BRAF and KRAS Mutations in Colorectal Hyperplastic Polyps and Serrated Adenomas

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ABSTRACT

Colorectal cancer is believed to progress through an adenoma–carcinoma sequence. However, recent evidence increasingly supports the existence of an alternative route for colorectal carcinogenesis through serrated polyps, a group that encompasses a morphological spectrum, including hyperplastic polyp (HP), admixed hyperplastic polyp/adenoma (HP/AD), and serrated adenoma (SA; the latter two manifest epithelial dysplasia). We have studied a large series of serrated polyps for BRAF and KRAS mutations. BRAF mutations were detected in 18 of 50 (36%) HPs, 2 of 10 (20%) HP/ADs, and 9 of 9 (100%) SAs. Twenty-six of 29 mutations caused amino acid substitutions at valine 599, the known hotspot. KRAS mutations were detected in 9 of 50 (18%) HPs, 6 of 10 (60%) HP/ADs, and 0 of 9 (0%) SAs. BRAF and KRAS mutations are mutually exclusive (P = 0.001). The associations of BRAF mutations with SAs (P < 0.001) and KRAS mutations with HP/ADs (P = 0.005) are statistically significant. A majority (90%) of the serrated polyps showing dysplasia had mutations in either BRAF or KRAS, significantly different from those without dysplasia (54%; P = 0.01). Our data highlight the important role of activation of the RAS-RAF-mitogen-activated protein/extracellular signal-regulated kinase pathway in the initiation and progression of serrated neoplasms. Acquisition of a BRAF mutation appears to be associated with the progression of HP to SA, whereas progression to HP/AD is predominantly associated with acquisition of a KRAS mutation. The high incidence of BRAF mutations in HPs and SAs is consistent with the notion that the group of colorectal cancers carrying BRAF mutations may harbor most that have progressed through the HP-SA-carcinoma pathway.

INTRODUCTION

RAS proteins participate in the RAS-RAF-MEK-ERK-MAP kinase pathway, which mediates cellular responses to growth signals (1). Somatic mutations of the RAS genes, leading to activation of this signaling pathway and malignant transformation, are common in human cancers. There are three RAS genes, each encoding cytoplasmic serine/threonine kinases that are regulated by binding to RAS (1, 2). We have shown previously that mutations of BRAF provide an alternative route for activation of this signaling pathway in human cancers (3). BRAF mutations can be found in malignant melanoma, colorectal cancers, and ovarian borderline (low malignant potential) tumors, and they tend to occur in a mutually exclusive relationship with RAS mutations (3–5). Subsequently, BRAF mutations have also been found in most benign melanocytic naevi and lung cancer (6–8). Mutations in BRAF occur in two regions of the BRAF kinase domain, the activation segment (which protects the substrate binding site), and, less commonly, the G loop (which mediates binding of ATP). Mutated forms of BRAF that have been studied thus far have elevated kinase activity and can transform NIH3T3 cells (3).

An adenoma–carcinoma sequence has long been recognized to constitute a major pathway of colorectal carcinogenesis (9). KRAS is known to play an important role in the progression along this pathway, predominantly occurring during the transformation of small to intermediate sized adenomas (10). We and others have demonstrated the involvement of BRAF in a similar phase of colorectal cancer development, albeit in a much smaller percentage of cases (3–10% of adenomas have BRAF mutations compared with ~30–60% with KRAS mutations; Refs. 4 and 5). Emerging evidence supports the existence of an alternative pathway of colorectal cancer development through the serrated polyp (for review, see Refs. 11 and 12). The serrated polyp encompasses a morphological spectrum, including HPs, HP/ADs, and SAs. HPs of the large intestine are found in 50 years of age (13, 14). Morphologically, they are characterized by elongated crypts with serrated architecture covered by nondysplastic colonic epithelial cells. It has been suggested that HPs arise because of hypermaturetation of glandular cells consequent on diminished apoptosis (15), but their pathogenesis and propensity for malignant progression remain controversial. Subsequently, two morphological variants of HPs that are associated with epithelial dysplasia have been recognized. The term SA has been used for polyps with serrated morphology (as seen in ordinary HPs) that show epithelial dysplasia throughout the lesion. HP/ADs show focal hyperplastic and focal adenomatous components (16, 17). Evolution of these lesions to invasive carcinomas has been reported (18–21).

Molecular studies have indicated that serrated polyps (including HPs) are likely to be clonal neoplasms, because mutations of KRAS and p53, MSI, and chromosome 1p loss have been found in variable proportions (22–30). However, mutations of adenomatous polyposis coli are uncommon (26, 30, 31). Overall, the molecular pathway of evolution in serrated polyps and its relative contribution to the incidence of colorectal cancer are unclear.

In our recent study of BRAF and KRAS mutations in colorectal polyps, one of three HPs showed a BRAF mutation (4). This finding has prompted us to examine a larger series of serrated polyps for BRAF and KRAS mutations with reference to their morphological classification, in particular the presence of dysplasia and MSI.

