An Unusual Immunohistochemical “Null” Pattern of Four MMRs Proteins of Gastric Cancer and Literature Review

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Case report

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Abstract

Background: Immunohistochemical (IHC) stainings for the mismatch repair (MMR) proteins are useful methods for the treatment and prognosis in gastric cancer. Different IHC staining patterns reflect the complex biological phenomena underlying MMR deficiency. We herein report a rare IHC staining pattern of four MMR-related proteins in gastric cancer.

Case presentation: An “null” IHC staining pattern of four MMR-related proteins in a 67-year-old man with gastric cancer, including MLH1, PMS2, MSH2, and MSH6. The results remained unchanged after repeated tests. We find the promoter hypermethylation of MLH1 subsequently. Moreover, next-generation sequencing showed that the four genes exhibited changes. One of these was the somatic mutation of the missing copy number in exon 14 of MSH2. Mutation analysis using peripheral blood showed no germline mutation in the these four genes. The patient had no personal or family tumor history. After finishing the above work, we classified this case as sporadic case. The patient returned to normal after the operation. 9 months after operation, patients were admitted to hospital and no signs of tumor metastasis and recurrence were found. After 6 cycles of adjuvant chemotherapy, the patients and was discharged home in a stable condition.

Conclusions: It is greatly important for the patient and their relatives to improve the understanding of abnormal IHC findings and the biological significance by clinicians. This observation revealed a rare but potential phenomenon in assessing MMR proteins, and explained this was a sporadic case that needed no clinical management implications for his family.

Introduction

Gastric cancer is one of the most common cancers and a main cause of cancer deaths worldwide. Its molecular and clinical characteristics are complicated by histological and etiological heterogeneity. Gastric adenocarcinomas are divided into four subtypes according to their molecular features: tumors exhibiting chromosomal instability, microsatellite instability high (MSI-H), Epstein–Barr virus (EBV) positivity, and genomic stability [1, 2]. Microsatellite instability (MSI) and molecular typing are important in the treatment and prognosis of gastric cancer [3, 4].

The maintenance of mismatch repair (MMR) pathway plays an important role in maintaining accurate DNA replication and genome stability. Base pair insertion or loss occurs in the microsatellite region due to mismatch repair system defects, the replication errors cause MSI [5, 6]. Four representative MMR-related proteins, including MLH1, PMS2, MSH2, and MSH6 tested by Immunohistochemistry(IHC), can direct testing to that specific gene and assist in the identification of patients with Lynch syndrome (LS). LS is an autosomal dominant disease caused by germline mutations of MMR related genes [7]. IHC is a convenient and affordable method for MSI analysis, and it has high sensitivity and specificity. Different IHC staining patterns reflect the complex biological phenomena underlying MMR deficiency. Therefore, it is greatly important to improve the understanding of abnormal IHC findings and their biological significance. We found an abnormal and rare expression of IHC in four MMR-related proteins by an unusual “null” pattern, and these MMR-related genes exhibited changes tested by gene testing.

Case Presentation

We present the case of a 67-year-old man with upper abdominal discomfort and vomiting for 4 months. He had no personal or family history of other tumor. Gastroscopy showed a large mass on gastric body. Total gastrectomy was performed. The result showed a 5 cm T3N3aM0 adenocarcinoma.

Paraffin-embedded block of gastric cancer was performed for pathologic diagnosis, IHC, and molecular analysis. The tumor showed an infiltrative growth pattern in gross (Fig. 1A). Histologically, the tumor was poorly differentiated. It has a solid pattern with necrosis. It was heterogeneous with glandular differentiation and prominent tumor-infiltrating lymphocytes (Fig. 1B and C). The tumor cells were positive for low-molecular-weight cytokeratin (AE1/AE3, Ventana) (Fig. 1D). Combined positive score (CPS) of Programmed death ligand 1 (PD-L1, 22C3, Dako) was high (Fig. 1E). EBV-encoded small RNA (EBER) was negative by in situ hybridization (Fig. 1F). MMR proteins, including MLH1 (M1, Ventana), PMS2 (A16-4, Ventana), MSH2 (G219-1129, Ventana), and MSH6 (SP93, Ventana) were analyzed by IHC. All these four proteins were completely negative in the tumor cells but positive in the positive control, stromal, and inflammatory cells. The results of IHC were coincident of other paraffin blocks (Fig. 2). HER2 (4B5, Ventana) was 1+, Ki-67 (30 – 9, Ventana) was 60%.

