Expression of p16<sup>+</sup> in Squamous Cell Carcinoma Arising from Porokeratosis

Furue, Masutaka
Department of anatomic pathology, graduate school of medical sciences, Kyushu University | Division of Skin Surface Sensing, Department of Dermatology, Kyushu University | Research and Clinical Center for Yusho and Dioxin, Kyushu University

Sato, Seisho
Department of Dermatology, Kyushu University

Tsukamoto, Karin
Department of Dermatology, Kyushu University

Nakamoto, Sumire
Department of Dermatology, Kyushu University

他

http://hdl.handle.net/2324/1901725

出版情報：福岡醫學雜誌. 108 (6), pp.176-182, 2017-06-25. 福岡医学会
バージョン：published
権利関係：
Expression of p16INK4a in Squamous Cell Carcinoma Arising from Porokeratosis

Masutaka FURUE1,2, Seisho SATO1, Karin TSUKAMOTO1, Sumire NAMAMOTO1, Shiori HIKICHI1, Ginju YOKOTE1, Long DUGU1 and Nobutoshi TAKE1
1) Department of Dermatology, Kyushu University, Fukuoka, Japan
2) Division of Skin Surface Sensing, Department of Dermatology, Kyushu University, Fukuoka, Japan
3) Research and Clinical Center for Yusho and Dioxin, Kyushu University, Fukuoka, Japan

Abstract
The expression of p16INK4a has been reported to induce cell cycle arrest and cellular senescence. We recently demonstrated that p16INK4a expression is an integral part of the pathogenesis of porokeratosis. In this study, we further examined the p16INK4a expression in squamous cell carcinoma (SCC) arising from disseminated porokeratosis. p16INK4a-expressing keratinocytes were detected in the porokeratotic epidermis and atypical keratinocytes in the SCC in situ developed from porokeratosis. The expression of p16INK4a was also detected in the invasive SCC cells; however, it was eventually lost in the tumoral nests. These results suggest that the aberrant expression of p16INK4a in affected keratinocytes may be related to the pro-oncogenic nature of porokeratosis. Its expression may be lost by subsequent tumor progression.

Key words: p16INK4a, porokeratosis, squamous cell carcinoma

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 lamella1). One of the characteristic features of porokeratosis is its carcinogenicity1). The malignant transformation of porokeratosis into in situ and invasive squamous cell carcinoma has been described, with a reported incidence of 7.5% to 11%1). In line with this, the porokeratotic cells revealed abnormal DNA ploidy with increased DNA indices1). Although it is believed that the clonal expansion of abnormal keratinocytes leads to the development of porokeratosis, the pathogenesis behind this remains unknown1).

We and others have demonstrated the aberrant expression of protein 16INK4a (p16INK4a) in porokeratosis and speculated that the p16INK4a+ keratinocyte clones are potentially involved in the malignant transformation of porokeratosis2,3). In this study, we further examined the spatial distribution of p16INK4a in a patient with disseminated porokeratosis who developed multiple squamous cell carcinoma. For the immunodetection of p16INK4a, antigen retrieval was performed using Heat Processor Solution pH6 (Nichirei Biosciences Inc., Tokyo, Japan) at 100°C for 40 min. The sections were then incubated with monoclonal antibody against p16INK4a (E6H4, CINtec ; Roche MTM Laboratories, MA, USA) at 4°C overnight, followed by incubation with secondary antibody, N-Histofine® Simple Stain
MAX-PO MULTI (Nichirei Biosciences Inc., Japan).

In the perilesional normal skin, p16INK4a was not expressed in the epidermal keratinocytes (Fig. 1). However, 16INK4a+ keratinocytes were detected in the porokeratotic epidermis inside the cornoid lamella (arrowhead) (Fig. 1). The staining pattern of p16INK4a was cytoplasmic and nuclear (Fig. 1). From a clinical perspective, malignant transformation was suspected in the erythematous and erosive area (Fig. 1). The increase of proliferative potential with age should suppress cancer, but cancer incidence increases nearly exponentially with age656. In accordance with p16INK4a playing a pivotal role in cell cycle arrest and senescence, the accumulation of p16INK4a is related to tumor generation6. The increase of p16INK4a-expressing keratinocyte clones has been demonstrated in lichen planus, which is also an oncogenic skin disorder9. In contrast, recent studies have revealed that the expression of p16INK4a was paradoxically associated with a favorable prognosis of patients with squamous cell carcinoma of oropharyngeal and genital region10-12, suggesting the existence of a complex interrelationship among senescence, carcinogenesis, and prognosis.

