Relationship between the Expression of MMP-2/TIMP-2 and Microvessel Density in Psoriasis Vulgaris

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Abstract

Objective: To study the relationship between the expression of matrix metalloproteinase-2 (MMP-2) and tissue inhibitors of metalloproteinase-2 (TIMP-2) and microvessel density (MVD) in patients with psoriasis vulgaris. Methods: The biopsies were taken from psoriatic lesions in 17 patients with active psoriasis vulgaris and the skin of 10 normal controls. The expression of MMP-2 and TIMP-2 were detected by the SP immunohistochemical method. MVD was detected by immunohistochemical method with monoclonal antibody specific for the endothelial marker CD34. Results: MVD in the lesions of psoriasis vulgaris was higher than that in normal control (P<0.01). MMP-2 and TIMP-2 were expressed moderate to intense staining in the psoriatic lesions, but very weak to absent in the normal skin. The expression level of MMP-2 was positive correlation with MVD (r=0.625, P<0.01), and TIMP-2 was negative associated with MVD in active psoriasis vulgaris (r=-0.424, P<0.01). Conclusion: The overexpression of MMP-2 could be responsible for cell-cell and cell-matrix changes noted in psoriatic epidermis. Downregulation of TIMP-2 that may serve as an aetiological factor in the development of psoriasis. The abnormal expression of MMP-2 and TIMP-2 protein are closely correlated with the increase of MVD in psoriatic lesions. These findings indicate that there is a close correlation between the state of
the superficial vasculature and the clinical status of psoriasis. It was concluded that MMP-2 \ TIMP-2 might play important roles in the occurrence and progression of angiogenesis in psoriasis. Further mechanistic investigations are required with the potential that MMP-2 \ TIMP-2 protein may help to explain the pathogenesis of microvascular abnormalities in psoriatic skin. The expanded superficial microvascular bed in psoriatic skin is a necessary component for maintaining clinical lesions and these blood vessels are thus a legitimate target for treatment.

**Key words**: psoriasis, microvessel density, matrix metalloproteinase-2, tissue inhibitors of metalloproteinase-2

**Introduction**

Microvascular abnormalities (capillary elongation, widening and tortuosity) are a characteristic feature of psoriasis and form one of the pathological diagnostic criteria. These changes occur early in the progression of a psoriatic lesions, before there is clinical or histological evidence of epidermal hyperplasia. The extracellular matrix (ECM) plays a critical role in angiogenesis by providing biochemical and positional cues, as well as by mechanically influencing microvessel cell behavior\(^{[1]}\). Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes collectively capable of degrading all major components of the ECM. They are the only group of enzymes that can degrade fibrillar collagen. Breakage or degradation of ECM is necessary in the process of keratinocyte hyperproliferation or abnormal differentiation and angiogenesis. ECM depends on the balance of the levels between activated MMPs and tissue inhibitors of metalloproteinases (TIMPs). Specially, MMP-2 and its tissue inhibitor (TIMP-2) take important roles in degradation of ECM \(^{[2]}\). They have been found in psoriasis\(^{[3-4]}\), but there have been few investigations of the relationship between the expression of MMP-2/ TIMP-2 and microvascular in psoriasis vulgaris. An effort is presently made to examine expression of MMP - 2 and TIMP - 2 proteins in psoriatic lesions, to study relationships between expression of them and microvessel density (MVD) in patients with psoriasis vulgaris and to evaluate their clinical significance.

**Materials and Methods**

**1. Tissue Samples**

Skin biopsies of psoriatic lesions were obtained from 17 patients with active psoriasis vulgaris (aged 19-68 years: 9 males, 8 females) at Zhe Jiang Province Tai Zhou Municipal Hospital (China). The patients who had received systemic treatment, phototherapy or skin-directed local therapies during the 3
months before biopsy were excluded from this study. Additionally, normal skin was obtained from 10 patients in plastic surgery (6 males, 4 females), and none of them was suffered from dermatosis and systemic diseases. All patients were given written consent before the trial. Each of biopsies was divided into two: one was subjected to the routine histopathological examination, and the other was subjected to the SP immunohistochemical.

2. Immunohistochemistry

Immunostaining was performed on serial sections. Four micrometer sections were cut from paraffin blocks of formalin-fixed tissue, deparaffinaged with xylene, and dehydrated by graded ethanol (70%~100%). Deparaffinaged sections were autoclaved (120°C 2 atm, 15 min) in 10 mmol/L citrate buffer (pH 6.0). Endogenous peroxidase activity was quenched by incubating with 0.3% hydrogen peroxidase. The samples were washed with phosphate buffer saline and blocked for 10 min with diluted normal goat serum (1:100). Immunohistochemical staining was conducted by a SP immunohistochemical method and strept avidin-biotin-enzyme complex-double staining method. Mouse monoclonal antibodies to TIMP-2, MMP-2, CD34, diluted 1:100, 1:100, 1:50, were provided by Meixing Company of Fujian (China). For negative control, the slides were treated with PBS in place of primary antibody.

