REVIEW

The premature ageing syndromes: insights into the ageing process

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Introduction

Ageing in man results from a complex interaction of genetic and environmental factors. Many overlapping and sometimes conflicting theories of ageing exist and the emergence of a unified theory still seems unlikely. Experimental studies of senescence are difficult to design because of the variable effect of diseases and other extrinsic factors.

The syndromes of accelerated ageing have been proposed as models to simplify the analysis of the ageing process, by restricting the focus to a more definable area [1]. Research in this field may give an insight into the nature of the genes that play a role in ageing and help to separate the boundary between ageing and age-related disease. Furthermore, the association of various cancers and vascular pathologies in many of these conditions has allowed a better understanding of the genetic or intrinsic components of these important causes of premature death.

Several rare conditions exist in man that exhibit certain phenotypic characteristics associated with senescence. Often referred to as 'segmental progeroid syndromes'[2], the most widely studied of these are Hutchinson-Gilford progeria, Werner's syndrome and Cockayne's syndrome, but the group also includes Bloom's syndrome, ataxia telangiectasia and Down's syndrome. Studies of these syndromes have focused on genetic and cytochemical analysis as well as descriptive approaches to tissue and organ changes. Recent identification of the gene responsible for Werner's syndrome has attracted widespread interest, and rapid developments in our understanding of the cytochemical basis of these diseases can be expected. Whilst Japan and the USA lead in terms of research output relating to these syndromes, European studies also make an important contribution. A recent Medline search revealed that between 1991 and 1995, the UK was the origin of one in five publications relating to the premature ageing syndromes within Europe.

This report is not intended to comprehensively review all aspects of these complex and fascinating conditions but rather to demonstrate the relevance of their study to the ageing process.

Theories of 'normal' ageing

It is pertinent first to outline briefly current concepts of senescence. As our knowledge base has grown, we have learnt that certain processes, such as osteoporosis, which were once seen as inevitable consequences of intrinsic ageing, have several external risk factors which may be modifiable in many cases [3]. In attempting to define ageing, it is not possible to ignore the fact that older people do actually suffer from diseases and not just simple physiological inadequacy. Consequently, ageing may be regarded as a progressive decline in physiological response to stress, which would include physical and chemical insults, infections, tumours and trauma.

Excellent attempts at classifying the many theories of ageing have been made, such as that by Medvedev [4] (see Table 1). Evolutionary theories were established from the observation of species-specific maximal life spans. Most geneticists argue that the senescent phenotype is non-adaptive [5]; in other words, that ageing emerges as a by-product of gene action or because the effects of natural selection decline with age. Examples include Williams' antagonistic pleiotropic genes [6], in which genes which have a beneficial effect when expressed early in life may have a deleterious effect when expressed late in life, and Kirkwood's 'disposable soma' theory [7], where

Table 1. Theories of ageing

Type of theory	Explanation
Genetic programme	Linked to development and morphogenesis; study of the genetic control over the rate of ageing and lifespan
Primary damage	Examples include free radical theory, error catastrophe theory, somatic mutation theory
Evolutionary	Designed to explain wide range of species-species variation in lifespan
Homeostatic failure by organ systems	Examples include glucocorticoid cascade hypothesis, immunological theory of ageing, neuroendocrine immune theory
Age changes	Involve studies of changes occurring throughout the whole lifespan, often involving deterioration of an anatomical/physiological nature

Adapted from Medvedev (Biol Rev 1990; 65: 375-398) [4] and Sweeney and Weiss (Gerontology 1992; 38: 139-52) [25].

somatic maintenance is optimized to the expected lifespan of the species. Martin argues that there are likely to be several thousand genes that play a role in the senescent phenotype, although it is probable that relatively few of these have a major impact [2]. Support for genetic variability in longevity comes from the observation that late-egg-laying *Drosophila* have a lifespan that is a mean 20 days more than early-laying flies [8, 9]. Therefore, understanding the role of single gene mutations on the ageing process, from study of conditions such as Werner's syndrome, could yield valuable information.

