CELLULAR SENESCENCE AND CANCER

واحد:ساز و کارهای سلولی و مولکولی سرطان

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INTRODUCTION

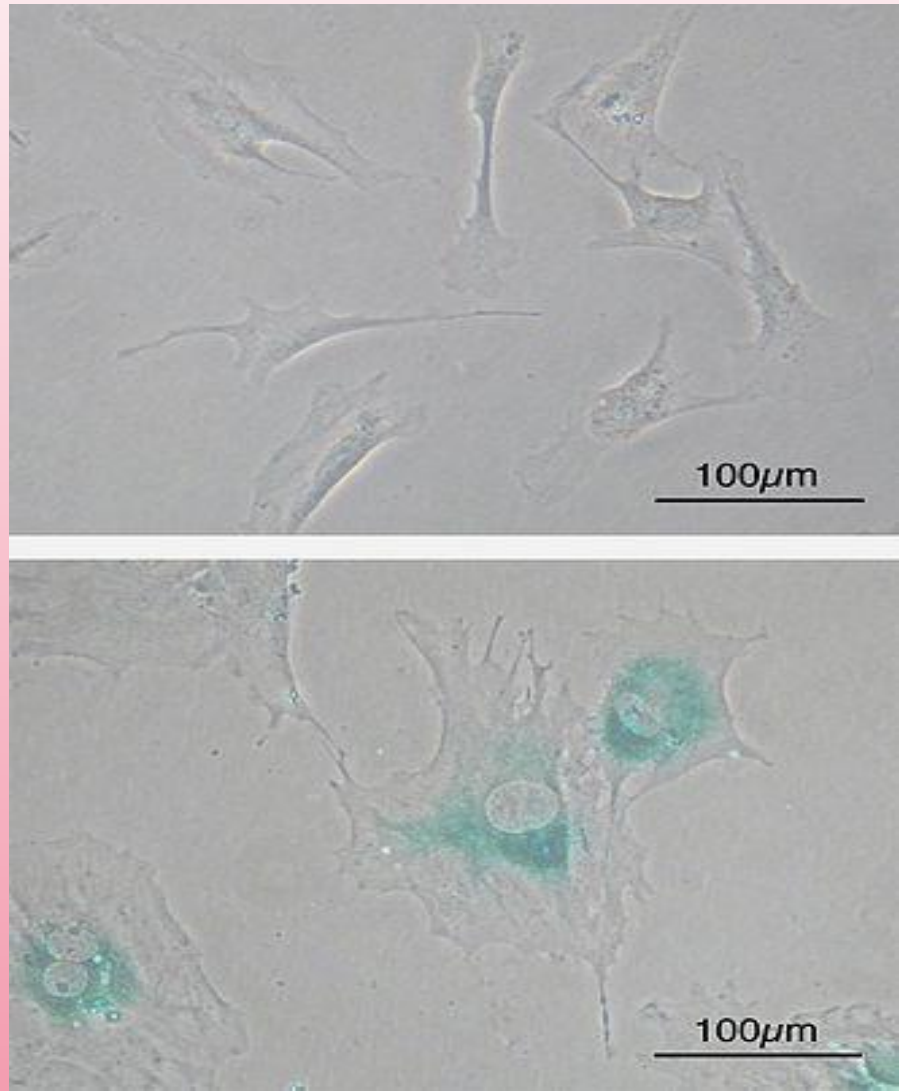
- For most species, aging promotes a host of degenerative pathologies that are characterized by debilitating losses of tissue or cellular function.
- However,especially among vertebrates, aging also promotes hyperplastic pathologies,the most deadly of which is cancer.
- In contrast to the loss of function that characterizes degenerating cells and tissues,malignant (cancerous) cells must acquire new (albeit aberrant) functions that allow them to develop into a lethal tumor.
- Despite seemingly opposite characteristics, the degenerative and hyperplastic pathologies of aging are at least partly linked by a common biological phenomenon:

a cellular stress response known as cellular senescence.

WHAT IS SENESCENCE?

- The senescence response is widely recognized as a potent tumor suppressive mechanism.

- However, recent evidence strengthens the idea that it also drives both degenerative and hyperplastic pathologies, most likely by promoting chronic inflammation.
- Thus,the senescence response may be the result of antagonistically pleiotropic gene action.



WHAT DEFINES A SENESCENT CELL?

- Permanent and naturally irreversible growth arrest at G1.
- not all senescent cells display all the senescence markers that have so far been identified. Thus, senescent cells are generally identified by a constellation of characteristics.
- a necessary but insufficient marker of senescent cells is an absence of proliferation markers.
- In addition, senescent cells generally enlarge, often doubling in volume, and, if adherent, adopt a flattened morphology.
- Histochemical staining for senescence-associated β-galactosidase (SA-Bgal) at PH6 is a commonly used marker for senescence cells. b-Galactosidase is found within lysosomes, where an acidic pH (4.0–4.5) is required for maximal enzymatic activity.pH 6 is an unfavorable condition that reduces its enzymatic activity by almost 99%.In this scenario, only cells with increased lysosomal content and therefore increased lysosomal b-galactosidase show positive staining.it was the first marker to permit the detection of senescent cells in situ in tissues, showing that senescent cells indeed increase with age in vivo.
- Another marker now used regularly to identify senescent cells in culture and tissues is the p16INK4a tumor suppressor protein. p16INK4a expression is low or undetectable in most normal cells and tissues but is readily detectable in cells induced to senesce by many stimuli.

SENESCENCE VS QUIESCENCE

- Both are forms of growth arrest, yet they differ in several important aspects:
- Quiescence usually originates from a lack of nutrition or mitogenic signals and is invariably reversible once these nutrients or signals are available again.
- In contrast, cellular senescence in general persists even after removal of the inducing stimulus,albeit senescent cancer cells may be the exception to this rule.
- resistance to apoptosis is a hallmark of cell senescence and they rely on autophagy.
- The study of cellular senescence has been addressed in three contexts:

developmentally programmed senescence (DPS),

stress-induced premature senescence (SIPS)

replicative senescence

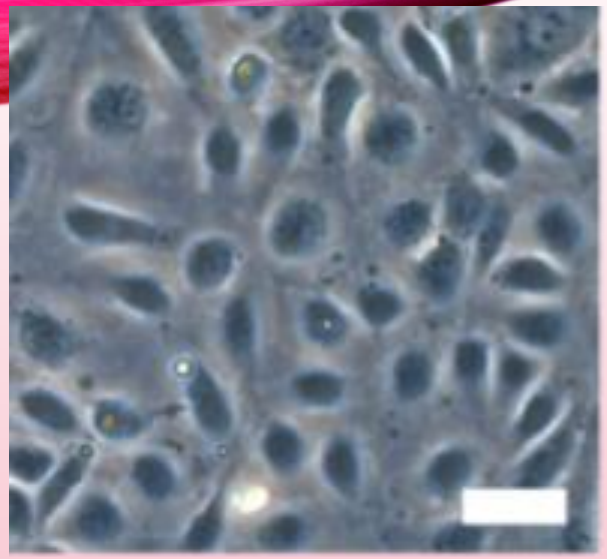
DEVELOPMENTALLY PROGRAMMED SENESCENCE

- Embryonic developmental structures of mammalians express cell senescence biomarkers, but in contrast to adult cells they mostly employ p21 as cell senescence effector and DNA damage is not required for the response to occur.
- DPS can be thought of as a primitive form of cell senescence.
- On the other side, senescent natural killer cells (NK) facilitate embryonic Implantation,they are the most abundant immune cells at the maternal/fetal interphase.
- during early pregnancy.The fetal trophoblast activates the CD158d receptor in senescent NK cells, initiating the p21 signaling pathway and inducing a secretory proteome that promotes vascular remodeling and angiogenesis.

- Other senescence markers include upregulated expression of the tumor suppressor proteins DEC1 (Deleted in Esophageal Cancer) and DcR2 (Decoy Receptor 2),both of which are targets of p53 transactivation.

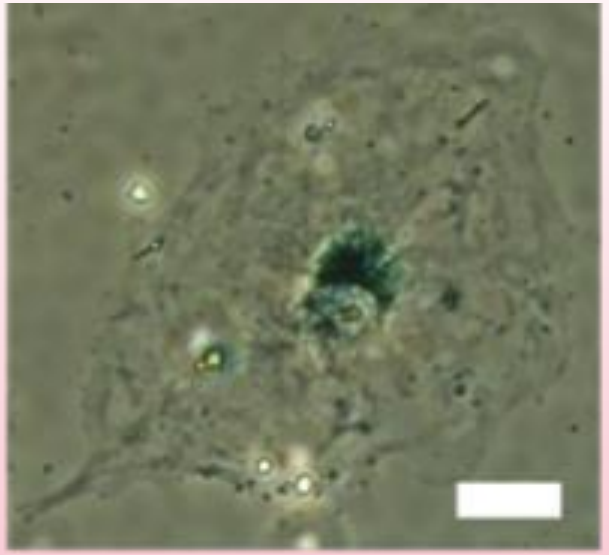
- Senescent cells also markedly downregulate expression of the nuclear lamina protein lamin B1 (LMNB1).

- These markers (and some others) are less widely used, probably because they are currently less extensively validated. DEC1 and DcR2 upregulation and LMNB1 downregulation have been validated in cultured cells and human or mouse tissues.

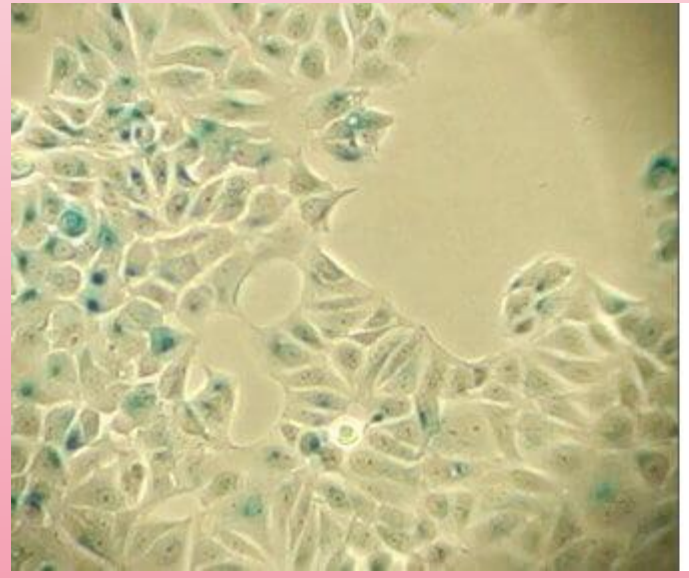


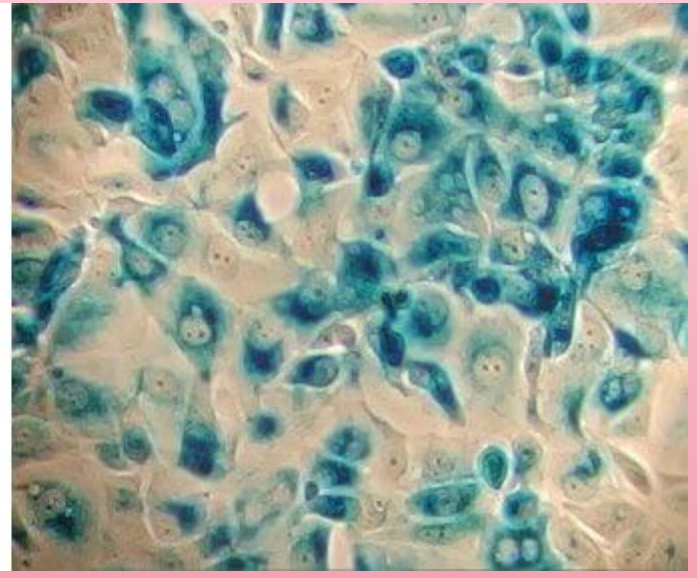
Senescence stimuli

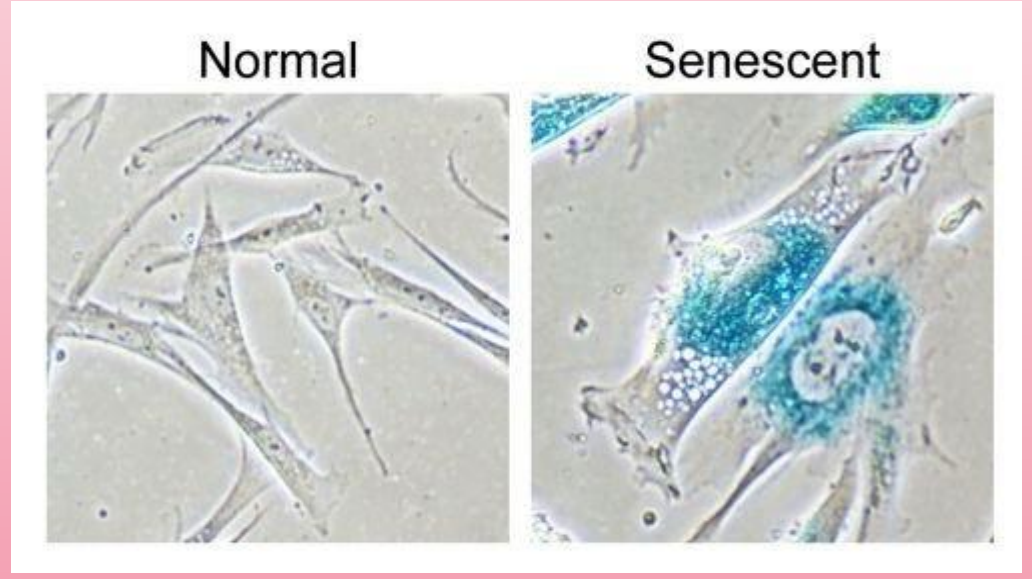
- Etoposide
- Ionizing radiation
- ROS inducers
- DNA-damaging agents



- Growth arrest
- SA-βGal
- SASP
- DNA foci
- Morphology
- Molecular profile
- Genetic profile