Subsequently, the methylation-specific polymerase chain reaction assay of the MLH1 promoter region was performed. There was promoter hypermethylation of MLH1. Many studies had shown that MSI-H is associated with MLH1 promoter methylation. However, the presence of MSI-H is not certain in gastric cancer patients with MLH1 promoter methylation, and not all patients with MSI-H gastric cancer have MLH1 promoter methylation [8, 9].

Simultaneously, next generation sequencing (NGS), which included 41 genes, was performed and demonstrated that MLH1, MSH2, MSH6, and PMS2 genes exhibited changes. There were the mutation of the clipping region in exon 12 of MLH1 (c.1039-13_1039-8del), missense mutation in exon 11 of PMS2 (c.1799T > C, p.Met600Thr), missing copy number in exon 14 of MSH2 (Fig. 3), and missense mutation in exon 4 of MSH6 (c.2693C > A, p.Pro898His). This mutation of MSH2 may lead to the shift of the subsequent coding frame of MSH2 gene, and then lead to the loss of protein expression. Mutation analysis using peripheral blood showed no germline mutation in the these four genes. MLH1 promoter methylation and the loss-of-function mutation of MSH2 support to MSI-H. The patient had no personal or family tumor history indicated that this MMR deficiency was highly likely sporadic in nature.

The tumor showed a high number of other mutations, including the copy number amplified by PIK3CA gene (Fig. 3). It may lead to up regulation of PIK3CA gene expression. The PTEN gene had a frameshift mutation in exon 7 (c.800del, p.Lys267fs) and nonsense mutation in exon 5 (c.388C > T, p.Arg130). The exon began to shift from the 267 amino acid lysine in exon 7. It is likely that terminators will be introduced into the new reading frame. The 130 amino acid of exon 5 was mutated from arginine to terminator. These two premature terminator may lead to meaningless mRNA degradation, resulting in protein loss. It had a missense mutation of p.g245s of the TP53 gene in exon 7, and mutations in the shear region of the ATM gene in exon 6 (c.497-5_497-4del) and MET gene in exon 12(c. 2584-13_2584-9del). The genes of PTEN, TP53, and ATM were identified as significantly mutated in all types of adenocarcinomas, but the mutation of the MET gene is relatively rare in gastric cancer [1]. Its EBER was negative. An increased frequency of mutant-pattern TP53 expression was found in EBV-negative carcinomas, consistent with this case. This case belongs to MSI-H of four subtypes of gastric adenocarcinomas [1]. PD-L1 is highly expressed due to the presence of more lymphoid stroma.

The patient returned to normal after the operation. 9 months after operation, patients were admitted to hospital and no signs of tumor metastasis and recurrence were found. After 6 cycles of adjuvant chemotherapy, the patients and was discharged home in a stable condition.

Discussion

This “null” IHC staining pattern of all four MMR proteins was first reported in colorectal cancer by Hagen [10] due to germline MSH2 mutation and somatic MLH1 hypermethylation in a 71-year-old woman with LS. The morphology under the microscope was similar to our case, but the gene expression was inconsistent. The patient had personal tumour history of colon cancer and ureteral cancer. Besides, she also had a strong family tumour history. All these demonstrated this was a patient with LS. But our case supported sporadic case.

Wang [11] et al deemed the four MMR proteins deficiency in an 80-year-old woman with colorectal cancer was highly likely sporadic, and no high-risk surveillance protocols were recommended to the patient or her family members. Its morphology and gene expression were similar to our case. They found promoter hypermethylation of MLH1 and double somatic truncating mutations in MSH2. They also found BRAFV600E mutation. We found promoter hypermethylation of MLH1 and missing copy number in exon 14 of MSH2. Westwood [12] found the the percentage of additional Partly or completely loss of MSH2 and MSH6 in MLH1-deficient CRC of patients was 0.48% (4/829). In addition, there only one case showed a complete null expression of MMR proteins by IHC in colorectal cancer, but it showed strong staining for all four proteins in rectal cancer. Unfortunately, there only biopsy was available for testing in colorectal cancer. Further genetic analysis showed MLH1 promoter hypermethylation and BRAFV600E mutation. Summary of “null” IHC staining pattern of all four MMR proteins reported in the literature can be seen in Table 1.

Research showed MSI tumors with MLH1 methylation were associated with BRAFV600E mutation only in the colon, not in the stomach [13]. Our case proved this point, the BRAFV600E mutation was not detected in our case. In addition, no abnormalities were found at the detection site of KRAS, NRAS, and HRAS. It's also different from colon cancer.

