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Research Article

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ABSTRACT

Matrix metalloproteinase 13 (MMP13) is a zinc-containing endopeptidase secreted by keratinocytes and skin fibroblasts and participates in many inflammatory diseases. Drugs for retinoic acid include tazarotene and acitretin. Tazarotene/acitretin and narrow-band ultraviolet B (NB-UVB) irradiation are used as a general treatment for psoriasis. However, their impact on MMP13 expression has yet to be determined. In this study, we measured the expression of MMP13 in patients with psoriasis, and investigated the effects of tazarotene and/or NB-UVB on MMP13 expression in a mouse model of psoriasis. After exposure to acitretin and/or NB-UVB, immortalized human HaCaT keratinocytes were analyzed for viability and MMP13 expression. Our results showed that MMP13 protein levels

increased in skin lesions and serum samples in patients with psoriasis. Treatment with acitretin and NB-UVB irradiation alone or in combination suppressed cell viability and MMP13 expression in HaCaT cells. Consistently, tazarotene treatment and/or NB-UVB irradiation attenuated imiquimod-induced psoriasis-like dermatitis and inhibited MMP13 expression in a mouse model. Taken together, these results indicate that tazarotene/acitretin and NB-UVB irradiation can inhibit the expression of MMP13 in keratinocytes and psoriasis mouse models. Targeting MMP13 may represent a promising therapeutic strategy against psoriasis.

Key words: psoriasis; retinoic acid；tazarotene; acitretin; narrow-band ultraviolet B; matrix metalloproteinase 13

INTRODUCTION

Psoriasis is a chronic recurrent inflammatory disease caused by abnormal proliferation and differentiation of keratinocytes[1-3]. Matrix metalloproteinases (MMPs) are structurally related zinc-dependent endopeptidases capable of degradation of extracellular matrix (ECM)[4; 5]. MMP13 has been reported to regulate multiple pathological processes such as arthritis, infection, and tumor progression[5-7]. Both topical and systemic vitamin A derivatives, are used in the treatment of psoriasis. Tazarotene is a topical agent and acitretin is a systemic retinoid[8]. Both are most effective in combination with other treatment modalities. Tazarotene and acitretin are selective retinoic acid receptor (RAR) agonists. They show the ability to regulate keratinocyte proliferation and differentiation and inhibit inflammation[8]. Narrow-band UVB (NB-UVB) that emits mostly 311/312 nm lights is commonly used in phototherapy[9]. NB-UVB has a more profound therapeutic efficacy

for psoriasis than conventional UVB, with a shorter period of time and fewer adverse effects. NB-UVB irradiation can result in lymphocyte apoptosis and decrease the number of Langerhans cells[10; 11]. Psoriasis resolves to a greater degree in patients treated with NB-UVB in combination with retinoic acid than in patients treated with either NB-UVB or retinoic acid alone[8; 12-14]. Despite these findings, the mechanism underlying the therapeutic benefits in psoriasis is still unclear.

In this study, we determined the expression of MMP13 in patients with psoriasis and investigated the influence of tazarotene/acitretin and NB-UVB irradiation on the expression of MMP13 in imiquimod (IMQ)-induced mice and keratinocytes.

MATERIALS AND METHODS

Ethics statement

All studies involving human patients were approved by the General Hospital of Tianjin Medical University (Tianjin, China). Participants gave their informed consent in writing, and the study was conducted according to the declaration of Helsinki.

Tissue sample collection

Eighteen cases of skin lesions were collected from patients with psoriasis who were diagnosed in General Hospital of Tianjin Medical University between May 2019 and August 2019. Exclusion criteria were ongoing systemic anti-inflammatory treatment, other rheumatological diseases, other inflammatory dermatoses, pregnancy, intense UV exposure two weeks prior to study start, chronic infectious diseases, exposure to photosensitizing drugs, and age < 18 years. Ten cases of normal skin samples were obtained from patients undergoing cosmetic surgery in General Hospital of Tianjin Medical University. Blood samples were taken for preoperative HIV screening, and serum samples were stored at -80˚C. Skin biopsies were paraffin-embedded and subjected to

immunohistochemistry.

Animal experiments

Twenty-five male BALB/c mice (6-8 weeks old) were purchased from Beijing Viton LiHua Co., LTD (Beijing, China). Mice were maintained at 24-25°C under a 12 h light-dark cycle with free access to food and water. The study was performed in accordance with the European guideline for care in animal research and approved by the Institutional Review Board of General Hospital of Tianjin Medical University.

All the mice were randomly divided into 5 groups: control group, IMQ-induced group, IMQ+ tazarotene group, IMQ+NB-UVB irradiation group, and IMQ+ tazarotene+ NB-UVB irradiation group. Control mice were untreated. The IMQ-induced group received 62.5 mg IMQ cream on the shaved back daily for 7 days to induce psoriasis-like skin inflammation[15]. IMQ + tazarotene group, IMQ + NB-UVB irradiation group, and IMQ + tazarotene + NB-UVB irradiation groups received the IMQ treatment daily for 7 days together with tazarotene treatment, 300mJ/cm^2 NB-UVB irradiation, with 50mJ/cm^2 increase on the next day, and tazarotene plus NB-UVB irradiation, respectively. The 2 × 2 cm^2 skin on the back of mice was selected as the observation area. At the end of the experiment, all mice were sacrificed. Blood was taken from the eyeball of mice. The dorsal skin sample of mice was paraffin-embedded and analyzed for MMP13 expression. The epidermal thickness was quantified using a computer-assisted image analyzer.

