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Short communication

Establishment and conventional cytogenetic characterization of three gastric cancer cell lines

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Abstract

Gastric cancer is the fourth most frequent type of cancer and the second most frequent cause of cancer mortality worldwide. Only a modest number of gastric carcinoma cell lines have been isolated thus far. Here we describe the establishment and cytogenetic characterization of three new gastric cancer cell lines obtained from primary gastric adenocarcinoma (ACP02 and ACP03) and cancerous ascitic fluid (AGP01) of individuals from northern Brazil. ACP02, ACP03, and AGP01 cell lines are presently in the 60th passage. The cell lines grew in a disorganized single layer with some agglomerations and heterogeneous divisions (bipolar and multipolar). All cell lines exhibited a composite karyotype with several clonal chromosome alterations. Trisomy 8 was the most frequent alteration. Chromosome 8 aneusomy was confirmed by fluorescence in situ hybridization. All cell lines also exhibited trisomy 7 and deletion of chromosome arm 17p. These results suggest that, although frequent chromosome alterations are commonly observed due to culture process, the ACP02, ACP03, and AGP01 cell lines and primary gastric cancer from individuals of northern Brazil share genetic alterations, supporting use of these cell lines as a model of gastric carcinogenesis in this population. © 2009 Elsevier Inc. All rights reserved.

1. Introduction

Gastric cancer (GC) is the fourth most frequent type of cancer and second most frequent cause of cancer mortality worldwide [1]. In the northern Brazil state of Pará, GC was the most common cause of cancer death in 2000. In Belém, the capital of Pará, the 5-year survival rate is 9 10% [2].

Gastric cancer is thought to result from a combination of environmental factors and the accumulation of generalized and specific genetic and epigenetic alterations, which affect oncogenes, tumor suppressor genes, and mechanisms that control genomic instability. Knowledge of the events that lead to these alterations in cancer cells in vivo is still limited. The study of cancer cell lines is useful in solving this problem. For GC, however, the establishment of cell lines is difficult, because of technical problems associated with the extraction of viable cells from surgical samples [3]. Only a modest number of gastric carcinoma cell lines have been isolated thus far. Almost all established GC cell lines have been developed in Asian countries. An earlier cell line, ACP01, established by our research group, was the first gastric adenocarcinoma cell line developed in Brazil [4].

Here, we describe the establishment and cytogenetic characterization of three new GC cell lines obtained from primary gastric adenocarcinoma (ACP02 and ACP03) and ascitic fluid (AGP01), each of which exhibited a composite karyotype with several clonal chromosome alterations.

2. Material and Methods

2.1. Sample

The ACP02 cell line was established from a primary gastric adenocarcinoma of a 66-year-old man. The tumor

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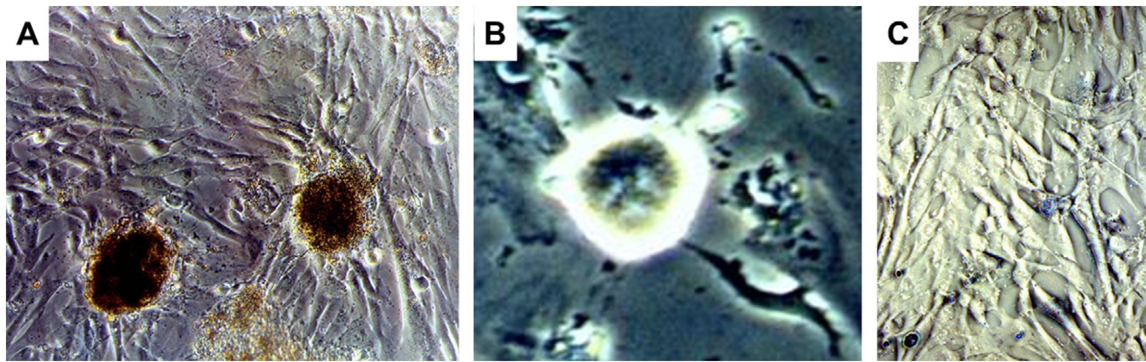

Fig. 1. Typical morphology of gastric cancer cell lines ACP02, ACP03, and AGP01 in culture. (A) Disorganized single layer growth with some agglom erations (×40). (B) Bipolar cell division (×100). (C) Disorganized single layer growth in AGP01 (×40).

was removed from the cardia region of the stomach. The GC sample was classified as diffuse type [5] and was staged as T3N2M0 [6].

The ACP03 cell line was established from a primary gastric adenocarcinoma of a 63-year-old man. The tumor was removed from antrum region of the stomach. The GC sample was classified as intestinal type and was staged as T4N1M0.

