World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v18.i33.4522

World J Gastroenterol 2012 September 7; 18(33): 4522-4532
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Loss of fragile histidine triad and amplification of 1p36.22 and 11p15.5 in primary gastric adenocarcinomas

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Received: December 19, 2011 Revised: February 1, 2012
Accepted: April 13, 2012
Published online: September 7, 2012

Abstract

AIM: To investigate the genomic copy number alterations that may harbor key driver genes in gastric tumorigenesis.

METHODS: Using high-resolution array comparative genomic hybridization (CGH), we investigated the genomic alterations of 20 advanced primary gastric adenocarcinomas (seventeen tubular and three mucinous) of Chinese patients from the Jilin province. Ten matching adjacent normal regions from the same patients were also studied.

RESULTS: The most frequent imbalances detected in these cancer samples were gains of 3q26.31-q27.2, 5p, 8q, 11p, 18p, 19q and 20q and losses of 3p, 4p, 18q and 21q. The use of high-resolution array CGH increased the resolution and sensitivity of the observed genomic changes and identified focal genetic imbalances, which included 54 gains and 16 losses that were smaller than 1 Mb in size. The most interesting focal imbalances were the intergenic loss/homozygous deletion of the fragile histidine triad gene and the amplicons 11q13, 18q11.2 and 19q12, as well as the novel amplicons 1p36.22 and 11p15.5.

CONCLUSION: These regions, especially the focal amplicons, may harbor key driver genes that will serve as biomarkers for either the diagnosis or the prognosis of gastric cancer, and therefore, a large-scale investigation is recommended.

Key words: Array comparative genomic hybridization; Amplicon; Gastric adenocarcinoma; Oncogene; Fragile histidine triad

Peer reviewers: Hitoshi Tsuda, MD, PhD, Diagnostic Pathology Section, Clinical Laboratory Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan; Dr. Debra F Saxe, Department of Pathology and Laboratory Medicine, Emory University, Atlanta, GA 30322, United States

Liu YY, Chen HY, Zhang ML, Tian D, Li S, Lee JY. Loss of fragile histidine triad and amplification of 1p36.22 and 11p15.5 in primary gastric adenocarcinomas. *World J Gastroenterol* 2012; 18(33): 4522-4532 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i33/4522.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i33.4522

INTRODUCTION

Gastric cancer is one of the leading causes of cancer-related death worldwide. Although its incidence has gradu-

ally decreased in many Western countries, the incidence of gastric cancer still remains high in South and Central America and is highest in Eastern Asia, specifically in China, South Korea and Japan[1,2]. The most common gastric malignancy is adenocarcinoma[3], which is characterized by multiple genetic instabilities, as are other adenocarcinomas. One of these genetic instabilities is chromosomal instability, a common consequence of a chromosomal or chromosome-segment abnormality, that causes DNA copy number changes during tumor progression. These alterations may lead to a loss of function of tumor suppressor genes (inactivation) and/or a gain of function of oncogenes (activation). High-level DNA copy number changes (amplification/amplicons) in tumors are frequently restricted to certain chromosomal regions containing well-known oncogenes that are also overexpressed or activated[4,5]. Some oncogenes, such as *NMYC*, *LMYC* and *GLI*, were originally discovered because of their genomic amplification in human tumors[4]. An analysis of the composition of DNA amplifications showed that human cancer can be classified *via* DNA copy number profiling because such amplifications are non-randomly selected with respect to the biological backgrounds of cancer[6]. Therefore, the detection and discovery of unidentified or incompletely described amplicons and relevant genes located within these amplicons can lead to the identification of genes putatively involved in growth control and tumorigenesis.

Recently available whole-genome array comparative genomic hybridization (CGH), a high-throughput genomic technology, facilitates the accumulation of high-resolution data of genomic imbalances associated with disease. In this study, we identified possible candidate genes that could provide insight into the pathology of gastric adenocarcinoma through the integration of genomic copy number changes.

MATERIALS AND METHODS

Tumor samples

This study included seventeen tubular and three mucinous adenocarcinomas of advanced primary stomach cancer samples from Jilin Province in North-Eastern China (Table 1). Of the twenty samples studied, thirteen were from males and seven were from females. The mean age was 62.1 (ranging from 52 to 76) years. The stage of each tumor was classified according to the tumor node metastasis classification of the International Union Against Cancer. The histopathological grades were as follows: Grade 1 (well-differentiated/low grade adenocarcinoma), no cases; Grade 2 (moderately differentiated/intermediate-grade adenocarcinoma), five (tubular) cases; and Grade 3 (poorly differentiated/high-grade adenocarcinoma), 15 (12 tubular and three mucinous) cases. Unfortunately, we were unable to obtain information concerning postsurgical pathological stages. Tumor samples were obtained surgically in the First Hospital of Jilin University; paired adjacent normal tissue was also collected as a control for comparison with the tumor. All patients had negative histories of exposure to either chemotherapy or radiotherapy before surgery, and there were no other diagnosed cancers. An informed consent with approval of the ethics committee of the First Hospital of Jilin University was obtained from all participating patients.

Twenty tumor samples and ten paired adjacent normal tissues were snap-frozen after surgical resection and stored at -80 ℃. DNA was isolated from the tumor tissue by proteinase K digestion followed by phenol-chloroform extraction according to standard protocols.

