KRAS and *BRAF* mutations and MSI status in precursor lesions of colorectal cancer detected by colonoscopy

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Abstract. Colorectal cancer (CRC) is one of the most frequent cancers worldwide. Adenoma is the main precursor lesion and, recently, the serrated polyps were described as a group of colorectal lesions with malignant potential. The morphologic and biologic characterizations of serrated polyps remain limited. The aim of the present study was to determine the frequency of KRAS and BRAF mutations and microsatellite instability (MSI) in CRC precursor lesions, to evaluate the association between molecular, pathologic and morphologic alterations in precursor lesions and to compare with the alterations detected in CRC. A series of 342 precursor lesions were removed from 155 patients during colonoscopy. After morphologic classification, molecular analysis was performed in 103 precursor lesions, and their genetic profile compared with 47 sporadic CRCs. Adenomas were the main precursor lesions (70.2%). Among the serrated polyps, the main precursor lesion was hyperplastic polyps (HPs) (82.4%), followed by sessile serrated adenomas (12.7%) and traditional serrated adenomas (2.0%). KRAS mutations were detected in 13.6% of the precursor lesions, namely in adenomas and in HPs, but in no serrated adenoma. BRAF mutations were found in 9 (8.7%) precursor lesions, mainly associated with serrated polyps and absent in adenomas (P<0.001). High MSI (MSI-H) was absent in precursor lesions. In the 47 CCR cases, 46.8% exhibited KRAS mutation, 6.5% BRAF mutations and 10.6% MSI-H. This study confirms the role of KRAS and BRAF mutations in CRC carcinogenesis, a crucial step in implementing CRC screening strategies.

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Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in men and the second in women worldwide. In Brazil, it is the third most frequent type of cancer with an estimated 30,000 new cases of CRCs in 2012 (1). In addition, CRC is the second leading cause of mortality worldwide and the fifth in Brazil (2,3). Therefore, it is highly important to improve strategies for CRC prevention and early detection aiming to decrease its incidence and mortality (4).

The majority of CRC cases develop through a stepwise evolution of normal mucosa to precursor lesions and ultimately to a malignant tumor. Adenoma is the principal precursor lesion of CRC (5,6) but, recently, serrated polyp was recognized as an alternative precursor lesion of CRC and follows an alternative pathway in which serrated polyp replaces the traditional adenoma as the precursor lesion to serrated CRC, accounting for ~10% of all CRCs (7,8). Serrated polyps form a heterogeneous group of colorectal lesions that include hyperplastic polyps (HPs), sessile serrated adenoma (SSA), traditional serrated adenoma (TSA) and a combination of two or more characteristics, formerly classified as mixed polyps (MP) (9). HPs are the most common serrated polyp and they have been increasingly suggested to be precursor lesions, since they may develop into other serrated polyps as SSA, TSA or MP to CRC (10).

Colonoscopy is considered the main method for detection and removal of precursor lesions during screening and surveillance of CRC (11). However, it can still miss up to 26% of adenomas and 2% of advanced adenomas (11). Therefore, novel and complementary approaches to detect these potential malignant lesions are required and the application of molecular biomarkers has been considered in the context of CRC screening (12). One of the most challenging issues in biomarker screening is the knowledge of the different molecular pathways implicated in colorectal carcinogenesis and hence the identification of relevant and reliable biomarkers for colorectal screening and surveillance.

The molecular mechanism underlying the adenoma-to-carcinoma sequence has been extensively studied and involves a cumulative acquisition of mutations in tumor suppressor

genes, such as *APC*, and oncogenes such as *KRAS* leading to a phenotype of genomic instability (5). On the other hand, the mechanisms related to serrated carcinoma development are less understood. Mutations of *BRAF* and, less frequently, *KRAS*, are likely to be the initiating events and serrated carcinomas are characterized by microsatellite instability (MSI) and/or CpG island methylator phenotype (CIMP) (7). MSI is a hallmark of CRC arising in the context of hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome (13). However, ~15-20% of sporadic CRCs are MSI (13,14). The MSI is caused by the loss of mismatch-repair genes, which leads to an increased susceptibility to accumulate mutations in genes with microsatellite regions (13,14). Both *KRAS* and *BRAF* encode kinases that belong to the mitogen-activated protein kinase (MAPK) cascade that mediates the cellular signaling involving cell proliferation, apoptosis and differentiation (15). In adenomas, mutations in *KRAS* occur during the early to advanced adenomas in the adenoma-to-carcinoma sequence. However, in the other precursor lesions, there is considerable variability in the frequency of *KRAS* and *BRAF* reported (7).

