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Expression status of GATA3 and mismatch repair proteins in upper tract urothelial carcinoma

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Abstract **GATA binding protein 3 (GATA3) and mismatch repair (MMR) deficiency contribute to the development of urothelial carcinoma. However, the combined expression of GATA3 and microsatellite instability (MSI) in upper tract urothelial carcinoma (UTUC) and its prognostic value have not been investigated. Here, we immunohistochemically stained GATA3 and MMR proteins in 108 UTUC samples. GATA3 was positive in 74 cases, and its expression was significantly lower than in adjacent benign urothelium ($P < 0.001$). Loss of GATA3 expression was statistically associated with adverse clinicopathologic parameters, such as advanced stage, lymphovascular invasion, neural invasion, lymph node metastasis, and extensive necrosis. Cancer-specific survival (CSS, $P = 0.028$) and disease-free survival (DFS, $P = 0.024$) were significantly shorter in patients with GATA3 negative tumors than in patients with GATA3 positive tumors. The absence of MMR proteins was observed in 8.3% of the cases, and focal staining was identified in 13.0%. When using "lax criteria" which resulted in counting cases as negative where MMR staining was in fact focally positive (< 5%), we found that GATA3 was inversely associated with MSI ($P = 0.005$). Moreover, GATA3$^-$/microsatellite stability (MS) tumors were correlated with advanced pT stage ($P < 0.001$) and poor outcome ($P = 0.019$ for CSS, $P = 0.016$ for DFS) compared with GATA3$^+$/MSI ones. The GATA3$^-$/MSI cases had unfavorable clinical outcomes compared with GATA3$^+$/MSI cases ($P = 0.008$ for CSS, $P = 0.023$ for DFS). This finding raises a question as to whether GATA3 interacts with MSI through the TGF-β signaling pathway and regulates UTUC progression.**

Keywords upper tract urothelial carcinoma; GATA binding protein 3; mismatch repair; microsatellite instability; prognosis

Introduction

Upper tract urothelial carcinoma (UTUC), derived from the urothelium of renal pelvis and ureter, is relatively uncommon and constitutes only 5%–10% of all cases of urothelial carcinomas in western countries [1]. By contrast, the prevalence of UTUC is as high as 20%–30% in some regions of China [2]. Compared with patients in western countries, patients in China show earlier age of onset, higher tumor grade, more advanced stage, higher rate of lymph node metastasis, and poorer survival [3,4]. UTUC is clinically much more aggressive than bladder cancer and has a worse prognosis. Thus, the identification of novel therapeutic targets for the management of UTUC is warranted.

GATA3 is a member of the GATA family of zinc finger transcription factors and was originally identified as a T-cell lineage-specific factor [5]. Subsequently, GATA3 has been implicated in the development and progression of breast cancer [6,7]. Since first reported in 2007 [8], GATA3 has been used as one of the most useful urothelial markers in diagnostic practice, and loss of GATA3 was found to promote bladder cancer cell migration and invasion [9]. A recent study demonstrated that GATA3 inhibits the metastasis of breast cancer by terminating the TGF-β signaling pathway [10]. However, the prognostic values of GATA3 expression in Chinese patients with UTUC and the regulatory mechanisms involving GATA3-interacting proteins remain unclear.

Microsatellite instability (MSI) is widely recognized as an important molecular event in the pathogenesis of

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colorectal cancer and useful in identifying the subgroups of colorectal cancer patients with differing survival rate. One study found that TGF-β signaling was ablated secondary to the high frequency of an inactivating mutation of the TGF-β receptor in MSI-H colorectal cancer [11]. Although previous studies [12–14] have shown that MMR deficiency contributes to the development of urothelial carcinomas, MSI status in Chinese patients with UTUC remains controversial. Therefore, the aim of this study is to investigate GATA3 expression and MSI status, as well as the prognostic value of the expression of GATA3 and MMR proteins in combination in Chinese patients with UTUC.

Study subjects

The medical records of 108 UTUC patients who underwent radical nephroureterectomy between January 2007 and March 2017 at Peking University Shougang Hospital and Peking University Third Hospital were retrieved. The cases were reviewed, and pathologic diagnoses were confirmed independently by two GU pathologists (Yue Wang and Huiying He). Data were collected during follow-ups until June 2017. A total of 14 patients were lost to follow-up after surgery, leaving 94 cases for final survival analysis.

Research design and methods

Tissue microarrays (TMA) were constructed from 108 urothelial tumor samples and 24 adjacent benign urothelium samples. A previous study suggested that $\geqslant 3$ cores from each sample gave an acceptable statistical analysis in TMAs in diverse tumor types [15]. Thus, three cores were punched from the marked area on the donor block and transferred to premade recipient paraffin block. Each core was 1 mm in diameter, and the cores were spaced 0.8 mm apart on a single glass slide.

Immunohistochemical staining (IHC) was performed on the TMA sections. The primary antibodies were mouse monoclonal antibody GATA3 (clone L50-823; 1:200), rabbit monoclonal antibody MSH2 (clone RED2; 1:200), rabbit monoclonal antibody MSH6 (clone EP49; 1:200), mouse monoclonal antibody MLH1 (clone ES05; 1:100), and rabbit monoclonal antibody PMS2 (clone EP51; 1:40). The antibodies were all obtained from Origene, Rockville, USA. Human colorectal cancer served as the positive control for MMR proteins, and tris-buffered saline omitting primary antibodies was used as the negative control.

