INVESTIGATIVE REPORT

Increased Expression of the Wnt Signalling Inhibitor Dkk-1 in Non-lesional Skin and Peripheral Blood Mononuclear Cells of Patients with Plaque Psoriasis

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Psoriasis is a common inflammatory skin disease characterised by abnormal keratinocyte proliferation, increased dermal angiogenesis and systemic inflammation. The cell signalling cascades provoked by Wnt proteins and their inhibitors, such as Dickkopf-1 (Dkk-1), play crucial roles to maintain homeostasis of a variety of tissues, including skin, and are also involved in angiogenesis and innate immunity. This study was designed to investigate the distribution of Dkk-1, in lesional and non-lesional skin, in serum and in peripheral blood mononuclear cell (PBMCs) of patients with psoriasis compared with healthy controls. Our results showed significantly increased mRNA and protein expression of Dkk-1 in non-lesional compared with lesional skin and healthy control skin. No significant differences of Dkk-1 serum levels were observed, but Dkk-1 protein expression was significantly increased in patients’ PBMC. Increased levels of Dkk-1 in PBMC suggest a possible role of Dkk-1 in the chronic systemic inflammation of psoriasis. Increased levels of Dkk-1 in non-lesional psoriasis skin offers new insights in the local inflammatory processes in psoriasis skin since Wnt signalling regulates angiogenesis. In conclusion, Dkk-1 may be a possible target for future treatment options.

Key words: psoriasis; Dickkopf-1; Wnt-proteins; peripheral blood mononuclear cells.

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MATERIAL AND METHODS

Study subjects

In all, 47 patients with mild to severe plaque type psoriasis and 25 normal healthy controls were enrolled for the study (male/female 29/18 and 7/18; mean ± SD age 55.3 ± 14.6 and 49.2 ± 17.0, respectively). Written informed consent was obtained from all subjects under protocols approved by the ethical committee Linköping University, Linköping, Sweden. This study was conducted in compliance with good clinical practice and according to the Declaration of Helsinki Principles. Sex, age and disease duration were recorded, as well as an assessment of disease severity using the Psoriasis Area and Severity Index.
(PASI) showed mean ± SD of 7.1 ± 6.5. Full-thickness punch biopsies were taken from non-lesional skin (with a distance of at least 10 cm from any psoriatic lesion; 4 mm diameter) and from the active margin of a psoriatic plaque (4 mm diameter) from every patient. Study subjects did not use any systemic anti-psoriatic treatments for 2 weeks prior to biopsy. One biopsy was obtained from corresponding anatomical sites from healthy controls. Immediately upon removal, biopsies were stored either in formalin for immunohistochemistry or RNA later for gene expression analysis (Ambion, Austin, USA) and stored at −80°C.

Blood samples were taken from patients with psoriasis and controls for gene expression analysis from peripheral blood (Tempus Blood RNA tubes, Life technologies corporation, Carlsbad, CA, USA) and Dkk-1 protein analysis in serum and from PBMCs. Blood samples were immediately stored at −80°C.

Controls for gene expression analysis from peripheral blood was purified using the Tempus Spin RNA Isolation Reagent kit (Life technologies). Concentration and purity was measured using a Nanodrop ND-1000 (Thermo Fisher Scientific Inc., Waltham, MA, USA), and RNA integrity was assessed using the RNA integrity number with a 2100 Bioanalyzer (Agilent technologies, Santa Clara, USA). RNA was reverse transcribed using the High capacity cDNA reverse transcription kit with RNase inhibitor (Life Technologies), according to the manufacturer’s instructions, and the resulting cDNA was stored at −80°C.

Expression of DKK1, and of 2 housekeeping genes, ACTB and GAPDH, were analysed on the 7500 Fast real-time PCR system (Applied Biosystems, City, Country) and the standard run mode using Taqman Gene Expression Assays (Hs00183740_m1, Hs99999903_m1, and Hs03929097_g1, respectively) and TaqMan Universal Master Mix no UNG (Applied Biosystems). For each assay and sample, cDNA based on 10 ng were analyzed in a total volume of 20 μl. Threshold cycle (CT) values were established using the ExpressionSuite software version 1.0.1 (Life Technologies). Missing CT values, due to low copy numbers, were replaced by the highest CT value available for the gene in question, increased by one cycle. For the target gene (DKK1) the resulting CT values were normalised using CT values of the selected reference genes, GAPDH in blood and ACTB in skin biopsies.

ELISA

Dkk-1 concentration in serum and in peripheral blood mononuclear cells was measured according to the manufacturer’s instructions using a commercially available ELISA kit (QuantiKine, Human Dkk-1 Immunoassay, R&D Systems, Minneapolis, USA). Duplicate 100 μl serum samples and 100,000 PBMCs after repeated freezing and thawing cycles were added to the assay.

Statistics analysis

Statistical evaluation of multiple groups was performed by Kruskal-Wallis ANOVA by Ranks and Mann-Whitney U test as a post hoc test and Student’s t-test was used to compare 2 groups. All statistical analysis was performed using Statistica 12 software (Statistica, Tulsa, OK, USA). All p < 0.05 were considered significant.

RESULTS

In normal skin of healthy controls immunohistochemistry showed weak epidermal staining of Dkk-1 and staining of endothelial cells and fibroblasts (Fig. 1A). In non-lesional psoriasis skin epidermal staining of Dkk-1 was more intense and prominent at the epidermal basal cell layer (Fig. 1B). In lesional psoriasis skin epidermal Dkk-1 staining was absent and intense positive Dkk-1 staining of the inflammatory perivascular infiltrate was present (Fig. 1C). Quantification revealed that Dkk-1 immunostaining signal (dermal and epidermal) in non-lesional skin of patients with psoriasis was significantly increased compared with lesional skin and control subjects (Fig. 2A).

