***KRAS* mutation of extraovarian implants of serous borderline tumor: prognostic indicator for adverse clinical outcome**

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In contrast to non-invasive extraovarian implants, invasive implants of ovarian serous borderline tumor/atypical proliferative serous tumor are associated with adverse outcome and have been reclassified as low-grade serous carcinoma. Mutations of *KRAS* and/or *BRAF* have been reported in up to 50% of serous borderline tumor/atypical proliferative serous tumor. We investigated *KRAS* and *BRAF* mutation frequencies in the two types of implants of serous borderline tumor/atypical proliferative serous tumor in correlation with clinical outcome. Forty-two implants of serous borderline tumor from 39 patients were included (invasive implants/low-grade serous carcinoma, $n = 20$; non-invasive implants, $n = 22$). *KRAS* mutation was found in 12 of 20 invasive implants (60%) and 3 of 22 non-invasive implants (14%). *BRAF* V600E mutation was found in 1 of 22 non-invasive implants (5%) and none in invasive implants (0%). Invasive implants were more frequently associated with higher stage disease. Nine of 14 patients (64%) with *KRAS* mutation were found to have stage IIIC disease, while 5 of 24 patients (20%) without the mutation had stage IIIC disease. Patients with invasive implants had higher recurrence rate compared to those with non-invasive implants (60 vs 14 %, $P = 0.0003$, log-rank test) and worse disease-specific survival ($P = 0.0008$, log-rank test). Regardless of the histological subtypes, patients with *KRAS* mutation positive implants had significantly higher recurrence rate than those without the mutation (71 vs 21%, $P = 0.0021$, log-rank test) and an unfavorable disease-specific survival ($P = 0.0104$, log-rank test). In conclusion, compared to those with non-invasive implants, patients with invasive implants present with higher stage of the disease, higher recurrence rate and worse survival. *KRAS* mutation, but not *BRAF* V600E mutation, is significantly associated with invasive implants of serous borderline tumor. Regardless of the histological subtypes of the implants, *KRAS* mutation is a significant prognostic indicator for high risk of tumor recurrence and worse disease-specific survival.

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Extraovarian implants are found in 13–30% of serous borderline tumors/atypical proliferative serous tumors (SBT/APST).^{1–6} It is crucial to correctly categorize tumor implants to either invasive or non-invasive subtype, as the former is associated with adverse clinical outcome and has been reclassified as low-grade serous carcinoma (LGSC) by the 2014 WHO classification.³ Although the molecular mechanism underlying the progression of serous borderline tumor/atypical proliferative serous tumor and their implants remains unclear, many studies

have suggested the importance of abnormal activation of mitogen-activated protein kinase (MAPK) pathway, as two key components of the pathway, that is, *KRAS* and *BRAF*, are frequently mutated in serous borderline tumor/atypical proliferative serous tumor.^{1,7,8} Approximately 50% of serous borderline tumor/atypical proliferative serous tumor and low-grade serous carcinoma harbor *KRAS* and *BRAF* mutations, and the incidence of *BRAF* mutations is higher in serous borderline tumor/atypical proliferative serous tumor than that of low-grade serous carcinoma.^{4,7,8} In one study, up to 70% of low-grade serous carcinomas were found to have *KRAS* mutation while only small numbers of serous borderline tumor/atypical proliferative serous tumors had detectable mutated *KRAS*.⁹ Moreover, vast majority of peritoneal implants, non-invasive and invasive, were found to have identical *KRAS* or *BRAF* mutations to their corresponding primary ovarian

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serous borderline tumor/atypical proliferative serous tumors, supporting the hypothesis of clonal progression.¹⁰ However, additional investigations into the roles of *KRAS* and *BRAF* mutations in the pathogenesis of extraovarian tumor implants are needed to solidify the theory, and the prognostic values of these mutations have not yet been fully explored. In this investigation, we examined the presence of *KRAS* exon 2 and *BRAF* V600E mutations in comparable numbers of invasive and non-invasive implants of serous borderline tumor/atypical proliferative serous tumors in correlation with tumor stage and patient outcome.

