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Detection of kinase amplifications in gastric adenocarcinomas

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Aim: To determine the incidences of copy number aberrations of receptor kinases and their relations in Turkish patients with gastric adenocarcinoma.

Materials and methods: The prevalence of genomic copy number aberrations of epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2)/topoisomerase IIa (TOP2A), centrosome-associated kinase aurora A (AURK A), centrosome-associated kinase aurora B (AURK B), and mesenchymal-epithelial transition factor (MET) genes and polysomies of related chromosomes were analyzed by fluorescent in situ hybridization (FISH) in tumor samples from 35 patients with gastric cancer.

Results: There were 28.6%, 65.7%, 20.0%, 17.1%, 60.0%, and 45.7% cases considered FISH-positive for EGFR, MET, HER2, TOP2A, AURK A, and AURK B genes, respectively. Statistically significant associations were determined in detection of amplifications of 1) EGFR gene with chromosome 7 polysomy, 2) MET gene in nonpolysomic chromosome 7 nuclei, 3) HER2/TOP2A genes in nonpolysomic chromosome 17 nuclei, 4) coamplification of HER2/TOP2A in poorly differentiated carcinomas, and 5) AURK A gene in nonpolysomic chromosome 20 nuclei. Most of the aberrations were predominantly seen in poorly differentiated tumors, but a high rate of the amplified MET gene was also detected in moderately differentiated carcinomas.

Conclusion: Chromosome 7 polysomy may be responsible for EGFR gene amplifications, and we concluded that MET and AURK A genes amplifications were commonly seen aberrations in gastric adenocarcinomas and may offer information about disease progression and administration of individualized treatment for gastric cancer patients.

Key words: Receptor protein-tyrosine kinases, aurora kinase, gastric adenocarcinomas

1. Introduction

Despite the worldwide incidence of gastric cancer decreasing over recent decades, it is still the second most common cause of cancer-related deaths in the world, with an estimated 989,600 new cases and 738,000 deaths annually (1). The incidence of gastric cancer in Turkey is between a lower incidence in the Western and higher incidence in the Eastern worlds. The incidence in men is 9.6 cases/100,000 of the population, and in women it is 6.89/100,000 of the population. In addition, it is the second highest cause of cancer-associated death in men, whereas it is the third in women after lung and breast cancers, consistent with the reported rates in developing countries. Gastric cancer has been reported predominantly in central, northeastern, and eastern parts of Turkey (2). Despite its high prevalence worldwide, the improvements in early diagnosis and the treatment of the gastric cancer are still quite unsatisfactory and it remains a challenge for physicians. Surgical resection of the stomach with lymph node dissection is currently the only effective treatment for the disease. Significant challenges remain in refining our understanding of the molecular basis of gastric cancer, which would bring diagnosis and treatment benefits to the patient.

Today, it is well known that gastric cancer results from complex gene–environment relations. Recently developed high-throughput techniques have revealed the heterogeneous and complex backgrounds of gastric

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cancers. Genetic and epigenetic mechanisms are known to play key roles in the development and progression of both diffuse and intestinal types. Genomic instability is one of the characteristics of gastric cancers and the instability has been reported as either microsatellite instability or chromosomal instability (3,4). The genome instability induced by various pathological mechanisms responsible for alterations in cellular signaling pathways is a characteristic feature of cells in carcinogenesis process. The receptor tyrosine kinase is one of the important cell-signaling pathways in cancer development and progression. Epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2) are membrane-associated cytokine receptors and initiate intracellular signaling cascade from membrane to nucleus. The amplification and overexpression of EGFR and HER2 have been reported in a variety of solid tumors and in gastric cancers, as well (5). The topoisomerase IIa gene (TOP2A) is located on chromosome 17q12-q21 near the HER2 oncogene and encodes an enzyme involved in DNA replication, and it is a molecular target for anthracyclines. The coamplification of HER2 and TOP2A has been described in different tumor types. In their study, Kanta et al. (6) analyzed EGFR, HER2, and TOP2A genomic copy alterations and their expressions in gastric cancers and showed significant association between TOP2A and HER2 gene amplification. Recently, the involvement of hepatocyte growth factor (HGF)–mesenchymal-epithelial transition factor (MET) has been reported in various cancer types, including gastric cancer (7,8). Gene rearrangement or amplification, somatic mutations, and transcriptional upregulation have been reported as the causes of constitutively active MET oncogenic tyrosine kinase activity. Previous studies have indicated the presence of amplification and overexpression of MET in about 20% of gastric adenocarcinomas and suggested an association among high MET expression, tumor invasiveness, and poor clinical prognosis (7).

