Lack of microsatellite instability in gastrointestinal stromal tumors

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Abstract. The microsatellite instability (MSI) phenotype may constitute an important biomarker for patient response to immunotherapy, particularly to anti-programmed death-1 inhibitors. MSI is a type of genomic instability caused by a defect in DNA mismatch repair (MMR) proteins, which is present mainly in colorectal cancer and its hereditary form, hereditary nonpolyposis colorectal cancer. Gastrointestinal stromal tumor (GIST) development is associated with activating mutations of KIT proto-oncogene receptor tyrosine kinase *(KIT)* or platelet-derived growth factor receptor α *(PDGFRA)*, which are oncogenes that predict the response to imatinib mesylate. In addition to *KIT*/*PDGFRA* mutations, other molecular alterations are important in GIST development. In GISTs, the characterization of the MSI phenotype is scarce and the results are not consensual. The present study aimed to assess MSI in a series of 79 GISTs. The evaluation of MSI was performed by pentaplex polymerase chain reaction comprising five markers, followed by capillary electrophoresis. The expression of MMR proteins was evaluated by immunohistochemistry. Regarding the *KIT*/*PDGFRA*/B-Raf proto-oncogene, serine/threonine kinase molecular profile of the 79 GISTs, 83.6% of the tumors possessed *KIT* mutations, 10.1% had *PDGFRA* mutations and 6.3% were triple wild-type. The mutated-*PDGFRA* cases were associated with gastric location and a lower mitotic index compared with *KIT*-mutated and wild-types, and these patients were more likely to be alive and without cancer. MSI analysis identified 4 cases with instability in one marker, however, additional evaluation of normal tissue and immunohistochemical staining of MMR proteins confirmed their microsatellite-stable nature. The results of

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the present study indicated that MSI is not involved in GIST tumorigenesis and, therefore, cannot serve as a biomarker to immunotherapy response in GIST.

Introduction

Microsatellite instability (MSI) status has drawn attention as a guide to immunotherapy against different types of tumor (1). Immune checkpoint inhibitors represent a significant advance in precision medicine, inducing durable tumor responses even in patients with late-stage cancer who have failed to respond to multiple previous lines of therapy (1,2). Anti-programmed death (PD)-1 inhibitors, including pembrolizumab, are humanized monoclonal antibodies which block the interaction between PD-1 and its ligands, PD-L1 and PD-L2, and allow T cells to kill the tumor cells (1).

Notably, a phase II study (NCT01876511) in metastatic carcinomas demonstrated that the MSI phenotype constituted an important biomarker for patient response to immunotherapy (1). Most notably, the study revealed that immune checkpoint proteins, including PD-1 and PD-L1, were significantly upregulated in tumors with MSI, enabling them to survive. In MSI colorectal cancer (CRC), PD-L1 is expressed on tumor-infiltrating lymphocytes and/or myeloid cells as opposed to tumor cells (1,2).

MSI is characterized by widespread somatic alterations in the length of nucleotide repeat sequences, which are known as microsatellites (3). The MSI phenotype is a marker of defects in the DNA mismatch repair (MMR) system during DNA replication (3,4). The MSI phenotype is present in all cases of hereditary nonpolyposis CRC syndrome, as well as ~15% of sporadic CRC, while it is less frequently observed in other tumors, including gastric, biliary tract, pancreas, ovary, prostate and small intestine tumors (1,5). In CRC, the presence of MSI is also associated with a number of clinicopathological features, including proximal location, poorly-differentiated tumors, low frequency of distant metastases and an improved prognosis (6).

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the gastrointestinal tract (7), with a global annual incidence of 11-18 per million (8,9). GISTs are considered to originate from the

interstitial cells of Cajal, or a common stem/precursor cell (8,10), and usually arise in the stomach (40-70%) or small intestine (20-40%), and less frequently in the esophagus, colon and rectum (8,11). GISTs also occur elsewhere within the abdominal cavity, primarily in the omentum, mesentery or retroperitoneum (<5% of all GISTs), and these are referred to as extra-gastrointestinal tract tumors (12,13). Histologically, the spectrum of morphology includes spindle, epithelioid or mixed cells (14).

