The Crucial Role of TGF-β in the Age-Related Alterations Induced by Ultraviolet A Irradiation

To the Editor:

The major changes associated with aging, such as wrinkles and solar lentigines, are due to excessive exposure to solar ultraviolet (UV), namely photoaging. Recent studies (Fisher et al, 1997; Krutmann, 2001; Varani et al, 2001) demonstrate that UV irradiation alters the relative proportion of extracellular components (collagen, elastin, and proteoglycans), or degradative pathways that render these molecules nonfunctional, can result in clinical manifestations such as skin aging (Yin et al, 2000; Varani et al, 2001). The molecular alteration in the dermis includes the decrease of collagen synthesis, induction of matrix metalloproteinases (MMP), abnormal accumulation of elastic fibers, and proteoglycans (Fisher et al, 1996; Yin et al, 2001). The photoaging process displays the prominent alterations in the skin through stimulation of multiple signal transduction pathways, which lead to activation of transcription factors or target genes (Fisher et al, 1998). Transforming growth factor (TGF)-β is a multifunctional cytokine that regulates cell proliferation and differentiation, tissue remodeling, and repair (Massague, 1998). TGF-β is a potent growth inhibitor of the epidermis, whereas in the dermis, TGF-β acts as a positive growth factor inducing the synthesis of extracellular matrix proteins. TGF-β first binds to TGF-β type II receptor (TbRII), resulting in the formation of a heteromeric complex between TGF-β type I receptor (TbRI) and TbRII (Wrana et al, 1992). Overexpression of either TbRI or TbRII increases collagen promoter activity in fibroblasts (Kawakami et al, 1998). TbRII is essential for the binding of TGF-β, and TbRI is necessary for downstream signal transduction induced by TGF-β binding to TbRII. Binding of TGF-β to TbRII is the first critical step in the TGF-β signaling because TbRI does not bind TGF-β in the absence of TbRII (Wrana et al, 1992). Therefore, TbRII expression should be of considerable biologic interest in the initial TGF signaling pathway. A recent report (Quan et al, 2001) showed that UV irradiation could cause downregulation of TbRII receptor mRNA and protein, and induction of Smad7 mRNA and protein in human skin. The modes of action by which UV radiation induces TGF-β-related aging-associated changes in the skin fibroblasts, however, have not been well investigated.

We initially investigated the effect of UV irradiation on TGF-β1 production, assayed by enzyme-linked immunosorbent assay, in the conditioned media of skin fibroblasts. As shown in Fig 1(A), UVA treatment significantly induced the amount of TGF-β1 in the conditioned media (p < 0.05). The amount of active TGF-β1 was found not to be different between UVA-treated and control cells. The latent TGF-β1, which was calculated from the total minus active one, was shown to increase in the UVA-treated cells as compared with the controls. These data indicated that UVA irradiation induced TGF-β1 signaling by increasing the secretion of latent TGF-β1. We also checked the mRNA expression of TbRII after a variety of treatments using the reverse transcriptase–polymerase chain reaction (reverse transcriptase–PCR). As shown in Fig 1(B), TbRII mRNA expression was increased after the treatment of recombinant human TGF-β1 (10 ng per ml) (R&D System, Minneapolis, MN). Consistent with the study of Quan et al (2001), TbRII receptors were prominently decreased by 40% as compared with control after UVA irradiation; however, TbRII was elevated about 20% in the treatment TGF-β1 (10 ng per ml) following UVA irradiation as compared with UVA treatment alone.

To examine the functional consequences of the alteration of TGF-β1 by UVA irradiation, we determined the collagen protein and MMP-1 mRNA expression in skin fibroblasts using western blot and reverse transcriptase–PCR, respectively (Fig 2A,B). UVA irradiation resulted in a 75% reduction of collagen protein as compared with the control, whereas TGF-β1 treatment showed an obvious increase. The fibroblasts treated with TGF-β1 following UVA irradiation display the protein level of collagen that was more than that of UVA-treated alone, suggesting that TGF-β1 could abrogate the inhibition of collagen production induced by UVA irradiation. To confirm the hypothesis that TGF-β modulate MMP gene expression during UVA irradiation, the MMP-1 expression was also examined in the fibroblasts. TGF-β1 resulted in a reduction of the MMP-1 signal by nearly 99%, whereas exposure to UVA resulted in a 1.5-fold increase in MMP-1 expression.
as compared with the control; however, TGF-β1 addition affected the MMP-1 expression, by 45% reduction as compared with the UVA treatment alone, indicating that MMP-1 expression led to response to the addition of TGF-β1 in skin fibroblasts. The results showed that MMP-1 mRNA expression was eliminated by incubation of exogenous active TGF-β1 suggesting that TGF-β1 is the critical component in age-associated modulation.

