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ORIGINAL ARTICLE

KRAS mutation in primary ovarian serous borderline tumors correlates with tumor recurrence

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Abstract

Oncogenic activation of the mitogen-activated protein kinase (MAPK) pathway due to *KRAS* or *BRAF* gain-of-function mutation is frequently found in ovarian serous borderline tumor (SBT) and their extraovarian implants. We investigated mutational status of *KRAS* and *BRAF* of the primary ovarian SBTs that had a high stage presentation in correlation with clinical outcome. Among 39 consecutive primary SBTs with either invasive implants (20 cases) or non-invasive implants (19 cases), *KRAS* and *BRAF* mutational analysis was informative in 34 cases. Sixteen cases (47%) harbored a *KRAS* mutation, while 5 cases (15%) had a *BRAF* V600E mutation. High-stage disease (IIIC) was seen in 31% (5/16) of patients with a *KRAS* mutation and 39% (7/18) of patients without a *KRAS* mutation ($p=0.64$). *KRAS* mutations were present in 9/16 (56%) tumors with invasive implants/LGSC versus 7/18 (39%) tumors with non-invasive implants ($p=0.31$). *BRAF* mutation was seen in 5 cases with non-invasive implants. Tumor recurrence was seen in 31% (5/16) of patients with a *KRAS* mutation, compared to 6% (1/18) of patients without a *KRAS* mutation ($p=0.04$). A *KRAS* mutation predicted an adverse disease-free survival (31% survival at 160 months) compared to those with wild-type *KRAS* (94% at 160 months; log-rank test, $p=0.037$; HR 4.47). In conclusion, *KRAS* mutation in primary ovarian SBTs is significantly associated with a worse disease-free survival, independent of the high tumor stage or histological subtypes of extraovarian implant. KRAS mutation testing of primary ovarian SBT may servce as a useful biomarker for tumor recurrence.

Keywords *KRAS* mutation · Ovarian serous borderline tumor · Disease-free survival

Introduction

Serous borderline tumor (SBT) of the ovary is a low-grade epithelial neoplasm affecting primarily reproductive-age women [1, 2]. While patients with stage I SBT have an excellent prognosis, 10 to 20% of SBTs present with extrauterine involvement in the form of tumor implants at the time of initial surgery [3–5]. A subset of patients will develop tumor recurrence, and in up to 7% of SBTs, the tumor progresses to low-grade serous carcinoma (LGSC) over time [6, 7]. Established risk factors for the development of subsequent carcinoma include micropapillary/cribriform histology, advanced stage, bilaterality, ovarian surface involvement, and residual disease after surgery [5, 8–13]. While implants are, by definition, non-invasive, the term "invasive implant" remains as a qualifier for the diagnosis of extra-ovarian LGSC in the setting of a primary SBT [7, 12].

In recent decades, studies have established that abnormal activation of the mitogen-activated protein kinase (MAPK) pathway is important for the pathogenesis of SBT. Two key components of the pathway, *KRAS* and *BRAF*, are frequently mutated in these tumors [14–18]. Other less frequent MAPK activation pathways have also been implicated [19, 20]. In recent studies of the extraovarian implants of SBT, *KRAS* mutation in implants of either invasive or noninvasive type ware found as a worse prognostic indicator for tumor recurrence and disease-specific survival. *BRAF* V600E mutation, however, may portend a lower risk for progression to carcinoma [13, 14, 21, 22]. Only limited data suggested that *KRAS* mutation in primary ovary SBTs is similarly associated with an unfavorable prognosis [23]. In this study, we examined the presence of *KRAS* and *BRAF* mutations in the primary

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tumors of ovarian SBTs of patients with at least stage IIA disease in correlation with clinical outcome.

Materials and methods

Study case selection and histological review

Consecutive cases of high stage SBTs were retrieved from pathology archives at a single institution accessioned between 1990 and 2020. Primary ovarian SBTs and their extraovarian lesions were histologically reviewed (AH and PH), and their extraovarian lesions were reclassified according to the 5th Edition 2020 WHO criteria [24] as either invasive implants/low-grade serous carcinoma or non-invasive implants. Briefly, tumor implants were assigned as invasive based on one or more of the following: destructive growth pattern at low magnification, presence of micropapillary architecture, and tumor cell nests surrounded by retraction artifact in dense fibrotic stroma (Fig. 1). A tumor implant without the aforementioned morphologic qualifiers was classified as non-invasive (Fig. 2), of either epithelial type (hierarchically branching papillae and detached clusters of cells without stromal response) or desmoplastic type (single cells or clusters of cells embedded in reactive-appearing or desmoplastic stroma). Patient demographics and clinical follow-up data were collected by medical record review. The study was performed under research protocols approved by the Institutional Review Board.