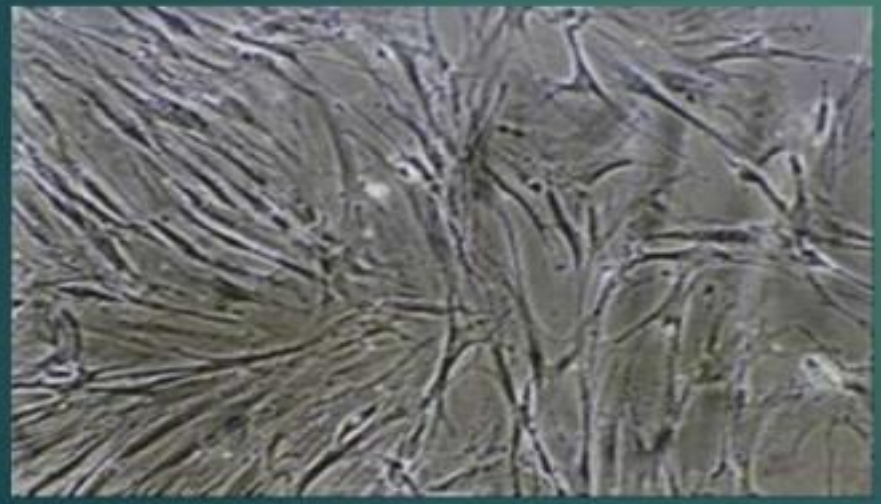


ARREST OF CELL CYCLE

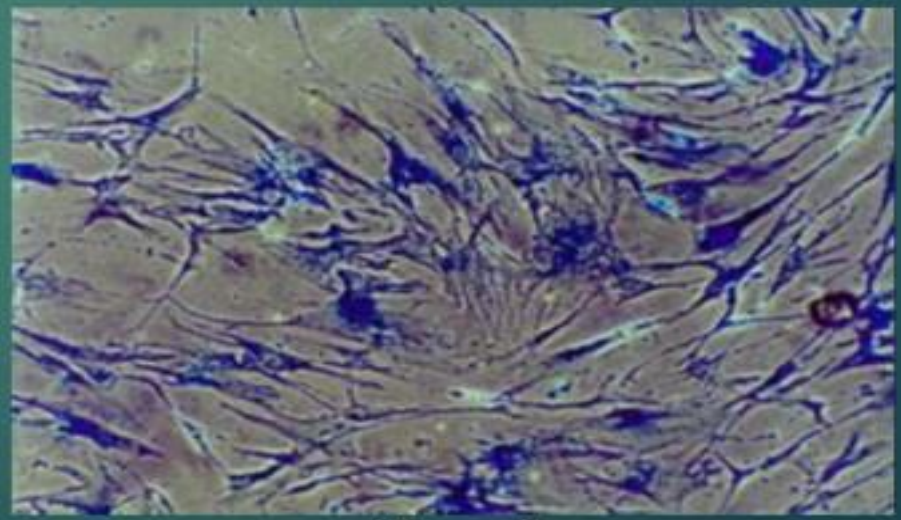
- The permanence of the senescence growth arrest enforces the idea that the senescence response evolved at least in part to suppress the development of cancer.
- The senescence arrest is considered irreversible because no known physiological stimuli can stimulate senescent cells to reenter the cell cycle. However, molecular biological manipulations, for example, the sequential inactivation of certain tumor suppressor genes, can cause senescent cells to proliferate.
- There may be as-yet-unrecognized physiological circumstances under which the senescence growth arrest is reversible.

Senescence morphology

- Senescent cells become flattened, enlarged and have increased β-galactosidase activity
- Increased size of nucleus and nucleoli
- Increased number of multinucleated cells
- Increased number of lysosomes, Golgi and cytoplasmic microfilaments



'Young'
Pre-senescent

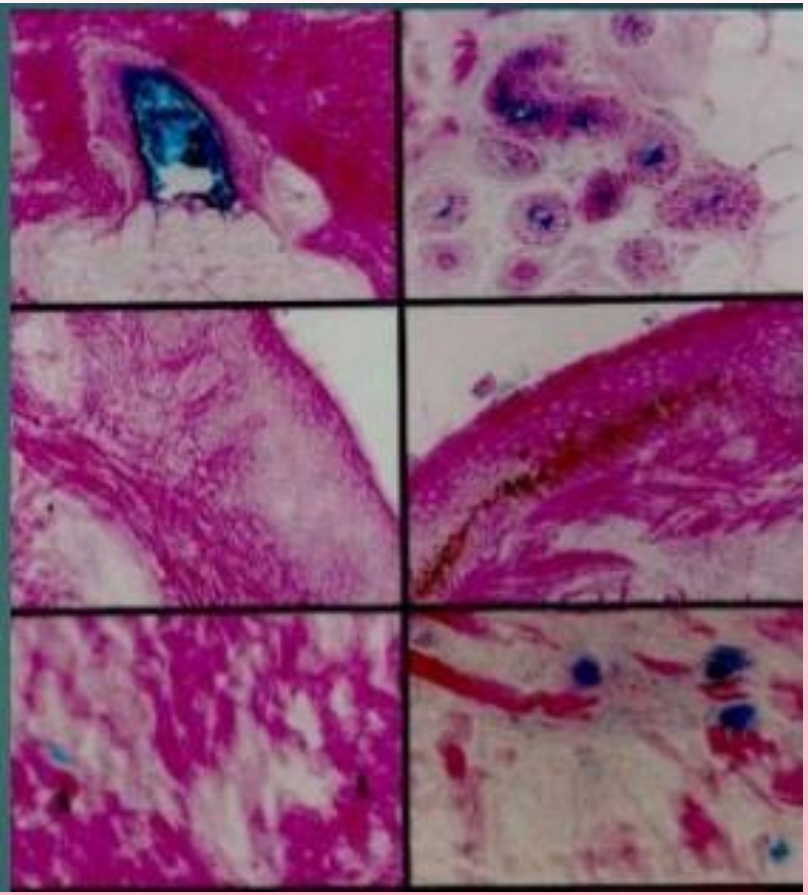


'Aged'
Senescent

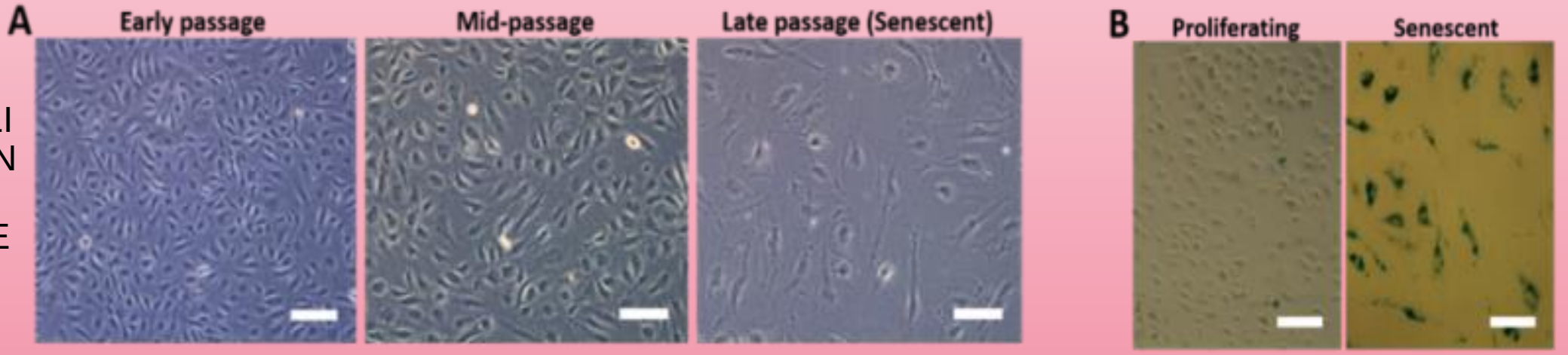
markers:

- *p16* expression
- Heterochromatic foci damage
- Telomeric-DNA damage
- DNA damage foci

