



Published in final edited form as:

J Invest Dermatol. 2013 May ; 133(5): 1362–1366. doi:10.1038/jid.2012.509.

Elevated Matrix Metalloproteinases and Collagen Fragmentation in Photodamaged Human Skin: Impact of Altered Extracellular Matrix Microenvironment on Dermal Fibroblast Function

Taihao Quan, Emily Little, Hehui Quan, John J. Voorhees, and Gary J. Fisher

Department of Dermatology, University of Michigan Medical School, Ann Arbor, Michigan, USA

Taihao Quan: thquan@umich.edu; Gary J. Fisher: gjfisher@umich.edu

Exposure of the human skin to acute solar ultraviolet (UV) irradiation induces several members of the matrix metalloproteinase family (MMPs), which degrade collagen fibrils and other components of the dermal extracellular matrix (ECM) (Fisher et al., 1996; Yaar and Gilchrist, 2007). Elevation of MMPs due to chronic sun exposure can promote accumulation of dermal ECM fragmentation, which contributes to impaired skin function and aged appearance of chronically sun-exposed skin (Fisher et al., 1997). Histological and ultrastructural studies have revealed that major alterations in dermal extracellular matrix, most notably the accumulation of amorphous elastin-containing material in the upper dermis, and disorganized collagen fibrils, which constitute the bulk (90% dry weight) of skin connective tissue.

Elevation of MMPs and consequent dermal collagen fibril fragmentation following acute UV irradiation in human skin is well characterized (Fisher et al., 1996; Quan et al., 2009). However, expression of MMPs in clinically-photodamaged human skin and the role of fragmented collagen microenvironment in cellular function have not been extensively studied. Here we quantified gene expression of all 23 known mammalian MMPs (Egeblad and Werb, 2002) in photodamaged forearm and subject-matched sun-protected underarm skin, from 19 individuals, and the effect of collagen fragmentation on dermal fibroblast function, the major cells responsible for collagen homeostasis in skin.

The relative expression levels of MMPs in underarm skin are shown in Figure 1a. Transcripts for MMP-8, -10, -13, -20, and -26 were not detected. MMP-14, -2, -3, -28, -7, and -15 were the most highly expressed, while remaining MMPs were expressed at lower levels. Among the 18 MMPs expressed in human skin, seven were significantly elevated in photodamaged forearm, compared to sun-protected underarm skin (fold-increase from high to low: MMP-9, 5.3-fold; MMP-27, 5.1-fold; MMP-3, 3.0-fold; MMP-11, 2.7-fold; MMP-17, 2.2-fold; MMP-1, 1.9-fold; MMP-2, 1.6-fold) (Figure 1b). To quantify the relative contributions to elevated MMPs, epidermis and dermis were separated by laser capture microdissection (LCM). Figure 1c shows that all MMPs that are elevated in photodamaged

Users may view, print, copy, and download text and data-mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use:http://www.nature.com/authors/editorial_policies/license.html#terms

CONFLICT OF INTEREST

The authors state no conflict of interest.

skin, except MMP-3, were primarily expressed in the dermis (MMP-27, 86%; MMP-9, 85%; MMP-2, 83%; MMP-11, 82%; MMP-17, 62%; MMP-1, 59%).

MMPs and TIMPs are often coordinately regulated as a means to control excess MMP activity, we also investigated whether TIMPs are elevated in photodamaged skin. We found that all four known TIMP genes (TIMP-1, -2, -3, and -4) are primarily expressed in human skin dermis, however, no differences in mRNA levels of TIMP-1, -2, -3, or -4 were found between sun-protected underarm and sun-exposed forearm skin (Figure 1d).

