ARTICLE

Mechanisms of Photoaging and Chronological Skin Aging

Gary J. Fisher, PhD; Sewon Kang, MD; James Varani, PhD; Zsuzsanna Bata-Csorgo, MD; Yinsheng Wan, PhD; Subhash Datta, PhD; John J Voorhees, MD

Human skin, like all other organs, undergoes chronological aging. In addition, unlike other organs, skin is in direct contact with the environment and therefore undergoes aging as a consequence of environmental damage. The primary environmental factor that causes human skin aging is UV irradiation from the sun. This sun-induced skin aging (photoaging), like chronological aging, is a cumulative process. However, unlike chronological aging, which depends on the passage of time per se, photoaging depends primarily on the degree of sun exposure and skin pigment. Individuals who have outdoor lifestyles, live in sunny climates, and are lightly pigmented will experience the greatest degree of photoaging. During the last decade, substantial progress has been made in understanding cellular and molecular mechanisms that bring about chronological aging and photoaging. This emerging information reveals that chronological aging and photoaging share fundamental molecular pathways. These new insights regarding convergence of the molecular basis of chronological aging and photoaging provide exciting new opportunities for the development of new anti-aging therapies. This article reviews our current understanding and presents new data about the molecular pathways that mediate skin damage by UV irradiation and by the passage of time. *Arch Dermatol. 2002;138:1462-1470*

Ultraviolet irradiation from the sun damages human skin, causing it to age prematurely. This premature aging process (photoaging) is cumulative with sun exposure, and preferentially affects individuals with lighter skin color. During the last 10 years, substantial progress has been made in understanding the molecular mechanisms responsible for photoaging in human skin. One major conceptual advance elaborated by this work is that UV irradiation invokes a complex sequence of specific molecular responses that damage skin connective tissue. These molecular processes derive from the ability of UV irradiation to exploit highly evolved cellular machinery that regulates responses of cells to physiological and environmental extracellular stimuli. The cellular machinery that mediates UV damage to human skin connective tissue includes cell surface receptors, protein kinase signal transduction pathways, transcription factors, and enzymes that synthesize and degrade structural proteins in the dermis that confer strength and resiliency to skin. Recruitment by UV irradiation of the cellular machinery that damages skin connective tissue is initiated by photochemical generation of reactive oxygen species (ROS). Ultraviolet-induced ROS also cause direct deleterious chemical modifications to cellular components (ie, DNA, proteins, and lipids). Chemical oxidation of cellular components and activation of cellular machinery, both brought about by UV-induced oxidative stress, act in concert to cause photoaging. Insights gained into the molecular basis of photoaging provide new opportunities for therapeutic intervention aimed at prevention.

From the Department of Dermatology, University of Michigan, Ann Arbor.

UV-INDUCED SIGNAL TRANSDUCTION PATHWAYS MEDIATE DAMAGE TO SKIN CONNECTIVE TISSUE

UV Irradiation Activates Cell Surface Growth Factor and Cytokine Receptors

Signal transduction pathways that are activated by UV irradiation in human skin are depicted in **Figure 1**. Ultraviolet

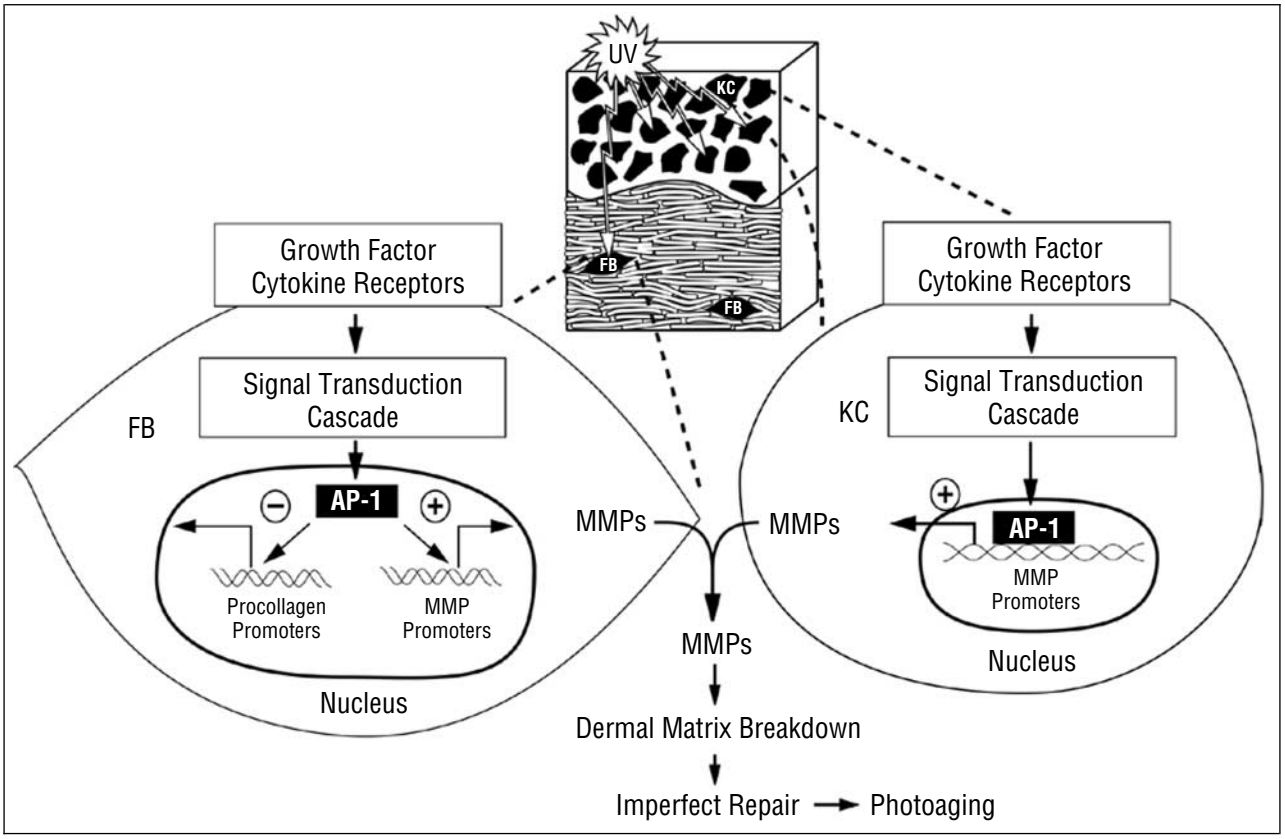

Figure 1. Model depicting solar UV irradiation damage to skin connective tissue. Ultraviolet irradiation (jagged arrows) activates growth factor and cytokine receptors on the surface of keratinocytes (KC) and fibroblasts (FB). Activated receptors stimulate signal transduction cascades that induce transcription factor AP-1, which stimulates transcription of matrix metalloproteinase (MMP) genes. In fibroblasts, AP-1 also inhibits procollagen gene expression. Matrix metalloproteinases are secreted from keratinocytes and fibroblasts and break down collagen and other proteins that comprise the dermal extracellular matrix. Imperfect repair of the dermal damage impairs the functional and structural integrity of the extracellular matrix. Repeated sun exposure causes accumulation of dermal damage that eventually results in characteristic wrinkling of photodamaged skin.

