

INVESTIGATIVE REPORT

Downregulation of SMAD2, 4 and 6 mRNA and TGF β Receptor I mRNA in Lesional and Non-lesional Psoriatic Skin

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Transforming growth factor beta (TGF β) has been suggested to be an effective inhibitor of the increased keratinocyte proliferation in psoriasis. Three TGF β isoforms are described (TGF β 1, 2 and 3), signalling via a heteromeric receptor complex of TGF β RI and TGF β RII. Receptor binding activates SMAD2, 3 and 4, which translocate into the nucleus and regulate TGF β -responsive genes. SMAD6 and 7 proteins represent a negative feedback loop inhibiting the TGF β -SMAD signalling pathway. As TGF β 1 overexpression inhibits keratinocyte proliferation, the aim of this study was to investigate with real-time RT-PCR the expression of TGF β 1, 2 and 3, TGF β RI and TGF β RII and SMAD2, 3, 4, 6 and 7 in lesional and non-lesional psoriatic skin from 13 patients with chronic plaque-type psoriasis as compared to skin from 10 healthy subjects. The study data demonstrate significantly downregulated TGF β RI and SMAD2, 4 and 6 mRNA expression in lesional and non-lesional psoriatic skin. SMAD7 mRNA expression was significantly decreased in lesional psoriatic skin compared with both non-lesional psoriatic skin and healthy skin. A significant TGF β 3 and TGF β RII mRNA upregulation exclusively in non-lesional psoriatic skin but no significant difference in the expression of TGF β 1 and 2 was found. The results of this study suggest that the expression of TGF β isoforms, receptors and SMADs may be involved in the increased proliferation of keratinocytes in psoriatic skin.
Key words: psoriasis; SMAD; TGF β .

(Accepted January 13, 2009.)

Acta Derm Venereol 2009; 89: 351–356.

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Psoriasis is a chronic immune-mediated inflammatory skin disease. While the cause of psoriasis remains unknown, it appears to result from a combination of genetic and environmental factors. Several studies emphasize the potential role of multiple genes conferring increased susceptibility to psoriasis. Genome-wide scans in the search for psoriasis genes reveal evidence that a major locus of the psoriasis susceptibility gene (PSORS1) is

located within the major histocompatibility complex (MHC) on the short arm of chromosome 6 (1). Recent studies highlight the importance of the interleukin (IL)-23 receptor gene as a risk gene not only for psoriasis but also for Crohn's disease (2, 3). Current concepts of psoriasis focus on dendritic cells skewing T cells in the direction of a Th17-differentiation. By production of IL-17 and IL-22 there is direct activation of keratinocytes, which, among other factors, produce antimicrobial peptides, the overexpression of which is a hallmark of psoriasis (4, 5).

Interestingly, the role of negative regulators of proliferation and activation is by far less well understood. Transforming growth factor beta (TGF β) is suggested to be an effective inhibitor of keratinocyte proliferation in psoriasis (6). TGF β exists in three isoforms (TGF β 1, TGF β 2 and TGF β 3). The biological function of TGF β is mediated by specific receptors (TGF β RI, TGF β RII and TGF β RIII). These receptors are transmembrane, heterodimeric complexes with serine/threonine-kinase activities. Binding of TGF β to its receptor activates the SMAD intracellular signalling pathway (7).

SMAD proteins represent the human homologues of genes first described in *C. elegans* (MAD: mother against decapentaplegic) and in *Drosophila* (SMA: small body size). Eight different SMAD proteins are described in humans and, according to their function, they are classified as "receptor-regulated SMADs" (SMAD1, 2, 3, 5 and 8), "common SMAD" (SMAD4), and "inhibitory SMADs" (SMAD6 and 7). The involvement of SMADs in TGF β signalling has been studied intensively in wound healing. SMAD3 knock-out mice showed that wound healing is accelerated, while local inflammatory responses are decreased (8). Studies suggest that the beneficial effect of 1 α ,25-dihydroxyvitamin D₃ (active derivative of vitamin D₃) in psoriasis is mediated at least in part by TGF β and SMAD signalling (9–11).