The induction of endogenous TGF-β1 from UVA-irradiated cells contributes to the intracellular defense capacity, and its effect can be functioned by the activation. The responses of fibroblasts to TGF-β1 are mediated through its active form binding to the cell surface receptor complex. Here we reported that UVA irradiation might disturb cellular responsiveness to TGF-β1 through the induction of the nonfunctional latent TGF-β and downregulation of the TGF-β receptor. As known in the photoaging skin, the abnormal elastic materials termed solar elastosis accumulate. The induction of latent TGF-β seemed to show a similar trend with elastic fibers. Gibson et al. (1995) reported that latent TGF-β-binding protein has a direct role in elastic fiber structure and assembly. This will lead to greater understanding of the molecular mechanism among the UV, elastin, and TGF-β signaling pathway. In terms of in vitro-aged fibroblasts, reduced expression of mRNA for TGF-β and TbRII and decreased TGF-β binding to the receptors was observed (Mori et al., 1998). According to the results presented here, exogenous TGF-β1 might be a useful candidate to stimulate collagen production or to protect against UV radiation. Further studies are under way to prove this concept.

**Figure 1.** UVA irradiation induces the latent TGF-β1 production and reduces TGF-β type II receptor (TbRII) mRNA in the skin fibroblasts. (A) The content of TGF-β1 after UVA irradiation. The cells used in this study were from primary cultures of dermal fibroblasts, which were obtained from the sun-exposed part of healthy individuals without smoking (nonsmokers). The fibroblasts at passages 3–18 were used. The data were from three independent experiments using cells from different donors (n = 3). The fibroblasts at near confluence were changed to starvation medium (without fetal calf serum) 2 h prior to stimulation with UVA (30 J per cm²). UVA irradiation was performed with a UVA1 Sellamed System Dr Sellmeier (Sellas, Gevelsburg, Germany). The media of cultured fibroblasts were harvested 24 h after UVA irradiation and TGF-β1 contents (pg per ml) were determined by enzyme-linked immunosorbent assay (EELISA, Immuno-assay system, Prometheus, WI). The total content TGF-β1 was performed following the procedure of acid treatment in the provided protocol. The biologically active TGF-β1 was directly assayed and latent form was given by the calculation. The amounts of TGF-β1 were normalized to the protein content of conditioned media. Protein content was measured by using a micro-BCA protein assay (Pierce, Rockford, IL). The data were expressed as TGF-β1 content per protein content of corresponding conditioned medium (pg per mg). The results are presented as the mean ± SD. The significance of difference was determined by Turkey–Kramer test and is indicated on the bar as compared with the corresponding control *p < 0.05. (B) The mRNA expression of TbRII after UVA irradiation. The skin fibroblasts were UV irradiated (30 J per cm²) and total RNA was prepared 24 h after irradiation. The mRNA expression of TbRII was analyzed by reverse transcriptase–PCR. PCR of TbRII was conducted with 24 cycles of amplification. The PCR was within the linear amplification range for cDNA. To ensure that similar amounts of cDNA were used for PCR, the samples were assessed for the expression of GAPDH as a housekeeping gene. The primers of TbRII were followed as sense: 5'-TGAGTT CAACCTGGGAAAACC-3', anti-sense: 5'-ACGTGGAAGTGAAG CAACTCC-3'. Bar heights indicate mean ± SE of TbRII mRNA levels. The numbers on the x-axis represent the ratios of the signal of each PCR product to the corresponding GAPDH PCR product. *p < 0.05.

**Figure 2.** UVA irradiation inhibits type I collagen protein and induces MMP-1 mRNA expression, and its effect could be abrogated by active TGF-β1 addition. (A) Type I collagen protein level. The protein level of type I collagen was quantified by western blot analysis [no. 1: no treatment; no. 2: TGF-β1 (10 ng per ml); no. 3: UVA 30 J per cm²; no. 4: UVA 30 J per cm² + TGF-β1 (10 ng per ml)]. The media were harvested 24 h after exposure to UVA/TGF-β1 and resolved on the 5–20% polyacrylamide ready gels (Bio-Rad, Hercules, CA) under reducing conditions. The same amount of protein (30 μg) was loaded each well. After the protein was transferred to a polyvinylidine difluoride membrane (Millipore, Billerica, MA), the membrane was then incubated with the primary antibody: anti-type I collagen (Polysciences, Warrington, PA), and reacted with anti-rabbit peroxidase-conjugated IgG (DAKO, Copenhagen) and finally developed by ECL system (Amersham Pharmacia Biotech, Uppsala, Sweden). (B) MMP-1 mRNA expression. The skin fibroblasts were treated with UVA (30 J per cm²) or TGF-β1 (10 ng per ml) and total RNA was prepared after the treatments. The MMP-1 mRNA expression was performed by reverse transcriptase PCR. PCR of MMP-1 was conducted with 24 cycles of amplification. PCR was within the linear amplification range for cDNA. To ensure that similar amounts of cDNA were used for PCR, the samples were assessed for the expression of GAPDH as a housekeeping gene. The primers of MMP-1 were followed as sense: 5'-GATGCGACACT TTCCTCCACTGC-3', anti-sense: 5'-GATGTCTGTTGACCCCTGAGC-3'. The data are mean ± SE of MMP-1 mRNA levels (normalized to GAPDH mRNA levels). *p < 0.05

**REFERENCES**


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