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Biology of Aging and Possibilities of Gerontomodulation

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After years of generating data describing age-related changes in organisms, organs, tissues, cells and macromolecules, biogerontologists are now able to construct general principles of aging and explore various possibilities of gerontomodulation. There is significant evidence to show that aging is characterized by a stochastic accumulation of molecular damage and a progressive failure of maintenance and repair, and the genes involved in homeodynamic pathways are the most likely candidate virtual gerontogenes. Several approaches are being tried and tested to modulate aging in a wide variety of organisms with the ultimate aim of improving the quality of human life in old age, and prolong their health-span. These approaches include gene therapy, hormonal supplementation, nutritional modulation and intervention by antioxidants and other molecules. A recent approach is that of applying hormesis in aging research and therapy, which is based on the principle of stimulation of maintenance and repair pathways by repeated exposure to mild stress.

Key Words: Age, Anti-aging, Homeostasis, Homeodynamics, Repair, Damage, Hormesis, Survival, Longevity

Introduction

Biogerontology, the study of the biological basis of aging, has now attained the status of being a fully matured field of scientific research. After decades of systematic collection of data describing age-related changes in organisms, organs, tissues, cells and macromolecules, biogerontologists are now in a position to construct general principles of aging and explore various possibilities of intervention using rational approaches. The highly complex phenomenon of aging is now reasonably well described and any missing details will be filled in sooner or later without really changing the overall picture that has emerged so far. There are several excellent books and monographs which provide comprehensive data and cross-references to original publications on age-related changes at all biological levels (see for example, Evans et al. 2000, Holliday 1995, Kanungo 1980, 1994, Masoro & Austad 2001, Rattan & Toussaint 1996).

The large body of published data clearly shows that aging has many facets. The progression or rate of aging is highly variable in different species, in organisms within a species, in organs and tissues within an organism, in cell types within a tissue, in sub-cellular compartments within a cell type, and in macromolecules within a cell (Rattan 2000a, b). Thus, there is neither a single way of defining aging, nor is there a single cause. Most importantly, these observations have led most biogerontologists to abandon the notion of aging being genetically programmed and to consider aging as being stochastic and individualistic (Hayflick 1994, 2000, Holliday 1995, 2000, Rattan 2000a).

Principles of Aging

Although the descriptive data about aging suggest that there are no universal markers of aging, some general principles can still be derived, which can be useful for future research and intervention. First, aging is considered to be an emergent phenomenon seen primarily in protected environments that allow survival beyond the natural lifespan in the wild. This is because most animals in the wild die due to accidents, infections and predation without showing

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significant signs of aging. Second, aging is the progressive failure of homeodynamics, which is the ability of all living systems to respond to internal and external stress, and to counteract by neutralization and/or by adaptation any disturbances threatening their survival (Rose 1997). A failure of homeodynamics leads to the impairment in functional ability at all levels of organization and increased possibilities of a plethora of diseases and eventual death. Third, unlike development, which is a highly programmed and well-coordinated genetic process in the evolutionary life history of an organism, there is no genetic program that determines the exact duration of survival of an organism.

The evolutionary theories of aging and longevity have developed sophisticated and convincing arguments against the existence of genes that may have evolved specifically to cause aging and to determine the lifespan of an organism (for a detailed analysis of evolutionary arguments, see Kirkwood & Austad 2000, Partridge 2001, Rose 1991). The role of genes in determining the duration of lifespan is primarily in terms of assuring what has been termed "essential lifespan" (ELS) of a species, defined as the time required to fulfill the Darwinian purpose of life, that is successful reproduction and continuation of generations (Rattan 2000 a,b). For example, species undergoing fast maturation and early onset of reproduction with large reproductive potential generally have a short ELS. In contrast, slow maturation, late onset of reproduction, and small reproductive potential of a species is concurrent with its long ELS (Finch 1990, 1998, Holliday 1994). Considered this way, the ELS for Drosophila is less than a week (Sørensen & Loeschcke 2002) and that for Homo sapiens is about 50 years (Holliday 1996a), even though in protected laboratory environments and in modern societies they can live for several months or for more than 120 years, respectively.

Therefore, genes that do influence longevity are those that have evolved in accordance with the life history of a species for assuring ELS. Such genes are termed longevity assurance genes (Jazwinski 1996) or vitagenes (Rattan 1998c). Several lines of evidence support the view that natural survival and longevity of a species is a function of maintenance and repair capacities. For example, positive correlations between species lifespan and the ability to repair DNA, to defend against reactive oxygen species, to respond and to counteract stress, and to proliferate and turnover the cells have been reported. In contrast, there is a negative correlation between longevity and the rate of damage accumulation, including mutations, epimutations, macromolecular oxidation and aggregation (for cross references to original publications, see Bürkle 2000, Holliday 1995, Levine 2002, Rattan 1989, 1995b, von Zglinicki et al. 2001). Thus, the manifestation of aging and the limits to lifespan are primarily due to the failure of maintenance and repair mechanisms.

