Tokai J Exp Clin Med., Vol. 30, No. 3, pp. 177-182, 2005

Expression of Carbohydrate Antigens (SSEA-1, Sialyl-Lewis X, DU-PAN-2 and CA19-9) and E-selectin in Urothelial Carcinoma of the Renal Pelvis, Ureter, and Urinary Bladder

Hiroshi KAJIWARA*, Masanori YASUDA*, Nobue KUMAKI*, **, Taro SHIBAYAMA*** and Yoshiyuki OSAMURA*

*Department of Pathology, Tokai University School of Medicine
** Division of Pathology, Isehara Kyodo Hospital
*** Department of Urology, Isehara Kyodo Hospital

(Received July 6, 2005; Accepted July 19, 2005)

Carbohydrate antigens and E-selectin play important roles in the invasion and metastasis of cancers. We examined the expression of these antigens and their ligand protein, E-selectin, in urothelial carcinomas to evaluate whether their staining is correlated with the grade and stage of cancer.

We studied the expression of carbohydrate antigens (type 1 and type 2 blood-group antigens) and E-selectin in urothelial carcinomas of the renal pelvis, ureter, and urinary bladder in 52 patients by staining SSEA-1 (Le^x), sialyl LeX (sLe^x), DU-PAN-2, CA19-9, and E-selectin with 5 different monoclonal antibodies (MAbs) to evaluate whether their staining correlated with cancer grade and stage. The differences between organs with regard to the degree of expression of these antigens were not evident. Type 2 antigens (SSEA-1 and sialyl Le^x) are frequently expressed in the tumor cells regardless of atypical grade. The expression level of type 1 antigens (DU-PAN-2 and CA19-9) is lower than that of type 2 antigens. However, the presence of DU-PAN-2 tends to correlate with the grade of atypia; however, that of CA19-9 is inversely proportional to the grade of atypia. The lack of CA19-9 and appearance of DU-PAN-2 in urothelial carcinoma implies a high malignant potential. The expression of E-selectin can be correlated with stage and grade of tumor atypia.

Type 2 antigen and E-selectin may be involved in tumor invasion and metastasis.

Key words: carbohydrate antigen, E-selectin, immunohistochemistry, urothelial cancers

INTRODUCTION

It has been reported that the expression of carbohydrate antigens and E-selectin correlates with atypical grade and clinical stage of cancer in some carcinomas. The carbohydrate antigens (Lewis blood group antigens) are classified as type 1 antigens and type 2 antigens (Fig. 1). Loss or expression of type 2 antigens (SSEA-1 and sLe^x) is correlated with increased invasiveness and higher tendency for the recurrence of transitional cell carcinoma of the bladder (TCCB) [1-3].

The type 1 antigens (CA19-9 and DU-PAN-2) are known as tumor markers of pancreatic cancers. In recent times, a relationship between CA19-9 and urothelial cancers has been indicated.

E and P-selectin mediate the adhesion of various cancer cells to the endothelium [4-6]. Most, but not all, ligands carry sialylated, sulfated, and/or fucosylated sequences that are normally found on carbohydrate antigens [7]. The sialyl Lewis A (sLe^a or CA19-9), sialyl Lewis C (sLe^c or DU-PAN-2), and sialyl Lewis X (sLe^X) carbohydrate antigens are the main ligands for selectins [5-7].

Fujii *et al*. have shown that there is an adhesion between E-selectin and sialyl Le^x

Hiroshi KAJIWARA, Department of Pathology, Tokai University School of Medicine, Bouseidai, Isehara, Kanagawa 259-1193, Japan E-mail: h-kaji@is.icc.u-tokai.ac.jp

