Charles University

Faculty of Science

Study programme: Molecular Biology and Biochemistry of Organisms

Andrea Franců

Dual role of senescent cells in carcinogenesis and regulation of their secretome using senomorphic agents

Dvojí role senescentních buněk v kancerogenezi a regulace jejich sekretomu látkami se senomorfní aktivitou

Bachelor´s thesis

Supervisor: RNDr. Milan Reiniš, CSc.

Prague, 2025

Poděkování:

Ráda bych poděkovala svému školiteli, RNDr. Milanu Reinišovi, CSc., za jeho přínosné rady, nekonečnou trpělivost a vstřícnost. Dále bych chtěla poděkovat všem, kteří mě během studia podporovali.

Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Umělá inteligence (AI) byla použita pouze ke korektuře textu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 29. 4. 2025 Andrea Franců

ABSTRAKT

Buněčná senescence je stresová odpověď buňky a představuje především ochranný mechanismus proti nádorové transformaci, avšak může také přispívat k růstu nádorů. Během senescence se aktivují signalizační dráhy, které regulují expresi a tvorbu se senescencí asociované sekrečního fenotypu (SASP). Komponenty sekretomu zprostředkovávají interakci s prostředím a ovlivňují imunitní systém. Některé protinádorové terapie vedou a využívají senescenci k inhibici růstu nádoru a k regulaci imunitní odpovědi. Zároveň však mohou způsobit senescenci v nenádorových buňkách, které také mohou podpořit růst nádorů a potlačit imunitní odpověď. Pokud nedojde k odstranění senescentních buněk nebo jejich vlivů, přetrvávající účinky sekretomu vedou k chronickému zánětu, podporují invazi do okolní tkáně a přispívají k progresi nádoru po remisi. Cílené odstranění senescentních buněk nebo modulace sekretomu představuje v posledních letech možné řešení pro potlačení vedlejších účinků protinádorových terapií. Senomorfika jsou látky, které cílí na fenotyp senescentních buněk a které většinou inhibují signalizační dráhy regulující sekretom bez toho, aniž by ovlivnily životaschopnost buněk (na rozdíl od senolytik). Dlouhodobé potlačení účinků sekretomu pomocí senomorfních agents by mohlo v budoucnu odvrátit negativní dopad senescentních buněk na nádorové mikroprostředí a přispívat k léčbě dalších nemocí spojených se stárnutím.

Klíčová slova: Buněčná senescence, kancerogeneze, sekretom, cytokiny, imunosuprese, senomorfní látky

ABSTRACT

Cellular senescence is a stress response that primarily serves as a protective mechanism against tumor transformation. However, it can also contribute to tumor growth. During senescence, signaling pathways that regulate the expression and production of senescence-associated secretory phenotype (SASP) are activated. Components of the secretome mediate interactions with the microenvironment and influence the immune system. Anticancer therapies induce and utilize senescence to inhibit tumor growth and regulate the immune response. Such therapies can also induce senescence in non-cancerous cells, which may support tumor growth and suppress the immune response. If senescent cells or their effects are not eliminated, the persistent presence of the secretome leads to chronic inflammation, promotes invasiveness and cancer relapse. Targeted removal of senescent cells or modulation of their secretome has emerged as a potential solution to alleviate the side effects of anticancer therapies. Senomorphic agents target the phenotype of senescent cells and typically inhibit the signaling pathways that regulate the secretome without affecting cell viability (unlike senolytics). Long-term modulation of secretome activity using senomorphic agents could help overcome the negative impact of senescent cells on the tumor microenvironment and contribute to the treatment of other age-associated diseases.

Key words: Cellular senescence, carcinogenesis, secretome, cytokines, immunosuppression, senomorphic agents

TABLE OF CONTENTS

LIST OF ABBREVIATION

TIS	therapy-induced senescence
OIS	oncogene-induced senescence
DDR	DNA damage response
SAHF	senescence-associated heterochromatic foci
ROS	reactive oxygen species
DAMP	damage-associated molecular patterns
SASP	senescence-associated secretory phenotype
SA-β-gal	senescence-associated β galactosidase
SCAPS	senescent cells anti-apoptotic pathways
TGF-β	transforming growth factor
cGAMP	cyclic-GMP-AMP
cGAS	cGAMP synthase
NF-κB	nuclear factor-κB
JAK	Janus kinase
STAT	signal transducer and activator of transcription
ATM	ataxia-telangiectasia mutated
IκB	inhibitor of NF-κB
IKK	IκB kinase complex
TAK1	TGF-β activated kinase 1
CCF	cytosolic chromatin fragments
IFN	interferon
IRF	IFN regulatory factor

mTOR	mechanistic/mammalian target of rapamycin
mTORC	mTOR complex
MAPK	mitogen-activated protein kinase
MAPKAPK2/MK2	MAPK-activated protein kinase 2
C/EBPβ	CCAAT/enhancer binding protein β
CD	cluster of differentiation
(G)-MDSC	(granulocytic) myeloid-derived suppressor cells
MHC	major histocompatibility complex
HLA	human leucocyte antigen E
NK	natural killer
Treg	regulatory T cells
EMT	epithelial-mesenchymal transition
FKBP	FK506-binding protein
CAR	chimeric antigen receptor
uPAR	urokinase-type plasminogen activator receptor
PD-1/PD-L1	programmed cell death 1/programmed cell death ligand 1
TAME	targeting aging by metformin
SIRT/sirtuin	silent information regulator
Rb	retinoblastoma protein
TME	tumor microenvironment
NEMO	NF-κB essential modulator
NBD	NEMO-binding domain
IL	interleukin

TNF	tumor necrosis factor
MICA	MHC class I chain-related protein A
RAS	Rat sarcoma
PTEN	phosphate and tensin homolog
$p21^{cip1}$	cyclin-dependent kinase inhibitor 1
$p16^{INK4a}$	cyclin-dependent kinase inhibitor 2A
BCL	B cell lymphoma
MCL-1	myeloid cell leukemia-1
GRO	growth-regulated oncogene
MCP	monocyte chemoattractant protein
MIP	macrophage inflammatory protein
GM-CSF	granulocyte-macrophage colony-stimulating factor
GATA4	GATA binding protein 4
SH2	Src homology 2
BRD4	bromodomain containing protein 4
MLL	mixed lineage leukemia
NKG2A	natural killer group 2 member A
NKG2D	natural killer group 2 member D
CDK	cyclin-dependent kinase

1. INTRODUCTION

Senescence, as one of the possible fates of a cell, is a complex process responding to physiological and pathological stimuli. Stressors induce irreversible cell cycle arrest and activate signaling pathways that lead to changes in gene expression. Many of these pathways contribute to the establishment and regulation of senescence-associated phenotype. Senescence-associated secretory phenotype (SASP), which is an integral part of phenotypic changes, is the main mediator between the cell and its environment, primarily interacting with the immune system. The phenotype is not universal and is dependent on the cell type, duration, intensity and type of the stimulus as well as other factors.

Initially, senescence was understood to be a defense mechanism against tumor transformation, but it was later discovered to be an integral part of other biological and pathological processes as well. Chronic inflammation caused by persistent SASP contributes to the development of age-related diseases. Anticancer therapies often aim to induce apoptosis or senescence as a strategy to suppress tumor growth. Senescent cancer cells promote immunosurveillance, leading to their subsequent elimination. However, senescent cells also stimulate an immunosuppressive environment, promoting the onset of age-related diseases and enhanced carcinogenesis. The main question is therefore how to preserve the beneficial properties of senescent cells while limiting the negative impact.

In the last decade, there has been significant progress in developing agents that target senescent cells. Senolytics and immunomodulators eliminate senescent cells, suggesting their potential for complementary use in anticancer therapies. Senomorphic agents target pathways that regulate SASP, thereby modulating SASP secretion and its effects on the surrounding microenvironment.

The objective of this work is to describe the key pathways regulating the senescence-associated secretome and to critically evaluate the controversial dual role of senescent cells in immunosurveillance and immunosuppression within the context of carcinogenesis. Another objective of this work is to evaluate how these findings could be used in the development and application of senotherapeutic (especially senomorphic) agents to counteract the adverse effects of cancer treatment and potentially delay the onset of age-related diseases.

2. CELLULAR SENESCENCE

Cellular senescence is defined as an irreversible arrest of the cell cycle, preventing the transmission of damage into the next generation of cells.[1] The concept of cellular senescence was originally set up when the limit of cell multiplication in human fibroblasts was discovered. The cycle arrest was first attributed to internal factors (replicative stress) that manifests as aging at cellular level.[2] However, senescence can be triggered not only by replicative stress, but also by many other external and internal stressors. There are several types of premature/accelerated cellular senescence, each triggered by different stimuli. These types include therapy-induced senescence (TIS) caused by therapeutic agents, oncogene-induced senescence (OIS, mainly *RAS* gene activation) resulting from oncogene activation, senescence associated with the loss of tumor suppressor genes (mostly *PTEN* gene depletion), stress-induced senescence due to factors like oxidative stress or ionizing radiation. These factors usually lead to cellular stress (genotoxic stress, endoplasmic reticulum stress, oxidative stress) and DNA damage that activates DNA damage response (DDR) and other signaling pathways.[3, 4] The mechanisms of regulation and phenotype of premature and replicative senescence overlap.[5] The maintenance and control over senescence are mostly controlled by p53/$p21^{cip1}$ and $p16^{INK4a}$/Rb tumor suppressor pathways.[6] The most abundant type of senescent cells in humans are fat cell progenitors (preadipocytes), endothelial cells, fibroblasts and epithelial cells.[7]

The senescence phenomenon plays a dual role in the organism – physiological and pathological. Senescence not only prevents uncontrolled cell proliferation, but it also contributes to key processes during development and embryogenesis. In mammalian embryos, senescence-associated markers are detectable in key developmental regions, including the neural tube and the forming forelimb.[5] Other beneficial functions of senescence include new tissue formation and remodeling, which occur as part of the repair response to tissue injury. Although senescence plays an important role against carcinogenesis, it may be involved in the development of dysfunctional immunosurveillance, age-related diseases (cardiovascular, fibrosis, diabetes) and can even promote carcinogenesis.[3]

