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E-cadherin: a marker for differentiation and invasiveness in prostatic carcinoma

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Summary. Considerable controversy exists concerning the value of histomorphological data in the assessment of the malignant potential of prostatic carcinomas. We investigated the expression pattern of E-cadherin in human prostate at the translational level. E-cadherin is a specific epithelial cell–cell adhesion molecule which has previously been found to be expressed in well-differentiated non-invasive carcinoma cell lines but is lost in many poorly differentiated invasive cell lines. The E-cadherin expression pattern in the prostate samples was correlated with histopathological findings in the same specimens. We found strong E-cadherin expression in normal prostate and benign prostatic hyperplasia. A decrease in or loss of E-cadherin was seen in 13 of 14 locally advanced and in 8 of 9 poorly differentiated prostatic carcinomas. We conclude that downregulation of E-cadherin expression plays a role in prostate carcinogenesis and invasiveness.

Key words: Cell–cell adhesion molecules – Differentiation – E-cadherin – Invasiveness – Prostatic carcinoma

Prostatic carcinoma is the second most common urological malignancy in men, with 132000 and 21000 new cases being reported during 1991 in the United States and Germany, respectively [1]. More than 95% of prostate cancers are adenocarcinomas [24, 33]. The ability of prostate tumors to invade and to metastasize is high: 40% of clinically diagnosed patients already have advanced disease [9, 11, 19, 26, 32, 38]. The following parameters of prostatic diseases are of prognostic and therapeutic value: age [24, 38] and performance status [38] of the patient, tumor differentiation [2, 3, 10, 14, 17, 26, 30], tumor stage [2, 26], and the level of expression of prostate-specific antigen [33].

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While uncontrolled proliferation is characteristic of metastatic diseases, it is increasingly apparent that loss of cell–cell adhesion and vigorous motility of prostate tumor cells are essential for their dissemination from primary to secondary sites [25]. Frixen et al. [13] examined the expression of the cell–cell adhesion molecule E-cadherin in various human carcinoma cell lines. They found that carcinoma cells with high E-cadherin expression generally showed an epithelioid (differentiated) phenotype and were non-invasive in vitro. Carcinoma cells with a reduced or absent expression of E-cadherin were fibroblastoid (poorly differentiated) and invasive in vitro. Dedifferentiation and invasiveness could be prevented by transfection with E-cadherin cDNA [13]. These results were confirmed for human and rat prostate cancer cell lines [7, 12, 27, 31]. Schipper et al. [29] found a strong correlation of the expression pattern of E-cadherin with the differentiation grade in squamous cell carcinomas of head and neck. On the basis of these in vitro and in vivo results it is reasonable to assume that downregulation of E-cadherin might also induce dedifferentiation and invasiveness in human prostatic carcinomas in vivo.

Materials and methods

Surgical specimens from 53 male patients, 42–78 years of age, with either normal prostate, benign prostatic hyperplasia (BPH), or prostatic carcinoma were studied.

Immunofluorescence

Frozen sections (6–8 μm) of human prostate material were fixed with ethanol (7 min, −20 °C), permeabilized with 0.5% Triton X-100 in phosphate-buffered saline (PBS), and washed four times with PBS. The coverslips were incubated for 60 min at 37 °C with 60 μl hybridoma supernatant 6F9, which contains monoclonal antibody against E-cadherin [13]. The coverslips were then washed and incubated with a fluorescein isothiocyanate (FITC)-labeled F(ab')2 rabbit anti-mouse immunoglobulin conjugate (Dako F313) for 30 min at 37 °C. Finally, the coverslips were washed and mounted on glass slides using *p*-phenylendiamine and viewed using an Orthoplan fluorescence microscope (Leitz, Wetzlar, Germany) at ×400 and ×630 magnification.

The following criteria were used for the evaluation of E-cadherin expression: +++, >90% of the carcinoma cells positively stained with a high intensity; ++, 10–90% of carcinoma cells positively stained with high or medium intensity; +, <10% of carcinoma cells positively stained; –, all tumor cells negative.

