

Chromosomal aberrations in ascetic fluid of metastatic gastric cancer patients: A clustering analysis

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To date, no specific pattern of chromosomal abnormalities has been established in gastric cancer (GC). Cytogenetic analysis was performed using G-banding and fluorescence in situ hybridization (FISH) in 9 ascetic fluids from GC patients, and the clustering patterns of chromosomal abnormalities were studied. Twenty-six different types of chromosomal abnormalities were identified. In contrast to structural abnormalities, the gain or loss of chromosomes was infrequent. Moreover, five main clusters of chromosomal abnormalities were identified by clustering analysis. Extensive cytogenetic complexity, specific chromosomal abnormalities and karyotype heterogeneity are the main characterizations of GC. Some of the recurrent and novel chromosomal abnormalities with distinct clustering patterns identified in this study may play important roles for GC initiation and progression and could serve as promising diagnostic and prognostic markers in GC patients.

Key words: chromosomal abnormalities, ascetic fluids, clustering analysis, gastric cancer

Gastric cancer (GC), despite poor survival rates, is one of the most prevalent cancers around the world, accounting for a considerable amount of cancer-related morbidity and mortality [1]. Although environmental factors such as *Helicobacter pylori* infections, diet, and smoking play critical roles in the pathogenesis of GC, it is widely accepted that this type of tumor arises from complex genetic and environmental interactions contributing to the initiation and progression of the cancer [2, 3].

Like other types of solid tumors, cytogenetic studies to detect specific and recurrent chromosomal abnormalities in GC may be helpful in identifying causal genes that contribute to tumorigenesis [4]. Moreover, recurrent chromosomal abnormalities may provide important markers for determining the prognosis and clinical outcome of gastric cancer patients.

Recent studies have shown that chromosomal aberrations are linked to the histological type, survival, and further clinicopathological parameters of GC patients [5–7]. Several CGH studies in GC patients have indicated that there were both concordant and discordant correlations between specific chromosomal abnormalities and clinicopathological features of patients [8, 9].

Because of technical limitations, minimal information has become available regarding specific chromosomal alterations in GC patients. Although numerous chromosomal aberrations comprising simple and complex chromosomal changes have been reported, a specific pattern of chromosomal aberrations has yet to be identified for GC patients [10]. Conventional karyotyping of cancerous effusions of GC patients indicated karyotype complexity with frequent rearrangements of chromosomes 3, 5 and 17, and the gain of chromosomes 7 and 13 [11]. Chromosomal analysis revealed that some GC patients have simple numerical chromosomal aberrations, like an isolated gain of chromosomes 8, 9, 19 and X, or simple structural chromosomal aberrations like an isolated del (7q) and i(8q). In other GC patients, complex karyotypes have been described [12, 13].

Through Giemsa-banding and fluorescence *in situ* hybridization (FISH), the chromosomes obtained from the cancerous ascetic fluids of 9 patients with GC were examined in this study. The results are analyzed herein together with the results of previously reported studies in order to identify recurrent or novel chromosomal abnormalities in GC. The clustering patterns of detected chromosomal abnormalities

were also studied in an attempt to identify the association patterns of specific aberrations.

This study was undertaken to determine the potential value of the cytogenetic analysis of ascites fluids and to characterize recurrent chromosomal breakpoints and translocations specific to human GC to better understand the genetic make-up of gastric tumors. This study shows the feasibility and advantage of using FISH and karyotyping to find complex abnormalities in the body fluid of these patients. Results from this study along with those of former studies demonstrate that extensive cytogenetic complexity and karyotype heterogeneity are the main cytogenetic characterizations of metastatic gastric cancer. Moreover, a variety of cytogenetic alterations in different clustering patterns may possibly have a key role in GC pathogenesis and progression.

Patients and methods

Patients. A total of nine patients diagnosed with metastatic gastric cancer were included in this study. The general and clinicopathological characteristics of the patients are presented in Table 1. The pathological staging of the disease was based on the revised tumor-node-metastasis classification system [14]. The histological diagnoses were based on the criteria of the World Health Organization [15]. All patients received 60 mg/m² of docetaxel, 50 mg/m² of oxaliplatin, followed by 2500 mg/m² daily of fluorouracil continuous infusions over 24 h for 5 days. The Ethics Committee of Tehran University of Medical Sciences (TUMS) approved this study, and informed consent was obtained from all participants prior to sampling.

