High-density Array Comparative Genomic Hybridization Detects Novel Copy Number Alterations in Gastric Adenocarcinoma

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Abstract. *Aim: To investigate frequent quantitative alterations of intestinal-type gastric adenocarcinoma. Materials and Methods: We analyzed genome-wide DNA copy numbers of 22 samples and using CytoScan® HD Array. Results: We identified 22 gene alterations that to the best of our knowledge have not been described for gastric cancer, including of v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 4 (ERBB4), SRY (sex determining region Y)-box 6 (SOX6), regulator of telomere elongation helicase 1 (RTEL1) and UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 5 (B4GALT5). The most significant alterations related to peritoneal invasion involved the regions 13q21.1 (gain) and 15q15.1, 17q23.1, 19q13.2 and 20q11.22 (loss of heterozygozity; LOH), where we found LOH of erythrocyte membrane protein band 4.1-like 1 (EPB41L1) gene. In relation to early age of onset, the most significant alterations were gains in the regions Xq26 and Xp22.31 and a loss in the region 11p15.4. Conclusion: These quantitative changes may play a role in the development of this type of neoplasia and may be used as markers in evaluating poor prognosis, as well as act as potential therapeutic targets for gastric cancer.*

Gastric cancer is the fourth most frequent type of cancer (1, 2) and the second cause of cancer mortality worldwide (3). In Northern Brazil, excluding non-melanoma skin cancer, gastric cancer is the second most frequent cancer in men and the third in women (4). The state of Pará has a high incidence of gastric adenocarcinoma and this disease is a public health problem, since mortality rates are above the Brazilian average (5).

This tumor can be classified into two histological types, intestinal and diffuse, according to Laurén (4, 6, 7). The intestinal type predominates in high-risk areas, such as Brazil, and arises from precursor lesions, whereas the diffuse type has a similar distribution in high- and low-risk areas and generally no precursor lesions are identified (8, 9).

Many of these tumors can exhibit features related to aggressiveness and poor outcome, such as early onset (less than 50 years old) (10) and peritoneal invasion (T4 stage), which leads to peritoneal carcinomatosis (11), a disease with a median survival of less than one year with systemic chemotherapy (12). The comprehension of such fundamental processes is very important in reducing morbidity and mortality rates associated with this neoplasia.

The majority of intestinal gastric adenocarcinomas, like other solid tumors, exhibit defects in the maintenance of genome stability, resulting in many DNA copy number alterations that can be analyzed in a genomic approach by array-comparative genomic hybridization (aCGH) (13).

These assays, mainly high-density ones, are a powerful high-throughput technology of molecular cytogenetics for detecting chromosomal copy number aberrations in cancer, aiming at identifying related critical genes from the affected genomic regions (14).

The majority of microarray studies examining gastric adenocarcinoma aim at developing exploratory gene profiles of gastric tumor or gastric cancer cell lines to identify gastric cancer-related genes, delineate molecular phenotypes, demonstrate tumor subtypes, and identify functional gene clusters as potential markers of biological behavior (15-21).

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Table I. *Clinicopathological data and genetic alterations of patients.*

