Histological and immunohistochemical study of the eyelid basal cell carcinomas

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Abstract

The eyelids represent a frequent site for numerous malignant tumors, which generally present subtle symptoms or can imitate benign tumors. Our study was carried on 80 patients, 48 males and 32 females aged between 48 and 92 years. The patients were hospitalized in the Ophthalmology Clinic of the Emergency County Hospital of Craiova, Romania. The study was conducted over five years, between 2010 and 2014. Our study included 80 basal cell carcinomas of the eyelids, of which 48 (60%) were nodular basal cell carcinomas, 15 (18.75%) were adenoid basal cell carcinomas, 10 (12.5%) were cystic and seven (8.75%) were morpheaform basal cell carcinoma. Our study showed a moderate expression of bcl-2 marker in the nodular type of basal cell carcinoma and a high expression in the other histopathological types, thus inducing an increased malignancy comparing to the nodular type. E-cadherin was absent in nodular, cystic and adenoid basal cell carcinomas and had a moderate expression in morpheaform basal cell carcinoma. Morpheaform and adenoid types presented 20% expression of Ki67 of the malignant cells nuclei, while the cystic type presented Ki67 expression in less than 10% of the malignant cells nuclei. Due to high morbidity and increasing incidence, basal cell carcinoma of the eyelid represents an important health issue nowadays.

Keywords: basal cell carcinoma, immunohistochemistry, bcl-2, Ki67, CK8.

Introduction

The eyelids represent a frequent site for numerous malignant tumors, which generally present subtle symptoms or can imitate benign tumors [1]. There are very many tumoral types that are localized in the periorcular and periorbital area, so that even an experienced oculoplastic surgeon can encounter serious issues [2]. Though rarely lethal, a late diagnosis of the eyelid malignant tumors requires a more invasive surgical technique, and thus also inducing an increased malignancy comparing to the nodular type. E-cadherin was absent in nodular, cystic and adenoid basal cell carcinomas and had a moderate expression in morpheaform basal cell carcinoma. Morpheaform and adenoid types presented 20% expression of Ki67 of the malignant cells nuclei, while the cystic type presented Ki67 expression in less than 10% of the malignant cells nuclei. Due to high morbidity and increasing incidence, basal cell carcinoma of the eyelid represents an important health issue nowadays.

Materials and Methods

Our study was conducted on a group of 80 patients,
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Table 1 – Primary antibodies used in the immunohistochemical study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Target</th>
<th>Dilution</th>
<th>Antigen retrieval</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>34BE12</td>
<td>Mouse, IgG1k</td>
<td>High molecular weight</td>
<td>1:100</td>
<td>EDTA, pH 9</td>
<td>M0630, Dako</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cytokeratin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bcl-2</td>
<td>Mouse, IgG1k</td>
<td>BCL2 oncogene</td>
<td>1:100</td>
<td>EDTA, pH 9</td>
<td>M0887, Dako</td>
</tr>
<tr>
<td>Cytokeratin</td>
<td>Rabbit, polyclonal</td>
<td>Low molecular weight cytookeratin</td>
<td>1:100</td>
<td>0.1 M Citrate, pH 6</td>
<td>ab5294, Abcam</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>Mouse, IgG1k</td>
<td>Adhesion molecule</td>
<td>1:50</td>
<td>0.1 M Citrate, pH 6</td>
<td>M3612, Dako</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proliferating cell marker</td>
<td>1:50</td>
<td>0.1 M Citrate, pH 6</td>
<td>M0879, Dako</td>
</tr>
</tbody>
</table>

Results

Our study totalized 80 cases of eyelid basal cell carcinoma, of which 55 (70%) were nodular (solid) basal cell carcinomas, 10 (12%) of them were the adenoid type, nine (12.5%) were cystic basal cell carcinomas and six (7%) were morpheaform (sclerosing) basal cell carcinomas (Figure 1, Table 2).

Table 2 – Frequency, histopathological types and characteristics of the studied basal cell carcinomas

<table>
<thead>
<tr>
<th>Basal cell carcinoma / histopathological type</th>
<th>Frequency, No. of cases / Percent</th>
<th>Histopathological characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodular</td>
<td>55 / 70%</td>
<td>Large tumor aggregates showing attachment to the epidermis; peripheral nuclear palisading.</td>
</tr>
<tr>
<td>Adenoid</td>
<td>10 / 12%</td>
<td>Excessive stromal mucin deposition giving rise to a pseudoglandular appearance; peripheral nuclear palisading.</td>
</tr>
<tr>
<td>Cystic</td>
<td>9 / 11%</td>
<td>Tumor cells may be compressed in one to two layers thick; the cystic space contains connective tissue type mucin; peripheral nuclear palisading.</td>
</tr>
<tr>
<td>Morpheaform / sclerosing</td>
<td>6 / 7%</td>
<td>Hyalinized or keloidal stroma; the tumor aggregates are typically compressed in narrow strands; peripheral nuclear palisading.</td>
</tr>
</tbody>
</table>

