Molecular Mechanisms in Skin Aging

Dana Sachs, MD; Yolanda Helfrich, MD; John Voorhees, MD

The molecular events underlying skin aging have been carefully studied. Signaling pathways induced by UV light lead to sequential events culminating in an increase in collagen degradation and a decrease in collagen formation. This article reviews the mechanisms underlying these pathways and discusses how various therapeutic modalities intervene at specific sites in the pathways.

As the population ages, there is great interest in addressing an aged appearance. Skin is the largest organ, and its appearance conveys one's health, lifestyle, and overall well-being. Aging skin is characterized by uneven pigmentation, laxity, and wrinkles. The mechanisms involved in skin aging have been well studied during the last decade. There is a fairly clear sequence of events that begins with UV light exposure and culminates with collagen fragmentation leading to an aged appearance. Comprehension of these events will help us to better understand the mechanisms of existing therapies and will aid in the development of targeted antiaging therapies. Current interventions in aging skin include sunscreens, topical retinoids, antioxidants, neurotoxins, soft tissue fillers, and laser and other light-based devices.

EPISODIC PHOTODAMAGE

Episodic photodamage will eventually culminate in permanent photoaging. Typically, humans are exposed to episodic UV exposure rather than to continuous UV irradiation. In addition, UVB (290–320 nm) and UVA (320–400 nm) irradiation results in increased reactive oxygen species (ROS), specifically hydrogen peroxide (H_2O_2), which is a source of hydroxyl radical. In fact, H_2O_2 is increased 3-fold following UV irradiation.[1] This increase in H_2O_2 is associated with 2 events: a decrease in collagen formation and an increase in collagen degradation (Figure 1).

Collagen formation proceeds through the transforming growth factor (TGF-β)/mothers against decapentaplegic (SMAD) pathway, with TGF-β binding to cell surface receptors on the fibroblast composed of TβRI and TβRII. Binding of TGF-β to TβRII activates the intrinsic serine/threonine kinase activity of the receptor, leading to phosphorylation of transcription factors SMAD2 and SMAD3. In the phosphorylated state, SMAD2 and SMAD3 combine with SMAD4 and translocate into the nucleus of fibroblasts. In the nucleus, the SMAD transcription factors regulate the transcription of type I procollagen, and UV irradiation has been demonstrated to impair type I procollagen by downregulation of TβRII.[2] In addition, UV irradiation stimulates SMAD7 formation, which blocks SMAD2/3 phosphorylation by TβRI. On immunohistology, TGF-β1 protein is reduced in the dermis of patients aged 80 years and older compared with young patients (aged 18–29 years).[3] In vitro, fibroblasts treated with collagenase demonstrate an increase in ROS evidenced by increased staining with RedoxSensor Red Probe. Collagenase-treated fibroblasts have red staining throughout the cytoplasm indicative of increased ROS.[3]

Collagen degradation is mediated via mitogen-activated protein (MAP) kinase signaling pathways, which are activated by hydroxyl radical. In the normal state, the epidermal growth factor receptor (EGFR) is dephosporylated and remains in this state due to phosphatases, which remove phosphates. In the presence of hydroxyl radical,

Dr. Sachs is Associate Professor; Dr. Helfrich is Assistant Professor; and Dr. Voorhees is Professor and Chair; all at the Department of Dermatology, University of Michigan Medical Center, Ann Arbor.

Dr. Voorhees holds patents for Quick-Med Technologies, Inc, and Cognis.

Correspondence: Dana Sachs, MD, 1910 Taubman Center, 1500 E Medical Center Dr, Ann Arbor, MI, 48109.

