

# Aberrant E-Cadherin and $\alpha$ -Catenin Expression in Prostate Cancer: Correlation with Patient Survival<sup>1</sup>

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## ABSTRACT

E-cadherin maintains the normal differentiated phenotype in epithelial cells; this function is partly mediated by  $\alpha$ -catenin, which links E-cadherin to the cell cytoskeleton. Dysfunction of E-cadherin *in vitro* and *in vivo* is associated with an invasive phenotype. However, the role of  $\alpha$ -catenin is largely undetermined. We analyzed the expression of E-cadherin and  $\alpha$ -catenin in prostate cancer to assess the relationship of abnormal expression to stage, grade and survival. E-cadherin expression was evaluated in 99 prostate cancers. In 79 of those specimens,  $\alpha$ -catenin was also assessed. In benign prostatic epithelium, both E-cadherin and  $\alpha$ -catenin were expressed uniformly at the cell membrane. Abnormal E-cadherin expression was found in 56% of cancer specimens, whereas  $\alpha$ -catenin expression was abnormal in 42%. Abnormal expression of each molecule was significantly correlated with Gleason score ( $P < 0.0001$ ) and the ratio of resection chippings infiltrated by tumor ( $P < 0.0001$ ). E-cadherin expression was also associated with the extent of disease on the initial bone scan ( $P = 0.017$ ). Univariate analysis showed significantly lower survival rate for patients with abnormal E-cadherin ( $P = 0.0003$ ) or  $\alpha$ -catenin expression ( $P = 0.031$ ). Multivariate regression analysis showed that the prognostic value of E-cadherin was independent of tumor grade but not of metastasis. These results suggest that perturbation of cell-cell adhesion is involved in the progression of prostate cancer and that analysis of E-cadherin expression may be clinically useful.

## INTRODUCTION

E-cadherin is the prime mediator of intercellular adhesion in epithelial cells (1). This transmembrane glycoprotein is localized mainly in the zonula adherens junctions and mediates by its extracellular domain cell-cell adhesion through calcium-dependent, homotypic interactions. Its carboxy cytoplasmic domain is associated with a group of undercoat proteins, termed catenins ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -catenin; Refs. 1 and 2). E-cadherin binds directly to either  $\beta$ -catenin or  $\gamma$ -catenin, whereas  $\alpha$ -catenin links the bound E-cadherin complex to the actin cytoskeleton (2). This binding is essential for the formation of stable cell-cell adhesion and is partly regulated by  $\alpha$ -catenin (3, 4).

Overwhelming evidence suggests that perturbation of E-cadherin-mediated cell adhesion is involved in tumor progression and metastasis. *In vitro*, decreased expression of E-cadherin is associated with an invasive behavior of cancer cells (5–7). *In vivo*, aberrant E-cadherin expression was correlated with poorly differentiated and invasive phenotype in several human cancers (8–13). Similarly, down-regulation of  $\alpha$ -catenin expression was found in esophageal (13), gastric (14), breast (15), and bladder (16) tumors. Furthermore, aberrant E-cadherin and  $\alpha$ -catenin expression appears to be of prognostic value in certain tumors (8, 10, 16).

In prostate cancer, reduced or absent E-cadherin expression has

been correlated with loss of differentiation, advanced clinical stage, and poor survival (17–21). Altered expression of  $\alpha$ -catenin was also found in several prostate cancer cell lines (22). In PC-3 prostate cancer cells, homozygous deletion of  $\alpha$ -catenin gene results in complete loss of  $\alpha$ -catenin expression. Interestingly, these cells express E-cadherin normally at the cell membrane. However, there are no *in vivo* studies on the coexpression of E-cadherin and  $\alpha$ -catenin in human prostate cancer. Therefore, we evaluated the expression of E-cadherin and  $\alpha$ -catenin in formalin-fixed, paraffin-embedded specimens from 99 patients with prostate cancer. In addition, we investigated the relationship between their expression and tumor grade and stage and patient survival.