Histology and immunohistochemistry

For histology, skin tissues were fixed in 4% buffered formalin, embedded in paraffin, and sectioned. Consecutive sections were stained with hematoxylin and eosin (H&E) and Safranin-O. For immunohistochemistry, sections were deparaffinized and rehydrated. Endogenous peroxidase was

blocked with 0.6% hydrogen peroxide in methanol. Sections were incubated with anti-MMP13 antibody overnight at 4˚C. An appropriate Bright Vision peroxidase system (Immunologic) was used. The staining was visualized with diaminobenzidine.

Enzyme-linked immunosorbent assay (ELISA) for MMP13

MMP13 concentrations in the serum samples and supernatants of cells were quantified using a commercially available MMP13 ELISA kit according to the protocols provided by the manufacturers. All analyses were performed in triplicate. Optical densities were measured at 450 nm using a microplate reader.

Cell culture and reagents

HaCaT cells at a density of 1×10^5 cells/well were cultured in DMEM (Gibco, USA) containing 10% FBS (Gibco), and 2 mM glutamine at 37°C under a humidified atmosphere of 5% CO_2. HaCaT cells at an 80% confluence were exposed to 1 μM acitretin (Chongqing Huapont Pharmaceuticals Co., Ltd., Chongqing, China) and/or 50-100mJ/cm^2 NB-UVB (312 nm UVB light source, Spectroline, USA). After irradiation, the cells were incubated for additional 24 h.

CCK-8 analysis

100μl of cell suspension (1×10^3 cells/well) was dispensed in a 96-well microtiter plate, and then pre-incubated in an incubator with humidified atmosphere and 5% CO^2 at 37 °C for 24h. HaCaT cells were exposed to 1μM acitretin and/or to 50-100mJ/cm^2NB-UVB, cell cultures were incubated for 24h in the incubator.10μl of CCK-8 solution (Solarbio Co., Ltd., Beijing, China) was added to each well and then incubated for 4h in the incubator. A microplate reader was used to measure the absorbance at 450nm.

Quantitative real-time PCR analysis

Total RNA from cells were extracted using Trizol RNA isolation reagent (Thermos Fisher Scientific, USA) according to the manufacturer's protocol. One microgram of total RNA was used for the synthesis of first-strand cDNA. Real-time PCR assays were carried out using AceQ Universal SYBR qPCR Master Mix. The PCR primers were used as follows: forward 5′-AACGCCAGACAAATGTGACC-3′ and reverse 5′-AAAACAGCTCCGCATCAACC-3′ for MMP13; forward 5′-GTCCACCGCAAATGCTTCTA-3′ and reverse 5′-TGCTGTCACCTTCACCGTTC-3′ for β-actin. The cycling conditions used were as follows: 95 °C for 5 min, followed by 40 cycles of denaturation at 95°C for 10s, annealing at 60°C for 30s, and extension at 72°C for 15s. The relative amount of MMP13 transcript was normalized by the amount of β-actin transcript. The results were analyzed using the formula $2^{-\Delta\Delta CT}$.

Western blot analysis

HaCaT cells were lysed in lysis buffer. The protein concentrations were measured by Bicinchoninic Acid (BCA) Protein Assay. Protein samples (20 μg) were separated by SDS-PAGE (10%) and transferred onto polyvinylidene fluoride (PVDF) membrane. The membrane was blocked with 5% fat-free milk and incubated overnight at 4°C with anti-MMP13 antibody (Abcam, USA). After washing, the membrane was then exposed to secondary antibodies coupled to horseradish peroxidase. Immunoreactivities were detected using ECL reagents (Biyuntian Biotechnology Co., LTD., shanghai, China). Protein bands were quantified using Image J software.

Statistical analysis

All quantitative data represent the results of three independent experiments conducted in triplicate. Each value is expressed as the mean ± standard deviation (SD). The data were analyzed with the unpaired, two-tailed Student's *t* test or one-way analysis of variance (ANOVA), using SPSS 23.0

and GraphPad Prism 8.0 software. Differences with *P* values of less than 0.05 were considered statistically significant.

RESULTS

Upregulation of MMP13 in patients with psoriasis

MMP13 staining was strongly positive in each layer of skin tissues in patients with psoriasis. In contrast, MMP13 staining was weak in normal skin tissues and mainly confined to the spinous and basal layers (**Figure 1A**). Consistent with the upregulation of MMP13 in skin tissues, we showed that the serum MMP13 level in patients with psoriasis was significantly higher than that in the control group (**Figure 1B**).

