Clinical Case Report

Medicine®

OPEN

Utility of *KRAS* mutational analysis in the preoperative diagnosis of synchronous pancreatic cancer and intrahepatic cholangiocarcinoma

A case report

Yuji Eso, MD, PhD[a,*], Norimitsu Uza, MD, PhD[a], Hiroko Yamagishi, MD[b], Kazuaki Imada, MD[c], Yuto Kimura, MD[a], Toshihiko Masui, MD, PhD[d], Yuzo Kodama, MD, PhD[a], Hiroshi Seno, MD, PhD[a]

Abstract

Rationale: It is often challenging to discriminate between intrahepatic cholangiocarcinoma (ICC) and metastatic liver tumors, especially when the hepatic tumor is small and of a mass-forming type.

Patient concerns: We report a 69-year-old woman presented at our hospital with a small solid tumor in the head of the pancreas that was previously discovered during a medical checkup.

Diagnoses: The patient was diagnosed with synchronous pancreatic cancer and ICC.

Interventions: The patient underwent clinical, histological, immunohistological, and *KRAS* mutational analysis.

Outcomes: Computed tomography revealed poorly enhanced small nodules in both the pancreatic head and liver. Biopsies of both nodules revealed adenocarcinoma; however, it was unclear whether the hepatic lesion was a metastasis of the pancreatic tumor or primary ICC. *KRAS* mutational analysis from FFPE biopsy samples revealed a discordance of mutation status between the tumors. Therefore, the patient was diagnosed with synchronous pancreatic cancer and ICC, whereupon she underwent hepatopancreatoduodenectomy.

Lessons: *KRAS* mutational analysis of FFPE biopsy samples can be utilized for differentiating between ICC and metastatic liver tumor.

Abbreviations: CECT = contrast-enhanced computed tomography, CK = cytokeratin, CRC = colorectal cancer, FFPE = formalin-fixed paraffin-embedded, HCC = hepatocellular carcinoma, ICC = intrahepatic cholangiocarcinoma, MRI = magnetic resonance imaging, PDAC = pancreatic ductal adenocarcinoma, US = ultrasound.

Keywords: intrahepatic cholangiocarcinoma, *KRAS* mutation, pancreatic cancer

1. Introduction

KRAS is a critical proto-oncogene involved in signal transduction, and plays a central role in cancer cell proliferation, invasion, and metastasis.[1] *KRAS* mutations are common in colorectal cancer, pancreatic ductal adenocarcinoma (PDAC), intrahepatic cholangiocarcinoma (ICC), and lung cancer.[2–5] Recent advancements in DNA extraction and mutation testing techniques have enabled the identification of *KRAS* mutation status from formalin-fixed paraffin-embedded (FFPE) needle biopsy samples.[6] Herein, we report a case of synchronous pancreatic cancer and ICC diagnosed by *KRAS* mutational analysis of FFPE needle biopsy samples.

2. Case report

A 69-year-old woman presented at our hospital with a small solid tumor in the head of the pancreas that was previously discovered during a medical checkup. She was a nonsmoker and had been treated for hypertension for the past 5 years with Nifedipine (20 x0200A;mg daily). She reported no symptoms, including no abdominal pain. Her serum carbohydrate antigen 19 to 9 level was markedly elevated (833.2 U/mL). A contrast-enhanced computed tomography (CECT) scan revealed a poorly enhanced nodule (9 mm in size) in the head of the pancreas (Fig. 1A). Additionally, a 14-mm nodule was also observed in the caudate lobe of the liver. The hepatic nodule exhibited low vascularity on CECT and was suspected of being either a pancreatic tumor metastasis or primary ICC (Fig. 1B). No lymph node or distant metastasis was detected on CECT. On gadolinium ethoxybenzyl

Editor: Andrea Ruzzenente.

Written informed consent for publishing this case report was obtained from the patient.

The authors have no conflicts of interest to disclose.

[a] Department of Gastroenterology and Hepatology, [b] Integrated Clinical Education Center, [c] Department of Cardiovascular Medicine, Kyoto University Hospital, [d] Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan.

