Liver Cancer

Case Report

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Genomic and Immune Features in an Intrahepatic Cholangiocarcinoma Patient with Microsatellite Instability-High Suffered Rapid Acquired Resistance to PD-1 Inhibitor

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Keywords
Intrahepatic cholangiocarcinoma · MSI-H · PD-1 inhibitor · Rapid acquired resistance

Abstract
Introduction: Intrahepatic cholangiocarcinoma (ICC) is a highly aggressive liver malignancy with poor prognosis. Recently, the development of immune checkpoint inhibitors (ICIs), such as programmed cell death 1 (PD-1) inhibitors, has emerged as a promising strategy in multiple tumor types, including ICC. Microsatellite instability-high (MSI-H) is an important biomarker for ICIs in solid tumors. The response rate in patients with MSI-H is significantly higher than in those with microsatellite stability/microsatellite instability-low. And approximately 80–90% of the patients with MSI-H could maintain sustained clinical benefits once they had an initial response. However, some patients could have primary resistance at the beginning, and some might have acquired resistance after long-term treatment. ***Case Presentation:*** We present the case of an ICC patient with MSI-H who suffered rapid progression after a short-term remission with camrelizumab, a kind of PD-1 inhibitor, as second-line treatment. The patient's genomic and immune features were analyzed by next-generation sequencing and multiplex immunofluorescence staining to explore the possible mechanisms of the rapidly acquired resistance of ICIs in this MSI-H case. ***Conclusion:*** The genomic and immunohistochemical analysis showed that TGFBR2 mutation, loss of HLA B44 supertype, carrying B62 supertype, and increased PD-L1^{+} cells, macrophages, and Tregs in the tumor microenvironment might be related to the nonsustain benefit of ICIs in this MSI-H patient.

Introduction

Intrahepatic cholangiocarcinoma (ICC) is a highly aggressive malignancy arising from the bile duct epithelium within the liver [1]. Although gemcitabine plus cisplatin has demonstrated significant antitumor activity as first-line therapy for advanced ICC, the 5-year survival rate was only 10% [2]. It is now widely recognized that the

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development of immune checkpoint inhibitors (ICIs), such as programmed cell death 1 (PD-1) inhibitors, has emerged as a promising strategy in multiple tumor types [3]. However, not all patient could benefit from ICI therapy.

Microsatellite instability-high (MSI-H) cancers are biologically marked by genomic instability and often associated with high mutation rates of frameshift and missense mutations. Mounting evidences suggested that tumors with MSI-H trend toward clinical benefit from PD-1 inhibitors [4]. And the US Food and Drug Administration (FDA) approved pembrolizumab for treatment of a variety of advanced solid tumors with MSI-H, which had progressed following prior treatment [5]. But the overall response rate varies from approximately 40–60% in tumors with MSI-H [6–9]. Although about 80–90% of patients with MSI-H who benefit from ICI therapy could have durable response for over 1 year or even longer [6, 9], there were still some patients showing primary or acquired resistance [10, 11]. Moreover, a small group of patients may even experience hyperprogressive disease (HPD), which defines as dramatic tumor growth following the initiation of the immunotherapy within 2 months [12]. And there has been no report of a rapid progression following a short-term disease remission in MSI-H patients treated with PD-1 inhibitor so far.

Here, we presented the case of an advanced ICC patient with MSI-H who received camrelizumab, a kind of PD-1 inhibitor, monotherapy after disease progression of chemotherapy. Surprisingly, the patient had a rapid progression following dramatic tumor regression after 5 cycles of camrelizumab treatment. The patient's genomic and immune features were analyzed by next-generation sequencing and multiplex immunofluorescence staining to explore the possible mechanisms of the rapid acquired resistance of ICIs in this MSI-H case.