To investigate the role of TGF β and SMAD signalling in psoriasis, biopsies were taken from lesional and non-lesional psoriatic skin, as well as from skin from healthy subjects, and mRNA expression of TGF β 1, 2, 3, TGF β RI and II and SMAD2, 3, 4, 6, and 7 were analysed by real-time reverse-transcriptase polymerase chain reaction (real-time RT-PCR).

MATERIALS AND METHODS

Patients

Thirteen patients (8 men, 5 women, mean age 42 years) with chronic plaque-type psoriasis were recruited for the study. None of the patients had received any treatment for at least 4 weeks prior to the investigation. Normal human skin tissue was obtained from 10 healthy subjects who underwent elective surgery for excision of benign naevi. All participants gave their written informed consent prior to participation, and the study followed the Declaration of Helsinki (<http://www.wma.net/e/policy/b3.htm>) and the amendments of the World Medical Association. The study procedure was approved by the local ethics committee.

Tissue samples

In each patient two punch biopsies (5 mm diameter) were taken from lesional and non-lesional skin after local anaesthesia using prilocaine 1%. Biopsies from lesional skin were taken from the margin of plaques. Psoriasis plaques and non-lesional skin were at least 5 cm apart. Control skin samples from healthy subjects were taken with a 5 mm punch biopsy immediately after surgical excision of the naevus from the surrounding skin. All tissue specimens were immediately immersed in RNA-later solution (Qiagen, Hilden, Germany), kept for 24 h at room temperature and finally stored at -20°C until further use.

RNA isolation

Total RNA of the biopsies was isolated using an RNA isolation kit (Stratagene, Heidelberg, Germany) according to the manufacturer's instructions. Briefly, the biopsy tissue was transferred to 300 μl lysis buffer containing 1% β -mercaptoethanol (Sigma, Deisenhofen, Germany). Thereafter, the tissue was homogenized for 2 sec in iced tubes using an ultrasonic apparatus (Bandelin Sonopuls GM70, Berlin, Germany). After adding 300 μl 70% (v/v) ethanol (Merck, Darmstadt, Germany) total RNA was immobilized on spin columns and treated with DNase before elution in diethylpyrocarbonate-treated (DEPC) water (Fluka, Buchs, Germany). The concentration and purity of RNA was determined by measuring the absorbance at 260 nm and 280 nm in a spectrophotometer. Ribosomal RNA (28S rRNA and 18S rRNA) was visualized on a 1% agarose gel following electrophoresis to demonstrate its integrity.

Real-time reverse transcriptase-polymerase chain reaction (real-time RT-PCR)

Equal amounts of total RNA from each sample were reverse transcribed into cDNA with standard reagents according to the recommendations of the manufacturer (Gibco-BRL, Karlsruhe, Germany). 1 μg RNA was incubated for 10 min at 70°C with 1 μl oligo dT (0.5 $\mu\text{g}/\mu\text{l}$) (Gibco-BRL). After incubation RNA was transferred into a reaction mixture containing 4 μl 5 $\times$ reverse transcriptase buffer (250 mmol/l Tris-HCl (pH 8.3), 50 mmol/l MgCl_2 and 300 mmol/l KCl), 2 μl DTT (100 mmol/l), 1 μl ribonuclease inhibitor (40 U/ml), 1 μl dNTPs (10 mmol/l) and 0.5 μl Superscript II (Gibco-BRL) and incubated at 42°C for 60 min. Transcription was stopped at 95°C for 5 min. Thereafter, the reverse-transcriptase (RT) product was diluted with 80 μl DEPC-water and stored at -20°C until further use.

