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Advances in Brief

Inverse Relation of E-Cadherin and Autocrine Motility Factor Receptor Expression as a Prognostic Factor in Patients with Bladder Carcinomas[1]

Thomas Otto, Walter Birchmeier,[2] Ulrich Schmidt, Axel Hinke, Jörg Schipper, Herbert Rübben, and Avraham Raz[3]

Department of Urology [T. O., A. H., H. R.], Institute of Cell Biology (Tumor Research) [W. B.], Institute of Pathology [U. S.], Department of Otorhinolaryngology [J. S.], University of Essen, Medical School, Hufelandstr. 55, 4300 Essen, Federal Republic of Germany, and Metastasis Research Program, Michigan Cancer Foundation, Departments of Pathology and Radiation Oncology, Wayne State University, Detroit, Michigan 48201 [A. R.]

Abstract

Down-regulation of E-cadherin, an intercellular adhesion molecule, and up-regulation of autocrine motility factor receptor (gp78) expressions have been shown to play a role in tumor cell invasion and metastasis. Monoclonal antibodies against E-cadherin and gp78 were used to stain serial snap-frozen sections of 12 normal bladder and 83 bladder carcinoma specimens (27 noninvasive, 53 invasive, and 3 metastases). In normal urothelium, E-cadherin is expressed while gp78 is not. Positive expression of E-cadherin and negative expression of gp78 were found to be associated with a low risk of clinical progression in the superficial bladder carcinoma patient group. While reduction in E-cadherin concomitantly with an increase in gp78 expression was associated with poor prognosis, 71% of the patients (n = 30) underwent rapid cancer progression, and 32% of the patients died of cancer-related disease at a median of 2 years after initial diagnosis. Thus, it is suggested that reduction of E-cadherin expression associated with an increase in the level of gp78 in bladder cancers may define a high risk group of patients. The dual use of these two antigens may improve early diagnosis of high risk bladder cancer patients and influence treatment decisions.

Introduction

Cancer of the urinary bladder is the fifth most common cancer in men and the second most common urological malignancy in Western society (1) with an incidence rate per year of 29.8 per 100,000 males. Bladder tumors are distinguished as either invasive or superficial; invasive tumors are generally associated with poor prognosis, while 20–30% of the superficial carcinomas will later recur and progress to become invasive and metastatic (1, 2). The most common prognostic factors for classification of urothelial cancer are staging and grading, which are based on morphological criteria. In the last decade, however, other factors have been developed as possible prognostic aids for better disease management such as expression of particular cell surface antigens, DNA content, chromosomal aberrations, gene rearrangements, and point mutations (1). Since most cancers of the bladder are carcinomas and are associated with dedifferentiation of epithelial cells, we questioned whether a reduced expression of the epithelium-specific cell-cell adhesion molecule E-cadherin might be related to progression. E-cadherin is a calcium-dependent cell adhesion molecule of the cadherin supergene family (3). Expression was found to be down-regulated in several human and murine carcinoma cell lines as well as in various human tumors and furthermore found to be associated with an increased tumor cell invasion *in vitro* (4–8). These results suggest that the loss of E-cadherin expression may play a role in the progression to malignancy of epithelial cells and may act as an invasion suppressor gene product (4–8).

The loss of cell-cell adhesion and the ability of cells to migrate are apparently fundamental to the acquisition of invasive properties of tumor cells. A tumor-derived cytokine, AMF,[4] was identified by its ability to induce direct and random cell migration via a receptor-mediated signaling pathway (9, 10). The receptor for AMF was identified as a cell surface glycoprotein of M_r 78,000 (gp78), homologous in part to p53, and its expression was regulated by cell contact in normal cells and was associated with tumor cell motility and metastasis (10–12). Due to the apparent opposing functional roles of E-cadherin and AMF receptor in tumor cell invasion and metastasis, we have initiated studies to evaluate the expression of these two cell surface antigens in bladder carcinomas and have correlated the results with tumor stage and patient survival.

Materials and Methods

A total of ninety-five patients, 45–81 years old, underwent surgery at the Department of Urology, University of Essen, and tissue samples were immediately frozen in liquid nitrogen. Normal control cystectomy specimens (n = 12) and bladder carcinoma tissues (n = 83) were examined. The bladder tissue was surgically removed either by transurethral resection (n = 39) or by radical cystectomy (n = 53). Metastases were removed by lymph node dissection (n = 2) and partial lung excision (n = 1). Tissues were directly obtained from the operating room, *i.e.*, samples were received within 10 min after surgical removal and were immediately snap-frozen in liquid nitrogen. Sections of the tissues were stained with hematoxylin and eosin and by immunofluorescence using monoclonal antibodies against E-cadherin (4) and gp78 (10). The stage of each tumor was classified according to the tumor-node-metastasis classification. For prospective study, specimens from 51 newly diagnosed patients were collected immediately after tumor resections. The expression of E-cadherin and gp78 was determined and correlated with the pathological grading, staging, and clinical follow-up for 24 months.