Considering the wide divergence in the frequency of *KRAS* and *BRAF* mutations in the precursor lesions of CRC and the absence of data in the Brazilian population, the aim of this study was to research the frequency of *KRAS* and *BRAF* mutations and MSI phenotype in precursor lesions of a Brazilian population referred for colonoscopy and to associate molecular alterations with histological and morphological characteristics. Moreover, we compared these findings with molecular alterations found in a series of Brazilian CRC.

Materials and methods

Patients. A total of 155 patients (>50 years old) referred to the Department of Endoscopy of Barretos Cancer Hospital for colonoscopy, from January to October 2011, were prospectively included in this study. A total of 342 lesions were endoscopically removed from 82 (52.9%) men and 73 (47.1%) women with a mean age of 66 years (range 50-89). The main indication for colonoscopy was surveillance after colectomy for CRC (36.4%), followed by surveillance after polypectomy (14.6%), CRC (12.6%) and abdominal pain (9.8%). Ninety-two (59.4%) patients had more than one lesion of the same or different histological type (mean 2.2; range 1-9). Patients with a known family history, hereditary CRC or bowel inflammatory disease were excluded.

For the comparative analysis of molecular alterations, 47 patients with sporadic colorectal adenocarcinoma were retrospectively retrieved from the Department of Pathology of the same hospital and randomly included in the study. The study was approved by the Ethics Committee of Barretos Cancer Hospital.

Endoscopic analysis and tissue specimens. All colonoscopies were performed with high-resolution magnification endoscopes (Fujinon 4400 and Olympus CV GIF 180; Tokyo, Japan) and with targeted dye spraying of the colon using 0.4% indigo carmine solution. The cecum was reached in all cases and all lesions detected were removed. The lesions were characterized according to Paris classification [type 0-I, polypoid (0-Is, sessile; 0-Isp, semi-pedunculated; 0-Ip, pedunculated); type 0-II, non-polypoid (0-IIa, slightly elevated; 0-IIb, flat; 0-IIc, slightly depressed; type 0-III, excavated); LST, laterally spreading type] (16). The site and size of each lesion was annotated and for the purpose of analysis, lesions located in the cecum, ascending colon and transverse colon were regarded as right colon and those from descending colon, sigmoid colon and rectum were regarded as left colon. All lesions removed during colonoscopy were submitted to histological analysis and re-evaluated in a blind manner from the initial pathology classification. The lesions were classified based on WHO criteria (17). The combination of more than one histological type in the same lesion was regarded as MPs. Advanced adenomas were classified if at least 10 mm size or with villous architecture or high-grade dysplasia. For molecular analysis, 103 lesions (one from each patient) were randomly selected, to have a balanced distribution of the different histological subtypes.

DNA isolation. Serial 5-μm unstained sections of formalin-fixed paraffin-embedded blocks were cut, and one adjacent hematoxylin and eosin-stained (H&E) section was taken for pathologist identification and selection of the precursor lesion and tumor tissue. DNA was isolated from 1 unstained section from each specimen as previously described (18). Briefly, tissues were deparaffinized at 80˚C and serial washed with xylene and ethanol (100, 70 and 50%). Selected areas of tumor or precursor lesions were macrodissected using a sterile needle (18G x 1 ½) (Becton Dickinson Ind Cirúrgicas Curitiba-PR, Brazil) and carefully collected into a microtube. DNA was extracted using QIAamp DNA Micro Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. DNA quantity and quality was evaluated by Nanodrop 2000 (Thermo Scientific, Wilmington, DE, USA). DNA samples were diluted to a final concentration of 50 ng/μl and stored at -20˚C for further molecular analysis.

Mutational analysis of KRAS and BRAF. The hotspots regions of the oncogenes *KRAS* (codons 12 and 13) and BRAF (codon 600) were analyzed by polymerase chain reaction (PCR), followed by direct sequencing, as previously described by our group (18,19).

