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## HIF-1 $\alpha$ may promote glycolysis in psoriasis vulgaris via upregulation of CD147 and GLUT1

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### ABSTRACT

**Objective:** To analyze the expressions and distributions of hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ), CD147, and glucose transporter 1 (GLUT1) in epidermis from psoriasis vulgaris and normal people, and to explore the associations among these proteins and their roles in hypoxic HaCaT cell line.

**Methods:** The expression levels of HIF-1 $\alpha$ , CD147, and GLUT1 were determined by immunohistochemistry staining in skin biopsies from 48 psoriasis vulgaris patients and 33 healthy subjects. Cobalt chloride (CoCl<sub>2</sub>) was added into the culture media of HaCaT cells to mimic hypoxia while RNA interference and transfection technologies were used to explore the association among these proteins by quantitative real-time polymerase chain reaction and Western blotting. Glycolytic capacity was detected by ATP and lactate measurements.

**Results:** HIF-1 $\alpha$ , CD147, and GLUT1 were highly expressed and the glycolytic capacity was increased in lesions of psoriasis vulgaris; HIF-1 $\alpha$  upregulated the expression of CD147 and GLUT1, increased the lactate production and decreased the ATP level in CoCl<sub>2</sub>-treated HaCaT cells, while CD147 and GLUT1 directly or indirectly bound to each other.

**Conclusion:** Glycolytic capacity increases in the injured keratinocytes of psoriasis vulgaris, suggesting that HIF-1 $\alpha$ , CD147, and GLUT1 are associated with glycolysis, which can be considered as the promising targets for psoriasis therapy.

### KEY WORDS

hypoxia-inducible factor-1 $\alpha$ ; glycolysis; psoriasis; CD147; glucose transporter 1

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# HIF-1 $\alpha$ 可能通过增加CD147和GLUT1的表达促进寻常型银屑病的糖酵解过程

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**[摘要]** 目的: 分析缺氧诱导因子1 $\alpha$ (hypoxia-inducible factor-1 $\alpha$ , HIF-1 $\alpha$ )、白细胞分化抗原147(CD147)及葡萄糖转运蛋白1(glucose transporter 1, GLUT1)在寻常型银屑病皮损和正常人表皮中的分布与表达, 并探索这些蛋白质之间的关联及其在缺氧的人永生表皮细胞(HaCaT细胞)中的作用。方法: 采用免疫组织化学法分析48例寻常型银屑病患者及33例正常人皮肤活检组织中HIF-1 $\alpha$ 、CD147和GLUT1蛋白质的表达; 在HaCaT细胞系中运用氯化钴诱导缺氧、RNA干扰和转染技术后, 通过实时RT-PCR和蛋白质印迹法探索蛋白质间的关联; 通过检测ATP和乳酸浓度反映皮肤组织和HaCaT细胞的糖酵解能力。结果: 寻常型银屑病皮损中HIF-1 $\alpha$ , CD147, GLUT1的表达增加及糖酵解能力增强; 在诱导HaCaT细胞缺氧的过程中, HIF-1 $\alpha$ 使CD147和GLUT1的表达增加, 同时乳酸浓度升高及ATP浓度降低, 而CD147和GLUT1之间也存在直接或间接的结合。结论: 糖酵解能力在寻常型银屑病皮损中增强, 提示与其有关的HIF-1 $\alpha$ , CD147, GLUT1可作为银屑病潜在的治疗靶点。

**[关键词]** 缺氧诱导因子1 $\alpha$ ; 糖酵解; 银屑病; CD147; 葡萄糖转运蛋白1

Psoriasis is a common chronic skin disease with an incidence rate between 2% and 3%. It is characterized by hyperproliferation, abnormal differentiation, inflammatory cell infiltration, and dilated microvessels. Its pathomechanism is complicated and still being explored. Gradually increased epidemiological studies suggest psoriasis is associated with serious comorbidities, including malignant tumors<sup>[1-2]</sup> like lymphoma, digestive system carcinoma, and so on. Molecules closely related with tumors such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), matrix metalloproteinases (MMPs), and intercellular adhesion molecule-1 (ICAM-1) are also highly expressed in psoriasis. Antineoplastic drugs such as methotrexate and bimegestrol can be used for psoriasis. These evidences imply that the growth microenvironment of keratinocytes in psoriatic lesions is similar to that of tumor cells, and other organs might be dysfunction in the microenvironment affected by psoriasis.

Warburg effect, a metabolic phenomenon best studied in a majority of cancer cells, exhibits elevated glucose uptake and lactate production, regardless of oxygen availability, also known as aerobic glycolysis. It not only produces ATP faster than oxidative phosphorylation (OXPHOS), but also provides intermediates for cell growth, and becomes a well-recognized hallmark of cancer metabolism. During this

process, glycolysis related proteins—glucose transporters (GLUTs) play important roles. It's well known that GLUTs facilitate the transport of glucose over a plasma membrane. One member of GLUTs is GLUT1, which is induced by hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ )<sup>[3]</sup>, highly expresses in various cancer cells<sup>[4-6]</sup>. HIF-1 $\alpha$  is an oxygen sensitive subunit of HIF-1 (an  $\alpha/\beta$  heterodimeric protein complex) and its expression is mainly induced under hypoxic conditions. It plays a vital role in metabolism, invasiveness, and angiogenesis in cancers. In Warburg effect, HIF-1 $\alpha$  can increase the expressions of the transporters necessary for the entry of glucose into the cell, active the most enzymes involved in glycolytic pathway<sup>[7]</sup>, decrease mitochondrial function<sup>[8]</sup>, and turn into glycolysis for energy production<sup>[9-11]</sup>.

It has been demonstrated that HIF-1 $\alpha$ <sup>[12-15]</sup> and GLUT1<sup>[16]</sup> are also highly expressed in psoriatic lesions, so we suppose that in early stage of psoriasis, keratinocytes excessively proliferate under multiple factors. Just like most cancer cells, keratinocytes may reprogram metabolism in the process of survival of the fittest.

