PATHOPHYSIOLOGY OF PREMATURE SKIN AGING INDUCED BY ULTRAVIOLET LIGHT

GARY J. FISHER, PH.D., ZENGQUAN WANG, PH.D., SUBHASH C. DATTA, PH.D., JAMES VARANI, PH.D., SEWON KANG, M.D., AND JOHN J. VOORHEES, M.D.

ABSTRACT

Background Long-term exposure to ultraviolet irradiation from sunlight causes premature skin aging (photoaging), characterized in part by wrinkles, altered pigmentation, and loss of skin tone. Photoaged skin displays prominent alterations in the collagenous extracellular matrix of connective tissue. We investigated the role of matrix-degrading metalloproteinases, a family of proteolytic enzymes, as mediators of collagen damage in photoaging.

Methods We studied 59 whites (33 men and 26 women, ranging in age from 21 to 58 years) with light-to-moderate skin pigmentation, none of whom had current or prior skin disease. Only some of the participants were included in each of the studies. We irradiated their buttock skin with fluorescent ultraviolet lights under standard conditions and obtained skin samples from irradiated and nonirradiated areas by keratome or punch biopsy. In some studies, tretinoin and its vehicle were applied to skin under occlusion 48 hours before ultraviolet irradiation. The expression of matrix metalloproteinases was determined by in situ hybridization, immunohistology, and in situ zymography. Irradiation-induced degradation of skin collagen was measured by radioimmunoassay of soluble cross-linked telopeptides. The protein level of tissue inhibitor of matrix metalloproteinases type 1 was determined by Western blot analysis.

Results A single exposure to ultraviolet irradiation increased the expression of three matrix metalloproteinases — collagenase, a 92-kd gelatinase, and stromelysin — in skin connective tissue and outer skin layers, as compared with nonirradiated skin. The degradation of endogenous type I collagen fibrils was increased by 58 percent in irradiated skin, as compared with nonirradiated skin. Collagenase and gelatinase activity remained maximally elevated (4.4 and 2.3 times, respectively) for seven days with four exposures to ultraviolet irradiation, delivered at two-day intervals, as compared with base-line levels. Pretreatment of skin with tretinoin (all-*trans*-retinoic acid) inhibited the induction of matrix metalloproteinase proteins and activity (by 70 to 80 percent) in both connective tissue and outer layers of irradiated skin. Ultraviolet irradiation also induced tissue inhibitor of matrix metalloproteinases-1, which regulates the enzyme. Induction of the inhibitor was not affected by tretinoin.

Conclusions Multiple exposures to ultraviolet irradiation lead to sustained elevations of matrix metalloproteinases that degrade skin collagen and may contribute to photoaging. Treatment with topical tretinoin inhibits irradiation-induced matrix metalloproteinases but not their endogenous inhibitor. (N Engl J Med 1997;337:1419-28.)

ULTRAVIOLET irradiation from the sun has deleterious effects in human skin, including sunburn, immune suppression,[1] cancer, and premature aging (photoaging). Sunburn and immune suppression occur acutely in response to excessive exposure to the sun, whereas skin cancer and photoaging result from accumulated damage caused by repeated exposures. Skin cancer, the most prevalent form of cancer in humans,[2] typically occurs in skin that is photoaged.[3] Photoaged skin is characterized by wrinkles, laxity, uneven pigmentation, brown spots, and a leathery appearance.[4] In contrast, chronologically aged skin that has been protected from the sun is thin and has reduced elasticity but is otherwise smooth and unblemished.[5]

Histologic and ultrastructural studies have shown that alterations in photoaged skin are found in dermal connective tissue.[6,7] The dermis lies below and provides mechanical support for the outer, protective layer of skin, the epidermis. The extracellular matrix in the dermis is composed primarily of type I collagen, with lesser amounts of type III collagen, elastin, proteoglycans, and fibronectin. Collagen fibrils are responsible for the strength and resiliency of skin.[8]

Dermal damage induced by ultraviolet irradiation is principally manifested histologically as the disorganization of collagen fibrils[9] and the accumulation of abnormal elastin-containing material.[5] Biochemical evidence of connective-tissue alterations in photoaged skin includes reduced levels of types I and III collagen precursors[10] and cross-links,[11] an increased ratio of type III to type I collagen,[12] and an increased level of elastin.[13] Improvement in the appearance of photoaged skin after treatment with tretinoin (all-*trans*-retinoic acid) is associated with the restoration of type I procollagen to levels approaching those in skin that has not been exposed to sun.[14] These observations have led to the belief that the aged appearance of sun-exposed skin results from alterations in the structure and composition of collagen and elastin in the dermal extracellular matrix. Mechanisms responsible for skin connective-tissue damage caused by ultraviolet irradiation are not known.

From the Departments of Dermatology (G.J.F., Z.Q.W., S.C.D., S.K., J.J.V.) and Pathology (J.V.), University of Michigan Medical School, Ann Arbor. Address reprint requests to Dr. Fisher at Medical Science I, Rm. 6447, 1150 W. Medical Center Dr., Department of Dermatology, University of Michigan, Ann Arbor, MI 48109-0609.

Matrix-degrading metalloproteinases are a family of proteolytic enzymes that specifically degrade collagens, elastin, and other proteins in connective tissue and bone.[15,16] Matrix metalloproteinases are critical for matrix remodeling during development and wound healing.[17] Their activities are regulated by tissue inhibitors of matrix metalloproteinases.[18] The expression of tissue inhibitor of matrix metalloproteinases type 1 often parallels that of matrix metalloproteinases, to prevent excessive matrix degradation.[19,20]

In a previous study, we demonstrated by biochemical methods that a single exposure to ultraviolet irradiation induces three matrix metalloproteinases in human skin in vivo — collagenase, 92-kd gelatinase, and stromelysin-1 — and that pretreatment of skin with tretinoin inhibits the induction of all three.[21] The combined actions of collagenase, 92-kd gelatinase, and stromelysin can fully degrade skin collagen.[15-17] This raises the possibility that matrix metalloproteinases induced by ultraviolet irradiation are largely responsible for dermal damage in photoaging and that tretinoin, by inhibiting the induction of matrix metalloproteinases, may prevent this damage. We performed the current study to demonstrate that matrix metalloproteinases induced by ultraviolet irradiation are expressed in the dermis (the site of damage in photoaging), where they degrade skin collagen; that the expression of matrix metalloproteinases remains maximally elevated after multiple exposures to ultraviolet irradiation; and that pretreatment of skin with tretinoin inhibits the expression of matrix metalloproteinases due to ultraviolet irradiation.

