Recently developed methods allowed IgE BP180 antibodies to be easily detected in BP patients [8,9]. Considering that IgG and IgE BP180 antibodies induce the different clinical symptoms, the evaluation of both classes of the antibodies may be useful to understand the treatment efficacy in relation to the individual symptoms. Further studies are necessary to explain the detailed mechanism underlying the induction of erythema by IgE BP180 antibodies.

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## Letter to the Editor

### A mouse model of skin aging: Fragmentation of dermal collagen fibrils and reduced fibroblast spreading due to expression of human matrix metalloproteinase-1

*Keywords:*
MMP-1; Collagen; Aging; Transgenic mice; Fibroblast; Skin

*Dear Editor,*

Fragmentation of dermal collagen fibrils, the major structural proteins in skin, is a prominent feature of human skin aging [1,2]. Elevated collagenase (MMP-1) is largely responsible for initiation of collagen fragmentation in aging human skin [1,3,4]. We previously reported that expression of mutant human MMP-1 (hMMP-1/V94G), which undergoes auto-activation in either young human skin in organ culture or fibroblasts cultured in 3D collagen lattices, causes collagen fibril fragmentation similar to that in aged human skin [5].

To further explore MMP-1 function in skin aging, we generated transgenic mice that express mutant auto-activating human hMMP-1/V94G, under control of an epithelial-specific keratin-5 promoter [6]. hMMP-1/V94G contains a valine to glycine mutation at amino acid 94 within the N-terminal inhibitory domain. This mutation alters the interaction between the inhibitory and catalytic domains, thereby allowing auto-cleavage of the inhibitory domain. The cleaved N-terminal is released thereby converting full length 54 kDa MMP-1 to 44 kDa catalytically active form [5].

Fig. 1A shows immunostaining of hMMP-1/V94G in the epidermis of transgenic mice (K5-hMMP-1/V94GTg). Immunostaining in non-transgenic (non-Tg) littermate mice skin was negative. As expected, hMMP-1/V94G mRNA was readily detected in skin of K5-hMMP-1/V94GTg mice, but absent in non-Tg littermate mice skin (Fig. 1B). Western blot analysis revealed the presence of both full length and cleaved active forms of hMMP-1/V94G, at the expected molecular weights of 54 kDa and 44 kDa, respectively, in K5-hMMP-1/V94GTg mice skin (Fig. 1C). Full length hMMP-1/V94G was the predominant form of the protein. The relative low level of cleaved hMMP-1/V94G may have been due to a relatively high rate of turnover, or reflect inefficient conversion. *In vitro*, cleaved hMMP-1/V94G undergoes further degradation [5], suggesting that the former possibility is more likely.

Skin of seven months old K5-hMMP-1/V94GTg mice had normal gross appearance (data not shown). However, Masson's trichrome staining of skin sections revealed reduced density and disorganization of collagen fibrils (Fig. 1D), compared to non-Tg littermate mice skin.

Atomic force microscopy (AFM) was used to assess nanoscale structure of dermal collagen fibrils [7]. Collagen fibrils in non-Tg littermate mice skin were intact, densely packed and well-organized (Fig. 2A, upper left panel). Characteristic D-band striations, representing periodic alignment of microfibrils that assemble to form larger fibrils, were readily apparent. Collagen fibrils in non-Tg littermate mice skin closely resemble collagen fibrils in young human upper inner arm (underarm) skin (Fig. 2A, lower left panel). In contrast, collagen fibrils in K5-hMMP-1/V94GTg mice skin were less densely packed, disorganized, and appeared to be fragmented (Fig. 2A, upper right panel). These alterations closely resemble disorganization and fragmentation of collagen fibrils that are observed in aged human forearm skin