Array CGH assay

Array CGH was performed according to the manufacturer's protocol with minor modifications on a 385k oligonucleotide chip (Roche/NimbleGen Systems Inc., Madison, WI). Commercially available pooled normal control DNA (Promega Corporation, Madison, WI) was used for reference. The patient DNA and the reference DNA were labeled with either cyanine 3 (Cy-3) or cyanine 5 (Cy-5) by random priming (Trilink Biotechnologies, San Diego, CA) and then hybridized to the chip *via* incubation in the MAUI hybridization system (BioMicro Systems, Salt Lake City, UT). After 18-h hybridization at 42 ℃, the slides were washed and scanned using a GenePix 4000B (Molecular Devices, Sunnyvale, CA).

Statistical analysis

Profile smoothing and breakpoint detection were performed with NimbleScan version 2.4 and SignalMap version 1.9 (NimbleGen Systems). If a smoothed copy number $\log_2$ ratio was above 0.15 or below -0.15 across five neighboring probes, it was defined as a gain or a loss, respectively. Amplifications were defined as those with a smoothed DNA copy number ratio above 0.5.

RESULTS

Overview of genomic imbalances in 20 primary gastric adenocarcinomas

An overview of genomic imbalances in the twenty advanced primary gastric adenocarcinomas is shown in Figure 1. Genomic copy number changes (gains, losses, amplifications or homozygous deletions) were detected in all cases except one. Net gains (15 cases) of genetic material were more frequent than net losses (4 cases). The sizes of the net genomic imbalances per sample ranged from a loss of 122.2 Mb (4.1% of genome) to a gain of 336.9 Mb (11.2% of genome) (Table 1). The mean number of gains per case was 9.0, ranging from 0 to 40, and the mean number of losses per case was 3.5, ranging from 0 to 14. The gain sizes ranged from 56.3 kb to 158.6 Mb, and the loss sizes ranged from 150.1 kb to 131 Mb. Approximately 28% (70/250) of the genomic imbalances were smaller than 1 Mb; from this subset, 21.6% (54/250) of the total imbalances were gains, and 6.4% (16/250) were losses. The most frequent

Table 1 Clinical characteristics, risk factors and overall genomic imbalances in 20 gastric adenocarcinomas

No.	ID	Sex/age (yr)	T/N/M stage	Tumor type	Histology grade (differentiated)	Tumor location	Smoke history	Drink history	Genomic size of total gain (Mb)	Genomic size of total loss (Mb)	Net imbalances (Mb) (%)[2]
1	T64	M/75	T3/N3/M0	Tubular	Poorly	Upper (CR), lower (AA)	Y	Y	290.7	147.3	+143.4 (4.8)
2	TW0800	M/65	T3/N3/M0	Tubular	Poorly	Upper (CR/GF), central (GB)	N	N	388.9	205.3	+183.6 (5.6)
3	T74	M/58	T3/N3/M0	Tubular	Poorly	Central (GB)	N	Y	290.1	412.3	-122.2 (4.1)
4	TW0784	M/75	T3/N1/M0	Tubular	Poorly	Upper (CR/GF)	N	N	3.4	0	+3.4 (0.1)
5	T66	M/50	T3/N2/M0	Tubular	Poorly	Lower (AA)	N	Y	34.2	0	+34.2 (1.1)
6	T78	F/59	T3/N1/M0	Tubular	Poorly	Lower (AA)	N	N	146.2	0	+107.4 (3.6)
7	T41	M/73	T3/N2/M0	Tubular	Poorly	Lower (AA/P)	Y	Y	7.2	0	+7.2 (0.2)
8	T47	F/52	T3/N1/M0	Tubular	Poorly	Lower (AA)	N	N	0.0	0.0	0.0 (0.0)
9	T38	F/53	T2/N1/M0	Tubular	Poorly	Lower (AA/P)	N	N	294.3	37.4	+256.9 (8.6)
10	TW0796	M/56	T3/N1/M0	Tubular	Poorly	Lower (AA/P)	Y	Y	224.4	11.9	+212.5 (7.1)
11	TW0807	M/54	T3/N0/M0	Tubular	Poorly	Lower (AA/P)	Y	Y	191.7	43.7	+148.0 (4.9)
12	TW0813	F/57	T3/N2/M0	Tubular[1]	Poorly	Lower (AA/P)	N	N	76.1	54.6	+21.5 (0.7)
13	T52	M/73	T3/N2/M0	Tubular	Moderately	Upper (CR/GF)	N	N	333.9	361.8	-27.9 (0.9)
14	TW0797	M/62	T3/N1/M0	Tubular	Moderately	Upper (CR/GF)	N	Y	161.1	0	+161.1 (5.4)
15	TW0782	M/53	T3/N2/M0	Tubular	Moderately	Upper (CR/GF)	Y	N	290.1	108.3	+181.8 (6.1)
16	TW0780	F/59	T2/N0/M0	Tubular	Moderately	Lower (AA/P)	N	N	4.1	4.16	-0.06 (0.0)
17	T75	M/69	T3/N2/M0	Tubular	Moderately	Lower (AA)	Y	Y	28	0	+28.0 (0.9)
18	T76	F/59	T3/N1/M0	Mucinous	Poorly	Upper (CR/GF)	Y	Y	99.1	0	+99.1 (3.3)
19	TW0789	M/64	T3/N1/M0	Mucinous	Poorly	Lower (AA/P)	N	N	504.5	167.6	+336.9 (11.2)
20	TW0774	F/76	T3/N3/M0	Mucinous	Poorly	Lower (AA/P)	N	N	41.4	90.4	-49.0 (1.6)

[1]Signet ring cell; [2]Percent of net imbalances calculated based on 3000 Mb of genome size. F: Female; M: Male; T: Tumor; N: Node; M: Metastasis ; CR: Cardiac region; GF: Gastric fundus; GB: Gastric body; AA: Antral area; P: Pylorus; Y: Yes; N: No.

genomic imbalances detected in these cancer samples were gains of 3q26.31-q27.2 (6/20), 5p (5/20), 8q [12/20: 8q22.2-q22.3 (10), 8q24.13-q24.22 (12)], 11p (6/20), 18p (4/20), 19q (8/20) and 20q (8/20) and losses of 3p14.2 (6/20), 4p15.1 (6/20), 18q21.2-q22.1 (4/20) and 21q21.1-q21.2 (4/20). However, no genomic imbalances were detected in the ten paired adjacent tissues, demonstrating that these genomic imbalances are tumor related.

