notably ATM and CHK2, which appear to play a role in the initiation and maintenance of IL-6 and IL-8 secretion (Rodier et al. 2009). To investigate a hypothetical regulation of ATF6α on DDR proteins, such as ATM or CHK2, we assessed the protein abundance of both total and phosphorylated forms of ATM and CHK2 three days after the last UVB exposure. This first investigation revealed a slight increase in these two DDR proteins' abundances in UVB-induced senescent NHDFs, but the invalidation of ATF6α did not appear to impact it. Nevertheless, further experiments could confirm this initial observation, and shorter

time intervals after the UVB exposure could be considered for analysis since the DDR is strongly activated a few hours after the stresses before diminishing and leading, in the context of senescence to a persistent DDR (Fumagalli et al. 2014).

Then, we hypothesized another potential link between ATF6α and mTOR, which has not yet been investigated in our model. In fact, the mTORC pathway has already been shown to play a major role in the establishment of senescent biomarkers. For instance, activation of the PI3K/AKT/mTORC pathway in senescent endothelial cells, where no major morphological changes were observed, induced their enlargement (Bent, Gilbert, and Hemann 2016). In addition, mTORC is known to regulate the SASP (R.-M. Laberge et al. 2015; Herranz et al. 2015), and it has been demonstrated that the addition of rapamycin, an mTORC inhibitor, prior to H_2O_2-induced senescence, significantly reduced the number of SA-βgal positive cells. Moreover, in contexts other than senescence, it has been shown that under ER stress, ATF6α is responsible for mTORC activation (Allen and Seo 2018; Schewe and Aguirre-Ghiso 2008). Interestingly, a recent study observed that in human colon cancer cells, the ATF6α/mTORC axis is responsible for sustaining the expression level of HSP90 and stabilizing BRCA-1 (a protein involved in DNA homologous repair), allowing for protection against DNA damage accumulation and cell death (Benedetti et al. 2022). Furthermore, several studies revealed that multiple components of the DDR, such as CHK1, are HSP90 clients (reviewed in; Pennisi, Ascenzi, and di Masi 2015). Thus, investigating the link between ATF6α and mTOR in the context of UVB-induced senescence appears to be an intriguing avenue to explore.

Finally, in various studies examining the link between senescence and UPR, the question of whether UPR is a cause or a consequence of senescence is almost always raised (reviewed in; Abbadie and Pluquet 2020). In this study, we chose to transfect NHDFs with siRNA against either ATF6α, PERK or IRE1α the day before the first UVB stress. Thus, our results suggest that the invalidation of ATF6α could have a preventive effect on most of the senescence biomarkers. PERK appears to slightly prevent the increased proportion of SA-βgal positive cells, and IRE1α the overexpression of *IL-1β, IL-8* and *MMP-3* and both prevent the increased expression of $p16^{INK4}$. Given these results, we could imagine that even if the establishment of the senescent phenotype is dependent on one of the UPR branches, its maintenance can be controlled by one or

both. In our case, we could for example, hypothesize that while ATF6α may regulate the appearance of several senescence biomarkers, PERK and IRE1α might be necessary for maintaining the long-term cell cycle arrest. This notion could be supported by evidence showing that PERK activation provokes a decreased expression of cyclin D1 and promotes cell-cycle arrest (Brewer and Diehl 2000).

Finally, the question regarding the cause or consequence effect of UPR in our model using HDFs treated with chemical inhibitors of the UPR directly after the last UVB exposure remains challenging. In fact, we have never examined the presence or abundance of senescent cells during SIPS or immediately afterward. Given the enrichment of senescent cells we observed after three days after the last UVB exposure, it is highly likely that only few cells should already be established in senescence immediately after the last stress. Nevertheless, it would be interesting to delve deeper into this, notably to determine whether this model prevents the establishment of the senescent phenotype from spreading or if it can reverse it. Furthermore, it might be feasible to add the chemical inhibitors three days after the last UVB exposure, to investigate their potential for reversal, as observed in the case of fibroblasts undergoing replicative senescence (Druelle et al. 2016).

B Is the UPR activation associated with skin ageing?

Our results indicating a dependence on ATF6α for the establishment of the senescent phenotype induced *in vitro* by UVB in fibroblasts, naturally prompt the question of how these observations could be translated to *in vivo* reality. Depending on various factors, such as model constraints and cell types utilized, the biological effect observed *in vitro* may sometimes be exaggerated or underestimated. In such cases, it becomes crucial to broaden the range of models used to support the relevance of the result.

As mentioned earlier, the detection of senescent cells in tissue samples remains challenging, especially in tissues like the skin where multiple cell populations coexist. The high inter-heterogeneity between different skin senescent cell populations and potential intra-heterogeneity within each, make it difficult to identify all of them in *ex vivo* tissues (reviewed in; González-Gualda et al. 2021). For instance, a single-cell

RNA-seq (scRNA-seq) analysis of human skin from individuals of different ages identified eleven skin cell types with unique gene expression profiles (Zou et al. 2021). Interestingly, another study using scRNA-seq as well, observed ten major cell types in mouse skin exposed to UVB, including a population of senescent fibroblasts (Qiang and Dai 2024).

However, techniques such as immunofluorescence to specifically identify senescent cells in tissues remain complicated. More than a technical aspect of the limitation of antibodies that can be used, some well-established markers *in vitro* are just undetectable *in vivo*, such as morphological changes. Through this work, we could benefit from access to a biobank of dermal skin samples from both young and elderly donors and from areas sun-exposed or not, which has enabled us to answer the question: do senescent cells accumulate in the dermis with age, and does chronic sun-exposure increase their accumulation? In our study, we detected an accumulation of 53BP1-positive fibroblasts with age, which was already observed in other studies (Nassour et al. 2016; Bauwens et al. 2023). However, we also demonstrated that this accumulation was further exacerbated in sun-exposed areas regardless of age. To strengthen this observation, we could look at another senescent biomarker such as lamin B1, as its loss has been recognized as a robust biomarker, enabling the detection of senescent cells in photoaged skin (A. S. Wang et al. 2017).

Among the physiological responses induced by chronic exposure to UV light in the skin, senescence is certainly not the only adaptive response triggered. Interestingly, in their study, Gupta *et al.,* showed that in UVB-exposed mice skin, the level of p-eiF2 was significantly increased and that treatment with rosmarinic acid alleviated ER stress (Gupta et al. 2023).

However, little is known about the *in vivo* status of UPR during skin ageing. For the first time, we demonstrated that ER stress and UPR-related proteins, such as PDI, HERPUD1 and XBP1 were increased in dermis samples of elderly compared to young donors and that sun-exposure could enhance their expression.

At first sight, these results may seem surprising, as the commonly accepted idea is that the expression and activity of ER chaperones and UPR decline with age, resulting in a dysfunctional ER. For instance, Nuss *et al.* demonstrated a decreased activity of

PDI and BiP in aged mouse livers (Nuss et al. 2008). In addition, several recent studies investigating the impact of age on the ER stress response in rats, suggest a reduced protective response and an increased pro-apoptotic signalling mediated by a prolonged UPR activation (Hussain and Ramaiah 2007; Paz Gavilán et al. 2006). However, this interpretation is not always corroborated for the three branches of the UPR. For instance, Kristensen et *al.* demonstrated that aged mouse liver is associated with increased activity of the ATF6α pathway. In addition, they demonstrated that regular exercise did not alter this observation, although it prevented the age-associated decline in BiP and IRE1 protein levels, suggesting that the regulation of the UPR pathway could vary with ageing (Kristensen et al. 2017). Finally, a recent study showed that the loss of ATF6α in *C. elegans* extends lifespan (Burkewitz et al. 2020). Once again, it appears that depending on the organism, the context and the tissue, the response to prolonged ER stress can be specific. Although the activity of the UPR could be diminished during ageing, its chronic activation could also be mandatory to manage SASP proteins in a senescence-prone context.

Thus, exploring the status of the UPR during skin ageing, influenced by both intrinsic and extrinsic factors, reveals its significance, and deserves further investigation. This could involve expanding the number of markers studied to have a clearer understanding of the activity within each of the three UPR pathways. Additionally, examining the UPR in the epidermis could provide valuable insights, allowing for comparisons with dermal results, to identify potential similarities or differences in the UPR activity between epidermal and dermal cells from young and elderly individuals.

Then, our subsequent question was: under what physiological and/or pathophysiological conditions could targeting the UPR, and more specifically ATF6α or one of its effectors, be beneficial?

C. What could be the physiological or pathophysiological relevance of targeting ATF6α?

As described above, a clear remodelling of the secretory profile can be observed in cells undergoing senescence. Besides, due to its diverse composition, SASP can have pleiotropic effects on the cellular environment.

In this work, we observed that one of the effects of ATF6α invalidation on NHDFs exposed to UVB, was a modulation of their SASP composition when compared to siNT UVB-exposed NHDFs. This investigation was initially carried out using RT-qPCR on well-known SASP genes, revealing a decrease in the expression of specific interleukins and MMPs, notably *IL-1β*, *IL-8*, *MMP-1*, and *MMP-3* in siATF6α UVB-exposed NHDFs.

To date, despite efforts to characterize the senescence phenotype and the composition of SASP, understanding their biological effects remains challenging. The paracrine effects of the SASP have notably been associated with potential pro-tumorigenic activity (reviewed in; Schmitt, Wang, and Demaria 2022). In these studies, pro-tumorigenic effects was often linked to one individual SASP factors, such as IL-6, IL-8, and CCL-5, which have been described as SASP effectors increasing cancer cell proliferation (Ortiz-Montero, Londoño-Vallejo, and Vernot 2017; Eyman et al. 2009), as well as chemerin and CXCL-12, promoting cancer cells migration and invasion respectively (Farsam et al. 2016; Y. H. Kim et al. 2017).

In light of these observations and considering the impact of ATF6α invalidation on the mRNA expression of interleukins and on the secretion of IL-8, our aim was to investigate how the SASP of UVB-induced senescent NHDFs modified the migration and invasion of skin cancer cells, and how the modifications of the SASP composition induced by ATF6α invalidation would impact these effects.

To achieve this, we collected conditioned media (CM) from NHDFs exposed to UVB or not, and invalidated or not for ATF6α, three days after the last UVB exposure. Subsequently, we assessed the migration and invasion potential of three melanoma cell lines, when grown in contact with the CM from our different experimental conditions. Among the various types of cancer cells that can originate in the skin, we used melanoma cells because, unlike carcinomas, they could easily penetrate in the dermis (McCarter 2018). Therefore, in the context of ageing, they are more likely to encounter the SASP of senescent fibroblasts.

Firstly, we observed that the conditioned media from UVB-induced senescent NHDFs did not specifically induce a pro-migratory or pro-invasive effect of melanoma-derived

cells, regardless of the technique used (e.g. scratch wound healing using Incucyte or Boyden chambers).

Although a limited number of studies have investigated the role of conditioned media (CM) from UVB-exposed skin cells, Toutfaire *et al*. also reported no significant pro-proliferative, pro-migratory, or pro-invasive effects of CM from UVB-exposed human dermal fibroblasts (HDFs) in squamous cell carcinoma (Toutfaire et al. 2018). Additionally, Bauwens *et al.,* demonstrated that although the secreted levels of IL-6 and IL-8 are significantly increased three days after UVB exposure in normal human epidermal keratinocytes (NHEKs), the CM did not enhance the migratory capacities of A431 epidermoid carcinoma cells. Conversely, the CM harvested at seven days after UVB exposure accelerated the migratory capacities of A431 cells, even though at this time point, no significant increase in the secretion of either IL-6 or IL-8 was detected (Bauwens et al. 2022). These findings, along with ours, suggest that despite the presence of interleukins known in other models to induce pro-tumorigenic effects, their presence in the UVB SASP is not sufficient to elicit similar effects (Malaquin, Tu, and Rodier 2019) .

Surprisingly, during the use of conditioned media (CM), we noticed a lack of consensus regarding the percentage of CM added in experiments, or the ratio between the number of senescent secretory cells per cancer cell that should be considered. This potential bias, which could significantly influence the biological outcomes observed, is never addressed in the articles (Demaria 2019). Conversely, in this work, we consistently standardized the number of secretory cells to facilitate comparisons, as recommended (Malaquin, Tu, and Rodier 2019).

Secondly, we found that invalidation of ATF6α prior to UVB exposure does not result in the production of a SASP that increases the pro-migratory or pro-invasive properties of the conditioned media. Hence, our results suggest that the modified SASP produced by siATF6α UVB NHDFs does not possess the potential to enhance the pro-tumorigenic effects of UVB-conditioned media. As a comparison, a study demonstrated that inhibiting ATF6α with Ceapin-A7 in chondrocytes stimulated with TNFα, IL-17, or IFNγ reduced the pro-migratory capacities of HUVECs (M. Ma et al. 2021), but this was done on another context than senescence and on normal cells.

As targeting ATF6α does not seem particularly relevant in a pathophysiological context, we focused our attention on its potential effects in a physiological context. To address this, we wondered which types of skin cells could be affected by senescent fibroblasts' secretome and devised methods to demonstrate this *in vitro*.

Given the essential crosstalk between dermal fibroblasts and epidermal keratinocytes in maintaining skin homeostasis (Jevtić et al. 2020), we sought to investigate how the altered SASP of siATF6α UVB-exposed NHDFs influenced the behavior of keratinocytes. However, once again, we observed that most studies using senescent fibroblasts' secretome focused on skin carcinogenic events. For example, CM from senescent NHDFs has been shown to increase the post-senescence emergence of NHEKs (Malaquin et al. 2013), and promote EMT-like phenotype in primary keratinocytes from elderly donors (Tinaburri et al. 2021).

This is why we decided to use a more sophisticated model of reconstructed human epidermis, employing normal and young primary keratinocytes (Frankart et al. 2012), to assess the impact of conditioned media (CM) on the behavior of keratinocytes, particularly on their proliferation and differentiation.

As keratinocyte differentiation can be influenced by some inorganic salts and vitamins found in culture media (Bikle et al. 2001), the effects of fibroblast-cultured media named Basal Medium Eagle (BME) in the reconstruction of the epidermis were first assessed. A mix ratio of 50% Epilife : 50% BME showed neither observable differences in H&E staining nor in BrdU incorporation. Consistent with this observation, we added the same volume of conditioned media from fibroblasts to classical reconstruction media.

Surprisingly, and probably due to the technical constraints, very few similar models have been used in the literature, most relying on the addition of cytokines directly into the reconstruction medium to mimic skin diseases such as atopic dermatitis (Hubaux, Bastin, and Salmon 2018) or moving directly to a more complete model including fibroblasts and keratinocytes in a reconstructed human skin model. This last option has indeed been considered to answer our question, however, given the long period of reconstruction required (Bernerd and Asselineau 2008), this necessitates the fibroblasts to be constitutively invalidated for ATF6α. To this end, we attempted to

establish a knock-out NHDFs cell line using CRISPR-Cas9. However, our first attempt based on the electroporation of NHDFs with the CRISPR-Cas9 ribonucleoparticles, led to their premature replicative senescence, due to the limiting dilution. Hence, it would be useful to repeat the procedure, either by employing CRISPR-Cas9 lentiviral particles to avoid the limited dilution step or by using immortalized fibroblasts to avoid premature entry into replicative senescence.

Nevertheless, using our model of RHE, we noted that CM from UVB-induced senescent NHDFs resulted in epidermal thickening without affecting the expression of differentiation markers. Unexpectedly, we discovered that this mechanism was mediated by an ATF6α/IL-8 dependent mechanism, and we confirmed this effect by adding neutralizing antibody against IL-8 on keratinocytes forming colonies. These observations align perfectly with prior studies that have described the influence of IL-8 on keratinocytes' proliferation (Tuschil et al. 1992; Steude, Kulke, and Christophers 2002). However, these observations were made by the exogenous addition of IL-8 to the culture medium, whereas in our case, the quantity of IL-8 in the MC is surely more representative of what happens *in vivo*.

For the first time, we demonstrated that the transient presence of SASP from senescent fibroblasts may initially stimulate processes such as keratinocytes' proliferation in a physiological context.

Furthermore, while the SASP composition can influence physiological and pathophysiological outcomes in the microenvironment, the long-term effects of these modifications within the tissue environment could potentially reciprocally modulate the SASP composition (Giroud et al. 2023). This hypothesis which remains to be investigated, suggests that the communication of senescent cells may not be unidirectional, and reinforces the idea that establishment and maintenance of senescence probably depend on different effectors (**Figure 32**).

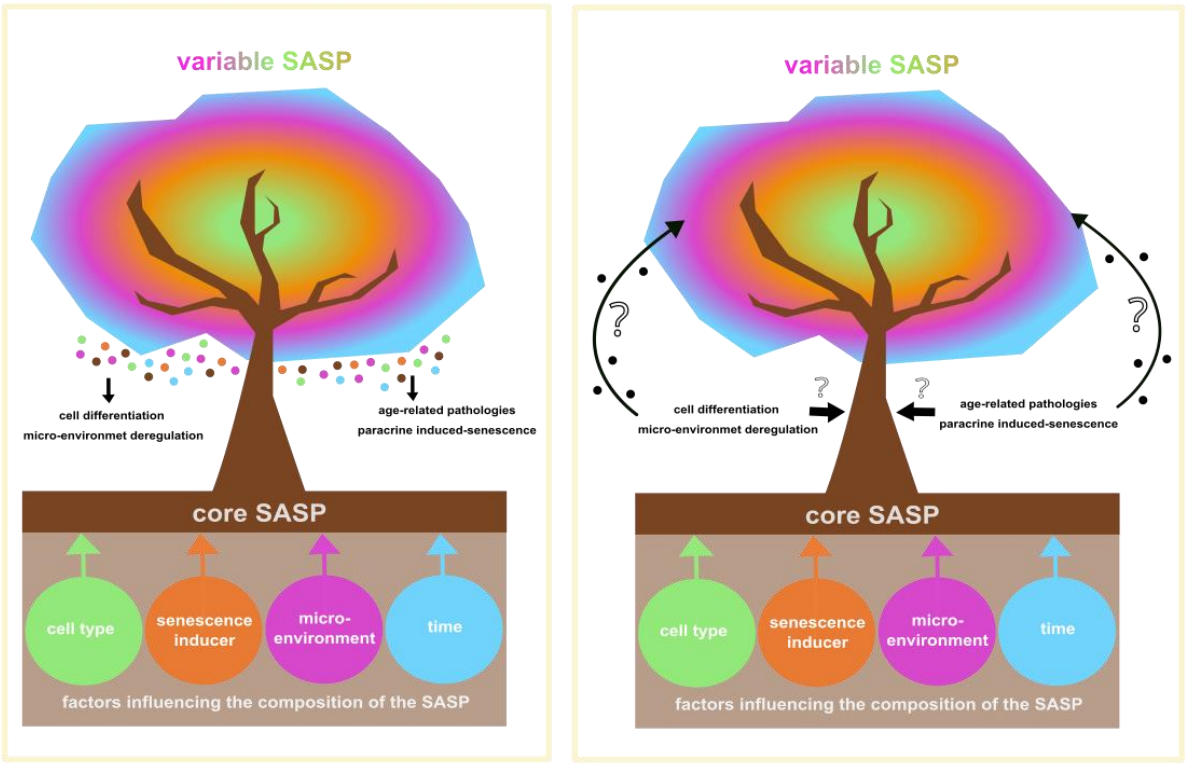


Figure 32. **The crosstalk between the senescent cells and their microenvironment through the SASP.** From Giroud *et al.,* 2023.

Finally, from the outset of the study, we were aware of the heterogeneity within our cell population of UVB-exposed NHDFs. Thus, it is possible that some of the potential effects of the UVB-induced SASP could be masked by the secretome of non-senescent or early senescent cells in our population that we cannot discriminate. Hence, to facilitate the identification of ATF6α effectors, we conducted transcriptomic and secretomic analyses.

Firstly, our transcriptomics results have enabled us to identify an association between the UVB profile and classical pathways of senescence, such as cell cycle regulation and chemokine signalling pathways. Indeed, the UVB-induced senescent signature appears to overlap with other signatures of senescence derived from various senescent models. Moreover, gene ontology predictions revealed the enrichment of biological processes that we successfully verified *in vitro*, thereby reinforcing our findings.

Analysis of the effect of siATF6α on the transcriptome is still ongoing. Surprisingly, our initial results seem to suggest that a few genes (less than 70) are differentially regulated by ATF6α in UVB-induced senescent NHDFs. As a comparison, another study demonstrated that in ATF6α-deficient mesenchymal stem cells at late passage, but not senescent, 48 genes were upregulated and 145 downregulated, and that the majority of these genes encode membrane and transmembrane proteins (S. Wang et al. 2018). In our senescent dataset, it appears that genes regulated by ATF6α also exhibit specificity in their function as most of them encode protein structures or are involved in the organization of the extracellular matrix.

Interestingly, even though we have encountered technical limitations in the development of the analysis of the UVB-induced senescence secretome, we also highlighted the potential impact of ATF6α on the extracellular matrix organization and structure. As we know that mass spectrometry would provide us with different information than the impact on cytokines, chemokines, and interleukins, we expected to identify other biological processes, and the intersection with results of transcriptomics is particularly intriguing.

Given the cross-referencing of these results at two levels of regulation (transcriptomic and secretomic), the impact of ATF6α on extracellular matrix (ECM) organization, composition, and effects deserves interest. Similar questions to those in our project could be investigated: How does the composition of matrices produced by senescent or young fibroblasts, whether deficient in ATF6α differ? Do the matrices impact the proliferation of cancer cells? Can they influence other cellular behaviors? Could they improve the visible effects of skin ageing?

In summary, our findings and the pursuit of these perspectives will enable us to confirm or refute the potential safe senomorphic nature of the ATF6α pathway, which would consist of inhibiting several of the senescent cell's characteristics by blocking their SASP, without killing them.

Conclusion

In conclusion, this work highlights the potential role of ATF6α in the establishment of the UVB-induced senescent phenotype and skin homeostasis maintenance. On the one hand, the effects mediated by the ATF6α/IL-8 deserve further investigation to understand the full regulatory mechanism. On the other, the contribution of ATF6α in maintaining skin homeostasis under stress could be expanded by the study of extracellular matrices which are known to be largely modified during skin ageing. In addition, our results suggest that targeting ATF6α would be a safe strategy that would not promote potential pro-tumorigenic effects (**Figure 33**).

Finally, since chemical inhibitors of ATF6α exist, they could be used in mouse models (young and old) to examine physiological and pathophysiological consequences on the skin and overall ageing.

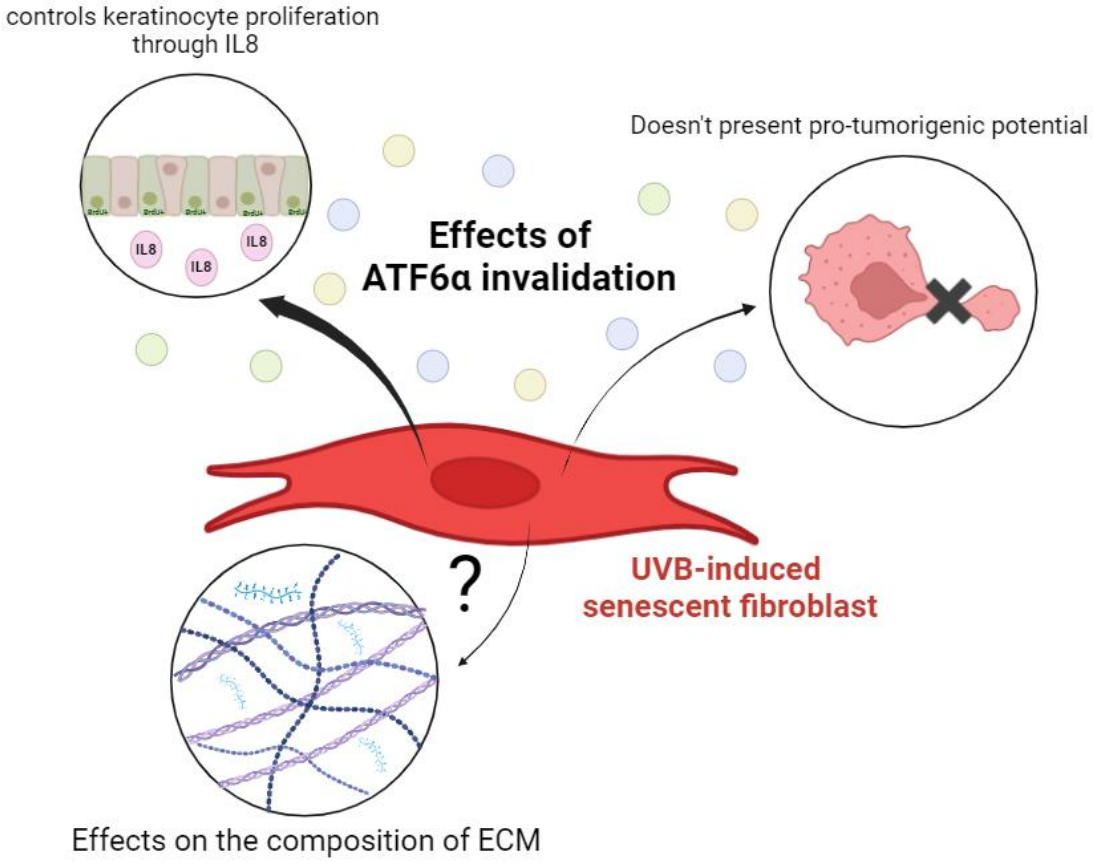


Figure 33. **The physiological and pathophysiological effects of ATF6α in the skin microenvironment.** Figure created using Biorender.

Bibliography

Abbadie, Corinne, and Olivier Pluquet. 2020. “Unfolded Protein Response (UPR) Controls Major Senescence Hallmarks.” *Trends in Biochemical Sciences* 45 (5): 371–74. https://doi.org/10.1016/J.TIBS.2020.02.005.

Acosta, Juan C., Ana O’Loghlen, Ana Banito, Maria V. Guijarro, Arnaud Augert, Selina Raguz, Marzia Fumagalli, et al. 2008. “Chemokine Signaling via the CXCR2 Receptor Reinforces Senescence.” *Cell* 133 (6): 1006–18. https://doi.org/10.1016/j.cell.2008.03.038.

Acosta, Juan Carlos, Ana Banito, Torsten Wuestefeld, Athena Georgilis, Peggy Janich, Jennifer P. Morton, Dimitris Athineos, et al. 2013. “A Complex Secretory Program Orchestrated by the Inflammasome Controls Paracrine Senescence.” *Nature Cell Biology* 15 (8): 978. https://doi.org/10.1038/NCB2784.

Adachi, Yusuke, Keisuke Yamamoto, Tetsuya Okada, Hiderou Yoshida, Akihiro Harada, and Kazutoshi Mori. 2008. “ATF6 Is a Transcription Factor Specializing in the Regulation of Quality Control Proteins in the Endoplasmic Reticulum.” *Cell Structure and Function* 33 (1): 75–89. https://doi.org/10.1247/csf.07044.

Adams, Peter D., Andre Ivanov, Jeff Pawlikowski, Indrani Manoharan, John Van Tuyn, David M. Nelson, Taranjit Singh Rai, et al. 2013. “Lysosome-Mediated Processing of Chromatin in Senescence.” *Journal of Cell Biology* 202 (1): 129–43. https://doi.org/10.1083/jcb.201212110.

Aird, Katherine M., Osamu Iwasaki, Andrew V. Kossenkov, Hideki Tanizawa, Nail Fatkhutdinov, Benjamin G. Bitler, Linh Le, et al. 2016. “HMGB2 Orchestrates the Chromatin Landscape of Senescence-Associated Secretory Phenotype Gene Loci.” *Journal of Cell Biology* 215 (3): 325–34. https://doi.org/10.1083/jcb.201608026.

Alcorta, D A, Y Xiong, D Phelps, G Hannon, D Beach, and J C Barrett. 1996. “Involvement of the Cyclin-Dependent Kinase Inhibitor P16 (INK4a) in Replicative Senescence of Normal Human Fibroblasts.” *Proceedings of the National Academy of Sciences of the United States of America* 93 (24): 13742–47. https://doi.org/10.1073/pnas.93.24.13742.

Alimbetov, Dauren, Terence Davis, Amy J.C. Brook, Lynne S. Cox, Richard G.A. Faragher, Talgat Nurgozhin, Zhaxybay Zhumadilov, and David Kipling. 2016. “Suppression of the Senescence-Associated Secretory Phenotype (SASP) in Human Fibroblasts Using Small Molecule Inhibitors of P38 MAP Kinase and MK2.” *Biogerontology* 17 (2): 305. https://doi.org/10.1007/S10522-015-9610-Z.

Allen, Dylan, and Jin Seo. 2018. “ER Stress Activates the TOR Pathway through Atf6.” *Journal of Molecular Signaling* 13: 1–9. https://doi.org/10.5334/1750-2187-13-1.

Alspach, Elise, Kevin C. Flanagan, Xianmin Luo, Megan K. Ruhland, Hui Huang, Ermira Pazolli, Maureen J. Donlin, et al. 2014. “P38MAPK Plays a Crucial Role in Stromal-Mediated Tumorigenesis.” *Cancer Discovery* 4 (6): 716–29. https://doi.org/10.1158/2159-8290.CD-13-0743.

Althubiti, M., L. Lezina, S. Carrera, R. Jukes-Jones, S. M. Giblett, A. Antonov, N. Barlev, et al. 2014. “Characterization of Novel Markers of Senescence and Their Prognostic Potential in Cancer.” *Cell Death & Disease 2014 5:11* 5 (11): e1528–e1528. https://doi.org/10.1038/cddis.2014.489.

Amor, Corina, Judith Feucht, Josef Leibold, Yu-Jui Ho, Changyu Zhu, Direna Alonso-Curbelo, Jorge Mansilla-Soto, et al. 2020. “Senolytic CAR T Cells Reverse Senescence-Associated Pathologies.” *Nature* 583: 127. https://doi.org/10.1038/s41586-020-2403-9.

Anand, Sanjay, Enakshi Chakrabarti, Hiroko Kawamura, Charles R Taylor, and Edward V Maytin. 2005. “Ultraviolet Light (UVB and UVA) Induces the Damage-Responsive Transcription Factor CHOP/Gadd153 in Murine and Human Epidermis: Evidence for a Mechanism Specific to Intact

Skin." *Journal of Investigative Dermatology* 125 (2): 323–33. https://doi.org/https://doi.org/10.1111/j.0022-202X.2005.23784.x.

Andrés Juan, Celia, José Manuel Pérez de la Lastra, Francisco J Plou, Eduardo Pérez-Lebeña, and Steffen Reinbothe. 2021. "Molecular Sciences The Chemistry of Reactive Oxygen Species (ROS) Revisited: Outlining Their Role in Biological Macromolecules (DNA, Lipids and Proteins) and Induced Pathologies." *Int. J. Mol. Sci* 22: 4642. https://doi.org/10.3390/ijms.

Araki, Kazutaka, and Kazuhiro Nagata. 2012. "Protein Folding and Quality Control in the ER." *Cold Spring Harbor Perspectives in Biology* 4 (8). https://doi.org/10.1101/cshperspect.a015438.

Arda, Oktay, Nadir Göksügür, and Yalçin Tüzün. 2014. "Basic Histological Structure and Functions of Facial Skin." *Clinics in Dermatology* 32 (1): 3–13. https://doi.org/10.1016/j.clindermatol.2013.05.021.

Avery, Simon V. 2011. "Molecular Targets of Oxidative Stress." *The Biochemical Journal* 434 (2): 201–10. https://doi.org/10.1042/BJ20101695.

Azazmeh, Narmen, Benjamin Assouline, Eitan Winter, Shmuel Ruppo, Yuval Nevo, Alexander Maly, Karen Meir, et al. 2020. "Chronic Expression of P16INK4a in the Epidermis Induces Wnt-Mediated Hyperplasia and Promotes Tumor Initiation." *Nature Communications* 11 (1): 1–13. https://doi.org/10.1038/s41467-020-16475-3.

B'chir, Wafa, Anne-Catherine Maurin, Valérie Carraro, Julien Averous, Céline Jousse, Yuki Muranishi, Laurent Parry, Georges Stepien, Pierre Fafournoux, and Alain Bruhat. 2013. "The EIF2α/ATF4 Pathway Is Essential for Stress-Induced Autophagy Gene Expression." *Nucleic Acids Research* 41 (16): 7683–99. https://doi.org/10.1093/nar/gkt563.

Baar, Marjolein P., Renata M.C. Brandt, Diana A. Putavet, Julian D.D. Klein, Kasper W.J. Derks, Benjamin R.M. Bourgeois, Sarah Stryeck, et al. 2017. "Targeted Apoptosis of Senescent Cells Restores Tissue Homeostasis in Response to Chemotoxicity and Aging." *Cell* 169 (1): 132-147.e16. https://doi.org/10.1016/j.cell.2017.02.031.

Bahamondes Lorca, Verónica A., and Shiyong Wu. 2023. "Ultraviolet Light, Unfolded Protein Response and Autophagy†." *Photochemistry and Photobiology* 99 (2): 498–508. https://doi.org/10.1111/php.13777.

Baker, Darren J, Bennett G Childs, Matej Durik, Melinde E Wijers, Cynthia J Sieben, Jian Zhong, Rachel A Saltness, et al. 2016. "Naturally Occurring P16 Ink4a-Positive Cells Shorten Healthy Lifespan." https://doi.org/10.1038/nature16932.

Baker, Darren J, Tobias Wijshake, Tamar Tchkonia, Nathan K Lebrasseur, Bennett G Childs, Bart Van De Sluis, James L Kirkland, and Jan M Van Deursen. 2011. "Clearance of P16 Ink4a-Positive Senescent Cells Delays Ageing-Associated Disorders." https://doi.org/10.1038/nature10600.

Banks, By Jim. 2020. "The 'Age' of Opportunity," no. APRIL 2017: 12–15.

Bansaccal, Nordin, Pauline Vieugue, Rahul Sarate, Yura Song, Esmeralda Minguijon, Yekaterina A. Miroshnikova, Dagmar Zeuschner, et al. 2023. *The Extracellular Matrix Dictates Regional Competence for Tumour Initiation*. *Nature*. Vol. 623. Springer US. https://doi.org/10.1038/s41586-023-06740-y.

Basisty, Nathan, Abhijit Kale, Ok Hee Jeon, Chisaka Kuehnemann, Therese Payne, Chirag Rao, Anja Holtz, et al. 2020. "A Proteomic Atlas of Senescence-Associated Secretomes for Aging Biomarker Development." *PLoS Biology* 18 (1): e3000599. https://doi.org/10.1371/JOURNAL.PBIO.3000599.

Bauwens, Emilie, Tom Parée, Sébastien Meurant, Inès Bouriez, Clotilde Hannart, Anne-Catherine Wéra, Alexis Khelfi, et al. 2022. "Senescence Induced by UVB in Keratinocytes Impairs Amino Acids Balance." *The Journal of Investigative Dermatology*, December. https://doi.org/10.1016/J.JID.2022.11.017.

Bell, Eugene, H Paul Ehrlich, David J Buttle, and Takako Nakatsuji. 1981. “Living Tissue Formed in Vitro and Accepted as Skin-Equivalent Tissue of Full Thickness.” *Science* 211 (4486): 1052–54. https://doi.org/10.1126/science.7008197.

Benedetti, Rossella, Maria Anele Romeo, Andrea Arena, Maria Saveria, Gilardini Montani, Livia Di Renzo, Gabriella D’orazi, and Mara Cirone. 2022. “ATF6 Prevents DNA Damage and Cell Death in Colon Cancer Cells Undergoing ER Stress.” *Cell Death Discovery 2022 8:1* 8 (1): 1–12. https://doi.org/10.1038/s41420-022-01085-3.

Benson, Erica K, Sam W Lee, and Stuart A Aaronson. 2010. “Role of Progerin-Induced Telomere Dysfunction in HGPS Premature Cellular Senescence.” *Journal of Cell Science* 123 (Pt 15): 2605–12. https://doi.org/10.1242/jcs.067306.

Bent, Eric H., Luke A. Gilbert, and Michael T. Hemann. 2016. “A Senescence Secretory Switch Mediated by PI3K/AKT/MTOR Activation Controls Chemoprotective Endothelial Secretory Responses.” *Genes and Development* 30 (16): 1811–21. https://doi.org/10.1101/gad.284851.116.

Bento-Lopes, Liliana, Luís C Cabaço, João Charneca, Matilde V Neto, Miguel C Seabra, and Duarte C Barral. 2023. “Melanin’s Journey from Melanocytes to Keratinocytes: Uncovering the Molecular Mechanisms of Melanin Transfer and Processing.” *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms241411289.

Berman, Brian, Clay J. Cockerell, and Panagiotis Zografos. 2013. “Pathobiology of Actinic Keratosis: Ultraviolet-Dependent Keratinocyte Proliferation.” *Journal of the American Academy of Dermatology* 68 (1 SUPPL.1): S10–19. https://doi.org/10.1016/j.jaad.2012.09.053.

Bernales, Sebastián, Sebastian Schuck, and Peter Walter. 2007. “ER-Phagy: Selective Autophagy of the Endoplasmic Reticulum.” *Autophagy* 3 (3): 285–87. https://doi.org/10.4161/auto.3930.

Berneburg, M, S Grether-Beck, V Kürten, T Ruzicka, K Briviba, H Sies, and J Krutmann. 1999. “Singlet Oxygen Mediates the UVA-Induced Generation of the Photoaging-Associated Mitochondrial Common Deletion.” *The Journal of Biological Chemistry* 274 (22): 15345–49. https://doi.org/10.1074/jbc.274.22.15345.

Berneburg, Mark, Heidi Plettenberg, Kathrin Medve-König, Annette Pfahlberg, H Gers-Barlag, Olaf Gefeller, and Jean Krutmann. 2004. “Induction of the Photoaging-Associated Mitochondrial Common Deletion in Vivo in Normal Human Skin.” *The Journal of Investigative Dermatology* 122 (5): 1277–83. https://doi.org/10.1111/j.0022-202X.2004.22502.x.

Bernerd, Françoise, and Daniel Asselineau. 2008. “An Organotypic Model of Skin to Study Photodamage and Photoprotection in Vitro.” *Journal of the American Academy of Dermatology* 58 (5 Suppl 2): S155-9. https://doi.org/10.1016/j.jaad.2007.08.050.

Bertolotti, A, Y Zhang, L M Hendershot, H P Harding, and D Ron. 2000. “Dynamic Interaction of BiP and ER Stress Transducers in the Unfolded-Protein Response.” *Nature Cell Biology* 2 (6): 326–32. https://doi.org/10.1038/35014014.

Bertrand-Vallery, Véronique, Nathalie Belot, Marc Dieu, Edouard Delaive, Noëlle Ninane, Catherine Demazy, Martine Raes, et al. 2010. “Proteomic Profiling of Human Keratinocytes Undergoing UVB-Induced Alternative Differentiation Reveals TRIpartite Motif Protein 29 as a Survival Factor.” *PLoS ONE* 5 (5). https://doi.org/10.1371/JOURNAL.PONE.0010462.

Bienkowska, Agata, Günter Raddatz, Jörn Söhle, Boris Kristof, Henry Völzke, Stefan Gallinat, Frank Lyko, et al. 2023. “Development of an Epigenetic Clock to Predict Visual Age Progression of Human Skin.” *Frontiers in Aging* 4 (January): 1–13. https://doi.org/10.3389/fragi.2023.1258183.

Bikle, D. D., D. Ng, C. L. Tu, Y. Oda, and Z. Xie. 2001. “Calcium- and Vitamin D-Regulated Keratinocyte Differentiation.” *Molecular and Cellular Endocrinology* 177 (1–2): 161–71. https://doi.org/10.1016/S0303-7207(01)00452-X.

Biran, Anat, Lior Zada, Paula Abou Karam, Ezra Vadai, Lior Roitman, Yossi Ovadya, Ziv Porat, and

Valery Krizhanovsky. 2017. “Quantitative Identification of Senescent Cells in Aging and Disease.” *Aging Cell* 16 (4): 661–71. https://doi.org/10.1111/acel.12592.

Birch, Jodie, and Jesús Gil. 2020. “Senescence and the SASP: Many Therapeutic Avenues.” *Genes & Development* 34 (23–24): 1565–76. https://doi.org/10.1101/GAD.343129.120.

Birlea, Stanca A, Ying Jin, Dorothy C Bennett, Deborah M Herbstman, Margaret R Wallace, Wayne T McCormack, E Helen Kemp, et al. 2011. “Comprehensive Association Analysis of Candidate Genes for Generalized Vitiligo Supports XBP1, FOXP3, and TSLP.” *The Journal of Investigative Dermatology* 131 (2): 371–81. https://doi.org/10.1038/jid.2010.337.

Blackwood, Erik A., Khalid Azizi, Donna J. Thuerauf, Ryan J. Paxman, Lars Plate, Jeffery W. Kelly, R. Luke Wiseman, and Christopher C. Glembotski. 2019. “Pharmacologic ATF6 Activation Confers Global Protection in Widespread Disease Models by Reprograming Cellular Proteostasis.” *Nature Communications* 10 (1): 1–16. https://doi.org/10.1038/s41467-018-08129-2.

Blanpain, Cédric, and Elaine Fuchs. 2006. “Epidermal Stem Cells of the Skin.” *Annual Review of Cell and Developmental Biology* 22: 339–73. https://doi.org/10.1146/annurev.cellbio.22.010305.104357.

Blanpain, Cédric, and Elaine Fuchs. 2009. “Epidermal Homeostasis: A Balancing Act of Stem Cells in the Skin.” *Nature Reviews Molecular Cell Biology* 10 (3): 207–17. https://doi.org/10.1038/nrm2636.

Blazanin, Nicholas, Jeongin Son, Alayna B. Craig-Lucas, Christian L. John, Kyle J. Breech, Michael A. Podolsky, and Adam B. Glick. 2017. “ER Stress and Distinct Outputs of the IRE1α RNase Control Proliferation and Senescence in Response to Oncogenic Ras.” *Proceedings of the National Academy of Sciences* 114 (37): 9900–9905. https://doi.org/10.1073/PNAS.1701757114.

Blond-Elguindi, Sylvie, Anne M. Fourie, Joseph F. Sambrook, and Mary Jane H. Gething. 1993. “Peptide-Dependent Stimulation of the ATPase Activity of the Molecular Chaperone BiP Is the Result of Conversion of Oligomers to Active Monomers.” *Journal of Biological Chemistry* 268 (17): 12730–35. https://doi.org/10.1016/s0021-9258(18)31449-2.

Bock, Stephanie, André Said, Gerrit Müller, Monika Schäfer-Korting, Christian Zoschke, and Günther Weindl. 2018. “Characterization of Reconstructed Human Skin Containing Langerhans Cells to Monitor Molecular Events in Skin Sensitization.” *Toxicology in Vitro* 46: 77–85. https://doi.org/https://doi.org/10.1016/j.tiv.2017.09.019.

Boilan, Emmanuelle, Virginie Winant, Elise Dumortier, Jean Pascal Piret, François Bonfitto, Heinz D. Osiewacz, Florence Debacq-Chainiaux, and Olivier Toussaint. 2013. “Role of P38MAPK and Oxidative Stress in Copper-Induced Senescence.” *Age* 35 (6): 2255–71. https://doi.org/10.1007/s11357-013-9521-3.

Bolland, Hannah, Tiffany S. Ma, Syafiq Ramlee, Kristijan Ramadan, and Ester M. Hammond. 2021. “Links between the Unfolded Protein Response and the DNA Damage Response in Hypoxia: A Systematic Review.” *Biochemical Society Transactions* 49 (3): 1251–63. https://doi.org/10.1042/BST20200861.

Bommiasamy, Hemamalini, Sung Hoon Back, Paolo Fagone, Kyungho Lee, Sasha Meshinchi, Elizabeth Vink, Rungtawan Sriburi, et al. 2009. “ATF6alpha Induces XBP1-Independent Expansion of the Endoplasmic Reticulum.” *Journal of Cell Science* 122 (Pt 10): 1626–36. https://doi.org/10.1242/jcs.045625.

Bonnans, Caroline, Jonathan Chou, and Zena Werb. 2014. “Remodelling the Extracellular Matrix in Development and Disease.” *Nature Reviews. Molecular Cell Biology* 15 (12): 786–801. https://doi.org/10.1038/nrm3904.

Borghesan, Michela, Juan Fafiá N-Labora, Olga Eleftheriadou, Anna Vossenkamper, Daniel Muñ Oz-Espin, Ana O’, and Loghlen Correspondence. 2019. “Small Extracellular Vesicles Are Key Regulators of Non-Cell Autonomous Intercellular Communication in Senescence via the Interferon Protein IFITM3.” *Cell Reports* 27: 3956–71.

https://doi.org/10.1016/j.celrep.2019.05.095.

Braig, Melanie, Soyoung Lee, Christoph Loddenkemper, Cornelia Rudolph, Antoine H.F.M. Peters, Brigitte Schlegelberger, Harald Stein, Bernd Dörken, Thomas Jenuwein, and Clemens A. Schmitt. 2005. “Oncogene-Induced Senescence as an Initial Barrier in Lymphoma Development.” *Nature* 436 (7051): 660–65. https://doi.org/10.1038/nature03841.

Branisteanu, Daciana Elena, Diana Stefania Dirzu, Mihaela Paula Toader, Daniel Constantin Branisteanu, Alin Codrut Nicolescu, Ilarie Brihan, Camelia Margareta Bogdanici, et al. 2022. “Phototherapy in Dermatological Maladies (Review).” *Experimental and Therapeutic Medicine* 23 (4): 259. https://doi.org/10.3892/etm.2022.11184.