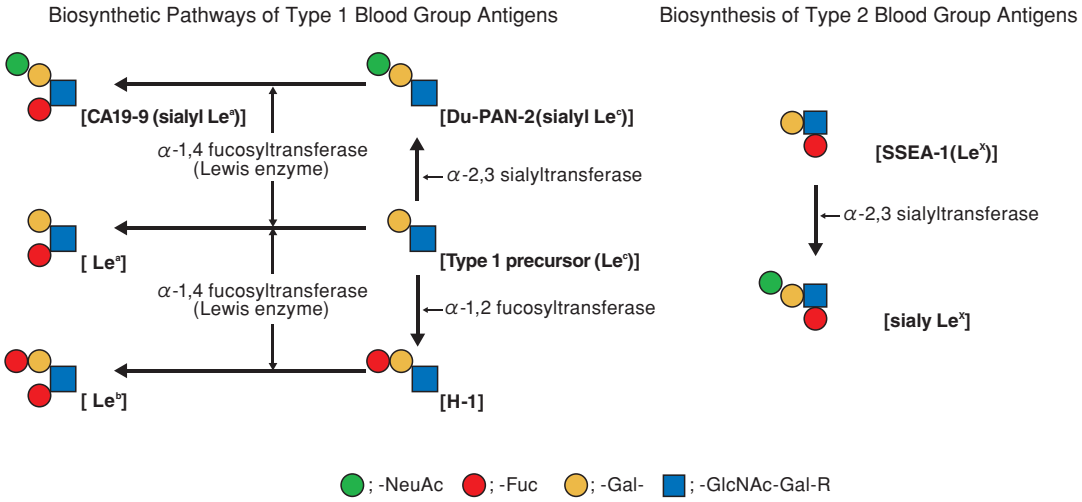

Fig. 1 Biosynthetic pathways of type 1 and type 2 blood group antigens

and/or sialyl Le^a in urothelial cancer. In some cancers, there is a correlation between the expression of E-selectin and clinical prognostic factors [8, 9]. The correlation between E-selectin and tumor grade in urothelial cancers had not been reported.

MATERIALS AND METHODS

Patients

A total of 52 tumors (renal pelvis cancer, 14; ureter cancer, 12; and urinary bladder cancer, 26) were included in the study. The mean age of the patients was 68 years (range, 52-90 years). The group consisted of 53 males and 15 females. Based on the TNM classification of invasive tumor stages, these cases included 15 of pT3 (extramuscular invasion); 6, pT2 (muscular invasion); 17, pT1 (lamina proprial invasion); and 14, pTa or pTis. With regard to tumor grade, there were 6 cases of G1; 25, G2; and 21, G3. Three patients had lymph node metastasis at the time of diagnosis (Table 1).

Tissues and Immunohistochemistry

All the patients who had undergone surgical resection of renal pelvis, ureter, or urinary bladder carcinomas at the Isehara Kyodo Hospital between 1990 and 2002 were identified, and a total of 52 patients were included in the study. Paraffin-embedded tissue blocks of the originally resected tumors were obtained, and 3 μm sections of these tissues were used for the study. For recurrent superficial tumors, only the primary or the first resected tumor was used. Using the high polymer method (Histofine SAB-PO Kit, Nichirei Co., Tokyo, Japan), sections of the paraffin-embedded tissues were immunohistochemically stained with monoclonal antibodies (MAbs) of anti-SSEA-1 (Kyowa Medex, Mishima, Japan), anti- sialyl Le^X (Kyowa Medex, Mishima, Japan), anti-CA19-9 (Fujirebio Diagnostic, Malvern, PA), anti-DU-PAN-2 (Kyowa Medex, Mishima, Japan), and anti-E-selectin (Novocastra, MA, USA). The paraffin-embedded tissue sections were deparaffinized with xylene and graded alcohol concentrations. They were then incubated with 3% hydrogen peroxide and methanol in a ratio of 1:3 to block endogenous peroxidase activity. Sections were also treated with 20% normal human serum diluted with phosphate buffered saline (PBS) buffer to block binding of nonspecific antibodies and background proteins. The sections were then incubated with primary antibodies. Following this, these sections were treated with a high polymer immunohistochemical detection kit (Histofine SAB-PO Kit, Nichirei Co., Tokyo, Japan). Slides were prepared using a 0.5 mg/mL solution of diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO, USA and 1.5 μL of 30% hydrogen peroxide for 2-8 min. All the incubations were performed at room temperature. Sections were counterstained with Gill's hematoxylin

