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# Differential regulation of IGF-II-induced IL-8 by extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinases in human keratinocytes☆

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**Abstract**

In order to study the relationship between insulin like growth factor-II (IGF-II) and interleukin-8 (IL-8) that are upregulated in psoriasis, we monitored IL-8 expression in IGF-II-treated human keratinocytes and explored the signaling pathways of IL-8 expression by IGF-II. IGF-II increased the IL-8 mRNA and protein levels in human keratinocytes. The upregulation of IL-8 expression by IGF-II was reduced by pretreatment with inhibitors of tyrosine kinase, Src, PI3-kinase, and ERK, but not by p38. Furthermore, IGF-II remarkably increased the DNA binding activities of NF-κB and AP-1, and the IL-8 promoter activity. However, cotransfection with IκB mutant blocked the IGF-II-induced IL-8 promoter activity. In addition, cotransfection with dominant negative MEK1 mutant, but not with dominant negative p38 mutant, blocked the IGF-II-induced IL-8 promoter activity. These results suggest that IGF-II is involved in the pathogenesis of psoriasis by inducing IL-8 gene expression through the tyrosine kinase-Src-ERK1/2-AP-1 pathway, and the PI3-kinase and NF-κB pathway.


*Keywords:* IGF-II; IL-8; ERK; p38; NF-κB; AP-1; Psoriasis

The prominence of dermal microvascular expansion in the psoriatic lesion suggests that psoriasis is angiogenesis-dependent [1]. Nickoloff et al. [2] examined the ability of epidermal extracts obtained from psoriatic patients to induce angiogenesis in the rat corneal bioassay and found that media conditioned by keratinocytes from psoriatic plaques induced an angiogenic response stronger than that from normal keratinocytes. It is now recognized that the keratinocytes of lesional skin are a major source of proangiogenic cytokines in psoriasis and studies have identified several angiogenic factors from psoriatic epidermis, including IL-8, TNF-α, endothelial cell stimulating angiogenesis factor, thymidine phosphorylase (TP), and vascular endothelial growth factor (VEGF) [2–7], thereby suggesting their roles in the pathogenesis of psoriasis.

IL-8 was originally identified as a neutrophil chemoattractant [8] and was later shown to be a potent proangiogenic factor inducing corneal vascularization [9] and angiogenesis, both in vitro and in vivo [10,11]. Basal production of IL-8 is normally low or undetectable but it can be rapidly induced by different stimuli such as cytokines, viruses, and reactive oxidants, in which case it is secreted from a variety of cells, including keratinocytes. IL-8 strongly attracts and activates neutrophils and T lymphocytes, and is involved in the pathogenesis of several inflammatory diseases [12–16]. It is present in high amounts in lesional biopsies in pustular diseases such as psoriasis palmoplantar pustulosis [12,14,17]. IL-8 could play an additional important part in keratinocyte hyperproliferation, neutrophil and T-lymphocyte infiltration, and epidermotropism [18–23].

It has been also reported that insulin-like growth factor-II (IGF-II) was increased in psoriatic lesions,

☆ *Abbreviations:* IGF-II, insulin like growth factor-II; IL-8, interleukin-8; VEGF, vascular endothelial growth factor; MAPK, mitogen-activated protein kinase; MEK, MAPK kinase; ERK, extracellular signal-regulating kinase; PLC, phospholipase C; NF-κB, nuclear factor-κB; AP-1, activating protein-1; EMSA, electrophoretic mobility shift assay.

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especially in the serum or blister fluid of psoriasis patients [24]. IGF-II, a polypeptide that has a structural homology with insulin and IGF-I, plays a role in regulating proliferation and differentiation in a variety of cell types [25]. IGF-II induced angiogenesis by increasing VEGF expression in hepatocellular carcinoma (HCC) cells [26]. Therefore, it is believed that IGF-II may play a role in the pathogenesis of psoriasis by regulating the expression of angiogenic factors such as VEGF and IL-8. However, the specific molecular function of IGF-II in promoting angiogenesis remains to be investigated. In the present study, we attempted to study the effects of IGF-II on the expression of IL-8 genes and IGF-II signaling pathways for the regulation of IL-8 in keratinocytes.

## Materials and methods

*Materials*. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and antibiotics (penicillin/streptomycin) were obtained from Gibco-BRL (Rockville, MD). [α-$^{32}$P]dCTP (3000 mCi/mmol) was purchased from Amersham (Piscataway, NJ), recombinant human IGF-II was from R&D Systems (Minneapolis, MN), Genistein was from Alexis (San Diego, CA), PP1 was from Biomol (Plymouth Meeting, PA), and SB203580, PD98059, and U0126 were from Calbiochem (San Diego, CA). All other chemicals including LY294002 were purchased from Sigma Chemicals (St. Louis, MO).

