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Calcitonin gene-related peptide regulates the expression of vascular endothelial growth factor in human HaCaT keratinocytes by activation of ERK1/2 MAPK

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Abstract

Psoriasis is a chronic disease characterized by abnormal epidermal proliferation, inflammation and angiogenesis. The pathogenetic process resulting in hypervascularity remains to be further investigated. It has been reported that a potent angiogenic factor, vascular endothelial growth factor (VEGF) is overexpressed in psoriatic epidermis and that the level of calcitonin gene-related peptide (CGRP) is elevated in psoriasis lesions and CGRP-containing neuropeptide nerve fibers are denser in the psoriatic epidermis. We hypothesized that CGRP might regulate the expression of VEGF by human keratinocytes. VEGF expression in the CGRP-treated human keratinocytes was investigated and the CGRP signaling pathways were examined with respect to VEGF expression. The mRNA and protein levels of VEGF by CGRP were increased in a concentration-dependent manner. However, this increase was abrogated by pretreatment with an extracellular signal-regulated kinase (ERK) inhibitor PD98059. The CGRP-mediated VEGF induction was also effectively inhibited by a pretreatment with the CGRP receptor antagonist CGRP 8–37. In addition, CGRP treatment induced rapid phosphorylation of ERK1/2, PD98059 and CGRP 8–37 were able to inhibit CGRP-induced ERK1/2 phosphorylation. These results suggest that CGRP regulates the expression of VEGF through the CGRP receptor and ERK1/2 MAPK signaling pathway in human HaCaT keratinocytes.

Keywords: Psoriasis; CGRP; VEGF; Angiogenesis; ERK1/2

1. Introduction

Calcitonin gene-related peptide (CGRP), a 37-amino acid peptide, is one of the most abundant neuropeptides in human and rodent skin. The receptor for CGRP has been cloned from various species including human, rat and pig [1]. The receptor displays seven transmembrane domains and shows significant homology with a subfamily of G-protein-coupled receptors that includes calcitonin, vasoactive intestinal peptide, secretin, glucagon and corticotropin releasing factor. CGRP has been previously described to influence proliferation of several cell types, such as endothelial cells [2], Schwann cells [3], and tracheal epithelial cells [4]. It has been demonstrated that the expression of CGRP is elevated in psoriasis lesions and CGRP-containing neuropeptide nerve fibers are denser in the psoriatic epidermis [5]. These findings suggest that CGRP may play a significant role in the pathophysiologic process of psoriasis. However, due to the absence until recently, of selective and high affinity antagonists [6], the precise role of CGRP in psoriasis has not been well clarified.

Psoriasis is a common, chronic skin disease characterized by abnormal keratinocyte proliferation and differentiation, inflammation, and angiogenesis [7,8]. Microvascular changes within plaques of psoriasis include dilatation, tortuosity, increased permeability, and endothelial cell proliferation within the venous limb of capillaries in the dermal papillae [9,10]. Vascular proliferation in psoriasis is in part influenced by angiogenic factors produced by the epidermis [11,12] and these include vascular endothelial growth factor (VEGF)/vascular permeability factor [13]. In normal human skin, VEGF is both expressed and secreted by epidermal keratinocytes. VEGF is a major angiogenic factor that regulates the growth of new capillaries from pre-existing

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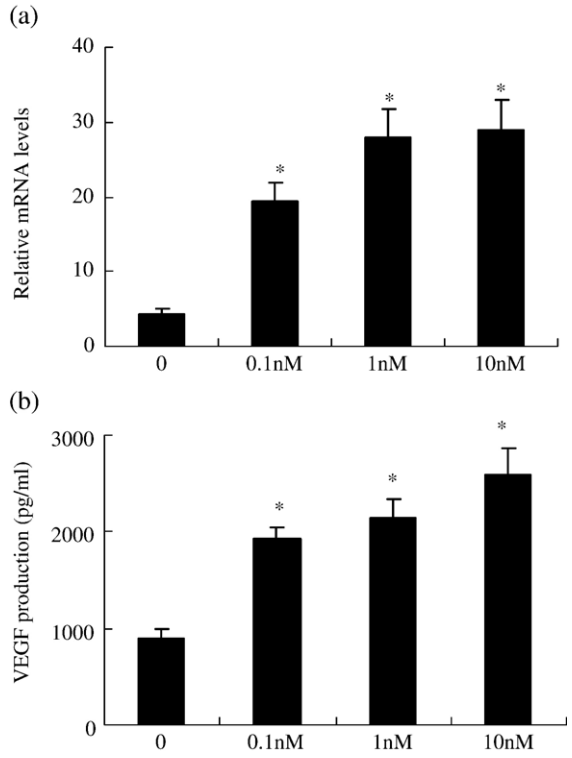