Real-time quantitative RT-PCR and data analysis was carried out using the LightCycler technique (Roche Diagnostic, Mannheim, Germany). Specific primers are shown in Table I. In these studies, one cDNA preparation that expressed each specific gene of TGF β , TGF β receptors or SMADs was arbitrarily employed as the assay standard. The level of expression of each mRNA and their esti-

mated crossing points in each sample was determined relative to the standard preparation using the LightCycler computer software. Total cellular RNA was used as a reference for data normalization (12). Amplification was performed in a total volume of 10 μl including 1 μl RT, 1 μl LightCycler-FastStart Reaction Mix SYBR Green 1 (Roche Molecular Biochemicals), 0.5 $\mu\text{mol/l}$ specific primer, 3 mmol/l MgCl_2 with 45 cycles of PCR. Each PCR cycle included denaturation for 1 min at 95°C , annealing of primers at primer specific temperatures (Table I) for 30 sec and elongation at 72°C for 2 min with a final extension at 72°C for 5 min.

To establish the specificity of DNA products, melting curve analysis was carried out after the PCR. For each primer set, agarose gel electrophoresis was also employed to establish the integrity of the PCR reaction and product size.

Statistical analysis

All values were calculated as mean and standard deviation (SD). Statistical significance between groups was analysed by Student's *t*-test. Student's paired *t*-test was used for analysis of the results obtained from lesional and non-lesional psoriatic skin, since we took a paired sample from each patient. We used the unpaired *t*-test for comparison of psoriasis and normal skin. A *p*-value below 0.05 was considered significant.

RESULTS

Expression of TGF β mRNA

The data obtained from real-time RT-PCR analysis did not reveal different expression of TGF β 1 and TGF β 2 mRNA in lesional or non-lesional psoriatic skin compared with skin from healthy subjects ($p > 0.05$, data not shown). TGF β 3 mRNA expression was significantly up-regulated in non-lesional psoriatic skin compared with lesional ($p < 0.01$) and control skin ($p < 0.001$, Fig. 1).

Table I. Sequence, size of primers and annealing temperature (T_m ($^{\circ}\text{C}$)) used in real-time RT-PCR assays

mRNA	Primer sequence (5'-3')	Size (bp)	T_m ($^{\circ}\text{C}$)
TGF β 1	S-TGG CGA TAC CTC AGC AAC C AS-CTC GTG GAT CCA CTT CCA G	405	58.8
TGF β 2	S-ATC CCG CCC ACT TTC TAC AGA C AS-ASCAT CCA AAG CAC GCT TCT TCC	565	62.1
TGF β 3	S-TAC TAT GCC AAC TTC TGC TC AS- AAC TTA CCA TCC CTT TCC TC	522	55.3
TGF β RI	S- ACG GCG TTA CAG TGT TCT G AS- GGT GTG GCA GAT ATA GAC C	358	56.7
TGF β RII	S- AGC AAC TGC AGC ATC ACC TC AS-TGA TGT CTG AGA AGA TGT CC	688	59.4
SMAD2	S- GGA GCA GAA TAC CGA AGG CA AS- CTT GAG CAA CGC ACT GAA GG	128	59.4
SMAD3	S- AGA AGA CGG GGC AGC TGG AC AS-GAC ATC GGA TTC GGG GAT AG	511	63.5
SMAD4	S- GCA TCG ACA GAG ACA TAC AG AS-CAA CAG TAA CAA TAG GGC AG	484	57.3
SMAD6	S- CAA GCC ACT GGA TCT GTC CGA AS- TTG CTG AGC AGG ATG CCG AAG	321	61.8
SMAD7	S- ATG DTG TGC CTT CCT CCG CT AS-CGT CCA CGG CTG CTG CAT AA	494	61.4

bp: base pairs; TGF: transforming growth factor; RT-PCR: reverse transcriptase-polymerase chain reaction.

Expression of *TGFβ* receptor mRNA

The *TGFβRI* mRNA expression was significantly decreased in lesional ($p < 0.01$) and non-lesional ($p < 0.01$) psoriatic skin compared with control skin. *TGFβRII* showed a significant increased mRNA expression in non-lesional psoriatic skin compared with lesional psoriatic skin ($p < 0.05$) and control skin ($p < 0.01$).

