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Beta-Catenin Nuclear Labeling is a Common Feature of Sessile Serrated Adenomas and Correlates with Early Neoplastic Progression Following *BRAF* Activation

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Abstract

Recent observations indicate that some sessile serrated adenomas (SSAs) have aberrant β -catenin nuclear labeling, implicating the Wnt pathway in the molecular progression of SSAs to colorectal carcinoma. We sought to expand upon this finding by characterizing β -catenin expression in the full spectrum of serrated colorectal polyps, and correlating these findings with the genetic status of *BRAF*, *KRAS* and *CTNNB1*. Immunolabeling for β -catenin confirmed the presence of abnormal nuclear accumulation in SSAs, with 35/54 (67%) SSAs showing nuclear labeling compared to 0/12 hyperplastic polyps (HPs). Abnormal nuclear labeling was also identified in 4/11 (36%) traditional serrated adenomas (TSAs) ($p=0.00001$). When SSAs were further analyzed with respect to the presence or absence of conventional epithelial dysplasia, nuclear β -catenin labeling was seen in 8/27 (29%) SSAs without dysplasia (SSA) but in 27/27 (100%) of SSAs with dysplasia (SSADs) ($p=0.000001$). Sequencing of genomic DNA extracted from a subset of HPs, SSAs, SSADs, TSAs and tubular adenomas (TAs) failed to identify any *CTNNB1* mutations to account for abnormal β -catenin nuclear labeling. However, abnormal nuclear labeling always occurred in the setting of a *BRAF* V600E mutation, indicating aberrant nuclear labeling occurs on a background of *BRAF* activation. Of interest, all six TSAs contained a *KRAS* mutation confirming that SSAs and TSAs are genetically distinct entities. These findings validate previous reports implicating activation of the Wnt signaling pathway in SSAs, and further indicate that Wnt pathway activation plays a role in the neoplastic progression of SSAs and TSAs to colonic carcinoma by mechanisms independent of *CTNNB1* mutation.

Keywords

serrated adenoma; hyperplastic polyp; Wnt pathway; beta-catenin; colon cancer

Introduction

Serrated polyps of the colorectum are comprised of hyperplastic polyps (HP), sessile serrated adenomas (SSA), and traditional serrated adenomas (TSA) (23). HPs are characterized by an average size <0.5 cm, a predominantly left sided location, and the lack of neoplastic potential.

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Histologically, HPs have uniformly shaped crypts that narrow towards the muscularis mucosae, a uniform proliferative zone confined to the lower half of the crypts, and prominent serrations towards the luminal surface. By contrast, SSAs are characterized by an average size >0.5 cm, a predominantly right sided location, and the potential to progress to infiltrating carcinoma. Unlike HPs, SSAs are notable for irregularly shaped crypts that widen towards the muscularis mucosae in association with basilar crypt branching, papillary formations, and an irregularly distributed proliferation zone (23). TSAs, the least frequent form of serrated polyp and that was first described by Longacre and Fenoglio-Preiser (15), are subsets of polyps with epithelial features of both an SSA and conventional tubular adenoma (TA). However, unlike HPs and SSAs, TSAs are the only form of serrated polyp to have ectopic crypt formation (24). Filiform serrated adenomas, described by Yantiss et al (30), likely correspond to TSAs with prominent exophytic features. Cytoplasmic eosinophilia is also a common finding in TSAs that is absent in HPs and only rarely encountered in SSAs (24). Not surprisingly, the distinction among these forms of serrated polyps is an important issue due to the different clinical recommendations for patients diagnosed with a HP versus an SSA or TSA at endoscopy (4,7,9).

