Different responses to anti-programmed cell death protein 1 (PD-1) immunotherapy in a patient with Lynch syndrome and metachronous urothelial and colon cancer: A case report

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Abstract. Lynch syndrome (LS), as a result of the germline mutations in DNA mismatch repair genes, is characterized by the increased risk of endometrium, colon, and urinary tract cancer. Individuals with this disorder may occasionally have multiple primary carcinomas. Regardless of tumor type, pembrolizumab was approved for the treatment of patients with unresectable or metastatic mismatch repair deficient tumors, which may be an optional therapeutic method for patients with LS with multiple primary carcinomas. This case study is of a MSH2-deficient patient with LS with metachronous urothelial and colon cancer, who received pembrolizumab treatment for 8 months. The responses of the two primary sites to immunotherapy differed. Based on the changes of tumor markers and tumor size illustrated by imageological examinations, no response was observed in the sigmoid colon lesion, whereas an immune-associated phenomenon known as pseudoprogression was detected in the ureteral lesion. Immunotherapy was innovatively applied to the patient with multiple primary carcinomas. This case proposes a novel concept in which immunotherapy may potentially control the cancer growth in patients with LS and multiple primary carcinomas. However, further large-scale investigations are required. Furthermore, it raises a challenge to monitor the effectiveness of immunotherapy.

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Introduction

Lynch syndrome (LS), also called hereditary nonpolyposis colorectal cancer (HNPCC), is a hereditary cancer predisposition syndrome that causes an increased risk for many types of cancers, including colorectal cancer and cancers of the endometrium, stomach, ovary, small bowel, as well as the urinary tract (1). The definitive diagnosis of LS is based on the identification of the germline pathogenic variants of mismatch repair (MMR) genes, mainly *MLH1*, *PMS2*, *MSH2* and *MSH6* (1). Defects in MMR proteins commonly result in the accumulation of genetic errors during DNA replication and therefore lead to high microsatellite instability (MSI-H) (1). Pembrolizumab, an anti-programmed cell death 1 (PD-1) antibody, shows promising efficacy for MSI-H or deficient MMR (dMMR) tumors (2,3). Through the pooled analysis of 5 single-arm clinical studies (KEYNOTE 016, 164, 012, 028, 158), the objective response rate (ORR) was 36% in MSI-H or dMMR patients with colorectal cancer (CRC) and 46% in 14 non-CRC cancers, which promoted the first tissue/site agnostic approval of pembrolizumab in unresectable or metastatic, MSI-H or dMMR solid tumors (4). The results obtained in the current study suggested that different cancers with MSI-H or dMMR have different responses to anti-PD-1 therapy. However, the underlying mechanism is unknown.

Synchronous or metachronous multiple primary carcinomas are found in a subset of patients with LS. It has been previously reported that >60% of patients with LS with rectal cancer develop colon cancer within 30 years (5). The 10-year cumulative risk of endometrial cancer for patients with LS firstly diagnosed with CRC was ~23% (6). Despite this, to the best of our knowledge, there is a lack of a well-established treatment mode, therefore, this case aimed to investigate the therapeutic choice for the rare concurrent urothelial and colon cancers. We reported the response of a patient with LS with metachronous urothelial and colon cancers to pembrolizumab treatment.

Case report

A 38-year-old male presented to the Cancer Hospital, Chinese Academy of Medical Sciences (Beijing, China), complaining

