Journal of Cellular Biochemistry 45:147–155 (1991)

Cellular Senescence: A Reflection of Normal Growth Control, Differentiation, or Aging?

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Abstract Normal cells, with few exceptions, cannot proliferate indefinitely. Cell populations—in vivo and in culture—generally undergo only a limited number of doublings before proliferation invariably and irreversibly ceases. This process has been termed the finite lifespan phenotype or cellular senescence. There is long-standing, albeit indirect, evidence that cellular senescence plays an important role in complex biological processes as diverse as normal growth control, differentiation, development, aging, and tumorigenesis. In recent years, it has been possible to develop a molecular framework for understanding some of the fundamental features of cellular senescence. This framework derives primarily from the physiology, genetics, and molecular biology of cells undergoing senescence in culture. Our understanding of senescence, and the mechanisms that control it, is still in its infancy. Nonetheless, recent data raise some intriguing possibilities regarding potential molecular bases for the links between senescence in culture and normal and abnormal growth control, differentiation, and aging.

Key words: cell proliferation, immortality, tumor suppression, c-fos, histone ribosomal protein

PURPOSE AND LIMITATIONS OF THIS PROSPECTIVE

In this prospective, we discuss some current ideas on the relationship between cellular senescence and biological processes as diverse as normal growth control, differentiation, aging, and tumorigenesis. Our discussion makes no pretense of being comprehensive. Good reviews of cellular senescence have been published recently [1–3]. Here, we will limit our discussion to some of the studies that have helped us to interpret our own data and that provide a context for future directions.

CELLULAR SENESCENCE

A fundamental feature of normal, higher eukaryotic cells is their limited ability to proliferate. This limitation exists for most differentiated cells, even rather primitive or developmentally immature cells. It occurs in vivo—although it has not been widely studied in vivo—and certainly occurs in culture, where it has been more extensively studied. Only some primitive stem cells—such as the inner cell mass of the early embryo—may have an unlimited proliferative potential in culture [4]; in vivo, of course, stem cell proliferation is restricted by differentiation.

Differentiated cells that can divide in vivo will, under appropriate culture conditions, go through an initial period of proliferation (used here interchangeably with growth). However, as the culture undergoes an increasing number of populations (PD), there is invariably a progressive decline in proliferative capacity. This progression has been termed the finite lifespan phenotype or cellular senescence.

Senescence was first systematically described more than 25 years ago in cultures of human fetal lung fibroblasts [5,6]. The fibroblasts that grew out of the tissue explants used in these early studies initially grew well. However, by roughly 60 PD, the proliferative capacity of the culture was exhausted, although the cells remained perfectly viable. Cellular senescence or the finite lifespan of cells has been observed in a wide variety of cultures established from dif-

Received August 8, 1990; accepted September 28, 1990.

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ferent tissues (and cell types), in donors of ages ranging from fetal to old and in several animal species [7–10].

What is the significance of cellular senescence? In recent years, senescence has been viewed from a number points of view that differ in their emphasis, but are not necessarily exclusive.

BIOLOGY OF CELLULAR SENESCENCE

Species-Dependence

Cellular senescence occurs in normal cell cultures from many species of animal cells [10]. However, whereas the *phenomenon* of senescence is widespread, the *stringency* with which it occurs depends upon the species of origin.

Senescence is particularly stringent in human cells. Immortal variants (cells having an unlimited lifespan in culture) very rarely arise spontaneously from human cell cultures (frequency $< 10^{-12}$). This is not the case for many other species, particularly several rodent species. Mouse and rat cell cultures, for example, nearly always give rise to clones of immortal cells at a low, but measurable, frequency (generally 10^{-5}–10^{-6}, for cultures established from embryos). Thus, for human cells, senescence is virtually complete and irreversible, whereas for rodent cells, the decline in proliferation eventually reverses as rare, immortal cells take over the culture [11–14].

Cell Types

Because fibroblasts are easy to obtain and grow as relatively pure cultures, they have been an excellent cell system in which to study both growth control and cellular senescence. Fibroblasts are, of course, differentiated cells, but little attention has been paid to this aspect of their phenotype. This is unfortunate because, as discussed below, there is evidence that senescence entails changes not only in cell proliferation, but also in the state of differentiation. Nonetheless, studies with fibroblasts have provided much of the information that support the concepts relating senescence to aging and transformation.

