CHAPTER 1

BIOLOGICAL CAUSES OF AGING AND AGE-RELATED DISEASES

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Abstract: Aging is a progressive accumulation of molecular damage in nucleic acids, proteins and lipids. The inefficiency and failure of maintenance, repair and turnover pathways is the main cause of age-related accumulation of damage, which is also the basis of all age-related diseases. Research in molecular gerontology is aimed at understanding the genetic and epigenetic regulation of molecular mechanisms at the levels of transcription, post-transcriptional processing, post-translational modifications, and interactions among various gene products. Concurrently, several approaches are being tried and tested to modulate aging. The ultimate aim of such studies is to improve the quality of human life in old age and prolong the health-span. Various gerontomodulatory approaches include gene therapy, hormonal supplementation, nutritional modulation and intervention by free radical scavengers 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. A combination of molecular, physiological and psychological modulatory approaches can be effective to prevent and/or treat various age-related diseases

Keywords: lifespan, survival, longevity, stress, hormesis, homeostasis, homeodynamics

1. INTRODUCTION

The significant increase in human life expectancy during the last three generations, achieved primarily by reducing birth-related parturient-deaths and infant-deaths, is however not matched by an equivalent improvement in the health-span in old age. As a biosocial issue, aging is the underlying basis of almost all major human diseases, such as atherosclerosis, cancer, cardiovascular defects, cataract, diabetes, dementia, macular degeneration, neurodegeneration, osteoporosis and sarcopenia.

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Although the optimal treatment of each and every disease, irrespective of age, is a social and moral necessity, preventing the onset of age-related diseases by intervening in the basic process of aging is the best solution for improving the quality of human life in old age.

Biogerontology, the study of the biological basis of aging, has so far unveiled mysteries of aging by describing age-related changes in organisms, organs, tissues, cells and macromolecules (Rattan). The large body of published data clearly shows that aging has many facets. Most significantly, the progression and 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. Thus, there is neither a single way of defining aging, nor is there a single cause. Furthermore, these observations have led most biogerontologists to abandon the notion of aging being genetically programmed and to consider it as being stochastic and individualistic. A combination of genes, environment and chance appear to determine the course and consequences of aging and the duration of survival of an individual (longevity) (Rattan and Clark, 2005).

2. 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 as an emergent phenomenon seen primarily in protected environments which allow survival beyond the natural lifespan in the wild. The natural lifespan of a species has also been termed "essential lifespan" (ELS) (Rattan, 2000), or the "warranty period" of a species (Carnes et al., 2003). ELS is defined as the time required to fulfil the Darwinian purpose of life, that is successful reproduction for the continuation of generations. 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. For example, the ELS of *Drosophila* is less than a week as compared with that of about 50 years of *Homo sapiens*, even though in protected environments (laboratories and modern societies), a large proportion of populations of both species can and do live for much longer than that. Therefore, the period of extended survival beyond ELS is also the period of aging.

Second, aging is characterized by a progressive accumulation of molecular damage in nucleic acids, proteins and lipids. The inefficiency and failure of maintenance, repair and turnover pathways is the main cause of age-related accumulation of damage. Since homeostasis or homeodynamic ability of a living system is primarily due to its maintenance and repair processes, it is the progressive failure of maintenance and repair mechanisms which is the universal biochemical basis of aging and age-related diseases (Holliday, 1995, 2000).

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 programme which determines the exact duration of survival of an organism. Furthermore, studies on establishing an association between genes and longevity have reported that the genetic heritability of variance in lifespan is less than 35% (Herskind et al., 1996; Finch and Tanzi, 1997; Korpelainen, 2000; Gudmundsson et al., 2000). 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 (Rose, 1991; Kirkwood andAustad, 2000; Gavrilov andGavrilova, 2001).

3. THE ROLE AND NATURE OF GERONTOGENES

Genes that do influence longevity are those that have evolved in accordance with the life history of a species for assuring ELS. Several lines of evidence support the view that natural survival and longevity of a species is a function of its 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 facilitate turnover of 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 (Holliday, 1995; Rattan, 1989; Rattan, 1995).

