Prognostic Value of Cadherin-associated Molecules (α-, β-, and γ-Catenins and pl20cav) in Bladder Tumors

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ABSTRACT

Loss of E-cadherin-mediated adhesion is an important step in the progression of many carcinomas. In model systems, it has been shown that cadherin function requires not only proper E-cadherin expression but also its linkage to the cytoskeleton through catenins. Hence, defects in catenins may cause defective E-cadherin function, and catenins as well as E-cadherin might constitute prognostic indicators. Here, we extend our previous study on E-cadherin in bladder cancer (Cancer Res., 53: 3241–3245, 1993). We have evaluated the expression of E-cadherin-associated cytoplasmic molecules (α-, β-, and γ-catennins and pl20cav) to clarify whether or not the pattern of their expression could provide additional prognostic information beyond that from E-cadherin alone. Forty-eight frozen bladder tumor specimens and 9 samples of normal urothelium were studied by immunohistochemistry. A discrepancy between the E-cadherin and catenin expression pattern was seen in 20.8% of cases. Abnormal expression of each molecule is significantly correlated with tumor grade (P < 0.01) and stage (P < 0.01). Reduced expression of all of the molecules correlates with poor survival (P < 0.01 for each variable). Proportional hazard regression analysis showed that β-catenin, E-cadherin, and α-catenin have strong predictive value, whereas plakoglobin and pl20cav have a somewhat lower predictive value. Within patients with invasive tumors, those with a normal staining for either E-cadherin, α-catenin, or β-catenin show a trend toward better survival. However, the difference in survival is significant only for E-cadherin (P < 0.05). Thus, β-catenin, E-cadherin, and α-catenin have similar prognostic values. Therefore, from a practical point of view, the expression of any of these proteins can be of prognostic value for patients with bladder cancer.

INTRODUCTION

Transitional cell carcinoma of the urinary bladder comprises a spectrum of diseases with diverse natural histories. Seventy to 80% of these tumors present as superficial lesions that recur in 30-90% of the patients (1, 2). Fifteen to 20% of these recurrences become invasive and/or metastatic (3). Patients with invasive carcinomas are usually treated by radical cystectomy. However, metastatic disease appears after surgery in approximately 50% of the cases (4-6). Adjuvant therapy, including chemotherapy and radiotherapy, is under investigation and shows encouraging results. Therefore, it is important to identify patients who might benefit from surgery with or without adjuvant therapy.

To invade the surrounding tissues and metastasize to distant organs, cancer cells have to detach from the primary lesion as an initial step. This involves disruption of normal cell-cell adhesion in epithelial tissues. In this respect, analysis of the molecular basis of cancer cell detachment is an important aspect of the study of cancer progression. Cadherins are a family of transmembrane glycoproteins involved in homotypic calcium-dependent intercellular adhesion. Several reports have indicated that E-cadherin, the epithelial-specific cadherin, is a key molecule for the maintenance of epithelial integrity and polarized function, and that the reduction of E-cadherin-mediated cell-cell adhesion favors the dispersion of cancer cells (7, 8). Indeed, for most carcinoma types, decreased E-cadherin immunoreactivity correlates with a lack of differentiation and aggressiveness of the tumors. In this regard, our previous study on bladder cancer revealed the potential usefulness of E-cadherin immunohistochemistry as a prognostic marker (9).

Cadherins are complexed via their cytoplasmic domain with a set of molecules termed catenins, which link cadherins to the actin-based cytoskeleton. Proper linkage to the cytoskeleton is indispensable for E-cadherin function (10-12). Recently, studies on esophageal, stomach, and breast cancers have suggested that assessment of α-catenin expression by immunohistochemistry might be a better prognostic factor than E-cadherin (13-15). As already mentioned, we have previously reported that reduced expression of E-cadherin is significantly associated with poor prognosis in patients with bladder carcinomas. In this paper, we extend our previous observation by comparing E-cadherin expression with catenin expression to clarify whether or not the expression pattern of catenins could provide additional prognostic value over E-cadherin alone in human bladder cancers. Furthermore, we have also studied the expression of another member of the β-catenin/plakoglobin family, pl20cav, that was also recently shown to coimmunoprecipitate with E-cadherin (16).