*Primary cell culture*. The normal human epidermal keratinocyte was obtained from Welskin (Seoul, Korea). The normal human epidermal keratinocyte was maintained in Keratinocyte Basal Medium (KBM; Clonetics, USA) containing $5 \times 10^{-7}$ M hydrocortisone, 5 ng/ml of an epidermal growth factor, 30 μg/ml of a bovine pituitary extract, 5 μg/ml insulin, 50 μg/ml gentamicin, and 50 ng/ml amphotericin B. The culture was maintained in a humidified atmosphere of 5% $CO_2$ in air at 37 °C. Generally, after 2–3 days, small clusters of adherent cells were apparent and the KBM was changed every 2–3 days with the subsequent appearance of progressively larger keratinocyte colonies. The cells were starved with supplement-free KBM for 24 h before addition of IGF-II.

*Cell culture*. The immortalized human keratinocyte cell line, HaCaT, was kindly provided by Professor N. Fusenig (German Cancer Research, Germany). Cells were maintained as monolayer cultures in DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in humidified atmosphere with 5% $CO_2$. The cells were seeded at a density of $1 \times 10^6$ cells/100-mm dishes. After 48 h, the cells were washed with serum-free medium and then replaced with media without FBS at least 24 h prior to experiments for treatment.

*Treatment of cells with inhibitors*. Cells were pretreated with either 100 μM Genistein (a tyrosine kinase inhibitor), 10 μM PP1 (a Src family specific inhibitor), 20 μM LY294002 (an inhibitor of PI3-kinase), 40 μM PD98059 (a selective MEK inhibitor), 5 μM SB203580 (a p38 inhibitor), or 10 μM U0126 (a specific MEK1/2 inhibitor) for 1 h in serum-free medium. IGF-II (100 ng/ml) was then added to the cells.

*Preparation of total RNA and Northern blot hybridization*. The total RNA was isolated from the cells treated with IGF-II according to the single step guanidine thiocyanate–phenol–chloroform extraction procedure using Trizol (Invitrogene, Carlsbad, CA) in line with the manufacturer's instructions. The total RNA (35 μg) was electrophoresed in a 1% agarose gel containing 2.2 M of formaldehyde, transferred to a nylon membrane (Osmonics, Westborough, MA), and covalently linked by UV-cross-linker (UV Stratalinker 1800; Stratagene, USA). Hybridization was performed with $^{32}$P-labeled cDNA probe prepared by random primer oligonucleotides (Rediprime DNA Labeling System; Amersham Pharmacia Biotech, Piscataway, NJ).

*Quantitative human IL-8 immunoassay*. The quantity of IL-8 secreted into the culture medium was analyzed using a Quantikine human IL-8 chemiluminescence kit (R&D Systems, Minneapolis, MN), according to the manufacturer's manual, for quantitative and specific sandwich enzyme immunoassay. Both the samples and standards were assayed in parallel.

*Electrophoretic mobility shift assays*. The double strand oligonucleotides containing the NF-κB and AP-1 sequences (Promega, Madison, WI) were 5′-end labeled with [γ-$^{32}$P]ATP using T4 polynucleotide kinase. The binding reactions were carried out in a buffer containing 10 mM Tris–HCl (pH 7.5), 50 mM NaCl, 0.5 mM DTT, 10 mM $MgCl_2$, 10% glycerol, 0.05% NP-40, and 2 μg poly(dI–dC) on ice for 15 min. The assay mixture was incubated with the radiolabeled oligonucleotides for 30 min at room temperature. After 6× dye solution (0.1% bromophenol and 30% glycerol) was added, the mixture was immediately loaded and electrophoresed on a nondenaturing 6% polyacrylamide gel in 0.25× TBE for 2 h at 150 V. The gels were then dried in a vacuum drier at 80 °C for 1 h and autoradiographed on Fuji RX X-ray films.

*Plasmid*. Human IL-8 promoter/pGL3 plasmid (hIL-8-promoter-luc) was kindly provided by Dr. M.A. Harrington (Indiana University School of Medicine and the Walther Cancer Institute, Indianapolis, Indiana) and the dominant negative IκB mutant plasmid (pcDNA/IκBα-SR) was gift from Dr. H. Nakshatri (Indiana University School of Medicine, Indianapolis, Indiana). The dominant negative MEK1 mutant plasmid pUSE-DN-MEK1, the dominant negative p38 mutant plasmid pEF6-DN-p38, and control vector were kindly provided by Dr. A.K. Yi (University of Tennessee Health Science Center, Memphis, Tennessee).

