Prognostic Value of p53 Protein Overexpression in Upper Tract Urothelial Carcinomas in Taiwan

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Abstract. *Background: p53 plays an important role in maintaining genomic stability and regulating the cell cycle. However, the accumulation of p53 protein has been reported to be involved in the carcinogenesis, progression, and metastasis of many types of human cancer. This study evaluates the clinical significance of p53 expression in upper tract urothelial carcinoma. Patients and Methods: One-hundred and twelve cases of upper tract urothelial carcinoma were included in this study. p53 expression was evaluated by immunohistochemistry and the association of p53 expression with clinicopathological variables was analyzed. Results: p53 expression was significantly correlated with patients who were undergoing hemodialysis (p=0.005) and had increased serum creatinine levels (p=0.001). High p53 expression was associated with poor progression-free (p=0.025) and cancer-specific survival (p=0.021), Cox regression analysis also revealed that p53 was an independent predictor of poor progression-free (hazard ratio=3.74, p=0.025) and cancer-specific (hazard ratio=5.87, p=0.030) survival. Conclusion: Our findings indicate that p53 expression is a potential biomarker for predicting patient survival. Further study is necessary to investigate the role of p53 in the carcinogenesis of upper tract urothelial carcinoma.*

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Urothelial carcinoma is the most common malignancy of the urinary tract. The incidence of renal pelvic and ureteral cancer is relatively low, accounting for only 4% of all urothelial tumors. The ratio of urothelial carcinoma in renal pelvis, ureter and bladder is reported to be 3:1:51 (1, 2). In the United States, renal pelvic urothelial carcinoma accounted for about only 8% of all renal tumors, and ureter cancer about 5% of all urothelial tumors in 2010 (3). In Taiwan, the ratio of renal pelvis, ureter, and bladder cancer has shifted to 1.1:0.9:8.0 (4), showing that the Taiwanese population may have specific genetic and environment factors contributing to upper tract urothelial carcinomas (UTUC). The clinical characteristics and prognosis are quite different for bladder cancer and UTUC. The upper urinary tract has anatomical characteristics, such as a thin muscle layer, proximity to the kidney and rich lymphatic drainage (5). Tumor invasion may significantly influence distant metastasis and progression in patients with UTUC. Our previous immunochemistry reports revealed the roles of cyclooxygenase-2 (6), osteopontin (7), hypoxia-induced factor-1α (8) and nuclear factor-κB (9) in the UTUC, and examined their potential as poor outcome predictors for the UTUC patients in Taiwan. Patients with UTUC almost always present with hydronephrosis or hematuria and are diagnosed by pathology. Pathological stage and pathological grade are the only factors to predict disease prognosis. However, the exact molecular mechanisms of tumor invasion, recurrence, and prognosis of this disease are not clear. There are no reliable biomarkers for diagnosis, outcome prediction, or treatment effect monitoring for UTUC. Moreover, different cancer behaviors are still observed even between patients with the same stage or grade of disease.

p53 is a well-known tumor suppressor gene located on chromosome 17p13.1 and encoding a nuclear phosphoprotein

of p53 kDa (10). Functional p53 plays an important role in maintaining genomic stability, regulating the cell cycle, and in inducing apoptosis (11). Loss of p53 expression disables one of the most important cellular mechanisms responsible for the elimination of damaged DNA (12). The abnormal p53 oncoprotein produced by the mutant gene has a longer in half-life and is more stable than the wild-type protein, and tends to accumulate in the cell (13). Non-functional mutated p53 accumulates in the nucleus of tumor cells and abolishes DNA binding, thereby abolishing its normal tumor-suppressor function (14). Immunohistochemical staining for p53 has been used as a popular surrogate marker for *p53* mutational status (15). However, there are few studies of p53 molecular mechanisms in UTUC in Taiwan. The purpose of this study was to evaluate the expression of p53 in association with clinical outcome and prognosis of patients with UTUC.