Brash, Douglas E., Jeffrey A. Rudolph, Jeffrey A. Simon, Anthony Lin, Gregory J. Mckenna, Howard P. Baden, Alan J. Halperin, and Jan Pontén. 1991. “A Role for Sunlight in Skin Cancer: UV-Induced P53 Mutations in Squamous Cell Carcinoma.” *Proceedings of the National Academy of Sciences of the United States of America* 88 (22): 10124–28. https://doi.org/10.1073/pnas.88.22.10124.

Breitkreutz, Dirk, Nicolae Mirancea, and Roswitha Nischt. 2009. “Basement Membranes in Skin: Unique Matrix Structures with Diverse Functions?” *Histochemistry and Cell Biology* 132 (1): 1–10. https://doi.org/10.1007/s00418-009-0586-0.

Breitling, Jörg, and Markus Aebi. 2013. “N-Linked Protein Glycosylation in the Endoplasmic Reticulum.” *Cold Spring Harbor Perspectives in Biology* 5 (8). https://doi.org/10.1101/cshperspect.a013359.

Breugel, H H van, and P R Bär. 1992. “Power Density and Exposure Time of He-Ne Laser Irradiation Are More Important than Total Energy Dose in Photo-Biomodulation of Human Fibroblasts in Vitro.” *Lasers in Surgery and Medicine* 12 (5): 528–37. https://doi.org/10.1002/lsm.1900120512.

Brewer, J W, and J A Diehl. 2000. “PERK Mediates Cell-Cycle Exit during the Mammalian Unfolded Protein Response.” *Proceedings of the National Academy of Sciences of the United States of America* 97 (23): 12625–30. https://doi.org/10.1073/pnas.220247197.

Brown, Marishka K., and Nirinjini Naidoo. 2012. “The Endoplasmic Reticulum Stress Response in Aging and Age-Related Diseases.” *Frontiers in Physiology* 3. https://doi.org/10.3389/FPHYS.2012.00263.

Brunk, U, J L Ericsson, J Ponten, and B Westermark. 1973. “Residual Bodies and ‘Aging’ in Cultured Human Glia Cells. Effect of Entrance into Phase 3 and Prolonged Periods of Confluence.” *Experimental Cell Research* 79 (1): 1–14.

Buettner, Dan, and Sam Skemp. 2016. “Blue Zones: Lessons From the World’s Longest Lived.” *American Journal of Lifestyle Medicine* 10 (5): 318–21. https://doi.org/10.1177/1559827616637066.

Bulteau, Anne-Laure, Marielle Moreau, Carine Nizard, and Bertrand Friguet. 2002. “Impairment of Proteasome Function upon UVA- and UVB-Irradiation of Human Keratinocytes.” *Free Radical Biology & Medicine* 32 (11): 1157–70. https://doi.org/10.1016/s0891-5849(02)00816-x.

Buonanno, Manuela, Veljko Grilj, and David J Brenner. 2019. “Biological Effects in Normal Cells Exposed to FLASH Dose Rate Protons.” *Radiotherapy and Oncology : Journal of the European Society for Therapeutic Radiology and Oncology* 139 (October): 51–55. https://doi.org/10.1016/j.radonc.2019.02.009.

Burkewitz, Kristopher, Gaomin Feng, Sneha Dutta, Charlotte A Kelley, Michael Steinbaugh, Erin J Cram, and William B Mair. 2020. “Atf-6 Regulates Lifespan through ER-Mitochondrial Calcium Homeostasis.” *Cell Reports* 32 (10): 108125. https://doi.org/10.1016/j.celrep.2020.108125.

Byers, H Randolph, Soniya Maheshwary, Dana M Amodeo, and Sarah G Dykstra. 2003. “Role of Cytoplasmic Dynein in Perinuclear Aggregation of Phagocytosed Melanosomes and Supranuclear Melanin Cap Formation in Human Keratinocytes.” *The Journal of Investigative Dermatology* 121 (4): 813–20. https://doi.org/10.1046/j.1523-1747.2003.12481.x.

Bygrave, F L, and A Benedetti. 1996. “What Is the Concentration of Calcium Ions in the Endoplasmic Reticulum?” *Cell Calcium* 19 (6): 547–51. https://doi.org/10.1016/s0143-4160(96)90064-0.

Byrd, Allyson L, Yasmine Belkaid, and Julia A Segre. 2018. “The Human Skin Microbiome.” *Nature Reviews Microbiology* 16 (3): 143–55. https://doi.org/10.1038/nrmicro.2017.157.

Cadet, Jean, André Grand, and Thierry Douki. 2015. “Solar UV Radiation-Induced DNA Bipyrimidine Photoproducts: Formation and Mechanistic Insights.” *Topics in Current Chemistry* 356: 249–75. https://doi.org/10.1007/128_2014_553.

Cadet, Jean, Evelyne Sage, and Thierry Douki. 2005. “Ultraviolet Radiation-Mediated Damage to Cellular DNA.” *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis* 571 (1-2 SPEC. ISS.): 3–17. https://doi.org/10.1016/j.mrfmmm.2004.09.012.

Calfon, Marcella, Huiqing Zeng, Fumihiko Urano, Jeffery H Till, Stevan R Hubbard, Heather P Harding, Scott G Clark, and David Ron. 2002. “IRE1 Couples Endoplasmic Reticulum Load to Secretory Capacity by Processing the XBP-1 MRNA.” *Nature* 415 (6867): 92–96. https://doi.org/10.1038/415092a.

Calì, Tito, Carmela Galli, Silvia Olivari, and Maurizio Molinari. 2008. “Segregation and Rapid Turnover of EDEM1 by an Autophagy-like Mechanism Modulates Standard ERAD and Folding Activities.” *Biochemical and Biophysical Research Communications* 371 (3): 405–10. https://doi.org/https://doi.org/10.1016/j.bbrc.2008.04.098.

Campisi, Judith, and Fabrizio D’Adda Di Fagagna. 2007. “Cellular Senescence: When Bad Things Happen to Good Cells.” *Nature Reviews Molecular Cell Biology 2007 8:9* 8 (9): 729–40. https://doi.org/10.1038/nrm2233.

Cao, He, Panpan Yang, Jia Liu, Yan Shao, Honghao Li, Pinglin Lai, Hong Wang, et al. 2023. “MYL3 Protects Chondrocytes from Senescence by Inhibiting Clathrin-Mediated Endocytosis and Activating of Notch Signaling.” *Nature Communications* 14 (1): 1–13. https://doi.org/10.1038/s41467-023-41858-7.

Caramelo, Julio J, Olga A Castro, Leonardo G Alonso, Gonzalo De Prat-Gay, and Armando J Parodi. 2003. “UDP-Glc:Glycoprotein Glucosyltransferase Recognizes Structured and Solvent Accessible Hydrophobic Patches in Molten Globule-like Folding Intermediates.” *Proceedings of the National Academy of Sciences of the United States of America* 100 (1): 86–91. https://doi.org/10.1073/pnas.262661199.

Castellani, Giorgia, Mariachiara Buccarelli, Maria Beatrice Arasi, Stefania Rossi, Maria Elena Pisanu, Maria Bellenghi, Carla Lintas, and Claudio Tabolacci. 2023. “BRAF Mutations in Melanoma: Biological Aspects, Therapeutic Implications, and Circulating Biomarkers.” *Cancers* 15 (16). https://doi.org/10.3390/cancers15164026.

Castro, Patricia, Dipak Giri, Dolores Lamb, and Michael Ittmann. 2003. “Cellular Senescence in the Pathogenesis of Benign Prostatic Hyperplasia.” *The Prostate* 55 (1): 30–38. https://doi.org/10.1002/PROS.10204.

Cecco, Marco De, Takahiro Ito, Anna P. Petrashen, Amy E. Elias, Nicholas J. Skvir, Steven W. Criscione, Alberto Caligiana, et al. 2019. “L1 Drives IFN in Senescent Cells and Promotes Age-Associated Inflammation.” *Nature 2019 566:7742* 566 (7742): 73–78. https://doi.org/10.1038/s41586-018-0784-9.

Celli, A, D S Mackenzie, D S Crumrine, C L Tu, M Hupe, D D Bikle, P M Elias, and T M Mauro. 2011. “Endoplasmic Reticulum Ca2+ Depletion Activates XBP1 and Controls Terminal Differentiation in Keratinocytes and Epidermis.” *The British Journal of Dermatology* 164 (1): 16–25. https://doi.org/10.1111/j.1365-2133.2010.10046.x.

Chaib, Selim, Tamar Tchkonia, and James L. Kirkland. 2022. “Cellular Senescence and Senolytics: The Path to the Clinic.” *Nature Medicine* 28 (8): 1556–68. https://doi.org/10.1038/s41591-022-01923-y.

Chainiaux, Florence, Joao Pedro Magalhaes, François Eliaers, José Remacle, and Olivier Toussaint. 2002. “UVB-Induced Premature Senescence of Human Diploid Skin Fibroblasts.” *International Journal of Biochemistry and Cell Biology* 34 (11): 1331–39. https://doi.org/10.1016/S1357-2725(02)00022-5.

Chalil, Sreeda, Nicolas Pierre, Astrid D. Bakker, Ralph J. Manders, Annelies Pletsers, Marc Francaux, Jenneke Klein-Nulend, Richard T. Jaspers, and Louise Deldicque. 2015. “Aging Related ER Stress Is Not Responsible for Anabolic Resistance in Mouse Skeletal Muscle.” *Biochemical and Biophysical Research Communications* 468 (4): 702–7. https://doi.org/10.1016/j.bbrc.2015.11.019.

Chang, B D, E V Broude, M Dokmanovic, H Zhu, A Ruth, Y Xuan, E S Kandel, E Lausch, K Christov, and I B Roninson. 1999. “A Senescence-like Phenotype Distinguishes Tumor Cells That Undergo Terminal Proliferation Arrest after Exposure to Anticancer Agents.” *Cancer Research* 59 (15): 3761–67.

Chang, Jianhui, Yingying Wang, Lijian Shao, Remi Martin Laberge, Marco Demaria, Judith Campisi, Krishnamurthy Janakiraman, et al. 2016. “Clearance of Senescent Cells by ABT263 Rejuvenates Aged Hematopoietic Stem Cells in Mice.” *Nature Medicine* 22 (1): 78–83. https://doi.org/10.1038/nm.4010.

Chen, Dan Dan, Xuyan Peng, Yuxuan Wang, Mingjun Jiang, Mengjiao Xue, Guohui Shang, Xuhui Liu, et al. 2021. “HSP90 Acts as a Senomorphic Target in Senescent Retinal Pigmental Epithelial Cells.” *Aging (Albany NY)* 13 (17): 21547. https://doi.org/10.18632/AGING.203496.

Chen, Hongshan, Penelope D. Ruiz, Wendy M. McKimpson, Leonid Novikov, Richard N. Kitsis, and Matthew J. Gamble. 2015. “MacroH2A1 and ATM Play Opposing Roles in Paracrine Senescence and the Senescence-Associated Secretory Phenotype.” *Molecular Cell* 59 (5): 719–31. https://doi.org/10.1016/j.molcel.2015.07.011.

Chen, Q. M., V. C. Tu, J. Catania, M. Burton, O. Toussaint, and T. Dilley. 2000. “Involvement of Rb Family Proteins, Focal Adhesion Proteins and Protein Synthesis in Senescent Morphogenesis Induced by Hydrogen Peroxide.” *Journal of Cell Science* 113 (22): 4087–97. https://doi.org/10.1242/jcs.113.22.4087.

Chen, Stella X, Ling-Juan Zhang, and Richard L Gallo. 2019. “Dermal White Adipose Tissue: A Newly Recognized Layer of Skin Innate Defense.” *Journal of Investigative Dermatology* 139 (5): 1002–9. https://doi.org/https://doi.org/10.1016/j.jid.2018.12.031.

Chen, Xi, Jingshi Shen, and Ron Prywes. 2002. “The Luminal Domain of ATF6 Senses Endoplasmic Reticulum (ER) Stress and Causes Translocation of ATF6 from the Er to the Golgi.” *Journal of Biological Chemistry* 277 (15): 13045–52. https://doi.org/10.1074/jbc.M110636200.

Chen, Zhenbang, Lloyd C. Trotman, David Shaffer, Hui Kuan Lin, Zohar A. Dotan, Masaru Niki, Jason A. Koutcher, et al. 2005. “Crucial Role of P53-Dependent Cellular Senescence in Suppression of Pten-Deficient Tumorigenesis.” *Nature* 436 (7051): 725–30. https://doi.org/10.1038/nature03918.

Childs, Bennett G., Martina Gluscevic, Darren J. Baker, Remi Martin Laberge, Dan Marquess, Jamie Dananberg, and Jan M. Van Deursen. 2017. “Senescent Cells: An Emerging Target for Diseases of Ageing.” *Nature Reviews Drug Discovery 2017 16:10* 16 (10): 718–35. https://doi.org/10.1038/nrd.2017.116.

Cho, Joon Ho, Deepak Kumar Saini, W. K.Ajith Karunarathne, Vani Kalyanaraman, and N. Gautam. 2011. “Alteration of Golgi Structure in Senescent Cells and Its Regulation by a G Protein γ Subunit.” *Cellular Signalling* 23 (5): 785–93. https://doi.org/10.1016/J.CELLSIG.2011.01.001.

Cho, Kyung A., Jin Ryu Sung, Sin Oh Yoon, Hyeun Park Ji, Weon Lee Jung, Hwang Phill Kim, Tae Kim Kyung, Soon Jang Ik, and Chul Park Sang. 2004. “Morphological Adjustment of Senescent Cells by Modulating Caveolin-1 Status.” *Journal of Biological Chemistry* 279 (40): 42270–78. https://doi.org/10.1074/jbc.M402352200.

Cho, Soyun, Min Jung Lee, Mi Sun Kim, Serah Lee, Yoon Kyung Kim, Dong Hun Lee, Chae Wook

Lee, Kwang Hyun Cho, and Jin Ho Chung. 2008. “Infrared plus Visible Light and Heat from Natural Sunlight Participate in the Expression of MMPs and Type I Procollagen as Well as Infiltration of Inflammatory Cell in Human Skin in Vivo.” *Journal of Dermatological Science* 50 (2): 123–33. https://doi.org/https://doi.org/10.1016/j.jdermsci.2007.11.009.

Choudhury, Subholakshmi, and Amitava Das. 2021. “Advances in Generation of Three-Dimensional Skin Equivalents: Pre-Clinical Studies to Clinical Therapies.” *Cytotherapy* 23 (1): 1–9. https://doi.org/10.1016/j.jcyt.2020.10.001.

Chung, Christina Lee, Ibiyonu Lawrence, Melissa Hoffman, Dareen Elgindi, Kumar Nadhan, Manali Potnis, Annie Jin, et al. 2019. “Topical Rapamycin Reduces Markers of Senescence and Aging in Human Skin: An Exploratory, Prospective, Randomized Trial.” *GeroScience* 41 (6): 861–69. https://doi.org/10.1007/s11357-019-00113-y.

Cichorek, Mirosława, Małgorzata Wachulska, Aneta Stasiewicz, and Agata Tymińska. 2013. “Skin Melanocytes: Biology and Development.” *Postepy Dermatologii i Alergologii* 30 (1): 30–41. https://doi.org/10.5114/pdia.2013.33376.

Cleland, W W. 1964. “DITHIOTHREITOL, A NEW PROTECTIVE REAGENT FOR SH GROUPS.” *Biochemistry* 3 (April): 480–82. https://doi.org/10.1021/bi00892a002.

Cnop, Miriam, Fabienne Foufelle, and Licio A Velloso. 2012. “Endoplasmic Reticulum Stress, Obesity and Diabetes.” *Trends in Molecular Medicine* 18 (1): 59–68. https://doi.org/10.1016/j.molmed.2011.07.010.

Cohn, Rachel L., Nathan S. Gasek, George A. Kuchel, and Ming Xu. 2023. “The Heterogeneity of Cellular Senescence: Insights at the Single-Cell Level.” *Trends in Cell Biology* 33 (1): 9–17. https://doi.org/10.1016/j.tcb.2022.04.011.

Coin, Frédéric, Etienne Bergmann, Alexandre Tremeau-Bravard, and Jean-Marc Egly. 1999. “Mutations in XPB and XPD Helicases Found in Xeroderma Pigmentosum Patients Impair the Transcription Function of TFIIH.” *The EMBO Journal* 18 (5): 1357–66. https://doi.org/https://doi.org/10.1093/emboj/18.5.1357.

Collado, Manuel, Jesús Gil, Alejo Efeyan, Carmen Guerra, Alberto J. Schuhmacher, Marta Barradas, Alberto Benguría, et al. 2005. “Tumour Biology: Senescence in Premalignant Tumours.” *Nature* 436 (7051): 642. https://doi.org/10.1038/436642a.

Cong, Yu-Sheng, Woodring E. Wright, and Jerry W. Shay. 2002. “Human Telomerase and Its Regulation.” *Microbiology and Molecular Biology Reviews* 66 (3): 407–25. https://doi.org/10.1128/mmbr.66.3.407-425.2002.

Connolly, T, and R Gilmore. 1993. “GTP Hydrolysis by Complexes of the Signal Recognition Particle and the Signal Recognition Particle Receptor.” *The Journal of Cell Biology* 123 (4): 799–807. https://doi.org/10.1083/jcb.123.4.799.

Contrepois, Kévin, Clément Coudereau, Bérénice A. Benayoun, Nadine Schuler, Pierre François Roux, Oliver Bischof, Régis Courbeyrette, et al. 2017. “Histone Variant H2A.J Accumulates in Senescent Cells and Promotes Inflammatory Gene Expression.” *Nature Communications* 8 (May). https://doi.org/10.1038/ncomms14995.

Coppé, Jean Philippe, Pierre Yves Desprez, Ana Krtolica, and Judith Campisi. 2010. “The Senescence-Associated Secretory Phenotype: The Dark Side of Tumor Suppression.” *Annual Review of Pathology* 5 (February): 99. https://doi.org/10.1146/ANNUREV-PATHOL-121808-102144.

Coppé, Jean Philippe, Christopher K. Patil, Francis Rodier, Y. Sun, Denise P. Muñoz, Joshua Goldstein, Peter S. Nelson, Pierre Yves Desprez, and Judith Campisi. 2008. “Senescence-Associated Secretory Phenotypes Reveal Cell-Nonautonomous Functions of Oncogenic RAS and the P53 Tumor Suppressor.” *PLoS Biology* 6 (12). https://doi.org/10.1371/JOURNAL.PBIO.0060301.

Corchado-Cobos, Roberto, Natalia García-Sancha, Rogelio González-Sarmiento, Jesús Pérez-Losada, and Javier Cañueto. 2023. “Cutaneous Squamous Cell Carcinoma: From Biology to Therapy.” *Advances in Surgical and Medical Specialties*, 393–424.

Cormenier, Johanna, Nathalie Martin, Julie Deslé, Clara Salazar-Cardozo, Albin Pourtier, Corinne Abbadie, and Olivier Pluquet. 2018. “The ATF6α Arm of the Unfolded Protein Response Mediates Replicative Senescence in Human Fibroblasts through a COX2/Prostaglandin E2 Intracrine Pathway.” *Mechanisms of Ageing and Development* 170 (July 2017): 82–91. https://doi.org/10.1016/j.mad.2017.08.003.

Correia-Melo, Clara, and João F. Passos. 2015. “Mitochondria: Are They Causal Players in Cellular Senescence?” *Biochimica et Biophysica Acta - Bioenergetics* 1847 (11): 1373–79. https://doi.org/10.1016/j.bbabio.2015.05.017.

Correia-Melo, Clara, Francisco DM Marques, Rhys Anderson, Graeme Hewitt, Rachael Hewitt, John Cole, Bernadette M Carroll, et al. 2016. “Mitochondria Are Required for Pro-ageing Features of the Senescent Phenotype.” *The EMBO Journal* 35 (7): 724–42. https://doi.org/10.15252/embj.201592862.

Corsi, Ann K., and Randy Schekmant. 1996. “Mechanism of Polypeptide Translocation into the Endoplasmic Reticulum.” *Journal of Biological Chemistry* 271 (48): 30299–302. https://doi.org/10.1074/jbc.271.48.30299.

Courtois-Cox, S., S. L. Jones, and K. Cichowski. 2008. “Many Roads Lead to Oncogene-Induced Senescence.” *Oncogene* 27 (20): 2801–9. https://doi.org/10.1038/sj.onc.1210950.

Courtois-Cox, Stéphanie, Sybil M. Genther Williams, Elizabeth E. Reczek, Bryan W. Johnson, Lauren T. McGillicuddy, Cory M. Johannessen, Pablo E. Hollstein, Mia MacCollin, and Karen Cichowski. 2006. “A Negative Feedback Signaling Network Underlies Oncogene-Induced Senescence.” *Cancer Cell* 10 (6): 459–72. https://doi.org/10.1016/j.ccr.2006.10.003.

Csekes, Erika, and Lucia Račková. 2021. “Csekes, E.; Racková, L. Skin Aging, Cellular Senescence and Natural Polyphenols. Int. J. Mol. Sci. 2021, 22, 12641. Https://Doi.Org/ 10.3390/Ijms222312641.” *International Journal of Molecular Sciences* 22 (23).

Csiszar, Anna, Danuta Sosnowska, Mingyi Wang, Edward G. Lakatta, William E. Sonntag, and Zoltan Ungvari. 2012. “Age-Associated Proinflammatory Secretory Phenotype in Vascular Smooth Muscle Cells From the Non-Human Primate Macaca Mulatta: Reversal by Resveratrol Treatment.” *The Journals of Gerontology: Series A* 67 (8): 811–20. https://doi.org/10.1093/GERONA/GLR228.

Cullinan, Sara B, Donna Zhang, Mark Hannink, Edward Arvisais, Randal J Kaufman, and J Alan Diehl. 2003. “Nrf2 Is a Direct PERK Substrate and Effector of PERK-Dependent Cell Survival.” *Molecular and Cellular Biology* 23 (20): 7198–7209. https://doi.org/10.1128/MCB.23.20.7198-7209.2003.

D’Adda Di Fagagna, Fabrizio. 2008. “Living on a Break: Cellular Senescence as a DNA-Damage Response.” *Nature Reviews Cancer* 8 (7): 512–22. https://doi.org/10.1038/nrc2440.

D’Adda Di Fagagna, Fabrizio, Philip M. Reaper, Lorena Clay-Farrace, Heike Fiegler, Philippa Carr, Thomas Von Zglinicki, Gabriele Saretzki, Nigel P. Carter, and Stephen P. Jackson. 2003. “A DNA Damage Checkpoint Response in Telomere-Initiated Senescence.” *Nature* 426 (6963): 194–98. https://doi.org/10.1038/nature02118.

D’Orazio, John, Stuart Jarrett, Alexandra Amaro-Ortiz, and Timothy Scott. 2013. “UV Radiation and the Skin.” *International Journal of Molecular Sciences* 14 (6): 12222–48. https://doi.org/10.3390/ijms140612222.

Dandekar, Aditya, Roberto Mendez, and Kezhong Zhang. 2015. “Cross Talk between ER Stress, Oxidative Stress, and Inflammation in Health and Disease.” *Methods in Molecular Biology* 1292: 205–14. https://doi.org/10.1007/978-1-4939-2522-3_15/COVER.

Darling, Nicola J, and Simon J Cook. 2014. “The Role of MAPK Signalling Pathways in the Response to Endoplasmic Reticulum Stress.” *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* 1843 (10): 2150–63. https://doi.org/https://doi.org/10.1016/j.bbamcr.2014.01.009.

Davalos, Albert R., Misako Kawahara, Gautam K. Malhotra, Nicholas Schaum, Jiahao Huang, Urvi Ved, Christian M. Beausejour, Jean Philippe Coppe, Francis Rodoer, and Judith Campisi. 2013. “P53-Dependent Release of Alarmin HMGB1 Is a Central Mediator of Senescent Phenotypes.” *The Journal of Cell Biology* 201 (4): 613. https://doi.org/10.1083/JCB.201206006.

Debacq-Chainiaux, Florence, Emmanuelle Boilan, Jérémie Dedessus Le Moutier, Geoffroy Weemaels, and Olivier Toussaint. 2010. “P38(MAPK) in the Senescence of Human and Murine Fibroblasts.” *Advances in Experimental Medicine and Biology* 694: 126–37. https://doi.org/10.1007/978-1-4419-7002-2_10.

Debacq-Chainiaux, Florence, Céline Borlon, Thierry Pascal, Véronique Royer, François Eliaers, Noëlle Ninane, Géraldine Carrard, et al. 2005. “Repeated Exposure of Human Skin Fibroblasts to UVB at Subcytotoxic Level Triggers Premature Senescence through the TGF-B1 Signaling Pathway.” *Journal of Cell Science* 118 (4): 743–58. https://doi.org/10.1242/JCS.01651.

Debacq-Chainiaux, Florence, Jorge D Erusalimsky, Judith Campisi, and Olivier Toussaint. 2009. “Protocols to Detect Senescence-Associated Beta-Galactosidase (SA-Bgal) Activity, a Biomarker of Senescent Cells in Culture and in Vivo.” *Nature Protocols 2009 4:12* 4 (12): 1798–1806. https://doi.org/10.1038/nprot.2009.191.

Debacq-Chainiaux, Florence, Cedric Leduc, Alix Verbeke, and Olivier Toussaint. 2012. “UV, Stress and Aging.” *Http://Www.Tandfonline.Com/Action/AuthorSubmission?JournalCode=kder20&page=instructions* 4 (3): 236–40. https://doi.org/10.4161/DERM.23652.

DeGregori, J, G Leone, A Miron, L Jakoi, and J R Nevins. 1997. “Distinct Roles for E2F Proteins in Cell Growth Control and Apoptosis.” *Proceedings of the National Academy of Sciences of the United States of America* 94 (14): 7245–50. https://doi.org/10.1073/pnas.94.14.7245.

Demaria, Marco. 2019. *Cellular Senescence Methods and Protocols: Methods and Protocols*. https://doi.org/10.1007/978-1-4939-8931-7.

Demaria, Marco, Naoko Ohtani, Sameh A. Youssef, Francis Rodier, Wendy Toussaint, James R. Mitchell, Remi Martin Laberge, et al. 2014. “An Essential Role for Senescent Cells in Optimal Wound Healing through Secretion of PDGF-AA.” *Developmental Cell* 31 (6): 722–33. https://doi.org/10.1016/j.devcel.2014.11.012.

Denoyelle, Christophe, George Abou-Rjaily, Vladimir Bezrookove, Monique Verhaegen, Timothy M. Johnson, Douglas R. Fullen, Jenny N. Pointer, et al. 2006. “Anti-Oncogenic Role of the Endoplasmic Reticulum Differentially Activated by Mutations in the MAPK Pathway.” *Nature Cell Biology* 8 (10): 1053–63. https://doi.org/10.1038/ncb1471.

Deruy, E, J Nassour, N Martin, C Vercamer, N Malaquin, J Bertout, F Chelli, A Pourtier, O Pluquet, and C Abbadie. 2014. “Level of Macroautophagy Drives Senescent Keratinocytes into Cell Death or Neoplastic Evasion.” *Cell Death & Disease* 5 (12): e1577. https://doi.org/10.1038/cddis.2014.533.

Despres, Julie, Yasmina Ramdani, Marine di Giovanni, Magalie Bénard, Abderrakib Zahid, Maité Montero-Hadjadje, Florent Yvergnaux, Thibaut Saguet, Azeddine Driouich, and Marie Laure Follet-Gueye. 2019. “Replicative Senescence of Human Dermal Fibroblasts Affects Structural and Functional Aspects of the Golgi Apparatus.” *Experimental Dermatology* 28 (8): 922–32. https://doi.org/10.1111/EXD.13886.

Dhomen, Nathalie, Jorge S. Reis-Filho, Silvy da Rocha Dias, Robert Hayward, Kay Savage, Veronique Delmas, Lionel Larue, Catrin Pritchard, and Richard Marais. 2009. “Oncogenic Braf Induces Melanocyte Senescence and Melanoma in Mice.” *Cancer Cell* 15 (4): 294–303. https://doi.org/10.1016/j.ccr.2009.02.022.

Dickey, Jennifer S., Brandon J. Baird, Christophe E. Redon, Mykyta V. Sokolov, Olga A. Sedelnikova, and William M. Bonner. 2009. “Intercellular Communication of Cellular Stress Monitored by γ-H2AX Induction.” *Carcinogenesis* 30 (10): 1686–95. https://doi.org/10.1093/carcin/bgp192.

Dimri, Goberdhan P, Xinhua Leet, George Basile, Meileen Acosta, Glynis Scorrt, Calvin Roskelley, Estela E Medrano, et al. 1995. “A Biomarker That Identifies Senescent Human Cells in Culture and in Aging Skin in Vivo (Replicative Senescence/Tumor Suppression/18-Galactosidase) Communicated by Arthur.” *Cell Bioiogy* 92: 9363–67.

Dörr, Jan R., Yong Yu, Maja Milanovic, Gregor Beuster, Christin Zasada, J. Henry M. Däbritz, Jan Lisec, et al. 2013. “Synthetic Lethal Metabolic Targeting of Cellular Senescence in Cancer Therapy.” *Nature 2013 501:7467* 501 (7467): 421–25. https://doi.org/10.1038/nature12437.

Dörr, Jan R, Yong Yu, Maja Milanovic, Gregor Beuster, Christin Zasada, J Henry, M Däbritz, et al. 2013. “Synthetic Lethal Metabolic Targeting of Cellular Senescence in Cancer Therapy.” *Nature* 501. https://doi.org/10.1038/nature12437.

Dou, Zhixun, Kanad Ghosh, Maria Grazia Vizioli, Jiajun Zhu, Payel Sen, Kirk J. Wangensteen, Johayra Simithy, et al. 2017. “Cytoplasmic Chromatin Triggers Inflammation in Senescence and Cancer.” *Nature 2017 550:7676* 550 (7676): 402–6. https://doi.org/10.1038/nature24050.

Douki, T., and E. Sage. 2016. “Dewar Valence Isomers, the Third Type of Environmentally Relevant DNA Photoproducts Induced by Solar Radiation.” *Photochemical and Photobiological Sciences* 15 (1): 24–30. https://doi.org/10.1039/c5pp00382b.

Douki, Thierry, Anne Reynaud-Angelin, Jean Cadet, and Evelyne Sage. 2003. “Bipyrimidine Photoproducts Rather than Oxidative Lesions Are the Main Type of DNA Damage Involved in the Genotoxic Effect of Solar UVA Radiation.” *Biochemistry* 42 (30): 9221–26. https://doi.org/10.1021/bi034593c.

Dreesen, Oliver, Alexandre Chojnowski, Peh Fern Ong, Tian Yun Zhao, John E Common, Declan Lunny, E Birgitte Lane, et al. 2013. “Lamin B1 Fluctuations Have Differential Effects on Cellular Proliferation and Senescence.” *The Journal of Cell Biology* 200 (5): 605–17. https://doi.org/10.1083/jcb.201206121.

Druelle, Clémentine, Claire Drullion, Julie Deslé, Nathalie Martin, Laure Saas, Johanna Cormenier, Nicolas Malaquin, et al. 2016. “ATF6α Regulates Morphological Changes Associated with Senescence in Human Fibroblasts.” *Oncotarget* 7 (42): 67699. https://doi.org/10.18632/ONCOTARGET.11505.

Drullion, Claire, Guillemette Marot, Nathalie Martin, Julie Desle, Laure Saas, Clara Salazar-Cardozo, Fatima Bouali, Albin Pourtier, Corinne Abbadie, and Olivier Pluquet. 2018. “Pre-Malignant Transformation by Senescence Evasion Is Prevented by the PERK and ATF6alpha Branches of the Unfolded Protein Response.” *Cancer Letters* 438 (September): 187–96. https://doi.org/10.1016/j.canlet.2018.09.008.

Durik, Matej, Daniel Sampaio Gonçalves, Coralie Spiegelhalter, Nadia Messaddeq, William M Keyes, and Bill Keyes. 2023. “Senescent Cells Deposit Intracellular Contents through Adhesion-Dependent Fragmentation.” *BioRxiv*, 2023.01.11.523642. https://www.biorxiv.org/content/10.1101/2023.01.11.523642v1%0Ahttps://www.biorxiv.org/content/10.1101/2023.01.11.523642v1.abstract.

Eckhart, Leopold, Saskia Lippens, Erwin Tschachler, and Wim Declercq. 2013. “Cell Death by Cornification.” *Biochimica et Biophysica Acta - Molecular Cell Research* 1833 (12): 3471–80. https://doi.org/10.1016/j.bbamcr.2013.06.010.

Egea, Pascal F., Robert M. Stroud, and Peter Walter. 2005. “Targeting Proteins to Membranes: Structure of the Signal Recognition Particle.” *Current Opinion in Structural Biology* 15 (2): 213–20. https://doi.org/10.1016/j.sbi.2005.03.007.

El-Domyati, M, S Attia, F Saleh, D Brown, D E Birk, F Gasparro, H Ahmad, and J Uitto. 2002. “Intrinsic Aging vs. Photoaging: A Comparative Histopathological, Immunohistochemical, and

Ultrastructural Study of Skin." *Experimental Dermatology* 11 (5): 398–405. https://doi.org/10.1034/j.1600-0625.2002.110502.x.

"Enhancing the Impact of Aging Research for Its Intended Beneficiaries." 2021. *Nature Aging* 1 (9): 749. https://doi.org/10.1038/s43587-021-00119-2.

Evangelou, Konstantinos, Nikolaos Lougiakis, Sophia V Rizou, Athanassios Kotsinas, Dimitris Kletsas, Daniel Muñoz-Espín, Nikolaos G Kastrinakis, et al. 2017. "Robust, Universal Biomarker Assay to Detect Senescent Cells in Biological Specimens." *Aging Cell* 16 (1): 192–97. https://doi.org/10.1111/acel.12545.

Eyerich, Stefanie, Kilian Eyerich, Claudia Traidl-Hoffmann, and Tilo Biedermann. 2018. "Cutaneous Barriers and Skin Immunity: Differentiating A Connected Network." *Trends in Immunology* 39 (4): 315–27. https://doi.org/10.1016/j.it.2018.02.004.

Eyman, D, M Damodarasamy, S R Plymate, and M J Reed. 2009. "CCL5 Secreted by Senescent Aged Fibroblasts Induces Proliferation of Prostate Epithelial Cells and Expression of Genes That Modulate Angiogenesis." *Journal of Cellular Physiology* 220 (2): 376–81. https://doi.org/10.1002/jcp.21776.

Fabregat, Antonio, Steven Jupe, Lisa Matthews, Konstantinos Sidiropoulos, Marc Gillespie, Phani Garapati, Robin Haw, et al. 2018. "The Reactome Pathway Knowledgebase." *Nucleic Acids Research* 46 (D1): D649–55. https://doi.org/10.1093/nar/gkx1132.

Farage, M. A., K. W. Miller, P. Elsner, and H. I. Maibach. 2008. "Intrinsic and Extrinsic Factors in Skin Ageing: A Review." *International Journal of Cosmetic Science* 30 (2): 87–95. https://doi.org/10.1111/j.1468-2494.2007.00415.x.

Farsam, Vida, Abhijit Basu, Martina Gatzka, Nicolai Treiber, Lars A. Schneider, Medhanie A. Mulaw, Tanja Lucas, Stefan Kochanek, Reinhard Dummer, Mitchell P. Levesque, Meinhard Wlaschek, and Karin Scharffetter-Kochanek. 2016. "Senescent Fibroblast-Derived Chemerin Promotes Squamous Cell Carcinoma Migration." *Oncotarget* 7 (50): 83554. https://doi.org/10.18632/ONCOTARGET.13446.

Fingar, Diane C., Sofie Salama, Christina Tsou, Ed Harlow, and John Blenis. 2002. "Mammalian Cell Size Is Controlled by MTOR and Its Downstream Targets S6K1 and 4EBP1/EIF4E." *Genes and Development* 16 (12): 1472–87. https://doi.org/10.1101/gad.995802.

Flament, Frederic, Roland Bazin, Sabine Laquieze, Virginie Rubert, Elisa Simonpietri, and Bertrand Piot. 2013. "Effect of the Sun on Visible Clinical Signs of Aging in Caucasian Skin." *Clinical, Cosmetic and Investigational Dermatology* 6: 221–32. https://doi.org/10.2147/CCID.S44686.

Flor, Amy C., Don Wolfgeher, Ding Wu, and Stephen J. Kron. 2017. "A Signature of Enhanced Lipid Metabolism, Lipid Peroxidation and Aldehyde Stress in Therapy-Induced Senescence." *Cell Death Discovery* 3 (1): 1–12. https://doi.org/10.1038/cddiscovery.2017.75.

Forouhan, M, K Mori, and R P Boot-Handford. 2018. "Paradoxical Roles of ATF6α and ATF6β in Modulating Disease Severity Caused by Mutations in Collagen X." *Matrix Biology : Journal of the International Society for Matrix Biology* 70 (September): 50–71. https://doi.org/10.1016/j.matbio.2018.03.004.

Fossel, Michael. 2019. "Cell Senescence, Telomerase, and Senolytic Therapy." *OBM Geriatrics* 03 (01): 34. https://doi.org/10.21926/obm.geriatr.1901034.

Foufelle, Fabienne, and Pascal Ferré. 2007. "[Unfolded protein response: its role in physiology and physiopathology]." *Medecine sciences : M/S* 23 (3): 291–96. https://doi.org/10.1051/medsci/2007233291.

Frankart, Aureélie, Jeéreémy Malaisse, Evelyne De Vuyst, Freédeéric Minner, Catherine Lambert de Rouvroit, and Yves Poumay. 2012. "Epidermal Morphogenesis during Progressive in Vitro 3D Reconstruction at the Air-Liquid Interface." *Experimental Dermatology* 21 (11): 871–75. https://doi.org/10.1111/exd.12020.

Frankart, Aurélie, Jérémy Malaisse, Evelyne De Vuyst, Frédéric Minner, Catherine Lambert de Rouvroit, and Yves Poumay. 2012. “Epidermal Morphogenesis during Progressive in Vitro 3D Reconstruction at the Air–Liquid Interface.” *Experimental Dermatology* 21 (11): 871–75. https://doi.org/https://doi.org/10.1111/exd.12020.

Frediani, Elena, Francesca Scavone, Anna Laurenzana, Anastasia Chillà, Katia Tortora, Ilaria Cimmino, Manuela Leri, et al. 2022. “Olive Phenols Preserve Lamin B1 Expression Reducing CGAS / STING / NF κ B- - Mediated SASP in Ionizing Radiation- - Induced Senescence,” no. June 2021: 2337–50. https://doi.org/10.1111/jcmm.17255.

Freund, Adam, Remi Martin Laberge, Marco Demaria, and Judith Campisi. 2012. “Lamin B1 Loss Is a Senescence-Associated Biomarker.” *Molecular Biology of the Cell* 23 (11): 2066–75. https://doi.org/10.1091/mbc.E11-10-0884.

Freund, Adam, Christopher K. Patil, and Judith Campisi. 2011. “P38MAPK Is a Novel DNA Damage Response-Independent Regulator of the Senescence-Associated Secretory Phenotype.” *The EMBO Journal* 30 (8): 1536–48. https://doi.org/10.1038/EMBOJ.2011.69.

Fulzele, Sadanand, Bharati Mendhe, Andrew Khayrullin, Maribeth Johnson, Helen Kaiser, Yutao Liu, Carlos M. Isales, and Mark W. Hamrick. 2019. “Muscle-Derived MiR-34a Increases with Age in Circulating Extracellular Vesicles and Induces Senescence of Bone Marrow Stem Cells.” *Aging* 11 (6): 1791–1803. https://doi.org/10.18632/aging.101874.

Fumagalli, Marzia, Francesca Rossiello, Chiara Mondello, and Fabrizio d’Adda di Fagagna. 2014. “Stable Cellular Senescence Is Associated with Persistent DDR Activation.” *PloS One* 9 (10): e110969. https://doi.org/10.1371/journal.pone.0110969.

Gangbè, Marcellin, and Francine Ducharme. 2006. “Le « Bien Vieillir » : Concepts et Modèles.” *Médecine/Sciences* 22 (3): 297–300. https://doi.org/10.1051/medsci/2006223297.

Gao, Li Bin, Ya Hong Wang, Zhi Hua Liu, Yu Sun, Peng Cai, and Qing Jing. 2021. “Identification of a Small Molecule SR9009 That Activates NRF2 to Counteract Cellular Senescence.” *Aging Cell* 20 (10). https://doi.org/10.1111/ACEL.13483.

Gee, Heon Yung, Shin Hye Noh, Bor Luen Tang, Kyung Hwan Kim, and Min Goo Lee. 2011. “Rescue of ΔF508-CFTR Trafficking via a GRASP-Dependent Unconventional Secretion Pathway.” *Cell* 146 (5): 746–60. https://doi.org/10.1016/j.cell.2011.07.021.

Gerland, Luc-Marie, Simone Peyrol, Christophe Lallemand, Robert Branche, Jean-Pierre Magaud, and Martine Ffrench. 2003. “Association of Increased Autophagic Inclusions Labeled for Beta-Galactosidase with Fibroblastic Aging.” *Experimental Gerontology* 38 (8): 887–95. https://doi.org/10.1016/s0531-5565(03)00132-3.

Ghadaouia, Sabrina, Marc Alexandre Olivier, Aurélie Martinez, Tibila Kientega, Jian Qin, Patrick Lambert-Lanteigne, Guillaume B. Cardin, Chantal Autexier, Nicolas Malaquin, and Francis Rodier. 2021. “Homologous Recombination-Mediated Irreversible Genome Damage Underlies Telomere-Induced Senescence.” *Nucleic Acids Research* 49 (20): 11690–707. https://doi.org/10.1093/nar/gkab965.

Ghetti, M, H Topouzi, G Theocharidis, V Papa, G Williams, E Bondioli, G Cenacchi, J T Connelly, and C A Higgins. 2018. “Subpopulations of Dermal Skin Fibroblasts Secrete Distinct Extracellular Matrix: Implications for Using Skin Substitutes in the Clinic.” *The British Journal of Dermatology* 179 (2): 381–93. https://doi.org/10.1111/bjd.16255.

Gil, Jesús. 2023. “The Challenge of Identifying Senescent Cells.” *Nature Cell Biology* 25 (11): 1554–56. https://doi.org/10.1038/s41556-023-01267-w.

Gire, Véronique, and Vjekoslav Dulic. 2015. “Senescence from G2 Arrest, Revisited.” *Cell Cycle* 14 (3): 297–304. https://doi.org/10.1080/15384101.2014.1000134.

Giroud, Joëlle, Inès Bouriez, Hugo Paulus, Albin Pourtier, Florence Debacq-Chainiaux, and Olivier Pluquet. 2023. “Exploring the Communication of the SASP: Dynamic, Interactive, and Adaptive

Effects on the Microenvironment." *International Journal of Molecular Sciences* 24 (13): 10788. https://doi.org/10.3390/ijms241310788.

Glück, Selene, Baptiste Guey, Muhammet Fatih Gulen, Katharina Wolter, Tae Won Kang, Niklas Arndt Schmacke, Anne Bridgeman, Jan Rehwinkel, Lars Zender, and Andrea Ablasser. 2017. "Innate Immune Sensing of Cytosolic Chromatin Fragments through CGAS Promotes Senescence." *Nature Cell Biology* 19 (9): 1061–70. https://doi.org/10.1038/ncb3586.

Godar, Dianne E. 2005. "UV Doses Worldwide." *Photochemistry and Photobiology* 81 (4): 736–49. https://doi.org/10.1562/2004-09-07-ir-308r.1.

Gonçalves, Susana, Kelvin Yin, Yoko Ito, Adelyne Chan, Ioana Olan, Sarah Gough, Liam Cassidy, et al. 2021. "COX2 Regulates Senescence Secretome Composition and Senescence Surveillance through PGE2." *Cell Reports* 34 (11): 108860. https://doi.org/10.1016/J.CELREP.2021.108860.

González-Gualda, Estela, Andrew G. Baker, Ljiljana Fruk, and Daniel Muñoz-Espín. 2021. "A Guide to Assessing Cellular Senescence in Vitro and in Vivo." *FEBS Journal* 288 (1): 56–80. https://doi.org/10.1111/febs.15570.

Gonzalez-Meljem, Jose Mario, John Richard Apps, Helen Christina Fraser, and Juan Pedro Martinez-Barbera. 2018. "Paracrine Roles of Cellular Senescence in Promoting Tumourigenesis." *British Journal of Cancer* 118 (10): 1283–88. https://doi.org/10.1038/s41416-018-0066-1.

González-Quiroz, Matías, Alice Blondel, Alfredo Sagredo, Claudio Hetz, Eric Chevet, and Rémy Pedeux. 2020. "When Endoplasmic Reticulum Proteostasis Meets the DNA Damage Response." *Trends in Cell Biology* 30 (11): 881–91. https://doi.org/10.1016/j.tcb.2020.09.002.

Gorgoulis, Vassilis, Peter D. Adams, Andrea Alimonti, Dorothy C. Bennett, Oliver Bischof, Cleo Bishop, Judith Campisi, et al. 2019. "Cellular Senescence: Defining a Path Forward." *Cell* 179 (4): 813–27. https://doi.org/10.1016/j.cell.2019.10.005.

Gorgoulis, Vassilis G., and Thanos D. Halazonetis. 2010. "Oncogene-Induced Senescence: The Bright and Dark Side of the Response." *Current Opinion in Cell Biology* 22 (6): 816–27. https://doi.org/10.1016/J.CEB.2010.07.013.

