A Switch from E-Cadherin to N-Cadherin Expression Indicates Epithelial to Mesenchymal Transition and Is of Strong and Independent Importance for the Progress of Prostate Cancer

Karsten Gravdal, Ole J. Halvorsen, Svein A. Haukaas, and Lars A. Akslen

Abstract

Purpose: Cell adhesion molecules are of crucial importance in cancer invasion and metastasis. Epithelial to mesenchymal transition, characterized by reduced E-cadherin and increased N-cadherin expression, has been recognized as a feature of aggressive tumors, but the importance of this phenotype has not been settled in human prostate cancer. We here present novel data, with special focus on the independent relationship between an E-cadherin to N-cadherin switch (EN-switch) and patient prognosis.

Experimental Design: Tissue microarray sections from a consecutive series of 104 radical prostatectomies during 1988 to 1994 with detailed clinical-pathologic data and long follow-up were studied immunohistochemically for the expression of E-cadherin, N-cadherin, P-cadherin, β-catenin, and p120CTN.

Results: Low E-cadherin expression was significantly associated with adverse clinical-pathologic features, whereas other biomarkers were mostly related to Gleason score. In univariate survival analyses, cadherin switching (high N-cadherin and low E-cadherin) showed strong and significant associations with multiple end points of progression and cancer-specific death. Expression of the “basal cell marker” P-cadherin was associated with shorter time to skeletal metastasis ($P = 0.036$). In multivariate analysis of time to clinical recurrence, the “EN-switch” (hazard ratio, 4.3; $P < 0.0005$) had strong and independent prognostic effect, together with Gleason score.

Conclusion: These novel data unravel the importance of epithelial to mesenchymal transition for prostate cancer progression, and demonstration of a switch from E-cadherin to N-cadherin expression could have significant effect on the care of prostate cancer patients.
On this background, the aim of our study was to examine a panel of cell adhesion molecules (E-cadherin, N-cadherin, P-cadherin, β-catenin, and p120CTN) with reference to clinico-pathologic phenotype and prognostic information in prostate cancer and with special focus on the significance of EMT.

Materials and Methods

Patients
As described previously (25, 26), a consecutive series of 104 men treated by radical prostatectomy for clinically localized prostate cancer during 1988 to 1994, with long and complete follow-up, was included. Clinical stage T1/T2 disease, negative bone scan, and generally good health were the prerequisites for radical retropubic prostatectomy. The majority of cancers in this series are clinical stage T2 and presented before the prostate-specific antigen (PSA) era started in Norway in the mid-1990s. Consequently, the prevalence of adverse prognostic factors, such as capsular penetration, seminal vesicle invasion, and positive surgical margins, is rather high compared with most contemporary series. No patients treated by radical prostatectomy received radiotherapy before biochemical failure or clinical recurrence.

Clinicopathologic variables
Two separate Gleason scores were recorded: one standard score from radical prostatectomy specimens and one local score on the 1 to 2 cm² tumor area from which tissue microarray samples were punched. Further, WHO histologic grade (26), largest tumor dimension, capsular penetration, seminal vesicle invasion, involvement of surgical margins, presence of lymph node and skeletal metastasis, clinical and pathologic stage, and serum PSA (s-PSA) level before and after surgical treatment were recorded. Tumor cell proliferation (Ki-67; ref. 27), microvessel density (26), vascular proliferation index (25), glomeruloid microvascular proliferation (28), and expression of
PTEN (29) and p27 (29) were included from previous studies for comparison.

**Tissue sampling**

The entire prostate was cut into 5-mm transverse serial sections. Based on H&E-stained slides, the area of highest histologic tumor grade was identified and cut out of the paraffin blocks, reembedded in paraffin, and sectioned for immunohistochemistry for estimation of Ki-67 (27), microvessel density (26), vessel proliferation by Ki-67/F-VIII double staining (25), and glomeruloid microvascular proliferation (28).

**Tissue microarrays**

The tissue microarray technique has been described and validated in several studies (30–33). This method has been used in our studies of prostate cancers (25, 29) and expression of cell adhesion markers in other tumors (34, 35). Representative tumor areas were identified on H&E slides, and three tissue cylinders (diameter of 0.6 mm) were punched from the donor block and mounted into a recipient paraffin block (30).