of progressive symptoms, including frequent and urgent micturition, and increased nocturia without apparent causes since February 2015. His maternal grandmother was diagnosed with carcinoma of the rectum at 39 years old. His mother was diagnosed with endometrial cancer at 39 and a metachronous adenocarcinoma of colon at 59 years old. His three maternal uncles were diagnosed with liver cancer. In December 2015, computed tomography (CT) imaging and magnetic resonance imaging (MRI) revealed the left ureteral mass (2.7x2.9 cm) and multiple enlarged lymph nodes. Paraaortic lymph nodes biopsy and histological examination confirmed the lesions to be urothelial carcinoma, and the tumor stage was evaluated as $T_4N_2M_X$ with suspected lung metastasis according to the 2004 World Health Organization classification (7). The patient received 4 cycles of gemcitabine and cisplatin chemotherapy between January 2016 and March 2016. CT scans in July 2016 suggested that the tumor became larger (4.5x3.8 cm). Therefore, the patient was treated with paclitaxel liposome combined with tegafur, gimeracil, and oteracil potassium capsules between July 2016 and August 2016. In September 2016, the patient underwent regular chest and abdomen/pelvis CT scans, and the results showed local thickening of sigmoid colon wall (the thickest part was ~1.6 cm, and ~6.7 cm in length) and multiple enlarged lymph nodes on the outside of the intestinal wall. Further colonoscopy revealed an ulcerative tumor on sigmoid colon. The hematoxylin and eosin (H&E)-stained slides were reviewed by pathologists to confirm the diagnosis of moderately differentiated adenocarcinoma (Fig. 1A). Briefly, tissue samples were placed in 10% formalin for 24 h at room temperature and embedded in paraffin. Immunohistochemical staining (IHC) was performed using automated standard procedures (EnVision™ Flex+ Detection System; cat. no. K8002; Dako; Agilent Technologies, Inc.). The standardized protocol supplied by the manufacturer was followed. The following primary antibodies were used: mutL homolog 1 (MLH1; cat. no. MAB-0642), mutS homolog 2 (MSH2; cat. no. MAB-0291), mutS homolog 6 (MSH6; cat. no. MAB-0643), PMS1 homolog 2, mismatch repair system component (PMS2; cat. no. MAB-0656), PD-1 (cat. no. MAB-0734), cytokeratin 20 (CK20; cat. no. Kit-0025), CD7 (cat. no. Kit-0021) and IL-12 P40 monomer (P40; cat. no. RMA-0815; all from Fuzhou Maixin Biotech Co., Ltd.), HER-2 (cat. no. 790-4493), BRAF-V600E (cat. no. 790-4855) and PD-L1 (cat. no. 741-4905; all from Roche Diagnostics) and caudal type homeobox 2 (CDX2; cat. no. ZA-0520; OriGene Technologies, Inc.). All antibodies were ready-to-use and were used without dilution. The IHC staining demonstrated that the tumor was positive for HER-2 (+), CDX2) (3+) and CK20 (3+), and was negative for CD7 and P40, which is significantly different from that of metastatic lymph nodes of urothelial carcinoma (HER-2: ++; fluorescence *in situ* hybridization revealed no amplification; CDX2: -; CK20: ++; CD7: +++; P40: +++). In addition, BRAF-V600E, PD-1 and PD-L1 for colon were not expressed. For MMR proteins, MSH2 was negative, however this was not the case for MLH1, MSH6 and PMS2 (Fig. 1). A diagnosis of primary moderately differentiated sigmoid adenocarcinoma was made.

Taking into consideration the clinical diagnosis, the MSH2-deficiency and the overwhelming family history,

the patient was suspected to harbor a germline mutation in MMR genes, especially in *MSH2*. A genetic test was performed using DNA from the patient's peripheral blood by next-generation sequencing (Geneplus-Beijing Institute). As a result, 43 somatic mutations were identified and a germline variant was detected on *MSH2* (Table SI; Fig. 2A). The *MSH2* c.1759+1G>T germline variant is a novel splice site mutation that may affect splicing process and result in a defective MSH2 protein (8,9). According to the American College of Medical Genetics and Genomics guidelines (10), the variant was judged as a likely pathogenic mutation. Sanger sequencing was performed to confirm the presence of *MSH2* c.1759+1G>T variant as previously described (11) (Fig. 2B). Therefore, the patient was finally diagnosed as MSH2-associated LS with urothelial and colon cancer. As the sigmoid colon lesions were observed 6 months after the diagnosis of urothelial carcinoma, it should be defined as metachronous carcinoma.

After 3 cycles of chemotherapy (irinotecan and pemetrexed) combined with bevacizumab treatment, the tumors progressed. The patient was subsequently administered pembrolizumab treatment (200 mg every three weeks) in December 2016. Compared with the lesions before pembrolizumab treatment (Fig. 3A and D), the imaging examinations in March 2017 suggested that the sigmoid colon lesion slightly increased and the ureteral lesion had progressed (Fig. 3B and E). In view of the notable level reduction of tumor markers detected by electrogenerated chemiluminescence (Table I), the patient was suspected to have pseudoprogression and therefore continued pembrolizumab treatment for another 4 months. The PET-CT performed in July 2017 showed the shrinkage of the left ureteral tumor. By contrast, progression was present on the sigmoid colon wall (Fig. 3C and F). According to the Response Evaluation Criteria In Solid Tumors v1.1 (12), the sigmoid colon lesion was evaluated as stable disease (SD) at the first evaluation, and progressive disease (PD) at the second evaluation, while the ureteral lesion was assessed as PD at the first evaluation and partial response (PR) at the second evaluation. Taking into consideration the unsatisfactory efficacy, the patient then returned to his local hospital to receive symptomatic and supportive treatment.

