Aging & Rehabilitation
An Interdisciplinary Research Seminar Series
Sponsors

**Department of Veteran Affairs**
- Rehabilitation Outcomes Research Center (RORC)
- Brain Rehabilitation Outcomes Research Center (BRRC)
- Geriatric Research, Education, and Clinical Center (GRECC)

**UF College of Medicine**
- Institute on Aging
- Department of Aging and Geriatric Research

**UF College of Public Health and Health Professions**
- Brooks Center for Rehabilitation Studies

**UF College of Liberal Arts and Sciences**
- Center for Gerontological Studies

**UF McKnight Brain Institute**

**UF College of Nursing**
Schedule

• August 29th, 2005 – May 22nd, 2006
• Mondays, 12:00 – 1:00
• HPNP Room – G103

CYBER SEMINAR VENUES
• VA RORC, Conference Room, Suite 350
• VA BRRC, VA Nursing Home, Room 271-12
• UF Brooks Center Conference Room, Jacksonville
Themes

- Basic Science (C. Leeuwenburgh)
- Clinical Science (R. Beyth)
- Outcomes / Health Policy (E. Andresen)
- Behavioral and Social Research (M. Marsiske)
- Cutting Edge / New Research (T. Foster/ J. Aris)
How Do Cells Age?

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Associate Professor

Department of Anatomy and Cell Biology
## Cell Aging - One Level Among Many

<table>
<thead>
<tr>
<th>Level</th>
<th>Theory of Aging</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecule</td>
<td>Metabolic error</td>
</tr>
<tr>
<td></td>
<td>Free radical (molecular damage)</td>
</tr>
<tr>
<td>Cell</td>
<td>Somatic mutation</td>
</tr>
<tr>
<td></td>
<td>Gene dysregulation</td>
</tr>
<tr>
<td>Tissue</td>
<td>Genome integrity</td>
</tr>
<tr>
<td></td>
<td>Defect accumulation</td>
</tr>
<tr>
<td>Organ</td>
<td>Cell senescence</td>
</tr>
<tr>
<td></td>
<td>Neuroendocrine</td>
</tr>
<tr>
<td>Organ system</td>
<td>Immunological</td>
</tr>
<tr>
<td>Individual</td>
<td>Disposable soma</td>
</tr>
<tr>
<td></td>
<td>Antagonistic pleiotrophy</td>
</tr>
<tr>
<td>Population</td>
<td>Post-reproductive selection shadow</td>
</tr>
</tbody>
</table>
Replicative and Chronological Aging

Aging in the **yeast** *S. cerevisiae* is the number of **buds** a mother cell produces.

Aging in the **worm** *C. elegans* is the number of **days** a worm lives.

Prod individuals daily to check for the ability to move any part of the body.

**Replicative Aging** of 1 MOTHER CELL

**Chronological Aging** of 959 MOTHER CELL

Aging and Senescence in Cells

Aging in Cells
Changes during "normal" life span that are:
Universally observed in species
Not due to a disease process
Usually irreversible
Progressive

Cell Senescence
Stage at which replicating cells are viable
but no longer capable of cell division as a
result of changes due to aging
Replicative Aging in Human Cells


Human fibroblasts from skin were grown through many population doublings in tissue culture until senescence.

Hayflick Limit

Hayflick limit - number of cell population doublings that are required to reach replicative senescence

Hayflick limit is a measure of replicative aging

Hayflick limits have been observed and measured in many cultured cell types from many different species

Hayflick limit for cells in culture correlates with the organism's chronological life span in many cases
Gompertz formula - relationship between age and mortality
Exponential increase in mortality rate with age
Similar mortality curves are characteristic of aging in cells
Werner Syndrome

A Japanese-American Werner patient as a teenager and at age 48. She had eight children, two of whom were also affected. At 48, she had hair loss and graying, thin extremities, atrophy of the skin, among other symptoms. She lived longer than most Werner patients, dying at 57. (Epstein et al, 1966, Medicine 45:177)

Werner gene was identified in 1996 (Chang-En et al, 1996, Science 272:258-262)

WRN encodes a RecQ family DNA helicase

In vivo function remains unknown
SGS1 is the yeast homolog of WRN

sgs1 mutants have a short life span and show signs of aging (sterility and genomic instability)

Replicative life span is limited and declines exponentially. SGS1 is the yeast homologue of human Werner (WRN) gene. sgs1 mutants show signs of aging (sterility, genomic instability).
Sardinia has highest proportion (1:1) of male centenarians

Worldwide ratio of women to men who live to 100 is 5:1

Genetic profiling of centenarians is underway

Longevity Genes

Yeast longevity gene - $UTH4$ ($SIR4$-42) is a semi-dominant gain-of-function allele that extends replicative life span.

