Cellular Aging and Senescence in Cancer

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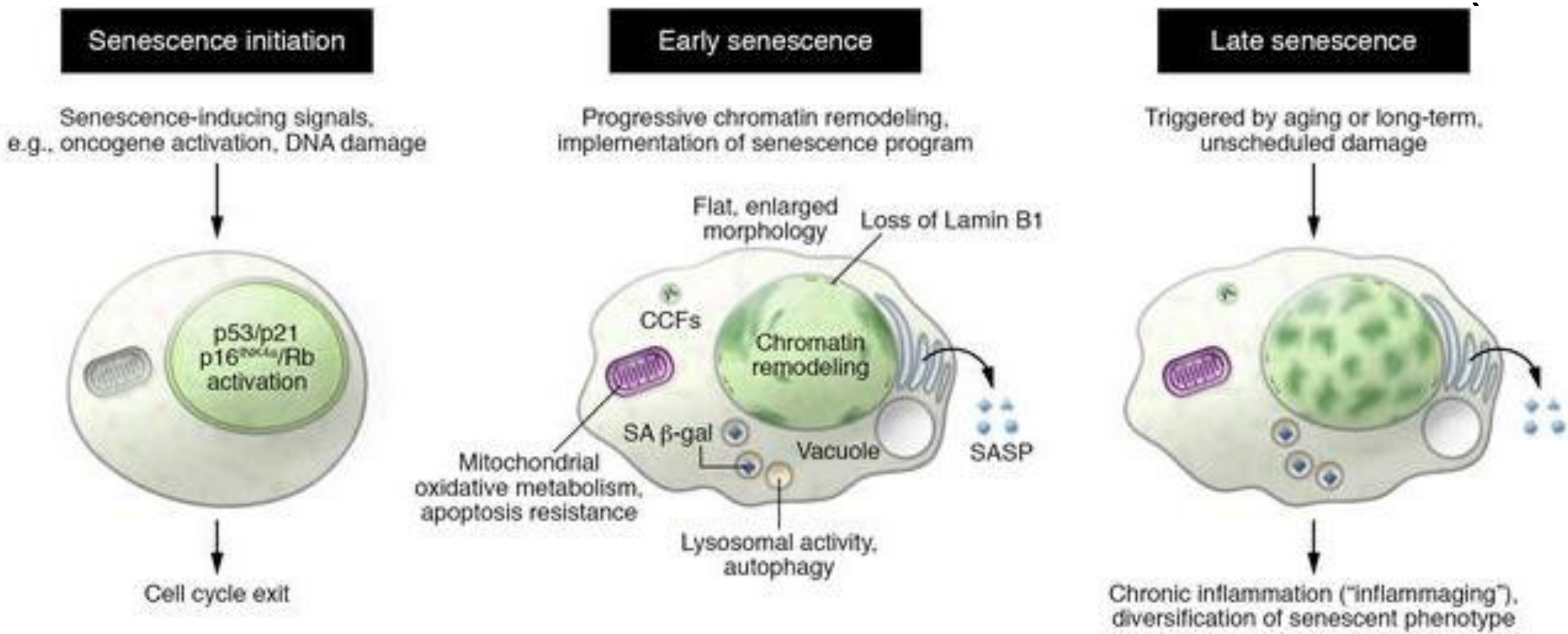
Introduction

- In 1961, Leonard Hayflick and Paul Moorhead published their findings that normal human cells in culture have a limited number of divisions before they stop proliferating, a phenomenon now known as the **Hayflick limit or cellular senescence**.
- This discovery disproved the idea of normal cell immortality and provided a model for studying cellular aging at the level of individual cells
- The Hayflick limit is considered a natural mechanism that acts as a defense against cancer by preventing cells from dividing uncontrollably.

https://www.sciencedirect.com/science/article/abs/pii/0014482761901926?via%3Dihub

