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Molecular Mechanisms of Skin Aging and Rejuvenation

Miri Kim and Hyun Jeong Park

Additional information is available at the end of the chapter

Abstract

The aging process in the skin is complex and influenced by more intrinsic and extrinsic factors than any other body organ. The effects of these two types of factors overlap for the most part. The combined effects of these two aging processes also affect dermal matrix alterations. The main clinical signs of skin aging include wrinkling and irregular pigmentation, which are influenced by a combination of intrinsic and extrinsic (e.g., UV radiation, heat, smoking, and pollutants) factors. Histologically, collagen decreases, and the dermis is replaced by abnormal elastic fibers as a cause of wrinkle formation through the loss of skin elasticity. There have been numerous studies of skin aging performed to elucidate the underlying molecular mechanisms and to develop various antiaging therapeutics and preventive strategies. We summarized the molecular mechanisms and treatments of skin aging. Mainly UV radiation induces ROS formation and DNA damage, leading to increased production of MMPs and decreased production of collagen in keratinocytes and fibroblasts, which reflect the central aspects of skin aging. Besides UV radiation exposure, extrinsic factors including tobacco smoking, exposure to environmental pollutants, infrared radiation, and heat contribute to premature skin aging. Like UV radiation, these factors cause ROS formation and increase expression of MMPs, thus accelerating skin aging by inducing extracellular matrix (ECM) degradation. Accumulated collagen fibrils inhibit the new collagen synthesis and account for the further degradation of the ECM through this positive feedback loop. Accumulating evidence for molecular mechanisms of skin aging should provide clinicians with an expanding spectrum of therapeutic targets in the treatment of skin aging.

Keywords: skin aging, photoaging, molecular mechanisms, antiaging treatments, rejuvenation

1. Introduction

Skin aging is a complex process affected by both genetic and environmental factors, and it is largely influenced by the cumulative damage from exposure to ultraviolet (UV) radiation. Chronic exposure of UV radiation on human skin leads to solar elastosis, degradation of the extracellular matrix (ECM), and wrinkle formation. Skin aging is affected by both intrinsic and extrinsic factors. Intrinsic or chronological skin aging results from the passage of time and is influenced by genetic factors. Extrinsic skin aging mainly results from UV irradiation, which is called photoaging. These two types of aging processes are superimposed in sun-exposed skin, and they have common clinical features caused by dermal matrix alterations that mainly contribute to wrinkle formation, laxity, and fragility of aged skin [1]. The dermal matrix contains ECM proteins such as collagen, elastin, and proteoglycans which is responsible for conferring strength and resiliency of the skin. Skin aging associated with dermal matrix alterations and atrophy can be caused by senescence of dermal cells such as fibroblasts, and decreased synthesis and accelerated breakdown of dermal collagen fibers [2]. Mouse models of skin aging have been developed extensively to elucidate intrinsic aging and photoaging processes, to validate *in vitro* biochemical data, and to test the effects of pharmacological tools for retarding skin aging because they have the advantages of being genetically similar to humans and are easily available. This review is focused on the molecular mechanisms of skin aging and antiaging treatment with a brief summary of the clinical and histological features of skin aging (**Figure 1**).
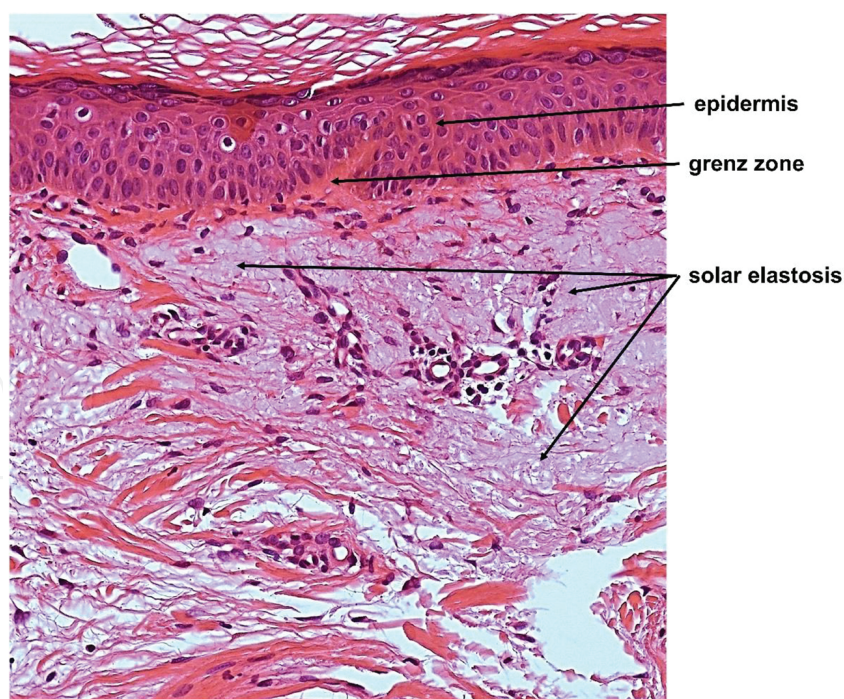
Figure 1. Histology of photoaged skin. The predominant histological finding of photodamaged skin is solar elastosis, which is basophilic degeneration of elastotic fibers in the dermis. Solar elastosis separates from the epidermis by a narrow band of normal-appearing collagen (grenz zone) with collagen fibers arranged horizontally (H&E stating. Original magnification ×400).

