

Original Article

Association of PIN1 promoter polymorphisms with colorectal carcinoma risk and clinicopathological parameters

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Abstract: Studies have reported the associations between the two single nucleotide polymorphisms (SNPs), -842G>C (rs2233678) and -667C>T (rs2233679), in the PIN1 promoter region and the risk of cancers. However, no studies have reported the association between the PIN1 polymorphisms and risk of colorectal carcinoma. In this study, we investigated the association between the two SNPs -842G>C and -667C>T in PIN1 promoter with risk of colorectal carcinoma (CRC) and clinicopathological parameter of CRC. The results indicated the frequencies of -842G>C and -667C>T genotypes and alleles were not significantly different between the colorectal cancer patients and controls. Interestingly, -667C>T SNP genotypes showed a significant association with clinical stage (P = 0.022). Compared to the -667CC genotype, the -667CT genotype was lower in the advance stage (rate in the advance stage, -667CC: 34/51, -667CT: 27/64). The -842G>C genotype was marginally associated with lymph node status (P = 0.099). In conclusion, our findings suggested that the polymorphic variants of -842G>C and -667C>T in PIN1 promoter were not associated with the risk of CRC but associated with the clinicopathological CRC.

Keywords: PIN1, colorectal carcinoma, polymorphism, risk

Introduction

PIN1 (peptidylprolyl cis/trans isomerase, NIMA-interacting 1), an approximate 18kDa protein consisting of a COOH-terminal catalytic domain and a WW amino-terminal protein-protein interaction domain, belongs to the evolutionarily conserved peptidyl-prolyl isomerase family of proteins [1] which can recognize and bind to specific phosphorylated Ser/Thr-Pro motifs and change conformation of phosphoproteins [2]. PIN1 plays a vital role in the development and progression of cancer by controlling the activity and/or stability of Pro-directed phosphoproteins [2]. PIN1 contributes to the development of cancer through various targets, such as β -catenin in prostate cancer [3], hepatitis B virus X-protein in liver cancer [4], estrogen receptor-alpha in breast cancer [5] and cyclin

D1 in cervical cancer [6]. Overexpression of PIN1 has been reported in various types of human cancers [7]. Higher expression of PIN1 is associated with tumor progression and prognosis in several cancers, such as esophageal carcinoma [8], lung cancer [9], prostate cancer [10] and colorectal cancer [11].

Studies have reported the associations between the two single nucleotide polymorphisms (SNPs), -842G>C (rs2233678) and -667C>T (rs2233679), in the PIN1 promoter region and the risk of cancers [12-17]. Compared to -842GG homozygote, only -842GC heterozygote but not -842CC homozygote has reported to show a significantly decreased cancer risk in breast cancer [13], squamous cell carcinoma of head and neck [14], lung cancer [15] and nasopharyngeal carcinoma [17]. On the SNP

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Table 1. Characteristics of colorectal carcinoma patients and controls

Characteristic	Patient	Control	P value
Age			
<40	34	40	0.052
≥40	121	84	
Gender			
Male	89	59	0.102
Female	66	65	
CEA			
<5 µg/L	100	-	
≥5 µg/L	43	-	
Primary tumor extension			
T1+T2	21	-	
T3+T4	113	-	
Lymph node status			
NO	55	-	
N1+N2+N3	71	-	
Metastasis			
NO	121	-	
YES	16	-	
Clinical stage			
I+II	60	-	
III+IV	72	-	

-667C>T, the -667T allele is suggested to be increased in hepatitis B and C co-infected patients [12]. Studies indicate that the -667TT homozygote have a significantly increased risk of oral squamous cell carcinoma [18] and nasopharyngeal carcinoma [17]. However, no studies have reported the association between the PIN1 polymorphisms and risk of colorectal carcinoma.

Colorectal cancer is the third most commonly diagnosed cancer in males and the second in females in the world. It is estimated that 1.4 million cases and 693,900 deaths occurred worldwide in 2012 [19]. Colorectal cancer is the 5 most commonly diagnosed cancers and the 5 leading causes of cancer death in China [20]. A few genes were reported to be associated with the risk of colorectal cancer [21, 22]. PIN1 is overexpressed in colorectal carcinoma and correlated with β -catenin expression [23]. Therefore, we speculated that there could be an association between PIN1 polymorphism and colorectal carcinoma risk. In this study, we genotyped the two common promoter SNPs -842G>C (rs2233678) and -667C>T (rs2233-

679) to test whether PIN1 polymorphisms were associated with risk of colorectal carcinoma. We also examined the associations between these polymorphisms and clinicopathological characteristics such as age, gender, serum carcinoembryonic antigen (CEA) level, primary tumor extension, nodal status, metastasis, and tumor stage.

