Clinicopathological characteristics of upper tract urothelial cancer with loss of immunohistochemical expression of mismatch repair proteins in universal screening

Tetsutaro Hayashi[1], Ikeda Kenichiro[1], Keisuke Goto[1], Shogo Inoue[1], Jun Teishima[1], Kazuhiro Sentani[2], Daiki Taniyama[2], Shintaro Akabane[2], Wataru Yasui[2], Hideki Yamamoto[3], Kazuya Kuraoka[3], Ryan S Hsi[4], Takao Hinoi[5] and Akio Matsubara[1]
The Department of Urology[1], Molecular Pathology[2], Clinical and Molecular Gentics[5], Hiroshima University, The Department of pathology[3], Kure Medical Center, The Department of Urology[4], Vanderbilt University Medical Center

BACKGROUND

- Lynch syndrome (LS) is an inherited cancer caused by germline mutations in DNA mismatch repair (MMR) genes. Upper tract urothelial cancer (UTUC) is the third most frequent cancer in LS, but little is known about the prevalence and clinico-pathological features of MMR deficient UTUC.
- The detection of MMR proteins by immunohistochemistry (IHC) as well as microsatellite instability (MSI) test is widely used as screening methods.
- Recent genomic studies have revealed that urothelial carcinoma can be classified into molecular subtypes, which helps precision medicine. Furthermore, it was previously shown that mismatch-repair status predicted clinical benefit of immune checkpoint blockade with anti-PD-1 antibody therapy .

OBJECTIVES

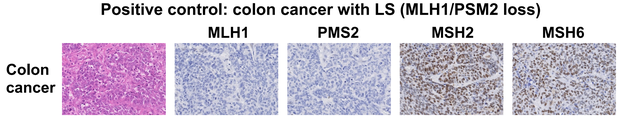
To assess the prevalence and clinico-pathological characteristics of UTUC with loss of mismatch repair (MMR) genes, we examined the expression of MMR proteins immunohistochemically and compared clinical and pathological features between patients with UTUC with and without MMR deficiency .

MATERIALS & METHODS

- We studied IHC of MLH1, MSH2, MSH6 and PMS2 in 118 cases of UTUC treated by radical nephroureterectomy without neoadjuvant chemotherapy.
- IHC on whole sections in nephroureterectomy specimens were performed.
- We defined MMR loss when tumors showed complete absence in at least one MMR, but retained expression of MMR in inflammatory cell and/or adjacent normal tissue as positive control.
- We defined MMR low as cases that had tumor with lower than 5% of at least one MMR staining.
- We analyzed cases of MMR loss and low as MMR-deficient UTUC.
- We used MMR antibodies below and colon cancers with LS as positive controls.
- We defined the positivity was any cancer cell staining for UPK3, over 10% and 20% of cancer cell staining for TP53 and GATA3 and all layers staining for CK5/6 and CD44v9. Any cancer or stroma cell staining for PD-L1 were positive, and we evaluated CD8+ T-cell infiltration into tumor or stroma.

Antibodies for MMR
MLH1: mouse monoclonal, ES05(code No.IR079), x100
MSH2: mouse monoclonal, FE11(code No.IR085), x100
MSH6: rabbit monoclonal, EP49(code No. IR086), x100
PMS2: rabbit monoclonal, EP51(code No. IR087), x50

Positive control: colon cancer with LS (MLH1/PSM2 loss)



RESULTS

- MMR deficient was detected in 15 / 118 (13%) of our UTUC cases.

MMR deficient	Number of cases
MSH2/MSH6	3
MLH1/PMS2	2
MLH1/PMS2/MSH6	1
MLH1/MSH6	1
MSH6	8

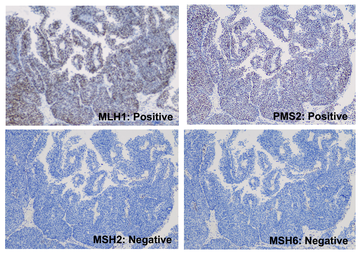
Case 1 : tumor with Loss of MSH2/MSH6

75 year-old, male
Past history: Colon cancer and gastric cancer
Present illness: during follow-up of colon cancer, CT scan pointed out bilateral ureter cancer. Left nephroureterectomy (1) and transureteral resection (TUR) of right ureteral tumor (2) were underwent. After 11 months, bladder tumor (3) was detected and treated by TUR of bladder tumor.