Relationship to Aging

An intriguing feature of cellular senescence is that it occurs after an intrinsically determined number of PD. That number depends upon the cell type, but, within a cell type, it is inversely proportional to the age of the tissue donor [8,9]. For example, under identical culture conditions, human fetal fibroblasts generally senesce after 50 or more PD, whereas fibroblasts from old adults may senesce after fewer than 25 PD. Moreover, although there is scatter in the data, fibroblasts from intermediate-aged humans generally senesce between 25 and 50 PD, also inversely dependent on the donor age. These observations have led to the view that senescence in culture is a manifestation, at a cellular level, of processes that occur during aging in vivo.

Relationship to Tumorigenesis

An important consequence of escape from senescence, or immortality, is an increased susceptibility to neoplastic transformation. Relative to cells having a finite lifespan, immortal cells are orders of magnitude more susceptible to neoplastic transformation. Since human cells are exceedingly resistant to immortalization, it is not surprising, then, that they are also exceedingly resistant to transformation by radiation, chemical carcinogens, and oncogenic viruses or activated oncogenes [11–14]. Taken together, these observations support another view of cellular senescence: that senescence constitutes a mechanism for curtailing or suppressing tumorigenesis. Moreover, many (but not all) tumor cells are immortal. Thus, escape from senescence may be a common event in tumorigenesis; a priori, it may be required for the establishment of metastases.

Relationship to Differentiation

Some of the phenotypic changes that occur when fibroblasts undergo senescence (discussed below) may have little to do with the loss of proliferative capacity. These observations have led to the idea that senescent fibroblasts are terminally differentiated [15,16]. Because there are no good markers for the differentiated states of fibroblasts, this idea is difficult to test critically. However, studies on cultured bovine adrenocortical epithelial cells have provided some insights into the relationship between senescence and differentiation. As these cultured cells senesce, expression of steroid 17α-hydroxylase, a specific differentiation marker, decreases in parallel with the decline in proliferation [17]; however, in individual cells, differentiated gene expression and proliferation decline independently [18]. Together, these observations suggest that the growth arrest associated with senes-

cence constitutes just one aspect of a more complex change in cell phenotype.

CELL BIOLOGY OF SENESCENCE

Phenotypic Changes

As noted above, senescent cells generally display a number of phenotypic changes in addition to the cessation of cell division. Depending on the cell type and the particular culture, these changes often include an increase in cell size, alterations in subcellular architecture, and extracellular matrix (reviewed in [1–3,15]), and qualitative, as well as quantitative, changes in the synthesis of some proteins [16,19,20]. Recent data suggest that, in some cases, there may be an alteration in cell function associated with senescence. These cases include the decline in steroid 17α-hydroxylase gene expression in adrenocortical cells [17,18] and an increase in collagenolytic activity in human dermal fibroblasts [20]. Currently, very little is known about how the senescent phenotype is related to the phenotypes that are expressed in vivo.

Growth Arrest

The most obvious, consistent feature that distinguishes early passage from senescent cells is the cessation of cell growth. Senescent fibroblasts remain viable for long periods of time (many months), during which they are metabolically active and continue to synthesize and turn over RNA and protein [16,21–23]. Thus, senescent cells are not dead or dying cells, and cellular senescence is not programmed cell death.

Senescent fibroblasts arrest growth with a G_1 DNA content [24]. In this regard, they resemble early passage cells that have arrested growth in a reversible, nonproliferating state termed quiescence or G_0. Early passage fibroblasts enter G_0 at high cell densities or when they are deprived of growth factors; quiescent fibroblasts leave G_0 and resume proliferation when replated at low cell densities or provided with growth factors [25,26]. A striking difference between quiescent and senescent cells is that the latter cannot be stimulated to enter the S phase of the cell cycle by any known combination of growth factors or physiological stimuli (reviewed in [1–3]).

