衰老及相关基因群*

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摘 要:综述20世纪与基因相关的衰老原理的探索及其进展.整体动物水平的衰老研究归纳了衰老的诸多表象但疏于对衰老本质的探讨.线粒体-自由基衰老学说阐述了线粒体DNA的损伤与衰老有很大的相关性.由Hayflick分裂限制衍生的端粒衰老学说给衰老机制提供了重要信息.目前狭隘的基因程序化衰老学说已和损伤衰老概念有机的联系在了一起.总之,自由基衰老学说得到了氧化衰老学说和糖基化衰老学说的补充逐渐形成了生化副反应与基因衰老学说的大统一衰老机制板块理论.

关键词:衰老;基因;Hayflick极限;氧化应激;糖基化

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Aging and Genes Related

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Abstract: Several up to date most important theories on aging mechanisms and gene-related aging studies that were developed during the 20's century were reviewed. The theories of aging at the whole animal level reveal important aging manifestations, but most of them lacking accurate explanations of the aging mechanisms. The mitochondria-free radical theory of aging based mainly on free radical theory of aging was closely gene related. The Hayflick Limit, the telomere theory of aging and DNA damage during stresses all provide specific knowledge about the cellular aging processes. Narrow-minded search for longevity-genes has been unsuccessful, ending up with a conjunction of programmed aging with stochastic aging concepts. The free radical theory of aging is supplemented by the oxidative stress theory of aging and the glycation/Maillard reaction theory of aging for covering a full scale of biological deteriorative side reactions.

Key words: aging; genes; Hayflick limit; oxidative stress; glycation

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1 Definition and common phenomena of aging[1,2]

Aging can be defined as a general decline in organ functions as well as a decrease in adaptiveness to change and to restore disrupted homeostasis. Aging changes occur to most (if not all) organs, to cells as well as to various bio-molecules, e.g. DNA, proteins, lipids and carbohydrates. Some gerontologists prefer to use the word senescence because the aging process implies that the passage of time resulting in deteriorations. However, diseases of old age, such as arthritis, osteoporosis, heart disease, cancer and Alzheimer disease, are often distinguished from aging itself. But even if the aging process is distinct from the diseases of aging, it is nonetheless true that the aging process causes changes that increase the probability that diseases may develop.

Some aging changes that typically occur with age are as follows. Declines of hearing ability and sensitivities to taste and smell. About half of those aged 70 have lost all teeth. Weight declines after age 55 due to loss of lean tissue, water and bone. Body fat increases after age 35. Muscle strength for men declines 30% ~ 40% from age 30 to age 80. Reaction time declines from age 30. Elderly people tend to sleep more lightly, more frequently and for shorter periods, with a reduction in rapid eye-movement sleep. There is a reduction of the thymus gland to 5% ~ 10% of its original mass by age 50. Levels of antibodies increase with aging. One third of men and half of women over 65 report some form of arthritis. The elderly require twice insulin to achieve the glucose uptake of the young. There is reduced sensitivity to growth factors and hormones due to fewer receptors and dysfunctional post-receptor pathways. The temperature needed to separate DNA strands increases with age.

Aging changes are frequently associated with an increase in likelihood of mortality, but this is not neeessarily the case. For example, graying of hair is a symptom of aging, but it does not increase likelihood of mortality. Aging changes which are not associated with a specific disease, but which are associated with a generalized increase in mortality would qualify as biomarkers of aging and would distinguish biological age from chronological age. Certain biomarkers would be better predictors of the increased likelihood of mortality than the chronological age. To date no biomarkers of aging, however, have been universally accepted.

2 Conparative animal aging, species specific lifespan[3~8]

Many questions have been raised and under vigorous debate in interpretation of aging mechanisms. Scientists have wondered whether aging is inherently multifaceted or whether a single cause lies behind all aging phenomena. Differences in lifespan between species raise a number of critical questions. Why human lives about 5 times longer than cats and cats lives 5 times longer than rats? Aren't the cells much the same? Why is it that at age 3, about 30% of rodents have had cancer, whereas at age 85, about 30% of humans have had cancer? Some species (such as lobsters, alligators and sharks) do not appear to age at all. Cancer cells, stem cells and human germ cells seem "immortal" when compared to other cells.

