Theories of Aging and Strategies to promote Healthy Aging

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Seminar for Vermont Medical College 2003
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Why do we age? What are good anti-aging interventions?

What is Aging?

Aging:
- Progressive
- Universal
- Post-maturation
- Irreversible?

Senescence:
- Deleterious
- Aging

Age-related diseases: are the clinical manifestations of senescence

Wallace DC

Leading Causes of Death

1900: 2003:
1) pneumonia, tuberculosis 1) heart disease
2) GI infections, diarrhea 2) cancer
3) heart disease 3) neurodegenerative diseases
4) sarcopenia related injury

Maximum Life-span
Mean Life-Span

- Mean life-span (MLS) is the average life-span age of a cohort studied. Often influenced by the environment, disease, and life-style
- Maximum life-span potential is the maximum age of one individual within a cohort studied. Mostly genetically determined (MLSP)

General Objectives

- What is the objective of The National Institute on Aging and various groups investigating mechanisms of aging and strategies for healthy aging.
  - Improved understanding of the mechanisms of longevity can be used to fight age-related diseases and disabilities to ensure a healthy, active, and independent life well into very old age

Molecular Gene Theories

- Codon restriction: Fidelity/accuracy of mRNA translation is impaired due to inability to decode codons in mRNA.
- Error catastrophe: Fidelity of gene expression declines with age, resulting in increased fraction of abnormal proteins.
- Somatic mutation: Accumulation of molecular damage, primarily to DNA/genetic material.
- Dysdifferentiation: Gradual accumulation of random molecular damage impairs regulation of gene expression.
- Gene regulation: Aging caused by changes in gene expression regulating both aging and development. Gene expression protein folding and activity
Cellular Theories

• **Free radical** - Oxidative metabolism produces highly reactive free radicals that subsequently damage protein and DNA. Mitochondrial DNA Damage (Mitochondrial Theory of Aging)

• **Wear and tear** - Accumulation of normal injury
  - Inflammation Theory of Aging
  - Glycoxidation Theory of Aging (products from glucose with proteins + oxidation; AGE (advanced glycation End-products)

• **Apoptosis** - Programmed cell death resulting from intrinsic damage and genetically determined events or genome crisis.

• **Senescence** - Phenotypes of aging are caused by an increase in frequency of senescent cells. Senescence may be the result of telomere loss (replicative senescence) or cell stress (cellular senescence).

System Theories

• **Rate-of-living** - Assumes a fixed amount of metabolic potential for every living organism (live fast, die young).

• **Neuroendocrine** - Alterations in neuroendocrine control of homeostasis results in age-related physiological changes. Neuroendocrine Theories of Aging

• **Immunologic** - Well documented decline of immune function with age results in increased incidence of disease. Immunological Theory of Aging

Evolutionary Theories

• **Disposable Soma** - Somatic cells are maintained only to ensure continued reproductive success, following reproduction the soma is disposable. (life span theory)

• **Antagonistic Pleiotropy** - Genes that are beneficial at younger ages are deleterious at older ages.

• **Mutation Accumulation** - Mutations that affect health at older ages are not selected against.
Opossums

- Mainland Opossums
  - ~80% die from predators in the first year; typically reproduce only once; Age very rapidly
- Sapelo Island Opossums
  - out in daylight (no predators)
  - reproduce twice (fewer offspring/litter)
  - longer average life span
  - "Sapelo island opossums live longer because they age more slowly than mainland opossums. Demonstrated by reduced levels of collagen cross-linking in Sapelo Island opossums when compared to mainland opossums. (Collagen X-linking measures the amount of molecular damage accumulated over time)"

There are clear Species-Specific Differences in Maximum Life-span.

