Clinicopathologic and Molecular Characteristics of Hepatoid Adenocarcinoma of Bladder: a case report

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Case Report

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Abstract

Background

Hepatoid adenocarcinoma of bladder (HACD) is extremely rarely reported. Only 10 cases of HACD have been reported to date in English literature and no molecular analyses of HACD have ever been reported

Case presentation:

This case was a 57-year-old man who complained of gross hematuria for 5 days and urinary tract ultrasound examination revealed a hypoechoic mass at the right lateral wall of the bladder. 10 months later, the tumor recurred and transurethral resection was performed again. Definitive diagnosis of HACD was made by morphological and immunohistochemical analysis. A comparison was made between those two resections by the next generation sequencing (NGS). Somatic mutations of TP53, RB1 and KMT2D and amplifications of CCND1 and FGFR1 were identified in both resections. FGFR3 amplification was detected in the recurrent resection.

Conclusions

Detection of amplifications of FGFR1 and FGFR3 suggests that the FGFR pathway may play an important role in HACD and could be a candidate for personalized treatment. In this report, we provide some useful molecular clues to the analysis of HACD, while the mechanism and treatment still need to be explored.

Background

Hepatoid adenocarcinoma (HAC) is a very rare subtype of adenocarcinoma of extra-hepatic origin with its histopathological and immunohistochemical characteristics mimicking that of primary hepatocellular carcinoma (HCC). Serum levels of alpha-fetoprotein (AFP) are elevated or hepatic markers of AFP, HepPar1, glypican-3 are over expressed in most cases but not in all of them. HAC has been reported in multiple organs and is frequently found in stomach (63%), ovary (10%), lung (5%), gallbladder (4%), pancreas (4%), uterus (4%) [1]. Primary adenocarcinoma accounts for less than 2% of all bladder malignancies and most adenocarcinomas are considered to be arising from urachal remnants [2]. Hepatoid adenocarcinoma of bladder (HACD) as a subtype is rarely reported and solid or papillary components can be easily mistaken for high grade urothelial carcinoma (HGUC). To our knowledge, only 10 cases of HACD have been reported in English literature and no molecular analyses of HACD have ever been reported [2–8]. In this study, we present a HACD case. We analyzed its clinicopathological features and investigated the molecular gene mutation by the next generation sequencing (NGS).

Case Presentation

The patient was a 57-year-old man without unusual clinical antecedents, who complained of gross hematuria for 5 days without any obvious inducement in March of 2019. Urinary tract ultrasound examination revealed a hypoechoic mass of 2.2 cm × 1.9 cm at the right lateral wall of the bladder with the presence of internal vascularity on the Doppler ultrasound. Subsequently, the patient underwent a transurethral resection (TUR) and cystoscopy revealed a pedunculated mass of 2.5cm × 3.0cm at the original site just above the right ureteral orifice. No significant abnormality was seen in the ureteral mucosa. Microscopically, the tumor cells were polygonal epithelial cells arranged in solid and papillary patterns (Fig. 1A). These tumor cells revealed wide eosinophilic or granular cytoplasm, marked nuclear atypia, visible nucleoli, notable pathologic mitoses (10/10HPF) (Fig. 1B) and hyaline globules in the cytoplasm and interstitial (Fig. 1C). Postoperative diagnosis was initially misdiagnosed as HGUC. 10 months later, the patient suffered a right lower abdominal pain for 2 months due to unknown reason. Urinary tract ultrasound examination revealed a hypoechoic mass of 0.6 cm × 0.5 cm and the patient underwent TUR again. Besides polygonal cells with atypia and hyaline globules, cribriform and trabecular structures were present compared with the first resection, and there was a transition between glands and solid nests as well (Fig. 1D). Immunohistochemical analysis confirmed that the tumor cells in the second resection were positive for AFP (Fig. 2A), Glypican-3 (Fig. 2B), SALL4(Fig. 2C), CDX-2, β-catenin (membrane expression) and focally positive for CK7, CD56, Syn, while negative for CK20, AR, NKX3.1, GATA3, P63, PSMA, P504S, CgA and PSA. The Ki67 proliferation index was 70% (Fig. 2D). Further studies were also performed in the first resection and showed that the tumor cells were positive for AFP, Glypican-3 and focally positive for HepPar-1 without any expression of GATA3 or P63.