In the classical adenoma-carcinoma pathway, biallelic inactivation of *APC* is the initiating event that is followed by the accumulation of mutations in key genes that largely include *KRAS* and *p53*. This sequence of events leads to the development of tubular adenomas and colorectal cancers characterized by chromosomal instability, and is epitomized by the genetic progression model first proposed by Fearon and Vogelstein in 1990 (6). In the second pathway, known as the serrated pathway, *BRAF* mutations appear to be an early or instigating event that is followed by the progressive increase in promoter methylation, leading to hypermethylation induced silencing of *MLH1* in advanced lesions (19,12,21,26). This sequence is associated with the development of SSAs, some of which may progress to colorectal cancers characterized by high levels of microsatellite instability (19,8). Of interest, while most have considered HPs as a distinct entity from SSAs based on differing rates of *KRAS* and *BRAF* mutation (3), the finding of similar protein expression profiles in both lesions have led some to posit that they are part of a continuum that differs by growth dynamics and mutational profiles rather than by cellular differentiation (2). The relationship of these two pathways to the development of TSAs remains an area of some uncertainty as features of both may be found in these polyps, leading some to propose a third “fusion” pathway to colorectal cancer (10).

We have previously reported our observation of aberrant nuclear labeling of β -catenin in a subset of SSAs, but not HPs, indicating that disruption of Wnt signaling may also play a role in the serrated pathway to colorectal carcinoma (27). In an effort to expand upon this observation, we now report our findings of β -catenin expression in a larger set of serrated polyps of the colorectum, as well as the relationship of β -catenin nuclear labeling to the genetic status of *BRAF*, *KRAS2* and *CTNNB1* (the gene coding for β -catenin protein).

Materials and Methods

Samples

To obtain serrated polyps for study, we performed a search of the Johns Hopkins Pathology Archives using the term “sessile serrated adenoma” and “sessile serrated adenoma and dysplasia” spanning January 1, 2006 to January 1, 2007. This time period was expanded from January 1, 2005 to January 1, 2008 for a search using the term “traditional serrated adenoma”. This identified 159 potential polyps for study. All slides were reviewed and 66 serrated polyps that were well oriented on routinely stained sections, that did not contain cautery artifacts and that had available paraffin blocks were selected for further study. All polyps were then reviewed and classified using proposed by criteria of Torlakovic et al (23,24). Sessile serrated adenomas were identified based on features of prominent basilar crypt dilation, abundant intracellular and extracellular mucin, dystrophic goblet cells, and abnormal proliferation. Polyps with mixed

features of sessile serrated adenoma and hyperplastic polyp were included in the sessile serrated adenoma category. Traditional serrated adenomas were identified based on the features of a serrated epithelial architecture, ectopic crypt formations and eosinophilic cytoplasm in association with features of conventional epithelial dysplasia (nuclear crowding, nuclear enlargement, pencillate nuclei, loss of nuclear polarity and loss of differentiation). In addition, paraffin-embedded samples of 12 hyperplastic polyps and 18 conventional tubular adenomas were also obtained from a one week period of in house signout of routine biopsies by one of the senior authors (C.I.D.). All hyperplastic polyps were microvesicular type and were identified based on the features of thickened surface basal membrane, thickening and extension of the muscularis mucosa, presence of Kulchitsky cells, and decreased overall architectural distortion (23). Clinicopathologic data of all patients whose polyps were used for the study were collected from the corresponding pathology reports. The project was approved by the Institutional Review Board.

Immunohistochemistry

Immunohistochemical labeling was performed using standard methods. Unstained 5- μ m sections were cut from paraffin blocks and the slides were deparaffinized by routine techniques followed by incubation in 1 $\times$ sodium citrate buffer (diluted from 10 $\times$ heat-induced epitope retrieval buffer, Ventana-Bio Tek Solutions, Tucson, AZ) before steaming for 20 minutes at 80 °C. Slides were cooled 5 minutes and incubated with beta-catenin monoclonal antibody (1:1000 dilution, Transduction, catalog #610154) using a Bio Tek-Mate 1000 automated stainer (Ventana-Bio Tek Solutions). Immunolabeling was detected per kit instructions (Ventana IVIEW Detection Kits, catalog #760091).

β -catenin labeling was evaluated with respect to membranous and/or nuclear localization. Membranous labeling was considered normal, whereas an abnormal labeling pattern for β -catenin was considered present when nuclear labeling accompanied by a loss of membranous labeling was seen outside the crypt bases where the β -catenin positive progenitor population normally resides (5).