2. Clinical manifestations of chronological aging and photoaging

Clinical features of skin aging vary among individuals with differences in both genetic factors and lifestyles, which result in various degrees of cutaneous outcomes. Clinical signs of chronological aging include thinning of the skin, cigarette paper-like wrinkles, xerosis, loss of elasticity, and development of benign vascular formations such as cherry angiomas and benign overgrowths such as seborrheic keratosis [3]. Chronological aging is mainly the result of loss of soft tissue volume from fat atrophy, gravity-induced soft tissue redistribution, and weakened facial skeletal support related to bone resorption [3]. Clinical signs of photoaging include wrinkling, laxity, and a leather-like appearance, which are mainly the result of structural changes in the connective tissue of the dermis. These changes include both enzymatic degradation and reduced de novo synthesis of collagen, which cause wrinkling of the skin. The cumulative UV irradiation dose and Fitzpatrick skin type are used to assign the degree of photoaging. Individuals with Fitzpatrick skin types I and II show atrophic skin changes with fewer wrinkles, focal depigmentation, dysplastic premalignant changes such as actinic keratosis and malignant skin cancer. By contrast, skin types III and IV skin display hypertrophic features with deep wrinkles, leathery appearance, and lentigines [4]. In addition, photoaging includes changes in the dermal vascular structure, which appear clinically as telangiectasia (**Figure 2**).
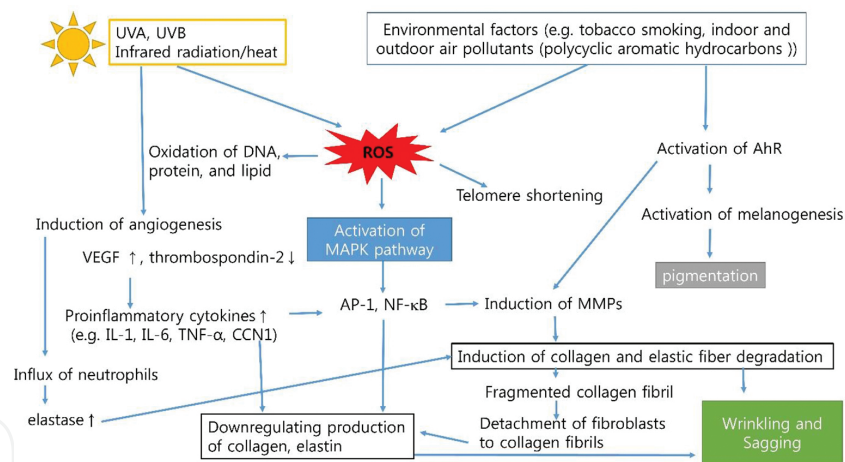
Figure 2. Schematic representation of pathogenesis of premature/extrinsic skin aging. ROS: reactive oxygen species, AhR: arylhydrocarbon receptor, NF-kB: nuclear factor kappa-B, IL-1: interleukin-1, TNF-α: tumor necrosis factor, CCN1: cysteine-rich protein 61, MAPK: mitogen-activated protein kinase, AP-1: activator protein 1, and MMPs: matrix metalloproteinases.

3. Histology of chronological aging and photoaging

Histological changes in chronologically aged skin are characterized by epidermal atrophy with reduced amounts of fibroblasts and collagen content in the dermis. The epidermal atrophy of intrinsically aged skin, which particularly affects the stratum spinosum, is related to lower epidermal turnover rate because of prolonged cell cycles [5]. Several studies of skin aging have

reported that the epidermis is hypocellular with decreased melanocytes, mast cells, and Langerhans cells [6]. After the age of 30 years, the number of melanocytes decreases by 8–20% per decade [7]. The number of Langerhans cells in the epidermis becomes markedly decreased with noticeable morphological alterations and functional impairment. In dermis of chronologically aged skin, the collagen fibers are loose, thin, and disorganized compared with those in the sun-protected skin of young people [8]. The dermis of chronologically aged skin shows fewer mast cells and fibroblasts than that of young skin, and the amounts of collagen and elastic fibers are decreased [9]. In a study of the chronological changes in collagen fibers, the synthesis of collagen was found to be decreased by 30% in the first 4 years of menopause, then by 2% per year [10].