[*] Correspondence: Yuji Eso, Department of Gastroenterology and Hepatology, Graduate School of Medicine, Kyoto University, 54 Kawahara-cho, Syogoin, Sakyo-ku, Kyoto, 606-8507, Japan (e-mail: yujieso@kuhp.kyoto-u.ac.jp).

Medicine (2017) 96:50(e9217)

Received: 10 March 2017 / Received in final form: 28 September 2017 / Accepted: 20 November 2017

http://dx.doi.org/10.1097/MD.0000000000009217

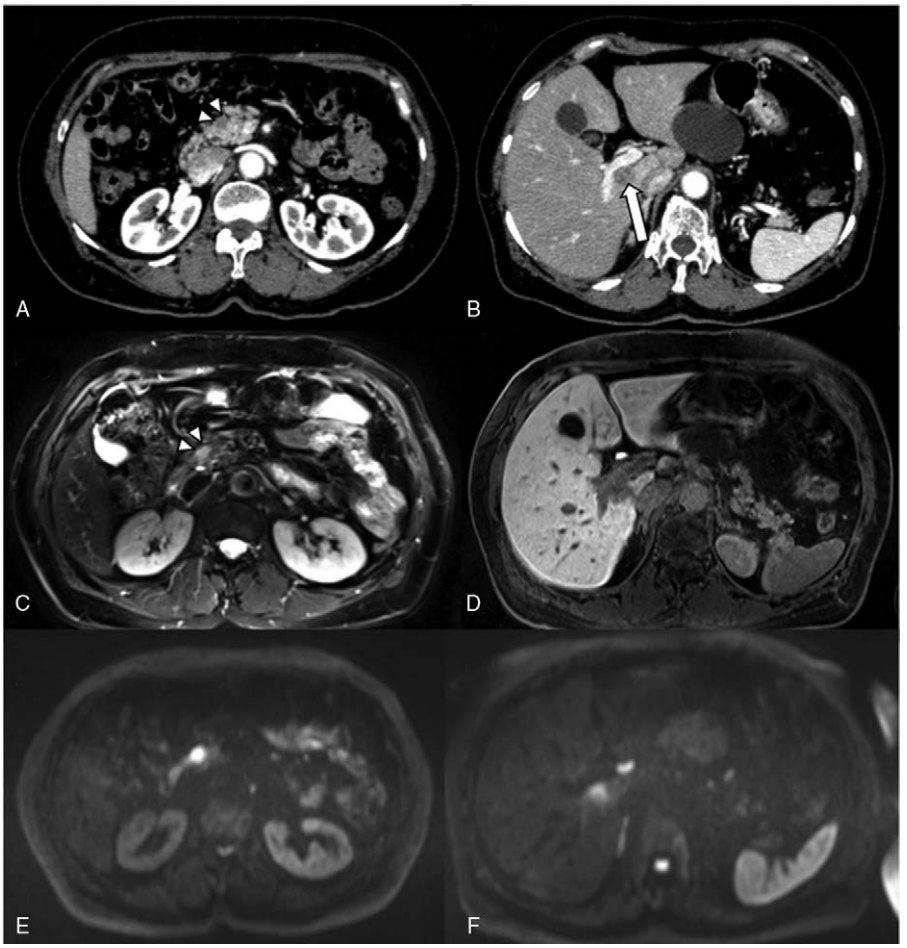

Figure 1. Findings of contrast-enhanced computed tomography (CECT) and Gd-EOB-DTPA-enhanced magnetic resonance imaging (EOB-MRI). (A) The CECT scan revealed a 9 mm poorly enhanced nodule in the head of the pancreas (white arrowheads). (B) A 14 mm poorly enhanced nodule is present in the caudate lobe of the liver (white arrow). (C) EOB-MRI revealed that the pancreatic nodule exhibits hyperintensity on T2-weighted imaging (white arrowheads). (D) The hepatic nodule demonstrated hypointensity in the hepatobiliary phase (white arrow). (E and F) The pancreatic nodule and hepatic nodules exhibited high signal intensity on diffusion-weighted MRI.

diethylenetriamine pentaacetic acid-enhanced magnetic resonance imaging (MRI), the pancreatic nodule showed hyperintensity on T2-weighted images, while the hepatic nodule demonstrated hypointensity in the hepatobiliary phase (Fig. 1C and D). Both nodules exhibited high signal intensity on diffusion-weighted MRI (Fig. 1E and F).