GENETICS OF SENESCENCE

Dominance

Cell fusion studies show clearly that finite lifespan is dominant and that immortality is recessive [27–29]. Thus, when normal human fibroblasts are fused to immortal human tumor cell lines, the hybrid cells generally have a finite proliferative lifespan. Moreover, when two different immortal cells are fused to each other, there is often—but not always—complementation, and the hybrid cells senescence. By fusing a variety of immortal and tumor cell lines with each other, four complementation groups for immortality have been defined [30]. Immortal cell lines assigned to the same complementation group give rise to immortal hybrid cells (no complementation), whereas cell lines assigned to different complementation groups give rise to finite life span hybrids. With the exception of cells immortalized by the SV-40 T antigen (discussed below), the mutation that conferred immortality in these cell lines, and thereby defines a complementation group, is unknown. It is particularly intriguing that the complementation group for immortality to which a tumor cell can be assigned does not depend on the cell type or embryonic germ layer of origin. However, cells transformed by the same oncogene can generally be assigned to the same complementation group. These findings suggest that cells can escape from senescence by more than one mechanism, and there may be common mechanisms that lead to senescence in cells of different lineages.

Genes That Reverse Senescence

The genes that control the lifespan of cells are not yet known. Physiological growth stimulators (e.g., serum, growth factors) fail to induce senescent fibroblasts to initiate DNA replication. In addition, most xenobiotics (e.g., chemical carcinogens, viruses) are also ineffective. However, the transforming genes of some DNA tumor viruses are at least partially effective. The best studied of these is the SV-40 virus large T antigen, a ~ 90 kDa multifunctional nuclear protein [31].

T antigen immortalizes and transforms rodent fibroblasts [32,33] and stimulates quiescent fibroblasts to enter S phase [34]. SV-40 infection induces about 30% of senescent human fibroblasts to initiate DNA synthesis, but the cells do not undergo mitosis [35]. In addition, T antigen extends the lifespan of human fibroblasts by about 20 PD. However, even T-antigen transfected human cultures do not proliferate indefinitely and immortal clones arise only rarely [36–38]. These data suggest that escape

from senescence or immortality requires at least two genetic changes. Recent studies on human fibroblasts carrying a conditional T antigen suggest that T antigen can accomplish only one of these changes [39,40]—presumably that which enables senescent cells to enter S phase.

Hereditary Diseases of Aging

The existence of genetic diseases of "premature aging," and the finding that cells from affected individuals have a decreased lifespan in culture, suggest that it may be possible to identify genes that affect both cellular senescence and aging. Premature aging syndromes in humans—progeria (Hutchinson–Gilford syndrome) [41], Werner's syndrome [42], and Cockayne's syndrome [43], for example—are all rare disorders. Patients with these diseases present at a young chronological age with some features reminiscent of aged people—graying hair, wrinkled skin, and cataracts. Most affected individuals die at a young age, usually from cardiovascular disease such as myocardial infarction or congestive heart failure [44].

There are serious questions about whether these syndromes are really premature aging. Nonetheless, fibroblasts from patients with either progeria or Werner's syndrome senesce after fewer PD than age-matched controls [43–49]. Moreover, when normal human fibroblasts are fused to fibroblasts from an individual with Werner's syndrome, the hybrids have a proliferative lifespan closer to that of the Werner's syndrome cells [50]. These results are consistent with the idea that cellular senescence and aging are related processes under the control of specific genes.

BIOCHEMISTRY AND MOLECULAR BIOLOGY OF SENESCENCE

Protein Metabolism

Among the metabolic changes shown by senescent cells is a decline in the overall rate of protein synthesis [21,23]. Since senescent cells are generally about twofold larger than are cells at early passages, the rate of protein degradation also declines [22,51]. The major proteins synthesized by early passage and senescent cells are similar. However, the synthesis of a few specific proteins changes with senescence [16–19], and recent data suggest that ornithine decarboxylase (odc) mRNA is translated with decreased efficiency in senescent human fibroblasts [52]. These findings suggest that the efficiency and specificity of the protein synthetic machinery may change during cellular senescence. It is noteworthy that similar changes in protein synthetic capacity occur in animal tissues as they age in vivo [53].