KRAS and *BRAF* mutational analysis

Formalin-fixed, paraffin-embedded tumor blocks were selected from the primary ovarian borderline tumors. One hematoxylin and eosin-stained slide (H&E) and additional unstained sections were created. Once confirmed by H&E slide review, the corresponding target tumor tissue from the unstained slides were microdissected into a microcentrifuge tube. DNA was extracted by hydrothermal pressure method of simultaneous deparaffinization and lysis of formalin-fixed paraffin-embedded tissue followed by conventional column purification to obtain high quality DNA [25].

KRAS and *BRAF* mutation analysis by the highly sensitive single strand conformation polymorphism (SSCP) technique was performed according to previously described methods [26]. Briefly, 5–20 ng of extracted DNA was amplified using PCR primers flanking the mutational hotspot of exon 2 of the *KRAS* gene (forward primer: 5′-GACTGAATATAAACTTGTGG-3′ and reverse primer: 5′-CTGTATCAAAGAATGGTCCT-3′) and *BRAF* V600E mutation (forward primer: 5′-CTCTT CAT AATGCTTGCTCTGATAGG-3′ and reverse primer: 5′-TAG TAACTCAGCAGCATCTCAGG-3′). The reaction was performed in a 50-µl solution containing 1×PCR buffer, 0.1-mM dNTP, 1.5-mM MgCl2, and 2.5 units of AmpliTaq Gold DNA polymerase. PCR started with initial denaturation at 95 °C for 8 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and synthesis at 72 °C for 2 min, with a final extension at 72 °C for 10 min (ABI Veriti Thermal Cycler, Applied Biosystem, Foster City, CA, USA). The PCR product was analyzed in duplicate by SSCP using MDE

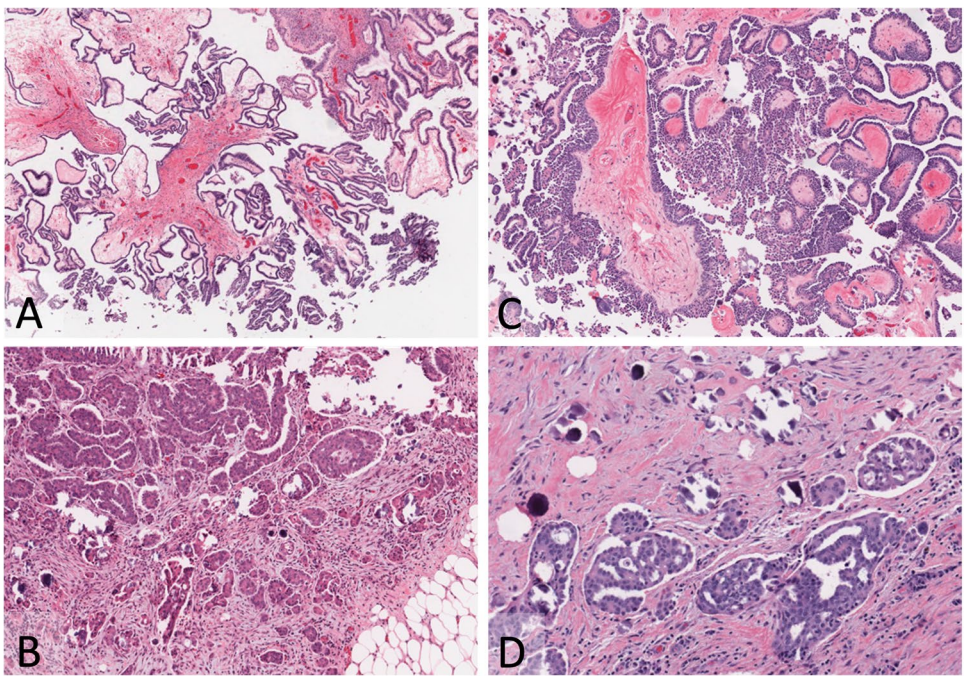

Fig. 1 Representative ovarian serous borderline tumors (**A**, **C**) and extraovarian invasive/LGSC implants (**B**, **D**) from patients #4 (**A**, B) and #12 (**C**, **D**) from Table 1. Note the presence of retraction artifact, in which solid nests and some micropapillae are densely packed together within clear, lacunar-like spaces

