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Loss of DNA mismatch repair proteins in prostate cancer

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Abstract

Recent studies have suggested an increased risk of prostate cancer in men with Lynch syndrome driven by germline mutations in mismatch repair (MMR) genes. However, the incidence and clinical implication of MMR deficiency in sporadic prostate cancers remain poorly understood. We immunohistochemically stained for MLH1, MSH2, MSH6, and PMS2 in a set of tissue microarray consisting of 220 radical prostatectomy specimens and evaluated the relationship between loss of their expression and available clinicopathological features. MLH1, MSH2, MSH6, and PMS2 were lost in 2 (0.9%), 6 (2.7%), 37 (16.8%), and 27 (12.3%) prostate cancers, respectively. Loss of at least 1 MMR protein was identified in 50 (22.7%) cases. There were no statistically significant associations between MMR deficiency and patient age, family history of prostate cancer, Gleason score, or pT/pN stage. Nonetheless, the levels of preoperative prostate-specific antigen (PSA) were significantly ($P = .015$) higher in patients with MMR deficiency (mean ± SD: 9.12 ± 9.01 ng/mL) than in those without abnormal MMR (5.76 ± 3.17 ng/mL). There were 15 (6.8%) cases showing loss of at least 2 MMR proteins, which was not significantly associated with PSA level or tumor grade/stage. Additionally, 5 and 2 cases showed losses of at least 3 MMR proteins and all 4 proteins, respectively. Kaplan–Meier analysis revealed no significant associations between loss of MLH1 ($P = .373$), MSH2 ($P = .348$), MSH6 ($P = .946$), or PMS2 ($P = .681$), or at least 1 ($P = .477$), 2 ($P = .486$), or 3 ($P = .352$) MMR proteins and biochemical recurrence. Further analyses of the data on programmed death-ligand 1 (PD-L1) expression previously stained in the same set of tissue microarray demonstrated associations between loss of ≥2 MMR proteins and a higher rate of PD-L1 expression in cancer cells (17.2% vs 5.2%; $P = .033$) as well as between cases showing both loss of ≥1 MMR protein(s) and PD-L1 expression in tumor-infiltrating immune cells vs a higher risk of biochemical recurrence ($P = .045$). MMR protein loss was seen in a subset of prostate cancers. Interestingly, it was associated with significantly higher levels of PSA. Moreover, immunohistochemical detection of MMR proteins together with other proteins, such as PD-L1, might be helpful in predicting tumor recurrence following radical prostatectomy.

Abbreviations: MMR = mismatch repair, MSI = microsatellite instability, PD-1 = programmed cell death protein 1, PD-L1 = programmed death-ligand 1, PSA = prostate-specific antigen, TMA = tissue microarray.

Keywords: immunohistochemistry, mismatch repair deficiency, prognosis, prostate cancer

1. Introduction

Prostate cancer is the most frequently diagnosed neoplasm in men, with an estimated 191,930 new cases and 33,330 deaths occurred in 2020, in the US.[1] Although radical prostatectomy for localized prostate cancer often provides cure of disease, tumor recurrence after the surgery remains a major clinical challenge. Importantly, there are only few molecular markers, other than clinicopathological features including Gleason score of the tumor and preoperative level of prostate-specific antigen (PSA), which are able to precisely predict disease progression.[2] Meanwhile, multi-omics-based approaches, using genomics, metabolomics, and/or proteomics, have identified potential molecular biomarkers that may be useful for early detection of localized prostate cancer and decision-making in its management.[3] Further identification of molecules that play a key role in prostate cancer outgrowth is thus required, which may successively offer novel prognosticators and/or novel targeted treatment for prostate cancer.