Case Presentation

A 75-year-old Chinese female without viral hepatitis, biliary stones, nonalcoholic fatty liver disease, or family history of disease was found to have liver-occupying lesions by color Doppler ultrasound during her routine physical examination in June 2020. Enhanced magnetic resonance imaging (MRI) of abdomen showed a lesion located in the caudate lobe, invaded portal vein and inferior vena cava around, measuring up to 6.7 cm (shown in Fig. 1a, b). Computed tomography scan of lung and pelvic cavity did not find any distal metastasis. Carcinoembryonic antigen was normal while cancer antigen 19-9 (CA19-9) was increased to 700.6 U/mL. The patient received a liver biopsy, which obtained three pieces of tumor tissues, in June 14, 2020, and the immunohistochemical analysis was shown as following: CK7 (+), CK19 (+), Muc-1 (+), Hep-1 (+), arginase (−), Gly-3 (−), GS (−), CK5/6 (+), and p40 (−), which suggested the diagnosis was ICC with stage of IIIB (cT4N0M0).

With no indication of radical surgery, this patient received gemcitabine (1,000 mg/m^2 on days 1 and 8) plus S-1 (80 mg daily on days 1–14 of a 21-day cycle) as first-line regimen. CA19-9 level fell rapidly to normal after the first cycle. Follow-up enhanced MRI of abdomen showed that the tumor size decreased about 71% without any new lesion emerging after five cycles treatment (shown in Fig. 1b). Because of serious thrombocytopenia, the patient refused the combination of gemcitabine and received S-1 oral-only regimen since the sixth cycle. Five months later, enhanced MRI of abdomen revealed tumor progression (shown in Fig. 1b).

With the patient's consent, the primary tumor tissue sample was obtained by biopsy. And the gene sequencing of her tumor tissue confirmed that this patient was MSI-H PD-1 inhibitor was recommended as an alternative regimen after first-line treatment failure. Camrelizumab, a kind of PD-1 inhibitor, had proved efficacy and safety in solid tumor patients with MSI-H and mismatch repair deficiency in a phase 2 clinical trial [13], was then initiated with 200 mg intravenously every 3 weeks. After 2 months of treatment, enhanced MRI scan showed that the targeted lesion had a dramatic regression, 44% shrinkage from baseline (shown in Fig. 1b). Unfortunately, a massive, rapidly disease progression burst out 1.5 months later, not only in liver but also spreading to lymph nodes and subcutaneous soft tissues (shown in Fig. 1c). And patient died 2 months later.

To explore the potential mechanism of this patient's rapid progression, we also obtained the progressive metastatic tumor tissue by surgery with the patient's consent. Multiple immunohistochemical staining showed that the metastatic tumor tissue was CK19, CK7, and Muc-1 positive, which could be diagnosed as cholangiocarcinoma metastasis (shown in Fig. 2a). A 769-gene panel next-generation sequencing was used to analyze the gene feature of both primary and progressive tumor tissues (shown in online suppl. Table S1; see online suppl. material at https://doi.org/10.1159/000530273). The results showed that 36 genes mutated both in primary and progressive tumor tissues. The mutation abundance was calculated from alternating allelic observations divided by the read depth at each position [14]. Interestingly, while the tumor mutation burden was similar before and after immunotherapy (26.05 and 24.36 Mut/Mb, respectively), the abundance of gene mutation was lower at the time of disease progression (shown in Fig. 2b). Among these mutated genes, there were some clinically significant pathogenic mutations, such as TP53, KRAS, ARID1A. No events were observed in JAK1, JAK2, JAK3, B2M, or PTEN, which are associated with acquired resistance to ICIs. However, these mutated genes were detected in MSI-H ICC patients who were sensitive to ICI treatment (shown in online suppl. Table S2, S3). Besides, TGFBR2 frameshift mutation was detected in both gene-sequencing results.

To estimate loss of heterozygosity (LOH) of human leukocyte antigen (HLA) class I at the somatic level, we used the LOHHLA [15] algorithm with default settings. Both primary tumor and metastatic tissue specimens had LOH in HLA-I alleles. In particular, this patient was found to harbor subclonal loss of HLA-B44 supertype while keeping HLA-B62 supertype (shown in Fig. 2c).