Patients and Methods

Surgical specimens and clinicopathological data. One-hundred and twelve formalin-fixed UTUC samples were obtained from the Department of Urology, Kaohsiung Medical University Hospital from 1997-2006. The pathological grade was classified according to World Health Organization (WHO) histological criteria (16), and tumor stage was determined according to the International Union Against Cancer tumor-node-metastasis classification (17). Recurrence-free survival was calculated from the date of surgery to the date of recurrence. Progression-free survival was calculated from the date of surgery to the date when the patient was defined as with invasion tumor or with distant metastasis. Cancer-specific survival was calculated from the date of surgery to the date when the patient died of cancer. The study protocol was reviewed and approved by the Institutional Review Board of the Kaohsiung Medical University (KMUH-IRB-20120031).

Immunohistochemistry. Five-micrometer-thick sections from representative tissue blocks were cut, de-paraffinized with xylene rinse, rehydrated with a graded alcohol series (100%, 95%, 85%, and 75%) for 5 min each, and then rinsed with distilled water. Antigen retrieval was enhanced by autoclaving slides in sodium citrate buffer (10 mM, pH 6.0) for 30 min. Endogenous peroxidase activity was quenched by incubation in 3% hydrogen peroxide/methanol buffer for 30 min. The slides were incubated with a monoclonal antibody against p53 (clone DO-7; Dako, Glostrup, Denmark) at a dilution of 1:500 overnight at 4˚C in humidified chambers. The slides were washed three times in phosphate-buffered solution and further incubated with a biotinylated secondary antibody for 30 min at room temperature. Antigen antibody complexes were detected by the avidin-biotin-peroxidase method using 3,3'-diaminobenzidine as a chromogenic substrate (Dako). Finally, the slides were counterstained with hematoxylin and then examined by light microscopy. Notably, only staining in tumor cells (approximately 1,000 cells in 3-4 high-power fields) was observed.

Evaluation of immunohistochemical staining. The scoring of p53 staining was based on the percentage of positively-stained cells in four quantitative categories: Score 1, ≤25% positive cells; score 2, 26-50% positive cells; score 3, 51-75% positive cells; and score 4, ≥76% positive cells. The staining for each specimen was determined separately by two pathologists and the rare cases with discordant scores were re-evaluated and scored on the basis of consensual opinion.

Statistical analysis. All statistical analyses were performed using the SPSS statistical package for PC (version 14.0, SPSS, Inc., Chicago, IL, USA). For p53 expression, tumor with scores 1 and 2 were categorized as low expression (*i.e.* ≤50% positively stained cells), and scores 3 and 4 were categorized as high expression (ie >50%). Chi-square test was applied to study the correlation of p53 expression with tumor stage, tumor grade, gender, age at diagnosis, body mass index (BMI), distant metastasis, hemodialysis, and serum creatinine level. Survival curves were generated using Kaplan–Meier estimates, and the significance of differences between curves was evaluated by the log-rank test. Furthermore, hazard ratios (HRs) and 95% confidence intervals (CIs) computed from univariate and multivariable Cox regression models were used for investigating the relationship between clinicopathological characteristics and survival. *p*-Values less than 0.05 were considered statistically significant.

Results

The expression of p53 in cancer tissues (n=112 patients) was examined by immunohistochemistry and stratified into four scores (quartiles) (score 1, n=26 patients; score 2, n=14 patients; score 3, n=40 patients; and score 4, n=32 patients). Based on the scores, tissues were further categorized into high (scores 3 and 4; 64.3%) –and low (scores 1 and 2; 35.7%)– expression groups (Figure 1 and Table I). To explore the potential role of p53 in UTUC tissues, the expression patterns of p53 were correlated with clinicopathological variables including tumor stage, tumor grade, gander, age at diagnosis, BMI, tumor distant metastasis, hemodialysis and serum creatinine level. High p53 expression in UTUC tissues was significantly associated with patients who were undergoing hemodialysis and had increased serum creatinine levels (p=0.005 and 0.001, respectively; Table I).