Sequencing

Regions of serrated epithelium and adenomatous epithelium from unstained sections were dissected from unstained 10 micron thick sections. Genomic DNA was extracted from each sample by phenol-chloroform and 20 ng used for PCR amplification of *KRAS2* exon 2, *BRAF* exon 15 and *CTNNB1* exon 3 using intronic primers flanking these exons (Table 1). PCR products were sequenced in both directions by use of a M13F primer (5'-GTAAAACGACGGCCAGT-3') and a M13R primer (5'-CAGGAAACAGCTATGACC-3') that were incorporated into the forward and reverse primer of each primer pair, respectively (Agencourt Bioscience Corporation, Beverly, MA). Sequence data were analyzed with Sequencher™ 4.8 software (Gene Codes, Ann Arbor, MI). Verification of all mutations was accomplished by bidirectional sequencing of a second PCR product derived independently from the original template.

Statistics

For comparing parametric distributions an ANOVA was used, and for frequency distributions a Chi-squared test was used. P values ≤ 0.05 were considered statistically significant.

Results

Clinical Features

A total of 12 HPs, 54 SSAs, 12 TSAs and 18 TAs were studied. Among the SSAs, 27 contained foci of conventional epithelial dysplasia/low grade dysplasia (SSAD). Clinicopathologic features of these polyps are shown in Figure 1 and Table 2. There was no difference in the mean age across each group of patients, nor was there a difference in the proportion of males and females. SSAs and SSADs were predominantly located in the right colon, whereas TSAs were more commonly located in the left colon ($p=0.00001$). The SSAs, SSADs and TSAs were also more commonly greater than 5 mm in diameter compared to the HPs or TAs ($p=0.0001$).

β -catenin Protein Expression in Colonic Polyps

Wu *et al* have previously shown that a subset of SSAs show nuclear accumulation of β -catenin (27). To validate this finding, we performed β -catenin immunolabeling of paraffin sections prepared from each type of polyp. Examples of β -catenin immunolabeling patterns are shown in Figure 2. All 12 HPs showed a normal membranous pattern, with scattered positive labeling nuclei seen at the crypt bases. In some HPs, positive membranous labeling was confined to the crypt bases with the remainder of the epithelial cells negative. This labeling pattern was identical to that of adjacent normal mucosa in all cases, consistent with physiologically active Wnt signaling within the progenitor population of intestinal epithelium (5). By contrast, abnormal nuclear accumulation of β -catenin was seen in 35/54 SSAs (67%), in 4/11 TSAs (36%) and in 18/18 (100%) TAs ($p=0.00001$). When SSAs were further analyzed with respect to the presence or absence of conventional epithelial dysplasia, abnormal nuclear β -catenin labeling was seen in 8/27 (29%) SSAs without dysplasia (SSA) but in 27/27 (100%) SSAs with dysplasia (SSADs) ($p=0.0000001$).

In addition to evaluating for the presence of abnormal β -catenin nuclear labeling, when the quality of nuclear labeling was considered two different patterns of abnormal nuclear labeling were noted (Figure 2). For example, in some serrated polyps abnormal nuclear accumulation was seen as weak intensity labeling of the nuclei that involved 20-50% of the epithelial cells spanning from the crypt base to the surface (Abnormal Pattern #1-weak nuclear). In this pattern, weak membranous labeling was also occasionally present, but less in intensity than that of adjacent normal mucosa within the same section. This type of labeling was characteristic of all eight SSAs, 3/4 TSAs (75%) and 10/27 SSADs (37%) with nuclear β -catenin labeling (Figures 2C, 2D). By contrast, in the remaining 17/27 SSADs (63%), 1/4 TSAs (25%) and all 18 TAs the abnormal nuclear accumulation was seen as intense positive nuclear labeling that was diffusely present within >80% to 100% of the epithelial cells (Abnormal Pattern #2-strong nuclear) (Figures 2E, 2F). In some polyps, the aberrant nuclear labeling was accompanied by intense positive cytoplasmic labeling as well. In TAs, this pattern of labeling is consistent with robust nuclear accumulation of β -catenin in association with genetic inactivation of *APC* early in colorectal carcinogenesis (6).

