EpCAM Overexpression is Associated with High-grade Urothelial Carcinoma in the Renal Pelvis

ANDREA BRUNNER[1], GEORG SCHAEFER[2], LOTHAR VEITS[1], BARBARA BRUNNER[3], MARTINA PRELOG[3] and CHRISTIAN ENSINGER[1]

[1]Institute of Pathology, Medical University of Innsbruck, Muellerstrasse 44, 6020 Innsbruck, Tirol; [2]Department of Urology and [3]Department of Pediatrics, Medical University of Innsbruck, Anichstrasse 35, 6020 Innsbruck, Tirol, Austria

Abstract. *Background: EpCAM serves as an attractive target for immunotherapy due to its expression on the surface of most epithelial cancer cells. Urothelial carcinoma of the renal pelvis (RP-UC) comprises 2.4-4.6% of tumors of the lower urinary tract. To assess the expression of EpCAM in RP-UC a retrospective study was performed. Patients and Methods: Tumor tissue from 42 patients with RP-UC was selected from the archives of the Institute of Pathology, Medical University of Innsbruck, Austria. EpCAM expression was demonstrated by immunohistochemistry using the mouse monoclonal antibody ESA. Results: EpCAM overexpression was significantly associated with high grade and invasive behaviour ($p=0.014$ and $p=0.029$) and the presence of lymph node metastases ($p=0.031$), but not with the extent of nodal involvement ($p=0.12$). Conclusion: In RP-UC, EpCAM overexpression is associated with an aggressive tumor phenotype. The association of EpCAM overexpression with the presence of lymph node metastasis may be of prognostic and therapeutic relevance.*

EpCAM is a type 1 transmembrane glycoprotein acting as a homophilic cell adhesion molecule and is expressed in most human epithelial cells (1). In addition, EpCAM has been detected in a variety of different epithelial carcinomas, including cancer of the tongue, thyroid, breast, lung, gallbladder, colon, stomach, ovary, kidney, prostate and bladder (2-8). EpCAM expression has been linked with clinicopathological parameters, such as grade, stage and presence of lymph node metastasis in some tumors (2, 4, 5, 9, 10), while others failed to show an association with stage and histological tumor differentiation (6, 8, 11). Overexpression of EpCAM was found to predict a worse overall survival in some cancer types (4-6, 11), while a better overall survival was observed in others (12, 13). In fact, the function of EpCAM is still under investigation. *In vitro* studies have shown that EpCAM is related to cell proliferation and differentiation, directly stimulating the cell cycle and proliferation by up-regulation of *c-myc* and cyclin E/A (14).

Recently, we have shown that EpCAM expression in urothelial carcinoma (UC) of the bladder is associated with high grade, advanced stage and poor outcome (9). UC, in fact, also arises in other parts of the lower urinary tract including the renal pelvis (RP). Among tumors of the lower urinary tract, RP-UCs comprise 2.4% of tumors in males and 4.6% in females (15). The most important prognostic factor in RP-UC is tumor stage, lymph node involvement, age and the presence of concurrent urothelial neoplasia (15).

Beside radical surgery and chemotherapy, there are not many treatment options for RP-UC. EpCAM, which is currently used as a target molecule in antibody-mediated therapies, would be an attractive target especially in advanced disease (1).

Therefore, to assess the expression of EpCAM in RP-UCs, we performed an immunohistochemical study including 42 patients diagnosed between 1992 and 2004.

Patients and Methods

Patients. Specimens were selected from 42 patients (32 males; 10 females), with a diagnosis of RP-UC between 1992 and 2004 at the Institute of Pathology, Medical University of Innsbruck, Austria. The mean age of patients was 70 years, ranging from 37 to 90 years. Tumors were located in the renal pelvis of 39 patients and in both, the renal pelvis and ureter, in 3 patients. All patients initially underwent nephrectomy or nephro-ureterectomy. Lymph node dissection was only performed in 14 patients with 6 patients having lymph node metastases at the time of the diagnosis. Follow-up information was available in 35 patients. Four patients developed distant metastasis. Eleven patients had a history of recurrent UC of the bladder prior to the diagnosis of RP-UC and 6 patients developed UC in the bladder

Correspondence to: Dr. Andrea Brunner, Institute of Pathology, Medical University of Innsbruck, Muellerstrasse 44, 6020 Innsbruck, Tirol, Austria. Tel: +43 512 9003 71306, Fax: +43 512 582088, e-mail: andrea.brunner@i-med.ac.at

Key Words: EpCAM, urothelial cancer, renal pelvis.

