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REVIEW

Progress and prospects: Gene therapy in aging

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Studies performed on various experimental model systems indicate that genetic interventions can increase longevity, even if in a highly protected laboratory condition. Generally, such interventions required partial or complete switching off of the gene and inhibiting the activity of its gene products, which normally have other well-defined roles in metabolic processes. Overexpression of some genes, such as stress response and antioxidant genes, in some model systems also extends their longevity. Such genetic interventions may not be easily applicable to humans without knowing their effects on human growth, development, maturation, reproduction and other characteristics. Studies on the association of single nucleotide polymorphisms and multiple polymorphisms (haplotype) in genes with human longevity have identified several genes whose frequencies increase or decrease with age. Whether genetic redesigning can be achieved in the wake of numerous and complex epigenetic factors that effectively determine the life course and the life span of an individual still appears to be a 'mission impossible'.

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In brief

Progress

- Biological basis of aging are well understood.
- Prevention is the most rational approach against aging.
- Mutations causing a loss of gene function increase life span in model systems.
- Overexpression of some genes increases life span in model systems.
- Human life span is associated with polymorphisms in genes.
- Several issues concerning gene therapy for extended longevity remain to be resolved.
- Gene therapy in aging also involves epigenetic interventions including stem cells.

Prospects

- The nature and the number of genes and their variants involved in determining the rate of aging and the life span potential of organisms will be determined.
- Experimental model systems will continue to have their use in identifying evolutionarily conserved longevity assuring or gerontogenic pathways; but there will also be human-specific gerontogenic pathways, which remain to be identified.
- The complexity of the epigenetic factors that influence the polygenic traits aging and longevity will be elucidated.
- The realization that aging at the molecular level is a progressive increase in molecular heterogeneity leading to interrupted, incomplete and illegitimate macromolecular interacting networks, will greatly determine what kind of interventions can be feasible or not.
- Gene therapy in aging will require both the prevention of loss of gene expression and the inhibition of gene expression in the right cell types at the right time.
- Understanding the trade-offs between efficient growth, development, maturation and reproduction on the one side and the extended survival of the body beyond the reproductive age on the other will determine the success and failure of potential antiaging interventions.

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Introduction

The inevitable consequence of aging is death. Whatever the duration of 'old age' before the final demise may be, this period is generally fraught with increasing chances

of occurrence and emergence of one or more diseases. Aging is the underlying cause of almost all major human diseases, such as atherosclerosis, cancer, cardiovascular defects, cataract, diabetes-2, dementia, macular degeneration, neurodegeneration, osteoporosis and excessive muscle loss leading to sarcopenia.[1,2] Although the optimal treatments for 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 may be the ideal solution for maintaining and/or improving the quality of human life and its dignity in old age. As genes are the fundamental units of information for biological processes, a simplistic view is that gene therapy can be the absolute antiaging treatment. While some people have suggested that gene therapy and other interventions for aging will be soon able to achieve exceptionally long and healthy life spans, others have judged such pronouncements as the extreme arrogance of antiaging medicine![3]

In this study, we review and evaluate the scientific basis for developing potential therapies and modes of intervention in the process of aging, which may or may not involve tinkering with genes. To fully appreciate these arguments, we first provide a brief overview of the biological understanding of aging, which is considered to be no longer an unresolved problem in biology.[4,5]

Biological basis of aging are well understood

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. The large body of descriptive data has led two of the pioneers of modern biogerontology, Leonard Hayflick and Robin Holliday, to declare that aging is no longer an unsolved problem in biology.[4,5] Their bold assertion underlines that biological basis of aging are well understood and a distinctive framework has been established, which can be the basis for translational research and interventions toward achieving a healthy old age. This framework depicts aging as an emergent, epigenetic and a metaphenomenon, which is not controlled by a single mechanism. A combination of genes, milieu and chance determine the course and consequences of aging and the duration of survival of an individual.[1]

