MLH1 Exon 12 Gene Deletion Leading to Lynch Syndrome: A Case Report

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Established Facts

- The most common causes of Lynch syndrome (LS) include nonsense and frameshift mutations. These mutations lead to termination codons in mismatch repair (MMR) genes, followed by a large number of deletions or insertions.
- Data from the NCCN guidelines support the use of checkpoint inhibitor therapy as first-line treatment for deficient MMR (dMMR) CRC. The NCCN guidelines also state that dMMR is a therapeutic biomarker for checkpoint inhibitor therapy and this therapy should be applied to dMMR patients only.

Novel Insights

- In this report, we demonstrate that c.1057_1060delGCTG, an MLH1 gene mutation never reported before, can result in abnormal splicing of the MLH1 gene during the development of LS. This patient died of multiple organ failure soon after undergoing checkpoint inhibitor therapy. We recommend checkpoint inhibitor therapy as the first-line treatment for LS patients.

Keywords

Lynch syndrome · Colorectal cancer · Mismatch repair · Germline mutation · PD-1 inhibitors

Abstract

Introduction: Deleterious heterozygous mutation of the MLH1 gene is an important cause of Lynch syndrome (LS), an autosomal dominant cancer caused by functional defects in the DNA mismatch repair (MMR) complex. **Case Report:** The proband was a 35-year-old patient with confirmed colorectal cancer (CRC). Immunohistochemical (IHC) staining revealed the absence of MLH1 and PMS2 expression in the colorectal tissue specimens of the patient. Genetic counseling and tumor gene testing were performed using next-generation sequencing technology. The genetic tumor verification report showed the deletion of 4 bases in exon 12 of

the tested MLH1 gene and a transcoding mutation. To our knowledge, this germline splice site mutation of MLH1 has not been reported before. The proband accepted several therapeutic regimens including PD-1 inhibitor and ultimately died of multiple organ failure. **Conclusion:** Nonsense mutations and frameshift mutations of MMR genes are the most common causes of LS. Common mutations include those in MSH2, MLH1, MSH6, and PMS2. We report a mutation of MLH1 that has never been reported before. We recommend that patients with a history of colon or rectal cancer receive universal MMR or MSI testing and checkpoint inhibitor therapy for the first-line treatment of deficient MMR CRC.

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Shiyun Cui and Xiao Zhang should be regarded as joint first authors for their equal contributions.

Introduction

Lynch syndrome (LS; hereditary nonpolyposis colorectal cancer [CRC]) is one of the most common causes of CRC. Hereditary CRC syndrome has a high penetrance and low incidence, accounting for 5% of CRC cases diagnosed annually [1]. However, many carriers with a high

risk of disease have not been identified, and it is estimated that in the USA, with appropriate screening, approximately 12,000 people can be diagnosed with hereditary nonpolyposis colon cancer (HNPCC) each year [2].

LS is acquired through autosomal dominant inheritance and it is caused by a germline mutation of one of the mismatch repair (MMR) genes. Common mutations

Fig. 1. Hematoxylin-eosin (HE) staining and IHC staining of colon cancer tissue specimens. Magnification $\times 100$. **A** HE staining. **B** Negative MHL1 expression. **C** Positive MSH2 expression. **D** Positive MSH6 expression. **E** Negative PMS2 expression. **F** IHC staining of PD-1.

include those in MSH2 [3], MLH1 [4], MSH6 [5], and PMS2 [6]. The MLH1-encoded protein can be heterodimerized to form MutL- α with the MMR endonuclease PMS2, which is part of the DNA MMR system [7]. When MutL binds to MutS and some helper proteins, the PMS2 subunit of MutL introduces a single-strand fracture near the DNA mismatch, providing an entry point for exonuclease degradation. The encoded proteins also participate

in DNA damage signal transduction and can form MutL, which is involved in meiosis after heterodimerizing with the DNA MMR protein MLH3. These MMR genes are thought to be a common site of mutations in HNPCC. Here, we used second-generation high-throughput sequencing technology to analyze variants (including point mutations and deletion/insertion mutations) in exons of genes related to inherited digestive tract tumors and their adjacent 10-bp intron regions (within 20 bp) in the proband and found a mutation of MLH1 that had not been reported previously. The patient refused to disclose private information, so we anonymized the information. This case report was prepared following CARE guidelines [8].

Case Report

The patient underwent radical resection of colon cancer at the age of 35 years on January 12, 2018, at our hospital. Pathological detection revealed poorly differentiated cancer, and the cancer tissue infiltrated the entire wall of the intestine to the serous layer (Fig. 1a). The pathological stage was pT4a pN2b pMx IIIC. The family tree is shown in Figure 2. His father was found to have pancreatic cancer at the age of 58 years, but he had no history of colorectal or stomach cancer. His mother and maternal uncle developed colon carcinoma at the age of 52 and 47 years, respectively.

Fig. 2. Pedigree of the patient. The arrow indicates the proband. Squares and circles denote males and females, respectively. Closed symbols indicate persons with cancer.

