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ORIGINAL RESEARCH

IL-27 rs153109 polymorphism increases the risk of colorectal cancer in Chinese Han population

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Abstract: IL-27, a new member of the IL-12 family, has been found to have antitumor effects in colorectal cancer (CRC); therefore, polymorphisms of this protein may modulate CRC carcinogenesis. So, we studied the association of single nucleotide polymorphisms of the *IL-27* gene with the risk of CRC occurrence using a case–control using 600 CRC patients and matched healthy controls. The IL-27 rs153109 polymorphism was analyzed with polymerase chain reaction-restriction fragment length polymorphism and DNA sequencing methods. Data indicate that GG, GA, and combined A-variant genotypes (GG + GA) conferred significantly greater risk of CRC (P=0.034, 0.002, and 0.001, respectively), and that G alleles were associated with higher susceptibility to CRC (P=0.001). However, no correlation was found between the IL-27 rs153109 polymorphism and particular clinical features. In conclusion, our data demonstrated a clear association of IL-27 rs153109 polymorphism and the risk of CRC development.

Keywords: IL-27, polymorphism, CRC

Introduction

Colorectal cancer (CRC) contributes greatly to cancer deaths worldwide.[1] Although the etiology of CRC is still unknown, researchers accept that CRC pathogenesis is intricate and that dietary, lifestyle, and genetic changes contribute to its incidence.[2] Growing evidence indicates that genetic polymorphisms are key to CRC development[3–5] and that several tumor cell cytokine genes can induce systemic immunity against CRC. Cytokines may activate immunocompetent cells and stimulate a cascade of immune processes to elicit antitumor activity.[6] IL-27, a novel member of the IL-12 family, was shown to be one such cytokine.[7]

IL-27 is a heterodimeric cytokine comprising a p40-related protein, EBI3, and a newly discovered IL-12 p35-related protein, p28. IL-27 is produced early after activation by antigen presenting cells, and has two distinct immune functions: an initiator of the TH1-type immune response to promote naïve T-cell proliferation and a potent inducer of IFN-γ production, participating in host defense against intracellular infection. IL-27 has not only antitumor immune activity via cytotoxic T lymphocytes or natural killer cells, but also has anti-angiogenic properties, a feature first depicted in a murine tumor model of colon carcinoma C26.[8–11] C26 cells transduced with single-chain IL-27 cDNA were modified to secrete IL-27 (C26-IL-27) and had minimal tumor growth in vivo. All mice inoculated with C26 cells survived and had complete tumor remission, indicating that IL-27 can potently induce antitumor activity and protective immunity.[10]

Recently, several studies suggest the role of IL-27 polymorphisms in cancer risk such as esophageal cancer,[12] ovarian cancer,[13] nasopharyngeal carcinoma,[14]

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hepatocellular carcinoma,[15] and glioma.[16] Most importantly, associations of the IL-27 rs153109 polymorphism and risk for several diseases such as asthma, COPD, and inflammatory bowel disease have been identified.[17–19] Little is known about the role of the IL-27 rs153109 polymorphism in the risk of CRC development. Thus, we investigated this association.

Materials and methods

Subjects

Six hundred CRC patients and 600 age- and sex-matched cancer-free controls were recruited in this study. Samples were collected prior to any anticancer treatment between January 2009 and April 2014 in the Department of Surgical Oncology of the First Hospital of Wenzhou Medical University. Diagnosis was established by histopathological examination of the biopsy. CRC patients were staged according to the American Joint Committee on Cancer/International Union against Cancer Tumor-Node-Metastasis (TNM) staging system. Healthy controls were recruited from routine examination from the Department of Health, First Hospital of Wenzhou Medical University. Stratified analyses were performed for combined genotypes (GG + GA versus AA) in CRC patients according to sex, age at admission, differentiation status, tumor growth pattern, lymph node metastasis, and TNM pathological stage. This study was approved by the hospital-based ethics committee and informed consent was obtained from all subjects. Subject data are shown in Table 1.

