RESEARCH NOTE

Association between the IFN-γ and IL-1 genetic polymorphisms and colorectal cancer in the Chinese Han population

BAO-YING FEI[1]*, HUO-XIANG LV[1], YONG-WEI CHENG[1] and JIANG-MING YANG[1]

[1]*Department of Gastroenterology,* [2]*Department of Clinical laboratory, and* [3]*Department of Coloproctology, Zhejiang Province People's Hospital, Hangzhou 310014, Zhejiang Province, People's Republic of China*

[Fei B.-Y., Lv H.-X., Cheng Y.-W. and Yang J.-M. 2014 Association between the IFN-γ and IL-1 genetic polymorphisms and colorectal cancer in the Chinese Han population. *J. Genet.* **93**, 235–239]

Introduction

Colorectal cancer (CRC) is one of the major causes of mortality and morbidity, and is the third most common cancer in men and the second most common cancer in women worldwide (Sameer 2013). Over a 20 years period, the incidence of CRC has been increasing rapidly in China. CRC develops through a specific series of events, from the transformation of normal colonic epithelium to an adenomatous intermediate, and then ultimately adenocarcinoma (Pino and Chung 2010). Colitis-associated cancer (CAC) is the CRC subtype that is associated with inflammatory bowel disease (IBD) (Rubin *et al*. 2012). The molecular mechanism of CRC is still not fully understood. Increased expression of proinflammatory cytokines and inflammatory infiltration were found in colorectal cancer (Lee *et al*. 2012; Moossavi and Bishehsari 2012).

IFN-γ is one of the main products of Th1-specific proinflammatory cytokines and has an effect on host defense and immune regulation, such as antiviral, antimicrobal, and antitumour activities. The functional SNP at position +874 (T/A) is located at the 5′-end of a CA repeat at the first intron of the human IFN-γ gene. It has been reported that the IFN-γ+874T/A single nucleotide polymorphism (SNPs) correlates with IFN-γ expression (Pravica *et al*. 2000).

Interleukin-1 (IL-1) is involved in various physiological and patho-physiological processes, such as inflammation, tissue injury, cell proliferation and angiogenesis. The IL-1β SNP associates with increased *in vitro* expression of IL-1β. A variable 86-bp sequence repeats is located at the second intron of the IL-1 receptor antagonist (*IL-1RN*) gene. Rafiq *et al*. (2007) demonstrated that two IL-1RA SNPs were strongly associated with different IL-1RA levels *in vivo*. Polymorphisms in IL-1RN influence IL-1RA mRNA expression (Korthagen *et al*. 2012).

Some studies reported that cytokines polymorphisms affected the risk of colorectal cancer development (Azimzadeh *et al*. 2011; Walczak *et al*. 2011). The influence of genetic polymorphism on diseases varied in different ethnic groups. In the present study, we investigate the association between IFN-γ, IL-1 and IL-1RN polymorphisms, and the risk of colorectal caner in a Chinese population.

Patients and methods

Patients studied

Subjects were 162 patients with colorectal cancer (104 males and 58 females; mean age was 54.3 ± 11.6 years) and 178 unrelated healthy individuals (115 males and 63 females; mean age was 51.6 ± 11.2 years) from a Han ethnic population, Zhejiang, China. The diagnosis of colorectal cancer was confirmed by histopathology. The study was approved by the Institutional Ethical Committee of Zhejiang Provincial People's Hospital. After informed consent blood samples were obtained from all patients.

Genomic DNA extraction

Genomic DNA was extracted from venous blood by the salting out procedure with minor modifications (Miller *et al*. 1988).

IFN-γ polymorphism typing

Amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) was carried out to amplify the

*For correspondence. E-mail: feibaoying@hotmail.com.

Keywords. IFN-γ; IL-1; polymorphism; colorectal cancer.

IFN-γ gene using two different PCR reactions for each sample. Each reaction involved a generic antisense primer and one of the two allele-specific sense primers for the detection of allele T or A, plus an internal control forward primers. The band length of the T or A gene was 116 bp and the internal control was 798 bp. The conditions for the PCR were as follows: initial denaturation at 94°C for 5 min, followed by 33 amplification cycles, each consisting of denaturation at 94°C for 50 s, annealing at 60°C for 50 s and extension at 72°C for 50 s, and final extension at 72°C for 5 min. The amplified products were separated by electrophoresis on a 1.5% agarose gel stained with ethidium bromide (figure 1).

