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Influence of the microenvironment on invasiveness of human bladder carcinoma cell lines

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Abstract To investigate the importance of the microenvironment in bladder cancer invasion, a panel of six bladder carcinoma cell lines (SD, RT112, JON, 1207, T24, and J82) was tested in both in vitro and in vivo invasion assays. Furthermore, invasiveness was correlated with the expression of components of the E-cadherin–catenin complex. The E-cadherin-negative cell lines, T24 and J82, displayed a high in vitro invasive capacity, whereas the E-cadherin-positive cell lines, SD and JON, completely lacked in vitro invasive capacity. In contrast, in vivo invasion was noted for all cell lines, with the exception of cell line JON. Most notably, SD formed highly invasive tumors in vivo. The in vivo invasiveness of the E-cadherin-positive bladder carcinoma cell lines was associated with a heterogeneous expression of the E-cadherin–catenin complex. The discrepancy between in vitro and in vivo invasive behavior implies that, in vivo, the microenvironment plays an important role in the establishment of the invasive phenotype. In addition, it was found that orthotopic xenografting of 1207 and T24 bladder carcinoma cells resulted in site-specific tumor take and an enhanced tumor outgrowth and invasiveness, respectively, compared with heterotopic (i.e., subcutaneous) inoculation. We conclude that the site-specific growth and invasion of the bladder carcinoma cell lines in vivo and the observed assay specific invasion (in vitro vs in vivo) points to an effect of the local (bladder) microenvironment on tumor cell behavior.

Keywords Bladder carcinoma · Microenvironment · Neoplasm invasiveness · E-cadherin · Catenins

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Introduction

Bladder cancer is the fifth most common malignancy in males in the Western world. From a clinical point of view, bladder cancers can be divided into superficial and deeply invasive tumors. Superficial bladder carcinomas have a relatively good prognosis despite a high recurrence rate after transurethral resection [18]. Only a small proportion of these superficial carcinomas progress to invasive disease, and distant metastases only seldom develop [19]. Invasive bladder carcinoma, however, has a much less favorable prognosis than superficial bladder carcinoma. Despite the use of aggressive therapies, invasive bladder carcinomas have a 5-year survival rate of 50% or less [30]. The key regulators involved in the invasion of bladder tumors into the bladder wall are only partially understood. A further elucidation of these processes requires relevant in vitro and in vivo tumor models.

A currently available in vitro model for tumor invasion is the well-documented embryonic chicken heart invasion assay, which has been used to study several epithelial tumor systems [2, 37, 38]. Since paracrine effects of the host tissue may regulate the expression of invasion promoting or suppressing molecules, it can be anticipated that in vitro observations may not necessarily be identical to in vivo findings. Furthermore, for some tumor systems, it was demonstrated that the site of tumor cell inoculation might influence tumor growth, invasion and metastasis [26]. Particularly, orthotopic xenografting has been shown to enhance tumor take, invasive properties, and metastatic behavior of a number of human carcinoma cell lines [27, 32]. Therefore, we compared the in vitro invasive properties of six human bladder carcinoma cell lines with their invasiveness after orthotopic and heterotopic inoculation in SCID (severe combined immunodeficiency syndrome) mice. SCID mice were chosen as a host strain, since it was demonstrated that these mice were more apt to permit xenograft take than nude mice [6, 8, 16].

An initial step in the invasive process is the detachment of tumor cells from the in situ carcinoma. It has

been proposed that loss of cell–cell adhesions is a prerequisite for detachment of cells from the primary tumor mass. The E-cadherin–catenin complex primarily determines epithelial cell–cell adhesion. In tumor models, loss of E-cadherin expression appears to be associated with the gain of the invasive phenotype. Similarly, loss of the invasion suppressor molecule E-cadherin is predictive for poor survival of patients with bladder cancer [4, 36]. Furthermore, abnormal expression of the cytoplasmatic, E-cadherin-associated proteins (α-, β-, γ-catenin and p120^{cas}) was also correlated with tumor grade, stage, and poor prognosis [35]. Because these studies suggest that the downregulation of the E-cadherin–catenin complex is involved in invasive bladder carcinoma, we also studied the expression of members of the E-cadherin–catenin complex during the in vitro and in vivo invasion assays.

Our results showed that the transitional cell carcinoma (TCC) cell lines which were used demonstrated a different cell biological behavior in the applied bioassays. In vitro, invasion of the TCC cell lines into the embryonic chicken heart fragments was strongly correlated with absent or heterogeneous expression of E-cadherin. However, transplantation of tumor cells into SCID mice resulted in an enhanced invasive capacity of the E-cadherin-positive TCC cell lines. Furthermore, two TCC cell lines (1207 and T24) exhibited site-specific growth and invasion. These results point to a specific influence of the (bladder) microenvironment on tumor growth and invasion.

