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Cellular aging and cancer

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Abstract

Aging is manifest in a variety of changes over time, including changes at the cellular level. Cellular aging acts primarily as a tumor suppressor mechanism, but also may enhance cancer development under certain circumstances. One important process of cellular aging is oncogene-induced senescence, which acts as an important anti-cancer mechanism. Cellular senescence resulting from damage caused by activated oncogenes prevents the growth or potentially neoplastic cells. Moreover, cells that have entered senescence appear to be targets for elimination by the innate immune system. In another aspect of cellular aging, the absence of telomerase activity in normal tissues results in such cells lacking a telomere maintenance mechanism. One consequence is that in aging there is an increase in cells with shortened telomeres. In the presence of active oncogenes that cause expansion of a neoplastic clone, shortening of telomeres leading to telomere dysfunction prevents the indefinite expansion of the clone because the cells enter crisis. Crisis results from fusions and other defects caused by dysfunctional telomeres and is a terminal state of the neoplastic clone. In this way the absence of telomerase in human cells, while one cause of cellular aging, also acts as an anti-cancer mechanism.

Keywords

Aging; senescence; telomeres; crisis; cancer development; tumor suppression; experimental tumorigenesis; inflammation; innate immunity

1 Introduction

It is often stated that, because cancer incidence is strongly age-related, cancer must be a disease of aging. In the past, this has not been a universally accepted view [1]. If cancer is not related to aging, then the age-related increase in cancer is explained by the facts that cancer takes several molecular steps for full development, and each step takes time; however, those steps are neither more nor less likely in an old individual than a young one. Although there is some validity to this view, it has also become clear over the past decade or so that aging impacts cancer initiation and progression in many ways. Aging comprises many time-dependent changes in organs and tissues; a variety of age-dependent changes occur at the cellular level in tissues. Collectively these changes are termed cellular aging. In this review the basic science of cellular aging and its impact on cancer are reviewed.

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Conflict of interest statement

The author has no conflict of interest.

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While the emphasis in this review is on specific aspects of cellular aging and their impact on cancer, it is important to place this in context. A very large variety of time-dependent changes take place in the human body and to varying extents cause the changes in the body that we term aging. While the aspects of cellular aging reviewed here are important, they no doubt form only a very small aspect of the total set of processes that comprise the aging process as a whole.

2.1 Telomere shortening in culture

The earliest described form of cellular aging comprised the phenomenon often associated with the name of its discoverer, Leonard Hayflick [2]. In the 1960s Hayflick showed that normal human cells could not divide indefinitely in culture. Decades later it was shown that this limit results from progressive cell division-dependent shortening of telomeres [3,4]. Telomeres shorten in most dividing human somatic cells because they lack activity of the enzyme complex telomerase, which is required for telomere maintenance [5,6]. The lack of telomerase activity results from the absence of expression of the reverse transcriptase subunit (TERT) of the telomerase ribonucleoprotein complex [7,8]. When cells divide in the absence of telomerase activity about 40–100 bp of the terminal telomeric repeat DNA is not replicated [5,6]. This amount is a constant for various types of human cells, thus providing a kind of mitotic counter [5,6].

While Hayflick called the limited replicative potential of normal human cells “aging under glass,” the term cellular senescence came into use as the standard term for the phenomenon. The process was understood as comprising two steps: first the progressive shortening of telomeres, causing telomere dysfunction, and second the state of permanent inability for cell division that results from telomere dysfunction. Subsequently it became evident that telomere shortening was only one of many ways in which cells could become senescent. In fact, many types of cellular stress can drive cells into a permanently nondividing state, now be recognized as cellular senescence [9]. In the 1990s it was shown that activated oncogenes can cause senescence in normal human cells [10]. The significance of oncogene-induced senescence is considered later in this review. In terms of cellular aging and cancer, there are two topics that must be considered separately: first, telomere biology, aging and cancer, and second, cellular senescence and cancer.

