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ORIGINAL ARTICLE

Study on the association between PI3K/AKT/mTOR signaling pathway gene polymorphism and susceptibility to gastric cancer

Lei Qi, Kewen Sun, Yun Zhuang, Jing Yang, Jianping Chen

Department of Gastroenterology, the Third Affiliated Hospital of Soochow University, Changzhou, China

Summary

Purpose: *Excessive activation of PI3K/AKT/mTOR signaling pathway is one of the most common changes in human cancers, and single nucleotide polymorphisms (SNPs) existing in its functional region can affect the occurrence process of a variety of cancers. This study aimed to screen out the SNPs associated with susceptibility to gastric cancer in the PI3K/AKT/mT0R signaling pathway.*

Methods: *In this case-control study, the tagging SNPs in the promoter region5'-UTR, exon region or 3'-UTR of PIK3CA, PIK3CB, PIK3R1, PIK3R2, PIK3R3, AKT1, AKT2, AKT3 and mTOR genes were screened out. The relationship between the genetic variation of PI3K/AKT/mT0R signaling pathway genes and the susceptibility to gastric cancer in Chinese Han population was investigated by this case-control study.*

Results: *The results showed that the polymorphisms of the two loci, PIK3R3 rs7536272 (Additive model: OR=1.16, 95% CI=1.01-1.35) and mTOR rs2295080 (GG vs TT: OR=0.75, 95% CI=0.60-0.94; Additive model: OR=0.78, 95% CI=0.66-0.93), were associated with the risk of gastric cancer in the studied population and there was a combined effect between the two loci (p_{trend}=0.005).*

Conclusions: *In conclusion, the polymorphisms of the two loci, PIK3R3 rs7536272 and mTOR rs2295080, on the PI3K/AKT/mTOR signaling pathway genes are associated with genetic susceptibility to gastric cancer in Chinese population.*

Key words: *gastric cancer, PI3K/AKT/mTOR, polymorphism*

Introduction

Gastric cancer is the fifth major tumor with a high incidence rate worldwide, ranking third in the common causes of death from tumors. According to the statistical data of the World Health Organization (WHO) in 2012, there were about 951,600 new cases of gastric cancer, leading to 723,100 deaths each year [1]. Studies have confirmed that the occurrence of gastric cancer is the result of the combined action of environmental and genetic factors. Helicobacter pylori (Hp) infection [2], poor eating habits [3,4], smoking and drinking [5] are the main risk factors for gastric cancer. Under the exposure to the same risk factors, the risk of gastric cancer is not exactly the same in individuals, suggesting that the individual susceptibility may play an important role in the occurrence and development of gastric cancer.

Phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB or AKT)/mammalian target of rapamycin (mTOR) signaling pathway is an important intracellular signaling pathway, which affects the occurrence, development, treatment and prognosis of tumors through regulating a variety of cell physiological processes [6]. Many studies have confirmed the PI3K/AKT/mTOR signaling pathway disorders in tumors, and particularly in the biological regulation of gastric cancer [7], liver cancer [8], breast cancer [9], colorectal cancer [10]

Correspondence to: Jianping Chen, MD. Department of Gastroenterology, the Third Affiliated Hospital of Soochow University, 185 Juqian Road, Changzhou, 213003 Jiangsu, China.
Tel: +86 013775000717, E-mail: 349599942@qq.com
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and prostate cancer [11], playing a role as proto-oncogene, which has become a hotspot of the new molecular biomarker-based and targeted therapy of tumors. Up to now, there have been more studies on the PI3K signaling pathway gene polymorphism. Li et al. found in Chinese population that the polymorphisms of PI3K/AKT/mTOR pathway genes in patients with non-small cell lung cancer can predict brain metastasis, which is instructive for the prevention of such metastasis in this cancer [12]. Lin et al. performed a case-control study to investigate whether the combined action of PI3K/AKT/mTOR pathway gene polymorphism and environmental factors would affect the occurrence and development of bladder cancer, and they found that the risk of bladder cancer in individuals with excessive energy intake, little exercise or several risk alleles of PI3K/AKT/mTOR pathway is increased by nearly 21-fold; these results have implications for the prevention of bladder cancer [13].

At present, a large number of studies on the PI3K/AKT/mTOR pathway gene polymorphism and tumors have been conducted, but only few studies explored the PI3K/AKT/mTOR pathway gene polymorphism and gastric cancer. Moreover, such studies are limited to the single pathway gene polymorphism and gastric cancer, and the study on the correlation between PI3K/AKT/mTOR pathway gene polymorphism and susceptibility to gastric cancer is lacking.

