Gerontology and drug development: the challenge of the senescent cell

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The efficacy of new pharmaceuticals is considerably more difficult to estimate before use in the elderly than in young patients, particularly because clinical trials frequently involve young volunteers and because elderly patients on multiple treatments are at greater risk of adverse side-effects. There is an urgent need to understand the basic processes of senescence, and a practical requirement to deal with their consequences. Here, the authors review current understanding of the ageing process, summarize the experimental data supporting a link between senescence in cell cultures and ageing in the various tissues of the body, examine the changes that occur in the senescent cell from a pharmacological perspective and highlight possible improvements to existing *in vitro* screens for drug efficacy.

Biological ageing, the persistent decline in age-specific functional competence of an organism resulting from internal physiological deterioration[1], is common to all higher animals and has also been studied in fruit flies, nematodes and other simple metazoans. Ageing is pre-eminently a characteristic of animal populations that are protected from environmental risks such as competition or predation. The ageing process as such confers no selective advantage to individuals because it occurs after the peak reproductive age and acts on a relatively small pool of surviving animals. It appears to be an inevitable consequence of the optimization of the organism for successful reproduction at sexual maturity. Several evolutionary theories have been proposed that account for the ageing process in these terms, of which the 'disposable soma' theory of Kirkwood and Holliday[2] remains the best developed. None, however, have yet received clear experimental support.

Age-related diseases

When the risk of predation or infectious disease is removed, increasing proportions of an animal population survive for longer periods of time. Observational data of this type are usually presented as a survival curve: the fraction of survivors from a population over time. Survival curves are available for a variety of human populations and can provide useful data on the relationship between life-expectancy, infectious disease and the endogenous limits to lifespan. In poor countries, such survival curves are marked by a high death rate in the early period, corresponding to childhood mortality, followed by a gradual population decline. Finally, a very small fraction of individuals remain with lifespans of ten decades or more. By contrast, in rich countries the survival curve is almost rectangular due to the removal of the major causes of early death (Figure 1).

The maximum lifespan of humans is unknown; however, it seems to reach a limit of about 120 years in all populations for which accurate data exist. Popular claims for people with lifespans in excess of this figure, usually located by the press

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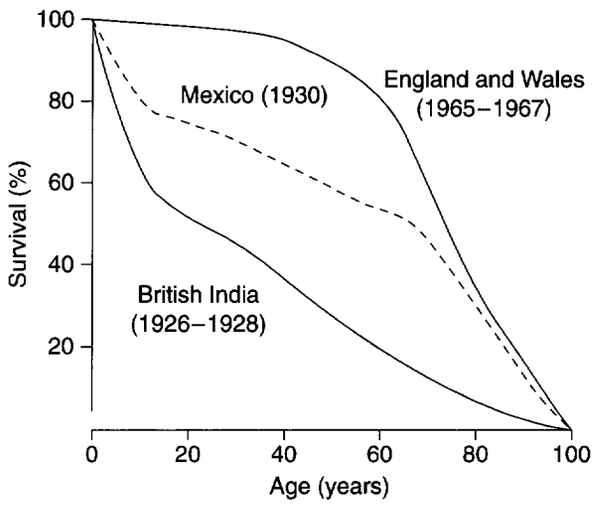

Figure 1. *Survival curves for sample developed and underdeveloped countries. Note the extremely high fraction of early deaths in British India due to elevated childhood mortality. By contrast, a relatively low fraction of deaths occurs during early life in developed countries with proficient health care and sanitation. Note that there is no variation in the maximum age reached by the survivors, indicating that there are intrinsic limits to the human lifespan, which are essentially unaffected by environmental limits. Reproduced from Ref. 74, with permission of Edward Arnold.*