3. Evaluation of immunohistochemical staining

Immunohistochemical staining was independently evaluated by two pathologists unaware of sample origin, there was disagreement in the assessment of immunostaining in <10% of slides examined and consensus was reached on further review. TIMP-2 and MMP-2 immunostaining was semi-quantitatively estimated based on the proportion (positive cell number) and intensity. The staining were subdivided into four score categories: 0, 0%; 1, 1% - 25%; 2, 26% - 50%; 3, 50 % positive cell. The intensity was classified into four categories as 0, negative; 1, weak; 2 (+), moderate; 3 (++), strong (+++). TIMP-2 and MMP-2 score for each case were generate by pulsing the values for the two variables.

MVD counting: searching for the single cell or cluster of cells of microvessels stained brown, locating the mostly occupied area of microvessels under the 40 multiple microscope. Counting the right cells in 3 view fields (area of every view field is 0.74 mm²) and the mean value was the microvessel density.

4. Statistical analysis

The test data were analyzed by SPSS11.5 software using ridit analysis. Spearman’s correlation test was used to analyzed the relationship between CAV-1 and MMP-2, P
Results

1. Positive MMP-2, TIMP-2 in psoriatic skin

15 of the 17 cases of psoriatic skin examined displayed moderate to intense staining for MMP-2 in the epidermis. It shows intense staining for MMP-2 in most keratinocytes of the suprabasal layer (Fig.1). 14 of the 17 cases of psoriatic skin examined TIMP-2 in the epidermis, showing TIMP-2 in a distinct pericellular pattern in suprabasal keratinocytes (Fig.2). Normal control skin were negative for MMP-2, TIMP-2 (Fig.3). TIMP-2 and MM-2 expression were summarized in table 1.

2. Relationship between expression of MMP-2, TIMP-2 and MVD in psoriasis

The expression level of CD34 in psoriasis (18.93±9.46) were significantly higher than that in the normal control (4.34±1.67) (t=8.36, P<0.01). There is extensive positivity of dermal capillary endothelium in psoriatic skin (Fig.4). The relationship between MMP-2 and MVD showed positive associated (r = 0.625, P<0.01) using Spearman’s correlation test. It was negative correlation between TIMP-2 and MVD (r=-0.424, P<0.01).

Table 1  The expression of MMP-2, TIMP-2 in psoriatic and normal skin

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<th>group</th>
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<th>MMP-2</th>
<th>TIMP-2</th>
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<td>-</td>
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<tr>
<td>Normal skin</td>
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<td>10</td>
<td>0</td>
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<tr>
<td>Psoriasis vulgaris</td>
<td>17</td>
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MMP-2 : R_{Normal}=0.533,R_{Psoriasis}=0.426,U=3.83>2.58,P<0.01;
TIMP-2 : R_{Normal}=0.481,R_{Psoriasis}=0.383,U=3.57>2.58,P<0.01．
Discussion

Psoriasis is believed to have a multiple-gene hereditary pattern involving various factors such as immunity, inflammation, cell proliferation and apoptosis, and neura media, and so on (5). It has been estimated that psoriasis affects about 2% of the population in West countries, 0.1% ~ 0.3% in the Far East, and is rather rare in the black race (6,7). The local pathological changes in skin lesions are the formation of Munro's microabscesses containing neutrophils, parakeratosis and the invasion of CD4 and CD8 T cells, mast cells, dendritic cells and macrophages in the dermis and epidermis. It has been believed for so many years that since the initial features of psoriasis are characterized as epidermal hyperplasia, there must be inner abnormalities in keratinocyte proliferation and differentiation in patients with psoriasis. Furthermore, the disease can be triggered or exacerbated by external factors such as trauma, infection, and drugs. There must be an activated proliferation as well as an apoptotic disorder. Alteration in cell-cell and cell-matrix adhesion versus an autoimmune process has been proposed as the primary defect. Some studies showed decreased adhesiveness between keractions and alterations of the BM or ECM at the epidermaldermal interface (8,9). The dynamic balance between the ECM degrading and remodeling is considered to be critical for many physiological processes including cell migration, differentiation, proliferation, reproduction and apoptosis as well as pathological processes, for examples, arthritis, tumor invasion and metastasis and wound healing. The alteration of the ECM in psoriatic skin is of considerable interest as these structure play a role in angiogenesis.

