Immunohistochemical Study of Cytokeratin Related Protein Expression in Patients with Trichilemmal Tumors and Squamous Cell Carcinoma of the Skin

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SUMMARY
Trichilemmal tumors can be either benign and malignant skin tumors arising from the outer root sheath of the hair follicle. It remains unknown whether malignant trichilemmal tumors can be differentiated from squamous cell carcinoma (SCC). In order to differentiate malignant trichilemmal tumors from SCC, we studied cytokeratin-related protein and involucrin expression immunohistochemically. Twenty-three trichilemmal carcinomas (TLC), 4 malignant proliferating trichilemmal tumors (MPTT) and 25 SCCs were studied. All the skin biopsy specimens were subjected to staining with anti-cytokeratin and anti-involucrin antibodies immunohistochemically, and the expression of cytokeratins and involucrin were investigated. The occurrence rate of cytokeratin 1 positive staining in TLC was significantly less than that in SCC (P < 0.05), while that of cytokeratin 19 positivity in SCC was significantly less than that in TLC (P < 0.01). These results demonstrate that immunohistochemical staining with anti-cytokeratin 1 and anti-cytokeratin 19 antibodies is useful for the differential diagnosis of TLC and SCC.

Key Words: trichilemmal carcinoma, squamous cell carcinoma, cytokeratin, involucrin, immunohistochemistry

INTRODUCTION
Trichilemmoma is a benign skin tumor arising from outer root sheath of the hair follicle as first described by Headington et al. in 1962. Then in 1976, he described trichilemmal carcinoma (TLC) as a malignant variant arising from outer root sheath. Much later he published the criteria for the diagnosis of TLC showing hair follicle differentiation by electron microscopic and immunohistochemical observations, but the details of these findings were not shown. He also described malignant proliferating trichilemmal tumor (MPTT) as another malignant variant of proliferating trichilemmal tumor. Reports of...
layer or the periphery of the tumor nest to clear and swollen cells and keratinized tissue. The structure in TLC is thought to reflect the differentiation of epidermal cells to that of outer root sheaths. Expression of keratin and involucrin protein is known to change along the differentiation of epithelial cells. We examined the expression of cytokeratin (CK) and involucrin protein to see if the differentiation into outer root sheath were involved in TLC.

MATERIALS AND METHODS

Twenty-three patients with trichilemmal carcinoma (TLC) (male:female = 16:7, mean age 74.1 years, age range 29-103 years), 4 patients with malignant proliferating trichilemmal tumor (MPTT) (male:female = 3:1, mean age 64.8 years, age range 17-85 years) and 25 patients with squamous cell carcinoma (SCC) (male:female = 13:12, mean age 84.1 years, age range 37-95 years) admitted to our hospital from 1987 to 2001 were studied. Histological diagnosis of TLC was based on the following features. The tumor was histologically invasive and consisted of atypical clear cells resembling those of the outer root sheath, which were in solid, lobular, or trabecular grown patterns with foci of pilar-type keratinization and peripheral palisading with subnuclear vac-
ulization. The tumor cell nuclei were hyperchromatic, pleomorphic, and very large. The cytoplasm contained glycogen, which is PAS-positive and diastase-sensitive. Areas of trichilemmal keratinization were frequently present \(^5\). (Figure 3). MPTT was defined as a tumor generally resembling TLC but specifically showing multiple pseudohornysts, trichilemmal keratinization and mild atypia \(^11\). Tumors with dyskeratosis, atypia, mitosis, cancer pearl and invasion into the deep dermis were defined as SCC. At the periphery, tumor cells of SCC did not exhibit palisading and their basement membrane were not distinct. Keratinization was of epidermal rather than trichilemmal type (Figure 4). While in TLC, at the periphery, tumor cells had a basophilic appearance and exhibited palisading. Lobules of tumor cells were bordered by a distinct basement membrane. Clear vacuolated cells showed trichilemmal keratinization (Figure 3). Grading of SCC was tabulated as follows: In grade 1, less than 75% of the cells were differentiated; in grade 2, less than 50%; in grade 3, less than 25% 12. Normal skins adjacent to the benign tumor biopsy specimens were used as a normal control. Skin biopsy specimens of 8 trichilemmomas male: female = 5:3, mean age 61.1 years, age range 28-75 years), and 11 trichilemmal cysts (male: female = 6:5, mean age 54.0 years, age range (A) 20-72 years) were also studied.

