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ORIGINAL ARTICLE

Basic Study

# Identification of *IL11RA* and *MELK* amplification in gastric cancer by comprehensive genomic profiling of gastric cancer cell lines

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## Abstract

***AIM***

To identify common copy number alterations on gastric cancer cell lines.

***METHODS***

Four gastric cancer cell lines (ACP02, ACP03, AGP01 and PG100) underwent chromosomal comparative genome hybridization and array comparative genome hybridization. We also confirmed the results by fluorescence *in situ* hybridization analysis using the bacterial artificial chromosome clone and quantitative real time PCR analysis.

***RESULTS***

The amplification of 9p13.3 was detected in all cell lines by both methodologies. An increase in the copy number of 9p13.3 was also confirmed by fluorescence *in situ* hybridization analysis. Moreover, the interleukin 11 receptor alpha (*IL11RA*) and maternal embryonic leucine zipper kinase (*MELK*) genes, which are present in the 9p13.3 amplicon, revealed gains of the *MELK* gene in all the cell lines studied. Additionally, a gain in the copy number of *IL11RA* and *MELK* was observed in 19.1% (13/68) and 55.9% (38/68) of primary gastric adenocarcinoma samples, respectively.

***CONCLUSION***

The characterization of a small gain region at 9p13.3 in gastric cancer cell lines and primary gastric adenocarcinoma samples has revealed *MELK* as a candidate target gene that is possibly related to the development of gastric cancer.


**Key words:** *IL11RA*; Gastric cancer; Genomic profiling; *MELK*; 9p13.3



**Core tip:** While the presence of alterations in the DNA copy number is one of the key hallmarks of carcinogenesis, in gastric cancer, the chromosomal regions with frequent gain and loss are still poorly defined. Array comparative genome hybridization is a high resolution tool that allows the simultaneous detection of sub-microscopic copy number changes across the genome. The characterization of a small gain or loss region in gastric cancer cell lines and primary gastric adenocarcinoma samples could reveal a candidate target gene that may possibly be linked to the development of gastric cancer.

Calcagno DQ, Takeno SS, Gigek CO, Leal MF, Wisnieski F, Chen ES, Araújo TMT, Lima EM, Melaragno MI, Demachki S, Assumpção PP, Burbano RR, Smith MC. Identification of *IL11RA* and *MELK* amplification in gastric cancer by comprehensive genomic profiling of gastric cancer cell lines. *World J Gastroenterol* 2016; 22(43): 9506-9514 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i43/9506.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i43.9506

## INTRODUCTION

Gastric cancer (GC) remains a major public health issues, as it is the fifth most common malignancy and the third leading cause of cancer death in both sexes worldwide[1]. The most common type of GC is adenocarcinoma, which can be further categorized into two main types, intestinal type and diffuse type, which are biologically different with distinct clinical and epidemiological profiles[2]. The difference in the clinicopathological characteristics between the histological types of gastric cancer indicate that gastric tumor development occurs through the progressive accumulation of distinct genetic alterations[2-5]. Thus, the characterization of these genomic abnormalities in gastric cancer may help to clarify the molecular pathogenesis of the disease and may unveil genetic markers of progression and for predicting treatment response or survival.

Genomic instability with frequent DNA copy number variations (CNVs) is one of the key hallmarks of gastric carcinogenesis[6]. Tumor progression seems to depend on the successive acquisition of chromosomal aberrations, leading to gains or losses of parts of the genome. However, there is no clear agreement on the genetic changes underlying gastric carcinogenesis.

In the last decades, chromosomal comparative genome hybridization (cCGH) and array CGH (aCGH) analyses of gastric tumors and gastric cell lines have revealed recurrent DNA CNVs[7-11]. Using cCGH, Burbano *et al*[3] showed that the copy number gain of 8q24.1, the locus containing the *MYC* oncogene, is a frequent alteration in GC. Further investigations by our group demonstrated that *MYC* amplification is a common finding in preneoplastic gastric lesions and tumors[4,5,12-15].

