Value of CD34 and CD10 Immunostaining in the Differentiation between Basal Cell Carcinoma and Trichoepithelioma

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Abstract

Purpose: Trichoepithelioma (TE) is a benign skin tumor with follicular differentiation, which is sometimes difficult to distinguish clinically and histologically from basal cell carcinoma (BCC). Both are characterized morphologically by the proliferation of basaloid cells; however, BCCs are clinically associated with a more aggressive behavior. It is important to distinguish these neoplasms for effective and appropriate therapeutic planning. The present study aims at investigating the role of CD10 & CD34 in differentiation between TE and BCC.

Patients and Methods: This study includes 30 cases of BCC and 10 cases of TE. Cases were collected from Pathology Department of Faculty of Medicine-Benha University and Egyptian National Cancer Institute (NCI) in the period 2008-2013. CD34 and CD10 immunohistochemistry was performed in all cases and the patterns of expression were analyzed.

Results: The pattern of CD34 expression in BCC compared to that of TE was statistically significant ($p<0.05$). Trichoepithelioma exhibited stromal expression in the immediate stromal cells adjacent to the tumor islands while in BCC, the surrounding stroma was immunoreactive to CD34 with a clear zone of negatively stained cells separating epithelial islands from the positive mesenchymal stromal cells. The pattern of CD10 expression in BCC and TE was statistically highly significant ($p<0.01$). Seventy percent of TE cases exhibited pure stromal cells staining while 70% of BCC cases showed staining of basaloid cells.

Conclusion: This study suggests CD10 and CD34 as useful markers for the differential diagnosis between TE and BCC with preference to CD10.

Key Words: CD34 – CD10 – Basal cell carcinoma (BCC) – Trichoepithelioma (TE) – Immunohistochemistry (IHC).

Introduction

WORLDWIDE Basal cell carcinoma (BCC) is the most common malignant cutaneous tumor, accounting for approximately 80% of non-melanoma skin cancers. BCC is of low grade malignancy, locally aggressive, and metastasis is very unusual [1,2].

In Egypt, the NCI registry data of years 2000 to 2011 revealed that BCC constitutes 43% of nonmelanoma skin cancers [3].

Trichoepithelioma (TE) is a benign tumor derived from basal cells in the hair follicle. It may be sporadic or as a part of a common genetic disorder called multiple familial trichoepithelioma characterized by the presence of many small tumors predominantly on face, inherited in an autosomal dominant pattern [4,5].

Trichoepithelioma in some instances may resemble BCC, so the differentiation of BCC from TE can be problematic based on clinical presentation and routine hematoxylin and eosin stained sections. Attempts have been made to identify immunohistochemical markers helpful in differentiating them [4,5]. The correct diagnosis of these tumors is important because the tumors are treated differently; basal cell carcinoma is a locally aggressive neoplasm and must be totally excised with safe margins. However, trichoepithelioma is a benign neoplasm, which may be partially excised by shaving [6,7].

CD34 is a 110-kDa transmembrane glycoprotein present on leukemic cells, endothelial cells, and stem cells [3]. In addition, it is localized on cells of the splenic marginal zone, dendritic interstitial cells around vessels, nerves, hair follicles, muscle bundles, and sweat glands in a variety of tissues and organs [7]. Its function is still unclear. It is used for leukemia diagnosis and subclassification and for diagnosis of vascular tumors. Antibodies
to CD34 also strongly label gastrointestinal stromal tumors, and the antigen is invariably found in solitary fibrous tumor and dermatofibrosarcoma protuberans [8].

CD10 is a cell-surface zinc metalloproteinase of 100 KD that is also known as common acute lymphoblastic leukemia antigen (CALLA). It was originally found to be expressed on the cell surface of most cases of acute lymphoblastic leukemia, and was soon found in many other types of neoplasms [9]. CD10 expression has been shown in tumors of follicular differentiation, including trichoepithelioma, pilomatrixoma, basaloïd follicular hamartoma and BCC [10]. A few studies have indicated its expression in BCC and TE because of the limited number of available studies [11].

The purpose of this study is to investigate the usefulness of CD 10 and CD34 in distinguishing BCC and TE.

