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A short evaluation of aging theories and focal points of aging studies

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The process of aging has been investigated for a long time. Numerous theories were described to understand actual mechanism of aging. External environmental factors, genomic component, somatic mutation, harmful effects of free radicals, decline of the immune response and deficiency in the endocrine system, accumulation of glycosylation end products (AEGs), decreasing amount of heat-shock proteins (HSPs), telomeric shortening and calorie intake are thought to be related. Many questions exist about the phenomenon "aging and life span". This article represents a brief evaluation of aging theories and fundamental focus point of aging studies.

Key words: aging theories, free radicals, telomeric shortening, calorie intake.

Aging and aging theories

More than 300 theories on aging were described by gerontologists (Merker et al., 2001). Theories of aging can be divided into two categories: "why" and "how". The former looks into the process of aging and the factors that are responsible for it, the latter strikes to explain the evolutionary background of the phenomenon "aging".

Many aging theories from the first category consist of two broad categories based on putative casual forces: intrinsic factors, referring to genomic components and processes and extrinsic factors, encompassing various external environmental influences on the organism. One theory, which explains why an organism ages, is the "longevity determinant gene hypothesis" (Merker et al., 2001). This theory proposes that aging is the result of the normal biological processes necessary for life. These processes may have long-term negative or aging effects on organisms. This thesis divides the process into two major categories: developmentally-linked senescent processes and continually-acting senescent processes. The common feature of these hypotheses is that they suggest all species share common aging causes and common mechanisms of regulating aging rate.

The "somatic mutation theory" explains how an organism ages and focuses on intrinsic factors. Harman (1981) proposed that somatic mutations in DNA are the key events leading to aging. These mutations can cause to occur the non-functional proteins/enzymes that no longer perform their essential functions. The accumulation of mutations leads to structural disabilities and finally to the death of the organisms. This process is termed "somatic mutation theory", because of the mutations in destructive properties especially in somatic tissues. In a transgenic mouse model, which was engineered to measure mutation frequencies *in vivo*, it was found that somatic mutations accumulate with age in liver but not in brain (Dollé et al.,

1997). Later work showed that mutations accumulate with age in both mouse heart and small intestine but there were distinct differences between the type of mutations and their rate of accumulation (Dollé et al., 2000). Remarkably, it was also found that calorie restriction, which is well known for increasing the life span (Mattson et al., 2001), reduced the age-related accumulation of mutations. This provides some of the best evidences for a relationship between aging and somatic mutation.

Another theory is "telomere-hypothesis of aging". The proliferative life span of cells is measured in cell divisions, thus cells must process a molecular "clock" which counts the number of division times. In the telomere model of senescense (Harley, 1991), telomere's length acts as the counting mechanisms. A comparison between young and old human fibroblast telomere length showed that the length in the young cells was 10–20 kb whereas in the old donor cells it was 5–10 kb (Iwana, 1998). This observation has been interpreted so that the telomere lengths of somatic cells are reduced during aging due to the end-replication problem. Telomere shortening might play a major role in the aging of the immune system (Merker et al., 2001).

A theory that is very closely related to the "free radical theory" is the concept called "rate of aging hypothesis". This theory comes from the observation that species with higher metabolic rates have shorter maximum life span potential and age faster (Smeal & Guarente, 1997). The realization of energy consumption of mitochondria may result in superoxide radical production joining the free radical theory and the rate of living theory. A faster rate of respiration, associated with a greater generation of oxygen radicals, hastens aging (Merker et al., 2001).