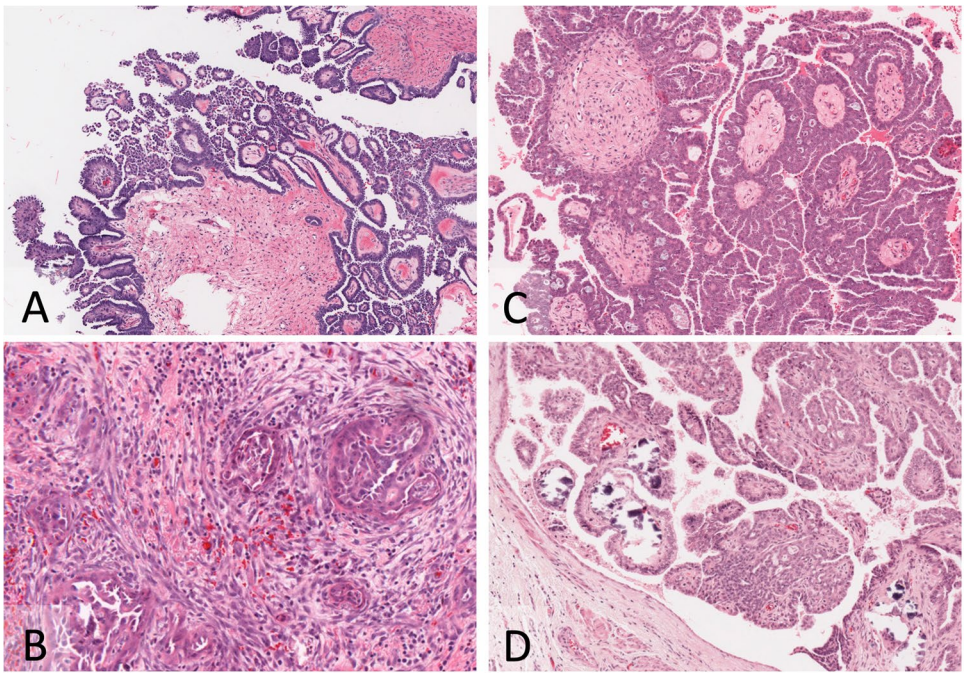

Fig. 2 Representative ovarian serous borderline tumors (**A**, **C**) and extraovarian non-invasive implants (**B**, **D**) from patients #22 (**A**, **B**) and #7 (**C**, **D**) from Table 1. A micropapillary pattern is defined as a 5 mm or greater area of small papillae with no fibrovascular cores that are at least 5 times as long as they are wide, often arising from a central, thicker papilla (**C**). Desmoplastic-type implants (**B**) show clusters of cells embedded within reactive-appearing desmoplastic stroma which predominates over the epithelial component. Epithelial-type implants (**D**) contain small to medium sized papillae, have detached clusters of cells not associated with stroma, and are within epithelium-lined spaces

non-denaturing gel. Electrophoresis was carried out on ice for 2 h 45 min at 325 V. The SSCP gel was then stained with a SYBR Gold (Molecular Probes, Invitrogen, Norwalk, CT, USA) 1:10 000 in TE buffer added for 20 min and imaged by a Biorad GelDoc UV System (BioRad, Hercules, CA, USA). The presence of *KRAS* or *BRAF* mutations were determined by comparing the SSCP banding patterns with those of known *KRAS* mutation or *BRAF* V600E mutation positive controls (Fig. 3A and B). Cases with indeterminant *KRAS* mutation status by SSCP were evaluated by a Sanger sequencing analysis of DNA purified from the abnormal SSCP gel bands (Fig. 3C). Briefly, a mixture of deoxynucleoside triphosphates (dNTPs) and fluorescently labeled dideoxynucleoside triphosphates (ddNTPs) were utilized to generate nested fragments by chain termination during the synthesis of complimentary DNA [27].

Statistical analysis

Statistical analyses were performed using the two-tailed Student's *t*-test for differences in the means of continuous variables and the Pearson chi-squared test for categorical variables. Statistical significance was determined by setting the level of $p < 0.05$ (alpha = 0.05) as significant. Follow-up time for disease-free survival calculation was measured in months from the day of initial surgery to the date of recurrence, defined as the date of surgical removal of tissue diagnosed as recurrent serous disease (SBT or LGSC), or date last known to be alive with or without disease. Recurrence was defined as tissue diagnosis of recurrent serous disease (either SBT or LGSC). The date of detection of tumor recurrence was defined as surgery date of the recurrent tumor. The Kaplan–Meier statistic method was used to generate a *p*-value using the Cox-Mantel log-rank test. The Mantel–Haenszel test was utilized to calculate relative risk in tests for which the Cox-Mantel log-rank test would fail (i.e., no recurrence within a subgroup).

Results

Clinicopathological characteristics of the study cohorts

A total of 39 cases of high stage SBT and follow-up data were included (Table 1). Patient age ranged from 26 to 79 years (mean 50.5, median 51). Laterality was unknown in 2 patients who had total hysterectomies with bilateral salpingo-oophorectomies at outside institutions. Bilateral ovarian SBTs were seen in 31 patients (84%), and unilateral tumors were seen in 6 patients. A micropapillary/cribriform pattern was seen in 6 primary ovarian tumors, and microinvasion was seen in 3 SBTs. Invasive implants/LGSC were present in 20 patients, and non-invasive implants were seen in 19 patients (Table 1). Two patients had both invasive and non-invasive implants. Pelvic lymph node involvement was seen in 14 patients (36%), and pelvic endosalpingiosis was seen in 15 patients (38%).

