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Targeting Senescent Cells: Possible Implications for Delaying Skin Aging: A Mini-Review

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Key Words

Cellular senescence · Wound healing · Senescence-associated secretory phenotype · Skin regeneration · Aging · Immune system · Therapy · Drugs · DNA damage · Mitochondria

Abstract

Senescent cells are induced by a wide variety of stimuli. They accumulate in several tissues during aging, including the skin. Senescent cells secrete proinflammatory cytokines, chemokines, growth factors, and proteases, a phenomenon called senescence-associated secretory phenotype (SASP), which are thought to contribute to the functional decline of the skin as a consequence of aging. Due to the potential negative effects of the SASP in aged organisms, drugs that selectively target senescent cells represent an intriguing therapeutic strategy to delay aging and age-related diseases. Here, we review studies on the role of senescent cells in the skin, with particular emphasis on the age-related mechanisms and phenotypes associated with excessive accumulation of cellular senescence. We discuss the aberrant behavior of senescent cells in aging and how the different signaling pathways associated with survival and secretion of senescent cells can be engaged for the development of targeted therapies.

Introduction

Cellular senescence is a tumor-suppressive mechanism wherein cells are permanently growth arrested even in the presence of strong mitogenic signals [1]. Cells are induced to senescence by a wide variety of cellular perturbations, including nuclear DNA damage and mitochondrial dysfunction [1, 2]. Senescent cells are identified by several nonexclusive markers, such as persistent elevated expression of the cell cycle inhibitors $p21^{WAF1}$ and $p16^{INK4A}$ and of DNA damage response proteins, increased activity of a lysosomal enzyme termed senescence-associated β-galactosidase, loss of nuclear HMGB1, decreased expression of lamin B1, and different chromatin-remodeling events [1]. Moreover, senescent cells are characterized by the secretion of several proinflammatory factors, a phenomenon called senescence-associated secretory phenotype (SASP) [3]. The accumulation of senescent cells with age is thought to contribute to impaired tissue homeostasis and to different age-related diseases [4]. Lack of cell proliferation in senescent cells hampers the ability of tissues to regenerate after chronic and persistent injury, resulting in tissue damage. The proinflammatory and tissue-remodeling activities of the SASP also create chronic inflammation and alter tissue structure, which are the two main causes of age-related pathology

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[4]. One fascinating hypothesis is that senescent cells might contribute in a cell and non-cell autonomous fashion to skin aging. Skin aging is associated with several pathologies, including lower protection from pathogens, increased irritation, loss of insulation, delayed wound healing and susceptibility to cancer, among others. Here, we summarize the evidence of the presence of senescent cells in the skin, and the potential for pharmaceutical interventions that eliminate the negative effects of senescent cells as methods to delay skin aging.

Senescent Cells in Skin Aging

Epithelial tissues in humans are constantly renewing, and the skin represents the gold standard example of an epithelial tissue continuously regenerating [5]. Persistent mitogenic signals lead to replicative senescence. Hence, it is not surprising that the number of senescent cells increases with age in the epidermal compartment of the skin [6]. Since proliferation of stem and differentiated cells is a major contributor to skin renewal, the accumulation of an excessive number of senescent cells may cause impairments in tissue regeneration with age [7]. The overexpression of $p16^{INK4A}$ in young human keratinocytes (isolated from 30 to 40-year-old patients) results in cellular senescence and in an atrophic layer of epidermis in organotypic cultures, reminiscent of that formed by old keratinocytes (isolated from 53 to 66-year-old patients) [8]. Interestingly, reducing $p16^{INK4A}$ expression in these old keratinocytes restored the normal thickness of the epidermis, similar to that formed by young keratinocytes. In mice, cellular senescence in the epidermis, caused by persistent mitochondrial damage and dysfunction, is also associated with epidermal thinning with age [9, 10].

Senescent cells can also act in a non-cell autonomous fashion through the secretion of several cytokines, chemokines, growth factors, and proteases, a phenomenon described as SASP. In recent years, different laboratories have shown the importance of transiently induced senescent cells and their secretory phenotype to promote optimal cutaneous wound healing. The removal or absence of these short-lived senescent cells during wound healing in young mice impairs granulation, reduces the number of myofibroblasts, results in fibrosis, and delays the time of wound closure [11, 12]. Thus, the presence of these short-lived senescent cells contributes to proper and timed tissue repair.