The malignant potential of GISTs ranges from entirely benign to aggressive tumors. However, ~40% of GISTs that are localized at the time of diagnosis eventually metastasize (13). The metastatic dissemination has a predilection to the liver, omentum, peritoneum and other intra-abdominal sites (13). The prognosis of patients with GISTs is based on criteria established by the Armed Forces Institutes of Pathology (AFIP) criteria (15), including tumor location, size and mitotic index. This criterion ranks the patients as benign, very low, low, intermediate and high risk (8).

The majority of GISTs are positive for the proto-oncogene receptor tyrosine kinase (KIT) protein (anti-CD117 is used to identify KIT), and this positivity acts as a crucial diagnostic marker for these tumors (8,16). KIT is a member of the type III receptor tyrosine kinase family, and the binding of its growth factor, stem cell factor (SCF), to the extracellular domain results in dimerization of the receptor and downstream activation of mitogen-activated protein kinase, phosphatidylinositol 3-kinase and Janus kinase/signal transducers and activators of transcription pathways (13).

KIT gene mutations are present in 70-80% of GIST cases (17). These oncogenic mutations result in the constitutive activation of the receptor and consequently, the activation of intracellular pathways (17). *KIT* mutations typically affect the juxtamembrane domain encoded by exon 11 (70% of cases), the extracellular domain encoded by exon 9 (6-15%) and the kinase I and II domains encoded by exons 13 and 17 (2%) (17,18). In particular, deletions have been associated with a worse clinical outcome compared with other types of exon 11 mutation, with shorter progression-free and overall survival times (9). In addition, GISTs harboring *KIT* exon 9 mutations are characterized by small bowel location, aggressive clinical characteristics (9,19) and decreased sensitivity to first line therapy compared with *KIT* exon 11 mutant tumors (9).

Another member of the tyrosine kinase receptor family, platelet-derived growth factor receptor α (PDGFRA), is also involved in GIST pathogenesis (16,20). Mutations in the *PDGFRA* gene occur in 5-7% of cases, in domains which are similar to those in the *KIT* gene (16,21). GISTs harbor mutations in the *PDGFRA* juxtamembrane domain (encoded by exon 12), the ATP-binding domain (encoded by exon 14) or the activation loop (encoded by exon 18) (21). The majority of GISTs with mutated-*PDGFRA* have a distinct phenotype, including gastric location, epithelioid morphology, variable/absent KIT expression as determined by immunohistochemistry and an indolent clinical course (22). In addition, mutations in exon 18 of *PDGFRA* are associated with a lack of response to imatinib therapy (21). Consistent with their functional overlap, *KIT* and *PDGFRA* mutations are mutually exclusive in GISTs (8,16).

Between 10-15% of GISTs are *KIT* or *PDGFRA* wild-type (22). These tumors form a heterogeneous group, a

Table I. Clinicopathological features of gastrointestinal stromal tumors.

Variable	Patients, n (%)
Sex	
Female	41 (46.6)
Male	47 (53.4)
Histological subtype	
Spindle	67 (81.7)
Epithelioid	12 (14.6)
Mixed	3 (3.7)
Primary localization	
Esophagus	1 (1.1)
Stomach	44 (50.0)
Small intestine	25 (28.4)
Rectum	6 (6.9)
Mesentery	1 (1.1)
Retroperitoneum	6 (6.9)
Colon	1 (1.1)
Others[a]	4 (4.5)
Tumor size	
≤5 cm	28 (37.3)
5.1-10 cm	22 (33.3)
>10 cm	25 (29.3)
Mitotic index	
≤5	39 (58.2)
>5	25 (37.3)
6-10	3 (4.5)
AFIP risk classification	
Benign	7 (11.3)
Very low	7 (11.3)
Low	7 (11.3)
Intermediate	9 (14.5)
High	32 (51.6)
Imatinib	
Yes	44 (95.7)
No	2 (4.3)
Local disease recurrence	
Absent	66 (77.6)
Present	19 (22.4)
Metastasis	
Absent	47 (54.7)
Present	39 (45.5)
***KIT/PDGFRA/BRAF* mutation status**	
KIT	66 (83.6)
PDGFRA	8 (10.1)
BRAF	0 (0.0)
Wild-type	5 (6.3)
Current status	
Mortality due to cancer	28 (31.8)

Table I. Continued.