Expression of *SMAD* mRNA

SMAD2 mRNA expression was significantly down-regulated in biopsies from lesional and non-lesional psoriatic skin (both $p < 0.01$) compared with healthy skin (Fig. 2). Our data did not reveal a significant difference in the expression of *SMAD3* mRNA (data not shown). The mRNA expression of *SMAD4* was significantly decreased in psoriasis plaques ($p < 0.001$) and non-lesional psoriatic skin ($p < 0.001$) compared with normal skin (Fig. 2). In lesional psoriatic skin ($p < 0.001$) and non-lesional psoriatic skin ($p < 0.001$) the mRNA expression of *SMAD6* was significantly decreased compared with healthy skin (Fig. 2). The expression of *SMAD7* mRNA was significantly down-regulated in lesional psoriatic skin compared with healthy skin ($p < 0.05$), whereas there was no significant difference in *SMAD7* mRNA expression between non-lesional skin and control skin (Fig. 2).

DISCUSSION

TGFβ is suggested to play a central role in various diseases. Mutations in the *TGFβ* genes, its receptors, and intracellular signalling molecules (*SMAD* proteins) are associated with the pathogenesis of cancer (13). Despite this important influence of *TGFβ* on the homeostasis of human tissue there have been only a few studies into the role of *TGFβ* and its intracellular signalling proteins (*SMAD*) in the pathogenesis of psoriasis (14–17). As psoriasis is characterized by epidermal hyperproliferation, and several studies showed anti-proliferative effects of *TGFβ* on keratinocytes (6, 18, 19), we investigated the mRNA expression of *TGFβ*, its receptors and signalling molecules (*SMADs*) in psoriatic skin.

Only a few studies describe the expression of *TGFβ* in psoriatic skin. Kane et al. (20) showed intracellular immunoreactivity of *TGFβ1* in basal and suprabasal layers of the epidermis in normal skin, but no staining in lesional psoriatic skin. These results are not in accordance with our findings, since we did not find any differences in *TGFβ1* mRNA expression between the analysed skin samples. Since we described mRNA transcript levels of *TGFβ1* in whole skin biopsies, it is difficult to evaluate these differing results. In a second study, *TGFβ1* was neither detected in normal skin, nor in non-lesional or lesional psoriatic skin,

