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Original Article

High endothelin-1 expression predicts good prognosis in upper tract urothelial carcinoma

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Abstract: Purpose: The survival of upper tract urothelial carcinoma has not changed significantly during the last two decades and there were also no adequate prognostic factors for the disease. In this study, we aim to evaluate the predictive value of endothelin expression on the prognosis of patients with upper tract urothelial carcinoma. Methods: One hundred and fifty-six UTUC patients who underwent radical nephroureterectomy (RNU) with bladder cuff excision were enrolled in the study. ET axis expression was analyzed by immunohistochemistry. The association between retrospectivelycollectedclinical parameters and ET axis expression was examined by using the χ^2 test. The Kaplan-Meier method was applied to estimate the effect of ET axis expression on metastasis-free survival (MFS), cancer-specific survival (CSS), and overall survival (OS). Hazard ratios of ET axis expression on survival were evaluated by the Cox regression model.Results: Endothelin-1 expression was strongly associated with superficial pathologic tumor stage (P=0.002), absence of lymph node involvement (P<0.001), and low pathologic tumor grade (P=0.040). Endothelin-1 overexpression was also associated with significantly better metastasis-free survival (HR=0.40, P=0.047), CSS (HR=0.21, P=0.002), and overall survival (HR=0.35, P=0.021). Conclusions: These findings indicate that endothelin expression could be a biomarker that predicts good survival and warrants further examination as anti-cancer treatment target. Studies focusing on the underlying mechanisms are needed to provide novel therapy for upper tract urothelial carcinoma.

Keywords: Endothelin, upper tract urothelial carcinoma, immunohistochemistry, prognosis, survival

Introduction

Upper tract urothelial carcinoma (UTUC) is tumors derived from the urothelium along the urinary tract and account for only 5-10% of urinary tract carcinomas [1, 2]. Previous reports [3, 4] have shown that the male to female ratio in UTUC is about 3:1, and the incidence of pyelocaliceal tumors is about twice as common as ureteral tumors. However, higher incidence of UTUC is observed with a slight female predominance, and ureteral tumors account for more than half of all UTUC [5, 6] in Taiwan. Radical nephroureterectomy (RNU) with bladder cuff excision, by either open or laparoscopic methods, remains the gold standard treatment [1-4, 6]. Retrospective studies [4, 7] have shown that the percentage of invasive tumors is higher in UTUC than in bladder urothelial carcinoma (UC). Furthermore, the clinical course of high stage UTUC is more aggressive than bladder UC. Despite optimal surgical treatment, patient outcomes are still not satisfactory. Previous prognostic factors for UTUC after RNU, such as pathological tumor stage and grade, are not adequate to define surgical outcome [6]. A previously published study has revealed that the disease-specific survival of UTUC has not changed significantly during the last two decades [8]. If more accurate markers of the tumor's biological behavior were available, newer treatment options for improving disease outcome could be offered to patients.

Table 1. The association of clinicopathologic parameters with endothelin immunoreactivity

	All patients	ET-1 expression		*p* value
		Negative	Positive	
Number of patients, no. (%)	156 (100)	27 (17.3)	129 (82.7)	
Age, years				*0.601*
Mean ± SD		65.0 ±13.6	66.3 ± 10.8	
Gender, no. (%)				*0.788*
Females	83 (53.2)	15 (55.6)	68 (52.7)	
Males	73 (46.8)	12 (44.4)	61 (47.3)	
Smoking, no. (%)				*0.714*
No	117 (75.0)	21 (77.8)	96 (74.4)	
Yes	39 (25.0)	6 (22.2)	33 (25.6)	
GFR, no. (%)				*0.453*
≥60	50 (32.1)	7 (25.9)	43 (33.3)	
<60	106 (67.9)	20 (74.1)	86 (66.7)	
Bladder tumor history				*0.850*
No	131 (84.0)	23 (85.2)	108 (83.7)	
Yes	25 (16.0)	4 (14.8)	21 (16.3)	
Surgery method, no. (%)				*0.157*
Open	116 (74.4)	23 (85.2)	93 (72.1)	
Laparoscopic	40 (25.6)	4 (14.8)	36 (27.9)	
Tumor number, no. (%)				*0.875*
Solitary	106 (67.9)	18 (66.7)	88 (68.2)	
Multiple	50 (32.1)	9 (33.3)	41 (31.8)	
Pathological tumor stage, no. (%)				*0.002*
≤T1	65 (41.7)	4 (14.8)	61 (47.3)	
>T1	91 (58.3)	23 (85.2)	68 (52.7)	
N stage, no. (%)				*<0.001*
N0	146 (93.6)	21 (77.8)	125 (96.9)	
N1	10 (6.4)	6 (22.2)	4 (3.1)	
Pathological tumor grade, no. (%)				*0.040*
Low	35 (22.4)	2 (7.4)	33 (25.6)	
High	121 (77.6)	25 (92.6)	96 (74.4)	