Attempts to classify theories of aging have led to the two major classifications programmed aging and environmental damage related aging. Programmed aging theories suggested that aging is due to something inside an organism's control system that forces elderliness and deterioration. By contrast aging due to damage is the sum effect of many kinds of environmental assaults, i.e., damages due to radiation, chemical toxins, metal ions, free-radicals, hydrolysis, glycation, disulfide-bond cross-linking, etc. Such damage can affect genes, proteins, cell membranes, enzyme function, blood vessels, etc.

The distinction between the two categories of aging is not as clear-cut as it might appear. When Pacific salmon have lived in the ocean for 2 or 3 years, they swim upstream in rivers until they find a place suitable for spawning. After spawning, the adrenal gland releases massive amounts of corticosteroids leading to rapid deterioration. Although this process is obviously "programmed", it is incorrect to describe it as "aging". As a matter of fact, programmed death, rather than programmed aging, is a common phenomenon

among animals that reproduce only once.

Comparison of species-specific lifespan provides convincing evidence that longevity is genetically influenced by different conditions. Some experiments on *Drosophilae* showed that flies bred by only using eggs that were laid toward the end of reproductive life for 15 generations achieved maximum lifespan 30% greater than that of controls. There was no evidence that lifespan would not have increased more if the experiment had continued. Human germ cells have arguably lived for millions of years through an DNA-repair enzymes, probably due to antioxidant enzymes and telomerase or some other reasons. Some species of jellyfish show no sign of aging.

3 Different organ systems aging at different speed

Aging in the reproductive system provides the best example of programmed aging in mammals. For different organs, particularly the heart, brain, lung and kidney, specific disease states associated with aging are of more significance than generalized deterioration. There is wide variation in the health status of specific organs amongst the elderly.

Skin, lungs, muscles, blood vessels and organ-function in general is adversely affected by protein cross-linking, which is increased in diabetes. Because most of those 65 years of age have at least some symptoms of subclinical diabetes and since most of the symptoms of aging are accelerated in diabetes, diabetes figures strongly when the elderly are described in terms of averages. Generalized reduction in blood flow due to atherosclerosis also has an adverse effect on most organ systems. Both protein cross-linking and cardiovascular deterioration are strongly influenced by genetics and environmental influences (diet, smoking, etc.).[9, 10]

The kidney provides perhaps the most striking example of individual variation in the effects of aging. On average, kidney weight declines about 15% between ages 40 and 80. The kidney's filtering capacity for the average 90-year-old is typically half what it is for the average 20 year old. But high blood pressure and diabetes are particularly damaging to kidney function. A 20-year longitudinal study showed no change at all among elderly men who had no health problems. If this result can be extrapolated it would mean that within the human maximum lifespan there is no significant deterioration in the absence of disease conditions.

Cardiovascular disease is the cause of most deaths among the elderly. The left ventricle of the heart increases in size with age (hypertropy) due to an increase in size of the heart muscle cells that must work harder to pump blood through a circulatory system that has narrower channels and reduced elasticity. Lipofuscin content of heart muscle cells increases from about 1% in the young to over 5% in the old[11, 12]. Arteries thicken with age such that about three-quarters of elderly people have increased blood pressure, both systolic and diastolic. However, about a quarter of elderly people do not have elevated blood pressure. While heart attacks from ischemia account for 40% of deaths for those 65~74 years of age, it accounts for only about 10% of deaths for that age group in Japan.

The claim that all people lose about 100 000 neurons per day has not been supported by modern research. On average, 2% of neurons are lost, between ages 20 and 90. Those over age 85 show a 10% decline in brain weight. But averaging can be misleading, since the elderly include many people with considerable dementia and others with little or none. Some researchers have found no decline in brain weight or IQ among the mentally normal elderly. Dementias are more common among the elderly who develop cardiovascular disease. Dramatic reduction in cerebral blood flow and in brain oxygen and glucose utilization is frequently seen after the 8th decade of life. Although most dementias are due to Alzheimer's disease, at least 20% of dementias are due to stroke.

