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Molecular Analyses of Early-Onset Gastric Cancer in Brazilian Patients: *TP*53 Mutations, Cadherin-Catenin and Mucins Proteins Expression

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ABSTRACT

Early gastric carcinomas may develop with a molecular profile differing from sporadic carcinomas occurring at a later age. In this study, we analyzed a retrospective series of 88 patients with gastric adenocarcinoma diagnosed before the age of 45 years for the presence of *TP*53 mutations, clinicopathological features and immunohistochemistry to evaluate the expression of markers considered to be important in gastric carcinogenesis (E-cadherin, *β*-catenin, MUC1, MUC2, MUC5AC, MUC6 and p53). The majority of proportion of tumors were diffuse-type (70%) and advanced stage (56%). Familial history of cancer was positive in 21% of the cases. There was a significant association between altered expression of E-cadherin and *β*-catenin, and between p53 expression and perineural invasion. *TP*53 mutations were detected in 14.5% of evaluated cases, including a germline mutation (p.R337H) in a 12-year-old patient. Overall survival analysis showed significant differences in relation with tumor stage and histopathology. The evaluated biomarkers did not present prognostic value in non-exploratory multivariate analyses. The low frequency of *TP*53 mutations in this series suggests these alterations are not a major molecular event in gastric cancer occurring at early age, although the identification of a case with germline p.R337H mutation is consistent with the hypothesis that a small proportion of early, apparently sporadic gastric cancer, may be associated with widespread Brazilian founder mutations. Further studies are needed to evaluate the prognostic significance of markers for specific groups of patients according to tumor histology and familial history.

Keywords: Early-Onset; Gastric Cancer; Young Patients, Cellular Adhesion; *TP*53 Mutations

1. Introduction

According to the Brazilian National Cancer Institute estimative for 2012, gastric cancer is the fourth most common cancer among men and the sixth among women in Southeast region of Brazil. It is estimated 12,670 new cases of the disease in males and 7,420 new cases in females, accounting for an estimated 13 and 7 new cases per 100,000 per year for men and women, respectively [1]. Its etiology is complex, involving genetic susceptibility, familial predisposition and combinations of dietary and environmental factors that drive the accumulation of genetic alterations associated with increasing genetic instability [2]. Gastric cancer occurs predominantly in older patients with a peak of incidence in the 6th and 7th decades. Only 2% to 15% of patients diagnosed with this disease are younger than 45 years, and these cases are defined as early-onset gastric cancer (EOGC) [3-5]. According to recent studies, these patients are thought to develop gastric carcinomas with a molecular genetic profile differing from that of sporadic tumors occurring at a later age. A prominent molecular mechanism is biological alterations in the E-cadherin/catenin adhesion complex. Abnormalities in this adhesion complex could lead to Wnt signaling pathway activation and consequently to the tumor progression and metastasis [6,7]. Loss of function mutations in the CDH1 gene encoding E-cadherin has been shown to be the main genetic basis of Hereditary Diffuse Gastric Cancer (HDGC) [8]. Studies have suggested that mucin alterations can be regarded as molecular biomarkers of malignant transformation of the

gastric mucosa. Gastric carcinomas were found to present a higher level of MUC1 expression than normal mucosa, decreased expression of mucins that are normally expressed in normal gastric mucosa (MUC5AC and MUC6), and expression of the intestinal mucin MUC2 [9,10]. Previously, our group has reported the results of a retrospective study evaluating a series of 515 tumors from patients with gastric adenocarcinoma, divided into two groups according to age (≤40 and >40 years) in order to compare the clinicopathological characteristics, immunohistochemical expression of markers related to cellular adhesion (E-cadherin and *β*-catenin) and differentiation (MUC1, MUC2, MUC5AC and MUC6), and overall survival. The results showed significant differences between the groups regarding the parameters evaluated, corroborating the hypothesis that young patients develop carcinomas by distinct genetic pathways in comparison to patients with gastric tumors in older age [11].

