BRIEF COMMUNICATION

Mutations in BRAF and KRAS Characterize the Development of Low-Grade Ovarian Serous Carcinoma

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Activating mutations in KRAS and in one of its downstream mediators, BRAF, have been identified in a variety of human cancers. To determine the role of mutations in BRAF and KRAS in ovarian carcinoma, we analyzed both genes for three common mutations (at codon 599 of BRAF and codons 12 and 13 of KRAS). Mutations in either codon 599 of BRAF or codons 12 and 13 of KRAS occurred in 15 of 22 (68%) invasive micropapillary serous carcinomas (MPSCs; low-grade tumors) and in 31 of 51 (61%) serous borderline tumors (precursor lesions to invasive MPSCs). None of the tumors contained a mutation in both BRAF and KRAS. In contrast, none of the 72 conventional aggressive high-grade serous carcinomas analyzed contained the BRAF codon 599 mutation or either of the two KRAS mutations. The apparent restriction of these BRAF and KRAS mutations to low-grade serous ovarian carcinoma and its precursors suggests that low-grade and high-grade ovarian serous carcinomas develop through independent pathways. [J Natl Cancer Inst 2003; 95:484–6]

The kinase cascade involving RAS, RAF, mitogen/extracellular signal-regulated kinase (MEK), extracellular signal-regulated kinase (ERK), and mitogen-activated protein kinase (MAPK) mediates the transmission of growth signals into the nucleus *(1).* One of the three RAF members, BRAF, has been recently reported to be activated by somatic mutation in many human cancers, with mutations in BRAF occurring at a particularly high rate in cutaneous melanoma and papillary carcinoma of the thyroid *(2,3).* All known BRAF mutations occur within the kinase domain, with a single substitution of A for the T at nucleotide position 1796 (1796T/A) accounting for at least 80% of BRAF mutations *(2,4,5).* This mutation converts a valine residue at amino acid position 599 to a glutamic acid (V599E); the mutant protein has elevated kinase activity and is able to transform NIH3T3 cells independent of RAS function *(2).* Similarly, activating mutations in codons 12 and 13 of KRAS occur frequently in carcinomas and result in constitutive activation of KRAS that contributes to tumorigenesis *(1).*

To investigate the role of BRAF and KRAS mutations in ovarian carcinoma, we analyzed different types of ovarian carcinomas for three common mutations in these genes—the BRAF mutation at codon 599 and the KRAS mutations at codons 12 and 13. Ovarian carcinoma, one of the major cancer types and the most lethal gynecologic malignancy, comprises a heterogeneous group of tumors with distinctly different histologic types, molecular features, and clinical behavior *(6–8).* The most common type of ovarian cancer is serous carcinoma which, in our previous study *(9)*, we further divided into high-grade conventional serous carcinoma and a low-grade tumor, invasive micropapillary serous carcinoma (MPSC). All serous carcinomas are believed to develop from ovarian surface epithelium or inclusion cysts *(10).* In contrast to conventional serous carcinoma, for which morphologically recognizable precursor lesions have not been identified, invasive MPSC develops in a stepwise fashion from a noninvasive group of neoplasms termed serous borderline tumors. Based on our extensive morphologic and molecular studies, serous borderline tumors include a benign precursor (atypical proliferative serous tumor) and a noninvasive carcinoma designated noninvasive MPSC *(9,11–14).* The non-serous types of ovarian carcinoma include endometrioid carcinoma and clear-cell carcinoma, which are less common than serous carcinoma and appear to develop from endometriosis *(15).*

Formalin-fixed, paraffin-embedded tissue samples of 182 ovarian tumor tissues were obtained from the surgical pathology file of the Johns Hopkins Hospital. Genomic DNA was purified from the microdissected tumor component, as previously described *(9).* The ovarian tumors (51 serous borderline tumors, 21 invasive MPSCs, 69 conventional serous carcinomas, 21 endometrioid carcinomas, and 20 clear-cell carcinomas), three conventional serous carcinoma cell lines (SKOV-3, OVCAR-3 and HTB-75), and one primary culture of an invasive MPSC were analyzed for the codon 599 mutation in BRAF and the codon 12 and 13 mutations in KRAS. Five normal ovarian tissues and 10 serous cystadenomas were also included in the mutation analysis. The KRAS mutation status of some of the tumor samples (22 of the serous borderline tumors, 15 of the invasive MPSCs, and 20 of the conventional serous carcinomas) has already been reported *(9).* Waiver of patients' consent was approved by the local Institutional Review Board. All the cases were reviewed by three gynecologic pathologists (R. J. Kurman, G. Singer, and I.-M. Shih), who concurred with the diagnoses before microdissection.

Analysis of the 1796T/A status in BRAF was performed using a polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) technique *(3).* For this method, the BRAF PCR product of exon 15, which contains nucleotide position 1796, was digested with TspR1 (New England Biolabs, Inc., Beverly, MA) at 65 °C for 3 hours. The PCR products were electrophoresed on a 10% polyacrylamide gel and were also sequenced to validate the RFLP results. As shown in Fig. 1, BRAF mutations were found

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Journal of the National Cancer Institute, Vol. 95, No. 6,

