Correlation of mismatch repair protein deficiency, PD-L1 and CD8 expression in high-grade urothelial carcinoma of the bladder

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ABSTRACT

Mismatch repair-deficient (d-MMR) tumours have been reported to show susceptibility to immune checkpoint inhibitors targeting programmed death-1/PD ligand-1 (PD-1/PD-L1). In this study, we sought to correlate the association of d-MMR, PD-L1 and CD8 expression in muscle invasive, high-grade urothelial carcinoma (HGUC) of bladder. A tissue microarray (TMA) was constructed from 201 cases and sequentially stained with PD-L1, CD8, MSH2, MSH6, MLH1 and PMS2. PD-L1 was assessed in tumour and immune cells. CD8 was assessed in a hotspot fashion with results averaged across cores. Loss of nuclear MMR expression on TMA sections was further assessed using corresponding whole tissue sections. d-MMR was identified in four cases (2%). The mean CD8 count was significantly higher in d-MMR tumours (10 vs 35, p=0.007) as was the proportion of PD-L1 positivity (75% vs 20%, p=0.031). d-MMR is uncommon in HGUC of bladder but shows strong correlation with cytotoxic T lymphocyte infiltration and PD-L1 tissue expression.

INTRODUCTION

High-grade urothelial carcinoma of bladder (HGUC) is a common and aggressive malignancy with approximately 20% of cases presenting with detrusor muscle invasion (≥pT2).[1] Therapeutic options in this setting have traditionally been limited with the gold-standard treatment being surgery in the form of radical cystectomy and pelvic lymph node dissection.[2 3] For the past three decades, systemic therapy has typically consisted of platinum-based chemotherapy (either adjuvantly or neoadjuvantly). The advent of immune checkpoint inhibition has opened a new therapeutic avenue for muscle invasive HGUC with multiple clinical trials demonstrating durable responses in the advanced disease and metastatic settings.[4–6]

Patients are currently triaged for programmed death-1/PD ligand-1 (PD-1/PD-L1) inhibitors based on tissue expression levels of PD-L1 with better overall response rates noted in patients who are PD-L1 high/positive. Despite this, PD-L1 is an imperfect biomarker as patients who are PD-L1 negative may respond to treatment and conversely, not every patient with positive PD-L1 expression will have a clinically meaningful response. Therefore, additional biomarkers that could better stratify patients for therapy are desirable. One such biomarker is mismatch repair deficiency (d-MMR). A number of publications have shown that patients with multiple tumour types exhibiting MMR protein loss may respond to PD-1/PD-L1 blockade[7 8]; because of this, an accelerated Food and Drug Administration approval was granted in the USA, allowing d-MMR tumours to be treated with immune checkpoint inhibitors agnostic of tissue/site or PD-L1 status.[9]

From a genitourinary pathology perspective, loss of MMR protein expression has been best characterised in the upper urothelial tract, given the association of these malignancies with Lynch syndrome. There are limited data pertaining to the rates of d-MMR in HGUC of bladder, although is has been shown that microsatellite instability is a feature of a small proportion of bladder cancers.[10] In this study, we sought to assess the frequency and histological features of d-MMR in a retrospective cohort of muscle invasive HGUC bladder and to correlate d-MMR with cytotoxic lymphocyte infiltration (assessed using CD8 expression) and tissue PD-L1 protein expression. We hypothesised that d-MMR tumours would contain a higher number of CD8 positive lymphocytes and would exhibit increased rates of PD-L1 tissue positivity compared with MMR intact HGUC.

MATERIALS AND METHODS

Patient cohort and tissue microarray creation

All cases were identified through a retrospective search of the laboratory information system, Sunquest CoPath (n=235), as previously described.[11] Briefly, the search criteria included cases of HGUC of the bladder treated by cystectomy between 1999 and 2015. Only cases that were muscle invasive with a predominant urothelial component (>50%) were selected and cases with a neuroendocrine carcinoma component were excluded. The electronic patient records (EPRs) were searched to document any history of Lynch syndrome. The following parameters were collected: age, sex, tumour histology, stage and clinical follow-up.

