Cellular Senescence, Inflammation, and Age-Related Disease: The Path to Translation

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Aging World Population

% of population
60 years or older

- <5
- 5-12.4
- 12.5-20
- >20

Today
2025
What is “Aging”? Universal
What is “Aging”?  

Universal

Intrinsic
What is “Aging”?  

Universal  
Intrinsic  
Progressive
Survival Curves
Human Maximum Life Span:
122.45 Years

Jeanne Calment
21 February 1875
– 4 August 1997
Successful Aging

Fitness

Healthspan

Compressing Period of Decline
Aging at the Nexus of Chronic Disease

AGING

- Osteoporosis
- Osteoarthritis
- Vascular disease
- Renal failure
- Neurodegeneration
- Dementia
- Stroke
- Macular degeneration
- Cataract
- IPF
- COPD
- Diabetes
- Cancer
- Infection susceptibility
- Sarcopenia
Aging: From Stimuli to Phenotypes

**Stimuli**
- Time (→random events; 2nd law)
- DNA mutations
- ROS
- Reactive metabolites
- Metabolic stress
- Repeated replication
- Toxins
- Infections

**Fundamental Aging Mechanisms**

**Phenotypes**
- Geriatric Syndromes:
  - Sarcopenia
  - Frailty
  - Immobility
  - MCI
- Chronic diseases:
  - Dementias
  - Atherosclerosis
  - Diabetes
  - Osteoporosis
  - Osteoarthritis
  - Renal dysfunction
  - Blindness
  - Chronic lung disease
- Deceased resilience:
  - Infections
  - Delirium
  - Delayed wound healing
  - Slow rehabilitation
Fundamental Aging Mechanisms Shared by Chronological Aging and Age-Related Chronic Diseases

- Inflammation (chronic, low-grade, sterile)
- Cellular Senescence
- Macromolecular Dysfunction (DNA, protein aggregation, autophagy, AGE’s, lipotoxicity)
- Stem Cell or Progenitor Dysfunction
Do We Have Interventions That Work?

Interventions that appear to be effective in mice:

**Lifespan and healthspan**
- Caloric restriction
- Exercise
- Rapamycin
- \(\alpha\)-estradiol
- ACE inhibitors, ARB’s
- Metformin
- **Senolytics**

**Healthspan**
- Flavonoids/Resveratrol/Sirtuin activators
- Senescence-associated secretory phenotype (SASP) inhibitors

**Lifespan**
- Acarbose
- NDGA (Nordihydroguaiaretic Acid)
- Methionine restriction
- Aspirin (median lifespan only), salicylic acid, salsalate (?)

**Potential**
- GDF8, GDF11 (?)
- Protein aggregation inhibitors
- Others (at least 35 potential strategies)
Cellular Senescence

Senescence Associated β-Galactosidase

25th passage human abdominal subcutaneous preadipocytes
Senescent Cells Can Even Appear in Blastocysts

\[ \gamma H2A.X \]

\[ SA \beta\text{-gal} \]
Hypothesis

Eliminating senescent cells alleviates multiple chronic diseases and enhances healthspan
Ink4a/Arf expression is a biomarker of aging

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The Ink4a/Arf locus encodes 2 tumor suppressor molecules, p16INK4a and Arf, which are principal mediators of cellular senescence. To study the links between senescence and aging in vivo, we examined Ink4a/Arf expression in rodent models of aging. We show that expression of p16INK4a and Arf markedly increases in almost all rodent tissues with advancing age, while there is little or no change in the expression of other related cell cycle inhibitors. The increase in expression is restricted to well-defined compartments within each organ studied and occurs in both epithelial and stromal cells of diverse lineages. The age-associated increase in expression of p16INK4a and Arf is attenuated in the kidney, ovary, and heart by caloric restriction, and this decrease correlates with diminished expression of an in vivo marker of senescence, as well as decreased pathology of those organs. Last, the age-related increase in Ink4a/Arf expression can be independently attributed to the expression of Ets-1, a known p16INK4a transcriptional activator, as well as unknown Ink4a/Arf coregulatory molecules. These data suggest that expression of the Ink4a/Arf tumor suppressor locus is a robust biomarker, and possible effector, of mammalian aging.