Definition

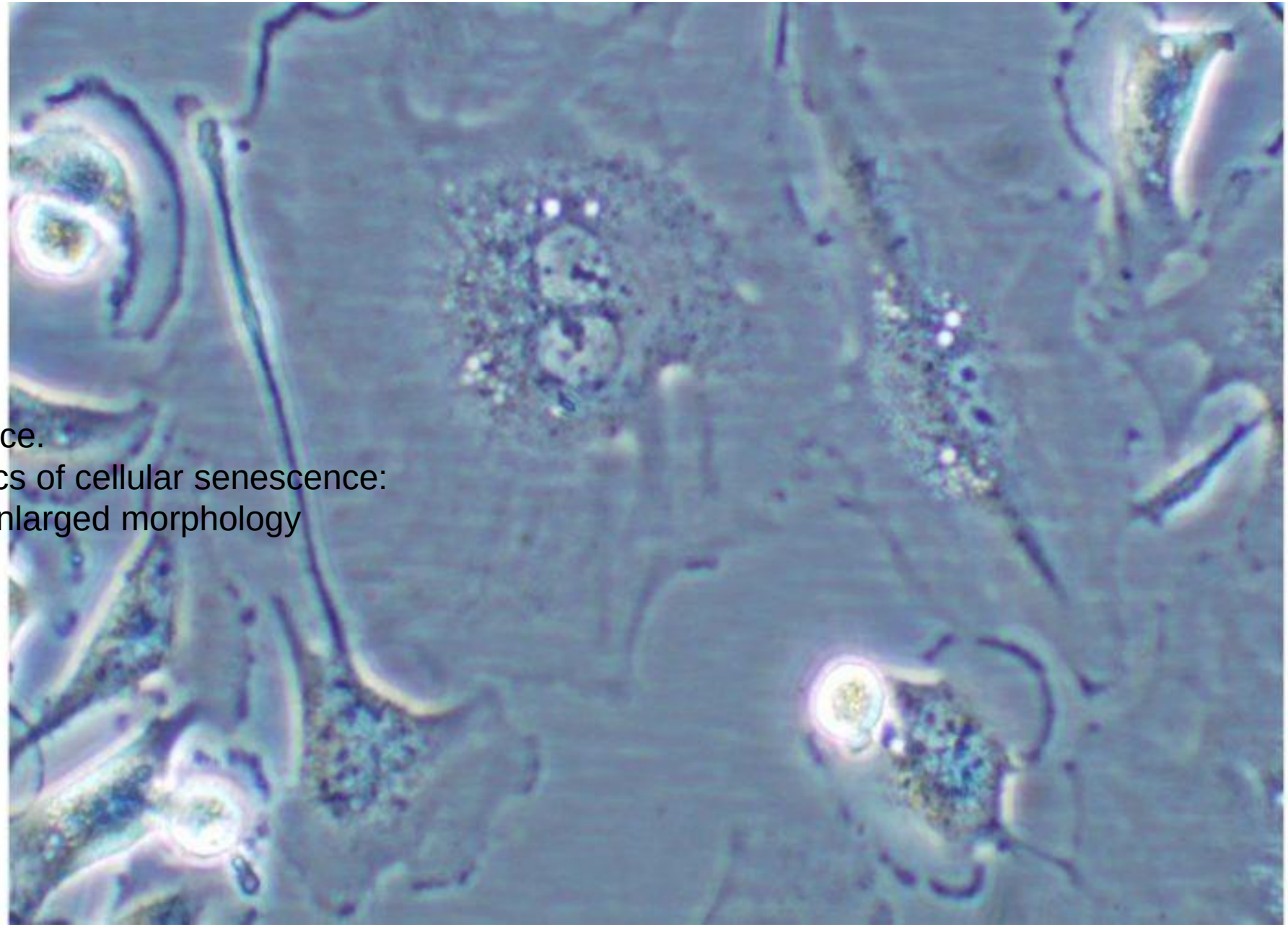
- Cellular senescence is a stress response aimed to eliminate unwanted, damaged, or aberrant cells
- This response consists of a stable proliferative arrest together with the development of a vigorous proinflammatory secretome known as senescence-associated secretory phenotype, or **SASP**
- Senescence defined by an irreversible cell-cycle arrest (unlike to apoptosis) enforced by upregulation of cyclin-dependent kinase inhibitors CDK OR include persistent DNA damage signaling, chromatin remodeling, altered metabolism, resistance to apoptosis, and the SASP, among others
- Through the SASP, senescent cells recruit immune cells to promote their own immune clearance, thereby restoring tissue homeostasis



phenotypic alterations

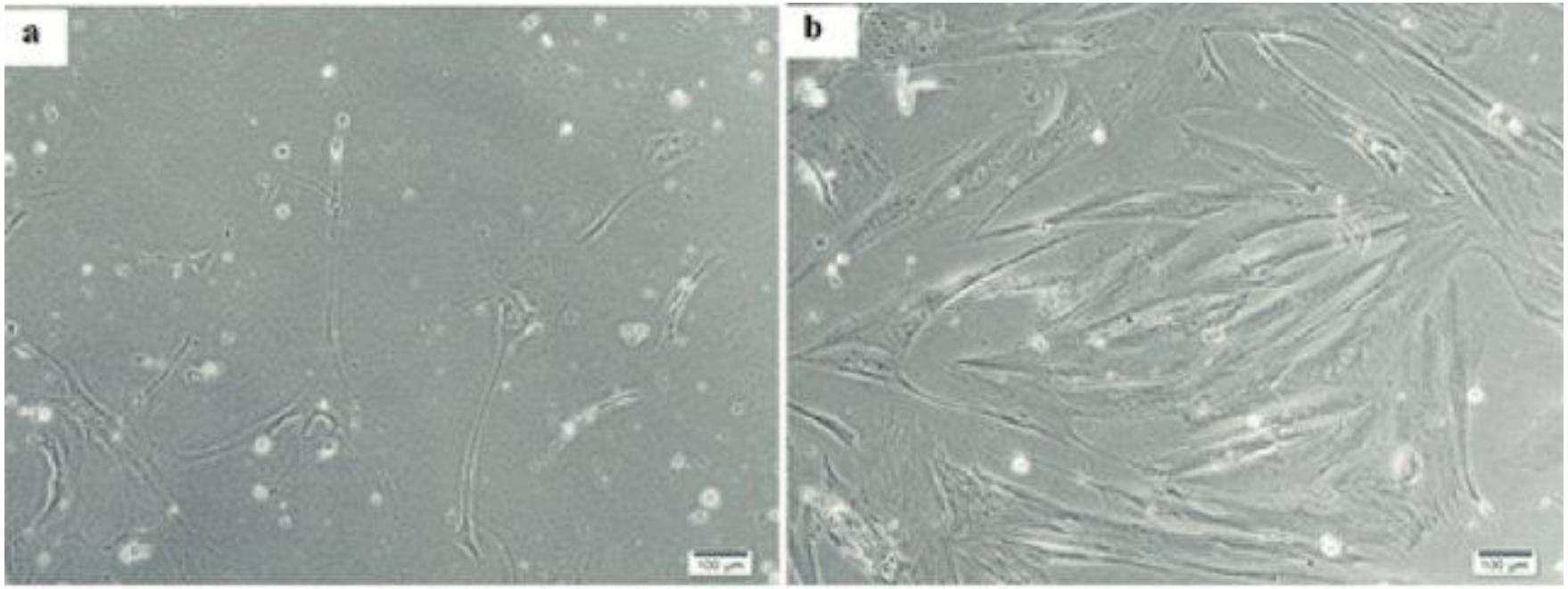
- Cellular senescence has been shown to promote or exacerbate age-associated diseases in humans through the induction of chronic inflammation and tissue dysfunction by the senescence-associated secretory phenotype (SASP).
- SASP proteins mediate an array of effects including mononuclear cell recruitment, fibroblast activation, and degradation of the extracellular matrix.
- Senescent cells, such as endothelial cells, astrocytes, and myocardiocytes, contribute to the progression of age-related diseases including atherosclerosis, neurodegeneration, and myocardial fibrosis, respectively.
- In addition, although cellular senescence inhibits tumor initiation by preventing the proliferation of damaged cells, persistence of senescent cells within the tumor microenvironment promotes tumor recurrence.
- Thus, cellular senescence and SASP are potential therapeutic targets in the treatment of disease in aged individuals.

