



## Case report

# Rapid recurrence of a ruptured mucinous borderline ovarian tumor harboring *K-RAS* mutation followed by progression into anaplastic carcinoma with *TP53* mutation

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## ABSTRACT

We describe the case of a young patient with a borderline mucinous ovarian tumor that progressed into ipsilateral ovarian anaplastic carcinoma in only 3 months with metastasis to the contralateral ovary and extensive spread in the pelvic and abdominal regions. The mucinous tumor harbored micro-foci of intraepithelial carcinoma, but no mural nodules, microinvasion, or invasive adenocarcinoma were detected. Notably, a rupture on the ovarian mass and low-grade pseudomyxoma peritonei were present. Next-generation sequencing identified an identical *KRAS* mutation in the mucinous tumor and anaplastic carcinoma, while the latter had *KRAS* gene amplification and *CDKN2A*, *MPL* and *TP53* mutations. These findings indicate the anaplastic carcinoma might have arisen via recurrence, malignant transformation and dedifferentiation of the former low-grade mucinous tumor. We consider that the mass rupture and pseudomyxoma peritonei were high-risk factors for recurrence, while genetic mutations were key drivers of progression. Accordingly, such cases may benefit from active surgical treatment and early chemotherapy.

## 1. Introduction

Mucinous borderline tumors (MBTs) of the ovary occur across a wide age range, even including childhood [1, 2]. Herein, we report a rare juvenile case in which 3 months after the resection of an ovarian MBT, a high-grade anaplastic carcinoma (AC) rapidly occurred in the ipsilateral ovary with extensive concurrent metastasis. We consider whether the clinical and genetic characteristics of the case indicate a pathogenesis of two independent neoplasms or the progressive dedifferentiation of the mucinous tumor.

## 2. Case report

A 15-year-old female patient was treated at our hospital for obvious abdominal distention. Physical examination showed a large abdominal mass. Tumor marker tests showed an elevated CA-125 level of 181.4 U/mL, an elevated CEA level of 58.07 U/mL, and an elevated CA19-9 level of 1021.17 U/mL, while the HE-4 level remained within the normal range. Gynecological ultrasound identified a cystic mass with an

irregular shape in front of the uterus, and ovarian carcinoma was suspected. In addition, the peritoneum was thickened with massive effusion in the pelvic region and abdominal cavity. Whole abdominal magnetic resonance imaging (MRI) showed a huge multilocular mass in the abdominal cavity, and the tumor was considered to be a cystadenoma arising from the left ovary. The patient underwent laparotomy. A giant cystic mass measuring 28 × 23 × 4 cm was found on the surface of the left ovary, and a 4-cm-long rupture was seen on the surface of the mass. The peritoneum of the left pelvic wall and rectouterine pouch was slightly thickened. A brittle neoplasm was observed on the surface of the right ovary. No abnormality was found in the bilateral fallopian tubes. Excision of the ovarian mass was performed.

Grossly, the tumor was a multiloculated cystic mass containing mucin. The majority of the capsule wall was smooth, and several small papillae with diameters of 0.5–0.8 cm were seen in the focal areas. There were no solid areas. No well-defined mural nodule was identified. The papillary areas were preferentially sampled for frozen-section examination. Microscopically, this tumor was composed of multiple cysts lined by gastrointestinal-type mucinous epithelium, presenting a structure of

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tufting and papillae, nuclear stratification, and low-grade nuclear atypia. A diagnosis of MBT was made based on the frozen section examination. Biopsies of the focal thickened peritoneum and the neoplasm on the surface of the right ovary were performed rather than salpingo oophorectomy in consideration of the patient's young age.