Discussion

LS is an autosomal-dominant disease caused by a pathogenic germline mutation in a DNA MMR, including *MLH1, MSH2, MSH6, PMS2,* or *EPCAM* gene (13,14). Individuals with LS may have increased risk for many types of cancer. Particularly, the lifetime risk of colorectal cancer and endometrial cancer are 42 and 35%, respectively (13,14). LS is also associated with an increased risk of gastric cancer, ovarian cancer, hepatobiliary tract cancer, urinary tract cancer, small bowel cancer, brain cancer, pancreatic cancer, as well as sebaceous neoplasms (13,14). *MSH2,* being the most frequently mutated gene, has a cumulative incidence of ureter and kidney cancers for patients with MSH2-associated LS of 17.8% by 75 years old (15). LS-associated hepatocellular carcinoma, although not common, was reported in a number of cases (16,17). In this case, a germline *MSH2* mutation was identified in a patient with metachronous urothelial and colon cancer. The cancerous manifestations of the patient and his family members were all
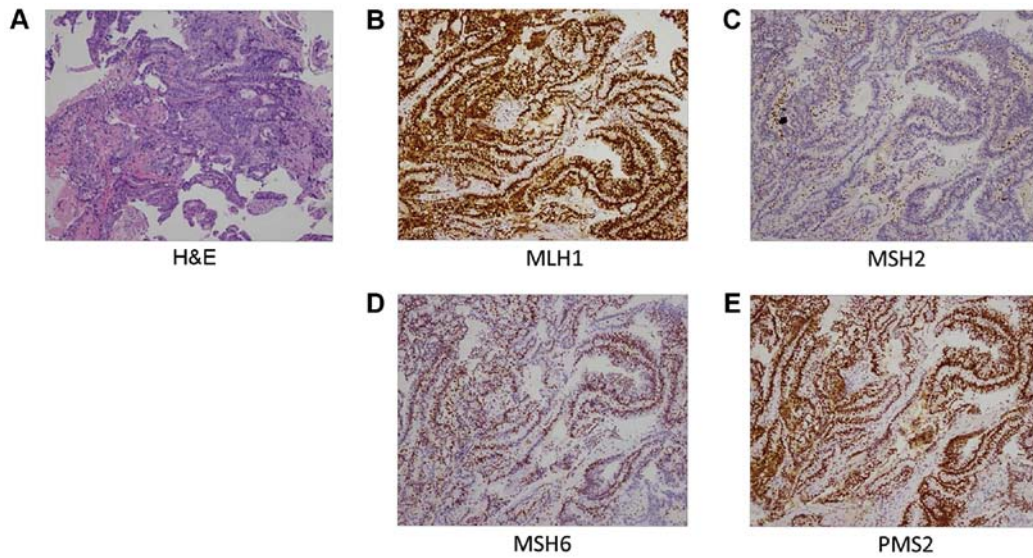
Figure 1. H&E and immunohistchemical staining of the sigmoid colon lesion. (A) H&E and immunohistochemical staining of (B) MLH1, (C) MSH2, (D) MSH6 and (E) PMS2 of the sigmoid colon lesion. MLH1, MutL homolog 1; PMS2, PMS1 homolog 2, mismatch repair system conponent; MSH6, mutS homolog 6; MSH2, MutS homolog 2. Magnification, x100.
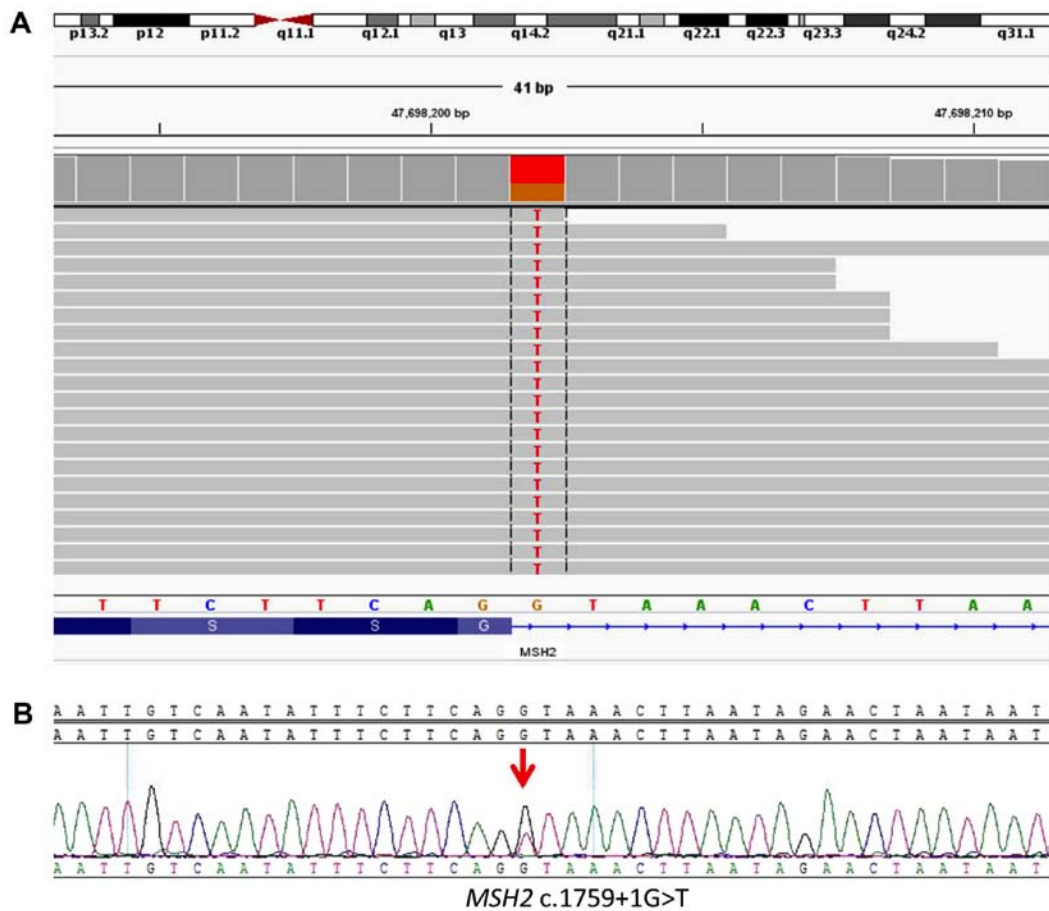