Photoaging causes several histological changes in the skin that are distinct from histological alterations that occur intrinsically during aging. In photoaging, the thickness of the epidermis and the morphology of epidermal is heterogeneous [11]. The epidermis of photodamaged skin is thicker than that of intrinsically aged skin, whereas the epidermis of severely photodamaged skin elicits epidermal atrophy [12]. Furthermore, increased numbers of atypical melanocytes and keratinocytes may be seen [4]. Melanogenesis is also upregulated and participates in the neutralization of free radicals induced by UV radiation exposure, which may act as a mechanism for protection from photodamage [13]. Major alterations of photoaged skin and their molecular effects occur primarily within the dermis and the dermoepidermal junction. There is an increased amount of glycosaminoglycans and proteoglycans within the aged dermis, possibly because of a rise in the level of matrix metalloproteinases (MMPs) with increased numbers of hyperplastic fibroblasts [14]. Unlike the hypocellular feature of chronologically aged skin, photoaged skin can present an increased number of inflammatory cells such as mast cells, eosinophils, and mononuclear cells [15]. In addition, the amount of extracellular matrix is decreased and breakdown of collagen fibers is increased, which may appear as wrinkles [16]. Meanwhile, the most pronounced histological feature of photoaging is the disintegration of elastic fibers (solar elastosis), which results in accumulation of amorphous, thickened, curled, and fragmented elastic fibers [17]. Elastotic material consists of elastin, fibrillin, glycosaminoglycans, particularly hyaluronic acid and versican, a large chondroitin sulfate proteoglycan. The pathogenesis of solar elastosis is assumed to be a result of both degradation and de novo synthesis, although it is not yet fully understood [18].

4. Molecular mechanisms of skin aging

4.1. Telomere shortening

Telomeres are tandem repeats of TTAGGG located at the distal ends of most eukaryotic organisms to protect chromosomes from degradation and from fusion with neighboring chromosomes [19]. Telomere shortening prevents aberrant cellular proliferation by limiting cellular division. A consequence of this protection is cellular senescence and aging [20]. Telomerase, also called telomere terminal transferase, is capable of adding the telomeric sequence TTAGGG to the 3′ end of telomeres [21]. In humans, telomerase plays a significant

role in the maintenance of skin aging and oncogenesis [22]. The regulation of telomerase activity may therefore have significant role in antiaging and anticancer therapy. Gradual shortening of telomeres can explain cellular senescence that is essentially caused by intrinsic aging because it results from cell division. However, UV radiation exposure may induce telomere shortening by producing reactive oxygen species (ROS) in the skin [23]. Because UV radiation induces the formation of ROS in the skin and telomere shortening is accelerated by ROS, it has been postulated that skin exposed to UV radiation may have shorter telomeres compared with skin protected from UV radiation [1, 23]. Oikawa et al. [23] demonstrated that UVA irradiation accelerated telomere shortening in human cultured fibroblast cell lines by site-specific DNA damage at the GGG sequence in the telomere sequence. By contrast, Sugimoto et al. reported that telomere length in the epidermis and dermis was reduced with age and telomere length was not significantly different between epidermis from sun-exposed and sun-protected areas. They could not confirm that telomere shortening was associated with photoaging [19]. Telomeres are metabolically active and possess a set of characteristics *in vitro* and *in vivo*, which are known as biomarkers of aging and cellular senescence. Among biomarkers of cellular senescence, telomere shortening is a rather elegant and frequently used biomarker. The validity of telomere shortening as a marker for cellular senescence is based on the theoretical and experimental data. Further studies to determine the relationships between telomere restriction fragment length in skin cells and lifestyle/genetic background are necessary to confirm the validity of this marker. The details of molecular events and especially the function of telomere shortening in skin aging are not understood completely. However, interesting and important research progress is expected because advances in this field of research are mostly recent (**Table 1**).