Histopathological grading and staging

Additional serial sections were stained with hematoxylin and eosin to determine the histopathological grading (G0–G3) as follows:
Grade 0, no atypia, normally differentiated. The nuclei are not enlarged and in basally situated. The glandular epithelium has two layers and the epithelium/stroma relation is normal, i.e. every glandular formation is surrounded by stroma.
Grade 1, well differentiated. The nuclei are slightly enlarged and the glandular epithelium has a single layer.
Grade 2, moderately differentiated. The nuclei are enlarged and the nucleus/cytoplasm ratio is approximately 1:4. The stromal bridges between the glands are reduced. The glands are "back to back" or close to each other and in a single layer.
Grade 3, poorly differentiated. The cells are small, the cytoplasm is clear. The nucleus/cytoplasm ratio is >1. The epithelium has a single layer with cuboidal cells. The nucleoli are enlarged and eccentrically placed. Solid tumor cell formations are predominant.
The staging (T0–T4) was defined according to the criteria of Union Internationale Contre le Cancer (UICC 1987:[16]).

Results

We examined the surgical specimens from 53 male Caucasian patients, 42–78 years of age, with either normal prostate ($n = 2$), benign prostatic hyperplasia (BPH; $n = 25$) or prostatic carcinoma ($n = 26$; Table 1). The prostate tissue was surgically removed by transurethral resection ($n = 30$), radical prostatectomy ($n = 19$) or radical cystoprostatectomy ($n = 2$). Metastases of prostate cancer were removed by lymph node dissection ($n = 1$) and by excision of a cutaneous metastasis ($n = 1$).

E-cadherin expression in normal prostate and benign prostatic hyperplasia

E-cadherin was, without exception, found to be strongly expressed in tissues of the normal prostate and BPH (Fig. 1, Table 2). Staining was confined to epithelial cells, with a typical staining at cell–cell contacts.

E-cadherin expression in prostatic carcinoma

The prostatic carcinomas were all adenocarcinomas and consisted of two well-differentiated (G1), 15 moderately differentiated (G2) and 9 poorly differentiated cases (G3). The two well-differentiated carcinomas expressed E-cadherin with a high staining intensity. E-cadherin was also expressed strongly in 3 of 15 moderately differentiated prostatic carcinomas (Table 2). However, 12 of 15 of the G2 carcinomas showed reduced E-cadherin expression (Fig. 2) and, furthermore, E-cadherin expression was decreased in 8 of 9 poorly differentiated prostatic carcinomas. Interestingly E-cadherin was not detectable in three G3 lesions and in one G2 lesion. When these tumors were grouped according the TNM system, E-cadherin was reduced in all but one cases of locally advanced prostatic carcinomas ($T \geq 3$) (Table 2). Three of eight pT2 carcinomas and two pT1 prostate cancers were strongly E-cadherin positive. One cutaneous metastasis was found to be E-cadherin positive; another metastasis from a lymph node showed reduced E-cadherin expression.

Table 1. Patient characteristics, histopathological results and operative procedure in the prostate specimens studied

Histopathological results	*n*	TUR	Prostatectomy[a]	Others[b]
Normal prostate	2	0	2	0
Benign prostatic hyperplasia	25	25	0	0
Prostatic carcinoma	24	5	19	0
Metastastic	2	0	0	2
Totals	53	30	21	2

TUR, Transurethral resection
[a] This material was obtained by radical cystoprostatectomies of 46- and 48-years-old patients with muscle invasive bladder cancer and normal prostate
[b] Metastases were obtained by lymph node excision and local excision of a cutaneous metastasis

Discussion

In the present study we examined the expression of E-cadherin in prostate specimens. We found strong E-cadherin expression in all specimens of normal prostate, BPH and well-differentiated prostatic carcinoma. E-

