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Whole exome sequence analysis of serous borderline tumors of the ovary

Jeff Boyd [a,b,c,*], Biao Luo [a,b], Suraj Peri [d], Beth Wirchansky [a], Lucinda Hughes [a], Caitlin Forsythe [a], Hong Wu [c]

[a] Cancer Genome Institute, Fox Chase Cancer Center, 333 Cottman Ave., Philadelphia, PA 19111, USA
[b] Cancer Biology Program, Fox Chase Cancer Center, 333 Cottman Ave., Philadelphia, PA 19111, USA
[c] Department of Pathology, Fox Chase Cancer Center, 333 Cottman Ave., Philadelphia, PA 19111, USA
[d] Biostatistics and Bioinformatics Core Facility, Fox Chase Cancer Center, 333 Cottman Ave., Philadelphia, PA 19111 USA

HIGHLIGHTS

- This study examined the entire exome of serous borderline tumors (SBTs) of the ovary for somatic genetic mutations.
- A very small number of mutations are characteristic of SBTs of the ovary.
- Novel candidate genes for the pathogenesis of ovarian SBT were identified.

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ABSTRACT

Objective. Serous borderline tumor (SBT) is a unique histopathologic entity of the ovary, believed to be intermediate between benign cystadenoma and invasive low-grade serous carcinoma. While somatic mutations in the *KRAS* or *BRAF*, and rarely *ERBB2*, genes have been well characterized in SBTs, other genetic alterations have not been described. Toward a more comprehensive understanding of the molecular genetic architecture of SBTs, we undertook whole exome sequencing of this tumor type.

Methods. Following pathologic review and laser capture microdissection to enrich for tumor cells, whole exomes were prepared from DNA of two independent SBTs and subjected to massively parallel DNA sequencing.

Results. Both tumors contained an activating mutation of the *BRAF* gene. A total of 15 additional somatic mutations were identified, nine in one tumor and six in the other. Eleven were missense mutations and four were nonsense or deletion mutations. Fourteen of the 16 genes found to be mutated in this study have been reported to be mutated in other cancers. Furthermore, 12 of these genes are mutated in ovarian cancers. The *FBXW7* and *KIAA1462* genes are noteworthy candidates for a pathogenic role in serous borderline tumorigenesis.

Conclusions. These findings suggest that a very small number of somatic genetic mutations are characteristic of SBTs of the ovary, thus supporting their classification as a relatively genetically stable tumor type. The mutant genes described herein represent novel candidates for the pathogenesis of ovarian SBT.

Introduction

The classification of "borderline" epithelial ovarian neoplasms was originally introduced to describe tumors that are noninvasive but that occasionally seem to behave in a malignant fashion [1]. Approximately 2% of all ovarian tumors of serous histology are borderline, as compared to 78% that are benign tumors and 20% that are invasive carcinomas [2]. There appears to exist a pathological range of serous borderline tumors (SBTs), with those at the lower end of the spectrum behaving in a benign fashion, and referred to as "atypical proliferative serous tumors" (APSTs) and those at the upper end of the spectrum behaving more like low grade invasive carcinomas and referred to as "micropapillary serous tumors" (MPSTs) (ref. [1]). The current consensus is that the terms "borderline" and "atypical proliferative" are synonymous, and that "low malignant potential" should not be used to describe borderline tumors [3].

An emerging theory suggests classification of ovarian neoplasms into two types, wherein borderline tumors represent an intermediate pathologic lesion between benign cystadenomas and low-grade carcinomas in the "type I" category [1,4,5]. In contrast, "type II" tumors consist of high grade serous and other histologic type carcinomas, with no well accepted precursor lesion. This model of ovarian cancer pathogenesis is supported not only by traditional morphologic observations but also by molecular genetic analyses of various ovarian tumor types [5,6]. Type II serous ovarian carcinomas are notable for the ubiquitous nature of *TP53* mutations [7,8], low but statistically recurrent somatic mutations in nine additional genes, and an average of 61 additional rare somatic

* Corresponding author at: Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia, PA 19111, USA. Fax: +1 215 214 4503.
E-mail address: jeff.boyd@fccc.edu (J. Boyd).