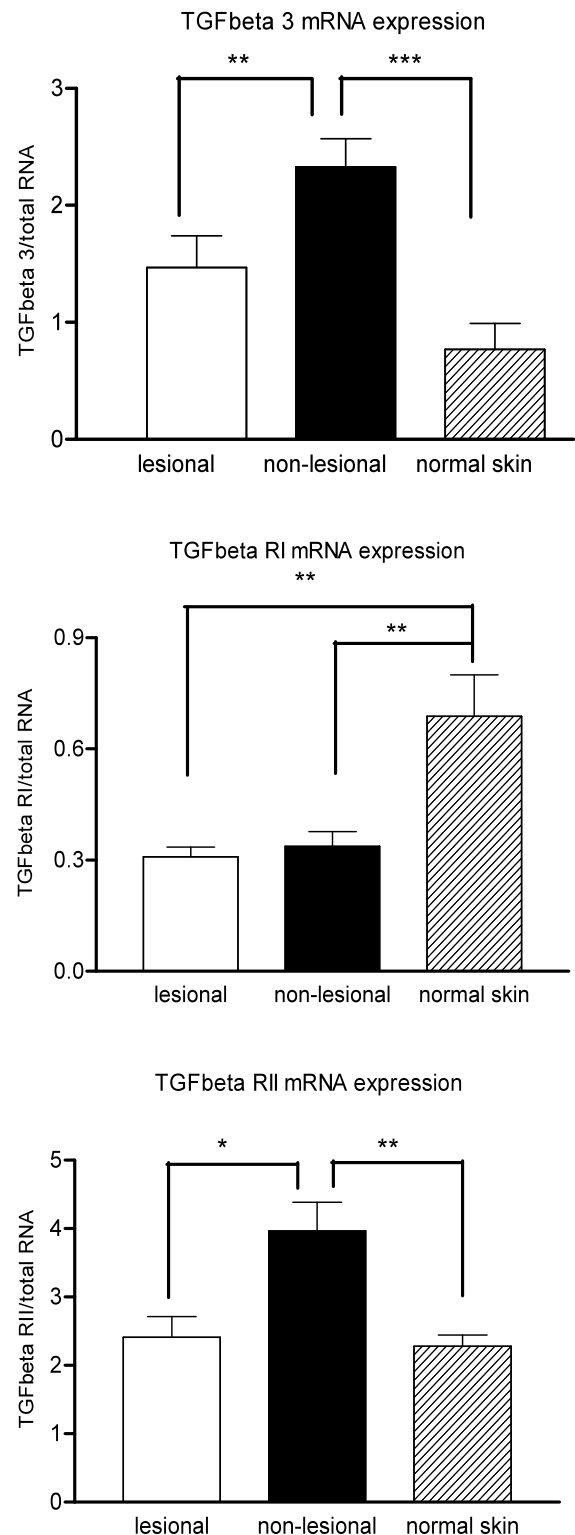


Fig. 1. Transforming growth factor (*TGFβ*) 3, *TGFβ* receptor (*TGFβR*) I and II mRNA expression analysed by real-time reverse transcriptase-polymerase chain reaction (real-time RT-PCR) using the LightCycler technique. The data represent mean $\pm$ standard deviation. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

whereas *TGFβ3* showed subepidermal staining in all skin samples (16). It is difficult to compare our results

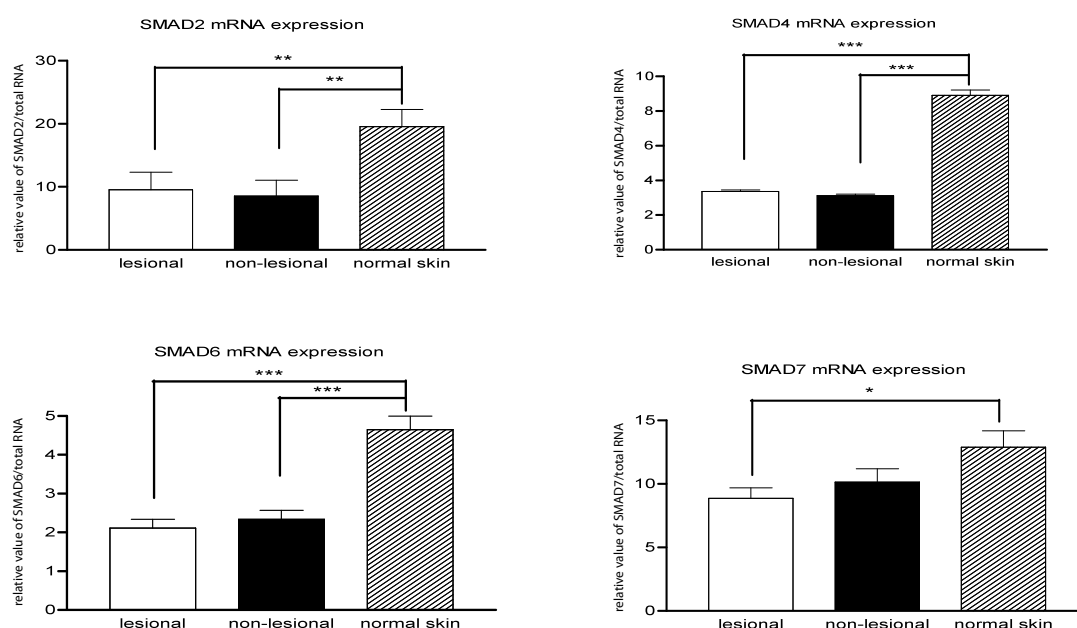


Fig. 2. SMAD2, 4, 6 and 7 mRNA expression analysed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) using the LightCycler technique. The data represent mean $\pm$ standard deviation. *Significant at $p < 0.05$; **significant at $p < 0.01$; and ***significant at $p < 0.001$.