A lack of specific gerontogenes which 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 et al. , 2000; Johnson, 2002), insects (Rogina et al., 2000; Tatar et al., 2001), rodents and humans that mutations in certain genes can either prolong or shorten the lifespan, and are the cause of premature aging syndromes (Arking et al., 2002; Kuro-o et al., 1997; Yu et al., 1996; Martin andOshima, 2000). 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 (Rattan, 2000; Johnson, 2002; Martin andOshima, 2000; Warner, 2005).

Additionally, genetic linkage studies for longevity in mice have identified major histocompatibility complex (MHC) regions (Gelman et al., 1998), and quantitative trait loci on chromosomes 7, 10, 11, 12, 16, 18 and 19 (Miller et al., 1998; De Haan 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. Similarly, several other studies have been published reporting an association between human longevity and single nucleotide polymorphisms in a variety of genes, including heat shock response,

immune response, cholesterol metabolism and others (Altomare et al., 2003; Tan et al., 2001; Singh et al., 2004; Bessenyei et al., 2004; Atzmon et al., 2005).

The diversity of the genes associated with longevity of different organisms indicates that whereas the common or "public" genes such as those involved in repair and maintenance pathways may be important from an evolutionary point of view, each species may also have additional "private" or specific 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 caloric 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 andAustad, 1996; Yu, 1999; Weindruch, 1996). 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 and Foley, 2000). An increase in lifespan of transgenic *Drosophila* containing extra copies of Cu-Zn superoxide dismutase (SOD) and catalase genes appears to be due primarily to enhanced defenses against oxidative damage (Orr andSohal, 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 and Hekimi, 1996; Larsen, 1993; 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 accumulate damage specifically, to cause age-related changes and to kill the organism. Since their involvement in influencing aging and longevity is also a biological fact, such genes have been termed "virtual gerontogenes" (Rattan, 1995, 1998). "Post-reproductive genetics" is another term used in order to explain different biological roles played at different ages by the same genetic variants (Franceschi et al., 2005).

4. MOLECULAR MECHANISMS OF AGING

A generalised 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,

researchers have proposed a large number of hypotheses 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, 1994), somatic mutation accumulation (Morley, 1995; Vijg, 2000), protein errors and modifications (Holliday, 1996), 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 (Holliday, 1995) 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.

Several theoretical and mathematical models are being developed in order to understand the interactive nature of the biological networks and trade-offs (Franceschi et al., 2000; Kowald and Kirkwood, 1996) Recently, the reliability theory of aging and longevity about the inevitable failure of complex systems such as cells and organisms (Gavrilov andGavrilova, 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, 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. Only then a complete understanding of the mechanistic aspects of aging will be achieved and better methods of intervention could be developed.

5. AGING AND AGE-RELATED DISEASES: THERAPY OR PREVENTION?

Unlike some other fields of research, it is central 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. According to the three principles of aging and longevity described above, having the bodies that we have developed after millions of years of evolution, occurrence of aging in the period beyond ELS, and the onset of one or more diseases before eventual death appear to be the “normal” sequence of events. This viewpoint makes modulation of aging different from the treatment of one or more specific diseases. In the case of a disease, such as a cancer of any specific kind, its therapy will, ideally, mean the removal and elimination of the cancer cells and

restoration of the affected organ/tissue to its original disease-free state. What will be the "treatment" of aging and to what original "age-free" stage one would hope to be restored – to day 1, year 1, 10, 30, 50 or what? Considering aging as a disease and then trying to cure that disease is unscientific and misguided. Similarly, although piecemeal replacement of non-functional or half-functional body parts with natural or synthetic parts made of more durable material may provide a temporary solution to the problems of age-related impairments, it does not modulate the underlying aging process as such.