**Immunohistochemistry**

Immunohistochemical staining was done on formalin-fixed and paraffin-embedded tissue using 5-μm sections from tissue microarray blocks as described (25). Western blot analysis confirming the specificity of the antibodies against E-cadherin, N-cadherin, P-cadherin, and β-catenin has been done and described in a recent report from our research group (35).

**E-cadherin.** After microwave antigen retrieval (boiling for 15 min at 350 W) in citrate buffer (pH 6.0), slides were incubated overnight at 4°C with the monoclonal mouse E-cadherin antibody M3612 (diluted 1:400; DAKO). Staining was done on TechMate 500 automated slide processing equipment (DAKO).

**N-cadherin.** After boiling in Tris-EDTA buffer (pH 9.0) for 20 min at 350 W, solutions were incubated for 1 h at room temperature with the monoclonal mouse antibody M3613 (diluted 1:25; DAKO).

**P-cadherin.** After boiling for 20 min at 350 W in target retrieval solution (DAKO) buffer (pH 6.0), an autostainer (DAKO) was used for staining, with incubation for 1 h at room temperature with the monoclonal mouse antibody C24120 (diluted 1:100; BD Transduction Laboratories).

**β-catenin.** After microwave retrieval in citrate buffer (pH 6.0; boiling for 15 min at 350 W), tissue microarray slides were incubated for 25 min at room temperature with the monoclonal mouse antibody clone 14 (diluted 1:800; BD Transduction Laboratories) using TechMate 500 automated slide processing equipment for staining.

**p120CTN.** After boiling for 15 min at 500 W in target retrieval solution buffer (pH 6.0), an autostainer was used with incubation for 1 h at room temperature with a monoclonal mouse antibody (clone 98; diluted 1:3,000; BD Transduction Laboratories).

Staining for N-cadherin, P-cadherin, and p120CTN was done using the EnVision-labeled polymer method, with commercial kits (DAKO). For E-cadherin and β-catenin, the standard avidin-biotin method was used. The peroxidase was localized by the diaminobenzidine tetra-chloride peroxidase reaction and counterstained with Mayer’s hematoxylin. Negative controls were obtained using isotypic mouse immunoglobulin (IgG1). Samples with known reactivity were used as positive controls (e.g., liver, colon, epidermis, endometrium, and multitissue sections).

**Evaluation of staining results in prostate tissues**

E-cadherin stained cell membranes consistently in benign and variably in malignant epithelium (Fig. 1A and B). N-cadherin was negative in benign epithelium; mostly incomplete but distinct membranous staining was found in a subgroup of cancers (Fig. 1C).

## Table 1. Associations between E-cadherin, N-cadherin, and P-cadherin expression and clinicopathologic features in 104 patients with clinically localized prostate adenocarcinoma (radical prostatectomies)

<table>
<thead>
<tr>
<th>Variable</th>
<th>E-cadherin*</th>
<th>N-cadherin*</th>
<th>P-cadherin*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (n (%))</td>
<td>High (n (%))</td>
<td>P (n (%))</td>
</tr>
<tr>
<td>Gleason score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤3 + 4</td>
<td>11 (22)</td>
<td>38 (78)</td>
<td>0.008</td>
</tr>
<tr>
<td>&gt;3 + 3</td>
<td>26 (47)</td>
<td>29 (53)</td>
<td></td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤31</td>
<td>24 (31)</td>
<td>54 (69)</td>
<td>0.05</td>
</tr>
<tr>
<td>&gt;31</td>
<td>13 (52)</td>
<td>12 (48)</td>
<td></td>
</tr>
<tr>
<td>Seminal vesicle invasion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>18 (26)</td>
<td>51 (74)</td>
<td>0.005</td>
</tr>
<tr>
<td>Present</td>
<td>19 (54)</td>
<td>16 (46)</td>
<td></td>
</tr>
<tr>
<td>Extra prostatic extension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>6 (19)</td>
<td>26 (81)</td>
<td>0.017</td>
</tr>
<tr>
<td>Present</td>
<td>31 (43)</td>
<td>41 (57)</td>
<td></td>
</tr>
<tr>
<td>Lymph node infiltration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>32 (33)</td>
<td>65 (67)</td>
<td>0.04</td>
</tr>
<tr>
<td>Present</td>
<td>5 (71)</td>
<td>2 (29)</td>
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<tr>
<td>pT2</td>
<td>5 (17)</td>
<td>25 (83)</td>
<td>0.01</td>
</tr>
<tr>
<td>≥pT3</td>
<td>32 (43)</td>
<td>42 (57)</td>
<td></td>
</tr>
</tbody>
</table>

*Membranous expression.

