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Cellular Senescence in Human Skin Aging: Leveraging Senotherapeutics

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Abstract

Background: As the largest organ in the human body, skin is continuously exposed to intrinsic and extrinsic stimuli that impact its functionality and morphology with aging. Skin aging entails dysregulation of skin cells and loss, fragmentation, or fragility of extracellular matrix fibers that are manifested macroscopically by wrinkling, laxity, and pigmentary abnormalities. Age-related skin changes are the focus of many surgical and non-surgical treatments aimed at improving overall skin appearance and health.

Summary: As a hallmark of aging, cellular senescence, an essentially irreversible cell cycle arrest with apoptosis resistance and a secretory phenotype, manifests across skin layers by affecting epidermal and dermal cells. Knowledge of skin-specific senescent cells, such as melanocytes (epidermal aging) and fibroblasts (dermal aging), will promote our understanding of age-related skin changes and how to optimize patient outcomes in aesthetic procedures.

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Author Contributions

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Conflict of Interest Statement

Patents on senolytic drugs to J.L.K. and T.T. are held by Mayo Clinic. S.P.W. has a nonrelevant financial interest in Rion LLC. The authors have no financial interest to declare in relation to the content of this article. This research has been reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with Mayo Clinic conflict of interest policies.

Key Messages: This review provides an overview of skin aging in the context of cellular senescence and discusses senolytic intervention strategies to selectively target skin senescent cells that contribute to premature skin aging.

Keywords

Cellular senescence; skin aging; age-related changes; epidermal senescence; dermal senescence

Introduction

Aging, associated with a time-dependent functional decline in most living organisms, has piqued the quest to slow or reverse biological aging throughout the history of humankind [1, 2]. Skin aging, akin to organismal whole-body aging, is characterized by gradual loss of function and regenerative capacity [3]. The human epidermis has the innate capacity to renew approximately every 40-56 days but slows with aging [4]. Intrinsic and extrinsic insults drive the skin aging process [5]. Intrinsic aging primarily reflects genetic background, whereas extrinsic aging reflects environmental triggers, such as ultraviolet (UV) exposure, air pollution, smoking, alcohol intake, and poor nutrition, among others [6], resulting in reduced regenerative potential. Clinically, skin aging is linked to reduced barrier protection, poor wound healing [7], increased inflammation [8], deficient water and thermal homeostasis [9], and susceptibility to skin disorders, including skin cancers [10]. Indeed, the interlinked hallmarks of whole-body aging, characterized by a progressive loss of physiological integrity, include genomic instability [11], telomere attrition [12], epigenetic alterations [13], loss of proteostasis [14], deregulated nutrient-sensing [15], mitochondrial dysfunction [16], cellular senescence [17], stem cell exhaustion [18], and altered intercellular communication [19] (Figure 1). In this review, we primarily focus on the role of cellular senescence in skin aging and regeneration.

Cellular senescence is an essentially permanent state of cell cycle arrest with both beneficial and detrimental effects in development and aging. Leonard Hayflick and Paul Moorhead originally hypothesized the connection between aging and senescence in 1961 after noticing limited proliferative capacity in serially-subcultured human primary fibroblasts [20]. While cellular senescence has an evolutionarily advantageous role in facilitating tissue remodeling during development and after injury, it can also play a damaging role in the aging process by impairing tissue regeneration, causing inflammation and fibrosis, and promoting tumor growth [21]. Senescent cells exhibit extensive alterations in chromatin architecture and gene expression in addition to growth arrest [22]. The senescence-associated secretory phenotype (SASP) is a prominent characteristic of senescent cells that can includes the secretion of several pro-inflammatory cytokines, chemokines, growth factors, proteases, bioactive lipids (bradykines, ceramides, prostenoids), non-coding nucleotides (*e.g.*, microRNA's and mitochondrial DNA), and other factors [23, 24, 25, 26, 27, 28]. The SASP portfolio, which includes factors that modulate immune cell proliferation and migration, allows senescent cells to activate, suppress, modulate, and/or evade the immune system [29] (shown in Fig. 1). Indeed, various types of cellular stressors can trigger cellular senescence *in vitro* [30]; yet, the identification of unique senescence markers, particularly *in vivo*, is still under investigation. Therefore, the field of translational geroscience continues to define the

senescent phenotype in specific tissues and identify new pathways for therapeutic removal of senescent cells directly relevant to the skin.