In addition to HDGC, gastric cancer occurs in other familial diseases with inherited cancer predisposition such as hereditary non-polyposis colorectal cancer (HNPCC) and, more rarely, in Li-Fraumeni (LFS) syndrome. The only known underlying genetic defect, observed in 30% of families with Li-Fraumeni features, is germline mutation of the *TP*53 gene [12,13]. In Southeast region of Brazil, a *TP*53 mutant at codon 337 (1010G→A, R337H) occurs at a frequency of about 1:300 individuals (0.3%), which is much higher than the estimated frequency of other germline *TP*53 mutations elsewhere in the world [14,15]. The frequent occurrence of this mutation is caused by a widespread founder effect [16]. Previous studies in Brazil have shown that p.R337H is present in the germline of subjects from families with a wide spectrum of inherited cancers, compatible with Li-Fraumeni-like patterns, and in subjects who are carriers of germline *TP*53 mutations in Brazil, the occurrence of gastric cancer appears to be relatively common [17]. Recently, we reported a rare case of p.R337H mutation in a 12 year-old male patient diagnosed with a T4N2M1 gastric adenocarcinoma in our institution [18].

In this study, we have analyzed a series of 88 patients with gastric adenocarcinomas diagnosed before the age of 45 years for the presence of mutations in the *TP*53 gene. Clinicopathological characteristics were reviewed and immunohistochemical analysis was performed to assess the expression pattern of a panel of biomarkers considered important in gastric carcinogenesis (E-cadherin, *β*-catenin, MUC1, MUC2, MUC5AC, MUC6 and p53).

2. Material and Methods

2.1. Samples

A total of 88 patients diagnosed with gastric adenocarcinoma (≤45 years old) that were admitted to Hospital AC Camargo (São Paulo, Brazil) for the management of adenocarcinoma of the stomach between 1988 and 2010 and who provided informed consent for inclusion of their tissues in the institutional tumor bank, were considered eligible for this retrospective study. Formalin-fixed, paraffin wax-embedded (FFPE) tissue and frozen tissue samples from these patients were retrieved from the archival tissue bank of the Anatomic Pathology Department. Details of patients' age and gender, familial history of cancer, location and histological type of tumor, depth of wall invasion (pT), lymph node metastasis (pN), distant metastases (pM), stage of disease were obtained from medical charts. Tumor staging followed the AJCC/UICC TNM staging manual, 7th edition [19]. Histological typing was based on Laurén's classification [20]. Tumor size was categorized according the medium size of all tumors (≤3 cm and >3 cm). Follow up data were obtained from the hospital records. Overall survival was defined as the time elapsed between primary treatment and death from gastric cancer or from other causes.

2.2. Immunohistochemical Analyses

Immunohistochemistry (IHC) was performed in 3 μm-thick sections from each FFPE sample block by streptavidin-biotin peroxidase technique, as previously described [11]. The following antibodies were used: DO-7 (DakoCytomation, Denmark) for p53 (1:100 dilution); 36 (BD Transduction) for E-cadherin (1:600); 14 (BD Transduction) for *β*-catenin (1:800); and all mucins by Novocastra, UK: MUC1 (1:500), MUC2 (1:1000), MUC5AC (1:500) and MUC6 (1:600). The p53 immunoreactivity was scored as positive when staining was seen in 10% or more tumor cells. The expression of E-cadherin and *β*-catenin in malignant cells was compared with that of non-malignant cells. Only the membrane pattern, which was stained as strongly as normal epithelial cells, was considered as normal. In contrast, the absence of a staining-pattern (loss of staining), cytoplasmic pattern (cytoplasmic staining with loss of membranous expression), and heterogeneous pattern (cytoplasmic staining with preservation of membranous expression) were categorized as altered pattern. Nuclear staining of *β*-catenin was also categorized as altered pattern. In the case of mixed patterns in some sections, the classification was based on the dominant pattern. The expression of the mucins MUC1, MUC2, MUC5AC and MUC6 was considered positive if at least 10% of the neoplastic cells were stained.

2.3. *TP*53 Sequencing Analyses

For FFPE tissues, DNA extraction was carried out using

the QIAamp Micro Kit (Qiagen), and a DNA extraction with phenol-chloroform protocol was performed for frozen tissues. Genomic DNA was screened for *TP*53 mutations at IARC, and direct sequencing of genomic DNA was performed according to the protocols described in the IARC *TP*53 database (http://p53.iarc.fr/). Mutations were screened on both DNA strands and confirmed in an independent PCR product. Tumors were classified as Wild-type or Mutated *TP*53 and the mutations were categorized according the IARC *TP*53 database.

2.4. Statistical Analyses

All statistical analyses were performed using the SPSS 20.0 (SPSS, Chicago, IL). The chi-square test and Fisher's exact test were used to analyze the association between clinicopathological parameters and molecular biomarkers expression. The five-year overall survival rates were estimated using the Kaplan-Meier method. Non-exploratory multivariate Cox models were performed to evaluate the prognostic value of biomarkers (variables of interest). The pathological stage, age and period of treatment were mandatory adjustable variables. In addition, pathological variables with a p value less than 0.10 were also tested in the model (perineural and lymphatic invasion). The level of significance was set at 5%.