To analyze the change of tumor microenvironment before and after immunotherapy, immunofluorescence staining, imaging, and

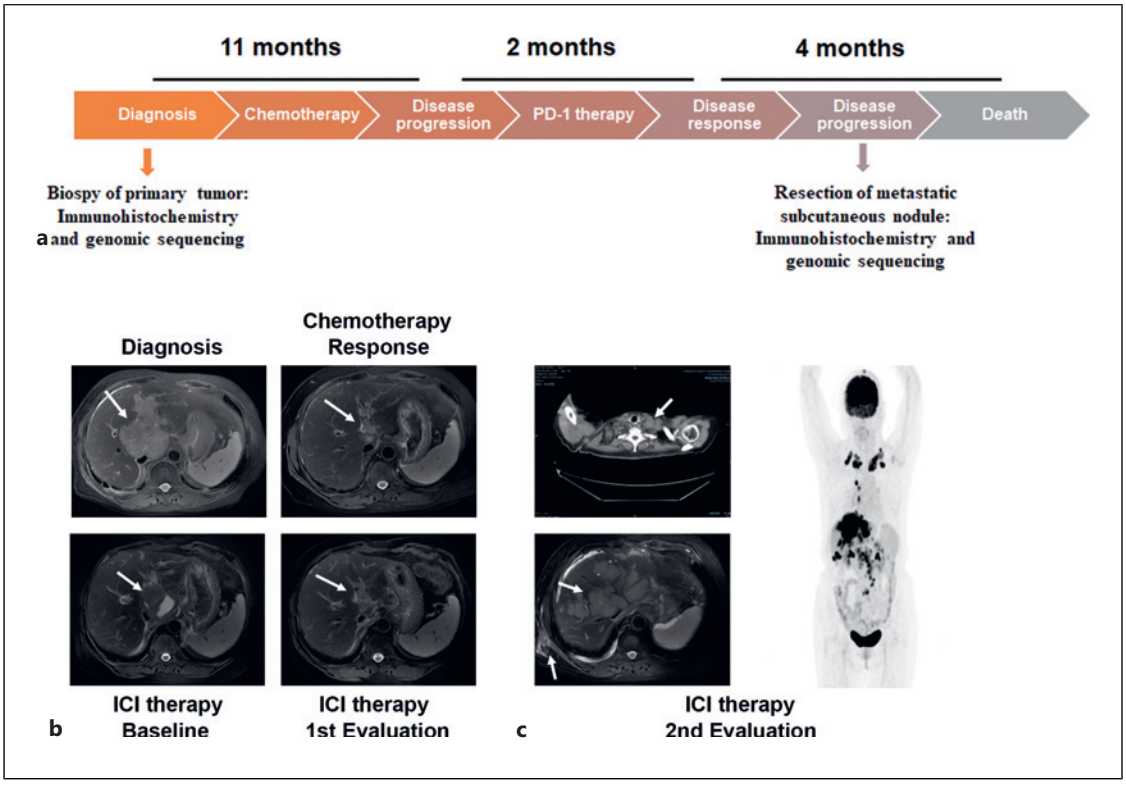

Fig. 1. Clinical course and radiologic images before and after camrelizumab treatment. **a** Clinical course and tissue collection for immunohistochemical assessment and gene sequencing. **b** Magnetic resonance imaging (MRI) of the changes of the targeted lesion during treatment before the immunotherapy progression. **c** Representative image of positron emission tomography-computed tomography (PET-CT), MRI, and chest CT examination at the time of immunotherapy progression.

quantification of simultaneous detection for multiplex molecules within the tumor immune microenvironment were performed by Tissue Cytometry Analysis System. The results showed that the proportion of PD-L1$^+$ cells in tumor was highly enhanced in the ICI-resistant metastatic tumor tissue. And the results also revealed that the infiltration percentages of the immune cells in the baseline primary tumor tissue were very low, with only CD68$^+$CD163$^-$ M1 macrophages having been detected. And a variety of immune cells infiltrated in the ICI-resistant metastatic tumor tissue had increased, including CD8$^+$PD-1$^+$ T cells, CD4$^+$PD-1$^+$ T cells, Foxp3$^+$CD4$^+$ Treg cells, CD68$^+$CD163$^-$ M1 macrophages, and CD68$^+$CD163$^+$ M2 macrophages (shown in Fig. 3). However, only the increase of Treg cells, M1 and M2 macrophages had statistical difference.

Discussion

This is the first reported case of a patient with advanced ICC of MSI-H who developed rapid, diffuse progression after a brief period of significant response with PD-1 inhibitors, surviving for only 2 months after progression. This is inconsistent with previous studies that immunotherapy could achieve sustained benefit for patients showing initial response. Therefore, we analyzed the primary tumor tissue before treatment and metastatic tumor tissue after the progression of immunotherapy by gene sequencing and multiplex immunofluorescence staining, respectively.

