Decreased E-Cadherin Expression Is Associated with Poor Prognosis in Patients with Prostate Cancer


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ABSTRACT

Decreased levels of the cell-cell adhesion molecule E-cadherin are associated with loss of differentiation in a number of human carcinomas. However, the value of E-cadherin as a prognostic marker in these cancers is largely undetermined. A previous study of E-cadherin levels in prostate cancer revealed that almost 50% of tumors examined had reduced or absent levels of this protein (Umbas et al., Cancer Res., 52: 5104–5109, 1992). To determine the potential prognostic significance of this finding, prostate cancer specimens from 89 patients were evaluated immunohistochemically for E-cadherin expression, and the results were related to histopathological grade, tumor stage, presence of metastases, and survival.

As previously observed, a significant inverse correlation was found between E-cadherin expression and tumor grade. Importantly, we also found significant correlations between E-cadherin expression and tumor stage and overall survival. Sixty-three percent of the tumors that extended beyond the prostate capsule (T4a) versus 33% of the tumors confined to the prostate (T1a–T3a) had aberrant expression ($\chi^2 = 8.1, P = 0.001$). Seventy-six percent of the primary tumors from patients that presented with metastases showed aberrant staining compared to 32% from patients without metastases ($\chi^2 = 14.9, P = 0.001$). The life table analysis showed a significantly higher survival rate for patients with normal staining compared to patients with aberrant expression ($\chi^2 = 20.4, P = 0.001$ by log rank test). Moreover, abnormal expression of E-cadherin correlated significantly with progression after radical prostatectomy ($P < 0.005$). These results suggest that E-cadherin expression can serve as a prognostic indicator for the biological potential of prostate cancer.

INTRODUCTION

Prostate cancer is one of the most common malignancies in men (1), and most patients will succumb to their disease once it has metastasized. Improvements in the diagnosis of prostate cancer have now resulted in screening programs that reveal many more organ confined lesions, with a concomitant increase in the number of radical surgeries. Whether this truly improves overall survival is not clear because of the unpredictable biological potential of these tumors. Not every prostate cancer will progress (2, 3); on the other hand, most patients will succumb to their disease once it has metastasized. Improvements in the diagnosis of prostate cancer have now resulted in screening programs that reveal many more organ confined lesions, with a concomitant increase in the number of radical surgeries. Whether this truly improves overall survival is not clear because of the unpredictable biological potential of these tumors. Not every prostate cancer will progress (2, 3); on the other hand, most patients will succumb to their disease once it has metastasized.

Prostate cancer has now gained considerable attention, albeit that few of these have yet established clinical value. One of these markers, the epithelial cell adhesion molecule, E-cadherin, is of particular interest since it can function as an invasion suppressor gene (8–10). In normal physiologic conditions, E-cadherin plays an important role in embryonic development, morphogenesis, and maintenance of epithelial integrity (11). Furthermore, loss of E-cadherin expression correlated well with the in vitro invasive phenotype of cancer cell lines (12, 13). Also, in human cancers, E-cadherin expression correlated inversely with tumor grade. Recently, we demonstrated a statistically significant correlation between aberrant expression of E-cadherin and increased grade of human prostate cancer. The staining pattern and intensity of all the well-differentiated tumors (Gleason score 4 and 5) were uniformly positive, whereas almost all of the poorly differentiated and undifferentiated tumors (Gleason score 9 and 10) had aberrant E-cadherin expression (14). In addition, the relationship between E-cadherin expression and patient survival was also studied. The results showed a significant inverse correlation between E-cadherin expression and patient survival ($\chi^2 = 20.4, P = 0.001$ by log rank test). These results suggest that E-cadherin expression can serve as a prognostic indicator for the biological potential of prostate cancer.