Plasma Membrane

The genetics of senescence suggest that senescent cells express one or more dominant inhibitors of cell proliferation. Such inhibitors may reside in the plasma membrane, since growth suppressing activity is found in membrane protein from senescent and quiescent cells [54]. Whatever the nature of the inhibitors, they probably do not block the occupancy or function of growth factor receptors. Early passage and senescent human fibroblasts have similar receptor numbers and affinities for the major mitogenic growth factors, including platelet-derived growth factor [55], epidermal growth factor [56], and insulin-like growth factor [57]. Moreover, there is no general breakdown in growth factor signal transduction in senescent cells. Protein kinase C activity does not change (H.Y. Park and J. Campisi, unpublished), and cAMP-dependent protein kinase is actually about threefold higher in senescent fibroblasts [58]. The most compelling evidence that growth factor receptors and signaling are intact is that many growth factor-inducible genes remain fully responsive in senescent cells [52,59,60].

Growth Factor-Inducible Gene Expression

Many genes are expressed and regulated similarly in senescent and early passage cells. When fibroblasts are deprived of growth factors (2–4 days) and then stimulated with serum or growth factors, the basal and induced levels of several mRNAs are similar whether the cells are at early or late passage [52,59,60]. These growth factor-inducible genes include the c-myc and c-ras-Ha protooncogenes, which appear to be essential for cell proliferation [61,62] and stimulate proliferation, in conjunction with other signals, when introduced into fibroblasts in an unregulated form [63,64]. Other mRNAs that are induced in senescent cells are those encoding ODC, the first and rate-limiting enzyme in polyamine biosynthesis, and the cytoskeletal proteins actin and vimentin [52,59]. Thus, senescent fibroblasts retain the growth factor signaling pathways needed to fully express several growth factor-inducible mRNAs.

ALTERED PATTERN OF GENE EXPRESSION IN SENESCENT FIBROBLASTS

Recently, we identified several changes in gene regulation in senescent human fetal lung fibroblasts (strain WI38) [60]. To study differences between quiescent and senescent cells, we deprived the cells of serum at early passage (75–85% growing cells) and near complete senescence (< 8% growing cells) for 3 days. The cells were then stimulated with serum-containing medium, and the level of specific mRNAs was determined by Northern blot analysis.

Down-Regulation of a Ribosomal Protein mRNA

As noted above [52,59,60], serum stimulation induced several mRNAs similarly in early passage and senescent cells. However, when we hybridized our Northern blots to a commonly used "control" gene, pHE-7, we found that senescent cells expressed five- to tenfold less pHE-7 mRNA. pHE-7 was cloned several years ago from a HeLa cell cDNA library [65]. The clone is a 300 bp insert that detects a prevalent ~ 1.5 kb mRNA whose translation product was unknown. In several cell systems, the level of pHE-7 mRNA does not change with growth state or phase of the cell cycle [59,60,65].

Because pHE-7 mRNA was down-regulated specifically in senescent cells—and not in quiescent cells—we sought to identify the gene. In vitro translation of pHE-7-hybrid-selected mRNA showed that it encoded a ~ 30 kDa basic protein. Recently, we isolated a cDNA clone containing most of the protein coding region of the mRNA. Sequence comparison of our clone with entries in DNA sequence databases revealed about 90% amino acid homology to the rat L7 ribosomal protein (T. Seshadri and J. Campisi, unpublished). Thus, the mRNA for a ribosomal structural protein was specifically down-regulated in senescent human fibroblasts. This finding may explain the decline in protein synthesis in senescent cells. We do not yet know whether other ribosomal protein genes are down-regulated in senescence or whether the decline in L7 expression is sufficient to depress the rate of protein synthesis.

There are two points worth noting about the partial repression of L7 in senescent fibroblasts. First, it is not simply related to growth state or rate of protein synthesis. Quiescent cells express as much L7 mRNA as do growing cells, even though the rate of protein synthesis is generally about threefold lower in quiescent cells. Second, preliminary evidence in skin fibroblasts suggests that L7 mRNA may decline with donor age (discussed below). Thus, repression of L7—and possibly other ribosomal proteins—may be a common marker of fibroblast senescence in culture and aging in vivo. It remains to be seen whether L7 expression changes during the senescence or aging of cell types other than fibroblasts.