Materials and methods

Cell lines

The human bladder cancer cell lines T24, SD, RT112, JON, and J82 were kindly provided by Prof. Dr. J.A. Schalken, Urological Research Laboratory, University Hospital Nijmegen, The Netherlands, and have been characterized previously [23, 29]. Bladder carcinoma cell line 1207 was obtained from Dr. W.I. De Boer, GETU Service d'Urologie, Paris, France [10]. Cells were maintained in DMEM supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 IU/ml penicillin and 100 µg/ml streptomycin at 37°C in a 5% CO_2 atmosphere.

Antibodies

Monoclonal antibody RCK108 directed against cytokeratin 19 (Eurodiagnostica, Arnhem, The Netherlands) or monoclonal antibody DC10 directed against cytokeratin 18 (Beckman Coulter, Fullerton, Calif.) was used to distinguish the human bladder carcinoma cells from murine tissue (in vivo assay) or embryonic chicken heart tissue (in vitro assay). This is based on the species specificity of these two antibodies for human cytokeratins. Monoclonal antibody DC10 was used for staining J82 cells, whereas the other cell lines were stained with monoclonal antibody RCK108. Nearly 100% of the bladder carcinoma cells of each cell line were labeled with RCK108 or DC10. The mouse monoclonal anti-bromodeoxyuridine (BrdU) antibody IIB5 (kindly donated by Dr. B. Schutte, University of Maastricht, The Netherlands) was used to visualize cells that had incorporated BrdU during the S-phase of the cell cycle. Expression of E-cadherin was demonstrated with the monoclonal antibody 5H9 (Eurodiagnostica) raised against an 80-kDa tryptic fragment of E-cadherin, derived from human A-431 carcinoma cells. This antibody also detects the 120-kDa mature E-cadherin protein [25]. The monoclonal antibodies against α- and β-catenin were obtained from Transduction Laboratories (Lexington, Ky.).

Immunohistochemistry

E-cadherin, α- and β-catenin expression by bladder tumor cells in vitro was determined as follows: cell lines were cultured on multichamber slides until confluency was reached and fixed in cold acetone (–20°C) for 1 h. After drying, the slides were incubated with the different primary antibodies, followed by visualization with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse immunoglobulins (Dako, Glostrup, Denmark).

For immunohistochemistry on paraffin-embedded tissues, 5-µm-thick paraffin sections were mounted on 3-amino-propyl-triethoxy-silane (Sigma, St. Louis, Mo.) coated slides and dried overnight at 37°C. Sections were deparaffinized, endogenous peroxidase was blocked in 3% H_2O_2 in methanol (20 min), and the sections were then rinsed in phosphate-buffered saline (PBS).

For antigen retrieval, sections were digested for 10 min in 0.1% pronase (Sigma; containing 1% $CaCl_2$ for E-cadherin staining) in PBS. Prior to α- and β-catenin staining, sections were boiled twice for 5 min in a solution of 0.01 M citrate (pH 6.0) in a microwave oven. Paraffin sections of normal human skin were used as positive controls for E-cadherin, α- and β-catenin staining. For BrdU-staining, after the antigen retrieval procedure was performed, slides were incubated in 2 N HCl for 30 min, followed by two 5-min washes with Borax buffer [34]. Prior to the application of the primary antibodies, non-specific binding was blocked with 10% normal goat serum diluted in PBS containing 1% bovine serum albumin. Incubation with the primary antibody was followed by a biotinylated goat anti-mouse antibody (Dako) and, subsequently, a horseradish peroxidase conjugated streptavidin-biotin complex (Biogenex, San Ramon, Calif.). Peroxidase activity was visualized with 0.03% H_2O_2 and 0.02% 3,3,-diaminobenzidine tetrahydrochloride (DAB; Fluka, Basel, Switzerland) diluted in PBS. The slides were counterstained with Mayer's hematoxylin, dehydrated, and mounted.

In vivo tumorigenicity of cell lines

The tumorigenicity of each cell line was tested in 6-week-old female SCID mice (B17/ICR Han Hsd-SCID), obtained from Harlan (Zeist, The Netherlands). Bladder carcinoma cells suspended in Hanks' buffered salt solution were injected both subcutaneously ($\pm 4\times10^6$ cells) in a volume of 200 µl and in the submucosa of the bladder ($\pm 1\times10^6$ cells) in a volume of 50 µl. For inoculation of tumor cells in the bladder wall, an abdominal incision was made in mice anaesthetized with avertine (0.25 mg/g body weight). One hour before the mice were sacrificed, they received an intraperitoneal injection of BrdU (40 mg/kg body weight) in 0.15 M NaCl. Under a dissecting microscope, tumor inoculation sites plus other relevant organs were removed and fixed in 4% phosphate-buffered formalin for 16 h before paraffin embedding.

