Vascular endothelial growth factor promotes sensitivity to ultraviolet B–induced cutaneous photodamage
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Introduction

A single exposure to ultraviolet B (UVB) irradiation (290-320 nm wavelength) induces skin alterations that include erythema, vascular hyperpermeability, dilation of dermal blood vessels, and epidermal hyperplasia. All are characteristic of a sunburn reaction. The sun is the most common source of UVB; prolonged exposure of human skin to UVB results in the formation of wrinkles, the degradation of matrix macromolecules, the development of elastosis, and an enhanced risk for epithelial skin cancer. Pronounced vascular changes observed after acute UVB irradiation indicate that the cutaneous vasculature plays an important role in the mediation of acute photodamage.

Several angiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor, and interleukin-8 (IL-8), are up-regulated after acute UVB irradiation of the skin. In contrast, we found that expression of the angiogenesis inhibitor thrombospondin-1 (TSP-1) was down-regulated by UVB irradiation,18 and VEGF transgenic mice develop chronic inflammatory skin damage associated with degradation of the dermal matrix and enhanced vascularization. Systemic treatment with an anti-VEGF blocking antibody reduced the sensitivity of wild-type mice to acute UVB irradiation without inhibiting post-UVB repair. Our results reveal that VEGF promotes the cutaneous damage that occurs after UVB exposure and that the VEGF signaling pathway might serve as a novel target for the prevention of UVB-induced photodamage. (Blood. 2005;105:2392-2399)

Acute ultraviolet B (UVB) irradiation of the skin results in erythema, vasodilation, edema, and angiogenesis, which is associated with the expression of vascular endothelial growth factor (VEGF) by epidermal keratinocytes. It is unclear, however, whether VEGF is required for the damage or repair process that occurs in the skin on UVB exposure. We subjected transgenic mice that overexpress VEGF, and their wild-type littermates, to graded doses of acute UVB irradiation. The skin of VEGF-overexpressing mice was highly photosensitive and became erythematic when exposed to half the UVB dose required to induce erythema in wild-type mice. Erythema was associated with proliferating dermal endothelial cells, cutaneous edema, and inflammatory cell infiltration. When subjected to 10 weeks of low-level UVB irradiation, no major changes were observed in wild-type mice, whereas VEGF transgenic mice developed skin damage associated with degradation of the dermal matrix and enhanced vascularization. Systemic treatment with an anti-VEGF blocking antibody reduced the sensitivity of wild-type mice to acute UVB irradiation without inhibiting post-UVB repair. Our results reveal that VEGF promotes the cutaneous damage that occurs after UVB exposure and that the VEGF signaling pathway might serve as a novel target for the prevention of UVB-induced photodamage. (Blood. 2005;105:2392-2399)

Angiogenesis—the formation of new blood vessels from pre-existing vessels—is characterized by increased microvascular permeability and by the migration and proliferation of vascular endothelial cells, leading to the formation of new capillaries. In healthy skin, angiogenesis is restricted to the peri-follicular vasculature during the growth phase of hair follicles. Skin can initiate a rapid angiogenic response after tissue injury and during inflammatory reactions, however, and skin carcinogenesis is associated with pronounced vascular proliferation. We have previously identified VEGF as a major skin angiogenesis factor that is strongly up-regulated in the hyperplastic epidermis of patients with inflammatory diseases such as psoriasis, with healing wounds, and with other skin diseases characterized by enhanced angiogenesis. Moreover, the targeted overexpression of VEGF in the epidermis of transgenic mice resulted in enhanced skin vascularization, and VEGF transgenic mice develop chronic inflammatory skin lesions after the acute induction of skin inflammation. Because vascular activation and cutaneous inflammation are key features of the acute and chronic UVB responses and because we and others have observed the up-regulation of epidermal VEGF expression after acute and chronic UVB irradiation in vivo and in vitro, we hypothesized that VEGF mediates the cutaneous damage that occurs after UVB irradiation.

