Mini-review

Inflammaging in skin and intrinsic underlying factors

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ABSTRACT

Aging of organs starts from the time of birth and continues throughout life. Aging of skin can be divided into two distinct types — intrinsic aging and extrinsic, based on the fact that the skin is the outermost organ exposed to the external environment. However, despite their different histological features and triggers, intrinsic and extrinsic aging share common biochemical mechanisms. β-galactosidase, p16[INK4a], and senescence-associated secretory phenotype (SASP) factors are detected in skin cells as biomarkers of senescence. In particular, inflammatory cytokines, the constituents of SASP, play pivotal roles in "inflammaging" which is a concept involving the relationship between aging and low-grade inflammation. In this review, the features of skin aging and its underlying mechanism of skin aging are summarized.
Keywords: SASP; Inflammaging; Skin

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1. Introduction

Aging of organs starts from the time when one is born and continues throughout life. Thus, the strategies to prevent chronological tissue dysfunction have become an important issue in increasing elderly societies. Since Hayflick and Moorhead reported the finite proliferative capacity of cultured normal human fibroblasts[1], researchers have contended the involvement of cell senescence in organ aging. However, accumulating evidence has established obvious roles of senescent cells, which are defined by irreversible cell-cycle arrest, resistance to apoptosis, and senescence-associated secretory phenotype (SASP), in physiological and pathological states[2-4].

In the skin, aging can be divided into two distinct types: intrinsic aging and extrinsic aging. Intrinsic aging is an inevitable physiological process and characterized by dry skin, fine wrinkles, and gradual dermal atrophy. On the other hand, extrinsic aging is induced by environmental factors, including air pollution and sun exposure, and is characterized by coarse wrinkles, loss of elasticity, and rough texture appearance[5,6]. Similar to other organs, senescent cells accumulate in intrinsically and extrinsically aged skin and contribute to skin aging. In this review, the skin environment of senescent cells is summarized.

2. Intrinsic skin aging

Intrinsic skin aging is a process of physiological change involving photo-protected areas. Intrinsically aged skin is clinically characterized by dryness, fine wrinkles, and a histologically thinner epidermis and flattened dermal-epidermal junction[7,8]. Clinical features are induced by a significant reduction in surface lipid production with

chronological aging, whereas stratum corneum hydration and transepidermal water loss are modestly lowered or unchanged[9,10]. Thinning of the epidermis is induced by reduction of basal keratinocyte proliferation dependent on reduction of the nutrient flux through age-related flattened dermal-epidermal junctions, in which the area of the available exchange surface is reduced[11,12]. In the dermis, there are fewer fibroblasts in aged skin than in young skin[13]. In addition, the production of type I procollagen in intrinsically aged human skin is reduced, depending on the downregulation of TGF-β/Smad signaling[14]. In addition to aging-induced structural changes, presumably the cutaneous immunity becomes defective with age. A variety of bacterial, fungal, and viral infections markedly increase with age[15-17]. Toll-like receptors (TLRs), which are crucial pathogen pattern recognition receptors, are expressed in keratinocytes, fibroblasts, and skin-resident immune cells. Once triggered by ligands, TLRs lead to the production of inflammatory cytokines and initiation of immune responses[18,19]. Shaw *et al.* reported that the expression and function of TLRs diminished with age[20]. Furthermore, TLR ligand-induced production of inflammatory cytokines is reduced in circulating dendritic cells in older individuals[21]. This indicates that pathogen pattern recognition, which is the primary process of the innate immune system, is attenuated with age. Dendritic cells (DCs), which are the sentinels of the immune system, bridge the innate and adaptive immune system by sequestration and presentation of antigens to T cells. Diverse populations of DCs, including dermal DCs, dermal macrophages in the dermis, and Langerhans cells (LCs) represent DCs in epidermis[22,23]. A previous study reported that the absolute number of DCs and their CD34+ precursors declined with age[24,25]. In addition to the reduction in the number of LCs in aged-skin, the migratory ability of LCs in aged skin is impaired because of attenuation of the responses to cytokine gradients, and the subsequent accumulation of LCs in regional lymph nodes is reduced[26-28]. In the whole skin of an average person, there are approximately 2×10^{10} T cells, including resident memory T cells (T_{RM}) and circulating memory T cells[29]. The CD4+/CD103− T_{RM} cells are located in the dermis, whereas CD4+/CD103+ and CD8+/CD103+ T_{RM} cells are enriched in epidermis. Both CD4+/CD103+ and CD8+/CD103+ T_{RM} cells have more potent effector functions than circulating T cells, but have less proliferative capacity than that of the CD103− T_{RM} cells[30]. To investigate antigen-specific T cell responses, delayed-type hypersensitivity reactions (DTHs) represent the most informative *in vivo* experimental models. Previous studies have demonstrated that DTHs are impaired in older humans and mice[18,31-33]. The proportion of memory phenotype T cells increases with age and becomes predominant after midlife, whereas the total number of T cells is maintained throughout life[34]. Repeated antigen exposure during the lifespan induces exhausted T cells characterized by telomere shortening and expression of exhaustion markers such as PD1 and LAG3[35]. Moreover, continuous homeostatic proliferation induces dysfunctional CD4+ T cells, named senescence-associated T cells, which are characterized by the expression of PD1 and LAG3, and abundant secretion of inflammatory cytokines. The proportion of senescence-associated T cells progressively increases with age[36,37]. Therefore, it is supposed that age-related dysfunction, including antigen recognition and presentation, and senescence in T cells reflects cutaneous immunity.

