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ORIGINAL ARTICLE

Positive correlation between interleukin-1 receptor antagonist gene 86bp VNTR polymorphism and colorectal cancer susceptibility: a case-control study

Mostafa Ibrahimi[1] · Maryam Moossavi[2,3] · Ehsan Nazemalhosseini Mojarad[4] · Mahsa Musavi[5] · Milad Mohammadoo-khorasani[1] · Zahra Shahsavari[6]

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Abstract

Colorectal cancer (CRC) is one of the most common malignancies worldwide. Genetic variations in cytokine genes and their receptors lead to the severity of the disease. The interleukin-1 receptor antagonist (IL1RN) is a cytokine that inhibits interleukin-1 (IL-1) activity by binding to IL-1 receptors. Also, interleukin-4 (IL-4) is an anti-inflammatory cytokine that can play an important role in several cancers. The present case-control study was aimed to evaluate the association of IL-4 and IL1RN VNTR polymorphisms with the susceptibility to CRC in a sample of Iranian population provided by the Research Center for Gastroenterology and Liver Disease at Taleghani Hospital, Tehran. A total of 123 patients diagnosed with CRC and 152 healthy controls were recruited in the present study. Genomic DNA was extracted by salting out method from whole blood and genotyping of IL1RN and IL-4 VNTR polymorphisms were determined by PCR-based technology. Our study manifested the frequency of 1/2 and 2/4 genotypes of IL1RN 68bp VNTR polymorphism are significantly different between both groups ($p = 0.0001$ and $p = 0.01$ respectively). However, we could not find any correlation between IL-4 VNTR polymorphism and CRC cancer. It seems that 1/2 and 2/4 genotypes of IL1RN are correlated with CRC susceptibility in our population, although, more studies are needed to confirm our results.

Keywords Colorectal cancer · Cytokines · VNTR polymorphism · IL-4 · IL1RN

Introduction

Cancer has been a heavy burden on public health systems and governments all over the world [1]. Among cancer types, colorectal cancer (CRC) (OMIM#114500) as the third widespread cancer in European and American societies is studied profoundly [2]. Also, in Middle-Eastern countries, notably Iran, the prevalence of this non-communicable disease is rising in various geographical regions [3–5]. Although, it is implied in many studies that complex interaction between genetic and environmental component plays an important role in causing CRC, as other types of cancer, the definite etiology of this type of cancer is still remained to be elucidated. Inflammatory biomarkers as a mean to trigger cancer have

Mostafa Ibrahimi and Maryam Moossavi have equally contributed as first author

✉ Milad Mohammadoo-khorasani
miladkh24@yahoo.com

✉ Zahra Shahsavari
z.shahsavari@sbmu.ac.ir

[1] Department of Clinical Biochemistry, Tarbiat Modares University, Tehran, Iran

[2] Department of Molecular Medicine, Birjand University of Medical Sciences, Birjand, Iran

[3] Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran

[4] Gastroenterology and Liver Disease Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[5] Department of Biostatistics and Epidemiology, Hamadan University of Medical Sciences, Hamadan, Iran

[6] Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

drawn the attention of various epidemiological studies [6]. Among these biomarkers, systemic and local alteration in cytokine profile as central inflammatory effectors in CRC received a great deal of research interest [7]. Moreover, evidences revealed that cancer susceptibility could be influenced by several types of polymorphisms placed in genes encoding cytokines and their receptors [8, 9]. IL-4 as an important cytokine in triggering different types of cancer is over-expressed in the early development of CRC [10]. The importance of interleukin-4 in etiology of CRC is due to its anti-inflammatory function whether in local or systemic inflammatory conditions which downregulates macrophage activity as well as inhibition of pro-inflammatory cytokine secretion especially IL-1 [11]. This phenomenon prepares a favorable microenvironment for cancer development. Interleukin-4 is mapped on chromosome 5q31.1 and includes four exons [12]. A variable number of tandem repeat (VNTR) of 70 base pair repeats in its third intronic region is responsible for predisposition to several types of cancer [13]. The other anti-inflammatory cytokine is interleukin-1 receptor antagonist well known as IL1RN (16 to 18 kD protein) related to interleukin-1 (IL1) cytokine family which competes with IL-1a and IL-1b for binding to IL-1 receptor as an endogenous inhibitor of IL-1. IL-1 family plays a controversial role in CRC development and polymorphisms in IL1RN are suggested to be associated with CRC risk [7, 14]. The genes encoding IL-1 family are located on chromosome 2q14 [15]. There is a variable number of tandem repeat (VNTR) of 86 base pair repeats in its second intronic region with three potential protein binding sites. It has been reported that these binding sites control cell proliferation activity [16]. Preliminary studies reported a positive association between gastric cancer risk and VNTR polymorphisms in IL1RN [17]. Also, it is shown by various studies that IL1RN VNTR alternative genotypes are highly linked to variety of cellular activities [18, 19]. Furthermore, by inhibiting hepatocyte growth factor (HGF) signaling pathway, IL1RN reduces the metastatic potential of colon cancer [20]. Due to these controversial results, we aimed to evaluate the association of VNTR polymorphisms of IL-4 and IL1RN with colorectal cancer risk in a sample of population in Tehran, Iran.