3. PHENOTYPE OF SENESCENT CELLS

Senescent cells may be enlarged, as they undergo cytoskeleton-mediated shape changes and they may vary in the composition of their plasma membrane.[8] However, the combination of these and below mentioned markers is an indication of the overall state of the cells and cannot be viewed as a universal list. The phenotype of senescent cells is heterogeneous and variable and can be influenced by several factors. It depends on the cell type, as well as the way in which senescence was induced.[1] It is important to understand how the specific phenotype of senescent cells can influence the environment and which markers help us to objectively evaluate and assess senescent cells from other cells. For example, terminally differentiated cells are also able to steadily withdraw from cell cycle as a response to physiological cues. Senescent cells can respond to physiological and pathophysiological stimuli.[4]

One of the observable markers is a cell cycle arrest which involves p53/$p21^{cip1}$ and $p16^{INK4a}$/Rb pathways. DDR stabilizes the transcription factor p53 levels, allowing upregulation and downregulation of its target genes, including *CDKN1A* gene encoding p21 protein. This cyclin-dependent kinase (CDK) inhibitor is essential for establishing cell cycle arrest and resistance to apoptosis. After the induction of senescence, p53 protein level drops and p16 is upregulated. The p21 and p16 inhibit CDK mediated phosphorylation of Rb, preventing cells to initiate S phase and preventing cells from dividing.[6] An important question is whether the cell cycle arrest is reversible. If replicative senescence is responsible for the arrest, telomerase in human cells cannot change the cell fate and the cycle cannot be restored. Only p16-negative cells are able to restore proliferation by inactivating p53. This suggest that p16 plays an important role in maintaining the irreversibility of cell cycle arrest in senescent cells and tumor suppression. Mutations disrupting these mechanisms lead to carcinogenesis.[9] Another key role of $p16^{INK4a}$/Rb pathway is the induction of SAHF formation.

Senescence-associated heterochromatic foci (SAHF) are facultative heterochromatin structures which are associated with proteins and contribute to the silencing of genes that are regulated by E2F transcription factor. The formation of SAHF and the stable silencing of E2F target genes require the activation of the tumor suppressor protein Rb. The chromatin changes can be visualized using DAPI staining. Number of DAPI foci can be spread throughout the nucleus and they correspond to SAHF.[10] SAHF mostly occurs in oncogene-induced senescent cells.[1] Another alteration affecting the nucleus is the reduced expression of lamin B1 leading to a disruption of the integrity of the nuclear envelope. This causes increased permeability of

nucleus and subsequent leakage of nuclear chromatin into the cytoplasm.[11] Cytosolic DNA signaling by cGAS/STING pathway regulates other phenotypic traits.

Another marker that can be observed is senescence-associated β-galactosidase (SA-β-gal). Elevated expression of this senescence-associated hydrolase has not been demonstrated in quiescence nor in terminally differentiated cells and it has not been detected in immortal cells. Its induction is determined by both replicative and physiological age, implying that it could act as a biomarker of aging.[12] SA-β-galactosidase staining is used as a visualization technique for demonstrating the onset of replicative senescence. SA-β-gal origin and function are still mostly unknown. The rise in SA-β-galactosidase activity corresponds with an increase in lysosomal mass. Thus, it originates most likely in lysosomes.[13]

Senescence-associated mitochondrial dysfunction is caused by dysregulated mitochondrial turnover. This impaired regulation results in increased mitochondrial mass and higher oxygen consumption. It can lead to decreased efficiency of oxidative phosphorylation and a higher production of reactive oxygen species (ROS). ROS activates the DNA damage signaling pathway which may result in the development of senescence.[14] Dysfunctional mitochondria may also release ROS, its DNA (mtDNA) and formyl peptides, which are recognized as intracellular danger-associated molecular patterns (DAMP). These signals are then recognized by NOD-like receptors 3, leading to the activation of inflammatory caspases and the subsequent production of pro-inflammatory cytokines IL-18 and IL-1β. This demonstrates the correlation between dysregulated mitophagy, accumulation of dysfunctional mitochondria and senescence-associated secretory phenotype (SASP) production.[15]

Changes in cellular signaling lead to changes in gene expression and protein secretion, regulating senescent cell anti-apoptotic pathways (SCAPs) or developing senescence-associated secretory phenotype (SASP). Research in some of these senescence markers in human and mouse tissue has revealed that senescence only occurs in premalignant lesions. Its absence in malignant lesions indicates that cellular senescence is primarily a tumor-suppressive process *in vivo*.[16]

3.1. SENESCENCE AND APOPTOSIS

Another protective mechanism against cell damage is apoptosis, programmed cell death. In order to survive DNA damage, metabolic flux and other apoptosis inducing factor, senescent and cancer cells had to develop mechanisms to escape cell death. This resistance of senescent

cells to apoptosis is attributed to the overexpression of negative regulators of apoptosis and anti-apoptotic and pro-survival signaling pathways.[17] The signaling includes anti-apoptotic BCL-2, BCL-XL, BCL-W and MCL-1 proteins.[18] These upregulated proteins prevent cytochrome c release from the permeabilized outer mitochondrial membrane counteracting caspase-2 activation driven by DNA damage-mediated p53 stabilization.[19] Some chemotherapies target highly proliferating cells by imposing genotoxic stress and activating apoptosis or senescence. There are ongoing studies developing alternative therapeutic agents clearing TIS cells by inducing apoptosis. These agents, so called senolytics, could alleviate adverse effects and serve as a complementary anticancer treatment.[20,17]

4. SASP, ITS COMPONENTS AND ITS REGULATION

Although senescent cells do not proliferate, they remain metabolically active. Senescent cells gradually undergo metabolic alterations that lead to different gene expression and subsequent secretion of proteins. This wide-range profile of proteins is cell and inducer type specific and can consist of cytokines, chemokines, signaling molecules, growth factors, regulators and proteases.[21] This profile is then collectively referred to as senescence-associated secretory phenotype (SASP). The production of SASP components is triggered by inflammasome and IL-1α signaling.[22]

Important components of SASP are pro-inflammatory cytokines, such as IL-1, IL-6, chemokine IL-8, GRO-A, MCP-1, MIP-3α, HGF, GM-CSF and many more.[21] Senescent cells also shed surface molecules (ICAMs, uPAR, TNF receptors) and survival factors.[23] IL-6 and IL-8 are able to not only maintain the pro-inflammatory state and attract immune cells, but they have also been controversially associated with reinforced growth arrest in surrounding cells.[24] Elevated IL-8 production affects and increases angiogenesis and vascularization.[25] The regulation of SASP occurs on an epigenetic level, at the transcriptional and post-transcription stage and on a protein level (cleavage of precursors).

4.1. CELLULAR PATHWAYS THAT REGULATE GENE EXPRESSION AND SASP

It is essential to mention that cell cycle arrest in senescent cells, following DNA damage, acts as a barrier against tumor transformation. To alert the environment about the cell's damage, various signaling pathways that regulate gene expression are activated (Figure 1). Upon activation of the DDR, chromatin can leak into the cytoplasm, forming cytoplasmic foci and trigger the cGAS/STING cytosolic DNA-sensing pathway. The activated transcription factors then regulate SASP production. The inflammatory response is further amplified by JAK/STAT signaling. Additionally, the mTOR pathway regulates DDR though regulation of the p53 and p21.[26] All these pathways activate major transcription factors (NF-κB, C/EBPβ, GATA4, STAT, IRF3) and contribute to the specific secretome of senescent cells.[27] The family of the master transcription factor known as nuclear factor-κB (NF-κB) includes the proteins p50, p52, p65/RelA, RelB, and c-Rel. Stimuli (RNA, DNA, lipopolysaccharide, cytokines) activating NF-κB signaling regulate cellular fate and immune response through inflammation. Dysregulation

may cause pathological changes.[28] Epigenetic regulators (SIRT1) have also been shown to modulate SASP expression.[1]

4.1.1. DNA DAMAGE RESPONSE

To handle the threats posed by DNA damage, cells have evolved mechanisms that form DNA damage response (DDR) signaling pathway. Upon detecting DNA damage, the cell sends a signal indicating DNA damage and initiates attempts to repair it. If effective repair occurs, the proliferation process resumes. If the repair is unsuccessful, the cell undergoes apoptosis or enters a state of senescence, depending on the significance and duration of the stimulus.[29]

Upon the site of DNA damage, a MRN complex senses double strand breaks, activating ataxia-telangiectasia mutated (ATM) kinase. ATM then phosphorylates checkpoint kinase 2, p53 and histone variant H2AX. This leads to a build-up of DNA repair proteins and chromatin-remodelling complexes near damaged sites. Phosphorylated H2AX (γH2AX) recruits Mdc1 protein that triggers the ATM to spread and propagate H2AX phosphorylation across surrounding damaged chromatin domains, forming γH2AX foci chromatin.[30]

Another target of ATM is ubiquitin ligase Mdm2. Phosphorylation of p53 and Mdm2 by ATM stabilizes p53 levels in the cell, allowing p53 to upregulate or downregulate the expression of genes involved in DNA repair, cell cycle arrest, apoptosis, senescence and quiescence.[6] A common feature of cancer cells is the presence of mutations in the *p53* gene, leading to loss of p53 function. Reactivation of p53 is responsible for tumor suppression caused either by inducing senescence or apoptosis.[31]

ATM also mediates the activation of TGF-β activated kinase 1 (TAK1) and IκB kinase complex (IKK). ATM forms complexes with ELKS, NEMO, IKK and other proteins that are important for binding TAK1. The complex of TAK1 and IKK promotes autophosphorylation of TAK1, followed by activation of IKK. Activated IKK phosphorylates the inhibitor of NF-κB (IκB), causing it to unbind from the NF-κB.[28] After NF-κB separates from IκB in the cytoplasm, NF-κB is released and forms dimers that are translocated to the nucleus. Dimers then bind to promoters of target genes and act as transcriptional factors. The formation of SASP (mainly inflammatory compounds) and aging mechanisms are linked to the activation of NF-κB.[32]

