

# The first successful treatment and genetic sequencing of primary hepatic adenocarcinoma with sarcomatous overgrowth: a case report

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Hanjie Hu<sup>1,2,\*</sup> , Qianqian Song<sup>2,3,\*</sup>,  
Hong Zhao<sup>1,2,\*</sup>, Bo Zheng<sup>2,4</sup> and Haizhen Lu<sup>2,4</sup>

## Abstract

Adenocarcinoma is a rare type of tumor with a mixture of epithelial and stromal components and often occurs in the female reproductive system. Primary hepatic adenocarcinoma (PHAS) is extremely rare, with only two cases reported so far. Both patients had poor outcomes. Here, we report the case of a 36-year-old man with pain under the xiphoid process who was diagnosed with a bile duct tumor. He was treated with adjuvant radiotherapy when surgery was performed on him. Pathologically, the tumor contained benign epithelial tissue, and the submucosa of the bile duct in the liver showed infiltrating growth of spindle cell components. The cells were dense, mildly heterotypic, and occasionally mitotic, and the patient was diagnosed with PHAS. Whole-exome sequencing results showed that a total of 12 mutations were shared by the two tissues. The patient received adjuvant radiotherapy and he was tumor-free until 31 months postoperatively. This case will provide some references of the disease to other researchers.

<sup>1</sup>National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Hepatobiliary Surgery, Beijing, China

<sup>2</sup>Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

<sup>3</sup>National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, State Key Laboratory of Molecular Oncology

<sup>4</sup>National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Department of Pathology, Beijing, China

\*These authors contributed equally to this work.

### Corresponding author:

Haizhen Lu, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Beijing, China.

E-mail: [haizhen\\_lu@qq.com](mailto:haizhen_lu@qq.com)



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## Keywords

Hepatic adenocarcinoma, overgrowth, sequencing, microdissection, whole-exome sequencing, diagnosis, oncology, liver cancer

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## Introduction

Adenocarcinoma is a rare type of tumor with a mixture of epithelial and stromal components.<sup>1</sup> It often occurs in the female reproductive system and is known as Mullerian adenocarcinoma, which is the most common type.<sup>1</sup> Mullerian adenocarcinoma was first described in 1974 and is often characterized by a benign, occasionally atypical glandular component and a sarcomatous stromal component, which is usually low-grade. When the sarcoma component accounts for at least one-quarter of the tumor, it is defined as a sarcomatous overgrowth, which has an aggressive clinical course.<sup>2</sup> Primary sarcoma of the liver represents less than 1% of all liver cancers.<sup>3</sup> Because of its various histological subtypes, no recognized authoritative clinical guidelines have been established.<sup>4</sup> It is not clear whether primary hepatic adenocarcinoma (PHAS) is a type of primary hepatic sarcoma. Primary adenocarcinomas of the digestive system, especially the liver, are rare.<sup>1</sup> Some previous reports of liver adenocarcinoma introduced rare cases of liver endometriosis. John E. Jelovsek's group reported a case in a 52-year-old woman who underwent a hysterectomy with bilateral salpingo-oophorectomy for endometriosis history in 2004.<sup>5</sup> Pathological findings of the liver mass were an endometrioma and retroperitoneum with a Mullerian adenocarcinoma component. This finding was extremely similar to the case of a 54-year-old woman that N'Senda et al. described in 2000.<sup>6</sup> However, in 2018, Meguro et al. described