Centrosome-associated kinase aurora A (AURK A) and centrosome-associated kinase aurora B (AURK B) are members of a family of mitotic serine/threonine kinases that contribute to the regulation of cell cycle progression and play an important role in maintaining genomic stability. They are associated with centrosomes and the spindle microtubules during mitosis and are involved in various mitotic events, such as establishment of mitotic spindle, centrosome duplication, chromosome segregation, chromosome alignment, centrosome separation, and cytokinesis. AURK A and B have been shown to be frequently amplified/overexpressed in solid tumors (9,10). The overexpression and importance of AURK A as a tumor progression marker has been demonstrated previously (11,12). However, the genomic copy number aberrations of these kinases in gastric tumors have not yet been reported in detail.

Dysregulation of all these receptor kinases has been demonstrated to contribute to the development and progression of gastric adenocarcinoma. Although gastric cancer is a public health problem in Turkey, data related to the prevalence of tyrosine kinases in gastric adenocarcinoma are very limited. This study was planned to determine the incidences of these receptor tyrosine kinase amplifications in Turkish patients with gastric adenocarcinoma, in order to provide more accurate data for future studies in the Turkish population.

2. Materials and methods

2.1. Patients

The present study contained 35 patients with gastric adenocarcinoma (15 women and 20 men) with a mean age of 65.4 ± 2.4 years. The patients were treated with curative surgery and none of them had synchronous multiple gastric cancers. The patients were not treated with any additional anticancer therapy other than curative surgery. The clinicopathological characteristics of the patients are given in Table 1. The collection of samples and research protocols were reviewed and approved by the Ethics Committee of Eskişehir Osmangazi University and written informed consent was obtained from all the patients regarding the investigation. Tyrosine receptor fluorescent in situ hybridization (FISH) analyses were performed on fresh tissues containing more than 85% tumor cells. Cancer staging was performed according to the TNM

Table 1. Clinical characteristics of the patients (N = 35).

	N = 35	**%**
Age		
<60 (42–60)	12	34.3
>60 (60–79)	23	65.7
Sex		
Female	15	42.9
Male	20	57.1
Histology		
Moderately differentiated adenocarcinoma	22	62.9
Poorly differentiated adenocarcinoma	13	37.1
Lauren classification		
Intestinal	23	65.7
Diffuse	7	20.0
Mixed	5	14.3
Stage		
T1	2	5.7
T2b	13	37.1
T3	15	42.9
T4	5	14.3

cancer staging system of the American Joint Committee of Cancer (Table 1).

2.2. FISH analysis

The sections of tissue containing at least 85% pure tumor cells were dissected from fresh tissues and used for FISH analysis. Following mechanical disaggregation and enzymatic digestion of the tissues, the cell suspensions were fixed by Carnoy's fixative and then slides were prepared for interphase-FISH analysis. The determination of EGFR, HER2/TOP2A, AURK A, AURK B, and MET copy number alterations in tumor samples was done using commercially available probes.