Direct chromosome preparation. Approximately 20 ml of ascitic fluid from the patients were collected into tubes containing transfer media (complete RPMI1640 media supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin). The collected specimens were centrifuged at 3000 rpm for 15 min. The supernatant was then discarded. Next, the cells were diluted 1:6 in a culture medium (RPMI1640 supplemented with a 20% FBS). Colcemid was added to the 25 cm² culture flasks with a final concentration of 0.05 µg/ml. The flasks were incubated at 37°C for 20–30

min followed by centrifugation at 3000 rpm for 15 min. The pelleted cells were resuspended in 10 ml of pre-warmed 0.075 M KCl and allowed to swell for 10–12 min. The cells were spun down, the supernatant was discarded, and the cells were fixed three times in chilled fixative (3:1, methanol: acetic acid). After 10 min, the cells were again centrifuged and resuspended and used for slide preparation by conventional air-drying methods. The chromosomes were G-banded using pancreatin and analyzed based on the International System for Human Cytogenomic Nomenclature (ISCN) 2016 [16].

Cell cultures for chromosome preparations. The ascites fluid was cultured (1×10⁶ cells/ml) in a 5 ml RPMI1640 medium supplemented with 20% FBS, 1% penicillin/streptomycin, and 1% L-glutamine. The long-term culture was conducted at 37°C for 48–96 h in a humidified atmosphere of 5% CO₂. Thereafter, 1 ml of trypsin-EDTA was added, and the culture was incubated for 3–5 min at 37°C. Next, the cells were exposed to colcemid (0.05 µg/ml) for 20 min, followed by processing in a pre-warmed 0.075 M KCl for 30 min, which subsequently underwent fixation. Slides were stained with a Giemsa solution pretreated with pancreatin. Accordingly, karyotyping was performed on slides with a range of 10 to 15 metaphases.

Fluorescence *in situ* hybridization (FISH). FISH was carried out to confirm chromosomal abnormalities detected by G-banding. The FISH probes used for confirmation are listed in Table 3. The G-banded slides were de-stained by methanol and then immersed into a 2× (SSC) saline sodium citrate buffer for 2 min at room temperature, followed by dehydration in a graded ethanol series (70%, 85%, 90%, and 100%, each for 1 to 2 min). Then, 1.5 µl of the probe mixture was spotted onto the marked metaphases and sealed with a cover slip thru a rubber glue solution. Denaturation was performed at 76°C for 5 min. For 13–15 h, the slides were hybridized at 37°C. Afterwards, the slides were washed in 0.4x SSC at 75°C for 2 min and were then immersed in 2× SSC, 0.1% NP40 (pH=7.0) solution at room temperature for one minute. After using DAPI (Cytocell, UK/Abott Molecular, USA) on each slide for staining the nuclei, FISH signals were evaluated on a minimum of 100 interphase nuclei using a fluorescence microscope (Olympus, BX51, Japan).

Table 1. Clinicopathological characteristics of gastric cancer patients with a diagnosis of adenocarcinoma.

Case No.	Sex	Histologic Grades	Tumor Location	Greatest tumor dimension	Vascular invasion	Lymphatic invasion	TNM	Stage	Survival status
1	Male	Poorly differentiated	Cardia	30 mm	+	+	T2N1M1	IV	Death (14 months after diagnosis)
2	Male	Well differentiated	Antropyloric	–	–	–	T3N0M0	III	Death (10 months after diagnosis)
3	Male	Well differentiated	Cardia	130 mm	+	–	T3N1M0	II	Death (8 months after diagnosis)
4	Male	Poorly differentiated	Cardia	65 mm	+	+	T3N1M1	IV	Alive
5	Male	Poorly differentiated	Fundus	8 mm	+	–	T3N3M0	II	Death (8 months after diagnosis)
6	Male	Poorly differentiated	Fundus	7 mm	+	–	T3N1M0	IV	Death (3 months after diagnosis)
7	Male	Poorly differentiated	Cardia	45 mm	+	+	T4N2M1	IV	Alive
8	Male	Poorly differentiated	–	–	+	+	T3N1M1	III	Alive
9	Male	Poorly differentiated	Cardia	50 mm	–	–	T2N0M0	III	Death (8 months after diagnosis)