3. Results

Of the 88 patients (12 - 45 years old, mean age of 34.6 years) included in this study, 19 (21.6%) were 30 years old or younger. **Table 1** summarizes the frequencies distribution of the clinicopathological variables and biomarkers expression patterns in all patients. There was a higher proportion of male patients, and 21.1% of the patients reported familial history of cancer. Most tumors were of advanced stage disease and diffuse-type gastric tumors. Similar distributions with respect to perineural and lymphatic involvement were observed, but only 14% of the cases presented vascular involvement. The association between p53 and the biomarkers expression pattern and the clinicopathological features (**Table 2**) showed that the frequency of perineural invasion was higher in cases with p53 positivity (p = 0.01). MUC1 positivity was significantly associated with p53 expression. No significant difference between the expression of p53 and the other biomarkers was observed.

*TP*53 mutations were detected in 11 of 76 (14.5%) of the evaluated cases. The age of the patients ranged from 12 to 40 years old with a predominance of male patients. Twelve out 88 patients were not considered for the sequencing analyses due to insufficient DNA quality precluding the complete analysis of all *TP*53 exons. Clinicopathological features, immunohistochemical expres-

Table 1. Clinicopathological characteristics and biomarkers expression of patients with early-onset gastric cancer.

Variables	Categories	N (%)
Gender	M	56 (57.7)
	F	41 (42.3)
Familial history of cancer	N	60 (78.9)
	Y	16 (21.1)
Depth of invasion	T1	19 (22.6)
pT	T2	18 (21.4)
	T3	43 (51.2)
	T4	4 (4.8)
Lymph node metastasis	Negative	37 (44)
pN	Positive	47 (56)
Distant metastasis	Negative	68 (81.0)
pM	Positive	16 (19.0)
Histological classification	Diffuse	61 (69.3)
(Laurén)	Intestinal	17 (19.3)
	Mixed	10(11.4)
Perineural involvement	Negative	46 (53.5)
	Positive	40 (46.5)
Vascular involvement	Negative	74 (86.0)
	Positive	12 (14.0)
Lymphatic involvement	Negative	47 (54.6)
	Positive	39 (45.3)
Tumor location	Cardia	8 (10.2)
	Body	33 (42.3)
	Antrum	29 (37.2)
	Linitis	8 (10.2)
Tumor size	≤3 cm	25 (53.2)
	>3 cm	22 (46.8)
E-cadherin	Altered	41 (55.4)
	Membranous	33 (44.6)
β-catenin	Altered	42 (56.0)
	Membranous	33 (44.0)
MUC1	Negative	37 (74.0)
	Positive	13 (26.0)
MUC2	Negative	25 (49.0)
	Positive	26 (51.0)
MUC5AC	Negative	16 (32.0)
	Positive	34 (68.0)
MUC6	Negative	29 (60.4)
	Positive	19 (39.6)
p53	≤10%	38 (50.7)
	>10%	37 (49.3)

Table 2. Association between p53 and biomarkers expressions and clinicopathological features in early-onset gastric cancer.

