Feature Review

CellPress

Special Issue: Immunity and Cancer

Senescence-associated inflammatory responses: aging and cancer perspectives

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Senescent cells, albeit not proliferating, are metabolically and transcriptionally active, thereby capable of affecting their microenvironment, notably via the production of inflammatory mediators. These mediators maintain and propagate the senescence process to neighboring cells, and then recruit immune cells for clearing senescent cells. Among the inflammatory cues are molecules with pronounced tumor-controlling properties, both growth and invasion factors and inhibitory factors, working directly or via recruited immune cells. These senescence–inflammatory effects also prevail within tumors, mediated by the senescent tumor cells and the senescent tumor stroma. Here, we review the course and impact of senescence-associated inflammatory responses in aging and cancer. We propose that controlling senescence-associated inflammation by targeting specific inflammatory mediators may have a beneficial therapeutic effect in treatment of cancer and aging-related diseases.

Introduction

Cellular senescence was formally described more than five decades ago, when Hayflick and colleagues showed that normal cultured cells stop dividing after a limited amount of passages [1]. This phenomenon, termed 'replicative senescence', was at variance with cell cultures derived from tumors, which often divide indefinitely, suggesting that senescence has a role in cancer prevention. Since then, senescence has been implicated in a variety of processes, such as aging [2], development [3–6], wound healing [7,8], tumor prevention [9,10], and tumor progression [11–13]. Whereas senescence born as a result of unresolved stress response was traditionally identified with irreversible dormancy, lately this concept has been challenged. We realized that, despite their growth arrest, senescent cells are not dormant, but rather are metabolically active and express a vast number of secreted proteins. This phenotype, termed the 'senescence-associated secretory phenotype' [12,14] (SASP; also known as senescence messaging secretome; SMS [15]) suggests that senescent cells both propagate the stress response and impact their microenvironment by communicating with neighboring cells. The SASP includes a large number of inflammatory cytokines and chemokines [12], providing a strong link between senescence and inflammation. This link has been strengthened recently by the identification of a different, unique type of inflammation, activated in senescent cells, the senescence inflammatory response (SIR) [16]. We also learned that inflammatory responses associated with senescence could, paradoxically, have both beneficial and detrimental roles in the organism. Thus, whereas senescent cells in tumors can recruit immune cells through the SASP and induce tumor clearance [10], prolonged SASP can also induce immune suppression and enhance tumor proliferation and invasion [14,17–19]. Here, we deliberate the dual role of senescence in tumor prevention and progression, focusing on the link between senescence and inflammation. We also discuss the therapeutic potential of targeting senescent cells and possible pharmacological means of suppressing senescence for cancer prevention.

Senescence: causes and types

Replicative senescence in normal cells is often attributed to telomere erosion, which triggers a continuous DNA damage response and persistent p53 activation [20,21]. Telomeres, the ends of chromosomes, become progressively shorter with each round of cellular division. Once they reach a critical length, the shortened telomeres trigger a DNA damage response (DDR), which eventually results in the permanent exit of the cell from the cell cycle pool (senescence) [21]. Cellular senescence can also be triggered following prolonged stress. Stressors such as DNA damage, hypoxia, or reactive oxygen species (ROS) activate the tumor suppressor p53, which can then activate either apoptosis or transient growth arrest through its target p21 [22]. If the stress is not resolved by cellular repair mechanisms, this transient growth arrest can progress to senescence, via activation of $p16^{INK4A}$. p16 activation leads to growth arrest, by inhibition of the kinases CDK4 and CDK6, which phosphorylate the retinoblastoma protein (Rb). Unphosphorylated Rb binds E2F transcription factors and inhibits them, thus arresting the cell in G1 [23,24].

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Keywords: senescence; inflammation; SASP/SIR; parainflammation; cancer; aging.