Moreover, Takeno *et al*[10] stated that diffuse-type GC shows a complex pattern of chromosomal alterations, especially chromosome region losses. Recently, Liang *et al*[16] suggested that the detection of DNA CNVs from tissue or blood samples may be a useful tool for guiding individualized treatment strategies and for identifying new drug targets in patients with GC.

In the current study, we analyzed the chromosomal abnormalities of four GC cell lines by cCGH and aCGH. The occurrence of the amplification of chromosomal region 9p13 in GC cell lines was validated by fluorescence *in situ* hybridization (FISH) and confirmed in primary gastric adenocarcinoma samples by quantitative polymerase chain reaction (qPCR). Among the genes within the 9p13 region, we chose two genes for validation in primary GC samples, interleukin 11 receptor alpha (IL11RA) and maternal embryonic leucine zipper kinase (MELK).

## MATERIALS AND METHODS

### Gastric cancer cell lines

The ACP02, ACP03 and AGP01 gastric adenocarcinoma

cell lines, which were previously established and characterized by our research group, were used in the present study[17,18]. Additionally, we used the GC cell line, PG100, obtained from the Rio de Janeiro Cell Bank (Rio de Janeiro, RJ, Brazil), which was previously characterized cytogenetically by our group[19]. All cell lines were cultured according to Lima *et al*[20].

### Primary gastric cancer samples

Quantitative gene copy number measurements were performed on 68 primary gastric adenocarcinoma samples that were obtained from patients who underwent surgery resection in João de Barros Barreto University Hospital (HUJBB), Belém, Pará, Brazil. In Pará, Brazil, the human population is composed of interethnic crosses between three main origin groups, European (mainly represented by Portuguese), Africans, and Amerindians[21].

All the patients had negative histories of exposure to either chemotherapy or radiotherapy before surgery, and there were no other diagnosed cancers. Signed informed consent, with the approval of the ethics committee of HUJBB, was obtained from all patients prior to the collection of samples.

### DNA isolation

DNA from the GC cells lines and gastric tumors were isolated using the QiAmp DNA isolation kit (Qiagen, Hilden, Germany) according to the manufacturer's recommended protocol. DNA concentration and purity were evaluated by Nanodrop (NanoDrop Technologies, Houston, TX, United States) and agarose gel electrophoresis. All DNA samples used had an A260/280 ratio of 1.8-2.0 and an A260/A230 ratio of > 1.5 and were visualized as a high molecular weight band on an agarose gel.

### cCGH

DNA samples from GC cell lines were labeled using the CGH Nick Translation Kit (Abbott Laboratories, IL, United States) with Control DNA (Promega, Madison, United States) according to the manufacturer's instructions. Hybridization was performed with CGH Metaphase Target Slides (Abbott Laboratories, Illinois, United States), following the manufacturer's protocols. The slides were analyzed by Corel Photo-Paint - Version 5.00 - Isis Zeiss® software, using an Axioskop Zeiss microscope (Carl Zeiss Inc. Canada, Don Mills, ON, Canada) equipped with an epi-illuminator and fluorochrome-specific optical filters.

The three-color images with red, green, and blue were acquired from 15 metaphases. Chromosome imbalances were detected on the basis of the deviation of the fluorescence ratio profile from the balanced value (FITC:rhodamine = 1). For each chromosome, the final ratio values were prepared from the mean values of at least ten chromosome homologues from separate metaphase spreads. The CGH results were plotted as a series of green to red ratio profiles.

### aCGH

To evaluate the complete genome of all the four cell lines studied, high density microarray analysis was performed using the AffymetrixR CytoScan™ HD Array platform (Affymetrix, Santa Clara, CA, United States). First, genomic DNA was digested by the *Nsp*I restriction enzyme, and the digested samples were ligated using the *Nsp*I adaptor. The fragments were amplified by PCR and run on a 2% agarose gel to verify that the PCR product size distribution was between 150 bp and 2000 bp. After PCR product purification and dilution, we performed the quantification of each sample using a NanodropR 1000 Spectophotometer (NanoDrop Technologies, Houston, TX, United States). The average purification yield for each sample was ⩾ 3.0 μg/μL.