Fig. 3. CT images of the patient. **A** First progression. **B** Second progression.

Fig. 4. A Results of gene detection (Sanger sequencing). The frameshift mutation c.1057_1060delGCTG (p.gly-354profs *12) was detected in the MLH1 gene of the proband (arrows). **B** Outcomes of high-throughput genome sequencing; the red vertical line represents gene mutation sites.

ly. The patient accepted oxaliplatin (225 mg on day 1) and capecitabine (3 tablets b.i.d. on days 1–14) for 4 cycles as postoperative therapy.

After 4 cycles of chemotherapy, the patient's condition was good, but examination of tumor markers from the serum on April 16, 2018, implied cancer progression. The CEA level was 33.38 ng/mL (it should be <4.70 ng/mL). A CT scan performed on May 10, 2018, also revealed the progression of cancer (Fig. 3a). Examination of CEA and CA724 levels verified progression, with a CEA level of 390.50 ng/mL and a CA724 level exceeding 300 U/mL. Because he met the criteria of the revised Bethesda guidelines for LS screening, the patient was referred for genetic counselling and provided written informed consent for the genetic analysis. The expression of MLH1, MSH2, MSH6, and PMS2 proteins in the resected tumor was analyzed by immunohistochemistry. Immunohistochemical (IHC) staining demonstrated the loss of MLH1 and PMS2 expression in colon cancer tissue specimens obtained from the patient (Fig. 1b, e) and revealed deficient MMR (dMMR). Then, we used second-generation high-throughput sequencing technology to analyze variants (including point mutations and de-

letion/insertion mutations) in the exons of genes related to hereditary digestive tract tumors and their adjacent 10-bp intron region variants (within 20 bp) in the proband and found the frameshift mutation c.1057_1060delGCTG (p.gly354profs *12) (Fig. 4b). Sanger sequencing was performed to verify the mutation (Fig. 4a). Four bases in exon 12 of the tested MLH1 gene were deleted, and a frameshift mutation occurred at position 365, which led to early termination of the coding protein and resulted in a truncated polypeptide chain; the normal gene encodes 756 amino acids. The functional and clinical significance of this mutation has not been reported in the literature. The same mutation was also found in blood samples of the patient's mother and uncle but not in the patient's aunt, who appeared to be cancer free (Fig. 5).

According to NCCN guidelines, we recommended PD-1 inhibitors for treatment [9]. IHC staining of PD-1 is shown in Figure 1f. The patient refused treatment because of his financial status and alternatively accepted second-line chemotherapy (bevacizumab, 300 mg i.v.d. on day 1, irinotecan hydrochloride at 300 mg i.v.d. on day 2, and 5-FU at 0.5 g on day 1 + 3.5 g on day 2; 46 h i.v.d.) [10] beginning on May 17, 2018. The CT scan on July 9, 2018, re-

Fig. 5. The results of family gene detection were analyzed by Sanger sequencing. **A** Heterozygous (Het) mutations were observed in the proband. **B** Het mutations were observed in the proband's uncle. **C** Genetic tests on the proband's maternal aunt showed normal results. **D** Het mutations were observed in the proband's mother. The red arrows show the Het mutation c.1057_1060delGCTG (p.gly354profs *12).

Table 1. Organization of the case into a timeline

Date	[T]/[S]/[E]
January 12, 2018	[T] Radical resection of colon cancer
January 2018	[E] Pathological detection
February 2018	[T] Four cycles of chemotherapy (oxaliplatin and capecitabine)
April 16, 2018	[E] Six items of tumor markers from serum (AFP, CEA, CA199, CA724, CYFRA21-1, and NSE), CEA 33.38 ng/mL, NSE 22.67 ng/mL
May 10, 2018	[E] CT detection revealed progression [E] Tumor markers from serum, CEA 390.50 ng/mL, CA724 exceeded 300 U/mL
May 2018	[E] Immunohistochemical staining, loss of MLH1 and PMS2 expression [E] Second-generation high-throughput sequencing and the Sanger method revealed the frame-shift mutation c.1057_1060delGCTG (p.gly354profs *12)
May 17, 2018	[T] Chemotherapy (bevacizumab, irinotecan hydrochloride, and 5-FU)
July 9, 2018	[E] CT scan revealed progression [E] Tumor markers from serum, CEA 411.80 ng/mL, CA724 253.50 U/mL
July 16, 2018	[T] PD-1 inhibitors therapy
July 23, 2018	[S] The patient died of multiple organ failure

[T], treatment; [S], symptoms; [E], examination.

vealed progression again (Fig. 3b), consistent with the elevated levels of CEA and CA724, which were 411.80 ng/mL and 253.50 U/mL, respectively. The patient finally accepted PD-1 inhibitor therapy (pembrolizumab at 200 mg q3w) on July 16, 2018. However, 1 week later, the therapy failed to stop tumor progression and the patient died of multiple organ failure (Table 1).