2.2.1. EGFR and MET FISH analysis

EGFR FISH analysis was carried out using the LSI EGFR Spectrum Orange/CEP 7 Spectrum Green FISH probe (Vysis, Abbott), whereas MET FISH analysis was performed using C-MET (7q31) and SE7 probes (Kreatech) according to manufacturer's specifications. Probes were denatured at 73 ± 1 °C for 5 min and then were applied immediately to the previously determined areas of the slides. Following overnight hybridization at 37 °C, posthybridization washes were performed and the slides were air-dried in darkness. The slides were counterstained using DAPI (4′-6′-diamidine-2-phenylindole) and stored at –20 °C in the dark. FISH analyses were performed independently by 2 authors. For EGFR and MET genes, EGFR/chromosome 7 and MET/chromosome 7 rates were determined by counting the EGFR/MET signals and Chr 7 signals in 100 nuclei using an Olympus BX61 fluorescence microscope (Olympus) and images were captured using an image analysis system (Applied Imaging). In each FISH experiment, known positive and negative controls were used.

Chromosome 7 polysomy and monosomy were defined as ≥3 signals and 1 signal in more than 30% of nuclei, respectively. In the evaluation of fluorescence spots specific to EGFR/MET genes and chromosome 7 centromeres, the gene copy phenotypes were classified into 6 categories as described elsewhere (13,14): disomy (≤2 copies in >90% of cells), low trisomy (≤2 copies in ≥40% of cells, 3 copies in 10%–40% of cells, and ≥4 copies in <10% of cells), high trisomy (≤2 copies in ≥40% of cells, 3 copies in ≥40% of cells, and ≥4 copies in <10% of cells), low polysomy (≥4 copies in 10%–40% of cells), high polysomy (≥4 copies in ≥40% of cells), and gene amplification (presence of tight EGFR gene clusters and a ratio of the EGFR gene to chromosome 7 of ≥2, or ≥15 copies of EGFR per cell in ≥10% of cells). The patients were put into 2 groups based on detected EGFR or MET gene copy number alterations, including the patients with EGFR/MET FISH negative or low gene copies and the patients with EGFR/MET FISH positive or high gene copies.

2.2.2. TOP2A/HER-2/CEP 17 FISH analysis

A 3-color FISH was performed using a probe set of LSI TOP2A SpectrumOrange, LSI HER2 SpectrumGreen, and centromere enumeration probe 17, labeled in the SpectrumAqua probe (Vysis, Abbott) to evaluate the relationship among TOP2A, HER2, and chromosome 17 copy number alterations in tumor samples. Absolute and relative numbers (relative to chromosome 17 copy number) of the individual genes were scored in 100 cancer nuclei per tumor. FISH was performed according to the manufacturer's instructions. In each FISH experiment, known positive and negative controls were used. In the FISH assessments, a HER2:CEP17 or TOP2A:CEP17 (centromeric probe 17) ratio of ≥2 was defined as positive for HER2 and TOP2A amplifications, whereas a ratio of <2.0 was considered to be negative for HER2 and TOP2A. The chromosome 17 polysomy was defined as >3 centromere 17 specific signals on average per cell, as described previously (15,16).

2.2.3. AURK A and AURK B FISH analysis

Dual color FISH was carried on using an AURK A (20q13) and 20q11 DNA probe (Kreatech Diagnostics) and an AURK B (17p13)/Alpha satellite 17 specific DNA probe (Kreatech Diagnostics) according to manufacturer's instructions. Absolute and relative numbers (relative to chromosome 17 copy number) of the individual genes were scored in 100 cancer nuclei per tumor. In each FISH experiment, known positive and negative controls were used. The genes (AURK A and AURK B) and chromosome-specific signals (centromere enumeration probes; CEP20 and CEP17) were counted per individual cell nucleus. The FISH ratio (AURK A to CEP20 and AURK B to CEP17) was calculated for each analyzed cell nucleus and the mean and standard deviation was calculated for each cell line. True gene-specific amplification was considered at a FISH ratio of >2.

2.3. Statistical analysis

All statistical analyses were performed using SPSS 18.0 for Windows (SPSS Inc.). Statistical analyses were performed using Fisher's exact test to identify significant clinicopathological differences between FISH-positive and FISH-negative tumors for EGFR, MET, HER2, TOP2A, AURK A, and AURK B genes; to determine relationships between the status of the genes and chromosome polysomy; and to examine the correlation between spontaneous HER2 amplification and TOP2A coamplification in nonpolysomic tumors. All P-values of less than 0.05 were considered significant.