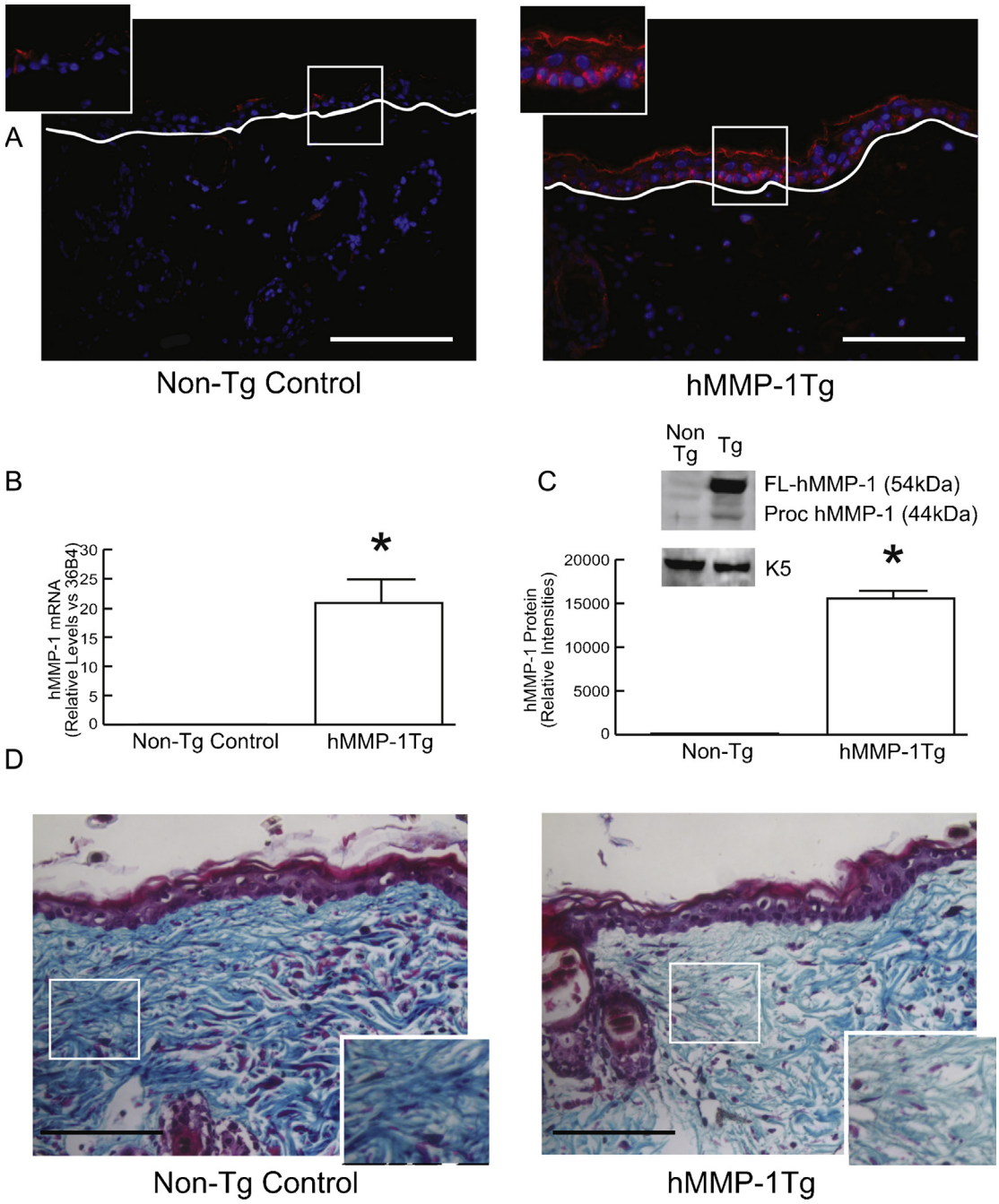


**Fig. 1.** Expression of human auto-activated MMP-1V94G in mouse skin alters dermal collagen fibrils. Full thickness skin biopsies were obtained from seven months old K5-hMMP-1/V94GTg transgenic (hMMP-1Tg) and non-transgenic littermates (non-Tg) mice. (A) Immunofluorescence staining of human MMP-1 (reddish fluorescence, MMP-1 antibody, Cat# ab38929, Abcam, Cambridge, MA, USA). Nuclei are stained with DAPI (blue fluorescence). White lines indicate the boundary between the epidermis (top) and dermis (bottom). Figures show representative images. $N = 6$. Bar = 100 μm. (B) MMP-1 mRNA levels. Human MMP-1 mRNA was determined by real-time RT-PCR and normalized to housekeeping gene (36B4, internal control). Data are means ± SEM. $N = 10$, $^*p < 0.001$. (C) Human MMP-1 protein expression. Representative Western blot shows full length (FL) and processed (Proc) human MMP-1. Immunoblotting of keratin 5 (K5) was used as loading control. Data are means ± SEM. $N = 3$, $^*p < 0.001$. (D) Masson's trichrome staining, representative images. $N = 6$. Bar = 100 μm.

(Fig. 2A, lower right panel). Quantitation of collagen fibril organization, measured by analysis of AFM topographical roughness data, indicated that dermal collagen fibrils were significantly more disorganized in K5-hMMP-1/V94GTg mice, compared to non-Tg control mice (87 ± 12.8 nm *vs* 41 ± 6.9 nm) (Fig. 2B, upper panel). A similar degree of disorganization is observed in aged human forearm skin, compared to young underarm skin (77.6 ± 15.6 nm *vs* 38.1 ± 8.3 nm) (Fig. 2B, lower panel).

Fibroblasts normally bind to intact collagen fibrils through specific cell surface integrins. This binding allows fibroblasts to spread and attain morphological and mechanical stability. Electron microscopy revealed densely packed, intact collagen fibrils in non-Tg littermate mice skin (Fig. 2C, upper left panel). Fibroblasts were in intimate contact with these fibrils and displayed spread morphology with abundant cytoplasm (Fig. 2C, lower left panel). In contrast, collagen fibrils were sparse and disorganized in K5-hMMP-1/V94GTg mice skin (Fig. 2C, upper right panel). Fibroblasts