Clinical	Histological	Molecular mechanism
Roughness	Increased compaction of stratum corneum, increased thickness of granular cell layer, reduced epidermal thickness and reduced epidermal mucin content	Decreased hyaluronan which plays an essential role in supporting tissue architecture and is also known as important factors to protect skin from dryness by its capacity to bind water
Irregular pigmentation		
Freckle	Reduced or increased number of hypertrophic, strongly dopa-positive melanocytes	
Solar lentigines	Elongation of epidermal rete ridges; increases in number and melanization of melanocytes	Mutations of keratinocyte and melanocyte genes which play a role in pigment formation and transfer. Stem cell factor mutations in keratinocyte have been suggested

Clinical	Histological	Molecular mechanism
		to be responsible for the development of solar lentigine
Guttate hypomelanosis	Decreased dopa oxidase-positive, KIT+, and melanocyte sand reduction in melanocytes	
Wrinkles	Thinned epidermis as well as less elastotic changes, tropoelastin, and collagen VII when compared to the surrounding photoaged skin	UVB radiation, infrared radiation, and heat produce and increase inflammatory cytokines, such as IL-1α, IL-6, and TNFα, which stimulate keratinocytes and dermal fibroblasts to produce matrix metalloproteinases (MMPs), leading to the degradation of extracellular matrix proteins and induction of wrinkles destruction of collagen and elastic fibers, and formation of wrinkles in sun-exposed skin. The expression of MMP-1 and MMP3 mRNA and protein levels is increased in dermal fibroblasts by the activation of ERK and JNK
Sagging	Loss of elastic tissue in the dermis and the remaining fibers were disorganized, shortened, and fragmented	UV radiation exposure causes keratinocytes to secrete GM-CSF, which triggers fibroblasts and stimulate the gene expression of MMP-1 to a greater extent than neprilysin by dermal fibroblasts and the secretion of IL-6, leading predominantly to sagging of the skin via the degradation of collagen I fibers by the enhanced activity of MMP-1
Inelasticity	Accumulated large amounts of homogenization and a dark blue amorphous elastotic material in the dermis, so-called solar elastosis	UV radiation, infrared radiation, and heat lead to an influx of neutrophils, which are packed with proteolytic enzymes (such as neutrophil elastase) that participate in extracellular matrix damage processes,

Clinical	Histological	Molecular mechanism
		mainly collagen and elastic fibers degradation. Neutrophil elastase is strongly associated with the development of solar elastosis resulting from continued exposure to UV radiation
Vascular changes		
Telangiectasia	Ectatic vessels often with atrophic walls	Acutely, UV exposure stimulates angiogenesis through vascular endothelial growth factor upregulation via MEK-ERK activation and thrombospondim-1 downregulation via P13K-Akt activation in human epidermis, although with chronic exposure blood vessels may be decreased in UV-damaged skin
Senile purpura	Extravasated erythrocytes and increased perivascular inflammation	Blood vessel fragility by decreased collagen and elastic fibers

Table 1. Clinical and histological features of photoaged skin and related molecular mechanisms.

4.2. Matrix metalloproteinases and signal transduction pathways

Matrix metalloproteinases (MMPs) are an important family of zinc-containing proteinases that have the capacity to degrade most of the extracellular matrix proteins that comprise the structure of the skin dermal connective tissue [24, 25]. During the past 20 years, a remarkable amount of research has been conducted into both skin aging and MMPs, which can play a significant role in the aging process in the skin. In 1996, Fisher et al. [26] observed that MMP expression was induced and activated after UVB irradiation of the human skin. The molecular model proposed by Fisher et al. suggests that UV radiation can activate various growth factor and cytokine receptors on the cell surface and stimulate mitogen-activated protein kinase (MAPK) signal transduction, which then upregulates transcription factors activator protein-1 (AP-1) composed of c-Jun and c-Fos proteins, and nuclear factor kappa B (NF-κB) in the nucleus. The induction of AP-1 elevates MMP expression, including MMP-1 (collagenase), MMP-3 (stromelysin-1), and MMP-9 (92 kDa gelatinase), and results in the degradation of extracellular matrix components in human skin *in vivo* [1, 27]. This degradation results in accumulation of fragmented, disorganized collagen fibrils, and these damaged collagen products downregulate new collagen synthesis, which suggests that collagen synthesis is negatively regulated by collagen breakdown [28, 29]. The combined actions of MMP-1, MMP-3, and MMP-9 degrade most of type I and III dermal collagen. Furthermore, AP-1 inhibits procollagen biosynthesis by suppressing gene expression of type I and III procollagen in the

dermis, which results in a reduced collagen content [25]. The data indicate that epidermal keratinocytes are a major cellular source of MMPs that are produced in response to exposure of human skin to solar UV irradiation. However, dermal cells may also play a role in epidermal production of MMPs, through indirect paracrine mechanisms, by release of growth factors or cytokines, which in turn modulate MMP production by epidermal keratinocytes.