Immunostaining results were evaluated independently by two pathologists (Yue Wang and Huiying He). Discrepancies in analysis were reconciled by a third reviewer (Jinxia Zhang). German Immunoreactive Score criteria (range 0–12) were used for the evaluation of GATA3. The percentage of immunoreactive cells (0%, 0; 1% – 10%, 1; 11% – 50%, 2; 51% – 80%, 3; 81% – 100%, 4) was multiplied by staining intensity (0, negative; 1, weak; 2, moderate; 3, strong) [16]. Cases with weighted scores of 2–12 were defined as positive. The immunohistochemical expression of four MMR proteins were scored as negative if the tumor showed complete absence of nuclear staining within tumor cells (stringent criteria). Mangold *et al*. [17] and Joost *et al*. [18] suggested that focal staining defined as nuclear staining in less than 5% of tumor cells indicates the presence of MSI-H (lax criteria). Thus, both of these cutoffs were independently used in this study.

SPSS software version 16.0 (SPSS, Chicago, IL) was used for data processing and statistical analysis. The relationships between IHC staining results and clinicopathologic parameters were analyzed with Pearson's chi-square test and Fisher's exact test. Cancer-specific survival (CSS) and disease-free survival (DFS) analysis were performed using the Kaplan–Meier method and Log-rank test. Multivariate Cox regression analysis was performed for multivariate predictive modeling. Qualifying criteria for inclusion in the Cox regression analysis were P value of $\leqslant 0.1$ or risk ratio $\leqslant 0.5$ or > 2 in univariate Cox analysis. Tumor necrosis was defined as necrosis greater than 10% of tumor area based on microscopic evaluation in UTUC [19]. CSS was defined as the interval between surgery and death from UTUC. Death was scored as an event, and patients who died from other causes or were still alive were censored at the time of the last follow-up. DFS was calculated from the date of surgery to the date of the first documented evidence of recurrent disease or last follow-up visit alive. For all statistical tests, a P value of < 0.05 represented a significant difference.

Clinical data

The clinicopathologic characteristics of the patients are summarized in Table 1. The median age at diagnosis was 70 years (range of 41–86 years). Of the 108 UTUC cases, 14 were low-grade and 94 were high-grade tumors, and the stage distribution was as follows: pTa, 14 cases (13.0%); pT1, 29 cases (26.8%); pT2, 27 cases (25.0%); pT3, 29 cases (26.9%); and pT4, 9 cases (8.3%). Median follow-up time was 28 months (range of 1–101 months). During the observation period, complete follow-up information was obtained from 94 patients, and 28 (29.8%) patients died of tumor progression.

Clinicopathologic significance of GATA3 expression and MSI in UTUC

Immunohistochemical staining for GATA3 and four MMR

Table 1 Patient characteristics at diagnosis

Variable	*n*	%	Variable	*n*	%
Patient age			Lymph node metastasis		
<65	40	37.0	No	98	90.7
⩾65	68	63.0	Yes	10	9.3
Sex			Concurrent CIS		
Male	62	57.4	No	93	86.1
Female	46	42.6	Yes	15	13.9
Laterality			Extensive necrosis		
Left	49	45.4	No	91	84.3
Right	58	53.7	Yes	17	15.7
Both	1	0.9	Glomerular sclerosis		
Tumor site			No	73	67.6
Renal pelvis	42	38.9	Yes	35	32.4
Ureter	55	50.9	Solitary or multifocal		
Transitional zone	10	9.3	Solitary	93	86.1
Renal pelvis & ureter	1	0.9	Multifocal	15	13.9
Tumor size			Bladder cancer		
<3.0 cm	60	55.6	No	71	75.6
⩾3.0 cm	48	44.4	Simultaneous	5	5.3
Tumor grade			Postoperative	16	17.0
Low grade	14	13.0	Preoperative	2	2.1
High grade	94	87.0	Median follow-up time (month)	28	
Pathological stage			Status		
pTa	14	13.0	Survival	66	70.2
pT1	29	26.8	Death	28	29.8
NMI (pTa + pT1)	43	39.8	Cancer-specific survival		
pT2	27	25.0	<1 year	19	
pT3	29	26.9	1−3 years	41	69.4% [a]
pT4	9	8.3	>3 years	34	
MI (pT2 + pT3 + pT4)	65	60.2	Disease-free survival		
Lympho-vascular involvement			<1 year	36	
No	87	80.6	1−3 years	31	67.5% [b]
Yes	21	19.4	>3 years	27	
Neural invasion					
No	99	91.7			
Yes	9	8.3			

Abbreviations: CIS, carcinoma *in situ*; NMI, non-muscle invasive; MI, muscle invasive.
[a] The three-year cancer-specific survival rate of UTUC. [b] The three-year disease-free survival rate of UTUC.

proteins was carried out in 108 UTUC samples and 24 corresponding normal urothelial tissues. Nuclear staining was considered a positive signal (Fig. 1). GATA3 expression was observed in 74 (68.5%) of 108 UTUC and all 24 (100%) normal tissues. The levels of GATA3 expression were significantly lower in tumor specimens than in normal urothelium ($P < 0.001$). After the evaluation of MMR protein expression, 1 of 108 (0.9%) UTUC cases demonstrated the absence of MSH2 staining, 1 (0.9%) showed the absence of MSH6, 6 (5.6%) showed the absence of MLH1, and 7 (6.5%) showed the absence of PMS2. The total loss of MMR protein was observed in 9 (8.3%) cases, and focal staining was observed in 14 (13.0%) cases. An inverse relationship between GATA3 expression and MSI (strict criteria) was observed but was not statistically significant ($P = 0.136$). However, the relationship became significant when lax criteria were used and focal staining cases were included ($P = 0.005$, Table 2).