Variables	Categories	p53 expression		p value
		≤10%	>10%	
		n (%)	n (%)	
Gender	Male	18 (42.9)	24 (57.1)	0.06
	Female	20 (60.6)	13 (39.4)	
Familial history of cancer	No	26 (51.0)	25 (49.0)	1
	Yes	6 (46.2)	7 (53.8)	
Depth of invasion	T1/T2	17 (58.6)	12 (41.4)	0.47
pT	T3/T4	20 (47.6)	22 (52.4)	
Lymph node metastasis	Negative	16 (55.2)	13 (44.8)	0.81
pN	Positive	21 (50.0)	21 (50.0)	
Distant metastasis	Negative	29 (52.7)	26 (47.3)	1
pM	Positive	8 (50.0)	8 (50.0)	
Histological classification	Diffuse	26 (52.0)	24 (48.0)	
(Laurén)	Intestinal	7 (41.2)	10 (58.8)	1
	Mixed/Unclassified	5 (62.5)	3 (37.5)	
Perineural involvement	Negative	24 (66.7)	12 (33.3)	0.01
	Positive	13 (35.1)	24 (64.9)	
Vascular involvement	Negative	32 (52.5)	29 (47.5)	0.54
	Positive	5 (41.7)	7 (58.3)	
Lymphatic involvement	Negative	20 (52.6)	18 (47.4)	0.81
	Positive	17 (48.6)	18 (51.4)	
Tumor location	Cardia	1 (20.0)	4 (80.0)	0.05
	Body	13 (46.4)	15 (53.6)	
	Antrum	17 (60.7)	11 (39.3)	
	Linitis	3 (50.0)	3 (50.0)	
Tumor size	≤3 cm	12 (57.1)	9 (42.9)	0.22
	>3 cm	11 (50.0)	11 (50.0)	
E-cadherin	Altered	18 (45.0)	22 (55.0)	0.16
	Membranous	15 (51.7)	14 (48.3)	
b-catenin	Altered	18 (43.9)	23 (56.1)	0.13
	Membranous	16 (55.2)	13 (44.8)	
MUC1	Negative	18 (50.0)	18 (50.0)	0.02
	Positive	2 (15.4)	11 (84.6)	
MUC2	Negative	10 (41.7)	14 (58.3)	0.22
	Positive	10 (38.5)	16 (61.5)	
MUC5AC	Negative	6 (37.5)	10 (62.5)	0.23
	Positive	14 (42.4)	19 (57.6)	
MUC6	Negative	12 (41.4)	17 (58.6)	0.23
	Positive	7 (38.9)	11 (61.1)	

sion of the biomarkers and description of the detected mutations in these eleven cases are listed in **Table 3**. Of these mutations, three were silent (at codons 216, 289 and 355) and seven were missense (codons 175, 274, 301, 365, 380, 385 and 337). A *TP*53 mutation at codon 337 (1010G→A, R337H) was detected in the tumor sample of a 12 year-old male patient diagnosed with advanced gastric adenocarcinoma. This rare occurrence was recently published as a Case Report [18].

In this series, 43 patients were alive with no evidence of disease and four were alive with the disease at the time of evaluation. Thirty-seven patients had died from the disease and three from other causes not connected with gastric cancer. Follow-up ranged from six days to 303.42 months, with a median of 43.5 months.

Univariate analysis (**Table 4**) showed that patients with advanced-stage disease ($p < 0.001$), perineural ($p = 0.003$) and lymphatic invasion ($p < 0.001$), and MUC5AC negativity ($p = 0.005$) had a worse survival rate. For the non-exploratory multivariate analysis (**Table 5**) the adjustable variables for each model were the pathologic stage (I + II vs. III + IV), period of treatment (before 2000 vs. as of 2000) and age (continuous variable). The confidence interval was 95%. None of the biomarkers was found to be independent prognostic factors of survival for the patients with gastric cancer.

4. Discussion

Current evidences on the epidemiological and clinicopathological features of gastric cancer in patients younger than 45 years old (defined as early-onset gastric cancer) have suggested that these cancers have distinct molecular and pathological profiles. Despite geographic or ethnic variations, common characteristics of EOGC including female predominance, tumor located at the oesophagogastric junction and in the fundus, diffuse growth types, undifferentiated histology (particularly signet-ring cell carcinoma), and advanced stage and poor prognosis. These characteristics are different from those of older patients [5,21,22].

In our series, there was a predominance of male patients (57.7%) and tumor located in the body and antrum regions, which is consistent with other studies [23,24]. Tumor location has been shown to be an independent prognostic factor in gastric carcinoma, with proximal carcinomas (including the gastric cardia and oesophagogastric junction junction) having a poorer prognosis than distal cancers [25], but this observation was not confirmed in the present study. It has been reported that the poor prognosis of patients with EOGC was due to late diagnosis and advanced disease stage, however some studies have shown that this delay did not interfere in the patient survival [26]. Furthermore, several studies showed

Table 3. Description of clinicopathological features and biomarkers expression of cases presenting *TP*53 mutations.