Materials and methods

Study population

A total of 155 patients who were diagnosed with colorectal carcinoma and 124 cancer-free controls at *Hunan Tumor Hospital*, were enrolled in this study. The healthy individuals were matched for age with the colorectal cancer patients. The age range for colorectal cancer patients was 21-67 and for controls was 22-65. Tumor size and status of regional lymph node metastasis were examined by magnetic resonance imaging (MRI) or computed tomography (CT) scan. Clinical stages were graded according to the criteria of Union for International Cancer Control (UICC). All the patients were diagnosed either by histopathology or imageology and received no treatment before the blood drawing. Blood samples were collected after the informed consent was obtained from all participants. This study was approved by the institutional review board.

SNP selection and genotyping

The genomic DNA was extracted using TIAN-amp Genomic DNA kit (TIANGEN BIOTECH, Beijing, China) according to manufacturer's instruction. The two SNPs (-842G>C, rs2233678 and -667C>T, rs2233679) genotypes were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as described by Lambert JC [24]. We used forward primer (5'-CGG GCT CTG CAG ACT CTA TT-3') and reverse primer (5'-AAA TTT GGC TCC TCC ATC CT-3') to amplify the fragment. PCR amplification was performed in a 15 µl reaction mixture containing 100 ng genomic DNA, 0.5 µl of each primers and 1 X PCR master mix (Promega). For genotyping of -842G>C and -667C>T polymorphisms, 2 µl of the PCR products were digested for 4 h at 37°C with 1 µl of BanII (New England BioLabs) or 0.5 µl of SacI (New England BioLabs) respectively in a total volume of 10 µl. Then the cleaved

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Table 2. Genotype and allele distribution of -667C>T and -842G>C in patients and controls

Polymorphism	Patient	Control	P value	OR	95% CI
-667C>T					
Genotype					
CC	58 (37.4)	45 (36.3)	0.835		
CT	70 (45.2)	60 (48.4)			
TT	27 (17.4)	19 (15.3)			
CT versus TT			0.611	1.207 ^a	0.585-2.491
CC versus TT			0.735	0.873 ^a	0.396-1.921
Allele					
C	186	150	0.908	0.980	0.697-1.379
T	124	98			
-842G>C					
Genotype					
CC	0 (0)	0 (0)	0.069		
GC	15 (9.7)	5 (4.0)			
GG	140 (90.3)	119 (96.0)			
CG versus GG			0.160	0.448 ^a	0.146-1.373
Allele					
C	15	5	0.075	2.471	0.885-6.898
G	295	243			

^aData were calculated by unconditional logistic regression with adjustment for age and gender.

products were separated on 3% agarose gel and identified by ethidium bromide staining. We confirmed the reproducibility of genotyping by direct sequencing using 10% of samples which were randomly selected.

Statistical analysis

Hardy-Weinberg equilibrium was analyzed using the Pearson's two-sided chi-square test in the cancer-free controls. The statistical difference of PIN1 genotype and allele frequencies between the colorectal cancer patients and controls was evaluated using the Pearson's two-sided chi-square test. The odds ratio (OR) and its 95% confidence interval (CI) was obtained by the logistic regression analysis to estimate the associations between PIN1 variants and colorectal cancer risk. The crude odds ratio was determined through univariate logistic regression with only the genotype or allele. The adjusted odds ratio was calculated using the multivariate logistic regression method with an adjustment for age and gender. Chi-square test or Fisher's exact test was performed to determine the association between the genotype frequencies and clinicopathologi-

cal parameters such as age, gender, serum carcinoembryonic antigen (CEA), primary tumor extension, nodal status, metastasis, and tumor stage in patients. All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant using the SPSS 16.0 software.

Results

Characteristics of the study population

This study included 155 colorectal cancer cases and 124 cancer-free controls. Demographic variables and risk factors of the patients and controls are listed in **Table 1**. Lack of statistical differences were observed in the distributions of age ($P = 0.052$, **Table 1**) and gender ($P = 0.102$, **Table 1**) between patients and controls.

Distribution of PIN1 genotypes and their associations with risk of colorectal carcinoma

Table 2 summarized the genotype and allele distributions of the PIN1 SNPs (-842G>C, rs2233678 and -667C>T, rs2233679) in patients and controls. The genotype frequency distributions of -667C>T and -842G>C both showed lack of statistical significance between study groups and controls ($P = 0.835$ and 0.069 , respectively, **Table 2**). No significant difference was shown in the distribution of allele frequency of -667C>T between patients and controls ($OR = 0.980$, $95\% CI = 0.697-1.379$, $P = 0.908$, **Table 2**). Nevertheless, a marginally statistical difference was found in the allele distribution of -842G>C between patients and controls ($OR = 2.479$, $95\% CI = 0.885-6.898$, $P = 0.075$).