Those with Lynch syndrome, an autosomal dominant genetic disorder driven by germline mutations in DNA mismatch repair (MMR) genes including *MLH1*, *MSH2*, *MSH6*, and *PMS2*, have been known to be at a substantially greater risk of developing malignancies, especially colorectal cancer, via microsatellite instability (MSI).[4] Recent studies have also indicated a link between MMR deficiency, particularly defects within the *MSH2* or *MSH6* gene, and the risk of prostate cancer.[5–7] Moreover, alterations of MMR genes have been found in men with prostate cancer.[8–10] In 2 of these studies, MMR deficiency has also been associated with favorable response to anti-programmed cell death protein 1 (PD-1) therapy[8] or the protein expression of a PD-1 ligand, programmed

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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death-ligand 1 (PD-L1), in tumors,[9] suggesting its role as a predictive biomarker for immune checkpoint blockade.

Several recent studies have immunohistochemically assessed the expression of MMR proteins in prostate cancer specimens.[9,10,12,13] However, the incidence and clinical implication of MMR protein loss in sporadic prostate cancers remain far from being fully understood. The present study aimed to determine the expression status of MMR proteins in prostate cancer tissue specimens and its prognostic implication.

2. Materials and methods

2.1. Prostate tissue microarray (TMA)

We retrieved 220 prostate tissue specimens obtained by radical prostatectomy performed at the University of Rochester Medical Center. Appropriate approval from the Institutional Review Board was obtained before construction and use of the TMA consisting of representative lesions of prostatic adenocarcinoma, as described previously.[14,15] The institutional review board also approved the request to waive the documentation of informed consent from the patients. Their mean age at presentation was 60.3 years (range: 42–78 years) and the mean follow-up after the surgery was 48.2 months (range: 3–116 months). None of the patients had received therapy with hormonal reagents, radiation, or other anti-cancer drugs pre- or post-operatively before clinical or biochemical recurrence. Biochemical recurrence was defined as a single PSA level of ≥0.2 ng/mL.

2.2. Immunohistochemistry

Immunohistochemical staining for MMR proteins was performed, using a primary antibody to MLH1 (clone G168–15; Biocare Medical, Concord, CA), MSH2 (clone FE11; Biocare Medical), MSH6 (clone BC/44; Biocare Medical), or PMS2 (clone A16–4; Biocare Medical), and a polymer detection system (Dako, Carpinteria, CA) on an automated staining system (Dako), on the sections (5 μm thick) from the prostate TMA, as described previously.[16] All stains were quantified independently by 2 pathologists (MS and HM.) who were blinded to sample identity. Convincing nuclear staining of each protein in at least 1% of tumor cells was considered to be positive. Cases with discrepancies in the positivity were re-reviewed simultaneously by the 2 pathologists until a consensus was reached.

2.3. Statistical analysis

The Fisher exact test or chi-square test was used to evaluate the association between categorized variables. Non-parametric 2-group comparisons were carried out, using Mann-Whitney *U* test, to assess differences in variables with ordered distribution across dichotomous categories. The rates of recurrence-free survival were calculated by the Kaplan–Meier method, and comparisons were made by the log-rank test. *P* values less than.05 were considered to be statistically significant.

3. Results

We immunohistochemically stained for four MMR proteins in a set of prostate TMA consisting of radical prostatectomy specimens (Fig. 1). Table 1 summarizes the loss of MMR proteins in 220 cases of prostatic adenocarcinoma. Overall, MLH1, MSH2, MSH6, and PMS2 were lost in 2 (0.9%), 6 (2.7%), 37 (16.8%), and 27 (12.3%) prostate cancers, respectively. Both cases with MLH1 loss concurrently lost other 3 proteins, while all 6 cases with MSH2 loss showed concurrent MSH6 loss. Thus, loss of at least one MMR protein was identified in 50 (22.7%) cases. Table 2 summarizes the associations between MMR deficiency and clinicopathological features. There were no statistically significant associations between loss of at least 1 MMR protein and patient age, family history of prostate cancer, Gleason score, or pT or pN stage. However, the levels of preoperative PSA were significantly elevated in patients with MMR deficiency, compared to those without abnormal MMR.

