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
- Decreased lung, kidney, etc function
- Diabetes, metabolic syndrome
- 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

metabolic imbalances

oncogenic mutations

organelle stress
Cellular senescence, a physiological response

Irreversible GROWTH ARREST

Tri-partite pheno-type

Multi-faceted SECRETORY PHENOTYPE

RESISTANCE to APOPTOSIS

embryonic development

tissue repair wound healing
Cellular senescence, a complex stress response

Irreversible GROWTH ARREST

Tri-partite phenotype

Multi-faceted SECRETORY PHENOTYPE

RESISTANCE to APOPTOSIS

Endogenous factors

Environmental factors
Cellular senescence, an evolutionary balancing act

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
How are senescent cells defined?
What defines a senescent cell?

- Dimri, *PNAS*, 1995;
- Beausejour, *EMBO J*, 2003;
- Narita, *Cell*, 2003;
- Wiley, unpublished

**GROWTH ARREST**

- Persistent DNA damage foci (DNA-SCARS/TIF)
- lamin B1 loss
- HMGB1 loss/
  secretion
- Heterochromatin foci (SAHF)

**SAVF**

- GATA4 stabilization
- p16INK4a
- SA-Bgal
- RIP
- Apoptosis resistance

**DAMPs**

- Cytokines, chemokines, growth factors, proteases' eicosanoids

**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.

*Senescent cells* disrupt normal structures, functions.

*Non-senescent (non-SEN) cells* disrupt stem cell functions.

*Senescent (SEN) cells* promote malignant phenotypes.

Parrinello et al., J Cell Sci., 2005; Chintar et al., unpublished collaboration with Andersen lab; Krtolica et al., PNAS, 2001; Coppe et al., PLoS One, 2010.
young tissue

-aged tissue
  - senescent cell
  - dysfunctional cell
    - SASP

-degenerating tissue

-premalignant cell
  - senescent cell

-neoplastic tissue
  - SASP
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-κB 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\(^{INK4a}\) promoter + inactivation of p14ARF

Mice have normal (diploid) copies of p16 and p14 genes

\[\text{p16 Promoter} \quad \text{renLuc} \quad \text{mRFP} \quad \text{HSV-tk}\]

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 $\rightarrow$ persistent senescent cells
Inject into inguinal mammary fat pad $\rightarrow$ multifocal mammary adenocarcinomas + lung/liver metastases

Senescent cells promote metastases

MMTV-PyMT breast cancer

MMTV-PyMT cells expressing firefly luciferase
Senescent cells promote metastases in mice with breast cancer

Control

GCV

breast cancer

DOXO

GCV

IMAGE

10 days

3 days

5 days

14-21 days

Control

GCV
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)

- Saline (no cancer) for 7 days
- DOXO (10mg/kg) for 3 days
- GCV for 5 days
- Metabolic cages for 3 days
### Behavior: mice + chemotherapy (no cancer)

<table>
<thead>
<tr>
<th></th>
<th>DOXO + PBS</th>
<th>DOXO + GCV</th>
<th>NT</th>
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<tbody>
<tr>
<td>EFODA</td>
<td>10.04 ± 3.28</td>
<td>9.02 ± 2.05</td>
<td>8.3 ± 2.22</td>
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<tr>
<td>TFODA</td>
<td>1.56 ± 0.33</td>
<td>2.29 ± 0.95</td>
<td>2.22 ± 0.54</td>
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<td>DWART</td>
<td>0.99 ± 0.71</td>
<td>2.046 ± 2.18</td>
<td>3.06 ± 3.79</td>
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<td>TWART</td>
<td>1.36 ± 2.01</td>
<td>1.59 ± 1.46</td>
<td>2.01 ± 1.81</td>
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<tr>
<td>WHEEL</td>
<td>9.58 ± 13.12</td>
<td>37.22 ± 14.29*</td>
<td>36.771 ± 18.13*</td>
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<tr>
<td>IHOME</td>
<td>1.86 ± 1.66</td>
<td>2.64 ± 3.34</td>
<td>7.026 ± 6.05</td>
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<td>THOME</td>
<td>1.15 ± 1.12</td>
<td>1.09 ± 1.58</td>
<td>1.91 ± 1.19</td>
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<td>LLNGE</td>
<td>61.46 ± 18.11</td>
<td>37.67 ± 15.81</td>
<td>27.53 ± 18.81*</td>
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<tr>
<td>SLNGE</td>
<td>6.94 ± 2.51</td>
<td>9.52 ± 2.13</td>
<td>11.17 ± 1.96*</td>
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Interaction with food hopper A (significant uptake found)
Interaction with food hopper A (no significant uptake)
Interaction with water dispenser (significant uptake found)
Interaction with water dispenser (no significant uptake)
Interaction with wheel (>= 1 revolution)
Entered habitat (stable mass reading)
Interaction with habitat (no stable mass reading)
Long lounge (> 60 sec, no non-XY sensor interactions)
Short lounge (5 - 60 sec, no non-XY sensor interactions)

% Total time

\[ p\text{-value: } *<0.05; **<0.01; ***<0.001 \]

\[ N=5 \]

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^{INK4a} increases in brains of human PD patients

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
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

↓↓↓↓↓↓

1 2 3 4 5

weeks after surgery

sham vehicle GCV

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

<table>
<thead>
<tr>
<th>% weight</th>
<th>no surgery</th>
<th>sham</th>
<th>veh</th>
<th>GCV</th>
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<tbody>
<tr>
<td></td>
<td>100%</td>
<td>120%</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60%</td>
<td>100%</td>
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ACLT
Cellular senescence, an evolutionary balancing act (why did the SASP evolve?)

- Irreversible GROWTH ARREST
- Tri-partite phenotypic resistance
- Multi-faceted SECRETORY PHENOTYPE

Phenotypes:
- Tissue remodeling/repair
- Tumor suppression
- Aging phenotypes
- Age-related disease (including cancer)

Aging phenotypes:
- Inflammation
- Oxidative stress
- DNA damage

Effects:
- Irreversible growth arrest
- Secretory phenotype
- Resistance to apoptosis

Benefits:
- Tumor suppression
- Tissue remodeling/repair
- Age-related disease prevention
Cellular senescence

Adverse effects of chemotherapy

Parkinson's disease and brain aging

Injury-induced osteoarthritis

Wound healing
Cellular senescence is induced during wound healing

Induction of p16^{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

Day 0 1 2 3 4 5 6 7 8 9 10 11

Punch

i.p. GCV / topical PDGF-AA

Wound size (%)
Cellular senescence, an evolutionary balancing act

Irreversible GROWTH ARREST

Tri-partite phenotype

Multi-faceted SECRETORY PHENOTYPE

Replace SnC factors

RESISTANCE to APOPTOSIS

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

Environmental factors

RESISTANCE to APOPTOSIS
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