2.2 Telomere-based crisis

In normal human cells in culture, telomere dysfunction leads to permanent growth arrest. The permanent cessation of growth that defines cellular senescence requires the operation of cell cycle checkpoints. In particular, cell cycle checkpoint machinery that depends on the p53 and pRB pathways must be functional for senescence to be able to take place. This complex topic has recently been comprehensively reviewed [9]. When the normal operation of those pathways is interrupted, under experimental conditions in culture, telomeres continue to shorten progressively and become increasingly dysfunctional. Unprotected telomeres cause the cell to enter a terminal state termed **crisis** [11]. Telomere-based crisis and telomere dysfunction-based senescence both result from telomere shortening, but in the former case there is no cessation of cell growth or of DNA replication. In crisis, short dysfunctional telomeres cause end-to-end chromosome fusions. Evidence for fusions is seen as the occurrence of anaphase bridges [12]. In cells with disrupted checkpoints, telomere fusions result in (i) breakage-fusion-bridge cycles, leading to increasing aneuploidy; and (ii) mitotic catastrophe, a failure of cytokinesis, resulting in tetraploidization, multipolar cell division, and gross aberrations in chromosome number [13–19]. Mitotic catastrophe leads to arrest in mitosis, or alternatively to the formation of cells with multiple nuclei or a single giant nucleus [15,16,19]. Whereas crisis is often termed a form of cell death, observations in the author's laboratory indicates that death of cells in crisis is nonspecific; cells become

extremely enlarged and appear to be unable to maintain a normal degree of contact with the plastic substratum [20]. When such cells are replated on a more adherent substratum, e.g. collagen-coated plastic, they re-attach and can be shown to be still living [unpublished observations]. In many previous studies of cells in crisis in culture, the fact that the cell population gradually decreased over time was interpreted as resulting from a progressive death of the cells [11,17–19]. However, these studies did not show a specific cell death mechanism in the cells being lost from the population, and in view of the author's observations it seems likely that the loss of cells from the population results from an inability to maintain cell attachment rather than cell death. Additionally, oncogenic mechanisms that allow the bypass of telomere dysfunction-based senescence would typically result in a loss of the ability of the cell to undergo apoptosis [11].

2.3 Role of telomere length and telomerase in experimental tumorigenesis

The evidence that crisis is a reliable barrier to continued growth of premalignant or pre-tumorigenic cells comes from experiments in which SV40 large T antigen and oncogenic Ras were expressed in normal human cells. SV40 is an oncogenic DNA virus that encodes several genes; of these the large T antigen gene is essential for the tumorigenic effects of SV40 while the others are to varying extents dispensable [21,22]. SV40 T antigen binds and inactivates p53 and pRB [21,22]. Ras is a small GTPase signal transduction protein that interacts with several intracellular pathways involved in a variety of cellular functions; when mutated such that it becomes GTP-independent it acts to drive cell proliferation and to confer malignant properties such as anchorage independence and invasiveness [23]. These two genes, SV40 T antigen and mutated Ras, form a convenient and effective combination to convert normal human cells to a tumorigenic state (Figure 1). To test the tumorigenic potential of these cells they were transplanted beneath the kidney capsule of immunodeficient mice [20,24]. This combination is tumorigenic in human adrenocortical cells [24], human skin fibroblasts [20], and primary colon smooth muscle cells [25].

In view of earlier data [26] it was surprising that these two genes were sufficient to convert normal human cells into aggressively growing invasive cancers. They were nevertheless not immortal; while tumors could grow to 1–2 cm in immunodeficient mice, they could not be serially transplanted. In all cases primary or secondary tumors entered crisis; no escapes from crisis via activation of telomerase or ALT (alternative lengthening of telomeres; see ref. 27) were observed in more than 200 animals that received transplants of these cells (ref. 20 and unpublished observations). The lack of escape by development of some form of telomere maintenance mechanism indicates that crisis reliably prevented the continued growth of the cancer. Thus, at least in a direct experimental test, crisis acts to prevent the continued growth of a clone that would otherwise form a lethal cancer.

2.4 Telomere biology in tissues in vivo

In cultured human cells and in experimental xenografts, the absence of telomerase activity acts as a barrier to continued growth of the tumor. If this is also to act in tissues in vivo, it will depend on the absence of telomerase activity in normal tissues in vivo. Humans as a species have exceptionally short telomeres. Although there is evidence for telomerase activity in some stem cells in vivo, most tissues and cells show a progressive shortening of telomeres in vivo as a function of age (reviewed in ref. 28). Thus many human cells are completely telomerase negative, and when stem cells have telomerase activity it is not at a level that is capable of preventing telomere shortening in the differentiated cells that are derived from stem cells. The earliest observations on an age-related decline in proliferative capacity, presumably resulting from telomere shortening, was on dermal fibroblasts, but these observations were later challenged [29–32]. However, most fibroblasts are probably proliferatively quiescent in vivo after maturity and undergo very low rates of cell division.

