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The clinicopathological characteristics of muscle-invasive bladder recurrence in upper tract urothelial carcinoma

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Abstract

This study aimed to clarify the clinical characteristics and oncological outcomes of patients with upper tract urothelial carcinoma (UTUC) who developed muscle-invasive bladder cancer (MIBC) after radical nephroureterectomy (RNU). We identified 966 pTa-4N0-2M0 patients with UTUC who underwent RNU and clarified the risk factors for MIBC progression after initial intravesical recurrence (IVR). We also identified 318 patients with primary pT2-4N0-2M0 MIBC to compare the oncological outcomes with those of patients with UTUC who developed or progressed to MIBC. Furthermore, immunohistochemical examination of p53 and FGFR3 expression in tumor specimens was performed to compare UTUC of MIBC origin with primary MIBC. In total, 392 (40.6%) patients developed IVR after RNU and 46 (4.8%) developed MIBC at initial IVR or thereafter. As a result, pT1 stage on the initial IVR specimen, concomitant carcinoma in situ on the initial IVR specimen, and no intravesical adjuvant therapy after IVR were independent factors for MIBC progression. After propensity score matching adjustment, primary UTUC was a favorable indicator for cancer-specific death compared with primary MIBC. Subgroup molecular analysis revealed high FGFR3 expression in non-MIBC and MIBC specimens from primary UTUC, whereas low FGFR3 but high p53 expression was observed in specimens from primary MIBC tissue. In conclusion, our study demonstrated that patients with UTUC who develop MIBC recurrence after RNU exhibited the clinical characteristics of subsequent IVR more than those of primary UTUC. Of note, MIBC subsequent to UTUC may have favorable outcomes, probably due to the different molecular biological background compared with primary MIBC.

Abbreviations: CIS, carcinoma in situ; CSS, cancer-specific survival; CT, computed tomography; HR, hazard ratio; IHC, immunohistochemistry; IVR, intravesical recurrence; LVI, lymphovascular invasion; MIBC, muscle-invasive bladder cancer; NMIBC, non-muscle-invasive bladder cancer; PS, propensity score; RC, radical cystectomy; RFS, recurrence-free survival; RNU, radical nephroureterectomy; TURBT, transurethral resection of bladder tumor; UTUC, upper tract urothelial carcinoma.

KEYWORDS
immunohistochemistry, intravesical recurrence, muscle-invasive bladder cancer, non-muscle-invasive bladder cancer, upper tract urothelial carcinoma

1 | INTRODUCTION

UTUC, which comprises cancer of the ureter and renal pelvis, is relatively uncommon and accounts for only approximately 5% of urothelial malignancies.[1] A major cause of concern for patients with UTUC is IVR after RNU, the incidence of which was reported to be approximately 15%-50%.[2,3] Many patients develop IVR within 2 y after RNU, but the majority of the relapses exhibit low grade, multiple, papillary-liked features, which are diagnosed as NMIBC.[4,5] Although most IVR pathologically results in pTa or pT1, some bladder cancers originating from UTUC develop as MIBC at the initial bladder tumor recurrence or progress to secondary MIBC after repeated IVR development.

Although the predictive factors for tumor progression in patients who developed IVR after RNU were previously investigated,[6,7] we do not yet have a sufficient understanding of whether these aggressive bladder cancers of secondary MIBC take over the characteristics of primary UTUC. Furthermore, the current guidelines lack evidence for the natural history of future oncological outcomes of patients with MIBC who had a previous history of primary UTUC.[1] Our specific aim was to clarify the clinicopathological characteristics and oncological outcomes of patients with MIBC who previously underwent RNU for UTUC.

2 | MATERIALS AND METHODS

2.1 | Samples from patients with UTUC (Cohort 1)

The present study was approved by the review boards of 8 institutions, consisting of Keio University Hospital and 7 affiliated facilities. After the data sets were combined, 966 patients with pTa-4N0-2M0 UTUC who underwent RNU between 1990 and 2016 were identified and included in the present study as cohort 1 (Figure 1).

RNU was performed according to the standard procedure,[8] involving extrafascial dissection of the kidney, with the entire length of the ureter and adjacent segment of the bladder cuff being removed. A small iliac incision (Gibson incision) was made to retrieve the kidney and ureter en bloc, and to resect the bladder cuff. Regional lymph node dissection was not performed unless there were suspicious lymph nodes on preoperative imaging or based on intraoperative findings.[9] We did not administer intravesical chemotherapy early (within 48 h) after RNU.

2.2 | Samples from patients with primary MIBC (cohort 2)

To evaluate the oncological outcomes of MIBC subsequent to UTUC, we identified 318 patients with primary pT2-4N0-2M0 MIBC who underwent RC between 2006 and 2016 at the same institutions, and defined them as cohort 2 (Figure 1). Due to inherent differences between primary MIBC and MIBC subsequent to UTUC, PS matching analyses were applied to adjust for baseline patient characteristics. All patients underwent standard RC by an open surgical method with urinary diversion, including ileal conduit, neobladder, or cutaneous ureterostomy. Regional lymphadenectomy, including bilateral internal iliac, external iliac, and obturator lymph nodes, was performed.