Among the molecular targets, the endothelin (ET) system has drawn attention in oncologic research, as emerging evidence suggests that it plays an important role in cancer biology [9, 10]. ET is an endothelial cell-derived vasoconstrictor peptide that has three isoforms ET-1, 2, and 3. ET along with its two G-protein-coupled receptors, endothelin-A (ET_AR) and endothelin-B (ET_BR), and endothelin-converting enzymes is commonly referred to as the ET axis. The three final biologically active products of ET peptides, ET-1, ET-2 and ET-3, all have 21 amino acids and are encoded by distinct genes. Each of the three endothelin peptides is expressed in various tissues within the body and is involved in many normal physiological processes [10-12].

Previous reports have demonstrated that endothelin has an influence on tumor invasion and metastasis [13, 14]. It also influences angiogenesis by potentiating hypoxia signaling via regulation of hypoxic inducible factor-1α (HIF-1α) [15], and is involved in regulating tumor-infiltrating immune cells by influencing their differentiation and trafficking [15]. However, whether the increase in plasma endothelin is the cause or consequence of tumor progression is still a matter of debate. Nelson et al. [16] reported that plasma endothelin is significantly elevated in men with metastatic prostate cancer, and the elevation may be related to the osteoblastic response of the bone to the metastatic prostate cancer. Wülfing et al. have

reported that increased expression of endothelin and its receptors correlated with several clinicopathological parameters characterizing aggressive types of breast cancer that were associated with higher vascularity and shorter disease-free and overall survival [17]. In urothelial carcinoma, overexpression of the ET axis has been demonstrated by previous reports [18-20]. ET-1 overexpression was found to be associated with an increased hazard ratio of death in non-metastatic, muscle-invasive bladder cancer [20]. On the contrary, another report showed that ET-1 expression was associated with tumor stage, histologic grade, low proliferation status, and longer overall survival in non-invasive or superficial invasive bladder tumor [19]. The authors concluded that the lack of ET-1 expression was related to poor overall survival, and lack of ET_BR may be independently related to worse recurrence-free survival [19]. Therefore, the role of the ET axis in urothelial carcinoma is not well established and needs further investigation. Until date, there are no studies investigating the role of the ET axis in UTUC both *in vivo* and *in vitro*. The purpose of the present study was to determine the predictive value of the expression of the proteins of the ET axis in patients with UTUC. To the best of our knowledge, this has not been studied before.

Patients and methods

Patients and tumor tissues

Between 2000 and 2013, 156 patients who underwent either open or laparoscopic RNU with bladder cuff excision for non-metastatic UTUC at the Kaohsiung Medical University Hospital were included. This study was reviewed by the review board of our institution (KMUH-IRB-20130211). We retrospectively collected information on clinical parameters including demographic characteristics, pathological features, oncologic follow-up, and the cause leading to mortality. We excluded patients who underwent neoadjuvant chemotherapy or radiotherapy, had concurrent muscle-invasive bladder tumor, acute blood disorders, or bone marrow diseases, and those with incomplete clinical information. Tumor stage was defined according to the 2002 American Joint Committee Cancer TNM system. Tumor tissues were reviewed by two pathologists and re-classified as low or high-grade using the 2004 WHO grading system. All the clinicopathologic parameters are summarized in **Table 1**.

Postoperative follow-up

After the operation, patients were followed up in the outpatient clinics every 3 months for the first 2 years, and every 6 months for the next 2 years. From the fifth year onward, annual follow-ups were arranged for patients with no evidence of the disease. Detailed history taking, physical examination, urine cytology, cystoscopy, and serial imaging survey were performed following the surveillance guidelines. Metastatic progression was defined as tumor recurrence at the operation site, regional lymph nodes, or distant organ. Tumors occurring in the bladder or the contralateral upper urinary tract were considered metachronous and were not categorized as disease progression.