With aging, the lens thickens and becomes yellowed[11]. Collagen and elastin in tendons and ligaments become less elastic and more fragmented as a men grows older, particularly due to non-enzymatic glycosylation (a diabetes-related deteriorative biological side-reaction). Articular cartilage becomes frayed and the synovial fluid between joints becomes "thinner". Decline in circulatory function contributes to this process. Collagen and elastin also cross-link in skin, resulting in a loss of elasticity. The protein keratin in fingernails

is also a component of the outer layer of skin, which provides "water-proofing". The epidermis thins with age, leading to wrinkles. Decreased secretion by sweat glands increases vulnerability to heat stroke. When the melanocytes associated with hair follicles cease functioning, hair turns white. Partial reduction of melanocyte function results in hair that appears "gray". Loss of flexibility of the proteins collagen and elastin in the lung results in loss of elastic recoil. It becomes difficult for full respiration, which reduces air exchange, reducing the capacity to do work.

Bone is composed of 30% cells and blood vessels and 45% mineral deposits. Most of the white ash remaining after cremation is calcium, lead, zinc and potassium from bone. Both men and women lose bone mass between the ages of 39 and 70 (osteoporosis), but women lose bone mass at twice the rate as men mainly due to the loss of estrogen at menopause. Bone loss is less rapid after the age of 70. Part of the problem is due to decreased calcium and Vitamin D absorption in the intestine. Young bones have been compared to green tree branches that can bend considerably before breaking and upon breaking does so with splintering. By contrast, old bone is like a dry stick that snaps upon bending. Hip fractures associated with osteoporosis are fatal to aged population.

After age 20, growth hormone (GH) levels in the blood decline[13]. Attempts have been made to use GH injections to restore youthful bone and muscle in a manner analogous to anabolic steroids, but without the negative side effects. While increased muscle mass and bone strength was achieved, these were associated with reduced insulin sensitivity and increased sodium retention. And the positive effects were inferior to those, which could be achieved by an exercise program. Many of the effects of GH are due to Insulin-like growth factor-1, which is produced in the liver as a result of GH stimulation. Insulin-like growth factor-1 causes neither water retention nor diabetes, but it can promote cancer. Though investigation and controversy continues, solid evidence for the use of GH for life extension in humans is still lacking.

4 Up and down of the free radical theory of aging[14]

Free radicals are highly reactive molecules that have an unpaired electron in their outer orbital. Free radicals can damage nucleic acids, proteins or lipids. For biological systems, oxygen free radicals are the most important, in particular superoxide ($O_2^{\cdot-}$), nitric oxide ($\cdot NO$) and the hydroxyl radical ($\cdot OH$). Although hydrogen peroxide (H_2O_2) and hypochlorite are not themselves free radicals, these oxygen-containing molecules can facilitate free-radical formation.

A superoxide ion ($O_2^{\cdot-}$) would result from the addition of an electron to a normal oxygen molecule (O_2). The hydroxyl radical ($\cdot OH$) is typically formed either directly from superoxide and hydrogen peroxide:

$$O_2^{\cdot-} + H_2O_2 \rightarrow \cdot OH + OH^- + O_2,$$

or by oxidation of a heavy metal ion by hydrogen peroxide:

$$Fe^{++} + H_2O_2 \rightarrow Fe^{+++} + \cdot OH + OH^-.$$

The last reaction, known as the Fenton Reaction, is the most dangerous because a cell's superoxide ions tend to be concentrated in the mitochondria and are much less frequently found in the nucleus than in the cytoplasm. But hydrogen peroxide (H_2O_2) molecules can drift into the nucleus where the Fenton reaction can produce hydroxyl radicals that can damage DNA.

Animal cells contain three important enzymes to deal with the superoxide and hydrogen peroxide: superoxide dismutase (SOD), glutathione peroxidase and catalase. Catalase catalyzes the formation of water and free oxygen from hydrogen peroxide. Catalase is present in membrane-limited organelles known as peroxisomes. Peroxisomes contain enzymes that degrade amino acids and fatty acids producing hydrogen peroxide as a by-product.

SOD is the most abundant of anti-oxidant enzymes in animals. The liver, in particular, is very high in SOD. Cellular concentration of SOD relative to metabolic activity is a very good lifespan predictor of animal species. Most mammals experience a lifetime energy expenditure of 200 000 calories per gram, but humans have an amazing 800 000 calories per gram. Humans

have the highest levels of SOD of all species studied.