Researchers have focused on two organ systems, the endocrine system and the immune system, in the investigation of the aging physiology. Scientists have known that the immune defense declines with age for a long time, and they try to explain the mechanisms of immune response. Recent studies have demonstrated that the age-related decline in T-cell-mediated immunity is a multifactor phenomenon affecting T-cell subset composition as well as several proximal events, such as protein tyrosine phosphorylation, generation of second messengers, calcium mobilization and translocation of protein kinase C; and distal events, such as lymphocyte proliferation and cytokine production of the T-cell activation pathway. Age-related T-cell immune deficiency is preceded by thymic involution and is influenced by several intrinsic as well as extrinsic factors. Further, the role of monocytes and macrophages in T-cell activation changes with advancing age (Chakravarti & Abraham, 1999). All dynamics of the relation between aging and immune system are not clear yet, but it is an important research focus for gerontologist. Also, another important point is the interaction of hormones and immune system. For example, dehydroepiandrosterone (DHEA) has been shown to revive immune response in aging. DHEA seems to be needed, for instance, to assist in the function and proliferation of immune cells. In the experiments in mice, DHEA-sulfate was shown to boost the levels of interleukine-2 in the older animals which is important in immune response (Daynes & Araneo, 1992). A study, in which the antioxidant properties of DHEA have been investigated, showed that a pre-treatment of rat primary mixed hippocampal cell cultures with DHEA protects against the toxicity induced by H_2O_2 and sodium nitroprusside. Moreover, DHEA was also able to prevent $H_2O_2/FeSO_4$-stimulated lipid peroxidation in both control and Alzheimer's disease hippocampal tissues (Bastianetto et al., 1999). It is thus suggested that DHEA may be useful in treating age-related central nervous system disease based on its protective effects (Bastianetto et al., 1999; Hu et al., 2000).

The chemical messengers of the body, hormones and other growth factors, are another study area for gerontologist to understand the causes of aging. We also know that when some declining hormones are replaced, various signs of aging diminish. For instance, growth hormone is still in the experimental stage but estrogen is used in medical practice to alleviate the discomforts of menopause. Estrogen replacement therapy is also used to prevent the accelerated bone loss that comes with menopause and may help prevent cardiovascular diseases. However, its protective and anti-aging effects have been investigated at the moment (Garcia-Segura et al., 2001). On the other hand, questions about cancer and other risks exist in estrogen replacement therapy and have not been resolved yet.

Oxygen radicals

Free radical theory of aging has been first proposed by Harman (1981). It was explained that the oxygen radicals are responsible for many degenerative changes that come with aging. Free radicals have been involved not only in aging but also in degenerative disorders, including cancer, atherosclero-

sis, cataracts and neurodegeneration (NORDBERG & ARNER, 2001).

Oxygen radicals are the product of normal oxidative metabolism and they are highly reactive molecules (STADTMAN & LEVINE, 2000). Several cytokines, growth factors, hormones and neurotransmitters use reactive oxygen species (ROS) as secondary messengers in the intracellular signal transduction (THANNICAL & FANBURG, 2000). Antioxidant system supplies main protection against to unstable and potential harmful molecules (HALIWEL & GUTTERRIDGE, 1990). It includes several enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (Gpx), catalase (CAT); and several nonenzymatic molecules, such as vitamins C and E and β-karoten. These molecules prevent mostly oxidative damage but not completely. Today it is commonly accepted that oxidative damage occurs little by little when we age, and is responsible for aging and several diseases (WICKENS, 2001). High levels of antioxidant, which long life organisms possess, have also been correlated with life span. Deficiencies in either the Cu, Zn-, or Mn-superoxide dismutases (SOD1 and SOD2) decrease yeast life span (BARKER et al., 1999). The common opinion of a gerontologist is that these high levels of antioxidant support a protection from oxidative damage during aging.

