Inhibition of Ultraviolet Radiation Mediated Extracellular Matrix Remodeling in Fibroblasts by Transforming Growth Factor-β

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Research Article

Inhibition of ultraviolet radiation mediated extracellular matrix remodeling in fibroblasts by transforming growth factor-β

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Abstract. The extracellular matrix is synthesized predominantly by fibroblasts and gives tissue its structural integrity. It is remodeled by ultraviolet radiation via the increased expression/activities of matrixmetalloproteinases (MMP) and inhibition of the tissue inhibitors of matrix metalloproteinases (TIMP). On the other hand, transforming growth factor-β (TGF-β) stimulates extracellular matrix deposition. The hypothesis of the research was the counteraction of UV radiation effects on fibroblasts by a TGF-β inducing agent (AT). Non-irradiated and UVB radiated fibroblasts were exposed to AT and examined for MMP-1 promoter activity, and expression of MMP-2, TIMP-1, and TIMP-2. AT inhibited MMP-1 promoter activity and dramatically stimulated MMP-2 expression; its effects were antagonized by UV radiation. The dramatic stimulation of TIMPs by AT predominated in non-irradiated and UV irradiated fibroblasts. TGF-β induction in fibroblasts is therefore an effective strategy to counteract UV radiation induced damage to the extracellular matrix, particularly through the induction of TIMPs.

Introduction

Chronic exposure of human skin to ultraviolet (UV) radiation results in photoaging that manifests as wrinkles, impaired wound healing and cancer. In photoaged skin there is degeneration of the extracellular matrix (ECM) from increased expression and/or activities matrix metalloproteinases (MMPs) that degrade the structural collagens (Uitto et al., 1989; Talvensaari-Mattliin et al., 1998; Scharffetter et al., 2000). The predominant MMPs are MMP-1 that cleaves interstitial collagen, and MMP-2 that degrades the basement membrane and damaged interstitial collagen. MMP-1 and MMP-2 are inhibited by tissue inhibitor of metalloproteinases (TIMPs), TIMP-1 and TIMP-2 (Khorramizadeh et al., 1999; Millis et al., 1992; Ricciarelli et al., 1999; Westermarck et al., 1999). Increased MMP expression/activity or lowered expression of TIMPs favors ECM remodeling that is characteristic of skin aging, photoaging and cancer.

Transforming growth factor-β (TGF-β) is a primary regulator of ECM. TGF-β reciprocally regulates MMPs (except MMP-2) and TIMPs to reduce the MMPs/TIMPs balance and strengthen the ECM in the dermis (Edwards et al., 1996). It inhibits MMP-1 while stimulating TIMPs and MMP-2, which removes damaged structural

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collagen (Overall et al., 1991; Mori et al., 1998; Wu et al., 2000). The reciprocal regulation of MMPs and TIMPs by TGF-β is by different mechanisms (Millis et al., 1999).

TGF-β can regulate its expression by feedback mechanisms. A polyclonal anti TGF-β antibody (AT) that neutralizes the activities of TGF-β1-3,5 (allisoforms; AB–100–NA, R&D Systems) stimulates dramatic release of TGF-β in dermal fibroblasts, epidermal keratinocytes, and a renal adenocarcinoma cell line; indicating feedback stimulation of TGF-β in these cell lines (Philip et al., 2002; Philips, 2003; Philips et al., 2004). AT exhibits TGF-β like regulation in these cells (Philip, et al., 2002; Philips, 2003; Philips et al., 2004). It inhibits MMP-1 and stimulates MMP-2 in fibroblasts, the predominant cells of the ECM (Philips et al., 2004). The effect of AT is much greater than that of TGF-β and is not mimicked by normal IgGs (Philips et al., 2004).

TGF-β is beneficial to skin health whereas UV radiation is damaging. The hypothesis of this research is the counteraction of the effects of UV radiation by AT (TGF-β inducing antibody) in fibroblasts.