4.3. Oxidative stress

Free radicals are considered the major contributors to the aging process of skin through the accumulation of ROS. The free radical theory postulates that extremely reactive chemical molecules are a major cause of the aging process [30]. The status of oxidant–antioxidant imbalance is referred to as oxidative stress. Oxidative stress occurring in cells can lead to the oxidation of cell membrane phospholipids, which results in the distortion of the transmembrane signaling pathway [31]. Generally, increased ROS production leads to the activation of MAPK. MAPK induces AP-1, which consequently increases the expression of MMPs, resulting in a decrease of collagen in aged skin [32]. Oxidative stress also contributes to the increased oxidation of macromolecules, such as cellular lipids, proteins, and DNA, which causes cellular dysfunction with age. Age-related accumulation of damaged, oxidized, and aggregated altered proteins might lead to aging [31, 33]. Oxidative protein damage is the most common molecular sign of aging and is observed in photodamaged skin through ROS-mediated protein damage in the upper dermis [33]. Cellular accumulation of lipofuscin, a large protein–lipid aggregate, gradually increases with age, eventually inhibiting proteasome function [31]. Disruption of the epidermal calcium gradient is observed in aged skin because of changes in the composition of the cornified envelope, which results in reduced epidermal barrier function [31].

4.4. Vascular alterations

Human skin is affected by various environmental conditions such as solar UV radiation, infrared (IR) radiation, and heat, and these stimuli can contribute to skin angiogenesis. Interestingly, although the exposure of acute UV radiation stimulates skin angiogenesis, blood vessels of skin are decreased in chronically photodamaged skin. These differential effects of acute and chronic UV radiation on skin angiogenesis remain unknown. Acute and chronic UV irradiation of skin influences angiogenesis. UV irradiation induces angiogenesis via the upregulation of vascular endothelial growth factor and inhibition of thrombospondin-1, a potent inhibitor of angiogenesis [34].

4.5. Cytokines in skin aging

Cytokines play a central role in the visible clinical signs of aging [35]. Tumor necrosis factor α (TNF-α), which has a key role in proinflammatory process in skin, inhibits collagen synthesis and induces the production of MMP-9 [35, 36]. 3-Deoxysappanchalcone inhibits MMP-9 expression through the suppression of AP-1 and NF-κB in human skin keratinocytes [36]. Furthermore, high concentrations of TNF-α are correlated with a decrease of collagen production via induction of collagenase activity in fibroblasts [37]. Levels of interleukin (IL)-1 and

IL-18 increase with age and promote skin inflammation, which causes age-related processes [38]. UV radiation exposure stimulated IL-1 receptor antagonist (IL-1ra), a competitive inhibitor of IL-1, although IL-1ra production in the skin decreased with age. IL-1ra has a regulatory role in the IL-1 related proinflammatory response, and may play a role in the regulation of IL-1-induced inflammatory responses, and maintain an appropriate balance between IL-1 and IL-1ra [38]. IL-18, an IL-1 superfamily cytokine, is a pleiotropic immune regulator that functions as an angiogenic mediator in inflammation [39]. IL-18 has been implicated as a strong proinflammatory mediator in the pathogenesis of age-related diseases by inducing interferon-γ [39]. In addition, another proinflammatory cytokine, IL-6, increases after menopause and is associated with the formation of skin wrinkles [40]. IL-6 levels are upregulated on exposure to UV radiation [41]. Increased levels of cysteine-rich protein 61 (CCN1) are observed in dermal fibroblasts with age, and CCN1 contributes to the skin connective tissue aging by collagen reduction and degradation in aged human skin *in vivo* [42]. Furthermore, CCN1 induced age-associated secretory proteins, including various proinflammatory cytokines and MMPs, which results in aging of connective tissue [42].