## PATIENTS AND METHODS

**Patients and Tissue Specimens.** We studied 99 patients with carcinoma of the prostate who underwent TUR-P<sup>3</sup> at the Royal Free Hospital between 1984 and 1992. We reviewed the clinical records and cases were included for study if follow-up data were available. Treatment for localized disease was either watchful waiting or radical radiotherapy ( $n = 35$ ); treatment for locally advanced or metastatic disease was androgen deprivation therapy ( $n = 64$ ). Patients were reviewed every 3 months for the first year and every 6 months after that. Total follow-up ranged from 1 to 114 months (mean, 41 months). The mean age at presentation was 74 years.

Archival blocks for each case were reviewed, and the percentage of resection chippings infiltrated with cancer cells was used for evaluation of the local tumor extent (23). Histopathological grading was assessed using the Gleason score (24). The initial staging isotope bone scans were reviewed and scored according to Soloway *et al.* (25). Ten specimens of benign prostatic hyperplasia from TUR-P were also examined.

**Antibodies.** A mouse monoclonal antibody (HECD-1) against human E-cadherin (British Biotechnology, Oxford, United Kingdom) was used at 1:1000 dilution. The  $\alpha$ -18 monoclonal anti- $\alpha$ -catenin antibody (4) was used at 1:40 dilution.

**Immunohistochemistry.** A standard streptavidin-biotin indirect immunoperoxidase method was used for immunostaining as described elsewhere (11, 26, 27). To enhance antigen retrieval, sections were microwave pretreated in 0.01 M citrate buffer (pH 6.0) at 700 W for 25 min. Thereafter, sections were incubated sequentially with the primary antibodies, biotinylated secondary antibodies, and streptavidin-biotin-peroxidase complex. The peroxidase reaction was visualized with a solution of 3,3'-diaminobenzidine tetrahydrochloride (Sigma, Poole, United Kingdom) supplemented with 0.01% hydrogen peroxide. Paraffin-embedded and fresh frozen sections of normal colonic epithelium of homogeneous immunophenotype for the studied antigens were included as positive controls. Negative controls had the primary antibody omitted and replaced by PBS.

**Evaluation of Immunostaining.** Sections were examined by two independent observers (P. J. M. R. and M. P.). The intensity and cellular localization of immunostaining were evaluated using the adjacent normal prostatic epithelium as an internal positive control. If the staining pattern in tumor cells was similar to that in normal epithelial cells (*i.e.*, membranous staining) the antigen expression was considered normal. Heterogeneous (*i.e.*, mixed areas of positive and negative cells), uniformly negative (*i.e.*, less than 10% of stained cells), and altered cellular distribution (*i.e.*, cytoplasmic or nuclear) of immunostaining were evaluated as abnormal as described previously (11, 17, 20, 26, 27).

**Statistical Analysis.** Correlations between antigen expression and tumor grade, stage, and metastasis were evaluated by Fisher's exact test and the  $\chi^2$

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

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test for trend. Survival rates were calculated by the Kaplan-Meier method, and the differences were evaluated by the log-rank test. The prognostic value of E-cadherin and  $\alpha$ -catenin expression in relation to other pathological variables was assessed by multivariate Cox regression analysis. *P* values less than 0.05 were considered statistically significant.

## RESULTS

**E-Cadherin and  $\alpha$ -Catenin Expression.** In benign prostatic epithelium, E-cadherin and  $\alpha$ -catenin expression was localized uniformly

to the cell membrane particularly at the intercellular junctions (Fig. 1, A and B). Forty-four (44%) of the tumors showed normal E-cadherin expression. Abnormal E-cadherin expression (heterogeneous or negative staining) was evident in 55 of 99 (56%) tumors. Seventy-nine tumor specimens were stained with the anti- $\alpha$ -catenin antibody. Forty-six (58%) of these tumors were evaluated as normal (Fig. 1C), whereas abnormal  $\alpha$ -catenin expression was seen in 33 of 79 cases (42%; Fig. 1, D and E). Results of E-cadherin and  $\alpha$ -catenin coexpression in 79 tumors revealed four immunophenotypes, as shown in