DNA-based NGS was performed by Macrogen USA (Rockville, MD) using the Ion Torrent (Life Technologies/Thermo Fisher Scientific, Waltham, MA) NGS platform. Bioinformatics analysis of NGS data was processed by Torrent Server Suite 4.2 and sequences aligned to human genome reference sequence HG-19 (The Genome Reference Consortium). The FATHMM (Functional Analysis Through Hidden Markov Models), SIFT (Sorting Intolerant from Tolerant), and PolyPhen (Polymorphism Phenotyping) scores predicting functional consequences of coding variants were either obtained from the COSMIC (Catalogue of Somatic Mutations in Cancer) at https:// cancer.sanger.ac.uk or assessed during bioinformatic analysis. Both resections were detected by DNA-based NGS and compared their difference (Table 1). Somatic mutations of TP53, RB1 and KMT2D and amplifications of CCND1 and FGFR1 were identified in both resections. AKT1 amplification and BRCA2 deletion were detected in the initial resection, while FGFR3 amplification was detected in the recurrent resection. Tumor mutation burden (TMB) was low of 2.81 and 3.37 Muts/Mb, respectively. No microsatellite abnormalities, gene rearrangements or fusions were detected. Moreover, low-frequency mutations of CHEK1, EP300, ATM, CREBBP, RAD51, FANCC in the first resection and MRE11 in the second resection were detected. In both resections, the tumor was confined to the lamina propria of the bladder. The patient was eventually diagnosed as HACD and received four and three cycles of intravesical perfusion of gemcitabine in a dose of 1600 mg before and after recurrence, respectively. The patient was in a good condition with a normal serum AFP levels till December 31, 2021.

Table 1
Molecular profiling of the first and the second resection of HACD.

Samples	Somatic mutation				Copy number variations			MSS/MSI	Rearrangements or fusions	TMB
	Gene	Alteration	AA change	Frequency	Gene	Deletion / Amplification	Copy numbers			
The first resection	TP53	c.225_240del16	p.A76fs*42	85.90%	CCND1	Amplification	7.25	MSS	Not detected	2.81
	RB1	c.975_978delTCTT	p.Y325*	73.20%	AKT1	Amplification	2.12			
	KMT2D	c.11035C > T,	p.Q3679*	45.60%	FGFR1	Amplification	2.07			
	KMT2D	c.11377C > T	p.Q3793*	42.10%	BRCA2	Deletion	0.59			
	SMARCA4	c.3277C > T	p.R1093*	1.40%						
The second resection	TP53	c.225_240del16	p.A76fs*42	82.60%	CCND1	Amplification	5.13	MSS	Not detected	3.37
	RB1	c.975_978delTCTT	p.Y325*	56.30%	FGFR1	Amplification	2.22			
	KMT2D	c.11035C > T	p.Q3679*	44.00%	FGFR3	Amplification	2.03			
	KMT2D	c.11377C > T	p.Q3793*	44.60%						

Discussion

HACD, as a rare site of HAC, shared the morphological and immunohistochemical similarity to primary HCC, together with elevated serum levels of AFP. We reviewed the previously reported cases of HACD and summarized them in Table 2. Including the present case, most of the patients were middle aged or elderly male adults of 45 to 89 years old and the median age at presentation was 68 years old, which was similar to HAC of other sites. Nearly 81.8% of patients with HACD were presented with hematuria as the main clinical manifestation. Among the 7 cases with a preoperative examination of serum AFP, 6 cases had increased serum AFP levels before operation. Tumor sites were reported in the right wall (3 cases), urachal site (1 case), posterior basal wall (1 case) and anterior wall (1 case) of bladder with a tumor size of 0.6cm to 11cm in diameter.

Table 2
Clinical characteristics of reported HACD cases.