Histopathological imaging of the endoscopic ultrasonography (US)-guided fine-needle aspiration biopsy sample from the pancreatic tumor revealed adenocarcinoma with anisonucleosis, nuclear enlargement, and hyperchromasia that were consistent with PDAC (Fig. 2A). The pancreatic tumor was immunohistologically positive for cytokeratin (CK) 7 and negative for CK20 (Fig. 2B and C). Moreover, US-guided percutaneous biopsy of the hepatic nodule revealed a moderately differentiated adenocarcinoma with ductal formation; the nodule was positive for CK7 and negative for CK20 (Fig. 2D–F). Discrimination between PDAC with liver metastasis and synchronous PDAC and ICC was essential for determining the course of treatment (i.e., systemic chemotherapy vs hepatopancreatoduodenectomy). However, such discrimination was not possible using CT/MRI images and histopathological analysis.

Accordingly, we investigated *KRAS* mutation statuses in both tumors. DNA was extracted from the FFPE biopsy samples of the pancreatic and hepatic tumors using the QIAamp DNA FFPE Tissue Kit (Qiagen, Foster City, CA) following the manufacturer's protocol. The oligonucleotide primers were designed to amplify the sequences of exon 2 and exon 3 of *KRAS* as follows: *KRAS* exon2S 5′-cttaagcgtcgatggaggag-3′, *KRAS* exon2AS 5′-agaatggtcctgcaccagtaa-3′, *KRAS* exon3S 5′-tcaagtcctttgcccatttt-3′, and *KRAS* exon3AS 5′-tgcatggcattagcaaagac-3′. Amplification of the *KRAS* gene was performed using Tks Gflex DNA Polymerase (Takara Bio, Shiga, Japan). Sequencing was performed using the Applied Biosystems 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA). This resulted in the detection of a *KRAS* G12D mutation in the pancreatic tumor; however, no codon 13, 59, and 61 mutations were detected. On the other hand, a *KRAS* Q61H mutation was detected in the hepatic tumor, although there were no codon 12, 13, or 59 mutations (Table 1). We confirmed that the same results were obtained by multiplex PCR assaying at an outsourcing laboratory (BML, Inc., Tokyo, Japan).

Based on these results, the patient was diagnosed with synchronous PDAC and ICC; she consequently underwent hepatopancreatoduodenectomy. Histopathological examination of the pancreatic tumor revealed a moderately differentiated ductal adenocarcinoma with vascular and perineural invasion