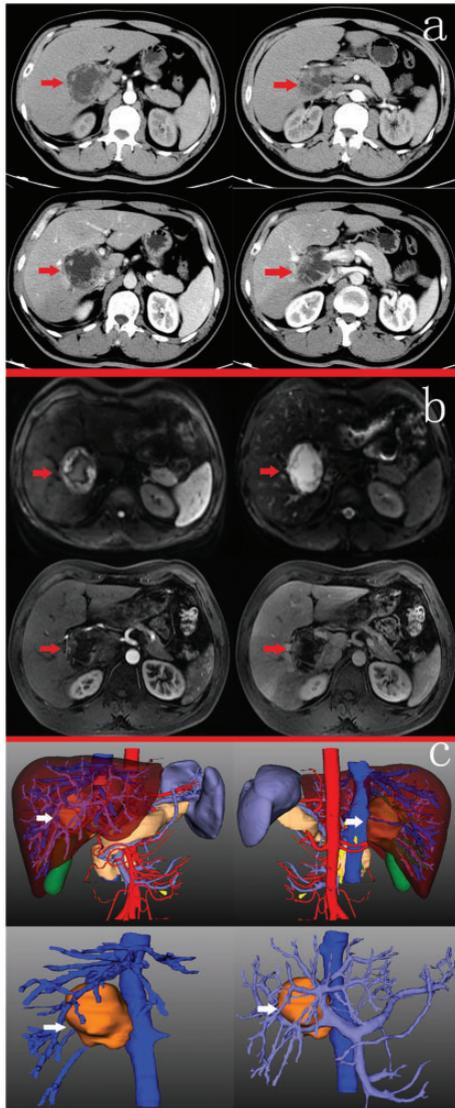
a new case of PHAS for the first time during an autopsy after the patient died with liver cirrhosis.<sup>1,6</sup> Oliveira et al. published the case of a 65-year-old female PHAS patient in 2020, and tumor recurrence appeared after 2 years of follow-up and death at 28 months after surgery.<sup>7</sup> This subtype of tumors cannot be classified on the basis of any recent criteria provided by the World Health Organization (WHO), and is very likely to have great aggressive potential. Here, we report a case of PHAS treated with hepatectomy and adjuvant radiotherapy. Additionally, we used laser capture microdissection (LCM) for whole-exome sequencing (WES) of different components of the tumor.

We present the following case in accordance with the CARE reporting checklist.

## Case report

### Clinical history

A 36-year-old man came to National Cancer Center/Cancer Hospital because of pain under the xiphoid process. Ultrasound imaging revealed a mass in the right lobe of the liver. Physical examination showed no special findings. Enhanced CT indicated that a 6.4 × 5.7 cm cystic solid mass (predominantly cystic) was in the right lobe of the liver with enhancement, which frequently are malignant. Multiple nodules were seen around the mass (Figure 1a). Gd-EOB-DTPA magnetic resonance imaging (MRI) showed no clear contrast agent



**Figure 1.** (a) CT: There was a mass in the right lobe of the liver with a maximum section of  $6.4 \times 5.7$  cm, with enhancement and clear edge. It was dominated by cystic density and nodular and cord-like soft tissue shadows were seen at the edge. (b) MRI: Cystic solid mass in the right lobe of the liver,  $6.5 \times 5.5$  cm, and multiple nodules with enhancement were seen in the capsule wall. T2WI/FS shows medium and high signal, and diffusion weighted imaging (DWI) diffusion was limited. The mass continued with the bile duct, close to the right portal vein. (c) Three-dimensional reconstruction showing the relationship between tumor and vessel.

uptake in the hepatobiliary stage. The mass was attached to the bile duct and closely related to the right ramus of the portal vein, which tend to be a cystadenoma of the bile duct (malignancy cannot be ruled out) (Figure 1b). The man smoked one pack of cigarettes per day for 20 years. He had no history of hepatitis or heavy drinking nor any clear genetic or family factors. We discussed this patient in a multidisciplinary team (MDT) meeting and decided to perform a radical operation. To further clarify the relationship between the lesion and its surrounding tissues, we performed three-dimensional CT reconstruction (Figure 1c).

After excluding the surgical contraindications and obtaining the patient's informed consent for treatment, the operation was performed smoothly. Through laparotomy, the size of the tumor was approximately 10 cm. The tumor was in the right hepatic duct with a length and diameter of approximately 8 cm and was cystic and solid. It extended along the intra-hepatic bile duct adjacent to the right branch of the portal vein, pressing the right hepatic artery and not involving the confluence of the left and right hepatic ducts. We inferred that it was a malignant tumor of the bile duct and performed right hepatectomy + cholecystectomy + hilar lymph node dissection. The operation lasted 6 hours and 35 minutes, and there was an approximately 500 mL bleeding event without blood transfusion. He was discharged from the hospital on the seventh day after the operation without significant complications.