Induction of a Novel Histone mRNA

In contrast to the normal induction of c-myc, odc, and c-ras-Ha mRNA in senescent cells, there was no induction of the replication-dependent (rd) H3 histone mRNA. This mRNA, along with the mRNAs for the other rd-histones (H2A, H2B, H4), is generally expressed only during the S phase of the cell cycle [66,67]. Thus, in quiescent early passage cells, serum induced H3 mRNA > 30-fold about 16 hr after stimulation, the time at which the cells entered S phase. In senescent cells, H3 mRNA was not expressed, and none of the rd-histone proteins were synthesized at any time after serum stimulation. Thus, it appears that there is repression of the rd-histone genes in senescent cells.

However, in the senescent fibroblasts only, we noted a slower migrating mRNA, faintly detectable in total cellular RNA, that hybridized to the (murine) H3 probe. This larger mRNA appeared to be closely related to the rd-H3 mRNA: The hybridization signal was lost only after high stringency washes, which caused a parallel loss of hybridization to the rd-H3 mRNA. In contrast to the rd-H3 mRNA, the H3-related mRNA in senescent cells was polyadenylated. Rd-histone mRNAs are not polyadenylated, but polyadenylated histone mRNAs are transcribed from unique genes that encode structurally similar variant or replacement histones; in addition, variant histone mRNAs often have larger 5′ or 3′ untranslated sequences [67]. The significance of these results is that, in other cell types, replacement histone genes are generally expressed only after terminal differentiation.

Repression of c-fos Protooncogene Expression

The most striking change that we find in senescent fibroblasts is that the c-fos protooncogene is under nearly complete transcriptional repression. In many early passage human and rodent fibroblast cultures, c-fos transcription and mRNA is transiently induced > 20-fold

within 30 min after stimulation by serum or certain mitogens [68–70]. However, in senescent human fibroblast cultures, c-fos mRNA is barely detectable at any time after serum stimulation. Moreover, epidermal growth factor and phorbol esters, which induced c-fos mRNA in early passage cells, fail to induce c-fos mRNA in senescent cells (A. RayChaudhury and J. Campisi, unpublished). Nuclear run-on assays show that the c-fos gene is suppressed at the level of transcription in senescent human fibroblasts.

Of the changes that occur in senescent cells, repression of c-fos is a good candidate for being causative in growth arrest. Several studies show that inhibition of c-fos expression—for example, by microinjection of antibodies or transfection of antisense vectors—prevents serum-stimulated fibroblasts from entering S phase and inhibits exponential growth [71–73]. Thus, a transcriptional repressor of c-fos could act as a dominant inhibitor of proliferation, a feature of senescent cells predicted by the genetic and biochemical studies described above.

GENE EXPRESSION IN FIBROBLASTS FROM YOUNG AND OLD DONORS

c-fos and c-myc Expression in Fibroblasts

Because c-fos and L7 expression declines when human fetal lung fibroblasts senesce in culture, and senescence and aging appear to be related, we explored the expression of these genes in skin fibroblasts from the inner arms of young (21–30 years) and old (65–90) humans. Initially, we used fibroblasts from ten randomly selected young-old donor pairs. The cells were used at early passage (6–10 PD) and made quiescent by serum deprivation. c-fos and c-myc mRNA was quantitated by Northern analysis at various times after serum stimulation. In all ten pairs, we found no significant differences in the extent and kinetics of c-fos and c-myc induction. However, we noted that the proliferative capacity of all the cultures was similar (40–60% growing cells). Thus, the inducibility of c-fos and c-myc may reflect the proliferative potential of a culture and not the age of a donor. These results demonstrate two difficulties in studying human aging using cell cultures. First, in order to grow enough cells from the biopsy for Northern analyses, there may be a selection for cells with a high proliferative potential and a dilution of cells near the end of their proliferative lifespan. Second, whereas fibroblast cultures from old individuals do tend to have a longer doubling time than do cultures from young individuals [8,9], some old donor cultures grow vigorously and some young donor cultures do not. Thus, variability between non-isogenic individuals introduces a large amount of scatter in the data.