Immunohistochemistry

The paraffin-embedded tissue blocks were sectioned into 2-5 μm slices and mounted on poly-L-lysine-coated slides. After deparaffinization, 3% H_2O_2 for 10-20 minutes was used to block the endogenous peroxidase activity in the sections, and 10% BSA in PBS for over 30 minutes was used to block non-specific binding of immunoglobulin. Samples were then incubated with primary antibodies against ET-1 (monoclonal mouse antibody antiendothelin-1 MAb [clone-TR.ET.48.5]; Affinity Bioreagents, Golden, CO, USA), ET_AR, and ET_BR (sheep polyclonal antibodies, ET_A-receptor antiserum [no. 210-507-C250]; ET_B-receptor antiserum [no. 210-506-C250]; Affinity Bioreagents, Golden, CO, USA) for 1 hour. They were rinsed twice in PBS for 5 minutes, after which the sections were incubated with secondary antibodies for 7 minutes at room temperature. After that, samples were again rinsed twice in PBS for 5 minutes, incubated in DAB peroxidase substrate solution (Zymed) for 3 minutes, and briefly rinse in distilled water. The slides were counterstained with Gill's hematoxylin solution for 30 seconds on desired and rinsed in running tap water for 10 minutes. They were dehydrated using 95% ethanol for 10 minutes and 100% ethanol for 10 minutes, twice. They were then cleared twice in xylene for 10 minutes. Subsequently, a coverslip was applied using permanent mounting medium. Results were recorded using a

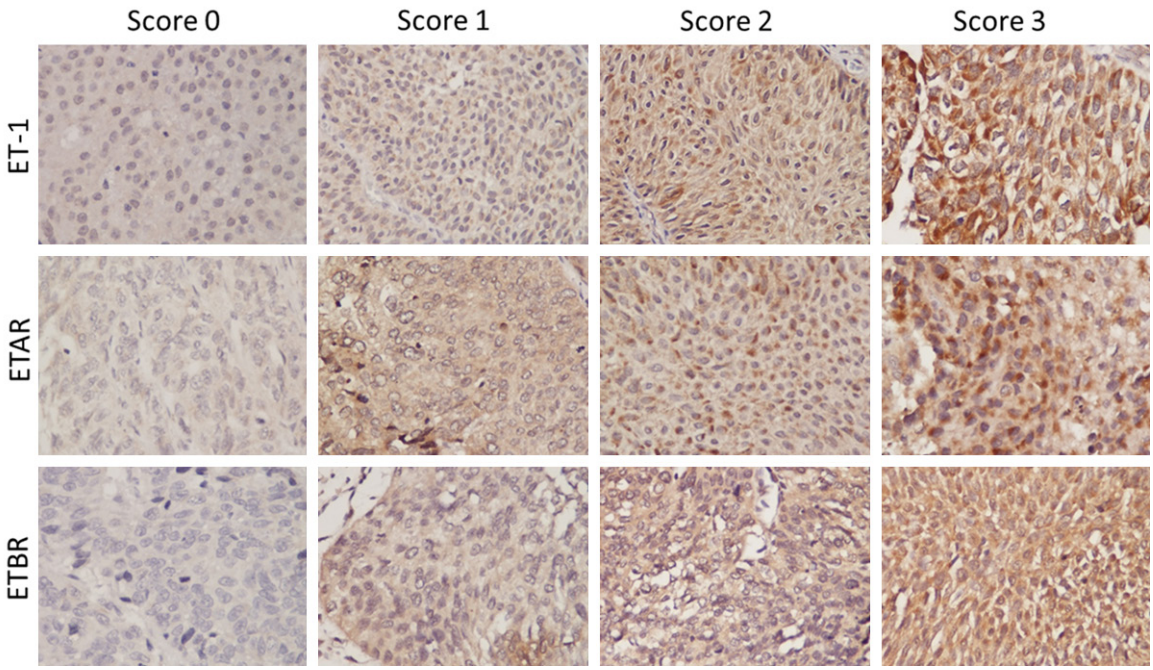

Figure 1. The staining intensity of ET-1, ET_AR, and ET_BR among different samples ranged from complete absence of staining (Score 0), moderate staining (Score 1, 2) and strong staining (Score 3).

magnifier digital camera. Controls included omitting or preabsorbing primary antibody and omitting secondary antibody.