*Transfections*. Transient transfections were performed using Lipofectin (Gibco-BRL, Rockville, MD) according to the manufacturer's protocol. Briefly $5 \times 10^5$ cells were plated 60 mm dish plate the day before transfection and grown to about 70% confluence. Two micrograms of plasmid DNA prepared by Qiagen kit (Qiagen, Santa Clara, CA) per plate was transfected. Transfections were allowed to proceed for 24 h. The transfected cells were washed with 4 ml PBS and then stimulated with 100 ng/ml human IGF-II. The cells continually cultured in serum-free DMEM until the cells were harvested. Transfection efficiency was corrected by cotransfection of 0.5 μg phRL-TK Vector (Promega, Madison, WI).

*Luciferase assay*. After experimental treatments, cells were washed twice with cold PBS, lysed in a passive lysis buffer provided in the dual luciferase kit (Promega), and assayed for luciferase activity using TD-20/20 luminometer (Turner Designs, Sunnyvale, CA) according to the manufacturer's protocol. All transfections were done in triplicate. The data were presented as a ratio between firefly and *Renilla* luciferase activity.

*Data analysis*. Scanning densitometry was performed using an Image Master VDS (Pharmacia Biotech, San Francisco, CA). The one-way analysis of variance procedure was used to assess the significant differences among the treatment groups. For each significant effect of treatment, the Newman–Keuls test was used for significance set at $p < 0.05$ or $p < 0.01$.

## Results

### *Induction of IL-8 genes by exogenous IGF-II in primary normal human keratinocytes and HaCaT cells*

Since IL-8 is known as an angiogenic factor similar to VEGF [27], and IL-8 and IGF-II are overexpressed in psoriasis [24,28], we hypothesized that IGF-II may affect

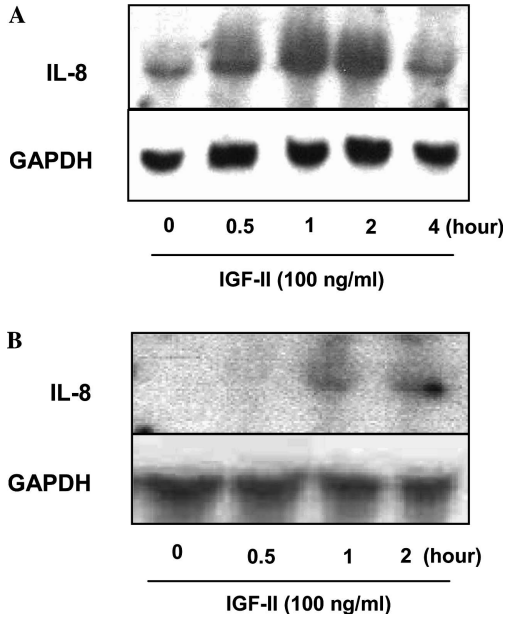


Fig. 1. Induction of IL-8 gene by exogenous IGF-II in primary normal human keratinocytes (A) and HaCaT (B) cells. (A) Northern blot analyses for IL-8 mRNA levels in a time-dependent manner in primary normal human keratinocytes. The IL-8 mRNA levels were detected in the total RNA fractions (35 μg per lane) isolated from primary normal human keratinocytes. The amount of RNA loaded in each lane was confirmed by rehybridization of the stripped membrane with a $^{32}$P-labeled probe complementary to GAPDH mRNA. (B) Induction of the IL-8 mRNA levels in a time-dependent manner in HaCaT cells. The data were confirmed by two independent experiments.

angiogenesis by inducing the expression of IL-8 in psoriasis. Thus, we investigated whether IGF-II was able to increase the IL-8 expression levels in primary normal human keratinocytes and the immortalized human keratinocyte cell line, HaCaT.

In the primary normal human keratinocytes treated with exogenous IGF-II (100 ng/ml), the IL-8 mRNA levels were increased 0.5, 1, and 2 h after treatment, but returned to control levels 4 h after treatment (Fig. 1A). When the HaCaT cells were treated with IGF-II (100 ng/ml), Northern blot analyses showed that the IL-8 mRNA level was significantly increased at 1 and 2 h (Fig. 1B).

*Implication of tyrosine kinase and the subsequent pathway (Src-ERK pathway and PI3-kinase) in the IGF-II-mediated IL-8 mRNA expression*

Since IL-8 expression by IGF-II showed the same pattern in both of primary normal human keratinocytes and HaCaT, HaCaT was used to investigate the possible mechanisms underlying IL-8 induction by IGF-II. IGF-II-associated receptor tyrosine kinases were investigated to determine if they are directly involved in the IL-8 expression caused by IGF-II in the mRNA level. When the HaCaT cells were pretreated with a tyrosine kinase inhibitor, Genistein (100 μM), 1 h prior to the IGF-II treatment, the IGF-II-mediated induction of IL-8 was decreased (Fig. 2A).