Gosselin, Karo, Sébastien Martien, Albin Pourtier, Chantal Vercamer, Peter Ostoich, Luc Morat, Laure Sabatier, et al. 2009. "Senescence-Associated Oxidative DNA Damage Promotes the Generation of Neoplastic Cells." *Cancer Res* 69: 7917–42. https://doi.org/10.1158/0008-5472.CAN-08-2510.

Goy, Erwan, Nathalie Martin, Claire Drullion, Laure Saas, Olivier Molendi-Coste, Laurent Pineau, David Dombrowicz, et al. 2023. "Flow Cytometry-Based Method for Efficient Sorting of Senescent Cells." *Bio-Protocol* 13 (7): e4612. https://doi.org/10.21769/BioProtoc.4612.

Gray-Schopfer, V. C., S. C. Cheong, H. Chong, J. Chow, T. Moss, Z. A. Abdel-Malek, R. Marais, D. Wynford-Thomas, and D. C. Bennett. 2006. "Cellular Senescence in Naevi and Immortalisation in Melanoma: A Role for P16?" *British Journal of Cancer* 95 (4): 496–505. https://doi.org/10.1038/sj.bjc.6603283.

Greenberg, S B, G L Grove, and V J Cristofalo. 1977. "Cell Size in Aging Monolayer Cultures." *In Vitro* 13 (5): 297–300. https://doi.org/10.1007/BF02616174.

Greenfield, J J, and S High. 1999. "The Sec61 Complex Is Located in Both the ER and the ER-Golgi Intermediate Compartment." *Journal of Cell Science* 112 (Pt 1 (May): 1477–86. https://doi.org/10.1242/jcs.112.10.1477.

Greider, Carol W., and Elizabeth H. Blackburn. 1985. "Identification of a Specific Telomere Terminal Transferase Activity in Tetrahymena Extracts." *Cell* 43 (2 PART 1): 405–13. https://doi.org/10.1016/0092-8674(85)90170-9.

Greussing, Ruth, Matthias Hackl, Pornpimol Charoentong, Alexander Pauck, Rossella Monteforte, Maria Cavinato, Edith Hofer, et al. 2013. "Identification of MicroRNA-MRNA Functional Interactions in UVB-Induced Senescence of Human Diploid Fibroblasts." *BMC Genomics* 14

(April): 224. https://doi.org/10.1186/1471-2164-14-224.

Groisman, Bella, Marina Shenkman, Efrat Ron, and Gerardo Z Lederkremer. 2011. “Mannose Trimming Is Required for Delivery of a Glycoprotein from EDEM1 to XTP3-B and to Late Endoplasmic Reticulum-Associated Degradation Steps.” *The Journal of Biological Chemistry* 286 (2): 1292–1300. https://doi.org/10.1074/jbc.M110.154849.

Gromkowska-Kępka, Krystyna Joanna, Anna Puścion-Jakubik, Renata Markiewicz-Żukowska, and Katarzyna Socha. 2021. “The Impact of Ultraviolet Radiation on Skin Photoaging — Review of in Vitro Studies.” *Journal of Cosmetic Dermatology* 20 (11): 3427–31. https://doi.org/10.1111/jocd.14033.

Grosse, Laurent, Nicole Wagner, Alexander Emelyanov, Clement Molina, Sandra Lacas-Gervais, Kay Dietrich Wagner, and Dmitry V. Bulavin. 2020. “Defined P16High Senescent Cell Types Are Indispensable for Mouse Healthspan.” *Cell Metabolism* 32 (1): 87-99.e6. https://doi.org/10.1016/j.cmet.2020.05.002.

Guan, Jingyan, Congxiao Wu, Yunfan He, and Feng Lu. 2023. “Skin-Associated Adipocytes in Skin Barrier Immunity: A Mini-Review.” *Frontiers in Immunology* 14: 1116548. https://doi.org/10.3389/fimmu.2023.1116548.

Guerriero, Christopher J, and Jeffrey L Brodsky. 2012. “The Delicate Balance between Secreted Protein Folding and Endoplasmic Reticulum-Associated Degradation in Human Physiology.” *Physiological Reviews* 92 (2): 537–76. https://doi.org/10.1152/physrev.00027.2011.

Guillon, Jordan, Coralie Petit, Marie Moreau, Bertrand Toutain, Cécile Henry, Henry Roché, Nathalie Bonichon-Lamichhane, et al. 2019. “Regulation of Senescence Escape by TSP1 and CD47 Following Chemotherapy Treatment.” *Cell Death and Disease* 10 (3). https://doi.org/10.1038/s41419-019-1406-7.

Guo, Jun, Xiuqing Huang, Lin Dou, Mingjing Yan, Tao Shen, Weiqing Tang, and Jian Li. 2022. “Aging and Aging-Related Diseases: From Molecular Mechanisms to Interventions and Treatments.” *Signal Transduction and Targeted Therapy* 7 (1). https://doi.org/10.1038/s41392-022-01251-0.

Gupta, Divya, Raghu Rai Sharma, Haroon Rashid, Aalim Maqsood Bhat, Malik Ahmad Tanveer, and Sheikh Tasduq Abdullah. 2023. “Rosmarinic Acid Alleviates Ultraviolet-Mediated Skin Aging via Attenuation of Mitochondrial and ER Stress Responses.” *Experimental Dermatology* 32 (6): 799–807. https://doi.org/10.1111/exd.14773.

Guyuron, Bahman, David J. Rowe, Adam Bryce Weinfeld, Yashar Eshraghi, Amir Fathi, and Seree Iamphongsai. 2009. “Factors Contributing to the Facial Aging of Identical Twins.” *Plastic and Reconstructive Surgery* 123 (4): 1321–31. https://doi.org/10.1097/PRS.0b013e31819c4d42.

Hai, T., F. Liu, W. J. Coukos, and M. R. Green. 1989. “Transcription Factor ATF CDNA Clones: An Extensive Family of Leucine Zipper Proteins Able to Selectively Form DNA-Binding Heterodimers.” *Genes and Development* 3 (12 B): 2083–90. https://doi.org/10.1101/gad.3.12b.2083.

Haigis, Marcia C, and Bruce A Yankner. 2010. “The Aging Stress Response.” https://doi.org/10.1016/j.molcel.2010.10.002.

Halbleib, Kristina, Kristina Pesek, Roberto Covino, Harald F. Hofbauer, Dorith Wunnicke, Inga Hänelt, Gerhard Hummer, and Robert Ernst. 2017. “Activation of the Unfolded Protein Response by Lipid Bilayer Stress.” *Molecular Cell* 67 (4): 673-684.e8. https://doi.org/10.1016/j.molcel.2017.06.012.

Halkoum, Rym, Virginie Salnot, Christophe Capallere, Christelle Plaza, Aurore L’honoré, Karl Pays, Bertrand Friguet, Carine Nizard, and Isabelle Petropoulos. 2022. “Glyoxal Induces Senescence in Human Keratinocytes through Oxidative Stress and Activation of the Protein Kinase B/FOXO3a/P27KIP1 Pathway.” *The Journal of Investigative Dermatology* 142 (8): 2068-2078.e7. https://doi.org/10.1016/J.JID.2021.12.022.

Hall, Michael J., Sara Lopes-Ventura, Matilde V. Neto, João Charneca, Patricia Zoio, Miguel C.

Seabra, Abel Oliva, and Duarte C. Barral. 2022. “Reconstructed Human Pigmented Skin/Epidermis Models Achieve Epidermal Pigmentation through Melanocore Transfer.” *Pigment Cell and Melanoma Research* 35 (4): 425–35. https://doi.org/10.1111/pcmr.13039.

Hamsanathan, Shruthi, and Aditi U. Gurkar. 2022. “Lipids as Regulators of Cellular Senescence.” *Frontiers in Physiology* 13 (March): 1–16. https://doi.org/10.3389/fphys.2022.796850.

Harding, Heather P, Isabel Novoa, Yuhong Zhang, Huiqing Zeng, Ron Wek, Matthieu Schapira, and David Ron. 2000. “Regulated Translation Initiation Controls Stress-Induced Gene Expression in Mammalian Cells.” *Molecular Cell* 6 (5): 1099–1108. https://doi.org/https://doi.org/10.1016/S1097-2765(00)00108-8.

Harding, Heather P, Yuhong Zhang, and David Ron. 1999. “Protein Translation and Folding Are Coupled by an Endoplasmic-Reticulum-Resident Kinase.” *Nature* 397 (6716): 271–74. https://doi.org/10.1038/16729.

Harding, Heather P, Yuhong Zhang, Huiquing Zeng, Isabel Novoa, Phoebe D Lu, Marcella Calfon, Navid Sadri, et al. 2003. “An Integrated Stress Response Regulates Amino Acid Metabolism and Resistance to Oxidative Stress.” *Molecular Cell* 11 (3): 619–33. https://doi.org/10.1016/s1097-2765(03)00105-9.

Harley, C B, A B Futcher, and C W Greider. 1990. “Telomeres Shorten during Ageing of Human Fibroblasts.” *Nature* 345 (6274): 458–60. https://doi.org/10.1038/345458a0.

Hart, Corey R., Zachary C. Ryan, Kyle T. Pfaffenbach, Surendra Dasari, Mojtaba Parvizi, Antigoni Z. Lalia, and Ian R. Lanza. 2019. “Attenuated Activation of the Unfolded Protein Response Following Exercise in Skeletal Muscle of Older Adults.” *Aging* 11 (18): 7587–7604. https://doi.org/10.18632/aging.102273.

Hasegawa, Tatsuya, Tomonori Oka, Heehwa G Son, Valeria S Oliver-García, Marjan Azin, Thomas M Eisenhaure, David J Lieb, Nir Hacohen, and Shadmehr Demehri. 2023. “Cytotoxic CD4+ T Cells Eliminate Senescent Cells by Targeting Cytomegalovirus Antigen.” *Cell* 186 (7): 1417-1431.e20. https://doi.org/https://doi.org/10.1016/j.cell.2023.02.033.

Hattori, Hirokazu, Kazuki Takaoka, Miho Ueta, Masayuki Oshitani, Joji Tamaoka, Kazuma Noguchi, and Hiromitsu Kishimoto. 2021. “Senescent RAW264.7 Cells Exhibit Increased Production of Nitric Oxide and Release Inducible Nitric Oxide Synthase in Exosomes.” *Molecular Medicine Reports* 24 (3). https://doi.org/10.3892/MMR.2021.12320.

Haydont, Valérie, Véronique Neiveyans, Philippe Perez, Élodie Busson, JeanJacques Lataillade, Daniel Asselineau, and Nicolas O Fortunel. 2020. “Fibroblasts from the Human Skin Dermo-Hypodermal Junction Are Distinct from Dermal Papillary and Reticular Fibroblasts and from Mesenchymal Stem Cells and Exhibit a Specific Molecular Profile Related to Extracellular Matrix Organization and Modeling.” *Cells* 9 (2). https://doi.org/10.3390/cells9020368.

Hayflick, L., and P. S. Moorhead. 1961. “The Serial Cultivation of Human Diploid Cell Strains.” *Experimental Cell Research* 25 (3): 585–621. https://doi.org/10.1016/0014-4827(61)90192-6.

HAYFLICK, L. 1965. “THE LIMITED IN VITRO LIFETIME OF HUMAN DIPLOID CELL STRAINS.” *Experimental Cell Research* 37 (March): 614–36. https://doi.org/10.1016/0014-4827(65)90211-9.

Haze, K, T Okada, H Yoshida, H Yanagi, T Yura, M Negishi, and K Mori. 2001. “Identification of the G13 (CAMP-Response-Element-Binding Protein-Related Protein) Gene Product Related to Activating Transcription Factor 6 as a Transcriptional Activator of the Mammalian Unfolded Protein Response.” *The Biochemical Journal* 355 (Pt 1): 19–28. https://doi.org/10.1042/0264-6021:3550019.

Haze, K, H Yoshida, H Yanagi, T Yura, and K Mori. 1999. “Mammalian Transcription Factor ATF6 Is Synthesized as a Transmembrane Protein and Activated by Proteolysis in Response to Endoplasmic Reticulum Stress.” *Molecular Biology of the Cell* 10 (11): 3787–99. https://doi.org/10.1091/mbc.10.11.3787.

He, B. 2006. “Viruses, Endoplasmic Reticulum Stress, and Interferon Responses.” *Cell Death and Differentiation* 13 (3): 393–403. https://doi.org/10.1038/sj.cdd.4401833.

Health World Organization. 2002. “Global Solar UV Index A Practical Guide.” *World Health*, 18.

Heckenbach, Indra, Garik V Mkrtchyan, Michael Ben Ezra, Daniela Bakula, Jakob Sture Madsen, Malte Hasle Nielsen, Denise Oró, et al. n.d. “Nuclear Morphology Is a Deep Learning Biomarker of Cellular Senescence.” Accessed March 22, 2023. https://doi.org/10.1038/s43587-022-00263-3.

Heilig, Rosalie, Mathias S. Dick, Lorenzo Sborgi, Etienne Meunier, Sebastian Hiller, and Petr Broz. 2018. “The Gasdermin-D Pore Acts as a Conduit for IL-1β Secretion in Mice.” *European Journal of Immunology* 48 (4): 584–92. https://doi.org/10.1002/eji.201747404.

Henis-Korenblit, Sivan, Peichuan Zhang, Malene Hansen, Mark McCormick, Seung-Jae Lee, Michael Cary, and Cynthia Kenyon. 2010. “Insulin/IGF-1 Signaling Mutants Reprogram ER Stress Response Regulators to Promote Longevity.” *Proceedings of the National Academy of Sciences of the United States of America* 107 (21): 9730–35. https://doi.org/10.1073/pnas.1002575107.

Hennies, Hans Christian, and Yves Poumay. 2021. “Skin Disease Models In Vitro and Inflammatory Mechanisms: Predictability for Drug Development.” *Handbook of Experimental Pharmacology* 265: 187–218. https://doi.org/10.1007/164_2020_428.

Hennings, H, D Michael, C Cheng, P Steinert, K Holbrook, and S H Yuspa. 1980. “Calcium Regulation of Growth and Differentiation of Mouse Epidermal Cells in Culture.” *Cell* 19 (1): 245–54. https://doi.org/10.1016/0092-8674(80)90406-7.

Herbig, Utz, Mark Ferreira, Laura Condel, Dee Carey, and John M. Sedivy. 2006. “Cellular Senescence in Aging Primates.” *Science* 311 (5765): 1257. https://doi.org/10.1126/science.1122446.

Herbig, Utz, Wendy A Jobling, Benjamin P C Chen, David J Chen, and John M Sedivy. 2004. “Telomere Shortening Triggers Senescence of Human Cells through a Pathway Involving ATM, P53, and P21(CIP1), but Not P16(INK4a).” *Molecular Cell* 14 (4): 501–13. https://doi.org/10.1016/s1097-2765(04)00256-4.

Hernandez-Segura, Alejandra, Tristan V. de Jong, Simon Melov, Victor Guryev, Judith Campisi, and Marco Demaria. 2017. “Unmasking Transcriptional Heterogeneity in Senescent Cells.” *Current Biology : CB* 27 (17): 2652. https://doi.org/10.1016/J.CUB.2017.07.033.

Hernandez-Segura, Alejandra, Jamil Nehme, and Marco Demaria. 2018. “Hallmarks of Cellular Senescence.” https://doi.org/10.1016/j.tcb.2018.02.001.

Hernandez, Lidia, Kyle J. Roux, Esther Sook Miin Wong, Leslie C. Mounkes, Rafidah Mutalif, Raju Navasankari, Bina Rai, et al. 2010. “Functional Coupling between the Extracellular Matrix and Nuclear Lamina by Wnt Signaling in Progeria.” *Developmental Cell* 19 (3): 413–25. https://doi.org/10.1016/j.devcel.2010.08.013.

Herranz, Nicolás, Suchira Gallage, Massimiliano Mellone, Torsten Wuestefeld, Sabrina Klotz, Christopher J. Hanley, Selina Raguz, et al. 2015. “MTOR Regulates MAPKAPK2 Translation to Control the Senescence-Associated Secretory Phenotype.” *Nature Cell Biology* 17 (9): 1205–17. https://doi.org/10.1038/ncb3225.

Herrmann, Gernot, Peter Brenneisen, Meinhard Wlaschek, Jutta Wenk, Katrin Faisst, Gudrun Quel, Christina Hommel, et al. 1998. “Psoralen Photoactivation Promotes Morphological and Functional Changes in Fibroblasts in Vitro Reminiscent of Cellular Senescence.” *Journal of Cell Science* 111 (6): 759–67. https://doi.org/10.1242/jcs.111.6.759.

Hersey, Peter, and Xu Dong Zhang. 2008. “Adaptation to ER Stress as a Driver of Malignancy and Resistance to Therapy in Human Melanoma.” *Pigment Cell and Melanoma Research* 21 (3): 358–67. https://doi.org/10.1111/j.1755-148X.2008.00467.x.

Hetz, Claudio. 2012. “The Unfolded Protein Response: Controlling Cell Fate Decisions under ER Stress and Beyond.” *Nature Reviews. Molecular Cell Biology* 13 (2): 89–102. https://doi.org/10.1038/nrm3270.

Hetz, Claudio, Eric Chevet, and Scott A Oakes. 2015. “Proteostasis Control by the Unfolded Protein Response.” *Nature Cell Biology* 17 (7): 829–38. https://doi.org/10.1038/ncb3184.

Hetz, Claudio, and Laurie H Glimcher. 2009. “Fine-Tuning of the Unfolded Protein Response: Assembling the IRE1alpha Interactome.” *Molecular Cell* 35 (5): 551–61. https://doi.org/10.1016/j.molcel.2009.08.021.

Hetz, Claudio, and Feroz R. Papa. 2018. “The Unfolded Protein Response and Cell Fate Control.” *Molecular Cell* 69 (2): 169–81. https://doi.org/10.1016/j.molcel.2017.06.017.

Hewitt, Graeme, Diana Jurk, Francisco D.M. Marques, Clara Correia-Melo, Timothy Hardy, Agata Gackowska, Rhys Anderson, Morgan Taschuk, Jelena Mann, and João F. Passos. 2012. “Telomeres Are Favoured Targets of a Persistent DNA Damage Response in Ageing and Stress-Induced Senescence.” *Nature Communications* 3. https://doi.org/10.1038/ncomms1708.

Hickson, La Tonya J., Larissa G.P. Langhi Prata, Shane A. Bobart, Tamara K. Evans, Nino Giorgadze, Shahrukh K. Hashmi, Sandra M. Herrmann, et al. 2019. “Senolytics Decrease Senescent Cells in Humans: Preliminary Report from a Clinical Trial of Dasatinib plus Quercetin in Individuals with Diabetic Kidney Disease.” *EBioMedicine* 47: 446–56. https://doi.org/10.1016/j.ebiom.2019.08.069.

Hiebert, Paul, Mateusz S. Wietecha, Michael Cangkrama, Eric Haertel, Eleni Mavrogonatou, Michael Stumpe, Heiko Steenbock, et al. 2018. “Nrf2-Mediated Fibroblast Reprogramming Drives Cellular Senescence by Targeting the Matrisome.” *Developmental Cell* 46 (2): 145-161.e10. https://doi.org/10.1016/J.DEVCEL.2018.06.012.

Higa, Arisa, Said Taouji, Stéphanie Lhomond, Devon Jensen, Martin E Fernandez-Zapico, Jeremy C Simpson, Jean-Max Pasquet, Randy Schekman, and Eric Chevet. 2014. “Endoplasmic Reticulum Stress-Activated Transcription Factor ATF6α Requires the Disulfide Isomerase PDIA5 to Modulate Chemoresistance.” *Molecular and Cellular Biology* 34 (10): 1839–49. https://doi.org/10.1128/MCB.01484-13.

Hildebrand, Dominic G., Simon Lehle, Andreas Borst, Sebastian Haferkamp, Frank Essmann, and Klaus Schulze-Osthoff. 2013. “α-Fucosidase as a Novel Convenient Biomarker for Cellular Senescence.” *Cell Cycle* 12 (12): 1922–27. https://doi.org/10.4161/cc.24944.

Hindi, Nagham Nafiz, Noha Elsakrmy, and Dindial Ramotar. 2021. “The Base Excision Repair Process: Comparison between Higher and Lower Eukaryotes.” *Cellular and Molecular Life Sciences* 78 (24): 7943–65. https://doi.org/10.1007/s00018-021-03990-9.

Hitomi, Kazuhiro, Ryo Okada, Tze Mun Loo, Kenichi Miyata, Asako J. Nakamura, and Akiko Takahashi. 2020. “DNA Damage Regulates Senescence-Associated Extracellular Vesicle Release via the Ceramide Pathway to Prevent Excessive Inflammatory Responses.” *International Journal of Molecular Sciences 2020, Vol. 21, Page 3720* 21 (10): 3720. https://doi.org/10.3390/IJMS21103720.

Hiyama, E., and K. Hiyama. 2007. “Telomere and Telomerase in Stem Cells.” *British Journal of Cancer* 96 (7): 1020–24. https://doi.org/10.1038/sj.bjc.6603671.

Ho, Chun Te, Yu Jia Chang, Li Xi Yang, Po Li Wei, Tsan Zon Liu, and Jun Jen Liu. 2015. “A Novel Microtubule-Disrupting Agent Induces Endoplasmic Reticular Stress-Mediated Cell Death in Human Hepatocellular Carcinoma Cells.” *PLoS ONE* 10 (9): 1–16. https://doi.org/10.1371/journal.pone.0136340.

Hoare, Matthew, and Masashi Narita. 2018. “The Power Behind the Throne: Senescence and the Hallmarks of Cancer.” *Annual Review of Cancer Biology* 2: 175–94. https://doi.org/10.1146/annurev-cancerbio-030617-050352.

Hollien, Julie, and Jonathan S Weissman. 2006. “Decay of Endoplasmic Reticulum-Localized MRNAs during the Unfolded Protein Response.” *Science (New York, N.Y.)* 313 (5783): 104–7. https://doi.org/10.1126/science.1129631.

Horiguchi, Michiko, Satoru Koyanagi, Akinori Okamoto, Satoshi O Suzuki, Naoya Matsunaga, and Shigehiro Ohdo. 2012. “Stress-Regulated Transcription Factor ATF4 Promotes Neoplastic Transformation by Suppressing Expression of the INK4a/ARF Cell Senescence Factors.” *Cancer Research* 72 (2): 395–401. https://doi.org/10.1158/0008-5472.CAN-11-1891.

Hovnanian, Alain. 2004. “Darier’s Disease: From Dyskeratosis to Endoplasmic Reticulum Calcium ATPase Deficiency.” *Biochemical and Biophysical Research Communications* 322 (4): 1237–44. https://doi.org/10.1016/j.bbrc.2004.08.067.

Hu, Qinchao, Jianmin Peng, Laibo Jiang, Wuguo Li, Qiao Su, Jiayu Zhang, Huan Li, et al. 2020. “Metformin as a Senostatic Drug Enhances the Anticancer Ef Fi Cacy of CDK4 / 6 Inhibitor in Head and Neck Squamous Cell Carcinoma.” *Cell Death and Disease*. https://doi.org/10.1038/s41419-020-03126-0.

Hubaux, Roland, Coralie Bastin, and Michel Salmon. 2018. “On the Relevance of an in Vitro Reconstructed Human Epidermis Model for Drug Screening in Atopic Dermatitis.” *Experimental Dermatology* 27 (12): 1403–7. https://doi.org/10.1111/exd.13810.

Hüls, Anke, Dorothea Sugiri, Kateryna Fuks, Jean Krutmann, and Tamara Schikowski. 2019. “Lentigine Formation in Caucasian Women-Interaction between Particulate Matter and Solar UVR.” *The Journal of Investigative Dermatology*. United States. https://doi.org/10.1016/j.jid.2018.09.034.

Hussain, Syed G, and Kolluru V A Ramaiah. 2007. “Reduced EIF2alpha Phosphorylation and Increased Proapoptotic Proteins in Aging.” *Biochemical and Biophysical Research Communications* 355 (2): 365–70. https://doi.org/10.1016/j.bbrc.2007.01.156.

Ifop, Etude, and Humasana Septembre. 2023. “LES FRANÇAISES ET LEUR RAPPORT À L ’ ÂGE ET AU VIEILLISSEMENT.”

Ito, Yoko, Matthew Hoare, and Masashi Narita. 2017. “Spatial and Temporal Control of Senescence.” *Trends in Cell Biology* 27 (11): 820–32. https://doi.org/10.1016/j.tcb.2017.07.004.

Jacquemyn, Julie, Ana Cascalho, and Rose E Goodchild. 2017. “The Ins and Outs of Endoplasmic Reticulum-controlled Lipid Biosynthesis.” *EMBO Reports* 18 (11): 1905–21. https://doi.org/https://doi.org/10.15252/embr.201643426.

Jafri, Mohammad A., Shakeel A. Ansari, Mohammed H. Alqahtani, and Jerry W. Shay. 2016. “Roles of Telomeres and Telomerase in Cancer, and Advances in Telomerase-Targeted Therapies.” *Genome Medicine* 8 (1). https://doi.org/10.1186/s13073-016-0324-x.

Jan, Rehmat, and Gul-E-Saba Chaudhry. 2019. “Understanding Apoptosis and Apoptotic Pathways Targeted Cancer Therapeutics.” *Advanced Pharmaceutical Bulletin* 9 (2): 205–18. https://doi.org/10.15171/apb.2019.024.

Jansen, Gregor, Pekka Määttänen, Alexey Y Denisov, Leslie Scarffe, Babette Schade, Haouaria Balghi, Kurt Dejgaard, et al. 2012. “An Interaction Map of Endoplasmic Reticulum Chaperones and Foldases.” *Molecular & Cellular Proteomics : MCP* 11 (9): 710–23. https://doi.org/10.1074/mcp.M111.016550.

Jevtić, Marijana, Anna Löwa, Anna Nováčková, Andrej Kováčik, Sabine Kaessmeyer, Gerrit Erdmann, Kateřina Vávrová, and Sarah Hedtrich. 2020. “Impact of Intercellular Crosstalk between Epidermal Keratinocytes and Dermal Fibroblasts on Skin Homeostasis.” *Biochimica et Biophysica Acta. Molecular Cell Research* 1867 (8): 118722. https://doi.org/10.1016/j.bbamcr.2020.118722.

Jeyapalan, Jessie C, Mark Ferreira, John M Sedivy, and Utz Herbig. 2007. “Accumulation of Senescent Cells in Mitotic Tissue of Aging Primates.” *Mechanisms of Ageing and Development*

128 (1): 36–44. https://doi.org/10.1016/j.mad.2006.11.008.

Johnson, A E, and M A van Waes. 1999. “The Translocon: A Dynamic Gateway at the ER Membrane.” *Annual Review of Cell and Developmental Biology* 15: 799–842. https://doi.org/10.1146/annurev.cellbio.15.1.799.

Jung, Tobias, Nicolle Bader, and Tilman Grune. 2007. “Lipofuscin: Formation, Distribution, and Metabolic Consequences.” *Annals of the New York Academy of Sciences* 1119 (November): 97–111. https://doi.org/10.1196/annals.1404.008.

Juzeniene, Asta, and Johan Moan. 2012. “Beneficial Effects of UV Radiation Other than via Vitamin D Production.” *Dermato-Endocrinology* 4 (2): 109–17. https://doi.org/10.4161/derm.20013.

Kale, Abhijit, Amit Sharma, Alexandra Stolzing, Alexandra Stolzing, Pierre Yves Desprez, Pierre Yves Desprez, and Judith Campisi. 2020. “Role of Immune Cells in the Removal of Deleterious Senescent Cells.” *Immunity and Ageing* 17 (1): 1–9. https://doi.org/10.1186/s12979-020-00187-9.

Kammeyer, A., and R. M. Luiten. 2015. “Oxidation Events and Skin Aging.” *Ageing Research Reviews* 21: 16–29. https://doi.org/10.1016/j.arr.2015.01.001.

Kampinga, Harm H, and Elizabeth A Craig. 2010. “The HSP70 Chaperone Machinery: J Proteins as Drivers of Functional Specificity.” *Nature Reviews. Molecular Cell Biology* 11 (8): 579–92. https://doi.org/10.1038/nrm2941.

Kang, Jian, Wenqi Chen, Jiping Xia, Yanhua Li, Bon Yang, Bin Chen, Weiling Sun, et al. 2008. “Extracellular Matrix Secreted by Senescent Fibroblasts Induced by UVB Promotes Cell Proliferation in HaCaT Cells through PI3K/AKT and ERK Signaling Pathways.” *International Journal of Molecular Medicine* 21 (6): 777–84. https://doi.org/10.3892/ijmm.21.6.777.

Kang, Tae-Won, Tetyana Yevsa, Norman Woller, Lisa Hoenicke, Torsten Wuestefeld, Daniel Dauch, Anja Hohmeyer, et al. 2011. “Senescence Surveillance of Pre-Malignant Hepatocytes Limits Liver Cancer Development.” *Nature* 479 (7374): 547–51. https://doi.org/10.1038/nature10599.

Kanitakis, Jean. 2002. “Anatomy, Histology and Immunohistochemistry of Normal Human Skin.” *European Journal of Dermatology : EJD* 12 (4): 390–91.

Karagöz, G. Elif, Diego Acosta-Alvear, and Peter Walter. 2019. “The Unfolded Protein Response: Detecting and Responding to Fluctuations in the Protein-Folding Capacity of the Endoplasmic Reticulum.” *Cold Spring Harbor Perspectives in Biology* 11 (9): 1–18. https://doi.org/10.1101/cshperspect.a033886.

Karali, Evdoxia, Sofia Bellou, Dimitris Stellas, Apostolos Klinakis, Carol Murphy, and Theodore Fotsis. 2014. “VEGF Signals through ATF6 and PERK to Promote Endothelial Cell Survival and Angiogenesis in the Absence of ER Stress.” *Molecular Cell* 54 (4): 559–72. https://doi.org/10.1016/j.molcel.2014.03.022.

Kaufman, Randal J. 1999. “Stress Signaling from the Lumen of the Endoplasmic Reticulum: Coordination of Gene Transcriptional and Translational Controls.” *Genes and Development*. https://doi.org/10.1101/gad.13.10.1211.

Kavanagh, Kylie, Chrissy Sherrill, Alistaire Ruggiero, Masha Block, Ravichandra Vemuri, Matthew Davis, and Amanda Olivier. 2021. “Biomarkers of Senescence in Non-Human Primate Adipose Depots Relate to Aging.” *GeroScience* 43 (1): 343–52. https://doi.org/10.1007/s11357-020-00230-z.

Kim, Eok Cheon, and Jae Ryong Kim. 2019. “Senotherapeutics: Emerging Strategy for Healthy Aging and Age-Related Disease.” *BMB Reports* 52 (1): 47–55. https://doi.org/10.5483/BMBREP.2019.52.1.293.

Kim, Hag Dong, Chang-Young Jang, Jeong Min Choe, Jeongwon Sohn, and Joon Kim. 2012. “Phenylbutyric Acid Induces the Cellular Senescence through an Akt/P21(WAF1) Signaling

Pathway." *Biochemical and Biophysical Research Communications* 422 (2): 213–18. https://doi.org/10.1016/j.bbrc.2012.04.086.

Kim, Hee Suk, Yongjin Kim, Min Jae Lim, Yun-Gyu Park, Serk In Park, and Jeongwon Sohn. 2019. "The P38-Activated ER Stress-ATF6α Axis Mediates Cellular Senescence." *The FASEB Journal* 33 (2): 2422–34. https://doi.org/10.1096/FJ.201800836R.

Kim, Jiyoon, Heon Yung Gee, and Min Goo Lee. 2018. "Unconventional Protein Secretion – New Insights into the Pathogenesis and Therapeutic Targets of Human Diseases." *Journal of Cell Science* 131 (12). https://doi.org/10.1242/jcs.213686.

Kim, Kyoung Mi, Ji Heon Noh, Monica Bodogai, Jennifer L. Martindale, Poonam R. Pandey, Xiaoling Yang, Arya Biragyn, Kotb Abdelmohsen, and Myriam Gorospe. 2018. "SCAMP4 Enhances the Senescent Cell Secretome." *Genes and Development* 32 (13–14): 909–14. https://doi.org/10.1101/gad.313270.118.

Kim, Mi-Sun, Yeon Kyung Kim, Kwang Hyun Cho, and Jin Ho Chung. 2006. "Regulation of Type I Procollagen and MMP-1 Expression after Single or Repeated Exposure to Infrared Radiation in Human Skin." *Mechanisms of Ageing and Development* 127 (12): 875–82. https://doi.org/10.1016/j.mad.2006.09.007.

Kim, Sung Young, Eun Jae Yang, Sung Bae Lee, Young Sam Lee, Kyoung A. Cho, and Sang Chul Park. 2020. "Global Transcriptional Downregulation of TREX and Nuclear Trafficking Machinery as Pan-Senescence Phenomena: Evidence from Human Cells and Tissues." *Experimental & Molecular Medicine 2020 52:8* 52 (8): 1351–59. https://doi.org/10.1038/s12276-020-00490-x.

Kim, William Y., and Norman E. Sharpless. 2006. "The Regulation of INK4/ARF in Cancer and Aging." *Cell* 127 (2): 265–75. https://doi.org/10.1016/j.cell.2006.10.003.

Kim, You Mi, Hae Ok Byun, Byul A. Jee, Hyunwoo Cho, Yong Hak Seo, You Sun Kim, Min Hi Park, Hae Young Chung, Hyun Goo Woo, and Gyesoon Yoon. 2013. "Implications of Time-Series Gene Expression Profiles of Replicative Senescence." *Aging Cell* 12 (4): 622–34. https://doi.org/10.1111/acel.12087.

Kim, Young Hwa, Yong Won Choi, Jeonghun Lee, Euy Young Soh, Jang-Hee Kim, and Tae Jun Park. 2017. "Senescent Tumor Cells Lead the Collective Invasion in Thyroid Cancer." *Nature Communications* 8 (May): 15208. https://doi.org/10.1038/ncomms15208.

Kimata, Yukio, Yuki Ishiwata-Kimata, Seiko Yamada, and Kenji Kohno. 2006. "Yeast Unfolded Protein Response Pathway Regulates Expression of Genes for Anti-Oxidative Stress and for Cell Surface Proteins." *Genes to Cells : Devoted to Molecular & Cellular Mechanisms* 11 (1): 59–69. https://doi.org/10.1111/j.1365-2443.2005.00921.x.

Kligman, A M. 1969. "Early Destructive Effect of Sunlight on Human Skin." *JAMA* 210 (13): 2377–80.

Kobbe, Cayetano Von. 2019. "Targeting Senescent Cells: Approaches, Opportunities, Challenges." *Aging* 11 (24): 12844–61. www.aging-us.com.

Koh, Jae Sook, Hoon Kang, Sung Woo Choi, and Hyung Ok Kim. 2002. "Cigarette Smoking Associated with Premature Facial Wrinkling: Image Analysis of Facial Skin Replicas." *International Journal of Dermatology* 41 (1): 21–27. https://doi.org/10.1046/j.1365-4362.2002.01352.x.

Kokame, Koichi, Hisao Kato, and Toshiyuki Miyata. 2001. "Identification of ERSE-II, a New Cis-Acting Element Responsible for the ATF6-Dependent Mammalian Unfolded Protein Response." *Journal of Biological Chemistry* 276 (12): 9199–9205. https://doi.org/10.1074/jbc.M010486200.

Komori, Ryota, Mai Taniguchi, Yoshiaki Ichikawa, Aya Uemura, Masaya Oku, Sadao Wakabayashi, Kazuhiko Higuchi, and Hiderou Yoshida. 2012. "Ultraviolet a Induces Endoplasmic Reticulum Stress Response in Human Dermal Fibroblasts." *Cell Structure and Function* 37 (1): 49–53. http://www.ncbi.nlm.nih.gov/pubmed/22251794.

Korolchuk, Viktor I, Satomi Miwa, Bernadette Carroll, and Thomas von Zglinicki. 2017. “Mitochondria in Cell Senescence: Is Mitophagy the Weakest Link?” *EBioMedicine* 21 (July): 7–13. https://doi.org/10.1016/j.ebiom.2017.03.020.

Korotkevich, Gennady, Vladimir Sukhov, Nikolay Budin, Maxim N. Atryomov, and Alexey Sergushichev. 2021. “Fast Gene Set Enrichment Analysis. BioRxiv.” *BioRxiv*, 1–29.

Koumenis, Constantinos. 2006. “ER Stress, Hypoxia Tolerance and Tumor Progression.” *Current Molecular Medicine* 6 (1): 55–69. https://doi.org/10.2174/156652406775574604.

Kreienkamp, Ray, and Susana Gonzalo. 2020. “Metabolic Dysfunction in Hutchinson-Gilford Progeria Syndrome.” *Cells* 9 (2): 1–17. https://doi.org/10.3390/cells9020395.

Kristensen, Caroline M, Christina T Brandt, Stine Ringholm, and Henriette Pilegaard. 2017. “PGC-1α in Aging and Lifelong Exercise Training-Mediated Regulation of UPR in Mouse Liver.” *Experimental Gerontology* 98: 124–33. https://doi.org/https://doi.org/10.1016/j.exger.2017.08.006.

Krutmann, Jean, Anne Bouloc, Gabrielle Sore, Bruno A. Bernard, and Thierry Passeron. 2017. “The Skin Aging Exposome.” *Journal of Dermatological Science* 85 (3): 152–61. https://doi.org/10.1016/J.JDERMSCI.2016.09.015.

Krutmann, Jean, Tamara Schikowski, Akimichi Morita, and Mark Berneburg. 2021. “Environmentally-Induced (Extrinsic) Skin Aging: Exposomal Factors and Underlying Mechanisms.” *Journal of Investigative Dermatology* 141 (4): 1096–1103. https://doi.org/10.1016/j.jid.2020.12.011.

Kuehn, Meta J, Johannes M Herrmann, and Randy Schekman. 1998. “COPII–Cargo Interactions Direct Protein Sorting into ER-Derived Transport Vesicles.” *Nature* 391 (6663): 187–90. https://doi.org/10.1038/34438.

Kuilman, Thomas, Chrysiis Michaloglou, Liesbeth C.W. Vredeveld, Sirith Douma, Remco van Doorn, Christophe J. Desmet, Lucien A. Aarden, Wolter J. Mooi, and Daniel S. Peeper. 2008. “Oncogene-Induced Senescence Relayed by an Interleukin-Dependent Inflammatory Network.” *Cell* 133 (6): 1019–31. https://doi.org/10.1016/J.CELL.2008.03.039.

Kulkarni, Ameya S., Sriram Gubbi, and Nir Barzilai. 2020. “Benefits of Metformin in Attenuating the Hallmarks of Aging.” *Cell Metabolism* 32 (1): 15–30. https://doi.org/10.1016/j.cmet.2020.04.001.

Kumari, Ruchi, and Parmjit Jat. 2021. “Mechanisms of Cellular Senescence: Cell Cycle Arrest and Senescence Associated Secretory Phenotype.” *Frontiers in Cell and Developmental Biology* 9 (March): 485. https://doi.org/10.3389/FCELL.2021.645593/BIBTEX.

Kurz, D. J., S. Decary, Y. Hong, and J. D. Erusalimsky. 2000. “Senescence-Associated β-Galactosidase Reflects an Increase in Lysosomal Mass during Replicative Ageing of Human Endothelial Cells.” *Journal of Cell Science* 113 (20): 3613–22. https://doi.org/10.1242/jcs.113.20.3613.

Kusama, Kazuya, Mikihiro Yoshie, Kazuhiro Tamura, Takahiro Nakayama, Hirotaka Nishi, Keiichi Isaka, and Eiichi Tachikawa. 2014. “The Role of Exchange Protein Directly Activated by Cyclic AMP 2-Mediated Calreticulin Expression in the Decidualization of Human Endometrial Stromal Cells.” *Endocrinology* 155 (1): 240–48. https://doi.org/10.1210/en.2013-1478.

Laberge, Remi-Martin, Yu Sun, Arturo V Orjalo, Christopher K Patil, Adam Freund, Lili Zhou, Samuel C Curran, et al. 2015. “MTOR Regulates the Pro-Tumorigenic Senescence-Associated Secretory Phenotype by Promoting IL1A Translation.” *Nature Cell Biology* 17 (8): 1049–61. https://doi.org/10.1038/ncb3195.

Lask, Gary, Nathalie Fournier, Mario Trelles, Monica Elman, Michael Scheflan, Michael Slatkine, Jenny Naimark, and Yoram Harth. 2005. “The Utilization of Nonthermal Blue (405-425 Nm) and near Infrared (850-890 Nm) Light in Aesthetic Dermatology and Surgery-a Multicenter Study.” *Journal of Cosmetic and Laser Therapy : Official Publication of the European Society for Laser Dermatology* 7 (3–4): 163–70. https://doi.org/10.1080/14764170500344302.

Lawrence, Karl P, Thierry Douki, Robert P E Sarkany, Stephanie Acker, Bernd Herzog, and Antony R Young. 2018. “The UV/Visible Radiation Boundary Region (385–405 Nm) Damages Skin Cells and Induces ‘Dark’ Cyclobutane Pyrimidine Dimers in Human Skin in Vivo.” *Scientific Reports* 8 (1): 12722. https://doi.org/10.1038/s41598-018-30738-6.

Lechler, Terry, and Elaine Fuchs. 2005. “Asymmetric Cell Divisions Promote Stratification and Differentiation of Mammalian Skin” 437 (September): 275–80. https://doi.org/10.1038/nature03922.

Lee, Andrew C., Brett E. Fenster, Hideki Ito, Kazuyo Takeda, Nancy S. Bae, Tazuko Hirai, Zu Xi Yu, Victor J. Ferrans, Bruce H. Howard, and Toren Finkel. 1999. “Ras Proteins Induce Senescence by Altering the Intracellular Levels of Reactive Oxygen Species.” *Journal of Biological Chemistry* 274 (12): 7936–40. https://doi.org/10.1074/jbc.274.12.7936.

Lee, Bo Yun, Jung A Han, Jun Sub Im, Amelia Morrone, Kimberly Johung, Edward C Goodwin, Wim J Kleijer, Daniel DiMaio, and Eun Seong Hwang. 2006. “Senescence-Associated Beta-Galactosidase Is Lysosomal Beta-Galactosidase.” *Aging Cell* 5 (2): 187–95. https://doi.org/10.1111/j.1474-9726.2006.00199.x.

Lee, Kwang M., Kyung H. Choi, and Michel M. Ouellette. 2004. “Use of Exogenous HTERT to Immortalize Primary Human Cells.” *Cytotechnology* 45 (1–2): 33–38. https://doi.org/10.1007/10.1007/s10616-004-5123-3.

Lee, Marcus C.S., Elizabeth A. Miller, Jonathan Goldberg, Lelio Orci, and Randy Schekman. 2004. “Bi-Directional Protein Transport between the ER and Golgi.” *Annual Review of Cell and Developmental Biology* 20: 87–123. https://doi.org/10.1146/annurev.cellbio.20.010403.105307.

Lefèvre-Utile, Alain, Camille Braun, Marek Haftek, and François Aubin. 2021. “Five Functional Aspects of the Epidermal Barrier.” *International Journal of Molecular Sciences* 22 (21): 1–14. https://doi.org/10.3390/ijms222111676.

Leiter, Ulrike, Thomas Eigentler, and Claus Garbe. 2014. “Epidemiology of Skin Cancer.” *Advances in Experimental Medicine and Biology* 810: 120–40. https://doi.org/10.1007/978-1-4939-0437-2_7.

Lerner, Alana G, John-Paul Upton, P V K Praveen, Rajarshi Ghosh, Yoshimi Nakagawa, Aeid Igbaria, Sarah Shen, et al. 2012. “IRE1α Induces Thioredoxin-Interacting Protein to Activate the NLRP3 Inflammasome and Promote Programmed Cell Death under Irremediable ER Stress.” *Cell Metabolism* 16 (2): 250–64. https://doi.org/10.1016/j.cmet.2012.07.007.

Li, Jingyi, Yuxuan Zheng, Pengze Yan, Moshi Song, Si Wang, Liang Sun, Zunpeng Liu, et al. 2021. “A Single-Cell Transcriptomic Atlas of Primate Pancreatic Islet Aging.” *National Science Review* 8 (2). https://doi.org/10.1093/nsr/nwaa127.

Liao, E. C., Y. T. Hsu, Q. Y. Chuah, Y. J. Lee, J. Y. Hu, T. C. Huang, P. M. Yang, and S. J. Chiu. 2014. “Radiation Induces Senescence and a Bystander Effect through Metabolic Alterations.” *Cell Death & Disease* 5. https://doi.org/10.1038/cddis.2014.220.

Liberzon, Arthur, Aravind Subramanian, Reid Pinchback, Helga Thorvaldsdóttir, Pablo Tamayo, and Jill P Mesirov. 2011. “Molecular Signatures Database (MSigDB) 3.0.” *Bioinformatics (Oxford, England)* 27 (12): 1739–40. https://doi.org/10.1093/bioinformatics/btr260.

Lim, Hyun, Haeil Park, and Hyun Pyo Kim. 2015. “Effects of Flavonoids on Senescence-Associated Secretory Phenotype Formation from Bleomycin-Induced Senescence in BJ Fibroblasts.” *Biochemical Pharmacology* 96 (4): 337–48. https://doi.org/10.1016/J.BCP.2015.06.013.

Lim, Jae Sung, Da Young Lee, Hyung Seok Kim, Sang Chul Park, Won Keun Oh, and Kyung A Cho. 2020. “L P Re Of.” *Mechanisms of Ageing and Development*, 111355. https://doi.org/10.1016/j.mad.2020.111355.

