

Cytokeratin Expression in Seborrheic Keratosis

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Background : Using biochemical and immunohistochemical studies, alterations of cytokeratin expression has been reported in seborrheic keratosis.

Objective : To further investigate the cytokeratin expression in seborrheic keratosis, we have done immunohistochemical staining using a panel of specific anti-keratin antibodies in this study. We also observed the cytokeratin expression in the hair, sebaceous gland and sweat gland of the some epidermis.

Methods : Twenty cases of seborrheic keratosis were collected from the pathologic files. The histological types included acanthotic type (13 cases), hyperkeratotic type (5 cases), and pigmented type (2 cases). All tissues had been fixed in formalin and then paraffin-embedded according to conventional procedures. Each section was mounted on a gelatin-coated glass slide, and incubated with various anti-keratin antibodies. The sections were then immunostained using the avidin-biotin-peroxidase complex system. The peroxidase reaction was visualized with diaminobenzidine (DAB).

Results :

1. Cytokeratin expression in seborrheic keratosis lesions

On staining with 34 β B4 (K1), several staining patterns in the suprabasal layers of the epidermis were observed in 10 out of 20 cases. Using the AE1 (K10,14,15), we observed focal staining in 2 cases. We observed several positive staining patterns in 5 cases with K13,16 antibody. On staining with K10 antibody, we observed focal or irregular staining patterns in 14 cases. Focal staining was also observed with K5,8 antibody in one case.

2. Cytokeratin expression in the hair, sebaceous gland and sweat gland

On immunoperoxidase staining of hair, there were positive reactions with CAM5.2 (K8,18) in 2 cases. There were positive reaction with K13,16 antibody in one case, with 34 β B4 (K1) and K10 antibody in 3 cases, and with K17 antibody in 2 cases.

On immunoperoxidase staining of sebaceous glands, there was one positive reaction with CAM5.2 (K8,18) in the suprabasal cells of sebaceous glands and with K13,16 antibody in sebaceous ducts. There were positive reactions with K17 antibody in the sebaceous ducts in 2 cases, and with K1 antibody in the sebaceous glands in one case.

Using 34 β B4 (K1), 4 out of 20 cases showed positive reactions in sweat glands. On staining with AE1 (K10,14,15), positive reactions were observed in 8 cases. Staining with CAM5.2 (K8,18) showed positive reactions in 14 cases. There were positive reactions with K19 antibody in 9 cases.

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Keratins are the largest intermediate filament (IF) group with at least 30 different protein chains, consisting of roughly 20 epithelial keratins and 10 hair keratins¹. The classification by Moll et al² is most widely used. It is based on dividing the different keratins according to molecular weight

Conclusion : Our data suggests that the predominant keratin expression in the tumor cells of seborrheic keratosis is high molecular weight keratin (K1/K10) rather than other lower molecular weight keratin. Tumor cells show some proliferative activity and monoclonal antibody K19 could be a marker for eccrine sweat glands like CAM5.2 (K8,18).
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Key Words : Anti-keratin antibody, Seborrheic keratosis

(MW) and isoelectric points with the assignment of a number to each keratin. Characteristics of type I epithelial keratins are acidic isoelectric points, members consist of Moll Nos. K10 through K19, and MW range of 40 to 56.5 kd. Features of type II epithelial keratins are neutral-basic isoelectric points, members consist of Moll Nos. K1 through K9, and MW range of 52 to 67 kd.

Using biochemical and immunohistochemical studies, alterations of cytokeratin expression has been reported in seborrheic keratosis^{3,6}, and recently Fujisawa et al⁷ reported that in seborrheic keratosis (acanthotic type), tumor cells throughout the entire tumor do not express keratin recognized by 34 β B4, and Nindl et al⁸ also showed partial lack of high MW cytokeratin (#1, #10) in all 10 cases of seborrheic keratosis.

To further investigate the cytokeratin expression in seborrheic keratosis, we have done immunohistochemical staining using a panel of specific anti-keratin antibodies in this study. We also observed the cytokeratin expression in the hair, sebaceous gland and sweat gland of the same epidermis.

MATERIALS AND METHODS

Biopsy material

Twenty cases of seborrheic keratosis were collected from the pathological files of the Department of Pathology, Hallym University Hospital. Hematoxylin and eosin stained sections of all lesions were reviewed and the diagnoses were confirmed. The histological types of 20 cases were acanthotic type (13 cases), hyperkeratotic type (5 cases), and pigmented type (2 cases). All tissues had been fixed in formalin and then paraffin-embedded according to the conventional procedure.

