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DF3 (CA15-3) Antibody as a Marker of Cutaneous Adnexal Tumors

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DF3(CA15-3) monoclonal antibody detects the 20 amino acid sequence of epithelial mucin. In normal cutaneous tissue DF3 antibody exhibits strong and constant positive reactivity with sebaceous and secretory segments of eccrine and apocrine sweat glands. All epidermal tumors are DF3 negative but poorly differentiated squamous cell carcinoma. Various adnexal tumors such as eccrine hidrocystoma, tubular apocrine adenoma, syringocystadenoma papilliferum, eccrine poroma, eccrine spiradenoma, clear cell hidradenoma, mixed cell tumor, eccrine porocarcinoma, extramammary Paget's disease and adenoid cystic carcinoma are DF3 positive, which indicates epithelial mucin production by these tumors. ***Key words: Epithelial mucin; Sweat gland tumors; Secretory segment.***

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DF3(CA15-3) is developed as a marker of breast cancer (1) and its distribution has been investigated immunohistochemically. In normal tissue CA15-3 distributes to secretory mammary epithelial cells, gastrointestinal gland cells, bile duct, pancreas duct (2) and nasal mucosa (unpublished data). In fetal tissues CA15-3 distributes not only to gland cells but also to gastrointestinal epithelial cells, liver and pancreas (2). Distribution to various tumors other than breast cancer is also reported (3, 4).

CA15-3 antigen is detected by two monoclonal antibodies, DF3 and 115D8 antibody; both antibodies detect the same antigen. Epitope of DF3 antibody is the 20 amino acid sequence which exists in the epithelial mucin core protein (5). Thus, DF3 antibody staining is supposed to reflect the activity of epithelial mucin production or an embryonic feature of tumor cells.

In our study of DF3(CA15-3) distribution in normal skin, we found sebaceous glands and secretory segments of eccrine or apocrine sweat glands being strongly positive. Ductal segment of sweat glands was occasionally weakly positive. Epidermis, hair follicles and other cutaneous components were all negative (6).

In the presented investigation the use of DF3 antibody as a marker of adnexal tumor differentiation was evaluated.

MATERIALS AND METHODS

Skin tumor tissues were obtained from patients who visited Teikyo University Mizonokuchi Hospital between 1988 and 1992. Some specimens were obtained from patients of Kantochuoh Hospital or Toranomon Hospital. Tumors included in this study are as follows (number of cases in parenthesis): seborrheic keratosis (2), actinic keratosis (2), basal cell epithelioma (4), squamous cell carcinoma (5), nevus sebaceous (2), syringoma (3), eccrine hidrocystoma (2), tubular apocrine adenoma (1), syringocystadenoma papilliferum (2), eccrine poroma (3), eccrine spiradenoma (1), clear cell hidroadenoma (1), mixed tumor (3), eccrine porocarcinoma (2), extramammary Paget's disease (3), adenoid cystic carcinoma (1).

All specimens were fixed in buffered formalin, embedded in paraffin, and sectioned at 4 μm thickness on a microtome. Sections were deparaffinized and dehydrated through xylene and graded series of alcohol. Endogenous-peroxidase activity was inhibited by the treatment of 10% H_2O_2 before immunoperoxidase staining. After incubation of 10% normal pig serum to block non-specific staining, sections were incubated with the first antibody, DF3 (Tore-Fuji Bionichs, Tokyo, Japan), diluted 1:100, or CEA as an antibody reacting with the ductal part of the sweat apparatus (Kyowa Medics, Tokyo, Japan), diluted 1:100 for 12 h at 4°C. They were washed three times in phosphate buffer solution (PBS), pH 7.4, for 10 min, and were then incubated with the second antibody, horseradish peroxidase-conjugated goat anti-mouse immunoglobulin (TAGO, Burlingam, CA, USA) for 1 h at 37°C at a dilution of 1:100. The sections were washed three times in PBS and incubated with 10 mg 3-3' diaminobenzidin in 16 ml PBS mixed with 2 ml 3% H_2O_2. For negative control the first antibody was omitted.

RESULTS

Results are summarized in Table I. Most epidermal tumors and keratinizing squamous cell carcinoma are DF3 and CEA negative, but poorly differentiated squamous cell carcinoma is positive.

In nevus sebaceous, all lobes are stained strongly by DF3 (Fig. 1) but not by CEA. Some CEA negative sweat gland tumors are DF3 positive. For example, cyst wall cells of eccrine hidrocystoma are strongly DF3 positive (Fig. 2) but CEA negative. DF3 also stains lumina margins of other benign sweat gland tumors but its distribution in each tumor is different from CEA. Syringocystadenoma papilliferum is stained by DF3; lu-

Table I. *Summary of DF3 and CEA staining of various skin tumors*

+: Weakly positive or limited area of specimen is positive, ++: Strongly positive, +−++: Staining strength varies among cases, −−+: Weakly positive in one of the cases, −: Negative.