The purified samples were then fragmented using DNAse I enzyme, and the products were run on a 4% agarose gel to verify that the majority of fragments had a size distribution between 25 and 125 bp.

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

During the hybridization step, each sample was hybridized onto a CytoScan® HD Array (Affymetrix, Santa Clara, CA, United States) and placed in a GeneChip® Hybridization Oven 640 (Affymetrix, Santa Clara, CA, United States) at 50 ℃ and 60 rpm for 16 to 18 h. The processes prior to scanning of arrays, washing and staining, were carried out at a Fluidics Station 450 (Affymetrix, Santa Clara, CA, United States). The arrays were scanned using GeneChip® Scanner 3000 7G (Affymetrix, Santa Clara, CA, United States).

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

### FISH

FISH was performed on nuclei and metaphase spreads of the cell lines, ACP02, ACP03 and AGP01. Metaphase spreads of lymphocytes from a healthy donor were used as a control. The bacterial artificial chromosome (BAC) clone, RP11-165H19, was obtained from *BAC/PAC Resources* (http://bacpac.chori.org/). Bacterial cultures and DNA isolation was performed using Qiagen Plasmid Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Alu-PCR products of the BAC were used as probes and were biotinylated using nick translation, as described previously[22].

### qPCR

For the validation of 9p13 amplification, we evaluated the copy number of two genes within this locus,

**Table 1 Overview of detected chromosomal aberrations by chromosomal comparative genome hybridization and array comparative genome hybridization on gastric cancer cell lines**

