Senescent cells and aging
Disclosure:

I am a scientific co-founder of UNITY Biotechnology
Aging = susceptibility to (chronic) disease
not a coincidence! caused by basic aging process(es)

Neurodegeneration, memory loss
Osteoporosis
Macular degeneration, hearing loss
Heart disease
Vascular disease
Sarcopenia, frailty
Diabetes, metabolic syndrome
Decreased lung, kidney, etc function

CANCER

Cellular senescence: a candidate basic aging process
What is cellular senescence?
Cellular senescence, a complex stress response

Irreversible GROWTH ARREST

Tri-partite phenotype

Multi-faceted SECRETORY PHENOTYPE

RESISTANCE to APOPTOSIS

(epi)genomic damage

oncogenic mutations

metabolic imbalances

organelle stress
Cellular senescence, a physiological response

Irreversible GROWTH ARREST

Tri-partite phenotype

Multi-faceted SECRETORY PHENOTYPE

RESISTANCE to APOPTOSIS

embryonic development

tissue repair wound healing
Cellular senescence, a complex stress response

Irreversible GROWTH ARREST

Endogenous factors

Tri-partite phenotype

Environmental factors

Multi-faceted SECRETORY PHENOTYPE

RESISTANCE to APOPTOSIS
Cellular senescence, an evolutionary balancing act

Irreversible GROWTH ARREST

Tri-partite phenotype

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RESISTANCE to APOPTOSIS

Tissue remodeling/repair

Tumor suppression

Aging phenotypes

Age-related disease (including cancer)
How are senescent cells defined?
What defines a senescent cell?

- Cytokines, chemokines, growth factors, proteases, eicosanoids
- DAMPs
- GATA4 stabilization
- p16INK4a
- SA Bgal
- ROS
- RIP apoptosis resistance
- Persistent DNA damage foci (DNA-SCARS/TIF)
- Heterochromatin foci (SAHF)
- HMGB1 loss/secretion
- Lamin B1 loss

ps – there are no senescence-SPECIFIC markers

When and where do senescent cells occur?
Senescent cells increase with age in many tissues

Human, non-human primates, rodents, zebrafish skin, retina, liver, spleen, aorta, kidney, lung, etc.

SA-Bgal staining, human skin

Dimri et al., PNAS, 1995
Senescent cells are present at sites of many age-related pathologies

Venous ulcers, atherosclerotic plaques, arthritic joints, COPD, visceral fat, AD brain, etc

Benign prostatic hyperplasia, pre-neoplastic lesions

Noureddine et al., Circulation, 2011

Senescent cells ....

Present at the right time and place to drive aging and multiple age-related diseases

How do senescent cells drive aging?
DO senescent cells drive aging?
How might they do it?

DAMPs
HMGB1
loss/secretion

SASP*

*SASP = senescence-associated secretory phenotype

Inflammation

destroys tissues
disrupts normal cell/tissue functions
prevents stem cell functions
promotes cancer
Senescent cells have potent paracrine activities on normal, premalignant and malignant cells.

- Non-SEN cells:
  - Disrupt normal structures, functions

- SEN cells:
  - Disrupt stem cell functions

- SEN CM:
  - Promote malignant phenotypes

Are you depressed yet?
Strategies to combat aging phenotypes and pathologies fueled by senescent cells

Suppress secretory phenotype
What are the pathways and molecules that drive the secretory phenotype?

(three pathways relevant to cancer and aging)
The DNA Damage Response (DDR) pathway
The p38MAPK-NF-kB pathway
The mTOR pathway

These are important pathways that are required for tissue homeostasis

Drugs that suppress the SASP require continuous dosing (a safe drug?)
Strategies to combat aging phenotypes and pathologies fueled by senescent cells

Suppress secretory phenotype

Kill/eliminate senescent cells
p16-3MR (tri-modal reporter) mice

BAC containing murine INK4a locus inserted into mouse genome

3MR knock-in: downstream of p16\textsuperscript{INK4a} promoter + inactivation of p14ARF
Mice have normal (diploid) copies of p16 and p14 genes

\[
\begin{array}{ccccc}
p16 \text{ Promoter} & \text{renLuc} & \text{mRFP} & \text{HSV-tk} \\
\end{array}
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3MR: renilla luciferase; modified red fluorescent protein; herpes simplex virus thymidine kinase

GCV = gancyclovir
Low affinity for cellular TK
High affinity for viral TK

Demaria et al, Dev Cell, 2014; Laberge et al, CDD
Senescent cells can be eliminated from naturally aged mice

**Luminescence and GCV treatment in aging p16-3MR mice**

parallel age-related increase in endogenous p16INK4a, 3MR, IL-6, etc; all reduced by GCV treatment
Senescent cells ..... 

