Lecture 27

Aging and Associated Diseases

Theories of aging
Experimental models
Approaches and Recent findings

Diseases associated with aging
Werner syndrome
Alzheimer’s disease
Improved understanding of the mechanisms of longevity can be used to fight age-related diseases and disabilities and to ensure a healthy, active, and independent life well into very old age.
Aging: progressive, universal, post-maturation, irreversible

Senescence: deleterious aging

Age-related diseases: clinical manifestation of senescence

Maximum life-span:
  between and within species genetically determined

Mean life-span:
  influenced by environment, disease, life-style etc

Leading Causes of Death:
1900 (pneumonia tuberculosis, GI infections, diarrhea; heart disease)
1988 (heart disease, cancer, neurodegenerative diseases, sarcopenia)
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.

In October of 1995, much press coverage announced that Jeanne had exceeded the lifespan of Shigechiyo (Chigechiyo) Izumi, who until then had held the claim to the longest lived human. In fact, work by John Wilmoth indicates that Izumi may have only been 105 when he died, meaning that Jeanne may have outlived Izumi in 1980. If that is accurate, Jeanne would have become the longest lived human in 1991 when she exceeded the longevity of Carrie White, who died at the age of 116.

Quotes attributed to Jeanne Calment:

• In life, one sometimes makes bad deals.

Comments on the notary public, Andre-Francois Raffray, who purchased her apartment, promising to pay $500 per month until Jeanne died. He paid twice the market value for the apartment before dying in December of 1995.

Comments on her vision of the future on her 120th birthday.

• I've been forgotten by a good God. (or L'Oubliée de Dieu?)

• I've only got one wrinkle and I'm sitting on it. (Je n'ai jamais eu qu'une seule ride et je suis assise dessus.)
Yoda and Princess Leia

World's oldest mouse helps unlock aging secrets

By Chloe Foster, Daily Staff Reporter on 4/15/04

Yoda lived four years and 12 days, the equivalent of more than 136 in human years. "I mean, he was old. That was the bottom line," Pobojewski said. "He was really, really old." An average lab mouse lives slightly more than two years.

Yoda, who received the name after researchers agreed he looked like the sage of the Star Wars movies, had genetic mutations that affected his pituitary and thyroid glands and reduced insulin production. The changes are suspected to have played a significant role in his longevity, and left him a third smaller than an average mouse and very sensitive to cold.

Researchers are studying the genetic mutants to determine how altered hormone levels can slow the aging process, with the hope of figuring out which methods, if any, eventually could be applied to humans.

He is survived by his companion mouse, Princess Leia.

Maximum Life Span:
human (120), Galapagos turtle (100), Indian elephant (70), Chinese alligator (52), Golden eagle (46), Gorilla (39), Toad (36), domestic cat (27) domestic dog (20), vampire bat (13), mouse (3)
Theories on Aging

**Programmed Theories**

**Programmed Senescence.** Aging is the result of the sequential switching on and off of certain genes, with senescence being defined as the time when age-associated deficits are manifested.

**Endocrine Theory.** Biological clocks act through hormones to control the pace of aging.

**Immunological Theory.** A programmed decline in immune system functions leads to an increased vulnerability to infectious disease and thus aging and death.

**Error Theories**

**Wear and Tear.** Cells and tissues have vital parts that wear out.

**Rate of Living.** The greater an organism's rate of oxygen basal metabolism, the shorter its life span.

**Crosslinking.** An accumulation of crosslinked proteins damages cells and tissues, slowing down bodily processes.

**Free Radicals.** Accumulated damage caused by oxygen radicals causes cells and eventually organs to stop functioning.

**Error Catastrophe.** Damage to mechanisms that synthesize proteins results in faulty proteins which accumulate to a level that causes catastrophic damage to cells, tissues, and organs.

**Somatic Mutation.** Genetic mutations occur and accumulate with increasing age, causing cells to deteriorate and malfunction.
Molecular Gene Theories

Codon restriction: translation fidelity impaired due to inability to decode mRNA.
Error catastrophe: fidelity declines with increase in abnormal protein
Somatic mutation: accumulation of DNA damage
Gene regulation: changes in gene expression regulating both aging and development. Protein folding.