| Cell line | cCGH | aCGH |
|---|---|---|
| ACP02 | +8p21-pter, +8q24, +9p12-p22, +9q21.1-q21.3, +15q11.1-q14, +16p, +16q, +17p11, +17q11.2, +17q23, +22q11.1-q12.3 | +1p13.2, +1p21.3, +1q21.2, +1q21.3, +2p11.2, +4p12, +4p13, +4q12, +5q12.1, +7q11.22, +7q11.23, +8p11.23, +8p21.3, +8q24.11, +8q24.22, -8q24.22, +8q24.23, +8q24.3, +9p13.2, +9p13.3, +9p11.3, +9q21.12, +10p11.22, +10p12.1, +10p12.31, +10p13, +10p14, +10q11.21, -11p11.12, +11p11.2, , -11q12.1, -11q12.2, -11q12.3, +12q12-q15, +13q12.11, +14q11.2, +15q11.2, +15q12, +15q14, +15q15.3, +15q24.1, +15q25.1, +15q26.1, +16p11.2, +16p12.3, +16q11.2, +16q13, +16q21, +16q22.1, +17p11.2, +17q11.2, +17q23.1, +18q11.2, +19q13.11, +20p11.1, +20p11.23, +20p12.2, +20q11.21, +20q11.23, +22q11.21,+ 22q11.23, +22q12.2, +22q12.3 |
| ACP03 | +4p15.1-pter, +6p22.3-p24, +6q25.1,q26, +8p22-pter, -8q11.1-q11.2, +9p12-p22, +10p12-p14, -11p11.1, -11q12, +15q11.1-q15, +15q23-q26.1, +16p12-p13.1, +16p21-p23, +22q11.1-q12.1 | +1p13.2, +1p13.3, +1p21.3, +1q21.1, +1q21.2, 1q21.3, +2p12, +4p12, +4p14-p13, +4p15.1, +5q12.1, +6p24.2, +7q11.21, +7q11.22, +7q11.23, +8p11.21, +8p11.23-p11.22, +8p21.2, +8p21.3, -8q11.21, +8q24.11, -8q24.12, +8q24.13, +8q24.21, +8q24.22, -8q24.22, +8q24.23, +8q24.3, +9p13.2, +9p13.3, +9q21.13, +9p23, +10p11.21, +10p11.22, +10p12.1, +10p12.2, +10p12.31, +10p12.33, +10p13, +10p14, +10q11.21, -11p11.12, +11q11.2, -11q12.1-q12.2, -11q12.3, +12q12-q15, +14q11.2, +14q13.2, +15q11.2, +15q12, +15q13.1, +15q14, +15q24.1, +15q25.1, +15q25.2, +15q26.1, +16p11.2, +16p12.3, +16q11.2, +16q12.1, +16q12.2, +16q21, +16q22.1, +17p11.2, +17q11.2, +17q23.1, +18q11.2, +18q12.1, +19q13.11, +20p11.1, +20p11.23, +20p12.2, +20q11.21, +20q11.22, +20q11.23, +22q11.21, +22q11.23, +22q12.2, +22q12.3 |
| AGP01 | +1p13-21, +1q12-q21.3, +2p11.2-p12, +4p11-p12, +4q12-q13.1, +5p11-p12, +5q11.2-q12, +6p11-p12, +6q12-q16.1, +7q11.1-q11.2, +9p12-p13, +9q13-q21.3, +10p11.2-p12.3, +10q11.1-q21.1, +11p11-p11.2, +12p11.1, +12q12, +13q11-q12, +14q11.1-q13, +15q11-q14, +16p11.2, +16q12, +17p11.2, +17q11.2, +18p11.2-p11.3, +18q11-q12, +19q12-q13.1, +20p11.2-p12, +20q11.1-q11.2 | +1p13.3, +1p21.3, +1q21.2, +1q21.3, +2p11.2, +2p12, +4p12, +4p14-p13, +5p12, +5q12.1, +6p24.2, +6q13, +7q11.21, +7q11.22, +7q11.23, +8p11.21, +8p11.23-p11.22, +8p21.3, -8q11.21, +8q24.11, -8q24.12, +8q24.21, +8q24.22, +8q24.23, +8q24.3, +9p13.2, +9p13.3, +9p22.3, +9q21.12, +9q21.13, +10p11.22, +10p12.1, +10p12.31, +10p13, +10p14, +10q11.21, -11p11.12, +11p11.2, -11q12.1, -11q12.2, -11q12.3, +12q12-q15, +13q12.11, +14q11.2, +15q12, +15q14, +15q15.1, +15q15.3, +15q24.1, +15q25.1, +15q25.2, +15q25.3, +15q26.1, +16p11.2, +16p12.3, +16q11.2, +16q12.1, +16q13, +16q21, +16q22.1, +17p11.2, +17q11.2, +17q23.1, +18q11.2, +19q13.11, +20p11.21, +20p11.1, +20p11.23, +20p12.2, 20q11.21, +20q11.23, +22q11.21, +22q11.23, +22q12.2, +22q12.3 |
| PG100 | +9p12-p23 | +1p13.2, +1p13.3, +1p21.3, +1q21.1, +1q21.2, +1q21.3, +2p11.2, +4p15.1, +6q13, +7q11.21, +7q11.22, +7q11.23, +8p11.21, +8p11.23-p11.22, +8p21.2, +8p21.3, -8q11.21, +8q24.11, +8q24.13, +8q24.22, -8q24.22, +8q24.23, +8q24.3, +9p13.2, +9p13.3, +9p22.3, +9q21.12, +9q21.13, +10p11.22, +10p12.1, +10p12.2, +10p12.31, +10p14, +10q11.21, +11p11.2, -11q12.1, -11q12.2, -11q12.3, +12q12-q15, +13q12.11, +14q11.2, +15q11.2, +15q12, +15q14, +15q15.1, +15q15.3, +15q24.1, +15q25.2, +15q25.3, +15q26.1, +16p11.2, +16p12.3, +16q11.2, +16q12.1, +16q21, +16q22.1, +17p11.2, +17q11.2, +17q23.1, +18q11.2, +19q13.11, +20p11.1, +20p11.23, +20p12.2, +20q11.21, +20q11.23, +22q11.21, +22q11.23, +22q12.1-q12.2, +22q12.2, +22q12.3 |

cCGH: Chromosomal comparative genome hybridization; aCGH: Array comparative genome hybridization.

