Association of Oncofetal Protein Expression with Clinical Outcomes in Patients with Urothelial Carcinoma of the Bladder

Evanguelos Xylinas, Eugene K. Cha, Francesca Khani, Luis A. Kluth, Malte Rieken, Björn G. Volkmer, Richard Hautmann, Rainer Küfer, Yao-Tseng Chen, Marc Zerbib, Mark A. Rubin, Douglas S. Scherr, Shahrokh F. Shariat* and Brian D. Robinson

From the Department of Urology (EX, EKC, LAK, MR, DSS, SFS, BDR), Division of Medical Oncology (SFS) and Department of Pathology and Laboratory Medicine (FK, YTC, MAR, BDR), Weill Cornell Medical College and Institute for Precision Medicine of Weill Cornell Medical College and New York Presbyterian Hospital (MAR), New York, New York, Institut National de la Santé et de la Recherche Médicale U955 Equipe 07, Université Paris-Est Créteil (EX), Créteil and Department of Urology, Cochin Hospital, Assistance Publique-Hôpitaux de Paris, Paris Descartes University (EX, MZ), Paris, France, and Departments of Urology, University Medical Center Hamburg-Eppendorf (LAK), Hamburg, Hospital Kassel (BGV), Kassel, University Hospital Ulm (RH), Ulm, Hospital am Eichert (RK), Göppingen, Germany, University Hospital Basel (MR), Basel, Switzerland, and Medical University of Vienna (SFS), Vienna, Austria

Abbreviations and Acronyms

IMP = insulin-like growth factor II mRNA-binding protein

MAGE-A3 = melanoma antigen group A protein 3

RC = radical cystectomy

TMA = tissue microarray

TPBG = trophoblast glycoprotein

UCB = bladder urothelial carcinoma

Accepted for publication August 19, 2013.

Study received institutional review board approval.

* Correspondence: Department of Urology, Medical University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria (telephone: +43 1 40400 2615; FAX: +43 1 40400 2332; e-mail: sfshariat@gmail.com).

Purpose: Oncofetal proteins are expressed in the developing embryo. Oncofetal protein expression correlates with the clinical outcome of nonmuscle invasive bladder urothelial carcinoma. IMP3, MAGE-A, glypican-3 and TPBG are oncofetal proteins that have not been well characterized in urothelial carcinoma of the bladder.

Materials and Methods: We investigated the expression of these 4 proteins and their association with clinical outcomes using tissue microarrays from 384 consecutive patients treated with radical cystectomy between 1988 and 2003 at 1 academic center. We stained for IMP3, MAGE-A, glypican-3 and TPBG. Univariable and multivariable Cox regression analyses were done to evaluate the association of oncofetal protein expression with disease recurrence and cancer specific mortality.

Results: IMP3, MAGE-A, glypican-3 and TPBG were expressed in 39.5%, 45%, 6% and 85% of urothelial bladder carcinomas, respectively. Expression was tumor specific and did not correlate with pathological features except for TPBG. At a median followup of 128 months 176 patients (46%) experienced disease recurrence, 175 (45.5%) had died of the disease and 96 (27.5%) had died of another cause. On univariable analysis IMP3 and MAGE-A expression was associated with an increased risk of disease recurrence ($p < 0.001$ and 0.03) and cancer specific mortality ($p = 0.004$ and 0.03, respectively). On multivariable Cox regression analysis adjusted for the effects of standard clinicopathological features IMP3 and MAGE-A expression was independently associated with disease recurrence ($p = 0.004$, HR 1.55, 95% CI 1.15–2.11 and $p = 0.02$, HR 1.44, 95% CI 1.05–1.99, respectively) but not with cancer specific mortality.

Conclusions: Oncofetal proteins are commonly and differentially expressed in urothelial carcinoma of the bladder compared to normal urothelium. IMP3 and MAGE-A expression was associated with disease recurrence and cancer specific mortality but glypican-3 and TPBG expression was not.

Key Words: urinary bladder, urothelium, carcinoma, oncofetal antigens, risk

0022-5347/14/1913-0830/0
THE JOURNAL OF UROLOGY®

http://dx.doi.org/10.1016/j.juro.2013.08.048
Vol. 191, 830-841, March 2014
Printed in U.S.A.

