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Cellular senescence as a tumor suppressor mechanism

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Organisms with renewable tissues had to evolve mechanisms to prevent the development of cancer. One such mechanism is cellular senescence, which irreversibly arrests the growth of cells at risk for neoplastic transformation. Recent findings have revealed the complexities of the senescent phenotype, and possibly unexpected consequences for the organism.

Cell division is essential for the survival of multicellular organisms that contain renewable tissues. However, cell division also puts organisms at risk for developing cancer. Genomes are continually damaged by environmental insults, oxidative metabolism, and, in dividing cells, errors in DNA replication and mitosis. Depending on the level and type of damage, cells may attempt repair, or die. In dividing cells, the major risk from genomic damage is mutations, which are generated by failures or mistakes in repair. If a mutation confers a growth or survival advantage, or causes the genome to become unstable (and thus hypermutable), the stage is set for the development of cancer (oncogenesis).

Complex organisms evolved at least two cellular mechanisms to suppress the proliferation (used here interchangeably with growth) of cells at risk for oncogenic transformation: apoptosis or programmed cell death, and cellular senescence or the senescence response. Cellular senescence irreversibly arrests cell growth, and is a major barrier that cells must overcome in order to progress to full-blown malignancy [1,2]. In this regard, cellular senescence is similar to apoptosis. However, whereas apoptosis kills and eliminates potential cancer cells, cellular senescence irreversibly arrests their growth.

Recent findings have shed new light on the causes of cellular senescence, the complexity of the senescent phenotype, and the potential consequences of cellular senescence for the organism. These findings are discussed here, with an emphasis on the senescence response of human cells.

Potentially oncogenic events cause cellular senescence

Cellular senescence was first recognized >40 years ago as a process that prevented normal human fibroblasts from growing indefinitely in culture [reviewed in 1,2]. In the last decade, we learned that this process, now known as replicative senescence, is driven by telomere shortening.

Telomeres, the repetitive DNA sequence (TTAGGG in vertebrates) and specialized proteins that cap the ends of linear chromosomes, are essential for chromosomal integrity. Owing to the biochemistry of DNA replication, 50-200 bp of telomeric DNA are not replicated during each S phase. Because telomerase, the enzyme that can synthesize telomeric DNA *de novo*, is not expressed by most human cells, telomeres shorten with each cell cycle. When the telomeres erode (from ~10 kb in the germ line) to 4-6 kb on average -- before chromosomal integrity is lost -- human cells irreversible arrest growth with a characteristic (senescent) phenotype [reviewed in 2,3]. The stringency of the senescence response, and whether short telomeres or other factors (discussed below) induce the response, is highly species-dependent.

Two points regarding replicative senescence are noteworthy. First, cells very likely respond to disruption of the telomere structure, rather than shortening *per se* [4]. Second, telomerase is expressed in germ line, early embryonic, and a few adult cells -- and most tumor cells. Expression of telomerase is the most common mechanism by which cancer cells stabilize their telomeres, and hence avoid replicative senescence [reviewed in 3].

Recently, stimuli having little or no impact on telomeres were shown to induce normal cells to arrest growth with a senescent phenotype [reviewed in 2]. These stimuli include DNA damage, chromatin remodeling and strong mitogenic signals. Thus, replicative senescence is an example of a more general process, termed here cellular senescence.

DNA damage -- double strand breaks or oxidation -- can induce cellular senescence [reviewed in 2]. This may explain why mouse cells senesce after many fewer doublings than human cells, despite having longer telomeres and, frequently, expression of telomerase: mouse cells may be more sensitive to the 20% oxygen in which cells are typically cultured. In addition, agents that open or decondense chromatin induce cellular senescence [reviewed in 2, 5], possibly by abolishing chromatin-mediated gene silencing, which can derange normal differentiation -- a common feature of cancer cells. Finally, normal human and mouse cells senesce in response to intense mitogenic signals -- for example, overexpression of the growth-stimulatory transcription factor E2F1, or activated forms of the growth factor signal transducing proteins RAS, MEK or RAF. To the extent it has been examined, these stimuli induce senescence after only a few cell divisions and without telomere shortening [6-8]. Moreover, cells that express telomerase nonetheless senesce in response to stimuli such as activated RAS [6]. Thus, diverse stimuli, not solely telomere shortening, induce a senescence response.