*IL11RA* and *MELK*. For this, we used the same DNA samples from GC cell lines that were used for cCGH and aCGH and from GC tissues. qPCR was performed using the FAM/MGB-labeled TaqMan probes (Life Technologies, Foster City, CA, USA) for *IL11RA* (Hs01842695_cn) or *MELK* (Hs05076287_cn). VIC/TAMRA-labeled TaqManCopy Number Reference Assay RNAse P (#4403326; Life Technologies, Foster City, CA, United States) was used as an internal control. All the real-time qPCR reactions were performed in quadruplicate with gDNA using a 7500 Fast Real-Time PCR system (Life Technologies, Foster City, CA, United States) as described previously[13]. The copy number of each sample was estimated by CNV analysis using Copy Caller Software V1.0 (Life Technologies, Foster City, CA, United States). Known Human Genomic DNA, G1471 and G1521 (Promega, Madison, United States), were used for calibration.

### Statistical analysis

The data on clinical features were compared by the $\chi^2$ test or two-tailed Fisher's exact test for categorical variables. All statistical analyses were performed with the statistical package SPSS for Windows (V.17.0, SPSS Inc, Chicago, IL, United States). *P* values of $\leqslant$ 0.05 were considered significant.

## RESULTS

### Recurrent regions of alterations

The ACP02, ACP03, AGP01 and PG100 cell lines showed multiple gains and losses by cCGH and aCGH. Most chromosomal aberrations detected in these cell lines by cCGH were confirmed by aCGH (Table 1), although aCGH analysis enabled the identification of many additional chromosomal gains and losses. On the other hand, the gain of 16p21-p23 in ACP03 and the gains of 6p11-p12, 12p11.1 and 18p11.2-p11.3 in AGP01 were detected only by cCGH.

Notably, the gain of chromosome region 9p13 was common in all cell lines and as such, this locus was selected for further investigation.

**Table 2 Number of copies of 9q13 locus by FISH analysis in gastric cancer cell lines *n* (%)**

| Cell line | 0 signal | 1 signal | 2 signals | 3 signals | 4 signals | ⩾ 5 signals |
|---|---|---|---|---|---|---|
| ACP02 | 12 (6.0) | 26 (13.0) | 112 (56.0) | 25 (12.5) | 22 (11.0) | 3 (1.5) |
| ACP03 | 22 (11.0) | 40 (20.0) | 99 (49.5) | 22 (11.0) | 6 (3.0) | 1 (0.5) |
| AGP01 | 19 (9.5) | 45 (22.5) | 99 (49.5) | 27 (13.5) | 10 (5.0) | - |
| PG100 | 18 (9.0) | 55 (27.5) | 87 (43.5) | 32 (16.0) | 7 (3.5) | 1 (0.5) |
| Control | 34 (17.0) | 68 (34.0) | 97 (48.5) | 1 (0.5) | - | - |