Sample	Gender	Age, years	Localization	TNM/Stage	Laurén classification	No. of genes altered			Mapd*
						Gain	Loss	LOH	
A	Female	46	Antrum and corpus	T3N3a/IIIB	Intestinal	24	337	1	0.22
B	Male	49	Corpus	T3N3b/IIIB	Intestinal	467	1037	5	0.162
C	Male	47	Antrum and pilorus	T3N2/IIIA	Intestinal	136	15	5	0.138
D	Male	49	Antrum and pilorus	T4bN3aM1/IV	Intestinal	355	148	10	0.159
E	Male	35	Antrum	T4bN2/IIIB	Intestinal	314	384	54	0.184
F	Female	36	Antrum, pilorus and corpus	T4aN2/IIIB	Intestinal	9	149	5	0.158
G	Female	50	Antrum	T4N3a/IIIA	Intestinal	122	12	4	0.148
H	Male	35	Corpus	T4bN3aM1/IV	Intestinal	381	144	8	0.152
I	Male	73	Antrum	T4bN2/IIIC	Intestinal	509	470	59	0.162
J	Male	63	Corpus	T4bN3a/IIIC	Intestinal	63	31	5	0.159
K	Male	63	Antrum	T2N1/IIA	Intestinal	264	274	8	0.19
L	Male	61	Corpus	pT4aN3bM1/IV	Intestinal	21	142	12	0.184
M	Male	66	Antrum and pilorus	T3N2/IIIA	Intestinal	316	376	6	0.146
N	Female	55	Antrum and corpus	T3N2/IIIA	Intestinal	8	11	3	0.15
O	Male	52	Corpus	T4aN2/IIIB	Intestinal	48	80	3	0.212
P	Male	68	Corpus	T3N1/IIB	Intestinal	448	500	6	0.156
Q	Male	67	All stomach	T4bN3a/IIIC	Intestinal	451	761	54	0.146
R	Male	64	Corpus and fundus	T4aN3aM1/IV	Intestinal	42	12	4	0.156
S	Male	55	Antrum	T4bN3M1/IV	Intestinal	356	95	14	0.183
T	Male	65	Antrum	T3N2/IIIA	Intestinal	12	30	8	0.156
U	Female	61	Corpus	T3N3a/IIIB	Intestinal	25	25	2	0.157
V	Female	51	Antrum and pilorus	T4bN3b/IIIC	Intestinal	81	1108	4	0.183
Mean						202.4	279.1	12.7	
Standard deviation						182.1	325.1	17.8	

LOH: Loss of heterozygosity. *Quality control for copy number analisys that should be less than 0.25.

Therefore, the objective of this study was to investigate through a high-density aCGH technique, in virtually all quantitative alterations of genome, the most frequent alterations of intestinal gastric adenocarcinoma in an attempt to identify genes that may play critical roles in the carcinogenesis of intestinal-type gastric cancer.

Materials and Methods

Samples. We analyzed 22 samples from patients with intestinal gastric adenocarcinoma, obtained from primary gastric tumors from the João de Barros Barreto University Hospital (HUJBB), located in Pará State, Brazil.

All samples were obtained before administration of chemical treatments or radiotherapy and all individuals signed a Consent Form allowing the use of biological samples and clinical data.

Histopathology. Histopathological data, such as histological subtype, degree of differentiation, depth of invasion, lymph node involvement and distant metastasis, were taken from pathology reports of the Department of Pathology of HUJBB. The histopathological analysis of tumor fragments was performed according to Laurén's classification (6).

DNA extraction. Genomic DNA extraction was performed using Gentra Puregene Kit (Qiagen®, Germantown, MD, USA), according to the manufacturer's instructions. The CytoScan™ Assay (Affymetrix, Santa Clara, CA, USA) requires a genomic DNA concentration of 50 ng/μl or greater. Therefore, the volume for each sample was adjusted accordingly to achieve the desired concentration, using Low EDTA TE buffer (Affymetrix, Santa Clara, CA, USA).

Array comparative genomic hybridization. We performed high-density microarray analyses through the Affymetrix® CytoScan™ HD Array platform, evaluating the complete genome of all 22 patients. This assay uses over 750,000 Single Nucleotide Polymorphisms probes and 1.9 million non-polymorphic copy number probes with a median spacing of 1.1 kb.

The standard protocol has eight general procedures until scanning: digestion of genomic DNA, ligation of *Nsp*I adapter, amplification of fragments by polymerase chain reaction (PCR), fragmentation of PCR products, labeling, hybridization, washing, staining and scanning.

Firstly, genomic DNA was digested by the *Nsp*I restriction enzyme, then the digested samples were ligated using the *Nsp*I adaptor. The fragments were amplified by PCR and then run on a 2% agarose gel to verify if the PCR product distribution was between 150 bp to 2000 bp.

