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Biogerontology: research status, challenges and opportunities

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Summary. Biogerontology is the study of the biological basis of ageing and age-related diseases. The phenomenon and the process of ageing are well understood in evolutionary and biological terms; and a conceptual framework has been established within which general principles of ageing and longevity can be formulated. The phenotype of ageing in terms of progressive loss of physical function and fitness is best seen during the period of survival after the evolution-determined essential lifespan (ELS) of a species. However, the ageing phenotype is highly heterogenous and individualistic at all levels from the whole body to the molecular one. Most significantly, the process and the progression of ageing are not determined by any specific gerontogenes. Ageing is the result of imperfect maintenance and repair systems that allow a progressive shrinkage of the homeodynamic space of an individual. The challenge is to develop and apply wholistic approaches to the complex trait of ageing for maintaining and/or improving health. One such approach is that of mild stress-induced physiological hormesis by physical, mental and nutritional hormetins. Biogerontological research offers numerous opportunities for developing evidence-based novel biomedical technologies for maintaining and improving health, for preventing the onset of age-related diseases, and for extending the health-span. (www.actabiomedica.it)

Key words: gerontogenes; health-span; homeostasis; homeodynamics, hormetin; longevity

Introduction

Biological ageing is no longer a mysterious, ununderstood and unresolved problem in biology (1, 2); and the science of biological ageing – biogerontology – is firmly rooted in its data-driven conceptual framework. The three pillars of biogerontology's foundation are:

- In the continuum of life, ageing starts after the end of the natural lifespan of a species, termed 'essential lifespan' (ELS), and is characterized by a progressive loss of physical function and fitness that culminates in death of an individual (3-5).
- There is neither a rigid programme nor any gerontogenes that have evolved with the specific role of causing ageing and death of an individual (6-8).
- The progression, rate and phenotype of ageing is different in different species, in individuals within a species, in organs and tissues within an organism, in cell types within a tissue, in subcellular compartments within a cell type, and in macromolecules within a cell (7, 9, 10).

Thus biological ageing is an emergent, epigenetic and a meta-phenomenon, which is not controlled by a single mechanism or a central regulator. Individually no tissue, organ or system becomes functionally totally exhausted until the death of a very old organism, it is the dynamic interaction and interdependence at all biological levels that determines the quality and the duration of life of an individual. Longevity-correlation

analyses performed on the data for the lifespan variance among siblings, and monozygotic and dizygotic twins indicates that the contribution of genes to the lifespan of an individual is about 25% (11). This means that non-genetic, epigenetic and environmental factors have more than 75% influence in determining the length of lifespan of an individual. This also implies that ageing, health-span and lifespan are not pre-determined and can be modulated.

The aim of this article is to take status of the biogerontological understanding of ageing and longevity, and to address the remaining research questions and challenges, along with the ongoing efforts and future opportunities for ageing interventions.

Evolutionary understanding of life and death

Sooner or later, all individuals die out even though the apparent immortality of a population or of the germ line may overshadow the mortality of its individual members. In nature, a vast variety in strategies for survival can be encountered and the spiral of life and death has innumerable variations. Rates of degenerative changes fall into three main categories – rapid, negligible and gradual, and these can explain most types of life histories that culminate in the death of an individual (12). The third category, found most commonly in animals, involves the growth and development of the organisms to adulthood and a period of reproduction followed by gradual and progressive ageing and senescence leading to death. Generally, species with repetitively reproducing (iteroparous) life histories experience ageing after completing a period of reproductive fitness. It is in this category of organisms, which includes human beings, that the phenomenon of progressive, intrinsic, and impairing ageing (13) is best manifested during the limited lifespan of the organism; and it is this kind of ageing which is the main focus of biogerontological studies.