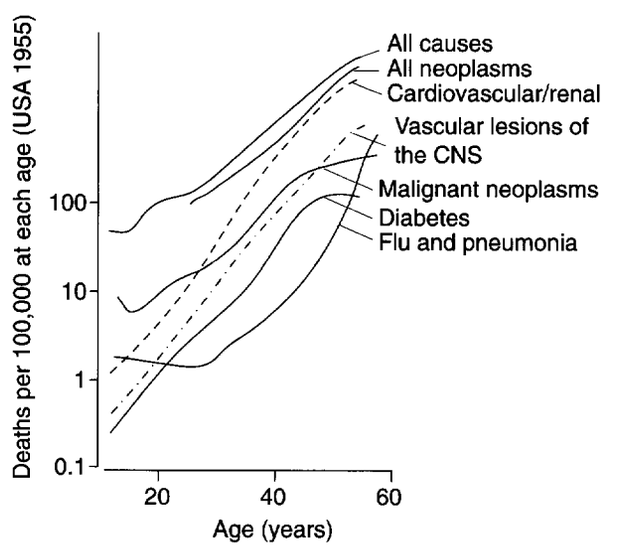

Figure 2. *Gompertz relationship between age and disease. Rates of death as a fraction of the population from a range of diseases are plotted against the age at death of the sufferers. Diseases that show an increase in specific death rate with advancing years are associated with the intrinsic ageing process. Note that the diseases responsible for the majority of deaths in the West today are age-associated. Reproduced from Ref. 74, with permission of Edward Arnold.*

in some exotic part of the world, have never been confirmed. The popular belief that the maximum human lifespan has increased over time is also untrue; historically verifiable examples of lifespans close to 100 years can be found at least as far back as the 5th century BC [for example, the Athenian orator Isocrates, who lived to be 98 years old (435–337 BC); for a listing of long-lived historical personalities, see Ref. 3] and at all times since. What has increased quite dramatically in the modern world is the individual's chance of attaining such a lifespan and consequently the fraction of people who are living to advanced years. This, coupled with a modest decline in the birth rate, has led to an increasing proportion of older people in the population today. Unfortunately, the major causes of death in the elderly appear to be intrinsic and linked to the underlying ageing process. Such age-related diseases, first recognized as such by the 19th century mathematician Gompertz (Figure 2), include atherosclerosis, dementia, arteriosclerosis and cancer. Again, these diseases are not a modern phenomenon [see, for example, the classical description of cancer in the works of Hippocrates of Kos (c. 460–c. 377 BC), and the death of the Byzantine Empress Theodora from throat cancer in 548 AD]; they simply kill more people now because almost nothing else is left to do so. (For a detailed treatment of age-related disease in history, see Perutz[3].)

Not only does increasing age carry a continuously increasing risk of death, but debilitating, chronic diseases also plague older people with increasing frequency. These chronic diseases can seriously compromise the quality of life of elderly patients and are frequent targets for repeat prescription. This increases the relative importance of drug side-effects in this fraction of the population.

Unfortunately, aged individuals have severe, adverse drug reactions roughly twice as frequently as the young[4]. The basis of many of these adverse reactions appears to be the differences in tissue physiology between old and young people. Active transport across the gut is often compromised, with negative consequences for drugs imported by an active route rather than by simple diffusion. The relative

composition of the blood also shifts with increasing age, with the potential to alter the binding of drugs to plasma proteins and erythrocytes. Human serum albumin, in particular, has been shown to decline significantly with age; this has been predicted to increase the bioavailability of several drugs to tissues in older people. The potential therefore exists for differences between old and young patients in the availability and metabolism, and hence the effect, of active agents. Overall, there is a broad shift in tissue balance with human ageing towards a relative deficit in lean body mass and an increase in body fat of 10–20%. Thus lipid-soluble drugs, in particular, have the potential to accumulate or exhibit prolonged effects in the older organism.

Cellular hypothesis of ageing

A principal concern of early gerontology, given the universal and degenerative nature of ageing in higher animals, was to determine whether the mechanism of ageing resided within the individual mammalian cell or operated at the higher levels of tissue organization represented by the organism. Work by one of the early pioneers of *in vitro* cell culture, Alexis Carrel, strongly supported the theory that the body tissues aged and failed rather than their component parts. Carrel initiated cultures of chick heart fibroblasts, which he claimed to have successfully grown in continuous culture for over 30 years[5]. The cultures were finally discarded shortly after Carrel's death. Subsequently, the failure to reproduce similar cultures of 'everlasting cells' was attributed to poor culture technique.