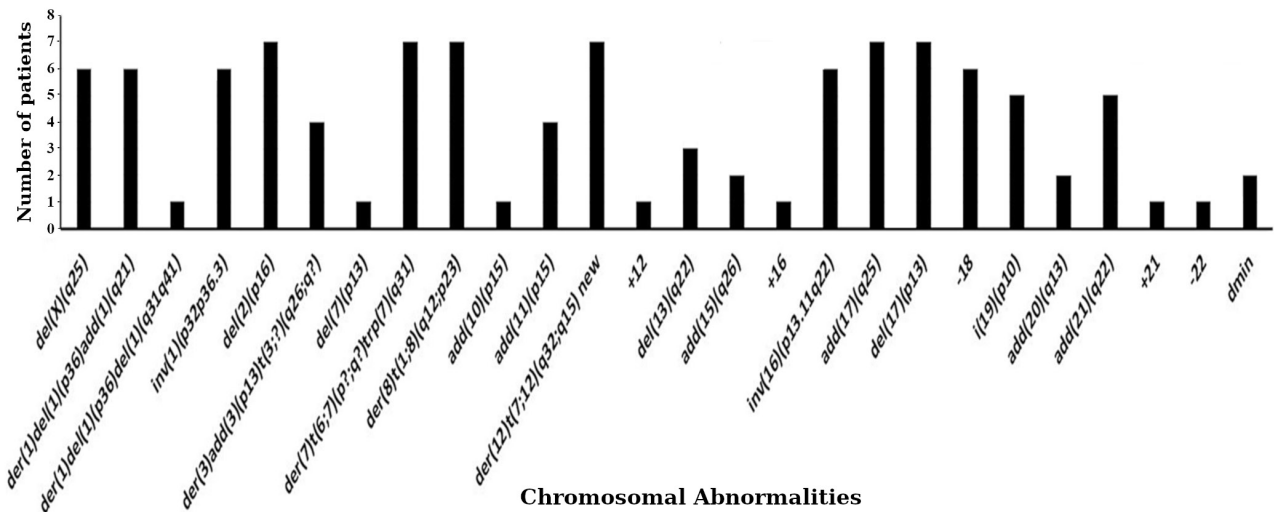


Figure 1. The distribution of chromosomal abnormalities in 9 metastatic gastric cancer patients. The y-axis shows the number of patients harboring relevant chromosomal abnormalities.

Table 2. Karyotypes obtained from 9 patients with gastric cancer.