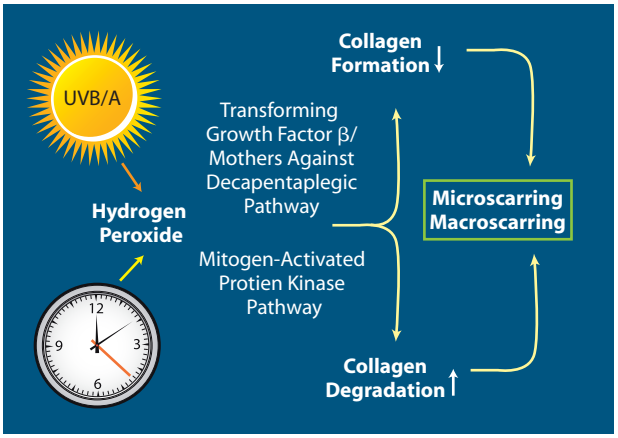

Figure 1. Increase in hydrogen peroxide following UV irradiation associated with a decrease in collagen formation and an increase in collagen degradation.

phosphates are not removed from the receptor rendering the EGFR constitutively active. Activated EGFRs lead to an increase in MAP kinase signaling.[4] In the presence of UV irradiation, the 3 MAP kinase pathways are activated: extracellular signal–regulated kinase (ERK), c-jun amino-terminal kinase, and the protein p38.[5] The ERK pathways are most associated with activation of growth factor receptors, whereas c-jun and p38 pathways are more closely associated with activation of cytokine receptors.[5] Activation of the MAP kinase pathways leads to induction of activator protein-1 (AP-1), which is comprised of c-fos and c-jun.[6] In turn, AP-1 activation leads to the transcription of matrix metalloproteinases (MMPs), which are enzymes responsible for collagen degradation. In human skin, MMP-1 (collagenase) degrades collagen types I and III; MMP-9 (gelatinase B) contributes to further breakdown of collagen following collagenase degradation; and MMP-3 (stromelysin) degrades collagen type IV.[7]

The imbalance between collagen formation and collagen degradation that results following episodic UV damage results in microscarring. Over time, repeated episodes of microscarring gives way to macroscarring resulting in perceived clinical changes of aging including wrinkling. Dermal matrix breakdown followed by repair yields imperfect repairs and the dermal matrix can never be perfectly restored. Compared with young skin, old skin demonstrates fragmented collagen fibers, and quantitative biochemical analysis reveals that the amount of fragmented collagen is more than 4 times greater in skin aged 80 years or older compared with young skin aged 21 to 30 years.[8]

MECHANICAL TENSION AND FIBROBLAST COLLAPSE

Sun-protected skin is characterized by long, intact collagen fibers on electron microscopy with stretched fibroblasts clinging to collagen fibers. Collagen is normal and collagenase levels are normal. In contrast, photoaged skin is characterized by fragmented collagen fibers, and the fibroblasts exist in a collapsed state unable to attach to the collagen network. Collagen is decreased by 70% and collagenase levels are increased 4-fold (Figure 2).[9] Fragmented collagen in the dermal matrix is a hallmark of aged skin and results from collagenase destruction of collagen fibers as previously described. Fibroblasts produce collagen, and in the setting of an altered dermal matrix, fibroblasts cannot attach to fragmented collagen fibers. Essentially, the scaffolding for fibroblasts is broken down and does not allow for proper attachment. When fibroblasts do not properly attach, they exist in a collapsed state rather than a stretched state. In this configuration, fibroblasts secrete low levels of collagen and high levels of MMPs. This leads to a self-perpetuating cycle in which collagen production is low and collagen degrading enzymes are elevated. Such an imbalance induces further stress on the fibroblasts, enhancing the aging process.[9]

NATURAL AGING VERSUS PHOTOAGING

Natural aging is characterized by fine wrinkles and a crepelike quality to the skin. It is seen in its purest form on sun-protected sites such as the upper, inner arm. When seen on the face or sun-exposed sites, it is usually seen in conjunction with photoaged skin. The epidermis is atrophic and skin tension is very lax. In old skin, mitochondrial production of ROS, including free oxygen radical, H_2O_2, and hydroxyl radical, leads to an increase in phosphorylated kinases. In turn, this leads to an increase in phosphorylated AP-1 causing an increase in collagenase. Fragmented collagen results from the increase in collagenase and as previously stated, there is a decrease in mechanical tension. This decrease in mechanical tension leads to an increase in ROS, and increased ROS blocks the TGF-β pathway, which leads to a decrease in procollagen.[12,13] In natural aging, the combined events of fewer fibroblasts, reduced fibroblast activity, and loss of mechanical tension results in a 70% decrease in new collagen production by fibroblasts.[10]