DDR in OIS cells induces degradation of G9a/GLP methyltransferase, leading to reduced levels of histone H3 monomethylated on lysine 9 and histone H3 dimethylated on

lysine 9. The (di)methylation acts as an epigenetic marker for euchromatic gene silencing. The change in histone methylation around IL-6 and IL-8 promoters leads to their transcriptional activation and expression.[33]

Prolonged or permanent DNA damage signaling is important for the induction and maintenance of senescence.[34] Some chemotherapeutic agents impose genotoxic stress to the cell, inducing DDR and other pathways that lead to senescence and modulate gene expression, using it to activate antitumor immunity.[20]

4.1.2. cGAS-STING PATHWAY

DNA damaging agents that induce senescence or spontaneous immortalization that impact nuclear envelope's integrity. The lamin B1 degradation results in the accumulation of cytosolic chromatin fragments (CCF), activating the cytosolic cyclic-GMP-AMP (cGAMP) synthase (cGAS).[35] cGAS acts as a cytosolic sensor of double-stranded DNA and, upon binding to dsDNA, induces the production of the second messenger cGAMP that activates the endoplasmic reticulum protein STING.[36] This adaptor protein recruits and activates IKK and TANK-binding kinase 1 and they activate NF-κB and interferon regulatory factor 3 (IRF3) transcription factors. Once activated, these transcription factors translocate to the nucleus, where they trigger the production of type 1 interferons (IFNs) and cytokines.[35]

4.1.3. JAK/STAT PATHWAY

Janus family of protein tyrosine kinases (JAKs) are non-covalently associated with cytokine receptors and they activate members of the signal transducer and activator of transcription (STAT) in cytoplasm. JAK kinases are activated by autophosphorylation upon ligand binding to its cytokine receptor. JAKs then mediate phosphorylation of tyrosine in the receptors, creating binding sites for SH2 domains of proteins (including STAT proteins). This leads to the recruitment and phosphorylation of STAT proteins. STAT monomers interact with each other, creating homodimers or heterodimers. Dimerization results in the translocation of STATs to the nucleus to initiate transcription of effector genes.[37] The JAK/STAT pathway plays a major role in cytokine production, hematopoiesis, immune response and many more.[38]

Janus kinase family includes four main members: JAK1, JAK2, JAK3 and tyrosine kinase 2 (TYK2). JAKs are composed of 7 homology domains, including kinase and pseudokinase domains, SH2 and FERM domains. The interaction between JAK and STAT is mediated through the pseudokinase domain.[38] The STAT family includes seven members:

STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6. The SH2 domain recognizes phosphorylated motifs in cytokine receptors, while the linking domain connects to the DNA-binding domain, which is responsible for regulating target genes as well as nuclear export and import.[39]

The inflammatory cytokine IL-6, controlled by NF-κB, is an activator of STAT3. This indicates that NF-κB, STAT3 and its upstream mediator JAK2 regulate a variety of oncogenic and inflammatory genes.[40] It has been shown that activated JAK 1 and JAK2/STAT3 signaling is a key factor for regulating the inflammation-SASP-senescence feedback loop.[41] STAT3 inactivation in *PTEN*-loss-induced senescent cancer cells led to a decline in immunosuppressive cytokine levels (IL-10, GM-CSF, M-CSF, IL-13) and therefore enabled immunosurveillance. This STAT3 inactivation reprograms SASP and activates antitumor immune response without affecting proliferation, apoptosis or NF-κB signaling.[42] Senomorphic therapies utilise the properties of JAK/STAT inhibition to avert chronic consequences of SASP.

4.1.4. mTOR PATHWAY

The mechanistic/mammalian target of rapamycin (mTOR) is a phosphatidylinositol 3-kinase-related serine/threonine protein kinase that participates in the regulation of autophagy, metabolism, protein and lipid synthesis and many more cell growth related functions. The mTOR complex 1 (mTORC1) is activated by intracellular signals, including DNA damage, and interacts with DDR. Dysregulation of mTOR signaling can impair DDR and promote carcinogenesis.[26]

The mTOR signaling pathway is responsible for enlarged morphology and production of SASP components.[43] The mTOR manages translation of mitogen-activated protein kinase-activated protein kinase-2 (MAPKAPK2), also known as MK2. The upregulated MK2, a downstream effector of p38MAPK signaling, phosphorylates and inhibits mRNA binding protein ZFP36L1. This phosphorylated protein is then unable to bind to the 3' untranslated region of mRNA of SASP components, preventing their degradation.[44] Inhibiting mTOR with rapamycin suppresses IL-1α translation causing a decrease in its autocrine signaling. The positive feedback loop between IL-1α and NF-κB is disrupted, leading to a decline in SASP protein levels.[45] Another transcription factor acting on IL-1α production is GATA4.[46] CCAAT/enhancer binding protein (C/EBPβ) transcription factor regulates the production of IL-6, IL-1β, IL-8 and many more.[24] BRD4 and MLL are both epigenetic regulators.[27]

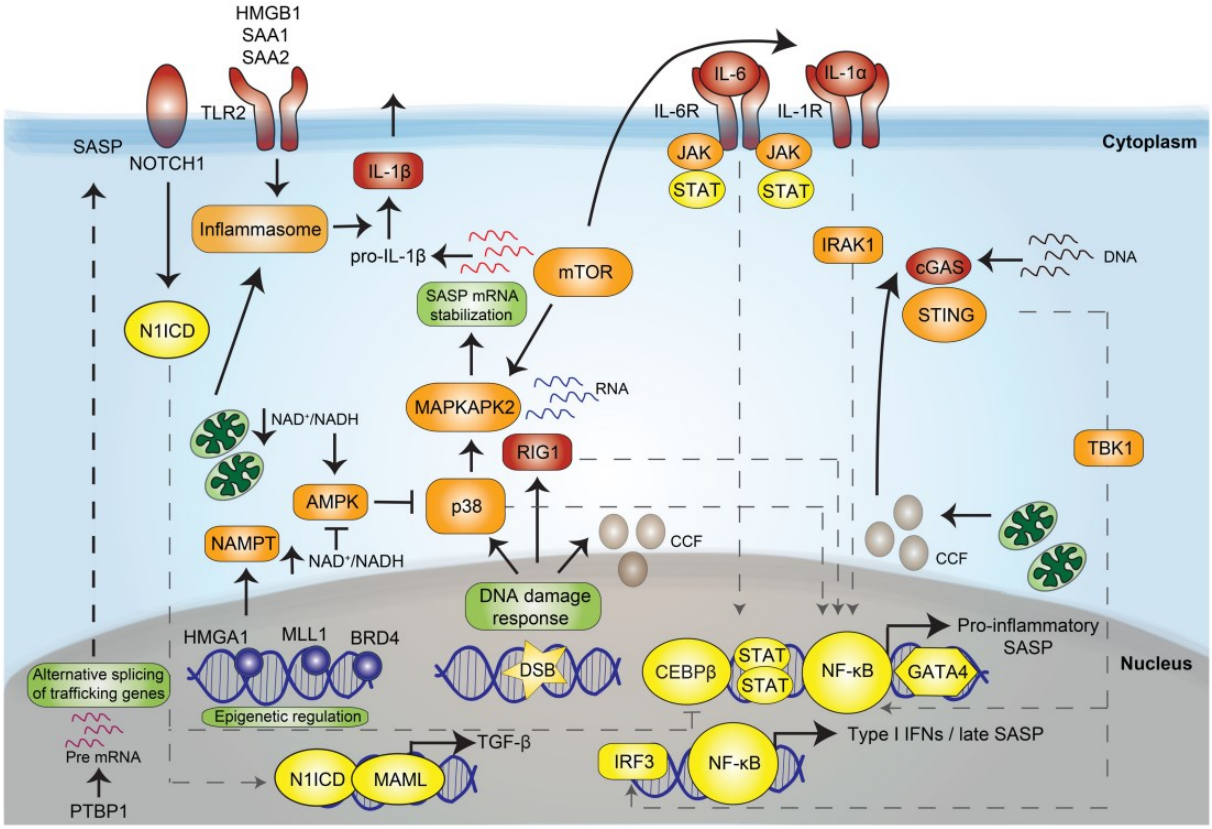

Figure 1: This scheme shows how the regulation of SASP is interconnected and complex. For better understanding, only a part of the mechanisms involved in the regulation was selected. The overall outcome is the expression of pro-inflammatory cytokines, type I IFN and other SASP components. Sensors, receptors and ligands (red) are connected through intracellular signaling molecules (orange) with transcriptional factors (yellow).[27]

5. THE DUAL ROLE OF CELLULAR SENESCENCE IN CANCER

Senescence affects individual cells, the microenvironment and the whole organism. Inducing senescence is primarily a barrier against cancer development. The response and elimination by the immune system is crucial for maintaining homeostasis. The clearance of senescent cells is mediated by innate and adaptive immune system. The SASP components recruit and coordinate natural killers (NK), antigen-presenting cells, T lymphocytes and others.[3]

5.1. SENESCENCE IN CANCER AND STROMAL CELLS

The context in which senescence is induced, including the specific cell type involved, is crucial for understanding its characteristics and the outcomes. Senescent cancer cells mediate strong antitumor response. After recruitment of immune cells, senescent cancer cells promote the activation and maturation of dendritic cells and therefore enhance the antigen-dependent activation of CD8+ T cells.[47] Many chemotherapeutic drugs inducing senescence intend to activate this antitumor response.[20] The upregulation of NKG2D and DNAM-1 ligands on senescent cancer cell surface after chemotherapy treatment also increases degranulation of NK cells.[48] The issue with these drugs seems to be the induction of senescence in noncancerous cells, contributing to the side effects associated with it.[49] Senescent stromal cells (fibroblasts, endothelial cells) recruit granulocytic myeloid-derived suppressor cells (G-MDSCs) that suppress CD8+ T cell function. This immunosuppression drives carcinogenesis and other pathologies.[50] The detrimental effects are the subject of ongoing senotherapy research.