Case No.	Modal Number	Karyotypes
1	Hypotriploid	67~68,XY,del(X)(25)[15], inv(1)(p32p36.3)×2[14], der(1)del(1)(p36)add(1)(q21)[13],del(2)(p16)[15], der(3)add(3)(p13)t(3;?)(q26;?)[13], der(7)t(6;7)(p;q?) trp(7)(q31)[15], der(8)t(1;8)(q12;p23)[15], add(11)(p15)[13], der(12)t(7;12)(q32;q15)[15],inv(16)(p13q22)[13], del(17)(p13)[15], add(17)(q25)[14],-18[13], i (19)(p10)[13][cp15]
2	Hypertriploid	70,XY, del(X)(q25)[6] inv(1)(p32p36.3)×2[13],der(1)del(1)(p36)add(1)(q21)[15], del(2)(p16)[15],der(7)t(6;7)(p;q?) trp(7)(q31) ×2[15],der(8)t(1;8)(q12;p23)[15], add(11)(p15)[9], der(12)t(7;12)(q32;q15)[11], del(13)(q22)[7], inv(16)(p13.11q22)[9], del(17)(p13)[10],+add(17)(q25)[15], i (19)(p10)[10], add(21)(q22)[6],+dmin[8][cp15]
3	Hypotriploid	68,XY, del(X)(q25)[15], inv(1)(p32p36.3)×2[1],der(1)del(1)(p36)add(1)(q21)[13],del(2)(p16)[15], der(7)t(6;7)(p;q?) trp(7)(q31)[15],der(8)t(1;8)(q12;p23)[15], add(11)(p15)[9],der(12)t(7;12)(q32;q15)[15], del(13)(q22)[8], inv(16)(p13.11 q22)[15], del(17)(p13)[10],add(17)(q25)[10], -18 [13], add(21)(q22)[4][cp15]
4	Hypotriploid	68,XY inv(1)(p32p36.3)×2[10],der(1)del(1)(p36)add(1)(q21)[8], del(2)(p16)[15],der(3)add(3)(p13)t(3;?)(q26;?)[8], der(7)t(6;7)(p;q?) trp(7)(q31)[15],der(8)t(1;8)(q12;p23)[12], der(12)t(7;12)(q32;q15)[10], inv(16)(p13.11 q22)[9],del(17)(p13)[15],add(17)(q25)[15], -18[10],i (19)(p10)[6],add(20)(q13)[7][cp15]
5	High Hyperdiploid	53,XY,+del(X)(q25)[10], +del(2)(p16)[15],+der(7)t(6;7)(p;q?)trp(7)(q31)[13], +der(8)t(1;8)(q12;p23)[12], +der(12)t(7;12)(q32;q15)[9], +del(17)(p13)[8], +add(17)(q25)[9],-18[5],+ add(21)(q22)[6][cp15]
6	Hyperdiploid	47,XY,+21[15]
7	Hyperdiploid	47,XY,+12[15]
8	Hypotriploid	68,XY,del(X)(q25)[8], inv(1)(p32p36.3)×2[13], der(1)del(1)(p36)add(1)(q21)[8], del(2)(p16)[15], der(3)add(3)(p13)t(3;?)(q26;?)[7], der(7)t(6;7)(p;q?) trp(7)(q31)[7], der(8)t(1;8)(q12;p23)[5], add(10)(p15)[5], der(12)t(7;12)(q32;q15)[6], del(13)(q22)[5], add(15)(q26)[4], inv(16)(p13.11q22)[10], del(17)(p13)[7], add(17)(q25)[7], -18[11], i(19)(p10)[9], add(21)(q22)[10][cp15]
9	Hypertriploid	73,XY, del(X)(q25)[15] inv(1)(p32p36.3)×2[13], der(1) del(1)(p36) del(1)(q31q41) [6], +der(1)del(1)(p36)add(1)(q21)[15], del(2)(p16)[15],der(3)add(3)(p13)t(3;?)(q26;?)[15], der(7)t(6;7)(p;q?)trp(7)(q31)[7], +del(7)(p13)[5], der(8)t(1;8)(q12;p23)[8], +add(11)(p15)[9], der(12)t(7;12)(q32;q15)[8], add(15)(q26)[10], inv(16)(p13.11 q22)×2[15],+16[15], del(17)(p13)[15], +add(17)(q25)[15], -18[6], i(19)(p10)[15], +add(20)(q13)[5], add(21)(q22)[6],-22[6],+dmin[6][cp15]

Hierarchical clustering. A cluster analysis was performed to assess the association of chromosomal aberrations based on the frequency and type of chromosomal abnormalities within different patients. Each abnormality was scored as being present or absent within the karyotype of patients. Hierarchical clustering was achieved using the gplots package with R statistics software. The distance matrices were calculated using the Euclidean distance. Ward’s method was used for hierarchical cluster analyses.

Results

Cytogenetic aberrations in metastatic gastric cancer. The modal chromosome number was hyperdiploid in two patients, high hyperdiploid in one patient, hypertriploid in two patients, and hypotriploid in four patients. Among the patients in this study, several recurrent structural chromosomal abnormalities were detected (Figure 1 and Table 2). In contrast to the structural abnormalities, the gain or loss of

