Loss of epithelial cell adhesion molecule (EpCAM) in infiltrative basal cell carcinoma

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Abstract: Basal cell carcinoma (BCC) is the most common type of skin cancer and expresses high protein levels of the epithelial cell adhesion molecule (EpCAM, syn. CD326). Though BCCs only rarely metastasize, infiltrative and destructive growth do occur. EpCAM has been studied extensively in the context of adhesion and carcinogenesis but results of studies relating EpCAM expression to invasive potential or patient prognosis have been inconsistent. In an attempt to link EpCAM expression with infiltrative potential, we retrospectively stained paraffin embedded tissue samples of nodular and infiltrative BCCs. A total of 96 samples comprising 48 nodular and 48 infiltrative BCC cases were immunochemically stained with anti-EpCAM clone BerEP4. Loss of EpCAM expression along the tumor invasive front was detected in 6 of 48 (12.5%) of the nodular BCC as compared to 29 of 48 (60.4%) of the infiltrative BCC cases (P < 0.0001). These results exemplify the important role of EpCAM for cell adhesion. BCC infiltration seems to be promoted by down-regulation of EpCAM along the tumor invasion front.

Keywords: Basal cell carcinoma, EpCAM, epithelial cell adhesion molecule, CD326

Introduction

Basal cell carcinoma (BCC) is the most common type of skin cancer accounting for approximately 70% of all skin malignancies [1]. Though BCCs are usually slow-growing, non-aggressive tumors, mostly cured by surgical treatment, a minority of cases shows an aggressive, rarely even metastatic behavior [2].

Ackermann classified BCC as trichoblastic carcinoma [3] but the cell of origin is still matter of debate. Most likely, the majority of BCCs arises from the lowermost layers of the epidermis but there has also been evidence that some BCCs may originate from the outer root sheath of the pilosebaceous unit [4, 5]. Interestingly, BCC is strictly stroma dependent, thus, an xeno-transplantation into mice is unsuccessful if the stroma is not included [6]. This stromal dependency is the most likely reason for the low incidence of metastasis of these tumors. The morphology of BCC is quite variable. Consequently, various histopathological subtypes have been defined including nodular (solid), micronodular, pigmented, keratotic, superficial (multifocal), cystic, adenoid, fibroepitheliomatous, infiltrating, sclerosing, infundibulocystic, metatypical, and basosquamous [7]. The non-aggressive nodular type accounts for approximately 70% of all cases whereas only approx. 5% represent the infiltrating type, characterized by invasive growth pattern with clinically indistinct borders [4]. Mixed patterns are quite common. The vast majority of BCC are closely attached to the basal layer of the epidermis while longer existing lesions usually extend into the lower dermis. Further growth usually occurs diffusely or along the cutaneous adnexae [8]. Perineural invasion is present in nearly 1% of all BCC cases with an increasing incidence in aggressive variants [9-11].

The epithelial cell adhesion molecule (EpCAM, syn. CD326) is frequently expressed in BCC [12]. Sellheyer and Krahl suggested that the expression of EpCAM could be a clue to the adnexal nature of BCC proposing that BCC is
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the most primitive follicular tumor [13]. EpCAM is a transmembrane cell surface glycoprotein that is expressed by developing and differentiated epithelia [14-16], carcinomas, tumor-initiating cells, circulating tumor cells, and stem cells [17, 18]. EpCAM has many faces and the literature regarding its function is extensive (for review [15, 19, 20]). Among the activities attributed to EpCAM, it has been reported that it mediates adhesion [21], that it reduces adhesion [22], and that it functions as an outside-in signaling molecule [23]. In humans, EpCAM mutations induce congenital tufting enteropathy, a rare diarrheal syndrome that is caused by severe intestinal epithelial dysplasia and loss of epithelial integrity [24]. The role of EpCAM for tumorigenesis is also ambiguous. EpCAM has been intensively studied as a tumor antigen that may represent a suitable therapeutical target [25], and because it may play a role in cancer pathogenesis [17]. In some settings, EpCAM may facilitate cancer cell invasion and metastasis [23], and its expression in tumors may indicate poor prognosis [15, 19, 20]. In contrast, other studies could demonstrate that in some tumors EpCAM expression appears to be beneficial [20, 26]. It seems likely that because EpCAM is a molecule that interacts with surrounding cells, tissue context and microenvironment are important.

