LETTER TO THE EDITOR

Tazarotene-Induced Gene 3 May Affect Inflammatory Angiogenesis in Psoriasis by Downregulating Placental Growth Factor Expression

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Dear Editor:

Histologically, the inflammatory angiogenesis that is associated with psoriasis differs from classical angiogenesis, with the former showing dilation of existing blood vessels accompanied by greater permeability and tortuosity, while the latter is associated with new blood vessels sprouting[1,2]. To date, various molecules, including interleukin-8, tumor necrosis factor (TNF)-α, vascular endothelial growth factor (VEGF), thymidine phosphorylase, basic fibroblast growth factor (bFGF), and chemokine ligand 2, have been reported to stimulate angiogenesis in psoriatic lesions. Furthermore, angiopoietin-1, angiopoietin-2, and the receptor tyrosine kinase Tie2 (Tie2) are overexpressed in psoriatic lesions compared with uninvolved skin[3-5]. Placental growth factor (PlGF), a homologue of VEGF, is usually expressed at very low levels in the skin compared with VEGF, but it is upregulated during events associated with neoangiogenesis, including wound healing and anagen-associated angiogenesis in hair follicles, and during acute cutaneous inflammation, and it is upregulated within primary and metastatic melanomas. In PlGF-deficient mice, the vascular leakage induced by skin damage, allergens, and neurogenic inflammation is reduced. The PlGF-mediated activation of VEGF receptor (VEGFR)-1 synergizes with the VEGF-mediated activation of VEGFR-2 to induce a stronger angiogenic response[6].

Previously, we undertook a comparative study that investigated the clinical efficacies of topical monotherapies administered twice each day for 12 weeks. Tazarotene gel (0.1%) (Tazorac®; Allergan, Irvine, CA, USA) was applied to psoriatic plaques on one side of the patient's body and calcitriol gel (3 μg/g) (Silkis®; Galderma, Lausanne, Switzerland) was applied to psoriatic plaques on the other side of the patient's body, and the lesions were scored over a 12-week period using the overall lesional assessment tool. We found that PlGF expression declined after 4 weeks and 12 weeks of treatment with tazarotene (Fig. 1A~C), and that Tie2 expression, which is high in the endothelial cells of untreated psoriatic lesions, only declined after 12 weeks of treatment with tazarotene, which marked the end of tazarotene treatment in the study. Furthermore, we found reduced levels of dilatation and tortuosity associated with the blood vessels within those psoriatic lesions that showed greater clinicopathologic improvements after tazarotene treatment[7]. To explore the mechanisms of action underlying the suppression of inflammatory angiogenesis by tazarotene in greater detail, we undertook in situ hybridization using the tazarotene-induced gene 3 (TIG3) riboprobe. TIG3 mRNA expression may be associated with antiangiogenic events at the molecular level that are induced by tazarotene. We also investigated the immunohistochemical expression of the p53 and B-cell

Received August 9, 2012, Revised December 30, 2012, Accepted for publication April 4, 2013

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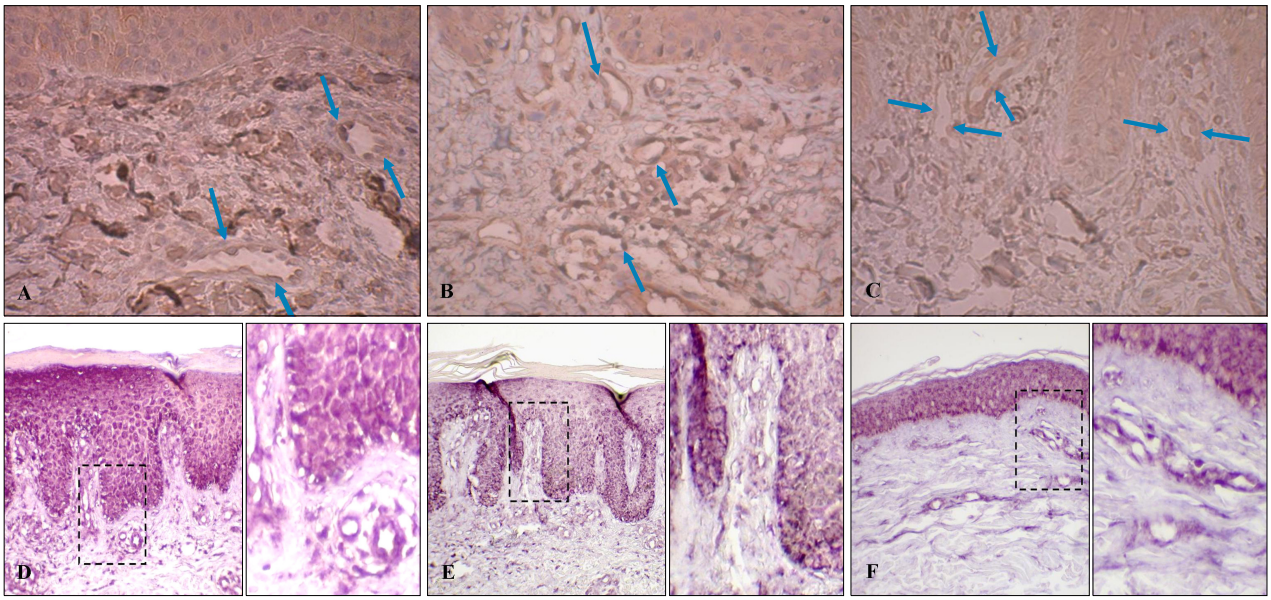