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mutations per tumor [8]. Notably, a recent study involving whole exome analysis of low-grade serous carcinomas of the ovary identified an average of only 10 somatic mutations per tumor in seven cases [9]. Thus, the genetic mutational landscape of type II serous tumors appears dramatically distinct from that of type I tumors.

Since the initial observation that SBTs frequently harbor *KRAS* mutations [10], subsequent studies have confirmed this observation and further demonstrated that *KRAS* and *BRAF* mutations are common in SBTs and low-grade serous carcinomas [11–14]. Mutation of *KRAS* and *BRAF* are mutually exclusive for a given tumor, and one or the other is present in approximately one-half to two-thirds of SBTs and low-grade serous carcinomas [6], although a more recent report suggests that the prevalence of these mutations in advanced-stage, low-grade serous carcinomas may be substantially lower [15]. Finally, a 12-bp insertion mutation in *ERBB2*, which ultimately results in *KRAS* activation, has also been described in a small proportion of SBTs that lack mutations in *KRAS* or *BRAF* [16,17]. Otherwise, the molecular genetic architecture of SBTs of the ovary remains unknown. The purpose of this study was to perform whole exome sequencing of SBTs to identify additional genetic alterations that may contribute to the initiation and/or progression of type I serous ovarian neoplasms.

Recent advances in technology, bioinformatics, and computational biology have led to a revolution in the mining of the cancer landscape. The application of second-generation DNA sequencing technologies, also known as next-generation sequencing, allowing for whole-genome, whole-exome, and whole-transcriptome tumor analyses, is rapidly transforming cancer genomics [18]. In the near-term, the complete molecular genetic dissection of individual tumors may be anticipated not only to impact mechanisms of cancer pathogenesis, but also to suggest novel approaches to diagnosis and therapeutic selection as well. To further these goals with respect to SBTs of the ovary, we sequenced the entire coding regions (exomes) of two independent tumors, SBT-s2, and SBT-s5.

