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Decreased RECK and Increased EMMPRIN Expression in Urothelial Carcinoma of the Bladder Are Associated with Tumor Aggressiveness

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Key Words
RECK · EMMPRIN · CD147 · Matrix metalloproteinase · Urothelial carcinoma of the bladder

Abstract
Objective: Urothelial bladder carcinomas show a divergent biological behavior, which significantly complicates risk stratification and clinical management. The MMP repressor RECK and the MMP activator EMMPRIN regulate the invasive potential by metalloproteinase-induced stromal degradation. Data on RECK in urothelial bladder cancer are lacking and information on EMMPRIN is sparse. This study aims to investigate the expression of RECK and EMMPRIN in urothelial carcinoma of the bladder and to correlate these findings with clinicopathological parameters. ***Methods:*** Our study included 127 specimens of urothelial carcinomas derived from 103 patients who underwent either TUR-B or cystectomy. Immunohistochemical expression analysis was performed for RECK, EMMPRIN, MMP-2, MMP-9 and MMP-14. Expression levels were graded for staining intensity and correlated with pT stage and WHO tumor grade. ***Results:*** Invasive (≥pT1) as well as WHO high-grade urothelial carcinomas showed a statistically significant and stepwise downregulation of RECK ($p < 0.001$) and concomitant upregulation of EMMPRIN ($p < 0.001$) compared to non-invasive and WHO low-grade tumors. No correlation was observed for the MMPs investigated. ***Conclusion:*** Decreased RECK and increased EMMPRIN expression are associated with increasing stage and grade. Both proteins may serve as molecular marker for the distinction between potentially invasive (≥pT1) and non-invasive tumors (≤pTa).

Abbreviations used in this paper

CIS	carcinoma in situ
EGFR	epidermal growth factor receptor
EMMPRIN	extracellular matrix metalloproteinase inducer
HE	hematoxylin and eosin
HER	human epidermal growth factor receptor
HER2/neu	human epidermal growth factor receptor 2
ISUP	International Society of Urological Pathology
MAPK	mitogen-activated protein kinase
MMP	matrix metalloproteinase
RECK	reversion-inducing cysteine-rich protein with Kazal motifs
TUR-B	transurethral resection of the bladder
WHO	World Health Organization

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Introduction

Urinary bladder cancer is the fourth most frequent malignancy in men after prostate, colorectal and lung cancer. In 2009, it was diagnosed over 71,000 times in the United States with approximately 14,000 deaths per year [1]. In Western countries, 90% of the carcinomas of the urinary bladder derive from the urothelial lining. A key problem in the therapeutic management of bladder tumors is the variable and hardly predictable clinical disease progression.

Papillary urothelial neoplasms of low malignant potential or well-differentiated papillary urothelial carcinomas that do not penetrate the basement membrane (pTa, low-grade) are rarely associated with the patient's death; however, disease recurrence is common and 10–15% develop into invasive disease [2]. By contrast, invasive urothelial carcinomas (≥pT1) and carcinomas with histological high-grade features are potentially life-threatening and harbor a high risk of progression to muscle invasion and pT2 tumors with only 50% survival at 5 years [3]. At present, determining tumor prognosis relies on prior recurrence rate, number of tumors, tumor size, presence of CIS, tumor stage and tumor grade [4, 5]. However, the latter two are in some cases difficult to assess by the pathologist due to incomplete sampling, small specimen sizes, cauterization artifacts, and other reasons. In an attempt to refine the applied diagnostic criteria, the old WHO/ISUP pathological grading system was replaced by a novel and simpler consensus classification in 2004. Aiming to better reflect the prognosis of urothelial carcinomas, the novel grading system discriminates only between low- and high-grade urothelial carcinomas [6].

However, despite these recent advancements, reliable prognostic information regarding the invasiveness and metastatic potential of a certain tumor is still limited [7]. Hence, new molecular markers that provide additional information on the biological potential of these tumors may allow a more precise and objective assessment and may also help to identify drugable targets especially for high-grade urothelial carcinomas. Numerous promising novel biomarkers have been investigated during the last couple of years [8], but only very few markers have been validated in prospective trials [9]. Until now, only immunohistochemical staining of p53 found its way into pathological routine diagnostics for selected cases [10, 11]. Since high-grade and invasive tumors (≥pT1) in particular are characterized by their invasive potential and ability to metastasize, this study focuses on two key players that are well known to orchestrate tissue invasion by tissue degrading MMPs: the MMP inhibitor RECK and the activating counterpart EMMPRIN (also known as CD147).