irradiation causes activation of cell surface cytokines and growth factor receptors. Epidermal growth factor (EGF), interleukin (IL) 1, and tumor necrosis factor α (TNF-α) receptors are activated within 15 minutes following UV exposure (twice the minimal erythema dose) in human skin in vivo. Functional activation of these receptors requires stimulation of distinct tyrosine kinase activities.[1] This addition of phosphate groups to tyrosine residues on the receptors and their associated adaptor proteins is the initial biochemical event in receptor activation and provides specific docking sites for molecules involved in signal propagation within the cell.[2]

The primary mechanism by which UV irradiation initiates molecular responses in human skin is by photochronical generation of ROS. These ROS include superoxide anion, peroxide, and singlet oxygen. The mechanism(s) of receptor activation by UV irradiation are not well understood. One possibility, which is supported by indirect experimental evidence, is that photochemical generation of ROS oxidize, and thereby inhibit, specific protein-tyrosine phosphatases, which function in opposition to receptor-activated protein-tyrosine kinases by removing phosphate group from receptors or their associated adaptor proteins.[3] The result would be a net increase in receptor phosphorylation (activation).[4] This mechanism of UV activation of cell surface receptors is supported by several publications,[5,6] although direct evidence that protein-tyrosine phosphatases regulate the activation state of cell surface receptors is lacking.

UV Irradiation Activates NADPH Oxidase, Which Generates Hydrogen Peroxide

Irrespective of mechanism, UV irradiation activates cell surface receptors, as does ligand binding, and triggers downstream signal transduction pathways. Several studies have demonstrated that UV irradiation stimulates production of hydrogen peroxide, which induces multiple signaling pathways, although the mechanism(s) by which hydrogen peroxide acts is not clear. In human skin in vivo and human keratinocytes, hydrogen peroxide levels are increased within 15 minutes following UV irradiation and continue to accumulate for approximately 60 minutes after UV exposure (**Figure 2**A). It is important to appreciate that this generation of hydrogen peroxide after UV exposure is distinct from photochemical generation of ROS described above, which occurs only during UV exposure and abates following UV exposure.

Phagocytes contain a multi-subunit complex, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which catalyzes the reduction of molecular oxygen to superoxide anion.[7] The superoxide produced is quantitatively converted to hydrogen peroxide, which is less damaging to cells and serves as a cosubstrate for per-

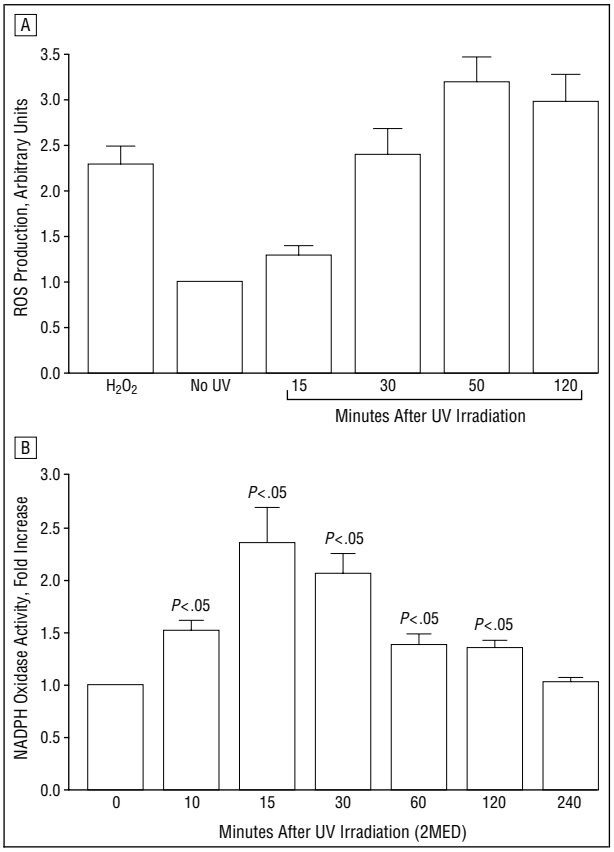

Figure 2. Ultraviolet irradiation stimulates generation of hydrogen peroxide (H_2O_2) in human keratinocytes. A, Time course for accumulation of H_2O_2 following UV irradiation from the UV-B/UV-A2 source of human keratinocytes. B, Time course of UV stimulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity in human skin in vivo. Skin samples were obtained at the indicated times after UV irradiation (UV-B/UV-A2 source) and analyzed for NADPH oxidase activity. Results are mean±SEM (error bars) of 5 experiments. ROS indicates reactive oxygen species; 2MED, twice the minimal erythema dose.

oxidases. In turn, hydrogen peroxide can be converted to other ROS, including hydroxyl radical and singlet oxygen. Human skin and human keratinocytes also express NADPH oxidase subunits, and contain NADPH oxidase activity that is induced following UV exposure. In keratinocytes, NADPH oxidase activity is induced 2-fold within 20 minutes following UV exposure (Figure 2B). Interestingly, pharmacological inhibition of UV-induced NADPH oxidase completely abrogates UV-induced hydrogen peroxide generation. Thus, NADPH oxidase is a major enzymatic source of hydrogen peroxide production following UV irradiation in human keratinocytes. The functional role of hydrogen peroxide in mediating UV responses in human skin remains to be determined.

UV Irradiation Activates Signaling Pathways

Ultraviolet irradiation activates protein kinase–mediated signaling pathways within 1 hour. These signaling pathways are maximal within 4 hours after UV exposure.[8-10] At this time, immunohistologic examination reveals activation (phosphorylation) of several of signaling kinases in cells throughout the epidermis (**Figure 3**). The activated kinases up-regulate expression and functional activation of the nuclear transcription factor, AP-1 (composed of Jun and Fos proteins), which then stimulates transcription of genes for matrix-degrading enzymes such as metalloproteinase (MMP) 1 (collagenase), MMP-3 (stromelysin 1), and MMP-9 (92-kd gelatinase).[11,12] Transcription factor AP-1 also interferes with collagen gene expression in human dermal fibroblasts (see the following section). Ultraviolet irradiation also activates the transcription factor NF-κB[13] that stimulates transcription of proinflammatory cytokine genes, including IL-1β, TNF-α, IL-6, and IL-8, and adhesion molecules including intercellular adhesion molecule-1.[14] Ultraviolet-induced cytokine gene products then act through their cell surface receptors to activate AP-1 and NF-κB and thereby amplify the UV response.