PBMCs preparation and DNA extraction

Peripheral blood samples were collected in vacuum tubes with 5% ethylenediaminetetraacetic acid (EDTA). PBMCs were isolated from blood samples by density gradient centrifugation using lymphocyte separation medium. Genomic DNA was extracted using a DNA Purification Kit (Tiangen Biotech, Beijing, People's Republic of China) according to kit instructions.

IL-27 genotyping

Genotyping of the IL-27 rs153109 polymorphism was performed by polymerase chain reaction (PCR)-restriction fragment length polymorphism. The forward primer 5-CTGATCCTGACCTCACT CAACGC-3 and the reverse primer 5-CTGACTGG GACTGGGACTCAGC-3 were used for PCR. A 20 μL PCR mixture contained 50–150 ng of genomic DNA and 10 μL 2× PCR mix (Tiangen Biotech). For PCR amplification, an initial denaturation at 94°C for 5 minutes was followed by 36 cycles at 94°C for 30 seconds, at 64°C for 30 seconds, at 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. BstU I (New England Biolabs Inc., Beverly, MA, USA) was used to detect the A-G transition. PCR products are shown in Figure 1. To confirm genotyping results, PCR-amplified DNA samples were examined by DNA sequence (Figure 2), and the results were 100% concordant.

Statistical analysis

Statistical calculations were performed with SPSS statistical software (version 18.0; SPSS Inc., Chicago, IL, USA).

Table 1 The clinical characteristics of the subjects including CRC and healthy control

Variable	Colorectal cancer N=600 (%)	Healthy control N=600 (%)	*P*-value
Age			
<50 years	186 (31.0)	204 (34.0)	0.27
≥50 years	414 (69.0)	396 (66.0)	
Sex			
Male	350 (58.3)	326 (54.3)	0.16
Female	250 (41.7)	274 (45.7)	
Alcohol drinking			
Yes	382 (63.7)	357 (59.5)	0.14
No	218 (36.3)	243 (40.5)	
Smoking			
Yes	338 (56.3)	327 (54.5)	0.52
No	262 (43.7)	273 (45.5)	
Family history of cancer			
Yes	38 (6.3)	42 (7.0)	0.64
No	562 (93.7)	558 (93.0)	
Family history of colorectal adenoma or polyp			
Yes	183 (30.5)	198 (33.0)	0.35
No	417 (69.5)	402 (67.0)	

Abbreviation: CRC, colorectal cancer.

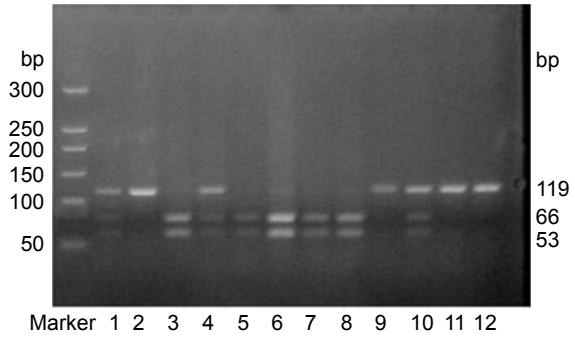

Figure 1 PCR products of IL-27 of the CRC patients (lane 1–6) and control (lane 7–12).

Genotype frequencies were analyzed with a chi-squared test. Differences were considered significant at $P<0.05$.

Results

Clinical characteristics of patients

CRC patients and healthy controls (N=600, each) were recruited for the present study. CRC patients (41.7% women) were diagnosed as TNM stage I plus II (38.0%) and stage III plus IV (62.0%).