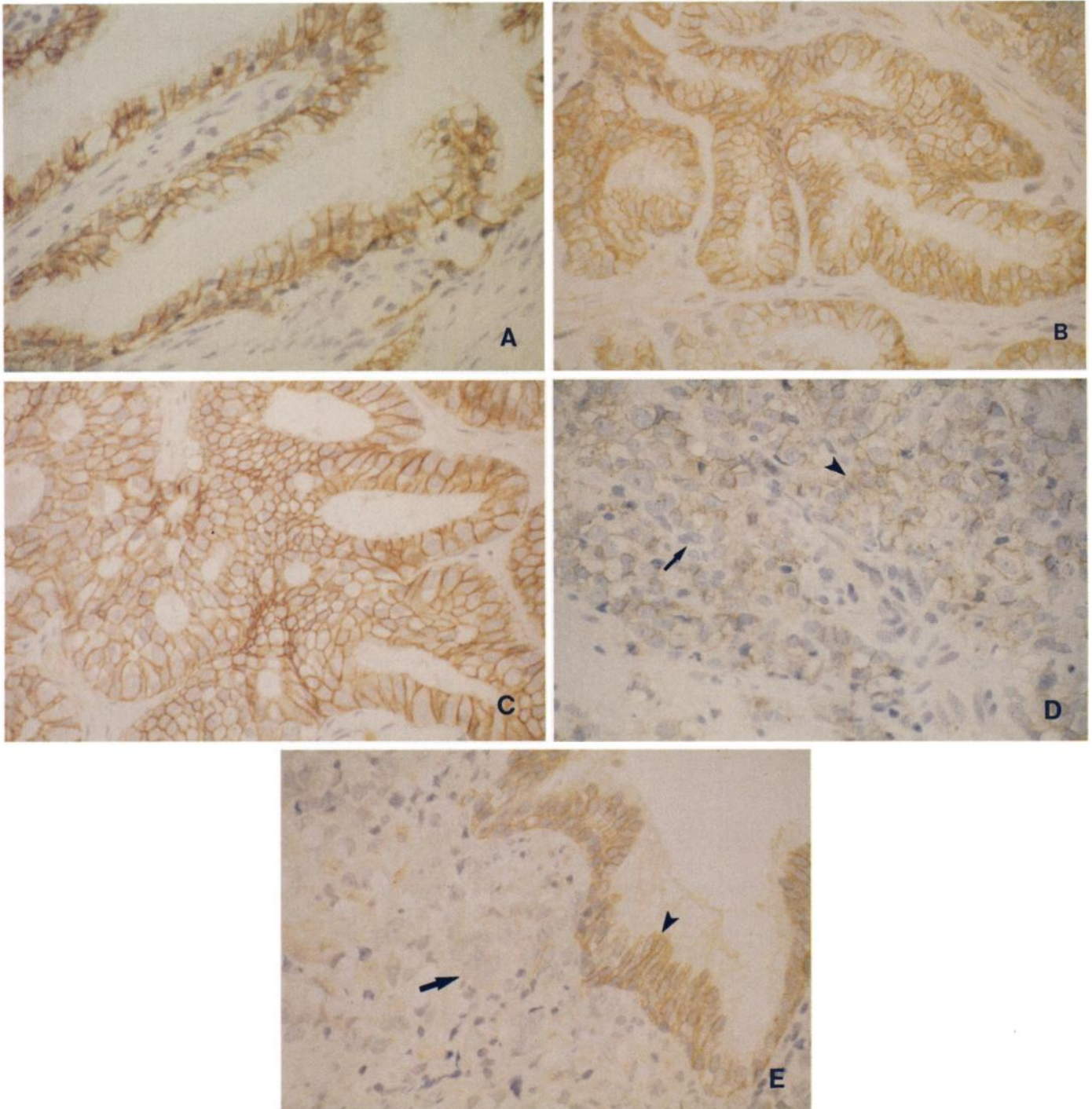


Fig. 1. A, normal membranous E-cadherin expression in benign prostatic hyperplasia. B, normal  $\alpha$ -catenin expression confined to the intercellular borders in normal prostatic epithelium. C, preserved membranous  $\alpha$ -catenin expression in a well-differentiated (Gleason score, 2) prostatic adenocarcinoma. D, heterogeneous immunostaining for  $\alpha$ -catenin in a poorly differentiated (Gleason score, 8) tumor; some cells show positive membranous staining (arrowhead), whereas other cells exhibit negative staining (arrow). E, completely negative immunostaining for  $\alpha$ -catenin in a poorly differentiated (Gleason score, 9) tumor (arrow). Note that the adjacent cells in an area of benign prostatic hyperplasia show normal membranous  $\alpha$ -catenin expression (arrowhead).  $\times 200$ .

Table 1 Relationship between E-cadherin and  $\alpha$ -catenin expression (n = 79)

E-cadherin expression	No.	$\alpha$ -Catenin expression		Significance
		Normal	Reduced	
Normal	33	32 (40) <sup>a</sup>	1 (1)	$\chi^2 = 34.97^b$ P < 0.0001
Reduced	46	14 (18)	32 (40)	
Total	79	46	33	

<sup>a</sup> Values in parentheses are percentages.

<sup>b</sup> Fisher's exact test.

Table 2 Relationship between E-cadherin expression and tumor grade, stage, and metastasis (n = 99)