After PCR product purification and dilution, we performed the quantitation of each sample using a Nanodrop® 1000 Spectophotometer (NanoDrop Technologies, Houston, TX, USA). The average purification yield for each sample was ≥3.0 μg/μl.

The purified samples were then fragmented using DNAse I enzyme, then the products were ran on a 4% agarose gel to verify if the majority of fragment distribution was between 25 to 125 bp.

Labeling was performed using terminal deoxynucleotidyl transferase (TdT) enzyme, which added biotinylated nucleotides at the 3' end of fragmented samples.

During hybridization, each sample was hybridized onto a CytoScan® HD Array and placed in a GeneChip® Hybridization Oven 640 at 50˚C and 60 rpm for 16 to 18 hours.

The processes prior to scanning of arrays, washing and staining, were carried out at a Fluidics Station 450 (Affymetrix, Santa Clara, CA, USA). The arrays were scanned using GeneChip® Scanner 3000 7G (Affymetrix, Santa Clara, CA, USA).

The copy number was deduced from the weighted $\log_2$ ratio and the aberration type was identified and confirmed using the allelic plot.

Statistical analysis. The analysis of copy number variation was performed using Affymetrix® Chromosome Analysis Suite Software v1.2.1 - ChAS (Affymetrix, Santa Clara, CA, USA). The association of results with clinicopathological data of the patients were assessed by Fisher's exact test, using the statistical program BioEstat® v5.0 (22). *p*-Values of ≤0.05 were considered significant.

Results

All samples showed multiple gains, losses and loss of heterozygosity (LOH) (Table I). The most frequent alterations observed in patients were amplifications involving 8q (55.5%), 20q (55.5%), 17q (50%), 1q (41%), 7p (41%), 6p (36.4%), 5p (36.4%), 13q (36.4%), 3q (32%), 7q (32%) e 20p (32%); deletions involving 3p (55.5%), 6q (50%), 2q (50%), 1p (45,5%), 5q (41%), 9p (36,4%), Xq (32%) and Xp (27.3%); and LOH involving 1q (36.4%) and 16p (77.3%).

In relation to the most frequent alterations, we found 29 genes that were altered in at least 50% of patients and which are described in literature as being correlated to carcinogenesis of many types of cancers (23-49), except for *KIAA0125* that has never been cited in cancer literature (Table II). It is noteworthy, 22 of these alterations have not been associated with gastric cancer.

Regarding the comparison between the clinicopathological data, stage (T4 *versus* T1-T3) and age (>50 *versus* ≤50 years old), we found a large number of significantly altered genes (Tables III and IV). We did not find significant results correlating any other clinicopathological data.

The most significant alterations related to peritoneal invasion (observed exclusively in T4 stage, p=0.023) involved the regions 13q21.1 (gain), 15q15.1 (LOH), 17q23.1 (LOH), 19q13.2 (LOH) and 20q11.22 (LOH). The majority of genes found inside these regions have not been described in cancer literature, however, among the alterations, we found LOH of erythrocyte membrane protein band 4.1-like 1 (*EPB41L1*), located at 20q11.22, which is well-discussed in cancer literature and is correlated with aggressiveness of other tumor types (50-51).

Table II. *The most frequent genetic alterations found in intestinal-type gastric cancer samples (n=22).*