5.2. BYSTANDER SENESCENCE

The senescent secretome acts in both cell-autonomous and non-cell-autonomous manner. A non-random accumulation of senescent cells in their surrounding had been observed. Induction of DNA damage in normal cells driven by ROS was found to be transmitted via gap junctions. This cell to cell contact induces bystander effect in neighbouring cells.[51]

Another possibility for inducing senescence in surrounding cells is by secreting transforming growth factor (TGF-β), IL-1 and other SASP components. This so called paracrine senescence is dependent on many factors (cell type, concentration of SASP components, proliferation) and can expand in premalignant lesion, preventing malignant transformation and enhancing immune clearance.[22]

5.3. ACTIVATION OF ANTITUMOR IMMUNITY

Senescent cells releasing DAMP molecules directly stimulate the innate immune response through pattern recognition receptors.[15] These danger signals are essential for the activation and enhancement of adaptive immune cells. In their absence, antigen presentation to T lymphocytes promotes peripheral tolerance and suppresses immune response.[47]

Immunosurveillance of senescent cells is also mediated by CD8+ T cells, as well as by NK cells. Senescent cells present increased levels of MHC-related proteins, including MICA and ULBP2, that are ligands of stimulatory cell receptor NKG2D on NK cells. This immunogenic feature may be suppressed by increased levels of non-classical MHC-I glycoproteins (HLA-E), ligands to inhibitory receptor NKG2A. The outcome of the immune response is dependent on the more prevalent inhibitory and stimulatory receptor signals, providing future therapeutic approaches.[52,53] HLA-E expression is induced by IL-6 and regulated by p38MAPK[52], further pointing to the interconnection between SASP, its signaling pathways (Figure 1) and the immune system. The elimination of target cells by NK and CD8+ T cells is mediated through the release of pore-forming protein perforin and protease granzyme, which induce apoptosis. The decline in NK cell cytotoxicity (decreased perforin binding) with age affects the accumulation of senescent cells.[54]

The produced type I IFNs stimulate increased major histocompatibility complex (MHC) class I presentation.[55] Enhanced presentation of stress associated self-peptides on MHC-I of senescent cells induces antigen-dependent activation of CD8+ T cells. The opposite happens in cancer cells, as they downregulate MHC-I presentation to escape adaptive immunity. Thus, this IFN mediated upregulation could act as a strategy to prevent escape from immune system and trigger antitumor adaptive immune response. The highly effective antitumor response induced by senescent cancer cells sparked new opportunities for anticancer vaccine development approaches.[47] Immunosurveillance mediated by CD4+ T helper 1 lymphocytes though antigen-presenting cells resulted in an elimination of premalignant senescent hepatocytes and melanocytes. This elimination is mediated through macrophages. [56]

5.4. NEGATIVE IMPACT

5.4.1. SYSTEMATIC

If senescent cells escape elimination or after TIS cells accumulate in the organism, pathological changes may occur.[57] A decline in the function of the immune system is believed

to be responsible for the build-up that happens especially with age, causing the development of age-related diseases (including cancer).[7] However, it is up for a debate whether the disease is a cause or a direct consequence. For example, senescent β-cells limit insulin secretion, increasing the risk of developing type 2 diabetes mellitus.[58] Chronic inflammation can lead to increased activity of enzymes like matrix metalloproteinases (extracellular matrix-degrading enzymes secreted by fibroblasts) which can damage the extracellular matrix.[59] At the same time, infiltrating immune cells can overproduce substances that promote uncontrolled proliferation.[60] Chronic inflammation caused by SASP is responsible for invasive behaviour of cells, angiogenesis and extracellular matrix remodelation,[23] causing atherosclerosis, fibrosis, cancer and many more age-related diseases.[21]

5.4.2. TUMOR MICROENVIRONMENT (TME)

Cellular senescence plays a role in promoting chemotherapy-related side effects and cancer relapse. Many chemotherapeutic drugs (doxorubicin, docetaxel) target cell cycle and limit the proliferation ability. Therapy-induced senescence (TIS) cells mobilize immune cells against the damaged and tumor cells area. The side effects also include an induction of senescence in noncancerous cells. The SASP of senescent noncancerous cells promotes the development of chronic inflammation, leading to local and systemic negative effects (cardiac dysfunction, bone marrow suppression) and many more age-related diseases.[49,61] If these TIS cells restore cell cycle through elevated expression of Cdc2/Cdk1 kinase[62], they display senescence-associated stemness. This can result in increased carcinogenesis initiation[63], more aggressive cancer growth and therapy resistance in comparison to cells that never underwent senescence.[64] Senescent fibroblasts secreting IL-8 and IL-6 contribute to the induction of epithelial-mesenchymal transition (EMT), enabling invasive and metastatic behaviour of epithelial cells through a basement membrane.[23] Removing TIS cells after chemotherapy lowered the possibility of relapse and metastasis of cancer and postponed the onset of age-related diseases.[49]

Secretome of senescent stromal cells increases inflammation and can attract granulocytic myeloid-derived suppressor cells (G-MDSC). MDSC are responsible for the induction of FOXP3+ T regulatory lymphocytes activity. Together, MDSC and Treg create immunosuppressive microenvironment by inducing apoptosis and suppressing the proliferation of CD8+ T lymphocytes, mediated by an inducible nitric oxide synthase mechanism. [65,50] Treg cells are also able to induce senescence in both naive and effector CD4+ and CD8+ T lymphocytes. The Treg-induced senescent T lymphocytes exhibit reduced expression of

costimulatory molecules CD27 and CD28 on cell membrane, limiting their activation. Paracrine senescence can be induced in surrounding T lymphocytes, further amplifying the ineffective elimination.[66] MDSC also inhibit NK cells granule exocytosis.[67] The limited ability of antitumor immunity to respond contributes to carcinogenesis.

MDSC also modulate the M1-M2 polarization of macrophages toward a M2-like phenotype. Macrophages exhibiting a M2-polarized phenotype are associated with tumor promotion and development, as well as increased metastasis in lung cancer.[65] The predominant cell type with senescent features within the lung TME are macrophages and endothelial cells. Senescent-like state in macrophages can be induced by inflammation and they promote invasion (BCA-1 secretion), metastasis and cancer (MCP-3 secretion).[68]

An increase in matrix metalloproteinase activity and a lower expression of extracellular matrix production components in senescent activated hepatic stellate cells helped to limit the expansion of fibrosis. This finding suggests the key role of cellular senescence in restraining the response leading to fibrosis during tissue repair.[53] In contrast, another study suggested that senescence contributed to accelerated development of idiopathic pulmonary fibrosis and chronic obstructive pulmonary disease due to stem cell exhaustion of progenitor cells, leading to improper tissue repair and fibrosis formation.[69]

6. EMERGING THERAPEUTIC APPROACHES

Some chemotherapies and radiotherapies induce senescence as a prevention for further replication of cells and to prevent the risk of invasive behaviour and activate antitumor immunity. The chemotherapeutic agents have plenty of targets, including topoisomerase I/II inhibitors (doxorubicin), alkylating agents (busulfan), platinum-based drugs, microtubule inhibitors (docetaxel), kinase inhibitors, mTOR inhibitors (rapamycin) and many more. An attractive approach is inducing senescence with chemotherapeutic agents, activating antitumor immune response which then targets senescent cells.

Baker et al. (2011) first presented evidence that linked senescence to age-related pathologies.[57] They treated an INK-ATTAC (inducing apoptosis through targeted activation of caspase in $p16^{INK4a}$ positive cells) transgenic mouse model lines with a synthetic drug AP20187. This drug caused dimerization of FKBP-caspase 8 protein which tehn activated it. This fusion protein allowed selective induction of apoptosis in p16-positive senescent cells. They observed reduced senescence markers (SA-β-gal, p16), improved tissue repair and postponed aging symptoms. This study sparked the possibility of therapeutic strategies that can eliminate senescent cells or block the SASP effect on tissue to postpone the onset of age-related phenotypes.[57] The findings of Baker et. al (2011) and the possible side effects of TIS may have imposed an idea of senotherapy usage in nongenetically modified organisms. The pharmacological strategies targeting senescence include immunotherapies, senolytic and senomorphics agents. While senolytics and immunotherapies eliminate and mediate clearance of senescent cells, senomorphics are modulating SASP production and its effects.

6.1. SENOLYTIC THERAPY

Zhu et al. (2015) first presented a class of senotherapeutic agents, so called senolytic, that selectively eliminate senescent cells by inducing apoptosis.[17] They observed elevated levels of apoptosis inhibitors, such as BCL proteins, in senescent cells, mediating their resistance to apoptosis. They also tested the use of an inhibitor of tyrosine kinases (dasatinib) and a natural flavonoid that targets and inhibits phosphatidylinositol-4,5-bisphosphate 3-kinases and other kinases (quercetin). Dasatinib, also approved as a treatment for chronic myelogenous leukemia, induces apoptosis of senescent preadipocytes. Quercetin induces apoptosis of senescent human umbilical vein cells and bone marrow-derived mesenchymal stem cells. Zhu et al. (2015) found that the treatment with these senolytics extended healthspan of a mouse model with accelerated aging.[17] This breakthrough laid foundation for the development of more senotherapeutic agents.

Since then, the use of senolytic agents like ABT-263[70] and ABT-737[18] (BCL-W, BCL-2 and BCL-xL inhibitors), AZD2014 and AZD8055 (mTOR inhibitors)[71], S63845 (MCL-1 inhibition)[72], cardiac glycosides family of compounds (Na^+/K^+ ATPase pump inhibitior)[73], panobinostat (histone deacetylases inhibitor)[74], geldanamycin and 17-AAG (heat shock protein 90 inhibitors)[75] has been further explored. These agents target different proteins and pathways involved in apoptosis. Some of these candidates showed a potential in post-chemotherapy usage for eliminating senescent cancer cells and preventing stem-cell related properties. Senotherapies were observed to be efficient in different cell lines and were associated with different effects on a wide-range of pathologies. The tissue-specific factors need to be properly studied.[74,64]

The side effects of senolytic elimination could compromise beneficial roles of senescence, including immune activation driven by senescent cancer cell and wound healing. Questionable potential problem could also be cell lysis syndrome.[17] An alternative solution to these obstacles could be the use of senomorphic compounds.