IL-1RN polymorphism typing

The conditions for the PCR were as follows: initial denaturation at 94°C for 5 min, followed by 33 amplification cycles, each consisting of denaturation at 94°C for 60 s, annealing at 60°C for 60 s and extension at 72°C for 60 s, and a final extension at 72°C for 5 min. Then, 10 μL of the PCR products were separated on 1.5% agarose gel and stained on ethidium bromide.

IL-1β+3953 polymorphism typing

The conditions for the PCR were as follows: initial denaturation at 94°C for 5 min, followed by 33 amplification cycles, each consisting of denaturation at 94°C for 50 s, annealing at 55°C for 50 s and extension at 72°C for 50 s, and final extension at 72°C for 5 min. Then, 10 μL of the PCR products were digested with 3 U of *Taq*I restriction enzyme for 3 h at 65°C. The digested products were then separated on 3.0% agarose gel and stained with ethidium bromide.

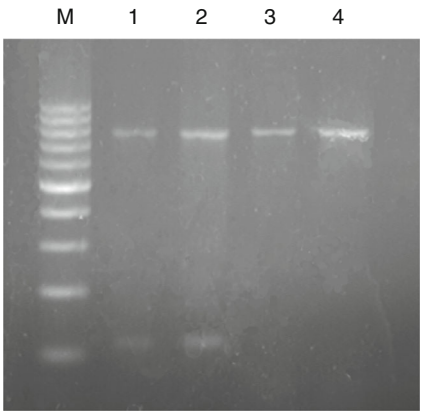

Figure 1. Electrophoresis pattern of IFN-γ+874 polymorphism by tetraprimer ARMS. M, 100-bp DNA ladder marker; (1 and 2), 116 bp fragment (T or A gene) and 798 bp internal control fragment; 3, 4: 798 bp internal control fragment.

Cloning and sequencing

The PCR products of the IFN-γ+ 874 allele were purified and ligated with a PGEM-T vector. High efficiency JM 109 competent cells were used in the process of transformation. The required transformants were obtained by blue/white colour screening and standard ampicillin selection. Recombinant plasmid DNA was isolated and identified. Sequencing was performed on an ABI 377 DNA sequencer.

Statistical analysis

Chi-square and Fisher's exact tests were used to test for a significant association between the two groups. Hardy–Weinberg equilibrium (HWE) was tested using a chi-square test. P value of < 0.05 was considered statistically significant.

Results

Association of IFN-γ+874 genotype and colorectal cancer

The allele and genotype distribution at the IFN-γ+874 locus in colorectal cancer and healthy controls are provided in table 1. When we used the IFN-γ+874 AA genotype as reference, we found that the IFN-γ+874 TA genotype was significantly lower in patients with colorectal cancer than in healthy controls (18.5% vs. 26.4, $x^2 = 4.33$, $P = 0.037$, OR = 0.571, 95% CI: 0.336–0.970). The frequency of the T allele was significantly lower in patients with colorectal cancer (20.4% vs. 29.5%, $x^2 = 7.50$, $P = 0.006$, OR = 0.612, 95% CI: 0.429–0.871). In a dominant model, the frequency of the IFN-γ+874 TA+TT genotype was significantly lower in patients with colorectal cancer than in healthy controls (29.6% vs. 40.7%, $x^2 = 6.25$, $P = 0.012$, OR = 0.565, 95% CI: 0.361–0.886). However, IFN-γ+874 genotypes were not related to age, gender, tumour location or Dukes grade in patients with colorectal cancer (table 2).

Association of IL-1+3953 and IL-1RN polymorphisms and colorectal cancer

There were three alleles and four kinds of genotypes (1/1, 1/2, 2/2 and 3/1) for the *IL-1RN* gene. There were two alleles and three kinds of genotypes (1/1, 1/2 and 2/2) at the IL-1β+3954 locus (figure 1). The distribution of the IL-1β+3953 and IL-1RN alleles and genotypes were similar and no significant differences were found between patients with colorectal cancer and healthy controls.

Sequence of the IFN-γ+874 allele

The 35→150 sequence in figure 2 was PCR amplified at the IFN-γ+874 locus. The 35→58 sequence was the same as the specific sense primers for the detection of allele T. The 131→150 sequence was completely complementary with the generic antisense primer.

Table 1. Genotype frequencies of the IFN-γ+874 locus in colorectal cancer and healthy controls.