The AGP01 cell line was established from cancer cells in the ascitic fluid of a 55-year-old man with gastric adenocarcinoma. The tumor was at the antrum and body region of the stomach. The GC sample was classified as intestinal type and staged as T3N2M1.

Samples were obtained at the Pará State João de Barros Barreto University Hospital (HUJBB). Informed consent with approval of the ethics committee of HUJBB was obtained. All patients were from northern Brazil, a region in which the human population is composed of interethnic crosses among three main origin groups: Europeans (mainly represented by the Portuguese), Africans, and Amerindians [7]. All patients had negative histories of exposure to either chemotherapy or radiotherapy before surgery, and there were no other diagnosed cancers.

2.2. Establishment, culture and morphology of the cell lines

Tumoral fragments were cut into small pieces and treated with 0.1% collagenase IV (Sigma Aldrich, St. Louis, MO). The tumoral fragments and ascitic fluid were cultured in HAM-F10 medium (Sigma Aldrich) supplemented with 20% fetal calf serum and antibiotics at 37°C. The culture was immortalized in vitro by spontaneous transformation. The morphological analysis of cell lines was performed using light microphotographs of cultures prepared daily and taken with an inverted Axiovert 25 microscope (Zeiss, Jena, Germany).

Table 1
Distribution of chromosome number in cells of three gastric cancer cell lines

	ACP02	ACP03	AGP01
Chromosome number, range	40–100	54–102	37–52
Haploid (23–34), %	0	0	0
Diploid (35–57), %	59	62	78
Triploid (58–80), %	36	30	12
Tetraploid (81–103), %	5	8	10
Modal number (mn)[a]	49	48	54
Metaphases with mn, no.	32	45	47

[a] Chromosomes of 100 metaphase spreads were counted to evaluate the modal number.

2.3. Conventional cytogenetic analysis

Cytogenetic analysis was performed as previously described [4] when the cell line had undergone 55 passages. Briefly, exponentially growing cells were synchronized [8] and blocked with 0.0016% colchicine. Cells were harvested with 0.05% trypsin, treated with hypotonic solution (KCl 0.0075 mol/L) for 20 minutes at 37°C, and fixed with 3:1 methanol acetic acid. Slides were banded using standard trypsin Giemsa banding (GTG-banding). Chromosomes of 100 metaphase spreads were counted to evaluate the modal number, and 30 metaphases were karyotyped from each cell line. The description of chromosome aberrations was according to ISCN 2005 [9].

2.4. Fluorescence in situ hybridization

For each cell line, fluorescence in situ hybridization (FISH) was performed on recently made slides from methanol acetic acid fixed cells of the 55th passage, as previously described [10], using chromosome 8 α-satellite DNA probe (Cytocell, Cambridge, UK). For each passage, 200 interphase nuclei were analyzed. Positive chromosome 8 signals appeared as green spots in the nucleus and were scored using the criteria of Hopman et al. [11]. To avoid misinterpretation due to technical error, normal lymphocyte nuclei were used as control.

3. Results

3.1. Establishing cell lines

We cultured 10 primary gastric adenocarcinomas and 10 cancerous ascitic fluid specimens, only 3 of which were

immortalized in vitro by spontaneous transformation. The ACP02, ACP03, and AGP01 cell lines are presently in their 60th passage; the first stored sample was prepared at the 6th passage. These cell lines are stored in liquid nitrogen, and they were cryopreserved in intercalated passages during their development.

3.2. Morphological analysis

The ACP02 and ACP03 cell lines grew in a disorganized single layer, similar to fibroblasts, with some agglomerations (Fig. 1A) and heterogeneous divisions (bipolar and multipolar) (Fig. 1B). The cell cycles of both cell lines were ~30 hours, with maximum confluence at 72 hours. On the other hand, the AGP01 cell line grew in suspension during the early passages; after the 15th passage, growth followed the same patterns as for ACP02 and ACP03 (Fig. 1C).

3.3. Conventional cytogenetic analysis

The analysis of the modal number revealed that the ploidy of ACP02, ACP03, and AGP01 cell lines as diploid, with most metaphases hyperdiploid. The distribution of chromosomal variation for the three cell lines is given in Table 1.

No normal 46,XY cells were observed in ACP02, ACP03, or AGP01. The composite karyotype of each cell line is presented in Table 2. In ACP02, the most frequent alteration was trisomy 8 (96.7% of cells) followed by chromosome 17p deletion (50% of cells). In ACP03, trisomy 8 was observed in all metaphases and trisomy 17 was observed in 90% of cells. In AGP01, trisomy 1 was observed in all metaphases, and trisomy 8 and trisomy 17 in 83.3% of cells. All cell lines exhibited trisomy 8 (83.3 100% of cells), trisomy 7 (~40% of cells), and deletion of chromosome arm 17p (36.7 50% of cells) (Fig. 2).