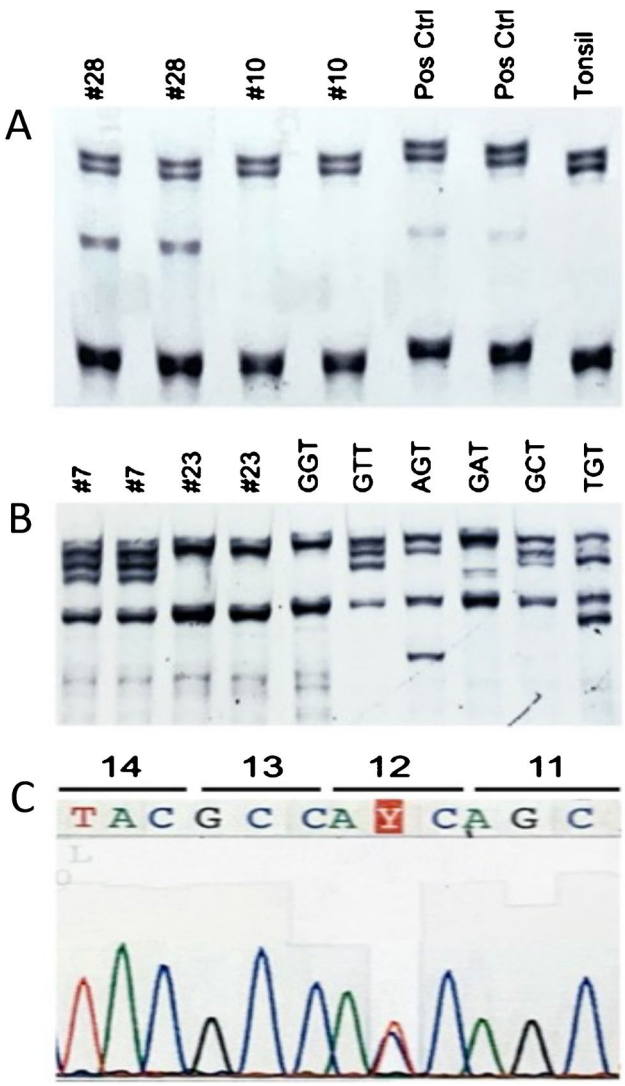

Fig. 3 Representative SSCP gels demonstrate positive *BRAF* V600E mutation (**A**) in the primary tumor of patient #28, wild-type status of patient #10, and appropriate positive and negative controls; and detection of *KRAS* exon 2, codon 12 mutation (GTT) (**B**) of the primary tumor from patient #7; wild-type *KRAS* (GGT) pattern from patient #23, and additional wild-type and common *KRAS* mutation controls (GTT, AGT, GAT, GCT, and TGT). All patient samples are run in duplicate for quality control. Representative Sanger sequencing of *KRAS* exon 2 (**C**) shows codons 11–14 with GGT to GTT mutation (G12V) from patient #20

KRAS and *BRAF* mutational analysis

KRAS and *BRAF* mutation analysis was informative in 34 of 39 cases (Table 2). *KRAS* mutation was detected in 47% (16/34) of primary SBTs, while 5 tumors harbored *BRAF* V600E mutations (15%). *KRAS* and *BRAF* mutations were mutually exclusive. Clinicopathological features were comparable between patients with *KRAS* mutation and those without *KRAS* mutation, including patient age, presence of bilateral tumor, types of extraovarian implants, and tumor stage at presentation (Table 3). Notably, *KRAS* mutation correlated significantly with the presence of pelvic endosalpingiosis: 63% (10/16) of cases with *KRAS* mutation versus 22% (4/18) of cases without *KRAS* mutation ($p=0.017$).

Correlation of implant types and disease progression

Of the 39 patients with follow-up data (ranging from 8 to 440 months), 8 patients experienced disease recurrence, 8 died of their disease, and 5 died of an unrelated cause. No statistically significant difference in terms of patient age or follow-up time was identified between patients with non-invasive implants and invasive/LGSC implants (Table 3). Similarly, no statistically significant difference in age was found between women with a *KRAS* mutation detected and women with wild-type *KRAS* tumor status.

Of the 39 patients, 20 had invasive/LGSC implants, whereas 21 had only non-invasive implants. Two patients had both invasive and non-invasive implants (Table 1). High-stage disease (IIIC) was seen in 70% (14/20) of patients with invasive/LGSC implants, whereas only 16% (3/19) with only non-invasive implants had high stage disease. When stratified by implant type, recurrence occurred in 40% (8/20) of patients with invasive/LGSC implants, compared to 0% (0/19) of patients with only non-invasive implants ($p=0.002$) (Table 3). The rate of disease-free survival at 160 months was 34% in patients with invasive/LGSC implants (95% confidence interval 0–56.9%), compared to 100% disease-free survival in patients with non-invasive implants. Patients with invasive/LGSC implants had a worse disease-free survival (log-rank test, p-value = 0.003; Mantel–Haenszel hazard ratio 8.49) than those with non-invasive implants (Fig. 4).