Determination of the proliferative activity of TCC in vivo

Proliferative activity was determined by counting BrdU-positive nuclei in a total of 1000 nuclei, at four random areas of the tumor. Proliferation is expressed as the labeling index: number of BrdU-positive nuclei/total number of nuclei ×100%.

Morphometry

The size of in vivo-formed tumors was estimated using image analysis. Serial sections were cut, and the estimated largest tumor

area was determined from the immunostainings with the RCK108 or DC10 antibody. Subsequently, these tumor areas were quantitated using a Hitachi CCTV camera equipped with the KS400 image analysis software package (Kontron Elektronik, Eching, Germany).

In vitro invasion assay

Chicken heart invasion assays were performed as described by Mareel et al. [22]. Briefly, human bladder tumor cells were confronted with precultured rounded fragments of embryonic chicken heart on soft agar for 24 h. Next, the fragments with attached bladder tumor cells were kept in suspension culture under gyrotory shaking (120 rpm, 37°C) in MEM REGA 3 medium (GibcoBRL/Life Technologies, Breda, The Netherlands), containing 10% FCS.

After 6 days of cocultivation, fragments were fixed in Bouin-Hollande's solution and embedded in paraffin. Invasion was scored on serial histological sections stained with hematoxylin/eosin. The tumor cells can be discerned from the heart tissue by their more basophilic staining. Additional confirmation of invasion was obtained using selective keratin (RCK108 and DC10) immunostaining of the human bladder carcinoma cells.

Morphology of tumor cells on Matrigel

The morphological appearance of bladder tumor cells on Matrigel was assessed. Therefore, 200 µl growth factor-reduced Matrigel (9.64 mg/ml; Becton Dickinson, Bedford, Mass.) per well was allowed to polymerize for 30 min at 37°C in a 24-well plate. Subsequently, the same Matrigel solution containing 1×10^4 tumor cells was poured on top of the polymerized Matrigel. After the gelation of the second Matrigel solution, standard culture medium was pipetted on top. After 1 week, morphology of the bladder tumor cells (epithelial or fibroblast-like) was recorded using microscopic examination.

Results

In vivo tumorigenicity

Tumor take, tumor size, muscle invasion, and proliferative activity after heterotopic (subcutaneous) and orthotopic inoculation of each of the six cell lines was examined (Table 1). A tumor take of 100% was observed for SD and RT112 bladder carcinoma cells both after orthotopic and heterotopic inoculation. Both cell lines clearly invaded the muscle tissue underlying the skin or bladder mucosa (Fig. 1A, B). The size of the subcutaneous tumors was four- to ten-fold larger than the bladder tumors, possibly reflecting the larger number of cells inoculated subcutaneously. SD and RT112 tumors occasionally penetrated through the bladder mucosa into the bladder lumen.

We observed a high take rate after both heterotopic and orthotopic inoculation of T24 cells in SCID mice (Table 1). Strikingly, subcutaneous T24 tumors were rather small, and no muscle invasion was noted (Table 1 and Fig. 1D). In contrast, T24 tumors, localized in the bladder, were about threefold larger, showed muscle invasion (Fig. 1C), and had a significantly higher proliferative activity than subcutaneous T24 tumors (Table 1).

Site-specific tumor take was seen for cell line 1207. Although in four out of five mice, orthotopically injected with 1207 cells, tumors had developed in the bladder wall (two were muscle-invasive), no tumors were found at the subcutaneous injection sites despite the larger number of subcutaneously inoculated tumor cells. Microscopic examination of the subcutaneous injection sites confirmed this observation. The two muscle invasive 1207 tumors also showed penetration into the bladder lumen.

JON cells demonstrated a high take rate both hetero- and orthotopically, but muscle invasion was completely lacking. The subcutaneous JON tumors showed an expanding growth pattern with sharp tumor borders. These tumors were associated with an inflammatory infiltrate. The tumor take of J82 was low, both after orthotopic and heterotopic inoculation. In one mouse, a large muscle invasive subcutaneous tumor was found.

In none of the injected mice were lymphogenic or distant metastases found, but regional lymph vessels in the loose connective tissue surrounding the bladders of mice injected with SD, RT112, or T24 bladder carcinoma cells occasionally contained isolated small tumor aggregates (Fig. 1A).