For *KRAS*, PCR reaction was performed in a final volume of 15 μl, under the following conditions: 1.5 μl buffer (Qiagen), 2 mM $MgCl_2$ (Qiagen), 100 mM dNTPs (Invitrogen, Carlsbad, CA, USA), 0.2 mM of both sense and anti-sense primers (Sigma Aldrich, St. Louis, MO, USA), 1 unit of HotStarTaq DNA polymerase (Qiagen) and 1 μl of DNA. The *KRAS* primers used were: GTGTGACATGTTCTAATATAGTCA (sense) and GAATGGTCCTGCACCAGTAA (anti-sense) (19). For *BRAF* the PCR reaction was realized in a final volume of 15 μl, under the following conditions: 1.5 μl buffer (Qiagen), 2 mM $MgCl_2$ (Qiagen), 100 mM dNTPs (Invitrogen), 0.3 mM of both sense and antisense primers (Sigma Aldrich, St. Louis, MO, USA), 1 unit of HotStarTaq DNA polymerase (Qiagen) and 1 μl of DNA. The *BRAF* primers used were: TCATAATGCTTGCTCTGATAGGA (sense) and GGCCAAAAATTTAATCAGTGGA (antisense) (18,19). The PCR was performed in Veriti Termociclador (Applied Biosystems, Austin, TX, USA) using Taq polymerase (Qiagen). The PCR products were evaluated by electrophoresis in agarose gel.

Table I. Endoscopic and histopathological characteristics of precursor lesions of colorectal cancer removed by colonoscopy.

	No. of lesions	%
Location		
Right colon	160	46.8
Left colon	182	53.2
Morphology (Paris classification)		
Polypoid	285	87.4
0-Is	257	78.9
0-Isp	21	6.4
0-Ip	7	2.1
Non polypoid	41	12.6
0-IIa	34	10.5
LST	7	2.1
Size (mm)		
<10	305	90.2
≥10	33	9.8
Histological type		
Adenomas	240	70.2
Tubular	226	66.1
Tubulovillous	14	4.1
Serrated	102	29.8
Hyperplastic polyp	84	24.5
MVHP	50	14.6
GCHP	34	9.9
SSA	13	3.8
TSA	2	0.6
MP	3	0.9

MVHP, microvesicular hyperplastic polyps; GCHP, goblet cell hyperplastic polyps; SSA, sessile serrated adenomas; TSA, traditional serrated adenomas; LST, lateral spreading tumor.

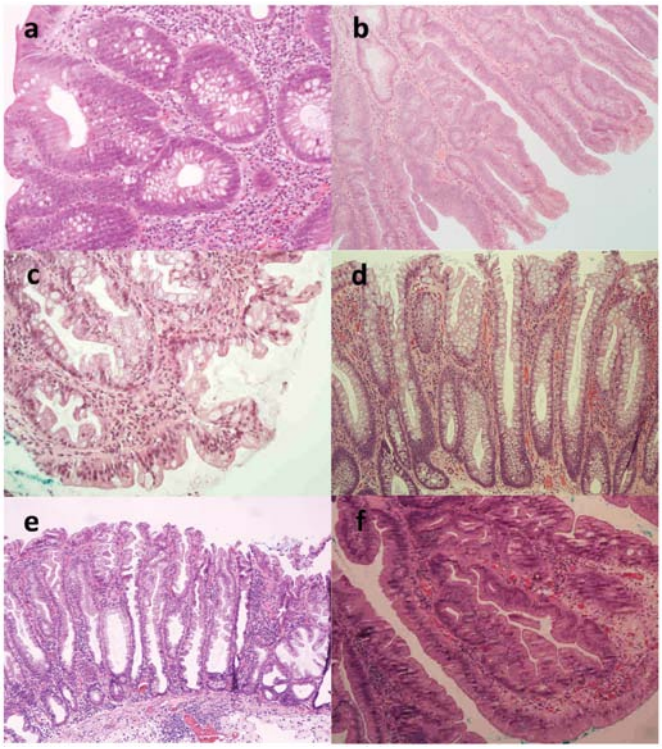

Figure 1. Images obtained from slides stained with H&E of representative cases of precursor lesions. (a) Tubular adenoma with low-grade dysplasia (x200); (b) tubulovillous adenoma with low-grade dysplasia (x200); (c) microvesicular hyperplastic polyp (MVHP) (x200); (d) goblet cell hyperplastic polyp (x100); (e) sessile serrated adenoma (x100); (f) traditional serrated adenoma (x100).