3. Results

To determine the incidences and relations of the previously reported dysregulated receptor kinases involved in the development and progression of gastric adenocarcinoma

in 35 Turkish patients, the genomic copy alterations of EGFR, MET, HER2, TOP2A, AURK A and AURK B genes and chromosome 7, 17, and 20 polysomies were analyzed by FISH analysis.

Of 35 cases, 10 patients (28.6%) were considered FISH-positive for the EGFR gene according to previously described criteria in Section 2, and the difference was statistically significant (P = 0.041; Table 2). As seen in Figure 1a, chromosome 7 polysomy was revealed in 11 tumors, of which concomitant EGFR higher polysomy/amplification phenotype was detected in 6 tumors. Of these, 4 had EGFR amplification and 2 showed higher EGFR polysomy. The remaining 4 tumors (EGFR amplification) were disomic for chromosome 7.

It was interesting that high polysomy and amplification of the MET gene were determined in 23 tumors (65.7%) and the difference was statistically significant (P = 0.003). The frequency of increased MET gene copy number aberrations and their microscopic image are given in Table 2 and Figure 1b. Of 7 tumors with chromosome 7 polysomy, 6 were determined as MET-FISH–negative, but in the remaining tumor, all analyzed nuclei displayed equal numbers of copies of MET and CEP7 (range: 3–10 copies). Of 28 tumors with disomic nuclei phenotype, high MET gene polysomy and/or amplification was revealed in 22 (78.6%) samples.

As shown in Table 3 and Figure 1c, no HER2- and/or TOP2A-positive phenotype was determined in polysomic tumors. The positive HER2 and TOP2A FISH phenotypes were determined in 20% and 17.1% of nonpolysomic tumors, respectively. TOP2A amplification without concomitant amplification of HER2 was only seen in 1 tumor. No tumor specimens had TOP2A deletions. There were 5 tumors with HER2 amplification that showed TOP2A coamplification (71.4%; Table 4), and based on HER2 positivity, a significant association (P < 0.001) was determined in the presence of concomitant amplification of TOP2A in the tumors.

In the comparison of the relations among the HER2, TOP2A, EGFR, and MET positive tumors, it was determined that, of 4 tumors with HER2 and TOP2A amplifications, high polysomies of EGFR and MET genes were also seen in 2 (50.0%) and 3 (75.0%) tumors, respectively. Higher EGFR polysomies were seen in 2 tumors with HER2 but without TOP2A amplification.

The frequencies of AURK A and AURK B copy number alterations are given in Tables 5 and 6, respectively. When the tumors were compared for AURK A and AURK B copy number alterations, it was determined that 60.0% and 45.7% of the tumors showed >2 AURK A and AURK B specific fluorescent signals, respectively. Chromosome 20 polysomy was seen in 8 tumors, of which 7 were negative for higher AURK A specific fluorescent spots (Table 5). As shown in Figure 1d, AURK A amplification was seen in 5 (23.8%) tumors, whereas the rest had higher gene copy numbers. A statistically significant difference was determined in the tumors depending on AURK A positivity and negativity (P = 0.003). Moreover, in the evaluation of AURK A positivity depending on the chromosome 20 copy number status, it seemed as if the increased AURK A gene copy number alterations were preponderantly seen in nonpolysomic chromosome 20 nuclei (74.1%).

In the AURK B FISH analysis, the chromosome 17 polysomy ratio was determined as 17.1% (6/35). The AURK B amplification was seen in 2 (33.3%) of the 6 chromosome 17 polysomic tumors, but the higher copy aberrations of AURK B were predominantly determined in disomic tumors (48.3%; Table 6).