Therefore it would not be surprising if little or no exhaustion of proliferative capacity were observed. Nevertheless, the progressive decline in telomere length has been observed in many other cell types [28].

2.5 The consequences of telomere dysfunction in vivo

The available data in experimental systems suggest that, in human cells, the combination of short telomeres (i.e. short as a species) and suppression of TERT expression may together provide an anti-cancer mechanism. Thus it is necessary to consider the evidence that telomere dysfunction can lead to either senescence or crisis in human tissues in vivo.

Based on work in cell culture, one might expect that short-telomere cells in tissues would stop dividing when they reach a telomere length that triggers senescence. However, observations made in the human genetic disease dyskeratosis congenita (DKC) suggest that perhaps this does not occur in vivo. DKC is a disease of impaired telomerase activity and shortened telomeres [33–35]. In one form of the disease (X-linked) the DKC1 gene is defective; its protein product, dyskerin, is required for proper RNA processing, including the RNA of the telomerase ribonucleoprotein complex. In an autosomal dominant form of DKC, telomerase RNA is mutated. In these syndromes there are proliferative defects in tissues known to have telomerase-positive stem cells (hematopoietic system and skin). DKC patients have very short telomeres in fibroblasts and white blood cells. They usually die of bone marrow failure at a young age. However, the disease is also associated with chromosomal abnormalities, suggesting that in this case telomere shortening in human tissues in vivo might lead to crisis rather than replicative senescence.

One of the key signatures of telomere dysfunction is the anaphase bridge, as described earlier. Cells with anaphase bridges, abnormal nuclei and other features of mitotic catastrophe are often observed in human cancers [15,16,19]. While anaphase bridges are often seen in human cancers, it is of great interest that they are also observed at low numbers in non-cancer tissues [36]. In non-neoplastic cells adjacent to esophageal cancer there is an inverse correlation between telomere length and anaphase bridge frequency [37]. In the oral epithelium there is also an inverse relationship of anaphase bridge frequency and telomere length, and there is an increase in anaphase bridges as a function of age of the donor [38].

These data suggest that even normal cells can undergo sufficient telomere shortening in tissues in vivo that the resultant telomere dysfunction causes fusions and anaphase bridges. Possibly such cells may even be considered to be in crisis. This is surprising; why do these normal cells not enter senescence as a result of telomere dysfunction, well before reaching a stage at which short telomeres result in chromosomal abnormalities? At least one reason may be that these cells have acquired multiple abnormalities; they may have acquired mutations that activate oncogenes and thereby inactivate cell cycle checkpoints that would normally cause the cells to undergo senescence or apoptosis. When senescence or apoptosis is blocked, cells continue to divide and may acquire further abnormalities, proceeding eventually to crisis [39]. Currently we do not know whether cells with indications of crisis that are observed in tissues in vivo harbor multiple abnormalities, either in normal individuals or individuals with genetic diseases that cause telomere dysfunction; more research is needed to determine this.

On the other hand, there is ample evidence that telomere dysfunction can lead to typical senescence, as expected for cells with normal cell cycle checkpoints. There is evidence for an association between telomere length and markers of senescence in tissues in vivo. In early studies, greater numbers of senescent cells were found in the skin of human subjects as a function of age of donor [40]. One of the most careful investigations of the increase in senescent cells in aging is in tissues of the baboon [41,42]. Fibroblasts in skin biopsies had

various markers of senescence: for one senescence marker, the fraction of positive fibroblasts increased linearly from ~20% at age 5 years to ~80% at age 29 years. Additionally, the fraction of fibroblasts in skin biopsies having telomere-dysfunction induced foci (TIF) rose from ~2% in the young animals to ~17% in the old. Thus in these observations there is evidence for multiple markers of senescence within individual cells and, moreover, there is an age-dependent increase in the number of cells that have both telomere damage together with other markers of senescence [41,42]. Although these data show damage to telomeres, it does not necessarily indicate that this is caused by telomere shortening. Damage might also result from a greater susceptibility of telomeres to DNA damage from causes such as oxidative stress [43]. As mentioned earlier, dermal fibroblasts generally have a low rate of cell division *in situ*.