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To directly investigate the role of VEGF in mediating UVB-induced photodamage, we first compared the photosensitivity of VEGF transgenic mice and of wild-type mice. We found that transgenic expression of VEGF specifically in the skin of mice enhanced their sensitivity to acute UVB-induced skin damage. Conversely, systemic blockade of VEGF markedly reduced the skin photosensitivity of wild-type mice. Moreover, chronic UVB exposure, at a dose that did not induce detectable skin damage in wild-type mice, resulted in pronounced cutaneous photodamage and skin wrinkling in VEGF transgenic mice. Together, these findings indicate that VEGF serves as a novel target for preventing acute UVB damage of the skin and cutaneous photoaging.

Materials and methods
UVB irradiation regime
Eight-week-old male FVB wild-type mice or transgenic mice that overexpress VEGF-A164 in the epidermis under control of the human keratin 14 promoter11,18 were exposed to graded doses of a single UVB irradiation, using a bank of 4 equally spaced fluorescent lamps (Southern New England Ultraviolet, Branford, CT).24 The height of the lamps was adjusted to deliver 0.35 mW/cm² at the dorsal skin surface. The minimal erythema dose (MED) was determined by irradiation of square areas of back skin with 7 different doses of UVB, ranging from 1.8 × 10⁻⁵ J/cm² to 1.26 × 10⁻³ J/cm² (n = 5 per group). An additional skin area was sham irradiated. Erythema formation was evaluated daily for up to 9 days by 2 independent observers, and ear thickness was measured daily as described.25

In additional experiments, wild-type mice were treated with 50 μg goat antimouse VEGF-neutralizing antibody (R&D Systems, Minneapolis, MN), or with 50 μg control isotype immunoglobulin G (IgG) by intraperitoneal injection 24 hours before and 24 hours after a single irradiation with 7 graded doses of UVB, as described in the previous paragraph (n = 5 per group). Moreover, 8-week-old male VEGF transgenic mice and their wild-type littermates were irradiated 3 times weekly for 10 weeks with single doses ranging from 1.8 to 2.4 × 10⁻² J/cm² (n = 10 per genotype). No acute sunburn reactions were observed. Control mice were sham irradiated. Samples of back skin were snap-frozen or fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS). The Massachusetts General Hospital Subcommittee on Research Animal Care approved all animal studies.

RT-PCR analysis of VEGF isoform expression
Female albino hairless mice (n = 30; Hoshino Laboratory Animals, Yashio, Japan) were exposed to single doses of UVB irradiation (200 mJ/cm²) at 8 weeks of age from Toshiba FL-20 SE fluorescent lamps (Toshiba, Tokyo, Japan) that deliver energy in the UVB range (280-340 nm), as described.10 Mice were humanely killed on days 2, 4, 6, 8, and 13 (n = 5 per time point) after irradiation. Total RNA was isolated from the dorsal skin of these mice and of 5 nonirradiated control mice using TRI reagent (Sigma, St Louis, MO). Thereafter, cDNA was reverse transcribed using the SuperScript first-strand synthesis system (Invitrogen, Carlsbad, CA), as described.10 Vascular endothelial growth factor (VEGF) and G3PDH were amplified using Platinum Taq DNA polymerase (Invitrogen) for 30 cycles at 58°C. Polymerase chain reaction (PCR) products were fractionated by gel electrophoresis. The following primers were used: VEGF, 5'-CAACATCAC-CAAGTGAGAA/GATCT/GAATTCC-3' and 5'-TCAACGGCCTTGGTGTGAC-3'; G3PDH, 5'-GAAGGTGAAGGTCGGAGTC-3' and 5'-GAACATGATGATGAGATGG-/GATTCC-3'. Gene expression levels of the 3 major VEGF isoforms—VEGF120, VEGF164, and VEGF188—were normalized to G3PDH expression levels and were quantified by using National Institutes of Health (NIH) Image 1.62 software. Their relative expression levels were analyzed by the unpaired Student t test and are expressed as mean ± SD (n = 5 per group).

