Beta–catenin expression in relation to the histological pattern of basal cell carcinoma

Abstract:
Beta–catenin expression in relation to the histological pattern of basal cell carcinoma

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Background: Beta–catenin is a member of E–cadherins/catenin membrane complex which plays a role in tumor differentiation and aggressive behavior of various malignancies.

Objective: To study the expression of beta–catenin in relation to the histological pattern of basal cell carcinoma (BCC)

Materials and methods: One–hundred and eight BCC samples obtained during January 1992 to December 2002 from Songklanagarind Hospital were investigated by immunohistochemical method using monoclonal beta–catenin antibody.

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Results: Beta–catenin membrane expression was reduced in 90 of 108 BCC cases (83.3%). The reactivity was diffusely reduced in 54 of 66 cases (81.8%) of the nodular type, in 25 of 30 cases (83.3%) of the infiltrative type and in all 6 cases of the superficial type. The staining completely disappeared in the center of the large tumor sheath of the nodular type, but was present around the keratotic center of follicular differentiation. No nuclear staining was detected in any subtypes. A faint cytoplasmic staining was found only in the infiltrative type.

Conclusion: A difference in pattern of expression of beta–catenin between undifferentiated and differentiated BCC was observed. By contrast, the levels of reduction appeared to be similar among the different growth patterns. Therefore, the beta–catenin may be implicated in tumor differentiation, but not for invasiveness of BCC.

Key words: beta–catenin expression, the histological pattern, basal cell carcinoma

Introduction

The incidence of basal cell carcinoma (BCC), the most common human cutaneous malignant neoplasm, has risen markedly during the last decades.1 2 Most BCCs have slow-growing and non-aggressive characteristics. However, some exhibit a local invasive behavior with a tendency to recur. At present, the biological processes responsible for this particular behavior have not yet been identified. It has been found that the particular histological growth pattern of this tumor is correlated with its aggressiveness.3 4 Therefore, it is of interest to investigate the association between biological alterations of this tumor and its growth patterns.

Beta–catenin, a crucial member of E-cadherins/catenin membrane complex, is a multifunctional protein, which functions in cell–cell adhesion.5 Either an alteration in cell–cell adhesion or a cell–matrix interaction is widely seen as an implication of tumor invasion and metastatic potential. Significant changes in expression or structure of one of these components can lead to adherens junction disassembly. As a consequence, this implicates the loss of tumor differentiation and the development of an invasive tumor phenotype.6

In addition to its role as an adhesion molecule, beta-catenin plays a key role in the Wnt signaling pathway. This includes mediation of many inductive events during the develop-
ment of the tumor. The up-regulation of this pathway has been found to be one of the processes in tumorigenesis. Upon signaling activation, beta-catenin ubiquitylation/degradation is inhibited, resulting in beta-catenin stabilization. This beta-catenin then enters the nucleus and interacts with Lef/TCF transcription factors, inducing a genetic program that can lead to cell transformation.

A few studies on the role of beta-catenin in BCC have been conducted and conflicting results with respect to subcellular localization have been reported. Two studies have demonstrated the nuclear expression of beta-catenin. However, the expression pattern in relation to tumor invasive pattern was evaluated in only one study, in which reduced membrane expression and increased cytoplasm/nuclear expression were seen in the infiltrative type. This work aims to confirm these findings using an immunohistochemical study, concentrating on cellular distribution and the relationship to the histological pattern of BCC.

Materials and methods

Cases studied

One hundred and eight specimens of formalin-fixed paraffin-embedded BCC tissue were collected from the Anatomical Pathology Unit, Department of Pathology, Faculty of Medicine, Prince of Songkla University, Thailand. These specimens were biopsied from patients of Songkla-nagarind Hospital during January 1992 to December 2002. The second excisions of residual tumors were excluded. All haematoxylin-eosin stained sections were reviewed and classified based on histological differentiation and tumor growth pattern. Clinical information including sex, age and site were recorded.