In BCC it has been demonstrated that islands of tumor cells along the tumor front are surrounded by a stroma that is different form the adjacent dermis and that BCC cells express decreased protein levels of basement membrane components (e.g. bullous pemphigoid antigens 1 and 2, integrins alpha6 and beta4, and beta3 chain of laminin), which may facilitate the capability of tumor cells to invade [6, 8]. Furthermore, a loss of expression of epithelial markers and junctional proteins, such as E-cadherin, beta-catenin, and desmoglein among the invasive tumor front has been shown in canine oral and cutaneous squamous cell carcinomas [27]. It is further known that the classical cadherins (primarily E-cadherin) and EpCAM are co-expressed in some tissues, and previously it has been reported that EpCAM can modulate cadherin-mediated adhesion [22].

In an attempt to link immunohistochemical EpCAM expression and infiltrative potential we retrospectively stained paraffin embedded tissue samples of nodular and infiltrative BCCs.

Material and methods

Formalin fixed paraffin-embedded (FFPE) BCC samples, that had been surgically removed between 2011 and 2012, were obtained from the Department of Dermatology and the Institute of Pathology of the University Medical Center Mannheim, University of Heidelberg. Clinical data sets included age, sex of the patients and histopathological features. Diagnosis of BCC was verified histopathologically. Additionally, selected cases were subjected to immunohistochemical staining with GATA3, EMA, Vimentin, and S100. All procedures were performed according to the principle of the Declaration of Helsinki and approved by the local medical ethics committee (2014-835R-MA).

A total of 121 BCC cases were included into the study. Of those, 25 cases had to be omitted because due to a mixed growth pattern, a clear classification into nodular or infiltrating BCC subtype was not possible. The remaining cases included 48 nodular BCC and 48 infiltrating BCC. Loss of EpCAM was defined as an obvious decrease (less than 50% staining intensity as compared to the rest of the tumor) of EpCAM staining intensity occurring on tumor borders or tumor islands infiltrating the dermis.

Immunohistochemistry

Tissue sections were stained for EpCAM (clone Ber-EP4, 1:50; cat # M0804, Dako, Agilent, Santa Clara, CA, USA), EMA (clone E29, 1:200;
Figure 2. Histopathological examination demonstrating the immunohistochemical features of two cases (A, B) of infiltrating BCC. Vimentin-staining depicts the stroma, GATA3-staining was used to unmask small tumor islands that...
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Table 1. Immunohistochemical features of infiltrative and nodular basal cell carcinoma

<table>
<thead>
<tr>
<th></th>
<th>Infiltrative BCC</th>
<th>Nodular BCC</th>
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<tbody>
<tr>
<td>Total no</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>EpCAM loss*</td>
<td>29</td>
<td>6</td>
</tr>
<tr>
<td>Perineural invasion</td>
<td>3 out of 12 cases</td>
<td>1 out of 24 cases</td>
</tr>
<tr>
<td>GATA3</td>
<td>7 positive out of 7 cases</td>
<td>10 positive out of 10 cases</td>
</tr>
<tr>
<td>EMA</td>
<td>7 negative out of 7 cases</td>
<td>10 negative out of 10 cases</td>
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</tbody>
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*Fisher’s exact test, P < 0.0001.

Statistics

Statistical analysis was performed using GraphPad Prism software version 7.03 (GraphPad Software, La Jolla, CA, USA, www.graphpad.com). Differences between groups were estimated by Fisher’s exact test. P < 0.05 was considered significant.