Enzyme-linked immunosorbent assay for VEGF-A
Skin lysates were obtained from ear skin 48 hours after UVB irradiation or sham irradiation (n = 3 per group). Tissues were homogenized, and murine VEGF-A levels were quantified by an enzyme-linked immunosorbent assay (ELISA) (Quantikine M; R&D Systems), as described.25 This ELISA also detected the transgenic product, murine VEGF164. Experiments were performed at least twice with comparable results. Statistical analysis was performed using the unpaired Student t test.

Immunofluorescence and computer-assisted morphometric analysis
Immunofluorescence analysis was performed on 5-μm frozen sections, as described previously,22 using a monoclonal rat antimouse CD31 antibody (BD Biosciences PharMingen, San Diego, CA), a monoclonal rat antimouse VEGF receptor (VEGFR)–2 antibody (BD Biosciences PharMingen), a rabbit anti-Ki-67 antibody (Novocastra Laboratories, Newcastle, United Kingdom), and corresponding secondary antibodies labeled with Alexa Fluor 488 or 594 (Molecular Probes, Eugene, OR). Representative sections were obtained from the skin of UVB-irradiated and of sham-irradiated mice (n = 5 per group) and were analyzed using a Nikon E-600 microscope (Nikon, Melville, NY) equipped with a Plan Fluor 10 × objective with an aperture of 0.30 (Nikon), a Plan Flur 20 × objective with an aperture of 0.50, and a Plan Flur 40 × objective of 0.75. The image medium was air, and images were acquired using Adobe Photoshop (Adobe Systems, San Jose, CA). Images were captured with a Spot 1.3.0 digital camera (Diagnostic Instruments, Sterling Heights, MI), and morphometric analyses were performed using IP-LAB software (Scanalytics, Fairfax, VA), as described previously.22 Three different fields of each section were examined at 10 × magnification, and the number of vessels per square millimeter, the average vessel size, and the relative area occupied by blood vessels were determined in the dermis, in an area within 200 μm distance from the epidermal–dermal junction. The unpaired Student t test was used to analyze differences in microvessel density and vascular size. In addition, paraffin sections were obtained from the skin of the same mice, and routine hematoxylin-eosin staining, Trichrome staining, and Luna aldehyde fuchsin (LUNA) staining were performed as described previously.26

Subcutaneous micropocket assay
Eight-week-old male FVB mice were implanted with pellets containing or not containing recombinant human VEGF 165 (500 ng/pellet) (R&D Systems) subcutaneously by surgical incision. Pellets were prepared as previously described.27,28 Five days after implantation, mice were irradiated with a single UVB dose of 5.4 × 10⁻² J/cm². Control mice were sham irradiated (n = 5 per each group). After 48 hours, back skin samples were snap-frozen or fixed in 4% paraformaldehyde for histologic analysis, as described above.

Results
VEGF164 is the predominant VEGF isoform in normal and in UVB-irradiated mouse skin
In healthy mouse skin, we found VEGF164 to be the predominantly expressed VEGF isoform, compared with low-level mRNA expression of VEGF120 and VEGF188 (Figure 1A). Forty-eight hours and 4 days after a single exposure of mouse skin to a dose of 200 mJ UVB, expression of VEGF164 was significantly (P < .001) up-regulated (day 2, 1.09 ± 0.26; day 4, 0.98 ± 0.19; nonirradiated, 0.48 ± 0.05). We also found enhanced expression of VEGF120 (day 2, 0.58 ± 0.11, P < .01; day 4, 0.39 ± 0.05, P < .001) and VEGF188 (day 2, 0.47 ± 0.05, P < .001; day 4, 0.61 ± 0.12, P < .01); however, VEGF164 was the most strongly expressed isoform at all time points studied. VEGF mRNA expression levels returned to background levels of nonirradiated skin at 8 days after
irradiation (Figure 1A). Together, these results reveal that VEGF164 is the predominant VEGF isoform induced by UVB irradiation of the skin.