Lister, R, M Chrysovergi, and D Lagares. 2022. “Ephrin-B2 Neutralizing Antibody Suppresses the SASP in Senescent Fibroblasts and Treats Lung Fibrosis in Aged Mice” 216: 147059.

Liu, Su, Harpreet Uppal, Marco Demaria, Pierre Yves Desprez, Judith Campisi, and Pankaj Kapahi. 2015. “Simvastatin Suppresses Breast Cancer Cell Proliferation Induced by Senescent Cells.” *Scientific Reports 2015 5:1* 5 (1): 1–11. https://doi.org/10.1038/srep17895.

Liu, Yaohua, Hiroaki Sakamoto, Masaaki Adachi, Shiguang Zhao, Wataru Ukai, Eri Hashimoto, Masato Hareyama, Tadao Ishida, Kohzoh Imai, and Yasuhisa Shinomura. 2012. “Heat Stress Activates ER Stress Signals Which Suppress the Heat Shock Response, an Effect Occurring Preferentially in the Cortex in Rats.” *Molecular Biology Reports* 39 (4): 3987–93. https://doi.org/10.1007/s11033-011-1179-2.

Lizardo, Darleny Y., Yen Lung Lin, Omer Gokcumen, and G. Ekin Atilla-Gokcumen. 2017. “Regulation of Lipids Is Central to Replicative Senescence.” *Molecular BioSystems* 13 (3): 498–509. https://doi.org/10.1039/c6mb00842a.

Logue, Susan E., Eoghan P. McGrath, Patricia Cleary, Stephanie Greene, Katarzyna Mnich, Aitor Almanza, Eric Chevet, et al. 2018. “Inhibition of IRE1 RNase Activity Modulates the Tumor Cell Secretome and Enhances Response to Chemotherapy.” *Nature Communications 2018 9:1* 9 (1): 1–14. https://doi.org/10.1038/s41467-018-05763-8.

López-Otín, Carlos, Maria A. Blasco, Linda Partridge, Manuel Serrano, and Guido Kroemer. 2013. “The Hallmarks of Aging.” *Cell* 153 (6): 1194. https://doi.org/10.1016/j.cell.2013.05.039.

Lourenço Dos Santos, Sofia, Isabelle Petropoulos, and Bertrand Friguet. 2018. “The Oxidized Protein Repair Enzymes Methionine Sulfoxide Reductases and Their Roles in Protecting against Oxidative Stress, in Ageing and in Regulating Protein Function.” *Antioxidants (Basel, Switzerland)* 7 (12). https://doi.org/10.3390/antiox7120191.

Lu, Min, David A Lawrence, Scot Marsters, Diego Acosta-Alvear, Philipp Kimmig, Aaron S Mendez, Adrienne W Paton, James C Paton, Peter Walter, and Avi Ashkenazi. 2014. “Opposing Unfolded-Protein-Response Signals Converge on Death Receptor 5 to Control Apoptosis.” *Science (New York, N.Y.)* 345 (6192): 98–101. https://doi.org/10.1126/science.1254312.

Lu, Phoebe D, Heather P Harding, and David Ron. 2004. “Translation Reinitiation at Alternative Open Reading Frames Regulates Gene Expression in an Integrated Stress Response.” *The Journal of Cell Biology* 167 (1): 27–33. https://doi.org/10.1083/jcb.200408003.

Luo, Rongjin, Shuai Li, Gaocai Li, Saideng Lu, Weifeng Zhang, Hui Liu, Jie Lei, et al. 2021. “FAM134B-Mediated ER-Phagy Upregulation Attenuates AGEs-Induced Apoptosis and Senescence in Human Nucleus Pulposus Cells.” *Oxidative Medicine and Cellular Longevity* 2021: 3843145. https://doi.org/10.1155/2021/3843145.

Ma, Mengjun, Hongyu Li, Peng Wang, Wen Yang, Rujia Mi, Jiahao Zhuang, Yuhang Jiang, et al. 2021. “ATF6 Aggravates Angiogenesis-Osteogenesis Coupling during Ankylosing Spondylitis by Mediating FGF2 Expression in Chondrocytes.” *IScience* 24 (7): 102791. https://doi.org/https://doi.org/10.1016/j.isci.2021.102791.

Ma, Wenjian, Meinhard Wlaschek, Christina Hommel, Lars Alexander Schneider, and Karin Scharffetter-Kochanek. 2002. “Psoralen plus UVA (PUVA) Induced Premature Senescence as a Model for Stress-Induced Premature Senescence.” *Experimental Gerontology* 37 (10–11): 1197–1201. https://doi.org/10.1016/s0531-5565(02)00143-2.

Ma, Yanjun, Yuichiro Shimizu, Melissa J Mann, Yi Jin, and Linda M Hendershot. 2010. “Plasma Cell Differentiation Initiates a Limited ER Stress Response by Specifically Suppressing the PERK-Dependent Branch of the Unfolded Protein Response.” *Cell Stress & Chaperones* 15 (3): 281–93. https://doi.org/10.1007/s12192-009-0142-9.

Macario, Alberto J L, and Everly Conway de Macario. 2002. “Sick Chaperones and Ageing: A Perspective.” *Ageing Research Reviews* 1 (2): 295–311. https://doi.org/10.1016/s1568-1637(01)00005-8.

Machado-Oliveira, Gisela, Cristiano Ramos, André R.A. Marques, and Otília V. Vieira. 2020. “Cell Senescence, Multiple Organelle Dysfunction and Atherosclerosis.” *Cells* 9 (10).

https://doi.org/10.3390/CELLS9102146.

Maestroni, Laetitia, Samah Matmati, and Stéphane Coulon. 2017. “Solving the Telomere Replication Problem.” *Genes* 8 (2): 1–16. https://doi.org/10.3390/genes8020055.

Magalhães, João Pedro De, Florence Chainiaux, José Remacle, and Olivier Toussaint. 2002. “Stress-Induced Premature Senescence in BJ and HTERT-BJ1 Human Foreskin Fibroblasts.” *FEBS Letters* 523 (1–3): 157–62. https://doi.org/10.1016/S0014-5793(02)02973-3.

Magalhães, João Pedro de, and João F. Passos. 2018. “Stress, Cell Senescence and Organismal Ageing.” *Mechanisms of Ageing and Development* 170 (March): 2–9. https://doi.org/10.1016/J.MAD.2017.07.001.

Magkouta, Sophia, Dimitris Veroutis, Athanasios Pousias, Angelos Papaspyropoulos, Kety Giannetti, Natassa Pippa, Nikolaos Lougiakis, et al. 2024. “One-Step Rapid Tracking and Isolation of Senescent Cells in Cellular Systems, Tissues, or Animal Models via GLF16.” *STAR Protocols* 5 (1): 102929. https://doi.org/10.1016/j.xpro.2024.102929.

Mahadevan, Navin R., Jeffrey Rodvold, Homero Sepulveda, Steven Rossi, Angela F. Drew, and Maurizio Zanetti. 2011. “Transmission of Endoplasmic Reticulum Stress and Pro-Inflammation from Tumor Cells to Myeloid Cells.” *Proceedings of the National Academy of Sciences of the United States of America* 108 (16): 6561–66. https://doi.org/10.1073/pnas.1008942108.

Maksimovic, Srdjan, Masashi Nakatani, Yoshichika Baba, Aislyn M. Nelson, Kara L. Marshall, Scott A. Wellnitz, Pervez Firozi, et al. 2014. “Epidermal Merkel Cells Are Mechanosensory Cells That Tune Mammalian Touch Receptors.” *Nature* 509 (7502): 617–21. https://doi.org/10.1038/nature13250.

Malaquin, Nicolas, Marc-Alexandre Olivier, Aurélie Martinez, Stéphanie Nadeau, Christina Sawchyn, Jean-Philippe Coppé, Guillaume Cardin, Frédérick A Mallette, Judith Campisi, and Francis Rodier. 2020. “Non-canonical ATM/MRN Activities Temporally Define the Senescence Secretory Program.” *EMBO Reports* 21 (10). https://doi.org/10.15252/EMBR.202050718.

Malaquin, Nicolas, Véronique Tu, and Francis Rodier. 2019. “Assessing Functional Roles of the Senescence-Associated Secretory Phenotype (SASP).” *Methods in Molecular Biology (Clifton, N.J.)* 1896: 45–55. https://doi.org/10.1007/978-1-4939-8931-7_6.

Malaquin, Nicolas, Chantal Vercamer, Fatima Bouali, Sébastien Martien, Emeric Deruy, Nicolas Wernert, Maggy Chwastyniak, Florence Pinet, Corinne Abbadie, and Albin Pourtier. 2013. “Senescent Fibroblasts Enhance Early Skin Carcinogenic Events via a Paracrine MMP-PAR-1 Axis.” *PLOS ONE* 8 (5): e63607. https://doi.org/10.1371/JOURNAL.PONE.0063607.

Mann, Tobias, Kerstin Eggers, Frank Rippke, Mirko Tesch, Anette Buerger, Maxim E Darvin, Sabine Schanzer, Martina C Meinke, Jürgen Lademann, and Ludger Kolbe. 2020. “High-Energy Visible Light at Ambient Doses and Intensities Induces Oxidative Stress of Skin-Protective Effects of the Antioxidant and Nrf2 Inducer Licochalcone A in Vitro and in Vivo.” *Photodermatology, Photoimmunology & Photomedicine* 36 (2): 135–44. https://doi.org/10.1111/phpp.12523.

Mariano, Alessia, Irene Bigioni, Anna Scotto d’Abusco, Alessia Baseggio Conrado, Simonetta Maina, Antonio Francioso, Luciana Mosca, and Mario Fontana. 2021. “Pheomelanin Effect on UVB Radiation-Induced Oxidation/Nitration of l-Tyrosine.” *International Journal of Molecular Sciences* 23 (1). https://doi.org/10.3390/ijms23010267.

Marteijn, Jurgen A, Hannes Lans, Wim Vermeulen, and Jan H J Hoeijmakers. 2014. “Understanding Nucleotide Excision Repair and Its Roles in Cancer and Ageing.” *Nature Reviews Molecular Cell Biology* 15 (7): 465–81. https://doi.org/10.1038/nrm3822.

Martic, Ines, Sophia Wedel, Pidder Jansen-Dürr, and Maria Cavinato. 2020. “A New Model to Investigate UVB-Induced Cellular Senescence and Pigmentation in Melanocytes.” *Mechanisms of Ageing and Development* 190 (September): 111322. https://doi.org/10.1016/j.mad.2020.111322.

Martien, Sébastien, Olivier Pluquet, Chantal Vercamer, Nicolas Malaquin, Nathalie Martin, Karo Gosselin, Albin Pourtier, and Corinne Abbadie. 2013. “Cellular Senescence Involves an Intracrine Prostaglandin E2 Pathway in Human Fibroblasts.” *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids* 1831 (7): 1217–27. https://doi.org/10.1016/j.bbalip.2013.04.005.

Martin, Nathalie, Clara Salazar-Cardozo, Chantal Vercamer, Louise Ott, Guillemette Marot, Predrag Slijepcevic, Corinne Abbadie, and Olivier Pluquet. 2014. “Identification of a Gene Signature of a Pre-Transformation Process by Senescence Evasion in Normal Human Epidermal Keratinocytes.” *Molecular Cancer* 13 (June): 151. https://doi.org/10.1186/1476-4598-13-151.

Martínez-Zamudio, Ricardo Iván, Pierre François Roux, José Américo N.L.F. de Freitas, Lucas Robinson, Gregory Doré, Bin Sun, Dimitri Belenki, et al. 2020. “AP-1 Imprints a Reversible Transcriptional Programme of Senescent Cells.” *Nature Cell Biology* 22 (7): 842–55. https://doi.org/10.1038/s41556-020-0529-5.

Martini, Hélène, and João F. Passos. 2023. “Cellular Senescence: All Roads Lead to Mitochondria.” *FEBS Journal* 290 (5): 1186–1202. https://doi.org/10.1111/febs.16361.

Matacchione, Giulia, Felicia Gurău, Andrea Silvestrini, Mattia Tiboni, Luca Mancini, Debora Valli, Maria Rita Rippo, et al. 2021. “Anti-SASP and Anti-Inflammatory Activity of Resveratrol, Curcumin and β-Caryophyllene Association on Human Endothelial and Monocytic Cells.” *Biogerontology* 22 (3): 297. https://doi.org/10.1007/S10522-021-09915-0.

Matai, Latika, Gautam Chandra Sarkar, Manish Chamoli, Yasir Malik, Shashi Shekhar Kumar, Umanshi Rautela, Nihar Ranjan Jana, Kausik Chakraborty, and Arnab Mukhopadhyay. 2019. “Dietary Restriction Improves Proteostasis and Increases Life Span through Endoplasmic Reticulum Hormesis.” *Proceedings of the National Academy of Sciences of the United States of America* 116 (35): 17383–92. https://doi.org/10.1073/pnas.1900055116.

Matos, Liliana, Alexandra Monteiro Gouveia, and Henrique Almeida. 2015. “ER Stress Response in Human Cellular Models of Senescence.” *The Journals of Gerontology: Series A* 70 (8): 924–35. https://doi.org/10.1093/GERONA/GLU129.

Matsui, Takeshi, and Masayuki Amagai. 2015. “Dissecting the Formation, Structure and Barrier Function of the Stratum Corneum.” *International Immunology* 27 (6): 269–80. https://doi.org/10.1093/intimm/dxv013.

McCarter, Martin D. 2018. “Chapter 68 - Melanoma.” In , edited by Alden H Harken and Ernest E B T - Abernathy’s Surgical Secrets (Seventh Edition) Moore, 311–18. Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-323-47873-1.00068-1.

McCracken, A A, and J L Brodsky. 1996. “Assembly of ER-Associated Protein Degradation in Vitro: Dependence on Cytosol, Calnexin, and ATP.” *The Journal of Cell Biology* 132 (3): 291–98. https://doi.org/10.1083/jcb.132.3.291.

McCullough, K D, J L Martindale, L O Klotz, T Y Aw, and N J Holbrook. 2001. “Gadd153 Sensitizes Cells to Endoplasmic Reticulum Stress by Down-Regulating Bcl2 and Perturbing the Cellular Redox State.” *Molecular and Cellular Biology* 21 (4): 1249–59. https://doi.org/10.1128/MCB.21.4.1249-1259.2001.

McDermott, Martina S.J., Neil Conlon, Brigid C. Browne, Adam Szabo, Naoise C. Synnott, Neil A. O’brien, Michael J. Duffy, John Crown, and Norma O’donovan. 2019. “HER2-Targeted Tyrosine Kinase Inhibitors Cause Therapy-Induced-Senescence in Breast Cancer Cells.” *Cancers* 11 (2). https://doi.org/10.3390/CANCERS11020197.

McHugh, Domhnall, and Jesús Gil. 2018. “Senescence and Aging: Causes, Consequences, and Therapeutic Avenues.” *Journal of Cell Biology* 217 (1): 65–77. https://doi.org/10.1083/jcb.201708092.

Medrano, E E, S Im, F Yang, and Z A Abdel-Malek. 1995. “Ultraviolet B Light Induces G1 Arrest in Human Melanocytes by Prolonged Inhibition of Retinoblastoma Protein Phosphorylation

Associated with Long-Term Expression of the P21Waf-1/SDI-1/Cip-1 Protein." *Cancer Research* 55 (18): 4047–52.

Mees, Alexandra, Tobias Klar, Petra Gnau, Ulrich Hennecke, Andre P M Eker, Thomas Carell, and Lars-Oliver Essen. 2004. "Crystal Structure of a Photolyase Bound to a CPD-Like DNA Lesion After in Situ Repair." *Science* 306 (5702): 1789–93. https://doi.org/10.1126/science.1101598.

Meier, Florian, Andreas-David Brunner, Scarlet Koch, Heiner Koch, Markus Lubeck, Michael Krause, Niels Goedecke, et al. 2018. "Online Parallel Accumulation-Serial Fragmentation (PASEF) with a Novel Trapped Ion Mobility Mass Spectrometer." *Molecular & Cellular Proteomics : MCP* 17 (12): 2534–45. https://doi.org/10.1074/mcp.TIR118.000900.

Meinhardt, Merve, Ronald Krebs, Angelika Anders, Ulrike Heinrich, and Hagen Tronnier. 2008. "Wavelength-Dependent Penetration Depths of Ultraviolet Radiation in Human Skin." *Journal of Biomedical Optics* 13 (4): 044030. https://doi.org/10.1117/1.2957970.

Mekahli, Djalila, Geert Bultynck, Jan B Parys, Humbert De Smedt, and Ludwig Missiaen. 2011. "Endoplasmic-Reticulum Calcium Depletion and Disease." *Cold Spring Harbor Perspectives in Biology* 3 (6). https://doi.org/10.1101/cshperspect.a004317.

Melnik, Bodo C. 2014. "Endoplasmic Reticulum Stress: Key Promoter of Rosacea Pathogenesis." *Experimental Dermatology* 23 (12): 868–73. https://doi.org/10.1111/exd.12517.

Menicacci, Beatrice, Caterina Cipriani, Francesca Margheri, Alessandra Mocali, and Lisa Giovannelli. 2017. "Modulation of the Senescence-Associated Inflammatory Phenotype in Human Fibroblasts by Olive Phenols." *International Journal of Molecular Sciences 2017, Vol. 18, Page 2275* 18 (11): 2275. https://doi.org/10.3390/IJMS18112275.

Menicacci, Beatrice, Francesca Margheri, Anna Laurenzana, Anastasia Chillà, Mario Del Rosso, Lisa Giovannelli, Gabriella Fibbi, and Alessandra Mocali. 2019. "Chronic Resveratrol Treatment Reduces the Proangiogenic Effect of Human Fibroblast 'Senescent-Associated Secretory Phenotype' on Endothelial Colony-Forming Cells: The Role of IL8." *Journals of Gerontology - Series A Biological Sciences and Medical Sciences* 74 (5): 625–33. https://doi.org/10.1093/gerona/gly175.

Meyer, Hemmo, Monika Bug, and Sebastian Bremer. 2012. "Emerging Functions of the VCP/P97 AAA-ATPase in the Ubiquitin System." *Nature Cell Biology* 14 (2): 117–23. https://doi.org/10.1038/ncb2407.

Micco, Raffaella Di, Marzia Fumagalli, Angelo Cicalese, Sara Piccinin, Patrizia Gasparini, Chiara Luise, Catherine Schurra, et al. 2006. "Oncogene-Induced Senescence Is a DNA Damage Response Triggered by DNA Hyper-Replication." *Nature* 444 (7119): 638–42. https://doi.org/10.1038/nature05327.

Micco, Raffaella Di, Valery Krizhanovsky, Darren Baker, and Fabrizio d'Adda di Fagagna. 2020. "Cellular Senescence in Ageing: From Mechanisms to Therapeutic Opportunities." *Nature Reviews Molecular Cell Biology 2020 22:2* 22 (2): 75–95. https://doi.org/10.1038/s41580-020-00314-w.

Micco, Raffaella Di, Valery Krizhanovsky, Darren Baker, and Fabrizio d'Adda di Fagagna. 2021. "Cellular Senescence in Ageing: From Mechanisms to Therapeutic Opportunities." *Nature Reviews. Molecular Cell Biology* 22 (2): 75. https://doi.org/10.1038/S41580-020-00314-W.

Michaloglou, Chrysiis, Liesbeth C.W. Vredeveld, Maria S. Soengas, Christophe Denoyelle, Thomas Kuilman, Chantal M.A.M. Van Der Horst, Donné M. Majoor, Jerry W. Shay, Wolter J. Mooi, and Daniel S. Peeper. 2005. "BRAFE600-Associated Senescence-like Cell Cycle Arrest of Human Naevi." *Nature* 436 (7051): 720–24. https://doi.org/10.1038/nature03890.

Michielon, Elisabetta, Marta López González, Judith L A Burm, Taco Waaijman, Ekaterina S Jordanova, Tanja D de Gruijl, and Susan Gibbs. 2020. "Micro-Environmental Cross-Talk in an Organotypic Human Melanoma-in-Skin Model Directs M2-like Monocyte Differentiation via IL-10." *Cancer Immunology, Immunotherapy : CII* 69 (11): 2319–31. https://doi.org/10.1007/s00262-

020-02626-4.

Mirzakhani, Kimia, Julia Kallenbach, Seyed Mohammad Mahdi Rasa, Federico Ribaudo, Martin Ungelenk, Marzieh Ehsani, Wenrong Gong, et al. 2021. “The Androgen Receptor—LncRNASAT1-AKT-P15 Axis Mediates Androgen-Induced Cellular Senescence in Prostate Cancer Cells.” *Oncogene 2021 41:7* 41 (7): 943–59. https://doi.org/10.1038/s41388-021-02060-5.

Misawa, Tomoka, Yoko Tanaka, Ryo Okada, and Akiko Takahashi. 2020. “Biology of Extracellular Vesicles Secreted from Senescent Cells as Senescence-Associated Secretory Phenotype Factors.” *Geriatrics and Gerontology International* 20 (6): 539–46. https://doi.org/10.1111/ggi.13928.

Moiseeva, Olga, Véronique Bourdeau, Antoine Roux, Xavier Deschênes-Simard, and Gerardo Ferbeyre. 2009. “Mitochondrial Dysfunction Contributes to Oncogene-Induced Senescence.” *Molecular and Cellular Biology* 29 (16): 4495–4507. https://doi.org/10.1128/mcb.01868-08.

Moll, Roland, Markus Divo, and Lutz Langbein. 2008. “The Human Keratins: Biology and Pathology.” *Histochemistry and Cell Biology* 129 (6): 705–33. https://doi.org/10.1007/s00418-008-0435-6.

Moon, Kyung-Chul, Jong-Phil Yang, Jae-Sun Lee, Seong-Ho Jeong, Eun-Sang Dhong, and Seung-Kyu Han. 2019. “Effects of Ultraviolet Irradiation on Cellular Senescence in Keratinocytes Versus Fibroblasts.” *The Journal of Craniofacial Surgery* 30 (1): 270–75. https://doi.org/10.1097/SCS.0000000000004904.

Moreci, Rebecca S., and Terry Lechler. 2020. “Epidermal Structure and Differentiation.” *Current Biology* 30 (4): R144–49. https://doi.org/10.1016/j.cub.2020.01.004.

Moujaber, Ossama, Francine Fishbein, Nawal Omran, Yue Liang, Inés Colmegna, John F. Presley, and Ursula Stochaj. 2019. “Cellular Senescence Is Associated with Reorganization of the Microtubule Cytoskeleton.” *Cellular and Molecular Life Sciences* 76 (6): 1169–83. https://doi.org/10.1007/s00018-018-2999-1.

Mound, Abdallah, Vesela Lozanova, Céline Warnon, Maryse Hermant, Julie Robic, Christelle Guere, Katell Vie, et al. 2017. “Non-Senescent Keratinocytes Organize in Plasma Membrane Submicrometric Lipid Domains Enriched in Sphingomyelin and Involved in Re-Epithelialization.” *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* 1862 (9): 958–71. https://doi.org/https://doi.org/10.1016/j.bbalip.2017.06.001.

Münch, Christian. 2018. “The Different Axes of the Mammalian Mitochondrial Unfolded Protein Response.” *BMC Biology* 16 (1): 1–9. https://doi.org/10.1186/s12915-018-0548-x.

Murtada, Sae Il, Nicole Mikush, Mo Wang, Pengwei Ren, Yuki Kawamura, Abhay B. Ramachandra, David S. Li, et al. 2023. “Lonafarnib Improves Cardiovascular Function and Survival in a Mouse Model of Hutchinson-Gilford Progeria Syndrome.” *ELife* 12: 1–17. https://doi.org/10.7554/elife.82728.

Muther, Charlotte, Lara Jobeili, Maëlle Garion, Sandrine Heraud, Amélie Thepot, Odile Damour, and Jérôme Lamartine. 2017. “An Expression Screen for Aged-Dependent MicroRNAs Identifies MiR-30a as a Key Regulator of Aging Features in Human Epidermis.” *Aging* 9 (11): 2376–96. https://doi.org/10.18632/aging.101326.

Narayanan, Deevya L., Rao N. Saladi, and Joshua L. Fox. 2010. “Ultraviolet Radiation and Skin Cancer.” *International Journal of Dermatology* 49 (9): 978–86. https://doi.org/10.1111/j.1365-4632.2010.04474.x.

Narita, Masashi, Sabrina Nũnez, Edith Heard, Masako Narita, Athena W. Lin, Stephen A. Hearn, David L. Spector, Gregory J. Hannon, and Scott W. Lowe. 2003. “Rb-Mediated Heterochromatin Formation and Silencing of E2F Target Genes during Cellular Senescence.” *Cell* 113 (6): 703–16. https://doi.org/10.1016/S0092-8674(03)00401-X.

Narzt, Marie Sophie, Vera Pils, Christopher Kremslehner, Ionela Mariana Nagelreiter, Markus

Schosserer, Emilia Bessonova, Alina Bayer, et al. 2021. “Epilipidomics of Senescent Dermal Fibroblasts Identify Lysophosphatidylcholines as Pleiotropic Senescence-Associated Secretory Phenotype (SASP) Factors.” *Journal of Investigative Dermatology* 141 (4): 993-1006.e15. https://doi.org/10.1016/J.JID.2020.11.020.

Nassour, Joe, and Corinne Abbadie. 2016. “A Novel Role for DNA Single-Strand Breaks in Senescence and Neoplastic Escape of Epithelial Cells.” *Molecular and Cellular Oncology* 3 (5): 1–3. https://doi.org/10.1080/23723556.2016.1190885.

Nassour, Joe, Sébastien Martien, Nathalie Martin, Emeric Deruy, Elisa Tomellini, Nicolas Malaquin, Fatima Bouali, et al. 2016. “Defective DNA Single-Strand Break Repair Is Responsible for Senescence and Neoplastic Escape of Epithelial Cells.” *Nature Communications* 7. https://doi.org/10.1038/ncomms10399.

Nesvizhskii, Alexey I, Andrew Keller, Eugene Kolker, and Ruedi Aebersold. 2003. “A Statistical Model for Identifying Proteins by Tandem Mass Spectrometry.” *Analytical Chemistry* 75 (17): 4646–58. https://doi.org/10.1021/ac0341261.

Neurohr, Gabriel E., Rachel L. Terry, Jette Lengefeld, Megan Bonney, Gregory P. Brittingham, Fabien Moretto, Teemu P. Miettinen, et al. 2019. “Excessive Cell Growth Causes Cytoplasm Dilution And Contributes to Senescence.” *Cell* 176 (5): 1083-1097.e18. https://doi.org/10.1016/j.cell.2019.01.018.

Ni, Chunya, Marie Sophie Narzt, Ionela Mariana Nagelreiter, Cheng Feng Zhang, Lionel Larue, Heidemarie Rossiter, Johannes Grillari, Erwin Tschachler, and Florian Gruber. 2016. “Autophagy Deficient Melanocytes Display a Senescence Associated Secretory Phenotype That Includes Oxidized Lipid Mediators.” *The International Journal of Biochemistry & Cell Biology* 81 (December): 375–82. https://doi.org/10.1016/J.BIOCEL.2016.10.006.

Nickel, Walter, and Catherine Rabouille. 2009. “Mechanisms of Regulated Unconventional Protein Secretion.” *Nature Reviews Molecular Cell Biology* 10 (2): 148–55. https://doi.org/10.1038/nrm2617.

Niehues, Hanna, Gijs Rikken, Ivonne M.J.J. van Vlijmen-Willems, Diana Rodijk-Olthuis, Piet E.J. van Erp, Patrick L.J.M. Zeeuwen, Joost Schalkwijk, and Ellen H. van den Bogaard. 2022. “Identification of Keratinocyte Mitogens: Implications for Hyperproliferation in Psoriasis and Atopic Dermatitis.” *JID Innovations* 2 (1): 100066. https://doi.org/10.1016/j.xjidi.2021.100066.

Nishio, Koji, Akira Inoue, Shanlou Qiao, Hiroshi Kondo, and Akio Mimura. 2001. “Senescence and Cytoskeleton: Overproduction of Vimentin Induces Senescent-like Morphology in Human Fibroblasts.” *Histochemistry and Cell Biology* 116 (4): 321–27. https://doi.org/10.1007/s004180100325.

Novoa, I, H Zeng, H P Harding, and D Ron. 2001. “Feedback Inhibition of the Unfolded Protein Response by GADD34-Mediated Dephosphorylation of EIF2alpha.” *The Journal of Cell Biology* 153 (5): 1011–22. https://doi.org/10.1083/jcb.153.5.1011.

Nuss, Jonathan E, Kashyap B Choksi, James H DeFord, and John Papaconstantinou. 2008. “Decreased Enzyme Activities of Chaperones PDI and BiP in Aged Mouse Livers.” *Biochemical and Biophysical Research Communications* 365 (2): 355–61. https://doi.org/10.1016/j.bbrc.2007.10.194.

O’Sullivan, Roderick J., and Jan Karlseder. 2010. “Telomeres: Protecting Chromosomes against Genome Instability.” *Nature Reviews Molecular Cell Biology* 11 (3): 171–81. https://doi.org/10.1038/nrm2848.

Oakes, Scott A. 2020. “Endoplasmic Reticulum Stress Signaling in Cancer Cells.” *The American Journal of Pathology* 190 (5): 934–46. https://doi.org/https://doi.org/10.1016/j.ajpath.2020.01.010.

Ogata, Yuichiro, Takaaki Yamada, Seiji Hasegawa, Ayumi Sanada, Yohei Iwata, Masaru Arima, Satoru Nakata, Kazumitsu Sugiura, and Hirohiko Akamatsu. 2021. “SASP-Induced Macrophage Dysfunction May Contribute to Accelerated Senescent Fibroblast Accumulation in the Dermis.”

Experimental Dermatology 30 (1): 84–91. https://doi.org/10.1111/exd.14205.

Ogata, Yuichiro, Takaaki Yamada, Seiji Hasegawa, Kazumitsu Sugiura, and Hirohiko Akamatsu. 2023. "Changes of Senescent Cell Accumulation and Removal in Skin Tissue with Ageing." *Experimental Dermatology* 32 (7): 1159–61. https://doi.org/10.1111/exd.14818.

Ohoka, Nobumichi, Satoshi Yoshii, Takayuki Hattori, Kikuo Onozaki, and Hidetoshi Hayashi. 2005. "TRB3, a Novel ER Stress-Inducible Gene, Is Induced via ATF4-CHOP Pathway and Is Involved in Cell Death." *The EMBO Journal* 24 (6): 1243–55. https://doi.org/10.1038/sj.emboj.7600596.

Olden, K, R M Pratt, C Jaworski, and K M Yamada. 1979. "Evidence for Role of Glycoprotein Carbohydrates in Membrane Transport: Specific Inhibition by Tunicamycin." *Proceedings of the National Academy of Sciences of the United States of America* 76 (2): 791–95. https://doi.org/10.1073/pnas.76.2.791.

Olivari, Silvia, and Maurizio Molinari. 2007. "Glycoprotein Folding and the Role of EDEM1, EDEM2 and EDEM3 in Degradation of Folding-Defective Glycoproteins." *FEBS Letters* 581 (19): 3658–64. https://doi.org/10.1016/j.febslet.2007.04.070.

Olumi, AF, GD Grossfeld, SW Hayward, PR Carroll, GR Cunha, P Hein, and TD Tlsty. 1999. "Carcinoma-Associated Fibroblasts Direct Tumor Progression of Initiated Human Prostatic Epithelium." *Cancer Research* 59 (19). https://doi.org/10.1186/BCR138.

Orjalo, Arturo V., Dipa Bhaumik, Bridget K. Gengler, Gary K. Scott, and Judith Campisi. 2009. "Cell Surface-Bound IL-1α Is an Upstream Regulator of the Senescence-Associated IL-6/IL-8 Cytokine Network." *Proceedings of the National Academy of Sciences of the United States of America* 106 (40): 17031–36. https://doi.org/10.1073/PNAS.0905299106/SUPPL_FILE/0905299106SI.PDF.

Ortiz-Montero, Paola, Arturo Londoño-Vallejo, and Jean-Paul Vernot. 2017. "Senescence-Associated IL-6 and IL-8 Cytokines Induce a Self- and Cross-Reinforced Senescence/Inflammatory Milieu Strengthening Tumorigenic Capabilities in the MCF-7 Breast Cancer Cell Line." *Cell Communication and Signaling : CCS* 15 (1): 17. https://doi.org/10.1186/s12964-017-0172-3.

Ostrowski, Matias, Nuno B. Carmo, Sophie Krumeich, Isabelle Fanget, Graça Raposo, Ariel Savina, Catarina F. Moita, et al. 2010. "Rab27a and Rab27b Control Different Steps of the Exosome Secretion Pathway." *Nature Cell Biology* 12 (1): 19–30. https://doi.org/10.1038/ncb2000.

Ozcan, Lale, and Ira Tabas. 2012. "Role of Endoplasmic Reticulum Stress in Metabolic Disease and Other Disorders." *Annual Review of Medicine* 63: 317–28. https://doi.org/10.1146/annurev-med-043010-144749.

Özcan, Servet, Nicola Alessio, Mustafa B. Acar, Eda Mert, Fatih Omerli, Gianfranco Peluso, and Umberto Galderisi. 2016. "Unbiased Analysis of Senescence Associated Secretory Phenotype (SASP) to Identify Common Components Following Different Genotoxic Stresses." *Aging (Albany NY)* 8 (7): 1316. https://doi.org/10.18632/AGING.100971.

Palmer, Raymond D. 2022. "Aging Clocks & Mortality Timers, Methylation, Glycomic, Telomeric and More. A Window to Measuring Biological Age." *Aging Medicine (Milton (N.S.W))* 5 (2): 120–25. https://doi.org/10.1002/agm2.12197.

Park, Kyungho, Sang Eun Lee, Kyong-Oh Shin, and Yoshikazu Uchida. 2019. "Insights into the Role of Endoplasmic Reticulum Stress in Skin Function and Associated Diseases." *The FEBS Journal* 286 (2): 413–25. https://doi.org/10.1111/febs.14739.

Park, Mineon, Jiyoung Na, Seo Young Kwak, Sunhoo Park, Hyewon Kim, Sun Joo Lee, Won Suk Jang, et al. 2022. "Zileuton Alleviates Radiation-Induced Cutaneous Ulcers via Inhibition of Senescence-Associated Secretory Phenotype in Rodents." *International Journal of Molecular Sciences 2022, Vol. 23, Page 8390* 23 (15): 8390. https://doi.org/10.3390/IJMS23158390.

Park, Soon Sang, Yong Won Choi, Jang Hee Kim, Hong Seok Kim, and Tae Jun Park. 2021. "Senescent Tumor Cells: An Overlooked Adversary in the Battle against Cancer." *Experimental & Molecular Medicine 2021 53:12* 53 (12): 1834–41. https://doi.org/10.1038/s12276-021-00717-5.

Park, Sung Min, Tae Il Kang, and Jae Seon So. 2021. “Roles of XBP1s in Transcriptional Regulation of Target Genes.” *Biomedicines* 9 (7): 1–26. https://doi.org/10.3390/biomedicines9070791.

Parrinello, Simona, Jean Philippe Coppe, Ana Krtolica, and Judith Campisi. 2005. “Stromal-Epithelial Interactions in Aging and Cancer: Senescent Fibroblasts Alter Epithelial Cell Differentiation.” *Journal of Cell Science* 118 (Pt 3): 485. https://doi.org/10.1242/JCS.01635.

Partridge, Linda, Joris Deelen, and P. Eline Slagboom. 2018. “Facing up to the Global Challenges of Ageing.” *Nature 2018 561:7721* 561 (7721): 45–56. https://doi.org/10.1038/s41586-018-0457-8.

Passos, João F., Gabriele Saretzki, Shaheda Ahmed, Glyn Nelson, Torsten Richter, Heiko Peters, Ilka Wappler, et al. 2007. “Mitochondrial Dysfunction Accounts for the Stochastic Heterogeneity in Telomere-Dependent Senescence.” *PLoS Biology* 5 (5): 1138–51. https://doi.org/10.1371/journal.pbio.0050110.

Passos, João F, Glyn Nelson, Chunfang Wang, Torsten Richter, Cedric Simillion, Carole J Proctor, Satomi Miwa, et al. 2010. “Feedback between P21 and Reactive Oxygen Production Is Necessary for Cell Senescence.” *Molecular Systems Biology* 6: 347. https://doi.org/10.1038/msb.2010.5.

Pathak, Rashmi Upadhyay, Mamilla Soujanya, and Rakesh Kumar Mishra. 2021. “Deterioration of Nuclear Morphology and Architecture: A Hallmark of Senescence and Aging.” *Ageing Research Reviews* 67 (May). https://doi.org/10.1016/J.ARR.2021.101264.

Paz Gavilán, M., José Vela, Angélica Castaño, Blanca Ramos, Juan C. del Río, Javier Vitorica, and Diego Ruano. 2006. “Cellular Environment Facilitates Protein Accumulation in Aged Rat Hippocampus.” *Neurobiology of Aging* 27 (7): 973–82. https://doi.org/10.1016/j.neurobiolaging.2005.05.010.

Pelham, H. R. 1988. “Evidence That Luminal ER Proteins Are Sorted from Secreted Proteins in a Post-ER Compartment.” *The EMBO Journal* 7 (4): 913–18. https://doi.org/10.1002/j.1460-2075.1988.tb02896.x.

Pennisi, Rosa, Paolo Ascenzi, and Alessandra di Masi. 2015. “Hsp90: A New Player in DNA Repair?” *Biomolecules* 5 (4): 2589–2618. https://doi.org/10.3390/biom5042589.

Peris, Ketty, Maria Concetta Fargnoli, Roland Kaufmann, Petr Arenberger, Lars Bastholt, Nicole Basset Seguin, Veronique Bataille, et al. 2023. “European Consensus-Based Interdisciplinary Guideline for Diagnosis and Treatment of Basal Cell Carcinoma-Update 2023.” *European Journal of Cancer (Oxford, England : 1990)* 192 (October): 113254. https://doi.org/10.1016/j.ejca.2023.113254.

Pfisterer, Karin, Lisa E. Shaw, Dörte Symmank, and Wolfgang Weninger. 2021. “The Extracellular Matrix in Skin Inflammation and Infection.” *Frontiers in Cell and Developmental Biology* 9 (July): 1–19. https://doi.org/10.3389/fcell.2021.682414.

Phillips, Melissa J, and Gia K Voeltz. 2016. “Structure and Function of ER Membrane Contact Sites with Other Organelles.” *Nature Reviews. Molecular Cell Biology* 17 (2): 69–82. https://doi.org/10.1038/nrm.2015.8.

Pluquet, Olivier, and Corinne Abbadie. 2021. “Cellular Senescence and Tumor Promotion: Role of the Unfolded Protein Response.” *Advances in Cancer Research* 150 (January): 285–334. https://doi.org/10.1016/BS.ACR.2021.01.001.

Pluquet, Olivier, Nicolas Dejeans, Marion Bouchecareilh, Stephanie Lhomond, Raphael Pineau, Arisa Higa, Maylis Delugin, et al. 2013. “Posttranscriptional Regulation of PER1 Underlies the Oncogenic Function of IREα.” *Cancer Research* 73 (15): 4732–43. https://doi.org/10.1158/0008-5472.CAN-12-3989.

Pluquet, Olivier, Albin Pourtier, and Corinne Abbadie. 2015. “A Review in the Theme: Cellular Mechanisms of Endoplasmic Reticu-Lum Stress Signaling in Health and Disease.” *Am J Physiol Cell Physiol* 308: 415–25. https://doi.org/10.1152/ajpcell.00334.2014.

Pobre, Kristine Faye R, Greg J Poet, and Linda M Hendershot. 2019. “The Endoplasmic Reticulum (ER) Chaperone BiP Is a Master Regulator of ER Functions: Getting by with a Little Help from ERdj Friends.” *Journal of Biological Chemistry* 294 (6): 2098–2108. https://doi.org/https://doi.org/10.1074/jbc.REV118.002804.

Polefka, Thomas, Thomas Meyer, Patricia Agin, and Robert Bianchini. 2012. “Effects of Solar Radiation on the Skin.” *Journal of Cosmetic Dermatology* 11 (June): 134–43. https://doi.org/10.1111/j.1473-2165.2012.00614.x.

Prattichizzo, Francesco, Angelica Giuliani, Rina Recchioni, Massimiliano Bonafè, Fiorella Marcheselli, Sabrina De Carolis, Anna Campanati, et al. 2016. “Anti-TNF-α Treatment Modulates SASP and SASP-Related MicroRNAs in Endothelial Cells and in Circulating Angiogenic Cells.” *Oncotarget* 7 (11): 11945–58. https://doi.org/10.18632/ONCOTARGET.7858.

Proksch, Ehrhardt, Johanna M Brandner, and Jens-Michael Jensen. 2008. “The Skin: An Indispensable Barrier.” *Experimental Dermatology* 17 (12): 1063–72. https://doi.org/10.1111/j.1600-0625.2008.00786.x.

Puthalakath, Hamsa, Lorraine A O’Reilly, Priscilla Gunn, Lily Lee, Priscilla N Kelly, Nicholas D Huntington, Peter D Hughes, et al. 2007. “ER Stress Triggers Apoptosis by Activating BH3-Only Protein Bim.” *Cell* 129 (7): 1337–49. https://doi.org/https://doi.org/10.1016/j.cell.2007.04.027.

Qiang, Mingyue, and Zijia Dai. 2024. “Biomarkers of UVB Radiation-Related Senescent Fibroblasts.” *Scientific Reports* 14 (1): 1–12. https://doi.org/10.1038/s41598-023-51058-4.

Quaas, Marianne, Gerd A. Müller, and Kurt Engeland. 2012. “P53 Can Repress Transcription of Cell Cycle Genes through a P21 WAF1/CIP1-Dependent Switch from MMB to DREAM Protein Complex Binding at CHR Promoter Elements.” *Cell Cycle* 11 (24). https://doi.org/10.4161/cc.22917.

Rabek, Jeffrey P, William H 3rd Boylston, and John Papaconstantinou. 2003. “Carbonylation of ER Chaperone Proteins in Aged Mouse Liver.” *Biochemical and Biophysical Research Communications* 305 (3): 566–72. https://doi.org/10.1016/s0006-291x(03)00826-x.

Rafehi, Haloom, Christian Orlowski, George T Georgiadis, Katherine Ververis, Assam El-Osta, and Tom C Karagiannis. 2011. “Clonogenic Assay: Adherent Cells.” *Journal of Visualized Experiments : JoVE*, no. 49 (March). https://doi.org/10.3791/2573.

Raffaele, Marco, and Manlio Vinciguerra. 2022. “The Costs and Benefits of Senotherapeutics for Human Health.” *The Lancet Healthy Longevity* 3 (1): e67–77. https://doi.org/10.1016/S2666-7568(21)00300-7.

Răileanu, Mina, Mihai Straticiuc, Decebal-Alexandru Iancu, Radu-Florin Andrei, Mihai Radu, and Mihaela Bacalum. 2022. “Proton Irradiation Induced Reactive Oxygen Species Promote Morphological and Functional Changes in HepG2 Cells.” *Journal of Structural Biology* 214 (4): 107919. https://doi.org/10.1016/j.jsb.2022.107919.

Rajesh, Kamindla, Andreas I Papadakis, Urszula Kazimierczak, Philippos Peidis, Shuo Wang, Gerardo Ferbeyre, Randal J Kaufman, and Antonis E Koromilas. 2013. “EIF2α Phosphorylation Bypasses Premature Senescence Caused by Oxidative Stress and Pro-Oxidant Antitumor Therapies.” *Aging* 5 (12): 884–901. https://doi.org/10.18632/aging.100620.

Randall, Matthew J., Astrid Jüngel, Markus Rimann, and Karin Wuertz-Kozak. 2018. “Advances in the Biofabrication of 3D Skin in Vitro: Healthy and Pathological Models.” *Frontiers in Bioengineering and Biotechnology* 6 (OCT). https://doi.org/10.3389/fbioe.2018.00154.

Rastogi, Rajesh P., Richa, Ashok Kumar, Madhu B. Tyagi, and Rajeshwar P. Sinha. 2010. “Molecular Mechanisms of Ultraviolet Radiation-Induced DNA Damage and Repair.” *Journal of Nucleic Acids* 2010. https://doi.org/10.4061/2010/592980.

Raykhel, Irina, Heli Alanen, Kirsi Salo, Jaana Jurvansuu, Van Dat Nguyen, Maria Latva-Ranta, and Lloyd Ruddock. 2007. “A Molecular Specificity Code for the Three Mammalian KDEL Receptors.”

The Journal of Cell Biology 179 (6): 1193–1204. https://doi.org/10.1083/jcb.200705180.

Reddy, K. L., J. M. Zullo, E. Bertolino, and H. Singh. 2008. “Transcriptional Repression Mediated by Repositioning of Genes to the Nuclear Lamina.” *Nature* 452 (7184): 243–47. https://doi.org/10.1038/nature06727.

Redmond, Matthew. 2022. “Living Too Long.” *Nineteenth-Century Literature* 77 (1): 29–55. https://doi.org/10.1525/ncl.2022.77.1.29.

Reggiori, Fulvio, and Maurizio Molinari. 2022. “Er-Phagy: Mechanisms, Regulation, and Diseases Connected To the Lysosomal Clearance of the Endoplasmic Reticulum.” *Physiological Reviews* 102 (3): 1398–1448. https://doi.org/10.1152/physrev.00038.2021.

