Immunology Letters 188 (2017) 59–63

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Association of interleukin-22 polymorphisms with the colon cancer: A case-control study

Lin Lin, Weili Xu, Guojian Zhang, Pengtao Ren, Jing Zhao, Qinghui Yan*

Department of Colorectal Anal Surgery, Second Hospital of Hebei Medical University, Shijiazhuang 050000, China

ARTICLE INFO

Keywords:
Colon cancer
Interleukin-22
Gene polymorphism
Case-control study

ABSTRACT

Introduction: Interleukin-22 (*IL-22*), an IL-10 family cytokine produced by T cells and innate lymphoid cells, is implicated in inflammation and tumorigenesis. In this study, we aimed to investigate the association of *IL-22* polymorphisms with the colon cancer in a Chinese population.

Materials and methods: Five hundred forty colon cancer cases and 540 healthy controls were recruited in the case-control study. The fluorogenic 5' exonuclease assays were used for genotype analysis of three common polymorphisms (−429C/T, +1046 T/A and +1995 A/C) of the *IL-22* gene.

Results: Colon cancer cases had a significantly higher frequency of *IL-22*–429 TT genotype [odds ratio (OR) = 1.69, 95% confidence interval (CI) = 1.24, 2.30; $P = 0.001$] and −429 T allele (OR = 1.35, 95% CI = 1.14, 1.60; $P = 0.001$) than healthy controls. The findings are still emphatic by the Bonferroni correction ($P < 0.017$). When stratifying by the differentiation of colon cancer, we found that colon cancer cases with poor differentiation had a significantly higher frequency of *IL-22*–429 TT genotype (OR = 1.45, 95% CI = 1.02, 2.07; $P = 0.04$). When stratifying by the tumor location, tumor size, growth pattern and TNM stage of colon cancer, we found no statistical association. The *IL-22* +1046 T/A and *IL-22* +1995 A/C gene polymorphisms were not associated with colon cancer.

Conclusion: Our findings suggested that the *IL-22* −429C/T gene polymorphisms might be associated with colon cancer.

1. Introduction

In 2015, the American Cancer Society approximated 93,090 new cases of colon cancer would be diagnosed, resulting in death for 49,700 of those people due to the disease [1]. The incidence and mortality of colorectal cancer have substantially increased in urban rather than rural areas over the past several decades in China [2]. Risk factors for colorectal cancer include lifestyle, older age, diet, smoking, alcohol, lack of physical activity, family history of colon cancer and colon polyps, presence of colon polyps, race, exposure to radiation, and inherited genetic disorders [3–7]. It was found that activation of innate immunity and inflammation resulted in the production of cytokines that could either stimulate or inhibit tumor growth and progression [8]. Inflammatory cytokines IL-17 and TNF-α up-regulate PD-L1 expression in colon cancer cells through activation of NF-κB signaling, in the presence of AKT activity [9].

Interleukin-22 (*IL-22*) is a member of the *IL-10* family, a class of potent mediators of cellular inflammatory responses [10]. *IL-22* is produced by activated dendritic cell and T cells and initiates innate immune responses against bacterial pathogens especially in epithelial cells such as gut epithelial cells [11–13]. *IL-22* is also associated with tumorigenesis and tumor progression in cancers [14,15]. *IL-22* expression has been reported to play critical roles in many types of cancer, including skin cancer [14], pancreatic cancer [16], lung cancer [17], liver cancer [18], papillary thyroid cancer [19], gastric cancer and colorectal cancer [20–22]. Several single nucleotide polymorphisms (SNPs) have previously been identified in the *IL-22* gene [19,23–28].

There has been a population-based case-control study to evaluate the association of seven tagging SNPs within *IL-22* with the colon cancer, but the population is predominantly of European descent (> 94%) [29]. The aim of this study was to investigate the association of *IL-22* polymorphisms with the colon cancer in a Chinese population.

2. Materials and methods

2.1. Subjects

This was a prospective hospital-based case-control study. Between

* Corresponding author at: Department of Colorectal Anal Surgery, Second Hospital of Hebei Medical University, No.215, West Heping Road, Shijiazhuang 050000, China.
E-mail address: qinghuiyan@outlook.com (Q. Yan).

http://dx.doi.org/10.1016/j.imlet.2017.06.007
Received 5 March 2017; Received in revised form 12 June 2017; Accepted 12 June 2017
Available online 15 June 2017
0165-2478/

June 2012 and June 2015, five hundred forty colon cancer cases were recruited at the Department of Colorectal Anal Surgery in the Second Hospital of Hebei Medical University. A total of 540 age- and gender-matched cancer-free healthy individuals were recruited as the control group, who undergoing colonoscopy as preventive measure and who had a normal colonoscopy. The healthy controls were recruited from a pool of healthy volunteers who visited the hospital during the same period. All participants were Chinese from the same geographic region. Pathology grades were determined according to the criteria of World Health Organization (WHO). Tumor pathology stages were classified according to the tumor-node-metastasis (TNM) classification of the Union Internationale Contra le Cancer (UICC). The patient or their families/surrogates were interviewed. The Institutional Ethical Committee of the Second Hospital of Hebei Medical University approved all parts of the study, and informed consent according to the Declaration of Helsinki was obtained from all participants or their families/surrogates.