Cellular Theories

Free radical: mitochondria ROS, protein DNA damage
Wear and tear:
  Glycoxidation theory (AGE, advanced glycation end-products)
  Inflammation theory
Senescence: accumulation of senescent cells due to replicative senescence (e.g. telomere shortening) or cellular senescence (e.g. stress)
System Theories

Rate-of-living: assumeing a fixed amount of metabolic potential for Every living organism (live fast, die young)
Neuroendocrine: alterations in neuroendocrine control of homeostasis Results in age0related physiological changes Immunologic: decline in immune function with age results in increased Incidence of disease

Evolutionary

Disposable soma: (Life span theory) soma is disposable following reproduction Antagonistic pleiotropy: genes beneficial at young age are deleterious at old age Mutation accumulation: mutations that affect health at old age are not selected against
Genetic connection and Longevity genes

*Drosophila melanogaster* or fruit flies, after breeding and selection of the long-lived stocks by selecting and mating flies late in life live for 70 ~ 80 days, nearly twice the average *Drosophila* life span. Yeast, worms, and mice can all be manipulated to enhance their MLS.

Yeast has normally 21 divisions. Yeast gene, *LAG-1*, influences the number of divisions in yeast. Overexpressing *LAG-1* gives 28 divisions, mutating it gives 12 divisions. Function is still a mystery. May be Encodes a membranes protein. Similar sequences have been found in human DNA, so a second investigative path is to clone the human gene and study its function.

Replicative genes and anti-replicative genes: (cancer and aging)

Telomerase and telomere: (cancer and aging)
Yeast as a model: *LAG-1, Sir2, PNC1*
UV → Disease → Nutrient limitation → Dehydration → Activation of sirtuin pathway → Life-cycle alteration
Stress resistance Longevity?

Plant stress signaling molecules → Activation of sirtuin pathway → Life-cycle alteration
Stress resistance Longevity

Resveratrol
sirtuin family deacetylases
Action of sir2

Young

Old

KYC
Telomeres cap the ends of chromosomes to protect them from inappropriate “repair” mechanism.

Each time a cell divides, the telomeres shed a number of bases, so telomere length gives some indication of how many divisions the cell has already undergone and how many remain before it becomes senescent.
Biochemistry and Aging

**ROS:** Demolishing proteins and damaging nucleic acids, ROS are thought to be the villains in cells. The free radical theory of aging, proposed by Denham Harman at the University of Nebraska, holds that damage caused by ROS is responsible for many of the bodily changes that come with aging. Free radicals have been implicated not only in aging but also in degenerative disorders, including cancer, atherosclerosis, cataracts, and neurodegeneration.

**AGEs:** Glucose attaches to proteins, setting in motion a chain of chemical reactions that ends in the proteins binding together or crosslinking, a process called non-enzymatic glycosylation or glycation, thus altering their biological and structural roles. The process is slow but increases with time. Crosslinks, which have been termed advanced glycosylation end products (AGEs), seem to toughen tissues and may cause some of the deterioration associated with aging. AGEs have been linked to stiffening connective tissue (collagen), hardened arteries, clouded eyes, loss of nerve function, and less efficient kidneys.

**DNA repair:** photoaging, mitochondria DNA

**HSPs:** Produced in response to stress, HSPs decline with age.

**Hormones:** Declining levels of these chemical messengers may trigger some aging processes.
Manipulation of the life span of worms

daf-2 $\rightarrow$ DAF-16 (FOXO family)
$\rightarrow$ SOD

daf-2/insulin and IGF-1 receptor

Insulin/IGF-1 pathway
Cynthia Kenyon: Science 302, 611, 2003
Alzheimer’s Disease (AD)

Dr. Alois Alzheimer
Observed and autopsied brain of patient with dementia

Dementia=means out of one’s mind. Clinical diagnosis usually made if three symptoms are present: **impairments in short-term memory**, in other areas of cognition (e.g., language), and in social or daily functioning.

Alzheimer Autopsied brain of AD patient: shrunken cerebral cortex, with damaged and dead cells.

A normal, healthy female brain usually weighs between 1100 and 1400 grams. AD brains shrink below 1000, as the disease destroys brain tissue.
Tightly packed ruts & grooves of cortex also change. More pronounced mountains and valleys, gaping spaces between them.
Morphology of normal and AD brain

In Alzheimer's disease, brain scans show shrinkage of the overall volume of the brain.

http://www.alzheimersdiseasetreatment.com/
Possible New Test Found for Alzheimer's Disease

As the population ages, physicians care more and more frequently find themselves facing the diagnostic dilemma posed by an elderly patient who has begun showing memory loss and declining mental function. Is this due to Alzheimer's disease, which is currently untreatable? Or is it due to some other condition, such as depression, vitamin deficiency, or drug medication, for which effective therapy is available? It can be a difficult call for the average physician—and that dilemma has fueled the search for a simple and reliable test for Alzheimer's disease.