Ritschka, Birgit, Mekayla Storer, Alba Mas, Florian Heinzmann, Mari Carmen Ortells, Jennifer P. Morton, Owen J. Sansom, Lars Zender, and William M. Keyes. 2017. “The Senescence-Associated Secretory Phenotype Induces Cellular Plasticity and Tissue Regeneration.” *Genes and Development* 31 (2): 172–83. https://doi.org/10.1101/GAD.290635.116/-/DC1.

Robinson, Mark D, Davis J McCarthy, and Gordon K Smyth. 2010. “EdgeR: A Bioconductor Package for Differential Expression Analysis of Digital Gene Expression Data.” *Bioinformatics (Oxford, England)* 26 (1): 139–40. https://doi.org/10.1093/bioinformatics/btp616.

Rochette, Patrick J, Jean-Philippe Therrien, Régen Drouin, Daniel Perdiz, Nathalie Bastien, Elliot A Drobetsky, and Evelyne Sage. 2003. “UVA-Induced Cyclobutane Pyrimidine Dimers Form Predominantly at Thymine-Thymine Dipyrimidines and Correlate with the Mutation Spectrum in Rodent Cells.” *Nucleic Acids Research* 31 (11): 2786–94. https://doi.org/10.1093/nar/gkg402.

Roczniak-Ferguson, Agnes, Constance S Petit, Florian Froehlich, Sharon Qian, Jennifer Ky, Brittany Angarola, Tobias C Walther, and Shawn M Ferguson. 2012. “The Transcription Factor TFEB Links MTORC1 Signaling to Transcriptional Control of Lysosome Homeostasis.” *Science Signaling* 5 (228): ra42. https://doi.org/10.1126/scisignal.2002790.

Roddy, Meagan, Thom Nelson, Daniel M Appledorn, Vince Groppi, Essen Bioscience, and Ann Arbor. 2017. “IncuCyte ® Scratch Wound 96-Well Real-Time Cell Migration and Invasion Assays,” 1–8.

Rodier, Francis, Jean Philippe Coppé, Christopher K. Patil, Wieteke A.M. Hoeijmakers, Denise P. Muñoz, Saba R. Raza, Adam Freund, Eric Campeau, Albert R. Davalos, and Judith Campisi. 2009. “Persistent DNA Damage Signaling Triggers Senescence-Associated Inflammatory Cytokine Secretion.” *Nature Cell Biology* 11 (8): 973. https://doi.org/10.1038/NCB1909.

Rodier, Francis, Denise P. Muñoz, Robert Teachenor, Victoria Chu, Oanh Le, Dipa Bhaumik, Jean Philippe Coppé, et al. 2011. “DNA-SCARS: Distinct Nuclear Structures That Sustain Damage-Induced Senescence Growth Arrest and Inflammatory Cytokine Secretion.” *Journal of Cell Science* 124 (1): 68–81. https://doi.org/10.1242/jcs.071340.

Rodvold, Jeffrey J., Kevin T. Chiu, Nobuhiko Hiramatsu, Julia K. Nussbacher, Valentina Galimberti, Navin R. Mahadevan, Karl Willert, Jonathan H. Lin, and Maurizio Zanetti. 2017. “Intercellular Transmission of the Unfolded Protein Response Promotes Survival and Drug Resistance in Cancer Cells.” *Science Signaling* 10 (482). https://doi.org/10.1126/scisignal.aah7177.

Römisch, Karin. 2017. “A Case for Sec61 Channel Involvement in ERAD.” *Trends in Biochemical Sciences* 42 (3): 171–79. https://doi.org/10.1016/j.tibs.2016.10.005.

Rorteau, Julie, Fabien P. Chevalier, Bérengère Fromy, and Jérôme Lamartine. 2020. “Functional Integrity of Aging Skin, from Cutaneous Biology to Anti-Aging Strategies.” *Medecine/Sciences* 36 (12): 1155–62. https://doi.org/10.1051/medsci/2020223.

Rösing, Sarah, Fabian Ullrich, Susann Meisterfeld, Franziska Schmidt, Laura Mlitzko, Marijana Croon, Ryan G Nattrass, et al. 2024. “Chronic Endoplasmic Reticulum Stress in Myotonic Dystrophy Type 2 Promotes Autoimmunity via Mitochondrial DNA Release.” *Nature Communications* 15 (1): 1534. https://doi.org/10.1038/s41467-024-45535-1.

Rossi, Stefania, Martina Cordella, Claudio Tabolacci, Giovanni Nassa, Daniela D'Arcangelo, Cinzia Senatore, Paolo Pagnotto, et al. 2018. "TNF-Alpha and Metalloproteases as Key Players in Melanoma Cells Aggressiveness." *Journal of Experimental & Clinical Cancer Research : CR* 37 (1): 326. https://doi.org/10.1186/s13046-018-0982-1.

Rovira, Miguel, Rebecca Sereda, David Pladevall-Morera, Valentina Ramponi, Ines Marin, Mate Maus, Julio Madrigal-Matute, et al. 2022. "The Lysosomal Proteome of Senescent Cells Contributes to the Senescence Secretome." *Aging Cell* 21 (10): e13707. https://doi.org/10.1111/ACEL.13707.

Rowe, John W, and Robert L Kahn. 1987. "Human Aging: Usual and Successful." *Science* 237 (4811): 143–49. https://doi.org/10.1126/science.3299702.

Rubio-Patiño, Camila, Jozef P. Bossowski, Eric Chevet, and Jean Ehrland Ricci. 2018. "Reshaping the Immune Tumor Microenvironment Through IRE1 Signaling." *Trends in Molecular Medicine* 24 (7): 607–14. https://doi.org/10.1016/j.molmed.2018.05.005.

Rübsam, Matthias, Joshua A. Broussard, Sara A. Wickström, Oxana Nekrasova, Kathleen J. Green, and Carien M. Niessen. 2018. "Adherens Junctions and Desmosomes Coordinate Mechanics and Signaling to Orchestrate Tissue Morphogenesis and Function: An Evolutionary Perspective." *Cold Spring Harbor Perspectives in Biology* 10 (11): 1–21. https://doi.org/10.1101/cshperspect.a029207.

Russell-Goldman, Eleanor, and George F. Murphy. 2020. "The Pathobiology of Skin Aging: New Insights into an Old Dilemma." *American Journal of Pathology* 190 (7): 1356–69. https://doi.org/10.1016/j.ajpath.2020.03.007.

Sabath, Niv, Flonia Levy-Adam, Amal Younis, Kinneret Rozales, Anatoly Meller, Shani Hadar, Sharon Soueid-Baumgarten, and Reut Shalgi. 2020. "Cellular Proteostasis Decline in Human Senescence." *Proceedings of the National Academy of Sciences of the United States of America* 117 (50): 31902–13. https://doi.org/10.1073/pnas.2018138117.

Saitou, Marie, Darleny Y Lizardo, Recep Ozgur Taskent, Alec Millner, Omer Gokcumen, and Gunes Ekin Atilla-Gokcumen. 2018. "An Evolutionary Transcriptomics Approach Links CD36 to Membrane Remodeling in Replicative Senescence." *Molecular Omics* 14 (4): 237–46. https://doi.org/10.1039/c8mo00099a.

Salamito, Mélanie, Benjamin Gillet, Delfien Syx, Elisabeth Vaganay, Marilyne Malbouyres, Catherine Cerutti, Nicolas Tissot, et al. 2023. "NRF2 Shortage in Human Skin Fibroblasts Dysregulates Matrisome Gene Expression and Affects Collagen Fibrillogenesis." *Journal of Investigative Dermatology* 143 (3): 386-397.e12. https://doi.org/10.1016/J.JID.2022.07.034.

Sancar, Aziz, Laura A. Lindsey-Boltz, Keziban Ünsal-Kaçmaz, and Stuart Linn. 2004. "Molecular Mechanisms of Mammalian DNA Repair and the DNA Damage Checkpoints." *Annual Review of Biochemistry* 73: 39–85. https://doi.org/10.1146/annurev.biochem.73.011303.073723.

Sander, Christina S, Hong Chang, Susann Salzmann, Cornelia S L Müller, Swarna Ekanayake-Mudiyanselage, Peter Elsner, and Jens J Thiele. 2002. "Photoaging Is Associated with Protein Oxidation in Human Skin In Vivo." *Journal of Investigative Dermatology* 118 (4): 618–25. https://doi.org/https://doi.org/10.1046/j.1523-1747.2002.01708.x.

Sardiello, Marco, Michela Palmieri, Alberto di Ronza, Diego Luis Medina, Marta Valenza, Vincenzo Alessandro Gennarino, Chiara Di Malta, et al. 2009. "A Gene Network Regulating Lysosomal Biogenesis and Function." *Science (New York, N.Y.)* 325 (5939): 473–77. https://doi.org/10.1126/science.1174447.

Saul, Dominik, Diana Jurk, Madison L Doolittle, Robyn Laura Kosinsky, David G Monroe, Nathan K LeBrasseur, Paul D Robbins, Laura J Niedernhofer, Sundeep Khosla, and João F Passos. 2023. "Distinct Secretomes in P16- and P21- Positive Senescent Cells across Tissues." *BioRxiv : The Preprint Server for Biology*. United States. https://doi.org/10.1101/2023.12.05.569858.

Saul, Dominik, Robyn Laura Kosinsky, Elizabeth J. Atkinson, Madison L. Doolittle, Xu Zhang, Nathan K. LeBrasseur, Robert J. Pignolo, et al. 2022. "A New Gene Set Identifies Senescent Cells and

Predicts Senescence-Associated Pathways across Tissues." *Nature Communications 2022 13:1* 13 (1): 1–15. https://doi.org/10.1038/s41467-022-32552-1.

Savignac, Magali, Marina Simon, Anissa Edir, Laure Guibbal, and Alain Hovnanian. 2014. "SERCA2 Dysfunction in Darier Disease Causes Endoplasmic Reticulum Stress and Impaired Cell-to-Cell Adhesion Strength: Rescue by Miglustat." *The Journal of Investigative Dermatology* 134 (7): 1961–70. https://doi.org/10.1038/jid.2014.8.

Sayers, Carly M, Ioanna Papandreou, David M Guttmann, Nancy L Maas, J Alan Diehl, Eric S Witze, Albert C Koong, and Constantinos Koumenis. 2013. "Identification and Characterization of a Potent Activator of P53-Independent Cellular Senescence via a Small-Molecule Screen for Modifiers of the Integrated Stress Response." *Molecular Pharmacology* 83 (3): 594–604. https://doi.org/10.1124/mol.112.081810.

Schadendorf, D, A Möller, B Algermissen, M Worm, M Sticherling, and B M Czarnetzki. 1993. "IL-8 Produced by Human Malignant Melanoma Cells in Vitro Is an Essential Autocrine Growth Factor." *The Journal of Immunology* 151 (5): 2667–75. https://doi.org/10.4049/jimmunol.151.5.2667.

Scherbov, Sergei, and Warren Sanderson. 2020. "Measures of Population Ageing." *World Population Ageing 2019*, no. February: 13–24. https://doi.org/10.18356/a812e16a-en.

Schewe, Denis M., and Julio A. Aguirre-Ghiso. 2008. "ATF6α-Rheb-MTOR Signaling Promotes Survival of Dormant Tumor Cells in Vivo." *Proceedings of the National Academy of Sciences of the United States of America* 105 (30): 10519–24. https://doi.org/10.1073/pnas.0800939105.

Schieke, StefanM, Helger Stege, Viola Kürten, Susanne Grether-Beck, Helmut Sies, and Jean Krutmann. 2002. "Infrared-A Radiation-Induced Matrix Metalloproteinase 1 Expression Is Mediated through Extracellular Signal-Regulated Kinase 1/2 Activation in Human Dermal Fibroblasts." *The Journal of Investigative Dermatology* 119 (6): 1323–29. https://doi.org/10.1046/j.1523-1747.2002.19630.x.

Schmitt, Clemens A. 2016. "The Persistent Dynamic Secrets of Senescence." *Nature Cell Biology* 18 (9): 913–15. https://doi.org/10.1038/ncb3403.

Schmitt, Clemens A., Boshi Wang, and Marco Demaria. 2022. "Senescence and Cancer — Role and Therapeutic Opportunities." *Nature Reviews Clinical Oncology* 19 (10): 619–36. https://doi.org/10.1038/s41571-022-00668-4.

Schmittgen, Thomas D, and Kenneth J Livak. 2008. "Analyzing Real-Time PCR Data by the Comparative CT Method." *Nature Protocols* 3 (6): 1101–8. https://doi.org/10.1038/nprot.2008.73.

Schmitz, Roland, George W. Wright, Da Wei Huang, Calvin A. Johnson, James D. Phelan, James Q. Wang, Sandrine Roulland, et al. 2018. "Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma." *The New England Journal of Medicine* 378 (15): 1396. https://doi.org/10.1056/NEJMOA1801445.

Schöley, Jonas, José Manuel Aburto, Ilya Kashnitsky, Maxi S. Kniffka, Luyin Zhang, Hannaliis Jaadla, Jennifer B. Dowd, and Ridhi Kashyap. 2022. "Life Expectancy Changes since COVID-19." *Nature Human Behaviour* 6 (12): 1649–59. https://doi.org/10.1038/s41562-022-01450-3.

Schreier, Wolfgang J, Tobias E Schrader, Florian O Koller, Peter Gilch, E Carlos, Vijay N Swaminathan, Thomas Carell, and Wolfgang Zinth. 2009. "Thymine Dimerization in DNA Is an Ultrafast Photoreaction" 315 (5812): 625–29. https://doi.org/10.1126/science.1135428.Thymine.

Scott, Timothy L., Perry A. Christian, Melissa V. Kesler, Kevin M. Donohue, Brent Shelton, Kazumasa Wakamatsu, Ito Shosuke, and John D'Orazio. 2012. "Pigment-Independent CAMP-Mediated Epidermal Thickening Protects against Cutaneous UV Injury by Keratinocyte Proliferation." *Experimental Dermatology* 21 (10): 771–77. https://doi.org/10.1111/exd.12012.

Serrano, Manuel, Athena W. Lin, Mila E. McCurrach, David Beach, and Scott W. Lowe. 1997. "Oncogenic Ras Provokes Premature Cell Senescence Associated with Accumulation of P53

and P16(INK4a).” *Cell* 88 (5): 593–602. https://doi.org/10.1016/S0092-8674(00)81902-9.

Shamma, Awad, Yujiro Takegami, Takao Miki, Shunsuke Kitajima, Makoto Noda, Takao Obara, Takahiro Okamoto, and Chiaki Takahashi. 2009. “Rb Regulates DNA Damage Response and Cellular Senescence through E2F-Dependent Suppression of N-Ras Isoprenylation.” *Cancer Cell* 15 (4): 255–69. https://doi.org/10.1016/j.ccr.2009.03.001.

Shay, J W, and W E Wright. 2000. “Hayflick, His Limit, and Cellular Ageing.” *Nature Reviews. Molecular Cell Biology* 1 (1): 72–76. https://doi.org/10.1038/35036093.

Shen, Jingshi, Xi Chen, Linda Hendershot, and Ron Prywes. 2002. “ER Stress Regulation of ATF6 Localization by Dissociation of BiP/GRP78 Binding and Unmasking of Golgi Localization Signals.” *Developmental Cell* 3 (1): 99–111. https://doi.org/https://doi.org/10.1016/S1534-5807(02)00203-4.

Shibatani, Toru, Larry L. David, Ashley L. McCormack, Klaus Frueh, and William R. Skach. 2005. “Proteomic Analysis of Mammalian Oligosaccharyltransferase Reveals Multiple Subcomplexes That Contain Sec61, TRAP, and Two Potential New Subunits.” *Biochemistry* 44 (16): 5982–92. https://doi.org/10.1021/bi047328f.

Shimizu, Akira, Kyoichi Kaira, Masahito Yasuda, Takayuki Asao, and Osamu Ishikawa. 2017. “Clinical and Pathological Significance of ER Stress Marker (BiP/GRP78 and PERK) Expression in Malignant Melanoma.” *Pathology Oncology Research : POR* 23 (1): 111–16. https://doi.org/10.1007/s12253-016-0099-9.

Shin, Eun-Young, Nak-Kyun Soung, Martin Alexander Schwartz, and Eung-Gook Kim. 2021. “Altered Endocytosis in Cellular Senescence.” *Ageing Research Reviews* 68: 101332. https://doi.org/https://doi.org/10.1016/j.arr.2021.101332.

Signaling, B-sasp, Jieun Woo, Seoungwoo Shin, Hyanggi Ji, Dehun Ryu, Eunae Cho, Youngseok Kim, Junoh Kim, Deokhoon Park, and Eunsun Jung. 2022. “Isatis Tinctoria L . Leaf Extract Inhibits Replicative Senescence.”

Smith, Heather L., Oliver J. Freeman, Adrian J. Butcher, Staffan Holmqvist, Ibrahim Humoud, Tobias Schätzl, Daniel T. Hughes, et al. 2020. “Astrocyte Unfolded Protein Response Induces a Specific Reactivity State That Causes Non-Cell-Autonomous Neuronal Degeneration.” *Neuron* 105 (5): 855-866.e5. https://doi.org/10.1016/j.neuron.2019.12.014.

Solano, Francisco. 2020. “Photoprotection and Skin Pigmentation: Melanin-Related Molecules and Some Other New Agents Obtained from Natural Sources.” *Molecules (Basel, Switzerland)* 25 (7). https://doi.org/10.3390/molecules25071537.

Song, Shuling, Eric W.F. Lam, Tamara Tchkonia, James L. Kirkland, and Yu Sun. 2020. “Senescent Cells: Emerging Targets for Human Aging and Age-Related Diseases.” *Trends in Biochemical Sciences* 45 (7): 578–92. https://doi.org/10.1016/j.tibs.2020.03.008.

Soto-Gamez, Abel, Wim J Quax, and Marco Demaria. 2019. “Regulation of Survival Networks in Senescent Cells: From Mechanisms to Interventions.” *Journal of Molecular Biology* 431 (15): 2629–43. https://doi.org/10.1016/j.jmb.2019.05.036.

Sprenger, Cynthia C., Stephen R. Plymate, and May J. Reed. 2010. “Aging-Related Alterations in the Extracellular Matrix Modulate the Microenvironment and Influence Tumor Progression.” *International Journal of Cancer* 127 (12): 2739–48. https://doi.org/10.1002/IJC.25615.

Sriram, Gopu, Paul Lorenz Bigliardi, and Mei Bigliardi-Qi. 2015. “Fibroblast Heterogeneity and Its Implications for Engineering Organotypic Skin Models in Vitro.” *European Journal of Cell Biology* 94 (11): 483–512. https://doi.org/10.1016/j.ejcb.2015.08.001.

Stearns-Reider, Kristen M., Antonio D’Amore, Kevin Beezhold, Benjamin Rothrauff, Loredana Cavalli, William R. Wagner, David A. Vorp, et al. 2017. “Aging of the Skeletal Muscle Extracellular Matrix Drives a Stem Cell Fibrogenic Conversion.” *Aging Cell* 16 (3): 518–28. https://doi.org/10.1111/acel.12578.

Stein, G H, L F Drullinger, A Soulard, and V Dulić. 1999. “Differential Roles for Cyclin-Dependent Kinase Inhibitors P21 and P16 in the Mechanisms of Senescence and Differentiation in Human Fibroblasts.” *Molecular and Cellular Biology* 19 (3): 2109–17. https://doi.org/10.1128/MCB.19.3.2109.

Stelzer, Gil, Naomi Rosen, Inbar Plaschkes, Shahar Zimmerman, Michal Twik, Simon Fishilevich, Tsippi Iny Stein, et al. 2016. “The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses.” *Current Protocols in Bioinformatics* 54 (June): 1.30.1-1.30.33. https://doi.org/10.1002/cpbi.5.

Steude, Judith, Reinhard Kulke, and Enno Christophers. 2002. “Interleukin-1-Stimulated Secretion of Interleukin-8 and Growth-Related Oncogene-α Demonstrates Greatly Enhanced Keratinocyte Growth in Human Raft Cultured Epidermis.” *Journal of Investigative Dermatology* 119 (6): 1254–60. https://doi.org/10.1046/j.1523-1747.2002.19616.x.

Stojic, Marija, Verónica López, Andrés Montero, Cristina Quílez, Gonzalo de Aranda Izuzquiza, Lucy Vojtova, José Luis Jorcano, and Diego Velasco. 2019. *Skin Tissue Engineering*. *Biomaterials for Skin Repair and Regeneration*. Elsevier Ltd. https://doi.org/10.1016/B978-0-08-102546-8.00003-0.

Sugiura, Kazumitsu, Yoshinao Muro, Kyoko Futamura, Kenji Matsumoto, Noriko Hashimoto, Yuji Nishizawa, Tetsuro Nagasaka, Hirohisa Saito, Yasushi Tomita, and Jiro Usukura. 2009. “The Unfolded Protein Response Is Activated in Differentiating Epidermal Keratinocytes.” *The Journal of Investigative Dermatology* 129 (9): 2126–35. https://doi.org/10.1038/jid.2009.51.

Sulli, Gabriele, Raffaella Di Micco, and Fabrizio D.Adda Di Fagagna. 2012. “Crosstalk between Chromatin State and DNA Damage Response in Cellular Senescence and Cancer.” *Nature Reviews Cancer* 12 (10): 709–20. https://doi.org/10.1038/nrc3344.

Sun, Peiqing, Naoto Yoshizuka, Liguo New, Bettina A. Moser, Yilei Li, Rong Liao, Changchuan Xie, et al. 2007. “PRAK Is Essential for Ras-Induced Senescence and Tumor Suppression.” *Cell* 128 (2): 295–308. https://doi.org/10.1016/j.cell.2006.11.050.

Tai, Shi, Jiaxing Sun, Yuying Zhou, Zhaowei Zhu, Yuhu He, Mingxian Chen, Hui Yang, et al. 2022. “Metformin Suppresses Vascular Smooth Muscle Cell Senescence by Promoting Autophagic Flux.” *Journal of Advanced Research* 41 (139): 205–18. https://doi.org/10.1016/j.jare.2021.12.009.

Tajima, S, L Lauffer, V L Rath, and P Walter. 1986. “The Signal Recognition Particle Receptor Is a Complex That Contains Two Distinct Polypeptide Chains.” *The Journal of Cell Biology* 103 (4): 1167–78. https://doi.org/10.1083/jcb.103.4.1167.

Takahashi, Akiko, Ryo Okada, Koji Nagao, Yuka Kawamata, Aki Hanyu, Shin Yoshimoto, Masaki Takasugi, et al. 2017. “ARTICLE Exosomes Maintain Cellular Homeostasis by Excreting Harmful DNA from Cells.” https://doi.org/10.1038/ncomms15287.

Takai, Hiroyuki, Agata Smogorzewska, and Titia de Lange. 2003. “DNA Damage Foci at Dysfunctional Telomeres.” *Current Biology : CB* 13 (17): 1549–56. https://doi.org/10.1016/s0960-9822(03)00542-6.

Takasugi, Masaki. 2018. “Emerging Roles of Extracellular Vesicles in Cellular Senescence and Aging.” *Aging Cell* 17 (2). https://doi.org/10.1111/ACEL.12734.

Tam, Arvin B, Lindsay S Roberts, Vivek Chandra, Io Guane Rivera, Daniel K Nomura, Douglass J Forbes, and Maho Niwa. 2018. “The UPR Activator ATF6 Responds to Proteotoxic and Lipotoxic Stress by Distinct Mechanisms.” *Developmental Cell* 46 (3): 327-343.e7. https://doi.org/10.1016/j.devcel.2018.04.023.

Tan, Qing, Na Liang, Xiaoqian Zhang, and Jun Li. 2021. “Dynamic Aging: Channeled Through Microenvironment.” *Frontiers in Physiology*. https://doi.org/10.3389/fphys.2021.702276.

Thastrup, O, P J Cullen, B K Drøbak, M R Hanley, and A P Dawson. 1990. “Thapsigargin, a Tumor

Promoter, Discharges Intracellular Ca2+ Stores by Specific Inhibition of the Endoplasmic Reticulum Ca2(+)-ATPase." *Proceedings of the National Academy of Sciences of the United States of America* 87 (7): 2466–70. https://doi.org/10.1073/pnas.87.7.2466.

Théry, Clotilde, Aled Clayton, Sebastian Amigorena, and and Graca Raposo. 2006. "Isolation and Characterization of Exosomes from Cell Culture Supernatants." *Current Protocols in Cell Biology*, 30:3.22:3.22.1–3.22.29.

Thuerauf, Donna J, Lisa E Morrison, Holly Hoover, and Christopher C Glembotski. 2002. "Coordination of ATF6-Mediated Transcription and ATF6 Degradation by a Domain That Is Shared with the Viral Transcription Factor, VP16." *The Journal of Biological Chemistry* 277 (23): 20734–39. https://doi.org/10.1074/jbc.M201749200.

Tigges, Julia, Heike Weighardt, Sandra Wolff, Christine Götz, Irmgard Förster, Zippora Kohne, Ulrike Huebenthal, et al. 2013. "Aryl Hydrocarbon Receptor Repressor (AhRR) Function Revisited: Repression of CYP1 Activity in Human Skin Fibroblasts Is Not Related to AhRR Expression." *Journal of Investigative Dermatology* 133 (1): 87–96. https://doi.org/10.1038/jid.2012.259.

Tinaburri, Lavinia, Carola Valente, Massimo Teson, Ylenia Aura Minafò, Sonia Cordisco, Liliana Guerra, and Elena Dellambra. 2021. "The Secretome of Aged Fibroblasts Promotes EMT-Like Phenotype in Primary Keratinocytes from Elderly Donors through BDNF-TrkB Axis." *Journal of Investigative Dermatology* 141 (4): 1052-1062.e12. https://doi.org/10.1016/J.JID.2020.08.019.

Tirasophon, W, K Lee, B Callaghan, A Welihinda, and R J Kaufman. 2000. "The Endoribonuclease Activity of Mammalian IRE1 Autoregulates Its MRNA and Is Required for the Unfolded Protein Response." *Genes & Development* 14 (21): 2725–36. https://doi.org/10.1101/gad.839400.

Tirasophon, W, A A Welihinda, and R J Kaufman. 1998. "A Stress Response Pathway from the Endoplasmic Reticulum to the Nucleus Requires a Novel Bifunctional Protein Kinase/Endoribonuclease (Ire1p) in Mammalian Cells." *Genes & Development* 12 (12): 1812–24. https://doi.org/10.1101/gad.12.12.1812.

Toussaint, Olivier, Patrick Dumont, Jean François Dierick, Thierry Pascal, Christophe Frippiat, Florence Chainiaux, Francis Sluse, François Eliaers, and José Remacle. 2000. "Stress-Induced Premature Senescence. Essence of Life, Evolution, Stress, and Aging." *Annals of the New York Academy of Sciences* 908: 85–98. https://doi.org/10.1111/j.1749-6632.2000.tb06638.x.

Toussaint, Olivier, José Remacle, Patrick Dumont, Jean-François Dierick, Thierry Pascal, Christophe Frippiat, Joao Pedro Magalhaes, and Florence Chainiaux. 2001. "Oxidative Stress-induced Cellular Senescence." *Encyclopedia of Life Sciences*, no. August. https://doi.org/10.1038/npg.els.0003441.

Toutfaire, Marie, Emilie Bauwens, and Florence Debacq-Chainiaux. 2017. "The Impact of Cellular Senescence in Skin Ageing: A Notion of Mosaic and Therapeutic Strategies." *Biochemical Pharmacology* 142: 1–12. https://doi.org/10.1016/j.bcp.2017.04.011.

Toutfaire, Marie, Elise Dumortier, Antoine Fattaccioli, Martine Van Steenbrugge, Charlotte M. Proby, and Florence Debacq-Chainiaux. 2018. "Unraveling the Interplay between Senescent Dermal Fibroblasts and Cutaneous Squamous Cell Carcinoma Cell Lines at Different Stages of Tumorigenesis." *International Journal of Biochemistry and Cell Biology* 98 (March): 113–26. https://doi.org/10.1016/j.biocel.2018.03.005.

Tremblay, L O, and A Herscovics. 1999. "Cloning and Expression of a Specific Human Alpha 1,2-Mannosidase That Trims Man9GlcNAc2 to Man8GlcNAc2 Isomer B during N-Glycan Biosynthesis." *Glycobiology* 9 (10): 1073–78. https://doi.org/10.1093/glycob/9.10.1073.

Trifunovic, Aleksandra, Anna Wredenberg, Maria Falkenberg, Johannes N Spelbrink, Anja T Rovio, Carl E Bruder, Mohammad Bohlooly-Y, et al. 2004. "Premature Ageing in Mice Expressing Defective Mitochondrial DNA Polymerase." *Nature* 429 (6990): 417–23. https://doi.org/10.1038/nature02517.

Trylcova, Jana, Petr Busek, Karel Smetana, Eva Balaziova, Barbora Dvorankova, Alzbeta Mifkova,

and Aleksi Sedo. 2015. “Effect of Cancer-Associated Fibroblasts on the Migration of Glioma Cells in Vitro.” *Tumor Biology* 36 (8): 5873–79. https://doi.org/10.1007/s13277-015-3259-8.

Tuschil, Andrea., Charles. Lam, Alexander. Haslberger, and Ivan. Lindley. 1992. “Interleukin-8 Stimulates Calcium Transients and Promotes Epidermal Cell Proliferation.” *Journal of Investigative Dermatology* 99 (3): 294–98. https://doi.org/https://doi.org/10.1111/1523-1747.ep12616634.

Tuttle, Camilla S.L., Mariette E.C. Waaijer, Monique S. Slee-Valentijn, Theo Stijnen, Rudi Westendorp, and Andrea B. Maier. 2020. “Cellular Senescence and Chronological Age in Various Human Tissues: A Systematic Review and Meta-Analysis.” *Aging Cell*. https://doi.org/10.1111/acel.13083.

Udono, Miyako, Kaoru Fujii, Gakuro Harada, Yumi Tsuzuki, Keishi Kadooka, Pingbo Zhang, Hiroshi Fujii, et al. 2015. “Impaired ATP6V0A2 Expression Contributes to Golgi Dispersion and Glycosylation Changes in Senescent Cells.” *Scientific Reports* 5 (June): 6–10. https://doi.org/10.1038/srep17342.

Upton, John-Paul, Likun Wang, Dan Han, Eric S Wang, Noelle E Huskey, Lionel Lim, Morgan Truitt, et al. 2012. “IRE1α Cleaves Select MicroRNAs during ER Stress to Derepress Translation of Proapoptotic Caspase-2.” *Science (New York, N.Y.)* 338 (6108): 818–22. https://doi.org/10.1126/science.1226191.

Urano, F, X Wang, A Bertolotti, Y Zhang, P Chung, H P Harding, and D Ron. 2000. “Coupling of Stress in the ER to Activation of JNK Protein Kinases by Transmembrane Protein Kinase IRE1.” *Science (New York, N.Y.)* 287 (5453): 664–66. https://doi.org/10.1126/science.287.5453.664.

Valerio, Hellen Paula, Felipe Gustavo Ravagnani, Graziella Eliza Ronsein, and Paolo Di Mascio. 2021. “A Single Dose of Ultraviolet-A Induces Proteome Remodeling and Senescence in Primary Human Keratinocytes.” *Scientific Reports* 11 (1): 1–16. https://doi.org/10.1038/s41598-021-02658-5.

Vassilakos, A, M Michalak, M A Lehrman, and D B Williams. 1998. “Oligosaccharide Binding Characteristics of the Molecular Chaperones Calnexin and Calreticulin.” *Biochemistry* 37 (10): 3480–90. https://doi.org/10.1021/bi972465g.

Venuta, Giuseppe La, Marcel Zeitler, Julia P Steringer, Hans-Michael Müller, and Walter Nickel. 2015. “The Startling Properties of Fibroblast Growth Factor 2: How to Exit Mammalian Cells without a Signal Peptide at Hand.” *The Journal of Biological Chemistry* 290 (45): 27015–20. https://doi.org/10.1074/jbc.R115.689257.

Victorelli, Stella, Anthony Lagnado, Jessica Halim, Will Moore, Duncan Talbot, Karen Barrett, James Chapman, et al. 2019. “Senescent Human Melanocytes Drive Skin Ageing via Paracrine Telomere Dysfunction.” *The EMBO Journal* 38 (23). https://doi.org/10.15252/EMBJ.2019101982.

Waaijer, Mariëtte E.C., David A. Gunn, Peter D. Adams, Jeff S. Pawlikowski, Christopher E.M. Griffiths, Diana Van Heemst, P. Eline Slagboom, Rudi G.J. Westendorp, and Andrea B. Maier. 2016. “P16INK4a Positive Cells in Human Skin Are Indicative of Local Elastic Fiber Morphology, Facial Wrinkling, and Perceived Age.” *Journals of Gerontology - Series A Biological Sciences and Medical Sciences* 71 (8): 1022–28. https://doi.org/10.1093/gerona/glv114.

Wang, Audrey Shimei, Peh Fern Ong, Alexandre Chojnowski, Carlos Clavel, and Oliver Dreesen. 2017. “Loss of Lamin B1 Is a Biomarker to Quantify Cellular Senescence in Photoaged Skin.” *Scientific Reports* 7 (1): 1–8. https://doi.org/10.1038/s41598-017-15901-9.

Wang, Boshi, Jaskaren Kohli, and Marco Demaria. 2020. “Senescent Cells in Cancer Therapy: Friends or Foes?” *Trends in Cancer* 6 (10): 838–57. https://doi.org/10.1016/j.trecan.2020.05.004.

Wang, E. 1995. “Senescent Human Fibroblasts Resist Programmed Cell Death, and Failure to Suppress Bcl2 Is Involved.” *Cancer Research* 55 (11): 2284–92.

Wang, Rong, Zhen Yu, Bharath Sunchu, James Shoaf, Ivana Dang, Stephanie Zhao, Kelsey Caples,

et al. 2017. “Rapamycin Inhibits the Secretory Phenotype of Senescent Cells by a Nrf2-independent Mechanism.” *Aging Cell* 16 (3): 564. https://doi.org/10.1111/ACEL.12587.

Wang, Si, Boqiang Hu, Zhichao Ding, Yujiao Dang, Jun Wu, Di Li, Xiaoling Liu, et al. 2018. “ATF6 Safeguards Organelle Homeostasis and Cellular Aging in Human Mesenchymal Stem Cells.” *Cell Discovery 2018 4:1* 4 (1): 1–19. https://doi.org/10.1038/s41421-017-0003-0.

Wang, Wengong, Xiaoling Yang, Isabel López de Silanes, David Carling, and Myriam Gorospe. 2003. “Increased AMP:ATP Ratio and AMP-Activated Protein Kinase Activity during Cellular Senescence Linked to Reduced HuR Function.” *The Journal of Biological Chemistry* 278 (29): 27016–23. https://doi.org/10.1074/jbc.M300318200.

Wang, Yuting, Liping Liu, Yifan Song, Xiaojie Yu, and Hongkui Deng. 2022. “Unveiling E2F4, TEAD1 and AP-1 as Regulatory Transcription Factors of the Replicative Senescence Program by Multi-Omics Analysis.” *Protein and Cell* 13 (10): 742–59. https://doi.org/10.1007/s13238-021-00894-z.

Webb, Ann R. 2006. “Who, What, Where and When—Influences on Cutaneous Vitamin D Synthesis.” *Progress in Biophysics and Molecular Biology* 92 (1): 17–25. https://doi.org/https://doi.org/10.1016/j.pbiomolbio.2006.02.004.

West, Heather C., and Clare L. Bennett. 2018. “Redefining the Role of Langerhans Cells as Immune Regulators within the Skin.” *Frontiers in Immunology* 8 (JAN): 1–8. https://doi.org/10.3389/fimmu.2017.01941.

Wiley, Christopher D., Su Liu, Chandani Limbad, Anna M. Zawadzka, Jennifer Beck, Marco Demaria, Robert Artwood, et al. 2019. “SILAC Analysis Reveals Increased Secretion of Hemostasis-Related Factors by Senescent Cells.” *Cell Reports* 28 (13): 3329-3337.e5. https://doi.org/10.1016/j.celrep.2019.08.049.

Wiley, Christopher D., Rishi Sharma, Sonnet S. Davis, Jose Alberto Lopez-Dominguez, Kylie P. Mitchell, Samantha Wiley, Fatouma Alimirah, et al. 2021. “Oxylipin Biosynthesis Reinforces Cellular Senescence and Allows Detection of Senolysis.” *Cell Metabolism* 33 (6): 1124-1136.e5. https://doi.org/10.1016/j.cmet.2021.03.008.

Wiley, Christopher D., Michael C. Velarde, Pacome Lecot, Su Liu, Ethan A. Sarnoski, Adam Freund, Kotaro Shirakawa, et al. 2016. “Mitochondrial Dysfunction Induces Senescence with a Distinct Secretory Phenotype.” *Cell Metabolism* 23 (2): 303. https://doi.org/10.1016/J.CMET.2015.11.011.

Wissler Gerdes, Erin O., Avanish Misra, Jair Machado Espindola Netto, Tamar Tchkonia, and James L. Kirkland. 2021. “Strategies for Late Phase Preclinical and Early Clinical Trials of Senolytics.” *Mechanisms of Ageing and Development* 200. https://doi.org/10.1016/j.mad.2021.111591.

Wlaschek, Meinhard, Iliana Tantcheva-Poór, Lale Naderi, Wenjian Ma, Lars Alexander Schneider, Ziba Razi-Wolf, Jutta Schüller, and Karin Scharffetter-Kochanek. 2001. “Solar UV Irradiation and Dermal Photoaging.” *Journal of Photochemistry and Photobiology B: Biology* 63 (1): 41–51. https://doi.org/https://doi.org/10.1016/S1011-1344(01)00201-9.

Woehlbier, Ute, and Claudio Hetz. 2011. “Modulating Stress Responses by the UPRosome: A Matter of Life and Death.” *Trends in Biochemical Sciences* 36 (6): 329–37. https://doi.org/10.1016/j.tibs.2011.03.001.

Wondrak, Georg T., Myron K. Jacobson, and Elaine L. Jacobson. 2006. “Endogenous UVA-Photosensitizers: Mediators of Skin Photodamage and Novel Targets for Skin Photoprotection.” *Photochemical and Photobiological Sciences* 5 (2): 215–37. https://doi.org/10.1039/b504573h.

Woo, Jieun, Seoungwoo Shin, Eunae Cho, Dehun Ryu, David Garandeau, Hanane Chajra, Mathilde Fréchet, Deokhoon Park, and Eunsun Jung. 2021. “Senotherapeutic-like Effect of Silybum Marianum Flower Extract Revealed on Human Skin Cells.” *PLOS ONE* 16 (12): e0260545. https://doi.org/10.1371/JOURNAL.PONE.0260545.

Wu, Chi-Hwa, Jan van Riggelen, Alper Yetil, Alice C Fan, Pavan Bachireddy, and Dean W Felsher.

2007. “Cellular Senescence Is an Important Mechanism of Tumor Regression upon C-Myc Inactivation.” *Proceedings of the National Academy of Sciences of the United States of America* 104 (32): 13028–33. https://doi.org/10.1073/pnas.0701953104.

Wu, Jun, D. Thomas Rutkowski, Meghan Dubois, Jayanth Swathirajan, Thomas Saunders, Junying Wang, Benbo Song, Grace D.Y. Yau, and Randal J. Kaufman. 2007. “ATF6α Optimizes Long-Term Endoplasmic Reticulum Function to Protect Cells from Chronic Stress.” *Developmental Cell* 13 (3): 351–64. https://doi.org/10.1016/j.devcel.2007.07.005.

Wu, Tianzhi, Erqiang Hu, Shuangbin Xu, Meijun Chen, Pingfan Guo, Zehan Dai, Tingze Feng, et al. 2021. “ClusterProfiler 4.0: A Universal Enrichment Tool for Interpreting Omics Data.” *Innovation (Cambridge (Mass.))* 2 (3): 100141. https://doi.org/10.1016/j.xinn.2021.100141.

Wynford-Thomas, David, and David Kipling. 1997. “The End-Replication Problem.” *Nature* 389 (6651): 551. https://doi.org/10.1038/39210.

Xin, Nan, Jenni Durieux, Chunxia Yang, Suzanne Wolff, Hyun Eui Kim, and Andrew Dillin. 2022. “The UPRmt Preserves Mitochondrial Import to Extend Lifespan.” *Journal of Cell Biology* 221 (7). https://doi.org/10.1083/jcb.202201071.

Xu, Ming, Tamara Tchkonia, Husheng Ding, Mikolaj Ogrodnik, Ellen R. Lubbers, Tamar Pirtskhalava, Thomas A. White, et al. 2015. “JAK Inhibition Alleviates the Cellular Senescence-Associated Secretory Phenotype and Frailty in Old Age.” *Proceedings of the National Academy of Sciences of the United States of America* 112 (46): E6301–10. https://doi.org/10.1073/PNAS.1515386112/SUPPL_FILE/PNAS.201515386SI.PDF.

Yamamoto, Keisuke, Takashi Sato, Toshie Matsui, Masanori Sato, Tetsuya Okada, Hiderou Yoshida, Akihiro Harada, and Kazutoshi Mori. 2007. “Transcriptional Induction of Mammalian ER Quality Control Proteins Is Mediated by Single or Combined Action of ATF6alpha and XBP1.” *Developmental Cell* 13 (3): 365–76. https://doi.org/10.1016/j.devcel.2007.07.018.

Yang, Nae-Cherng, and Miao-Lin Hu. 2005. “The Limitations and Validities of Senescence Associated-Beta-Galactosidase Activity as an Aging Marker for Human Foreskin Fibroblast Hs68 Cells.” *Experimental Gerontology* 40 (10): 813–19. https://doi.org/10.1016/j.exger.2005.07.011.

Yao, Xuefeng, Huaqiong Li, Liping Chen, and Lay Poh Tan. 2022. “UV-Induced Senescence of Human Dermal Fibroblasts Restrained by Low-Stiffness Matrix by Inhibiting NF-KB Activation.” *Engineered Regeneration* 3 (4): 365–73. https://doi.org/10.1016/j.engreg.2022.08.002.

Ye, Jin, Robert B. Rawson, Ryutaro Komuro, Xi Chen, Utpal P. Davé, Ron Prywes, Michael S. Brown, and Joseph L. Goldstein. 2000. “ER Stress Induces Cleavage of Membrane-Bound ATF6 by the Same Proteases That Process SREBPs.” *Molecular Cell* 6 (6): 1355–64. https://doi.org/10.1016/S1097-2765(00)00133-7.

Yi, Yuxin, Hongfu Xie, Xiao Xiao, Ben Wang, Rui Du, Yingzi Liu, Zibo Li, et al. 2018. “Ultraviolet A Irradiation Induces Senescence in Human Dermal Fibroblasts by Down-Regulating DNMT1 via ZEB1.” *Aging* 10 (2): 212–28. https://doi.org/10.18632/aging.101383.

Yoon, Young-Sil, Dong-Sun Yoon, In Kyoung Lim, Soo-Han Yoon, Hae-Young Chung, Manuel Rojo, Florence Malka, Mei-Jie Jou, Jean-Claude Martinou, and Gyesoon Yoon. 2006. “Formation of Elongated Giant Mitochondria in DFO-Induced Cellular Senescence: Involvement of Enhanced Fusion Process through Modulation of Fis1.” *Journal of Cellular Physiology* 209 (2): 468–80. https://doi.org/10.1002/jcp.20753.

Yosef, Reut, Noam Pilpel, Ronit Tokarsky-Amiel, Anat Biran, Yossi Ovadya, Snir Cohen, Ezra Vadai, et al. 2016. “Directed Elimination of Senescent Cells by Inhibition of BCL-W and BCL-XL.” *Nature Communications* 7: 1–11. https://doi.org/10.1038/ncomms11190.

Yoshida, H, K Haze, H Yanagi, T Yura, and K Mori. 1998. “Identification of the Cis-Acting Endoplasmic Reticulum Stress Response Element Responsible for Transcriptional Induction of Mammalian Glucose-Regulated Proteins. Involvement of Basic Leucine Zipper Transcription Factors.” *The Journal of Biological Chemistry* 273 (50): 33741–49. https://doi.org/10.1074/jbc.273.50.33741.

Yoshida, H, T Matsui, A Yamamoto, T Okada, and K Mori. 2001. “XBP1 MRNA Is Induced by ATF6 and Spliced by IRE1 in Response to ER Stress to Produce a Highly Active Transcription Factor.” *Cell* 107 (7): 881–91. https://doi.org/10.1016/s0092-8674(01)00611-0.

Yoshida, Hiderou. 2007. “ER Stress and Diseases.” *The FEBS Journal* 274 (3): 630–58. https://doi.org/10.1111/j.1742-4658.2007.05639.x.

Yu, Guangchuang, Li-Gen Wang, Guang-Rong Yan, and Qing-Yu He. 2015. “DOSE: An R/Bioconductor Package for Disease Ontology Semantic and Enrichment Analysis.” *Bioinformatics (Oxford, England)* 31 (4): 608–9. https://doi.org/10.1093/bioinformatics/btu684.

Zeng, Ji ping, Bo Bi, Liang Chen, Ping Yang, Yu Guo, Yi qun Zhou, and Tian yi Liu. 2014. “Repeated Exposure of Mouse Dermal Fibroblasts at a Sub-Cytotoxic Dose of UVB Leads to Premature Senescence: A Robust Model of Cellular Photoaging.” *Journal of Dermatological Science* 73 (1): 49–56. https://doi.org/10.1016/j.jdermsci.2013.08.013.