Variable	Patients, n (%)
Current status	
Mortality due to other causes	2 (2.3)
Alive with cancer	27 (30.7)
Alive without cancer	28 (31.8)

AFIP, Armed Forces Institutes of Pathology; KIT, KIT proto-oncogene receptor tyrosine kinase; PDGFRA, platelet-derived growth factor receptor α. [a]Other localizations included rectovagina (2/4), vagina (1/4), and unknown (1/4).

number of which are driven by oncogenic mutations acting downstream of the receptor kinases, such as B-Raf proto-oncogene, serine/threonine kinase (*BRAF*) mutations (described in 1.3% of all tumors) (23,24). A previous study demonstrated that wild-type GISTs exhibit a different genetic background, including mutation in succinate dehydrogenase (21). In either of these cases, there is poor response to first line therapy (9).

Molecular-targeted agents are being utilized as first line treatment for GISTs, including imatinib mesylate and sunitinib maleate. These two agents are KIT/PDGFRA competitive inhibitors that stabilize the inactivated form of the receptors, inhibiting downstream signaling activation (25-27). The median survival time for patients with advanced disease treated with imatinib is 5 years, with 34% of patients surviving >9 years (8). Despite this, the vast majority (≥80%) of patients eventually develop secondary resistance (13). Acquired mutations in *KIT* or *PDGFRA* usually occur in the kinase domain and interfere with drug binding, causing resistance (9,21). The majority of mutations in exon 9 are 6-nucleotide duplications encoding Ala502-Tyr503, which require twice the normal dose of imatinib (800 mg/day) for optimal clinical results. In the *PDGFRA* gene, the most common mutation is a missense mutation in exon 18, which leads to substitution of Asp to Val (termed D842V) (19,28). This mutation is usually resistant to treatment with imatinib (19,28).

In GISTs, the characterization of MSI is limited and the results are controversial (29-31). Therefore, the present study aimed to assess the presence and frequency of MSI using an accurate methodology in a series of 88 Brazilian GISTs, and investigated the association with clinicopathological features of patients.

Patients and methods

Patient population and tissue samples. The present study analyzed 88 patients submitted to resection at Barretos Cancer Hospital (São Paulo, Brazil) between January 1989 and December 2012. A total of 79 primary GISTs were included in the KIT/PDGFRA molecular test and MSI analysis. The other 9 cases were excluded due to poor DNA quality and lower quantity. Clinicopathological data of patients were retrospectively obtained, including age, sex, tumor localization and risk classification (according to AFIP criteria), local disease recurrence, metastasis, chemotherapy and follow-up

status (as of March 2015). In addition, information concerning GIST molecular status (*KIT, PDGFRA* and *BRAF* mutations) was previously reported for 60 cases (32,33). The clinical and molecular data are summarized in Table I.

The average age of the individuals was 57±12.4 years old. The most common histological subtype was spindle cells, and the most common primary localization was the stomach, followed by small intestine, rectum and retroperitoneum, (Table I). The tumors were classified as high risk in 51.6% of cases and the majority of patients were treated with an oral administration of 400 mg of imatinib. Only 2 patients were treated with 5-fluorouracil and/or etoposide. The majority of patients (54.7%) did not experience local recurrence or metastasis. Of those that did, liver (66.7%) and lung (7.7%) were the most common sites of metastasis (Table I).

The present study was approved by the local ethical committees (approval no. 554/2011) of Barretos Cancer Hospital. The ethics committee of our institution authorized that no patient consent was required due to the retrospective nature of the study.

DNA isolation. DNA from samples that had been fixed in 10% formalin for 12-24 h at room temperature and then paraffin-embedded was retrieved from 5-μm cuts, following careful macrodissection of the tumor area and ensuring the presence of >75% of neoplastic cells. DNA extraction was performed using the QIAamp DNA Micro kit (Qiagen, Inc., Valencia, CA, USA), following the manufacturer's protocol, quantified by NanoDropVR 2000 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and stored at -20°C until subsequent genetic analysis.

