CASE REPORT

A case report of response to crizotinib in chemotherapy-refractory metastatic gallbladder cancer with met amplification and acquired resistance resulting from the loss of MET amplification

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Abstract

Gallbladder cancer (GBC) is a highly invasive disease and the most prevalent malignancy of the biliary system. Patients with GBC are commonly diagnosed at a late stage and have an unfavorable prognosis. Palliative chemotherapy has been the standard care for recurrent or metastatic disease in the past decades. Recently, several targeted therapies have been investigated in advanced biliary tract cancer (BTC) including inhibitors of genes or pathways such as FGFR2 fusions or rearrangements, IDH1 mutations, and NTRK gene fusions. Also, several clinical studies involving molecular stratification have been performed in defined patient groups, for example, BRAF V600E and HER2. Mesenchymal epithelial transition (MET) encodes a tyrosine kinase receptor and its ligand hepatocyte growth factor is a proto-oncogene. Targeting the MET signaling pathway is an effective strategy in numerous cancer types. However, the poor efficacy of MET inhibitors has been demonstrated in several phase II studies, but currently no reports have explained the potential mechanisms of resistance to MET inhibitors in BTC. In this article, we report a case of metastatic GBC with MET amplification that exhibited a rapid response to crizotinib after the failure of two lines of chemotherapy. After the patient had progressed and discontinued crizotinib, cabozantinib was introduced. Analysis of circulating tumor DNA (ctDNA) by next-generation sequencing (NGS) indicated a loss of MET amplification status. To our knowledge, this is the first case study demonstrating the use of NGS in ctDNA to monitor the development of acquired resistance during anti-MET treatment in GBC.
Key words: metastatic gallbladder cancer; MET amplification; targeted therapy; ctDNA; acquired resistance; case report

Introduction

Gallbladder carcinomas (GBCs) are the most prevalent malignancies in the biliary tract and are highly invasive diseases with late diagnosis and an unfavorable prognosis. The five-year survival rate of GBC is 5% for patients with stage III disease and 1% for patients with stage IV disease. Radical surgery remains the standard treatment for patients with locally resectable tumors. Palliative chemotherapy is the standard of care for patients with recurrent or metastatic disease; however, outcomes remain poor, with a median overall survival (OS) of <12 months. The poor outcomes in GBC are mainly due to low responses to first-line chemotherapy and the absence of effective second- or third-line therapeutic strategies. Recently, novel targeted therapies have been developed for the management of advanced biliary tract cancer (BTC) that have used next-generation sequencing (NGS) technology for patient stratification. Several targeted agents have been approved for advanced BTC by the FDA (Food and Drug Administration) including inhibitors of particular genes or pathways such as FGFR2 fusions or rearrangements, IDH1 mutations, NTRK gene fusions and MSI-H status. These alterations are present in around 0.1%–20% of patients with BTC and are less frequent in patients with GBC. Several precision medicine trials are ongoing, for example, BRAF V600E and HER2. These data suggest that it is very necessary to carry out accurate treatment for BTC based on molecular stratification.

Mesenchymal epithelial transition (MET) encodes a receptor tyrosine kinase that is activated by binding of its ligand, hepatocyte growth factor (HGF). Dysregulation of the MET pathway involves amplification, fusion, overexpression, and mutation. Several studies have indicated MET overexpression in 11%–43% of intrahepatic cholangiocarcinoma (IHCC) and 16%–80% in extrahaepatic cholangiocarcinoma (EHCC). MET amplification has been reported with a fairly low frequency ranging from 0% to 7% in IHCC and EHCC, and from 0% to 18% in GBC. Activation of MET signaling is involved in tumor angiogenesis, metastatic progression, and acquired resistance to anti-EGFR treatment. In a study by Hida and colleagues, expression of MET was also strongly correlated with tumor location, T category, AJCC stage, and perineural invasion. These factors can lead to successive local recurrence and poor prognosis in extrahepatic BTC. Therefore, targeting the MET signaling pathway is a potential treatment strategy. Combinations of multiple kinases or selective tyrosine kinase MET inhibitors (TKIs) and anti-MET antibodies, antibody-drug conjugates have been studied. For example, selective MET TKIs including capmatinib and the multi-kinase MET inhibitor crizotinib have shown promising efficacy in lung cancer patients with MET amplification and MET exon 14-alterations. However, the effectiveness and safety of MET inhibitors in GBC with MET amplification or mutations remains unknown.