J Clin Invest 114:1299-307, 2004
Inguinal Fat From Wild Type Mice is More Highly SA $\beta$-Gal$^+$ Than From Ames Mice (deficient prophet of pituitary transcription factor-1)

Wild type

Ames

Age 20 months
Representative of 3 experiments

Data from 2005
Senescent Cells Accumulate with Aging and Obesity in Rats

Data from 2005
Senescent Cells Accumulate in Human Fat in Obesity

BMI=23.53; 37 years old
SA-βGal quantification: 0.221 OD/g
Abdominal subcutaneous fat

BMI=32.8; 30 years old
SA-βGal quantification: 2.18 OD/g
Abdominal subcutaneous fat

A. Bouloumie
Pathway Analysis - Metacore

1. Cell adhesion_ECM remodeling
2. Immune response_Alternative complement pathway
3. Cell adhesion_Cell-matrix glycoconjugates
4. Cell adhesion_Chemokines and adhesion
5. Immune response_Classical complement pathway
6. Immune response_Lectin induced complement pathway
7. Immune response_Oncostatin M signaling via MAPK in human cells
8. Immune response_IL-17 signaling pathways
9. Blood coagulation_Blood coagulation
10. Cell adhesion_Endothelial cell contacts by non-junctional mechanisms
Further Support For Our Hypothesis

Human Diseases and Age-Related Conditions Associated with Cellular Senescence

Diabetes
Obesity
Vascular Hyporeactivity
Atherosclerosis
Hypertension
Alzheimer’s Disease
Parkinson’s Disease
ALS
COPD
Idiopathic Pulmonary Fibrosis
Tobacco
Primary Biliary Cirrhosis

HIV, Latent Viruses
Prostatic Hypertrophy
Macular Degeneration
Cataract
Chronic Renal Disease
Psoriasis
Progerias
Frailty
Sarcopenia
Osteoporosis
Osteoarthritis
Cancers
Cancer Predisposition Syndromes
Chemotherapy
Radiation

Palmer et al., Diabetes 64: 2289, 2015
Ness et al., Cancer 121:1540-1547, 2015
Senescent cells, like cancer cells, are resistant to apoptosis (E. Wang, 1995)

**ATTAC**: (apoptosis through targeted activation of caspase)

*ATTAC* expresses a fusion protein including caspase-8 and vFKBP (Phe36Val mutant FKBP). The monomeric fusion protein is myristoylated can be dimerized by AP20187, a drug with little effect on wild type FKBP

Oligomerized caspase-8 activates the executioner caspases (caspases-3,6,7)
Targeting Senescent Cells *In Vivo*

- **Senescence-activated promoter**
- **ATTAC**
- **GFP**
- **FKBP Caspase 8-Flag**

*Senescent activated promoter*

- **p16**
- **p53**

Accelerate accumulation of senescent cells

Healthspan, not maximum lifespan, as key outcome

Nature 479:232, 2011
**INK-ATTAC;BubR1^{H/H}**

- **Ink promoter**
- **ATTAC**
- **IRES**
- **GFP**

-2617 FKBP Caspase 8-
Flag

- Cross with *BubR1^{H/H}*
- Transgenic as opposed to knock-in

AP20187

JvD after 6/2008

Nature 479:232, 2011
Activated **ATTAC** kills senescent cells *in vitro* and in mice, so senescent cells are susceptible to the caspase apoptosis execution cascade downstream of caspase 8.

Nature 479:232, 2011
Apoptosis Pathways

Survival Factors (e.g., IGF1) → GPCR → RTK → PLC → G-Protein

Chemokines, Hormones, Transmitters (e.g., interleukins, serotonin, etc.) → Growth Factors (e.g., TGFα, EGF) → Integrins → cdc42 → Ras/raf

Extracellular Matrix → Fyn/Shc → FAK/Src → Dishevelled → GSK-3β

Cytokines (e.g., EPC) → Cytokine Receptor → JAKs → STAT3,5

Apoptosis Pathways

Gene Regulation

Cell Proliferation

Death factors (e.g., FasL, Tnf)

FADD → Bad → Abnormality Sensor → Bim

FasR

Death factors (e.g., FasL, Tnf)