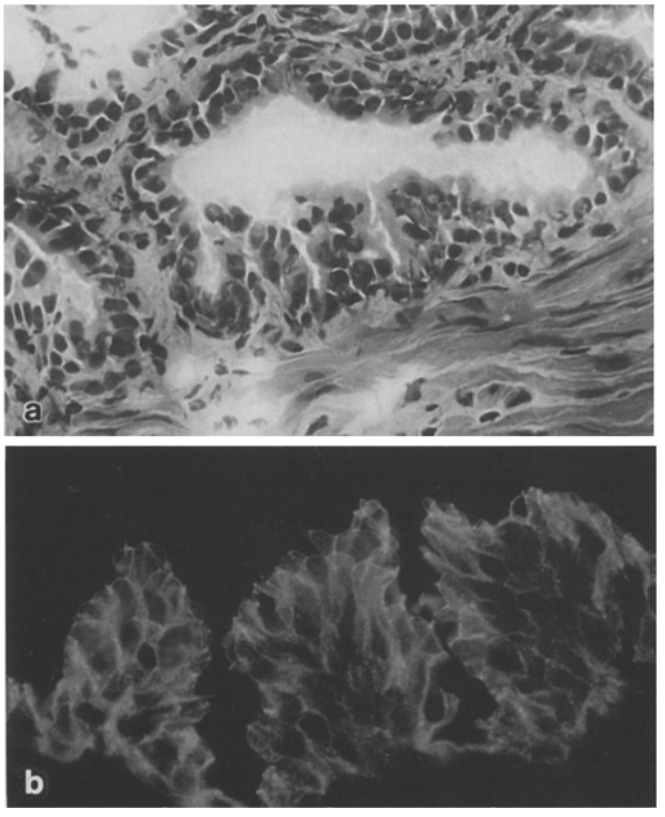

Fig. 1. a Benign prostatic hyperplasia (BPH). H&E, ×150. **b** BPH: immunofluorescence staining of E-cadherin shows a strong expression pattern. ×400

Table 2. E-cadherin expression in normal prostate, benign prostatic hyperplasia (BPH) and prostatic carcinoma

	n	E-cadherin expression[a]			
		+++	++	+	−
Normal prostate tissue	2	2	0	0	0
BPH	25	25	0	0	0
Prostatic carcinoma	26	6	8	8	4
Grade G1	2	2	0	0	0
Grade G2	15	3	8	3	1
Grade G3	9	1	0	5	3
Stage T1	2	2	0	0	0
Stage T2	8	3	3	2	0
Stage T3	8	0	3	4	1
Stage T4	6	1	1	1	3
Stage M1	2	0	1	1	0

[a] Expression was measured by immunofluorescence as described in Material and Methods

cadherin expression was reduced in 89% of poorly differentiated carcinomas and in 93% of locally advanced prostatic carcinomas. These results provide strong evidence that E-cadherin plays a crucial role in maintaining the epithelial phenotype in vivo and are consistent with findings in head and neck squamous cell carcinomas [29].

It has been shown in several in vitro studies that the disturbance or loss of E-cadherin induces new properties, i.e. the cells change their morphology from an epithelioid to a fibroblastoid phenotype and the in vitro invasiveness increases for collagen and heart tissue [4–6]. There are major differences between in vitro assays and the invasion of tumor cells into underlying tissues and adjacent organs in vivo. In particular, hormonal effects and the influence of the environment play a crucial role in invasion and metastasis of prostatic carcinomas [3, 7]. Therefore, human carcinoma tissues need to be examined in order to gain insights into the role of E-cadherin in dedifferentiation, invasivness and metastasis in the in vivo situation. Umbas and coworkers [37] demonstrated a decreased level of E-cadherin in 40 of 84 prostate tumors. In this study all of the well-differentiated and 85% of the moderately differentiated carcinomas showed a strong E-cadherin expression pattern. Fifty per cent of the poorly differentiated and 55% of the anaplastic carcinomas showed a reduced expression pattern. Interestingly, E-cadherin expression decreased in 6 of 8 metastatic lesions. These results are therefore largely in agreement with our observations. In squamous cell carcinomas of head and neck a strong correlation of the expression pattern of E-cadherin with the differentiation grade was found [29]. All poorly differentiated carcinomas were E-cadherin negative, as were all but one lymph node metastases. Shimoyama et al. [31] examined the E-cadherin expression in gastric carcinomas. They found decreased E-cadherin expression in 17% only. We recently investigated the expression of E-cadherin in 40 human bladder cancers [23].