## Pathology

### Macroscopic features

A mass measuring  $7.8 \times 5 \times 5$  cm was observed near the hilum of the liver. The structure of the tumor was cystic solid mixture, in which the solid component

predominated. The surface of the cystic area showed papillary growth, and the tumor appeared to grow along the lumen.

### Microscopic features

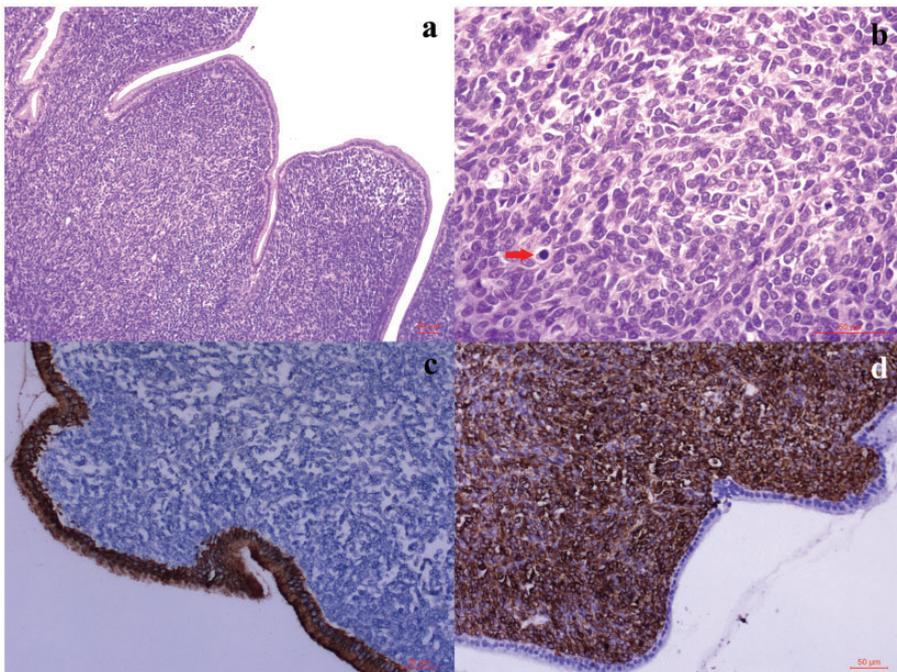
The submucosa of the bile duct in the liver showed infiltrating growth of spindle cell components. The cells were dense, mildly heterotypic, and occasionally mitotic. The tumor grew along the intrahepatic bile duct system with no clear involvement of liver tissue. No tumor was seen at the basal margin.

### Immunohistochemistry analysis

AE1/AE3 (epithelium+), Desmin (-), Ki-67 (hotspot 20%+), S-100(-), Vimentin (3+), CK18 (-), LCA (-), CK19 (epithelium+), CK7 (epithelium+), CD56 (1+),

SMA (-), CD34 (-), CD31 (-), Bcl-2 (2+), ER (-), P53 (-), PR (1+), Inhibin (-), CD10 (1+), Vimentin (matrix+), and EMA (epithelium+) (Figure 2).

**Diagnosis and differential diagnosis.** The pathological diagnosis was hepatic adenosarcoma. The differential diagnoses were as follows: 1: Undifferentiated carcinoma, also known as sarcomatoid carcinoma or oncosarcoma, is a highly malignant tumor with obvious atypia of tumor components. The tumor easily exhibits nuclear mitosis, generally without a lobulated structure or differentiated mature epithelial coating, and expresses epithelial markers. 2: Adenofibroma, a benign tumor, is generally similar to adenosarcoma. Under the microscope, the surface of the lobulated structure



**Figure 2.** Pathology: (a) Hematoxylin and eosin (H&E)-100x: at lower magnification, the tumor showed phyllodes-like configuration lining with mucinous columnar epithelial cells. (b) H&E-400x: at higher magnification, the stromal cells were mild to moderate cytological atypia with mitosis (red arrow). (c) Immunohistochemistry (IHC) 200x: CK19 staining was highly positive in the epithelial cells. (d) IHC 200x: Vimentin staining was strongly positive in the stromal cells.

is covered by glandular epithelium. The stromal components have no obvious atypia, the cells are relatively sparse, and the mitotic phase is rare.