MATERIALS AND METHODS

Materials. Pathology records and histological slides with a diagnosis of either HP, HP/AD, or SA from 1997 to 2002 in the Department of Pathology, Queen Mary Hospital were reviewed independently by two pathologists with an interest in gastrointestinal neoplasia (S. Y. L. and S. T. Y.). Six-μm-thick sections were cut from paraffin-embedded tissue and stained with 0.1% methylene blue. The lesions were microdissected under a light microscope, and only areas with the pathological lesion were taken for DNA extraction. Apart from scanty amounts of contaminating inflammatory and stromal cells, we estimated that ≥70% of the resulting DNA was from the epithelial components of the serrated polyps. DNA was extracted using standard protocols. In total, 69 sporadic serrated polyps from 63 individuals generated adequate DNA of...
quality suitable for BRAF and KRAS analysis. This study was approved by the Ethics Committee of the University of Hong Kong.

**Mutation Screening of BRAF and KRAS.** The complete coding sequences of exons 11 (G loop region) and 15 (activation segment) of BRAF and exon 2 of KRAS were amplified using intronic primers and directly sequenced on both strands using the DYEnamic ET Terminator Cycle Sequencing Kits (Amersham Pharmacia) and analyzed by the Applied Biosystems 377 or 3700 automated sequencer. These covered most of the mutation hot spots known previously of the two genes. All mutations were reconfirmed by independent PCR reactions and sequencing. The primers for BRAF sequencing of exon 15 were similar as described previously (3). The primers for exon 11 of BRAF (forward- TTC TGT TTT GCT TGA CTF GAC TT and reverse- ACT TGT CAC AAT GTC ACC ACA TT) and exon 2 of KRAS (forward- CTG AAA ATG ACT GAA TAT AAA CTT GT and reverse- ATA TGC ATA TTA AAA CAA GAT TTA CC) were designed to amplify a shorter fragment suitable for use in paraffin DNA. In each case with mutation, the percentage of mutant peak height over mutant plus wild-type peak height.

**Analysis of MSI.** Because corresponding normal tissue was not available, microsatellite analysis was performed using three monomorphic microsatellite markers, including BAT25, BAT26, and transforming growth factor 

**Statistical Analysis.** Categorical variables were compared with the use of the chi-square test with Yates correction or Fisher’s exact test as appropriate.

### RESULTS

Sixty-nine sporadic serrated polyps from 63 patients were suitable for the present study. These included 50 typical HPs, 10 HP/ADs, and 9 SAs. Thirty-nine polyps were from male patients, and 30 were from female patients. Twenty-two were from the right colon, and 46 were from the left colon (the tumor location in one case was not known). The 50 HPs showed no epithelial cell dysplasia. Of the 19 HP/ADs and SAs, 14 showed mild, 4 moderate, and 1 severe dysplasia. The 9 SAs. Thirty-nine polyps were from male patients, and 30 were from female patients. Twenty-two were from the right colon, and 46 were from the left colon (the tumor location in one case was not known). The 50 HPs showed no epithelial cell dysplasia. Of the 19 HP/ADs and SAs, 14 showed mild, 4 moderate, and 1 severe dysplasia. The size of these polyps ranged from 2 to 20 mm in maximal dimension (mean = 6.57 mm).

The incidence of BRAF and KRAS mutations and their relationship to clinicopathological features is shown in Table 1. Of the 69 serrated polyps studied, there were 29 cases (42%) with BRAF mutations and 15 cases (21.7%) with KRAS mutations. The spectra of BRAF and KRAS mutations found are shown in Table 2. Representative sequence chromatographs are shown in Fig. 1. Twenty-eight of the 29 BRAF mutations are in exon 15 with only one located within exon 11, and 26KRAS mutations found are shown in Table 2. Representative sequence chromatographs are shown in Fig. 1. Twenty-eight of the 29 BRAF mutations are in exon 15 with only one located within exon 11, and 26

![Mutation spectrum of BRAF and KRAS in 69 serrated polyps](image)

**Fig. 1.** Sequence chromatographs of serrated polyps with BRAF or KRAS mutations. A, a SA with V599E (T1796A) mutation of BRAF. B, an HP without BRAF mutation. C, an SA with V599M (G1795A) mutation of BRAF. D, an HP with D586E (C1758G) mutation of KRAS. E, an admixed HP/AD with G12D (G34T) mutation of KRAS. F, an HP with no KRAS mutation.

