Mechanism of UVB-Induced Wrinkling of the Skin: Paracrine Cytokine Linkage between Keratinocytes and Fibroblasts Leading to the Stimulation of Elastase

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In clinical studies, the formation of facial wrinkles has been closely linked to the loss of elastic properties of the skin. Repetitive irradiation of animal skin with UVB radiation at suberythemal doses significantly reduces its elastic properties, resulting in the formation of wrinkles. Repetitive UVB irradiation elicits a marked alteration in the three-dimensional structure of elastic fibers, which is closely associated with a subsequent reduction in the elastic properties of the skin. Although UVB irradiation stimulates the activity of fibroblast elastases in the dermis, a synthetic inhibitor specific for fibroblast elastases prevents wrinkle formation. The close interrelationships among wrinkle formation, elastic properties, and elastic fiber linearity are revealed by the effects of different concentrations of the elastase inhibitor ($R^2 > 0.9$), suggesting that enhanced elastase activity by dermal fibroblasts plays a pivotal role in the UVB wrinkling mechanism. In *in vitro* studies we identified a paracrine linkage between keratinocytes and fibroblasts that leads to wrinkle formation through the upregulation of fibroblast elastases. These studies support our hypothesis for a mechanism of wrinkle formation by which cytokine expression is activated in epidermal keratinocytes by UVB radiation and triggers dermal fibroblasts to increase their expression of elastase.

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INTRODUCTION

Facial wrinkles are the most prominent characteristic by which skin aging is recognized. Histologically, in aged and wrinkled skin, there is an accumulation of altered elastic fibers and degradation or degeneration of collagen bundles in the dermis. However, there is no direct evidence that solar elastosis from sunlight causes wrinkle formations, and there is no published study showing that the dermis underlying facial wrinkles, especially at the most susceptible site, the corner of the eye, includes elastosis. Repetitive exposure to sunlight has been thought to be the most plausible factor producing wrinkles because wrinkles frequently appear on the face. However, little is known about the precise physicochemical and biological mechanism(s), which lead to the formation of wrinkles due to sunlight exposure. This review focuses on our long-term research directed towards clarifying the mechanism of formation of UVB-induced wrinkles in an evidence-based fashion.

Wrinkling results from the loss of skin elasticity

Our previous studies in mice and rats (Imayama *et al.*, 1994; Imokawa *et al.*, 1995; Takema and Imokawa, 1998) suggested that cumulative UVB radiation causes degeneration in the three-dimensional structure of elastic fibers, which results in the loss of skin elasticity, and eventually leads to wrinkle formation. Figure 1 shows the marked wrinkling of hairless mouse skin induced by 2 weeks of daily UVB irradiation at a suberythemal dose, where the wrinkle score continued to increase up to 12 weeks (Takema and Imokawa, 1998). When changes in various elastic parameters were measured by a commercial instrument (Cutometer SEM 474, Courage and Khazaka, Cologne, Germany) after 14 weeks of daily UVB irradiation (Tsukahara *et al.*, 2001a), all elastic parameters (Ue, Uf, Ur and Uv) decreased significantly, which suggests that the loss of skin elasticity is directly associated with wrinkle formation. The close relationship between loss of elasticity and wrinkle formation was corroborated by our other studies using human facial skin (Takema *et al.*, 1995; Akazaki and Imokawa, 2001; Akazaki *et al.*, 2002). To clarify why the corners of the eyes are most susceptible to wrinkle formation among facial sites (Takema *et al.*, 1995), we measured the thickness and elasticity of skin on the face and on the ventral forearm of 170 women using a Cutometer, and evaluated the effects of age and exposure to sunlight (Takema *et al.*, 1994). Skin thickness decreased with

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Abbreviations: SEM, scanning electron microscopy

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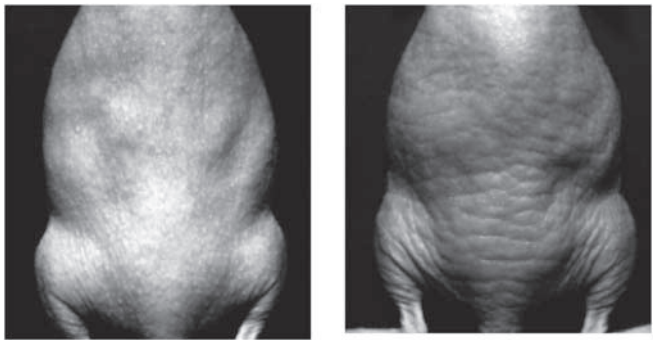