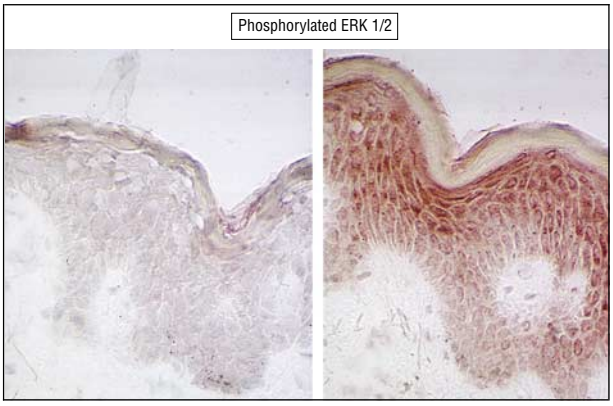

Figure 3. Ultraviolet irradiation activates intracellular kinases in human skin in vivo. Skin samples (A, No UV; B, UV) were obtained 4 hours after UV irradiation (twice the minimal erythema dose, UV-B/UV-A2 source) and analyzed for kinase (extracellular signal-related kinase [ERK] 1/2) activation by immunohistologic examination. The antibodies used specifically detect the activated, phosphorylated kinase. Results are representative of 5 subjects.

UV-Induced MMPs Degrade Skin Collagen

Ultraviolet-induced MMP-1 initiates cleavage of fibrillar collagen (type I and III in skin) at a single site within its central triple helix. Once cleaved by MMP-1, collagen can be further degraded by elevated levels of MMP-3 and MMP-9.[15] Metalloproteinase 1, MMP-3, and MMP-9 activities have been shown to colocalize with collagen in the dermis, following UV irradiation of human skin in vivo.[10,16] Type I collagen molecules are stabilized by intermolecular covalent cross-links. Depending on the extent of degradation, partially degraded collagen can remain cross-linked within the insoluble collagen matrix. These insoluble collagen fragments are susceptible to proteolytic cleavage, in vitro, by proteases with broad specificity such as chymotrypsin. When sun-protected human skin was irradiated and biopsy performed, results showed the level of partially degraded collagen 24 hours following UV irradiation increased 3-fold (**Figure 4**). Thus, UV-induced MMPs degrade skin collagen and thereby impair the structural integrity of the dermis. In the absence of perfect repair, MMP-mediated collagen damage is expected to accumulate with each successive UV exposure. Such cumulative collagen damage is likely a major contributor to the phenotype of photoaged human skin.

UV IRRADIATION INHIBITS PRODUCTION OF TYPE I AND TYPE III PROCOLLAGEN

In addition to degrading mature dermal collagen, UV irradiation impairs ongoing collagen synthesis, primarily through down-regulation of type I and type III procollagen gene expression.[17] Two mechanisms contribute to reduced procollagen gene expression. As described earlier, UV irradiation induces the transcription factor AP-1. By binding and sequestering factors that are part of a transcriptional complex required for procollagen transcription, AP-1 interferes with collagen production.[11,18] Transcription factor AP-1 has also been shown to decrease collagen synthesis by blocking the effects of transforming growth factor β (TGF-β), a major profibrotic cytokine, and sequestering one of the signaling proteins it activates both directly and indirectly.[18-22]

Ultraviolet irradiation also interferes with TGF-β–dependent type I procollagen gene expression by down-regulating type II TGF-β receptor, within 8 hours of irradiation, rendering the cells unresponsive to TGF-β effects.[23] In cultured human fibroblasts, UV-induced down-regulation of type II TGF-β receptor and subsequent loss of TGF-β responsiveness results in substantial reduction of type I procollagen gene expression. These data suggest that down-regulation of type II TGF-β receptors, in addition to AP-1–mediated transcriptional repression, contribute to reduced procollagen gene expression observed in human skin in vivo, following UV irradiation.

SKIN PIGMENT PROTECTS AGAINST UV-INDUCED RESPONSES THAT LEAD TO COLLAGEN DEGRADATION

It is well established that skin pigment provides a significant degree of protection against actinic damage. For a given exposure to UV irradiation, persons with less pigment (lighter skin color) will exhibit greater erythema and less tanning than persons with more pigment (darker skin pigment). This inverse relationship between skin color and UV-induced skin reddening (sunburn) forms the basis of the skin phototype classification system. This classification system identifies 6 skin phototypes, with light-skinned persons who sunburn easily and do not tan (classified as skin phototype I) and dark-skinned persons who do not sunburn and tan readily as phototype VI. However, quantitative assessment of the effects of these pigment-associated phototypes on molecular responses of skin to UV irradiation is lacking. Therefore, the effects of skin color on UV-induced MMP-1 gene expression and formation of thymine dimers were investigated in human skin in vivo.

Subjects were placed in one of 2 groups based on quantitative measure of their surface skin color, as determined by a color meter (Chroma Meter; Minolta Camera Co Ltd, Osaka, Japan). Persons with Chroma Meter values greater than 65 were placed in one group (lightly pigmented), and persons with Chroma Meter values less than 55 were placed in a separate group (darkly pigmented). These 2 groups approximately correspond to skin phototypes I and II and phototypes V and VI, respectively. Subjects were exposed to either a UV-B/UV-A2 source (λmax = 311 nm, filtered to remove UV-C) or a UV-A1 source (λmax = 359 nm, 366 nm, and 374 nm) (Sellas 2000 Watt High Pressure Unit; Sellas GmbH, Gevelsberg, Germany). Lightly pigmented subjects were exposed to twice their minimal erythema dose from the UV-B/UV-A2 source (average minimal erythema dose was 640 mJ/cm^2 total UV irradiation). Darkly pigmented subjects were exposed to 2 and 4 times the average minimal erythema dose of the lightly pigmented group (640 mJ/cm^2 to 1280 mJ/cm^2). Skin samples were obtained 24 hours after UV irradiation for determination of MMP-1 messenger RNA (mRNA) levels and DNA photoproducts. Ultraviolet-B/UV-A2 exposure resulted in substantial induction of MMP-1 mRNA (**Figure 5**) and formation of thymine dimers (Figure 5) in lightly pigmented subjects. In contrast, twice the average exposure of the lightly pigmented group produced only modest MMP-1 mRNA induction (Figure 5) or DNA damage (Figure 5) in the darkly pigmented group.

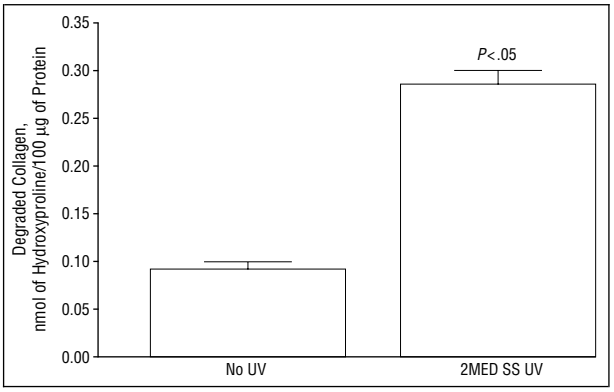

Figure 4. Ultraviolet irradiation causes degradation of collagen in human skin in vivo. Skin samples were obtained 24 hours after UV irradiation (twice the minimal erythema dose [2MED], UV-B/UV-A2 source). Insoluble, partially degraded collagen in the dermis was quantified. Results are mean ± SEM (error bars) from 6 subjects. SS indicates solar simulator.