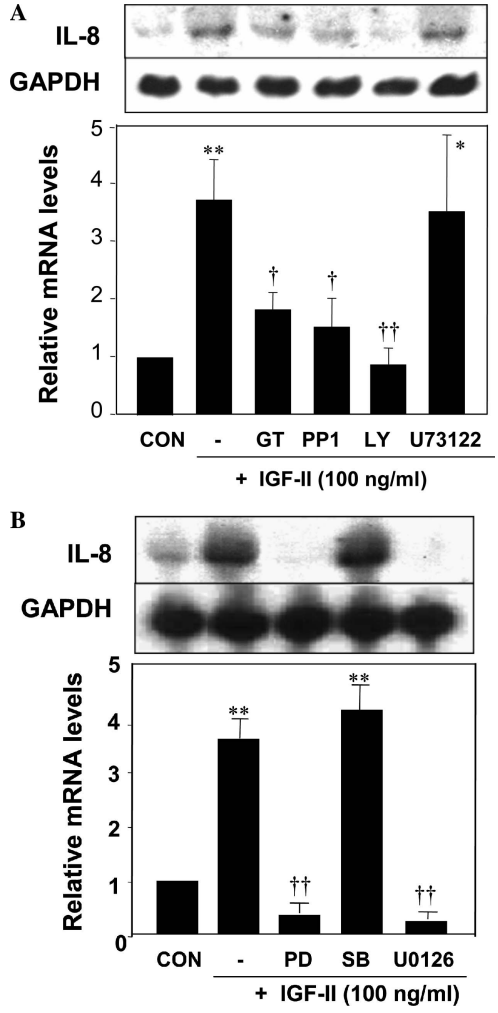


Fig. 2. IL-8 expression mediated by IGF-II is implicated in tyrosine kinase and the subsequent pathways (Src-PI3-kinase pathway (A) and ERK1/2 pathway (B)). Northern blot analysis for IL-8 mRNA and the relative IL-8 mRNA levels. The IL-8 mRNA levels were detected in the total RNA fractions (35 μg per lane). The HaCaT cells were incubated for 1 h with the inhibitors (the tyrosine inhibitor, Genistein (GT; 100 μM); the Src inhibitor (PP1; 10 μM); the PI3-kinase inhibitor, LY294002 (20 μM); the PLC inhibitor, U73122 (5 μM); the MEK inhibitor, PD98059 (40 μM); the p38 inhibitor, SB203580 (5 μM); or the MEK1/2 inhibitor, U0126 (10 μM)). IGF-II (100 ng/ml) was then added to the cells. Two hours later, the total RNA was extracted from the cells and Northern blot analyses were done. The data are presented as means ± SEM of three independent experiments. One-way analysis of variance was used to compare the multiple group means followed by Newman–Keuls test (significance compared to the control, $^{*}p < 0.05$ or $^{**}p < 0.01$; significance compared with IGF-II, $^{\dagger}p < 0.05$ or $^{\dagger\dagger}p < 0.01$) (control level = 1).

We then investigated the possibility that the IGF-II-mediated activation of the receptor tyrosine kinases elicits the phosphorylation of Src and PI3-kinase, which in turn activates phospholipase C (PLC), thereby altering the expression of the IL-8 gene using various

protein kinase inhibitors. Pretreatment of the cells with the Src inhibitor, PP1 (10 μM), and the PI3-kinase inhibitor, LY294002 (20 μM), significantly decreased the IGF-II-induced IL-8 expression, but not with the PLC inhibitor, U73122 (Fig. 2A). These results suggest that IGF-II induces IL-8 expression by mediating the activation of the receptor tyrosine kinases which elicit the phosphorylation of Src and PI3-kinase. The signal transduction pathway, which mediates the increased IL-8 expression by IGF-II, was further investigated. IL-8 mRNA overexpression 2 h after IGF-II treatment was significantly diminished following the addition of the selective MEK inhibitor, PD98059 (40 μM), and the specific MEK1/2 inhibitor, U0126 (10 μM). However, pretreatment with the P38 inhibitor, SB203580 (5 μM), did not moderate the IGF-II-induced IL-8 expression (Fig. 2B). This indicates that the induction mechanism of IL-8 by IGF-II is implicated in the ERK pathway.

*Induction of IL-8 protein levels by exogenous IGF-II through tyrosine kinase and the subsequent pathway (Src-ERK pathway) as well as PI3-kinase*

ELISA was performed to detect the IL-8 protein using the supernatant from the IGF-II-treated HaCaT. IL-8 production started to increase 2 h after the IGF-II treatment in a time-dependent manner (Fig. 3A). When the cells were pretreated with these kinase inhibitors, tyrosine kinase inhibitor, Genistein, Src inhibitor, PP1, and PI3-kinase, LY294002 completely inhibited the IGF-II-induced IL-8 secretion. The MEK inhibitor, PD98059, and the MEK1/2 inhibitor, U0126, also significantly inhibited the increase of IL-8 protein level by IGF-II, whereas the PLC inhibitor, U73122, and the p38 MAPK inhibitor, SB203580, failed to block the increase of IL-8 protein level by IGF-II (Fig. 3B). These results suggest that the induction mechanism of IL-8 by IGF-II is implicated in the tyrosine kinase-Src-ERK1/2 pathway and PI3-kinase.