Gene	Localization	Copy number state	N (%)
*TP53TG3B**	16p11.2	LOH	18 (82%)
*TP53TG3**	16p11.2	LOH	18 (82%)
*ZNF267**	16p11.2	LOH	18 (82%)
*ERBB4**	2q34	1	16 (73%)
FHIT	3p14.2	1	13 (59%)
*LUZP2**	11p14.3	1	13 (59%)
*CDH8**	16q21	1	13 (59%)
LRP1B	2q22.2	1	12 (54.5%)
*GBE1**	3p12.2	1	12 (54.5%)
ROBO2	3p12.3	1	12 (54.5%)
*ADAM3A**	8p11.22	1	12 (54.5%)
*NRG3**	10q23.1	1	12 (54.5%)
*SOX6**	11p15.1	1	12 (54.5%)
*GPC5**	13q31.3	1	12 (54.5%)
*KIAA0125**	14q32.33	3	13 (59%)
*ADAM6**	14q32.33	4	13 (59%)
*RTEL1**	20q13.33	3	11 (50%)
TNFRSF6B	20q13.33	3	11 (50%)
*ZGPAT**	20q13.33	3	11 (50%)
*SLC2A4RG**	20q13.33	3	11 (50%)
*ZBTB46**	20q13.33	3	11 (50%)
*TPD52L2**	20q13.33	3	11 (50%)
*PRPF6**	20q13.33	3	11 (50%)
SOX18	20q13.33	3	11 (50%)
*ASXL1**	20q13.33	3	11 (50%)
*RGS19**	20q13.33	3	11 (50%)
*B4GALT5**	20q13.13	3	11 (50%)
CYP24A1	20q13.2	3	11 (50%)
PTPN1	20q13.13	3	11 (50%)

LOH: Loss of heterozygosity. *Alterations that have never been described in gastric cancer.

In relation to early age of onset, the most significant alterations, found in patients aged 50 years or less, were a gain in the regions Xq26 (cancer/testis antigen family 45, member A4 - CT45A4, p=0.0096), Xp22.31 (steroid sulfatase (microsomal), isozyme S - STS, p=0.0096) and a loss in the region 11p15.4 (olfactory receptor, family 52, subfamily N, member 5 - *OR52N5* and *OR52N1*, p=0.0023). However, to our knowledge, there are no studies in literature correlating these genes with carcinogenesis.

Moreover, we found amplification of the genes ubiquitin B (*UBB*) and transient receptor potential cation channel, subfamily V, member 2 (*TRPV2*) (p=0.0364) in patients aged 50 years old or less, which although not among the most significantly altered genes, are correlated is some studies with progression of carcinogenesis (52-56), which leads us to suspect that these alterations may play a key role in the development of this neoplasia in younger individuals, where gastric cancer is not as common.

Table III. *Alterations differentially observed in the patients with T4 tumor versus those with T1-T3 tumor.*