Of interest, in 17 of the 27 SSADs in this study, there were regions of the polyp with classical features of SSA but lacking conventional epithelial dysplasia, indicating the development of dysplasia occurred within a pre-existent SSA. A comparison of β -catenin immunolabeling patterns in the regions of SSA versus SSAD within these same polyps indicated a transition to increasingly aberrant nuclear labeling in 14 of 17 (82%) cases. For example, in five polyps the regions of SSA showed normal β -catenin labeling, whereas the regions of SSAD showed either weak positive nuclear labeling (Abnormal Pattern #1, $n=2$) or strong positive and diffuse nuclear labeling (Abnormal Pattern #2, $n=3$). In the remaining 9 polyps, the regions of SSA showed weak positive nuclear labeling (Abnormal Pattern #1) whereas the foci of SSAD showed strong positive diffuse nuclear labeling (Abnormal Pattern #2) (Figure 3). Finally, in

three polyps, strong positive nuclear labeling (Abnormal Pattern #2) was seen in both the regions with and without conventional epithelial dysplasia (Figure 3), suggesting that increasing abnormal nuclear accumulation of β -catenin precedes the development of conventional dysplasia.

Genetic Features of Serrated Polyps with Nuclear β -catenin Labeling

To determine the genetic features that are characteristic of serrated polyps with abnormal nuclear β -catenin labeling, we extracted gDNA from 10 HPs, 9 SSAs, 13 SSADs, 6 TSAs and 3 TA for which immunolabeling was performed and sequenced them for mutations in exon 15 of *BRAF*, exon 2 of *KRAS* and exon 3 of *CTNNB1* (Figure 4 and Table 3). These polyps were used solely for the ability to obtain high quality gDNA for study from available paraffin sections.

Mutations of *BRAF* (V600E) were found in 26/40 serrated polyps (65%) representing 5/10 HPs (50%), 9/9 SSAs (100%) and 12/12 SSADs (100%) but in 0/6 TSAs and 0/3 TAs ($p=0.00001$). For one SSAD, the PCR for *BRAF* was repeated two times but failed. By contrast, mutations of *KRAS* were found in 8/41 serrated polyps (20%). All eight *KRAS* mutations were located within codon 12. These eight polyps corresponded to 2/10 HPs (20%) and 6/6 TSAs (100%) ($p=0.0006$). *KRAS* mutations were generally mutually exclusive with *BRAF* mutations, with seven *KRAS* mutations occurring in serrated polyps that were WT for *BRAF*. However, in one HP both a *BRAF* V600E and a *KRAS* G12D mutation were found. Histologic review of this polyp confirmed it was a conventional microvesicular HP (23). We did not find *KRAS* mutations in any of the three TAs analyzed. However, all three were less than 0.5 cm in size, consistent with the infrequent rate of *KRAS* mutations typically seen in early TAs (25).

Abnormal nuclear β -catenin labeling was present in 22/41 (54%) polyps corresponding to 4/9 SSAs (44%), 13/13 SSADs (100%), 2/6 TSAs (33%) and 3/3 TAs (100%). However, no *CTNNB1* mutations were found in any of the 41 polyps analyzed to account for the abnormal labeling patterns. This analysis included two SSADs in which both the regions of SSA and conventional epithelial dysplasia were independently microdissected and sequenced. A comparison of the relationship of *BRAF* or *KRAS* mutation to abnormal β -catenin labeling in serrated polyps with neoplastic potential indicated that aberrant nuclear labeling always occurred in the presence of a *BRAF* mutation (16/21 SSAs or SSADs, 72%) or a *KRAS* mutation (2/6 TSAs, 33%), suggesting that Wnt pathway activation occurs in the setting of *BRAF* or *KRAS* activation in the serrated neoplasia pathway.