KIT/PDGFRA/BRAF mutations. *KIT* and *PDGFRA* mutational status was analyzed by polymerase chain reaction (PCR) amplification and subsequent DNA sequencing of exons 9, 11, 13 and 17 to *KIT* and exon 12, 14 and 18 to *PDGFRA*, as previously described (32,33).

Tumors with wild-type *KIT* and *PDGFRA* mutations were analyzed for the presence of exon 15 *BRAF V600E* mutations as previously described (24). The quality of PCR products was confirmed with 1% agarose gel electrophoresis. DNA sequencing of the PCR product was performed using the BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) and an ABI 3500XL Genetic Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.) in accordance with manufacturer's protocol.

MSI analysis. The MSI evaluation was performed using a multiplex PCR comprising five quasi-monomorphic mononucleotide repeat markers (BAT-25, BAT-26, NR-21, NR-24 and NR-27) as previously reported (34-36). The primer sequences used were described in previous studies (34,35). Each antisense primer was end labeled with a fluorescent dye: 6-carboxyfluorescein for BAT-26 and NR-21; 2'-chloro-7'-phenyl-1,4-dichloro-6-carboxyfluorescein for BAT-25 and NR-27; and 2,7,8-benzo-5-fluoro-2,4,7-trichloro-5-carboxyfluorescein for NR-24. PCR was performed using the Qiagen Multiplex PCR kit (Qiagen, Inc.), with 1 μl DNA at 50 ng/ml and the following thermocycling conditions:

Table II. Association between *KIT*/*PDGFRA* mutation status and clinicopathological features of gastrointestinal stromal tumors.

Variable	*KIT* mutation, n (%)	*PDGFRA* mutation, n (%)	Wild-type, n (%)	P-value[a]
Sex				1.000
Female	32 (48.5)	4 (50.0)	2 (40.0)	
Male	34 (51.5)	4 (50.0)	3 (60.0)	
Primary localization				0.398
Esophagus	1 (1.5)	0	0	
Stomach	29 (43.9)	8 (100)	4 (80)	
Small intestine	20 (30.3)	0	0	
Rectum	5 (7.6)	0	1 (20)	
Mesentery	1 (1.5)	0	0	
Retroperitoneum	6 (9.1)	0	0	
Other	4 (6.1)	0	0	
Tumor size				0.963
≤5 cm	19 (37.3)	4 (50.0)	2 (50.0)	
5.1-10 cm	13 (25.5)	2 (25.0)	1 (25.0)	
>10 cm	19 (37.3)	2 (25.0)	1 (25.0)	
Mitotic index				0.018
≤5	24 (51.1)	6 (75.0)	3 (60.0)	
5.1-10 cm	1 (2.1)	2 (25.0)	0 (0.0)	
>10	22 (46.8)	0 (0.0)	2 (40.0)	
AFIP risk classification				0.198
Benign	3 (7.1)	1 (12.5)	1 (20.0)	
Very low	5 (11.9)	2 (25.0)	0 (0.0)	
Low	5 (11.9)	1 (12.5)	0 (0.0)	
Intermediate	4 (9.5)	2 (25.0)	2 (40.0)	
High	25 (59.5)	2 (25.0)	2 (40.0)	
Metastasis				0.097
Absent	34 (52.3)	7 (87.5)	4 (80.0)	
Present	31 (47.7)	1 (12.5)	1 (20.0)	
Status at last follow-up				0.010
Alive without cancer	16 (25.4)	7 (87.5)	3 (60.0)	
Alive with cancer	24 (38.1)	0 (0.0)	0 (0.0)	
Mortality due to cancer	21 (33.3)	1 (12.5)	2 (40.0)	
Mortality due to other causes	2 (3.2)	0 (0.0)	0 (0.0)	