Prognostic correlation with *KRAS* and *BRAF* mutation status

Among the 34 cases with informative *KRAS* and *BRAF* mutational analysis and clinical follow-up data, stage IIC disease or above was seen in 75% (12/16) of patients with *KRAS* mutation and 72% (13/18) of the patients without *KRAS* mutation ($p=0.85$). Similarly, the presence of high-stage disease (IIIC) at presentation was seen in 31% (5/16) of patients with *KRAS* mutations and 39% (7/18) of patients without *KRAS* mutations ($p=0.64$). *KRAS* mutations were present in 56% (9/16) of tumors with invasive implants/LGSC, compared to 39% (7/18) of tumors with non-invasive implants ($p=0.311$). Tumor recurrence was seen in 31% (5/16) of patients with *KRAS* mutations, compared to 6% (1/18) of patients without *KRAS* mutations ($p=0.04$). Independent of the tumor stage and the histologic subtypes of implants, *KRAS* mutation in the primary tumors predicted a worse disease-free survival (31%

Table 1 Clinicopathologic parameters of the study cohort ($n=39$)

Pt #	Age	Laterality	FIGO Stage	Implant	Surgery	FU (mo)	Recur-rence (mo)	Status	Endo	Nodal disease
1	30	L	IIB	Non-invasive	TAH/LSO/s	142	No	NED	Yes	No
2	53	Bi	IIIA2	Invasive (LGSC)	TAH/BSO/s	31	No	NED	Yes	No
3	37	Bi	IIB	Non-invasive	TAH/BSO/s	73	No	NED	Yes	No
4	34	Bi	IIIA	Invasive (LGSC)	TAH/BSO/s	255	Yes (46)	DOD	Yes	No
5	34	Bi	IIA	Non-invasive	TAH/BSO/s	18	No	NED	Yes	No
6	40	R	IIIB	Non-invasive	TAH/BSO/s	8	No	NED	Yes	No
7[a]	50	Bi	IIC	Non-invasive	TAH/BSO/s	45	No	NED	Yes	No
8	34	Bi	IIIB	Non-invasive	TAH/BSO/s	168	No	NED	Yes	Yes
9[a]	47	Bi	IIIC	Invasive (LGSC)	TAH/BSO/s	58	Yes (3)	DOD	Yes	Yes
10[b]	38	Bi	IIIB	Invasive (LGSC)	TAH/BSO/s	60	No	NED	No	No
11[a]	55	Bi	IIIC	Invasive (LGSC)	TAH/BSO/s	136	Yes (63)	AWD	No	Yes
12	51	Bi	IIIC	Invasive (LGSC)	TAH/BSO/s	54	Yes (10)	DOD	No	No
13	26	R	IIIC	Non-invasive	TAH/BSO/s	102	No	NED	No	Yes
14	65	Bi	IIIB	Non-invasive	TAH/BSO/s	184	No	DUC	No	No
15	58	Bi	IIIC	Invasive (LGSC)	TAH/BSO/s	52	Yes (29)	DOD	Yes	Yes
16	61	Bi	IIIC	Invasive (LGSC)	TAH/BSO/s	53	No	NED	No	No
17	65	Bi	IIB	Non-invasive	TAH/BSO/s	102	No	NED	No	Yes
18	66	Bi	IIIA	Invasive (LGSC)	TAH/BSO/s	58	No	NED	No	No
19	52	Bi	IIIC	Invasive (LGSC)	TAH/BSO/s	254	Yes (144)	DOD	No	No
20	62	Bi	IIA	Non-invasive	TAH/BSO/s	14	No	NED	Yes	No
21[a,b]	58	Bi	IIIB	Invasive (LGSC)[c]	TAH/BSO/s	192	No	DUC	Yes	Yes
22	77	Bi	IIIA	Non-invasive	TAH/BSO/s	131	No	DUC	Yes	Yes
23	55	Bi	IIIB	Non-invasive	TAH/BSO/s	129	No	NED	Yes	No
24	43	L	IIIB	Non-invasive	TAH/BSO/s	106	No	NED	No	Yes
25	41	Bi	IIIB	Invasive (LGSC)	TAH/BSO/s	60	No	NED	No	No
26	62	Bi	IIIC	Invasive (LGSC)	TAH/BSO/s	47	No	NED	No	Yes
27	67	Bi	IIB	Non-invasive	TAH/BSO/s	97	No	NED	No	No
28	44	Bi	IIB	Non-invasive	TAH/BSO/s	43	No	NED	No	No
29[a]	34	L	IIIC	Non-invasive	TAH/LSO/s	26	No	NED	No	Yes
30	63	Bi	IIIC	Invasive (LGSC)	TAH/BSO/s	28	No	NED	No	Yes
31[a]	68	Bi	IIIC	Invasive (LGSC)	TAH/BSO/s	42	No	DUC	No	Yes
32	79	Bi	IIB	Non-invasive	TAH/BSO/s	121	No	NED	No	No
33	46	Bi	IIIC	Invasive (LGSC)	TAH/BSO/s	200	No	NED	No	No
34[b]	36	Bi	IIB	Non-invasive	TAH/BSO/s	47	No	NED	No	No
35[d]	59	N/A	IIIC	Invasive (LGSC)	TAH/BSO/s	98	No	NED	Yes	No
36[d]	72	N/A	IIIC	Invasive (LGSC)	TAH/BSO/s	440	No	DOD	No	Yes
37	60	Bi	IIIC	Non-invasive	TAH/BSO/s	180	No	DUC	No	No
38	43	R	IIIC	Invasive (LGSC)	TAH/BSO/s	333	Yes (156)	DOD	No	No
39	39	Bi	IIIC	Invasive (LGSC)[c]	TAH/BSO/s	186	Yes (159)	DOD	No	No