The PCR products of each analyzed exon were firstly purified with EXO-SAP (GE Technology, Cleveland, OH, USA), then, PCR products were submitted to a sequencing reaction using 1 μl of BigDye (Applied Biosystems), 1.5 μl of sequencing buffer (Applied Biosystems) and 1 μl of primer. The sequencing reaction was followed by post-sequencing purification with EDTA, alcohol and sodium citrate. The products of PCR were eluted in HiDye (formamide) and incubated at 95˚C for 5 min and at -4˚C for at least 5 min. Direct sequencing was realized in 3500 series Genetic Analyzer (Applied Biosystems).

All lesions with mutations were confirmed twice with a new PCR and direct sequencing. Additionally, for quality control, in 10% of cases, a new DNA isolation and further mutation analyses were performed.

Analysis of MSI. The MSI evaluation was performed using a multiplex PCR comprising five quasimonomorphic mononucleotide repeat markers (NR27, NR21, NR24, BAT 25 and BAT26), as described by our group (20). The MSI status of the lesion was analyzed using GeneMapper 4.1 software (Applied Biosystems). Cases exhibiting instability at two or more markers were considered to have high MSI (MSI-H), those with instability at one marker were defined as having low MSI (MSI-L) and finally those that showed no instability were defined as microsatellite stable (MSS). In cases with MSI-H, DNA was isolated from adjacent normal tissue and instability of markers was assessed. DNA from cell lines HCT15 (MSI-H) and DNA of healthy people (MSS) were used as controls. Analyses of samples with an abnormal profile were repeated twice.

Statistical analyses. Statistical analyses were performed in SPSS Software® for Windows, version 19.0. The casuistic was characterized by means of descriptive statistics. Categorical variables were compared using the chi-square or Fisher's exact tests, depending on the expected values in the contingency tables. The significance level was set at 5%.

Results

Endoscopic and histopathological features of the colorectal precursor lesions removed by colonoscopy. The endoscopic and histopathological characteristics of the colorectal precursor lesions removed by colonoscopy are summarized in Table I and are illustrated in Fig. 1. For association analysis, serrated polyps were stratified in two groups: SAs (SSA and TSA) (Fig. 1E and F) and HPs (Fig. 1C and D) based on malignant potential differences between them. Due to the presence of more than

Table II. Association between histological types and endoscopic characteristics of precursor lesions of colorectal cancer.

		Serrated polyps		
	Adenomas n (%)	SAs n (%)	HPs n (%)	P-value
Location				
Right colon	130 (54.2)	7 (46.7)	22 (26.2)	<0.001
Left colon	110 (45.8)	8 (53.3)	62 (73.8)	
Morphology				
Polypoid	203 (88.6)	10 (66.7)	69 (87.3)	0.060
Non polypoid	26 (11.4)	5 (33.3)	10 (12.7)	
Size (mm)				
<10	211 (88.3)	12 (80.0)	79 (97.5)	0.009
≥10	28 (11.7)	3 (20.0)	2 (2.5)	

SAs, serrated adenomas; HPs, hyperplastic polyps.

Table III. Histological types and frequency of mutation among precursor lesions of colorectal cancer.

	KRAS	*BRAF*	MSI	
Histological type	Mutated n (%)	Mutated n (%)	MSI-L n (%)	MSI-H n (%)
Adenoma (n=50)	7 (14.0)	0 (0.0)	3 (6.0)	0 (0.0)
Tubular (n=44)	3 (6.8)	0 (0.0)	2 (4.5)	0 (0.0)
Tubulovillous (n=6)	4 (66.7)	0 (0.0)	1 (16.7)	0 (0.0)
Serrated polyps (n=53)	7 (13.2)	9 (17.0)	1 (1.9)	0 (0.0)
Hyperplastic (n=38)	7 (18.4)	3 (7.9)	0 (0.0)	0 (0.0)
MVHP (n=18)	4 (22.2)	1 (5.6)	0 (0.0)	0 (0.0)
GCHP (n=20)	3 (15.0)	2 (10.0)	0 (0.0)	0 (0.0)
SAs (n=15)	0 (0.0)	6 (40.0)	1 (6.7)	0 (0.0)
SSA (n=13)	0 (0.0)	4 (30.8)	0 (0.0)	0 (0.0)
TSA (n=2)	0 (0.0)	2 (100.0)	1 (50.0)	0 (0.0)