In this research, the key genes of the whole PI3K/AKT/mTOR pathway were studied based on the Thousand Talents Program Database, 5 tagging SNPs in the functional region of PIK3CA, PIK3CB, PIK3R1, PIK3R2, PIK3R3, AKT1, AKT2, AKT3 and mTOR genes were screened out, and a case-control study was performed to investigate the association between the selected loci and gastric cancer risk in 574 patients with gastric cancer and 912 control subjects.

Methods

Study objectives

A total of 574 patients with new-onset gastric cancer who were treated in the Third Affiliated Hospital of Soochow University from March 2014 to January 2017 were enrolled in this study, while a total of 912 healthy subjects in the same hospital and time period served as controls. All patients were diagnosed with gastric cancer by biopsy and did not suffer from other cancers or receive any radiotherapy or chemotherapy before enrollment. Cases and controls were matched according to age and gender, and the age difference was ±5 years. Informed consent was obtained from all individuals; 5 mL venous blood was collected using vacuum anticoagulant tube containing ethylene diamine tetraacetic acid (EDTA) and stored at -20°C. The basic information of all subjects, such as gender, age, tumor type and tumor site, was registered. This study was approved by the Ethics Committee of Soochow University.

SNPs selection strategy

According to the UCSC gene browser, the tagging SNPs in the promoter region, 5'-untranslated region (5'-UTR), exon region or 3'-untranslated region (3'-UTR) of PIK3CA, PIK3CB, PIK3R1, PIK3R2, PIK3R3, AKT1, AKT2, AKT3 and mTOR were selected and screened out using Haploview4.0 software according to Pairwise tagging and r^2 threshold of 0.8. The minor allele frequency (MAF) of the selected loci was higher than 0.10.

Genotyping

DNA was extracted from all samples using the traditional ammonia-chloroform method, and the DNA genotype was identified via ABI7900HT fluorescent quantitative PCR instrument using TaqMan genotyping method.

Statistics

Chi-square test was used for the frequency distribution of genotyping in the control subjects to ensure that they met the Hardy-Weinberg equilibrium (HWE). Pearson's chi-square test was used for the demographic characteristics, age and gender matching involved in the case-control study. The odds ratio (OR) and 95% confidence interval (CI) of different alleles in clinicopathologic staging were analyzed using the univariate and multivariate logistic regression analysis. SAS9.2 software was used for the data analysis. All tests were two-sided. $P<0.05$ suggested that the difference was statistically significant.

Results

General characteristics of the study subjects

A total of 1486 cases and controls (574 patients with gastric cancer and 912 healthy controls) were enrolled, and the main characteristics are shown in Table 1. There were no differences in age and gender between cases and controls (p=0.418; p=0.115, respectively). There were 214 (37.3%) patients with gastric cardia cancer and 360 (62.7%) patients with non-gastric cardia cancer. There were also 328 (57.1%) patients with diffuse type (57.1%) and 246 (42.9%) patients with intestinal type.

Analysis of the correlation between the SNPs and the susceptibility to gastric cancer

All loci selected met HWE (p>0.05) and MAF was higher than 0.1 (Table 2). Univariate logistic regression analysis results are shown in Table 3. After adjustment for age and gender, PIK3R3

rs7536272 was found to be associated with increased risk of gastric cancer (Additive model: OR=1.16, 95%CI=1.01-1.35), and mTOR rs2295080 was associated with decreased risk of gastric cancer (GG vs TT: OR=0.75, 95%CI=0.60-0.94; Additive model: OR=0.78, 95%CI=0.66-0.93). The stratified analysis results based on age, gender, lesion site and pathological type showed that rs2295080 could reduce the onset risk of the elderly, female, non-gastric cardia cancer and intestinal type gastric cancer patients (Table 4). The conjoint analysis showed that rs7536272 and rs2295080 had a combined effect, and the onset risk of gastric cancer was significantly increased with the increase of risk base (p_{trend}=0.005). Compared with that in the group carrying 0-2 risk bases, the incidence rate of gastric cancer in the group carrying 3-4 risk bases was increased by 1.28. In addition, it was not found that the three tagging SNPs, rs3730089, rs3756668 and rs1130233, were associated with the onset risk of gastric cancer (p>0.05) (Table 5).