Immunoperoxidase staining

An immunohistochemical study was performed on 5 μ m sections from formalin-fixed, paraffin-em-

bedded biopsy specimens. After deparaffinization with xylene and rehydration of the tissues with ethanol, endogenous peroxidase activity was blocked with 3% hydrogen peroxide in absolute methanol for 5 minutes. Each section was mounted on gelatin-coated glass slides, and incubated with various anti-keratin antibodies. The sections were then immunostained using the avidin-biotin-peroxidase complex system, as previously described⁹. The peroxidase reaction was visualized with diaminobenzidine (DAB), and the slide was counterstained with hematoxylin and mounted. The immunohistochemical reagents and their sources and specificities are listed in Table 1.

Table 1. The sources of the monoclonal anti-keratin antibody used in this study

Anti-keratin antibody	Source
34 β B4(K1)	ENZO
AE1(K10,14,15)	Bio-Genex
CAM5.2(K 8,18)	Boeringer Mannheim
K13,16	ICN
K17	DAKO
K19	DAKO
K10	DAKO
K5,8	ICN

RESULTS

Cytokeratin expression in seborrheic keratosis lesions (Table 2)

On staining with 34 β B4 (K1), several staining patterns in suprabasal layers of the epidermis were observed in 10 out of 20 cases. There was homogeneous staining(Fig. 1) in 5 cases, peripheral staining(Fig. 2) in 2 cases, and heterogeneous staining, with immunoreactive cells scattered between non-immunoreactive cells in 3 cases. Using the AE1 (K10,14,15), we observed focal staining in 2 cases. In cases of K13,16 antibody staining, several staining

Fig. 1. Homogeneous staining of tumor cells with K1 antibody. ($\times 400$).

Fig. 2. Cells staining with K1 antibody are predominantly located on the periphery of tumor ($\times 40$).

Fig. 3. Homogeneous staining of tumor cells with K13, 16 antibody ($\times 400$).

Fig. 4. Positive staining of hair with K17 antibody ($\times 100$).

Fig. 5. Positive staining of eccrine sweat glands with K8, 18 antibody ($\times 400$).

Fig. 6. Positive staining of eccrine sweat glands with K19 antibody ($\times 400$).

Table 2. Cytokeratin expression in seborrheic keratosis lesions

Anti-keratin antibody	Histologic Type(20)			Hair (20)	Sebaceous gland(20)	Sweat gland(20)
	ACA(13)	HYP(5)	PIG(2)			
34 β B4(K1)	7	2	1	3	1	4
AE1(K10,14,15)	1	1				8
CAM5.2(K8,18)				2	1	14
K5,8	1					
K10	10	4		3		
K13,16	4	1		1	1	
K17				2	2	
K19						9

ACA : acanthotic type, HYP : hyperkeratotic type

PIG : pigmented type

() : number of subtotal or total cases

number : number of cases showing positive reactions

patterns (diffuse homogeneous staining and peripheral or focal staining)(Fig. 3) were observed in 5 cases. On staining with K10 antibody, we observed focal or irregular staining in 14 cases. Focal staining was also observed with K5,8 antibody in one case.

There was no positive immunoreactive cell on staining with CAM5.2 (K8,18), K17 and K19 antibodies. There was no specific cytokeratin expression pattern according to the type of seborrheic keratosis.

Cytokeratin expression in the hair, sebaceous glands and sweat glands (Table 2)

1) Immunoperoxidase staining of hair

On immunoperoxidase staining of hair, there were positive reactions with CAM5.2 (K8,18) in two cases. There were positive reactions with K13,16 antibody in one case, with 34 β B4 (K1) and K10 antibody in 3 cases, and with K17 antibody in 2 cases(Fig. 4).

2) Immunoperoxidase staining of sebaceous gland

On immunoperoxidase staining of sebaceous glands, there was one positive reaction with CAM5.2 (K8,18) in the suprabasal cells of sebaceous glands and with K13,16 antibody in sebaceous ducts. There were positive reactions with K17 antibody in the sebaceous duct in 2 cases, and with K1 antibody in one sebaceous gland of one specimen.