As classically recognized, the nodular type of BCC was characterized by the existence of varied-sized nests of basaloid cells infiltrating the dermis, with a palisadal disposition of the epithelial cells at the edges of the tumor areas (Figure 2A). Frequently, the lobules were accompanied by slit-like retraction of the surrounding stroma, otherwise not evidently fibroplastic. Mitoses were not frequent and apoptotic cells were only rarely observed in the atypical basaloid tumor areas. Frequently, a rich lympho-plasmocytic inflammatory infiltrate unseathed the BCC nodules but dissipated fast with increasing distance in the dermis.

In adenoid BCC, the tumor cells were arranged in an adenoid and lace-like pattern (Figure 2B). Palisading was noted at the periphery of some of the nests, and retraction spaces were seen around only a few of the nests. On occasion, the centre of the adenoid structures showed the accumulation of amorphous eosinophilic material (Figure 2B).
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Cystic BCC followed in general a compact architecture too, but with some tumor areas comprising cavities with necrotic material, resulting probably from the disintegration of tumor cells or because of sebaceous differences (Figure 2C).

Morpheaform or sclerosing BCC was characterized by narrow and angulated columns of basaloid cells of one to two cells thick surrounded by a denser fibroblastic stroma (Figure 2D). Tumor nests showed a more evident infiltrative pattern in the surrounding dermis compared to the other types of BCC, had less numerous tumor-epithelium retraction clefts and were surrounded by a diffuse-like lympho-plasmocytic inflammatory infiltrate. Cellular pleomorphism and apoptosis were more frequently encountered compared to the other types described above.

Bcl-2 had a high expression in adenoid, cystic and nodular basal cell carcinomas (more than 75% of the tumoral cells) and a moderate expression in nodular basal cell carcinoma (60% of the tumoral cells). This immunomarker had a moderate/low expression in the peritumoral inflammatory infiltrate (Figure 3).

Figure 2 – Basal cell carcinoma – histopathological types: (A) Nodular; (B) Adenoid; (C) Cystic; (D) Morpheaform. HE staining, ×100.

Figure 3 – bcl-2 expression in basal cell carcinomas: (A) Nodular; (B) Adenoid. bcl-2 immunostaining, ×200.
In our study, E-cadherin (E-cad) presented low/moderate expression in nodular, adenoid and morpheaform BCCs, and a low expression in cystic basal cell carcinomas (Figure 4).

PCNA proliferation-associate marker was expressed in varied percentages in all the described types of BCC. Morpheaform BCC showed a proliferation index of more than 60% for the epithelial tumor nuclei, followed by nodular BCC (40% of tumor nuclei), adenoid (20% of tumor nuclei) and cystic types (less than 10% of tumor nuclei) (Figure 5). Most of the PCNA-positive nuclei occurred in cells situated at the periphery of the tumor areas and not in the cores of these nests.

CK34βE12, a high molecular weight cytokeratin, presented a high expression in the cytoplasm of the tumoral epithelium cells in all histopathological types of BCC, especially in the adenoid BCC, and dense-diffuse in morpheaform BCC. In nodular BCC, the most intense expression was seen in the peripheral cells of the tumoral islands, thus contrasting the morpheaform BCC, where the most intense expression was seen at the core of the tumoral columns (Figure 6).

With only very scant stained foci, CK8 (a low molecular cytokeratin) was not expressed in these series of tumors (Figure 7).
Figure 5 – PCNA expression in basal cell carcinomas: (A) Nodular; (B) Adenoid; (C) Cystic; (D) Morpheaform. PCNA immunostaining, ×200.

Figure 6 – CK34βE12 expression in basal cell carcinomas: (A) Nodular; (B) Adenoid; (C) Cystic; (D) Morpheaform. CK34βE12 immunostaining, ×200.
Discussion

Our study highlights the predominance of nodular BCC (70% of the cases) comparing the other histopathological types of BCC. Berking et al. (2014) proves that nodular basal cell carcinoma is the most frequent of all BCC histopathological types [17, 18].