The role of oxidants in senescence was originally proposed by Harman in 1956 [10] and, subsequently, processes such as damage to DNA bases, mutagenesis and lipid peroxidation have been shown to ensue from free radical attack [11]. Oxidative damage is a continuous process in all human tissues and may be ameliorated by a variety of repair mechanisms, such as the enzyme superoxide dismutase. The effectiveness of the free radical repair system is therefore likely to be of importance in determining the aged phenotype.

Research in cellular ageing and Werner's syndrome has often focused on the lifespan of cells (usually fibroblasts) *in vitro*, to determine the classic 'Hayflick limit' first described in 1961 [12], showing that cells have only a limited capacity to replicate *in vitro*. The mechanisms for this, such as loss of length of telomeres (the repetitive DNA sequences located at the ends of linear chromosomes) have been highlighted in an excellent review by Hayflick himself [13].

In most tissues, cells are continually turning over and therefore only have a relatively short residence time, potentially limiting any damage that may result from processes such as endogenous oxidation. However Hayflick also demonstrated that older cells have fewer replicative cycles than younger cells [14], which would increase their residence time and potentially permit greater oxidative damage. Presumably stem cells, believed to exist in most tissues and the non-dividing post-mitotic cells, such as neurons, muscle cells and fat cells may be the most susceptible to this type of

damage. Cell culture techniques have limited observations to mainly one cell type and studies are of disproportionately long duration. However, the absence of endocrine and other regulatory influences renders cells autonomous, making it possible to form correlations between biochemical defects and postulated ageing processes without these external influences.

Clinical features of the premature ageing syndromes

Table 2 lists the main characteristics of a selection of progeroid syndromes, some of which are marked by clinical heterogeneity.

Progeria is a rare genetic disease, originally described by Johnathan Hutchinson in 1886 and Hastings Gilford in 1904, having a birth incidence of approximately 1 in 8 million and probably caused by a sporadic dominant mutation of unknown origin [1], although there is still some doubt about this. Affected individuals show several characteristics of accelerated ageing (Figure 1), exhibiting severe growth retardation in infancy, associated baldness, loss of subcutaneous tissue, eyebrows and eyelashes [15]. Skeletal abnormalities, including diffuse osteoporosis and resorption of distal phalanges are marked and sometimes predominate [16]. Intelligence is unaffected and most deaths are due to the consequences of severe atherosclerosis, particularly myocardial infarction and heart failure, which occurs in the early teenage years. However, other features typically associated with the ageing process, such as tumours, diabetes mellitus, neuronal changes and cataract formation, are seldom seen.

Werner's syndrome is an autosomal recessive disorder first described in 1904 and, with an estimated prevalence rate of 1-10 per million population, being more common in Japan and Sardinia. Unlike progeria, the phenotype is not usually apparent until the third or fourth decade—'progeria of the adult'. The most notable features include shortness of stature, a

Table 2. Characteristics of some of the premature ageing syndromes[a]

	Syndrome				
	Progeria	Werner's	Cockayne's	Bloom's	Ataxia telangiectasia
Onset	Infancy	3rd decade	2nd year	Infancy	Infancy
Death (decade)	2nd	5th/6th	2nd	2nd/3rd	Childhood
Stunted growth	■	■	■	■	■
Abnormalities					
Skin	■	■	■	■	□
Skeletal	■	■	■	■	□
Muscular	■	■	■	□	□
Visual impairment	□	■	■	□	–
Mental deficiency	□	□	■	–	–
Photosensitivity	□	□	■	■	■
Cancer risk	□	■	□	■	■

■, present; □, absent; –, not reported.
[a] Other premature ageing syndromes include Rothmund–Thompson syndrome, myotonic dystrophy, Down's syndrome, familial Alzheimer's disease, Wiedemann–Rautenstrauch syndrome and acrogeria.
Adapted from Mills and Weiss (Gerontology 1990; 36: 84–98) [1].

bird-like appearance with a beak shaped nose, premature loss and greying of hair, scleroderma-like skin changes and skin ulceration [17]. Osteoporosis and profound wasting of the limb musculature is characteristic. In contrast to progeria, conditions such as diabetes, cataracts and cancers of mesodermal origin

Figure 1. Example of progeria: typical facial appearance, exhibiting baldness, prominent scalp vessels and loss of eyebrows and eyelashes.

are common, although death still usually results from a vascular origin at a median age of 47.