MATERIALS AND METHODS

Twenty-two superficial and 26 invasive bladder cancer specimens were included in this study. The male:female ratio of the patients was 39:9, and the age ranged from 42-88 years, with an average of 66.5 years. Tumor grade 1:2:3 ratio was 5:21:22. The patients’ profiles are detailed in Table 1. Normal urothelium was obtained from five normal ureters from patients with renal cell carcinoma and four bladder samples from patients with nonurothelial tumors. Antibodies used were HEC1D (dilution 1:20) for E-cadherin (Takara), anti-β-catenin (1:400) and anti-pl20 (1:200) from Transduction Laboratories, and PG5.1 (1:10) for plakoglobin (Progen Biotechnik GmbH). For α-catenin staining, we developed a polyclonal mouse antiserum (used at the dilution 1:1000). For this, we cloned nucleotides 454-1981 of human α-catenin cDNA (a kind gift from Dr. W. B. Issacs, Johns Hopkins Hospital, Baltimore, MD) into the pRc26 plasmid. The α-catenin/glutathione S-transferase fusion protein was purified and used as an antigen. The serum of one BALB/c female mouse after the second boost was used in this study. Before use, the antiserum was checked by Western blotting and found to recognize a single 120,000 band comigrating with α-catenin (data not shown). Four-μm frozen sections were fixed in 3% paraformaldehyde and then permeabilized in 0.2% Triton X-100 in PBS. Immunohistochemistry was performed using biotinylated secondary antibodies (1:200) and streptavidin biotin peroxidase complex (1:100) from Amersham.

Evaluation of the staining was carried out by three independent observers (T. S., P. P. B., and L. A. G.) without prior knowledge of the tumor stage and patient profile. If the staining pattern in cancer samples was similar to that in normal urothelium (i.e., staining at virtually all intercellular borders) it was evaluated as normal. Abnormal staining was divided into negative (i.e., com-
complete absence of immunoreactivity) and heterogeneous (i.e., tumor composed of positive and negative areas), as described previously (9).

One of the serial sections was stained with H&E for histological evaluation. The grading and staging of the tumor were based on WHO classification (17) and TNM classification of International Union Against Cancer (18), respectively. Correlations among cadherin/catenin expression and tumor grade or stage were evaluated by χ² test. Differences in survival were assessed by log-rank test. To evaluate clinical relevancy, the proportional hazard regression procedure supported by Statistical Analysis System was used.

RESULTS

In normal urothelium, catenin staining outlines the intercellular borders. The luminal membrane and the parts of the cells in contact with the basement membrane do not react with the antibodies (Fig. 1A, D, G, and J). A more pronounced staining at the apical junctional complexes of the superficial cell layer can be seen. The protein p120cat follows the same staining pattern as catenins. These patterns of staining are very similar to those observed with E-cadherin (Ref. 14; Fig. 1M).

Detailed results for each tumor are given in Table 1. As previously found for E-cadherin, the most prevalent abnormal staining is not a complete absence of catenin expression but a heterogeneous immunoreactivity. Normal staining was found in 30 cases for α-catenin, 29 cases for β-catenin, 28 cases for γ-catenin, 30 cases for p120cat, and 26 cases for E-cadherin. Overall, a good correlation was found between E-cadherin expression and the expression of each catenin (Fig. 1B-D except for D, G, J, and M). However, in 10 cases, discrepancies were noticed. This included heterogeneous E-cadherin staining with normal staining for one or several catenin(s) in seven cases (cases 1, 2, 4, 17, 19, 27, and 40 in Table 1), and normal E-cadherin staining with heterogeneous staining for at least one catenin in three cases (cases 13, 31, and 46 in Table 1). Discrepancies between E-cadherin expression and that of p120cat were more frequent.

