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Coexistence of BRAF V600E and TERT promoter mutations in low grade serous carcinoma of ovary recurring as carcinosarcoma in a lymph node: report of a case

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Abstract

Low-grade serous carcinomas only rarely coexist with or progress to high-grade tumors. We present a case of low grade serous carcinoma with transformation to carcinosarcoma on recurrence in the lymph node. Identical BRAF V600E and TERT promoter mutations were identified in both the original and recurrent tumor. Given that TERT promoter mutations are thought to play a role in progression of other tumor types, the function of TERT mutations in BRAF mutated low-grade serous carcinoma deserves investigation.

Introduction

Serous carcinomas of gynecologic origin (ovarian, fallopian tube, primary peritoneal) can be divided into low-grade and high-grade subtypes based on morphologic and molecular features. Low-grade serous carcinomas (LGSC) demonstrate uniform nuclei with infrequent mitotic figures and often arise from either adenofibromas or serous borderline tumors, particularly those with micro-papillary features, and may show BRAF and KRAS mutations [1,2]. LGSC do not respond to conventional chemotherapy and patients experience multiple recurrences over time [3]. In contrast, high-grade serous carcinomas (HGSC) demonstrate pleomorphic, enlarged nuclei and numerous mitotic figures; they are usually not associated with precursor borderline tumors and frequently show TP53 mutations [1, 2]. The median survival is much lower in HGSC although they tend to respond to platinum based chemotherapy [3]. The pathogenesis of HGSC is characterized by rapid development, TP53 mutations, a high level of chromosomal instability, and in hereditary carcinomas, BRCA germline mutations. Notably, KRAS and BRAF mutations are absent. Serous borderline tumors (SBT) can progress to LGSC which is associated with poor prognosis. However, LGSC tend to preserve their low-grade histology at recurrence [4]. Occasional transformation of LGSC to high-grade carcinomas many years after initial diagnosis is a rare phenomenon but has been reported [5–14]. Reported 5-year survival rates range from 40% to 56% for LGSC, and 9% to 34% for HGSC [5, 15]. BRAF V600E mutation is present in 35% of LGSC and is associated with significantly longer survival [16]. We present a case of

LGSC with transformation to carcinosarcoma on recurrence with attention being paid to molecular alterations.

Case Summary

Our patient is a 67-year-old female, with a history of unilateral oophorectomy for dermoid cyst in 1980, who developed abdominal pain and was found to have a 22cm intraperitoneal complex cystic mass with multiple septations, appearing to originate from right adnexa with multiple enlarged cardiophrenic lymph nodes (largest 2.3 cm), retroperitoneal adenopathy with punctate calcifications, as well as axillary, pelvic and inguinal bulky lymphadenopathy. Her CA-125 was elevated (173 U/mL) and CEA was normal (1 U/mL). She had no history of abnormal pap smears and history of 3 pregnancies with no live births. Her family history was significant for maternal aunt with breast cancer and a sister with endometrial cancer. Fine needle aspiration of the axillary node showed an abundantly cellular smear with papillary groups of malignant epithelial cells with enlarged nuclei and moderate amount of cytoplasm associated with psammomatous calcifications. Immunohistochemical stains showed the tumor cells were strongly positive for CK7, with weak and focal reactivity for nuclear WT-1. The cells showed focal reactivity for ER and were negative for PAX8, PR, and calretinin. CK5/6 highlighted basal cells in the papillary structures. Despite the absence of PAX8 expression, the findings best supported involvement by an ovarian serous neoplasm, and the differential diagnosis included a LGSC as well as a SBT. No high grade cytologic features were seen. The lack of diffuse expression of CK 5/6, equivocal D2-40, and absent calretinin expression provided no definitive support for a mesothelial process.

She was taken for an exploratory laparotomy, right salpingo-oophorectomy with removal of her symptomatic pelvic mass and omentectomy. A lymph node dissection and complete cytoreduction was not attempted as she was found to have extensive unresectable retroperitoneal disease and extra abdominal lymphadenopathy. The right salpingo-oophorectomy specimen showed a 23cm low-grade serous neoplasm in the ovary with extensive necrosis and hemorrhage, consistent with torsion. Most of the cystic mass was necrotic, with only small foci of residual serous neoplasm. The ovarian neoplasm was at least a serous cystadenofibroma, but assessment of epithelial stratification in the papillary structures was severely limited due to necrosis. We did not see definite invasive carcinoma in the ovarian mass; however, most of the neoplasm was necrotic. Immunohistochemically stains demonstrated tumor cells that were positive for PAX-8, MOC-31, and BERE4 (focal) but negative for CK20, D2-40 and calretinin, supporting the morphologic impression of a mullerian rather than mesothelial tumor. In the omentum, umbilical nodule, and umbilical hernia sac specimens; however, the tumor was clearly invasive LGSC (Figure 1). Molecular studies using a targeted 130 gene panel revealed a BRAF V600E mutation (c.1799T>A) and a Telomerase reverse transcriptase (TERT) promoter mutation (C228T; c.-124C>T) in an omental implant. A variant of uncertain significance in nuclear factor (erythroid derived 2)-like 2 (NFE2L2) was also identified. No additional mutations were identified on our panel in the recurrent specimen as compared to the initial specimen.