What do these stimuli have in common? All have the potential to cause or contribute to cancer. Telomere erosion inevitably leads to genomic instability, and thus hypermutability. Likewise, DNA damage can cause mutations (in oncogenes or tumor suppressor genes), chromosomal aberrations and genomic instability. Chromatin disruption, particularly loss of silencing, can derange normal differentiation, causing unregulated growth, invasiveness and other properties typical of tumor cells. And supraphysiological mitogenic signals can, of course, drive unregulated growth. Thus, cellular senescence appears to be a mechanism for irreversibly arresting the growth of cells at risk for tumorigenesis.

Tumor suppressors control cellular senescence

Consistent with its role in suppressing cancer, cellular senescence is controlled by several tumor suppressor genes [reviewed in 9,10]. The most critical of these encode the p53 and pRB proteins, which lie at the heart of two major tumor suppressor pathways. Together, p53 and pRB are the most commonly lost functions in mammalian cancers. p53 is a transcriptional activator and repressor that controls the expression of genes that cause cell cycle arrest or apoptosis in response to genomic damage. pRB regulates transcription indirectly, by interacting with transcription factors and recruiting chromatin remodeling proteins to genes that control cell cycle progression and differentiation. The pathways controlled by p53 and pRB are essential for cells to establish and maintain the senescence growth arrest in response to diverse stimuli.

p53. p53 activity, and in some cases protein levels, increase when cells senesce [reviewed in 11]. The mechanisms responsible for this activation are incompletely understood. One cause appears to be an increase in the expression of $p14^{ARF}$, a tumor suppressor encoded by the INK4a locus. $p14^{ARF}$ ($p19^{ARF}$ in mice) stimulates p53 activity because it sequesters MDM2, preventing negative feedback regulation of p53 by MDM2 [reviewed in 9]. $p14^{ARF}$ is induced by E2F1, oncogenic RAS and DNA damage. It is repressed by TBX2, a transcription factor and potential oncogene [12]. The mechanisms that alleviate repression of $p14^{ARF}$ by TBX2 in response to senescence-inducing signals are not known. Another cause for the increase in p53 activity may be the PML (promyelocytic leukemia) tumor suppressor. PML is induced by replicative senescence and oncogenic RAS [13,14] by as yet unknown mechanisms. PML interacts with CBP/p300 acetyltransferases (CBP/p300), which acetylates p53 and stimulates its activity [13] (Fig. 1).

pRB. pRB exists only in its active (hypophosphorylated) growth-inhibitory form in senescent cells. This is because senescent cells express high levels of p21, p16 and, in some cases, p27 [reviewed in 9]. These proteins inhibit the cyclin-dependent protein kinases (CDKs) that

phosphorylate and inactivate pRB during cell cycle progression. It is not known why p27 increases in senescent cells, but it may be a consequence of increased activity of the PTEN tumor suppressor [reviewed in 9]. p21 is elevated at least partly because the gene is a direct target of p53 transactivation [11], although p53-independent, posttranscriptional mechanisms also contribute to the rise in p21 [15]. p16, a second tumor suppressor encoded by the INK4a locus [reviewed in 9,10], increases in part because Ets1, a transcription factor that stimulates p16 expression, accumulates in senescent cells, while Id1, which negatively regulates Ets activity, declines [16]. The resulting increase in Ets activity presumably overcomes the repression of p16 by BMI-1, an oncogene and member of the Polycomb family of chromatin remodeling proteins [17]. Oncogenic RAS may induce cellular senescence by activating the mitogen-activated protein kinase cascade, which stimulates Ets activity [16] (Fig. 2). It is not known how other senescence inducers stimulate Ets activity, or how Id1 is repressed in response to senescence signals.