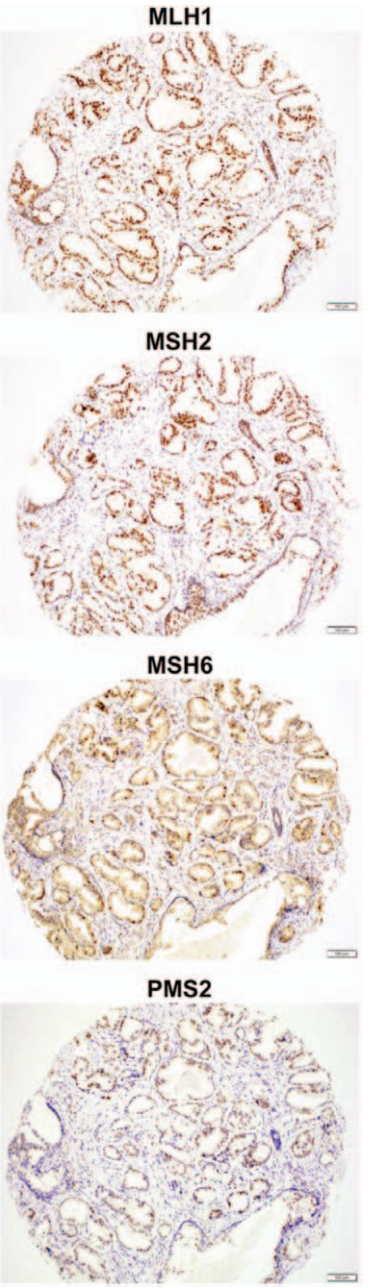

Figure 1. Immunohistochemistry of mutations in mismatch repair proteins in prostate cancer tissue. Representative images (original magnification: ×100) show MLH1/MSH2/MSH6/PMS2 expression primarily in the nucleus of benign or malignant cells from a single case.

Table 1

Loss of MMR proteins.

Proteins	N (out of 220 cases)
MLH1	2 (0.9%)
MSH2	6 (2.7%)
MSH6	37 (16.8%)
PMS2	27 (12.3%)
At least 1 Protein	50 (22.7%)
At least 2 Proteins	15 (6.8%)
At least 3 Proteins	5 (2.3%)
All 4 Proteins	2 (0.9%)

MMR = mutations in mismatch repair.

There were 15 (6.8%) cases showing loss of at least 2 MMR proteins, which was not significantly associated with PSA level or tumor grade/stage. Additionally, 5 (2.3%) and 2 (0.9%) cases showed losses of at least three MMR proteins and all four proteins, respectively.

Kaplan–Meier analysis coupled with log-rank test was performed to assess the prognostic values of MMR deficiency (Fig. 2). Of the 220 patients, 39 (17.7%) had clinical or biochemical recurrence following radical prostatectomy. However, loss of MLH1 ($P=.373$), MSH2 ($P=.348$), MSH6 ($P=.946$), or PMS2 ($P=.681$), or at least 1 ($P=.477$), 2 ($P=.486$), or 3 ($P=.352$) MMR proteins showed no strong association with disease recurrence.

We recently stained for PD-L1 in the same set of TMA and showed that PD-L1 was positive in 29 (13.2%) of prostate cancers and in tumor-infiltrating lymphocytes or macrophages in 33 (15.0%) of cases.[16] Analyses of these data, along with the current results, demonstrated associations between loss of at least 2 MMR proteins and a higher rate of PD-L1 expression in cancer cells (17.2% [vs 5.2%]; $P=.033$), but not between loss of at least 1 MMR protein and PD-L1 positivity in cancer cells [31.0% (vs 21.5%); $P=.340$] or immune cells (18.2% [vs 23.5%]; $P=.653$) as well as between loss of at least 2 MMR proteins and PD-L1 positivity in immune cells (0% [vs 8.0%]; $P=.135$). Furthermore, cases showing both loss of at least 1 MMR protein and PD-L1 expression in tumor-infiltrating immune cells had a significantly higher risk of biochemical recurrence (Fig. 3; $P=.045$). There were no significant associations of PD-L1 positivity in cancer cells, as well as loss of at least one MMR protein ($P=.213$) or at least 2 MMR proteins ($P=.543$), with patient outcomes.