	DF3	CEA
Seborrheic keratosis	−	−
Basal cell epithelioma	−	−
Squamous cell carcinoma	−−+	−−+
Nevus sebaceous	++	−
Syringoma	−	++
Eccrine hidrocystoma	++	−
Tubular apocrine adenoma	+	++
Syringocystadenoma papilliferum	++	+
Eccrine poroma	+−++	+
Eccrine spiradenoma	+	−
Clear cell hidradenoma	++	−
Mixed tumor	++	−−+
Eccrine porocarcinoma	+	−
Extramammary paget's disease	++	++
Adenoid cystic carcinoma	++	−

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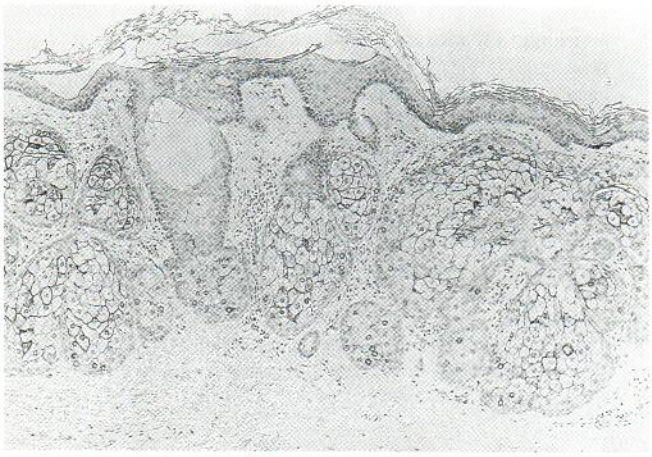

Fig. 1. Nevus sebaceous stained by DF3.

mina margins and excreted material are positive, especially in the central or deep area of the tumor. CEA only stains the upper portion of invagination. DF3 stains not only lumina margins but also cell membranes of the solid part of eccrine poroma and clear cell hidradenoma; this staining pattern is different from CEA. Malignant adnexal tumors, shown in Table I, are strongly stained by DF3 in cytoplasm (Fig. 3). Among these, adenoid cystic carcinoma is not stained by CEA.

DISCUSSION

Several antibodies have been studied as the markers of skin appendages and they have different distributions (7–9).

One of the important observations is the reverse result of DF3 staining between syringoma and eccrine hidrocystoma. By the observation of electron microscopy (EM), syringoma lumina are lined by ductal cells (10). DF3 does not react with the cell, indicating the absence of epithelial mucin. Similar to syringoma, Spelling & Sakas (11) found no Golgi apparatus in eccrine hidrocystoma. On the contrary, Ebner & Erlach (12) described Golgi apparatus and numerous vacuoles in the lumen cell layer of this tumor, so DF3 positive reactivity may reflect the epithelial mucin secretion by the tumor cells.

In clear cell hidradenoma and eccrine poroma, rough-surfaced endoplasmic reticulum and Golgi complexes are observed by EM, which suggests the mucin production potential (13, 14). Positive DF3 staining coincides with this observation and loss of lumen polarity in these tumors suggests the embryonic character of these tumor cells. However, in contrast to malignant tumors, these tumors exhibit a tendency of stronger staining in cell membrane than in cytoplasm.

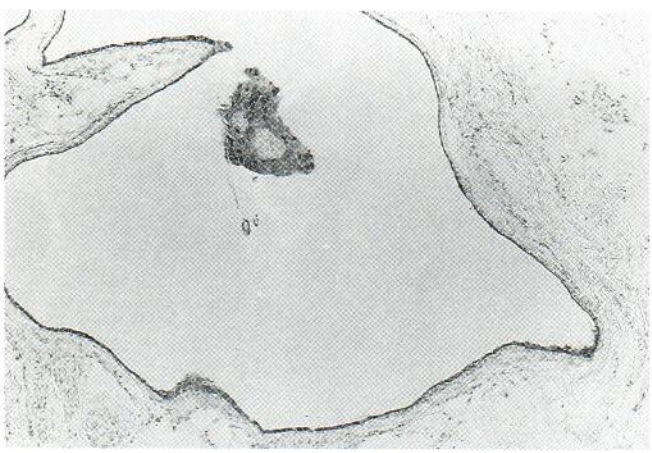

Fig. 2. Eccrine hidrocystoma stained by DF3.