There is much supporting evidence that shows that survival and longevity of a species are a function of the ability of its maintenance and repair mechanisms to keep up with damage and wear and tear. The complex processes of maintenance and repair involve hundreds of genes whose products and their interactions give rise to a 'homeodynamic space', or 'buffering capacity', which is the ultimate determinant of an individual's chance and ability to survive and maintain a healthy state.[6,7] Table 1 gives a list of the key molecular pathways and processes operating in cells, which are quintessential components of the homeodynamic machinery. Theoretically, a fully functional and well-maintained homeodynamics could make a biological system immortal.[8] However, a progressive shrinkage of the homeodynamic space is the universal characteristic of aging.[6,7]

Table 1 Main molecular pathways of maintenance and repair, which comprise the homeodynamic space

1. Antioxidative and enzymic defences against reactive oxygen species
2. Stress response
3. Protein repair and chaperoning
4. Removal and turnover of defective proteins and other cellular components
5. Nucleic acid repair

Prevention is the most rational approach against aging

Rational antiaging strategies based on scientific evidence aim to slow down the aging process by preventing and/or delaying physiological decline and regaining lost functional abilities. Such approaches may be either short-term dealing with one body part at a time or long-term interventions against the process of aging itself. Such short-term interventions include organ replacement, stem cell injections and external supplementations of hormones, nutriceuticals and micronutrients. Although some of these therapies have shown some clinical benefits in alleviating problems associated with severe deficiencies in old age, none of these temporary interventions really act on and modulate the aging process.[9–12] Long-term or near permanent prevention of aging will require effective gene-based interventions to increase the homeodynamic space, and to prevent or reduce the rate of its shrinkage that occurs because of the accumulation of unrepaired molecular damage.

Mutations causing a loss of gene function increase life span in model systems

It is almost 20 years ago that the first gene mutation resulting in the extended life span of an organism was identified as the so-called age-1 mutant in the nematode, *Caenorhabditis elegans*. Since then a large number of putative gerontogenes or longevity genes have been reported in yeast, *C. elegans*, *Drosophila* and rodents, which, when experimentally mutated to lose function, result in the extension of average and maximum life span of the organism. The methods used for the identification of such genes include the induction of mutations and deletions by irradiation and chemical mutagens, alterations in gene expression by knockout, by homologous recombination and reduction in gene expression by RNAi-induced abrogation of translation.[13–15]

It is important to note that in these studies, an extension of life span occurred when one or multiple interventions resulted in the reduction or total inhibition of the activity of one or more genes. Some of the main pathways whose 'loss-of-function' is shown to associate with extended period of survival are (i) energy generation and utilization in mitochondrial respiratory chain; (ii) nutrition and hormonal sensing and signaling including insulin/insulin-like growth factor-1 (IGF-1) and its target forkhead transcription factor FOXO, transcriptional silencing by sirtuin-mediated histone deacetylase and (iii) translational interference through target of rapamycin, which is a kinase that integrates

signals from nutrients and growth factors, and regulates cell growth and the progression of cell cycle.[16–18] Similarly, several mutant mice strains with defects in growth hormone (GH) pathways, including deficiencies of GH levels and GH receptor, have extended life spans.[19,20] In *C. elegans*, two of the main genes in which induction of mutation(s) leading to the loss-of-function results in the modulation of life span are IGF-1 tyrosine kinase receptor *daf-2* and forkhead transcription factor *daf-16*, which appear to work antagonistically. For example, whereas a loss of *daf-2* activity extends longevity, the loss of *daf-16* suppresses that effect. Further comparative analyses of such genetic mutants in worms, flies and rodents indicate that the pathway of insulin/IGF-1 signaling may be a key regulator of longevity.[19,20] Table 2 gives a selective list of genes whose manipulation extends the life span of animal model systems including nematodes, insects and rodents. For the latest informa-

Table 2 A selective list of putative gerontogenes whose manipulation extends life span in animal model systems[a]

