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CASE REPORT

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Immunotherapy-induced microsatellite instability status shift in recurrent perihilar cholangiocarcinoma: A case report

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ABSTRACT

Immunotherapy revolutionized the treatment of biliary tract tumors and tumors with high microsatellite instability (MSI-H). This paper reports a 52-year-old woman with recurrent perihilar cholangiocarcinoma. The tumor was initially microsatellite stable (MSS) and proficient mismatch repair (pMMR) but shifted to MSI-H and deficient mismatch repair (dMMR) after combined immunotherapy. Following laparoscopic radical resection for jaundice, stage IV recurrence was diagnosed. Genetic testing revealed the MSS status. Subsequent treatment with camrelizumab and lenvatinib led to a partial response. Ovarian metastases, removed due to abdominal symptoms, exhibited dMMR and MSI-H. The mismatch in MSI status between the primary tumor and metastases suggests tumor heterogeneity and the influence of spatial or temporal factors. This shift can have important clinical significance since MSI-H is associated with significant responses to immune checkpoint inhibitors. MSI-H should be systematically tested in tumors and metastases to personalize treatments. MSI heterogeneity is not only rare but potentially has implications for treatment personalization and prognosis in patients with cholangiocarcinoma. This case highlights the dynamic changes in tumor characteristics during immunotherapy.

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Introduction

Perihilar cholangiocarcinoma is a highly aggressive malignancy of the biliary tract system. It is associated with a dismal 5-year survival of 10%.[1] The proximity to the hepatic hilum blood vessels complicates surgery and increases the recurrence risk, leading to a meager 30% five-year survival rate.[2]

Cancers with microsatellite instability (MSI) have a better prognosis than those without,[3,4] respond better to immunotherapy,[5] and can predict the response to some adjuvant chemotherapies.[3,4] MSI can be categorized into three types: high MSI (MSI-H), low MSI (MSI-L), and microsatellite stable (MSS).[6] MSI-H is associated with defective DNA mismatch repair (dMMR) protein function and demonstrates a significant response to immune checkpoint inhibitors, including programmed death 1 (PD-1)/programmed death ligand 1 (PD-L1) antibodies.[7] Immunotherapy may be more effective against MSI-H tumors.[8–11] The predictive value of MSI-H for immunotherapy efficacy transcends specific cancer types.[12] Tumors with proficient mismatch repair (pMMR) or MSS show low immune response and poor immune escape characteristics. Such tumors are difficult to be recognized and attacked by the immune system.[3,4]

Tumor heterogeneity refers to the changes in molecular biology or genes in tumor cells during the evolution of tumors, giving rise to different cell clones with differences in growth rate, invasion ability, and sensitivity to drugs of different tumor cells.[13] In a heterogeneous tumor, different clones can display different MSI statuses.

Considering the limited efficacy of chemotherapy in perihilar cholangiocarcinoma, immunotherapy (lenvatinib and pembrolizumab) is an interesting option.[14] Lenvatinib and camrelizumab are also possible.[15,16] Lenvatinib combined with PD-1 inhibitors exerts synergistic antitumor effects.[17–19] Therefore, MSI/MMR status assessment emerged as a critical indicator of potential immunotherapy benefits[20] and for treatment selection in biliary tract cancer.[21,22] Herein, in this report, we present a case of perihilar cholangiocarcinoma that exhibited a dynamic shift in MSI phenotype.

Patient presentation

The presentation of this case was approved by the Hospital's ethics committee, and the patient provided written informed consent for the publication of the case. A 52-year-old female was admitted to the Hepatobiliary Surgery Department of Hunan Provincial People's Hospital in September 2019, complaining of yellow skin and dark urine for more than 1 month.

The patient had a previous medical history of percutaneous nephrolithotomy for renal calculus in 2009. Physical examination revealed severe jaundice of skin and sclera and mild epigastric tenderness. Laboratory examination showed that carbohydrate antigen 19–9 (CA19–9) was 53.81 U/mL (reference range: 0–37 U/mL), total bilirubin (TBIL) was 161.5 μmol/l (reference range: 0–21 μmol/L), direct bilirubin (DBIL) was 122.5 μmol/l (reference range: 0–5 μmol/L), alanine transaminase (ALT) was 139 U/L (reference range: 0–40

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U/L), aspartate aminotransferase (AST) was 89 U/L (reference range: 0–40 U/L), and albumin (ALB) was 47.4 g/L (reference range: 35.0–55.0 g/L). Abdominal CT plain and enhanced scans showed a thickening and abnormal enhancement of the hilar bile duct and dilatation of the intrahepatic bile duct. Hilar cholangiocarcinoma was considered. Abdominal B-mode ultrasonography showed that the hilum had a slightly hyperechoic area of 27 × 26 mm. Magnetic resonance cholangiopancreatography showed that the hilar bile duct wall was thickened, and the intrahepatic bile duct was dilated, highly suggesting hilar cholangiocarcinoma. Treatment involved percutaneous transhepatic cholangial drainage (PTCD) followed by laparoscopic radical resection. Pathological evaluation established the diagnosis of moderately differentiated cholangiocellular carcinoma. Adjuvant therapy with oral tegafur was administered for 6 months. The initial laparoscopic radical resection and adjuvant therapy led to jaundice resolution and alleviation of the abdominal pain.