Radiation produces the hydroxyl radical, but most of the oxygen free radicals are byproducts of cell metabolism, particularly in the mitochondria, the lysosomes and the peroxisomes. One of the reasons why organelles are surrounded by membranes may be to protect the cell from the free radicals they generate. DNA may be sequestered in the nucleus, in part, as additional protection against free radicals. Nonetheless, free radicals contribute to DNA damage and mutation. Hydrogen peroxide is particularly dangerous to nuclear DNA because it is more stable and can diffuse into the nucleus.

In addition to enzymes, the animal cell uses many other chemicals to protect against oxygen free radicals. Vitamin E is the main free-radical trap in the membranes. Beta-carotene is a primary defense against singlet oxygen and is another free-radical trap in the membranes. Uric acid can act as an anti-oxidant. Vitamin C can act as both an anti-oxidant and a pro-oxidant. Melatonin, a hormone produced by the pineal gland in decreasing quantities with aging, efficiently crosses membranes and is effective against hydroxyl radicals.

Mammals fed anti-oxidants show up to a 30% increase in average lifespan, but no increase in maximum lifespan. Anti-oxidants are most valuable for animals that are cancer-prone, or subjected to radiation or chemical toxins. There are evidently homeostatic mechanisms in cells that govern the amount of allowable anti-oxidant activity. For example, increased level of Vitamin E in the diet correlates with reduced levels of glutathione peroxidase activity, and vice versa.

More and more evidences indicate that free radicals are involved in diseases both acute and chronic, which accelerate aging process, the free radical theory of aging itself, however, is facing many challenges such as the importance of free radicals (e.g. NO) in essential biology, their antibiotic function in immune process (respiration burst). The disappointed results of vitamin therapy in a few large scare antioxidant supplemental studies and diabetes speeded aging symptoms all put questions to the validity of the theory as a sole explanation of aging mechanism. The free radical damage should be, with overwhelming experimental observations, one of the most importance culprits of aging and appears being both a cause and consequence during aging.

5 Mitochondrial DNA and aging[15]

The mitochondria are the cellular organelles that generate energy from aerobic metabolism. Each cell may contain 100 to 1 000 mitochondria. Mitochondria are the only cellular organelles with their own DNA. Each cell contains many mitochondria, but the total mitochondrial DNA in a cell represents less than 1% of the amount of DNA found in the nucleus. Most mitochondrial proteins must be imported from outside the organelle. The mitochondrial DNA is derived almost entirely from the mother. Human mitochondrial DNA are 5~10 circular strands and composed of 16 570 nucleic acids.

Five mitochondria protein complexes form the respiratory chain, which creates energy through conversion of oxygen to water molecules. These protein complexes are nominated as Complex I, II, III and IV. More than a quarter of the Complex I proteins are coded-for by mitochondrial DNA, whereas Complex II is entirely coded by nuclear DNA. An associated protein complex, Complex V produces high-energy phosphate molecules ATP. Each DNA strand codes for 13 proteins, many of which are part of Complexes IIV. Complex I and Complex II supply independently electrons to Complex III via Coenzyme Q (CoQ). CoQ is also known as ubiquinone, because it is "ubiquitous" in almost all cellular organisms. CoQ is an essential component of the mitochondrial respiratory chain. Up to 5% of oxygen used by mitochondria will normally "leak out" from the respiratory chain to form superoxide. CoQ forms an important antioxidant defense against these reactive oxygen species. Associated with aging is a decline in the amount of CoQ in organs. A person 80 years old will typically have about half as much CoQ10 in the heart, lungs and spleen as a 20-year-old.

During oxidative stresses free radicals continuously damage the complexes in respiration chain located on mitochondrial inner membrane. An estimated 2%~5% of the oxygen consumed by mitochondria is divert-

ed to generating the free-radical-producing species superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2). The "mitochondrial theory of aging" postulates that damage to mitochondrial DNA and organelles by free radicals leads to loss of mitochondrial function and loss of cellular energy with loss of cellular function. Mutations in mitochondrial DNA occur at 16-times the rate seen in nuclear DNA. Mitochondrial DNA has neither protective histone proteins nor DNA-repair enzymes. A comparison of 7 non-primate mammals (mouse, hamster, rat, guinea-pig, rabbit, pig and cow) showed that the rate of mitochondrial superoxide and hydrogen peroxide production in heart and kidney were inversely correlated with maximum life span. A comparison of the heart mitochondria in rats (4-year lifespan) and pigeons (35-year lifespan) showed that the pigeon mitochondria leak only 30% as many free-radicals as the rat mitochondria. Free-radicals from mitochondria result in also damage to cellular proteins, lipids and DNA throughout the cell. This damage has been implicated as a cause of aging. If the fatty acids entering the mitochondria for energy-yielding oxidation have been peroxidized in the blood, this places an additional burden on antioxidant defenses. The greatest damage occurs in the mitochondria themselves, including damage to the respiratory chain protein complexes. With aging, there is a decline in the number of mitochondria in most tissues.