†Pearson χ².

‡Standard Gleason score in radical prostatectomy specimens.

§Largest tumor dimension in prostatectomy specimen.

‖Capsular penetration.

¶Pelvic lymph node infiltration at radical prostatectomy.

**Pathologic stage, tumor-node-metastasis (pTNM).
and D). Cytoplasmic N-cadherin staining with variable intensity was also recorded. P-cadherin stained basal cells. In malignant cells, positive cases showed mixed cytoplasmic and membranous staining (Fig. 1F); other cases were weak or negative (Fig. 1E). β-Catenin showed a mixed cytoplasmic and membranous staining in benign epithelium; both membranous and nuclear staining were found in malignant epithelium (Fig. 1G and H), and these were positively correlated (P = 0.008). p120CTN showed membranous staining particularly in basal cells, and variable membranous staining was also recorded. P-cadherin stained basal cells. In malignant cells, positive cases showed mixed cytoplasmic and membranous staining (Fig. 1F); other cases were weak or negative (Fig. 1E).

Follow-up
Postoperatively, s-PSA, locoregional tumor recurrences, distant metastases, and patient survival were recorded (26). Time from surgery until biochemical failure (defined as persistent or rising s-PSA level of >0.5 ng/mL in two consecutive blood samples) was noted. Further, a tumor in the prostatic fossa or evidence of distant metastasis on bone scan, X-ray, or magnetic resonance imaging was recorded as clinical recurrence. The last time of follow-up was December 2001 (25).

Median follow-up time was 95 months (7.9 years). No patients were lost because of insufficient data. Sixty-seven patients experienced biochemical failure, 31 patients had clinical recurrence, and 9 patients died of prostate cancer.

Statistics
Associations between variables were assessed by Pearson’s χ² test or the Mann-Whitney U test. Univariate survival analysis was done by the Kaplan-Meier method (log-rank test), and multivariate survival analysis was done by the proportional hazards method (likelihood ratio test). Model assumptions were examined by log-log plots. The Statistical Package for the Social Sciences statistical package 13.0 (SPSS, Inc.) was used.

Results

Clinicopathologic characteristics
Largest tumor dimension (median) was 28 mm (range, 10-45 mm). Fifteen tumors (14.4%) had a standard Gleason score of 0 to 6, 34 tumors (32.7%) had a Gleason score of 3 + 4, 33 tumors (31.7%) had a Gleason score of 4 + 3, and 22 tumors (21.2%) had score of 8 to 10. Standard Gleason score was significantly correlated with local Gleason score (Spearman’s rho = 0.65; P < 0.0005). Capsular penetration was shown in 72 cases (69.2%), seminal vesicle invasion in 35 cases (33.7%), and positive surgical margins in 55 cases (52.9%). Pelvic lymph node infiltration was found in seven patients (6.7%) at time of surgery. The median preoperative s-PSA was 11.2 ng/mL (range, 1.8-70.0). More details are available (26).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low (%)</th>
<th>High (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>M</strong>-β-catenin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤≤ ≤ ≤ 4</td>
<td>15 (31)</td>
<td>34 (69)</td>
<td>0.008</td>
</tr>
<tr>
<td>≥ ≥ ≥ ≥ 4 3</td>
<td>31 (56)</td>
<td>24 (44)</td>
<td></td>
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<tr>
<td>Diameter (mm)**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤≤ ≤≤ 31</td>
<td>32 (41)</td>
<td>46 (59)</td>
<td></td>
</tr>
<tr>
<td>&gt; &gt; &gt; &gt; 31</td>
<td>14 (56)</td>
<td>11 (44)</td>
<td></td>
</tr>
<tr>
<td>Seminal vesicle invasion</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Present</td>
<td>15 (43)</td>
<td>20 (57)</td>
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</tr>
<tr>
<td>Extra prostatic extension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>13 (41)</td>
<td>19 (59)</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>33 (46)</td>
<td>39 (54)</td>
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</tr>
<tr>
<td>Lymph node infiltration**</td>
<td></td>
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<tr>
<td>Absent</td>
<td>42 (43)</td>
<td>55 (57)</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>4 (57)</td>
<td>3 (43)</td>
<td></td>
</tr>
<tr>
<td>Pathologic stage **† †††</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>pT2</td>
<td>12 (40)</td>
<td>18 (60)</td>
<td></td>
</tr>
<tr>
<td>≥ ≥ pT3</td>
<td>34 (77)</td>
<td>40 (23)</td>
<td></td>
</tr>
<tr>
<td><strong>N</strong>-β-catenin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>24 (49)</td>
<td>25 (51)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>31 (56)</td>
<td>24 (44)</td>
<td></td>
</tr>
<tr>
<td><strong>p120CTN</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>17 (35)</td>
<td>32 (65)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>35 (64)</td>
<td>20 (36)</td>
<td></td>
</tr>
</tbody>
</table>