Table 1 Clinicopathological profile of the cases

No	age /sex		Histologic type	Grade	pathological stage (LN meta)
1	73/F	BO	TCC	G1	pTa
2	56/M	BO	TCC	G1	pT1a
3	60/M	BO	TCC	G1	pTa
4	75/M	BO	TCC	G1	pTa
5	73/M	BO	TCC	G1	pTa
6	62/M	BO	TCC	G1	pTa
7	74/M	BO	TCC papillary	G2	pT1a, ly1, v+ (1/5)
8	65/M	BO	TCC	G2	pTa
9	73/M	BO	TCC	G2	pTa
10	56/M	BO	TCC	G2	pT1
11	81/M	BO	TCC	G2	pT1a
12	69/M	BO	TCC	G2	pTa
13	80/M	BO	TCC	G2	pTa
14	64/M	BO	TCC>>AC	G2	pTa
15	71/M	P	TCC	G2	pT3
16	79/F	P	TCC	G2	pT1
17	58/F	P	TCC	G2	pT3 (0/5)
18	61/M	P	TCC	G2	pT1 (0/5)
19	68/F	P	TCC	G2	pT3
20	69/M	P	TCC	G2	pTis
21	67/M	P	TCC	G2	pT1
22	61/M	P	TCC	G2	pT1, pL0, pV0
23	61/M	P	TCC	G2	pT1, pL0, pV0
24	73/F	P	TCC	G2	pT1, pL0, pV0
25	88/F	U	TCC>SCC	G2	pT3
26	82/F	U	TCC	G2	pT1 (0/1)
27	64/M	U	TCC	G2	pT2, pL0, pV0
28	64/F	U	TCC	G2	pTa, pL0, pV0
29	67/F	U	TCC	G2	pT2
30	57/M	U	TCC>AC	G2	pT1
31	52/F	U	TCC	G2	pT2
32	65/M	BO	TCC	G3	pT3a pL2, pV0
33	54/F	BO	SCC>>TCC	G3	pT3b
34	53/M	BO	TCC	G3	pT2, pL1, pV1 (0/29)
35	64/M	BO	TCC=SCC>AC	G3	pT2, pL0, pV0
36	64/M	BO	TCC	G3	pT3b (1/11)
37	45/M	BO	TCC	G3	pT3a, pL1, pV1 (0/3)
38	90/M	BO	TCC trabecular	G3	pT3b, pL2, pV1
39	69/F	BO	TCC	G3	pT3b, pL0, pV1 (0/1)
40	76/M	BO	TCC	G3	pT3a
41	73/M	BO	TCC	G3	pTa
42	72/M	BO	TCC	G3	pT1b
43	83/M	BO	TCC	G3	invasion +
44	78/F	P	TCC	G3	pT1
45	52/M	P	TCC>AC	G3	pT3 (0/12)
46	79/M	P	TCC	G3	pTa, pL0, pV0
47	69/M	P	TCC	G3	pT1
48	67/F	U	TCC	G3	pT3
49	59/M	U	TCC	G3	pT2 (0/9)
50	74/M	U	TCC	G3	pT1, pL1, pV0
51	76/F	U	TCC	G3	pT3
52	66/M	U	TCC trabecular	G3	pT3, pL1, pV1 (2/2)

B; Bladder tumor、 P; Pelvic tumor、 U; Ureter tumor

and mounted using Permount.

Each stained tumor section was analyzed for both presence and extent of staining. The extent of staining was classified into one of four phases, namely, −(negative), +(<50%), ++ (50-75%), or +++(75-100%) depending on the percentage of cells that exhibited staining. Statistical analysis was performed using the Wilcoxon-Mann-Whitney test; $P<0.05$ was regarded as significant.

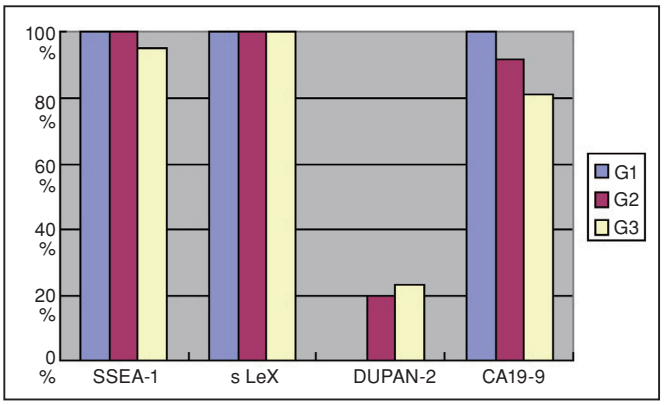

Fig. 2 Percentage expression of carbohydrate antigens according to tumor grade.