Gerontogenes: Real or Virtual?

A lack of specific genes that cause aging does not imply that genes do not or cannot influence survival, longevity and the rate of aging. There is ample evidence from studies performed on yeast, fungi (Jazwinski 1999), nematodes (Johnson 2002, Johnson et al. 2000), insects (Rogina et al. 2000, Tatar et al. 2001), rodents and humans (Arking et al. 2002, Kuro-o et al. 1997, Yu et al. 1996) that mutations in certain genes can either prolong or shorten the lifespan, and can be the cause of premature aging syndromes. Interestingly, these genes cover a wide range of biochemical pathways, such as insulin metabolism, kinases and kinase receptors, transcription factors, DNA helicases, membrane glucosidases, GTP-binding protein coupled receptors, and cell cycle arrest pathways with little or no overlap among them (Guarente et al. 2000, Jazwinski 1999, Johnson 2002, Johnson et al. 2000, Martin & Oshima 2000, Rattan 2000b). Additionally, genetic linkage studies for longevity in mice have identified major histocompatibility complex (MHC) regions (Gelman et al. 1988), and quantitative trait loci on chromosomes 7, 10, 11, 12, 16, 18 and 19 (De Haan et al. 1998, Miller et al. 1998) as putative genes for aging. In human centenarians, certain alleles of HLA locus on chromosome 6 (Gelman et al. 1988), regions of chromosome 4 (Puca et al. 2001), different alleles of APO-E and APO-B, and DD genotype of angiotensin converting enzyme (ACE) have been linked to exceptional longevity (Frisoni et al. 2001, Heijmans et al. 2000, Perls 2001, Tan et al. 2001).

The diversity of the genes associated with aging and longevity of different organisms indicates that

whereas the genes involved in repair and maintenance pathways may be important from an evolutionary point of view, each species may also have additional "private" gerontogenic pathways which influence its aging phenotype (Martin 1997). Further evidence that the maintenance and repair pathways are crucial determinants of natural survival and longevity comes from experiments performed to retard aging and to increase the lifespan of organisms. For example, anti-aging and life-prolonging effects of calorie restriction are seen to be accompanied by the stimulation of various maintenance mechanisms. These include increased efficiency of DNA repair, increased fidelity of genetic information transfer, more efficient protein synthesis, more efficient protein degradation, more effective cell replacement and regeneration, improved cellular responsiveness, fortification of the immune system, and enhanced protection from free-radical- and oxidation-induced damage (Masoro 1995, Masoro & Austad 1996, Weindruch 1996, Yu 1999). Genetic selection of Drosophila for longer lifespan also appears to work mainly through an increase in the efficiency of maintenance mechanisms, such as antioxidation potential (Luckinbill & Foley 2000). An increase in lifespan of transgenic Drosophila containing extra copies of Cu-Zn superoxide dismutase (SOD) and catalase genes is due primarily to enhanced defenses against oxidative damage (Orr & Sohal 1994). The identification of long-lived mutants of the nematode Caenorhabditis elegans, involving various genes provides other examples that increased lifespan is accompanied by an increased resistance to oxidative damage, an increase in the activities of SOD and catalase enzymes, and an increase in thermotolerance (Lakowski & Hekimi 1996, Larsen 1993, Lithgow 1996, Lithgow et al. 1995). In contrast, reduced activity of the tumour suppressor defense gene p53 induces premature aging in mice (Tyner et al. 2002). A comparative analysis of oxidative stress resistance ability of cells isolated from a variety of animals also showed that species lifespan was directly related to the cellular antioxidative defense ability(Kapahi et al. 1999).

What is clear from the identification of the genes influencing aging and longevity is that whatever their normal function and mechanism of action may be, these gerontogenes did not evolve to specifically accumulate damage, to cause age-related changes and to kill the organism. Since their involvement in influencing aging and longevity cannot be denied, they have been termed "virtual gerontogenes" (Rattan 1995a).