Fig. 1. Calcitonin gene-related peptide (CGRP) induces the mRNA expression and production of vascular endothelial growth factor (VEGF) in HaCaT kertinocytes. (a) HaCaT cells were harvested after CGRP treatment for 6 h. Total RNA was extracted, and VEGF mRNA transcript levels were determined by real-time RT-PCR. (b) Cells were treated with indicated concentration of CGRP for 12 h. Cell supernatants were collected and then VEGF concentration was measured by ELISA, each performed in triplicate. Data are mean±SD (*$P<0.05$).

blood vessels and a process which involves the extravasation of the plasma proteins, the degradation of the extracellular matrix, and endothelial cell migration and proliferation, as well as capillary tube formation [14]. VEGF is produced in several different isoforms created by differential splicing of its mRNA product. In the human, four different isoforms have been identified, VEGF121, VEGF165, VEGF186 and VEGF206 [15]. These isoforms differ in their bioavailability such as VEGF189 and 206 being limited by their binding to extracellular matrix proteins, while the soluble extracellular isoforms, VEGF121 and 165, are associated with more widespread activity [16].

Several studies have demonstrated that VEGF expression is increased in lesional psoriatic skin [17]. A major role of VEGF in the pathogenesis of psoriasis was further corroborated by the phonotype of transgenic mice with epidermis-specific overexpression of VEGF. VEGF transgenic mice show enhanced skin vascularity and vascular permeability [18]. At about 6 months of age, these mice spontaneously develop chronic inflammatory skin lesions that histologically closely resemble human psoriasis [19]. It is of interest that selective targeting of skin vessels via epidermal overexpression of an angiogenesis factor was able to reproduce the complete psoriatic phenotype, including epidermal hyperplasia and altered epidermal differentiation, upregulation of adhesion molecules, accumulation of CD4-positive T-lymphocytes within the dermis and of CD8-positive cells within the epidermis. Moreover, VEGF transgenic mice show the characteristic Koebner phenomenon, with induction of chronic psoriasis-like lesions by unspecific skin irritation [19]. In addition, the gene for VEGF is located on chromosome 6p.21 [20,21], close to PSORS 1 [22,23]. These findings indicate VEGF might play an important role in the pathogenesis of psoriasis.

The aim of this study was to determine whether CGRP can modulate VEGF production at the protein and mRNA levels in the human keratinocyte cell line HaCaT, and identify intracellular transduction pathways involved in the effect of CGRP.

2. Materials and methods

2.1. Materials

Human α-calcitonin gene-related peptide and human á-calcitonin gene-related peptide-(8–37) were purchased from Sigma (Saint Louis, MO), PD98059 was obtained from Biosource (Camarillo, CA). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco (Carlsbad, CA). VEGF ELISA kit was purchased from Biosource (Camarillo, CA). Monoclonal mouse antiphospho-ERK1/2 (p-

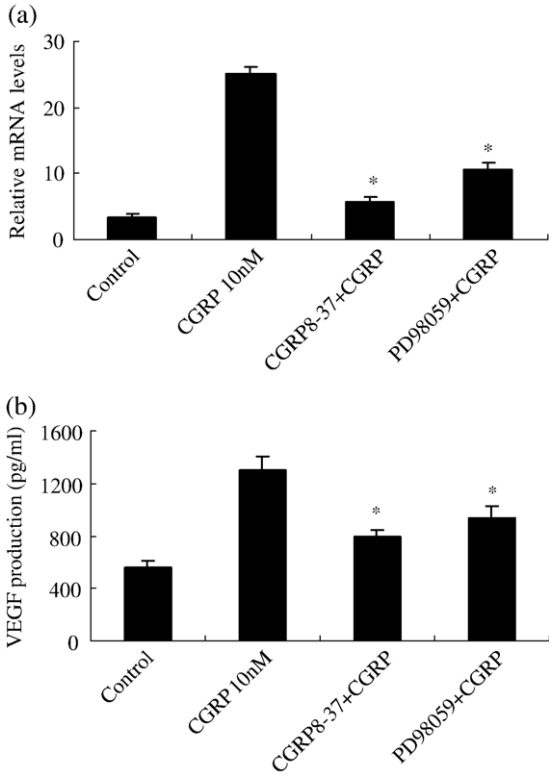

Fig. 2. The CGRP receptor antagonist CGRP 8–37 and the ERK1/2 inhibitor PD98059 inhibit the mRNA expression and production of VEGF in CGRP-treated HaCaT kertinocytes. (a) HaCaT cells were stimulated with or without 10 nM CGRP for 6 h after treatment with CGRP 8–37 or PD98059 (10 μM) for 2 h. Total RNA was extracted, and VEGF mRNA transcript levels were determined by real-time RT-PCR. (b) Cells were incubated with CGRP 8–37 or PD98059 (10 μM) for 2 h prior to the addition of CGRP (10 nM) for 12 h. Cell supernatants were collected and then VEGF concentration was measured by ELISA, each performed in triplicate. Data are mean±SD (*$P<0.01$).