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Review

From cells to ageing: a review of models and mechanisms of cellular senescence and their impact on human ageing

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Abstract

Given the duration of ageing in humans, cell culture studies are a promising approach to the study of human ageing. It is reasonable to assume that human ageing has, at least partly, a cellular origin. The question is how we can replicate in vitro the age-related changes that occur in human cells in vivo. In this review, widely used models for studying ageing in cell culture, such as Hayflick's, are interpreted in the context of the human ageing process. The mechanisms behind cellular senescence such as telomere disruption and DNA damage are reviewed and their relation to human ageing debated. A system-level examination of these mechanisms suggests that cell culture models are useful for studying cancer and certain age-related pathologies. There is little evidence, however, that cellular senescence is a significant factor in human ageing or that the mechanisms responsible for in vitro cellular senescence are a causative factor in human ageing in vivo. Therefore, novel approaches for studying human ageing at a cellular level are necessary and some suggestions are put forward.

Keywords: Ageing; Cellular proliferation; Cytogerontology; Hayflick's limit; Replicative senescence; SIPS

Introduction

Human ageing can be defined as the collection of changes that render human beings progressively more likely to die [1]. The duration of ageing in humans makes it almost impossible to perform in vivo studies and so the mechanisms involved remain largely a mystery. One simpler alternative is to study ageing in cellular cultures. It is reasonable to assume that human ageing is, at least in part, of cellular origin. In other words, changes occurring at a cellular level contribute to render us more likely to die as we age. The question is how we can replicate in vitro the age-related changes that occur in human cells in vivo.

I will briefly introduce the most widely used models of cellular senescence, such as Hayflick's. Since this and other models have been recently reviewed [2–6], cellular models will not be presented in detail. Rather, the aim of this review is to place cellular models and their mechanisms in the context of human ageing. I will review the current knowledge of the mechanisms involved, how cellular changes could be a factor in human ageing, and then follow a systems biology approach to examine their relevance to human ageing in vivo. Lastly, I will present some novel cell culture methodologies that may serve as useful alternatives to cellular senescence in understanding the human ageing process.

Replicative senescence

Hayflick's model

In 1961, Leonard Hayflick and Paul Moorhead discovered that human fibroblasts derived from embryonic tissues could only divide a finite number of times in culture, usually around 50 CPDs or cumulative population doublings [7], a phenomenon herein called replicative senescence (RS). RS

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has been described in cells derived from adults of all ages and in different cell types as well as in cells taken from several animals including mice, chickens, and the Galapagos tortoise. Exceptions exist and certain human and animal cell lines never reach RS. These are said to be "immortal" and include embryonic germ cells and most cell lines derived from tumours, such as HeLa cells [8].

Briefly, the phenotype of RS in human diploid fibroblasts (HDFs) is characterized by a series of biomarkers [2]. The most obvious biomarker is growth arrest, that is, cells stop dividing. In culture, the percentage of senescent cells progressively increases until all cells are growth arrested [9]. The growth arrest in RS is irreversible in the sense that growth factors cannot stimulate the cells to divide [10].

Other biomarkers exist such as cellular morphology [11] and senescence associated β-galactosidase activity (SA β-gal). The lysosomal hydrolase β-galactosidase is normally active at pH 4. As cells in culture approach their replicative life span, the percentage of cells with β-galactosidase active at pH 6 increases. In immortal cell lines such as HeLa cells, however, the percentage of cells staining positive for SA β-gal does not correlate with CPDs [12]. Lastly, the expression levels of several genes change during in vitro cellular ageing [2,13].

Cell cycle regulation by the telomeres

During ageing in vitro, the telomeres shorten at each subcultivation [14]. The telomerase enzyme serves to lengthen the telomeres, but normal human somatic cells do not have telomerase activity. Expression of the catalytic subunit of human telomerase (hTERT) in both retinal pigment epithelial cells and foreskin fibroblasts avoids RS [15]. HDFs immortalized with hTERT divide vigorously, do not show increased staining for SA β-gal, and do not show signs of transformation [16]. Even expression of hTERT in old HDFs appears to reverse the loss of function characteristic of senescent cells [17].