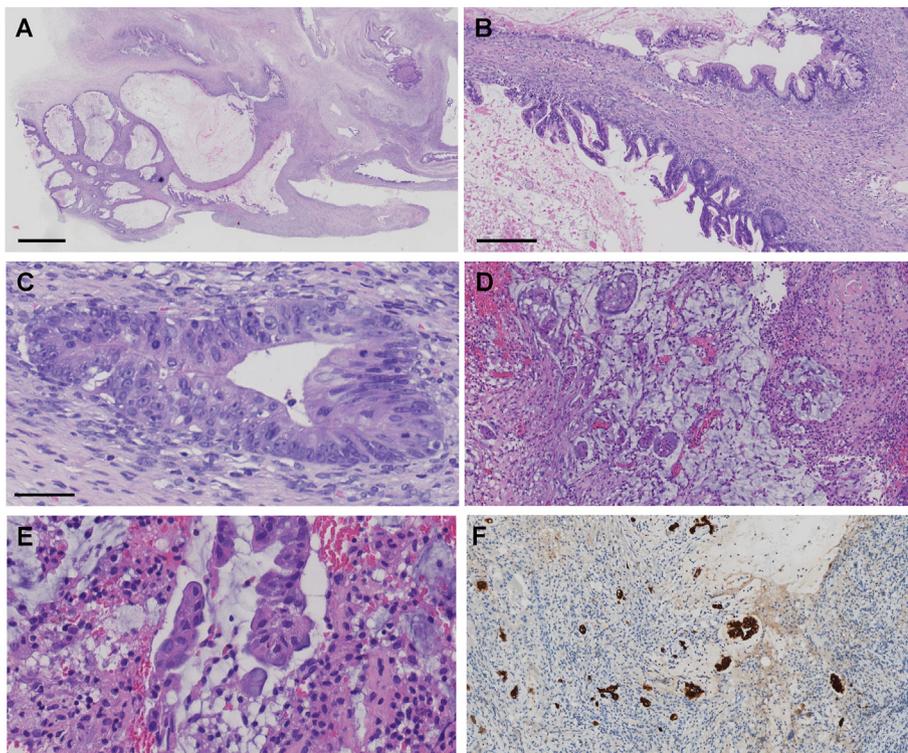
The tumor was subsequently processed. Two sections per 1 cm were sampled and examined. Microscopically, the morphological features were similar to those seen in the frozen sections. Additionally, micro-foci of the epithelium presented as high-grade nuclear atypia, warranting a diagnosis of intraepithelial carcinoma (IEC) (Fig. 1A-C). There was no evidence of stromal invasion in multiple sections. The biopsy of the peritoneum of the rectouterine pouch showed hypocellular mucinous deposits containing a few mucinous tumor cells with low-grade cytological atypia within the peritoneum (Fig. 1D, E). The tumor cells were immunoreactive for CK7 (Figure 1F) and immunonegative for CK20, CDX2, and SATB2. A diagnosis of pseudomyxoma peritonei (PMP) (grade 1, i.e., "mucinous carcinoma peritonei, grade 1") mostly likely arising from the mucinous ovarian tumor was supported. The biopsy results for the peritoneum of the left pelvic wall and the neoplasm on the right ovary showed these tissues to be free of tumor cells. Cytopathologic examination of the abdominal dropsy was carried out twice. Some atypical cells were observed that might have been reactive mesothelial cells. Although no definite tumor cells were identified, the possibility that they were tumor cells could not be completely excluded, and thus, repetition of the examination was recommended. The definitive diagnosis was a MBT of the ovary with focal IEC and low-grade PMP. The tumor was graded as FIGO stage IIB. Because the ovarian tumor was predominantly a borderline phenotype, no further treatment was given but the patient was advised to return for early follow-up.

