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# Decrease of galectin-3 in keratinocytes: A potential diagnostic marker and a critical contributor to the pathogenesis of psoriasis

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ABSTRACT

Psoriasis-specific proteins dysregulated in keratinocytes and involved in the pathophysiological process of psoriasis remains elusive. We report here that epidermal galectin-3 expression is significantly downregulated in lesional skin, but not in non-lesional skin in psoriasis patients, nor in a group of diseases known as psoriasiform dermatitis clinically and histologically similar to psoriasis. The deficiency of epidermal galectin-3 is sufficient to promote development of psoriatic lesions, as evidenced by more severe skin inflammation in galectin-3 knockout (gal3$^{-/-}$) mice, compared to wild-type mice, after imiquimod treatment, and in skin from gal3$^{-/-}$ mice grafted onto wildtype mice. The development of psoriatic-like lesions is attributable to 1) the spontaneously tuning up of psoriasis signatures in keratinocytes through JNK pathway; and 2) neutrophil accumulation caused by the enhanced leukocyte-recruiting capacity associated with overexpression of S100A7-9 and CXCL-1, 8 in keratinocytes with impaired galectin-3 expression. Psoriasis-like skin inflammation is significantly improved in gal-3$^{-/-}$ mice both by inhibition of neutrophils accumulation with a selective CXCR2 antagonist of SB225002, and by intracutaneous injection of recombinant galectin-3. Overall, these findings offer promising galectin-3-related diagnostic and therapeutic resolutions of psoriasis.



## 1. Introduction

The diagnosis of psoriasis is primarily relied on the clinical morphologies typically presented as red and well-demarcated papules and plaques with white to slivery scales. Histologically, psoriatic lesions are characterized by focal aggregates of neutrophils in superficial epidermis (known as Munro's microabscesses), epidermal hyperplasia, dermal infiltration and neovascular generation [1]. However, sometimes it is rather challenging even for experienced physicians to distinguish atypical psoriasis cases from the other psoriasiform dermatitis, because all are characterized by scaly red rashes and epidermal hyperplasia. Therefore, it would be very useful to develop a specific and sensitive molecular markers for the diagnosis of psoriasis [2].

The pathogenesis of psoriasis is complex and remains elusive, but there is no doubt that the interplay between keratinocytes and immune cells plays an essential role [3]. However, whether psoriatic skin lesions arise from a primary defect in epidermal keratinocytes or in immune cells is still under discussion [4–6]. Several transgenic mouse strains generated by altering gene expression in the epidermis were shown to spontaneously develop, or be susceptible to stimuli-induced psoriasis-like skin inflammation, supporting the concept of keratinocytes as the primary driver of psoriasis. For instance, transgenic mice with inducible epidermal depletion of *JunB/c-Jun* or with constitutively active epidermal expression of *Stat3* spontaneously developed psoriasis-like skin

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inflammation [7,8], while mice with inducible epidermal depletion of *TNIP1* or microRNA-31 (miR-31) were more susceptible to imiquimod- or IL-17A-triggered skin inflammation [9,10]. JunB and Stat3 are transcription factors widely expressed in various organs and tissues. TNIP1 inhibits, while miR-31 is regulated by the widely expressed transcription factor NF-κB. Thus, it is possible that these transcription-related molecules may also participate in the development of the other skin diseases with epidermal hyperplasia. Moreover, the psoriatic skin inflammation in these transgenic mouse models is all dependent on the presence of T cells, as well as cytokines they produce, like IL-17, IFN-α, TNF-α and IL-22, whereas the contribution of neutrophils remains largely unknown.

Galectin-3 is one of the most extensively studied member of the galectin family of β-galactoside-binding lectins and widely distributed in various organs and tissues [11]. It is involved in the pathological process of certain skin diseases, such as atopic dermatitis, contact dermatitis and various skin cancers [11]. Our recent research established that galectin-3 is targeted by anti-galectin-3 antibody and associated with skin damage in systemic lupus erythematosus [12]. Galectin-3 was reported to be decreased in psoriatic skin by a few studies, however, no further research was performed to investigate the expression and involvement of galectin-3 in psoriasis development since then [13,14].

Here we found that galectin-3 was significantly decreased in epidermis in lesional skin in psoriasis patients, but not in lesional skin in the other psoriasiform dermatitis. The specific deficiency of galectin-3 in skin is capable to drive psoriasis-like skin inflammation, which is at least partly contributed by neutrophil accumulation in epidermis associated with the enhanced chemotactic capacity toward neutrophils by keratinocytes via JNK pathway. Interestingly, intracutaneous injection of recombinant galection-3 suppressed psoriatic inflammation induced by imiquimod in galectin-3 knockout mice. These findings not only extend the insight of the pathogenesis of psoriasis, but also offer new diagnostic and therapeutic targets for the disease.