The membrane-anchored glycoprotein RECK is known as an inhibitor of MMP-2, MMP-9 and MMP-14, and is ubiquitously expressed throughout normal human tissue [12, 13]. While tumor cells were reported to downregulate RECK, thereby enhancing their invasive potential [14], forced expression of RECK in cancer cells conversely resulted in reduced angiogenesis, invasion and metastasis in animal xenografts [14, 15]. The MMP activator EMMPRIN is a transmembraneous and pleiotropic glycoprotein that is particularly critical for mammalian reproduction, neuronal and lymphatic development and maintenance as well as for tissue repair and remodeling in general [16]. Although EMMPRIN expression is not exclusively restricted to tumor cells, there is ample evidence that EMMPRIN is an important promoter of tumor cell invasion [16, 17].

Although several studies demonstrated both proteins to be implicated in the development and progression of numerous cancer entities [12, 18, 19], the data available on urological tumors, especially on urothelial carcinomas of the bladder, are limited. Therefore, this study aims to investigate the expression profile of RECK, EMMPRIN and RECK's targets MMP-2, MMP-9 and MMP-14 in 127 urothelial carcinomas of the bladder, particularly with respect to stage and grade of the tumor. To our knowledge, this is the first study demonstrating RECK in the urinary bladder and urothelial carcinoma.

Material and Methods

Tissues and Patients

A total of 127 urothelial carcinomas of the bladder from 103 patients who were diagnosed at the Institute of Pathology at Charité University Hospital (Berlin, Germany) between 2006 and 2009 were included in this study with permission of the local ethics committee (table 1). The patients were treated by TUR-B (110 cases) or cystectomy (17 cases) and did not receive chemotherapy or radiation prior to the surgery. CIS was present in 9 cases. Twenty-four of the 127 carcinomas were relapses occurring in 20 of the 103 patients. Initial diagnoses were existent for all 103 patients. Tumor numbers were known for 41 carcinoma samples, 24 of them were singular and 17 were multifocal carcinomas. Tumor size in diameter was known for 14 cystectomies and 1 TUR-B sample, 2 of them were <3 cm, the remaining 13 were larger. Tissue samples were fixed in 4% buffered formaldehyde, embedded in paraffin and histological diagnoses were established on standard HE-stained sections. Tumor stage and grade were determined according to the WHO classification from 2004 [6].

Table 1. Clinical and histopathological data of 103 patients with 127 urothelial carcinomas undergoing TUR-B and/or cystectomy

	n (%)
Patient characteristics (n = 103)	
Age, years[1]	
≤50	6 (6)
51–60	13 (13)
61–70	32 (31)
≥71	52 (50)
Sex	
Male	75 (73)
Female	28 (27)
Tumor characteristics (n = 127)	
pT classification	
pTa	63 (50)
pT1	35 (27)
pT2	15 (12)
pT3	8 (6)
pT4	6 (5)
WHO grade	
Low	53 (42)
High	74 (58)
Operative method	
TUR-B	110
Cystectomy	17

[1] Median age 71 years, range 34–90 years.

Immunohistochemistry

From every formalin-fixed paraffin-embedded tissue, serial histological sections were taken. The first set of sections was routinely HE stained. Immunostaining was performed on the following sections, as described previously [20]. In brief, tissue sections of 2–3 μm were incubated with the primary antibody against RECK (rabbit monoclonal antibody, Cat. No. 3433 [clone D8C7]; Cell Signaling Technology Inc., Boston, Mass., USA, for 1 h at room temperature, 1:50) [21], EMMPRIN (rabbit polyclonal antibody, Cat. No. 34-5600; Invitrogen, Karlsruhe, Germany, for 1 h at room temperature, 1:250) [22], MMP-2 (mouse monoclonal antibody, Cat. No. MAB13431 [clone A-Gel VC2]; Chemicon International Inc./Millipore, Temecula, Calif., USA, over night at 4°C, 1:25), MMP-9 (rabbit polyclonal antibody, Cat. No. RB-9234-P1; Lab Vision Corp., Fremont, Calif., USA, 1 h at room temperature, 1:100), and MMP-14 (rabbit monoclonal antibody, Cat. No. ab51074 [clone EP1264Y]; Abcam, Cambridge, Mass., USA, for 1 h at room temperature, 1:100). Slides were subsequently incubated with a biotinylated anti-mouse/anti-rabbit secondary antibody mix using a multilink biotin-streptavidin-amplified detection system (LSAB2 System-AP; Dako, Hamburg, Germany). Staining was visualized using fast-red chromogen (Sigma-Aldrich, Munich, Germany).

Dilution series were done on representative sections in order to determine the optimal concentration of the primary antibodies. Positive and negative controls guaranteed persistent quality of the staining procedure. Negative controls were performed with missing primary antibody. Specificity of the primary antibodies was checked by Western blot analyses (see below).