Zhang, Rugang, Maxim V. Poustovoitov, Xiaofen Ye, Hidelita A. Santos, Wei Chen, Sally M. Daganzo, Jan P. Erzberger, et al. 2005. “Formation of MacroH2A-Containing Senescence-Associated Heterochromatin Foci and Senescence Driven by ASF1a and HIRA.” *Developmental Cell* 8 (1): 19–30. https://doi.org/10.1016/j.devcel.2004.10.019.

Zhang, Wei, Wen Zeng, Aofei Jiang, Zhi He, Xiaoping Shen, Xian Dong, Jianglong Feng, and Hongguang Lu. 2021. “Global, Regional and National Incidence, Mortality and Disability-Adjusted Life-Years of Skin Cancers and Trend Analysis from 1990 to 2019: An Analysis of the Global Burden of Disease Study 2019.” *Cancer Medicine* 10 (14): 4905–22. https://doi.org/10.1002/cam4.4046.

Zhao, Min, Jinqiang Luo, Bin Xiao, Hongyan Tang, Fanjun Song, Xiaolei Ding, and Guilan Yang. 2020. “Endoplasmic Reticulum Stress Links Psoriasis Vulgaris with Keratinocyte Inflammation.” *Postepy Dermatologii i Alergologii* 37 (1): 34–40. https://doi.org/10.5114/ada.2020.93382.

Zhou, Jiahai, Chuan Yin Liu, Sung Hoon Back, Robert L. Clark, Daniel Peisach, Zhaohui Xu, and Randal J. Kaufman. 2006. “The Crystal Structure of Human IRE1 Luminal Domain Reveals a Conserved Dimerization Interface Required for Activation of the Unfolded Protein Response.” *Proceedings of the National Academy of Sciences of the United States of America* 103 (39): 14343–48. https://doi.org/10.1073/pnas.0606480103.

Zhu, Bokai, Christina H. Ferry, Lauren K. Markell, Nicholas Blazanin, Adam B. Glick, Frank J. Gonzalez, and Jeffrey M. Peters. 2014. “The Nuclear Receptor Peroxisome Proliferator-Activated Receptor-β/ δ (PPARβ/δ) Promotes Oncogene-Induced Cellular Senescence through Repression of Endoplasmic Reticulum Stress.” *Journal of Biological Chemistry* 289 (29): 20102–19. https://doi.org/10.1074/jbc.M114.551069.

Zhu, C, F E Johansen, and R Prywes. 1997. “Interaction of ATF6 and Serum Response Factor.” *Molecular and Cellular Biology* 17 (9): 4957–66. https://doi.org/10.1128/MCB.17.9.4957.

Zhu, Yi, Tamara Tchkonia, Heike Fuhrmann-Stroissnigg, Haiming M. Dai, Yuanyuan Y. Ling, Michael B. Stout, Tamar Pirtskhalava, et al. 2016. “Identification of a Novel Senolytic Agent, Navitoclax, Targeting the Bcl-2 Family of Anti-Apoptotic Factors.” *Aging Cell* 15 (3): 428–35. https://doi.org/10.1111/acel.12445.

Zhu, Yi, Tamara Tchkonia, Tamar Pirtskhalava, Adam C. Gower, Husheng Ding, Nino Giorgadze, Allyson K. Palmer, et al. 2015. “The Achilles’ Heel of Senescent Cells: From Transcriptome to Senolytic Drugs.” *Aging Cell* 14 (4): 644. https://doi.org/10.1111/ACEL.12344.

Zhu, Yukun, Xuewen Liu, Xuelu Ding, Fei Wang, and Xin Geng. 2019. “Telomere and Its Role in the Aging Pathways: Telomere Shortening, Cell Senescence and Mitochondria Dysfunction.” *Biogerontology* 20 (1): 1–16. https://doi.org/10.1007/s10522-018-9769-1.

Zimmermann, Robert, Juliane G Strauss, Guenter Haemmerle, Gabriele Schoiswohl, Ruth Birner-Gruenberger, Monika Riederer, Achim Lass, et al. 2004. “Fat Mobilization in Adipose Tissue Is Promoted by Adipose Triglyceride Lipase.” *Science (New York, N.Y.)* 306 (5700): 1383–86.

https://doi.org/10.1126/science.1100747.

Zou, Zhiran, Xiao Long, Qian Zhao, Yandong Zheng, Moshi Song, Shuai Ma, Yaobin Jing, et al. 2021. “A Single-Cell Transcriptomic Atlas of Human Skin Aging.” *Developmental Cell* 56 (3): 383-397.e8. https://doi.org/https://doi.org/10.1016/j.devcel.2020.11.002.

Zuber, Christian, James H Cormier, Bruno Guhl, Roger Santimaria, Daniel N Hebert, and Jürgen Roth. 2007. “EDEM1 Reveals a Quality Control Vesicular Transport Pathway out of the Endoplasmic Reticulum Not Involving the COPII Exit Sites.” *Proceedings of the National Academy of Sciences of the United States of America* 104 (11): 4407–12. https://doi.org/10.1073/pnas.0700154104.

Annexes

International Journal of
Molecular Sciences

MDPI

Review

Exploring the Communication of the SASP: Dynamic, Interactive, and Adaptive Effects on the Microenvironment

Joëlle Giroud [1,2], Inès Bouriez [1], Hugo Paulus [1], Albin Pourtier [2], Florence Debacq-Chainiaux [1,*] and Olivier Pluquet [2,*]

[1] Laboratory of Biochemistry and Cell Biology (URBC), Namur Research Institute for Life Sciences (NARILIS), University of Namur, 5000 Namur, Belgium; joelle.giroud@unamur.be (J.G.); ines.bouriez@unamur.be (I.B.); hugo.paulus@student.unamur.be (H.P.)
[2] University of Lille, CNRS, Inserm, Pasteur Institute of Lille, UMR9020-U1277-CANTHER-Cancer Heterogeneity Plasticity and Resistance to Therapies, 59000 Lille, France; albin.pourtier@univ-lille.fr
* Correspondence: florence.chainiaux@unamur.be (F.D.-C.); olivier.pluquet@ibl.cnrs.fr (O.P.)

Abstract: Cellular senescence is a complex cell state that can occur during physiological ageing or after exposure to stress signals, regardless of age. It is a dynamic process that continuously evolves in a context-dependent manner. Senescent cells interact with their microenvironment by producing a heterogenous and plastic secretome referred to as the senescence-associated secretory phenotype (SASP). Hence, understanding the cross-talk between SASP and the microenvironment can be challenging due to the complexity of signal exchanges. In this review, we first aim to update the definition of senescence and its associated biomarkers from its discovery to the present day. We detail the regulatory mechanisms involved in the expression of SASP at multiple levels and develop how SASP can orchestrate microenvironment modifications, by focusing on extracellular matrix modifications, neighboring cells' fate, and intercellular communications. We present hypotheses on how these microenvironmental events may affect dynamic changes in SASP composition in return. Finally, we discuss the various existing approaches to targeting SASP and clarify what is currently known about the biological effects of these modified SASPs on the cellular environment.

Keywords: ageing; senescence; intercellular communication; senescence-associated secretory phenotype (SASP); microenvironment; age-related disease; senomorphics

check for updates

Citation: Giroud, J.; Bouriez, I.; Paulus, H.; Pourtier, A.; Debacq-Chainiaux, F.; Pluquet, O. Exploring the Communication of the SASP: Dynamic, Interactive, and Adaptive Effects on the Microenvironment. *Int. J. Mol. Sci.* **2023**, *24*, 10788. https://doi.org/10.3390/ijms241310788

Academic Editor: Claudiu T. Supuran

Received: 30 April 2023
Revised: 20 June 2023
Accepted: 25 June 2023
Published: 28 June 2023

1. Introduction

Cellular senescence was first described as a stable and irreversible cellular state, in which cells permanently stop proliferating while remaining metabolically active. Since then, our understanding and definition of cellular senescence and its various roles have constantly evolved. Currently, senescence is presented as a complex and partially heterogeneous phenotype that can occur in response to the exhaustion of the proliferative capacity of the cell, and/or as a result of exposure to intrinsic and extrinsic stressors [1–5].

Since there is no universal marker for senescence, a combination of specific biochemical markers and phenotypic features is necessary to identify senescent cells. However, there is no consensus on the number and type of markers required to identify senescent cells, as one senescent cell is not equivalent to another [6]. Despite this, multiple pieces of evidence in the literature suggest that senescent cells share several characteristics, including a strong or prolonged growth arrest, an altered metabolism [3,4], and a specific senescence-associated secretory phenotype (SASP) [7–10]. Although SASP is a hallmark shared by various types of senescence, it is heterogeneous and can evolve in conjunction with changes in gene and protein expression, impacting biochemical features, trafficking, and intercellular signaling [3]. The diversity and the dynamic nature of SASP make it a complex process to understand, which is well demonstrated by its close association with both beneficial and detrimental effects depending on the physiological context [1,11–15].

These diverse effects are commonly linked with key SASP proteins whose secretion is increased in different senescence models. However, the variable component of SASP, which is modulated according to context, is often neglected in current knowledge, despite its importance.

Here, we first present the complexity of the senescence phenotype and the new biomarkers that have recently been revealed. Then, we describe the heterogeneity and plasticity of SASP composition, as well as its different levels of regulation, and highlight its contribution to both ageing and cancer. Additionally, we emphasize the importance of further investigating the complex crosstalk between the microenvironment and the SASP. Finally, we discuss how controlling SASP might be a suitable approach to treating age-related diseases.

1.1. The Senescent Phenotype: A Large Definition

Cellular senescence was identified more than 60 years ago by Hayflick and Moorhead who observed in vitro that normal human fibroblasts grown in optimal culture conditions have a limited proliferative capacity [16]. Nowadays, the "Hayflick limit" is widely accepted, especially since the discovery of telomere shortening-induced genomic instability [17]. This type of senescence is therefore referred to as replicative senescence (RS).

In recent years, progress has been made to suggest that senescence could be an adaptive stress response, resulting in the persistence of irreparable damages and mainly in a prolonged cell division arrest. Indeed, senescence can be induced by the activation of oncogenes or repression of tumor suppressor genes, known as oncogene-induced senescence (OIS) [18], or by repeated exposures to oxidative or genotoxic stress, known as stress-induced premature senescence (SIPS) [19]. Since the range of potential senescence-inducing stresses is wide, it is commonly labeled based on the nature of the inducer such as irradiation-induced senescence (IRIS) or therapy-induced senescence (TIS). One common feature of these different senescence inducers is the generation of irreparable DNA damage at the origin of the sustained cell cycle arrest. However, senescence induction can occur independently of DNA damage, such as senescence induced by sodium butyrate, a histone deacetylase inhibitor (HDACi) [20], or by mitochondrial dysfunction, known as MiDAS (Mitochondrial Dysfunction-Associated Senescence), which is associated with decreased NAD+/NADH ratios [21]. Finally, cellular senescence can also be triggered by epigenetic modifications, perturbed proteostasis, and autophagy impairment [22].

1.2. Biomarkers and Characteristics of Senescence

The scientific community has faced challenges in identifying robust and specific markers that characterize the senescent state, likely due to the heterogeneity of cellular senescence. While growth arrest is the main characteristic of senescent cells, it is not sufficient to distinguish them from other non-proliferative cell states, such as quiescence or terminal differentiation. Interestingly, prolonged quiescence induces a lack of response to proliferative stimuli and progressively leads to senescence [23]. Nevertheless, the establishment of the senescent phenotype is accompanied by a set of features and alterations that are now accepted as "standard hallmarks" (Figure 1).

To ensure the senescent state of a cell, several of these hallmarks must be validated in combination.

1.2.1. Standard Hallmarks

As displayed in Figure 1, the cell cycle arrest that characterizes the senescent cell often depends on the activation of two main pathways, p53/p21^{WAF1} and/or p16^{INK4}/pRb. Senescent cells are enlarged and adopt a flat shape and their nuclei undergo structural and functional alterations [3]. These alterations include a decreased expression of lamin B1, a structural protein of the *nuclear lamina*, impairment of their structural integrity due to epigenetic alterations, and the formation of condensed heterochromatin regions, termed senescence-associated heterochromatic foci (SAHF) [24–27]. Senescent cells also

exhibit alterations in histones (de)methylations and persistent DNA damage [28,29]. Their metabolic profile is deregulated, mainly through mitochondrial dysfunction, increased production of reactive oxygen species, elevated lysosomal activity (resulting in the SA-βgal activity), increased autophagy, and activation of the AMPK signaling pathway [30]. Apoptosis resistance via the upregulation of ephrins and anti-apoptotic proteins of the Bcl-2 family is also used as a senescence biomarker [31]. Finally, senescent cells have a particular secretory phenotype including soluble pro-inflammatory factors as well as growth factors, regulatory components of the MEC, bioactive lipids, and extracellular vesicles referred to as the senescence-associated secretory phenotype and further developed in Section 2.

While these biomarkers are commonly encountered in the literature, it is not unusual to observe variations in their expression and effectors.

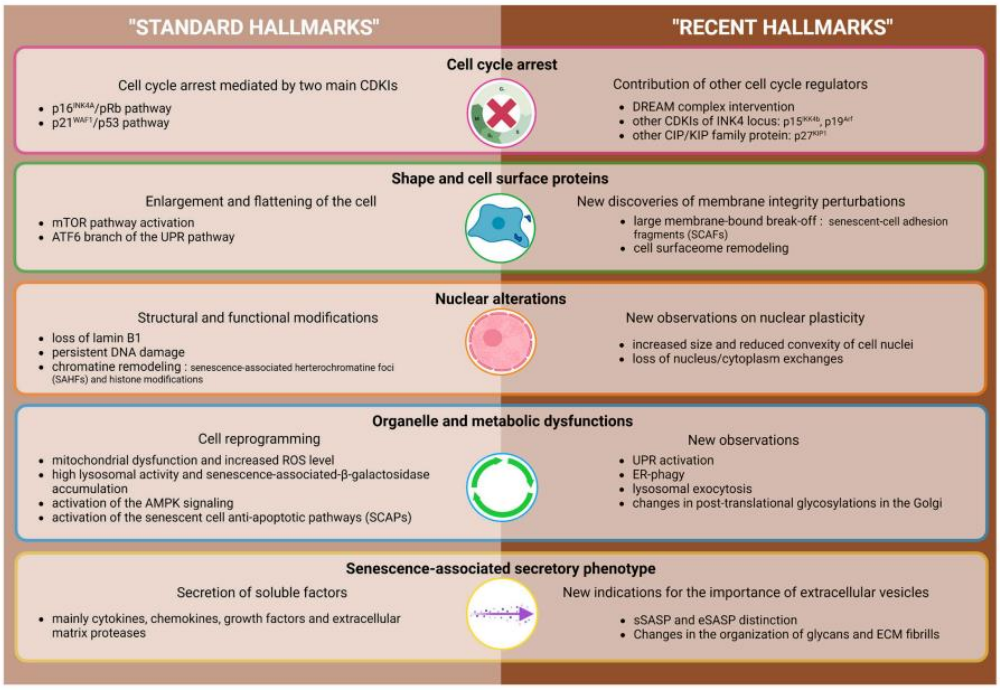


Figure 1. The updating hallmarks of senescence. The scheme compiles the major hallmarks of senescent cells, which are classified into five characteristic groups: cell cycle arrest, shape and cell surface proteins, nuclear alterations, organelle and metabolic dysfunctions, and senescence-associated secretory phenotype. The light and dark brown portions indicate the well-described standard hallmarks and the more recent ones, respectively. CDKIs, cyclin-dependent kinase inhibitors; UPR, unfolded protein response; ER, endoplasmic reticulum; ERAD, ER-associated degradation. This figure was created using BioRender.com.

1.2.2. Other Pathways Involved in the Senescence-Mediated Cell Cycle Arrest

The cell cycle arrest mediated by the p53, $p21^{WAF1}$, and $p16^{INK4}$ results in the hypophosphorylation of pRb. This leads to the subsequent sequestration of E2F and prevents the expression of genes necessary for cell cycle progression. Interestingly, the $p53/p21^{WAF1}$ pathway is also involved in assembling the repressive DREAM complex, which serves the same purpose, thereby reinforcing the role played by p53 [32,33]. However, other cell cycle regulators are also involved in this senescence-associated cell cycle arrest. Additionally, the actors of the cell cycle arrest

can vary over time, with some being expressed at the onset of senescence and others during its late phases. As an example, in glyoxal-induced senescent keratinocytes, the cell cycle arrest is first mediated by the protein kinase B-FOXO3a-$p27^{KIP1}$ pathway but is sustained over time by the $p16^{INK4}$/pRb pathway [34]. Furthermore, in therapy-induced senescence in positive breast cancer cell lines, the tyrosine kinase inhibitor Lapatinib can induce senescence by increasing the expression of $p27^{KIP1}$ and $p15^{IKK4b}$ [35] and in prostate cancer cells, supraphysiological androgen levels regulate the establishment of senescence through $p15^{IKK4b}$ [36]. Thus, it is important to extend the study of the expression of other proteins involved in cell cycle arrest and to verify their expression over time. It would be beneficial to decipher the actors involved in the cell cycle arrest and their expression patterns over time according to the cell type and senescence inducer. Currently, it is still unclear which pathways act concurrently and whether there are differences in the hierarchy and kinetics of the events, as well as which pathways are dispensable. It is worth noting that while the senescence-associated cell cycle arrest has historically been considered "irreversible", primarily studied in fibroblasts, under certain conditions and contexts, both tumoral and normal senescent cells can resume proliferation and thus escape senescence [37,38].

1.2.3. Surfaceome

Since any cell population is inherently heterogeneous, the induction of senescence only affects a fraction of the population, which therefore becomes enriched in senescent cells. Therefore, the identification, sorting, and targeting of these senescent cells using surface markers is of crucial importance. Althubiti et al. [39] were the first to perform proteomic analyses of plasma membrane-associated proteins in an EJ bladder cancer cell line with regulating expression of $p21^{WAF1}$ or $p16^{INK4}$. They identified 107 potential protein markers and demonstrated that a set of 10 proteins (DEP1, NTAL, EBP50, STX4, VAMP3, ARMX3, B2MG, LANCL1, VPS26A, and PLD3), when combined, could serve as markers of senescence and facilitate the detection of senescent cells in various human tissues [39]. Since this first analysis, other proteins that contribute to the surfaceome of senescent cells have been described such as the dipeptidyl peptidase 4 (DPP4) [40] and the urokinase-type plasminogen activator receptor uPAR [41]. B2MG, DPP4, and uPAR are therefore used as targets for the specific clearance of senescent cells in vitro and in vivo [41–43].

1.2.4. Alterations of Nuclear Shape and Nucleus-Cytoplasm Exchanges

Senescence is also associated with alterations of the nuclear architecture. Besides the well-known modification of the *nuclear lamina* partly linked to lamin B1 loss [44], other nuclear features including nuclear matrix, nucleolus, heterochromatin, and even nuclear shape and size can also be altered [45]. In particular, the increased nuclear shape is a promising biomarker to predict the senescent state [46]. By using machine learning algorithms, the group of Heckenbach demonstrated that nuclear morphology could predict senescence following different senescent inducers, in different cell types and species. Deep learning could therefore be used as a tool to predict the occurrence of senescent cells in clinical contexts and to study their potential negative effects, paving the way for the prevention of the harmful effects of senescence [47].

Moreover, the nucleus–cytoplasm exchanges are altered in senescence, as the transcription-export (TREX) machinery and the nucleocytoplasmic trafficking (NCT) are downregulated [48,49]. Interference in exchanges between the nucleus and the cytoplasm of senescent cells leads to a reduction in the transmission of extrinsic signals toward the nucleus and alters the nucleus-to-cytoplasm protein–RNA transport, resulting in the establishment of a "nuclear barrier" [48,49].

1.2.5. UPR and ER Control Quality

The endoplasmic reticulum (ER) is a dynamic structure playing a major role in the folding, synthesis, processing, and quality control of secreted and transmembrane proteins. When ER homeostasis is perturbed, an adaptive mechanism called UPR (Unfolded Protein Response) aims to restore ER homeostasis and promote cell survival [50]. It is now well

accepted that different cell types undergoing senescence upon various inducers promote UPR activation [47,51]. We and other groups have shown evidence that the different arms of the UPR pathway may control several senescence hallmarks. Indeed, UPR inducers promote a premature senescence phenotype [52,53]. Moreover, the ATF6α arm of the UPR controls cell size and enlargement in replicative senescent fibroblasts [52]. In addition, the expression of autophagic component MAP1LC3-II in glioblastoma cells undergoing therapy induced-senescence is triggered by the PERK/ATF4 arm [54], and in Hras-induced senescence, production of SASP induces the UPR activation via proteotoxic stress [55,56]. The role of the UPR regarding SASP is discussed in Section 3.

How senescence affects the other ER quality control systems, namely, the ERAD (Endoplasmic reticulum-associated protein degradation) and ER-phagy, is poorly understood. To date, only one study has reported a relationship between ER-phagy and senescence. The authors demonstrated activation of the ER-phagy mediated by FAM134B, an ER-phagy receptor, upon advanced glycation end product (AGE) stress-induced senescence in primary human nucleus pulposus (NP) cells [57]. They also found that enhancement of ER-phagy by FAM134B overexpression reduces the percentage of SA-β Gal positive cells, while the suppression of FAM134B exacerbates it upon AGEs stress in NP cells [57]. The role of ERAD and ER-phagy deserves to be further explored in senescence.

2. Characteristics of SASP

During its lifespan, a cell can release a range of molecules into its surrounding extracellular environment. The secretory profile is constantly influenced by intrinsic characteristics, which depend on the cell type and its differentiation stage, as well as extrinsic factors such as the change in the cellular environment. Indeed, a clear remodeling of the secretory profile can be observed in cells undergoing senescence [58]. Examining the secretome of senescent cells reveals modifications in the levels of soluble, insoluble, and extracellular vesicle (EV)-related components. Senescence establishment can cause these components to be either exacerbated or partially depleted, and can also lead to the secretion of new components when compared to proliferative cells [59].

2.1. Reported SASP Factors

Senescent cells exhibit a distinct and dynamic secretome different from their exponentially growing counterparts [60]. This SASP is complex and is composed of hundreds of different proteins and non-protein signaling molecules [60,61]. Despite the diversity of the factors secreted, a core protein secretome can be distinguished from the soluble part of SASP (sSASP) (Table 1). In human primary fibroblasts subjected to various senescence inducers (IR, RAS, atazanavir (ATV)), this core protein sSASP includes, among others, STC1 (stanniocalcin 1), chemokines such as CXCL1 (C-X-C motif ligand 1), and proteases such as MMP-1 (matrix metalloproteinase 1) [60]. Hemostasis-related factors, another class of bioactive compounds, show a marked increase in secretion into the extracellular medium by senescent human primary fibroblasts when exposed to different inducers (IR, doxorubicin (DOX), and MiDAS) [61]. Non-protein signaling molecules, including various bioactive oxidized lipid metabolites, prostaglandins, and nitric oxide can also be found enriched in the sSASP of senescent cells [62–65]. While empirical research has focused on soluble factors secreted by senescent cells, new studies show evidence that EVs are also a substantial and effective part of SASP [66]. EVs are lipid membrane vesicles containing cytosol from the secreting cells and are released by multiple cell types. According to their origin, biological function, and secretion, EVs can be classified into two main subtypes: exosomes and microvesicles (MVs) [67]. In this context, it has been evidenced in multiple cell types that EV secretion increases after exposure to different senescence inducers, with changes in cargo composition including proteins and genomic content such as microRNAs (miRNAs) and lipids [63,68,69].

Table 1. Common SASP proteins shared between different cell types under different inducers of senescence.

SASP Factor	Cell Type	Inducer	Detection	References
IL-6	Human fibroblasts (WI-38, IM90, BJ) Prostate epithelial cells (PrECs) Human keratinocytes (NHEK)	Irradiation-induced senescence Replicative Senescence Ras overexpression UVB-induced senescence	Antibodies array ELISA	[9,29,70]
CXCL1-2-3	Human fibroblasts (IMR90, HCA2) Prostate epithelial cells (PrECs)	Irradiation-induced senescence Replicative Senescence	Antibodies array	[9,29]
IL-8	Human fibroblasts (WI-38, IM90, BJ), Prostate epithelial cells (PrECs) Renal epithelial cells (ATCC) Human keratinocytes (NHEK)	Irradiation-induced senescence Replicative Senescence UVB-induced senescence Glyoxal-induced senescence	Antibodies array Mass spectrometry ELISA	[9,29,34,60,70]
IGFBP-2	Human fibroblasts (IMR90, WI-38, HCA-2, BJ) Renal epithelial cells (ATCC)	Irradiation-induced senescence	Antibodies array Mass spectrometry	[29,60]
IL-7	Human fibroblasts (WI-38, HCA-2, BJ) Prostate epithelial cells (PrECs)	Irradiation-induced senescence	Antibodies array	[9]
GDF15	Human fibroblasts (IMR-90) Epithelial renal cells (ATCC)	Irradiation-induced senescence Replicative Senescence	Mass spectrometry	[60]
Macrophage migration inhibitory factor (MIF)	Human fibroblasts (IMR-90, WI-38) Epithelial renal cells (ATCC) Human keratinocytes (NHEK) Prostate epithelial cells (PrECs) Bone marrow MSC	Irradiation-induced senescence UVA-induced senescence RAS overexpression Chemical-induced senescence (ATZ) H_2O_2-induced senescence	Mass spectrometry Antibodies array	[9,60,71,72]
Filamin B	Human fibroblasts (IMR-90) Epithelial renal cells (ATCC) Human keratinocytes (NHEK) Prostate epithelial cells (PrECs) Bone marrow MSC	Irradiation-induced senescence UVA-induced senescence RAS overexpression Chemical-induced senescence (ATZ) H_2O_2-induced senescence	Mass spectrometry	[9,60,71,72]
Cathepsin D	Mesenchymal stem cells	Replicative Senescence Chemical-induced senescence (doxorubicin) H_2O_2-induced senescence Irradiation-induced senescence	Mass spectrometry	[72]

The core secretome is a concept based on a limited number of established cellular models used to study senescence, such as human primary fibroblasts, and represents only a portion of the complete picture. Hence, the application of a core SASP should be considered as a tool to evaluate the acquisition of the senescent phenotype in a standardized manner, rather than a way to generalize the effects of SASP on the cellular microenvironment.

Indeed, the bioactive effects of SASP may be more closely linked to specific and possibly subtle variations in the secretome that result from the combination of a specific cell type and a particular senescence inducer, rather than the shared components.

2.2. SASP Heterogeneity and Plasticity

Coppé and colleagues demonstrated initially that only a subset of SASP proteins was shared between fibroblasts and prostate epithelial cells upon irradiation-induced senescence (IRIS) [9]. A subsequent large-scale proteomic analysis of SASP then revealed only 58 shared SASP factors between fibroblasts and renal epithelial cells in IRIS [60]. When considering other proteomic studies on various cell types and senescence inducers, such as UVA-induced senescent keratinocytes and IRIS mesenchymal stem cells [71,72], the number of shared SASP factors drops to 19, suggesting that only a handful of proteins are commonly secreted across all types of senescent cells. Given the differences in experimental parameters such as EV isolation methods, detection techniques, and time points assessed after senescence induction, comparing these studies remains challenging.

Secondly, some SASP factors are secreted at different times depending on the cell type. For instance, in IRIS, the *IL-1β* gene is overexpressed on days 10 and 20 in fibroblasts, but only on day 10 in keratinocytes, and on day 20 in melanocytes [73]. This point emphasized SASP plasticity over time. Moreover, we showed an increased secretion of IL-6 and IL-8 in UVB-induced senescent keratinocytes on day 3 that disappeared on day 7 following senescence induction [70]. In addition, HDFs in replicative senescence also exhibit two distinct phases of SASP gene expressions. The first phase is often characterized by an overexpression of SASP genes related to inflammation, such as *IL-6* and *IL-8*, while the second phase is more characterized by changes in the expression of genes associated with extracellular matrix modulation, such as *MMPs* [74]. This is related to the temporal regulation of SASP, further developed in Section 3. Further experiments are required to better understand the kinetics of SASP factors and their physiological relevance in the short and long term.

Thirdly, SASP composition is also influenced by the senescence inducers. Senescent IMR-90 fibroblasts present a different secretome profile depending on whether the senescence was induced by X-rays, ATZ, or RAS overexpression [60]. Similar results were observed on mesenchymal stem cells in senescence induced by oxidative stress, doxorubicin treatment, X-ray irradiation, or replicative exhaustion [72].

Finally, the matrix and cellular microenvironment of senescent cells can impact their secretome composition. The substrate stiffness impacts the NF-κB phosphorylation status in UV-induced senescence in fibroblasts [75], suggesting that the ECM composition could have an impact on SASP composition. Moreover, it has been demonstrated that co-cultivating squamous cell carcinoma and RS fibroblasts exacerbate some SASP gene expressions, such as *IL-8*, *IL-1β*, or *CCL2*, in RS fibroblasts [76]. These findings demonstrate the need of developing cellular models that consider the influence of the ECM and other cell types within the same tissue. Such models would allow a better understanding of the complex interplay between SASP and its microenvironment, which can affect SASP composition over time.

3. Regulation of SASP

The regulation of SASP involves transcriptional, post-transcriptional, epigenetic, and translational mechanisms. In addition, the secretion of SASP components is regulated through intracellular trafficking, and many compartments of secretion are altered during

senescence. These alterations could potentially affect the dynamic and heterogeneous composition of SASP.

3.1. Transcriptional Regulation

Multiple signaling pathways have been identified to activate transcription factors that play a crucial role in regulating the expression of inflammatory cytokines.

First, there is a clear link between the expression of SASP and the DNA Damage Response (DDR) pathway, as several DDR proteins (ATM, Chk2, and NBS1) are necessary for the initiation and maintenance of the cytokine response in IRIS fibroblasts [29]. It has recently been described that in the absence of DNA damage, such as after sodium butyrate treatment, the SASP of fibroblasts still relies on the non-canonical activation of DDR and the accumulation of ATM, MRE11, and NF-kB on chromatin [77]. However, the expression of SASP factors seems independent of the cell-cycle regulators p53 and pRb, as their invalidation or inactivation even promotes IL-6 secretion [9,29]. On the other hand, it has been recently demonstrated that the knockdown of p16 in fibroblasts decreases the expression of IL-6 and IL-8 in OIS and etoposide-induced senescence [78].

Then, the NF-κB and C/EBPβ transcription factors were identified to be involved in the regulation of CXCR2 ligands expression, including IL8 in fibroblasts in OIS [79]. The activation of NF-κB in fibroblasts has been demonstrated to depend on GATA4, whose regulation by p62 is suppressed during senescence but mediated by DDR-related ATM and ATR [80]. NOTCH1 plays a dual role in the regulation of SASP. It is positively associated with early SASP expression in OIS in fibroblasts but then represses late SASP expression by suppressing C/EBPβ expression [81].

Regarding the regulation of inflammatory cytokine expression, the activation of another signaling pathway involved in inflammation, JAK/STAT, has also been demonstrated in a PTEN-deficient prostate cancer mouse model [82], as well as in senescent preadipocytes [83].

Finally, the cGAS/STING pathway has been highlighted to be involved in the regulation of inflammatory SASP factors, notably, IL-6 and CXCL10 secretion, via NF-κB activation in vitro and in vivo [84], following the detection of cytoplasmic chromatin fragments (CCFs) [85] associated with a loss of nuclear integrity following Lamin B1 (*LMNB1*) downregulation [86]. It has recently been demonstrated that COX2 plays an important role in regulating the expression of several inflammatory SASP components in OIS through an autocrine feedback loop involving prostaglandin E2 (PGE2) binding to EP4, but the downstream pathways of PGE2 and EP4 remain unknown. Nevertheless, the COX2 pathway is thought to be able to activate major SASP transcriptional regulators, such as NF-κB, C/EBPβ, and GATA4 [87].

3.2. Post-Transcriptional Regulation

While early SASP is mainly regulated at the transcriptional level, its long-term SASP expression is mainly driven by post-transcriptional mechanisms. This has been demonstrated by the lack of impact of actinomycin D treatment, an inhibitor of transcription, on the expression of several SASP factors [88]. $P38^{MAPK}$ appears to be an important factor in the temporal regulation of SASP. If it is first activated after the induction of senescence, it enables the expression of SASP factors, such as IL-6 and IL-8, through NF-κB activation in IRIS fibroblasts [89]. It is also involved in the subsequent post-transcriptional regulation of SASP by restricting the binding of AUF1 to the 3′-UTRs of several SASP mRNAs, including *IL-6* and *IL-8*, thereby preventing their destabilization, as demonstrated in bleomycin-induced senescent fibroblasts [88]. The mTOR pathway is also involved in the post-transcriptional regulation of SASP. Specifically, mTOR activates the translation of MK2 (or MAPKAPK2), which can phosphorylate and inhibit the RNA-binding protein ZFP36L1, also involved in the destabilization of several SASP mRNAs [90]. The mTORC1 kinase has also been shown to modulate senescence-induced inflammation and SASP [91].

As previously mentioned, studies on the regulation of SASP have primarily focused on the transcriptional and post-transcriptional regulation of inflammatory cytokines. However, there is a limited understanding of the regulatory mechanisms underlying other SASP factors, such as growth factors and proteases. A recent study on fibroblasts has described that E2F4, TEAD1, and AP-1 transcription factors are major regulators of RS [92]. Moreover, AP-1 is involved in the expression of *IL-6*, *IL-1β*, and *MMP-10*, as their expression is abrogated when expressing a dominant-negative isoform of c-Jun, one of the subunits of AP-1, during OIS in fibroblasts [93]. Future studies focusing on the regulation of non-inflammatory SASP factors would be valuable.

3.3. Epigenetic Regulation

The physical clustering of SASP genes, such as *MMPs* (*MMP-1*, *-3*, *-10*, and *-12*) or chemokines (*CXCLs* and *CCLs*), suggests that the regulation of their expression may depend, at least in part, on broader changes in chromatin conformation [94]. Indeed, several histone variants can influence the expression of SASP genes. For example, the relocation of the macroH2A1 histone variant away from SASP genes following ER stress response-mediated activation of ATM in fibroblasts in OIS is involved in the maintenance of SASP gene expression [56]. Moreover, the increased expression of histone variant H2A.J in fibroblasts undergoing etoposide-induced senescence enhances the expression of multiple genes associated with inflammation and immune response. This effect is likely attributed to the interaction of H2A.J with other factors [95]. In addition, nuclear HMGBs bind to DNA, facilitating the access of transcription factors to promoter regions. In fibroblasts in RS or IRIS, HMGB1 can be released into the extracellular space and act as an alarmin to activate NF-kB, which subsequently upregulates the expression of pro-inflammatory target genes [96]. Furthermore, HMGB2 preferentially localizes to SASP gene regions during OIS in fibroblasts, protecting them from being incorporated in transcriptionally repressed SAHF regions [97].

3.4. Secretory Control: Compartments of Secretion and Vesicular Trafficking

Even though most organelles are morphologically or functionally affected during senescence, their proportion increases in senescent cells due to various signaling defects. In addition to nuclear and mitochondrial dysfunction, the endoplasmic reticulum, Golgi apparatus, and lysosomal compartments are strongly involved in the generation, processing, and release of SASP factors [91].

The ER is the site of membrane biosynthesis used in secretory and excretory pathways. It is responsible for folding and maturating secreted proteins, making it the first compartment of secretion. Recently, it has been proposed that ER stress and the subsequent activation of the unfolded protein response upon senescence could contribute to the modified secretome of senescent cells [98]. While there are multiple connections between the UPR and inflammation [99,100], the UPR and normal or tumoral secretome [101–103], as well as the UPR and direct control of MMPs [104], the data directly linking ER stress with SASP are scarce. Our group demonstrated that knocking-down ATF6 in RS fibroblasts decreased *IL-6* mRNA levels [57]. Chen et al. [56] proposed that UPR induction in RAS-mediated senescence led to macroH2A1 expression, which, in turn, induces the expression of various SASP-associated genes in fibroblasts. Moreover, Dorr et al. [55] suggested that OIS and TIS induce proteotoxic stress and UPR activation to ensure SASP production. Nevertheless, the exact role of ER and UPR must be further addressed to confirm a central role in the establishment and composition of SASP.

The Golgi structure is also altered in senescent cells [105]. These alterations can not only be mediated by the translocation of a G protein γ subunit from the plasma membrane to the Golgi [106] but also by the impaired expression of the vacuolar ATPase ATP6V0A2, which acidifies organelles such as Golgi, endosomes, or lysosomes [107]. This results in deep changes in post-translational glycosylation in the Golgi, impacting SASP compounds. In addition, the trans-Golgi network (TGN) appears to be increased in senescent cells. A blockade of TGN components such as the protein kinase D1 (PKD1) is

associated with the intracellular accumulation of some SASP factors including IL-6 and IL8 in OIS fibroblasts [108].

Lysosomes are at the crossroads of endocytic and exocytic pathways, and their increased abundance in senescent cells may be associated with the exacerbation or deregulation of these pathways. Besides their partnership with the Golgi apparatus and the endosomal compartments, lysosomes are also important for the clearance of cytoplasmic chromatin fragments (CCFs). CCFs may leak from the nucleus in the cytoplasm of senescent cells and induce an SASP; both CCFs and SASP inductions would be related to a retrograde mitochondrial–nucleus signaling pathway associated with the mitochondrial increase in ROS species [91].

In melanoma cells, the lysosomal exocytosis mediated by the small GTPase RAB27A has also been shown to be upregulated in TIS and to participate in SASP factors secretion, including the chemokines CCL-2 and CXCL-12 [109]. Along with this enhanced lysosomal secretion, senescent cells exhibit a remodeling of their lysosomal proteome with selective enrichment in some lysosomal resident proteins such as those implicated in vesicular transport and fusion [109].

Small EV and exosome secretions are now part of the specific secretory phenotype. The release of senescence-associated exosomes is linked to RAB27A expression, as silencing of *RAB27A* leads to decreased exosome secretion in fibroblasts undergoing RS or OIS [110]. Rab27 GTPases are associated with the connection of multivesicular endosomes and the secretion of exosomes [111]. The enhanced biogenesis of EVs and their release by senescent cells have been demonstrated to be associated with the extent of DNA damage generated by the senescence inducer, as well as the activation of the ceramide synthetic pathway [112]. EVs and exosomes also contribute to SASP and its paracrine impact. For example, EVs from senescent stromal cells can enhance the proliferation of cancer cells by promoting the activation of the ephrin-A2 tyrosine kinase receptor, which interacts with overexpressed ephrin-A1 on the surface of the cancer cells, thereby boosting an Erk-dependent proliferation pathway [110,113]. In addition to being components of SASP, the release of senescence-associated EVs seems to be a mechanism used by senescent cells to discard cytoplasmic chromatin DNA fragments (CCFs), thus limiting DNA damage accumulation caused by major stress exposure and potentially modulating SASP [110,112]. Finally, EVs and exosomes are opening a new era of a multifunctional SASP due to their wide range of potential contents, as well as the specificities of the cellular niches and partnerships in which they operate.

4. Senescence and SASP In Vivo

Senescent cells accumulate in tissues with age. A meta-analysis showed that even if the proportion of senescent cells in 14 different human tissues is correlated with chronological age, it varies depending on the tissue type and the senescence marker used [114]. Moreover, the accumulation of senescent cells is also detected at pathological sites due to various stress signals regardless of age [115]. As a result, there is a wide diversity of senescent cells throughout the body. Furthermore, several studies have indicated that the elimination of senescent cells using transgenic mice, such as the INK-ATTAC and p16-3MR mouse models that both specifically target the elimination of p16-positive cells [15,116], or through the use of small pharmacological molecules called senolytics (which kill senescent cells) or senomorphics (which suppress some or all of their phenotype/properties) has shown improvements in healthspan, alleviated several age-associated conditions, delayed tumor formation, and mitigated the side effects of chemotherapy [117–120]. These findings highlight senescence as a significant contributor to ageing and associated pathologies. Therefore, SASP profiles may contribute to developing senescence biomarkers in human plasma or other biofluids, as well as assessing the efficacy of senescence-targeted therapies (see Section 6). Basisty and colleagues defined in senescent culture cells a core SASP including GDF15, STC1, SERPINE1/PAI-1, and MMP1, which are also reported to be significantly increased among the plasma markers of ageing in humans [60]. Another

study showed that doxorubicin-induced senescence enriched the SERPINE1/PAI-1 SASP factor in plasma in vivo [61]. However, these markers can also serve as biomarkers for several diseases such as cardiovascular, metabolic, neurodegenerative, and malignant diseases, regardless of age. This makes them indicators of a "state of ageing" rather than a chronological accumulation of senescent cells. Surprisingly, the production of SASP factor IL-6 is increased in in vitro senescent models, but the circulating levels of IL-6 are not significantly different between young and elderly subjects [121,122]. Interestingly, Markov et al. identified, by using machine learning on a human cohort, immune biomarkers to predict brain ageing and suggested that intervention on these biomarkers could prevent brain ageing [123]. Regarding the use of senolytics for treating human cellular senescence-associated diseases, it is worth noting that the SASP factors assayed or detected can vary significantly from one study to another [124,125]. These variations are undoubtedly specific to the origin of senescent cells, which makes them difficult to use as markers in clinical settings. Further studies are needed to be able to robustly consider SASP factors, or a subset of them, as reliable biomarkers and to determine how SASP could be effectively translated into clinical applications.

5. Pleiotropic Roles of SASP

Due to its diverse composition, SASP can have pleiotropic effects on the cellular environment, which can be either beneficial or deleterious. Several recent publications have extensively covered this topic [10,58,126]. However, in this section, we focus on certain physiological and pathophysiological consequences that SASP can cause. Specifically, we examine how SASP influences extracellular matrix remodeling, intercellular communication modification, ageing, and the development of cancer.

5.1. Extracellular Matrix Remodeling

Collagen alterations in the dermal ECM have been associated with the decline in human skin structure and function during ageing. This emphasizes the overexpression of cysteine-rich protein 61 (CCN1) and MMP-1 expression in the SASP of senescent fibroblasts [127]. Changes in ECM composition and ECM-degrading molecules produced by SASP also disrupt elastin and collagen fiber networks and basement membranes in ageing tissue [128]. Therefore, SASP-related changes in ECM components have a notable effect on cell functions and fates by altering the tortuosity of collagen or by increasing the stiffness of the ECM as ageing progresses.

In the context of tissue injury, senescent cells can play a role in regeneration by accelerating wound healing or limiting fibrosis. For instance, the transient secretion of PDGF-AA (Platelet-derived growth factor AA) from senescent fibroblasts is necessary for effective healing following skin injury [15]. Strikingly, the short-term presence of miR-23a-3p in EVs derived from senescent fibroblasts allows a faster wound closure of epidermal keratinocytes [129]. However, to date, few studies have investigated the link between senescent cells and ECM since the matricellular protein CCN1 has been shown to induce ROS-induced senescence in fibroblasts during wound healing [130]. In addition, the secretome of senescent HSCs (hepatic stellate cells) plays an important role in fibrotic degradation and maintenance of liver tissue homeostasis [131]. Moreover, the elimination of senescent $p16^{High}$ LSECs (Liver Sinusoïd Endothelial Cells) in mice induces fibrosis [132]. Recently, efforts have been made to better characterize the changes in the matrisome of senescent cells and their effects on the environment. Hierbert and colleagues [133] described that the activation of Nrf2 in fibroblasts triggers the production of a senescence-promoting ECM via the expression and secretion of certain ECM proteins, such as PAI-1. This can accelerate wound closure and promote re-epithelization in vivo. In addition, Nrf2 inhibition in fibroblasts reduces the production of collagen I and alters ECM deposition [134]. Furthermore, when muscle stem cells are seeded onto decellularized ECM maintained by senescent fibroblasts, their responses and functions are affected, resulting in enhanced expression of fibrogenic markers and reduced myogenic markers [135].

5.2. Tumor Suppression and Promotion

While cellular senescence is widely recognized as an anti-tumor barrier, there is growing evidence to suggest that senescence may also have a tumor-promoting role.

Senescent cells have been observed at sites of benign tumors, such as prostatic hyperplasia and melanocytic naevi [136,137]. The factors secreted by these cells influence the tissue microenvironment and impact cellular differentiation and proliferation, notably in cancer cells [136,138]. Moreover, the first studies pointing out the role of the cellular microenvironment in the promotion of cancer progression highlighted the role of Carcinoma-Associated Fibroblasts (CAFs) in prostate cancer progression [139]. It has been subsequently reported that senescent fibroblasts share many features with CAFs, and can have a similar impact on the differentiation of epithelial cells initiated by cancer, and on tumor growth both in vitro and in vivo [13,140]. Co-culture systems and xenograft models have shown that SASP from senescent fibroblasts promotes the tumorigenesis of premalignant epithelial cells [141], induces epithelial–mesenchymal transition (EMT), and increases tumor vascularization, which suggests pro-tumorigenic properties [98].

We and others have shown that Normal Human Mammary Epithelial Cells (HMECs) and Normal Human Epidermal Keratinocytes (NHEKs) can spontaneously and systematically escape from the senescent state [142]. Some rare senescent cells re-enter the cell cycle via a process called Post-Senescence Neoplastic Escape (PSNE) with characteristics similar to those observed during the early stages of tumor initiation [52,143–146]. More importantly, when xenografted in nude mice, these PSNE cells developed into disseminated skin lesions such as hyperplasia and small non-melanoma skin carcinoma, evidencing their tumorigenic potential [144,145]. SASP from senescent dermal fibroblasts promotes neoplastic escape from normal human keratinocytes and increases markers of EMT as well as the migration of emerging cells [145]. This was attributed to the activation of the membrane PAR-1/Thrombin receptor by MMPs among SASP of senescent fibroblasts [145]. Furthermore, a recent study has identified a BDNF-TrkB axis as being associated with the role of SASP of aged fibroblasts in promoting EMT initiation in primary keratinocytes from aged donors [147].