2.3 | Evaluation of urothelial carcinoma after surgical management

Surgical specimens were processed according to standard pathological procedures at each institution. All specimens, including primary UTUC, subsequent IVR, and MIBC, were histologically confirmed to be urothelial carcinoma. Tumors were staged according to the 2002 American Joint Committee on Cancer/Union Internationale Contre le Cancer TNM classification and graded according to the 2004 World Health Organization classification.[10] Lymphovascular invasion (LVI) was defined as the unequivocal presence of tumor cells within endothelial-lined lymphatic and vascular channels based on the criteria in the "WHO Classification of Tumours of the Urinary System and Male Genital Organs."[11] Tumor multifocality was defined as pathologically confirmed tumors with more than or equal to 2 distinct locations within the upper urinary tract involving the renal pelvis and ureter.[12]

2.4 | Follow-up regimen

Patients were generally followed up routinely 3-6 mo after surgery, every 6 mo during the first 5 y, and annually thereafter. The follow-up consisted of a history, physical examination, routine blood work, urinary cytology, chest radiography, and cystoscopic evaluation of the urinary bladder. Radiographic evaluations of the contralateral upper urinary tract using CT, magnetic resonance imaging, or excretory urography were conducted every 6 mo for the first 5 y, and annually thereafter. Elective bone scans and chest CT were performed when clinically indicated. Patients with MIBC received RC and were generally followed up at least every 3-4 mo for 2 y, then every 6 mo until 5 y, and annually thereafter. Radiographic evaluations were conducted in the same flow

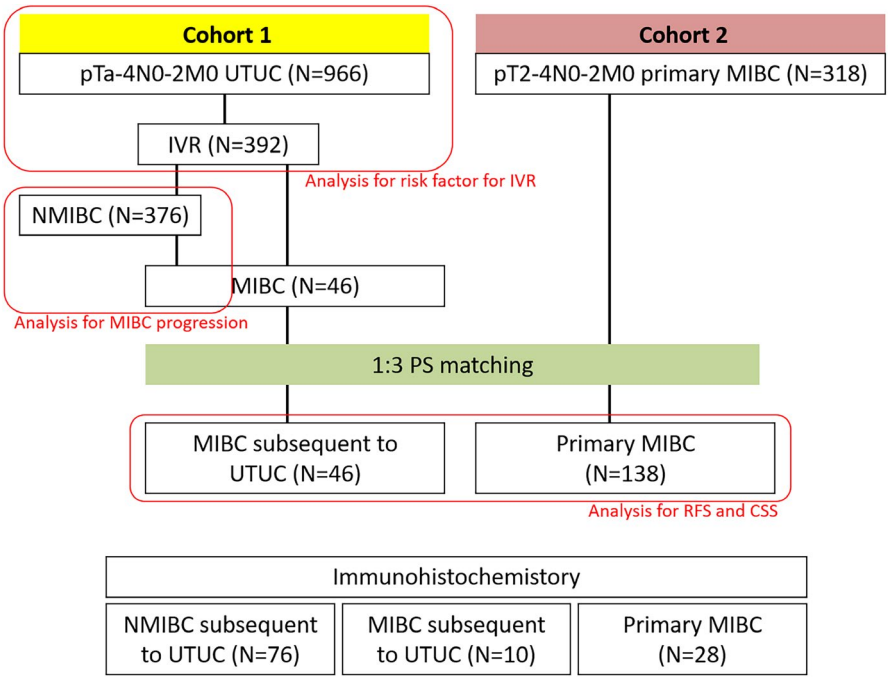


FIGURE 1 Flow chart of the study population. In total, 966 pTa-4N0-2M0 patients with UTUC who underwent RNU were included in cohort 1. Among them, 392 developed IVR. Sixteen patients were diagnosed with MIBC at initial IVR, 30 patients progressed to subsequent MIBC after repeated IVR, and 346 (35.8%) patients developed NMIBC during the follow-up period. We also identified 318 pT2-4N0-2M0 patients with primary MIBC who underwent RC between years 2006 and 2016 at the same institutions, and defined them as cohort 2. The RFS and CSS between patients with primary MIBC and those who developed MIBC after RNU were analyzed after 1:3 PS adjustments. Tissue samples for immunohistochemistry were obtained from 86 IVR tumor specimens, including specimens from 76 patients with NMIBC and 10 patients with MIBC that subsequently developed after RNU, and 28 paired tissues of TURBT and RC specimens from patients with primary MIBC

as for UTUC. RFS was calculated as the duration from RC to the date when disease recurrence, including extravesical local recurrence and/or distant metastasis, was detected. CSS was defined as the period from RC to cancer-related death from urothelial carcinoma.

2.5 | Tissue samples and immunohistochemical examination

Tissue samples were obtained from consenting patients in the present study, which was approved by the Keio University Ethics Committee. We examined 86 IVR tumor specimens, including specimens from 76 patients with NMIBC and 10 patients with MIBC who subsequently developed after RNU, and 28 paired tissues of TURBT and RC specimens from patients with primary MIBC. All specimens were fixed in 10% formalin and embedded in paraffin, and all slides were re-reviewed by genitourinary pathologists. Sections (4 μm thickness) of formalin-fixed and paraffin-embedded tissues were evaluated. Sections were deparaffinized in xylene, and rehydrated in graded alcohol and distilled water. After antigen retrieval with citric acid (pH 6.0) at 120°C for 10 min, endogenous peroxidase activity was blocked by 1% hydrogen peroxide for 15 min, followed by washing with distilled water. To bind non-specific antigens, sections were incubated at room temperature for 15 min with 5% skimmed milk in PBS. Sections were then incubated at 4°C overnight with anti-FGFR3 mouse monoclonal antibody (Ab) (1:150 dilution; Origene, Rockville, MD, USA) or anti-p53 rabbit polyclonal Ab (1:100 dilution; Abcam, Cambridge, MA, USA). After washing with PBS, tissue sections were incubated with the secondary Ab for 60 min. Color was developed using 3,3′-diaminobenzidine tetrahydrochloride in 50 mmol/L Tris-HCl (pH 7.5) containing 0.005% hydrogen peroxide. Sections were then counterstained with hematoxylin.