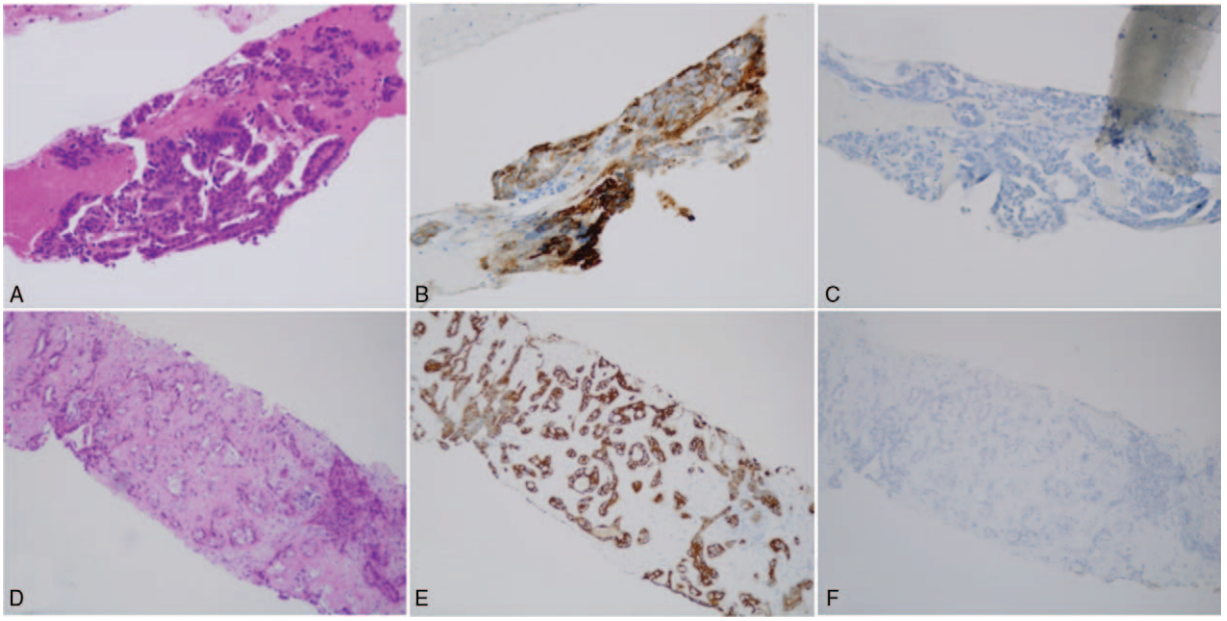

Figure 2. Histopathological findings following ultrasonography-guided fine-needle aspiration biopsy sample of the pancreatic and hepatic tumor. (A) The pancreatic tumor was adenocarcinoma with anisonucleosis, nuclear enlargement, and hyperchromasia that were consistent with pancreatic ductal adenocarcinoma (hematoxylin and eosin stain). (B) The pancreatic tumor was immunohistologically positive for CK7. (C) The pancreatic tumor was negative for CK20. (D) The hepatic tumor was a moderately differentiated adenocarcinoma with ductal formation (hematoxylin and eosin stain). (E) The hepatic tumor was immunohistologically positive for CK7. (F) The hepatic tumor was negative for CK20.

(Fig. 3A). The hepatic tumor was diagnosed as a moderately differentiated adenocarcinoma with ductal formation that was consistent with ICC (Fig. 3B). Additional *KRAS* sequencing of the surgical specimen was not performed.

3. Discussion

ICC normally presents as an adenocarcinoma with ductal formation, and is classified into mass-forming, periductal-infiltrating, and intraductal-growth types.[7] On the other hand, the liver is a common site of metastasis, most frequently from gastrointestinal, pancreatic, lung, and breast cancers; many such metastases present as an adenocarcinoma. Therefore, discriminating between ICC and metastatic liver tumors can often be difficult, especially when the tumor is small and of the mass-forming type.

Immunostaining using antibodies with high organ specificity often plays an important role in the differential diagnosis of metastatic liver tumors.[8] In particular, immunostaining with CK is helpful for discriminating between hepatocellular carcinoma (HCC) and other types of liver tumors, and for determining the primary tumor site once the diagnosis of adenocarcinoma has been established. Normal and neoplastic hepatocytes express CK8 and 18, and are generally negative for CK7, 19, and 20. On the other hand, normal and neoplastic cholangiocytes express CK7, 8, 18, and 19, and are usually negative for CK20.[8] Noting these CK profiles, Shimonishi et al[9] reported that immunostaining of CK7 and 20 is helpful for differentiating HCC and ICC from metastatic adenocarcinomas in the liver. However, the CK profiles of ICC and PDAC are similar[9]; hence, immunostaining with CK7 and CK20 is of limited value when discriminating ICC from PDAC liver metastasis.

Table 1

***KRAS* mutational analysis of pancreatic and hepatic tumor.**

	Pancreatic tumor	Hepatic tumor
Exon 2		
codon 12	G12D	(–)
codon 13	(–)	(–)
Exon 3		
codon 59	(–)	(–)
codon 61	(–)	Q61H

