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Numerical aberrations of chromosome 8 detected by conventional cytogenetics and fluorescence in situ hybridization in individuals from northern Brazil with gastric adenocarcinoma

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Abstract Gastric cancer is the third most frequent type of neoplasia and the second most important cause of cancer-related death in the world. In northern Brazil, the state of Pará shows a high incidence of this disease and the capital ranks among cities with the highest incidence of stomach cancer in the world. To evaluate chromosomal aberrations implicated in gastric carcinogenesis, we analyzed 16 samples of gastric adenocarcinoma by fluorescence in situ hybridization using a chromosome 8 α-satellite probe and by direct chromosomal analysis techniques. All lesions were classified as at advanced stages according to the recommendations of the Union Internationale Contre le Cancer (UICC). Trisomy 8 was the main finding of this study, observed in all cases. There was no significant difference between chromosome 8 ploidy and localization, stage, or histological type of adenocarcinoma in our sample. The high incidence of alterations we found in chromosome 8 may be a regional characteristic, related to the high incidence of this neoplasm in Pará state and a strong influence of external factors, such as eating habits. This aberration may comprise a cytogenetic subgroup of this neoplasm. Additional investigations are necessary to confirm the involvement of chromosome 8 and to identify genes in this chromosome related to gastric carcinogenesis.

1. Introduction

Gastric cancer is one of the most common tumors in the world [1,2], but there are few published conventional cytogenetic studies on gastric carcinoma, with only 119 cases reported to date [3], mainly due to technical difficulties related to in vitro cell culture [4]. Nevertheless, several chromosome alterations have been reported in gastric cancer, involving different chromosomes, such as polysomy X; aneuploidy 3, 5, 6, 8, 9, 12, and 19; del(7q); and i(8q), as well as complex abnormalities involving chromosomes 1, 3, 6, 7, 11, 13, and 17 [5–12].

Gastrointestinal tract tumors are notorious for being difficult to analyze with conventional cytogenetic techniques [6,12–15]. Fluorescence in situ hybridization (FISH) technique with centromere-specific DNA probes allows rapid detection of numerical aberrations in interphase nuclei in tumor cells. FISH studies have shown numerical aberrations of chromosomes 1, 7, 8, 9, 17, 20, X, and Y to be common in gastric cancer [16–21].

Our objective was to investigate the existence of numerical alterations of chromosome 8 in gastric cancer samples from the state of Pará, with conventional cytogenetic and FISH techniques, and to correlate these findings with histopathological characteristics, which can represent prognostic parameters in this type of malignancy.

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Table 1
Histological diagnosis with chromosomal alterations from conventional cytogenetics (GTG banding technique) and FISH analysis (interphase nuclei) of chromosome 8 centromere copy number