CD147, also named extracellular matrix metalloproteinase inducer (EMMPRIN), is a multifunctional transmembrane glycoprotein belongs to immunoglobulin superfamily. It can interact with various regulators such as integrins<sup>[17]</sup>, no obesity

diabetes 2 (NOD2)<sup>[18]</sup>, and EGF receptor<sup>[19]</sup>, and is suggested to be used to determine the different cellular functions involved in a wide range of other biological activities including invasiveness, proliferation, and immunological responses, etc<sup>[20]</sup>. Our group previously confirmed that CD147 is one of psoriasis susceptibility genes<sup>[21]</sup>. It is highly expressed in psoriatic lesions and peripheral blood cells<sup>[22]</sup>, which is closely related with disease activity<sup>[23-24]</sup>, and may become a therapeutic target for psoriasis. CD147 is also found over-expressed in a number of cancerous specimens, and its expression gradually increases from benign tumors to malignancy in solid tumors<sup>[25-26]</sup>. Ke et al<sup>[27]</sup> demonstrated the upregulation of CD147 could be induced under hypoxia, which is mainly mediated by a combined effect of transcription factor HIF-1 and specificity protein 1 (Sp1) on the activation of CD147 promoter in epithelial solid tumors. It also has been demonstrated that the silencing of CD147 can decrease glycolytic rate and lactate efflux in carcinoma cell line, indicating that CD147 is essential in tumor glycolysis<sup>[28-30]</sup>.

The aim of the present study is to analyze the expressions and distributions of key proteins mentioned above that related to psoriasis and glycolysis in psoriasis vulgaris or normal human epidermis, and to explore the relationship among these proteins and their roles in hypoxic HaCaT cell line.

## 1 Materials and methods

### 1.1 Cell culture

The spontaneously immortalized HaCaT cell line was purchased from American Type Culture Collection (ATCC, USA). Cells were maintained in DMEM medium (Thermo Scientific, MA, USA) with 10% fetal bovin serum (Thermo Scientific, MA, USA) and 100 U/mL penicillin/streptomycin (Dingguo, Beijing, China) at 37 °C in a humidified incubator (Thermo Scientific, MA, USA) under 5% CO<sub>2</sub>. When the cells were at 80%–90% confluence, they were washed three times with phosphate-buffered saline (PBS) and exchanged with fresh medium. Then 250  $\mu$ mol/L CoCl<sub>2</sub> (Sigma, CA, USA) or dimethyl sulfoxide (New England Biolabs, MA, USA) was added to induce hypoxia or be a negative control. The cells were harvested for further experiments.

### 1.2 Immunohistochemistry and immunofluorescence

Paraffin-embedded tissue blocks of primary lesions and normal skins were sectioned at a thickness of 5  $\mu$ m, heated at 60 °C for 30 min, dewaxed in turpentine, rehydrated through graded concentrations of alcohol, and incubated in 3% hydrogen peroxide for 10 min to block endogenous peroxidase. Antigen retrieval was achieved by microwaving tissue sections in 0.01 mol/L citrate buffer solution (pH 6.0) for 8 min (Galanz microwave, 800 W), and cooled for 20 min at room temperature. The sections were incubated overnight at 4 °C with primary antibodies, including HIF-1 $\alpha$  (1:100, Epitomics, CA, USA), CD147 (1:300, Abcam, MA, USA), and GLUT1 (1:400, Abcam, MA, USA). Negative controls were incubated with PBS instead of primary antibodies. A 2-step plus poly-HRP anti-mouse/rabbit IgG detection system (Zhongshan Co., Beijing, China) was performed according to manufacturer's instructions, using diaminobenzidine chromogen (Zhongshan Co., Beijing, China) as substrate for visualization. Sections were counterstained with hematoxylin and mounted in neutral balsam.

For immunofluorescence, paraffin-embedded tissue blocks from primary lesions were incubated overnight with primary rabbit anti-CD147 (1:100, Santa Cruz, California, USA) and mouse anti-GLUT1 (1:300, Abcam, MA, USA) at 4 °C, visualized by incubation in Alexa Fluor<sup>®</sup> conjugated secondary antibodies (1:150, Invitrogen, CA, USA) for 2 h at room temperature, and covered with VECTASHIELD<sup>®</sup> mounting medium containing DAPI (Vector Laboratories, CA, USA) to preserve the fluorescence and counterstain nuclei. Images were acquired through an inverted fluorescence microscope (Leica, Solms, Germany).

### 1.3 Immunohistochemical scoring

Two independent dermatological pathologists performed microscopic examination without any prior knowledge of each patient's clinical information. The staining cells were mainly in basal layer and spinous layer in epidermis. Both layers were evaluated for the proportion and intensity of staining cells. Staining intensity was graded as follows: 0 point was negative, 1 point was light, 2 points was moderate, and 3 points was intensive. The proportion of stained cells in each layer was graded as follows: 0 point was non-staining cells, 1

point was less than 25% stained cells, 2 points was 25%–50% stained cells, and 3 points was more than 50% stained cells. An immunostaining-intensity-distribution index was then computed for each specimen as follows: the score for the overall staining intensity was multiplied by the score of the proportion of staining cells in each layer, and the result scores of both layers then added. The average score (0–18) was classified into 0 (0), 1 (<6), 2 (6–12), and 3 (>12). 0 and 1 were considered as weak expression and 2 and 3 were as high expression.