Biopsy specimens were fixed in 10% formaldehyde, and embedded in paraffin. The tissue blocks were cut into sections of 3 μm-thick sections which were then deparaffinized, rehydrated, rinsed in tris-buffered saline (TBS), and stained immunohistochemically. Anti-cytokeratin (CK) 1 antibody (34 β B4, Medac, Wedel, Germany), anti-CK 19 antibody (Ks19.1, Progen, Heidelberg, Germany), anti-CK 10, 14 ~ 16, 19 antibody (AE1, Chemicon, Temecula, USA) and anti-involucrin antibody (Castra, Newcastle, UK) were used for immunohistochemically (Table 1). Involucrin is known as a marker for keratinocyte terminal differentiation 13. These antigens were stained by LSAB (labeled streptavidin-biotin, DAKO, Carpinteria, USA) method. Sections for CK 1, CK 19 and CK 10, 14 ~ 16, 19 staining were pretreated with 0.01 mol/L citrate buffer at 95°C for 40 min, and then incubated for 20 minutes at room temperature. Slides were incubated 5 minutes in a solution of 3% hydrogen peroxide to consume endogenous peroxidase activity. Anti-CK 1 antibody and anti-CK 19 antibody were applied for 90 min at 40 times dilution in phosphate-buffered saline (PBS). Anti-CK 10, 14 ~ 16, 19 antibody was applied for 60 min at 500 times dilution with PBS. Sections for involucrin staining were pretreated with 0.01 mol/L trypsin 250 (Beckton Dickinson, New York, USA) in TBS buffer at 37°C for 30 min. Anti-involucrin antibody was applied for 120 min at 100 times dilution in PBS. The sections were washed three times in PBS and secondary antibodies (biotinylated goat antimouse immunoglobulin G and goat anti-rabbit immunoglobulin G) were applied at 37°C for 15 min. After three more washes in PBS, color was developed with dianobenzidine tetrahydrochloride (DAB) as a substrate. After light counterstaining with Mayer’s hematoxylin, the sections were cover-slipped with Acrytol.

Difference in occurrence rate was examined with the chi-square analysis.

**RESULTS**

Cytokeratin (CK) 1, CK 19, CK 10, 14 ~ 16, 19 and involucrin staining in normal controls, trichilemmal tumors and squamous cell carcinoma

In normal skin tissue, suprabasal cells in epidermis and follicular infundibulum were positive for CK 1 (Figure 5). In 5 of 8 trichilemmomas, clear cells stained positive for CK 1. In 9 of 11 trichilemmal cysts, stainings for CK 1 was negative and in10 of 23 trichilemmal carcinomas (TLCs) and 3 of 4 malignant proliferating trichilemmal tumors (MPTTs), cytokeratin 1 staining was positive (Figure 6). In 19 of 25 squamous cell carcinomas (SCCs), atypical cells around cancer pearls were positive for CK 1 (Figure 7). In normal skin tissue, CK 19 was positive for acinus of sweat glands and outer root sheath, but not for epidermis. In 7 of 8 trichilemmomas (A) and 10 of 11 trichilemmal cysts, CK 19 was negative. In 13 of 23 TLCs and 2 of 4 MPTTs, tumor cells were positive for anti-cytokeratin19 antibody (Figure 8) as were positive in 4 of 25 SCCs (Figure 9). Of the 10 cases of TLC not

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<tr>
<th>Antibody</th>
<th>Specificity</th>
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<th>Concentration</th>
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<tbody>
<tr>
<td>34 β B4</td>
<td>CK1</td>
<td>Medac</td>
<td>1 : 40</td>
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<tr>
<td>Ks19.1</td>
<td>CK19</td>
<td>Progen</td>
<td>1 : 40</td>
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<tr>
<td>AE1</td>
<td>CK10, 14 to 16, 19</td>
<td>Chemicon</td>
<td>1 : 500</td>
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<td>Involucrin</td>
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<td>Castra</td>
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\( \text{CK} = \text{cytokeratin} \)
stained with CK 19, 2 cases were positive for CK 1. In normal skin tissue, basal cells, the outer root sheath and sweat glands were positive for anti-cytokeratin 10, 14 ~ 16,19 antibodies. In all the trichilemmomas, trichilemmal cysts, TLCs, MPTTs, and SCCs, tumor cells stained positively for involucrin. In 4 of 11 trichilemmal cysts, and in all trichilemmomas, TLCs, MPTTs and SCCs, clear cells were positive for involucrin (Figure 10).