						Interphase FISH signals, no.(%)				
Case	Age	Location	LAU	UICC/AJCC	Karyotype	1	2	3	4	⩾5
1	47	Antrum	Diff.	T3N0M0	45~47,X,−Y,+8,−9[cp 12]/46,XY[3]	7 (3.5)	116 (58)	71 (35.5)	4 (2.0)	2 (1.0)
2	59	Antrum	Intest.	T4N1M0	44~47,XY,−3,+8,−9[cp 7]/46,XY[3]	8 (4.0)	126 (63.0)	57 (28.5)	3 (1.5)	4 (2.0)
3	57	Antrum	Diff.	T3N2M0	44~47,XY,+8,−9,−12[cp 11]/46,XY[4]	4 (2.0)	120 (60.0)	68 (34.0)	6 (3.0)	2 (1.0)
4	71	Antrum/body	Intest.	T3N2M1	44~47,X,−Y,+8,−10[cp 11]/46,XY[2]	0 (0.0)	111 (55.5)	89 (44.5)	0 (0.0)	0 (0.0)
5	47	Antrum	Intest.	T3N2M0	44~47,X,−Y,+8,−16[cp 10]/46,XY[4]	2 (1.0)	123 (61.5)	69 (34.5)	4 (2.0)	4 (2.0)
6	51	Body	Intest.	T3N2M0	45~47,X,−Y,+8,−9[cp 8]/46,XY[2]	5 (2.5)	125 (62.5)	57 (28.5)	7 (3.5)	6 (3.0)
7	55	Antrum/body	Diff.	T3N2M1	45~47,XY,−6,+8[cp 7]/46,XY[3]	6 (3.0)	135 (67.5)	57 (28.5)	2 (1.0)	0 (0.0)
8	50	Cardia	Diff.	T3N1M0	45~47,XY,−7,+8[cp 8]/46,XY[3]	6 (3.0)	130 (65.0)	47 (23.5)	8 (4.0)	9 (4.5)
9	41	Antrum	Intest.	T3N2M1	45~46,XY,+8,−17[cp 8]/46,XY[3]	9 (4.5)	126 (63.0)	56 (28.0)	6 (3.0)	3 (1.5)
10	62	Antrum	Diff.	T3N1M0	45~46,X,−Y,+8,−9[cp 10]/46,XY[2]	10 (5.0)	130 (65.0)	50 (25.0)	7 (3.5)	3 (1.5)
11	76	Antrum	Intest.	T2N1M0	—[a]	5 (2.5)	112 (56.0)	71 (35.5)	9 (4.5)	3 (1.5)
12	77	Antrum	Intest.	T2N1M0	—[a]	7 (3.5)	109 (54.5)	69 (34.5)	10 (5.0)	5 (2.5)
13	74	Antrum	Intest.	T2N1M0	—[a]	2 (1.0)	120 (60.0)	57 (28.5)	14 (7.0)	7 (3.5)
14	58	Antrum	Diff.	T1N0M0	—[a]	4 (2.0)	92 (46.0)	82 (41.0)	17 (8.5)	5 (2.5)
15	58	Antrum/body	Intest.	T1N1M0	—[a]	8 (4)	97 (48.5)	77 (38.5)	15 (7.5)	3 (1.5)
16	48	Antrum/body	Intest.	T3N0M0	—[a]	9 (4.5)	112 (56.0)	65 (32.5)	10 (5.0)	4 (2.0)
Control	37	Lymphocytes			46,XY[20]	4 (2.0)	192 (96.0)	4 (2.0)	0 (0.0)	0 (0.0)

Abbreviations: AJCC, American Joint Committee on Cancer; Diff., diffuse; Intest., intestinal; LAU, Lauren classification; UICC, Union Internationale Contre le Cancer.

[a] Data could not be evaluated.

2. Materials and methods

Sixteen samples of primary tumors submitted to surgical resection were obtained from 16 male patients at the Pará State João de Barros Barreto University Hospital (HUJBB). Patients in this study had never received chemotherapy or radiotherapy prior to surgery, nor had they any other diagnosed cancer. This study was approved by the Ethics Committee of HUJBB.

Tissue specimens were collected from fresh, surgically resected tumors; routine histopathological examination followed. Tumors were evaluated at the Pathology Department and were classified according to Union Internationale Contre le Cancer (UICC) criteria [22] and Lauren classification [23] for gastric adenocarcinoma. A small portion of each resected tumor was directly processed for cytogenetic study [24]. For 10 of the 16 samples, specimens were analyzed with direct chromosomal analysis technique as described by Xiao et al. [9] and with GTG banding of metaphases as described by Scheres [25]. Chromosomes were identified and classified according to ISCN 1995 [26].

FISH analysis was performed with recently made slides from methanol–acetic acid fixed cells of all patients. Interphase cells were hybridized with chromosome 8 α-satellite DNA probe D8Z2 (LPE 08G, Aquarius Probes; Cytocell, Cambridge, UK) corresponding to chromosome region 8p11.1~q11.1. FISH procedures were performed according to modified protocols [16,27]. Nuclei were counterstained with ethidium bromide. Molecular cytogenetic analysis was carried out under a Zeiss Axiophot fluorescence microscope with double FITC/TRICT filter and ISIS capture and image analysis system. For each case, 200 interphase nuclei were analyzed. Positive chromosome signals appeared as green spots in the nucleus and were scored using criteria of Hopman et al. [28]. To avoid misinterpretation due to technical error, normal lymphocyte nuclei were used as control. A chi-square test was used for all statistical evaluation.