	Case (%) (n = 162)	Control (%) (n = 178)	OR (95% CI)	P
IFN-γ+874 genotype				
AA	114 (70.4)	102 (57.3)	1 (reference)	
TA	30 (18.5)	47 (26.4)	0.571 (0.336–0.970)	0.037
TT	18 (11.1)	29 (16.3)	0.555 (0.291–1.059)	0.072
Dominant model				
AA	114 (70.4)	102 (57.3)	1 (reference)	
TA+TT	48 (29.6)	76 (42.7)	0.565 (0.361–0.886)	0.012
Recessive model				
AA+TA	144 (88.9)	149 (83.7)	1 (reference)	
TT	18 (11.1)	29 (16.3)	0.642 (0.342–1.207)	0.167
Allele				
A	258 (79.6)	251 (70.5)	1 (reference)	
T	66 (20.4)	105 (29.5)	0.612 (0.429–0.871)	0.006

Table 2. Association between the IFN-γ+874 polymorphism and clinical features of colorectal cancer (n = 162).

Variables	n	AA (n = 114)	TA (n = 30)	TT (n = 18)	x^2	P
Age group						
≤ 50	59	36	13	10	4.169	0.099
>50	103	78	17	8		
Gender						
Female	58	37	12	9	2.364	0.307
Male	104	77	18	9		
Tumour location						
Colon	86	65	15	6	3.642	0.162
Rectum	76	49	15	12		
Dukes grade						
A+B	102	76	18	8	3.431	0.18
C+D	60	38	12	10		

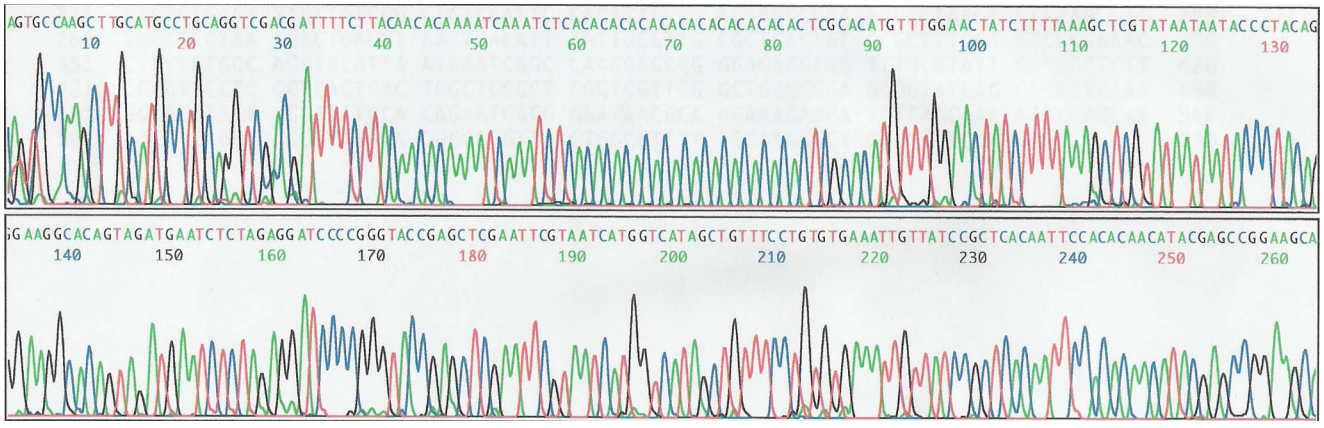

Figure 2. The sequence of the IFN-γ+874 allele (35→150).

Discussion

Cytokines play an important role in the regulation of inflammation and the pathogenesis of cancer. It is widely recognized that inflammation can contribute to tumour formation and growth, and that the strongest association of chronic inflammation with malignant diseases is in colon carcinogenesis arising in IBD patients. Inflammation can exacerbate *APC*-driven colon tumourigenesis. Genetic polymorphisms are largely responsible for individual differences in cytokine expression and lead to differences in immune response and susceptibility to colorectal cancer.