The comparison of copy number alterations of EGFR, MET, HER2, TOP2A, AURK A, and AURK B genes and the clinicopathological parameters of the tumors are shown in Table 7. Based on the age of the patients, no significant association was identified in the number of the patients with the positive FISH aberrations. However, significant associations were identified in the copy number aberrations of the analyzed genes with respect

Table 2. Relationship among copy number alterations of EGFR, MET genes, and chromosome 7.

	EGFR FISH				MET FISH			
Chr. 7	Negative		Positive (High polysomy + amp.)		Negative		Positive (High polysomy + amp.)	
	N	%	N	%	N	%	N	%
No polysomy	20	83.3	4	16. 7	6	21.4	22	78.6
Polysomy	5	45.5	6	54.6	6	85.7	1	14.3
Total	25	71.4	10	28.6	12	34.3	23	65.7
P-value	0.041*				0.003**			

*: P < 0.05; **: P < 0.01.

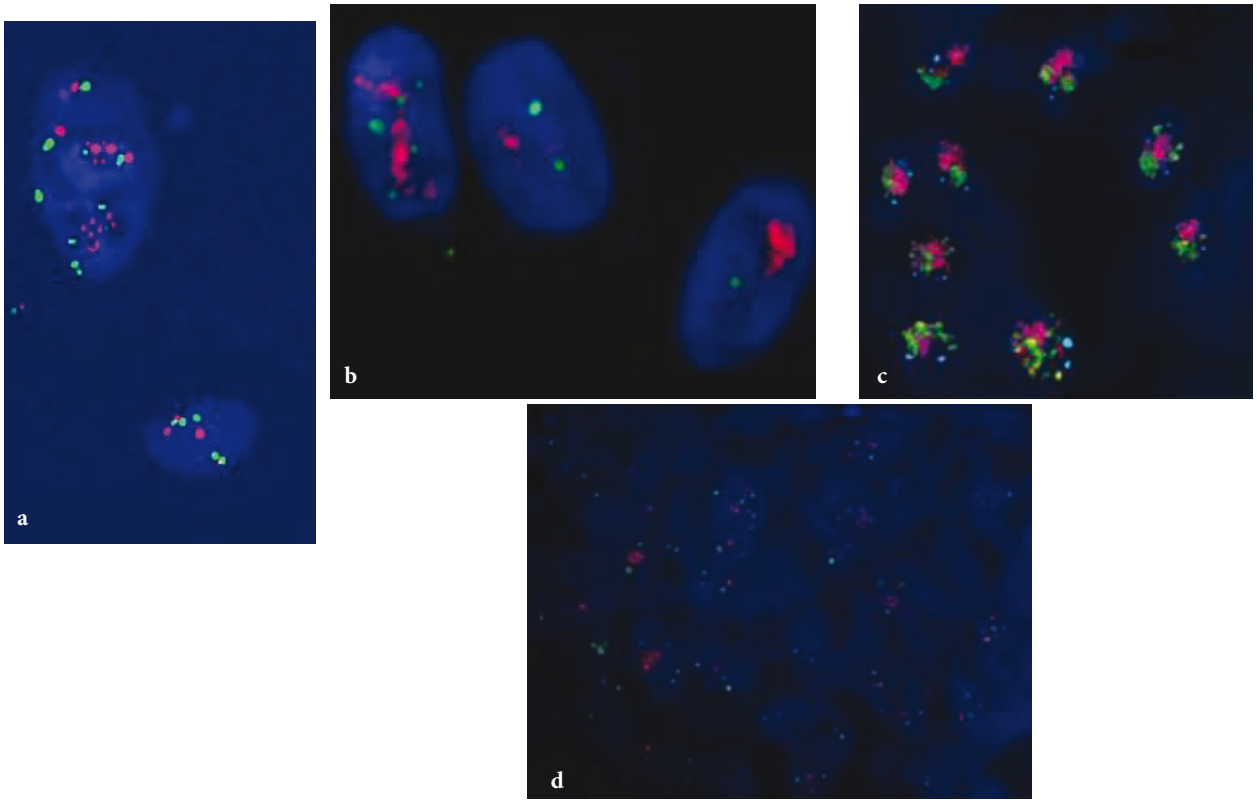

Figure 1. The FISH-detected copy number aberrations of: **a)** polysomy of chromosome 7 (green) and increased number of EGFR gene copies (red) (LSI EGFR Spectrum Orange/CEP7 Spectrum Green FISH probe); **b)** c-MET amplifications (red) with disomic chromosome 7 centromere specific (green) signals [C-MET (7q31) Red & SE7 DNA probe Green]; **c)** HER2/TOP2A amplifications (green and red, respectively) with disomic chromosome 17 specific (blue) signals (LSI TOP2A SpectrumOrange/ LSI HER2 SpectrumGreen/CEP17 Spectrum Aqua); **d)** AURK A amplifications (red) with chromosome 20 centromere specific (green) signals [AURK A (20q13) Red/20q11 Green DNA probe] in the analyzed nuclei of gastric tumor tissues.

to pathological features. Most of the aberrations were preponderantly seen in poorly differentiated tumors, except the MET gene. Of the moderately differentiated tumors, 59.1% had higher copy numbers of the MET gene; that was the only gene with higher copy numbers in an earlier stage of the tumors.

4. Discussion

The dietary habits, life conditions, and differences in delivery of health care between regions are the main risk factors of gastric cancer in Turkey. Although physicians are well aware of the gastric cancer problem in Turkey and are experienced in the management of this cancer, gastric cancer remains an important public health problem (2). The higher expressions and/or increased copy number aberrations of EGFR, MET, HER2, AURK A, and AURK B genes, and their important roles in gastric cancer development and progression, have been well documented previously. However, the status of these genes their prevalence, and relations with each other could not be clarified before in gastric cancer patients of the Turkish population. Therefore, in the present study, we decided to focus our investigations on the altered copy number aberrations of these genes in tissues from Turkish gastric cancer patients. Although