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Original Article

Immunohistochemical evaluation of CD10, podoplanin (D2-40), and Ki-67 expression in basaloid epidermal proliferation of nevus sebaceous with comparative analysis of basal cell carcinoma and trichoepithelioma

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Abstract: This study was designed to evaluate whether CD10, podoplanin (D2-40), and Ki-67 could reliably differentiate trichoepithelioma (TE) from basal cell carcinoma (BCC) and whether these proteins could be used as markers of basaloid epidermal proliferation (BEP) of nevus sebaceous (NS). The clinico-pathological characteristics of 11 TE, 19 BCC, and 23 NS cases with BEP were analyzed. CD10, podoplanin, and Ki-67 expression was evaluated by immunohistochemistry. Eight out of 11 (72.7%) TE cases expressed CD10. In 7 cases, CD10 was expressed only in the stroma, while stromal expression of CD10 was detected in 1 (5.3%) case of BCC. Twelve (63.2%) BCC cases only expressed CD10 in tumor cells. Seven out of 11 (63.6%) TE cases presented D2-40 staining. The staining was diffused in 4 cases (36.4%) and focally positive in 3 cases (27.3%). In BCC, only 6 of 19 (31.6%) cases stained for D2-40 and most cases were focally positive (26.3%). Mean Ki-67 labeling index of TE cases was 15.5, while that of BCC cases was 36.6. Cases presenting NS with BEP were less positive for CD10 (34.8%) and D2-40 (26.1%) compared to TE and BCC cases. Six cases presenting NS with BEP (26.1%) demonstrated CD10 positivity only in the stroma, and two cases (8.7%) presented CD10 positivity only in tumor cells. Mean Ki-67 labeling index of NS with BEP was 10.9. In conclusion, immunohistochemistry analysis demonstrated that CD10 and Ki-67 are reliable markers to differentiate TE from BCC. Additionally, NS presented a less aggressive biologic behavior in the BEP areas.

Keywords: Basal cell carcinoma, CD10, D2-40, Ki-67, nevus sebaceous, trichoepithelioma

Introduction

Nevus sebaceous (NS), first described in 1895 by Jadassohn, is a benign hamartoma with epidermal, follicular, and apocrine elements [1]. Clinically, it usually presents at birth as solitary yellowish, waxy hairless plaques, or linear lesions, and as verrucous nodules in adults [2]. Various appendageal tumors develop from NS in the adult stage. The most commonly associated appendageal tumors are trichoblastoma and syringocystadenoma papilliferum, but many tumors, including trichoepithelioma (TE) and basaloid epidermal proliferation (BEP) are associated with NS. Approximately 6.5-14% of cases with NS present BEP, but its malignancy potential and characteristic correlation with basal cell carcinoma (BCC) are controversial [2, 3]. BCC is the most common malignant neoplasm that arises from NS [4]. In most cases, distinguishing TE from BCC is not difficult. However, when the biopsy specimen is insufficient or both tumors are merely composed of nests of basaloid cells with follicular differentiation, their differentiation can become exceedingly difficult.

Differentiating TE and BCC is important, as there is significant differences in prognosis and treatment are exist. TE is a benign tumor and requires only conservative treatment including shaving or simple excision. However, BCC is a malignant tumor with a tendency for recurrence and locally destructive growth pattern, which requires complete excision [5]. Due to their conflicting prognosis, large number of immunohistochemical studies was conducted.

CD10 is a cell-surface zinc metalloproteinase of 100 KDa that is expressed on the cell surface of acute lymphoblastic leukemia and in many other types of neoplasms [6]. A few studies have demonstrated its usefulness in differentiating BCC from TE [5, 7-9].

D2-40 antibody is directed against podoplanin, a lymphovascular marker that also reacts in the basal layer of the outer root sheath of hair follicles, peripheral germinative cells of sebaceous glands, and some cutaneous neoplasms [10]. Plaza *et al*. [11] investigated its reactivity in TE and BCC and concluded that D2-40 could be utilized to differentiate TE from BCC. However, additional studies are required to confirm its usefulness.