Secretion of several proinflammatory and tissue-remodeling factors by senescent cells might contribute to the loss of tissue homeostasis and unbalanced tissue structure [4]. While short-lived senescent cells may act as positive regulators of wound healing, the presence of long-lived senescent cells may exacerbate pathological diseases in the skin. Indeed, the chronic secretion of MMPs by senescent cells might be an important contributor to the degradation of collagen and other extracellular matrix components in the dermal connective tissue, a hallmark of skin aging [13]. Moreover, chronic wounds are associated with the presence of long-lived senescent cells in wound areas [14, 15]. A persistently elevated number of senescent cells may disrupt cell signaling responses and prevent wound repair to progress through the different stages of the healing process, one of the main features of chronic wounds [16]. Consistent with this idea, cellular senescence also persists in skin fibroblasts after exposure to DNA-damaging agents, such as X-rays, ultraviolet light, and cigarette smoke, resulting in the secretion of factors that may contribute to aging phenotypes [17, 18]. Increased cellular senescence has also been observed in the skin of patients with different accelerated-aging phenotypes, including Werner syndrome, xeroderma pigmentosum, and Hutchinson-Gilford progeria syndrome [19]. These hereditary disorders are associated with defects in DNA damage repair or nuclear organization that may explain the high frequency of senescent cells in these patients.

Eliminating Senescent Cells

The impact of senescent cells on animal pathology was directly demonstrated when eliminating senescent cells through a suicide gene in a premature aging mouse model reduced selected age-related pathologies such as sarcopenia, cataracts, and loss of subdermal adipose tissue [20]. Interfering with senescent cells may be beneficial for the overall health of the animal, and the development of specific interventions that target senescent cells may serve as a therapy to delay aging, including skin pathologies. This strategy can be achieved using three different approaches: (1) selective induction of cell death; (2) improvement of the immune system, and (3) inhibition of the SASP (fig. 1).

Selective Induction of Cell Death

Two different papers reported efficient strategies to specifically eliminate senescent cells. In the first report, Di Mitri and Alimonti [21] used a metabolic approach based on increased glucose uptake and ATP production

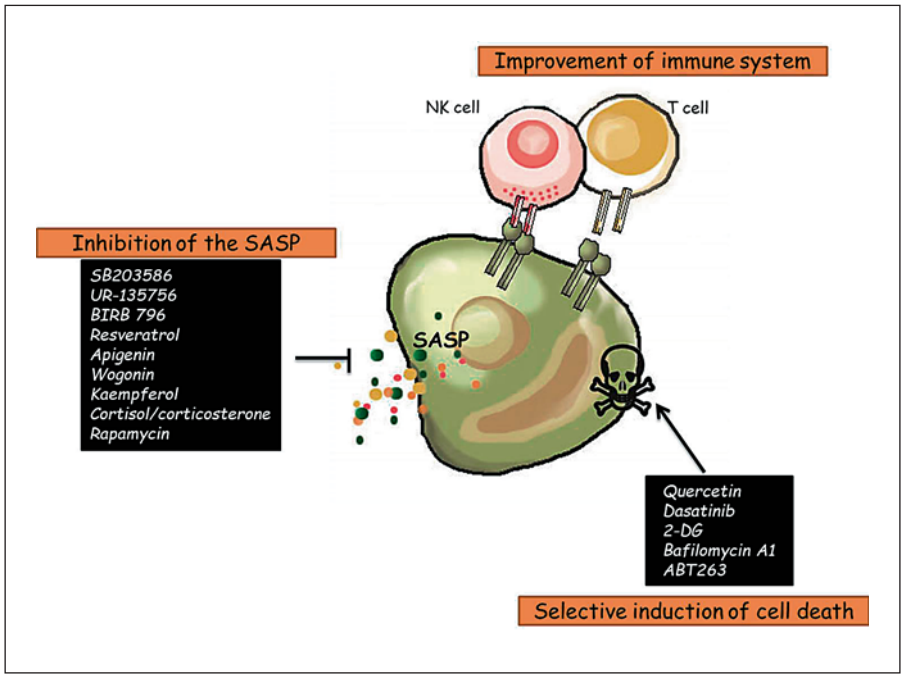

Fig. 1. Eliminating senescent cells and inhibiting the SASP as therapeutic strategy to slow down skin aging. Different drugs have been demonstrated to interfere with signaling pathways that are specific to senescent cells. These drugs show specificity in blocking signals associated with cell survival or secretion of senescent cells, leading to selective cell death or inhibition of the SASP. Another potential approach to target senescent cells relies on enhancing the clearance or increasing the number of NK cells and T cells.