Original H&E slides for each case were retrieved from our departmental archive and reviewed by a genitourinary pathologist (MRD). Only cases with more than one available tumour block were selected for inclusion in the tissue microarray (TMA). The tumour inflammation was semiquantitatively assessed as previously reported.[11]

Table 1 Clinicopathological variables of study cohort (n=201)

Variable	N (%)
Sex	
Male	145 (72.1)
Female	56 (27.9)
Age (years)	
Median (range)	71(33–93)
Histotype	
Usual	139 (69.2)
Variant/divergent differentiation	62 (30.8)
Pathologic stage	
pT2	31 (15.4)
pT3	111 (55.2)
pT4	59 (29.4)
Nodal metastases	
Present	83 (41.3)
Absent	109 (54.2)
No nodal dissection	9 (4.5)
Chemotherapy	
Neoadjuvant	38 (19)
Adjuvant	44 (21.9)
Follow-up	
Alive	79 (39.3)
Dead of disease	48 (23.9)
Not available	74 (36.8)

Triplicate core TMAs were constructed from 201 of the available 235 cases. Technical details regarding construction of the TMAs have been previously described.[12] A H&E section was examined from each TMA block to confirm tumour adequacy.

Immunohistochemistry staining and assessment

Four-micron thick unstained sections were sequentially cut from the TMA blocks and stained with CD8 (SP57 clone Ventana), PD-L1 (SP263 clone Ventana) and MSH2 (FE11 clone Dako/Agilent), MSH6 (EP49 clone Dako/Agilent), MLH1 (ES05 clone Dako/Agilent) and PMS2 (EP51 clone Dako/Agilent).

CD8 was assessed in a hotspot fashion (1 representative 40x field/core; scores averaged across 3 cores). PD-L1 was interpreted according to manufacturer's instructions in both tumour cells (TC) and immune cells (IC). Twenty-five per cent TC and/or IC expression was deemed PD-L1 positive. The identification of TCs and ICs was based on morphological features alone with assistance of H&E slides. MMR protein expression was assessed in tumour nuclei with complete nuclear loss required for a case to be called d-MMR. Peritumoural and intratumoural lymphocytes served as an internal control.

Any case that demonstrated loss of MMR protein expression on the TMA was further evaluated using repeat MMR and PD-L1 staining of the whole tumour section from which the TMA cores had been sampled.

Statistical analysis

The SPSS statistical software V.24.0 (IBM) was used. The correlations between d-MMR and PD-L1 and CD8 expression were calculated using Fisher's exact test. A $p<0.05$ was considered statistically significant.

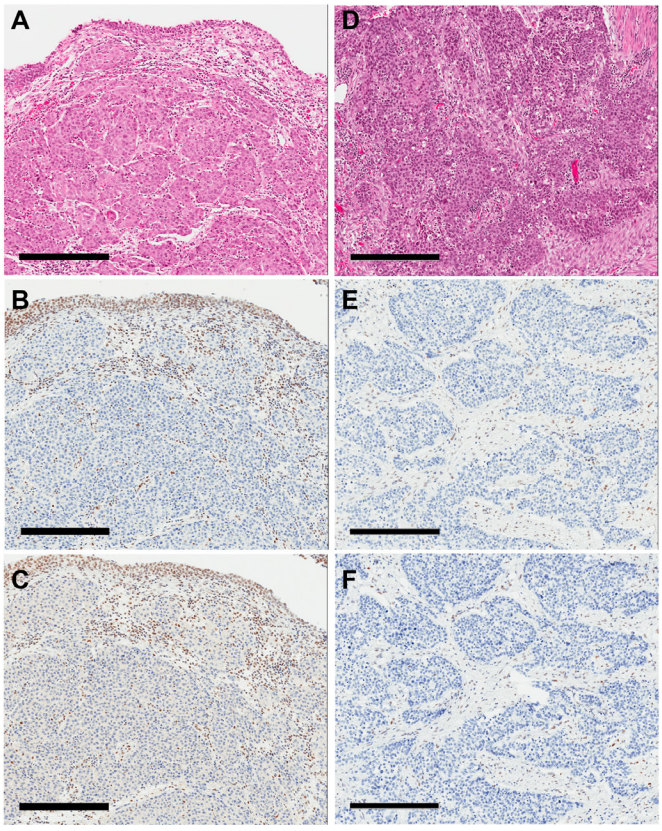

Figure 1 Mismatch repair protein loss in high-grade urothelial carcinoma of the bladder. (A) Urothelium overlying invasive high-grade urothelial carcinoma of the bladder. (B) Loss of MLH1 and (C). PMS2 expression in tumour cells. (D) Invasive high-grade urothelial carcinoma of the bladder in a patient with concurrent papillary urothelial carcinoma of the renal pelvis (not shown). (E) Loss of MSH2 and (F) MSH6 expression in tumour cells. Note the internal positive control in both cases. Scale bar=300 µm.