For histopathological and immunofluorescence examination, frozen bladder tissues were serially cut (5 μm) and (*a*) stained with hematoxylin and eosin to establish grading according to the criteria of the Union International Contre Cancer (13); and (*b*) stained by specific antibodies followed by immunofluorescence. For this purpose, sections were fixed with ethanol (7 min at −20°C), permeabilized with 0.5% Triton X-100 in PBS, pH 7.2, washed four times with PBS, and incubated for 60 min at 37°C with the 6F9 anti-E-cadherin monoclonal antibody (4). The sections were then washed in the same buffer and stained with a fluorescein isothiocyanate-labeled rabbit anti-mouse IgG conjugate (Dako F313) for 30 min at 37°C. Finally, the sections were washed in PBS and mounted onto glass slides using *p*-phenylenediamine. The criteria used for the evaluation of E-cadherin expression was as previously described (6): normal, ++, > 90% of the cells positively stained with a high density; +, heterogeneous staining with a considerable fraction of E-cadherin-negative cells; –, virtually all cells E-cadherin negative. The immunofluorescence

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[2] Present address: Max Delbruck Cancer Center, Robert-Rossler St., 10, 12132 Berlin, Federal Republic of Germany.

[3] To whom requests for reprints should be addressed, at Metastasis Research Program, Michigan Cancer Foundation, 110 East Warren Avenue, Detroit, MI 48201.

[4] The abbreviations used are: AMF, autocrine motility factor; PBS, phosphate-buffered saline.

localization of gp78 was performed at room temperature as described previously (12). Briefly, frozen tissue sections on glass slides were fixed for 15 min with 3% paraformaldehyde in PBS. After being washed, the sections were incubated with the anti-gp78 monoclonal antibody for 30 min, followed by washing with PBS and staining with fluorescein isothiocyanate-labeled rabbit anti-rat antibody for 30 min. The sections were washed and mounted as described above. The criteria for gp78 expression were: ++, > 50% of the bladder cancer cells AMF-receptor positive with large, multiple spots; +, 10–50% of the bladder cells gp78 positive with single, small spots; normal, –, no significant gp78 expression. The specimens were visualized using a Leitz Orthoplan equipped with ×40 and ×63 planapo objectives fluorescence microscope. Slides were classified independently in a blind fashion by three investigators.

For statistical analysis, standard univariate and multivariate statistical methods including asymptotic (Pearson-Yates correlation) and exact (Fisher) tests for difference (two-sided) were used to analyze the histopathological findings. A logrank test was used to analyze E-cadherin and gp78 expression in relation to the clinical data, *i.e.*, time of progression.

Results

In a retrospective analysis of 1926 patients with bladder carcinoma in the registry of urinary tract tumors at the University of Essen, several prognostic parameters like stage, grade, age, tumor size, dysplasia, multiplicity, and hydronephrosis were evaluated in relation to clinical outcome. It was found that tumor stage was the most reliable prognostic factor (Fig. 1; $P < 0.001$). However, the prediction of the patient's outcome based solely on histopathological classifications was found to be inaccurate in about 40% of the cases (Fig. 1; Ref. 2). This poses an obvious predicament of how to decide which patient may be at risk for recurrence and progression and should undergo aggressive therapy. In an attempt to improve the detection of high risk tumors, we have analyzed the expression of two cell surface antigens suggested to be involved in cell-cell adhesion and cell motility and to play a role in tumor cell invasion and metastasis, *i.e.*, E-cadherin and gp78. Their expression was correlated with the histopathological classification of the same tissue samples and with the patient's clinical outcome.