of chemotherapy-induced senescent cells. Senescent cells are induced by standard anti-cancer genotoxic therapies, but despite their ability to improve long-term outcomes, they are also thought to promote several potential harmful properties, particularly through the SASP. Mice bearing lymphomas showed therapy-induced senescence when challenged with the cytotoxic drug cyclophosphamide. Induction of senescence was followed by a hypermetabolic phenotype, which included enhanced glycolysis, fatty acid catabolism, ATP-boosting oxidative phosphorylation, lysosomal protein degradation, and autophagy. Importantly, interference with this aberrant metabolism by treatment with either 2-DG, a false substrate for the glycolytic metabolism, or bafilomycin A1, a specific inhibitor of the lysosomal V-ATPases, was sufficient to reduce the survival of therapy-induced senescent cells and to improve survival of chemotherapy-treated mice bearing lymphomas.

Another recent paper reported the specific elimination of senescent cells upon treatment with the drugs dasatinib and quercetin [22]. Dasatinib, which is known to inhibit ephrin B (EFNB)-dependent suppression of apoptosis, preferentially decreases viability and increases cell death in senescent compared to nonsenescent human preadipocytes. Quercetin, which inhibits phosphoinositide 3-kinase, preferentially induced cell death in senescent relative to nonsenescent HUVEC. Combination of dasatinib and quercetin reduced senescent cells in fat and liver tissues of old mice, as well as in muscle and fat tissues of irradiated mice. Moreover, this combined treatment alleviated several age-related pathologies, such as impaired cardiovascular function and extended health span of the *Ercc1*$^{-/\Delta}$ progeroid mouse model, supporting the therapeutic potential of eliminating senescent cells at old age.

The ability of dasatinib and quercetin to selectively eliminate senescent cells suggests that there are different signaling pathways which regulate cell survival in senescent versus nonsenescent cells. Dasatinib and quercetin inhibit EFNB and phosphoinositide 3-kinase signaling pathways, respectively. Consistent with this, knockdown of mRNA transcripts to EFNB1 and EFNB3 ligands and phosphatidylinositol-4,5-bisphosphate 3-kinase delta catalytic subunit (PIK3CD) triggered cell death in senescent cells with little effect on proliferating, quiescent, and differentiated nonsenescent cells [22], demonstrating the importance of these pathways in targeting the elimination of senescent cells. Knockdown of other mRNA transcripts, such as p21^{WAF1}, plasminogen-activated inhibitor-2 (PAI-2), and BCL-xL, also selectively killed senescent relative to nonsenescent cells [22]. Identifying molecules that can target these pathways may help classify senolytic specific agents with minimal effect on nonsenescent cells. Indeed, ABT263, which targets both the antiapoptotic proteins BCL-xL and BCL-2, has been

recently shown to specifically induce apoptosis in senescent cells [23].

Improvement of the Immune System

Another possible way of eliminating senescent cells is to increase the number and/or activity of immune cells that can selectively recognize and remove senescent cells. Indeed, natural killer (NK) cells and T cells trigger cytolytic responses on senescent cells [24]. Moreover, the CD4+ T cell-mediated adaptive immune response initiates an immune-dependent clearance of senescent cells termed senescence surveillance [25]. The decline in immune function with age is consistent with the high number of senescent cells at old age [4], further supporting the idea that the immune system may limit the number of senescent cells through clearance of these cells. Hence, it may be worth developing a strategy that boosts the immune cells capable of specifically eliminating senescent cells.

Inhibition of the SASP

Apart from the removal of senescent cells, decreasing the effect of the SASP may potentially be an alternative strategy to dampen the negative effects of long-lived senescent cells. Overexpression of oncogenic RAS, which causes genotoxic stress and cellular senescence, is a strong inducer of the SASP [3]. RAS activation can trigger a phosphorylation cascade involving activation of the mitogen-activated protein kinase (MAPK) family of proteins. Consistent with this, the MAPK family member p38MAPK is an important regulator of the SASP [26]. Treatment with the p38MAPK inhibitor SB203580 potently suppressed SASP expression in senescent cells. Two next-generation p38 inhibitors UR-13756 and BIRB 796, which are more selective and specific compared to SB203580, can also dampen the SASP [27]. BIRB 796 has reached phase III clinical trials, suggesting that p38MAPK may be a potential therapeutic target to suppress the SASP in vivo. Inhibition of MK2 kinase (also MAPKAPK2), a downstream target of p38MAPK, can also inhibit the SASP [27]. MK2 is implicated in phosphorylation of the RNA-binding protein ZFP36L1, which degrades transcripts of several SASP components. Hence, MK2 kinase inhibition is also being investigated as a potential pharmacological strategy to dampen senescence-associated inflammation.