Previously, it was generally believed that there is a species-specific maximum lifespan. However, this belief has frequently been challenged on the basis of both demographic-statistical analyses and experimental studies with very large cohorts of insect populations (14-16). In the case of human beings too, attempts at estimating the upper limits to human lifespan have failed to reach any definite conclusion (17-21). There are several genetic and non-genetic correlates of human lifespan, including parental and grand-parental lifespan, age of parents at the time of birth, reproductive history, marital and educational status, and other factors (22). Therefore, a concept such as "species-specific maximum lifespan" is of not much value when no reliable estimate of maximum achievable lifespan by an individual can be made.

Another way of talking about the lifespan is in terms of evolution. The evolutionary forces of natural selection have resulted in evolving mechanisms of maintenance that operate in concert with the complete structural (anatomical) and functional (physiological) design of the organism and assure certain period of survival of the body until reproduction. This duration has been termed "essential lifespan", (ELS) of a species (3). ELS can be considered as the natural lifespan of a species as "required" by evolution, and is distinct (and usually several-fold shorter) from the average lifespan for a cohort, and from the maximum lifespan observed for a single member of a species. For example, ELS for human species is considered to be about 50 years (23), whereas the average lifespan in economically developed countries is already between 80 and 85 years, and the maximum lifespan for a human being, recorded so far, is 122 years, 5 months and 14 days (24).

The studies discussed above show precisely that, whereas no absolute limit to longevity can be inferred from the data, there is still a practical limit to lifespan, and no fly could realistically attain longevity characteristics comparable to, say, those of mouse or man. Furthermore, such a measure of the practical limit makes room for the possibility of alteration in maximum achievable lifespan with changing conditions of life, which, in the case of human beings, include social, psychological and cultural elements.

Genetics and epigenetics of ageing

In the context of evolution, it is incorrect to assume that ageing and limited lifespan of an individual had some purpose or adaptive significance in terms of being advantageous for the species. In natural wild

populations the probability of death by accidental causes, including disease and predation, is so high that there is never a significant number of long-lived individuals left that might require special mechanisms to terminate life for the sake of newly born individuals. Even if there were any life-terminating mechanisms that operated after a long period of survival, these would not be capable of resisting the spontaneous origin and evolution of non-ageing and immortal "mutants", which in a given population would soon take over (25).

In contrast to the adaptive theories of the evolution of ageing and lifespan, the non-adaptive theories state that ageing occurs either because natural selection is insufficient to prevent it, owing to its post-reproductive nature, or that senescence is a by-product of the expression of genes with early beneficial traits but deleterious and pleiotropic effects at later stages. Two major schools of thought (whose ideas are not mutually exclusive) in the non-adaptive theories of the evolution of ageing and lifespan are represented by antagonistic pleiotropy theory (26) and the disposable soma theory based on the Weismann's distinction between the soma and the germ line (27). According to these theories, evolutionary forces have optimised conditions for efficient and successful reproduction either by (i) selecting for "good" early genes that later have "bad" effects, or (ii) selecting for efficient maintenance and repair of the germ cells at the cost of somatic maintenance.

As regards the nature of genes involved in determining or regulating ageing and lifespan, a lot of effort has been put in to discover such genes, termed gerontogenes (28). Although evolutionary theories of ageing and longevity discount the notions of an adaptive nature of ageing and the diversity of the forms and variations in which age-related alterations are manifested suggest that the progression of ageing is neither programmed nor deterministic, there appears to be a genetic component of some kind. The role of genes in ageing is indicated by: (1) an apparent limit to lifespan within a species (19, 29); (2) some heritability of lifespan as evident from studies on twins (30); (3) human genetic mutants of premature ageing syndromes (31, 32); and (4) some gene association with extreme longevity (33).