All of the above three literatures were found in colorectal cancer, none in gastric cancer. Junhun [14]. found 5 cases showed all four MMR proteins negative and none showed a single MMR protein loss of 580 cases by IHC, further genetic testing was not carried out except PCR tests of MMR genes. But We retrospectively collected 2808 cases of postoperative gastric cancers from the Fourth Hospital of Hebei Medical University, from May, 2017 to August, 2020. Only this case was completely negative. The current incidence is 0.0356%. And 15 cases showed only negative of PMS2, and 3 cases only negative of MSH6. What are the reasons for the differences? Junhun used the standards that completely loss or < 20% focal weak equivocal nuclear staining. We consider that the negative judgment standards used for our cases were more strict, so the total negative case is more rare. In addition, we tested more cases, so we found the cases with single negative of MMR protein. In another 464 cases of gastric cancer study, the co-negative percent of MLH1 and MSH2 was 4.4% [15]. They used tissue microarray for test. However, there was a potential heterogeneity in the use of tissue microarray. The in-depth genetic testing was not performed. Therefore, our case first revealed this rare IHC staining pattern of four MMR proteins in gastric cancer and showed the difference of gene expression with colorectal cancer. We do not think it is a common phenomenon in gastric cancer, even has not been reported in detail in the stomach before.

The patients with MSI-H gastric cancer frequently are older, majority of them are women, the cancer is mostly in the distal part of the stomach, most of them are intestinal type in Lauren's classification, and less lymph node metastasis. They have lower recurrence rate, and the best prognosis compared with other subgroups [1]. But our patient is old man, the cancer located near cardia. Although the tumor are heterogeneous, but the intestinal type part of Lauren's classification only occupied a small part in the tumour. Kawazoe [16] showed that PD-L1 expression (22C3) in immune cells (ICs) was associated with EBV positivity and lymph-node metastasis. Our case showed the expression of PD-L1 only on ICs and high CPS with EBV negative and lymph-node metastasis. MSI status is considered to be a practical alternative to immunotherapy response. The immunotherapy is recommended for this MSI-H patients [17]. But the patient did not receive immunotherapy. However, the patient's condition is still stable after 9 months, and there was no sign of recurrence and metastasis. Besides, this patient had the large tumor size, many lymph node metastasis and the specificity of gene expression. These are unusual compared with others, so the patient was asked to follow up regularly just to be on the safe side.

In summary, we reported a sporadic case with unusual MMR IHC and genes pattern in gastric adenocarcinoma, needed no clinical management implications for his family. The intimate understanding of the abnormal expression of MMR is helpful for individualized follow-up treatment.

Abbreviations

IHC: Immunohistochemical; MMR: Mismatch repair; MSI: Microsatellite instability; MSI-H: Microsatellite instability high; EBV: Epstein–Barr virus; LS: Lynch syndrome; NGS: Next generation sequencing; CPS: Combined positive score; PD-L1: Programmed death ligand 1; EBER: EBV-encoded small RNA; IC: immune cells

Declarations

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Authors' contributions

YM and LQ collected the patient's clinical data, YM performed literature review, and drafted the whole manuscript. LJ assisted in data collection and literature review. LY helped YM with revision of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

This was approval by the ethics committee of the Fourth Hospital of Hebei Medical University.

Conflict of interest

The authors declare that they have no competing interests.

Consent for publication

Written consent was obtained from the the patient for publication of this case report and accompanying photos and images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

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Tables

Table 1 Summary of Clinicopathological features of null MMR IHC staining pattern reported in the literature.								
Case No.	Age	Sex	Location	MLH1 promoter methylation status	MSH2 status	BRAF status	Specimen tested	Reference
1	72	female	colorectal cancer	hypermethylation	germline G587R mutation	negative	Resection	Hagen et al., 2011[10]
2	80	female	colorectal cancer	hypermethylation	three somatic mutations (MSH2 c.1861 C>T (p.R621*) in exon 12, c.298G>A (p.V100I) and c.633dupG(p.K212Efs*20)	mutation	Resection	Wang et al., 2017 [11]
3	76	male	Ascending colon	hypermethylated	unknown	mutation	Biopsy	Westwood et al., 2019[13]

Figures

Figure 1

A-F Pathological characteristics. Gross appearance of tumor (A), Microscopic appearance of tumor with necrosis (B), Microscopic appearance of tumor with solid pattern and lymphatic stroma (C), The tumor cells are positive for cytokeratin AE1/AE3 by IHC (D), High expression of PDL1(22C3) CPS>1 (E), Epstein Barrvirus encoded small RNA(EBER) (F) was negative (figures at 100-200× magnification)

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Figure 2

A-D The expression of MMR-related proteins by IHC. The tumor cells are completely negative of MLH1(A), PMS2(B), MSH2(C) and MSH6(D), but with positive staining in stromal and inflammatory cells (all figures at 200× magnification)

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