The expression patterns of p53 in UTUC tissues were further correlated with the recurrence-free, progression-free, and cancer-specific survival of the patients by Kaplan–Meier estimates. Patients were followed-up for recurrence-free, progression-free, and cancer-specific survival status with a median follow-up of four years (range 0.3-16 years). There were no significant differences in recurrence-free survival among the patients in the low- and high-p53 expression groups (Figure 2A). Increased progression-free and cancer-specific survival rates were observed in the low-p53-expressing group (p=0.025 and 0.021, respectively), as determined by the log-rank test (Figure 2B and 2C).

To evaluate the factors related to p53 expression in cancer of the renal pelvis cancer, HRs were estimated by univariate and multivariable Cox regression, as shown in Tables II-IV. In the univariate analysis, the significant factors associated

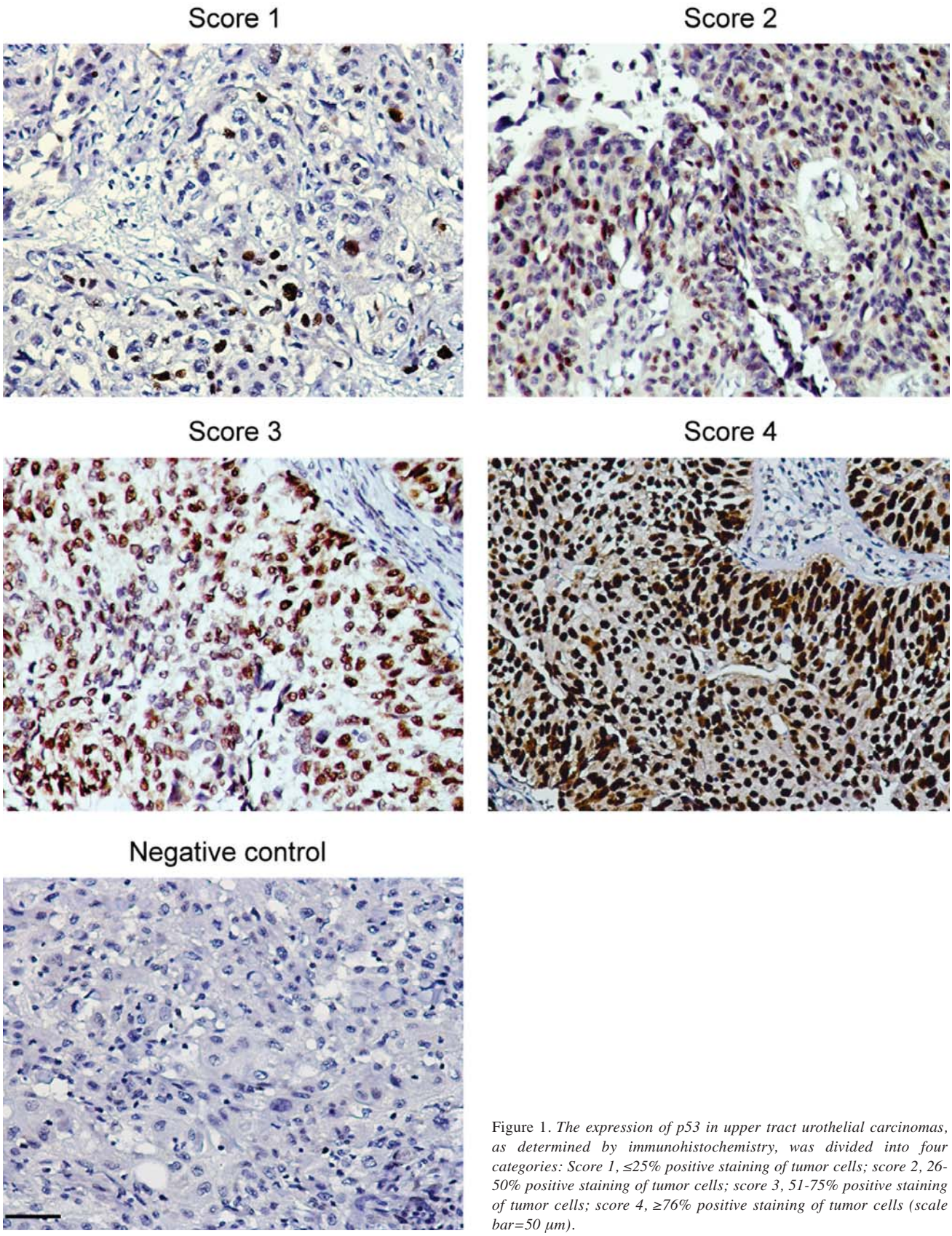

Figure 1. *The expression of p53 in upper tract urothelial carcinomas, as determined by immunohistochemistry, was divided into four categories: Score 1, ≤25% positive staining of tumor cells; score 2, 26-50% positive staining of tumor cells; score 3, 51-75% positive staining of tumor cells; score 4, ≥76% positive staining of tumor cells (scale bar=50 μm).*

