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BRAF Mutation is Associated with Early Stage Disease and Improved Outcome in Patients with Low-Grade Serous Ovarian Cancer

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Abstract

Background—Low-grade serous (LGS) ovarian cancer (OC) is a chemoresistant disease that accounts for 10% of serous ovarian cancers. Prior studies have reported that 28–35% of serous borderline (SB)/LGS ovarian tumors harbor a BRAF mutation, suggesting that BRAF inhibitors may be a rational therapeutic approach for this disease. We sought to determine if BRAF or KRAS mutation status is associated with disease stage and/or histology in patients with SB and LGS ovarian cancer.

Methods—We genetically profiled 75 SB and LGS ovarian tumors for mutations in BRAF and KRAS. The incidence and identity of BRAF and KRAS mutations were defined and the results were correlated with stage, response to treatment, and overall survival.

Results—Of 75 samples examined, 56(75%) were SB and 19(25%) LGS histology. Fifty-seven percent of tumors harbored either a KRAS mutation (n=17) or a BRAF V600E mutation (n=26). BRAF V600E mutation was significantly associated with early stage disease (Stage I/II, $P<0.001$) and serous borderline histology ($P=0.002$). KRAS mutation was not significantly associated with stage or histology. Of the 22 (29%) patients who required treatment with chemotherapy, 20 were KRAS/BRAF wild-type (WT), 2 were KRAS mutant, and none had tumors harboring a BRAF mutation. All BRAF mutant patients were alive at a median follow-up of 3.6 years (range 1.9–129.3 months).

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Conclusions—V600E BRAF mutations are present in 35% of SB/LGS ovarian cancers. The presence of BRAF V600E mutation in SB/LGSOC is associated with early stage disease and improved prognosis. Patients with SB/LGSOC who require systemic therapy are unlikely to have BRAF mutant tumors.

Introduction

Mutations in BRAF and KRAS, components of the RAS/RAF/MEK/ERK cascade, are common in low-grade serous and serous borderline ovarian tumors. In contrast, they are present in less than 1% of patients with high-grade serous ovarian cancer [1–6]. Conversely, p53 mutations are found in 96% of cases of high-grade serous ovarian cancer but are rare in low-grade serous tumors [1, 7].

On the basis of these clinical and genetic differences between low-grade and high-grade serous ovarian cancer, a two-tiered grading system has been created [8–11]. This dualistic model of ovarian carcinogenesis proposes that high-grade disease arises *de novo* from distal fallopian tube epithelium, whereas low-grade serous ovarian tumors evolve through a step-wise progression from a benign serous cystadenoma to a serous borderline neoplasm (SB) to an invasive low-grade serous carcinoma (Figure 1) [12]. Serous borderline neoplasms can further be sub-classified as atypical proliferative serous tumors or non-invasive micropapillary serous carcinomas. When micropapillary serous carcinomas develop invasion they become synonymous with low-grade serous carcinomas [13, 14]. The presence of micropapillary features in serous borderline neoplasms is associated with an increased frequency of bilateral ovarian disease, peritoneal implants, and recurrent disease, when compared to SB neoplasms without micropapillary features[15, 16].In contrast to patients with high-grade disease, patients with low-grade serous ovarian cancer present at a younger age (45–57 years) and their tumors typically display a low tumor mitotic index and are largely resistant to chemotherapy (Table 1)[17–20]

Contrary to prior findings, it has recently been reported that BRAF mutation is rare in advanced stage low-grade serous ovarian cancer and that patients with BRAF or KRAS mutation may have an improved clinical outcome [7].

The primary objective of the present study was to determine if BRAF or KRAS mutation status is associated with disease stage and/or histology in patients with low-grade serous and serous borderline ovarian cancer. To achieve this objective, we retrospectively analyzed tumor samples and associated clinical data from all patients with low-grade serous or serous borderline ovarian cancer surgically treated at MSKCC between 2000 and 2010.