*Activation of DNA binding activity of NF-κB and AP-1 by IGF-II and its involvement in the induction of IL-8 by IGF-II*

The IL-8 gene promoters contain both NF-κB and AP-1 binding sites, and their activation appears to be associated with angiogenesis by expressing IL-8 [29–32]. In order to investigate that the induction of the IL-8 genes by IGF-II occurs through the NF-κB or AP-1 pathway, we performed electrophoretic mobility shift assays (EMSAs). The nuclear extracts were isolated from the keratinocytes of IGF-II treatment, and NF-κB and AP-1 oligomers were used as probes. As shown in Fig. 4, the DNA binding activities of NF-κB and AP-1 were dramatically increased 30 min after the IGF-II

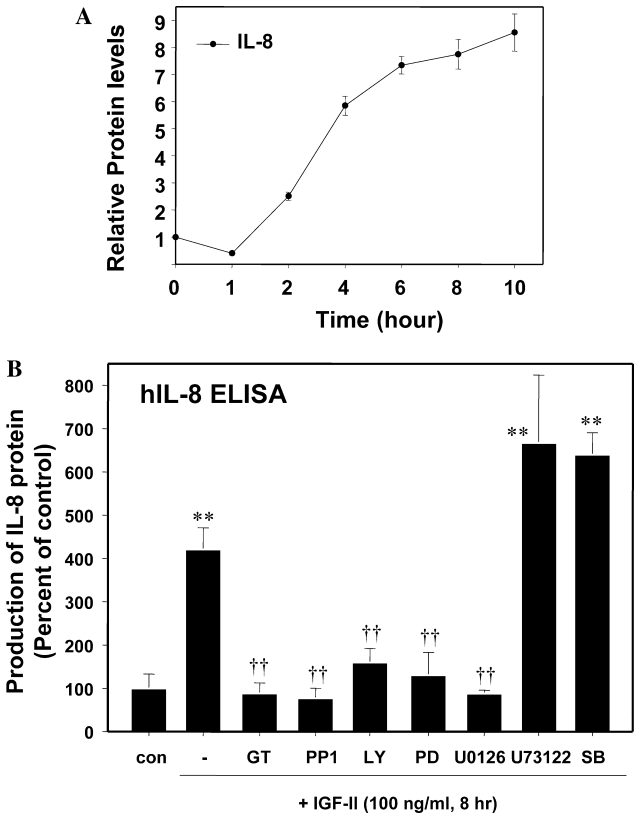


Fig. 3. IGF-II-induced IL-8 secretion. ELISA was performed using the supernatant from the IGF-II-treated HaCaT in a time-dependent manner (A) or from the IGF-II-treated HaCaT for 8 h (B). The inhibitors were pretreated 1 h before. The data are presented as means ± SEM of three independent experiments. One-way analysis of variance was used for comparisons of the multiple group means followed by Newman–Keuls test (significance compared to the control, $^{**}p < 0.01$; significance compared with IGF-II, or $^{\dagger\dagger}p < 0.01$) (control level = 1).

treatment. NF-κB and AP-1 activated by IGF-II were engaged in the gene expression by binding in the promoter of the target gene, IL-8.

To further determine whether NF-κB activated by IGF-II is involved in the expression of IL-8, we performed luciferase reporter assay using IκBα-SR, in which the IκB phosphorylation site is mutated. HaCaT cells transfected with hIL8-promoter-luc were cotransfected with either pcDNA/IκBα-SR or pcDNA3. Then, the transfectants were exposed to IGF-II (100 ng/ml) for 8 h. IGF-II increased IL-8 promoter activity approximately 3-fold higher than that of the untreated control (Fig. 5A). However, when the mutant pcDNA/IκBα-SR was transfected, it did not change the luciferase activity by IGF-II (Fig. 5B). It is well known that NF-κB activation is regulated by its inhibitory protein, IκB, and that most NF-κB activators induce IκB degradation by phosphorylation of IκB. Therefore, these results suggest that IGF-II activates IL-8 promoter via NF-κB.