1471-4906/
 http://dx.doi.org/10.1016/j.it.2015.02.009

Transformed cells have developed many mechanisms to escape, or altogether avoid senescence. For example, reactivation of telomerase prevents telomere shortening and induction of senescence [25,26]. Loss of p53 activity, a frequent event in tumors [27], and loss of $p16^{INK4A}$ [28,29] lead to elimination of the senescence response. Transformed cells can still undergo senescence in response to oncogene activation (oncogene-induced senescence; OIS) or loss of tumor suppressors. OIS was first described by Lowe and colleagues, who reported that the activation of an oncogenic mutant rat sarcoma viral oncogene homolog (RAS) in cultured cells resulted in senescence [9]. Later reports demonstrated that *in vivo* activation of oncogenes, or loss of tumor suppressors, can lead to senescence in both mouse models and human premalignant lesions [30–36]. Different oncogenes can induce senescence through different mechanisms: activation of RAS leads to increased proliferation rates, which can cause DNA damage, eventually leading to senescence [9]. Similarly, activation of v-raf murine sarcoma viral oncogene homolog B (BRAF) also induces senescence, but through a different mechanism involving p16 induction, upregulation of the inflammatory cytokines interleukin (IL)-6 and IL-8 [37], and activation of pyruvate dehydrogenase (PDH) [38]. PDH activation then leads to increased mitochondrial metabolism, which, in turn, leads to increased generation of ROS. Interestingly, BRAF-induced senescence can be maintained even following loss of p16 or p53 [31,36]; melanocytic nevi often escape senescence via inactivation of phosphatase and tensin homolog (PTEN), which results in the activation of the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathway [39]. Similarly to oncogene activation, loss of a tumor suppressor can also trigger senescence: loss of the tumor suppressor Rb induces senescence via activation of the DDR [40], while loss of the tumor suppressor PTEN can induce senescence in prostate lesions via mammalian target of rapamycin complex 1 (MTORC1) and p53 activation [33,41]. Interestingly, PTEN loss triggers senescence without DDR activation or hyper-replication and can also trigger senescence in quiescent cells [41]. Following induction of OIS, cells must acquire an additional mutation in p53 or in p16 to escape senescence and develop into full-blown malignant cells [42,43].

Several recent studies have shown that senescence is not only a stress response, but also occurs during normal embryonic development, where it contributes to patterning of the embryo [3–5]. Unlike OIS, developmental senescence occurs independently of DNA damage or p53 activation, but is dependent on p21 activation and is mediated by transforming growth factor (TGF)-β/SMAD and PI3K/Forkhead box O (FOXO) [3,5]. Surprisingly, however, p21-deficient animals show minimal to no developmental defects [44,45], indicating that other mechanisms can substitute for senescence-based developmental processes. Indeed, another form of developmental senescence was also described recently, where natural killer (NK) cells in the placenta undergo senescence and secrete various cytokines that promote vascular remodeling and angiogenesis [4]. Remarkably, despite the different mechanisms leading to senescence, senescent cells in the embryo and cells undergoing senescence due to oncogene activation share similar gene expression signatures. The identification of developmental senescence indicates that senescence, similarly to apoptosis, is a normal developmental process, which can be appropriated in the adult as a stress response. Senescence also entwines with aging: aged cells accumulate damage generated by various causes over the years and, thus, are prone to senesce. Indeed, examination of aged tissues revealed accumulation of senescent cells, thought to contribute to tissue degeneration during aging [2,46,47].

Senescence was originally thought to be an irreversible outcome of stress responses. However, it seems that, in some cases, senescence can be escaped. Campisi and colleagues have shown that levels of p16 at onset of senescence determine the ability of senescent cells to later escape senescence and resume growth: cells that express low levels of p16 at onset of senescence will then be able to escape senescence if p53 is lost [48]. Cells that express high levels of p16 at onset of senescence will not escape senescence upon loss of p53, but will resume growth if Rb is lost. Yet, the mechanism governing the reversibility or irreversibility of senescence at the molecular level is still unclear; an appealing hypothesis is that an irreversible growth arrest should be mediated by epigenetic changes. Indeed, heterochromatin foci, which are maintained by Rb, are associated with repression of E2F target genes and have been shown to be a hallmark of senescent cells [49]. Thus, these foci were thought to harbor epigenetic modifications that govern maintenance of senescence. However, several studies [30,50] have shown that cells that escape senescence still display these foci, suggesting that they are not sufficient for maintenance of senescence. Thus, in-depth analysis of the transcriptomic and epigenetic profile of senescent cells will be necessary to fully elucidate the molecular mechanism governing senescence. Such profiling requires a robust method for identification of senescent cells, which remains a challenge.

Identification of senescent cells *in vivo* is based on a few markers thought to be common to all senescent cells. However, most of these markers were identified via *in vitro* studies carried out in fibroblasts and, thus, might not be relevant *in vivo*. In addition, hallmarks of senescence could differ from tissue to tissue, or vary according to the nature of the process triggering senescence. For example, cultured senescent cells undergo morphological changes and become larger and flat [9,51–54]; however, *in vivo* senescent cells retain their normal morphology and do not appear different from other cells in the same tissue [55]. To overcome this problem, the *in* vivo detection of senescent cells is usually based on a combination of markers, most common of which is senescence-associated β-galactosidase staining [56], based on the fact that senescent cells are characterized by increased lysosomal content [57]. Recently, it was reported that staining of lysosomal aggregates by Sudan Black could also serve to identify senescent cells [58]. Another common feature of senescent cells is downregulation of cell cycle markers, such as KI67 or low bromodeoxyuridine (BrdU) incorporation, along with upregulation of markers for growth arrest, such as p53, p21, p16, and unphosphorylated Rb. Senescent cells also undergo chromatin reorganization, giving rise to heterochromatin foci (senescence-associated heterochromatin