Genomic regions with amplification: Possible diagnostic marker loci

The most prominent feature in this study was the amplicons of 11q13 (two tubular and one mucinous), 18q11.2 (three tubular) and 19q12 (three tubular), as well as the novel amplicons 1p36.22 (two tubular) and 11p15.5 (three tubular) (Table 2 and Figure 2). Amplification of 11q13 had the smallest region of overlap (SRO) of 343.7 kb, which included the *CCND1*, *ORAOV1* and *FGF19* genes. Amplification of 18q11.2 had an SRO of 625.0 kb, which included the *GATA6* and *CTAGE* genes. Amplification of 19q12 had an SRO of 1.4 Mb, which included nine genes: *LOC148145*, *LOC10050583*, *UQCRFS1*, *LOC284395*, *VSTM2B*, *POP4*, *PLEKHF1*, *C19orf12* and cyclin E1 (*CCNE1*). The SRO of the novel amplification 1p36.22 was 418.7 kb and included *FEX14*, *CASZ1*, *C1orf127* and *TARDBP*. The SRO of the other novel amplification, 11p15.5, was 343.7 kb and included *MUC5B*, *LOC255512*, *TOLLIP*, *BRSK2*, *HCCA2*, *DUSP8* and *LOC338865*. Other regions in which amplification was detected were 8p23.1, 8q24.21, 10q26.12q26.13, 11q13, 12p12.1, 12q15, 17q12 and 20q13.2; these regions are summarized in Table 2.

The most common losses involved the fragile histidine triad/FRA3B and PDCH7 genes

Six cases had a loss of the fragile histidine triad (*FHIT*) gene, which maps to the common fragile site 3p14.2. Five of these were intergenic losses of the *FHIT* gene,

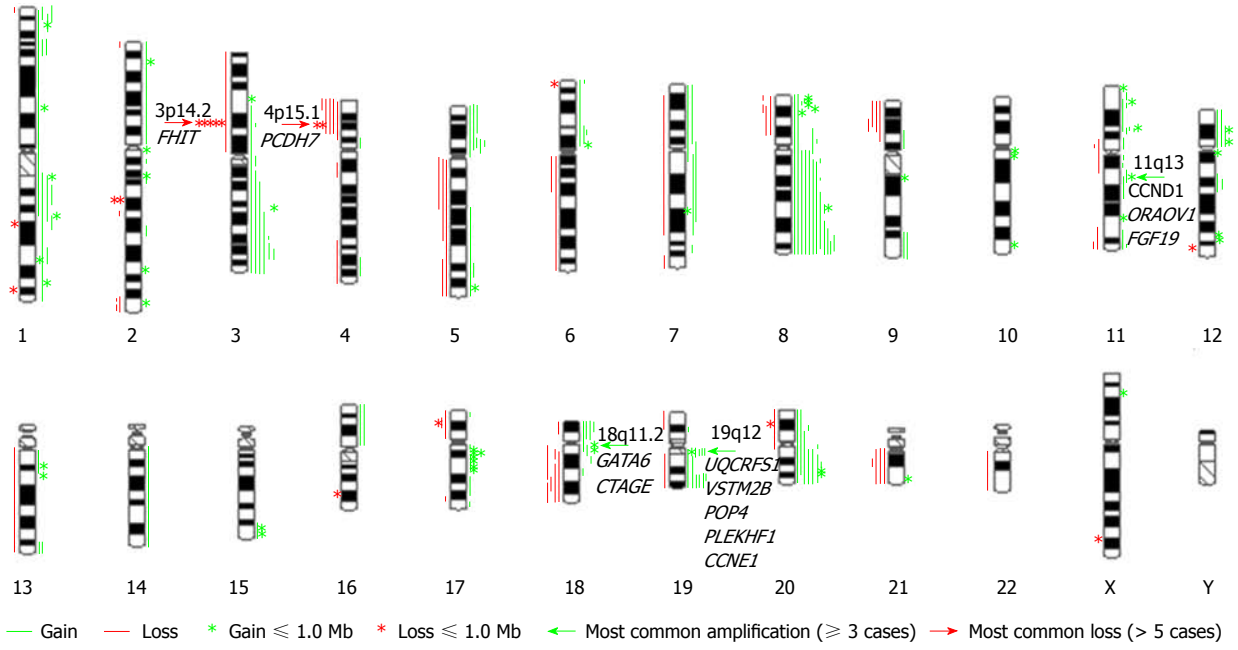

Figure 1 An overview of genomic imbalances in 20 primary gastric adenocarcinomas. The total number of gains was 180 (54 ⩽ 1 Mb), and the total number of losses was 70 (16 ⩽ 1 Mb). Partial or whole gains of 8q (12/20), 19q (8/20), and 20q (8/20) were most frequent. The most common amplicons were of the 11q13, 18q11.2 and 19q12 regions (green arrows). The loss of the *FHIT* gene and the partial loss of 4p with the smallest region of overlap including the *PCDH7* gene were the most frequent losses (red arrow).

and one of these five cases (T38) showed a homozygous deletion. The sizes of these losses ranged from 88.3 kb to 762.5 kb (Table 3 and Figure 3). Another common loss identified in all six cases was the 4p15.1 region (Table 3 and Figure 3). Two cases (TW789 and TW782) had an intergenic loss of the *PDCH7* gene.