IL-27 rs153109 polymorphism results

The genotype and allele frequency of the IL-27 rs153109 polymorphism was detected by PCR-restriction fragment length polymorphism and the GG, GA, and combined G variant genotypes (GG + GA) conferred significantly greater risk of CRC (P=0.034, 0.002, and 0.001, respectively). Moreover, G alleles were associated with higher susceptibility to CRC (P=0.001) (Table 2). Stratified analyses for combined genotypes (GG + GA versus AA) in CRC patients according to sex, age at admission, differentiation status, tumor growth pattern, lymph node metastasis, and TNM pathological stage, were performed to correlate clinical stages with genotypes, and no significant differences were observed (Table 3).

Discussion

CRC is one of the most common cancers and its incidence and mortality is increasing in the People's Republic of China annually.[20] Genetic screening for specific cancer risks is useful but more work is needed to identify susceptibility genes involved in cancer.[21] Single nucleotide polymorphisms are the most common sources of human genetic variation, and these may contribute to an individual's susceptibility to CRC.[3–5]

IL-27, a newly discovered heterodimeric cytokine of the IL-12 family, displays not only anti-proliferative and anti-angiogenic effects by directly acting on cancer cells,

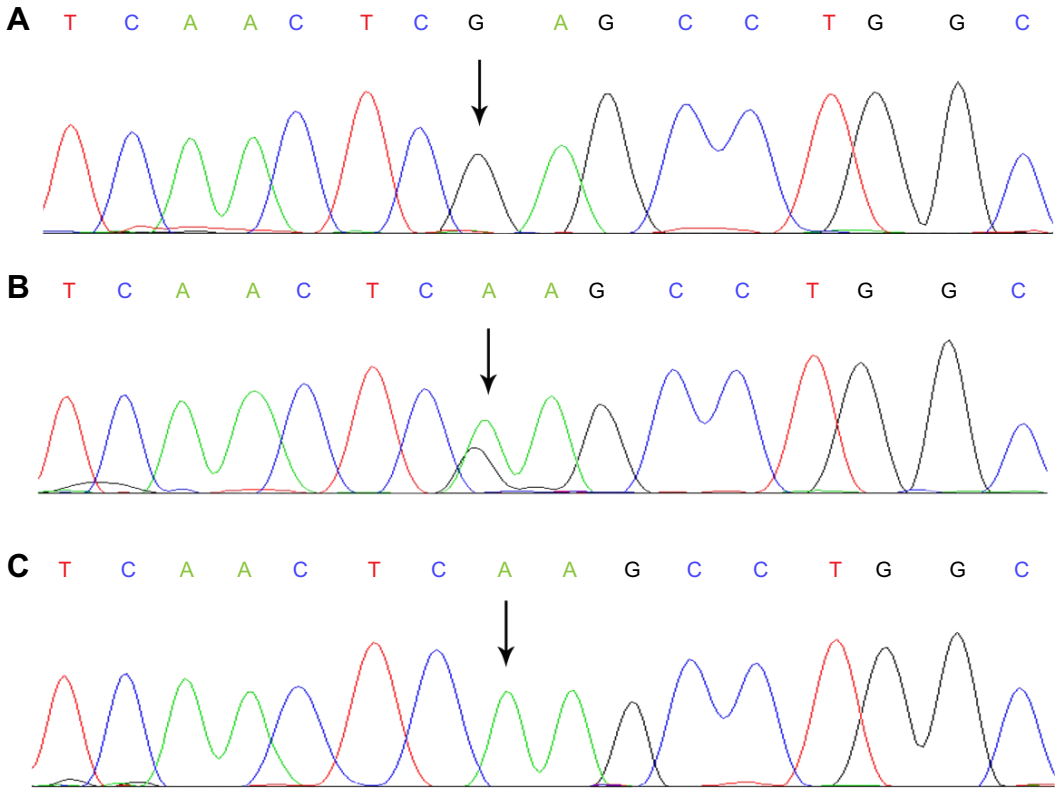

Figure 2 Sequencing map of genotype for IL-27 rs153109 polymorphism.
Notes: The arrow in (**A–C**) show GG, AG and AA genotypes, respectively.