MVHP, microvesicular hyperplastic polyps; GCHP, goblet cell hyperplastic polyps; SA, serrated adenomas; SSA, sessile serrated adenomas; TSA, traditional serrated adenomas; HPs, hyperplastic polyps, MSI, microsatellite instability; MSI-L, low level of microsatellite instability; MSI-H, high level of microsatellite instability.

one histopathological type, MP cases were not considered in the association analysis. HPs were located predominantly in the left colon when compared with adenomas (Fig. 1A and B) and SAs (P<0.001, Table II). Non-polypoid type was more likely to be more frequent among SAs compared to adenomas or HPs (P=0.06). A significant association between lesion size and histological type was observed (Table II). Lesions >10 mm were more common among SAs than HPs and adenomas (P=0.009, Table II).

Molecular alterations in colorectal precursor lesions removed by colonoscopy. After the morphological characterization of all lesions, we selected 103 lesions (one from each patient) consisting of 50 adenomas and 53 serrated polyps (13 SSAs, TSAs and 38 HPs) for *KRAS* and *BRAF* mutation analysis and MSI status analysis. The frequency and mutation description are summarized in Table III and Table IV.

Mutations in *KRAS* and *BRAF* were respectively detected in 14 (13.6%) and 9 (8.7%) out of 103 lesions and they were mutually exclusive events (Fig. 2). None of the precursor lesions exhibited MSI-H phenotype.

KRAS mutations were observed in 7 (14.0%) out of 50 adenomas and in 7 (13.2%) out of 53 serrated polyps. None of the SAs were *KRAS* mutated (P=0.223; Tables IV and V). The majority of *KRAS* mutations were found in codon 12 (86.7%), and the most frequent mutation type was Gly12Asp, observed in 8 cases (61.5%) (Table IV). A tubular adenoma had two *KRAS* mutations (Gly12Ala and Gly13Asp).

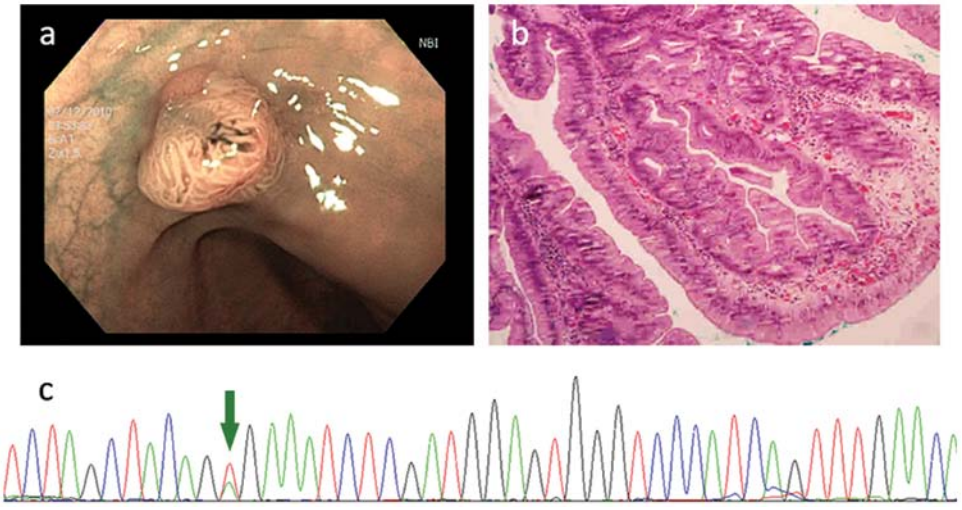

Figure 2. Representative case of traditional serrated adenoma (TSA). (a) Endoscopic view (sessile polyp, 0-Is); (b) H&E at x200 with villiform and complex growth pattern; (c) partial sequence of *BRAF* gene with V600E mutation (arrow).

Table IV. Clinicopathological characteristics of patients with *KRAS*/*BRAF* mutations among precursor lesions of colorectal cancer.