6.2. IMMUNOTHERAPIES

Other approaches developed to eliminate senescent cell use antibodies, vaccines and chimeric antigen receptor (CAR) T cells. A cell surface urokinase-type plasminogen activator receptor (uPAR) can be upregulated during senescence (part of SASP) and can also be observed as a signaling receptor of tumor cells. Mice treated with uPAR-specific CAR T lymphocytes exhibited a reduction in senescent cells and an increased infiltration of CD4+ and CD8+ cells.[76] Development of a vaccine targeting senescent T cells expressing CD153 on their surface improved obesity-induced metabolic disorders (insulin resistance).[77] Blocking PD-1 interaction with PD-L1 using monoclonal antibodies led to the elimination of PD-L1+ senescent cells.[78]

6.3. SENOMORPHICS

Senomorphic agents represent a wide range of substances that can modulate the phenotype of senescent cells by interfering with related regulatory signaling pathways and their secretome without inducing apoptosis. Secretome modulators may help suppress chronic inflammation and restore or enhance immunosurveillance. However, they should not interfere with growth arrest, an important phenotypic trait.[75] A lot of the below mentioned inhibitors were identified before the senomorphic/senotherapy terminology was introduced. Therefore, there are many opinions about which molecules fit the senomorphic definition. The agents that are preserving cell viability are targeting NF-κB, p38MPAK, mTOR, IL-1α and other signaling

molecules (Figure 2). Research conducted on these compounds used various senescence inducers and cell lines/organism models. These variables are highly relevant for their potential future clinical application. The results may vary between *in vivo* and *in* vitro systems. Due to the complexity, these variables will not be discussed further here.

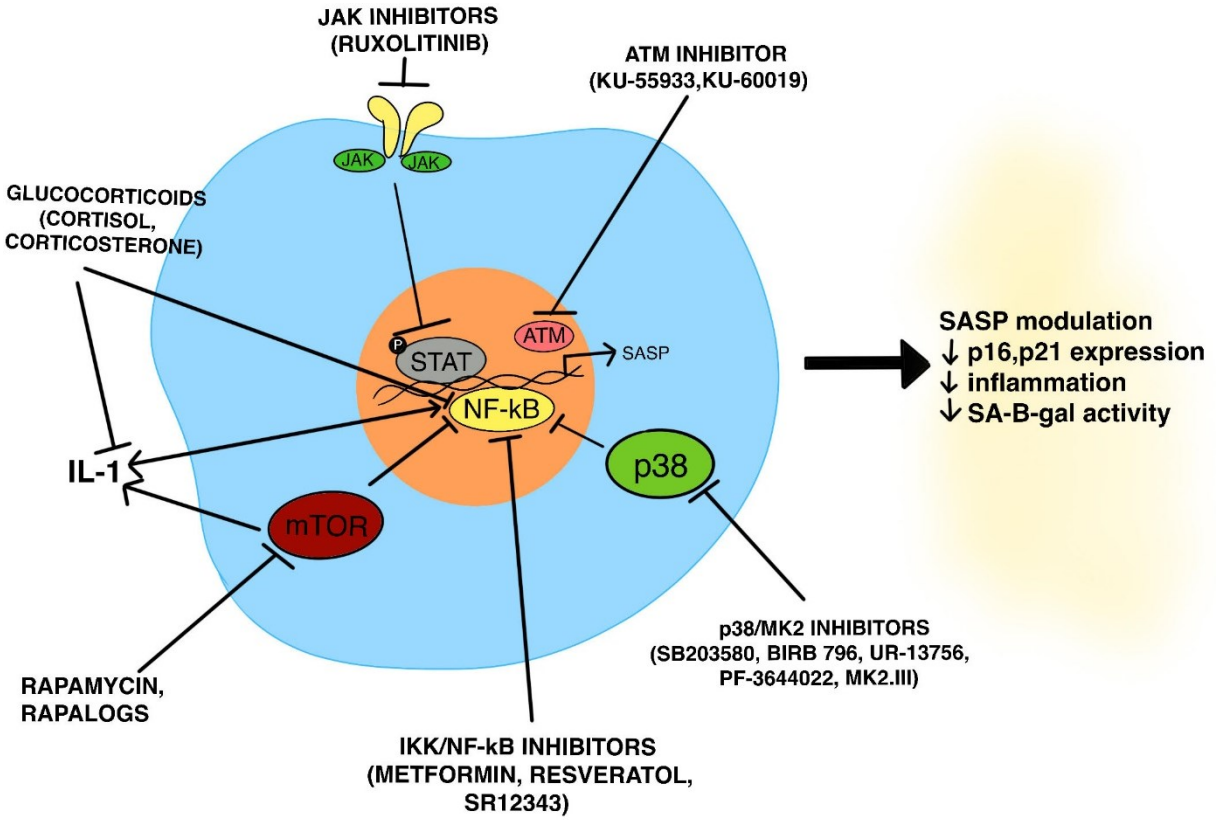

Figure 2: Selected senomorphic agents, their targets and possible outcomes. (own work)

6.3.1. RAPAMYCIN

Rapamycin is known as a macrolide compound and it suppresses senescent phenotype by interfering with mTOR pathway. It was isolated from *Streptomyces hydroscopicus* and was initially studied for its antifungal properties. Its immunosuppressive and anticancer properties (inhibition of cell growth) were studied and rapamycin was approved as an antirejection transplantation and pancreatic cancer drug. Besides rapamycin's ability to reduce SASP secretion, it can delay senescence onset (reduced p16 expression, SA-β-gal activity), decrease aging impact and decrease tumor growth.[79] In contrast, another study suggested that high doses of rapamycin can cause complete inhibition of 4E-BP1 phosphorylation, thereby inducing apoptosis in cancer cells. [80]

The mTOR pathway consists of two multiprotein complexes: mTORC1 and mTORC2. The mTORC1 controls translation by phosphorylating its downstream effectors 4E-BP1 and S6K1 proteins. The mTORC1 has an effect on gene expression related to growth and metabolism. Rapamycin binds to a cytosolic FKBP12 protein which mediates mTORC1 inhibition. This prevents mTORC1 from activating translation regulators S6K1 and 4E-BP1, inhibiting protein synthesis and growth [81] Rapamycin was shown to reduce secretion of IL-6 and IL-8 by impairing helicase machineries. Rapamycin reduces surface protein levels of IL-1α, compromising IL-1α signaling to degrade IRAK1 and IκBα proteins, not allowing NF-κB to translocate. Therefore, rapamycin indirectly affects NF-κB transcription activity. This SASP modulation could repress carcinogenesis and metastasic behaviour. [45]

Adverse effects of rapamycin are caused by the off-target inhibition of mTORC2. Metabolic disruption, hyperlipidemia and other side effects could be avoided by rapamycin analogs (rapalogs). These rapalogs (sirolimus, everolimus, temsirolimus) and other mTOR inhibitors have to be highly selective to mTORC1 inhibition while maintaining senomorphic functions.[82]

6.3.2. METFORMIN

Metformin is a biguanide derivative extracted from the plant *Galega officinalis*. It is also an already approved drug used for the treatment of type 2 diabetes. However, some data showed that metformin is associated with reduced cancer risk, likely because of its effect on energy metabolism.[83] Metformin inhibits phosphorylation of IKKa/IKKb, not affecting p38MAPK signaling or IRF3/7 transcription activity, modulating pro-inflammatory cytokine production by NF-κB and not affecting interferon secretion in OIS cells.[84] Long term treatment with metformin in mice revealed promising benefits in lowering chronic inflammation and oxidative stress (lowering ROS production[84]), therefore delaying aging processes. [85]

Metformin is the first drug against aging on the way to be tested in a clinical trial, but the TAME (Targeting Aging by Metformin) trial has not started yet due to funding. Studies show mixed data results on metformin. Evidence of rapamycin's effects demonstrates more potential against aging pathologies/phenotypes.[86]

6.3.3. GLUCOCORTICOIDS

Another class of agents shown to be SASP modulators, cortisol and corticosterone, are targeting IL-1α/NF-κB signaling loop, mainly suppressing IL-6 production. On the other hand,

they do not interfere with DNA damage foci in nuclei and have no effect on other markers of senescence (SA-β-gal), the morphology nor the growth arrest. Glucocorticoids selectively suppress some SASP components (IL-1α, IL-8, IL-6, GM-CSF) and they limit invasive ability. Glucocorticoids are already approved for asthma, allergies and autoimmune diseases treatment, showing promising future for senomorphic use.[87]

6.3.4. JAK/STAT INHIBITORS

Ruxolitinib is a JAK 1/JAK 2 inhibitor and is also approved for myelofibrosis treatment. Targeting the JAK/STAT pathway by JAK1/2 inhibitors can disrupt inflammatory signaling, leading to the SASP composition reprograming and improved antitumor immune response.[42] The JAK1/2 inhibitors lead to a decrease of phosphorylated STAT3 levels, lowering systematic and adipose tissue inflammation and improving physical function in aged mice, suggesting its senomorphic properties.[41] Ruxolitinib also prevents progerin-induced senescence and reduces premature aging phenotypes, and is being considered as a potential therapy for for Hutchinson-Gilford progeria syndrome.[88]

STAT3 inhibitor Stattic and its analogues (K1823, K1836) target and bind to the SH2 domain, blocking its phosphorylation, followed dimerization and translocation to the nucleus. This leads to a significant reduction of inflammatory microenvironment, potentially serving as senotherapy or antitumor agents.[89]

6.3.5. IKK INHIBITORS

The effect of inhibiting NK-κB activation on senescence was proved before senotherapy became a recognized concept. The NF-κB essential modulator (NEMO)-binding domain (NBD) was used to reduce ROS production and cellular damage.[90] Small molecule SR12343 is an inhibitor of NF-κB signaling pathway that acts as a mimetic to NBD. NF-κB activation is dependent on a IκB kinase (IKK) complex, composed of IKKα, IKKβ and IKKγ/NEMO subunits. SR12343 was designed to disrupt the interaction between IKKβ and IKKγ. IκBα is then not phosphorylated and therefore does not allow NF-κB to dissociate, translocate and target genes.[91] Chronic treatment with SR12343 reduced inflammatory SASP compounds (IL-6, IL-1α, TNFα), slowed progression of age-related pathologies and extended healthspan in mice models with accelerated aging.[92] These senomorphic properities of NK-κB/IKK inhibitors shows potential for developing future treatments for age-associated diseases, given the interconnection with other signaling pathways and their inhibitors influencing NF-κB activity.