After 3 months, the girl complained of distending pain in her lower abdomen. MRI and positron emission tomography (PET)/computed tomography (CT) investigations revealed an approximately 9×5-cm solid-cystic mass in the left adnexal area strongly suspicious for adenocarcinoma, accompanied by multiple metastases in the perihepatic areas, perisplenic areas, peritoneum and omentum majus. The findings during operation were a greyish white, solid mass measuring 7 × 5 × 3 cm in the

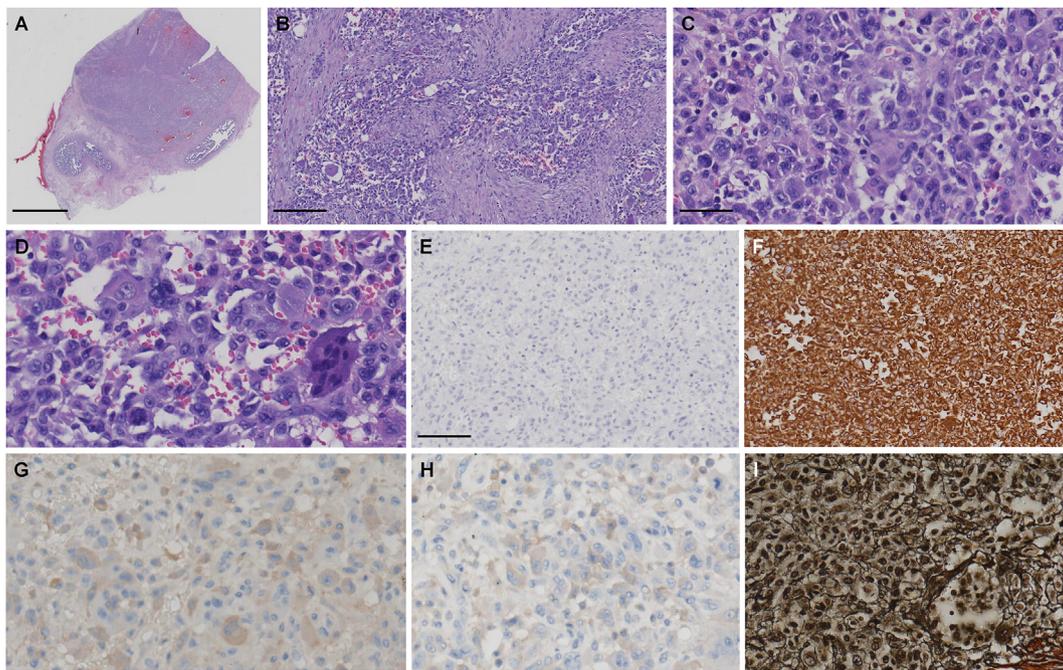
left adnexal area and a mass with a similar appearance measuring 8 × 4 × 4 cm in the right adnexal area, as well as multiple grayish white nodules with diameters of 0.3–0.7 cm on the hilum and serosal surface of the right ovary. Additionally, tumor nodules were observed on the uterus, bladder, upper segment of the rectum, sigmoid colon, descending colon, descending mesocolon, jejunum, ileum, bilateral diaphragm, liver, spleen, omentum, pelvic wall, and abdominal wall, as well as the pre-sacral and paraaortic lymph nodes. No visible tumors were seen on the appendix. The diagnosis based on intraoperative frozen sections sampled from the right ovarian mass was malignant tumor likely derived from ovarian epithelial cells. The tumor was graded as FIGO stage IVB. She subsequently underwent total hysterectomy with bilateral salpingo oophorectomy, resection of bladder lesions and repair of the bladder, omentectomy, splenectomy, appendectomy, partial resection and repair of the bilateral diaphragm, excision of pelvic peritoneum neoplasms, extended left hemicolectomy supplemented with coloproctostomy, partial jejunectomy and anastomosis, partial ileectomy and anastomosis, and excision of liver lesions.

The postoperative pathological examinations demonstrated that tumor cells with a consistent morphology were present in all the inspected tissues except the appendix. These tumor tissues displayed features completely identical to those of a high-grade, poorly differentiated, malignant neoplasm composed of round to polygonal cells arranged in sheets with ample cytoplasm, highly atypical nuclei and rapid mitosis, intermingled with tumor giant cells and reactive multinucleated giant cells, and surrounded by fibrous stroma (Fig. 2A-D). Immunohistochemical analysis of the ovarian tumor showed negative staining for pan-cytokeratin and positive, diffuse staining for vimentin, while staining for CK8/18 and EMA was weakly positive (Fig. 2E-H). Negative results were obtained for other markers including CK7, CK20, E-cadherin, PAX8, WT1, ER, PR, CD34, ERG, actin, desmin, MyoD1, and myogenin. Reticular fibers surrounded nests of tumor cells, instead of individual cells (Figure 2I). The final diagnosis was AC, possibly arising from the recurrence and high-grade transformation of the prior MBT.