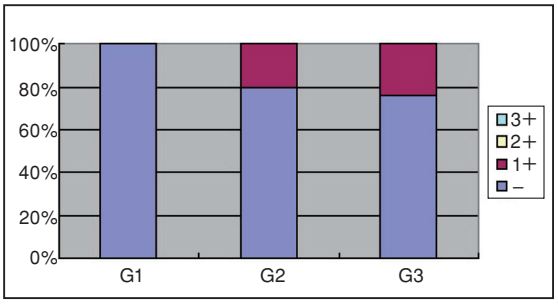

Fig. 3 Expression of DU-PAN-2 according to tumor grade

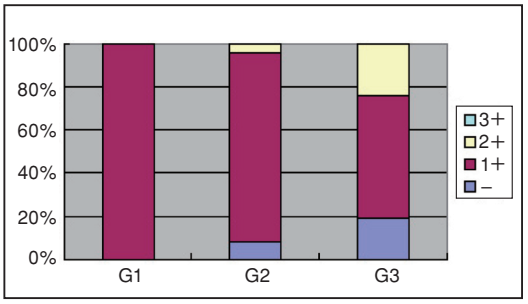

Fig. 4 Expression of CA19-9 according to tumor grade

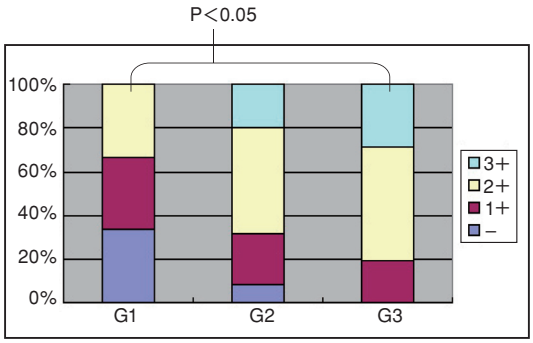

Fig. 5 Expression of E-selectin according to tumor grade

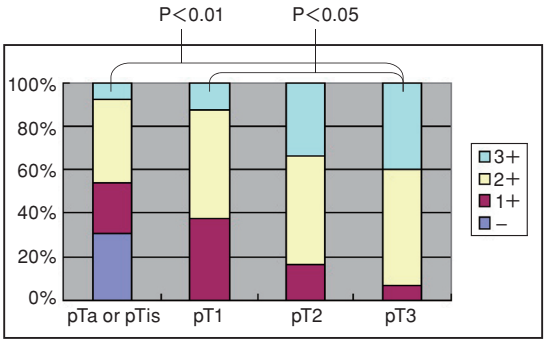

Fig. 6 Expression of E-selectin according to clinical stage

RESULTS

Immunohistochemical staining of SSEA-1, Sialyl Le^X, DU-PAN-2, CA19-9, and E-selectin was observed in 51/52 (98%), 52/52 (100%), 46/52 (88%), 10/52 (19%), and 48/52 (92%) tumors, respectively.

The expression rate of SSEA-1 according to tumor grade was 100% in G1 and G2, and 95% in G3; that of sialyl Le^X was 100% in all the grades; that of DU-PAN-2 was 0% in G1, 20% in G2, and 23% in G3; that of CA19-9 was 100% in G1, 92% in G2 and 81% in G3; and that of E-selectin was 67% in G1, 92% in