4.6. Other environmental stressors in skin aging

4.6.1. Tobacco smoke

Like UV radiation exposure, smoking can result in extrinsic skin aging. Findings from large epidemiological studies imply that there is a link between tobacco smoking and premature skin aging [43–45]. Skin damage from long-term smoking can result in a "smoker's face" and can cause facial skin to appear grayish and lines to develop around the eyes and mouth, through damage to collagen fibers and elastin in the dermis [46]. Significantly increased levels of MMP-1 mRNA are observed in the dermal connective tissue of smokers compared with nonsmokers [47]. Increased MMP-1 leads to the degradation of collagen and elastic fibers, which are major extracellular matrix proteins in the dermis. To confirm the pathogenic role of tobacco in skin aging, Morita et al. [46] showed that topical application of water-soluble tobacco smoke extract to the backs of mice led to a loss of collagen bundles and a concomitant increase of damaged collagen in the upper dermis, which mimicked age-related skin. Furthermore, several *in vitro* studies provided possible pathomechanisms for the association between tobacco smoke extract and skin aging. Tobacco smoke extract reduces procollagen types I and III and induced the production of MMP-1 and 3, which degrades extracellular matrix proteins, and also results in abnormal regulation of extracellular matrix deposition in human cultured skin fibroblasts [48]. Tobacco smoke extract also inhibited cellular responsiveness to transforming growth factor-β (TGF-β), a key mediator of collagen synthesis through the induction of a nonfunctional form of TGF-β, and downregulation of the TGF-β receptor in supernatants of cultured skin fibroblasts, leading to decreased synthesis of extracellular matrix [49]. Moreover, tobacco smoke is a major source of polycyclic aromatic hydrocarbon exposure in humans. In this regard, it has been shown that tobacco smoke extract induced MMP-1 expression via activation of the aryl hydrocarbon receptor (AhR) signaling pathway in human fibroblasts and keratinocytes [50].

4.6.2. Infrared radiation and heat

Heat energy may be transmitted by IR radiation, which increases skin temperature. Lee et al. [51] found that the temperature of human skin can increase to about 40°C under IR irradiation following the conversion of absorbed IR radiation into heat energy. Chronic exposure of skin to heat may cause premature skin aging, just like UV radiation. The expression of MMP-1 and MMP-3 is induced by heat shock in cultured normal human skin fibroblasts through ERK and JNK activation [52]. Decreased type I procollagen levels and increased MMP-1 expression were observed in human skin exposed to IR radiation, which suggests that chronic IR irradiation can cause skin wrinkling [53]. MMP-12, which is the most active MMP against elastic fiber network in human skin, was induced after heat treatment *in vivo* and thereby contributed to the development of solar elastosis in photoaged skin, thereby contributing to premature skin aging [54]. IR irradiation induces an angiogenic switch by the upregulation of vascular endothelial growth factor and downregulation of thrombospondin-2, which leads to angiogenesis and inflammation in human skin *in vivo* [55].

4.6.3. Environmental pollutants

Exposure to outdoor air pollution from traffic and industry is associated with an increased risk of signs of extrinsic skin aging, in particular pigmented spots and wrinkles in white women of European ancestry [56]. Polycyclic aromatic hydrocarbons are the major participants and trigger the AhR signaling pathway, because their lipophilicity enables them to penetrate the skin easily. Activation of the AhR pathway increases MMP-1 expression in the normal human keratinocytes [57]. In addition, AhR signaling pathway could contribute to the modulate melanogenesis by upregulating tyrosinase enzyme activity [58]. These findings suggest that polycyclic aromatic hydrocarbon-induced AhR activation may play a significant role in the formation of dark pigmentation and coarse wrinkles, which are clinical hallmarks of extrinsic aging. Indoor air pollution released by the combustion of solid fuels for heating is a major environmental and public health challenge in developing countries [59]. Recently, Li et al. [60] reported that indoor air pollution from cooking with solid fuels was significantly associated with 5–8% deeper facial wrinkles and folds and an increased risk of developing fine wrinkles on the dorsum of hands in Chinese women. It is thus likely that exposure to indoor combustion of solid fuels might induce the same molecular pathways in skin cells as outdoor pollution and thereby cause wrinkle formation.

5. Molecular aspects of antiaging treatments

5.1. Topical retinoids

Topical retinoids (vitamin A derivatives, also known as retinol) are the main stay of treatment of patients gradually photoaging [61]. Tretinoin and tazarotene are the only two topical retinoids that are currently U.S. Food and Drug Administration (FDA) approved for the treatment of photoaging. The detailed mechanism of action of retinoids has been elucidated.