**Lymph node condition.** No lymph node metastasis was observed (0/10).

**Adjuvant therapy and follow-up.** To improve the local control rate and reduce the postoperative recurrence rate, we discussed MDTs again. In addition, 6 mV-X-ray volumetric intensity modulated radiotherapy (VMAT) was performed. Specific plan: 95% planning target volume (PTV) 50 Gy/2.0 Gy/25 f. clinical target volume (CTV): The tumor bed and metal marker (placed during the surgery) were externally placed 1 cm, including an 8, 9, 12, 13, 16a lymphatic drainage area. The PTV:CTV outlay was 0.5 cm.

The patient was followed up according to the conventional strategy: the first review was performed 1 month after surgery and then every 3 months for the first 2 years, then every 6 months for 2 to 5 years. To date, the patient has been followed up to 31 months after the operation, and no signs of recurrence have been found (Figure 3). The patient could carry out normal life and work, and no abnormal results were found in the hematology test.

### WES through LCM

Because there were two components of the tumor, benign columnar epithelium and

malignant sarcoma, we planned to perform WES of the two components separately aiming to better analyze the origin and molecular characteristics of the tumor.

### Sample collection

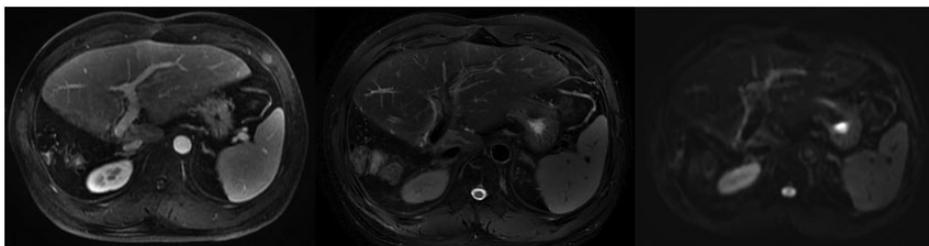
All tissue samples were retrieved from the National Cancer Center/Cancer Hospital under institutional ethics committee approval and written informed consent of the patient. All specimens were obtained during surgery and frozen at  $-40^{\circ}\text{C}$ , then removed and used to generate frozen sections after 2 years.

### LCM

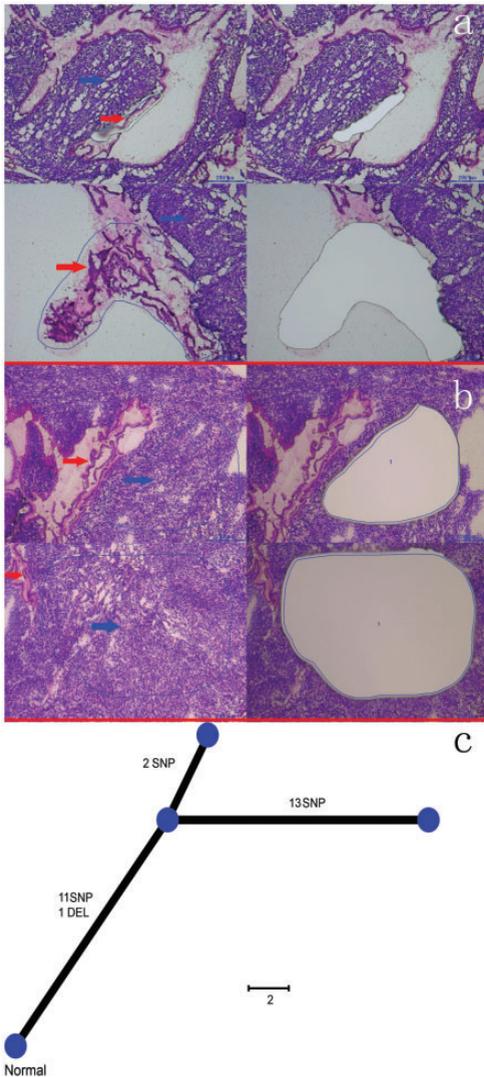
Tissue samples were cut into consecutive sections at a thickness of 10  $\mu\text{m}$ . Rapid hematoxylin and eosin (H&E) staining was performed on each section to ensure the differentiation of tissues. LCM was performed immediately after H&E. Microscopically, the epithelial tissue was distinct from the sarcomatous tissue, and some epithelial tissue has been shed. We used LCM to collect epithelial and sarcoma tissues separately (Figure 4a, 4b).