Ki-67 is a nuclear proliferation marker expressed during the active phases of the cell cycle, including G1, M, G2, and S phases [12]. Measuring Ki-67 reactivity allows us to predict the tumor proliferative potential.

In this study, immunohistochemical studies were performed to determine the usefulness of CD10, D2-40, and Ki-67 in differentiating TE and BCC, which occur in NS, but are somewhat difficult to differentiate. Additionally, we determined whether CD10, D2-40, and Ki-67 could be used as markers of basaloid epidermal proliferation (BEP) of nevus sebaceous (NS).

Materials and methods

Specimens

Specimens from 53 cases were used in this study, including 11 cases with TE, 19 cases with BCC, and 23 cases with NS. Specimens were retrieved from the archives of Daegu Catholic University Medical Center histopathology and Yeungnam University Medical center histopathology laboratories. Medical information, including patient's age, sex, and site of lesion were recorded.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue blocks were cut into 4-μm thick sections and mounted onto poly-L-lysine-coated slides. All specimens were incubated with antibodies to CD10 (1:100 dilution, SIGNET, Covance, San Diego, CA, USA), podoplanin (D2-40; 1:50 dilution, Biocare, Concord, CA, USA), and Ki-67 (1:200 dilution, Cell Marque, Rocklin, CA, USA) and incubated for 60 minutes at room temperature. Positive staining was visualized using DAKO EnVision Plus-HRP detection kit (DAKO, Glostrup, Denmark).

Evaluation of immunoreactivity

Two dermatopathologists (J. Park and JB. Park) and one dermatologist (Y. Kim), blinded to the clinical information, analyzed the pattern of CD10 and D2-40 staining as well as the Ki-67 labeling index with consensus. For each case stained with the markers, 5 fields were evaluated at high magnification (×400). Positive immunoreactivity is defined as the percentage of positive cells being over 10%. Cases positive for CD10 were further classified by location as tumor cell only, stroma only, and both. Cases positive for D2-40 were further classified as focal positive (positive cells corresponding to less than 50% of the tumor) and diffuse positive (positive cells corresponding to more than 50% of the tumor). The fraction of Ki-67-positive cells is referred to as the Ki-67 labeling index (LI).

Statistical analysis

The data were collected and statistically analyzed. CD10 and D2-40 immunostaining were compared between cases presenting TE, BCC, and NS with BEP using a two sample t-test. Ki-67 LI was analyzed using the Chi-square test, employing the Statistical Package for the Social Sciences (SPSS ver 19.0, Chicago, IL, USA). A $P<0.05$ was considered statistically significant.

Results

Clinical data

This study included 11 cases of TE, 19 cases of BCC, and 23 cases of NS with BEP. The patients with TE included 5 males (45.5%) and 6 females (54.5%). The mean age was 50.4 years. TE was observed on the following sites: nose (3), scalp (2), forehead (2), cheek (2), and nasolabial fold (2). Patients with BCC included 6 males (31.6%) and 13 females (68.4%). The mean age was 74.1 years. BCC was observed on the following sites: cheek (9), nose (8), forehead (1), and nasolabial fold (1). Patients with