Normal epithelia of the urothelium were stained positive for E-cadherin (12 of 12) as described previously (14) while being negatively stained for gp78 expression (12 of 12; Table 1). Muscle and connective tissue of the bladder were negative for both E-cadherin and gp78 expression (28 of 28). A high percentage of the bladder carcinoma specimens showed reduced or negative staining for E-cadherin and an increased expression of gp78. In bladder carcinomas, 67% (18 of 27) and 48% (13 of 27) of the noninvasive tumors (T_A/T_1) expressed reduced E-cadherin and elevated gp78 expression, respectively. This trend is further amplified in the poorly differentiated invasive carcinoma (T_2-T_3), whereby 92% (49 of 53) and 81% (43 of 53) were negative for E-cadherin and positive for gp78 expression, respectively. The three metastases were characterized by a complete down-regulation of E-cadherin accompanied with up-regulation of gp78 expression. When the tumors were grouped according to the differentiation grade, about 50% (6 of 12) of the G_1 cases showed reduced E-cadherin expression and increased gp78 expression (5 of 12); the numbers increased to 86% (38 of 44) and 66% (29 of 44) in the G_2 and to over 90% (25 of 27) in the G_3 tumors, respectively (Table 1). Fig. 2 summarized the observed relation inverse between the down-regulation of E-cadherin and the up-regulation of gp78 expression in these bladder cancer specimens.

Fig. 3 depicts an example of serial sections of a poorly differentiated bladder carcinoma specimen whereby E-cadherin is barely detected (Fig. 2*b*) while gp78 is highly expressed in a polarized pattern (Fig. 3*c*), similar to the pattern described for a bladder carcinoma cell line in culture (12).

A 24-month follow-up revealed a correlation between reduced E-cadherin and increased gp78 expression to cancer progression and to the mortality of patients. In bladder cancers (T_A/T_1) which showed

Table 1 *Immunodetection of E-cadherin and AMF receptor expression in bladder tissue specimens*

		E-Cadherin negative[a]			gp78 positive[a]		
Type of tissue	T	*n*	(%)	*P*	*n*	(%)	*P*
Normal urothelium	12	0	(0)		0	(0)	
Bladder carcinomas	83	70	(84)	<0.0001	59	(71)	<0.0001
T_A/T_1	27	18	(67)		13	(48)	
T_2-T_3	53	49	(92)	<0.008	43	(81)	<0.01
M_1	3[b]	3	(100)		3	(100)	
G_1	12	6	(50)		5	(42)	
G_2	44	38	(86)	<0.001	29	(66)	<0.0001
G_3	27	26	(96)		25	(92)	

[a] As defined in "Materials and Methods." T, total number of patients in the group; *n*, number of patients.

[b] Represents one lymph node metastasis, one cutaneous metastasis, and one lung metastasis.

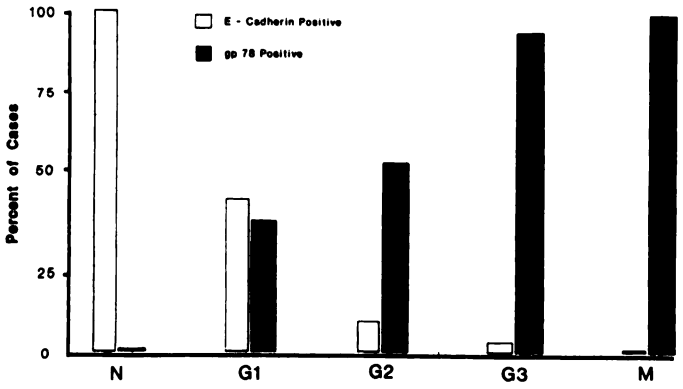

Fig. 2. The relative expression of E-cadherin and gp78 in bladder carcinomas according to the data from Table 1. *N*, normal urothelium; *G1*, well differentiated carcinomas; *G2*, moderately differentiated carcinomas; and *G3*, poorly differentiated carcinomas; *M*, metastasis.

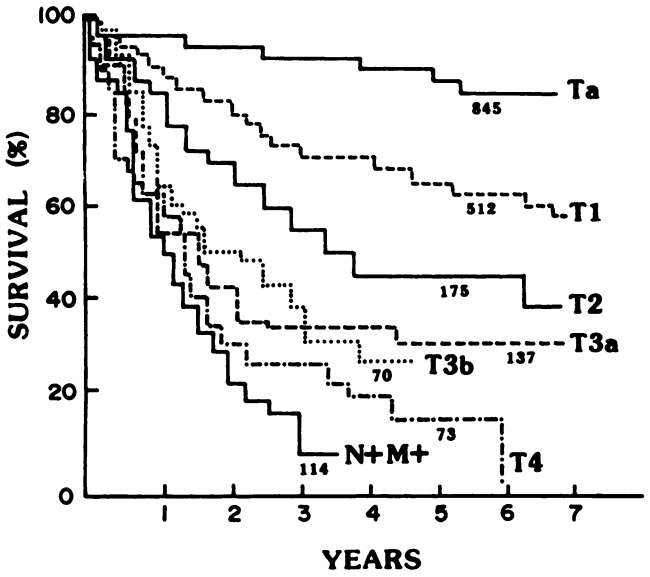

Fig. 1. Survival of patients with bladder carcinoma as related to tumor stage. Patient data from the University of Essen and the members of the registry of urinary tract tumors in Essen. Staging was performed according to the criteria of the Union International Contre Cancer as described in "Materials and Methods."