Material and Methods

The retrospective studied group included random 30 cases of BCC and 10 cases of TE, collected from histopathologic archive of files of Pathology Department of Faculty of Medicine-Benha University and Egyptian National Cancer Institute (NCI) in the period 2008-2013. Paraffin-embedded tissue sections were obtained from archival tissue blocks of the hospital. Hematoxylin and eosin sections were reviewed to confirm diagnosis.

**Immunohistochemical staining:** Paraffin-embedded tissue sections, 3-4 micron thick were mounted on positively-charged slides and heated at 60°C for 30 minutes then deparaffinized and rehydrated through a series of xylene and alcohol before staining. After antigen retrieval with microwave treatment in 10mM citrate buffer (Neo-Markers, Cat. # AP-9003), pH 6.0, endogenous peroxidase was blocked with 3% hydrogen peroxide for 20 minutes. Sections were washed 3 times with cold 0.01 M phosphate buffered saline (PBS). After blocking with 10% normal rabbit serum, sections were incubated with mouse monoclonal antibody against CD34 (Lab Vision, Thermo scientific, USA, Cat. # MS-363-R7, ready to use), mouse monoclonal CD 10 (Lab Vision, Thermo scientific, USA, Cat. # MS-728-R7, ready to use). Slides were incubated for 90 minutes with each antibody at room temperature. The freshly prepared DAB-substrate-chromogen solution was applied and incubated for 5-15 minutes until color intensity has been reached. Lastly, sections were counterstained with Mayer’s hematoxylin.

For CD 10, normal intestinal biopsy was used as positive control. CD10 stained the cytoplasm of the surface epithelial cells of small intestine. Sections of the tonsil for CD34 were used as a positive control 15. Negative controls were performed by omitting the primary antibody step.

**Interpretation of immunohistochemical staining of CD34:**

All specimens were examined under a light microscope. Positive reactions for CD34 appeared as a brownish cytoplasmic sometimes nuclear, granular staining of the tumor cells [18]. The amount of immunopositive tumor cells and stromal cells were evaluated by using a scale of [0] to [2+] as follows: [0], negative (<10% positive cells); [1+], regionally positive (10-50% positive cells); [2+], diffusely positive (>50% positive cells) [2].

**Interpretation of immunohistochemical staining of CD10:**

Positive CD 10 staining was identified as brown cytoplasmic staining with or without cell membrane staining. For each case, 10 fields were examined at high magnification (x400), and the percent of positive cells was calculated as follow: 0-10% was judged as negative, 10-50% was judged as low expression, and >50% was judged as high expression. Localization of anti-CD10 to the stroma and/or tumor cells was determined in cases with immunoreactivity [1,11].

**Statistical analysis:**

Statistical analysis was performed using the SPSS (version 16.0 for windows) software package according to Sperman’s correlation coefficient. Correlation between several variables was computed using Fisher’s exact test. p-value less than 0.05 (<0.05) was considered significant and <0.01 was highly significant.

**Results**

**A- Immunohistochemical results of CD34 staining:**

Among the 30 cases of BCC, 20 cases (66.7%) were positive to CD34 expression in stromal cells. In 16 cases (53.3%) of them, the immediate tumor stroma did not stain for CD34. A clear demarcation was found between the mesenchymal component intermixed with the epithelial tumor islands being
negative for CD34 and those stromal cells peripheral to the tumor (surrounding stroma) being CD34-positive (Fig. 2). The other 4 cases (13.3%) stained both immediate and surrounding stroma. Among the 20 positive cases, 17 cases showed focally positive CD34 expression and the other 3 cases were diffusely positive to it. The remaining 10 cases (33.3%) were negative for CD34 immunostaining.

This study included 10 cases of TE, 8 of them (80%) were diffusely positive for CD34 expression in the form of stromal expression in the immediate stromal cells adjacent to the tumor islands. There was no buffer zone of negatively stained cells separating epithelial islands from the positive mesenchymal stromal cells (Fig. 2). Two cases (20%) were non-immunoreactive for CD34.

This pattern of CD34 expression in BCC and TE was statistically significant ($p<0.05$), the pattern of CD34 expression in stromal cells was useful for differentiating BCC from TE (Table 1).

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>Total</th>
<th>CD34 Pattern of expression</th>
<th>$p$-value</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Negative Immediate stroma Surrounding stroma Both immediate and surrounding stroma</td>
<td></td>
</tr>
<tr>
<td>BCC</td>
<td>30</td>
<td>10 (33.3%) 0 16 (53.3%) 4 (13.3%)</td>
<td>$p&lt;0.05$</td>
</tr>
<tr>
<td>TE</td>
<td>10</td>
<td>2 (10%) 8 (80%) 0 0</td>
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BCC: Basal cell carcinoma.  
TE: Trichoepithelioma.  
N.B: The pattern of CD34 expression in BCC and TE was statistically significant ($p<0.05$).