Methods

Patient Samples

Following Institutional Review Board approval, clinical data were collected on all patients with a diagnosis of low-grade serous or serous borderline ovarian cancer who underwent surgery at MSKCC between the years 2000 and 2010. All included patients were required to have formalin-fixed, paraffin-embedded (FFPE) tissue available from at least one prior staging or debulking operation.

The original pathology reports were reviewed to determine stage based on the AJCC staging system for ovarian and primary peritoneal cancer (7^{th} ed., 2010). Patients' records were also reviewed for determination of clinical status, date of diagnosis, date of last follow-up, date of recurrence(s), and treatment history. For all specimens, tumor histology was reviewed and confirmed by a reference specialty pathologist (K.G or D.D.).

Tissue Analysis—FFPE tissue samples were macro-dissected to remove stromal contamination and ensure tumor cellularity of ≥80%. Tumor DNA was extracted using the DNeasy tissue kit according to manufacturer's instructions (Qiagen, Valencia, CA). Each specimen was analyzed using a custom iPLEX assay (Sequenom, Inc, San Diego, CA) to detect KRAS and BRAF hotspot mutations[21]. Each variant detected was manually reviewed. Those tumors harboring a mutation and with sufficient DNA underwent confirmation of mutation status with an orthogonal method. All primer sequences are available upon request.

Statistical Analysis—This was a single institution retrospective analysis of archived tumor tissues and associated clinical variables. For the purposes of this analysis patients were grouped into early stage (stage I or II disease at presentation) or advanced stage (stage III or IV disease at presentation) based on AJCC 7th ed. staging. Fisher-Exact tests were used to determine the association between mutation status (KRAS mutant, KRAS WT, BRAF mutant, BRAF WT, or KRAS/BRAF WT) and stage at presentation, and the association between mutation status and histology (serous borderline or low-grade serous). Overall survival intervals were calculated from the date of diagnosis to the date of death or last follow-up and were estimated using the method of Kaplan and Meier.

Results

Twelve patients were excluded due to inadequate quantity of tumor DNA. Seventy-five patients were included in the final analysis. Eighty percent (n=60) of samples were collected from a primary staging or debulking operation, 16% (n=12) from a secondary debulking, 3% (n=2) from a tertiary debulking, and 1% (n=1) from a quaternary debulking. Seven patients have died and 68 remain alive. The median follow-up for the living patients was 35.9 months (0.8–129.3 months) (Table 2).

Fifty-seven percent of tumors harbored either a BRAF (n=26) or KRAS (n=17) mutation. Figure 2 displays a representative mass spectrometry trace of a BRAF V600E mutation from one of the tumor specimens. The same mutation was detected using Sanger sequencing. BRAF and KRAS mutations were mutually exclusive. All BRAF mutations were V600E. KRAS mutations were all either G12D (n=11) or G12V (n=6) (Figure 3A). All 11 KRAS G12D mutations were confirmed by Sanger sequencing or a second Sequenom assay. Two out of 6 KRAS G12V mutations and 7 of 26 BRAF V600E mutations were unable to be confirmed by Sanger sequencing or a second Sequenom assay due to insufficient DNA amount. BRAF V600E mutation (n=26) was significantly associated with early stage disease (P<0.001) and serous borderline histology (P=0.002) when compared to BRAF WT tumors (n=49) (Table 3). Presence of BRAF V600E mutation was also significantly associated with early stage (P<0.001) and serous borderline histology (P<0.001) when compared to KRAS/BRAF WT tumors (n=32). In contrast, KRAS mutation (n=17) was not significantly associated with stage or histology when compared to KRAS WT (n=58) or KRAS/BRAF WT tumors (n=32). Eleven out of the 56 (20%) SB tumors had micropapillary features. Two of the 11 (18%) with micropapillary features harbored BRAF mutations, while 23/45 (51%) of those without micropapillary features were found to have a BRAF mutation.

