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ORIGINAL REPORT

Clinicopathological Significance of BAT26 Instability in 184 Patients with Colorectal Cancer

Fabricio Carvalho[1]; Erika M.Monteiro Santos[2], RN, MS; Sergio D.Pena[3], MD, PhD; André Vettore[1], PhD; Ademar Lopes[2], MD, PhD; Fabio O.Ferreira[2], MD, PhD; Samuel Aguiar Junior[2], MD, MS; Wilson T.Nakagawa[2], MD, MS; Benedito M.Rossi[2], MD, PhD

1 Ludwig Institute for Cancer Research – Sao Paulo Branch - São Paulo, Brazil

2 Pelvic Surgery Department – A.C.Camargo Cancer Hospital – São Paulo, Brazil

3 Department of Biochemistry – Federal University of Minas Gerais - Belo Horizonte, Brazil

ABSTRACT

Microsatellite instability (MSI) occurs in 90% of colorectal cancer (CRC) from HNPCC patients and 15% of sporadic CRC. Patients with CRC and MSI have a distinct phenotype. OBJECTIVE: this study evaluates the isolated clinicopathological significance of BAT26 instability by itself in patients with CRC. METHODS: From 1995 to 2000, 184 patients submitted to CRC surgery were selected at random. Medical records were studied in order to determine clinical data and BAT26 analysis was carried out. RESULTS: BAT26 instability was found in 22 (12%) of the 184 cases and was correlated to proximal colon tumors ($p<0.001$); CRC from HNPCC patients ($p=0.002$); poor cell differentiation ($p=0,025$); and mucinous component ($p=0,007$). BAT26 instability tumors have shown a slight trend toward absence of metastases ($p=0,082$). The five-year cancer-specific survival was 65% and 85% for stable and instable BAT26, respectively, with no statistical significance. CONCLUSION: BAT26 instability should be considered a useful screening method to select CRC patients for MSI.

Key words: Microsatellite Repeats. Colorectal Neoplasms / pathology.

INTRODUCTION

Colorectal cancer (CRC) is the sixth in incidence in Brazil. In Southeastern Brazil, though, it is the second most frequent cancer in males, behind prostate cancer, and the third in females, behind breast and uterine cervix cancer. Regardless gender, CRC is the second most common, just after breast cancer, with an estimate of 9,400 new cases per year,[1] whereas in the USA estimated number is 130,200.[2]

Familial Adenomatous Polyposis (FAP) and Hereditary Non-Polyposis Colorectal Cancer (HNPCC) are the two major inherited syndromes related to the development of CRC. In Brazil, FAP and HNPCC are responsible for about 1% and 3% to 8% of CRC cases, respectively. Although there is no data on the incidence of HNPCC, it might be around 752 new cases per year against 10,416 in USA.[3,4]

Besides these two classical syndromes, there still are other ones like recessive familial adenomatous polyposis, linked to *MYH* gene, and hamartomatous polyposis like Peutz-Jeghers syndrome. Although not typically characterized as inherited or sporadic, one very important family group comprises CRC cases and other types of cancer including those associated to HNPCC. This group represents 20%-30% of new CRC cases and their study may help to characterize new genes and syndromes. Therefore, this problem should be carefully investigated especially within the Brazilian population, with such peculiar genetics influenced by Africans, Native Brazilians, and Europeans.

Microsatellite instability (MSI) is recognized as genome–wide alterations in repetitive DNA sequences caused by defects in mismatch repair (MMR) genes, mainly *hMLH1*

Correspondence
Erika M. Monteiro Santos
Departament of Pelvic Surgery
A.C. Camargo Cancer Hospital
Rua Prof. Antonio Prudente, 211
01509-900 São Paulo, Brazil
Phone: 55 11 32070500
E-mail: emonteiro@hcancer.org.br

and *hMSH2*. MSI occurs in about 90% of HNPCC tumors and 15% of sporadic CRC.[5-7] Patients with CRC and MSI have a distinct phenotype: proximal tumor location, poor differentiation, mucinous component, older onset of the disease, increased number of tumors and better prognosis, although a controversial result in some studies.[8]

Bethesda guidelines[9] have established that a panel with five microsatellites should be done for HNPCC suspected patients with CRC: BAT25; BAT26; D2S123; D5S346 and D17S250. If two or more of the five microsatellite sequences in the tumor DNA have been mutated, then the tumor is named MSI-high (MSI-H). If only one of the five have been mutated, then the tumor is named MSI-low (MSI-L). If none of the sequences have been mutated, then the tumor is named microsatellite stable (MSS).[9-10] In general, patients with MSI-L tumors are classified as MMS, but it is recommended an additional panel of microsatellite sequences for testing accurately the tumor characterization. Some authors have suggested that, BAT26 should be the first only test to screen suspected MSI CRC tumors[11], and even that, somatic mutations in the BAT26 sequence accumulate in peripheral blood lymphocytes far before tumor diagnosis in MMR mutation carriers[12].