Immunostainings were examined independently by two pathologists (D.W. and A.S.) blinded for all clinical and pathological data. Ambiguous cases were discussed at a multi-headed microscope until consensus was achieved. Staining intensities of RECK, EMMPRIN, MMP-2, MMP-9 and MMP-14 were quantified in the area of the tumor's invasion front and classified on a scale from 0 to 3. For statistical analyses and graphic design, staining intensities of 0 and 1 were categorized as weak, those of 2 and 3 as strong staining intensity.

Western Blot

HCV-29 cells derived from normal human urothelium were kindly provided by R. Knüchel-Clarke, Regensburg, Germany. The human bladder carcinoma cell lines RT-4 and RT-112 were obtained from the 'Deutsche Sammlung von Mikroorganismen und Zellkulturen' (DSMZ), the human bladder carcinoma cell line J82 was purchased from the American Type Culture Collection (ATCC). Cells were lysed in a buffer of 50 mM Tris (hydroxymethyl-)aminomethane, 1% (w/v) sodium dodecyl sulfate, 1 mM ethylene diaminetetraacetic acid, 1 mM phenylmethylsulfonyl fluoride, 100 μg/ml soybean trypsin inhibitor, 10 μg/ml aprotinin, pH 6.8, and sonicated. After centrifugation, 20 μg protein of the supernatant each were separated on a 7.5% (RECK) or 10% (EMMPRIN) sodium dodecylsulfate polyacrylamide gel and transferred onto a polyvinylidene difluoride membrane (Millipore Corp., Bedford, Mass., USA). After blocking with a solution of 2% ECL Advance Blocking Agent (GE Healthcare, Munich, Germany) in Tris-buffered saline/0.1% Tween-20, the membrane was incubated with the RECK antibody (1:1,000) or the EMMPRIN antibody (1:5,000) for 1 h in this solution (antibody data: see above). Horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (1:2,000; Dako, Hamburg, Germany) served as secondary antibody. Bands were detected by enhanced chemiluminescence (ECL Advance Western Blotting Detection Kit, GE Healthcare) in a Fluor-S MultiImager (Bio-Rad Laboratories, Hercules, Calif., USA).

Statistical Analyses

Statistical calculations were performed with SPSS version 18.0 (SPSS, Chicago, Ill., USA) and GraphPad Prism version 5.00 (GraphPad Software, La Jolla, Calif., USA). Spearman's bivariate correlation, Fisher's exact test and χ^2 test according to Pearson were used for determining statistical significance. p values <0.05 were considered significant, all p values were two-sided.

Results

Immunostaining and Localization of RECK, EMMPRIN, MMP-2, MMP-9 and MMP-14

Blinded for all clinical and pathological data, 127 tumor samples of 103 patients were investigated for the expression of RECK, EMMPRIN, MMP-2, MMP-9 and MMP-14. The staining intensities of each protein expres-