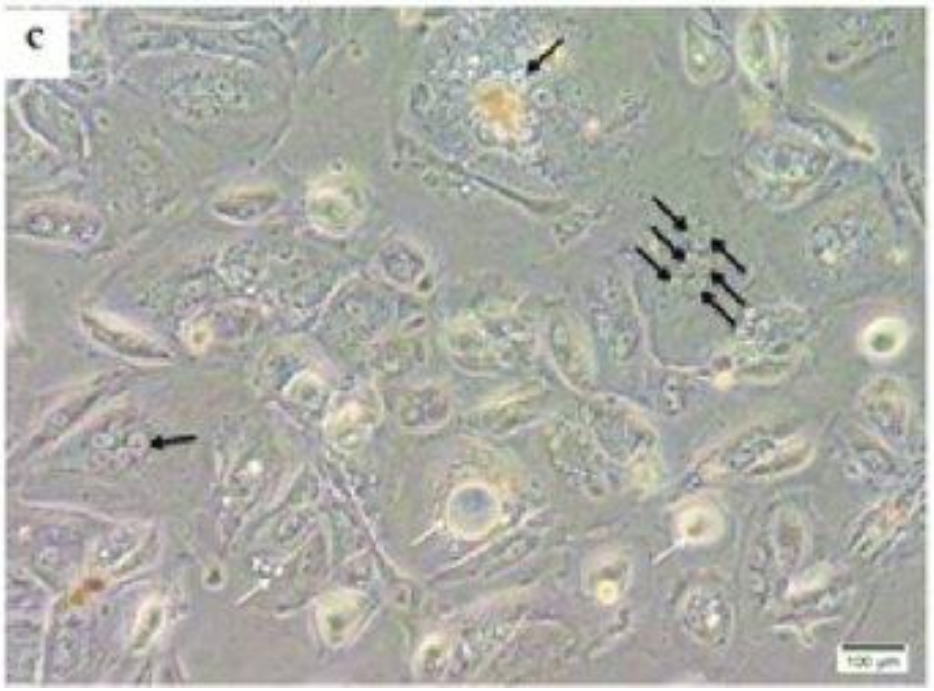
- Unlike apoptotic cells, which undergo a tightly regulated process leading to irreversible cell elimination, senescent cells (SnCs) are unable to divide or undergo cell death, yet are still metabolically active and thus impact their microenvironment
- Throughout organismal lifespan, these cells accumulate in different tissues and contribute to both beneficial and detrimental effects on the body and contribute to diverse pathological conditions
- In cancer, senescence works as a tumor-suppressive barrier by halting the proliferation of damaged cells, preventing the onset of tumors, yet can also promote tumor progression
- This duality arises from complex interactions among SnCs, their senescence-associated secretory phenotype (SASP), and the tumor microenvironment (TME).



Cell undergoing senescence.
Cell showing characteristics of cellular senescence:
binucleated and flat and enlarged morphology

- Cancer cells are usually exposed to a multitude of stressors known to trigger senescence, including oncogenic signaling, replicative stress, hypoxia, reactive oxygen species, nutrient deprivation, and exposure to cytokines present in the tumor microenvironment
- Senescent tumor cells SnCs are also characterized by an increased resistance to cell death. This resistance is mediated by the upregulation of pro-survival pathways, allowing SnCs to persist in a viable but non-dividing state.
- Senescent tumor cells often evade apoptosis by expressing anti-apoptotic proteins
- a variety of anticancer therapies can also induce senescence in cancer cells e.g., senolytics or senostatic compounds
- Consequently, tumors, both before and after therapy, generally contain a variable fraction of senescent cells





Different types of senescent cells, as diverse as their primary morphology.

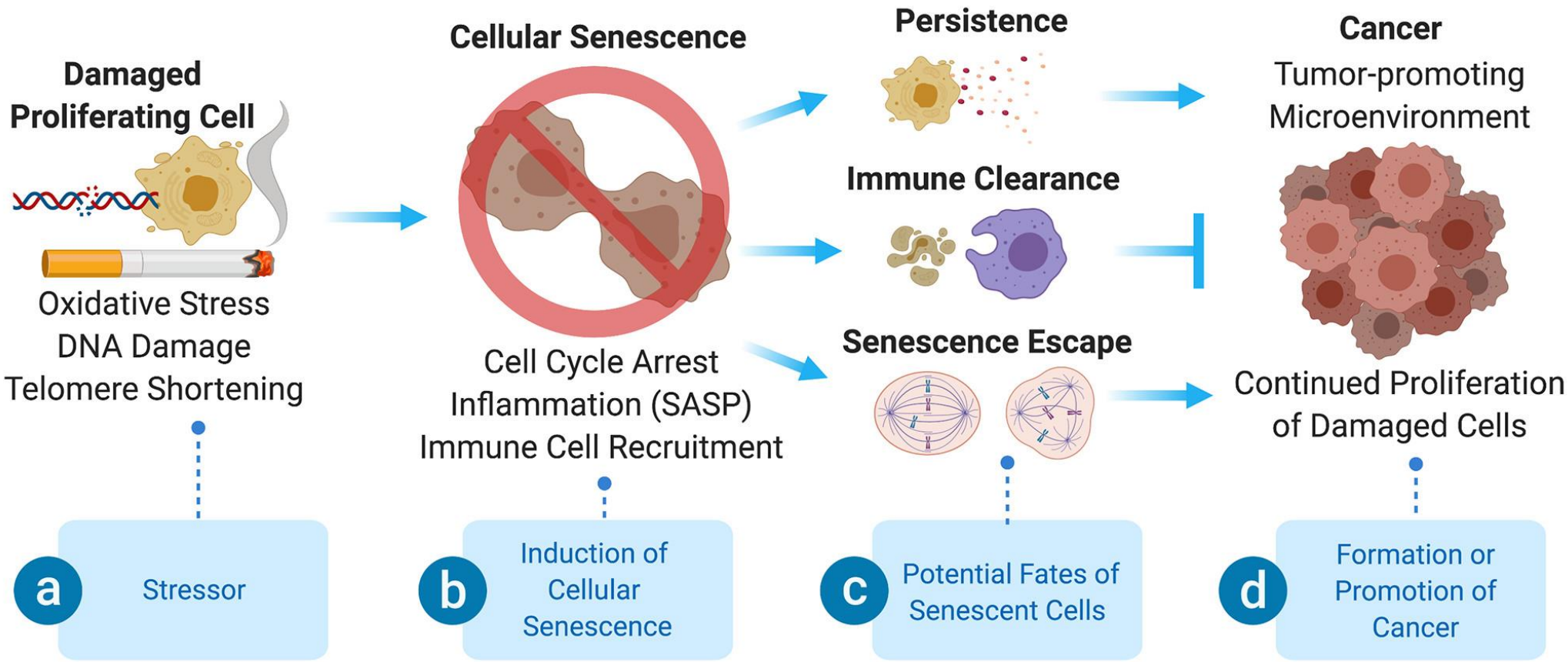
(a) Neuronal-like morphology with cytoplasmic projections in senescent fibroblastoid cells at the 17th passage.