Either using telomerase or not, all known immortal cell lines must stabilize their telomeres [18]. Telomerase inhibition can induce senescence in cancer cells [19] and defects in telomere replication have been shown to trigger senescence in unicellular eukaryotes such as yeast [20]. Although the details are not yet clear, it appears that telomere length contributes to the stabilization of the telomeres and is the key in avoiding RS. Therefore, telomere length has been considered the molecular clock that keeps track of CPDs and originates RS [21].

How telomere dysfunction induces senescence

Apart from hTERT expression, it is also possible to immortalize HDFs by viral-mediated inactivation of the tumour suppressor protein p53 and the retinoblastoma protein pRb [22,23]. Inhibition of p53 and pRb by antisense technology enabled cells to achieve more 50 CPDs than normal [24]. These findings led to the present concept that two pathways are responsible for inducing senescence.

In agreement with its anti-oncogenic profile, pRb is a central regulator of cell cycle progression [25]. Hyperphosphorylated pRb allows the cell cycle to proceed while hypophosphorylated pRb prevents cell cycle progression. pRb presumably operates through inactivation of the E2F family of transcription factors, responsible for transcription of several genes involved in G1/S transition and DNA synthesis [2,26]. Succinctly, the phosphorylation of pRb is dependent on cyclin-dependent kinases (CDKs) that govern the progression through the various phases of the cell cycle [27]. Inactivation of the G1 CDKs, responsible for the phosphorylation of pRb, prevents transition from phase G1 to phase S and blocks the cell cycle, originating, for example, RS.

Cyclin-dependent kinase inhibitors (CDKIs), as the name implies, inhibit the activity of CDKs. The CDKI $p16^{INK4a}$ disrupts and inhibits the activities of CDK4 and CDK6, thus preventing cell cycle progression [28]. Immortalization may also be achieved by disruption of both $p16^{INK4a}$ and p53 [29], suggesting $p16^{INK4a}$ acts upstream of pRb in regulating RS. Another important CDKI is $p21^{WAF1}$, which also has the ability to block the cell cycle by inhibiting CDK2, CDK4, and CDK6 and thus preventing pRb phosphorylation [30]. Both $p21^{WAF1}$ and $p16^{INK4a}$ can induce senescence [31]. Since $p21^{WAF1}$ expression levels increase in pre-senescent cells before $p16^{INK4a}$ overexpression, $p21^{WAF1}$ likely triggers senescence before $p16^{INK4a}$ [32]. In contrast, $p16^{INK4a}$ remains overexpressed in senescent cells while $p21^{WAF1}$ levels wane [31].

Overexpression of p53 leads to cell cycle arrest or apoptosis [33]. The induction of p53 by DNA-damaging agents led to the suggestion that p53 is a checkpoint factor that prevents cells from accumulating mutations by inducing apoptosis or growth arrest [34]. Although it is possible that other downstream targets of p53 exist, p53 induces $p21^{WAF1}$ [35] and it is likely that $p21^{WAF1}$ is involved in RS mediated by p53.

Increased levels of p53 have been associated with critically short telomeres [36] and p53-deficiency attenuated the phenotype of telomere dysfunction [37]. Thus, p53 is probably responsible for recognizing dysfunctional telomeres—for example, critically short telomeres—as DNA damage and triggering RS. Indeed, activation of p53 occurs as HDFs approach senescence [36]. Therefore, p53 appears to be the major initiator of senescence, while $p16^{INK4a}$ presumably maintains senescence [38].

