Accumulation of Matrilysin (MMP-7) and Macrophage Metalloelastase (MMP-12) in Actinic Damage

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Photodamage is characterized by degradation of collagen and accumulation of abnormal elastin in the superficial dermis and several matrix metalloproteinases have previously been implicated in this process. Using immunohistochemistry and in situ hybridization, we have studied the localization of two elastolytic matrix metalloproteinases, matrilysin (matrix metalloproteinase-7) and human macrophage metalloelastase (matrix metalloproteinase-12) in solar damage. Human macrophage metalloelastase protein was detected in the superficial dermis in areas of elastotic material. Matrix metalloproteinase-7 was seen in the mid-dermis in regions with less damaged elastic fibers and morphologically better preserved collagen as well as in a band-like pattern below basal keratinocytes in eight of 18 solar elastoses. In samples taken from healthy volunteers 3 d after repeated ultraviolet A exposure, however, basal keratinocytes were matrix metalloproteinase-7 immunopositive, explaining the linear immunostaining below basal keratinocytes noted particularly in ultraviolet B treated 3 d specimens. Upregulation of metalloelastase was also demonstrated in the skin of hairless mice after repeated ultraviolet exposure. In normal skin, no staining for human macrophage metalloelastase or matrix metalloproteinase-7 was observed in association with elastin. The amount of immunoreactivity for the substrates of matrix metalloproteinase-7, versican, and tenascin, was clearly increased in solar elastosis and photoprovoked skin; versican but not tenascin was detected in the same areas as matrix metalloproteinase-7. Our results suggest that both matrix metalloproteinase-7 and -12 may contribute to remodeling of elastotic areas in sun-damaged skin. Key words: elastin/keratosis/tenascin/versican. J Invest Dermatol 113:664–672, 1999

Repeated exposure of skin to solar light leads to dermal photoaging characterized by alterations in the composition of the dermal matrix such as increase in the amounts of elastin, fibrillin, and versican (Bernstein et al, 1994, 1995a) and reduced amounts of type I collagen and decorin (Bernstein et al, 1995a, 1996). The matrix metalloproteinase (MMP) family consists of at least 16 enzymes that can synergistically digest all matrix macromolecules (Birkedal-Hansen et al, 1993; Llano et al, 1997; Pendas et al, 1997). Certain members of this enzyme family have been suggested to have a role in dermal photoaging. Ultraviolet (UV) B induces expression of MMP-1, -3, and -9 in normal human epidermis in vivo (Koivukangas et al, 1994; Fisher et al, 1996). UVA induces expression of MMP-1 by dermal fibroblasts in vivo and expression of MMP-1, -2, and -3 in culture (Scharffetter et al, 1991) and it may well be that these MMP take part in the degradation of type I collagen seen in chronically photoaged skin (Bernstein et al, 1996; Fisher and Voorhees, 1998).

Human macrophage metalloelastase (HME, MMP-12) is an MMP that was initially found in alveolar macrophages of cigarette smokers (Shapiro et al, 1993). On a molar basis, it is clearly the most active MMP against elastin (Shapiro and Senior, 1998). HME has a broad substrate specificity, however, being able to degrade also type IV collagen, laminin, fibronec tin, vitronectin, entactin, and heparan and chondroitin sulfates (Chandler et al, 1996; Gronski et al, 1997). In vivo HME has been shown to participate in the degradation of elastic fibers in the pathogenesis of atherosclerosis and in emphysema (Halpert et al, 1996; Hautamäki et al, 1997). HME mRNA and protein are also expressed by accumulations of macrophages in granulomatous skin disorders (Vaalamo et al, 1999).

In addition to degrading elastic tissue, metalloelastase has been shown to aid macrophage migration in other tissues; macrophages from metalloelastase deficient (−/−) mice are unable to penetrate reconstituted basement membranes (BM) in vivo and in vitro (Shipley et al, 1996; Gronski et al, 1997). Our recent findings on HME expression in macrophages under the shedding intestinal epithelium in inflammatory bowel diseases support its role in the degradation of BM components (Vaalamo et al, 1998). Furthermore, HME
expression may also be associated with macrophage migration through BM in certain inflammatory skin diseases such as dermatitis herpetiformis or pityriasis lichenoides (Vaalamo et al, 1998).