(b) AF-type cells at the beginning of senescence.

(c) Flat, circular E-type cells at the 5th passage when they start being multinuclear (black arrows), granular, and vacuolar (white arrows).

CELLULAR SENESCENCE:

Mechanisms of Tumor Suppression and Promotion



Mechanisms of tumor suppression and promotion

(a) Induction of cellular senescence is an important mechanism of tumor suppression which prevents tumor initiation by inhibiting proliferation of damaged cells.

(b) Senescent cells undergo a permanent cell cycle arrest and adopt the senescence-associated secretory phenotype (SASP), which recruits leukocytes to remove senescent cells.

(c) There are several potential fates for cells undergoing senescence. Normal tissue function can be restored if macrophages remove senescent cells; however, this process is not always efficient, leading to the persistence of senescent cells that can promote tumor recurrence and the side effects of cancer therapy through SASP.

Some reports also suggest that tumor cells can either escape or inhibit the induction of cellular senescence allowing them to reenter the cell cycle leading to tumor initiation further underscoring the contrasting functions of cellular senescence in tumor suppression and promotion.

- Senescent cancer cells **SCC**, due to their low or null proliferative capacity, do not contribute to tumor growth; however, they contribute to modify the tumor microenvironment through their SASP
- SASPome includes extracellular vesicles, non-coding RNA, bioactive lipids, and other mediators that collectively shape the TME, propagate senescence signals, modulate inflammation, and remodel extracellular architecture
- The SASP produced by intratumoral senescent cells has complex and often opposite effects on tumor behavior depending on multiple factors that partly reflect the intrinsic heterogeneity of cancer and the response to cancer therapies

Table 1. The SASPome

Component	Description	Reference
Cytokines and interleukins	Secretion of IL-6 and IL-8, IL-1α, IL-1β, and TNF-α reinforces growth arrest in an autocrine manner and can induce senescence in neighboring cells through paracrine signaling.	Coppe et al.[18]; Coppe et al.[19]; Acosta et al.[20]; Orjalo et al.[21]
Chemokines	CXCL1, CXCL8, CCL2, and GROα, which contribute to the pro-inflammatory environment. These chemokines recruit immune cells, including macrophages and neutrophils, to the site of senescence, thereby modulating tissue remodeling and immune responses. While chemokine secretion by senescent cancer cells can be beneficial, it may also support tumor progression	Di Mitri et al.[22]; Kim et al.[23]; Ruan et al.[24]; Liu et al.[25]; Langhi Prata et al.[26]
Growth factors	These factors, including amphiregulin, EGF, FGFs, and HGF, contribute to an inflammatory microenvironment that influences tumor progression. While some growth factors promote tissue repair and regeneration, others can support cancer cell survival, proliferation, and resistance to therapy by altering the extracellular matrix and attracting pro-tumorigenic immune cells.	Guccini et al.[15]; Shang et al.[27]; Igarashi et al.[28]; Coutu & Galipeau[29]; Xu et al.[30]
Extracellular matrix components	ECM proteins secreted by SnCs play a crucial role in tissue remodeling by modifying the extracellular landscape. Among these proteins, fibronectin and osteopontin facilitate cell adhesion, migration, and proliferation therefore promoting cell invasion and metastasis while also fostering a pro-inflammatory microenvironment.	Brina et al.[31]
Extracellular vesicles (EVs)	SnCs exhibit increased production of EVs, including exosomes and microvesicles. These vesicles serve as carriers for proteins, lipids, and nucleic acids, facilitating intercellular communication. EVs derived from SnCs can propagate senescence to recipient cells and modulate immune responses, thereby influencing tissue	Meng et al.[32]; Takasugi et al.[33]