In conclusion, the aberrant expression of p16INK4a in affected keratinocytes may be related

As previously reported2, the lesional keratinocytes did express p16INK4a in non-malignant porokeratosis in the present study. The atypical keratinocytes also expressed p16INK4a in the epidermis of porokeratosis with malignant transformation. Moreover, the expression of p16INK4a was strongly detected in the invasive keratinocytes in the dermal columnar tumor nests. However, the atypical keratinocytes in the solid, tumorous nests lost p16INK4a positivity. These results suggest the possibility that the p16INK4a-expressing keratinocyte clones may contribute to the malignant transformation: however, p16INK4a expression is likely to be lost in further tumor progression. Similar findings have been reported by Genders et al.7. They reported that the p16INK4a expression was found in 19% of actinic keratosis, 92% of Bowen’s disease and 35% of squamous cell carcinoma7. In addition, the expression pattern of p16INK4a was focal and patchy in actinic keratosis and squamous cell carcinoma, while that of Bowen’s disease was diffuse and strong7. In squamous cell carcinoma, the p16INK4a expression was present not only in invasive front but also in central tumor nest, suggesting again that its expression is not likely to be related to invasiveness78.

Senescence and carcinogenesis are paradoxical phenomena. The loss of proliferative potential with age should suppress cancer, but cancer incidence increases nearly exponentially with age56. In accordance with p16INK4a playing a pivotal role in cell cycle arrest and senescence, the accumulation of p16INK4a is related to tumor generation6. The increase of p16INK4a-expressing keratinocyte clones has been demonstrated in lichen planus, which is also an oncogenic skin disorder9. In contrast, recent studies have revealed that the expression of p16INK4a was paradoxically associated with a favorable prognosis of patients with squamous cell carcinoma of oropharyngeal and genital region10-12, suggesting the existence of a complex interrelationship among senescence, carcinogenesis, and prognosis.

In conclusion, the aberrant expression of p16INK4a in affected keratinocytes may be related
Fig. 1 Clinical features, and H&E and p16INK4a staining in porokeratosis. Arrowhead indicates cornoid lamella. Bar: 100 μm.

Fig. 2 Clinical features, and H&E and p16INK4a staining in porokeratosis. Arrowhead indicates cornoid lamella. Bar: 100 μm.
**Fig. 3** Clinical features, and H&E and p16$^{INK4a}$ staining in erythematous erosive lesion suspected of squamous cell carcinoma in situ arising from porokeratosis. Bar: 100 μm.

**Fig. 4** Clinical features, and H&E and p16$^{INK4a}$ staining in erosive, indurated lesion of invasive squamous cell carcinoma arising from porokeratosis. Bar: 100 μm.
to the pro-oncogenic nature of porokeratosis. Its expression may be lost by subsequent tumor progression. However, further studies are required to confirm this.

Conflicts of Interest

The authors have no conflicts of interest to declare.

References

5) Mowlà SN, Lam EW and Jat PS: Cellular senescence and aging: the role of B-MYB.
10) Satgunaseelan L, Virk SA, Lum T, Gao K, Clark JR and Gupta R: p16 expression independent of human papillomavirus is associated with lower


(Received for publication June 8, 2017)
汗孔角化症由来有棘細胞癌におけるp16INK4aの発現

古江増隆1)−3), 佐藤清象1), 塚本華倫1), 仲本すみれ1), 挽地史織1), 横手銀珠1), 独孤龍1), 武信肇1)

1)九州大学大学院医学研究院皮膚科学分野
2)九州大学大学院医学研究院皮膚科学分野寄附講座体表感知学
3)九州大学病院病院職員診療センター

p16INK4aは細胞増殖を停止させ細胞老化を誘導することが知られている。我々は以前、汗孔角化症を構成する表皮細胞内にp16INK4a陽性クローンが存在することを明らかにし、本症に認められる発癌に関与するのではないかと推定した。今回、播種性汗孔角化症に有棘細胞癌が多発した症例を経験し、本症例の汗孔角化症、有棘細胞癌におけるp16INK4aの発現パターンを免疫組織学的に検討した。p16INK4aは健常皮膚では発現は認められず、汗孔角化症内表皮細胞にはp16INK4a陽性クローンが混在していた。本症から発症した表皮内および浸潤性有棘細胞癌細胞のほとんどはp16INK4a陽性であった。一方、腫瘍形成に至る有棘細胞癌細胞ではp16INK4aの消失を認めた。以上の結果は、p16INK4a陽性表皮細胞クローンが汗孔角化症の発癌に関与していると推定されるが、腫瘍細胞の癌化が進展する中でp16INK4a陽性所見は陰転化する可能性があると考えられた。

キーワード：p16INK4a，汗孔角化症，有棘細胞癌