Gastroenterology and Hepatology From Bed to Bench.

ORIGINAL ARTICLE

Association of co-stimulatory human B-lymphocyte antigen B7-2 (CD86) gene polymorphism with colorectal cancer risk

Pedram Azimzadeh, Sara Romani, Hanieh Mirtalebi, Seyed Reza Fatemi, Shabnam Kazemian, Mahsa Khanyaghma, Seyed Reza Mohebbi

Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ABSTRACT

Aim: This study investigated the role of CD86 +237 G/C polymorphism in intensifying the risk of CRC development.

Background: Colorectal cancer (CRC) is a multi-factorial diseases. Genetic background could affect the susceptibility of individuals to CRC development. CD86 is a co-stimulatory factor on antigen-presenting cells that plays key roles in several cancer related mechanisms such as autoimmunity, transplantation and tumor immunity.

Patients and methods: A total of 300 individuals**,** 150 known CRC patients and 150 healthy control individuals, were subjected for the study. CD86 rs17281995 single nucleotide polymorphism (SNP) was genotyped using Allelic Discrimination method.

Results: A statistically significant difference was found among CD86 gene polymorphism (rs17281995) and risk of CRC development. The frequency of GG, GC and CC in control subjects was determined as 38%, 57.3% and 4.7% respectively and in CRC subjects were determined as 42%, 85% and 23% respectively. The data shows a significant association between CC genotype (P=0.007) and C allele (P=0.017) of the studied polymorphism and risk of CRC. CC genotype and C allele are also more frequent in female patients when the data is stratified according to gender status.

Conclusion: Our results suggest that CD86 gene alteration could affect the individual's risk for developing CRC among Iranian population and could be used as an important prognostic factor associated with risk of CRC.

Keywords: CD86, Single nucleotide polymorphism, Colorectal cancer.

(Please site as: **Azimzadeh P, Romani S, Mirtalebi H, Fatemi SR, KazemianS, Khanyaghma M, et al. Association of co-stimulatory human B-lymphocyte antigen B7-2 (CD86) gene polymorphism with colorectal cancer risk. Gastroenterol Hepatol Bed Bench 2013;6(2):86-91).**

Introduction

Colorectal cancer is the fourth commonest form of cancer with approximately 783,000 new cases a year and the third common cause of cancer-related deaths worldwide (1, 2). In the Iranian population CRC is the fourth most common cancer among both sexes and has increased in incidence in recent years (3). Molecular epidemiology studies suggest that genetic alterations such as single nucleotide polymorphisms could significantly affect the risk of disease development, particularly in cancer patients. There are many publications showing that allelic and genotype frequencies of single nucleotide polymorphisms (SNPs) are much different among different populations and ethical groups. Thus these genetic factors can be investigated as population specific prognostic factors for early diagnosis or screening programs (4-10).

Received: 29 October 2012 *Accepted*: 18 February 2013
Reprint or Correspondence: Seyed Reza Mohebbi, PhD. Gastroenterology and Liver Diseases Center, Shahid Beheshti University of Medical sciences, Iran
E-mail: srmohebbi@gmail.com

Inflammatory reactions have long been connected with the progression of cancer. Such reactions could change the microenvironment around a cell and modify the physiologic procedures such as homeostasis and repair (9). Variation occurred in production of immune system receptors such as CD86 could change the functional cells of body's immunity such as T cells, and might resulted in systemic inflammatory responses (9, 11).

Cluster of Differentiation 86 (CD86) is a protein expressed in immune system cells and it has been involved in the pathogenesis of a variety of inflammatory and inflammation related disorders. Co-stimulatory signals that have been made by such receptors are essential for activation and survival of T cells (12, 13). This molecule that is also called B7-2 can modulate individual's susceptibility to cancer by modification of T cell response (14). CD86 gene is located on the human chromosome 21, and has eight exons and a 3' untranslated region (UTR) regulatory domain (15). The purpose of our study was to investigate the association of +237 G/C polymorphism located on the 3'UTR region of CD86 gene sequence with risk of CRC among Iranian patients.

Patients and Methods

This case-control study was performed on a total of 300 Iranian individuals. The case group consisted of 150 patients with diagnosis of colorectal cancer referred to gastroenterology ward of Taleghani hospital, Tehran, Iran. The patients included met the criteria of positive colonoscopy and pathology results for colorectal carcinoma and the control individuals were 150 healthy volunteers with eligible colonoscopy results and negative family history for gastrointestinal diseases. Ethics committee of research center for gastroenterology and liver diseases (RCGLD), Shahid Beheshti University of medical sciences approved the sample collection and acknowledged the consents of subjects of the study.