Enhanced cutaneous photosensitivity in VEGF164 transgenic mice

We next investigated whether targeted overexpression of VEGF164 might play a biologic role in the mediation of acute cutaneous photodamage. Therefore, we determined the MED, a parameter used to quantify cutaneous photosensitivity, at 48 hours after a single UVB irradiation of the dorsal skin. Graded doses of UVB were applied to wild-type and to VEGF164-overexpressing mice (Figure 1B). The skin of wild-type mice did not show signs of erythema until it was exposed to 7.2 × 10^{-2} J/cm² UVB, but VEGF-overexpressing mice developed skin erythema on irradiation with 3.6 × 10^{-2} J/cm². At doses of 7.2 × 10^{-2} J/cm² UVB and higher, VEGF-overexpressing mice developed further symptoms, including edema (Figure 1B). These mice also developed erythema and edema of the ear skin after receiving only 3.6 × 10^{-2} J/cm² UVB (Figure 2B,D), whereas their wild-type littermates did not (Figure 2A,C). Hence, the MED is 7.2 × 10^{-2} J/cm² UVB for wild-type mice and 3.6 × 10^{-2} J/cm² UVB for VEGF-overexpressing mice.

Forty-eight hours after UVB irradiation with one MED, the ear skin of VEGF transgenic mice showed characteristic features of acute photodamage, including epidermal hyperplasia, single keratinocyte necrosis, marked dermal edema, vessel dilation, and inflammatory cell infiltration (Figure 2F). In contrast, wild-type skin exposed to the identical UVB dose did not show any major histologic changes (Figure 2E).

Immunohistochemical analysis with antibodies against the endothelial cell membrane molecule CD31 and the proliferation marker Ki-67 revealed enlarged cutaneous vessels in the skin of VEGF-overexpressing mice by 48 hours after irradiation with 3.6 × 10^{-2} J/cm² UVB and pronounced proliferation of epidermal keratinocytes and endothelial cells (Figure 2H) in these vessels. In contrast, CD31⁺ vessels were not dilated in the skin of wild-type mice that received the same dose of UVB, and no proliferating endothelial cells were detected (Figure 2G). The ears of VEGF-overexpressing mice were also significantly thicker, whereas no ear swelling was detected after treatment with the lower dose of 1.8 × 10^{-2} J/cm² UVB (P < .001; Figure 2I). Ear thickness returned to baseline levels 7 days after irradiation (Figure 2I). No ear swelling response was seen in wild-type mice after irradiation with 3.6 × 10^{-2} J/cm² UVB (Figure 2I). Together, these findings revealed that the skin of VEGF transgenic mice had greater photosensitivity than that of wild-type controls.

To quantify the amount of VEGF overproduction in the skin of transgenic mice, we measured VEGF protein levels in skin lysates. Using a specific ELISA for murine VEGF, we observed a significant increase in the level of this protein in the skin of the transgenic mice.
mice by 48 hours after irradiation with $3.6 \times 10^{-2}$ J/cm$^2$ UVB (138.1 $\pm$ 13.0 pg/mg protein), compared with nonirradiated transgenic skin (53.2 $\pm$ 6.6 pg/mg) ($P < .001$; Figure 2J). In wild-type mice, UVB irradiation with the same dose did not significantly increase cutaneous VEGF protein levels (25.8 $\pm$ 7.3 pg/mg) compared with nonirradiated skin (14.8 $\pm$ 5.9 pg/mg) (Figure 2J). VEGF protein levels were also significantly higher in untreated transgenic mice than in untreated wild-type mice ($P < .01$).