2.2. Genotyping analysis

For genetic analyses, genomic DNA was isolated from peripheral blood using Blood Kit (Macherey-Nagel, Germany). The fluorogenic 5' exonuclease assays (TaqMan) were used for genotype analysis of three common polymorphisms (-429C/T, +1046 T/A and +1995 A/C) of the *IL-22* gene. Table 1 gave the details of primer and probe sequences for 5'-exonuclease assays for *IL-22* polymorphisms. The polymerase chain reaction (PCR) was performed in a Primus 96 plus thermal cycler using a total volume of 5 µl containing 2.5 µl of Universal-MasterMix, 0.125 µl 40 x Assay-by- Design mix, 0.375 µl H_2O and 2 µl DNA. Reactions were overlaid with 15 µl of mineral oil. Cycling parameters were: 10 min at 94 °C for primary denaturation, followed by 40 cycles of 20 s at 92 °C and 1 min at 60 °C. Fluorescence was measured in a lambda Fluoro 320 Plus plate reader (MWG Biotech AG, Germany).

2.3. Statistical analysis

The existence of differences in genotypic frequencies between groups was assessed by means of Pearson x^2 test and calculating the odds ratio (OR) with the 95% confidence intervals (CI). Data are presented as percentages for categorical variables or as means ± standard deviation (SD). Differences between categorical variables were evaluated using Pearson x^2 test, while those between continuous variables were assessed by Student's *t* test. Stratification analysis was used to study subgroups by tumor location, tumor size, growth pattern, differentiation, and TNM stage. Statistical significance was set at $P < 0.05$. Statistical analyses were carried out using the STATA program, version 9.0 (StataCorp LP, College Station, TX, USA) for Windows®.

3. Results

3.1. Baseline characteristics of the participants

General characteristics of colon cancer cases and healthy controls were shown in Table 2. There were no statistically significant difference between cases and controls regarding sex ($P = 0.58$) or age ($P = 0.10$). Mean cases age was 59.7 years and 58.9 years for the controls. The tumor location, tumor size, growth pattern, differentiation and TNM stage of colon cancer were shown in Table 2.

Table 2
General characteristics of colon cancer cases and healthy controls.

Variables	Cases	Controls	*P* value
Number of subjects	540	540	
Sex (Men/Women)	287/253	278/262	0.58
Age (Years)	59.7 ± 8.1	58.9 ± 7.9	0.10
Tumor location			
Left colon	230(42.6)		
Right colon	310(57.4)		
Tumor size			
< 4 cm	318(58.9)		
> 4 cm	222(41.1)		
Growth pattern			
Ulcerative	291(53.9)		
Protruding	249(46.1)		
Differentiation			
Good	223(41.3)		
Moderate	178(33.0)		
Poor	139(25.7)		
TNM stage			
I	233(43.1)		
II	190(35.2)		
III	67(12.4)		
IV	50(9.3)		

Abbreviations: TNM, tumor-node-metastasis.

3.2. Hardy–Weinberg equilibrium

In both patients and controls, the observed genotype distributions of all investigated polymorphisms were in accordance with those predicted by the Hardy–Weinberg equilibrium (Table 3).

3.3. IL-22 −429C/T gene polymorphisms and colon cancer

Colon cancer cases had a significantly higher frequency of *IL-22*–429 TT genotype (OR = 1.69, 95% CI = 1.24, 2.30; $P = 0.001$) and −429 T allele (OR = 1.35, 95% CI = 1.14, 1.60; $P = 0.001$) than healthy controls (Table 3). The findings are still emphatic by the Bonferroni correction ($P < 0.017$). When stratifying by the differentiation of colon cancer, we found that colon cancer cases with poor differentiation had a significantly higher frequency of *IL-22*–429 TT genotype (OR = 1.45, 95% CI = 1.02, 2.07; $P = 0.04$) (Table 4). When stratifying by the tumor location, tumor size, growth pattern and TNM stage of colon cancer, we found no statistical association (Table 4).

3.4. IL-22 +1046 T/A gene polymorphisms and colon cancer

The *IL-22* +1046 T/A gene polymorphisms were not associated

Table 1
Primer and probe sequences for 5'-exonuclease assays for *IL-22* polymorphisms.

SNPs	rs2227485	rs1182844	rs1179246
Exchange	−429C/T	+1046T/A	+1995A/C
Forward Primer	AAAATGAGTCCGTGACCAAAATGC	CCACCTATGAGACTTCCCTATCAGT	GAAAAAGCCTTCCTGCCTAATGG
Reverse Primer	ACACAATTGTTTTGTCTTAGTAGAGTTCAGAT	CACTAAAGGAAAAGGAAAGCTGTGTTT	GGTGCTGCCTAAAGGTCAGA
Wildtype-Probe	FAM-CTCCTATAGTGACTGAGTAA-NFQ	VIC-AAACTTACTAGTAGGTATGACTCNFQ	VIC-TGAACAGAGTTATCTGCCTC−NFQ
Mutant-Probe	VIC-CTCCTATAGTGGCTGAGTAA-NFQ	FAM-CTTACTAGTAGGAATGACTC−NFQ	FAM-AACAGAGTTAGCTGCCTC−NFQ

Abbreviations: SNP, single nucleotide polymorphisms.