Bcl-2 is an important apoptotic gene that codifies a protein, which produces apoptosis inhibition. Abnormal expression of bcl-2 gene may lead to a control loss of modified cells, thus increasing predisposition to neoplasia [14, 19]. Bcl-2 expression in basal cell carcinomas (of any type) represents a good sign for aggressive disease. All these tumors present moderate to high bcl-2 expression. Clinically aggressive types (sclerosing/morpheaform) have a more intense bcl-2 expression, comparing to nodular type [14, 20]. In our study, bcl-2 expression was moderate in nodular basal cell carcinoma and high in the other histopathological types, thus inducing a more aggressive disease comparing to nodular basal cell carcinomas.

E-cadherin (E-cad) represents a calcium-dependent cellular adhesion molecule, which mediates homotypic and hemophilic adhesion between cells in contact [21, 22]. E-cad is expressed in almost all tumoral cells of basal cell carcinomas [21, 23]. One study highlights that numerous nodular basal cell carcinomas and some of the superficial basal cell carcinomas present a reduced E-cad expression, though [21]. In our study, E-cadherin presented low/moderate expression in nodular, adenoid and morpheaform BCCs, and a low expression in cystic basal cell carcinomas.

Previous studies considered PCNA as an alternative to other markers, in order to determine cell proliferation in blocked tumor samples [14, 24]. In cutaneous carcinomas that are related to UV light exposure, PCNA expression is enhanced [14, 25]. Other authors described the high rate of positivity for PCNA as being the cause of low sensitivity for investigation of the proliferative index, comparing to other markers [14, 20]. It is not possible to determine whether the increased number of PCNA-positive tumoral cells is due to high turnover or does it indicate a large proportion of cells dividing in a prolonged cycle, without knowing the exact moment of the cell cycle [14, 15, 20].

The long half-life may be a cause of the increased variation in the intensity of staining for PCNA-positive cells. It is suggested that all cells should be considered as marked, whether they are low, high or moderate in staining intensity, because of the lack of certainty whether the less-stained cells are in cycle or not [14–16]. Our study highlights a moderate PCNA expression (40% of the nuclei) in nodular basal cell carcinomas, thus suggesting a quite increased proliferation index. Adenoid and morpheaform BCCs presented a 20% of the tumoral cells nuclei PCNA expression, while cystic BCCs presented the lowest PCNA-expressed proliferation index: below 10% of the tumoral nuclei.
Cytokeratins (CK) represent intermediate filaments and essential intracellular components, which highlight different cellular properties, and reflect differentiation stages in epithelial tissues. The proteins that belong to the cytokeratins family are either acid or base epithelial polypeptides, with low or high molecular weight [26, 27]. The intermediate filaments are central components of the intracellular matrix. The expression of this type of proteins is specific to every tissue and it is conserved during carcinogenesis. The intermediate filaments are interpreted as specific markers of cell regulation mechanisms. Several studies associate the changes in these proteins expression with an aberrant cell behavior [28, 29]. In our study, the immunohistochemical expression of CK34βE12 was intense in all basal cell carcinomas, but with different patterns: in nodular BCCs, the expression was most intense at the periphery of the malignant cell nests, comparing to the morpheaform BCCs, where the most intense expression was at the center of the malignant cell columns.

In our study, CK8 was not expressed in any type of basal cell carcinoma. There is recent prove that the induction of CK8/18 expression (low molecular weight cytokeratin) in non-malignant cells of the oral mucosa lead to significant changes of the phenotypic characters after CK8/18 transfection [26, 30]. These modifications included increased cell mobility, fact that may indicate increased tumor aggressiveness and poor prognosis. These findings could not be correlated to clinical outcome, though. Therefore, the signification of these data in clinical context, especially regarding disease prognosis, is not clear [26].

BCC recurrences are mostly associated with histological aggressive-growth variants [31]. Sexton et al. [32] described the overall recurrence rate of 26% in infiltrative type, and only of 6.4% in nodular type. Zagrodnik et al. [33] demonstrated similar recurrence rates: 27.7% for sclerosing (infiltrative) basal cell carcinomas and 8.2% for nodular BCCs. A better perspective of BCC pathogenesis and evolution can be gained by comparing individual morphological and immunobiological features of these lesions [31].

Conclusions

Through high morbidity and increased incidence, eyelid basal cell carcinoma represents a topic of serious interest. Due to high bcl-2 expression and moderate PCNA expression, morpheaform basal cell carcinoma represents the most aggressive BCC histopathological type in our study. Other markers are also present in morpheaform BCC, also suggesting an aggressive disease that correlates clinically with large invasion, fast local growth and recurrence risk.

Conflict of interests

The authors declare that they have no conflict of interests.

Contribution note

All authors have equally contributed to this paper.

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