KRAS mutations are common in colorectal cancer (CRC), PDAC, ICC, and lung cancer.[2–5] In the case described herein, *KRAS* mutations were detected in both the pancreatic and hepatic tumors. However, the type of mutation was different in each case; the pancreatic tumor showed G12D whereas the hepatic tumor had Q61H. Several studies have shown that intratumoral heterogeneity of *KRAS* mutational status and *KRAS* heterogeneity between primary tumor and metastasis is rare in PDAC.[10,11] Furthermore, *KRAS* mutations are detectable in 70% to 93% of PDACs.[11,12] According to previous studies, the most frequent *KRAS* mutation in PDAC is G12D (46.5–55.2%), followed by G12V (11.1–37.9%), G12R (3.4–14.8%), G12A (2.3–3.7%), G12C (3.4–3.7%), and G12S (3.7%).[13–15] On the other hand, it was reported that *KRAS* mutations are detectable in 11.0% to 31.6% of ICCs, with G12D being the most frequently reported; however only mutations at codons 12 and 13 (exon 2) were investigated in these reports.[4,16–19] In this case, the discovery of a Q61H mutation in exon 3 of hepatic tumor DNA ruled out PDAC liver metastasis.

The *KRAS* mutational analysis assay using FFPE samples is well-established and widely utilized for predicting the response to anti-epidermal growth factor receptor monoclonal antibodies (cetuximab and panitumumab) in CRC.[6,20–22] Therefore, *KRAS* mutational analysis can also be applied to FFPE samples from other organs. In fact, Krasinskas et al[23] reported the utility of *KRAS* mutational analysis for distinguishing pancreatic meta-

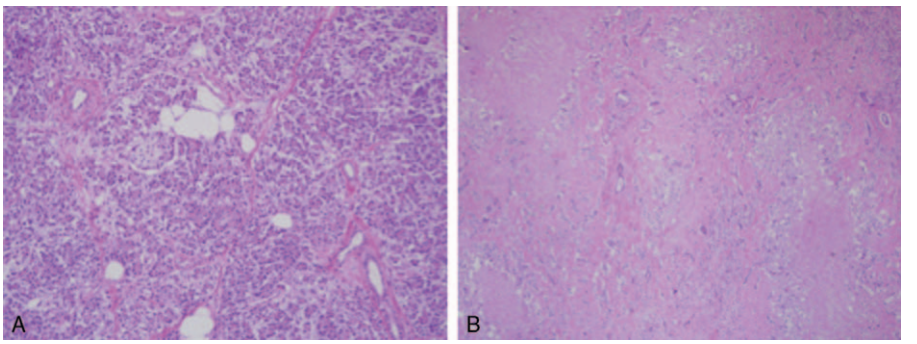

Figure 3. Histopathological findings of the resected pancreatic and hepatic tumor. (A) The pancreatic tumor was a moderately differentiated ductal adenocarcinoma with vascular and perineural invasion (hematoxylin and eosin stain). (B) The hepatic tumor was a moderately differentiated adenocarcinoma with ductal formation that was consistent with intrahepatic cholangiocarcinoma (hematoxylin and eosin stain).

static adenocarcinomas from primary lung adenocarcinomas. To our knowledge, this is the first case report of synchronous PDAC and ICC diagnosed by *KRAS* mutational analysis; our findings suggest that such analyses from FFPE needle biopsy samples may be utilized to differentiate between primary hepatic tumors and metastasis to the liver.

This case report was prepared in accordance with the CARE Statement.[24]

Acknowledgments

The authors would like to thank Dr. H. Maeda, M. Sakaguchi, Y. Iemura, A. Yoshizawa, S. Minamiguchi, T. Sakurai, and H. Haga (Department of Diagnostic Pathology, Kyoto University Hospital) for histopathological diagnosis.