On page 105, a research team led by Leonard Scivo and Huntington Potter of Harvard Medical School proposes a promising candidate as a test that distinguishes Alzheimer’s patients from normal subjects.

But as hopeful as the early results seem, previous failures of a series of highly touted potential diagnostic tests have made Alzheimer's research more than usually cautious. If the new test works, it "would be marvelous," says Leon Thal, who helped Alzheimer's at the University of California, San Diego. But, he adds, "it is a very important and preliminary study." Caution aside, one reason for enthusiasm over the current test is the hope that it may be able to identify Alzheimer's before symptoms become apparent—which could be a boon toward effective treatments become available.

At present, the only definitive way of confirming a diagnosis of Alzheimer's is by autopsy. But there is also a relatively reliable, albeit controversial, method for diagnosing the disease in a living patient, using a complex battery of neurological and psychological exams. And recently researchers at Albert Einstein College of Medicine in the Bronx announced a psychological test that can tell whether a person is at risk for developing Alzheimer's in the next 4 or 5 years, but despite these advances, the search goes on for an even cheaper and simpler diagnostic tool, "something on the level of a pregnancy test," says Albert Einstein's director, Dave Davies of Albert Einstein. The test, if it proves reliable, could possibly be such a test, says John Trojanowski, who directs the Alzheimer's research center at the University of Pennsylvania School of Medicine. "It has a lot of appeal because of its simplicity and low cost."

"Voter came up with the idea for the eye scan by reviewing the literature on patients with Down's syndrome. He chose that literature because people with Down's who reach middle age invariably develop a condition whose neuropathology is identical to that of Alzheimer's. Potter was looking for a so-called "pathological marker" of Down's—characteristics that can be easily measured without brain scans or psychological tests—that might also be present in Alzheimer's patients and could be used as a diagnostic indicator.

Potter's search was productive. He found a marker that seemed promising. Several studies had found that Down's patients are hyper-sensitive to drugs that block the effects of the neurotransmitter acetylcholine. The next step was to see whether Alzheimer's patients show a similar effect, and if so, whether it is specifically associated with the disease. To answer these questions, Potter joined forces in 1989 with Elaine Marder, a neuroscientist at the University of Pennsylvania, and the two surgeons, then at Beth Israel Hospital in Boston. Together, they developed a simple assay for the hyper-sensitivity that measures pupil dilation in response to a very light illumination of the eye.

The Harvard team found that the eyes of normal subjects hardly responded to this stimulus when it is diluted to one hundredth of the standard concentration. But 18 out of 20 patients with probable Alzheimer's disease, as determined by neurological and psychological tests, were hyperresponsive to the drug, their pupils dilating 1.3% or more. "You get this very dramatic separation between the two groups," says Potter. Even more intriguing, Potter and Scivo suggest that their test may be able to predict who will get Alzheimer's even before symptoms develop. In two patients, one of whom is described in the current paper, the pupil test was positive in the year before the first Alzheimer's symptoms developed. Scivo says: "It can be found that half the neurodegeneration in Alzheimer's, a predic-tive test would be very valuable. "The biggest bang for your buck with a therapy may come in a patient who is in a premorbid stage, before the damage to the brain is too extensive," says Potter's Trojanowski.

The current test is intriguing to some researchers who say that it makes biological sense, as acetylcholine-blocking drugs, like the one used in the test, "would make very well explain the hyperr-sensitivity to cholinergic agents in the eye."

But given the dismal record of other proposed Alzheimer's tests (there have been various skin-prick tests, blood tests, and brain scans that initially looked good, then failed in larger trials), extreme caution must be maintained until the eye test is validated in trials that go far beyond the present total of 58 subjects and controls. "We need to test 10 to 100 times the number of subjects in that paper," says Elizabeth Davis. "Let's make sure it works in people without dementia, with dementias, and with eye diseases that cause pupil dilation... We need to know what limits the utility of this test."

Potter and Scivo agree that their test needs confirmation in larger trials. "We hope our colleagues all over the country will try their patients," says Potter. And they are getting, their wish. Trojanowski says the Alzheimer's center at Penn is setting up to test the eye exam, and they expect other researchers to follow suit.