Table I. *Clinicopathological characteristics of the 42 patients included in the study.*

Number of patients (%)	Stage				
	pTa	pT1	pT2	pT3	pT4
Grade					
Low	13 (31%)	0	0	0	0
High	4 (9%)	4 (9%)	1 (2%)	13 (31%)	7 (17%)
Location					
Renal pelvis	16 (38%)	4 (9%)	0	12 (29%)	7 (17%)
Renal pelvis and ureter	1 (2%)	0	1 (2%)	1 (2%)	0
Lymph node metastasis					
NX	13 (31%)	3 (7%)	1 (2%)	8 (19%)	4 (9%)
N0	4 (9%)	1 (2%)	0	2 (5%)	0
N1	0	0	0	2 (5%)	1 (2%)
N2	0	0	0	1 (2%)	2 (5%)
Distant metastasis*					
Yes	0	1 (3%)	0	1 (3%)	2 (6%)
No	14 (40%)	2 (6%)	1 (3%)	9 (26%)	5 (14%)
Concomitant UC*					
pr. d. yes	6 (17%)	0	0	3 (8%)	2 (6%)
pr. d. no	8 (23%)	3 (8%)	1(3%)	7 (20%)	5 (14%)
po. d. yes	3 (8%)	1 (3%)		1 (3%)	1 (3%)
po. d. no	11 (31%)	2 (6%)	1(3%)	9 (26%)	6 (17%)

*Data only available for 35 patients; pr. d., prior to the diagnosis of RP-UC, po. d., post diagnosis of RP-UC.

after the diagnosis of RP-UC (4 primary diagnoses, 2 recurrent tumors). In 2 patients, cystectomy was performed due to recurrent or concomitant UC in the bladder. Histological sections were re-evaluated by two pathologists (A.B., C.E.) and graded according to the WHO 2004 classification (16). Finally, the study group consisted of 17 pTa, 4 pT1, 1 pT2, 13 pT3 and 7 pT4 tumors. The clinicopathological characteristics of all patients are summarized in Table I.

Immunohistochemistry. EpCAM overexpression was demonstrated by immunohistochemistry using the mouse monoclonal antibody ESA (NovoCastra, Medac GmbH, Hamburg, Germany), as described elsewhere (3, 9). The evaluation of slides was independently performed by two pathologists (A.B., C.E.). EpCAM expression was calculated as a total score: product of intensity and proportion score, ranging from 0 to 12. The intensity score represents the estimated staining intensity, 0: no staining, 1: weak, 2: moderate, 3: strong reaction intensity; the proportion score describes the estimated fraction of positively stained tumor cells, 0: no visible reaction, 1: 0%-10, 2: 11%-50%, 3: 51%-80%, 4: more than 80% of tumor cells stained. EpCAM overexpression was defined as a total score of >4, as described elsewhere (3).

Statistical analysis. Data were analysed using SPSS for Windows (Version 15.0 for Windows, SPPS Inc., Chicago, Illionois, USA) statistical software. For continuous variables, comparison of means with ANOVA was used, while Fisher's exact test was performed to test the association of EpCAM overexpression with clinicopathological parameters including tumor grade, stage and presence of lymph node metastases. $P<0.05$ was considered significant.

Results

EpCAM expression was found in 32 tumor specimens, while 10 (23%) specimens showed no expression. The mean percentage of EpCAM-expressing cells was 41.5%, ranging from totally negative to 100% positivity; the mean total score was 5.3 (range 0-12). EpCAM overexpression, as defined as a score of >4, was found in 26 tumors (62%). There was a significant association of EpCAM overexpression with high grade and invasive behaviour ($p=0.014$ and $p=0.029$; Table II). No significant association was detected with tumor stage, though there was a tendency towards higher EpCAM expression in high stage tumors ($p=0.087$; Table II). In 14 histologically examined lymph nodes, EpCAM overexpression was associated with the presence of lymph node metastasis ($p=0.031$), but not with extend of the lymph node involvement ($p=0.12$). Neither tumor stage, presence of distant metastases, age nor gender showed any relationship with EpCAM expression. However, though data did not reach statistical significance, EpCAM overexpression was seen in all but 1 patient with distant metastases. The results are summarized in Table II.