	microenvironment.	
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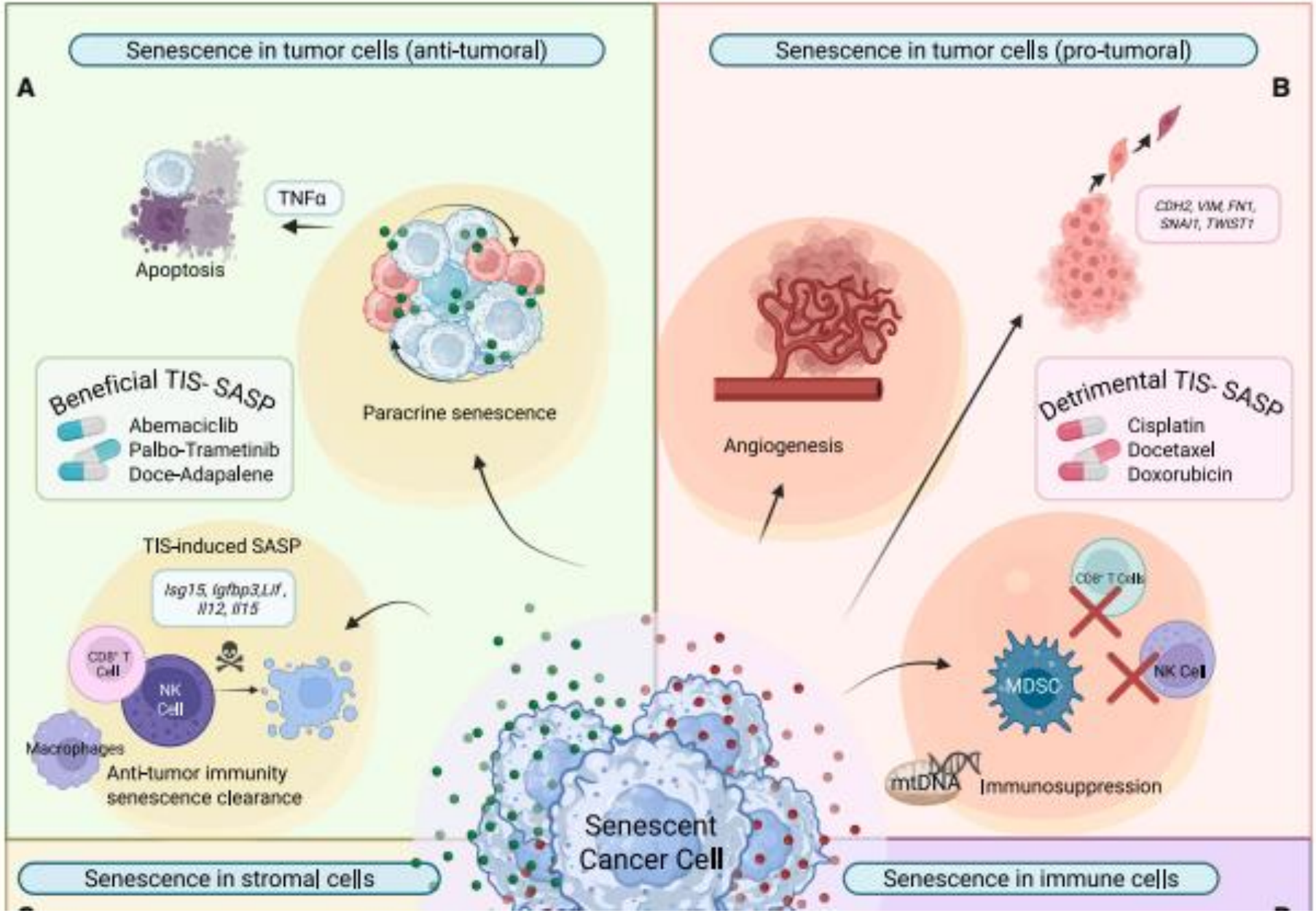
Mitochondrial and genomic DNA	SnCs can release fragments of mitochondrial DNA and genomic DNA into the cytosol and extracellular space, either freely or encapsulated within EVs. These cytosolic and extracellular DNA fragments can act as DAMPs, regulating the SASP or directive activating immune cells such as myeloid-derived suppressor cells (MDSCs), thereby contributing to the pro-inflammatory environment characteristic of senescent tissues.	Victorelli et al.[34]
Lipid mediators	Products of COX2 enzymatic activity, such as PGE_2, are well understood to affect inflammation and have recently been described as upregulated by SnCs in both aging and cancer contexts. Considering the broad biological activity of lipid mediators in various disease states, further exploration should reveal additional lipids that are consistent with the SASPome.	Loo et al.[35]; Goncalves et al.[36]; Chen et al.[37]
microRNA	MicroRNA are small noncoding fragments of RNA that have been studied for their role in senescence modulation both intracellularly and as disease biomarkers in systemic circulation. miR-146a is consistently upregulated in senescent fibroblasts, and miR-146a-GFP reporter constructs have been utilized for their consistency in monitoring senescence *in vitro*. Although this has mainly been described in aging, its upregulation and extracellular circulation should be further explored the cancer SASPome.	Kang et al.[38]


Senescence in tumor cells (anti-tumoral)
A
Apoptosis
TNFα
Paracrine senescence
Beneficial TIS- SASP
Abemaciclib
Palbo-Trametinib
Doce-Adapalene
TIS-induced SASP
Isg15, Igfbp3,Lif, Il12, Il15
CD8+ T Cell
NK Cell
Macrophages
Anti-tumor immunity
senescence clearance
Senescence in tumor cells (pro-tumoral)
B
CDH2, VIM, FN1, SNAI1, TWIST1
Angiogenesis
Detrimental TIS- SASP
Cisplatin
Docetaxel
Doxorubicin
CD8+ T Cells
MDSC
NK Cell
mtDNA
Immunosuppression
Senescent Cancer Cell
Senescence in stromal cells
Senescence in immune cells