targeted therapies have emerged as a new hope in cancer management during recent years, several drugs have been studied in gastric cancer and most reports focus on kinase gene alterations. However, there is a lack of detailed information about the formation of the gene amplifications and their status in tissues. Kiyose et al. (17) stated that the gene amplifications may be the cause of tumor progression, or they may be inducers of severe structural changes in the genome. EGFR amplification has been described in many human tumors, including gastric cancer. The role of EGFR in gastric cancer is still contentious. While some authors have suggested the suitability of EGFR as a target for receptor tyrosine kinase inhibitors because of higher expression of EGFR in gastric cancers (18,19), others (20,21) have reported EGFR overexpression/amplification as a rare event. Besides, other genetic mutations, including point mutations (22) or truncated mutations that are very important to

Table 3. Relationship among copy number alterations of HER2, TOP2A genes, and chromosome 17.

Chr. 17 copy number	HER2 FISH				TOP2A FISH			
	Negative		Positive		Negative		Positive	
	N	%	N	%	N	%	N	%
No polysomy	20	74.1	7	25.9	22	78.6	6	21.4
Polysomy	8	100.0	-	0.0	7	100.0	-	0.0
Total	28	80.0	7	20.0	29	82.9	6	17.1
P-value	0.166				0.311			

determine therapeutic drug resistance for hepatocellular carcinoma and lung cancer (23), are also rarely seen in gastric cancer (24). In this study, EGFR amplification prevalence was in agreement with the previously reported studies. However, the polysomy 7 prevalence was relatively higher. Therefore, our data also supported the possible responsibility of chromosome 7 polysomy for increased EGFR gene copy number in gastric carcinomas.

The range of the MET gene amplification prevalence was between 3% and 30%, (17,25,26), but it was interesting that a relatively higher rate was determined in our series. Because of the higher rate, the samples were analyzed twice. MET is known to be overexpressed in various cancer tissues and associated with disease progression (14,27). Previous reports have presented the increased MET copy number aberrations and its correlation with increased depth of tumor invasion and increased metastatic potential (26,28–30). Although 10 of the 13 MET positive samples of our study were poorly differentiated tumors, higher copy aberrations were also seen at higher frequencies in moderately differentiated tumors. No MET gene copy aberration was seen in control samples. According to our results, we also suggest that the amplified MET gene may be a predictor of poor prognosis, and because of its putative tumor progression marker potential, further analyses targeting this gene may provide new possibilities in the treatment of gastric cancer.