Materials and Methods

Fibroblasts (Cascade Biologics, 2 donors) were not-irradiated or exposed to UVB (UV) radiation (2.5mJ) and dosed with AT (TGF-β inducing antibody, 0-3 μg/ml), in four independent experiments, for 24 hours. The cells were examined for cell viability, membrane damage, and expression of MMP-2, TIMP-1 and TIMP-2. For MMP-1 expression, cells were transfected with MMP-1 promoter-chloramphenicol acetyl transferase (CAT) plasmid prior to dosing with 0-3 μg/ml AT and analyzed for CAT expression by direct ELISA (Roche).

Cell viability/membrane damage

The cells were examined for cell viability by CellTiter 96® AQueous One or MTS assay [tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS) + electron coupling reagent (phenazine ethosulfate; PES; Promega). The medium was collected, and cells were incubated with 500μl aliquots of MTS mixture (yellow) for 2 hours at 37°C. Viable cells reduce the MTS mixture to produce a color change (brown), read spectrophotometrically at 490 nm.

The media was examined for lactate dehydrogenase (LDH) that is indicative of membrane damage. Aliquots of experimental media were incubated with a mix of LDH substrate, cofactor and dye and analyzed spectrophotometrically for product (color) formation (Sigma, Tox-7).

Protein levels: MMP and TIMPs

The media was examined for MMP–2, TIMP-1, and TIMP-2 proteins by indirect ELISA (Kirkguaard and Perry Laboratories, Inc). 100μl of medium from each sample was added to an independent well of a 96 well plate for 24 hours at 4°C. The wells were blocked with bovine serum albumin, and then incubated with MMP-2, TIMP-1 or TIMP-2 antibody (Sigma) for 1 hour at room temperature. The plate was washed with wash buffer, incubated with secondary antibody linked to peroxidase for 1 hour at room temperature, washed and subsequently incubated with peroxidase substrate until color development, which was measured spectrophotometrically at 405 nm.

MMP-1 promoter activity

Fibroblasts were co-transfected with the MMP-1 promoter -CAT (chloramphenicol acetyl transferase) plasmid, a gift from Dr. William Parks, Washington University School of Medicine, St. Louis, Missouri 63110, (10 μg per 33 mm dish) and RSVZ-gal (2 μg per 33 mm dish) with ESCORT (Sigma) prior to experimental treatments. Cells were lysed by repeated freeze/thaw cycles, measured for total protein, beta-galactosidase and CAT expression. The total protein levels were measured by BCA (bicinchoninic acid) protein assay by incubating aliquots of cell lysates with BCA/copper sulphate mix and quantitating the BCA-copper-protein complex spectrophotometrically (Pierce). Aliquots of the cells containing equal proteins were examined for CAT expression (indicator of promoter

activity) by ELISA as described above, and for β-GAL activity (for normalization of transfection/CAT ELISA data) by incubation with a colorless substrate (0-nitrophenyl β-D-galactopyranaside) that is converted by β-GAL to colored product (o-nitrophenol), read spectrophotometrically (Roche).

Data analysis

The effects of each of the AT (TGF-β inducing antibody) concentrations on non-irradiated cells were analyzed relative to control (cells not dosed with AT represented at 100% in the figures). The effect of UV radiation is stated in the text (not represented in the figures) and has been reported (Philips et al., 2003). The effect of each of the AT concentrations on UV radiated cells are presented relative to the UV effect alone (UV respective control, represented as 100% in the figure). The data was statistically analyzed by ANOVA and student t-test at 95% confidence interval (relative to the respective controls, and relative to non-irradiated and UVB radiated fibroblasts for each AT concentration).

Results

The results support our hypothesis of the photoprotective effect of AT (TGF-β inducer) on the ECM's structural integrity. Non-irradiated or UV radiated fibroblasts were treated with or without AT and examined for cell viability, membrane integrity and expression of MMPs that compromise the structural integrity of the ECM and TIMPs, which inhibit MMPs.

The cell viability and membrane integrity was not altered in non-irradiated or UV radiated cells in the absence or presence of AT (Philips et al., 2003; Philips et al., 2004).

UVB radiation at 2.5mJ did not significantly alter MMP-1 expression (Philips et al., 2003). Relative to control (no additives; 600pg/ml of CAT expression at 100%), 3 μg/ml of AT significantly inhibited MMP-1 promoter activity to 70% of control in non-irradiated cells ($p<0.05$), but not in the UVB radiated cells (Figure 1a).