The patient underwent chemotherapy with carboplatin and taxol every three weeks for 6 cycles which was well tolerated. A PET/CT following the completion of chemotherapy

showed persistent nodal disease. The patient declined endocrine therapy as she was asymptomatic of her residual disease. Eighteen months after the surgery, she developed a rapidly growing right axillary mass causing pressure, numbness and pain locally. A breast MRI showed worsening bilateral axillary lymphadenopathy. The largest right axillary nodal mass measured 6.7×3.9 cm and appeared to have engulfed previously noted enlarged lymph nodes. The mass also appeared to abut the pectoralis muscle with mass effect, but did not appear to invade the muscle. Other enlarged lymph nodes were seen in the superior aspect of the right axilla associated with the surrounding edema. Multiple enlarged lymph nodes were seen in the left axilla with the largest measuring 2.5 cm. The patient underwent a palliative resection of the axillary/breast lymphadenopathy which revealed a large solid and cystic necrotic mass. The cystic component contained LGSC which was intimately associated with a malignant spindle cell neoplasm diffusely infiltrating the tissue (Figure 2). Most of the spindled tumor was extensively necrotic with foci of well-preserved tumor centered around the blood vessels. The spindle cells were very atypical with enlarged, elongated nuclei, coarse chromatin, scant cytoplasm and numerous mitoses. Numerous calcifications were present scattered in the necrotic areas. The malignant spindle cells were negative for GATA-3, BRST-2, Pax-8, CKmix, S100, SOX-10, CK5/6, ER, p63, CK7, CK20, CD34, ERG, TTF-1, desmin, myogenin, D2-40, calretinin, EMA, and WT-1. TP53 showed a normal expression pattern (Figure 3). INI was intact. CD31 was patchy positive. LGSC accounted for 20% of the mass and the necrotic, sarcomatous component accounted for about 80%. Mutational analysis was performed as part of a targeted somatic mutation panel and demonstrated NFE2L2 (c.802G>A, p. V268M) and BRAF V600E (c.1799T>A) mutations and a TERT promoter mutation (c.-124C>T). A restaging PET/CT showed widely metastatic disease, including lungs and bone. In light of her new findings and the associated poor prognosis the patient elected to proceed with best supportive care and died shortly after entering home hospice.

Discussion

This case illustrates an unusually aggressive clinical course for LGSC with the recurrent disease demonstrating a high-grade sarcomatous component. Although unusual, SBT and LGSC can coexist with or progress to a high-grade neoplasm (HGSC, sarcomatoid carcinoma, or carcinosarcoma) at the time of presentation or in the setting of a recurrence [13]. The presence of high grade component, not surprisingly, is associated with poor prognosis [13]. As summarized in table 1, Malpica et al. reported one case of SBT associated with HGSC among their total 50 cases [5]. In a large series of 210 HGSC, 6 tumors were associated with low grade serous neoplasms, 3 with SBT and 3 with invasive LGSC [6]. Quddus, et al. also reported a case of HGSC arising in association with SBOT and LGSC [7]. Parker et al. reported 2 cases of SBT with early recurrences, one as HGSC and one as poorly differentiated carcinoma with sarcomatoid areas, in axillary lymph nodes and hernia sac after initial diagnosis [8]. There are also four reported cases of “sarcoma like mural nodules” in association with SBT [9–12]. Garg et al reported three cases of SBT that progressed to high-grade malignancy at the time of recurrence. Two patients were initially diagnosed with SBT, and recurred in the pelvis 3 and 10 years later with sarcomatoid carcinoma. The third patient presented with LGSC arising in a micropapillary SBT, and

recurred with a carcinosarcoma 3 years later. All 3 patients had an aggressive clinical course [13]. Boyd et al reported a series of 7 cases in whom ovarian low-grade serous neoplasms were associated with a high-grade carcinoma, either HGSC or undifferentiated carcinoma [14]. Regarding the relationship of the primary tumor and the recurrence in our case, we initially considered the possibility that the recurrent tumor could represent a collision tumor of LGSC with a malignant phyllodes or metaplastic breast cancer. However, the lack of phyllodes architecture and negative keratin staining, respectively, made these unlikely. Additionally, the molecular findings of identical TERT promoter and BRAF mutations in both the initial LGSC and the predominantly sarcomatous recurrence suggest a shared clonal process between these tumors. Given the patient was not optimally debulked at her initial presentation, another possibility is that the sarcomatous component could have been present from the beginning. However, at recurrence the low grade serous carcinoma was intimately associated with the sarcoma component and the mass contained predominately neoplastic tissue without any significant lymph node or adipose tissue. Taken together with the shared pathogenic mutations of the initial and recurrent tumors we favor this to be carcinosarcoma arising from LGSC as opposed to a de novo sarcoma.

