ABSTRACT

Background: CD10 is a zinc-dependent metalloprotease known as common acute lymphoblastic leukemia antigen (CALLA). Although CD10 expression has been investigated in some cutaneous tumors, to our knowledge, data regarding its expression in cutaneous epithelial neoplasms are very limited. We aimed to determine the immunohistochemical expression of CD10 in basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) and to associate it with the available clinicopathological parameters in both tumors.

Patients and Methods: This study included 16 SCC and 21 BCC cases (17 solid type, 2 morphea type and 2 adenoid basal types). BCC cases were divided into 12 cases with microscopic infiltrative base and 9 cases with well-circumscribed base. The localization of anti-CD10 to the tumor and/or stromal cells was determined in each case.

Results: Positive CD10 staining was identified as brown cytoplasmic, with or without cell membrane staining. In all the 16 SCC cases, tumor cells failed to stain with CD10 in contrast to the stromal cells that showed CD10 expression in 13 cases (81%). In BCC cases, the expression of CD10 was noted in tumor cells in 10 cases (47.6%) and in stromal cells of 20 cases (95.24%). Most of CD10+ (7/10) BCC showed well-circumscribed deep margin, however, most of CD10- cases (9/11) showed infiltrating base (p=0.030). BCCs with infiltrating deep margins (12 cases) tended to show CD10 negative basaloid cells (9/12) and CD10 positive stromal cells (12/12) (p=0.0003).

Conclusion: From our results we suggest that CD10 might be a useful immunohistochemical marker to differentiate between BCC and SCC. At least, if tumor cells were CD10 positive, this would favor BCC over SCC. Absence of CD10 in all the SCC and in infiltrating BCC together with its overexpression in the surrounding stromal cells might confer invasive properties to such tumors. However, its relation to other poor prognostic factors needs larger studies to be confirmed.

Key Words: CD10 – Immunostaining – Skin.

INTRODUCTION

Approximately 80 percent of nonmelanoma skin cancers are basal-cell carcinomas (BCC), and 20 percent are squamous-cell carcinomas (SCC) [1]. According to Cancer Pathology Registry 2003-2004, in Egypt, BCC and SCC constitute 45.5% and 37% of malignant skin tumors respectively [2]. BCC is one of the most common cutaneous cancers worldwide and is of low-grade malignancy. It is locally aggressive and metastasis is very unusual [3]. The term "basal cell carcinoma" is derived from the cytological similarity between the tumor cells and the normal basal cells of the epidermis and BCC was believed traditionally to arise from the basal cells. However, the suggestion that BCC might represent a primitive "adnexal" carcinoma or trichogenic carcinoma has been made over the years. BCC is clearly different biologically from SCC, which is derived from epidermal keratinocytes [4].

CD10 is a zinc-dependent metalloprotease known as common acute lymphoblastic leukemia antigen (CALLA). CD10 is a useful marker in classification and diagnosis of leukemia/lymphoma [5]. CD10 has been reported in both epithelial (bladder, hepatocellular, renal cell carcinomas) [6,7,8] and mesenchymal neoplasms (endometrial stromal sarcoma of the uterus and stromal cells of ovarian epithelial neoplasms [9,10]. In cutaneous tumors, CD10 has been expressed with some frequency in xanthomatous neoplasms of the skin [11]. Baharami et al.,

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suggested that CD10 has a potential role in differentiating cutaneous metastatic renal cell carcinoma from adenexal neoplasm with eccrine and apocrine differentiation but not sebaceous differentiation [12]. CD10 expression had been suggested to be helpful in distinguishing between atypical fibroxanthomas (strong diffuse expression in 94% of cases) and SCC (weak and patchy expression in 50% of cases) [13]. Also, Kanitakis et al., suggested that the expression of CD10 in cutaneous mesenchymal tumors including dermatofibromas, dermatofibrosarcoma protuberans and neurofibromas might be a marker for tumor progression [14]. Bilalovic et al., found that expression of CD10 was significantly higher in metastatic than in primary melanomas and the more advanced primary tumors had higher CD10 expression in the tumor cells [15].