*Membranous expression.**
† Nuclear expression.
‡ Pearson χ².
§ Standard Gleason score in radical prostatectomy specimens.
¶ Largest tumor dimension in prostatectomy specimen.
† Capsular penetration.
** Pelvic lymph node infiltration at radical prostatectomy.
†† Tumor-node-metastasis (pTNM).
Cell adhesion markers

**E-cadherin.** Weak membrane expression (36%) was significantly associated with all adverse clinicopathologic variables (Table 1), including poor WHO histologic grade ($P = 0.001$). Positive membranous expression (34%) was associated with poor WHO histologic differentiation ($P = 0.030$), seminal vesicle invasion ($P = 0.006$), and pelvic lymph node infiltration ($P = 0.029$; Table 1). Local Gleason score $\geq 4 + 3$ showed a trend ($P = 0.083$). Cytoplasmic N-cadherin expression was associated with increased proliferation by Ki-67 ($P = 0.012$).

**P-cadherin.** Positive membranous expression (44%) was significantly related to increased local Gleason score $\geq 4 + 3$ ($P = 0.002$) and to the poorly differentiated group by WHO ($P = 0.04$).

**β-catenin.** Weak membranous expression (44%) was significantly related to standard Gleason score $\geq 4 + 3$ ($P = 0.008$; Table 2), poor WHO histologic grade ($P = 0.036$), and increased preoperative s-PSA ($P = 0.019$). Positive nuclear expression (47%) was related to the poorly differentiated group by WHO ($P = 0.033$) and presence of glomeruloid microvascular proliferations ($P = 0.05$).

**p120^{CTN}.** Weak membranous expression (50%) was significantly associated with standard Gleason score $\geq 4 + 3$ ($P = 0.003$), pelvic lymph node infiltration ($P = 0.013$; Table 2), and advanced clinical stage ($P = 0.047$).

Associations among cell adhesion markers

Weak expression of membranous E-cadherin was strongly associated with presence of membranous N-cadherin ($P < 0.0005$), presence of membranous P-cadherin ($P < 0.0005$), weak membranous expression of β-catenin ($P < 0.0005$), and weak expression of membranous p120^{CTN} ($P < 0.0005$). A nonsignificant trend was observed between presence of membranous P-cadherin and presence of membranous N-cadherin ($P = 0.14$). Both presence of membranous N-cadherin ($P = 0.002$) and presence of P-cadherin ($P = 0.006$) were related to weak expression of membranous p120^{CTN}. Weak membrane staining of β-catenin was significantly associated with weak membrane expression of p120^{CTN} ($P < 0.0005$).

**Associations with other biomarkers and tumor-associated angiogenesis**

Significant alterations of p27 and PTEN have previously been reported in this series (29). Weak E-cadherin ($P < 0.0005$) and weak membranous β-catenin expression ($P < 0.0005$), as well as positive N-cadherin ($P = 0.001$) and P-cadherin expression ($P = 0.006$), were all related to low expression of p27. Weak E-cadherin ($P = 0.015$) and positive membranous P-cadherin expression ($P = 0.039$) were associated with low PTEN expression.

The prognostic significance of microvessel density and vascular proliferation index has previously been studied in this material (25, 26), and these angiogenic markers were not associated with any of the five cell adhesion proteins.

**Univariate survival analysis**

**E-cadherin.** Weak membrane expression was strongly associated with shorter time to biochemical failure, clinical recurrence (Table 3; Fig. 2), locoregional recurrence ($P = 0.011$), skeletal metastasis ($P = 0.003$), and cancer-specific death ($P = 0.012$).