Figure 1. **Marked wrinkling in the dorsal skin of hairless mice after 14 weeks of UVB irradiation.** A suberythemal dose of 50–65 mJ cm^{-2} was administered five times a week (reprinted with permission from Tsuji *et al.*, 2001).

age in ventral forearm skin, which has limited exposure to sunlight, but increased significantly in skin on the forehead, at the corners of the eyes and on the cheeks, which are exposed markedly to sunlight. Although skin elasticity (Ur/Uf) decreased with age on both the face and forearm, we found that during age-dependent changes in skin elasticity at various facial sites, the corner of the eye had the strongest age-dependent decline (Takema *et al.*, 1994). To further elucidate the susceptibility of the corners of the eyes to wrinkle formation, we evaluated the effects of temporary skin fixation on wrinkle formation after UVB irradiation using the dorsal skin of hairless mice (Takema *et al.*, 1996). In mice treated with UVB radiation immediately after production of an artificial groove parallel to the midline using a cyanoacrylate resin, wrinkles formed parallel to the midline, an uncommon direction for wrinkle formation in this mouse model. These wrinkles did not disappear even when the skin was stretched. No such changes were observed in mice in which the temporary groove was produced, but without UVB irradiation. This result indicates that temporary grooves or wrinkles, seen as laugh grooves in the corners of the eyes, may eventually become permanent as a result of the loss of skin elasticity due to repetitive UVB irradiation. These findings suggest strongly that wrinkle formation is closely associated with the loss of skin elasticity, which is induced by frequent sunlight exposure.

For a quantitative evaluation of facial wrinkles, a system was developed wherein the morphology of the skin surface could be evaluated directly in three dimensions (Akazaki and Imokawa, 2001; Akazaki *et al.*, 2002). This system employs a non-invasive device that utilizes white light of halogen bulb origin and allows the computation of wrinkle depth and width, and other parameters of skin surface morphology. An optical system was devised so that light is transmitted through a slit and can measure not only replicas of the skin but also the skin surface directly. The measured area is 6.4×6.4 mm, and the theoretical resolution with a 50-magnification lens is less than 12.5 μm. The system was applied to study age-related changes in the morphology of wrinkles in the corners of the eyes in women at varying ages. Representative analytical data obtained (Akazaki *et al.*, 2002) demonstrated that wrinkles rapidly increased in depth in women of 40 years or older and plateaued at the age of 60 years. Surface morphology parameters yielded results similar to age-related changes in wrinkles. When the depth and the width of wrinkles in the corners of the eyes were plotted against age, a close correlation was found between these parameters and chronological age (Akazaki *et al.*, 2002). Therefore, it was of considerable interest to determine whether severity of wrinkles correlated with the reduction in elasticity of human facial skin. When the elasticity of the skin at the corner of the eye was measured in 36 women aged 60 years or older, using a Cutometer and was compared with the depth or the width of wrinkles at the same sites, a significant inverse correlation was found between the depth of wrinkles and skin elasticity (Akazaki *et al.*, 2002) suggesting that wrinkle formation results from the preceding loss of skin elasticity.

Skin elasticity is closely linked to the function of elastic fibers due to their three-dimensional configuration

We used scanning electron microscopy (SEM), combined with perfusion, resin injection, and selective digestion, to determine the effects of UVB irradiation on the three-dimensional architecture of elastic fibers in rat hind limb skin (Imayama *et al.*, 1994). These SEM observations (Tsukahara *et al.*, 2001b), showed that elastic fibers in the dermal connective tissue of unexposed animals have an orderly pattern of relatively straight fibers arranged in multiple dense layers. The fibers in each layer are oriented differently from the fibers in adjacent layers, producing a meshwork appearance. This orderly arrangement is maintained until 15 weeks of age, when maturation is essentially complete. SEM further showed that, after 6 weeks of UVB irradiation, elastic fibers in the exposed skin became tortuously deformed (Tsukahara *et al.*, 2001b). Fibers in the superficial layer became increasingly tortuous, whereas those in the deep layer remained straight. After 12 weeks of UVB irradiation, the fine branches developed into an irregular network by anastomosing with each other, or by interconnecting with the tortuous fibers in the superficial layer of the dermal connective tissue.