Patient	Age	Gender	Indication	Location	Paris	Size (mm)	Histology	Codon	Mutation type
KRAS mutation									
19	71	F	Post polypectomy	Left	0-Is	<10	Tubulovillous	12	Gly12Asp
8	71	M	Bleeding	Left	0-Isp	<10	Tubulovillous	12	Gly12Ser
14	82	M	Post colectomy	Left	0-Is	<10	Tubulovillous	12	Gly12Val
121	82	M	Rectum neoplasia	Right	0-Is	<10	Tubulovillous	12	Gly12Asp
181	64	M	Post polypectomy	Left	0-Is	<10	Tubular	12	Gly12Asp
151	76	M	Post colectomy	Left	0-Is	<10	Tubular	12	Gly1Val
40	77	M	Post colectomy	Right	0-Is	<10	Tubular	12/13	Gly12Ala+ Gly13Asp
87	64	F	Post polypectomy	Left	0-Is	<10	MVHP	13	Gly13Asp
70	64	F	Rectum neoplasia	Left	0-Is	<10	MVHP	12	Gly12Val
46	60	F	Post colectomy	Left	0-IIa	<10	MVHP	12	Gly12Asp
208	73	M	Rectum neoplasia	Left	0-Is	<10	MVHP	12	Gly12Asp
17	70	F	NR	Left	0-IIa	<10	GCHP	12	Gly12Asp
68	76	M	Post colectomy	Left	0-Is	<10	GCHP	12	Gly12Asp
201	65	F	Ascendent colon neoplasia	Left	0-Is	<10	GCHP	12	Gly12Asp
BRAF mutation									
105	61	F	NR	Right	0-Is	<10	MVHP	600	Val600Glu
36	56	F	Abdominal pain	Right	0-Is	<10	GCHP	600	Val600Glu
30	65	F	Post colectomy	Right	0-Is	≥10	GCHP	600	Val600Glu
64	72	M	Post colectomy	Left	0-Is	≥10	TSA	600	Val600Glu
58	60	M	Post polypectomy	Left	0-Is	<10	TSA	600	Val600Glu
59	89	F	Post colectomy	Left	0-Is	<10	SSA	600	Val600Glu
60	64	M	Abdominal pain	Right	0-Is	<10	SSA	600	Val600Glu
64	70	M	Post colectomy	Left	0-Is	<10	SSA	600	Val600Glu
249	73	F	Loss of weight	Left	0-IIa	<10	SSA	600	Val600Glu

F, female; M, male; Paris, Paris classification.

Table V. Relationship between histological subtypes and molecular alterations in precursor lesions of colorectal cancer.

			Serrated polyps		
	Total	Adenomas	HP	SSA/TSA	P-value
KRAS mutation					
Mutated	14 (13.6)	7 (14.0)	7 (18.4)	0 (0.0)	0.223
Wild type	89 (86.4)	43 (86.0)	31 (81.6)	15 (100.0)	
BRAF mutation					
Mutated	9 (8.7)	0 (0.0)	3 (7.9)	6 (40.0)	<0.001
Wild type	94 (91.3)	50 (100.0)	35 (92.1)	9 (60.0)	

HP, hyperplastic polyp; SSA/TSA, sessile serrated adenomas/traditional serrated adenoma.

BRAF mutations were found in 9 (17.0%) out of 53 serrated polyps and in no adenoma. All *BRAF* mutations were V600E (Val600Glu) (Table IV). *BRAF* mutations were significantly associated with SAs when compared with adenomas and HPs (P<0.001; Table V).

We further analyzed the association between *KRAS* and *BRAF* status and endoscopic characteristics (Table VI). Twelve (85.7%) lesions with *KRAS* mutations were located in the left colon and only 2 (14.3%) in the right colon, while 52 (58.4%) wild-type *KRAS* were located in the left colon and 37 (41.6%)

Table VI. Association between molecular alterations and endoscopic characteristics of precursor lesions of colorectal cancer.

	KRAS			*BRAF*		
	Wild-type	Mutated	P-value	Wild-type	Mutated	P-value
Location						
Right colon	37 (41.6)	2 (14.3)	0.050	35 (37.2)	4 (44.4)	0.727
Left colon	52 (58.4)	12 (85.7)		59 (62.8)	5 (55.6)	
Morphology						
Polypoid	71 (80.7)	12 (85.7)	0.999	75 (80.6)	8 (88.9)	0.999
Non polypoid	17 (19.3)	2 (14.3)		18 (19.4)	1 (11.1)	
Size (mm)						
<10	77 (88.5)	14 (100.0)	0.349	84 (91.3)	7 (77.8)	0.218
≥10	10 (11.5)	0 (0.0)		8 (8.7)	2 (22.2)	

Table VII. Clinicodemographic characteristics of patients with colorectal adenocarcinomas.