Organism	*Gene name (symbol)*	*Normal function*	*Genetic intervention*	*Effect on life span*
Caenorhabditis elegans	Aging alteration (*age-1*)	Phosphoinositide-3-kinase; a central component of insulin like signaling pathway; lying downstream of daf-2 and upstream of daf-16	Mutation	Loss-of-function; a recessive allele of age-1 increases life span up to 100%; mutants have a 40–65% increase in the mean life span and a 65–110% increase in the maximum life span
	Abnormal dauer Formation daf-2 (*daf-2*)	Insulin-/Insulin-like growth factor-1 (IGF-1) tyrosine kinase receptor; regulator of dauer formation	Mutation	Loss-of-function; increase in life span by more than 100%; life span extension requires the activity of daf-16
	Abnormal dauer Formation daf-16 (*daf-16*)	Forkhead transcription factor; acts in insulin-mediated pathway to affect dauer formation	Mutation	Suppresses life extension caused by mutations in daf-2
			Overexpression	Modestly increases life span (~20%)
	Tachykinin receptor family (*tkr-1* or *old-1*)	Tyrosine kinase receptor	Overexpression	Increases resistance to environmental stress and extends life span up to 65%
	Eating: abnormal pharyngeal pumping (*eat-2*)	Nicotinic acetylcholine receptor subunit	Mutation	Loss-of-function; extends life span up to 20–30%
	Coenzyme Q7 homolog (*clk-1*)	Required for ubiquinone biosynthesis	Mutation	Loss-of-function; increases adult life span by 30%; *clk-1* and *daf-2* mutants have fivefold increased life span as compared to the wild type
	Caffeine-induced death protein (*cid-1*)	Checkpoint protein	Mutation and RNAi inhibition	Extends life span up to 20%
	hsp70-F	Stress protein	Overexpression	Extends life span up to 45%
	Heat–shock transcription factor 1 (*hsf-1*)	Transcriptional factor regulating heat–shock response	Overexpression	Extends life span up to 22%
Drosophila	Insulin receptor substrate-1 (*Chico*)	Insulin receptor substrate that functions in an insulin/IGF signaling pathway	Mutation	Loss-of-function; extends fruit fly median life span up to 48% in homozygotes and 36% in heterozygotes
	Superoxide dismutase (*Sod1*)	Encodes the oxygen radical metabolizing enzyme CuZn superoxide dismutase (SOD1)	Overexpression	Overexpression of a single gene, in a single cell type, the motorneuron, extends life span up to 40%
	Superoxide dismutase 2 (*Sod2*)	SOD2 encodes a mitochondrial manganese superoxide dismutase	Overexpression	Overexpression in motor neurons extends life span up to 30%
	Protein-L-isoaspartate (D-aspartate) O-methyltransferase (*Pcmt*)	Protein carboxyl methyltransferase important for repair of abnormal protein aspartyl residues	Overexpression	Extends life span up to 35%
	Methuselah (*Mth*)	Member of the seven transmembrane domain (7-TM) protein superfamily; homology to several guanosine triphosphate-binding 7-TM protein-coupled receptors	Mutation	35% increase in average life span and enhanced resistance to various forms of stressors