We quantitated the deformation by measuring the linearity of individual fibers every 3 weeks for 15 weeks, and confirmed that the tortuosity of the fibers increased in accordance with the total time of UV exposure (Imayama *et al.*, 1994). Generally, the elastic properties of unexposed control skin decreased in the first 2 weeks, when the animals were 3–5 weeks old, and then maintained at a certain level until 25 weeks of age, shortly after maturation. In contrast, the elastic properties of UVB-irradiated skin decreased relatively steeply in the first 2 weeks and showed a further gradual decrease over the remainder of the irradiation period. There was a significant difference in the curve for one elastic parameter, the immediate distension (Ue), between control and UVB-irradiated skin. Curves for the other elastic parameters, such as delayed distension (Uv), immediate retraction (Ur), and final distension (Ue), each showed

features resembling those of the immediate distension (Ue) curve, which consisted of the initial, purely elastic component. Thus, exposure to UVB produces tortuous deformation, together with fraying of elastic fibers in rat skin. These deformations may be responsible for the decline in skin elasticity *in situ*, because the tight fit of rat skin may be attributed to the ability of elastic fibers to resume a short, straight configuration after being stretched (Gibson and Kenedi, 1970; Cua *et al.*, 1990). Thus, it is likely that the tortuous appearance of elastic fibers suggests a loss of their original elasticity (Imayama and Bravermann, 1989). The three-dimensional alterations revealed by these SEM studies correlated with previous observations using light and electron microscopy (Smith *et al.*, 1962; Mitchell, 1967; Stevanovic, 1976; Montagna and Carlisle, 1979). The pathogenic relationships among the three-dimensional alterations of elastic fibers, loss of skin elasticity, and wrinkle formation is also corroborated by other animal studies in which induced wrinkles were repaired by CO_2 laser (Tsukahara *et al.*, 2001c) or retinoic acid treatment (Tsukahara *et al.*, 1999).

UVB irradiation specifically stimulates elastase activity in the dermis

To determine what matrix proteases are linked to the loss of skin elasticity, the activities of several matrix proteases in the dermis of UVB-irradiated mouse skin were measured at week 14 (Tsukahara *et al.*, 2004a). There was a significant increase in the activity of elastase in UVB-irradiated skin compared with non-irradiated skin. The increase in dermal elastase activity appeared 2 weeks after the onset of irradiation and continued until 18 weeks (Tsuji *et al.*, 2001). In contrast, there was a slight, but not significant, increase in the activity of collagenase I in the dermis at week 14 in irradiated skin compared with non-irradiated skin. On the other hand, there was a decrease in the activity of collagenase IV in irradiated skin at the same time compared with non-irradiated skin. Thus, it is likely that enhanced elastase activity in the dermis of irradiated skin plays an important role in the degeneration of elastic fibers, which eventually results in the loss of skin elasticity.

A specific inhibitor of human skin fibroblast elastase prevents the UVB-induced formation of wrinkles and maintains the linear configuration of elastic fibers and skin elasticity

We designed an inhibitor specific for human skin fibroblast elastase to determine whether that enzyme is responsible for the degeneration of elastic fibers, leading to a loss of elasticity in the skin. Comparing the effects of various protease inhibitors (Tsuji *et al.*, 2001) revealed that neutrophil elastase is inhibited by serine protease inhibitors, such as PMSF and elastatinal, but is not inhibited by the other inhibitors tested. In contrast, fibroblast elastase is inhibited remarkably by metal-chelating agents (such as EDTA and phenanthrolin) and by a metalloprotease inhibitor (phosphoramidon), but it is not inhibited by the serine protease inhibitors (PMSF or elastatinal), by a thiol protease inhibitor (leupeptin) or by a carboxyl protease inhibitor (pepstatin A). These results indicate that fibroblast elastase does indeed belong to the metalloprotease family.

