Bcl-2, and stromal cells next to tumor cells stain positive for CD34. Moreover, Ki-67, a proliferative marker associated with mitosis, shows relatively weak nuclear positivity[1]. These features help differentiate BFH from basal cell carcinoma, especially infundibulocystic type, in that this presents with deeper infiltration, strong nuclear positivity for Ki-67, negativity for CD34, and prominent cytoplasmic positivity for Bcl-2. Other differential diagnoses include trichoepithelioma, which shows more abundant and highly fibrocytic stroma, and frequently involves follicular bulbs and papillae[5].

In conclusion, we report a case of BFH with clinical presentation of localized grouped papules on the chest of an adult that has not been reported.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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p16^{INK4a} Expression in Porokeratosis

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Dear Editor:

Porokeratosis is a chronic skin disorder of aberrant epidermal keratinization, clinically manifesting as patches with an elevated peripheral keratotic ridge that corresponds histologically to the cornoid lamella. The lesions have a tendency toward peripheral expansion[1]. The cornoid lamella is a column of tightly packed parakeratotic cells with pyknotic nuclei. In the cornoid lamella, the granular layer is usually lost and the keratinocytes beneath the parakeratotic column show vacuolated or eosinophilic degenerative cytoplasm in association with mild superficial dermal mononuclear cell infiltration[1]. According to the number, size, and distribution of the lesions, at least six clinical variants have been described, including disseminated superficial porokeratosis (DSP) and porokeratosis of Mibelli (PM)[1]. The malignant transformation of porokeratosis into Bowen's disease and squamous cell carcinoma has been described, with a reported incidence of

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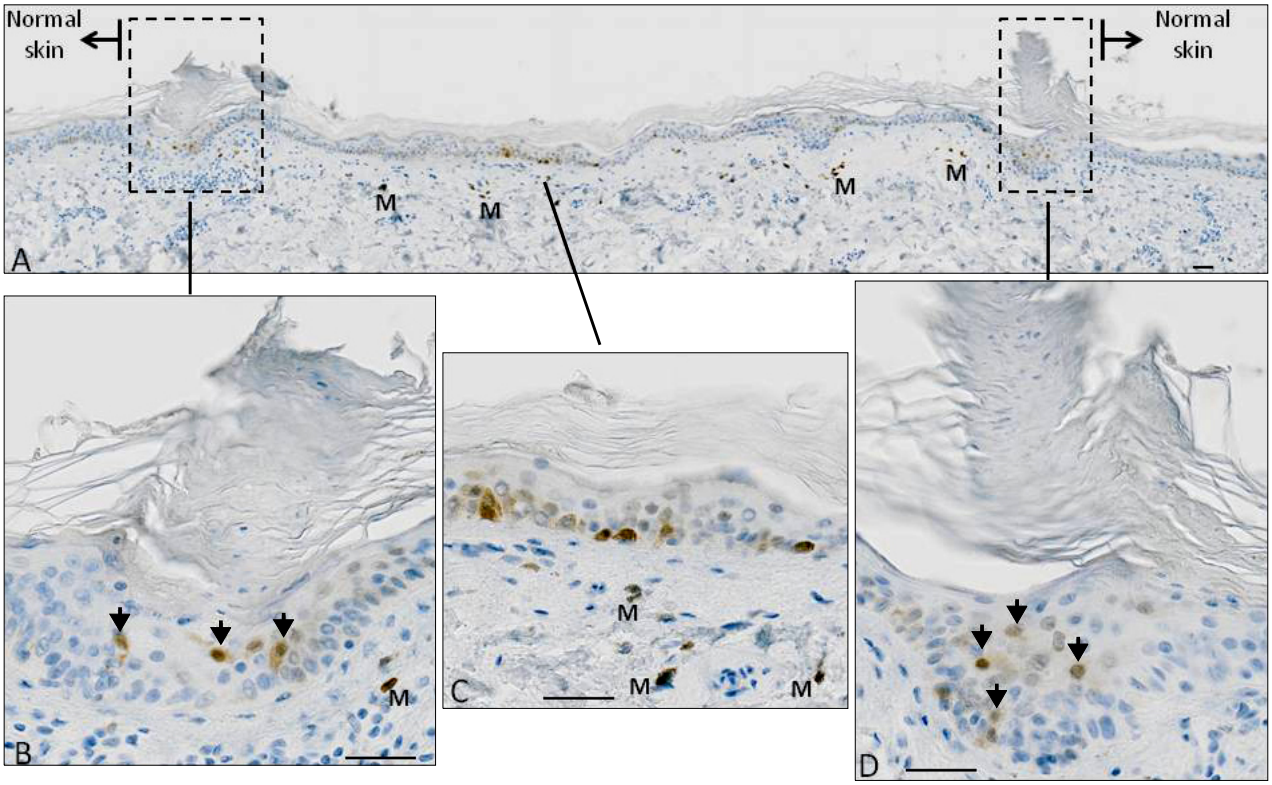