3.4. FISH analysis

Numerical alteration of chromosome 8 in ACP02, ACP03, and AGP01 was confirmed by FISH (Table 3).

Two signals for the chromosome 8 centromere were observed in 95% of peripheral blood lymphocytes. In GC cell lines, trisomy 8 was the most frequent alteration, ranging from 74.5% to 91.5% of cells (Fig. 3). We also observed clonal tetrasomy 8, in 5 14.5% of cells.

4. Discussion

Normal cells can divide only a limited number of times in vitro, because of replicative senescence. Some tumor cells, however, can become immortal and therefore they do not undergo senescence when cultured in vitro [12]. According to the Hayflick limit, the maximum number of passages that a normal cell attains before senescence is ~50 [13,14]. In the present study, we established three new GC cell lines that, as of writing, had reached the 60th passage.

The ACP02, ACP03, and AGP01 cell lines grew in a disorganized single layer with some agglomerations and heterogeneous divisions (bipolar and multipolar). These characteristics had been previously observed in the ACP01 cell line, which was established from a primary gastric adenocarcinoma of an individual from northern Brazil [4]. These morphological characteristics are also similar to other primary gastric adenocarcinoma cell lines (OACP4C, MKN7, MKN74, and MKN28) and from a lymph node metastasis (OACM4.1 C) [15,16]. Walen [17] suggested that these morphological characteristics might be indicators of the in vitro immortalization process.

The disorganized growth and the formation of agglomerates in a cell line is caused by the loss of contact inhibition or density-based growth control [17,18]. We previously observed that all GC samples from our population exhibited *CDH1* methylated sequences [19]. E-cadherin, the *CDH1* product, is a known homophilic cell adhesion protein that mediates cell cell adhesion; it also has a possible role in modulating intracellular signaling, thus promoting tumor growth [20]. Methylation of the *CDH1* gene is the most common mechanism responsible for loss of E-cadherin expression, and it can contribute to disorganized growth.

The heterogeneous divisions (bipolar and multipolar) observed in all three cell lines, as well as in ACP01, suggest that proliferation control errors can contribute to the generation of chromosomal instability. Jin et al. [21] showed that cells with a high frequency of multipolar mitosis and no anaphase bridge had a high number of single (nonclonal) cells, with chromosome numbers deviating strongly from the modal number. Thus, the presence of multipolar division in the present three new cell lines may contribute to the large variation in chromosome number.

Chromosomal instability is characterized by changes in chromosome copy number (aneuploidy) and alterations in chromosomal regions, which may induce oncogene activation, tumor suppressor gene inactivation, or both.

Table 2
Composite karyotype for three gastric cancer cell lines

Cell line	Karyotype
ACP02	42~50,XY,+3[10],+7[12],+8[29],+11[3],del(17)(p?)[15],+21[7][cp30]
ACP03	43~49,XY,del(5)(p?)[7],+7[13],+8[30],+16[8],+17[27],del(17)(p?)[12],+18[4][cp30]
AGP01	44~57,XY,+1[30],+2[8],+3[6],+5[9],+7[12],+8[25],del(9)(p?)[11],+12[8],+14[3],+17[25],del(17)(p?)[11],+19[4],+22[8][cp30]