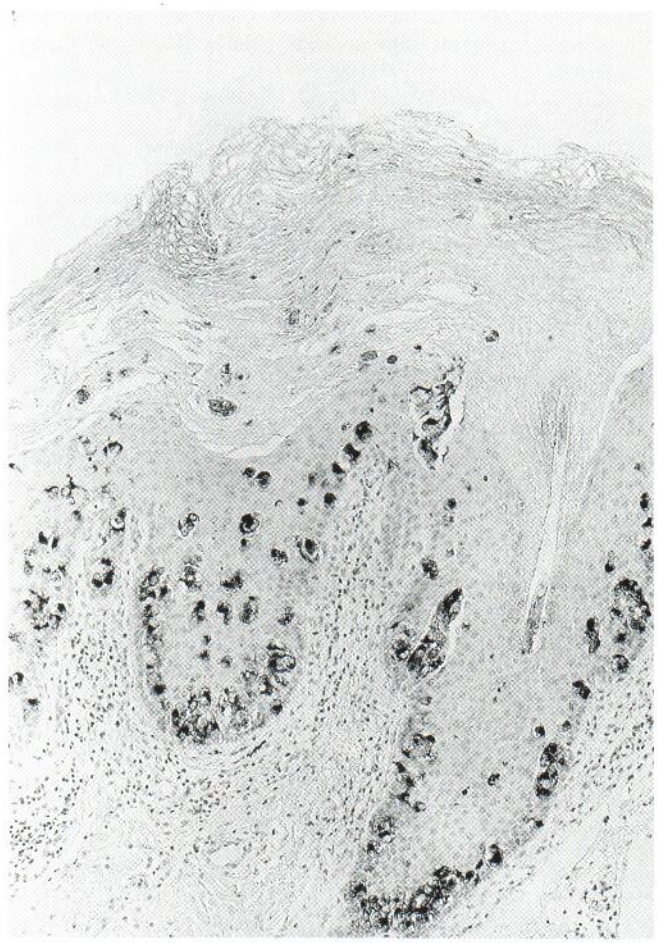

Fig. 3. Extrammary Paget's disease is DF3 positive.

In the observation of mammary tissue, DF3 antigen localizes along the outer surface of apical cell membrane in the benign tissue but DF3 accumulates in rough endoplasmic reticulum, Golgi apparatus and cytosole etc in malignant tissue (15). A similar loss of polarity is observed in extramammary Paget's disease and eccrine porocarcinoma.

Our results show the usefulness of DF3 antibody in revealing the adnexal lineage of cutaneous tumors. DF3 distribution is different from CEA. Some CEA negative adnexal tumors are DF3 positive and in the tumors that are both CEA and DF3 positive, the distribution is different. In conclusion, DF3 should be utilized in combination with CEA to disclose the nature of cutaneous adnexal tumors.

REFERENCES

1. Kufe D, Inghirami G, Abe M, Hayes D, Justi-Wheeler H, Schlom J. Differential reactivity of a novel monoclonal antibody (DF3) with human malignant versus benign breast tumors. Hybridoma 1984; 3: 223–232.
2. Matsuura N, Higashiyama S, Kadota T, et al. Evaluation of CA15-3

as a tumor marker of digestive cancer. Proceedings of 7th meeting of tumor marker investigation society. Tumor Marker Investigation Society. Tokyo, 1987: 98–100.
3. Kusaka K, Asano M, Moro I, et al. Tumor markers in human renal cell carcinoma. Tumor Biol 1991; 12: 189–197.
4. Lertprasertsuke N, Tsurumi, Y. Alpha-fetoprotein-producing urachal adenocarcinoma. Acta Pathol Jpn 1991; 41: 318–326.
5. Abe M, Kufe D. Structural analysis of the DF3 human breast carcinoma-associated protein. Cancer Res 1989; 49: 2834–2839.
6. Ando I, Yasui S, Kukita A, Hino H. CA15-3(DF3) antibody reacts to normal sweat and sebaceous glands. JEADV, in press.
7. Nakama T. Carcinoembryonic antigen in cutaneous tumors. Jpn J Dermatol 1983; 93: 1271–1279.
8. Inaba Y, Egawa K, Kageshita T, Tomomichi O. Expression of CA50 in normal human skin: comparative study of CEA and CA19-9. J Dermatol (Tokyo) 1992; 19: 592–597.
9. Sweat apparatus tumors. In: Mehregan AH, Hashimoto K, eds. Pinkus' guide to dermatohistopathology. 5th edn. A publishing division of Prentice hall. Appleton & Lange, 1991; 563–582.
10. Hashimoto K, Gross BG, Lever WF. Syringoma, histochemical and electron microscopic studies. J Invest Dermatol 1966; 46: 150–166.
11. Spelling LC, Sakas EL. Eccrine hidrocystomas. J Am Acad Dermatol 1982; 7: 763–770.
12. Ebner VH, Erlach E. Ekkrine Hidrozystome. Dermatol Monatsschr 1975; 161: 739–744.
13. Hashimoto K, Lever WF. Eccrine poroma, histochemical and electron microscopic studies. J Invest Dermatol 1964; 43: 237–247.
14. Hashimoto K, Dibella RJ, Lever WF. Clear cell hidradenoma. Histological, histochemical, and electron microscopic studies. Arch Dermatol 1967; 96: 18–38.
15. Ohtani H, Nagura H. Immunoelectron microscopic localization of DF3 cancer-associated antigen in human breast cancer. Pathol Res Pract 1991; 187: 1014–1019.