Discussion

The current understanding of serrated polyps of the colorectum indicates that HPs and SSAs are molecularly distinct entities with contrasting potential for neoplastic progression but with overlapping morphologic features (23,29,13,19). Our data support these prior observations, in that we found *BRAF* mutations in all serrated polyps with neoplastic potential. By contrast, *BRAF* or *KRAS* mutations were found in a subset of HPs. Both *BRAF* and *KRAS* mutations in HPs have been described (12,29,19), with *KRAS* mutations relatively more common among the goblet cell variant (19). Although only a small number of HPs were sequenced in this study, we nonetheless believe our data regarding the frequency and spectrum of *BRAF* and *KRAS* mutations in serrated polyps are in keeping with those observations.

Our data now also confirm prior observations of aberrant β -catenin expression in SSAs (27), and further implicate disruption of the Wnt signaling pathway as an additional molecular feature that segregates these two classes of serrated polyps. In human colon cancer, inactivating mutations of the adenomatous polyposis coli gene (*APC*) form the basis for the hereditary colon cancer syndrome familial adenomatous polyposis (14,18). Biallelic inactivation of *APC* is

found in the majority of sporadic colon cancers as well, and in both instances inactivation of *APC* leads to the inappropriate stabilization of β -catenin and activation of Wnt target genes. By contrast, activating mutations in *CTNNB1*, and not *APC* genetic inactivation, are frequent features of microsatellite-instability (MSI+) colon cancers that similarly cause stabilization of β -catenin and aberrant Wnt pathway activation (17,16,20). While we found frequent β -catenin nuclear labeling in SSAs, we found no mutations in *CTNNB1* as a mechanism for Wnt activation. However, as *CTNNB1* mutations have been described as a specific feature of HNPCC-associated but not sporadic MSI+ colon cancers (11), our findings support claims that SSAs are a precursor of sporadic MSI+ colon cancer (1). Of note, Suzuki *et al* provided compelling evidence of Wnt pathway activation in colorectal cancer cell lines by hypermethylation of Wnt pathway antagonists *SFRP1*, *SFRP2* and *SFRP5* (22). Thus, it is conceivable that loss of expression of Wnt antagonists by promoter hypermethylation, and not genetic alterations of *APC* or *CTNNB1*, may be an underlying mechanism of Wnt pathway activation in SSAs. Additional studies will be required to address this possibility.

In addition to the confirmation of β -catenin nuclear labeling in serrated polyps with neoplastic potential, we also now report that aberrant nuclear labeling, and hence Wnt pathway activation, is a pervasive feature of SSAs that have progressed to development of conventional epithelial dysplasia (SSAD). However, there was no relationship of the degree of abnormal β -catenin nuclear accumulation to the presence of conventional dysplasia in these polyps, nor was abnormal β -catenin nuclear accumulation specific to the regions of conventional dysplasia in SSADs, indicating Wnt activation preceded the development of conventional dysplasia. This is consistent with the observations by Wu *et al* (27) who found prominent β -catenin nuclear accumulation (now classified as Abnormal Pattern #2) in an SSA without conventional epithelial dysplasia (Figure 3B in that paper). These patterns of abnormal β -catenin expression differ from that previously reported for MLH1 protein expression in SSAs in which loss of expression was exclusively seen in regions of conventional low or high grade epithelial dysplasia and/or infiltrating carcinoma, but not in the adjacent SSA (21). Thus, the identification of nuclear β -catenin labeling may aid identification of SSAs with neoplastic potential that have not yet lost MLH1 expression.

Of interest, we also found that the molecular features of TSAs are distinct from SSAs in that all TSAs had a *KRAS* mutation whereas *BRAF* mutations were only seen in SSAs. This concept is not novel (19,10,13), yet conclusive studies of the molecular genetics of TSAs have been limited by the heterogeneity of polyps classified in this category. By contrast, we have utilized the criteria recently reported by Torlakovic *et al* for classification of TSAs (24) resulting in a highly homogeneous population of polyps with classic features of traditional serrated adenoma. Moreover, while the number of TSAs analyzed was not extensive, our results nonetheless indicate that Wnt pathway activation, seen as nuclear labeling for β -catenin, is also a feature of a subset of TSAs. However, whereas nuclear labeling was most frequent in SSAs with conventional epithelial dysplasia, there was no correlation of nuclear labeling to the presence or absence of low grade conventional epithelial dysplasia in TSAs. Of interest, Yamamoto *et al* also evaluated β -catenin expression in serrated polyps and concluded that β -catenin does not play a role (28). However, review of the immunostaining presented in Figure 3B of that paper does in fact indicate scattered positive nuclear labeling consistent with the Abnormal Pattern #1/weak labeling pattern that we also observed in TSAs.