### Sequencing and identification of somatic mutations

Whole genome libraries of tumors and matching white blood cell (WBC) DNA were enriched for those covering the exome regions with Agilent SureSelectXT



**Figure 3.** No tumor was found by MRI (25 months after operation).



**Figure 4.** (a, b) Laser capture microdissection (LCM) images. The red arrows show epithelial tissue and the blue arrows show the sarcoma tissue. (c) The evolutionary tree of the patient. Eleven single nucleotide polymorphisms (SNPs) and one deletion (DEL) were shared by both tissues.

Human All Exon V5 probe and reagents (Agilent, Santa Clara, CA, USA). The captured and amplified libraries were sequenced on an Illumina HiSeqX Ten with 150-bp paired-end sequencing.

Raw reads were then trimmed for adapter contamination with Trimmomatic version 0.33. Trimmed reads were aligned to the hg19 human genome with BWA MEM software for both tumor and normal samples. Polymerase chain reaction duplications of each BAM file were marked with Picard software (version 1.103 <https://broadinstitute.github.io/picard/>). The BAM files were locally realigned, and the base quality scores were recalibrated with GATK (version 3.1).<sup>8</sup> Single nucleotide variants and insertions/deletions (indels) were identified by MuTect (version 1.1.6) and Strelka (version 1.0.14) separately with default parameters.<sup>9,10</sup> Mutations were subsequently filtered with the following criteria: tumor sample coverage  $\geq 15X$ ; normal sample coverage  $\geq 10X$ ; tumor frequency  $> 0.02$ ;  $\geq 4$  distinct reads supporting the mutation in tumor sample; normal frequency  $< 0.05$ ; normal frequency/tumor frequency  $< 0.1$ . Then, the filtered somatic variants were further annotated with Variant Effect Predictor (version 83).<sup>11</sup> The variants were filtered to remove all noncoding and synonymous variants, retaining only nonsynonymous single-nucleotide variants, splice site variants, and coding indels. The selected variants were then filtered against databases including dbSNP, the 1000 Genomes Project, Exome Sequencing Project (6500), and Exome Aggregation Consortium (ExAC) to remove common germline variants. All the filtered somatic variants were further validated by visual inspection in IGV (version 2.3.34). Then, a phylogenetic tree for this case was built using the software tool PHYLIP (version 3.1).

### Sample quality

Genomic DNA was extracted from three samples, the epithelium (sample A), sarcoma (sample B), and somatic cells (sample N), using a KAPA HyperPlus Kit (Kapa Biosystems, Wilmington, MA, USA).

Samples A and B were collected through LCM and sample N was collected from gall bladder tissue, which was removed during surgery. The total amounts of DNA extracted were 31.75 ng (sample A), 192.08 ng (sample B), and 24,480 ng (sample N). The sequencing data of the three samples were 37.1 GB (sample A), 15.8 GB (sample B), and 17.9 GB (sample N). The average sequencing depths were 171.1X (sample A), 172.3X (sample B), and 190X (sample N).

### ***Bioinformatic analysis***

There were 25 somatic mutations found in sample A, among which 24 single-nucleotide polymorphisms (SNPs) and one deletion (DEL) were identified. Fourteen somatic mutations were detected in sample B, and there were 13 SNPs and 1 DEL. Eleven SNPs and one DEL were shared by both tissues (Figure 4c). The details of the mutations are shown in Supplemental Table 1.