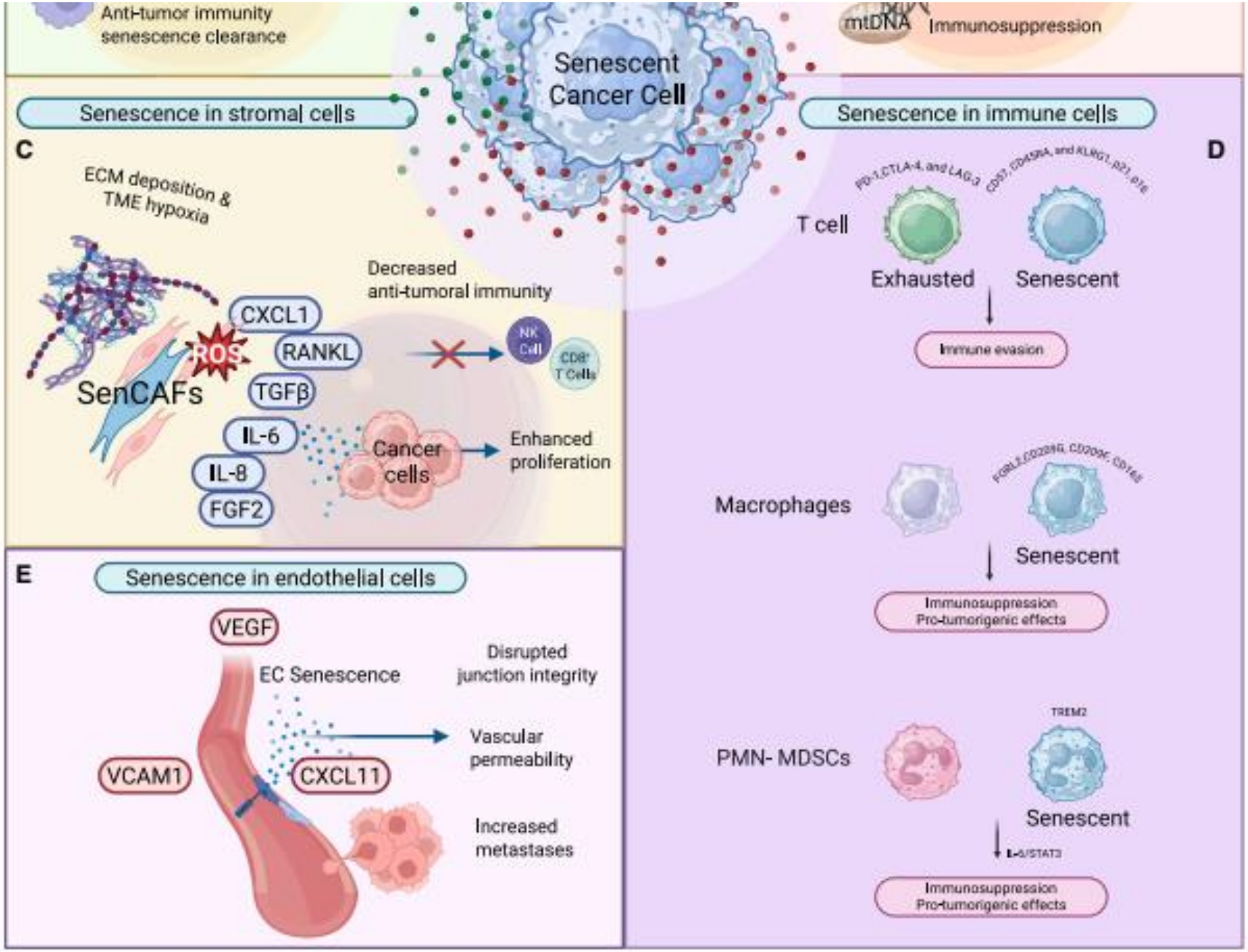

Anti-tumor immunity
senescence clearance
Senescent
Cancer Cell
mtDNA
Immunosuppression
Senescence in stromal cells
C
ECM deposition &
TME hypoxia
Decreased
anti-tumoral immunity
CXCL1
RANKL
ROS
TGFβ
IL-6
IL-8
FGF2
SenCAFs
NK Cell
CD8+ T Cells
Cancer cells
Enhanced proliferation
Senescence in immune cells
D
T cell
Exhausted
Senescent
Immune evasion
Macrophages
Senescent
Immunosuppression
Pro-tumorigenic effects
PMN- MDSCs
TREM2
Senescent
IL-6/STAT3
Immunosuppression
Pro-tumorigenic effects
E
Senescence in endothelial cells
VEGF
EC Senescence
Disrupted
junction integrity
Vascular
permeability
VCAM1
CXCL11
Increased
metastases

Table 2. Clinical application of pro-senescence, senomorphic, and senolytic compounds

	Compound	Type	Target(s)	Clinical applications	FDA approval	EMA approval
Pro-senescence	**Docetaxel**	Small molecule	Tubulin	Head and neck cancer, non-small-cell lung adenocarcinoma, prostatic cancer, stomach cancer, bladder cancer, and breast neoplasms	Head and neck neoplasms carcinoma, non-small-cell lung adenocarcinoma, prostatic neoplasms, stomach neoplasms, bladder neoplasm (intravesical instillation), breast neoplasms	Head and neck neoplasms carcinoma, non-small-cell lung adenocarcinoma, prostatic neoplasms, stomach neoplasms, bladder neoplasm (intravesical instillation), breast neoplasms
	Paclitaxel	Small molecule	Tubulin	Sarcoma, kaposi carcinoma, non-small-cell lung, ovarian neoplasms, breast neoplasms	Sarcoma, kaposi carcinoma, non-small-cell lung, ovarian neoplasms, breast neoplasms	Sarcoma, kaposi carcinoma, non-small-cell lung, ovarian neoplasms, breast neoplasms
	Nab-Paclitaxel	Protein-bound small molecule	Tubulin	Breast cancer, pancreatic cancer, lung cancer	Breast neoplasms, pancreatic neoplasms, carcinoma, non-small-cell lung adenocarcinoma	Breast neoplasms, pancreatic neoplasms, carcinoma, non-small-cell lung adenocarcinoma
	Cabazitaxel	Small molecule	Tubulin	Prostatic neoplasms	Prostatic neoplasms	Prostatic neoplasms
	Suberoylanilide hydroxamic acid (SAHA)	Small molecule	Histone deacetylase enzymes (HDAC1, HDAC2, HDAC3, HDAC6)	Clinical trial for advanced metastatic and/or local chest wall recurrent HER2-amplified breast cancer (phase I/II). clinical trial in breast cancer		
	Vorinostat	Small molecule	Histone deacetylase enzymes (HDAC1, HDAC2, HDAC3, HDAC6)	metastatic colorectal cancer, non-small-cell lung carcinoma, prostate cancer, bladder cancer, urothelial carcinoma	Refractory mycosis fungoides (MF) or Sézary syndrome (SS)	Lymphoma, T cell, cutaneous (EMA withdrawn)
	Ribociclib	Small molecule	Cyclin-dependent kinases 4 and 6 (CDK4/6)	Breast cancer	Breast neoplasms	Breast neoplasms
	Palbociclib	Small molecule	Cyclin-dependent kinases 4 and 6 (CDK4/6)	Breast cancer	Breast neoplasms	Breast neoplasms
	Abemaciclib	Small molecule	Cyclin-dependent kinases 4 and 6 (CDK4/6)	Breast cancer	Breast neoplasms	Breast neoplasms