Figure 2. *MSH2* germline mutation identified by NGS and confirmed by Sanger sequencing. (A) The reads map of *MSH2* c.1759+1G>T germline mutation detected by NGS. (B) Sanger sequencing of the *MSH2* c.1759+1G>T germline mutation for this patient, indicated by the red arrow. MSH2, MutS homolog 2; NGS, next-generation sequencing.

considered as part of the tumor spectrum of LS, though this case was unable to get access to other family members' DNA samples.

The traditional two-hit model is widely used to describe the genesis of LS. A germline loss-of-function mutation accompanied with somatic inactivation of the other allele

Table I. Tumor markers of the patient between November 2016 and July 2017 as detected by electrogenerated chemiluminescence.

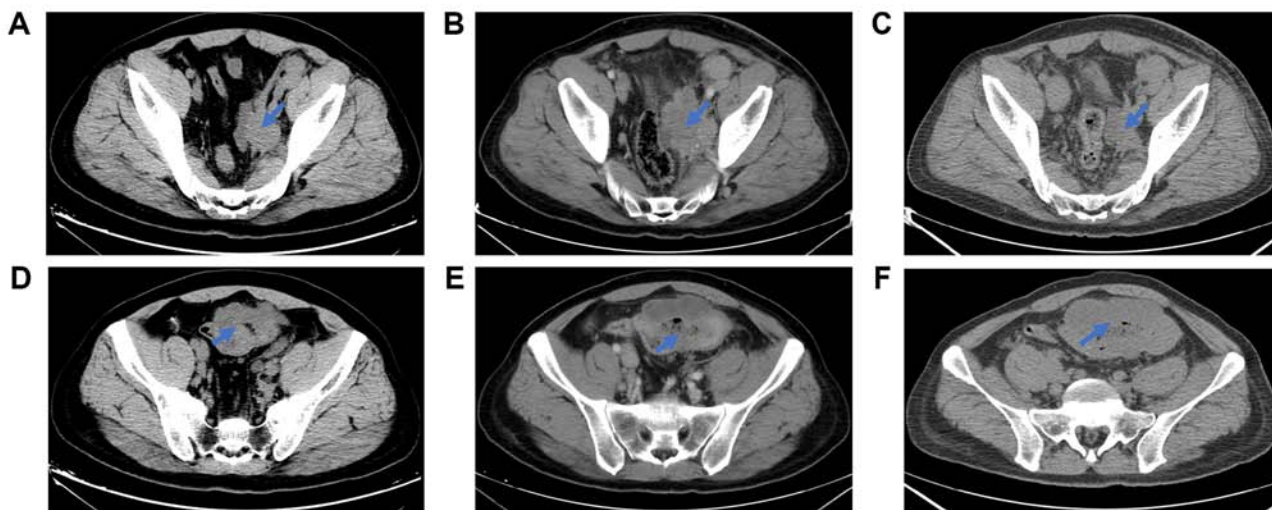| Markers (unit)	11/28/2016	03/20/2017	07/05/2017	Upper limit of the normal range
CA19-9 (U/ml)	307.9	133.3	116.7	37
CA72-4 (U/ml)	82.04	41.84	56.48	9.8
CEA (ng/ml)	216.2	159.5	327	5