The paradoxical situation of the genetic aspects of ageing and longevity on one hand, and the stochastic nature of the progression of the ageing phenotype on the other, can be resolved by developing radically novel views about the nature of gerontogenes. The proposed term gerontogenes does not refer to any real genes, which have evolved specifically to cause ageing; and that is why the modified term "virtual gerontogenes" is more appropriate, and it reflects the altered state of other genes, giving the appearance of being the genes for ageing (6). This notion of virtual genes also applies to several so-called disease-causing genes. For example, the Werner gene, which is considered to "cause" the premature ageing syndrome, is in reality a DNA helicase gene whose normal role in DNA replication and repair prevents the emergence of Werner's syndrome, and it is only when this gene is altered by mutation that the disease phenotype emerges (34). The same applies to most of the so-called oncogenes, which are cancer-causing only when they are mutated and cannot perform their normal function (35).

Two kinds of gene action are postulated to be responsible for the emergence of the ageing phenotype. The first considers the role of late-acting mutations, which are already present at the time of fertilization and birth, and show their deleterious effects after the period of growth, development and maturation (36, 37). The second category of gene action is referred to as the antagonistic pleiotropic genes, which involves genes selected for some beneficial effects during early development but which have harmful effects in post-reproductive life when they escape the force of natural selection (25). In both cases, these genes were not selected as the real genes that cause ageing, but manifest themselves as virtual gerontogenes owing to their eventual involvement in the progression of age-related changes (8).

There is a large body of evidence showing that the maintenance and repair pathways are one of the main determinants of ELS. Such evidence comes from comparative studies performed on species with widely varying lifespans, and from experiments performed to slow down ageing and prolong the lifespan. Such genes are referred to as the longevity assurance genes (LAG) or vitagenes that determine the ELS of a species (38). These longevity assurance genetic pathways include

the efficiency of deoxyribonucleic acid (DNA) repair (39, 40), the fidelity of genetic information transfer (41), the efficiency of protein degradation (42), the extent of cellular responsiveness to stress (43), and the capacity to protect from damage induced by free radicals and oxidation (44).

The diversity of the genes associated with ageing and longevity of different organisms indicates that at the molecular level there are no universal pathways affecting ageing and longevity. Whereas the genes involved in repair and maintenance pathways may be important from an evolutionary point of view as the longevity assurance genes, each species may also have additional gerontogenic pathways which influence its ageing phenotype. Such genetic pathways have been termed as public and private pathways, respectively (45).

In addition to the genetic aspects of ageing and longevity, there is a lot of interest in unraveling the epigenetic aspects of ageing (46, 47). This is because although genes are the foundation of life, genes in themselves are non-functional entities. It is the wide variety of gene products, including coding and non-coding RNAs, proteins and other macromolecules, which constitute the biochemical and biophysical milieu for life to exist. Epigenetics is the most commonly used broad term to explain the consequences of the intracellular and extracellular milieu, which establish and influence the structural and functional stability of genes. These epigenetic effects and alterations generally remain uninherited from one generation to the next, but have strong deterministic effects on the health, survival and ageing of the individual.

Various intracellular epigenetic markers include methylated cytosines, oxidatively modified nucleotides, alternatively spliced RNAs, and post-translationally modified proteins, including protein folding (48). The full spectrum of epigenetics of ageing is yet to be unraveled and at present it is one of the most attractive and challenging areas of research in biogerontology (49-51). A major reason for the apparent difficulties in fully understanding the epigenetics of ageing is the existence of several orders higher complexity and diversity of the constituting components, such as physical, chemical, biological and environmental factors, including psychological factors in human beings. Furthermore, a lot of epigenetic modifications can occur and even become reversed on a daily basis depending on several lifestyle factors (52, 53).

Ageing as the shrinkage of the homeodynamic space

Living systems have the intrinsic ability to respond, to counteract and to adapt to the external and internal sources of disturbance. This is what makes them different from the inorganic and non-living systems. The traditional conceptual model to describe this ability is homeostasis, which, however, is not totally correct. The main reason for the incompleteness of the homeostasis model is its notion of "stability through constancy", which does not take into account the dynamic nature of information and interaction networks that underlie the complexity of the biological systems. Instead of homeostasis, the term homeodynamics encompasses the fact that, unlike machines, the internal milieu of biological systems is not permanently fixed, is not at equilibrium, and is a dynamic regulation and interaction among various levels of organization (54).