Bcl-2 → FasR

Caspase 8

Caspase 9

Cytochrome C

BAD

FADD

Death factors (e.g., FasL, Tnf)

Bcl-2

FasR

Abnormality Sensor

Bim

BAD

FADD
Hypothesis-Driven Senolytic Drug Development

1) Senescent cells can resist apoptotic stimuli, implying increased pro-survival and anti-apoptotic defenses

2) In some respects, senescent cells are like cancer cells that do not divide
Anti-Apoptotic Gene Networks Are Active in Senescent Cells

Negative Regulation of Apoptosis

Anti-Apoptosis

GSEA Gene Sets

Aging Cell, 2015
siRNA’s Against Anti-Apoptotic Regulators Selectively Decrease Senescent Cell Viability

Radiation-induced senescent cells
Day 0 vs. 4

Aging Cell, 2015

Selected from 39 pro-survival transcripts targeted by siRNA, 17 of which affected senescent cell viability
Networks of Anti-Apoptotic Regulators Conferring Resistance to Apoptosis in Senescent Cells
D Acts on Senescent Human Preadipocytes, Q on Senescent HUVECs

ATP Lite; validated by crystal violet; abdominal subcutaneous preadipocytes from 4 healthy kidney transplant donors; for HUVEC’s N=5 replicates. Day 0 vs. 3
D+Q Are Synergistic in MEFs

SA β-Gal+ cells; N=3; ***P< 0.005; t-test

Ercc1-deficient MEFs

Aging Cell, 2015
D+Q Reduce Senescent Cells in Inguinal Fat From Old Mice

SA β-Gal

24 month old mice; 5 days after single dose

Also: mouse quadriceps muscle p16 mRNA and inguinal fat SA β-Gal^+ cells after leg radiation; mouse adipose tissue p16 mRNA and SA β-Gal^+ cells after high fat diet; TAF’s in mouse tissues with aging and after high fat diet; monkey skin p16 mRNA

Aging Cell, 2015
D+Q Are Synergistic in Eliminating Senescent Cholangiocytes From Old Mice

p16 FISH

24 month mice, single dose of D+Q, livers analyzed after 5 days
N=8 animals/ group. *P<0.05

N. LaRusso lab
Senolytics Enhance Cardiac and Vascular Function in Old Mice

24 month old mice

J Miller
Senolytics Delay Neurologic Dysfunction in Progeroid Mice

L Niedernhofer

Aging Cell, 2015
Senolytics Delay Frailty in Progeroid Mice

N=7-8 mice/group; *P<0.05; **P<0.01

L Niedernhofer

Aging Cell, 2015
Senolytics Delay Osteoporosis in Progeroid Mice

S Khosla

Aging Cell, 2015
Senolytics Delay Nucleus Pulposus GAG Loss in Progeroid Mice

GAG content normalized by DNA

Vehicle | D + Q
---|---
1.0 | 1.5

* L Niedernhofer
  P Robbins

Aging Cell, 2015
Off-Target Effects of Candidate Senolytics: Association vs. Causation

1) Association: ↓ senescent cells + ↓ phenotypes

2) Strong association: drug phenocopies genetic clearance

3) Very strong association: epistasis (pitfalls: only valid at doses below ceiling effect on phenotype; SASP inhibitors, anti-inflammatory, combining distinct senolytic targets may exhibit epistasis)

4) Intimate association, almost causation: phenotypes persist after drug clearance

5) Causation: phenotype alleviation prevented by constitutively expressing the drug’s target
D+Q Phenocopies Effects of Genetic Clearance on Glucose Tolerance in Diet-Induced Obese Mice

A. Palmer (with J Campisi)
Transplanting Senescent Cells Into Lean, Young Rats Causes Glucose Intolerance
A Single Dose of Senolytics Alleviates Radiation-Induced Gait Disturbance for 7 Months

N=6-9 mice/group; 
* P<0.05;
** P<0.001

Aging Cell, 2015
Intermittent Treatment

• Single or intermittent doses of senolytics appear to alleviate at least some age- or senescence-related conditions

• This suggests that intermittent treatment may eventually be feasible in humans, perhaps given during periods of good health

• If so, this would reduce side effects

• Senescent cells do not divide, so drug resistance as with antibiotics or anti-cancer drugs is unlikely
New Senolytics