Histology of Skin Aging

Skin consists of three layers: the epidermis, dermis, and subcutaneous tissue. The epidermis, comprised of multiple cell types including keratinocytes, melanocytes, Langerhans cells, and Merkel cells, is a stratified squamous epithelium that undergoes continuous renewal [31]. Histologically, skin aging results in epidermal thinning with flattening of the dermal-epidermal junction [32]. This manifests as increased skin fragility and reduced nutrient transfer between dermis and epidermis, attributed to the loss of surface area of the dermal-epidermal interface. Furthermore, epidermal cell turnover decreases with age [33], which accounts for less effective desquamation and reduced wound healing.

Beyond the epidermis, the dermis experiences the most significant ultrastructural change with age [34]. The dermis, divided into the more superficial papillary dermis and the deeper reticular dermis, consists of extracellular matrix (ECM) fibers, which are crucial for maintaining skin's structural integrity [35]. Deterioration causes the dermis to separate from the epidermis, resulting in skin laxity and decreased epidermal stem cell renewal [36]. Fibroblasts, the most prevalent cells in the dermis, deposit the collagen and elastic fibers of the ECM [37]. Throughout the aging process, fibroblasts synchronously decrease in number and function [38]. Young dermal fibroblasts produce glycosaminoglycans and extracellular matrix fibers, including elastin and type I collagen, which make up approximately 90% of the extracellular matrix [39]. As the number and diameter of collagen fibers decrease with age, the ratio of type III collagen to type I collagen increases [40]. Furthermore, aged skin is associated with dermal collagen and elastin fragmentation, which presents as decreased skin elasticity and turgor [41]. Together, these age-dependent ultrastructural changes account for the physical manifestations of cutaneous aging [42, 43].

Molecular Biomarkers of Skin Aging

Markers of cellular senescence in skin, including nuclear and SASP markers, have been used to detect senescent cells in aging and disease. Upregulation or downregulation of various cellular senescence markers have been used to characterize cellular senescence burden in skin. Increase in SA-β-galactosidase has been applied extensively as a marker of cellular senescence [44, 45]. Similarly, the cell cycle markers $p16^{INK4a}$ and $p21^{CIP1/WAF1}$ have been used to study senescent fibroblasts and melanocytes in skin [46, 47, 48]. Alterations in the level of lamin B1 have been implicated as an early senescence marker in multiple tissues, including skin [49, 50, 51, 52]. Particularly, reductions in lamin B1 were found in dermal fibroblasts and keratinocytes from older donors [53], keratinocytes in photoaged skin [52], and melanocytes in melanocytic nevi [51]. Senescent fibroblasts have also been demonstrated to secrete HMGB1 before developing a SASP [54]. On the other hand, melanocytes and keratinocytes from older donors expressed reduced HMGB1 [55]. In addition to these, numerous biomarkers have been developed for skin aging, such as telomere-associated foci [48].

Epidermal Aging: Role of Keratinocytes

Keratinocytes are the most abundant cells in the epidermis and directly contribute to the skin barrier. As skin ages, there is a shift in keratinocyte morphology that contributes to epidermal thinning. Basal keratinocytes become shorter and larger, and corneocytes, which are terminally differentiated keratinocytes, also grow larger due to reduced epidermal turnover [56]. The notion of whether keratinocytes can acquire senescent phenotypes has been questioned given their highly proliferative state. $p16^{INK4a}$, a marker of cellular senescence, was detected in human skin biopsies of sun-exposed areas [57, 58, 59]. Skin tissues from photoprotected areas of young and old donors showed that $p16^{INK4a}$-positive cells were predominantly melanocytes and not keratinocytes in the epidermal layer, highlighting the differences in senescence phenotypes given sun exposure [55, 60]. However, senescent cell markers were detected in keratinocytes from actinic keratoses, UV-associated lesions. Specifically, actinic keratosis was associated with increased $p16^{INK4a}$ and reduced lamin B1 and HMGB1, and $p16^{INK4a}$ expression was associated with development of squamous cell carcinoma [61, 62].