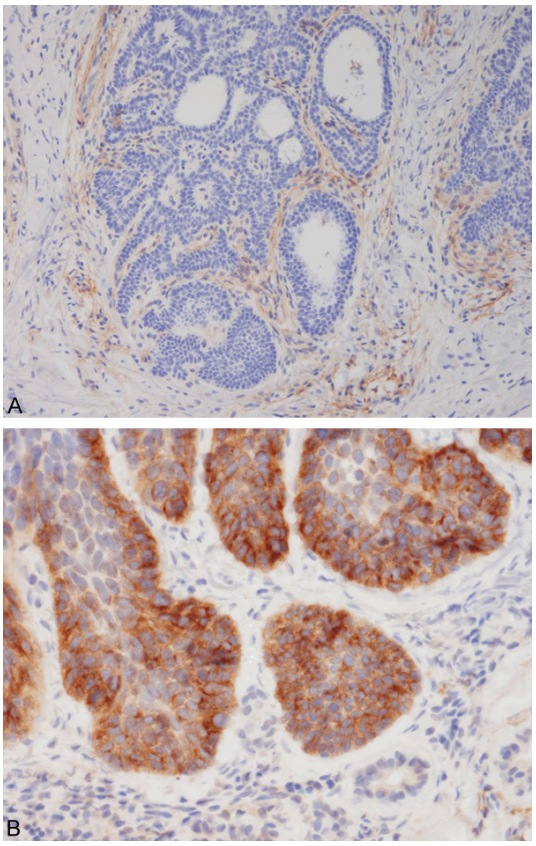

Figure 1. A. CD10 expression in stromal cells in TE specimens. No staining is observed in tumor cells (CD10, ×200). B. Diffuse expression of CD10 in tumor cells and negative staining in stromal cells in BCC specimens (CD10, ×400).

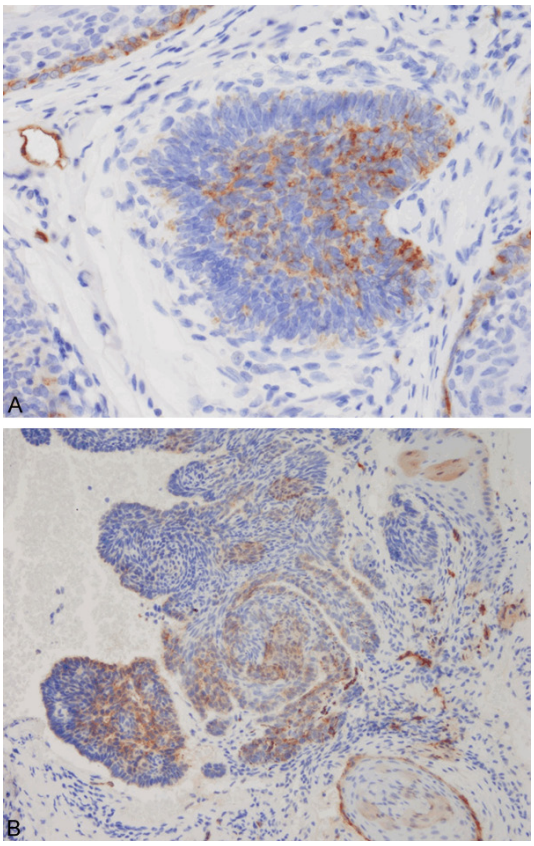

Figure 2. D2-40 staining of TE and BCC specimens. Diffuse staining in TE specimens (A) and focal staining in BCC specimens (B) are detected, but no statistical difference was observed (P=0.07) (A: D2-40, ×400, B: D2-40, ×200).

NS included 15 males (65.2%) and 8 females (34.8%). The mean age was 40.0 years. NS was observed on the following sites: scalp (19), forehead (3), and nose (1).

Immunohistochemical findings

The percentage of CD10 expression in TE and BCC specimens was 72.7% and 73.7% respectively. Seven TE cases (63.6%) demonstrated strong CD10 staining in the stroma surrounding nests without staining of basaloid cells (**Figure 1A**). In BCC cases, only 1 case (5.3%) showed CD10 reactivity in the stroma, but 12 cases (63.2%) showed CD10 reactivity in basaloid cells (**Figure 1B**). Reactivity to D2-40 was higher in TE cases (63.6%) than in BCC cases (31.6%). Four TE cases (36.4%) were diffusely positive and 3 cases (27.3%) were focally positive (**Figure 2A**), while 1 BCC case (5.26%) was diffusely positive and 5 cases were focally positive (**Figure 2B**). The difference in pattern of D2-40 staining between TE and BCC was not statistically significant (P=0.07).

Ki-67 LI in TE and BCC specimens was 15.5 and 36.6, respectively (**Figure 3**). Ki-67 positive cells were located mainly in the peripheral layer of tumor islands in TE specimens, but, in BCC, Ki-67 was more intensely reactive throughout the tumor islands. Overall, CD10 staining pattern and Ki-67 LI were different between TE and BCC (P=0.001 and P<0.001 respectively) (**Table 1**).