Senescent cells

Hayflick and Moorehead[6], building on earlier work by Swim and Parker, demonstrated in a landmark paper that 25 separate strains of normal human fibroblasts each possessed a limited lifespan in culture. After a defined number of passages the cultures became composed entirely of cells in a non-growing state, described as *senescence*. The work was rapidly confirmed in other laboratories, and media composition, latent viruses and metabolite depletion were all excluded as explanations for the failure to grow[7]. A subsequent paper[8] demonstrated that cells derived from adults had a significantly decreased proliferative potential, now usually measured in population doublings, compared with those derived from an embryo. This paper[8] also proposed a cellular theory of ageing, which hypothesized that the finite lifespan of cells was an intrinsic property of normal cells, that cell growth *in vitro* was somehow mechanistically related to human ageing and that cultures of such primary cells were a useful and legitimate model system for the study of *in vivo* ageing. Hayflick's discovery has been confirmed in literally hundreds of instances over the last 30 years and cell culture systems are now widely used as models of the ageing process.

Programmed or unprogrammed ageing

Explanations of *how* the ageing process works, as opposed to *why* the ageing process occurs, now fall into two major categories with little overlap between them. Theories can generally be described either as unprogrammed theories, in which ageing is a result of environmental accidents or general 'wear and tear', or programmed theories, in which ageing is attributed to a predetermined intrinsic process, such as differentiation. Both types of theory can be tested in tissue culture systems (Hayflick had initially proposed an unprogrammed mechanism to account for his observations), and a very large body of data has been generated concerning the validity of each type of theory (see Ref. 9 for a review). In general, attempts to prove that the finite lifespan of fibroblasts *in vitro* is due to the random accumulation of DNA damage, free radical damage, non-enzymatic glycation or generalized errors have not been convincing. Although it is certainly true that all these reactions do occur, their causative role in the limited lifespan is unproven. A major argument against unprogrammed theories of cell ageing that invoke damage mechanisms was the discovery that permanently non-dividing 'senescent' cells do not die over any meaningful period. Such senescent cells are highly metabolically active, synthesizing both RNA and protein at a high rate[10]. It is a little difficult to understand how a cell can sustain a damage load great enough to prevent it from dividing but still remain viable. Random damage theories are clearly useful in explaining the death of constitutively non-dividing cells such as neurons, muscle and lens fibre cells, but do not appear to be the primary cause of senescence in cell populations that have an intrinsic capacity for replacement.

By contrast, theories of ageing that suppose that some key part of the process is biologically programmed have gained considerable support from the study of fibroblasts undergoing senescence in culture. A relatively early discovery was that, after fusion of senescent cells with growing cells, further proliferation in the hybrid was suppressed. Fusions of senescent cells derived from different fibroblast cell strains also failed to yield growing hybrids[11]. Moreover, fusions of pairs of cell strains with defined but different lifespans

always gave hybrids whose lifespan was the arithmetic average of the parent cells[12]. It was therefore possible to propose that senescence was an active process requiring gene expression on the part of the senescent cell, and that the mechanism of senescence was common between cell strains and was not just a consequence of the random accumulation of damage.