Fig. 1. Immunohistological expression of $p16^{INK4a}$ in disseminated superficial porokeratosis. (A) $p16^{INK4a}$ expression is present in the periphery (dotted rectangle) and the center of the lesion. The perilesional normal epidermis lacks positive signals. (B) Cornoid lamella is negatively stained with $p16^{INK4a}$. Some keratinocytes beneath the cornoid lamella are positive for $p16^{INK4a}$ (arrows). (C) $p16^{INK4a}$-immunopositive keratinocytes are detected in the central area of the lesion. (D) Cornoid lamella is negatively stained with $p16^{INK4a}$. Some keratinocytes beneath the cornoid lamella are positive for $p16^{INK4a}$ (arrows). M: melanin granules. Bar: 100 μm.

7.5% to 11%[1]. In line with this, the porokeratotic cells revealed abnormal DNA ploidy with increased DNA indices[1]. Although it is believed that the clonal expansion of abnormal keratinocytes leads to the development of porokeratosis, the pathogenesis remains unknown[1].

Cyclin-dependent kinases 4 and 6 are critical regulatory enzymes that drive cell cycle progression from the G_0 or G_1 phase into the S phase, so their activity is under stringent control to ensure successful cell division[2,3]. The protein 16^{INK4a} ($p16^{INK4a}$) downregulates the kinase activity of cyclin-dependent kinases 4 and 6 and acts as an inhibitor of cell cycle progression[2]. Moreover, $p16^{INK4a}$ is strongly associated with the cellular senescence process and a diverse array of aging stimuli upregulate its expression[2,3]. To the best of our knowledge, only one report has described the transcriptional overexpression of $p16^{INK4a}$ in a single case of congenital unilateral linear porokeratosis[4]. However, the immunohistological localization of $p16^{INK4a}$ has never been reported.

We examined formalin-fixed and paraffin-embedded tissues of five cases of normal skin and seventeen cases of porokeratosis (DSP, n=9; PM, n=8). Sections were deparaffinized with xylene for 10 min and rehydrated through a graded ethanol series. Antigen retrieval was performed using Heat Processor Solution pH6 (Nichirei Biosciences Inc., Tokyo, Japan) at 100°C for 40 min, and endogenous peroxidase was blocked by incubating the sections with 3% H_2O_2 (Nichirei Biosciences Inc.). The sections were then incubated with monoclonal antibody against $p16^{INK4a}$ (E6H4, CINtec; Roche MTM Laboratories, Westborough, MA, USA) at 4°C overnight, followed by incubation with secondary antibody, N-Histofine® Simple Stain MAX-PO MULTI (Nichirei Biosciences Inc.). Immunodetection was conducted with 3,3-diaminobenzidine as a chromogen, followed by light counterstaining with hematoxylin. Sections stained without primary antibody served as a negative control. All of the sections were negatively stained with anti-human papilloma virus antigen antibody (K1H8; Abcam, Cambridge, MA, USA). This study was approved by the ethical committee of Kyushu University Hospital (IRB no. 27-157).

