Senescence and Immortalization

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What are we expected to learn today?

- What is cellular Senescence?
- What is the nature of Senescence phenotype?
- What are the intrinsic and extrinsic factors that dictate Senescence?
- What has senescence got to do with Cancer?
- What is the physiological relevance of Senescence?
- Can we target Senescence for cancer therapy?
- Emerging new faces of Senescence
What is Senescence?
Irreversible growth-arrested state that depends on the age or cell doublings of a cell – cannot be reversed by physiological stimuli

Leonard Hayflick and Paul Moorhead (1961)

Normal cells tracks division and stops after finite number of divisions -“Hayflick limit” or “Replicative Senescence”
Phase I - Primary culture that divides actively to cover the culture dish.

Phase II - Confluent cell culture that stopped growing, but will continue to grow when subcultured.

Phase III - Cells stopped dividing (Senescent or growth arrested)

Normal cells have predetermined number of population doublings dictated by the species of origin, tissue of origin, age of the donor.

Exceptions: Embryonic stem cells and terminally differentiated cells
Loss of Proliferative Capacity with Age

\[ PDM = 67.2 - 0.165 \times \text{age in years} \]

Population doublings vs. donor age (years)

- Grey: foreskin
- Red: chest
- Orange: face
- Green: leg
- Blue: arm

18 years old vs. 76 years old
Unlimited cellular division (escape from senescence) can be only achieved by cancer cells.

Cessation of cell growth in culture may be related to aging in vivo.

Hayflick linked senescence to tumor suppression and aging half a century ago!

Why should normal cells lack immortality?
- Anticancer defense mechanism

Normal cells need to bypass this barrier of senescence to become cancer cells.

Understanding of senescence helps to device strategies to interfere with immortality in cancer cells.

What are the factors that regulate senescence in normal cells?
• How Senescence cells differ from their normal counterparts?
What are the features of Senescent cells?

Senescent cells acquire complex phenotypes

- Permanent growth arrest – cannot be reversed by physiological stimuli
- Increased cell size – flat appearance with huge cytoplasm
- Increased cytoplasmic granularity
- Express senescence associated (SA) beta galactosidase
- (reflects increased lysosomal mass)
- Metabolically active (dead….but live?)

..........and more
Cumulative oxidative damage (ROS) can accelerate senescence.
Human foreskin keratinocytes have extended life span when cultured with feeder fibroblasts (low induction of senescence marker p16INK4a)
Cyclin-dependent kinase inhibitors (CDKIs) - critical for cell cycle-regulatory phosphorylation events.

\( p21^{\text{CIP1/WAF1}} (\text{CDKN1A}) \), induced in response to genomic damage is a direct target of p53 transactivation crucial for establishing and maintaining the p53-mediated senescence growth arrest.

\( p16^{\text{INK4a}} (\text{CDKN2A}) \) can be induced by stress that does not entail DNA damage. Inhibits phosphorylation of pRB by cyclin D/CDK4/6 which results in growth arrest.

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<td>7</td>
<td>42</td>
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Ectopic expression of p16INK4a induces Senescence Phenotype
Two major Tumor Suppressors, p53 and pRb control Senescence

Inactivation of both p53 and pRb is needed to escape from senescence

In majority of human cancers of both p53 and pRb are inactivated
Inappropriate activation of mitogenic signaling pathways by RasV12 can trigger Premature Senescence, which can be overcome by p53 suppression.
Bypassing Senescence vs Attaining Immortalization

Senescence
Halt of proliferation with retention of cell viability

Crisis
Involves apoptosis

Immortalization
Rare variants that escape will be immortalized

10-20 PDs

SV40-LT
Telomeres function as Molecular Counters of Cell division

Ends of chromosomes had to be capped by a special structure termed the telomere to prevent chromosome fusion.