#### 1.4 Western blotting

At the end of treatments, HaCaT cells were harvested and solubilized in lysis buffer (containing 1% Triton X-100, 50 mmol/L Tris balanced to pH 7.4, 150 mmol/L NaCl, 10 mmol/L EDTA, 100 mmol/L NaF, 1 mmol/L  $\text{Na}_3\text{VO}_4$ , 1 mmol/L phenylmethylsulfonyl fluoride, and 2 mg/L aprotinin) at approximately 80% confluence, left on ice for 30 min, centrifuged at 12 000 g for 15 min at 4 °C. The supernatant protein concentration was relatively quantified by the BCA protein assay (Santa Cruz, CA, USA). Cell lysates were mixed with lysis buffer and loading buffer, and boiled at 95 °C for 10 min, loaded equal amount proteins into 10% polyacrylamide gel for separation and transferred to polyvinylidene difluoride membrane (Millipore, MA, USA). The membrane was blocked in 5% fat-free milk, incubated with indicating primary antibodies: HIF-1 $\alpha$  (1:1 000), CD147 (1:1 000), GLUT1 (1:800, Santa Cruz, USA), washed three times and incubated with HRP-linked secondary antibodies (1:10 000, Sigma, USA), visualized by chemiluminescence detection system (Bio-Rad, CA, USA). Protein levels in each lane were normalized to that of  $\beta$ -actin (1:10 000, Sigma, USA).

#### 1.5 Immunoprecipitation

Hypoxia HaCaT cells were harvested and solubilized in a special immunoprecipitation (IP) lysis buffer on ice for 30 min, centrifuged at 6 000 r/min for 4 min at 4 °C. Supernatant containing 100  $\mu\text{g}$  of protein was added to 1  $\mu\text{g}$  of CD147, GLUT1 or control IgG and then with protein A+G agarose beads. The specimen was incubated overnight at 4 °C with shaking, washed three times with lysis buffer, added lysis buffer and 5 $\times$  loading buffer, boiled in SDS sample buffer and loaded

into a 10% SDS-polyacrylamide gel. The immunoprecipitates were analyzed by Western blotting.

#### 1.6 Tissue source

A total of 48 paraffin-embedded archival tissue blocks from patients (aged 18–60 years) with psoriasis vulgaris were obtained between 2012 and 2013, and 33 normal skin tissue blocks were derived from uninvolved skin of people under mole resection at Department of Dermatology in Xiangya Hospital of Central South University (Changsha, Hunan, China). The involved skin and uninvolved skin were biopsied for 5 mm according to the criterion that they had not received any treatment in the last 4 weeks before taking biopsies, and normal skin samples were derived from uninvolved skin of mole resection. Informed consent was obtained from each patient. All experimental procedures were approved by the Regional Committee for Medical Research Ethics (No. 201212087).

#### 1.7 ATP assay

The concentration of ATP was measured by the luciferin-luciferase method following the protocol of ATP detection kit (Beyotime, Beijing, China). Treated HaCaT cells and skin epidermis [normal skin epidermis (N), psoriatic non-lesional epidermis (PN) and psoriatic lesional epidermis (PS)] were grinded with 200  $\mu\text{L}$  lysis buffer on ice, centrifuged at 12 000 g for 5 min at 4 °C. The supernatant protein concentration was relatively quantified by the BCA protein assay (Santa Cruz, USA), in 96-well plates, 100  $\mu\text{L}$  of each supernatant was mixed with 100  $\mu\text{L}$  ATP detection working dilution. Luminance was measured by a enzyme microplate reader (Beckman DTX880, CA, USA). The standard curve of ATP concentration was prepared from a known amount (1 nmol/L–1  $\mu\text{mol/L}$ ). Total ATP levels were expressed as nmol/mg protein. All the experiments were carried out with triplicates.

#### 1.8 Lactate concentration measurements

Lactate was detected following the protocol of lactate colorimetric assay kit (Biovision, CA, USA). Equal culture medium of differently treated HaCaT cells and skin epidermis (N, PS, PN) were added to assay buffer, centrifuged three times to remove insoluble materials, and transferred 50  $\mu\text{L}$  supernatant to 96-well plates, added 50  $\mu\text{L}$  reaction mix to each well, incubated



the reaction for 30 min at room temperature, measured at OD 450 nm in a microplate reader. Standard curves were also generated and the protein concentration of each group was determined using the BCA protein assay (Santa Cruz, USA). Total lactate concentration was expressed as nmol/mg protein. All the experiments were carried out with triplicates.

### 1.9 Cell transfection

For the transient transfection, cells were seeded in 3.5 cm culture dishes. At 50%–70% confluence, siRNA (HIF-1 $\alpha$  siRNA: sense 5'-UAUUUGUUCACAUUAUC-AGAA-3', antisense 5'-CUGAUAAUGUGAACAAAU-ACA-3'; CD147 siRNA: sense 5'-CAUACACUCC-UUCUUUUUA-3', antisense 5'-AAAAAGAAGGAA-GUGUAUGAU-3'; GLUT1 siRNA: sense 5'-AUAGAA-GACAGCGUUGAUGCC-3', antisense 5'-CAUCAAC-GCUGUCUUCUAUUA-3'; or scramble siRNA: sense 5'-UUCUCCGAACGUGUCACGUTT-3', antisense 5'-ACGUGACACGUUCGGAGAATT-3' as negative controls) synthesized by GenePharma Company (Shanghai, China), was transfected with RNAi-max (Invitrogen, CA, USA) according to manufacturers' instructions.

In same conditions, the HaCaT cell line was transfected with the pGL3-basic plasmids (Promega, WI, USA) containing the entire EMMPRIN or GLUT1 sequence using TurboFect Transfection Reagent (Thermo Scientific, USA) according to manufacturers' instructions. Control transfectants were generated by transfection with empty pGL3 vector.