In vitro embryonic chicken heart invasion assay and tumor cell morphology on Matrigel

In vitro invasion of bladder carcinoma cells was examined after 4–6 days of cocultivation with embryonic

Table 1 Heterotopic and orthotopic tumorigenicity of bladder carcinoma cell lines. *Htr* heterogeneous; *Hom* homogeneous; *Neg* absent expression at the cell membrane; *labeling index* percentage of BrdU (anti-bromodeoxyuridine)-positive cells; – no subcutaneous tumors

Cell line	Mean follow-up period in days (range)	Bladder		Subcutaneous		E-cadherin in vivo	Labeling index		Estimated largest area	
		Tumor take	Muscle invasion	Tumor take	Muscle invasion		Bladder (mean±SD)	Subcutaneous (mean±SD)	Bladder (mean±SD)	Subcutaneous (mean±SD)
SD	39.5 (35–53)	4/4	4/4	4/4	4/4	Htr	13.4±9.3	16.3±3.4	6.0±6.5	21.2±10.8[a]
RT112	37.5 (27–53)	4/4	3/4	4/4	3/4	Htr	22.0±1.6	19.4	3.5±4.1	34.6±19.3[a]
T24	58 (35–74)	4/4	3/4	3/4	0/3	Neg	10.3±2.9[a]	2.0±0.7	4.0±1.1[a]	1.2±1.6
1207	50.6 (29–53)	4/5	2/4	0/5	0/0	Htr	16.1±3.8	–	2.2±2.6	–
Jon	50.2 (31–57)	5/5	0/5	4/5	0/4	Hom	16.3±2.6	13.2±4.3	1.5±1.5	2.6±1.6
J82	42 (40–53)	2/5	0/2	1/5	1/1	Neg	21.0±0.0	17.3[b]	3.1±4.1	53.2

[a] Significantly different in bladder compared with subcutaneous (*t* test; $P<0.05$)
[b] Only one tumor was available for analysis