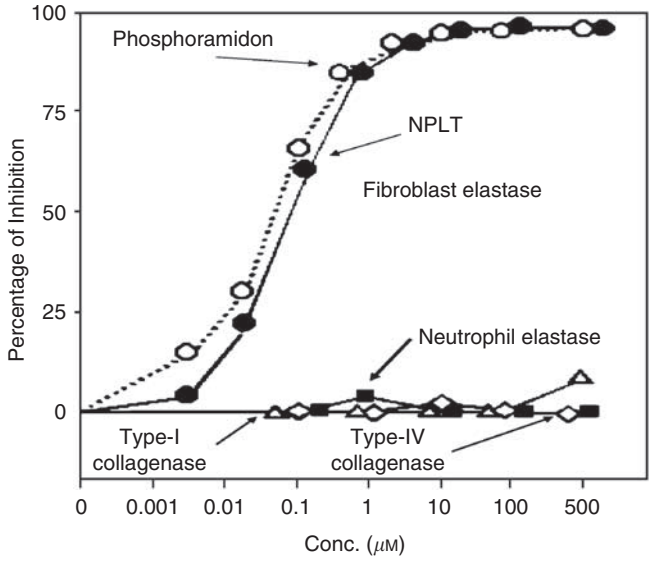

Figure 2. **Concentration-dependent inhibition of skin fibroblast elastase by NPLT and phosphoramidon and the inhibitory profile of NPLT on several proteinases (reprinted with permission from Tsuji *et al.*, 2001).** An enzyme solution obtained from a lysate of human fibroblasts was used at a concentration of 100 μg ml^{-1}. Elastase activity was measured using STANA as a substrate in the presence of NPLT (—●—) or phosphoramidon (· · · ○ · · ·) at the indicated concentrations. Neutrophil elastase (—■—), type I collagenase (—Δ—) and type IV collagenase (—◇—) were used at concentrations of 5 μg ml^{-1} (0.1 U ml^{-1}), 0.5 U ml^{-1}, 0.5 U ml^{-1}, respectively. Each enzymatic activity was measured using each substrate according to the instruction guide of the agency. NPLT was used at the indicated concentrations. Data represent means ± SD.

In designing a specific inhibitor for skin fibroblast elastase, it should be noted that phosphoramidon is known as a typical inhibitor of metalloprotease, but that it has poor permeability through the skin because of its hydrophilic rhamnose residue. To enhance the cutaneous permeability of phosphoramidon, we synthesized a derivative (designated here as NPLT) by replacing the hydrophilic rhamnose residue with a phenethyl residue (Tsuji *et al.*, 2001). NPLT was the best suited derivative tested and was able to inhibit skin fibroblast elastase significantly, with an IC_{50} of 50 nM, but it did not affect the activity of neutrophil elastase or collagenases Type I or Type IV (Figure 2) (Tsuji *et al.*, 2001).

To determine whether NPLT inhibits elastase in mouse skin, UVB-exposed and non-exposed mouse skin was examined for elastase activity *in vitro*. The elastase activity stimulated by UVB irradiation was reduced significantly by NPLT, to a level similar to that of non-irradiated mouse skin in a dose-dependent manner (Tsuji *et al.*, 2001). This indicates that NPLT can inhibit UVB-inducible elastase activity and that the enhanced elastase activity in UVB-exposed skin is associated with an NPLT-sensitive metalloprotease. When NPLT was applied daily for 18 weeks at a concentration of 1 mM to the dorsal skin of hairless mice, immediately after each suberythemal UVB irradiation, the wrinkle formation was diminished significantly compared with ethanol-treated controls (Tsuji *et al.*, 2001). Similar

treatment with a UVB sunscreen (p-MCX) had no effect. Comparison of wrinkle scores in that study revealed that NPLT suppressed wrinkle formation significantly after 15 to 18 weeks of irradiation, compared with ethanol-treated controls. In contrast, similar treatment with the UVB sunscreen p-MCX had no suppressive effect, indicating that the sunscreen effect played no role in the prevention of wrinkle formation by NPLT. This study, using a specific inhibitor of skin fibroblast elastase, revealed that the enhanced activity of skin fibroblast elastases from UVB radiation is involved primarily in the loss of skin elasticity and subsequent wrinkle formation.