Other conditions also deserve attention as models for accelerated ageing. Cockayne's syndrome (Figure 2) is a rare autosomal recessive disorder [18] which usually becomes apparent around the second year of life (although onset in the first few months after birth has been described [19]) and was first described in 1936. It is characterized by growth retardation, skeletal and retinal abnormalities, progressive neurological degeneration and severe photosensitivity, although cancer rates are low. Patients may survive into their teens or early twenties. A review of 140 cases by Nance and Berry is extremely comprehensive [20]. Bloom's syndrome is characterized by severe pre- and postnatal growth deficiency, a sun-sensitive erythematous face, well-demarcated hyper- or hypopigmented skin lesions and variable immunodeficiency [21]. Cancer, chronic lung disease and diabetes mellitus are the main complications. Although rare, this condition, like ataxia telangiectasia, has been intensely studied, primarily to establish the genetic basis of various cancers.

Biochemical and cellular characteristics

Various avenues have been explored to establish the fundamental cellular defect in these syndromes. In progeria and Werner's syndrome most biochemical abnormalities are associated with connective tissue, principally of mesodermal origin. The most thoroughly investigated is the finding of 10–20 times greater urinary excretion of hyaluronic acid in patients with progeria compared with controls [22, 23]. Similar results have also been obtained in patients with Werner's syndrome [24]. Hyaluronic acid is an

Figure 2. Cockayne's syndrome: features are mental retardation, microcephaly, deep-set orbits, sensorineural deafness and photosensitivity.

unsulphated glycosaminoglycan known to be involved in maintaining the integrity of skeletal, muscular and vascular systems.

Alterations in hyaluronic acid concentrations are linked to processes such as scleroderma-like skin changes, collagen hardening and calcification of arterial walls [25]. Brown found that hyaluronic acid levels increase with age in normal subjects [22] from around 1% of total glycosaminoglycan content to around 5-6% over the age of 70, possibly increasing further at very advanced ages. This compares with 10-20% of glycosaminoglycan content in Werner's syndrome [24]. Whether this is a primary or secondary phenomenon is not known, but hyaluronic acid excretion could be a consequence of mesodermal cells at the end of their replicative lifespan. It does seem unlikely, however, that all the complex changes associated with age are due to this one macromolecule. Indeed many other abnormalities, such as increased levels of elastin production at the protein and mRNA

level in progeroid skin fibroblasts [26] and reduced decorin expression in Wiedemann-Rautenstrauch syndrome [27], have also been demonstrated. Plasma lipid concentrations increase with age and hypercholesterolaemia is a common finding in patients with Werner's syndrome [28], resulting in the accumulation of cholesterol esters which may contribute to atherosclerosis.

The relationship of changes in oxidative stress to the development of the premature ageing syndromes has not been explored in detail [11]. Cultured cells from patients with Werner's syndrome contain normal levels of superoxide dismutase and glutathione peroxidase [29] and the rate of lipid peroxidation is normal. Nevertheless, Oliver *et al.* have shown significantly higher levels of oxidatively modified proteins compared with age- and sex-matched controls [30] and in Bloom's syndrome superoxide dismutase activity is two to three times higher than expected [31]. Down's syndrome demonstrates many of the important physical features of a premature ageing syndrome and has been regarded as a 'segmental progeroid' syndrome by Martin [32]. Recent reports suggest free radical involvement in the pathogenesis of this condition. Superoxide dismutase activity has been shown to be increased in fibroblast cell lines derived from patients with Down's syndrome [33], and superoxide dismutase overexpression with a paradoxical increase in hydrogen peroxide (and subsequently hydroxyl radical) formation has been proposed by Ceballos-Picot *et al.* [34] as a possible mechanism for cerebral toxicity in Down's syndrome. These authors developed transgenic mice that expressed high levels of human copper-zinc superoxide dismutase which led to higher levels of lipid peroxidation in transgenic brains compared with controls.