The observed preferential induction of MMPs relative to TIMPs suggests that MMP activities are elevated in photodamaged skin. To assess MMP activity, we performed *in situ* zymography, in which unfixed skin sections are placed over a layer of fluorescently-labeled collagen. As shown in figure 1e, elevated MMP activity in photodamaged skin resulted in breakdown of the collagen, resulting in loss of fluorescence. In addition, production of type I collagen, the major structural protein in skin, was significantly reduced in LCM-captured photodamaged forearm dermis, compared to sun-protected underarm dermis (Figures 1f and 1g). These data demonstrate aberrant collagen homeostasis, i.e. increased multiple MMPs and reduced collagen production, in photodamaged dermis.

Given that dermal fibroblasts are primarily responsible for collagen production and turnover *in vivo*, data presented above suggest that impaired dermal fibroblast function contributes to aberrant collagen homeostasis in photodamaged skin. We have previously reported that in standard monolayer culture, collagen and MMP-1 expression in fibroblasts from photodamaged and sun-protected skin are similar (Varani et al., 2001), suggesting that the fragmented extracellular microenvironment within photodamaged dermis may trigger abnormal fibroblast function.

To examine this possibility, we cultured dermal fibroblasts in intact or fragmented three dimensional (3D) collagen lattices to model sun-protected and sun-exposed dermis, respectively. Collagen lattices were fragmented by controlled exposure to purified human MMP-1 (Fisher et al., 2009). Atomic force microscopy (AFM) indicated that intact (Figure 2a, upper left panel) and MMP-1-fragmented (Figure 2a, upper right panel) collagen lattices resemble collagen fibrils in sun-protected underarm (Figure 2a, lower left panel) and sun-exposed forearm (Figure 2a, lower right panel), respectively. Measurement of all 23 known mammalian MMPs indicated that transcripts for MMP-13, MMP-20 and -26, which are undetectable in human skin *in vivo*, and non-fibroblast cell-type-specific MMPs (MMP-8, neutrophil collagenase; MMP-9, 92kDa gelatinase-B; MMP-28, epilysin) were not detected in human dermal fibroblasts in 3D collagen lattices. Interestingly, all MMPs that were found to be elevated in photodamaged dermis *in vivo*, except MMP-17, were elevated in fibroblasts cultured in fragmented 3D collagen lattices (fold-increase from high to low: MMP-1, 4.5-fold; MMP-27, 3.2-fold; MMP-11, 3.1-fold; MMP-2, 2.4-fold; MMP-3, 1.9-fold) (Figure 2b). Consistent with above *in vivo* observations, no differences in mRNA levels of TIMP-1, -2, -3, or -4 were found between cells cultured in fragmented versus intact 3D collagen lattices (Figure 2c). Type I collagen mRNA (Figure 2d) and protein (Figure 2e) levels were significantly reduced in fibroblasts cultured in fragmented 3D collagen lattices, as is observed in photodamaged forearm dermis. Thus, collagen fragmentation recreates many of

the abnormalities seen in photodamage *in vivo*. These data suggest that fragmentation of the collagenous ECM in photodamaged dermis alters collagen homeostasis by influencing the function of dermal fibroblasts.

MMPs comprise a large family of proteinases that are capable of degrading every type of dermal ECM protein. Studies conducted by us and others over the past several years have shown that acute UV irradiation transiently induces expression of only three MMPs in resident human skin cells *in vivo*, i.e., interstitial collagenase (MMP-1), stromelysin-1 (MMP-3), and 92kDa gelatinase (MMP-9) (Brenneisen et al., 2002; Fisher et al., 1996; Quan et al., 2009). Our data indicate that compared to acute UV irradiation, a larger variety of MMPs, including UV-inducible MMPs, are constitutively elevated in photodamaged skin. Interestingly, compared to acute UV irradiation, in which the epidermis is the major source of transiently induced MMPs (Quan et al., 2009), the dermis is the major source of elevated MMPs in photodamaged skin. Elevated MMPs in photodamaged dermis can be divided into following groups: collagenases, MMP-1; gelatinases, MMP-2; stromelysins, MMP-3, MMP-9, MMP-11; membrane-associated: MMP-17, and recently identified MMP-27. Additionally, compared to acute UV irradiation, in which TIMP-1 is significantly induced (Fisher *et al.*, 1997), elevated MMPs in photodamaged skin are not accompanied by alterations of TIMPs expression. It is tempting to speculate that the combined actions of the wide variety of MMPs that are constitutively elevated in photodamaged dermis are involved in progressive degradation of dermal ECM.