To assess FGFR3 and p53 staining, cancer cells with positive staining in the cell cytoplasm were counted in at least 10 representative fields, and the mean percentage of positive cancer cells (0-100) and staining intensity stratified from 0 to 3 (0: no staining 1: low staining, 2: moderate staining, 3: strong staining) were estimated. The histoscore (H-score) was calculated by applying the following formula: mean percentage × intensity (range 0-300).[13]

2.6 | Statistical analysis

Medians and interquartile ranges were generated for continuously coded variables, and the Mann-Whitney U test and chi-square test were used to assess the significance of differences between medians and proportions, respectively. One-way ANOVA analysis was conducted

TABLE 1 Patient and tumor characteristics of patients with UTUC who underwent RNU

Patient characteristics		Total n = 966, (%)	Patients with UTUC without IVR n = 574, (%)	Patients with UTUC who developed IVR n = 392, (%)	*P*-value
Follow-up duration	Mean ± SD	62.7 ± 39.2	59.8 ± 35.5	66.9 ± 43.7	<.001
Age	Mean ± SD	69.7 ± 10.2	70.4 ± 10.6	68.6 ± 9.6	.008
Sex	Male	707 (73.2)	414 (72.1)	293 (74.7)	.204
	Female	259 (26.8)	160 (27.9)	99 (25.3)	
ECOG-PS	0-1	706 (73.1)	407 (70.9)	299 (76.3)	.038
	2	260 (26.9)	167 (29.1)	93 (23.7)	
Tumor location	Pelvis	523 (54.2)	296 (51.6)	227 (57.9)	.057
	Ureter	443 (45.8)	278 (48.4)	165 (42.1)	
Tumor histology	Pure UC	857 (88.7)	508 (88.5)	349 (89.0)	.442
	Non-pure UC	109 (11.3)	66 (11.5)	43 (11.0)	
Pathological T stage	<3	496 (51.3)	276 (48.1)	220 (56.1)	.008
	≥3	470 (48.7)	298 (51.9)	172 (43.9)	
Pathological N stage	0	901 (93.3)	534 (93.0)	367 (93.6)	.412
	1, 2	65 (6.7)	40 (7.0)	25 (6.4)	
Tumor grade	Low	418 (43.3)	255 (44.4)	163 (41.6)	.209
	High	548 (56.7)	319 (55.6)	229 (58.4)	
LVI	Absent	610 (63.1)	354 (61.7)	256 (65.3)	.410
	Present	356 (36.9)	220 (38.3)	136 (34.7)	
Concomitant CIS	No	845 (87.5)	509 (88.7)	336 (85.7)	.103
	Yes	121 (12.5)	65 (11.3)	56 (14.3)	
Tumor multifocality	No	850 (88.0)	519 (90.4)	331 (84.4)	.004
	Yes	116 (12.0)	55 (9.6)	61 (15.6)	
Systemic adjuvant chemotherapy	No	785 (81.3)	464 (80.8)	321 (81.9)	.373
	Yes	181 (18.7)	110 (19.2)	71 (18.1)	
Previous history of bladder cancer	No	847 (87.7)	505 (88.0)	342 (87.2)	.403
	Yes	119 (12.3)	69 (12.0)	50 (12.8)	

Abbreviations: UTUC, upper tract urothelial carcinoma; RNU, radical nephroureterectomy; IVR, intravesical recurrence; SD, standard deviation; ECOG-PS, Eastern Cooperative Oncology Group-Performance Status; UC, urothelial carcinoma; LVI, lymphovascular invasion; CIS, carcinoma in situ.

to compare 3 or more continuous variables. PS matching was calculated for each patient using a multivariable logistic regression model including age, sex, Eastern Cooperative Oncology Group-Performance status, number of TURBT procedures, clinical and pathological tumor stage, tumor grade, lymph node involvement, LVI, the administration of systemic neoadjuvant and adjuvant chemotherapy, and concomitant CIS. We applied the nearest-neighbor method with caliper matching, adopting a 1:3 matching ratio to maintain a large sample size, which maximized the statistical power for maintaining a balance in the present cohort.[14] Kaplan-Meier analyses with the log-rank test were conducted to draw the RFS and CSS curves. Univariate and multivariate Cox regression models were used to calculate proportional HRs for investigating prognostic factors for MIBC development, RFS, and CSS. In all statistical analyses, tests were two-sided and a P-value < .05 was considered to indicate significance. All statistical analyses were performed using the Statistical Package of Social Sciences software, v.24.0 (SPSS, Chicago, Illinois, USA).

3 | RESULTS

3.1 | Clinical and pathological characteristics of patients with UTUC

The median age was 70 (42-91) y old, with a median follow-up term of 59 (4-234) mo. Among the 966 patients with UTUC, 392 (40.6%) developed IVR, and the median duration from RNU to IVR was 12.9 mo. The clinicopathological indicators and tumor characteristics of primary UTUC are shown in Table 1. The primary UTUC locations were the renal pelvis in 523 (54.2%) and the ureter in 443 (45.8%). A previous history of bladder cancer before RNU was observed in 119 (12.3%) patients. Regarding the pathological results, 470 (48.7%) patients had pT3 or higher stage, 65 (6.7%) were lymph node-positive, 548 (56.7%) had high-grade disease, 356 (36.9%) were LVI positive, 121 (12.5%) had concomitant CIS, and 116 (12.0%) demonstrated tumor multifocality in their RNU specimens.