Characteristics	n (%)
Mean age ± standard deviation	66±7.5
Gender	
Female	20 (42.6)
Male	27 (57.4)
Location	
Right colon	25 (53.2)
Left colon	22 (46.8)
TNM stage	
I	2 (4.3)
II	18 (38.3)
III	14 (29.7)
IV	13 (27.6)
Differentiation	
Well	15 (32.6)
Moderate	27 (58.7)
Poor	4 (8.7)
Neoadjuvant treatment	
No	39 (83.0)
Yes	8 (17.0)

in the right colon (P=0.05). Regarding morphology and size of the lesions, no association was found with *KRAS* status. On the other hand, *KRAS* mutations were significantly more common in advanced adenomas (33.3%) than in non-advanced adenomas (5.7%) (P=0.020).

No association was found between *BRAF* status and localization, morphology or size of the lesions.

Comparison of precursor CRC lesions and colorectal adenocarcinomas. We further compared the frequency of molecular alterations found in precursor lesions of CRC with molecular findings in 47 CRCs from the same institution. All CRCs were adenocarcinomas, and their clinical and clinical-pathological characteristics are detailed in Table VII. The description of clinical and demographic characteristics of adenocarcinomas harboring *KRAS* or *BRAF* mutations is shown in Table VIII. *KRAS* mutations were detected in 22 (46.8%) cases and *BRAF* mutations were found in 3 (6.5%) colorectal adenocarcinomas. *KRAS* mutations were significantly more frequent in colorectal adenocarcinomas than in precursor lesions (P<0.001). As found in precursor lesions, the most frequent mutations in *KRAS* were at codon 12 (81.8%), and the Gly12Asp was the most frequent mutation (44.5%). All *BRAF* mutations were V600E (Val600Glu), as well as in precursor lesions. MSI-H was detected in 10.6% of all cancers and in no precursor lesions.

Discussion

Colorectal cancer (CRC) is a major health problem in Brazil with an increase in its incidence in the last decade. Approximately 25,000 new cases of CRCs were expected in 2006 and 30,000 in 2012 (1). The only way to change this is through prevention strategies with early detection and resection of their precursor lesions. The morphological and molecular characterization of these lesions has helped us in the understanding of the sequence of events by which normal cells develop into cancer. In line with that, this study sought to contribute to the morphologic and molecular characterization of the different types of colorectal precursor lesions removed during colonoscopy in a Brazilian population with an increased risk for CRC. To the best of our knowledge, this is the first study to describe molecular alterations in colorectal precursor lesions in a Brazilian population.

In our series, adenomas were the most frequent (70.2%) colorectal precursor lesion removed during colonoscopy, as described by other authors in different populations (51-67%) (21-24). Tubular adenomas were more prevalent than tubulovillous and villous adenomas. HPs accounted for ~24% of all serrated lesions followed by SSAs (3.8%), TSAs (<0.6%) and MPs (21,24-26). Most HPs were left-sided and <10 mm. There was a significantly higher number of SAs and conventional adenomas in the right colon than in HPs. In addition, SAs had a

Table VIII. Clinicodemographic characteristics of patients with colorectal adenocarcinomas with KRAS/BRAF mutation.

Patient	Age	Gender	Location	TNM stage	Differentiation	Codon	Mutation type
KRAS mutation							
20	65	M	Left	IIB	Poor	13	Gly13Asp
36	59	M	Right	IIIB	Well	12	Gly12Val
16	64	M	Right	IIIB	Moderate	12	Gly12Asp
45	63	M	Left	IIIB	Poor	13	Gly13Asp
42	64	M	Left	IV	Moderate	12	Gly12Ser
41	58	M	Right	IIB	Moderate	12	Gly12Val
39	57	M	Right	IIIC	Moderate	12	Gly12Asp
37	69	M	Left	IIIC	Moderate	12	Gly12Asp
34	60	F	Right	IIA	Moderate	12	Gly12Val
32	76	F	Left	IIIB	Moderate	12	Gly12Asp
29	68	M	Left	IIIC	Moderate	12	Gly12Ala
28	58	M	Left	IIA	Poor	12	Gly12Asp
26	60	M	Left	IIIB	Well	12	Gly12Asp
24	65	M	Left	IV	Moderate	12	Gly12Ser
18	71	F	Right	IIA	Moderate	12	Gly12Cys
12	82	F	Right	IIA	Moderate	12	Gly12Asp
8	64	F	Right	IV	Well	12	Gly12Asp
7	70	M	Right	IV	Well	12	Gly12Val
5	65	M	Right	IV	Well	13	Gly13Asp
3	63	F	Right	IIIC	Moderate	12	Gly12Glu
48	56	F	Right	IIA	Moderate	13	Gly13Asp
21	57	F	Left	I	Well	12	Gly12Val
BRAF mutation							
17	73	M	Right	IV	Well	600	Val600Glu
6	67	M	Right	IIA	Well	600	Val600Glu
35	80	M	Left	IIA	-	600	Val600Glu