AFIP, Armed Forces Institutes of Pathology; KIT, KIT proto-oncogene receptor tyrosine kinase; PDGFRA, platelet-derived growth factor receptor α. [a]Fisher's exact test.
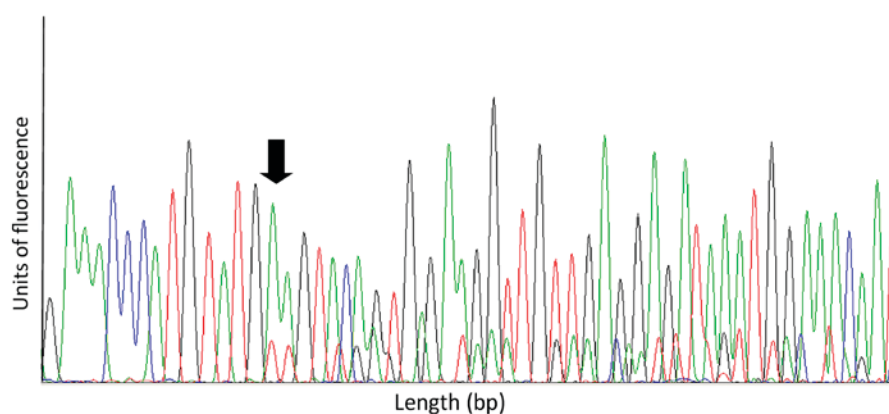
Figure 1. Electropherogram of *KIT*-mutated gastrointestinal stromal tumor (exon 11, p.Glu554_Val559del).
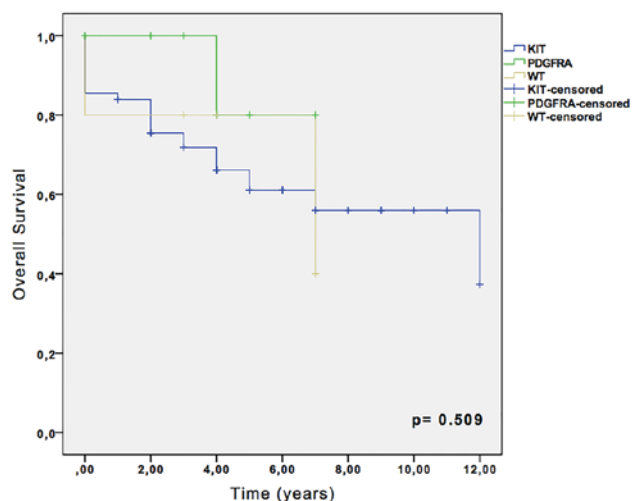
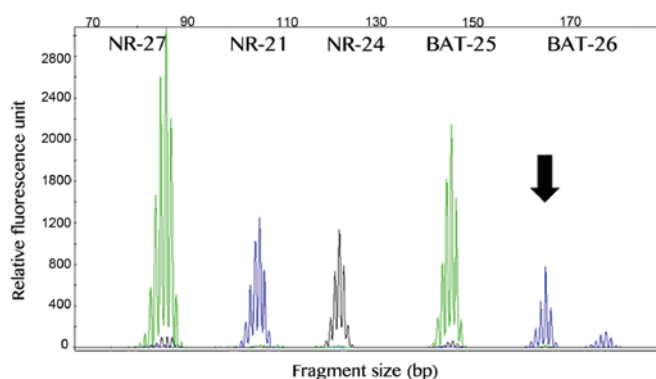Figure 2. Overall survival for KIT and PDGFRA-mutated and wild-type GISTs.

Figure 3. Electropherogram of fragment analysis in a representative gastrointestinal stromal tumor case, with the marker BAT-26 altered (9 nucleotides deletion, arrow).

15 min at 95˚C; 40 cycles of 95˚C for 30 sec; 55˚C for 90 sec and 72˚C for 30 sec; and a final extension at 72˚C for 40 min. PCR products were then submitted to capillary electrophoresis on an ABI 3500XL Genetic Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol, and the results were analyzed using GeneMapper v4.1 software (Applied Biosystems; Thermo Fisher Scientific, Inc.). In all analyses, the DNA from the HCT-15 cell line (ATCC® CCL-225™; American Type Culture Collection, Manassas, VA, USA) (MSI-high) was used as a positive control for MSI.