TABLE 2 Univariate and multivariate Cox regression analyses for determining risk factors for IVR development in patients with UTUC

	Univariate			Multivariate		
Clinical indicators	**HR**	**95% CI**	***P*-value**	**HR**	**95% CI**	***P*-value**
Age (≥70 vs. <70)	1.28	1.02-1.60	.030	1.19	0.96-1.50	.113
Sex (male vs. female)	1.05	0.88-1.22	.563			
ECOG-PS (2 vs. 0-1)	0.88	0.69-1.11	.270			
Tumor location (ureter vs. renal pelvis)	2.48	1.93-3.18	<.001	2.08	1.83-3.03	<.001
Tumor histology (pure UC vs. non-pure UC)	1.00	0.73-1.38	.984			
Pathological T stage (T2 ≤ vs. T3 ≥)	1.22	1.01-1.49	.048	1.25	1.02-1.53	.029
Pathological N stage (N0 vs. N1,2)	1.15	0.77-1.73	.494			
Tumor grade (high vs. low)	0.99	0.81-1.21	.899			
Concomitant CIS (yes vs. no)	1.16	0.88-1.54	.295			
Tumor multifocality (yes vs. no)	1.49	1.13-1.96	.004	1.25	1.05-1.65	.025
LVI (positive vs. negative)	0.87	0.70-1.07	.176			
Systemic adjuvant chemotherapy (yes vs. no)	0.89	0.69-1.15	.373			
Previous history of bladder tumor (yes vs. no)	1.10	0.82-1.48	.525			

Abbreviations: IVR, intravesical recurrence; UTUC, upper tract urothelial carcinoma; HR, hazard ratio; CI, confidence interval; ECOG-PS, Eastern Cooperative Oncology Group-Performance Status; UC, urothelial carcinoma; CIS, carcinoma in situ; LVI, lymphovascular invasion.

3.2 | Risk factors for IVR development in patients with UTUC who underwent RNU

We conducted univariate and multivariate Cox regression analyses to clarify the risk factors for developing subsequent IVR after RNU, with these listed in Table 2. Based on the univariate analysis, age ≥ 70, tumor location, pT ≤ 2, and tumor multifocality were significantly associated with subsequent IVR. Furthermore, multivariate analysis revealed pT2 or lower-stage (HR 1.25, $P = .029$), ureteral cancer (HR 2.08, $P < .001$), and tumor multifocality (HR 1.25, $P = .025$) to be independent risk factors for IVR development after RNU.

3.3 | Risk factors for MIBC recurrence in patients with UTUC who developed IVR

We conducted subgroup analysis to clarify the risk factors for subsequent MIBC progression after initial IVR. In total, 392 patients who developed primary IVR were included in this subgroup analysis. Overall, 46 (4.7%) developed or progressed to MIBC after RNU. Sixteen (1.7%) patients were diagnosed with MIBC at initial IVR, 30 (3.1%) patients progressed to subsequent MIBC after repeated IVR, and 346 (35.8%) patients had NMIBC during the follow-up period. The details of IVR tumor specimens are listed in Table S1. Based on the pathological findings of the first TURBT specimens, 250 (63.8%) were diagnosed with pTa, 126 (32.1%) were diagnosed with pT1, and 16 (4.1%) were diagnosed with pT2. In total, 146 (37.2%) exhibited high-grade disease and 30 (7.7%) had CIS involvement. Intravesical bacillus Calmette and Guérin (BCG) or chemoagents were selected for 254 (64.8%) patients, whereas 138 (35.2%) received neither.

Cox regression analysis was conducted to investigate the clinical and pathological indicators associated with secondary MIBC progression after IVR development in patients with UTUC (Table 3). According to the multivariate analysis, a previous history of bladder cancer (HR 3.03, $P = .002$), pT1 stage of the IVR tumor (HR 2.02, $P = .029$), concomitant CIS of the bladder (HR 2.65, $P = .003$), and the absence of intravesical therapy (HR 2.56, $P = .037$) were independent risk factors for secondary MIBC progression.

3.4 | Oncological analysis of RFS and CSS in patients with UTUC who developed MIBC

To clarify the further oncological outcomes of patients with UTUC who developed MIBC, we compared factors for RFS and CSS between patients with primary MIBC (n = 318) and those who developed MIBC (n = 46) after RNU. After PS adjustments, 138 (75.0%)

TABLE 3 Univariate and multivariate analyses for assessing secondary MIBC progression in patients with UTUC