Fig. 1. Placental growth factor (PlGF) (A～C) and tazarotene-induced gene 3 (TIG3) (D～F) expression in psoriatic lesions. (A) PlGF was expressed in the keratinocytes and endothelial cells, and was overexpressed before tazarotene treatment. PlGF expression reduced after treatment with tazarotene at (B) 4 weeks and (C) 12 weeks[7]. (D～F) TIG3 expression within the epidermal keratinocytes and the dermal vascular endothelial cells (D) before treatment with tazarotene and (E) after 4 weeks of tazarotene treatment. (F) TIG3 expression increased after 12 weeks of tazarotene treatment. (A～C) PlGF stain, ×400; (D～F) TIG3 stain, ×200; dotted lines, ×400.

lymphoma 2 (Bcl-2) proteins to determine whether TIG3 is involved in processes other than the direct suppression of PlGF and Tie2, including the induction of apoptosis within the dermal vascular endothelial cells. Each 4-㎛ paraffin-embedded tissue section was treated with proteinase K (InnoGenex Universal ISH kit; InnoGenex, San Ramon, CA, USA) at 60°C for at least 12 hours, and then the tissue was incubated with the preheated hybridization buffer (Biognostik, Göttingen, Germany), which involved heating the tissue and the buffer to 95°C followed by maintenance at 30°C for 3 hours. The tissues were subsequently incubated with the TIG3 (5-GTTGACCCGATAGCAACAGCCT-3) fluorescein isothiocyanate (FITC)-labeled probe (Biognostik) at 30°C for 12 hours. The sections were then incubated with the biotinylated anti-FITC antibody (InnoGenex Universal ISH kit) at room temperature for 60 minutes and the 4-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate mixture for color development. The tissues were photographed using an optical microscope. For the immunohistochemical studies, the epitopes were retrieved by autoclaving the 4-㎛ deparaffinized sections at 121°C for 10 minutes in citrate-buffered saline (pH 6.0). Immunoperoxidase staining procedures were carried out in the usual manner using the following monoclonal antibodies: mouse anti-human p53 protein diluted 1 : 100 (Dako, Glostrup, Denmark) and mouse anti-human Bcl-2 oncoprotein diluted 1 : 100 (Dako).

Compared to the expression in the lesions before tazarotene treatment, TIG3 mRNA expression increased within the keratinocytes and in the dermal vascular endothelial cells after 4 weeks and 12 weeks of tazarotene treatment, the latter marking the end of treatment (Fig. 1D～F). Furthermore, increased levels of p53 expression were present within the keratinocytes, but not within the dermal vascular endothelial cells in the tazarotene-treated lesions. In contrast, Bcl-2 was not expressed in either the epidermal keratinocytes or the dermal vascular endothelial cells within the psoriatic lesions after tazarotene treatment (Fig. 2).

Based on the results of this study, we consider that tazarotene may affect the inflammatory angiogenesis associated with psoriasis. TIG3 is isolated from human keratinocytes treated with tazarotene, which is a retinoic acid receptor-selective retinoid. The expression of TIG3 is reduced in psoriatic skin and its expression is induced in psoriatic lesions after topical tazarotene treatment. It is known that within psoriatic lesions treated with tazarotene, TIG3 plays an important role in the crosslinking that occurs within keratinocytes. Recently, TIG3 has been reported to downregulate VEGF within ovarian cancer cells through the human epidermal growth factor receptor 2 and the Akt/mammalian target of rapamycin signaling pathways[8,9]. Furthermore, the expression of c-fos-induced