Here, we report a case of terminal GBC with MET amplification that had metastasized to the lymph nodes and liver. The patient showed a rapid response to crizotinib after the failure of two lines of chemotherapy. Moreover, the MET amplification status was negative after the failure of anti-MET therapy.

Case presentation

In August 2019, a 55-year-old woman visited our clinic with mild right upper abdominal pain on a numerical rating scale (NRS) of 2–3 with a poor appetite and weight loss (Fig. 1A). Magnetic resonance cholangiopancreatography of the abdomen discovered gallbladder lesions, retroperitoneal lymphadenopathy, and enlarged intraperitoneal lymph nodes. The serum CA19-9 level was 555 U/ml (normal range 0–22 U/ml) and the carcinoembryonic antigen (CEA) level was 316 ng/ml (normal range, 0–3 ng/ml). The patient had no history of chronic diseases. On October 29, 2019, the patient underwent palliative resection of a gallbladder lesion and intraperitoneal lymph node biopsy. Histologically, the gallbladder lesion was diagnosed as poorly differentiated adenocarcinoma that had invaded the outer membrane and the lymph nodes were metastatic. The patient was diagnosed with GBC at pT2N2M1 and stage IVB.

One month after surgery, the patient had a new palpable hard nodule in the left neck that was 2 cm in diameter and had back pain. An enhanced abdomen computed tomography (CT) scan displayed a new nodule in the liver and the level of serum CA19-9 increased to over 1000 U/ml. CEA levels had also increased (Fig. 1B). The performance status (PS) score of the patient was 1. The patient was administered 2 cycles of GEMOX chemotherapy (gemcitabine plus oxaliplatin) as a first-line treatment on December 21, 2019. The patient then received 2 cycles of GA (gemcitabine plus paclitaxel-albumin) as a second-line treatment. Unfortunately, the best response to both regimens was progression disease according to the Response Evaluation Criteria in Solid Tumors version 1.1. The patient experienced intensified back pain to NRS 7–8. Fentanyl transdermal patches were used every 72 h and the PS score decreased to 2. CT evaluation after two lines of chemotherapy (pre-crizotinib) is shown in (Fig. 1B).

Genetic variations in the operative tumor specimens were assessed using NGS technology with a 733-gene panel (3D medicines Inc.) performed in a CLIA and CAP-certificated laboratory. Amplification of four genes (MET, CDK4, MDM2, and FRS2) was identified and the copy numbers were 5, 7, 5, and 6, respectively. NGS also revealed
Figure 1. A. Timeline of treatment management and genetic changes in MET amplification of the patient with metastatic GBC. B. The levels of the tumor marker CEA and representative computed tomography (CT) images of tumor burden before chemotherapy and after two lines of chemotherapy and before crizotinib. Partial response to crizotinib was observed one month later with a continued partial response to crizotinib observed for 2 months. The figures include two lesions of the retroperitoneal lymph nodes and liver metastases.

Abbreviations: CEA, carcinoembryonic antigen; FFPE, formalin-fixed and paraffin-embedded; ctDNA, circulating tumor DNA; NGS, next-generation sequencing; MET, mesenchymal epithelial transition; CDK4, cyclin dependent kinase 4; MDM2, murine double minute 2; FRS2, recombinant fibroblast growth factor receptor substrate 2; PD, progression disease; PR, partial response.
that the tumor harbored two mutations, ATM p.G2765S
Exon57 and KEAP1 p.I145Hfs+29 Exon2. After obtaining
informed consent for the use of off-label targeted ther-
apy, the patient started treatment with crizotinib at an
initial dose of 250 mg q.d. in March 2020. After 2 months
of treatment, the back pain was significantly reduced
(NRS 2–3) and the left supraclavicular lymph node had
almost disappeared. The serum CA19-9 level was sig-
nificantly reduced to 383 U/ml. A partial response (PR)
was observed by abdominal CT scan on reexamination
with a significant reduction in the mass located in the
liver as well as the retroperitoneal lymph node (Fig. 1B).
However, the patient did not choose the recommended
dose (250 mg b.i.d.) and finally discontinued crizotinib
due to intolerable treatment-associated adverse effects
including nausea (grade 4) and vomiting (grade 4) despite
being prescribed antivomiting drugs. Supportive treat-
ment was then given. A CT scan was performed and
showed no evidence of disease progression.

