

Case Report

A Case of BRAF V600E Mutation and Targeted Therapy in Borderline Ovarian Serous Cystadenoma

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Abstract

Background: Borderline ovarian serous cystadenoma with 30% *BRAF* mutation is common in young women. Although surgery is generally the treatment of choice, surgery or repeated surgery can reduce a woman's fertility and ovarian reserve. Therefore, it is critical to explore other treatment measures besides surgery.

Case Presentation: We herein describe the clinical outcome and significance of a 41-year-old patient with a *BRAF* V600E mutation in borderline ovarian serous cystadenoma after treatment with a *BRAF* inhibitor.

Conclusion: A *BRAF* inhibitor may be effective in patients with borderline ovarian serous cystadenoma with *BRAF* V600E mutation.

Keywords: *BRAF* V600E; Targeted therapy; Borderline ovarian cystadenoma; Mutation; Dabrafenib

Introduction

Borderline epithelial ovarian tumor is also known as low-grade malignant potential tumor or atypical proliferation tumor without obvious infiltration [1], and the primary pathological types are serous and mucinous [2]. Although investigators have in recent years detected the expression of an increasing number of abnormal genes in borderline ovarian serous tumors, there are no reports of targeted therapy for the disease. This report outlines a case of borderline ovarian serous cystadenoma with a *BRAF* V600E mutation, and the subsequent successfully targeted treatment using a *BRAF* inhibitor.

Case Presentation

We herein describe a 41-year-old woman who underwent right adnexectomy for "right ovarian mass" under laparoscopy in 2013. The postoperative pathology was borderline serous tumor of the right adnexa, with no specific abnormality of the fallopian tube. In 2016, an abnormal ultrasonogram revealed a left cystic solid ovarian mass 3 cm in diameter; and on 11 January 2018, gynecological ultrasonography showed left adnexal

mass, which was the same as the imaging result before primary surgery. This suggested the possibility of borderline ovarian tumor recurrence (Figure 1). We detected the somatic mutation c.1799T>A (*p. V600E*) in the *BRAF* gene (NM_004333.6) of the patient using Whole-Exome Sequencing (WES) [3]. Peripheral blood samples were used as germline controls, and the sequencing depth at this locus was 64X, with a mutation frequency of 9.38%. We also noted wild-type *KRAS*, which was consistent with the conclusion that the two oncogenes were mutually exclusive.

In May 2018, the patient was administered dabrafenib (a *BRAF* inhibitor) at a dosage of 50 mg orally twice per day for 3 months, and at follow-up, the tumor size shrank from 4.1cm×2.7cm to 2.9cm×2.7cm×2.6cm. Adverse reactions were grade I hair loss and mild discomfort at the waist, with no other side-effects. The dabrafenib was ultimately halted due to drug cost. After the patient stopped taking it, ultrasonographic examination was rechecked every 3 months. The tumor gradually increased from 3.2cm×2.8cm×2.8cm in Nov 2018 to 4.7cm×3.9cm×3.4cm in Dec 2019. On 9 June 2020, ultrasono-

graphic examination showed a cystic solid mass of the left adnexa 4.6cm×3.4cm×3.4cm in (Figure 2).

On 11 June 2020, the woman who had no fertility desire underwent laparoscopic surgery for borderline ovarian tumor-staging, with removal of the entire uterus + left adnexectomy + pelvic lymph-node dissection + omentectomy + appendectomy + adhesiolysis. During the operation, we determined that the left ovarian cyst was enlarged to 5 cm with a clear borderline, but with no tumors on the ovarian surface. The right adnexa were absent, and adhesions were observed in the pelvic cavity. Postoperative pathology showed borderline left ovarian serous cystadenoma, but no tumor cells were found in other tissues such as the omentum or appendix, or in abdominal- or pelvic-lavage fluid.

After surgery, the patient was examined at regular follow-ups by gynecological ultrasonography for 2 years without signs of recurrence.