**N-cadherin.** Positive membrane expression was associated with shorter time to biochemical failure, clinical recurrence

![Table 3](https://www.aacrjournals.org/doi/fig/10.1158/1078-0432.CCR-07-0028)
Fig. 2. Univariate survival analyses according to the Kaplan-Meier method by E-cadherin (A), N-cadherin (B), P-cadherin (C), β-catenin (D), and p120CTN (E), with clinical recurrence in prostate cancer as endpoint.
(Table 3; Fig. 2), and skeletal metastasis \( (P = 0.046) \). No significant differences were found for cytoplasmic expression.

**EN-switch.** Univariate survival analyses were also done for the EN-switch (weak E-cadherin and positive N-cadherin, \( n = 23; \) 22\%). This subgroup was strongly associated with shorter time to all five end points (Fig. 1).

**P-cadherin.** Positive membrane staining was significantly associated with shorter time to skeletal metastases \( (P = 0.036) \), and nonsignificant trends with shorter time to biochemical failure and clinical recurrence (Table 3; Fig. 2) were observed. No significant differences were found for cytoplasmic expression.

**\( \beta \)-Catenin.** No significant survival differences were observed (Table 3; Fig. 2).

\( p120^{\text{CTN}} \). Weak membrane expression was associated with shorter time to biochemical failure, clinical recurrence (Table 3; Fig. 2), locoregional recurrence \( (P = 0.034) \), and cancer-specific death \( (P = 0.023) \).

### Multivariate survival analysis

Cell adhesion markers (E-cadherin, N-cadherin, P-cadherin, \( \beta \)-catenin, and \( p120^{\text{CTN}} \)) with \( P \) values of <0.15 in univariate survival analyses were included together with preoperative s-PSA, standard Gleason score \( (\leq 3 + 4 \text{ versus } \geq 4 + 3) \), and pathologic stage.

When including individual variables in a simultaneous model (excluding the combined EN-status), E-cadherin [hazard ratio \( (HR) \), 2.5; \( P = 0.019 \)], N-cadherin \( (HR) \), 5.6; \( P = 0.003 \), and standard Gleason score \( (HR) \), 4.3; \( P = 0.002 \) were all independent predictors of time to clinical recurrence, whereas E-cadherin \( (HR) \), 1.8; \( P = 0.02 \), standard Gleason score \( (HR) \), 2.9; \( P < 0.0005 \), and pathologic stage \( (HR) \), 2.7; \( P = 0.001 \) all had an independent prognostic effect on time to biochemical failure. In contrast, P-cadherin, \( \beta \)-catenin, or \( p120^{\text{CTN}} \) did not show independent prognostic effect for any of these end points.

In a final simultaneous model, excluding E-cadherin and N-cadherin, the EN-status (weak E-cadherin and strong N-cadherin), as an indication of EMT in prostate cancers, consistently showed an independent prognostic effect, stronger than for E-cadherin and N-cadherin separately, together with Gleason score \( (\leq 3 + 4 \text{ versus } \geq 4 + 3) \) using both biochemical failure and clinical recurrence as end points (Table 4).

### Discussion

The prognosis of patients with clinically localized prostate cancer cannot be accurately predicted by standard variables such as preoperative s-PSA, Gleason score, and pathologic stage alone, and there is a need for supplementary prognostic factors (2). The aim of the present study was to explore the significance of cell adhesion markers in comparison with the above-mentioned “triad.” As a novel finding, increased expression of N-cadherin was a strong and independent predictor of clinical recurrence after radical prostatectomy.

We here show an independent prognostic significance of reduced E-cadherin expression as found by others (9–11, 37–40). Reduced expression of E-cadherin in prostate cancer may be caused by DNA hypermethylation \( (41) \) and transcriptional \( (42) \) and posttranslational mechanisms \( (43) \) but probably not DNA mutations \( (38) \). E-cadherin repression seems to be a dynamic and partly reversible process, and reexpression has been observed in metastatic prostate cancer \( (38, 43) \).

In our series, E-cadherin was not associated with preoperative s-PSA. One explanation might be that lowered E-cadherin is a marker of biological aggressiveness, whereas s-PSA is only a marker of tumor volume. Indeed, high preoperative s-PSA predicted advanced pathologic stage \( (pT3) \) but was without independent prognostic effect in predicting clinical recurrence after surgical treatment.