Scientific and rational anti-aging strategies aim to slow down aging, to prevent and/or delay the physiological decline, and to regain lost functional abilities. 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 (Boia, 2004). 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 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, dehydroepiandrosterone (DHEA), melatonin and estrogen, and nutritional supplementation with synthetic and natural antioxidants in purified form or in extracts prepared from plant and animal sources (Rattan, 2003; Ferrari, 2004). 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 (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, 2005).

In contrast to this, nutritional modulation through caloric restriction (CR) has been shown to be an effective anti-aging and longevity extending approach in rodents and monkeys, with possible applications to human beings (Roth et al., 2004). But, this is a highly debatable issue at present both in terms of the practicalities of defining CR and of applying CR in human beings in physiological and evolutionary contexts (Demetrius, 2004).

Some studies have reported an extension of lifespan of experimental animals by gene manipulation. For example, overexpression of superoxide dismutase and catalase genes and of heat shock protein (hsp) genes have resulted in the increase in average lifespan in *Drosophila* and nematodes, respectively (Orr andSohal, 1994; Yokoyama et al., 2002). Such a gene-therapy approach for gerontomodulation requires redesigning the blueprint for structural and functional units of the body at

the level of genes, gene products, macromolecular interactions, molecular-milieu interactions, and so on. Considering how little information and knowledge we have at present about all those interacting variants of genes, molecules, milieu and chance, it is not clear what this approach really means in practical and achievable terms. Similarly, although piecemeal replacement of non-functional or half-functional body parts with natural or synthetic parts made of more durable material may provide a temporary solution to the problems of age-related impairments, it does not modulate the underlying aging process as such.

5.1 Hormesis

In a more realistic and near-future scenario, a promising approach in aging intervention and prevention is based in making use of an organism's intrinsic homeodynamic property of self maintenance and repair. 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 anti-aging and longevity-promoting effects. Such a phenomenon in which stimulatory responses to low doses of otherwise harmful conditions improve health and enhance lifespan is known as hormesis.

Although the phenomenon of hormesis has been defined variously in different contexts, for example in toxicology, pharmacology and radiation biology (Calabrese and Baldwin, 2000; Parsons, 2000), hormesis in aging is characterized by the beneficial effects resulting from the cellular responses to mild repeated stress (Rattan, 2001). The paradigm of hormesis is moderate exercise which is well known to have numerous beneficial effects despite it being a generator of free radicals, acids, and other damaging effects (McArdle et al., 2002).

During the last few years, research done in our labs has shown hormetic effects of mild stress. We have demonstrated the hormetic effects of repeated mild stress (RMS) on human cells undergoing aging in culture. Using a mild stress regime of exposing human skin fibroblasts to 41°C for 1 hr twice a week throughout their replicative lifespan *in vitro*, several beneficial and anti-aging effects have been observed (Rattan et al., 2004). It is important to note that whereas several age-related alterations, such as accumulation of oxidized proteins, levels of various hsp, proteasome activities, and stress resistance, were affected by RMS, there was no change in the proliferative behaviour of cells. This has implications in separating the phenomenon of aging from longevity. It appears that the progression of cellular aging *in vitro* as the increased molecular disorder can be slowed down without upsetting the regulatory mechanisms of cell cycle arrest (Rattan et al., 2004; Rattan et al., 2003). Thus the quality of life of the cell in terms of its structural and functional integrity can be improved without pushing these cells in to potentially carcinogenic hyperproliferative mode.

Other chemical, physical and biological treatments can be used to unravel various pathways of maintenance and repair whose sustained activities improve the physiological performance and survival of cells and organisms. Stresses that have been reported to delay aging and prolong longevity in various systems (for example, yeast, *Drosophila*, nematodes, rodents and human cells) include temperature shock, irradiation (UV-, gamma- and X-rays), heavy metals, pro-oxidants, acetaldehyde, alcohols, hypergravity, exercise and CR (Minois, 2000; Hercus et al., 2003; Rattan, 2004). Hormesis-like beneficial effects of chronic but mild undernutrition have been reported 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 food calories, had higher DNA repair capacity and higher levels of DNA polymerase <??>, which were also maintained during aging (Raji et al., 1998). Intermittent fasting has been reported to have beneficial effects on glucose metabolism and neuronal resistance to injury (Anson et al., 2003).