**Implantation of VEGF-releasing pellets enhances UVB-induced cutaneous angiogenesis in wild-type mice**

Because UVB-induced vascular alterations in VEGF164 transgenic mice might have been the result of short-term UVB-induced up-regulation and of stromal changes induced by long-term delivery of transgenic VEGF, we next implanted VEGF165 slow-releasing pellets subcutaneously into FVB wild-type mice, followed by irradiation with a single UVB dose of $5.4 \times 10^{-2}$ J/cm$^2$ after 5 days. VEGF165 is the human homolog of murine VEGF164. Two days after UVB irradiation, histologic analyses revealed marked tissue edema in mice implanted with VEGF165-releasing pellets (Figure 3D) but only minor edema in sham-irradiated mice bearing VEGF-releasing pellets (Figure 3B) and in UVB-irradiated mice bearing control pellets (Figure 3C). No major changes were seen in sham-irradiated mice bearing control pellets (Figure 3A). Immunofluorescence stains for CD31 revealed pronounced neovascularization and vessel enlargement in mice bearing VEGF-releasing pellets 48 hours after UVB irradiation (Figure 3H), whereas less pronounced vascular enlargement was found in sham-irradiated mice with VEGF pellets (Figure 3F) and in UVB-irradiated mice bearing control pellets (Figure 3G). No major vascular changes were observed in sham-irradiated mice implanted with control pellets (Figure 3E).

Morphometric analyses of CD31-stained skin sections confirmed these findings. We observed a significant increase in the cutaneous area that was covered by vessels (8.26% $\pm$ 0.61%; $P < .001$; Figure 3I), the average vessel size (554.9 $\pm$ 86.2 $\mu$m$^2$; $P < .05$; Figure 3J), and the average vessel density (151.1 $\pm$ 20.3 vessels/mm$^2$; $P < .01$; Figure 3K) in UVB-irradiated mice implanted with VEGF165-releasing pellets compared with UVB-irradiated mice implanted with control pellets (average vessel area, 4.37% $\pm$ 0.86%; vessel size, 414.1 $\pm$ 49.4 $\mu$m$^2$; vessel density, 103.2 $\pm$ 6.5 vessels/mm$^2$). Sham-irradiated mice implanted with VEGF-releasing pellets showed a significant increase in average cutaneous area covered by vessels (4.16% $\pm$ 0.54%; $P < .01$; Figure 3I) and in vessel size (348.4 $\pm$ 33.6 $\mu$m$^2$; $P < .05$; Figure 3J), but not in vessel density (119.6 $\pm$ 9.3 vessels/mm$^2$; Figure 3K) compared with mice bearing control pellets (vessel area, 2.16% $\pm$ 0.45%; vessel size, 241.3 $\pm$ 45.5 $\mu$m$^2$; vessel density, 102.6 $\pm$ 6.1 vessels/mm$^2$). Taken together, the UVB-induced vascular changes were most pronounced in the presence of temporarily increased local levels of VEGF165, indicating that VEGF165 contributes to the mediation of UVB effects on the skin, in accordance with the results obtained in the VEGF164 transgenic mice.

**Systemic blockade of VEGF activity reduces skin sensitivity to acute UVB irradiation but does not inhibit tissue repair**

To investigate whether VEGF is necessary for the acute cutaneous UVB response, we treated wild-type mice with a neutralizing anti–VEGF antibody 24 hours before and 24 hours after irradiation with a single dose of UVB. Marked tissue edema was detected in control IgG-treated mice 48 hours after irradiation with $5.4 \times 10^{-2}$ J/cm$^2$ UVB (Figure 4C), but not in anti–VEGF antibody-treated mice (Figure 4D). After treatment with a dose of $7.2 \times 10^{-2}$ J/cm$^2$ UVB or higher, tissue edema and inflammatory cell infiltration were slightly reduced in VEGF-antibody-treated mice (Figure 4E-F) compared with control IgG-treated mice. Furthermore, UVB-induced damage of collagen fibers was reduced in the anti–VEGF antibody-treated mice (Figure 4H) compared with IgG-treated controls (Figure 4G).