Barbara McClintock
1983 Nobel Laureate in Physiology or Medicine,
Telomeres function as a 'molecular clock'. Once the telomeres are shortening of telomeres below a threshold length triggers senescence and crisis.
Mechanism of breakage-fusion-bridge cycles
Mechanism of breakage-fusion-bridge cycles
non-telomeric DNA of chromosome (many megabase pairs long)

double-stranded region of telomeric DNA (5–10 kbp long)

single-stranded 3’ overhang of G-rich strand of telomeric DNA (several hundred bases long)
**telomere length control**

TRF1 complex
- TRF1
- PINX1
- TIN2
- Pot1
- tankyrase 1/2

**telomere protection**

TRF2 complex
- WRN
- hRap1
- Mre11
- Rad50
- Nbs1
- ERCC1/XPF
- TRF2
- T-loop

3' TTAGGG

5' AATCCC
Loss of TRF2 leads to massive end to end fusion

Fluorescence in situ hybridization (FISH) using Fluorescent DNA probe that recognizes telomere repeat
How does Telomere shortening occur with cell division?
Arises due to the inability of the general DNA replication machinery to completely replicate the very ends of the chromosomes.
How cancer cells escape telomere erosion?

Telomerase:
Reverse transcriptase with its own RNA template solves the end replication problem
Immortalization of cells with hTERT

Expression of telomerase prevent crisis
85-90% human tumors are telomerase positive

Human Keratinocytes and Mammary epithelial cells are immortalized by hTERT alone
Evidence for Alternative Lengthening of Telomere (ALT)

early passage cells

40 population doublings later
Alternative Lengthening of Telomere (ALT)

Copy choice mechanism

[Diagram showing the mechanism of telomere lengthening through alternative lengthening of telomere (ALT).]
Telomerase also protects from premature senescence.
• Introduction of viral oncogenes (SV40 – T Ag, Papilloma virus E6, E7)
• Inactivation of Rb and p53
• Further shortening of telomere
• Crisis (genomic instability and massive apoptosis)
• Rare variants (1 in 10 millions) - maintain stable length of telomeres and form immortalized clones.
• Telomere erosion........DNA damage response...p53 activation...... Growth arrest and senescence.

• Senescent cells can have DNA damage in non telomeric sites which generate persistent growth arrest

• Double stranded DNA breaks

• HDAC inhibitors ....... relaxed chromatin structure....no DNA damage...ATM and P53 DNA damage response (DDR)

• P53 activation , which arrests cell proliferation largely through p21^{CIP1/WAF1}. At low levels of damage, the DDR is transient, but high levels cause chronic low level DDR signaling and p53 activation, which maintain a senescence growth arrest.

• DDR activates a subset of the SASP, but independent of p53 activity; loss of p53 function amplifies the SASP, suggesting that p53 restrains this phenotype. The SASP is additionally regulated by microRNAs, the cytokine receptor CXCR2, IL-1 receptor signaling, the transcription factors C/EBPβ and NF-KB, and the JAK/STAT signaling pathway.
Factors that influence Senescence

- Immortal cells
- Primary cell
- Replicative senescence
- Senescent cell
- Terminal arrest
- Immortal cells

Factors:
- Oxidative Stress (SIS)
- Oncogenic Stress (OIS)
- Telomeric erosion
- DNA damage
- Epigenetic change or stochastic mutation/s
- Culture stress

Emerging senescence phenotypes

- Express tumor suppressor p16INK4a

- Activated DDR proteins, phospho-ATM and dysfunctional telomeres

- Persistent DDR signaling lead to nuclear foci termed “DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS)”

- Senescence Associated Heterochromatin Foci (SAHF) (not commonly expressed in quiescent or terminally differentiated cells)

- Resist apoptosis

- Increased expression of secreted proteins Senescence Associated Secretary Phenotype (SASP)

- inflammatory cytokines, growth factors, and proteases

No universal marker of senescence identified so far
Not all SC express all possible markers
Hallmarks of Senescent Cells

Senescence stimuli → growth arrest

SA-Bgal
DNA damage foci (DNA-SCARS/TIF)
Heterochromatin foci (SAHF)

p16INK4a
SASP
Stimulation of tissue repair and age-associated pathologies by altering the tissue microenvironment (found at sites of age-related pathologies, osteoarthritis, atherosclerosis, hyperproliferative lesions such as benign prostatic hyperplasia and melanocytic naevi)

Numerous cell culture and mouse xenograft studies support the idea that senescent cells secrete factors that can disrupt tissue structure and function and promote cancer progression.