Another point is that the alteration in the secretion of ECM components and regulators by senescent prostate cells generates a favorable environment for tumor development [148]. UVB-induced senescent fibroblasts were shown to produce an ECM that promotes proliferative signaling pathways of preneoplastic HaCaT epidermal keratinocytes [149]. Enhanced collagen deposition has been described along breast cancer progression, with dysregulated architecture and increased reticulation via abnormal expression of lysyl oxidase and MMP-resistant collagen isoforms, contributing to carcinoma progression [150]. Moreover, increased collagen matrix stiffness has been shown to control the cell fate of normal breast cells in 3D models. The increase in collagen concentration led to the overexpression of the $\alpha 6\beta 4$ integrin pair, profound disruption of the architecture from regular normal-like acini and tubes to a tumor-like mass of increasing size, and decreased differentiation [151].

Taken together, the combination of SASP factors responsible for the pro-tumorigenic effects remains poorly understood. However, progress has been made in defining how context-dependent (such as cellular partnerships, specific soluble proteins, or membrane receptors) can influence the effects of SASP in cancer promotion [152].

5.3. Senescence Induction and Reinforcement

In addition, SASP primarily influences the induction and reinforcement of senescence. It is now clear that senescent cells maintain their phenotype through an autocrine positive feedback loop in which the main factors identified are cytokines such as IL-6 and IL-8 [79,153]. Similarly, the same SASP factors and many others such as TGF-β family ligands, VEGF, and chemokines such as CCL2 and CCL20 also play an important role in inducing paracrine senescence in neighboring cells [154]. The intensity of SASP can impact local homeostasis paracrine through signals that propagate the senescent state, exacerbating local stress, and inducing ROS-mediated damage in neighboring cells. This is the so-called

SMS effect of SASP. Hence, conditioned media (CM) of cells exposed to UV radiations (UVA, B, and C) initiate bystander DNA damage in non-exposed neighboring cells [155]. Moreover, since the recent interest in deciphering the role of eSASP, new studies have shown the important contribution of microvesicles in the propagation of the senescent phenotype, for example, via the transfer of interferon or miRNA cargo factors [156,157].

5.4. Other Functions of SASP

SASP can modulate the fate of neighboring cells in several ways and can even impact the differentiation of surrounding cells.

For example, Wiley et al. [21] have shown that CM harvested from fibroblasts whose mtDNA has been depleted (rho0) can block adipogenesis in preadipocytes but promote keratinocyte differentiation. A recent study also showed that TGF-β secreted by senescent cells can influence the differentiation of T helper cells during the response to influenza infection in mice [158]. A proteomic analysis of CM of fibroblasts in IRIS identified a role for SASP in hemostasis, platelet activation, and degranulation [61]. Moreover, transient exposure of primary mouse keratinocytes to SASP of OIS keratinocytes led to enhanced plasticity via the increased expression of stemness markers and better regenerative capacities in vivo, while long-term exposure promoted senescence, reducing regenerative stimuli [159]. Therefore, SASP may induce cellular plasticity and tissue regeneration capacities according to its intensity and duration, and may promote cellular reprogramming in neighboring cells [160]. In parallel, ECM stiffness is also of particular importance in stem cell response and can lead to considerable changes in cell signaling, shape, and differentiation status [161]. Decellularized matrices form cardiac explants of donors of various ages, differentially impacting the cell fates and functions of IPSC-derived cardiomyocytes (ICMs) cultured on top of the matrices. In fact, matrices from young donors can enhance the proliferation and functions of young ICMs, while matrices from aged donors promoted their senescence [162]. In addition, SASP can reduce muscle stem cell expansion. In damaged muscle, senescent cells altered their normal niche to create an age-related-inflamed microenvironment that impairs regeneration [163].

Finally, Saul et al. [164] conducted a bioinformatic analysis and identified a panel of genes, called SenMayo, which is enriched in elderly vs. young women. They evaluated the applicability of SenMayo across tissues and species. Using their tool, they were able to evaluate intercellular communication patterns of senescent cells with other cells in their microenvironment at the single-cell level. Overall, they showed that senescent hematopoietic and mesenchymal cells interact with neighboring cells mainly through the Macrophage Migration Inhibitory Factor (MIF) pathway.

6. Developing Strategies to Block SASP or Its Specific Effects

Given the importance of senescence in physiological processes, it is reasonable to think that there is a threshold beyond which the accumulation of senescent cells induces a microenvironment conducive to the development of pathologies via SASP. The accumulation of senescent cells can also occur when the immune system ages, altering the ability of immune cells to clear senescent cells.

Elimination of senescent cells by senolytics demonstrated a contributive role of senescent cells in ageing and age-related diseases [165] and paved the way for the development of senotherapeutic approaches. Therefore, over the past 5 years, senotherapeutic research has emerged to slow down the ageing phenotypes. Current senotherapeutic strategies targeting senescent cells are mainly based on drugs that specifically kill senescent cells (senolytics) and components that suppress the detrimental effects of SASP without inducing senescent cell death (senomorphics, also known as senostatics) [166–172]. Other senotherapeutic strategies include prodrugs, protein degraders, nanocarriers, and immunotherapies [173]. It is worth noting that a recent study showed that eliminating senescent cells by using chimeric antigen receptor (CAR) T cells that specifically target senescence-specific surface antigens such as uPAR improved the survival of mice with lung adenocarcinoma and re-

stored tissue homeostasis in a chemical-induced liver fibrosis mouse model [41]. Emerging preclinical evidence has highlighted the significant potential of these approaches [27,28,124]. However, further analyses are necessary to rule out the potential adverse effects of long-term administration. Additionally, there are ongoing efforts to evaluate combinations of senotherapies in individuals with multiple age-related diseases [174].

Nevertheless, in this section, we will not cover all senotherapeutic strategies, especially as excellent reviews have recently been published on senolytic developments [171,175–177], but rather focus on those with senomorphic activities (Table 2), based on their ability to block SASP components.

A first strategy would consist in using neutralizing antibodies, recognizing and blocking specific surface proteins upregulated at senescence. Secretion of IL-6 has been decreased in senescent HUVECs and fibroblasts treated with anti-TNFα or anti-ephrin B2 antibodies, respectively [178,179]. Several other surface proteins are known to play a role in the regulation of SASP profiles, including SCAMP4, Notch, and CD36 [40,81,180]. However, it has not yet been reported that the use of neutralizing antibodies targeting SCAMP4, Notch, or CD36 can impact the composition of SASP and, therefore, arrive at a conclusion regarding their senomorphic properties. In a model of bleomycin-induced senescence, the secretion of certain SASP factors (including IL-6 and IL-8) can be directly inhibited with neutralizing antibodies such as those against the membrane-bound IL-1α [181]. It would be interesting to investigate the impact of other neutralizing antibodies directed against other major SASP factors such as circulating IL-1β-, IL-6, or their receptors [182], on their abilities to alter the chemical composition of SASP, impair SASP-mediated effects, and attenuate other features of senescence in different cell types.

A second strategy would be to use pharmacological and natural compounds. Many senomorphics are polyphenols (including flavonoids, phenolic acids, lignans, and stilbenesenes) that possess antioxidant activities, but their modes of action have not been thoroughly studied. Other senomorphics are plant extracts consisting of a mixture of terpenes, alkaloids, and polyphenols. The biological effects of these compounds are multiple, ranging from the activation of antioxidant enzymes to the reduction in interleukin or MMP expression, and the inhibition of MAPKs. Data in Table 2 show that most senomorphics modulate the senescent phenotypes to disrupt the proinflammatory nature of senescent cells.

Table 2. Senomorphics that block SASP components at the secreted level only (measured by ELISA, antibody arrays, and multiplex array). Arrows mean that the secretion of all written factors is decreased upon treatment with the corresponding compound, compared to untreated senescent cells.

Compound	Function	Cell Type	Inducer	Effect on SASP Factors	References
Adalimumab (monoclonal antibody)	TNFα inhibitor	HUVECs	Replicative senescence	IL-6 ↓	[178]
Anti-ephrin B2 antibody (clone B11)	Ephrin B2 inhibitor	Human fibroblasts	Chemical-induced senescence Irradiation-induced senescence	IL-6 ↓	[179]
Apigenin (flavonoid)	NF-κB inhibitor	BJ fibroblasts	Bleomycin-induced senescence	IL-6; IL-8; IL-1β ↓	[183]
Avenanthramicine C	AMPK activator p38/NF-κB inhibitor	Human fibroblasts (HDFs)	Replicative Senescence	IL-6; IL-8; TGF-β ↓	[184]
BIRB796	p38 inhibitor	Human fibroblasts (NHDFs)	Replicative Senescence	IL-6 ↓	[185]
Cortisol/corticosterone	Glucocorticoids	Human fibroblasts (HCA2)	X-irradiation induced senescence	IL-6 ↓	[186]
Hydroxytyrosol (olive phenolic compound)	NF-κB inhibitor	Human fibroblasts (NHDFs , MRC5)	Replicative senescence	IL-6; MMP-2; MMP-9 ↓	[187]
IPI-504	HSP90 inhibitor	ARPE-19	H_2O_2-induced senescence	IL1-β; IL-8 ↓	[188]
Isatis tinctoria L. Leaf extract (ITE)	mTOR/MAPK/ NF-κB inhibitor	Human fibroblasts (HDFs)	Replicative Senescence	IL-6; IL1-β; IL-8 ↓	[189]
Kaempferol (flavonoid)	NF-kB inhibitor	BJ fibroblasts	Bleomycin-induced senescence	IL-6; IL-8; IL-1β ↓	[183]
Lamivudine	Nucleoside reverse transcriptase inhibitor	Human fibroblasts	Replicative Senescence	IFN-1 ↓	[190]
Metformin	Several pathways	Human HNSCC cell line Cal27	LY2835219 (CDK4/6 inhibitor)- induced senescence	NT3; MCP-1; IL-6; IL-8; GRO; IGFBP1; BMP4; BLC ↓	[191]

Table 2. *Cont.*

Compound	Function	Cell Type	Inducer	Effect on SASP Factors	References
Metformin	Several pathways	Primary VSMCs from the aortas of elderly patients	Ang II-induced premature senescence	MMP-2; IL-6; TGFβ ↓	[192]
Mix of bioCurcumin, Polydatin and liposomal-b-caryophyllene	Several pathways	HUVECs	Replicative Senescence Doxorubine-induced senescence	IL-6; IL-1β ↓	[193]
MK2.III	MK2 kinase inhibitor	Human fibroblasts (NHDFs)	Replicative Senescence	IL-6 ↓	[185]
Oleuropein (olive phenolic compound)	NF-κB inhibitor	Human fibroblasts (NHDFs) MRC5	Replicative senescence γ-irradiation-induced senescence	IL-6; MMP-2; MMP-9↓ IL-6; IL-8; MCP-1; RANTES ↓	[187,194]
Simvastatin	HMG-CoA reductase inhibitor	Normal Human Fibroblasts (HCA2)	γ-irradiation-induced senescence	IL-6 ↓	[195]
Rapamycin	mTOR inhibitor	Normal Human Fibroblasts (HCA2)	γ-irradiation-induced senescence	IL-6; CSF2; CCL7; CCL8; IGF1; TGFB3; IL-8; BMP4; IL-10 ↓	[196]
Rapamycin	mTOR inhibitor	Murine MEFs	H_2O_2-induced senescence	TNFα; LIX; Leptin R; MIP-1α	[197]
Resveratrol	SIRT1 activator NF-κB inhibitor NRF2 activator	Arterial VSMCs derived from aged rhesus monkeys	Chronological age	MCP-1; TNFα, VEGF	[198]
Ruxolitinib	JAK1/2 inhibitor	Preadipocytes from healthy human kidney transplant donors	γ-irradiation-induced senescence Replicative senescence	IL-6; GM-CSF; G-CSF; IL-10; CXCL-1; MIP-1a; IL-8, MCP-1; RANTES, MCP-3; PAI-1; MIP-1β; TNFα; IFN-α2; IL-1α; VEGF; CCL-11; PDGF-AA IL-6; IL-8; MCP-1; PAI-1 ↓	[83]

Table 2. *Cont.*

Compound	Function	Cell Type	Inducer	Effect on SASP Factors	References
SB203580	p38 inhibitor	Human fibroblasts (NHDFs) Normal Human Fibroblasts (HCA2) Normal Human Fibroblasts (HCA2)	Replicative Senescence γ-irradiation-induced senescence Ras-induced senescence	IL-6 ↓ GRO; IL-6; IL-8; MCP-2; MCP-1; GCP-2; GM-CSF; IL-10; GDNF; IGFBP4, CNTF; GROα; TGF-β1, Angiogenin; IL-2; Eotaxin; IL-7; MIG; IL-1α; TNFα; IL-5; TNFβ; Sgp130; Osteoprotegerin IL-6; IL-8; GM-CSF ↓ GM-CSF; IL-6; GRO; MIP-1α; IL-1β; ENA78; GROα; IL-8; MCP-3; HGF; ICAM3; MIP-1β; uPAR; Dtk; IGF-1SR; IL-1α; Sgp130; IL-12 p40; IL-4; TIMP1; IL-11; PIGF; IL-15; IL-2; RANTES; IL-2 Rα; Oncostatin M; GDNF; MIP-3α; IL-12 p70; Thrombopoietin	[89,185]
Silybum marianum flower extract (SMFE)	Unknown	Human fibroblasts (HDFs)	Replicative Senescence	IL-6; MMP-1 ↓	[199]
SR9009	Reduces ROS level via the activation of the NRF2 pathway	Human fibroblasts (HDFs)	Doxorubicin-induced senescence	IL-1α; IL-1β ↓	[200]
UR-13756	p38 inhibitor	Human fibroblasts (NHDFs)	Replicative Senescence	IL-6 ↓	[185]
Wogonin (flavonoid)	NF-κB inhibitor	BJ fibroblasts	Bleomycin-induced senenscen	IL-6; IL-8; IL-1β ↓	[183]
Zileuton	5-LO inhibitor	Human fibroblasts (HDFs)	γ-irradiation-induced senescence	IL-6 ↓	[201]

Most studies, however, have only assessed a few SASP major factors (such as IL-6, IL-1β, and MMPs) following senomorphic treatments, which is not representative of SASP as a whole. Moreover, the impact of senomorphics on the secretion of ECM components, microvesicles, and complex lipids remains largely unexplored. Senomorphics can act on multiple targets depending on the context, the nature, and the model of senescence. In some cases, we cannot rule out that they might even increase the secretion of some detrimental factors. This raises the concern that SASP resulting from senomorphic treatment should probably be less deleterious and should be considered as modified rather than non-senescent-like. In addition, few studies are using CM from senescent cells treated with senomorphics to examine the biological effects of the modified SASP (such as the pro-tumoral impact or differentiation) on other cell types. A study demonstrated that CM from senescent HUVECs treated with anti-TNFα reduced the migration and mammosphere formation of MCF7 cells compared to the CM obtained from untreated senescent HUVECs [178]. Other authors showed that CM from senescent preadipocytes treated with a JAK inhibitor (ruxolitinib) prevented macrophage migration, reduced inflammation in non-senescent preadipocytes [83], and attenuated osteoclast differentiation [202]. CM collected from simvastatin-treated senescent fibroblasts suppressed breast cancer cell proliferation by reducing the phosphorylation of ERK1/2 [195]. Furthermore, CM of senescent cancer cells treated with metformin attenuated the sphere-forming ability of growing cancer cells [191]. Metformin has also been shown to reverse the decrease in the migration of senescent VSMCs [192]. Therefore, in the absence of more extensive data, it is difficult to assess the real effectiveness of senomorphics on SASP.

7. Conclusions and Future Directions

Despite the efforts to characterize the senescence phenotype and the composition of SASPs, understanding the biological effects at a given time for a given cell type and senescence inducer remains challenging. Senescent cells are very heterogeneous, therefore, comparing the effects and status of senescence between senescent cells is a complex operation. Indeed, senescence evolves based on the microenvironment and time elapsed. This modulation suggests that the factors responsible for initiating senescence may not be the same as those involved in maintaining the senescent phenotype. In other words, the "initial/early" senescence is not the same as the "terminal/late" senescence. Once senescence is reached, senescent cells can correct some characteristics of their phenotype via the microenvironmental context. This indicated that the tissue's physiological and pathological context contributes to the extent, intensity, and regulation of senescence. In turn, the new information received can prompt senescent cells to adapt and produce an SASP that is more attuned to the context, meaning that the communication of the senescent cells is not unidirectional (Figure 2). This gives rise to a large variety of SASPs, and the different combinations of SASP components could generate distinct « barcodes » that may dictate SASP-mediated responses. For this reason, machine learning might be a promising tool to help the better characterization of SASPs [203].

Although efforts have been made to understand the effects of secreted factors, particularly, pro-inflammatory factors, little is known about the effects of changes in the ECM as well as direct cell-to-cell contacts. Secreted factors are only one of the means by which cells communicate with their matrix and cellular microenvironment [13]. Moreover, most studies on senescence have been carried out on fibroblasts, a model that, in essence, does not give rise to direct intercellular communication. Furthermore, the precise role of each class of secreted molecules in mediating the biological effects of SASP remains unclear. While the pro-inflammatory portion is known to be related to the pro-oncogenic effects of SASP, the role of secreted matrix proteins is not well understood. Models of decellularized matrices from senescent cells could help to fill this gap in our knowledge.

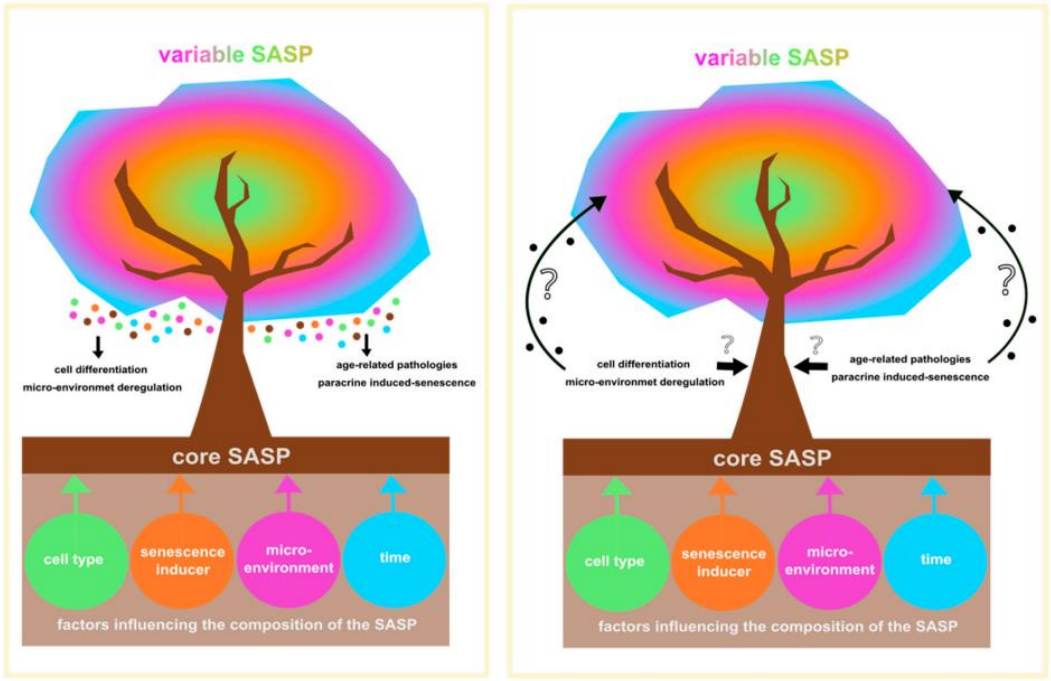


Figure 2. The crosstalk between the senescent cells and their microenvironment through the SASP. The core SASP is defined as the common SASP components shared between different senescent cells regardless of the cell type, senescence inducer, micro-environment, or time-point. The final composition of SASP (colored dots) is the combination of the core SASP and the variable SASP, which is defined by the four different factors mentioned above. Altogether, the whole SASP will influence different physiological and pathophysiological processes, such as cell differentiation, micro-environment deregulation, paracrine induced-senescence, and age-related pathologies (**left** panel). However, the question remains, are those modulations of the cellular environment influencing, in turn, the composition of SASP, and to what extent the core and variable SASP are affected (**right** panel)?

Beyond the fact that SASP may evolve and that we lack knowledge on temporal aspects, data on the transcriptomic, proteomic, and secretomic concordance are necessary. There are limited secretomic data available to support conclusions about the effects of SASP. In addition, co-culture models could be of great interest for modeling cellular interactions and the evolution of SASP, as the presence of other cell types can influence the secretome of senescent cells, either by slowing it down or stimulating its production. Several studies have attempted to define a core SASP, which is very useful to understand the common aspects following different senescence inducers or cell types. However, from a therapeutic point of view, it would be more interesting to understand what differentiates two cell types that have undergone the same senescence inducer. As an example, when two SASPs have a 70% similarity, it raises questions about the potential impact of the remaining 30% difference. This highlights the potential for the selective use of SASP blockers. Regarding SASP blocking molecules, their senomorphic properties have been essentially based on their ability to inhibit the transcription of SASP genes. However, concordance between transcriptomics, proteomics, and secretomics has not been demonstrated for all SASP genes.

Further experiments are needed to validate this concordance as well as data using CM from senescent cells treated with senomorphics.

In conclusion, there is still much to be discovered regarding how SASP communicates with its environment and network, and it is only through multidisciplinary approaches that we can better understand and decode them. Finally, it is still challenging to critically evaluate the use of SASP in vivo, as modulating its components has demonstrated limited reliability on human health. To be continued in the next "What SASP" discussion.

Funding: This work was supported by the Centre National de la Recherche Scientifique, the Institut National de la Santé et de la Recherche Médicale, the Université de Lille, the Ligue contre le Cancer (Comité du Nord), the Institut Pasteur de Lille, and the Contrat Plan État Région—CTRL (Centre Transdisciplinaire de Recherche sur la Longévité) and the Contrat de Plan Etat Region CPER Cancer 2015–2020 to O.P. JG is a recipient of a co-funded FSR fellowship (UNamur) and doctoral fellowship (Univ Lille), IB is a recipient of an FSR fellowship (UNamur). FDC is a research associate at Fonds de la Recherche Scientifique-FNRS.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the article.

Conflicts of Interest: The authors declare that they have no competing interest.

References

1. Roger, L.; Tomas, F.; Gire, V. Mechanisms and Regulation of Cellular Senescence. *Int. J. Mol. Sci.* **2021**, *22*, 13173. [CrossRef] [PubMed]
2. Niklander, S.E.; Lambert, D.W.; Hunter, K.D. Senescent Cells in Cancer: Wanted or Unwanted Citizens. *Cells* **2021**, *10*, 3315. [CrossRef] [PubMed]
3. Hernandez-Segura, A.; Nehme, J.; Demaria, M. Hallmarks of Cellular Senescence. *Trends Cell Biol.* **2018**, *28*, 436–453. [CrossRef] [PubMed]
4. Abbadie, C.; Pluquet, O.; Pourtier, A. Epithelial Cell Senescence: An Adaptive Response to Pre-Carcinogenic Stresses? *Cell. Mol. Life Sci.* **2017**, *74*, 4471–4509. [CrossRef]
5. Toussaint, O.; Medrano, E.E.; Von Zglinicki, T. Cellular and Molecular Mechanisms of Stress-Induced Premature Senescence (SIPS) of Human Diploid Fibroblasts and Melanocytes. *Exp. Gerontol.* **2000**, *35*, 927–945. [CrossRef]
6. Toutfaire, M.; Bauwens, E.; Debacq-Chainiaux, F. The Impact of Cellular Senescence in Skin Ageing: A Notion of Mosaic and Therapeutic Strategies. *Biochem. Pharmacol.* **2017**, *142*, 1–12. [CrossRef]
7. Malaquin, N.; Martinez, A.; Rodier, F. Keeping the Senescence Secretome under Control: Molecular Reins on the Senescence-Associated Secretory Phenotype. *Exp. Gerontol.* **2016**, *82*, 39–49. [CrossRef]
8. Nacarelli, T.; Liu, P.; Zhang, R. Epigenetic Basis of Cellular Senescence and Its Implications in Aging. *Genes* **2017**, *8*, 343. [CrossRef]
9. Coppé, J.P.; Patil, C.K.; Rodier, F.; Sun, Y.; Muñoz, D.P.; Goldstein, J.; Nelson, P.S.; Desprez, P.Y.; Campisi, J. Senescence-Associated Secretory Phenotypes Reveal Cell-Nonautonomous Functions of Oncogenic RAS and the P53 Tumor Suppressor. *PLoS Biol.* **2008**, *6*, e301. [CrossRef]
10. Birch, J.; Gil, J. Senescence and the SASP: Many Therapeutic Avenues. *Genes Dev.* **2020**, *34*, 1565–1576. [CrossRef]
11. Campisi, J.; Andersen, J.K.; Kapahi, P.; Melov, S. Cellular Senescence: A Link between Cancer and Age-Related Degenerative Disease? *Semin. Cancer Biol.* **2011**, *21*, 354. [CrossRef]
12. Yoshimoto, S.; Loo, T.M.; Atarashi, K.; Kanda, H.; Sato, S.; Oyadomari, S.; Iwakura, Y.; Oshima, K.; Morita, H.; Hattori, M.; et al. Obesity-Induced Gut Microbial Metabolite Promotes Liver Cancer through Senescence Secretome. *Nature* **2013**, *499*, 97–101. [CrossRef]
13. Krtolica, A.; Parrinello, S.; Lockett, S.; Desprez, P.Y.; Campisi, J. Senescent Fibroblasts Promote Epithelial Cell Growth and Tumorigenesis: A Link between Cancer and Aging. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 12072–12077. [CrossRef] [PubMed]
14. Muñoz-Espín, D.; Cañamero, M.; Maraver, A.; Gómez-López, G.; Contreras, J.; Murillo-Cuesta, S.; Rodríguez-Baeza, A.; Varela-Nieto, I.; Ruberte, J.; Collado, M.; et al. Programmed Cell Senescence during Mammalian Embryonic Development. *Cell* **2013**, *155*, 1104. [CrossRef] [PubMed]
15. Demaria, M.; Ohtani, N.; Youssef, S.A.; Rodier, F.; Toussaint, W.; Mitchell, J.R.; Laberge, R.M.; Vijg, J.; VanSteeg, H.; Dollé, M.E.T.; et al. An Essential Role for Senescent Cells in Optimal Wound Healing through Secretion of PDGF-AA. *Dev. Cell* **2014**, *31*, 722–733. [CrossRef]
16. Hayflick, L.; Moorhead, P.S. The Serial Cultivation of Human Diploid Cell Strains. *Exp. Cell Res.* **1961**, *25*, 585–621. [CrossRef]
17. Maciejowski, J.; De Lange, T. Telomeres in Cancer: Tumour Suppression and Genome Instability. *Nat. Rev. Mol. Cell Biol.* **2017**, *18*, 175–186. [CrossRef] [PubMed]

18. Gorgoulis, V.G.; Halazonetis, T.D. Oncogene-Induced Senescence: The Bright and Dark Side of the Response. *Curr. Opin. Cell Biol.* **2010**, *22*, 816–827. [CrossRef]
19. Ott, C.; Jung, T.; Grune, T.; Höhn, A. SIPS as a Model to Study Age-Related Changes in Proteolysis and Aggregate Formation. *Mech. Ageing Dev.* **2018**, *170*, 72–81. [CrossRef]
20. Pazolli, E.; Alspach, E.; Milczarek, A.; Prior, J.; Piwnica-Worms, D.; Stewart, S.A. Chromatin Remodeling Underlies the Senescence-Associated Secretory Phenotype of Tumor Stromal Fibroblasts That Supports Cancer Progression. *Cancer Res.* **2012**, *72*, 2251–2261. [CrossRef]
21. Wiley, C.D.; Velarde, M.C.; Lecot, P.; Liu, S.; Sarnoski, E.A.; Freund, A.; Shirakawa, K.; Lim, H.W.; Davis, S.S.; Ramanathan, A.; et al. Mitochondrial Dysfunction Induces Senescence with a Distinct Secretory Phenotype. *Cell Metab.* **2016**, *23*, 303. [CrossRef]
22. Gorgoulis, V.; Adams, P.D.; Alimonti, A.; Bennett, D.C.; Bischof, O.; Bishop, C.; Campisi, J.; Collado, M.; Evangelou, K.; Ferbeyre, G.; et al. Cellular Senescence: Defining a Path Forward. *Cell* **2019**, *179*, 813–827. [CrossRef] [PubMed]
23. Fujimaki, K.; Yao, G. Cell Dormancy Plasticity: Quiescence Deepens into Senescence through a Dimmer Switch. *Physiol. Genomics* **2020**, *52*, 558–562. [CrossRef]
24. Sen, P.; Shah, P.P.; Nativio, R.; Berger, S.L. Epigenetic Mechanisms of Longevity and Aging. *Cell* **2016**, *166*, 822–839. [CrossRef] [PubMed]
25. O'Sullivan, R.J.; Kubicek, S.; Schreiber, S.L.; Karlseder, J. Reduced Histone Biosynthesis and Chromatin Changes Arising from a Damage Signal at Telomeres. *Nat. Struct. Mol. Biol.* **2010**, *17*, 1218–1225. [CrossRef]
26. Rai, T.S.; Cole, J.J.; Nelson, D.M.; Dikovskaya, D.; Faller, W.J.; Vizioli, M.G.; Hewitt, R.N.; Anannya, O.; McBryan, T.; Manoharan, I.; et al. HIRA Orchestrates a Dynamic Chromatin Landscape in Senescence and Is Required for Suppression of Neoplasia. *Genes Dev.* **2014**, *28*, 2712–2725. [CrossRef]
27. Schmeer, C.; Kretz, A.; Wengerodt, D.; Stojiljkovic, M.; Witte, O.W. Dissecting Aging and Senescence—Current Concepts and Open Lessons. *Cells* **2019**, *8*, 1446. [CrossRef]
28. Sidler, C.; Kovalchuk, O.; Kovalchuk, I. Epigenetic Regulation of Cellular Senescence and Aging. *Front. Genet.* **2017**, *8*, 138. [CrossRef] [PubMed]
29. Rodier, F.; Coppé, J.P.; Patil, C.K.; Hoeijmakers, W.A.M.; Muñoz, D.P.; Raza, S.R.; Freund, A.; Campeau, E.; Davalos, A.R.; Campisi, J. Persistent DNA Damage Signaling Triggers Senescence-Associated Inflammatory Cytokine Secretion. *Nat. Cell Biol.* **2009**, *11*, 973. [CrossRef] [PubMed]
30. Wiley, C.D.; Campisi, J. The Metabolic Roots of Senescence: Mechanisms and Opportunities for Intervention. *Nat. Metab.* **2021**, *3*, 1290–1301. [CrossRef]
31. Childs, B.G.; Baker, D.J.; Kirkland, J.L.; Campisi, J.; Deursen, J.M. Senescence and Apoptosis: Dueling or Complementary Cell Fates? *EMBO Rep.* **2014**, *15*, 1139–1153. [CrossRef]
32. Quaas, M.; Müller, G.A.; Engeland, K. P53 Can Repress Transcription of Cell Cycle Genes through a P21 WAF1/CIP1-Dependent Switch from MMB to DREAM Protein Complex Binding at CHR Promoter Elements. *Cell Cycle* **2012**, *11*, 4661–4672. [CrossRef] [PubMed]
33. Kumari, R.; Jat, P. Mechanisms of Cellular Senescence: Cell Cycle Arrest and Senescence Associated Secretory Phenotype. *Front. Cell Dev. Biol.* **2021**, *9*, 485. [CrossRef] [PubMed]
34. Halkoum, R.; Salnot, V.; Capallere, C.; Plaza, C.; L'honoré, A.; Pays, K.; Friguet, B.; Nizard, C.; Petropoulos, I. Glyoxal Induces Senescence in Human Keratinocytes through Oxidative Stress and Activation of the Protein Kinase B/FOXO3a/P27KIP1 Pathway. *J. Investig. Dermatol.* **2022**, *142*, 2068–2078.e7. [CrossRef] [PubMed]
35. McDermott, M.S.J.; Conlon, N.; Browne, B.C.; Szabo, A.; Synnott, N.C.; O'brien, N.A.; Duffy, M.J.; Crown, J.; O'donovan, N. HER2-Targeted Tyrosine Kinase Inhibitors Cause Therapy-Induced-Senescence in Breast Cancer Cells. *Cancers* **2019**, *11*, 197. [CrossRef]
36. Mirzakhani, K.; Kallenbach, J.; Rasa, S.M.M.; Ribaudo, F.; Ungelenk, M.; Ehsani, M.; Gong, W.; Gassler, N.; Leeder, M.; Grimm, M.O.; et al. The Androgen Receptor—LncRNASAT1-AKT-P15 Axis Mediates Androgen-Induced Cellular Senescence in Prostate Cancer Cells. *Oncogene* **2021**, *41*, 943–959. [CrossRef]
37. Saleh, T.; Tyutyunyk-Massey, L.; Gewirtz, D.A. Tumor Cell Escape from Therapy-Induced Senescence as a Model of Disease Recurrence after Dormancy. *Cancer Res.* **2019**, *79*, 1044–1046. [CrossRef] [PubMed]
38. Martínez-Zamudio, R.I.; Stefa, A.; Nabuco Leva Ferreira Freitas, J.A.; Vasilopoulos, T.; Simpson, M.; Doré, G.; Roux, P.F.; Galan, M.A.; Chokshi, R.J.; Bischof, O.; et al. Escape from Oncogene-Induced Senescence Is Controlled by POU2F2 and Memorized by Chromatin Scars. *Cell Genom.* **2023**, *3*, 100293. [CrossRef]
39. Althubiti, M.; Lezina, L.; Carrera, S.; Jukes-Jones, R.; Giblett, S.M.; Antonov, A.; Barlev, N.; Saldanha, G.S.; Pritchard, C.A.; Cain, K.; et al. Characterization of Novel Markers of Senescence and Their Prognostic Potential in Cancer. *Cell Death Dis.* **2014**, *5*, e1528. [CrossRef]
40. Kim, K.M.; Noh, J.H.; Bodogai, M.; Martindale, J.L.; Pandey, P.R.; Yang, X.; Biragyn, A.; Abdelmohsen, K.; Gorospe, M. SCAMP4 Enhances the Senescent Cell Secretome. *Genes Dev.* **2018**, *32*, 909–914. [CrossRef]
41. Amor, C.; Feucht, J.; Leibold, J.; Ho, Y.-J.; Zhu, C.; Alonso-Curbelo, D.; Mansilla-Soto, J.; Boyer, J.A.; Li, X.; Giavridis, T.; et al. Senolytic CAR T Cells Reverse Senescence-Associated Pathologies. *Nature* **2020**, *583*, 127. [CrossRef] [PubMed]

42. Poblocka, M.; Bassey, A.L.; Smith, V.M.; Falcicchio, M.; Manso, A.S.; Althubiti, M.; Sheng, X.B.; Kyle, A.; Barber, R.; Frigerio, M.; et al. Targeted Clearance of Senescent Cells Using an Antibody-Drug Conjugate against a Specific Membrane Marker. *Sci. Rep.* **2021**, *11*, 20358. [CrossRef] [PubMed]
43. Rossi, M.; Abdelmohsen, K. The Emergence of Senescent Surface Biomarkers as Senotherapeutic Targets. *Cells* **2021**, *10*, 1740. [CrossRef] [PubMed]
44. Freund, A.; Laberge, R.M.; Demaria, M.; Campisi, J. Lamin B1 Loss Is a Senescence-Associated Biomarker. *Mol. Biol. Cell* **2012**, *23*, 2066. [CrossRef]
45. Pathak, R.U.; Soujanya, M.; Mishra, R.K. Deterioration of Nuclear Morphology and Architecture: A Hallmark of Senescence and Aging. *Ageing Res. Rev.* **2021**, *67*, 101264. [CrossRef]
46. Heckenbach, I.; Mkrtchyan, G.V.; Ben Ezra, M.; Bakula, D.; Sture Madsen, J.; Hasle Nielsen, M.; Oró, D.; Osborne, B.; Covarrubias, A.J.; Laura Idda, M.; et al. Nuclear Morphology Is a Deep Learning Biomarker of Cellular Senescence. *Nat. Aging* **2022**, *2*, 742–755. [CrossRef]
47. Abbadie, C.; Pluquet, O. Unfolded Protein Response (UPR) Controls Major Senescence Hallmarks. *Trends Biochem. Sci.* **2020**, *45*, 371–374. [CrossRef]
48. Kim, S.Y.; Yang, E.J.; Lee, S.B.; Lee, Y.S.; Cho, K.A.; Park, S.C. Global Transcriptional Downregulation of TREX and Nuclear Trafficking Machinery as Pan-Senescence Phenomena: Evidence from Human Cells and Tissues. *Exp. Mol. Med.* **2020**, *52*, 1351–1359. [CrossRef]
49. Park, S.S.; Choi, Y.W.; Kim, J.H.; Kim, H.S.; Park, T.J. Senescent Tumor Cells: An Overlooked Adversary in the Battle against Cancer. *Exp. Mol. Med.* **2021**, *53*, 1834–1841. [CrossRef] [PubMed]
50. Kaufman, R.J. Stress Signaling from the Lumen of the Endoplasmic Reticulum: Coordination of Gene Transcriptional and Translational Controls. *Genes Dev.* **1999**, *13*, 1211–1233. [CrossRef] [PubMed]
51. Pluquet, O.; Pourtier, A.; Abbadie, C. The Unfolded Protein Response and Cellular Senescence. A Review in the Theme: Cellular Mechanisms of Endoplasmic Reticulum Stress Signaling in Health and Disease. *Am. J. Physiol. Cell Physiol.* **2015**, *308*, 415–425. [CrossRef]
52. Druelle, C.; Drullion, C.; Deslé, J.; Martin, N.; Saas, L.; Cormenier, J.; Malaquin, N.; Huot, L.; Slomianny, C.; Bouali, F.; et al. ATF6α Regulates Morphological Changes Associated with Senescence in Human Fibroblasts. *Oncotarget* **2016**, *7*, 67699. [CrossRef] [PubMed]
53. Kim, H.S.; Kim, Y.; Lim, M.J.; Park, Y.-G.; Park, S.I.; Sohn, J. The P38-Activated ER Stress-ATF6α Axis Mediates Cellular Senescence. *FASEB J.* **2019**, *33*, 2422–2434. [CrossRef]
54. Wang, J.; Qi, Q.; Zhou, W.; Feng, Z.; Huang, B.; Chen, A.; Zhang, D.; Li, W.; Zhang, Q.; Jiang, Z.; et al. Inhibition of Glioma Growth by Flavokawain B Is Mediated through Endoplasmic Reticulum Stress Induced Autophagy. *Autophagy* **2018**, *14*, 2007–2022. [CrossRef]
55. Dörr, J.R.; Yu, Y.; Milanovic, M.; Beuster, G.; Zasada, C.; Däbritz, J.H.M.; Lisec, J.; Lenze, D.; Gerhardt, A.; Schleicher, K.; et al. Synthetic Lethal Metabolic Targeting of Cellular Senescence in Cancer Therapy. *Nature* **2013**, *501*, 421–425. [CrossRef]
56. Chen, H.; Ruiz, P.D.; McKimpson, W.M.; Novikov, L.; Kitsis, R.N.; Gamble, M.J. MacroH2A1 and ATM Play Opposing Roles in Paracrine Senescence and the Senescence-Associated Secretory Phenotype. *Mol. Cell* **2015**, *59*, 719–731. [CrossRef]
57. Lu, S.; Song, Y.; Luo, R.; Li, S.; Li, G.; Wang, K.; Liao, Z.; Wang, B.; Ke, W.; Xiang, Q.; et al. Ferroportin-Dependent Iron Homeostasis Protects against Oxidative Stress-Induced Nucleus Pulposus Cell Ferroptosis and Ameliorates Intervertebral Disc Degeneration In Vivo. *Oxid. Med. Cell. Longev.* **2021**, *2021*, 6670497. [CrossRef]
58. Tan, Q.; Liang, N.; Zhang, X.; Li, J. Dynamic Aging: Channeled Through Microenvironment. *Front. Physiol.* **2021**, *12*, 702276. [CrossRef] [PubMed]
59. Matos, L.; Gouveia, A.M.; Almeida, H. ER Stress Response in Human Cellular Models of Senescence. *J. Gerontol. Ser. A* **2015**, *70*, 924–935. [CrossRef] [PubMed]
60. Basisty, N.; Kale, A.; Jeon, O.H.; Kuehnemann, C.; Payne, T.; Rao, C.; Holtz, A.; Shah, S.; Sharma, V.; Ferrucci, L.; et al. A Proteomic Atlas of Senescence-Associated Secretomes for Aging Biomarker Development. *PLoS Biol.* **2020**, *18*, e3000599. [CrossRef]
61. Wiley, C.D.; Liu, S.; Limbad, C.; Zawadzka, A.M.; Beck, J.; Demaria, M.; Artwood, R.; Alimirah, F.; Lopez-Dominguez, J.A.; Kuehnemann, C.; et al. SILAC Analysis Reveals Increased Secretion of Hemostasis-Related Factors by Senescent Cells. *Cell Rep.* **2019**, *28*, 3329–3337.e5. [CrossRef] [PubMed]
62. Ni, C.; Narzt, M.S.; Nagelreiter, I.M.; Zhang, C.F.; Larue, L.; Rossiter, H.; Grillari, J.; Tschachler, E.; Gruber, F. Autophagy Deficient Melanocytes Display a Senescence Associated Secretory Phenotype That Includes Oxidized Lipid Mediators. *Int. J. Biochem. Cell Biol.* **2016**, *81*, 375–382. [CrossRef] [PubMed]
63. Narzt, M.S.; Pils, V.; Kremslehner, C.; Nagelreiter, I.M.; Schosserer, M.; Bessonova, E.; Bayer, A.; Reifschneider, R.; Terlecki-Zaniewicz, L.; Waidhofer-Söllner, P.; et al. Epilipidomics of Senescent Dermal Fibroblasts Identify Lysophosphatidylcholines as Pleiotropic Senescence-Associated Secretory Phenotype (SASP) Factors. *J. Investig. Dermatol.* **2021**, *141*, 993–1006.e15. [CrossRef]
64. Wiley, C.D.; Sharma, R.; Davis, S.S.; Lopez-Dominguez, J.A.; Mitchell, K.P.; Wiley, S.; Alimirah, F.; Kim, D.E.; Payne, T.; Rosko, A.; et al. Oxylipin Biosynthesis Reinforces Cellular Senescence and Allows Detection of Senolysis. *Cell Metab.* **2021**, *33*, 1124–1136.e5. [CrossRef] [PubMed]