Abbreviations: *AWD*, alive with disease; *Bi*, bilateral; *DOD*, died of disease; *DUC*, died of unrelated cause; *Endo*, endosalpingiosis; *FIGO*, International Federation of Gynecology and Obstetrics; *FU*, follow-up; *L*, left; *LGSC*, low-grade serous carcinoma; *LSO*, left salpingo-oophorectomy; *mo*, month; *mut*, mutant; *N/A*, unknown; *NED*, no evidence of disease; *Pt*, patient; *R*, right; *s*, staging; *TAH/BSO*, total abdominal hysterectomy/bilateral salpingo-oophorectomy; wt, wild-type

[a]Patients 7, 9, 11, 21, 29, and 31 had micropapillary morphological features in the primary tumors

[b]Patients 10, 21, and 34 had microinvasion in the primary ovarian tumor

[c]Patients 21 and 39 had both invasive (LGSC) and non-invasive implants

[d]The laterality of primary ovarian disease could not be determine for patients 35 and 36

Table 2 Primary serous borderline tumors with informative *KRAS* and *BRAF* mutation data

		Invasive implant $n=16$	Non-invasive implant $n=18$	Total $n=34$
KRAS	Mutated	9 (56%)	7 (39%)	16 (47%)
	Wild type	7 (44%)	11 (61%)	18 (53%)
BRAF	Mutated	0 (0%)	5 (28%)	5 (15%)
	Wild type	16 (100%)	13 (72%)	29 (85%)

at 160 months) compared to those with wild-type *KRAS* in the primary ovarian tumor (94% at 160 months; log-rank test, $p=0.037$; HR 4.47). *BRAF* mutation was only seen in 5 cases with non-invasive implants.

Discussion

The two most prevalent genes involved in the pathogenesis of ovarian low-grade serous tumors (SBT and LGSC) are *KRAS* and *BRAF* [15, 16]. Mutations in the two genes result in abnormal gain of function, leading to uncontrolled activation of the MAPK signaling pathway. Close to 50% of SBTs and LGSCs harbor *KRAS* or *BRAF* mutations [15, 17, 28]. *KRAS* and *BRAF* mutations are mutually exclusive in the vast majority of cases [15, 29]. In the current study of at least stage IIA ovarian SBTs, *KRAS* mutation was identified in the primary tumors of 16/34 patients (47%), while *BRAF* V600E mutation was identified in 5/34 patients (15%), consistent with the existing literature.

Limited studies have suggested that *KRAS* mutation in primary ovarian tumors is associated with unfavorable prognoses and disease progression to LGSC [23]. Focusing on *KRAS* and *BRAF* mutation status of the primary ovarian SBTs in this study, the outcome analysis found that *KRAS* mutation detectable in the primary ovarian SBTs is a significant prognostic indicator for tumor recurrence: SBTs harboring a *KRAS* mutation had a considerably higher recurrence rate than those without the mutation (31% vs 6%) and a significantly worse disease-free survival (31% vs 94% at 160 months, log-rank test, *p*-value 0.037, hazard ratio 4.47). Remarkably, such prognostic value is independent of the high tumor stage and histologic subtypes

Table 3 Statistical analysis of the patient cohort with available follow-up data stratified by implant type ($n^a=39$) and *KRAS* mutation in the primary ovarian tumors ($n^b=34$)