Gene	Localization	Copy number state	No. of cases		*p*-Value
			T4	T1-T3	
ABCA13	7p12.3	3	5	0	0.049
BAIAP2L1, BRI3	7q21.3	3	5	0	0.049
C7orf69	7p12.3	3	5	0	0.049
CD36, GNAI1	7q21.11	4	5	0	0.049
GLI3	7p14.1	3	5	0	0.049
CHRAC1	8q24.3	3	5	0	0.049
ALG5, C13orf36, CCNA1, CSNK1A1L, EXOSC8, FAM48A, RFXAP, SMAD9, SPG20	13q13.3	3	5	0	0.049
ATP8A2, CDK8, GPR12, RNF6, SHISA2, WASF3	13q12.13	3	5	0	0.049
C13orf15	13q14.11	3	5	0	0.049
C13orf23	13q13.3	3	5	0	0.049
CKAP2	13q14.3	3	5	0	0.049
COG6	13q13.3	3	5	0	0.049
ENOX1	13q14.11	3	5	0	0.049
FREM2	13q13.3	3	5	0	0.049
GTF3A	13q12.13	3	5	0	0.049
GUCY1B2	13q14.3	3	5	0	0.049
HMGB1	13q12.3	3	5	0	0.049
HNRNPA1L2	13q14.3	3	5	0	0.049
KIAA0564	13q14.11	3	5	0	0.049
LECT1	13q14.3	3	5	0	0.049
LHFP	13q13.3	3	5	0	0.049
LOC100188949	13q12.3	3	5	0	0.049
MIR4305	13q13.3	3	5	0	0.049
MIR548F5	13q13.3	3	5	0	0.049
MIR759	13q14.3	3	5	0	0.049
MTIF3	13q12.2	3	5	0	0.049
MTMR6	13q12.13	3	5	0	0.049
MTUS2	13q12.3	3	5	0	0.049
NAA16	13q14.11	3	5	0	0.049
NBEA	13q13.2	3	5	0	0.049
NUPL1	13q12.13	3	5	0	0.049
OLFM4	13q14.3	3	5	0	0.049
OR7E37P	13q14.11	3	5	0	0.049
PCDH8	13q14.3	3	5	0	0.049
PDX1	13q12.2	3	5	0	0.049
POLR1D	13q12.2	3	5	0	0.049
PRR20E, PRR20B, PRR20A, PRR20D, PRR20C	13q21.1	3	6	0	0.023
RFC3	13q13.2	3	5	0	0.049
SERP2	13q14.11	3	5	0	0.049
SLC7A1	13q12.3	3	5	0	0.049
SPERT	13q14.13	3	5	0	0.049
STOML3	13q13.3	3	5	0	0.049
SUGT1	13q14.3	3	5	0	0.049
THSD1	13q14.3	3	5	0	0.049
TPTE2P3	13q14.3	3	5	0	0.049
TRPC4	13q13.3	3	5	0	0.049
TSC22D1	13q14.11	3	5	0	0.049
USPL1	13q12.3	3	5	0	0.049
VPS36	13q14.3	3	5	0	0.049
KIAA0125, ADAM6	14q32.33	3	0	6	0.0011
NCRNA00226	14q32.33	3	0	4	0.0172
PCSK5	9q21.13	1	0	4	0.0172
ARNT, SETDB1	1q21.3	LOH	5	0	0.049
CAPN3, GANC, ZFP106	15q15.1	LOH	5	0	0.049
HAUS2, LRRC57	15q15.1	LOH	6	0	0.023
APPBP2, BCAS3, CA4, CLTC, DHX40, DHX40P1, HEATR6, LOC645638, LOC653653, MIR21, PPM1D, PTRH2, RPS6KB1, SCARNA20, RNFT1, TMEM49, TUBD1, USP32	17q23.1	LOH	6	0	0.023
BZRAP1, C17orf47, C17orf64, C17orf71, HSF5, LPO, MIR301A, MIR454, MKS1, MPO, MTMR4, PPM1E, PRR11, RAD51C, RNF43, SEPT4, SKA2, SUPT4H1, TEX14, TRIM37, YPEL2	17q22	LOH	5	0	0.049
CEACAM5, CEACAM6, CEACAM3, LYPD4, CD79A, ARHGEF1, RABAC1, ATP1A3, GRIK5, ZNF574, POU2F2, MIR4323, DEDD2, ZNF526, GSK3A, ERF, CIC, PAFAH1B3, PRR19, TMEM145, MEGF8, CNFN, LIPE, CXCL17, CEACAM1, CEACAM8	19q13.2	LOH	6	0	0.023
PSG1, PSG10, PSG3, PSG6, PSG7, PSG8	19q13.2	LOH	5	0	0.049
PSMC4	19q13.2	LOH	5	0	0.049
CPNE1, RBM12, NFS1, ROMO1, RBM39, PHF20, SCAND1, C20orf152, LOC647979, EPB41L1, C20orf4, DLGAP4, MYL9, TGIF2, C20orf24, SLA2, NDRG3, DSN1, C20orf117	20q11.22	LOH	6	0	0.023

LOH: Loss of heterozygosity.

Discussion

Although gastric cancer is a highly lethal global disease, the causes are not entirely known. What is clear is that gastric cancer initiation and progression are the outcomes of a stepwise accumulation of genetic alterations. Among these, gene amplification and aberrant expression of oncogenic proteins, as well as deletion or inactivation of tumor-suppressor genes, represent hallmark steps (57-61).