Materials and methods

Study Case Selection and Histological Review

Forty-two extraovarian implants of serous borderline tumors in 39 patients were included in this study. All cases were retrieved from pathology archives at a single institution. The primary ovarian serous borderline tumors and their extraovarian lesions were histologically reviewed by all authors and reclassified according to the 2014 WHO criteria into 20 invasive implants/low-grade serous carcinoma and 22 non-invasive implants.^{2,3,6,11} Briefly, tumor implants were classified as invasive based one of the following: low-power destructive growth pattern, presence of micropapillary architecture and tumor cell nests surrounded by retraction artifact in dense fibrotic stroma (Figure 1). A tumor implant without the aforementioned morphologic qualifiers was considered non-invasive. 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.

DNA Extraction and Mutational Analysis

Formalin-fixed, paraffin-embedded blocks containing target lesional tissues were selected from each case. One hematoxylin and eosin-stained section and additional unstained sections were created on glass slides from the paraffin blocks. Areas of target tumor foci were confirmed to constitute at least 5% of tumor cells upon hematoxylin and eosin-stained slide review and corresponding tissue areas from the unstained slides were scraped with scalpel into microcentrifuge tubes. 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.¹²

KRAS and *BRAF* mutation analysis by the high sensitive single strand conformation polymorphism (SSCP) was performed according to previously described method.¹³ Briefly, 5–20 ng of extracted DNA was amplified using PCR primers flanking the hotspot mutations of exon 2 of the *KRAS* gene

(forward 5'-GACTGAATATAAACTTGTGG-3' and reverse 5'-CTGTATCAAAGAATGGTCCT-3') and *BRAF* V600E mutation (forward primer: 5'-CTCTT CATAATGCTTGCTCTGATAGG-3' and reverse primer: 5'-TAGTAACTCAGCAGCATCTCAGG-3'). The reaction was performed in a 50 µl solution containing 1x 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, and finished by a final extension at 72 °C for 10 min (ABI Veriti Thermal Cycler, Applied Biosystem, Foster City, CA, USA). The PCR product was analyzed by single strand conformation polymorphism (SSCP) using MDE non-denaturing gel. Electrophoresis was carried out on ice for 2 h and 45 min at 325 V. The SSCP gel was then stained with SYBR Gold (Molecular Probes, Invitrogen, Norwalk, CT, USA) 1:10 000 in TE buffer added for 20 min and imaged by 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 (Figure 2).

Statistical Analysis

Statistical analysis was performed according to the nature of the data, including Fisher exact test (2-tail), Student *t*-test, and Pearson X² test. The statistical significance was determined by setting level of *P* < 0.05. The overall survival was measured in months from the day of initial diagnosis to the date when the patient died of the tumor. The date of detection of tumor recurrence was used for measuring disease-free survival. The Kaplan–Meier statistic method was used to generate *P*-value using log-rank test.

Results

Clinicopathological Characteristics of the Study Cohorts

The overall patient demographic and clinicopathologic characteristics of the two study cohorts are presented in Table 1. Among 42 implants of serous borderline tumor/atypical proliferative serous tumor, 20 were classified as invasive and 22 were non-invasive, including one implant with indeterminate invasive morphology (Figure 1f, case 3). Three patients had two implants analyzed in this study that either developed concurrently or at tumor recurrence (cases 2, 3, and 4, Table 1). Overall 20 patients had invasive implants and 19 had non-invasive implants.

BRAF and *KRAS* Mutations Detected by SSCP

Using the PCR-SSCP detection methods, *BRAF* mutation analyses were informative in all cases and

Figure 1 Extraovarian tumor implants of SBT/APST. The tumor implants are classified as invasive based one of the following: low-power destructive growth pattern (a); the presence of micropapillary architecture (b) and tumor cell nests surrounded by retraction artifact in dense fibrotic stroma (c). Non-invasive implants demonstrate either peritoneal surface growth without destructive invasive and without aforementioned features of invasive implants (d,e). Note, the implants presented in c and f are from the same patient (case 3, Table 1). The initial tumor implant (f) displayed a well-circumscribed nodule involving the omental adipose tissue as indeterminate implant according to recent criteria.^{2,6}

Figure 2 SSCP detection of *KRAS* mutation in implants of SBT/APST. Samples 1, 2, 3, and 6 show wild-type *KRAS* (GGT), while samples 4 and 5 show mutant *KRAS*, GAT, and GTT, respectively. Samples 7–12 are *KRAS* wild-type control (GGT) followed by five common *KRAS* mutation controls (GTT, AGT, GAT, GCT, and TGT).