3. Extrinsic skin aging

Extrinsic aging is caused by several exogenous factors such as tobacco smoke, air pollution and ultraviolet (UV) rays. Out of these factors, UV affects aging the most; therefor, extrinsic aging is referred to as photoaging. UV is classified as UVA, UVB and UVC, depending on the wavelength. UVA (320–400 nm) and UVB (280–320 nm) reach the surface of the earth, while UVC (100–280 nm) is absorbed by the ozone layer. Although UVB has higher energy, UVB is mostly absorbed by the epidermis, owing to its shorter wavelength, while UVA, which has a lower energy, can penetrate into the dermis. Therefore, UVB is responsible for acute sunburn reactions in the epidermis, and UVA is considered as a major factor in chronic dermal photoaging. UV-irradiated epidermis thickens, in contrast to the thinner epidermis, is observed in intrinsically aged skin[38]. Tissue renewal in the epi-

dermis is dependent on proliferative cells in the basal layer, which include keratinocyte stem cells (KSCs) and transit amplifying (TA) cells[39]. Although the expression of integrin β1, which is a KSC marker, is reduced and the ratio between involucrin, a differentiation marker of keratinocytes, and integrin β1 is increased, aberrant suprabasal integrin β1 expression and enhanced expression of Ki-67, expressed in proliferating cells, are detected in chronic sun-exposed skin of the elderly. In addition, flow cytometric analysis revealed that integrin α6^{bri} CD71^{bri} cell numbers are greater in sun-exposed epidermis than in sun-protected epidermis, suggesting that the proliferation of TA cells is increased in sun-exposed epidermis[40,41]. These results suggest that UV exposure induces a hyperproliferative state of epidermis in photoaged skin. Another clinical characteristic is the presence of coarse wrinkles in photoaged skin. Studies have demonstrated that the reduction of collagen type I formation in photodamaged human skin, depending on UV irradiation-induced matrix metalloproteinase (MMP) expression and synthesis inhibition by damaged collagen, contributes to UV irradiation-induced wrinkle formation[42-44]. Similarly, the suppression of collagen type IV, a component of the basement membrane, and collagen type VII, an anchoring fibril connecting fibroblasts to the basement membrane, affects wrinkle formation, because of weakening of the dermal-epidermal junction[45,46].

4. Senescent cell biomarkers

Cellular senescence was first described as the finite proliferative capacity of cultured normal human fibroblasts[1]. Irreversible cell growth arrest occurs due to DNA damage, telomere shortening[47], and oncogenic stress[48]. As removing senescent cells from aging tissues can delay tissue dysfunction and lead to prolonged lifespan, obvious biomarkers to identify senescent cells have been sought. The activity of β-galactosidase (β-gal) at pH 6 is increased in middle-late passage cultured fibroblasts and keratinocytes, whereas terminally differentiated keratinocytes do not express β-gal at pH 6. Activity in skin sections from the different age groups increases with age, suggesting that se-