### 1.10 Real-time RT-PCR

Total RNA was extracted using the TRIzol (Invitrogen, CA, USA) procedure. First-strand cDNA was synthesized from 2  $\mu$ g RNA using Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) according to manufacturers' instructions. The sequences of PCR primers were as follows: GAPDH sense 5'-CTGCACCACCAACTGCTTAG-3'; antisense 5'-AG-GGAG-GGGAGCCGGCTGTC-3'; HIF-1 $\alpha$  sense 5'-CCATTA-GAAAGCAGTTCCGC-3', antisense 5'-TG-GGTAGGA-GATGGAGATGC-3'; CD147 sense 5'-GCAGCGGTT-GGAGGTTGT-3', antisense 5'-AGCC-AGGGATGCCC-AGGAAGG-3'; GLUT1 sense 5'-CTTCTCTGTGGG-CCTTTTCGT-3', antisense 5'-CAA-

AGGACTTGCCC-AGTTTCG-3'. Real-time PCR was performed using the SYBR Green PCR Master Mix kit (Takara Bio, Dalian, China) and ABI7500 real time system (Applied Biosystems, CA, USA). Each sample was run in triplicate and in 3 independent experiments. All data were normalized to GAPDH expression comparing different groups determined by the ddCt comparative threshold ( $\Delta\Delta$ Ct) method. For all real-time PCRs, the conditions were as follows: 40 cycles, 94  $^{\circ}$ C for 25 s, 60  $^{\circ}$ C for 25 s, and 72  $^{\circ}$ C for 25 s.

### 1.11 Statistical analysis

All statistical analyses were performed using the SPSS 17.0 statistical software package (SPSS, IL, USA) and the GraphPad Prism 5.01 (GraphPad Software, CA, USA) statistical packages. Spearman's rank correlation coefficient test was used to evaluate the pairwise association between different markers.  $\chi^2$  test was used to detect the relationship between each marker and psoriasis. Significance was determined by  $P < 0.05$ .

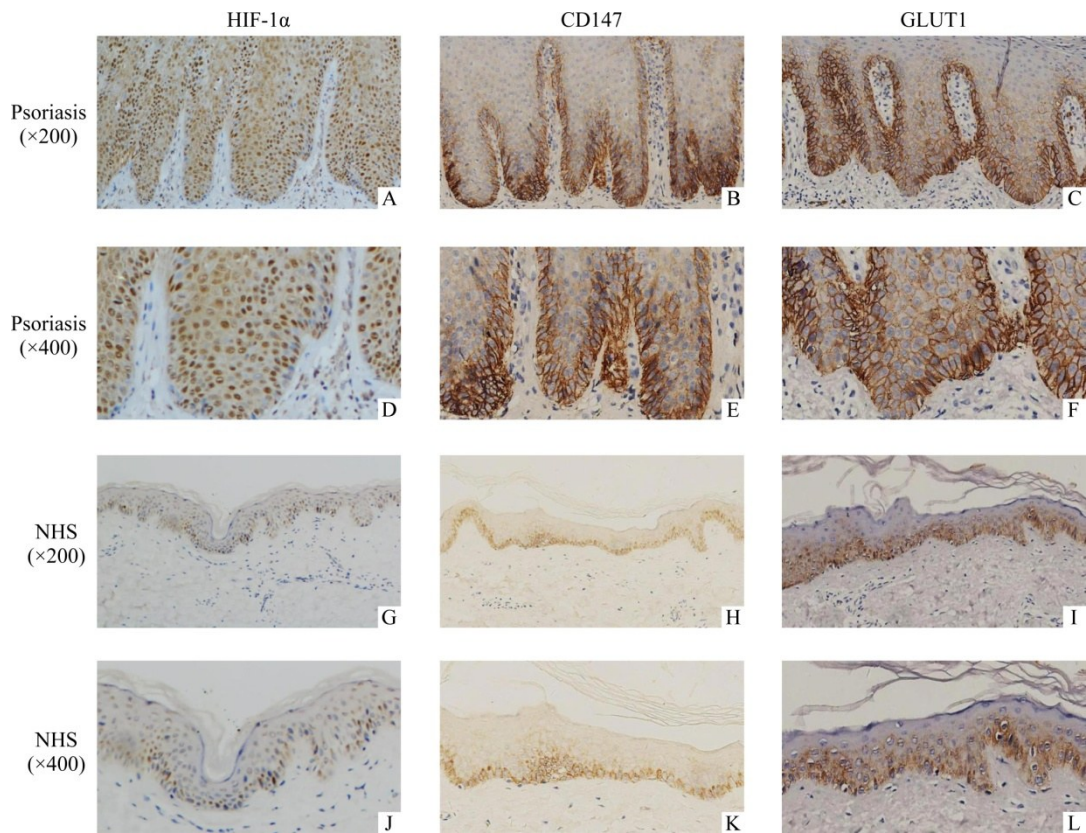
## 2 Results

### 2.1 Expressions of HIF-1 $\alpha$ , CD147, and GLUT1 in normal and psoriatic epidermis

HIF-1 $\alpha$  was predominantly expressed in the nuclear throughout the psoriatic epidermis, while mainly in the basal layer in normal epidermis (Figure 1A, 1D, 1G, 1J). CD147 (Figure 1B, 1E, 1H, 1K) and GLUT1 (Figure 1C, 1F, 1I, 1L) showed mainly membranous expression in basal and spinous layer in psoriatic epidermis and almost in basal layer in normal epidermis. The differences in the expressions of HIF-1 $\alpha$ , CD147 and GLUT1 between normal and psoriatic human epidermis were significant ( $P < 0.01$ , Table 1).

### 2.2 Correlations among expressions of HIF-1 $\alpha$ , CD147 and GLUT1 in psoriatic epidermis

The result of Spearman's rank test analyses for the immunohistochemical scores in psoriatic epithelium showed that HIF-1 $\alpha$  expression was positively correlated with the expressions of CD147 and GLUT1 ( $r > 0$ ,  $P < 0.05$ ), and the CD147 expression was also positively correlated with the GLUT1 expression ( $r > 0$ ,  $P < 0.05$ ).