Case/reference	Age	Gender	Symptoms	Sites in bladder	Tumor size (cm)	Serum AFP levels	Arrangements	Hyaline globules	Bile pigment	Mitosis (/10HPF)
1 Sinard [3]	68	F	Hydronephrosis	Anterior wall	2.5	NA	solid, tubular	+	+	10–15	TUR
2 Yamada [4]	89	F	Hematuria	Right wall	6.5	12700	solid	NA	NA	NA	TUR + TC
3 Burgues [2]	71	M	Hematuria	Posterior basal wall	NA	Normal	organoid,solid, tubular	+	-	≥10	TUR
4 Lopez [5]	66	M	Hematuria	NA	6.5	1065	NA	+	+	14	TC
5 Lopez [5]	85	M	Hematuria	NA	80g	NA	NA	+	+	15	TUR + TC
6 Lopez [5]	61	M	Hematuria	NA	5	2025	NA	+	+	10	TC
7 Lopez [5]	68	M	Hematuria	NA	1.5	1070	NA	-	-	8	TUR
8 Kawamura [6]	79	M	Hematuria	Right wall	1	39	NA	NA	NA	NA	TUR
9 Sekino [9]	49	M	No symptoms	NA	0.6	NA	solid,trabeculae	+	NA	NA	TUR
10 Fernando [8]	51	M	Hematuria and abdominal pain	Urachal site	11	Elevated	nest,trabeculae	+	NA	≥10	PC
11 present case	57	M	Hematuria	Right wall	3	NA	solid, tubular, papillary	+	-	≥10	TUR

F: female, M: male, NA: Not available, TUR: transurethral resection, TC: total cystectomy, PC: partial cystectomy, NED: no evidence of disease, DOD: died of di

Histologically, origin of HACD remained controversial but the histological features were distinctive. It was comprised of large polygonal cells with abundant eosinophilic or clear cytoplasm and a variable proportion of nest, cords, solid, tubular, papillary or trabeculae structures could be present (Table 2). Most cases of HACD showed high-grade nuclei, central nuclei and prominent nucleoli. Pathologic mitosis was notable (≥10/10HPF). Intracellular and intercellular hyaline globules and intracytoplasmic bile pigment could also be occasionally seen. AFP, Glypican-3, SALL4, HepPar-1 and Arginase-1 were recognized as diagnostic markers in most cases of HACD. One of the important steps for diagnosis was the possibilities of ruling out metastases of HCC, HAC and adenocarcinoma of

other sites before diagnosis. Newly reported gastric adenocarcinoma with enteroblastic differentiation had also to be differentiated as its distinction from HAC is extremely difficult [10]. Therefore, a close systemic examination and long-term follow-up was required. Further more, HGUC with solid and papillary arrangements had histological overlaps and might mimic HACD just like this case which was initially misdiagnosed in the first resection. Primary urothelial carcinoma of bladder with hepatoid features or yolk sac tumor differentiation had also to be distinguished from HACD and a close pathologic sampling and examination was necessary [11].

The molecular changes associated with HACD were poorly understood. We reported the earliest NGS analysis of HACD and showed that somatic mutations of TP53, RB1 and KMT2D and amplifications of CCND1 and FGFR1 in both resections had been detected. This case had a low TMB and was microsatellite-stable. Gene rearrangements or fusions were not detected. TP53 and RB1 mutations were frequently reported in a lot of tumors and simultaneously loss function of RB1 and TP53 was considered to be the initial event of tumorgenesis [12]. TP53 mutation was also found in other sites of HAC. Tsuruta[13] reported about 27% of all cases of gastric HAC had TP53 mutation, while few cases had KRAS and CTNNB1 mutation and no BRAF mutation was observed. Only 6% of the gastric HAC cases had a MLH1 loss [13]. Besides, high-frequency mutations of CEBPA, RPTOR, WISP3, MARK1 and CD3EAP were also identified within 10–20% of gastric HAC [14]. Mutations of TP53, RB1 and FGFR3 were relatively frequent in the bladder cancers and mutations in FGFR3 had a close relationship with bladder cancers [15–17]. Besides, no abnormality was found by systemic imaging examinations which indicated that this patient should suffer a primary bladder cancer instead of secondary or metastatic HAC. Yamada [4] reported a HACD with urothelial carcinoma in situ in the mucosa proximal to the main tumor and more and more urothelial carcinoma with hepatoid features were reported [11, 18, 19]. Vail[20] reported that 72% of urothelial carcinoma with glandular differentiation had TERT promoter mutations, while primary adenocarcinoma of bladder had no TERT promoter mutations. TERT promoter mutations were thought to have a differential diagnosis value and suggested that primary adenocarcinoma of bladder might have different origin or carcinogenesis. Nevertheless, further exploration was still needed to determine whether a relationship existed between HACD and urothelial carcinoma. KMT2D was essential for early embryonic development and loss of function could lead to genomic instability. It encoded a highly conserved histone lysine methyltransferase of the SET1 family, which was frequently mutated in lymphoma [21, 22]. KMT2D mutation had high mutation abundance in this case which was not reported previously in HACD.