KRAS test was successful in all but one non-invasive implant sample (case 23, Table 1). As shown in Table 2, *KRAS* mutation was found in 12 of 20 invasive implants (60%) and 3 of 22 non-invasive implants (14%) ($P=0.0014$; X^2 test). *BRAF* V600E mutation was seen in 1 of 22 non-invasive implants (5%) and none of the 20 invasive implants (0%). PCR-SSCP demonstrated high specificity of calling at 6 genotypes of *KRAS* at codons 12 and 13, including wild-type GGT and 5 common *KRAS* mutations, that is, GTT, AGT, GAT, GCT, and TGT (Figure 2).¹⁴ Among the 15 implants with *KRAS* mutations, AGT was the most frequent mutation genotype (observed in 5 implants, 33%). Four implants (27%) carried GAT mutation, while GTT (20%) and GCT (20%) were each seen in three tumor implants. Only one

non-invasive implant showed detectable *BRAF* V600E mutation.

Invasive tumor implants were more often seen in older patients compared with non-invasive implants (41.0 vs 55.3, $P=0.0016$). A similar age distribution was found between patients with *KRAS* mutation (55.6 years) and those without the mutation (42.7 years, $P=0.0071$). In terms of tumor stage, both invasive tumor implants and the presence of *KRAS* mutation were associated with a higher stage disease (Table 3). However, the laterality of ovarian primary disease was not related to either the morphology or mutation status of implants.

Three patients (cases 2, 3, and 4, Table 1) had two implants analyzed, one invasive and one non-invasive. In case 2, *BRAF* mutation was seen in the non-invasive implant whereas a wild-type *KRAS* was seen in both the invasive and non-invasive implants. Case 4 had bilateral ovarian serous borderline tumors/atypical proliferative serous tumors with non-invasive implants on the uterine surface (Stage IIA) at the initial staging surgery, and 13 years later the patient developed recurrent disease with both invasive and non-invasive implants, of which *KRAS* mutation was found only in the invasive implant but not in the non-invasive implant. Case 3 presented initially with an omental implant consisting of a well-circumscribed nodular proliferation of papillary tumor nests without destructive growth (Figure 1f), which may be considered as non-invasive by earlier criteria,³ or indeterminate category by the most recent classification.^{2,6} However,

Table 1 Overall clinicopathologic features and mutation status of study cases ($n = 39$)

