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INVITED EDITORIAL

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Prognostic factors for bladder cancer

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Abstract Cancer of the urinary bladder is the fifth most common cancer in men and the second most urological malignancy in Western society [17], with an incidence rate per year of 29.8/100 000 males. Bladder tumors are distinguished as either invasive or superficial: invasive tumors are generally associated with poor prognosis, while 20–30% of superficial carcinomas recur and progress to become invasive and metastatic [26, 27]. The most common prognostic factors for classification of urothelial cancer are staging and grading, which are based on morphological criteria. In the past decade, however, other criteria have been developed as a possible prognostic aid to better disease management, such as expression of specific cell surface antigens, DNA content, chromosomal aberrations, gene rearrangements and point mutations [26, 7]. Since most tumors of the bladder are carcinomas and are associated with dedifferentiation and high metastatic capability, we investigated whether reduced expression of so-called differentiation factors in combination with increased cell motility might be correlated with tumor progression.

Key words Bladder carcinoma · Cell adhesion · Cell motility · Cell migration

Multivariate analysis of superficial bladder cancer uses prognostic factors such as stage, grade, tumor size, dysplasia, multiplicity and hydronephrosis. After patients have been stratified according to these parameters, only concomitant carcinoma in situ is of independent prognostic value [22]. Until now depth of infiltration and differentiation grade have been the most important prognostic parameters for tumor progression and survival. However, these parameters fail in up to 36% of patients, even those with superficial bladder carcinoma. We found similar data in patients with locally advanced bladder carcinoma. There is a subgroup of patients (less than 50%) who survive after radical cystectomy. The major problem is the selection of those patients who are at risk from tumor recurrence and progression and who may benefit from adjuvant treatment modalities. In order to define a high-risk bladder cancer group and to improve the diagnosis and treatment decisions for individual patients several other criteria and/or indicators have been developed which describe: (1) cell adhesion, (2) cell motility and (3) cell migration.

However, the value of these parameters needs to be determined in prospective trials and multivariate analysis and correlated with histopathological findings, i.e., differentiation grade, invasiveness and tumor stage. This paper reviews the currently underway studies which meet these criteria in order to define those prognostic factors which are independent.

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Cell adhesion

Since most cancers are associated with dedifferentiation of epithelial cells, we questioned whether reduced expression of the epithelium-specific cell-cell adhesion molecule E-cadherin was correlated with progression. E-cadherin is a calcium-dependent cell adhesion molecule of the cadherin supergene family [31]. Expression was found to be downregulated in several human and murine carcinoma cell lines [1–6, 8]. Downregulation of E-cadherin expression was also observed in various human tumors [12, 6, 28, 29, 32]. Decreased expression of E-cadherin was furthermore found to be associated with the characteristic of increased tumor cell invasion in vitro [3, 8]. We recently determined the expression of E-cadherin in prostatic carcinoma, normal prostate and beningn prostate hyperplasia (BPH), finding strong E-cadherin expression in all specimens of normal prostate. BPH and well-differentiated prostatic carcinoma. E-cadherin was reduced in 89% of poorly differentiated and in 93% of locally advanced prostatic carcinomas [23]. These results suggest that E-cadherin may play a role in the progression of epithelial cells and may act as an invasion suppressor molecule [3, 8, 21, 33].

Cell motility

A tumor-derived cytokine, autocrine motility factor (AMF), was recently identified by its ability to induce direct and random cell migration via a receptor-mediated signaling pathway [14, 20]. The receptor for AMF was identified as a cell surface glycoprotein with a molecular weight of 78 000 (gp78), which shows homology to p53 [35] and which may be associated with metastasis [34]. Furthermore, it was suggested that retinoic acid-induced differentiation and suppression of invasion of melanoma cells is related, at least in part, to downregulation of autocrine motility factor receptor expression [15].

The monoclonal antibody against the receptor for AMF (anti-gp78 mAb) was found to mimic the physiological effect of AMF, and the enhanced motility induced by anti-gp78 mAb was mediated by a pertussis toxin sensitive G-protein pathway as has been described for other motility factors [30]. We found a different expression pattern of gp78hAMFR in various bladder cancer cell lines. The poorly differentiated, highly motile cell line EJ 28 expressed a high amount gp78hAMFR as measured by the immunofluorescence technique. On the other hand, the well-differentiated bladder papilloma cell-line RT4 and the normal fetal urothelial cell-line HTBFS160 showed a decreased expression pattern for gp78hAMFR. On motile cells, i.e., EJ28 cell line, gp78hAMFR was localized by immunofluorescence to the leading lamella as well as to the trailling edge, suggesting shuffling of gp78hAMFR during cell migration [19, 30].