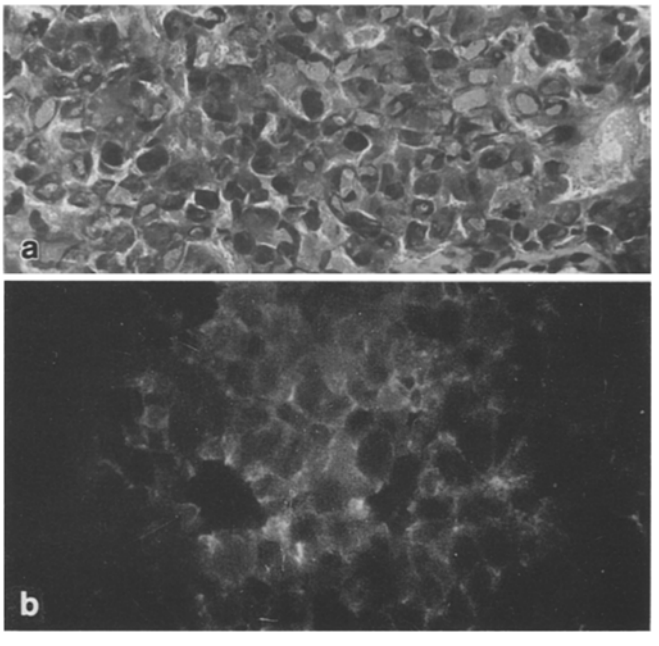

Fig. 2. a Prostatic carcinoma, G2. H&E, ×400. **b** Prostatic carcinoma, G2: immunofluorescence staining of E-cadherin is reduced or negative. ×400

There was a strong correlation of E-cadherin expression with tumor stage and grade: 75% of the poorly differentiated (G3) bladder cancers and 81% of the muscle-invasive transitional cell carcinomas showed a decreased E-cadherin expression pattern. A major question remains as to the level at which E-cadherin expression is downregulated. The E-cadherin gene is on chromosome 16q22.1, a location where an important, but as yet not cloned tumor suppressor gene is found [8, 13, 28]. Prostatic carcinomas show a frequency of allelic loss on chromosome 16q in 30% of cases [8, 28]. Allelotype analysis of breast cancer showed comparable results: allelic loss in 45% of the primary tumor and in 67% of lymph node metastases [27]. Similar results were found for carcinomas of the liver [22, 36]. The fact that allelotype analysis of prostatic carcinomas, breast cancers and liver carcinomas revealed the frequent deletion of the part of chromosome 16 thought to harbor a suppressor gene supports the hypothesis that E-cadherin is a candidate tumor suppressor gene.

We found strong E-cadherin expression in 1 of 2 metastatic lesions of prostate cancer. Normal E-cadherin expression was also detected in 2 of 8 metastatic deposits of prostatic carcinomas [37]. It is therefore increasingly apparent that additional factors are essential for their dissemination from primary to secondary sites [34, 35, 39]. Liotta and coworkers [18] identified the autocrine motility factor (AMF), which induces a strong motility response in various human epithelial cell lines. Consistent with the above, the content of AMF was increased in urine samples of bladder cancer patients [15]. We recently determined the expression of the AMF receptor [20, 21] in bladder cancer specimens and found a strong correlation not only with tumor grade and stage but also with E-cadherin expression. An inverse correlation between AMF receptor and E-cadherin expression at the translational level was found [23].

The combined investigations on human prostatic, head and neck and bladder carcinomas strongly support the hypothesis that loss of cell–cell adhesion and vigorous cell motility are essential requirements of tumor invasion and metastasis.