3. Results

Histopathological characteristics of resected gastric tumors, chromosomal alterations using the GTG banding technique, and findings from the FISH analysis are given in Table 1. There were several numerical but no structural clonal aberrations. Chromosome 8 trisomy was the main finding, present in all samples (Fig. 1, left panel). Monosomy of chromosomes 9 and Y was found in five cases. Other chromosomal aberrations, nonclonal (e.g., monosomy of chromosomes 3, 6, 7, 10, 12, 16, and 17), were observed only once.

Numerical aberrations of chromosome 8 were observed in 16 primary tumors. Trisomy 8 was detected in all cases varying from 23.5% (case 8) to 44.5% (case 4). Tetrasomy 8 was observed in 93.75% of the cases varying from 1% (case 7) to 8.5% (case 14). Presence of five or more signals for chromosome 8 was observed in 14 cases (87.5%), with the highest frequency was found in case 8 (4.5%). In peripheral blood lymphocytes, 96% of analyzed nuclei had two signals for the chromosome 8 probe. A representative example of FISH analysis is shown in Fig. 1 (right panel). No statistically significant difference ($P > 0.05$) was observed between level of chromosome 8 ploidy and site, stage, or histological type of the adenocarcinomas studied.

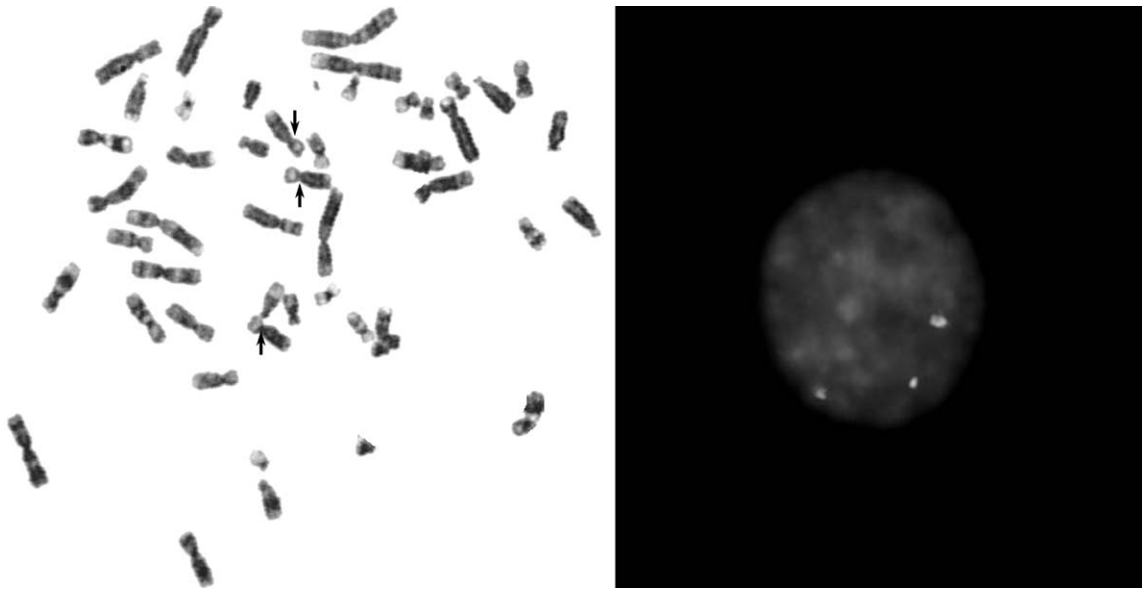

Fig. 1. *Left*: Metaphase with GTG banding. Arrows point to chromosome 8 trisomy. *Right*: Interphase nuclei showing chromosome 8 trisomy (white signals) by FISH.