METHODS

Ultraviolet Irradiation and Tissue Samples

We studied 59 white adults (33 men and 26 women; mean age, 35.3 years; range, 21 to 58), without current or prior skin disease and with light-to-moderate pigmentation. We irradiated buttock skin with four fluorescent ultraviolet lights (F36T12 ERE-VHO). A Kodacel filter (TA401/407; Kodak, Rochester, N.Y.) was mounted 4 cm in front of the tubes to remove wavelengths of less than 290 nm (ultraviolet C). The intensity of irradiation was monitored with the use of a phototherapy radiometer (model 443, International Light, Newburyport, Mass.) and a photodetector (SED240/UVB/W, International Light). Spectroradiometry was performed with the OL 754 system (Optronic Laboratories, Orlando, Fla.). The total irradiation (290 to 800 nm) 17 inches from the light source was 1.49×10^{-3} W per square centimeter. The distribution of power output was 47 percent ultraviolet B (290 to 320 nm), 18 percent ultraviolet A2 (320 to 340 nm), 9 percent ultraviolet A1 (340 to 400 nm), and 26 percent visible and near-infrared (400 to 800 nm). The dose causing minimal erythema was determined 24 hours after irradiation. Irradiated and nonirradiated skin samples were obtained from each subject by keratome or punch biopsy, as previously described.[22] Tretinoin and its vehicle (70 percent ethanol, 30 percent polyethylene glycol, and 0.05 percent butylated hydroxytoluene) were applied to skin, to which a protective covering was then applied, 48 hours before exposure to ultraviolet irradiation. For studies with multiple time points or treatments, tissue was obtained from each subject for each time point or treatment. Twenty-five of the 105 subjects who donated skin specimens for studies described in a prior report[21] also donated skin specimens for the studies reported here. The study was approved by the institutional review board at the University of Michigan, and all subjects provided written informed consent.

Measurements of Matrix Metalloproteinases

Skin specimens from irradiated and nonirradiated sites were homogenized in 20 mM TRIS–hydrochloric acid (pH 7.6) and 5 mM calcium chloride and centrifuged at $3000 \times g$ for 10 minutes. Supernatants were used to measure collagenase and gelatinase activity by hydrolysis of tritium-labeled fibrillar collagen[17] (100 μg per assay) and gelatin zymography[23] (20 μg per assay), respectively.

In Situ Hybridization

Digoxigenin-containing sense and antisense riboprobes to detect human collagenase, 92-kd gelatinase, and stromelysin-1 messenger RNA (mRNA) were synthesized with the use of T3 and T7 ribonucleic acid polymerases. Frozen skin sections (5 μm) were mounted, fixed, treated, and hybridized as described elsewhere.[24] Hybridization signals were detected immunohistochemically with the use of alkaline phosphatase–conjugated antidigoxigenin antibody.

Immunohistologic Analysis

Immunohistologic analysis of collagenase, gelatinase, and stromelysin was performed as described elsewhere.[14] Collagenase and gelatinase were detected with affinity-purified polyclonal IgG antibody (Cambio, Cambridge, United Kingdom). Stromelysin was detected with mouse monoclonal antibody (a gift from Dr. Constance E. Brinckerhoff, Dartmouth Medical School, Hanover, N.H.). Sheep IgG and mouse IgG (Sigma Chemical, St. Louis) were used as controls.

In Situ Zymography

In situ zymography with the use of fluorescein isothiocyanate–gelatin and resorufin–casein substrates has been described elsewhere.[25] The same procedures were followed with the use of fluorescein–collagen (Elastin Products, Owensville, Mo.) as substrate, except that fluorescein–collagen (4 mg per millimeter) was coated directly onto slides, without the addition of agarose.

Radioimmunoassay for Type I Collagen Telopeptide

Punch-biopsy specimens were homogenized in 150 mM sodium chloride, 50 mM TRIS (pH 7.5), 0.02 percent sodium azide, 2 mM phenylmethylsulfonyl fluoride, and 10 μg of aprotinin per milliliter and centrifuged at $25,000 \times g$ for 30 minutes. Supernatants were used to measure soluble type I collagen cross-linked telopeptides with the use of a commercial radioimmunoassay kit[26] (Incstar, Stillwater, Minn.).

Western Blot Analysis

Levels of tissue inhibitor of matrix metalloproteinases-1 and stromelysin were determined in supernatants from skin homogenates, as described elsewhere.[21] Samples (100 μg) were incubated for 16 hours with antibodies (Cambio). Immunoreactive stromelysin and tissue inhibitor of matrix metalloproteinases-1 were visualized with enhanced chemiluminescence.

Statistical Analysis

Data were analyzed with paired t-tests (for the telopeptide data and the comparison of tretinoin-treated skin and vehicle-treated skin) or repeated-measures analysis of variance and with Tukey's studentized range test[27] (for the data on multiple exposures and different doses of radiation). All P values are two-tailed, and differences were considered significant when P values were less than or equal to 0.05.

RESULTS

Ultraviolet Irradiation and Induction of Matrix Metalloproteinases

Collagenase mRNA was minimally detectable in nonirradiated skin (Fig. 1A). Twenty-four hours after exposure to ultraviolet irradiation, collagenase mRNA was induced in all keratinocytes throughout the epidermis (Fig. 1B). Ultraviolet irradiation also induced collagenase mRNA in cells in the connective tissue, although to a lesser extent than in keratinocytes (Fig. 1B, inset). Hybridization of irradiation-exposed skin with a sense probe, a control for nonspecific hybridization, yielded no detectable signal (data not shown). Collagenase protein was minimally detectable in nonirradiated skin (Fig. 1C). Exposure to ultraviolet irradiation induced the expression of collagenase protein throughout the epidermis and in connective tissue (Fig. 1D). Immunoreactivity in the epidermis and dermis was detected intracellularly in keratinocytes and fibroblasts, respectively, and extracellularly. Extracellular staining was especially noticeable in the dermis, where immunoreactivity was seen throughout the collagenous matrix (reddish-brown staining in Fig. 1D). Staining of irradiation-exposed skin specimens with control IgG revealed minimal background staining (data not shown). The level of in situ collagenase activity was very low in nonirradiated skin (Fig. 1E). In irradiated skin, collagenase activity was detectable throughout the epidermis and dermis (Fig. 1F). The addition of tissue inhibitor of matrix metalloproteinases-1 or a cation chelator (EDTA) to irradiated skin sections reduced the level of collagen breakdown to control values (Fig. 1E), as expected for collagen degradation by collagenase (data not shown). In sections of irradiated skin incubated at 4°C as a control, collagenase activity was not detectable (data not shown).