DNA was extracted from the paraffin-embedded sections, and mutation analysis was performed for the two specimens. The MBT



**Figure 1. The initial mucinous tumor.** (A–C) Ovarian MBT. (A) The mucinous glands were arranged in a multi-cystic structure. (B) The cyst lining showed some villous architecture and epithelial stratification. (C) Focal intraepithelial carcinoma. The epithelium exhibited stratification, with high-grade nuclear atypia characterized by enlarged nuclei, a high N:C ratio, vesicular chromatin and prominent nucleoli. (D–F) Peritoneal mucinous tumor. (D) Low-power view showed multi-focal mucin with some small cohesive epithelial cell clusters. (E) High-power view of this tumor deposit showed mucinous epithelium with low-grade cytological atypia. (F) Peritoneal tumor cells were positive for CK7 on immunohistochemical staining. Scale bars: (A) 1.25 mm; (B), (D) and (F) 200  $\mu$ m; (C) and (E) 50  $\mu$ m.



**Figure 2.** The recurrent malignant tumor. (A) Low-power view showed adnexal tumor with destruction of ovarian tissue. (B) The tumor cells were arranged in solid sheets surrounded by fibrous tissues. (C) The tumor cells showed an anaplastic appearance characterized by ample cytoplasm and marked nuclear pleomorphism as well as atypia. (D) Numerous multinucleated giant cells were present, including tumor giant cells and reactive giant cells. (E) Immunohistochemical staining for pancytokeratin was completely negative in tumor cells. (F) Vimentin staining was diffuse and strongly positive. CK8/18 (G) and EMA (H) staining were both weakly positive. (I) Reticular fiber staining showed that the tumor cells were arranged in nests. Scale bars: (A) 5 mm; (B) 200  $\mu$ m; (C), (D), (G)–(I) 50  $\mu$ m; (E) and (F) 100  $\mu$ m.

demonstrated the p.Gly12Val, c.35G>T mutation in exon 2 of the *KRAS* gene. The AC not only displayed the same *KRAS* mutation, but also had mutations of *CDKN2A* (p.Asp84Gly, c.251A>G), *MPL* (p.Arg90Ter, c.268C>T), and *TP53* (p.Ser269\_Phe270dup, c.804\_809dup). Additionally, the *KRAS* gene was amplified 3.2 times in the AC (Table 1).

Two weeks after the operation, PET/CT scanning showed multiple nodules in her liver, peritoneum, great omentum, pelvis and abdomen, which were considered as tumor recurrence and metastasis. The patient received chemotherapy with paclitaxel, carboplatin, and doxorubicin hydrochloride.

### 3. Discussion

According to previously published clinical data, MBT may relapse after cystectomy or adhesions, or in cases of capsule rupture before surgery and spillage of the contents [3, 4, 5]. Tumor stage is the most significant prognostic factor for mucinous ovarian tumors. The majority of patients with MBTs have a favorable prognosis, while the recurrence rate is higher among cases with later-stage disease [1, 6]. Additionally, rupture is particularly important in early-stage tumors, as it can increase the risk of recurrence [4, 6]. By retrospective analysis of the disease

