Int J Clin Exp Pathol 2017;10(1):645-650
www.ijcep.com /ISSN:1936-2625/IJCEP0041099

Original Article

G801A polymorphism within SDF-1 predicts prognosis of colorectal carcinoma in a Chinese population

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Received October 2, 2016; Accepted October 20, 2016; Epub January 1, 2017; Published January 15, 2017

Abstract: The purpose of current study was to evaluate the association between SDF-1 G801A polymorphism (rs1801157) and the risk of colorectal carcinoma (CRC) in a Chinese population. A total of 452 CRC patients and 530 non-cancer controls were included in the case-control study recruited in hospital. SDF-1 G801A polymorphism was identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Logistic regression was used to analyze the effect of SDF-1 G801A polymorphism on CRC risk. *SDF-1* G801A polymorphism was correlated with CRC risk. Compared with individuals carrying GG genotypes, those subjects carrying AA genotypes had a markedly increased risk of colorectal cancer (OR: 1.89, 95% CI: 1.18-3.02, *P*=0.01). Similar results were also betrayed in recessive model and additive model. Furthermore, in stratification analysis based on tumor stage, significant associations were found in tumor III+IV stage (AA versus GG, OR: 1.98, 95% CI: 1.18-3.33, *P*=0.01; GA+AA versus GG, OR: 1.35, 95% CI: 1.01-1.80, *P*=0.04; AA versus GG+GA, OR: 1.88, 95% CI: 1.15-3.09, *P*=0.02; A allele versus G allele, OR: 1.37, 95% CI: 1.09-1.71, *P*=0.01). Plasma ELISA demonstrated that the polymorphism increased plasma *SDF-1* expression in patients with higher stage CRC. Our findings suggested that SDF-1 G801A polymorphism might be a promising biomarker for CRC prognosis. However, larger population studies are required to validate our results.

Keywords: SDF-1, polymorphism, CRC, risk, tumor stage, prognosis

Introduction

Colorectal carcinoma (CRC) is one of the most common digestive cancers and claims the fourth-leading cancer death globally. Recently, the incidence rate of CRC was seen increasing in several countries within Eastern Asia, such as main land China [1]. Although the pathogenesis of CRC is still not completely elucidated, epidemiological studies have suggested that besides dietary intake, genetic factors also play a pivotal role in the pathogenesis of CRC [2, 3]. Thus, identification of new functional genetic variations within oncogenes or tumor suppressor genes would help to facilitate comprehension of colorectal carcinogenesis.

Chemokines are 8 to 12 kDa peptides, its function was involved in cellular activation, progenitor cells differentiation, and membrane trafficking. Stromal cell-derived factor-1 (SDF-1) is a homeostatic chemokine and plays an important role in modulation of hematopoietic cell trafficking and secondary lymphoid node construction. SDF-1 has been reported to be rich in lymph nodes, hepatocytes, pulmonary tissue and bone marrow, but poorly expressed in the epithelium of small intestine, nephron, skin, cerebrum and skeletal muscle, which facilitate locations confirmation of cancer metastasis [4]. SDF-1 can bind its cognate receptors CXCR4 and CXCR7 and affects several common signaling transduction pathways relevant to cellular survival, hyperplasia and cell locomotion [5-9]. The gene encoding SDF-1 protein is sited on chromosome 10q11.1 and has a regulatory polymorphism identified within the 3'UTR (G801A, rs1801157). Previous studies showed that *SDF-1* G801A polymorphism had an influence on the susceptibility of cancer, such as

Table 1. The main characteristics of the subjects included in the study

Characteristics	Cases N (%)	Controls N (%)	*P*-value
Total	452	530	
Age (Mean ± SD)	59.4 ± 9.7	58.6 ± 10.1	0.21
Gender			
Male	325 (71.9)	399	
Female	127 (28.1)	131	0.23
Tumor site			
Colon	208 (46.0)	-	
Rectum			
Tumor stage	244 (54.0)	-	
I	40 (8.8)	-	
II	131 (29.0)	-	
III	168 (37.2)	-	
IV	113 (25.0)	-	

breast cancer, pancreatic cancer, laryngeal cancer and prostate cancer [10-13]. However, the relationship of SDF-1 G801A polymorphism with CRC risk was not reported in Chinese Han population. Therefore, a case-control study based on hospital was performed to assess whether this association was positive or not.

Materials and methods

Enrolled subjects

A total of 452 CRC patients and 530 cancer-free controls were enrolled from The Affiliated Hospital of Xuzhou Medical University from July 2013 to December 2015. All recruited participants were non-related Chinese Han population. CRC patients were diagnosed through colonoscopy and CT imaging, exploration of interoperation and final pathological examination. Our enrolled controls matched the CRC cases in sex and age. Related data, such as age at diagnosis, gender, tumor site and tumor stage, were gathered from CRC patients' in-hospital records and pathological reports. We collected informed agreement from every subject. The current investigation was authorized by the ethical committee of the local hospital.