Heterogenous cell cultures

Another early revelation concerned the composition of the primary cultures being studied. Hayflick's original theoretical description of primary fibroblast cultures intrinsically assumed that the cultures being studied were composed of homogeneous (unimodal) populations of cells, which were either all growing (described as phases I and II by Hayflick) or all non-growing (so-called phase III cells). Subsequent experiments showed that a fibroblast culture could not be considered a single, homogeneous population of cells. Hayflick and Smith showed that a fibroblast culture was very heterogeneous with respect to the growth potential of the individual cells of which it was composed[13]. Furthermore, Cristofalo and Scharf[14,15] incubated embryonic human fibroblasts with tritiated thymidine and estimated the fraction of cells that entered DNA synthesis (S phase) by autoradiography. They observed that unlabelled cells were present in very young cultures and that labelled cells were present in very old cultures, a finding which contradicted Hayflick's original assumption of unimodality. They also showed that the fraction of unlabelled cells increased smoothly with serial passage of the culture. Thus, it appeared likely that cultures of fibroblasts *in vitro* were a heterogeneous mixture of dividing and non-dividing, senescent, cells. A variety of cloning experiments directly demonstrated that primary cell cultures were mixtures of senescent and growing cells, the proportions of which altered in a highly predictable manner as the culture aged[12,16]. Further experiments also excluded a gradual lengthening of the cell cycle as a cause of senescence[17]. This behaviour is difficult (but not impossible) to explain as general damage, but is quite reminiscent of a simple differentiation pathway or a similar biologically determined mechanism. A recent and attractive hypothesis links this declining replicative behaviour to a counting mechanism based on the shortening of chromosomal telomeres (ends) with concomitant gene activation. Unfortunately, rates of telomere shortening have not yet been directly correlated with the rate of senescence as measured by markers of the S phase, such as tritiated thymidine[18].

Although most early work in the field of cell ageing involved the use of fibroblasts, there is now an increasing quantity of data that shows that essentially all normal cell types capable of cell division in the body have a finite growth potential *in vitro*. Lens epithelial cells[19], human epidermal keratinocytes[20], glial cells[21], arterial endothelium[22], mammary epithelia[16], peritoneal mesothelium[23] and corneal cells[24,25] have all been shown to undergo changes characteristic of cellular senescence. In a review designed to dispel prejudices of the type originally encountered by Hayflick 20 years earlier, Effros and Walford[26] suggested that cells of the haemopoietic system also display a limited lifespan. Subsequently, Perillo and coworkers[27] demonstrated a limited lifespan for human T cells *in vitro*, and a significant difference in the number of population doublings achieved by cells from adult versus neonatal donors was observed, analogous to that seen by Hayflick for fibroblasts. These findings have been confirmed and a limited lifespan for B cells has been reported[28,29]. The available data strongly suggest that all normal cell types that divide within the body have a limited lifespan *in vitro*.

Senescent cells and ageing bodies

A question that has not been addressed in great detail by adherents of the cellular senescence theory of ageing is the exact relationship between the appearance of senescent cells *in vitro* and the behaviour of aged tissue *in vivo*. An inverse correlation between donor age and the number of population doublings (PD) achieved *in vitro* has been reported[30]. These authors used fibroblast cultures derived from 100 subjects with an age range from fetal to 90 years and obtained a regression line indicating a decline in replicative potential of 0.20 PD per year of donor life. An extension of this work included more donors over the age of 60 years. No significant change in the slope of the regression line was noted[31].

Schneider and Mitsui[32], in extensive studies of the *in vitro* growth of fibroblasts derived from 'young' (21–36 years) and 'older' (63–92 years) donors, observed a decline in fibroblast migration rate, in *in vitro* lifespan, in cell population growth rate and in saturation density at confluence. Colony-forming efficiency was also reduced in cultures from old donors compared with those from young donors, confirming the earlier work. However, none of these differences was as great as those found between 'young' (approx. 20 PD) and 'old' (approx. 40 PD) human embryonic fibroblasts.

Premature ageing syndromes

Cell cultures initiated from human donors suffering from premature ageing or progeroid syndromes, such as Werner's syndrome and Hutchinson–Gilford progeria, display an attenuated growth potential[33]. In Werner's syndrome the major causes of death are atherosclerosis, arteriosclerosis, diabetes and cancer, and the reduction in fibroblast growth is particularly severe. At least 90% of Werner's syndrome cultures fail to complete 20 PDs (Ref. 34). Recent work from our laboratory has demonstrated that Werner's syndrome fibroblasts have a greatly increased rate of transition from a growing to a senescent state *in vitro*, leading to cultures that have more senescent cells at each passage[35]. Goldstein and coworkers[36] cloned a wide range of messages from Werner's syndrome cultures and showed them to be overexpressed in normal senescent cells. This study demonstrated that Werner's syndrome is a genuine model of normal cellular senescence, rather than a consequence of damage-related growth arrest or some other phenocopy of the process. Recently, Norwood and other workers have pointed out the relevance of such senescence-specific messages to the age-related pathologies seen in the normal elderly[22,37,38]. It is therefore reasonable to suppose that the accelerated accumulation of senescent cells *in vivo* is responsible for the aged pathology seen in Werner's syndrome and also, by implication, that the accumulation of such cells in normal people contributes to the normal ageing process. Proof of this would be the strongest possible support for the notion that cellular senescence contributes to normal human ageing.