Interacting pathways. From the above, it is clear that cellular senescence entails the activation of several tumor suppressor proteins and inactivation of several oncoproteins, each of which ultimately engages either the p53 or pRB pathway (Figs. 1 and 2). This is not to say that the p53 and pRB pathways are independent; rather, they interact at multiple levels [9,10,18]. For example, p21, which is induced by p53, inhibits CDKs that inactivate pRB, and pRB binds MDM2, preventing it from facilitating p53 degradation. Thus, senescence is delayed or abrogated not only when either p53 or pRB are inactivated, but also when key components of either pathway (e.g., p21 or INK4a proteins) are inactivated [reviewed in 1,2,9-11]. Moreover, ectopic overexpression of p21, p16 or $p14^{ARF}$ causes cells to arrest growth with a senescent phenotype [19,20]. Potential cancer cells must lose p53 and/or pRB function in order to overcome the proliferative barrier imposed by cellular senescence. This can occur by mutation or epigenetic silencing of one or more key components of the pathways.

Cellular senescence suppresses tumorigenesis in vivo

Although much of the evidence that links cellular senescence and tumor suppressor pathways derives from cell cultures, there is substantial supporting evidence from intact organisms. Perhaps the best evidence derives from mice in which genes encoding p53 or INK4a proteins are inactivated in the germline. Cells derived from these animals fail to senesce in response to multiple stimuli. In all cases, the animals develop cancer at an early age [21]. There are several other genetically modified mice in which cells resist or fail to respond to senescence signals. In large measure, these animals are highly cancer-prone [21]. By contrast, a genetic manipulation that causes premature senescence of mammary epithelial cells suppresses the development of breast cancer in young mice exposed to the mouse mammary tumor virus [22].

Human cells are markedly more resistant to neoplastic transformation than mouse cells. Nonetheless, mutations that disrupt the senescence response in humans generally lead to increased cancer incidence. For example, fibroblasts from humans with Li-Fraumeni syndrome -- a hereditary cancer-prone syndrome caused by mutations in p53 -- immortalize at a frequency that is well above the vanishingly low immortalization frequency of normal fibroblasts [23]. In addition, most, if not all, human tumors harbor mutations in one or more components of the p53 and/or pRB pathways. Of course, inactivation of these pathways confers many advantages to tumor cells, such as genomic instability and resistance to growth inhibitory signals and apoptosis. But the inactivation also allows tumor cells to ignore senescence-inducing signals.

The senescent phenotype

Cellular senescence entails many changes in gene expression, only some of which are involved in the growth arrest. Thus, some cells (e.g., human fibroblasts and T lymphocytes) also

become resistant to apoptotic death upon senescence. Moreover, all cells show changes in function when they senesce [reviewed in 2]. The functional changes are best characterized in human fibroblasts. Senescent fibroblasts overexpress many genes that encode secreted proteins, such as metalloproteinases, inflammatory cytokines, and growth factors. These secreted factors can destroy the local tissue structure, attract cells that cause inflammation, and stimulate neighboring cells to grow. In this regard, senescent fibroblasts appear to be constitutively "activated". Normally, fibroblasts are activated only transiently during wound healing, although, interestingly, they can also become activated when adjacent to epithelial tumors [reviewed in 24].

It is easy to understand why cellular senescence, in suppressing tumorigenesis, causes an irreversible growth arrest (a damaged, mutant or potentially neoplastic cell cannot produce a tumor if it cannot proliferate). It is less easy to understand why it causes functional changes, particularly detrimental changes. One possibility is that cellular senescence is antagonistically pleiotropic [see 2,24]. Because the force of natural selection declines with age, the antagonistic pleiotropy hypothesis suggests that some genes or processes that were selected to maintain fitness in young organisms (suppressing tumorigenesis, for example) can have <u>unselected</u> effects that are harmful (tissue disruption, for example) in aged organisms. The senescence growth arrest may be the selected trait, which prevents potential cancer cells from proliferating. The functional changes, by contrast, may be an unselected trait. As such, it would have little impact on young organisms, where senescent cells are relatively rare. However, it could be deleterious in older organisms, where senescent cells are more abundant [reviewed in 2,24].