4. Discussion

In solid tumors, detection of recurrent genetic alterations by conventional cytogenetics was hampered by complexity of chromosomal abnormalities and difficulty in preparing adequate metaphase spreads. FISH techniques have been valuable in solving some of these problems [16]. Direct chromosomal analysis has been used widely due to the possibility of observing cells immediately after obtaining the samples from tumors [7,11,12].

Ferti-Passantonopoulou et al. [6], studying few cases by conventional staining techniques, found that numerical aberrations of chromosomes 8 and 9 are frequent in gastric cancer, and the authors also discussed the possibility of tumors from different sites of gastrointestinal tracts exhibiting similar cytogenetic findings. Xia et al. [12] reported an association of trisomies of chromosomes 8 and 9 might represent a cytogenetic subgroup of gastric cancer. In our samples, numerical aberration of chromosome 9 was the monosomy present in five cases. Our team is currently performing other cytogenetic approaches to this aberration, with the aim of evaluating whether it is a regional feature, and to investigate what clinical relevance, if any, is related to this monosomy.

Panani et al. [11] supposed that trisomy of chromosome 8 should be related to tumors with better prognostic, although some authors had described it in advanced cases. Xiao et al. [9] have also observed trisomy 8 in a case with minimal chromosomal changes, suggesting that this abnormality might be a non-random event in gastric tumorigenesis.

In a recent study, Panani et al. [16] analyzed 33 gastric tumor samples with FISH, using a chromosome 8 α-satellite probe. Numerical aberrations involving chromosome 8 were observed in 62.16% of the studied samples, where trisomy was detected in 43.24%, tetrasomy in 10.81%, and monosomy in 8.10%. Our results confirm that trisomy of chromosome 8 is a common biological phenomenon in adenocarcinoma of stomach and can be used as a gastric mucosa malignancy marker. In our study, 100% of samples presented gain of chromosome 8 as clonal alteration. This fact corroborates previous data obtained by our research team in an ACP01 gastric adenocarcinoma cell line, where this trisomy was found in 60% of analyzed cells [29]. Many authors had considered numerical aberrations at this chromosome as an important event to gastric cancer [6,9,11,12,16,30]. A larger sample number appears to be necessary, but, if this finding is confirmed, it may represent either a regional characteristic or a cytogenetic subgroup of this neoplasia.

Interphase FISH analysis revealed an increased chromosome 8 copy number in our entire sample. Presence of three signals was observed in all cases, four signals in 93.75% and five or more signals in 87.5%. Han et al. [31], using FISH analysis of 18 paraffin-embedded gastric adenocarcinomas, detected polysomy 8 in 27.8% and monosomy in 5.5%. In another study [19] with centromeric probes for chromosomes 7, 8, 11, 17, and Y on 40 deparaffinized sections of gastric tumors, polysomy 8 was found in 62.5% of cases. Comparative genomic hybridization studies have show gains on 8q material in approximately 18–56% of cases [32–35].

Numerical abnormalities of chromosome 8 are suggested to be an important mechanism to increase copy number of *MYC* (alias *c-MYC*). Trisomy of chromosome 8 (whether associated with other chromosomal aberrations or not) could occur in less advanced stages of disease, possibly prior to the occurrence of metastases [12].

The possibility that numerical aberrations of chromosome 8 might reflect alterations of other genes implicated in genesis and progression of gastric cancer could not be excluded. Our study has not focused on any *MYC* alterations reflected by polysomy 8.

A correlation of chromosome 8 numerical aberrations with certain histopathological characteristics representing prognostic factors in gastric cancer was evaluated, although the number of cases we studied was small. Our results did not reveal any association of chromosome 8 with histological type, tumor aggressiveness, or invasion. Alterations found in this study have been previously described in the literature, but in our sample the frequencies were higher. External factors, such as eating habits and other environmental agents, have a direct influence on development of this neoplasm, and many of the genetic alterations found may be regional characteristics of a given population.

An increased copy number of chromosome 8 needs to be better investigated in other stages of gastric neoplasias, to clarify whether it is an etiologic cause of malignant transformation or a consequence of the proliferation process.

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