Matrilysin (MMP-7) is the smallest of the MMP lacking the hemopexin domain. It is frequently produced by glandular epithelial cells in different organs (Saraihlo-Kere et al, 1995) and is often overexpressed in epithelial tumor cells of cancers of various origins (Nagashima et al, 1997). Furthermore, it has recently been shown to function in the repair of intestinal and airways epithelium (Saraihlo-Kere et al, 1996; Dunsmore et al, 1998). MMP-7 is also produced by blood monocytes (Busiek et al, 1995) and, at least in atherosclerotic lesions, by tissue macrophages (Halpert et al, 1996). MMP-7 has a very broad substrate specificity in vitro being able to degrade insoluble elastin, fibronectin, gelatins, type IV collagen, laminin-1, entactin, fibulins, vitronectin, and aggrecan (Murphy et al, 1991). Like HME, it can cleave plasminogen and generate angiostatin molecules (Patterson and Sang, 1997). Its substrates also include two proteoglycans, tenascin and versican, the latter of which is also efficiently degraded in vivo by MMP-7 secreted by lipid-laden macrophages of atherosclerotic lesions (Halpert et al, 1996). In diseased skin, expression of MMP-7 has only been detected in benign sweat gland tumors (Saraihlo-Kere et al, 1995) and aggressive basal and squamous cell carcinomas (Karelina et al, 1996). No previous reports exist on the effects of UV light on the regulation of this protease or of versican.

Long-term sun exposure results in wrinkled, inelastic skin, the histologic examination of which reveals loss of collagen with concomitant accumulation of elastotic material. As photoaging is also associated with changes in elastic tissue, we decided to study whether two elastolytic MMP, MMP-7 and −12, are expressed in solar elastosis and keratosis and demonstrate here increased immunoreactivity for these proteins in abnormal elastic fibers of chronically photoaged skin. We also show that the putative substrate for MMP-7, tenasin, is upregulated in solar damage, but does not colocalize with MMP-7 protein unlike versican.

MATERIALS AND METHODS

Tissues Formalin-fixed, paraffin-embedded specimens of solar elastosis (n = 18, mean age 74 y, head and arm), solar keratosis (n = 8, mean age 67 y, head and ankle), anetoderma (n = 5, mean age 33 y, back and thigh), and mid-dermal elastolysis (n = 5, mean age 31 y, abdomen, arm) were obtained from the Department of Dermatopathology, University of Helsinki. Several samples of normal skin from two groups of adults (n = 7, mean age 23 y, back, buttock, and neck; n = 7, mean age 72 y, abdomen, back, and face) and children under 5 y of age (n = 3; neck, back, and face) that were photoaged were also examined.

Eight healthy individuals, aged 23–44 y, volunteered for photoprovocation and skin biopsies. The photoprovocation was performed during winter months as previously described (Hasan et al, 1997) with UVASUN (emission spectrum 340–400 nm) and with Philips (Eindhoven, The Netherlands) TL 20W/12 (emission spectrum 280–370 nm) light sources. Briefly, a 5 × 8 cm area of intact back skin was irradiated on 3 consecutive days with 100 J/cm2 total irradiation except for MMP-7 and versican. Skin biopsies were obtained from six volunteers 3–5 d after the last repeated provocation, fixed in formalin, and embedded in paraffin. Two subjects were biopsied 24 h after a single provocation and the samples were snap-frozen immediately in liquid nitrogen and stored at 70°C until analysis. The study protocol was approved by the Ethical Committees of Oulu and Tampere University Hospitals.

SKH-1 hairless mice were exposed to the sun spectrum of 90% UVA and 10% UVB radiation as previously described (Starcher et al, 1999). Briefly, groups of five mice were exposed for 30 s three times a week with 0.09 J per cm2 of total irradiation (equivalent to about 1 minimal erythema dose). As previously described only occasional immunohistochemistry. In situ hybridization was performed on sections serial to those used for in situ hybridization using Weigert’s elstanin stain (Weigert’s Resorcin-Fuchsin). Morphologic damage to collagen was assessed by van Gieson’s staining.