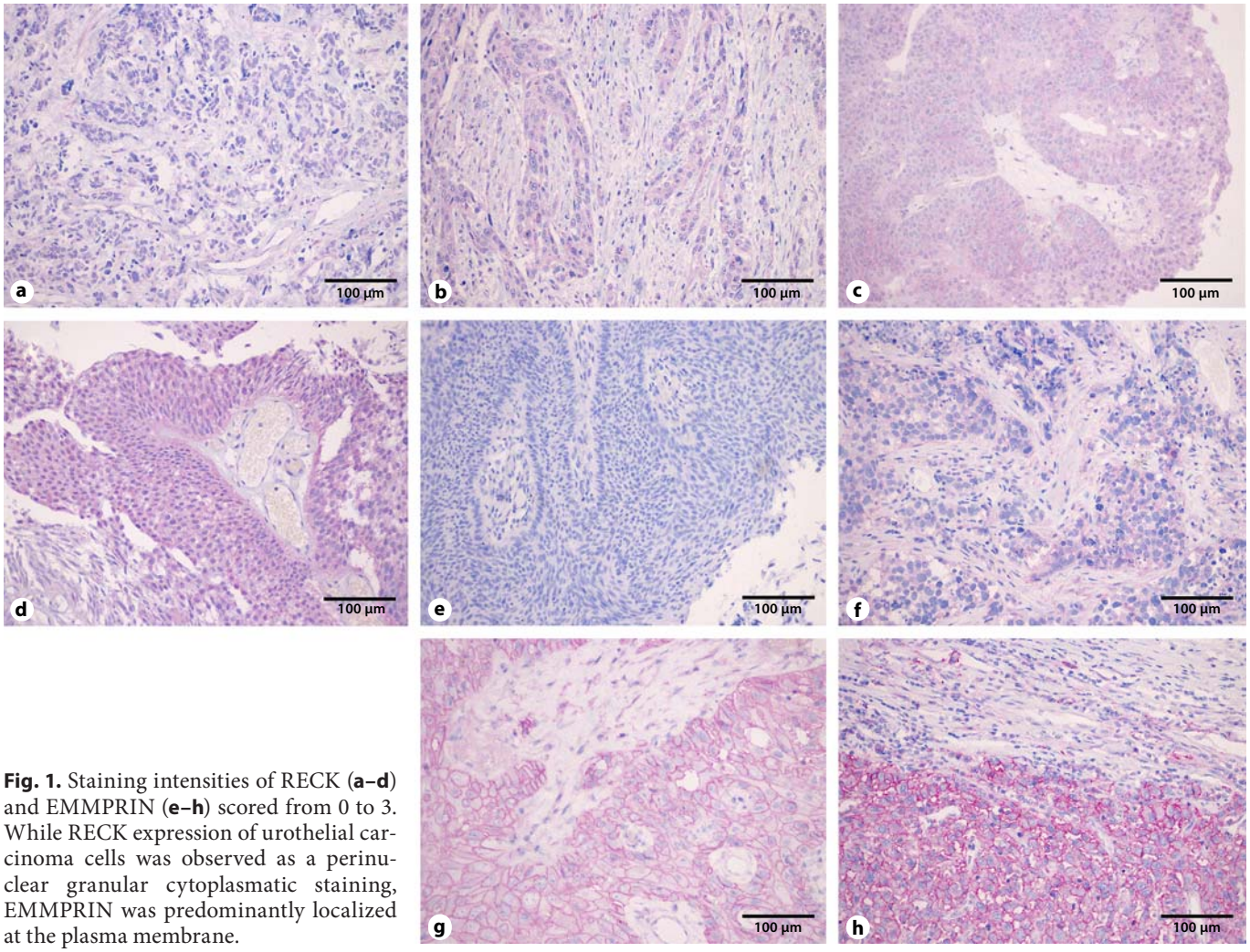

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Fig. 1. Staining intensities of RECK (**a–d**) and EMMPRIN (**e–h**) scored from 0 to 3. While RECK expression of urothelial carcinoma cells was observed as a perinuclear granular cytoplasmatic staining, EMMPRIN was predominantly localized at the plasma membrane.

sion were subdivided into four different classes ranging from 0 to 3 as described above. Clinicopathological parameters of the patients and tumors are shown in table 1.

RECK was detected in 104 of 127 tumors (82%). RECK expression was observed predominantly as a perinuclear granular cytoplasmatic staining with maximal staining intensity localized at the invasion frontline of the tumor (fig. 1a–d) as well as in adjacent normal urothelium. Rarely, immunostaining of nuclei and/or nucleoli occurred. Some stromal cells revealed a weak expression.

EMMPRIN was detected in 96 of 127 tumors (76%). EMMPRIN expression was found to be localized at the plasma membrane and attenuated within the cytoplasm (fig. 1e–h). Rarely, immunostaining of nuclei and/or nucleoli occurred. Stromal cells as well as some leukocytes partly also revealed weak expression. Both antibodies, anti-RECK and anti-EMMPRIN, were highly specific as demonstrated by Western blot (fig. 2).

Immunodetection of MMP-2, MMP-9 and MMP-14 showed a granular cytoplasmatic staining pattern (data not shown). Peripheral stromal cells were observed to have weak expression as well.

Immunohistochemical Expression of RECK Correlates with Tumor Stage and Grade

The data obtained from the assessment of the staining intensity of RECK expression were analyzed with regard to pT tumor stage. RECK expression was significantly related to the decreasing invasiveness of the tumor. Noninvasive urothelial carcinomas (pTa) were associated with stronger RECK expression, whereas invasive tumors (≥pT1) showed a stepwise decrease in RECK levels