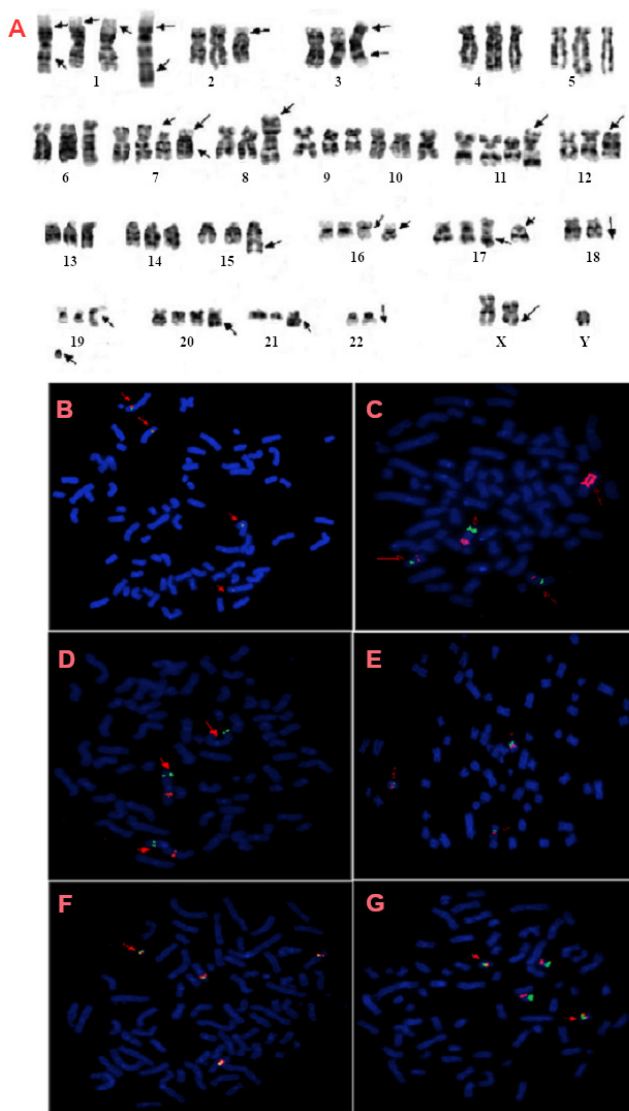


Figure 2. GTG-banded metaphase spread, karyotype and FISH obtained from case No. 9: A) An abnormal hypertriploid complex karyotype complement with the following chromosomal abnormalities: 73,XY, del(X)(q25)[15] inv(1)(p32p36.3)×2[13], der(1) del(1)(p36) del(1)(q31q41) [6],+der(1)del(1)(p36)add(1)(q21)[15],del(2)(p16)[15],der(3) add(3)(p13)t(3;?)(q26;?) [15], der(7)t(6;7)(p;q?)trp(7)(q31)[7],+del(7)(p13)[5], der(8)t(1;8)(q12;p23)[8], +add(11)(p15)[9], der(12)t(7;12)(q32;q15)[8], add(15)(q26)[10], inv(16)(p13.11 q22)×2[15],+16[15], del(17)(p13)[15], +add(17)(q25)[15], -18[6], i(19)(p10)[15], +add(20)(q13)[5], add(21)(q22)[6],-22[6], +dmin[6][cp15]. Arrows indicate abnormal chromosomes. B) FISH result by the Cytocell IGK break apart probe consisting of yellow-labeled IGK (2p11.2). C) FISH result by the Cytocell C-MET amplification probe consisting of red-labeled C-MET (7q31.2) and green-labeled D7Z1, 7p11.1-q11.1. D) FISH result by the Kreatech 1q21/ SRD(1p36) probe consisting of red-labeled SRD (1p36) and green-labeled 1q21. E) FISH result by the Cytocell MDM2 amplification probe consisting of red-labeled MDM2 (12q15) and green-labeled D12Z1, 12p11.1-q11.1. F) FISH result by the Cytocell C-MYC break apart probe consisting of yellow-labeled C-MYC (8q24). G) FISH result by the Kreatech CFBF t(16;16); inv(16) break apart probe consisting of red-labeled CFBF (16q22) and green-labeled MYH11 (16p13.11) probe. A break signal is defined when the red/green signals apart from each other. Arrowheads of their respective colors in representative metaphase indicate hybridization signals.

a whole chromosome was infrequently observed. Except for the loss of chromosome 18 that was frequent and detected in 6 patients, the loss of chromosome 22 and the gain of chromosomes 12, 16, and 21 each were detected in only one patient. The gain of chromosomes 12 and 21 was detected as the sole chromosomal abnormality in two cases (cases 6 and 7).