### Table 1. Incidence of BRAS and KRAS mutations and their relationship to clinicopathological features of serrated polyps

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total</th>
<th>No. of BRAF mutation (%)</th>
<th>No. of KRAS mutation (%)</th>
<th>No. of BRAF or KRAS mutation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>39</td>
<td>15 (39)</td>
<td>9 (23)</td>
<td>24 (62)</td>
</tr>
<tr>
<td>F</td>
<td>30</td>
<td>14 (47)</td>
<td>6 (20)</td>
<td>20 (67)</td>
</tr>
<tr>
<td>Tumor side</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>22</td>
<td>10 (46)</td>
<td>3 (14)</td>
<td>13 (59)</td>
</tr>
<tr>
<td>Left</td>
<td>46</td>
<td>19 (41)</td>
<td>12 (26)</td>
<td>31 (67)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP</td>
<td>50</td>
<td>18 (36)</td>
<td>9 (18)</td>
<td>27 (54)</td>
</tr>
<tr>
<td>HP/AD</td>
<td>10</td>
<td>2 (20)</td>
<td>6 (60)</td>
<td>8 (80)</td>
</tr>
<tr>
<td>SA</td>
<td>9</td>
<td>9 (100)</td>
<td>0 (0)</td>
<td>9 (100)</td>
</tr>
<tr>
<td>Dysplasia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nil</td>
<td>50</td>
<td>18 (36)</td>
<td>9 (18)</td>
<td>27 (54)</td>
</tr>
<tr>
<td>Mild</td>
<td>14</td>
<td>10 (71)</td>
<td>3 (21)</td>
<td>13 (93)</td>
</tr>
<tr>
<td>Moderate</td>
<td>4</td>
<td>1 (25)</td>
<td>2 (50)</td>
<td>3 (75)</td>
</tr>
<tr>
<td>Severe</td>
<td>1</td>
<td>0 (0)</td>
<td>1 (100)</td>
<td>1 (100)</td>
</tr>
</tbody>
</table>

1. Fisher’s exact test, HP/ADs versus HPs, P = 0.009; HP/ADs versus HPs + SAs, P = 0.005.
2. Fisher’s exact test, SAs versus HPs, P < 0.001; SAs versus HPs + HP/ADs, P < 0.001.
3. Fisher's exact test, dysplasia Nil versus mild to severe, P = 0.014.

![Mutation spectrum of BRAF and KRAS in 69 serrated polyps](image)

**Fig. 1.** Sequence chromatographs of serrated polyps with BRAF or KRAS mutations. A, a SA with V599E (T1796A) mutation of BRAF. B, an HP without BRAF mutation. C, an SA with V599M (G1795A) mutation of BRAF. D, an HP with D586E (C1758G) mutation of KRAS. E, an admixed HP/AD with G12D (G34T) mutation of KRAS. F, an HP with no KRAS mutation.

F594L. The V599M and D586E are novel mutations that have not been reported before. In 72% of cases with BRAF mutation, the mutant allele constitutes 35–66% of the total mutant and wild-type signal, suggesting that these are heterozygous mutations present in
most of the epithelial cells within the lesion. The remaining eight cases harbor mutant BRAF alleles that account for 20–32% of the total signal. The five cases with the lowest percentage of mutant alleles (20–24%) are all HPs. It is likely that these lower signals represent mutations present in a subpopulation of the lesion. For the 15 serrated polyps with KRAS mutations, the percentage of mutant alleles ranged from 20 to 71%. None of the polyps that carry a BRAF mutation have a KRAS mutation \((P = 0.001, \chi^2 \text{ with Yates correction})\).

Thirty-six percent (18 of 50) of HPs, 20% (2 of 10) of HP/ADs, and all (100%, 9 of 9) of SAs have BRAF mutations. Eighteen percent (9 of 50) of HPs, 60% (6 of 10) of HP/ADs, and none (0%, 0 of 9) of SAs have KRAS mutations. The associations of BRAF mutations with SAs (Fisher’s exact test, SAs versus HPs, \(P < 0.001\); SA versus HP+HP/ADs, \(P < 0.001\) and KRAS mutations with HP/ADs (Fisher’s exact test, HP/AD versus HP, \(P = 0.009\); HP/AD versus HP + SA, \(P = 0.005\)) are statistically significant. Overall, 64% of serrated polyps (44 of 69) harbor mutations in either BRAF or KRAS. Ninety percent (17 of 19) of the serrated polyps showing dysplasia had mutations (11 BRAF and 6 KRAS), significantly different from those without dysplasia (54%, 27 of 50; \(\chi^2\) test with Yates correction, \(P = 0.014\)). All 69 serrated polyps were microsatellite stable.

**DISCUSSION**

We have shown that BRAF mutations are present in 42% of serrated polyps. Similar to most other tumors, conversion of valine 599 to glutamic acid is the most common mutation found in serrated polyps (86% of mutations observed). This substitution results in the insertion of a negatively charged residue adjacent to a site of regulatory phosphorylation at T598 which may mimic normal regulatory phosphorylation and lead to constitutive activation of BRAF independent of a negatively charged residue adjacent to a site of regulatory glutamic acid is the most common mutation found in serrated polyps. Similar to most other tumors, conversion of valine 599 to glutamic acid is the most common mutation found in serrated polyps (86% of mutations observed). In most other tumors, conversion of valine 599 to glutamic acid is the most common mutation found in serrated polyps (86% of mutations observed).

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**REFERENCES**

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