nescent cells accumulate *in vivo* with age[49]. Thus, the β-gal activity is termed senescence-associated β-gal (SA-β-gal) activity and remains the gold standard for identifying senescent cells in culture and in tissue samples. As senescent cells are irreversibly arrested, cell cycle regulators are usually employed to detect senescent cells. p16^{INK4a}, encoded by the Ink4a/Arf locus, is a tumor growth suppressor. In normal human keratinocytes, p16^{INK4a}, which is upregulated by single or repeated UVB irradiation, plays a role in cell cycle regulation[50]. The expression of p16^{INK4a} markedly increases with advancing age in mice and humans, suggesting that p16^{INK4a} is a cellular senescence marker[51,52]. A previous study showed that the number of p16^{INK4a} positive cells increases with age in the skin and that numerous cardiovascular diseases are significantly associated with tertiles of p16^{INK4a} positive cells in epidermal cells, suggesting an association between cell senescence and age-related pathology[53]. Previous studies have demonstrated that nuclear senescence-associated events such as heterochromatin loss, remodeling of the nuclear lamina, and DNA methylation are involved in cell proliferation[54-57]. Senescent cells secrete senescence-associated secretory phenotype (SASP) factors, including inflammatory cytokines, chemokines, MMPs and growth factors. The presence of SASP factors such as MMP3, MMP9, IL-6, IL-8, and insulin-like growth factor binding protein 7 has been used as a marker for senescent dermal fibroblasts and melanocytes[58-60]. The release of SASP factors is facilitated by the translocation of high mobility group box-1 (HMGB1) proteins from the nucleus to the cytoplasm and extracellular space in senescent cells[61,62]. While molecular hallmarks of cell senescence have been characterized *in vitro*, Lupa *et al.* demonstrated a correlation between SASP expression and age in intrinsically-aged human dermal fibroblasts, suggesting that SASP expression is upregulated along with chronological aging *in vivo*[63]. Collectively, senescent cells, which are considered passive bystanders, modulate their environment by secretion of SASP factors in both *in vitro* and *in vivo* (**Figure 1**).
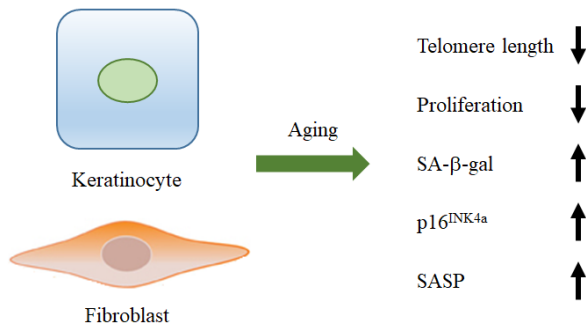
Figure 1. Senescent cell biomarkers. Shortened telomeres, reduction of cell proliferative capacity, and increased SA-β-gal activity, p16^{INK4a} expression, and SASP production.

5. Inflammaging

Since Franceschi *et al.* used the term "Inflamm-aging"[64], similar terms such as "inflammaging" and various other concepts have been proposed[65-70]. Despite of confusion regarding definitions and terminology, there is consensus that inflammaging refers to low-grade, chronic, and asymptomatic inflammation, and that the primary feature of inflammaging is an increase in proinflammatory status with advancing age[71]. Several factors are involved in the underlying mechanism of inflammaging[69,72-77]. Inflammatory cytokines, which are constituents of SASP, play pivotal roles in inflammation. Previous studies have suggested that complex processes are involved in the upregulation of inflammatory cytokine expression. Although p16^{INK4a} is sufficient to induce senescent cell cycle arrest[78], precipitating DNA damage leads to the upregulation of inflammatory cytokines, suggesting that the mechanism of inflammatory cytokine production is distinct from that of senescent cell cycle arrest. Studies have also demonstrated that the upregulation of inflammatory cytokine expression is triggered by activated ataxia telangiectasia mutated (ATM)-mediated DNA damage responses[79,80]. In addition to the DNA-damage response pathway, p38MAPK and protein kinase D1 are involved in the production of inflammatory cytokines. DNA-damage responses and kinases induce phosphorylation of NFκB p65/RelA subunit, followed by translocation to the nucleus where it binds to the promoters of inflammatory cytokine genes, regulating their induction during senescence[81-83]. In addition to NFκB, CCAAT/ enhancer binding protein β (CEBPβ), which is regulated by the mitogen-activated protein kinase (MAPK) pathway, participates in inflammatory cytokine production. Sebastian *et al.* showed that CEBPβ is critical for cell senescence in mouse embryonic fibroblasts[84]. Following the activation of transcription factors, it is supposed that IL-1α paracrinally regulates the production of SASP production as an upstream modulator. Previous studies have shown that IL-1α is essential for inducing the production of IL-6 and IL-8[85,86]. Consequently, low-grade chronic inflammation reinforces senescence via cell growth arrest and disruption of stem cell function[58,59,87,88]. Similar to the regulation of SASP production in intrinsic aging, previous studies have demonstrated that UVB irradiation, which is a major stimulus in extrinsic aging (photoaging), is involved in SASP regulation, such as the production of IL-6 and IL-8, activation of NFκB, activation of insulin-like growth factor-1 receptor, and HMGB1 release[89-92]. In contrast, sirtuin 1 (SIRT1), which is one of the regulators of wound healing[93], suppresses inflammatory cytokines by binding to the promoter regions of inflammatory cytokine genes[93,95]. However, because these studies were performed *in vitro*, it is necessary to perform *in vivo* experiments with a dose of UVB that meets the definition of inflammaging as asymptomatic (**Figure 2**).