B- Immunohistochemical results of CD10 staining:

Among the 30 cases of BCC, 29 cases (96.7%) were positive to CD 10 expression. Of these positive cases, there were 21 cases (70%) showed staining of basaloid cells (Fig. 3), of them 15 cases with high CD 10 expression and 6 cases with low CD 10 expression. Four cases (13.3%) showed staining of stromal cells and 4 cases (13.3%) showed staining of both basaloid and stromal cells with high expression of CD10 (Fig. 4). Only one case (3.3%) was negative for CD10 immunostaining.

The 10 cases of TE, 9 of them (90%) were highly positive for CD10 expression. Seven cases (70%) exhibited pure stromal cells staining (Fig. 3), 2 cases exhibited staining of both stromal and basaloid cells, while no case (0%) showed staining of basaloid cells alone. One case (10%) was non-immunoreactive for CD10 (Table 2).

This pattern of CD10 expression in BCC and TE was statistically highly significant ($p<0.01$), CD10 expression in stromal cells around basaloid nests was useful for differentiating TE from BCC. In contrast, CD10-positive basaloid cells and negative stromal cells were diagnostic for BCC (Table 2).

<table>
<thead>
<tr>
<th>Type of lesion</th>
<th>Total</th>
<th>CD10 Pattern of expression</th>
<th>$p$-value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Negative Basaloid cells Stromal cells Both basaloid and stromal cells</td>
<td></td>
</tr>
<tr>
<td>BCC</td>
<td>30</td>
<td>1 (3.3%) 21 (70%) 4 (13.3%) 4 (13.3%)</td>
<td>$p&lt;0.01$</td>
</tr>
<tr>
<td>TE</td>
<td>10</td>
<td>1 (10%) 0 7 (70%) 2 (20%)</td>
<td></td>
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</tbody>
</table>

N.B: The pattern of CD10 expression in BCC and TE was statistically highly significant ($p<0.01$).
Fig. (1): A- A case of TE revealed well–circumscribed lesion composed of basaloid cells in small or large nodules within a variably cellular stroma; the basaloid cells are encircled by fibroblasts (H&E. x400). B- A case of BCC revealed multiple tumor islands occupying the upper dermis of different sizes & shapes composed of basaloid cells with peripheral palisading of the outer row of the basaloid cells in the tumor islands which are separated from the stroma by a cleft (H&E. x400).

Fig. (2): A- CD34 expression in immediate stroma of TE ( ). B- CD34 expression in surrounding stroma of BCC ( ) (IHC, DAB x400).

Fig. (3): A- CD10 expression in stroma of TE ( ). B- CD10 expression in basaloid cells ( ) (IHC, DAB x400).

Fig. (4): CD10 expression in both basaloid cells ( ) and stromal cells ( ) in BCC ( IHC, DAB x400).
Discussion

Trichoepithelioma is a benign skin tumor with follicular differentiation, whose distinction from basal cell carcinoma is sometimes difficult, clinically and histologically. Both tumors are composed of nests of basaloid cells with follicular differentiation. Sometimes it may be impossible to make a histopathologic differentiation on the basis of routine hematoxylin and eosin staining. Such distinction is clinically important because of the differences in prognosis and treatment of these tumors. Therefore, several laboratory techniques have been investigated as an aid in this differentiation. In these instances, immunohistochemical examinations may provide further information [4].

In the current study, 66.7% of BCC cases were positive to CD34 expression in stromal cells. In 53.3% of cases, there was CD34 expressions in the surrounding stroma of BCC nests with the adjacent zones (the immediate tumor stroma) which were not stained with CD34 and 13.3% of cases stained both immediate and surrounding stroma. As regard TE, 80% were diffusely positive for CD34 expression in the form of stromal expression in the immediate mesenchymal stromal cells adjacent to the tumor island (Fig. 2). This pattern of CD34 expression in BCC and TE was statistically significant (p<0.05).

