

The Immunohistochemical Study of MAPKs Expression in Psoriatic Epidermis

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Background: Mitogen-activated protein kinases (MAPKs), serine/threonine protein kinases which are known to mediate cell proliferation, differentiation and apoptosis are composed of ERK (extracellular signal-regulated protein kinase), JNK (stress-activated c-Jun N-terminal kinase) and p38 kinase. PKC (protein kinase C) is one of the major biochemical factors in the pathogenesis of psoriasis. Recently, several studies have reported that MAPKs are associated with abnormal differentiation of psoriatic epidermis with PKC.

Objective: This study was performed to examine the MAPKs expression pattern in normal and psoriatic epidermis, using immunohistochemical staining.

Methods: Fifteen patients with psoriasis and 5 normal subjects as a control were selected. Tissue specimens were fixed in formalin and embedded in paraffin. We examined MAPKs expression (pan ERK, JNK-2 and p-JNK) by using the standard ABC (avidin-biotin-complex) system. We performed immunohistochemical analysis based on the staining of the nuclei of cells under light microscopy.

Results: Pan ERK was expressed in the cytoplasm and nuclei of the basal and spinous layer of psoriatic epidermis. In the 5 cases with severe papillomatosis, dense staining with pan ERK was found in the keratinocytes above deep rete ridges. JNK-2 was expressed diffusely in the cytoplasm of spinous cell layer, but p-JNK was rarely expressed in psoriatic epidermis.

Conclusions: We suggest that ERK and JNK seem to be related to the hyperproliferation and JNK may not be associated with the abnormal differentiation in psoriatic epidermis. (*Ann Dermatol* 16(3) 99~104, 2004)

Key Words: MAPKs, Psoriasis

INTRODUCTION

Psoriasis is a chronic relapsing disease of the skin characterized by abnormal proliferation of keratinocytes and affects about 1 to 2 percent of the population. The pathogenesis of psoriasis is not fully understood, although genetic factor, biochemical factor, abnormal epidermal kinetics and others are considered¹.

MAPKs (mitogen-activated protein kinases) are serine/threonine protein kinases which are known to mediate various biochemical reactions; cell proliferation, differentiation and apoptosis in mammalian cells². The exogenous stimuli are able to activate a variety of intracellular signal transduction pathways and one of the activated signal pathways is the MAPKs pathway^{2,3}. In humans, MAPKs are composed of ERK (extracellular signal-regulated kinase), JNK (stress-activated c-Jun N-terminal kinase) and p38 kinase^{2,4}. PKC (protein kinase C) is one of the major biochemical factors in the pathogenesis of psoriasis⁵⁻⁸. Recent studies disclosed that abnormal differentiation of psoriatic epidermis, such as increased cystatin A and involucrin expression is regulated by PKC-MAPK pathway^{9,10}. These results suggested that MAPKs may be associated with the path-

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ogenesis of psoriasis and mediate psoriatic epidermal hyperproliferation or abnormal differentiation.

In the present study, we have studied the expression of pan ERK, JNK-2 and p-JNK in normal and psoriatic epidermis using immunohistochemical staining.

MATERIALS AND METHODS

1. Patients

Fifteen patients with psoriasis confirmed by clinical and histopathological findings at the Chosun University Hospital Dermatology Clinic and 5 normal subjects as a control were selected. All patients with other cutaneous diseases, except psoriasis, were excluded. Tissue specimens were fixed in formalin and embedded in paraffin.

2. Methods

We used mouse anti-human pan ERK monoclonal antibody (BD Transduction Laboratories, San Jose, CA, USA), mouse anti-human JNK-2 monoclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, California, USA) and mouse anti-human p-JNK monoclonal antibody (Santa Cruz Biotechnology Inc.) as the primary antibodies and they were diluted 1:400, 1:50 and 1:50 in PBS, respectively. Immunohistochemical staining was performed by using the standard ABC (avidin-biotin complex) system. The sections of 5 μ m thickness were deparaffinized and hydrated. They were then incubated for 5 minutes at 95°C with antigen retrieval solution (Dako A/S, Denmark) to unmask antigens of tissue specimens which were then pretreated with 3% hydrogen peroxide for 30 minutes. The sections were then incubated for 3 hours at room temperature with pan ERK, JNK-2 and p-JNK as the primary antibodies. Biotinylated goat anti-mouse IgG (Vector, CA, USA) was used as the secondary antibody, and immune reaction was visualized by avidin-biotin-peroxidase complex (Vector) with 3-3'-diaminobenzidine as a chromogen. Finally, the sections were counterstained with hematoxylin and mounted. To examine the expression pattern of activated MAPKs translocating into the nuclei of keratinocytes, we performed immunohistochemical analysis based on the staining of the nuclei of cells under light microscopy.

