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Pancreatic cancer 'mismatch' in Lynch syndrome

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ABSTRACT

Objective Immune therapy with the PD1 inhibitor pembrolizumab has been approved to treat unresectable/metastatic solid tumours exhibiting mismatch repair (MMR) deficiency. Lynch syndrome (LS), caused by autosomal dominant germline mutations of a MMR gene, predisposes to the development of MMR-deficient cancers. We report a case of *MSH2*-LS with an MMR-intact pancreatic ductal adenocarcinoma (PDAC) ineligible for treatment with pembrolizumab.

Design Immunohistochemistry of MMR proteins was performed in each malignancy developed in a *MSH2*-LS patient to determine MMR status.

Results The patient carried a pathogenic *MSH2* germline mutation and had a history of LS-type cancers, including endometrial carcinoma, colorectal adenocarcinoma, urothelial carcinoma of the bladder and PDAC. Three malignancies (endometrial, colorectal, urothelial) lacked MSH2 and MSH6 expression, consistent with *MSH2*-associated tumorigenesis. However, MSH2 and MSH6 expression were intact in the PDAC, suggesting the sporadic occurrence of the pancreatic tumour unrelated to the germline *MSH2* mutation. These inconsistent MMR statuses among the tumours rendered the patient ineligible for the immunotherapy pembrolizumab.

Conclusion Testing for MMR protein expression is recommended for each tumour in patients with LS, especially pancreatic, as discordant results may have profound effects on treatment opportunities. To our knowledge, this is the first documented case of MMR-intact PDAC in a patient with *MSH2*-LS.

INTRODUCTION

Tandem repeat sequences in the genome, termed microsatellites, accumulate alterations in length due to errors inherent in DNA replication. Generally, these alterations are corrected post-replication by the DNA mismatch repair (MMR) process. Defects in MMR produce a condition of genomic hypermutability, particularly affecting repeat regions and giving rise to the microsatellite instability-high (MSI-H) molecular phenotype.[1] MMR-deficient tumours are identifiable by traditional MSI testing of a designated panel of microsatellite loci by PCR or by immunohistochemistry (IHC) of the MMR proteins to detect loss of expression. More recently, next-generation sequencing (NGS) coupled with bioinformatics approaches have been applied to simultaneously identify the hypermutator (high mutation rate) and MSI phenotypes as biomarkers of MMR deficiency.[2] In 2017, the PD1 inhibitor, pembrolizumab, was approved by the U.S. Food and Drug Administration for the treatment of MMR-deficient cancers, irrespective of site, given the high rates of response to immune therapy specifically among patients with a MMR-deficient cancer.[3 4] This is an encouraging development for patients with MMR-deficient pancreatic cancer, given the typically dismal prognosis. Testing for a biomarker of MMR deficiency has thus become crucial for identifying patients eligible for immune therapy.

Older studies reported high rates of MSI in pancreatic ductal adenocarcinomas (PDACs).[5] However, these studies were limited by small sample sizes and varying methodologies, hence the incidence of MSI in pancreatic cancer may have been overestimated.[6] More recently, the Cancer Genome Atlas study of PDAC found the rate of MSI to be just 0.5%, although the selection of cases for inclusion may have been biased.[7] In a large case series of PDAC in which NGS and bioinformatics algorithms were used to identify the hypermutated and MSI-H phenotypes, in addition to IHC of the MMR proteins, 7/833 (0.8%) cases were identified as MMR-deficient and germline testing of the MMR genes revealed all seven cases had Lynch syndrome (LS).[8]