Effects of pharmacological treatment and NB-UVB irradiation on pathological changes in a mouse model of psoriasis

we investigated the effect of different treatments with mice. As shown in **Figure 2A**, the skin changes in mice after treatment with tazarotene and/or NB-UVB irradiation. Compared to the control group, IMQ-induced mice showed increased skin thickness, hypertrophic spinous layer, and prolonged epidermal ridge (**Figure2C**). IMQ-induced epidermal thickness was significantly attenuated by treatment with tazarotene and/or NB-UVB irradiation. The combination of tazarotene and NB-UVB irradiation led to a more profound improvement in skin thickness than each treatment alone (**Figure 2B**).

Assessment of MMP13 expression in psoriatic mice

Compared to control mice, skin lesions from IMQ-induced psoriatic mice had increased MMP13 expression. Treatment with tazarotene or NB-UVB irradiation alone or in combination markedly prevented the IMQ-induced elevation of MMP13 in skin tissues (**Figure 2D**). Similarly,

IMQ-induced mice had significantly greater levels of serum MMP13 than control mice. Serum MMP13 levels were lowered by treatment with tazarotene and/or NB-UVB irradiation (**Figure 2B**).

Acitretin and NB-UVB irradiation inhibit cell viability and MMP13 expression in HaCaT cells

Next, we investigated the effect of different treatments on HaCaT cells. As shown in **Figure 3A**, the HaCaT cell viability was reduced after treatment with acitretin and/or NB-UVB irradiation. Moreover, MMP13 expression was remarkably suppressed by acitretin and/or NB-UVB irradiation (**Figures 3B-3D**).

DISCUSSION

In this study, we showed that the levels of MMP13 in skin lesions and serum samples of patients with psoriasis were higher than those in the normal group. Combined treatment with acitretin and NB-UVB irradiation inhibited MMP13 expression in keratinocytes. Similarly, tazarotene and NB-UVB irradiation led to synergistic inhibition of MMP13 expression in an IMQ-induced psoriasis-like mouse model.

MMPs mediate the degradation of different components of extracellular matrix and regulate cell adhesion, proliferation, migration, and differentiation. MMP13 can be released by keratinocytes and play a key role in psoriasis[16]. Given the finding that MMP13 can be repressed by acitretin or NB-UVB, we investigated the effect of these treatments on the proliferation of keratinocytes. As expected, acitretin treatment and NB-UVB irradiation markedly inhibited the proliferation of HaCaT keratinocytes. The combination of acitretin and NB-UVB irradiation yielded synergistic effects on the proliferation of keratinocytes. In vivo studies confirmed that tazarotene and NB-UVB treatment suppressed MMP13 expression in skin lesions and blood samples of IMQ-induced mice. Increased expression of MMP13 may promote the degradation of the ECM and thus affect the

proliferation of keratinocytes. The ECM is the microenvironment of keratinocytes[17; 18], and the strong expression of MMP13 in psoriasis skin lesions may lead to the degradation of the ECM of the skin, leading to changes in the keratinocytes microenvironment and epidermal dynamics, thereby affecting the proliferation and differentiation of keratinocytes[19; 20]. The changes of cells to cells and cells-matrix adhesion in the epidermis of psoriasis may be related to the abnormal increase of MMP13, so MMP13 may be involved in the formation of psoriasis skin lesions and development of psoriasis. Therefore, we speculate that tazarotene/acitretin treatment and NB-UVB irradiation attenuate the psoriasis-like phenotype through reduction of MMP13 expression.

However, this study has several limitations. Although we show that tazarotene/acitretin and NB-UVB irradiation can suppress MMP13 expression, the underlying molecular mechanism is still unclear. Our results reveal the reduction of both MMP13 mRNA and protein levels by acitretin and NB-UVB, indicating a regulation of MMP13 expression at a transcriptional level. Ongoing studies are conducted to explore the key transcriptional factor(s) mediating the regulation of MMP13 expression by tazarotene/acitretin and NB-UVB.

CONCLUSION

In summary, we demonstrate that MMP13 expression is increased in skin lesions and serum samples of patients with psoriasis. Tazarotene treatment plus NB-UVB effectively inhibits MMP13 expression in a mouse model of psoriasis. Moreover, Acitretin and NB-UVB treatment suppress HaCaT cell proliferation and MMP13 expression. Our findings suggest that tazarotene/acitretin and NB-UVB exert their synergistic beneficial effects on psoriasis likely through regulation of MMP13 expression and secretion. Further study is necessary to reveal the detailed molecular mechanism involved in the improvement of psoriasis by tazarotene/acitretin and NB-UVB.

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CONFLICT OF INTEREST: None declared.