RESULTS

Solar elastosis Solar elastosis manifests sunlight-induced damage of the elastic fibers in the upper and mid-dermis. Immunostaining for HME was clearly seen in all the 18 samples examined within the fragmented, distorted, and thickened elastic fibers of the upper dermis staining abnormally with Weigert’s elstanin stain (Fig 1a, d). MMP-7 protein was detected in thinner, but also histologically abnormal-looking elastic fibers, located deeper towards mid-dermis (Fig 1b, g). Van Gieson staining done in eight samples revealed that the morphology of collagen was better preserved in MMP-7 than HME-positive areas (data not shown). In eight of 18 samples band-like staining for MMP-7 under the BM was also evident (Fig 1e). Furthermore, staining was occasionally detected in hair follicles (Fig 1e) in accordance with the data of Karelina et al (1994). Immunoreactivity for the MMP-7 substrate, versican, was detected in large areas of the upper and mid-dermis partly colocalizing with MMP-7-positive regions (Fig 1f, g). Versican was only detected as a linear band under the BM, but not at any elastotic areas of the upper dermis (Fig 1h). In contrast to immunostaining for MMP-7 and −12, elastic areas did not show MMP-9 immunoreactivity (data not shown).

Solar keratosis In solar keratosis the upper dermis shows actinically damaged fibers whereas keratinocytes manifest with variable degrees of atypia. The pattern of immunostaining for HME and MMP-7 was very similar compared with that in the specimens of solar elastosis. HME was detected in the upper dermis in areas of damaged and fragmented or amorphous elastic fibers (Fig 2a, g), whereas MMP-7 was seen in areas of less pronounced clumping and thickening of elastin in the mid-dermis (Fig 2c, d). In contrast to findings in solar elastosis, however, only one of eight samples demonstrated staining for MMP-7 under the BM (Fig 2c, e). In situ hybridization experiments did not reveal any positive epidermal or dermal cells for HME or MMP-7 in either elastosis or keratosis (Fig 2f); as previously described only occasional

containing 0.25% acetic anhydride. After hybridization at 50°C or 55°C, the slides were washed under stringent conditions, including treatment with RNase A to remove unhybridized probe. Following 30–40 d of autoradiographic exposure, the photographic emulsion was developed, and the sections were stained with hematoxylin and eosin. The sections chosen for presentation in this report were exposed for 30–35 d. Samples previously positive for HME and MMP-7 (breast carcinomas) were used as positive controls in each experiment and each specimen was processed in at least two experiments.

Antibodies HME was immunostained using affinity-purified rabbit polyclonal antibodies (Shapiro et al, 1993; Belaouaj et al, 1995; Curci et al, 1998). Matrilysin protein was detected by using affinity-purified antibodies raised in rabbits against a synthetic peptide corresponding to amino acids 93–108 (Busiek et al, 1992; Saraihlo-Kere et al, 1995). A third elastolytic MMP, 92 kDa gelatine MMP-9), was immunolocalized as described previously (Vaalamo et al, 1999). Versican and tenasin were immunolocalized by using mouse monoclonal antibodies (no. 270428, Seikagaku, Tokyo, Japan; no. 1927, Chemicon, Temecula, CA, respectively). Controls were performed with normal mouse immunoglobulins or with rabbit preimmune serum.

Immunohistochemistry This was performed on sections serial to those used for in situ hybridization. Anti-HME antibodies were diluted 1:200; MMP-7 1:800, MMP-9 1:500, versican 1:500, and tenasin 1:1000. The peroxidase–antiperoxidase technique was applied using dianisobenzidine as a chromogenic substrate and Harris hematoxylin as counterstain, as described in detail (Saraihlo-Kere et al, 1993). Sections were pretreated with 10 mg/ml trypsin except for MMP-7 and versican. MMP-7 immunoanalysis was also performed on acetone-fixed frozen sections and the antibody was diluted 1:1000 and reacted at 4°C overnight. These results were confirmed with the commercial MMP-7 antibody (IM40L, Calbiochem, Cambridge, MA) diluted 1:50.

Elastin and collagen staining Staining for revealing elastic fibers was performed on sections serial to those used for in situ hybridization using Weigert’s elstanin stain (Weigert’s Resorcin-Fuchsin). Morphologic damage to collagen was assessed by van Gieson’s staining.