Dermal fibroblasts are the primary cells that are responsible for collagen production and turnover in skin. Our data support the concept that fragmentation of the dermal collagenous ECM alters dermal fibroblast function to shift the balance to produce more MMPs and less collagen in photodamaged skin. A wealth of evidence indicates that tissue microenvironment controls a variety of cellular processes including signal transduction, gene expression, and tissue homeostasis (Bissell and Hines, 2011; Fisher et al., 2009; Spencer et al., 2007; Varani et al., 2004). One important finding of our study is that alterations of the dermal ECM microenvironment brought about by chronic exposure to solar UV irradiation have significant consequences on regulation of collagen homeostasis by fibroblasts. Currently, mechanisms by which alter ECM microenvironment in photodamaged human skin control fibroblast function are not well understood. We previously reported that fragmented collagen is unable to support normal cell shape and mechanical tension within fibroblasts, and this loss of cell shape and mechanical tension is closely associated with increased transcription factor AP-1 (Fisher *et al.*, 2009). Given that AP-1 functions as a major driving force for multiple MMPs and potent negative regulator of type I procollagen expression, it is conceivable that AP-1 activity induced by fragmented collagen microenvironment significantly contributes to elevated MMPs and loss of type I collagen expression in photodamaged skin. Additionally, fragmented collagen in photodamaged skin may also impair mechanical properties of the dermal collagen network, collagen-integrin mechanical sensing, and subsequent integrin signaling events associated with aberrant collagen homeostasis.

In summary, photodamaged dermis constitutively expresses elevated levels of several MMPs and reduced production of type I collagen, which likely lead to chronic, progressive

degradation of the dermal collagenous ECM and loss of collagen in photodamaged human skin. This aberrant collagen homeostasis largely results from altered fibroblast function due to long-term consequences of fragmented dermal collagen microenvironment in photodamaged human skin.

Acknowledgments

We thank Diane Fiolek for graphics and administrative assistance and Suzan Rehbine for the procurement of tissue specimens. This work was supported by the National Institute of Health (AG025186 to G Fisher, AG19364 to G Fisher and T Quan).

Abbreviations

UV	ultraviolet
MMPs	matrix metalloproteinase family
ECM	extracellular matrix
COL-1	type I collagen
AFM	atomic force microscopy
3D	three dimensional

References

- Bissell MJ, Hines WC. Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression. *Nat Med.* 2011; 17:320–9. [PubMed: 21383745]
- Brenneisen P, Sies H, Scharffetter-Kochanek K. Ultraviolet-B irradiation and matrix metalloproteinases: from induction via signaling to initial events. *Ann N Y Acad Sci.* 2002; 973:31–43. [PubMed: 12485830]
- Egeblad M, Werb Z. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer.* 2002; 2:161–74. [PubMed: 11990853]
- Fisher GJ, Datta SC, Talwar HS, Wang ZQ, Varani J, Kang S, et al. Molecular basis of sun-induced premature skin ageing and retinoid antagonism. *Nature.* 1996; 379:335–9. [PubMed: 8552187]
- Fisher GJ, Quan T, Purohit T, Shao Y, Cho MK, He T, et al. Collagen fragmentation promotes oxidative stress and elevates matrix metalloproteinase-1 in fibroblasts in aged human skin. *Am J Pathol.* 2009; 174:101–14. [PubMed: 19116368]
- Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultraviolet light. *N Engl J Med.* 1997; 337:1419–28. [PubMed: 9358139]
- Quan T, Qin Z, Xia W, Shao Y, Voorhees JJ, Fisher GJ. Matrix-degrading metalloproteinases in photoaging. *J Invest Dermatol Symp Proc.* 2009; 14:20–4.
- Spencer VA, Xu R, Bissell MJ. Extracellular matrix, nuclear and chromatin structure, and gene expression in normal tissues and malignant tumors: a work in progress. *Adv Cancer Res.* 2007; 97:275–94. [PubMed: 17419950]
- Varani J, Schuger L, Dame MK, Leonard C, Fligiel SE, Kang S, et al. Reduced fibroblast interaction with intact collagen as a mechanism for depressed collagen synthesis in photodamaged skin. *J Invest Dermatol.* 2004; 122:1471–9. [PubMed: 15175039]
- Varani J, Spearman D, Perone P, Fligiel SE, Datta SC, Wang ZQ, 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; 158:931–42. [PubMed: 11238041]
- Yaar M, Gilchrist BA. Photoaging: mechanism, prevention and therapy. *Br J Dermatol.* 2007; 157:874–87. [PubMed: 17711532]