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Figure legends

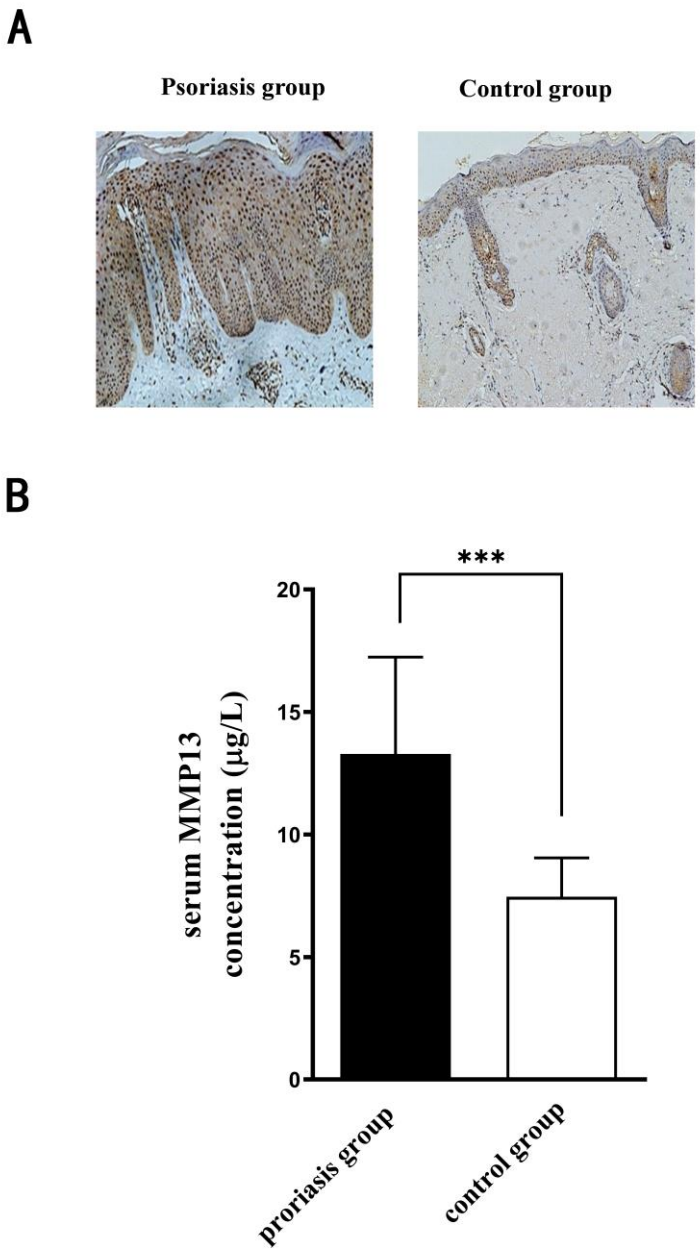
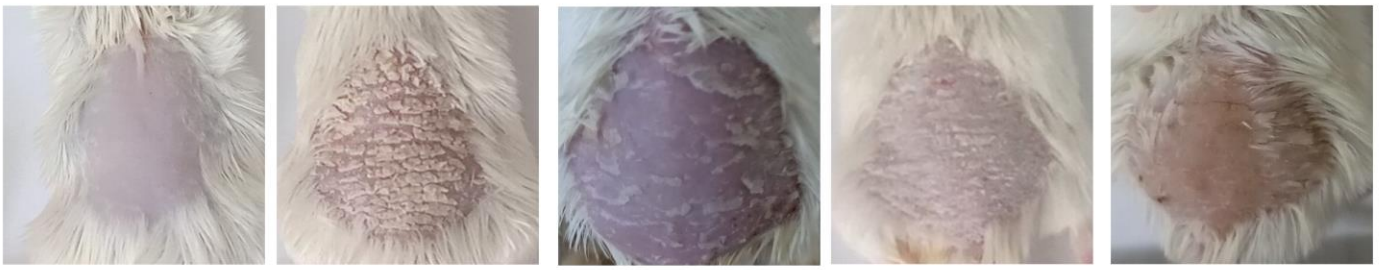


Figure 1. Expression of MMP13 in patients with psoriasis. (A) Immunostaining for MMP13 in psoriasis and normal skin tissues. Representative images of MMP13 staining are shown (×100). (B) Quantification of serum MMP13 levels in patients with psoriasis and control subjects. Data represent the mean ± standard deviation (SD). $^{***}P<0.001$. The data were analyzed with the unpaired, two-tailed Student's *t* test.