Expression of cell adhesion and motility factors in bladder cancer

Due to the apparent opposite roles of E-cadherin and autocrine motility factor receptor in tumor cell invasion and metastasis, we initiated a study to evaluate the expression of these two cell surface antigens in bladder carcinomas and correlated these findings with tumor stage, tumor grade and survival [24, 25].

We found that the normal epithelia of the urothelium were highly positive for E-cadherin (12/12), while they were negative for gp78 (0/12) expression. The criteria used for the evaluation of E-cadherin expression were positive (normal) if >90% of the urothelial cells stained positively with high density and negative (pathological) if there was heterogeneous staining with a considerable fraction of E-cadherin negative cells. The criterion used for gp78hAMFR evaluation was positive (pathological) if >50% of the cancer cells were gp78hAMFR positive with large, multiple spots.

A large proportion of the bladder carcinomas showed reduced or negative staining for E-cadherin and positive staining for gp78hAMFR. Muscle and connective tissue of the bladder were negative for both E-cadherin and gp78hAMFR expression. In the case of bladder carcinomas, 67% (18/27) and 52% (14/27) of the noninvasive tumors were reduced for E-cadherin and gp78hAMFR, respectively. However, in the case of muscle-invasive carcinoma, 92% (49/53) and 19% (10/53) were negative for E-cadherin and positive for gp78hAMFR (Figs. 1, 2). The

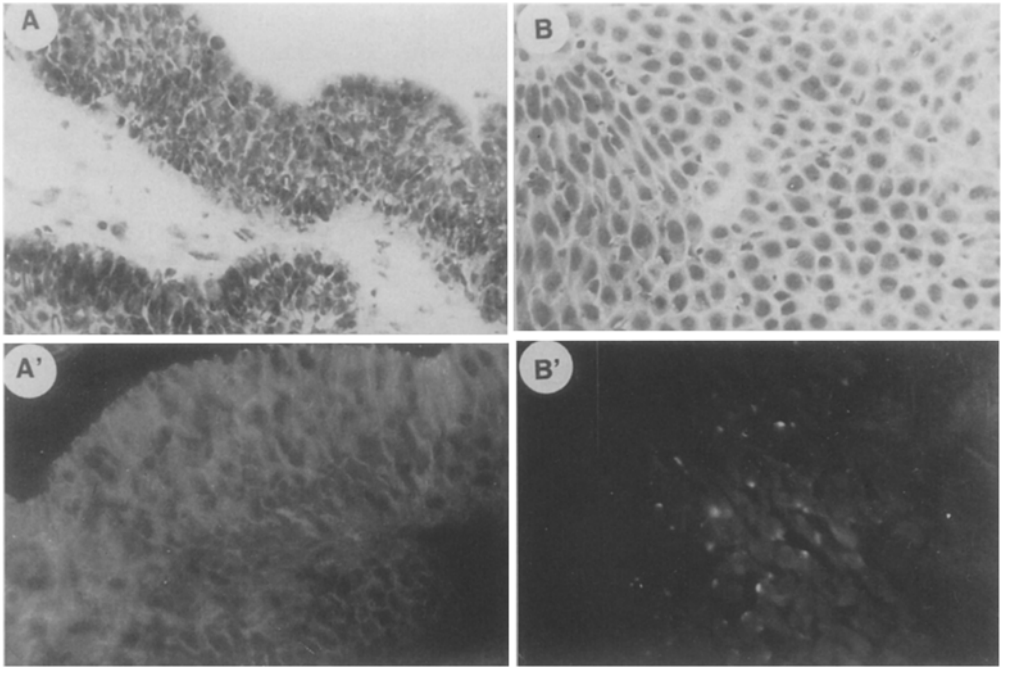

Fig. 1 **A** TCC, TAG1, H&E, **A'** TCC, TAG1. 150. Immunoflourescence staining of E-cadherin shows a strong expression pattern, 450. **B** TCC, TAG1, H&E staining, 450. **B'** TCC, TAG1. Immunoflourescence staining of gp78hAMFR shows a low expression pattern, 450