DISCUSSION

In this study, we investigated gene/segmental genomic copy number alterations in twenty advanced primary gastric adenocarcinomas (seventeen tubular and three mucinous) *via* whole-genome array CGH. We observed that the total number of gains/amplifications (180) was 2.6 times the total number of losses (70). Nineteen out of the 20 cases had genomic imbalances; fifteen of these had net genomic gains (3.4-336.9 Mb), and four of these had net genomic losses (0.06-122.2 Mb), indicating that genomic gains are more common than losses (Figure 1). These findings are compatible with previous findings determined *via* conventional CGH[7-51]. However, we discovered cryptic genomic imbalances smaller than 1.0 Mb in 28% (70/250) of the total imbalances (with an average of 3.5 per case) and narrowed down the SROs of losses or of gains/amplifications to those including interesting genes or focal genomic segments. These findings are explained by the resolution of the array that we used. The most interesting cryptic imbalances were losses of 3p14.2 and 4p15.1 (Figure 3) and amplifications of 1p36.22, 11p15.5, 11q13, 18q11.2 and 19q12 (Figure 2).

The loss of 3p14.2 in six of our cases encompassed the *FHIT* gene, which was discovered at the FRA3B locus on the short arm of chromosome 3 and is the most active common chromosome fragile site in the human genome. *FHIT*'s loss of function as a tumor suppressor gene due to breakage, allelic loss, occasional homozygous deletion and promoter hypermethylation has been evaluated in different types of epithelial tumors, including gastric cancer, which is strongly associated with direct or indirect exposure to environmental carcinogens[52-54]. The FHIT protein is absent in more than 50% of the observed cases, both in gastric cancer cell lines and in primary gastric adenocarcinomas, irrespective of any specific histotype, indicating that alterations of the *FHIT* gene can play a role in tumorigenicity as an important and preliminary genetic alteration in the cell[55]. However, subsequent additional genetic changes involving other tumor suppressor genes or oncogenes may be necessary for tumor progression. In our six cases who had lost the *FHIT* gene, five showed an intergenic loss of *FHIT*, ranging in size from 150 kb to 762.4 kb. One case had a homozygous deletion of part of the *FHIT* gene (case T36); however, no deletion of this region was found in the normal adjacent tissue of the same case (date not shown). This finding suggests that *FHIT* loss, not normal copy number variation, is clearly linked to tumorigenicity. However, copy number variations, particularly losses including the *FHIT* gene, have been reported in the normal population[56], raising the question of whether constitutional copy number loss of the *FHIT* gene increases tumorigenicity susceptibility.

The other most common loss was of 4p15.1, detected in six cases and encompassing the *PDCH7* gene. PDCH7 is an integral membrane protein that is thought

Table 2 Amplification segments and the genes involved

Chr. region	Amp	Gain	SRO, bp (hg 18)	Size (kb)	Genes	Selected references
1p36.22[1]	2	1	10 587 540-11 006 299	418.7	*PEX14, CASZ1, C1orf127, TARDBP*	N/A
1q21.2	1	3	148 737 723-149 062 575	324.9	*TARS2, ECM1, ADAMTSL4*[3], *MCL1*[3], *ENSA*[2], *GOLPH3L, HORMAD1*[3], *CTSS, CTSK, ARNT*[2]	Gastric cancer[7], adenocarcinoma of the gastroesophageal junction[8][3], basal/luminal breast cancer[9][3], hepatocellular carcinoma[10]
8p23.1	2	3	10 475 239-10 562 632	87.4	*RP1 L1*	Gastric cancer[7,11], esophageal adenocarcinoma[12,13][3],[14],[15][3], adenocarcinomas of the gastroesophageal junction[16][3], small bowel adenocarcinoma[17]
			10 681 251-10 943 920	262.7	*PINX1, XKR6*	
			11 250 228-11 887 602	637.4	*TDH, FAM167A, BLK, GATA4*[2,3,4], *NEIL2*[2], *FDFT1*[2,3], *CTSB*[2,3]	
8q24.21	1	11	128 331 422-128 837 626	506.2	*POU5F1B, LOC727677, MYC*[2,3]	Various cancer[5], esophageal adenocarcinoma[14], gastric cancer[18],[19][2],[20], papillary renal cell carcinoma[21][3]
10q26.12	1	0	121 881 486-123 931 430	2050.0	*PPAPDC1A, LOC283089, WDR11, FGFR2*[3], *ATE1*[3], *NSMCE4A*[3], *TACC2*[3]	Breast cancer[22][3], gastric carcinoma[20]
10q26.13			126 212 750-126 362 686	149.9	*LHPP, FAM53B*	
11p15.5[1]	1	0	1 175 114-1 556 281	381.2	*MUC5B, LOC255512, TOLLIP, BRSK2, HCCA2, DUSP8, LOC33865*	N/A
11p13	2	1	34 675 094-35 068 916	393.8	*APIP*[3], *PDHX*[3]	Breast cancer[23], gastric cancer cell line[24], head and neck squamous cell carcinoma cell line[25][3]
11q13.2q13.3	3	0	68 912 663-69 256 388	343.7	*CCND1*[2,3], *ORAOV1*[2,3], *FGF19*[3]	Various cancers[5], gastric cancer[7,11,20],[26][2], hepatocellular carcinoma[27][2], esophageal adenocarcinoma[14], esophageal and gastric cancer[26], esophageal squamous cell carcinoma[28][3], laryngeal/pharyngeal cancer[29][3]
12p12.1	2	2	25 150 060-25 437 736	287.7	*LRMP*[3], *CASC1, LYRM5, KRAS*[2,3]	Various cancers[5], esophageal adenocarcinoma[14], gastric cancer[11,20],[30][2], ovarian cancer[31][3]
12q15	2	0	67 475 003-67 875 102	400.1	*MDM2*[2], *CPM*	Various cancers[5], esophageal adenocarcinoma[14], gastric cancer[20,32], liposarcomas[33][3], melanoma cell line[34][3]
			68 037 717-68 318 830	281.1	*YEATS4 (GAS41)*[3], *FRS2*[3], *CCT2*[3], *LRRC10*	
17q12	2	1	35 000 176-35 150 077	149.9	*NEUROD2, PPP1R1B*[2], *STARD3*[2], *TCAP, PNMT*[2], *PERLD1*[2], *ERBB2*[2], *C17orf37, GRB7*[2]	Various cancers[5], gastric cancer[11,20],[35-37][2],[38],[39][2]
18q11.2	3	2	17 800 202-18 425 167	625.0	*GATA6*[3,4], *CTAGE1*	Pancreatic carcinoma[40,41][3], esophageal adenocarcinoma[14],[42][3]
19q12	3	3	33 606 476-35 037 396	1340.9	*LOC148145, LOC10050583, UQCRFS1*[2], *LOC284395, VSTM2B, POP4*[2], *PLEKHF1*[2], *C19orf12*[2], *CCNE1*[2]	Gastric cancer[43][2],[44],[45][2], esophageal/gastric cardiac adenocarcinoma[12]
20q13.2	1	6	51 568 862-51 993 797	424.9	*ZNF217*[3], *SUMO1P1, BCAS1*[3]	Esophageal adenocarcinoma[14], adenocarcinoma of the gastroesophageal junction[46][3], breast cancer[47], gastric adenocarcinoma[48], glioblastoma[49], various cancers[50]