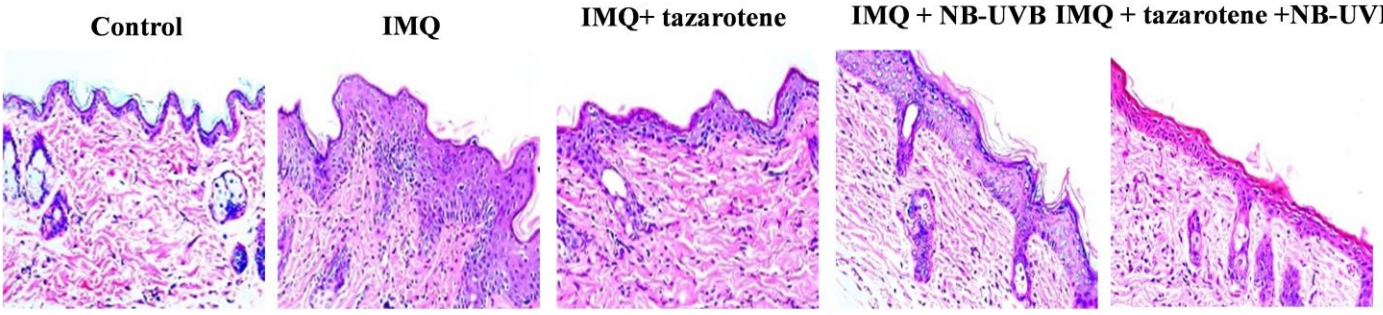
A



B

	the epidermal thickness （μm）	MMP13 concentration （μg/L）
control	1.26 ± 0.04	185.76 ± 7.22
IMQ	7.93 ± 0.59[a]	215.98 ± 15.17[a]
IMQ+ tazarotene	3.56 ± 0.37[b]	197.39 ± 3.92[b]
IMQ+ NB-UVB	3.83 ± 0.39[b]	196.13 ± 11.76[b]
IMQ+tazarotene+NB-UVB	2.14 ± 0.34[b]	183.21 ± 14.99[b]
F value	208.617	6.331
P value	<0.001	<0.01

C



D

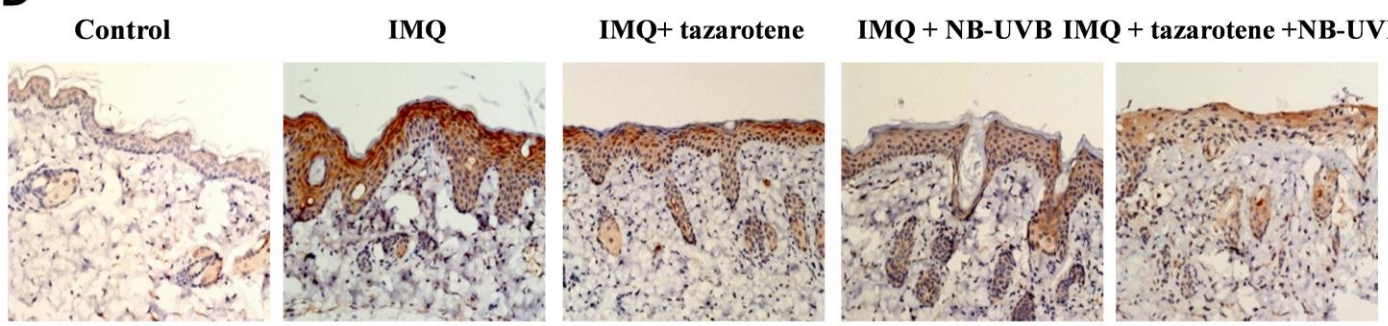
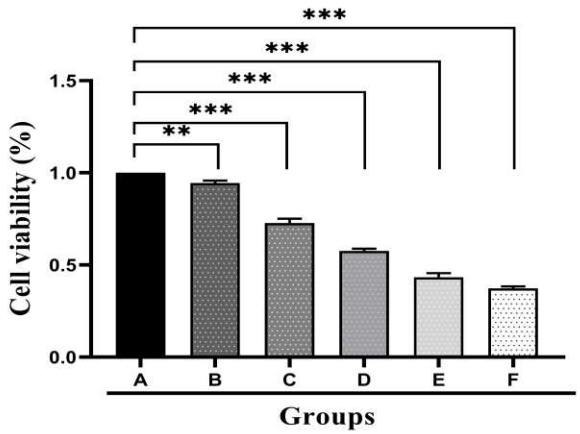


Figure 2. Effects of pharmacological treatment and NB-UVB irradiation on pathological changes in a mouse model of psoriasis. (A) Representative photographs showing mice after indicated treatments. (B) Quantification of skin thickness of mice with indicated treatments and measurement of serum MMP13 levels in treated as in (A). [a] $P<0.05$ relative to control group, [b] $P < 0.05$ relative to IMQ group. Data represent the mean ± standard deviation (SD). The data were analyzed with One-way ANOVA with Least-Significant Difference test was used. (C) Pathological changes in skin tissues from mice in treated as in (A). Representative HE staining images are shown (×200). Assessment of MMP13 expression in psoriatic mice. (D) Histochemical analysis of MMP13 expression in skin tissues from mice with indicated treatments. Representative images of MMP13 staining are shown (×200).

A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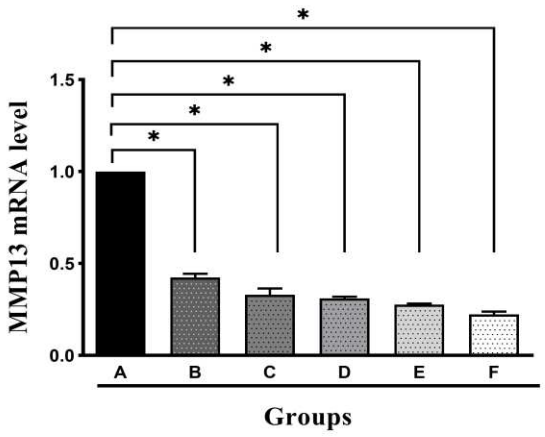
Cell viability (%)
1.5
1.0
0.5
0.0
A
B
C
D
E
F
Groups