In situ hybridization The production and specificity of the 650 bp HME, 800 bp HME, and 800 bp matrilysin probes have been described previously (McDonnell et al, 1991; Shapiro et al, 1992; Vaalamo et al, 1998). In situ hybridization was performed on 4 μm sections as described in detail (Prosser et al, 1989; Saraihlo-Kere et al, 1993). All samples were treated with proteinase K and were washed in 0.1 M trisethanamine buffer
Figure 1. HME and MMP-7 protein are detected in damaged elastin fibers of solar elastosis. (a) Immunostaining for HME in superficial dermis of a sample of solar elastosis. (b) Immunostaining for MMP-7 in the mid-dermis of the same specimen. (c) Higher magnification of the MMP-7 positive area in b. (d) Serial section stained for elastin. Arrows depict corresponding areas. (e) Immunostaining for MMP-7 under epidermis and in a hair follicle in another sample of solar elastosis. (f) Immunostaining for versican in solar elastosis. (g) Serial section immunostained for MMP-7. (h) Immunostaining for tenascin in solar elastosis. Counterstaining with hematoxylin (all panels except d). Scale bars: (a, b, d-h) 40 µm; (c) 20 µm.

Sweat gland cells were positive for MMP-7 (Saarialho-Kere et al, 1995) (Fig 2e). Staining for versican partly colocalized both with HME and MMP-7 (Fig 2a, b, c). As in solar elastosis, tenascin was detected as a continuous line under the BM, but not in the dermis and it did not essentially colocalize with MMP-7 immunoreactivity (Fig 2e, f).
Figure 2. HME and MMP-7 proteins are detected in different areas of the dermis in actinic keratosis. (a) Immunostaining for HME in the superficial dermis of a sample of actinic keratosis. (b) Immunostaining for versican in the same sample. (c) Immunostaining for MMP-7 in the mid-dermis of the same specimen. Arrows depict corresponding areas. (d) Higher magnification of the MMP-7 positive area in (c). (e) Immunostaining for MMP-7 in another specimen of actinic keratosis. (f) Immunostaining for tenascin in the same sample. (g) Higher magnification of an HME positive area in a. (h) Control section for HME processed with rabbit preimmune serum. (i) In situ hybridization dark-field for HME mRNA of the same specimen. Counterstaining with hematoxylin and eosin (i) or with hematoxylin (a–h). Scale bars: (a–c, e, f) 200 µm; (d, g–i) 20 µm.

UVA/UVB exposed human skin The skin of healthy volunteers was biopsied 3 d after either UVA or UVB provocation (see Materials and Methods). Histologically slightly dilated capillaries, increase in the number of lymphocytes and melanocytes as well as parakeratosis were frequently detected. Particularly in UVB-irradiated skin shrinkage of keratinocytes was seen. In situ hybridization (data not shown) revealed no HME expression after our acute UVA or UVB provocation. Immunosignal was detected in a few stromal fibroblast/macrophage-like cells, but not in the epidermis (Fig 3a, b). Sweat gland epithelium showed MMP-7 mRNA and immunoreactivity, but no positive stromal cells were detected in the samples (Fig 3c–e, inset e). The intensity of staining under
BM, however, clearly increased after the UVB provocation compared with the MMP-7 immunoreactivity of samples obtained after UVA irradiation (Fig 3c, d, g, k). This could be explained by the finding of MMP-7 immunopositive basal keratinocytes in two samples of frozen tissue obtained 1 d after UVB exposure (Fig 3f).

Versican was demonstrated in the same area as MMP-7 under the BM (Fig 3g, h, j). The area of positive immunostaining for tenascin clearly increased after UVB compared with UVA provocation (Fig 3l, m).