The effect of skin pigment on induction of MMP-1 mRNA and DNA photoproducts by UV-A1 exposure was investigated next. In the lightly pigmented group, 110 mJ/cm^2 UV-A1 caused substantial induction of MMP-1 mRNA expression and DNA photoproducts (**Figure 6**). The response to UV-A1 was similar to that observed in response to UV-B/UV-A2 described above (Figure 5). In contrast, 110 mJ/cm^2 UV-A1 had little effect on the level of MMP-1 gene expression or DNA photoproducts (Figure 6) in the darkly pigmented group. These data demonstrate that pigmentation provides substantial protection against deleterious effects of both UV-B/UV-A2 and UV-A1 irradiation or is at least correlated with enhanced protection. These findings provide quantitative support for the clinical observation that photoaging is less severe in the darkly pigmented population compared with the lightly pigmented population. These data are also consistent with the low incidence of skin cancer in the darkly pigmented population.

It is worth noting the difference between lightly and darkly pigmented populations in depth from the surface where DNA photoproducts were observed. In the lightly pigmented group, DNA photoproducts were observed in cells throughout all layers of the epidermis and in the upper dermis (Figures 5 and 6). In the darkly pig-

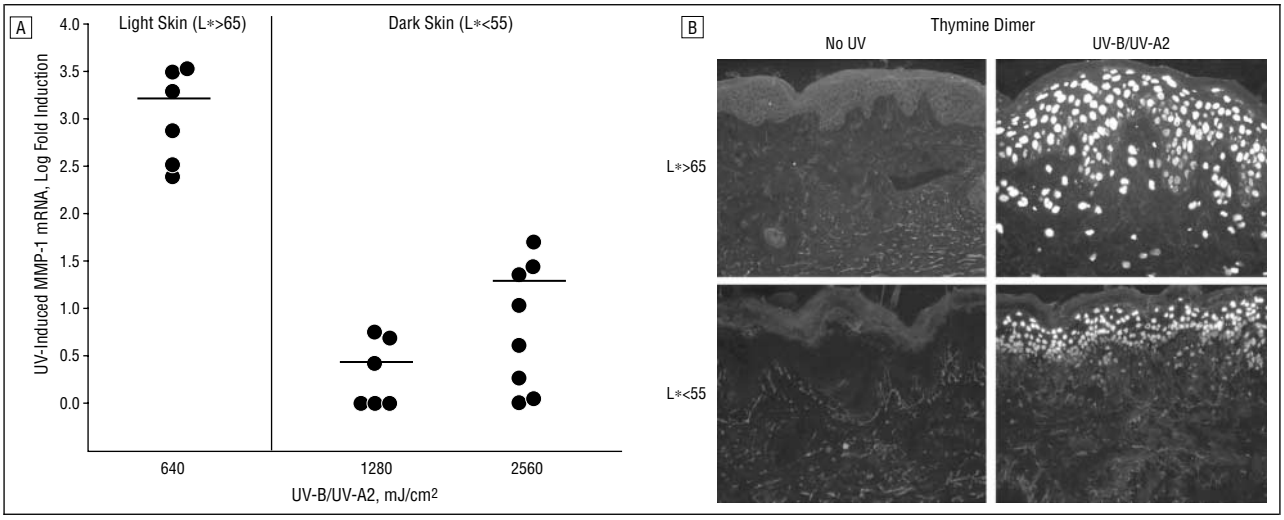

Figure 5. A, Ultraviolet B/UV-A2 irradiation induction of matrix-degrading metalloproteinase-1 (MMP-1) and DNA damage are greater in light skin compared with dark skin. Human skin color value (L*) was determined by a color meter (Minolta CR200 Chroma Meter). L* is a measure of color value (lightness/darkness), and varies between 0 (dark) and 100 (light). Subjects with skin L* greater than 65 (light skin) were exposed to twice the minimal erythema dose of UV-B/UV-A2 (average exposure, 640 mJ/cm^2 total UV irradiance). Subjects with L* less than 55 (dark skin) were exposed to 1280 mJ/cm^2 total UV irradiance (equivalent to 4 times the minimal erythema dose for the light skinned group) and 2560 mJ/cm^2 total UV irradiance (equivalent to 8 times minimal erythema dose for the light skinned group). Skin samples were obtained 24 hours after UV irradiation and analyzed for matrix MMP-1 messenger RNA (mRNA) levels by real-time reverse transcriptase polymerase chain reaction, and DNA thymine dimer photoproducts, by immunofluorescence. B, Thymine dimer immunofluorescence data are representative of 5 light skinned subjects exposed to twice the minimal erythema dose and 5 dark skin subjects exposed to 2560 mJ/cm^2 (equivalent to 8 times the minimal erythema dose for light skinned group).

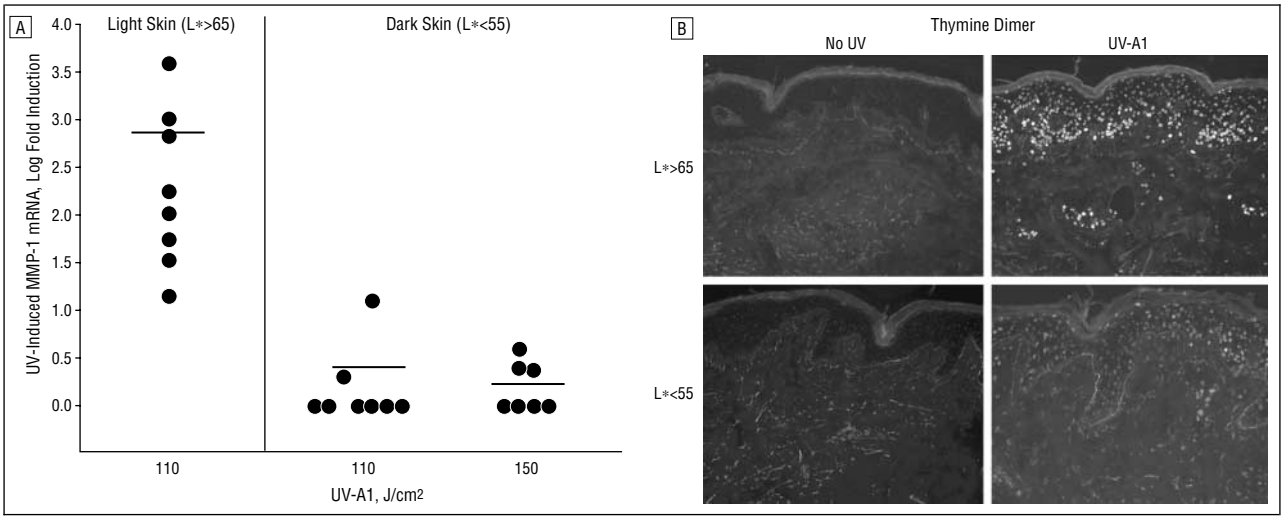

Figure 6. A, Ultraviolet A1 irradiation induction of matrix-degrading metalloproteinase-1 (MMP-1) and DNA damage are greater in light skin compared with dark skin. Human skin color value (L*) was determined as described in the legend to Figure 5. Subjects were exposed to the indicated doses of UV-A1, and skin samples were obtained 24 hours after UV irradiation. Matrix metalloproteinase-1 messenger RNA (mRNA) levels were quantified by real-time reverse transcriptase polymerase chain reaction, and DNA thymine dimer photoproducts were visualized by immunofluorescence. B, Thymine dimer immunofluorescence data are representative of 8 light skinned and 8 dark skinned subjects exposed to 110 J/cm^2.