	No.	E-Cadherin expression		Significance
		Normal	Reduced	
All cases	99	44 (44) <sup>a</sup>	55 (56)	$\chi^2 = 22.49^b$ P < 0.0001
Gleason score				
2-4	13	12 (92)	1 (8)	
5-7	58	28 (48)	30 (52)	$\chi^2 = 28.72^b$ P < 0.0001
8-10	28	4 (14)	24 (86)	
Ratio of TUR-P chips <sup>c</sup>				
<0.05	19	17 (89)	2 (11)	$\chi^2 = 5.65^b$ P = 0.017
0.05-0.50	31	18 (58)	13 (42)	
0.50-0.75	16	3 (19)	13 (81)	
>0.75	33	6 (18)	27 (82)	
Metastasis <sup>d</sup>				$\chi^2 = 5.65^b$ P = 0.017
Absent	57	32 (56)	25 (44)	
1-5 lesions	21	8 (38)	13 (62)	
≥6 lesions	16	4 (25)	12 (75)	

<sup>a</sup> Values in parentheses are percentages.

<sup>b</sup>  $\chi^2$  test for trend.

<sup>c</sup> Ratio of the number of chips with cancer to the total number of chips.

<sup>d</sup> As assessed by bone scintigraphy in 94 patients.

Table 1. All E-cadherin normal tumors except one also showed normal  $\alpha$ -catenin expression. Of the 46 tumors with aberrant E-cadherin expression, 32 (70%) also showed abnormal  $\alpha$ -catenin expression, whereas the remaining 14 (30%) expressed normal  $\alpha$ -catenin immunoreactivity. However, 32 of 79 (40%) tumors showed concomitantly either normal or aberrant expression of both E-cadherin and  $\alpha$ -catenin. The correlation between E-cadherin and  $\alpha$ -catenin expression was highly significant (P < 0.0001 by Fisher's exact test).