**Figure 3.** HME and MMP-7 expression in UVA/UVB treated skin of healthy volunteers. (a) Immunostaining for HME in UVA-treated skin. (b) Higher magnification from the dermis of the same specimen. Arrows depict HME-positive cells. (c) Immunostaining for MMP-7 in a UVB-treated specimen. (d) Immunostaining for MMP-7 after UVB provocation in a sample from the same person. (e) In situ hybridization dark-field for MMP-7 mRNA in UVB-treated skin of another volunteer. (inset e’) Signal for MMP-7 mRNA in sweat glands. (f) Immunostaining for MMP-7 in frozen tissue specimen obtained 1 d after UVB exposure. (g) UVB-treated skin from the volunteer in (e) stained with MMP-7 antibodies. (h) Immunostaining for versican in the same specimen. (i) A nearby section immunostained with MMP-7 preimmune serum. (j) Immunostaining for versican in UVA-treated skin. (k) Immunostaining for MMP-7 and for tenascin (l) in the same specimen. (m) Immunostaining for tenascin in a UVB-treated sample from the same volunteer as (l). Counterstaining was performed with hematoxylin and eosin (e) or with hematoxylin (a-d, f–m). Scale bar: (a–h) 40 μm; (a, f) 20 μm; (b) 10 μm.

**Figure 4.** MMP-7, versican and tenascin in normal control skin. (a) Immunostaining for MMP-7 at the control back skin of a 2 y old child. (b) Staining for elastic fibers. (c) Staining with MMP-7 preimmune serum. (d) Immunostaining for MMP-7 in the control buttock skin of a 23 y old male. (e) Staining for elastin in the same specimen. (f) Immunostaining for versican in (d). (g) Immunostaining for tenascin in the same specimen. Arrows depict corresponding areas. Counterstaining with hematoxylin (a, c, d, f–h). Scale bar: (a–h) 40 μm.

**UV-irradiated hairless mouse skin**  The hairless SKH-1 mouse has long been an experimental model to study the effects of chronic low-dose UVA and UVB irradiation on the histologic changes that occur during solar damage (see Starcher et al., 1999). MME-positive stromal cells were infrequently detected in the mid-dermis of age-matched non-UV-irradiated control mice (Fig 5A). In samples taken 12 d after starting repeated UV administration, signal for MME was seen more frequently in the stromal cells of the mid-dermis in the vicinity of dermal cysts characteristic of the hairless
mice (Fig 5B). Abundant signal for MME was further observed in biopsies obtained 8 and 11 wk after starting repeated UV irradiation. Signal was not detected in the epidermis or the epithelium of keratinizing cysts but in plump (Fig 5A) macrophage-like or spindle fibroblast-like (Fig 5C, D) stromal cells.

Normal skin Seventeen samples of normal skin from different parts of the body and patients of different ages (see Materials and Methods) were examined. HME positive areas/cells were not detected either by immunohistochemistry or in situ hybridization. Staining for MMP-7 in sweat glands and the luminal surface of sweat ducts was evident in all the samples. Fibroblast-like staining below basal keratinocytes was seen particularly in the samples of young adults (buttock, back) (Fig 4d) and it became fainter in the specimens of children (Fig 4a) and controls over 70 y of age. In normal skin, staining for versican (Fig 4f) and elastin (Fig 4b, c) was detected in MMP-7-positive areas under the BM. Tenascin was seen as a partly interrupted line immediately below basal keratinocytes in the papillary dermis, not co-localizing with MMP-7 (Fig 4d, g).

Neither HME nor MMP-7 proteins were detected in anetoderma or mid-dermal elastolysis (data not shown). MMP-7 immunoreactivity was seen in occasional sweat glands but no staining was ever seen under the BM in these disorders. No immunoreactivity was detected with control mouse immunoglobulins or with rabbit preimmune serum (Figs 2h, 3i, and 4c).

DISCUSSION

Development of a solar scar due to repeated exposure to sunlight may be mediated by either direct induction of dermal fibroblast MMP expression by UVA or induction of MMP by UVB in epidermal keratinocytes. Latent MMP-1 derived from keratinocytes or dermal fibroblasts could be maximally activated by MMP-3 and ultimately initiate degradation of collagen types I and III. Simultaneous expression of MMP-2, -3, and -9 could lead to degradation of noncollagenous extracellular matrix, including BM and proteoglycans (see Kähäri and Saarialho-Kere, 1997; Fisher and Voorhees, 1998). In this paper we report that two additional MMP, HME, and MMP-7, may also participate in the pathobiology of solar damage.