As has been reported previously[5], the expression of $p16^{INK4a}$ was not detected in the perilesional normal epidermis (Fig. 1A, 2A). In all cases of both DSP (Fig. 1) and

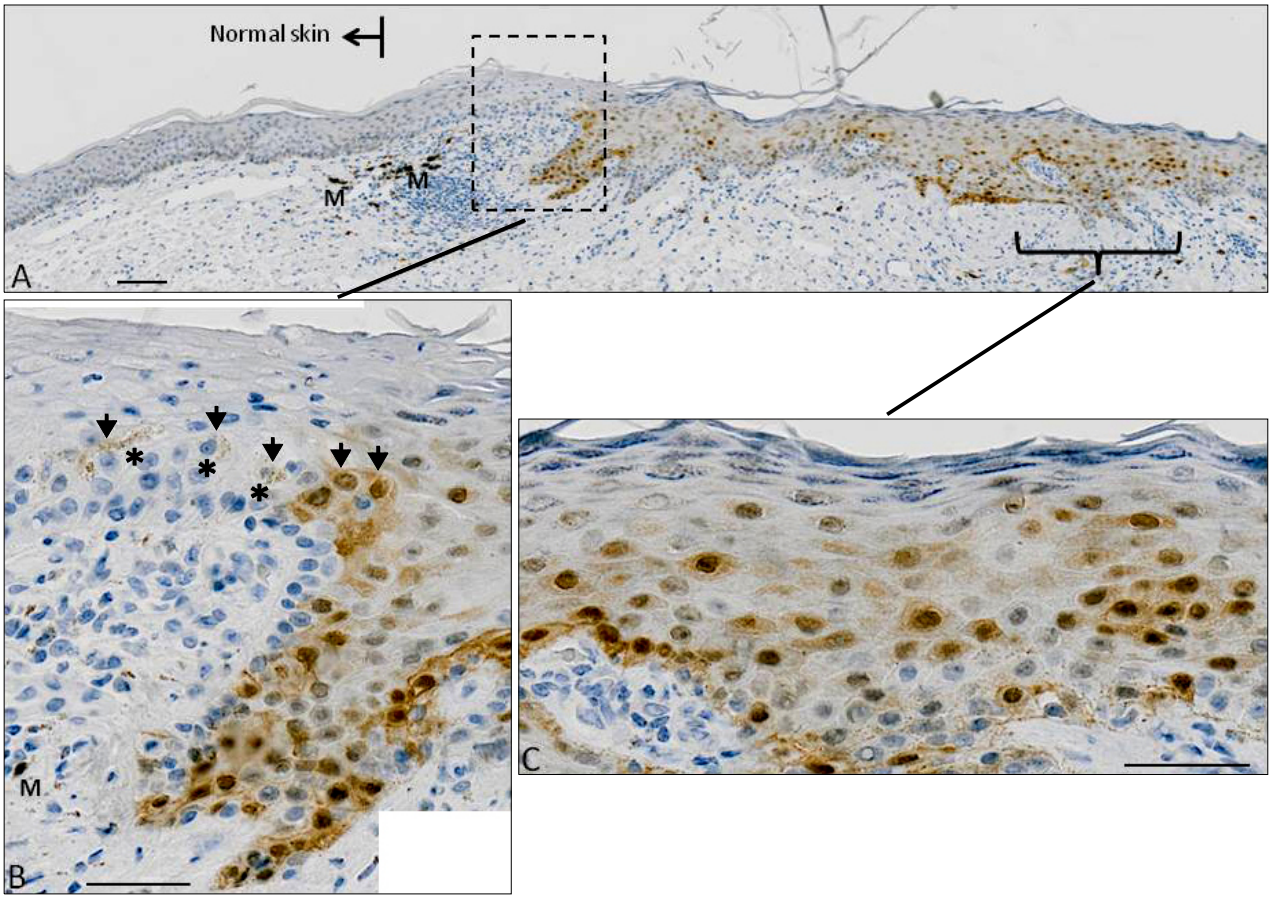

Fig. 2. Immunohistological expression of $p16^{INK4a}$ in porokeratosis of Mibelli. (A) $p16^{INK4a}$ expression is present in the periphery (dotted rectangle) and the center of the lesion. The perilesional normal epidermis is completely negative for $p16^{INK4a}$. (B) Cornoid lamella is negatively stained with $p16^{INK4a}$. Some keratinocytes beneath the cornoid lamella are positive for $p16^{INK4a}$ (arrows). Vacuolated keratinocytes (asterisks) are also positively stained. (C) $p16^{INK4a}$-immunopositive keratinocytes are detected in the central area of the lesion. M: melanin granules. Bar: 100 μm.

PM (Fig. 2), parakeratotic cornoid lamella was negative for $p16^{INK4a}$. However, a population of keratinocytes beneath and around the cornoid lamella were positively stained with $p16^{INK4a}$ (Fig. 1B, D, 2B, Supplementary Fig. 1). Some of them were vacuolated cells (Fig. 2B). In the central area of porokeratosis, variable numbers of keratinocytes were also immunolabeled with $p16^{INK4a}$ (Fig. 1C, 2C). The central $p16^{INK4a}$ immunoreactivity was separate from (Fig. 1) or continuous with (Fig. 2) the cornoid lamella area. The staining pattern of $p16^{INK4a}$ was both nuclear and cytoplasmic. Central immunostaining for $p16^{INK4a}$ was detected in 6 of 9 DSP cases and 7 of 8 PM cases.

The present study is the first to reveal the immunolabeling of $p16^{INK4a}$ in porokeratosis. $p16^{INK4a}$ immunopositivity was observed in the keratinocytes underlying the cornoid lamella as well as those in the central portion of the lesion. These results coincide with the upregulated expression of $p16^{INK4a}$ mRNA in a single case of congenital unilateral linear porokeratosis[4]. Besides $p16^{INK4a}$, another molecular effector