(1) Left ureteral tumor

Urothelial carcinoma, Grade 2, pT1

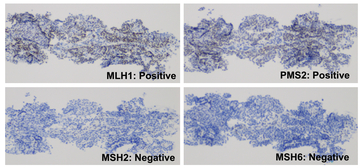
Loss of MSH2/MSH6



(2) Right ureteral tumor

Urothelial carcinoma, Grade 2, pTa

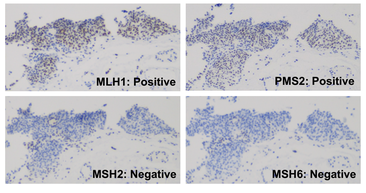
Loss of MSH2/MSH6



(3) Bladder tumor

Urothelial carcinoma, Grade 2, pTa

Loss of MSH2/MSH6



Clinical and pathological characteristics of MMR deficient UTUC

1) Comparison of patient characteristics between MMR deficient and normal

Factors		MMR deficient (n=15)	MMR normal (n=103)	P-value
Gender	Male	11 (73%)	77 (75%)	>0.9999
	Female	4	26	
Age (Ave ± SD)		65.7 ± 9.6	71.8 ± 10.0	0.0256
BMI (Ave ± SD)		24.2 ± 3.1	22.5 ± 3.1	0.0943
Side	Right	7 (47%)	50 (49%)	0.8919
	Left	8	53	
History of cancer	Positive	9 (60%)	32 (34%)	0.0830
	Negative	6	62	
Bladder cancer	Positive	6 (40%)	34 (33%)	0.3103
	Negative	9	69	

Patients with deficient MMR of UTUC were detected at younger age.

2) Comparison of blood test between MMR deficient and normal UTUC

Factors		MMR deficient (n=14)	MMR normal (n=99)	P-value
White blood cells (WBC)		6523 ± 2182	6471 ± 2080	0.9193
Neutrophils (Neu)		3833 ± 1272	4035 ± 1620	0.8181
Lymphocytes (Lym)		2191 ± 946	1760 ± 762	0.1028
NLR (Neu to Lym ratio)		1.88 ± 0.62	2.55 ± 1.12	0.0377
Hemoglobin (Hb)		13.1 ± 1.22	12.9 ± 1.90	0.7349
Platelet (PLT)		20.95 ± 4.871	22.29 ± 7.457	0.5763
Albumin (Alb)		4.15 ± 0.42	4.17 ± 0.38	0.8151
Creatinine (Cre)		1.03 ± 0.62	1.20 ± 1.13	0.1973
CRP	< 0.3	11 (79%)	70 (71%)	0.7537
	0.3 ≦	3	29	

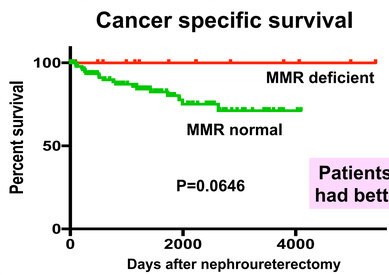
Patients with deficient MMR of UTUC showed lower NLR.

3) Comparison of pathological factors between MMR deficient and normal

Factors		MMR deficient (n=15)	MMR normal (n=103)	P-value
Tumor location	Renal	11 (73%)	48 (49%)	0.0993
	Ureter	4	50	
	Renal/Ureter	0	5	
Tumor morphology	Papillary	12 (80%)	57 (55%)	0.0702
	Nodular/Flat	3	46	
Histological classification	Pure UC	14 (93%)	94 (91%)	>0.9999
	Variants	1	9	
With CIS	Positive	3 (20%)	46 (45%)	0.0128
	Negative	12	57	
Histological grade	G1/2	14 (93%)	63 (61%)	0.0180
	G3	1	40	
pT stage	pTis/a/1	12 (80%)	45 (44%)	0.0117
	pT2/3/4	3	58	
pN stage	pN0/X	15 (100%)	98 (95%)	>0.9999
	pN1/2	0	5	

Tumors with deficient MMR showed lower grade and lower stage with less CIS than those with MMR normal.

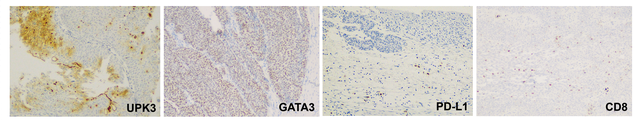
4) Comparison of prognosis between MMR deficient and normal



Patients with MMR deficient UTUC had better cancer specific survival.