Overall, whatever changes occur during telomere dysfunction, the mechanisms triggering growth arrest appear to involve DNA damage pathways. Moreover, recent results confirm that the chromosome ends of senescent cells contribute to the DNA damage response and that uncapped telomeres directly associate with many DNA damage response proteins [39]. As such, the most likely explanation is that dysfunctional telomeres are recognized as DNA

damage. Although unidentified players may also be involved, the most widely accepted hypothesis is that the p53 and pRb/$p16^{INK4a}$ pathways collaborate to stop cellular proliferation. Probably, the p53 pathway involving $p21^{WAF1}$ is activated beforehand, while $p16^{INK4a}$ prevails under strong physiological stimuli or stress and to maintain cells growth arrested (Fig. 1).

RS as a stress-response mechanism

Cell culture does not mimic in vivo conditions

There is ample evidence that cell culture does not mimic the conditions cells encounter in vivo [4,40]. Cells in vivo are subject to controlled pH and temperature levels that cannot be replicated in vitro. Even more critically, cell culture conditions normally feature 20% oxygen (O_2), which is much higher than O_2 at typical physiological conditions. It is not surprising then that an inverse correlation is witnessed between CPDs and O_2 culture conditions [41]. Recent results also show that oxygen sensitivity limits the proliferative capacity of mouse fibroblasts [42]. Together, these results strongly argue that oxidative stress in cell culture is a factor not present in vivo.

Cell culture typically involves the serial passage of cells through flasks as cells proliferate [7,8]. Each time cells in culture are subcultivated, however, they are exposed to proteolytic insult by trypsin. This procedure, not normally encountered in vivo, may have grave consequences on cellular performance by degrading receptors and other molecules with extracellular domains [43,44].

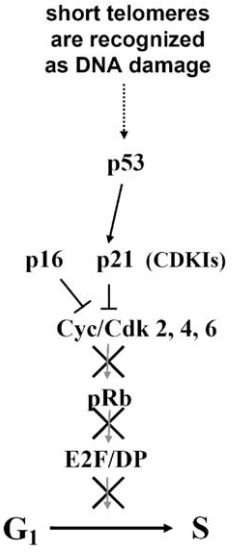

Fig. 1. From critically short telomeres to irreversible growth arrest at the G1/S transition. Telomere dysfunction causes an activation of DNA repair pathways, such as the activation of p53. In turn, p53 activates $p21^{WAF1}$ that blocks the actions of several CDKs preventing the phosphorylation of pRb. Without hyperphosphorylated pRb the transcription of several critical genes in the G1/S transition does not take place, thus blocking the cell cycle.

Probably, the biggest difference between in vivo and in vitro conditions is that cells in culture are deprived of interactions with other cell types via, for example, growth factors and hormones. For instance, differences in cell behavior have been reported between 2-D and 3-D culture systems [45], showing how the microenvironment is essential to cell behavior and function [46]. Mina Bissell's work on 3-D culture systems is a good example of the limitations of typical culture conditions [47]. Equally important, the interaction with other cell types is crucial for the regulation of cell proliferation, differentiation, and apoptosis. In cell culture, these interactions are absent.

Models of cellular senescence

Clearly, cell culture is an artificial, harsher environment for the cell that may be considered as stressful [40,48]. When HDFs are cultured at 3% O_2, which is closer to physiological conditions, they achieve a further 20 CPDs [49]. In contrast, different types of human cells cultured above 20% O_2—but below 50%, which is cytotoxic—displayed a reduced growth rate and reached RS at lower CPDs [50]. Not only is proliferate life span shortened, but subcytotoxic stress, either by oxygen or through other stressors, appears to accelerate the senescent phenotype [5,6,50]. In 1999, at the EMBO workshop of Molecular and Cellular Gerontology in Olivone, Switzerland, the term stress-induced premature senescence (SIPS) was coined [51].