Discussion

The most common causes of LS include nonsense mutations and frameshift mutations, which lead to the early appearance of termination codons in MMR genes, followed by a large number of deletions or insertions [11]. The spliceosome (5'-gctg-3') affects the splicing of mRNA, causing a change in the peptide chain from Gly at 354 and termination of the peptide chain at 365 (it should have been 756 amino acids). In this report, we demonstrated that c.1057_1060delGCTG resulted in abnormal splicing of the MLH1 gene. Since this genetic change is not a highly conserved base mutation at the regular position of gt-ag (the intron starts with the dinucleotide "gt" and ends with the dinucleotide "ag" – the so-called gt-ag rule), we evaluated the effects of this 4-bp deletion on splicing. According to the prediction, skipping exon 12 will lead to a frameshift and eventually stop translation at exon 12, which is consistent with our detection results. The members of this family were subjected to predictive tests. Regardless of the family history of gastric cancer, all carriers accepted the HNPPC monitoring plan each year (starting from the age of 25 years, but the earliest tumor in the family was diagnosed at least 5 years before onset), which included the following: annual check-up, colonoscopy, abdominal ultrasound, colposcopy (in the Department of Gynecology), ultrasound, and gastroscopy. The MLH1-encoded protein can heterodimerize with the MMR endonuclease PMS2, which is part of the DNA MMR system, to form MutL- α . The combined loss of MLH1 and PMS2 is characteristic of patients with MLH1 mutations, while isolation and deletion of the PMS2 protein in tumor tissue indicates a germline mutation of PMS2 [7].

According to NCCN guidelines for colon cancer (<https://www.nccn.org/>), it is recommended that all patients with a personal history of colon or rectal cancer undergo universal MMR or MSI (microsatellite instability) testing. In addition to its role as a predictive marker for immunotherapy use in the advanced CRC setting, MMR/MSI status can also help to identify individuals with LS.

Limited data referred to by the NCCN guidelines support the use of checkpoint inhibitor therapy for the first-line treatment of dMMR CRC. An abstract on the phase II CheckMate-142 trial reported results for checkpoint inhibitors in 45 patients with previously untreated dMMR CRC [12]. The objective response rate (ORR) was found

to be 60% (95% CI 44.3–74.3%), with a median follow-up of 13.8 months. After 19.9 months of follow-up, the investigator-assessed ORR was 64% (95% CI 49–78%), the disease control rate was 84% (95% CI 71–94%), and the duration of response was not reached. Pembrolizumab has been approved by the FDA to treat MSI-H or dMMR solid tumors because of the 39.6% ORR among 149 patients with 15 different tumor types (95% CI 31.7–47.9), with a 7% complete response rate [13]. A report revealed a case in which 1 MSI-H CRC patient progressed after checkpoint inhibitor therapy and accepted combination immunotherapy with nivolumab plus ipilimumab. This therapy has been proven to be successful in metastatic melanoma patients and controls progression. This case may have reference significance for future treatment [14]. The NCCN guidelines also state that dMMR status is a biomarker for checkpoint inhibitor therapy and emphasize that this therapy should be applied to dMMR patients only. A review summarized relevant studies to confirm this view [15]. An effective therapeutic regimen is urgently required for non-dMMR/MSI-H patients (95% in the metastatic setting) [16]. LS is caused by a germline mutation of the MMR gene, which could result in dMMR. Therefore, checkpoint inhibitor therapy is a good choice for LS patients, as revealed by genetic analyses. In this case, the best time for the patient to accept PD-1 inhibitor therapy was after the genetic analysis, which revealed dMMR. If the patient had accepted PD-1 inhibitor therapy sooner, lethal progression might not have occurred.

Although the patient died of multiple organ failure, we cannot exclude the possibility of disease overprogression (HPD). This concept was first defined in a study by French scholars, who reported that the tumor growth rate after immunotherapy was more than twice as high as that before treatment, with an incidence of 9% [17]. Patients with certain mutations, such as MDM2 gene amplification [18], are at risk of HPD. We hypothesize that the death of the patient in this case might have been related to HPD, but we do not have gene detection results; therefore, the results cannot be verified.

To our knowledge, the mutations detected in this study have not been previously reported. In addition, at the date of writing, these mutations had not been included in the International Society for Gastrointestinal Inherited Tumor database (<https://www.insight-group.org/>) or in the Human Gene Mutation Database (<http://www.hgmd.org/>).

Statement of Ethics

This study protocol was reviewed and approved by the Ethics Committee of The First Affiliated Hospital of Nanjing Medical University (approval No. 2021-QT-07). Written informed consent was obtained from the participants prior to this study.

Conflicts of Interest Statement

The authors have no conflict of interests to declare.

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Author Contributions

Jing Sun: acquisition of funding and general supervision of a research group. Shiyun Cui: acquisition of funding and general administrative support. Xiao Zhang: editing. Ruihan Zou: writing assistance. Fan Ye: technical editing. Yutong Wang: language editing.

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