The property of homeodynamics of the living systems is based in a wide range of maintenance and repair systems (MARS) at all levels of organization. Some of the main MARS are: nuclear and mitochondrial DNA repair; anti-oxidative enzymes and free radical scavengers; degradation of damaged DNA, RNA, proteins and other organelles; apoptosis; detoxification of harmful chemicals and metabolites; Immune responses; wound healing and tissue regeneration, and other higher order processes such as thermal regulation, neuroendocrine balance, and circadian rhythms.

All these processes involve hundreds of survival-assurance genes whose products and their interactions give rise to a "homeodynamic space", which is the ultimate determinant of an individual's chance and ability to survive and maintain a health (9, 55). Ageing, age-related diseases and eventual death are the result of a failure of homeodynamics. This fact is also reflected in the definition of ageing as a progressive shrinkage of the homeodynamic space (9, 55).

At the molecular level, the theories of the mechanisms of ageing are mostly centered on the occurrence and accumulation of molecular damage (55-57). Some

other views, such as continuous growth leading to a kind of quasi-programme of ageing (58), and entropy are also put forward (59). An age-related increase in the levels of damage in various macromolecules, including DNA, RNA, proteins, carbohydrates and lipids is well established (9, 55, 60). Therefore, the occurrence and accumulation of molecular damage as the basis of age-related failure of homeodynamics is considered as a unified explanation for biological ageing (55, 57).

The biological consequences of increased levels of molecular damage are wide ranging (57), and include altered gene expression, genomic instability, mutations, molecular heterogeneity, loss of cell division potential, cell death, impaired intercellular communication, tissue disorganization, organ dysfunctions, and increased vulnerability to stress and other sources of disturbance (57). What is not clear at present is the relationship between the extent of a molecular damage and its physiological and functional consequences. How much damage a cell, tissue and an organism tolerate or compensate without becoming harmful; and how much damage a system needs to repair or remove in order to regain health, functionality and extended heal-span, are the two most challenging basic questions to be resolved in molecular biogerontology.

Interventional approaches and opportunities

Biogerontolology has revealed that ageing is an emergent phenotype due to the failure of homeodynamics and not due to the action of any life-limiting and death-causing mechanisms. Therefore, this understanding should transform our approach towards ageing interventions from being "anti-ageing" in the sense of reversion and rejuvenation, to maintaining health, preventing age-related diseases and achieving "healthy ageing". However, such a shift towards ageing interventions is yet to happen universally.

One of the most prevalent biomedical approaches to ageing intervention is what one may call as the piecemeal remedies. The basic principle behind this approach is to "fix what is broke"; and this ranges from cosmetics to the tissue/organ repair or transplantation, targeted treatments with stem cells, and rejuvenation with young blood/plasma transfusion (61-63). More recently, elimination of senescent cells by potential senolytic compounds is becoming an increasingly appealing approach (64-67). Although such interventions often have life-saving effects in acute situations, these benefits are often transient, limited and require recurring interventions.

The second most common ageing interventional approach is that of replenishing the loss, tested mostly in animal model systems. This approach is often based on the naïve understanding that age-related decline in the levels of enzymes, hormones or other metabolites is always harmful, and that these changes should be reset to some normal, youthful and healthy levels. Biogerontological studies have, however, repeatedly shown that numerous age-induced changes in the immune system, hormone levels and other proteins and enzymes are the sign of constant remodeling and adaptation for survival and health (68, 69). For example, experimental studies on the extension of lifespan of various model systems by genetic and non-genetic means clearly show that a reduction in the levels of various hormones and their intermediates and receptors is almost always a requirement (70). Therefore, unnecessary supplementation with hormones, antioxidants and other such nutritional replenishments have little, none or even harmful effects in normal healthy model systems and in humans (71-74).