The objective of this study is to evaluate the clinicopathological significance of BAT26 instability, as the only marker, in 184 patients with CRC.

Material and Methods

Patients

From 1995 until 2000, 184 patients were selected at random from 554 consecutive patients who underwent surgery for CRC at the Department of Pelvic Surgery, A.C. Camargo Cancer Hospital, Sao Paulo, Brazil.

Patient medical records were studied in order to determine clinical data including: age, gender, tumor location, surgery intention (paliative or curative), recurrence, level of CEA and CA 19.9. The pathological characteristics included: mucinous component, cell differentiation, vascular and lymphatic embolization, perineural invasion and tumor stage.

Microsatellite Instability Analysis

Tumor tissue was grossly dissected from adjacent normal tissue from one single paraffin block per case. Genomic DNA from 6 formalin-fixed, paraffin-embedded sections was extracted with the Nucleon HT kit (Amersham Biosciences), according to the manufacturer's instructions.

Microsatellite analysis was carried out using BAT26 marker. The primers used for amplification of the BAT26 adenine mononucleotide repeat located within intron 5 of the *hMSH2* gene were 5'-TGACTACTTTTGACTTCAGCC-3' (sense) and 5'-ACCATTCAACATTTTTAACC-3' (antisense). The sense primer was labeled with the fluorescein 6-FAM.

PCR amplification of DNA was performed in a final volume of 25 µl containing 50-100 mg of genomic DNA, 1X PCR buffer, 3 mM $MgCl_2$, 200 µM of each dNTP, 0.4 µM of each primer, 2 units of Platinum *Taq* DNA polymerase (Invitrogen). The thermal conditions were 94°C for 5 min followed by 40 cycles (94°C for 1 min, 50°C for 1 min and 72°C for 1 min) and a final extension at 72°C for 7 min.

The dye-labelled PCR products were analyzed on ABI PRISM 3100 Genetic Analyser using Genescan 3.7 software (Applied Biosystems).

Statistical Analysis

Statistical analysis was performed using the SPSS software (version 10.0). The Fisher exact test was used to investigate the associations of BAT26 status with independent variables. Two-tailed $p<0.05$ was considered statistically significant.

Survival data was obtained using the Kaplan-Meier method, and comparison of survival was made using the log-rank test, with $p<0.05$ as the significant limit.

Results

Among 184 patients with CRC, 22 have shown BAT26 instability (12%). The average and median age was 61 and 64 year-old, respectively (29 to 92). Relationships between BAT26 status and clinical and pathological variables are presented in Table 1 and Table 2, respectively.

Table 1 – Clinical characteristics of colorectal cancer patients according to BAT26 status

Characteristic		Stable	Instable	Total	p*
Age	≤ 60 y.o.	72 (44.4%)	7 (31.8%)	79 (42.9%)	0.359
	> 60 y.o.	90 (55.6%)	15 (68.2%)	105 (57.1%)	
Gender	Men	72 (44.4%)	6 (27.3%)	78 (42.4%)	0.168
	Women	80 (55.6%)	16 (72.7%)	106 (57.6%)	
HNPCC	No	160 (98.8%)	18 (81.8%)	178 (96.7%)	0.002
	Yes	2 (1.2%)	4 (18.2%)	6 (3.3%)	
Tumor location	Proximal	29 (17.9%)	18 (81.8%)	47 (25.5%)	<0.001
	Distal	133 (82.1%)	4 (18.2%)	137 (74.5%)	
Type of surgery	Curative	132 (81.5%)	21 (95.5%)	153 (83.2%)	0.132
	Paliative	30 (18.5%)	1 (4.5%)	31 (16.8%)	
CEA**	≤ 5 ´g/L	64 (62.7%)	11 (73.3%)	75 (64.1%)	0.587
	> 5 ´g/L	38 (37.3%)	4 (26.7%)	42 (35.9%)	
CA19.9***	≤ 37 U/ml	72 (84.7%)	9 (81.8%)	81 (84.4%)	0.681
	> 37 U/ml	13 (15.3%)	2 (18.2%)	15 (15.6%)	
Recurrence#	No	102 (79.1%)	19 (90.5%)	121 (80.7%)	0.370
	Yes	27 (20.9%)	2 (9.5%)	29 (19.3%)	

* p: Fisher exact test; ** 67 patients without information; *** 88 patients without information; # pacientes submmited to palliative surgery were excluded