L7 Expression in Fibroblasts

In a second series of young-old donor pairs, we examined the level of L7 mRNA. In four pairs tested, L7 mRNA was reduced five- to 20-fold in fibroblasts from old donors compared to those from young donors (M. Peacocke, X. Lee, B. Gilchrest, unpublished). These old donor cells, however, were all significantly less proliferative than were the young donor cells ($< 20\%$ vs. 40–60% proliferating cells). Whether the repression of L7 reflects in vivo age or the reduced proliferative potential of the old donor cells must still be determined.

L7 Expression in Keratinocytes

The relationship between senescence and differentiation is difficult to study in fibroblasts because few good markers of differentiation exist. In contrast, human epidermal keratinocytse grow well in culture [74] and there are good markers for various states of differentiation. Human keratinocytes senesce in culture [74], and cultures from old donors are less responsive to mitogens than are young donor cultures [75]. Terminal differentiation can be induced in culture by high extracellular Ca^{+2} or phorbol esters. Thus, in this cell system, it will be possible to study how molecular events associated with senescence relate to aging and differentiation.

In our first experiments, we asked whether L7 mRNA changed during terminal differentiation of human neonatal keratinocytes. Proliferating keratinocytes were induced to differentiate by addition of either calcium chloride or phorbol esters. The mRNA for involucrin [76], a well-known marker of terminal keratinocyte differentiation, was induced within 6 h and continued to rise for 24 h after addition of differentiating agents. Over the same interval, the level of L7 mRNA declined four- to tenfold (J. Younus and M. Peacocke, unpublished). Although our results are still preliminary, collectively, our data suggest that L7 expression declines with senescence, aging, and terminal differentiation, but not with quiescence.

DISCUSSION AND SUMMARY

Proliferation

A universal feature of senescent cells, regardless of cell type, is an inability to proliferate. What is the nature of this growth arrest? Senescent fibroblasts, from which much of the data about the growth arrest have derived, cannot enter S phase, and it has been suggested that they are blocked in G_1 or at the G_1/S boundary [2,3,59]. Although senescent and quiescent fibroblasts have some similarities (e.g., a G_1 DNA content, similar levels and inducibility of c-myc, c-ras, odc mRNAs), this idea is difficult to reconcile with our recent data. Two temporally separated, growth-related genes are repressed in senescent cells (c-fos—normally expressed early in G_0/G_1, and the rd-H3 histone—normally expressed in S phase), and two genes (L7 and a histone variant) are expressed differently in senescent and quiescent cells. These data suggest that senescent fibroblasts arrest growth in a state that is distinct from the reversible, G_0 state in which early passage fibroblasts arrest.

Differentiation

Because there are differences between senescent and quiescent fibroblasts, it has been suggested that senescence is the terminally differentiated state of fibroblasts [15,16,60,77,78]. As used here, differentiation is an essentially irreversible, but not immutable, program of gene expression that defines cell function and phenotype. We suggest that current evidence is consistent with the idea that senescence in fibroblasts is a process of terminal differentiation. First, as noted above, senescent fibroblasts arrest growth in a state that is distinct from the growth arrest states shown at early passage. Second, quantitative (c-fos, rd-histone, L7) and qualitative (H3-variant histone) changes in the pattern of gene expression occur during the senescence of fetal lung fibroblasts [60]. Third, senescent human skin fibroblasts express higher levels of collagenase, and lower levels of collagenase inhibitor, than at early passage [20], suggesting that senescence results in a functional change in skin fibroblasts (increased in collagenolytic activity). As far as we know, the changes in gene expression that accompany fibroblast senescence are essentially irreversible.

Cultured fibroblasts display a range of phenotypes, depending on the tissue and developmental stage of origin, and may differ in their growth rates and amounts and types of extracellular matrix components that they produce [79,80]. Unfortunately, it is not known whether differences among fibroblast populations are due to distinct states of differentiation, with in vivo counterparts, or whether they reflect the plasticity of a single differentiated state. It is also not known whether fibroblasts undergo terminal differentiation in vivo. However, the data from adrenocortical epithelial cells suggest that some, but certainly not all, differentiated functions change with senescence, but that this is not tightly coupled to the loss of proliferative capacity [17,18]. Therefore, for these—and possibly other cell types—senescence appears to result in an *altered* state of differentiation that is not necessarily linked to the loss of proliferative potential. For all cell types, whether the senescent phenotype exists as part of a normal differentiation process in vivo or whether senescence is a unique differentiated state is still very much a matter of speculation.