In all the patients with UTUC, loss of GATA3 expression was associated with tumor site ($P = 0.048$), pathological stage ($P = 0.001$), lymphovascular involvement ($P = 0.035$), neural invasion ($P = 0.004$), lymph node metastasis ($P = 0.010$), and extensive necrosis ($P < 0.001$). In cases with muscle invasion, loss of GATA3 expression was more frequently observed in

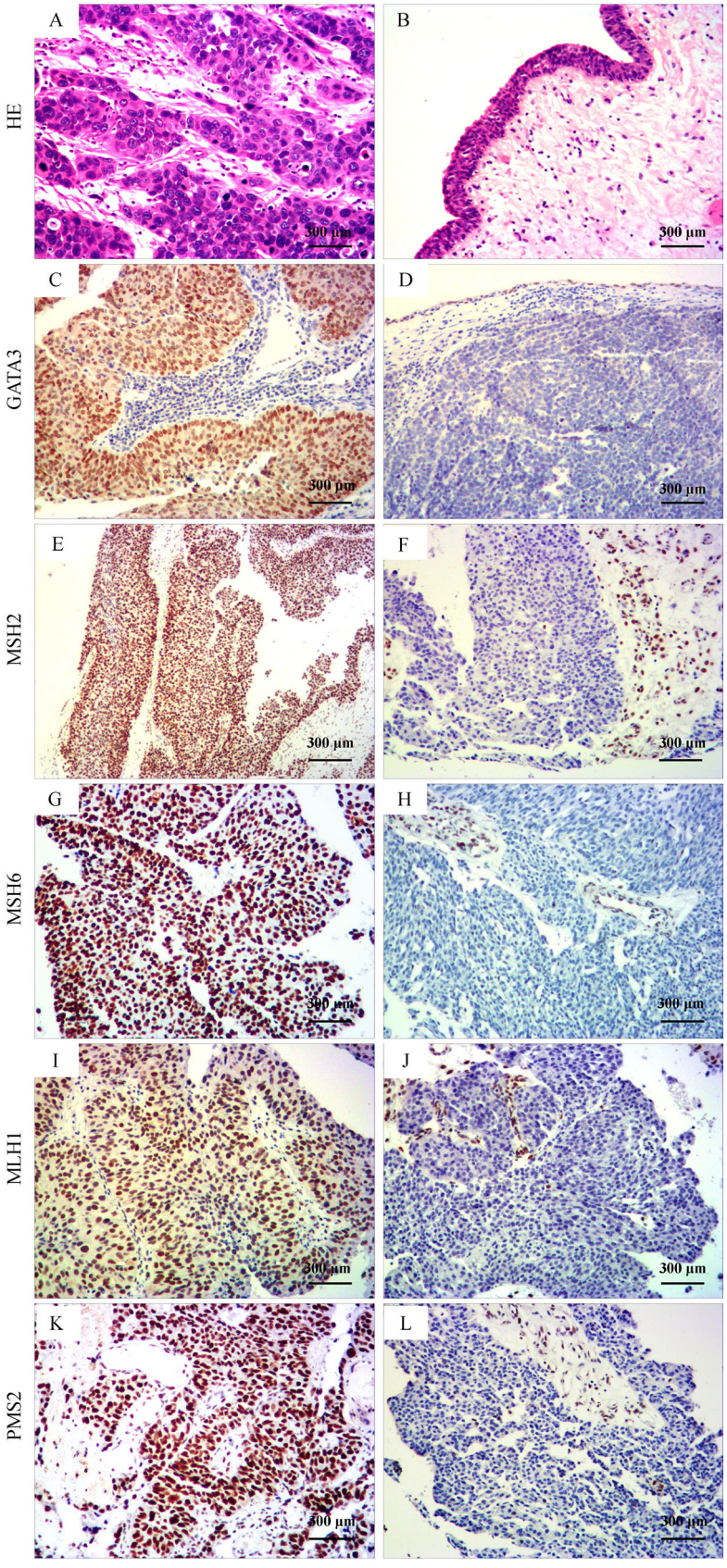

Fig. 1 Representative staining of GATA3 and MMR proteins in UTUC. (A) Representative H&E images of UTUC. (B) Representative H&E images of normal urothelium. (C) Representative examples of positive staining of GATA3 in UTUC. (D) Representative examples of negative staining of GATA3 in UTUC. (E – L) Representative examples of positive (left panel) and negative (right panel) staining of MSH2, MSH6, MLH1, PMS2 in UTUC, respectively. Magnification, 100×.

Table 2 Correlation of GATA3 and MMR proteins in UTUC

	n	MMR			*P*	
		−	focal +	+	− vs. focal +/+	−/focal + vs. +
GATA3$^-$	34	5	8	21	0.136	0.005*
GATA3$^+$	74	4	6	64		

Abbreviations: UTUC, upper tract urothelial carcinoma; MMR, mismatch repair.

cases that also showed neural invasion (P = 0.032) or extensive necrosis (P = 0.011). However, neither absence nor focal staining of MMR protein showed an association with clinicopathologic parameters in the two UTUC groups (Table 3).

In our study cohort, the loss of GATA3 expression demonstrated strong association with poor survival in the CSS assay (P = 0.028) and DFS assay (P = 0.024) (data not shown). However, neither focal staining nor absence of MMR proteins showed any significant association with patient outcome.

In multivariate Cox regression analysis, only GATA3 met the qualifying criteria for inclusion in predictive modeling. Although the absence of GATA3 expression was shown to be a negative indicator for survival status in this UTUC cohort overall, the association was not observed in the multivariate analysis. Moreover, neither GATA3 nor MSI was significantly correlated with survival status in patients with muscle-invasive UTUC. However, as expected, advanced pT stage and the presence of extensive necrosis were independent prognostic factors for poor outcome (Table 4).