Photoaging is characterized by wrinkles, redness, and dyspigmentation and is best seen on the skin of sun-exposed sites such as the face, posterior neck, and forearms. In one variant of photoaging, fine wrinkles, epidermal atrophy, erythema, and dyspigmentation, along with precancerous lesions and skin cancers, predominate. In a less common variant, deep coarse wrinkles with less dyspigmentation predominate, and patients tend to have fewer skin cancers. In photoaging, UV light drives increased AP-1 activity along with MMP-1

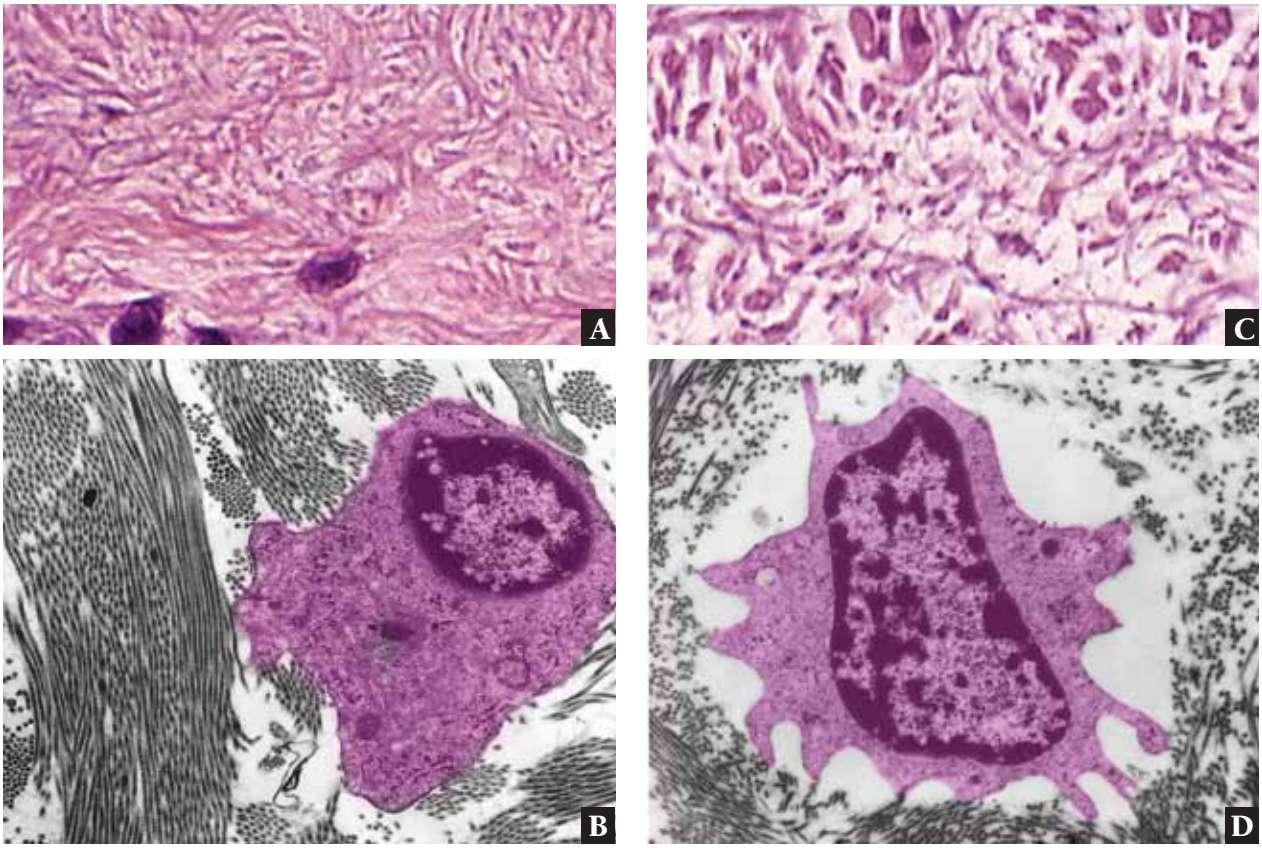

Figure 2. Young skin (<30 years of age) is characterized by long, intact collagen fibers (A) (H&E original magnification ×490), with fibroblasts existing in a stretched state and anchored to a healthy collagen scaffold seen on electron microscopy (H&E original magnification ×2050) (B). In contrast, older skin (>80 years of age) displays fragmented collagen fibers and the collagen content is decreased by 70% (C) (H&E original magnification ×490). In addition, collagenase activity is increased 4-fold (D) (H&E original magnification ×2050).