**Correlation between E-Cadherin and  $\alpha$ -Catenin Expression and Clinicopathological Features.** A significant inverse correlation was found between E-cadherin expression and tumor differentiation (Table 2). The majority (92%) of well-differentiated tumors (Gleason score, 2-4) showed normal E-cadherin expression. In contrast, 52% of moderately (Gleason score, 5-7) and 86% of poorly (Gleason score, 8-10) differentiated tumors showed aberrant E-cadherin expression (P < 0.0001 by the  $\chi^2$  test for trend). E-cadherin expression was also significantly correlated with the local tumor extent as assessed by the ratio of tumor-infiltrated resection chips. There was a distinct trend toward reduced E-cadherin expression as the local extension of the tumor increased (P < 0.0001 by the  $\chi^2$  test for trend). In 94 patients, a staging bone scan was performed. Abnormal E-cadherin expression in primary tumors was significantly correlated with the presence and the number of metastatic lesions on the bone scan (P = 0.017 by the  $\chi^2$  test for trend; Table 2).

Preoperative serum prostate-specific antigen values were available in only 30 of the patients in this study; it is of interest that the median prostate-specific antigen for those cases with aberrant E-cadherin expression was 45 ng/ml (n = 10) versus 14 ng/ml (n = 20) with normal expression.

The relationship between  $\alpha$ -catenin expression and clinicopathological features is shown in Table 3. As already shown for E-cadherin, there was a significant inverse correlation between  $\alpha$ -catenin expression and tumor grade. Abnormal  $\alpha$ -catenin expression was found more frequently in poorly differentiated than in moderately or well-

differentiated tumors (P < 0.0001 by the  $\chi^2$  test for trend). A significant correlation between abnormal  $\alpha$ -catenin expression and the degree of local tumor extent was also found (P = 0.0001 by the  $\chi^2$  test for trend). There was no statistically significant correlation between abnormal  $\alpha$ -catenin expression and the presence of metastases on bone scan (Table 3).

**Correlation between E-Cadherin and  $\alpha$ -Catenin Expression and Survival.** Results from E-cadherin and  $\alpha$ -catenin immunostaining were combined to analyze the effect on survival of both markers alone and in combination. Survival analysis showed that abnormal  $\alpha$ -catenin expression was correlated with poor survival (P = 0.031 by the log-rank test; Fig. 2) as was abnormal E-cadherin expression (P = 0.0003 by the log-rank test; Fig. 3). Concerning the four immunophenotypes, survival curves for the phenotypes in which either E-cadherin or  $\alpha$ -catenin or both were abnormal were virtually identical, and therefore, these three groups were combined into one. The survival rate of patients with normal expression of both E-cadherin and  $\alpha$ -catenin was higher when compared to those with aberrant expression of either one or both of the markers (Fig. 4). In multivariate regression analysis, when only Gleason score and E-cadherin expression were considered in Cox's model, E-cadherin expression was a significant prognostic factor for survival independ-

Table 3 Relationship between  $\alpha$ -catenin expression and tumor grade, stage, and metastasis (n = 79)

	No.	$\alpha$ -Catenin expression		Significance
		Normal	Reduced	
All cases	79	46 (58) <sup>a</sup>	33 (42)	$\chi^2 = 16.58^b$ P < 0.0001
Gleason score				
2-4	22	20 (91)	2 (9)	
5-7	39	21 (54)	18 (46)	$\chi^2 = 16.11^b$ P < 0.0001
8-10	18	5 (28)	13 (72)	
Ratio of TUR-P chips <sup>c</sup>				
<0.05	20	18 (90)	2 (10)	$\chi^2 = 1.88^b$ P = 0.17 <sup>c</sup>
0.05-0.50	19	13 (68)	6 (32)	
0.50-0.75	9	4 (44)	5 (56)	
>0.75	31	11 (35)	20 (65)	
Metastasis <sup>d</sup>				$\chi^2 = 1.88^b$ P = 0.17 <sup>c</sup>
Absent	45	28 (62)	17 (38)	
1-5 lesions	15	11 (73)	4 (27)	
≥6 lesions	14	5 (36)	9 (64)	

<sup>a</sup> Values in parentheses are percentages.

<sup>b</sup>  $\chi^2$  test for trend.

<sup>c</sup> Ratio of the number of chips with cancer to the total number of chips.