Table 2 Continued

Organism	*Gene name (symbol)*	*Normal function*	*Genetic intervention*	*Effect on life span*
	I am not dead yet protein (*Indy*)	Sodium-dependant citrate transporter; homologous to mammalian sodium dicarboxylate cotransporter.	Mutation	Five independent P-element insertional mutations in a single gene resulted in a near doubling of the average adult life span
	Insulin-like 1 receptor (*InR*)	Insulin/IGF receptor; regulates cell growth and proliferation through the dP13K/dAkt pathway	Mutation	Loss-of-function; yields dwarf females with up to 85% extension of adult longevity, and dwarf males with reduced late age-specific mortality
	Heat–shock protein 70 (*Hsp70*)	Heat–shock response chaperone	Overexpression	Extends life span up to 20–30%
	Forkhead box, subgroup O (*dFoxo*)	Transcriptional regulator	Overexpression	Increases life span and decreases fecundity
Rodents	Prophet of Pit-1 (*Prop1*)	Transcription factor involved in hormonal regulation and development	Knockout	Loss-of-function; yields dwarves who live approximately 1 year longer than controls. Homozygous $Prop1^{df/df}$ (Ames dwarf) male and female mice have 49 and 68% increase in life span, respectively
	POU domain, class 1, transcription factor-1 (*Pit-1*)	Pituitary specific transcription factor. Pit-1 is required for normal development of the anterior pituitary	Knockout	Loss-of-function; homozygous $Pit\text{-}1^{dw/dw}$ (Snell dwarf) mice show a 42% increase in mean life span
	Growth hormone releasing hormone receptor (*Ghrhr*)	Growth hormone releasing hormone receptor	Knockout	$Ghrhr^{lit/lit}$ mice have a 20% increase in life span
	SHC (Src homology 2 domain containing) transforming protein 1 ($Shc1/p66^{shc}$)	Regulation of intracellular redox levels, signal transduction and apoptosis	Knockout	30% increase in life span
	Klotho (*Kl*)	Calcium metabolism involved in the suppression of several aging phenotypes	Mutation	Reduced life span that resembles premature aging in humans
	Transformation-related protein 53 (*Trp53*)	Tumor suppressor/DNA-binding transcription factor important for apoptosis	Mutation	Partial deletion of gene shows signs of premature aging and reduces life span
	Growth hormone receptor (*Ghr*)	Growth hormone receptor/ growth hormone-binding protein	Knockout	Loss-of-function; mice homozygous for disruption of Ghr have a life span that is 40–50% longer than wild type
	IGF-1 receptor (*IGF-1r*)	IGF; homologous of tyrosine kinase receptors InR and DAF-2	Knockout	$IGF\text{-}1r^{(+/-)}$, heterozygous knockout mice live on average 26% longer than their wild type

[a]For cross references, refer to online databases: http://genomics.senescence.info/genes/longevity.html, http://wormbase.org/db/misc/site_map?format=searches, http://sageke.sciencemag.org/index.dtl.

tion on such genes identified in a variety of model systems and for cross references, one should refer to various online databases, for example, http://genomics.senescence.info/genes/longevity.html, http://wormbase.org/db/misc/site_map?format=searches and http://sageke.sciencemag.org/index.dtl.

Overexpression of some genes increases life span in model systems

Studies have also been performed in which the effects of adding one or multiple copies of various genes, that leads to the increased expression of their gene products, has resulted in the extension of life span in model systems worms, fruitflies, rodents and cultured cells. Some such transgenic manipulations include the addition of gene(s) antioxidant genes superoxide dismutase and catalase, NAD^+-dependent histone deacetylases sirtuins, forkhead transcription factor FOXO, heat–shock proteins (HSP), heat–shock factor, protein repair methyltransferases and klotho, which is an inhibitor of insulin and IGF-1 signaling.[19–21] Another system in which genetic interventions have been tested is the Hayflick system of limited proliferative life span of normal diploid differentiated cells in culture.[22] 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 life span of cells by bypassing the cell cycle check points.[23,24] One of the most widely used genetic interventions in extending indefinitely the replicative life span of normal cells has been the ectopic expression of telomerase in a wide variety of cells. However, continuous proliferation by such genetically modified cells often leads to their genomic instability, transformation and cancer-forming activity when injected *in vivo*.[23,24] In the case of animals, whereas telomerase-negative mice show reduced life span and some other abnormalities after six generations, overexpression of telomerase in the skin increases myc-induced hyperplasia[25] without any extension of life span. Thus, it appears that genetic interventions to bypass the Hayflick limit of restricted proliferative potential of normal cells may lead to carcinogenesis.