References

[1] Downward J. Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer 2003;3:11–22.
[2] Onozato W, Yamashita K, Yamashita K, et al. Genetic alterations of K-ras may reflect prognosis in stage III colon cancer patients below 60 years of age. J Surg Oncol 2011;103:25–33.
[3] Zhang C, Guo W, Wu J, et al. Differential high-resolution melting analysis for the detection of K-ras codons 12 and 13 mutations in pancreatic cancer. Pancreas 2011;40:1283–8.
[4] Fujimoto A, Furuta M, Shiraishi Y, et al. Whole-genome mutational landscape of liver cancers displaying biliary phenotype reveals hepatitis impact and molecular diversity. Nat Commun 2015;6:6120.
[5] Guibert N, Ilie M, Long E, et al. KRAS mutations in lung adenocarcinoma: molecular and epidemiological characteristics, methods for detection, and therapeutic strategy perspectives. Curr Mol Med 2015;15:418–32.
[6] Angulo B, Lopez-Rios F, Gonzalez D. A new generation of companion diagnostics: cobas BRAF, KRAS and EGFR mutation detection tests. Expert Rev Mol Diagn 2014;14:517–24.
[7] Nakanuma Y, Miyata T, Uchida T. Latest advances in the pathological understanding of cholangiocarcinomas. Expert Rev Gastroenterol Hepatol 2016;10:113–27.
[8] Kakar S, Gown AM, Goodman ZD, et al. Best practices in diagnostic immunohistochemistry: hepatocellular carcinoma versus metastatic neoplasms. Arch Pathol Lab Med 2007;131:1648–54.
[9] Shimonishi T, Miyazaki K, Nakanuma Y. Cytokeratin profile relates to histological subtypes and intrahepatic location of intrahepatic cholangiocarcinoma and primary sites of metastatic adenocarcinoma of liver. Histopathology 2000;37:55–63.
[10] Hashimoto D, Arima K, Yokoyama N, et al. Heterogeneity of KRAS mutations in pancreatic ductal adenocarcinoma. Pancreas 2016;45:1111–4.
[11] Cancer Genome Atlas Research NetworkIntegrated genomic characterization of pancreatic ductal adenocarcinoma. Cancer Cell 2017;32:185.e13–203.e13.
[12] di Magliano MP, Logsdon CD. Roles for KRAS in pancreatic tumor development and progression. Gastroenterology 2013;144:1220–9.
[13] Miglio U, Oldani A, Mezzapelle R, et al. KRAS mutational analysis in ductal adenocarcinoma of the pancreas and its clinical significance. Pathol Res Pract 2014;210:307–11.
[14] Immervoll H, Hoem D, Kugarajh K, et al. Molecular analysis of the EGFR-RAS-RAF pathway in pancreatic ductal adenocarcinomas: lack of mutations in the BRAF and EGFR genes. Virchows Arch 2006;448:788–96.
[15] Scarpa A, Capelli P, Villaneuva A, et al. Pancreatic cancer in Europe: Ki-ras gene mutation pattern shows geographical differences. Int J Cancer 1994;57:167–71.
[16] Churi CR, Shroff R, Wang Y, et al. Mutation profiling in cholangiocarcinoma: prognostic and therapeutic implications. PLoS One 2014;9:e115383.
[17] Zou S, Li J, Zhou H, et al. Mutational landscape of intrahepatic cholangiocarcinoma. Nat Commun 2014;5:5696.
[18] Hsu M, Sasaki M, Igarashi S, et al. KRAS and GNAS mutations and p53 overexpression in biliary intraepithelial neoplasia and intrahepatic cholangiocarcinomas. Cancer 2013;119:1669–74.
[19] Xie D, Ren Z, Fan J, et al. Genetic profiling of intrahepatic cholangiocarcinoma and its clinical implication in targeted therapy. Am J Cancer Res 2016;6:577–86.
[20] Allegra CJ, Rumble RB, Hamilton SR, et al. Extended RAS gene mutation testing in metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy: American Society of Clinical Oncology Provisional Clinical Opinion Update 2015. J Clin Oncol 2016;34:179–85.
[21] Ciombor KK, Wu C, Goldberg RM. Recent therapeutic advances in the treatment of colorectal cancer. Annu Rev Med 2015;66:83–95.
[22] Bando H, Yoshino T, Shinozaki E, et al. Simultaneous identification of 36 mutations in KRAS codons 61 and 146, BRAF, NRAS, and PIK3CA in a single reaction by multiplex assay kit. BMC Cancer 2013;13:405.
[23] Krasinskas AM, Chiosea SI, Pal T, et al. KRAS mutational analysis and immunohistochemical studies can help distinguish pancreatic metastases from primary lung adenocarcinomas. Mod Pathol 2014;27:262–70.
[24] Gagnier JJ, Kienle G, Altman DG, et al. The CARE guidelines: consensus-based clinical case report guideline development. J Clin Epidemiol 2014;67:46–51.