Molecular Mechanisms of Aging

A generalized definition of aging as the failure of homeodynamics still requires mechanistic explanation(s) as to why such a failure occurs in the first place and what controls the rate of failure in different species. Over the last fifty years a large number of hypotheses have been put forward, which attempt to explain how the observed age-related changes in macromolecules, cells, tissues, organs and systems may occur. Main examples of such hypotheses include altered gene regulation (Kanungo 1980, 1994), somatic mutation accumulation (Morley 1995, Vijg 2000), protein errors and modifications (Holliday 1996b), reactive oxygen species and free radicals (Harman 1994), immune-remodeling and neuroendocrine dysfunctioning (Franceschi et al. 2000). At the cellular level, the so-called telomere loss theory (Harley et al.1992, Olovnikov 1996), and epimutation theory of progressive loss of DNA methylation (Holliday1995) are other examples of providing mechanistic explanations for the loss of proliferative potential of normal, differentiated and diploid cells *in vitro* and *in vivo*.

These and other related hypotheses, which provide a variety of explanations for understanding the observed age-related alterations at a specific level, can be quite useful within their area of focus. However, in order to answer the question why the occurrence of detrimental and eventually lethal changes cannot be avoided completely, one has to appeal to the evolutionary theories of aging and longevity, as discussed above. To recapitulate, the evolutionary life history of a species is what determines the extent of activity and stability of its maintenance and repair networks required for its ELS. For example, a nocturnal species can easily survive with less efficient repair mechanisms against UV-induced damage than a species exposed to the sunlight. Similarly, a species evolved to live at high altitude and low ambient oxygen concentration does not have to have as efficient antioxidative defense mechanisms as compared with species living in an

high oxygen-containing environment. A progressive failure of the network of maintenance and repair mechanisms in the period beyond ELS is purely a stochastic event, but within the constraints of the design of the network (Carnes & Olshansky 1997).

Several theoretical and mathematical models are being developed for understanding the interactive nature of the biological networks and trade-offs (Franceschi et al. 2000, Kowald & Kirkwood 1994, 1996). Recently, the reliability theory of aging and longevity about the inevitable failure of complex systems such as cells and organisms (Gavrilov & Gavrilova 2001) has reiterated the fundamental law that no process can be one-hundred-percent accurate one-hundred-percent of the time, and it is the interactive nature of genes, milieu and chance that effectively determines how long a system can survive. Therefore, in order to resolve the issue of widely varying rates of aging in nature, it is important to undertake comparative studies on various aspects of the aging process in a variety of organisms with widely differing life-history scenarios (Barja 2002). Only then a complete understanding of the mechanistic aspects of aging will be achieved and better methods of intervention could be developed.

Gerontomodulation

Unlike some other fields of research, it is integral to biogerontology that effective means of intervention are found, developed and applied for modulating human aging in order to prevent the onset of age-related diseases and improving the quality of life in old age. This is because, whatever its academic and intellectual importance, aging is a highly emotive and health issue for human beings. It has been argued that the experience of aging and age-related diseases may be one of the basis for the origin of human cultural aspects including religion and moral codes of conduct (Holliday 2001).

During the last one hundred years, progress in biomedicine and healthcare have resulted in a steady increase in human life expectancy throughout the world, primarily by minimizing childhood deaths. This has made the survival beyond the Darwinian ELS a reality for human beings in large numbers that were never seen before in human history. However, this increase in lifespan has not been accompanied by an improvement in health-span of the elderly who often go through a long period of physical and mental disability and disease before their ultimate demise. Therefore, gerontomodulation to maintain the functional ability or to slow down its loss is a challenging and a high priority social, political and economic issue throughout the world (Holliday 2000, Wilmoth 2000).

However, the history of anti-aging research and therapy is replete with fraud, pseudoscience and charlatanism, and has often given a bad name to the whole field. Claims for miraculous remedies and promises for extremely long lifespan are prevalent even today. Recently, highly critical analyses of such approaches have been made by biogerontologists with a view to educate and inform people about the science and non-sense of aging-intervention research (Olshansky & Carnes 2001, Olshansky et al. 2002).

While not giving serious consideration to the claims made by charlatans, it cannot be ignored that several researchers are making genuine attempts to test and develop various means of intervention for the prevention and treatment of age-related diseases, for regaining the functional abilities and for prolonging the lifespan of experimental organisms. Some of the main anti-aging approaches include supplementation with hormones including growth hormone (Wolfe 1998), dehydroepiandrosterone (DHEA) (Baulieu 1996), melatonin (Reiter 1995) and estrogen (Miller & Franklin 1999), and nutritional supplementation with synthetic and natural antioxidants in purified form or in extracts prepared from plant and animal sources. Although some of these approaches have been shown to have some clinical benefits in the treatment of some diseases in the elderly, none of these really modulate the aging process itself (Hayflick 2000, Olshansky et al. 2002).