mented group, the modest level of photoproducts was restricted to post–mitotic cells in the upper epidermis. Because UV-B–induced DNA photoproducts are produced by direct absorption of UV-B irradiation by DNA, these data indicate that skin pigment substantially attenuates penetration of UV irradiation into skin, which was expected owing to its broad UV absorption spectrum[24,25] and preferential distribution in supranuclear "caps" in the path of incident UV irradiation.[26]

Based on optical properties of the skin, it is often argued that penetration of UV-B is restricted to the epidermis, while penetration of longer wavelength UV-A1 irradiation extends deep into the dermis. While this distinction of skin penetration between UV-B and UV-A irradiation may be generally true, our data on the localization of photoproduct formation indicate that, in lightly pigmented individuals, penetration of UV-B/UV-A2 irradiation extends definitely into the upper dermis, a site of major changes in photoaging.

RETINOIDS REPAIR AND PREVENT PHOTOAGING

Many cosmetic and cosmeceutical products claim to improve the appearance of photoaged skin. In general, however, objective evidence that would allow evaluation of

these claims is not available. In contrast, a large number of controlled clinical studies have been published demonstrating that topical application of 0.025% to 0.1% all-*trans* retinoic acid (tRA) improves the appearance of photoaged skin.[27,28] In spite of extensive knowledge regarding the molecular mechanisms by which tRA acts to regulate expression of certain genes, details regarding the molecular basis of tRA action in photoaging remain largely unknown. Type I collagen is the most abundant protein in skin, and type I and type III collagen fibrils provide strength and resiliency to skin. Photoaged skin contains an abundance of degraded, disorganized collagen fibrils and has a reduced production of type I and type III procollagen. All-*trans* retinoic acid has been shown to induce type I and type III procollagen gene expression in photoaged human skin.[29] Because procollagen is the precursor to collagen, it is likely that increased production of procollagen results in increased deposition of collagen fibrils. This laying down of newly synthesized collagen fibrils may lie at the heart of the ability of tRA to improve the appearance of photoaged skin.

If so, then how does tRA induce new collagen synthesis? One clue is the observation that topical tRA induces TGF-β in human and mouse skin.[30,31] As described earlier, TGF-β induces production of type I and type III procollagen and other components of the dermal extracellular matrix. Thus, it is reasonable to envision that tRA induction of TGF-β plays a primary role in tRA's mechanism of action in photoaging. However, this possibility remains to be investigated.

A major obstacle to understanding the actions of tRA in photoaging is lack of suitable in vitro models. In monolayer cultures, human skin fibroblasts continuously produce high levels of type I procollagen and TGF-β1. These cultures are largely insensitive to exogenous TGF-β and tRA. In 3-dimensional collagen gel cultures, human skin fibroblasts express less procollagen than in monolayer culture, but they respond similarly poorly to exogenous TGF-β and tRA. It has recently been demonstrated that fibroblasts cultured from photoaged skin produce amounts of type I procollagen equivalent to fibroblasts from sun-protected skin.[32] These data indicate that reduced type I procollagen expression observed in photoaged skin in vivo stems from contextual influences, which are alleviated in culture in vitro. This observation suggests that a suitable in vitro model to investigate the mechanisms by which tRA regulates type I procollagen synthesis in photoaged skin may be difficult to achieve without additional knowledge regarding how alterations in the epidermis and/or extracellular matrix in photodamaged skin affect fibroblast function.

Photoaging involves complex alterations to many important structural components of the dermal extracellular matrix besides collagen. The elastin fiber network is grossly disrupted, and modifications in the structure and composition of anchoring fibrils, proteoglycans, and glycoaminoglycans have been reported.[33-35] It is likely that the mechanisms by which tRA improves photoaged skin involve actions on other dermal components as well as collagen. In addition to its ability to improve existing photoaging, tRA has also been shown to interfere with responses of skin to acute UV irradiation that bring about collagen degradation.[16] Treatment of human or mouse skin with tRA prior to UV exposure inhibits induction of transcription factor c-Jun.[10] c-Jun is necessary to form an active AP-1 complex, which, as described above, both stimulates transcription of MMP-1, MMP-3, and MMP-9, and inhibits expression of type I procollagen and type III procollagen. All-*trans* retinoic acid does not inhibit UV induction of c-Jun mRNA, but rather blocks accumulation of c-Jun protein.[10] Whether tRA inhibits c-Jun protein synthesis and/or stimulates c-Jun protein degradation is not known.

Regardless of mechanism, suppression by tRA of UV induction of c-Jun protein reduces MMP activities that degrade skin connective tissue and prevents loss of procollagen expression. In essence, tRA is predicted to impede photoaging by mitigating disruption of collagen homeostasis caused by UV irradiation. The metabolic precursor of tRA is vitamin A (all-*trans* retinol [tROL]). Skin keratinocytes and fibroblasts, as well as most other cell types, possess the ability to convert vitamin A (which is taken up from the circulation) into tRA. However, cellular tRA levels are constrained by catabolic inactivation mediated by specific members of the cytochrome P450 family. Human keratinocytes also possess the ability to esterify tROL. All-*trans* retinol esters reside in cellular membranes and are considered a stored form of tROL. Under appropriate conditions, tROL esters can be enzymatically hydrolyzed to provide substrate for conversion to tRA, which binds nuclear retinoic acid receptors that tROL does not bind.

