

this, we observed that the downstream effectors of the Wnt pathway, i.e., c-myc and Skp2 were not expressed in KSCs by reverse transcription-PCR (Figure 1b). Further, probing cell lysates of WIF1-arrested keratinocytes in western blots revealed that Wnt3A/WIF1 treatment resulted in increased p21 protein levels (Figure 2o) demonstrable quantitatively (Figure 2p). Thus, WIF1 may achieve its cell cycle arrest in keratinocytes at least in part through derepression of p21 transcription.

In conclusion, we report that WIF1 is, to our knowledge, previously unreported as a marker of interfollicular KSCs, and that it inhibits cell cycle progression in human keratinocytes even in the presence of activating Wnt signals (Wnt3A). Although canonical Wnt signaling appears to be dispensable during development in the interfollicular epidermis (Huelsen et al., 2001; Nguyen et al., 2009), our data suggest that inhibition of Wnt signaling may be required for keeping interfollicular stem cells quiescent and differentiating cells from proliferating during homeostasis.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Serum Gastrin-Releasing Peptide Levels Correlate with Pruritus in Patients with Atopic Dermatitis

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TO THE EDITOR

Bombesin and two major bombesin-like peptides in mammals, gastrin-releasing peptide (GRP) and neuromedin B, have been shown to elicit various physiological effects. GRP elicits gastrin release and

regulates gastric acid secretion and motor function (Merali et al., 1999). This peptide is also involved in the biology of the circadian system. Interestingly, intradermal injections of GRP elicit scratching in mice (Andoh et al., 2011). GRP is

expressed in a subset of peptidergic dorsal root ganglion neurons, whereas GRP receptor (GRPR) is expressed in lamina I of the dorsal spinal cord (Sun and Chen, 2007). When lamina I neurons expressing GRPR in the spinal cord were selectively ablated, the mice showed profound scratching deficits in response to all of the itching stimuli tested, irrespective of their histamine dependence (Sun et al., 2009). These data support the labeled line

Abbreviations: AD, atopic dermatitis; GRP, gastrin-releasing peptide; GRPR, GRP receptor; PGP, protein gene product; VAS, visual analog scale

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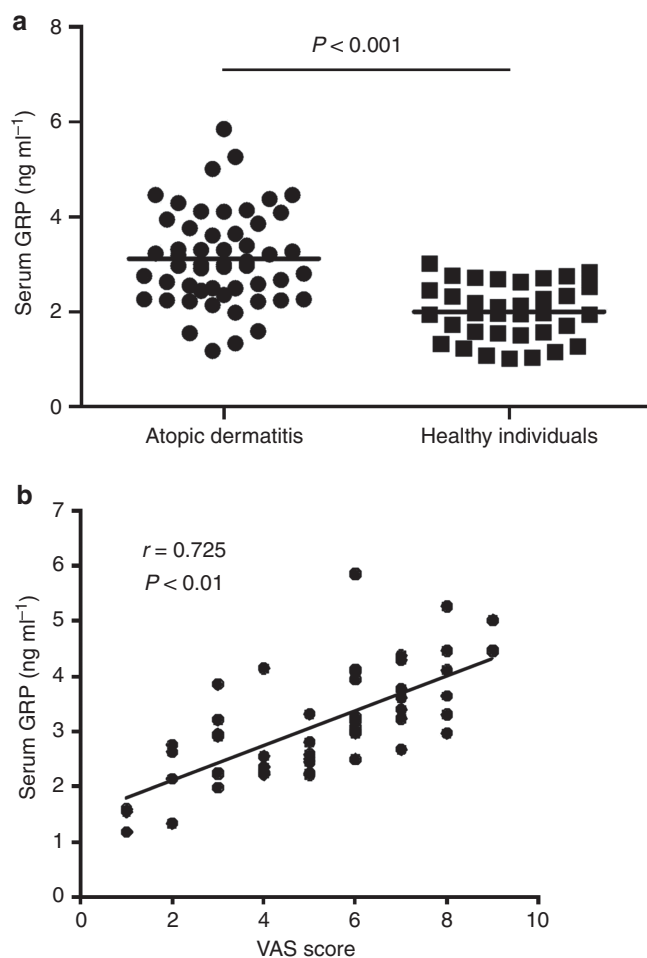