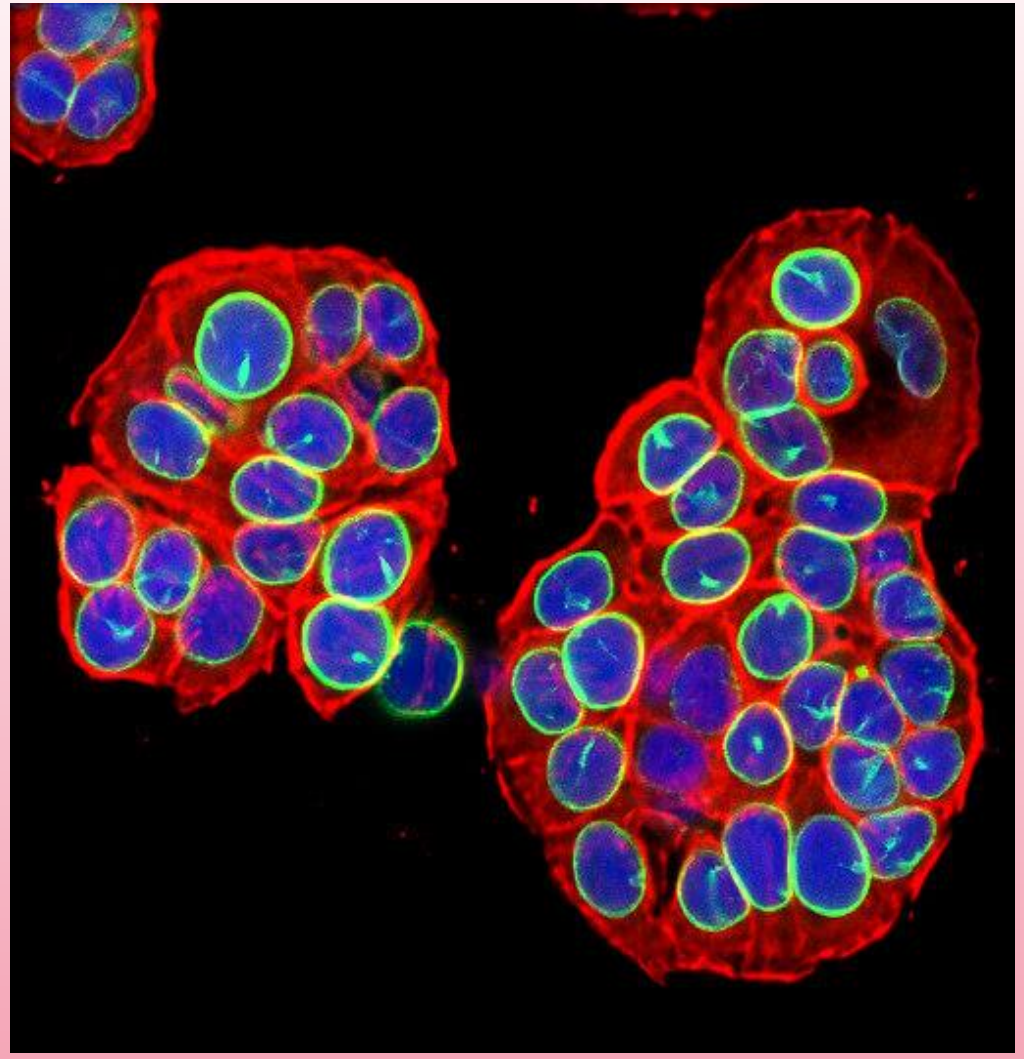
SA B-Gal staining in human skin



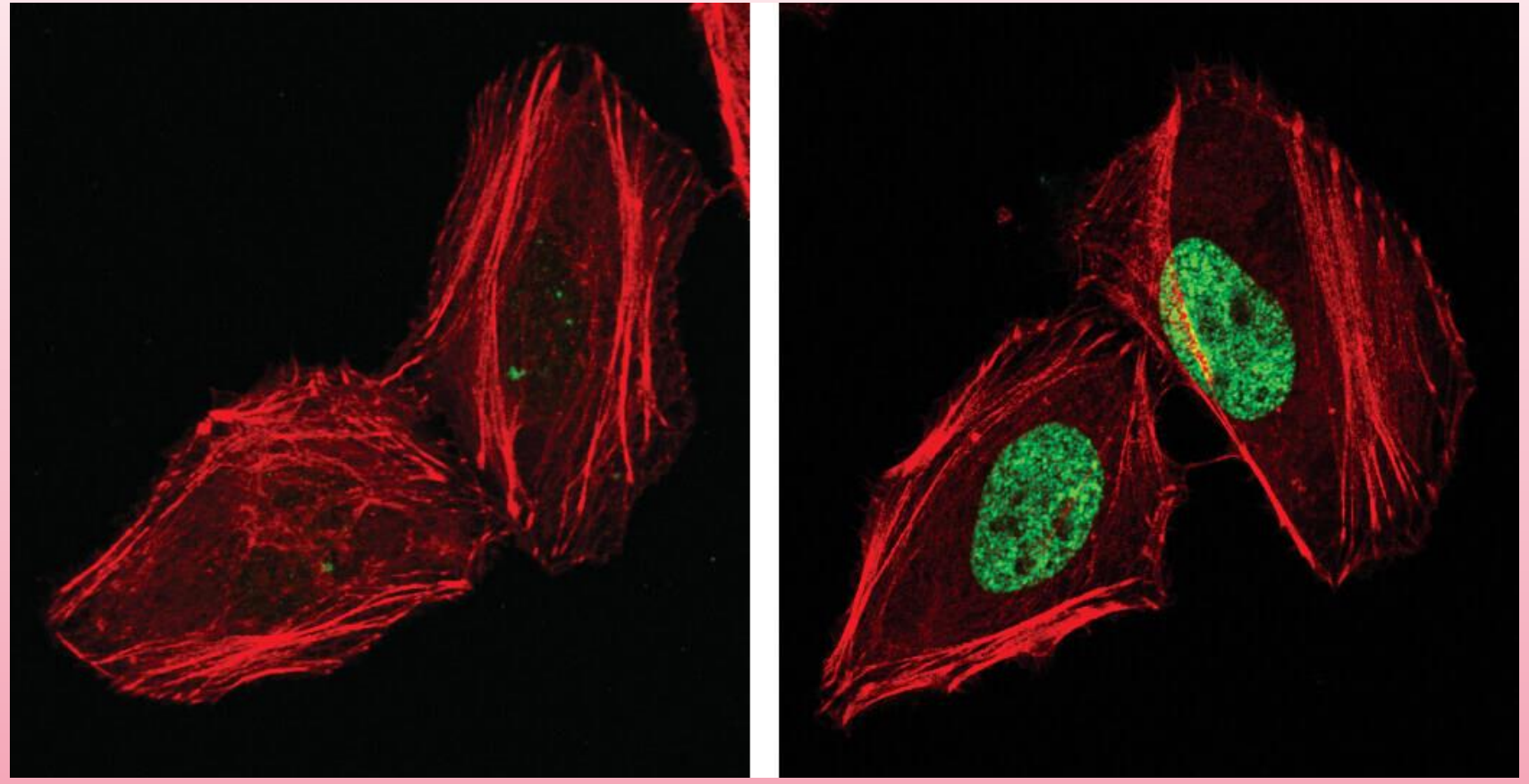
HUMAN RETINAL ENDOTHELIAL CELLS IN A MONOLAYER CELL CULTURE



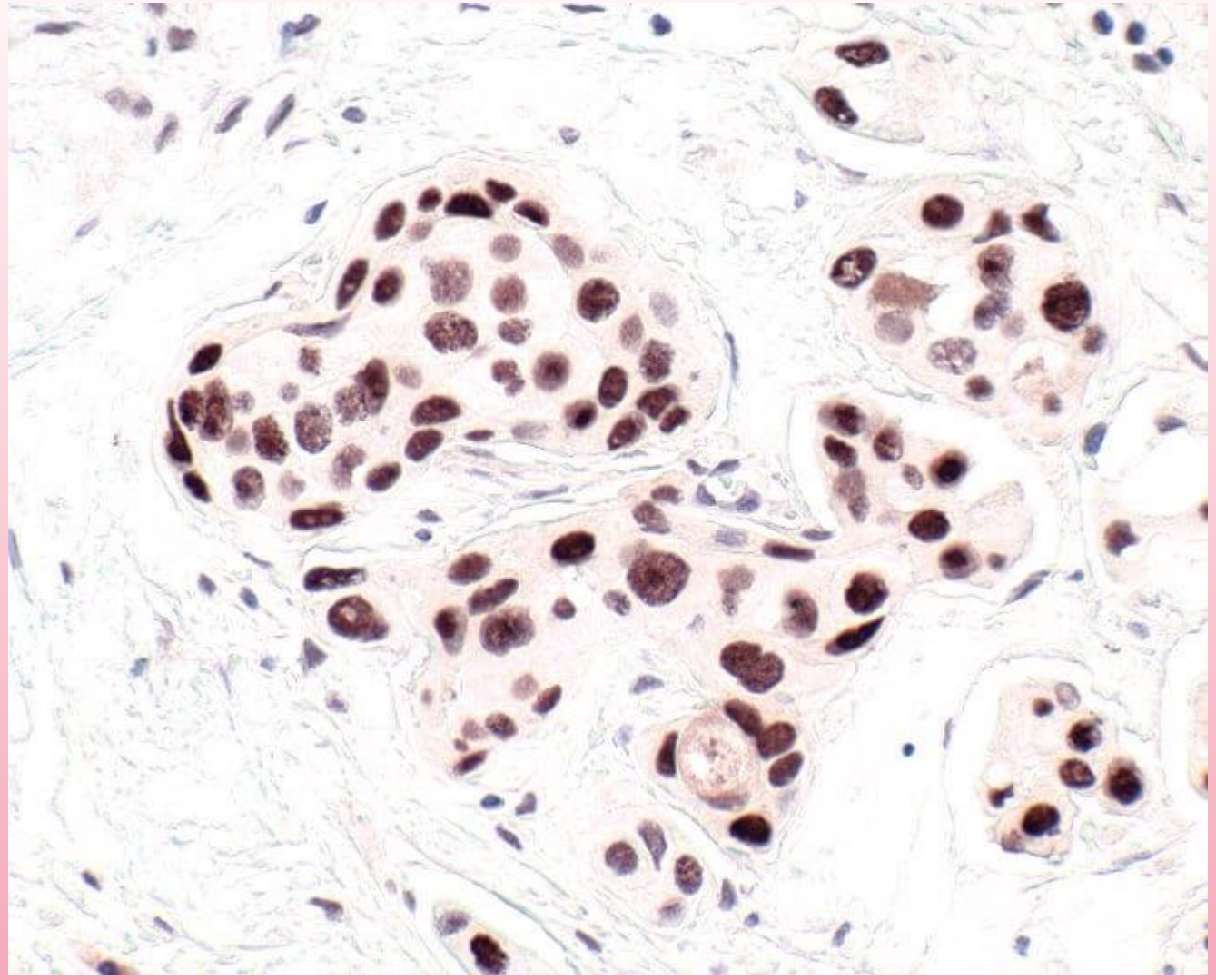
LOSS OF LAMIN B1



- The presence of DNA double-strand breaks is revealed by the occurrence of phosphorylated histone c-H2AX foci (Ser139)in their vicinity. These c-foci are characteristic of cells from aged animals, and of senescing human cells in vitro.



- Immunohistochemical analysis of HP1 expression in paraffin-embedded human breast carcinoma using HP1 alpha antibody.



Protein or Marker	Role in Senescence
Senescence-associated β-galactosidase	Increased activity at pH 6.0 in senescent cells
p53	Activation can trigger cell cycle arrest
Rb (Retinoblastoma tumor suppressor protein)	Inhibition triggers cell cycle arrest
$p21^{CIP1}$	Inhibits cyclin dependent kinases; downstream of p53
$p16^{INK4A}$	Inhibits phosphorylation and inactivation of pRb
Bcl-2	Increased expression in senescent cells, inhibits apoptosis
macroH2A1.1	macroH2A1 isoform; marker of SAHF
macroH2A1.2	macroH2A1 isoform; marker of SAHF
H3K9Me2/3 (lysine 9 di-or-tri-methylated histone H3)	Marker of SAHF
HP1 (heterochromatin protein 1)	Marker of SAHF
Phospho-Histone H2A.X (Ser139)	Marker of DNA damage
Lamin B1	Expression reduced in senescent cells leading to disruption of nuclear envelope
HMGB1 (High mobility group protein B1)	SASP component
IL-6 (Interleukin 6)	SASP component
TNF-α (Tumor necrosis factor α)	SASP component
MMP3 (Matrix metalloproteinase-3)	SASP component

SASP

- The SASP has powerful paracrine activities, the nature of which suggests that the senescence response is not solely a mechanism for preventing cancer. Rather, cellular senescence and the SASP likely evolved both to suppress the development of cancer and to promote tissue repair or regeneration in the face of injury.
- the paracrine activities of senescent cells can be either beneficial or deleterious, depending on the physiological context.
- The SASP is arguably the most striking feature of senescent cells because it has the potential to explain the role of cellular senescence in organismal aging and age-related pathology.
- SASP components include a large number of cytokines, chemokines, growth factors, and proteases, the details of which have been.
- Whereas some SASP factors are known (or suspected) to fuel the deleterious effects of senescent cells, other factors—or even the same factors—may have beneficial effects.

- The SASP can stimulate cell proliferation, owing to proteins such as theGROs (growth-regulated oncogenes) and amphiregulin, as well as stimulate new blood vessel formation, owing to proteins such as VEGF (vascular endothelial growth factor).
- However, the SASP also includes proteins that have complex effects on cells—for example, the biphasic WNT modulator SFRP1 (secreted frizzled related protein 1) and interleukins IL-6 and IL-8, which can stimulate or inhibit WNT signaling and cell proliferation, respectively, depending on the physiological context.Chronic WNT signaling can drive both differentiated and stem cells into senescence.
- In addition, some SASP factors induce an epithelial-to-mesenchymal transition in susceptible cells others (for example, SFRP1, GROα, and IL-6) can alter stem cell proliferation or differentiation or modify stem cell niches.
- Of particular relevance to the role of cellular senescence in aging and age-related disease,many SASP components directly or indirectly promote inflammation. For example: IL-6 and IL-8; a variety of MCPs (monocyte chemoattractant proteins) and MIPs(macrophage inflammatory proteins); and proteins that regulate multiple aspects of inflammation,such as GM-CSF (granulocyte/macrophage colony–stimulating factor). The secretion of these Chronic inflammation, of course, is a cause of—or an important contributor to—virtually every major age-related disease, both degenerative and Hyperplastic.
- Finally, the SASP is a plastic phenotype. That is, proteins that are included in the SASP vary among cell types and, to some extent, with the stimulus that induced the senescence response,yet proinflammatory cytokines are the most highly conserved feature.

WHAT CAUSES SASP

- The SASP is primarily a property of cells that senesce owing to, or accompanied by, genomic damage or epigenomic perturbation. Thus, normal cells that senesce owing simply to the ectopic overexpression of p21 or p16INK4a do not express a SASP, despite displaying other characteristics of senescent cells.

- In contrast, cells that senesce owing to DNA damage, dysfunctional telomeres, epigenomic disruption, mitogenic signals, oxidative stress, and other senescence-inducing stimuli develop a SASP of varying qualities and robustness.

- these findings suggest that one function of the SASP may be to ensure that damaged cells communicate their compromised state to neighboring cells to prepare the tissue for repair; another function may be to stimulate the clearance of such damaged cells by the immune system.

Table 1. The senescence-associated secretory phenotype (SASP) factors (based on [24,34]).

Interleukins and Other Inflammatory Molecules	IL-1; IL-1β; IL-6; IL-7; IL-8; IL-13; IL-15; TGFβ; GM-CSF; G-CSF; CSF-1; IFN-γ; BLC; MIF
Chemokines and Growth Factors/Regulators	CXCL1; CXCL2; CXCL5; CXCL12; CCL2; CCL5; CCL8; CCL13; MIP-1α; MIP-3α; HCC-4; eotaxin/eotaxin-3; TECK; ENA-78; Amphiregulin; epiregulin; heregulin; EGF; bFGF; HGF; KGF (FGF7); VEGF; angiogenin; SCF
Receptors and Ligands	ICAM-1/3; OPG; TNFα; sTNFRI; sTNFRII; TRAIL-R3; Fas; uPAR; SGP130; EGF-R
Proteases and Extracellular Matrix Proteins	MMP-1/3/10/12/13/14; TIMP-1/2; PAI-1/2; tPA; uPA; cathepsin B
Non-Protein Molecules and Insoluble Factors	Nitric oxide; ROS; PGE2; fibronectin; collagens; laminin

