

Dermal Periostin: A New Player in Itch of Prurigo Nodularis

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Prurigo nodularis (PN) features hyperkeratotic and intensely pruritic multiple nodules that mainly appear on the extensor surfaces of the limbs and the trunk. Itch in PN impairs patients' quality of life and is the most burdensome aspect of this disease's symptoms instead of the visibility of skin lesion (1). However, treating itch in PN is challenging, because its etiology is largely unknown (2).

Several itch mediators that are Th2-mediated are reported to be responsible for PN-associated itch, including interleukin (IL)-31 and its receptor subunits (IL-31 receptor α (IL-31RA) and oncostatin M receptor β (OSMR β)) (3), and thymic stromal lymphopoietin (TSLP) and TSLP receptor signaling (4). Recently, an extracellular matrix protein named periostin that promotes a Th2 allergic inflammation has been identified as a novel itch mediator (5). Therefore, we sought in this study to elucidate whether periostin is correlated with itch in PN.

MATERIALS, METHODS, AND RESULTS

This study was approved by the Institutional Review Board of the University of Miami (20180898). We collected lesional skin samples and clinical data from 12 PN patients (59 \pm 15 years (19–77); 4 males and 8 females) and 10 healthy individuals (38 \pm 13 years (20–67); 5 males and 5 females) at the University of Miami Dermatology outpatient clinic. Their diagnoses were confirmed based on clinical and histological findings. Itch intensity was measured with numerical rating scale (NRS) scores from zero (no itch) to 10 (the worst itch) at the time and location of the biopsy. NRS scores were obtained from 8 PN patients ranging from 4 to 10 (median, 7; average 7.12), and all healthy individuals had no itch (NRS, zero).

Formalin-fixed paraffin-embedded skin samples of 5- μ m thickness were deparaffinized and subjected to antigen retrieval with DAKO target retrieval solution (Dako, Glostrup, Denmark) at 60°C overnight. Samples were next treated with PBS containing 5% normal donkey serum and 0.2% Triton X-100 at room temperature for 2 hours. Then, incubated with primary Abs for periostin (ab14041, Abcam, Cambridge, UK; dilution, 1:200), IL-31 (ab102750, Abcam; dilution, 1:200), IL-31RA (ab113498, Abcam; dilution, 1:200), and OSMR (LS-B11477, LifeSpan BioSciences, Seattle, WA, USA; dilution, 1:200) at 4°C overnight, followed by reaction with Alexa Fluor 488-conjugated secondary Ab (Molecular Probes, Eugene, OR, USA) and mount with Vectashield with DAPI (Vector Laboratories, Burlingame, CA, USA). Negative control for periostin staining was performed by omitting primary antibody (Fig. S1[†]). For quantification, photomicrographs were captured with DM6000 microscope (Leica Microsystems, Wetzlar, Germany). The deposition of periostin in the entire dermis was measured as fluorescence intensity in arbitrary units (AU) normalized by area

and background fluorescence using Image J software (National Institute of Health, Bethesda, MD, USA). The number of immunoreactive cells near the dermo-epidermal junction (in the papillary and subpapillary dermis) was quantified with Image J software (6). To compare differences between two groups, Mann-Whitney's *U* test were used. For detecting correlation, we calculated Spearman's rank correlation coefficient (*r*) using a statistical software "EZR" (7). Statistical significance was set at *p*<0.05.

We found that dermal deposition of periostin was enhanced in PN compared with healthy controls (Mann-Whitney's *U*-test, *p*<0.05), but immune-reactivity for periostin was not detected at the epidermal layer. Dermal periostin was significantly correlated with itch intensity (Spearman's correlation, *r*=0.524, *p*<0.05) (Fig. 1A). In addition, dermal expression of periostin tended to correlate with dermal IL-31RA(+) cells (Spearman's correlation, *r*=0.412, *p*=0.0581), but we did not detect any significant correlation of dermal periostin with dermal IL-31(+) cells and OSMR β (+) cells (Spearman's correlation, *r*=0.304, *p*=0.168, and *r*=-0.0234, *p*=0.921, respectively) (Fig. 1B).

DISCUSSION

Various allergic skin diseases with itch show enhanced dermal expression of periostin, including atopic dermatitis (8), scabies (9), stasis dermatitis (10), and bullous pemphigoid (11). In addition to these diseases, we revealed, for the first time, the direct correlation between dermal periostin and itch intensity in PN. In the skin, not only dermal fibroblasts (8) but also epidermal keratinocytes are capable of secreting periostin (5). We detected massive deposition of periostin in the entire dermis, while no immune-reactivity was confirmed for periostin at the epidermal layer. Accordingly, a major cellular origin of periostin in PN may be dermal fibroblasts.