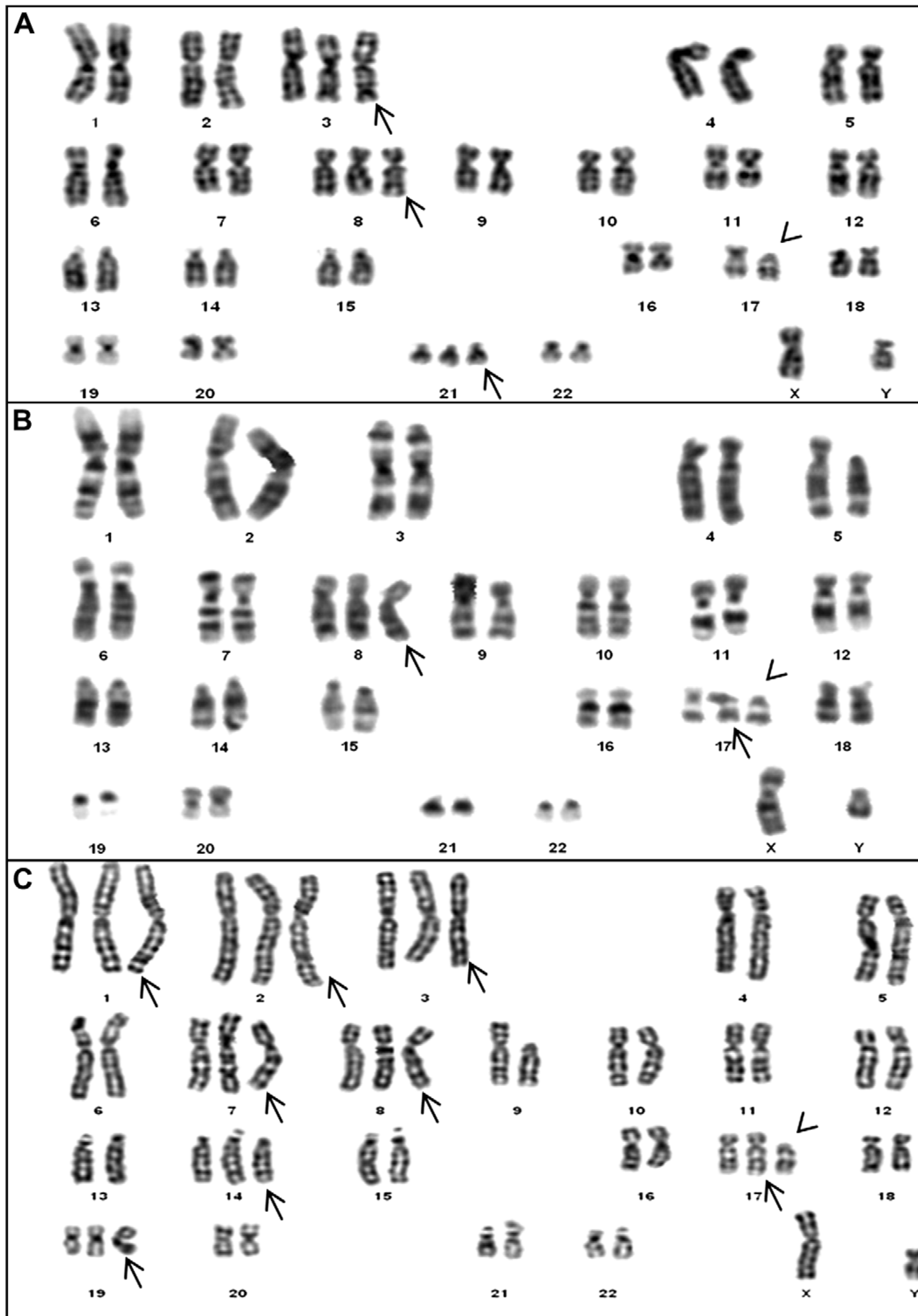

Fig. 2. Karyograms of a GTG banded metaphase spread from each of the three gastric cancer cell lines: (A) ACP02, (B) ACP03, and (C) AGP01. Arrows indicate trisomy; arrowheads indicate del(17)(p).

Chromosomal instability is one of the two major genomic instability pathways involved in gastric carcinogenesis [22]. Our research group previously reported several frequent aneusomies (e.g., involving chromosomes 8, 9, 17, and X) in GC samples and cell line from individuals of northern Brazil, which suggests a genomic instability [4,10,23 28]. These findings support the importance of the establishment and study of GC cell lines in this population to understand the mechanisms that lead to a chromosomal instability phenotype.

In the present study, we performed cytogenetic analysis when ACP02, ACP03, and AGP01 were in the 55th passage (i.e., the early passages after cell line immortalization). No normal karyotype was found in any of the cell lines, and all

Table 3
Interphase nucleus FISH analysis of chromosome 8 centromere copy number in three gastric cancer cell lines and in normal lymphocytes

Sample	Nuclei exhibiting chromosome 8 signals, no. (%)[a]				
	1 signal	2 signals	3 signals	4 signals	⩾5 signals
ACP02	5 (2.5)	9 (4.5)	149 (74.5)	29 (14.5)	8 (4.0)
ACP03	3 (1.5)	7 (3.5)	161 (80.5)	26 (13.0)	3 (1.5)
AGP01	2 (1.0)	4 (2.0)	183 (91.5)	10 (5.0)	1 (0.5)
Lymphocytes	7 (3.5)	190 (95.0)	3 (1.5)	0 (0)	0 (0)

[a] The FISH analysis was performed on 200 interphase nuclei.

three cell lines exhibited a composite karyotype with several clonal chromosomal alterations.