These results were in agreement with results reported by Zaky & Abdel-Rahman, [8] who found that in BCC cases, the immediate tumor stroma together with the spindle cells intermixing the tumor nests were CD34 negative, but in TE cases the immediate tumor stroma as well as the spindle cells surrounding the tumor islands were focally CD34 positive. Sengul et al., [2] reported a significant difference in CD34 expression between the two groups included in their study; stromal expression of CD34 for benign tumors of cutaneous appendages originating from hair follicles was observed just at the adjacent to the tumor islands; it was not like that for BCC. There was CD34 expressions in the surrounding stromas of BCCs with the adjacent zones which were not stained with CD34.

Another study by Basarab, et al., [12] found that CD34 staining of the immediate peritumoral spindle-shaped cells was observed in only 20% of TE compared with 7% of BCC. Kirchmann, et al., [13] reported that the spindle-shaped cells surrounding the islands of trichoepithelioma cells were focally strongly positive for CD34. In all basal cell carcinomas, the spindle-shaped cells surrounding the nests of tumor cells were negative. However, these studies concluded CD34 can be reliably used to differentiate between TE and BCC.

CD 10 immune staining was performed on 10 cases of trichoepithelioma and 30 cases of basal cell carcinoma. Of these, 90% of trichoepithelioma and 96.7% of basal cell carcinoma biopsies were immunoreactive for CD10. The most frequently labeled were the tumor stroma, with positive readings in 70% of trichoepithelioma and 13.3% of basal cell carcinoma biopsies (Fig. 3). CD 10 staining was present in both the basaloid cells and the stroma in 20% of trichoepithelioma and 13.3% of basal cell carcinoma biopsies. CD 10 staining of only the basaloid cells was observed in 70% of basal cell carcinoma biopsies but not in any case of trichoepithelioma. The pattern of staining of basaloid cells and stromal cells in BCC and trichoepithelioma was highly statistically different (p<0.001).

The current results were in agreement to results reported by Sengul et al., [14] and Naglaa & Naglaa, [15] who found that the pattern of staining with CD 10 in TE is stromal, whereas, in BCC lesions, it is cytoplasmic in epithelial cells, without expression in the surrounding stroma. This staining is more remarkable in the outermost basaloid cells. Also, Pham et al., [16] found that (92%) TE showed positive stromal immunoreactivity, and (8%) showed positivity of the basaloid cells. No TE demonstrated epithelial expression alone. On the other hand, expression of CD 10 by basaloid cells was identified in (87%) cases of BCC. Stromal positivity was also identified in three cases of BCC. Condensation of CD10-positive stromal cells around basaloid nests was statistically significant in differentiating TE from BCC (p<0.0001). Conversely, CD 10-positive basaloid cells were seen predominantly in BCC (p<0.0001).

Similarly, Heidarpour et al., [11] reported that in BCC cases, the expression of CD10 was noted in tumoral cells in (83.2%). Of these, 3 cases showed positivity of the stromal and basaloid cells, two cases demonstrated stromal expression alone and two BCCs were not immunoreactive. On the other hand, (83.3%) TEs showed positive stromal immunoreactivity. Of these, one case also showed positivity of the basaloid cells. One TE demonstrated epithelial expression alone and one TE was not immunoreactive. The pattern of staining of basaloid cells and stromal cells in BCC and trichoepithelioma was statistically different (p<0.001). Also, Aslani et al., [17] found that Comparison of CD10 expression between the BCC and TE groups dem-
onstrated a significant difference in both the tumor and stromal cells (p<0.001).

Kirchmann et al., [18] speculated that benign TE had a stroma which components were closely resembled the mesenchymal component of stroma surrounding hair follicles in normal skin. On the contrary, BCC were surrounded by a stroma containing cells not normally found in the dermis. In the stroma surrounding BCC, there is a deposition of mucinous material and often cleft formation. Stromal differences, fibronectin, mucin production of glucoseaminoglycan, and mesenchymal bodies can cause that different staining pattern between BCC and TE [8,14].

Tebcherani et al., [7] reported that despite the use of panels of immune markers, histopathological criteria associated with clinical data certainly remain the best guideline for the differential diagnosis of trichoepithelioma and basal cell carcinoma. However this study can suggest CD10 and CD34 as useful markers that aid in the differential diagnosis between TE and BCC with preference to CD 10. The limited number of the cases in this work was the handicap. So, larger series, and different research techniques, are necessary for more accurate diagnoses.

References