Table 2 IL-27 rs153109 polymorphism in CRC patients and healthy controls

	CRC patient N=600 (%)	Control N=600 (%)	OR (95% CI)	*P*-value
Genotype frequency				
GG	140 (23.3)	127 (21.2)	1.382 (1.024–1.864)	0.034
GA	243 (40.5)	201 (33.5)	1.515 (1.170–1.962)	0.002
AA	217 (36.2)	272 (45.3)	1.00 (reference)	
Allele frequency				
Allele G	523 (43.6)	455 (37.9)	1.265 (1.074–1.489)	0.001
Allele A	677 (56.4)	745 (62.1)	1.00 (reference)	
Genotype frequency				
GG or GA	383 (63.8)	328 (54.7)	1.797 (1.344–2.404)	0.001
AA	217 (36.2)	272 (45.3)	1.00 (reference)	
Genotype frequency				
GG	140 (23.3)	127 (21.2)	1.134 (0.863–1.488)	0.367
AA or GA	460 (76.7)	473 (78.8)	1.00 (reference)	

Abbreviations: CRC, colorectal cancer; OR, odds ratio; CI, confidence interval.

but also has indirect antitumor effects driven by immune stimulatory activity in many cancers.[6–11] Recently published data confirmed that the IL-27 rs153109 polymorphism was associated with cancer risk, but how this contributes to CRC is unknown.[12–16] Thus, we investigated this association.

We found that GG, GA, and combined A variant genotypes (GG + GA) conferred significantly greater risk of CRC and that G alleles were associated with higher susceptibility to CRC. Stratified analyses for combined genotypes (GG + GA versus AA) did not reveal differences within a CRC patient subgroup according to sex, age at admission, differentiation status, tumor growth pattern, lymph node metastasis, and TNM pathological stage. So, we did link the IL-27 rs153109 polymorphism and CRC risk but larger multicenter studies are needed to confirm our data and assess the validity of this single nucleotide polymorphism as a cancer screening marker.

Acknowledgment

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Table 3 Association between IL-27 rs153109 polymorphism and clinicopathological characteristic of CRC patients

Characteristic	Cases N=600 (%)	Genotype no		OR (95% CI)	*P*-value
		N=383 GA + GG (%)	N=217 AA (%)		
Age					
<50 years	186 (31.0)	114 (29.8)	72 (33.2)	0.854 (0.597–1.220)	0.385
≥50 years	414 (69.0)	269 (70.2)	145 (66.8)	1.00 (reference)	
Sex					
Male	350 (58.3)	228 (59.5)	122 (56.2)	1.145 (0.818–1.605)	0.430
Female	250 (41.7)	155 (40.5)	95 (43.8)	1.00 (reference)	
Growth pattern					
Ulcerative	226 (37.7)	139 (36.3)	87 (40.1)	0.851 (0.605–1.199)	0.356
Protruding	374 (62.3)	244 (63.7)	130 (59.9)	1.00 (reference)	
Differentiation					
Good	274 (45.7)	172 (44.9)	102 (47.0)	0.919 (0.658–1.284)	0.620
Poor + moderate	326 (54.3)	211 (55.1)	115 (53.0)	1.00 (reference)	
Lymph node metastasis					
Yes	348 (58.0)	226 (59.0)	122 (56.2)	1.121 (0.800–1.570)	0.506
No	252 (42.0)	157 (40.9)	95 (43.8)	1.00 (reference)	
TNM pathological stage					
I and II	228 (38.0)	140 (36.6)	88 (40.6)	0.845 (0.600–1.189)	0.332
III and IV	372 (62.0)	243 (63.4)	129 (59.4)	1.00 (reference)	

Abbreviations: CRC, colorectal cancer; OR, odds ratio; CI, confidence interval; TNM, Tumor-Node-Metastasis.

Disclosure

The authors report no conflicts of interest in this work.

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