The percentage of CD10 expression in NS with BEP specimens was much lower (34.8%) than that observed in TE and BCC specimens. Six cases (26.1%) presenting NS with BEP showed CD10 stromal reactivity to CD10 and 2 cases (8.7%) showed tumor cell reactivity, which resembled to the CD10 staining pattern

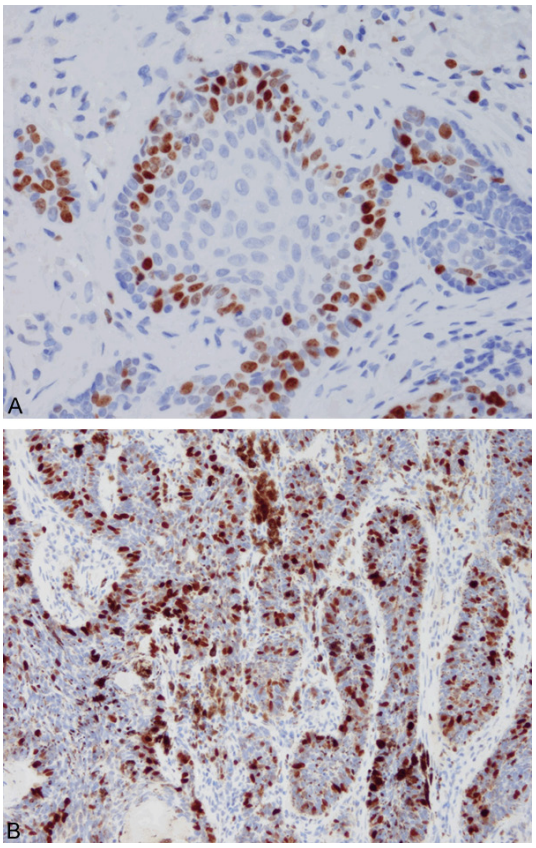

Figure 3. A. Focal Ki-67 expression in tumor islands of TE specimens. Note that Ki-67 is reactive along the periphery of tumor islands (Ki-67, ×400). B. Strong, diffuse Ki-67 expression in both central and peripheral regions of tumor islands in BCC specimens (Ki-67, ×200).

observed in TE specimens (**Figure 4A**). In contrast, CD10 reactivity pattern was much different from that observed in BCC specimens (P=0.01). Overall D2-40 reactivity in NS with BEP specimens was lower (26.1%) than that observed in TE and BCC specimens. Five cases (21.7%) presented focal reactivity and 1 case (4.35%) showed diffuse reactivity to D2-40. These results were statistically different from those observed in TE specimens (P=0.03) (**Figure 4B**; **Table 2**). Ki-67 LI in NS with BEP specimens was 10.9, which was statistically lower than that in BCC specimens (P<0.001) (**Figure 4C**; **Table 3**).

Discussion

Before evaluating the biologic behavior of NS with BEP, we determined whether TE and BCC could be differentiated immunohistochemically

Table 1. TE and BCC immunohistochemical comparison

Marker	Group		*P*-value
	TE (n=11)	BCC (n=19)	
CD10, n (%)			
Stroma	7 (63.6)	1 (5.3)	0.001
Tumor	0 (0)	12 (63.2)	
S+T	1 (9.09)	1 (5.26)	
Positive, total	8 (72.7)	14 (73.7)	
D2-40, n (%)			
Diffuse	4 (36.4)	1 (5.26)	0.07
Focal	3 (27.3)	5 (26.3)	
Positive, total	7 (63.6)	6 (31.6)	
Ki-67, mean (SD)			
Labeling index	15.5 (14.8)	36.6 (7.7)	0.000