Table IV. *Alterations differentially observed in the patients ≤50 years old versus those >50 years old.*

Gene	Localization	Copy number state	Age		*p*-Value
			≤50 Years	>50 Years	
LOC340094	5p15.33	3	3	0	0.0364
PLEKHG4B	5p15.33	3	4	1	0.0393
AQPEP	5q23.1	1	3	0	0.0364
CEP120	5q23.2	1	3	0	0.0364
LOC644100	5q23.1	1	3	0	0.0364
OR52N5, OR52N1 UBB, TRPV2, NCRNA00188, SNORD49B, SNORD49A, SNORD65,	11p15.4	1	6	1	0.0023
C17orf76	17p11.2	3	3	0	0.0364
CT45A4	Xq26.3	3	4	0	0.0096
CXorf56	Xq24	3	3	0	0.0364
CYLC1	Xq21.1	3	3	0	0.0364
DACH2	Xq21.2	3	3	0	0.0364
KIAA2022	Xq13.3	3	3	0	0.0364
MIR651	Xp22.31	3	3	0	0.0364
PNPLA4	Xp22.31	3	3	0	0.0364
SEPT6	Xq24	3	3	0	0.0364
SMS	Xp22.11	3	3	0	0.0364
STS	Xp22.31	3	4	0	0.0096

In the present study, we identified 29 frequently altered genes in a cohort of patients with intestinal gastric cancer using a high-density aCGH method (Table II). Among them, we highlighted the 22 alterations that to our knowledge have never been described in gastric cancer, however, we chose to discuss only the alterations in v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 4 (*ERBB4*), SRY (sex determining region Y)-box 6 (*SOX6*), regulator of telomere elongation helicase 1 (*RTEL1*) and UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 5 (*B4GALT5*) genes, since there is not sufficient literature regarding the other alterations to develop a consistent discussion.

Although there are many studies regarding *ERBB4* as an oncogene, including of gastric cancer (62-65), we found that 73% of patients had a deletion of this gene. It is noteworthy that *ERBB4* has a controversial role in carcinogenesis, since some studies have reported a tumor-suppressor function of this gene in breast cancer (66, 67). Suo *et al*. demonstrated that ERBB4 expression was associated with favorable outcome in a study of 100 patients with mammary carcinoma, in contrast to ERBB2 (68). Similarly, Witton *et al*., in an analysis of 220 primary breast cancer biopsies, stated that, unlike epidermal growth factor receptor (EGFR), ERBB2 and ERBB3 overexpression, ERBB4 overexpression was associated with estrogen-receptor-positive, lower grade, and significantly better outcome (69).

In a study of 129 cases of ductal carcinoma *in situ*, the absence of ERBB4 predicted recurrence within a 5-year-period, and co-expression of ERBB2 and ERBB4 resulted in a lower risk of recurrence that expression of ERBB2 alone (70).

Taken together, these results support a possible association of ERBB4 overexpression with favorable outcome in breast cancer, and underexpression with a more aggressive tumor phenotype (71). Despite there being no studies showing a tumor-suppressor function of *ERBB4* in gastric cancer, our results, in accordance with the studies cited above, gives new insight of the role of *ERBB4* in intestinal gastric carcinogenesis, which deserves considerably better investigation.

Additionally, we observed a copy number loss of *SOX6* gene in 54.5% of the samples and recent studies have demonstrated that *SOX6* functions either as a tumor suppressor or as an oncogene in different types of human cancer (72). The aberrant expression of *SOX6* has been demonstrated to be involved in tumorigenesis and tumor progression in esophageal squamous cell carcinoma, endometrial cancer, glioma (72-74) and hepatocellular carcinoma (42).

In a recent study, Guo *et al*. observed that both SOX6 mRNA and protein levels were significantly decreased in hepatocellular carcinoma tissues compared to adjacent non-neoplastic liver tissues, conferring a poor prognosis in this type of cancer (42). These findings support the hypothesis that *SOX6* may function as a tumor suppressor in hepatocellular carcinoma (42). Additionally, another study revealed that this gene was frequently down-regulated in primary esophageal squamous cell carcinoma (72).