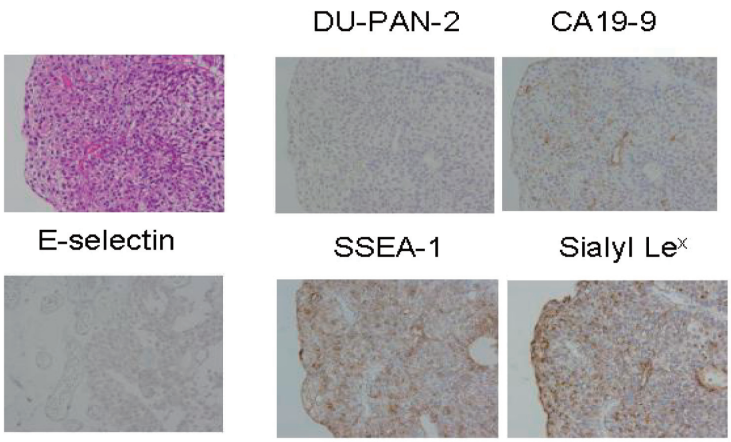

Fig. 7 Non-invasive cancer

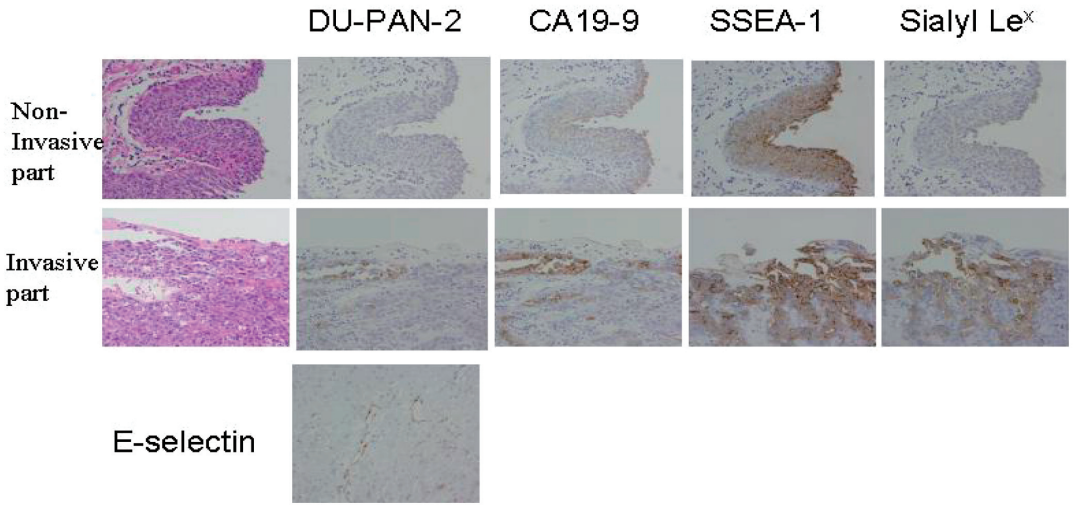

Fig. 8 Invasive cancer

G2, and 100% in G3 (Figs. 2-5). The number of positive cells that expressed E-selectin also correlated with the pathological stage (Fig. 6). In the E-selectin, significant difference was noted between G1 and G3 cancers, and pT3 and pT1 or non-invasive cancers.

Immunohistochemical findings of the carbohydrate antigens and E-selectin are shown in Fig. 7 and Fig. 8.

DISCUSSION

In our immunohistochemical study, we confirmed the expression of carbohydrate antigens in various tumors and its correlation with clinical stage of the cancer and tumor grade [10-12].

Our results indicate that type 2 antigens (SSEA-1 and sialyl Le^{x}) are frequently expressed in urothelial cancers, irrespective of the tumor grade and pathological stage. Shirahama *et al.* [13] and Konety [14] have shown a significant correlation between tumor grade, stage, and expression of SSEA-1. A progressive increase in the expression of SSEA-1 along with an increase in tumor grade is suggestive of an upregulation in antigen expression in higher grade tumors. Our findings and the observations of Sheinfeld *et al.* did not substantiate their results [15]. Sheinfeld *et al.* found that there was a downregulation of the expression of the SSEA-1 antigen with increasing tumor grade. Immunostaining of the type 2 antigen is not useful to detect poor prognostic cancers

because of the markers are frequently positive for high-grade tumors.

Only a few cases showed the expression of DU-PAN-2 after staining, and the expression of DU-PAN-2 slightly correlated with tumor grade. The numbers of cases in which CA19-9 disappears increase in the high-grade tumors, but high expressed cases also increase. Chuang *et al.* have shown that CA19-9 is a promising candidate for being used as a biomarker that detects and monitors low-grade bladder cancer [16]. Sashide *et al.* have revealed that CA19-9 is a serum marker in urothelial carcinoma that indicates poor prognosis [17]. In our study, CA19-9 expression was frequently observed in the low-grade tumors. However, non-neoplastic urothelial cells were also stained for CA19-9, and the number of positive cells increased in high-grade tumors. The expression of DU-PAN-2 has not been reported in urothelial carcinomas. When higher-grade tumors were monitored for the expression of DU-PAN-2, the expression of CA19-9 and a decrease of DU-PAN-2 (precursor of CA19-9) were observed. The involvement of α-1,4-fucosyl transferase has been implicated in this decrease.