**Figure 1** Expressions of HIF-1 $\alpha$ , CD147, and GLUT1 protein in normal human skin (NHS) and psoriasis (immunohistochemistry,  $\times 200$ ,  $\times 400$ , respectively)

A, D, G, J: HIF-1 $\alpha$  is expressed in nucleus; B, E, H, K: CD147 is expressed in membranes; C, F, I, L: GLUT1 is expressed in membranes. Positive signals appear brown; blue counter staining with hematoxylin.

**Table 1** Differences in expressions of HIF-1 $\alpha$ , CD147, and GLUT1 between psoriatic and normal epithelium

Groups	<i>n</i>	Expression	Protein level		
			HIF-1 $\alpha$	CD147	GLUT1
Psoriasis	48	Weak	14	14	2
		High	34	34	46
Normal	33	Weak	10	28	12
		High	23	5	21
<i>P</i>			<0.001	0.001	<0.001

### 2.3 HIF-1 $\alpha$ induces the expressions of CD147 and GLUT1 in HaCaT cells under hypoxia

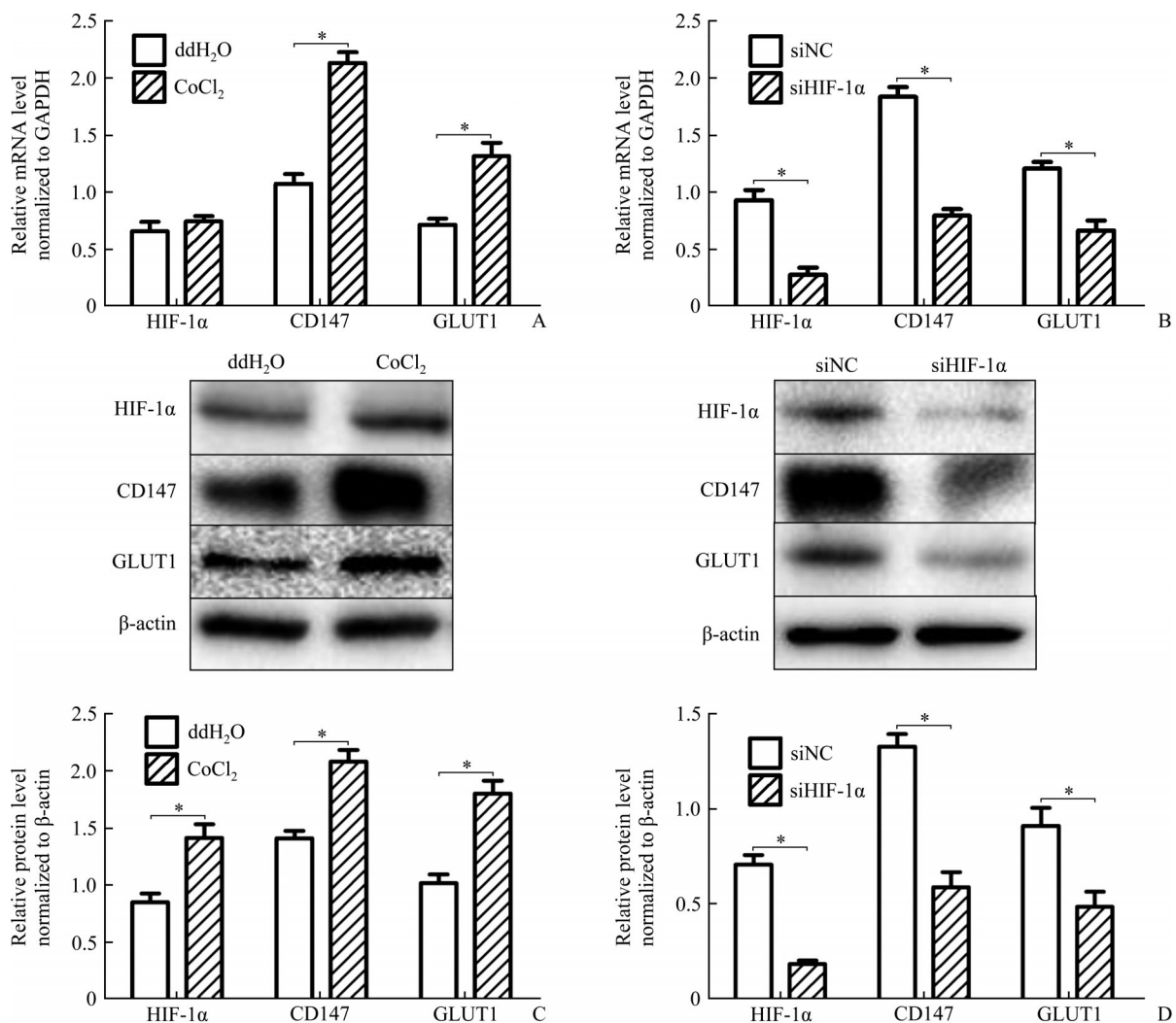
The results of real-time RT-PCR and Western blotting showed a clear induction of HIF-1 $\alpha$ , CD147, and GLUT1 at both mRNA and protein level (Figure 2), while HIF-1 $\alpha$  mRNA level had no significant difference between hypoxia and normoxia (Figure 2A, 2C). After siRNA against HIF-1 $\alpha$  was used to specifically knock

down HIF-1 $\alpha$ , then treated cells with same hypoxia conditions, the results of real-time PCR and Western blotting showed a reduction of CD147 and GLUT1 (Figure 2B, 2D).

### 2.4 Co-localization and interaction of CD147 and GLUT1

The immunohistochemical results of CD147 and GLUT1 in normal and psoriatic human epidermis showed that the expressions and locations of these two proteins were nearly the same. Then immunofluorescence was performed in psoriatic epidermis in order to show the location more clearly (Figure 3A). Co-localization of proteins, as revealed by yellow immunostaining of merged images, was evident in the epidermis.

The results of Western blotting showed no changes in expressions of CD147 and GLUT1 proteins (Figure 3B, 3C).