Twenty-two patients (29%) were treated with chemotherapy (4 serous borderline, 18 low-grade serous) either in the adjuvant or recurrent setting; 2 were KRAS mutant, 0 were BRAF mutant, and 20 were KRAS/BRAF WT (Figure 3B). The need for chemotherapy treatment was significantly associated with BRAF mutation status (p< 0.001). All BRAF mutant patients remain alive at a median follow-up of 43.3 months (range 1.9–129.3 months), with the suggestion of improved overall survival as compared to KRAS mutant or KRAS/BRAF

WT patients. Median survival has not yet been reached in any of the mutation cohorts (Figures 4A and 4B).

Discussion

Our findings indicate that within the disease continuum of serous borderline and low-grade serous ovarian cancer V600E BRAF mutation is associated with early stage at presentation, serous borderline histology, and improved outcome. We postulate that the presence of a BRAF mutation in patients with serous borderline disease prevents progression to more aggressive disease. This is in contrast to papillary thyroid cancer where the presence of a V600E BRAF mutation is associated with advanced stage and poor prognosis, and metastatic colon cancer where V600E BRAF mutation is also associated with poor prognosis, indicating that BRAF mutation status has tumor lineage specific prognostic implications [22–27].

Serous borderline and low-grade serous ovarian cancer is typically a chemotherapy resistant disease, with reported response rates to cytotoxic chemotherapy of 4% in the neoadjuvant setting and 2.1%–4.9% in the recurrent setting [18, 19]. Given the high prevalence of BRAF and KRAS mutations in serous borderline and low-grade serous ovarian cancer, there has been recent interest in testing inhibitors targeting the MAP kinase pathway in patients with advanced disease. Twenty-nine percent of the patients in this study received at least one line of systemic chemotherapy to date; however none of the patients who required systemic therapy had a BRAF mutant tumor. These findings suggest that patients with aggressive low-grade serous ovarian cancers, the population most in need of novel effective systemic therapies, are unlikely to harbor BRAF mutant tumors. As the selective RAF inhibitor vemurafenib lacks activity in KRAS mutant and BRAF/RAS wild-type patients, our data imply that this agent will be of limited utility in this disease[28].

Notably, a recently completed phase II trial of the MEK inhibitor AZD6244 in women with recurrent low-grade serous carcinoma of the ovary or peritoneum reported a radiographic response rate of 15.4%. Out of the 34 patients with sufficient DNA for mutation analysis only 2 tumors harbored BRAF mutations, supporting our finding that BRAF mutation is rare in those patients requiring systemic therapy. A correlation between RAS or BRAF mutation status and disease response was not observed [29]. This lack of correlation between BRAF and RAS mutation status and AZD6244 response may have been the results of the presence of occult genomic events in the BRAF and RAS wild-type cohort which phenocopy the effects of RAS mutation such as NF1 mutation or loss. It is notable, however, that the 15.4% response rate observed following treatment with AZD6244 is markedly higher than that previously reported with cytotoxic chemotherapies in patients with low-grade serous ovarian cancer. These results indicate that a subset of low-grade serious ovarian cancer tumors are dependent upon MEK activity and that further evaluation of MEK inhibitors is warranted in this disease.

In summary, low-grade serous ovarian cancer is a chemotherapy resistant disease with limited systemic treatment options available. Our results suggest that the finding of a BRAF mutation predicts for a favorable outcome in surgically treated patients. Testing for BRAF mutation in newly diagnosed patients with serous borderline histology may serve as a powerful prognostic tool in determining those patients who are unlikely to progress to more aggressive histology and advanced disease.

Our finding that BRAF mutations are rarely found in patients who require systemic therapy suggests that highly selective RAF inhibitors may have limited utility in this disease. Further studies of MEK and ERK inhibitors are warranted given the promising early clinical results

with the selective MEK inhibitor AZD6244. A detailed exploration of the genetic basis of BRAF/KRAS wild-type serous borderline and low-grade serous ovarian cancer is also warranted as such efforts may result in the identification of novel therapeutic targets in the cohort of patients most likely to suffer ovarian cancer specific mortality.