Interestingly, human fibroblasts possess little if any capacity to esterify tROL and therefore must depend on uptake of tROL from the circulation for tRA biosynthesis. In human keratinocytes, the rate and capacity of tROL esterification is hundreds of times greater than that of oxidation to tRA.[36] Thus, esterification functions to sequester tROL from conversion to tRA. The lack of esterification activity in fibroblasts should permit higher rates of tRA synthesis than that in keratinocytes. Given the capacity of human skin to metabolically convert topical tROL to tRA, a sufficient concentration of tROL (approximately 0.25%-0.5%) should provide therapeutic and preventative benefits equivalent to those of tRA. Comparisons of similar biologically active concentrations of topical tROL and tRA demonstrated less erythema and scaling with tROL than with tRA. Because the severity of tRA adverse effects is concentration dependent, the stringent control of tROL conversion to tRA, as described earlier, likely accounts for the observed difference

SIMILARITIES BETWEEN CHRONOLOGICAL AGING AND PHOTOAGING

Skin, like all organs, ages with the passage of time. Compared with photodamaged skin, sun-protected aged skin appears thinner, more evenly pigmented, laxer, and more finely lined. Often, photoaging and chronological aging have been considered separate entities. However, recent evidence indicates that they share some important molecular features. Photoaging is the superposition of UV irradiation from the sun on intrinsic aging. To exert its biological effects, UV irradiation must be absorbed by mol-

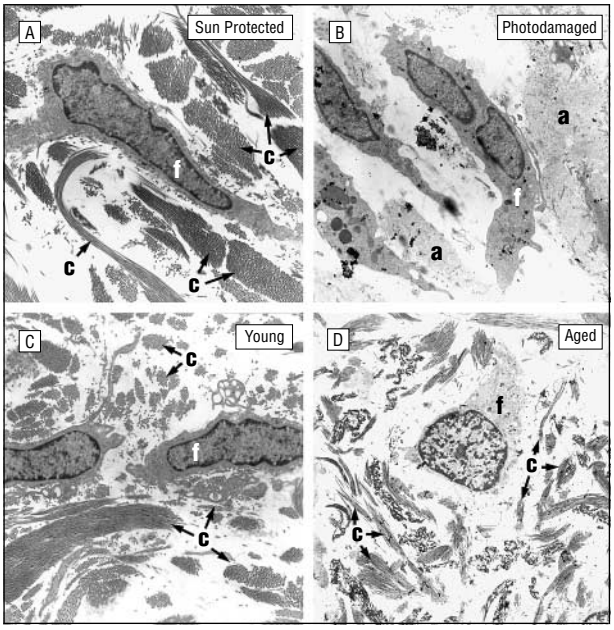

Figure 7. Collagen fibrils are damaged in photoaged and chronologically aged human skin. Transmission electron micrographs reveal that collagen fibrils (c) are fragmented and disorganized in photoaged and chronologically aged compared with sun-protected and young human skin. In sun-protected and young skin, intact collagen fibrils are observed as long strands (cut parallel) or circular arrays (cut perpendicular) in close proximity to fibroblasts (f). In photodamaged and aged skin, collagen fibrils are fragmented and replaced by amorphous material (a) surrounding fibroblasts. Sun-protected hip skin (A) and photodamaged forearm skin (B) were obtained from a 67-year-old subject; young skin (C) was from the hip of a 25-year-old subject; and aged skin (D) was from the hip of an 83-year-old subject.

ecules (chromophores) in the skin, and the absorbed energy must be converted into chemical reactions. Depending on the chromophore, absorbed energy may cause direct chemical modification of the chromophore itself, or the energy may be transferred from the chromophore to another molecule, which undergoes chemical modification. For example, UV-B absorption by DNA causes cross-linking of adjacent pyrimidines, whereas UV-A–absorbing skin chromophores transfer energy to oxygen to generate ROS, which oxidize cellular constituents including proteins, lipids, and DNA. Photoaging is mediated by direct UV absorption and ROS-mediated photochemical reactions. The cause of chronological aging is far less clear than that of photoaging. Many theories have been advanced to explain chronological aging. One, the free radical theory, states that aging results from accumulation of cellular damage that results from excess ROS that are generated as a consequence of oxidative metabolism.[37,38] Age-associated cellular damage includes oxidation of DNA resulting in mutations, oxidation of proteins resulting in reduced function, and oxidation of membrane lipids resulting in reduced transport efficiency and possibly altered transmembrane signaling. The main source of excess ROS implicated in aging is mitochondrial oxidative energy generation. As a result of accumulated damage, the aged cell has reduced antioxidant capacity,[39,40] further exacerbating ROS-mediated damage and the aged phenotype.[41]

Given the central role of ROS in both photoaging and chronological aging, it is possible that the 2 processes have common molecular mediators. As described earlier, transcription factor AP-1 is a critical mediator of acute photodamage that is involved in both overexpression of MMPs and reduction of type I procollagen. In human skin, AP-1 activity is limited by c-Jun expression, since c-Fos is continuously expressed.[10] Interestingly, while c-Fos expression in young (18-28 years old) and aged (>80 years old) skin does not differ, c-Jun expression is elevated in aged compared with young skin.[42] Both c-Jun mRNA and protein levels are elevated in aged skin, a finding similar to that in UV-irradiated skin. In addition, the activity of the upstream activator of c-Jun, c-Jun N-terminal kinase, is also elevated in aged compared with young skin.[42] These data suggest that AP-1 activity is increased in aged skin. In support of this possibility, AP-1–regulated MMP-1 and MMP-9 activities are also increased in aged human skin in vivo.[43]

Increased MMP activity would be expected, over time, to degrade dermal connective tissue. Consistent with this view, insoluble partially degraded collagen, as a percentage of total collagen, is increased 4-fold in aged compared with young human skin (data not shown; unpublished data, 2002). This increased percentage of partially degraded collagen in aged skin is similar to that observed in photoaged skin compared with sun-protected skin. These data indicate that imperfect repair of elevated MMP-mediated collagen breakdown in aged skin, as in UV-irradiated skin, results in accumulation of collagen fragments. Transmission electron microscopy reveals the fragmented nature of collagen fibrils in photoaged and aged human skin in vivo (**Figure 7**).

DAMAGED COLLAGEN AS A REGULATOR OF TYPE I PROCOLLAGEN SYNTHESIS: IMPLICATIONS FOR PHOTOAGING AND CHRONOLOGICAL AGING

In addition to reduction of type I procollagen synthesis following immediate UV irradiation as described earlier, ongoing procollagen synthesis is significantly reduced in severely photodamaged and chronologically aged skin, independent of recent UV exposure.[29,43] Mechanisms underlying the sustained reduction in procollagen synthesis are not fully understood. In full-thickness photoaged skin, total collagen content is only modestly reduced.[34] However, procollagen gene and protein expression are substantially reduced in approximately the upper one third of the dermis.[44] This restricted localization of procollagen reduction likely reflects the depth of penetration of UV irradiation into the skin, which mitigates more extensive reduction of total collagen. In photodamaged skin, the number of fibroblasts in vitro is similar to that in sun-protected skin of the same subjects, and the capacity to synthesize type I procollagen is the same for fibroblasts cultured from both areas.[43] Thus, the reduced procollagen observed in photodamaged skin likely results from down-regulation of fibroblast procollagen synthesis by factors within the dermal milieu rather than from inherent alterations in the fibroblasts.

