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SHORT REPORT

Low frequency of *TERT* promoter mutations in gastrointestinal stromal tumors (GISTs)

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Somatic mutations in the promoter region of *telomerase reverse transcriptase (TERT)* gene, mainly at positions c. – 124 and c. – 146 bp, are frequent in several human cancers; yet its presence in gastrointestinal stromal tumor (GIST) has not been reported to date. Herein, we searched for the presence and clinicopathological association of *TERT* promoter mutations in genomic DNA from 130 bona fide GISTs. We found *TERT* promoter mutations in 3.8% (5/130) of GISTs. The c. – 124C > T mutation was the most common event, present in 2.3% (3/130), and the c. – 146C > T mutation in 1.5% (2/130) of GISTs. No significant association was observed between *TERT* promoter mutation and patient's clinicopathological features. The present study establishes the low frequency (4%) of *TERT* promoter mutations in GISTs. Further studies are required to confirm our findings and to elucidate the hypothetical biological and clinical impact of *TERT* promoter mutation in GIST pathogenesis.
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INTRODUCTION

The *telomerase reverse transcriptase (TERT)* gene encodes the catalytic subunit of telomerase that is crucial to maintenance and regulation of the telomeres.[1,2] In normal somatic adult tissues, telomerase activity is restricted to stem cells, and telomerase reactivation was proposed to be one of cancer hallmarks.[3] Recently, hotspot somatic mutations in the promoter region of *TERT*, located − 124 and − 146 bp upstream from the ATG start site (c. – 124C > T and c. – 146C > T) were reported in several human cancers, including bladder (~85% of mutated cases), gliomas (~50%), thyroid (~15%) and melanoma (22–85%).[4–9] It was proposed that both c. – 124C > T and c. – 146C > T mutations create new binding motif sites (GGAA) of ETS transcription factors leading to upregulation of *TERT* levels and protein activity.[4,5]

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumor on the gastrointestinal tract characterized by hotspot mutations in *KIT* and *PDGFRA* genes, which are predictive of imatinib-based therapy response.[10,11] Somatic *BRAF* mutations[12–14] and germinative *SDHx* mutations were reported in a subset of *KIT*/*PDGFRA* wild-type GIST.[15,16] Increased telomerase activity was reported in GISTs and was associated with poor prognosis.[17] Yet, *TERT* promoter mutation has not been reported in GIST. Herein, we searched for the presence and clinicopathological association of the c. – 124C > T and c. – 146C > T *TERT* promoter mutations in a series of 130 bona fide GISTs.[12,18–20]

MATERIALS AND METHODS

One hundred and thirty cases of GIST were selected from the files of the Department of Pathology from Barretos Cancer Hospital, Brazil, Centro Hospitalar S. João and Garcia de Orta Hospital, Portugal. The cases were retrospectively re-evaluated and classified according to the WHO classification,[21] and were assessed for the mean age, primary localization, tumor size, National Comprehensive Cancer Network (NCCN) risk classification,[22] metastasis and overall survival. The mean age of the patients was 59.8 years, 52.3% were male and the tumors were located mainly in the stomach (50%) and the small intestine (32.7%). Most tumors had tumor size > 5 cm, high malignancy risk and metastatic potential (Table 1).

The characterization of the mutational status for *KIT* and *PDGFRA* was performed in all GISTs.[12,18–20] In addition, the *BRAF* mutation status was evaluated in *KIT/PDGFRA* wild-type GISTs ($n = 9$) from Barretos Cancer Hospital and the *SDH* genes status was evaluated in *KIT/PDGFRA/BRAF* wild-type GISTs ($n = 18$) from Centro Hospitalar S. João.[15,16]