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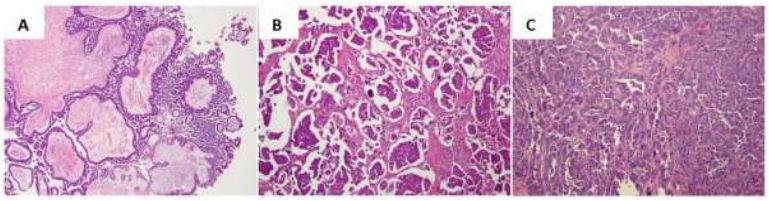

Figure 1.

A: Serous borderline tumor (SB) with papillary architecture and abundant micropapillae growing on the surface of the large, papillary fronds. Like LGS, SB tumors display low nuclear grade and a low mitotic index but lack stromal invasion. B: Low-grade serous carcinoma (LGS) with the typical invasion pattern of micropapillae embedded in stroma surrounded by an artifactual cleft. LGS carcinomas possess low nuclear grade and a low mitotic index compared to HGS. C: High grade serous carcinoma (HGS) with high nuclear grade and focal anaplasia, a markedly elevated mitotic index, and glandular architecture with abundant tufting and budding of cells.

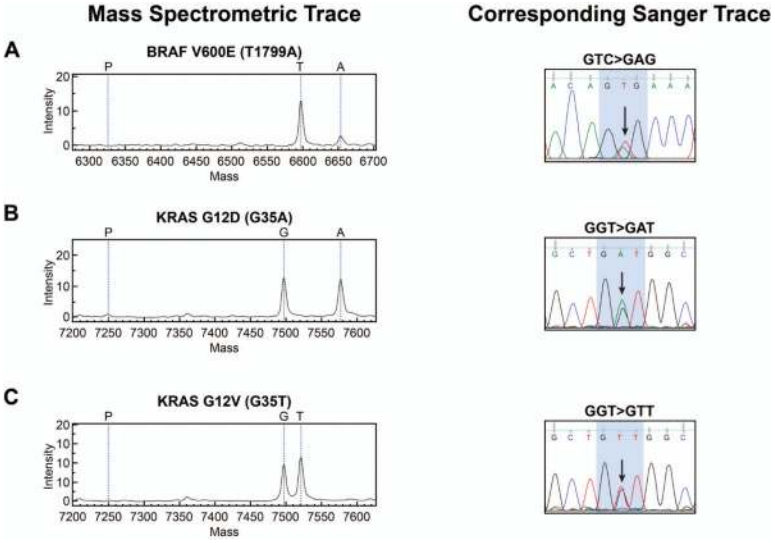

Figure 2.

Representative mass spectrometry (MS) trace and corresponding Sanger trace for three tumors harboring (A) BRAF V600E, (B) KRAS G12D, and (C) KRAS G12V mutations.