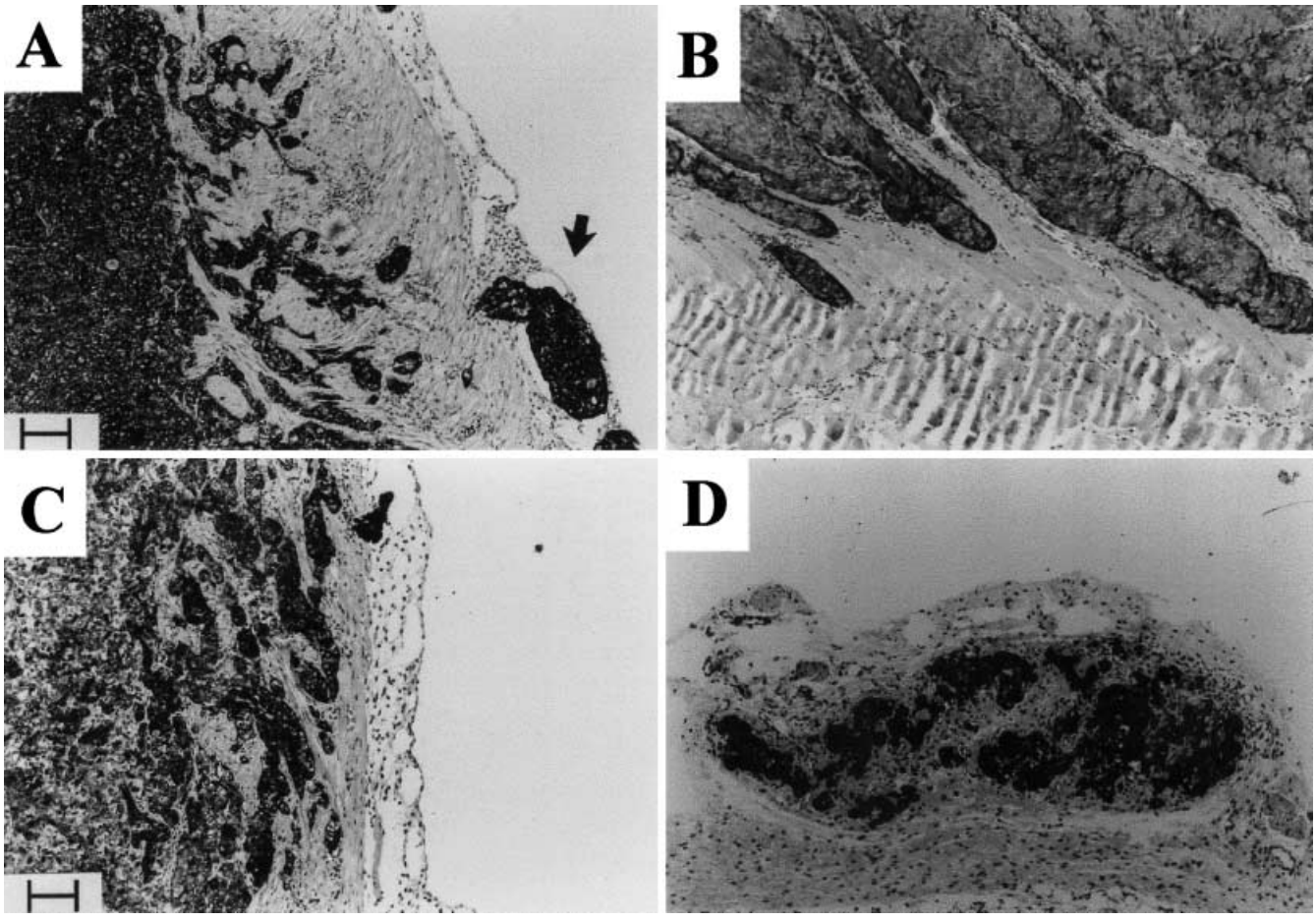

Fig. 1 Tumorigenicity of bladder tumor cells. Tumor cells were selectively stained with RCK108. Invasion of RT112 cells into the submucosa and muscularis of the bladder (**A**) and the skeletal muscle underneath the skin (**B**). Note the clump of RT112 cells in a perivesicular lymph vessel of the bladder (**A**; *arrow*). Part of a large orthotopic tumor of T24 cells in the bladder (**C**) and, as a reference, the small tumor in the subcutis (**D**). *Scale bar* 100 µm

Table 2 In vitro invasion of transitional cell carcinoma (TCC) cell lines. *Htr* heterogeneous; *Hom* homogeneous; *Neg* absent expression at the cell membrane; *cytopl/cell-membr* cytoplasm/cell membrane; *I* invasive; *TC* tight colonies, criteria adapted from [35]

Cell line	E-cadherin expression in vitro	α-catenin expression in vitro	β-catenin expression in vitro	In vitro confronting cultures Adherent[a]	In vitro confronting cultures Invasive[b]	Morphology Matrigel
SD	Hom	Hom	Hom	37/38 (97%)	0/37 (0%)	TC Epithelial
RT112	Hom	Hom	Hom	9/15 (60%)	3/9 (33%)	TC Epithelial
T24	Neg	Htr Cytopl/cell-membr	Hom	18/30 (60%)	16/18 (89%)	I Fibroblastic
1207	Htr	Hom	Hom	28/45 (62%)	11/28 (39%)	TC epithelial
JON	Hom	Hom	Hom	8/15 (53%)	0/8 (0%)	TC Epithelial
J82	Neg	Htr Cytopl/cell-membr	Hom	9/17 (53%)	8/9 (89%)	I Fibroblastic

[a] Number of adherent/total number of confronted embryonic chicken heart cultures
[b] Number of invasive/number of confronted cultures with adherent tumor cells