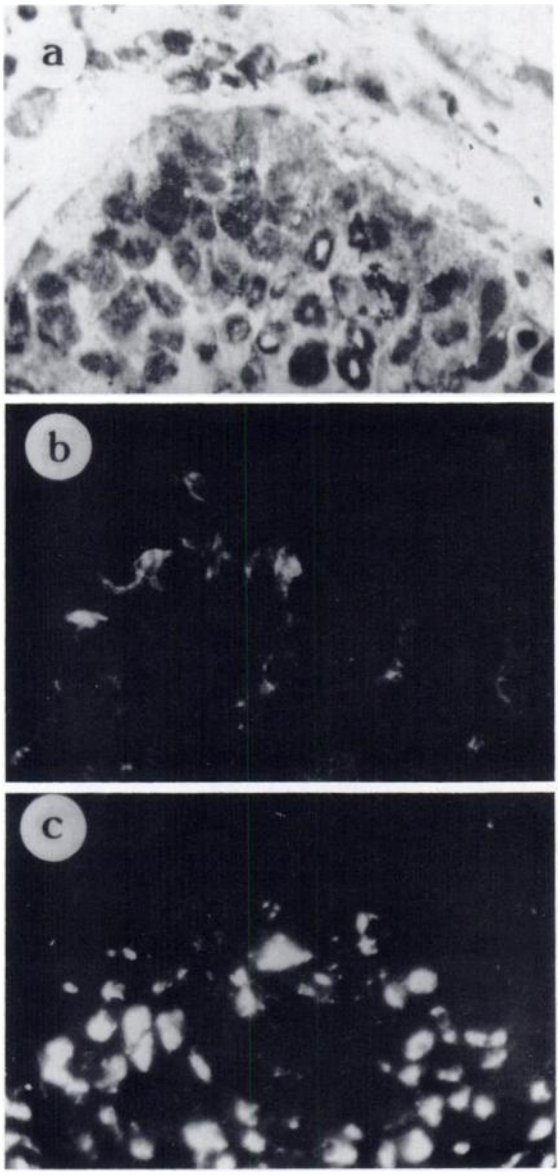

Fig. 3. Serial sections of a G_3 bladder carcinoma. The hematoxylin and eosin staining is shown in *a*, and the immunohistochemical localizations of E-cadherin in *b* and of gp78 in *c*. × 630.

a strong E-cadherin expression and were negative for gp78 staining, a low risk for tumor progression was found, and none of these patients (0 of 12) died during this period (Table 2). Even patients with locally advanced carcinoma (T_2-T_3) with strong E-cadherin and negative gp78 staining were found to belong to a low risk group; only 1 of 7 cancers progressed, and none of the patients died during the 24-month follow-up. In sharp contrast, most of the patients diagnosed with reduced E-cadherin (22 of 35) and increased gp78 (23 of 32) cancers progressed, and one-third of the patients (11 of 32) died during the 24-month follow-up. Moreover, some of the patients with superficial bladder carcinoma (T_A/T_1) who were E-cadherin negative and gp78 positive underwent rapid cancer progression (2 of 5) or died from the disease (1 of 5; Table 2).

Discussion

It is well accepted that patients diagnosed with well differentiated, superficial noninvasive bladder carcinoma belong to a low risk group. The 5-year progression rate is less than 5%, and 5-year survival is 96% (2, 15). In contrast, superficial invasive bladder carcinoma, carcinoma *in situ*, and muscle-invasive bladder cancer were found to be associated with high risk. In the latter group, the 5-year survival was less than 65% in superficial bladder cancer (2, 15), and it was suggested that transurethral resection may not be sufficient as a treatment of choice since these patients have an increased risk of local recurrence (95%) and progression (38%). Randomized trials have not shown any efficacy of systemic or intravesical chemotherapy in high grade superficial bladder carcinoma (16). Adjuvant immunotherapy with *Bacillus Calmette-Guérin* decreases the frequency of recurrence compared to transurethral resection alone (17), but the influence on tumor progression is limited. Taken together, these results indicate that after optimal organ- preserving therapy, one-third of the patients with high grade superficial bladder cancer are still at risk to develop muscle invasive disease or distant metastases, which highlight the need for a better prognostic parameter for discriminating those patients which should undergo aggressive disease management.