Pt#	Age	Laterality	Stage	Implant	KRAS	BRAF	Surgery	Follow-up (mo)	Recurrence	Status
1	28	R	IIIC	Non-invasive	wt	wt	RSO/Tu resec	168	No	NED
2 ^a	58	Bi	IIIB	Invasive	wt	wt	TAH/BSO staging	144	No	NED
				Non-invasive	wt	mut				
3 ^b	52	Bi	IIIC	Invasive	mut (GTT)	wt	TAH/BSO staging	151	Yes	DOD
				Indeterminate ^b	mut (GTT)	wt				
4 ^c	39	Bi	IIA	Non-invasive	wt	wt	TAH/BSO staging	186	Yes	DOD
				Invasive	mut (GCT)	wt				
5	55	Bi	IIIA	Invasive	wt	wt	TAH/BSO staging	24	Yes	DOD
6 ^d	48	n/a	IIIA	Non-invasive	wt	wt	Tumor excision	48	No	NED
7	66	Bi	IIIA	Invasive	mut (GCT)	wt	TAH/BSO staging	10	No	NED
8 ^e	38	Bi	IIIB	Invasive	mut (AGT)	wt	TAH/BSO staging	60	No	NED
9 ^e	47	Bi	IIIC	Invasive	wt	wt	TAH/BSO staging	58	Yes	DOD
10	46	Bi	IIIC	Invasive	wt	wt	TAH/BSO staging	24	No	NED
11	41	Bi	IIIB	Invasive	wt	wt	TAH/BSO staging	60	No	NED
12 ^e	55	Bi	IIIC	Invasive	mut (AGT)	wt	TAH/BSO staging	136	Yes	AWD
13	58	Bi	IIIC	Invasive	mut (GTT)	wt	TAH/BSO staging	52	Yes	DOD
14	75	Bi	IIIC	Invasive	mut (AGT)	wt	TAH/BSO staging	24	Yes	DOD
15	26	R	IIIC	Non-invasive	wt	wt	TAH/BSO staging	52	No	NED
16	51	Bi	IIIC	Invasive	mut (AGT)	wt	TAH/BSO staging	54	Yes	DOD
17 ^d	72	n/a	IIIC	Invasive	mut (GAT)	wt	Tumore debulking	96	Yes	DOD
18	43	R	IIA	Invasive	mut (GCT)	wt	TAH/BSO	205	Yes	AWD
19	68	Bi	IIIC	Invasive	wt	wt	TAH/BSO staging	42	Yes	DOD
20	77	Bi	IIIA	Non-invasive	wt	wt	TAH/BSO staging	48	No	NED
21	34	Bi	IIIB	Non-invasive	wt	wt	TAH/BSO staging	132	No	NED
22	41	Bi	IIIB	Non-invasive	wt	wt	TAH/BSO staging	168	No	NED
23	65	Bi	IIIB	Non-invasive	failed	wt	TAH/BSO staging	184	No	DUC
24 ^f	34	Bi	IIIA	Non-invasive	wt	wt	TAH/BSO staging	216	Yes	AWD
25	61	Bi	IIIC	Invasive	mut (GAT)	wt	TAH/BSO staging	5	No	NED
26	23	Bi	IIIB	Non-invasive	wt	wt	BSO	336	No	NED
27 ^e	47	Bi	IIIC	Non-invasive	mut (GAT)	wt	TAH/BSO staging	133	Yes	DOD
28 ^d	60	n/a	IIIC	Invasive	mut (AGT)	wt	Tumor debulking	180	Yes	DOD
29	37	Bi	IIIB	Non-invasive	wt	wt	TAH/BSO staging	73	No	NED
30	26	R	IIIA	Non-invasive	wt	wt	RSO	60	Yes	DUC
31	30	L	IIIB	Non-invasive	wt	wt	LSO	94	No	NED
32	55	Bi	IIIB	Non-invasive	wt	wt	TAH/BSO staging	80	No	NED
33	67	L	IIIB	Invasive	wt	wt	TAH/BSO	48	No	NED
34	65	Bi	IIIB	Non-invasive	mut (GAT)	wt	TAH/BSO staging	52	No	NED
35	54	Bi	IIIB	Invasive	wt	wt	TAH/BSO staging	48	No	NED
36	15	Bi	IIIA	Non-invasive	wt	wt	TAH/BSO staging	108	No	NED
37	31	Bi	IIIA	Non-invasive	wt	wt	TAH/BSO staging	252	No	NED
38	37	L	IIIA	Non-invasive	wt	wt	TAH/BSO staging	57	No	NED
39	35	Bi	IIIA	Non-invasive	wt	wt	TAH/BSO	384	No	NED

Abbreviations: AWD, alive with disease; Bi, bilateral; DOD, dead of disease; DUC, dead of unrelated cause; L, left; LSO, left salpingo-oophorectomy; mo, month; mut, mutant; n/a, unknown; NED, no evidence of disease; Pt, patient; R, right; RSO, right salpingo-oophorectomy; TAH/BSO, total abdominal hysterectomy/bilateral salpingo-oophorectomy; wt, wild type.

^aCase 2 had invasive implants at initial staging surgery and after 60 days, developed small bowel obstruction with non-invasive implant on the surface of resected small bowel.

^bCase 3 had an indeterminate implant (Figure 1f) and the patient had recurrence with invasive implant (Figure 1c) 13 years later.

^cCase 4 had non-invasive implants on the uterine surface (Stage IIA) at the initial staging surgery, and later the patient developed recurrent disease with both invasive and non-invasive implants.

^dCases 6, 17, and 28 had prior surgical history of TAH/BSO for unknown primary tumors, and the laterality of primary ovarian disease can not be determined.

^eCases #8, 9, 12, and 27 had micropapillary morphological features in either primary ovarian tumors or extraovarian implants.

^fCases #24 had KRAS (G12V) in primary ovarian tumor detected by next-generation sequencing assay.