Epidermal Aging: Role of Melanocytes

Melanocytes, or pigment-producing cells derived from the neural crest, are in spatial proximity to keratinocytes in the epidermal layer. It has been postulated that cellular senescence may provide an evolutionary protection against malignant transformation of melanocytes, as pigmentation is a strong defense against melanoma [63]. As such, melanin accumulation in the epidermis, through α-melanocyte stimulating hormone or cholera toxin, can induce melanocyte senescence through the p16/CDK4/pRB pathway [64]. Studies have also shown that $p16^{INK4a}$-positive melanocytes accumulate in aged human epidermis. A correlation between increased numbers of $p16^{INK4a}$-positive melanocytes and facial aging phenotypes, such as wrinkles, morphological changes in elastic fibers, and dysfunctional telomeres, has been reported [55, 65, 66, 67]. In addition, UV-irradiated melanocytes enter premature senescence with downregulation of DNA repair programs such as nucleotide excision repair (NER) pathway genes, especially genes involved in DNA damage recognition (RAD23B, XPC, ERCC3, ERCC8, and RPA1) [68]. Moreover, senescent melanocytes could result in tissue-level disruption. p16-positive melanocytes induce gamma-H2A-X foci in neighboring keratinocytes, indicating telomere dysfunction, and exposure to senescent-melanocyte-conditioned media induced telomere damage in fibroblasts [55]. Interestingly, clearance of senescent melanocytes with ABT-737, a BCL-2 inhibitor, or MitoQ, a mitochondrial-targeted antioxidant, attenuated telomere dysfunction [55]. Yet, caution must be utilized in its clearance as p16 has a function in suppressing or limiting growth of melanocytic nevi (moles) and germline mutations in p16 are often associated with dysplastic nevi and even melanomas [69].

Dermal Aging: Role of Fibroblasts

Fibroblasts, as the most abundant cell type that resides in the dermis, largely contribute to hallmarks of skin aging [70]. Dermal fibroblasts subjected to *in vitro* aging protocols accumulate double-strand breaks [71], oxidative DNA damage, chromosomal and epigenetic

abnormalities, telomere shortening or oxidation, and impaired DNA repair mechanisms [72]. Senescent fibroblasts garner defects in protein synthesis, folding, and degradation, in addition to defects in post-translational modifications such as oxidation and cross-linking, which affect protein homeostasis (quantitative and qualitative of the cellular proteome). These changes cause senescent fibroblasts to display biomarkers such as increased senescence-associated-beta-galactosidase (SA-β-gal), $p16^{INK4a}$, and $p21^{CIP1/WAF1}$ [73, 74, 75].

Indeed, senescent fibroblasts in the skin can cause harmful effects through different mechanisms. UV-induced dermal senescence can alter the extracellular matrix as well as the function of adjacent cells, increasing the risk of carcinogenesis. For example, the cytokines IL-1α, IL-1β, IL-6, and TNF-α, are highly secreted by senescent cells and have been reported to induce skin carcinogenesis. Furthermore, the secretion of MMPs as a consequence of photodamage leads to collagen degradation, epithelial-mesenchymal conversion, angiogenesis, and inflammation [76, 77]. In cultured fibroblasts, UVA and/or a combination of UVA and UVB upregulate MMP-1 [78, 79], leading to skin aging phenotypes.

It has also been reported that an age-dependent increase in human fibroblast senescence occurs as indicated by $p16^{INK4a}$ and SA-β-gal expression in skin biopsies from donors across the age groups of 0-20 years, 21-70 years, and 71-95 years [80]. Analysis of primary human dermal fibroblasts in multiple *in vitro* aging models, including UVB irradiation and accelerated proliferation of human dermal fibroblasts in young *vs.* elderly donors, revealed reduced cell growth rate and premature senescence [81]. Further reports indicate that young skin is more resilient to wound healing, particularly in the context of chronic wounds that accumulate senescent cell phenotypes [82, 83]. However, a transient induction of senescent cells occurs in normal acute wound healing and could be beneficial [84]. These findings implicate senescent fibroblasts as a potential target for reducing the negative effects on extracellular matrix due to SASP factors and for enhancing dermal skin rejuvenation.