MATERIALS AND METHODS

Patients. Eighty-nine prostate cancer patients, treated between January 1987 and December 1991, were included in this study. The mean age at the time of diagnosis was 70 years (range, 42 to 87 years). Clinical tumor staging was determined according to the classification of the Union Against Cancer (15). Bone scan was performed in order to detect metastatic lesions, and further evaluation by radiographs was done in case of a positive bone scan. The treatment options were radical prostatectomy for early stage and hormonal therapy, either medical or surgical, for advanced disease. Only in four cases was external beam radiotherapy with linear acceleration given in combination with hormonal treatment. The patient characteristics are summarized in Table 1.

Surgical Specimens. Transurethral resection or radical prostatectomy specimens were snap frozen. Sections 4–6 μm thick were cut on a cryostat, air-dried, and stored at −20°C until use. One section from each patient was stained with hematoxylin and eosin to assess the histopathological grade according to Gleason (16). In this group of patients, tumor grade ranged from Gleason scores 4 to 10.

Immunohistochemistry. Indirect immunoperoxidase staining was performed as described previously (14) using either anti E-cadherin (Eurodiagnostica BV, Apeldoorn, the Netherlands) or HECD-1 (Takara, Berkeley, CA) monoclonal antibodies. E-cadherin staining is localized on the membrane, particularly at areas of cell-cell contact. In the specimens analyzed, the area of worst differentiation was used for immunohistochemical analysis, i.e., the sample was not randomly chosen. To assess the staining, we used the following criteria: uniformly positive, uniformly negative, or heterogeneous (mixed populations of positive and negative stained cells) as described by Schipper et al. (17). Besides positive or negative staining, some tumors showed a cytoplasmic staining, which was also considered to be abnormal and included in the criteria for heterogeneous staining. Uniformly positive staining patterns were regarded as normal, while uniformly negative and heterogeneous stainings were considered as aberrant expression.

Statistical Analysis. The correlation of E-cadherin expression with tumor grade, clinical stage, pathological stage, and metastases was evaluated by the $\chi^2$ test, adjusted where necessary with the Yates correction. The value of significance was taken as $P < 0.05$. Actuarial survival rate of patients with...
normal and decreased E-cadherin expression were evaluated according to Kaplan-Meier (18), and the differences were tested with a log-rank test. The statistical software used was Statistical Package for the Social Sciences (SPSS/PC+ 4.0, Chicago, IL).

RESULTS

Eighty-nine snap-frozen prostate cancer specimens were stained with the anti-E-cadherin monoclonal antibodies anti-E-cadherin or HECD-1 and scored as described previously (14). The scoring system is based on the biological functional relation between loss of E-cadherin expression and invasiveness, i.e., the presence of a negative subpopulation is considered to have important biological significance. In Fig. 1A, an example of normal expression can be seen with uniformly positive staining on the membrane at cell-cell contacts. Abnormal patterns comprise partially positive and partially negative stainings on the membrane or a cytoplasmic staining (Fig. 1B). Occasionally, no E-cadherin expression at all was found in the entire tumor area evaluated (Fig. 1C). Both of the latter staining patterns were regarded as aberrant expression of this molecule.

Correlation between Decreased E-Cadherin Expression and Local and Distant Progression of the Tumor. In a previous study, we found that E-cadherin expression correlated significantly with tumor grade (Gleason score) in human prostate cancer (14). Considering the putative consequences of loss of E-cadherin function, i.e., acquisition of an increased invasive potential, we evaluated whether aberrant E-cadherin expression also correlated with stage and the presence of metastasis. Indeed, there was a correlation between degree of local extension of the tumor and aberrant expression of E-cadherin (Table 2). All well-differentiated tumors (n = 11) showed normal expression, while 28 and 79% of the moderately and poorly differentiated tumors, respectively, showed aberrant E-cadherin expression. The correlation between aberrant E-cadherin expression and local extension of the tumor (clinical tumor stage) was statistically significant (P < 0.005). Only 33% of the T1–3 tumors (organ confined) showed aberrant E-cadherin expression compared to 63% of T3–4 tumors (locally invasive; Table 2). Moreover, the presence of metastases was significantly correlated with aberrant E-cadherin expression (Table 2; P < 0.001). In tumor tissue from patients without metastatic disease, E-cadherin expression was normal in 68% and aberrant in 32%. On the other hand, E-cadherin expression was normal in 24% and aberrant in 76% of the patients that presented with metastases (Table 2).