The most frequent structural chromosomal rearrangements were: del(17)(p13), der(7)t(6;7)(p;q?)trp(7)(q31), del(2)(p16), add(17)(q25) and der(8)t(1;8)(q12;p23), and der(12)t(7;12)(q32;q15) in 7 cases; followed by der(1)del(1)(p36)add(1)(q21), inv(16)(p13.11 q22), inv(1)(p32p36.3), and del(X)(q25) in 6 cases, i(19)(p10) and add(21)(q22) in 5 cases, der(3)add(3)(p13)t(3;?)(q26;?) and add(11)(p15) in four cases, del(13)(q22) in 3 cases, add(20)(q13) and add(15)(q26) in 2 cases as well as del(7)(p13) and der(1)del(1)(p36)del(1)(q31q41) in one case (Figure 1).

FISH results. FISH was performed using commercially available probes to confirm the presence or absence of particular cytogenetic aberrations as well as to identify the occurrence of translocation breakpoints (Figure 2). The results of the FISH signals for each chromosomal abnormality along with their associated chromosomal abnormalities are summarized in Table 3.

The clustering of chromosome aberrations. The clustering of cytogenetic abnormalities for all analyzed metaphases from the GC patients revealed five main clusters (Figure 3). The first cluster was characterized by frequently presented chromosomal abnormalities including: del(17)(p13), add(17)(q25), der(12)t(7;12)(q32;q15), der(8)t(1;8)(q12;p23), der(7)t(6;7)(p;q?)trp(7)(q31), del(2)(p16), and loss of chromosome 18. The second cluster was identified by: der(1)del(1)(p36)add(1)(q21), inv(16)(p13.11q22), inv(1)(p32p36.3), i(19)(p10), der(3)add(3)(p13)t(3;?)(q26;q?), the third cluster by: del(X)(q25) and add(21)(q22), add(11)(p15) and del(13)(q22), the fourth cluster by less frequent abnormalities including: gain of chromosome 16, loss of chromosome 22, del(7)(p13), der(1)del(1)(p36)del(1)(q31q41), and the fifth one by rare abnormalities including: add(15)(q26), add(10)(p15), and gain of chromosomes 12 and 21.

Discussion

In this study, chromosomal abnormalities and their clustering patterns in the cancerous ascetic fluids obtained from GC patients were analyzed. Structural aberrations of chromosome 17 as del(17)(p13)/loss of TP53 (in 7 cases) and add(17)(q25) (in 7 cases) were the most frequent structural rearrangements in the cohort of patients in this study. In the case of GC, structural and numerical (often loss) abnormalities of chromosome 17 have been previously reported [13, 17, 18]. Formerly, Kobayashi et al. determined 17p13/TP53 alterations in GC patients using FISH analysis and reported TP53 deletion in 39% of tumors [19]; however, the frequency

Table 3. The results of the FISH signals and their associated chromosomal abnormalities.

Chromosome aberration	FISH probe	Result (FISH signal)
inv(1)(p32p36.3)×2, der(1) del(1)(p36) del(1)(q31q41), der(1)del(1)(p36)add(1)(q21)	SRD(1p36R/1q12G)	2R3G
del(2)(p16)	IGK Break apart(2p11.2 Y)	4Y
add(3)(p13)?t(3;?)(q26;?)	PPARG (3p25Y)	3Y
der(7)t(6;7)(p?;q?)trp(7)(q31)	MET amp(7q31R/CEP7G)	7R 3G
der(8) t(1;8)(q12;p23)	SRD(1p36R/1q12G)	2R3G
der(12) t(7;12)(q32;q15)	MDM2 Amplification(12q15R/CEP12G)	3R3G
inv(16)(p13.11q22)	CBFB/MYH11 Dual fusion(16q22R/16p13G)	2R2G2Y
+add(17)(q25)	HER2 amplification(17q12R/CEP17G)	4R4G
del(17)(p13)	P53 deletion(17p13R/CEP17G)	2R3G
-18	BCL2 Break apart(18q21 Y)	2Y
i(19)(p10)	E2A break apart(19p13.3 Y)	4Y
add(20)(q13)	Satellite Enumeration probe 20(R)	3R
+add(21)(q22)	Satellite Enumeration probe 21(R)	3R
del(X)(q25)	X/Y (DXZ1R/DYZ1 G)	3R