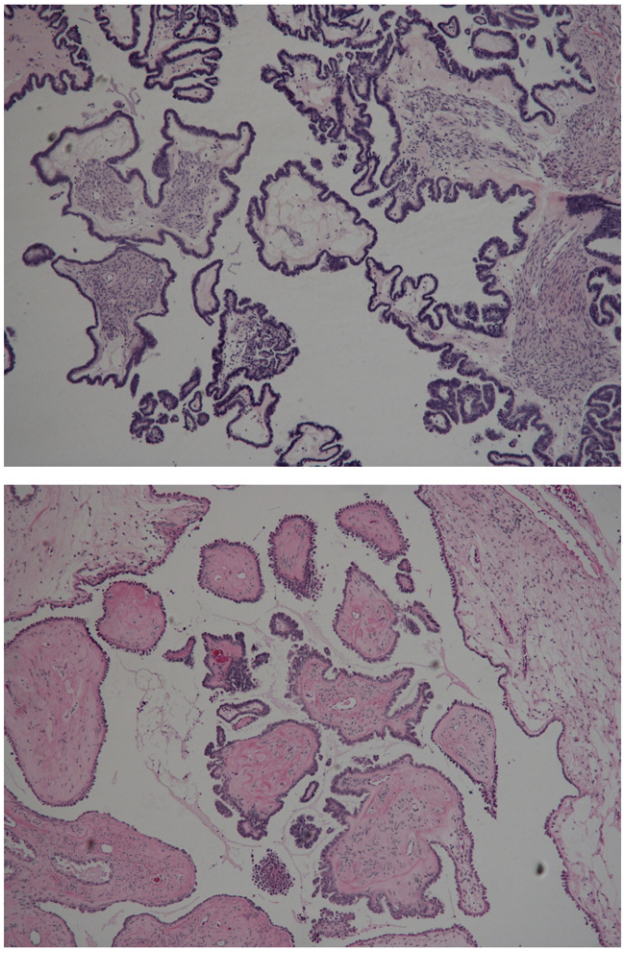

Fig. 1. Serous borderline tumors SBT-s2 (top) and SBT-s5 (bottom). Characteristic features of SBTs include hierarchical branching of micropapillae emanating from larger, more centrally located papillae, tufting, and epithelial stratification [1].

Methods

Tumor specimens

The tumor and corresponding blood samples used in this study were obtained from the Fox Chase Cancer Center Biosample Repository Facility under a protocol approved by the Institutional Review Board. Two tumors, SBT-s2 and SBT-s5, were initially identified from pathology reports to meet the criteria of SBTs. Additional pathologic review confirmed the original diagnosis. Photomicrographs of H&E-stained sections of the tumors are shown in Fig. 1. Both tumors were unilateral from the left ovary, and in neither case was there evidence of additional pathology in the reproductive tract. Both tumors SBT-s2 and SBT-s5 were removed from 49-year-old patients, both approximately two years prior to this study. The tumors were flash frozen in liquid nitrogen following pathologic processing at the time of surgery, and embedded in OCT medium prior to preparation for laser capture microdissection.

Laser capture microdissection and DNA isolation

Tissue sections of 7 μm thickness were prepared from embedded tumors with a cryostat and adhered to uncharged Superfrost microscope slides (Fisher Scientific, Pittsburgh, PA). The first, last, and every tenth tissue section from each tumor specimen ($n = 30$ for SBT-s2 and $n = 75$ for SBT-s5) was stained with H&E and subjected to pathologic review to confirm the clinical diagnosis and homogeneity of the tissue specimen. For laser capture microdissection, slides were stained with H&E immediately prior to loading into an Arcturus Veritas instrument (Life Technologies, Carlsbad, CA). Tumor cells were selected and laser captured onto CapSure Macro LCM Caps (MDS Analytical Technologies, Sunnyvale, CA), which were then placed onto sterile nuclease-free 0.5 ml PCR tubes (Eppendorf) and stored at −80 °C. Genomic DNA was isolated from the cells using the QIAamp DNA Micro kit (Qiagen, Valencia, CA) according to the manufacturer's protocol for isolation of genomic DNA from laser microdissected tissue, with one exception. After the addition of lysis buffer and proteinase K, tubes were incubated at 56 °C for 16 h before proceeding with the protocol. Purified DNA was eluted in 25 μl of TE buffer, quantitated with a Nanodrop ND 1000 spectrophotometer (Fisher Scientific), and stored at 4 °C.

Library construction and exon capture

Samples of DNA were adjusted to a concentration of 20 μg/ml with 1× TE buffer, and 100 μl of each sample were placed in a Covaris microTUBE 6 × 16 mm round bottom glass tube, AFA fiber, and pre-slit snap-cap system (Covaris, Woburn, MA). Fragmentation of DNA to 300-bp was accomplished by sonication with a Covaris S2 sonicator using the following parameters: duty cycle 10%; intensity 4; cycles per burst 200; and time 120 s. Sheared DNA was purified and concentrated using the Agencourt AMPure XP system (Beckman Coulter, Beverly, MA) and eluted into nuclease-free water according to the manufacturer's instructions.

Libraries were prepared using reagents from the NEBNext DNA Sample Prep Master Mix Set 1 (New England Biolabs, Ipswich, MA) and custom designed adaptors and primers (Integrated DNA Technologies, Coralville, IA) for paired-end library construction as described [18]. Damaged ends of the fragmented DNA were repaired and a single A base was added to the 3′-ends using the End Repair and dA-Tailing Modules, respectively, according to the manufacturer's protocols (New England