Interestingly, exposure of cultured fibroblasts from either photodamaged or sun-protected skin to partially degraded type I collagen, produced by in vitro treat-

ment of collagen with a mixture of MMPs from human skin, inhibited procollagen synthesis.[32] These results suggest that elevated levels of degraded collagen observed in photodamaged skin act to down-regulate type I procollagen synthesis. Of the different MMPs, MMP-1 was the most effective collagenase, followed by MMP-8 and MMP-13.[32] As expected, gelatinolytic enzymes (MMP-2 and MMP-9) did not degrade intact collagen and did not inhibit procollagen synthesis, but the combination of MMP-1 and MMP-9 broke down collagen to small peptides.[32] Interestingly, these small fragments did not inhibit procollagen synthesis, but rather the larger breakdown fragments of type I collagen negatively regulated its synthesis. Taken together, these data suggest that the high-molecular-weight fragments of type I collagen serve as negative regulators of type I collagen synthesis and that further breakdown of MMP-1–cleaved collagen by MMP-9 can alleviate this inhibition. Thus, UV-induced MMPs damage the dermis by 2 related mechanisms: direct degradation of collagen and indirect inhibition of collagen synthesis by MMP-generated collagen degradation products.

In contrast to photodamaged skin, in aged sun-protected skin, both the number of fibroblasts and their capacity to synthesize type I procollagen are reduced compared with young skin.[43] In addition, aged skin, similar to photoaged skin, contains elevated levels of partially degraded collagen. Therefore, it is likely that the inhibitory effects of collagen fragments observed in photoaged skin are also operative in aged skin and are superimposed on an intrinsic decline in collagen synthetic activity.

Reduced mechanical tension may also contribute to diminished number of fibroblasts in aged skin because the loss of mechanical tension results in increased apoptosis in model cell systems.[45,46] How MMP-damaged collagen functions to inhibit new collagen synthesis in vivo is not known, but collagen synthetic activity is regulated by mechanical tension on fibroblasts resulting from attachment to a firm substratum.[47] Fibroblasts exert contractile forces on the collagen extracellular matrix, and the physical resistance of the matrix to this contraction generates mechanical tension on the fibroblast. The rate of collagen synthesis is proportional to the level of mechanical tension.[48,49] We envision that damaged collagen fibrils are more pliable than native fibrils. As fibroblasts interact with damaged collagen fibrils, the cells experience less resistance and therefore less mechanical tension, resulting in reduced procollagen synthesis. How mechanical tension regulates procollagen synthesis is poorly understood, although many cellular functions are affected by mechanical tension, including cell surface receptor activation, signal transduction, gene expression, and cell growth.[46,47,50] The observed disruption in the structure of the collagenous extracellular matrix and attendant reduction of mechanical tension on fibroblasts offer an attractive explanation, at least in part, for reduced procollagen expression in aged and photoaged human skin.

CONCLUSIONS

The pathophysiological process of photoaging and skin aging derives largely from aberrant regulation of a multitude of finely tuned molecular mechanisms, which have evolved to maintain the structural integrity of skin connective tissue. These molecular mechanisms enable skin cells to communicate with each other and their environment. Knowledge regarding relevant signal transduction pathways and their effectors provides exciting opportunities for therapeutic intervention to prevent and repair age-related skin damage. Two critical mediators of skin aging are the transcription factor AP-1 and AP-1–regulated MMPs, both of which play critical roles in tumor formation, inflammation, and fibrosis, which are all subjects of intensive basic and clinical research. The promise of this research is the development of new drugs that target these critical mediators of disease. The challenge lies in the application of these new therapies to skin aging.

Accepted for publication July 23, 2002.

This work was supported in part by grant AG139364-01-GJF from the National Institutes of Health, Bethesda, Md, and grants from Johnson & Johnson, New Brunswick, NJ, and the Mary Reynolds Babcock Foundation, Winston-Salem, NC.

All procedures involving human subjects were approved by the University of Michigan institutional review board, and all subjects provided written informed consent.

Corresponding author and reprints: Gary J. Fisher, PhD, Medical Science I, R6447, Department of Dermatology, University of Michigan, Ann Arbor, MI 48109 (e-mail: dianemch@umich.edu).

REFERENCES

1. Ullrich A, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. *Cell.* 1990;61:203-212.
2. van der Geer P, Hunter T, Lindberg RA. Receptor protein-tyrosine kinases and their signal transduction pathways. *Annu Rev Cell Biol.* 1994;10:251-337.
3. Gross S, Knebel A, Tenev T, et al. Inactivation of protein-tyrosine phosphatases as mechanism of UV-induced signal transduction. *J Biol Chem.* 1999;374:26378-26386.
4. Barford D, Jia Z, Tonks NK. Protein tyrosine phosphatases take off. *Nat Struct Biol.* 1995;2:1043-1053.
5. Denu JM, Tanner KG. Specific and reversible inactivation of protein tyrosine phosphatases by hydrogen peroxide: evidence for a sulfenic acid intermediate and implications for redox regulation. *Biochemistry.* 1998;37:5633-5642.
6. Meng T-C, Fukada T, Tonks NK. Reversible oxidation and inactivation of protein tyrosine phosphatases in vivo. *Mol Cell.* 2002;9:387-399.
7. Babior BM, Lambeth JD, Nauseef W. The neutrophil NADPH oxidase. *Arch Biochem Biophys.* 2002;397:342-344.
8. Cobb MH. MAP kinase pathways. *Prog Biophys Mol Biol.* 1999;71:479-500.
9. Garrington TP, Johnson GL. Organization and regulation of mitogen-activated protein kinase signaling pathways. *Curr Opin Cell Biol.* 1999;11:211-218.
10. Fisher GJ, Talwar HS, Lin JY, et al. Retinoic acid inhibits induction of c-Jun protein by ultraviolet radiation that occurs subsequent to activation of mitogen-activated protein kinase pathways in human skin in vivo. *J Clin Invest.* 1998;101:1432-1440.
11. Karin M, Liu Z-G, Zandi E. AP-1 function and regulation. *Curr Opin Cell Biol.* 1997;9:240-246.
12. Angel P, Szabowski A, Schorpp-Kistner M. Function and regulation of AP-1 subunits in skin physiology and pathology. *Oncogene.* 2001;20:2413-2423.
13. Senftleben U, Karin M. The IKK/NF-κB pathway. *Crit Care Med.* 2002;30(1 suppl):S18-S26.
14. Yamamoto Y, Gaynor RB. Therapeutic potential of inhibition of the NF-κB pathway in the treatment of inflammation and cancer. *J Clin Invest.* 2001;107:135-142.
15. Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol.* 2001;17:463-516.
16. Fisher GJ, Voorhees JJ. Molecular mechanisms of photoaging and its preven-