Neurons are the largest cells in the body and have the highest metabolic demands, with 70% of ATP produced required to maintain the sodium-potassium pump. Clinically, damage to brain and muscle tissue are the first symptoms of mitochondrial disease. Mitochondria in the brain tissue of Alzheimer's patients are particularly damaged. Therapy has included the B-vitamins that act as coenzymes in the respiratory chain (thiamine, riboflavin, niacinamide) and CoQ10.

6 The glycation theory of aging[16~18]

Proteins are long chains of amino acids (amino acid polymers, or polypeptides). In general, 20 different amino acids occur in animal proteins.

Proteins can be damaged both by free radicals and by glycation. Glycation (also called the Maillard reaction, or non-enzymatic glycosylation) is a reaction by which sugars become attached to proteins without the assistance of an enzyme. This attachment occurs at the free amine group of lysine or arginine, which is not involved in the peptide bond. The reaction between glucose and a lysine amino acid in a protein molecule can finally result in the formation of advanced glycation end-products (AGEs).

AGEs attached to LDL-cholesterol accelerate oxidation and subsequent atherosclerosis. The irreversible cross-linked proteins of AGEs in vessel collagen also contribute to atherosclerosis, as well as to kidney failure, conditions worsened in diabetes. AGEs aggregate protein cross-linking in the plaques and tangles of Alzheimer's disease, thereby accelerating neuron death. AGEs in tissues increase the rate of free radical production to 50-times the rate of free-radical production by unglycated proteins.

Lens crystallines, collagen and basement membrane are the proteins most vulnerable to cross-linking and AGE formation because they are the most long-lived proteins, with a slow rate of turnover. Collagen accounts for about a third of total body protein in mammals. Collagen cross-linking in skin, muscle and organs throughout the body leads to the sinewy, inelastic tissue characteristic of aging. The higher glycation rate in diabetics is undoubtedly related to the fact that they appear to "age faster".

Cell proteins are continuously being produced and broken-down. This may seem wasteful, but proteins accumulate damage and become defective with time. Proteins made defective by racemization, or Amadori products, are not so difficult to remove, but AGEs and some other defective proteins can be very difficult to remove. Protein degradation are catalyzed by degradative enzymes called proteases. The 76-amino-acid straight-chain peptide ubiquitin gets its name from being ubiquitous in eukaryotic cells being not much different in yeast or humans. Ubiquitin plays a role in both regulatory and degradative protease activity. Regulatory proteins controlling the cell cycle are marked for protease destruction by ubiquitin attachment. Ubiquitin also attaches itself to non-regulatory proteins to mark them for protease destruction. Ubiquitin can be found in the

proteinaceous plaques and tangles of Alzheimer's Disease in a failed attempt to destroy these abnormal proteins. AGEs which are resistant to proteases can also be covered with ubiquitin.

Cross-linking of proteins makes connective tissue lose elasticity, increases arteriosclerosis, reduces kidney function, slows wound healing, reduces the vital capacity of the lung and contributes to cataracts. In fact, cross-linking of collagen is widely regarded as a biomarker of aging. Glucose is not the most active sugar for glycation. Galactose is 5 times more reactive than glucose, deoxyglucose is 25 times more reactive, ribose 100 times more reactive and deoxyribose 200 times more reactive.

7 Cell dividing and the hayflick limit[19]

Animal cells can be classified as germ cells, stem cells and somatic cells. Somatic cells are either non-dividing after birth (like neurons or muscle cells) or dividable cells that continue to divide (stem cells and most somatic cells). But the number of times that most dividing cells can divide is limited.

One of the most well known experiments in cellular aging study was done by Dr. Leonard Hayflick. He observed that embryonic fibroblasts in tissue culture would divide about 50 times before they ceased dividing. This 50-division limit seemed to be a property of the cell nucleus or DNA. If an old nucleus is transplanted to a young cell, the nucleus still divides no more than 50 times. And if a young nucleus is transplanted to an old cell, the nucleus still divides 50 times.