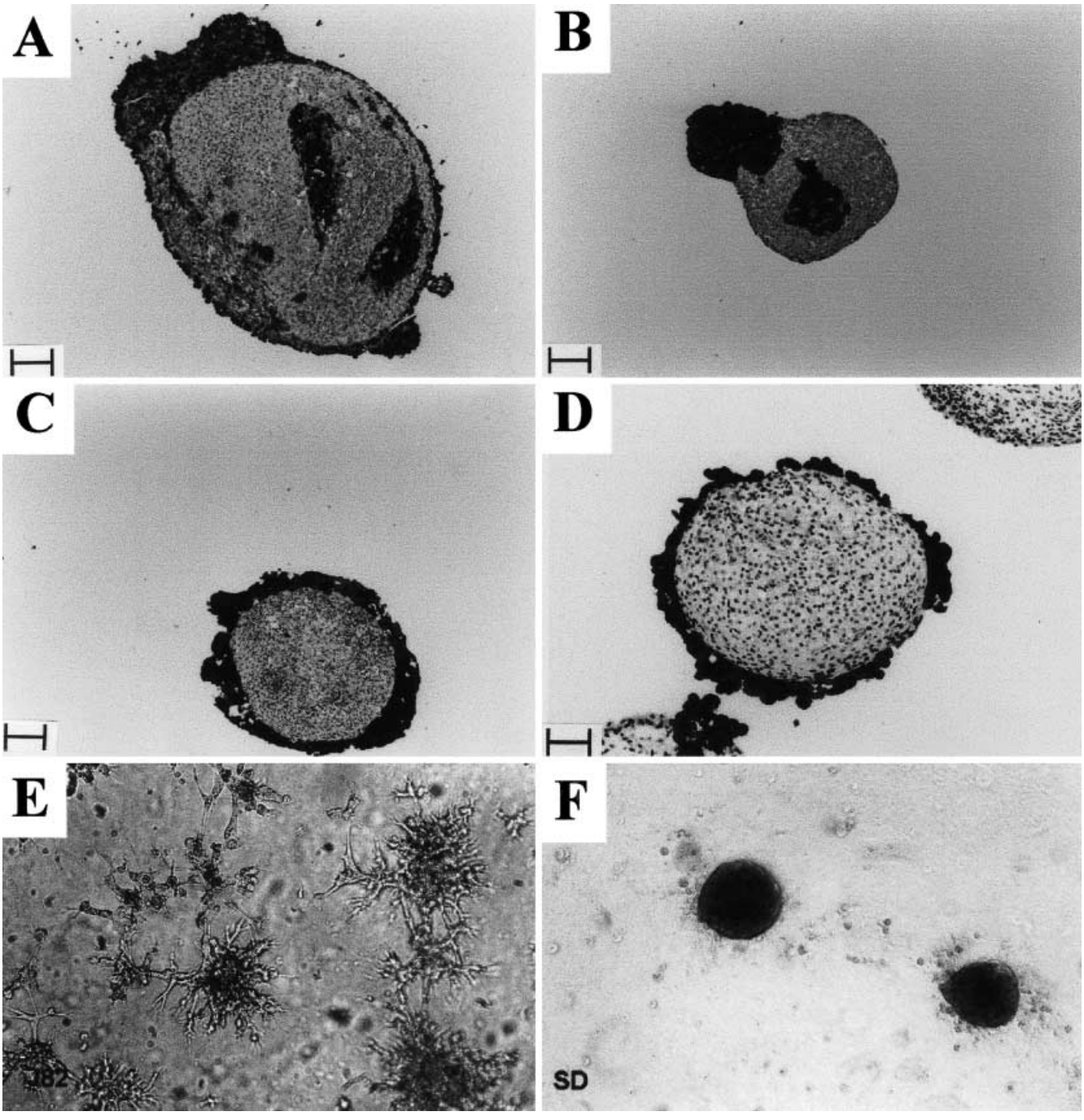

Fig. 2 Confronting cultures of invasive and noninvasive human transitional cell carcinoma (TCC) cells with embryonic chicken heart fragments. Photomicrographs from paraffin sections of embryonic chick heart fragments confronted in organ culture with the cell lines T24 (**A**) and 1207 (**B**) that invaded the fragments and two noninvasive cell lines SD (**C**) and JON (**D**); the former engulfs the chicken heart tissue. Bladder tumor cells could be discerned from the chick tissue using a selective staining with the human-specific antibody RCK108. *Scale bar* 100 µm; except **D,** *scale bar* 50 µm. Phase-contrast photomicrographs of colonies formed in solid Matrigel. Examples of colonies with fibroblastic appearance formed by cell line J82 (**E**) and of epithelioid colonies formed by cell line SD (**F**)

chicken heart fragments. Adhesion of tumor cells to chicken heart fragments varied from 53% of the cases for JON and J82 cells to 97% of the cases for the SD cells (Table 2). T24 and J82 cells showed the strongest invasive capacity. After successful adhesion, nearly all embryonic chicken heart fragments were infiltrated by these E-cadherin-negative tumor cells (Fig. 2A). RT112 and 1207 (Fig. 2B) cells had a moderate capacity (33% and 39% of the cases, respectively) for invading heart fragments, whereas SD (Fig. 2C) and JON (Fig. 2D) cells were not able to invade the embryonic chicken heart fragments at all. The SD cell line formed a clear