Based on a variety of physiological and biochemical similarities, diabetes mellitus has also been proposed as a model of premature ageing [35, 36]. These include impairment of cognitive performance, decreased blood-brain barrier transport of glucose and choline, increases in collagen cross-links in skin, dura and tendon and increased capillary basement membrane thickening [37]. Abnormalities of oxidative stress have been increasingly recognized in this metabolic disorder and include both increased plasma lipid peroxidation and reduced antioxidant protection, particularly ascorbate [38], providing additional but indirect support for free radicals playing a role in the morphological changes accompanying ageing.

The enormous variety of biochemical changes that occur in these syndromes clearly lend support to the ageing theories based on physiological deterioration and primary damage.

Genetic and chromosomal abnormalities

Cultured fibroblasts from patients with progeria and

Werner's syndrome show a markedly reduced potential for *in vitro* growth and have a shorter replicative life span compared with control cultures [39]. Kill and colleagues, using antibodies that identify proliferating cells only, confirmed that the rate of loss of replicating cells in Werner's syndrome is increased five- to sixfold compared with normal human fibroblasts [40]. The cell cycle defect appears to be due to impaired S-phase transit [41] and decreased expression of regulators such as platelet-derived growth factor β-receptors may be partially responsible [42]. However not all features correlate with normal ageing; for example, expression of the c-*fos* proto-oncogene, essential for cell proliferation, appears to be preserved in Werner's syndrome fibroblasts unlike normal senescing cells [43]. In contrast to Werner's syndrome and progeria, cells from patients with Cockayne's and Down's syndromes do not show a defect in replicative capacity and neural defects predominate in these syndromes, possibly as a result of oxidative damage to these post-mitotic cells.

One problem in cell culture work in progeroid syndromes is that due to the short replicative lifespan of these cells, fibroblasts are often immortalized by viral transformation so that they can be adequately studied. Although this raises questions about the validity of findings from these abnormal cells and whether they can be extrapolated to the behaviour of cells *in vivo*, they have nevertheless provided highly valuable information pertinent to the ageing process.

Recently, an exciting development has been the identification of the gene defect responsible for Werner's syndrome on chromosome 8 [44]. The gene has seven different domains and different types of mutation have been found in different ethnic groups. The significance of this finding is that the gene resembles DNA helicase, implying that defective DNA metabolism is the principal cause of the condition. Reduced DNA repair ability has also been proposed in cells from progeria patients [45]. DNA helicase uncoils DNA for a variety of replicative, transcriptional and repair processes, with failure resulting in increased genomic instability. Werner's syndrome cells exhibit a variety of chromosomal aberrations, including reciprocal translocations, deletions and inversions [46]. Mutation rates (mainly deletions) at the HPRT locus in Werner's syndrome are increased by 10-fold or more [47].

Similar patterns of chromosomal instability have been found in other premature ageing syndromes, such as Bloom's syndrome where chromosomal breaks are readily apparent [48]. These patients are homozygous for the BLM gene on the long arm of chromosome 15, which has also been recently shown to encode a DNA helicase [49]. Thus, the occurrence of higher rates of malignancy in this condition and Werner's syndrome are likely to be due to chromosomal abnormalities resulting from reduced cell cycle regulation [50].

Cells from patients with ataxia telangiectasia show spontaneous chromosomal instability and are hypersensitive to the effects of ionizing radiation, exposing patients to a variety of cancers. The gene defect may result in failure to monitor DNA damage and activate DNA repair resulting in accumulation of genomic damage and consequent cell death [51]. However, Cockayne's syndrome, also associated with abnormalities of DNA repair (nucleotide excision damage), does not predispose to cancer [20], although fibroblasts are known to be hypersensitive to the cytotoxic effects of UV light. Venema and colleagues demonstrated that the genetic defect in Cockayne's syndrome is associated with inefficient repair of ultraviolet-induced DNA damage in transcriptionally active DNA rather than inactive genomic sequences [52], resulting in relatively normal somatic cell mutation frequencies which may explain the low cancer rates [53].