B


*
*
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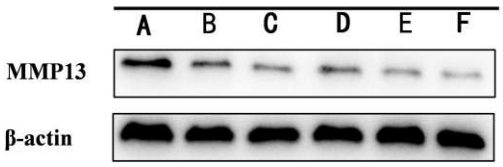
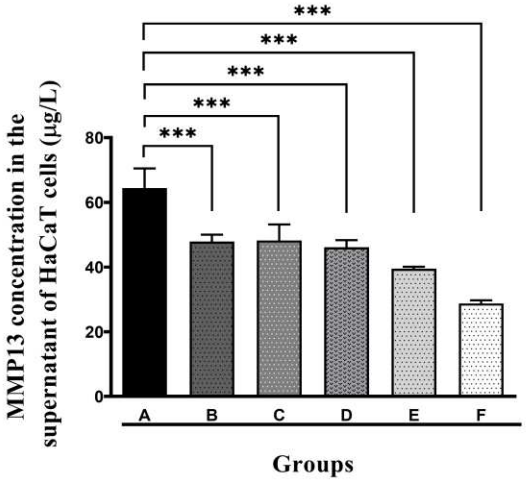
MMP13 mRNA level
1.5
1.0
0.5
0.0
A
B
C
D
E
F
Groups

Groups

C


A
B
C
D
E
F
MMP13
β-actin

D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MMP13 concentration in the supernatant of HaCaT cells (μg/L)
80
60
40
20
0
A
B
C
D
E
F
Groups

Figure 3. Effects of acitretin treatment or NB-UVB irradiation on HaCaT cells. Groups A-F: control group,1μM acitretin group,50mJ/cm^{2}NB-UVB group,1μM acitretin+50mJ/cm^{2}NB-UVB group, 100mJ/cm^{2}NB-UVB group and 1μM acitretin+100mJ/cm^{2}NB-UVB group, respectively. (A) Analysis of HaCaT cell viability after indicated treatments. (B) Analysis of MMP13 mRNA levels in HaCaT cells treated as in (A). (C) Western blot analysis of MMP13 protein levels in HaCaT cells after indicated treatments. (D) ELISA analysis of MMP13 concentration in the supernatant of HaCaT cell. Data represent the mean ± standard deviation (SD). * P<0.05, ** P < 0.01, *** P < 0.001 relative to control group. The data were analyzed with One-way ANOVA with Dunnett's multiple test was used.