Most patients with HACD had TUR and 3 cases had cystectomy. 4 patients were staged T3 and 8 patients had relapsed. The most common site of metastasis was the lung and 3 cases died within 12 to 19 months. Among the 7 cases with staged Ta, T1 and T2, 6 cases had gross hematuria and none of them died from the disease except for one lost to follow up. Though HACD was thought to be an aggressive tumor and have a poor prognosis, prognosis of HACD seemed to be slightly better than HAC of other sites as early clinical symptoms of gross hematuria might contribute to the early detection and treatment. Nowadays, there was no recommended treatment in the HACD. In this case, NGS found FGFR1 amplification in both resections and FGFR3 amplification in the recurrent resection. FGFR signaling pathway was confirmed to play key roles in promoting the proliferation, differentiation, and migration of tumor cells. Thus, FGFR signaling pathway was regarded as one of the therapeutic targets [23]. More and more FGFR inhibitors had gone clinical or pre-clinical trials and some achieved great success in tumor-targeted therapies not just in bladder cancers [24]. Meanwhile, it remained to be demonstrated whether HACD patients with FGFR1 and FGFR3 amplifications might benefit from FGFR inhibitors like other bladder cancers.

Conclusions

HACD is indeed rare and it is crucial for pathologists to be aware of the histological overlaps between HGUC and HACD. This case showed the partial morphological and immunohistochemical similarity to primary HCC. NGS showed somatic mutations of TP53, RB1 and KMT2D and gene amplifications of CCND1 and FGFR1. FGFR amplifications may play an important role in HACD and might benefit from FGFR inhibitors treatment. Our research tries to provide more clues for the molecular basis of HACD, while further researches in large cases will be needed.

Abbreviations

HACD: hepatoid adenocarcinoma of bladder; HGUC: high grade urothelial carcinoma; TUR: transurethral resection; HPF: high power field; NGS: next generation sequencing; HAC: hepatoid adenocarcinoma; HCC: hepatocellular carcinoma; AFP: alpha-fetoprotein; FGFR: fibroblast growth factor receptors; H&E: hematoxylin and eosin staining; IHC: immunohistochemical staining

Declarations

Acknowledgments

Not applicable.

Authors ' contributions

JJW was a major contributor in drafting the manuscript. NS participated in the histological evaluations. FNN participated in the clinical data collection. FXS and YF participated in the study conception and histological evaluation. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

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

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The original data generated or analyzed in the study are included in the article; further inquiries can be directed to the corresponding author.