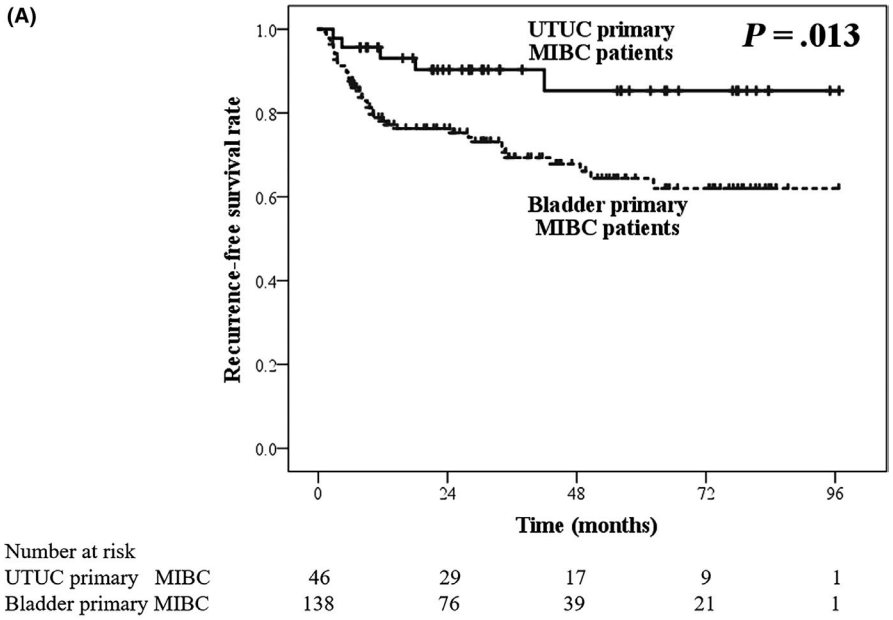
	Tumor progression to secondary MIBC					
	Univariate			Multivariate		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Clinical indicators and tumor characteristics of UTUC						
Age (≥75 vs. <75)	2.38	0.89-6.33	.082			
Sex (male vs. female)	1.00	0.45-2.20	.998			
ECOG-PS (2 vs. 0-1)	0.66	0.23-1.89	.435			
Surgical procedure (laparoscopy vs. open)	1.78	0.63-5.01	.277			
Tumor location (ureter vs. renal pelvis)	2.36	1.12-4.95	.023	1.88	0.63-3.01	.159
UTUC tumor histology (UC vs. non-UC)	2.43	0.78-7.59	.126			
UTUC pathological T stage (T2 ≤ vs. T3 ≥)	2.99	1.28-6.99	.012	1.99	0.87-3.99	.119
UTUC pathological N stage (N0 vs. N1,2)	3.95	0.47-13.3	.207			
UTUC tumor grade (high vs. low)	1.59	0.70-3.61	.268			
UTUC concomitant CIS (yes vs. no)	1.69	0.46-6.17	.437			
Tumor multiplicity (yes vs. no)	2.19	0.77-6.21	.203			
LVI (positive vs. negative)	0.58	0.22-1.43	.223			
Previous history of bladder tumor (yes vs. no)	4.48	1.59-12.6	.005	3.03	1.49-6.13	.002
Systemic adjuvant chemotherapy (yes vs. no)	1.54	0.74-3.22	.253			
Tumor characteristics of initial IVR						
IVR pathological T stage (T1 vs. Ta)	2.23	1.08-4.92	.032	2.02	1.08-3.81	.029
IVR tumor grade (high vs. low)	1.32	0.59-2.96	.495			
Concomitant CIS of bladder (yes vs. no)	3.58	1.15-11.1	.028	2.65	1.40-5.01	.003
Intravesical therapy (none vs. chemoagents/BCG)	2.17	1.01-6.25	.049	2.56	1.05-6.25	.037

Abbreviations: MIBC, muscle-invasive bladder cancer; UTUC, upper tract urothelial carcinoma; HR, hazard ratio; CI, confidence interval; ECOG-PS, Eastern Cooperative Oncology Group-Performance Status; UC, urothelial carcinoma; CIS, carcinoma in situ; LVI, lymphovascular invasion; IVR, intravesical recurrence; BCG, bacillus Calmette and Guérin.

TABLE 4 Univariate and multivariate analyses evaluating the prognostic factors associated with oncological outcomes in patients with MIBC after propensity score matching adjustment (n = 184)

	Disease recurrence						Cancer-specific death					
	Univariate			Multivariate			Univariate			Multivariate		
Clinical indicators	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Age (≥70 vs. <70)	1.25	0.70-2.26	.452				1.78	0.92-3.42	.085			
Sex (male vs. female)	1.41	0.56-3.57	.469				1.20	0.47-3.07	.704			
ECOG-PS (2 vs. 0-1)	1.14	0.69-1.90	.607				1.04	0.54-1.64	.821			
UTUC tumor histology (UC vs. non-UC)	1.98	1.00-3.89	.050				1.69	0.80-3.54	.168			
Systemic neoadjuvant chemotherapy (yes vs. no)	0.91	0.54-1.95	.934				0.97	0.49-1.95	.939			
Pathological T stage (≥3 vs. <3)	6.54	3.23-13.2	<.001	4.96	2.04-12.0	<.001	4.29	2.17-8.45	<.001	2.42	0.99-5.90	.052
Pathological N stage (1, 2 vs. 0)	2.34	1.19-4.63	.014	2.40	0.77-2.58	.170	3.17	1.61-6.24	.001	2.17	1.11-4.24	.024
Tumor grade (high vs. low)	1.11	0.52-2.37	.796				1.10	0.49-2.48	.824			
Concomitant CIS (yes vs. no)	1.20	0.54-2.69	.656				1.68	0.66-4.31	.277			
LVI (positive vs. negative)	4.69	2.55-8.65	<.001	2.90	0.88-4.10	.100	4.82	2.51-9.28	<.001	3.12	1.33-7.34	.009
Systemic adjuvant chemotherapy (yes vs. no)	2.12	1.15-3.94	.017	1.80	0.84-3.86	.129	1.76	0.89-3.46	.102			
Primary origin of UTUC (yes vs. no)	0.33	0.13-0.83	.018	0.23	0.09-0.60	.003	0.38	0.15-0.98	.045	0.26	0.10-0.68	.006

Abbreviations: MIBC, muscle-invasive bladder cancer; HR, hazard ratio; CI, confidence interval; ECOG-PS, Eastern Cooperative Oncology Group-Performance Status; UTUC, upper tract urothelial carcinoma; UC, urothelial carcinoma; CIS, carcinoma in situ; LVI, lymphovascular invasion.