The findings for 92-kd gelatinase and stromelysin in nonirradiated and irradiated skin were very similar to those for collagenase. Ultraviolet irradiation induced the expression of mRNA for 92-kd gelatinase and stromelysin predominantly in the epidermis, whereas gelatinase and stromelysin proteins were induced in the connective tissue and the epidermis (data not shown). Gelatinase activity was induced throughout the dermis and epidermis, whereas stromelysin activity was predominantly located in the dermis (data not shown).

Ultraviolet Irradiation and Degradation of Endogenous Collagen

The induction of dermal matrix metalloproteinase activity in skin exposed to ultraviolet irradiation would be expected to cause increased degradation of endogenous collagen. To examine this hypothesis, we quantified soluble type I collagen C-terminal cross-linked telopeptides, a reliable measure of endogenous destruction of type I collagen,[26,28] in non-irradiated and irradiated skin. The level of soluble cross-linked telopeptides was elevated by 58 percent 24 hours after ultraviolet irradiation (mean [±SE] level, 30.2 ± 4.6 ng per 100 μg of protein in five specimens, vs. 19.0 ± 2.2 ng per 100 μg of protein in five specimens of nonirradiated skin; $P = 0.02$) and remained elevated for at least 72 hours (mean level, 27.0 ± 3.4 ng per 100 μg of protein; $P = 0.02$).

Multiple Exposures to Ultraviolet Irradiation

Since photoaging results from the accumulation of damage caused by repeated exposures to ultraviolet light, we investigated the effect of single and multiple exposures on collagenase and 92-kd gelatinase activity in vivo. After a single exposure, collagenase and gelatinase activity increased within 8 hours, peaked within 24 hours, and returned to nearly basal (nonirradiated) levels within 72 hours (data not shown). For studies of multiple exposures, each subject was exposed to ultraviolet irradiation at four separate sites, with each site exposed one, two, three, or four times to irradiation delivered at 48-hour intervals. Skin specimens were obtained from each irradiated site 24 hours after the last exposure at that site and were also obtained from nonirradiated (control) sites for analyses. After a single exposure to ultraviolet irradiation, collagenase and 92-kd gelatinase activity was elevated 4.4 ± 0.2 times the values in nonirradiated skin (in eight specimens) and 2.3 ± 0.4 times (in five specimens), respectively (Fig. 2). Collagenase and gelatinase activity remained maximally elevated after the second, third, and fourth exposures on days 3, 5, and 7, respectively (Fig. 2). Thus, multiple exposures led to the sustained induction of matrix metalloproteinases.

Pretreatment with Tretinoin

Topical pretreatment with tretinoin inhibits the induction of matrix metalloproteinases, detected biochemically, in skin exposed to ultraviolet irradiation in vivo.[21] This finding suggests that tretinoin may prevent dermal damage. To do so, tretinoin must block the induction of matrix metalloproteinases in the dermis. We used immunohistologic methods and in situ zymography to localize the effects of tretinoin on the induced expression of collagenase, 92-kd gelatinase, and stromelysin in skin exposed to ultraviolet irradiation.

The treatment of skin with tretinoin, or its vehicle alone, did not alter the low basal levels of collagenase, 92-kd gelatinase, or stromelysin proteins or activity (data not shown). However, treatment with tretinoin before exposure to ultraviolet irradiation substantially reduced the subsequent induction of all three matrix metalloproteinases. Tretinoin blocked the epidermal and dermal expression of collagenase, 92-kd gelatinase, and stromelysin proteins (data not shown) and activity (Fig. 3).
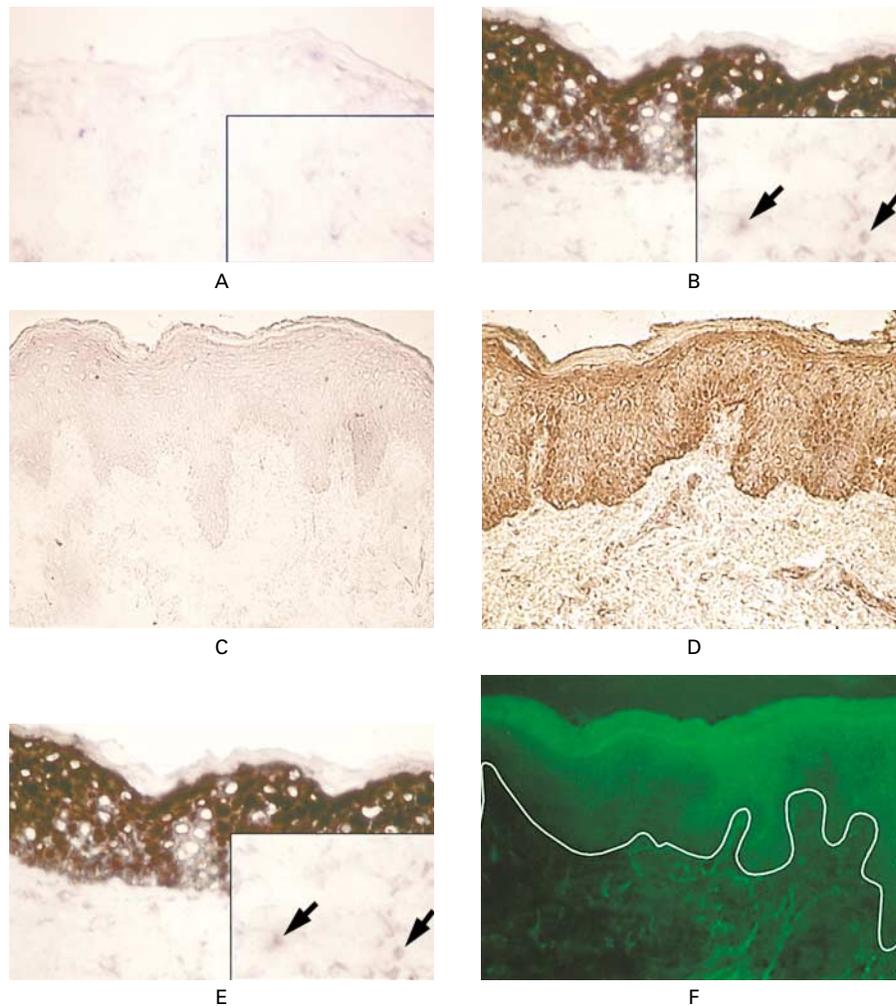
Figure 1. Induction of Collagenase Messenger RNA, Protein, and Activity in Skin Specimens from Three Subjects Exposed to Ultraviolet Irradiation.