Histologically, photoaging causes accumulation of so-called elastotic material, composed of elastin and versican (Bernstein et al, 1995a, b), in the upper and mid-dermis. This is accompanied by degeneration of surrounding collagenous meshwork, but due to the inability of HME and MMP-7 to degrade fibrillar collagens, they probably do not participate in that event. We found abundant immunostaining for HME in the areas of abnormal elastic fibers in both solar elastosis and keratosis. This corresponds the upper dermal region, where UV-induced damage can be expected to be most prominent. While this study was in progress, Curci et al (1998) reported that in selected regions of abdominal aortic aneurysms, HME protein localizes to residual elastic fiber fragments. In accordance with their negative results in intact elastin fibers, we did not find any immunoreactivity for HME in normal skin, anetoderma, or mid-dermal elastolysis, suggesting that HME does not bind to normal elastin or that in these conditions no abnormal accumulations of macrophages exist, as possible sources of HME.

It has been postulated that the elastotic material accumulating in photoaged skin results from direct UV-mediated damage to elastic fibers and fibroblasts (Bernstein et al, 1994). HME possibly takes part in a reparative remodeling process by trying to cleave this abnormal elastotic material or fibrillin (Ashworth et al, 1999). Granulocyte-macrophage colony-stimulating factor, induced at least in keratinocytes by UV light (Schwarz and Lugner, 1989), is able to upregulate HME production by macrophages (Kumar et al, 1996) and could thus be one of the candidate cytokines to stimulate HME expression in solar damage. UV irradiation causing abnormal changes in elastin might lead to accumulation of macrophages that try to cleave both abnormal elastin by secreting elastases as well as activate elastin/collagen synthesis by releasing, e.g., transforming growth factor (TGF)-β (Quaglino et al, 1990). Interestingly, abundant staining for latent TGF-β binding protein-1 and TGF-β colocalize with HME staining in solar elastosis and keratosis (Karonen et al, 1997). TGF-β is not likely to upregulate HME expression, as it usually downregulates MMP function and at least in mouse peritoneal macrophages, TGF-β is not able to induce metalloelastase (Kumar et al, 1996). We cannot, however, exclude that HME participates in the proteolytic release of this growth factor from the extracellular matrix. TGF-β could further augment the deposition of abnormal elastin, which is unable to assemble into functional elastic fibers due to the influence of proteolytic enzymes and UV radiation.

Staining for MMP-7 protein was detected deeper in the dermis than that for HME. The elastic bundles were less thickened and distorted and collagen better preserved in these areas than in superficial dermis of sun-damaged skin. MMP-7 is only rarely made by fibroblasts or stromal cells, and thus dermal macrophages would be the most natural source of this enzyme (Busiek et al, 1995; Halpert et al, 1996). MMP-7 can be induced by interleukin-1β and activated by stromelysin-1 (Wilson and Matrisian, 1998),
which both may be induced by UVB light. Both as an elastase and an inactivator of leukocyte elastase inhibitors, MMP-7 may enhance elastin degradation in photodamaged skin (Wilson and Matrisian, 1998). In vitro MMP-7 is also able to degrade decorin, a small proteoglycan binding to collagen (see Bernstein et al., 1995a). Incubation of decorin–TGFB complex with MMP-7 results in release of TGFB from this complex (Imai et al., 1997) and, thus, MMP-7 might contribute to TGFB activation.

No in situ expression for HME or MMP-7 mRNA was detected in fibroblasts or inflammatory cells in the specimens of solar elastosis and keratosis, although there was abundant immunostaining in the matrix. The MMP are usually not constitutively expressed but upregulated in response to specific signals; in general they are secreted as soon as they are synthesized. The absence of these mRNA would suggest that dermal macrophages secrete these enzymes over a long period of time and that the proteins accumulate after years of exposures of skin to UV light. Besides, it is rather typical for MMP-7 expression in vivo that the protein is detected more frequently than MMP-7 mRNA (Saarialho-Kere et al., 1995; Dunsmore et al., 1998).