<sup>d</sup> As assessed by bone scintigraphy in 74 patients.

<sup>e</sup> Not significant.

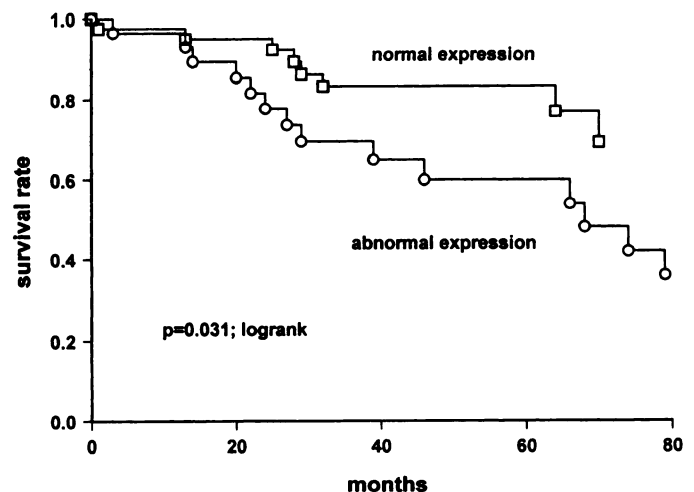


Fig. 2. Kaplan-Meier survival curve in relation to  $\alpha$ -catenin expression in 79 patients. Data points, prostate cancer-associated deaths.

ent of tumor grade ( $P = 0.015$  by the log-rank test; Table 4). When the bone scan data were also taken into account, neither E-cadherin expression nor Gleason score was independently predictive of survival. Similarly, the predictive values of abnormal  $\alpha$ -catenin expression, as well as the combination of abnormal E-cadherin and  $\alpha$ -catenin expression, were not independent of tumor grade, stage, or metastases (data not shown).

## DISCUSSION

In this study, we evaluated the expression of E-cadherin and  $\alpha$ -catenin immunohistochemically in microwave-treated, formalin-fixed, paraffin-embedded tissue specimens of prostate cancer. Previous studies have shown that microwave antigen retrieval is an efficient and reliable method to detect E-cadherin protein in a variety of formalin-fixed, paraffin-embedded tissues (10–12, 26, 27). More recently, it was also used for the detection of E-cadherin in paraffin-embedded prostatic tissue (19).

In the present study, 56% of tumors showed abnormal E-cadherin expression, which is comparable to previous studies in cryostat sections (17, 18). The frequency of abnormal  $\alpha$ -catenin was similar (42%), with high concordance between E-cadherin and  $\alpha$ -catenin expression. E-cadherin and  $\alpha$ -catenin expression was concomitantly

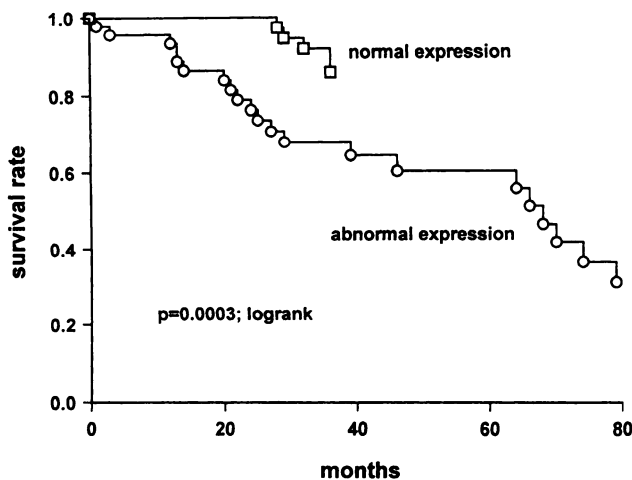


Fig. 3. Kaplan-Meier survival curve in relation to E-cadherin expression in 99 patients. Data points, prostate cancer-associated deaths.