**Table 3 *MELK* and *IL11RA* gene copy number and clinicopathological features of 68 gastric cancer patients**

| | *MELK* | | | *IL11RA* | | |
|---|---|---|---|---|---|---|
| | 2 copies (*n* = 38) | ⩾ 3 copies (*n* = 30) | *P* value | 2 copies (*n* = 55) | ⩾ 3 copies (*n* = 13) | *P* value |
| Age (yr) (mean ± SD) | | | | | | |
| > 50 (64.5 ± 6.9) | 23 | 25 | 0.0748 | 39 | 9 | 0.7461 |
| ⩽ 50 (42.5 ± 5.2) | 15 | 5 | | 16 | 4 | |
| Gender | | | | | | |
| Male | 23 | 21 | 0.5781 | 34 | 9 | 0.7544 |
| Female | 15 | 9 | | 21 | 4 | |
| Histopathology | | | | | | |
| Intestinal | 23 | 20 | 0.7886 | 32 | 11 | 0.1110 |
| Diffuse | 15 | 10 | | 23 | 2 | |
| Depth of tumor invasion | | | | | | |
| pT1-pT2 | 10 | 11 | 0.5137 | 18 | 3 | 0.4076 |
| pT3-pT4 | 28 | 19 | | 36 | 10 | |
| Lymph node metastasis | | | | | | |
| Absent | 10 | 7 | 1.0000 | 11 | 6 | 0.1091 |
| Present | 28 | 23 | | 44 | 7 | |
| Stage | | | | | | |
| Ⅰ-Ⅱ | 25 | 17 | 0.6049 | 33 | 9 | 0.7525 |
| Ⅲ-Ⅳ | 13 | 13 | | 22 | 4 | |

### *Validation of the amplified pericentromeric region, 9p13*

The presence of the 9p13 amplification in the GC cell lines was confirmed by metaphase FISH using a BAC clone (Figure 1). We observed signal gain in all cell lines, and only ACP02 showed high amplification of this region (Table 2).

Based on gene location and annotated gene function, we selected the *MELK* and *IL11RA* genes for validation in GC cell lines and in 68 primary gastric adenocarcinoma by qPCR. We detected two copies of *IL11RA* and three copies of *MELK* in all GC cell lines. By analyzing the CNV of these two genes in gastric tumors, we observed that 19.1% (13/68) and 55.9% (38/68) of gastric tumors had ⩾ 3 copies of *IL11RA* and *MELK*, respectively. No association was found between the clinicopathological characteristics of patients and the number of copies of the studied genes (Table 3).

## DISCUSSION

aCGH is a high resolution tool that allows the simultaneous detection of sub-microscopic copy number changes across the genome, thus overcoming the several limitations of cCHG[23]. In this study, most of the copy number changes observed in ACP02, ACP03, AGP01 and PG100 by cCGH were confirmed by aCGH. ACP02, ACP03 and AGP01 are gastric adenocarcinoma cell lines from diffuse and intestinal types and cancerous ascitic fluid and were previously established and characterized by our research group[17,18], while PG100 is a commercially available primary gastric adenocarcinoma cell line[19]. Furthermore, aCGH analysis enabled the identification of many additional chromosomal gains and losses. On the other hand, the gain of the 16p21-p23 region in ACP03 and the gains of the 6p11-p12, 12p11.1 and 18p11.2-p11.3 regions in AGP01 were only detected by cCGH. This may be due to technical reasons, as cCGH is more sensitive than aCGH for detecting large chromosome regions, as previously discussed by Kamradt *et al*[24].

When comparing the GC cell lines, only a few differences in cytogenetic composition were found by cCGH and aCGH. The gain on 9p13.3 was found in all cell lines, and the presence of this amplicon in these gastric cell lines was confirmed by metaphase FISH, using a BAC clone for the amplified region. It is noteworthy that high levels of this amplification were only found in ACP02.

Genetic alterations in the short arm of chromosome 9 are commonly observed in different cancer types[25]. In GC, losses of 9p have been frequently described[26-29]. Fan *et al*[29] (2012) observed a homozygous deletion at 9p21, which encompasses the *P16INK4A* tumor suppressor gene, in 11% (8/72) of the gastric tumors studied. To our knowledge, this is