Association between PIN1 genotypes and clinicopathological parameter

Tables 3 and **4** summarized the relationship between -667C>T and -842G>C genotype and clinicopathological parameters, respectively. Genotype in the two SNPs showed no signifi-

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Table 3. Association between the genotype frequencies of -667C>T and clinicopathological characteristics of colorectal carcinoma patients

Parameters	-667C>T			P value
	CC	CT	TT	
Age				
<40	10	18	6	0.514
≥40	48	52	21	
Gender				
Male	36	34	19	0.100
Female	22	36	8	
CEA				
<5 µg/L	37	43	20	0.208
≥5 µg/L	15	24	4	
Primary tumor extension				
T1+T2	9	9	3	0.807
T3+T4	40	54	19	
Lymph node status				
N0	22	25	8	0.794
N1+N2+N3	25	33	13	
Metastasis				
NO	43	58	20	0.947
YES	6	7	3	
Clinical stage				
I+II	17	37	6	0.022
III+IV	34	27	11	

Table 4. Association between the genotype frequencies of -842G>C and clinicopathological characteristics of colorectal carcinoma patients

Parameters	-842G>C		P value
	GG	GC	
Age			
<40	34	1	0.193
≥40	106	14	
Gender			
Male	82	7	0.376
Female	58	8	
CEA			
<5 µg/L	90	10	0.771*
≥5 µg/L	38	5	
Primary tumor extension			
T1+T2	18	3	0.460*
T3+T4	102	11	
Lymph node status			
N0	46	9	0.099
N1+N2+N3	66	5	
Metastasis			
NO	108	13	1.000*
YES	15	1	
Clinical stage			
I+II	54	6	0.836
III+IV	64	8	

*Two-sided Fisher's exact test was used.

cant associations with parameters including age, gender, serum carcinoembryonic antigen (CEA), primary tumor extension, lymph node status, and metastasis in patients. Interestingly, -667C>T SNP genotype showed a significant association with clinical stage ($P = 0.022$). Compared to the -667CC genotype, the -667CT genotype was lower in the advance stage (rate in the advance stage, -667CC: 34/51, -667CT: 27/64). The -842G>C genotype was marginally associated with lymph node status ($P = 0.099$).

Discussion

Previous studies reported the association between PIN1 polymorphism and cancer risk [12-17], such as breast cancer [12], squamous cell carcinoma of the head and neck [14], nasopharyngeal carcinoma [17] and lung cancer [15]. Our study investigated the association between PIN1 polymorphism and colorectal carcinoma risk. Our data indicated that the frequencies of -667C>T and -842G>C genotypes

and alleles were not significantly different between the colorectal cancer patients and controls. In the study of breast cancer [13], squamous cell carcinoma of the head and neck [12] and lung cancer [15], -842GC heterozygote had a significantly decreased cancer risk comparing to -842GG homozygote. Yet, no significant difference on the frequency of -842G>C genotype was observed in a study of breast cancer [16] and a study of hepatocellular carcinoma [12]. Furthermore, there was no evidence that -667C>T polymorphism was significantly associated with cancer risk in the study of breast cancer [13, 16], squamous cell carcinoma of the head and neck [14] and lung cancer [15]. However, decreased risk of cancer was found to be associated with the -842C variant allele in the study of breast cancer [13] and the study of squamous cell carcinoma of the head and neck [14]. The SNP -667T allele increased in the patients group of hepatocellular carcinoma with HBV and HCV co-infection [12].

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Although the sample size is small, our results suggest that -842G>C and -667C>T polymorphic variants might not have an influence on colorectal cancer risk.

It was interesting that -667C>T genotype showed significant association with clinical stage and -842G>C genotype was marginally associated with lymph node status in colorectal cancer. These data suggested that the two genotypes might be involved in the progression of colorectal cancer, but not in the initiation of cancer. Tumor stage and lymph node status were predictors of survival in colorectal carcinoma patients [25]. We infer that PIN1 genotype might be a predictor of survival in colorectal carcinoma patients. The probable reason for why the previous studies did not show an association between PIN1 genotype and clinicopathological parameter was that these associations were not examined in most of the studies [12-17].

In conclusion, our study suggest that the polymorphic variants of -842G>C and -667C>T in PIN1 promoter were not significantly associated with the risk of colorectal carcinoma. However, -667C>T genotype was associated with clinical stage and -842G>C genotype was marginally associated with lymph node status in colorectal cancer. These data suggested that the two genotypes might be involved in the progression of colorectal cancer and be a predictor of clinicopathological parameter of colorectal cancer. Further validation of our finding in larger population-based studies in diverse ethnic groups is needed.

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Disclosure of conflict of interest

None.

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