Importantly, a “cadherin switch” with increased N-cadherin and reduced E-cadherin expression had an independent prognostic effect on time to both biochemical failure and clinical recurrence in multivariate survival analyses, stronger than for E-cadherin or N-cadherin separately. Survival data for N-cadherin have not been previously reported for prostate cancer. N-cadherin expression contributes to a stroma-oriented cellular adhesion profile with increased tumor cell motility and invasive properties, indicating a possible EMT \( (6–8) \). Thus, cell adhesion molecules might add prognostic information beyond that presently given by histologic evaluation alone \( (44) \). Supporting our findings, soluble E-cadherin and N-cadherin were recently studied as serum biomarkers in prostate cancer, suggesting increased level of N-cadherin as a marker of ongoing EMT and tumor progress \( (45, 46) \). The higher frequency of positive N-cadherin staining compared with two other studies \( (11, 12) \) may be a result of increased detection using a different antibody validated by Western blot \( (35) \) in our lab.

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### Table 4. Final multivariate survival analysis according to Cox proportional hazards method for patients with clinically localized prostate cancer using biochemical failure or clinical recurrence as end points

<table>
<thead>
<tr>
<th>Variable</th>
<th>( n )</th>
<th>HR (95% confidence interval)</th>
<th>( P^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biochemical failure</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preoperative s-PSA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>73</td>
<td>1.0</td>
<td>0.017</td>
</tr>
<tr>
<td>High</td>
<td>25</td>
<td>2.1 (1.2-3.8)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Gleason score ( \leq 3 + 4 )</td>
<td>43</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>( \geq 4 + 3 )</td>
<td>55</td>
<td>3.3 (1.9-5.9)</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical recurrence</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gleason score ( \leq 3 + 4 )</td>
<td>49</td>
<td>1.0</td>
<td>0.001</td>
</tr>
<tr>
<td>( \geq 4 + 3 )</td>
<td>55</td>
<td>3.5 (1.5-8.2)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td><strong>EN-switch</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>77</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td><strong>EN-switch</strong></td>
<td>21</td>
<td>2.6 (1.4-4.7)</td>
<td></td>
</tr>
</tbody>
</table>

*Likelihood ratio test.

1 Six cases lacking information on preoperative s-PSA.

2 Upper quartile \( (\geq 20 \text{ ng/mL}) \).

3 Standard Gleason score in radical prostatectomy specimens.

4 Subgroup with combined weak E-cadherin and positive N-cadherin expression.

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Strong P-cadherin staining was found in the basal cell compartment, and this is in concert with earlier studies (12, 17 - 19). Further, P-cadherin was found to be reexpressed in a subset of prostate cancers, indicating a worse outcome although without independent prognostic power. This seems to be similar to P-cadherin as a myoepithelial marker in benign breast tissues, being reexpressed in the basaloid phenotype of more aggressive breast cancer (16). According to our demonstration of significant associations between both N-cadherin and P-cadherin with reduced E-cadherin-expression, the term cadherin switch should also include reexpression of P-cadherin in some prostate cancers. However, no independent prognostic effect was observed for P-cadherin, β-catenin, or p120^CTN, and these factors seem to be less important for prognostication.

In the present study, we asked whether there was an association between alterations of cell adhesion molecules, as evidence of EMT, and activation of the vascular system. Interestingly, one study showed a correlation between increased hypoxia-inducible factor and down-regulated E-cadherin in renal cell carcinoma (47), indicating a relationship between these pathways. However, we could not find any evidence for such a relationship, as none of the cadherins were linked to vascular related factors, such as microvessel density or vascular proliferation.

As a novel finding, our present data strongly suggest the importance of EMT (increased N-cadherin and decreased E-cadherin expression) for the progression and patient prognosis of human prostate cancer. Whereas previous findings have indicated reduced E-cadherin expression in prostate cancer subgroups (9, 10), an EMT phenotype and its association with outcome data have not been presented. Because this marker could have significant effect on the care of prostate cancer patients, including prospects of targeted therapy (48, 49), we suggest larger prospective studies to further validate our findings.

Acknowledgments
We thank Karen Bøhm-Nilsen, Gerd Lillian Hallseth, Bendik Nordanger, and Grethe Waaler for excellent technical assistance.

References
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