Discussion

In our study, the association of EpCAM expression with high grade and invasive behaviour in UC was further supported. Recently, we showed that in UC of the bladder, EpCAM

Table II. *Association of EpCAM overexpression and clinicopathological parameters.*

EpCAM overexpression	Number of cases		*p*-value
	No	Yes	
Age	16 (mean age 68)	26 (mean age 70)	0.88[1]
Gender			
Male	12	20	1.00
Female	4	6	
Grade			
Low	9	4	0.014
High	7	22	
Stage			
pTa	10	7	0.087
pT1	1	3	
pT2	1	0	
pT3	2	11	
pT4	2	5	
Invasion			
Non-invasive (pTa)	10	7	0.029
Invasive (pT1-4)	6	19	
Lymph node metastasis[2]			
Yes		6	0.031
No	5	3	
Distant metastasis[3]			
Yes	1	3	0.63
No	13	18	

[1]ANOVA; [2]including 14 patients with histological evaluated lymph nodes; [3]follow-up information available for 35 patients.

expression is associated with high grade, advanced stage and poor overall survival (9). In RP-UC, EpCAM expression is similarly associated with high-grade tumors. In fact, several studies on UCs, also using different antibodies, observed an increased EpCAM expression in high-grade tumors (9, 17, 18). We did not observe a significant relationship with tumor stage, which may be due to the number and heterogeneity of cases in our study. But when comparing invasive and non-invasive tumors, EpCAM expression was significantly higher in invasive tumors ($p=0.029$). This finding suggests a relationship with invasive behaviour in RP-UC and supports the reported association of EpCAM overexpression with enhanced proliferation and malignant potential (19). In addition, we showed a significant association between the presence of lymph node metastases and EpCAM overexpression ($p=0.031$). Strong EpCAM expression was significantly related to the presence of lymph node metastasis. However, the extent of lymph node involvement was not related to EpCAM expression. Several authors have reported a direct relation of EpCAM overexpression and the presence of lymph node involvement (4, 10).

Since it has been shown that the presence of EpCAM-positive cells in lymph nodes is associated with a poor survival, Piyathylake et al. suggested that EpCAM is at least partially involved in the development of metastastic disease (10). However, other authors were unable to find an association of EpCAM expression and nodal involvement, and metastastic cells do not always show EpCAM overexpression, indicating different mechanisms involved in the formation of metastasis (11, 19). Nevertheless, our data support an association of EpCAM expression with an aggressive behaviour of RP-UCs. EpCAM interferes with cadherin-dependent adhesion resulting in loss of cohesiveness (19). Increased EpCAM expression may be related with a decrease of cadherin-dependent adhesions facilitating the escape of tumor cells from the epithelium (19). Therefore, it seems likely that EpCAM overexpression is associated with an increased risk of lymph node involvement.

In conclusion, we showed that EpCAM overexpression is associated with high grade, invasive behaviour and the presence of lymph node metastasis in RP-UCs. This finding may be of prognostic value. In addition, we provide the possibility of applying EpCAM-based immunotherapy as an additional treatment for patients with locally advanced tumors as well as with metastatic disease.

References

1 Baeuerle PA and Gires O: EpCAM (CD326) finding its role in cancer. Br J Cancer *96(9)*: 417-423, 2007.

2 Yanamoto S, Kawasaki G, Yoshitomi I, Iwamoto T, Hirata K and Mizuno A: Clinicopathologic significance of EpCAM expression in squamous cell carcinoma of the tongue and its possibility as a potential target for tongue cancer gene therapy. Oral Oncol *43(9)*: 869-877, 2007.

3 Ensinger C, Kremser R, Prommegger R, Spizzo G and Schmid KW: EpCAM overexpression in thyroid carcinomas: a histopathological study of 121 cases. J Immunother *29(5)*: 569-573, 2006.

4 Spizzo G, Went P, Dirnhofer S, Obrist P, Simon R, Spichtin H, Maurer R, Metzger U, von Castelberg B, Bart R, Stopatschinskaya S, Kochli OR, Haas P, Mross F, Zuber M, Dietrich H, Bischoff S, Mirlacher M, Sauter G and Gastl G: High Ep-CAM expression is associated with poor prognosis in node-positive breast cancer. Breast Cancer Res Treat *86(3)*: 207-213, 2002.

5 Spizzo G, Went P, Dirnhofer S, Obrist P, Moch H, Baeuerle PA, Mueller-Holzner E, Marth C, Gastl G and Zeimet AG: Overexpression of epithelial cell adhesion molecule (Ep-CAM) is an independent prognostic marker for reduced survival of patients with epithelial ovarian cancer. Gynecol Oncol *103(2)*: 483-488, 2006.