6.3.6. p38MAPK INHIBITORS

The p38 mitogen-activated protein kinase (MAPK) pathway is prominently activated in *RAS*-induced senescence and it utilizes 3 signaling molecules: p38, JNK and ERK. Activation of p38MAPK is mediated by phosphorylation after cellular stress, establishing growth arrest by activating p16/Rb and p53 pathways.[93] IKK activation and p38MAPK signaling are downstream effectors of TAK1, a kinase involved in DDR.[84] p38MAPK increases the NF-κB activity, SASP production and stability, with the possibility of doing it independently on DDR. A small molecule SB203580 inhibits the ability of p38MAPK to phosphorylate target proteins by shifting ATP in its ATP binding pocket. p38MAPK inhibition lowered the ability of invasiveness. [94] Recently studied more efficient inhibitors are UR-13756 and BIRB 796. An inhibition of downstream effector proteins of p38MAPK like MK2 kinase (activates stress fibres in senescent cells) using MK2.III or PF-3644022 showed the possibility of SASP suppression by inhibition of non-essential pathways for survival.[95]

6.3.7. ATM KINASE INHIBITORS

Upon DDR activation, double strand breaks recruit MRN complex to mediate ATM autophosphorylation and activation. Inhibition of ATM phosphorylation with KU-55933[96] or KU-60019[97] molecule showed reduced senescence and SASP markers. These agents suppressed ROS production, leading to improved mitochondrial function and a reduction in abnormal nuclear morphology. Inhibition of ATM downregulated NF-κB activity, thereby affecting the SASP. Given the role of ATM in DNA repair and apoptosis induction, long term inhibition could impact the homeostasis and could increase the risk of uncontrolled proliferation.[97]

6.3.8. RESVERATROL

Naturally occurring polyphenol, resveratrol, is an activator of silent information regulator 2 homolog 1 (sirtuin1/SIRT1). Resveratrol has been shown to induce mitochondrial dysfunction, leading to an increase in ROS production. This oxidative stress can contribute to DNA damage, which is linked to an increased expression of DCL1 protein and activation of p38MAPK. This affects p21 activity, contributing to cell cycle arrest and senescence in cancer cells.[98] On the other hand, SIRT1, an NAD^+-dependent protein deacetylase, regulates NF-kB signaling by deacetylating p65/RelA protein. This leads to a decreased NF-kB activity, modulating SASP and inflammation.[99] This conflicting evidence of resveratrol on cellular senescence may depend on its concentration and organism model. At low doses, resveratrol exhibits senomorphic prosperities, whereas higher doses may promote senescence.

6.3.9. OTHER COMPOUNDS

Pharmacologically targeting and inhibiting other signaling molecules or using natural compounds with complex modes of action have also gained research attention. More compounds that had shown anti-inflammatory activities and SASP modulation properties are summarized in table 1.

Other compounds with possible senomorphic effects		
class	**target**	**compound**
natural compounds	NF-kB signaling	apigenin[100]
	NF-kB signaling	kaempferol[100]
	suppresses p53 acetylation	Epigallocatechin gallate (EGCG)[101]
	Ca+ channel blockers	loperamide, niguldipine[101]
	cGAS	RU.521[102]
	STING	H-151[103]
	BRD4	iBET-762[104]

Table 1: List of other agents with possible senomorphic activity and their targets. (own work)

7. DISSCUSSION AND CONCLUSION

The objective of this work was to summarize and highlight key signaling pathways that regulate the senescence-associated secretory phenotype (SASP), with a particular focus on its dual role in antitumor immune response (Figure 3). These findings pointed to the potential use of senotherapy as a targeted therapeutic approach.

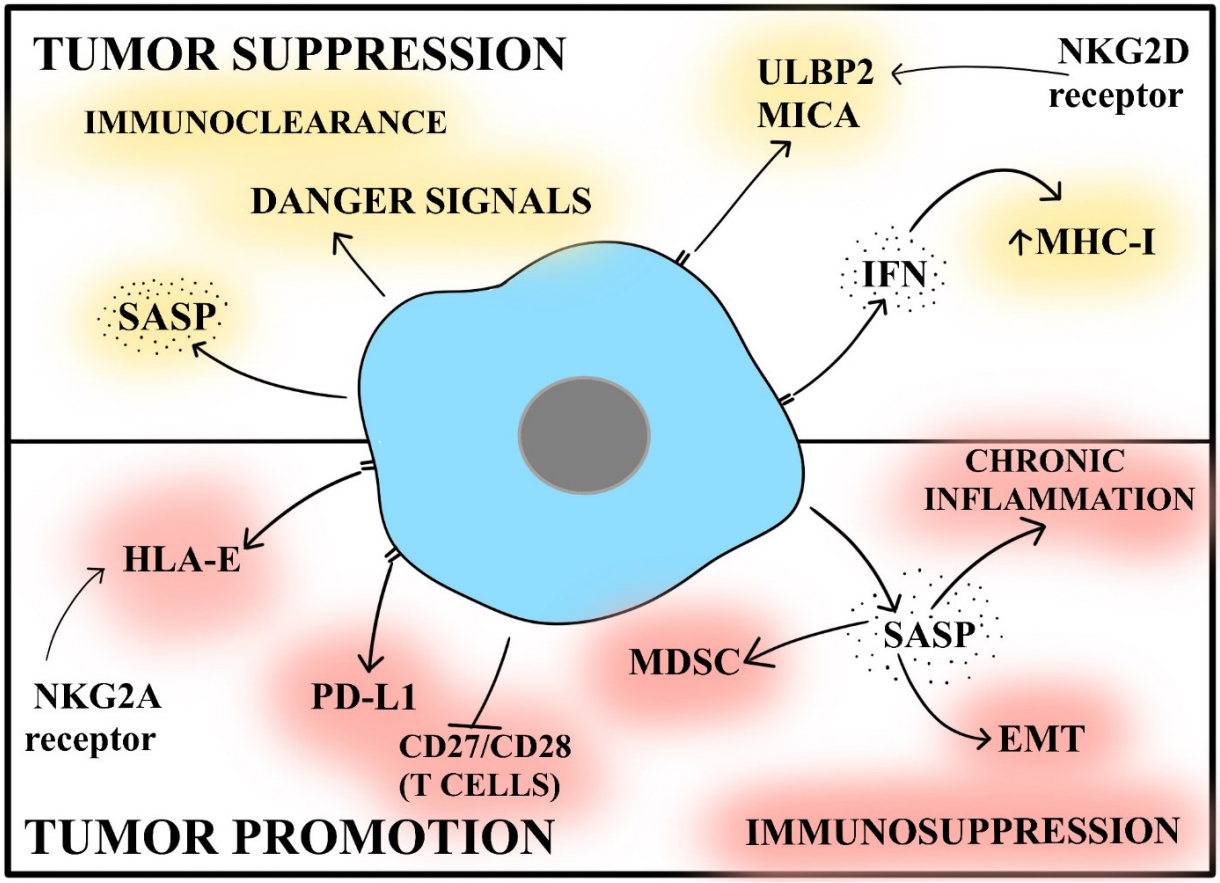

Figure 3: Dual role of senescent cells in carcinogenesis. (own work)

Another topic up for debate is whether senescence is truly irreversible. The absence of senescent cells in malignant lesions could be explained by the fact that cells were unable to induce OIS or could escape senescence. The p53 and p16 pathways that induce and maintain senescence are responsible for reversible cell cycle arrest during quiescence. Evidence suggests that degradation of MYC protein is important for maintaining irreversibility. Overexpression or mutation in this gene that could cause constitutive presence of MYC and enable cells to bypass or exit senescence in premalignant lesions.[105] Further investigation could provide promising approaches.

Senescent cancer and noncancerous cells have a dual effect on carcinogenesis as they can both stimulate and inhibit it (Figure 3). The challenge now lies in removing the harmful effects of senescent cells without impairing their beneficial roles in tumor suppression, wound healing and tissue remodelling. Recent studies have pointed to a promising future for senotherapies. Senolytic and senomorphics remain the most researched compounds of senotherapies, but which solution is better?

The distinction between senomorphics and senolytics is not clearly defined and may be influenced by the concentration upon administration (for example, rapamycin). Apoptosis-inducing senolytics eliminate senescent cells. For effective clinical applications, these therapies must be tissue specific. Senolytics appear to be a promising choice as complementary therapy to anticancer therapy. However, elimination of senescent cell from the TME can also inhibit some positive effects of senescent cells, such as antitumor immunity induction, that can theoretically interfere with immunotherapy using anti-PD-1 antibodies. Future studies need assess how senolytic therapies affect tissue structure and the TME over time.

Senomorphics, on the other hand, suppress or modulate SASP without eliminating cells. By targeting important signaling pathways, senomorphics reduce inflammation and limit SASP-driven carcinogenesis. However, the requirement of chronic administration may lead to off-target side effects and disrupt physiological roles. Currently, repurposing approved drugs with established safety profiles have showed the greatest potential for senomorphic use. Because the SASP drives many of the negative effects of senescence, using senomorphics agents could be an alternative in situations where clearing senescent cells might threaten tissue structure or function. Senomorphics may reduce the probability of epithelial-mesenchymal transition if administrated with anticancer therapies. However, senomorphics have the greatest potential to delay the onset of age-related diseases and prolong healthspan.

Senescent cells are not uniform, both in function and in molecular markers, making it challenging to evaluate the safety, tolerance and efficacy of senotherapies. The effectiveness of these therapies and the dependence on cell type and environment needs to be evaluated. Future research could explore how to create delivery systems that would target specific tissue. These delivery technologies could improve treatment outcomes, reduce potential side effects and enhance overall efficiency of senotherapies. While some senolytic agents have been presented

in clinical trial (dasatinib, quercetin), senomorphics are approved for clinical use and they can theoretically be introduced to clinical practise.[101]

Given the complexity of cellular senescence and its interactions with the surrounding environment, it was not possible to discuss all relevant aspects in detail. The primary objective was to illustrate the most important controversies and their clinical relevance.