Enzyme-linked immunosorbent assay

Peripheral vein blood was sampled before surgical operation and isolated by centrifugation for 30 minutes. Plasma was collected and stored at -80°C for enzyme-linked immunosorbent assay (ELISA). All plasma samples from the patients and control subjects were stored at -80°C for enzyme-linked immunosorbent assay (ELISA) with ELISA kit (R&D Systems) according to the instructions of the manufacturer. The plasma SDF-1 protein concentration was expressed as picograms per millilitre (pg/ml).

DNA extraction and genotyping

Human genomic DNA of peripheral vein blood samples were from isolation by genomic DNA extraction kit (Qiagen). Amplification, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method assay and allelic discrimination was applied with the previously reported protocol [10]. In order to validate the genotyping data, about 10% samples of the CRC cases and cancer-free controls were gathered randomly and tested in replication by two independent researchers.

Statistical analysis

All statistical analyses were carried out with the statistical software package SPSS 18.0. Differences in SDF-1 plasma level of CRC cases and healthy controls were compared with the Mann-Whitney U test. χ^2 tests and Student's *t*-tests were adopted to evaluate differences in the distributions of gender and age, respectively. The Hardy-Weinberg equilibrium (HWE) in controls was tested with a goodness-of-fit χ^2 test. Unconditional logistic regression analysis was applied to measure the odds ratios (ORs) and 95% confidence intervals (CIs) after adjusted by for age and sex status. $P<0.05$ were taken as statistically significant in the current study.

Results

Characteristics of enrolled subjects

The main characteristics of the enrolled subjects enrolled in the study are collected in **Table 1**. There was no marked differences between cases and controls in frequency distributions of age or gender ($P=0.21$ and 0.23, respectively), indicating that the case-control enrolled subjects had adequate matching in age and sex status.

Correlation of SDF-1 G801A polymorphism with CRC risk

The genotypic and allelic frequencies of the *SDF-1* G801A polymorphism in CRC cases and

Table 2. Association between SDF-1 G801A polymorphism and CRC risk

Comparison	Cases N (%)	Controls N (%)	OR (95% CI)	*P*-value	P-trend
Codominant model					
GG	231 (51.1)	303 (57.2)	1.00 (Reference)		
GA	172 (38.1)	192 (36.2)	1.17 (0.89-1.53)	0.28	0.01
AA	49 (10.8)	35 (6.6)	1.89 (1.18-3.02)	0.01	
P_{HWE}		0.54			
Dominant model					
GG	231 (51.1)	303 (57.2)	1.00 (Reference)		
GA+AA	221 (48.9)	227 (42.8)	1.27 (0.99-1.64)	0.06	
Recessive model					
GG+GA	403 (89.2)	495 (93.4)	1.00 (Reference)		
AA	49 (10.8)	35 (6.6)	1.72 (1.09-2.70)	0.02	
Additive model					
G allele	634 (70.1)	798 (75.3)	1.00 (Reference)		
A allele	270 (29.9)	262 (24.7)	1.30 (1.06-1.58)	0.01	

Table 3. Stratified analysis on the association between *SDF-1* G801A polymorphism and CRC risk based on tumor stage

Comparison	Cases N (%)	Controls N (%)	OR (95% CI)	*P*-value	*P*-trend
	I+II	Controls			
Codominant model					
GG	91 (53.2)	303 (57.2)	1.00 (Reference)		0.25
GA	64 (37.4)	192 (36.2)	1.09 (0.76-1.58)	0.64	
AA	16 (9.4)	35 (6.6)	1.52 (0.80-2.87)	0.20	
Dominant model					
GG	91 (53.2)	303 (57.2)	1.00 (Reference)		
GA+AA	80 (46.8)	227 (42.8)	1.17 (0.82-1.67)	0.37	
Recessive model					
GG+GA	155 (90.6)	495 (93.4)	1.00 (Reference)		
AA	16 (9.4)	35 (6.6)	1.45 (0.80-2.68)	0.24	
Additive model					
G allele	246 (71.9)	798 (75.3)	1.00 (Reference)		
A allele	96 (28.1)	262 (24.7)	1.19 (0.90-1.56)	0.22	
	III+IV	Controls			
Codominant model					
GG	140 (49.8)	303 (57.2)	1.00 (Reference)		0.01
GA	108 (38.4)	192 (36.2)	1.22 (0.90-1.67)	0.20	
AA	33 (11.7)	35 (6.6)	1.98 (1.18-3.33)	0.01	
Dominant model					
GG	140 (49.8)	303 (57.2)	1.00 (Reference)		
GA+AA	141 (50.2)	227 (42.8)	1.35 (1.01-1.80)	0.04	
Recessive model					
GG+GA	248 (88.3)	495 (93.4)	1.00 (Reference)		
AA	33 (11.7)	35 (6.6)	1.88 (1.15-3.09)	0.02	
Additive model					
G allele	388 (69.0)	798 (75.3)	1.00 (Reference)		
A allele	174 (31.0)	262 (24.7)	1.37 (1.09-1.71)	0.01	