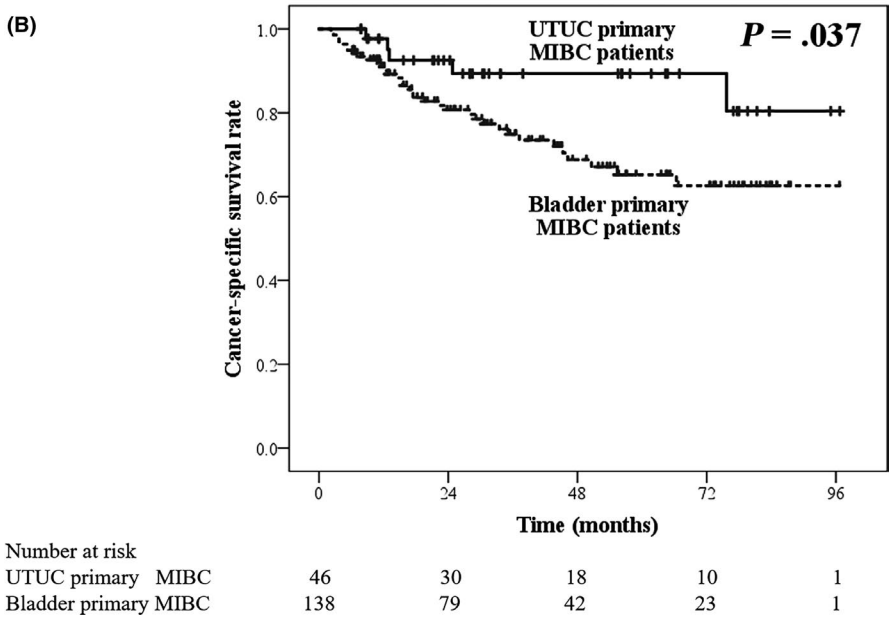
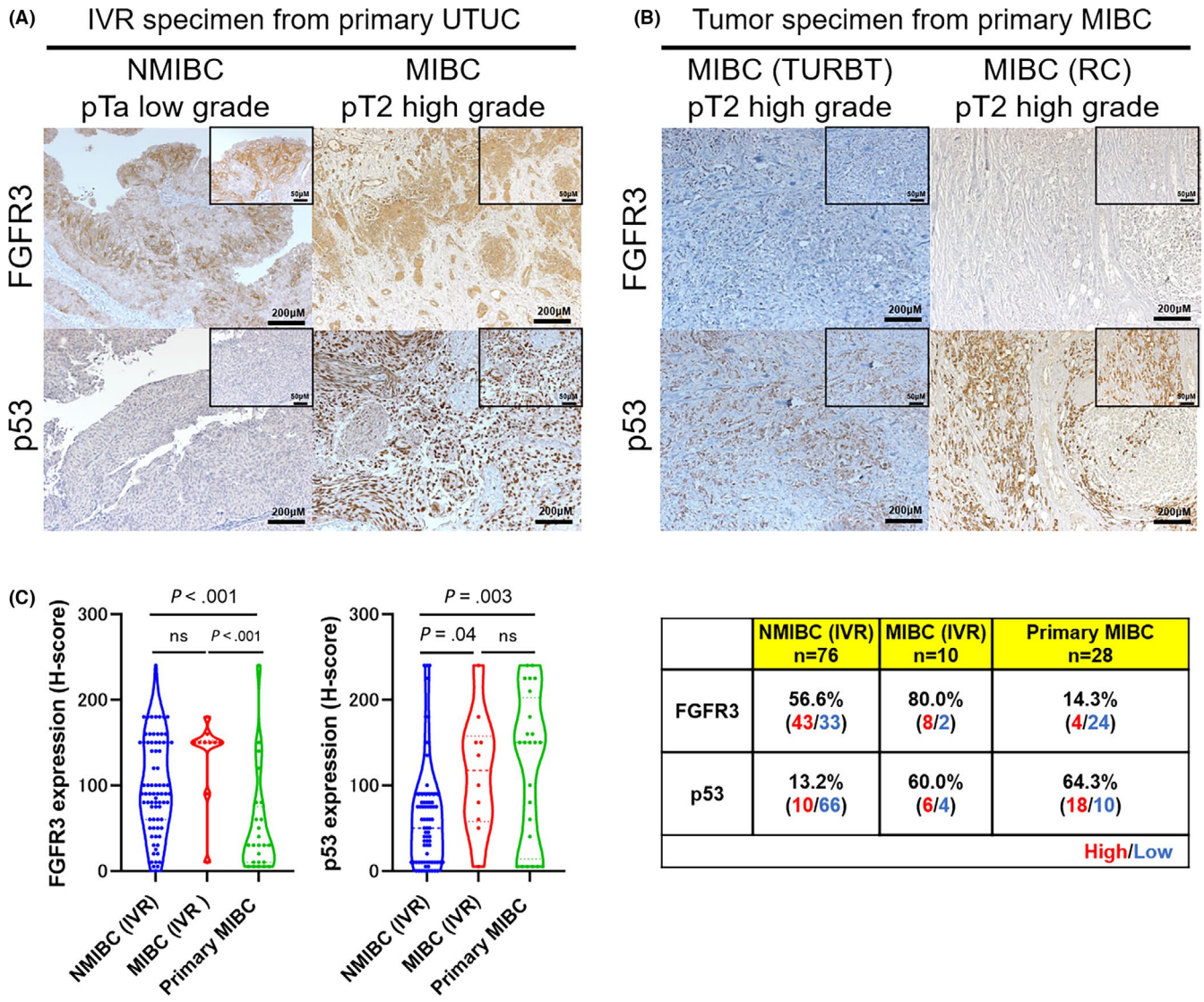


FIGURE 2 Kaplan-Meier curve analysis between patients who developed MIBC subsequent to UTUC and those with primary MIBC. The 3-y RFS rate (A) was 90.3% for MIBC subsequent to UTUC, which was significantly higher than that for primary MIBC (69.8%, $P = .013$). Moreover, the 3-y CSS rate (B) was 89.3% for MIBC subsequent to UTUC, which was significantly higher than that for primary MIBC (74.8%, $P = .037$)