Figures

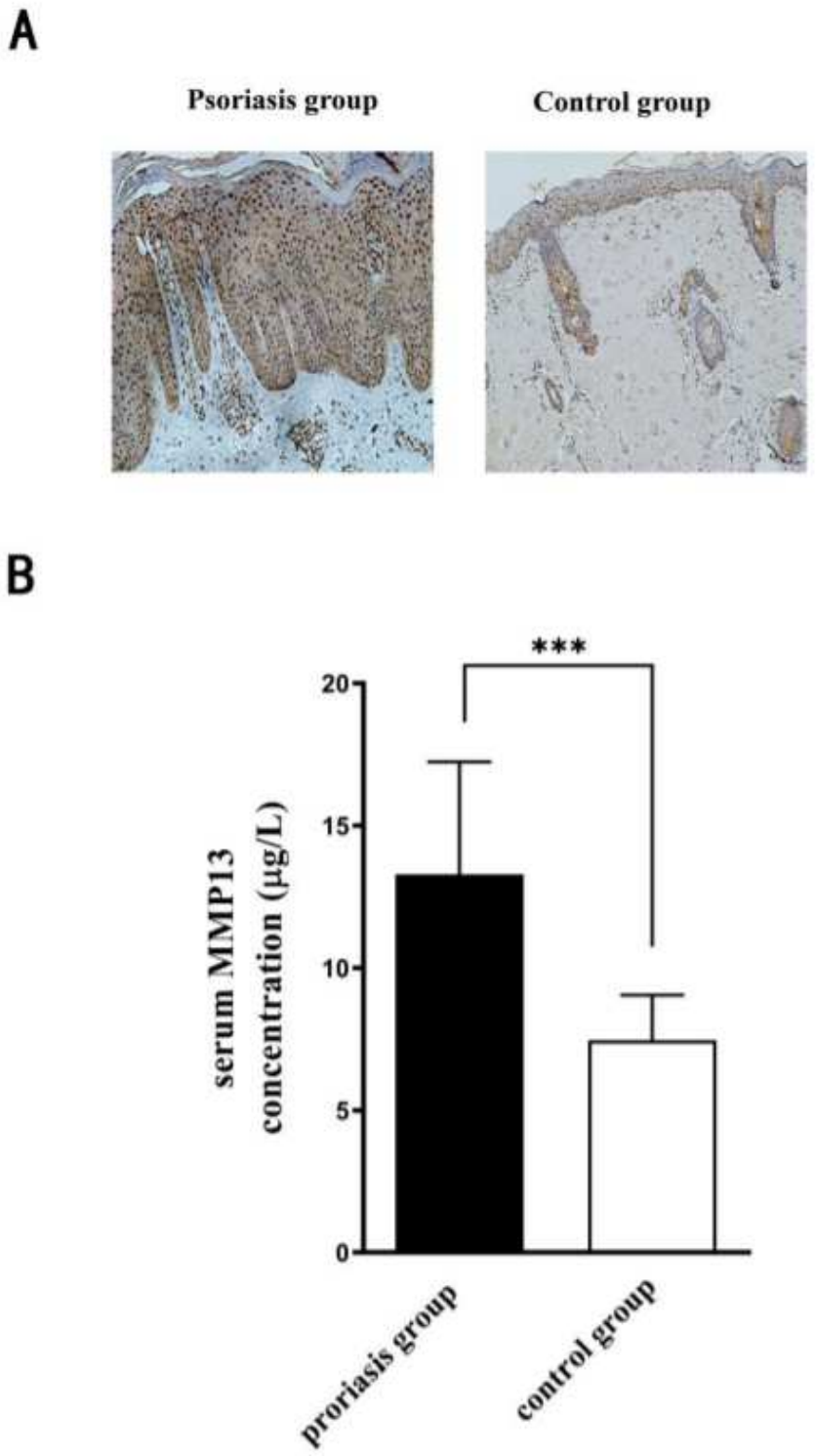

Figure 1

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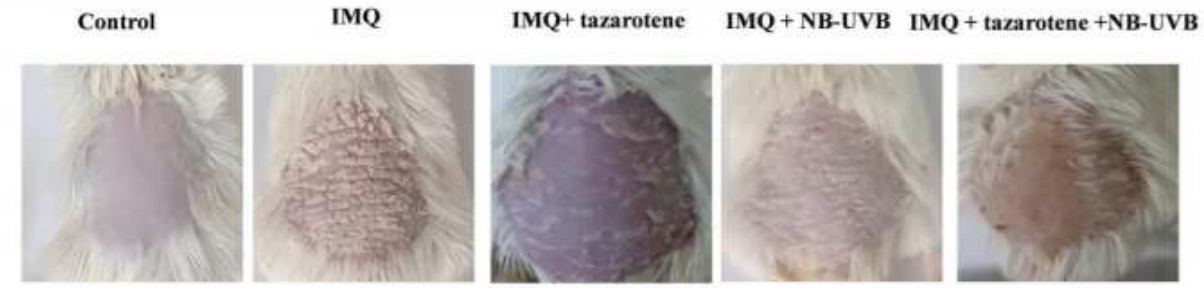
A



B

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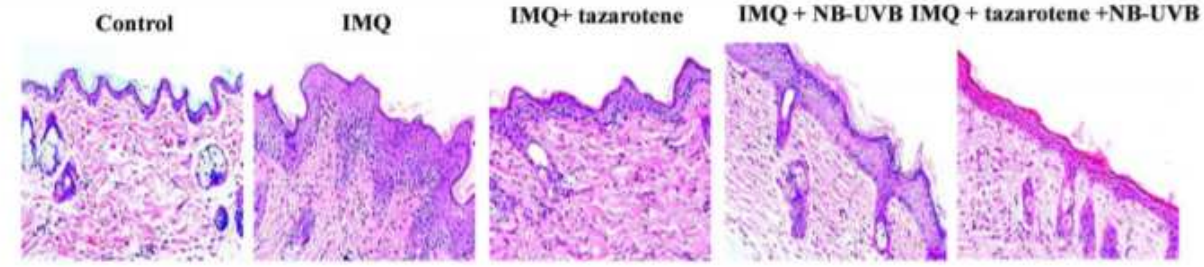
C



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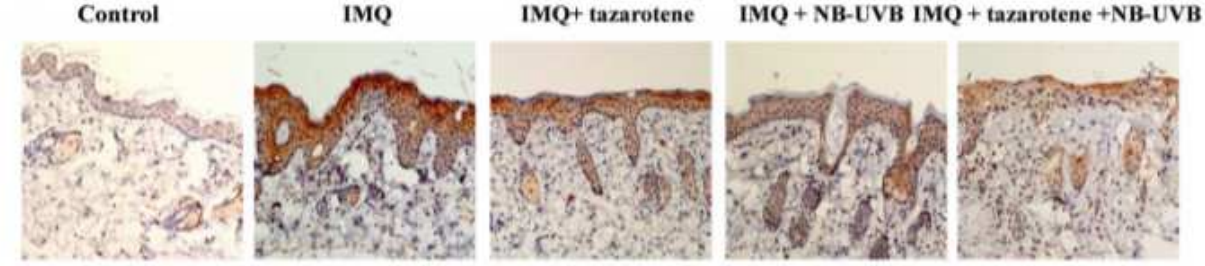

Figure 2

Effects of pharmacologic al treatment and NB UVB irradiation on pathological changes in a mouse model of psoriasis . (Representative photographs showing mice after indicated treatments. B Quantification of skin thickness of mice with indicated treatments and measurement o f serum MMP13 levels in treated as

in (A). a P <0.05 relative to control group , b P < 0.05 relative to IMQ group Data represent the mean ± standard deviation (SD). The data were analyzed with One way ANOVA with Least Significant Difference test was used. C Pathological changes in skin tissues from mice in treated as in (A). Representative HE staining images are shown (×200). Assessment of MMP13 expression in psoriatic mice. (D) Histochemical analysis of MMP13 expression in skin tissues from mic e with indicated treatment s. Representative images of MMP13 staining are shown (×200)