Biogerontologists are increasingly realizing that "single molecule, single target"-oriented approaches for ageing intervention are severely limited because these neglect the highly dynamic, interactive and networking nature of life. Therefore, whole body level holistic or more accurately "wholistic", (in order to distinguish science-based approaches from the "everything goes" holistic claims) approaches are being tested and developed as promising ageing interventions. One such wholistic interventionary approach is that of hormesis that encompasses food, physical activity and mental engagement, which strengthen the homeodynamic space (75, 76).

Hormesis in health maintenance and improvement is defined as the life-supporting beneficial effects resulting from the cellular and organismic responses to repeated and transient exposure to mild stress (77-79). Moderate physical exercise is the paradigm for

stress-induced hormesis, which initially increases the production of free radicals, acids and aldehydes. Other stressors that have been reported to modulate ageing in cells and animals include heat shock, irradiation, heavy metals, pro-oxidants, acetaldehyde, alcohols, hypergravity, polyphenols, flavonoids, terpinoids, infections, and dietary restriction, including intermittent fasting (77-80). An important observation in studies of hormesis is that a single stressor, such as heat shock or exercise, can strengthen the overall homeodynamics and enhance other abilities, such as immune response, robustness, resilience, cognition and memory, by initiating a cascade of processes resulting in a biological amplification and eventual beneficial effects (81-83).

All such conditions, which bring about biologically beneficial effects by initially causing low level stress, are termed as hormetins (84-86). Hormetins are further categorized as: (1) physical hormetins, such as physical exercise, heat and radiation; (2) biological and nutritional hormetins, such as micronutrients, phytochemicals in spices and other natural and synthetic food sources; and (3) psychological or mental hormetins, such as increased brain activity through cognitive games and challenges, including solving puzzles, social engagement, focused attention and meditation (87-89).

It should also be pointed out that several so-called anti-oxidants, including numerous plant components, some vitamins, and micronutrients are actually stress-inducing hormetins, and that their biological effects as being antioxidative are not due to the compounds themselves being direct antioxidants (90-97). Discovering novel hormetins as modulators of ageing and longevity is a promising area of research offering numerous opportunities in the aesthetic-, healthcare- and food-industry (98, 99).

Another experimental ageing interventional approach being tested is that of so-called gene therapy. One of the earlier experimental studies demonstrated that an induced mutation in a single gene increased the lifespan of the nematode *C. elegans* (100, 101). Since then hundreds of putative gerontogenes or longevity genes have been reported in *C. elegans*, *Drosophila* and rodents, which when mutated result in the extension of average and maximum lifespan of the organism. The methods used for the identification of such genes include induction of mutations and deletions by irradiation and chemical mutagens, alterations in gene expression by knockout, homologus recombination, or by gene addition, and reduction in gene expression by RNAi-induced abrogation of translation (for the latest information on such genes, refer to various online databases, such as: http://genomics.senescence.info/genes/) (33).

It is important to realize that in almost all such cases longevity extension had occurred when one or multiple interventions resulted in the reduction or total inhibition of the activity of one or more genes. Similarly, there are other examples which show that several mutant mice strains with defects in growth hormone (GH) pathways including deficiencies of GH levels and GH receptor have extended lifespans (102-104). Application of RNAi technology, together with the role of circulating, and small noncoding RNAs, has further identified numerous genes whose normal levels of activities are lifespan restricting, and can be a target for gene therapy.

Studies have also been performed in which the effects of adding one or multiple copies of genes, that leads to the increased expression of their gene products, has resulted in the extension of lifespan. Some such transgenic manipulations in model systems include the addition of gene(s) for one of the protein elongation factors (105), antioxidant genes superoxide dismutase and catalase (106-109) sirtuin (110), forkhead trascription factor FOXO (111), heat shock proteins (112-114) heat shock factor, (115, 116), protein repair methyltransferase (117), and klotho, which is an inhibitor of insulin and IGF1 signalling (118). Although theses studies have demonstrated longevity-extending effects of various genes in controlled laboratory conditions, there is very little information available on the basic process of ageing in terms of the rate and extent of occurrence and accumulation of macromolecular damage and its physiological consequences in these animals. There is also little information available as to what is the physiological price paid for inactivating such genes whose normal function is a part of the general metabolism and signaling (119, 120). For example, laboratory-protected longevity mutants in *C. elegans* have reduced Darwinian fitness when competing with the wild type worms under nutritionally challenging