3) Immunoperoxidase staining of sweat gland

Using 34 β B4 (K1), 4 out of 20 cases showed

positive reactions in eccrine sweat glands. On staining with AE1 (K10,14,15), positive reactions were observed in 8 cases. Staining with CAM5.2 (K8,18) showed positive reactions in 14 cases(Fig. 5). There were positive reactions with K19 antibody in 9 cases(Fig. 6).

DISCUSSION

The cytokeratin pairs K5/K14 predominated in the basal cell layer, and K1/K10 in the suprabasal cell compartment in normal adult skin^{10,11}. Disorders with greatly increased epidermal turnover yield altered patterns of keratin expression, and K6/K16 replaces K1/K10 in the suprabasal compartment. Thus expression of the keratin K6/K16 is associated with a hyperproliferative epidermis¹²⁻¹⁵. The small cells seen in the acanthotic type of seborrheic keratosis are related to cells of the epidermal basal cells rather than basalioma cells of basal cell epithelioma¹⁶. Moll et al⁵ observed positive reactions to K1, K2, K5, K6, K10, K11, K14, and K15, but not to K16 and K17. Fujisawa et al⁷ reported that in seborrheic keratosis (acanthotic type), positively stained cells and negatively stained cells with keratin 1 (K1) recognized by monoclonal antibody 34 β B4 were mixed throughout the entire tumor and Nindl et al⁸ also showed partial lack of high molecular weight cytokeratin (#1, #10) in all 10 cases of seborrheic keratosis. In 20 cases of the present study, we observed positive reactions with 34 β B4 (K1) (10 cases), AE1 (K10,14,15) (2 cases), K10 (14

cases), K13,16 (5 cases), and K5,8 (1 case) antibodies. Our data was similar to that of previous authors^{7,8}, but different from Moll's report⁵ in terms of positive reactions with K13,16 antibodies in 5 out of 20 cases. Our data suggests that tumor cells of seborrheic keratosis are somewhat different from epidermal basal cells in terms of cytokeratin expression and show some proliferative activity. Nindl *et al*⁸ also suggested that the altered cytokeratin expression in seborrheic keratosis might be attributable to de-differentiation of tumor cells or potential re-differentiation towards embryonic keratinocytes.

On immunoperoxidase staining of hair, there were two cases of positive reactions with CAM5.2 (K8,18), one case of positive reactions with K13,16 antibody, and 3 cases of positive reactions with 34 β B4 and K10 antibody, and two cases of positive reactions with K17 antibody in outer root sheath of hair. Wilson *et al*¹⁷ studied the cytokeratin expression in psoriatic scalps and they reported that there were keratin 1, 5, 6, 10, 14, 16, 17, but not keratin 8 in normal epidermis. They suggested that keratin expression in scalp was generally unaffected by psoriasis, except for widespread expression of K8 and K17 in suprabasal interfollicular psoriatic scalp epidermis and K17 was induced suprabasally during epidermal hyperproliferation. Our data was similar to that of Wilson *et al*¹⁷, apart from data of less frequent positivity and negative staining for keratin 5, 10, 14. On immunoperoxidase staining of sebaceous glands, there was one positive reaction with CAM5.2 (K8,18) in the suprabasal cells of sebaceous glands and with K13,16 antibody in sebaceous ducts. There were positive reactions with K17 antibody in the sebaceous duct in 2 cases, and with 34 β B4 (K1) antibody in the sebaceous gland in one case. Wilson *et al*¹⁷ reported that there were keratin 1, 5, 6, 7, 10, 14, 16, 17, but not keratin 4, 8, 13, 18 and CAM5.2 in normal sebaceous glands. Our data was somewhat different to normal cytokeratin expression of Wilson *et al*'s¹⁷ report in terms of positive staining with CAM5.2 (K8,18) in the suprabasal cells of sebaceous glands.

On immunoperoxidase staining of sweat glands, there were positive reactions in 4 cases with 34 β B4 (K1), in 8 cases with AE1 (K10,14,15), in 14 cases with CAM5.2 (K8,18), and in 9 cases with K19 antibody. Cho *et al*¹⁸ also observed positive reactions to

34 β B4 in sweat glands. It is well-known that normal epidermis can be reacted with K1, K10, K14, K15 antibodies, but not with K8, K18, and K19 antibodies. These findings suggest that monoclonal antibody K19 could be a marker for eccrine sweat glands like CAM5.2 (K8,18).

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