Epithelial–Mesenchymal Transition Protein Expression in Basal Cell Adenomas and Basal Cell Adenocarcinomas

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Abstract Basal cell adenomas and basal cell adenocarcinomas show marked histomorphologic similarity and are separated microscopically primarily by the invasive characteristics of the adenocarcinomas. We wished to explore potential differences in the expression of epithelial–mesenchymal transition associated proteins in these two tumor types. A tissue microarray was constructed utilizing 29 basal cell adenomas and 16 basal cell adenocarcinomas. Immunohistochemical expression of E-cadherin, beta-catenin, Twist 1 and vimentin were investigated. Both tumors expressed all proteins in a relatively similar manner. Nuclear beta-catenin was essentially limited to the abluminal cell populations in both tumor types. E-cadherin was limited largely to luminal locations but was more prevalent in the adenocarcinomas as compared to the adenomas. Primarily abluminal expression for vimentin was seen, sometimes present in an apical dot-like pattern. Distinct populations of cellular expression of these four markers of epithelial mesenchymal transition were present but were similar in locations in both tumors with no patterns discerned to separate basal cell adenoma from basal cell adenocarcinoma. Given these findings, the mechanisms by which basal cell adenocarcinoma is able to invade while its counterpart, basal cell adenoma can not, may be more complex than in other tumor types.

Keywords Basal cell adenoma · Basal cell adenocarcinoma · E-cadherin · Beta-catenin · Twist 1 · Vimentin

Introduction

Basal cell adenoma and basal cell adenocarcinoma represent uncommon basaloid salivary gland neoplasms. These tumors appear morphologically very similar to one another and the primary means of histologic separation require observation of invasion into the surrounding normal gland or adjacent tissues in the carcinomas, a finding that is nearly always associated with increased mitotic rate in the carcinomas. We wished to determine if there were differences in the expression of epithelial–mesenchymal transition (EMT) markers between the benign and malignant counterparts of two cyto-morphologically similar neoplasms and to determine if expression of EMT markers could help separate benign and malignant tumors.

Materials and Methods

We have previously reviewed the pathologic features of 69 neoplasms diagnosed as basal cell adenoma or basal cell adenocarcinoma gathered from our institution [1]. From these tumors we constructed tissue microarrays using 29 basal cell adenomas and 16 basal cell adenocarcinomas with each tumor replicated in triplicate with 0.2 cm diameters, selecting different areas of the paraffin block.

The arrays were then stained with antibodies to vimentin, Twist 1, E-cadherin and beta-catenin. The immunochemical reactions were carried out as follows. Tissue sections of 4 microns were sectioned from the

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formalin fixed paraffin embedded tissue for immunohistochemistry. Sections were deparaffinized and then brought through xylenes and alcohols to distilled water. Heat epitope induced retrieval (Biocare Decloaking Chamber, Concord, CA, USA) was carried out for all antibodies. The antibodies sources, dilutions and incubation times were as follows: vimentin (Dako, Carpinteria, CA, USA; 1:300, 15 min at room temperature); Twist1 (Abcam, Cambridge, MA, USA; 1:250 for 60 min at room temperature); E-cadherin (Dako, Carpinteria, CA, USA; 1:50 for 30 min at room temperature); Beta-catenin (BD Biosciences, San Jose, CA, USA; 1:6000 for 60 min at room temperature). Negative controls were performed with non-specific antibody. After exposure to antibody, the slides were washed in buffer and then Dako Mouse Envision System HRP (Dako) was applied for 30 min at room temperature. After washing with buffer, Dako DAB Plus (Dako) was applied followed by Dako DAB enhancer (Dako). After washing in buffer, the slides were counterstained with hematoxylin. Staining was assessed for absence or presence as well as location within the tumor cell nests/trabeculae.

Statistical analysis was performed using Stata software, Version 12 (Stata, College Station, TX, USA). The local Institutional Review Board approved these studies.

Results

General Findings

Distinct localization of staining as to abluminal or luminal areas was seen in many tumors, including those that appeared on hematoxylin and eosin staining to be composed of relatively similar basaloid cells.

B-Catenin Expression

Basal Cell Adenomas

Aberrant beta-catenin expression was seen in abluminal cells with 19/29 (66 %) tumors showing nuclear staining. No nuclear staining was present in the luminal cells of the adenomas.

Basal Cell Adenocarcinomas

The basal cell adenocarcinomas had identical staining patterns to the adenomas although less overall expression was seen. Five carcinomas (31 %) had nuclear staining of the abluminal cells (Fig. 1). No nuclear staining was seen in the luminal cells of the carcinomas.

Fig. 1 Beta-catenin is staining the nuclei of the abluminal cells in the basal cell adenocarcinoma

E-Cadherin Expression

Basal Cell Adenomas

E-cadherin expression was seen in 9/29 (31 %) tumors. The expression was preferentially seen in the luminal aspects of the tumor cell nests (Fig. 2).