Therefore, many of these syndromes do support the hypothesis that at a DNA level, ageing results, in part, from an imbalance between DNA damage and repair, in some cases causing such severe chromosomal aberrations that malignant transformation occurs. Genomic and particularly mitochondrial DNA in normal subjects is continuously exposed to high levels of oxidative damage and a critical delay in repair has been suggested as playing a key role in cell senescence [54]. So, genetic theories of ageing and theories of primary damage can be viewed as not being mutually exclusive, but as part of a much more complicated interaction of internal and external factors.

The place of telomeres in this process has not yet been fully established. Telomeres are the repetitive DNA sequences (TTAGGG) at the end of linear chromosomes which circumvent the inability of DNA polymerase to completely replicate chromosomal ends. The observation that telomere length of somatic chromosomes progressively declines with increasing age and that progeria cells have short telomeres lends support to the idea of a cellular 'clock', preventing further replication at a critically reduced length and therefore allowing DNA damage to occur unrepaired [55].

Other models of ageing—the senescence-accelerated mouse model

Animal studies remain a further experimental approach to the investigation of biological ageing and these have concentrated on characterizing changes in morphology and function with normal ageing or contrasting differences in longevity among species [56].

The senescence-accelerated mouse model (variant SAM-P/8), developed by Takeda and colleagues [57], shows early onset and rapid advancement of senescence revealed by analysis of ageing dynamics such as Gompertz function and survival curves and exhibits a significant age-related deterioration of memory and learning abilities. The fact that this model ages quickly

(average lifespan is 12 months—less than 50% of the average lifespan of a moderately long-lived mouse strain) allows investigators to generate life tables rapidly and creates opportunities for ageing intervention studies to be undertaken. Evidence to support the use of the senescence-accelerated mouse model as a valid one for research on effects of ageing includes the development of 'age-dependent' geriatric disorders such as osteoporosis, degenerative joint disease, cataract and hearing impairment.

However, despite the obvious value of animal models, such as the senescence-accelerated mouse, in studying particular aspects of ageing, some concerns have been raised as to their relevance to the 'normal' ageing process. In addition, accelerated ageing may be controlled by only a few genes whereas normal ageing may be influenced by many more. Therefore, short-lived strains may not display the wide range of clinical features seen in a normal ageing population. Senescence-accelerated mice, for instance, show a much lower rate of thymic lymphoma than normal mice.

Conclusion

Substantial progress has been made over the last few years in our understanding of the genetic and cellular abnormalities of the progeroid syndromes, especially Werner's syndrome. There are clearly many similarities to the 'normal' ageing process, but also some differences. Reduced cellular replication and impaired DNA repair may result in increased oxidative damage, chromosomal abnormalities and malignancy in these syndromes. All of these are features associated with 'normal' ageing. However phenotypic expression of senescence in these diseases varies, due to the differing effects of single gene defects, rather than the involvement of multiple gene loci in the normal ageing process. We must accept that the complex and variable nature of ageing limits the use of any one disease as a model of 'global' ageing. Yet it is exactly the segmental nature of these conditions which has provided the opportunity for scientists to study a specific sector of the ageing process.

Further areas of research will involve understanding the precise role of DNA helicase in these conditions, unravelling the telomere hypothesis and the identification of progeroid heterozygotes who may have higher cancer risks than normal.

Acknowledgements

Key points

- Since the complex interaction of diseases and other external environmental factors makes it difficult to design experimental studies of ageing, the premature ageing syndromes have been advanced as models of ageing.
- These conditions are characterized by a number of features (such as vascular pathology, cataracts, skin changes and increased cancer rates)—although not all features typically associated with ageing are present in all cases.
- Research in this field allows insight into the nature of the genes that have a role in ageing and helps to separate the boundary between ageing and age-related disease.

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