Evaluation of the immunohistochemical staining

Tumor immunostaining was evaluated independently by two experienced pathologists. If there were discrepancies in scoring an image, the two pathologists reviewed the sample jointly to reach a consensus. Endothelin axis expression was determined using the percentage of positive stained cells. Specimens with less than 50% (Score 1) of the cells stained positive were classified as having low endothelin expression, while those with more than 50% (Score 2, 3) of the cells stained were classified as having high endothelin expression.

Statistical analyses

Differences between categorical parameters were assessed using a χ^2 or Fisher's exact test. The Kaplan-Meier method was applied to estimate the effect of ET axis expression on metastasis-free survival (MFS), disease-specific survival (DSS), and overall survival (OS). Survival rates were recorded from the day of RNU to metastatic progression, cancer-specific death, or the latest visit. Survival curves were compared using the log-rank test. All prognostic factors in the univariate analysis were included in the multivariate Cox proportional hazard model to identify independent predictors for MFS, CSS, and OS. Statistical significance was set at $P<0.05$. SPSS 20.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

Result

Immunohistochemical analysis of expression of endothelin axis

The staining intensity of ET-1, ET_AR, and ET_BR among different samples ranged from complete absence of staining (Score 0), moderate staining (Score 1, 2) and strong staining (Score 3) (**Figure 1**). ET-1 staining (Score 1, 2, 3) was observed in 129 (82.7%) tumors, expression of ET_AR in 145 (92.9%) tumors, and expression of ET_BR in 142 (91.0%) tumors.

ET axis' association with clinicopathologic parameters

The association of clinicopathologic parameters with endothelin immunoreactivity is shown in **Table 1**. ET-1 expression was strongly associated with superficial pathologic tumor stage

Table 2. The association between endothelin receptors expression and clinicopathologic parameters

Characteristic	ET_AR			ET_BR		
	Positive	Negative	*p*	Positive	Negative	*p*
Number of patients, no. (%)	145 (92.9)	11 (7.1)		142 (91.0)	14 (9.0)	
Gender			*0.246*			*0.757*
Female	79 (54.5)	4 (36.4)		75 (52.8)	8 (57.1)	
Male	66 (45.5)	7 (63.6)		67 (47.2)	6 (42.9)	
Smoking			*0.857*			*0.746*
No	109 (75.2)	8 (72.7)		106 (74.6)	11 (78.6)	
Yes	36 (24.8)	3 (27.3)		36 (25.4)	3 (21.4)	
GFR			*0.097*			*0.758*
≥60	44 (30.3)	6 (54.5)		45 (31.7)	5 (35.7)	
<60	101 (69.7)	5 (45.5)		97 (68.3)	9 (64.3)	
Bladder tumor			*0.515*			*0.852*
No	121(83.4)	10 (90.9)		119 (83.8)	12 (85.7)	
Yes	24(16.6)	1 (9.1)		23 (16.2)	2 (14.3)	
Surgical method			*0.192*			*0.792*
Open	106 (73.1)	10 (90.9)		106 (74.6)	10 (71.4)	
Laparoscopic	39 (26.9)	1 (9.1)		36 (25.4)	4 (28.6)	
Tumor number			*0.751*			*0.131*
Solitary	99 (68.3)	7 (63.6)		99 (69.7)	7 (50)	
Multiple	46 (31.7)	4 (36.4)		43 (30.3)	7 (50)	
Tumor stage			*0.369*			*0.107*
≤T1	59 (40.7)	6 (54.5)		62 (43.7)	3 (21.4)	
>T1	86 (59.3)	5 (45.5)		80 (56.3)	11 (78.6)	
N stage			*0.098*			*0.016*
N0	137 (94.5)	9 (81.8)		135 (95.1)	11 (78.6)	
N1	8 (5.5)	2 (18.2)		7 (4.9)	3 (21.4)	
Tumor grade			*0.69*			*0.151*
Low	32 (22.1)	3 (27.3)		34 (23.9)	1 (7.1)	
High	113 (77.9)	8 (72.7)		108 (76.1)	13 (92.9)	