A follow-up positron emission tomography (PET)-CT examination in October 2020 showed liver lesions, lymph node metastases, soft tissue nodules in the right perirenal space, and a mass in the right ovary (Figure 1a). The patient was asymptomatic. Next-generation sequencing (NGS) of the primary tumor indicated MSS status, while immunohistochemistry (IHC) confirmed pMMR status with positivity for MLH1, MSH2, MSH6, and PMS2. PD-L1 expression was positive, and the combined positive score (CPS) was <1. The patient received combined immunotherapy and targeted therapy. Reexamination showed a partial response (PR) (Figure 1b).

In March 2022, the patient developed intermittent distending pain in the right lumbar area and lower abdomen. CT showed a regression of the lesions, except the cystic lesion in the right adnexa that was larger (118 × 104 mm). The patient underwent laparoscopic surgery for the adnexal lesions, revealing a moderately differentiated adenocarcinoma, MLH1 (-) (Figure 2a), MSH2 (++) (Figure 2b), MSH6 (++) (Figure 2c), and PMS2 (-) (Figure 2d), suggesting dMMR (Table 1). Genetic testing confirmed the MSI-H status. During combined immunotherapy, the CA199 levels exhibited a decreasing trend (Figure 3).

In summary, the tumor was initially MSS and pMMR. The patient was treated with laparoscopic radical resection, lenvatinib, and camrelizumab. The patient achieved a partial response. The patient eventually developed ovarian metastases that exhibited dMMR and MSI-H. When writing those lines, the patient was still alive and under treatment.

Discussion

Conventional chemotherapy for cholangiocarcinoma offers limited efficacy, with gemcitabine combined with cisplatin (GP) as the first-line choice.[23–25] The combination of anlotinib and sintilimab as second-line treatment demonstrated a remarkable objective response rate (ORR) and disease control rate (DCR).[26] However, the median progression-free survival (mPFS) remains relatively short.

Pembrolizumab yielded a high ORR in advanced dMMR/MSI-H cholangiocarcinoma.[27] In the case reported here, the patient achieved durable efficacy with camrelizumab plus lenvatinib combined therapy, substantially improving the quality of life. A shift in MSI status was observed between the primary liver lesion and ovarian metastases. The case highlights the importance of monitoring MSI status throughout treatment. Considering that the MSI and MRR statuses are associated with different sensitivity to immunotherapy,[3–5] regular MSI/MMR re-assessment in cancers undergoing immunotherapy can be relevant for clinical practice by personalizing the treatments dynamically in time. A retrospective series of 40 patients with colorectal cancer revealed that 36 and four patients had pMMR and dMMR primary tumors, respectively, while among the liver metastases, 30 were pMMR, and 10 were dMMR.[28] A study revealed that the primary and metastatic colorectal tumors had the same MSI status in 77% of the cases,[29] while another study revealed only 3.4% of mismatch between primary colorectal cancer and the matched metastases.[30]

Several factors may contribute to this shift. The heterogeneity within the tumor may lead to different cell populations having different MSI states, thus affecting the biological behavior and therapeutic response of the tumor. The most aggressive clone within a tumor is the one most likely to induce metastases. By changing the tumor microenvironment and