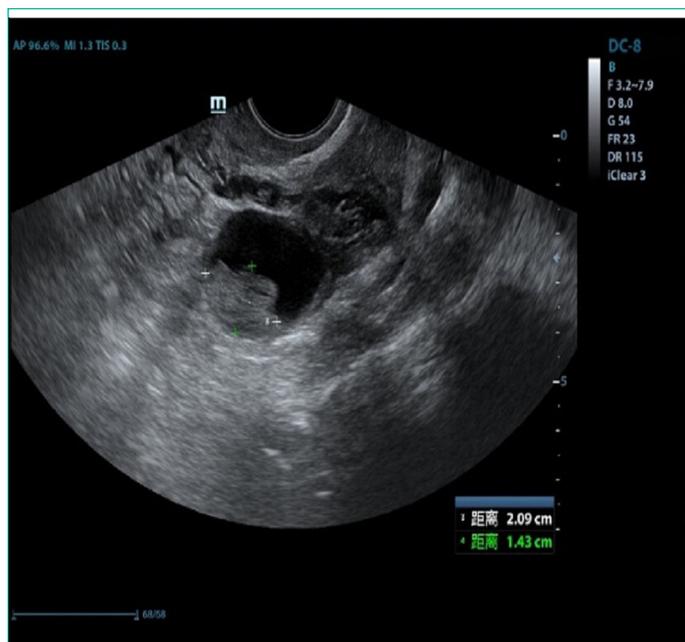


Figure 1:

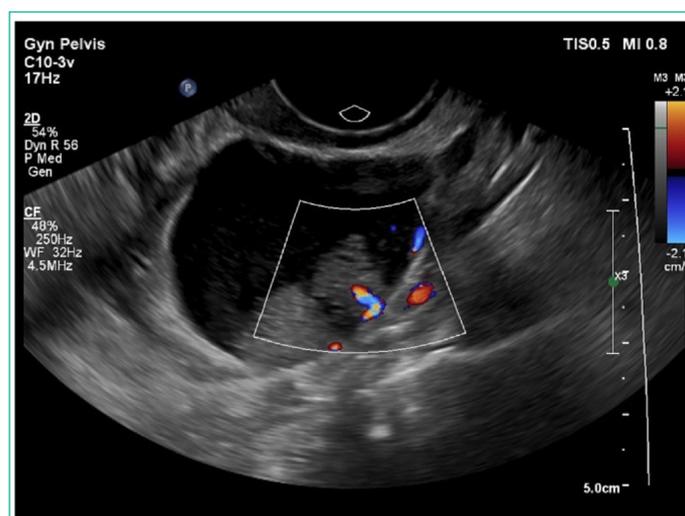


Figure 2:

Methods

Generation of Whole-Exome Sequencing (WES) Data

Genomic DNA From Formalin-Fixed Paraffin-Embedded (FFPE) tumor samples was extracted using a GeneRead DNA FFPE kit (QIAGEN) or Maxwell[®]FFPE Plus DNA Kit (Promega); and a blood sample from this patient was extracted using a TGuide Blood Genomic DNA Kit (TIANGEN). Then, 0.1–1 μ g of DNA was sheared into 200–300-bp fragments using a Covaris kit (Covaris, MA, USA), and the resulting DNA fragments were repaired and 3' poly A-tailed. Adapters were ligated to both ends of the fragments, followed by size selection, and the size-selected fragments were amplified by Polymerase Chain Reaction (PCR). We conducted exome capture using the Sure Select Human All Exon V6 platform (Agilent) according to the manufacturer's protocol, followed by PCR amplification. Libraries were prepared using the KAPA Library Quantification Kit (Kapa Biosystems, MA, USA), and validated DNA libraries were sequenced on an Illumina XTEN or NovaSeq 6000 at a mean sequencing depth for the tumor at 243 \times and for normal samples at 108 \times .