using antibodies against CD10, podoplanin (D2-40), and Ki-67. Each of these tumors presents characteristic histopathologic features. Histologic features that favor a TE diagnosis include lobular arrangement of neoplastic islands with association with papillary mesenchymal bodies, well-circumscribed, symmetrical lesion, highly fibrotic stroma, and clefts within the stroma and between the stroma [11, 13]. BCC exhibits peripheral layers of columnar cells with palisaded nuclei, necrosis, clefting between tumor islands and the surrounding mucin-rich stroma [13]. However, these tumors share common histologic features, including dermal infiltration of neoplastic islands composed of basaloid cells. Furthermore, in recent years, increasing usage of small shave biopsy for cosmetic reasons makes the differentiation of these tumors difficult. Previous studies used diverse immunohistochemical markers, including Bcl-2, Ki-67, CD10, PCNA, P53, CD34, CK20, and D2-40 in attempts to differentiate TE from BCC [11, 13-17]. Our results indicate that CD10 and Ki-67 are useful markers to differentiate TE from BCC. The CD10 staining patterns of these tumors differed. TE specimens presented with stromal CD10 staining, while BCC specimens presented with tumor cell CD10 staining. These results are consistent with previous studies [5, 7-9, 18]. The difference in CD10 expression pattern between TE and BCC may reflect their difference in stromal morphology and disparity in tumor growth regulation or host response of these tumors [5]. Further evaluation is required to identify the exact biological differences.

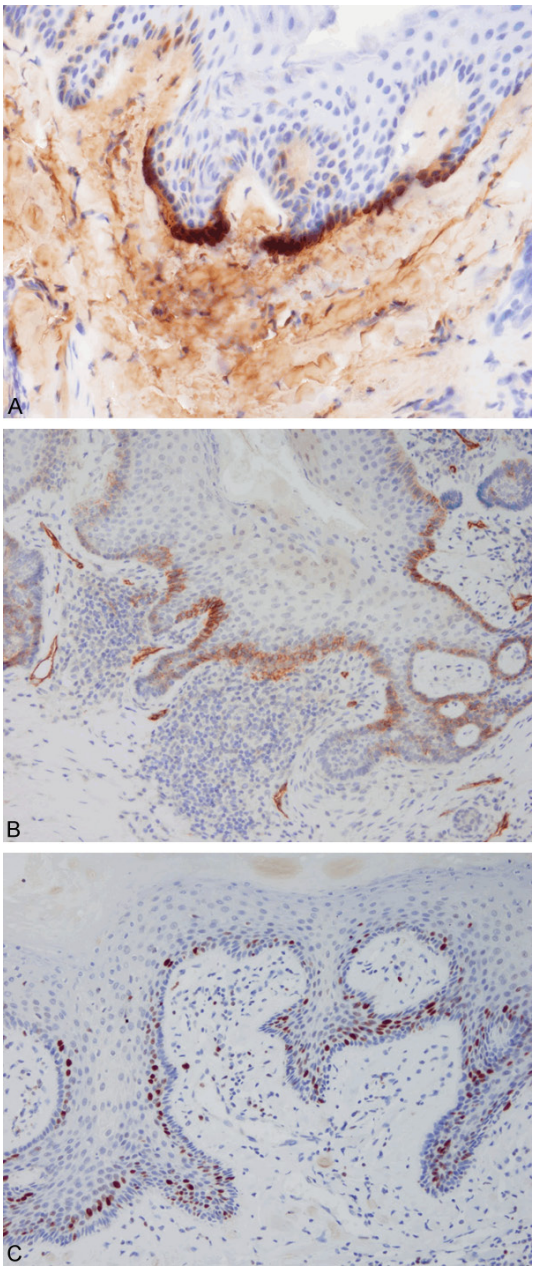

Figure 4. A. Expression of CD10 by stromal cells underneath BEP in NS specimens (CD10, ×400). B. D2-40 expression along the periphery of NS with BEP specimens (D2-40, ×200). C. Focal Ki-67 expression along the periphery of NS with BEP specimens (Ki-67, ×200).

The exact function of Ki-67 antigen is unknown, but it can be used as a marker to predict proliferative activity [19]. In our study, Ki-67 LI of TE specimens was significantly lower than that of BCC specimens and the reactive pattern was different; Positive cells were located mainly in the peripheral layer of tumor islands in TE specimens, while, in BCC specimens, Ki-67 was more intensely reactive throughout the tumor islands. The difference in the level of Ki-67 index and the pattern of reactivity indicates that TE has a much lower proliferative capability, which mainly localizes in the peripheral layer of tumor islands.