Tumor genomic DNA was extracted from formalin-fixed and paraffin-embedded tissues using the QIAamp DNA MicroKit (Qiagen, Hilden, Germany), following the manufacturer's instructions.[19] A fragment of the *TERT* promoter was amplified with PCR using primers 5′-AGTGGATTCGCG GGCACAGA-3′ and 5′-CAGCGCTGCCTGAAACTC-3′, resulting in a PCR product of 235 bp, which contained the chr5.hg19:g.1295228C > T and Chr5. hg19:g.1295250C > T sites of mutations. Alternatively, gene mutations can be designated based on their upstream location to the ATG initiation codon of *TERT*, as c. – 124C > T, and c. – 146C > T, as previously described.[7] PCR was performed with an initial denaturation at 95 °C for 15 min, followed by 40 cycles of 95 °C denaturation for 30 s, 64 °C annealing for 90 s and 72 °C elongation for 30 s and 72 °C final elongation for 7 min. Quality of PCR products was confirmed with gel electrophoresis. DNA sequencing of the PCR product was performed using the BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 3500 xL Genetic Analyzer (Applied Biosystems). The chromatograms were compared with the reference sequence (GeneBank, TERT:

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ENST00000310581). The SPSS 19.0 software (IBM Corp, Armonk, NY, USA) was used for all statistical analysis. To assess the relationship between variables, we used the Fisher's exact test. The *P*-value established for the statistics significance was <0.05.

Table 1 Association of *TERT* promoter mutation status and clinicopathological and molecular features of GISTs

Variable	*TERT wild type*	*TERT mutated*	P-*value*
Mean Age	59.6	65.9	0.351
Primary localization			
Esophagus	1 (0.9)	0	0.132
Stomach	62 (53)	3 (60)	
Small intestine	37 (31.6)	0	
Large intestine	2 (1.7)	1 (20)	
Rectum	4 (3.4)	1 (20)	
Mesentery	2 (1.7)	0	
Retroperitoneum	6 (5.1)	0	
Esophagus/stomach	1(0.9)	0	
Other	2 (1.7)	0	
Tumor size			
≤ 5 cm	41 (36.3)	1 (20)	0.654
>5 cm	72 (63.7)	4 (80)	
NCCN risk classification[a]			
Benign	2 (2.2)	0	0.646
Very low	19 (21.3)	0	
Low	18 (20.2)	2 (40)	
Intermediate	10 (11.2)	0	
High	40 (44.9)	3 (60)	
Metastasis			
Absent	78 (70.9)	4 (80)	1
Present	32 (29.1)	1 (20)	
Overall survival			
Dead	23 (27.4)	1 (20)	0.398
Alive	61 (72.6)	4 (80)	
KIT/PDGFRA/BRAF status			
KIT mutated	83 (66.4)	2 (40)	0.186
PDGFRA mutated	16 (12.8)	0	
Wild type	26 (20.8)	3 (60)	

Abbreviations: NCCN, National Comprehensive Cancer Network.
[a]Miettinen and Lasota.[22]

RESULTS

We found *TERT* promoter mutations in 3.8% (5/130) of the GISTs (Table 2 and Figure 1). The identified mutations are described in the LOVD database (https://research.cchmc.org/LOVD2/home.php; patient IDs 819–823). The c.−124C>T mutation was the most common event, present in 2.3% (3/130), and the c.−146C>T mutation in 1.5% (2/130) of GISTs. The two mutations occur in a mutually exclusive manner. No statistical correlation was found between *TERT* mutation and GIST clinical or molecular features (Table 1). Yet, *TERT* mutations appeared in tumors of slightly older patients, and no TERT-mutated cases were detected in benign/very-low malignancy risk GISTs (Table 1).

DISCUSSION

This study describes for the first time the occurrence of *TERT* promoter mutations (c.−124C>T and c.−146C>T) in GISTs, being present in ~4% of the cases. As paired blood or constitutive DNA of the tumors analyzed in the present study was not available, we cannot confirm the somatic nature of the c.−124 or c.−146 mutations identified. However, germline mutations at these hotspots were not reported in the various TERT studies that performed such paired (tumor *versus* normal) analysis.[4–7,23–25] In addition, in the COSMIC database,[26] these mutations are described as somatic, and they are not present in the 1000 Genomes database.[27] Therefore, we can almost certainty assume that the mutations observed in GISTs were somatically acquired.