Indicators of cell senescence

A clear demonstration of the presence of senescent cells *in vivo* has been hampered by the absence of a reliable indicator of senescence that does not also identify transiently growth-arrested cells (a state referred to as quiescence). Generally, senescent fibroblasts are larger than their growing counterparts and produce more fibronectin and collagen, providing a potential biomarker of the ageing process in connective tissue[39]. Labat-Robert and coworkers were able to demonstrate a very considerable increase in fibronectin expression in skin biopsies from a Werner's syndrome patient[39]. This evidence, although convincing, still did not involve the direct visualization of senescent fibroblasts.

However, last year, Campisi and coworkers modified an indigogenic histochemical assay for an endogenous mammalian β-galactosidase so that the presence of the enzyme can now also be demonstrated in rather large fibroblasts. In tissue culture, this modified assay provides a highly selective staining for senescent cells. It is of considerable interest that, in tissue sections derived from donors of increasing age, increasing numbers of β-galactosidase-positive cells were detected[40]. It may therefore be tentatively concluded that senescent cells do increase in number in human connective tissue with age and that, to an increasing degree, the physiological responses of this aged tissue are determined by the phenotype of the senescent cells that compose it. A similar picture is now emerging regarding human endothelium, based partly on the expected phenotype caused by genes expressed in senescent fibroblasts[41] and partly on culture experiments demonstrating a markedly decreased lifespan in culture of endothelial cells from arteriosclerotic vessels[42]. This is a potentially fascinating area of work, but is currently hampered by the lack of a reliable marker for the senescent state. β-galactosidase staining cannot be relied on unequivocally because it is dependent on the relationship between the increase in cell size and senescence. Our own observations suggest that in mesothelial cells this relationship is not a simple linear one[23]. The β-galactosidase staining technique is also not useful in T lymphocytes, which have been shown not to shift their nuclear/cytoplasmic ratio during cell ageing[43]. Identification of an unambiguous and reliable indicator of the senescent state remains a major priority in cellular gerontology.

Phenotype of senescence

A growing body of evidence indicates that cell senescence contributes to the decreasing physiological function of aged tissues. Because it is often the case that active drugs are required to function in ageing tissues, it is useful to examine the phenotype of senescence with regard to the pharmacology, pharmacodynamics and drug-targeting aspects of the permanently growth-arrested state. Rather surprisingly, until now, this has not been an active area of work in gerontology. However, enough data have been gleaned from academic research on the biology of cell senescence to indicate that many parameters of interest, from a pharmaceutical perspective, differ between growing and senescent cells. The majority of the examples that follow concern fibroblasts, with which most work has been carried out. A number of other cell types of more immediate physiological importance show similar changes, which will be discussed where appropriate.

Changes in cell size and surface

In general, adherent cells increase in size as they enter the senescent state, although it is not known whether this happens as they approach senescence or while they are in the postmitotic state. This is true for lens epithelial cells, fibroblasts, microglia and mesothelial cells[44–46]. In fibroblasts, this increase in size is associated with a decline in the net negative charge on the cell surface, independent of the cell size. This decline is of the order of 30% (Ref. 46) and is associated with decreased levels of cell surface sialic acid. Membrane fluidity and composition appear to remain constant in fibroblasts, but in ageing lens cells a clear shift in phospholipid content occurs[47]. Various changes in glycosaminoglycans occur with ageing, the increased urinary secretion of hyaluronic acid being one of the most spectacular[13]. Increased surface affinity for *Ricinus communis* lectin, which is specific for β-D-galactosyl residues is particularly noteworthy. Increased surface β-D-galactosyl reactivity has been reported both for normal aged fibroblasts and for endothelial cells from patients with Fuchs' dystrophy, an ocular disease in which the endothelium undergoes changes reminiscent of normal ageing[48]. Similar surface antigen shifts occur in ageing erythrocytes with the expression of senescent cell antigen, signalling their physical removal from the circulating population[49].