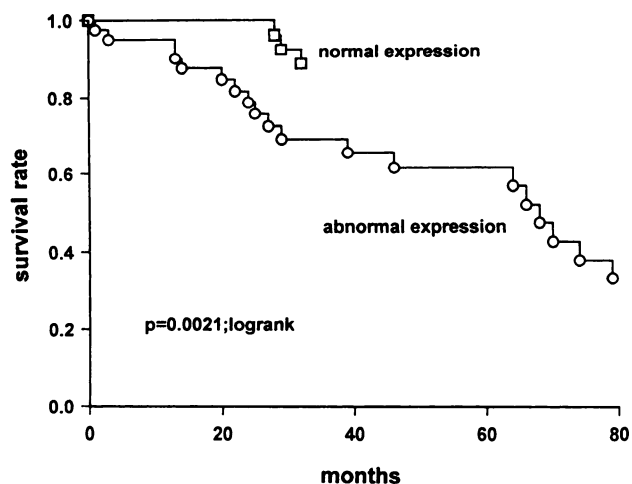


Fig. 4. Kaplan-Meier survival curve in relation to combined E-cadherin and  $\alpha$ -catenin expression in 79 patients. Data points, prostate cancer-associated deaths.

Table 4 Prostate cancer associated deaths as a function of E-cadherin expression and tumor grade in 93 patients<sup>a</sup>

Gleason score	E-Cadherin expression	
	Normal	Reduced
≤7	5/39 (13) <sup>b</sup>	11/29 (38)
≥8	0/3 (0)	10/22 (45)

<sup>a</sup> In six patients, status was unknown.

<sup>b</sup> Values represent prostate cancer-associated deaths; percentages are in parentheses.

either normal or abnormal in 80% of tumors. Interestingly, abnormal  $\alpha$ -catenin expression always coexisted with altered E-cadherin expression. Only one tumor showed abnormal expression of  $\alpha$ -catenin in the presence of normal E-cadherin expression. *In vitro* studies have shown that L-cells lacking endogenous cadherin also have a minimal amount of  $\alpha$ -catenin, despite the normal expression of  $\alpha$ -catenin mRNA (28). However, their transfection with E-cadherin cDNA resulted not only in cadherin up-regulation but also in  $\alpha$ -catenin up-regulation without changes in  $\alpha$ -catenin mRNA. When carboxy-tail truncated E-cadherin, which cannot bind to  $\alpha$ -catenin, was used for transfection,  $\alpha$ -catenin expression did not increase (28). These findings suggest that  $\alpha$ -catenin binding to E-cadherin is essential for  $\alpha$ -catenin protein expression. It is likely that reduced expression of  $\alpha$ -catenin *in vivo*, like that observed in this study, could be a consequence of E-cadherin down-regulation. This hypothesis is supported by our finding that reduced  $\alpha$ -catenin expression was seen only in one tumor specimen in which E-cadherin was still normally expressed on the cell membrane.

Results from immunohistochemical studies on esophageal and gastric cancer suggested that  $\alpha$ -catenin expression might be a better indicator of tumor invasion and metastasis than E-cadherin expression. (13, 14). This has not been confirmed in prostate cancer. In this study, abnormal  $\alpha$ -catenin expression was significantly correlated with tumor grade and local tumor extent but not with metastases. In contrast, abnormal E-cadherin expression was a more sensitive indicator of tumor differentiation and local invasion in accordance with previous reports (17–20) and was also associated with the presence and the extent of distant metastases. Survival analysis showed a similar prognostic value for E-cadherin,  $\alpha$ -catenin, and their combination in univariate analysis. In multivariate regression analysis, neither E-cadherin nor  $\alpha$ -catenin (alone or combined) nor Gleason score were independently predictive of prognosis when allowing for bone scan data. If Gleason score and E-cadherin expression are considered without clinical staging data, then E-cadherin is of important prognostic value. In practice, clinical staging information undoubtedly would be available, but the observations in this study highlight the important role that loss of E-cadherin-mediated cell adhesion has in the molecular biology of prostate cancer progression (29).

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