Material and method

Patients and sample collection

After Ethics Committee of Shahid Beheshti University of Medical Sciences approval (ethics code: IR.SBMU.RETECH.REC.1395.563), this case-control study was conducted at the Research Center for Gastroenterology and Liver Disease at Taleghani Hospital, Tehran, Iran. This study included 123 CRC patients that were diagnosed by gastroenterologists and then confirmed by pathologists and also 152 control healthy volunteers.

Genomic DNA extraction and genotyping

Samples are provided by the Research Center for Gastroenterology and Liver Disease of Shahid Beheshti University of Medical Sciences, Tehran, Iran. Genomic DNA was extracted from peripheral blood leukocytes using salting out method [21] and the quality of the extracted DNA was evaluated based on OD 260/280; this ratio was between 1.7–1.9 in all samples. Polymerase chain reaction was performed in 25 μL final volume contained 25 pmol of each primer, 0.1 mmol of dNTP, 0.5 μg of genomic DNA, 1.5 mmol/L of Mgcl2 and 2.5 μL of PCR buffer, and 1.5 unit of Taq DNA polymerase (Fermentas, Thermo Fisher Scientific) according to the following protocol: initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 45 s, annealing at 61 °C for IL-4 or 55 °C for IL1RN for 30 s, and extension at 72 °C for 45 s; and final extension at 72 °C for 5 min. PCR products were separated by electrophoresis on a 2.5% agarose gel and visualized by DNA Green Viewer (Pars tous) staining. The PCR primer sequences are described previously [11, 22].

Statistical analysis

Data were analyzed using statistical software SPSS 18 (SPSS, Chicago, IL). The differences between groups were analyzed by independent sample *t* test, Chi square test, or Fisher's exact test, whenever appropriate. Direct gene counting method was used to determine the allele frequency. The genotypes and alleles frequency were compared between CRC patients and controls by χ^2 test and Fisher's exact test. The odds ratio (OR) and 95% confidence intervals (CI) were also estimated. Values of $p < 0.05$ were considered statistically significant.

Results

Demographic characteristics of CRC patients and controls are shown in Table 1 and there were no significant statistical differences in age, gender, smoking state, and ethnicity between case and control subjects. The genotypic and allelic frequencies of IL-4 and IL1RN VNTR polymorphism are listed in Table 2. According to this table, there were no significant differences in genotypic and allelic frequencies of IL-4 VNTR polymorphism between CRC patients and the control group. Moreover, we observed six genotypes of IL1RA VNTR polymorphisms (1/1, 1/2, 1/3, 1/4, 2/2, 2/4) in our population of which, the frequency of

Table 1 Demographic characteristics of CRC patients and controls

Characteristics	Patient $N = 123$	Control $N = 152$	p value
Mean age at diagnosis, mean (± SD)	61 ± 10.39	60 ± 10	$p > 0.05$
Gender			$p > 0.05$
Females	57	70	
Males	66	82	
Tumor location			
Colon	78	–	
Rectum	36	–	
Cecum	9	–	
TNM			
I	19	–	
II	6	–	
IIA	41	–	
IIB	9	–	
III	7	–	
IIIA	2	–	
IIIB	17	–	
IIIC	12	–	
IV	10	–	
Smoking			$p > 0.05$
Yes	87	107	
NO	18	45	
Ethnicity			$p > 0.05$
Fars	68	85	
Gilak	5	5	
Kord	11	14	
Turk	24	30	
Lor	9	11	
Mazani	6	7	

1/2 and 2/4 genotypes is significantly different between groups. We observed higher frequencies of these two genotypes in CRC patients which are hence, correlated positively with CRC susceptibility ($p = 0.0001$ and $p = 0.01$ respectively). Furthermore, allele 2 frequency was significantly different between the two groups ($p = 0.001$). The mentioned allele is associated with CRC risk in our population.

Discussion

Cancer which has been a major health problem since long ago is triggered by genetic, immunologic, and environmental complex interaction [23]. Aggregation of genetic and epigenetic alterations stimulates distinctive neoplastic molecular pathways in every tumor. Immune cells residing in the tumor microenvironment offer a considerable opportunity for tumor development, hence, providing a great deal of research interest in the therapeutic filed [24]. Epidemiological studies have shown that chronic inflammation serves as a risk factor for different types of cancer. Since, cytokines are chief mediators of the inflammation process, variations located in genes encoding cytokines and their receptors can influence cancer susceptibility [25]. Molecular pathological epidemiology (MPE) as a novel field of scientific research merged by epidemiological research and pathological research disciplines. The former evaluate how an event ascent or descent the danger of cancer development. The latter describe the molecular features of tumors in order to forecast prognosis and therapy [26, 27].