Recent data have introduced HER2 as a molecular diagnostic test for gastric cancer (31). The FISH data of our study seemed to be compatible with the present approach. The expression of HER2 has been reported at enormously variable rates, ranging between 8% (32) and 91% (33), and there seems to be a consensus that higher HER2 expression is related with tumor morphology and is predominantly seen in intestinal type gastric cancers. In our study, the prevalence of a higher amplification of HER2 in disomic nuclei was detected (25.9%) within the previously reported range, and most of the samples with increased HER2 copy were poorly differentiated tumors. One of the recent focuses of oncology in the past few years has been the use of target enzyme TOP2A for specific anticancer drugs, anthracyclines, especially in breast carcinomas. TOP2A is almost exclusively coamplified with HER2, with the frequency ranging between 29.0% and 44.0% in breast carcinomas. The prevalence rate of FISH-detected TOP2A gene amplifications in gastric tumors is not known well. Previously reported rates range from 3.0% (13) to 63.0% (34). In our data set, the increased TOP2A copy number rate was 17.1% and 5 cases were seen with the coamplification of HER2 in higher grade

Table 4. Significance of TOP2A gene positivity depending on HER2 copy number aberrations in nonpolysomic tumors.

HER2	TOP2A			
	Negative		Positive	
	N	%	N	%
Negative	27	96.4	1	3.6
Positive	2	28.6	5	71.4
Total	29	82.9	6	17.1
P-value	0.001***			

***: $P \leq 0.001$.

Table 5. Relationship between copy number alterations of AURK A and chromosome 20.

Chromosome 20	AURK A			
	Negative		Positive	
	N	%	N	%
No polysomy	7	25.9	20	74.1
Polysomy	7	87.5	1	12.5
Total	14	40.0	21	60.0
P-value	0.003**			

**: $P < 0.01$.

Table 6. Relationship between copy number alterations of AURK B and chromosome 17.

Chromosome 17	AURK B			
	Negative		Positive	
	N	%	N	%
No polysomy	15	51.7	14	48.3
Polysomy	4	66.7	2	33.3
Total	19	54.3	16	45.7
P-value	0.666			

tumors. Although Kataoka et al. (35) reported that HER2 overexpression/amplification was less frequently seen in resectable gastric cancer than in metastatic gastric cancer, the prevalence of HER2 amplification in our data set from curatively resected gastric tumors was 20.0%. We suggest that geographic and ethnic heterogeneity of tumor-associated aberrations may help to explain the differences in HER2 amplification in various studies. Although some studies reported significant heterogeneity in both gene amplification and protein overexpression (36,37), others, including our own, showed homogeneous FISH-detected HER2 amplification within a tumor (38,39). The limitation of our study was the lack of the presentation of the relationship between HER2 amplification and HER2 overexpression. However, we should note that virtually all cells of the samples with HER2-positive phenotype showed HER2 amplification, and tumoral homogeneity was also seen in the samples with HER2 and TOP2A coamplification. However, we think that further comparative analyses in larger populations are necessary to determine whether patients with heterogeneous HER2-positive tumors show a different response to trastuzumab compared with patients with homogeneous HER2-positive samples.

As far as we know, the present study is the first report presenting the prevalence of FISH-detected AURK A and AURK B genomic copy number changes in gastric cancers and their relation with clinicopathological features of the tumors. Chromosomal instability frequently occurs in gastric tumors and manifests with genomic and gene specific copy number alterations, as well as tumoral aneuploidy. Although there are still unexplained chromosome instability mechanism(s), relations between changes in proteins involved in mitotic spindle functions and the development of aneuploidy in tumor cells have been reported previously (40). AURK A gene amplification and/or over-expression has been shown to be involved in genetic instability in several human carcinomas,

Table 7. Comparison of copy number alterations of EGFR, MET, HER2, TOP2A, AURK A, and AURK B genes and clinicopathological parameters.