A further question arises as to why some tumors remain localized whereas others that appear morphologically identical become invasive and metastatic. Cancer progression in the bladder as diagnosed by staging or grading is generally associated with increasing chromosomal anomalies, and aneuploidy in the population seems to be an indicator for tumor progression. No correlation appears to exist between the expression of certain oncogenes or the loss of tumor suppressor genes and clinical behavior. Furthermore, no specific marker is associated with transitional cell carcinoma of the bladder, although there have been attempts to correlate the presence of various antigens, *i.e.*, ABO antigen, CK-18, epidermal growth factor receptor, transferrin receptor, cancer antigens Ca-50 or CA19–9, and β-human chorionic gonadotropin, with the stage of disease (reviewed in Ref. 1).

It has been shown in several *in vitro* and *in vivo* studies that alteration or loss of E-cadherin expression is associated with a change in cell morphology, increased cell migration, and invasion (4–8). Schipper *et al.* (6) found a complete down-regulation of E-cadherin gene expression in all cases of poorly differentiated squamous cell carcinomas of the head and neck, and furthermore, all infiltrated lymph nodes were E-cadherin negative with one exception. Recently, positive E-cadherin expression was reported in all specimens of normal prostate, benign prostatic hyperplasia, and well-differentiated prostatic carcinoma, while E-cadherin expression was reduced in 90% of poorly differentiated and in 93% of locally advanced prostatic carcinomas (18, 19).

It was reported that the presence of AMF in the urine is a marker of transitional cell carcinoma of the bladder (20). Recently, the differential expression pattern of gp78 in various human bladder cancer cell lines was reported (12). The poorly differentiated, highly motile cell line EJ28 expressed a high amount of the protein, whereas the well-differentiated bladder papilloma cell line RT4 and the normal fetal urothelial cell line FHs738BL showed decreased expression. On motile EJ28 cells, gp78 was localized by immunofluorescence to the

Table 2 *Progression and death from cancer in relation to E-cadherin and gp78 expression in patients with bladder cancer*[a]

		E-cadherin expression[b]			gp78 expression[b]		
Stage		(++)	(−/+)	*P*	(−/+)	(++)	*P*
T_A/T_1	prog[c]	0/11	2/5	0.05	0/12	2/4	0.05
	dod	0/11	1/5	n.s.	0/12	1/4	n.s.
T_2-T_3	prog	1/5	20/30	0.05	1/7	21/28	0.05
	dod	0/5	10/30	0.05	0/7	10/28	0.05

[a] Median follow-up of 24 months.
[b] As defined in "Materials and Methods"; n.s., not significant.
[c] prog, progression; dod, dead of disease.

leading lamella as well as to the trailing edge, suggesting shuffling of gp78 during cell migration.

While this work was in progress, it was reported that a decrease in E-cadherin expression correlates with poor survival in patients with bladder carcinoma (14). Our results support the above findings. However, from a practical point of view, the lack of antigen immunoreactivity might pose the dilemma of whether negative immunolabeling is indeed a positive result or a technical artifact, suggesting that the sole usage of E-cadherin immunoreactivity of bladder cancers may be of limited use. Thus, we propose that the codetection of E-cadherin and gp78 expression should increase confidence, leading to a better prognostic evaluation of bladder carcinoma patients.

As yet it is unclear how down-regulation of E-cadherin is related to up-regulation of gp78 expression. Interestingly, both E-cadherin and gp78 were mapped to the long arm of the human chromosome 16 at the q22.1 and q21 bands, respectively (12, 21). Whether alterations in 16q result in coordinate and reciprocal changes in the regulation of E-cadherin and gp78 gene expressions are not yet known. Alterations and allelic deletions of chromosome 16q were reported in several human epithelial cancers including those of the prostate and the breast (22, 23).

In summary, we have analyzed the expression of gp78 and E-cadherin in 95 bladder tissue specimens and found an inverse correlation between the two markers. Longer follow-up time and a more expanded list of patients should refine the above analysis and should lead to a better understanding of how the differential expression of E-cadherin and gp78 may assist in predicting the clinical outcome of patients with carcinoma of the bladder.

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