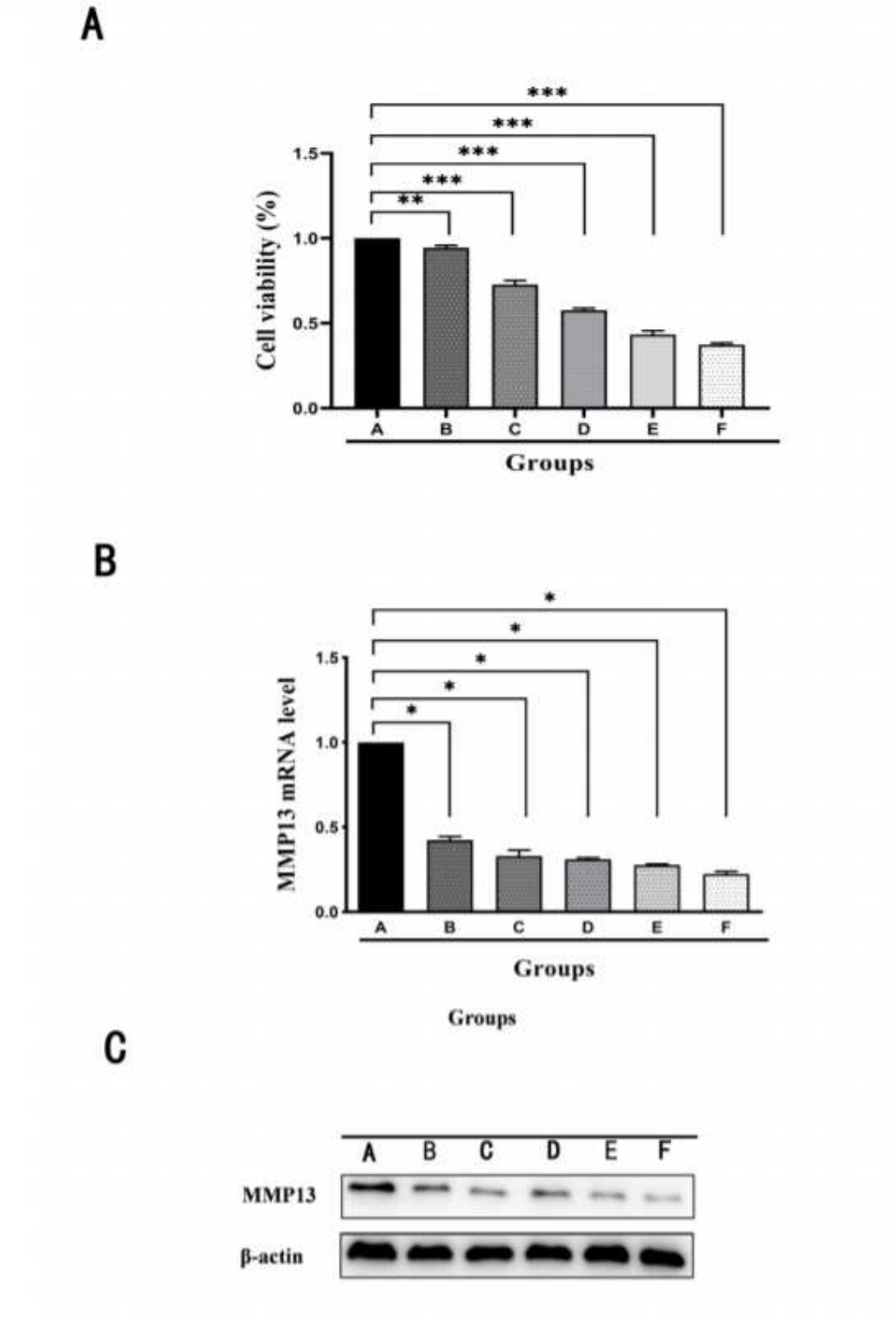

Figure 3

Effects of acitretin treatment or NB UV B irradiation on HaCaT cell s . Groups A F : control group, 1 μM acitretin group,50m J /cm 2 NB UVB group, 1 μM acitretin +50 m J cm 2 NB UVB g roup, 10 0m J /cm 2 NB UVB group and 1 μM acitretin +100 m J /cm 2 NB UVB group , respectively. (A) Analysis of HaCaT cel l viability after indicated treatments. (B) Analysis of MMP13 mRNA levels in HaCaT cell s treated as in (A). (C) Western blot analysis of MMP13 protein levels in Ha CaT cell s after indicated treatments. D E LISA anal y sis of MMP13 concentration in the supernatant of HaCaT cel l Data represent the mean ± standard deviation (SD). P <0.0 5 **P < 0.0 1, ***P < 0.001 relative to control group. The data were analyzed with O ne way ANOVA with Dunnett's multiple test was used.