MMPs are zinc-dependent endopeptidases involved in the remodeling of the ECM and play an important role in morphogenesis, angiogenesis, wound, healing, and in certain disorders. As a member of this family, MMP-2 widely degrades components of the basement membrane and connective tissue. In addition, MMP-2 is also capable of degrading laminins (10). MMP-2 is constitutively expressed in many cells and has a ubiquitous tissue distribution; its promoter lacks a conventional TATA box, AP-1, and PEA-3 site enhancers; and it is not stimulated by serine proteases or tissue plasminogen activator (11). In addition, MMP-2 responds poorly to growth factors or cytokines, although it can be moderately stimulated by transforming growth factor-beta-1 (12). TIMPs, as the specific inhibitors of MMPs, have such ability to form tight binding, noncovalent inhibitory complexes with multiple members of MMP family that they inhibit MMPs activity of ECM degradation and have anti-metastasis function (13). Tissue inhibitors of
MMP-2 (TIMP-2), besides acting as a specific inhibitor for MMP-2, has other biologic functions involving regulation of cell proliferation and survival\(^\text{14}\). The TIMP-2 plays a dual role in the regulation of MMP-2 activity. On the one hand, the haemopexin domain at the C-terminal of latent MMP-2 is bound to TIMP-2 to form a complex\(^\text{15}\). The Pro-MMP-1-TIMP-2 complex is believed to be critical for member associated activation process latent MMP-2\(^\text{16}\). On the other hand, if TIMP-2 is bound to the haemopexin domain at the C-terminal of active MMP-2 to form a complex which allows the N-terminal domain of TIMP-2 efficiently blocking up the enzyme’s active site\(^\text{15}\).

Our study showed abnormal expression of MMP-2 and TIMP-2 in patients with active psoriasis vulgaris. The expression of MMP-2 and TIMP-2 were higher than that in normal control (P<0.01). The activity form of MMPs are specifically inhibited by TIMPs and other proteinase inhibitors in vivo. The TIMP-2 can be bound to active MMP-2 with high affinity in a 1:1 molar ratio resulting in loss of proteolytic activity\(^\text{17}\). Therefore, the balance between MMP-2 \& TIMP-2 play an important role in determining the site and extent of degrading ECM component. But there were no relationship between expression of MMP-2 and TIMP-2 in psoriatic skin. TIMP-2 might not fully inhibits activity of MMP-2 in psoriasis which leads to ECM changing in psoriatic skin. In this experience, it was founded that MVD was positive correlation with MMP-2 and negative correlation with TIMP-2. It has been founded that MMP-2 promotes factors in angiogenesis and TIMP-2 inhibits in angiogenesis\(^\text{18}\). This found may explain the reason of microvascular abnormalities in psoriasis. This report represents the first to identify an association between MMP-2 \& TIMP-2 expression and MVD in psoriasis. The study provides preliminary data supporting that the overexpression of MVD in the hyperproliferative compartment of lesional skin may represent an important aetiological factor in the pathogenesis of the active psoriatic disease state. It has been shown that angiogenesis is a new potential target for the therapy of psoriasis\(^\text{19,20}\). We hope that future research to define more precisely the biologic roles in psoriasis may enhance our knowledge on keratinocyte biology and also engender new therapeutic approaches for this disease. It may exist to exploit novel strategies in the therapy of psoriasis.

（投稿日期：2009 年 11 月 2 日）

References


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尋常性銀屑病（Psoriasis Vulgaris）患者體內基質金屬蛋白酵素（MMP-2）及組織內基質金屬蛋白酵素抑制因子（TIMP-2）與顯微血管密度（MVD）的關係

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摘要

研究尋常性銀屑病（Psoriasis Vulgaris）患者體內基質金屬蛋白酵素（MMP-2）及組織內基質金屬蛋白酵素抑制因子（TIMP-2）與顯微血管密度（MVD）的關係。

方法：在17位患者病灶處及10位正常人身上取樣作為對照組。基質金屬蛋白酵素（MMP-2）及基質金屬蛋白酵素抑制因子（TIMP-2）藉由SP免疫組織化學染色法。顯微血管密度（MVD）使用CD34單株抗體用免疫組織化學染色法測定。

結果：顯微血管密度在尋常性銀屑病患者身上高於正常人（P<0.01）。基質金屬蛋白酵素及基質金屬蛋白酵素抑制因子在患者的表現中強度，但正常人身上非常低甚至於沒有。基質金屬蛋白酵素與顯微血管密度表現正相關（r = 0.625, P<0.01），基質金屬蛋白酵素抑制因子與顯微血管密度表現負相關（r = -0.424, P<0.01）。

結論：在患者表皮上，基質金屬蛋白酵素可過度表現於細胞與細胞間，細胞與間質間，而患者身上基質金屬蛋白酵素抑制因子的負調控可視為病原因子。患者身上基質金屬蛋白酵素及基質金屬蛋白酵素抑制因子的不正常表現，與顯微血管密度增加極為相關。本研究指出，表淺血管狀態與臨床患者狀況非常相關。總之，患者癥病開始局部血管增生時，基質金屬蛋白酵素及基質金屬蛋白酵素抑制因子扮演非常重要的角色。這需要更進一步的研究，或許能解釋患者微血管不正常病理表現基質金屬蛋白酵素及基質金屬蛋白酵素抑制因子。患者皮膚擴大的表皮血管床是維持症狀必須要的，因此這些血管是治療時真實而合理的目標。