Table 2 – Pathological characteristics of colorectal tumors according to BAT26 status

Characteristic		Stable	Instable	Total	p*
T stage**	1 and 2	44 (27.5%)	3 (14.3%)	47 (26.0%)	0.290
	3 and 4	116 (72.5%)	18 (85.7%)	134 (74.0%)	
N status**	Negative	98 (61.6%)	12 (54.5%)	110 (60.8%)	0.642
	Positive	61 (38.4%)	10 (45.5%)	71 (39.2%)	
M status	Negative	127 (78.4%)	21 (95.5%)	148 (80.4%)	0.082
	Positive	35 (21.6%)	1 (4.5%)	36 (19.6%)	
Differentiation***	Well/ Moderate	155 (98.1%)	19 (86.4%)	174 (96.7%)	0,025
	Poor	3 (1.9%)	3 (13.6%)	6 (3.3%)	
Venous invasion#	No	114 (72.6%)	18 (81.8%)	132 (73.7%)	0.445
	Yes	43 (27.4%)	4 (18.2%)	47 (26.3%)	
Lymphatic invasion#	No	77 (49.0%)	13 (59.1%)	90 (50.3%)	0.496
	Yes	80 (51.0%)	9 (40.9%)	89 (49.7%)	
Neural invasion##	No	107 (69.9%)	19 (86.4%)	126 (72.0%)	0.132
	Yes	46 (30.1%)	3 (13.6%)	49 (28.0%)	
Mucinous component	No	147 (90.7%)	15 (68.2%)	162 (88.0%)	0.007
	Yes	15 (9.3%)	7 (31.8%)	22 (12.0%)	

*p: Fisher exact test; ** 3 patients without information; *** 4 patients without information; #5 patients without information; ## 9 patients without information

BAT26 instability was related to proximal colon tumors (p<0.001), HNPCC patients (p=0.002) and mucinous component of the tumor (p=0.007). BAT26 instability has shown a slight trend towards absence of metastases (p=0.082). The cancer-specific survival was 65% versus 80% for stable and unstable BAT26, respectively. p=0.2287).

DISCUSSION

The molecular profile of CRC is likely to be a determinant of clinical outcome. CRC MMR-deficient and MMR-competent have significant differences between their biology.[13]

Emterling et al[14] have detected MSI in 13% of 438 CRC cases using the marker BAT26 alone and the rate was the same or similar to most previous studies of unselected or sporadic CRC using a panel of 5 markers, as suggested by Bethesda criteria.[9] Other authors have shown that BAT26 alone can constitute a specific and sensitive tool for identifying tumors with MSI with a certainty of 86% to 99.4%.[15,16]

Some clinicopathological characteristics of the CRC patients in the present study were significantly correlated to BAT26 alteration: proximal location of the tumor in the colon, Amsterdam criteria for HNPCC patients, poor differentiation and mucinous cell component. Lim SB et al[17] have shown similar results regarding proximal tumor location and poor histological differentiation. The relationship with proximal location and poor differentiation has been reported in other studies[17-19]. Some authors have reported relationships between younger age and female gender and MSI[20,21], but in the present study we have not found such correlation. The relationship between HNPCC and MSI (panel of 5 markers) is widely known[22], but our results have indicated that, when BAT26 is altered alone, the same association can be demonstrated. Wu AH et al[23], in a MSI analysis of 323 sporadic colon cancer cases using 10 microsatellite markers have shown a very high specificity for identifying MSI-H tumors for a number of histopathologic characteristics, including mucinous cell component.

Many studies have found that MSI (panel of 5 markers) is related to better survival rates in CRC patients[5,7]. The results of the present study have shown that the survival rates had a trend to be better in altered BAT26 CRC cases, although with no statistical significance (Figure 1), probably due to the small number of cases, resulting in an insufficient statistical power.

Samowitz et al[24] have performed a population-based study with more than 1,000 individuals with sporadic colon cancer. They have shown a relationship between MSI and better prognosis, independently of other pathological findings. This population-based study has reported that most of the reduction in risk of cancer death associated with MSI has occurred in patients with positive lymph nodes. Some authors have suggested that the better survival associated to MSI has occurred due to chemotherapy[25], whereas other studies have not found the same relationship.[26]

In conclusion, understanding differences in the natural history of tumors arising from the same tissue of origin provides an important clue to clarify molecular and cell biological influences on tumor initiation and progression. CRC is an excellent model to investigate this process and its relationship with potential prognostic factors. Furthermore, CRC has been the subject of various research projects on carcinogenesis. MSI is one of these streams, and BAT26 alone should be seen just as a part of this process, but the results presented here have shown that it could be an important part in this complex puzzle.

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