Table 1. Selected characteristics in gastric cancer cases and controls

Variables	*Cases*	*Controls*	*p value* [a]
	n (%)	*n (%)*	
All subjects	574 (100)	912 (100)	
Age, years			0.418
≤65	337 (58.7)	516 (56..6)	
≥65	237 (41.3)	396 (43.4)	
Gender			0.115
Male	361 (62.9)	610 (66.9)	
Female	213 (37.1)	302 (33.1)	
Tumor site			
Cardia	214 (37.3)		
Other site	360 (62.7)		
Pathological type			
Diffuse	328 (57.1)		
Intestinal	246 (42.9)		

[a] Two-sided chi-square test

Table 2. Primary information and minor allele frequencies (MAFs) of selected SNPs

SNP	*Gene*	*Position*	*Base change*	*HWE*	*MAF in our controls*
rs3730089	PIK3R1	Exon	G >A	0.236	0.286
rs3756668	PIK3R1	3'UTR	G >A	0.651	0.433
rs7536272	PIK3R3	Promoter	A>G	0.163	0.433
rs1130233	AKT1	Exon	A>G	0.211	0.424
rs2295080	mTOR	Promoter	T>G	0.908	0.282

HWE: Hardy-Weinberg equilibrium, MAF: minor allele frequency, SNP: single nucleotide polymorphism

Table 3. Univariate logistic regression analysis for associations between selected SNPs and gastric cancer risk

SNP	*Genotype*	*Cases (%)*	*Controls (%)*	*Adjusted OR* [a] *(95%CI)*	*p value* [a]
rs3730089	GG	307(53.5)	472(51.8)	1	
	AG	211(36.8)	358(39.3)	0.91(0.66-1.04)	0.389
	AA	56(9.8)	82(9.0)	1.03(0.86-1.24)	0.770
	Additive model			0.98(0.83-1.15)	0.787
rs3756668	GG	161(28.0)	304(33.3)	1	
	AG	279(49.5)	427(46.8)	0.97(0.77-1.23)	0.786
	AA	101(22.5)	181(19.8)	0.94(0.80-1.09)	0.396
	Additive model			0.94(0.81-1.09)	0.435
rs7536272	AA	194(33.8)	297(32.6)	1	
	GA	279(48.6)	441(48.4)	1.15(0.99-1.34)	0.059
	GG	101(17.6)	174(19.1)	1.26(0.99-1.61)	0.064
	Additive model			1.16(1.01-1.35)	0.043
rs1130233	AA	177(30.8)	293(32.1)	1	
	GA	284 (49.5)	464(50.9)	1.01(0.80-1.28)	0.933
	GG	113(19.7)	155(17.0)	1.09(0.94-1.28)	0.251
	Additive model			1.08(0.93-1.26)	0.300
rs2295080	TT	194(33.8)	297(32.6)	1	
	TG	279(48.6)	441(48.4)	0.81(0.65-1.01)	0.058
	GG	101(17.6)	174(19.1)	0.75(0.60-0.94)	0.013
	Additive model			0.78(0.66-0.93)	0.005

[a] Adjusted by age and sex

Table 4. Stratification analysis for association between variant genotypes and gastric risk

Variables	*rs7536272*		*Adjusted OR* [a]	*p* [a]	*rs2295080*		*Adjusted OR* [a]	*p* [a]
	Cases	*Controls*	*(95%CI)*		*Cases*	*Controls*	*(95%CI)*	
	AA /AG/GG	*AA /AG/GG*			*TT/TG/GG*	*TT/TG/GG*		
Age, years								
≤65	100/156/81	174/239/103	1.17 (0.97-1.41)	0.111	195/122/20	265/208/43	080 (0.64-1.00)	0.05
>65	61/128/48	130/188/78	1.17 (0.93-1.47)	0.186	13890/9	204/163/29	0.75 (0.57-0.99)	0.041
Sex								
Male	61/103/49	102/148/52	1.27 (0.99-1.64)	0.06	310/251/49	159/120/23	0.82 (0.61-1.08)	0.16
Female	104/181/76	202/279/129	1.09 (0.91-1.30)	0.376	206/140/15	119/480/306	0.76 (0.61-0.95)	0.014
Tumor site								
Cardia	60/106/48	304/427/181	1.16 (0.94-1.43)	0.164	120/81/13	469/371/72	1.22 (0.88-1.70)	0.235
Other site	105/178/77	304/427/181	1.12 (0.95-1.33)	0.187	51/126/78	213/131/16	0.74 (0.61-0.91)	0.005
Pathological type								
Diffuse	94/161/73	304/427/18	1.15 (0.96-1.37)	0.127	181/127/20	469/371/72	0.86 (0.70-1.06)	0.157
Intestinal	71/123/52	304/427/18	1.13(0.93-1.38)	0.228	152/85/9	469/371/72	0.67 (0.53-0.86)	0.001