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References

1. American cancer society (1992) Cancer facts and figures 1992
2. Barnes R, Hirst A, Rosenquist R (1976) Early carcinoma of the prostate: comparison of stages A and B. J Urol 115:404
3. Bartsch G, Hohlbrugger G, Mikuz G, Marberger H (1983) Transurethral resection in prostatic carcinoma: a cause of accelerated metastatic growth. World J Urol 1:36
4. Behrens J, Birchmeier W (1990) Specific activity of the arc-1/uvomorulin promoter in epithelial cells. J Cell Biol 11:157a
5. Behrens J, Birchmeier W, Goodman SL, Imhof BA (1985) Dissociation of Madin-Darby canine kidney epithelial cells by the monoclonal antibody anti-arc-1: mechanistic aspects and identification of the antigen as a component related to uvomorulin. J Cell Biol 1001:1307
6. Behrens J, Mareel MM, Roy FM van, Birchmeier W (1989) Dissecting tumor cells invasion: epithelial cells acquire invasive properties after loss of uvomorulin-mediated cell–cell adhesion. J Cell Biol 108:2435
7. Bussemakers MJG, Mooreselaar RJA van, Giroldi LA, Ichikawa T, Isaacs JT, Takeichi M, Debruyne FMJ, Schalken JA (in press) Decreased expression of E-cadherin in the progression of rat prostatic cancer. Cancer Res
8. Carter BS, Ewing CM, Ward WS, Treiger BF, Aalders TW, Schalken JA, Epstein JI, Isaacs WB (1990) Allelic loss of chromosomes 16q and 10q in human prostate cancer. Proc Natl Acad Sci USA 87:8751
9. Chlewbowski RT, Hestorff R, Sardorff L, Weiner J, Bateman JR (1978) Cyclophosphamide (NSC 26 271) versus the combination of adriamycin (NSC 123 127), 5-fluorouracil (NSC 19 893), and cyclophosphamide in the treatment of metastastic prostatic cancer: a randomized trial. Cancer 42:2546
10. Correa RJ Jr, Anderson RG, Gibbons RP, Mason JT (1974) Latent carcinoma of the prostate: why the controversy? J Urol 111:644
11. Eagan RT, Hahn RG, Myers RP (1976) Adriamycin (NSC 127 127) versus 5-fluorouracil (NSC 19 893) and cyclophosphamide (NSC 26 271) in the treatment of metastatic prostate cancer. Cancer Treat Rep 60:115
12. Eidelman S, Damsky CH, Wheelock MJ, Damjanov I (1989) Expression of the cell–cell adhesion glycoprotein cell CAM 120/80 in normal human tissue and tumor. Am J Pathol 135:101
13. Frixen UH, Behrens J, Sacks M, Eberle G, Voss B, Warda A, Lachner D, Birchmeier W (1991) E-cadherin mediated cell–cell adhesion prevents invasiveness of human carcinoma cells. J Cell Biol 113:173
14. Greene LF, Simon HB (1955) Occult carcinoma of the prostate: clinical and therapeutic study of eighty-three cases. JAMA 158:1494
15. Guirguis R (1988) Detection of autocrine motility factor in urine as a marker of bladder cancer. J Natl Cancer Inst 80:1203
16. Hermanek P (1986) Neue TNM/pTNM-Klassifikation and Stadieneinteilung urologischer Tumoren ab 1987. Urologe [B] 26:193
17. Khalifa NM, Jarman WB (1976) A study of 48 cases of incidental carcinoma of the prostate followed 10 years or longer. J Urol 116:329
18. Liotta LA (1986) Tumor cell autocrine motility factor. Proc Natl Acad Sci USA 83:3302
19. Muss HB, Howard V, Richards F, White DR, Jackson DV, Cooper MR, Stuart JJ, Resnick MI, Brodkin R, Spurr CL (1981) Cyclophosphamide versus cyclophosphamide, methotrexate, and 5-fluorouracil in advanced prostatic cancer: randomized trial. Cancer 47:1949
20. Nabi IR, Watanabe H, Raz A (1990) Identification of B16-F1 melanoma autocrine motility-like factor receptor. Cancer Res 50:409
21. Nabi IR, Watanabe H, Stilletti S, Raz A (1991) Tumor cell autocrine motility factor receptor. In: Goldberg ID (ed) Cell motility factors. Birkhäuser, Basel, p 164