Immunofluorescence analysis of CD31 expression revealed that by 48 hours after irradiation with $5.4 \times 10^{-2}$ J/cm$^2$ UVB, the dermal blood vessels of IgG-treated control mice were greatly enlarged (Figure 4I), whereas no such changes were observed in the skin of anti–VEGF antibody-treated mice (Figure 4J). These findings were confirmed by computer-assisted morphometric analysis of CD31-stained sections. We observed a significant reduction in the percentage of total tissue area covered by blood vessels (2.28 $\pm$ 0.19%; $P < .05$; Figure 4O) and in the average vessel size (211.1 $\pm$ 16.8 $\mu$m$^2$; $P < .01$; Figure 4P) of the anti–VEGF antibody-treated mice compared with controls (3.14% $\pm$ 0.29% of area; vessel size, 452.3 $\pm$ 40.1 $\mu$m$^2$). No differences in vessel density (number of vessels/mm$^2$) were observed between the treatment groups (Figure 4Q). Accordingly, the MED at 48 hours after UVB irradiation was higher in anti–VEGF antibody-treated mice (7.8 $\pm$ 2.08 $\times 10^{-2}$ J/cm$^2$) than in control IgG-treated mice (6.0 $\pm$ 1.04 $\times 10^{-2}$ J/cm$^2$).
We next investigated whether inhibition of VEGF activity might impair the natural cutaneous repair processes after UVB irradiation. At 14 days after UVB irradiation with a single dose of 7.2 × 10^{-2} J/cm², no edema and other histologic signs of cutaneous damage were observed in the skin of mice treated with control IgG (Figure 4K) or with anti-VEGF antibody (Figure 4L). Moreover, no differences in extracellular matrix structure were found between the control IgG-treated (Figure 4M) and the anti-VEGF antibody-treated mice (Figure 4N). Immunofluorescence staining revealed epidermal hyperplasia, inflammatory cell infiltration, and degradation of the collagen matrix in these mice (Figure 5I) but not in constitutively VEGF-overexpressing mice (Figure 7B, F).

Overexpression of VEGF promotes chronic UVB-induced skin damage

We next investigated what effect elevated levels of cutaneous VEGF would have on the skin after chronic low-dose UVB irradiation, such as that associated with premature aging in humans. After 10 weeks of irradiation, 3 × per week, with 1.8 × 10^{-2} J/cm² UVB, the skin of wild-type mice did not show any signs of UVB damage and was comparable to nonirradiated skin (Figure 5A-C, E). In contrast, the skin of VEGF-overexpressing mice contained many wrinkles (Figure 5B, F). Histologic analysis revealed epidermal hyperplasia, inflammatory cell infiltration, tissue edema, and degradation of the collagen matrix in these mice (Figure 5I) but not in constitutively VEGF-overexpressing mice that did not undergo irradiation (Figure 5H) or in wild-type mice that did or did not undergo irradiation (Figure 5G, I). Moreover, degradation of elastic fibers was regularly detected in skin samples taken from chronically VEGF-overexpressing mice after chronic irradiation (Figure 5N) but not in transgenic mice that did not undergo irradiation (Figure 5L) or in wild-type mice that did or did not undergo irradiation (Figure 5K, M).

At a macroscopic level, the skin of VEGF-overexpressing mice that received chronic UVB treatment was more vascularized (Figure 6D) than the skin of transgenic mice that had not undergone irradiation (Figure 6B) or of wild-type mice (Figure 6A, C). Immunohistochemical analysis of CD31 revealed a significant number of enlarged CD31+/VEGFR-2+ vessels in the skin of VEGF-overexpressing mice after chronic irradiation (Figure 7D, H, L) whereas little or no VEGFR-2 expression was found on CD31+ vessels in nonirradiated transgenic mice (Figure 7B, F, J) or in wild-type mice (Figure 7A, C, E, G, I, K). Wild-type mice also showed no increase in the number of CD31+ vessels after chronic UVB irradiation (Figure 7A, C). An increased number of tortuous vessels were found in the skin of nonirradiated VEGF-overexpressing mice, in agreement with previous results.