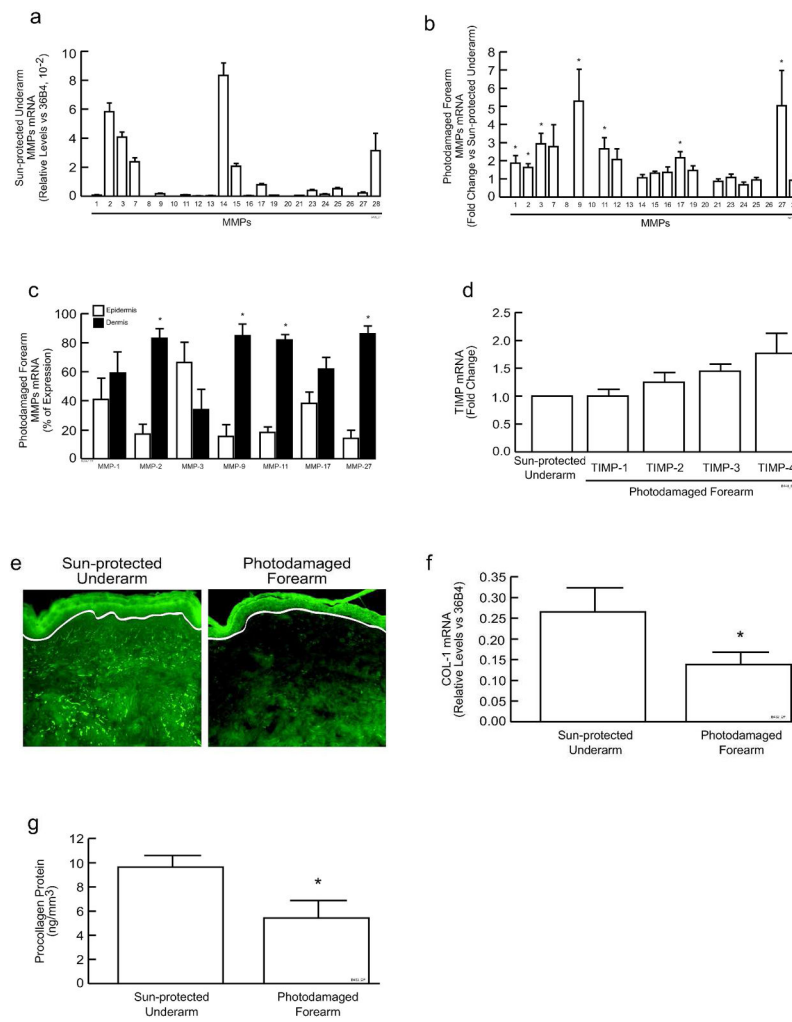


Figure 1. Elevated expression of multiple MMPs and reduced production of type I procollagen in photodamaged forearm human dermis