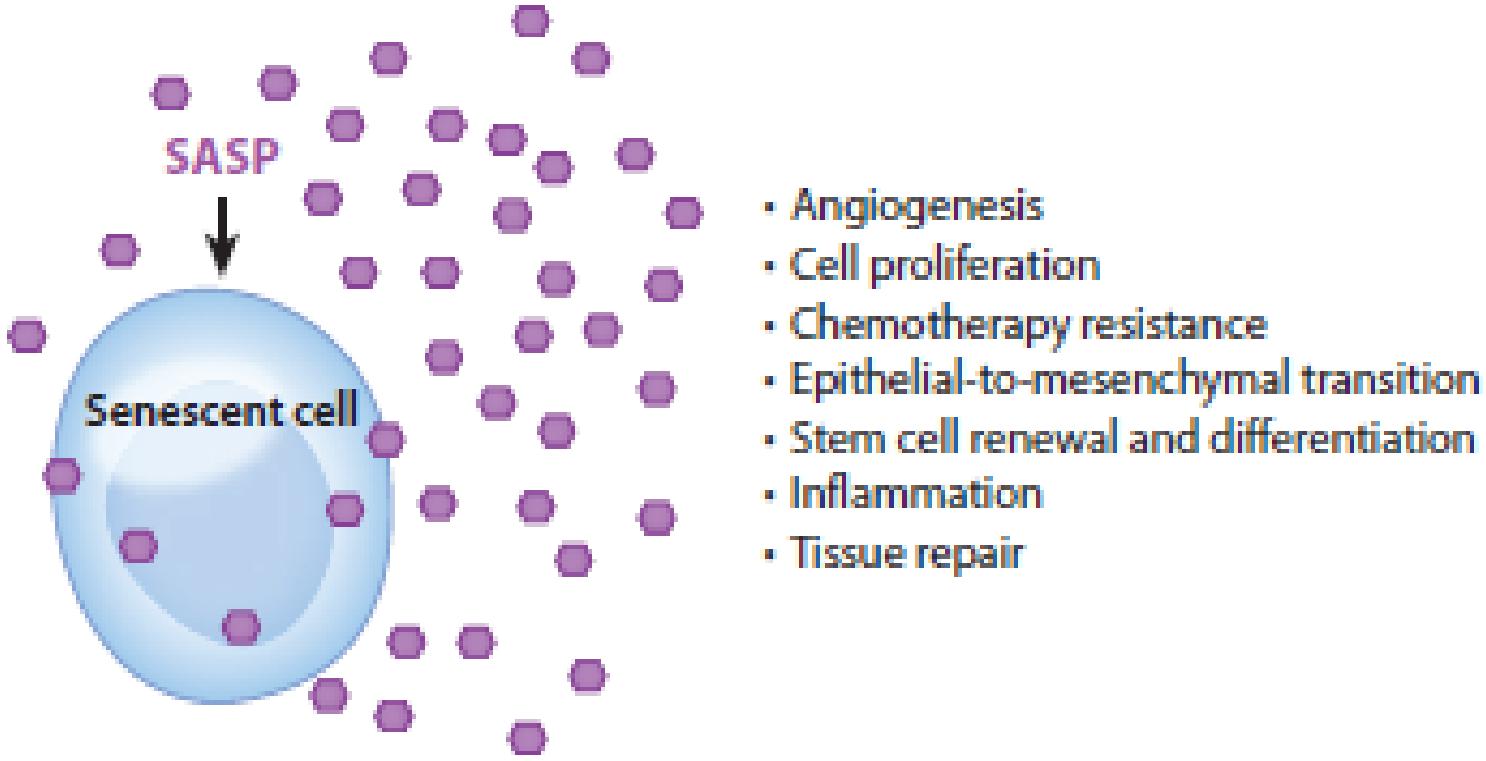


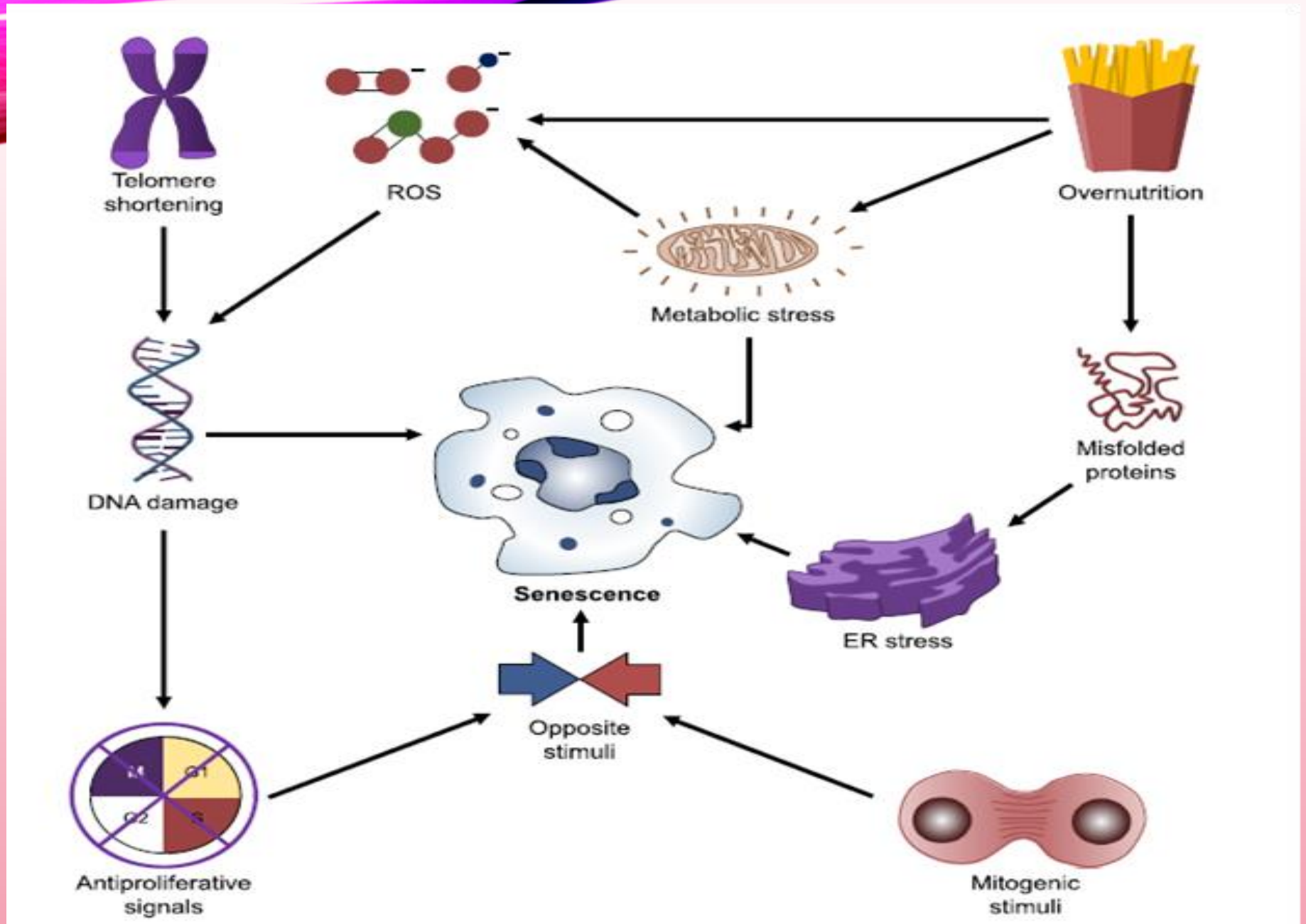
Figure 3

The myriad activities of the senescence-associated secretory phenotype (SASP). The many factors that compose the SASP have numerous biological activities, all highly dependent upon physiological context. These activities include stimulation of angiogenesis, stimulation and inhibition of cell proliferation, creation of a chemoresistant niche during cancer chemotherapy, stimulation of an epithelial-to-mesenchymal transition, chronic inflammation, alterations to stem cell renewal and/or differentiation, and optimization of tissue repair. Hexagons represent SASP factors that act within and outside the senescent cell.

REGULATION OF THE SASP

- Many, but not all, SASP components are positively regulated by the DDR proteins ATM, NBS1 (Nijmegen breakage syndrome 1), and CHK2 (checkpoint kinase 2)
- These proteins act upstream of p53, which does not positively regulate the SASP.
- Of particular importance, these DDRproteins stimulate the SASPonly after persistent DDR signaling has been established. That is, the rapid robust DDR that occurs immediately after DNA damage does not induce a SASP; rather, the SASP develops slowly—over several days in culture—and only after the initial DDR subsides .
- DNA-SCARS and TIF are particularly important for the effects of the DDR on the SASP. These nuclear structures contain the activated DDR proteins that ensure the persistent DDR signaling.
- Little is known about precisely how DDR signaling promotes the expression of the genes that encode the DDR-sensitive SASP components.
- TheSASP is also positively regulated by the transcription factors nuclear factor κB(NF-κB) and C/EBP-β.
- These transactivators are downstream of signaling cascades that control inflammatory cytokine gene expression, primarily in immune cells.

- in senescent cells,an early response to senescence-inducing stimuli is increased expression of IL-1α.
- This plasma membrane–associated cytokine binds its plasma membrane–associated receptor (IL1R), which in turn initiates a signaling cascade that ultimately activates NF-κB. NF-κB, in turn, induces the transcription of genes encoding inflammatory mediators such as IL-6 and IL-8.
- In contrast to positive regulation by the DDR, p53 negatively regulates or, more accurately, restrains the SASP.
- In normal senescent cells that express a SASP, inactivation of p53—for example, by RNA i—causes a striking hyperincrease in the secretion of several SASP factors, due primarily to an increase in mRNA abundance.
- Furthermore, p53 inactivation in cells that do not express p16INK4a, which renders the senescence growth arrest irreversible, causes cells to resume proliferation, but the SASP remains active.
- Such cells,Not only express a SASP, which can drive aging phenotypes in neighboring cells, but also are most likely (epi)genomically unstable and hence at risk for malignant transformation.
- Once IL-6 binds its receptor, a complex is formed with two GP130 molecules, leading to activation of the JAK-STAT signaling pathway.IL-6 is a direct inductor of cell cycle arrest and a pro-mitogenic factor in oncogene-induced cell senescence.
- both IL-1a and IL-1b are secreted by and expressed at the surface of senescent cells.They further extend the SASP profile by promoting NF-jB and C/EBPb DNA-binding capacity and promoting IL-6 and IL-8 secretion.



Triggers of cellular senescence

PATHWAYS

- the senescence arrest is established and maintained by at least two major tumor suppressor pathways—the p53/p21 and p16INK4a/pRB pathways—and is now recognized as a formidable barrier to malignant tumorigenesis.

- Consistent with this view, cells undergo senescence in response to a host of potentially oncogenic stimuli or their sequelae.

- In addition to arrested growth, senescent cells show widespread changes in chromatin organization and gene expression.
- These changes include the secretion of numerous proinflammatory cytokines, chemokines, growth factors, and proteases, a feature termed the senescence-associated secretory phenotype (SASP).

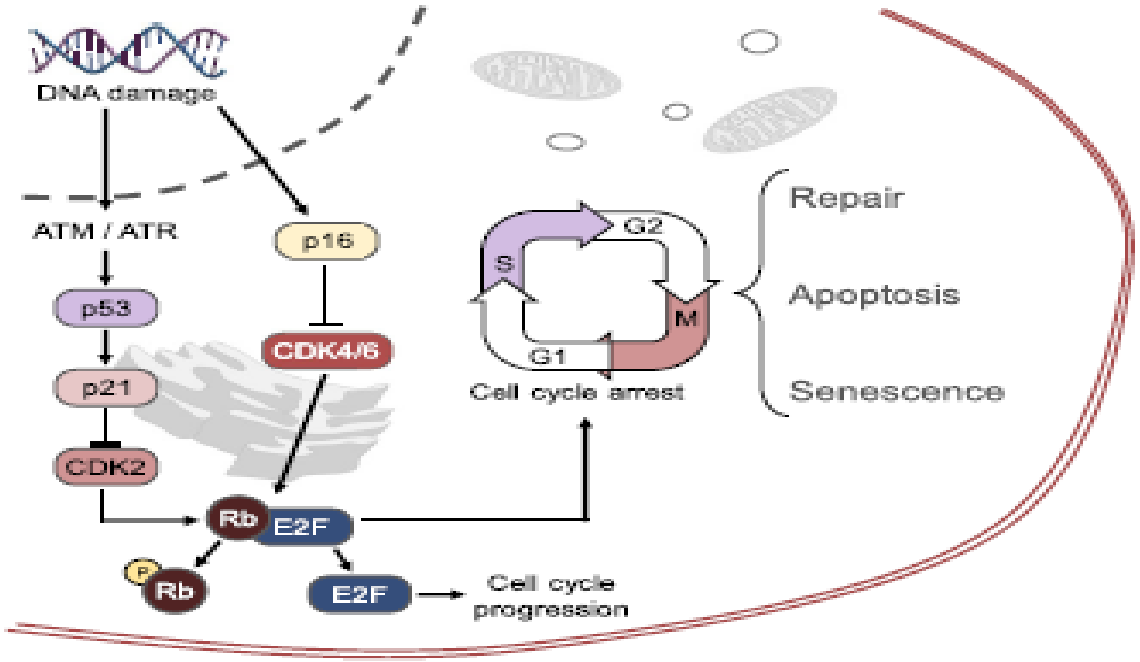
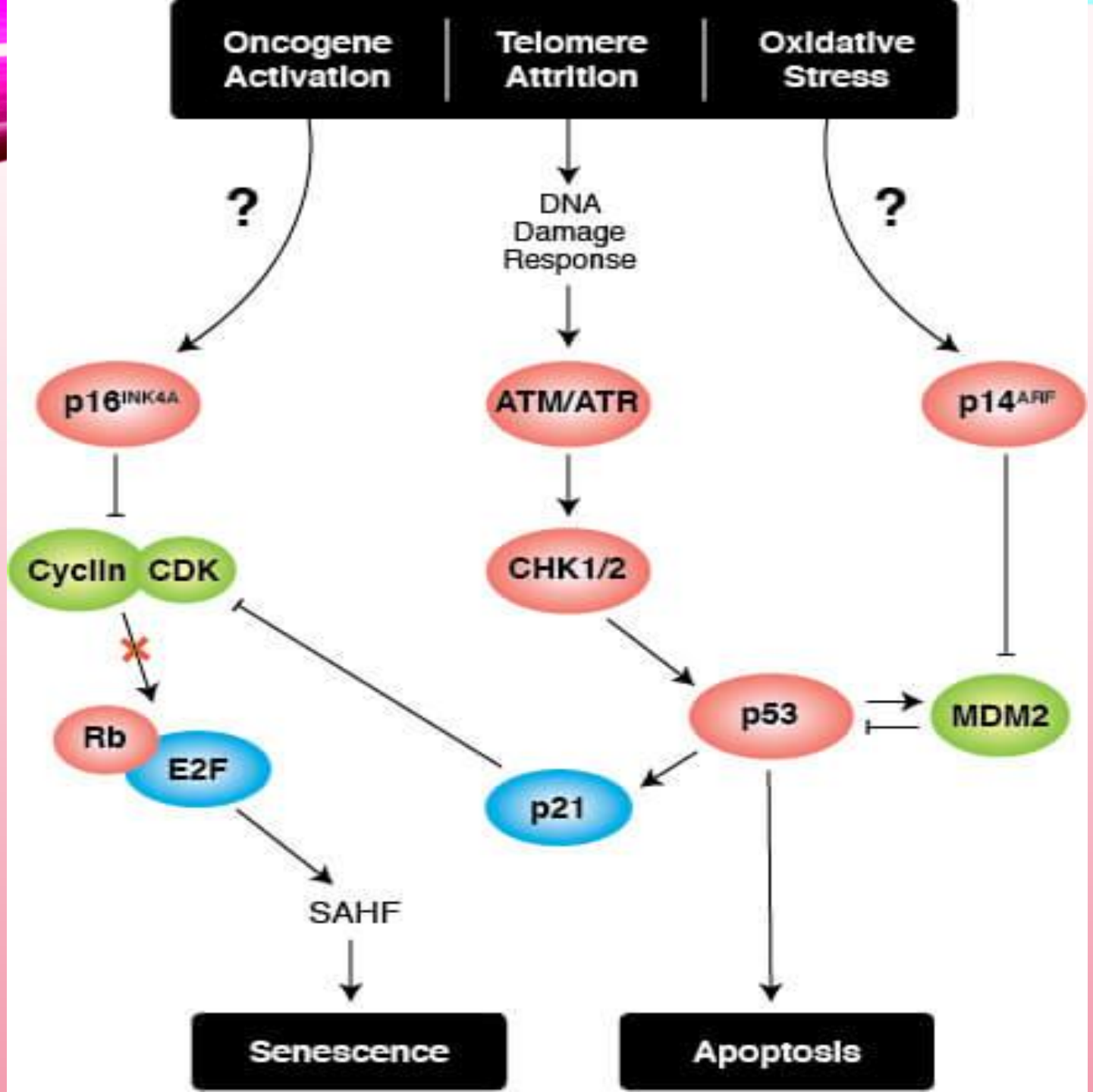


Fig. 1 Pathways leading from the DNA damage response (DDR) to cell senescence. E2F is a transcription factor that stimulates the transition from G1 to S phase of the cell cycle. The retinoblastoma protein (Rb) is normally complexed with E2F, inhibiting it and preventing mitosis. When Rb is phosphorylated by a cyclin-dependent kinase (CDK), it releases E2F and cell division ensues. DNA damage activates the ATM or ATR kinases, stabilizing p53 and subsequently stimulating the expression of the protein p21, which is an inhibitor of the cyclin-dependent kinase CDK2. When CDK2 is inhibited by p21 as a consequence of the DDR, Rb remains complexed with E2F, and the cell enters a state of apoptosis or senescence. The DDR also leads to enhanced expression of p16, which inactivates CDK4 and CDK6, also preventing Rb phosphorylation and cell cycle progression

ACTIVATION OF TUMOR SUPPRESSORS

- Stimuli that induce cellular senescence,establish and/or maintain the senescence growth arrest largely by engaging either or both of the p53/p21 and p16INK4a/pRB tumor suppressive pathways.Both pathways are complex; each has multiple upstream regulators, downstream effectors, and modifying side branches.
- Moreover, the pathways cross-regulate each other.Both pathways control the senescence response mainly by implementing widespread changes in gene expression. p53 and pRB are master transcriptional regulators.
- P21 is a downstream effector of p53, whereas p16INK4a is a positive upstream regulator of pRB; both are cyclin-dependent kinase inhibitors and potent negative regulators of cell cycle progression.
- There may be other, as yet poorly characterized p53- and pRB-independent pathways that can establish or maintain the senescence growth arrest, but these two are clearly of major importance.
- Chronic activation or overexpression of p53, pRB, p21, or p16INK4a is generally sufficient to induce a senescence growth arrest.