Although theses studies have shown longevity-extending effects of various genes in controlled laboratory conditions, there is very little information available on the basic process of aging in terms of the rate and extent of occurrence and accumulation of macromolecular damage and its physiological consequences in these animals. There is also almost no 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. There is some evidence that laboratory-protected longevity mutants in *C. elegans* have reduced Darwinian fitness when competing with the wild-type worms under nutritionally challenging conditions.[26,27] Similarly, klotho-induced insulin resistance and the paradox of the insulin/IGF-1 signaling pathways in longevity extension seriously question the practicality of such gene manipulations in humans.[26,28]

Human life span is associated with polymorphisms in genes

The evidence that genes do influence, to some extent, the life span in human beings has mainly come from the epidemiological studies performed on centenarians, their siblings and their children, on monozygotic and dizygotic twins and on clusters of long-living families.

By analyzing the variation in life spans of a cohort of people born about 100 years ago, the value of the genetic determinant of longevity is calculated to be around 25%.[29] Furthermore, using a genealogical approach, a clustering of families with long life spans has been reported in Sardinia, Italy, which also indicates the genetic influences on longevity.[30] On the other hand, several single gene mutations are known which lead to an exaggerated phenotype of accelerated aging, early onset of various age-related disorders and significantly reduced life span (for example, Werner's syndrome and progeria).[31]

Another powerful approach that has been developed and adopted for identifying virtual gerontogenes or longevity genes in human beings is by studying variations in single nucleotide polymorphisms in candidate genes with human longevity.[32] Most of the data available currently on the genetics of human longevity come from the candidate gene approach, measuring the frequencies of different alleles, genotypes and haplotypes between old cases and young controls.[32,33] Although polymorphisms in more than 80 genes have been reported which are associated with human longevity in various populations internationally, no reproducible association, except for the apolipoprotein-E gene involved in the cholesterol metabolism, has been found across the globe.[29,34] Some other genes whose allelic variations have been shown to associate with long life span are components of the immune system and the human leukocyte antigen region. These include the human leukocyte antigen haplotypes, mitochondrial haplotypes, inflammation pathway genes and cytokines.[29,32,35]

Some of the genes originally identified in animal model systems for their longevity-promoting effects in gene transfer experiments have been studied for their association with human longevity. These genes are human homolog of *Drosophila* gene *Indy*, superoxide dismuatases 1 and 2, *GH1, IGF-1* receptor, interleukin-6, sirtuin Sir2 homolog *SIRT3*, but not *SIRT1*, tumor suppressor protein *p53, HSP70*, forkhead transcription factor *FOXO*, and a functional variant of *KLOTHO*.[29,32,35–37] Recently, network of genes involved in cellular and molecular repair and defense mechanisms, such as DNA repair genes and HSP genes, have been analyzed for their association with human longevity. Whereas no association of seven polymorphisms in four DNA repair genes with longevity was observed,[38] association of *HSP* gene variants with longevity have been reported.[39]

At present, a serious limitation of gene-association studies with longevity is the lack of data for the relevance at the level of physiological or cellular function. It is not clear as to what extent variations in the frequency of occurrence of certain alleles in long-lived individuals translate into the amount, activity and other parameters of molecular function of their respective gene products. However, such analyses are now beginning to be performed, and one of the first studies to do so reported an association of reduced heat–shock response in human mononuclear cells with CC genotype of *HSP70-1* gene.[39,40] This gene was also reported to be negatively associated with survival and longevity in Danish long-lived individuals including centenarians.[39,40] Therefore, it is very important that polymorphic variations in genes are also studied for their functional effects at the level of gene products and other biological markers. This is extremely important for developing any potential gene therapeutic interventions in terms of which alleles are most effective and desirable.

Several issues concerning gene therapy for extended longevity remain to be resolved

Longevity is a polygenic trait, and at present, there is little information available about how many genes and their variants determine the potential longevity of a species or that of an individual. One of the reasons for this is that, until now, the vast majority of studies in the field have focused on individual genes/proteins, without adequately addressing the possible role of interactions between them. The system-biology approaches for the

analysis of the links between longevity-associated genes and genes involved in age-related diseases are being developed,[41,42] which will be necessary to select potential targets of intervention.