A previous study by our group determined the quasimonomorphic variation range of each marker for the Brazilian population (36). Accordingly, samples were considered MSI-high when two or more markers were altered, MSI-low when one marker was altered and microsatellite stable (MSS) in the absence of instability. In the MSI-low cases, validation by analysis of normal tissue or the immunohistochemistry of the MMR enzymes in tumor tissue was recommended (36).

MMR immunohistochemistry. Briefly, 10% formalin fixed (for 12-24 h at room temperature) paraffin-embedded tissue specimens were cut into 4-μm sections, which were deparaffinized by heating (75˚C for 4 min) and then were transferred to Autostainer Link 48 equipment (Dako; Agilent Technologies, Inc. Santa Clara, CA, USA) (37). The antigen retrieval process was performed in Tris-EDTA buffer (pH 9.0) at 97˚C for 20 min. The EnVision™ FLEX Wash Buffer (Dako; Agilent Technologies, Inc.) contained Tris with Tween-20 (pH 7.6). Endogenous peroxidases were blocked at room temperature with EnVision™ FLEX Peroxidase-Blocking reagent for 20 min. The primary rabbit polyclonal anti-human antibodies used in the present study were as follows: Anti-mutL homolog 1 (MLH1; dilution, 1:100; clone G168-728, ref. 285M-1); anti-mutS homolog 2 (MSH2; dilution, 1:100; clone G219-1129, ref. 286M-1); anti-PMS1 homolog 2, mismatch repair system component (PMS2; dilution, 1:25; clone EPR3947, ref. 288R-1); and anti-mutS homolog 6 (MSH6; dilution, 1:600; clone 44, ref. 287M-1). All primary antibodies were obtained from Dako (Agilent Technologies,

Inc.) and were incubated at room temperature for 20 min. The secondary antibody was the EnVision™ FLEX/horseradish peroxidase anti-rabbit and anti-mouse IgG (<10 μg/ml) in 10% animal serum in TBS (ref. RE7111; Agilent Technologies, Inc.), which was incubated at room temperature with the samples for 20 min. EnVision DAB solution was used for immunostaining visualization, and was incubated at room temperature with the samples for 10 min. Slides were counterstained with Hematoxylin of Harris (EP-101071; EasyPath, São Paulo, Brazil) at room temperature for 5 min, according to manufacturer's protocol. A light microscope was used to analyze all specimens at magnification, x100-400.

Statistical analysis. Associations between molecular and clinical data from patients were analyzed using the χ^2 test or Fisher's test. Cumulative survival probabilities were calculated using the Kaplan-Meier method. Differences between survival rates were tested with the log-rank test. SPSS 19.0 software (IBM SPSS, Armonk, NJ, USA) was used for all statistical analysis. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Molecular and clinical profile of GISTs. Of the 88 GISTs analyzed, 9 cases were excluded due to poor DNA quality and lower quantity, leaving a total of 79 GIST cases. *KIT* mutations were observed in 83.6% (66/79) of cases and 10.1% (8/79) of cases exhibited *PDGFRA* mutations (Table I). None of the remaining cases (n=5) exhibited *BRAF* mutations, leading to a frequency of 6.3% (5/79) wild-type cases. The *KIT* mutation was located at exon 11 in 58 cases (87.9%), exon 9 in 6 cases (9.1%) and exon 17 in 2 cases (3.0%) Fig. 1 depicts a representative electropherogram of a mutation in exon 11. Regarding *PDGFRA,* 5 cases were mutated at exon 18 (62.5%), 1 case was mutated at exon 12 (12.5%) and 2 cases were mutated at exons 12 and 18 (25.0%).