During tumorogenesis, cancerous cells interact with tumor microenvironment cells including extracellular matrix and other host cells (mesenchymal cells, vascular endothelial cells, and inflammatory cells). Inflammatory and immune cells are presented in various levels in the tumor microenvironment that can manifest in the pathologic

Table 2 Genotypic and allelic frequencies of IL-1 and IL-4 VNTR polymorphisms in CRC patients and controls

Genotype	Case ($n = 123$)	Control ($n = 152$)	p value	OR (95% CI)
IL1RN				
1/1	19	61		Ref = 1
1/2	85	52	0.0001*	5.27 (2.9–9.8)
13	7	1/3	0.24	1.6 (0.58–4.4)
1/4	0	7	0.14	0.9 (0.83–0.98)
2/2	9	19	0.25	0.38 (0.48–0.6)
2/4	3	0	0.01*	–
Allele				
1	130	194		Ref = 1
2	106	90	0.001*	1.75 (1.22–2.51)
3	7	13	0.419	0.8 (0.31–2.06)
4	3	7	0.38	0.64 (0.16–2.51)
IL-4				
RP1/RP1	104	122		Ref = 1
RP1/RP2	19	30	0.22	0.74 (0.395–1.39)
Allele				
RP1	227	274		
RP2	19	30	0.23	0.74 (0.395–1.39)

* $p < 0.05$

practice. This microenvironment provides oxygen, cytokines, cancerous growth factors, and other nutrients that are necessary for tumor development, survival, and ultimately metastasis [24]. Moreover, various studies implicated the role of cytokines in the regulation of cell or tumor cell growth, cell signaling and differentiation of tumor cells, and its development to metastatic state [28]. Among anti-inflammatory cytokines, IL1RN is a natural protein related to IL-1 family that blocks IL-1R effects on T lymphocytes and fibroblasts competitively. This protein is under investigation as a putative novel treatment in cancer therapy by decreasing tumor growth, tumor angiogenesis, and metastases [29]. IL-4 is a cytokine of Th2 family which is observed in tumor microenvironment in patients affected with different types of cancer. This cytokine increases survival of epithelial cancer cells in order to support tumor growth [30]. Studies reported that, VNTR polymorphism in IL-4 gene notably, 2R allele enhances the IL-4 production or activity by T cells and can be a risk factor for cancer [25]. In CRC, IL-4 is overexpressed early in the development of the disease. Several polymorphisms located in this gene stimulate a higher level of IL-4 production [7]. In the present study, we evaluated the correlation between IL-4 70bp and IL1RN 86bp variable number tandem repeat (VNTR) gene polymorphisms and the risk of colorectal cancer in a sample of Iranian population. The major finding of our investigation was the statistically significant association of 2R allele and 2R carrier genotypes (1/2 and2/4) with an increased risk of colorectal cancer, indicating the probable association of IL1RN VNTR polymorphism with CRC vulnerability in our population. However, we could not find any significant association with IL-4 VNTR polymorphism and CRC risk. Since allele 2 with 2 repeats (2R) is associated with prolonged inflammation, our study confirms the association of this genetic variation with CRC susceptibility. El-Omar et al. first reported a positive association between gastric cancer risk and VNTR polymorphisms in IL1RN [17]. Similar to our finding, Viet HT et al. (2005) reported that IL1RN polymorphism is associated with colorectal carcinogenesis [14]. A meta-analysis (2012) showed that IL1RN VNTR polymorphisms may contribute to gastric cancer susceptibility [18]. Moreover, Amira Ben Ahmed et al. (2014) indicated that allele 1 is associated with an increased risk of ovarian cancer development in Tunisian women [31]. And again consistent with our data regarding IL-4 gene VNTR polymorphism and CRC risk, Yang et al. (2014) found 2R/2R associated with early-stage oral and pharyngeal carcinoma risk (OPSCC) [32]. Also, Amar Chand Bhayal et al. reported the positive association of 2R allele and 2R carrier genotypes in the pathogenesis of gastric cancer in South Indian population [33]. Constant inflammatory responses can lead to tumor development, since, aspirin as anti-inflammatory drug reduces the risk of colorectal cancer (CRC) by hindering tumor pathway that relies on

inflammatory responses [34]. In summary, it seems that VNTR polymorphism of IL1RN gene predisposes CRC by influencing functions of this protein. In conclusion, more studies in different populations with higher sample sizes are needed to confirm and further investigate our findings.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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