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Original contribution

Detection of the *BRAF* V600E mutation in serous ovarian tumors: a comparative analysis of immunohistochemistry with a mutation-specific monoclonal antibody and allele-specific PCR

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Summary Mutations of components of the mitogen-activated protein kinase pathway, mainly *BRAF*, are common in serous ovarian borderline tumors, whereas high-grade serous ovarian carcinomas rarely show this feature. With the advent of specific kinase inhibitors active against *BRAF*-mutated cancers, rapid and sensitive detection of the *BRAF* V600E, by far the most common mutation of this gene, is of great practical relevance. Currently, *BRAF* mutations are detected by DNA-based techniques. Recently, a monoclonal antibody (VE1) specific for the BRAF V600E protein suitable for archival tissues has been described. In this study, we compared detection of the V600E mutation in serous ovarian tumors by VE1 immunostaining and by allele-specific polymerase chain reaction. All 141 cases of high-grade serous ovarian cancer showed negative or rarely weak, diffuse background VE1 immunostaining, and *BRAF* wild type was confirmed by molecular analysis in all tested cases. In contrast, 1 (14%) of 7 low-grade serous carcinomas and 22 (71%) of 31 serous borderline tumors revealed moderate to strong VE1 positivity. Immunostaining was clearly evaluable in all cases with sufficient tumor cells, and only rare cases with narrow cytoplasm were difficult to interpret. The V600E mutation was confirmed by allele-specific polymerase chain reaction and sequencing in all VE1-positive cases. Two VE1-positive cases with low epithelial cell content required repeat microdissection to confirm the presence of the mutation. Immunohistochemistry with the VE1 antibody is a specific and sensitive tool for detection of the *BRAF* V600E mutation in serous ovarian tumors and may provide a practical screening test, especially in tumor samples with low epithelial content.

1. Introduction

The serine threonine kinase v-RAF murine sarcoma viral oncogene homolog B1 (*BRAF*) proto-oncogene is an

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activator of the mitogen-activated protein kinase (MAPK) pathway and is commonly mutated in a variety of cancers. More than 95% of mutated cases carry the V600E point mutation, which results in constitutive tyrosine kinase activity [1,2]. *BRAF* V600E mutations are present in virtually all cases of hairy cell leukemia and are common in papillary thyroid cancer, malignant melanoma, and colon cancer of the alternative pathway [3-6]. Serous tumors of the ovary, the most common group of ovarian neoplasms, show a dichotomous distribution of *BRAF* mutations [7-13]. Whereas they are virtually absent in high-grade serous carcinoma, V600E is the most common oncogenic mutation in serous ovarian tumors of low malignant potential and also occurs in low-grade serous carcinoma [14-16].

The advent of specific inhibitors of mutant *BRAF* with clinical activity in malignant melanoma [17] and preclinical data indicating that inhibition of the MAP kinase pathway in *BRAF*-mutated ovarian cancer may yield clinical benefit suggest that the reliable and sensitive detection of the V600E mutation may soon be of clinical relevance in serous ovarian tumors [18,19]. Currently, detection of *BRAF* mutations relies on molecular methods, including conventional sequencing, pyrosequencing, or allele-specific polymerase chain reaction (PCR) with melting curve analysis, requiring access to a molecular diagnostic laboratory. Recently, a monoclonal mouse antibody (VE1) specifically detecting the mutated (V600E) BRAF protein in formalin-fixed, paraffin-embedded tissues has been described and may represent a practical tool for screening [20-22].

In the present study, we compared the reliability of VE1 immunostaining and allele-specific PCR and sequencing for the detection of the *BRAF* V600E mutation in a large series of serous ovarian carcinoma and serous borderline tumors.

2. Material and methods

2.1. Patients

The study collective comprised consecutive patients diagnosed with low-grade and high-grade serous ovarian cancer (SOC), and serous borderline ovarian tumors (SBT), treated at the University Women's Hospital Tübingen, Germany, between the years 2000 and 2008. All patients had undergone surgical resection according to their clinical stage and had received, if indicated, platinum-containing chemotherapy based on current German guidelines (http://www.ago-online.de). Follow-up data were obtained from the patient registry of the Comprehensive Cancer Center Tübingen. The clinical follow-up ranged from 1 to 121 months, with an average of 62 months. The 31 patients with serous borderline tumors had undergone surgical treatment only. None of these patients showed progression to a high-grade carcinoma or advanced disease. The study had been approved by the local ethics committee.

2.2. Construction of tissue microarrays

All slides were retrieved from the archives of the Institute of Pathology and reviewed by a pathologist with subspecialty training in gynecologic pathology (A.S). For each patient, the histologic subtype and grading were recorded. Adequate paraffin material for construction of a tissue microarray was available from 141 patients with high-grade SOC, 7 patients with low-grade SOC, and 31 women with SBT.

For tissue microarray construction, tumor areas with more than 70% tumor cells were circled on hematoxylin and eosin–stained sections. From the corresponding paraffin blocks, cores with diameters of 0.6 mm (cases of SOC) and 1 mm (SBT) were punched with a semiautomatic tissue array instrument (Beecher Instruments, Sun Prairie, WI) and placed into recipient blocks. Six cores were obtained per patient, with 3 cores from each side in cases with bilateral tumors. Normal ovarian and tubal tissue cores were included as controls. Four-micrometer-thick sections were cut from recipient blocks and mounted onto SuperFrost slides (Menzel, Braunschweig, Germany).

2.3. Immunohistochemistry

The BRAF V600E–specific monoclonal mouse antibody VE1 was generated against an 11-amino acid synthetic peptide representing the mutated *BRAF* sequence as previously described [20]. Immunostaining was performed on a Ventana BenchMark XT immunostainer (Ventana Medical Systems, Tucson, AZ) [20-22]. The tissue sections were pretreated with CC1 buffer solution (pH 8.2) for 60 minutes. The undiluted hybridoma supernatant was incubated at 37°C for 32 minutes. Primary antibody detection was performed using the ultraView Universal DAB Detection Kit (Ventana) and signal amplification (Amplification Kit, Ventana), followed by counterstaining with hematoxylin.

VE1 staining is exclusively cytoplasmic. The intensity of immunostaining was graded from 0 to 3, with 0 as negative staining, grade 1 as weak background staining which was also occasionally observed in control tissues, grade 2 as moderate,

Fig. 1 Results of BRAF V600E immunostaining in serous ovarian tumors. A, Borderline tumor/wild-type BRAF with weak, nonspecific staining of surface cilia, grade 1 (G1). B, BRAF V600E–mutated borderline tumor with moderate cytoplasmic staining, grade 2. C, BRAF V600E–mutated borderline tumor with strong cytoplasmic staining, grade 3 (G3). Weak (wild-type BRAF) (D) and moderate (V600E mutation) staining of SOC G1 (E, F). Negative (G) and weak, nonspecific staining of SOC G3 (both wild-type) (H). Note that tissue cores for the SBT (A-C) were bigger (10 mm diameter) than the cores for the SOC (6 mm). Original magnification: A-E, G, and H, ×100; F and inserts, ×400, immunoperoxidase.