The associations between *KIT/PDGFRA* mutation status and GIST clinicopathological features are listed in Table II. All *PDGFRA*-mutated GISTs had a gastric location and *PDGFRA*-mutation status was significantly associated with lower mitotic index (P=0.018; Table II). The average follow-up period was 4.3±3.2 years, and 87.5% of patients with *PDGFRA*
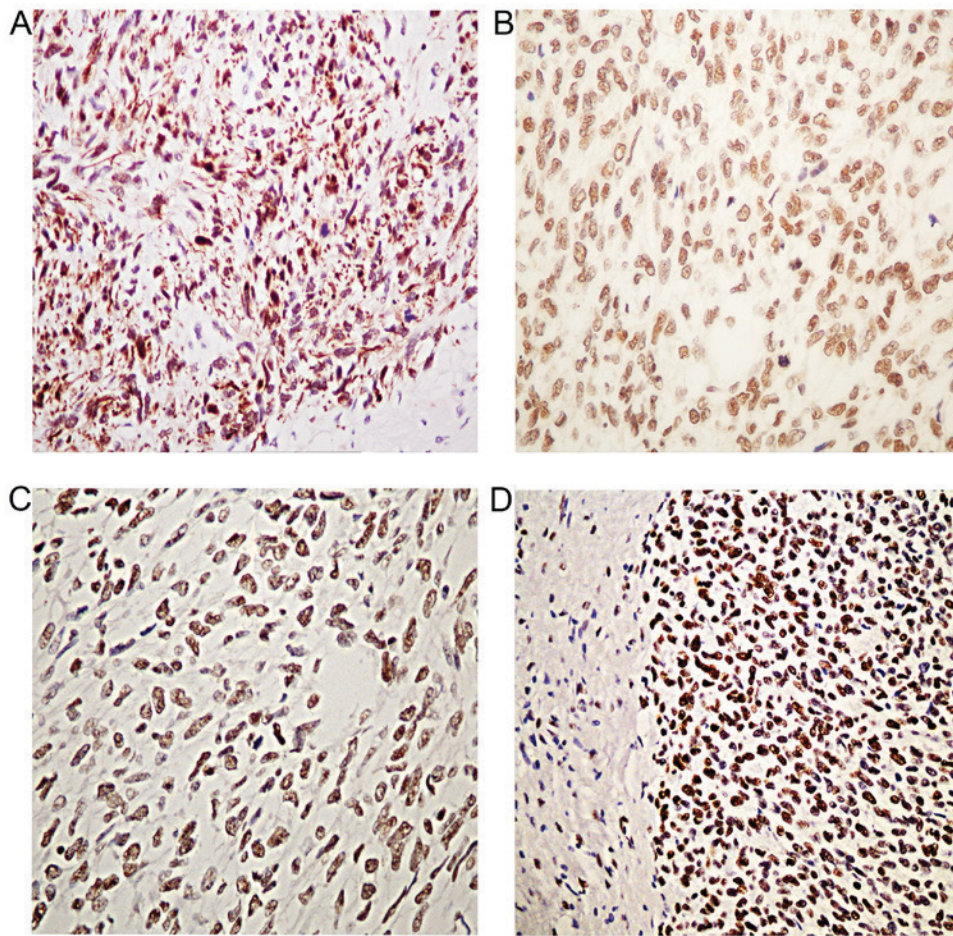
Figure 4. Immunohistochemistry of mismatch repair proteins for 1 case with instability in one marker. Slides were visualized using DAB and counterstained using hematoxylin. (A) mutL homolog 1, (B) PMS1 homolog 2, (C) MSH6 and (D) MSH2. Magnification, x200. MSH, mutS homolog.

mutations were alive with no evidence of cancer, compared with 25.4% of patients with *KIT*-mutations (P=0.010). All *KIT* exon 9-mutated cases exhibited tumor progression following imatinib treatment, while 44.4% of the *KIT* exon 11-mutated cases had stable disease subsequent to chemotherapy (data not shown).

Kaplan-Meier survival analysis revealed that, despite the absence of statistical significance, the 5-year overall survival rate was 66.1% for *KIT*-mutated cases, and 80% for *PDGFRA* and wild-type cases (Fig. 2). No significance was observed in recurrence-free survival analysis among *KIT*, *PDGFRA* and wild-type groups (data not shown).