Periostin involves both direct and indirect pathways to evoke itch. In a direct pathway, periostin directly acts on itch-sensitive nerve fibers via periostin receptor integrin α V β 3 (5). In an indirect pathway, periostin stimulates immune cells (e.g. macrophages and eosinophils) to release IL-31 and other itch mediators (9, 10). It may depend on a disease type which pathway plays a more active role in periostin-associated itch. The current study showed that dermal periostin was not significantly correlated with other major itch mediators including IL-31, IL-31RA, and OSMR β in the dermis. This finding may suggest that periostin might have a direct effect on itch in PN; further studies examining the role of periostin in itch are required to elucidate this association.

Periostin is also closely involved in allergic inflammation. PN is a Th2 skewed dermatosis with upregulated lesional expression of Th2 cytokines (12). These cytoki-

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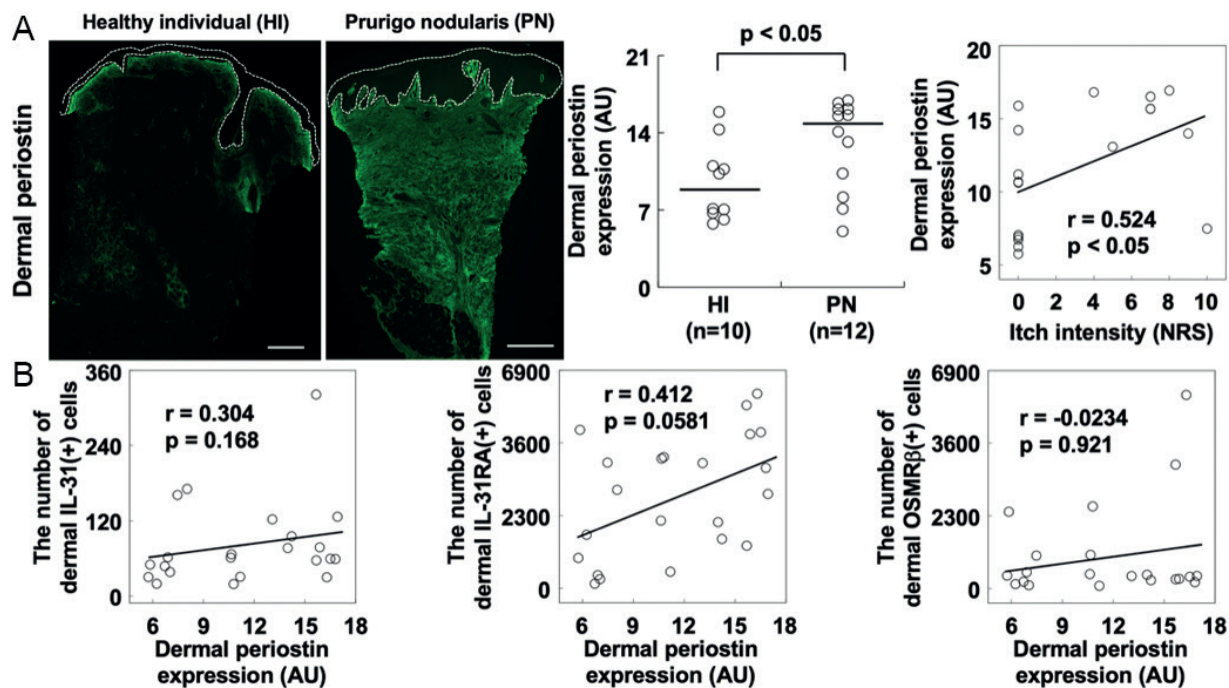


Fig. 1. Periostin in prurigo nodularis (PN). Representative images of PN lesions and healthy individual (HI) skin with quantification of staining. (A) Dermal expression of periostin was significantly enhanced in PN lesions and correlated with itch intensity. White dotted circles indicate the epidermis excluding the stratum corneum. Black lines in the center panel represent medians. (B) Dermal periostin did not correlate with the number of dermal IL-31(+) cells, IL-31RA(+) cells, and OSMRβ(+) cells. Bar = 1 mm. AU: arbitrary unit; r: Spearman's rank correlation coefficient; NRS: numerical rating scale.

nes induce periostin production from dermal fibroblasts. Furthermore periostin exacerbates secretion of Th2 cytokines causing a vicious cycle (5, 8, 13). Targeting periostin or its receptor integrin $\alpha V\beta 3$ may be a good therapeutic option for not only itch but also inflammation itself in PN.

The key limitation of this study was the relatively small number of the samples and the measurement of protein expression exclusively through immunofluorescence staining analysis. However, this study provides a novel insight into the mechanism of PN-associated itch in regard to periostin, which will help guide management of itch in PN.

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