Only a few cytogenetic studies of GC have been reported. An extra X chromosome, trisomy of 8, 9, or 19, del(7q), and i(8q) have been described as frequent simple chromosomal abnormalities in GC by conventional cytogenetics. Molecular cytogenetic studies have also shown that gains at 3q, 7p, 7q, 8q, 13q, 17q, 20p, and 20q and losses at 4q, 9p, 17p, and 18q are recurrent chromosomal alterations. (For a review, see reference [29].)

The presence of trisomy 8 was a common and frequent alteration in all three cell lines, ACP02, ACP03, and AGP01, corroborating our previous conventional and molecular cytogenetic studies in the ACP01 cell line [4,10] and in primary GC samples [23 26]. Among chromosome 8 genes, *MYC*, located at 8q24, has been the most studied. *MYC* is a regulator of the cell cycle and plays a major role in the control of cell growth, differentiation, apoptosis, and neoplastic transformation [30]. Several studies demonstrated an increased *MYC* expression in precancerous gastric lesions, suggesting that *MYC* alteration is important to the beginning of gastric carcinogenesis [31]. We also previously reported *MYC* amplification and its protein expression in all GC samples, including early GC, from individuals of northern Brazil [10,23,26]. Many authors have considered chromosome 8 numerical aberrations an important event in GC samples and cell lines from distinct populations [3,32 38]. In our population from northern Brazil, however, the frequency of this alteration seems to be higher.

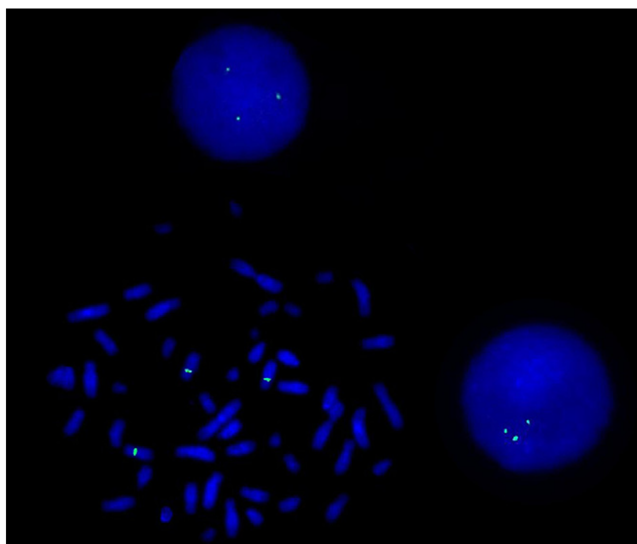

Fig. 3. Fluorescence in situ hybridization analysis of interphase and meta phase cells indicates trisomy 8.

Trisomy 7 was observed in ~40% of cells in the three cell lines. This aneusomy was also observed in the 6th passage of the ACP01 cell line [4]. Okada et al. [39] observed that four of five diffuse GC cell lines exhibited gain of chromosome 7 regions. Chun et al. [40] described the partial gain of chromosome 7 as the most frequent marker, which was present in three of five GC cell lines from Korean patients. Matturri et al. [41] observed trisomy of chromosome 7 in 40% of 126 GC samples. Kokkola et al. [42] reported that the most common gains involved chromosomes 7 and 8 in adenoma, a preneoplastic lesion. These data suggest that oncogenes in chromosomes 7 and 8 are important at the beginning of the gastric carcinogenesis process.

MET, a gene located at 7q31, encodes the tyrosine kinase receptor for the hepatocyte growth factor (HGF) and controls genetic programs leading to cell growth, invasion, and protection from apoptosis. The amplification and overexpression of *MET* was reported in GC samples [43 48]. Several strategies to block the activation of *MET* are under development, such as the use of tyrosine kinase inhibitors or monoclonal antibodies, and some of these compounds have already been used in clinical trials [49].

Another common chromosome alteration observed in all three cell lines was the deletion of 17p, which includes the locus for the *TP53* tumor suppressor gene. *TP53* somatic alteration is described in ~50% of human cancers, including GC [50]. Our research group previously observed that 20 of 20 GC samples exhibited loss of chromosome 17 and loss of *TP53* at different frequencies by FISH assay [51].

Our results suggest that, although frequent chromosome alterations are commonly observed because of culture process, the ACP02, ACP03, and AGP01 cell lines and primary GC from individuals of northern Brazil share genetic alterations, supporting the use of these cell lines as a model of gastric carcinogenesis in this population. Oncogenes in chromosomes 8 and 7, as well as suppressor genes in chromosome arm 17p, are important for gastric

carcinogenesis; however, other genes are important to the heterogeneity observed among gastric cancers of different stages and cell lines.

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