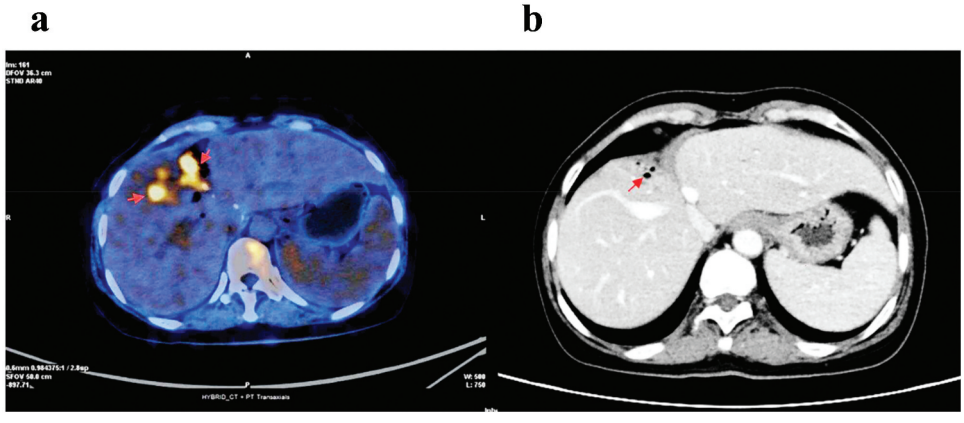

Figure 1. Positron emission tomography (pet)-computed tomography (CT) assessment of dynamic change of recurrent hepatic lesion during combined immunotherapy. (a) Diagram of the recurrent hepatic foci at the first surgical recurrence (2020-10-21). (b) The latest CT imaging evaluation after combined treatment (March 09, 2023). The red arrows show the cancer lesions.

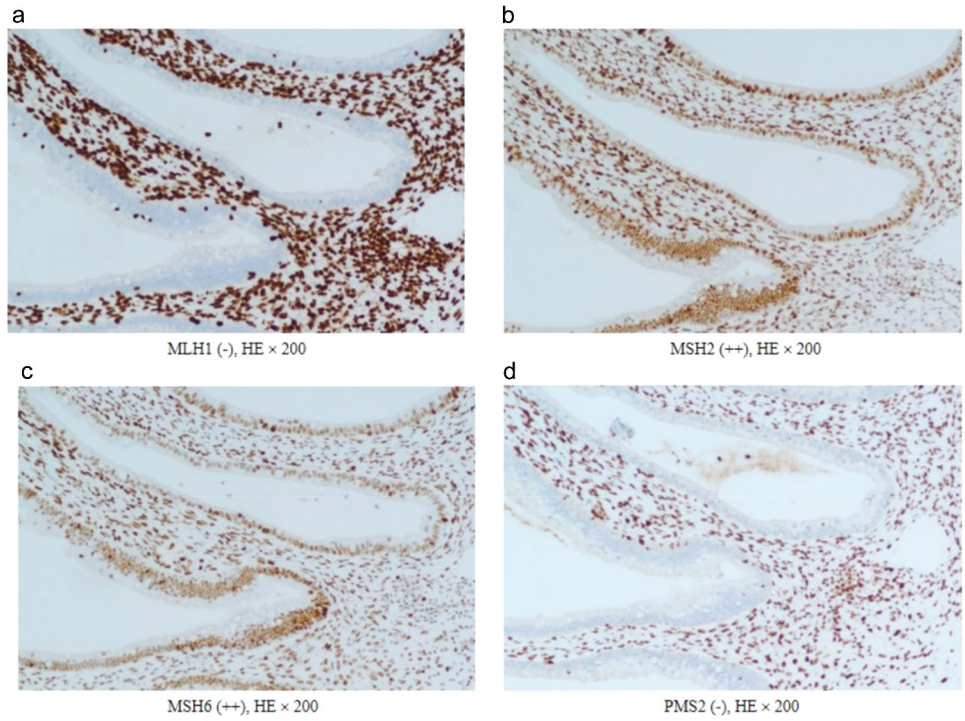

Figure 2. Immunohistochemistry for deficient mismatch repair (dMMR) markers from March 2022 showing (a) MLH1 (-), (b) MSH2 (++), (c) MSH6 (++), and (d) PMS2 (-). Magnification: 200 × .

Table 1. Summary of the MMR markers by immunohistochemistry.

MMR\Tumor	MLH1	MSH2	MSH6	PMS2
Primary	(+)	(+)	(+)	(+)
Metastasis	(-)	(++)	(++)	(-)