Table 2 Histological subtypes of implants and KRAS and BRAF mutation status ($n = 42$)

Implant type	Total	KRAS mutation n(%)	BRAF mutation n(%)	Wild type n(%)	P-value
Non-invasive ^a	22	3 (14%)	1 (5%)	17 (77%)	0.0014
Invasive	20	12 (60%)	0 (0%)	8 (40%)	

KRAS mutation analysis failed in one non-invasive tumor implant.

^aIncluding one implant of indeterminate type (case 3, Table 1).

Table 3 Statistical analyses of the patient cohorts (N=39)

	Non-invasive (n = 19)	Invasive (n = 20)	P-value	KRAS mutant ^a (n = 14)	Wild type (n = 24)	P-value
Age (mean)	41.0	55.3	0.0016	55.6	42.7	0.0071
FIGO stage						
IIA	0 (0%)	2 (10%)	0.0207	2 (14%)	0 (0%)	0.0259
IIB	4 (21%)	1 (5%)		1 (7%)	4 (17%)	
IIIA	8 (42%)	2 (10%)	> 0.05	1 (7%)	9 (38%)	> 0.05
IIIB ^b	4 (21%)	4 (20%)		1 (7%)	6 (25%)	
IIIC	3 (16%)	11 (55%)		9 (64%)	5 (20%)	
Bilateral ovarian disease	13 (68%)	16 (80%)	> 0.05	11 (79%)	17 (71%)	> 0.05
Recurrence	3 (14%)	12 (60%)	0.0003	10 (71%)	5 (21%)	0.0021
Follow-up months (median)	48–384 (132)	5–186 (56)	0.0008	5–186 (75)	24–384 (87)	0.0104

^a The same *KRAS* mutation was detected in two implants in one patient (Case 3, Table 1). ^b *KRAS* mutation analysis failed in one IIIB stage case with non-invasive tumor implant. (Case 23, Table 1).

the patient developed recurrence 13 years later with unequivocal invasive implants (Figure 1c). Nonetheless, identical GTT mutation at codon 12 of *KRAS* was seen in both the initial omental implant and the invasive implant at recurrence (Table 1).

Evaluation of Prognostic Value of Mutation Status of Tumor Implants

Overall, the study cohort had a median follow-up of 87 months with intervals ranging between 5 to 384 months (Table 3). Twenty patients with invasive implants had a significantly higher recurrence rate (12/20, 60%) compared with those with non-invasive implants (3 of 22, 14 %) ($P=0.0003$, log-rank test) (Figure 3a). Independent of implant histological subtypes, patients carrying *KRAS* mutation in their implants had a significantly higher recurrence rate than those without the mutation (71 vs 21 %, $P=0.0021$, log-rank test) (Figure 3b). Among 5 recurrent cases without detectable *KRAS* mutation in the extraovarian implant, one of cases (Table 1) was found to carry *KRAS* (G12V) in primary ovarian tumor by next-generation sequencing assay at a commercial laboratory, and in another case the primary ovarian tumor had micropapillary architecture and stage IIIC ovarian borderline tumor.

As expected, patients with invasive implants had a worse disease-specific survival than those with non-invasive implants ($P=0.0008$, log-rank test; Figure 4a). Regardless of the histological types of implants, the presence of *KRAS* mutation was also associated with an unfavorable disease-specific survival ($P=0.0104$, log-rank test; Figure 4b). Two patients had GTT (G12V) mutation and both died of the disease. Three of the five patients with AGT (G12S) mutation also died of their disease.

Discussion

One of most challenging diagnostic issues of serous borderline tumors/atypical proliferative serous tumors is the histological classification of the

Figure 3 Disease-free survival analyses. (a) Patients with invasive implants (gray) had a higher recurrence rate compared with those with non-invasive implants (black). (b) Independent of implant subtypes, patients with implants harboring *KRAS* mutations (gray) had a significantly higher recurrence rate than those without *KRAS* mutation (black).

extraovarian tumor involvement into invasive or non-invasive implants. Precise classification of implants is crucial for patient management and prognostication. In contrast to non-invasive implants, invasive implants of serous borderline tumors/atypical proliferative serous tumors are associated with adverse outcome and are currently reclassified as low-grade serous carcinoma by the 2014 WHO classification.³ However, diagnostic separation of extraovarian disease of serous borderline tumors/atypical proliferative serous tumors into invasive and non-invasive implants can be difficult solely based on histological features.^{11,15,16} Recently investigations of large cohorts of serous borderline