Figure 1. Serum gastrin-releasing peptide (GRP) levels in atopic dermatitis patients. (a) Serum GRP levels in 53 atopic dermatitis patients and 35 healthy individuals. Each bar indicates the mean of each group. (b) Correlations between serum GRP levels and visual analog scale (VAS) itch scores.

for itching in the spinal cord and provide an important cellular basis for explaining how a pruritogenic stimulus is perceived by the brain as a major sensation.

Atopic dermatitis (AD) is an inflammatory pruritic skin disorder. Mechanical trauma resulting from extensive scratching that is precipitated by intensive itching results in skin barrier dysfunction and exacerbation of AD (Barnes, 2010). So far, severe pruritus in AD has been attributed to increased release of substance P, nerve growth factor, neurotrophin-3 and 4, brain-derived neurotrophic factor, histamine, and IL-31 (Arck and Paus, 2006; Lee *et al.*, 2006; Scuri *et al.*, 2010; Lee and Yu, 2011). It was recently reported that a proportion of protein gene product (PGP) 9.5⁺ nerve fibers expressed GRP in both the epidermis and the dermis of NC/Nga mice, the most popular AD model mice (Tominaga *et al.*, 2009). In this study, we examined serum GRP levels in AD and their correlation with visual analog scale (VAS) itch scores.

A total of 53 AD patients (mean $\pm$ SD age: 36.4 ± 12.6 years, 31 men and 22 women) and 35 healthy controls (41.6 ± 16.0 years, 11 men and 24 women) were enrolled in this study. All AD patients were given diagnoses according to the criteria of Hanifin and Rajka (1980). We rated itch by VAS 0–10, asking the patients to mark a point on the

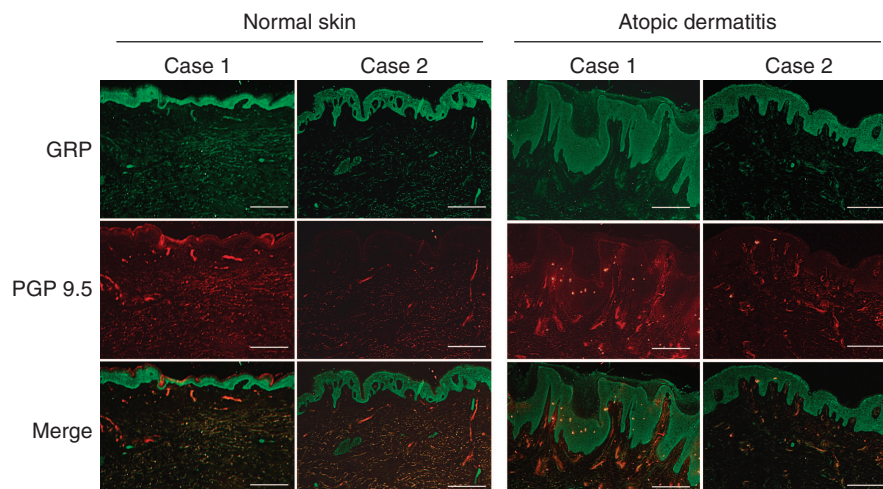


Figure 2. Double immunofluorescence staining for gastrin-releasing peptide (GRP) and protein gene product (PGP) 9.5. Dermal PGP 9.5⁺ nerve fibers (red) expressed GRP (green). Increased numbers of double-positive fibers (yellow) in lesional skin of atopic dermatitis compared with normal skin. Representative two cases in each group. Bar = 100 μ m.