The gene-sequencing results showed that both primary and ICI-resistant tumors were MSI-H and had a high number of gene mutations. Among these mutated genes, TGFBR2 frameshift mutation was detected in both gene-sequencing results. TGFBR2 is one of the isoforms of the TGF-β receptor [16]. An analysis of patients treated with ICIs or chemotherapy in a public cohort has shown that TGFBR2 mutations could predict resistance to ICIs in NSCLC patients [17]. In a gastrointestinal cancer patient with MSI-H, who developed HPD after immunotherapy, TGFBR2 mutation was also observed [18]. We obtained external data of ICC cohorts to analyze the relationship between TGFBR2 mutation and prognosis [19, 20].

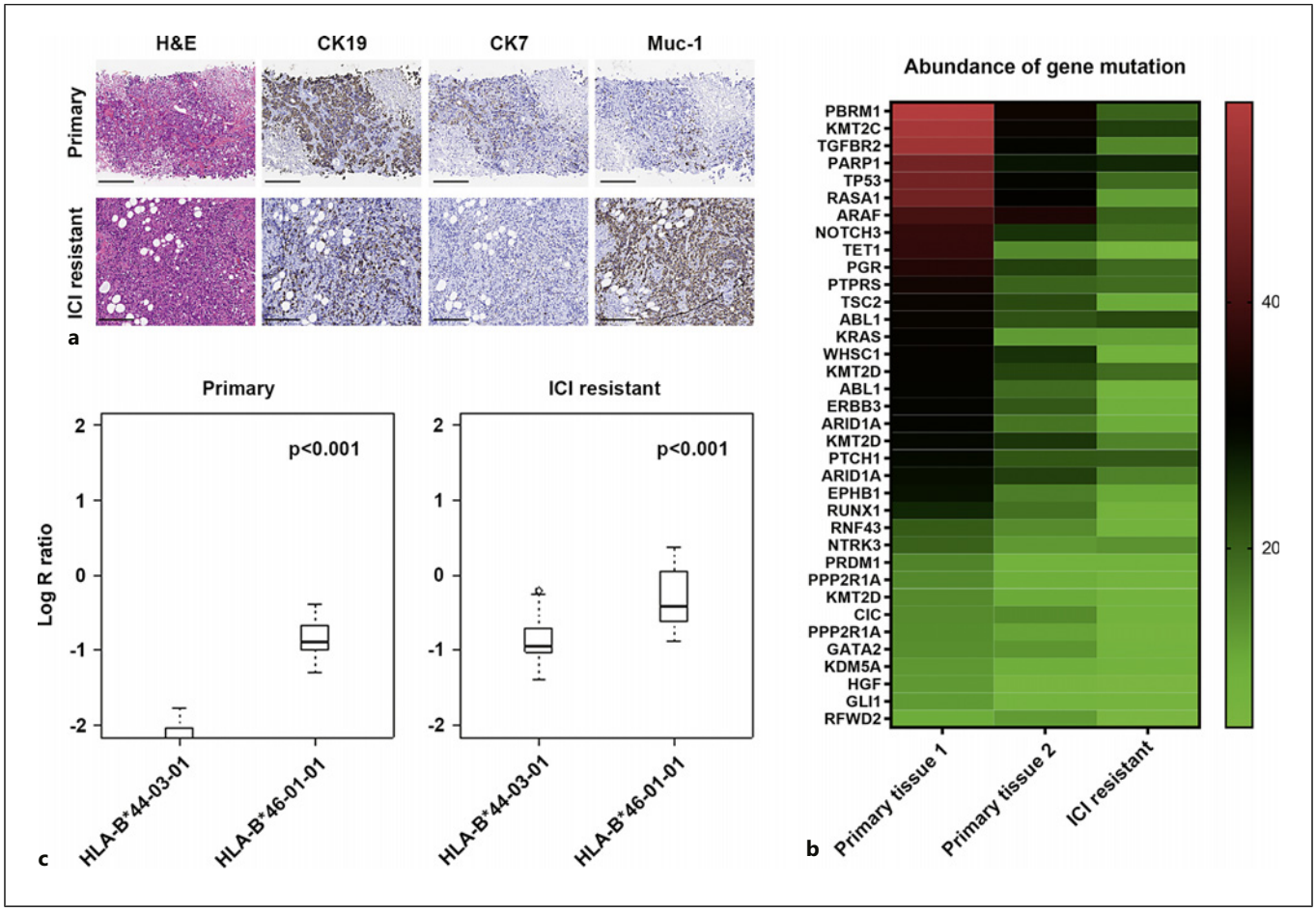

Fig. 2. Gene-sequencing results in tumors before and after camrelizumab treatment. **a** Representative images of H&E and cholangiocarcinoma markers including CK19, CK7, and Muc-1 in the primary and ICI-resistant tumors. **b** Abundance of gene mutation compared between the primary and ICI-resistant tumors. **c** Coverage comparison of HLA-B44 supertype and HLA-B46 supertype.

However, the proportion of TGFBR2 mutation was very low (about 1.5%) in ICC and showed a trend toward worse prognosis with no statistical significance (shown in online suppl. Fig. S1). Although the progression of the patient in this case did not meet the definition of HPD, whether the uncommon rapid diffuse progression and short-term death after remission with immunotherapy are associated with TGFBR2 mutation needs to be further investigated.

Previous studies have demonstrated that individual-specific hereditary HLA class I molecule (HLA-A/B/C) genotypes may influence the efficacy of ICI therapy in tumor patients. Patients with a greater variety of HLA class I molecule alleles are more likely to benefit from immunotherapy. In two separate melanoma cohorts, patients with HLA-B44 supertypes had a better survival benefit with immunotherapy, whereas the HLA-B62 supertype or LOH at HLA class I was associated with poor outcome [21]. Analysis of HLA molecules in this case showed that the patient was found to have the HLA-B62 supertype but lost the HLA-B44 supertype. This might be one of the reasons that the patient had rapid progression after the benefit of immunotherapy. However, it is inconclusive whether PD-1 inhibitors are contraindicated in MSI-H cancers once the HLA-B62 supertype is detected, and more studies are needed to confirm this.

PD-L1 expression is a useful predictive biomarker of ICIs in patients with a variety of tumor types [22]. However, the association between PD-L1 expression and clinical benefit of ICIs in biliary tract cancers (BTCs) is