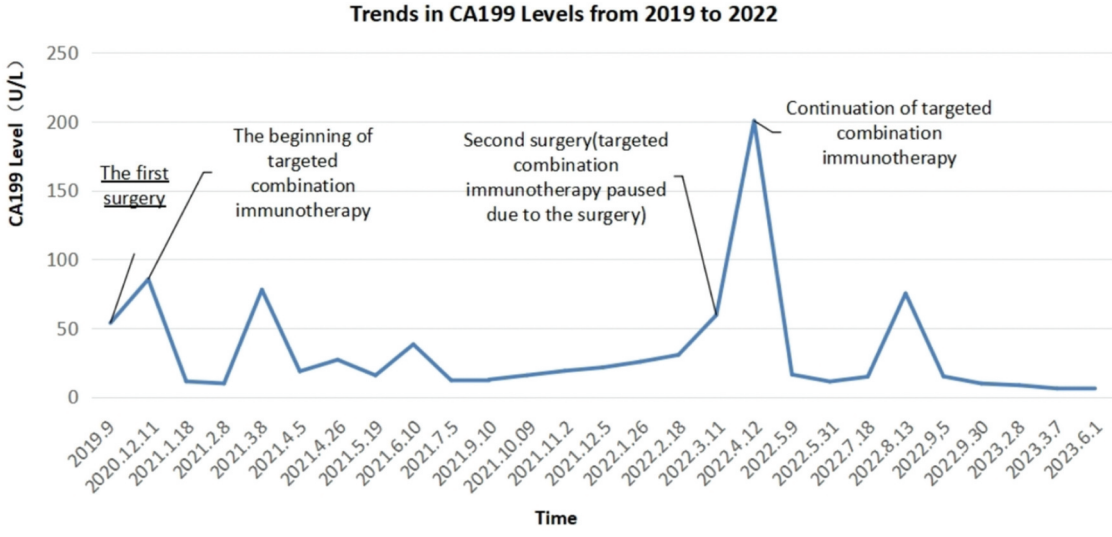

Figure 3. Line chart of the changes in CA199 from 2020 to 2021, along with the clinical events.

immune escape mechanism, immunotherapy may lead to dynamic changes in MSI status, thus affecting the therapeutic effect. It is also possible that the combined treatment could put selection pressure, favoring the predominance of a specific clone. The data from this case do not provide insights into the exact reason for the shift. Tumor heterogeneity gives rise to

different clones growing within the same tumor.[13] Still, tumor heterogeneity remains difficult to assess precisely, especially for rare and underrepresented clones.[31–33] Tumor heterogeneity affects diagnosis and treatment, monitoring, drug resistance, and prognosis.[34,35] An in-depth analysis of the tumor is helpful in revealing the dynamic process of tumor evolution, from the recognition of tumor morphological heterogeneity to the revelation of molecular mechanisms.[34,35] Deep sequencing of single cells or whole genomes of tumors in different regions can be performed in space or time.[36,37] PD-L1 expression and tumor mutational burden (TMB) can also be used to monitor treatment susceptibility and response.[38,39] PD-L1 expression suggests that the tumor will respond to immune checkpoint inhibitors.[40–43] TMB can also be used to tailor treatments since tumors with a high TMB also have a higher likelihood of responding to immunotherapy.[44–47] PD-L1 expression and TMB indicate different mechanisms through which immunotherapy acts on cancer cells.[39] Although the two biomarkers have been shown to correlate in lung cancer, variations that can affect tumor response have been observed.[48] New treatment patterns could emerge since cases of patients with MSS and no PD-L1 expression but responding to immunotherapy have been reported.[49,50] Dynamic monitoring of MSI/MMR status is theoretically feasible, but if each patient is subjected to re-biopsy of metastases plus immunohistochemical detection, it will be difficult, expensive, and will repeatedly expose the patients to the risks of re-puncture, all of which could lead to poor patient compliance. In addition, NGS is expensive, and it is not suitable for repeated measurements and dynamic monitoring. Determining the expression of specific MSI/MMR markers using PCR would be more feasible.[51,52]

There are several potential causes of tumor heterogeneity, including genetic drift, therapy-induced selection pressure, or different microenvironments within the same tumor.[31,53–55] Lenvatinib combined with PD-1 inhibitors will affect the tumor cells in several ways: effector T cell activation and regulatory T cell depletion in the tumor microenvironment, antigen-presentation and dendritic cell maturation, inhibition of immune-suppressive signaling, and normalization of tumor blood vessels.[17–19] Since tumor cells with MSI-H would be more sensitive to immunotherapy,[8–11] lenvatinib and camrelizumab could exert selection pressure on the most sensitive cells.[31,53–55] The changes in the tumor microenvironment could also lead to the selection or the activation of a specific clone that could participate more in recurrence and metastasis.[31,53–55] Nevertheless, the present study cannot provide data to determine the exact mechanisms involved.

Ovarian metastases from biliary tract tumors are exceedingly rare, and this case demonstrates that surgical intervention can be beneficial. This approach could be considered for ovarian metastasis in patients with cholangiocarcinoma.

In conclusion, this case highlights that the MSI status can shift in cholangiocarcinoma and that a durable clinical response is achievable through combined immunotherapy. This case emphasizes the need to consider the heterogeneity of MSI status and advocates for assessing both primary tumors and metastases, along with dynamic surveillance. It underscores the importance of understanding the dynamic nature of the MSI status in guiding treatment decisions. Nevertheless, further research is needed to elucidate the mechanisms contributing to MSI status heterogeneity and to identify more patients who could benefit from immunotherapy. Such studies should be undertaken in cholangiocarcinoma but also in other solid tumors, as common patterns could emerge.

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Author's contributions

Hailing Yu, Hongbing Liu, and Tan Deng participated in data acquisition and analysis and drafted the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Consent to participate

Written informed consent was obtained from the patient for the publication of the case report.

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