Unfortunately, the patient developed rapid jaundice
10 days after discontinuation of crizotinib and under-
went percutaneous transhepatic catheter drainage. One
week later, bilirubin levels dropped and due to adverse
effects another anti-MET targeted-drug (cabozantinib)
was prescribed. Cabozantinib was administered only for
18 days because of disease progression with bilateral
supraclavicular lymph node growth. To reevaluate the
dynamic change of the genetic characteristics, a periph-
eral blood sample was assessed for circulating tumor
deoxyribonucleic acid (ctDNA) using NGS with an array
of 61 genes (3D medicines Inc.) as the patient could not
tolerate tissue biopsy. The ctDNA analysis was nega-
tive for MET amplification, and mutation of gene GNAS
p.R201HExon8. The condition of the patient (PS 3–4)
rapidly deteriorated and the best supportive care was
then given. A CT scan was performed and showed no evidence of disease progression.

Discussion

In this case study, the patient was treated with crizo-
tinib after the failure of two lines of chemotherapy.
NGS genomic profiling of the operative tumor specimen
revealed MET amplification that confirmed a rapid PR
within 2 months. The patient rapidly progressed with
cabozantinib treatment potentially due to tumor hetero-
genosity. After the patient had progressed, crizotinib was
continued and cabozantinib was introduced. ctDNA
analysis was performed by NGS and showed that MET
amplification was lost. In this case, it was indicated
that MET inhibitors might be effective in harboring MET
amplification advanced GBC offering a potential targeted
therapy option in patients with GBC carrying this lesion.
Also, the loss of MET amplification may be a mechanism
that can promote resistance to subsequent anti-MET
therapy, although the exact mechanism remains unclear.
Mutation profiling of the patient’s ctDNA by NGS revealed
posttreatment dynamic changes in genomic status and
may be used as a novel clinical strategy for personalized
therapy in refractory BTC.

Traditionally, systemic chemotherapy with cisplatin
plus gemcitabine has been the standard of care for
patients with terminal BTC. Recently, targeted therapies
have been proposed in the treatment of metastatic BTC.
Several targeted agents have been approved by the FDA
for advanced BTC including inhibitors of special genes or
pathways such as FGFR2 fusions or rearrangements, IDH1
mutations, HER2 amplifications and NTRK gene fusions.
Precision therapies in BTC are still being explored. Tar-
geted therapy, particularly agents targeting angiogen-
esis, have provided encouraging results, for example,
regorafenib has been approved for advanced BTC by the
FDA.

MET is an attractive new target that is involved in
the pathogenesis of some malignancies. In recent years,
MET inhibitors have been increasingly investigated and
evaluated for the treatment of advanced BTC. Crizo-
tinib is a small molecule tyrosine kinase inhibitor that
inhibits ALK12, ROS1,13 and MET.13 HCC patients har-
boring EHB1-MET fusions present a continuous partial
response for 8 months with crizotinib.14 The first phase
II clinical trial of caboazantinib in terminal BTC patients
using caboazantinib as a second- or third-line treatment
demonstrated limited clinical efficacy in advanced
refractory BTC with a median OS of 5.2 months and a
median progression-free survival of 1.8 months.15 The
MET inhibitor, tivantinib, has been combined with gem-
citabine in BTC patients in a phase I study and patients
demonstrated a partial response at 46% and stable dis-
ease at 27%.16 A randomized phase 2 study of meres-
tinib is being conducted in patients with terminal BTC
(NCT02711553).

Crizotinib has shown promise as a targeted therapy in
lung cancers with MET amplification, yet the devel-
opment of acquired resistance remains a major prob-
lem. Mechanisms of resistance to targeted drugs have
been investigated in patients who initially benefited from
MET inhibitors; for example, those with bypass signaling
and second-site mutations, MET kinase domain muta-
tions, and MET amplifications.17 However, the mecha-
nism of drug resistance to MET inhibitors in advanced
BTC patients with MET amplification remains unknown.
In this case, the patient progressed after being treated
with MET inhibitors and ctDNA analysis by NGS showed
that MET amplification status was lost. Similarly, loss of
MET amplification has been reported in some lung cancer
patients who received first- or third-generation EGFR-TKI
and crizotinib at progression.18 We hypothesize that loss
of MET amplification may contribute to the occurrence of
drug resistance and disease recurrence.