[1]Novel amplicon; [2]Gene and references that are overexpressed when amplified in gastric cancer; [3]Genes (and references) that are overexpressed when amplified in types of cancer other than gastric cancer; [4]Underexpression has been reported in gastric cancer. SRO: Smallest region of overlap; N/A: Not available.

to function in cell-cell recognition and adhesion. Loss of heterozygosity (LOH) or deletion of this region has been reported in hepatocellular carcinoma (HCC) and in some head and neck squamous cell carcinomas[57,58]. Recently, a genome-wide analysis revealed a single-nucleotide polymorphism in *PDCH7* whose risk allele affects overall survival in early-stage non-small-cell lung cancer[59].

Gene/chromosomal segment amplifications are thought to reflect genetic instabilities in solid-tumor cells[60]. Such amplifications commonly consist of double minutes (DMs) or homogeneous staining regions or are dispersed at the genomic level; they are usually correlated with protein levels of genes[61]. It has been proposed that the activation of proto-oncogenes by amplification plays an important role in the development of many human solid tumors. Therefore, detection of specific gene amplifications in tumor cells can lead to the identification of genes putatively involved in growth control and tumorigenesis. In our study, we identified the novel amplicons 1p36.22 and 11p15.5 as well as prominent amplicons 11q13, 18q11.2 and 19q12 (≥ 3 cases with amplification).

LOH or the loss of the short arm of chromosome 1, which includes band p36, has been reported in vari-