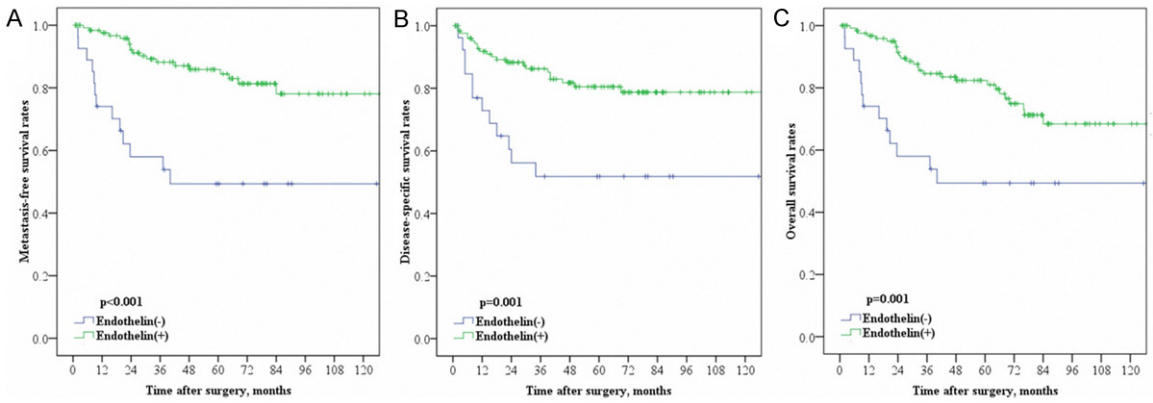

Figure 2. Kaplan-Meier curves for MFS(A), DSS(B), and OS(C) in patients with UTUC.

(*P*=0.002), absence of lymph node involvement (*P*<0.001), and low pathologic tumor grade (*P*=0.040). Endothelin-1 negative tumors were characterized by invasiveness, lymph node in-

Table 3. Univariate analysis predicting MFS, DSS, and OS in patients with UTUC

n=156	Metastasis free survival		Disease specific survival		Overall survival	
	Univariate analysis HR (95% CI)	*p*	Univariate analysis HR (95% CI)	*p*	Univariate analysis HR (95% CI)	*p*
Age (years)						
≤66	Reference	*0.663*	Reference	*0.302*	Reference	*0.175*
>66	1.01 (0.98-1.04)		1.02 (0.98-1.05)		1.02 (0.98-1.05)	
Gender						
Female	Reference	*0.878*	Reference	*0.706*	Reference	*0.503*
Male	1.05 (0.54-2.07)		0.88 (0.44-1.75)		0.81 (0.43-1.51)	
Smoking						
No	Reference	*0.664*	Reference	*0.851*	Reference	*0.85*
Yes	1.18 (0.56-2.46)		1.08 (0.50-2.33)		0.93 (0.46-1.91)	
GFR						
≥60	Reference	*0.037*	Reference	*0.025*	Reference	*0.02*
<60	2.56 (1.06-6.19)		2.98 (1.15-7.75)		2.65 (1.17-5.99)	
Bladder tumor						
No	Reference	*0.149*	Reference	*0.229*	Reference	*0.508*
Yes	1.79 (0.81-3.97)		1.68 (0.72-3.90)		1.32 (0.58-2.99)	
Surgical method						
Open	Reference	*0.535*	Reference	*0.359*	Reference	*0.357*
Laparoscopic	0.78 (0.35-1.72)		0.68 (0.29-1.56)		0.71 (0.34-1.48)	
Tumor number						
Solitary	Reference	*0.01*	Reference	*0.002*	Reference	*0.001*
Multiple	2.42 (1.23-4.76)		3.04 (1.52-6.10)		2.74 (1.47-5.10)	
Tumor stage						
≤T1	Reference	*<0.001*	Reference	*<0.001*	Reference	*<0.001*
>T1	6.76 (2.38-19.21)		13.38 (3.19-56.03)		5.15 (2.16-12.28)	
N stage						
N0	Reference	*<0.001*	Reference	*<0.001*	Reference	*<0.001*
N1	17.68 (7.04-44.38)		29.11 (11.10-76.36)		25.17 (9.83-64.44)	
Tumor grade						
Low	Reference	*0.016*	Reference	*0.021*	Reference	*0.037*
High	11.62 (1.59-85.01)		10.36 (1.41-75.95)		3.01 (1.07-8.46)	
ET-1						
Negative	Reference	*0.002*	Reference	*<0.001*	Reference	*0.002*
Positive	0.32 (0.16-0.65)		0.246 (0.12-0.50)		0.35 (0.18-0.69)	
ET_AR						
Negative	Reference	*0.277*	Reference	*0.611*	Reference	*0.932*
Positive	0.56 (0.20-1.59		0.73 (0.22-2.41)		0.95 (0.29-3.08)	
ET_BR						
Negative	Reference	*0.427*	Reference	*0.293*	Reference	*0.239*
Positive	0.66 (0.23-1.86)		0.57 (0.20-1.63)		0.57 (0.22-1.45)	