Caloric Restriction (CR)

Yu et al., 1985, *J. Gerontol.* 40:657
Caloric Restriction (CR)

Cell Aging

- Cell life span (replicative & chronological) is limited
- Cell life span exhibits Gompertz-like statistical behavior
- Cell life span is subject to genetic regulation
- Cell life span is regulated by single genes (suggesting that life span is controlled by a hierarchical regulatory network with a limited number of control points)
- Cell life span can be extended by caloric restriction
Goals for Studies of Cell Aging

- Understand molecular processes that underlie aging
- Identify genes that specifically influence aging
- Understand pathways that regulate aging
- Devise strategies to delay (prevent?) aging
Theories of Aging

**Metabolic error** - inherent error rate in complex biological processes (e.g., transcription, translation) increases with age and leads to decline in fidelity of these processes.

**Free radical** - molecular damage, primarily due to reactive oxygen species (ROS), cause progressive loss of function.

**Somatic mutation** - mutations in DNA due to damage accumulate progressively and are inherited by progeny cells.

**Gene dysregulation** - changes in patterns of gene expression, due to damage or a genetic program, cause aging.

**Genome integrity** - maintenance of genomic integrity is a balance between pro-aging and pro-longevity processes.

**Defect accumulation** - errors accumulate and lead to cell dysfunction and senescence (normal "wear and tear" theory).
Metabolic Error Theory

Young
Complex process (transcription, translation)
- Inherent error rate
- Impaired function
- Gene products required for complex process

Old
Complex process (transcription, translation)
- Inherent error rate
- Impaired function
- Gene products required for complex process
Free Radical Theory

Damage to cells and their parts is due to free radicals.

Damage to cells accumulates over the life span.

Damage results in cell dysfunction and senescence.
Free Radicals

Free radical - molecule or atom with unpaired (odd) electron(s)

Free radicals are highly reactive and promiscuous

Free radicals form covalent bonds with other molecules (i.e., form adducts that can block or impair function)

Free radicals initiate chain reactions that are propagated as molecules react with others in an attempt to pair electrons

Free radical damage to biological molecules, proteins and lipids and others, has been shown to take place during aging

Free radicals are formed by endogenous (e.g., metabolic) and exogenous mechanisms (e.g., X-rays, UV)
Reactive Oxygen Species (ROS)

Most free radicals in cells are formed from oxygen. Superoxide ($O_2^-$) and peroxide ($O_2^{2-}$) are highly reactive. Hydrogen peroxide ($H_2O_2$) is membrane permeant.
Mitochondrion

**Function**
- ATP production
- Heat production
- Metabolic precursor synthesis
- Oxidation of fatty acids

**Structure**
- Outer membrane (OM)
- Inner membrane (IM), cristae
- Intermembrane space
- Matrix

**Genetics**
- mtDNA - encodes 13 proteins
tRNAs, and rRNAs in human
- Most (>98%) proteins are encoded by nuclear genes
Aerobic Respiration in Mitochondria

Oxygen is final electron acceptor in aerobic metabolism in mitochondria.

- ~95-99% of O₂ is reduced by cytochrome c oxidase complex to form metabolic H₂O.

- ~1-5% of O₂ forms ROS.

Mitochondria are prone to ROS damage - most ROS form in mitochondria, where they react with mtDNA, lipids, proteins.
Lipid peroxidation - radicals react with lipids to yield modified lipids that can react with other membrane components. Adverse effects on membranes - reduced fluidity, decreased activity of membrane proteins, altered membrane permeability.
ROS Damage to DNA

DNA oxidation - chemical modification of bases in DNA
Alters coding potential for replication and transcription

8-Oxo-guanosine
ROS Damage to Proteins

Protein oxidation - chemical modification, carbonylation
Modifications alter function and trigger degradation
Oxidative Stress Response

Cells constitutively express proteins that inactivate ROS

Cells induce expression of proteins that inactivate ROS

Superoxide dismutase (SOD)
\[ \text{O}_2^- + \text{O}_2^- + 2 \text{H}_2\text{O} \rightarrow \text{SOD} \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]

Catalase
\[ 2 \text{H}_2\text{O}_2 \rightarrow \text{Catalase} \rightarrow 2 \text{H}_2\text{O} + \text{O}_2 \]

Glutathione (GSH) and glutathione peroxidase (GPX)
\[ 2 \text{H}_2\text{O}_2 + 2 \text{GSH} \rightarrow \text{GPX} \rightarrow 2 \text{H}_2\text{O} + \text{GSSG} \]

Antioxidants (vitamins C and E) can neutralize radicals
Oxidative Stress and Life Span

*Drosophila* that overexpress SOD2 (Cu, Zn SOD) and catalase show less oxidative damage to proteins and DNA.

*Drosophila* that overexpress SOD2 and catalase have a life span extended by approximately 1/3 compared to control flies (Orr and Sohal, 1994, *Science*, 263:1128).