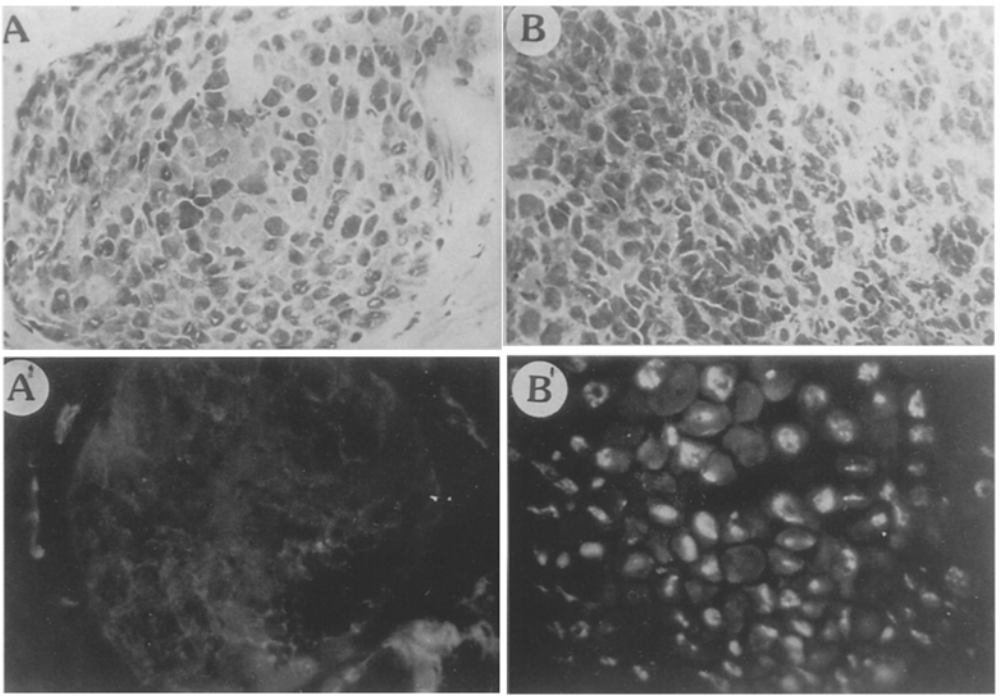

Fig. 2 **A** TCC, T2G3, H&E, 450. **A'** TCC, T2G3. Immunoflourescence staining of E-cadherin shows a low and partially negative expression pattern, 450. **B** TCC, T2G3, H&E staining, 450. **B'** TCC. T2G3. Immunofluorescence staining of gp78hAMFR shows a strong expression pattern, 450

Table 1 Expression of E-cadherin, human autocrine motility factor receptor and CD44 variants in human bladder specimens (% percentage of positively stained samples)

	n	E-cadherin (%)	hAMFR (%)	CD44v (%)	DI (%)	DIII (%)	VFF7 (%)	VFF (%)
Normal bladder tissue	16	100	7	66	31	36	47	33
Bladder carcinoma	27	11	79	89	70	73	81	78
T A/1	11	37	44	100	82	100	100	100
*T*2/3	16	0	85	81	63	57	71	65
P value[a]		<0.001	<0.001	>0.5	>0.5	>0.5	>0.5	>0.5

[a] Standard univariate and multivariate statistical methods including asymptotic (Pearson-Yates correlation) and exact (Fisher's) tests for differences (two-sided) were used to analyze the histopathological findings

three metastases showed a complete downregulation of E-cadherin (0/3), which was associated with upregulation of gp78hAMFR expression (3/3).

After a median follow-up of 24 months, a strong inverse relationship of reduced E-cadherin and increased gp78-hAMFR expression to cancer progression, and to patient mortality was noted. In bladder cancers (TA/T1) which showed strong E-cadherin expression and were negative for gp78hAMFR staining, we found a low risk for tumor progression (0/12), and none of these patients (0/12) died during the observation period. Even patients with locally advanced carcinomas (T2/T3) with strong E-cadherin and negative gp78hAMFR staining belonged to a low-risk group; only 1/7 patients developed tumor progression and none of these patients died from cancer-related causes. However, most of the patients with E-cadherin-reduced (22/35) and gp78hAMFR-increased (23/32) tumors showed progression, and nearly one-third of the patients (11/32) died of cancer. Even patients with superficial bladder carcinoma (TA/T1) underwent rapid tumor progression (2/5) or died from cancer-related causes (1/5).

Cell migration

In a similar context (cell adhesion, cell migration) the function of certain varaiants of the surface glycoprotein CD44 can be seen. The expression CD44 variants was recently demonstrated to be necessary and sufficient to transfert so-called spontaneous metastatic behavior onto a non-metastatic rat pancreatic adenocarcinoma cell-line, as well as onto a nonmetastatic rat fibrosarcoma cell-line [9]. These findings indicate that variant forms of CD44 play an important, but as yet unknown, role in tumor development and progression. Since it is known that neoplastic growth is a multifactorial process of tumorigenesis, we investigated whether there was a relationship between the expression of the above-described molecules E-cadherin and gp78hAMFR and the expression if variant CD44 in bladder tumors.