The data on experimental tumorigenesis discussed earlier suggest that telomerase is not a requirement for initial cancer formation, but is required when tumors have grown to the extent where telomere shortening limits their growth. In light of these experimental results, it is relevant to ask why telomerase-negative cancers that have a history of self-limiting growth are not observed clinically. There may be a few cancers that do grow extensively and then stop because of lack of telomerase [44]. Probably more frequently cancers that lack telomerase and do not acquire sufficient telomerase activity never grow large enough to be clinically detectable. The exception to that statement may be dermatological cancers, which have a greater likelihood of being detected at very early stages. Small squamous cell carcinomas may lack a telomere maintenance mechanism [45]. In a mouse, a 2-gram cancer that is not immortal can grow large enough to kill the animal [20]. In a human a similarly sized cancer may well be clinically undetectable, and after the cells enter crisis and eventually die little trace of the neoplasm's existence may remain. Although cells in experimental tumors that enter crisis do not die by apoptosis they do eventually die via nonspecific necrosis that occurs after the tumor stops enlarging [20]. Another reason cancers lacking a telomere maintenance mechanism are not more often detected is that experimentally, using genetic modification, one can "instantly" confer on a previously normal cell the properties that make it tumorigenic. Thereafter the cell clone can expand extensively before crisis ensues. However, in a more clinically realistic scenario, a neoplastic or pre-neoplastic clone acquires many random mutations, many of which do not confer growth or survival properties on the cells. Many or most of the cells are lost by various means -- death, senescence or differentiation -- before crisis is reached [18]. Thus the clone may still be very limited in size when it reaches crisis and dies out. As early detection of cancer improves, it may become more common to find very small malignant lesions that lack telomere maintenance mechanisms.

If, at some point during the growth of the clone or at crisis, cells within the clone acquire a sufficient level of telomerase activity for telomere maintenance then crisis can be bypassed [11,17,18]. Most cancer cells have activated mechanisms of telomere maintenance, either as a result of increased expression of telomerase reverse transcriptase (hTERT) or activation of ALT [11,46]. Mechanisms by which hTERT gene expression is activated are partially understood [47]. One potential mechanism is via mutational or epigenetic activations of oncogenes such as c-Myc, which promote the transcription of the hTERT gene [47]. ALT is poorly understood and, similarly, the process by which ALT is activated in tumorigenesis is unclear [48]. However, in the great majority of abnormal pre-malignant clones neither activation of hTERT nor activation of ALT occurs. Thus in human somatic cells the lack of a telomere maintenance mechanism, resulting from the lack of sufficient telomerase activity to permit indefinite growth, exerts a significant barrier to the formation of a lethal cancer from a clone of cells that otherwise has a set of mutations that give it malignant properties.

3.1 Senescence resulting from the action of oncogenes rather than telomere shortening

Although senescence was first described as the result of telomere shortening, it was later recognized that many cellular events can drive cells into senescence. Although these events are often described as premature stress-induced senescence, the term premature does not have any real meaning here -- senescence as an end-point can be attained by many stresses, one of which is telomere dysfunction. Among these stresses, as a cause of senescence, is the action of activated forms of oncogenes [49–51]. There is much evidence that oncogene-induced senescence occurs *in vivo* and is an important protective mechanism. For example, it is likely that senescence protects against melanoma *in vivo*. Most nevi have a mutation of the BRAF oncogene and also have markers of cellular senescence [52,53], although they do not have short telomeres [53]. BRAF is homolog B1 of the Raf murine sarcoma viral oncogene; it is a serine/threonine protein kinase involved in the regulation of the MAP kinase pathways that affect many cellular processes [54].

There may be a common pathway for the action of activated oncogenes and DNA damage, including telomere dysfunction. High levels of activation of members of the E2F family, which act downstream of several oncoproteins, cause the formation of nuclear foci that indicate the presence of double strand breaks, with characteristic proteins such as γ -H2AX and 53BP1 [55,56]. Early stage cancers also have similar DNA damage foci [57,58]. High levels of stimulation of the cell cycle machinery by oncoproteins may cause *replication stress*, a partially characterized process in which DNA damage is an indirect consequence of imbalances in the cellular replication machinery [51,55–57,59,60]. Potential mechanisms of oncogene induced senescence and DNA replication stress have been reviewed in detail [61]. These include (1) direct interactions of oncogenic proteins with DNA replication origins; (2) increased numbers of active DNA replication origins; (3) increased rates of fork stalling; (4) DNA re-replication, i.e. re-firing of the same origin before chromosome segregation. It is possible that excessive origin firing triggers the DNA damage response by depleting limiting DNA replication factors or by generating regions of single-stranded DNA, which activate the ATR-dependent checkpoint [61]. This is a very active area of research in which new insights may be expected [62].