LS is an autosomal dominant cancer predisposition condition caused by heterozygous germline mutations in one of four MMR genes (*MLH1*, *MSH2*, *MSH6* or *PMS2*) and is characterised by MSI in the tumours.[9] LS confers a high lifetime risk for the development of multiple cancer types, most frequently colorectal and endometrial cancers. Pancreatic ductal adenocarcinoma

has historically been included in the spectrum of LS-associated cancers.[10] There are two lines of evidence in support of this. First, the incidence of pancreatic cancer has been shown to be higher in patients with LS than in the general population. The cumulative risk of pancreatic cancer by age 70 years in patients with LS in the USA was estimated to be 3.7%, 8.6-fold higher than in the general population.[11] Similarly, in a recent prospective study of 3119 European patients with LS, the cumulative incidence of pancreatic cancer by age 75 years was 6.2%, 0.5%, 1.4% and 0%, respectively, for carriers of *MLH1*, *MSH2*, *MSH6* and *PMS2* germline mutations.[12] The second line of evidence is the demonstration of MMR deficiency in the tumour, consistent with a role for the germline MMR mutation in tumorigenesis. A few reports of pancreatic cancer in LS have shown concordant loss of MMR protein staining in the tumour tissue.[2 6 8] Thus, while it is clear that pancreatic cancer does occur in the context of LS, the incidence is rare.

CASE HISTORY

Here, we report a patient with a history of LS-type cancers. The patient was first diagnosed with stage IC endometrial cancer at the age of 44 years and underwent a total abdominal hysterectomy and bilateral salpingo-oophorectomy, followed by radiation therapy and adjuvant chemotherapy. The patient developed a colorectal adenocarcinoma (rectosigmoid) aged 64 and underwent a low-anterior resection. Routine IHC screening of this tumour for MMR deficiency identified dual loss of MSH2 and MSH6 expression, suggesting a primary defect in MSH2. Germline testing at that time identified a pathogenic deletion of exons 4–6 in *MSH2*, which was predicted to result in truncation of the MSH2 protein. When a urothelial carcinoma of the bladder was found at age 66, it was treated definitively with fulguration. In 2017, after presenting with abdominal pain, the patient aged 67 years was subsequently found to have a locally advanced pancreatic adenocarcinoma. CT of the abdomen showed extension into the retroperitoneum and involvement of the mesenteric vasculature, duodenum and mesenteric lymph nodes. Endoscopic ultrasound revealed severe stricture of the duodenum, and biopsy showed ductal adenocarcinoma. Unfortunately, the biopsy volume was inadequate for IHC testing of the MMR proteins or PCR-based MSI testing. After starting chemotherapy, the patient underwent another biopsy of the pancreatic cancer and IHC of all four MMR proteins was performed successfully. Surprisingly, the pancreatic cancer showed intact protein staining of all four MMR proteins, including MSH2 and MSH6 (figure 1). This finding suggested a sporadic occurrence of the tumour apparently unrelated to the germline *MSH2* mutation. Unfortunately, attempts to determine MSI status and mutational burden via NGS failed due to insufficient tumour material in the PDAC biopsy sample, hence we were unable to corroborate the IHC findings