Buttock skin was irradiated with twice the dose of ultraviolet irradiation that caused minimal erythema. Specimens of irradiated and nonirradiated buttock skin were obtained from each subject 24 hours after irradiation. Collagenase messenger RNA (mRNA) was detected with the use of digoxigenin-riboprobe in situ hybridization in nonirradiated skin (Panel A) and irradiated skin (Panel B). Collagenase mRNA is stained purple by this technique. The inset in Panel A shows the dermis at greater magnification. The inset in Panel B shows collagenase mRNA (arrows) in cells in the dermis. The specimens are from one subject and are representative of the findings in nonirradiated and irradiated tissue from six subjects.

Collagenase protein was detected by peroxidase immunohistologic techniques in nonirradiated skin (Panel C) and irradiated skin (Panel D). Collagenase protein is stained reddish brown by this technique. The specimens are from one subject and are representative of the findings in nonirradiated and irradiated tissue from six subjects.

Collagenase activity was detected by in situ zymography in nonirradiated skin (Panel E) and irradiated skin (Panel F). The green color is fluorescein-labeled collagen, which was coated onto a glass slide. The skin section was laid on top of the slide and incubated for 24 hours to allow collagenase in the tissue to degrade the fluorescein-labeled collagen on the slide. Darkened areas, especially noticeable in the specimen of irradiated skin (Panel F), are due to the degradation of fluorescein–collagen substrate. The white lines demarcate the boundary between the epidermis (top) and the dermis (bottom). The specimens are from one subject and are representative of the findings in nonirradiated and irradiated tissue from five subjects.
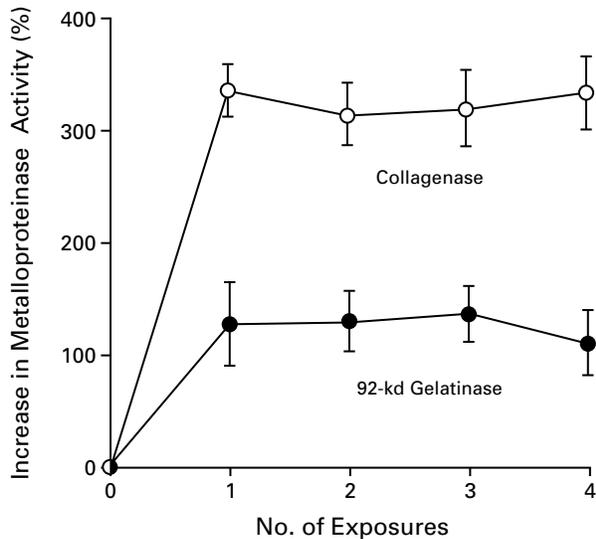
Figure 2. Sustained Elevations in Collagenase and 92-kd Gelatinase Activity after Multiple Exposures to Ultraviolet Irradiation.

Skin was obtained from five separate sites on the buttock. The first site was not irradiated, the second site was irradiated one time (on day 0), the third site was irradiated a second time (on day 2), the fourth site was irradiated a third time (on day 4), and the fifth site was irradiated a fourth time (on day 6). Skin was irradiated with half the dose that caused minimal erythema; doses were given at 48-hour intervals. Tissue specimens were obtained 24 hours after the last exposure and assayed for collagenase activity (in eight specimens) and 92-kd gelatinase activity (in five specimens). The values shown are means, with standard errors indicated by the bars. For each set of exposures, metalloproteinase activity was significantly higher in irradiated tissue than in nonirradiated tissue ($P<0.05$).

Induction of Tissue Inhibitor of Matrix Metalloproteinases-1

Since the regulation of matrix metalloproteinases and the regulation of their tissue inhibitors is coordinated, we investigated whether tissue inhibitors of matrix metalloproteinases are induced in skin exposed to ultraviolet irradiation. Nonirradiated skin expressed mRNA for tissue inhibitor of matrix metalloproteinases types 1 and 2. In irradiated skin, mRNA for tissue inhibitor of matrix metalloproteinases-1 was induced in a time- and dose-dependent manner. Induction occurred within 8 hours after exposure, peaked within 16 to 24 hours, and remained elevated for at least 72 hours (data not shown). The time course for induction of tissue inhibitor of matrix metalloproteinases-1 protein mirrored that of its mRNA (data not shown). The expression of mRNA for tissue inhibitor of matrix metalloproteinases-2 was not induced by ultraviolet irradiation (data not shown).

Tissue inhibitor of matrix metalloproteinases-1 was significantly induced by 1/10 the amount of ultraviolet irradiation that caused minimal erythema (0.1

minimal-erythema dose) and maximally induced by one minimal-erythema dose (Fig. 4A). The induction of stromelysin protein showed a similar dose dependence (Fig. 4A). Pretreatment of skin for 48 hours with tretinoin did not alter either basal or irradiation-induced values for tissue inhibitor of matrix metalloproteinases-1 mRNA (data not shown) or protein levels (Fig. 4B). In contrast, tretinoin significantly inhibited the induction of stromelysin mRNA (data not shown) and protein in irradiated skin (Fig. 4B).

DISCUSSION

We found that ultraviolet irradiation induced matrix metalloproteinases in the epidermis and dermis (these substances degrade collagen in the dermis) and that their induction was sustained with multiple exposures. These results support the concept that matrix metalloproteinases are primary mediators of connective-tissue damage in skin exposed to ultraviolet irradiation and of the premature aging of skin that results.

Figure 5 shows a model of the pathophysiology of dermal damage caused by ultraviolet irradiation and leading to skin wrinkling in photoaging. The model does not address aspects of photoaging that occur in the epidermis and lead to alterations in skin pigmentation and surface texture. We offer the hypothesis that agents that block the induction of matrix metalloproteinases by ultraviolet irradiation, such as tretinoin and its metabolic precursor, retinol,[29,30] or direct inhibitors of matrix metalloproteinases may prevent the dermal damage that leads to photoaging. An evaluation of this hypothesis will require long-term clinical trials.