line corresponding to mean itch during the last 7 days before blood draw. The medical ethical committee of the University of Tokyo approved all described studies, and the study was conducted according to the Declaration of Helsinki Principles. Informed consent was obtained from participants. The 35 healthy controls had no history of allergy or pruritus. Enzyme immunoassay was performed using GRP EIA kit obtained from Phoenix Pharmaceuticals (Burlingame, CA). Biopsy specimens obtained from AD patients ($n=5$) and healthy controls ($n=6$) were snap-frozen, cut into 5- μ m-thick cryostat sections, and fixed in acetone. These sections were then stained with rabbit anti-human GRP antibody (number H-027-07, Phoenix Pharmaceuticals) and mouse anti-human PGP 9.5 mAb (13C4/13C4, Abcam plc, Cambridge, UK), followed by corresponding secondary antibodies labeled with Alexa-Fluor 488 or Alexa-Fluor 594 (Molecular Probes, Eugene, OR). The numbers of double-positive fibers per $\times 100$ field were counted independently by two investigators in a blinded manner. The χ^2 goodness-of-fit test was used to evaluate normality for all parameters. The F-test was used to evaluate the equality of variance between two groups. Welch's t test was used for comparison between two groups. Correlation coefficients were determined by using Spearman's rank correlation test. $P<0.05$ was considered significant.

Serum GRP levels in AD patients were significantly higher than in healthy individuals (3.11 ± 0.98 and 2 ± 0.58 ng ml $^{-1}$, respectively; $P<0.001$, Figure 1a). When serum GRP levels higher than the mean + 2 SD of the control serum samples were considered to be elevated, the percentage of elevated samples in AD were 45.2%. We compared serum GRP levels with other laboratory data reflecting disease activity of AD, such as serum levels of IgE, sIL-2R, IL-31, CCL17, and numbers of eosinophils in peripheral blood. There was no significant relation between serum GRP levels and these markers (data not shown). By contrast, there was a significant correlation between serum GRP levels and VAS itch scores ($r=0.725$, $P<0.01$, Figure 1b). We next performed double immunofluorescence

staining for GRP and PGP 9.5. In both AD lesional skin and normal skin, dermal PGP 9.5 $^{+}$ nerve fibers expressed GRP (Figure 2), as was previously reported in NC/Nga mice. Absorption experiments suggested that immunoreactivity in the epidermis was false-positive (data not shown). The number of GRP $^{+}$ nerve fibers in AD skin (5.0 ± 1.2 per $\times 100$ field) was significantly larger than that of normal skin (2.7 ± 0.8 per $\times 100$ field).

We clearly demonstrated that serum GRP levels in AD patients were significantly higher than in healthy individuals, and that they correlated with VAS itch scores. Transepidermal water loss and serum levels of β -endorphin and IgE are useful biomarkers for itch intensity in AD patients (Lee *et al.*, 2006). IL-31 is also regarded as a key cytokine for the development of AD-induced skin inflammation and pruritus (Bilsborough *et al.*, 2006). However, serum IgE and IL-31 levels did not correlate with VAS itch scores in this study. Although further studies with a large number of cases are required, serum GRP levels could be a better biomarker of itch in AD patients. No significant correlation between serum GRP levels and serum IgE or IL-31 levels in this study has also suggested multiple pathways for itch sensation. GRP-induced scratching was inhibited by the μ -opioid receptor antagonist naltrexone hydrochloride, the GRPR antagonist RC-3095, the H $_1$ histamine receptor antagonists fexofenadine hydrochloride and chlorpheniramine maleate, and the PAR $_2$ proteinase-activated receptor antagonist FSLLRY-NH $_2$ (Andoh *et al.*, 2011). It has recently been reported that central GRPR and neuromedin B receptor act independently to elicit scratching behavior and that there is an additional, unidentified receptor mechanism underlying bombesin-elicited scratching (Su and Ko, 2011). In summary, elevated serum GRP levels and increased numbers of dermal GRP $^{+}$ nerve fibers suggested that this peptide could be a therapeutic target for itch in AD patients.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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