4. Discussion

Impairment of MMR genes has been linked to the risk of prostate cancer in men with Lynch syndrome[5–7] and has also been recently studied in sporadic cases of prostate cancer.[8–11] MMR deficiency in sporadic prostate cancer has indeed been associated with worse patient outcomes,[9] while favorable response to immune checkpoint blockade in those with MMR deficiency has also been reported.[8] However, the clinical impact of MMR deficiency, especially that detected by immunohistochemistry, in patients with prostate cancer remains largely unknown. In the present study, we immunohistochemically assessed the expression status of 4 MMR proteins in 220 cases of prostate cancer and its prognostic significance.

A few immunohistochemical studies have reported the incidence of MMR deficiency in prostate cancer (eg, 1.2% for MSH2 loss[12]; 5.0% for MLH1 loss, 8.0% for MSH2 loss, and 2.0% for PMS2 loss[13]). In the former study,[12] MSH2 loss was significantly more often seen in tumors with Gleason score 9–10/Grade Group 5 than in those with Gleason score $\leq$8/Grade Group $\leq$4. In the latter study,[13] however, no significant associations between MLH1/MSH2/PMS2 loss and Grade Groups were identified, while PMS2 loss was associated with a higher risk of biochemical recurrence ($P=.011$). Meanwhile, in these studies, MMR deficiency was not assessed as to its relationship with other clinicopathological features, such as tumor stage. Instead, elevated expression of MLH1, MSH6, and PMS2 in prostate cancer detected by immunohistochemistry was shown to associate with higher Gleason score or pT stage, lymph node metastasis, or earlier biochemical recurrence.[17] We here found that the incidence of MMR deficiency in sporadic prostate

Table 2

Associations of MMR deficiency with clinicopathological features.

	MMR Proficient (N=170)	MMR Deficient (N=50)	*P* value
Age (mean±SD, year)	60.2±7.1	60.2±6.7	.964
PSA (mean±SD, ng/mL)	5.76±3.17	9.12±9.01	.015
Family history of prostate cancer			.584
Yes	15 (8.8%)	6 (12.0%)	
No	155 (91.2%)	44 (88.0%)	
Gleason score			
6 (Grade Group 1)	68 (40.0%)	18 (36.0%)	.626 (GG 1 vs 2–5)
3+4 (Grade Group 2)	64 (37.6%)	21 (42.0%)	1.000 (GG 1–2 vs 3–5)
4+3 (Grade Group 3)	19 (11.2%)	6 (12.0%)	1.000 (GG 1–3 vs 4–5)
8 (Grade Group 4)	13 (7.6%)	5 (10.0%)	.341 (GG 1–4 vs 5)
9–10 (Grade Group 5)	6 (3.5%)	0 (0%)	
pT			
2/2+	132 (76.6%)	34 (68.0%)	.191 (2 vs 3)
3a	25 (14.7%)	10 (20.0%)	0.390 (2/3a vs 3b)
3b	13 (7.6%)	6 (12.0%)	
pN			.459 (0 vs 1)
0	107 (62.9%)	31 (62.0%)	
1	7 (4.1%)	4 (8.0%)	
X	56 (32.9%)	15 (30.0%)	

MMR = mutations in mismatch repair, PSA = prostate-specific antigen.