But that doesn't mean answers will emerge soon, says Trojanowski, as definitive confirmation of the test's diagnostic value will depend on following a certain number of trial subjects through to death and autopsy. If those longer trials confirm the initial results, though, says Alzheimer's researcher John Blum of Cornell University Medical College, "then they have given us a wonderful advance for diagnosis."

Marcia Bartinga
Alzheimer's Disease Pathway

AD pathway

KYC
Alzheimer also found abnormal clumps (now called senile or neuritic plaques) and tangled bundles of fibers (now called neurofibrillary tangles). These plaques and tangles in the brain are hallmarks of AD.

- **Plaques:** are made of a normally harmless protein called amyloid-beta. It's believed plaque deposits form between neurons early in the disease process, before neurons begin to die and symptoms develop. Although the ultimate cause of neuron death in AD isn't known, amyloid-beta protein may be the problem.

- **Tangles:** The internal support structure for brain neurons depends on the normal functioning of a protein called tau. In AD, threads of tau protein become twisted. Many researchers believe this may seriously damage neurons, causing them to die. Tangles not unique to AD, but high density of tangles is.

• Note: Some researchers see the plaques as the major problem and some focus on the tangles, or both.
Baptists: β-amyloid due to secretase action (unknown)
Taoists: tau protein (microtubule)
Centralists: APOE lipoprotein (cholesterol transport)

Early onset: APP (#21); pre-senilin-1 (#14), presenilin-1 (#1)
Late onset: APOE4 (319)
Intracellular tangles

Hyperphosphorylated Tau Protein

Glycosylation (glycosylated)
PHF (paired helical fragment)
Ubiquinated

Normal

Alzheimer's

Neurofibrillary tangles

Amyloid plaques

Neuron
Cascade to dementia? The conversion of Ab1-42 peptides to toxic amyloid fibrils, which begins a long process of calcium influx and gene expression—possibly the first steps in Alzheimer's disease—may be disrupted by decoy peptides generated by Vernon Ingram and colleagues. (Adapted from Blanchard, B. J., et al. J. Alz. Dis. 2000, 2, 137-149.)
How one scientist and 678 sisters are helping unlock the secrets of Alzheimer's?

The Nun Study

David Snowdon, epidemiologist at the University of Kentucky medical center

Book: Aging with Grace.

Sample: Began with 678 women.

Gives them a battery tests every 1-2 years, including tests of memory, concentration, language, visual-spatial abilities, and orientation to time and place (e.g., date, season of the year, time of day, State they are living in).

e.g., test of memory-given list of 10 words (leg, cheese etc.), distracted for 5 minutes then asked to recall again.

80% of sample agree to have brain studied after they die.
Earlier marker for predicting AD

Idea density in early autobiographies and AD late in life
Idea density defined as the average number of ideas expressed per 10 words.

25 Autopsied brains
10 of 25 had AD brains
90% of those with AD brains in the low density group (bottom third)
13% of those without AD brains in the low density group.

74 autopsied brains
correlated idea density and number of neurofibrillary tangles: -.59 in the frontal lobe, -.48 in the temporal lobe, and -.49 in the parietal lobe (get similar though slighter lower correlations with number of senile plaques).

Those who meet the brain criteria for AD have lower mean density scores than those who don’t meet these criteria (4.9 vs. 6.1).
Idea density

A Short Sketch of My Life

When I was first told that I saw the light of day on a Tuesday noon, there automatically ran through my mind the old nursery rhyme pretending to predict one's fate by making it depend on the day of the week on which one was born. It goes something like this:

Bramley's child is fair of face,
Tuesday's child is full of grace.

Now, I don't want to argue that I had dreamed of being a nurse from the age of reason, but it at least was good encouragement and something to strive for as an ideal. I remember little of my baby days and what little I have had to take on seriously from all accounts I was perfectly normal with regard to mischief and being the first of my four parents offspring, might have been a bad lad had I not bled them.

A Way with Words

Analyzing autobiographical sketches written by the sisters in their 20s, before they took their vows, Snowden discovered that the number of ideas they packed into their sentences was a powerful predictor of who would develop Alzheimer's 60 years later.

HIGH IDEA DENSITY, LOW RISK
When I was first told that I saw the light of day on a Tuesday noon, there automatically ran through my mind the old nursery rhyme pretending to predict one's fate by making it depend on the day of the week on which one was born.
Strokes, AD brains & dementia

Among those with AD brain:
93% had dementia if they had at least one stroke in subcortical areas such as thalamus or the basal ganglia.