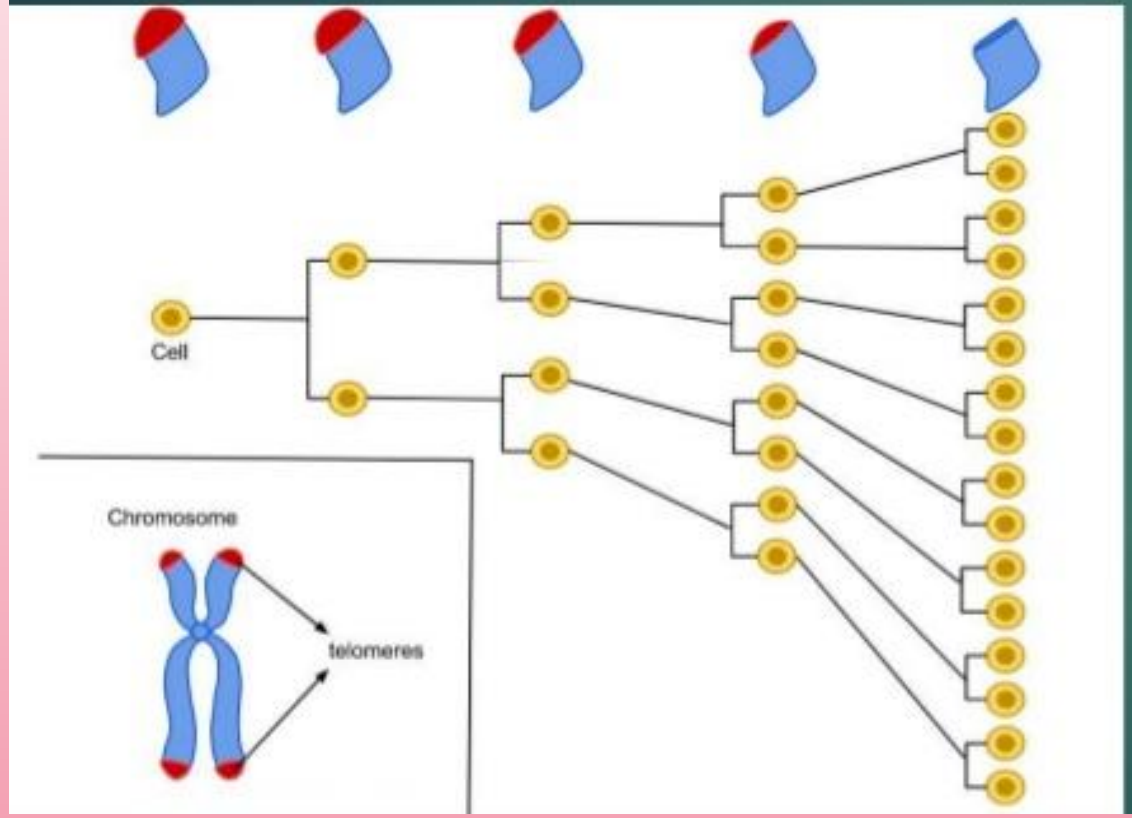

Oncogene Activation
Telomere Attrition
Oxidative Stress
?
DNA Damage Response
?
p16INK4A
ATM/ATR
p14ARF
Cyclin
CDK
CHK1/2
Rb
E2F
p53
MDM2
p21
SAHF
Senescence
Apoptosis

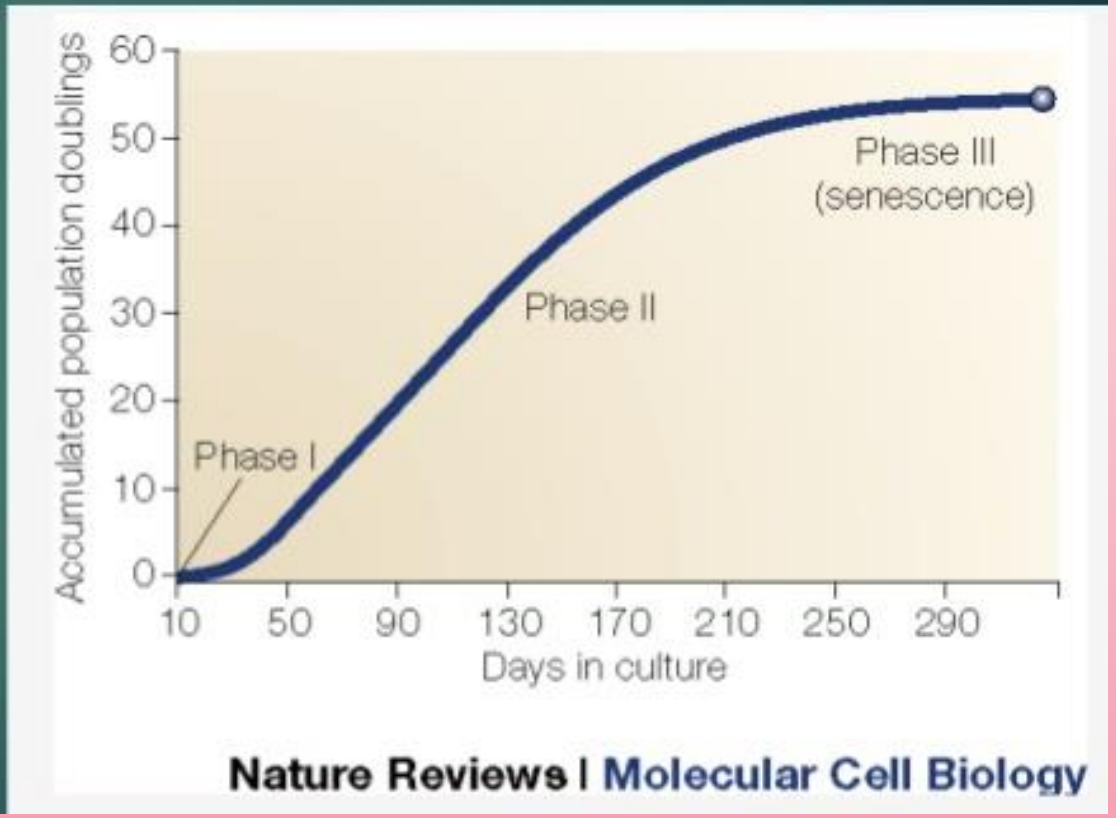
DISCOVERY OF SENESCENCE

- Cellular senescence was first formally described approximately five decades ago when Hayflick and colleague showed that normal human cells (in this case fibroblasts) did not proliferate indefinitely in culture.
- These cells were said to have a finite replicative life span, and, later, to undergo replicative or cellular senescence (sometimes termed replicative or cellular aging).
- The number of divisions that cells complete upon reaching the end of their replicative life span has been termed the Hayflick limit.
- The link between the Hayflick limit and aging was, for many years, conjectural and tenuous—largely on the basis that replicatively senescent cells appeared to be degenerated, although they remained viable and metabolically active.
- The link to cancer was more obvious. Even 50 years ago, it was evident that most cancer cells do not have a finite replicative life span,Hence, the idea that the senescence response is tumor suppressive, although still speculative 50 years ago, was more firmly grounded.

The Hayflick's paradigm

Hayflick limit or **Hayflick** phenomenon is the number of times a normal human cell population will divide until cell division stops.



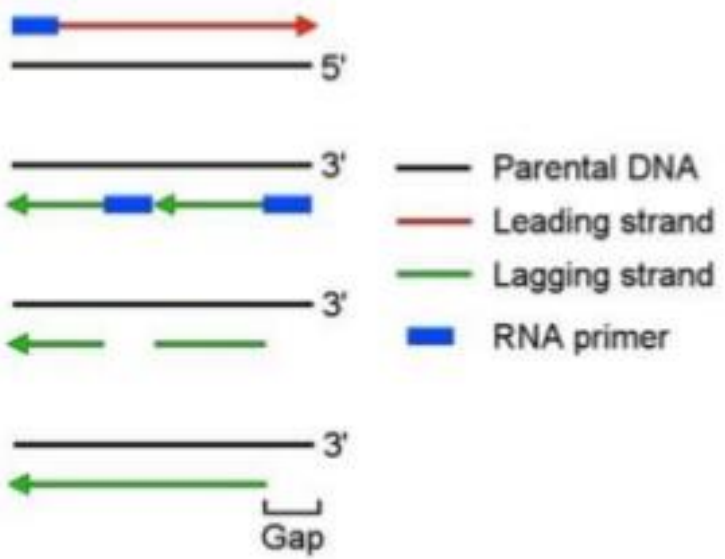


WHY IS REPLICATION LIMITED?

- The mechanism behind the finite replicative life span of normal cells is now understood.
- Because polymerases that copy DNA templates are unidirectional and require a labile primer, the ends of linear DNA molecules cannot be completely replicated .
- Thus, telomeres, the DNA-protein structures that cap the ends of linear chromosomes, shorten with each cell division.
- Telomere shortening does not occur in cells that express telomerase, the reverse transcriptase that can replenish the repetitive telomeric DNA de novo.
- The numbers and types of telomerase-expressing cells vary widely among species. In mice, for example, many cells in the adult animal are telomerase positive.
- In humans, however,such cells are rare. Telomerase positive human cells include most cancer cells, embryonic stem cells, certain adult stem cells, and a few somatic cells (for example, activated T cells).

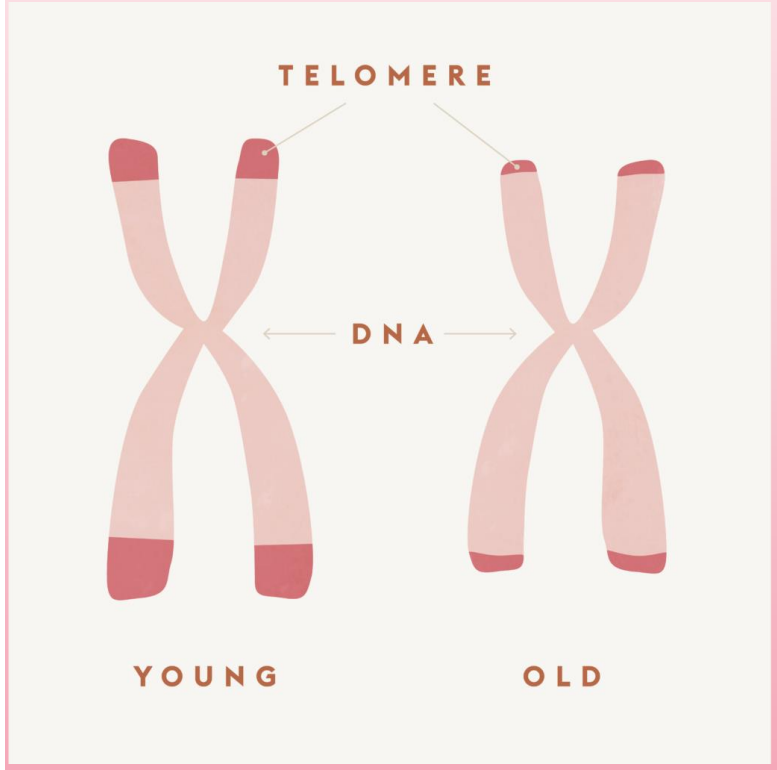
Closer Look: Telomere Shortening

- Telomere length and telomere capping **both** contribute to cellular senescence



- DNA Polymerase cannot fully synthesize 3' end of lagging strand: End Replication Problem
- Telomerase synthesizes short repeating sequence TTAGGG added to 3' end of DNA lagging strand
- Telomere shortening could act as a cellular time keeper

http://www.senescence.info/telomeres_telomerase.html



DNA DAMAGE RESPONSE

- Functional telomeres prevent DNA repair machineries until appropriate time,like DNA double-strand breaks (DSBs), to which cells rapidly respond and attempt repair.
- In the case of telomeres, repair followed by cell division will cause rampant genomic instability through cycles of chromosome fusion and breakage—major risk factors for developing cancer.
- Thus,repeated cell division in the absence of telomerase eventually causes one or more telomeres to become critically short and dysfunctional.
- Dysfunctional telomeres elicit a DNA damage response (DDR) but suppress attempted DNA repair. The DDR, in turn, arrests cell division primarily through activities of the p53 tumor suppressor, thereby preventing genomic instability.
- Dysfunctional telomeres appear to be irreparable; consequently, cells with such telomeres experience persistent DDR signaling and p53 activation,which enforce the senescence growth arrest.
- DDR signaling also establishes and maintains the SASP.

- many senescence inducers cause genomic damage, resulting in lasting DNA damage foci and DDR signaling. The persistent foci are termed telomere dysfunction–induced foci (TIF) when present at telomeres or, more generally, DNA-SCARS (DNA segments with chromatin alterations reinforcing senescence).
- They contain several markers of DNA damage foci, such as 53BP1, but are distinct from foci that form immediately after DNA damage.
- DNA SCARS often partially colocalize with promyelocytic leukemia protein (PML) nuclear bodies and contain the activated DDR proteins, such as phospho-CHK2, that are needed for the SASP.
- When cells arrested by DNA damage and p21 expression are exposed to mitogenic stimuli, they lose their proliferative potential,but also fail to apoptose.
- One example of such opposing stimuli is high-level RAS signaling,which provides a proliferation signal while also activating CDKN2a locus via “replication stress”

GENOMIC DAMAGE,A CAUSE OF SENESCENCE

- Telomere dysfunction is one of Many
- cells undergo senescence in response to severely damaged DNA,regardless of the genomic location.Many types of cytotoxic chemotherapies are severe DNA-damaging agents that can induce senescence in both tumor cells and surrounding normal cells.

- Cellular senescence can also be induced by strong, chronic, or unbalanced mitogenic signals,consistent with its role in suppressing tumorigenesis.

- The best-studied examples are the senescence responses that are provoked by certain oncogenes. The first report of what is now termed oncogene-induced senescence showed that an oncogenic form of H-RAS (H-RASV12),which chronically stimulates the mitogen-activated protein kinase (MAPK) signaling pathway,provokes senescence in normal cells . Several other MAPK pathway components have sincebeen shown to induce senescence when overexpressed or present in oncogenic forms.
- Likewise, cells senesce in response to overexpressed growth factor receptors such as ERBB2,chronic stimulation by cytokines such as interferon-β , loss of PTEN (which truncates growth factor signaling).
- *Pten* heterozygosity promotes tumour initiation and proliferation in the prostate but, paradoxically, the complete loss of *Pten* can oppose prostate tumorigenesis by triggering a powerful p53-dependent cellular senescence response3.

EPIGENOMIC DAMAGE

- Cellular senescence entails widespread changes in chromatin organization, including the formation of repressive heterochromatin at several loci that encode proproliferative genes.
- Perturbations to the epigenome can elicit a senescence response.

For example, global chromatin relaxation (such as that caused by broad-acting histone deacetylase inhibitors) induces senescence, often by derepressing the p16INK4a tumor suppressor, which promotes the formation of senescence-associated heterochromatin.

- Other inducers, for example, suboptimal c-MYC or p300 histone acetyltransferase activity, also appear to act by perturbing chromatin organization and inducing p16INK4a expression.
- p16INK4a, is both a tumor suppressor and a biomarker of aging
- Finally,under some circumstances, epigenomic perturbations can elicit a DDR in the absence of physical DNA damage. For example, histone deacetylase inhibitors activate the DDR protein ATM (ataxia-telangiectasia-mutated), which initiates a DDR without DNA damage.