There is a general consensus that the number of growth factor receptors per unit area (their density) and the affinity of the peptide ligands for the cognate receptors are unchanged during *in vitro* cell ageing[12]. However, receptor-mediated transport of several significant macromolecules is altered during cell senescence. As expected, the transferrin receptor (CD71) is downregulated due to the tight linkage of this transport system with the active cell cycle. Low-density lipoprotein (LDL) receptor transport is altered in senescent fibroblasts, the incorporation and degradation of the ligand being significantly reduced with a constant binding affinity[50]. By contrast, in senescent human smooth muscle cells, binding and incorporation of LDL remains normal, but degradation is significantly decreased[51]. This finding emphasizes the necessity to study a variety of ageing cells if a true picture of aged-tissue physiology is to emerge. The uptake of human serum albumin (HSA) is increased in senescent human fibroblasts, a significant finding in the light of reduced HSA levels in the aged body[52]. Bulk fluid movement by macropinocytosis is abolished in ageing glial cells, with related changes occurring in senescent human fibroblasts. The transport of a number of metabolites, such as 2-deoxy-D-glucose, uridine and zinc, is also altered by senescence, although without a consistent pattern of change[46].

Changes in gene expression

Cell senescence is, at least in part, an active, biologically determined genetic process that triggers changes in the expression of many genes that have the potential to affect drug responses, either actively by altering transcription of the target enzyme or passively by broad changes in the cellular environment. An illuminating example is the vascular endothelium, in which the expression of angiotensin converting enzyme, a well-known pharmacological target, is transcriptionally repressed in senescent endothelial cells[53]. Endothelial nitric oxide, the key secondary messenger in the regulation of vascular tone, is not produced by human umbilical vein cells once they have become senescent[54]. Similarly, the enzyme cyclooxygenase, which is also present in vascular endothelial cells, is non-inducible in senescent cells[55]. Major alterations to the β-adrenoceptor system occur with ageing, and similar changes have been reported in senescent cells[56,57]. A systematic study of senescence in bovine adrenocortical cells has revealed a stochastic repression of a wide range of enzymes during *in vitro* ageing. The enzyme activity of both 17α- and 11β-steroid hydroxylase declines with senescence[58]. Similarly, the response of adrenocortical cells to ACTH is significantly attenuated by replicative senescence. The authors suggested that these changes would generate heterogeneity among cells in an aged tissue, producing a mosaic of physiologically functional and non-functional cells[58]. Thus, as adrenocortical tissue ages, it would progressively shift towards being both non-dividing and non-functional. The stochastic repression of adrenal-specific enzymes indicates a mechanism that engenders a functional deficit while still maintaining some reproductive potential.

Calcium metabolism is also altered in many ageing tissues, including muscle and brain[59]. In different areas of the brain, the activity of the calcium-binding protein, calmodulin, increases or declines with advancing age. These changes have the potential to affect a range of calcium-dependent enzymes and processes, including the activity of Ca^{2+}-ATPase and protein kinase II. The activity of a calcium-dependent current has recently been studied in senescent fibroblasts. Expression of a senescence-specific message (WS3.10) abolishes calcium-dependent potassium currents in aged fibroblasts[60]. This gene has significant homology to calmodulin and may represent a 'dampener' of

calcium-dependent activity throughout the senescent cell. Altered activity of chloride channels has recently been reported in aged muscle[61]. It is therefore likely that many cell membrane events that are dependent on ion flow are altered in the senescent state.