Immunohistochemistry

Demonstration of the beta-catenin expression of BCC was performed by the immunohistochemical technique as follows. Paraffin embedded tissues were cut and the sections were deparaffinized and rehydrated. High temperature antigen retrieval was performed following the method of Alman, et al. by microwaving for 15 minutes in 0.01 mol L\(^{-1}\) citrate buffer pH 6.0 at 750 W. The sections were cooled to room temperature and immersed in 1.5% \(\text{H}_2\text{O}_2\) solution to block endogenous peroxidase for 15 minutes. Sections were then incubated with normal rabbit serum for 30 minutes; and subsequently incubated with 1 : 50 mouse monoclonal beta-catenin antibody (IgG1, clone 14, Transduction Laboratories, USA) for 60 minutes; and subsequently with 1 : 400 Rabbit Anti-Mouse Biotinylated secondary antibody (DAKO) for 30 minutes. The sections were then incubated in StrepABCComplex/HRP (DAKO) for 30 minutes. The labelled complex was further developed with diaminobenzidine (DAB, 0.5 mg/ml) for 5 to 8 minutes at room temperature.

The staining of the cell membrane, cytoplasm and nucleus were scored independently and counted per 1,000 tumor cells. The percentage of positively stained cells was recorded. The adjacent normal skin was regarded as an internal positive control. Reduced expression was defined when less than 75% stained tumor cells were observed.

Results

One hundred and eight BCC specimens were obtained from 107 patients. The mean age of the patients was 65 years with a range from 13 to 92 years. All except one of the patients were older than 30 years. Fifty-one of the patients were males and 56 were females. Most of the lesions were located on sun-exposed areas (93 of 107). Based on growth patterns, there were 66 cases of nodular type, 30 infiltrative, 6 superficial and 6 cases of mixed types. Based on histological differentiation, there were solid circumscribed (40 cases) and solid infiltrative (23 cases) of undifferentiated type and follicular (21 cases) and adenoid (8 cases) of differentiated type. Both classifications were closely related. The nodular growth pattern included all cases of solid circumscribed and adenoid types and some cases of follicular type.

In the normal epidermis of all cases, strong immuno-staining of beta-catenin was observed in the cell membrane of stratum spinosum and granulosum but not stratum corneum. Neither cytoplasmic nor nuclear staining was seen (Figure 1).
The distribution of membrane expression of BCCs is shown in Table 1. The reduced expression, positive staining of less than 70%, was found in 90 of 108 cases (83.3%). The correlation between membrane expression and histological growth pattern is shown in Table 2.

In the nodular growth pattern, the membrane staining was diffusely reduced in 54 of 66 cases (81.8%). In particular, the staining completely disappeared on the center of the large tumor sheath (Figure 2). In cases of follicular differentiation, the membrane staining was present around the keratotic center (Figure 3). In the adenoid type, the membrane reactivity was diffusely reduced in 7 of 8 cases. Neither cytoplasmic nor nuclear staining was observed in cases of this nodular growth pattern.

In the infiltrative type, membrane reactivity was diffusely reduced in 25 of 30 cases (83.3%). The staining of positive cases was not sharply demarcated on the cell membrane but appeared to be dispersed to the cytoplasm as a faint staining (Figure 4). Nuclear staining was not observed.

In all cases of superficial type, the membrane reactivity was diffusely reduced. Neither cytoplasmic nor nuclear staining was found.

Figure 1 Strong immunostaining of beta-catenin in the cell membrane of stratum spinosum and granulosum of normal epidermis

Figure 2 The complete disappearance of immunostaining is observed in the center of the tumor nodule of nodular BCC

Figure 3 Membrane staining is present around the keratotic center of follicular BCC

Figure 4 Staining is not sharply demarcated on the cell membrane but appears to be dispersed to the cytoplasm as a faint non specific expression of the infiltrative BCC
Discussion

Although BCC is a slow-growing tumor, a certain growth pattern (infiltrative type) has been demonstrated to confer particular tumor behavior. This has led to attempts to further investigate the molecular events underlying this behavior. In the present study, the expression of beta-catenin in relation to the histological pattern was examined. We found that there was a diffuse reduction of membrane expression in BCC compared to normal epidermis.