3.2 Potential clearance of senescent cells by the innate immune system

It has been realized for many years that senescence of cells is accompanied by a variety of changes in gene expression. This has been termed the senescence-associated phenotype or the SAS (senescence associated secretome) and the range of changes in gene expression is similar in different types of senescence (e.g. senescence resulting from telomere shortening and oncogene-induced senescence) [63,64]. This altered state, in which cells resemble the state of fibroblasts in inflammation, provokes clearance of senescent cells by the innate immune system, principally natural killer (NK) cells [64,65]. A specific mechanism has been described in a multiple myeloma (MM) cell line in culture, which undergoes senescence when treated with DNA-damaging agents. Senescence is accompanied by the induction of NKG2D and DNAM-1 ligands. NK cell degranulation is enhanced on interaction with drug-treated MM cells [66]. The results support a model in which senescence promotes tumor cell recognition and elimination by NK cells. NKG2D preferentially recognizes premalignant lesions early stages of tumorigenesis that are associated with oncogene induced senescence (reviewed in ref. 66). Thus NK cells may exert immunosurveillance toward premalignant cells undergoing senescence under the action of activated oncogenes. This is an attractive hypothesis: that the essential function of senescence is to initiate a reaction by the immune system, resulting in the disappearance from the body of oncogene-activated cells that could be a threat to the survival of the organism. Much more investigation is needed to provide definitive proof for this concept, however. Certainly in some cases the clearance of senescent cells seems extremely slow;

nevi, comprising senescent melanocytes, do disappear over time, but this may take decades [67].

3.3 Potential enhancement of cancer initiation and progression by senescent cells

Nevertheless, the changes in gene expression in senescent cells might also increase cancer incidence in tissues. Clearly senescence of cells prevents those cells from initiating a cancer, yet potentially could promote cancer development in neighboring cells [68]. The potentially pro-tumorigenic effect of senescent cells may represent one form of the influence of inflammation and chronic injury on the development of cancer [69]. Experimental evidence for the tumor-promoting effects of senescent cells has been obtained in xenograft models in immunodeficient mice [70,71] but not yet in models in which senescent cells are generated in tissues *in situ* rather than by cell transplantation. It should be noted that only some xenograft models show a clear effect of senescence of co-transplanted fibroblasts. Co-transplanted mesenchymal cells in general have stimulatory effects on xenograft growth, via a variety of mechanisms [72]. The additional stimulation of xenograft growth resulting from senescent cell products, originating from the co-transplanted cells, is seen only under certain experimental conditions [73]. This is an area of research in which creative new models are needed to elucidate these issues.

4 Conclusion

This review has emphasized the role of telomere biology as a major factor in the anti-cancer action of cellular aging. Continued research in this area will yield more insights into the role of this aspect of aging at the cell level in preventing human cancer. Additionally, oncogene-induced senescence forms an important barrier to cancer development. Recent progress in understand the consequences of senescence in tissues have emphasized the role of the innate immune system. For both forms of cellular aging, increased understanding of the how they act as tumor suppressors has the potential to lead to new aspects of prevention and therapy in human cancer.

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Biography

Dr. Peter Hornsby obtained a Ph.D. in Cell Biology at the Institute of Cancer Research of the University of London. He has held faculty positions at the University of California San Diego, the Medical College of Georgia, and Baylor College of Medicine. Currently he is Professor in Department of Physiology and Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center, San Antonio.

Figure 1.

Simplified model for the cooperation of activated Ras and SV40 T antigen used in the authors' experiments described in the text. Constitutively activated (mutated) Ras has a broad variety of downstream molecular targets and effects on cell behavior. Some of those are indicated in the box on the right (increased cell proliferation, lowered dependence on external mitogens, loss of dependence on anchorage for growth; in vivo, Ras promotes invasiveness and tumor formation, e.g. in immunodeficient animals). However, at levels of expression that exert these effects, the hyper-replication caused by Ras results in senescence via mechanisms discussed in the text. Senescence occurs via the activation of the p53 and Rb pathways. SV40 TAg acts to overcome senescence by interrupting these pathways, thereby permitting the expression of the full spectrum of pro-tumorigenic effects of Ras, although it also probably directly contributes to some of those properties. See text (Sections 2.3 and 3.1) for further details.