Succinctly, one key biomarker of SIPS is growth arrest: after exposure to H_2O_2, HDFs show a decrease in CPDs. Exposure to H_2O_2 also induces a senescent-like morphology [52]. Moreover, an increase in the proportion of SA β-gal-positive cells in SIPS has been reported for many types of cells under different stressors, namely in HDFs submitted to oxidative stress [53]. Several genes differentially expressed in RS show similar expression changes in SIPS as a result of oxidative stress [54]. Telomere shortening may also be accelerated under SIPS [50,55].

The mechanisms of RS and SIPS appear to overlap. In parallel to what happens in RS, pRb plays a crucial role in SIPS [56], as does p53 [54]. DNA damage may activate p53, which in turn triggers $p21^{WAF1}$. In addition to the p53/$p21^{WAF1}$ pathway, $p16^{INK4a}$ is likely involved in SIPS since $p16^{INK4a}$ is induced by DNA damage [57]. Taken together, these results suggest that DNA damage triggers a set of molecular pathways similar to those involved in RS, though it is possible other pathways also have the ability to cause SIPS. For example, many signalling pathways activated by stress, such as reactive oxygen species, can contribute to p53's activation [58].

SIPS has been observed for many stressors—for example, ethanol, ionizing radiations and mitomycin C—in many types of proliferative cells such as lung and skin fibroblasts, endothelial cells, melanocytes, and retinal pigment epithelial cells [5]. In addition to DNA damage—either caused by

oxidative stress or not—cellular senescence can also be induced by several oncogenes such as ras, again probably involving the p53 and $p16^{INK4a}$ pathways [3,59].

Complementary pathways of cellular senescence

Cellular senescence is primarily characterized by a loss of proliferative potential. RS occurs when a cell population cannot divide indefinitely in vitro. Since the percentage of cells actively dividing decreases with CPD [9], it is normal that the cell population ages, without changes other than more cells becoming growth arrested. Thus, a cell population ages in vitro when there is an increase in the percentage of growth-arrested cells: a mitotic coefficient (M_{coef}) of (mitotic)/(quiescent) cells < 0.5 per population doubling, not taking into account cell death.

In WI-38 HDFs, $p16^{INK4a}$ is widely overexpressed in presenescent and senescent WI-38 HDFs. Interestingly, WI-38 HDFs reach senescence with relatively long telomeres, suggesting that neither telomere shortening nor p53 cause RS in WI-38 HDFs [60]. In contrast, BJ cells, which are more resistant to oxidative stress [61], reach RS due to critically short telomeres without an overexpression of $p16^{INK4a}$ [60,62]. Also, the immortalization of human epithelial cells requires inactivation of $p16^{INK4a}$—or E7 expression to inhibit pRb—in addition to hTERT activity [63]. Yet under 2% O_2, epithelial cells can be immortalized with hTERT activity alone, suggesting that oxygen sensitivity activates $p16^{INK4a}$ and induces senescence independently of the telomeres [64,65]. Indeed, if $p16^{INK4a}$ is inactivated, the proliferative capacity of epithelial cells is increased and senescence is only reached when telomeres are critically short [66].

Overall, these studies indicate that the mechanisms of cellular senescence, including RS and SIPS, can then be seen as complementary in the sense they cause M_{coef} to fall below 0.5/PD. Some cell lines reach senescence not due to short telomeres but because of their increased sensitivity to O_2. RS and SIPS should then be seen as examples of a broader process termed cellular senescence [3]. The question then is whether the study of cellular senescence is relevant to human ageing.

Cellular senescence in ageing and cancer

The cellular component of organismal ageing

In the liver of mice, it was observed that ageing lowered the apoptotic response [67]. Similar results have been obtained in human lymphocytes [68]. A decline in fibroblast proliferation during human ageing has been reported [8,69], though results are contradictory [70]. In some tissues, such as the immune system, decreased proliferative ability may play a role in age-related degeneration [71,72]. Likewise, neuronal death appears to contribute to brain ageing [73]. In mice, mitotic cells decline with age in the lens epithelium [74]. Lastly, old stem cells have a diminished function when subjected to stress, suggesting intrinsic changes [75]. Taken together, these results hint that changes occur with age at a cellular level and are likely play a role in human ageing [3,72,73].