57% who had AD brain but no strokes evidenced dementia.

If strokes-- required fewer tangles in the neocortex to show signs of dementia than if stroke-free.

Strokes alone (without AD brain) associated with dementia only 2.5% of the time.
Idea Density in Early Life and Longevity

(Snowdon, Greiner, Kemper, Nanayakkara & Mortimer (1999)

(KYC)
Quartile Ranking of Number of Positive Emotion Sentences in Autobiographies Written in Early Life

(Danner, Snowdon, Friesen, 2001)
Median Age of Death of Individuals over 75 and Number of Positive Emotions in Autobiographies

(Danner, Snowdon, Friesen, 2001)
In 1903, as medical student at the ophthalmology clinic at the University of Kiel, Otto Werner was invited to examine four siblings in their early to late 30s with very unusual symptoms. He noted they had cataracts, premature greying and loss of hair, as well as skin changes he referred to as scleroderma. He presented these cases in his "Inaugural-Dissertation" in 1905, at the age of 25.

Thirty years later, Oppenheimer and Kugel (USA) described a similar case of what they termed "Werner's syndrome", an inherited disease in which patients develop symptoms post-puberty that resemble rapid aging. Clinical features of Werner's syndrome are now known to include: short stature, thin extremities, graying and loss of hair in their teens, cataracts in their 20's, a change of voice, osteoporosis, bone deformities, wrinkled, dry skin, diabetes, atherosclerosis, ankle ulcers, malignancies.
German patient 36 yr

American patient 50 yr

Japanese-American Werner patient
Teen age vs. 48 yr old
The gene responsible for Werner syndrome: The *WRN* gene encodes a DNA helicase of the RecQ family, *in vivo* function unknown. *In vitro*, WRN protein unwinds double-stranded DNA and has a high affinity for quadruplex "G-DNA", a structure that may form at telomeres, ribosomal DNA (rDNA) and other GC-rich sequences. The yeast homolog of WRN is Sgs1p. Yeast *sgs1* mutants have a short life span and rapidly show signs of aging such as a fragmented nucleoli (encompassing the rDNA) and sterility due to loss of silencing at the mating-type locus. One cause of the premature aging phenotype of *sgs1* strains was shown to result from increased instability of the genome, leading to lethal ERCs. An "Achilles heel" of this organism appears to be an inability to maintain repeated parts of the genome. The same may be true for humans.
KYC

Extent of G8 probe

Polymorphic HindIII sites

Haplotypes

1 2

A

B

C

D

CAGCAGCAGCAGCAGCAGCAGCAGCAGCAGGTCGTCGTCGTCGTCGTCGTCGTCGTCGTCGTCGTC

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KYC

Fob1-mediated replication fork-block

35S RNA transcription

Stalled fork prone to nicking and DSB formation

DSB

Rad52-mediated recombination between homologous sequences on same chromatid

Rad52

Generation of a single 9 kb ERC starts "aging clock"

ERCs replicate each S phase and stay within mother cell, thereby accumulating exponentially

1 cell division

2 cell divisions

3 cell divisions

15 cell divisions result >500 ERCs and death

KYC
1 obtain healthy young cells
2 Biotinylate the surface of approx. 100 million cells
3 Grow 12-15 hours in rich medium. Young to old cell ratio = 1:100,000,004
4 Add streptavidin-coated paramagnetic iron beads at a cell:bead ratio of 1:5
5 Place cells near strong magnet and wash away young cells
What Alzheimer's Does to the Brain

Spreading from the bottom to the top

The disease is characterized by the gradual spread of sticky plaques and clumps of tangled fibers that disrupt the delicate organization of nerve cells in the brain. As brain cells stop communicating with one another, they atrophy—causing memory and reasoning to fade.

1. Tangles and plaques first develop in the entorhinal cortex, a memory-processing center essential for making new memories and retrieving old ones.

2. Over time, they appear higher, invading the hippocampus, the part of the brain that forms complex memories of events or objects.

3. Finally, the tangles and plaques reach the top of the brain, or neocortex, the "executive" region that sorts through stimuli and orchestrates all behavior.

GRAY MATTERS

A brain ravaged by Alzheimer's, right, shrinks in size and weight as the disease destroys neural tissue. The once tightly packed ruts and grooves on the surface of a healthy cerebral cortex, left, become visibly pitted with gaps and crevices.