Rate of positive staining for CK 1, CK 19, CK 10, 14 ~ 16, 19 and involucrin in normal controls, trichilemmal tumors and squamous cell carcinomas

The rates of positivity for CK 1 in TLC and trichilemmal cyst were significantly lower than that in SCC (P < 0.05 and P < 0.01, respectively). When comparisons were made among the remaining lesions, no significant differences were found in the rate of occurrence of positivity for CK 1. Positivity for CK 19 was significantly lower in SCC, trichilemmoma and trichilemmal cyst than that in TLC (P < 0.01, P < 0.05 and P < 0.05, respectively). When comparisons were made among the remaining lesions, no significant differences were found in the rate of occurrence of positivity for CK 19. CK 10, 14 ~ 16, 19 were positive in all subjects. The rates of involucrin-positive staining in trichilemmal cysts were significantly lower than those of trichilemmoma, SCC and TLC (P < 0.05, P < 0.01 and P < 0.01, respectively). When comparisons were made among the remaining lesions, no other significant differences were found for the distribution of involucrin-positive staining (Table 2).
DISCUSSION

Trichilemmal carcinoma (TLC) was first described as the malignant counterpart of trichilemmoma arising from outer root sheath of the hair follicle by Headington in 1976. Later he published a criteria consisting of 6 items for its diagnosis. There were 1) continuity with a co-existing benign epithelial tumor (trichilemmoma); 2) continuity with the outer sheath epithelium of a co-existing hair follicle; 3) light microscopic evidence suggestive of outer sheath architecture, e.g., glycogen-rich epithelium, peripheral cell palisading, and prominent basement membrane zone; 4) focal epithelial differentiation (keratinization) in a trichilemmal mode, e.g., absent or minimal granular layer, abrupt single cell keratinization, and formation of dense non-lamellar keratin; 5) electron microscopic cellular detail similar to that found in either normal sheath epithelium or trichilemmomas; and 6) immunocytochemical similarities to normal outer sheath epithelium or to trichilemmomas e.g., positive for monoclonal antibodies to hair-associated keratins or other antigens of the outer sheath. The first and second items were not considered to be mandatory, because many malignant skin tumors do not always arise from benign tumors and the differentiation of tumors is more significant than the origin. The significance of the fifth item remains unknown. Accordingly, the third, fourth and sixth items were considered to be essential. The expression of keratin protein has been known to change according to the epithelial cells differentiation. In our study, we noted that cytokeratin (CK) 19 was stained on the outermost layer of cells in outer root sheath, not on the epidermis, while CK 1 was stained on the epidermis and suprabasal cells in follicular infundibulum for the differentiation of malignant trichilemmal tumors and squamous cell carcinoma (SCC). In the previous studies, the
tumors have been reported to be TLC that had histology continuity with the epidermis and/or hair follicles, clear atypical tumor cells containing glycogen, trichilemmal keratinization and palisading arrangement at the periphery of tumors. In tumors diagnosed as TLC according to these findings, it remains unknown whether these findings reflect the differentiation of cells to that of outer root sheaths. In addition, tumors composed of clear cells with trichilemmal keratinization and containing glycogen can be observed in SCC and Bowen's disease.

Two studies have been reported regarding immunohistochemistry of trichilemmal tumors. A study reported 7 TLCs and showed that cytokeratin AE 1–AE 3 was positively stained, but not EMA nor CEA. Another study reported that 2 out of 8 TLCs were positively stained with EMA and cytokeratin (Dako CK 1)10, and the results were inconsistent with each other. We speculated that the result showing that TLC was not stained with CK 1 in the present study did not represent a contradiction, because in the normal epidermis, both the epidermis and suprabasal cells in the follicular infundibulum were stained.

In the present study, in normal skin tissue, suprabasal cells in the epidermis were positive for CK 1 and negative for CK 19. In the outer root sheath of hair follicles, positivity was noted for CK 19 but not for CK 1. The histological feature of TLC reflects that of the outer root sheath. The result of the present study showed that TLC exhibits significantly higher CK 19 expression than SCC, which seems to reflect differentiation to the outer root sheath. In contrast, SCC demonstrated significantly higher CK1 expression than TLC, presumably reflecting characteristics of squamous cells in the epidermis. In 13 of 23 (57%) cases of TLC, there was differentiation in the outer root sheath. Of the 10 (43%) cases of TLC without differentiation in the outer root sheath, 2 (20%) were positive for cytokeratin 1, a result which may indicate that the tumors were SCC.

In conclusion, many cases of TLC could be differentiated from SCC with the immunohistochemical method. Cytokeratin 1 and cytokeratin 19 staining is useful in the differential diagnosis of TLC and SCC.

REFERENCES