Figure 4 Disease-specific survival analyses. (a) Patients with invasive implants (gray) had a worse overall survival compared with those with non-invasive implants (black). (b) Independent of implant subtypes, patients with implants harboring *KRAS* mutations (gray) had an unfavorable overall survival compared with those without *KRAS* mutation (black).

tumors/atypical proliferative serous tumors with extraovarian involvement found that among various histological parameters, destructive invasion of underlying normal tissue, micropapillary architecture, and solid epithelial nests surrounded by clefts should be classified as 'invasive,' whereas those lacking all three features were classified as 'non-invasive.'^{2,11} The new WHO classification formally advocates using these histological criteria for classifying such extraovarian lesions of serous borderline tumors/atypical proliferative serous tumors as invasive implants/low-grade serous carcinoma.^{2,3,11} Accordingly, the pattern of destructive growth was considered the most important morphologic feature of invasive implant, and micropapillary architecture and tumor cell nests surrounded by retraction artifact in dense fibrotic stroma were used as the next critical criteria for classifying invasive implants in our study. Corroborating the existing data, our data confirm that invasive implants are more frequently associated with high stage disease (stage IIIC) and higher recurrence rates, and have less favorable overall survival compared with non-invasive implants.

The origin of tumor implants remains enigmatic: independently developed process vs metastatic or clonally derived lesions from the primary tumor. Recent molecular genetic studies have suggested that tumor implants represent clonal extension/spread from the primary ovarian serous borderline tumors/

atypical proliferative serous tumors, as the tumor cells from both sites share the same genetic alterations such as *BRAF* and *KRAS* mutations.^{4,9,10,17} Approximately 50% of serous borderline tumors/atypical proliferative serous tumors and low-grade serous carcinoma harbor *KRAS* and *BRAF* mutations, and the incidence of *BRAF* mutations is higher in serous borderline tumors/atypical proliferative serous tumors than that of low-grade serous carcinoma.^{4,7,8} Although it is not clear if extraovarian tumor implants were subjected to mutational analysis in every investigation, various studies have shown similar *KRAS* mutations rates ranging from 57 to 78% in low-grade serous carcinoma or advanced stage serous borderline tumors/atypical proliferative serous tumors with invasive implants.^{4,9,10} In our study, *KRAS* and *BRAF* mutation status were simultaneously analyzed in comparable cohorts of two subtypes of extraovarian tumor implants. Consistent with the literature, *KRAS* mutation is significantly more associated with invasive implants than non-invasive ones (60 vs 14%). Of the two patients (cases 2 and 4, Table 1) who each had two implants undergoing mutation analysis (one invasive and one non-invasive), *KRAS* mutation was seen only in the invasive implant but not in the non-invasive implant in one patient; while the other patient had *BRAF* mutation present only in the non-invasive implant but wild-type *KRAS* gene in both implants. One patient (case 3, Table 1) presented initially with a nodular, well-circumscribed implant involving omental adipose tissue (Figure 1f) without destructive growth. While interpreted as non-invasive implant according to the original pathology report, the lesion was reclassified in the current study as indeterminate implant according to recent publications.^{2,6} Interestingly, the patient developed unequivocal invasive implants 13 years later (Figure 1c) and identical *KRAS* mutation was detected in both the original omental implant and invasive implants at recurrence, consistent with clonal tumor progression.

Interestingly, one recent long-term population-based study demonstrated that even non-invasive implants carry the statistically significant risk for subsequent development of carcinoma.¹⁸ Non-invasive implants in our study had a low frequency (14%) of *KRAS* mutation rate. While a similar low rate of *KRAS* mutation was observed in a prior study (23%),⁸ one recent study found that 28 of 55 non-invasive implants (51%) had *KRAS* mutation,¹⁰ which is even higher than the previously reported mutation rates (17–39.5%) in serous borderline tumors/atypical proliferative serous tumors and falls into the range of low-grade serous carcinoma (19–54.5%).⁴ It is worth noting that among 55 patients examined in that study,¹⁰ 5 patients had foci of low-grade serous carcinoma at the primary tumor sites and 4 patients also had concurrent invasive implants. It is possible that different inclusion