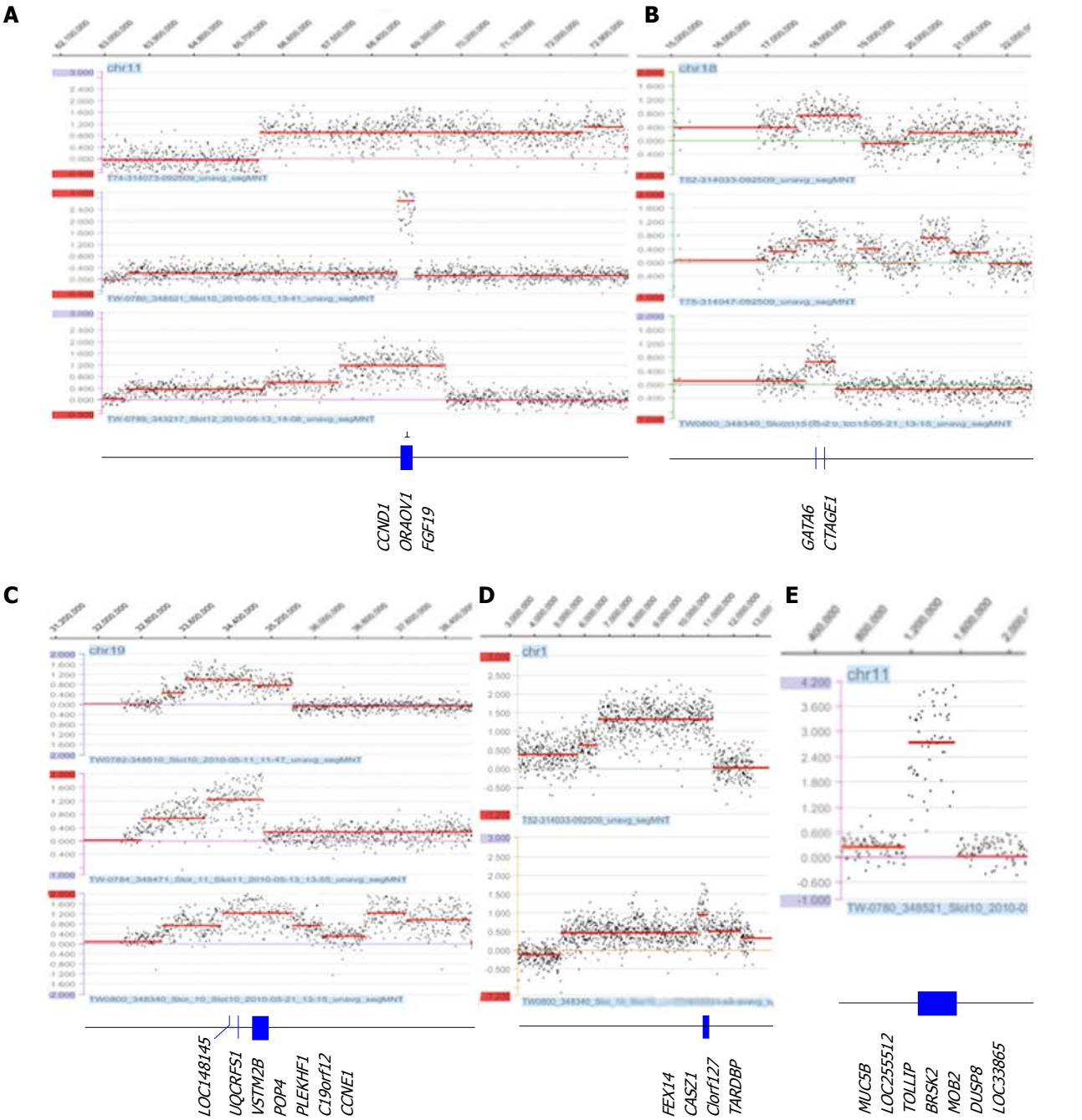

Figure 2 Representative amplifications detected by array comparative genomic hybridization and the genes that are located in the smallest region of overlap. The most common amplicons at 11q13 (A), 18q11.2 (B) and 19q12 (C) and novel amplicons at 1p36.22 (D) and 11p15.5 (E) region (log2 > 0.5). The x-axis indicates the genomic location, and the y-axis indicates the log2 ratio.

ous cancers[62], supporting the possibility that this region encompasses at least one tumor suppressor gene, as opposed to one or more oncogenes. The *CASZ1* gene on 1p36, which was amplified in our cases, has been implicated as a candidate tumor suppressor gene in neuroblastomas[63]. However, our study revealed three cases with a gain of 1p36.22. Of these, two cases with high-level amplification of a 418.7 kb SRO, which includes the *PEX14*, *CASZ1*, *C1orf127* and *TARDBP* genes, had not previously been reported. Further studies could determine whether this amplification induces overexpression of proteins. The other novel amplicon, found in one case, was the 11p15.5 region, which is 343.7 kb and includes the *MUC5B*, *TOLLIP*, *BRSK2*, *HCCA2*, *DUSP8* and *LOC33865* genes. The *MUC5B* gene encodes a member of the mucin family of proteins, which are highly glycosylated macromolecular components of mucus secretions. This family member is the major gel-forming mucin in mucus. The expression of this gene has been associated with a type of gastric carcinoma but not with the clinical-biological behavior of the tumors[64,65]. The *TOLLIP* gene encodes a ubiquitin-binding protein that interacts with several toll-like receptor (TLR) signaling cascade components. The TOLLIP