course in the present case, we speculated that the focal rupture on the surface of the mass may have been a high-risk factor for recurrence. However, recurrent MBTs are usually borderline and only in rare cases are carcinoma [3]. The latter has been suggested to be correlated with inadequate sampling of the primary tumors [4, 5], and AC often arises from pre-existing mural nodules. Histologically, mural nodules can be classified as reactive sarcoma-like lesions, AC, or sarcoma [7]. AC, which may present with a rhabdoid, spindle cell, or pleomorphic morphology and represents a form of dedifferentiation, is the most common type of mural nodules, and it usually exhibits aggressive behavior [8]. In the present case, the secondary tumor showed the morphological characteristics and immunophenotype of pure AC, and it was accompanied by extensive metastasis at diagnosis, suggesting the typically poor prognosis. However, through sufficient sampling of the primary mass, we found only foci of IEC, and no microinvasion, invasive adenocarcinoma, or mural nodules in all the samples taken. Additionally, we observed that the mucinous tumor cells were present on the peritoneum, which was diagnosed as “PMP, grade 1” following WHO grading criteria. PMP can originate from primary ovarian mucinous tumors with spontaneous or iatrogenic rupture, including teratoma-associated ovarian mucinous tumors [9]. The WHO Classification defines its morphology code as “/6”,

**Table 1.** Molecular features of MBT and AC.

	Genetic alterations				TMB	Microsatellite analysis
	Gene	Exon	Alterations	Abundance		
<b>MBT</b>	<i>KRAS</i>	2	c.35G>T (p.Gly12Val)	0.9%	0.5 mutations/Mb	MSS
<b>AC</b>	<i>KRAS</i>	2	c.35G>T (p.Gly12Val)	69.4%	1.9 mutations/Mb	MSS
	<i>KRAS</i>	-	amplification	3.2x		
	<i>CDKN2A</i>	2	c.251A>G (p.Asp84Gly)	14.9%		
	<i>MPL</i>	3	c.268C>T (p.Arg90Ter)	10.8%		
	<i>TP53</i>	8	c.804_809dup (p.Ser269_Phe270dup)	13.4%		

MBT, mucinous borderline tumor; AC, anaplastic carcinoma; TMB, tumor mutational burden; MSS, microsatellite stability.

which refers to “malignant tumors, metastatic site” and support the alternative terminology “mucinous carcinoma peritonei, grade 1”. These definitions clearly show its nature was malignant neoplasm metastasis in the peritoneum. According to the small amount of data available, MBTs with IEC or PMP do not seem to be associated with adverse outcomes [10, 11]; however, the relevant experience is very limited.

We questioned why this case of MBT progressed to high-grade carcinoma in such a short period. The current understanding is that tumorigenesis is driven by the step-wise accumulation of genetic events [12]. Based on molecular pathogenesis, benign mucinous tumors and MBTs have been considered as putative precursor lesions for mucinous carcinoma of the ovary (MCO) [13, 14]. Cheasley et al. reported a progressive model of evolution from benign to MBT to localized low-grade MCO and progressively through to high-grade and/or metastatic MCO [15]. Benign tumors often initiate with a common early event of either *KRAS* mutation or copy-number loss of *CDKN2A*. MBTs have a greater probability of having both events and may have additional copy number alterations. Low-grade MCO often has mutations similar to those in MBTs but with greater frequency and a higher tumor mutation burden. High-grade mutation of *TP53* occurs in most cases of mucinous carcinoma [13, 15, 16]. Furthermore, epidemiological evidence and data from genome-wide association studies support a common origin for MBTs and MCO tumors, as the risk factors and single nucleotide polymorphisms are shared between the two diseases [17]. Although *KRAS* mutations are identified mainly in low-grade mucinous tumors, Desouki et al. reported a case with a well-to-moderately differentiated ovarian mucinous adenocarcinoma with poorly differentiated mural nodules of AC. The two divergent components of that tumor revealed the same *KRAS* mutation (p.G12V, c.35G4T mutation in codon 12), suggesting that *KRAS* mutations can be present in dedifferentiated tumor and the two components might have the same clonal origin [18]. In the present case, the MBT and AC also displayed an identical *KRAS* mutation (both p.Gly12Val, c.35G>T mutation in exon 2), indicating that the AC shared an overlapping molecular setting and a common initiating genetic event with the prior MBT. By molecular detection, no additional genetic alterations were found in the MBT, whereas the AC had novel mutations of *CDKN2A*, *MPL* and *TP53* as well as amplification of *KRAS*. According to the study from Cheasley et al., *TP53* mutation and high copy number alterations are key drivers of invasive progression, metastasis, and a higher tumor grade [15].