Genomic DNA extraction was performed on 4 ml of whole blood and standard phenol-chloroform technique (16). Genotype determination was performed using TaqMan 5' nuclease assay (Applied Biosystems, Foster City, CA). Two TaqMan probes were used, one for each allele. Data analysis and genotyping was performed using Allelic Discrimination (AD) method and ABI PRISM 7500 instrument and SDS 1.1 software (Applied Biosystems, Foster City, CA). Genotyping PCR reaction was consisted of TaqMan Universal PCR Master Mix 2X, TaqMan predesigned primers and probes mixture (assay ID C_441625_10, Applied Biosystems, Foster City, CA), deionized water and 20 ng of purified genomic DNA.

Statistical analysis was performed using the software SPSS version 13 (SPSS, Chicago, IL, USA). Significant differences of the observed frequencies of alleles and genotypes in patients with CRC and in healthy blood donors were evaluated by Chi-Square test. Adjustment of data for removing the effect of confounder variables was performed using logistic regression. P-values under the 0.05 were considered as significant.

Results

The study population consisted of 150 patients with CRC (70 female and 80 male) with mean age of 56.2 ± 12.1 and 150 healthy control subjects (72 female and 78 male) with mean age of 45.7 ± 16.6 who undergo colonoscopy in gastroenterology ward of Taleghani hospital, Tehran. Distribution of male and female subjects was not significantly different among both case and control groups (P=0.45). Due to the fact that the cases had significantly higher age in comparison with the healthy controls (P<0.001), we adjusted our genotyping data according to the age status to remove the confounding effect of this variable.

There was no significant difference between two studied groups when BMI (P=0.45) and smoking behavior (P=0.11) were investigated. General characteristics of the study population are summarized in table 1.

Genotype distribution of CD86 gene +2379G/C (rs172899) polymorphism in our samples were GG (28%), GC (56.7%) and CC (15.3%) in CRC patients' group and GG (38%), GC (57%) and CC (4.7%) in healthy controls group. There was a significant difference between two studied groups (OR: 3.811 CI: 1.446-10.043, P=0.007). Furthermore, frequency of C allele was significantly higher in CRC group (OR: 1.536, CI: 1.081-2.182, P=017) (Table 2).

Moreover, after stratification of genotyping data according to the gender status we found a significant difference between case and control groups. Frequency of CC genotype among female patients with CRC was higher in comparison with healthy female subjects (OR: 11.489 CI: 3.660-36.060, P<0.0001). Investigation of allele distribution of CD86 gene +2379G/C polymorphism showed significant difference among gender groups. Frequency of C allele was significantly higher in female patients (2.055, CI: 1.243-3.399, P=0.005) (Table 3).

Discussion

SNP investigation association studies are suitable for the investigation of probable genetic prognostic factors and molecular biomarkers, especially in the study of genetic basis of human complex diseases (17). Due to their direct effect on the expression of corresponding genes, SNPs located on the regulatory regions such as 3'UTR (18) it is thought that they could have priority for SNP association studies. In addition further functional studies are considered necessary for more understanding of the effect of polymorphisms located in regulatory region at the 3'UTR of genes (19).

According to our literature review there are two common SNPs in the CD86 gene sequence that majority of studies focused on, +1057 G/A alanine / threonine substitution at coding region of 8th exon, and the +2379 G/C polymorphism located in 3'UTR. Due to the regulatory role of 3'UTR sequence of genes, SNPs located in these regions could affect the expression and function of corresponding protein (20). In the present study we investigated the association of rs17281995 polymorphism located on the 3'UTR regulatory region of CD86 gene sequence with risk of CRC in a sample of Iranian patients.

Table 1. Characteristics of study population

Variables	Controls (n=150)	Cases (n=150)	P Value
Age (Mean ± SD)[a]	45.7 ± 16.6	56.2 ± 12.1	**<0.0001**
BMI [a]	26.1 ± 5.5	25.6 ± 4.9	0.45
Sex			0.45
Female [b]	72 (48)	70 (46.7)	
Male	78 (52)	80 (53.3)	
Smoking			0.11
Smoker	10 (6.7)	17 (11.3)	
Non-Smoker	140 (93.3)	133 (88.7)	
Tumor Location			-
Colon	-	107 (71.3)	
Rectum	_	43 (28.7)	

[a] According to the Student's t-test results .
[b] According to The chi-square test results

Table 2. Associations of the CD86 SNP with CRC risk in our study population.