tion by retinoic acid: ultraviolet irradiation induces MAP kinase signal transduction cascades that induce AP-1-regulated matrix metalloproteinases that degrade human skin in vivo. *J Invest Dermatol.* 1998;3(suppl):S61-S68.
17. Fisher GJ, Datta S, Wang ZQ, et al. c-Jun-dependent inhibition of cutaneous procollagen transcription following ultraviolet irradiation is reversed by all-*trans* retinoic acid. *J Clin Invest.* 2000;106:663-670.
18. Chung KY, Agarwal A, Uitto J, Mauviel A. An AP-1 binding sequence is essential for regulation of the human alpha2(I) collagen (COL1A2) promoter activity by transforming growth factor-beta. *J Biol Chem.* 1996;271:3272-3278.
19. Verrecchia F, Tacheau C, Schorpp-Kistner M, Angel P, Mauviel A. Induction of the AP-1 members c-Jun and JunB by TGF-beta/Smad suppresses early Smad-driven gene activation. *Oncogene.* 2001;20:2205-2211.
20. Pessah M, Marais J, Prunier C, et al. c-Jun associates with the oncoprotein Ski and suppresses Smad2 transcriptional activity. *J Biol Chem.* 2002;277:29094-29100.
21. Massagué J. TGF-β signal transduction. *Annu Rev Biochem.* 1998;67:753-791.
22. Massagué J. How cells read TGF-β signals. *Nat Rev Mol Cell Biol.* 2000;1:169-178.
23. Quan T, He TY, Voorhees JJ, Fisher GJ. Ultraviolet irradiation blocks cellular responses to transforming growth factor-β by down regulating its type-II receptor and inducing Smad7. *J Biol Chem.* 2001;276:26349-26356.
24. Simon JD. Spectroscopic and dynamic studies of the epidermal chromophores trans-urocanic acid and eumelanin. *Acc Chem Res.* 2000;33:307-313.
25. Ortonne J. Photoprotective properties of skin melanin. *Br J Dermatol.* 2002;146 (suppl 61):7-10.
26. Jimbow K, Fitzpatrick TB, Wick MM. Biochemistry and physiology of melanin pigmentation. In: Goldstein LA, ed. *Physiology, Biochemistry, and Molecular Biology of the Skin.* Vol 2. New York, NY: Oxford University Press Inc; 1991: 873-909.
27. Kang S, Voorhees JJ. Photoaging therapy with topical tretinoin: an evidence-based analysis. *J Am Acad Dermatol.* 1998;39(2 pt 3):S55-S61.
28. Gilchrest BA. Treatment of photodamage with topical tretinoin: an overview. *J Am Acad Dermatol.* 1997;36(3 pt 2):S27-S36.
29. Griffiths CE, Russman AN, Majmudar G, Singer RS, Hamilton TA, Voorhees JJ. Restoration of collagen formation in photodamaged human skin by tretinoin (retinoic acid). *N Engl J Med.* 1993;329:530-535.
30. Kim HJ, Bogdan NJ, D'Agostaro LJ, Gold LI, Bryce GF. Effect of topical retinoic acids on the levels of collagen mRNA during the repair of UV-B-induced dermal damage in the hairless mouse and the possible role of TGF-beta as a mediator. *J Invest Dermatol.* 1992;98:359-363.
31. Glick AB, Flanders KC, Danielpour D, Yuspa SH, Sporn MB. Retinoic acid induces transforming growth factor-beta 2 in cultured keratinocytes and mouse epidermis. *Cell Regul.* 1989;1:87-97.
32. Varani J, Spearman D, Perone P, et al. Inhibition of type I procollagen synthesis by damaged collagen in photoaged skin and by collagenase-degraded collagen in vitro. *Am J Pathol.* 2001;158:931-942.
33. Lavker RM. Cutaneous aging: chronologic versus photoaging. In: Gilchrest B, ed. *Photodamage.* Vol 1. Cambridge, England: Blackwell Science Inc; 1995:123-135.
34. Bernstein EF, Uitto J. The effect of photodamage on dermal extracellular matrix. *Clin Dermatol.* 1996;14:143-151.
35. Yaar M, Gilchrest BA. Skin aging: postulated mechanisms and consequent changes in structure and function. *Clin Geriatr Med.* 2001;17:617-630.
36. Kurlandsky SB, Xiao JH, Duell EA, Voorhees JJ, Fisher GJ. Biological activity of all-*trans* retinol requires metabolic conversion to all-*trans* retinoic acid and is mediated through activation of nuclear retinoid receptors in human keratinocytes. *J Biol Chem.* 1994;269:32821-32827.
37. Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. *Science.* 1996;273:59-63.
38. Hensley K, Floyd R. Reactive oxygen species and protein oxidation in aging: a look back, a look ahead. *Arch Biochem Biophys.* 2002;397:377-383.
39. Chamougrand N, Rigoulet M. Aging and oxidative stress: studies of some genes involved both in aging and in response to oxidative stress. *Respir Physiol.* 2001; 128:393-401.
40. Ma W, Wlaschek M, Tantcheva-Poor I, et al. Chronological aging and photoaging of the fibroblasts and the dermal connective tissue. *Clin Exp Dermatol.* 2001; 26:592-599.
41. Herman D. Aging: overview. *Ann N Y Acad Sci.* 2001;928:1-21.
42. Chung J-H, Kang S, Varani J, Lin JY, Fisher GJ, Voorhees JJ. Decreased extracellular-signal-regulated kinase and increased stress-activated MAP kinase activities in aged human skin in vivo. *J Invest Dermatol.* 2000;114:177-182.
43. Varani J, Warner RL, Gharaee-Kermani M, et al. Vitamin A antagonizes decreased cell growth and elevated collagen-degrading matrix metalloproteinases and stimulates collagen accumulation in naturally aged human skin. *J Invest Dermatol.* 2000;114:480-486.
44. Talwar HS, Griffiths CE, Fisher GJ, Hamilton TA, Voorhees JJ. Reduced type I and type III procollagens in photodamaged adult human skin. *J Invest Dermatol.* 1995;105:285-290.
45. Huang S, Ungber D. Shape-dependent control of cell growth, differentiation, and apoptosis: switching between attractors in cell regulatory networks. *Exp Cell Res.* 2000;261:91-103.
46. Grinnell F, Zhu M, Charlson M, Abrams J. Release of mechanical tension triggers apoptosis of human fibroblasts in a model of regressing granulation tissue. *Exp Cell Res.* 1999;248:608-619.
47. Grinnell F. Fibroblast-collagen-matrix contraction: growth-factor signalling and mechanical loading. *Trends Cell Biol.* 2000;10:362-365.
48. Lambert C, Colige A, Lapiere C, Nusgens B. Coordinated regulation of procollagens I and III and their post-translational enzymes by dissipation of mechanical tension in human dermal fibroblasts. *Eur J Cell Biol.* 2001;80:479-485.
49. Kessler D, Dethlefsen S, Haase I, et al. Fibrolasts in mechanically stressed collagen lattices assume a "synthetic" phenotype. *J Biol Chem.* 2001;279:36575-36585.
50. Huang S, Ingber DE. The structural and mechanical complexity of cell-growth control. *Nat Cell Biol.* 1999;1:E131-E138.