Results

All BCC cases tested immunohistochemically positive for the expression of EpCAM. Loss of EpCAM along the tumor front/infiltrating islands was observed in 29 of 48 (60.4%) infiltrative BCC and in 6 of 48 (12.5%) nodular BCC (P < 0.0001) (Figure 1). EpCAM loss was mainly restricted to the invasive front. EpCAM staining demonstrated a heterogeneous expression pattern with strong EpCAM expression in superficial and central tumor parts and weak to total EpCAM loss at the deeper infiltrating tumor fingers including the invasive front (Figure 2). Perineural invasion as demonstrated by tumor cells being adjacent to S100 positive neural structures was observed in three of 12 (25.0%) infiltrative BCC and one of 24 (4.2%) nodular BCC. GATA3 and EMA expression were exemplarily tested in a subset of 10 cases in order to investigate their function as helpful tools in ambiguous BCC cases. GATA3 staining was seen as homogenous staining in seven of seven (100%) infiltrative BCC and 10 of 10 (100%) nodular BCC. EMA expression was absent in seven of seven (100%) infiltrative BCC and 10 of 10 (100%) nodular BCC (Table 1). Vimentin counter staining of the stroma was used for a better identification of small BCC tumor islets (Figure 2).

Discussion

To our knowledge, the loss of EpCAM along the invasive front of infiltrative BCC has not been described so far. We found a statistically relevant loss of EpCAM expression in infiltrating BCC as compared to nodular BCC. EpCAM loss was mainly restricted to the tumor invasion front as well as deeper infiltrating tumor islands. Immunohistochemical stainings for GATA3 and EMA showed an universal positivity and negativity in 10 cases, respectively. Thus, especially in infiltrative BCC cases the use of GATA3 can facilitate detecting single tumor islands that have lost their EpCAM expression and are otherwise too small in order to be detected in conventional H&E staining.

Loss of other intercellular adhesion molecules and junctional proteins, such as E-cadherin, beta-catenin, and desmoglein among the tumor invasion front has been described in squamous cell carcinoma [27] but not in BCC. It is known that loss of those molecules is associated with an increase of tumor cell invasion; thus, they are considered useful prognostic markers in human carcinomas [28-31]. At least one of those cell adhesion markers, E-cadherin, is known to be co-expressed with EpCAM and it has been illustrated that EpCAM can modulate cadherin-mediated adhesion [22]. Akin et al. could demonstrate that...
EpCAM modulates adhesion and tight junction function by regulating intracellular localization and degradation of selected claudins, especially claudin-7 and claudin-1 [32]. Our results underline the positive role of EpCAM expression for cell adhesion in BCC while its loss at the tumor front presumably facilitates tumor invasion.

Besides, the downregulation of EpCAM has been associated with epithelial-mesenchymal transition (EMT) [33, 34], a process, that plays a key role in carcinoma progression and is necessary for invasion and metastasis [35-37]. Interestingly, in contrast to other highly malignant EpCAM expressing tumors, such as Merkel cell carcinoma [38], even though the majority of BCC strongly expresses EpCAM [12], BCC only rarely metastasize.

It has been demonstrated that for BCC tumorigenesis a histological continuum exists that moves from low-risk superficial and nodular BCC subtypes via less frequent transitional, mixed types toward the high-risk micronodular, morpheic, and infiltrating types [39]. The host immune response and stromal alterations accompany this progression.

Thus, when considered in conjunction with the results reported herein, our data suggests that dynamic changes of EpCAM expression facilitate infiltration along the tumor invasion front and may be accompanied by histological changes towards more aggressive BCC subtypes.

Detection of EpCAM loss along the invasive BCC front could therefore serve as prediction marker for a destructive BCC growth pattern leading to substantial tissue damage. Tentatively and still theoretical, in cases where EpCAM loss is detected it might be reasonable to choose a larger safety margin.

Further studies on adhesion/junctional markers known to be associated with EpCAM (e.g. claudins and cadherins) are needed to shed further light on the mechanisms of EpCAM loss along the tumor invasion front.

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Disclosure of conflict of interest

None.

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