Targeting Cellular Senescence in Skin Aging

Initial reports that conveyed an inverse association between senescent cell burden and healthspan led to the advent of senolytics – a class of drugs that selectively clears senescent cells [85, 86, 87]. The impact of senescent cell accumulation was demonstrated when killing senescent cells *via* a suicide gene in a mouse model of premature aging reduced age-related diseases, such as sarcopenia, cataracts, and loss of subdermal adipose tissue in progeroid mice [88] and adipose and metabolic dysfunction in naturally-aged mice [89]. Therapeutic interventions that target senescent cells are categorized as senotherapeutics. Specifically, modulation of cellular senescence can be achieved by selective induction of cell death (senolytics) or SASP inhibition (senomorphics). Skin presents as an ideal site for senotherapeutic testing due to its accessibility and established characterization. However, translation of senotherapeutics has been limited by the need for better *in vitro* models of skin aging for testing. Few models of skin aging have been described, and they are limited by cell type [90, 91, 92].

A hypothesis-driven, mechanism-based drug discovery approach, stemming from the observation that senescent cells resist apoptosis, led to the development of the first senolytic drugs [85]. In particular, agents that transiently decrease anti-apoptotic regulators, such as Src kinases or Bcl-xL or other BCL-2 family members, were effective in disabling defenses of senescent cells against their own pro-apoptotic SASP, causing them to undergo apoptosis [87, 93]. Next, bioinformatics approaches were utilized to find compounds whose mechanisms of action targeted these senescent cell anti-apoptotic pathways (SCAPs). These agents included Dasatinib (D), the Src tyrosine kinase inhibitor, and Quercetin (Q), a naturally occurring flavonoid found in apple peels that targets other SCAP pathways. First-generation senolytics also include fisetin, luteolin, curcumin, navitoclax (ABT263), and procyanidin C1 among others [85, 87, 94, 95, 96, 97](PMID: 34873338). Second generation senolytic agents are being identified through other drug discovery methods, including random high-throughput drug library screens, vaccines, toxin-loaded nanoparticles preferentially lysed by senescent cells, and immunomodulators [45, 98, 99, 100]. In particular, the first-generation senolytic Dasatanib and Quercetin (D+Q) showed trends of reducing p16 and p21 expression in human epidermis, suggesting its potential efficacy [101].

Senomodifiers or senomorphics are drugs that suppress the adverse effects of senescent cells without directly clearing them, such as JAK inhibitors [102] or rapamycin [103]. Rapamycin, which targets the mTOR pathway regulating cell growth, metabolism, protein synthesis, and autophagy, has been found to reduce senescent cells in human skin, specifically dermal fibroblasts [57, 104], possibly because the SASP spreads senescence [93] and, hence, inhibiting the SASP could reduce senescent cell burden. Rapamycin inhibits the upregulation of IL-1α in senescent fibroblasts, which subsequently blocks IL-1α-induced secretion of other pro-SASP factors [105, 106, 107]. In accordance, rapamycin reduced signs of cellular skin aging in murine skin fibroblasts following UVB irradiation [106]. Indeed, 5 μM rapamycin significantly decreased SA-β-gal-positive cells, preserved elongated fibroblast morphology, and attenuated irradiation-induced reactive oxygen species release [106]. This was also described with the senomorphic and mTOR inhibitor, AZD8055, in foreskin fibroblasts [108]. Taken together, these observations suggest that targeting cellular senescence, in part, may contribute to skin rejuvenation and overall skin health. In addition, senotherapeutics could potentially block cancer pathways associated with cellular senescence, making them candidates to treat or prevent precancerous lesions, such as actinic keratoses.

Conclusions

This review highlights the importance of understanding cellular mechanisms of skin aging, especially, cellular senescence. Age-dependent physiological consequences of epidermal (keratinocytes and melanocytes) aging and dermal (fibroblasts) aging considerably affect skin health in the elderly population. Targeting cellular senescence as a driver of biological aging may allow modulation of age-related dysfunction to alleviate multimorbidity. However, there is need for future studies to evaluate senescent cell types and interactions in skin using large scale datasets and bioinformatics. With deeper understanding of cellular senescence in skin aging, applications of senotherapeutics for skin aging raise many possibilities. Can senotherapeutics reverse skin aging phenotypes resulting from

premature senescence and/or photoaging? How can we selectively target senescent cells that compromise skin tissue functionality while retaining the evolutionary benefit of senescence as a barrier to tumorigenesis? These questions warrant further research into testing senotherapeutics in the context of human skin aging.