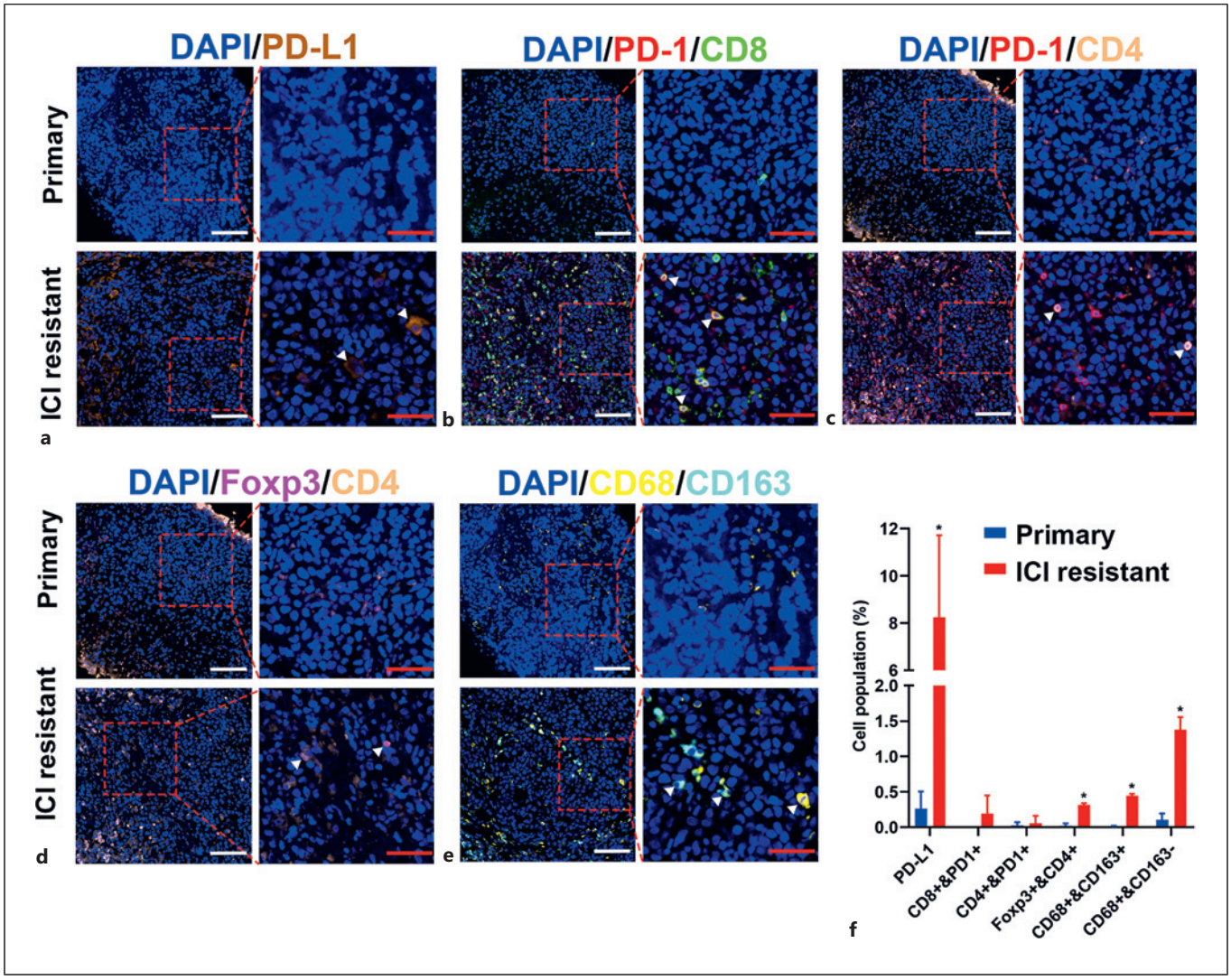

Fig. 3. Changes of TIICs before and after camrelizumab treatment. **a** The images of PD-L1-positive cells in primary and ICI-resistant tumor samples were shown. Right, the magnification of the representative region; white scale bar, 100 μm; red scale bar, 50 μm. White arrow indicated the targeted cells. **b** The images of PD-$1^{+}CD8^{+}$ cells in primary and ICI-resistant tumor samples were shown. Right, the magnification of the representative region; white scale bar, 100 μm; red scale bar, 50 μm. White arrow indicated the targeted cells. **c** The images of PD-$1^{+}CD4^{+}$ cells in primary and ICI-resistant tumor samples were shown. Right, the magnification of the representative region; white scale bar, 100 μm; red scale bar, 50 μm. White arrow indicated the targeted cells. **d** The images of $Foxp3^{+}CD4^{+}$ cells in primary and ICI-resistant tumor samples were shown. Right, the magnification of the representative region; white scale bar, 100 μm; red scale bar, 50 μm. White arrow indicated the targeted cells. **e** The images of $CD68^{+}$ $163^{+/-}$ cells in primary and ICI-resistant tumor samples were shown. Right, the magnification of the representative region; white scale bar, 100 μm; red scale bar, 50 μm. White arrow indicated the targeted cells. **f** The percentage of PD-L1-positive cells and TIICs in primary and ICI-resistant tumor samples was calculated. The fluorescent strength was set as default and analyzed by the Tissue Cytometry Analysis System. Each group was chosen three region of interest (ROI) to analyze, and the proportion of targeted cells was calculated by targeted cells relative to all viable tumor cells present in the ROI. The significance of mean values between two group of compared cells was analyzed by two-tailed Student's *t* test. * indicates $p < 0.05$.

controversial. In a Korean clinical study, the results showed that high PD-L1 expression (≥50%) was significantly associated with better response to pembrolizumab in patients with BTC [23] while another multicenter retrospective study found no relationship between PD-L1 expression and response in BTC patients treated with pembrolizumab [24]. Furthermore, a study also showed that high expression of PD-L1 in ICC patients had a

worse prognosis [25]. In this case, the expression of PD-L1 in ICI-resistant tumor tissue was significantly higher than primary tumor tissue. This finding suggests that PD-L1 expression may have played a role in the resistance to ICIs in this patient.