Morphometric analyses of CD31-stained skin sections confirmed these findings. We observed a significant increase in the cutaneous area covered by vessels (8.36% ± 0.85%; P < .001; Figure 7M), in average vessel size (523.3 ± 42.5 μm²; P < .001; Figure 7N), and in average vessel density (251.3 ± 26.6 vessels/mm²; P < .001; Figure 7O) in chronically UVB-irradiated mice after chronic VEGF-overexpression. On the other hand, tissue edema and inflammatory cell infiltration were pronounced in control IgG (E) and anti–VEGF antibody-treated mice (F). Hematoxylin-eosin-stain (A-F), and immunofluorescence for CD31 (G-I) and VEGFR-2 (J-L) were used. Scale bars, 200 μm (A-F, K, L); 100 μm (G-J, M, N). A Plan Fluor 10 objective with an aperture of 0.30 was used for panels A-F, K, and L. A Plan Fluor 20 objective with an aperture of 0.50 was used for panels G-J, M, and N.

Computer-assisted morphometric analysis revealed reduced vessel area (O) and reduced vessel size (P) in anti–VEGF antibody-treated mice (Q) compared with control IgG-treated mice. 48 hours after UVB exposure. Vessel density (number of vessels per square millimeter) was not significantly different between each group (Q). Data are expressed as mean ± SD (n = 5). **P < .01; *P < .05.
One of the major acute effects of UVB irradiation is the induction of erythema, cutaneous inflammation, vascular leakage, and edema formation, all of which are characteristics of sunburn. Recent studies have revealed that UVB irradiation of the skin induces an angiogenic switch, associated with the up-regulation of proangiogenic factors such as VEGF and with the down-regulation of endogenous angiogenesis inhibitors such as TSP-1 and interferon-β (IFN-β). However, it has remained unclear whether the acute vascular activation is part of a physiologic repair mechanism or whether the angiogenic response causally contributes to cutaneous photodamage. Surprisingly, the results of our study reveal that the induction of skin angiogenesis leads to enhanced photosensitivity and that VEGF plays a major causal role in the induction of cutaneous photodamage.

We show that targeted overexpression of VEGF164 in epidermal keratinocytes—the major cellular source of VEGF after UVB irradiation of wild-type mice—leads to enhanced photosensitivity. Immunofluorescence analysis with antibody against CD31 (A-L) demonstrated prominent vascularization in the papillary dermis of VEGF-overexpressing mice (D). Scale bars, 3 mm. Images were taken using a Nikon Coolpix 950 digital camera.

Figure 6. Increased vascularization in the skin of VEGF-overexpressing mice after long-term UVB irradiation. Compared with their wild-type littermates (A), VEGF-overexpressing mice that did not undergo irradiation had a slight increase in cutaneous vascularization (B). No changes were observed in wild-type mice (C) after long-term UVB irradiation, but increased cutaneous vascularization, with prominent enlargement of blood vessels, was observed after chronic UVB treatment of the skin of VEGF-overexpressing mice (D). Scale bars, 3 mm. Images were taken using a Nikon Coolpix 950 digital camera.
irradiation of normal murine and human skin\textsuperscript{9,10,20-23}—is sufficient to induce all the characteristic features of cutaneous photodamage at a subthreshold UVB dose. This indicates that selective up-regulation of VEGF mediates the damaging effects of acute UVB irradiation of the skin. Although protein levels of the distinct VEGF isoforms were not evaluated in the present study, we found that, at the mRNA level, VEGF164 is the predominantly expressed VEGF isoform in healthy mouse skin and after UVB irradiation. In contrast, the other major VEGF isoforms, VEGF120 and VEGF188, were expressed only at lower levels, though all 3 isoforms were up-regulated by UVB at similar ratios. Together with our findings that temporally enhanced local levels of VEGF165—the human homolog of murine VEGF164—promoted the cutaneous vascular response to UVB irradiation, these results indicate that VEGF164 is the major player involved in the mediation of UVB-induced cutaneous photodamage. They also validate the use of the VEGF164 transgenic mouse model, though combined transgenic overexpression of all 3 major VEGF isoforms ideally might provide an even more appropriate model to mimic the normal UVB response. Moreover, systemic blockade of VEGF activity markedly reduced the photosensitivity of wild-type mice, revealing that VEGF is required for the acute UVB response of the skin. Importantly, we found that the inhibition of VEGF activity did not impair the physiologic repair processes after UVB irradiation, in agreement with previous results obtained in wound healing models. Together, these findings identify VEGF as a novel therapeutic target for the prevention of acute UVB damage/sunburn reactions.