	FISH positivity					
	N = 35	EGFR, N = 10	MET, N = 23	HER2, N = 7	TOP2A, N = 6	AURK A, N = 21	AURK B, N = 16
Age							
<60 (42–60)	12	4	10	2	2	9	5
>60 (60–79)	23	6	13	5	4	12	11
Sex							
Female	15	4	10	2	2	9	8
Male	20	6	13	5	4	12	8
Pathology							
Moderately diff. TAC	22	3	13	2	-	10	7
Poorly diff. TAC	13	7*	10	5*	6*	11*	9*
Stage							
T1	2	-	2	-	-	-	1
T2b	13	1	8	2	-	7	5
T3	15	6	9	3	4	9	6
T4	5	3	4	2	2	5	4

*: Statistically significant differences between the pathology of the tumors and aberrant copy numbers of the genes.
EGFR gene, P = 0.010; HER2 gene, P = 0.047; TOP2A gene, P = 0.014; AURK A gene, P = 0.007; AURK B gene, P = 0.021.

including gastric cancers. The correlation of AURK A expression changes with malignant phenotype and tumor progression has been evaluated in a few studies (40,41). They reported a positive correlation between the higher expression of AURK A and aneuploid formation and poor prognosis in gastric cancers. AURK A is located on 20q13, which has been previously reported as a frequently amplified chromosome arm in gastric cancers. However, there is a lack of information about its amplification and/or overexpression and its relation to chromosome 20 polysomy. In our data set, AURK A amplification and chromosome 20 polysomy were detected in 60.0% and 22.9% of the tumors, respectively. The detection rate of the AURK A amplification in the tumors with chromosome 20 disomic nuclei was in a relative high percentage, and they were predominantly seen in poorly differentiated tumors. Most of the tumors with nonpolysomic chromosome 20 and AURK A amplifications also had nuclei with polysomic chromosomes 7 and 17 and gene-specific amplifications examined in the current study. Therefore, we suggest that AURK A amplification is not a rarely seen aberration in gastric cancers and our results also support the involvement of AURK A in aneuploidy formation. The other point is the usability of AURK A as an independent prognostic marker in gastric cancer. Recently, Wang et al. (41) reported a significant association between AURK A expression and TNM stages of the tumors, and they suggested that it is an independent prognostic factor in the identification of the patients with worse outcomes. Of the samples, 70.0% (14/21) with AURK A amplification were poorly differentiated tumors and there was a statistically significant association between increased copy number of AURK A and tumor stages. Moreover, it is necessary to analyze AURK A amplification and its relation with expression levels in tumor samples because recent evidence (42) has shown the antiapoptotic potential of AURK A to gastrointestinal cells by regulating levels of P53 through the AKT/HDM2 pathway, and AURK A kinase inhibitors might be a therapeutic option for patients with AURK A-positive expression. An experimental study has shown that deletion of AURK A by AURK A-specific small interference RNA could be a potential therapeutic method in the treatment of gastric cancers (43).

In conclusion, although our results should be interpreted guardedly due to the limited number of patients included and the lack of the relation of the analyzed gene amplifications with their expressions, our limited data allow us to suggest the following in Turkish patients: 1) The copy number changes of receptor kinases in Turkish patients were generally within the previously reported ranges. 2) Not only immunohistochemistry but also FISH analysis should be used in the determination of the status of the receptor kinases in tumor tissues to see the heterogeneity of tumors; this is very important in determining those patients who may benefit most from anticancer therapies and to discriminate tissues where receptor kinase amplifications are not associated with related chromosome polysomies. 3) Genomic copy aberrations of AURK A seem to be frequently seen in gastric cancers. Previous studies, including our study, show the potential of AURK A to offer information for disease progression and administration of individualized treatment for gastric cancer patients.

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