They bind to and activate the retinoic acid receptors (RARs), which are present in a nuclear and work in pairs. These nuclear receptors have two binding sites, which is one for the ligands (retinoid), and one for specific DNA sequences of a target gene. In the presence of the ligands, those heterodimers can recognize a DNA sequence and regulate transcription. Their signal transduction is believed to be implicated in the synthesis of procollagen, thus increasing the formation of type I and III collagen, and inhibiting MMPs [1, 26]. Although the precise mechanisms are unknown, the induction of dermal collagen appears to be a crucial factor. Retinoic acid stimulated production of type VII collagen (anchoring fibrils) and type I collagen [4]. Clinically, retinoids reduce the appearance of fine lines, improve skin texture, correct tone and elasticity, and slow the progression of photoaging. The clinical improvements of topical retinoid therapy are typically seen after several weeks of therapy [62, 63]. A 24-month, double-blind, vehicle-controlled, multicenter study of tretinoin cream versus vehicle in the treatment of photoaged facial skin was conducted. Topical administration of tretinoin cream for 24 months has proved to be effective in treating clinical signs including fine wrinkling at 2 months, hyperpigmentation at 4 months, coarse wrinkling at 1 month, and sallowness at 4 months [64]. In addition to these clinical benefits, the following histopathological effects are well documented: epidermal hyperplasia and thickening, a decrease of epidermal melanin content, an increase of collagen synthesis, and a decrease of dermal collagen breakdown by inhibiting MMPs and a decrease of p53 expression [65]. Topical retinoids may cause an irritant reaction such as erythema, scaling, burning sensation, and dermatitis, and these reactions cause to decrease patient compliance. Therefore, retinoids should be initiated at the lowest effective dose to minimize adverse reactions [66]. Although tazarotene is the only topical retinoid with a category X designation, topical retinoids are not recommended during pregnancy or lactation [67].

5.2. Cosmeceuticals

Cosmeceuticals encompass a heterogeneous category of nonprescription topical products, including antioxidants, vitamins, hydroxy acids, and plant extracts [68]. Cosmeceuticals are marketed to the consumer based on the claims of their antiaging effects. Although some products may have scientific rationale and produce visible results in the treatment of photo-aging, they are not classified as drugs. Most have not been studied thoroughly, especially on human skin, and these products are not subject to the rigorous testing or regulation by agencies such as the FDA. However, the most cosmeceuticals serve a role in keeping the skin moisturized, maintain the homeostasis of the skin, and many are combined with a topical retinol to enhance their antiaging benefits. As mentioned above, UV and IR irradiation of skin leads to free radical formation. It has been hypothesized that antioxidants scavenge these free radicals and thus protect cells from damage. The antioxidants include coenzyme Q, lipoic acid, vitamin C, and vitamin E [69–71]. Among the many cosmeceuticals, there is increasing interest in botanical antioxidants. Representatively, green tea polyphenols (GTPs) have gained attention because of their potent antioxidant activities. In animal models, UV radiation-induced cutaneous edema and cyclooxygenase activity could be significantly inhibited by feeding GTPs to the animals. After topical application of creams containing GTPs, GTPs on mouse skin decreased the UV-induced hyperplasia, edema, and myeloperoxidase activity. Epigallocate-

chin-3-gallate (EGCG), which is a polyphenolic constituent of GTP, has been reported to have the inhibitory effect on the expression and activity of MMPs and leukocyte elastase, which has an important role in tumor invasion and metastasis. In addition, GTPs have been shown to decrease the levels of oxidative stress, prevent UV radiation-induced DNA damage, their potential immunosuppressive effects, and skin cancers [72, 73]. Like GTPs, other botanic extracts have been described to contain potent antioxidant components, including curcumin (turmeric), silymarin (milk thistle), apigenin, resveratrol (red grape), and genistein (soy bean) [74]. In fact, recently, it has been demonstrated that these components can inhibit the inflammatory response by downregulation of various proinflammatory mediators, inhibition of activated immune cells, or inhibiting inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) via its inhibitory effects on NF-κB or AP-1 [75]. Further investigations are needed to focus on the potential application of cosmeceuticals and determine how they can contribute to improvements in skin aging at the maximum.

5.3. Cytokines and growth factors

Cytokines play a crucial role in the development of clinical features of skin aging. Numerous proinflammatory cytokines including TNF-α, IL-1, and IL-18 can affect the aging process by triggering collagen breakdown via upregulation of MMP-9. These inflammatory cytokines can lower the skin immunity and thus increases the risk of skin infections in old age [35]. Many growth factors are involved in wound healing and have the ability to increase fibroblast and keratinocyte proliferation within the dermis, thus inducing extracellular matrix formation [76]. Growth factors are produced and secreted by many skin cell types, including fibroblasts, keratinocytes, and melanocytes. These secreted growth factors are those that regulate the immune system, also known as cytokines [77, 78]. It is important to combine growth factors and cytokines (referred to collectively as GFs) because they work together and regulate each other via inter- and intracellular signaling pathways. The defined role of GFs in healing of skin wounds allows a parallel model to be developed for the role of GFs in skin rejuvenation. Like the skin wound-healing process, topical and injectable GFs have the potential to modify complex cellular network leading to the increase of collagen synthesis and decrease of collagen degradation. Therefore, they have emerged as an intriguing therapeutic modality to address skin aging through the stimulation of cell regeneration [79, 80]. In fact, topical GFs can cross the skin barrier and bind to cell surface receptors, which trigger a signaling cascade and lead to stimulate keratinocyte proliferation [81]. However, a limited number of controlled studies demonstrate that topically applied GFs can stimulate collagen synthesis and epidermal thickening, which is associated with clinical improvement in signs of photoaging. Because the use of GFs is still in an early stage, continued study to determine their efficacy is needed.