patients with primary MIBC were matched with 46 (25.0%) patients with UTUC who developed subsequent MIBC. Significant differences were observed among age, clinical T stage, and number of TURBT procedures between the 2 groups before adjustment; however, no significant differences were noted after PS matching (Table S2). Among 184 patients, the median age was 70 (44-87) y old and the median follow-up period was 30 (3-153) mo. Overall, 46 (25.0%) exhibited disease recurrence and 40 (21.7%) died due to urothelial carcinoma. As shown in Table 4, multivariate analysis revealed pT stage ≥ 3 (HR 4.96, $P < .001$) to be an independent risk factor for disease recurrence. Regarding CSS, pathological N stage ≥ 1 (HR 2.17, $P = .024$) and LVI-positive status (HR 3.12, $P = .009$) were independent prognostic factors. Conversely, patients who developed MIBC after primary UTUC had favorable oncological outcomes (HR 0.23, $P = .003$ for disease recurrence, and HR 0.26, $P = .006$ for cancer-specific death, respectively). Indeed, based on the Kaplan-Meier curve, the 3-y RFS rate was 90.3% for MIBC subsequent to UTUC, which was significantly higher than that for primary MIBC (69.8%, $P = .013$) (Figure 2A). Moreover, the 3-y CSS rate was 89.3% for MIBC subsequent to UTUC, which was significantly higher than that for primary MIBC (74.8%, $P = .037$) (Figure 2B).

3.5 | Molecular analysis of NMIBC and MIBC subsequent to UTUC compared with primary MIBC

We further conducted immunohistochemical examinations to confirm FGFR3 and p53 expression in NMIBC and MIBC tumor specimens at the time of IVR. We identified 86 IVR tumor



	NMIBC (IVR) n=76	MIBC (IVR) n=10	Primary MIBC n=28
FGFR3	56.6% (43/33)	80.0% (8/2)	14.3% (4/24)
p53	13.2% (10/66)	60.0% (6/4)	64.3% (18/10)
			High/Low

FIGURE 3 Immunostaining and molecular analysis of p53 and FGFR3 expression in MIBC subsequent to UTUC and primary MIBC. Representative immunostaining of p53 and FGFR3 in surgical specimens from (A) NMIBC and MIBC tumor specimens from primary UTUC, and (B) TURBT and RC specimens from primary MIBC. Low-power field scale bar = 200 μm and high-power field scale bar = 50 μm. C, Violin plots are shown on the left to compare the amount and distribution of the histoscore (H-score). The H-score was calculated by applying the following formula: mean percentage × intensity (range, 0-300). The numbers of specimens with low and high expression of p53 and FGFR3 are shown on the right. Receiver operating curve analysis was applied to determine the cut-off values of FGFR3 and p53. H-scores of 90 for FGFR3 and 120 for p53 were set as the cut-off values to divide low and high expression

specimens (including 76 NMIBC and 10 MIBC) and 28 paired tissues from patients with primary MIBC after TURBT and RC. The patient background of the IHC subgroup represented the overall study cohort (Tables S3-S5). The representative FGFR3 and p53 staining patterns of tumor samples are shown in Figure 3A,B. We compared the H-scores of FGFR3 and p53 expression among the 3 groups: NMIBC subsequent to UTUC, MIBC subsequent to UTUC, and primary MIBC specimens. As a result, the mean H-scores of FGFR3 staining were 98.2 ± 55.2 in NMIBC (IVR) specimens, 134.0 ± 49.1 in MIBC (IVR) specimens subsequent to UTUC, and 51.9 ± 11.9 in primary MIBC specimens. The H-score was significantly lower in primary MIBC specimens than in NMIBC (IVR) or MIBC (IVR) subsequent to UTUC specimens ($P < .001$ and $P < .001$). In contrast, regarding p53 expression, the mean H-scores were 44.8 ± 16.3 in NMIBC (IVR) specimens, 115.0 ± 69.5 in MIBC (IVR) specimens, and 121.9 ± 86.6 in primary MIBC specimens. Thus, higher p53 expression was observed in MIBC specimens (both MIBC subsequent to UTUC and primary MIBC) than in NMIBC (IVR) specimens ($P = .03$ and $P = .004$, respectively).

Lastly, we performed Kaplan-Meier analysis of oncological outcome data of 38 patients with primary MIBC who underwent IHC analysis of p53 and FGFR3 expression. As shown in Figure S1, no significant differences were found between high and low FGFR3 expression regarding RFS ($P = .508$), but patients with high FGFR3 expression had a more favorable CSS than their

counterparts ($P = .130$). Conversely, regarding p53 expression, patients with high p53 expression had a slightly lower RFS and CSS than their counterparts ($P = .170$ and $P = .034$, respectively) (Figure S2).

4 | DISCUSSION

Regarding the high incidence of IVR development after RNU, a recent meta-analysis of available data identified numerous predictors of bladder recurrence after RNU.[4,15] However, few studies have addressed the clinical characteristics of patients with UTUC after bladder recurrence and there is insufficient information concerning MIBC progression after developing IVR. Abe and colleagues investigated the clinical outcomes of patients who developed bladder recurrence among 74 patients with UTUC who developed IVR, and 20% had bladder progression during their follow-up period.[16] They also revealed the observation of CIS in the first TUR specimens to be an independent risk factor for bladder progression, but the major concern with regard to this study was the relatively small population to lead to a definitive conclusion. Furthermore, we previously reported the natural history of IVR recurrence in 241 patients with UTUC, but the risk factors for MIBC development or further oncological outcomes were not examined.[17]

To investigate the clinical indicators in patients with UTUC who subsequently develop MIBC, our study first described the risk factors for developing IVR. Pathological T2 or lower UTUC, ureteral cancer, and tumor multifocality were found to be independent risk factors, consistent with previous reports. These indicators are also well known factors that support the intraluminal seeding hypothesis, which represents the predominant mechanism of IVR after RNU.[15,18] In the present study, patients with a lower pathological stage were more likely to develop IVR, probably because patients with UTUC at an advanced stage often have a poor prognosis before the detection of subsequent IVR.[19] Conversely, patients with lower-stage UTUC were more likely to develop subsequent IVR after RNU, probably due to longer survival.