**Short-lived Species**

- *Caenorhabditis Elegans* (14-21 Days)
- *Drosophila Melanogaster* (1-3 Months)
- *Rattus* (2-3 Years)
- *Fischer-344*

**Long-lived Species; Maximum Life-span, Mean Life-span, Species-Specific Differences.**

- *Bats (10-30 years)*: High Metabolism, but long life-span and maybe a reduced radical production?
- *Monkeys (20-30 years)*: Homology 98.7%
- *Elephants*: 70 Years in the wild (~10y Zoo)
- *Humans (~77-M~80-F years)*: Maximum ~122y
  - Moreover most centenarians are females!!!
- *Jeanne Louise Calment* was born in Arles, France on February 21, 1875. She once met Vincent Van Gogh in her father’s shop. Her genes may have contributed to her longevity as her father lived to the age of 94 and her mother to the age of 86. She married a distant cousin at the age of 21. Her only grandson died in 1963. She rode a bicycle to the age of 100.

Proteonomics; Protein Expression and Folding:


-Although humans and chimpanzees are 98.7% genetically similar.......
-Investigators have taken genomics one step further by using DNA microarrays to measure the expression levels (that is, mRNA levels) in the liver and brain of humans and chimpanzees. The distance between one of the human samples and the others is greater than the overall distance between humans and chimpanzees,.......
-Altered gene expression could explain the genetic difference between human and chimp and could therefore provide alternative explanation for life-expectancy differences within humans and between primates and humans.
The Free Radical Theory of Aging

Oxygen free radicals generated cause cumulative oxidative damage, resulting in structural degeneration (extra cellular material, protein aggregation, apoptosis, etc.) functional decline, and age-related diseases.

Some believe that oxidative stress is the predominant cause of age-associated degenerative change.

• First some basic questions?
  • What are free radicals or better named oxidants?
  • Where are these oxidants produced?
  • What type of oxidants are produced?

Mitochondrial Dysfunction and Aging

Mitochondrial Theory of Aging

- Oxidative stress
- mt-DNA damage
- mt-DNA deletions
- Oxidized proteins
- Lipid peroxidation
- Lipid-adduct formation
- Decrease in repair systems

Radical Reactions

Ground-state O₂

\[ O_2 + e^- \rightarrow O_2^- \]  (Equation 1)

\[ O_2 + 2e^- + 2H^+ \rightarrow H_2O_2 \]  (Equation 2)

\[ O_2^- + O_2^- + 2H^+ \rightarrow SOD \rightarrow H_2O_2 + O_2 \]  (Equation 3)

\[ H_2O_2 + 2GSH \rightarrow GPX \rightarrow H_2O + GSSG + ROH \]  (Equation 4)

\[ 2H_2O_2 \rightarrow \text{Catalase} \rightarrow 2H_2O + O_2 \]  (Equation 5)

\[ Fe^{2+} + H_2O_2 \rightarrow HO^- + HO^+ + Fe^{3+} \]  (Equation 6)

\[ O_2^- + NO \rightarrow ONOO^- \]  (Equation 7)

\[ O_2^- + Mn^{2+} \rightarrow O_2 + Mn^{3+} \]  (Equation 8)

Aging

selectively decreases the rate of oxidative phosphorylation in the intercalated population of cardiac mitochondria (IPM) located among the myofibers, whereas subsarcolemmal mitochondria (SSM) located beneath the plasma membrane remain unaffected. Lesnefsky EJ, Guda TL, Wightman AR, Mighty-OT, Hoppel CL, Turkaly PJ, Hoppel CL. Mol Cell Cardiol. 2001 Jan;33(1):37-47.
There is an inverse relationship between superoxide production and longevity in both insects and mammals (Ku et al. 1993).

Animals and MLSP:
- Mouse: 3.5 years
- Hamster: 4 years
- Rat: 4.5 years
- Guinea pig: 7.5 years
- Rabbit: 18 years
- Pig: 27 years
- Cow: 30 years

Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals.

GUSTAVO BARJA1
ASUNCIÓN HERRERO

Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals.

Bats (10-30 years) and Birds (30-100) have a high metabolism (similar to rats (2-4 years), but are long lived! A reduced radical production has been detected in these long-lived species. (“life fast die young”)

What is important is the production of mitochondrial oxidants (ROS) and not O2 consumption. (In general it correlates negatively with MLSP).