Additionally, a recent study from Wang et al. revealed the mutational landscape of ovary-originating PMP as well as the potential factors that could be used to predict patient survival [19]. They observed that each PMP patient carried at least one cancer driver gene with mutations, and most patients carried multiple driver genes with mutations. The cancer driver genes associated with ovary-originating PMP included some known cancer driver genes, such as *FGFR2*, *KRAS*, *RBI*, *BRAF*, *EGFR*, *NRAS*, *PIK3CA*, *ATRX*, *EP300*, *NOCR1* and *PTEN*. Among all cancer driver genes, *ATM*, *SETD2* and *TP53* were the genes that exhibited the highest frequencies (25%). Their Kaplan-Meier survival analysis showed that *TP53*, *PTPRK* and *DICER1* might have predictive value for patient survival, especially *PTPRK*. Another interesting finding is that presurgical blood levels of CA125 and CA19-9 were strong predictors for patient survival. The peritoneal cancer index (PCI), completeness of cytoreduction (CCR), CA19-9 and *PTPRK* were identified as factors with significant correlation with survival, and *TP53*, *DICER1* and CA125 were identified as factors with potentially significant correlation with survival on univariate Cox analysis. In the present case, the patient had an extremely high serum level of CA19-9 preoperation. Moreover, the cytoreduction may have been incomplete due to failure to carry out hyperthermic intraperitoneal chemotherapy (HIPEC) immediately after the operation. Those factors may predict a poor prognosis. Regrettably, we cannot analyze the mutational state in the PMP due to inadequate sample availability.

In conclusion, the present report describes a rare case in which a mucinous tumor may have rapidly progressed into AC, possibly via a

form of dedifferentiation and potentially carrying an extremely adverse prognosis. We speculate that the AC arose from the prior MBT or PMP. The additional genetic alterations including *CDKN2A*, *MPL* and *TP53* mutations and increased copy number of the *KRAS* gene may have contributed to the rapid recurrence of the mucinous tumor and malignant progression to AC.

Mhaweche-Fauceglia et al. reported a case in which a 36-year-old woman suffered from a MBT of the ovary with foci of AC within a sarcoma-like mural nodule. Three months after her initial diagnosis, their patient returned with metastatic AC and died less than 1 week after chemotherapy [20]. The clinical course was very similar to our case, but a single 2-cm mural nodule containing a microfocus of AC measuring 0.5 cm was detected in her initial ovarian mass, while no evidence of the existence of even small foci of AC were found in the first MBT of our case. As such, the rapid progression of disease in our patient is very unusual and the first of its kind to be reported in the literature.

Moreover, although MBTs in children and adolescents can be treated conservatively to preserve patients' fertility [3, 6], our case indicates that for patients with ruptured MBTs along with PMP, unilateral adnexectomy, complete cytoreduction, hyperthermic intraperitoneal chemotherapy, and even early postoperative intraperitoneal chemotherapy may be beneficial. For patients with advanced or recurrent mucinous tumors, a personalized molecular therapeutic approach may be more practical and crucial to improving outcomes. In conclusion, the pathogenesis of AC is still poorly understood, and the exploration of the optimal treatment measures remains an important task for future research.

### Ethics approval

This study was approved by the Institutional Research Ethics Committee of Sun Yat-sen University (approval number: [2019]089). Written informed consent to participate in this study was provided by the participant's legal guardian. Written informed consent was obtained from the patient's legal guardian for the publication of any potentially identifiable images or data included in this article.

### Declarations

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All authors listed have significantly contributed to the investigation, development and writing of this article.

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#### Data availability statement

Data included in article/supp. material/referenced in article.

#### Declaration of interests statement

The authors declare no conflict of interest.

#### Additional information

No additional information is available for this paper.

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