with the study of Wataya-Kaneda et al. (16), as in the latter TGF β precursor-specific antibodies were used. Interestingly, irradiation of HaCaT-keratinocytes with narrowband ultraviolet (UV)-B induced upregulation of TGF β 1 in keratinocytes (21) and downregulation in fibroblasts (22). UV-B treatment leads to TGF β 1 induction in epidermal psoriatic skin, which partly explains the beneficial effect in psoriasis. As our data showed upregulation of TGF β 3 mRNA only in non-lesional psoriatic skin, we suggest that increased TGF β 3 levels in non-lesional psoriatic skin might be involved in pathomechanisms responsible for inhibiting epidermal hyperproliferation. TGF β 3 is known as an inhibitory factor in wound healing and is a potential treatment to prevent and reduce scarring (23). The role of TGF β 3 in the development of psoriatic lesions has not been described previously, and studying the effect of TGF β 3 protein on the proliferation of keratinocytes would be highly interesting. As adhesiveness of T-lymphocytes to dermal microvascular endothelial cells can be blocked by TGF β 1, reduction of TGF β 1 expression and function may contribute to lymphocyte infiltration into psoriatic plaques (17). The role of TGF β 1 in psoriasis remains unclear, and further studies investigating TGF β 1 protein expression in psoriatic skin are needed.

The studies of Nockowski et al. (14), Flisiak et al. (15) and Li et al. (18) further support a possible pathogenetic role of TGF β in psoriasis. Nockowski et al. (14) observed increased serum concentrations of TGF β 1 in patients with psoriasis. Flisiak et al. (15) showed a positive correlation between TGF β 1 plasma levels and the Psoriasis Area and Severity Index (PASI), but their results revealed that TGF β 1 plasma levels do not differ

from healthy controls. Flisiak et al. further found a significant correlation between TGF β 1 concentrations in psoriatic scales and disease duration and sedimentation rate (24). These contrasting results of TGF β 1 plasma levels emphasize the importance of new larger studies analysing the protein expression of TGF β 1 in plasma and skin of patients with psoriasis. Li et al. (18) found that transgenic mice overexpressing TGF β 1 in the epidermis develop inflammatory skin lesions with a gross appearance of psoriasis-like plaques and reveal enhanced SMAD signalling in epidermis and dermis (25).

The expression of TGF β receptors (TGF β R) in psoriasis were investigated by Leivo et al. (26). The authors found intense immunohistochemical staining for both receptors in basal and suprabasal epidermal keratinocytes in normal and non-lesional psoriatic skin, but no staining in the epidermis of lesional psoriatic skin. Our results showed a decreased TGF β RI mRNA expression in lesional and non-lesional psoriatic skin and increased TGF β RII mRNA expression in non-lesional psoriatic skin compared with normal skin. It is difficult to compare these results as Leivo et al. (26) analysed protein expression in the epidermis. However, the results of both studies suggest a possible role of the TGF β receptors in psoriasis. If TGF β 1 has an antiproliferative effect on the epidermis TGF β receptors are needed to mediate this effect. Decreased expression of both TGF β receptors could be a reason for diminished perception of TGF β 1 signalling. This may play an important role in the pathogenesis of psoriasis.

To our knowledge, investigations of SMAD mRNA expression in psoriasis have not yet been performed. The data obtained in our study showed a significant decrease

in the mRNA expression of the inhibitory SMAD6, of the common SMAD4 and of the receptor-regulated SMAD2 in lesional and non-lesional psoriatic skin compared with normal skin. The potential involvement of decreased SMAD6 and 7 expressions in the pathogenesis of psoriasis is substantiated by *in vivo* experiments using narrow-band UV-B exposure of normal human skin. After UV-B irradiation, increased expression of SMAD7 could be detected by *in situ* hybridization (27). In the light of these results, it is interesting that our study showed decreased SMAD7 mRNA expression in psoriatic skin compared with normal skin. These data suggest that activation of the TGF β -SMAD signalling pathway might be involved in the clinical improvement of psoriasis.