To assess how wrinkle formation, loss of skin elasticity, and degeneration in the three-dimensional configuration of elastic fibers are linked, we conducted a similar wrinkling study using rat hind limb skin at different concentrations of the skin fibroblast elastase inhibitor (Tsukahara *et al.*, 2001b). When NPLT was applied topically for 6 weeks at various concentrations, 1 hour or 24 hours after each UVB exposure, wrinkle formation was remarkably suppressed at concentrations of NPLT greater than 0.5 mM compared with ethanol-treated controls (Figure 3) (Tsukahara *et al.*, 2001b). In contrast, similar treatment with a UVB sunscreen (p-MCX) did not suppress wrinkle formation, indicating little involvement of a sunscreen effect. When assessed by image analysis of skin replicas, NPLT treatment reduced wrinkle formation significantly at concentrations greater than 0.5 mM, reaching a plateau at concentrations greater than 1 mM, whereas the UVB sunscreen p-MCX had little suppressive effect. In measurements of skin elasticity using a Cutometer, whereas 6 weeks of UVB irradiation decreased skin elasticity markedly (expressed as the parameters, Ue, Uf, Ur, and Uv), 6 weeks of treatment with NPLT after exposure prevented the decreases in skin elasticity at concentrations greater than 0.1 mM (for Ue, Uf, and Ur) or 1.0 mM (for Uv) (Tsukahara *et al.*, 2001b). In contrast, similar treatment with the UVB sunscreen p-MCX did not prevent the decrease in skin elasticity. In parallel, electron microscopic observations of the three-dimensional structure of elastic fibers revealed that although 6 weeks of UVB irradiation caused a marked disruption of the three- dimensional structure of elastic fibers, treatment with NPLT after UVB exposure over the 6 weeks prevented the disruption of elastic fibers, at concentrations greater than 0.1 mM (Figure 4) (Tsukahara *et al.*, 2001b). In contrast, similar treatment with the UVB sunscreen p-MCX did not prevent the disruption of elastic fibers. Quantitative measurements by image analysis of the disruption of elastic fibers based on elastic fiber linearity revealed that although 6 weeks of UVB irradiation induced a distinct decrease in elastic fibers with a high linearity, 6 weeks of treatment with NPLT during that cumulative UVB exposure prevented that decrease, in a dose-dependent manner and at concentrations greater than 0.1 mM, reaching a plateau at concentrations greater than 1 mM. In contrast, similar treatment with the UVB sunscreen p-MCX did not prevent the decrease in elastic fibers, with high linearity. Thus, there is a close and significant interrelationship ($R^2 > 0.9$) among wrinkle formation, elasticity, and elastic fiber linearity at different concentrations of NPLT (Figure 5).

These NPLT inhibition studies suggest strongly that the enhanced elastase activity of dermal fibroblasts plays an important role in the degeneration of elastic fibers following

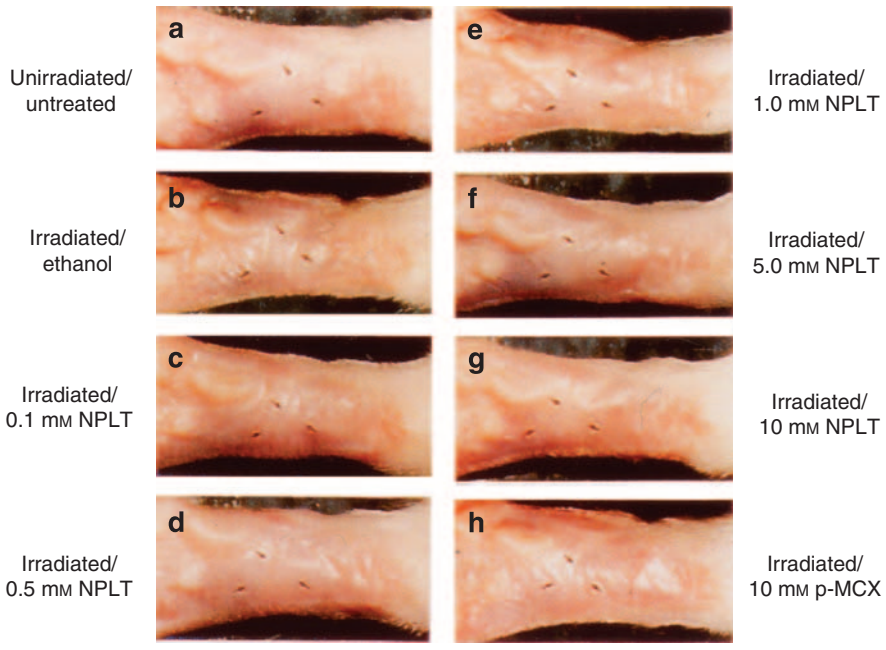

Figure 3. **Close-up photos after topical application (five times weekly for 6 weeks) during the period of UVB irradiation (three times weekly for 6 weeks) on rat hind limb skin (Tsukahara *et al.*, 2001c).** (**a**) Unirradiated and Untreated group; (**b**) Irradiated and ethanol-treated group; (**c**) Irradiated and 0.1 mM NPLT-treated group; (**d**) Irradiated and 0.5 mM NPLT-treated group; (**e**): Irradiated and 1.0 mM NPLT-treated group; (**f**): Irradiated and 5.0 mM NPLT-treated group; (**g**) Irradiated and 10.0 mM NPLT-treated group; (**h**) Irradiated and 10 mM p-MCX-treated group. Arrows represent areas where wrinkles appear.