[a] Adjusted by age and sex

Table 5. Combined effects of rs7536272 and rs2295080 on gastric cancer risks

No. of risk allele [a]	*Cases*	*Controls*	*Adjusted OR(95%CI)* [b]	*p* [b]
	n (%)	*n (%)*		
0-1	85 (14.8)	178 (19.5)	1	
2	209 (36.4)	345 (37.8)	1.27 (0.93-1.73)	0.132
3	205 (35.7)	295 (32.3)	1.47 (1.07-2.01)	0.017
4	75 (13.1)	94 (10.3)	1.67 (1.11-2.47)	0.013
Trend			1.18 (1.05-1.33)	0.005
Binary classification				
0-2	294 (51.2)	523 (57.3)	1	
3-4	280 (48.8)	389 (42.7)	1.28 (1.04-1.59)	0.020

[a] The rs7536272 G and rs2295080 T allele were assumed as risk alleles based on main effect of individual locus;
[b] Adjusted by age and sex

Discussion

In this study, the association of PI3K/AKT/mT0R signaling pathway gene polymorphism with the risk of gastric cancer in Chinese population was investigated based on the Thousand Talents Program Database using the candidate genes. The results showed that PIK3R3 rs7536272 and mTOR rs2295080 were associated with the onset risk of gastric cancer.

In the present study reported were the correlations between the loci rs7536272 and rs2295080 and the genetic susceptibility to gastric cancer. There is no report on PIK3R3 rs7536272 in cancer, but rs2295080 has been reported in prostate cancer [11] and colorectal cancer [10]. Hildebrandt et al. [14] reported the mTOR rs2295080 polymorphism in 2009 for the first time and discussed the effect of this locus in the prognosis of esophageal cancer patients in Europe and the United States who received radiotherapy and chemotherapy, but no correlation between rs2295080 polymorphism and the prognosis of patients with esophageal cancer was found [14]. Cao et al. found in Chinese population that the risk of renal cell carcinoma in individuals carrying rs2295080 TG/GG genotype was significantly reduced compared with that in individuals carrying TT genotype (OR=0.74, 95%CI=0.59-0.91); the mTOR mRNA of renal cancer tissues carrying G allele was significantly decreased, and the G allele could reduce the mTOR transcriptional activity and affect the expression

of mTOR [15]. Chen et al. found in Chinese population that the risk of prostate cancer in individuals carrying rs2295080 TG/GG genotype was significantly reduced compared with that in individuals carrying TT genotype (OR=0.77, 95%CI=0.62-0.96) [16]. In addition, Xu et al. have also found that the rs2295080 polymorphism plays a similar role in colorectal cancer [17]. The results of the present study are consistent with those previous reports.

In a number of cancer studies it has been shown that PI3K/AKT/mTOR signaling pathway does not act independently as a bridge molecule connecting extracellular signaling and intracellular response effects, but affects the downstream signaling molecules and the cell growth, proliferation, apoptosis, migration and other physiological functions under the combined action of many upstream and/or bypass signaling molecules, thus affecting the occurrence and regression of cancer. Tribbles pseudokinase-3 (TRIB3) is a tumor suppressor gene that can inhibit the expression of AKT. Downregulation of TRIB3 expression can promote the phosphorylation of AKT protein, enhance the activity of AKT downstream target gene FOXO3 and Bcl2 associated agonist of cell death (BAD) and promote the tumor growth [18]. Qiu et al. found that the silencing of eukaryotic elongation factor 1 alpha2 (eEFlα2) can inhibit the activity of PI3K/AKT/mT0R signaling pathway, lead to proliferation, migration and invasion of hepatocellular carcinoma cells and cause cell cycle arrest, so eEFlα2 may play a role as proto-oncogene through the PI3K/AKT/mT0R signaling pathway [19]. AKT is lowly expressed in the AKT-knockout liver cell lines, which can directly act on BAD, upregulate the BAD expression and promote apoptosis of hepatocellular carcinoma cells [20].

There are also some shortcomings in this study. First, the sample size was too small; considering the power of statistical analysis, some SNPs that met the screening criteria but with too small MAF were not included in the study. In addition, the power of the results might be affected because of the too small sample size, so the results need to be verified in future larger population-based samples. Second, only SNPs in the functional region were selected in this study, so a small number of potential associations with gastric cancer might be ignored. In addition, the study population was from the hospital, so selective and information bias were inevitable.

Conclusions

In conclusion, the results of our study showed that there is a significant correlation between the polymorphisms of the locus rs7536272 in the PIK3R3 gene promoter region and the locus rs2295080 in the mTOR gene promoter region and the risk of gastric cancer in Chinese population. Therefore, rs7536272 and rs2295080 are expected to be useful molecular biomarkers of gastric cancer, but the possible molecular mechanism of the SNPs of the two loci in the occurrence and development of gastric cancer needs further study.

Conflict of interests

The authors declare no conflict of interests.

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