p38MAPK activation of the SASP is largely due to an increase in nuclear factor-κB (NF-κB) transcriptional activity independently of the canonical DNA damage response [26]. NF-κB is thought to be an important inducer of the SASP. It is one of the transcription factors that re-enforces the SASP response [28]. Many signaling pathways that modulate the SASP seem to converge with NF-κB. For example, accumulation of GATA4 in senescent cells, as a consequence of its impaired degradation by p62-mediated autophagy, leads to activation of NF-κB and subsequent upregulation of the SASP [29]. Unlike p38MAPK, which is independent of the DNA damage response, GATA4-induced SASP is dependent on the DNA damage response, but independent of p53 and $p16^{INK4A}$ [29]. Chronic resveratrol treatment also dampens the SASP [30], likely through its ability to decrease IκB kinase activity, which leads to decreased IκBα phosphorylation and subsequent NF-κB activation [31]. Other natural compounds, such as apigenin, wogonin, and kaempferol, inhibit the SASP by blocking IκBζ expression and reducing NF-κB activity [32].

Another important activator of NF-κB and the SASP is interleukin-1 alpha (IL1A or IL-1α). Increased expression of plasma membrane-bound IL1A in senescent cells activates the plasma membrane-bound IL-1 receptor in juxtacrine cells, resulting in upregulation of several transcripts associated with inflammation [33]. IL1A-blocking antibody or knockdown of IL1A by RNA interference diminished SASP expression in senescent cells [33]. Moreover, exogenous IL1A restored IL-6 and IL-8 secretion to IL1A-knockout cells, further supporting a direct role of IL1A in SASP regulation [33]. Compounds that disrupt IL1A receptor signaling may serve as a strategy to dampen the SASP. Indeed, the glucocorticoids cortisol and corticosterone suppress IL1A signaling in senescent cells and decrease expression of the SASP components, but cotreatment with recombinant IL1A reestablishes the SASP [34]. Overexpressing the microRNA miR-146a/b, which knocks down the levels of the IL1A downstream target IRAK1 (also an upstream regulator of NFκB), resulted in decreased SASP expression [35]. Treatment with the mTOR inhibitor rapamycin also caused downregulation of IL-6 and other SASP factors, partly due to suppressing translation of the membrane-bound IL1A [36]. Additionally, rapamycin-induced SASP is also thought to result from inhibition of MK2 translation and inactivation of ZFP36L1 activity [37].

Other regulators of the SASP include p53, C/EBPβ, Sirt1, and NAD+/NADH redox balance. Functional loss of the p53 protein exacerbates the SASP [3, 38]. C/EBPβ is implicated in upregulating the SASP and may cooperate with IL-6 to amplify the SASP [28, 39]. Sirt1 suppresses SASP expression through histone deacetylation at the promoter regions of SASP factors [40]. Interestingly,

Sirt1 is also pharmacologically activated by resveratrol [41], which is capable of dampening the SASP [30]. The activation of Sirt1 by resveratrol should be explored further, particularly with regard to p53 inhibition [42]. Imbalance in NAD+/NADH redox due to impaired mitochondrial activity results in reduced expression of selected SASP factors [43]. Designing drugs that target SASP regulators may help in developing pharmaceutical therapies to limit the negative side effects of the SASP.

Conclusion

Removal of senescent cells and reducing the SASP are being considered as therapeutic strategies to delay the onset of age-related pathologies. Several drugs have already been identified that selectively target senescent cells (fig. 1). Some of them might have toxic effects when administered systemically for a long period of time: for example, ABT263 can cause thrombocytopenia in patients treated with an oral form of the compound. However, the toxicity might be highly reduced by developing drugs for topical treatment, an approach that would be suitable for skin interventions. The contribution of senescent cells to skin function is complex because they may be both beneficial and detrimental depending on the context; it is still unclear whether senolytic drugs will delay skin aging [6]. Senescent cells are important for proper wound healing through their secretion of the SASP factor PDGF-AA and through their capacity to limit fibrosis [12], while chronic induction of cellular senescence through mitochondrial dysfunction may contribute to stem cell loss with age [10]. Hence, proper testing of dosage and timing must be investigated to determine if these drugs would indeed reduce the negative impact on skin aging. Nonetheless, the possibility of selectively targeting senescent cells through pharmacological interventions posits a potential new solution to the functional decline associated with skin aging.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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