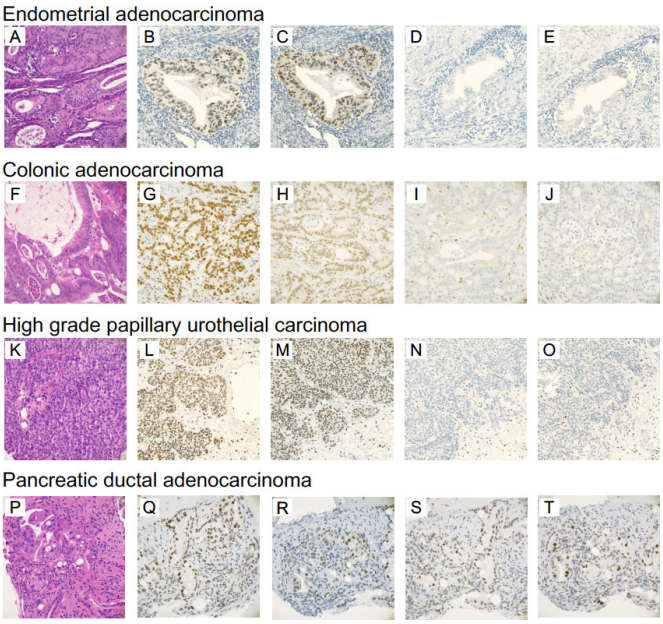

Figure 1 Immunohistochemical staining of the mismatch repair proteins in the patient's tumours. (A–E) Endometrial adenocarcinoma: H&E staining (A), with intact MLH1 (B) and pms2 (C), and dual loss of MSH2 (D) and MSH6 (E) immunostaining in tumour cell nuclei. (F–J) Colonic adenocarcinoma: H&E staining (F), with intact MLH1 (G) and pms2 (H), and dual loss of MSH2 (I) and MSH6 (J) immunostaining in tumour cell nuclei. (K–O) High-grade papillary urothelial carcinoma: H&E staining (K), with intact MLH1 (L) and pms2 (M), and dual loss of MSH2 (N) and MSH6 (O) immunostaining in tumour cell nuclei. (P–T) Pancreatic ductal adenocarcinoma: H&E staining (P) with intact MLH1 (Q), pms2 (R), MSH2 (S) and MSH6 (T) immunostaining in tumour cell nuclei. All photomicrographs taken at ×400 magnification.

with other biomarkers of MMR status. Given the unexpected IHC result in the PDAC, we undertook retrospective IHC testing of the MMR proteins in each of the prior malignancies (endometrial carcinoma, colorectal adenocarcinoma and bladder carcinoma) from the patient. All three other tumours had dual loss of MSH2 and MSH6 staining, consistent with *MSH2*-LS-associated tumorigenesis in them.

An alternative hypothesis is that haploinsufficiency for *MSH2* may have contributed to tumorigenesis of the PDAC. In support of this, reduced expression of *MSH2* mRNA has been shown to significantly decrease MMR efficiency using in vitro assays,[13] to promote cell-cycle progression and decrease the rate of apoptosis in the proliferation of pituitary adenomas,[14] and to cause low-level MSI in patients with LS patients without a cancer diagnosis.[15]

Finally, we considered the possibility that intact IHC staining of the MMR proteins in the PDAC may have represented a false-negative IHC test result (lack of sensitivity to detect true MMR deficiency). Rare instances of intact IHC staining of a MMR protein have been observed in LS-associated tumours despite loss of function of both genetic alleles, if one of the pathogenic mutations was a

missense mutation.[16] This presumably allowed for antibody binding in the IHC test, even if the encoded protein was malfunctional.[16] However, we considered this unlikely in the PDAC of our patient, given IHC staining of MSH6 was also intact. MSH2 and MSH6 are binding partners in the functional MutSα heterodimer, and retention of the MSH6 protein is dependent on stable binding with functional MSH2. If MSH2 function was compromised, loss of staining of its dependent binding-partner MSH6 would be anticipated. Our finding raises the question of which biomarker is optimal for determining MMR status in rarer and extracolonic tumours. In this case scenario, given the PDAC arose in the context of known *MSH2*-LS, and only a limited biopsy sample was available, we considered expression status of MSH2 and its binding partner MSH6 by IHC would be the most informative for determining MMR status.

Irrespective of the mechanistic basis for tumour development, or which test may prove optimal for determining MMR status in limited biopsy samples, the finding of intact MMR protein expression in the PDAC at the time of clinical treatment decision-making deemed the patient ineligible for immune therapy with pembrolizumab. This is, to our knowledge, the first documented case of an MMR-intact pancreatic ductal adenocarcinoma in the setting of *MSH2*-LS, which is associated with high lifetime risks for the full spectrum of Lynch-type cancers. Interestingly, another study has described MMR-intact PDAC in two patients with *PMS2* germline mutations, although one of these patients also carried a germline mutation of *BRCA2*.[8] Germline *PMS2* mutations are associated with lower lifetime risks and later age of cancer onset than *MSH2* mutations, hence sporadic occurrences of cancer in *PMS2* mutation carriers may be less surprising. Based on our experience, we recommend that each tumour in patients with LS be tested for MMR protein expression, as discordant results may have profound effects on treatment opportunities.

Contributors AEH wrote the manuscript, performed literature searches, cared for the patient, obtained consent for images to be used herein and provided the case history. BKL and RR prepared the figures and analysed the histological images. MG contributed to parts of the manuscript and performed literature searches. JG, VP, RT and MH edited the manuscript prior to submission.

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