Table 2. TE and NS with BEP immunohistochemical comparison

Marker	Group		*P*-value
	TE (n=11)	NS with BEP (n=23)	
CD10, n (%)			
Stroma	7 (63.6)	6 (26.1)	0.05
Tumor	0 (0)	2 (8.7)	
S+T	1 (9.09)	0 (0)	
Positive, total	8 (72.7)	8 (34.8)	
D2-40, n (%)			
Diffuse	4 (36.4)	1 (4.35)	0.03
Focal	3 (27.3)	5 (21.7)	
Positive, total	7 (63.6)	6 (26.1)	
Ki-67, mean (SD)			
Labeling index	15.5 (14.8)	10.9 (6.4)	0.213

Table 3. BCC and NS with BEP immunohistochemical comparison

Marker	Group		*P*-value
	BCC (n=19)	NS with BEP (n=23)	
CD10, n (%)			
Stroma	1 (5.3)	6 (26.1)	0.01
Tumor	12 (63.2)	2 (8.7)	
S+T	1 (5.26)	0 (0)	
Positive, total	14 (73.7)	8 (34.8)	
D2-40, n (%)			
Diffuse	1 (5.26)	1 (4.35)	0.926
Focal	5 (26.3)	5 (21.7)	
Positive, total	6 (31.6)	6 (26.1)	
Ki-67, mean (SD)			
Labeling index	36.6 (7.7)	10.9 (6.4)	0.000

D2-40 is a lymphovascular marker that also reacts with some of cutaneous neoplasms. Therefore, few studies suggest D2-40 as a reliable marker for differentiation. Plaza *et al.* [11] investigated 49 cases of TE and BCC and suggested that TE and BCC can be differentiated by D2-40 reactivity. In their study, TE showed high reactivity (95.5%) with diffusely positive

staining localized to the basal cell layer of the tumor nests, while BCC showed low reactivity (22.2%) with focally positive staining at the periphery of the tumor cell aggregates. However, our results differ in term of TE and BCC reactivity and reactive patterns. TE reactivity was 63.6%, which is much lower than that observed in the previous study. Moreover, the pattern of D2-40 staining in TE and BCC specimen did not allow the reliable distinction between TE and BCC.

The incidence of BEP arising in NS varies from 6.5 to 14%, but the pathogenesis is not clearly understood [3]. BEP represents benign adnexal proliferation with follicular differentiation and, in most cases, presents a benign biologic behavior [2]. The malignant potential of BEP is controversial [20, 21]. In our study, immunostaining of NS with BEP specimens was compared to those of TE and BCC specimens. The staining pattern and reactivity of CD10 and Ki-67 in NS with BEP showed no significant difference when compared to those in TE. A similar CD10 reactive pattern, stromal staining, and Ki-67 LI were observed in both tumor types. Considering that, histologically, both BEP and TE are follicular differentiated neoplasms, the similarity between TE and BEP is thought to be important. BEP presents a benign biologic behavior like TE. In contrast, NS with BEP showed clear differences when compared to BCC. Both the CD10 staining pattern and Ki-67 LI were significantly different from those of BCC. Considering that elevated proliferative rates could increase the chance of genetic error, leading to the development of malignant transformation, the lower Ki-67 LI in NS with BEP specimens than in BCC indicates its benign biologic behavior [3]. D2-40 staining pattern of NS with BEP specimens was similar to the pattern of BCC, but the reliability remains questionable since D2-40 showed no statistical significance in differentiating TE from BCC in this study (P=0.07).

In conclusion, there were significant differences in CD10 and Ki-67 expression patterns between TE and BCC specimens. Therefore, this study supports the use of CD10 and Ki-67 LI to differentiate TE from BCC when histologic findings are inconclusive. Furthermore, considering the CD10 staining pattern and Ki-67 LI, NS with BEP presents biologic characteristics similar to those of TE, which presents limited proliferative capability with a benign biologic behavior. The D2-40 staining pattern did not reliably demonstrate any similarities between NS with BEP and TE or BCC. Additional quantitative studies are necessary to confirm our conclusions.

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Disclosure of conflict of interest

None.

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