8. REFERENCES

* indicates a secondary source/review

1. *Hernandez-Segura, A., Nehme, J. & Demaria, M. Hallmarks of Cellular Senescence. *Trends in Cell Biology* **28**, 436–453 (2018).
2. Hayflick, L. & Moorhead, P. S. The serial cultivation of human diploid cell strains. *Experimental Cell Research* **25**, 585–621 (1961).
3. *Huang, W., Hickson, L. J., Eirin, A., Kirkland, J. L. & Lerman, L. O. Cellular senescence: the good, the bad and the unknown. *Nat Rev Nephrol* **18**, 611–627 (2022).
4. *Kuilman, T., Michaloglou, C., Mooi, W. J. & Peeper, D. S. The essence of senescence. *Genes Dev* **24**, 2463–2479 (2010).
5. Storer, M. *et al.* Senescence Is a Developmental Mechanism that Contributes to Embryonic Growth and Patterning. *Cell* **155**, 1119–1130 (2013).
6. Chang, B.-D. *et al.* Role of p53 and p21waf1/cip1 in senescence-like terminal proliferation arrest induced in human tumor cells by chemotherapeutic drugs. *Oncogene* **18**, 4808–4818 (1999).
7. *Tchkonia, T. *et al.* Fat tissue, aging, and cellular senescence. *Aging Cell* **9**, 667–684 (2010).
8. Druelle, C. *et al.* ATF6α regulates morphological changes associated with senescence in human fibroblasts. *Oncotarget* **7**, 67699–67715 (2016).
9. Beauséjour, C. M. *et al.* Reversal of human cellular senescence: roles of the p53 and p16 pathways. *EMBO J* **22**, 4212–4222 (2003).
10. Narita, M. *et al.* Rb-Mediated Heterochromatin Formation and Silencing of E2F Target Genes during Cellular Senescence. *Cell* **113**, 703–716 (2003).

11. Ivanov, A. *et al.* Lysosome-mediated processing of chromatin in senescence. *Journal of Cell Biology* **202**, 129–143 (2013).

12. Dimri, G. P. *et al.* A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 9363–9367 (1995).

13. Kurz, D. J., Decary, S., Hong, Y. & Erusalimsky, J. D. Senescence-associated β-galactosidase reflects an increase in lysosomal mass during replicative ageing of human endothelial cells. *Journal of Cell Science* **113**, 3613–3622 (2000).

14. *Korolchuk, V. I., Miwa, S., Carroll, B. & von Zglinicki, T. Mitochondria in Cell Senescence: Is Mitophagy the Weakest Link? *EBioMedicine* **21**, 7–13 (2017).

15. *Salminen, A., Kaarniranta, K. & Kauppinen, A. Inflammaging: disturbed interplay between autophagy and inflammasomes. *Aging (Albany NY)* **4**, 166–175 (2012).

16. Braig, M. *et al.* Oncogene-induced senescence as an initial barrier in lymphoma development. *Nature* **436**, 660–665 (2005).

17. Zhu, Y. *et al.* The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. *Aging Cell* **14**, 644–658 (2015).

18. Yosef, R. *et al.* Directed elimination of senescent cells by inhibition of BCL-W and BCL-XL. *Nat Commun* **7**, 11190 (2016).

19. Yang, J. *et al.* Prevention of Apoptosis by Bcl-2: Release of Cytochrome c from Mitochondria Blocked. *Science* **275**, 1129–1132 (1997).

20. *Saleh, T. *et al.* Therapy-Induced Senescence: An "Old" Friend Becomes the Enemy. *Cancers (Basel)* **12**, 822 (2020).

21. *Coppé, J.-P., Desprez, P.-Y., Krtolica, A. & Campisi, J. The Senescence-Associated Secretory Phenotype: The Dark Side of Tumor Suppression. *Annu Rev Pathol* **5**, 99–118 (2010).

22. Acosta, J. C. *et al.* A complex secretory program orchestrated by the inflammasome controls paracrine senescence. *Nat Cell Biol* **15**, 978–990 (2013).

23. Coppé, J.-P. *et al.* Senescence-Associated Secretory Phenotypes Reveal Cell-Nonautonomous Functions of Oncogenic RAS and the p53 Tumor Suppressor. *PLoS Biol* **6**, e301 (2008).

24. Kuilman, T. *et al.* Oncogene-Induced Senescence Relayed by an Interleukin-Dependent Inflammatory Network. *Cell* **133**, 1019–1031 (2008).

25. Sparmann, A. & Bar-Sagi, D. Ras-induced interleukin-8 expression plays a critical role in tumor growth and angiogenesis. *Cancer Cell* **6**, 447–458 (2004).

26. Ma, Y., Vassetzky, Y. & Dokudovskaya, S. mTORC1 pathway in DNA damage response. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **1865**, 1293–1311 (2018).

27. *Birch, J. & Gil, J. Senescence and the SASP: many therapeutic avenues. *Genes Dev.* **34**, 1565–1576 (2020).

28. *Wang, W., Mani, A. M. & Wu, Z.-H. DNA damage-induced nuclear factor-kappa B activation and its roles in cancer progression. *J Cancer Metastasis Treat* **3**, 45–59 (2017).

29. Spallarossa, P. *et al.* Doxorubicin induces senescence or apoptosis in rat neonatal cardiomyocytes by regulating the expression levels of the telomere binding factors 1 and 2. *American Journal of Physiology-Heart and Circulatory Physiology* **297**, H2169–H2181 (2009).

30. *Maréchal, A. & Zou, L. DNA Damage Sensing by the ATM and ATR Kinases. *Cold Spring Harb Perspect Biol* **5**, a012716 (2013).

31. Ventura, A. *et al.* Restoration of p53 function leads to tumour regression in vivo. *Nature* **445**, 661–665 (2007).

32. Ohanna, M. *et al.* Senescent cells develop a PARP-1 and nuclear factor-κB-associated secretome (PNAS). *Genes Dev.* **25**, 1245–1261 (2011).

33. Takahashi, A. *et al.* DNA Damage Signaling Triggers Degradation of Histone Methyltransferases through APC/CCdh1 in Senescent Cells. *Molecular Cell* **45**, 123–131 (2012).

34. Fumagalli, M., Rossiello, F., Mondello, C. & d'Adda di Fagagna, F. Stable Cellular Senescence Is Associated with Persistent DDR Activation. *PLoS One* **9**, e110969 (2014).

35. Yang, H., Wang, H., Ren, J., Chen, Q. & Chen, Z. J. cGAS is essential for cellular senescence. *Proceedings of the National Academy of Sciences* **114**, E4612–E4620 (2017).

36. Sun, L., Wu, J., Du, F., Chen, X. & Chen, Z. J. Cyclic GMP-AMP Synthase is a Cytosolic DNA Sensor that Activates the Type-I Interferon Pathway. *Science* **339**, 10.1126/science.1232458 (2013).

37. *Meyer, S. C. & Levine, R. L. Molecular Pathways: Molecular Basis for Sensitivity and Resistance to JAK Kinase Inhibitors. *Clin Cancer Res* **20**, 2051–2059 (2014).

38. *Hu, X., Li, J., Fu, M., Zhao, X. & Wang, W. The JAK/STAT signaling pathway: from bench to clinic. *Sig Transduct Target Ther* **6**, 1–33 (2021).

39. *Yu, H., Pardoll, D. & Jove, R. STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat Rev Cancer* **9**, 798–809 (2009).

40. Lee, H. *et al.* Persistently-activated Stat3 maintains constitutive NF-κB activity in tumors. *Cancer Cell* **15**, 283–293 (2009).

41. Xu, M. *et al.* JAK inhibition alleviates the cellular senescence-associated secretory phenotype and frailty in old age. *Proc Natl Acad Sci U S A* **112**, E6301–E6310 (2015).

42. Toso, A. *et al.* Enhancing Chemotherapy Efficacy in *Pten*-Deficient Prostate Tumors by Activating the Senescence-Associated Antitumor Immunity. *Cell Reports* **9**, 75–89 (2014).

43. Bent, E. H., Gilbert, L. A. & Hemann, M. T. A senescence secretory switch mediated by PI3K/AKT/mTOR activation controls chemoprotective endothelial secretory responses. *Genes Dev.* **30**, 1811–1821 (2016).

44. Herranz, N. *et al.* mTOR regulates MAPKAPK2 translation to control the senescence-associated secretory phenotype. *Nat Cell Biol* **17**, 1205–1217 (2015).

45. Laberge, R.-M. *et al.* MTOR regulates the pro-tumorigenic senescence-associated secretory phenotype by promoting IL1A translation. *Nat Cell Biol* **17**, 1049–1061 (2015).

46. *Lopes-Paciencia, S. *et al.* The senescence-associated secretory phenotype and its regulation. *Cytokine* **117**, 15–22 (2019).

47. Marin, I. *et al.* Cellular Senescence Is Immunogenic and Promotes Antitumor Immunity. *Cancer Discovery* **13**, 410–431 (2023).

48. Soriani, A. *et al.* ATM-ATR–dependent up-regulation of DNAM-1 and NKG2D ligands on multiple myeloma cells by therapeutic agents results in enhanced NK-cell susceptibility and is associated with a senescent phenotype. *Blood* **113**, 3503–3511 (2009).

49. Demaria, M. *et al.* Cellular Senescence Promotes Adverse Effects of Chemotherapy and Cancer Relapse. *Cancer Discovery* **7**, 165–176 (2017).

50. Ruhland, M. K. *et al.* Stromal senescence establishes an immunosuppressive microenvironment that drives tumorigenesis. *Nat Commun* **7**, 11762 (2016).

51. Nelson, G. *et al.* A senescent cell bystander effect: senescence-induced senescence. *Aging Cell* **11**, 345–349 (2012).