Cancer cells are often "immortal" and offer clues about the mechanism of the Hayflick Limit. This requires a review of the nature of DNA. Cell structure and metabolism operates under the direction of genes, which are located in the DNA of the chromosomes of the animal cell nucleus. At the ends of chromosomes are long non-functional strands of DNA called telomeres. Telomeres consist of the six-base repeating sequence TTAGGG (2 Thymines, 1 Adenine and 3 Guanines). With each cell division, some of the telomere is lost. The length of the remaining telomere is a good indicator of how many divisions a dividing cell has left. Once the telomere is gone, functional genetic DNA is lost with each cell division, and the cells are soon missing essential proteins. Gametes (i.e., germ cells, as distinct from the "somatic" cells of the body), stem cells (for skin, intestine and blood cells), "immortalized" cancer cells contain an enzyme called telomerase that replaces lost telomeres, thus preventing them from experiencing a Hayflick Limit. Hydrocortisone also can increase the Hayflick Limit, but at the cost of increased mutation.

It seems that there is a certain correlation between maximum lifespan of a species and the number of fibroblast doublings for that species. Mice with a 3-year maximum lifespan show 15 doublings, chickens with a 12-year lifespan show 25 doublings and the Galapagos tortoise with a 175-year lifespan shows 130 doublings. These species not only differ in initial telomere length, but also in the number of telomeres lost at each cell division. If the maximum lifespan was determined by the Hayflick Limit alone, these species would have a lifespan 2~3 times greater than what is observed. It should be noted that fibroblasts are only a type of dividing cells whereas postmitotic cells may aging quite differently.

8 Telomeres and aging[20, 21]

The end of each chromosome in a cell is composed of thousands of base pairs with the precise sequence TTAGGG (2 Thymines, 1 Adenine and 3 Guanines). At conception each telomere is about 10 000 base pairs long (i.e., about 1 666 TTAGGG repeats), and the typical chromosome is about 13 thousand times longer (130 million base-pairs). At birth, the average telomere is half as long as it was at conception. Telomeres lose an average of eight TTAGGG subunits per cell division, so it seems that half of the telomere length was lost due to the cell divisions of embryonic development.

If cells continue to divide after having lost their telomeres, they not only become misfunctional due to lost genes, but the chromosome ends may start sticking to other chromosomes increasing the number of abnormalities. Probably a cell may invoke apoptosis or other

"senescent" changes that prevent the cell from dividing or becoming cancerous. Hence it has been suggested "the Hayflick Limit itself may be a means of preventing cancer".

Some study shows that diseases that resemble accelerated aging may involve telomeres. In Hutchinson-Gilford syndrome, a child is born with abnormally short telomeres. In Werner's syndrome, the telomeres are of normal length initially, but shorten at an abnormally high rate. In Down's syndrome the telomeres of the immune system cells shorten at an abnormally high rate. Down's syndrome, among the three, most resembles accelerated aging. Down's syndrome is caused by an extra copy of chromosome 21. One birth in 700 is a Down's baby, most frequently seen in the babies of women giving birth in their 30 s or 40 s. The disease accounts for one-third of all cases of mental retardation in industrialized countries. Down's syndrome victims are very vulnerable to infection, due to the rapid shortening of the telomeres of their leukocytes (white blood cells). Almost all Down's syndrome victims have Alzheimer's disease by age 50, probably because chromosome 21 carries the amyloid gene. Chromosome 21 also carries Cu/Zn Superoxide Dismutase gene, resulting in increased production of hydrogen peroxide (H_2O_2) which (without catalase or glutathione peroxidase) can lead to more hydroxyl radicals. The incidence of diabetes is 5~ 10 times greater for Down's syndrome victims than for age-matched controls.

Childhood progeria occurs once per 8-million births. At age 5 the telomeres of a Hutchinson-Gilford syndrome child are about as long as those of a very elderly person. Most often these children die of cardiovascular disease (average age of death is 13) without the usual causes of high blood pressure or high blood cholesterol. Degeneration of blood vessel endothelial cells is the likely cause. These children do not have the high rates of cancer, osteoporosis or Alzheimer's disease often seen in the elderly.