Fruit flies that over-express copper-zinc superoxide dismutase (SOD2) and catalase (the first line of defense against oxidative damage) show less oxidative damage to DNA and protein and live up to 34% longer than controls (Orr and Sohal, Science 1994).

Engineering of Genes to increase Antioxidant Defenses

Long-lived Species; Maximum Life-span, Mean Life-span, Species-Specific Differences.

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Ubiquitous Overexpression of CuZn Superoxide Dismutase Does Not Extend Life Span in Mice

Ting-Ting Huang, Elder I. Caball, June Marie Glikson, Yiping Shi, and Charles J. Billing

Genetic Engineering has not worked in Longer-lived species
Oxidative Stress and Aging

- What happens when oxidant production is greater than antioxidant defenses?

  **Oxidative Stress**
  - DNA damage
  - Protein damage
  - Lipid Damage

Free Radical Theory

- Oxidative metabolism produces highly reactive free radicals that subsequently damage protein and DNA.
- Evidence from model organisms...
- Superoxide dismutase (SOD) transgenes can extend the life span of Drosophila.
- Chemicals that mimic catalase (peroxidase) activity can extend *C. elegans* life span.
- Long-lived mutants are typically stress resistant, including resistant to drugs (i.e. paraquat stress, which induces increases in free radicals)
- Life span extension by insulin-like signaling mutants in *C. elegans* requires catalase activity.

There are “Age” Genes

- Evidence for genetic regulation of life span,
- Mutations that reduce insulin-like signaling can extend *C. elegans* lifespan significantly.
- A conserved transcription factor is required for life span extension, indicating a regulated genetic response to reduced insulin-like signaling.
- Conserved in *Drosophila* and mouse.
- Insulin levels rise when we consume food, and drop in the absence of food, because reducing insulin-like signaling in *C. elegans* extends life span scientists are investigating if calorie restriction extends life span by reducing insulin-like signaling, does it?

Increasing life-span in *C. Elegans* and *Drosophila Melanogaster*

- Studies in invertebrates have led to the identification of a number of genes that regulate lifespan, some of which encode components of the insulin or insulin-like signaling pathways.
- Examples include the related tyrosine kinase receptors InR (Drosophila melanogaster) and DAF-2 (Caenorhabditis elegans) that are homologues of the mammalian insulin-like growth factor type 1 receptor (IGF-1R).

- Evidence for genetic regulation of life span.
- Mutations in certain genes in the *C. elegans* can also increase life span. For example, *daf--2* mutants extend a metabolic slowed, non-aging state. This occurs naturally when food is limited or there is stress.
- Mutations cause an 3-4 fold increases of life span.

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- To investigate whether IGF-1R also controls longevity in mammals, these scientists inactivated the IGF-1R gene in mice (*Igf1r*).
- Here, using heterozygous knockout mice because null mutants are not viable, we report that *Igf1r*+/2 mice live on average 26% longer than their wild-type littermates (*P* < 0.02).
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Insulin-like signaling in *C. elegans*

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Wear and tear - Accumulation of “normal” injury

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Sources of Oxidants
Chronic Inflammation and Oxidant Stress

Inflammation theory of aging

- The Molecular inflammation hypothesis of aging is based on specific anti-aging mechanism of calorie restriction, such as the reduction in TNF-α / NF-κB signaling

Inflammation theory of aging

- The inflammation theory of aging goes hand-in-hand with the free radical theory of aging and the glycooxidation theory of aging

Glycoxidation Theory of Aging

- Cooking and Browning Reaction
  - Cooking of foods accelerates the Browning or Maillard reaction
  - The toxic substances formed can lead to mutations and cancer
The Glycosylation (oxidation) Theory of Aging suggests that cross-links generated in proteins and nucleic acids by nonenzymatic glycosylation may contribute to age-related declines in the functioning of cells and tissues.