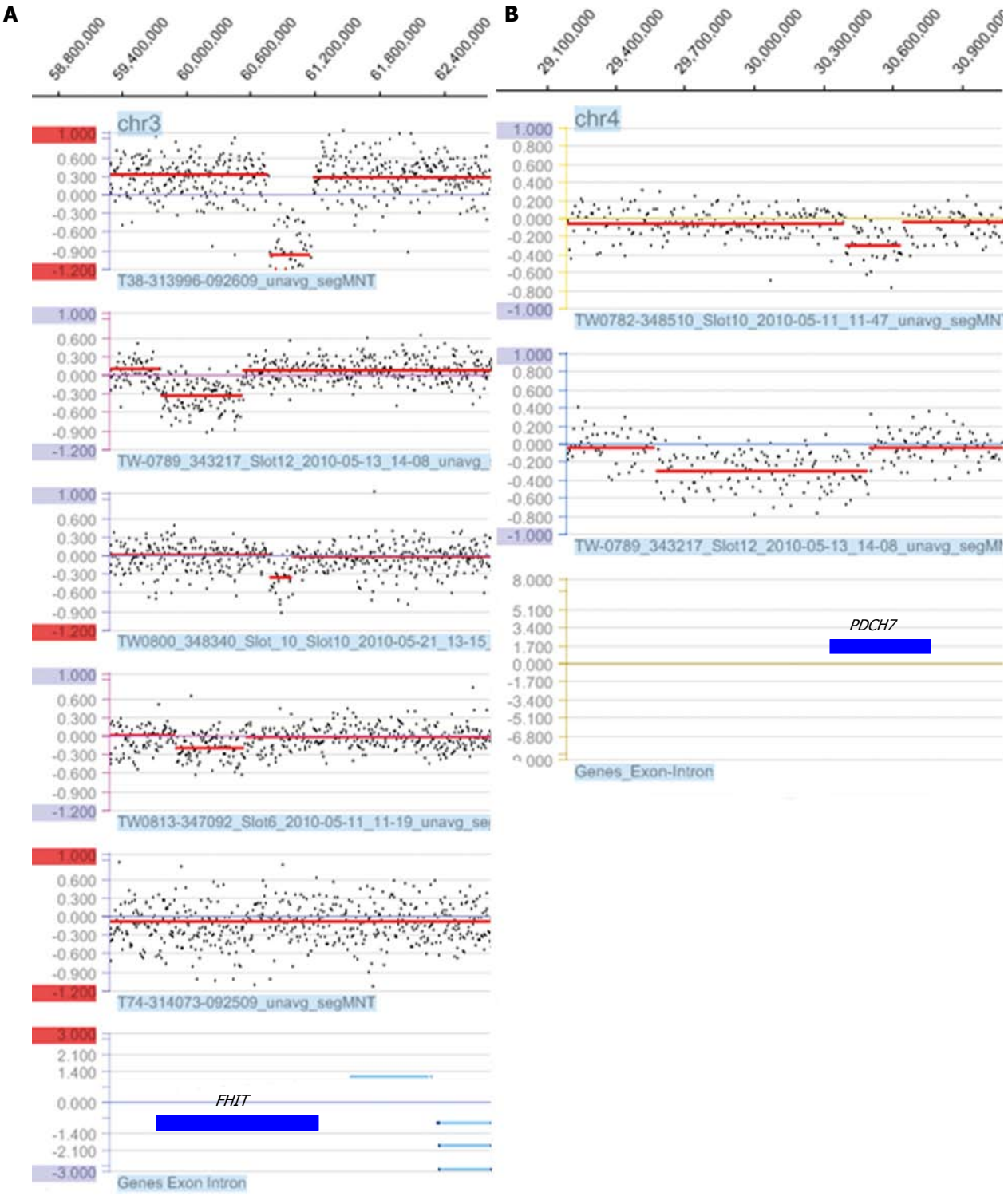

Figure 3 Intergenic loss of *FHIT*/FRA3B on 3p14.2 (A) and *PDCH7* on 4p15.1 (B) detected by array comparative genomic hybridization. The x-axis indicates the genomic location, and the y-axis indicates the log2 ratio.

protein regulates inflammatory signaling and is involved in interleukin-1 receptor trafficking and the turnover of the IL1R-associated kinase; a possible association with human cancer development has been suggested[66]. The BR serine/threonine kinase 2 (*BRSK2*) gene acts as a checkpoint kinase upon DNA damage induced by UV irradiation or methyl methane sulfonate[67]. Clinical implications of *BRSK2* expression in pancreatic ductal adenocarcinoma have been suggested[68]. The hepatocellular carcinoma-associated gene 2 (*HCCA2*) gene was initially identified as a HCC-specific protein. It was subsequently found to interact with *MAD2L2* and might function in cell cycle regulation[69]. The dual specificity phosphatase 8 (*DUSP8*) gene is a member of the *DUSP* subfamily.

Table 3 Summary of losses of *FHIT* at 3p14.2 and of *PDCH7* at 4p15.1

Gene	Chromosome region	Case	Genomic coordinates (NCBI build 36.3; hg18)	Size (kb)
FHIT (HD)	3p14.2	T38	60 775 135-61 162 550	387.4
FHIT	3p14.2	TW0789	59 762 657-60 525 130	762.5
FHIT intron	3p14.2	TW800	60 781 394-60 987 631	206.2
FHIT	3p14.2	TW813	59 900 085-60 543 794	437.5
FHIT etc.	3pter-p11.2	T74	37 570-88 331 466	88 300.0
FHIT intron	3p14.1	T782	60 343 805-60 493 871	150.1
PDCH7 etc.	4p16.3-p13	T64	2 431 351-44 331 469	41 900.0
PDCH7	4p15.1	TW789	29 575 024-30 487 701	912.7
PDCH7 etc.	4pter-p14	TW807	191-37 525 019	37 500.0
PDCH7 etc.	4pter-p14	TW774	191-38 356 422	38 400.0
PDCH7	4p15.1	TW782	30 393 830-30 637 746	243.9
PDCH7 etc.	4pter-p14	TW813	191-38 993 759	39 000.0

HD: Homozygous deletion.

DUSPs inactivate their target kinases by dephosphorylating both the phosphoserine/threonine and phosphotyrosine residues. These genes negatively regulate members of the mitogen-activated protein kinase (MAPK) superfamily (MAPK/ERK, SAPK/JNK, p38), which are involved in cellular proliferation and differentiation. The roles of the DUSPs in the regulation of MAPK activities have been highlighted as part of the oncogenic process[70]. Overall, any of the genes in this region are likely to play an important role in the progression of cancer.