RESULTS

1. Normal skin

Pan ERK was expressed in basal and lower suprabasal cell layer (Fig. 1A). JNK-2 was weakly ex-

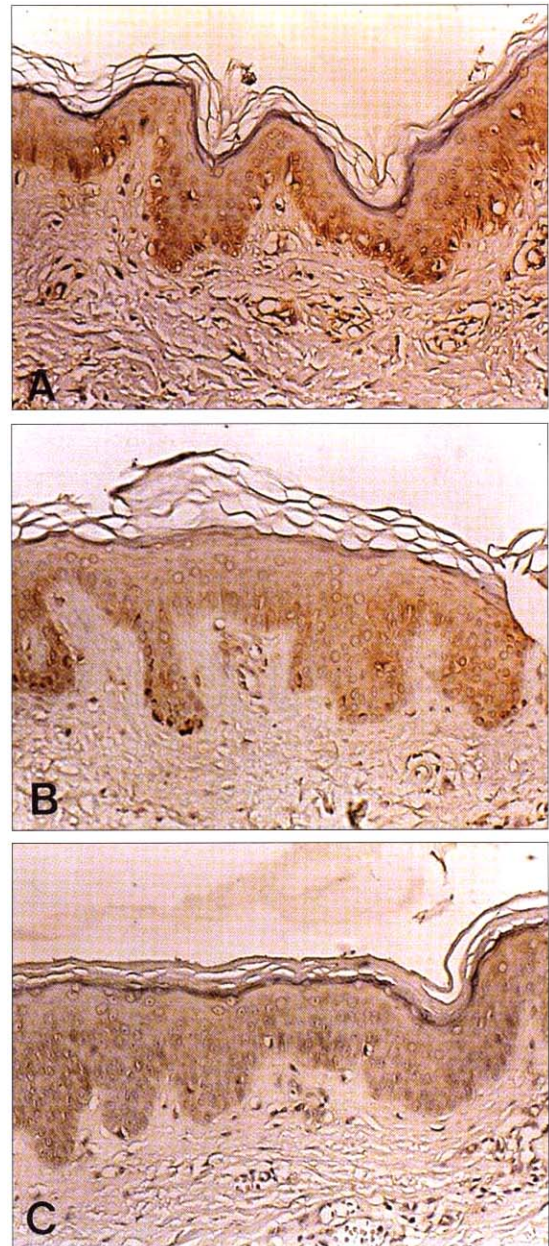


Fig. 1. Immunohistochemical localization of pan ERK, JNK-2 and p-JNK in normal epidermis; Positive staining in basal and lower suprabasal cell layer for pan ERK (A). Weak cytoplasmic staining in spinous cell layer for JNK-2 (B). A few keratinocytes with positive staining in granular cell layer for p-JNK (C).

pressed in the cytoplasm of spinous cell layer (Fig. 1B) and p-JNK was expressed in a few keratinocytes of granular cell layer (Fig. 1C).

2. Psoriatic skin

In all 15 cases of psoriatic epidermis, pan ERK was expressed in the cytoplasm and nuclei of basal and spinous cell layer (Fig. 2A). In the 5 cases with severe papillomatosis, it was highly expressed in the keratinocytes above deep rete ridges (Fig. 2B), and in 4 cases, it was expressed in the inflammatory cells of spongiotic pustule. JNK-2 was expressed diffusely in the cytoplasm of spinous cell layer (Fig. 3A), but p-JNK was not expressed in keratinocytes (Fig. 3B) except staining in the inflammatory cells of the spongiotic pustules in 4 cases.