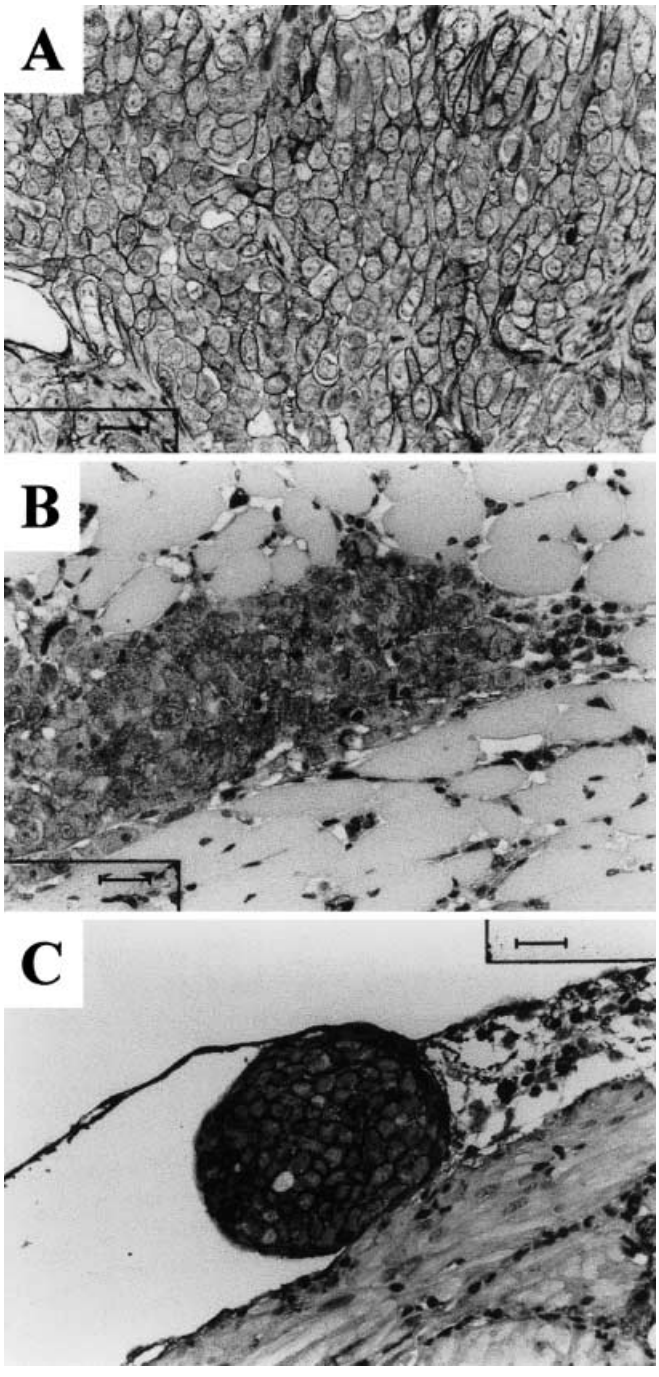

Fig. 3 Expression of E-cadherin in vivo. Normal expression of E-cadherin in a SD tumor (**A**). Reduced expression of E-cadherin in an invasive region of a subcutaneous SD tumor (**B**). RT112 cells in a perivesicular lymph vessel of the bladder, with normal expression of E-cadherin (**C**). *Scale bar* 25 μm

epithelioid cell layer around the embryonic heart tissue (Fig. 2C), a feature characteristic for cells with a functionally intact E-cadherin–catenin complex [37].

To determine the epithelial or mesenchymal morphotype of the used cell lines, cell lines were cultured in solid Matrigel substrate (method adapted from [37]). After 1 week of culture in solid Matrigel (Table 2), the constitutively E-cadherin-negative T24 and J82 (Fig. 2E) cells had a fibroblastic morphology. In contrast, the E-cadherin-positive cell lines, SD (Fig. 2F), RT112, and JON, expressed an epithelial morphology with formation of densely packed colonies. Bladder carcinoma cell line 1207, with a heterogeneous expression of E-cadherin, had an epithelial morphology when cultured in Matrigel.

In vitro and in vivo expression of E-cadherin and α- and β-catenin

Data on expression of E-cadherin and α- and β-catenin on confluent cultures of bladder carcinoma cell lines visualized using immunohistochemistry are summarized in Table 2. Expression patterns of E-cadherin were compared between the in vivo and in vitro assays. Cell lines T24 and J82 lacked E-cadherin expression in vivo and in vitro (Table 1 and Table 2). The cell lines, SD and RT112, which display a homogeneous E-cadherin expression in vitro, displayed a heterogeneous cell membrane immunostaining in vivo both in the subcutaneous tumors and in the tumors located in the bladder wall. Regions with normal expression of E-cadherin were seen in the central areas of these tumors (Fig. 3A). Reduced expression of E-cadherin was primarily seen in the invasive borders of these tumors (Fig. 3B). Strikingly, the isolated small aggregates of SD and RT112 tumor cells in lymph vessels had a normal expression of E-cadherin (Fig. 3C). The expression patterns of either α- or β-catenin was similar to the expression pattern of E-cadherin in the different tumors.

Discussion

The six human bladder carcinoma cell lines studied in this paper display a considerable heterogeneity of cell biological characteristics with respect to their invasive potential both in vitro and in vivo. SCID mice proved to be a very suitable host strain, allowing successful grafting of all six examined human bladder carcinoma cell lines. This was emphasized by xenografting T24 cells in SCID mice. Tumor take of T24 cells in nude mice is poor or absent both after orthotopic and heterotopic inoculation [23; unpublished]. Strikingly, T24 tumor growth, as reflected by tumor size, proliferative activity, and tumor invasiveness, was enhanced by the orthotopic inoculation (Table 1). Cells from cell line 1207 exhibited site-specific outgrowth, since 1207 tumor take only occurred after orthotopic transplantation. No signs of distant metastases for any of the xenografted TCC cell lines were found. Although isolated (RT112, SD, and T24) tumor aggregates were found in the perivesicular loose connective tissue, no lymph-node metastases were found. The short follow-up period of 2 months may account for this.