The amplification of 11q13 ranged from 343.7 kb to 20.5 Mb and had an SRO of 343.7 kb, which included the *CCND1*, *ORAOV1* and *FGF19* (*BLC6*) genes. The variously sized 11q13.3 amplicon containing *CCND1* is one of the most frequent amplification events in human tumors. Computational genome-wide approaches to identify driver genes have reported *CCND1* as one of the most common somatic focal amplifications in human cancers[5]. As shown in our 11q13 amplification, the *ORAOV1* and *FGH19* genes lie within 15 kb of *CCND1*, and they are invariably co-amplified with *CCND1*. However, the limited data show that their expression levels depend on the type of tumor, indicating that the driver gene can be tissue-type dependent/specific. The co-expression of *FGH19* with or without *CCND1* has been found in HCC, but an absence of *FGH19* expression has been found in breast cancer and oral cancer[27]. Future work can determine whether *FGH19* is co-expressed with *CCND1* in primary gastric adenocarcinoma, even given that recently published whole-genome expression data did not show a significant correlation or co-expression of amplification/expression of *CCND1* and *FGH19*[35,43]. *ORAOV1* overexpression has been found in all amplified HCCs; however, *ORAOV1* does not promote tumorigenicity in $p53^{-/-}$; Myc hepatoblasts, nor is it cooperative with *FGFR1* or *CCND1*[27]. A total of four cases in this study had a gain of the 18q11.2 region. Of these, three cases had amplifications ranging from 625.0 kb to 1.3 Mb with an SRO of 625.0 kb, which includes the *GATA6* and *CTAGE* genes. This 18q11.2 amplification, along with expression and epigenetic studies, supports the oncogenic function of *GATA6* in esophageal carcinoma, colon cancer and pancreatic cancer[40,42,71,72] but not in gastric adenocarcinoma. Upregulation of *GATA6* has been reported in the transition from normal esophageal epithelium to Barrett adenocarcinoma and adenocarcinoma[42,71]. A 0.36 kb amplification including *GATA6* and *CTAGE1* has been found in pancreatic carcinoma[40,41], and dysregulation (overexpression) of *GATA6* contributes to colorectal tumorigenesis and tumor invasion[72]. Moreover, in that study *GATA6* was overexpressed not only in the cases with an amplification of *GATA6* but also in the cases without amplification[72]. However, *CTAGE* was rarely overexpressed, indicating that *GATA6* is the driver in this amplicon[41] and suggesting that *GATA6* overexpression may play a role in the early stages of tumor development. A contradictory result showing underexpression of the *GATA6* gene has been reported in gastric adenocarcinoma[36], demonstrating that the oncogenic function of *GATA6* in gastric adenocarcinoma needs to be investigated. Our observed amplification of 19q12 with an SRO of 1.4 Mb includes the nine genes *LOC148145*, *LOC10050583*, *UQCRFS1*, *LOC284395*, *VSTM2B*, *POP4*, *PLEKHF1*, *C19orf12* and *CCNE1*. This 19q12 amplification has been found in gastric cancer and esophageal adenocarcinoma[12,44,45]. Of these genes, *CCNE1*, an E type cyclin, has traditionally been considered the target of the 19q amplification, which is also one of the most common amplification products in various tumor types[5,12]. However, a comprehensive analysis of the 19q12 amplification in gastric cancer has revealed clustered overexpression of *CCNE1* as well as *UQCRFS1*, *POP4*, *C19orf12* and *RMP*, indicating potential functions of other genes in this region in tumor development[45]. In ovarian cancer, it has been suggested that the *CCNE1* gene is the key driver in this 19q12 amplicon and is correlated with the gain in the 20q11 region that includes the *TPX2* gene[73]. It is not clear whether the *CCNE1* gene is a key driver in other types of tumors, including gastric cancer tumors, because no detailed study has been conducted; it is also possible that this gene is tissue specific, as shown in 19q12. However, the gain of the 20q11 region is one of the most

common findings in gastric cancer, and the correlation between 19q12 and 20q11 in gastric cancer needs to be investigated.

Other amplifications found were 1q21.2 (1 amp/3 gains), 8p23.1 (2 amps/3 gains), 8p23.1 (2 amps/3 gains), 8q24.21 (1 amp/11 gains), 10q26.12q26.13 (1 amp), 11p13 (2 amps/1 gain), 12p12.1 (2 amps/2 gains), 12q15 (2 amps), 17q12 (2 amps/1 gain) and 20q13.2 (1 amp/6 gains). We summarized these amplifications and the expression of the gene(s) corresponding to these amplicons in various primary epithelial tumor and cell lines (Table 2). *MCL1* on 1q21.1, *MYC* on 8q24.21, *KRAS* on 12p12.1, *MDM2* on 12q15, *ERBB2* on 17q12 and *ZNF217* on 20q13.2 are well-acknowledged oncogenes in several tumors[5]. Recently published data suggest that the same amplifications do not necessarily induce the same gene expression in different tissue types[27], implying that driver genes can be tissue-type specific and making it necessary to acquire and investigate both amplification and overexpression information for different tumor types, even in the case of well-validated oncogenes.

In summary, the array CGH technique allows for comprehensive, rapid and reliable analysis of the whole genome of primary gastric adenocarcinomas and enables the refined and detailed study of amplifications and regions of recurrent copy number change. This approach makes it possible to identify putative oncogenes and tumor suppressor genes that may deserve further investigation. In this context, we identified candidate target genes/genomic segments of amplification that may help to direct therapeutics against gastric cancer.

COMMENTS

Background

Gastric cancer is one of the leading causes of cancer-related death worldwide. Although its incidence has gradually decreased in many Western countries, the incidence of gastric cancer still remains high in South and Central America and is highest in Eastern Asia, specifically in China, South Korea and Japan. Searching for biomarkers for gastric cancer has proven to be quite challenging.

Research frontiers

Cancer is a genetic disease that involves multiple genetic alterations. Understanding the molecular profile of tumor tissue is crucial for efficiently targeting cancer cells. In this study, the authors identified possible candidate target genes that could provide insight into the pathogenesis of gastric adenocarcinoma through the integration of genomic copy number changes.

Innovations and breakthroughs

Recent reports have highlighted the oncogenic addiction associated with oncogene amplification as a driver gene in carcinogenesis and target treatments. In this research paper, the authors identified novel amplicons that have not been published previously and that could be new target sites for potential diagnosis, prognosis and treatment.

Applications

To learn how these new amplicons are induced and change gene expression, this study may represent a future strategy for therapeutic intervention in the treatment of patients with gastric adenocarcinoma.

Peer review

The paper was designed to be a proof of principle for using cytogenomic microarrays on a larger study to ascertain biomarkers for various gastric neoplasms. The authors did a very thorough analysis of the genetic alterations and of the discussion of the possible genes involved. As claimed, they showed the power of the cytogenomic microarray in detecting common genetic alterations in various gastric tumors. The study was organized in a very clear manner and each common abnormality was compared in a thoughtful manner.

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S- Editor Cheng JX **L- Editor** A **E- Editor** Li JY