Although irradiation-induced expression of the genes for collagenase, 92-kd gelatinase, and stromelysin-1 occurred predominantly in the epidermis, matrix metalloproteinase proteins and their enzymatic activity were abundant in both the dermis and the epidermis. Therefore, a substantial fraction of the matrix metalloproteinases synthesized in the epidermis was transported to the dermis. This was not an unexpected finding, since collagenase, 92-kd gelatinase, and stromelysin-1 are secreted proteins, and the basement-membrane zone, which separates the epidermis from the dermis, readily allows the passage of proteins between the two compartments.[31,32]

Collagenases are the only mammalian proteases capable of hydrolyzing fibrillar collagen, within its triple helical domain, and are required for normal collagen turnover in adult animals.[33] Once cleaved, collagen is further broken down by gelatinases and stromelysins. In situ hydrolysis of collagen, which we observed in skin exposed to ultraviolet irradiation, therefore presumably reflects the combined activities of collagenase and other proteinases, including 92-kd gelatinase and stromelysin-1, which were also el-
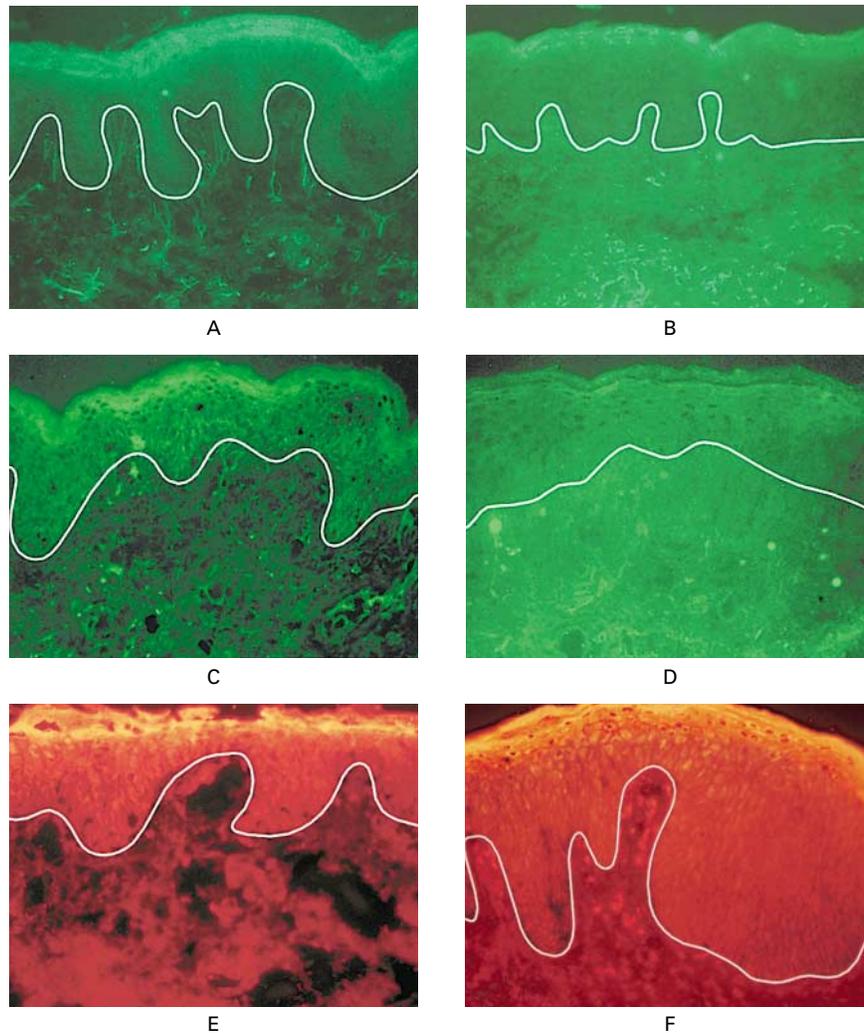
Figure 3. Induction of Collagenase, 92-kd Gelatinase, and Stromelysin Activity in Skin Specimens from Three Subjects Pretreated with Tretinoin and Exposed to Ultraviolet Irradiation.

In each subject, one site was treated with the vehicle and one site with 0.1 percent tretinoin 48 hours before ultraviolet irradiation with two times the dose that caused minimal erythema. Specimens of treated skin were obtained 24 hours after irradiation, and collagenase, 92-kd gelatinase, and stromelysin activity was assessed by in situ zymography.

Collagenase activity in irradiated skin pretreated with the vehicle (Panel A) and in irradiated skin pretreated with tretinoin (Panel B) was measured with the use of fluorescein-labeled collagen as substrate. The specimens are from one subject and are representative of the findings in tissue from five subjects. Gelatinase activity in irradiated skin pretreated with the vehicle (Panel C) and in irradiated skin pretreated with tretinoin (Panel D) was measured with the use of fluorescein-labeled gelatin as substrate. The specimens are from one subject and are representative of the findings in tissue from five subjects. Stromelysin activity in irradiated skin pretreated with the vehicle (Panel E) and in irradiated skin pretreated with tretinoin (Panel F) was measured with the use of resorufin-labeled casein as substrate. The specimens are from one subject and are representative of the findings in tissue from five subjects. Darkened areas, especially noticeable in Panels A, C, and E, are due to the degradation of colored substrates. The white lines demarcate the boundary between the epidermis (top) and the dermis (bottom).
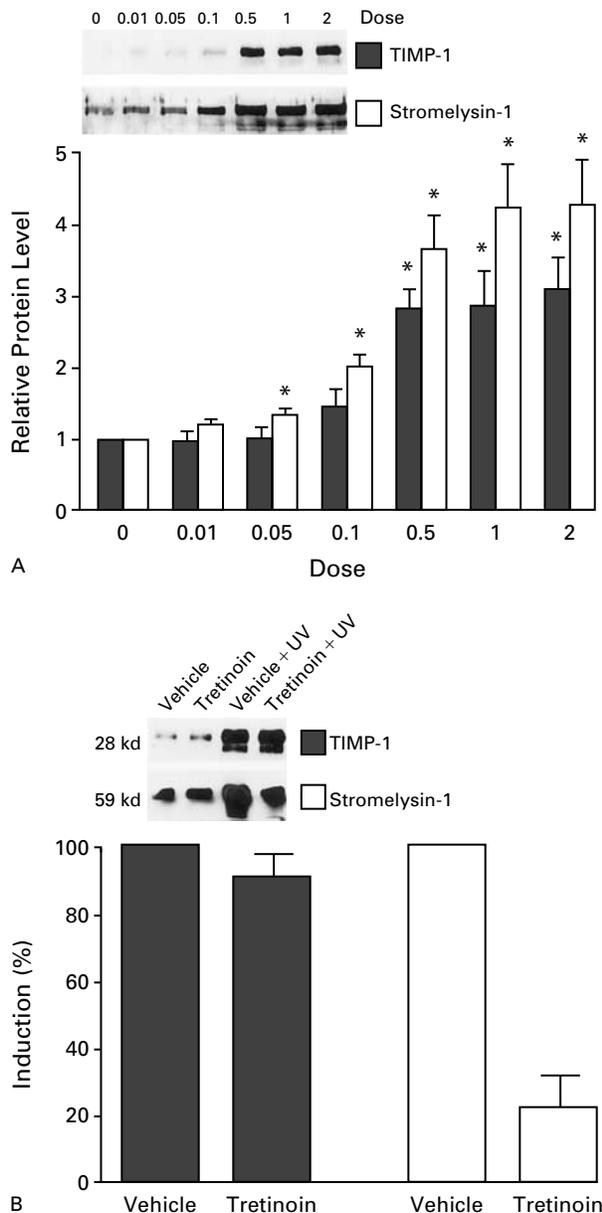
Figure 4. Effect of Ultraviolet Irradiation (UV) and Treatment with Tretinoin on the Induction of Tissue Inhibitor of Matrix Metalloproteinases-1 (TIMP-1) and Stromelysin-1.