UV radiation stimulated MMP-2 expression to 121% of control (70ng/ml at 100%). Relative to respective controls, AT significantly stimulated MMP-2 synthesis and the increase was significantly inhibited by UV radiation at concentrations of 0.3 μg/ml (non-irradiated: 580% of respective control; UV irradiated: 217% of respective control), 1 μg/ml (non- irradiated: 552% of respective control; UV irradiated: 435% of respective control), and 3 μg/ml (non-irradiated: 528% of respective control; UV irradiated: 421% of respective control; $p<0.05$, relative to respective controls, and between non-irradiated

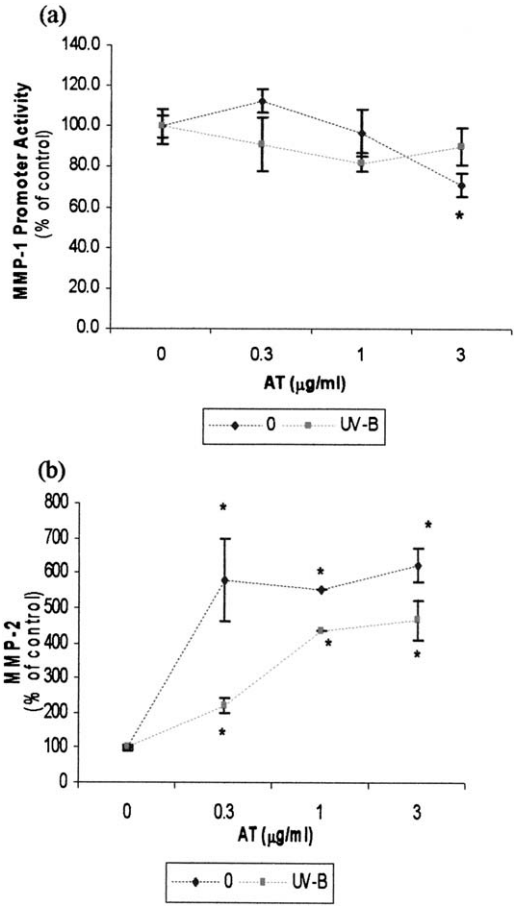

Figure 1. Effects of AT (TGF-β inducing antibody) on the regulation of expression of MMPs in non-irradiated and UVB radiated fibroblasts. MMP-1 promoter activity (a), and MMP-2 protein levels (b) were analyzed in non-irradiated (dark blue lines) or UVB irradiated fibroblasts (sky blue lines) exposed to AT (0, 0.3, 1, 3 μg/ml) for 24 hours. Data are represented relative to respective controls, cells not treated with AT in non-irradiated (0) and UVB irradiated (UV-B) cells, respectively. * = $p<0.05$, relative to respective controls. Error bars represent standard deviation, n=4.

and UVB radiated fibroblasts for each AT concentration; Figure 1b).

UV radiation inhibited TIMP-1 and TIMP-2 to about 80% of control (100%: 75 ng/ml of TIMP-1; 70ng/ml of TIMP-2). In non-irradiated and UV irradiated fibroblasts AT significantly increased TIMP-1 expression at 0.3 μg/ml (approximately 225% of respective controls), 1 μg/ml (approximately 400% of respective controls), and 3 μg/ml (non-irradiated: 528% of respective control; UV irradiated: 421% of respective control; $p<0.05$, relative to respective controls; Figure 2a). The effects of AT on TIMP-1 expression were similar in non-irradiated and UVB radiated fibroblasts, except for inhibition of TIMP-1 in UV radiated fibroblasts at 3 μg/ml ($p<0.05$, relative to non-irradiated cells at 3 μg/ml AT; Figure 2a). In non-irradiated fibroblasts and UV irradiated fibroblasts, AT similarly and significantly increased TIMP-2 expression at all concentrations; to about 320%, 620% and 670% of respective control at 0.3, 1, and 3 μg/ml, respectively ($p<0.05$, relative to respective controls; Figure 2b).