Staining for MMP-7 in a rather faint fibril-like pattern was localized in normal skin in the area of subepidermal elastin arcades in different parts of the body (Fig 4a, d). This staining became stronger and band-like if it was seen in samples of solar elastosis and keratosis (Fig 4d). The immunoreactivity was always more intense after acute UVB than UVA exposure. No MMP-7 mRNA or protein was detected in keratinocytes above this staining in samples collected 3 d after UV exposure. In frozen tissue taken 1 d after irradiation, however, the basal keratinocytes were repeatedly positive. This discrepancy may reflect either different sensitivity of the antibodies when working on frozen tissue versus paraffin embedded or true time–dependent gene regulation. MMP-7 is able to cleave selectively the β4-integrin present as α6β4 in hemidesmosomes at the basal side of keratinocytes (von Bredow et al., 1997) and, thus, degradative changes occurring after UV exposure at the BM zone could result from its presence.

Co-localization of versican and elastin has been shown previously (Zimmerman et al., 1994) as well as the upregulation of versican in photoaged skin (Bernstein et al., 1995a). We found positive immunostaining for versican and MMP-7 in the same areas of the mid-dermis both in solar elastosis and keratosis. Furthermore, staining for versican and MMP-7 was very similar in the papillary layer of normal skin (Fig 4d, f). Versican is associated with oxytalan, elastin, and elastic fibers and it is possible that MMP-7 and elastin might have interactions.

We were not able to detect substantial mRNA expression for HME or MMP-7 in biopsies taken 1 d after acute UVA/UVB provocation. Occasional HME-immunopositive spindle fibroblast/macrophage-like cells, however, were detected in the upper dermis. Agreeing with our data, Chung et al.1 very recently published a preliminary report on the induction of HME in UV-irradiated human skin. Based on their northern analysis findings that UV can induce HME also in fibroblast cultures, we cannot exclude this cell type as a possible source of HME also in our samples. Based on the structure of HME and MMP-7 promoter regions, UV-activated AP-1 can stimulate transcription of both of them. Maximal induction of AP-1 occurs after 1 min erythema dose (Fisher et al., 1996), which is actually less than the dose given by us. A single UVB exposure can cause acute induction of MMP which returns to basal levels 48–72 h thereafter. The induction of at least MMP-1, -2, and -9 is mediated by MAP-kinases, which are activated within 1 h after UV irradiation, maximally active at 4 h and return to baseline at 24 h (see Fisher and Voorhees, 1998). We cannot exclude the possibility that had we obtained paraffin-embedded tissue from volunteers biopsied 1 d instead of 3 d after photoprovocation, we would have seen increased in situ expression of HME or MMP-7 mRNA in the dermis. In any case our data suggests that long-term exposure of skin to sunlight is needed to upregulate these MMP in the dermis.

Upregulation of metalloelastase was demonstrated in UV-irradiated hairless mice skin. Repeated treatment of this mouse strain with UVB results in clinical and histologic changes similar to those observed in photodamaged human skin and in this model there is coexistence of an inflammatory infiltrate and elastosis (Kligman et al., 1982; Schwartz, 1988). Compared with human skin, however, there are differences in the amount and distribution of elastin, characteristics of the papillary dermis and mid-dermis (keratinizing cysts) that warrant caution when extrapolating results between species. Most of the signal for MME was detected in mid-dermis, where increased collagen mRNA levels and presence of inflammatory cells, particularly after some weeks of repeated UV treatment, were reported in a previous study (Starcher et al., 1999). UV irradiation is known to cause elastotic changes in the deep dermis and to enhance granulomatous reaction near dermal cysts in SKH-1 mice (Kligman et al., 1982). Our results suggest that particularly UV-induced tissue remodeling gives rise to MME production in macrophage/fibroblast-like cells.

In this work we showed, how tenascin was variably present in the BM region of normal skin and how its amount is increased in solar keratos and elastosis as well as UVA/UVB irradiated skin. This is in agreement with previous data on tenasin in solar keratos (see Tuominen et al., 1997) and with the very recent paper of Filsell et al. (1999) in samples of solar elastosis. Although MMP-7 is able to degrade tenasin at least in vitro, the distinct localizations of these two proteins in our in vivo samples, do not substantiate such an interaction in solar damage.

In conclusion, we demonstrate here for the first time that MMP-12 is present in abnormal elastic fibers in chronically sun-damaged skin. Furthermore, MMP-7 can be found in the BM zone and deeper in the dermis associated with areas of moderate actinic damage. Thus, these MMP may play an important part in remodeling events occurring in the dermal connective tissue during long-term exposure to sunlight.

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