Panel A shows the relation between the dose of ultraviolet irradiation and induction of TIMP-1 and stromelysin-1. The level of protein in untreated skin was assigned a value of 1. Levels measured after treatment are expressed in relation to that level. Each subject was irradiated on seven separate sites of the buttock with one of the indicated dose levels. One dose was defined as the amount of irradiation causing minimal erythema. Specimens of treated skin were obtained 24 hours after irradiation, and the levels of TIMP-1 (in specimens from three subjects) and stromelysin-1 (in specimens from nine subjects) were determined by Western blot analyses. Asterisks denote values that differ significantly from those in nonirradiated specimens (P<0.05). Three of the subjects were the same for the two analyses.

Panel B shows the effect of tretinoin on the induction of TIMP-1 and stromelysin-1 in irradiated skin. In each subject, separate sites were treated with the vehicle or 0.1 percent tretinoin 48 hours before irradiation at two times the dose causing minimal erythema. Specimens of treated skin were obtained 24 hours after irradiation and analyzed for TIMP-1 (in specimens from seven subjects) and stromelysin-1 (in specimens from nine subjects) by Western blot analyses. Three of the subjects were the same for the two analyses. The induction of stromelysin-1 was significantly decreased in specimens treated with tretinoin, as compared with those treated with the vehicle alone (P<0.001).

Bands from representative Western blot analyses are shown at the top of each panel. The values shown are means, with standard errors indicated by the bars.

evated in the dermis in skin exposed to ultraviolet irradiation.

Type I collagen fibrils are stabilized by intermolecular covalent cross-links,[34] which connect N- or C-terminal telopeptide domains to central triple helical domains on adjacent type I collagen molecules. Cross-linked telopeptides are released in soluble form after collagen has been broken down.[26,28] Levels of type I collagen C-terminal telopeptide cross-links were significantly higher in skin exposed to ultraviolet irradiation than in nonirradiated skin. Taken together, the results of in situ zymography and cross-linked telopeptide measurements demon-

strated that metalloproteinases induced by ultraviolet irradiation degrade dermal collagen in vivo.

Collagenase activity and 92-kd gelatinase activity, measured after four consecutive exposures to ultraviolet irradiation at 48-hour intervals, were induced to the same maximal level as that observed after a single exposure. Thus, there was no evidence of a lessened response over time. These studies, involving whites with light-to-moderate skin color, used a dose of ultraviolet irradiation that does not cause reddening of the skin. The dose used is equivalent to 5 to 15 minutes of exposure to noonday sun. In practical terms, this means that brief exposure to sun
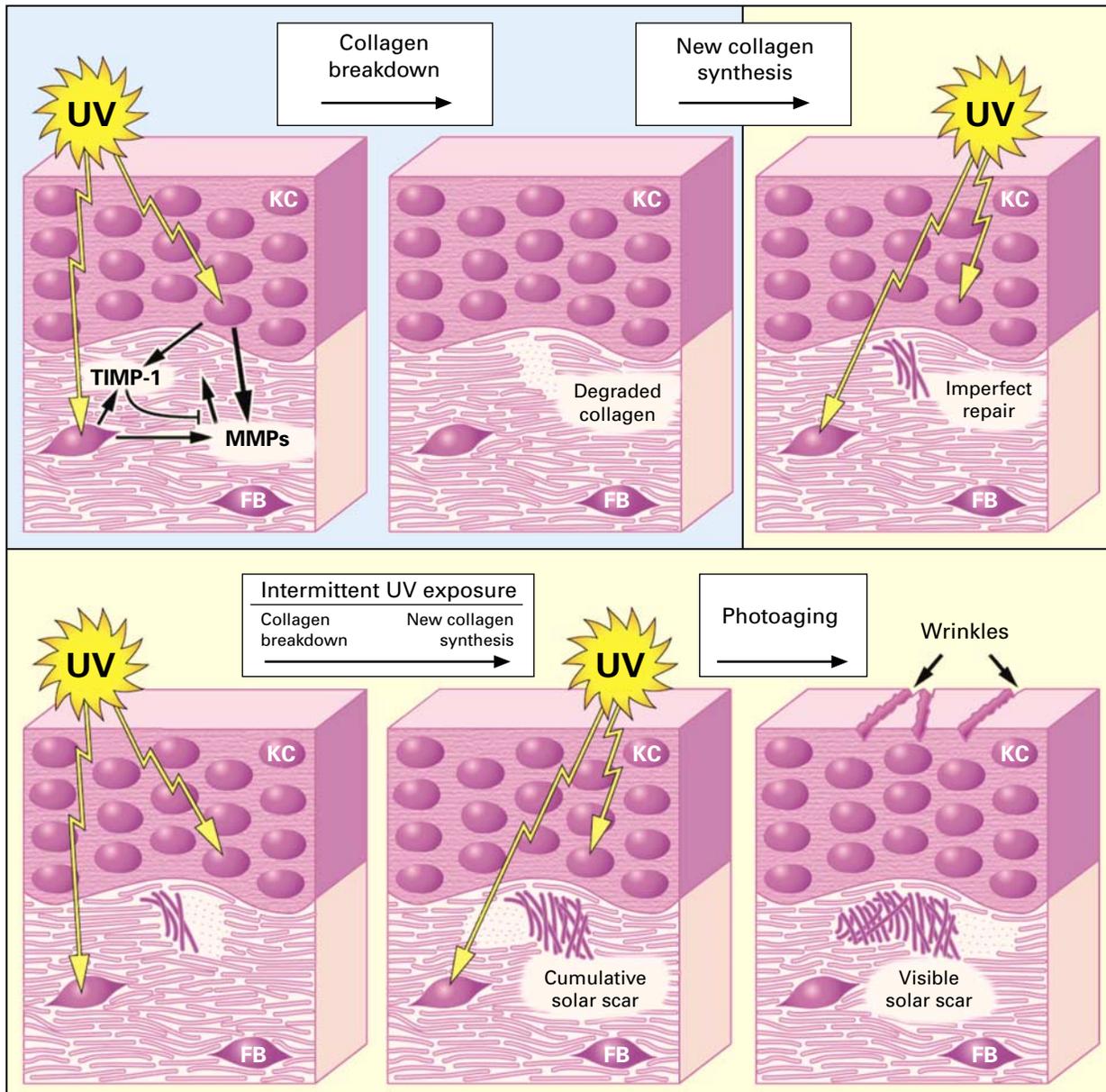
Figure 5. Hypothetical Model of the Pathophysiology of Dermal Damage and Photoaging Induced by Ultraviolet Irradiation.