**Figure 2** CD147 and GLUT1 were mediated by HIF-1 $\alpha$  under hypoxia

A: HaCaT cells were treated with 250  $\mu$ mol/L CoCl<sub>2</sub> for 12 h or ddH<sub>2</sub>O without previous siRNA transfection and transcripts of HIF-1 $\alpha$ , CD147, and GLUT1 were quantified by real-time RT-PCR. B: HaCaT cells were treated with 250  $\mu$ mol/L CoCl<sub>2</sub> for 12 h or ddH<sub>2</sub>O with previous siRNA transfection [siRNA against human HIF-1 $\alpha$  or scrambled siRNA (siNC) for 48 h], transcripts of HIF-1 $\alpha$ , CD147, and GLUT1 were quantified by real-time RT-PCR. C: Western blotting was performed with indicated antibodies for HaCaT cells only treated with 250  $\mu$ mol/L CoCl<sub>2</sub> or ddH<sub>2</sub>O. D: Western blotting was performed with indicated antibodies for HaCaT cells transfected with siRNA in hypoxia induced by 250  $\mu$ mol/L CoCl<sub>2</sub>. \* $P$ <0.05.

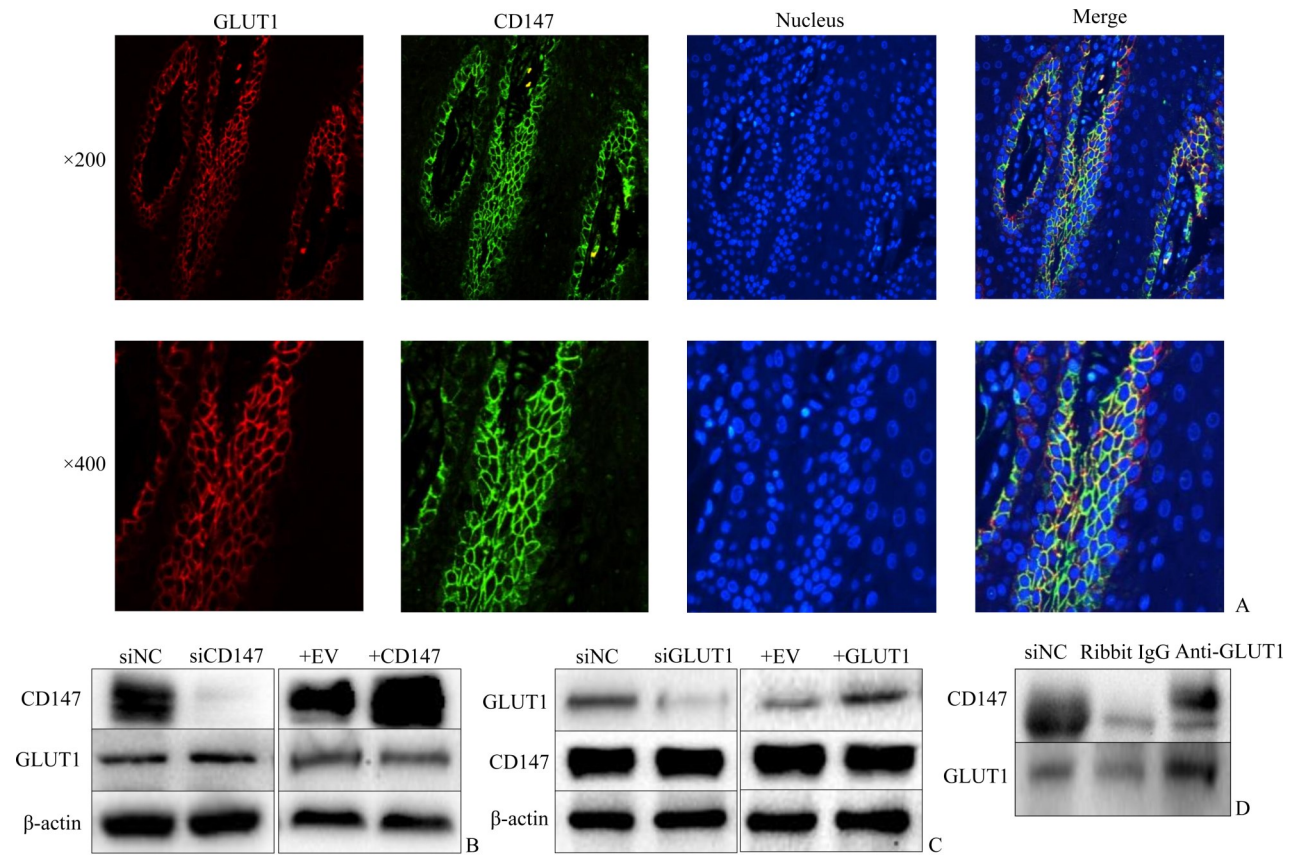
Next co-IP was performed in hypoxic HaCaT cells, cell lysates were then analyzed by co-IP using the polyclonal anti-GLUT1 antibody. As shown in Figure 3D, GLUT1 is co-IPed with CD147. However, it is failed to use the anti-CD147 antibody to pull down GLUT1, probably because monoclonal CD147 antibody only binds part of the mutiple epitopes of GLUT1, and the protein level isn't enough to visualize as a band.