- Non-enzymatic addition of glucose to proteins may gradually slow down the protein function.
- Non-enzymatic addition of glucose to nucleic acids may gradually damage DNA.

Advanced Glycosylation End products (AGEs)

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Hormone Theories
Decline in Estrogen and Testosterone

- **DHEA** dehydroepiandrosterone is secreted by the adrenal glands and called a master hormone because it is converted into many other hormones involved in growth and strength.
- Supplements of DHEA, which declines with age in both men and women, have been shown in animal studies to reverse many debilitating signs of aging.
- Risks of cancer with long term use and risk for prostate cancer etc. unknown.

Immunological Theory of Aging (Decline in Function Resistance to Stress)

- In general, humoral immunity declines with age, and the onset can occur as early as when an individual reaches sexual maturity.
- Aging appears to affect T cell number/function.
- Decline could lead to increase in risk to viral infections and cancer.
- Cell loss (Apoptosis), shift in the proportion of sub-populations, and qualitative cellular changes have all been detected.

Summary
Glycooxidation

- Cross-linking sugar related to the aging process? Yes
- Thus the aging process is also governed by certain chemical molecules and structure formations that are likely to participate in reactions to “slow you down”, which are not intended by genes.

Apoptosis Theory of Aging

- Apoptosis (programmed cell death) could be activated “prematurely” in post-mitotic cells and contribute to the aging process.
- What is the mechanism with age and what mechanisms can life-long exercise prevent the age-related loss in post-mitotic cells?
Myocardial Aging, Sarcopenia, Neurodegeneration, Oxidative Stress and Apoptosis

- Decrease in total number of skeletal and heart myocytes as well as neurons with age
- May lead to accelerated decline in cardiac functional capacity, sarcopenia, neurodegenerative diseases
- Oxidative Stress and Apoptosis may be one major factor

Therapies-Strategies to promote Healthy Aging and to Prolong Life

- Interventions that may promote health and prolong maximum life-span:
  - Life-Long-Caloric Restriction
  - Life-Long-Exercises
  - Anti-inflammatory Compounds (Aspirin)
  - Antioxidant Therapies (flavonoids, etc)
- *Genetic Engineering and Organ Transplant*

Caloric Restriction

- Reduces oxidant
- Reduces cytokines
- Reduces glycoxidation
- Reduces Pathology
Oxidative Damage in Skeletal Muscle is still increased with Aging and is Reduced by Caloric Restriction


Oxidative Stress

Dityrosine/Tyrosine (mmol/mol)

Age (Months)

Caloric Restricted
Heart
Ad Libitum

Caloric Restricted
Vastus Lateralis
Ad Libitum


Reduction of the Incidence of tumors following restriction of food intake

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rodents</th>
<th>Rodents with Tumors</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>89</td>
<td>43</td>
<td>48</td>
</tr>
<tr>
<td>Food Restricted</td>
<td>77</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control/Radiated</td>
<td>102</td>
<td>91</td>
<td>89</td>
</tr>
<tr>
<td>Restricted/Radiated</td>
<td>128</td>
<td>29</td>
<td>23</td>
</tr>
</tbody>
</table>


Hot spots for free radical attack:

Oxidative DNA damage in the Mitochondria and Nucleus

<table>
<thead>
<tr>
<th>Age (Months)</th>
<th>12.5</th>
<th>25.5</th>
<th>38.5</th>
</tr>
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<tbody>
<tr>
<td>12.5</td>
<td>1.0</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>25.5</td>
<td>1.5</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>38.5</td>
<td>2.0</td>
<td>2.5</td>
<td>3.0</td>
</tr>
</tbody>
</table>


Restrictive meal

calories: 750

Tasty but not fulfilling

Reducing calories is key to the diet.

Calories: 1,250

Tasty, filling, healthy.

Reducing additional calories needs to be considered.

Arthritis.

“Work, you tuberclosis in all the skin. After we are in a normal deficiency study, and then’s doing the effects of alcohol on the body.”
Antioxidant Supplementation

- Is there evidence that antioxidant supplementation prolongs maximal life-span?
- We would all like a pill to live longer……, but

What is a good Antioxidant?