Exposure to levels of ultraviolet (UV) light that cause no detectable sunburn induces the expression of matrix metalloproteinases (MMPs) in keratinocytes (KC) in the outer layers of skin, as well as fibroblasts (FB) in connective tissue; these metalloproteinases degrade collagen in the extracellular matrix of the dermis. The extent of matrix destruction is limited by the simultaneous induction of tissue inhibitor of matrix metalloproteinases-1 (TIMP-1), which partially inhibits the activity of matrix metalloproteinases. The breakdown of collagen is followed by synthesis and repair, which, as with all types of wound healing, is imperfect and leaves subtle, clinically undetectable deficits in the organization or composition of the extracellular matrix, or both. Matrix damage, followed by imperfect repair, occurs with each ensuing exposure to the sun, leading to the accumulation of altered matrix (solar scar) and, eventually, observable photoaging (wrinkles). The upper panels with the blue background depict the processes examined in our study, and the upper and lower panels with the yellow background depict hypothetical processes that are consistent with our results.

every other day should maintain elevated levels of matrix metalloproteinases, in the absence of skin reddening, in comparable persons. Although direct evidence is lacking, this mechanism may lead to persistent breakdown of skin connective tissue and accelerated premature skin aging.

Pretreatment of skin with tretinoin substantially inhibited the induction of collagenase, 92-kd gelatinase, and stromelysin-1, in both the epidermis and the dermis, in skin exposed to ultraviolet irradiation. The transcription of the genes for collagenase, 92-kd gelatinase, and stromelysin-1 is dependent on the transcription factor activating protein 1 (AP-1),[35-38] which is activated by ultraviolet irradiation and antagonized by tretinoin in human skin.[21] Pretreatment of skin with tretinoin did not inhibit induction of tissue inhibitor of matrix metalloproteinases-1. By inhibiting the induction of matrix metalloproteinases but not the induction of tissue inhibitor of matrix metalloproteinases-1, tretinoin alters their ratio in favor of the inhibition of matrix metalloproteinase. This may account for our observation that tretinoin substantially inhibited the induction of matrix metalloproteinase activities. The transcription of tissue inhibitor of matrix metalloproteinases-1, like that of the matrix metalloproteinases,[37-39] is regulated by AP-1.[19] The fact that tretinoin does not inhibit the induction of tissue inhibitor of matrix metalloproteinases-1 suggests that other transcription factors activated by ultraviolet irradiation, besides AP-1, stimulate the expression of tissue inhibitor of matrix metalloproteinases-1 in human skin. It is also possible that residual AP-1 activity in tretinoin-treated, ultraviolet-irradiated skin (approximately 30 percent of that in untreated skin[21]) may be sufficient for the full induction of tissue inhibitor of metalloproteinases-1 by ultraviolet irradiation.

Topical tretinoin improves the appearance of photoaged skin by reducing fine lines and wrinkles[40] and lightening brown spots.[41] Topical tretinoin preparations are available by prescription in the United States and several other countries for the treatment of visible photoaging. Our data suggest that tretinoin may also have a role in preventing the aspect of photoaging attributable to matrix metalloproteinase-mediated dermal damage (i.e., wrinkling). We used alcoholic solutions of tretinoin, not prescription products, in our study, and it was designed to test the concept that tretinoin may prevent photoaging rather than to assess its effectiveness in practice.

Whether topical tretinoin can in fact prevent photoaging must be determined by carefully controlled clinical trials. Such trials should also address the merits of topical tretinoin in relation to sunscreens, which are generally believed to provide protection against photoaging, and the widely held belief, based on anecdotal observations, that tretinoin increases the sensitivity of the skin to sun.

REFERENCES

1. Cooper KD, Oberhelman L, Hamilton TA, et al. UV exposure reduces immunization rates and promotes tolerance to epicutaneous antigens in humans: relationship to dose, CD1a-DR+ epidermal macrophage induction, and Langerhans cell depletion. Proc Natl Acad Sci U S A 1992;89:8497-501.
2. Weinstock MA. Epidemiology of nonmelanoma skin cancer: clinical issues, definitions, and classification. J Invest Dermatol 1994;102:4S-5S.
3. Kaminer MS. Photodamage: magnitude of the problem. In: Gilchrest BA, ed. Photodamage. Cambridge, Mass.: Blackwell Science, 1995:1-11.
4. Gilchrest BA, Yaar M. Ageing and photoageing of the skin: observations at the cellular and molecular level. Br J Dermatol 1992;127:Suppl 41:25-30.
5. Lavker RM. Cutaneous aging: chronologic versus photoaging. In: Gilchrest BA, ed. Photoaging. Cambridge, Mass.: Blackwell Science, 1995:123-35.
6. Smith JG Jr, Davidson EA, Sams WM Jr, Clark RD. Alterations in human dermal connective tissue with age and chronic sun damage. J Invest Dermatol 1962;39:347-50.
7. Warren R, Gartstein V, Kligman AM, Montagna W, Allendorf RA, Ridder GM. Age, sunlight, and facial skin: a histologic and quantitative study. J Am Acad Dermatol 1991;25:751-60. [Erratum, J Am Acad Dermatol 1992;26:558.]
8. Uitto J. Collagen. In: Fitzpatrick TB, Eisen AZ, Wolff K, Freedberg IM, Austen KF, eds. Dermatology in general medicine. 4th ed. Vol. 1. New York: McGraw-Hill, 1993:299-314.
9. Bernstein EF, Chen YQ, Kopp JB, et al. Long-term sun exposure alters the collagen of the papillary dermis: comparison of sun-protected and photoaged skin by Northern analysis, immunohistochemical staining, and confocal laser scanning microscopy. J Am Acad Dermatol 1996;34:209-18.
10. 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;105:285-90.
11. Yamauchi M, Prisayanh P, Haque Z, Woodley DT. Collagen cross-linking in sun-exposed and unexposed sites of aged human skin. J Invest Dermatol 1991;97:938-41.
12. Schwartz E, Cruickshank FA, Christensen CC, Perlish JS, Lebwohl M. Collagen alterations in chronically sun-damaged human skin. Photochem Photobiol 1993;58:841-4.
13. Calderone DC, Fenske NA. The clinical spectrum of actinic elastosis. J Am Acad Dermatol 1995;32:1016-24.
14. Griffiths CEM, 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;329:530-5.
15. Birkedal-Hansen H, Moore WG, Bodden MK, et al. Matrix metalloproteinases: a review. Crit Rev Oral Biol Med 1993;4:197-250.
16. Matrisian LM, Hogan BL. Growth factor-regulated proteases and extracellular matrix remodeling during mammalian development. Curr Top Dev Biol 1990;24:219-59.
17. Birkedal-Hansen H. Catabolism and turnover of collagens: collagenases. Methods Enzymol 1987;144:140-71.
18. Denhardt DT, Feng B, Edwards DR, Cocuzzi ET, Malyankar UM. Tissue inhibitor of metalloproteinases (TIMP, aka EPA): structure, control of expression and biological functions. Pharmacol Ther 1993;59:329-41.
19. Logan SK, Garabedian MJ, Campbell CE, Werb Z. Synergistic transcriptional activation of the tissue inhibitor of metalloproteinases-1 pro-