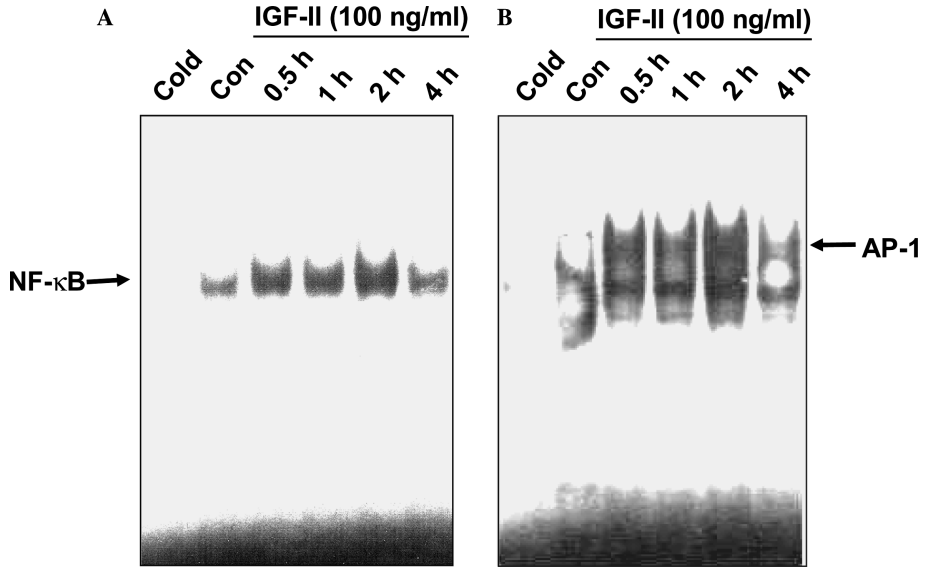


Fig. 4. IGF-II increases the DNA binding activities of NF-κB (A) and AP-1 (B) in HaCaT cells. The nuclear extracts from the cells treated with IGF-II (100 ng/ml) at the indicated times were incubated with [$\gamma$-$^{32}$P]ATP-labeled NF-κB and the AP-1 probe, and analyzed by EMSA. The specificity of the bands was verified by adding a 10-fold excess of a completing unlabeled NF-κB and AP-1 probe (cold probe) to the 2 h IGF-II treated nuclear proteins. The results were confirmed by two independent experiments.

*Upregulation of IL-8 promoter activity by IGF-II through an ERK-dependent pathway*

In our previous paper [33], we showed that IGF-II activated ERK1/2 phosphorylation in HaCaT. To further determine whether ERK1/2 activated by IGF-II is directly involved in IL-8 expression, HaCaT cells were transiently cotransfected with hIL-8-promoter-luc reporter and expression vectors encoding DN-MEK1 or DN-p38. The transfected cells were stimulated with IGF-II for 16 h and then luciferase activity was measured. As shown in Fig. 6A, IGF-II-induced IL-8 promoter-luciferase activity was substantially reduced by overexpression of DN-MEK1. However, overexpression of DN-p38 did not inhibit IGF-II-induced IL-8 promoter-luciferase activity (Fig. 6B). These results demonstrate that ERK1/2 activated by IGF-II contributes to IL-8 expression.

## Discussion

Psoriasis is characterized by the hyperproliferation of keratinocytes, the increase in angiogenesis, and the inflammation mediated by dermal infiltrates of leukocytes. The proliferation and differentiation of keratinocytes are controlled by a complex network of growth factors and cytokines [34]. Increased cytokine expression in inflammatory skin diseases is believed to mediate the skin inflammatory immune responses and leukocyte chemotaxis [35,36]. Several previous studies have indicated that keratinocytes probably produce IL-8 in psoriasis [14,17,27]. IL-8 causes chemotaxis of neutrophils and T-lymphocytes [37], and promotes epidermal proliferation and angiogenesis [10]. These findings suggest that IL-8 plays a role as a psoriasis-promoting factor.

It has been also reported that the IGF-II levels are higher in psoriatic lesions [24]. In addition, Wraight et al. [38] reported that inhibition of IGF-I receptor (IGF-IR) expression in a psoriatic lesion reverses psoriatic epidermal hyperplasia by slowing the rate of keratinocyte cell division. This suggests that IGF-IR stimulation is a rate-limiting step in psoriatic epidermal hyperplasia. However, the precise cause and the relationship between the cytokines involved in psoriasis are unclear.

There are some reports on the different responses between normal primary keratinocytes and HaCaT [39,40], in which p53 is mutated and abnormally overexpressed compared to primary keratinocytes. However, HaCaT exhibits most of the characteristics of the basal keratinocytes [41,42], and importantly, it exhibits hyperproliferation that is typical of epidermal keratinocytes in psoriatic lesion [43]. The HaCaT cell line has been widely used for the in vitro testing of antipsoriatic compounds [44–46]. Therefore, we investigated the IL-8 expression in IGF-II-treated human keratinocytes and explored the signaling pathways of IL-8 expression by IGF-II in HaCaT. In this study, we demonstrated the IGF-II induced IL-8 mRNA expression and secretion through the activation of tyrosine kinase, Src, PI3-kinase, and ERK pathway. In addition, we found the differential regulation of ERK and p38 on the IGF-II-induced IL-8 expression.