### 2.5 ATP level was associated with expression of HIF-1 $\alpha$ in both HaCaT cells and human epidermis

We have confirmed that HIF-1 $\alpha$  induced expressions of CD147 and GLUT1 in HaCaT cells under

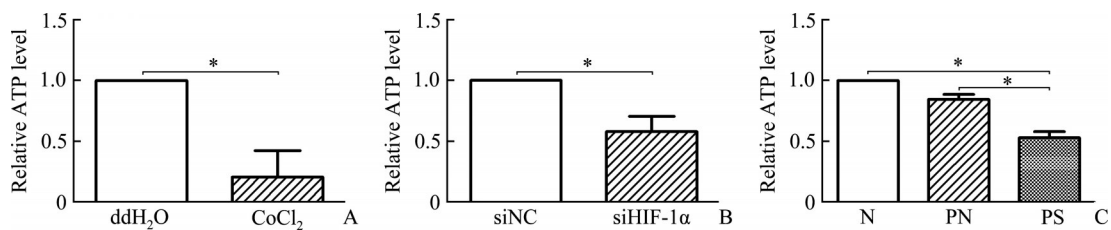
hypoxia. Next, ATP level was detected in identically treated HaCaT cells. It showed that ATP level significantly decreased in hypoxic HaCaT cells induced by CoCl<sub>2</sub> compared with negative control cells ( $P$ <0.05, Figure 4A), while in hypoxic siHIF-1 $\alpha$  HaCaT cells, ATP level decreased in a greater degree compared with hypoxic siNC cells ( $P$ <0.05, Figure 4B). At last, ATP level was detected in equal weight and body surface area of human epidermis ( $P$ <0.05), the results showed ATP level significantly decreased in psoriatic epidermis, while no significant change was found between non-lesional and normal epidermis ( $P$ <0.05, Figure 4C).





**Figure 3 Interaction between CD147 and GLUT1**

A: Co-localization of CD147 and GLUT1 in psoriatic epidermis (immunofluorescence,  $\times 200$ ,  $\times 400$ , respectively). B: HaCaT cells were transfected with siRNA against human CD147 and scrambled siRNA as negative control (siNC), or CD147 cDNA plasmid and empty vector (EV), after 48 h, then treated with 250  $\mu\text{mol/L}$   $\text{CoCl}_2$  for 12 h. Protein expressions of GLUT1 and CD147 were analyzed by Western blotting. C: HaCaT cells were transfected with siRNA against human GLUT1 or GLUT1 cDNA plasmid (negative controls and hypoxia condition as described before). Western blotting analysis was performed. D: HaCaT cells were treated with  $\text{CoCl}_2$  for 12 h, and cell lysates were performed co-IP. Lysates were immunoprecipitated with antibodies of GLUT1 and rabbit IgG, and subjected to antibodies of CD147 and GLUT1 for immunoblotting. Anti-GLUT1 isolated CD147 while rabbit IgG did not.



**Figure 4 Relative level of ATP in hypoxic HaCaT cells and different kinds of skin epidermis**

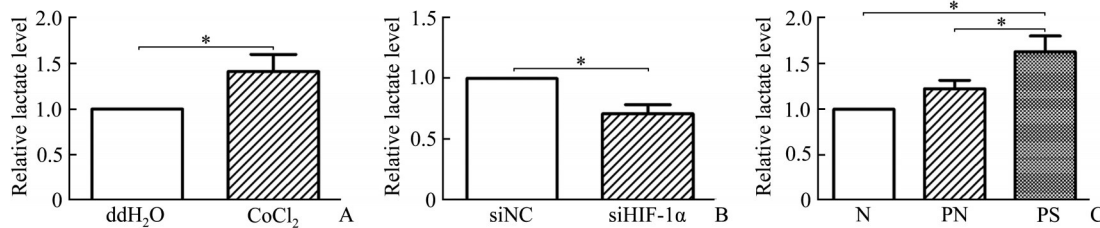
A: Cellular ATP level in hypoxic HaCaT cells induced by 250  $\mu\text{mol/L}$   $\text{CoCl}_2$  for 12 h, or in negative control with  $\text{ddH}_2\text{O}$ . B: HaCaT cells were treated with 250  $\mu\text{mol/L}$   $\text{CoCl}_2$  for 12 h with previous siRNA transfection [siRNA against human HIF-1 $\alpha$  or scrambled siRNA (siNC) for 48 h]. Cellular ATP level was detected. C: Cellular ATP level in normal skin epidermis (N), psoriatic non-lesional epidermis (PN), and psoriatic lesional epidermis (PS). Values present as mean $\pm$ SD of 3 independent experiments. \* $P < 0.05$ .



## 2.6 Lactate level was associated with expression of HIF-1 $\alpha$ both in HaCaT cells and human epidermis

Under the same treatments with ATP detection, lactate level was detected in HaCaT cells and human epidermis. The results showed that lactate level significantly increased in medium of hypoxic HaCaT cells induced by CoCl<sub>2</sub> compared with negative control

cells ( $P < 0.05$ , Figure 5A), and significantly decreased in medium of hypoxic siHIF-1 $\alpha$  HaCaT cells than that of hypoxic siNC cells ( $P < 0.05$ , Figure 5B). Also in human epidermis, lactate level significantly increased in psoriatic epidermis, and no significant change was found between non-lesional and normal epidermis ( $P < 0.05$ , Figure 5C).



**Figure 5** Relative level of lactate in hypoxic HaCaT cells and different kinds of skin epidermis

A: Cellular lactate level in hypoxic HaCaT cells induced by 250  $\mu\text{mol/L}$  CoCl<sub>2</sub> for 12 h, or in negative control with ddH<sub>2</sub>O. B: HaCaT cells were treated with 250  $\mu\text{mol/L}$  CoCl<sub>2</sub> for 12 h with previous siRNA transfection [siRNA against human HIF-1 $\alpha$  or scrambled siRNA (siNC) for 48 h]. Cellular lactate level was detected. C: Cellular lactate level in normal skin epidermis (N), psoriatic non-lesional epidermis (PN), and psoriatic lesional epidermis (PS). Values present as the mean $\pm$ SD of 3 independent experiments. \* $P < 0.05$ .

## 3 Discussion

In 4 classifications of psoriasis, psoriasis vulgaris is the most common form. Regardless of form, psoriasis is always recurrent and refractory, and brings an enormous physical and psychological distress to patients. Multiple factors such as genetic and environmental factors are involved in its complicated pathomechanism<sup>[31-33]</sup>.