IFN-γ can inhibit angiogenesis and exert direct antiproliferative and antimetabolic effects on a wide variety of tumour cells. IFN-γ showed the highest production at the 10th cycle between chronic colonic inflammation and its progression to adenocarcinoma. It showed an early induction of proinflammatory factors, which may have contributed to the development of colon cancer (Sánchez-Fidalgo *et al.* 2011). A number of molecular epidemiological studies have been conducted to evaluate the association between the IFN-γ+ 874 A/T polymorphism and tumour risk in diverse populations. The IFN-γ AA genotype was found to be associated with the susceptibility to chronic lymphocytic leukaemia (Urbanowicz *et al*. 2010). The IFN-γ AA genotype which is a low producer of IFN-γ, was associated with an increased risk of cervical cancer in a north-Indian population (Gangwar *et al.* 2009). This study suggests that the IFN-γ+874 T/A polymorphism is responsible for the genetic differences in IFN-γ production and may influence the human papillomavirus (HPV) clearance and cervical malignant progression in a population of Brazilian women (von Linsingen *et al*. 2009). The frequency of the IFN-γ+874 T/T genotype was significantly higher in breast cancer patients compared to those of controls, which indicates that Iranian women carrying the IFN-γ+874 T/T genotype may be exposed to an increased risk of breast cancer development (Kamali-Sarvestani *et al*. 2005).

Two studies reported the negative association of the IFN-γ polymorphism and colorectal cancer. Talseth *et al*. (2007) demonstrated no significant difference between hereditary nonpolyposis colorectal cancer patients and unaffected mismatch repair gene mutation carriers for IFN-γ SNPs in Australia and Poland populations. Lee *et al*. (2010) showed no significant associations between the IFN-γ (5644) polymorphism and the risk of colorectal cancer in a Korean population. In the study, the frequencies of the IFN-γ+874 TA genotype and T allele were significantly higher in patients with colorectal cancer than in healthy individuals. This suggests that the IFN-γ+874 TA genotype and T allele may be associated with resistance to colorectal cancer. However, IFN-γ+874 genotypes were not related to age, gender, tumour location or Dukes grade in patients with colorectal cancer. The association between the IFN-γ genetic polymorphism and colorectal cancer could be interpreted by IFN-γ polymorphism influencing IFN-γ secretion.

In recent years, the roles of the IL-1β and its counterpart IL-1RA have been studied in cancer. IL-1 polymorphisms have been investigated in a variety of malignancies. There were a few studies on the association between IL-1 polymorphisms and colorectal cancer, and the results remained contradictory. For colorectal carcinoma, IL-1β-511 heterozygotes had a significantly lower risk of carcinoma which suggests that the IL-1β-511C/T and T carrier state may indicate less risk for gastric and colorectal carcinoma in the Japanese population (Ito *et al*. 2007). Polymorphisms in IL-1β, IL-1RN, and VEGFA as well as the IL-1β/IL-1RN haplotype analysis may serve as molecular markers for tumour recurrence in stage II colon cancer (Lurje *et al*. 2009). The IL-1RA genotype status was significantly associated with Karnofsky performance status (KPS) in metastatic colorectal cancer (Graziano *et al*. 2009). However, Lee *et al*. (2010) reported no significant associations between IL-1β (+3962, –511) polymorphisms and the risk of colorectal cancer in a Korean population (Lee *et al*. 2010). In this study, no association was found between IL-1β (+3954) and IL-1RN polymorphisms and colorectal cancer in a Chinese population. The discrepancy may be caused by a genetic trait difference among different ethnic groups.

In conclusion, our results suggest that the IFN-γ+874 TA genotype and T allele may be associated with resistance to colorectal cancer. However, IFN-γ+874 genotypes were not related to age, gender, tumour location or Dukes grade in patients with colorectal cancer. In addition, IL-1β (+3954) and IL-1RN polymorphisms did not play a role in the pathogenesis of colorectal cancer.

References

Azimzadeh P., Romani S., Mohebbi S. R., Kazemian S., Vahedi M., Almasi S. *et al.* 2011 Interleukin-16 (IL-16) gene polymorphisms in Iranian patients with colorectal cancer. *J. Gastrointestin Liver Dis.* **20**, 371–376.

Gangwar R., Pandey S. and Mittal R. D. 2009 Association of interferon-gamma +874A polymorphism with the risk of developing cervical cancer in north-Indian population. *BJOG* **116**, 1671–1677.

Graziano F., Ruzzo A., Canestrari E., Loupakis F., Santini D., Rulli E. *et al.* 2009 Variations in the interleukin-1 receptor antagonist gene impact on survival of patients with advanced colorectal cancer. *Pharmacogenomics J.* **9**, 78–84.

Ito H., Kaneko K., Makino R., Konishi K., Kurahashi T., Yamamoto T. *et al.* 2007 Interleukin-1beta gene in esophageal, gastric and colorectal carcinomas. *Oncol. Rep.* **18**, 473–481.