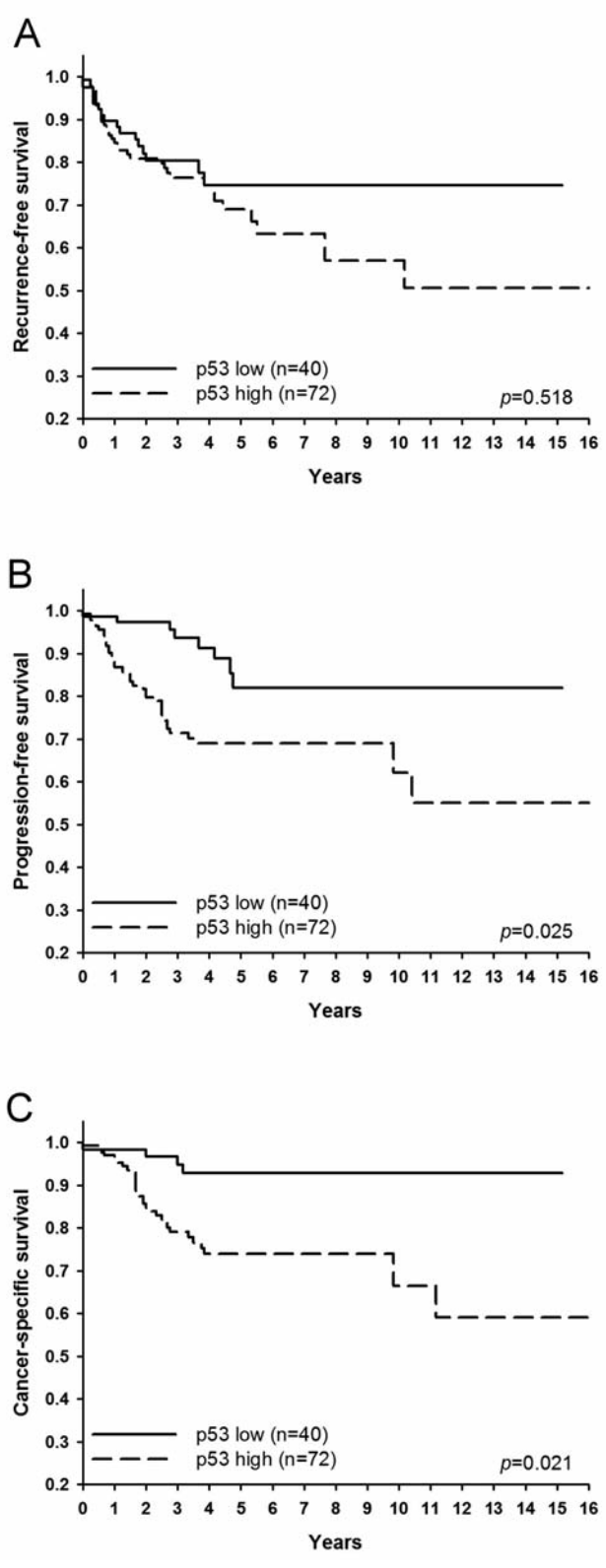

Figure 2. *Kaplan-Meier survival curves for recurrence-free (A), progression-free (B) and cancer-specific (C) survival of patients with low and high p53 expression in upper tract urothelial carcinoma.*

Table I. *Clinicopathological characteristics of patients with upper tract urothelial carcinoma and association with p53 expression.*

		p53		
		Low	High	
Variable[a]	Patient, no. (%)	n (%)	n (%)	*p*-Value[b]
No.	112 (100)	40 (35.7)	72 (64.3)	
Stage				
I/II	44 (40.0)	17 (38.6)	27 (61.4)	0.569
III/IV	66 (60.0)	22 (33.3)	44 (66.7)	
Grade				
Low	41 (36.6)	17 (41.5)	24 (58.5)	0.335
High	71(63.4)	23 (32.4)	48 (67.6)	
Gender				
Male	66 (58.9)	19 (28.8)	47 (71.2)	0.067
Female	46 (41.1)	21 (45.7)	25 (54.3)	
Age (years)				
<60	29 (25.9)	8 (27.6)	21 (72.4)	0.289
≥60	83 (74.1)	32 (38.6)	51 (61.4)	
BMI (kg/m^2)				
<25	73 (66.4)	25 (34.2)	48 (65.8)	0.710
≥25	37 (33.6)	14 (37.8)	23 (62.2)	
Distant metastasis				
Negative	100 (89.3)	38 (38.0)	62 (62.0)	0.145
Positive	12 (10.7)	2 (16.7)	10 (83.3)	
Hemodialysis				
Negative	95 (84.8)	39 (41.1)	56 (58.9)	0.005
Positive	17 (15.2)	1 (5.9)	16 (94.1)	
Creatinine (mg/dl)				
≤1.5	66 (60.0)	31 (47.0)	35 (53.0)	0.001
>1.5	44 (40.0)	7 (15.9)	37 (84.1)	

[a]Undetermined in small cases. [b]*p* by the chi-square test. BMI: Body mass index.

with recurrence-free survival included tumor grade (p=0.002) and gender (p=0.046; Table II), whereas the significant factors associated with progression-free survival included tumor stage (p=0.007), tumor grade (p=0.028), distant metastasis (p<0.001) and p53 expression (p=0.034; Table III). Moreover, the significant factors associated with cancer-specific survival included tumor stage (p=0.008), tumor grade (p=0.038), distant metastasis (p<0.001) and p53 expression (p=0.037; Table IV).

However, after adjusting for tumor stage, gender, age at diagnosis, BMI, hemodialysis and serum creatinine level in multivariable Cox regression analysis, only tumor grade (p=0.015) and distant metastasis (p=0.010) were significantly independent predictors of recurrence-free and progression-free survival in patients with cancer of the renal pelvis respectively (Tables II and III). Furthermore, p53 expression was a significant independent predictor of progression-free and cancer-specific survival (p=0.025 and p=0.030, respectively) in patients with cancer of the renal pelvis (Tables III and IV).