Aging

The relationship between senescence in culture and aging in vivo is still tenuous. As discussed earlier, there are a number of problems inherent in studying aging using cell cultures. However, as progress is made in identifying molecular changes that occur during the senescence of various cell types in culture, it will become more feasible to determine whether the senescent phenotype exists in vivo and whether its occurrence increases as organisms age. The cell culture studies suggest that both proliferation and differentiated characteristics change when cells senesce and that these changes need not occur coordinately.

For some cell types—certainly for fibroblasts—it is most unlikely that aging results from a loss of proliferative capacity. For example, skin biopsies from even very old individuals frequently contain plenty of proliferative fibroblasts. It is doubtful that skin fibroblasts reach the end of their proliferative lifespan very often in vivo.

On the other hand, a progressive change in differentiated state or function could result in at least some of the features associated with aging. For example, some cells may lose a differentiated function with age, as suggested by the studies on adrenocortical cells [17,18]. This type of change could explain the age-associated decline in hormone production [81]. Other cells

may induce or increase a differentiated function, as suggested by the elevation in collagenase that occurs in senescent skin fibroblasts [20]. In vivo, high collagenolytic activity could contribute to the degeneration of dermal tissue matrix that is so common in human aging. In addition, more universal cell functions, such as overall protein synthetic capacity, may change with age, as suggested by the decline in L7 expression that occurs in senescent and old donor fibroblasts and in terminally differentiated keratinocytes.

Tumorigenesis

As discussed earlier, there is evidence to suggest that senescence constitutes a suppressive mechanism for tumorigenesis. However, while senescence may play a role in limiting the growth of metastases, it is doubtful that the loss of proliferative capacity serves to curtail or prevent the growth of primary tumors. Considering fibroblasts again, most biopsies from old adults contain fibroblasts that can undergo > 20 PD in culture, and any cell that can undergo 15–20 PD is certainly capable of giving rise to a palpable tumor. However, if senescence is a process leading to terminal differentiation, it is possible that, as cells progress toward a more terminal state, they become more refractory to transformation. Immortality and transformation may interrupt this progressive change, as they are known to interrupt other differentiation processes. Of course, once cells have terminally differentiated, they are virtually resistant to transformation.

It has been known for some time that normal growth is under both positive and negative control [82] and that a non-random loss of chromosomal DNA occurs in many tumor cells [13]. The idea that tumor suppressor genes exist and function in normal cells has recently gained strong molecular support [83]. A reasonable hypothesis is that the genes responsible for the establishment and maintenance of cellular senescence are good candidates for being tumor suppressor genes.

Future Directions

A molecular understanding of cellular senescence and its relationship to differentiation, aging, and tumorigenesis is still much in its infancy. However, at this point, there are a number of rather specific, testable questions that can be addressed.

First, it should now be possible to define the senescent state of cell types other than fibroblasts, with an emphasis on determining how growth- and differentiation-specific gene expression changes. Are there genes whose expression is invariably altered with senescence, regardless of cell type, or does the senescent phenotype very much depend on the cell type of origin? If there are genes whose expression is universally altered by senescence, do the senescence-associated alterations also occur during well-characterized instances of terminal differentiation?

It will also be important to determine whether the senescent phenotype occurs in vivo. As good markers of senescence are identified, it will be possible to explore their expression in tissue, preferably by techniques like immunocytochemistry or in situ hybridization. This approach abrogates the need to culture cells, which, as discussed earlier, exerts selective pressure on the starting cell population.

Finally, it is now at least theoretically feasible to begin to identify the genes that control the molecular changes that occur in senescent cells. A good example is the putative repressor of c-fos transcription in senescent fibroblasts, which may be a good candidate for controlling at least some features of the senescent phenotype. Thus, by understanding the mechanisms by which specific changes in gene expression occur in senescent cells, we are more likely to identify genes that are of critical importance in growth control, aging, and tumorigenesis.

ACKNOWLEDGMENTS

We wish to acknowledge Drs. R. Miller, B. Gilchrest, and T. Seshadri for support and helpful discussions during the tenure of a Program Project Grant from the National Institute on Aging (AG07114).

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