The role of SMADs as an intracellular signalling pathway transducing the TGF β -signal is well established. Binding of TGF β 1 and 2 to the TGF β receptor complex (TGF β RI and II) leads to increased expression of SMAD2, 4, 6 and 7 (7). Our data revealed decreased expression of SMAD2, 4 and 6 in lesional and non-lesional psoriatic skin compared with normal skin. This points towards decreased TGF β -signalling. As we found no differences in TGF β 1 and 2 mRNA levels between psoriatic and normal skin, we hypothesize that the decreased expression of TGF β RI is the reason for the decreased SMAD2, 4 and 6 mRNA expression in lesional and non-lesional psoriatic skin.

Further studies are needed to decide whether the TGF β -SMAD signalling pathway is directly involved in the development of psoriasis or is a side-effect of the immunological processes in psoriatic skin. The interpretation of the results of our study is limited due to the fact that we analysed mRNA transcript levels and we cannot determine what kind of cell might be responsible for changes in transcript levels. Therefore, further studies are needed to describe the protein expression of SMAD and TGF β in psoriatic skin. The choice of PCR normalization method is crucial in analysing and interpreting PCR results. The PCR data obtained in this study are based on PCR normalization to total RNA. According to Bustin this is the least unreliable method (12), but there still are limitations and risks in interpreting PCR results. An important consideration when using total RNA for normalization is the lack of internal control for RT or PCR inhibitors. All quantitative methods assume that the RNA targets are reversely transcribed and subsequently amplified with similar efficacy. Normalization to total RNA content requires accurate quantification of the RNA sample and, in addition, this method does not provide reliable information about the quality of the RNA, a key consideration when quantitating mRNA levels in fresh tissue. Further problems in analysing PCR data may be associated with methodological errors (cDNA synthesis and PCR analysis). These limitations have been taken into account when interpreting the data obtained in this study.

ACKNOWLEDGEMENT

Part of this work was presented as a poster during the 3rd International Conference on Psoriasis: "Psoriasis: from Gene to Clinic", London, November 2002, and was awarded with the prize for the best scientific poster.

REFERENCES

1. Capon F, Munro M, Barker J, Trembath R. Searching for the major histocompatibility complex psoriasis susceptibility gene. *J Invest Dermatol* 2002; 118: 745–751.
2. Capon F, Di Meglio P, Szaub J, Prescott NJ, Dunster C, Baumber L, et al. Sequence variants in the genes for the interleukin-23 receptor (IL23R) and its ligand (IL12B) confer protection against psoriasis. *Hum Genet* 2007; 122: 201–206.
3. Cargill M, Schrodi SJ, Chang M, Garcia VE, Brandon R, Callis KP, et al. A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. *Am J Hum Genet* 2007; 80: 273–290.
4. Lowes MA, Bowcock AM, Krueger JG. Pathogenesis and therapy of psoriasis. *Nature* 2007; 445: 866–873.
5. Schröder JM, Harder J. Antimicrobial skin peptides and proteins. *Cell Mol Life Sci* 2006; 63: 469–486.
6. Doi H, Shibata MA, Kiyokane K, Otsuki Y. Downregulation of TGF β isoforms and their receptors contributes to keratinocyte hyperproliferation in psoriasis vulgaris. *J Dermatol Sci* 2003; 33: 7–16.
7. Derynck R, Zhang YE. Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* 2003; 425: 577–584.
8. Ashcroft GS, Yang X, Glick AB, Weinstein M, Letterio JL, Mizel DE, et al. Mice lacking Smad3 show accelerated wound healing and an impaired local inflammatory response. *Nat Cell Biol* 1999; 1: 260–266.
9. Yanagi Y, Suzawa M, Kawabata M, Miyazono K, Yanagisawa J, Kato S. Positive and negative modulation of vitamin D receptor function by transforming growth factor-beta signaling through smad proteins. *J Biol Chem* 1999; 274: 12971–12974.
10. Yanagisawa J, Yanagi Y, Masuhiro Y, Suzawa M, Watanabe M, Kashiwagi K, et al. Convergence of transforming growth factor-beta and vitamin D signaling pathways on SMAD transcriptional coactivators. *Science* 1999; 283: 1317–1321.
11. Oyama N, Iwatsuki K, Satoh M, Akiba H, Kaneko F. Dermal fibroblasts are one of the therapeutic targets for topical application of 1 α ,25-dihydroxyvitamin D3: the possible involvement of transforming growth factor-beta induction. *Br J Dermatol* 2000; 143: 1140–1148.
12. Bustin SA. Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *J Mol Endocrinol* 2000; 25: 169–193.
13. Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor beta in human disease. *N Engl J Med* 2000; 342: 1350–1358.
14. Nockowski P, Szepietowski JC, Ziarkiewicz M, Baran E. Serum concentrations of transforming growth factor beta 1 in patients with psoriasis vulgaris. *Acta Dermatovenereol Croat* 2004; 12: 2–6.
15. Flisiak I, Porebski P, Flisiak R, Chodynicka B. Plasma transforming growth factor beta1 as a biomarker of psoriasis activity and treatment efficacy. *Biomarkers* 2003; 8: 437–443.
16. Wataya-Kaneda M, Hashimoto K, Kato M, Miyazono K, Yoshikawa K. Differential localization of TGF-beta-