	Non-invasive ($n=19$)[a]	Invasive ($n=20$)[a]	*p*-value	*KRAS* wild type ($n=18$)[b]	*KRAS* mutant ($n=16$)[b]	*p*-value
Age (mean)	26–79 (49)	34–72 (53)	0.34	26–79 (53)	34–66 (49)	0.48
FIGO stage						
IIA	2 (11%)	0 (0%)		0 (0%)	2 (13%)	
IIB	7 (37%)	0 (0%)		5 (28%)	2 (13%)	
IIC	1 (5%)	0 (0%)		0 (0%)	1 (6%)	
IIIA	1 (5%)	3 (15%)		1 (6%)	3 (19%)	
IIIB	5 (26%)	3 (15%)		5 (28%)	3 (19%)	
IIIC	3 (16%)	14 (70%)	0.0006	7 (39%)	5 (31%)	0.64
Invasive implant				7 (39%)	9 (56%)	0.311
Bilateral ovarian disease	14 (74%)	17/18 (94%)	0.086	15 (83%)	15 (94%)	0.35
Endosalpingiosis	9 (47%)	6 (30%)	0.27	4 (22%)	10 (63%)	0.017
Recurrence	0 (0%)	8 (40%)	0.0019	1 (6%)	5 (31%)	0.049
Median disease-free survival (mo, rate at 160 months)	Median not reached (100%)	156 months (34%)	0.003	Median not reached (94%)	144 months (31%)	0.037
Nodal involvement	6 (32%)	8 (40%)		7/17 (41%)	5 (31%)	
Follow-up (mo, median)	8–182 (102)	28–440 (60)	0.173	26–200 (99.5)	8–255 (58)	0.65

Abbreviations: *mo*, months; *FIGO*, International Federation of Gynecology and Obstetrics

p-values for differences in means are two-tail for two sample *t*-test assuming unequal variances (alpha = 0.05). *p*-values for differences between categorical values represent chi-square tests of independence (alpha = 0.05)

[a]A total of 39 patients had implant status and follow-up information available, from which 19 had non-invasive implants and 20 had invasive/low-grade serous carcinoma implants

[b]A total of 34 patients had KRAS mutational data and follow-up information available, from which 16 had mutant KRAS tumors and 18 had wild-type KRAS tumors

[c]A total of 5 cases for which implant and follow-up data were available but KRAS mutational data was not available were comprised of 1 non-invasive implant and 5 invasive implants

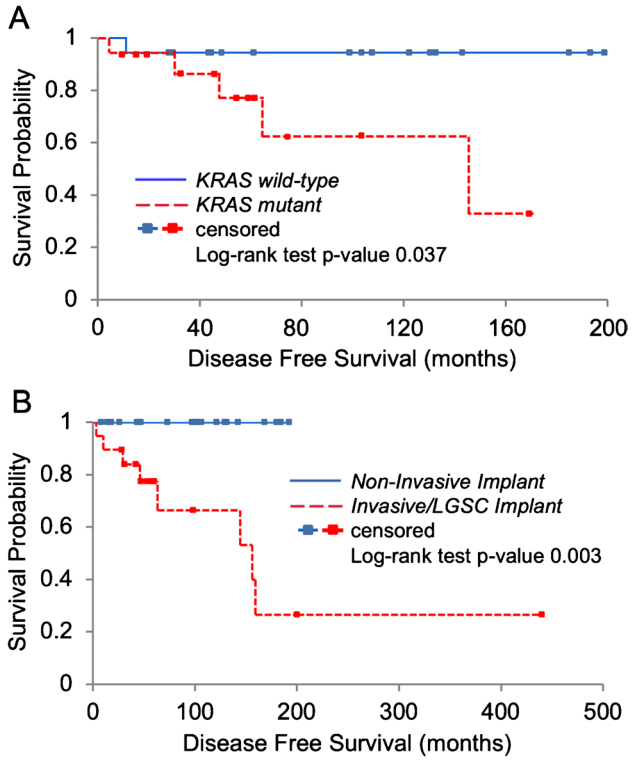

Fig. 4 **A** Disease-free survival represented graphically by Kaplan–Meier survival curve compared by the log-rank test (alpha = 0.05). **A** Patients with *KRAS* mutations in the primary tumor had a worse disease-free survival ($n = 34$, log-rank test, p-value = 0.037, hazard ratio 4.47) than those with wild-type *KRAS*. **B** Patients with invasive/LGSC implants had a worse disease-free survival ($n = 39$, log-rank test, p-value = 0.003; Mantel–Haenszel hazard ratio 8.49) than those with only non-invasive implants