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References

1. Metzgeroth G, Ströbel P, Baumbusch T, et al. Hepatoid Adenocarcinoma - Review of the Literature Illustrated by a Rare Case Originating in the Peritoneal Cavity. Onkologie. 2010;33(5):263–9. doi: 10.1159/000305717.
2. Burgues O, Ferrer J, Navarro S, et al. Hepatoid adenocarcinoma of the urinary bladder. An unusual neoplasm. Virchows Arch. 1999;435(1):71–5. doi: 10.1007/s004280050398.
3. Sinard J, Macleay L, Jr., Melamed J. Hepatoid adenocarcinoma in the urinary bladder. Unusual localization of a newly recognized tumor type. Cancer. 1994;73(7):1919–25. doi: 10.1002/1097-0142(19940401)73:7<1919:aid-cncr2820730724>3.0.co;2-l.
4. Yamada K, Fujioka Y, Ebihara Y, et al. Alpha-fetoprotein producing undifferentiated carcinoma of the bladder. J Urol. 1994;152(3):958–60. doi: 10.1016/s0022-5347(17)32623-x.
5. Lopez-Beltran A, Luque RJ, Quintero A, et al. Hepatoid adenocarcinoma of the urinary bladder. Virchows Archiv An International Journal of Pathology. 2003;442(4):381–7. doi: https://doi.org/10.1007/s004280050398.
6. Kawamura N, Hatano K, Kakuta Y, et al. A case of hepatoid adenocarcinoma of the urinary bladder. Hinyokika kiyo Acta urologica Japonica. 2009;55(10):619–22.
7. Sekino Y, Mochizuki H, Kuniyasu H. A 49-year-old woman presenting with hepatoid adenocarcinoma of the urinary bladder: a case report. J Med Case Rep. 2013;7:12. doi: 10.1186/1752-1947-7-12.
8. Fernando GD, Carlos MO, Jimenez CA, et al. Hepatoid Adenocarcinoma of the Urachus. Case Rep Pathol. 2016;2016:1871807. doi: 10.1155/2016/1871807.
9. Marchegiani G, Gareer H, Parisi A, et al. Pancreatic Hepatoid Carcinoma: A Review of the Literature. Digestive Surgery.2013;30(4–6):425–33. doi: 10.1159/000355442.
10. Kwon MJ, Byeon S, Kang SY, et al. Gastric adenocarcinoma with enteroblastic differentiation should be differentiated from hepatoid adenocarcinoma: A study with emphasis on clear cells and clinicopathologic spectrum. Pathology Research & Practice. 2019;215(9):152525. doi: 10.1016/j.prp.2019.152525.
11. Espejo-Herrera N, Enric C-M. Yolk sac tumor differentiation in urothelial carcinoma of the urinary bladder: a case report and differential diagnosis. Diagnostic Pathology. 2020;15(68):1–7. doi: 10.21203/rs.2.24486/v2.
12. Sherr CJ, McCormick F. The RB and p53 pathways in cancer. Cancer Cell. 2002;2(2):103–12. doi: 10.1016/S1535-6108(02)00102-2.
13. Tsuruta S, Ohishi Y, Fujiwara M, et al. Gastric hepatoid adenocarcinomas are a genetically heterogenous group; most tumors show chromosomal instability, but MSI tumors do exist. Human Pathology. 2019;88:27–38. doi: 10.1016/j.humpath.2019.03.006.
14. Wang Y, Sun L, Li Z, et al. Hepatoid adenocarcinoma of the stomach: a unique subgroup with distinct clinicopathological and molecular features. Gastric Cancer. 2019;22(6):1183–92. doi: 10.1007/s10120-019-00965-5.
15. Warrick JI, Knowles MA, Yves A, et al. Report From the International Society of Urological Pathology (ISUP) Consultation Conference On Molecular Pathology Of Urogenital Cancers. II. Molecular Pathology of Bladder Cancer: Progress and Challenges. The American Journal of Surgical Pathology. 2020;44:e30-e46. doi: 10.1097/PAS.0000000000001453.
16. Knowles MA. FGFR3 – a Central Player in Bladder Cancer Pathogenesis? Bladder Cancer. 2020;6(1):1–21. doi: 10.3233/BLC-200373.
17. Lima NC, Atkinson E, Bunney TD, et al. Targeting the Src Pathway Enhances the Efficacy of Selective FGFR Inhibitors in Urothelial Cancers with FGFR3 Alterations. International Journal of Molecular Sciences. 2020;21(9):3214. doi: 10.3390/ijms21093214.
18. Friedman P, Lai JP. Liver Metastasis of Urothelial Carcinoma with Hepatoid Features: An Unusual Morphological Finding. Anticancer Res. 2017;37(2):801–4. doi: 10.21873/anticanres.11380.
19. Samaratunga H, Samaratunga D, Dunglison N, et al. Alpha-fetoprotein-producing Carcinoma of the Renal Pelvis Exhibiting Hepatoid and Urothelial Differentiation. Anticancer Research. 2012;32(11):4987–91. doi: 10.1158/1535-7163.MCT-12-0358.
20. Vail E, Zheng X, Zhou M, et al. Telomerase reverse transcriptase promoter mutations in glandular lesions of the urinary bladder. Annals of Diagnostic Pathology. 2015;301–5. doi: 10.1016/j.anndiagpath.2015.06.007.

21. Froimchuk E, Jang Y, Ge K. Histone H3 lysine 4 methyltransferase KMT2D. Gene. 2017;627:337–42. doi: 10.1016/j.gene.2017.06.056.
22. Pasqualucci L, Trifonov V, Fabbri G, et al. Analysis of the coding genome of diffuse large B-cell lymphoma. Nature Genetics. 2011;43(9):830–7. doi: 10.1038/ng.892.
23. Luca A, Abate R, Rachiglio A, et al. FGFR Fusions in Cancer: From Diagnostic Approaches to Therapeutic Intervention. International Journal of Molecular Sciences. 2020;21:6856. doi: 10.3390/ijms21186856.
24. Krook MA, Reeser JW, Ernst G, et al. Fibroblast growth factor receptors in cancer: genetic alterations, diagnostics, therapeutic targets and mechanisms of resistance. British Journal of Cancer. 2021;124(5):880–92. doi: 10.1038/s41416-020-01157-0.