Alzheimer's and Parkinson's disease  
Atherosclerosis  
Cardiovascular dysfunction  
Cancer metastasis and recurrence  
Chemotherapy (HAART) cardiotoxicity, fatigue  
Cognitive decline/loss of neurogenesis  
Diabetes  
Myeloid → lymphoid skewing  
Osteoarthritis  
Sarcopenia/frailty  
Wound healing, tissue regeneration

* published; * unpublished
Cellular senescence

Adverse effects of chemotherapy

Parkinson's disease and brain aging

Injury-induced osteoarthritis

Wound healing
Many cancer + other therapies → DNA damage

DNA damage → senescence/SASP

DNA damaging therapies → long- and short-term adverse side effects

"Among adult survivors of childhood cancer, the prevalence of adverse health outcomes was high ...... medical assessment identified a substantial number of previously undiagnosed problems that are more prevalent in an older population."

Clinical ascertainment of health outcomes among adults treated for childhood cancer
Hudson et al, JAMA, 2013
DNA damaging therapies → persistent senescent cells
Senescent cells promote metastases

**MMTV-PyMT breast cancer**

MMTV-PyMT cells expressing firefly luciferase

Inject into inguinal mammary fat pad → multifocal mammary adenocarcinomas + lung/liver metastases
Senescent cells promote metastases in mice with breast cancer
Cardiotoxicity often limits chemotherapy
Senescent cells contribute to chemotherapy-induced cardiotoxicity

21 days post-doxo
No effect 7 days post
Chemotherapy-induced loss of activity (fatigue?)
Mice without breast cancer treated with chemotherapy (doxorubicin)
**Behavior: mice + chemotherapy (no cancer)**

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**% Total time**

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p-value: *<0.05; **<0.01; ***<0.001
\]

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N=5
\]

**Measurements at night**

Demaria et al, in progress
Cellular senescence

Adverse effects of chemotherapy

Parkinson's disease and brain aging

Injury-induced osteoarthritis

Wound healing
Senescence marker $p16^{\text{INK4a}}$ increases in brains of human PD patients

**p16 mRNA**

![Bar chart showing p16 mRNA expression in normal and PD brains](chart)

**IL-6 and IL-1**

![Bar charts showing IL-6 and IL-1 mRNA expression in control and PD samples](chart)

**IL-8 and MMP3**

![Bar charts showing IL-8 and MMP3 mRNA expression in control and PD samples](chart)

Chintar, Demaria, Andersen et al; unpublished
Paraquat (PQ) causes Parkinson's disease in mice and humans.

PQ causes astrocytes to undergo senescence.

Chinta et al, submitted
PQ reduces motor neuron function; restored by GCV

Fig. 5 B

Rearing behaviour

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<th>Saline</th>
<th>PQ</th>
<th>GCV</th>
<th>PQ + GCV</th>
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<td>Number of Rears per min.</td>
<td>10.5 ± 1.2</td>
<td>5.2 ± 1.1</td>
<td>8.9 ± 1.3</td>
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*Significant difference compared to Saline
#Significant difference compared to PQ
Cellular senescence

Adverse effects of chemotherapy

Parkinson's disease and brain aging

Injury-induced osteoarthritis

Wound healing
Surgical cut in anterior cruciate ligament

Vehicle or GCV

↓↓↓↓↓↓

weeks after surgery

Sham  Vehicle  GCV

28 days

p16  (fold change)

Time after surgery (days)

Response time (s)

p16-3MR mice undergoing post-traumatic OA.

Jeon, Elisseeff; unpublished
Surgical cut in anterior cruciate ligament: eliminating senescent cells restores function

% weight

120%
100%
80%
60%
40%

no surgery  sham  veh  GCV

ACLT

***  ***

40%  60%  80%  100%  120%
Cellular senescence, an evolutionary balancing act (why did the SASP evolve?)

Irreversible GROWTH ARREST

Tri-partite phenotype

Multi-faceted SECRETORY PHENOTYPE

RESISTANCE to APOPTOSIS

Tissue remodeling/repair

Aging phenotypes

Age-related disease (including cancer)

Tumor suppression
Cellular senescence

Adverse effects of chemotherapy

Parkinson's disease and brain aging

Injury-induced osteoarthritis

Wound healing
Cellular senescence is induced during wound healing

Wound healing: 4 days after punch biopsy

Induction of p16\textsuperscript{INK4a}, 3MR, IL-6 expression

Demaria et al, Dev Cell, 2014
Senescent cells are present transiently during wound healing.
WOUND HEALING IS RETARDED BY ELIMINATING SENESCENT CELLS

GCV 0-5 days after biopsy

female mice
Topical PDGF-AA topical rescues slow wound healing in GCV-treated 3MR mice.
Cellular senescence, an evolutionary balancing act

Irreversible GROWTH ARREST

Tri-partite phenotype

Multi-faceted SECRETORY PHENOTYPE

RESISTANCE to APOPTOSIS

Replace SnC factors

Kill SnCs

Tissue remodeling/repair

Aging phenotypes

Age-related disease (including cancer)

Tumor suppression
Cellular senescence, a complex stress response

Endogenous factors

Irreversible GROWTH ARREST

Tri-partite phenotype

Multi-faceted SECRETORY PHENOTYPE

RESISTANCE to APOPTOSIS

Environmental factors
THANKS!

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