RESEARCH ARTICLE

Are PIK3CA Mutation and Amplification Associated with Clinicopathological Characteristics of Gastric Cancer?

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Abstract

Alterations in mitochondrial DNA (mtDNA) have been studied in various cancers. However, the clinical value of mtDNA copy number (mtCN) alterations in gastric cancer (GC) is poorly understood. In the present study, we investigated whether alterations in mtCNs might be associated with clinicopathological parameters in GC cases. mtCN was measured in 109 patients with GC by real-time PCR. Then, correlations with clinicopathological characteristics were analyzed. mtCN was elevated in 64.2% of GC tissues compared with paired, adjacent, non-cancerous tissue. However, the observed alterations in mtCN were not associated with any clinicopathological characteristics, including age, gender, TN stage, Lauren classification, lymph node metastasis, and depth of invasion. Moreover, Kaplan-Meier survival curves revealed that mtCN was not significantly associated with the survival of GC patients. In this study, we demonstrated that mtCN was not a significant marker for predicting clinical characteristics or prognosis in GC.

Keywords: Gastric cancer - mitochondrial DNA - copy number - prognosis

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Introduction

Gastric cancer (GC) is highly prevalent in Asia and is a leading cause of cancer death worldwide. Its development has been shown as a multi-step process, ranging from chronic gastritis to atrophy, intestinal metaplasia, dysplasia, and finally, invasive cancer (Correa and Shiao, 1994; Leung et al., 2000). A better understanding of the molecular mechanisms of this progression may provide excellent survival outcome by suggesting new potential new novel treatment strategies.

The development of GC was characterized by multiple genetic events (Correa, 1992; Ottini et al., 2006). One of the most important pathways is the recently found phosphatidylinositol 3-kinase (PI3K) signaling pathway (Michl and Downward, 2005; Yu et al., 2008; Qu et al., 2009). The phosphoinositide-3-kinase, catalytic, alpha polypeptide (PIK3CA) gene encodes the catalytic subunit p110 alpha of phosphatidylinositol 3-kinase (PI3K) belonging to class 1A of PI3Ks. In various cancers, PIK3CA mutation stimulates cell growth by stimulating AKT pathway and has been reported in 6.5% of early gastric cancers (Campbell et al., 2004; Lee et al., 2005; Velho et al., 2005). PIK3CA amplification is found more frequently than PIK3CA mutation in various cancers and it promotes another mechanism for PI3K/AKT pathway (Bertelsen et al., 2006; Kato et al., 2007; Yamamoto et al., 2008). In GC, PIK3CA amplification was found in 67% of cancers and associated with poor prognosis (Shi et al., 2012). However, there was no study about PIK3CA mutation and amplification in Korean patients with GC.

In the present article, we examined the frequency of mutation in the exons 9 and 20 of PIK3CA gene and PIK3CA amplification in GC. And then, clinicopathological characteristics and prognostic value of GC patients with long-term follow-up were analyzed.

Materials and Methods

Patients

We recruited 121 patients who underwent gastrectomy for gastric adenocarcinoma from archives of paraffin blocks at Keimyung University Dongsan Hospital from October 1995 to December 1999. Tissue samples were fixed in formalin and embedded in paraffin. All cases were reviewed by an expert panel of pathologists according to the current criteria of the WHO classification for morphological features and immunohistochemical results. The clinical data and pathological reports of the patients with gastric adenocarcinoma were collected from the medical records in Keimyung University Dongsan Hospital.

Subsequently, the selected areas from paraffin embedded tissues were used for DNA extraction. DNA was isolated by using DNA extraction Kit (AbsoluteTM DNA extraction Kit, BioSewoom, Korea) according to

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the manufacturer's instructions.

PIK3CA mutation

Two hot spot-regions (exons 9 and 20) of PIK3CA mutation were investigated, because more than 75% of PIK3CA missense mutations cluster were found within these regions (Samuels et al., 2004). The polymerase chain reaction (PCR) amplification of the PIK3CA was performed as described previously with minor modification (Yamamoto et al., 2011). PCR was done using AmpliTaq Gold (Applied Biosystems, USA). The PCR conditions were as follows: 1 cycle of 95°C for 11 min, 40 cycles of 95°C for 30 sec, 55°C for 40 sec, and 72°C for 1 min, followed by 1 cycle of 72°C for 10 min. The PCR products were electrophoresed on 1.5% agarose gel and stained with ethidium bromide to confirm the size of the bands. Then, direct DNA sequencing for PIK3CA mutation was performed using the ABI 3730 DNA sequencer by Bionics Inc, Korea.