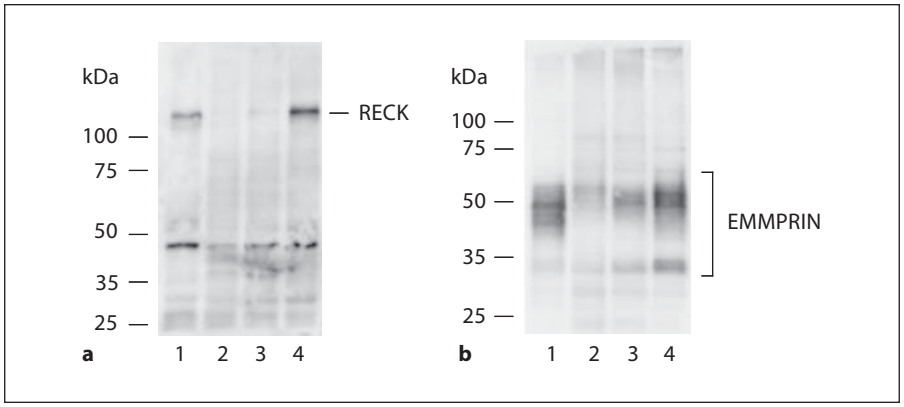

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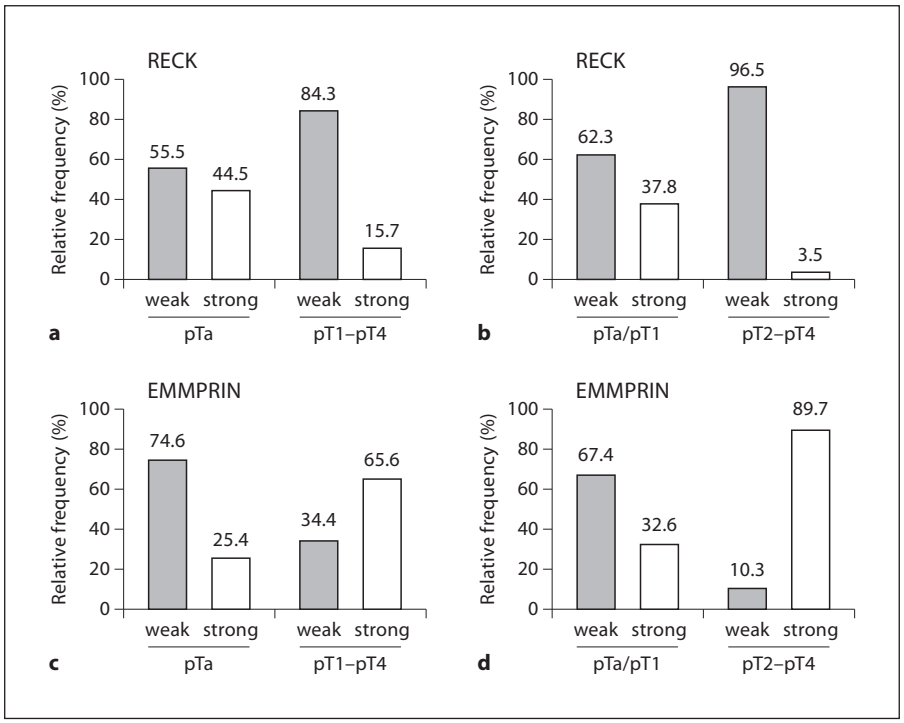
Fig. 2. Specificity of RECK and EMMPRIN antibodies in Western blots. Proteins of the four human bladder cell lines HCV-29 (lane 1), RT-4 (lane 2), RT-112 (lane 3) and J82 (lane 4) were separated by SDS-PAGE. **a** The Western blot shows a 125-kDa band corresponding to glycosylated RECK in HCV-29, RT-112 (weak) and in J82 cells. The 45-kDa band is supposed to be a degraded form that also occurs with another RECK antibody (data not shown). **b** EMMPRIN staining in HCV-29, RT-4 (weak), and in J82 cells represents highly (about 45–65 kDa) and less glycosylated (30 kDa) forms as described previously. Both antibodies seem to be highly specific in Western blots. Protein standard is given in kDa.

Fig. 3. a–d Percentage of RECK and EMMPRIN expression at the invasive front of 127 urothelial carcinomas of the bladder shown for different pT stages. Staining scores of 0 and 1 are pooled in weak staining intensity and staining scores of 2 and 3 are pooled in strong staining intensity of the respective antigen. The percentage of weak RECK expression (**a**) and the percentage of strong EMMPRIN expression (**c**) significantly increase in pT1–pT4 stages compared to pTa carcinomas. Analysis of pTa/pT1 vs. pT2–pT4 reveals a significant increase in weak RECK expression (**b**) and strong EMMPRIN expression (**d**) as well.