5.4. Neurotoxins and dermal fillers

Over the past 10 years, the use of botulinum toxin and dermal fillers for aesthetic purposes has risen sharply. Botulinum toxin is an injectable neuromodulator used to reduce fine lines and wrinkles. The toxin functions by inhibiting acetylcholine release at the neuromuscular junction, resulting in flaccid paralysis of targeted muscles [82]. It has been demonstrated that

botulinum toxin decreased both collagen synthesis and the expression of MMP-9 in human dermal fibroblasts *in vitro*, leading to decreases collagen degradation [83]. Furthermore, botulinum toxin can also stimulate the expression of type I collagen in human dermal fibroblasts, suggesting that botulinum toxin can stimulate remodeling of ECM, an essential for the rejuvenation of skin aging [83].

Dermal filler injections are used to improve coarse wrinkles and gradual loss of tissue volume [84]. Regardless of trade name, products with smaller particles (softer consistency) are the most useful for superficial injection to address fine lines, whereas larger particle (stiffer) hyaluronic fillers (HA fillers) are the best suited for deeper injection to treat volume loss and deep rhytids [85]. In general, superficial "fine-line" products last approximately 6 months or more, whereas larger particle products last for 6–12 months. In recent years, combination treatment with botulinum toxin and fillers was recommended to achieve superior clinical outcomes and greater patient satisfaction [86, 87].

Several studies demonstrated that fibroblasts in aged skin could be "rejuvenated" by enhancing structural support and mechanical force by injection of dermal filler, cross-linked hyaluronic acid, into the skin [88]. In addition, injection of dermal HA filler can stimulate fibroblasts to produce type I collagen associated with increase in mechanical forces. Enhanced mechanical support of the ECM also stimulates the fibroblast, endothelial cells, and keratinocytes proliferation, resulting in increase of vasculature and epidermal thickness mediated by upregulation of TGF-β receptor and various growth factors. Consistent with these observations in human skin, injection of dermal filler into dermal equivalent cultures *in vitro* induces elongation of fibroblasts and type I collagen synthesis by enhancing structural support of the ECM [88].

5.5. Lasers and other novel devices

Laser resurfacing is a skin rejuvenation technology developed to target the cutaneous signs of photodamage. Lasers and other light source procedures are divided into ablative and nonablative resurfacing. Laser procedures are based on the theory of selective photothermolysis [89]. Ablative lasers were first used in the 1980s and markedly improved rhytids, dyspigmentation, skin laxity, and other signs of photoaging by inducing dermal collagen remodeling. The traditional ablative lasers consisted of the 10,600-nm CO_2 and 2940-nm erbium-doped yttrium aluminum garnet (Er:YAG) lasers. Ablative laser resurfacing employs CO_2 and Er:YAG lasers, which both target water as their chromophore. CO_2 lasers emit light at 10,600 nm in the far-IR electromagnetic spectrum [90, 91]. Er:YAG lasers, introduced in 1996, emit light at 2940 nm, closer to the absorption peak of water, which results in very superficial absorption of laser light [92]. When compared with a CO_2 laser, the absorption coefficient for the Er:YAG laser is 16 times higher [93]. Ablative laser resurfacing provides significant improvement of photodamaged skin, photoinduced rhytids, dyschromias, and scars. However, because of the relatively high risk of adverse effects and long recovery period, fractional ablative and other nonablative lasers have since been developed. Nonablative lasers have been developed as an alternative to traditional ablative resurfacing with the aim to comply with patients' new demands for short healing time and reduced discomfort and are widely used for dermal

collagen remodeling [94, 95]. Long-pulsed 1064-nm neodymium-doped (Nd): YAG laser treatment increased dermal collagen and decreased the expression of MMP-1 associated with the increased expression of TGF-β [96]. Fractional radiofrequency therapy is a novel, noninvasive method of tissue tightening. Radiowave energy is delivered deep into the skin, which causes water in the skin cells to heat up, stimulating the production of heat-shock proteins and promoting the wound-healing response [97]. Side effects including dyspigmentation, keloids, or thermal injury can occur and need to be considered. Therefore, in managing photoaging, treatments should be tailored to target the specific clinical features in different skin types and ethnic origin of the patients, and sufficient epidermal cooling after the treatment is required [98].

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