Table II. *Univariate and multivariable analysis of recurrence-free survival for patients with upper tract urothelial carcinoma.*

Variable	Univariate			Multivariable		
	Hazard ratio	95% Confidence interval	*p*-Value	Hazard ratio	95% Confidence interval	*p*-Value
Stage						
III/IV	0.72	(0.38-1.37)	0.319	0.45	(0.18-1.11)	0.082
I/II	1.00			1.00		
Grade						
High	3.34	(1.56-7.18)	0.002	4.45	(1.34-14.82)	0.015
Low	1.00			1.00		
Gender						
Female	1.79	(1.01-3.16)	0.046	1.79	(0.72-4.44)	0.211
Male	1.00			1.00		
Age (years)						
≥60	1.32	(0.66-2.65)	0.436	1.87	(0.58-6.05)	0.296
<60	1.00			1.00		
BMI (kg/m^2)						
≥25	0.92	(0.48-1.74)	0.791	0.68	(0.27-1.68)	0.397
<25	1.00			1.00		
Distant metastasis						
Positive	1.27	(0.50-3.22)	0.619	1.36	(0.40-4.64)	0.627
Negative	1.00			1.00		
Hemodialysis						
Positive	0.75	(0.27-2.08)	0.575	1.98	(0.36-10.82)	0.429
Negative	1.00			1.00		
Creatinine (mg/dl)						
>1.5	1.06	(0.58-1.94)	0.842	0.31	(0.09-1.04)	0.058
≤1.5	1.00			1.00		
p53						
High	1.32	(0.57-3.03)	0.520	2.00	(0.73-5.45)	0.177
Low	1.00			1.00		

BMI: Body mass index.

Discussion

Taiwan has a remarkably high incidence of UTUC, and is well-known for its widespread use of Aristolochia herbal remedies, which may be closely-related to the cause of increased risk of developing end-stage renal disease or urothelial carcinoma (18). Interestingly, about 43% of UTUC are located in the upper urinary tract. Therefore, Taiwanese may have a unique etiology of UTUC, together with several unique markers for different stages of carcinogenesis (19). The pathological stage of the primary tumor, lymph node status, the presence of distant metastases, and tumor grade are important prognostic factors for UTUC (20). Given that disease recurrence and progression rates are high in patients with UTUC, a better understanding of prognostic parameters might lead to the identification, and hence improved counseling, of patients who stand to benefit from intensified therapy and monitoring.

Mutation of the *p53* gene is a common genetic alternation in malignant human tumors and can be inferred from the immunohistochemical detection of the accumulated mutant gene product. Nuclear accumulation of this protein is also of prognostic value in patients with UTUC (21). Some studies have shown p53 oncoprotein immunoreactivity to be associated with invasiveness and high grade in transitional cell carcinoma of the urinary bladder (22-24). In a previous study, immunoreactivity for the p53 oncoprotein tended to be associated with a shorter length of both disease-free survival and overall survival in transitional cell carcinoma of the upper urinary tract (25). In bladder cancer, patients with strong positive staining of p53 >60% had a much lower 5-year biochemical recurrence-free survival rate than patients with p53 staining <60% and this study also indicated that a strong positive staining for p53 >60% and tumor grade were significant predictors for recurrence (26). In colorectal cancer, p53 appears to be a prognostic factor in predicting recurrence-free survival (27). In Taiwan, there are still no studies of p53 overexpression and UTUC. To our knowledge, the present study is one of the only studies to evaluate the prognostic significance of p53 expression in UTUC, and is the only study that has assessed p53 levels within tumors. This study demonstrated p53 to be an independent predictor of worse recurrence-free and progression-free survival in UTUC.

Table III. *Univariate and multivariable analysis of progression-free survival for patients with upper tract urothelial carcinoma.*