PIK3CA Amplification

Copy number of PIK3CA gene was analyzed by quantitative real-time (qRT) PCR. For the quantitative determination of PIK3CA content relative to nDNA, primers for specific amplification of exon 20 in PIK3CA gene and nDNA-encoded ß-actin gene were selected according to previous study. Real-time PCR was then carried out on an LightCycler 480 II system (Roche Diagnostics, Germany) with a total volume of 20 μl reaction mixture containing 10 μl SYBR Green Master MIX (Takara, Japan), 8 pmol of each primers, and DNA (50 ng). The PCR conditions were 95°C for 1 min, followed by 40 cycles of 95°C for 15 s, and 60°C for 30 s. The threshold cycle number (Ct) values of the ß -actin gene and PIK3CA gene were determined. The copy number of PIK3CA in each tested specimen was then normalized against that of ß -actin gene to calculate the relative PIK3CA copy number. Each measurement was repeated in triplicate and 5 serially diluted control samples were included in each experiment. Copy amplification of PIK3CA gene was defined by a copy number ≥ 3.

Statistical Analysis

The SPSS statistical package, version 19.0 for Windows, was used for all statistical analyses. Correlation between PIK3CA mutations or amplification and clinicopathological characteristics was analyzed by Fisher's exact test or Pearson's Chi square test. Disease free survival was measured from the date of diagnosis to the date of recurrence or the last follow-up. Overall survival was measured from the date of diagnosis to the date of death or the last follow-up visit. Disease-free and overall survivals were measured according to the Kaplan Meier method. Differences between curves were analyzed using the log-rank test. P values <0.05 were considered to indicate statistically significant results, and all p values correspond to two-sided significance tests.

Results

We conducted a sequencing analysis to investigate the frequency of PIK3CA mutation and amplification in 121 GCs. Then we analyzed the clinicopathological characteristics of GC comparing with the presence of the PIK3CA mutation and amplification (Table 1). We found the 8 PIK3CA mutations of the 114 GCs (7.7%). However there were no significant association between the presence of PIK3CA mutation and any other clinicopathological characteristics.

To analyze PIK3CA amplification, we performed real-time PCR of the 110 GCs. With a gene copy number of 3 or more defined as amplification, we found 23 PIK3CA amplifications in 110 GCs (22.3%). There were no significant association between presence of PIK3CA amplification and any other clinicopathological characteristics. Although the frequency of PIK3CA amplifications was higher in the advanced stage of GCs, it was not significantly correlated with the stage of GC (p=0.09). And we analyzed association between PIK3CA mutation and amplification, there was no statistical significance (p=0.62; Table 2).

To examine whether the survival was associated with the mutation and the amplification of PIK3CA or not, we generated the over-all survival curves by using the Kaplan-Meier method. After a median follow-up duration of 82.2 months (3.7-158.8 months), a 5-year overall survival rate was 79.3%. There was no significant association

Table 1. The Frequency of PIK3CA Mutation and Amplification in Gastric Cancers

	PIK3CA mutation			PIK3CA amplification		
	Wild-type	Mutation	p	(-)	(+)	p
Total	96 (92.3)	8 (7.7)		87 (77.7)	23 (22.3)	
Age			0.91			0.48
< 60	50 (92.6)	4 (7.4)		45 (81.8)	10 (43.5)	
≥ 60	46 (92.0)	4 (8.0)		42 (76.4)	13 (56.5)	
Gender			0.89			0.76
Male	74 (92.5)	6 (7.5)		65 (78.3)	18 (21.7)	
Female	22 (91.7)	2 (8.3)		22 (81.5)	5 18.5)	
Type			0.36			0.41
Diffuse	22 (88.0)	3 (12.0)		22 (84.6)	4 (15.4)	
Intestinal	73 (93.6)	5 (6.4)		64 (77.1)	19 (22.9)	
T stage						
1	39 (92.9)	3 (7.1)		40 (87.0)	6 (13.0)	
2	21 (87.5)	3 (12.5)		18 (69.2)	8 (30.8)	
3	2 (100)	0 (0.0)		1 (50.0)	1 (50.0)	
4	34 (94.4)	2 (5.6)		28 (77.8)	8 (22.2)	
N Stage						0.27
0	55 (90.2)	6 (9.8)		53 (79.1)	14 (20.9)	
1	21 (100)	0 (0)		19 (90.5)	2 (9.5)	
2	11 (91.7)	1 (8.3)		9 (75.0)	3 (25.0)	
3	9 (90.0)	1 (10.0)		6 (60.0)	4 (40.0)	
Stage			0.86			0.09
Early	39 (92.9)	3 (7.1)		40 (87.0)	6 (13.0)	
Advanced	57 (91.9)	5 (8.1)		47 (73.4)	17 (26.7)	