Somatic Mutation-Rate Analysis

WES sequencing read pairs were trimmed, and only read pairs with ≤ 15 N bases and $>50\%$ high-quality bases were kept for subsequent analyses. The resulting high-quality reads were aligned to the human reference genome (Homo_sapiens_assembly19) using the Burrows-Wheeler Aligner (0.7.17) [4]. To improve alignment accuracy, we employed the Genome Analysis Toolkit (version 3.8.1) [5] to process BAM files, which included marking duplicates, and the local realignment around high-confidence insertions and deletions. To call somatic mutations accurately, we used the variant-calling pipeline developed by TCGA MC3 project. Briefly, this pipeline employs five callers to call substitution mutations and three callers to identify small indels, with detailed annotation. We only retained substitution mutations and indels supported by at least two callers for further analyses. All mutations were retained for subsequent analyses if their positions were $\geq 10\times$ in both normal and tumor samples. To calculate the Tumor-Mutation Burden (TMB) values, we herein used only missense or ORF shift mutations in the overlapping targeted regions with the Sure Select Human All Exon V6 platform and those regions defined in TCGA MC3 project [6]. We exploited MANTIS to call the MSI status for each tumor based on 2539 loci from the mSINGS package [3].

Discussion

Early symptoms of borderline serous cystadenoma of the ovary may be similar to those of benign tumors; ascites can be found in the late stages [7], and over 50% of the pelvic masses in patients are palpable [7]. Many methods are helpful with diagnosis— including gynecological ultrasonography, MRI, and laboratory examination. As the accuracy of intraoperative frozen pathology is 87% [8], surgery has evolved into the most important treatment modality for borderline ovarian tumors. A majority of recurrences entail some types of tumors and can be resected, but adjuvant therapy post-operatively is not effective in controlling disease recurrence.

Types of Genetic Mutations in Borderline Ovarian Tumors

Borderline tumors have not been as widely examined as ovarian cancer with respect to genetic mutations. However, *BRAF* and *KRAS* mutations are acknowledged to be the most

common in borderline ovarian serous tumors, with rates above 30% and 20% [9], respectively. The two tumor types are mutually exclusive, as wild-type *KRAS* was observed in all *BRAF*-mutated tumors, and vice versa [10]. Mutations in other genes such as *PIK3CA*, *BRCA1*, *EGFR*, *CTNNB1*, *PTEN*, *ERBB2*, and *AKT2* [11] are relatively infrequent, and reports of them are limited.

***BRAF* Mutation and its Significance in the Treatment of Other Tumors**

BRAF is a protooncogene located on chromosome 7q34 [12], and it encodes a serine- threonine protein kinase that plays an important role in the RAS-RAF-MEK-ERK- signaling pathway. *BRAF* links receptors and RAS proteins on the cell surface with nuclear transcription factors via MEK and ERK and initiates the participation of a variety of factors in the regulation of numerous biological events in cells—including cellular proliferation, differentiation, and apoptosis. Most mutations in *BRAF* occur in exon 15, where the thymine at nucleotide 1799 is converted to adenine, resulting in the mutation of valine at codon 600 (*V600E*) to glutamate [13] and leading to the continuous activation of MEK and ERK. Ultimately, the cells continue to proliferate, and this reflects a critical function in the formation of some tumors such as melanoma, colon adenocarcinoma, and ovarian serous tumors.

The mutation rates of *BRAF* in primary and metastatic melanoma are approximately 40%–60% and 50% [13], respectively, and the principal *BRAF* mutation site is *BRAF V600E* [14]. Current studies have revealed that *BRAF* mutations in melanoma patients portend lower Overall Survival (OS) and a poorer prognosis than those for patients with wild-type *BRAF* [14]. In view of the poor prognosis for patients with unresectable or metastatic melanoma and the limited benefits of standard chemotherapy, the emergence of small-molecule *BRAF* inhibitors has completely transformed the treatment of melanoma. *BRAF* inhibitors such as vemurafenib and dabrafenib are now used to treat unresectable or metastatic melanoma, with a response rate of approximately 50% [13]. Compared with dacarbazine, *BRAF* inhibitors such as vemurafenib can increase the OS at 6 months by 20% and reduce the relative risk of death by 63% and the risk of tumor progression by 74% [15]. These same investigators also described a Median Progression-Free Survival (mPFS) of 5.3 months in the vemurafenib group and 1.6 months in the dacarbazine group [15].