Kamali-Sarvestani E., Merat A. and Talei A. R. 2005 Polymorphism in the genes of alpha and beta tumor necrosis factors (TNF-alpha and TNF-beta) and gamma interferon (IFN-gamma) among Iranian women with breast cancer. *Cancer Lett.* **223**, 113–119.

Korthagen N. M., van Moorsel C. H., Kazemier K. M., Ruven H. J. and Grutters J. C. 2012 IL1RN genetic variations and risk of IPF: a meta-analysis and mRNA expression study. *Immunogenetics* **64**, 371–377.

Lee Y. S., Choi H. B., Lee I. K., Kim T. G. and Oh S. T. 2010 Association between interleukin-4R and TGF-β1 gene

polymorphisms and the risk of colorectal cancer in a Korean population. *Colorectal Dis.* **12**, 1208–1212.

Lee Y. S., Choi I., Ning Y., Kim N. Y., Khatchadourian V., Yang D. *et al.* 2012 Interleukin-8 and its receptor CXCR2 in the tumour microenvironment promote colon cancer growth, progression and metastasis. *Br. J. Cancer* **106**, 1833–1834.

Lurje G., Hendifar A. E., Schultheis A. M., Pohl A., Husain H., Yang D. *et al.* 2009 Polymorphisms in interleukin 1 beta and interleukin 1 receptor antagonist associated with tumor recurrence in stage II colon cancer. *Pharmacogenet. Genomics* **19**, 95–102.

Miller S. A., Dykes D. D. and Polesky H. F. 1988 A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acid Res.* **16**, 1215.

Moossavi S. and Bishehsari F. 2012 Inflammation in sporadic colorectal cancer. *Arch. Iran Med.* **15**, 166–170.

Pino M. S. and Chung D. C. 2010 The chromosomal instability pathway in colon cancer. *Gastroenterology* **138**, 2059–2072.

Pravica V., Perrey C., Stevens A., Lee J. H. and Hutchinson I. V. 2000 A single nucleotide polymorphism in the first intron of the human IFN-gamma gene: Absolute correlation with a polymorphic CA microsatellite marker of high IFN-gamma production. *Hum. Immunol.* **61**, 863–866.

Rafiq S., Stevens K., Hurst A. J., Murray A., Henley W., Weedon M. N. *et al.* 2007 Common genetic variation in the gene encoding interleukin-1-receptor antagonist (IL-1RA) is associated with altered circulating IL-1RA levels. *Genes Immun.* **8**, 344–351.

Rubin D. C., Shaker A. and Levin M. S. 2012 Chronic intestinal inflammation: inflammatory bowel disease and colitis-associated colon cancer. *Front Immunol.* **3**, 107.

Sameer A. S. 2013 Colorectal cancer: molecular mutations and polymorphisms. *Front Oncol.* **3**, 114.

Sánchez-Fidalgo S., Villegas I, de la Lastra C. A., Illanes M. and Motilva V. 2011 Role of different inflammatory and tumor biomarkers in the development of ulcerative colitis-associated carcinogenesis. *Inflamm. Bowel Dis.* **17**, 696–710.

Talseth B. A., Meldrum C., Suchy J., Kurzawski G., Lubinski J. and Scott R. J. 2007 Lack of association between genetic polymorphisms in cytokine genes and disease expression in patients with hereditary non-polyposis colorectal cancer. *Scand. J. Gastroenterol.* **42**, 628–632.

Urbanowicz I., Mazur G., Stacherzak-Pawlik J., Bogunia-Kubik K., Wróbel T., Woźniak M. *et al.* 2010 IFN gamma gene polymorphism may contribute to the susceptibility to CLL. *Pathol. Oncol. Res.* **16**, 213–216.

von Linsingen R., Bompeixe E. P., Maestri C. A., Carvalho N. S. and da Graça Bicalho M. 2009 IFNG (+874 T/A) polymorphism and cervical intraepithelial neoplasia in Brazilian women. *J. Interferon Cytokine Res.* **29**, 285–288.

Walczak A., Przybyłowska K., Trzciński R., Sygut A., Dziki L., Dziki A. *et al.* 2011 Association of -1112 c/t promoter region polymorphism of the interleukin 13 gene with occurrence of colorectal cancer. *Pol. Przegl. Chir.* **83**, 27–31.

Received 31 May 2013, in revised form 9 September 2013; accepted 26 November 2013
Published on the Web: 24 April 2014