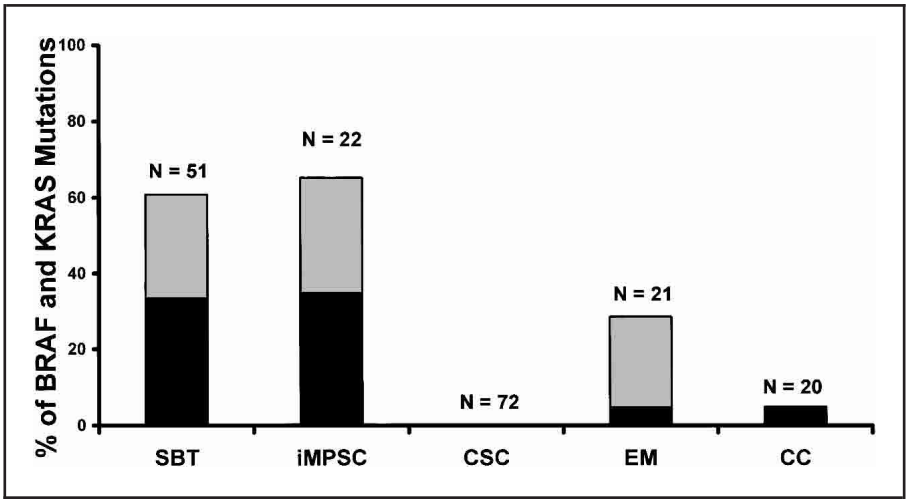

Fig. 1. Mutations of BRAF and KRAS in ovarian carcinomas. Mutational analysis of codon 599 of BRAF (**gray bars**) and codons 12 and 13 of KRAS (**black bars**) was performed in several types of ovarian neoplasm, including serous borderline tumors (SBT), invasive micropapillary serous carcinomas (iMPSC, low-grade carcinomas), conventional high-grade serous carcinomas (CSC), endometrioid carcinomas (EM), and clear-cell carcinomas (CC). The number of tumors of each type that were analyzed is indicated. None of the tumors showed both BRAF and KRAS mutations. iMPSCs and their precursor lesions, serous borderline tumors, demonstrate the highest frequency of mutations in BRAF and KRAS.

in 33% of the invasive MPSCs (including the primary culture of an invasive MPSC) and in 28% of their precursor lesions, serous borderline tumors. The BRAF mutation was not detected in the histologically normal-appearing cyst epithelium adjacent to a borderline tumor that contained the BRAF mutation (data not shown), indicating that BRAF mutations occur during progression of serous borderline tumors.

KRAS mutational status at codon 12 or 13 was analyzed either by digital PCR *(9,16,17)* or direct sequencing. In combination with our previous results *(9)*, KRAS mutations were found in 35% of invasive MPSCs and 33% of serous borderline tumors. None of the tumors contained a mutation in both BRAF and KRAS; thus, considering the two genes together, a mutation in one of them was found in 68% of invasive MPSCs and in 61% of serous borderline tumors. There was no correlation between the presence of the BRAF or KRAS mutations and patient age, clinical stage, tumor size, and mismatch repair deficiency status (two-sided Spearman's rank-order correlation) (data not shown). In contrast to invasive MPSCs and their precursors, all 69 specimens of clinically aggressive conventional serous carcinomas, as well as three well-established cell lines, contained wild-type BRAF and KRAS sequences at the analyzed sites in both genes. As controls, all five normal ovarian tissues and all 10 serous cystadenomas analyzed contained wild-type BRAF and KRAS.

The mutually exclusive nature of BRAF at codon 599 and KRAS mutations at codons 12 and 13 in ovarian carcinoma is consistent with a similar finding in melanoma and colorectal carcinoma and lends strong support for the view that BRAF and KRAS mutations have equivalent effects on tumorigenesis *(2,5)*. Although the possibility that other members of the RAF family or downstream targets of RAF are mutated in conventional high-grade serous carcinomas must be investigated, it would appear that the development of high-grade conventional serous carcinomas involves a pathway distinct from the RAS signaling pathway. For example, mutations in TP53 are common in high-grade ovarian serous carcinomas *(18)*.

We also analyzed codon 599 of BRAF and codons 12 and 13 of KRAS in less common, non-serous types of ovarian cancer, including endometrioid and clear-cell carcinomas. We did not include mucinous carcinomas involving the ovary because we had previously found that most such carcinomas are metastases from other primary sites *(19,20)*. We detected BRAF mutations in 24% of endometrioid carcinomas but in none of the clear-cell carcinomas. No other gene has such a high mutation rate in ovarian endometrioid carcinomas, except PTEN, which is mutated in 20% of ovarian endometrioid carcinomas *(21)*. Only one clear-cell carcinoma and one endometrioid carcinoma had a KRAS mutation. This finding is similar to that in a previous report, which also analyzed KRAS in a small number of cases *(22)*. Again, among the tumors we analyzed, the BRAF mutation and KRAS mutations were never both present in the same tumor.

Our results demonstrate that the mutational status of BRAF and KRAS is distinctly different among various histologic types of ovarian serous carcinoma, occurring most frequently in invasive MPSC, a clinically indolent neoplasm, and its precursors, serous borderline tumors. Thus, it appears that different histologic types of ovarian carcinomas have distinctive molecular pathways in tumor development. In addition, our analysis has extended a previous finding of BRAF mutations in four of 10 "low malignant potential" and one of 25 "malignant epithelial" ovarian neoplasms *(2)*. Our results also have potential implications for the treatment of invasive MPSC; such lesions, unlike conventional high-grade serous carcinomas, generally do not respond well to conventional chemotherapy. Conceivably, blocking KRAS–BRAF signaling may provide more effective therapy *(2)*.

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NOTES

G. Singer, R. Oldt III, and Y. Cohen contributed equally to this work.

Supported by research grant OC010017 from the Department of Defense; Public Health Service grant CA97527 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services; and the Swiss National Science Foundation (GS).

Manuscript received September 23, 2002; revised January 7, 2003; accepted January 15, 2003.