We compared the in vivo invasiveness of the various TCC cell lines with the results of the in vitro assay. The results of the in vitro embryonic chicken heart invasion

assay did not match the in vivo invasive behavior of some of the tested TCC cell lines, most notably SD. The SD cell line was not invasive in vitro, but after subcutaneous injection and after inoculation in the bladder wall, SD cells developed muscle-invasive tumors. Similar discrepancies between the embryonic chicken heart invasion assay and in vivo invasiveness were reported earlier in a study on colorectal carcinoma cell lines [11, 12]. Our results support the view that the microenvironment may regulate invasive behavior of bladder carcinoma cells in vivo, for instance by influencing the expression of E-cadherin, as suggested by Mareel et al. [21] or by induction of synthesis of extracellular matrix degrading proteins [33]. More recent studies have shown that there is a link between cell–cell adhesion and proteinase production that promotes invasiveness of tumor cells [20, 28].

We studied the immunochemical expression of the E-cadherin–catenin complex in vivo and in vitro and its correlation with invasiveness. The observed in vitro expression of the various members of the E-cadherin–catenin complex in the TCC cell lines was confirmed by means of Western blotting by Giroldi et al. [14]. In vivo, a heterogeneous E-cadherin and catenin expression by SD and RT112 cells was observed, in contrast to their homogeneous E-cadherin–catenin expression in vitro. Loss or reduced expression of the E-cadherin–catenin complex was primarily seen in the invasive regions of these tumors. These findings and the normal E-cadherin and α- and β-catenin expression by small aggregates of SD and RT112 tumor cells in lymph vessels (early sign of metastasis) suggests that the microenvironment may transiently reduce the expression of the E-cadherin–catenin complex during formation of invasive bladder tumors in SCID mice. Transient downregulation of E-cadherin expression in vitro is described previously and could possibly be accomplished through activation of exogenous *c-fos* [31], via c-erb2 [13] or transforming growth factor-β [24]. Furthermore, expression could be regulated by methylation of the E-cadherin gene or via tissue-specific responsive elements in the E-cadherin promoter [9]. Recent investigations have identified the transcription factor Snail, which could bind to E boxes in the human E-cadherin promoter and, thereby, repress transcription of E-cadherin [1, 7]. Furthermore, Keirsebilck et al. described that the in vivo transient downregulation of E-cadherin could be caused by instability of the E-cadherin messenger (m)RNA [17]. Recently, Bringuier et al. showed that abnormal E-cadherin immunoreactivity in bladder tumors is associated with mRNA downregulation or post-transcriptional downregulation of E-cadherin. In the same series of bladder tumors, no structural alterations of the E-cadherin gene were detected [5]. These findings corroborate with our observations on the transient reduction of E-cadherin expression in invasive bladder tumors.

A strong correlation was found between the in vitro expression of E-cadherin of bladder carcinoma cells and their ability to invade embryonic chicken heart fragments. Similar results were reported for cell lines of other origin [2, 37, 38]. The two E-cadherin-negative cell lines T24 and J82 were generally capable of invasion of the embryonic chicken heart, whereas the (in vitro) homogeneously E-cadherin-positive SD and JON cells showed no invasion. The epithelial morphology of these E-cadherin-positive cell lines grown on Matrigel is in accordance with their functionally intact membrane-bound E-cadherin–catenin complex [37].

The in vitro invasive cell lines, T24 and J82, had a fibroblastic morphotype when cultured on solid Matrigel. This is in accordance with the observation that the epithelial-to-mesenchymal transition is correlated with gain of motility and invasive disease [15]. The 1207 cells with a heterogeneous expression of E-cadherin had a rather limited infiltrative capacity in the embryonic chicken heart invasion assay. Surprisingly, RT112 cells with in vitro homogeneous E-cadherin and catenin expression were also capable of embryonic chicken heart invasion in one-third of the samples. Booth et al. showed that RT112 cells could invade the subepithelial capillary bed after culture on urinary tract stroma. In the same paper, they showed that these RT112 cells had partially lost their E-cadherin expression [3]. A similar in vitro mechanism could have occurred in our embryonic chicken heart invasion assay.

In conclusion, we have studied the invasive properties of six bladder carcinoma cell lines with the use of in vitro and in vivo invasion assays. We have shown that the use of SCID mice allows a high tumor take for bladder cancer cell lines, including those which proved to be poorly if at all tumorigenic in nude mice. Bladder tumor cell lines 1207 and T24, on the basis of their site-dependent tumor take and tumor outgrowth, respectively, may be considered to resemble more closely organ-confined human bladder carcinomas with regard to their response to growth-modulating factors and extracellular matrix proteins. The view that the microenvironment influences the induction of bladder tumor invasion is supported by the observation that the results of the in vitro invasion assay of E-cadherin-positive bladder carcinoma cell lines did not correlate well with their invasive properties in vivo. Transient downregulation of the E-cadherin–catenin complex may in part explain the observed invasive capacity in vivo of the latter cell lines.

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