In summary, we now provide compelling evidence for frequent Wnt pathway activation in SSAs, and in doing so further define the molecular progression of this class of colorectal polyp. These data also indicate that, in a minority of cases, the presence of abnormal β -catenin nuclear labeling may provide support for recommendations regarding the most appropriate management of patients with an isolated and problematic serrated polyp encountered at endoscopy.

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Figure 1. Histologic Features of Serrated Polyps

A. Microvesicular Hyperplastic Polyp. The serrations of the epithelium are confined to the upper half of the crypts, and the numbers of goblet cells are decreased relative to the adjacent normal mucosa. Proliferation is orderly with mitotic figures confined to the basal zones of the crypts. **B. Sessile Serrated Adenoma.** In contrast to the hyperplastic polyp shown in A, the architecture of the crypts is distorted, seen as basal crypt dilatation and crypt branching. The serrations of the crypt epithelium also extend to the base of the distorted crypts. **C. Sessile Serrated Adenoma with Focus of Conventional Epithelial Dysplasia.** In this polyp, the crypt epithelium is again distorted with crypt dilatation, crypt branching and extension of serrated epithelial changes to the base of the distorted crypts. In addition, arrows indicate a focus of

conventional epithelial dysplasia with features of nuclear enlargement and crowding, decreased maturation, loss of mucin, and eosinophilic cytoplasm. *D. Traditional Serrated Adenoma*. This exophytic polyp shows serrated epithelial changes in association with cytoplasmic eosinophilia, nuclear enlargement and crowding, and ectopic crypt formations (ECFs). Examples of ECFs are shown at higher power in E. *F. Tubular Adenoma*. This adenoma shows a top down distribution of adenomatous changes, seen as nuclear enlargement, nuclear crowding, frequent mitotic figures, and loss of mucin.

Figure 2. Patterns of β -Catenin Immunolabeling in Serrated Polyps

A. Sessile Serrated Adenoma with normal pattern of β -catenin labeling. Membranous expression is confined to the base of the distorted crypts. B. Traditional Serrated Adenoma with normal pattern of membranous β -catenin labeling. C. Abnormal Pattern #1 β -catenin labeling in a sessile serrated adenoma. Nuclear accumulation (indicated by arrows) is present in association with a loss of membranous expression. Note that the intensity of expression is relatively weak and present in ~30% of the nuclei. D. Abnormal Pattern #1 β -catenin labeling in a traditional serrated adenoma. Similar to that seen in C, nuclear accumulation (indicated by arrows) is present in association with a loss of membranous expression. E. Abnormal Pattern #2 β -catenin labeling in a sessile serrated adenoma. Strong positive expression is seen, and

virtually all nuclei have accumulation of β -catenin protein. This pattern is similar to that of the tubular adenoma shown in F.

Figure 3. Relationship of β -Catenin Expression to Neoplastic Progression within a Sessile Serrated Adenoma

A. Low power view of a SSAD illustrating the locations of both the background SSA component (discontinuous outline) and the focus of conventional epithelial dysplasia (solid outline) that arose within this polyp. These two regions are shown in higher power in Panels B and D, respectively. Nuclear accumulation of β -catenin protein is present in both the SSA component (C) and the focus of conventional epithelial dysplasia (E).

Figure 4. Genetic Features of Serrated Polyps

A. Example of sequencing results for *BRAF*, *KRAS* and *CTNNB1* in a SSAD. A heterozygous *BRAF* V660E mutation is present within this polyp (indicated by arrow), whereas *KRAS* is wild type. Despite the presence of nuclear accumulation of β -catenin in this polyp, no activating mutations in *CTNNB1* were found. B. Example of sequencing results for *BRAF*, *KRAS* and *CTNNB1* in a TSA. Unlike the SSA shown in A, no *BRAF* mutations were found in this TSA. By contrast, an activating *KRAS* mutation is present (indicated by arrow). However, similar to the SSA, no *CTNNB1* mutations were found despite the presence of nuclear accumulation of β -catenin in this TSA.