levels leading to degraded collagen. Additionally, type I procollagen synthesis is reduced. In contrast to natural aging, collagen production is decreased by fibroblasts in photoaged skin mainly due to the loss of mechanical tension.[11-13] The interplay of increased collagen destruction paired with decreased collagen production leads to decreased dermal collagen and, presumably, the clinical changes previously described.

The pathways of natural aging and photoaging converge with similar molecular events following ROS induction.

MMP-1 AND COLLAGEN

Collagenases are enzymes capable of breaking down type I collagen. These enzymes degrade the proteins, particularly collagen, in the extracellular matrix of dermis known as MMPs. In human skin, MMP-1 is induced by UV light and is responsible for collagen fragmentation related to chronic sun exposure.[8,14] Increased MMP-1 levels are associated with increased age and are thought to cause the fragmented dermal collagen fibers.[15] Compared with skin younger than 30 years, skin older than 80 years has substantially increased MMP-1 activity.[3]

SUNSCREENS

Sunscreens work by protecting skin from UVB and UVA rays and have been demonstrated to prevent UV-induced upregulation of MMPs and subsequent collagen breakdown (J. Voorhees, G. J. Fisher, unpublished data, 1995).

N-ACETYLCYSTEINE

N-acetylcysteine (NAC) is an amino acid derivative that gives rise to glutathione, an endogenous antioxidant. Its clinical uses include treatment of acetaminophen intoxication, treatment of intravenous contrast–induced nephropathy, and as a mucolytic. Recent work has shown that topical NAC applied to human skin under occlusion increases the reduced form of glutathione and eliminates the oxidized glutathione. In the reduced state, glutathione is a potent scavenger of ROS; however, NAC does not function as a sunscreen and does not reduce UV-induced skin reddening.[1]

When skin is pretreated with topical NAC, there is inhibition of UV-induced c-jun in human skin and subsequent upregulation of AP-1 and MMPs. This results in a decrease in UV-induced collagen breakdown. In related

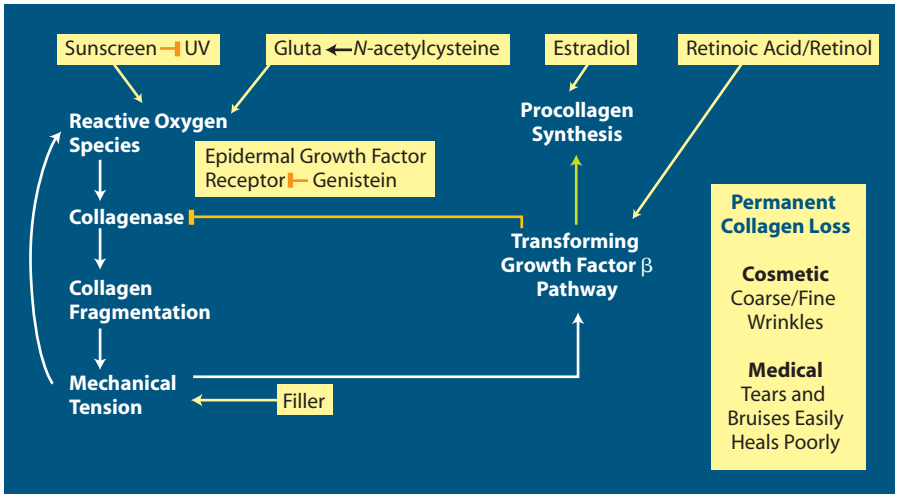

Figure 3. Therapeutic interventions for aging skin are shown at their sites of action.

experiments, skin pretreated with NAC has less collagenase induction than vehicle-treated skin.[1] Collagenase is needed for type I collagen breakdown.