ZKT6: potent, but cell type-specific

ZKT10: distinct target not on published interactome

ZKT11: narrow therapeutic window
Emerging Evidence for Effects of Senescent Cells or Their Removal On:

- Diabetes/ Obesity
- Age-Related Lipodystrophy
- Cardiac Dysfunction
- Vascular Hyporeactivity
- Aortic Lipid Deposits
- Frailty/ Sarcopenia
- Response to Chemotherapy
- Response to Radiation
- Cancer
- Cognition/ Alzheimer’s/ Parkinson’s/ ALS
- Renal Dysfunction
- Osteoporosis/ Osteoarthritis
- COPD/ Idiopathic Pulmonary Fibrosis/ Tobacco
- Primary Biliary Cirrhosis
- Progerias
- Cataracts/ Macular Degeneration
- HIV
- Prostatic Hypertrophy
- Skin Disorders
Clinical Trials Considerations

- Measurable, clinically-relevant outcomes appropriate for older populations
- Biomarkers (pharmacokinetic, pharmacodynamic, mechanism, surrogate endpoint)
- Symptomatic or imminently at-risk
- Short-term
- Compelling clinical need
- Benefit that justifies risk
- Multiple co-morbidities
- Acceptable for regulatory agency registration
Geroscience Network

Albert Einstein
Buck Institute
EU/University of Groningen/Newcastle/ MOUSEAGE
Harvard
Hopkins
Mayo
Scripps
Stanford
University of Alabama
University of Arkansas
University of Colorado
University of Connecticut
University of Florida
University of Michigan
University of Minnesota
University of Texas San Antonio
University of Southern California
University of Washington
Wake Forest

Many other groups in retreats and faculty exchanges

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Co-PI’s: N Barzilai, S Austad
Clinical Scenarios for Testing Agents That Target Aging Processes (e.g., Cellular Senescence or the SASP)

Simultaneous Alleviation of Co-Morbidities
- 3 or more of: diabetes, atherosclerosis, hypertension, MCI, sarcopenia, osteoarthritis, etc.
- Delay in 2nd or later co-morbidities

“Accelerated Aging” Conditions
- Childhood cancer survivors
- Bone marrow transplant survivors
- Progeroid syndromes
- Diabetes due to obesity
- HIV (dementia, frailty)

Conditions with Localized Cellular Senescence
- Osteoarthritis
- Fracture non-union
- Atherosclerotic plaques
- Radiation
- Idiopathic pulmonary fibrosis
- COPD/ tobacco

J. Gerontol. 48:1-5, 2013
Exp. Gerontol. 2014
Clinical Scenarios for Testing Agents That Target Aging Processes (e.g., Cellular Senescence or the SASP)

Otherwise Fatal Conditions
- Idiopathic pulmonary fibrosis
- Primary biliary cirrhosis
- Cancers
- HIV dementia

Resilience/ Clinical Stresses in Pre-frail Subjects
- Chemotherapy
- Radiation
- Elective surgery
- Bone marrow transplantation
- Rehabilitation after MI
- Immunization
- Recovery after pneumonia

Frailty
- Slow gait/ decreased strength/ sarcopenia
- Loss of independence in moderately frail subjects

J. Gerontol. 48:1-5, 2013
Exp. Gerontol. 2014
Clinical Trials of Interventions Targeting Fundamental Aging Processes

Rapamycin and related agents for Alzheimer’s (several underway)

Rapamycin and related agents for frailty (several underway)

Rapamycin and related agents for recovery after MI (underway)

Rapalogs to increase response to influenza vaccine (completed)

Rapalogs in Hutchinson Guilford (IRB approval pending)

GDF agents (underway)

Metformin (TAME, planned)

JAK inhibitors (planned)

Brief caloric restriction before chemotherapy (2 underway)
How will Geriatric Medicine be Practised in the Future?

A transformation in geriatrics is possibly close

Currently:

- Tertiary prevention
- Complications of chronic diseases
- Aides and devices
- Geriatric syndromes, frailty, social consequences

In 10 years:

Delay of chronic diseases and geriatric syndromes with compression of morbidity using interventions based on recent advances in the biology of aging
Acknowledgements