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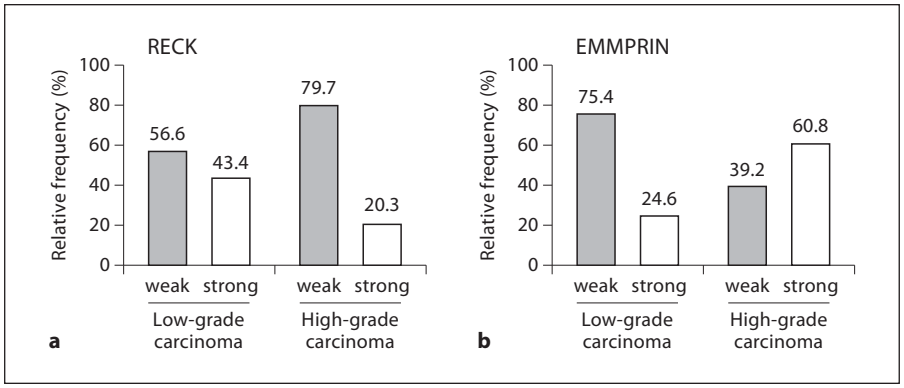
(fig. 3a; Fisher's exact test $p < 0.001$). Additionally, analysis of the difference of RECK expression between clinically so-called 'superficial' (≤pT1) and 'muscle-invasive' (≥pT2) urothelial carcinomas was found to be statistically significant (fig. 3b; Fisher's exact test $p < 0.001$). Furthermore, comparison of RECK expression with the WHO tumor grade also revealed an association of statistical significance (fig. 4a; Fisher's exact test $p = 0.006$). While high-grade urothelial carcinomas were rather associated with decreased RECK expression, low-grade tumors increasingly expressed RECK. We created a more detailed classification modeled after Sylvester [5], which took into account subgroups of lower pT stages including WHO grade and CIS to better estimate the risk of progression to muscle-invasive disease for these cases. This 'extended classification' was grouped in (a) pTa low grade, (b) pTa high grade, (c) pT1 low grade, (d) pT1 high grade, (e) pTa/1 with CIS, (f) pT2, (g) pT3, (h) pT4. RECK expression in the 127 carcinomas was negatively correlated to this classification (Spearman's rank correlation coefficient r_s –0.292, $p = 0.001$).

Immunohistochemical Expression of EMMPRIN Correlates with Tumor Stage and Grade

As well as in the analyses of RECK expression, the intensity scores of the EMMPRIN expression were corre-



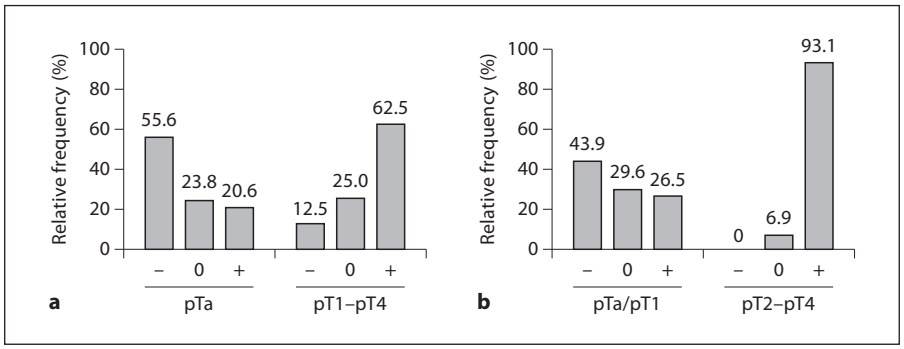


Fig. 4. Percentage of RECK and EMMPRIN expression at the invasive front of 127 urothelial carcinomas of the bladder shown for each WHO grade. Staining scores of 0 and 1 are pooled in weak staining intensity and staining scores of 2 and 3 are pooled in strong staining intensity of the antigen. The percentage of weak RECK expression (**a**) and the percentage of strong EMMPRIN expression (**b**) significantly increase in high-grade carcinomas compared to low-grade carcinomas.

Fig. 5. EMMPRIN/RECK balance in the invasive front of urothelial bladder carcinoma, dependent on pT stage. The percentage of scores is shown for negative EMMPRIN/RECK balance (–), for equation (0), and for positive EMMPRIN/RECK balance (+). **a** In comparison to pTa stages, positive EMMPRIN/RECK balance significantly increases in pT1–pT4 stage carcinomas. **b** An increase is shown as well dividing into stages of pTa/pT1 vs. pT2-pT4.

lated with pT tumor stage. In contrast to the RECK data, EMMRPIN expression was found to be significantly associated with increasing invasiveness of the tumor. Noninvasive urothelial carcinomas (pTa) were strongly related to weak EMMPRIN expression compared to invasive tumors (≥pT1) (fig. 3c; Fisher's exact test $p < 0.001$). Furthermore, the EMMPRIN expression also differed between clinically so-called 'superficial' (≤pT1) and 'muscle-invasive' (≥pT2) urothelial carcinomas with high statistical significance (fig. 3d; Fisher's exact test $p < 0.001$). As well as RECK, EMMPRIN expression was also significantly related to the WHO tumor grade but exactly in a converse modality (fig. 4b; Fisher's exact test $p < 0.001$). High-grade tumors were associated with increased EMMPRIN expression, whereas low-grade tumors were not. Additionally, EMMPRIN expression in all 127 carcinomas was positively correlated to our 'extended classification' as defined above (Spearman's rank correlation coefficient r_s 0.516, $p < 0.001$).