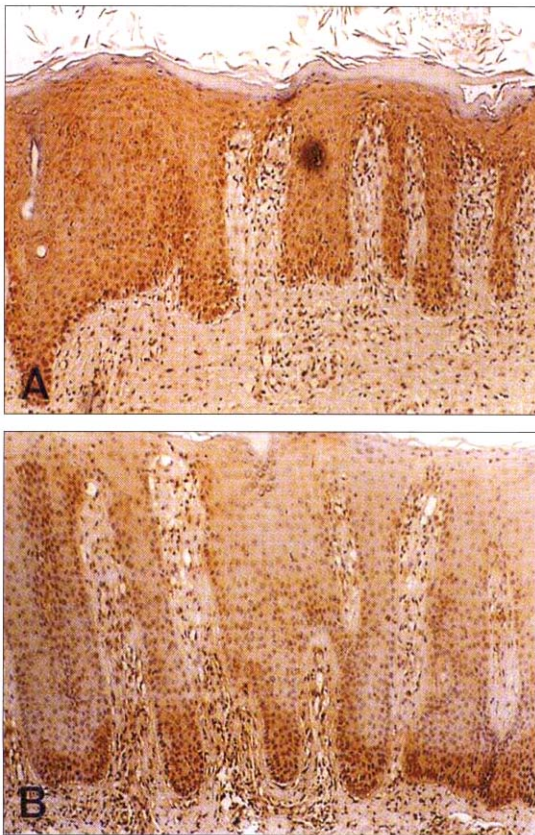


Fig. 2. Immunohistochemical localization of pan ERK in psoriatic epidermis; Positive cytoplasmic and nuclear staining in basal and spinous cell layer (A). In the 5 cases with severe papillomatosis, dense positive staining of the keratinocytes above deep rete ridges (B).

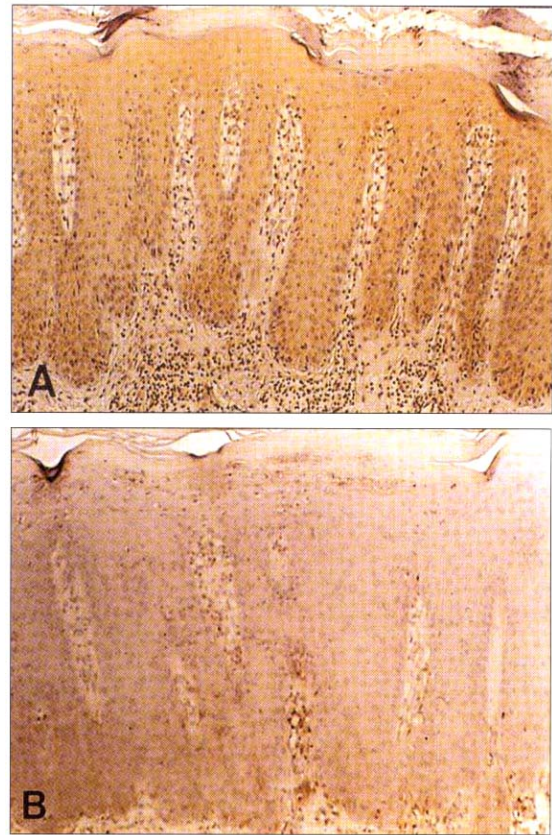


Fig. 3. Immunohistochemical localization of JNK-2 and p-JNK in psoriatic epidermis; Diffuse cytoplasmic staining of spinous cell layer for JNK-2 (A), but negative staining for p-JNK (B).

DISCUSSION

MAPKs are protein kinases utilized by cells to transduce extracellular signals to the nucleus. By phosphorylation, activated MAPKs lead to activation of nuclear transcription factors that affect DNA transcription^{2,3}. MAPKs are activated by cytokines, growth hormones, T cell antigens and cellular stresses in mammalian cells, and regulate various cellular processes; cell proliferation, differentiation and apoptosis. In humans, there are three subtypes of MAPKs including ERK, JNK and p38 kinase^{2,4}. ERK is activated mainly through epidermal growth factor stimulation¹¹ and may play an important role in cross communication of various transduction systems within the cell. JNK is stimulated by cellular stresses such as heat shock, inflammatory cytokines, osmotic imbalance and UV radiation². JNK influ-

ences cell proliferation^{12,13}, differentiation^{14,15} and apoptosis¹⁶⁻¹⁸. Several isoform families of JNK, JNK-1 and JNK-2 have been isolated, and JNK-2 binds c-Jun 25 times more efficiently than JNK-1¹². p38 kinase is activated by lipopolysaccharide molecules and by cellular stress associated with high osmolarity².