Correlations between the staining pattern for each antigen studied and the classical pathological data (grade and stage) are detailed in Table 2. As already shown for E-cadherin, a strong inverse correlation was found between immunoreactivity for members of the E-cadherin complex and both grade and stage.
CADHERIN-ASSOCIATED MOLECULES IN BLADDER TUMORS

Fig. 1. Expression of α-catenin (A, B, and C), β-catenin (D, E, and F), γ-catenin (G, H, and I), pl20Cα+ (J, K, and L), and E-cadherin (M, N, and O) in normal urothelium and bladder cancers. Original magnification, ×400. In normal urothelium, α-catenin (A), β-catenin (D), γ-catenin (G), pl20Cα+ (J), and E-cadherin (M) stainings outline the intercellular borders, with the luminal membrane of the superficial cells being devoid of staining. Note the pronounced staining right below the luminal surface, which corresponds to the apical junctional complex. E-cadherin staining has been repeatedly found weaker than catenin and pl20Cα+ staining in normal uroter. In a tumor with a normal staining pattern, α-catenin (B), β-catenin (E), γ-catenin (H), pl20Cα+ (K), and E-cadherin (N) expression is preserved at the intercellular borders. In heterogeneous tumors, some tumor cells show distinct expression of α-catenin (C), β-catenin (F), γ-catenin (I), pl20Cα+ (L), and E-cadherin (O) at intercellular borders, whereas other areas are negative.

Survival analysis showed that reduced expression of all of the antigens studied here correlates with poor survival (Table 3). Actually, the survival curves for E-cadherin and each of its associated molecules are very similar (Fig. 2). However, after stratification by stage, only E-cadherin remained a significant prognostic factor for patients with invasive disease. In this group of patients, decreased expression of α- and β-catenin is associated with poor survival, but without reaching the classical 5% significance threshold. Importantly, no association between survival and plakoglobin or pl20Cα+ expression was found in this group (Table 3). Within the patients with superficial disease, no correlation was found between survival and expression of any of the molecules of the adhesion complex. Proportional hazard regression confirmed that β-catenin, E-cadherin, and α-catenin have a strong predictive value, similar to grade and stage. Plakoglobin and pl20Cα+ staining have a somewhat lower predictive value (Table 4).

DISCUSSION

It has now become clear that the loss of E-cadherin-mediated adhesion is associated with the progression of many carcinomas. Accordingly, in vitro, E-cadherin has been shown to suppress the invasive potential of malignant cells (19, 20). We found previously that decreased E-cadherin expression correlates with poor prognosis for patients with bladder cancer (9). Because proper anchorage to the cytoskeleton is necessary for E-cadherin function, a lack of expression

Table 2: Comparison of E-cadherin and catenin expression with stage (superficial versus invasive) and grade (WHO classification)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Stage</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\chi^2$</td>
<td>$P$</td>
</tr>
<tr>
<td>α-catenin</td>
<td>14.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>β-catenin</td>
<td>21.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>γ-catenin</td>
<td>18.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>pl20Cα+</td>
<td>14.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>18.5</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

α, β, γ, and pl20Cα+ are not significant.

Table 3: Correlation of E-cadherin and catenin expression with survival (log-rank test)

<table>
<thead>
<tr>
<th>Variables</th>
<th>All tumors</th>
<th>Invasive tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\chi^2$</td>
<td>$P$</td>
</tr>
<tr>
<td>α-catenin</td>
<td>16.3</td>
<td>0.0001</td>
</tr>
<tr>
<td>β-catenin</td>
<td>26.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>γ-catenin</td>
<td>12.6</td>
<td>0.0004</td>
</tr>
<tr>
<td>pl20Cα+</td>
<td>6.8</td>
<td>0.009</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>17.0</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

α, not significant.
or defect of the molecules involved in this anchorage can also be responsible for cancer cell invasion. We thus studied catenin expression and compared it with E-cadherin expression to determine what specific prognostic information can be obtained from each antigen. We show that in bladder tumors, decreased expression of each catenin, including $\beta_{120}$, is significantly correlated with poor prognosis.