moter via functional interaction of AP-1 and Ets-1 transcription factors. J Biol Chem 1996;271:774-82.

20. Borden P, Heller RA. Transcriptional control of matrix metalloproteinases and the tissue inhibitors of matrix metalloproteinases. Crit Rev Eukaryot Gene Expr 1997;7:159-78.

21. Fisher GJ, Datta SC, Talwar HS, et al. Molecular basis of sun-induced premature skin ageing and retinoid antagonism. Nature 1996;379:335-9.

22. Fisher GJ, Esmann J, Griffiths CE, et al. Cellular, immunologic and biochemical characterization of topical retinoic acid-treated human skin. J Invest Dermatol 1991;96:699-707. [Erratum, J Invest Dermatol 1991; 96:814.]

23. Hibbs MS, Hasty KA, Seyer JM, Kang AH, Mainardi CL. Biochemical and immunological characterization of the secreted forms of human neutrophil gelatinase. J Biol Chem 1985;260:2493-500.

24. Fisher GJ, Reddy AP, Datta SC, et al. All-trans retinoic acid induces cellular retinol-binding protein in human skin in vivo. J Invest Dermatol 1995;105:80-6.

25. Galis ZS, Sukhova GK, Libby P. Microscopic localization of active proteases by in situ zymography: detection of matrix metalloproteinase activity in vascular tissue. FASEB J 1995;9:974-80.

26. Risteli J, Elomaa I, Niemi S, Novamo A, Risteli L. Radioimmunoassay for the pyridinoline cross-linked carboxy-terminal telopeptide of type I collagen: a new serum marker of bone collagen degradation. Clin Chem 1993;39:635-40.

27. Comparing more than two groups of observations: analysis of variance for comparing groups. In: Woolson RF. Statistical methods for the analysis of biomedical data. New York: John Wiley, 1987:314-63.

28. Autio P, Risteli J, Kiistala U, Risteli L, Karvonen J, Oikarinen A. Serum markers of collagen synthesis and degradation in skin diseases: altered levels in diseases with systemic manifestation and during systemic glucocorticoid treatment. Arch Dermatol Res 1993;285:322-7.

29. Kang S, Duell EA, Fisher GJ, et al. Application of retinol to human skin in vivo induces epidermal hyperplasia and cellular retinoid binding proteins characteristic of retinoic acid but without measurable retinoic acid levels or irritation. J Invest Dermatol 1995;105:549-56.

30. 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;269:32821-7.

31. Kazama T, Yaoita E, Ito M, Sato Y. Charge-selective permeability of dermo-epidermal junction: tracer studies with cationic and anionic ferritins. J Invest Dermatol 1988;91:560-5.

32. Okubo T, Sano S. Functional aspects of dermo-epidermal junction. Acta Derm Venereol Suppl (Stockh) 1973;73:121-8.

33. Liu X, Wu H, Byrne M, Jeffrey J, Krane S, Jaenisch R. A targeted mutation at the known collagenase cleavage site in mouse type I collagen impairs tissue remodeling. J Cell Biol 1995;130:227-37.

34. Woodley DT, Yamauchi M, Wynn KC, Mechanic G, Briggaman RA. Collagen telopeptides (cross-linking sites) play a role in collagen gel lattice contraction. J Invest Dermatol 1991;97:580-5.

35. Crawford HC, Matrisian LM. Mechanisms controlling the transcription of matrix metalloproteinase genes in normal and neoplastic cells. Enzyme Protein 1996;49:20-37.

36. Gack S, Vallon R, Schaper J, Ruther U, Angel P. Phenotypic alterations in fos-transgenic mice correlate with changes in Fos/Jun-dependent collagenase type I expression: regulation of mouse metalloproteinases by carcinogens, tumor promoters, cAMP, and Fos oncoprotein. J Biol Chem 1994;269:10363-9.

37. Quinones S, Buttice G, Kurkinen M. Promoter elements in the transcriptional activation of the human stromelysin-1 gene by the inflammatory cytokine, interleukin 1. Biochem J 1994;302:471-7.

38. Sato H, Seiki M. Regulatory mechanism of 92 kDa type IV collagenase gene expression which is associated with invasiveness of tumor cells. Oncogene 1993;8:395-405.

39. Tremble P, Damsky CH, Werb Z. Components of the nuclear signaling cascade that regulate collagenase gene expression in response to integrin-derived signals. J Cell Biol 1995;129:1707-20.

40. Ellis CN, Weiss JS, Hamilton TA, Headington JT, Zelickson AS, Voorhees JJ. Sustained improvement with prolonged topical tretinoin (retinoic acid) for photoaged skin. J Am Acad Dermatol 1990;23:629-37.

41. Rafal ES, Griffiths CEM, Ditre CM, et al. Topical tretinoin (retinoic acid) treatment for liver spots associated with photodamage. N Engl J Med 1992;326:368-74.

RECEIVE THE *JOURNAL*'S TABLE OF CONTENTS EACH WEEK BY E-MAIL

To receive the table of contents of the *New England Journal of Medicine* by e-mail every Thursday morning, send an e-mail message to:

listserv@massmed.org

Leave the subject line blank, and type the following as the body of your message:

subscribe TOC-L

You can also sign up through our website at: http://www.nejm.org

---