R: Red, Y: Yellow, G: Green

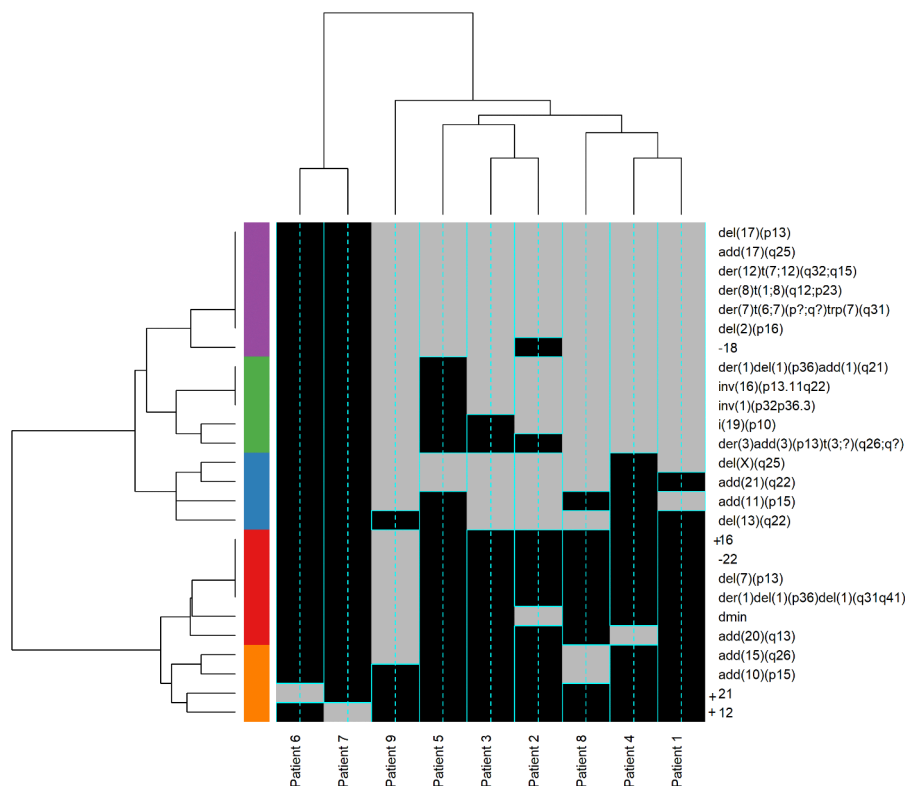


Figure 3. Hierarchical clustering based on the presence or the absence of chromosomal abnormalities detected in the gastric cancer patients. Each column represents a patient and each row denotes a chromosomal abnormality. Grey indicates the presence of each abnormality, and black indicates their absence.

of TP53 mutations varies greatly between different studies [20]. The TP53 deletion was detected in 7 out of 9 metastatic GC patients in the current study, suggesting its contributing role to the progression of GC. The results from cluster analysis revealed that chromosome 17 aberrations tend to be associ-

ated with del(2)(p16) and trp(7)(q31), suggesting the possibility of a synergism between these aberrations to accelerate gastric tumorigenesis. The TP53 tumor suppressor gene, c-MET proto-oncogene, and the MSH6 DNA mismatch repair gene were located in 17p13, 7q31, and 2p16, respectively. A

study by Young et al. previously demonstrated that the *TP53* tumor suppressor and *MSH6* DNA mismatch repair gene act together in accelerating genomic instability and tumorigenesis in mice models [21]. These results suggest that the loss of genes involved in the maintenance of genomic stability, such as *TP53* and *MSH6*, may lead to the progression of GC.

The other most common structural abnormality detected in patients in the current study was amplification of 7q31, which contains the *c-MET* gene. Results from CGH array studies have shown that 7q copy-number alterations are one of the most frequent types of chromosomal abnormalities, which preferentially occur in advanced GC [22, 23]. In this study, gains of 7q were found in 7 of the cases. This data confirms that *c-MET* amplification plays an important role in the progression of GC.

In addition, several non-reciprocal translocations were detected in the patients in the current study. Chromosomal bands repeatedly involved in non-reciprocal translocations comprised 1p36, 1q21, 3p13, 11p15, 15q26, 17q25, 20q13 and 21q22. The breakpoints detected in 3p13, 11p15, 15q26, 17q25, 20q13 and 21q22 were previously reported in GC cell lines and GC patients using SKY analysis by Yamashita et al. [24].

