学 位 論 文 の 要 旨

氏 名 張 燕

学 位 論 文 名 Vascular Endothelial Growth Factor 121 is the Predominant Isoform in Psoriatic Scales

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著 者 名 Yan Zhang, Hiroaki Matsuo, and Eishin Morita

論 文 内 容 の 要 旨

INTRODUCTION

Psoriasis is a chronic inflammatory skin disease characterized by accelerated proliferation of epidermis and morphological changes of the capillary system in superficial dermis, including pronounced dilatation, tortuosity and increased permeability. Mast cell-derived histamine is believed to be a major vasoactive mediator, but H1-receptor antagonists are less effective to those progressive changes in psoriatic lesions. This observation prompts speculation that there may be other contributing factor(s).

Vascular endothelial growth factor (VEGF) is a multifunctional cytokine that not only promotes angiogenesis, but also enhances vascular permeability. In humans, VEGF is a homodimeric 36-46 kD protein consisting of six differentially-spliced variants, giving rise to mature isoforms containing 121, 145, 165, 183, 189 and 206 amino acids. Immunohistochemical studies revealed that VEGF production is upregulated in the lesional epidermis of psoriasis and over-produced VEGF may play a pathogenic role in the progression of psoriatic lesions. Our purpose is to assess the VEGF obtained from the lesional stratum corneum of patients with psoriasis.

MATERIALS AND METHODS

Patients Thirteen patients with psoriasis vulgaris were enrolled in the study, who received neither corticosteroids treatment nor vitamin D3 analogues for at least 3 weeks prior to the study.

Immunohistochemistry Frozen sections were fixed, blocked, incubated with anti-VEGF mouse monoclonal antibodies at a concentration of 2 μg/ml overnight at 4°C and processed with immunoperoxidase kit. Sections were counterstained with hematoxylin. The specificity of the staining was proved with irrelevant isotype-matched mouse antibodies.

Preparation of scale extracts and detection of VEGF contents Water-soluble components were extracted from the scales and quantified by ELISA kit.

Western blotting Four mg of protein scale lysates per sample were immunoprecipitated with agarose-conjugated anti-VEGF rabbit polyclonal antibodies. The precipitates were subjected to SDS-PAGE and electroblotted onto a polyvinylidene difluoride microporous membrane. After being blocked, the membrane was incubated overnight at 4°C with goat anti-human VEGF polyclonal antibodies at a concentration of 0.2 μg/ml, then reacted with horseradish peroxidase-conjugated donkey anti-goat IgG antibodies at a concentration of 0.2 μg/ml for 1 h at room temperature. Signals were imaged by ECL plus Western Blotting Detection Reagents™. Recombinant VEGF 165 isoform and recombinant VEGF 121 isoform were used as controls.

Partial purification of VEGF from scale extracts VEGF in patients' scale extracts was partially purified by ammonium sulfate precipitation followed by Phenyl Sepharose chromatography.

Gel filtration The partially-purified VEGF preparation was separated by means of a TSKgel G2000SWxL column. VEGF was trailed by VEGF ELISA kit.

Assay of VEGF activity Biological activity of VEGF in the samples was determined by proliferation assay with the cultured human umbilical vein endothelial cells. Recombinant VEGF isoforms or partially-purified VEGF sample were added, and then the cells were counted on a Ceres 900 Hdi plate reader.

RESULTS AND DISCUSSION

In the present study, the immunohistochemical examination revealed that VEGF expression is

upregulated in the keratinocytes in the psoriatic lesions. In addition, we found that the amount of VEGF produced in lesional scales was at least 50 times higher than that in normal stratum corneum. On immunoblotting, 20 and 15 kDa bands were specifically detected as immunoreactive VEGF protein in lesional psoriatic scales, corresponding to VEGF 165 isoform and VEGF 121 isoform, respectively. The main peak of 28 kDa upon Gel-filtration profile confirmed the finding that VEGF 121 isoform is predominantly produced in psoriatic lesions. In the cell-proliferation analysis, recombinant VEGF 165 isoform elicited marked proliferation of vascular endothelial cells, whereas recombinant VEGF 121 isoform had no activity. Our results also revealed that the VEGF recovered from the lesional scales is still biologically active to induce the proliferation of vascular endothelial cells. However, compared to recombinant VEGF isoforms, the activity of partially-purified VEGF was significantly lower than that of recombinant VEGF 165 isoform, but higher than that of recombinant VEGF 121 isoform at a concentration of 5 ng/ml. This may reflect the fact that scale VEGF consists of mostly VEGF 121 isoform, but is possibly contaminated with VEGF 165 isoform.

A previous report showed that psoriatic skin lesions were pathologically characterized by elongation and enlargement of cutaneous microvessels not the formation of new vessel sprouts. Recent investigations have shown that in contrast to VEGF 165 isoform, VEGF 121 isoform is not able to induce angiogenic sprouting, but causes extensive dilation and hyperpermeability of pre-existing blood vessels. Accordingly, overproduction of VEGF 121 isoform may contribute to the onset of psoriasis by functional and structural changes of microvessels without angiogenesis.

CONCLUSION

VEGF 121 isoform is overproduced in psoriatic lesions, and the isoform mainly participates in the process leading to vessel dilatation and hyperpermeability in the lesions. The VEGF-induced vascular changes may increase extravasation of nutrients to the keratinocytes. This may cause rapid turnover in the epidermis, and possibly extravasation of leukocytes, which can result in epidermis microabscess formation. In this regard, modulation of VEGF secretion or blockade of VEGF action is likely to become an exciting new therapeutic strategy for psoriasis.