6. Conclusions

Despite their different histological features and triggers, intrinsic and extrinsic aging share common biochemical mechanisms. In particular, regulation of inflammatory cytokine production is considered an important therapeutic strategy not only for acute inflammation caused by UV irradiation, but also for anti-aging. On the other hand, from a literature search regrading senescent cells, a question arose that cellular aging in the epidermis and aging in other organs should be distinguished. According to the definition of senescent cells, the cells are denoted exhibiting stable and long-term loss of proliferative capacity, and are distinguished from terminally differentiated cells. As KSCs and TA cells still have proliferative capacity, whereas senescence biomarkers are detected in the epidermis, the microenvironment surrounding KSCs and TA cells should

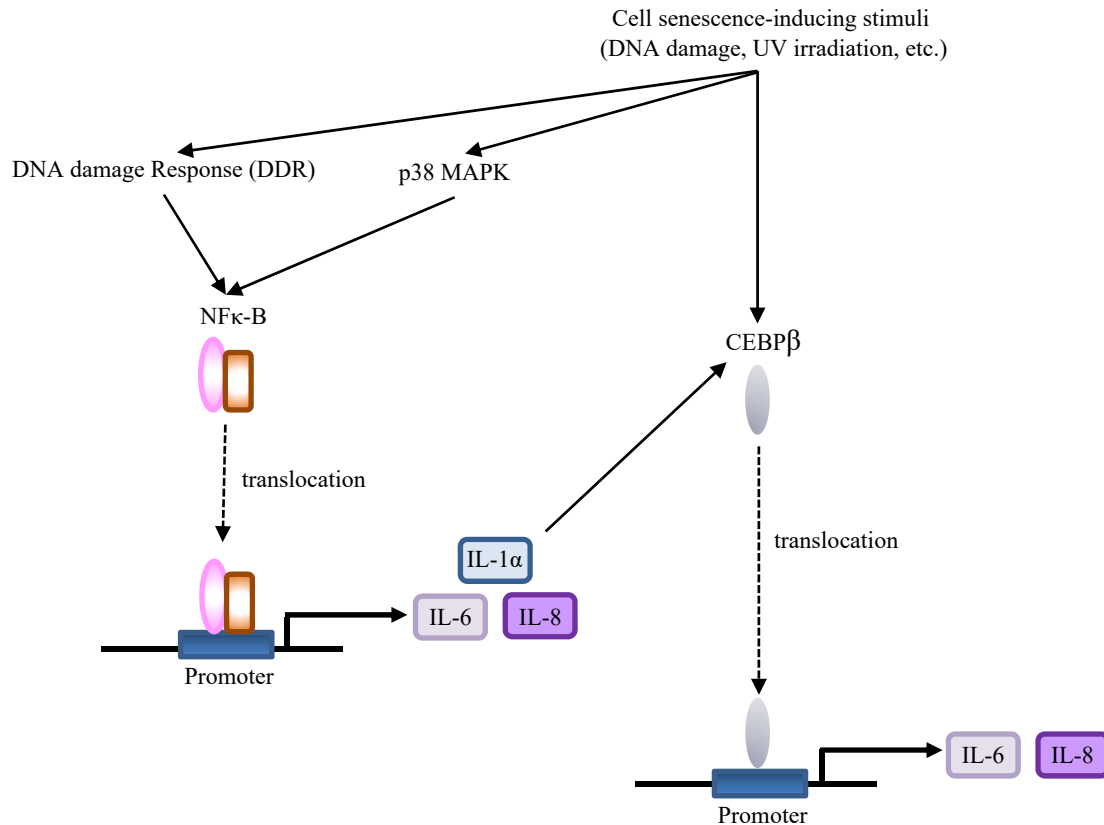be addressed to explore the specific conditions involving the epidermis in the future.

Figure 2. Inflammatory cytokine regulatory pathways. Senescence-inducing stimuli induce DDR and p38MAPK activation, followed by phosphorylation of NFκ-B. Phosphorylated NFκ-B translocates to the nucleus and then binds to the promoter regions of inflammatory cytokines genes. Consequently, the production of inflammatory cytokines is enhanced. Senescence-inducing stimuli and IL-1α, which is induced in the NFκ-B pathway, directly activate CEBPβ. Activated CEBPβ translocates to the nucleus and induces the expression of IL-6 and IL-8 expression.

Conflict of interest

There is no conflict of interest in this review.

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