Variable	Univariate			Multivariable		
	Hazard ratio	95% Confidence interval	*p*-Value	Hazard ratio	95% Confidence interval	*p*-Value
Stage						
III/IV	3.75	(1.42-9.88)	0.007	1.72	(0.56-5.27)	0.346
I/II	1.00			1.00		
Grade						
High	2.20	(1.09-4.46)	0.028	1.37	(0.40-4.64)	0.617
Low	1.00			1.00		
Gender						
Female	1.21	(0.67-2.18)	0.520	0.76	(0.28-2.11)	0.601
Male	1.00			1.00		
Age (years)						
≥60	0.60	(0.32-1.10)	0.097	0.49	(0.17-1.41)	0.188
<60	1.00			1.00		
BMI (kg/m^2)						
≥25	0.90	(0.46-1.75)	0.755	1.23	(0.51-2.96)	0.649
<25	1.00			1.00		
Distant metastasis						
Positive	4.76	(2.36-9.60)	<0.001	4.64	(1.44-14.97)	0.010
Negative	1.00			1.00		
Hemodialysis						
Positive	0.49	(0.12-2.05)	0.330	0.18	(0.02-1.69)	0.132
Negative	1.00			1.00		
Creatinine (mg/dl)						
>1.5	0.99	(0.54-1.83)	0.974	0.74	(0.25-2.22)	0.591
≤1.5	1.00			1.00		
p53						
High	3.21	(1.09-9.46)	0.034	3.74	(1.18-11.87)	0.025
Low	1.00			1.00		

BMI: Body mass index.

Nephropathy is a chronic tubulointerstitial disease frequently accompanied by UTUC. The association of chronic renal insufficiency, uremic oxalosis, long-term hemodialysis, acquired cystic kidney disease and development of variable precursor intratubular and intracystic lesions progressing to several papillary adenomas and multifocal renal cell carcinomas has been determined. The development of renal cell carcinoma in chronic renal disease and hemodialysis is much more common than in the normal kidney (28-30). Epidermal cells in patients with chronic renal failure on hemodialysis failed to respond to physiologically-produced p53 apoptotic signals, resulting in its continuous production and accumulation of p53 (31). In our study, p53 expression in UTUC was significantly associated with hemodialysis, suggesting that targeting p53 inhibition may be a promising approach for cancer treatment in the future.

In conclusion, using a cohort of one-hundred and twelve patients with UTUC, we observed a positive association between p53 expression and malignant UTUC behavior. High p53 expression was found to be independently-associated with poor recurrence-free and progression-free survival under multivariable Cox regression analysis. Further investigations are required to explore the detailed mechanisms of p53 signaling in UTUC development and to establish new diagnostic and therapeutic strategies using p53 as a target.

Conflicts of Interest

All Authors declare that they have no conflicting interests.

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Table IV. *Univariate and multivariable analysis of cancer-specific survival for patients with upper tract urothelial carcinoma.*

Variable	Univariate			Multivariable		
	Hazard ratio	95% Confidence interval	*p*-Value	Hazard ratio	95% Confidence interval	*p*-Value
Stage						
III/IV	7.32	(1.70-31.57)	0.008	4.46	(0.82-24.30)	0.084
I/II	1.00			1.00		
Grade						
High	2.42	(1.05-5.59)	0.038	1.16	(0.25-5.46)	0.851
Low	1.00			1.00		
Gender						
Female	1.14	(0.58-2.24)	0.706	1.22	(0.40-3.76)	0.724
Male	1.00			1.00		
Age (years)						
≥60	0.82	(0.39-1.73)	0.607	0.96	(0.28-3.33)	0.945
<60	1.00			1.00		
BMI (kg/m^2)						
≥25	0.64	(0.28-1.44)	0.280	0.55	(0.18-1.72)	0.307
<25	1.00			1.00		
Distant metastasis						
Positive	4.71	(2.14-10.37)	<0.001	3.16	(0.84-11.97)	0.090
Negative	1.00			1.00		
Hemodialysis						
Positive	0.74	(0.18-3.15)	0.687	0.37	(0.04-3.63)	0.396
Negative	1.00			1.00		
Creatinine (mg/dl)						
>1.5	1.19	(0.59-2.41)	0.623	0.81	(0.24-2.74)	0.730
≤1.5	1.00			1.00		
p53						
High	4.80	(1.10-20.99)	0.037	5.87	(1.19-29.10)	0.030
Low	1.00			1.00		

BMI: Body mass index.

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