In addition, *BRAF* mutations have been uncovered in ~10%–15% of colorectal cancers, with the mutation in *BRAF V600E* being the primary mutation site. This gene is mutated early in colorectal cancer and comprises an independent adverse predictor using multivariate analysis [13] because of its poor response to standard chemotherapeutic drugs and its rapid progression. With further elucidation of the *BRAF*-signaling pathway, triplet therapy (irinotecan, cetuximab, and vemurafenib) has achieved better rates of PFS (4.4 months vs. 2.0 months), objective remission (16% vs. 4%), and disease-control (67% vs. 22%, respectively) than double therapy (irinotecan and cetuximab) [16].

The aforementioned data showed that *BRAF* inhibitors achieved favorable therapeutic effect in other tumors, and we therefore also employed dabrafenib as the experimental treatment for this patient; and its therapeutic effect was proven by the clinical reduction in tumor size.

***BRAF* Gene Mutation in Borderline Ovarian Tumor**

The most common *BRAF* mutation is *BRAF V600E*, and in-

vestigators depicted *BRAF* mutations as related to specific cell types that showed aging characteristics in borderline ovarian serous tumors [9]; such mutations prevented the disease from developing into malignancy, and thus, the *BRAF V600E* mutation was positively correlated with borderline ovarian tumor prognosis. However, there was no significant effect of *BRAF V600E* mutation on five-year survival rate [17]. Only a small number of serous cystadenomas develop into borderline serous tumors, with the cystadenoma epithelium adjacent to the borderline tumor similar to normal ovarian epithelium and lacking cytologic atypia. In addition, *BRAF* mutation in borderline tumors is also found in adjacent tumor cells. These lines of evidence therefore strongly indicate that *BRAF* mutation is an early tumor event that precedes borderline tumorigenesis [10]. However, whether the early treatment of *BRAF*-gene mutation can alter the clinical outcome of patients requires further evaluation, as we showed in our present case report.

A histological diagnosis of borderline ovarian serous cystadenoma was confirmed in the present study by surgery in 2013, and after 3 years of follow-up, a recurrence was detected in the ovary. In 2018, after 3 months of treatment with oral dabrafenib, the tumor size was reduced significantly from 4.1cm×2.7cm to 2.9cm×2.7cm×2.6cm. However, after the drug was halted due to its cost to the patient, the tumor size gradually increased from 2cm×2.8cm×2.8cm to 4.7cm×3.9cm×3.4cm; and in 2020, we confirmed at surgery that borderline ovarian serous cystadenoma recurred. However, the fact that the tumor size was significantly reduced during the targeted drug therapy indicated that the drug could control borderline ovarian serous cystadenoma.

The population prone to borderline ovarian tumors tends to be younger, with ~1/3 of them being less than 40 years of age [18]. According to a recent study, only 22% of the patients achieved pregnancy after repeated surgery, with only 38% of the late-stage patients undergoing successful pregnancy [19]. Such high infertility rates therefore necessitate fertility preservation, especially for those patients whose recurrence surgery further attenuates ovarian reserve. Repeated surgery might thus be avoided—and ovarian function preserved as much as possible—in patients with *BRAF* mutations by using *BRAF* inhibitors to control the disease in such circumstances. We posit that research in this field will be moving in this direction in the near future. However, due to the case reported herein, the effectiveness of this protocol still requires replication in a much larger sample of clinical data.

Conclusion

The use of a *BRAF* inhibitor may be effective in patients with borderline ovarian serous cystadenoma and who express a *BRAF V600E* mutation.

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