Table 2. *Continued*

	Compound	Type	Target(s)	Clinical applications	FDA approval	EMA approval
Senolytic	**Navitoclax (ABT-263)**	Small molecule	Bcl-2, Bcl-xl, Bcl-w inhibitor	Used in clinical trials for treating chronic lymphocytic leukemia and small lymphocytic lymphoma	–	Myelofibrosis (orphan drug)
	Venetoclax (ABT-199)	Small molecule	Bcl-2 inhibitor	Approved for treating chronic lymphocytic leukemia and acute myeloid leukemia	Acute myeloid leukemia, chronic lymphocytic leukemia, small lymphocytic lymphoma	Acute myeloid leukemia, chronic lymphocytic leukemia
	Dasatinib	Small molecule	Kinase inhibitor	Approved for treating chronic myeloid leukemia and acute lymphoblastic leukemia	Chronic myeloid leukemia and acute lymphoblastic leukemia	Chronic myeloid leukemia, acute lymphoblastic leukemia
	CAR T cells	Engineered chimeric antigen receptor	Senescent cells' markers	Used in immunotherapy for treating leukemia and lymphoma		
	Piperlongumine	Alkaloid	ROS, NF-κB, NLRP3 inflammasome, PI3K/Akt/ mTOR pathway	Anticancer and anti-inflammatory effects		
	Fisetin	Flavonoid	BCL-XL, VEGF, NF-κB inhibitor	Anti-inflammatory, antioxidant, and anticancer properties.		
	Nicotinamide riboside	Vitamin-like	NAD+ Levels	Anti-aging, metabolic health, and neuroprotection		
	Danazol	Synthetic steroid	Telomeres	Approved for treating endometriosis, fibrocystic breast disease, and hereditary angioedema.	Endometriosis and hereditary angioedema	
Senomorphic	**Sirolimus/ Rapamycin**	Small molecule	Mechanistic target of rapamycin (mTOR) pathway, specifically mTORC1	Used as an immunosuppressant to prevent organ transplant rejection	Graft rejection, kidney transplantation	Graft rejection, kidney transplantation
	Sirolimus Protein Bound Particles	Protein-bound small molecule	Mechanistic target of rapamycin (mTOR)	Used in cancer therapy	Locally advanced unresectable or metastatic malignant	

Machine learning and AI in senescence research

Novel computational approaches compatible with both bulk RNA sequencing and single-cell RNA sequencing (scRNA-seq) have been developed to facilitate the identification and characterization of SnCs in tumor tissues:

- The senescence identification tool (SIT)
- SENCAN—a machine learning-based gene expression classifier
- Cancer SENESCopedia
- SenMayo gene set
- scRNA-seq
- the cellular senescence network (SenNet) program

A

Overview

Basic research → Translational → Clinic

- Basic research: SIT, SENCAN
- Translational: SenMayo, SenNet; Screening
- Clinic: AI-driven pathology?

B

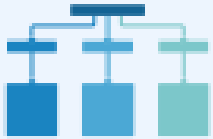
Morphological features

Deep-SeSMo
Accurately identified senescent cells based on morphological features.

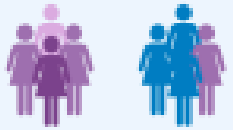
SA-β-Gal evaluation
Extracted 154 features with CellProfiler. Trained a random forest classifier for robust SA-β-Gal scoring.

Morphology-based Cascade R-CNN deep learning system
Correlated morphology with known markers: p16, p21, and SASP.

SAMP
Used hierarchical clustering to distinguish senescent from proliferative cells *in vitro* and *in vivo*.



Patient Stratification
Deep learning models analyzed senescence-associated morphologies to predict invasive breast cancer risk.

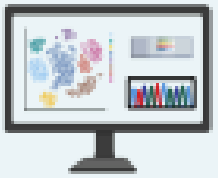


C

Genetic features

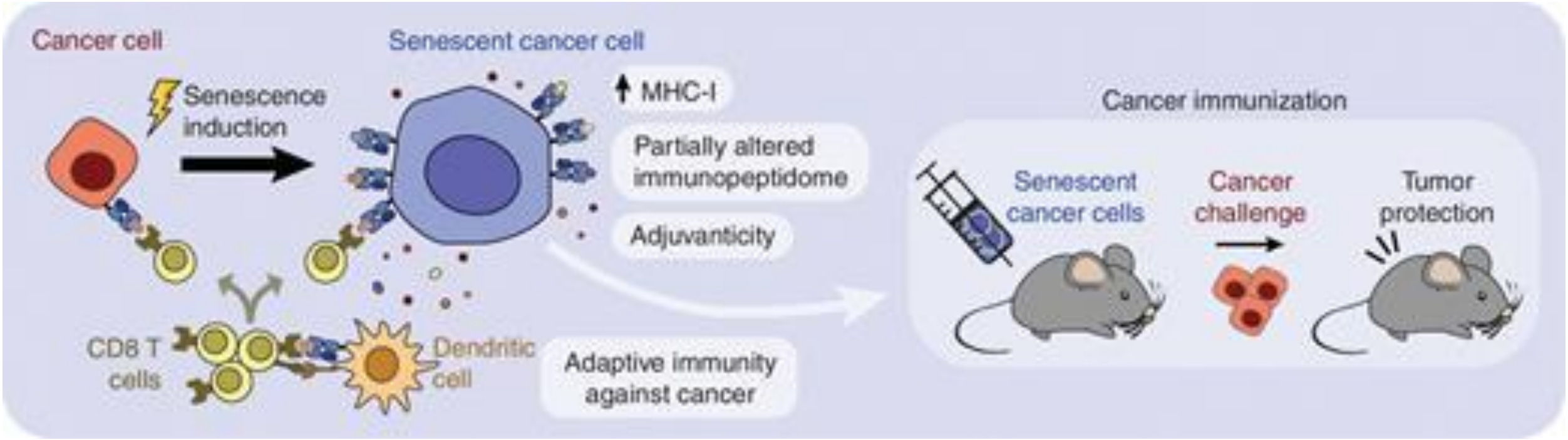
SenPred
Machine-learning tool based on single-cell RNA sequencing, detects senescent dermal fibroblasts, offering valuable insights into tumor progression.