RESULTS

The study population was composed of 201 patients with HGUC of bladder (≥pT2) treated by cystectomy (1999–2015). The median age of the cohort was 71 years (range 33–93 years). The male:female ratio was 2.6:1 (145 male:56 female). The clinicopathological parameters of the cohort are summarised in table 1. Thirty-eight patients (18%) received neoadjuvant chemotherapy and 44 (21%) received adjuvant chemotherapy with a platinum compound. None of the patients were treated were immunotherapy. Review of the EPR did not identify any patient with known or suspected Lynch syndrome in the cohort.

All 201 cases included in the TMAs were evaluable. There was loss of MMR protein expression in 15 cases (7.5%) on the TMA cores as follows: isolated MSH2 loss (n=6), isolated PMS2 loss (n=1), combined MSH2/MSH6 loss (n=3), combined MLH1/PMS2 loss (n=4) and one case with loss of MSH2 and PMS2. Whole section staining was repeated for the relevant MMR proteins. The two cases showing loss of MSH2 or PMS2 alone were determined to have intact MMR on whole sections. Only four cases (2%) had d-MMR on the whole sections of which three cases showed combined MLH1/PMS2 loss and one case showed MSH2/MSH6 loss (figure 1).

The clinicopathological features of the four d-MMR cases are summarised in table 2. All cases were pT3 tumours with three showing focal squamous differentiation. Only one of the

Table 2 Characteristics of the four MMR-deficient cases

	Case 1	Case 2	Case 3	Case 4
MMR result	MLH1/PMS2 loss	MLH1/PMS2 loss	MLH1/PMS2 loss	MSH2/MSH6 loss
Age	73	86	64	48
Sex	Male	Female	Male	Male
Pathology	5.5 cm, urothelial with sarcomatoid and squamous, pT3b, 0/4 LNs involved, no CIS	4.5 cm, urothelial with squamous, pT3a, 0/10 LNs involved, no CIS	3.5 cm, urothelial with squamous, pT3a, 2/9 LNs involved, no CIS	1.5 cm, pure urothelial, pT3b, 0/28 LN lymph nodes involved, no CIS Right nephro-u: high-grade papillary urothelial, 1 cm, pT3
Invasive edge	Pushing edge	Infiltrative edge	Pushing edge	Pushing edge
Inflammation	Low	High	High	High
Mean CD8 count	4	239	61	129
PD-L1 result	High	High	High	Low
Follow-up	Dead 3 months postoperatively	NA	NA	Dead 9 months postoperatively

CIS, carcinoma in situ; LN, lymph node; MMR, mismatch repair; NA, not available; nephro-u, nephro-ureterectomy; PD-L1, programmed death ligand 1.

four cases had lymph node involvement (MMR intact group, 41.6% nodal metastases). None of the four cases had carcinoma in situ (present in 91 MMR intact cases, 46.2%). Three of the four cases were highly inflamed within the tumour and at the invasive front. Three cases had a circumscribed 'pushing border' and one had a ragged, infiltrative edge (no significant difference compared with MMR intact cases, p=1.000). One patient with MSH2/MSH6 loss had a concomitant nephroureterectomy at the time of cystectomy, which showed a high-grade papillary urothelial carcinoma of the renal pelvis. Follow-up was available on only two patients, both of whom succumbed to disease within 1 year of surgery.

CD8 expression was evaluable in 196/201 cases. The mean CD8 expression assessed as hotspot counting in the four d-MMR cases was 108 cells/case. In comparison, the mean CD8 expression in the MMR intact cases was 35 cells/case (p=0.007).

PD-L1 was interpretable in 196/201 TMA cases. Overall, 44 cases were scored as PD-L1 high (22%). Three of the four d-MMR cases were PD-L1 positive (75%). The pattern of PD-L1 expression was in both the TC and IC but IC staining was more prominent. In comparison, within the MMR intact HGUC cases, the rate of PD-L1 positivity was 20% (40/197) with a predominance of TC positivity (25 cases positive on TC staining, 11 on IC staining and 4 both TC and IC). There was a significant correlation of PD-L1 positivity with MMR protein loss (p=0.031) (figure 2).