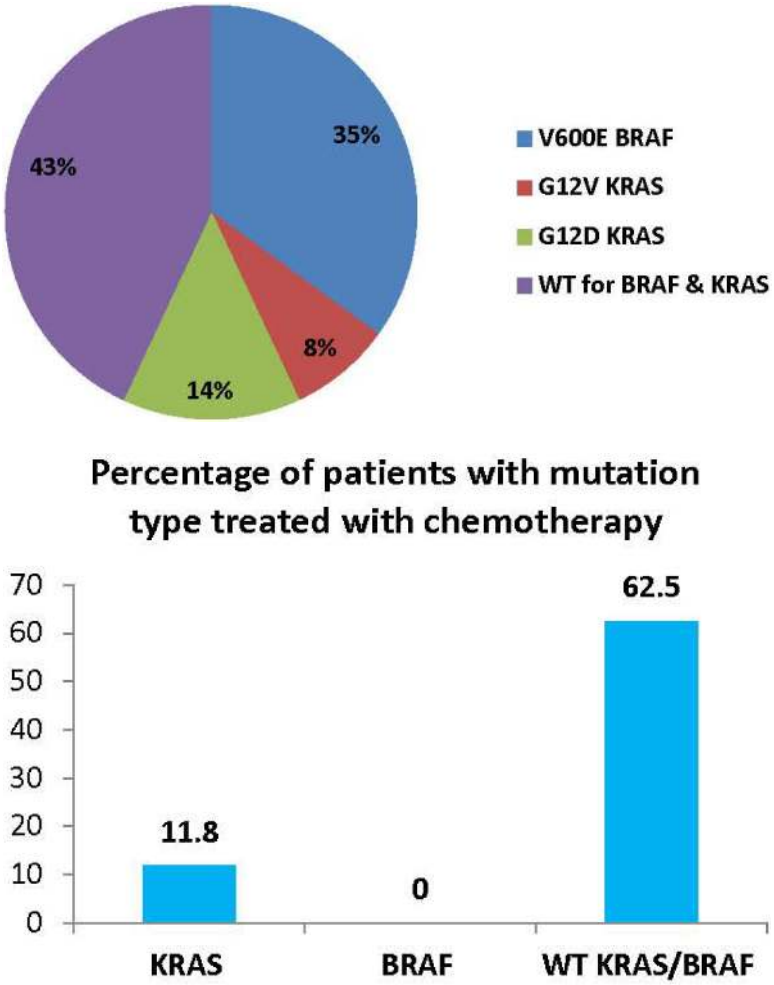

Figure 3.

Figure 3A: Out of 75 patients with low-grade serous or serous borderline ovarian cancer, 17 harbored a KRAS mutation (G12D=11; G12V=6), 26 harbored a BRAF mutation, and 32 were WT for KRAS and BRAF mutation. Figure 3B: 62.5% of patients that were WT for KRAS and BRAF received chemotherapy either in the adjuvant or recurrent setting, compared to 11.8 % of KRAS mutant patients and 0% BRAF mutant patients.