Relation between E-Cadherin Expression and Survival. Previous studies on the role of stage and grade as prognostic factors for prostate cancer are ambiguous, in large part due to the limited accuracy of clinical staging procedures. Once pathological staging is taken into account, stage becomes an important prognostic factor (7). Pathological staging is only available in the case of radical surgery; hence,
additional tools are urgently needed. Therefore, we investigated the relationship between E-cadherin expression and survival. The average follow-up time for patients who were still alive at the time of evaluation was 36 months (range, 12 to 71 months). The 3-year overall survival rate of patients with normal E-cadherin expression was significantly higher than for patients with aberrant expression ($X^2 = 20.4, P < 0.001$ by log rank test; Fig. 2). The group of patients described above is heterogeneous; hence, we stratified according to treatment, i.e., radical prostatectomy versus palliative TUR-P.

**Progression after Radical Prostatectomy.** The characteristics of the patients treated by radical prostatectomy are summarized in Table 3. Eleven of 42 patients (26%) had evidence of disease progression (clinical or biochemical progression, i.e., prostate-specific antigen $\geq 0.5$ ng/ml) after 8 to 42 months. All of them but one (91%) showed aberrant E-cadherin expression in their tumor specimens (Table 3). Further analysis showed a significant correlation ($X^2 = 9.4, P < 0.005$ by log rank test) between aberrant E-cadherin staining and progression as presented in Fig. 3.

Among the patients which have capsular penetration (pT $\geq 3$), eight had aberrant E-cadherin expression and seven of them progressed, whereas five had normal E-cadherin expression and only one of them progressed (Table 3).

**Progression after Palliative TUR-P.** Forty-seven patients with advanced disease were treated by TUR-P. We can divide this group into primary TUR-P, followed by hormonal treatment and/or occasionally radiotherapy, and TUR-P after hormone-escaped disease (Table 1).

In the primary TUR-P group, the analysis of survival within 3 years after tumor resection shows that aberrant staining is significantly correlated with poor prognosis ($X^2 = 15.1, P < 0.001$ by log rank test; Fig. 4A).

The mean survival of patients with hormone-escaped disease who underwent a TUR-P was 25 months for patients with normal E-cadherin expression and 17 months for patients with decreased expression. However, the 3-year survival analysis of this group shows no significant correlation between decreased immunoreactivity and survival ($X^2 = 1.2, P < 0.3$ by log rank test; Fig. 4B).

**DISCUSSION**

The relation between E-cadherin expression and grade is now well documented for several carcinomas (17, 19-23) including prostate cancer (14). To evaluate the potential use of E-cadherin immunohistochemistry as a prognostic factor for prostatic cancer patients, we

### Table 2 Relationship of E-cadherin expression to tumor grade, clinical stage, and metastases

<table>
<thead>
<tr>
<th>E-Cadherinexpression</th>
<th>Normal</th>
<th>Decreased</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>(%)</td>
<td>No.</td>
</tr>
<tr>
<td>Gleason score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>11 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>5-7</td>
<td>28 (72)</td>
<td>11 (28)</td>
</tr>
<tr>
<td>8-10</td>
<td>8 (21)</td>
<td>31 (79)</td>
</tr>
<tr>
<td>Clinical stage</td>
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<td></td>
</tr>
<tr>
<td>T1-2</td>
<td>31 (67)</td>
<td>15 (33)</td>
</tr>
<tr>
<td>T3-4</td>
<td>16 (37)</td>
<td>27 (63)</td>
</tr>
<tr>
<td>Metastases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>40 (68)</td>
<td>19 (32)</td>
</tr>
<tr>
<td>+</td>
<td>7 (24)</td>
<td>22 (76)</td>
</tr>
</tbody>
</table>

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3 The abbreviation used is: TUR-P, transurethral resection of the prostate.