**Aim of the work:**

The aim of this study was to determine the immunohistochemical expression of CD10 in BCC and SCC and to associate it with the available clinicopathological parameters in both tumors.

**MATERIAL AND METHODS**

This study included 21 cases of BCC and 16 SCC. Excision biopsies with safety margins were obtained from all patients after signing an informed consent. We include all patients presented to Dermatology and Andrology outpatient clinic of Faculty of Medicine, Menofiya University during the time period from February 2006 to May 2007.

Paraffin-embedded blocks were prepared at the Pathology Department, Faculty of Medicine, Menofiya University. From each representative block, three contiguous 4-µm-thick sections were cut and mounted on glass slides, one for routine hematoxylin and eosin (Hx & E) staining and two on poly-L-lysine – pre-coated slides for immunostaining (one as a test slide and the other as a negative control).

**Clinical examination:**

Patients’ data including age at time of diagnosis, sex, size of the tumor, site and lymph node status were recorded for basal and squamous cell carcinoma.

**Histopathological examination:**

Histological examination of Hx & E-stained sections was performed to confirm the clinical diagnosis of the cases and verify the histological types and grades. BCC types were diagnosed according to the criteria detailed by Kirkham, 2005 [4]. Grading of SCC was performed in the most aggressive area (medium magnification field). Cases were divided into well differentiated, moderately differentiated or poorly differentiated.

**Immunohistochemical staining:**

After de-paraffinization and re-hydration, sections were incubated in hydrogen peroxide (3% H₂O₂ in absolute methyl alcohol) for 10-15 minutes in humidity chamber. For antigen retrieval: Heat induced epitope retrieval (HIER) procedure was used (New Marker immune histopathology catalogue, 2000). Ultra V Block was applied for 5 minutes. Sections were incubated overnight with monoclonal primary antibody raised against CD10 (diluted at a 1:50), Zymed, Cat. No.18-2287 (Clone 56C6). After washing with PBS, biotinylated secondary anti-immunoglobulin (LSAB 2 system-HRP, Dako-cytomation (Copenhagen) was applied 40 min at room temperature. The specimens were washed in phosphate buffered saline (PBS) and incubated with streptavidin peroxidase for 10 minutes. While the slides were in PBS, the diaminobenzidine (DAB) chromogen substrate was prepared and then, applied on slides for 3 minutes. Finally, the specimens were counterstained with Mayer’s hematoxylin.

Normal intestinal biopsy was used as positive control. CD10 stained the cytoplasm of the surface epithelial cells of small intestine. Negative control was performed by omitting the primary antibody step.

**CD10 immunostaining interpretation:**

Positive CD10 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 percentage of positive cells was calculated as follow: 0-10% was judged as negative, 10-50% as low expression and >50% as high expression [16]. The localization of anti-CD10 to the stromal and/or tumor cells was determined in cases with immunoreactivity.
Statistical analysis:

The data were collected, tabulated and statistically analyzed, using a personal computer with "Statistical Package for the Social Sciences (SPSS), version 11". Fisher exact test was used for comparison between qualitative variables. Mann-Whitney U test was used to compare between quantitative variables. Differences were considered statistically significant when (p≤0.05) and highly significant when (p≤0.01).

RESULTS

Clinicopathological data:

This study included 16 cases of SCC, their ages ranged from 12-84 years, with median 46 and mean ± SD (40.8±19.8). Most of the SCC cases were males (75%). The size of the lesions was available in 11 cases and ranged from 1-21cm, mean ± SD (8.3±7.1). Half of the lesions aroused on chronic ulcers (morjolein ulcers). Histopathologic examination revealed 3 cases with poorly differentiated SCC, 9 cases with moderately differentiated and well differentiated SCC in 4 cases. Dissection of palpable lymph nodes was performed in 6 cases, four of them were involved by metastatic deposits (N1), however only two were free of involvement (N0).