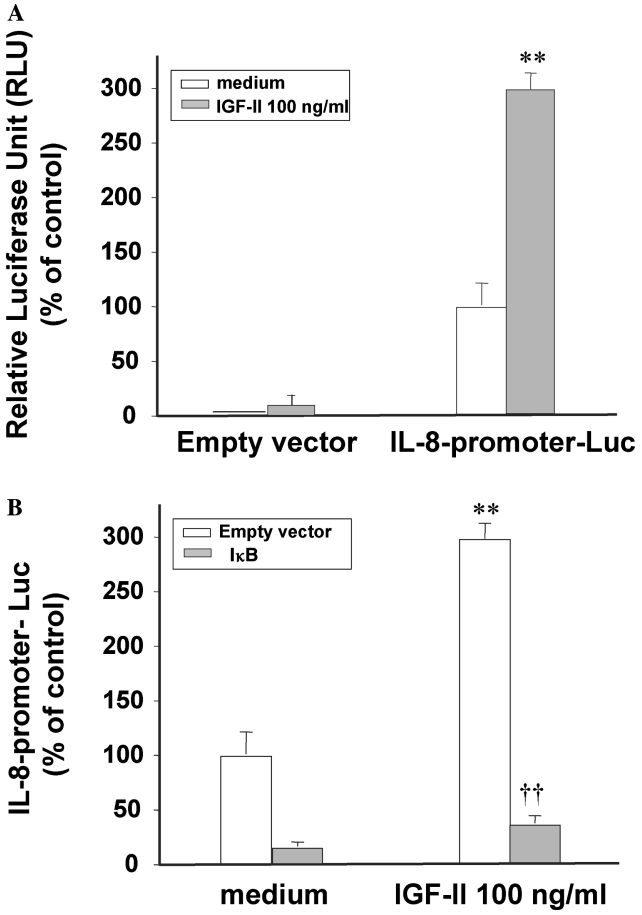


Fig. 5. IGF-II activates human IL-8 promoter in HaCaT cells. Cells were transfected with empty vector or 1 μg hIL-promoter-luciferase + 0.5 μg pRL-TK-luciferase (A), and empty vector or 1 μg pcDNA/IκBα-SR and 1 μg hIL-promoter-luciferase + 0.5 μg pRL-TK-luciferase (B). Cells were allowed to recover for 24 h and then treated with 100 ng/ml IGF-II. Cells were harvested 8 h post-treatment. Luciferase activities are presented as the fold activation relative to that of the untreated cells. The data are presented as means ± SD of three independent experiments. One-way analysis of variance was used to compare the multiple group means followed by Newman–Keuls test (significance compared to the control, $^{**}p < 0.01$; significance compared with IGF-II, $^{\dagger\dagger}p < 0.01$) (control level = 1).

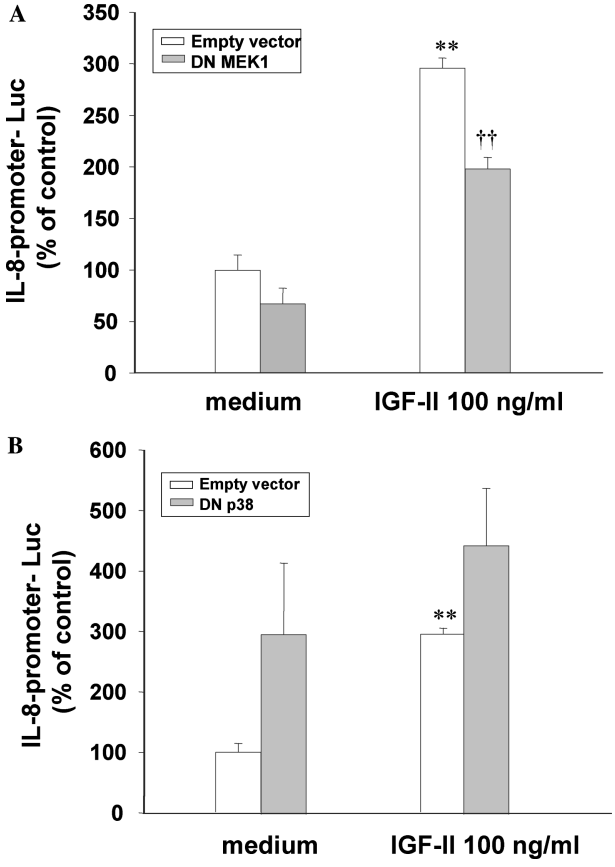


Fig. 6. IGF-II induces IL-8 promoter activity through an ERK-dependent signaling pathway. HaCaT cells were transiently cotransfected with empty vector or plasmids encoding DN-MEK1 and hIL-8 promoter-luciferase + pRL-TK-luciferase (A), and empty vector or DN-p38 and hIL-8 promoter-luciferase + pRL-TK-luciferase (B). Transfected cells were washed and then incubated in serum-free media for 24 h to allow expression of DN-MEK1 or DN-p38. Cells were stimulated with IGF-II (100 ng/ml) for 8 h. Luciferase activities are presented as the fold activation relative to that of the untreated cells. The data are presented as means ± SD of three independent experiments. One-way analysis of variance was used to compare the multiple group means followed by Newman–Keuls test (significance compared to the control, $^{**}p < 0.01$; significance compared with IGF-II, $^{\dagger\dagger}p < 0.01$) (control level = 1).