Basal Cell Adenocarcinomas

E-cadherin expression was seen in 10/16 (63 %) tumors. Similar to basal cell adenomas, these tumors exhibited preferential staining in the luminal areas of the tumor cell nests.

Fig. 2 Basal cell adenoma with expression of E-cadherin limited to the luminal cells of this trabecula

While the overall staining patterns and location of the staining was similar between basal cell adenomas and basal cell adenocarcinoma, more carcinomas showed E-cadherin expression than adenomas ($p \leq .04$).

Overall, E-cadherin expression was weaker compared to the other EMT markers in both tumor types.

Twist 1 Expression

Basal Cell Adenomas

All but one tumor expressed Twist 1 and both abluminal and luminal cell staining was present, often in both areas simultaneously. However, there was a slight preponderance for staining to be seen in the luminal cells (18/29) of the tumor cell nests versus those of the abluminal cells (13/29). The neoplastic cells exhibited both nuclear and cytoplasmic staining.

Basal Cell Adenocarcinomas

All 16 of the neoplasms exhibited Twist 1 staining (Fig. 3). Similar to basal cell adenomas the expression was slightly more common in the luminal cells (10/16) compared to the abluminal cells (8/16).

Vimentin Expression

Basal Cell Adenomas

Vimentin expression was seen in 25 of 29 basal cell adenomas. Only 4 tumors showed luminal cell staining. While most of the individual cell staining was seen to be

Fig. 3 In this basal cell adenocarcinoma, Twist 1 expression is seen limited to the luminal aspects of the tumor cells

Fig. 4 Basal cell adenoma with vimentin expression primarily confined to the abluminal cells

circumferential and cytoplasmic (Fig. 4). A focal/apical pattern in abluminal cells was seen in 8 tumors in the region of the cell that bordered the adjacent basement membrane (Fig. 5).

Basal Cell Adenocarcinomas

Vimentin expression was seen in 13 of 16 basal cell adenocarcinomas. Only 2 of the 13 positive tumors showed luminal staining with the remainder showing an abluminal staining pattern.

Fig. 5 Vimentin expression was sometimes seen in the abluminal cells in a dot like apical pattern adjacent to the surrounding basement membrane, as seen in the basal cell adenoma

Discussion

One of the qualities for a tumor to be considered malignant is its ability to invade. The proteins that are thought to accomplish invasion are those that are involved in embryogenesis whereby immature epithelial cells are able to transition into mesenchymal cells that then transit through the extracellular matrix [2].

Two major protein families are involved in preventing normal cells from migrating. Cadherins are a family of calcium dependent, cytoplasmic plaque proteins that act as links to the actin cytoskeleton which have cell proliferation and signal transduction actions. Of these two families, the most investigated proteins are E-cadherin and beta-catenin, respectively. Both E-cadherin and beta-catenin expression changes are seen in a multitude of benign and malignant tumors [3]. E-cadherin, a transmembrane protein present only in epithelial tissues, is a member of the cadherin family. When E-cadherin is decreased, loss of cellular adhesion can occur with resulting invasion and metastasis [4, 5]. E-cadherin controls cell motility and adherence by linkages of the cytoplasm to the actin cytoskeleton by cytoplasmic plaque proteins termed catenins [6]. Catenins consist of alpha-catenin, beta-catenin and gamma-catenin. Beta-catenin is involved in embryonic gastrulation whereby the bi-layer embryonic disc is separated into a tri-laminar disc with ectoderm, mesoderm and endoderm are formed as well as other developmental functions.

Previous work by our group has demonstrated that basal cell adenocarcinomas had significantly higher mitotic and proliferation rates but ultimately the distinction between the two tumors is made possible upon finding invasion in basal cell adenocarcinoma, but not basal cell adenoma [1]. We wished to study the expression of EMT markers in basal cell adenomas and basal cell adenocarcinomas as they may provide a platform to explore differences between benign and malignant glandular neoplasms that share very similar histologic morphology. We also wished to determine if EMT markers could help in distinguishing benign and malignant counterparts.

Our findings in EMT proteins in basal cell adenomas and adenocarcinomas reflect similar findings that Economopoulou et al. [7] found in expression of E-cadherin in other salivary gland tumors. These authors concluded the relationship seen between E-cadherin expression and cell polarity/glandular organization in breast and colon does not appear to exist for salivary gland neoplasms in which the diversity of architectural patterns precludes detection of any simple relationship.