Dkk-1 mRNA expression was significantly increased in non-lesional skin from patients with psoriasis compared with lesional skin and control samples (Fig. 2B). Analysis of PBMCs showed increased expression of Dkk-1 protein in patients with psoriasis compared to controls (p < 0.01, Fig. 3).

Our data revealed no significant differences in serum levels of Dkk-1 protein or in Dkk-1 gene expression in PBMC of patients with psoriasis and controls (Fig. S1^1).

DISCUSSION

Abnormal Wnt signalling has been associated with many human diseases, ranging from cancer to degenerative

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^1http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1970
disorders. The Wnt pathway has also been suggested to play a distinct role in inflammation and in linking innate and adaptive immunity to infections (2, 15). In the present study we report increased gene and protein expression of Dkk-1, a regulatory molecule of the Wnt pathway, in non-lesional skin of patients with psoriasis compared with lesional skin and healthy controls. Wnt signalling is known to induce angiogenesis by increased synthesis of vascular endothelial growth factor (VEGF) (16). Increased Dkk-1 expression in non-lesional psoriatic skin may inhibit angiogenesis by antagonising Wnt signalling thus sustaining the clinical picture of uninvolved skin. Owing to the significance of Wnt signalling in angiogenesis, Wnt antagonists, such as Dkk-1 have been considered potential treatments for neovascular disorders (17). Interestingly, DKK-1 is a pro-apoptotic gene and may play an important role in connecting the oncogenic Wnt and p53 tumour suppressor pathways (18). The pro-apoptotic effect of Dkk-1 may lead to sustained skin homeostasis in clinical non-lesional psoriatic skin and decreased Dkk-1 expression in psoriasis skin may therefore contribute to pathological activation of canonical Wnt signalling. Epidermal Langerhans’ cells (LCs) have pivotal roles in initiating immunity by acquiring antigens that are encountered in skin. Recent research showed that Dkk-1 reduces LC proliferation in mice (19). It is tempting to speculate that increased Dkk-1 in non-lesional psoriatic skin may inhibit LC proliferation and hence diminish inflammation in psoriasis; but further studies are needed to test this hypothesis.

PBMCs are main actors in inflammatory processes and they are linked to many diseases, such as atherosclerosis and psoriasis (20–22). Our study revealed significantly enhanced levels of Dkk-1 protein in PBMCs of patients with psoriasis. Recent findings identify Dkk-1 as a novel mediator in platelet-mediated endothelial cell activation and Ueland et al. (23) showed higher Dkk-1 expression in carotid plaques suggesting a role for Dkk-1-mediated inflammation in atherosclerotic lesions. In the light of these findings it would be interesting to...

Fig. 1. Expression of Dkk-1 protein (brown) in skin from healthy controls (A), in non-lesional (B) and lesional skin (C) from patients with psoriasis analysed by immunohistochemistry. 20× magnification (A and B) and 10× magnification (C).

Fig. 2. (A) Increased expression of Dkk-1 protein in non-lesional skin from patients with psoriasis compared with lesional skin and skin from healthy controls (controls \( n = 10 \), psoriasis \( n = 11 \)). (B) Increased Dkk-1 gene expression in non-lesional skin from patients with psoriasis compared with lesional skin and skin from healthy controls (controls \( n = 21 \), psoriasis \( n = 31 \)). (box = mean \( \pm 0.95 \) confidence interval, whiskers = mean \( \pm SD \), \( * p < 0.05 \), \( *** p < 0.001 \)).

Fig. 3. Significant increased expression of Dkk-1 protein in peripheral blood mononuclear cells of patients with psoriasis (\( ** p < 0.01 \), controls \( n = 15 \), psoriasis \( n = 45 \)).
further investigate the role of increased Dkk-1 PBMC levels in the increased cardiovascular risk profile of patients with psoriasis. Activation of the innate immune system is a key event in the early steps of psoriasis. Interestingly, recent data link Wnt signalling to innate immune functions. Wnt5a and its receptor Frizzled-5 regulate inflammatory responses of human mononuclear cells induced by microbial stimulation suggesting a role of Wnt proteins in human immune defence against infections (2). Further studies are needed to elucidate the effect of increased Dkk-1 in PBMCs on innate immunity and psoriasis.

Treatment of ankylosing spondylitis and rheumatoid arthritis with TNF-α inhibitors was accompanied by decreased Dkk-1 serum levels (24, 25). These studies emphasise Dkk-1 as a protagonist in chronic immune mediated diseases and Dkk-1 may serve as a biomarker for the activity of these diseases. This is less likely to be the case for psoriasis since our data did not reveal a significant difference in serum levels between patients and controls. However, the serum results are not concordant with the observed increased Dkk-1 levels in non-lesional skin and in PBMCs of patients with psoriasis. This discordance might be due to low serum secretion and intracellular storage of Dkk-1 or post translational modifications of secreted Dkk-1 complicating serum detection.

In conclusion, the present study describes alterations in the expression of Dkk-1 in patients with psoriasis compared to controls, yet the role of Dkk-1 in the complex immune mediated pathogenesis of psoriasis is still unclear. Investigating the effect of Dkk-1 substitution to psoriasis skin or inhibiting Dkk-1 in PBMCs has to show whether or not Dkk-1 plays a role in the development of psoriasis.

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REFERENCES