Immunohistochemical Expression of MMP-2, MMP-9 and MMP-14 Does Not Correlate with Tumor Stage and Grade

The data obtained from the assessment of the staining intensity of MMP-2, MMP-9 and MMP-14 were also analyzed with regard to pT tumor stage, WHO grade and our 'extended classification'. However, in contrast to RECK and EMMPRIN, the MMPs did not reveal any statistically significant association to any of the parameters (data not shown).

Calculation of 'Proteolytic Balance'

MMPs are proteolytic enzymes involved in the remodeling of almost all protein components. We aimed to determine the proteolytic balance using EMMPRIN as a surrogate marker for MMP activity and RECK as its counterpart. Considering EMMPRIN as promoter of proteolysis and RECK as a negative factor for proteolysis, we calculated a sum score of the staining scores for EMMPRIN (positive score) and RECK (negative score) for each case. In the following, the respective difference was called 'EMMPRIN/RECK balance'. The EMMPRIN/RECK balance obtained for pTa and pT1–pT4 tumors (fig. 5a; χ^2 test according to Pearson $p < 0.001$) as well as for summarized pTa/pT1 and pT2–pT4 tumors (fig. 5b; χ^2 test according to Pearson $p < 0.001$) revealed a statistically significant increase in proteolytic balance for higher pT stages. A shift to positive EMMPRIN/RECK balance was also observed from WHO low-grade to WHO high-grade carcinomas (χ^2 test according to Pearson $p < 0.001$, data not shown).

EMMPRIN/RECK balance in all 127 carcinomas was positively correlated to our 'extended classification' (Spearman's rank correlation coefficient r_s 0.546, $p < 0.001$).

Discussion

Urothelial carcinomas of the bladder show a rather divergent biological behavior leading to challenging problems regarding the clinical management of these tumors. Although much progress has been made in the understanding of the molecular alterations in bladder cancer in recent years, we certainly do not know all relevant factors that drive urothelial tumors into a life-threatening disease. Novel molecular markers are warranted to aid clinical risk stratification and therapy regimens.

Our study of 127 urothelial carcinomas reveals decreased RECK and increased EMMPRIN expression to be associated with increasing tumor aggressiveness. No significant correlation could be established regarding the MMPs investigated. The expression profile was assessed by means of immunohistochemistry, an easily accessible method that guarantees feasible validation of our results in a prospective setting. As competing peptides were not available for the RECK and EMMPRIN antibodies used, antibody specificities were validated by Western blotting. Very low background in the range of about 25–200 kDa implied high specificities for immunohistochemistry. To our best knowledge and in good accordance with the data on other tumor entities, this is the first study that demonstrates an inverse correlation of RECK expression and tumor aggressiveness in bladder cancer.

Although RECK is described to be membrane-anchored, immunostaining was predominantly found as perinuclear granular cytoplasmatic staining. RECK staining in the cytoplasm was already found in tissue of non-small cell lung cancer [23] and of esophageal squamous cell carcinoma [24]. In normal and neoplastic odontogenic and prostatic tissues, cytoplasmatic staining was found together with membrane staining [25, 26]. RECK was also detected in secretory granules of a subclass of macrophages [27], and it gave relatively abundant signals around the perinuclear region in mouse embryo fibroblast-derived NIH3T3 cells [28]. We assume that RECK granular/perinuclear staining indicates a vesicle-localized type of RECK which could be a precursor of the mature membrane-bound RECK or whose cellular function is hitherto unknown.

With respect to clinical risk stratification, RECK and EMMPRIN expression were correlated with pT tumor stage in order to study their relevance for diagnostic borderline cases with variable and hardly predictable clinical disease progression. In our study, EMMPRIN and RECK were found to be differentially expressed in non-invasive (pTa) compared to invasive tumors (≥pT1) as well as between clinically called 'superficial' (≤pT1) and 'muscle-invasive' (≥pT2) tumors. Urologists rely on this distinction to determine the appropriate therapy regimen (TUR-B, cystectomy, chemotherapy). Our data demonstrate a 2.75-fold increase in EMMPRIN expression and a concomitant 10.8-fold decrease in RECK expression for muscle-invasive compared to non-muscle-invasive urothelial bladder cancer. Moreover, compared to low-grade tumors, high-grade carcinomas showed an increase by 150% in strong EMMPRIN expression and concomitant reduction of the RECK expression level by more than 50%. Hence, a combined pathological approach that determines both tumor stage and grade as well as the level of RECK and EMMPRIN expression may help to guide and optimize clinical management in difficult settings (i.e. in pT1 G3 tumors).