### ***Ethics approval and consent to participate***

The study has been granted ethical exemptions by the Ethics Committee of National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital because it was a case report article. Written informed consent was obtained from the patient for the publication of this case report and accompanying images. The reporting of this study conforms to CARE guidelines.<sup>12</sup>

### **Discussion**

Adenosarcoma, especially hepatic adenosarcoma, is a rare tumor. Only two cases of primary hepatic adenosarcoma have been reported thus far.<sup>1,7</sup> For diagnosis, our preoperative examination showed that

this tumor was a primary malignant tumor of the liver, with a large number of cystic regions and atypical enhancement characteristics. Intraoperative exploration showed that the tumor was closely related to the bile duct system, which was considered to be atypical bile duct cell carcinoma. Therefore, we conducted further hilar lymph node dissection, and the final pathology revealed that none of the lymph nodes had metastasized. Because of its close proximity to intrahepatic vessels, we conducted postoperative adjuvant radiotherapy to reduce the risk of the tumor resection margin being close to the left hepatic vessel preservation.

Pathologically, we found that the tumor grew along the biliary system and contained epithelial components positive for CK19 and other markers. It also had a large number of dense, messy, heterogeneous sarcomas with mitotic items growing in the subepithelium, which is consistent with the diagnosis of typical adenosarcoma. To apply the definition of adenosarcoma of the uterus, a sarcoma component exceeding 25% of the tumor volume means sarcomatous overgrowth. This value was significantly higher than 25% in this patient, suggesting the possibility of poor prognosis. We completely retained the patient's tissue specimens, imaging data, and three-dimensional reconstruction, but unfortunately, intraoperative photos and general images were lost.

Previous literature shows that adenosarcoma is a rare tumor that mainly occurs in the female reproductive system, especially in the uterus and is usually of low malignancy.<sup>2,5,13–17</sup> It may also be secondary to endometriosis in other areas, such as the liver, colon/rectum, breast, and retroperitoneum. Secondary hepatic adenosarcomas were reported by Jelovsek et al. and N'Senda et al. in 2004 and 2000, respectively.<sup>5,6</sup> In the current case, the patient was male and the immunohistochemical ER

and PR staining results were not consistent with endometriosis. It is even rarer to have a primary disease elsewhere. In 2018, Meguro et al. first reported a case of primary hepatic adenocarcinoma found at autopsy,<sup>1</sup> and the specimens were recorded in detail and stained using immunohistochemistry. Another case was recorded in 2020 by Oliveira et al.,<sup>7</sup> and the patient survived tumor-free for 24 months and died at 28 months after radical surgery. In our case, the patient was first diagnosed in 2018 and has been followed up for more than 2 years. No recurrence or metastasis was found, and the patient's quality of life was not affected. This appears to be the first case of a successful treatment, and we hope to provide more experience for this disease through our report.

Adenocarcinomas are characterized by a mixture of benign epithelium and malignant sarcomas. Many scholars think of adenocarcinoma as a type of sarcoma. From this point of view, it appears that they should relapse as malignant sarcomas. If so, we may be able to refer to the treatment of sarcomas, and whether it can be classified as a sarcoma needs further study. A review of the literature shows that this belief is not true. Many recurrent adenocarcinomas still have epithelial components,<sup>14,18</sup> suggesting that they are essentially different from a simple sarcoma. Another question is whether the tumor originated from a single tissue. To further understand the genetic characteristics of the disease, which will help us understand the origin, occurrence, and treatment, we sequenced the specimens for the second generation. We divided the sequencing into three parts: the malignant component sarcoma, somatic control from the gallbladder, and benign component epithelium. Because of the small proportion of epithelial components in tumors and the distribution of monolayer cells around a large amount of mesenchymal cells, it is difficult to obtain pure epithelial cell DNA.

LCM was used for cell dissection to address this problem. The final sequencing results showed that the two components of the tumor had more than half the mutations in common, although the total number of mutations detected was small. This finding suggests the possibility of two components diverting from a single tissue. Moreover, this result may support the epithelium's potential to transform into sarcomas. However, no characteristic genes were found in the list of mutations, and the tumor mutation burden was not high.

