VEGF Induces IL-23 Expression in Keratinocytes through p38 Signaling

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Abstract

Background: Psoriasis is a chronic inflammatory skin disorder which is associated with increased cutaneous vascular endothelial growth factor (VEGF) expression. Several studies demonstrate that VEGF plays an important role in psoriasis pathogenesis by linking angiogenesis and inflammation. We aimed to study the molecular functions of VEGF in psoriasis and dissect a link between VEGF and pro-inflammatory cytokine expression in human keratinocytes.

Methods: VEGF expression was evaluated in sections from lesional psoriatic skin by immunohistochemistry. Cultured keratinocytes were transfected with a VEGF expression plasmid and changes in pro-inflammatory cytokine expression were investigated by qPCR, ELISA and dot-blot. Protein phosphorylation profiles in lysates from cells over-expressing VEGF were analysed to identify the underlying signaling pathways. Specific inhibitors or siRNA knockdown were used in confirmatory experiments.

Results: In lesional psoriatic skin, VEGF and the pro-inflammatory cytokine IL-23 are both strongly expressed by epidermal keratinocytes. VEGF over-expression in cultured keratinocytes resulted in increased IL-23 and IL-6 mRNA transcript abundance and protein expression. At the same time VEGF over-expression strongly increased phosphorylation of p38 MAPK, CREB and Hsp27. Inhibition of p38 MAPK by SB203580 blocked VEGF induced IL-23 expression while siRNA mediated knockdown of CREB or Hsp27 showed no effect.

Conclusions: VEGF up-regulates pro-inflammatory IL-23 and IL-6 secretion through p38 MAPK in epidermal keratinocytes in psoriasis. Targeting VEGF and/or p38 MAPK could lead to novel anti-inflammatory treatments for this chronic skin disease.

Keywords: Psoriasis; Vascular endothelial growth factor; p38 MAPK; Interleukin 23

Abbreviations: VEGF: Vascular Endothelial Growth Factor; NHEK: Normal Human Epidermal Keratinocytes; IL-23: Interleukin 23; IL-6: Interleukin 6; MAPK: Mitogen-Activated Protein Kinase; PBGD: Porphobilinogen Deaminase

Introduction

Psoriasis is a chronic inflammatory skin disease which is driven and maintained by multiple components of the immune system [1]. While most recent studies highlight the role of Th17 cells in psoriasis pathogenesis there is also evidence that in the course of the disease disturbed angiogenesis and skin inflammation are closely linked [2]. Alterations of the cutaneousvasculature and microcirculation such as increased permeability and dilatation of dermal capillaries are among the earliest detectable histological features during the development of psoriatic plaques [3].

From this perspective, vascular endothelial growth factor (VEGF) might play an important role in the pathophysiology of psoriasis as VEGF influences vascular permeability and mediates pro-inflammatory activity by inducing vascular leakage [4,5]. Already, in non-involved, non-lesional skin significant over-expression of several VEGF isoforms was observed in psoriasis patients as compared with healthy skin of volunteers [6]. Among resident cells in skin keratinocytes are a major source of VEGF and in psoriatic plaques epidermal VEGF expression is strongly increased [3].

A major role of VEGF in the pathogenesis of psoriasis was further corroborated by the phenotype of transgenic mice with epidermis-specific over-expression of VEGF. These mice showed enhanced skin vascularity and capillary permeability and the characteristic Koebner phenomenon with induction of chronic psoriasis-like lesions by unspecified skin irritation [3,7,8]. In addition, in a different psoriasis model, anti-VEGF treatment of mice, already displaying disease symptoms, resulted in an overall improvement of the psoriatic lesions [9]. Moreover, some patients with psoriasis receiving anti-VEGF treatment for cancer showed complete remission of their cutaneous symptoms [10]. Taken together, these findings indicate that VEGF may play an important role in the pathogenesis of psoriasis. However, the exact mechanisms of VEGF induced inflammation in psoriasis are unknown.

Materials and Methods

Study design

To study the pro-inflammatory function of VEGF in psoriasis, the expression levels of VEGF and IL-23p19 in human psoriatic skin biopsies were analysed by immunohistochemistry. Subsequently, primary human keratinocytes (NHEK) were transfected with a VEGF expression vector. Pro-inflammatory cytokines in primary keratinocyte over-expressing VEGF were profiled by qPCR, ELISA and dot-blot. Analyses of protein phosphorylation profiles in lysates from cells over-expressing VEGF were performed to identify the underlying signaling pathways which were then confirmed by siRNA knockdown.

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Discussion

Our observations suggest that VEGF up-regulates IL-23 and IL-6 expression in epidermal keratinocytes in psoriasis thereby linking cutaneous angiogenesis and T-cell mediated immune responses. As a mechanism, VEGF over-expression triggers pro-inflammatory cytokine release in keratinocytes through the p38 MAPK signaling pathway.

It is well established that in lesional psoriatic skin the activity of the p38 MAPK is increased suggesting that p38 MAPK signalling may play a role in the pathogenesis of psoriasis [13,14]. Moreover, Johansen and colleagues demonstrated that anti-inflammatory therapy in psoriasis is associated with a reduction in p38 MAPK phosphorylation and a
Figure 1: Human keratinocytes over-expressing VEGF increase IL-23 and IL-6. Immunohistochemical analyses demonstrate increased VEGF and IL23 (IL-23p19) protein expression in epidermal keratinocytes in lesional psoriatic skin in vivo (a). In vitro, primary human keratinocytes (NHEK) transfected with a VEGF expression plasmid show increased IL-23 and IL-6 mRNA abundance compared to NHEK transfected with a control plasmid (b). Data are means ± SD of a single experiment performed in triplicate and are representative of two to three independent experiments. In (c) induction of IL-23p19 protein in VEGF over-expressing NHEK was confirmed by dot blot. Increased release of IL-6 into cell culture supernatants could be demonstrated by ELISA.

Figure 2: VEGF increases IL-23 and IL-6 through p38 MAPK signaling in primary human keratinocytes. Primary human keratinocytes (NHEK) were transfected with a VEGF plasmid or a control plasmid at 1 µg/ml and treated with the specific p38 MAPK inhibitor, SB203580 (1µM), for 24h. p38 MAPK inhibition blocked IL-23 and IL-6 mRNA expression in VEGF over-expressing NHEK (as measured by qPCR) (a). Inhibition of CREB1 or Hsp27 by specific siRNA had no effect on VEGF induced IL-23 and IL-6 transcript abundance (as measured by qPCR) (b). Data are means ± SD of a single experiment performed in triplicate and are representative of three independent experiments.
subsequent decrease in the expression of p38 MAPK regulated genes [15]. Consequently, p38 MAPK was suggested as possible target for novel therapies for psoriasis. Indeed, specific inhibitors of p38 MAPK block the secretion of cytokines such as IL-6 from human keratinocytes [16].

In our study, p38 MAPK inhibition in VEGF over-expressing keratinocytes blocked VEGF induced IL-6 but also IL-23 expression. IL-23 is a key cytokine in psoriasis pathogenesis and responsible for subsequent Th17 cell responses. This study is the first to suggest that VEGF is involved in this pro-inflammatory cascade in psoriasis through p38 MAPK. Thus, blocking protein kinases could be a possible novel approach to psoriasis treatment that warrants further research. Furthermore, it will be interesting to investigate the factors responsible for VEGF induction in psoriatic skin. As another novel treatment option, inhibition of VEGF expression or activity could lead to amelioration of cutaneous inflammation. As VEGF is already over-expressed in developing psoriatic lesions an early anti-VEGF intervention might represent the most promising strategy.

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