Several recent studies suggested that proliferation and differentiation of the keratinocytes in psoriatic epidermis are regulated by the activation of PKC-MAPK pathway^{9,10}. Dimon-Gadal *et al.*¹⁹ reported that ERK activity was higher in cultured psoriatic fibroblasts than in normal fibroblasts. Haase *et al.*²⁰ reported increased expression of ERK in the cell membranes, cytoplasm and nuclei of the basal and lower suprabasal layer of psoriatic epidermis. Takahashi *et al.*²¹ reported that phosphorylated ERK was highly expressed in the basal and spinous cell layer, exclusively in the nuclei of keratinocytes of psoriatic skin. Similar to the result of Hasse *et al.*, we showed that the expression of ERK was increased in the cytoplasm and nuclei of the basal and spinous layer of psoriatic skin. In some cases, ERK was highly expressed in the keratinocytes above deep rete ridges. It was consistent with the model of Lavker and Sun²² about epidermal stem cells, in which epidermal stem cells and transient amplifying cells are located at the tips of the deep rete ridges. Therefore, we suggest that increased ERK expression may be associated with the hyperproliferation of psoriatic epidermis.

PKC is a phospholipid-dependent serine/threonine protein kinase and transduces extracellular signals to the nucleus of the cell. PKC is thought to be involved in proliferation²³ and differentiation²⁴ in mammalian cells and plays a significant role in hyperproliferation and abnormal differentiation of psoriatic epidermis. In psoriatic epidermis, TGF- α (transforming growth factor α) is overexpressed²⁵ and increased TGF- α interacts with the EGF (epidermal growth factor) receptor of keratinocytes, which activates PKC, in turn, activates MAPKs². The PKC activity of psoriasis may be varied with the severity of the disease. PKC activity in the membranes of psoriatic fibroblasts is first increased and then decreased in more severe disease or growth-arrested cells⁶. On the other hand, Horn *et al.*⁸ found that there was no significant difference of PKC activity between the involved and uninvolved epidermis from psoriasis patients, and its activity in the

epidermis of psoriasis patients was significantly lower statistically than in those of control subjects.

Recently, it has been reported that abnormal differentiation of keratinocytes in psoriasis is related to the increased expression of involucrin and cystatin A with both being regulated by PKC-MAPK pathway^{9,10}. Takahashi *et al.*⁹ revealed, in normal human skin, that ERK was expressed in the nuclei of basal cell layer, while JNK was differentially expressed in both cytosol and nuclei of upper spinous and granular cell layer. Cystatin A expression was stimulated by JNK and was suppressed by ERK. They found, in psoriatic epidermis, JNK expression which increases cystatin A expression was highly increased in both spinous and granular cell layer, but p38 expression which activates involucrin expression was not different between the involved and uninvolved skin²¹. Also, Takahashi *et al.*²¹ reported that, in contrast to JNK expression, ERK inhibiting cystatin A expression was increased in the basal and spinous cell layer of psoriatic epidermis. In our study, p-JNK was rarely expressed in psoriatic epidermis except staining in the inflammatory cells of spongiotic pustule. Several recent studies suggested that JNK may be responsible for apoptosis¹⁶⁻¹⁸. We also believed the results of usual JNK expression in the dyskeratotic cells or granular cell layer of epidermis may be related to apoptosis. Consequently, further investigations which disclose the mutual influences between involucrin and cystatin A expression associated with abnormal differentiation of epidermis and PKC-MAPK pathway will be performed in the future.

In the present study, JNK-2 was not expressed in the nuclei but diffusely in the cytoplasm of the spinous cell layer in psoriatic epidermis. Consistent with this result, several studies proposed that MAPKs may not be translocated into the nuclei of cells, regulate protein kinases such as cPLA2 (cytosolic phospholipase A2)²⁶ and RSK (ribosomal S6 kinase 2)²⁷ in the cytoplasm of cells, and then induce the hyperproliferation of epidermis. We suggest JNK seems to be related to the hyperproliferation, but not to the abnormal differentiation in psoriatic epidermis.

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