One possibility is that these are effects rather than causes of ageing since some studies suggest cellular degeneration with age is a result of extrinsic factors [8,40,76]. For example, the serial transplantation of bone marrow in mice indicates that changes in ageing are not related to intrinsic changes [77]. In contrast, organ transplantation in human patients suggests, at least to a certain degree, intrinsic changes during ageing. Bone marrow transplantation from older donors affects patient survival [78]. Liver transplants from older donors (>70 years) are associated with increased mortality [79] and patient mortality decreases when younger heart donors are used [80]. Lastly, kidney transplants may be influenced by donor age [81] and diminished functional reserves in kidneys from older donors are observed [82].

Cells taken from patients with progeroid syndromes, which appear to suffer from accelerated ageing, behave differently in culture: Werner syndrome patients' cells divide slower while cells from Hutchinson–Gilford syndrome patients divide faster than normal [83,84]. Cells from both of these progeroid diseases display more apoptosis than normal cells and are more susceptible to certain forms of stress [84,85]. Therefore, progeroid syndromes may be explained by alterations at a cellular level [84].

As a whole, these observations suggest that intrinsic cellular changes occur as we age, at least in some organs. The major question then, for the purpose of this review, is whether cellular senescence is appropriate to study these changes.

Cellular senescence in mammalian ageing

Senescent cells and senescence-associated biomarkers can be found in vivo. Briefly, HDFs cultured from distal lower extremities of patients with venous reflux, which precedes the development of venous ulcers, display characteristics of senescent cells [86]. Similar results also relate cellular senescence to atherosclerosis [87] and benign prostatic hyperplasia, a common age-related male pathology [88]. Senescence and inflammatory processes may be related to age-related pathologies such as osteoarthritis [89] and skin ageing [17]. Even though telomere length varies widely among individuals and between different tissues, telomere length has been linked to pathology in the elderly [90]. A study in rat kidneys showed $p16^{INK4a}$ levels increase dramatically with growth and ageing. As a whole, these results indicate that cellular senescence and its mechanisms appear in vivo and could contribute to at least some clinical aspects of human ageing [4,6,72,73].

Obviously, looking in vivo for biomarkers of senescence initially identified in vitro, often in HDFs, may be incorrect. It is not surprising then that many of these biomarkers have been recently attacked: at least post-partum, there is no

relation between the number of CPDs at RS and the age of the donor [91]. Studies in centenarians also failed to find a decrease in the CPDs of cells at RS [70]. In addition, they raised doubts on whether telomere shortening occurs in vivo and whether senescence-associated genes in vitro are also differentially expressed in vivo [92]. Though SA β-gal has been reported to increase in vivo [12], its role in vivo has also been attacked [93]. Moreover, there is a lack of quantification in these studies. For example, it has not been determined how many senescent cells are necessary to cause a physiological decline in vivo.

Cells from INK4a-deficient mice have increased proliferation and yet these animals not appear to age more slowly [94]. Likewise, cells taken from patients with DNA repair defects often show growth parameters similar to that of progeroid syndromes and yet these patients do not suffer from accelerated ageing [95]. In fact, cells from progeroid syndromes immortalized with viral-oncogenes or hTERT maintain their characteristic phenotypic properties of slow growth, diminished stress resistance, etc. [95,96], raising doubts on whether RS and cellular immortalization are accurate approaches for studying the mechanisms of these diseases.

Overall, these results cast doubts on whether RS is related to human ageing. Clearly, a major misconception derives from the usage of the term "immortality" when referring to cell cultures that do not cease division [3,40]. Many non-dividing cells are essential to the organism as loss of proliferative capacity in vivo is frequently not a sign of senescence or functional decline but rather a consequence of normal differentiation. Thus, neurons that lose their proliferative capacity early in development as part of their differentiation program and survive for decades in man are by no means senescent.