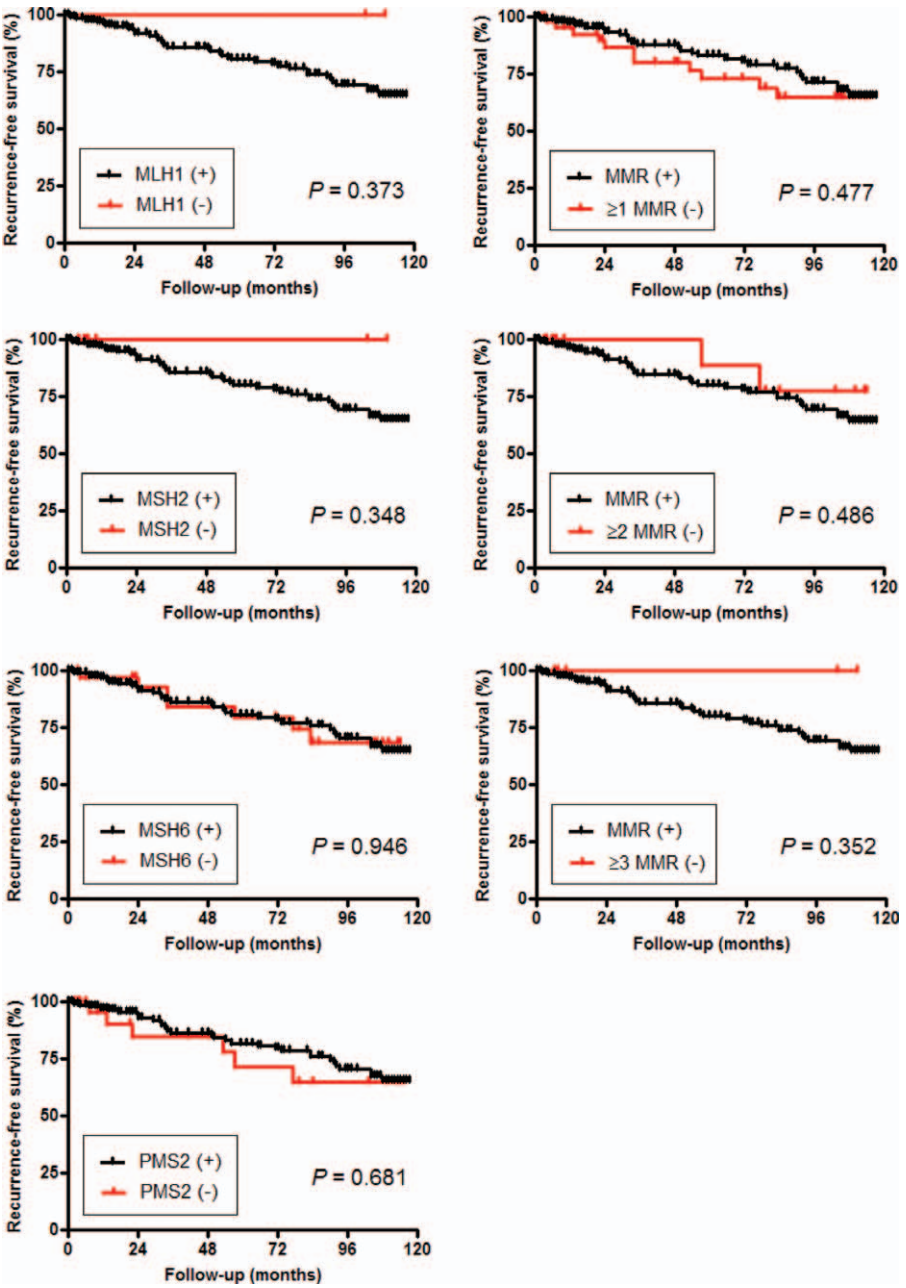

Figure 2. Kaplan–Meier curves for recurrence-free survival after radical prostatectomy according to the loss of mutations in mismatch repair protein(s).

cancer patients varied from 0.9% (MLH1) to 16.8% (MSH6) and that there were no significant associations of MMR deficiency in prostate cancer with patient age, family history of prostate cancer, Gleason score/Grade Group, pT or pN stage, or the risk of disease recurrence after radical prostatectomy. In accordance with previous observations[12] and the fact that the dimerization of MSH2 with MSH6 is required for stabilizing the 2 proteins,[18] all of our 6 cases with MSH2 loss showed concurrent loss of MSH6. Moreover, the levels of preoperative PSA in patients with MMR deficiency were significantly higher than those without MMR deficiency.