TOPICAL RETINOIDS

Despite the wide availability of topical preparations and cosmeceuticals for aging skin, only retinoids have substantial clinical evidence to support their use. Retinoids are vitamin A–derived drugs used for photoaging (and other cutaneous diseases including, but not limited to, acne) and include tretinoin, retinol, retinal, and tazarotene. Tretinoin, which is available in the United States by prescription only, has been the most extensively studied topical retinoid. A 16-week, double-blind, vehicle-controlled study of topical tretinoin in photoaged forearms and facial skin demonstrated reversal of photodamaged skin with clinical and histological end points.[16] In another study, 10 to 12 months of tretinoin use for photoaging demonstrated increased extracellular collagen I staining by 80% above baseline compared with a 14% reduction in vehicle-treated skin.[17] In an open-label study of subjects with photoaged skin, daily treatment with retinoic acid 0.05% for at least 6 months was associated with reduced melanocytic and keratinocytic atypia and a doubling in thickness of the collagen band in the papillary dermis.[18] All-*trans*-retinol (retinol) is a precursor to retinoic acid and is available in the United States without a prescription. It has been associated with increased tolerability due to reduced skin irritation, yet has the potential to approach retinoidlike effects in human skin.[19] More recently, a randomized, double-blind, vehicle-controlled, left- and right-arm comparison study demonstrated significant improvement in fine wrinkling in retinol-treated skin compared with vehicle, as well as increased glycosaminoglycan expression and procollagen I immunostaining.[20]

ESTROGENS

Postmenopausal women's skin is associated with increased dryness and decreased skin laxity.[21,22] Women who take hormone replacement therapy in the form of estrogen therapy have been noted to have better skin hydration, fewer wrinkles, and more collagen fibers compared with nonusers of estrogen therapy.[23,24] Recently, a study of topical estradiol demonstrated increased procollagen production in sun-protected skin of postmenopausal women in a dose-dependent manner and in men, but to a lesser extent. Interestingly, no increase in procollagen production in photodamaged skin of the forearms and face was observed.[25] The observation of no increase in procollagen production in photoaged skin was not due to a lack of estrogen receptors (ERs). In fact, there were no differences in the levels of expression of 3 types of ERs (ER-α, ER-β, GPR30) between sun-protected sites and photoaged sites in women or men.[25]

HYALURONIC ACID FILLERS

Dermal fillers enhance the appearance of wrinkles, lines, and acne scars by their space-occupying properties. They continue to gain popularity in the United States and worldwide. Temporary, semipermanent, and permanent fillers are available. Hyaluronic acid (HA) fillers are widely available temporary fillers produced by several different companies with differences mainly related to cross-linking properties. Rapidly degraded in skin, HA's stability is increased by chemical cross-linking.[26] Studies with a popular, nonanimal, stabilized hyaluronic acid (NASHA) demonstrated that NASHA injections stimulate de novo production of type I collagen. This induction is mediated by mechanical stretching and activation of dermal fibroblasts, which are collagen-producing cells.[13] Saline-injected skin samples were used as controls, and when stained for HA they stained positive for endogenous HA. In contrast, NASHA-injected skin samples demonstrated well-circumscribed spaces in the middle and lower dermis, containing residual filler that stained positive for HA. Tissue samples were stained for markers of newly synthesized type I collagen. Procollagen is a soluble precursor, and increased intracellular and extracellular dermal staining with an antibody to the C-terminal domain of type I procollagen was demonstrated in NASHA-treated skin samples compared with controls.[13]

CONCLUSION

The molecular events in aging skin are fairly well understood. Episodic UV damage leads to microscarring. The repair process is imperfect, and repeated UV damage and microscarring culminates in macroscarring, which is clinically manifested by wrinkles. Natural aging and photoaging follow common pathways in the cascade of events, with MMP-1 levels significantly elevated in elderly skin. This leads to downstream signaling and events associated with an increase in collagen fragmentation. While skin cannot fully recover from UV-mediated damage, therapeutic options exist to help restore skin to its predamaged state. Medical and procedural interventions target different events in the molecular cascades. Sunscreens block the production of ROS, and the antioxidant NAC prevents ROS in UV-irradiated skin. Topical retinoids increase extracellular collagen. Topical estradiol increases procollagen in sun-protected, but not photodamaged, skin. In addition, NASHA fillers promote new type I collagen formation via fibroblast activation mediated through mechanical stretching (Figure 3).

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