The mechanism of transformation from LGSC to a high-grade tumor is poorly understood. Whole exome sequencing (WES) and targeted analysis of LGSC has shown frequent mutations in BRAF, KRAS, and ERBB2, while HGSC is strongly associated with TP53 mutations and typically arises as a distinct process [17]. Dehari et al found identical KRAS mutations in the low and high-grade components in 2 of their 6-concurrent high and low grade serous carcinomas, indicative of a clonal relationship. However, they did not find TP53 mutations in any of the high grade components [6]. In contrast, Quddus et al reported the presence of strong and diffuse p53 staining in the high grade component of their coexistent low and high grade serous carcinoma [7]. The Cancer Genome Atlas (TCGA) has reported that 100% of *de novo* HGSC contain TP53 somatic mutations or deletions, except for cases that arise from a low-grade serous tumor [18]. Vang et al proposed that lack of molecular alterations of TP53 are essentially inconsistent with the diagnosis of HGSC and that tumors diagnosed as such should be rigorously reassessed to achieve correct classification. They also found pathogenic TP53 mutations in 96.7% of high grade serous tumor cases. [19] The remaining cases showed evidence of p53 dysfunction associated with copy number gain of MDM2 or MDM4, or were re-classified as LGSC or carcinoma of uncertain primary site. [19] Our patient's high grade tumor did not show p53 overexpression on immunohistochemical studies and TP53 was not found to be mutated on our targeted sequencing panel. In addition to efforts of TCGA and others to characterize serous carcinoma, WES has also been performed on small series gynecologic carcinosarcomas [20]. Although this study had only a small number of ovarian carcinosarcoma cases, they observed recurrent mutations in ARID1A and the PI3-kinase pathway as well as occasional mutations in KRAS and TP53. Of note, our case did not harbor mutations in these genes, perhaps further supporting the notion that the sarcomatous component was clonally related to the LGSC as opposed to a de novo process. In our case, identical BRAF V600E and TERT promoter mutations were identified in the original debulking specimen with LGSC and in the recurrence with high-grade sarcoma. The NFE2L2 mutation is characterized as a variant of uncertain significance and most likely represents a germline variant given the

allele fraction near 50% and the presence of this variant in large, germline polymorphism databases, whereas the BRAF and TERT promoter mutations are considered deleterious [21, 22].

TERT promoter mutations have been recently reported in gynecologic and non-gynecological malignancies such as ovarian clear cell carcinoma, LGSC, melanomas, liposarcomas, hepatocellular carcinomas, urothelial carcinomas, squamous cell carcinomas of the tongue, medulloblastomas, and subtypes of gliomas [23, 24]. Mutations in BRAF are common in SBT and LGSC but rare in advanced LGSC and HGSC [22]. In Papillary thyroid carcinoma (PTC), BRAF V600E mutations significantly increased the risk for recurrence but not cancer mortality [25]. However, in melanoma, the independent prognostic role of BRAF V600E mutation is controversial [26]. The coexistence of BRAF V600E and TERT promoter mutations has been identified as an important step in progression of melanoma and papillary thyroid carcinoma (PTC) [27]. One recently described mechanism for this synergistic effect is that TERT promoter mutations create new binding sites for ETS-domain transcription factors that are upregulated by BRAF signaling, thus increasing TERT expression. [28] Additionally, it has been suggested that TERT promoter mutations increase the replicative potential (immortalization) of the tumor [28] and BRAF mutations promote more aggressive behavior [29]. Based on such findings in other tumor types, it is interesting to speculate that a synergistic effect of the TERT promoter mutation and a BRAF V600E mutation in this patient's LGSC may have contributed to the aggressive clinical course and high-grade, transformation. Future studies are warranted to evaluate whether TERT promoter mutations in BRAF mutated LGSC might be associated with disease progression and poor prognosis.

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Figure 1.
Low grade serous carcinoma in ovary (left panels) and omentum (right panels)

Figure 2.
Recurrence in the lymph node with both carcinomatoid and sarcomatoid components

Figure 3.
Wild type P16 stain identified in lymph node

Table 1

Serous borderline tumors/low grade serous carcinoma with high grade components: Summary of literature

Authors	Year	Original Tumor	Recurrence
McCullough et al	1988	Four cases of sarcoma like mural nodules in association with SBT	
DeRosa et al	1991		
Andrews et al	2008		
Gungor et al	2010		
Malpica et al	2004	SBT associated with HGSC (1 of 50 SBT cases)	
Parker et al	2004	2 cases of SBT	One HGSC in lymph node
			One poorly differentiated carcinoma with sarcomatoid areas in hernia sac
Dehari et al	2007	3 SBT associated with HGSC (3/210 total cases)	
		3 invasive LGSC associated with HGSC (3/210 total cases)	
Quddus et al	2009	One case of HGSC arising from SBT and LGSC	
Garg et al	2012	3 cases of SBT	High grade malignancy
Boyd et al	2012	SBT and LGSC	LGSC and UC
		SBT, LGSC, UC	
		LGSC and HGSC	
		SBT and LGSC	HGSC
		SBT and HGSC	
		SMBT with microinvasion	HGSC
		SBT and HGSC	

SBT: Serous borderline Tumor; HGSC: High Grade Serous Carcinoma; LGSC: Low Grade Serous Carcinoma; UC: Undifferentiated carcinoma; SMBT Seromucinous borderline tumor