Analysis of GATA3 and MSI in combination

The patients were divided into four groups according to the expression patterns of GATA3 and MMR proteins as follows: GATA3$^+$/MSI (n = 9), GATA3$^+$/MS (n = 57), GATA3$^-$/MSI (n = 11), and GATA3$^-$/MS (n = 17). However, only a near-significant trend of survival difference was found within the four groups (P = 0.056 for CSS, P = 0.050 for DFS, Fig. 2). Upon further comparison of every set of two groups, the GATA3$^-$/MS cohort was associated with advanced pT stage ($P < 0.001$) and poor outcome (P = 0.019 for CSS, P = 0.016 for DFS, Fig. 3) as compared with the GATA3$^+$/MSI cases. GATA3$^-$/MSI patients showed unfavorable clinical outcome (P = 0.008 for CSS, P = 0.023 for DFS, Fig. 4) in contrast to cases with GATA3$^+$/MSI. No significant difference in any parameter was found between the other groups. The GATA3$^-$/MS group showed poorer outcome than the GATA3$^+$/MSI cohort. Within the MSI group, cases without GATA3 expression showed unfavorable clinical outcome compared with cases with GATA3 expression.

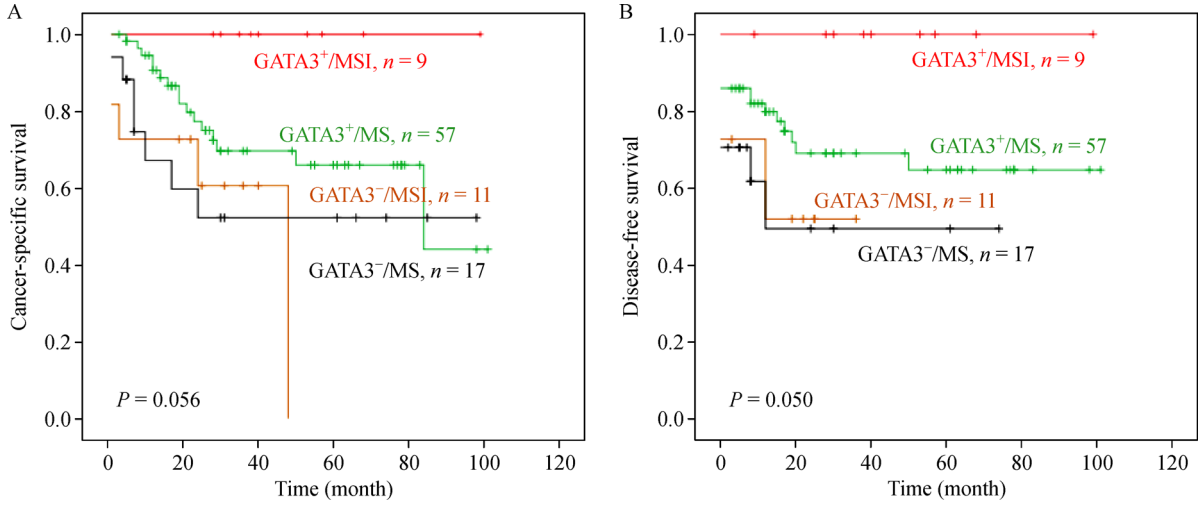

Fig. 2 Cancer-specific survival (A) and disease-free survival (B) according to different groups of GATA3 and MMR protein expression status.

Table 3 Association of clinicopathologic characteristics with GATA3 expression and MSI

	UTUC, total = 108					MI UTUC, total = 65				
	n	GATA3$^+$	*P*	MSI	*P*	*n*	GATA3$^+$	*P*	MSI	*P*
Patient age										
<65	40	29	0.528	2	0.480	23	12	0.608	1	1.000
⩾65	68	45		7		42	25		3	
Sex										
Male	62	43	0.837	4	0.491	37	20	0.622	2	1.000
Female	46	31		5		28	17		2	
Laterality										
Left	49	35	0.358	2	0.244	31	18	0.693	1	0.637
Right	58	39		7		33	19		3	
Both	1	0		0		1	0		0	
Tumor site										
Renal pelvis	42	28	0.048*	5	0.478	25	14	0.331	2	1.000
Ureter	55	42		3		31	20		2	
Transitional zone	10	4		1		8	3		0	
Renal pelvis & ureter	1	0		0		1	0		0	
Tumor size										
<3.0 cm	60	44	0.298	3	0.182	36	22	0.463	2	1.000
⩾3.0 cm	48	30		6		29	15		2	
Tumor grade										
Low grade	14	10	1.000	1	1.000	1	0	0.431	0	1.000
High grade	94	64		8		64	37		4	
Pathological stage										
NMI (pTa–pT1)	43	37	0.001*	5	0.479		–	–	–	–
MI (pT2–pT4)	65	37		4			–		–	
LVI										
No	87	64	0.035*	7	1.000	46	29	0.170	2	0.574
Yes	21	10		2		19	8		2	
Neural invasion										
No	99	72	0.004*	7	0.164	56	35	0.032*	2	0.089
Yes	9	2		2		9	2		2	
Lymph node metastasis										
No	98	71	0.010*	9	1.000	55	34	0.086	4	1.000
Yes	10	3		0		10	3		0	
Concurrent CIS										
No	93	62	0.381	7	0.609	55	30	0.495	2	0.109
Yes	15	12		2		10	7		2	
Extensive necrosis										
No	91	69	<0.001*	8	1.000	48	32	0.011*	3	1.000
Yes	17	5		1		17	5		1	
Glomerular sclerosis										
No	73	53	0.268	7	0.715	45	25	0.792	4	0.303
Yes	35	21		2		20	12		0	
Solitary or multifocal										
Solitary	93	65	0.550	7	0.609	52	30	1.000	2	0.176
Multifocal	15	9		2		13	7		2	
Bladder cancer[a]										
No	61	46	0.653	6	0.612	37	25	0.164	2	1.000
Simultaneous	5	4		0		4	3		0	
Postoperative	16	10		0		7	2		0	
Preoperative	2	2		0		2	2		0	

Abbreviations: MSI, microsatellite instability; UTUC, upper tract urothelial carcinoma; MI, muscle-invasive; CIS, carcinoma *in situ*; LVI, lympho-vascular involvement. [a] 84 cases with detailed bladder cancer recurrence information for total UTUC, and 50 cases for MI-UTUC. * $P < 0.05$.