**Paradoxical characteristics of senescence – Good or Bad?**

**Good**

Suppression of cancer by blocking the proliferation of damaged cells

**Bad**

Stimulation of tissue repair and age-associated pathologies by altering the tissue microenvironment (found at sites of age-related pathologies, osteoarthritis, atherosclerosis, hyperproliferative lesions such as benign prostatic hyperplasia and melanocytic naevi)
Biological Activities of Cellular Senescence

- Tumor suppression: cell autonomous growth arrest
- SASP (secretory action of senescent cells)
- Depletion of stem/progenitor cell pools

- Tumor promotion
- Aging
- Tissue repair

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Targeting senescence for cancer therapy

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**Oncogene stress** (ras, raf, MEK, Akt, etc)

**Telomeric erosion**

**Telomerase**

**DNA damage sensor**

**Oxidative stress**

**ARF/INK4 locus**

**CDK4/6**

**CDK2/4**

**p53**

**p21**

**E2F1**

**Rb**

**Telomeric erosion**

**Telomerase**

**Epigenetic changes**: e.g. DNA methylation p16<sup>INK4A</sup>

**miR-372, 373, 378, etc**

**Anti-miR**

**DNA methyltransferases inhibitors**

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**Ets, Tbx-2, Zbtb7, Pokemon, etc**

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**miR-372, 373, 378, etc**
Temporal Organization of Senescence Phenotype

Senescence stimulus

Increasing complexity of senescent phenotypes

time

decision initiate SASP express SASP express SASP tune down SASP

IL-1α

NF-κB C/EBPβ

IL-6, IL-8 MMPs etc

growth arrest

tumor suppression
tissue repair

tumor progression

immune clearance

aging

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Primary cells reach **Replicative Senescence** after several passages in culture (telomere erosion).

- DNA damage and oxidative stress can induce senescence (SIS or **Stress induced senescence**).
- Oncogenic stimuli also induces senescence (OIS or **Oncogene induced senescence**).
- OIS and SIS can be referred as **Premature Senescence** as they do not depend on telomere erosion.
- A senescence cell can remain arrested for a long period of time (terminally arrested).
- Alternatively, epigenetic changes and/or stochastic mutation/s can lead it to escape from senescence.

Senescent cells arrest growth owing to cell autonomous mechanisms, imposed by the p53 and p16INK4a/pRB tumor suppressor pathways, and cell nonautonomous mechanisms, imposed by some of the proteins that comprise the SASP. The growth arrest is the main feature by which cellular senescence suppresses malignant tumorigenesis but can contribute to the depletion of proliferative (stem/progenitor) cell pools. Additionally, components of the SASP can promote tumor progression, facilitate wound healing, and, possibly, contribute to aging.
Upon experiencing a potentially oncogenic insult, cells assess the stress and must “decide” whether to attempt repair and recovery, undergo apoptosis or senesce (decision period).

After this interval the senescence growth arrest becomes essentially permanent, effectively suppressing the ability of the stressed cell to form a malignant tumor.

One early manifestation of the senescent phenotype is the expression of cell surface–bound IL-1α. This cytokine acts in a juxtacrine manner to bind the cell surface–bound IL-1 receptor, which initiates a signaling cascade that activates transcription factors (NF-κB, C/EBPβ).

These transcription factors subsequently stimulate the expression of many secreted (SASP) proteins, including increasing the expression of IL-1α and inducing expression of the inflammatory cytokines IL-6 and IL-8. These positive cytokine feedback loops intensify the SASP until it reaches levels found in senescent cells. SASP components such as IL-6, IL-8, and MMPs can promote tissue repair, but also cancer progression.

Some SASP proteins, in conjunction with cell surface ligands and adhesion molecules expressed by senescent cells, eventually attract immune cells that kill and clear senescent cells (immune clearance).
A late manifestation of the senescent phenotype is the expression of microRNAs (mir-146a and mir-146b), which tune down the expression IL-6, IL-8, and possibly other SASP proteins, presumably to prevent the SASP from generating a persistent acute inflammatory response. Despite this dampening effect, the SASP can nonetheless continue to generate low-level chronic inflammation.

The accumulation of senescent cells that either escape or outpace immune clearance and express a SASP at chronic low levels is hypothesized to drive aging phenotypes. Thus, senescent cells, over time (yellow line), develop a phenotype that becomes increasingly complex (blue triangle), with both beneficial (tumor suppression and tissue repair) and deleterious (tumor promotion and aging) effects on the health of the organism.