In psoriatic lesion, the proliferation of keratinocytes increases oxygen consumption and epidermal thickening could lead to impaired oxygen supply. It's reasonable to speculate that as the oxygen level decreases, the generation of ATP shifts from the OXPHOS pathway to the oxygen-independent pathway of glycolysis. HIF-1 $\alpha$  and GLUT1 were significantly higher expressed in psoriasis vulgaris lesional epidermis than in normal epidermis. We also found that ATP level decreases and lactate production increases in psoriatic epidermis, indicating that psoriatic keratinocytes indeed mainly metabolize through glycolytic pathway. CD147, a multifaceted protein involved in the growth, survival, invasion, angiogenesis, and metastasis of tumors, mainly via MMP induction and interaction with various ligands

involved in the neoplastic cell behavior, while recently Marchiq et al<sup>[34]</sup> proposed that the strongest pro-tumoural action of CD147 was mediated through the control of fermentative glycolysis, and CD147 could play a role as a cell recognition molecule in a cell dependant manner. The studied evidence for CD147 participation in glycolysis is that it controls tumor metabolism by facilitating lactic acid export through the two monocarboxylate transporters MCT1 and hypoxia-inducible MCT4, and serves as a chaperone required for the plasma membrane translocation of MCTs<sup>[35-37]</sup>. Ke et al<sup>[27, 30]</sup> also confirmed that hypoxia-induced CD147 upregulation significantly promoted glycolytic switch and tumor cell survival and invasion. We've already confirmed CD147 is closely related to psoriasis both in genetics and molecular mechanisms<sup>[22, 38-39]</sup>. In this study, we demonstrated that CD147 was strongly related to HIF-1 $\alpha$  and GLUT1 in lesional epidermis and hypoxic HaCaT cell line, although not experimented in primary psoriatic keratinocytes, CD147 co-localized with GLUT1 in psoriatic cell membranes and interacted with each other in CoCl<sub>2</sub> induced hypoxic HaCaT cells under HIF-1 $\alpha$  control. Our study offers a new insight of

CD147 involving in glycolysis, that may make contribution to psoriasis pathogenesis.

HIF-1 $\alpha$  is found to drive the expressions of hundreds of genes involved in many biological processes, including glycolysis, angiogenesis, survival/apoptosis, migration, invasion, and metastasis<sup>[40-41]</sup>. In psoriasis, the main mechanism about HIF-1 $\alpha$  is that it could differentially promote angiogenesis by upregulating vascular endothelial growth factor (VEGF)<sup>[42-46]</sup> and its receptor Flt-1<sup>[45]</sup>, and VEGF upregulation has been demonstrated to be an early and important step in the pathophysiology of psoriasis<sup>[47]</sup>. In our study, we verified a significant increase of HIF-1 $\alpha$  protein level after CoCl<sub>2</sub> induction, probably CoCl<sub>2</sub> reduces hydroxylase activity, allowing HIF-1 $\alpha$  to escape from both destruction and blockade of coactivator recruitment, and enter the nucleus to heterodimerise with HIF-1 $\beta$ , further recruiting CBP/p300 co-activator, thereby upregulating downstream genes involved in cellular and systemic hypoxia responses<sup>[48]</sup>. We found that CD147 and GLUT1 were regulated by HIF-1 $\alpha$  and interacted with each other, but we did not observe a notable change in protein expression of GLUT1 or CD147 after one another's expression was changed. It remains unclear whether CD147 binds to GLUT1 directly or indirectly through other molecules and what the specific roles of this interaction in psoriasis are. Our team previously confirmed that CD147 interacts with GLUT1 at D105-199, CD147 silencing downregulates GLUT1 level via inhibiting PI3K/Akt signaling and decreases glucose uptake in melanoma<sup>[49]</sup>. From the above, we speculate that in psoriasis, the metabolism of proliferated cell mainly depends on glycolysis: regional hypoxia induces HIF-1 $\alpha$  expression, increases GLUT1 and CD147 expressions, and they consequently dependently or independently participate in the highly complex network involving overlapping mechanisms contributed to pathogenesis of psoriasis.

Skin has been suggested to be a hypoxic tissue<sup>[12]</sup>, and hypoxia is further intensified in psoriasis, that is most likely related to the metabolic switch. While in Warburg effect, cells show increased rates of glycolysis despite the presence of adequate O<sub>2</sub> levels due to irreversible damages to OXPHOS<sup>[50-51]</sup>, environmental acidosis<sup>[52]</sup>, etc. Though the drive factors of glycolysis are possibly different in cancers and psoriasis, the most

probable purpose is to generate ATP faster and provide carbon skeletons for biosyntheses<sup>[53]</sup>. Since active glycolysis may play a crucial role in psoriatic pathomechanisms, it provides new insights for therapies of psoriasis, but we should also concern on the side effects in normal tissues that also rely on glycolysis. However, to date, no selective HIF-1 $\alpha$  inhibitor has been clinically approved, partially due to the requirements of targeting protein-protein interactions without affecting other pathways. Needless to say, highly selective GLUT1 and CD147 inhibitors are very promising in psoriasis therapy although no direct inhibitors have been found yet. Finally, combination strategies may be more effective in inhibiting metabolic changes as well as simultaneously inhibiting other important processes such as angiogenesis.

In summary, our study showed demonstrated that glycolytic capacity increases in lesional keratinocytes of psoriasis vulgaris, and confirms that CoCl<sub>2</sub>-mimicked hypoxia in HaCaT cells leads to up-regulate HIF-1 $\alpha$  protein expression, further increase CD147 and GLUT1 expressions, thereby elevate cell glycolytic capacity. These results indicate the potential of glycolysis associated proteins as promising targets in psoriasis therapy. Future studies are needed to elucidate the specific roles of these proteins participated in the metabolic change in psoriasis.

**Conflict of interest:** The authors declare that they have no conflicts of interest to disclose.

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