A system-level understanding of cellular senescence

Just because two processes parallel each other does not imply a causal relation in any direction. One way to infer the impact on ageing of the pathways described herein is using a system-level approach. By perturbing each component of a pathway under study and integrating the observed effects it is possible to discriminate causes from effects and formulate new hypothesis [97]. What follows is a system-biology approach of the pathways previously described based on several perturbations of the pathway's components (Table 1).

Knocking-out telomerase in mice through deletion of the RNA component (TERC) from the germline, while not preventing cancer [98], appears to increase cancer resistance [99]. On the other hand, TERT overexpression in mice promoted cancer development [100]. Mutations in p53 have been associated with Li–Fraumeni syndrome, which is characterized by increased cancer incidence [101]. Germline mutations in $p16^{INK4a}$ have too been implicated in familial melanoma [102], CDK4 mutations appear to induce tumour formation [103], and polymorphisms in $p21^{WAF1}$ have been associated with cancer [104]. The retinoblastoma gene is also a class of cancer gene [105]. Clearly, the mechanisms involved in cellular senescence in vitro are related to cancer (Table 1).

More dubious is the role of cellular senescence and the telomeres in animal ageing (Table 1). The basic argument is that tissue function declines with age because the capacity for renewal or repair is progressively lost. Disruption of CDK4 in mice led to diabetes mellitus, a common age-related pathology, but this appears to be due to the degeneration of pancreatic beta cells [106]. Importantly, mice lacking TERC were viable up to six generations. Telomeres gradually shortened leading to several pathologies, with a special emphasis on highly proliferative tissues [98]. On the other hand, telomerase overexpression does not affect ageing in mice [100]. Mice also have long telomeres and feature high telomerase activity in many organs, in contrast to humans [107]. Therefore, telomere length and telomerase activity do not explain why humans age slower than mice but it helps explain why mice have a much higher cancer incidence than men [108]. Although changes in p53 activity have been

Table 1
Perturbations affecting the components of the pathways regulating in vitro cellular senescence

Protein name	Perturbation	Organism	Phenotype	Cancer	Ageing	References
TERC	Disruption (−/−)	Mouse	Increase in cancer resistance	+	−	[98,99]
TERT	Overexpression	Mouse	Cancer promotion	+	−	[100]
TERC	Mutation	Human	Dyskeratosis congenita	+/−	−	[111]
p53	Mutation	Human	Li–Fraumeni syndrome	+	−	[101]
p53	Disruption (−/−)	Mouse	Increase in cancer incidence	+	−	[109]
p53	Activated p53 mutation	Mouse	Decrease in cancer incidence but early-ageing signs	+	+	[109]
$p21^{WAF1}$	Polymorphisms	Human	Tumour-associated	+	−	[104]
$p16^{INK4a}$	Germline mutations	Human	Familial melanoma	+	−	[102,103]
INK4a	Deletion (−/−)	Mouse	Tumour induction	+	−	[94]
CDK4	Mutation	Human	Tumour induction	+	−	[103]
CDK4	Disruption (−/−)	Mouse	Growth retardation, infertility and diabetes mellitus	+	+/−	[106]
pRb	Mutation	Human	Retinoblastoma	+	−	[105]
E2F1	Disruption (−/−)	Mouse	Tumour induction	+	−	[119]

linked with murine ageing [109], Li–Fraumeni syndrome does not appear to alter the human ageing process [101]. Of course that these are mostly disruptive changes, so maybe other types of interventions affect ageing and not solely cancer, as demonstrated with p53 [109].