52. Pereira, B. I. *et al.* Senescent cells evade immune clearance via HLA-E-mediated NK and CD8+ T cell inhibition. *Nat Commun* **10**, 2387 (2019).

53. Krizhanovsky, V. *et al.* Senescence of activated stellate cells limits liver fibrosis. *Cell* **134**, 657–667 (2008).

54. Hazeldine, J., Hampson, P. & Lord, J. M. Reduced release and binding of perforin at the immunological synapse underlies the age-related decline in natural killer cell cytotoxicity. *Aging Cell* **11**, 751–759 (2012).

55. Morris, A., Cooley, M. & Blackman, M. The interaction of interferon with the immune response. *Journal of Hepatology* **3**, S161–S169 (1986).

56. Kang, T.-W. *et al.* Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. *Nature* **479**, 547–551 (2011).

57. Baker, D. J. *et al.* Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature* **479**, 232–236 (2011).

58. Guo, N. *et al.* Short Telomeres Compromise β-Cell Signaling and Survival. *PLoS One* **6**, e17858 (2011).

59. *Egeblad, M. & Werb, Z. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* **2**, 161–174 (2002).

60. *Bissell, M. J. & Radisky, D. Putting tumours in context. *Nat Rev Cancer* **1**, 46–54 (2001).

61. Hudson, M. M. *et al.* Clinical Ascertainment of Health Outcomes among Adults Treated for Childhood Cancer: A Report from the St. Jude Lifetime Cohort Study. *JAMA* **309**, 2371–2381 (2013).

62. Roberson, R. S., Kussick, S. J., Vallieres, E., Chen, S.-Y. J. & Wu, D. Y. Escape from Therapy-Induced Accelerated Cellular Senescence in p53-Null Lung Cancer Cells and in Human Lung Cancers. *Cancer Research* **65**, 2795–2803 (2005).

63. Ritschka, B. *et al.* The senescence-associated secretory phenotype induces cellular plasticity and tissue regeneration. *Genes Dev* **31**, 172–183 (2017).

64. Milanovic, M. *et al.* Senescence-associated reprogramming promotes cancer stemness. *Nature* **553**, 96–100 (2018).

65. Xia, S. *et al.* Gr-1+ CD11b+ Myeloid-derived Suppressor Cells Suppress Inflammation and Promote Insulin Sensitivity in Obesity. *J Biol Chem* **286**, 23591–23599 (2011).

66. Ye, J. *et al.* Human regulatory T cells induce T-lymphocyte senescence. *Blood* **120**, 2021–2031 (2012).

67. Eggert, T. *et al.* Distinct Functions of Senescence-Associated Immune Responses in Liver Tumor Surveillance and Tumor Progression. *Cancer Cell* **30**, 533–547 (2016).

68. Haston, S. *et al.* Clearance of senescent macrophages ameliorates tumorigenesis in KRAS-driven lung cancer. *Cancer Cell* **41**, 1242-1260.e6 (2023).

69. Chilosi, M., Carloni, A., Rossi, A. & Poletti, V. Premature lung aging and cellular senescence in the pathogenesis of idiopathic pulmonary fibrosis and COPD/emphysema. *Translational Research* **162**, 156–173 (2013).

70. Chang, J. *et al.* Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. *Nat Med* **22**, 78–83 (2016).

71. Wang, C. *et al.* Inducing and exploiting vulnerabilities for the treatment of liver cancer. *Nature* **574**, 268–272 (2019).

72. Troiani, M. *et al.* Single-cell transcriptomics identifies Mcl-1 as a target for senolytic therapy in cancer. *Nat Commun* **13**, 2177 (2022).

73. Triana-Martínez, F. *et al.* Identification and characterization of Cardiac Glycosides as senolytic compounds. *Nat Commun* **10**, 4731 (2019).

74. Samaraweera, L., Adomako, A., Rodriguez-Gabin, A. & McDaid, H. M. A Novel Indication for Panobinostat as a Senolytic Drug in NSCLC and HNSCC. *Sci Rep* **7**, 1900 (2017).

75. Fuhrmann-Stroissnigg, H. *et al.* Identification of HSP90 inhibitors as a novel class of senolytics. *Nat Commun* **8**, 422 (2017).

76. Amor, C. *et al.* Senolytic CAR T cells reverse senescence-associated pathologies. *Nature* **583**, 127–132 (2020).

77. Yoshida, S. *et al.* The CD153 vaccine is a senotherapeutic option for preventing the accumulation of senescent T cells in mice. *Nat Commun* **11**, 2482 (2020).

78. Wang, T.-W. *et al.* Blocking PD-L1–PD-1 improves senescence surveillance and ageing phenotypes. *Nature* **611**, 358–364 (2022).

79. *Selvarani, R., Mohammed, S. & Richardson, A. Effect of rapamycin on aging and age-related diseases—past and future. *GeroScience* **43**, 1135–1158 (2020).

80. Yellen, P. *et al.* High-dose rapamycin induces apoptosis in human cancer cells by dissociating mTOR complex 1 and suppressing phosphorylation of 4E-BP1. *Cell Cycle* **10**, 3948–3956 (2011).

81. *Bjedov, I. & Rallis, C. The Target of Rapamycin Signalling Pathway in Ageing and Lifespan Regulation. *Genes (Basel)* **11**, 1043 (2020).

82. Schreiber, K. H. *et al.* A novel rapamycin analog is highly selective for mTORC1 in vivo. *Nat Commun* **10**, 3194 (2019).

83. *Pollak, M. N. Investigating metformin for cancer prevention and treatment: The end of the beginning. *Cancer Discovery* **2**, 778–790 (2012).

84. Moiseeva, O. *et al.* Metformin inhibits the senescence-associated secretory phenotype by interfering with IKK / NF -κ B activation. *Aging Cell* **12**, 489–498 (2013).

85. Martin-Montalvo, A. *et al.* Metformin improves healthspan and lifespan in mice. *Nat Commun* **4**, 2192 (2013).

86. The TAME Trial for Metformin Remains Only Partially Funded. *Fight Aging!* https://www.fightaging.org/archives/2024/04/the-tame-trial-for-metformin-remains-only-partially-funded/ (2024).

87. Laberge, R. *et al.* Glucocorticoids suppress selected components of the senescence-associated secretory phenotype. *Aging Cell* **11**, 569–578 (2012).

88. Griveau, A., Wiel, C., Ziegler, D. V., Bergo, M. O. & Bernard, D. The JAK1/2 inhibitor ruxolitinib delays premature aging phenotypes. *Aging Cell* **19**, e13122 (2020).

89. Mikyskova, R. *et al.* STAT3 inhibitor Stattic and its analogues inhibit STAT3 phosphorylation and modulate cytokine secretion in senescent tumour cells. *Mol Med Rep* **27**, 81 (2023).

90. Tilstra, J. S. *et al.* NF-κB inhibition delays DNA damage–induced senescence and aging in mice. *J Clin Invest* **122**, 2601–2612 (2012).

91. Zhao, J. *et al.* Development of novel NEMO-binding domain mimetics for inhibiting IKK/NF-κB activation. *PLoS Biol* **16**, e2004663 (2018).

92. Zhang, L. *et al.* Novel small molecule inhibition of IKK/NF-κB activation reduces markers of senescence and improves healthspan in mouse models of aging. *Aging Cell* **20**, e13486 (2021).

93. Iwasa, H., Han, J. & Ishikawa, F. Mitogen-activated protein kinase p38 defines the common senescence-signalling pathway. *Genes to Cells* **8**, 131–144 (2003).

94. Freund, A., Patil, C. K. & Campisi, J. p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. *EMBO J* **30**, 1536–1548 (2011).

95. Alimbetov, D. *et al.* Suppression of the senescence-associated secretory phenotype (SASP) in human fibroblasts using small molecule inhibitors of p38 MAP kinase and MK2. *Biogerontology* **17**, 305–315 (2016).

96. Zhao, J. *et al.* ATM is a key driver of NF-κB-dependent DNA-damage-induced senescence, stem cell dysfunction and aging. *Aging (Albany NY)* **12**, 4688–4710 (2020).

97. Kuk, M. U. *et al.* Alleviation of Senescence via ATM Inhibition in Accelerated Aging Models. *Mol Cells* **42**, 210–217 (2019).

98. Ji, S. *et al.* Resveratrol promotes oxidative stress to drive DLC1 mediated cellular senescence in cancer cells. *Experimental Cell Research* **370**, 292–302 (2018)

99. Liu, S. *et al.* Resveratrol reduces senescence-associated secretory phenotype by SIRT1/NF-κB pathway in gut of the annual fish Nothobranchius guentheri. *Fish & Shellfish Immunology* **80**, 473–479 (2018).

100. Lim, H., Park, H. & Kim, H. P. Effects of flavonoids on senescence-associated secretory phenotype formation from bleomycin-induced senescence in BJ fibroblasts. *Biochemical Pharmacology* **96**, 337–348 (2015).

101. *Lagoumtzi, S. M. & Chondrogianni, N. Senolytics and senomorphics: Natural and synthetic therapeutics in the treatment of aging and chronic diseases. *Free Radical Biology and Medicine* **171**, 169–190 (2021).

102. Schuliga, M. *et al.* A cGAS-dependent response links DNA damage and senescence in alveolar epithelial cells: a potential drug target in IPF. *American Journal of Physiology-Lung Cellular and Molecular Physiology* **321**, L859–L871 (2021).

103. Herbstein, F. *et al.* The SASP factor IL -6 sustains cell-autonomous senescent cells via a cGAS - STING - NFκB intracrine senescent noncanonical pathway. *Aging Cell* **23**, e14258 (2024).

104. Thompson, P. J., Shah, A., Apostolopolou, H. & Bhushan, A. BET Proteins Are Required for Transcriptional Activation of the Senescent Islet Cell Secretome in Type 1 Diabetes. *IJMS* **20**, 4776 (2019).

105. Afifi, M. M. *et al.* Irreversible cell cycle exit associated with senescence is mediated by constitutive MYC degradation. *Cell reports* **42**, 113079 (2023).

Figure 1: Birch, J. & Gil, J. Senescence and the SASP: many therapeutic avenues. *Genes Dev.* **34**, 1565–1576 (2020).