While most epithelial neoplasms show decreased E-cadherin expression in carcinomas when compared to benign tumors, E-cadherin was seen in only one third of the

basal cell adenomas in our cases. On the other hand, E-cadherin was seen in 63 % of the adenocarcinomas. Similar to our findings of E-cadherin preferentially staining the luminal cells, Furuse et al. [8] also found that pleomorphic adenomas showed weak to no expression for E-cadherin in the myoepithelial cells but did show expression in ductal cells. Variability of E-cadherin expression in salivary gland tumors is commonly reported in the literature [7, 9]. In a series of salivary gland tumors, E-cadherin expression was found overall to be decreased in malignant salivary gland tumors versus benign [10]. Although more data is required, we believe that the increased incidence of E-cadherin in the basal cell adenocarcinomas may be related to what type of cell, luminal or abluminal, is dominant in these malignant tumors. Our data might suggest that in these malignant salivary gland tumors, the luminal cells are more likely the predominant cells.

Beta-catenin is considered a proto-oncogene and mutations of this gene are commonly found in a variety of cancers including carcinomas of the colon, ovary, breast, lung and liver. Beta-catenin, coded by the *CTNNB* gene, is usually held in E-cadherin adherens junctions and free beta-catenin is depleted by ubiquitin-mediated degradation. However, free beta-catenin can accumulate due to beta-catenin mutations or losses of APC or Axin function [6]. Increased cytoplasmic beta-catenin can move to the nucleus where it can modulate other transcription factors alone or in tandem with other factors, such as transcription factor T cell factor/lymphoid enhancer factor. These factors can then impact the expression of c-Myc and cyclin D1 [6]. In the present study, beta-catenin staining was more commonly seen in the abluminal cells in the adenomas, a finding that has been described in other salivary gland neoplasms [8, 11–13].

In our study, both the adenomas and adenocarcinomas expressed beta-catenin nuclear staining in the abluminal cells, with a higher percentage (66 %) seen in the adenomas when compared to the adenocarcinomas (31 %). Aberrant nuclear staining for beta catenin was also described in the abluminal cells of epi-myoeplithelial carcinomas in the study of Mantesso [14]. Kawahara et al. [12] described finding nuclear beta-catenin expression in 21 of 22 basal cell adenomas, with the expression localized in what was termed basaloid myoeplithelial cells. In that study, CTNNB1 mutation analysis was performed in the 21 adenomas showing nuclear beta-catenin expression and mutations were seen in 11 tumors. One of the three basal cell adenocarcinomas these investigators studied also showed nuclear beta-catenin expression. Interestingly, nuclear beta-catenin expression was not seen in a diverse mix of 154 other salivary gland tumors these authors

studied. Other investigators have reported beta-catenin staining in a variety of salivary gland tumors [15, 16].

Twists 1 and 2 are important regulators of cell interactions in embryogenesis as well as in carcinogenesis. Both proteins may act with oncoproteins, such as Ras or ErbB2, leading to EMT in epithelial cells [17]. In conjunction with hypoxia-inducible factor-2 α and SIP1, Twist 2 has been suggested to correlate with metastasis in adenoid cystic carcinoma [18].

It is thought that overexpression of Twist 1 promotes the metastatic properties of malignant cells by downregulating E-cadherin [19]. Twist, along with Slug and Snail, can bind to the E-box regulatory regions of e-cadherin, with resultant downregulation [20].

In our study, all but one basal cell adenoma expressed Twist 1 while all of the adenocarcinomas expressed this protein. However, it was the luminal cells that were more commonly seen to be staining as opposed to the abluminal cells. Thus, in basal cell adenomas and basal cell carcinomas, Twist expression may be associated with the luminal or ductal-type cells. It is also interesting that the widespread expression of Twist 1 in both adenomas and carcinomas, where both tumor types exhibited less E-cadherin expression overall, when compared to most neoplasms. This has been suggested previously in the literature, whereby expression of Twist is associated with down-regulation of E-cadherin expression.

Vimentin has traditionally been considered a marker of cellular mesenchymal phenotypes but is now being understood as an important finding in malignant epithelial neoplasms, likely related to EMT [21]. While many normal epithelial cells show no or minimal evidence of vimentin expression, numerous epithelial malignancies overexpress this intermediate filament. Our data show that both the basal cell adenomas and basal cell adenocarcinomas express vimentin, nearly exclusively in the abluminal cells. An interesting observation was the focal expression of vimentin by the abluminal cells at their border with the surrounding basement membrane.

Conclusion

As seen in this study, even though basal cell adenomas and adenocarcinomas are composed of similar appearing cells, there are distinct functional cell components within these tumors when observing EMT associated protein expression. While distinct populations of cellular expression of these markers was present, their expression was similar in both tumors. No distinct staining pattern was discerned to reliably separate basal cell adenoma from basal cell adenocarcinoma using these four markers of epithelial mesenchymal transition.

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Compliance with Ethical Standards

Conflict of interest The authors have no conflicts of interest to report.

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