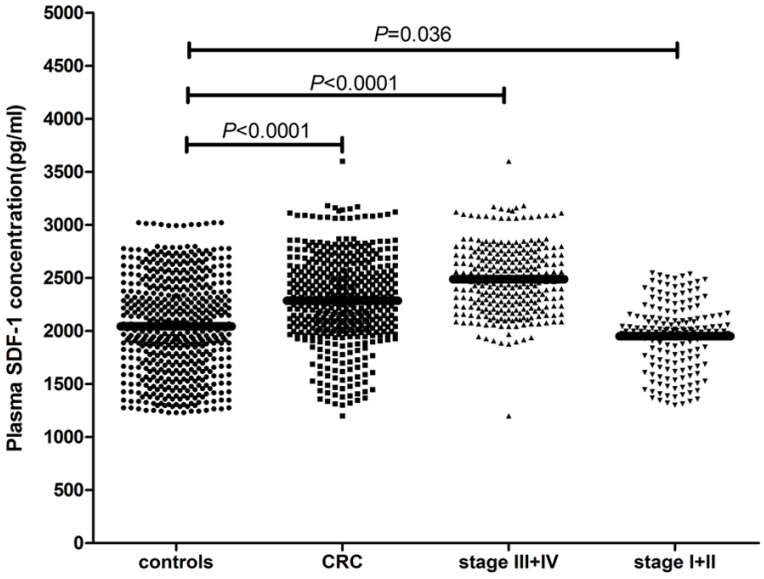

Figure 1. Plasma SDF-1 in CRC cases and healthy controls. CRC patients have significantly higher concentrations when compared with controls. Medians are shown by horizontal bars.

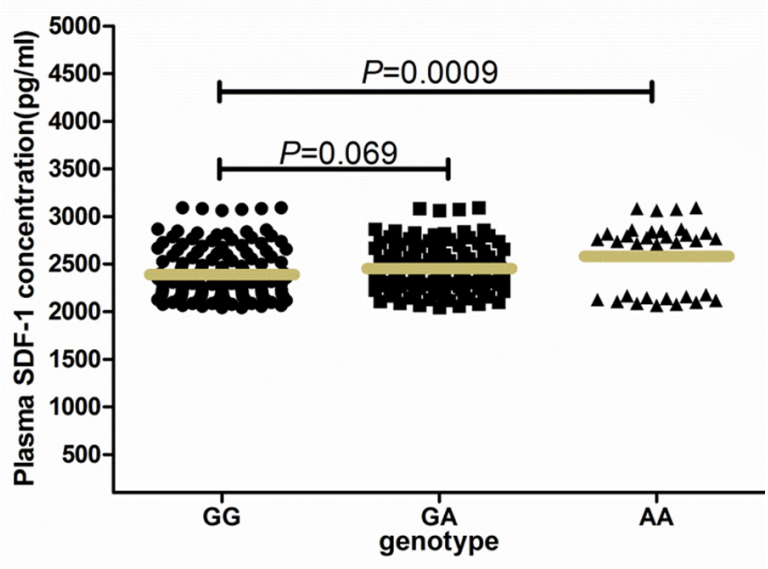

Figure 2. Plasma SDF-1 in CRC cases with higher stage CRC. Medians are shown by horizontal bars.

controls are presented in **Table 2**. The genotype frequency distribution in controls was in accordance with HWE (*P*=0.54). There was a remarkable difference in the genotype frequency between cases and controls (*P*trend=0.01). Cases with AA genotype was correlated with a markedly increased risk of CRC, compared with the genotype GG in codominant model (OR: 1.89, 95% CI: 1.18-3.02, *P*=0.01). When compared with the genotype GG and GA in recessive model, the genotype GG was also associated with a significantly increased risk of CRC (OR: 1.72, 95% CI: 1.09-2.70, *P*=0.02). In addition, additive model showed that the A allele is more frequent in patients compared to controls (OR: 1.30, 95% CI: 1.06-1.58, *P*=0.01).