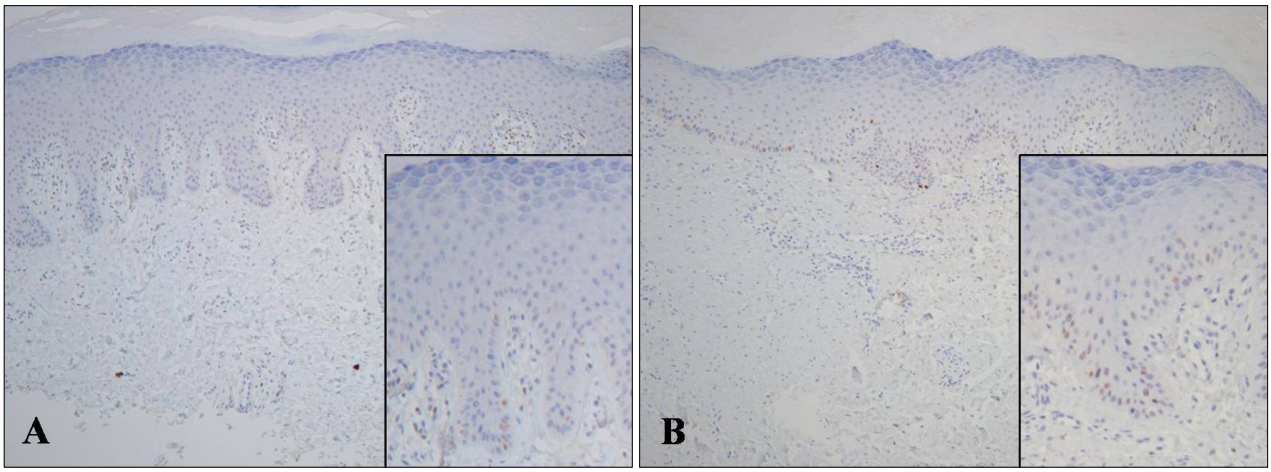

Fig. 2. The expression of p53 in psoriatic lesions (p53 stain, ×200; insets, ×400). (A) Dermal endothelial cells and epidermal keratinocytes within untreated psoriatic lesions did not express p53. (B) An increased expression of p53 was evident within the epidermal keratinocytes of psoriatic lesions treated with tazarotene for up to 12 weeks.

growth factor, a member of the VEGF family, may also be downregulated by TIG3. Moreover, PlGF may induce VEGF secretion from peripheral blood mononuclear cells and be co-expressed with VEGF in the synovial fluid in rheumatoid arthritis[10]. This observation concurs with our findings that treatment with tazarotene increases the expression of TIG3 mRNA within keratinocytes and dermal vascular endothelial cells and reduces the expression of PlGF within the same cells.

We found that the downregulation of PlGF was followed by a reduction in the expression of Tie2, which appeared to block the initiation of inflammatory angiogenesis within the upper dermal vascular endothelial cells. We propose that PlGF could be another regulatory factor in the angiopoietin-1 to angiopoietin-2 switch and that it might regulate the initiation of inflammatory angiogenesis, in the same way that VEGF, bFGF, and TNF-α regulate classical angiogenesis. In addition, we suggest that the apoptosis of endothelial cells is not part of the antiangiogenic action of tazarotene, because p53 and Bcl-2 were not expressed within the dermal vascular endothelial cells in any of the psoriatic lesions after tazarotene treatment.

Given that TIG3 mRNA is inducible via a topical route and that it may have a weaker regulatory action than VEGF, tazarotene could be considered a safer candidate for targeting angiogenesis in milder cases of psoriasis. Tazarotene may not have been able to induce apoptosis because the level of percutaneous penetration was insufficient, but this is unlikely because even systemic bexarotene does not appear to induce apoptosis within nonproliferating endothelial cells.

In conclusion, we suggest that an increase in the expression of TIG3 mRNA may suppress inflammatory angiogenesis by downregulating PlGF expression, resulting in the clinical improvement of psoriatic lesions. Furthermore, the molecular events surrounding tazarotene's antiangiogenic effect may not be associated with the p53-mediated apoptotic pathway in inflammatory angiogenesis.

ACKNOWLEDGMENT

This study was supported by research funds from Dong-A University.

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http://dx.doi.org/10.5021/ad.2014.26.4.520

Eosinophilic Pustular Folliculitis: Association with Long-Term Immunosuppressant Use in a Solid Organ Transplant Recipient

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Dear Editor:

Eosinophilic pustular folliculitis (EPF) is an inflammatory disease that is characterized by pruriticfollicular papules and pustules. Several reported cases showing an association between EPF and hematological diseases as well as a high proportion of EPF cases in HIV-positive patients suggest that immunological aberrations might play a role in the development of EPF. A 56-year-old man who underwent kidney transplantation 18 years ago presented with multiple papules on cheeks (Fig. 1). He was treated with cyclosporine (150 mg daily) and mycophenolate mofetil (MMF; 1,500 mg daily). Blood analysis yielded a white blood cell count of 12,000 cells/mm^3 (12.1% eosinophils). Tests for HIV were also negative. Skin biopsies led to the

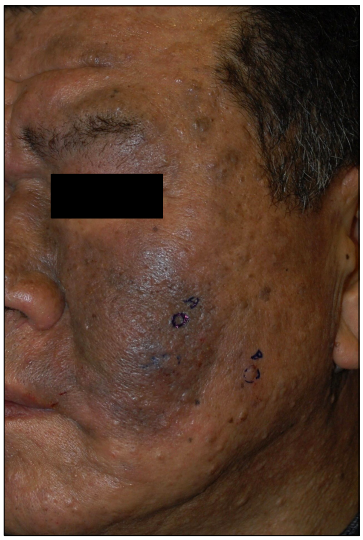

Fig. 1. Multiple papule and plaque with hyperpigmentation and induration on face.

Received June 4, 2013, Revised July 9, 2013, Accepted for publication July 16, 2013

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