Our study also included 21 cases with BCC. Their ages ranged from 16-77 years, with median 57 and mean ± SD (56.7±12.5). Twelve cases (57%) were females and 9 were males (43%). All the lesions were diagnosed in face and scalp. The size of the lesions was available in 17 cases and ranged from 0.4-5cm, mean ± SD (1.6±1.1). Five cases (24%) presented with chronic ulcers and the rest (76%) showed solitary lesions. Histopathologic examination revealed that 17 cases (81%) showed solid type. The other 4 cases were divided into 2 morphea type and 2 adenoid basal types. According to the criteria described by Kirkham, (2005), cases were divided into that showing microscopic infiltrative base (12 cases) and those, showing well-circumscribed base (9 cases).

Comparing both tumor groups revealed that our SCC cases showed more aggressive clinical features than BCC cases as the age in SCC was significantly less than that of BCC cases and the tumor size was significantly larger in the SCC cases (Table 1).

Immunohistochemical staining:

CD10 expression was detected focally in the basal layer of the normal skin, in folliculo-sebaceous structure staining the inner root sheath, hair matrix and perifollicular fibrous sheath. Sebaceous lobules showed focal cytoplasmic positivity. Eccrine glands were negatively stained. Inflammatory cells showed strong cytoplasmic positivity.

Comparing SCC and BCC groups with regard to CD10 expression, there was a significant difference between CD10 expression in tumor cells (p=0.002) but not in stromal cells (p=0.296) in both groups (Table 1).

In all the SCC cases (16), CD10 was negative in almost all tumor cells whereas, it was positive in stromal cells (fibroblastic and inflammatory cells) in 13 cases (81%), with expression more than 50% in 11 of them (Fig. 1). CD10 was positive in the stroma of 3 out of 4 cases of well differentiated SCC (75%) and 7 cases out of 9 of moderately differentiated tumors (78%) and in all the 3 cases of poorly differentiated tumors G3 (100%). Four cases (30.7%) were associated with lymph node metastasis and CD10 was expressed in stroma in 3 of them.

The expression of CD10 was noted in tumor cells in 10 cases of BCC (47.6%). Seven cases showed high expression (>50%) and the remaining 3 showed low expression (10-50%). Positive tumor cells showed immunostaining in the cytoplasm and focally in the cell membrane. CD10 staining was detected more at the periphery of tumor nests rather than centrally (Fig. 2).

The intratumoral and peritumoral stroma also showed CD10 expression in 95.24% of BCC cases (20/21). Twelve cases (60%) showed infiltrative pattern and 8 cases (40%) showed circumscribed pattern but with no significant difference between the two patterns (p=1.000). CD10 expression in the stroma was more than 50% in 16/20 cases.

In BCC, comparing CD10-positive and CD10-negative cases, there was a significant association between CD10 expression and status of deep margin (p=0.030) (Table 2). Seven out of 9 tumors with well-circumscribed deep margin showed positive CD10 staining. However, most of tumors with infiltrating base (9/12)
showed CD10-negative expression. There was no significant difference regarding sex, sites, multiplicity or histological type of tumors ($p<0.05$) (Table 2).

We observed a trend towards an inverse relationship between CD10 expression in tumor cells and stromal cells in our SCC cases and in BCC with infiltrating margins. BCCs with infiltrating deep margins (12 cases) tended to show CD10 negative basaloid cells (9/12) and CD10 positive stromal cells (12/12) and the difference was statistically highly significant ($p=0.0003$).

Table (1): Clinical and immunohistochemical comparison between BCC and SCC cases.