SNP (Gene ID)	Variable	Controls (n=150) n (%)	Cases (n=150) n (%)	Adjusted* OR (95%CI), P_{value}
rs17281995 (*CD86*)				
	Genotypes			
	GG	57 (38.0)	42 (28.0)	1.00 (Reference)
	GC	86 (57.3)	85 (56.7)	1.472 (0.864-2.508), 0.155
	CC	7 (4.7)	23 (15.3)	**3.811 (1.446-10.043), 0.007**
	Alleles			
	G	200 (66.7)	169 (56.3)	1.00 (Reference)
	C	100 (33.3)	131 (43.7)	**1.536 (1.081-2.182), 0.017**

* Adjusted for Age as a confounder variable

Table 3. Stratification genotyping data of CD86 polymorphism according to gender groups.

SNP	Variable	Female			male		
		Controls (n=72)	Cases (n=70)	Adjusted* OR (95% CI), P_{value}	Controls (n=78)	Cases (n=80)	Adjusted* OR (95% CI), P_{value}
rs17281995 (*CD86*)	Genotypes	n (%)	n (%)		n (%)	n (%)	
	GG	56 (38.9)	34 (24.3)	1.00 (Reference)	58(37.2)	50(31.3)	1.00 (Reference)
	GC	84(58.3)	76 (54.3)	1.708 (0.98-2.976), 0.059	88(56.4)	94(58.8)	1.283 (0.760- 2.167) 0.350
	CC	4 (2.8)	30 (21.4)	**11.489 (3.660-36.060), <0.0001**	10(6.4)	16(10.0)	1.410 (0.551- 3.610), 0.474
Alleles, n (%)	G	98 (68.1)	72 (51.4)	1.00 (Reference)	102(65.4)	97(60.6)	1.00 (Reference)
	C	46 (31.9)	68 (48.6)	**2.055 (1.243-3.399), 0.005**	54(34.6)	63(39.4)	1.168 (0.709-1.925), 0.542

* Logistic regression analysis values are adjusted for age as a confounder variable

There are several association studies conducted on the relationship between CD86 gene SNPs and different diseases. Ma et al. in 2010 reported that CD86 gene +1057 G/A polymorphism is not associated with chronic artery disease (CAD) in a sample of Chinese population (21).

Pan et al. in 2010 performed a study on important polymorphism of CD86 gene +1057 and reported a significant association between this SNP and increased risk of CRC. They also stratified the data according to gender status and have found that A allele in female patients is significantly higher than male patients with CRC (13). Results of the study of Liu et al. in 2010 showed that CD86 +1057 G/A polymorphism is associated with chronic obstructive pulmonary disease (COPD) (12). Wang et al. in 2011 performed a case control study in a Chinese population and suggested that the +1057G/A polymorphism located in CD86 gene sequence is associated with increased susceptibility to Ewing's Sarcoma (14). To the best of our knowledge there are limited publications about relationship of CD86 gene +2379 G/C polymorphism with risk of CRC development worldwide and there is no published data from frequency of CD86 gene polymorphisms among Iranian patients with CRC, so far.

The main limitations of present study that need to be taken into account were limited sample size and novelty of this variation and its relationship with cancer development.

The association of CD86 gene +2379 G/C polymorphism with CRC and COPD had previously been investigated. Landi et al. in 2008 found that both the GC and CC genotypes for the rs17281995 polymorphism of CD86 gene were associated with an increased risk of CRC in

population from Czech Republic. They suggested that the effect of this SNP is related to the modification of miRNA binding sites in the 3'UTR of CD86 gene (18). Our findings are in concordance with previous studies suggesting this SNP may have a functional role in regulating the expression of CD-86 protein acting at the level of the 3'UTR.

In conclusion we have analyzed 300 Iranian individuals to determine the frequency and distribution of alleles and genotypes of CD86 gene +2379 G/C polymorphism and our results suggested that the selected polymorphism might have a strong contribution to genetic susceptibility to CRC in Iranian patients.

References

1. Boyle P,Langman JS. ABC of colorectal cancer: Epidemiology. BMJ 2000; 321: 805-808.

2. Parkin DM. Global cancer statistics in the year 2000. Lancet Oncol 2001; 2: 533-43.

3. Moghimi-Dehkordi B, Safaee A, Zali MR. Prognostic factors in 1,138 Iranian colorectal cancer patients. International journal of colorectal disease 2008; 23: 683-88.