Microsatellite instable colorectal cancers have been shown to associate with an increased expression of immune checkpoint molecules, including PD-1 and PD-L1.[19] These findings have accelerated recent approval of 2 PD-1 inhibitors by the US Food and Drug Administration for the treatment of not only colorectal cancer, but also a variety of other malignancies, with MMR deficiency or high MSI, although prostate cancer has not been included. Similarly, elevation of PD-L1 expression in prostate cancer with MMR deficiency was documented.[9] Moreover, patients with MMR deficient prostate cancer were found to be more sensitive to anti-PD-1 therapy. Thus, MMR deficiency has been suggested to be a predictive marker for therapeutic response to immune checkpoint blockade. In our previous[16] and current studies, the status of either PD-L1 or MMR expression alone was not significantly associated with the risk of disease recurrence in prostate cancer patients who underwent radical prostatectomy. Nonetheless, their combination could offer considerable prog-

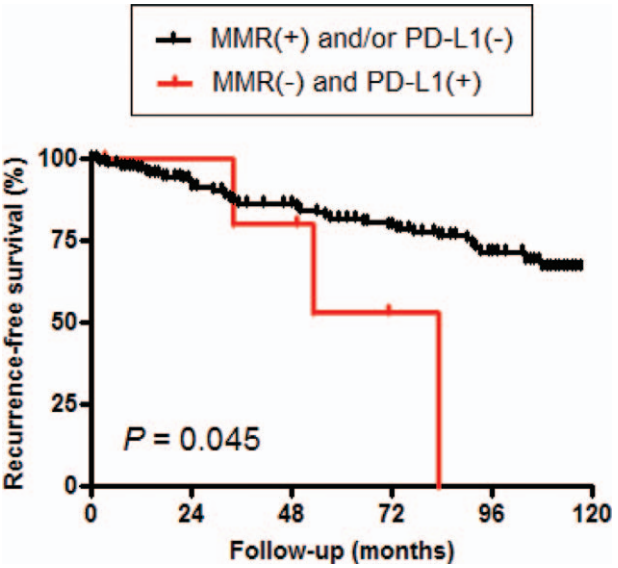

Figure 3. Kaplan–Meier curves for recurrence-free survival after radical prostatectomy in cases showing both loss of at least 1 MMR protein and positivity of PD-L1 in tumor-infiltrating immune cells (n = 7) vs no loss of MMR proteins and/or no expression of PD-L1 in tumor-infiltrating immune cells (n = 213). MMR = mutations in mismatch repair.

nostic information, although PD-L1 expression in tumor-infiltrating immune cells concurrently showing MMR loss in tumor cells appeared to be relatively uncommon (ie, 7 [3.2%] of 220 cases in the present study).

There are several limitations in our investigation, including its retrospective design which is subject to potential selection bias, although consecutive prostatectomy cases were included in our prostate TMA. A potentially more problematic issue is the stains in the TMA consisting of 1-mm tissue cores that may not be representative of the lesion of interest in each case. This may thus have produced false-negative results. Indeed, in a recent immunohistochemical study,[9] a discrepancy between TMA cores (ie, MLH1-negative and/or PMS2-negative) and large sections (ie, both positive) was found in 3 of 9 cases. It is thus interesting to perform further MSI testing by using, for instance, a polymerase chain reaction-based assay in our MMR deficient cases. In addition, the power of survival analysis, especially in those with loss of 3 or more MMR proteins (n = 5), may be limited due to such rare events.

In conclusion, MMR protein loss was detected in a subset of hormone-naïve prostate cancers in the current study. Interestingly, it was associated with significantly higher levels of PSA. However, immunohistochemical detection of MMR proteins alone is found to be not very useful for predicting tumor recurrence in patients with prostate cancer undergoing radical prostatectomy. Further validation studies with larger cohorts are thus warranted. In addition, the precise functional role of MMR proteins and related signaling pathways in the development and progression of prostate cancer needs to be further investigated.

Author contributions

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Formal analysis: Meenal Sharma, Zhiming Yang, Hiroshi Miyamoto.
Methodology: Meenal Sharma, Hiroshi Miyamoto.
Supervision: Hiroshi Miyamoto.
Validation: Hiroshi Miyamoto.
Writing – original draft: Meenal Sharma.
Writing – review & editing: Zhiming Yang, Hiroshi Miyamoto.

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