Inwards from the membrane, it has been established that the gene c-*fos* is transcriptionally repressed in senescent fibroblasts[62], and probably also in lymphocytes[63]. This means that the cell cannot form the most active AP1-transcriptional complex required for a wide range of cell cycle processes. However, c-*fos* repression does not seem to be the prime mediator of fibroblast senescence. In transfection experiments, strong ectopic expression of c-*fos* failed to overcome the block to continued cell cycling characteristic of cell senescence[64]. Also, many of the cell cycle genes, including cyclin A, cyclin B and *cdc2*, are also transcriptionally repressed in senescence[65]. At the same time, most of the important cyclin-dependent kinase (CDK) inhibitors, such as p15, p16 (Ref. 66), p21, p27 and p57, are constitutively overexpressed in the senescent cell. The CDK inhibitors, p16 and p27, seem to be especially important; indeed, the p21 inhibitor was first identified among this group of important regulators as a senescent cell dependent inhibitor[67]. These CDK inhibitors have already become the target of intensive research and development in the pharmaceutical industry as targets for the regulation of cell growth and division[68]. Nonpeptide CDK inhibitors are now known and are being vigorously examined by several large commercial laboratories.

The senescent state is also associated with the transcription of a wide variety of messages that are either entirely absent from growing cells, such as WS3.10, or present at appreciably lower levels. A recent study, using subtractive hybridization, generated 15 overexpressed cDNA clones, including fibronectin, SPARC, nm23, eIF-2B, EF-1a and acid sphingomyelinase[69]. This latter enzyme is of particular interest because of its putative role in the development of multidrug resistance[70]. A further ten cDNA clones coded for unknown proteins, including a secreted EGF-like protein and a novel DNA-binding protein. Similarly, the expression of mitochondrial cytochrome b and the NADH dehydrogenase 4/4L subunit have been shown to be strongly upregulated in senescent MRC-5 lung fibroblasts[71]. In human fibroblasts, senescence is accompanied by a decrease in mitochondrial number but with an increase in overall respiratory activity; the elevated expression of the above genes may be partly responsible for this. A marked decline in mitochondrial function is known to occur in aged human muscle and liver and is usually attributed to a dramatic loss of mitochondrial DNA. These changes suggest once again that age-related changes, although ubiquitous, do not necessarily have identical causes in dividing and non-dividing human tissues[72]. Differentially expressed sequence tags were recently isolated from aged versus young brain, but have not yet been characterized further[73].

Pharmaceutical potential

There is clearly a substantial and growing body of evidence indicating that senescent cells make a significant contribution to the physiological function of aged tissue. In addition, there is a welter of data detailing the behaviour of such cells. It is therefore very important to consider how this new information should be incorporated within strategies of drug discovery, design and development.

A helpful starting premise is to divide the parameters to be studied into those that might influence drug targeting and those that might influence drug design and usage. For example, the alteration in surface charge as cells age is likely to have profound effects on the efficiency of drugs relying on charge differential to enter senescent cells. Similarly, the altered uptake kinetics for many compounds such as LDL may also affect the choice of drug delivery route for chemicals designed principally for use in geriatric patients. A case in point is the transferrin receptor, a drug delivery vehicle that is heavily downregulated in senescent non-dividing cells. Drug delivery systems utilizing this vehicle are likely to encounter significant difficulties in supplying meaningful concentrations of the active agent to senescent tissues.

A potential way to rectify some of these problems is to expand the existing repertoire of cell lines used to assay candidate drugs to include the use of various senescent cell types. This new approach would have the advantage that drug assay techniques optimized for immortal cell lines could be easily transferred to normal cell strains. However, such a strategy requires the provision of well-characterized strains of human cells from many different differentiated tissues. Some well-characterized fibroblast strains already exist, but strains derived from the more important target tissues still require development, characterization, or both. Broad physical changes, such as increased cell size or altered surface charge, can already be studied in the available fibroblast strains. The investigation of more specific changes in aged cells, such as alterations in gene expression

and consequent alterations in drug metabolism and availability of important targets, would require the characterization of new workhorse strains.

In summary, the use of senescent human cells in the strategy for the discovery, design, screening and development of new drugs has the potential not only to identify age-related adverse drug side-effects at an early stage, but also to screen successfully for compounds designed to have novel effects in older patients.

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