Stratified analysis on the correlation of SDF-1 G801A polymorphism with CRC risk based on tumor stage

The correlation of *SDF-1* G801A polymorphism with CRC risk was further evaluated by stratification analysis based on tumor stage (Lower stage I+II, Higher stage III +IV) in four genetic models. As was shown in **Table 3**, Significant associations were only observed in tumor III + IV stage (AA versus GG, OR: 1.98, 95% CI: 1.18-3.33, *P*=0.01; GA+AA versus GG, OR: 1.35, 95% CI: 1.01-1.80, *P*=0.04; AA versus GG+GA, OR: 1.88, 95% CI: 1.15-3.09, *P*=0.02; A versus G, OR: 1.37, 95% CI: 1.09-1.71, *P*=0.01).

The polymorphism increased plasma SDF-1 expression in patients with higher stage CRC

We found the polymorphism could predict prognosis by influence tumor staging, then we hypothesized that the SDF-1 up regulated to enhance the ability of tumor cells to migration and metastasis. Thus, we examined the possible difference in plasma. As shown in **Figure 1**, plasma SDF-1 was obviously higher (*P*<0.0001) in CRC cases [median 2285 (range 1200-3181) pg/ml] than in controls [median 2043 (range 1231-3021) pg/ml]. When divided into two subgroups by different tumor stage, SDF-1plasma concentration of

higher stage tumor cases increased significantly than controls (median 2488 pg/ml, $P<0.0001$), while lower stage cases showed statistically significant increase ($P=0.036$). As was betrayed in **Figure 2**, further comparison on different genotype groups in higher stage tumor indicated that the polymorphism increased *SDF-1* expression in patients with higher stage CRC.

Discussion

Colorectal carcinogenesis investigation has leaped to genome-wide association study (GWAS) era, which strengthens the conception that genetic variations may provide new strategy to CRC therapy and risk prediction. However, as a commercial platform, not all polymorphisms were recorded in the study. New functional gene variations identification was of great help to play as a beneficial supplement to GWAS investigations. Recently, the important role of chemokines during tumor development is increasingly gaining interest. Our target gene *SDF-1* is a part of C-X-C subfamily of chemokine and plays a crucial role in not only human common embryonic growth and cell locomotion and tumor metastasis [14, 15]. *SDF-1* G801A polymorphism is at locus 801 of the 3'UTR and has been reported to be involved in risk of multiple cancers [10-13].

In the case-control study of 452 CRC cases and 530 controls conducted in hospital and in Chinese Han population, our investigation found that the *SDF-1* G801A polymorphism was significantly correlated with CRC risk in Chinese Han population. Compared with individuals carrying GG genotypes, those subjects with AA genotype had a markedly increased risk of CRC. We noticed that in codominant model of **Table 2**, GA vs. GG betrayed no marked difference, its confidential interval was (0.89-1.53), dominant model data followed the same trend, but recessive model and additive model demonstrated marked difference ($P=0.02$ for recessive model and $P=0.01$ for additive model). This phenomenon may be due to A allele with a recessive effect, dose-dependent effect was more obvious in homozygote with double A allele. In addition, stratification analysis showed a significant association between higher tumor stage cases and controls, demonstrating that *SDF-1* may be involved in the occurrence of advanced CRC. Furthermore, higher stage of CRC means poor prognosis, thus, we could easily conclude that the polymorphism could influence CRC prognosis.

It was interesting that several investigations found that no salient association between *SDF-1* G801A polymorphism and CRC [16-18], while the similar investigation in Taiwan [19] and latest meta-analysis [20] may indicate that the potential correlation is positive in Chinese Han population. However, the power limited sample size and lack of other center replication validation was the main weakness the investigation conducted in Taiwan. Our current investigation demonstrated that the *SDF-1* G801A polymorphism could influence CRC risk in Chinese Han population, furthermore, the polymorphism could indicate CRC prognosis for its aberrant distribution between low and high stage CRC patients. Previous *in vitro* report demonstrated that the SDF-1 could modulate cell migration and tumor growth [21], which facilitated our present findings from stratified analysis and plasma ELISA.

As far as we knew, this was the first molecular epidemiological report to investigate the correlation between *SDF-1* G801A polymorphism and CRC prognosis in a Chinese Han population. However, a major limitation should be addressed. Namely, functional experiments investigating the detailed molecular mechanisms under this association were not carried out.

In conclusion, our findings suggest that *SDF-1* G801A polymorphism may be a potential marker for CRC prognosis. However, large sample investigations from other centers and other populations are needed to confirm our current findings.

Acknowledgements

The current study was funded by Natural Science Foundation of China (No.81502428), Natural Science Foundation of Jiangsu Province (No.BK20140222, No.15KJB310024 and No.BK20150220), and scientific research fund for talents of Xuzhou Medical University (No. D2015018 and No.D2015019).

Disclosure of conflict of interest

None.

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