There are some limitations of this report. We did not provide a photograph of the gross specimen because it has not been properly preserved. Gene sequencing included only single-nucleotide variants, splice site variants, and coding indels, and the specimens were not fresh.

## **Conclusions**

Here, we report a new type of rare primary liver tumor and its diagnosis and treatment. Adenocarcinomas may be misdiagnosed and overlooked when they occur outside the female reproductive system, which can lead to bad clinical outcomes. We hope that documenting our experience will give researchers some references for this disease.

## **Authors' contributions**

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by HH and QS, and they contributed equally to this article. BZ prepared the pathological specimens. HZ and HL coordinated the project.

## **Declaration of conflicting interests**

The authors declare that there is no conflict of interest.

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### ORCID iD

Hanjie Hu  <https://orcid.org/0000-0003-3417-1839>

### Supplemental material

Supplemental material for this article is available online.

### References

- Meguro S, Yamazaki S, Matsushima S, et al. A case of a primary hepatic so-called adenocarcinoma with heterotopic ossification: possibly of biliary adenofibroma origin. *Hum Pathol* 2018; 73: 108–113.
- Duggal R, Nijhawan R, Aggarwal N, et al. Mullerian adenocarcinoma (heterologous) of the cervix with sarcomatous overgrowth: a case report with review of literature. *J Gynecol Oncol* 2010; 21: 125–128.
- Lin YH, Lin CC, Concejero AM, et al. Surgical experience of adult primary hepatic sarcomas. *World J Surg Oncol* 2015; 13: 87.
- Weitz J, Klimstra DS, Cymes K, et al. Management of primary liver sarcomas. *Cancer* 2007; 109: 1391–1396.
- Jelovsek JE, Winans C, Brainard J, et al. Endometriosis of the liver containing mullerian adenocarcinoma: case report. *Am J Obstet Gynecol* 2004; 191: 1725–1727.
- N'Senda P, Wendum D, Ballardur P, et al. Adenocarcinoma arising in hepatic endometriosis. *Eur Radiol* 2000; 10: 1287–1289.
- Oliveira RC, Terracciano L and Cipriano MA. Primary biliary adenocarcinoma of the liver—a special and new entity. *Virchows Arch* 2020; 477: 461–466.
- DePristo MA, Banks E, Poplin R, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet* 2011; 43: 491–498.
- Cibulskis K, Lawrence MS, Carter SL, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nat Biotechnol* 2013; 31: 213–219.
- Saunders CT, Wong WS, Swamy S, et al. Strelka: accurate somatic small-variant calling from sequenced tumor-normal sample pairs. *Bioinformatics* 2012; 28: 1811–1817.
- McLaren W, Gil L, Hunt SE, et al. The Ensembl Variant Effect Predictor. *Genome Biol* 2016; 17: 122.
- Gagnier JJ, Kienle G, Altman DG, et al. The CARE guidelines: consensus-based clinical case reporting guideline development[J]. *Headache* 2013; 53: 1541–1547.
- Ulrich UA and Denschlag D. Uterine Adenocarcinoma. *Oncol Res Treat* 2018; 41: 693–696.
- Li D, Yin N, Du G, et al. A Real-World Study on Diagnosis and Treatment of Uterine Sarcoma in Western China. *Int J Biol Sci* 2020; 16: 388–395.
- Nathanson MJ and Conley AP. Prognostic factors for uterine adenocarcinoma: a review. *Expert Rev Anticancer Ther* 2018; 18: 1093–1100.
- Nathanson MJ, Ravi V, Fleming N, et al. Uterine Adenocarcinoma: a Review. *Curr Oncol Rep* 2016; 18: 68.
- Pinto A and Howitt B. Uterine Adenocarcinoma. *Arch Pathol Lab Med* 2016; 140: 286–290.
- Sullivan MW, Gockley A, Lo YC, et al. Superior Vena Cava Syndrome associated with recurrent uterine adenocarcinoma. *Gynecol Oncol Rep* 2020; 33: 100613.