conditions (121-123). Similarly, extension of murine lifespan by the addition of *klotho* gene induces insulin resistance and disruption of insulin/IGF-1 signalling pathway (119, 120, 124, 125).

Another system in which genetic interventions have been tried as ageing interventions is the Hayflick system of limited proliferative lifespan of normal diploid differentiated cells in culture (126). Almost all the genetic interventions by transient or permanent transfection and ectopic expression of various genes on this model system have focused on extending the replicative lifespan of cells by bypassing the cell cycle check-points (127-129). One of the most widely used genetic interventions in extending the replicative lifespan of normal cells has been the ectopic expression of telomerase in a wide variety of cells (130, 131). However, continuous proliferation by such genetically modified non-ageing cells often leads to their genomic instability, transformation and cancer-forming activity (132, 133). In the case of animals, whereas telomerase negative mice show reduced lifespan and some other abnormalities after six-generations (134), overexpression of telomerase in the skin increases myc-induced hyperplasia (135) without any extension of lifespan.

In the case of humans, although several single gene mutations are known which lead to accelerated ageing and significantly reduced lifespan (32, 136), no gene mutations have yet been identified which increase the human lifespan. A strategy that has been used extensively to identify potential longevity genes is by gene-association analysis of genetic polymorphisms with human longevity (137). The full list of genes associated with human longevity, generally identified by both single nucleotide polymorphism (SNP) analysis or by genome wide association studies (GWAS) can be retrieved from http://genomics.senescence.info/genes/. To what extent this information can be used to develop gene-based ageing interventions in humans is not yet clear.

Some future scenarios for ageing interventions include intelligent redesigning either by the so-called strategies for engineered negligible senescence (SENS) (138), or by post-humanistic or trans-humanistic enhancements through robots and cyborgs combining both organic and biomechatronic body parts (139). Such interventions, if successful, raise several ethical issues such as the social and environmental consequences of extreme longevity, and the basic understanding of what it means to be human (140, 141)

Conclusions

According to the principles of ageing and longevity discussed above occurrence of biological ageing is inevitable owing to the imperfections of survival mechanisms. Whereas optimal treatment of each and every disease, irrespective of age, is a social and moral necessity, maintaining health and improving the quality of human life in old age require a shift in approach from ageing as a disease to ageing as a life condition that can be modulated. Ageing must be approached as a stage in life history of an individual, which is served best by biomedical, technological and social interventions, which could diminish the severity of age-related frailty, along with a possible extension of health-span.

Biogerontologists are beginning to narrow down the potential ageing pathways, including insulin/IGF-1 growth axis, mTOR activity, and stress resistance, which could be amenable to manipulation (33, 142). There is evidence that those and other metabolic pathways can be effectively modulated by life-style alterations, such as intermittent food restriction, exercise and nutritional and pharmacological interventions (74). However, one major challenge still is to translate the information gathered from studies performed on experimental model systems of insects, nematodes, rodents and others to human beings.

Another challenge for biogerontologists trying to develop effective means of ageing intervention is to come out of the reductionistic mode of doing experiments. The history of ageing intervention research has shown that taking this or that single compound of natural or synthetic origin, force-feeding it to some experimental model system, and analysing one or few molecular targets has, so far, not lead to any really useful practical interventions for human beings. The three pillars of health – food, physical activity, and mental and social engagement – require a change in the way biogerontologists design and perform experiments. And most importantly, biogerontologists also need to be clear as to what is the ultimate aim of such research:

is it to eliminate ageing and death for ever, and should we do that?

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