volvement, and high tumor grade. There was no association between ET_AR expression and clinicopathologic parameters. ET_BR expression was strongly associated with the absence of lymph node involvement (**Table 2**).

Survival analysis

Thirty-four patients (21.8%) experienced disease progression in our study cohort. Thirty-two (20.5%) patients died because of the can-

Table 4. Multivariate analysis predicting MFS, DSS, and OS in patients with UTUC

n=156	Metastasis free survival		Disease specific survival		Overall survival	
	Multivariate analysis HR (95% CI)	*p*	Multivariate analysis HR (95% CI)	*p*	Multivariate analysis HR (95% CI)	*p*
Age (years)						
≤66	Reference	*0.919*	Reference	*0.518*	Reference	*0.558*
>66	0.99 (0.97-1.03)		1.01 (0.98-1.05)		1.01 (0.98-1.04)	
Gender						
Female	Reference	*0.802*	Reference	*0.664*	Reference	*0.374*
Male	1.12 (0.47-2.63)		0.66 (0.27-1.61)		0.70 (0.32-1.54)	
Smoking						
No	Reference	*0.758*	Reference	*0.462*	Reference	*0.719*
Yes	1.17 (0.44-3.08)		1.48 (0.52-4.20)		1.20 (0.46-3.07)	
GFR						
≥60	Reference	*0.105*	Reference	*0.172*	Reference	*0.106*
<60	2.34 (0.84-6.55)		2.18 (0.71-6.67)		2.17 (0.85-5.56)	
Bladder tumor						
No	Reference	*0.015*	Reference	*0.013*	Reference	*0.108*
Yes	3.13 (1.25-7.84)		3.48 (1.30-9.29)		2.09 (0.85-5.16)	
Surgical method						
Open	Reference	*0.198*	Reference	*0.144*	Reference	*0.280*
Laparoscopic	1.83 (0.73-4.57)		2.03 (0.79-5.25)		1.57 (0.69-3.59)	
Tumor number						
Solitary	Reference	*0.061*	Reference	*0.002*	Reference	*0.004*
Multiple	2.04 (0.97-4.32)		3.68 (1.59-8.52)		2.88 (1.39-5.95)	
Tumor stage						
≤T1	Reference	*0.029*	Reference	*0.008*	Reference	*0.007*
>T1	3.59 (1.14-11.30)		8.49 (1.77-40.76)		4.41 (1.51-12.86)	
N stage						
N0	Reference	*<0.001*	Reference	*<0.001*	Reference	*<0.001*
N1	7.82 (2.56-23.94)		9.78 (3.26-29.32)		9.90 (3.36-29.17)	
Tumor grade						
Low	Reference	*0.215*	Reference	*0.703*	Reference	*0.53*
High	3.82 (0.46-31.83)		1.53 (0.17-13.45)		0.67 (0.19-2.37)	
ET-1						
Negative	Reference	*0.047*	Reference	*0.002*	Reference	*0.021*
Positive	0.40 (0.16-0.98)		0.21 (0.08-0.57)		0.35 (0.15-0.85)	
ET_AR						
Negative	Reference	*0.398*	Reference	*0.796*	Reference	*0.977*
Positive	0.39 (0.12-1.34)		0.82 (0.18-3.72)		1.02 (0.25-4.25)	
ET_BR						
Negative	Reference	*0.073*	Reference	*0.122*	Reference	*0.327*
Positive	3.18 (0.90-11.29)		2.62 (0.77-8.85)		1.73 (0.58-5.13)	

