Appendix S1

SUPPLEMENTARY MATERIALS AND METHODS

Formalin-fixed paraffin-embedded skin samples (5-μm thickness) from porokeratosis patients and non-itchy individuals with no porokeratosis lesions were deparaffinized and subjected to antigen retrieval with target retrieval solution (Dako, Glostrup, Denmark) at 60°C overnight. Samples were then treated with Protein Block serum-free (Dako) at room temperature for 10 min. Samples were incubated with primary antibodies (Abs) for IL-31 (Cat#, ab102750; Abcam, Cambridge, UK), IL-31RA (ab113498; Abcam), 2D7 (ab155577; Abcam), AAI (ab2378; Abcam), eotaxin-3 (ab217328; Abcam), TSLP (ab188766; Abcam), periostin (ab14041; Abcam), major basic protein (MBP) (NBP1-42140-1mL; Novus Biologicals, Centennial, Canada), β-III tubulin (Tuj1) (MO15013; Neuromics, Minneapolis, MN, USA), or CD203c (bs-1568R; Bioss Antibodies, Woburn, MA, USA) at 4°C overnight, followed by reaction with Alexa Fluor 488- or 568-conjugated secondary Ab and mounted with Fluoroshield with DAPI (GeneTex, Irvine, CA, USA). For quantification, photomicrographs were captured with a BZ-X710 microscope (Keyence, Osaka, Japan). Expression levels of IL-31, IL-31RA, TSLP, and eotaxin-3 in the entire epidermis and periostin in the dermis were measured as fluorescence intensity in arbitrary units normalized by area and background fluorescence, using Image J software (National Institute of Health, Bethesda, MD, USA) (1,2). The numbers of cells that expressed IL-31, IL-31RA, 2D7 or MBP in the dermis were manually counted by two independent researchers (S.O. and T.H.). To compare differences between HI and NIP, or between NIP and IP, the Mann-Whitney U-test (U-test) was used. For detecting correlations, we calculated Spearman's rank correlation coefficient (r) using EZR statistical software (3). Statistical significance was set at the level of $p < 0.05$. Skin samples were collected at the National Defense Medical College Hospital. This study was approved by the institutional review board of the National Defense Medical College (approval no. #4477).

REFERENCES

1. Awatani K, Hashimoto T, Satoh T. Eruptive pruritic papular porokeratosis accompanied by eosinophilic and basophilic infiltrate with upregulation of epidermal CCL26/eotaxin-3 and thymic stromal lymphopoietin. J Dermatol 2021; 48: e382–383.
2. Hashimoto T, Moriyama Y, Satoh T. Linear porokeratosis with severe itch accompanied by lesional upregulation of interleukin 31, thymic stromal lymphopoietin, and periostin. Eur J Dermatology 2021; 31: 570–572.
3. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 2012; 48: 452–458.