## 2. Material and methods

### 2.1. Patient samples

Lesional and non-lesional skin samples were collected from each individual patient with psoriasis vulgaris, and lesional skin samples were also collected from patients with psoriatic pustulosa, psoriatic erythroderma and the other diseases of psoriasiform dermatitis. Lesions at the progressive stage were defined as large erythematous plaques or papules with silvery scales that were not treated with any medicine for at least for 2 weeks before the biopsy. Lesions at the regressive stages were defined as erythematous patches with or without fine scales that were treated with topical and/or systemic medicine for more than 2 weeks. Normal skin samples were gathered from the peripheries of removed nevi. Serum samples were collected from twenty patients with psoriasis vulgaris and twenty age-matched healthy controls. The disease severity was measured by psoriasis area and severity index (PASI). The study was approved by the research ethics board of Sun Yat-sen Memorial Hospital and informed consent of donors was obtained.

### 2.2. Imiquimod and IL-23-induced mouse model of psoriasis

C57BL/6 mice (8–11 weeks old) were purchased from the Sun Yat-sen University laboratory animal Center, Guangzhou, China. Galectin3−/− (gal3$^{-/-}$) mice were purchased from The Jackson Laboratory. All experiments were performed according to the Animal Care and Use Committee guidelines of Sun Yat-sen university.

For imiquimod induced psoriasis model, the mice received a daily topical dose of 62.5 mg of a commercially available imiquimod cream (5%) (Aldara; 3M Pharmaceutical) on the shaved back consecutively for 6 days, as described previously [15]. The control mice were treated similarly with Vaseline. For IL-23 challenged psoriasis model, mice were removed back hair with electric clippers and a cream depilatory three days in advance, intradermal injected with IL-23 (eBioscience, #14–8231) or sterile saline in two locations on either side of the back for a total of 1 μg protein per mouse for 4 consecutive days [16,17].

### 2.3. Immunohistochemistry

Immunohistochemistry was performed as we previously described [18]. Briefly, paraffin-embedded tissue sections (4 mm) from human and mice were cut from routine blocks, deparaffinized with xylene, rehydrated, and subjected to heat-induced epitope retrieval methods before incubation with the appropriate antibody. Sections were immersed in 1 mM EDTA (pH 8) and subsequently heated in a pressure cooker for 8*2 min. After they were rinsed in PBS, the sections were incubated overnight at 4 °C with indicated primary antibody. The primary antibodies were mouse anti-galectin-3 antibody (1:40, Thermo Fisher Scientific, #MAI-940), rabbit anti-galectin-3 antibody (1:10000, Abcam, #ab76245), rat anti-Ly6G antibody (1:80, Abcam, #ab25377), rabbit anti-CD3 antibody (1:50, Abcam, #ab16669) and rat anti-F4/80 antibody (1:50, Abcam, #ab6640). Isotype-control staining was performed to check for nonspecific binding. The following day, sections were incubated with appropriate horseradish peroxidase (HRP) conjugated secondary antibody, developed in 3,3′-diaminobenzidine (DAB) kit and counterstained with hematoxylin.

### 2.4. Western blot analysis

For the total protein extraction, HaCaT cells were lysed with a radioimmunoprecipitation assay (RIPA) buffer with protease and phosphatase inhibitors (Roche, Germany). A cytoplasmic and nuclear protein extraction kit (Boster, China) was used to achieve nuclear-cytoplasmic separation. Antibodies against galectin-3 (1:6000, Abcam, #ab76245), S100A7 (1:1000, Abcam, #ab13680), p-P65 (1:5000, Epitomics, #2220), P65 (1:1000, Cell Signaling Technology, #8242S), p-AKT(1:1000,Cell Signaling Technology, #13038),AKT(1:1000, Cell Signaling Technology, #9272), IKB-α(1:1000, Cell Signaling Technology, #4812), p-ERK (1:2000, Cell Signaling Technology, #4370), ERK (1:1000, Cell Signaling Technology, #4695), p-P38 (1:1000, Cell Signaling Technology, #4511), P38 (1:1000, Cell Signaling Technology, #8690), p-JNK (1:1000, Cell Signaling Technology, #4668), JNK (1:1000, Cell Signaling Technology, #9252), Lamin B1 (1:5000, Epitomics, #6581-S), β-tublin (1:1000, Cell Signaling Technology, #2146), GAPDH (1:1000, Cell Signaling Technology, #5174P) and Actin (1:1000, Proteintech, #20536) were used for immunoblot analysis according to the manufactures' protocols.

### 2.5. RNA reverse transcription and qPCR

Total RNA was extracted from keratinocytes and skin biopsies using the RNAiso Plus reagent (Takara, #9109). For epidermis and dermis RNA extraction, skin samples were incubated overnight at 4 °C in 0.25% dispase II (Roche, #04942078001) to completely separate epidermis from dermis. Complementary DNA was synthesized using Prime Script RT Master Mix (Perfect Real Time) (Takara, #RR036A). Quantitative PCR (qPCR) was carried out with the LightCycler® 480 SYBR Green I Master (Roche,#4707516001) in a ViiA 7 Real-Time PCR system (Applied Biosystems). The relative expression of target genes was confirmed using quantity of target

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