criteria may have contributed to a high *KRAS* mutation rate in the study.¹⁰

Our study primarily focused on extraovarian implants of serous borderline tumors/atypical proliferative serous tumors, therefore, majority of patients in our study had high stage disease. Among the 42 extraovarian tumor implants, 14 implants were associated with stage IIIC tumor (Table 3). 9 of 14 patients (64%) with *KRAS* mutation detectable in their tumor implants had stage IIIC disease, while only 3 patients with stage IIA and IIB had *KRAS* mutation. Independent of the histological subtypes of implants, *KRAS* mutation is a significant prognostic indicator for tumor recurrence as the patients carrying *KRAS* mutation had a significantly higher recurrence rate than those without the mutation (71 vs 21%, $P=0.0021$, log-rank test). A worse disease-specific survival was also observed for patients with *KRAS* mutation positive implants compared with those without the mutation ($P=0.0104$, log-rank test) regardless of the histological subtype of implants. To our knowledge, this is the first report that *KRAS* mutation is found statistically associated with worse disease-free and disease-specific survival for patients of serous borderline tumors/atypical proliferative serous tumors with extraovarian tumor implants. Our data strongly indicate that *KRAS* mutation analysis may be a clinically useful marker for prediction of recurrence and survival of patients with extraovarian tumor implants of serous borderline tumor.

In several previous studies, GTT (G12V), GAT (G12D), and GCT (G12A) were found as common *KRAS* mutation variants in serous borderline tumors/atypical proliferative serous tumors and low-grade serous carcinoma.^{9,14} One recent study indicates that patients carrying *KRAS* G12V mutation (GTT mutation) have a significantly shorter overall survival than those with *KRAS* G12D mutation in recurrent low-grade serous carcinomas.⁹ *KRAS* G12V mutation has also been associated with unfavorable survival in colorectal and lung cancers.^{19,20} Consistently, the two patients with G12V mutation in the current study died of the disease. However, G12S (AGT mutation) instead of G12D was the most frequent mutation in extraovarian tumor implants in our study. Three of five patients carrying G12S also died of the disease, suggesting unfavorable impact of G12S mutation on patient survival. Biologically, an amino acid change from the wild-type glycine to serine residue—as a result of G12S mutation—is potentially relevant to the tumor biology as serine is one of the major biological phosphorylation sites in oncogenes, such as *KRAS*. Further investigations into larger cohorts of patients are needed to ascertain the biological implication of various *KRAS* mutations.

Previous data have suggested that *BRAF* mutation is primarily found in serous borderline tumors/atypical proliferative serous tumors without extraovarian involvement and in non-invasive implants.

BRAF mutation is uncommon in invasive implants and low-grade serous carcinoma. Consistently, in the current study, *BRAF* V600E mutation was seen only in 1 of 22 non-invasive implants (5%) and none of the 20 invasive implants harbored *BRAF* V600E mutation (0%). Previous studies showed 23 to 48% of serous borderline tumors/atypical proliferative serous tumors carrying the *BRAF* mutation while the rate dropped to 0 to 33% in low-grade serous carcinomas.⁴ Regarding *BRAF* mutation status in implants, one study found that 14 of 63 (22%) non-invasive implants and none of 7 invasive implants had *BRAF* mutation by either PCR-Sanger sequencing or immunohistochemistry analysis.¹⁰ Similarly, none of 23 recurrent low-grade serous carcinoma had *BRAF* mutation but 5 of 13 noncurrent cases had the mutation in another study.⁹

In conclusion, patients with invasive implants of serous borderline tumors/atypical proliferative serous tumors more frequently present at a higher disease stage, and incur a higher recurrence rate and a worse overall survival compared with those with non-invasive implants. *KRAS* mutation, but not *BRAF* V600E mutation, is significantly associated with the presence of invasive implants of serous borderline tumors/atypical proliferative serous tumors. Regardless of the histological subtypes of the implants, *KRAS* mutation is a significant prognostic indicator for high risk of tumor recurrence and worse disease-specific survival. Future studies of large patient cohorts are important to establish the clinical validity of *KRAS* mutation testing for diagnostic and prognostic applications in patients with ovarian serous borderline tumor/atypical proliferative serous tumors.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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