It has been suggested that in esophageal cancer, $\alpha$-catenin is better correlated with invasion and metastasis than is E-cadherin (13). Our proportional hazard regression analysis does not confirm this for bladder tumors. Actually, we found that $\beta$-catenin, E-cadherin, and $\alpha$-catenin have very similar prognostic values. Thus, any of these molecules can be used in practice to assess bladder tumor prognosis. The clinical potential of this study is demonstrated by the correlation we have found between these three parameters and survival in the subgroup of patients with invasive disease. This correlation is significant for E-cadherin, whereas $P$ does not reach the classical 5% significance threshold for $\beta$- and $\alpha$-catenin. In patients with superficial disease, no correlation was found, probably because most superficial bladder tumors are not life-threatening. Correlation with progression would be more relevant here, but it is difficult to study because it requires a large number of patients to obtain adequate numbers of end points. However, the prognostic value of plakoglobin and $p120^{alt}$ is clearly lower.

In seven cases, heterogeneous staining for E-cadherin was found, whereas $\alpha$-, $\beta$-, and $\gamma$-catenins seemed normally expressed (although the evaluation of intercellular border staining on frozen sections may be difficult in some cases). Because it has been demonstrated that both plakoglobin and $\alpha$-catenin are unstable in L cells lacking cadherin, and that they can be stabilized by either desmosomal cadherins or E-cadherin, respectively (21, 22), the staining pattern found in these seven cases might indicate that catenins are stabilized by molecules other than E-cadherin. Actually, we have evidence that in model

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Table 4: Comparison of the prognostic value of tumor stage, grade, and cell adhesion protein expression using the proportional hazard regression analysis for survival

<table>
<thead>
<tr>
<th>Variable</th>
<th>$X^2$ score</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage</td>
<td>20.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>Grade</td>
<td>15.4</td>
<td>0.0001</td>
</tr>
<tr>
<td>$\alpha$-catenin</td>
<td>16.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>$\beta$-catenin</td>
<td>27.0</td>
<td>0.0001</td>
</tr>
<tr>
<td>$\gamma$-catenin</td>
<td>13.0</td>
<td>0.0003</td>
</tr>
<tr>
<td>$p120^{alt}$</td>
<td>7.0</td>
<td>0.008</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>17.4</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
systems, other members of the classical cadherin family can form a complex with catenins.\(^3\) However, the role of these cadherins in epithelial differentiation and cancer progression is not yet clear.

In three cases, the opposite observation was made; E-cadherin staining was normal, whereas catenin immunoreactivity indicated that no functional complexes were present. In these cases, one can assume epithelial differentiation and cancer progression is not yet clear.

The correlation between E-cadherin expression and \(\text{p}120^{\text{cad}}\) is not as tight as the correlation between E-cadherin and catenin expression. Actually, very little is known about \(\text{p}120^{\text{cad}}\). In particular, its function in the E-cadherin complex is not yet well understood. Recently, it has been shown that \(\text{p}120^{\text{cad}}\) can interact with E-cadherin but not \(\alpha\)-catenin (23). Thus, \(\text{p}120^{\text{cad}}\) does not occupy a place similar to its structural homologues plakoglobin and \(\beta\)-catenin that bridge E-cadherin to \(\alpha\)-catenin.

In conclusion, we have compared the prognostic value of the expression of several catenins with that of E-cadherin in bladder tumors. Although \(\alpha\)-catenin has been studied in several cancers, data on the expression of \(\beta\)-catenin, plakoglobin, and \(\text{p}120^{\text{cad}}\) were previously missing. To our knowledge, this is the first report correlating catenin expression to survival. Interestingly, we have found that \(\beta\)-catenin, E-cadherin, and \(\alpha\)-catenin have a similar prognostic values. Thus, from a practical point of view, staining for any of these antigens can be used as a prognostic indicator. This is of particular interest because it seems difficult to obtain reliable E-cadherin staining on routinely processed (formalin-fixed, paraffin-embedded) samples\(^4\) (15, 24), indicating that staining for catenins may be a more reliable diagnostic tool.

ACKNOWLEDGMENTS

We thank Dr. G. Miller for a critical reading of the manuscript.

REFERENCES


\(^3\) Unpublished observations.

\(^4\) Rayter, personal communication.