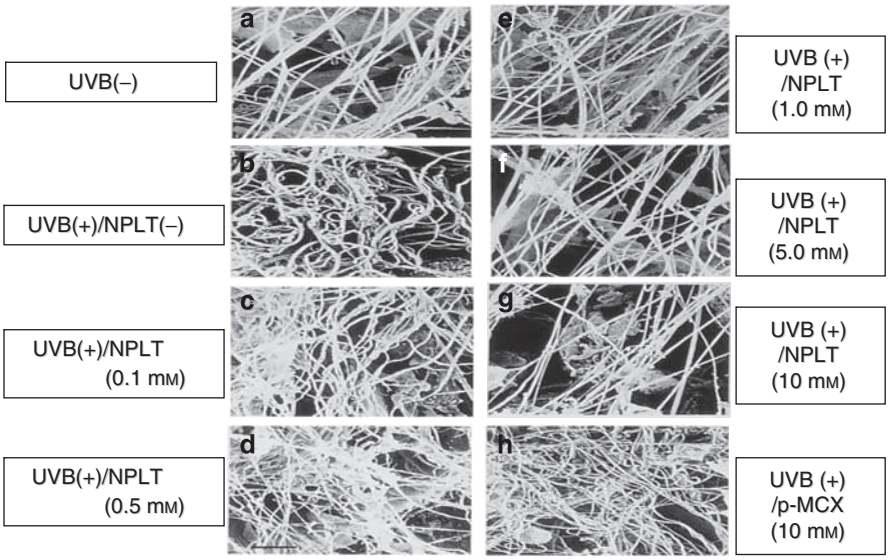

Figure 4. **Scanning electron micrographs after topical treatment followed by intravascular injection and selective digestion at 9 weeks (reprinted with permission from Tsukahara et al., 2001b).** (**a**) Unirradiated and Untreated group; (**b**) Irradiated and ethanol-treated group; (**c**) Irradiated and 0.1 mM NPLT-treated group; (**d**) Irradiated and 0.5 mM NPLT-treated group; (**e**) Irradiated and 1.0 mM NPLT-treated group; (**f**) Irradiated and 5.0 mM NPLT-treated group; (**g**) Irradiated and 10.0 mM NPLT-treated group; (**h**) Irradiated and 10 mM p-MCX-treated group. Bar represents 9 μm.

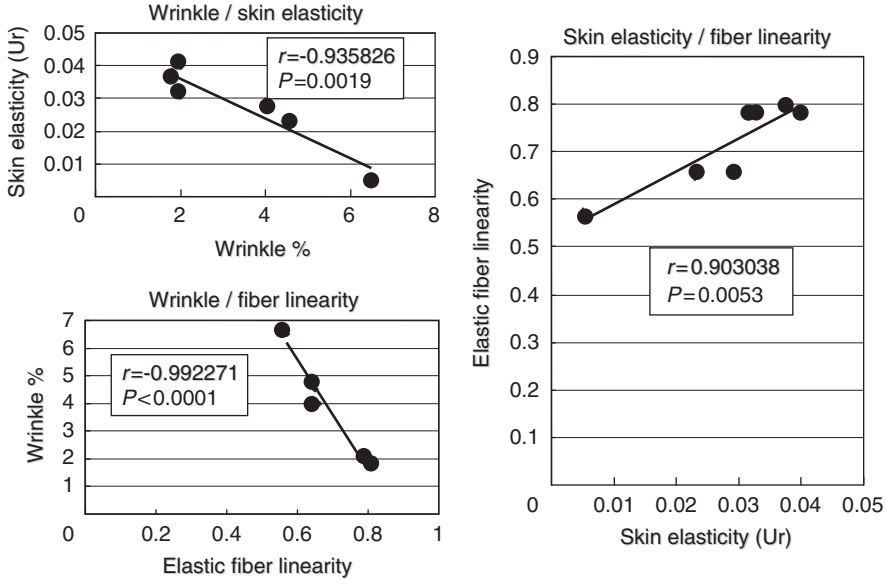

Figure 5. **Interrelationship among wrinkle formation, skin elastic property and elastic fiber linearity attained at different concentrations of NPLT.**