Previously, we analyzed a series of 36 GISTs and did not identify any *TERT* promoter mutation.[7] Likewise, Killela *et al*[6] also analyzed nine GISTs and did not found any *TERT* promoter mutation. As identical methodologies were used in all studies, one plausible reason for this discrepancy is the small number of cases analyzed in the previous studies.[6,7]

TERT promoter mutations seem to be widespread in cancer, although showing tissue specificity. Killela *et al*[6] suggested that cancers developing in tissues that are regularly self-renewing, such as in the gastrointestinal tract, skin and bone marrow, are not likely to harbor telomere-maintaining mutations, as telomerase is already epigenetically activated in their precursor cells. In contrast, cancers arising from cells that are not regularly self-renewing might harbor such mutations. GISTs fit to the second setting, as they are assumed to originate from the low-renewal Cajal cells or their precursors.[28] GISTs are prone to exhibit a high risk of disease relapse and metastasis spreading to distant organs such as the liver, peritoneal surface and lung.[10] Previous reports associate telomerase activity in GIST with higher tumor malignancy risk, metastasis and worse prognosis.[29–31] We found a low frequency of *TERT* mutations in GIST, but any

Table 2 Clinicopathological and molecular data of the GISTs with *TERT* promoter mutation (clinicopathological and molecular features of *TERT* promoter-mutated GISTs)

ID	*Hospital*	*Age (years)*	*Gender*	*Primary localization*	*Tumor size (cm)*	*NCCN risk classification*[a]	*Metastasis*	*Status at last follow-up*	*Follow-up time (years)*	*KIT/PDGFRA/BRAF mutation status*	*TERT promoter mutation*
Case 44	Barretos	77	Female	Stomach	7	Intermediate	Absent	Alive without cancer	10.71	*KIT*	c.−146C>T
Case 75	Barretos	47	Male	Rectum	4	Intermediate	Present	Alive with cancer	11.71	*KIT*	c.−124C>T
Case 159	S. João	49	Male	Stomach	6.5	High	Absent	Alive without cancer	22 .63	Wild type	c.−124C>T
Case 215	S. João	76	Male	Stomach	6	Intermediate	Absent	Alive without cancer	8.78	Wild type	c.−124C>T
Case 216	S. João	81	Male	Large intestine	12.5	High	Absent	Death due to cancer	0.02	Wild type	c.−146C>T

Abbreviation: NCCN, National Comprehensive Cancer Network.
[a]Miettinen and Lasota.[22]

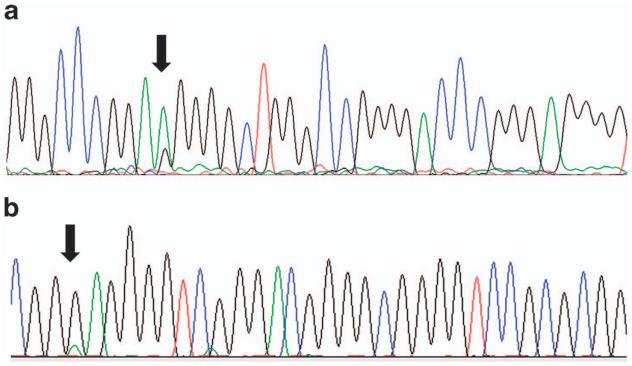

Figure 1 Electropherogram of *TERT* promoter mutations. (**a**) Heterozigotic c.−124C>T mutation (arrow); (**b**) Heterozigotic c.−146C>T mutation (arrow).

statistical association was found with tumor aggressiveness; however, most TERT-mutated GISTs displayed high recurrence risk features. Although our series is undersized to allow definitive conclusions, it would be of interest to further evaluate whether *TERT* promoter mutations associate with a higher expression of telomerase in GISTs, and to assess whether *TERT* promoter mutations associate with poor prognosis as reported in other cancers such as cancers of the thyroid,[32] melanoma[9] and brain.[6]

On the whole, our study establishes the presence of *TERT* promoter mutations in a subset of GISTs (4%). Future studies are required to validate our findings and to elucidate the potential biological and clinical impact of *TERT* promoter mutation in GIST pathogenesis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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