tctDNA is composed of short double-stranded DNA
fragments that are released into the bloodstream by
tumor cells during apoptosis or necrosis. ctDNA sam-
ple can be noninvasively and conveniently obtained in
real-time during the disease and can be used to detect
the genetic information of the tumor. This information can be used to predict treatment responses,
monitor disease relapse, and track mechanisms of therapeutic resistance.\textsuperscript{19} Previous studies have shown that positive results on ctDNA NGS are highly consistent with NGS analysis of tissues in oncogenic driver alterations. Furthermore, in the study of Ikeda et al.,\textsuperscript{20} ctDNA NGS showed higher rates of MET alterations (including MET amplification) than the tissue NGS testing. ctDNA can avoid temporal and spatial (intratumor and intertumor) heterogeneity caused by tissue biopsy. Also, ctDNA may overcome the limitations of NGS in tissue biopsies including insufficient quantities of tissue and risks associated with repeated invasive procedures.\textsuperscript{21} Liquid biopsy provides the possibility to monitor genetic changes during crizotinib treatment of terminal GBC. A similar approach has been reported in non-small cell lung, colorectal, and breast cancer. These data indicate that dynamic monitoring of ctDNA is useful in selecting the appropriate therapeutic strategies to extend patient survival.

In addition to detecting MET amplification of GBC, there was ATM mutation of this patient. ATM mutation has been reported by Zhang et al. as a good target by olaparib in GBC,\textsuperscript{22} there may be a change of response by combining PARP inhibitors. However, this patient was in poor health and she discontinued crizotinib due to intolerable treatment-associated adverse effects despite being prescribed the relevant drugs. During the late stage of disease development, the patient could not tolerate anticancer therapy as the physical status score was 3–4. Nevertheless, this is a good choice for patients of good health; meanwhile, this combined treatment strategy deserves to be explored for its efficacy and safety in subsequent studies. This patient was treated with a reduced dose of crizotinib due to poor health status. A similar case has been reported in the study of Yang et al.,\textsuperscript{13} where an elderly female patient harboring ROS1 rearrangement non-small cell lung cancer achieved a stable disease for 14 months on a reduced dose of crizotinib treatment.

This report has several limitations. Tissue was prioritized for genetic profiling after disease progression. We used peripheral blood samples to assess ctDNA using NGS because the patient could not tolerate tissue biopsy. However, ctDNA is the best alternative for when a conventional biopsy is unavailable or when insufficient quantities of DNA are available for sequencing. Also, the difference in the panel size of the genes in two tests can influence the depth of sequencing coverage. Also, the difference in the panel size of the genes in two tests can influence the depth of sequencing coverage. Furthermore, in the study of Ikeda et al.,\textsuperscript{20} ctDNA NGS showed higher rates of MET alterations (including MET amplification) than the tissue NGS testing. ctDNA can avoid temporal and spatial (intratumor and intertumor) heterogeneity caused by tissue biopsy. Also, ctDNA may overcome the limitations of NGS in tissue biopsies including insufficient quantities of tissue and risks associated with repeated invasive procedures.\textsuperscript{21} Liquid biopsy provides the possibility to monitor genetic changes during crizotinib treatment of terminal GBC. A similar approach has been reported in non-small cell lung, colorectal, and breast cancer. These data indicate that dynamic monitoring of ctDNA is useful in selecting the appropriate therapeutic strategies to extend patient survival.

In summary, this is the first report using NGS of ctDNA to monitor acquired resistance during anti-MET treatment in advanced GBC. Our data indicate that ctDNA can be a useful tool for tracking acquired therapeutic resistance and to analyze mechanisms of resistance. In the era of precision therapy, the accurate detection and identification of therapeutic targets should be prioritized in advanced BTC cases that have failed conventional treatments. This is a successful clinical case report of a GBC patient with MET amplification who received crizotinib as a monotherapy. Our findings suggest that crizotinib may be a promising therapeutic option for GBC patients with MET amplification and warrants further clinical investigation. In this study, the patient unavoidably became resistant to MET inhibitors and the mechanisms of resistance to MET inhibitors in patients with MET amplification remain unknown and require study.

**Ethics statement**

The informed consent for publication of this case report has been obtained from the next of kin of the patient.

**Author contributions**

SHN and LXF collected and analyzed the patients’ data and drafted the manuscript. DS treated the patients and contributed to the figure of the images and treatment process. SXD contributed to the collection of the genetic test results. QM supervised the project and revised the manuscript. All authors read and approved the final manuscript.

**Acknowledgements**

This work was supported by the National Key Development Plan for Precision Medicine Research (Grant No. 2017YFC0910004).

**Conflict of interest**

The authors declare that they have no competing interests.

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