Figures

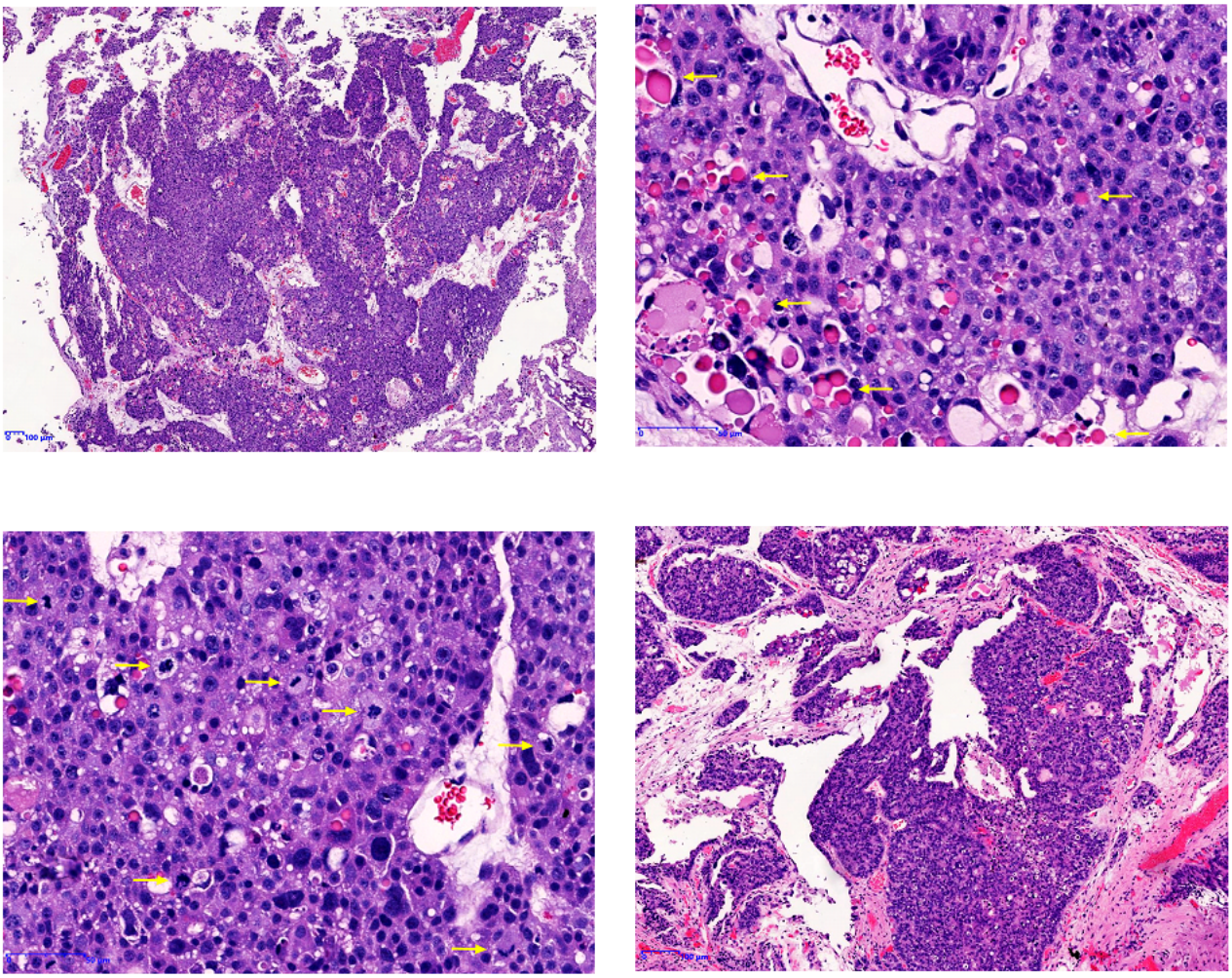

Figure 1

A In the first resection, the tumor cells grew in solid nests and complex papillae with a edematous fibrovascular axis (H&E×25).

B The tumor cells revealed wide eosinophilic cytoplasm, marked nuclear atypia, visible nucleoli, notable pathologic mitoses (Right arrow, H&E×200).