Mutations in hTERC are associated with the autosomal dominant form of dyskeratosis congenita [110], an inherited disease mostly affecting proliferative tissues [111]. Features of dyskeratosis congenita include bone marrow failure, which is the most usual cause of death, abnormal skin pigmentation, leukoplakia, and nail dystrophy [112]. As judged from the phenotype of dyskeratosis congenita and the telomerase knockout mice, telomeres are crucial in rapidly proliferating tissues but it is unclear whether telomere shortening is involved in human ageing. If RS measures cellular proliferation, then it is normal that most research on RS has focused on cancer and the mechanisms of cellular proliferation. Yet an understanding of human ageing cannot be complete if only the most rapidly dividing tissues are studied. Indeed, there is a great deal of variation in vivo in terms of mitotic rate. For instance, ageing occurs in the heart even though it is a mostly post-mitotic organ while kidney and liver cells have a much lower cell turnover than epithelial cells [8].

In conclusion, these observations strongly argue in favor of seeing cellular senescence as a tumour-suppressor mechanism [3,73]. The most likely hypothesis is that the increase in the proportion of senescent cells in vivo is a consequence rather than a cause of ageing. Overall, cellular senescence in vitro merely investigates the proliferative capacity of the most proliferating tissues, giving insights into cancer and maybe some age-related diseases affecting rapidly dividing tissues but barely increasing our knowledge of human ageing.

Beyond Hayflick's limit: a look ahead

As argued above, although human ageing appears to have a cellular component, cellular proliferation is not a good marker. Therefore, alternative methodologies to cellular senescence and proliferation are necessary to study the essence of cellular changes with age. Firstly, cellular studies should be done from the perspective of human ageing in vivo, not the other way around. For instance, recent gene expression experiments may allow us to find new biomarkers of senescence in vivo which can then be studied in vitro.

One frequent feature of life-extension in model organisms is increased stress resistance at a cellular and organismal level [113]. For example, the disruption of $p66^{shc}$ in mice not only increases longevity but also renders mouse embryonic fibroblasts more resistant to different types of stressors [114]. Stress resistance in vitro also correlates with mammalian longevity [115]. Moreover, as mentioned above, cells taken from patients with progeroid syndromes are more susceptible to stress [83,84]. Therefore, an association appears to exist between cellular stress resistance and organismal ageing. Studying this association is a possible alternative to RS.

One recent study in mice reported a correlation between the longevity of different strains of mice, oxidative stress resistance, and survival of senescent cells [116], suggesting that other cellular models besides RS and SIPS may be useful in studying ageing. Studying cellular responses to external stressors and signals could be useful. For example, in vivo changes in the brain's gene expression were partly mimicked in culture by inducing DNA damage [117]. Lastly, it has been suggested that that the response of an intact organism to radiation is largely determined by the sensitivity of stem cells rather than differentiated cells [118]. As such, studying stem cells rather than HDFs in vitro may be more useful to understand human ageing in vivo.

As mentioned above, a major issue is whether cell culture adequately reflects in vivo conditions. For cellular senescence to be informative about ageing, it is important that the laboratory conditions mimic the organismal conditions. Though mostly fostered by cancer research, 3-D culture systems may be an alternative to study cellular ageing in vitro [45].

Concluding remarks

The mechanisms described in this review show a decrease in functionality and survival of a cell population. Advocates of these models argue that similar changes occur at a cellular level in vivo. Yet there is little evidence RS or SIPS occur widely in normal human ageing and no causal connection between cellular senescence in vitro and human ageing in vivo has been established. The most likely scenario is that the mechanisms of cellular senescence evolved as an anti-cancer mechanism to prevent uncontrollable cellular growth, DNA damage, or other oncogenic signals.

In conclusion, the mechanisms behind in vitro cellular senescence, as derived from the study of RS and SIPS, appear to be causal factors in tumorigenesis, could be involved in certain age-related pathologies, but do not appear to be major players in human ageing. Cellular changes occur with age but the essence of those changes remains unknown and so new approaches are necessary to investigate ageing at a cellular level. The issue is what mechanisms occur in vivo and how we can experiment these in vitro. Finding novel models and parameters to measure remains one of the challenges of cytogerontology.

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