Tumor-infiltrating immune cells are critical components of the tumor microenvironment and have predictive effects on prognosis and treatment in cancer patients [26]. Although studies have shown that the presence of $CD8^+PD\text{-}1^+$ T cells in peripheral and tumor tissues prior to or after immunotherapy is correlated with a favorable response to ICIs [27, 28], the $CD4^+PD\text{-}1^+$ T cells might had opposite effect. A clinical study with NSCLC patients revealed that the accumulation of $CD4^+FOXP3^-PD\text{-}1^+$ T cells within the tumor and peripheral blood correlated with higher tumor burden [29]. High frequencies of macrophages and Treg cells also showed suppressive effect on ICIs treatment response [30, 31]. A recent study even revealed that PD-1 blockade may facilitate the proliferation of highly suppressive $PD\text{-}1^+CD4^+$ T cells in HPD [32]. In this case, the infiltration percentages of the immune cells in the baseline primary tumor tissue were very low, with only $CD68^+CD163^-$ M1 macrophages having been detected. However, when the patient had rapid progression following remission of immunotherapy, a variety of immune cells infiltrated in the metastatic subcutaneous nodules had increased, including $CD8^+PD\text{-}1^+$ T cells, $CD4^+PD\text{-}1^+$ T cells, $Foxp3^+$ $CD4^+$ Treg cells, $CD68^+CD163^-$ M1 macrophages, and $CD68^+CD163^+$ M2 macrophages. And the frequency of macrophages and Treg cells was higher than that of $CD8^+PD\text{-}1^+$ T cells and $CD4^+PD\text{-}1^+$ T cells. This suggested that the patient was indeed in the microenvironment that was not conducive to immune response when immunotherapy failed.

As a case report, the present results were far from providing guidance to clinical practice as there are some limitations to our study. First, chemotherapy can affect the efficacy of immunotherapy by modulating the immune microenvironment in a complex way [33]. However, we did not collect and test tissue samples after chemotherapy resistance, so the potential effect of chemotherapy on subsequent immunotherapy response may have been overlooked. Second, ICC has been reported of high heterogeneity, and more than half of ICCs have intratumoral heterogeneous immune infiltration, which posing a great challenge for the prediction of immunotherapeutic efficacy by single biopsy [34]. Besides, it has been observed that metastases that originate from different organs may have distinct molecular and immunological profiles, even if they share similar genomic characteristics with the primary tumor [35]. Therefore, the immune microenvironment changes in this case might be some bias due to tumor heterogeneity.

In conclusion, we report the case of an ICC patient with MSI-H who died of rapid progression after a short-term remission with anti-PD-1 monotherapy. Comparative analysis of primary and ICI-resistant tumor tissues suggests that TGFBR2 mutation, loss of HLA B44 supertype, carrying B62 supertype, and increased $PD\text{-}L1^+$ cells, macrophages, and Tregs in the tumor microenvironment might be possible factors for the subsequent nonbenefit of immunotherapy. Whether these factors can negate the use of immunotherapy in patients with MSI-H needs to be investigated more thoroughly in a larger sample cohort.

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Statement of Ethics

This study was approved by the Institutional Review Board of the Eastern Hepatobiliary Surgery Hospital on May 9, 2020 (approval number: EHBHKY2020-01-008). All procedures were conducted according to the Declaration of Helsinki. Written informed consent was obtained from the patient's next-of-kin for publication of the details of her medical case and any accompanying images.

Conflict of Interest Statement

Author Di Liu is employed by Genecast Biotechnology Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author Contributions

Zhengang Yuan conceived the idea of the article. Zhuo Cheng and Tianmei Zeng mainly takes charge of writing and

figure making. Zhuo Cheng, Tianmei Zeng, Guang Yang, Di Liu, and Zhi Zheng contributed to the data collection. Zhengang Yuan and Tianmei Zeng contributed to article revising. All authors contributed to the article and approved the submitted version.

Data Availability Statement

The raw data supporting the conclusions of this article will be made available by the authors without undue reservation. All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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