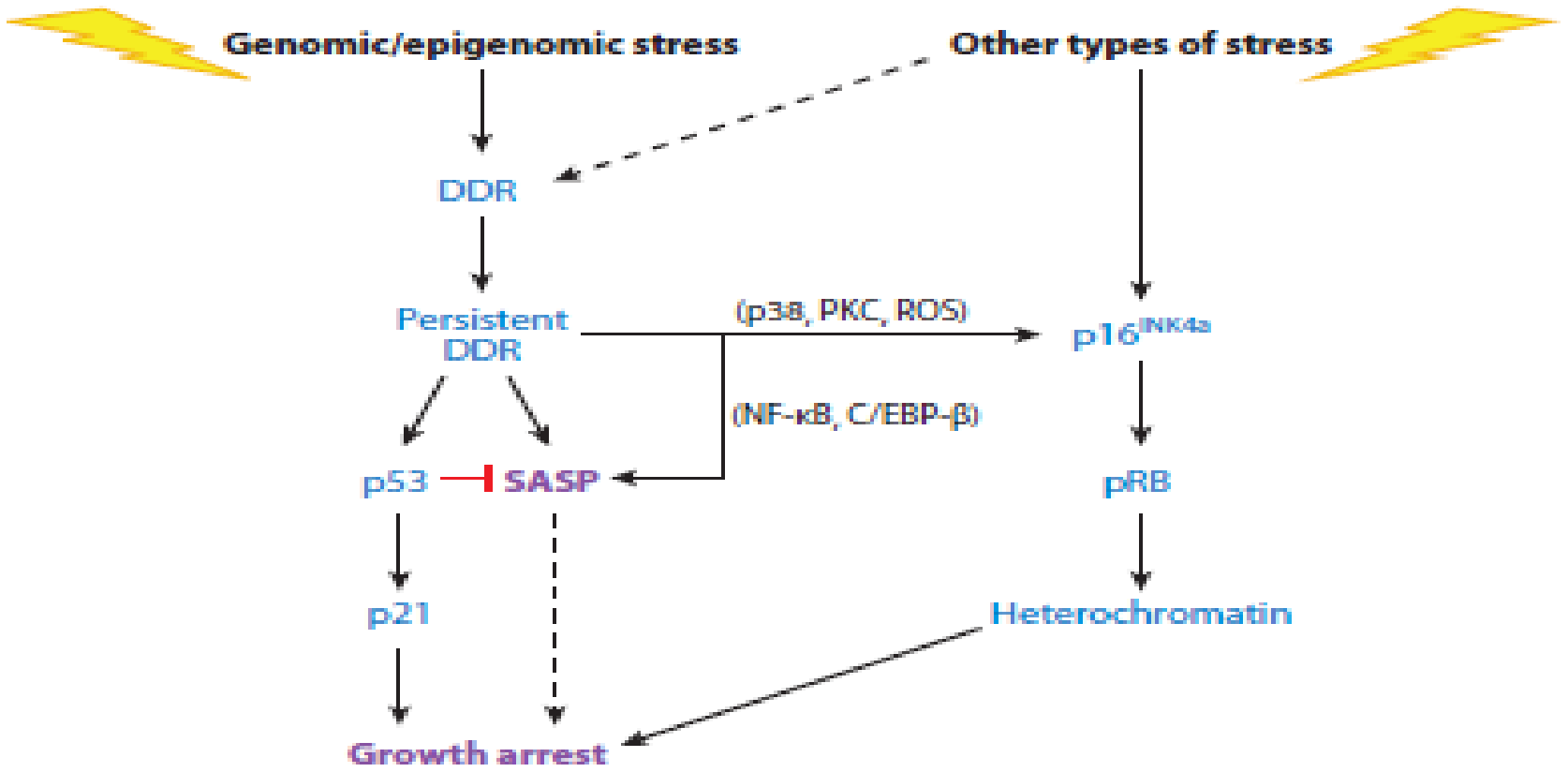


Figure 2

Regulation of senescence growth arrest and the senescence-associated secretory phenotype (SASP). Cellular senescence is initiated by genomic or epigenomic damage, which activates a DNA damage response (DDR). The DDR ultimately becomes persistent or chronic, which leads to activation of p38MAPK and protein kinase C (PKC) and increased reactive oxygen species (ROS) and, ultimately, expression of the $p16^{INK4a}$ tumor suppressor. Stress that does not entail direct genomic or epigenomic damage can also induce $p16^{INK4a}$ expression and in some cases can indirectly trigger a DDR (*dashed line*). $p16^{INK4a}$ activates the pRB tumor suppressor, which silences certain proproliferative genes by heterochromatinization, thereby instituting a stringent arrest of cell proliferation. Persistent DDR signaling also induces the SASP and activates the p53 tumor suppressor, which restrains the SASP. p53 also causes growth arrest, principally by inducing expression of the cell cycle inhibitor p21. In some forms of oncogene-induced senescence, the SASP reinforces the senescence growth arrest (*dashed line*). NF-κB denotes nuclear factor κB.

SHIFT IN GLUCOSE METABOLISM

- tumour cells often show a shift in glucose metabolism from mitochondrial oxidative phosphorylation to glycolysis, even in the presence of oxygen (the Warburg effect).
- Emerging evidence indicates that senescent cells also shift glucose metabolism, but in a way that is distinct from proliferating cancer cells
- Kaplon *et al.* recently showed that increased mito-chondrial oxidative phosphorylation is crucial for the induction of OIS.Cells in culture expressing either oncogenic BRAF-V600E or RAS-G12V that undergo OIS have increased PDH activity.
- Downregulation of PDH activity abrogated BRAF-V600E-induced senescence, whereas activation of PDH was sufficient for senescence induction in normal cells.

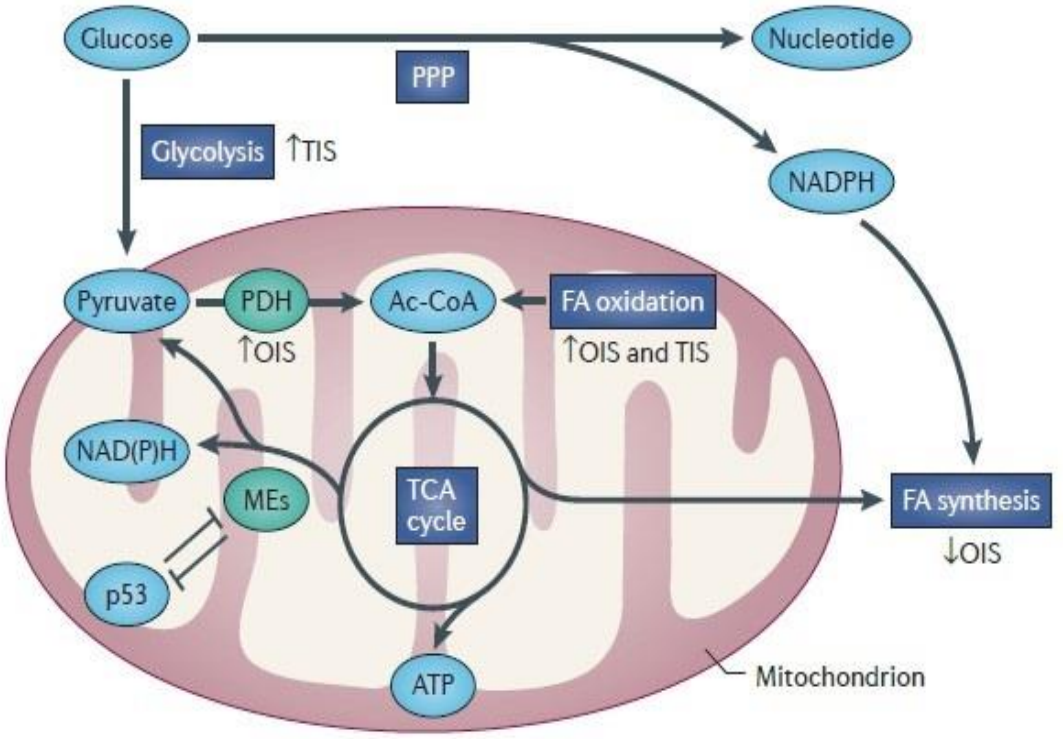


Figure 1 | **Glucose metabolism in oncogene-induced senescence (OIS) and therapy-induced senescence (TIS).** In contrast to the metabolic shift to glycolysis in cancer, active mitochondrial metabolism has been shown in OIS[41,53] and TIS[43]. In TIS, active glucose uptake is maintained. The activity of pyruvate dehydrogenase (PDH) is positively and negatively regulated by PDH phosphatases (PDPs) and PDH kinases (PDKs), respectively. PDH is activated in cells in which senescence has been induced by oncogenic BRAF or RAS, although activation of PDH is accompanied by the induction of PDP2 and the suppression of PDK1 in BRAF-driven senescence but not in RAS-driven senescence[41].

WITHOUT ACTUAL DNA DAMAGE!

- Some oncogenes and strong mitogenic stimuli cause DNA damage and persistent DDR signaling, possibly as a consequence of inappropriate replicon firing and replication fork collapse (which creates DNA DSBs).This mechanism cannot, however, explain all instances of senescence.

- For example, hyperactivation of p38MAPK, a stress-responsive MAPK pathway component, induces senescence by a DDR-independent mechanism.

- Likewise,activation of ATR, a DDR protein that responds to replication stress, can induce senescence in the absence of actual DNA damage.
- Whatever the initiating event, mitogenic signals ultimately engage the p53/p21 and/or p16INK4a/pRB pathways.

ADDITIONAL DETAILS OF SAID PATHWAYS

- The p53/p21 and p16INK4a/pRB pathways also regulate several—although not always all—other features of senescent cells.
- Genomic damage, including dysfunctional telomeres, activates the DDR, which engages the p53/p21 pathway. This engagement is biphasic. The initial response is rapid (generally within minutes to an hour), robust, and transient (generally subsiding within 24–48 h), which is typical of the p53 response to many forms of DNA damage.
- However, if the damage is severe or irreparable—enough to elicit a senescence response—low-level p53 activation and p21 expression persist once the robust rapid phase declines (42, 43, 75).
- Persistent DDR signaling appears to initiate the senescence growth arrest (as opposed to a transient damage-induced growth arrest).
- Such signaling is also accompanied by the slow (occurring over days) activation of other signaling pathways, such as those governed by the stress-responsive p38MAPK and protein kinase C pathways, and increased reactive oxygen species,which also participate in signaling pathways .
- These pathways are initiated by poorly understood mechanisms. These additional signaling pathways, then, stimulate the expression of p16INK4a, which, acting through pRB, ensures the essential irreversibility of the growth arrest.

WHAT IS AGING?

- The most prominent feature of aging is a gradual loss of function—or degeneration—that

occurs at the molecular, cellular, tissue, and organismal levels.

Age-related loss of function is a feature of virtually all organisms that age, ranging from single-celled creatures to large, complex animals.

- In mammals, age-related degeneration gives rise to well-recognized pathologies, such

as sarcopenia, atherosclerosis and heart failure, osteoporosis, macular degeneration, pulmonary insufficiency, renal failure, neurodegeneration etc.

- Among multicellular organisms with renewable (that is, repairable or regenerative) tissues, aging entails another feature: gain-of-function changes that allow cells to proliferate inappropriately(hyperplasia).
- Furthermore, through genomic instability, these changes allow cells to acquire phenotypes

that increase their abilities to proliferate, migrate, and colonize ectopic sites; to survive

hostile tissue environments; and to evade attack by the immune system. These phenotypes are, of course, hallmarks of lethal cancers.

CANCER AND AGING

- Cancer, like the age-related degenerative diseases, increases in incidence with nearly exponential kinetics beginning at approximately the mid-point of the life
- In this regard, cancer is no different from the other diseases of

aging, despite very different manifestations**(coincidence Or a common process?)**

- There is mounting evidence that at least one **process—a stress response termed cellular senescence**—links multiple pathologies of aging, both degenerative and hyperplastic.
- Cellular senescence is unlikely to explain all aging phenotypes. Nonetheless, a surprisingly large number of aging pathologies have been linked, directly or indirectly, to the senescence response.

SENESCENT CELLS AND DEGENERATIVE PHENOTYPES

- In three-dimensional cultures of breast epithelial cells, for example, the presence of senescent fibroblasts disrupted alveolar and branching morphogenesis, as well as milk protein production .the effects of the senescent fibroblasts were due primarily to their secretion of matrix metalloproteinases (MMPs), which are prominent SASP components .
- senescent pulmonary artery smooth muscle cells stimulated the proliferation and migration of neighboring smooth muscle cells, in part due to their secretion of IL-6, IL-8, and other factors, also causing hypertrophy of the pulmonary arteries, which result in pulmonary hypertension.
- senescent cells were seen with increased frequency in normal and premature aging skin.There, they are thought to cause or contribute to dermal and epidermal thinning and loss of collagen, perhaps owing to the secretion of MMPs.
- Indirect evidence suggests that the senescence and associated SASP of astrocytes can promote the age-related neurodegeneration that gives rise to cognitive impairment, as well as to Alzheimer’s and Parkinson’s diseases.
- In the central nervous system, fractalkine is a fundamental mediator of the communication between glial cells and neurons.this chemokine is decreased in aged hippocampal cells and has been attributed both beneficial and deleterious roles in the aging brain and neurodegenerative diseases
- the presence and SASP of senescent chondrocytes,which are prominent in age-related osteoarthritic joints and degenerated intervertebral discs, are thought to play a role in the etiology and promotion of these pathologies.

EVIDENCE!

- In a transgenic mouse model in which senescent cells could be eliminated by administering
- a drug,termed INK-ATTAC, a p16INK4a promoter element drives expression of caspase 8 fused to the FK506-binding protein; the fusion protein dimerizes in response to the drug AP20187, thereby activating caspase 8 activity and causing apoptosis. Thus,this model allowed administration of a drug to specifically eliminate p16INK4a-expressing cells

- INK-ATTAC mice were crossed with a progeroid mouse in which a hypomorphic form of the BubR1 checkpoint protein (BubR1H/H) was expressed constitutively and caused premature aging and death (due primarily to heart failure). Although drug-treated BubR1H/H;INK-ATTAC mice did not live longer, they were remarkably protected from several other age-related pathologies, including cataracts, sarcopenia, and loss of subcutaneous fat.

- This study provided the first direct evidence that senescent cells can, at least in a premature aging mouse model, drive degenerative age-related pathology.