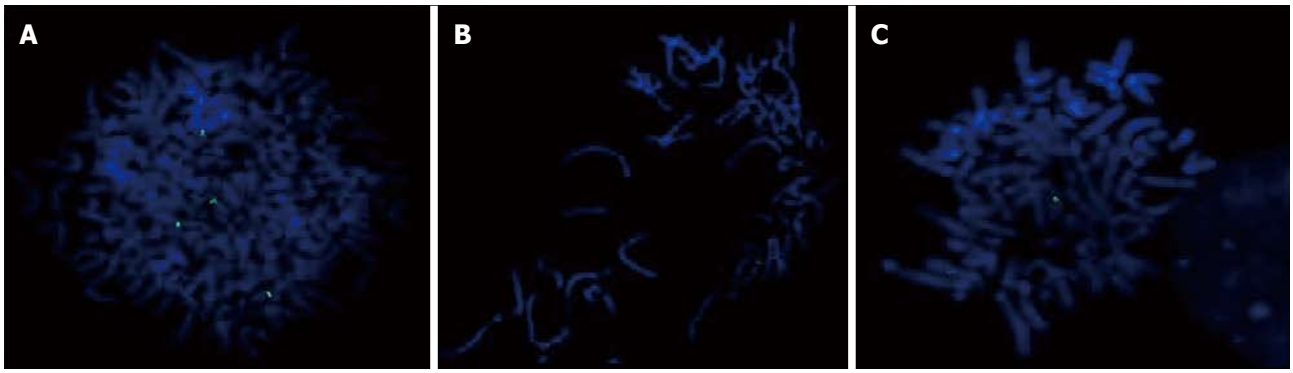


**Figure 1 9p13 amplification by fluorescence *in situ* hybridization analysis in ACP02 (A), ACP03 (B) and AGP01 (C) cell lines.** Magnification of × 60.

the first study that describes gains at 9p in GC.

Amplifications on 9p have been reported in esophageal cancer[30], lung sarcomatoid carcinoma[31] and breast cancer[32]. Towle *et al*[33] found that 16.6% (36/217) of the cell lines carried regions of genomic gain spanning part of chromosome 9p13. Additionally, 1.8% (4/217) harbored high-level DNA amplification of this region, including a ductal breast carcinoma line (B0T-474), a tongue squamous cell carcinoma line (SCC-9), a melanoma line (WM-115), and an osteosarcoma line (MG-63).

Because this region harbors several tumor-related genes, several studies in the literature have correlated gene copy number alterations of 9p13 with cancer[24,34]. Sarhadi *et al*[34] observed that the gain of chromosome 9p13 encompasses many genes, such as *KIAA1161*, *C9orf24*, *C9orf25*, *DNAI1*, *ENHO*, *CNTFR*, *LOC415056*, *C9orf23*, *DCTN3*, *ARID3C*, *SIGMAR1*, *GALT*, *IL11RA*, *CCL27*, *CCL19*, *CCL21* and *FAM205A*, in different types of cancer.

In this study, we selected the *IL11RA* and *MELK* genes to validate this amplification region in GC cell lines and primary gastric adenocarcinoma. The results showed an increase in the copy number of the *MELK* gene in ACP02, ACP03, AGP01 and PG100. Moreover, 19.1% (13/68) and 55.9% (38/68) of gastric tumors showed $\geqslant$ 3 copies of *IL11RA* and *MELK*, respectively.

Kamradt *et al*[24] analyzed a small amplicon at 9p13.3 in prostate cancer cell lines and validated *IL11RA* copy number gain in 75% (15/20) of prostate tumors. In addition, it has been demonstrated that *IL11RA* is overexpressed in GC, colon cancer, breast cancer, prostate cancer and osteosarcoma[35-41]. *IL11RA* encodes a specific receptor for IL11, and the IL11/IL11RA signaling pathway is involved in the regulation of several biological activities, such as adipogenesis, osteoclastogenesis, neurogenesis, and megakaryocyte maturation and platelet production[42,43].

With regard to *MELK*, the other gene that was selected for validation, this study describes, for the first time, that the copy number gain of the *MELK* gene occurs in cancer. To our knowledge, only one previous study on astrocytoma samples has investigated *MELK* amplification, and they did not find any *MELK* copy number gain[44].