To further clarify the biological foundation of MIBC development in patients with UTUC, we conducted a subgroup analysis for patients who developed primary IVR with NMIBC. According to multivariate analysis, the clinicopathological characteristics of primary UTUC were not significantly associated with subsequent MIBC progression. Instead, the pathological tumor characteristics of the first IVR bladder cancer were dominant for determining further bladder tumor progression. The current result appears to suggest that the development of invasive bladder cancer cells is associated with the tumor characteristics of bladder cancer after IVR, not from primary UTUC. We further conducted molecular analysis using IVR specimens to clarify whether the tumor characteristics of MIBC are of UTUC origin or from subsequent IVR. As a result, FGFR3 was highly expressed in both NMIBC and MIBC specimens, but p53 expression was higher at the MIBC stage than at the NMIBC stage. As the pathogenesis of IVR after RNU is based upon 2 theories, which are intraluminal seeding and field cancerization,[20-22] the present study suggests that the aggressive tumor subclones that progress to MIBC result from the accumulation of gene mutations during repeated IVR but not directly from the seeded tumor cells from the upper urinary tract. In this regard, we can assume that intraluminal seeding and pan-urothelial field defect mechanisms are both involved and overlap in bladder recurrence in patients with UTUC. However, as it is highly challenging to identify the biological origin of bladder tumors at the time of TUR in the clinical setting, guidelines similar to those for risk classification of primary bladder urothelial carcinoma should be followed for patients with UTUC who develop IVR to prevent MIBC progression.[23] As additional intravesical therapy is considered to be effective for preventing secondary bladder tumor recurrence, the present study suggested that not only a single post-operative dose of intravesical chemotherapy, but also specific regimens of repeated chemotherapy or BCG intravesical therapy during the follow-up period are useful after IVR.[24,25]

Of note, our present study also revealed the favorable survival outcomes of MIBC subsequent to UTUC compared with those of primary MIBC. Although many preoperative factors before RNU impact further oncological outcomes in patients with UTUC,[26] data regarding the prognosis of patients with MIBC with a previous history of UTUC are limited. To the best of our knowledge, no study has compared oncological outcomes between primary MIBC and MIBC subsequent to UTUC. Although our study has a limitation due to its retrospective nature, we have several explanations for the observed favorable outcome. First, patients with UTUC who underwent RNU are under strict management, including routine cystoscopy and radiological examination every 3-6 mo to detect early disease recurrence. Thus, detailed follow-up regimens using multiple imaging devices may enable us to detect MIBC in an earlier phase in the evolution of invasive bladder cancer compared with primary MIBC. Second, the total number of perioperative systemic chemotherapy cycles patients received may have positively impacted their prognosis. In our present study, 34 of 46 (73.9%) patients with MIBC subsequent to UTUC received perioperative systemic chemotherapy during RNU or RC. Conversely, 60 of 138 (43.5%) patients with primary MIBC received perioperative chemotherapy, including neo- and adjuvant chemotherapy. Therefore, the mean number of treatment cycles per patient receiving any type of systemic chemotherapy was 4.0 ± 1.4 (2-8) for patients with MIBC subsequent to UTUC, which was significantly greater than the 2.3 ± 1.1 (1-6) cycles for patients with primary MIBC ($P < .001$). Third, clinicopathologically advanced UTUC generally results in a poor prognosis because local or distant metastasis is likely to develop instead of bladder tumor recurrence. Therefore, the UTUC in patients who reached the MIBC stage after RNU may have had a lower malignant potential than primary MIBC, which may have resulted in favorable outcomes. Last, the differences in molecular biological background between bladder UC and UTUC may also affect the treatment response and/or further prognosis.[27] As previous studies have indicated, altered expression of TP53 and RB1 is more frequent in bladder MIBC, which plays an important role in urothelial carcinoma proliferation

or poor prognosis.[28] In contrast, FGFR3 mutations, which are associated with favorable outcomes in patients with urothelial carcinoma, are reported to be more frequent in UTUC and are related to longer survival.[29] Although our molecular analysis was performed using a limited number of tumor specimens, it supports the results of previous studies, suggesting that FGFR3 expression is dominant in IVR tumors in NMIBC and maintained in MIBC of UTUC origin. In contrast, high p53 but low FGFR3 expression was observed in both TURBT and RC specimens from patients with primary MIBC. Thus, the different genetic basis demonstrated by this molecular study may explain the favorable oncological outcomes of patients who developed MIBC subsequent to primary UTUC. However, further investigations are warranted to clarify this issue.

We acknowledge several limitations in the present study. The study population was relatively small and was examined retrospectively. It is premature to conclude that MIBC subsequent to UTUC has a lower malignant potential than primary MIBC because patients with UTUC who have aggressive tumor features may not survive until the MIBC stage after RNU because of their poor prognosis, which may cause selection bias. We did not include information related to other prognostic factors such as smoking history or the number of comorbidities. Different surgeons, and different surgical techniques and experiences may have led to a certain bias. BCG instillation and second-look TUR were not practiced for some patients after IVR. However, we clarified the overall clinical characteristics and oncological outcomes of UTUC-derived patients with MIBC from RNU to MIBC treatment. Considering the relatively rare nature of UTUC, our study may provide useful information considering the mechanism of bladder tumor progression in patients with UTUC.

In conclusion, our study demonstrated that patients with UTUC who develop MIBC recurrence after RNU take on the clinical characteristics of subsequent IVR more than those of primary UTUC. Although MIBC is considered to be an advanced disease, MIBC subsequent to UTUC may have more favorable outcomes than primary MIBC by comprising a different molecular biological background compared with primary MIBC.

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CONFLICT OF INTEREST

All authors state that there are no conflict of interests in the subject matter or materials discussed in the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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