to regulate senescence is p53[2]. Previous studies have revealed that the p53 expression is also upregulated in the keratinocytes underlying and surrounding cornoid lamella[6,7]. These results prompted us to speculate that the abnormal porokeratotic clones are senescence-prone keratinocytes which are generated beneath the cornoid lamella extending into the central lesion during lesional expansion to the periphery, which is in sharp contrast to the complete negativity of $p16^{INK4a}$ in the perilesional normal epidermis.

Although $p16^{INK4a}$ immunolabeling has been validated as an accurate surrogate marker for determining the concomitant infection of human papilloma virus in vulvar squamous cell carcinoma[8], none of the present cases exhibited immunopositivity to human papilloma virus antigen. In accordance with a pivotal role of $p16^{INK4a}$ in cell cycle arrest and senescence, melanomas expressing

$p16^{INK4a}$ exhibit slower growth than those without $p16^{INK4a}$ expression[9]. In addition, the loss of $p16^{INK4a}$ in affected T cells correlates with disease progression in mycosis fungoides[10]. Senescence and carcinogenesis are paradoxical phenomena. The loss of proliferative potential with age should suppress cancer, but cancer incidence, like other degenerative diseases of aging, increases nearly exponentially with age. There is now increasing evidence that the increase in cancer incidence is due to senescent cells secreting factors that create a tissue microenvironment that promotes tumor formation[3]. The aberrant expression of $p16^{INK4a}$, as well as p53, in affected keratinocytes may be related to the pro-oncogenic nature of porokeratosis. However, further studies are required to confirm this hypothesis.

SUPPLEMENTARY MATERIALS

Supplementary data can be found via http://anndermatol.org/src/sm/ad-29-373-s001.pdf.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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