As cited above, this is the first report of *SOX6* loss in gastric cancer and, since this alteration is related to poor prognosis, it is important to better investigate the impact of the loss of this gene in intestinal gastric carcinogenesis.

In our study, the long arm of chromosome 20 was frequently amplified and several studies have reported the occurrence of this alteration in cervical, gastric, prostate, colon, melanoma, bladder, breast and pancreatic cancer (75-82), suggesting that 20q amplification may play a causal role in tumorigenesis.

According to Tabach *et al*., 20q amplification may induce tumor initiation (83), which leads us to suggest that the frequent gain in the 20q arm (55.5% of patients) may be involved in the onset of gastric cancer in these patients, therefore, the study of the genes involved in such amplification is important in order to investigate the potential of novel biomarkers for early diagnosis.

In this context, it is important to note, due the high density of the assay, that we are the first to identify recurrent amplifications of *RTEL1, TNFRSF6B,* zinc finger, CCCH-type with G patch domain (*ZGPAT*), SLC2A4 regulator (*SLC2A4RG*), zinc finger and BTB domain containing 46 (*ZBTB46*), tumor protein D52-like 2 (*TPD52L2*), pre-mRNA processing factor 6 (*PRPF6*), additional sex combs like

transcriptional regulator 1 (*ASXL1*), regulator of G-protein signaling 19 (*RGS19*) and *B4GALT5* genes, located in the 20q region, in association with intestinal gastric carcinogenesis.

Several recent studies have established an essential role of *RTEL1* in the maintenance of telomere length and genomic stability (82, 32). Given that telomere dysfunction is dramatically mutagenic and plays an important role in tumor initiation and progression (83), *RTEL1* up-regulation is expected to have a tumorigenic function.

The *RTEL1* genomic locus (20q13.3) is frequently amplified in several types of human cancers, including gastric cancer (84-88). Wu *et al*. stated that up-regulation of *RTEL1* activity could also be important for tumorigenesis (32).

We also observed that 50% of the samples had a copy number gain in *B4GALT5* gene. In agreement with these results, Scotto *et al*. identified a total of 26 overexpressed genes as a consequence of 20q gain in cervical cancer, including a number of functionally important genes in cell-cycle regulation, such as *B4GALT5* (30). Furthermore, high gene expression was associated with multidrug resistance in patients with leukemia, probably by regulating the hedgehog pathway and the expression of p-glycoprotein and multidrug resistance-associated protein 1 (43).

In summary, these alterations, being the more frequent, may have an important role in the development and progression of intestinal gastric adenocarcinoma in these patients; therefore, it is imperative to carry-out further studies, to understand the consequence of these alterations in the pathogenesis of this type of neoplasia.

Furthermore, we found that amplification of *TRPV2* and *UBB* genes were significantly associated with patients aged 50 years or less and *EPB41L1* gene was significantly associated with peritoneal invasion. Gastric adenocarcinoma has a peak reported incidence in patients aged from 50 to 70 years. Although the prevalence of gastric cancer has decreased gradually during the last 50 years, the overall trend masks important age-specific characteristics, for example, the proportion of young patients is increasing year-on-year (89). According to Zheng *et al*., gastric cancer in young patients is highly malignant, with a lower rate of curative resection and poorer prognosis (90). Fewer than 10% of patients present with the disease before 45 years of age and these young patients are thought to develop carcinomas with a different molecular genetic profile from that occurring at a later age (91).

Hierarchical cluster analysis of aCGH data on patients with gastric cancer (including young patients) revealed clusters with genomic profiles that correlated significantly with age (92). Gains in chromosomes 17q, 19q and 20q have been found in young patients with CGH (93) and LOH findings have also shown that losses are infrequent in this group of patients (91).