65. Hattori, H.; Takaoka, K.; Ueta, M.; Oshitani, M.; Tamaoka, J.; Noguchi, K.; Kishimoto, H. Senescent RAW264.7 Cells Exhibit Increased Production of Nitric Oxide and Release Inducible Nitric Oxide Synthase in Exosomes. *Mol. Med. Rep.* **2021**, *24*, 1–9. [CrossRef]
66. Takasugi, M. Emerging Roles of Extracellular Vesicles in Cellular Senescence and Aging. *Aging Cell* **2018**, *17*, e12734. [CrossRef]
67. Abels, E.R.; Breakefield, X.O. Introduction to Extracellular Vesicles: Biogenesis, RNA Cargo Selection, Content, Release, and Uptake. *Cell. Mol. Neurobiol.* **2016**, *36*, 301–312. [CrossRef]
68. Wallis, R.; Mizen, H.; Bishop, C.L. The Bright and Dark Side of Extracellular Vesicles in the Senescence-Associated Secretory Phenotype. *Mech. Ageing Dev.* **2020**, *189*, 111263. [CrossRef]
69. Lee, A.H.; Ghosh, D.; Koh, I.L.; Dawson, M.R. Senescence-Associated Exosomes Transfer MiRNA-Induced Fibrosis to Neighboring Cells. *Aging* **2023**, *15*, 1237–1256. [CrossRef]
70. Bauwens, E.; Parée, T.; Meurant, S.; Bouriez, I.; Hannart, C.; Wéra, A.-C.; Khelfi, A.; Fattaccioli, A.; Burteau, S.; Demazy, C.; et al. Senescence Induced by UVB in Keratinocytes Impairs Amino Acids Balance. *J. Investig. Dermatol.* **2022**, *143*, 554–565.e9. [CrossRef]
71. Valerio, H.P.; Ravagnani, F.G.; Ronsein, G.E.; Di Mascio, P. A Single Dose of Ultraviolet-A Induces Proteome Remodeling and Senescence in Primary Human Keratinocytes. *Sci. Rep.* **2021**, *11*, 23355. [CrossRef] [PubMed]
72. Özcan, S.; Alessio, N.; Acar, M.B.; Mert, E.; Omerli, F.; Peluso, G.; Galderisi, U. Unbiased Analysis of Senescence Associated Secretory Phenotype (SASP) to Identify Common Components Following Different Genotoxic Stresses. *Aging* **2016**, *8*, 1316. [CrossRef] [PubMed]
73. Hernandez-Segura, A.; de Jong, T.V.; Melov, S.; Guryev, V.; Campisi, J.; Demaria, M. Unmasking Transcriptional Heterogeneity in Senescent Cells. *Curr. Biol.* **2017**, *27*, 2652. [CrossRef]
74. Kim, Y.M.; Byun, H.O.; Jee, B.A.; Cho, H.; Seo, Y.H.; Kim, Y.S.; Park, M.H.; Chung, H.Y.; Woo, H.G.; Yoon, G. Implications of Time-Series Gene Expression Profiles of Replicative Senescence. *Aging Cell* **2013**, *12*, 622–634. [CrossRef]
75. Yao, X.; Li, H.; Chen, L.; Tan, L.P. UV-Induced Senescence of Human Dermal Fibroblasts Restrained by Low-Stiffness Matrix by Inhibiting NF-KB Activation. *Eng. Regen.* **2022**, *3*, 365–373. [CrossRef]
76. Toutfaire, M.; Dumortier, E.; Fattaccioli, A.; Van Steenbrugge, M.; Proby, C.M.; Debacq-Chainiaux, F. Unraveling the Interplay between Senescent Dermal Fibroblasts and Cutaneous Squamous Cell Carcinoma Cell Lines at Different Stages of Tumorigenesis. *Int. J. Biochem. Cell Biol.* **2018**, *98*, 113–126. [CrossRef]
77. Malaquin, N.; Olivier, M.; Martinez, A.; Nadeau, S.; Sawchyn, C.; Coppé, J.; Cardin, G.; Mallette, F.A.; Campisi, J.; Rodier, F. Non-canonical ATM/MRN Activities Temporally Define the Senescence Secretory Program. *EMBO Rep.* **2020**, *21*, e50718. [CrossRef]
78. Buj, R.; Leon, K.E.; Anguelov, M.A.; Aird, K.M. Suppression of P16 Alleviates the Senescence-Associated Secretory Phenotype. *Aging* **2021**, *13*, 3290–3312. [CrossRef] [PubMed]
79. Acosta, J.C.; O'Loghlen, A.; Banito, A.; Guijarro, M.V.; Augert, A.; Raguz, S.; Fumagalli, M.; Da Costa, M.; Brown, C.; Popov, N.; et al. Chemokine Signaling via the CXCR2 Receptor Reinforces Senescence. *Cell* **2008**, *133*, 1006–1018. [CrossRef] [PubMed]
80. Kang, C.; Xu, Q.; Martin, T.D.; Li, M.Z.; Demaria, M.; Aron, L.; Lu, T.; Yankner, B.A.; Campisi, J.; Elledge, S.J. The DNA Damage Response Induces Inflammation and Senescence by Inhibiting Autophagy of GATA4. *Science* **2015**, *349*, aaa5612. [CrossRef]
81. Hoare, M.; Ito, Y.; Kang, T.W.; Weekes, M.P.; Matheson, N.J.; Patten, D.A.; Shetty, S.; Parry, A.J.; Menon, S.; Salama, R.; et al. NOTCH1 Mediates a Switch between Two Distinct Secretomes during Senescence. *Nat. Cell Biol.* **2016**, *18*, 979. [CrossRef]
82. Toso, A.; Revandkar, A.; DiMitri, D.; Guccini, I.; Proietti, M.; Sarti, M.; Pinton, S.; Zhang, J.; Kalathur, M.; Civenni, G.; et al. Enhancing Chemotherapy Efficacy in Pten-Deficient Prostate Tumors by Activating the Senescence-Associated Antitumor Immunity. *Cell Rep.* **2014**, *9*, 75–89. [CrossRef]
83. Xu, M.; Tchkonia, T.; Ding, H.; Ogrodnik, M.; Lubbers, E.R.; Pirtskhalava, T.; White, T.A.; Johnson, K.O.; Stout, M.B.; Mezera, V.; et al. JAK Inhibition Alleviates the Cellular Senescence-Associated Secretory Phenotype and Frailty in Old Age. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E6301–E6310. [CrossRef] [PubMed]
84. Glück, S.; Guey, B.; Gulen, M.F.; Wolter, K.; Kang, T.W.; Schmacke, N.A.; Bridgeman, A.; Rehwinkel, J.; Zender, L.; Ablasser, A. Innate Immune Sensing of Cytosolic Chromatin Fragments through CGAS Promotes Senescence. *Nat. Cell Biol.* **2017**, *19*, 1061–1070. [CrossRef] [PubMed]
85. Dou, Z.; Ghosh, K.; Vizioli, M.G.; Zhu, J.; Sen, P.; Wangensteen, K.J.; Simithy, J.; Lan, Y.; Lin, Y.; Zhou, Z.; et al. Cytoplasmic Chromatin Triggers Inflammation in Senescence and Cancer. *Nature* **2017**, *550*, 402–406. [CrossRef] [PubMed]
86. Sadaie, M.; Salama, R.; Carroll, T.; Tomimatsu, K.; Chandra, T.; Young, A.R.J.; Narita, M.; Pérez-Mancera, P.A.; Bennett, D.C.; Chong, H.; et al. Redistribution of the Lamin B1 Genomic Binding Profile Affects Rearrangement of Heterochromatic Domains and SAHF Formation during Senescence. *Genes Dev.* **2013**, *27*, 1800–1808. [CrossRef] [PubMed]
87. Gonçalves, S.; Yin, K.; Ito, Y.; Chan, A.; Olan, I.; Gough, S.; Cassidy, L.; Serrao, E.; Smith, S.; Young, A.; et al. COX2 Regulates Senescence Secretome Composition and Senescence Surveillance through PGE2. *Cell Rep.* **2021**, *34*, 108860. [CrossRef]
88. Alspach, E.; Flanagan, K.C.; Luo, X.; Ruhland, M.K.; Huang, H.; Pazolli, E.; Donlin, M.J.; Marsh, T.; Piwnica-Worms, D.; Monahan, J.; et al. P38MAPK Plays a Crucial Role in Stromal-Mediated Tumorigenesis. *Cancer Discov.* **2014**, *4*, 716–729. [CrossRef]
89. Freund, A.; Patil, C.K.; Campisi, J. P38MAPK Is a Novel DNA Damage Response-Independent Regulator of the Senescence-Associated Secretory Phenotype. *EMBO J.* **2011**, *30*, 1536–1548. [CrossRef]

90. Herranz, N.; Gallage, S.; Mellone, M.; Wuestefeld, T.; Klotz, S.; Hanley, C.J.; Raguz, S.; Acosta, J.C.; Innes, A.J.; Banito, A.; et al. MTOR Regulates MAPKAPK2 Translation to Control the Senescence-Associated Secretory Phenotype. *Nat. Cell Biol.* **2015**, *17*, 1205–1217. [CrossRef]
91. Machado-Oliveira, G.; Ramos, C.; Marques, A.R.A.; Vieira, O.V. Cell Senescence, Multiple Organelle Dysfunction and Atherosclerosis. *Cells* **2020**, *9*, 2146. [CrossRef] [PubMed]
92. Wang, Y.; Liu, L.; Song, Y.; Yu, X.; Deng, H. Unveiling E2F4, TEAD1 and AP-1 as Regulatory Transcription Factors of the Replicative Senescence Program by Multi-Omics Analysis. *Protein Cell* **2022**, *13*, 742–759. [CrossRef] [PubMed]
93. Martínez-Zamudio, R.I.; Roux, P.F.; de Freitas, J.A.N.L.F.; Robinson, L.; Doré, G.; Sun, B.; Belenki, D.; Milanovic, M.; Herbig, U.; Schmitt, C.A.; et al. AP-1 Imprints a Reversible Transcriptional Programme of Senescent Cells. *Nat. Cell Biol.* **2020**, *22*, 842–855. [CrossRef] [PubMed]
94. Coppé, J.P.; Desprez, P.Y.; Krtolica, A.; Campisi, J. The Senescence-Associated Secretory Phenotype: The Dark Side of Tumor Suppression. *Annu. Rev. Pathol.* **2010**, *5*, 99. [CrossRef]
95. Contrepois, K.; Coudereau, C.; Benayoun, B.A.; Schuler, N.; Roux, P.F.; Bischof, O.; Courbeyrette, R.; Carvalho, C.; Thuret, J.Y.; Ma, Z.; et al. Histone Variant H2A.J Accumulates in Senescent Cells and Promotes Inflammatory Gene Expression. *Nat. Commun.* **2017**, *8*, 14995. [CrossRef] [PubMed]
96. Davalos, A.R.; Kawahara, M.; Malhotra, G.K.; Schaum, N.; Huang, J.; Ved, U.; Beausejour, C.M.; Coppe, J.P.; Rodoer, F.; Campisi, J. P53-Dependent Release of Alarmin HMGB1 Is a Central Mediator of Senescent Phenotypes. *J. Cell Biol.* **2013**, *201*, 613–629. [CrossRef]
97. Aird, K.M.; Iwasaki, O.; Kossenkov, A.V.; Tanizawa, H.; Fatkhutdinov, N.; Bitler, B.G.; Le, L.; Alicea, G.; Yang, T.L.; Johnson, F.B.; et al. HMGB2 Orchestrates the Chromatin Landscape of Senescence-Associated Secretory Phenotype Gene Loci. *J. Cell Biol.* **2016**, *215*, 325–334. [CrossRef]
98. Pluquet, O.; Abbadie, C. Cellular Senescence and Tumor Promotion: Role of the Unfolded Protein Response. *Adv. Cancer Res.* **2021**, *150*, 285–334. [CrossRef]
99. Dandekar, A.; Mendez, R.; Zhang, K. Cross Talk between ER Stress, Oxidative Stress, and Inflammation in Health and Disease. *Methods Mol. Biol.* **2015**, *1292*, 205–214. [CrossRef]
100. Schmitz, R.; Wright, G.W.; Huang, D.W.; Johnson, C.A.; Phelan, J.D.; Wang, J.Q.; Roulland, S.; Kasbekar, M.; Young, R.M.; Shaffer, A.L.; et al. Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma. *N. Engl. J. Med.* **2018**, *378*, 1396. [CrossRef]
101. Smith, H.L.; Freeman, O.J.; Butcher, A.J.; Holmqvist, S.; Humoud, I.; Schätzl, T.; Hughes, D.T.; Verity, N.C.; Swinden, D.P.; Hayes, J.; et al. Astrocyte Unfolded Protein Response Induces a Specific Reactivity State That Causes Non-Cell-Autonomous Neuronal Degeneration. *Neuron* **2020**, *105*, 855–866.e5. [CrossRef] [PubMed]
102. Logue, S.E.; McGrath, E.P.; Cleary, P.; Greene, S.; Mnich, K.; Almanza, A.; Chevet, E.; Dwyer, R.M.; Oommen, A.; Legembre, P.; et al. Inhibition of IRE1 RNase Activity Modulates the Tumor Cell Secretome and Enhances Response to Chemotherapy. *Nat. Commun.* **2018**, *9*, 3267. [CrossRef]
103. Rubio-Patiño, C.; Bossowski, J.P.; Chevet, E.; Ricci, J.E. Reshaping the Immune Tumor Microenvironment Through IRE1 Signaling. *Trends Mol. Med.* **2018**, *24*, 607–614. [CrossRef]
104. Zhu, H.; Chen, X.; Chen, B.; Chen, B.; Song, W.; Sun, D.; Zhao, Y. Activating Transcription Factor 4 Promotes Esophageal Squamous Cell Carcinoma Invasion and Metastasis in Mice and Is Associated with Poor Prognosis in Human Patients. *PLoS ONE* **2014**, *9*, e103882. [CrossRef]
105. Despres, J.; Ramdani, Y.; di Giovanni, M.; Bénard, M.; Zahid, A.; Montero-Hadjadje, M.; Yvergnaux, F.; Saguet, T.; Driouich, A.; Follet-Gueye, M.L. Replicative Senescence of Human Dermal Fibroblasts Affects Structural and Functional Aspects of the Golgi Apparatus. *Exp. Dermatol.* **2019**, *28*, 922–932. [CrossRef]
106. Cho, J.H.; Saini, D.K.; Karunarathne, W.K.A.; Kalyanaraman, V.; Gautam, N. Alteration of Golgi Structure in Senescent Cells and Its Regulation by a G Protein γ Subunit. *Cell. Signal* **2011**, *23*, 785–793. [CrossRef]
107. Udono, M.; Fujii, K.; Harada, G.; Tsuzuki, Y.; Kadooka, K.; Zhang, P.; Fujii, H.; Amano, M.; Nishimura, S.I.; Tashiro, K.; et al. Impaired ATP6V0A2 Expression Contributes to Golgi Dispersion and Glycosylation Changes in Senescent Cells. *Sci. Rep.* **2015**, *5*, 6–10. [CrossRef]
108. Yuanyuan, S.; Sun, Z.; Xu, C.; Li, G. Protein Kinase D1-Mediated Classical Protein Secretory Pathway Regulates Oncogene Ras-Induced Senescent Response. *Artic. J. Cell Sci.* **2018**, *131*, jcs207217. [CrossRef]
109. Rovira, M.; Sereda, R.; Pladevall-Morera, D.; Ramponi, V.; Marin, I.; Maus, M.; Madrigal-Matute, J.; Díaz, A.; García, F.; Muñoz, J.; et al. The Lysosomal Proteome of Senescent Cells Contributes to the Senescence Secretome. *Aging Cell* **2022**, *21*, e13707. [CrossRef] [PubMed]
110. Takahashi, A.; Okada, R.; Nagao, K.; Kawamata, Y.; Hanyu, A.; Yoshimoto, S.; Takasugi, M.; Watanabe, S.; Kanemaki, M.T.; Obuse, C.; et al. ARTICLE Exosomes Maintain Cellular Homeostasis by Excreting Harmful DNA from Cells. *Nat. Commun.* **2017**, *8*, 15287. [CrossRef] [PubMed]
111. Ostrowski, M.; Carmo, N.B.; Krumeich, S.; Fanget, I.; Raposo, G.; Savina, A.; Moita, C.F.; Schauer, K.; Hume, A.N.; Freitas, R.P.; et al. Rab27a and Rab27b Control Different Steps of the Exosome Secretion Pathway. *Nat. Cell Biol.* **2010**, *12*, 19–30. [CrossRef]

112. Hitomi, K.; Okada, R.; Loo, T.M.; Miyata, K.; Nakamura, A.J.; Takahashi, A. DNA Damage Regulates Senescence-Associated Extracellular Vesicle Release via the Ceramide Pathway to Prevent Excessive Inflammatory Responses. *Int. J. Mol. Sci.* **2020**, *21*, 3720. [CrossRef]
113. Misawa, T.; Tanaka, Y.; Okada, R.; Takahashi, A. Biology of Extracellular Vesicles Secreted from Senescent Cells as Senescence-Associated Secretory Phenotype Factors. *Geriatr. Gerontol. Int.* **2020**, *20*, 539–546. [CrossRef]
114. Tuttle, C.S.L.; Waaijer, M.E.C.; Slee-Valentijn, M.S.; Stijnen, T.; Westendorp, R.; Maier, A.B. Cellular Senescence and Chronological Age in Various Human Tissues: A Systematic Review and Meta-Analysis. *Aging Cell.* **2020**, *19*, e13083. [CrossRef]
115. Song, S.; Lam, E.W.F.; Tchkonia, T.; Kirkland, J.L.; Sun, Y. Senescent Cells: Emerging Targets for Human Aging and Age-Related Diseases. *Trends Biochem. Sci.* **2020**, *45*, 578–592. [CrossRef] [PubMed]
116. Kaur, G.; Sundar, I.K.; Rahman, I. P16-3MR: A Novel Model to Study Cellular Senescence in Cigarette Smoke-Induced Lung Injuries. *Int. J. Mol. Sci.* **2021**, *22*, 4834. [CrossRef] [PubMed]
117. Baker, D.J.; Wijshake, T.; Tchkonia, T.; Lebrasseur, N.K.; Childs, B.G.; Van De Sluis, B.; Kirkland, J.L.; Van Deursen, J.M. Clearance of P16 Ink4a-Positive Senescent Cells Delays Ageing-Associated Disorders. *Nature* **2011**, *479*, 232–236. [CrossRef]
118. Baker, D.J.; Childs, B.G.; Durik, M.; Wijers, M.E.; Sieben, C.J.; Zhong, J.; Saltness, R.A.; Jeganathan, K.B.; Casaclang Verzosa, G.; Pezeshki, A.; et al. Naturally Occurring P16 Ink4a-Positive Cells Shorten Healthy Lifespan. *Nature* **2016**, *530*, 184–189. [CrossRef] [PubMed]
119. Chang, J.; Wang, Y.; Shao, L.; Laberge, R.M.; Demaria, M.; Campisi, J.; Janakiraman, K.; Sharpless, N.E.; Ding, S.; Feng, W.; et al. Clearance of Senescent Cells by ABT263 Rejuvenates Aged Hematopoietic Stem Cells in Mice. *Nat. Med.* **2016**, *22*, 78–83. [CrossRef]
120. Baar, M.P.; Brandt, R.M.C.; Putavet, D.A.; Klein, J.D.D.; Derks, K.W.J.; Bourgeois, B.R.M.; Stryeck, S.; Rijksen, Y.; van Willigenburg, H.; Feijtel, D.A.; et al. Targeted Apoptosis of Senescent Cells Restores Tissue Homeostasis in Response to Chemotoxicity and Aging. *Cell* **2017**, *169*, 132–147.e16. [CrossRef]
121. Beharka, A.A.; Meydani, M.; Wu, D.; Leka, L.S.; Meydani, A.; Meydani, S.N. Interleukin-6 Production Does Not Increase with Age. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **2001**, *56*, B81–B88. [CrossRef]
122. Jeon, O.H.; Mehdipour, M.; Gil, T.H.; Kang, M.; Aguirre, N.W.; Robinson, Z.R.; Kato, C.; Etienne, J.; Lee, H.G.; Alimirah, F.; et al. Systemic Induction of Senescence in Young Mice after Single Heterochronic Blood Exchange. *Nat. Metab.* **2022**, *4*, 995–1006. [CrossRef]
123. Markov, N.T.; Lindbergh, C.A.; Staffaroni, A.M.; Perez, K.; Stevens, M.; Nguyen, K.; Murad, N.F.; Fonseca, C.; Campisi, J.; Kramer, J.F.D. Age-Related Brain Atrophy Is Not a Homogenous Process: Different Functional Brain Networks Associate Differentially with Aging and Blood Factors. *Proc. Natl. Acad. Sci. USA* **2017**, *120*, 2017. [CrossRef]
124. Zhu, Y.; Prata, L.G.P.L.; Gerdes, E.O.W.; Netto, J.M.E.; Pirtskhalava, T.; Giorgadze, N.; Tripathi, U.; Inman, C.L.; Johnson, K.O.; Xue, A.; et al. Orally-Active, Clinically-Translatable Senolytics Restore α-Klotho in Mice and Humans. *eBioMedicine* **2022**, *77*, 103912. [CrossRef]
125. Hickson, L.T.J.; Langhi Prata, L.G.P.; Bobart, S.A.; Evans, T.K.; Giorgadze, N.; Hashmi, S.K.; Herrmann, S.M.; Jensen, M.D.; Jia, Q.; Jordan, K.L.; et al. Senolytics Decrease Senescent Cells in Humans: Preliminary Report from a Clinical Trial of Dasatinib plus Quercetin in Individuals with Diabetic Kidney Disease. *eBioMedicine* **2019**, *47*, 446–456. [CrossRef]
126. Hoare, M.; Narita, M. The Power Behind the Throne: Senescence and the Hallmarks of Cancer. *Annu. Rev. Cancer Biol.* **2018**, *2*, 175–194. [CrossRef]
127. Quan, T.; Fisher, G.J. Role of Age-Associated Alterations of the Dermal Extracellular Matrix Microenvironment in Human Skin Aging. *Gerontology* **2015**, *61*, 427. [CrossRef] [PubMed]
128. Frantz, C.; Stewart, K.M.; Weaver, V.M. The Extracellular Matrix at a Glance. *J. Cell Sci.* **2010**, *123*, 4195–4200. [CrossRef]
129. Terlecki-Zaniewicz, L.; Pils, V.; Bobbili, M.R.; Lämmermann, I.; Perrotta, I.; Grillenberger, T.; Schwestka, J.; Weiß, K.; Pum, D.; Arcalis, E.; et al. Extracellular Vesicles in Human Skin: Cross-Talk from Senescent Fibroblasts to Keratinocytes by MiRNAs. *J. Investig. Dermatol.* **2019**, *139*, 2425–2436.e5. [CrossRef] [PubMed]
130. Jun, J.-I.; Lau, L.F. The Matricellular Protein CCN1/CYR61 Induces Fibroblast Senescence and Restricts Fibrosis in Cutaneous Wound Healing. *Nat. Cell Biol.* **2010**, *12*, 676. [CrossRef] [PubMed]
131. Lujambio, A.; Akkari, L.; Simon, J.; Grace, D.; Tschaharganeh, D.F.; Bolden, J.E.; Zhao, Z.; Thapar, V.; Joyce, J.A.; Krizhanovsky, V.; et al. Non-Cell-Autonomous Tumor Suppression by P53. *Cell* **2013**, *153*, 449–460. [CrossRef]
132. Grosse, L.; Wagner, N.; Emelyanov, A.; Molina, C.; Lacas-Gervais, S.; Wagner, K.D.; Bulavin, D.V. Defined P16High Senescent Cell Types Are Indispensable for Mouse Healthspan. *Cell Metab.* **2020**, *32*, 87–99.e6. [CrossRef] [PubMed]
133. Hiebert, P.; Wietecha, M.S.; Cangkrama, M.; Haertel, E.; Mavrogonatou, E.; Stumpe, M.; Steenbock, H.; Grossi, S.; Beer, H.D.; Angel, P.; et al. Nrf2-Mediated Fibroblast Reprogramming Drives Cellular Senescence by Targeting the Matrisome. *Dev. Cell* **2018**, *46*, 145–161.e10. [CrossRef] [PubMed]
134. Salamito, M.; Gillet, B.; Syx, D.; Vaganay, E.; Malbouyres, M.; Cerutti, C.; Tissot, N.; Exbrayat-Héritier, C.; Perez, P.; Jones, C.; et al. NRF2 Shortage in Human Skin Fibroblasts Dysregulates Matrisome Gene Expression and Affects Collagen Fibrillogenesis. *J. Investig. Dermatol.* **2023**, *143*, 386–397.e12. [CrossRef]
135. Stearns-Reider, K.M.; D'Amore, A.; Beezhold, K.; Rothrauff, B.; Cavalli, L.; Wagner, W.R.; Vorp, D.A.; Tsamis, A.; Shinde, S.; Zhang, C.; et al. Aging of the Skeletal Muscle Extracellular Matrix Drives a Stem Cell Fibrogenic Conversion. *Aging Cell* **2017**, *16*, 518–528. [CrossRef]

136. Castro, P.; Giri, D.; Lamb, D.; Ittmann, M. Cellular Senescence in the Pathogenesis of Benign Prostatic Hyperplasia. *Prostate* **2003**, *55*, 30–38. [CrossRef]
137. Michaloglou, C.; Vredeveld, L.C.W.; Soengas, M.S.; Denoyelle, C.; Kuilman, T.; Van Der Horst, C.M.A.M.; Majoor, D.M.; Shay, J.W.; Mooi, W.J.; Peeper, D.S. BRAFE600-Associated Senescence-like Cell Cycle Arrest of Human Naevi. *Nature* **2005**, *436*, 720–724. [CrossRef]
138. Takasugi, M.; Okada, R.; Takahashi, A.; Virya Chen, D.; Watanabe, S.; Hara, E. Small Extracellular Vesicles Secreted from Senescent Cells Promote Cancer Cell Proliferation through EphA2. *Nat. Commun.* **2017**, *8*, 15729. [CrossRef]
139. Olumi, A.; Grossfeld, G.; Hayward, S.; Carroll, P.; Cunha, G.; Hein, P.; Tlsty, T. Carcinoma-Associated Fibroblasts Direct Tumor Progression of Initiated Human Prostatic Epithelium. *Cancer Res.* **1999**, *59*, 5002–5011. [CrossRef] [PubMed]
140. Parrinello, S.; Coppe, J.P.; Krtolica, A.; Campisi, J. Stromal-Epithelial Interactions in Aging and Cancer: Senescent Fibroblasts Alter Epithelial Cell Differentiation. *J. Cell Sci.* **2005**, *118 Pt 3*, 485. [CrossRef] [PubMed]
141. Krtolica, A.; Ortiz De Solorzano, C.; Lockert, S.; Campisi, J. Quantification of Epithelial Cells in Coculture with Fibroblasts by Fluorescence Image Analysis. *Cytometry* **2002**, *49*, 73–82. [CrossRef] [PubMed]
142. Gosselin, K.; Martien, S.; Pourtier, A.; Vercamer, C.; Ostoich, P.; Morat, L.; Sabatier, L.; Duprez, L.; T'kint De Roodenbeke, C.; Gilson, E.; et al. Senescence-Associated Oxidative DNA Damage Promotes the Generation of Neoplastic Cells. *Cancer Res* **2009**, *69*, 7917–7942. [CrossRef] [PubMed]
143. Romanov, S.R.; Kozakiewicz, B.K.; Holst, C.R.; Stampfer, M.R.; Haupt, L.M.; Tlsty, T.D. Normal Human Mammary Epithelial Cells Spontaneously Escape Senescence and Acquire Genomic Changes. *Nature* **2001**, *409*, 633–637. [CrossRef]
144. Gosselin, K.; Deruy, E.; Martien, S.; Vercamer, C.; Bouali, F.; Dujardin, T.; Slomianny, C.; Houel-Renault, L.; Chelli, F.; De Launoit, Y.; et al. Senescent Keratinocytes Die by Autophagic Programmed Cell Death. *Am. J. Pathol.* **2009**, *174*, 423–435. [CrossRef] [PubMed]
145. Malaquin, N.; Vercamer, C.; Bouali, F.; Martien, S.; Deruy, E.; Wernert, N.; Chwastyniak, M.; Pinet, F.; Abbadie, C.; Pourtier, A. Senescent Fibroblasts Enhance Early Skin Carcinogenic Events via a Paracrine MMP-PAR-1 Axis. *PLoS ONE* **2013**, *8*, e63607. [CrossRef] [PubMed]
146. Martin, N.; Salazar-Cardozo, C.; Vercamer, C.; Ott, L.; Marot, G.; Slijepcevic, P.; Abbadie, C.; Pluquet, O. Identification of a Gene Signature of a Pre-Transformation Process by Senescence Evasion in Normal Human Epidermal Keratinocytes. *Mol. Cancer* **2014**, *13*, 151. [CrossRef]
147. Tinaburri, L.; Valente, C.; Teson, M.; Minafò, Y.A.; Cordisco, S.; Guerra, L.; Dellambra, E. The Secretome of Aged Fibroblasts Promotes EMT-like Phenotype in Primary Keratinocytes from Elderly Donors through BDNF-TrkB Axis. *J. Investig. Dermatol.* **2021**, *141*, 1052–1062.e12. [CrossRef]
148. Sprenger, C.C.; Plymate, S.R.; Reed, M.J. Aging-Related Alterations in the Extracellular Matrix Modulate the Microenvironment and Influence Tumor Progression. *Int. J. Cancer* **2010**, *127*, 2739–2748. [CrossRef]
149. Kang, J.; Chen, W.; Xia, J.; Li, Y.; Yang, B.; Chen, B.; Sun, W.; Song, X.; Xiang, W.; Wang, X.; et al. Extracellular Matrix Secreted by Senescent Fibroblasts Induced by UVB Promotes Cell Proliferation in HaCaT Cells through PI3K/AKT and ERK Signaling Pathways. *Int. J. Mol. Med.* **2008**, *21*, 777–784. [CrossRef] [PubMed]
150. Egeblad, M.; Rasch, M.G.; Weaver, V.M. Dynamic Interplay between the Collagen Scaffold and Tumor Evolution. *Curr. Opin. Cell Biol.* **2010**, *22*, 697–706. [CrossRef]
151. Paszek, M.J.; Zahir, N.; Johnson, K.R.; Lakins, J.N.; Rozenberg, G.I.; Gefen, A.; Reinhart-King, C.A.; Margulies, S.S.; Dembo, M.; Boettiger, D.; et al. Tensional Homeostasis and the Malignant Phenotype. *Cancer Cell* **2005**, *8*, 241–254. [CrossRef] [PubMed]
152. Faget, D.V.; Ren, Q.; Stewart, S.A. Unmasking Senescence: Context-Dependent Effects of SASP in Cancer. *Nat. Rev. Cancer* **2019**, *19*, 439–453. [CrossRef] [PubMed]
153. Kuilman, T.; Michaloglou, C.; Vredeveld, L.C.W.; Douma, S.; van Doorn, R.; Desmet, C.J.; Aarden, L.A.; Mooi, W.J.; Peeper, D.S. Oncogene-Induced Senescence Relayed by an Interleukin-Dependent Inflammatory Network. *Cell* **2008**, *133*, 1019–1031. [CrossRef] [PubMed]
154. Acosta, J.C.; Banito, A.; Wuestefeld, T.; Georgilis, A.; Janich, P.; Morton, J.P.; Athineos, D.; Kang, T.W.; Lasitschka, F.; Andrulis, M.; et al. A Complex Secretory Program Orchestrated by the Inflammasome Controls Paracrine Senescence. *Nat. Cell Biol.* **2013**, *15*, 978. [CrossRef]
155. Dickey, J.S.; Baird, B.J.; Redon, C.E.; Sokolov, M.V.; Sedelnikova, O.A.; Bonner, W.M. Intercellular Communication of Cellular Stress Monitored by γ-H2AX Induction. *Carcinogenesis* **2009**, *30*, 1686–1695. [CrossRef]
156. Borghesan, M.; Fafiá N-Labora, J.; Eleftheriadou, O.; Vossenkamper, A.; Muñ Oz-Espin, D.; O', A.; Correspondence, L. Small Extracellular Vesicles Are Key Regulators of Non-Cell Autonomous Intercellular Communication in Senescence via the Interferon Protein IFITM3. *Cell Rep.* **2019**, *27*, 3956–3971. [CrossRef]
157. Fulzele, S.; Mendhe, B.; Khayrullin, A.; Johnson, M.; Kaiser, H.; Liu, Y.; Isales, C.M.; Hamrick, M.W. Muscle-Derived MiR-34a Increases with Age in Circulating Extracellular Vesicles and Induces Senescence of Bone Marrow Stem Cells. *Aging* **2019**, *11*, 1791–1803. [CrossRef]
158. Lorenzo, E.C.; Torrance, B.L.; Keilich, S.R.; Al-Naggar, I.; Harrison, A.; Xu, M.; Bartley, J.M.; Haynes, L. Senescence-induced Changes in CD4 T Cell Differentiation Can Be Alleviated by Treatment with Senolytics. *Aging Cell* **2022**, *21*, e13525. [CrossRef]
159. Ritschka, B.; Storer, M.; Mas, A.; Heinzmann, F.; Ortells, M.C.; Morton, J.P.; Sansom, O.J.; Zender, L.; Keyes, W.M. The Senescence-Associated Secretory Phenotype Induces Cellular Plasticity and Tissue Regeneration. *Genes Dev.* **2017**, *31*, 172–183. [CrossRef]

160. Mosteiro, L.; Pantoja, C.; Alcazar, N.; Marión, R.M.; Chondronasiou, D.; Rovira, M.; Fernandez-Marcos, P.J.; Muñoz-Martin, M.; Blanco-Aparicio, C.; Pastor, J.; et al. Tissue Damage and Senescence Provide Critical Signals for Cellular Reprogramming in Vivo. *Science* **2016**, *354*, aaf4445. [CrossRef]
161. Watt, F.M.; Fujiwara, H. Cell-Extracellular Matrix Interactions in Normal and Diseased Skin. *Cold Spring Harb. Perspect. Biol.* **2011**, *3*, a005124. [CrossRef]
162. Ozcebe, S.G.; Bahcecioglu, G.; Yue, X.S.; Zorlutuna, P. Effect of Cellular and ECM Aging on Human IPSC-Derived Cardiomyocyte Performance, Maturity and Senescence. *Biomaterials* **2021**, *268*, 120554. [CrossRef]
163. Moiseeva, V.; Cisneros, A.; Sica, V.; Deryagin, O.; Lai, Y.; Jung, S.; Andrés, E.; An, J.; Segalés, J.; Ortet, L.; et al. Senescence Atlas Reveals an Aged-like Inflamed Niche That Blunts Muscle Regeneration. *Nature* **2022**, *613*, 169–178. [CrossRef] [PubMed]
164. Saul, D.; Kosinsky, R.L.; Atkinson, E.J.; Doolittle, M.L.; Zhang, X.; LeBrasseur, N.K.; Pignolo, R.J.; Robbins, P.D.; Niedernhofer, L.J.; Ikeno, Y.; et al. A New Gene Set Identifies Senescent Cells and Predicts Senescence-Associated Pathways across Tissues. *Nat. Commun.* **2022**, *13*, 4827. [CrossRef] [PubMed]
165. Zhu, Y.; Tchkonia, T.; Pirtskhalava, T.; Gower, A.C.; Ding, H.; Giorgadze, N.; Palmer, A.K.; Ikeno, Y.; Hubbard, G.B.; Lenburg, M.; et al. The Achilles' Heel of Senescent Cells: From Transcriptome to Senolytic Drugs. *Aging Cell* **2015**, *14*, 644. [CrossRef]
166. Kim, E.C.; Kim, J.R. Senotherapeutics: Emerging Strategy for Healthy Aging and Age-Related Disease. *BMB Rep.* **2019**, *52*, 47–55. [CrossRef] [PubMed]
167. Zhang, L.; Pitcher, L.E.; Prahalad, V.; Niedernhofer, L.J.; Robbins, P.D. Targeting Cellular Senescence with Senotherapeutics: Senolytics and Senomorphics. *FEBS J.* **2023**, *290*, 1362–1383. [CrossRef]
168. Niedernhofer, L.J.; Robbins, P.D. Senotherapeutics for Healthy Ageing. *Nat. Rev. Drug Discov.* **2018**, *17*, 377. [CrossRef]
169. Sun, Y.; Li, Q.; Kirkland, J.L. Targeting Senescent Cells for a Healthier Longevity: The Roadmap for an Era of Global Aging. *Life Med.* **2022**, *1*, 103–119. [CrossRef]
170. Wissler Gerdes, E.O.; Zhu, Y.; Tchkonia, T.; Kirkland, J.L. Discovery, Development, and Future Application of Senolytics: Theories and Predictions. *FEBS J.* **2020**, *287*, 2418–2427. [CrossRef] [PubMed]
171. Chaib, S.; Tchkonia, T.; Kirkland, J.L. Cellular Senescence and Senolytics: The Path to the Clinic. *Nat. Med.* **2022**, *28*, 1556–1568. [CrossRef]
172. Pignolo, R.J.; Passos, J.F.; Khosla, S.; Tchkonia, T.; Kirkland, J.L. Reducing Senescent Cell Burden in Aging and Disease. *Trends Mol. Med.* **2020**, *26*, 630–638. [CrossRef]
173. Zhang, L.; Gao, S.; White, Z.; Dai, Y.; Malik, A.B.; Rehman, J. Single-Cell Transcriptomic Profiling of Lung Endothelial Cells Identifies Dynamic Inflammatory and Regenerative Subpopulations. *JCI Insight* **2022**, *7*, 6. [CrossRef]
174. Wissler Gerdes, E.O.; Misra, A.; Netto, J.M.E.; Tchkonia, T.; Kirkland, J.L. Strategies for Late Phase Preclinical and Early Clinical Trials of Senolytics. *Mech. Ageing Dev.* **2021**, *200*, 111591. [CrossRef]
175. Kirkland, J.L.; Tchkonia, T. Senolytic Drugs: From Discovery to Translation. *J. Intern. Med.* **2020**, *288*, 518–536. [CrossRef] [PubMed]
176. Morsli, S.; Doherty, G.J.; Muñoz-Espín, D. Activatable Senoprobes and Senolytics: Novel Strategies to Detect and Target Senescent Cells. *Mech. Ageing Dev.* **2022**, *202*, 111618. [CrossRef] [PubMed]
177. Paez-Ribes, M.; González-Gualda, E.; Doherty, G.J.; Muñoz-Espín, D. Targeting Senescent Cells in Translational Medicine. *EMBO Mol. Med.* **2019**, *11*, e10234. [CrossRef] [PubMed]
178. Prattichizzo, F.; Giuliani, A.; Recchioni, R.; Bonafè, M.; Marcheselli, F.; De Carolis, S.; Campanati, A.; Giuliodori, K.; Rippo, M.R.; Brugè, F.; et al. Anti-TNF-α Treatment Modulates SASP and SASP-Related MicroRNAs in Endothelial Cells and in Circulating Angiogenic Cells. *Oncotarget* **2016**, *7*, 11945–11958. [CrossRef] [PubMed]
179. Lister, R.; Chrysovergi, M.; Lagares, D. Ephrin-B2 Neutralizing Antibody Suppresses the SASP in Senescent Fibroblasts and Treats Lung Fibrosis in Aged Mice. *Am. Thorac. Soc.* **2022**, *216*, 147059.
180. Chong, M.; Yin, T.; Chen, R.; Xiang, H.; Yuan, L.; Ding, Y.; Pan, C.C.; Tang, Z.; Alexander, P.B.; Jia, Y.; et al. CD36 Initiates the Secretory Phenotype during the Establishment of Cellular Senescence. *EMBO Rep.* **2018**, *19*, e45274. [CrossRef]
181. Orjalo, A.V.; Bhaumik, D.; Gengler, B.K.; Scott, G.K.; Campisi, J. Cell Surface-Bound IL-1α Is an Upstream Regulator of the Senescence-Associated IL-6/IL-8 Cytokine Network. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 17031–17036. [CrossRef]
182. Di Micco, R.; Krizhanovsky, V.; Baker, D.; d'Adda di Fagagna, F. Cellular Senescence in Ageing: From Mechanisms to Therapeutic Opportunities. *Nat. Rev. Mol. Cell Biol.* **2020**, *22*, 75–95. [CrossRef]
183. Lim, H.; Park, H.; Kim, H.P. Effects of Flavonoids on Senescence-Associated Secretory Phenotype Formation from Bleomycin-Induced Senescence in BJ Fibroblasts. *Biochem. Pharmacol.* **2015**, *96*, 337–348. [CrossRef] [PubMed]
184. Lim, J.S.; Lee, D.Y.; Kim, H.S.; Park, S.C.; Oh, W.K.; Cho, K.A. Identification of a novel senomorphic agent, avenanthramide C, via the suppression of the senescence-associated secretory phenotype. *Mech. Ageing Dev.* **2020**, *192*, 111355. [CrossRef]
185. Alimbetov, D.; Davis, T.; Brook, A.J.C.; Cox, L.S.; Faragher, R.G.A.; Nurgozhin, T.; Zhumadilov, Z.; Kipling, D. Suppression of the Senescence-Associated Secretory Phenotype (SASP) in Human Fibroblasts Using Small Molecule Inhibitors of P38 MAP Kinase and MK2. *Biogerontology* **2016**, *17*, 305. [CrossRef]
186. Laberge, R.M.; Zhou, L.; Sarantos, M.R.; Rodier, F.; Freund, A.; de Keizer, P.L.J.; Liu, S.; Demaria, M.; Cong, Y.S.; Kapahi, P.; et al. Glucocorticoids Suppress Selected Components of the Senescence-Associated Secretory Phenotype. *Aging Cell* **2012**, *11*, 569–578. [CrossRef] [PubMed]

187. Menicacci, B.; Cipriani, C.; Margheri, F.; Mocali, A.; Giovannelli, L. Modulation of the Senescence-Associated Inflammatory Phenotype in Human Fibroblasts by Olive Phenols. *Int. J. Mol. Sci.* **2017**, *18*, 2275. [CrossRef]
188. Chen, D.D.; Peng, X.; Wang, Y.; Jiang, M.; Xue, M.; Shang, G.; Liu, X.; Jia, X.; Liu, B.; Lu, Y.; et al. HSP90 Acts as a Senomorphic Target in Senescent Retinal Pigmental Epithelial Cells. *Aging* **2021**, *13*, 21547. [CrossRef] [PubMed]
189. Signaling, B.; Woo, J.; Shin, S.; Ji, H.; Ryu, D.; Cho, E.; Kim, Y.; Kim, J.; Park, D.; Jung, E. *Isatis Tinctoria* L. Leaf Extract Inhibits Replicative Senescence. *Nutrients* **2022**, *14*, 1979.
190. De Cecco, M.; Ito, T.; Petrashen, A.P.; Elias, A.E.; Skvir, N.J.; Criscione, S.W.; Caligiana, A.; Brocculi, G.; Adney, E.M.; Boeke, J.D.; et al. L1 Drives IFN in Senescent Cells and Promotes Age-Associated Inflammation. *Nature* **2019**, *566*, 73–78. [CrossRef]
191. Hu, Q.; Peng, J.; Jiang, L.; Li, W.; Su, Q.; Zhang, J.; Li, H.; Song, M.; Cheng, B.; Xia, J.; et al. Metformin as a Senostatic Drug Enhances the Anticancer Ef Fi Cacy of CDK4/6 Inhibitor in Head and Neck Squamous Cell Carcinoma. *Cell Death Dis.* **2020**, *11*, 925. [CrossRef]
192. Tai, S.; Sun, J.; Zhou, Y.; Zhu, Z.; He, Y.; Chen, M.; Yang, H.; Xiao, Y.; Tu, T.; Tang, L.; et al. Metformin Suppresses Vascular Smooth Muscle Cell Senescence by Promoting Autophagic Flux. *J. Adv. Res.* **2022**, *41*, 205–218. [CrossRef]
193. Matacchione, G.; Gurău, F.; Silvestrini, A.; Tiboni, M.; Mancini, L.; Valli, D.; Rippo, M.R.; Recchioni, R.; Marcheselli, F.; Carnevali, O.; et al. Anti-SASP and Anti-Inflammatory Activity of Resveratrol, Curcumin and β-Caryophyllene Association on Human Endothelial and Monocytic Cells. *Biogerontology* **2021**, *22*, 297. [CrossRef]
194. Frediani, E.; Scavone, F.; Laurenzana, A.; Chillà, A.; Tortora, K.; Cimmino, I.; Leri, M.; Bucciantini, M.; Mangoni, M.; Fibbi, G.; et al. Olive Phenols Preserve Lamin B1 Expression Reducing CGAS/STING/NF κ B-Mediated SASP in Ionizing Radiation-Induced Senescence. *J. Cell. Mol. Med.* **2022**, *26*, 2337–2350. [CrossRef]
195. Liu, S.; Uppal, H.; Demaria, M.; Desprez, P.Y.; Campisi, J.; Kapahi, P. Simvastatin Suppresses Breast Cancer Cell Proliferation Induced by Senescent Cells. *Sci. Rep.* **2015**, *5*, 17895. [CrossRef] [PubMed]
196. Laberge, R.M.; Sun, Y.; Orjalo, A.V.; Patil, C.K.; Freund, A.; Zhou, L.; Curran, S.C.; Davalos, A.R.; Wilson-Edell, K.A.; Liu, S.; et al. MTOR Regulates the Pro-Tumorigenic Senescence-Associated Secretory Phenotype by Promoting IL1A Translation. *Nat. Cell Biol.* **2015**, *17*, 1049. [CrossRef] [PubMed]
197. Wang, R.; Yu, Z.; Sunchu, B.; Shoaf, J.; Dang, I.; Zhao, S.; Caples, K.; Bradley, L.; Beaver, L.M.; Ho, E.; et al. Rapamycin Inhibits the Secretory Phenotype of Senescent Cells by a Nrf2-independent Mechanism. *Aging Cell* **2017**, *16*, 564. [CrossRef] [PubMed]
198. Csiszar, A.; Sosnowska, D.; Wang, M.; Lakatta, E.G.; Sonntag, W.E.; Ungvari, Z. Age-Associated Proinflammatory Secretory Phenotype in Vascular Smooth Muscle Cells From the Non-Human Primate Macaca Mulatta: Reversal by Resveratrol Treatment. *Journals Gerontol. Ser. A* **2012**, *67*, 811–820. [CrossRef]
199. Woo, J.; Shin, S.; Cho, E.; Ryu, D.; Garandeau, D.; Chajra, H.; Fréchet, M.; Park, D.; Jung, E. Senotherapeutic-like Effect of Silybum Marianum Flower Extract Revealed on Human Skin Cells. *PLoS ONE* **2021**, *16*, e0260545. [CrossRef] [PubMed]
200. Gao, L.; Wang, Y.H.; Liu, Z.H.; Sun, Y.; Cai, P.; Jing, Q. Identification of a Small Molecule SR9009 That Activates NRF2 to Counteract Cellular Senescence. *Aging Cell* **2021**, *20*, e13483. [CrossRef]
201. Park, M.; Na, J.; Kwak, S.Y.; Park, S.; Kim, H.; Lee, S.J.; Jang, W.S.; Lee, S.B.; Jang, W.I.; Jang, H.; et al. Zileuton Alleviates Radiation-Induced Cutaneous Ulcers via Inhibition of Senescence-Associated Secretory Phenotype in Rodents. *Int. J. Mol. Sci.* **2022**, *23*, 8390. [CrossRef] [PubMed]
202. Farr, J.N.; Xu, M.; Weivoda, M.M.; Monroe, D.G.; Fraser, D.G.; Onken, J.L.; Negley, B.A.; Sfeir, J.G.; Ogrodnik, M.B.; Hachfeld, C.M.; et al. Targeting Cellular Senescence Prevents Age-Related Bone Loss in Mice. *Nat. Med.* **2017**, *23*, 1072–1079. [CrossRef] [PubMed]
203. Hughes, B.K.; Wallis, R.; Bishop, C.L. Yearning for Machine Learning: Applications for the Classification and Characterisation of Senescence. *Cell Tissue Res.* **2023**, 1–6. [CrossRef] [PubMed]