IGF-II, a polypeptide having a structural homology with insulin and IGF-I, is known to play a role in regulating the proliferation and differentiation in a variety of cell types [25]. The insulin receptor protein family is a widely expressed tyrosine kinase family that mediates insulin and IGF-I signaling [47–49]. The biological actions of both IGF-I and IGF-II have been thought to be mediated primarily by IGF-IR. Both insulin receptor and IGF-IR are expressed in skin keratinocytes [50–52]. IGF-IR is expressed at the cell surface as a tetramer of two α-subunits and two β-subunits. Ligand binding to the extracellular α-subunits leads to the activation of the intrinsic tyrosine kinases of the transmembrane β-subunits. Subsequent phosphorylation of the β-subunits and associated adapter molecules, such as insulin receptor substrates (IRS) and the SH2-containing protein Src, serves to recruit other signaling molecules. Tyrosine phosphorylated IRS is able to recruit other molecules via its SH2 domains. Recruitment of the 85 kDa regulatory subunit of PI3-kinase results in the activation of the enzyme to phosphorylate phosphoinositides at the 3-position. This leads to the activation of phosphoinositide-dependent kinase-1, which in its turn activates protein kinase B (PKB, also known as Akt). In addition, PI3-kinase lipid products support ins(1,4,5)P3 production by promoting translocation and phosphorylation of PLCγ1 and by direct stimulation of both PLCγ isoforms [53]. Another pathway that emerges after tyrosine phosphorylation of IGF-I receptor involves the activation of ERK1 and ERK2, which are isoforms of the mitogen-activated

protein kinase (MAPK) family. Phosphorylation of Src leads to the recruitment of Grb2, which binds SOS, a guanine nucleotide exchange factor that in turn converts inactive ras GDP into active ras GTP. Subsequently, GTP-bound ras recruits the raf-1 serine kinase that activates MAPK kinases (MEK1/2) [54].

Promoter of the IL-8 gene contain recognition sites for the transcription factors NF-κB and AP-1 [30–32]. Both NF-κB and AP-1 may be modulated by the inhibitor κB kinase (IKK) and the MAPK signaling pathway [29].

Based on these findings, our results demonstrated that IGF-II binding to the extracellular subunits of IGF-IR activates the intrinsic tyrosine kinase, which elicits the Src and PI3-kinase activation. In another way, the IGF-II-mediated activation of tyrosine kinase leads to Src activation, followed by ERK1/2 activation. The IGF-II-mediated activation of PI3-kinase and ERK may regulate the NF-κB or AP-1 DNA binding activity, which finally increases the IL-8 promoter activity. In the present study, cotreatment with PD98059 or U0126 blocked the increase of IL-8 mRNA expression and secretion (Figs. 2B and 3B), but the effect of the promoter activity by DN-MEK1 (almost 30% decrease) in Fig. 6A was little, compared to that by IκBα-SR (almost 83% decrease). Therefore, it seems that IL-8 expression by IGF-II is regulated mainly by NF-κB, and partially by ERK pathway. As mentioned above, IGF-II activates multiple signaling pathways including tyrosine kinase-Src-PI3-kinase and tyrosine kinase-Src-ERKs, which may contribute to stimulation of NF-κB-dependent transcription by IGF-II.

Previously, we demonstrated that IGF-II enhanced the phosphorylation of ERK1/2. Phospho-p38 was also increased by IGF-II treatment, as was total p38 [33]. Thus, it is not likely that phospho-p38 is involved in the gene regulation by IGF-II. In the present study, we focused on the differential role of MAPKs, especially, ERK and p38 on the expression of IL-8 by IGF-II. ERK, but not p38, played an important role in the IGF-II-induced IL-8 mRNA level, protein level, and promoter activity. Since the JNK components, Jun and Fos, regulate the DNA binding activity of AP-1, further study is required to determine the role of JNK in the IGF-II-induced IL-8 expression.

In conclusion, our results suggest that IGF-II induces the expression of an angiogenic factor, IL-8, which is involved in the pathogenesis of psoriasis, through the tyrosine kinase-Src-ERK1/2 pathway and the tyrosine kinase-PI3-kinase pathway followed by AP-1 and NF-κB.

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