The vanilloid receptor family (TRPV) is a sub-group of the transient receptor potential (TRP) superfamily of ion channels, and six members (TRPV1-6) have so far been identified (94). TRP channels constitute a novel area of research in oncology. Malignant transformation of cells is the result of enhanced proliferation, aberrant differentiation and impaired ability to die, resulting in abnormal tissue growth, which can eventually turn into uncontrolled expansion and invasion, characteristic of cancer. Such transformation is often accompanied by changes in ion channel expression and, consequently, by abnormal progression of the cellular responses with which they are involved (95).

High expression levels of members of the TRPV family were correlated with the emergence or progression of certain types of epithelial cancer, such as prostate cancer and melanoma (52-55).

Monet *et al*. stated that *TRPV2* transcript levels were 12-times higher in patients with metastatic prostate cancer (stage M1) compared to primary solid tumors (stages T2a and T2b) (96). Moreover, silencing of this channel drastically reduced the migration of prostate cancer cells, whereas its overexpression increased their migration. Monet *et al*. also found *TRPV2* contributes to enhanced cancer cell migration by induction of expression of key metalloproteinases MMP2 and MMP9, and cathepsin B, which are related to the invasive potential of cancer cells (96).

It is noteworthy that alterations involving the *TRPV2* gene in gastric carcinogenesis have never been described in literature, although some studies have reported the amplification of the 17p region, where this gene is located (97, 98).

The *UBB* gene encodes ubiquitin, one of the most conserved proteins known. The ubiquitin system is extremely versatile and can play multiple essential roles in various cellular processes by regulating not only protein stability but also protein interactions, trafficking, and activation. Therefore, it is not surprising that alterations in the ubiquitin system have been observed in many types of human cancers and that many of its components, when de-regulated, have been found to play key roles in cellular processes relevant to tumorigenesis (99, 100).

The elevated level of ubiquitin has been observed in most, if not all, cancer cells (101-105). In addition, a positive relationship between ubiquitin levels and the progression of hepatocellular carcinoma has been reported (106).

Oh *et al*. demonstrated that ubiquitin levels are efficiently reduced by small interfering RNA (siRNA), which effectively inhibited the survival and proliferation of cancer cells (56), suggesting that it has potential as a new therapeutic intervention for cancer treatment.

Therefore, these quantitative changes in the genetic material of tumors may be involved in gastric carcinogenesis, and may have a key role in the development of this neoplasia in younger individuals.

The progression of epithelial tumors to invasive carcinomas involves changes in cell polarity, adhesion and motility that permit the detachment of cancer cells from the epithelial layer, their invasion into adjacent tissue layers, and eventually their spread throughout the body. These processes require reorganization of the cellular cytoskeleton and altered expression of proteins that connect it to the cell membrane as well as remodeling of the extracellular matrix, including changes in the composition and processing of its constituents (107).

The ability of EPB41L1 proteins to bind very structurally diverse interaction partners *via* their different protein domains enables them to participate in many different physiological processes in a variety of cell types and tissues (108). These EPB41L1 proteins contribute to the organization of cell polarity, adhesion, motility, and affect transport through the membrane and responses to growth factors (107). The EPB41L1 protein was detected in various cell types and tissues, however, its functions in non-erythroid cells are not as clear (51).

Zhenyu *et al*. stated that EPB41L1 potentially serves as an inhibitor of migration and invasion by restoring the membrane cytoskeleton (51). Additionally, the expression of EPB41L1 also was moderately, but significantly decreased in prostate cancer tissues (107).

In our study, we frequently found LOH for this gene, which is a clear mechanism for gene inactivation (109). Taken together, these studies and our results suggest that *EPB41L1* may have had an important role in progression and invasion of gastric cancer in the group of patients with peritoneal invasion.

Through the microarray technique, we were able to identify several quantitative changes in the genome of intestinal gastric adenocarcinoma and novel genes associated with gastric carcinogenesis. A better investigation of these findings could provide useful pathway maps for the future understanding of the molecular pathogenesis of this malignancy, which can represent efficient tools in evaluating poor prognosis, as well as potential therapeutic targets for gastric cancer.

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