Table 1 Cell membrane beta-catenin expression of BCC cases

<table>
<thead>
<tr>
<th>% Positive staining</th>
<th>Number of cases</th>
<th>Percent of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>14</td>
<td>12.96</td>
</tr>
<tr>
<td>1-10</td>
<td>16</td>
<td>14.82</td>
</tr>
<tr>
<td>11-20</td>
<td>13</td>
<td>12.04</td>
</tr>
<tr>
<td>21-30</td>
<td>10</td>
<td>9.26</td>
</tr>
<tr>
<td>31-40</td>
<td>12</td>
<td>11.11</td>
</tr>
<tr>
<td>41-50</td>
<td>6</td>
<td>5.56</td>
</tr>
<tr>
<td>51-60</td>
<td>4</td>
<td>3.70</td>
</tr>
<tr>
<td>61-70</td>
<td>15</td>
<td>13.89</td>
</tr>
<tr>
<td>71-80</td>
<td>10</td>
<td>9.26</td>
</tr>
<tr>
<td>81-90</td>
<td>5</td>
<td>4.63</td>
</tr>
<tr>
<td>91-100</td>
<td>3</td>
<td>2.78</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>108</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Table 2 Correlation between cell membrane expression and histological growth pattern

<table>
<thead>
<tr>
<th>Histological growth pattern</th>
<th>staining &lt;75% number (%)</th>
<th>staining &gt;75% number (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodular</td>
<td>54 (81.8)</td>
<td>12 (18.2)</td>
<td>66</td>
</tr>
<tr>
<td>Infiltrative</td>
<td>25 (83.3)</td>
<td>5 (16.7)</td>
<td>30</td>
</tr>
<tr>
<td>Superficial</td>
<td>6 (100.0)</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Mixed</td>
<td>5 (83.3)</td>
<td>1 (16.7)</td>
<td>6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>90 (83.3)</strong></td>
<td><strong>18 (16.7)</strong></td>
<td><strong>108</strong></td>
</tr>
</tbody>
</table>

Beta-catenin was first identified as a component of the cell–cell adhesion junction. It binds directly to cadherins and also associates with alpha-catenin, providing a link between the actin cytoskeleton and cell–cell junctions.5 Our results show that the membrane beta-catenin expression was diffusely reduced in most cases of BCC while it was strongly expressed in the normal epidermis. The results are consistent with other studies evaluating beta-catenin expression in BCC.11-14 This may suggest a role of altered adhesion function of beta-catenin in BCC carcinogenesis. In addition, a different membrane expression was observed between undifferentiated and differentiated type. The central cells of undifferentiated nodular type showed a loss of membrane expression while the differentiated cells of follicular type showed a retention of membrane expression. However, both aggressive and non-aggressive growth patterns showed a similar level of membrane reduction. These results indicated that adhesion function of beta-catenin may be involved in tumor differentiation rather than as an invasive property.

In our study, none of the BCC examined showed nuclear expression. The absence of nuclear staining confirmed the study of Boonchai, et al.11 and Lo Muzio, et al.13, but contradicted the studies of Yamazaki, et al.12 and El-Bahrawy, et al.14 Yamazaki, et al. detected the expression using an immunofluorescence method, while we used the immunoperoxidase method. The different technique may have caused this discrepancy. However both El-Bahrawy’s and our studies used the same semiquantitative evaluation. In the study of El-Bahrawy, et al.14, nuclear staining in some subtypes and cytoplasmic staining in infiltrative BCC were found whereas our study showed only a faint cytoplasmic staining in the infiltrative type. As activation of the Wnt signaling pathway can lead to nuclear translocation of beta-catenin18-19, the role of the Wnt signaling pathway in BCC is inconclusive. The expression of beta-catenin in our study was reduced in almost all of the superficial and adenoid cases. However, as the number of cases was small, the relationship with beta-catenin expression of these subtypes is still unclear.
Conclusion

The beta-catenin expression was different between undifferentiated and differentiated BCC but showed similar levels of reduction among the growth patterns. This observation emphasized that the cell-to-cell adhesion function of beta-catenin may play a role in tumor differentiation, but not in the invasiveness of BCC.

References