Figure 3. Image examinations demonstrate the changes of left ureteral and colon tumors during the period of pembrolizumab treatment. The arrow marks the location of the tumors. (A) represents the changes of left ureteral tumors before pembrolizumab treatment, (B) at 4 months after treatment and (C) at 8 months after treatment. (D) represents the changes of colon tumors before pembrolizumab treatment, (E) at 4 months after treatment and (F) at 8 months after treatment. The colon wall increased in thickness steadily, while the ureteral lesion increased in size at 4 months, and markedly decreased at 8 months.

promotes tumorigenesis (18). In this case, the patient carried a germline *MSH2* mutation, while no *MSH2* somatic mutation was detected. Biallelic inactivation of *MSH2* may be caused by other mechanisms, such as loss of heterozygosity (19). IHC revealed the loss of expression of MSH2 protein and made the conclusion of dMMR.

Given that MMR deficiency is a validated biomarker for immunotherapy (2,20), the patient switched to pembrolizumab treatment after failure of chemotherapy. The lesion on the sigmoid colon appeared to have no response to pembrolizumab with continuous aggravation, whereas the ureteral lesion was evaluated as PD at the first response evaluation, and as PR at the second evaluation, which was considered as pseudoprogression.

Pseudoprogression is an immunotherapy-associated phenomenon. Increased tumor size, revealed by imaging examination, may reflect tumor cells that are infiltrated with lymphocytes and macrophages (21). These patients may benefit from immunotherapy, however may switch to other treatment regimens based on the imaging evaluation. These inflammatory-based processes have prompted the development of immune-related response criteria (irRC), which can identify 10% of patients with pseudoprogression (22). However, recent studies demonstrated that circulating tumor DNA monitoring can accurately distinguish pseudoprogression from true progression in patients with lung adenocarcinoma and melanoma treated with immunotherapy (23,24).

The response to PD-1 inhibition varies among different tumor types (25). The efficacy of pembrolizumab in patients with previously treated urothelial carcinoma was evaluated in study KEYNOTE-045. For patients treated with pembrolizumab, ORR was 21% and overall survival time was 10.3 months (26). A phase 2 clinical study evaluated the efficacy of pembrolizumab in metastatic carcinomas with or without dMMR (19). ORR for dMMR colorectal cancers was 40%. For MMR proficient colorectal cancers, ORR was 0 (19). The objective response rate of pembrolizumab in MSI-H or dMMR non-CRC cancers (36%) is higher than in patients with CRC (46%) (4,25). In this case, no response was observed on sigmoid colon lesion, while the ureteral lesion achieved PR after pseudoprogression Immunotherapy-associated biomarkers may contribute to the different responses. IHC analysis of colon lesion showed negative expression of MSH2 and PD-L1. However, the expression of these proteins in ureteral lesion was unknown and no tumor tissue was left to allow the performance of PD-1/PD-L1 IHC staining. One research investigated the states of MSI, PD-L1 and TMB in 11,348 patients with cancer (27). High MSI, PD-L1 and TMB were identified in 5.7, 6.7 and 7.2% of colorectal adenocarcinoma (1,395 patients), and in 0.0, 16.8 and 42.7% of bladder cancer (143 patients) (27). Compared with bladder cancer, the percentage of MSI was higher in colorectal carcinoma. On the contrary, TMB-H and PD-L1 positivity were present

more often in bladder cancer, which may explain the different responses of the two primary sites (27).

This case presents the treatment course in a challenging case of a patient with MSH2-associated LS manifested with metachronous ureteral urothelial cancer and colon adenocarcinoma. Taking into consideration dMMR confirmed by IHC staining, the patient was treated with immune checkpoint inhibitor pembrolizumab, after disease progression on chemotherapy. The response patterns of the two primary lesions to pembrolizumab differed. This case report discussed the potential explanations underlying this phenomenon; however, further clinical investigations are required.

Availability of data and materials

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

Authors' contributions

YF, YC and XH were major contributors in writing the manuscript. YF and XH were responsible for the analysis of clinical information. MY and RC were responsible for genetic analysis and literature review. MY edited the manuscript. YC and XJ conducted imaging examinations and analysis. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Written informed consent was obtained from the patient for publication of the clinical data and any accompanying images.

Competing interests

The authors declare no conflicts of interest.

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