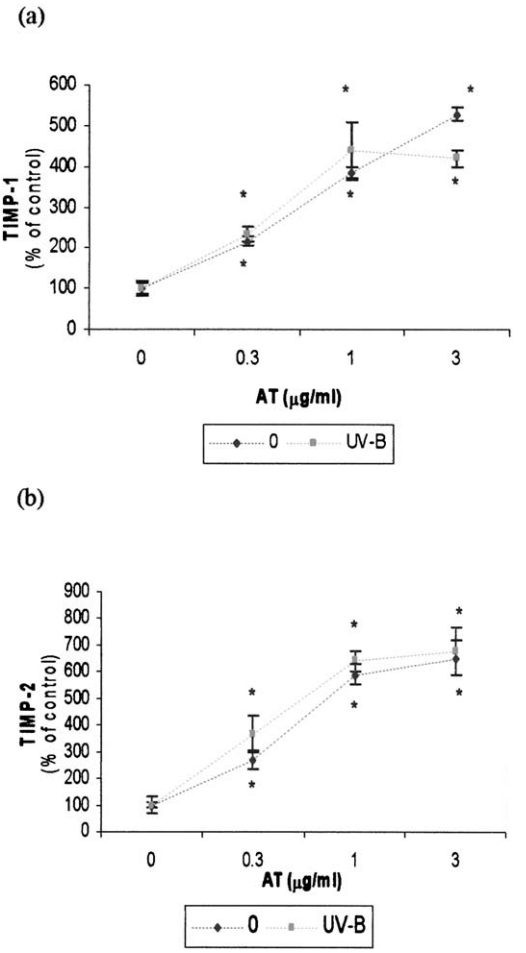

Figure 2. Effects of AT (TGF-β inducing antibody) on expression of TIMPs in non-irradiated and UVB radiated fibroblasts. TIMP-1 (a), and TIMP-2 (b) protein levels were analyzed in non-irradiated (dark blue lines) or UVB irradiated fibroblasts (sky blue lines) exposed to AT (0, 0.3, 1, 3 μg/ml) for 24 hours. Data are represented relative to respective controls, cells not treated with AT in non-irradiated (0) and UVB irradiated (UV-B) cells, respectively. * = $p<0.05$, relative to respective controls. Error bars represent standard deviation, n=4.

Discussion

Photoaging from exposure to UV radiation is associated with remodeling or degradation of the ECM due to an increase in MMP/TIMP balance. TGF-β is a potent inhibitor of ECM degradation; it inhibits the MMP/TIMP ratio in favor of ECM deposition. Hence we examined the hypothesis that induction of TGF-β in fibroblasts would inhibit damage from UV radiation with regards to ECM remodeling or degradation by counteracting the stimulation of MMP-1 and in addition stimulating TIMPs. The stimulation of TIMPs to counteract ECM damage by MMPs is a target for photoprotection.

The MMP-1 promoter activity was significantly altered by AT at 3 μg/ml but not at the lower AT concentrations (0.3 or 1 μg/ml). The inhibitory effect of 3 μg/ml AT on MMP-1 promoter activity was not observed in UV radiated cells, suggesting counteraction of the AT effect by UV radiation. UV radiation also antagonized MMP-2 stimulation by AT, though the final effect was that of AT in the UV radiation exposed fibroblasts. UV radiation counteracts the effects of AT by various mechanisms such as the down regulation of TGF-β receptors and TGF-β transducers (Taihao et al., 2004; Gambichler et al., 2007; Quan et al., 2002). Further, UV radiation stimulates AP-1 transcription factor, which directly activates MMP-1 promoter, and Smad-7, which inhibits TGF-β signaling (Taihao et al., 2004). Interestingly, the UV radiation's counteraction

of the MMP-2 induction by AT may be beneficial in protecting the basement membrane while sufficient to remove damaged interstitial collagen.

TGF-β had a predominant effect in the stimulation of TIMPs. Hence the stimulation of TIMPs by AT provides an effective means to counteract the induction of MMP-1 in photoaging or intrinsic aging and several pathologies, especially cancer associated with over expression of MMPs.

In summary, TGF- β induction via AT had overall predominant effects in combination with UV radiation in fibroblasts. It may be beneficial in counteracting UV radiation or cancer associated up-regulation of MMPs, especially through dramatic stimulation of TIMPs.

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