of the extraovarian implants (Table 3). It is worth noting that a recent study of 215 cases of low-grade serous carcinoma concluded that the tumors with MAP kinase pathway gene mutations had an improved overall survival comparing with those without the mutations of these genes [30]. This parodoxical findings may be explained by the major difference in the study cohorts where the majority of the cases were conventional low-grade serous carcinoma (175 cases) and only 39 cases were associated with ovarian serous borderline tumor. Moreover, the observed survival advantage was based on the total number of mutations of all MAP kindase genes including KRAS, NRAS, BRAF, etc.), and therefore, it is unclear the prognostic impact of individual gene mutations in this study as mutations of different MAP kinase genes may have different biological impact. For example, BRAF V600E mutation appears to be associated with SBTs and is uncommon in low-grade serous carcinoma including SBT with invasive implants. Previous studies showed 23–48% of serous borderline tumors carrying the BRAF mutation while the rate dropped to 0–33% in low-grade serous carcinomas [31]. Regarding BRAF mutation status in implants of SBT, one study found that 14 of 63 (22%) noninvasive implants and none of 7 invasive implants had BRAF mutation [29] and a similar finding was found in our previous study as well [21]. Similarly in another study, none of 23 recurrent low-grade serous carcinomas had BRAF mutation but 5 of 13 noncurrent cases had the mutation [23]. Our current investigation focused only on SBTs in correlation with KRAS or BRAF mutations and conventational low-grade serous carcinoma was not included. While we found KRAS mutation is signficantly assoicatedd with a worse diseae recurrence free survial, future long-term follow up studies are needed to ascertain if the presence of KRAS mutation in serous borderline tumors may eventually impact the the patient overall survival.

KRAS mutation in the primary SBT is not significantly correlated with the histological subtypes of implants: KRAS mutations were present in 9/16 (56%) tumors with invasive implants/LGSC versus 7/18 (39%) tumors with non-invasive implants ($p = 0.311$). The finding of *KRAS* mutation status in non-invasive implants may explain why non-invasive implants also carry a significant risk for subsequent development of LGSC, though lower than invasive/LGSC implants [7, 12]. Consistent with existing data [7, 12], high-stage disease (IIIC) was seen in 70% (14/20) of patients with invasive/LGSC implants, in contrast to 16% (3/19) with non-invasive implants. When the histologic subtype of extraovarian implants was considered, tumor recurrence occurred in 8 of 20 patients with invasive/LGSC implants (40%), compared to 0 of 19 patients with only non-invasive implants. Consistently, patients with invasive/LGSC implants had a worse disease-free survival with 34% survival at 160 months compared to 100% survival at 160 months in those with non-invasive implants (log-rank test, $p = 0.003$; Mantel–Haenszel hazard ratio 8.49).

KRAS mutation in primary ovarian SBTs correlates significantly with the presence of peritoneal endosalpingiosis in this study, present in 63% (10/16) of cases with *KRAS* mutation versus 22% (4/18) of cases without *KRAS* mutation ($p = 0.017$). While the overwhelming majority of studies support a theory of clonality between SBTs and implants and progression of SBTs to LGSC [13], the possibility of alternative mechanisms of concomitant pathogenesis may also occur. It is possible that some implants may arise not from an ovarian primary tumor but from endosalpingiosis. The documented higher frequency of endosalpingiosis in SBTs associated with subsequent carcinoma relative to those without subsequent carcinoma suggests that endosalpingiosis may be a direct precursor of primary peritoneal LGSC [32]. Future studies are required to ascertain the biological relationship between *KRAS* mutation of the primary ovarian SBT and peritoneal endosalpingiosis with respect to tumor recurrence or progressive transformation.

BRAF V600E mutation was seen in only 5 primary ovarian SBTs with only non-invasive extraovarian implants in this current study. *BRAF* mutations have been shown to be less prevalent in LGSC than SBTs [33]. Similarly, *BRAF* mutation is rare in advanced-stage LGSC [34]. In SBTs, *BRAF* mutation is more common in low-stage tumors and is associated with improved prognosis and lower frequencies of tumor recurrence [22, 23]. It has been proposed that *BRAF* mutation may have a prohibitive effect on tumor recurrence related to cellular senescence [35].

In conclusion, *KRAS* mutation present in the primary ovarian serous borderline tumors is significantly associated with a greater risk of tumor recurrence and a shorter disease-free survival, independent of their high tumor stage at presentation and the histologic subtypes of extraovarian implant. Additional studies of larger cohorts with longer clinical follow up are important to solidify the value of *KRAS* mutation testing of primary ovarian serous borderline tumors as a prognostic biomarker.

Author contribution Dr. Austin McHenry conducted study case selection, data collection and analysis, and drafted the manuscript. Dr. Douglas Rottmann conducted initial study case selection and data collection. Dr. Natalia Buza conducted study data review and contributed to the writing of the manuscript. Dr. Pei Hui designed the study, conducted data review and analysis, and finilized the manucirpt.

Declarations

Ethics approval This study has been performed in compliance with the institutional ethical standards.

Conflict of interest The authors declare no competing interests.

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