Our 'extended classification' of tumor stages including WHO grades and the presence of CIS, modeled after Sylvester [5], should estimate the potential of RECK and EMMPRIN to serve as progression markers. This means 'progression to muscle-invasive disease' for lower tumor stages and 'disease progression defined by tumor stage only' for stages pT2 and higher. Decreased RECK expression and increased EMMPRIN expression correlated with an increasing likelihood of progression. Our data are in line with the observations of several other groups in other tumor types. In this context, recent studies imply a predictive role of RECK in the clinical outcome of several other cancers, including colorectal, lung, breast, liver, pancreas and also prostate. These studies revealed a correlation of high RECK expression with a prolonged recurrence-free survival time [12, 13, 23, 29–32]. With respect to prostate cancer, our group recently proved decreased RECK expression to be associated with higher tumor aggressiveness and as independent prognostic factor for increased risk of PSA recurrence [26]. Our data regarding EMMPRIN are consistent with the results of Als et al. [33] who investigated a cohort of 124 patients with advanced bladder cancer having received a cisplatin-based therapy regimen. Patients with EMMPRIN-negative bladder cancer were found to have a 5-year survival rate of 22.5%, whereas the cohort with EMMPRIN-positive cancer had a significantly poorer survival at 5 years (14.6%). A smaller cohort of 58 patients also showed significant correlation of high EMMPRIN expression with poor outcome [17].

A limitation of this study is the lack of follow-up data. Although a few cases were known to be relapses, comprehensive data on prior recurrence rate, time to recurrence and progression after surgery were not available. This is mainly due to the fact that selected cases for this study were taken from daily routine diagnostics between 2006 and 2009. The essential criterion for case selection was to reflect routine diagnostic cases including those that require a clear-cut diagnosis regarding the level of invasion (pTa/pT1 and pT1/pT2) but are considered difficult to evaluate due to small specimen sizes, incomplete sampling and cauterization artifacts. Since 77% of the cases of our cohort were pTa/pT1 tumors with a rather good prognosis, the follow-up period was too short to survey endpoint data regarding survival of these patients. Therefore, the prior recurrence rate as well as the parameters tumor diameter and tumor number were hardly available, hence prognosis with regard to recurrence [5] was impossible to assess.

Besides the improvement of diagnostic reasoning, the investigation of the expression profile of factors that either promote or repress tissue invasion may also aid in the understanding of molecular factors that promote tumor aggressiveness in general. Over the last couple of years, EGFR and HER2/neu have evolved as promising therapeutic targets in breast cancer, colon cancer, glioblastomas and recently lung and gastric cancer [34, 35]. Both tyrosine kinase receptors share several intracellular signaling cascades including the MAPK pathway. Interestingly, several studies found high levels of HER2/neu expression to correlate with poor outcome in bladder cancer [8, 36]. Mellon et al. [37] reported on the association of overexpressed EGFR with disease progression and poor survival in high-grade pT1 urothelial bladder cancer. Notably, there is in vitro evidence that upon ligand binding, EGFR is critical for the induction of EMMPRIN and concomitant MMP-2 und MMP-9 expression [38]. Conversely, RECK expression was shown to be repressed by Ras and ERK via Sp transcription factors [39, 40]. Disappointingly, a clinical phase II trial failed to show any benefit from the combination of the tyrosine kinase inhibitor gefitinib added to gemcitabine and cisplatin chemotherapy in patients with advanced urothelial tract carcinomas and had to be halted due to high toxicity [41]. However, in combination with the other studies on EMMPRIN and RECK in urothelial bladder cancer, our data provide additional evidence and a new rationale for therapeutic targeting of HER2/neu and/or EGFR. Inhibition of MAPK signaling in urothelial bladder cancer by targeted therapy approaches using, e.g., cetuximab or trastuzumab should lead to direct repression of invasion by downregulation of EMMPRIN and upregulation of RECK. Since our study provides evidence that in contrast to pTa tumors, pT1 tumors, which penetrate the basement membrane but have not yet acquired the competence for muscle invasion, express higher levels of EMMPRIN and low levels of RECK, one may hypothesize that patients with early stages of disease will benefit most from EGFR and/or HER2/neu targeting.

To conclude, our study reveals RECK and EMMPRIN as prognostic indicators for tumor progression to muscle invasion and metastasis. We suggest them to be important factors in the tumor biology of urothelial carcinoma. Assessment of the expression level of both proteins may help to discriminate potentially muscle-invasive from non-muscle-invasive tumors, thereby improving pathological diagnosis, risk stratification and subsequent clinical management. Our data also point towards therapeutic interference with EGFR and HER2/neu-mediated MAPK-activity, which operates EMMPRIN and RECK for tissue invasion. Prospective studies are warranted to confirm these data.

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