Glucose and glycosylation end products

Several researches have focused on glucose and its effects on the life span. In a process called non-enzymatic glycosylation or glycation, glucose molecules attack to proteins, setting in a chain of chemical reaction that ends in the protein's binding together or cross-linking, thus, their biological and structural roles alter. Glycosylation-end-products (AEGs) may be formed by oxidative and non-oxidative reactions, in some cases identical to advanced lipoxidation-end-products (ALEs) formed in protein during lipid peroxidation reactions. AEGs affect the biochemical and physical properties of proteins and the extracellular matrix, including the charge, hydrophobicity, turnover and elasticity of collagen, and the cell adhesion, permeability and pro-inflammatory properties of the extracellular matrix (BAYNES, 2001). Diabetes, in fact, is sometimes considered as an accelerated model of aging. These complications in diabetes also imitate the physiological changes that can accompany old age. Thus, many studies about cross-links have been focused on its relation between diabetes and aging. Body has its own defence system against cross-linking; these are immune system cells called macrophages. A number of scavenger and AEG-specific receptors have been identified that may mediate the turnover of AEG-proteins, catalyse the local production of reactive oxygen species and attract and activate tissue macrophages (BAYNES, 2001). The effects of glycosylation and its end products on aging interest gerontologists for several reasons. AEGs in proteins have been explained probably correlative, rather than causative, with respect to aging and they accumulate to high levels in tissues in age-related chronic diseases, such as atherosclerosis, diabetes, arthritis and neurodegenerative diseases. Moreover inhibition of AEG formation in these diseases may limit oxidative and inflammatory damage in tissues, retarding the progression of pathophysiology and improve the quality of life during aging (BAYNES, 2001).

Heat shock proteins

Heat shock proteins (HSPs), produced in response to stress, are important research focus for gerontologists because it declines with age (REA et al., 2001). Despite their name, heat shock proteins are produced when cells are exposed to various stresses, not only to heat. Their expression can be initiated by exposure to toxic substances, such as heavy metals, chemicals and even behavioural and psychological stress. Yet, the role of HSPs in the aging is not exactly clear. They are known to help the cell dissemblance, disposal of damaged proteins, facilitation of making and transporting of new proteins. But what proteins are involved and how they relate to aging is still unknown completely.

The action of HSP-70 in specific sites, such as adrenal cortex, was investigated. It was observed that in adrenal cortex, in blood vessels and in possibly other sites, the expression of HSP-70 appeared to be closely related to hormones released in response to stress, such as glucocorticoids and catecolamines (BLAKE et al., 1991). HSP-70 was reported to protect neurons against excitotoxic and oxidative injury (YU et al., 1999). It was demonstrated that the induction of HSP-70 mRNA levels by alveolar macrophages, adherent to plastic culture plates, decrease approximately 70% with age, and HSP-70 induction is greater in alveolar macrophages isolated from dietary restriction rats; this difference is observed statistically in young rats. The induction of HSP-70 by heat shock was also decreased with age in the adherent alveolar macrophages, and dietary restric-

tion increased the induction of HSP-70 expression three-to-fourfold in adherent alveolar macrophage in both young and old rats (MOORE et al., 1998).

Longevity genes

Scientist are isolating specific genes, cloning them, mapping them to chromosomes and studying their products to learn what they do and how they influence longevity. Life spans were extended with selective breeding or genetic engineering studies (ROSE, 1984, 1999). The antioxidant enzyme, SOD, can be thought to be involved as well. It was explained that the life span of *Drosophila* sp. can be significantly increased by over-expressing the antioxidant enzyme Cu/Zn SOD (PARKES et al., 1998).

Specific genes that increase life span were found first in the nematode *Caenorhabditis elegans* by JOHNSON (1987). Longevity genes in nematodes comprise: *age-1*, which encodes a phosphatidyl inositol-3' kinase and confers increased heat tolerance; *daf-2*, which encodes a tyrosine kinase (KIMURA et al., 1997); *daf-16*, which encodes a transcription factor (OGG et al., 1997). Daf-12 has three predicted isoforms of a member from the nuclear steroid/thyroid hormone receptor family (ANTEBI et al., 2000). Another gene, *tkr-1*, which encodes a tyrosine kinase receptor, increases longevity from 40% to 100% and increases resistance to heat and UV radiation (MURAKAMI & JOHNSON, 1998).

Researchers have reached several clues about aging and longevity in yeast that have some genetic similarities to human cells. JAZWINSKI (1999) has found *Lag-1* gene influencing the number of divisions in yeast; its product was a protein located in cell membrane. Yeast *Lag-1* has homologues in higher eukaryotes, including humans (JAZWINSKI, 2001), and it has been implicated in the transport of glycosylinositolphospholipid-anchored proteins from the endoplasmic reticulum to the Golgi (BARZ & WALTER, 1999).