The majority of structural chromosomal changes detected so far in GC patients are unbalanced; however, Panani et al. [18] previously described balanced translocations in 5 of their 15 primary GC cases, which involved several chromosomes including t(1;7), t(7;14), t(6;17) and t(5;14). In the current study, two balanced chromosomal aberrations were detected in our patients as inv(16)(p13.11 q22) and inv(1)(p32p36.3) and confirmed these aberrations by FISH method.

The inv(16)(p13.11 ;q22) is one of the most common chromosomal aberrations detected in acute myeloblastic leukemia (AML). This rearrangement disrupts the myosin heavy chain (*MYH11*) gene at the short arm of chromosome 16 (16p13.11) and the core binding factor β (*CBFB*) gene at the long arm of this chromosome (16q22) [25]. Li et al. previously reported that the binding of the *CBFB* to the transcription factor *RUNX3* is required for gastrointestinal development, proliferation and differentiation [26]. Furthermore, a previous study showed that *CBFB* was downregulated in gastric cancer, suggesting that *CBFB* may play an important role in the development of GC [27]. However, further investigations are essential to provide the possible relation between inv(16)(p13.11;q22) and tumorigenesis as well as the clinicopathological features of GC patients.

Moreover, partial deletions of chromosome 2, X, 13, and 7 as del(2)(p16), del(X)(q25), del(13)(q22), and del(7)(p13) were detected in 7, 6, 3, and one of the cases, respectively.

Chromosome 13 alterations are very frequent in GC. A loss of heterozygosity at loci 13q is found in 59% of human stomach cancers [28]. The current most likely candidate gene at 13q22 is the *EDNRB*. Hypermethylation and low expression of the *EDNRB* gene were previously reported in GC patients [29]. These results suggest that the loss of 13q22 may lead to the deletion of the *EDNRB* gene, thus, playing

an important role in GC pathogenesis and progression. In contrast to the structural chromosomal aberrations, the gain and loss of a whole chromosome were infrequently observed except for the loss of chromosome 18 revealed in 6 patients.

A previous study showed that loss of chromosome 18q was associated with tumor progression in patients with primary gastric cancer [30]. Several well-known tumor suppressor genes such as DCC (Deleted in Colorectal Carcinoma), SMAD2 (SMAD Family Member 2), and SMAD4 (SMAD Family Member 4) are also located on chromosome 18, and their loss might play an important role in GC initiation and progression.

Previous gastric cancer studies have revealed a number of numerical chromosomal changes. The most frequent numerical aberrations previously reported in GC are copy number alterations of chromosomes 1, 4, 5, 6, 7, 8, 9, 17, 20, X and Y [13, 31]. Moreover, CGH analysis on GC cell lines have demonstrated that chromosomal gains are frequent in these cells [32].

There are conflicting results about the role of numerical chromosomal aberrations in GC patients. While some studies found no association between the numerical chromosomal aberrations and clinicopathological characteristics of GC patients [33, 34], other studies indicated an association between these two parameters [31, 35]. Moreover, clustering analysis showed that a set of chromosomal abnormalities were similar in all metastatic GC patients with consistent complex cytogenetic abnormalities. These findings suggest that these chromosomal abnormalities may be an early event in GC progression and, through further studies in large cohorts of patients, they may be considered as diagnostic and prognostic markers for GC patients.

In conclusion, the present study demonstrated several chromosomal abnormalities with specific clustering patterns in ascites fluid of metastatic GC as a non-invasive source for karyotyping. Very little is known about the genetics of gastric tumors; thus, these findings may help us achieve a deep understanding of the genetic make-up of these patients and validate the chromosomal copy number changes in larger studies to find reliable diagnostic or prognostic markers. In addition, further studies of the GC genomic profiles and its correlation with clinicopathological features, may introduce chromosomal copy number changes as predictive marker for lymph node metastasis and survival status in this group of patients. Stable chromosomal aberrations may also be used as minimal residual disease markers to follow-up on response to therapy. The chromosomal breakpoints defined in the current study may also contain critical genes, which are involved in the multistage carcinogenesis of GC and, thus, can serve as landmarks for crucial regions that warrant understanding the molecular basics of this cancer.

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