Chemicals that mimic the activity of catalase can extend the life span of *C. elegans*. Other mutations in *C. elegans* that extend life span require catalase activity.
Stress Response Signaling Pathways

Longo & Finch, 2003, Science 299:1342
Non-Enzymatic Glucosylation

Glucose added non-enzymatically to proteins, nucleic acids

Generates Advanced Glycation End products (AGEs)

Also known as free radical addition of glucose

Modification of collagen and elastin can lead to reduced function and covalent crosslinking of these proteins, which can lead to changes in properties of connective tissue

Intracellular protein glucosylation forms modified proteins resistant to protein degradation (i.e., form residual bodies)
AGE Formation

Reducing sugars such as glucose react with protein amino groups to yield Schiff bases. Schiff bases undergo rearrangement to yield Amadori products such as ketoamine. Amadori products introduce carbonyl groups into proteins, which disrupt both structure and function.
Somatic Mutation Mechanism

Mutations in somatic cell DNA accumulate over the life span

Mutations result from exogenous or endogenous mechanisms

Mutations are passed on to progeny in mitotic cell types (promotes “accumulation” of mutations in mitotic cell types)

Mutations may lead to alterations in:
- Chromatin structure (e.g., silencing)
- Promoter function (either increase or decrease)
- RNA processing, stability, or export
- Translation (e.g., rate and fidelity)
- Protein function, targeting, or stability
Gene Dysregulation Theory

Complex networks of regulatory pathways are sensitive to mutations and metabolic errors that accumulate over the life span and have a disproportionate effect on these pathways.

Genome Integrity Mechanism

Telomere - DNA/protein complex at chromosome end

Sequence - repeated short oligonucleotide (hexameric TTAGGG repeat in human)

Length - $10^3$ - $10^5$ bp, depending on species

T-loop structure - free single-strand 3'-end forms triple helix loop stabilized by telomere associated proteins

Protects chromosome ends from nucleolytic attack, non-homologous end joining (NHEJ), etc.

Attrition - telomeres shorten with each cell cycle because the lagging strand can't be replicated by DNA polymerase
Telomerase - ribonucleoprotein complex

Catalyzes lagging strand end-replication by reverse transcription (RNA used as template for synthesis)

Telomerase - functional in germ and certain stem cells
Defect Accumulation Theory

Aberrant molecules fail to be degraded and accumulate.

Lipofuscin - classic "age pigment" observed histologically.

Lipofuscin - complex mixture of modified proteins, lipids, metals, small molecules that is resistant to degradation.

Residual bodies - store lipofuscin and other substances.

Incomplete degradation of mitochondria may form lipofuscin.

Failed degradation mechanisms may lead to cell dysfunction.
Apoptosis

Apoptosis - programmed cell death

Apoptosis is regulated by endogenous & exogenous pathways

Endogenous pathway - cytochrome c release by mitochondria

Mitochondria damaged by oxidative stress have been implicated in the initiation of apoptosis

Apoptosis is also mediated by a p53 dependent pathway that senses damage to DNA (e.g., double strand breaks)

Inappropriate apoptosis may be the result of impaired regulation of pro- and anti-apoptotic pathways in old cells
Budding is an asymmetric cell division process
Mother (M) cells give rise to daughter (D) cells
Isolation of Old Yeast Cells

Yeast cell (in $G_1$) +B → Biotinylated cell wall

Cell decorated with S-avidin magnetic beads +M → New membrane and cell wall

Magnetic Sorting → Repeat growth and sorting

Bud ring in mitotic cell

Bud scars in old cells
Serial Sorting - Flow Cytometry

Sort Mean Intensity
1 294
2 581
3 900
4 1301
Serial Sorting - Bud Scar Counts

W303 (WT)

- W-1
- W-2
- W-3
- W-4

fob1Δ (long-lived)

- F-1
- F-3
- F-3
- F-4

Number of bud scars

0 2 4 6 8 10 12 14 16 18

1 3 5 9 11 13 17 19 21 23 25 27 29 31 33 35

Number of bud scars

1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35
Old Yeast Cell

Bud scars stained with WGA-Alexafluor488
Acknowledgements

Alaric Falcon

Natalie Rios

Ellison Medical Foundation

NIH NIA R21
Yeast - Good Model for Aging in Cells (as well as aging in cellars)
ERC Model

Sinclair & Guarente, 1997, Cell 91:1033
Recombinant Plasmids

Diamonds - ARS1

Squares - ARS1, CEN4

Triangles - 2 µ ori

Circles - no plasmid

Fraction of Cells Viable

Generations
Acetylation/Deacetylation Circuit

\[
\text{NAD} + \text{acetyl-K-histone} \xrightarrow{\text{Sir2}} \text{NAM} + \text{O-acetyl-ADP-ribose} + \text{K-histone}
\]

\[
\text{Acetate} + \text{CoA} + \text{ATP} \rightarrow \text{Acetyl-CoA Synthetase}
\]

\[
\text{K-histone} + \text{Acetyl-CoA} \rightarrow \text{Histone Acetyl Transferase (HAT)}
\]

\[
\text{Acetyl-K-histone} + \text{CoA}
\]

\[
\text{NAD}^+\quad \text{2'-O-acetyl-ADP-ribose}
\]