Table 2. Association between PIK3CA Mutation and Amplification

		PIK3CA amplification	
		(-)	(+)
PIK3CA mutation	Wild-type	78 (82.1)	17 (17.9)
	Mutation	6 (75.0)	2 (25.0)

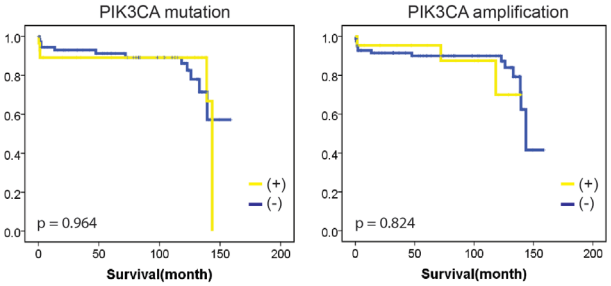

Figure 1. Survival Analysis of PIK3CA Mutation and Amplification in Gastric Cancers (A) PIK3CA Mutation (B) PIK3CA Amplification

between the mutation and the amplification of PIK3CA and the over-all survival in GC (Log-rank test, p=0.964 and p=0.824; Figure 1).

Discussion

PI3K/Akt pathway is well known that it plays important role in cancer cell proliferation, catabolism, cell adhesion and apoptosis. And the PIK3CA amplification and mutations frequently has been found in various human cancers such as breast, cervical cancers, ovarian cancers, thyroid cancers, and pituitary tumors (Shayesteh et al., 1999; Ma et al., 2000; Samuels et al., 2004; Garcia-Rostan et al., 2005; Velho et al., 2005; Wu et al., 2005; Lin et al., 2009; Kandula et al. 2013). In present study, however, we found relatively low frequency of PIK3CA mutations in the GC (7.7%). The low frequency of PIK3CA mutations is comparable with the previous studies in GC which were reported the frequency from 3 to 42% (Samuels et al., 2004; Shi et al., 2012; Cancer Genome Atlas Research, 2014). Recent study which was conducted in large scale reported that Epstein-Barr virus(EBV)-related subtype of GC was related with high frequency of PIK3CA mutation (Cancer Genome Atlas Research, 2014). In contrast to EBV-related GC, PIK3CA mutation was detected 3 to 42% in other subtypes of GC. Given that the prevalence of EBV-positive GC has been reported less than 10%, the 7.7% PIK3CA mutation of GC in our data is relevant.

Instead of PIK3CA mutation, we found 23 PIK3CA amplifications in 110 gastric cancers (22.3%). The inverse relationship between PIK3CA amplification and mutation has reported not only in the gastric cancer but also in various cancers (Campbell et al., 2004; Yamamoto et al., 2008; Shi et al., 2012). Furthermore genomic amplification, rather than gene mutations, has been suggested as major signature of neoplastic transformation and tumor progression (Gray and Collins, 2000). Chromosome copy number abnormalities have been frequently identified in gastric cancer, including PIK3CA amplification (Byun et al., 2003; Zhang et al., 2011). Therefore, the PIK3CA amplification may be a common mechanism in the activation of PI3K/Akt signaling pathway rather than PIK3CA mutation.

To investigate the possibility of applying PIK3CA amplification and mutation as prognostic marker in the gastric cancer, we scrutinized clinicopathological characteristics and prognostic value of recruited patients and analyzed the correlation with PIK3CA mutation and amplification. In agreement with the previous study (Shi et al., 2012), the presence of PIK3CA mutation was not correlated with clinicopathological characteristics and survival rate. In contrast to the previous study which was reported that PIK3CA amplification was significantly associated with poor survival (Shi et al., 2012), however, there was no significant relationship between PIK3CA amplification and clinicopathological characteristics or survival rate in the gastric cancer. The discrepancy between our data and the previous report might be caused by the several reasons. First of all is the high survival rate of recruited patients in over 150 month follow-up. Especially the high survival state of patients who have the GC detected PIK3CA mutation or amplification might be the one of the causes. The reason of the high survival rate of the patient would be related with early detection of GC which is resulted by high rate of early endoscopic diagnosis in Korea. In contrast to 14% of GC in T1 stage which was reported by the Shi et al. (2012), the proportion of early gastric cancer (EGC) was about 40% in our data. The high rate of EGC also might affect the difference with the previous study.

Considering the high rate of EGC which might be the outcome of the National Cancer Screening Program done by the National Cancer Center of Korea since 1999, our results suggest that PIK3CA mutation and amplification of GC would be negligible in Korean clinical environment. Although our results showed non-significant of PIK3CA mutation or amplification in GC, based on the importance of PI3K/Akt pathway in tumourigenesis, detailed further study would be needed relationship between PIK3CA mutation or amplification and development of GC to advanced stage.

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