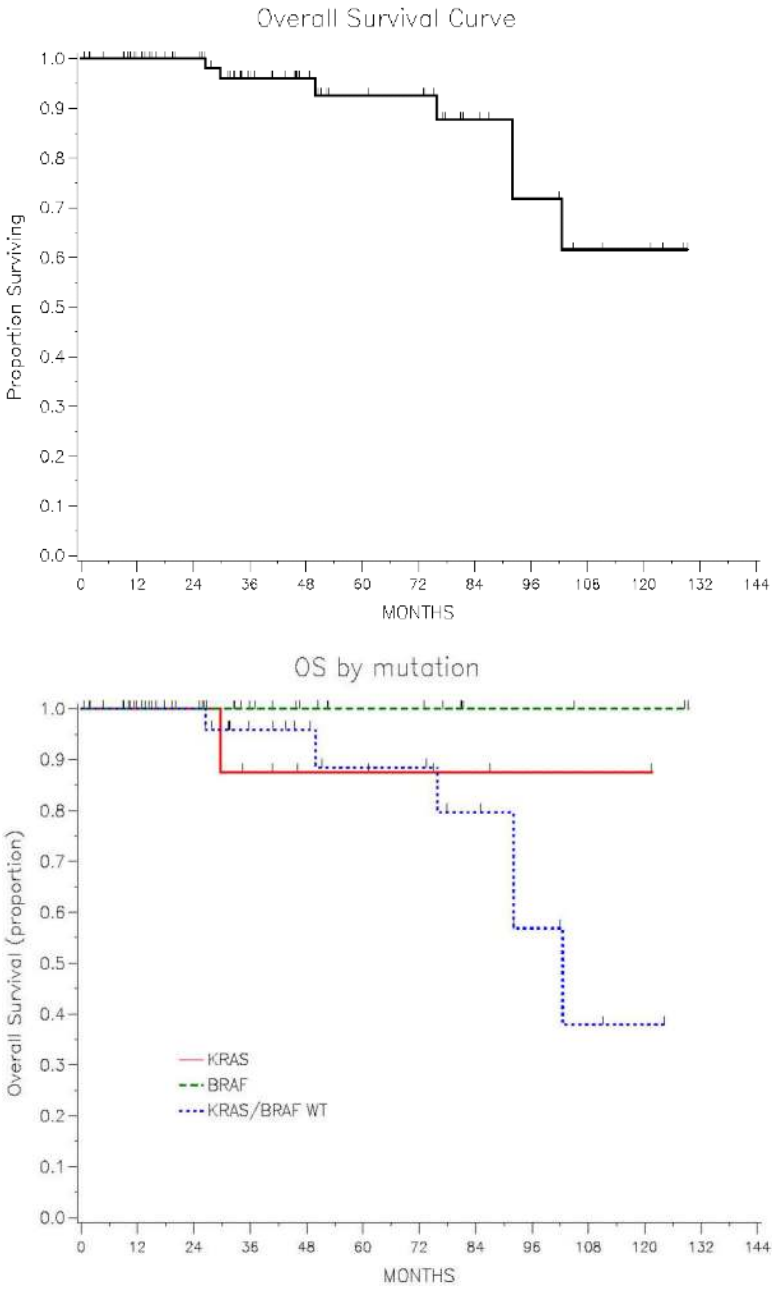

Figure 4.

Figure 4A: Kaplan-Meier curve of overall survival (OS) for all 75 patients. Seven patients have died. Figure 4B: Kaplan-Meier curve of proportion of patients surviving by mutation type. Median OS has not yet been reached for any group.

Table 1

Clinical Features of LGS vs. HGS Ovarian Cancer

	Low-Grade Serous	High-Grade Serous
Age in years (Median)[1]	45–57	55–65
% of Serous Ovarian Cancer Cases[8]	~10	~90
Growth Pattern[1]	Micropapillary-rich	Large papillae, solid and glandular growth
Nuclear Atypia[4]	Mild to moderate atypia	Marked atypia
Mitotic Rate[4]	≤12 mitoses per 10 HPFs	>12 mitoses per 10 HPFs
5-year Survival for Stage >1[1]	40–56%	9–34%
RR to Platinum-based Neoadjuvant Chemotherapy[8]	4%	80%

HPFs: high-power fields. RR: response rate.

Table 2

Population Characteristics

	N	%
Total	75	
Age at Diagnosis		
Median (Mean)	47(46.3)	
Range in Years	15–79	
Stage		
I/II	45	60.0
III/IV	30	40.0
Histology		
Serous Borderline	56	74.7
Low-Grade Serous	19	25.3
Mutation		
KRAS (G12D/G12V)	17	22.7
BRAF (V600E)	26	34.7
WT for KRAS & BRAF	32	42.7
Clinical Status		
Alive at Last F/U	68	90.7
Deceased	7	9.3

WT: wild type. F/U: follow-up

Table 3

BRAF Mutation Is Significantly Associated With Stage and Histology

	Total	Stage I/II	Stage III/IV	p-value	SB	LGS	p-value
BRAF V600E+	26	24(53.3%)	2(6.7%)	<0.001	25(44.6%)	1(5.3%)	0.002
BRAF WT	49	21(46.7%)	28(93.3%)		31(55.4%)	18(94.6%)	
KRAS G12D+/G12V+	17	10(22.2%)	7(23.3%)	1.000	14(25%)	3(15.8%)	0.534
KRAS WT	58	35(77.8%)	23(76.7%)		42(75%)	16(84.2%)	