GCY	*TP53* MUT	Gender	Age	Familial history of cancer	Tumor location	pT	pN	pM	pM1 Location	Laurén Histological classification	Differentiation grade	pIPN	pIVS	pIVL	Status	E-cadherin	ß-catenin	p53
4	R175H	M	19		cardia	2	1	0		Intestinal	Poorly	Pos	Pos	Pos	NED	Alt	Alt*	pos
7	A355A	M	35	colorectal, prostate and uterus	body	1	0	0		Diffuse	Poorly	Neg	Neg	Neg	NED	Alt	Alt	pos
8	V274I	F	36		linitis	3	1	1	Liver	Diffuse	Poorly	Pos	Neg	Pos	AWD	Alt	Alt*	pos
9	V216V, F385L	F	33		body	2	0	0		Diffuse	Poorly	Neg	Neg	Neg	NED	Alt	Memb	pos
18	R175H	M	36		linitis	3	3	1	Peritoneum	Diffuse	Poorly	Pos	Pos	Pos	DOD	Memb	Memb	pos
21	H380Y	M	32		body	3	2	1	Peritoneum	Diffuse	Poorly	Pos	Neg	Pos	DOD	Memb	Memb	neg
45	H365R	M	34			2	1	0		Unclassified	Poorly	Neg	Neg	Neg	DOD	Alt	Alt*	neg
51	L289L	F	37		body	1	0	0		Diffuse	Poorly	Neg	Neg	Neg	NED	Alt	Memb	pos
61	F385L	M	31		antrum	2	0	0		Intestinal	Well	Pos	Neg	Neg	DOD	Alt	Alt*	neg
71	P301S, R337H	M	12		cardia	4	2	1		Intestinal	Moderate	Pos	Neg	Pos	DOD	Memb	Memb	pos
89	R175H	F	40	stomach	linitis	3	3	0		Diffuse	Poorly	Pos	Neg	Neg	DOD	Alt	Alt*	pos

Abbreviations: GCY = sample ID; NED = no evidence of disease; AWD = alive with disease; DOD = dead of disease; Alt = altered expression; Memb = membranous expression; *b-catenin nuclear expression.

Table 4. Five-year overall survival rates according to clinicopathological features and biomarkers expression in early-onset gastric cancer.

Variables	Category	n	5-yr survival rate (%)	p value
Gender	M	47	58.5	0.89
	F	38	61	
Familial history of cancer	No	58	62.9	0.73
	Yes	16	66.2	
Histologic Type	Diffuse	59	57.3	0.87
	Intestinal	16	67	
	Mixed/Unclassified	10	60	
pT	I/II	37	91.4	<0.001
	III/IV	47	36.2	
pN	Negative	37	88.4	<0.001
	Positive	47	37.1	
pM	Negative	68	72.7	<0.001
	Positive	16	7.5	
Stage	Early	57	86.2	<0.01
	Advanced	27	4.3	
Perineural invasion	Negative	46	76.8	0.003
	Positive	39	41.8	
Vascular invasion	Negative	73	61.6	0.54
	Positive	12	50	
Lymphatic invasion	Negative	47	77	<0.001
	Positive	38	39	
E-cadherin	Altered	41	62.6	0.26
	Membranous	30	48.1	
β-catenin	Altered	41	57.4	0.92
	Membranous	31	56.7	
MUC 1	Negative	36	65.5	0.26
	Positive	11	40	
MUC 2	Negative	24	57.6	0.85
	Positive	24	59.2	
MUC 5AC	Negative	16	30.5	0.005
	Positive	32	71	
MUC 6	Negative	28	51.4	0.07
	Positive	18	76.5	
p53	Negative	37	61.3	0.45
	Positive	35	47.4	
*TP*53 mutations	Wild-type	63	60.6	0.65
	Mutated	10	70	

Table 5. Non-exploratory multivariate models in order to evaluate the prognostic value of biomarkers in early-onset gastric cancer.

Model (*)	Number of events	Biomarker	Category	N	HR	95%CI
#1	30	p53	≤10%	37	1	Reference
			>10%	34	0.6	0.3 - 1.5
#2	28	E-cadherin	Preserved	29	1	Reference
			Altered	41	0.6	0.3 - 1.4
#3	28	*β*-catenin	Preserved	30	1	Reference
			Altered	41	0.7	0.3 - 1.6
#4	25	*TP*53	Wild-type	62	1	Reference
		status	Mutated	10	0.6	0.2 - 2.0
#5	17	MUC1	Negative (<10%)	36	1	Reference
			Positive (>10%)	10	0.7	0.2 - 2.2
#6	18	MUC2	Negative (<10%)	23	1	Reference
			Positive (>10%)	24	0.9	0.3 - 2.8
#7	16	MUC6	Negative (<10%)	27	1	Reference
			Positive (>10%)	18	0.5	0.1 - 2.1
#8	18	MUC5AC	Negative (<10%)	15	1	Reference
			Positive (>10%)	32	0.7	0.2 - 3.3

(*) The adjustable variables for each model were: pathologic stage (I + II vs. III + IV), period of treatment (before 2000 vs. as of 2000) and age (continuous variable). 95%CI: Confidence interval (95%).

similar prognosis between young and elderly patients, as long as the disease was diagnosed at an early stage [21,27-29].