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![Figure 2](https://example.com/image2.png)  
**Fig. 2.** Kaplan-Meier overall survival rate related to E-cadherin expression. Bars, censored data. $X^2 = 20.4, P < 0.001$ by log rank test.

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![Table 3](https://example.com/table3.png)  
**Table 3** Characteristics of patients who underwent radical prostatectomy ($n = 42$)

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![Table 4](https://example.com/table4.png)  
**Table 4** Characteristics of patients who underwent radical prostatectomy ($n = 42$)

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4 PSA, prostate-specific antigen; A, alive without disease; B, alive with disease; C, death without disease.

5 With seminal vesicle involvement.

6 After preoperative adjuvant hormonal therapy.
analyzed 89 prostate cancer specimens. We show that aberrant E-cadherin expression (heterogeneous, cytoplasmic, or negative) was found in 33% (15 of 46) of the tumors that were clinically found to be organ confined (T1a–T2) and in 63% (27 of 43) of the lesions that extended beyond the prostatic capsule (T3a–T4). Moreover, 76% of the patients that were diagnosed with metastatic disease showed aberrant E-cadherin expression patterns. This is in good agreement with a study by Schipper et al. (17) and Mattijssen et al. (24) on squamous cell carcinoma of the head and neck that revealed a close correlation, not only between aberrant E-cadherin expression and grade, but also with the presence of lymph node metastases. Furthermore, we now show that overall survival correlated significantly with aberrant E-cadherin expression. When we stratified according to treatment, i.e., radical prostatectomy for localized disease versus palliation in case of locally or distantly metastatic prostate cancer, E-cadherin immunohistochemistry still had prognostic value. It was most striking to see that progression after radical prostatectomy occurred in 67% (10 of 15) patients with aberrant E-cadherin expression and only in 4% (1 of 27) of patients with normal E-cadherin staining. The correlation between aberrant E-cadherin expression and poor prognosis was also evident within the group of patients with tumors that extended beyond the prostatic capsule. Thus, this is the first study that suggests E-cadherin immunohistochemistry to be of value as a prognostic factor in prostate cancer.

It should be noted that in not all of the patients was E-cadherin expression indicative of progression. This may be explained by impaired catenin function through which E-cadherin is anchored to the cytoskeleton (8, 25). A recent study by Shimoyama et al. (26) suggests that loss of α-catenin expression can be causally related to dysfunction of cadherin-mediated interaction in a lung cancer cell line (PC9). More recently, Morton et al. (27) found that, in a prostate cancer cell line (PC3), impaired E-cadherin function could be explained by homozygous deletion of the α-catenin gene. This phenomenon might explain the discrepancy between normal E-cadherin expression and the presence of metastasis at the time of diagnosis. Other mechanisms that can lead to dysfunction of cadherin-mediated interaction are increased tyrosine phosphorylation of β-catenin (28) or mutational inactivation of the E-cadherin gene itself. The latter possibility is particularly interesting since the chromosomal segment to which E-cadherin is mapped (16q21) is frequently lost in prostate cancer development (29, 30). As yet, the mutational inactivation of E-cadherin function has not been found in human prostate cancer and was only reported for a subset of endometrial carcinomas (31) and gastric cancers (32).

Nevertheless, irrespective of the exact mechanism, the loss of E-cadherin function apparently marks the loss of epithelial integrity, which in turn can be an important step in the progression of cancer. It should be noted that, although these findings seem convincing, the follow-up is relatively short. Considering the urgent need for progression markers in prostate cancer, a large scale prospective study is hereby suggested to establish the place of E-cadherin immunohistochemistry in prostate cancer prognoses.

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REFERENCES

E-CADHERIN EXPRESSION AND PROSTATE CANCER PROGNOSIS


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