4. Abuli A, Fernandez-Rozadilla C, Alonso-Espinaco V, Munoz J, Gonzalo V, Bessa X, et al. Case-control study for colorectal cancer genetic susceptibility in EPICOLON: previously identified variants and mucins. BMC Cancer 2011; 11: 339.

5. Azimzadeh P, Romani S, Mohebbi SR, Kazemian S, Vahedi M, Almasi S, et al. Interleukin-16 (IL-16) Gene Polymorphisms in Iranian Patients with Colorectal Cancer. J Gastrointestin Liver Dis 2011; 20: 1-6.

6. Azimzadeh P, Romani S, Mohebbi SR, Mahmoudi T, Vahedi M, Fatemi SR, et al. Association of polymorphisms in microRNA-binding sites and colorectal cancer in an Iranian population. Cancer Genetics 2012; 205: 501-07.

7. Azmy IA, Balasubramanian SP, Wilson AG, Stephenson TJ, Cox A, Brown NJ, et al. Role of tumour necrosis factor gene polymorphisms (-308 and -238) in breast cancer susceptibility and severity. Breast Cancer Res 2004; 6: 24.

8. Bai RK, Leal SM, Covarrubias D, Liu A, Wong LJ. Mitochondrial genetic background modifies breast cancer risk. Cancer Res 2007; 67: 4687-94.

9. Rakoff-Nahoum S. Why cancer and inflammation? Yale J Biol Med 2006; 79: 123-30.

10. Karimi K, Arkani M, Safaei A, Pourhoseingholi MA, Mohebbi SR, Fatemi SR, et al. Association of leptin receptor gene Gln223Arg polymorphism with susceptibility to colorectal cancer. Gastroenterol Hepatol Bed Bench 2011; 4: 192-98.

11. Lu H, Ouyang W, Huang C. Inflammation, a key event in cancer development. Mol Cancer Res 2006; 4: 221-33.

12. Liu Y, Liang WB, Gao LB, Pan XM, Chen TY, Wang YY, et al. CTLA4 and CD86 gene polymorphisms and susceptibility to chronic obstructive pulmonary disease. Hum Immunol 2010; 71: 1141-6.

13. Pan XM, Gao LB, Liang WB, Liu Y, Zhu Y, Tang M, et al. CD86 +1057 G/A polymorphism and the risk of colorectal cancer. DNA Cell Biol 2010; 29: 381-6.

14. Wang J, Zhou Y, Feng D, Yang H, Li F, Cao Q, et al. CD86 +1057G/A Polymorphism and Susceptibility to Ewing's Sarcoma: A Case-Control Study. DNA Cell Biol 2011; 26: 26.

15. Jellis C, Wang S, Rennert P, Borriello F, Sharpe A, Green N, et al. Genomic organization of the gene coding for the costimulatory human B-lymphocyte antigen B7-2 (CD86). Immunogenetics 1995; 42: 85-89.

16. Sambrook J, Molecular cloning : a laboratory manual / Joseph Sambrook, David W. Russell, ed. D.W. Russell,L. Cold Spring Harbor2001, Cold Spring Harbor, N.Y. :: Cold Spring Harbor Laboratory.

17. Ramensky V, Bork P, Sunyaev S. Human non□synonymous SNPs: server and survey. Nucleic Acids Research 2002; 30: 3894-900.

18. Landi D, Gemignani F, Naccarati A, Pardini B, Vodicka P, Vodickova L, et al. Polymorphisms within micro-RNA-binding sites and risk of sporadic colorectal cancer. Carcinogenesis 2008; 29: 579-84.

19. Landi D, Moreno V, Guino E, Vodicka P, Pardini B, Naccarati A, et al. Polymorphisms affecting micro-RNA regulation and associated with the risk of dietary-related cancers: A review from the literature and new evidence for a functional role of rs17281995 (CD86) and rs1051690 (INSR), previously associated with colorectal cancer. Mutation Research 2010; 30: 30.

20. Pelletier C,Weidhaas JB. MicroRNA binding site polymorphisms as biomarkers of cancer risk. Expert Rev Mol Diagn 2010; 10: 817-29.

21. Ma XN, Wang X, Yan YY, Yang L, Zhang DL, Sheng X, et al. Absence of association between CD86 +1057G/A polymorphism and coronary artery disease. DNA Cell Biol 2010; 29: 325-28.