In 2013 bladder cancer was the second most common genitourinary cancer with an estimated 72,570 new cases and 15,210 bladder cancer related deaths in the United States.[1] Despite advances in surgical technique and improved systemic therapies the outcome of muscle invasive UCB remains poor.[2–4] Better prognostication of clinical outcomes and prediction of the response to therapy could help with patient counseling and treatment selection as well as timely administration of optimal local and systemic therapy in patients with UCB. While several tissue based molecular markers were suggested to improve UCB staging and outcome prediction,[5] none is used in daily practice to date.[6,7]

Oncofetal proteins are normally expressed during fetal development and absent in normal adult tissues but they may be aberrantly expressed in malignant neoplasms. Oncofetal proteins are clinically useful as markers or have functional significance for pathogenesis in solid malignancies.[8–11] Currently, limited studies have explored the expression of oncofetal proteins in UCB. We and others previously reported that survivin is associated with disease recurrence and mortality in UCB.[12] Sitnikova et al recently characterized IMP3 expression in non-muscle invasive UCB and noted that expression was associated with an increased risk of disease recurrence.[13] MAGE-A3 is expressed in high grade/high stage UCB.[14] Glypican-3 and TPBG are other oncofetal proteins whose prognostic significance was investigated for various malignancies but not for UCB.[8–11]

We evaluated the association of the expression of these oncofetal proteins with oncologic outcomes in a large, well characterized, homogeneous cohort of patients treated with RC and lymphadenectomy for UCB with long-term followup.

MATERIALS AND METHODS

Patient Population and Specimen Collection

This was an institutional review board approved study. Participating sites provided the necessary institutional data sharing agreements before initiation. The study analyzed specimens of 384 consecutive patients treated with RC and lymphadenectomy for UCB between 1988 and 2003 at a single center. Another 50 specimens of normal adjacent urothelium served as controls. Patients with muscle invasive UCB or prostatic stromal invasion, and/or recurrent Ta or T1 or carcinoma in situ refractory to transurethral resection with or without intravesical chemotherapy or immunotherapy were referred for RC. No patient received chemotherapy or radiotherapy preoperatively and none had known metastatic disease at surgery. A total of 35 patients (9.1%) received adjuvant chemotherapy at clinician discretion based on tumor stage, overall health status and patient preference.

Pathological Evaluation

Genitourinary pathologists initially assigned tumor grade according to the 1973 WHO grading system. Tumor stage was reassigned according to the 2002 TNM staging system.

Immunohistochemistry and Scoring

TMAs were constructed in triplicate from formalin fixed, paraffin embedded radical cystectomy specimens, consisting of primary tumors from 384 patients with UCB,[15] corresponding lymph node metastases from 117 of these patients and adjacent benign urothelium from 50 controls. The supplementary methods (http://jurology.com/) detail immunohistochemical staining for IMP3, glypican-3, TPBG and MAGE-A.

Two pathologists (FK and BDR) blinded to clinicopathological parameters and patient outcomes evaluated all stained tissue sections. For each antibody cells with any detectable staining were considered positive. The percent of positive cells was recorded for each sample along with average staining intensity, which was considered weak—1+, moderate—2+ or marked—3+. The combined score was calculated by multiplying the intensity and the percent. Samples with any percent of positivity regardless of intensity were considered to show aberrant expression of that particular oncofetal protein.

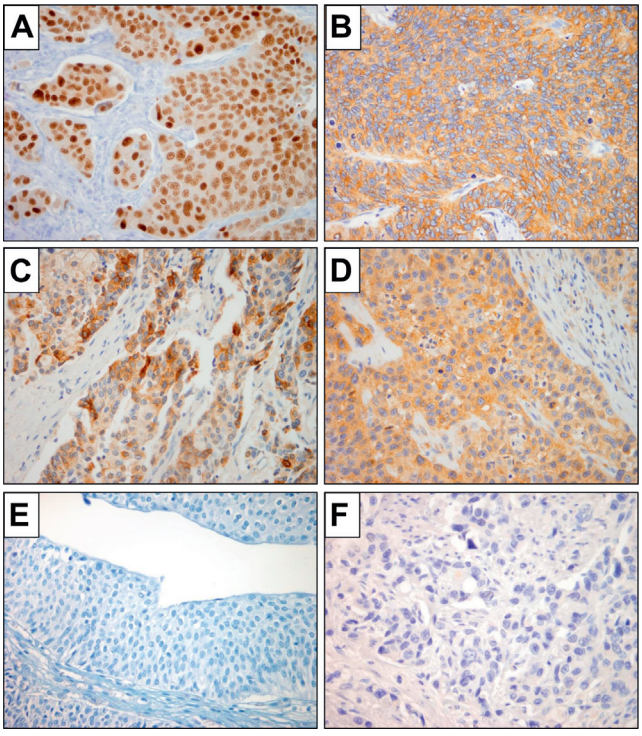

Figure 1. Immunohistochemical staining of oncofetal proteins in urothelial carcinoma. Positive nuclear staining for MAGE-A (*A*), and positive cytoplasmic staining for IMP3 (*B*), glypican-3 (*C*) and TPBG (*D*). Reduced from ×400. Negative staining in benign urothelium adjacent to tumor (*E*). MAGE-A stain, reduced from ×400. Negative staining in invasive carcinoma (*F*). Glypican-3 stain, reduced from ×400.