MSI analysis. The MSI analysis was successful in all 79 GIST cases. A total of 75 (~95%) samples exhibited a stable profile, while 4 primary GISTs exhibited instability in one marker. In total, 2 cases exhibited alteration of the BAT-26 marker, 1 case exhibited alteration of the NR-21 marker and 1 case demonstrated instability in the BAT-25 marker (Fig. 3). Our previous study reported that the presence of instability in one marker in the Brazilian population may be due to polymorphic variants (36). Therefore, it was proposed that analysis of the MMR immunohistochemistry or the MSI analysis of paired normal DNA should be performed for these cases to accurately determine the MSI status of these patients. The investigation of MMR immunohistochemistry

revealed positive staining for all MMR (MLH1, MSH2, MSH6 and PMS2) proteins analyzed (Fig. 4). In addition, the MSI analysis of paired normal DNA in all 4 cases revealed the same genotype in normal and tumor DNA. Thus, these results indicated that all 4 cases were MSS.

Discussion

Determination of MSI status appears to be a marker for novel treatments, and it may serve as a predictive marker for the selection of patients who may benefit from pembrolizumab, an anti-PD-1 immunotherapy (1). The data from this phase II trial support the hypothesis that MMR-deficient tumors are more responsive to PD-1 blockade compared with MMR-proficient tumors (1). However, there is still no data on clinical trials evaluating PD-1 agents in GISTs, despite the growing interest.

The MSI phenotype in GISTs is poorly-characterized and reports are not consensual. In the present study, MSI was analyzed in 79 GIST samples using a multiplex PCR comprising five quasi-monomorphic mononucleotide repeat markers. In the 4 cases that exhibited alteration in only one marker, MSI analysis was performed in paired normal DNA and MMR immunohistochemistry was performed, which revealed the MSS nature of these samples. Therefore, MSI was not present in the present series of GISTs. These findings are in accordance

with the first study addressing the presence of MSI in GISTs by Lopes *et al* (31), which analyzed 33 GISTs. However, other authors reported the presence of MSI in 5% (3/62) and 50% (10/22) of cases (29,30).

It was proposed that these discrepant results may have several causes. First, the number of cases analyzed in the aforementioned two studies was too small for consistent results (27,28). The present study examined 79 cases, which is the largest series that has undergone MSI status evaluation using molecular techniques. Secondly, distinct methodologies for MSI assessment were used, and the accuracy of MSI detection is known to be highly dependent on the techniques selected. Kose *et al* (30) used the BAT-26 marker in analysis of MSI, only in tumor DNA. Fukasawa *et al* (29) evaluated the loss of heterozygosity as well as MSI in paired normal and tumor DNA using dinucleotide markers dispersed on several chromosomes. Tissues were considered MSI-positive when one or more markers were altered. Notably, the two studies evaluated MSI in Japanese populations. This is particularly important due to the quasimonomorphic nature and the effect of the ancestry of the MSI markers. Buhard *et al* (34,35) studied the global population and identified polymorphisms in the BAT-26 marker in up to 3.3% of the Asiatic populations, whereas in Caucasian populations this marker exhibited a monomorphic nature.

In GISTs, the molecular profile serves as a classification system that is useful for diagnostic, prognostic and treatment planning purposes (19,22,38). In the present study, the *KIT* and *PDGFRA* profiles of the 79 GIST cases and their clinicopathological associations were similar to those previously reported in the literature (22). Mutations in *KIT* exon 11 were the most common oncogenic mutations observed in GISTs, followed by *KIT* exon 9. Exon 18 was also revealed to be the most frequently mutated *PDGFRA* region. *PDGFRA*-mutant GISTs frequently possessed characteristics of low-risk GIST, including a gastric primary site and a low mitotic index, as previously reported in the literature (19,22). In addition, a tendency for patients with *PDGFRA* mutations and those with wild-type GISTs to have a smaller risk of recurrence compared with patients with *KIT* mutations was observed.

In conclusion, using accurate MSI methodologies widely used for the assessment of CRC, a large series of confirmed GISTs was analyzed for the presence of genetic instability phenotypes. No cases with MSI were observed, and so it was concluded that the MMR system is proficient in patients with GISTs, and that MSI does not appear to be involved in GIST tumorigenesis.

Acknowledgements

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