Table 1
Summary of *KRAS*, *BRAF* and *CTNNB1* primers

Set	Exon/Gene	PCR Product Size (bp)	Temp (°C)	Primers (5' – 3')
1	2/ <i>KRAS</i>	112	54.6	FWD, AGGCCTGCTGAAAATGACTG REV, TTGGATCATATTCGTCCACAA
2	15/ <i>BRAF</i>	111	53.1	FWD, CTTCATGAAGACCTCACAGT REV, CATCCACATAATGGATCCAG
3	3/ <i>CTNNB1</i>	132	55	FWD, AAGCGGCTGTTAGTCACTGG REV, GGA CT TGGGAGGTATCCACA

Table 2

Clinicopathologic Features

	Hyperplastic Polyp (HP)	Sessile Serrated Adenoma (SSA)	Sessile Serrated Adenoma with Dysplasia (SSAD)	Traditional Serrated Adenoma (TSA)	Tubular Adenoma (TA)	P Value
Total Patients (n=94)	12	26	27	12	17	-
Total Polyps (n=96)	12	27	27	12	18	-
Age (yrs)	62.2±9.9	64.3±9.6	64.5±10.8	59.8±15.2	67.2±10.6	0.450
Gender (M:F)	5:7	10:16	15:11 ^a	8:4	6:11	0.295
Location						
Right	5	24	24	2	11	0.00001
Left	7	3	3	9	7	
Size (cm)						0.001
<0.5	12	12	15	2	10	
≥0.5	0	15	12	9	8	

^aThe gender of one patient was unavailable.

Table 3
Genetic Features of Serrated Polyps

Sample	BRAF	KRAS	CTNNB1	β-catenin IHC
<i>Hyperplastic polyps (HP)</i>				
1	V600E	G12D	WT	Normal
2	WT	WT	WT	Normal
3	V600E	WT	WT	Normal
4	WT	WT	WT	Normal
5	WT	WT	WT	Normal
6	V600E	WT	WT	Normal
7	V600E	WT	WT	Normal
8	WT	WT	WT	Normal
9	WT	G12D	WT	Normal
10	V600E	WT	WT	Normal
<i>Sessile Serrated Adenomas (SSA)</i>				
11	V600E	WT	WT	Normal
12	V600E	WT	WT	Normal
13	V600E	WT	WT	Normal
14	V600E	WT	WT	Normal
15	V600E	WT	WT	Normal
16	V600E	WT	WT	Abn 1
17	V600E	WT	WT	Abn 1
18	V600E	WT	WT	Abn 1
19	V600E	WT	WT	Abn 1
<i>Sessile Serrated Adenomas with Dysplasia (SSAD)</i>				
20	V600E	WT	WT	Abn 2
21	fail	WT	WT	Abn 2
22	V600E	WT	WT	Abn 1
23	V600E	WT	WT	Abn 2
24	V600E	WT	WT	Abn 2
25	V600E	WT	WT	Abn 1
26	V600E	WT	WT	Abn 1
27	V600E	WT	WT	Abn 1
28	V600E	WT	WT	Abn 1
29	V600E	WT	WT	Abn 1
30	V600E	WT	WT	Abn 1
31	V600E	WT	WT	Abn 2
32	V600E	WT	WT	Abn 2
<i>Traditional Serrated Adenomas (TSA)</i>				
33	WT	G12V	WT	Abn 2
34	WT	G12D	WT	Normal
35	WT	G12D	WT	Abn 1
36	WT	G12D	WT	Normal
37	WT	G12D	WT	Normal
38	WT	G12V	WT	Normal
<i>Tubular Adenoma (TA)</i>				
39	WT	WT	WT	Abn 2
40	WT	WT	WT	Abn 2
41	WT	WT	WT	Abn 2