How does VEGF promote UVB-induced skin damage? Our results indicate that in response to UVB exposure, VEGF promotes vessel dilation and vascular proliferation (as shown by the detection of Ki-67–positive proliferating endothelial cells) and vascular hyperpermeability (as shown by increased ear swelling and edema formation). Together, these changes represent the major vascular abnormalities observed in the skin after acute UVB irradiation. Moreover, we previously showed that overexpression of VEGF in the skin leads to a proinflammatory state, with enhanced leukocyte rolling and adhesion in the skin of VEGF transgenic mice,\textsuperscript{18} and to an increased response to acute proinflammatory stimuli.\textsuperscript{19,29} These findings are in agreement with our present results, showing that VEGF overexpression in the skin increases the inflammatory response after UVB irradiation. Although transgenic VEGF overexpression creates a proangiogenic environment that makes the skin more photosensitive, endogenous VEGF is also up-regulated in the skin within 48 hours of UVB irradiation, and blockade of VEGF in wild-type mice markedly reduces photosensitivity. These findings indicate that the up-regulation of endogenous VEGF is an important mediator of the UVB response in healthy skin.

The finding that systemic blockade of VEGF in wild-type mice inhibits acute photodamage and angiogenesis at lower (but not higher) doses of UVB indicates that additional factors might contribute to the angiogenic UVB response. Up-regulation of basic fibroblast growth factor, a potent proangiogenic mediator, and down-regulation of IFN-\(\beta\), a cytokine with antiangiogenic activity, have been previously found after a single dose of UVB irradiation of mouse ear skin.\textsuperscript{7} The endogenous angiogenesis inhibitor TSP-1 is expressed by epidermal keratinocytes in healthy skin\textsuperscript{30} and is thought to contribute to the quiescence of the cutaneous vasculature in healthy human skin.\textsuperscript{31} TSP-1 expression was dramatically down-regulated after acute UVB irradiation of the skin, and the acute UVB response was found to be increased in TSP-1–deficient mice. These findings all indicate that UVB exposure shifts the balance between the levels of proangiogenic and antiangiogenic mediators, leading to an angiogenic switch from normal vascular quiescence to a highly proangiogenic environment. The direct functional contributions of other mediators of angiogenesis, such as IL-8 and TSP-2, remain to be investigated.

Importantly, our results show that VEGF also plays a crucial role in the mediation of chronic cutaneous photodamage. A 10-week course of irradiation with a subthreshold dose of UVB, which did not induce any detectable alterations in wild-type skin, resulted in the formation of cutaneous wrinkles and in the classical features of chronically UVB-damaged skin. These features included epidermal hyperplasia, degradation of collagen and elastic fibers within the dermis, and inflammatory cell infiltration.

We have previously shown that transgenic overexpression of TSP-1 in the mouse epidermis reduced dermal damage, wrinkle formation, and angiogenesis after chronic UVB irradiation.\textsuperscript{32} Together with our current findings, angiogenesis inhibition appears to be a novel approach for the prevention of chronic UVB-induced skin aging. Systemic anti-VEGF therapy of cancer patients has been shown to be associated with a number of adverse effects\textsuperscript{33,34}; therefore, it does not appear to represent a feasible strategy for protecting healthy persons from the effects of UVB. However, it is conceivable that VEGF activity in the skin might be blocked by the synthetic small molecule inhibitors of VEGF signaling under investigation in cancer clinical trials.\textsuperscript{33,34} The molecular properties and the small size of these drugs indicate that they might efficiently penetrate the skin barrier after topical treatment. Additional studies are needed to investigate whether the inhibition of cutaneous angiogenesis, by blockade of VEGF activity or by overexpression of endogenous angiogenesis inhibitors such as TSP-1, might also prevent skin carcinogenesis induced by chronic UVB irradiation.

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References


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