UVB irradiation and that the loss of skin elasticity occurs as a direct result of the altered elastic fiber configuration, which subsequently triggers wrinkle formation. The inhibitory effect of NPLT on wrinkle formation was corroborated by a human clinical study using an extract of *Zingiber officinale (L.) Rose,* which is capable of inhibiting skin fibroblast elastase (with an IC_{50} of 0.013%, residue weight percentage) but not neutrophil elastase (Tsukahara *et al.*, 2006). A 1 year clinical study (Tsukahara *et al.*, 2004b; Imokawa, 2008) on human facial skin to determine the efficacy of the *L. Rose* extract demonstrated that it inhibits UV-induced decreases in skin elasticity and prevents or improves wrinkle formation in skin around the corners of the eyes without changing the water content of the stratum corneum. The subjects studied were 20 healthy Japanese men with a mean age of 37.6 years (Tsukahara *et al.*, 2004b). A 1% extract of *Zingiber officinale (L.) Rose* or a placebo was applied topically to each half of the face in a double-blind manner twice daily for 12 months. Before and 12 months after the initiation of topical treatment, wrinkles were assessed visually, replicas were collected, and skin elasticity and water content of the stratum corneum were measured. The replicas were used to measure wrinkles by

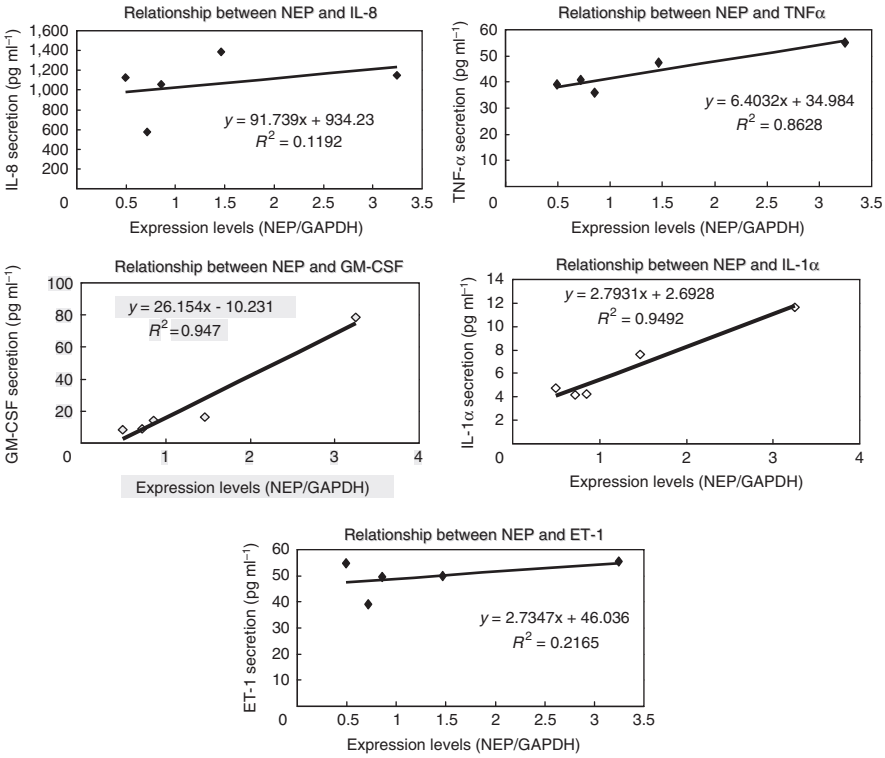

Figure 6. **Relationship between levels of cytokines released by UVB-exposed human keratinocytes at different UVB doses and the gene expression of neutral endopeptidase at 4 hours after incubation with the conditioned medium of UVB-exposed human keratinocytes.**

image analysis. After 1 year of topical application of the extract, the wrinkle score and the image analysis area ratio (%) of wrinkles and fine wrinkles had increased at the corners of the eyes and at the lower eyelid areas on the placebo-treated side but were significantly less in those areas on the side of the face treated with the extract. Skin elasticity, evaluated in terms of Ur/Uf, had significantly decreased after 1 year only on the placebo-treated side, and that decrease was prevented on the extract-treated side. The water content of the stratum corneum did not differ between the extract- and placebo-treated sides throughout the measurement period. These results suggest that the extract of *Zingiber officinale (L.) Rose* inhibits the decrease in skin elasticity and diminishes wrinkle formation in areas around the corners of the eyes by inhibiting fibroblast elastase activity, but without affecting the water content of the stratum corneum.

Identification of skin fibroblast elastase

Skin fibroblast elastase is a 94 kDa membrane-bound type metalloprotease with a neutral optimum pH (Szendri *et al.*, 1984; Schwartz *et al.*, 1986, 1988; Nakagawa *et al.*, 1987; Godeau and Hornebeck, 1988; Homsy *et al.*, 1988; Croute *et al.*, 1991; Mecham *et al.*, 1997). Although there are several candidate enzymes for skin fibroblast elastase, such as 92, 72 kDa type IV collagenase, neutrophil elastase, cathepsin G and protease 3, none of these have properties which match the above characteristics. Recently, we observed similarities between fibroblast elastase and neutral endopeptidase (Fulcher *et al.*, 1982) in terms of their size (MW 97,000), both being membrane-bound metalloproteases, and in their inhibitory profiles. Immunoprecipitation and western blotting using an antibody to neutral endopeptidase revealed that skin fibroblast elastase is identical to neutral endopeptidase (Moriwaki *et al.*, 1995).