- What is an antioxidant?
- A substance when present in trace (small) amounts inhibits the oxidation of the bulk
- What are considered good antioxidants?
  - Relatively un-reactive (antioxidant)
  - Repaired Rapidly
  - Decays to harmless products

The effect of antioxidant supplementation mean and maximum life-span

<table>
<thead>
<tr>
<th>SHORT-LIVED-SPECIES</th>
<th>(Mean)</th>
<th>(Max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tocopherol-p-chloro-phenoxyacetate</td>
<td>(13.0)</td>
<td>(13.0)</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>(31.4)</td>
<td>(23.2)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>(16.8)</td>
<td>(15.4)</td>
</tr>
<tr>
<td>Sulphydryl agent</td>
<td>(28.0)</td>
<td>NaN</td>
</tr>
<tr>
<td>N-acetylcysteine</td>
<td>(26.6)</td>
<td>NaN</td>
</tr>
<tr>
<td>SOD – Mimetics</td>
<td>(30-40)</td>
<td>(30)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>LONGER-LIVED-SPECIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercaptoethyamine 0.05%</td>
</tr>
<tr>
<td>Mercaptoethyamine 1.00%</td>
</tr>
<tr>
<td>Santoquin (0.5%)</td>
</tr>
<tr>
<td>Vitamin E, Vitamin C, b-carotene</td>
</tr>
</tbody>
</table>

Catalytic antioxidants tested in Sod2-- mice, and in aging paradigms

- Mean lifespan increase of 45%
- Maximum lifespan was increased up to 105%
- No dose dependence
- No differential between EUK-8 or EUK-134
Antioxidants have no effect on longevity.

Exercise increase mean life-span.

What are the cellular adaptations of LIFE LONG EXERCISE to explain increase in mean life-span?

To Increase Maximum Life-Span...

- A key may be to... reduce ENDOGENOUS chronic oxidant production.
- A key may be to... reduce ENDOGENOUS levels of specific cytokines.
- A key may be to... reduce ENDOGENOUS accumulation of protein aggregates and glycoxidation products.
- Reduce mitochondrial and nuclear DNA damage and/or increase repair.

Primary Interventions Could Effect Primary Aging:

- **Life-style**
  1) Diet (Caloric Restriction)
     - Avoid Restaurants (a treat now and then is ok)
     - Learn how to cook healthy yourself
     - Shop Smart
     - “Empty refrigerator” before going to the store
     - Some evenings just have a salad
     - Food actually taste better when you caloric restrict
     - You will not be miserable... life is short and you want to stay away from the doctor during the last 15-20 years of your life-span
     - Comes down to how much discipline you have
  2) Moderate exercise ?? (mean life span)
     - Increase daily activities and enjoy your exercise or walking program
  3) Take your antioxidants ?? (maybe mean live span)
     - Eat a diet high in fruits, veggies, and herbs

Secondary Interventions May be beneficial if you first follow primary interventions and these will also prevent secondary aging:

- Wine (1 serving) and Dark Beer (1 serving)
- Aspirin (COX, TNF-α / NF-κB inhibitor)
- AGE-breakers (amino guanidine)?
- ACE-inhibitors; reduces inflammation in patients with heart disease.
- Hormone therapy; DHEA after ~60years?? Estrogen is out-in?, Testosterone…. when levels decline??

We will investigate mitochondrial efficiency and deletions as well as oxidant and antioxidant balance?

Fig. 5. Survival curves for the four groups. The survival curves for the groups are significantly different from those of the sedentary group in both sets of animals.

Exercise have no effect on longevity.

Exercise increase mean life-span.
Why these?

- Currently we don’t need a genetic miracle to prolong healthy life. Stop smoking, perform regular exercise, effective stress management, stay lean and provide yourself with a heart-healthy diet. This could increase your mean-life-span by:

**20 to 25 HEALTHY years beyond the age of 70**