Considering that the molecular cause of aging is the progressive accumulation of macromolecular damage and increased molecular heterogeneity,[7] there are at least three major targets for antiaging genetic interventions: (1) increasing the repair of damaged macromolecules (for example, DNA repair pathways), (2) increasing the removal of damaged macromolecules (for example, proteasomal and lysosomal pathways) and (3) decreasing the source of damaging agents (for example, reactive oxygen species, other free radicals and reactive sugar metabolites). Whereas the first two targets basically imply achieving genetic enhancement or genetic improvement, the third target requires resetting the metabolic pathways. Potential 'master genes' in the third category may be the genes involved in nutrition and hormonal sensing and signaling including insulin/IGF-1 and its target forkhead transcription factor FOXO and transcriptional silencing by sirtuin-mediated histone deacetylase, manipulation of which may affect a large number of other genes.

Although there are several approaches in development for gene-based 'intelligent redesigning' for the enhancement of physical strength, endurance, appearance and memory, there are serious technical limitations and ethical and safety concerns that remain to be resolved.[43] Chances of such an 'intelligently redesigned' and directed evolution to succeed in competition with the Darwinian natural selection from much larger random variations and combinations are practically none.

Gene therapy in aging also involves epigenetic interventions including stem cells

The next level of complexity for gene manipulation is the epigenetics, which is the sum total of interactions of genes and the milieu in which genes happen to operate.[1] Both the 'regulated epigenetics', such as 5-methylation of cytosines, histone code through acetylation and noncoding RNAs[44] and 'stochastic epigenetics', such as numerous modifications of DNA and RNA nucleotides and of proteins,[45–47] effectively determine the success and failure of a gene action. Some of the major factors comprising epigenetic modulators are prenatal maternal health, nutritional and hormonal status, and postnatal access to nutrition and exposure to viruses, bacteria and other germs, especially until and around the age of puberty.[17,48–51]

Therefore, an ideal strategy for the prevention or modulation of aging for extended health span incorporates genetic and epigenetic interventions. Defective or inefficient genetic pathways, which either lead to the emergence of specific diseases or significantly enhance the chances of a disease surely require successful application of the main stream gene therapy approaches including stem cells. However, treating one or more age-related diseases by stem cells is only a short-term treatment, which is overshadowed by the systemic aging of the whole body, and requires repeated interventions.[52,53] Another approach in epigenetic intervention in aging is by targetting the homeodynamic space by repeated challenge through mild stress, termed hormesis.[54]

Prospects

Gene therapy or interventions in aging are, both in principle and in qualitative terms, different from the more familiar approaches of gene therapy against one or more diseases. Aging and longevity are polygenic traits for which neither the number of genes involved, their variants and the extent of interactions are known, nor is the complexity of the epigenetic factors that influence these traits are elucidated at present. Aging at the molecular level is a progressive increase in molecular heterogeneity leading to interrupted, incomplete and illegitimate macromolecular interacting networks,[6,7] whose exact nature and implications are yet to be understood.

Yet, studies performed on various experimental model systems do indicate that aging and longevity are amenable to modulation, even if in a highly protected laboratory condition. Even in those cases, the elements of epigenetic and chance events often determine the quality and duration of life span.[55] Furthermore, majority of the putative longevity genes identified so far in model systems require to be switched off, or their activities to be highly reduced to extend longevity. However, all these genes are part and parcel of normal metabolic processes evolved for normal growth, development and survival. Similarly, other genes whose enhanced expression by genetic interventions leads to extended longevity are also poorly understood for the evolutionary trade-off and for the metabolic price to be paid for tinkering with them. Although genetic interventions for slowing down aging and extending health span and longevity may be technically a relatively less problematic issue, epigenetic modulators of aging and longevity bring forth a level of complexity yet to be comprehended.

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