HYPERPLASTIC PATHOLOGIES OF SENESCENT CELLS

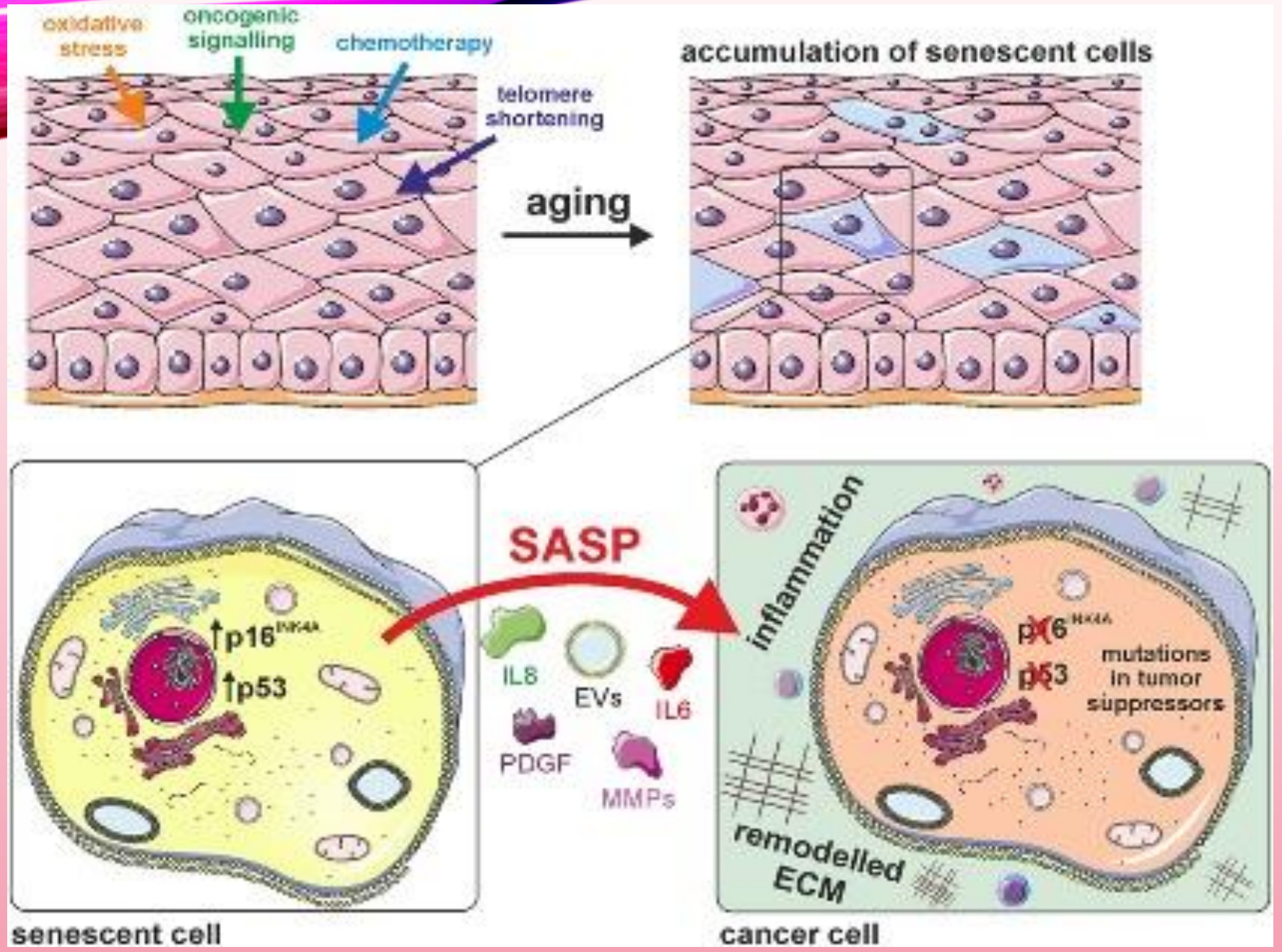
- In a xenograft studies,Coinjection of senescent, but not nonsenescent, fibroblasts significantly stimulated the proliferation of mouse and human epithelial tumor cells in immunocompromised mice.This stimulation is due in part to the SASP components MMP3 (stromelysin) which also promotes tumor cell invasion, and VEGF,which promotes tumor-driven angiogenesis.
- SASP factors can stimulate malignant phenotypes in culture. One such phenotype is the epithelial-to-mesenchymal transition.This morphological transition enables transformed epithelial cells to invade and migrate through tissues and is critical in the development of metastatic cancer. Senescent fibroblasts induce said transition in premalignant epithelial cells and nonaggressive cancer of epithelial cells in part through the secretion of IL-6 and IL-8.
- In summary,senescent cells accumulate with age, creating a tissue microenvironment that is permissive for the development, or at least the progression, of cancer.
- Senescent cells may also promote cancer initiation,through SASP-derived factors, can stimulate the infiltration of leukocytes, which produce reactive toxic moieties that can cause DNA damage.

IRONY!

- senescent cells can fuel malignant phenotypes and tumor growth. After all, cells enter a senescent state to prevent the proliferation of damaged cells, which is a major risk factor for the development of cancer. Even more ironic is the finding that senescent cells, particularly those that senesce in response to DNA-damaging radiation or chemotherapeutic agents, secrete factors that can protect neighboring tumor cells from being killed by those same chemotherapeutic agents.These chemoprotective SASP factors include WNT16B, IL-6, and TIMP-1 (tissue inhibitor of metalloproteinases-1).
- The effects of senescent cells within the tumor microenvironment are complex and highly dependent on physiological context. For example, global suppression of the SASP (through NF-κB inhibition) promoted resistance to chemotherapy in a mouse lymphoma model!

So especially within the context of DNA-damaging cancer therapies, it may be particularly important to consider adjuvant therapies aimed at eliminating senescent cells, both normal and tumor derived.

- Such therapies could enhance tumor cell killing by preventing the development of chemoresistant
- They could also inhibit cancer recurrence by preventing senescent cells from stimulating the proliferation of any residual cancer cells.


oxidative stress
oncogenic signalling
chemotherapy
telomere shortening
aging
accumulation of senescent cells
SASP
IL8
EVs
IL6
PDGF
MMPs
inflammation
mutations in tumor suppressors
remodelled ECM
senescent cell
cancer cell

BENEFICIAL EFFECTS OF CELLULAR SENESCENCE AND THE SASP

- There is little doubt that the senescence growth arrest suppresses the development of cancer.
- certain SASP components can apparently act in an autocrine fashion to buttress such growth arrest.In human cells, IL-6, IL-8, and IGFBP7 (insulin-like growth factor–binding protein 7) reinforce the senescence growth arrest caused by the oncogenic forms of RAS and BRAF(cytoplasmic proteins that participate in transducing growth factor and other extracellular signals to the cell interior)the genes that encode both proteins are frequently mutated in human cancer.
- Likewise, GROα, a potent mitogen that is a SASP component and is induced by oncogenic RAS, promotes the senescence of normal human ovarian fibroblasts.
- secreted WNT16B is an important enforcer of the senescence growth arrest of human
- Following injury, senescent fibroblasts and endothelial cells secrete a variety of proteins, including PDGF-AA which promotes myofibroblast differentiation and production of granulation tissue to initiate wound repair. In addition, SASP-associated proteases help to control excess fibrosis. Further, pharmacologic inhibition of cellular senescence *in vivo* inhibits wound healing.
- Additionally, inhibition of p53-mediated cellular senescence in hepatic stellate cells promotes fibrosis and tumorigenesis. This is thought to be due to the loss of critical functions mediated by senescent stellate cells such as promotion of anti-tumor macrophages and inhibition of hepatocyte transformation.This suggests that cellular senescence and SASP may limit fibrosis in specific contexts.These findings in the liver also demonstrate the potential anti-tumor functions of cellular senescence.
- Basically,at the same time,SASP can suppress tumour in one tissue and promote tumour in another!

Approach	Genetic background	Inducer of senescence	Tumour phenotype
Molecular induction of senescence			
Restoration of *Trp53* activity	LSL-*Trp53*; Rosa26-Cre-ERT2	Endogenous p53 re-expression	Regression of sarcomas
	Liver progenitor expressing ectopic $Hras^{G12V}$ and *Trp53* RNAi	Endogenous p53 re-expression	Regression of liver tumours
	Eμ-*Myc*; $Trp53^{fl}$; ectopic *Bcl2*	p53 reactivation	Senescence induction in lymphoma
Telomerase dysfunction	Eμ-*Myc*; ectopic *Bcl2*	*mTR* ablation	Suppression of Burkitt's lymphoma
Oncogene inactivation	Inducible *MYC* allele	MYC suppression	Suppression of lymphoma, osteosarc and hepatocellular carcinoma
	LSL-$Kras^{G12D}$; Ad-Cre lung infection	Omomyc expression	Regression of lung tumours
	Inducible *MYC* allele in haematopoietic system	MYC suppression	Regression of T cell acute lymphoblas lymphoma
	Inducible *BCR–ABL* allele in haematopoietic system	BCR–ABL suppression	Regression of pro-B cell leukaemia
Cell cycle deregulation	$Pten^{+/-}$	*Skp2* ablation	Lymphadenopathy and adrenal tumo abrogation
	$Pten^{lox/lox}$; Pb-Cre	*Skp2* ablation	Prostate cancer restriction
	Eμ-*Myc*	*Cdk2* ablation	Lymphomagenesis delay
	LSL-$Kras^{G12V}$; $RERT^{+/ERT}$	*Cdk4* ablation	Suppression of non-small-cell lung carcinoma development
Pharmacological induction of senescence			
DNA damage	Eμ-*Myc*; ectopic *Bcl2*	Cyclophosphamide	Lymphoma regression
	Eμ-$Nras^{G12V}$; ectopic *Bcl2*	Doxorubicin	Lymphoma regression
PTEN inhibitor	Human prostate cancer xenograft	VO-OHpic	Tumour growth inhibition
MDM2 inhibitor	$Pten^{lox/lox}$; Pb-Cre	Nutlin 3	Prostate tumour size decreased
CDK4 inhibitor	LSL-$Kras^{G12V}$; $RERT^{+/ERT}$	PD0332991	Suppression of non-small-cell lung carcinoma development
SKP2 inhibitor	Human lung and prostate cancer xenografts	SKP2 inhibitor#25	Tumour growth inhibition
mTOR inhibitor	Eμ-*Myc*	Everolimus	Lymphoma regression
Immunotherapeutic-induction of senescence			
T_H1 cell cytokines	RIP-Tag2	IFNγ and TNF	Pancreatic β-cell tumour regression

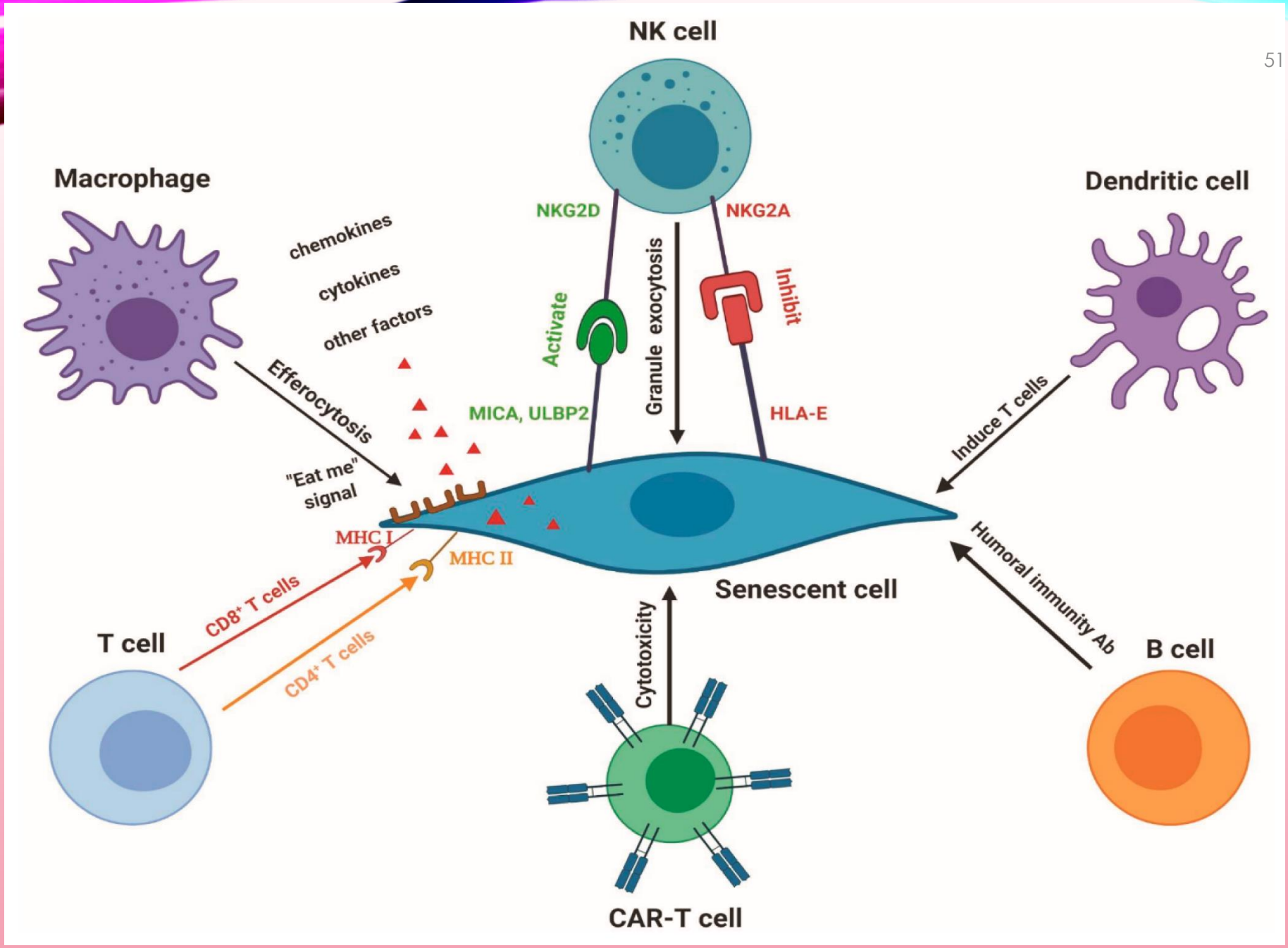
SENESCENCE AND THE IMMUNE SYSTEM

- Given the proinflammatory nature of the SASP, it is not surprising that senescent cells can attract immune cells, including destructive leukocytes of the innate and adaptive immune systems.
- One function of this immune reaction appears to be the killing and eventual clearance of senescent cells.
- Another function appears to be the stimulation of a local immune reaction to eliminate oncogene-expressing cells.
- In addition,genomic damage—a common cause of cellular senescence—induces expression of the membrane-bound ligands for the major natural killer cell receptor NKG2D.Thus, senescent cells appear to be programmed to mobilize the immune system to ensure their eventual elimination
- lymphotactin (XCL-1) is secreted along with other chemokines by aged CD4 and CD8 T cells and is thought to participate in T cell chemokine-dependent diseases of aging like rheumatoid arthritis and atherosclerosis.
- The only known CX3C chemokine, fractalkine(CX3CL-1), is overexpressed in senescent biliary epithelial cells and it is thought to exacerbate ductal inflammation in primary biliary cholangitis.
- It is found that the recognition of retinoic acid early inducible 1ε (RAE1ε; a ligand expressed by tumour cells) by NKG2D (an activating receptor expressed in all NK cells) is crucial for the elimination of senescent tumours.These ligands are induced in response to stress pathways that are associated with tumorigenesis and, indeed, RAE1ε in liver tumour cells was highly expressed before and after p53 restoration.