There have been over a thousand cases of Werner's syndrome since it was first reported. Most of these have been in Japan, with 25% of cases due to family intermarriage. The disease first becomes evident in the late teens or early 20 s, and typically results in death by age 50 by cardiovascular disease. Werner's syndrome victims have defective helicase, the enzyme that causes DNA to unwind and is involved in some forms of DNA repair. Arteriosclerosis is severe, along with calcification of soft tissues. Cancer, diabetes, osteoporosis and cataracts are extremely common.

For those who believe that telomeres are a biological clock that cause aging by shortening, there has been the hope that human aging can be stopped by somehow adding active telomerase to all somatic cells. An experiment transfected human somatic cells with a reverse transcriptase subunit of telomerase thereby forcing the cells to express telomerase. The cells exhibited 20 population doublings beyond their Hayflick Limit and continued to exhibit normal, healthy and youthful cellular appearance and activity. This experiment was done not only for fibroblasts, but also for retinal epithelial cells and vascular endothelial cells. This creates hope that it may someday be possible to preserve youth by a form of gene therapy that either induces the expression of telomerase in somatic cells or adds additional genetic material to cells consisting of an engineered telomerase superior to the natural form. A person undergoing such therapy might first take a dose of telomerase destroyers to prevent any incipient cancers from being nourished by the treatment that would follow.

9 Aging and DNA[5, 20]

As shown by Thomas Johnson the potential for programmed aging was very powerfully. He found that a single gene mutation on the *C. elegans* nematode can double its maximum lifespan. Of the approximately 100 000 genes in the human genome, it is estimated that only 1% of these are different from those of a chimpanzee, which has half the estimated maximum lifespan of a human. It has been suggested the longevity difference could be due to as few as a hundred genes or less.

Moreover, "wear and tear" can also operate at the level of DNA mainly in two types of alterations: mutation and DNA damage. An analogy illustrates the "alterations": the word "LOOK" can be mutated to the word "BOOK" by the substitution of a letter, whereas if the letter "O" is lost or altered, damage occurs, re-

sulting in a new word "LOK" or "LOEK". Substitution of a Thymine for an Adenine would be a mutation, whereas loss of an Adenine or methylation of a Guanine would be damage. The phenomena are not independent, however, because methylated Guanine is known to be mutagenic. Moreover, 85% of chemicals known to be mutagenic in bacteria are carcinogenic in animals, which is suggestive of a relationship between aging and cancer. There are an estimated 80 000 DNA damage events per mammalian cell per day. If DNA repair enzymes did not exist, most cells would soon be nonfunctional. DNA repair enzymes can replace the missing nucleic acid in the case of depurination or depyrimidation by "checking" the complement. Other repair enzymes can, on the other hand, "sew-up" a single-strand break.

The methyl groups may be de-methylated by enzymes and transferred from the nucleotide base to their own cysteine residues. A DNA crosslink involving (e.g. the formation of abnormal Thymine and Adenine) bases may not be repaired, nor can a somatic cell double-strand break. Although double-strand damage is rare, the absence of repair mechanisms makes them much injured for proliferation of somatic cells. Microtechniques may someday repair double-strand damage in somatic cells, but in mammals such repair can only occur in the meiosis of germ cells when complementary chromosomes exist.

Even a "wear and tear" theory like DNA damage is subject to a programmed aging interpretation. The deer mouse lives 2.5 times longer than the house mouse (20 vs 10 Hayflick doublings) and has a DNA repair capability that is 2.5 times greater. In general, DNA repair tends to lag behind DNA damage to a greater extent in short-lived species, and the amount of lag can constitute the degree of "programmed aging". DNA replication in the *E. coli* bacterium by DNA polymerase involves a "proofreading" step to replace misplaced bases. But proofreading requires energy, and a tradeoff between accuracy and speed. An "optimum" error tolerance is achieved for the survival of the species rather than the individual. RNA replication by RNA polymerase is less accurate than DNA replication by DNA polymerase.

10 Genes found related with aging[20,22~25]

Michael Rose demonstrated first longevity genes from breeding fruit flies, although the identity of those genes was not determined. Geneticist Tom Johnson of the University of Colorado discovered a mutant gene in *C. elegans* that at 25 ℃ mean life span was increased by 65% and maximum lifespan about 110%. *C. elegans* is a nematode worm of the size of a comma that is widely used in research as a model organism. Johnson named the gene age-1 in the expectation that other genes for aging would be found. Caloric restriction further extends the lifespan of age-1 mutants. Age-1 mutants were shown to have elevated Cu/Zn SOD and catalase (nematodes, unlike vertebrates, do not have glutathione peroxidase). Age-1 mutants show a lower rate of deletions in the mitochondrial genome than wild types.