SIT, **SENCAN**, **SenMayo**, and the **SenNet program**
Characterize senescent cell heterogeneity.
Support therapy development through vulnerabilities identification.



DNAmSen
DNA methylation-based predictor, accurately tracks age and senescence across multiple tissues, including cancer-affected ones, by identifying 88 CpG markers linked to various senescence triggers.

Application



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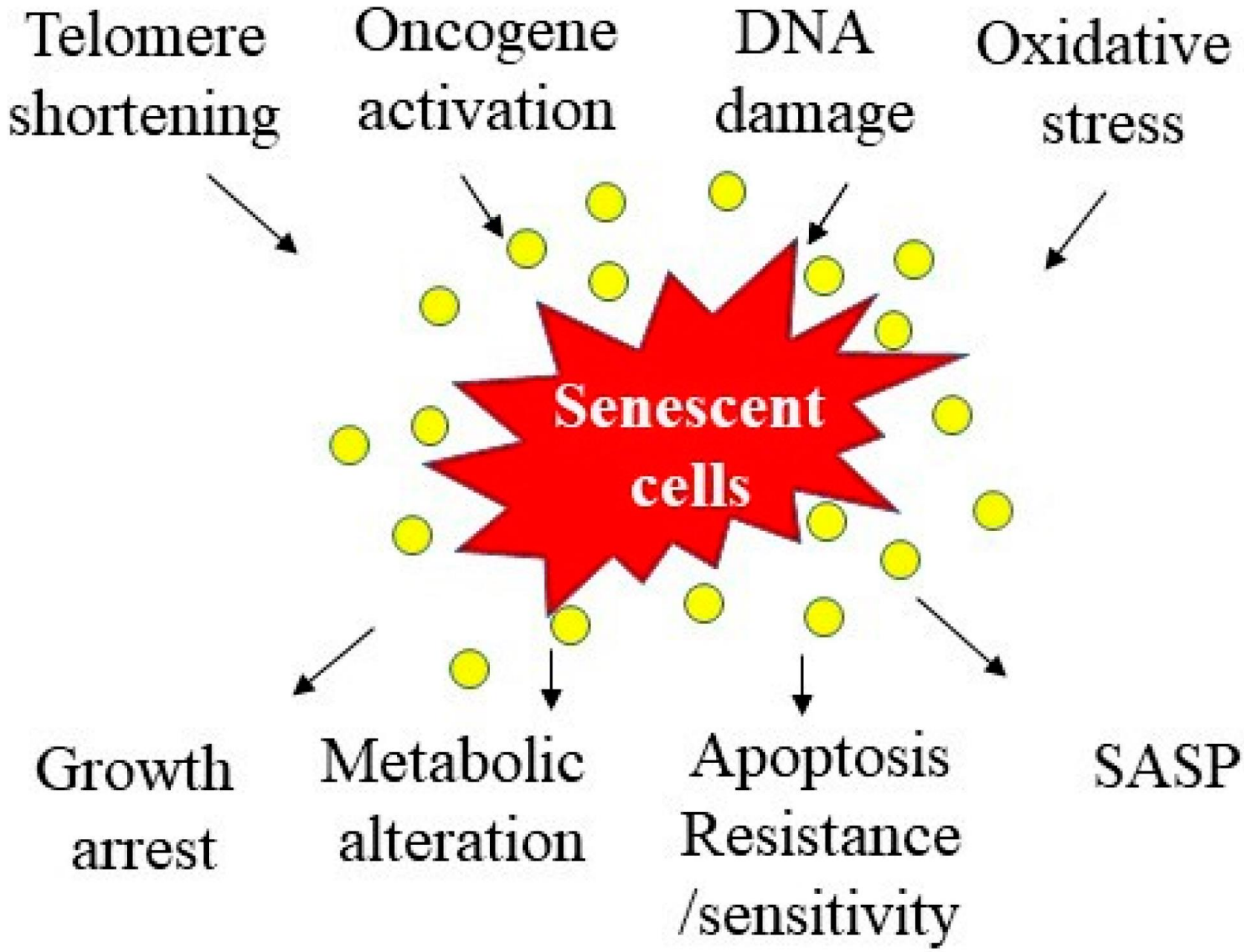
Conclusion

- **Senescent tumor cells STCs include nuclear enlargement, the release of cytoplasmic chromatin fragments (CCFs), and persistent DNA damage signaling, often driven by oncogenic or oxidative stress. STCs display mitochondrial dysfunction, resulting in metabolic reprogramming**
- **Cellular stress responses, such as autophagy, are upregulated in parallel with senescence**
- **Potential to enhance therapeutic efficacy and patient outcomes.**
- **Elucidate the mechanisms, markers, and functional consequences of immune cell senescence in cancer**
- **Clarifying how immune cells acquire the senescent phenotype within the TME and how they impact tumor progression could offer insights for the development of immune-senolytics for cancer therapy**
- **Translational implementation of the preclinical research to clinical evaluation of patient senescence status, use of AI-based diagnostic tools, and senolytic and senomorphic therapeutics should also be considered.**
- **Advance therapies for age-related diseases, neurodegeneration, fibrosis, and regenerative medicine**


Telomere shortening
Oncogene activation
DNA damage
Oxidative stress
Senescent cells
Growth arrest
Metabolic alteration
Apoptosis Resistance /sensitivity
SASP