CANCER AND SENESCENCE COMPLICATIONS

- secreted IL-8 recruits leukocytes and reinforces cancer cell senescence through their upregulated CXCR2 receptors, underscoring a tumor-suppressive role of SASP.
- Studies in animals and humans have shown that although chemotherapy is initially advantageous in virtue of its ability to either kill cancer cells by apoptosis or induce permanent cell cycle arrest of cancer cells by inducing cancer cell senescence,it can subsequently fuel cancer development given the capacity of chemotherapeutic drugs to induce senescence of immune,stromal, and cancer cells.
- In colonic carcinomas, dysregulation of p53 isoforms and p53 mutations are thought to inhibit the induction of p53-dependent cellular senescence and facilitate senescence escape.
- in the skin, senescent cells facilitate recruitment of immunosuppressive myeloid cells which inhibit anti-tumor T cell responses.
- radiation-induced pneumocyte senescence activates fibroblasts to promote pulmonary fibrosis, a late effect of radiation treatment. Radiation therapy also has been shown to induce endothelial cell senescence, which may contribute to cardiovascular disease in cancer survivors.
- In pediatric leukemia patients, increased expression of p16 in non-tumor cells has been suggested as a potential biomarker for radiation- induced cellular senescence that may be associated with the late effects of cancer therapy.These findings leave open the possibility that persistent senescent cells and SASP-associated inflammation are treatment-induced and can exacerbate chronic disease in cancer survivors.

IN ADDITION, THE SASP CAN ATTRACT IMMUNE-SUPPRESSIVE MYELOID-DERIVED SUPPRESSOR CELLS (MDSCS) INTO THE TUMOR.



EXAMPLE SENESCENT CELL TYPES

- Senescence of immune cells blunts the anti-tumorigenic properties of the immune system
- senescent stromal and cancer cells can release SASP factors that stimulate the proliferation of non-senescent cancer cells that survived chemotherapy.
- Most importantly,when senescent cancer cells were allowed to escape following acute inactivation of senescence-essential gene moieties, such as Suv39h1 or p53, they exhibited a much higher tumour-initiation potential than a cell population that had never entered senescence.
- Strikingly,non-stem leukaemia cells gained de novo leukaemia-initiating capacity after passaging through temporary senescence as the pivotal reprogramming state.
- The key stemness-associated Wnt signaling pathway was enhanced in senescent cells due to epigenetic activation and remained constitutively active in a small but stable subset of the cancer cells at the post-senescent state, indicating their lasting role as tumour-reinitiating cells in vitro and in vivo.
- senescence of neural progenitor cells, as can occur in multiple sclerosis, may inhibit oligodendrocyte-mediated remyelination.
- Increased numbers of senescent endothelial cells are thought to promote atherosclerosis and disrupt the blood-brain interface.

WHY DO SENESCENT CELLS INCREASE AND PERSIST WITH AGE?

- One possibility is that There is a striking, well-documented age-related decline in the adaptive immune system, particularly in the ability to mount functional T cell–mediated responses.This decline is largely responsible for the heightened susceptibility to infection in the elderly.
- moreover, the aged innate immune system is more likely to show a loss of proper regulation than a loss of function.
- Another possibility is that, with age, senescent cells are produced at a higher frequency.Indeed, aging tissues show a steady accumulation of cells that harbor DNA damage foci,similar to the foci that are found in senescent cells.
- Finally, the SASP also includes proteins that can help senescent cells evade immune recognition and clearance.For example MMPs,proteaseses can cleave both the cell surface ligands on natural killer target cells and the cell surface receptors on natural killer cells, thereby preventing natural killer cells from targeting and killing senescent cells. There may be a subpopulation of senescent cells that secrete high levels of MMPs, and these cells increase with age. Alternatively, the aging tissue may contain fewer inhibitors of MMPs or other proteases, thereby promoting immune evasion.

TISSUE REPAIR BY SENESCENT CELLS

- In a mouse model of acute liver injury, the injury induced the senescence of hepatic stellate cells,which were eventually cleared by the immune system.When the injury was performed on mice that were deficient in the p53/p21 and p16INK4a/pRB pathways—that is,mice deficient in undergoing a senescence response—healing was accompanied by a marked increase in fibrosis.
- These results provide a causal explanation for earlier findings showing that the presence of senescent hepatic stellate cells correlates with increased inflammation but reduced fibrosis.
- Given that senescent cells increase with age and age-related pathology, why does tissue repair not improve with age?
- One possibility is that senescent cells are beneficial when present only transiently. In acute liver injury, senescent cells are cleared by the innate immune system.
- In this case,senescent cells are not chronically present, which is the case during aging and at the sites of age-related pathologies.
- In the skin, for example, senescent cells clearly promote optimal wound healing.However, when chronically present, they may promote phenotypes associated with skin aging.
- The same is true for the plethora of age-related pathologies in which senescent cells are chronically present.More research is needed to define when and where senescent cells are beneficial.

SENESCENT AND LIFE SPAN

- Evidence from mouse models suggests that clearance of senescent cells improves health span and life span, limiting age associated organ dysfunction.
- Several small-molecule compounds(senolytics) have proven to lead to senescent cell clearance, and some of them are being tested in human trials.
- Results from many studi results proved that naturally occurring senescent cells shorten healthy life span by promoting age-dependent changes in vital organs. The results were independent of sex and genetic backgrounds.

REMOVAL OF SENESCENT CELLS

- Strategies against cell senescence that can be translated into therapies for use in humans
- May be classified into the following groups:

(1) nonpharmacological interventions that prevent the accumulation of senescent cells

(2) pharmacological therapies aimed at reducing the amount of SASP molecules produced by already existing senescent cells

(3) pharmacological therapies aimed at reducing the number of senescent cells in the organism (true senolytics)

- The challenge with the second strategy lies in the ability to block the deleterious effects of SASP proteins, without interfering with their anti-oncogenic properties.
- Within the first group, studies in mice have shown that a 3-month, 26% calorically restricted diet started at adulthood can reduce the number of senescent cells in rapidly proliferating(intestinal) and slowly proliferating (hepatic) tissues.A study of the human colonic mucosa showed that adults who had been voluntarily exposed to a 30% caloric restriction(with appropriate nutrition) for over 10 years had significantly reduced expression of p16INK4a and reduced local concentrations of IL-6.

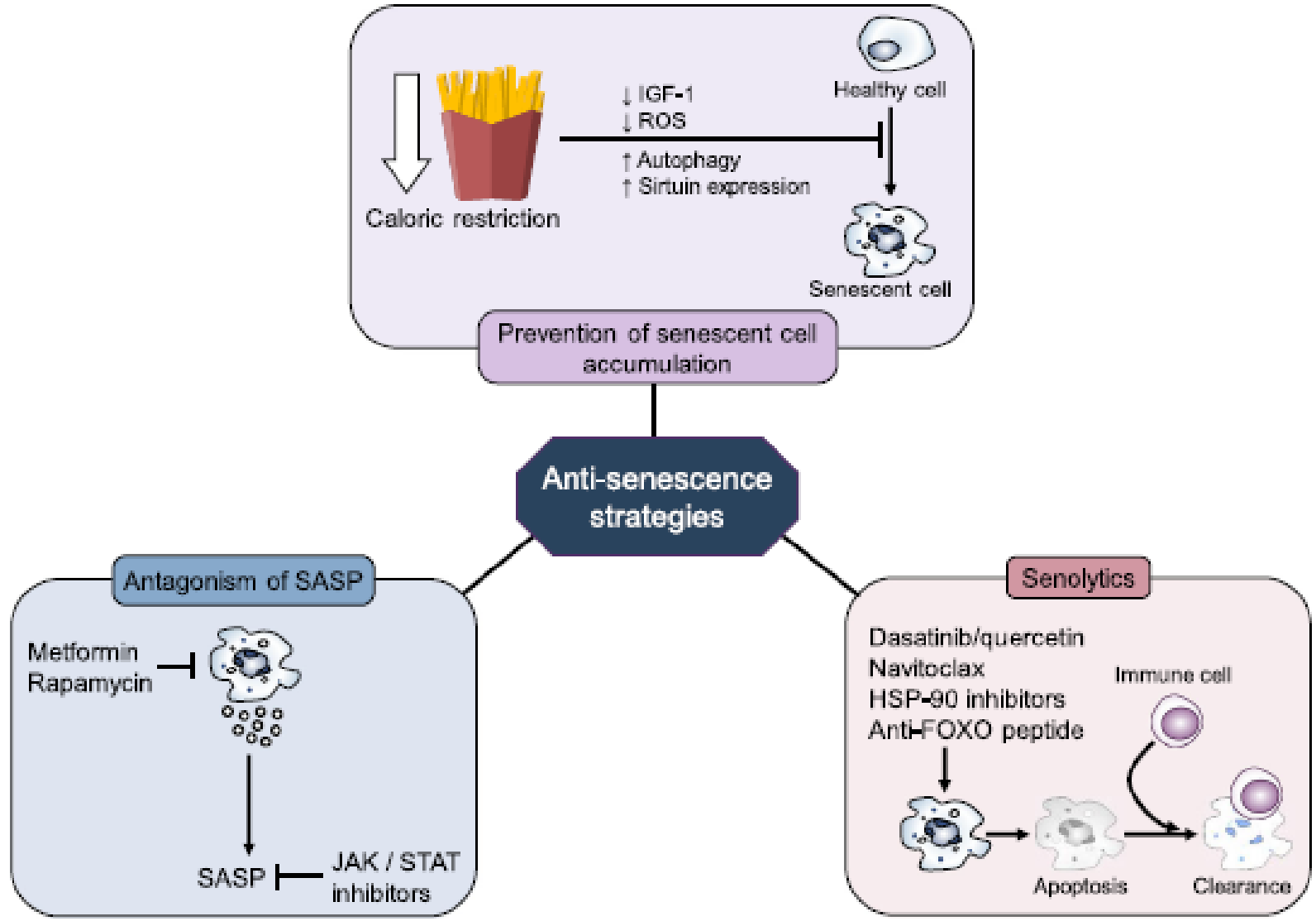


Fig. 6 Therapeutic strategies to counter the effects of senescent cells. IGF-1 insulin-like growth factor 1, ROS reactive oxygen species, SASP senescence-associated secretory phenotype, JAK/STAT Janus kinase/signal transducers and activators of transcription, HSP-90 heat-shock protein 90, FOXO forkhead box proteins O

ANTI-SASP DRUGS

- The category of anti-SASP compounds contains mostly molecules approved for other indications and that have demonstrated antisenescence properties in vitro, in animal models,or in translational studies in humans.
- Among them is the antidiabetic metformin,which is able to block the SASP in transformed fibroblasts.Chronic metformin use is associated with extended life span and health span, independent of its antihyperglycemic efficacy.
- Another long-time used medication is the immunosuppressant rapamycin (sirolimus). By binding to the intracellular signaling molecule mammalian target of rapamycin (mTOR), the drug inhibits transcription of several SASP members and increases autophagy of senescent cells
- A third group of approved drugs with anti-SASP features is JAK-STAT inhibitors.The JAK-STAT pathway is an intracellular signaling cascade responsible for many pro-inflammatory responses. Inhibition of this pathway has an anti-SASP effect in vitro.Since several JAK inhibitors are available for the treatment of autoimmune diseases,further assessment is needed.

- HSP90 Inhibitors:Geldanamycin,tanespimycin,alvespimycin(a water-soluble derivative of Geldanamycin)

- Forkhead Box Proteins O (FOXO) Targeting Peptide:A peptide capable of preventing the FOXO4/p53 interaction excludes p53 from the nucleus and causes preferential apoptosis of senescent cells.

- Fisetin:Fisetin is a polyphenolic compound naturally present in multiple fruits and vegetables.
- Immunotherapies:A different emerging approach involves the enhancement of the natural capacity of immune cells to clear senescent cells. Example:the autologous transplantation of immune cells after being challenged ex vivo with senescent cell-specific antigens, or the use of antibodies that target senescent cells for removal by natural killer cells

SENOLYTICS

- Most efforts, have been placed in the development of senolytics, compounds with the potential to eliminate or reduce the numeric burden of senescent cells.
- Compared to the blockade of SASP, this seems to be a more efficient strategy, as in theory it would only need to be used sporadically in order to eliminate the senescent cells accumulated over a period of time.
- Dasatinib is a tyrosine kinase inhibitor currently approved for the treatment of chronic myelogenous leukemia.
- the natural polyphenolic antioxidant quercetin inhibit key transcriptional nodes used by senescent cells for survival.
- The combination of dasatinib+quercetin greatly reduced senescent cells in mice with radiation exposure, increased DNA damage, or, most importantly, chronological ageing.
- In the Ercc1-/D mouse model of accelerated ageing, dasatinib + quercetin successfully prolonged health span and life span.
- Later studies from the same group found that the cocktail also prevents the accumulation of senescent cells in human adipose tissue explants. Plasma levels of various SASP interleukins and metalloproteinases were also reduced.
- Bcl Family Inhibitors:Navitoclax is an anticancer drug that acts by inhibiting several Bcl family members and promoting the release of pro-apoptotic factors. In contrast to dasatinib + quercetin, navitoclax lacks senolytic effect in adipose tissue,but displays a strong effect on endothelial cells and fibroblasts.

Table 2. Senolytic-induced nitric oxide (NO) modulations.

Senolytic Drugs	NO Changes	Model	References
Dasatinib	Increased NO	Pulmonary artery endothelial cells and smooth muscle cells	[124]
	Increase iNOS expresion	Intestinal and bone morrow-derived macrophages	[125]
	Decrease iNOS expresion	Silicotic macrophages	[126]
Quercetin	Inhibition of iNOS		[127]
	Inhibition of mRNA iNOS	Human hepatocyte-derived cell line	[128]
	Inhibition of NO production and iNOS expression	Livers of CCl4-treated mice	[129]
		In vitro (rat hepatocyte)	[130]
		In vitro and in vivo (RAW 264.7 macrophages, primary peritoneal macrophages and Balb/c mice)	[131]
		Chronic cadmium nephrotoxicity in rats	[132]
		Lung adenocarcinoma cell lines	[133]
Hsp90 inhibitor	Reduction of NO production	Endothelial cells	[134]
	Blocked VEGF-induced increase in eNOS activity	Endothelial cells	[135]

6.2. Bcl-2 Inhibitors

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