22. Nagafuchi A, Shirayoshi Y, Okazaki K, Yasuda K, Takeichi M (1987) Transformation of cell adhesion properties by exogenously introduced E-cadherin cDNA. Nature 329:341
23. Otto T, Birchmeier W, Raz A, Rübben H (1992) Motilitäts- und Adhäsionsfaktoren: Progressionsassozierte Parameter bein Harnblasenkarzinom. Urologe [A] 31:31
24. Paulson DF (1987) Management of prostatic malignancy. In: DeKernion JB, Paulson DF (eds) Genitourinary cancer management. Lea & Febiger, Philadelphia, p 107
25. Raz A, Silletti S (1992) Tumor cell autocrine motility factor receptor and its relation to metastasis. Clin Exp Metastasis 10 [Suppl]:13
26. Rübben H, Altwein JE (1987) Das Prostatakarzinom: ein therapeutisches Dilemma? Urologe [A] 26:7
27. Sato T, Tanigami A, Yamakawa K, Akiyama F, Kasumi F, Sakamoto G, Nakamura Y (1990) Allelotype of breast cancer: cumulative losses promote tumor progression in primary breast cancer. Cancer Res 50:7184
28. Schalken JA, Bussemakers MJG, Isaacs WB, Carter BS, Veen WJM van de, Debruyne FMJ (1991) E-cadherin is a candidate tumor suppressor gene implicated in prostate cancer. In: Société Internationale d'Urologie, 22nd congress 425:352
29. Schipper JH, Frixen UH, Behrens J, Unger A, Jahnke K, Birchmeier W (1991) E-cadherin expression in squamous cell carcinomas of head and neck: invasive correlation with tumor dedifferentiation and lymph node metastasis. Cancer Res 51:6328
30. Sheldon CA, Williams RD, Fraley EE (1980) Incidental carcinoma of the prostate: a review of the literature and critical reappraisal of classification. J Urol 124
31. Shimoyama Y, Hirohashi S, Hirano S, Noguchi M, Shimosato Y, Takeichi M, Abe O (1989) Cadherin cell-adhesion molecules in human epithelial tissues and carcinomas. Cancer Res 49:2128
32. Soloway MS, DeKernion JB, Gibbons RP, Johnson DE, Loening SA, Pontes JE, Prout GR, Schmidt JD, Scott WW, Chu TM, Gaeta JF, Slack NH, Murphy GP (1981) Comparison of estramustine phosphate and vincristine alone or in combination for patients with advanced hormone refractory previously irradiated carcinoma of the prostate. J Urol 125:664
33. Stamey TA, Kabalin IN (1989) Prostate specific antigen in diagnosis and treatment of adenocarcinoma of the prostate. J Urol 141:1070
34. Stoker M, Gherardi E, Perryman M, Gray W (1987) Scatter factor is a fibroblast-derived modulator of epithelial cell motility. Nature 327:239
35. Stracke ML, Guirguis R, Liotta LA, Schiffmann E (1987) Pertussis toxin inhibits stimulated motility independently of the adenylate cyclase pathway in human melanoma cells. Biochem Biophys Res Commun 146:339
36. Tsuda H, Zhang W, Shimosato Y, Yokota Y, Terada M, Sugimura T, Miyamura T, Hirohashi S (1990) Allele loss on chromosome 16 associated with progression of human hepatocellular carcinoma. Proc Natl Acad Sci USA 87:6791
37. Umbas R, Bussemakers MJG, Isaacs WB, Aalders TW, Carter BS, Debruyne FMJ, Schalken JA (1993) Decreased expression of E-cadherin in high grade prostate cancer. Cancer Res (in press)
38. Voogt H de, Sucin S, Sylvester R, Pavone-Macaluso M, Smith PH, Pauw M de (1989) Multivariate analysis of prognostic factors in patients with advanced prostatic cancer: results from 2 European Organization for Research on Treatment of Cancer trials. J Urol 141:883
39. Weidner KM, Behrens J, Vandekerckhove J, Birchmeier W (1990) Scatterfactor: molecular characteristics and effect on the invasion of epithelial cells. J Cell Biol 111:2097