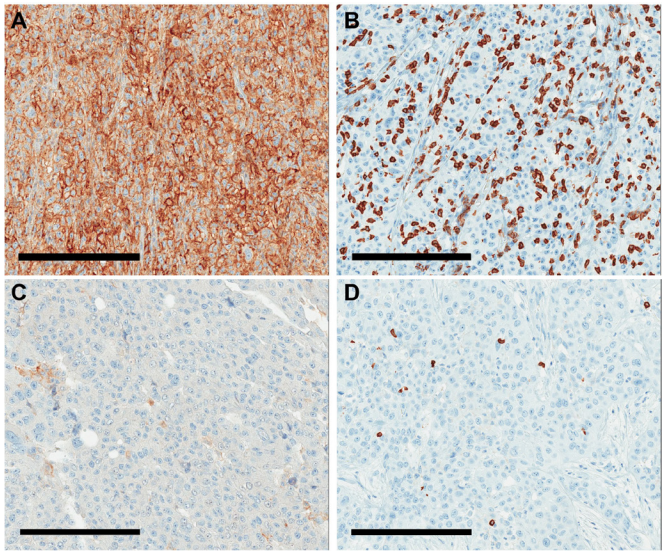

Figure 2 PD-L1 and CD8 immunoexpression in mismatch repair protein-deficient and proficient cases of high-grade urothelial carcinoma of the bladder. (A) High PD-L1 and (B). Increased CD8 expression in a mismatch repair protein-deficient case, compared with (C). Low PD-L1 and (D). Minimal CD8 expression mismatch repair protein-proficient case. Scale bar=200 µm. PD-L1, programmed death ligand-1.

DISCUSSION

Upper tract urothelial carcinoma (UTUC) is the third most common malignancy in patients with Lynch syndrome with up to 5% of all cases occurring in this setting.[13] The association of bladder cancer with Lynch syndrome is less well defined, however, emerging data have suggested that urothelial bladder cancers may be a component of the syndrome. Analysis of Lynch syndrome registry databases has suggested that the rate of bladder cancer in these patients is in the order of 2%–4.5% with loss of MMR protein expression seen in 86% of cases and microsatellite instability high in 20%. Bladder cancer risk appears to be greatest in those with *MSH2* mutations.[14 15]

The frequency of MMR loss in (presumed) sporadic bladder cancer has only been studied in a few instances[16–18] and the association of MMR loss with PD-L1 and CD8 tissue expression has not yet been reported in the primary disease setting (whereas the relationship between only PD-L1 and CD8 is well documented).[19] Given the frequent description of inflammation and infiltrating ICs in Lynch-associated malignancies and the recent developments in immune checkpoint inhibition therapy in the setting of tumoural MMR loss, this is a relevant area of study.

Prior publications looking at UTUC have proposed certain histological features to be suggestive of Lynch syndrome cases including papillary or villous architecture, pushing tumour edge, inverted papilloma-like growth, lack of pleomorphism and an increased amount of intratumoural lymphocytes.[20–22] We identified some similar features in our d-MMR cases with a predominance of pushing borders and increased intratumoural inflammation. However, there was a similar frequency of pushing borders in the MMR intact cases. All of our cases were high-grade and exhibited nuclear pleomorphism. No appreciable papillary architecture was identified in any of the tumours. Given the low frequency of d-MMR in our large cohort, there were insufficient cases to fully characterise the morphology of these cases.

Inflammation is commonly noted in colorectal and endometrial carcinomas associated with Lynch syndrome and is also a phenomenon that has been described in UTUC cases.[20] Inflammation is not infrequent in HGUC of bladder and while not a specific feature associated with d-MMR in this setting, we did note increased inflammation on both H&E sections and specifically in the context of CD8 staining. It is, therefore, potentially not surprising that d-MMR carcinomas in our cohort showed a significantly higher rate of PD-L1 expression compared with the MMR intact cases.

There are limitations to this study, which include its retrospective design, data from a single institution, inclusion of only muscle invasive HGUC and the small number of d-MMR tumours that were identified. Assessment of larger cohorts from multiple

institutions would be helpful in determining the frequency of MMR loss in the setting of presumed non-hereditary bladder cancer. This would allow a larger group of tumours to be evaluated in terms of morphological features, rates of MMR loss and PD-L1 expression in both low-grade and high-grade tumours in addition to non-muscle invasive carcinomas.

Based on our data, we conclude that loss of MMR protein loss by immunohistochemistry is uncommon in muscle invasive HGUC of bladder. There was a predominance of highly inflamed carcinomas with a pushing invading edge associated with increased cytotoxic T lymphocytes and PD-L1 expression in the d-MMR cases. The PD-L1 expression was marked in the IC component. It is unlikely that routine MMR staining of primary HGUC muscle invasive carcinomas would yield significant clinical benefit but larger studies are required to fully investigate the potential clinical utility of d-MMR as a predictive biomarker of immune therapy response.

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