cer and 40 (26.0%) patients died during follow-up. The 5-year MFS, DSS, and OS rates were 81.8%, 84.4%, and 81.0% respectively in the ET-1 expression group compared to 51.8%, 49.4%, and 49.4% in the ET-1 negative group. Kaplan-Meier analysis indicated that MFS, DSS, and OS rates were significantly influenced by ET-1 expression (**Figure 2A-C**, $P<0.001$, 0.001, and 0.001 respectively). Neither ET_AR nor ET_BR expression was associated with MFS,

DSS, or OS. Univariate analysis showed that poor renal function, multiple tumors, invasive tumors, lymph node involvement, higher tumor grades, and absence of ET-1 expression were associated with worse MFS, DSS, and OS (**Table 3**). In multivariate analysis, the history of a previous bladder tumor was identified as an independent risk factor for lower MFS and DSS. Multiple tumors were independently associated with bad DSS and OS. Advanced tumor stage, lymph node involvement, and absence of ET-1 expressionwere found to be independent risk factors for poor MFS, DSS, and OS (**Table 4**).

Discussion

ET-1 may directly influence tumor growth and paracrine stimulation in tumor cells. Studies on prostatic, ovarian, renal, pulmonary, colorectal, cervical, and breast carcinoma, Kaposi's sarcoma, brain tumors and melanoma have also demonstrated specific crosstalk between tumor invasion and cell survival [21]. ET-1 and ET_AR were found to be overexpressed in muscle invasive disease and can serve as prognostic markers for lung metastasis in a mouse model [22]. ET-1 from tumor cells enhances tumor migration and invasion of bothtumor cells and macrophages by elevating the levels of inflammatory cytokines and proteases through ET_AR. However, an ET_AR antagonist showed the opposite result. The process of metastatic lung colonization facilitated by ET-1 and ET_AR is dependent on macrophage infiltration of the lung. The expression of ET-1 and ET_AR activity in the tumor are less important in primary or metastatic tumor growth. Hence, cancer treatment targeting ET_AR might be more effective as an adjuvant therapeutic agent rather than treatment for the primary or metastatic disease [22]. Overexpression of the ET axis was reported in both invasive and noninvasive bladder cancer [18-20]. Studies on noninvasive and superficially invasive bladder cancer showed that higher ET-1 expression was associated with longer overall survival but not as an independent prognostic factor. However, the lack of ET-1 expression was predictive of poor overall survival [19]. ET_AR positive tumors showed favorable recurrence-free survival but the lack of ET_AR was not associated with statistically significant difference in recurrence-free survival. In muscle invasive bladder cancer, the results are divergent. ET-1 overexpression is associated with an increased hazard ratio of death in non-metastatic muscle-invasive bladder cancer [20]. In contrast, Wülfing et al. [18] showed that neither ET-1 nor ET_AR expression influenced survival. ET_BR positive tumors were of types that were more favorable and were associated with longer disease-free survival. The loss of ET_BR may be associated with higher tumor stages, higher histological grades, and tumor progression. Interestingly, we showed that the expression of ET-1 in UTUC is associated with favorable parameters, including lower pathologic tumor grade, lower tumor stage, and better survival, similar to the findings in superficial bladder cancer. However, ET_AR expression does not show the same results as those seen in superficial bladder cancer. There is no association between ET_AR expression and survival, implying that the better prognosis in tumors that express ET-1 may not simply be through the activation of ET_AR.

ET axis was also reported to modify immune response with a consequent effect on tumor survival. The most important antigen-presenting cells, the dendritic cells (DCs), which are intrinsic to the development of immune responses, particularly an immune function, produce large amounts of ET-1 and significantly increase endothelin receptors expression upon maturation [23]. Selective ET_AR antagonist significantly reduces the expression of the mature-DC marker CD83, decreases the production of the immunostimulatory cytokine interleukin-12, down-regulates DC's ability to stimulate T cells, and promotes DC apoptosis. On the other hand, blocking the ET_BR resulted in increased expression of CD83 and improved DC survival [23]. Another report showed that the overexpression of ET_BR was associated with a decrease in tumor-infiltrating lymphocytes and poor patient survival [24]. ET-1 suppresses T-cell adhesion and homing onto tumors via ET_BR by decreasing endothelial intercellular adhesion molecule 1 expression [23, 24]. However, the crosstalk between the ET axis and immune cells and their effect on tumor cells is not clearly understood and needs further investigation. Though ET_AR and ET_BR expression were not associated with survival in our study, among patients with tumors expressing ET-1, ET_AR, and ET_BR, a trend toward better prognosis was noted in patients with ET_BR dominant tumors (data not