Table 4 Univariate and multivariate analysis of cancer-specific survival and disease-free survival in patients with UTUC

	Cancer-specific survival						Disease-free survival					
	Univariate			Multivariate			Univariate			Multivariate		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
All cases (n = 108)												
GATA3	0.441	0.208–0.935	0.028*	0.684	0.308–1.517	0.350	0.448	0.210–0.955	0.024*	0.751	0.326–1.731	0.502
MSI	0.744	0.176–3.143	0.688	–	–	–	0.697	0.165–2.943	0.623	–	–	–
Tumor grade	25.684	0.244–2.699E3	0.031*	2.395E5	–	0.975	25.217	0.220–2.890E3	0.028*	3.748E5	–	0.980
pT stage	9.463	2.245–39.891	<0.001*	4.126	0.921–18.492	0.064	9.007	2.135–37.988	<0.001*	4.328	0.973–19.251	0.054
LVI	2.379	1.089–5.200	0.024*	1.307	0.585–2.919	0.514	2.345	1.082–5.086	0.019*	1.201	0.531–2.720	0.660
Neural invasion	1.994	0.687–5.787	0.194	–	–	–	1.785	0.617–5.165	0.252	–	–	–
LN metastasis	4.734	1.751–12.802	0.001*	1.680	0.554–5.094	0.359	3.793	1.422–10.123	0.002*	1.349	0.457–3.984	0.587
Concurrent CIS	0.883	0.305–2.554	0.817	–	–	–	0.882	0.306–2.545	0.806	–	–	–
Extensive necrosis	6.288	2.864–13.804	<0.001*	2.986	1.229–7.256	0.016*	5.410	2.424–12.074	<0.001*	2.682	1.068–6.733	0.036*
MI UTUC (n = 65)												
GATA3	0.607	0.280–1.317	0.206	–	–	–	0.622	0.284–1.362	0.235	–	–	–
MSI	1.181	0.278–5.022	0.822	–	–	–	1.026	0.242–4.346	0.972	–	–	–
Tumor grade	21.096	0.001–8.890E5	0.395	–	–	–	21.110	0.000–1.089E6	0.378	–	–	–
LVI	1.550	0.695–3.454	0.277	–	–	–	1.626	0.737–3.587	0.185	–	–	–
Neural invasion	1.178	0.402–3.447	0.764	–	–	–	1.075	0.369–3.137	0.885	–	–	–
LN metastasis	2.848	1.046–7.756	0.032*	1.498	0.489–4.590	0.479	0.418	0.155–1.123	0.047*	1.372	0.463–4.070	0.568
Concurrent CIS	0.971	0.333–2.831	0.957	–	–	–	0.939	0.323–2.727	0.900	–	–	–
Extensive necrosis	3.757	1.683–8.383	0.001*	3.310	1.355–8.087	0.009*	3.316	1.463–7.519	0.001*	2.990	1.212–7.377	0.017*

Abbreviations: UTUC, upper tract urothelial carcinoma; LVI, lympho-vascular involvement; MSI, microsatellite instability; MI, muscle-invasive; LN, lymph node; CIS, carcinoma *in situ*.
* $P < 0.05$.

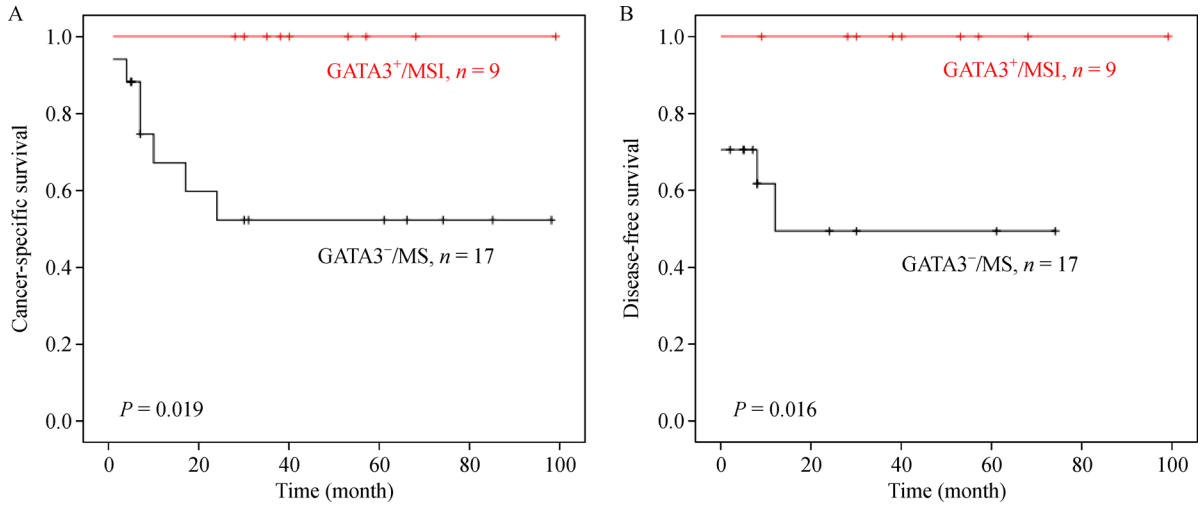

Fig. 3 Cancer-specific survival (A) and disease-free survival (B) comparing GATA3^{+}/MSI and GATA3^{-}/MS groups.