Drosophilae fruit flies also have the natural antioxidant enzymes SOD and catalase. Transgenic Drosophilae were created with (1) extra Cu/Zn-SOD genes (2) extra catalase genes and (3) extra Cu/Zn-SOD and extra catalase genes. Only the flies in the third category, having both extra genes showed extended lifespan. These transgenic flies showed 26% greater SOD activity, 73% greater catalase activity and 34% longer lifespan.

C. elegans gene that extends lifespan is also found related with clk-1 (the "clock" gene), which somehow alters growth rate, cell cycle time and other "timed" events in the nematode life-cycle. Another *C. elegans* mutant, daf-2 (dauer formation gene) causes the nematode to go into the developmentally arrested "dauer" state and extends lifespan. The mechanism of action is not entirely clear, but it may not be entirely due to arrested metabolism. Intriguingly, daf-2 is similar to the human insulin-receptor gene.

Heat-shock protein response is reduced in aging cells and is elevated in the cells of CRAN organisms leading to suspicions that CRAN may function partially by altering genetic switches. The production of heat-shock proteins (HSPs) can be increased by a transient elevation of temperatures that could ordinarily kill the

cell. When the temperature drops, HSP production falls. Although originally discovered in *Drosophilae* in response to heat, the HSPs are now known to also function against other cell stresses such as metal poisons and oxidation, and are of remarkably similar structure in nearly all cells, including those from baeteria, plants and mammals. Some HSPs evidently act by binding to incompletely folded metabolic proteins, protecting them in an inactive state until the traumatic stress has passed. Another explanation may be that higher HSPs in animals are just another manifestation of reduced damage due to aging by oxidation and glycation.

Apoptosis, so called programmed cell suicide is triggered when a cell becomes mutated, cancerous or defective. Apoptosis may be developed for protecting the organism from damage by malfunctioning cells. There are numerous genes/proteins that are associated with apoptosis. Suspicion has been raised that such genes could contribute to programmed aging. However, injecting the protein Apo-1 into a cell will trigger apoptosis. But the protein Bcl-2 can rescue a cell from apoptosis.

Another possible indication of programmed aging is the fact that aging cells do not make Fos, a protein that is produced when young cells are given growth faetors. Fos is a transcription factor that attaches itself to RNA polymerase and helps the polymerase attach to a region of DNA so transcription can begin.

11 Cancer and aging[26]

There is, as a subject of age, a general and exponential increase in the likelihood of cancer. An increased cumulative effect of DNA mutations and a decline in immune-system function was seen during aging. However, as a cause of death the relative incidence of cancer increases only to age 65 and decreases thereafter. At age 65, 30% of North American deaths are due to cancer, whereas at age 80 only 12% of deaths are due to cancer. These are mainly because of the relative increase of cardiovascular and Alzheimer's disease is faster than the increase in cancer with age. Smoking and dietary factors can play a significant role in the incidence of cancer, as indicated by the fact that breast cancer in North American women is ten times more common than those in Japan.

Because the damaging theory of aging emphasizes DNA-damage and decline in DNA-repair, the fact that cancer results from DNA-damage and mutation of DNA gives it a theoretical significance for aging. Animals subjected to sub-lethal radiation show increased mutations, chromosomal breakage and shortened life span that are independent of cancer death. But the resultant diseases and atrophy, such as kidney degeneration, are more focused than the symptoms of senescence. Nonetheless, cancer is aging related rather than aging dependant, more detailed studies as well as analysis of the differences and similarities between cancer and aging will be useful to understand the nature and the causes of aging.

12 Understanding multigenomic aging process[27,28]

It can be expected that in the coming years there would be hundreds of genes to be found to relate with animals aging and life span. Much of these are going to rely on studies of protenomics when the genetic architecture has been basically constructed since the establishment of human genomic base pair diagram. Most of such genes should undoubtedly associate with proteins, which play defending, repairing and balancing role in preventing biological stress-enhanced side (deteriorative) reactions during life.

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