<table>
<thead>
<tr>
<th></th>
<th>BCC (n=21)</th>
<th>SCC (n=16)</th>
<th>Test of significance</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age in years:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>57 (16-77)</td>
<td>46 (12-84)</td>
<td>U=74.5</td>
<td>0.004 HS</td>
</tr>
<tr>
<td><strong>Size of tumor (cm):</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>1.5 (0.4-5)</td>
<td>6.5 (1-21)</td>
<td>U=24.4</td>
<td>0.0006 HS</td>
</tr>
<tr>
<td><strong>Sex:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>9 (43%)</td>
<td>12 (75%)</td>
<td>Fisher exact test</td>
<td>0.093</td>
</tr>
<tr>
<td>Female</td>
<td>12 (57%)</td>
<td>4 (25%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tumor cells:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>10 (47.6%)</td>
<td>0 (0%)</td>
<td>Fisher exact test</td>
<td>0.002 HS</td>
</tr>
<tr>
<td>Negative</td>
<td>11 (52.4%)</td>
<td>16 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Stromal cells:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>20 (95.2%)</td>
<td>13 (81%)</td>
<td>Fisher exact test</td>
<td>0.296</td>
</tr>
<tr>
<td>Negative</td>
<td>1 (4.8%)</td>
<td>3 (19%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$U = $ Mann-Whitney $U$ test.
HS = Highly significant.

Table (2): Relationship between CD10 expression and clinicopathological parameters in BCC (n=21).

<table>
<thead>
<tr>
<th></th>
<th>Total (n=21)</th>
<th>+ve (n=10)</th>
<th>-ve (n=11)</th>
<th>Test of significance</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years):</strong></td>
<td>58.8±4.7</td>
<td>54.7±16.8</td>
<td>U=46.5</td>
<td>0.557</td>
<td></td>
</tr>
<tr>
<td><strong>Size (n=17):</strong></td>
<td>1.8±1.4</td>
<td>1.5±0.8</td>
<td>U=33.5</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td><strong>Sex:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>9 (33%)</td>
<td>6 (77%)</td>
<td>Fisher exact</td>
<td>0.387</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>12 (58%)</td>
<td>5 (42%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Site:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Face</td>
<td>13 (54%)</td>
<td>6 (46%)</td>
<td>Fisher exact</td>
<td>0.659</td>
<td></td>
</tr>
<tr>
<td>Scalp</td>
<td>8 (37.5%)</td>
<td>5 (62.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Single:</strong></td>
<td>16 (56%)</td>
<td>7 (44%)</td>
<td>Fisher exact</td>
<td>0.310</td>
<td></td>
</tr>
<tr>
<td><strong>Multiple:</strong></td>
<td>5 (20%)</td>
<td>4 (60%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Solid &amp; Adenoid:</strong></td>
<td>19 (42%)</td>
<td>11 (58%)</td>
<td>Fisher exact</td>
<td>0.214</td>
<td></td>
</tr>
<tr>
<td><strong>Morphea:</strong></td>
<td>2 (100%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Circumscribed base:</strong></td>
<td>9 (78%)</td>
<td>2 (22%)</td>
<td>Fisher exact</td>
<td>0.030*</td>
<td></td>
</tr>
<tr>
<td><strong>Infiltrating base:</strong></td>
<td>12 (25%)</td>
<td>9 (75%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$U = $ Mann-Whitney $U$ test.
* = Statistically significant.
DISCUSSION

Although, CD10 expression has been investigated in some cutaneous tumors, to our knowledge, data regarding its expression in cutaneous epithelial neoplasms are very limited. In the present study, we investigated the expression of CD10 in basal and squamous cell carcinomas.

The distinction between squamoid basal cell carcinoma and basaloïd squamous cell carcinoma is usually made readily on the basis of defined histological criteria. However, occasionally it can pose difficult morphological problems [4]. Such distinction is clinically important because the risk of progressive disease is significantly higher with squamous carcinoma of the skin than with basal cell carcinoma.