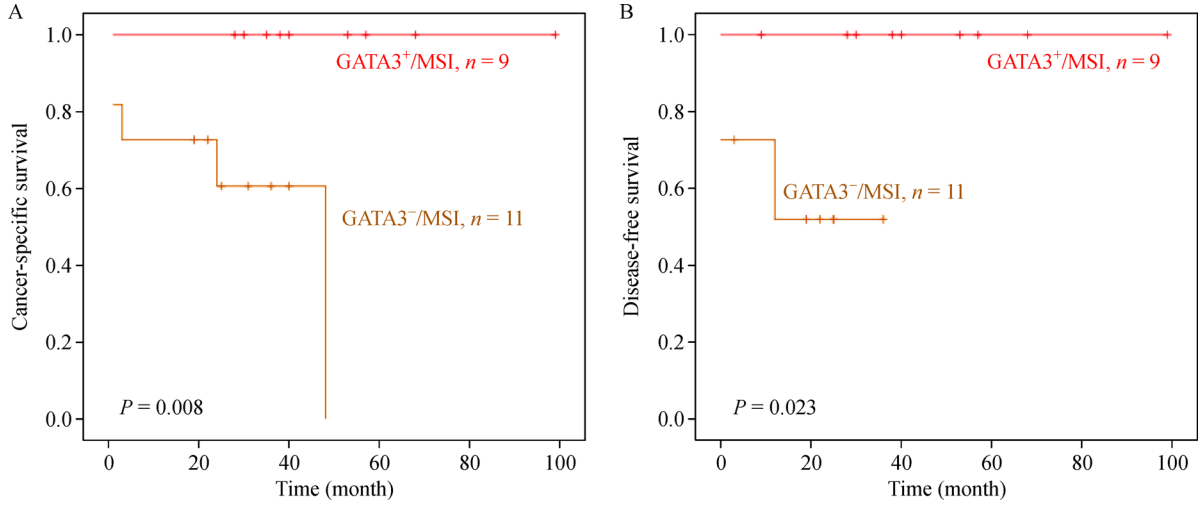

Fig. 4 Cancer-specific survival (A) and disease-free survival (B) analysis comparing GATA3^{+}/MSI and GATA3^{-}/MSI groups.

Discussion

GATA3 has been used widely as a marker for urothelial differentiation [20], and MMR proteins have been considered involved in the development of urothelial carcinoma [21]. However, to the best of our knowledge, these markers have not been evaluated together in a large number of UTUC cases in China, and whether there is a relationship between GATA3 expression and MSI status in UTUC remains unknown.

GATA3 is a well-established transcription factor marker for luminal muscle invasive bladder cancer and has been reported in several studies on bladder cancer molecular subtypes [22–24]. Similar to the findings on the cases of UTUC in western countries [16], our findings show that GATA3 expression is considerably downregulated relative to non-neoplastic urothelial tissues. GATA3 expression is considerably reduced when tumors with and without muscle-invasive UTUC are separately analyzed (data not shown).

In agreement with a previous study, which reported that GATA3 loss is related to muscle-invasive UTUC [16], our findings showed that the absence of GATA3 is associated with advanced stage UTUC. Meanwhile, no correlation was obtained among the UTUC cases. This finding is inconsistent with the findings of studies on bladder cancer [25], which demonstrated that loss of GATA3 expression is associated with high-grade tumor. This might explain the more aggressive behavior of UTUC relative to that of bladder cancer [26], thus resulting in a limited proportion

of cases of low-grade UTUC. Although numerous studies have demonstrated the prognostic role of GATA3 in urothelial carcinoma or in breast carcinoma [7,25,27], the prognostic value of GATA3 has not been explored in UTUC cases in China. Inoue *et al.* reported the loss of GATA3 expression is an independent predictor of poor outcome in Japanese patients with UTUC [16]. Accordingly, we found that the absence of GATA3 indicates poor CSS and poor DFS in the univariate log rank test, but GATA3 was not identified as an independent marker of adverse prognosis in multivariate Cox regression analysis.

Although some researchers have insisted that only stains that are completely negative for MMR proteins should be interpreted as MSI tumors, more recent studies have suggested that lax criteria should be used in practice. As mentioned above, Mangold *et al.* [17] and Joost *et al.* [18] showed that tumors with focal MMR staining (less than 5% of tumor cells positive) are in fact MSI-H cases upon the evaluation with PCR-gene scan assay. In view of these controversial results, stringent criteria and lax criteria were tested in our study. When the lax criteria were used, MMR-negative staining cases were presented in 21.3% UTUC patients. This finding is similar to the findings from western countries, indicating a high level of MSI (25%, 46%) in UTUC [12,28]. However, the correlation between MSI status and any clinicopathlogic parameter or prognosis in our UTUC cases was nonsignificant regardless of the criteria used. This result is inconsistent with the results of previous studies from other countries, which demonstrated that high MSI or inactivation of MMRs is associated with the presence of low-grade tumors and good prognosis in UTUC [28–31]. These might be explained by the fact that Chinese UTUC exhibits unique characteristics in pathogenesis and clinicopathologic features. Although stringent MSI evaluation has no significant association with GATA3 expression, we did find an inverse correlation between lax MSI evaluation and GATA3 expression. Subsequently, in the survival analysis, a near-significant trend between patient outcome and each of the four combined expression patterns of GATA3/MSI was observed only when lax MSI criteria were used. Thus, the use of lax criteria may be effective in the evaluation of MSI status in UTUC. Focal MMR protein staining cases, which may likely contain MSI-H, should be further experimentally assessed.

We evaluated the associations among the combined expression patterns of GATA3 and MSI status to assess their predictive value for survival. The prognostic values of GATA3 expression and MSI status have been separately documented in numerous studies, but the prognostic value in combination had not been explored. As expected, the $GATA3^{-}$/MS group was statistically associated with tumor progression and poor outcome as compared with the $GATA3^{+}$/MSI cohort. At the same time, the $GATA3^{-}$/MSI group was statistically correlated with tumor progression and poor outcome as compared with $GATA3^{+}$/MSI counterparts. This correlation suggests that GATA3 is a critical factor in the evaluation of MSI patient outcome in UTUC.