The dark side of cellular senescence

Because senescent cells can, in principle, disrupt local tissue integrity, they might also contribute to age-related pathology. Moreover, because they can alter the microenvironment

surrounding preneoplastic cells, they might actually stimulate tumorigenesis. This would be favored late in life, when both senescent cells and cells with preneoplastic mutations accumulate [reviewed in 2]. Recent evidence supports this idea. Senescent human fibroblasts were shown to stimulate preneoplastic, but not normal, human epithelial cells to proliferate in culture, and also to progress to tumorigenicity in mice. Much of this stimulation was due to factors secreted by the senescent cells [24].

There is an emerging, although still largely speculative, idea that cellular senescence is a two-edged sword, having both selected beneficial effects and unselected detrimental effects (antagonistic pleiotropy). Thus, as discussed above, cellular senescence is clearly is important for suppressing the development of cancer in young organisms, but it may facilitate tumorigenesis in old organisms. Another possible example of the antagonistic pleiotropy of cellular senescence was recently proposed for psoriasis [25]. This skin disease is characterized by the overgrowth of epidermal keratinocytes, which form thick dysfunctional plaques and become resistant to apoptosis. Psoriatic keratinocytes appear to be senescent, suggesting that the plaques are composed of dysfunctional, senescent keratinocytes. Interestingly, cancer is exceedingly rare in psoriatic lesions, although cancers may develop in unaffected skin adjacent to these lesions [25].

The factors that senescent cells secrete are incompletely characterized, and little is known about the mechanisms responsible for their overexpression. It is possible, however, that at least some of these factors are induced -- directly or indirectly -- by the tumor suppressor genes that control cellular senescence. Recent studies have used cDNA microarrays, which permits monitoring the expression of hundreds or thousands of genes in a single experiment, to identify genes controlled by tumor suppressors such as p53. The results showed that overexpression of either p53 or p21 induces many changes in gene expression, some of which overlap with those

shown by senescent cells [26-28]. This is particularly true of the genes induced in response to p21 overexpression. Many of these genes encode secreted factors, and are also induced by replicative senescence; interestingly, many also have the potential to contribute to age-related pathologies, including cancer [28]. These findings suggest that p21 and p53 (and possibly other tumor suppressors, such as pRB) have pleiotropic effects, some of which may explain the functional changes that accompany cellular senescence.

Conclusions

Since its first formal description four decades ago, much has been learned about the causes and characteristics of cellular senescence. There are still, however, many unanswered questions about how senescence signals are transmitted and how they are implemented. In addition, we are just beginning to understand whether and how senescent cells impact the intact organism. Future studies will need to determine whether cellular senescence is indeed antagonistically pleiotropic, and, if so, whether we can devise strategies to eliminate senescent cells or their deleterious side effects.

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Figure Legends

Figure 1.

Control of cellular senescence by the p53 pathway.

Shown are the consequences of senescence-inducing signals on the oncogenes (highlighted in green) and tumor suppressor genes (highlighted in red) in the p53 tumor suppressor pathway. Broken arrows indicated effects that are presumed or hypothesized. Solid arrows indicate effects that are supported by experimental evidence. See text for explanation and discussion.

Figure 2.

Control of cellular senescence by the pRB pathway.

Shown are the consequences of senescence-inducing signals on the oncogenes (highlighted in green) and tumor suppressor genes (highlighted in red) in the pRB tumor suppressor pathway. Broken arrows indicated effects that are presumed or hypothesized. Solid arrows indicate effects that are supported by experimental evidence. See text for explanation and discussion.