- precursor isotypes in psoriatic human skin. *J Dermatol Sci* 1996; 11: 183–188.
17. Cai JP, Falanga V, Taylor JR, Chin YH. Transforming growth factor-beta receptor binding and function are decreased in psoriatic dermal endothelium. *J Invest Dermatol* 1996; 106: 225–231.
 18. Li AG, Lu SL, Han G, Hoot KE, Wang XJ. Role of TGFbeta in skin inflammation and carcinogenesis. *Mol Carcinog* 2006; 45: 389–396.
 19. Moses HL. TGF-beta regulation of epithelial cell proliferation. *Mol Reprod Dev* 1992; 32: 179–184.
 20. Kane CJ, Knapp AM, Mansbridge JN, Hanawalt PC. Transforming growth factor-beta 1 localization in normal and psoriatic epidermal keratinocytes in situ. *J Cell Physiol* 1990; 144: 144–150.
 21. Quan T, He T, Kang S, Voorhees JJ, Fisher GJ. Ultraviolet irradiation alters transforming growth factor beta/smad pathway in human skin in vivo. *J Invest Dermatol* 2002; 119: 499–506.
 22. Choi CP, Kim YI, Lee JW, Lee MH. The effect of narrowband ultraviolet B on the expression of matrix metalloproteinase-1, transforming growth factor-beta1 and type I collagen in human skin fibroblasts. *Clin Exp Dermatol* 2007; 32: 180–185.
 23. Occleston NL, Lavery HG, O'Kane S, Ferguson MW. Prevention and reduction of scarring in the skin by transforming growth factor beta 3 (TGFbeta3): from laboratory discovery to clinical pharmaceutical. *J Biomater Sci Polym Ed* 2008; 19: 1047–1063.
 24. Flisiak I, Chodyncka B, Porebski P, Flisiak R. Association between psoriasis severity and transforming growth factor beta(1) and beta (2) in plasma and scales from psoriatic lesions. *Cytokine* 2002; 19: 121–125.
 25. Li AG, Wang D, Feng XH, Wang XJ. Latent TGFbeta1 overexpression in keratinocytes results in a severe psoriasis-like skin disorder. *EMBO J* 2004; 23: 1770–1781.
 26. Leivo T, Leivo I, Kariniemi AL, Keski-Oja J, Virtanen I. Down-regulation of transforming growth factor-beta receptors I and II is seen in lesional but not non-lesional psoriatic epidermis. *Br J Dermatol* 1998; 138: 57–62.
 27. Quan T, He T, Voorhees JJ, Fisher GJ. Ultraviolet irradiation blocks cellular responses to transforming growth factor-beta by down-regulating its type-II receptor and inducing Smad7. *J Biol Chem* 2001; 276: 26349–26356.