C Hyaline globules were visible in the cytoplasm and interstitium (Left arrow, H&E×200).

D In the second resection, the tumor cells had complex cribriform and trabecular structures. Focally, there was a transition between glands and solid nests (H&E×50).

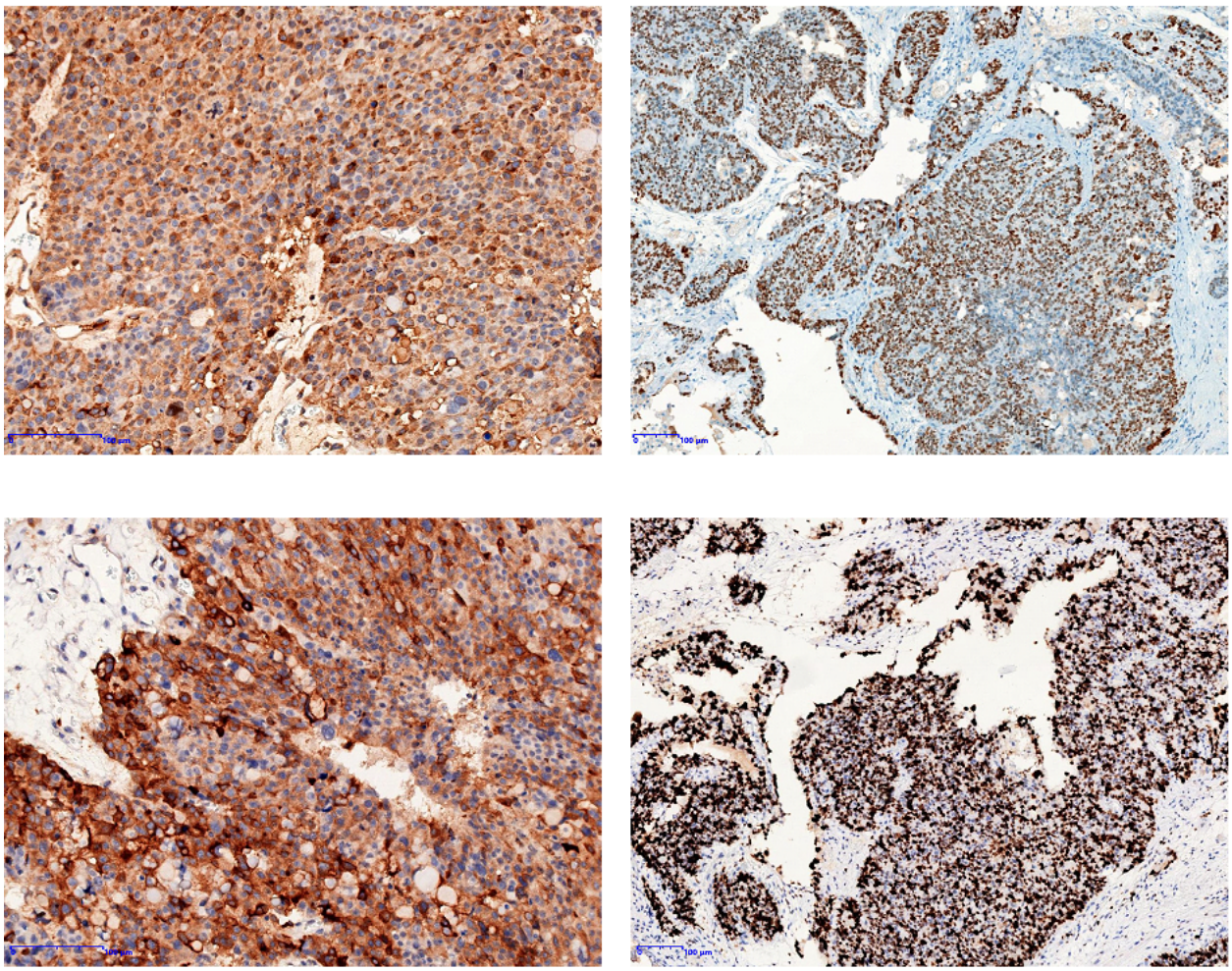

Figure 2

A AFP was strongly positive in the first resection (IHC×100).

B Glypican-3 was strongly positive in the first resection (IHC×100).

C SALL4 was diffusely positive in the second resection (IHC×50).

D The Ki67 proliferation index was 70% (IHC×50).