tendency to be non-polypoid lesions compared with adenomas and HPs. These findings are in agreement with previous published studies, which showed that HPs are the most common serrated polyp of the colon accounting for 10-15% of all polyps of the colon and SSAs account for approximately 3-9% of all the colorectal polyps (21-23). Previous studies have also shown that most of the HPs are small (<5 mm) and located in the distal colon (75-80% in the rectosigmoid) and SSAs are generally located in the right colon (27,28). HPs located in the distal colon have been considered indolent lesions, without the need of removal or further endoscopic vigilance. On the other hand, HPs >0.5 cm and located in the right side colon have been associated with increased cancer risk and their removal has been recommended (29). In contrast, SAs should be submitted to the same vigilance as patients with conventional adenomas (30).

In the classic adenoma-carcinoma sequence model of colorectal tumorigenesis proposed by Fearon and Vogelstein, HPs were described as harmless non neoplastic lesions with no malignant potential (5). This concept was challenged since the description of cancer occurrence in patients with hyperplastic polyposis syndrome (31) and in sporadically occurring serrated polyps (32). Approximately 10% of sporadic CRCs, known as serrated adenocarcinoma, will arise via serrated polyp-carcinoma sequence (13). In this context, HPs were recently recognized as neoplastic lesions included in the serrated group and may predispose to cancer. Therefore, efforts have been made to better differentiate serrated lesions without malignant potential from those with high risk. Herein, we performed an analysis of *KRAS* and *BRAF* mutations, and MSI status, to better understand the malignant potential of such lesions.

In the literature, there is considerable variability in *KRAS* and *BRAF* mutation frequencies among colorectal precursor lesions, mainly among serrated polyps. In our series, *KRAS* mutations were detected only in adenomas and HPs. Notably, all mutation types found in precursor lesions were those usually detected in CRC. *KRAS* mutations were significantly associated with advanced adenomas, which have a greater risk of developing into malignant tumors than non-advanced adenomas. Yadamsuren *et al* demonstrated that 57.5% of advanced adenomas harbored *KRAS* mutations compared with 31.0% of non-advanced adenomas in a series of 164 sporadic adenomas (33). These findings are in agreement with the Fearon and Vogelstein model where *KRAS* mutation is responsible for the intermediate stage of adenoma progression (33). At variance, we did not observe *KRAS* mutations in SAs, contrasting with some studies that report the presence of *KRAS* mutations, yet at lower frequencies (8-16,5%) (24,34). This

discrepancy in *KRAS* mutations status can be justified by the small number of cases studied, methodology issues or it can be related to differences in patient ethnic population pertaining to the distinct genetic background of patients. Nonetheless, the absence of *KRAS* mutations in SAs may be an indication that, in this Brazilian population, *KRAS* is not responsible for the serrated pathway.

Our results indicated that *BRAF* is a prevalent marker in the serrated pathway. We observed *BRAF* mutation in ~40% of SAs and this is in line with previous studies that demonstrated a frequency of *BRAF* mutations (V600E) ranging from 32 to 82.9% (33). Collectively, our findings, as well as those of others, are in agreement with a recent study using *BRAF V600E* knock-in murine models that demonstrate the pivotal role of *BRAF* mutations in the initiation of the serrated pathway (35).

In the present study, MSI-H was not detected among colorectal precursor lesions, in accordance with international literature, suggesting that MSI is a late event in the serrated adenocarcinoma progression (13).

In conclusion, the present clinical and molecular characterization of colorectal lesions may contribute to the identification of molecular diagnostic biomarkers as a tool for strategies of screening and early detection of CRC in the Brazilian population. Nevertheless, further studies are required to validate the present findings in a large number of patients, and to extend it not only to high risk CCR populations, as in our study, but also to average risk populations.

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