There was a predominance of infiltrative tumors and lymph node involvement, as well as the diffuse type tumors (Laurén), which is consistent with other studies [21,30]. Lymphatic, vascular, or perineural invasion have been shown to be adverse prognostic factors [25,31]. Regarding the dissemination of gastric adenocarcinoma it has been demonstrated lymphatic spread is more prevalent in gastric cancer than hematogenic spread [32]. In our series, vascular invasion was observed in a small number of cases (14%) compared to the frequency observed for perineural and lymphatic invasion (49.5 and 45.3%, respectively). The presence of perineural and lymphatic invasion was significantly associated with 5-year overall survival, although these factors were not independent prognostic factors in multivariate analysis.

A recent study performed in our institution compared 515 patients stratified in two groups according to the age (≤40 and >40 years), and demonstrated a high frequency of alterations in the biomarkers expression in the evaluated gastric carcinomas samples. However, the younger patients presented a significantly higher percentage of diffuse-type tumors, higher frequency of preserved biomarkers and better survival rates than the older patients. These findings supported the hypothesis that young patients develop carcinomas with a different genetic pathway compared to tumors occurring at a later age [11].

Consistent with our previous report [11], the patterns of expression of biomarkers in our series were only marginally altered as compared to normal expression patterns. A significant association between the altered expression of E-cadherin and *β*-catenin was observed, confirming the importance of the stability of these two proteins in the maintenance of the functional cellular adhesion system, as reported by other studies [6,33].

In addition to the biomarkers associated to cellular adhesion and differentiation, the expression of p53 as demonstrated by immunohistochemistry was observed in 49.3% of EOGC. Overexpression of p53 has been reported in 17% - 91% of invasive tumors, whereas the reported incidence of *TP*53 mutations in invasive carcinomas ranges from 0% to 77% [34-36].

Clinicopathological features and biomarkers were evaluated according p53 immunopositivity in this study. Positivity for p53 has been related to the proliferating activity and serosal invasion, lymph node metastasis and overall poor prognosis [37,38]. Expression of p53 was also significantly associated with MUC1 expression, independently of overall survival. Furthermore, an association was observed between p53 positivity and perineural invasion, and it was also significantly associated with overall survival. The prognostic significance of perineural invasion in gastric cancer has been previously investigated in few studies with conflicting results [39-41].

Assessment of the role of p53 in gastric cancer in relation to prognosis has produced conflicting results [42-45], largely due lack of consensus in using immunohistochemistry of mutation detection as biomarker.

In 5-year overall survival analyses, tumor characteristics such as pTNM staging, perineural and lymphatic invasion were associated with overall survival in early-onset gastric cancer. The expression of MUC5AC was significantly associated with better prognosis, consistent with previously reported data [46], but except by pTNM staging, these variables were not found to be independent prognostic factors for patients with gastric cancer diagnosed at a young age.

The frequency of a positive familial history of cancer in young patients points to the need to better identify high-risk individuals for screening [47]. Interestingly, none of the reported familial histories fulfilled the criteria for hereditary diffuse gastric cancer (HDGC) or extra-colonic form of Lynch's syndrome, suggesting the possibility of yet unidentified patterns of inheritance.

*TP*53 mutations were found to be rare events, occurring in 14.5% of the evaluated cases, regardless of familial history. This frequency is clearly lower than reported in gastric cancer in general suggesting that mutation of *TP*53 is not a major event in EOGC in this population. This result suggests that other pathways than p53 may play a prominent role. However, their exact nature remains to be determined. One case of EOGC, the youngest patient in our series, was found to be carrier of p.R337H, a common germline *TP*53 variant due to a widespread founder effect in the population of Southeast Eastern Brazil. This observation is compatible with data on families carrying this mutation suggesting that gastric cancer is common diagnosis in these families, and underlines the need for including the risk of gastric cancer in surveillance programs for carriers of the p.R337H mutation.

In summary, our results confirm that patients with EOGC have distinct patterns of biomarkers expression and also indicate that *TP*53 mutation appears to be an infrequent event, adding further evidence that EOGC may represent a specific subtype of gastric cancer from the point of view of molecular mechanisms and, perhaps, genetic risk factors. Although the current series lacked power for assessing multiple biomarkers in multivariate analyses, our observations point to the fact that EOGC has unique molecular features that deserve more detailed investigation in order to identify suitable biomarkers for improving prognosis in this specific group of patients.

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