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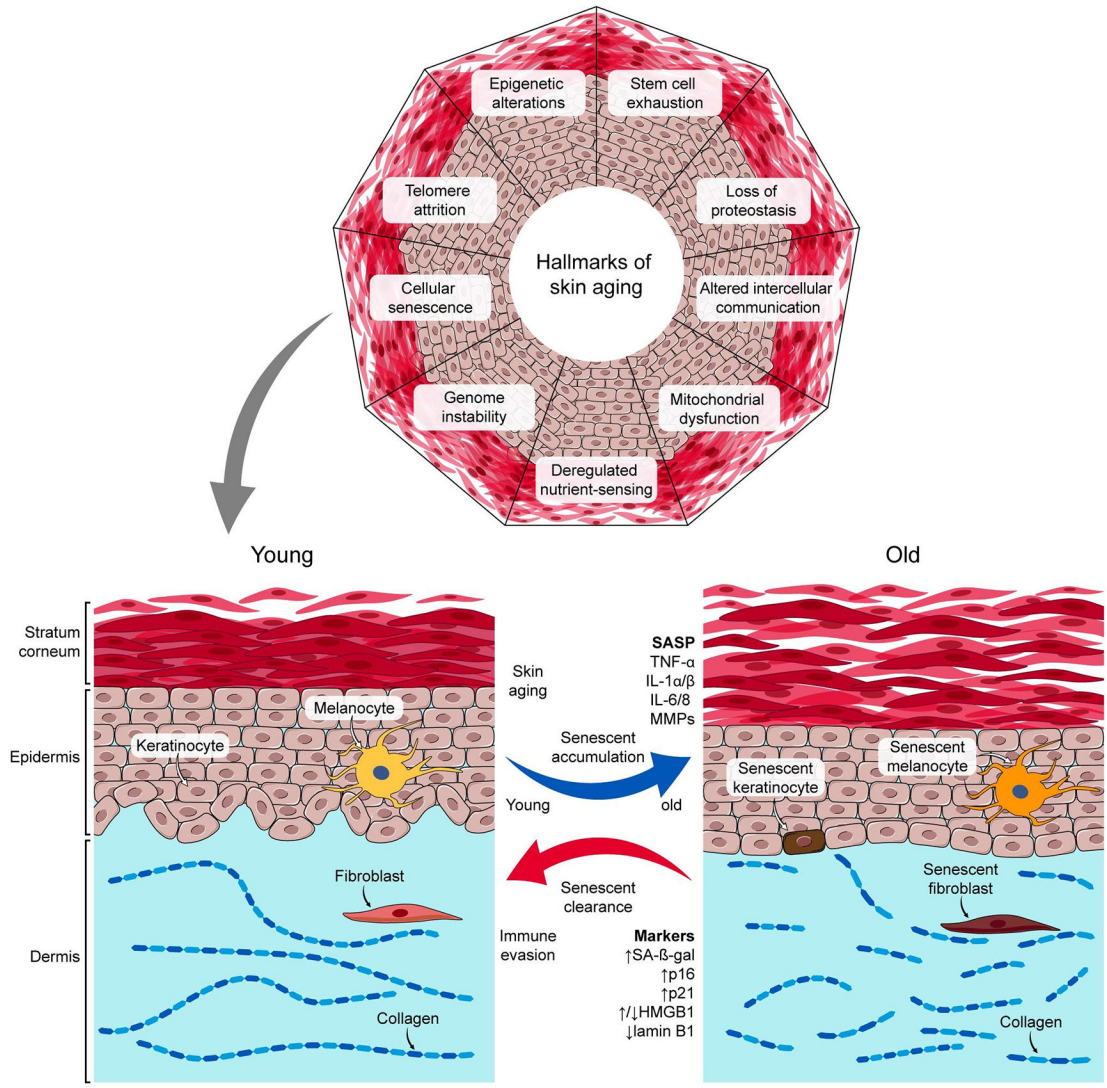

Fig. 1. Hallmarks of Skin Aging.

An illustration of skin senescent cell accumulation and corresponding senescence associated secretory phenotype (SASP) factor release in young *vs.* old human skin models.