Low Frequency of **BRAF** Mutations in Endometrial and in Cervical Carcinomas

**To the Editor:** **BRAF**, which encodes a RAF family member that functions downstream of RAS, has been reported to be somatically mutated in a number of human cancers, most frequently at codon 599 (1). Activating mutations of **BRAF** have been frequently observed in colorectal carcinomas with microsatellite instability (MSI; ref. 2). In addition, mutations of **BRAF** and **KRAS** have been reported as being mutually exclusive in colorectal carcinomas (2). Mutations in **BRAF** have been studied in gynecological cancer. They are common in low-grade ovarian serous carcinoma, in which they provide an alternative route for activation of the RAS signaling pathway (3). In contrast, **BRAF** mutations have not been found in either other histological types of ovarian carcinomas or in cervical carcinomas (4).

In the September 2005 issue of *Clinical Cancer Research*, Feng et al. reported a 21% incidence of **BRAF** mutations among 97 endometrial, 78 endometrioid (EEC), and 19 nonendometrioid carcinomas (5). In addition, one out of nine (11%) atypical endometrial hyperplasias also featured a **BRAF** mutation, most of which were located at previously unreported sites. In this series, there were no apparent differences in the prevalence of **BRAF** mutations among different stages, histological subtypes, and grades. The authors found 2 mutations in nonendometrioid carcinomas (11%) and 18 mutations in EECs (23%). Interestingly, **BRAF** mutations were more frequently found among tumors that negatively stained for hMLH1 (12 out of 32; 41%), suggesting an association between these **BRAF** mutations and MSI.

These findings are in clear contradiction with those of three recent series exhibiting a low frequency of **BRAF** mutations in EEC. Thus, Mutch et al. (6) reported a single mutation among 146 EECs, which were also evaluated for **KRAS** mutations and MSI. This mutation occurred in 1 of the 81 MSI-positive cases. Salvesen et al. (7) studied **BRAF** mutations in 48 endometrial carcinomas and found one mutation in an MSI-negative EEC. In addition, Pappa et al. (4) found no mutations among the 67 endometrial carcinomas they analyzed.

We have determined the incidence of **BRAF** mutations in exons 11 and 15 by PCR/single-strand conformational polymorphism and sequencing in a series of endometrial lesions previously characterized for **KRAS**, **PTEN**, and **β-catenin** mutations and MSI status (8). **BRAF** mutations were found in 1 of 19 atypical endometrial hyperplasias (5.6%) at codon 598 (T598I) as well as in 2 out of 94 ECs (2.1%) at codons 470 (S470F) and 614 (G614E), respectively. We also analyzed a series of 61 cervical carcinomas (19 squamous cell carcinomas and 42 adenocarcinomas) but did not find any **BRAF** mutations in the exons studied.

With respect to cervical cancer, our results are in accordance with those reported by Pappa et al., who did not find any **BRAF** mutations among 47 cases, including squamous cell carcinomas and adenocarcinomas. Concerning endometrial carcinomas, our data are in close agreement with those of Pappa et al. (4), Mutch et al. (6) and Salvesen et al. (7), which indicate a low prevalence of **BRAF** mutation in these tumors. Differences between these series and that reported by Feng et al. (5) are difficult to explain because all of them featured an analysis of exons 11 and 15. One possible explanation may be the different ethnicity of the populations studied, whereby **BRAF** seems to have a more important role in Chinese patients. This is improbable, however, because the frequency of other molecular alterations, such as **RAS** and MSI, was very similar in all studies.

In conclusion, most of the series analyzed thus far have shown a low prevalence of **BRAF** mutations in endometrial carcinomas, and an absence of association with other frequent molecular alterations, such as **KRAS**, **PTEN**, and MSI.

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In response: We thank Moreno-Bueno et al. for the letter in response to our recent article in *Clinical Cancer Research* (1). With regard to the differences in the incidence of **BRAF** mutations compared with other reports, we consider the following reasons:

1. Single-Strand Conformational Polymorphism Analysis

Single-strand conformational polymorphism was used in two (2, 3) of three previous articles cited (2–4) and in the report by Moreno-Bueno et al. Although single-stand
conformational polymorphism has been used widely for the screening of gene mutations, its sensitivity and efficiency are not completely satisfactory. There are many factors that influence the efficiency of single-strand conformational polymorphism, including the nucleotide sequence, PCR product size, percentage of polyacrylamide, etc. (5). Several studies have reported that the sensitivity of single-strand conformational polymorphism for detecting mutation is 50% to 60% (6–8). Therefore, the frequency of BRAF mutations in studies using single-strand conformational polymorphism is likely to be lower than those using direct sequencing for all cases.

2. Method of DNA Extraction

Microdissection of cells is of particular importance when analyzing genetic differences in tumorous compared with normal tissue. Failure to separate normal and tumor cell types can result in masking or false results. For example, use of the PCR to identify a loss of heterozygosity or mutation in tumor cell DNA may be missed without the use of microdissection as DNA from the normal cell population may greatly dilute DNA from the tumor cells (9). We have done the microdissection technique using paraffin-embedded tissue. However, in Mutch et al. (2) and in some cases in Salvesen et al. (3), DNA was extracted from frozen tissue of which the histologic diagnosis may have been less accurate. In Pappa et al. (4), the extraction method was not described in detail. Therefore, we think that differences in the DNA extraction method may have affected the sensitivity of mutation detection.

3. Exons Examined for BRAF Mutation

Most BRAF mutations have been reported in exons 11 and 15. In a previous article, mutations in only exon 15 were examined (4). This may have resulted in the low detection rate observed.

4. Distribution of the Patients

We also examined the K-ras/p53 mutation in our study, and the K-ras and p53 mutation ratio was 19% and 23%, being consistent with previous reports. In addition, we have confirmed the absence of these mutations in normal endometria adjacent to affected carcinomas. Collectively, we think that the design of the present study and the results obtained are reasonable. As Moreno-Bueno et al. have pointed out, however, we do not deny the possibility of a chance accumulation of patients with BRAF mutations, possibly due to ethnicity.

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