To date, the relationship between GATA3 and MSI and the mechanisms underlying the coordinate function of these molecules has not been described. A previous study found that GATA3 inhibited metastases of breast cancer through abolition of the TGF-β signaling pathway [10]. Interestingly, studies on MSI-H colorectal cancers demonstrated that TGF-β signaling is ablated secondary to the high frequency of an inactivating mutation of the TGF-β receptor. Thus, the process is involved in tumorigenesis and tumor progression [11,32,33]. Whether GATA3 interacts with MSI through the TGF-β signaling pathway to regulate UTUC progression requires further investigation.

Summary

In contrast to the absence of MMR proteins, loss of GATA3 expression was statistically associated with adverse clinicopathologic parameters and poor survival of Chinese patients with UTUC. The $GATA3^{-}$/MS group was correlated with advanced pT stage and poor outcome compared with $GATA3^{+}$/MSI cohort. This finding raises a question as to whether GATA3 interacts with MSI through the TGF-β signaling pathway and subsequently regulates UTUC progression.

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Compliance with ethics guidelines

Yue Wang, Jinxia Zhang, Yunfan Wang, Shufang Wang, Yu Zhang, Qi Miao, Fei Gao, and Huiying He declare no conflicts of interest. All the procedures were in accordance with the ethical standards of the responsible committee on human experimentation (Peking University Shougang Hospital Institutional Review Board) and the *Helsinki Declaration* of 1975, as revised in 2000.

References

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin 2017; 67(1): 7–30
2. Chen XP, Xiong GY, Li XS, Matin SF, Garcia M, Fang D, Wang TY, Yu W, Gong K, Song Y, He ZS, He Q, Zhou LQ. Predictive

factors for worse pathological outcomes of upper tract urothelial carcinoma: experience from a nationwide high-volume centre in China. BJU Int 2013; 112(7): 917–924

3. Singla N, Fang D, Su X, Bao Z, Cao Z, Jafri SM, Xiong G, Zhang L, Hutchinson R, Sagalowsky A, Lotan Y, Li X, Zhou L, Raman JD, Margulis V. A multi-institutional comparison of clinicopathologic characteristics and oncologic outcomes of upper tract urothelial carcinoma in China and the United States. J Urol 2017; 197(5): 1208–1213
4. Xiong G, Liu J, Tang Q, Fan Y, Fang D, Yang K, Xie F, Zhang M, Zhang L, Liu L, Zhang C, Yao L, Yang L, Ci W, Zhao W, Gong Y, He Q, Gong K, He Z, Wang G, Li X, Guo Y, Zhou L. Prognostic and predictive value of epigenetic biomarkers and clinical factors in upper tract urothelial carcinoma. Epigenomics 2015; 7(5): 733–744
5. Ho IC, Vorhees P, Marin N, Oakley BK, Tsai SF, Orkin SH, Leiden JM. Human GATA-3: a lineage-restricted transcription factor that regulates the expression of the T cell receptor α gene. EMBO J 1991; 10(5): 1187–1192
6. Asselin-Labat ML, Sutherland KD, Barker H, Thomas R, Shackleton M, Forrest NC, Hartley L, Robb L, Grosveld FG, van der Wees J, Lindeman GJ, Visvader JE. Gata-3 is an essential regulator of mammary-gland morphogenesis and luminal-cell differentiation. Nat Cell Biol 2007; 9(2): 201–209
7. Gonzalez RS, Wang J, Kraus T, Sullivan H, Adams AL, Cohen C. GATA-3 expression in male and female breast cancers: comparison of clinicopathologic parameters and prognostic relevance. Hum Pathol 2013; 44(6): 1065–1070
8. Higgins JP, Kaygusuz G, Wang L, Montgomery K, Mason V, Zhu SX, Marinelli RJ, Presti JC Jr, van de Rijn M, Brooks JD. Placental S100 (S100P) and GATA3: markers for transitional epithelium and urothelial carcinoma discovered by complementary DNA microarray. Am J Surg Pathol 2007; 31(5): 673–680
9. Li Y, Ishiguro H, Kawahara T, Kashiwagi E, Izumi K, Miyamoto H. Loss of GATA3 in bladder cancer promotes cell migration and invasion. Cancer Biol Ther 2014; 15(4): 428–435
10. Sun J, He H, Pillai S, Xiong Y, Challa S, Xu L, Chellappan S, Yang S. GATA3 transcription factor abrogates Smad4 transcription factor-mediated fascin overexpression, invadopodium formation, and breast cancer cell invasion. J Biol Chem 2013; 288(52): 36971–36982
11. de Miranda NF, van Dinther M, van den Akker BE, van Wezel T, ten Dijke P, Morreau H. Transforming growth factor β signaling in colorectal cancer cells with microsatellite instability despite biallelic mutations in TGFBR2. Gastroenterology 2015; 148(7): 1427–37.e8
12. Amira N, Rivet J, Soliman H, Cancel-Tassin G, Le Duc A, Janin A, Cussenot O. Microsatellite instability in urothelial carcinoma of the upper urinary tract. J Urol 2003; 170(4 Pt 1): 1151–1154
13. Skeldon SC, Semotiuk K, Aronson M, Holter S, Gallinger S, Pollett A, Kuk C, van Rhijn B, Bostrom P, Cohen Z, Fleshner NE, Jewett MA, Hanna S, Shariat SF, Van Der Kwast TH, Evans A, Catto J, Bapat B, Zlotta AR. Patients with Lynch syndrome mismatch repair gene mutations are at higher risk for not only upper tract urothelial cancer but also bladder cancer. Eur Urol 2013; 63(2): 379–385
14. Mylona E, Zarogiannos A, Nomikos A, Giannopoulou I, Nikolaou I, Zervas A, Nakopoulou L. Prognostic value of microsatellite instability determined by immunohistochemical staining of hMSH2 and hMSH6 in urothelial carcinoma of the bladder. APMIS 2008; 116(1): 59–65
15. Rubin MA, Dunn R, Strawderman M, Pienta KJ. Tissue microarray sampling strategy for prostate cancer biomarker analysis. Am J Surg Pathol 2002; 26(3): 312–319
16. Inoue S, Mizushima T, Fujita K, Meliti A, Ide H, Yamaguchi S, Fushimi H, Netto GJ, Nonomura N, Miyamoto H. GATA3 immunohistochemistry in urothelial carcinoma of the upper urinary tract as a urothelial marker and a prognosticator. Hum Pathol 2017; 64: 83–90
17. Mangold E, Pagenstecher C, Friedl W, Fischer HP, Merkelbach-Bruse S, Ohlendorf M, Friedrichs N, Aretz S, Buettner R, Propping P, Mathiak M. Tumours from MSH2 mutation carriers show loss of MSH2 expression but many tumours from MLH1 mutation carriers exhibit weak positive MLH1 staining. J Pathol 2005; 207(4): 385–395
18. Joost P, Veurink N, Holck S, Klarskov L, Bojesen A, Harbo M, Baldetorp B, Rambech E, Nilbert M. Heterogenous mismatch-repair status in colorectal cancer. Diagn Pathol 2014; 9(1): 126–135
19. Seitz C, Gupta A, Shariat SF, Matsumoto K, Kassouf W, Walton TJ, Fritsche HM, Otto W, Tritschler S, Bastian PJ, Carballido J, Ficarra V, Karakiewicz PI, Artibani W, Mazzoleni G, Novara G. Association of tumor necrosis with pathological features and clinical outcome in 754 patients undergoing radical nephroureterectomy for upper tract urothelial carcinoma: an international validation study. J Urol 2010; 184(5): 1895–1900
20. Paner GP, Annaiah C, Gulmann C, Rao P, Ro JY, Hansel DE, Shen SS, Lopez-Beltran A, Aron M, Luthringer DJ, De Peralta-Venturina M, Cho Y, Amin MB. Immunohistochemical evaluation of novel and traditional markers associated with urothelial differentiation in a spectrum of variants of urothelial carcinoma of the urinary bladder. Hum Pathol 2014; 45(7): 1473–1482
21. Bai S, Nunez AL, Wei S, Ziober A, Yao Y, Tomaszewski JE, Bing Z. Microsatellite instability and TARBP2 mutation study in upper urinary tract urothelial carcinoma. Am J Clin Pathol 2013; 139(6): 765–770
22. Inamura K. Bladder cancer: new insights into its molecular pathology. Cancers (Basel) 2018; 10(4): 100
23. Warrick JI, Walter V, Yamashita H, Chung E, Shuman L, Amponsa VO, Zheng Z, Chan W, Whitcomb TL, Yue F, Iyyanki T, Kawasawa YI, Kaag M, Guo W, Raman JD, Park JS, DeGraff DJ. FOXA1, GATA3 and PPARγ cooperate to drive luminal subtype in bladder cancer: a molecular analysis of established human cell lines. Sci Rep 2016; 6(1): 38531
24. Dadhania V, Zhang M, Zhang L, Bondaruk J, Majewski T, Siefker-Radtke A, Guo CC, Dinney C, Cogdell DE, Zhang S, Lee S, Lee JG, Weinstein JN, Baggerly K, McConkey D, Czerniak B. Meta-analysis of the luminal and basal subtypes of bladder cancer and the identification of signature immunohistochemical markers for clinical use. EBioMedicine 2016; 12: 105–117
25. Bahria-Sediki IB, Yousfi N, Paul C, Chebil M, Cherif M, Zermani R, El Gaaied AB, Bettaieb A. Clinical significance of T-bet, GATA-3, and Bcl-6 transcription factor expression in bladder carcinoma. J Transl Med 2016; 14(1): 144–154
26. Shi B, Su B, Fang D, Tang Y, Xiong G, Guo Z, He Q, Yang X, Zhao W, Guo Y, Li X, Zhou L. High expression of KPNA2 defines poor prognosis in patients with upper tract urothelial carcinoma treated with radical nephroureterectomy. BMC Cancer 2015; 15(1): 380–