6 Varga M, Obrist P, Schneeberger S, Muhlmann G, Felgel-Farnholz C, Fong D, Zitt M, Brunhuber T, Schafer G, Gastl G and Spizzo G: Overexpression of epithelial cell adhesion molecule antigen in gallbladder carcinoma is an independent marker for poor survival. Clin Cancer Res *10(9)*: 3131-3136, 2004.

7 Went P, Dirnhofer S, Salvisberg T, Amin MB, Lim SD, Diener PA and Moch H: Expression of epithelial cell adhesion molecule (EpCam) in renal epithelial tumors. Am J Surg Pathol *29(1)*: 83-88, 2005.

8 Went P, Vasei M, Bubendorf L, Terracciano L, Tornillo L, Riede U, Kononen J, Simon R, Sauter G and Baeuerle PA: Frequent high-level expression of the immunotherapeutic target Ep-CAM in colon, stomach, prostate and lung cancers. Br J Cancer *94(1)*: 128-135, 2006.

9 Brunner A, Prelog M, Verdorfer I, Tzankov A, Mikuz G and Ensinger C: EpCAM is predominantly expressed in high grade and advanced stage urothelial carcinomas of the bladder. J Clin Pathol Doi: 10.1136/jcp.2007.049460, Epub ahead of print.

10 Piyathilake CJ, Frost AR, Weiss H, Manne U, Heimburger DC and Grizzle WE: The expression of Ep-CAM (17-1A) in squamous cell cancers of the lung. Hum Pathol *31(4)*: 482-487, 2000.

11 Stoecklein NH, Siegmund A, Scheunemann P, Luebke AM, Erbersdobler A, Verde PE, Eisenberger CF, Peiper M, Rehders A, Esch JS, Knoefel WT and Hosch SB: Ep-CAM expression in squamous cell carcinoma of the esophagus: a potential therapeutic target and prognostic marker. BMC Cancer *6*: 165, 2006.

12 Seligson DB, Pantuck AJ, Liu X, Huang Y, Horvath S, Bui MH, Han KR, Correa AJ, Eeva M, Tze S, Belldegrun AS and Figlin RA: Epithelial cell adhesion molecule (KSA) expression: pathobiology and its role as an independent predictor of survival in renal cell carcinoma. Clin Cancer Res *10(8)*: 2659-2669, 2004.

13 Songun I, Litvinov SV, van de Velde CJ, Pals ST, Hermans J and van Krieken JH: Loss of Ep-CAM (CO17-1A) expression predicts survival in patients with gastric cancer. Br J Cancer *92(9)*: 1767-7172, 2005.

14 Munz M, Kieu C, Mack B, Schmitt B, Zeidler R and Gires O: The carcinoma-associated antigen EpCAM up-regulates c-myc and induces cell proliferation. Oncogene *23(34)*: 5748-5758, 2005.

15 Delahunt B, Amin MB, Hofstädter F, Hartmann A and Tyczynski JE: Tumours of the renal pelvis and ureter. *In*: Tumours of the Urinary System and Male Genital Organs. Eble JN, Sauter G, Epstein JI and Sesterhenn AI (eds.). Lyon, IARC Press, pp. 150-153, 2004.

16 Montironi R and Lopez-Beltran A: The 2004 WHO classification of bladder tumors: a summary and commentary. Int J Surg Pathol *13(2)*: 143-153, 2005.

17 Zorzos J, Zizi A, Bakiras A, Pectasidis D, Skarlos DV, Zorzos H, Elemenoglou J and Likourinas M: Expression of a cell surface antigen recognized by the monoclonal antibody AUA1 in bladder carcinoma: an immunohistochemical study. Eur Urol *28(3)*: 251-254, 1995.

18 Anagnostaki E, Skarlos D, Tamvakis N, Psaropoulou P, Blana E, Bamias A, Legaki S, Aravantinos G, Deliveliotis C and Dimopoulos K: Immunohistochemical and immunocytochemical study of bladder carcinomas using the epithelium-specific, tumour-associated monoclonal antibodies HMFG1 and AUA1. Br J Cancer Suppl *10*: 52-56, 1990.

19 Winter MJ, Nagtegaal ID, van Krieken JH and Litvinov SV: The epithelial cell adhesion molecule (Ep-CAM) as a morphoregulatory molecule is a tool in surgical pathology. Am J Pathol *163(6)*: 2139-2148, 2003.

Received September 14, 2007
Revised November 15, 2007
Accepted December 11, 2007