MELK is a highly conserved serine/threonine kinase that was first found to be expressed in a wide range of early embryonic cellular stages, and as a result, it has been implicated in embryogenesis and cell cycle control[45]. Additionally, several studies have identified MELK overexpression in stem cell populations and several human cancers, including aggressive astrocytoma, breast cancer, prostate cancer, melanoma and GC[44-49].

Preclinical studies have suggested MELK as a potential therapeutic target for multiple cancers. Since then, novel therapeutics that selectively inhibit MELK have been developed, such as OTSSP167, which is currently in a Phase I trial for patients with solid tumors and who have not responded to treatment[45,50-53].

Li *et al*[54] observed MELK overexpression more frequently in GC lesions than in the corresponding noncancerous mucosa and that higher MELK levels were associated with lymph node involvement, distant metastasis, and poor prognosis in patients with GC. In addition, these authors demonstrated that reducing MELK expression or inhibiting its kinase activity resulted in growth inhibition, G2/M arrest, apoptosis and the suppression of the invasive capability of GC cells *in vitro* and *in vivo*. *MELK* knockdown also led to alterations in the levels of epithelial mesenchymal transition (EMT)-associated proteins. Furthermore, in GC patient-derived xenograft models, targeted treatment with OTSSP167 showed anticancer effects. These results suggest that MELK may be a promising target for GC treatment.

In conclusion, our results from generating genome wide DNA copy number profiles in GC cell lines and validation in primary gastric adenocarcinoma specimens revealed genomic aberrations redundancies, indicating that the cell lines retain the gross genomic architecture of primary tumors. Moreover, the characterization of a small gained region at 9p13.3 in GC cell lines and primary gastric adenocarcinoma samples revealed *MELK* as a candidate target gene this region that may possibly be linked to the development of GC. Therefore, we hypothesize that the copy number gain of *MELK* may be a mechanism of gene overexpression

and may represent an interesting therapeutic target in gastric carcinogenesis.

## COMMENTS

### Background

Despite alterations in DNA copy number is one of the key hallmarks of carcinogenesis, the chromosomal regions with frequent gain and loss are still poorly defined in gastric cancer. The characterization of a small gain or loss region in gastric cancer cell lines and primary gastric adenocarcinoma samples could reveal a candidate target gene that may possibly be linked to the development of gastric cancer.

### Research frontiers

DNA copy number profiles in gastric cancer cell lines and validation in primary gastric adenocarcinoma specimens revealed genomic aberrations redundancies, indicating that the cell lines retain the gross genomic architecture of primary tumors. Moreover, the characterization of a small gained region at 9p13.3 in gastric cancer cell lines and primary gastric adenocarcinoma samples revealed *MELK* as a candidate target gene that is possibly related to the development of gastric cancer.

### Innovations and breakthroughs

Several studies in the literature have correlated gene copy number alterations of 9p13 region. A study described a small amplicon at 9p13.3 in prostate cancer cell lines and validated *IL11RA* copy number gain in 75% (15/20) of prostate tumors. However, this is the first time that the copy number gain of the *MELK* gene was described in tumor. Furthermore, in gastric cancer patient-derived xenograft models, targeted treatment with OTSSP167 (a MELK inhibitor) showed anticancer effects. These results suggest that MELK may be a promising target for gastric cancer treatment.

### Applications

The authors suggested that the copy number gain of *MELK* may be a mechanism of gene overexpression and may represent an interesting therapeutic target in gastric carcinogenesis in the future.

### Terminology

Copy number variation (CNV) is a type of structural variation characterized by duplication or deletion of sections of the genome, which in turn can result in phenotypic alterations. Array comparative genomic hybridization (aCGH) is a technology developed for a high-resolution evaluation of DNA copy number alterations associated with chromosome abnormalities.

### Peer-review

The authors tried to identify common copy number alterations by using chromosomal comparative genome hybridization and array comparative genome hybridization in four gastric cancer cell lines. They concluded MELK as a candidate target gene that is possibly related to the development of gastric cancer.

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