Biological mechanisms underlying UVB-induced upregulation of skin fibroblast elastase

Although the enhanced activity of neutral endopeptidase in skin fibroblasts may be responsible for a UVB-induced cascade of biological events in which the degeneration of elastic fibers reduces skin elasticity, which in turn leads to wrinkle formation, little is known about the mechanism(s) by which skin fibroblast elastase activity in dermal fibroblasts is stimulated by UVB irradiation. Based on the poor penetration of UVB into the dermis, the most plausible mechanism is that UVB causes the production of a basement membrane-permeable biological factor(s), which stimulates the expression of elastases by dermal fibroblasts. To test this hypothesis, we asked whether conditioned medium from UVB-exposed human keratinocytes stimulates the gene expression of matrix proteins or matrix metalloproteases in human fibroblasts (Imokawa, 2008) as follows: Real-time reverse transcriptase (RT)-PCR for neutral endopeptidase mRNA reveals that the conditioned medium from UVB-exposed keratinocytes stimulates gene expression for neutral endopeptidase at 4–6 hours and 24 hours post-incubation. Real-time RT-PCR for elastin mRNA reveals that conditioned

medium from UVB-exposed keratinocytes downregulates the gene expression of elastin at 4 hours post-incubation. Real-time RT-PCR for collagenase type I mRNA reveals that the conditioned medium from UVB-irradiated keratinocytes stimulates gene expression for collagenase type I at 4–12 and 24 hours post-incubation. Real-time RT-PCR for collagen mRNA reveals that the conditioned medium from UVB-irradiated keratinocytes downregulates gene expression for collagen only slightly at 12 and 24 hours post-incubation. Further, western blotting of conditioned medium-treated fibroblasts reveals that the conditioned medium from UVB-exposed keratinocytes increases protein levels of neutral endopeptidase at 48 hours post-incubation. The above findings indicate that paracrine factors are secreted by UVB-exposed keratinocytes, which may penetrate into the dermis to trigger dermal fibroblasts to generate fibroblast elastase activity.

In a comparison between *in vivo* profiles for matrix proteins and proteases and *in vitro* cellular effects, it is evident that the effects of conditioned medium from UVB-irradiated keratinocytes on fibroblasts may mimic the *in vivo* situation for matrix proteins and proteases in wrinkled skin. This suggests that the enhanced activity of skin fibroblast elastase in UVB-irradiated skin is mediated by basement membrane-permeable soluble factors secreted by keratinocytes. These results support our hypothesis for a mechanism of wrinkle formation by which cytokines are released by keratinocytes following UVB irradiation, triggering dermal fibroblasts to generate neutral endopeptidase. The neutral peptidase results in deterioration of the three-dimensional architecture of the elastic fibers, reducing skin elasticity, and eventually leading to the formation of wrinkles.

Identification of keratinocyte-derived cytokines responsible for increased gene expression of skin fibroblast elastase

To determine which cyotokine(s) secreted from human keratinocytes following UVB irrradiation are responsible for the increased gene expression of skin fibroblast elastase, we compared the gene expression of skin fibroblast elastase (measured by real-time RT-PCR) and levels of cytokines released into the medium by UVB-exposed human keratinocytes (HaCaT cells) at different UVB doses (Figure 6). There was a close correlation ($R^2 > 0.75$) between the secreted levels of IL-1α and GM-CSF and gene expression of the elastase, but no correlation with TNFα, IL-8, or ET-1. This suggests that both IL-1α and GM-CSF may play roles involved in the stimulated gene expression of skin fibroblast elastase (Imokawa, 2008), although additional studies are required to reach a final conclusion.

Thus, we propose a UVB-induced wrinkling mechanism as follows: Repetitive UVB exposure causes keratinocytes to secrete IL-1α which triggers GM-CSF secretion in an autocrine fashion. Secreted IL-1α and GM-CSF penetrate into the dermis to stimulate the expression of skin fibroblast elastase, which then cleave elastic fibers surrounding the fibroblasts, leading to the deterioration of the three-dimensional configuration of elastic fibers. This results in a loss of skin elasticity and subsequently to wrinkle formation.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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