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–canceroma 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 polyoid (HP), admixed hyperplastic polyoid/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/AD, 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% vs. 40.14%). Our data highlight the important role of activation of the RAS-RAF-mitogen-activated protein/extracellular signal-regulated kinase-mitogen-activated protein 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-MEK3-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 BRF 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 BRF occur in two regions of the BRF 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–canceroma 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 ~12% of individuals >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 hypermaturation 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).

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 \textit{BRAF} and \textit{KRAS} analysis. This study was approved by the Ethics Committee of the University of Hong Kong.

**Mutation Screening of \textit{BRAF} and \textit{KRAS}**. The complete coding sequences of exons 11 (G loop region) and 15 (activation segment) of \textit{BRAF} and exon 2 of \textit{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 \textit{BRAF} sequencing of exon 15 were similar as described previously (3). The primers for exon 11 of \textit{BRAF} (forward- TTC TGT TTT GCT TGA CTT GAC TT and reverse- ACT TGT CAC AAT TTC ACC ACA TT) and exon 2 of \textit{KRAS} (forward- CTG AAA ATG ACT GAA TAT AAA CTT GT and reverse- ATG TGA ATA TAA 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 \textit{B} II. The procedure was similar to those described in our previous publications (4, 32).

**Statistical Analysis**. Categorical variables were compared with the use of the \textit{c}2 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 size of these polyps ranged from 2 to 20 mm in maximal dimension (mean = 6.57 mm). The incidence of \textit{BRAF} and \textit{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 \textit{BRAF} mutations and 15 cases (21.7%) with \textit{KRAS} mutations. The spectra of \textit{BRAF} and \textit{KRAS} mutations found are shown in Table 2. Representative sequence chromatographs are shown in Fig. 1. Twenty-eight of the 29 \textit{BRAF} mutations are in exon 11 with only one located within exon 11, and 26 \textit{KRAS} mutations are in exon 15 with another 25 V599E, 1 V599M, shown previously to be a mutation hot spot.

**Table 1**. Incidence of \textit{BRAF} and \textit{KRAS} mutations and their relationship to clinicopathological features of serrated polyps

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total</th>
<th>No. of \textit{BRAF} mutation (%)</th>
<th>No. of \textit{KRAS} mutation (%)</th>
<th>No. of \textit{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 (66)</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)b</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)c</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>

\( ^a \) Fisher’s exact test, HP/ADs versus HPs, \( P = 0.009 \); HP/ADs versus HPs + SAs, \( P = 0.005 \).

\( ^b \) Fisher’s exact test, SAs versus HPs, \( P < 0.001 \); SAs versus HPs + HP/ADs, \( P < 0.001 \).

\( ^c \) \( \chi^2 \) test with Yates correction, dysplasia Nil versus mild to severe, \( P = 0.014 \).

**Table 2**. Mutation spectrum of \textit{BRAF} and \textit{KRAS} in 69 serrated polyps

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>\textit{BRAF} mutations*</th>
<th>HP ( n = 50 )</th>
<th>HP/AD ( n = 10 )</th>
<th>SA ( n = 9 )</th>
<th>Total ( n = 69 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1796A</td>
<td>V(599)E</td>
<td>15</td>
<td>2</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>G1795A</td>
<td>V(599)M</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>G1403C</td>
<td>G468A</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>C1758G</td>
<td>D586E</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>T1782G</td>
<td>F594L</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

\( ^a \) \textit{BRAF} and \textit{KRAS} mutations are mutually exclusive, \( \chi^2 \) with Yates correction, \( P = 0.001 \).

\( ^b \) Novel \textit{BRAF} mutations.

**Fig. 1**. Sequence chromatographs of serrated polyps with \textit{BRAF} or \textit{KRAS} mutations. A, a SA with V599E (T1796A) mutation of \textit{BRAF}. B, an HP without \textit{BRAF} mutation. C, an SA with V599M (G1795A) mutation of \textit{BRAF}. D, an HP with D586E (C1758G) mutation of \textit{BRAF}. E, an admixed HP/AD with G12D (G35A) mutation of \textit{KRAS}. F, an HP with no \textit{KRAS} mutation.

F594L. The V599M and D586E are novel mutations that have not been reported before. In 72% of cases with \textit{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, χ² 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; χ² 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 RAS. KRAS and BRAF mutations appear to be mutually exclusive, confirming previous suggestions that they have similar biological consequences (3–5). The combined incidence of BRAF and KRAS mutations in serrated polyps is 64%, indicating that activation of the RAS-RAF-MEK-ERK-MAP kinase pathway constitutes a highly significant event in the pathogenesis of this group of lesions. The occurrence of either BRAF or KRAS mutations in 54% of otherwise histologically “innocuous” HPs suggests that these may be early steps in the initiation of the serrated neoplasia pathway. However, the presence of epithelial dysplasia is associated with even higher rates (90%) of mutation in either BRAF or KRAS, highlighting the importance of activation of the RAS-RAF-MEK-ERK-MAP kinase pathway in the progression of serrated neoplasms.

There is strong evidence in support of a pathway to colorectal carcinogenesis through serrated polyps: (a) morphological studies have reported evolution of HP to invasive carcinoma (sometimes through intermediate lesions of SA and HP/AD; Refs. 18–21) and have shown that 11% of SAs harbor foci of intramucosal cancer (17); and (b) patients affected by the rare hyperplastic polyposis syndrome usually small and can easily be destroyed by overgrowth of the cancers that subsequently develop. Nevertheless, by identifying remnant SA elements at the edge of invasive colorectal cancers, it has been estimated that ~6% of sporadic colorectal cancers originate from an SA (18). Molecular studies have also yielded few insights because there have been previously no known molecular changes that specifically characterize the serrated neoplasm–carcinoma pathway. The high frequency of BRAF mutations in HP (30%) and SA (100%) reported here is in sharp contrast to the low incidence observed in classical ADs (2.8%; Ref. 4). We therefore propose that the group of colorectal cancers carrying BRAF mutations (estimated at between 5 to 10%) may have evolved, at least in part, from HP through the intermediate stage of SA. However, the high incidence of BRAF mutations in HPs and particularly SAs compared with that observed in colorectal cancer suggests that the majority of colorectal cancers do not evolve through this pathway. HPs that progress through HP/ADs predominantly have KRAS mutations. This makes the adenomatous components of HP/ADs indistinguishable from classical ADs both at the morphological and molecular levels. Hence, their contribution to colorectal cancer is more difficult to assess.

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**REFERENCES**


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