(a) Basal gene expression of MMP family members in human underarm skin. N=19. (b) Multiple MMPs elevated in photodamaged forearm skin relative to sun-protected underarm skin. N=19, *p<0.05. (c) Elevated MMPs in the dermis of photodamaged forearm skin. N=6, *p<0.05. (e) Elevated collagenase activity in the dermis of photodamaged forearm skin determined by in situ zymography. Loss of green fluorescence in photodamaged dermis indicates degradation of fluorescein-collagen substrate. White lines indicate boundary between the epidermis (top) and dermis (bottom). N=6. (d) Similar TIMP gene expression in sun-protected and photodamaged skin, N=10. (f) Reduced type I procollagen gene expression in photodamaged forearm dermis. N=19, *p<0.05. (g) Reduced type I procollagen protein levels in photodamaged forearm dermis. N=19, *p<0.05. (c, f, g) Dermis was isolated by laser capture microdissection. All results are means±SEM.

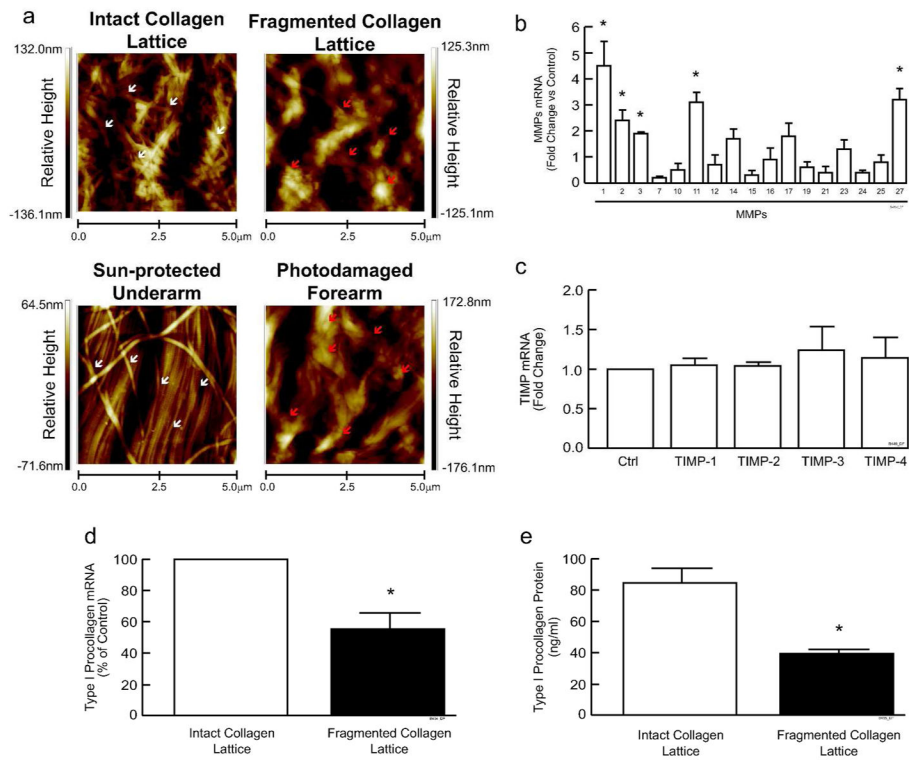


Figure 2. Collagen fibril fragmentation alters dermal fibroblast collagen homeostasis
(a) Nanoscale collagen fibrils were imaged by atomic force microscopy. The white and red arrows indicate intact and fragmented/disorganized collagen fibrils, respectively. Images are representative of six independent experiments. **(b)** MMPs that are elevated in photodamaged dermis are induced in fibroblasts cultured in MMP-1-fragmented collagen lattices. N=3, *p<0.05. **(c)** TIMPs gene expression are not elevated in fibroblasts cultured in MMP-1-fragmented collagen lattices. N=4. **(d)** Type I procollagen mRNA levels are reduced in the fibroblasts cultured in MMP-1-fragmented collagen lattices. N=9, *p<0.05. **(e)** Type I procollagen protein levels are reduced in the fibroblasts cultured in MMP-1-fragmented collagen lattices. N=5, *p<0.05. All results are means±SEM.