In our study, almost all the tumor cells in SCC cases were completely negative for CD10 similar to several previous reports both in skin and in oral cavity SCC [16,17]. In BCC, 10 out of 21 cases (47.6%) were positive for CD10, most of them showed positivity in more than 50% of tumor cells. Our results are in accordance with those of previous reports in BCC, Yada et al., (86%) [16], Pham et al., (87%) [18], Wagoner et al., (100%) [19]. We observed a significant difference between CD10 expression in tumor cells in SCC and BCC ($p=0.002$ HS). Our results reflect the fact that BCC is biologically different from SCC.

The belief that BCCs represent primitive adnexal carcinomas has been made over the years and is supported by the marked immunohistochemical similarity between BCC and tumors of hair follicle derivation [20,21]. In our study, CD10 showed predilection for the basal layer of epidermis, inner root sheath, hair matrix and perifollicular fibrous sheath. CD10 staining in BCC was detected more at the periphery of tumor nests rather than centrally, a pattern that was previously reported to be similar to that in some adnexal tumors [16]. Thus, we support the hypothesis that derivation of BCC is from an adnexal origin.

We assume that in addition to the well-defined histological criteria, CD10 might be a useful immunohistochemical marker, in difficult cases, to differentiate between BCC and SCC. At least, if tumor cells were CD10 positive, this would favor BCC over SCC. Wagoner et al., has recently reported that CD10 was strongly expressed in 14 out of 14 superficial BCCs and failed to be expressed in 13 out of 13 superficially invasive SCC and SCC in situ [19]. These findings strongly support our results and suggest the utility of CD10 in the differentiation between BCC and SCC.

In our BCC cases, CD10 expression was more frequently detected in tumors with well-circumscribed base, however, those with infiltrating base showed more frequent CD10 negativity ($p=0.030$). Interestingly, most of our BCCs with infiltrating deep margins (12 cases) tend to show CD10 negative basaloïd cells (9/12) and CD10 positive stromal cells (12/12) and the difference was statistically highly significant ($p=0.0003$). This observed pattern is similar to that noted in most of our SCC cases. Thus, our results reflect an inverse relationship between CD10 expression in tumor cells and...
stromal cells of both SCC and BCC. Therefore, we suggest that absence of CD10 in cutaneous epithelial tumor cells and its overexpression in the stromal cells might be associated with an invasive capacity of such tumors. Supporting our results, Yada et al., studied CD10 expression in BCC cases and found that sclerosing BCC tended to have more frequent CD10 – immunopositive stromal cells than other types [16].

It was postulated that due to structural similarities of CD10 to matrix metalloproteinases (MMPs), CD10 could create a microenvironment that facilitates cancer cell invasion and metastasis [22]. Increased stromal expression of CD10 had been related to tumor progression and metastasis in different tumors. For example, CD10 expression in the intratumoral stromal cells of malignant melanoma was higher in primary tumors with higher Clark level and tumor thickness according to Breslow [15]. In oral cavity SCC, CD10 stromal positivity was correlated to presence of metastasis, local recurrence and high tumor grade [17]. Ogawa et al., reported that the expression of CD10 in stromal cells in colorectal tumors was significantly correlated with the accumulation of p53 and with larger tumor size and might be related to invasion and metastasis by such tumors [23]. Iwaya et al., showed that stromal expression of CD10 in breast cancer is an important prognostic factor for recurrence and overall survival [24]. CD10 expression by stromal cells seems to promote invasion and metastasis of differentiated gastric carcinoma [25].

Conclusion:

From our results we suggest that CD10 might be a useful immunohistochemical marker to differentiate between BCC and SCC. At least, if tumor cells were CD10 positive, this would favor BCC over SCC. Absence of CD10 in all the SCC and in infiltrating BCC together with its overexpression in the stromal cells might confer invasive properties to such tumors. However, its relation to other poor prognostic factors needs larger studies to be confirmed.

REFERENCES