Furthermore, claims for the benefits of intake of high doses of vitamins and various antioxidants and their supposed anti-aging and life-prolonging effects have very little scientific evidence to back them (Le Bourg 2001). Some experiments have been performed demonstrating the extension of lifespan of Drosophila by overexpression of superoxide dismutase and catalase genes (Orr & Sohal 1994), but the possibilities of a successful gene therapy for aging is considered as a mission impossible (Rattan 1997, 1998a). In contrast to this, nutritional modulation through calorie restriction has been shown to be an effective anti-aging and longevity extending

approach in rodents and monkeys, with possible applications to human beings (Lane et al. 2002, Le Bourg 2001, Masoro 2000, Roth et al. 2002).

Hormesis A recent approach in geronto-modulation is rooted in making use of the fundamental characteristic of living systems, the homeodynamic property of self-maintenance and repair, as discussed above. Since aging is characterized by a decrease in the adaptive abilities due to progressive failure of homeodynamics, it has been hypothesized that if cells and organisms are exposed to brief periods of stress so that their stress response-induced gene expression is upregulated and the related pathways of maintenance and repair are stimulated, one should observe some anti-aging and longevity-promoting effects. Such a phenomenon in which stimulatory responses to low doses of otherwise harmful conditions improve the functional ability of cells and organisms is known as hormesis.

Although the phenomenon of hormesis has been defined variously in different contexts (Calabrese & Baldwin 2000a, Parsons 2000), hormesis in aging is characterized by the beneficial effects resulting from the cellular responses to mild repeated stress (Rattan 2001). Stresses that have been reported to delay aging and prolong longevity in various systems (for example, yeast, Paramecium, Drosophila, nematodes, rodents and human cells) include temperature shock, irradiation, heavy metals, pro-oxidants, acetaldehyde, alcohols, hypergravity, exercise and calorie restriction (Calabrese & Baldwin 2000b, Le Bourg et al. 2000, Masoro 1998, 2000, Minois 2000, Neafsey 1990, Parsons 1989, Shama et al. 1998). Hormesis-like beneficial effects of chronic but mild undernutrition have also been observed for human beings (Raji et al. 1998). For example, it was reported that peripheral blood lymphocytes isolated from people with low body mass index, representing a group with natural intake of restricted calories, had higher DNA repair capacity and higher levels of DNA polymerase ?, which were also maintained during aging (Raji et al. 1998).

During the last few years, research done in our labs have also shown hormetic effects of repeated mild heat shock (RMHS) on human cells. Using RMHS regime of exposing serially passaged human fibroblasts to 41°C for 1 hr twice a week throughout their replicative lifespan in vitro, we have reported several beneficial anti-aging effects (Fonager et al. 2002, Rattan 1998b, Verbeke et al. 2000, 2001, Verbeke et al. 2002). These effects included a reduction in the accumulation of oxidized and glycoxidized proteins, an increase in the levels of various heat shock proteins (hsp70, hsc70 and hsp27), an increase in proteasomal activities, an increase in antioxidative abilities, and increased resistance to ethanol, hydrogen peroxide and UV-A irradiation (Fonager et al. 2002, Verbeke et al. 2001, Verbeke et al. 2002). An important aspect of these studies is the observation that anti-aging and beneficial effects of repeated mild heat shock on human cells were observed without inducing additional cell proliferation. It appears that the progression of cellular aging *in vitro* in terms of accumulation of molecular damage can be slowed down without escaping the regulatory mechanisms of cell cycle check-points.

Studies on the application of hormesis in aging research and therapy are only beginning to be performed and other chemical, physical and biological treatments need to be tested to unravel various pathways of maintenance and repair whose sustained activities improve the physiological performance and survival of cells and organisms. However, there are several issues that remain to be resolved before mild stress can be used as a tool to modulate aging and prevent the onset of age-related impairments and pathologies (Rattan 2001). Some of these issues are: (1) how to establish biochemical and molecular criteria for determining the hormetic levels for different stresses; (2) how to identify differences and similarities in stress response pathways initiated by different stressors; (3) how to quantify the extent of various stress responses; (4) how to determine the interactive and pleiotropic effects of various stress response pathways; (5) how to adjust the levels of mild stress for age-related changes in the sensitivity to stress; and (6) how to determine the biological and evolutionary costs of repeated exposure to stress.

Finally, it must be emphasized that the goal of research on aging is not to increase human longevity regardless of the consequences, but to increase active longevity free from disability and functional dependence. Although substantial information about the descriptive aspects of biological aging has

been gathered, effective means of gerontmodulation are still not in sight. The ultimate solution to the problem of aging and death requires unravelling the complex network of genes influencing aging and longevity, and complete understanding of all components of the milieu in which genes and gene products function.

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