The isolated genes are only a few but scientists think hundreds of longevity and aging-related genes might exist. The big question for many gerontologists is whether there are counterparts in people (human homologues) of the genes that were found in laboratory animals. Increasingly, gerontologists are also asking how alterations in the process of gene expression itself may affect aging. Some proteins, such as antioxidants, appear to prevent cells damage to cells, and others may repair damaged DNA or help cells respond to stress. Other gene products are thought to control cell senescence, a process that is proved to be a key piece in the puzzle of aging and longevity.

Telomeres and telomerase

Every chromosome has tails named telomeres at the end, and they get shorter as a cell divides. All the tails have the same, short sequence of DNA bases, repeated thousands of times (PARDUE & DE BARYSHE, 1999). Telomeres do not carry genetic information, so they can act as a disposable part. It has been considered that they may play a more active role or possibly these chromosome ends regulate cellular life span and aging in some way (KLAPPER et al., 2001).

Telomere research is another territory where cancer and aging study merge. In immortal cancer cells, telomeres act abnormally: they stop shrinking with each cell division. In the studies about this phenomenon, it was focused on an enzyme called telomerase, which is a reverse transcriptase. It has also been proposed to possess anti-aging properties (MATTSON, 2000). Elevated telomerase levels are not only found in almost all cancers (and at very low level in surrounding cells), but levels also frequently correlate with disease's progression and metastatic state as well (SANO et al., 1998). The precise role of telomerase in tumorigenesis is not fully elucidated although there is abundant evidence that enzyme plays a major role in cell proliferation in many cell types (BODNAR et al., 1998). It was also declared that the telomerase is a major target for cancer chemotherapy (NEIDLE, 2000). The telomerase protein catalytic subunit TERT was cloned in human cells and it was established that telomerase activation is a critical step in tumor progression (WRIGHT & SHAY, 2000).

Dietary restriction and aging

The researches of the aging physiology try to understand the characteristics of normal aging in the absence of disease. In these studies, behavioural factors, such as diet are also investigated.

It was declared that dietary restriction (DR) have modulated the life span and prolonged the mean and maximum life span (MATTSON et al., 2001). This has been shown in many organisms, including the yeast *S. cerevisiae* (LIN et al., 2000), the roundworm *C. elegans* (SZE et al., 2000), mice and rats (WEINDRUCH & SOHAL, 1997), and monkeys (LANE et al., 1999). DR has been shown to retard the decline of several physiological functions and delay the onset of some age-related pathologies (AKSENOVA, 1998). It was shown that DR

act at many levels; reduced free radical generation, increased many scavengers, and protected membranes (KRISTAL, 1994). These effects can be explained by the fact that mitochondia in DR animals had lower basal levels of lipid peroxidation, and generated less lipid peroxidation upon challenges and detoxified harmful lipid peroxidation by products at higher rates from *ad libitum* fed animals (KRISTAL & YU, 1998). In mammals, DR reduced the development of age-related cancers (RAFFOUL et al., 1999), cardiovascular diseases (MAEDA et al., 1985) and deficit in immune function (SPAULDING et al., 1997). Clinical and epidemiological studies of humans were entirely consistent with beneficial effects of DR. Overeating was demonstrated to increase the risk of many age-related disease in humans including cardiovascular diseases, diabetes and cancer (BROCHU et al., 2000). A possibility was indicated that DR improves mitochondrial respiration and therefore decreases free radical production, and it may decrease the rate of protein synthesis and in result may help to restore the level of active enzyme levels including those of the proteosome (AKSENOVA, 1998). The related factors about calorie intake were declared as: decreasing body temperature, altered metabolism, increasing metabolic efficiency, aberrant gene expression, changes in neuroendocrine or hormonal regulation, and decreased DNA damage or increased DNA repair (MERKER at al., 2001). More recently it has been shown that DR stimulates the production of growth factors and stress proteins that may increase the resistance of cell to the age-related diseases (MATTSON et al., 2001).

In conclusion, gerontology is a multidisciplinary area, which includes several sciences, and essentially we know that all studies serve to understand the biggest secret of nature: long life. The clues that we have at the moment are not enough to solve this secret entirely but at least will provide a qualified longevity for human being.

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