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27. Du F, Yuan P, Wang T, Zhao J, Zhao Z, Luo Y, Xu B. The significance and therapeutic potential of GATA3 expression and mutation in breast cancer: a systematic review. Med Res Rev 2015; 35(6): 1300–1315
28. García-Tello A, Ramón de Fata F, Andrés G, Ropero S, López JI, Angulo JC. DNA repair genes and prognosis in sporadic forms of urothelial carcinoma of the upper urinary tract. Actas Urol Esp 2014; 38(9): 600–607
29. Harper HL, McKenney JK, Heald B, Stephenson A, Campbell SC, Plesec T, Magi-Galluzzi C. Upper tract urothelial carcinomas: frequency of association with mismatch repair protein loss and lynch syndrome. Mod Pathol 2017; 30(1): 146–156
30. Li Q, Bagrodia A, Cha EK, Coleman JA. Prognostic genetic signatures in upper rract urothelial carcinoma. Curr Urol Rep 2016; 17(2): 12–22
31. Rouprêt M, Fromont G, Azzouzi AR, Catto JW, Vallancien G, Hamdy FC, Cussenot O. Microsatellite instability as predictor of survival in patients with invasive upper urinary tract transitional cell carcinoma. Urology 2005; 65(6): 1233–1237
32. Parsons R, Myeroff LL, Liu B, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B. Microsatellite instability and mutations of the transforming growth factor β type II receptor gene in colorectal cancer. Cancer Res 1995; 55(23): 5548–5550
33. Lee J, Ballikaya S, Schönig K, Ball CR, Glimm H, Kopitz J, Gebert J. Transforming growth factor β receptor 2 (TGFBR2) changes sialylation in the microsatellite unstable (MSI) Colorectal cancer cell line HCT116. PLoS One 2013; 8(2): e57074