Our findings indicate a correlation between expression of E-selectin and clinical prognostic factors in urothelial cancers as well as other carcinomas.

As a summary, a positive correlation was found between the expression of E-selectin and clinical stage and tumor grade.

These results are considered to be useful to predict the clinical outcome of the urothelial carcinomas in surgical pathology.

ACKNOWLEDGMENT

This work was supported by the Department of Urology, Isehara Kyodo Hospital.

REFERENCE

1) Bergman S, Javadpour N. The cell surface antigen A, B or O(H) as an indicator of malignant potential in stage A bladder carcinoma: preliminary report. J Urol 119: 49-51, 1978.
2) Lange PH, Limas C, Fraley EE. Tissue blood-group antigens and prognosis in low stage transitional cell carcinoma of the bladder. J Urol 119: 52-5, 1978.
3) Coon JS, McCall A, Miller AW 3rd, *et al.* Expression of blood-group-related antigens in carcinoma in situ of the urinary bladder. Cancer 56: 797-804, 1985.
4) Luscinskas FW, Cybulsky MI, Kiely JM, *et al.* Cytokine-activated human endothelial monolayers support enhanced neutrophil transmigration via a mechanism involving both endothelial-leukocyte adhesion molecule-1 and intercellular adhesion molecule-1. J Immunol 146: 1617-25, 1991.
5) Takada A, Ohmori K, Yoneda T, Tsuyuoka K, *et al.* Contribution of carbohydrate antigens sialyl Lewis A and sialyl Lewis X to adhesion of human cancer cells to vascular endothelium. Cancer Res 53: 354-61, 1993.
6) Iwai K, Ishikura H, Kaji M, *et al.* Importance of E-selectin (ELAM-1) and sialyl Lewis(a) in the adhesion of pancreatic carcinoma cells to activated endothelium. Int J Cancer 54: 972-7, 1993.
7) Varki A. Selectin ligands. Proc Natl Acad Sci USA 91: 7390-7, 1994.
8) Eichbaum MH, de Rossi TM, Kaul S, *et al.* Serum levels of soluble E-selectin are associated with the clinical course of metastatic disease in patients with liver metastases from breast cancer. Oncol Res 14: 603-10, 2004.
9) Saito K, Fujii Y, Kawakami S, *et al.* Increased expression of sialyl-Lewis A correlates with poor survival in upper urinary tract urothelial cancer patients. Anticancer Res 23: 3441-6, 2003.
10) Yasuda M, Saito K, Kobayashi Y, *et al.* Serum carbohydrate antigen elevations in endometrial adenocarcinomas: characterization of DU-PAN-2 expression as a tumor marker. J Obstet Gynaecol Res 30: 59-64, 2004.
11) Kamoshida S, Satoh Y, Yasuda M, *et al.* Immunohistochemical heterogeneity of type 1 blood group antigen expressions in testicular germ cell tumors. Oncol Rep 9: 845-51, 2002.
12) Muramatsu T, Yasuda M, Osamura RY, *et al.* Clinicopathological Analysis of DU-PAN-2 as a tumor marker for endometrial adenocarcinoma in comparison with CA19-9. Acta Histochem Cytochem 35: 193-9, 2002.
13) Shirahama T, Ikoma M, Muramatsu T, *et al.* Expression of SSEA-1 carbohydrate antigen correlates with stage, grade and metastatic potential of transitional cell carcinoma of the bladder. J Urol 148: 1319-22, 1992.
14) Konety BR, Ballou B, Jaffe R, *et al.* Expression of SSEA-1 (Lewis(x)) on transitional cell carcinoma of the bladder. Urol Int 58: 69-74, 1997.
15) Sheinfeld J, Reuter VE, Melamed MR, *et al.* Enhanced bladder cancer detection with the Lewis X antigen as a marker of neoplastic transformation. J Urol 143: 285-8, 1990.
16) Chuang CK, Liao SK. Evaluation of CA19-9 as a tumor marker in urothelial malignancy. Scand J Urol Nephrol 38: 359-65, 2004.
17) Sashide K, Isobe H, Wakumoto Y, *et al.* CA19-9 as a serum marker for poor prognosis in urothelial carcinoma. Urol Int 72: 112-7, 2004.