The normal epithelial cells of the urothelim were highly positive for E-cadherin but negative for gp78hAMFR expression. Of the superficial carcinomas, 63% and 56% were

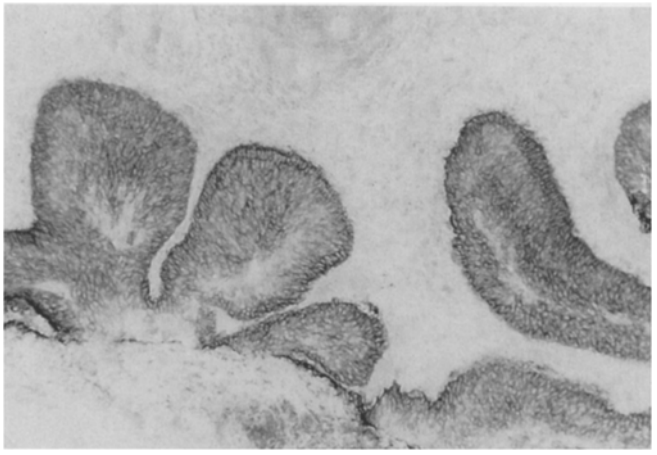

Fig. 3 TCC, TAG2. Immunohistochemical staining of CD44v shows a strong expression pattern of all urothelial cells and partially of the underlying tissue

negative for E-cadherin and gp78hAMFR, respectively ($P< 0.001$). Findings with invasive carcinomas were completely different from those with normal labeling; 100% and 15% of the specimes were negative for E-cadherin and gp78hAMFR ($P< 0.001$). All tested antibodies directed against the variant region of CD44 showed a positive reaction (positive reaction criterion was >90% of the urothelial cells positively stained) both on normal bladder tissue and on bladder carcinomas. The expression in carcinomas, however, was enhanced, i.e., a higher percentage of positively stained samples was observed ($P>0.5$) (Table 1, Figure 3). In contrast to the findings with the antivariant-CD44 antibodies, immunofluorescence staining with anti-E-cadherin and anti-hAMFR antibodies revealed a clear statistical difference between normal bladder tissue and tumors. Expression of E-cadherin was dramatically reduced in the carcinomas, whereas hAMFR expression was clearly increased in comparison to normal tissue (Table 1).

Conclusion

Until now depth of infiltration and differentiation grade have been the most important prognostic parameters for tumor progression and survival. However, these parameters fail in up to 36% of patients, even those with superficial bladder carcinoma [22, 27]. The major problem is the selection of those patients who are at risk from tumor recurrence and progression and who may benefit from adjuvant treatment modalities. In order to define a high-risk group we examined cell biological factors which are known to be involved in the steps necessary for tumor progression and metastasis formation. Here we investigated the expression pattern of the cell adhesion molecules E-cadherin and variant CD44 and the human autocrine motility factor receptor hAMFR in 43 specimes of bladder carcinoma and normal bladder tissue. Bladder tumors showed a similar high expression of variant CD44 to that observed in other types of carcinoma, i.e., in adenocarcinomas of the colon, stomach and breast [10]. In contrast to the results obtained with normal colon and breast tissue, normal bladder epithelium showed a clear reaction with the antibodies directed against the variant portion of the CD44 molecule, indicating that normal urothelim belongs to the group of CD44v-positive tissues. The reaction pattern with the different variant CD44-specific antibodies leads to the conclusion that the variant CD44 exons v3, v5 and v6 are expressed both in normal tissue and in the tumors. Thus, the CD44 variants expressed in bladder tissue might be similar to those found on normal skin keratinocytes but differ markedly from the variants detected in colon, stomach and breast tumors [10]. Interestingly, noninvasive tumors show a higher CD44v expression than invasive specimes. We determined an inverse correlation between gp78hAMFR and E-cadherin. Poorly differentiated, muscle-invasive bladder carcinomas were characterized by increased gp78hAMFR staining (85%) and a decreased E-cadherin expression pattern (100%). There was a clear distinction between superficial and muscle-invasive tumors: 37% and 100% were found to be E-cadherin reduced, respectively, whereas 44% and 85%, were found to be gp78hAMFR increased, respectively.

Furthermore, in 51 patients after a median follow-up of 24 months we determinded a correlation of decreased E-cadherin and increased gp78hAMFR expression to progression and survival [23, 24]. Clearly, we need a longer follow-up and more patients, but the different expressions of cell adhesion molecules and motility factors do go some way toward answering some of the perplexing questions about the biology of bladder tumors.

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