shown). Therefore, the ratio between ET_AR and ET_BR might play an important role in UTUC.

Currently, there are no reports elaborating the mechanisms underlying the relationship between ET axis expression and survival in patients suffering from UTUC. When tumors become large, tissue oxygenation becomes important for tumor growth, and the production of new blood vessels through the process of angiogenesis triggered by hypoxic tumor cells becomes crucial for survival. HIF-1α, which is known to be increased in tumor tissue, has been found to be increased during the carcinogenesis and progression of UTUC. Nakanishi et al. [25] showed that over half of the UTUC patients were found to express HIF-1α, and the protein was within the nucleus in tumor cells. Further analysis by Ke et al. [26] showed that a higher HIF-1α score was predictive of cancer-specific survival (HR 2.23, *P*=0.004) and tumor recurrence (HR 1.58, *P*=0.036) in UTUC. ET-1 expression has been reported to increase microvessel density and vascular endothelial growth factor (VEGF) level and this can be amplified under hypoxic conditions [15]. ET-1 potentiates hypoxia signaling by interfering with HIF-1α regulation. ET-1 has been shown to stabilize HIF-1α, resulting in the activation of HIF-1α-regulated angiogenic genes. Hypoxia induced endothelin transcription was also seen in various tumor cells. A functioning proximal hypoxia response element in the antisense strand of the promoter of ET-1, and induction of endothelin expression by hypoxia via HIF-1 was reported by Aversa et al. [27]. Tumor microenvironment can influence ET-1 expression. The microenvironment can also be modified through the actions of HIF-1α. This study demonstrated that the expression of ET-1 is related to lower tumor stage, lower tumor grade, and better survival. However, the relationship between ET-1 expression and HIF-1α protein in UTUC needs further evaluation. Under normoxic conditions, ET-1 also interferes with cyclooxygenase (COX)-1 and COX-2 expression, and increases prostaglandin E2 (PGE2) concentration. ET-1-induced VEGF and PGE2 production can be down regulated by the inhibition of COX enzymes that influence the activity of matrixmetalloproteinases (MMP), and cell invasion [28]. We also demonstrated that among patients with positive COX-2 expression in stromal cells, the stage of cancer was associated with cancer-specific survival (*P*=0.0109) and cancer recurrence (*P*=0.0235). However, COX-2 expression in the UTUC tumor cells was not related to either cancer-specific survival or recurrence-free survival [29]. The mechanism by which ET-1 influences COX-2 expression in stromal cells and UTUC tumor cells is unclear. Efforts are hence needed to clarify the same to aid the development of newer therapeutic targets for UTUC. To our knowledge, this is the first report to demonstrate the overexpression of ET axis in UTUC. We also showed, for the first time, that ET-1 overexpression was strongly associated with better MFS, DSS, and OS in UTUC. Although the role in ET_AR and ET_BR remains unclear, further studies may provide directions for newer therapeutic approaches in UTUC.

There were several limitations to our study. First, thiswas a retrospective analysis of a single-center series. Second, the enrolled patients were treated by different surgeons over a 13-year period. Third, we did not detect endothelin expression in the serum of UTUC patients to show whether it can be utilized as a serological prognostic biomarker in clinical settings. Moreover, we did not compare endothelin expression between normal urothelium and tumor tissue from UTUC patients. In addition, those who received neoadjuvant chemotherapy or radiotherapy were not included in our cohort.

Conclusion

Our results suggest that endothelin-1 is a promising prognostic biomarker for patients with UTUC. Higher expression of endothelin-1 is associated with better MFS, DSS, and OS. Further studies are needed for the elucidation of the mechanisms by which the endothelin axis mediates UTUC development and progression.

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Disclosure of conflict of interest

None.

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