Although at present there are only a few studies performed which utilize mild stress as a modulator of aging and longevity, hormesis can be a useful experimental approach in biogerontology. 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. Some of these issues are: (1) to establish biochemical and molecular criteria for determining the hormetic levels for different stresses; (2) to identify differences and similarities in stress response pathways initiated by different stressors; (3) to quantify the extent of various stress responses; (4) to determine the interactive and pleiotropic effects of various stress response pathways; (5) to adjust the levels of mild stress for age-related changes in the sensitivity to stress; (6) to determine the biological and evolutionary costs of repeated exposure to stress; and (7) to determine the biological significance of relatively small hormetic effects, which may or may not have large beneficial effects during the entire lifespan. Resolution of these issues requires much more research on hormesis than being carried out at present.

The proof of the hormetic principle has now been provided by experiments with a wide variety of biological systems and by using a range of physical, chemical and biological stressors. Two of the main lifestyle interventions, exercise and reduced food intake, both of which bring their beneficial and anti-aging effects through hormesis (McArdle et al., 2002; Singh, 2002; Masoro, 1998, 2000; Yu and Chung, 2001), are being widely recognized and increasingly practiced as an effective means of achieving a healthy old age.

One can also expect the availability of certain nutriceutical and pharmacological hormetic agents to mimic the HS response and CR. For example, bimoclomal, a nontoxic, hydroxylamine derivative with hsp-inducing activity and cytoprotective effects is under Phase II clinical trials (Vigh et al., 1997; Vigh et al., 1998). Celastrol, a quinone methide triterpene which is an active component of certain Chinese medicinal herbs is another hsp-inducing hormetic agent under test for its cytoprotective effects (Westerheide et al., 2004). Curcumin, an Indian yellow spice,

has also been shown to have cytoprotective effects through its hormetic action in stimulating the synthesis of hsp (Dunsmore et al., 2001). Similarly, various chemical mimetics of CR, such as 2-deoxy-D-glucose and its analogues (Lane et al., 2002), and resveratrol, which is a polyphenol found in red wine, are being tested for their use as anti-aging hormetic agents (**?**Lamming et al., 2004; Wood et al., 2004).

Another small molecule, N^6-furfuryladenine or kinetin, has been shown to have significant anti-aging (Rattan and Clark, 1994; Rattan, 2002), and anti-thrombotic (Hsiao et al., 2003) effects in human cells. Kinetin is considered to work both as a direct antioxidant (Olsen et al., 1999; Verbeke et al., 2000), and as a hormetic agent by inducing the synthesis of other protective enzymes and hsp (Rattan, 2002; Barciszewski et al., 1999; Holmes-Davis et al. , 2001). Although at present the use of kinetin has been limited to being a cosmeceutical ingredient in a range of cosmetics products, its usefulness as a hormetic nutriceutical agent is under investigation.

In the consideration of irradiation as a hormetic agent, epidemiologic studies of the public, medical cohorts, and occupational workers confirm that low doses of radiation are associated with reduced mortality from all causes, decreased cancer mortality, and reduced mutation load observed in aging and cancer (Pollycove and Feinendegen, 2001). Increasing use of low-dose total body irradiation as an immunotherapy for cancer (Safwat, 2000) also has its basis in hormesis, which, in the not-so-distant future, will be developed into a safe and preventive strategy against a variety of age-related diseases. Hormesis through mental challenge and through mind-concentrating meditational techniques (Bierhaus et al., 2003; De Nicolas, 1998; Kyriazis, 2003) may be useful in stimulating inter- and intra-cellular debris-removal processes, and thus preventing the neuronal loss that leads to the onset of age-related neurodegenerative diseases.

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. Healthy old age is an achievable goal that however requires significantly more research support and efforts in biogerontology.

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Chapter-01

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