5) Comparison of IHC markers between MMR deficient and normal UTUC

IHC of subtype markers and prognostic/predictive markers in MMR deficient case.



IHC Markers		MMR deficient (n=15)	MMR normal (n=103)	P-value
Uroplakin 3	Positive	7 (47%)	29 (28%)	0.2277
	Negative	8	74	
GATA3	Positive	12 (80%)	92 (89%)	0.3845
	Negative	3	11	
Cytokeratin 5/6	Positive	5 (33%)	20 (19%)	0.3067
	Negative	10	83	
TP53	Positive	2 (13%)	33 (32%)	0.2254
	Negative	13	70	
CD44v9	Positive	1 (7%)	27 (26%)	0.1158
	Negative	14	76	
PD-L1 (Tumor)	Positive	1 (7%)	16 (16%)	0.6932
	Negative	14	87	
PD-L1 (Stroma)	Positive	3 (20%)	34 (33%)	0.3846
	Negative	12	69	
CD8+ T cell (Tumor)	Positive	9 (60%)	27 (26%)	0.0141
	Negative	6	76	
CD8+ T cell (Stroma)	Positive	15 (100%)	98 (95%)	>0.9999
	Negative	0	5	

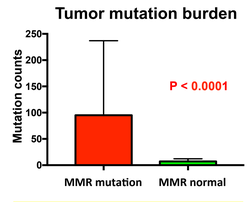
Tumors with deficient MMR showed no difference in the expression of subtype markers, prognostic marker, stem cell marker and PD-L1 compared to those with MMR normal. But tumors with deficient MMR showed more tumor infiltration of CD8+ T lymphocytes.

MMR mutation in public database of UTUC and MIBC

1) UTUC (MSK, 2019) datasets from cBioPortal

MMR mutation was detected in 15/119 (13%) of cases.

MMR mutation	Number of cases
MLH1/MSH2/MSH6	1
MSH2/MSH6	1
MLH1	4
MSH2	6
MSH6	2
PMS2	1

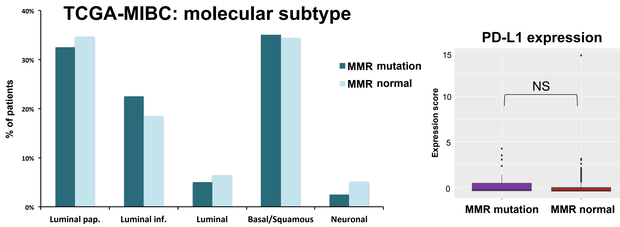


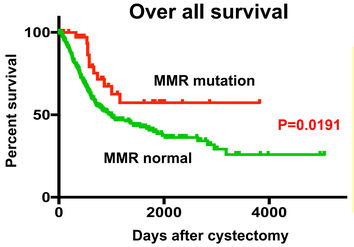
Factors	MMR mutation (n=15)	MMR normal (n=104)	P-value
Age	62.3 ± 11.4	69.3 ± 9.9	0.0316

Patients with MMR mutation showed younger and tumor mutation burden (TMB).

2) Muscle invasive bladder cancer: MIBC (TCGA) datasets from cBioPortal

MMR mutation was detected in 40/412 (9.7%) of MIBC cases.





- MIBCs with deficient MMR were similar in molecular subtypes and PD-L1 expression as MIBCs with MMR normal.
- MIBCs with deficient MMR showed better prognosis.

Summary

- MMR deficiency was detected in 13% of UTUC cases.
- Patients with deficient MMR tumor were younger and showed lower NLR compared to non-MMR deficient tumors .
- Tumors with deficient MMR showed lower grade and lower stage and had better prognosis compared to non-MMR deficient tumors.
- Tumors with deficient MMR showed similar percentage of molecular subtypes as those with non-deficient MMR, but showed higher tumor infiltration of CD8+ T-cells probably because of TMB.

CONCLUSIONS

We identified a prevalence of 13% of UTUC cases with potential Lynch syndrome. Compared to non-MMR deficient UTUC, patients with MMR deficient UTUC associated with younger age and lower NLR. Tumors with deficient MMR were associated with less pathological aggressive features with infiltration of CD8 positive lymphocytes. Patients with MMR deficient UTUC had better cancer specific survival. These findings suggest that determining MMR status may be helpful for clinical-decision making among patients with UTUC.

COI Disclosure Information: Tetsutaro Hayashi
I have no financial relationships to disclose