Annals of Colorectal Research
www.colorectalresearch.com

Association of FoxP3/Scurfin Germline Polymorphism (C-2383T/rs3761549) with Colorectal Cancer

Zahra Mojtahedi [1], Nasrollah Erfani [1, *], Mohammad Reza Haghshenas [1], Seyed Vahid Hosseini [2], Abbas Ghaderi [1]

[1] Cancer Proteomics and Biomarkers Laboratory, Shiraz Institute for Cancer Research, School of Medicine, Shiraz University of Medical Sciences, Shiraz, IR Iran

[2] Colorectal Research Center, Faghihi Hospital, Shiraz University of Medical Sciences, Shiraz, IR Iran

*Corresponding Author: Nasrollah Erfani, Shiraz Institute for Cancer Research,Shiraz University of Medical Sciences, Shiraz, IR Iran. Tel./Fax: +98-711 230 3687, E-mail: erfanin@sums.ac.ir

ABSTRACT

Background: FoxP3 gene encodes a transcription factor with crucial roles in the development and function of regulatory T cells.
Objectives: The present study was conducted to investigate whether the C-2383T (rs3761549) polymorphism in the promoter region of the FoxP3 gene is associated with colorectal cancer.
Patients and Methods: The study groups consist of 108 patients (52 men and 56 women) with colorectal cancer and 187 (126 men and 61 women) healthy individuals. They were genotyped for the FoxP3 polymorphism at position -2383 C >T using polymerase chain reaction-restriction fragment length polymorphism method. Frequencies of the gene variants were analyzed separately in men and women since FoxP3 is an X-linked gene.
Results: The numbers of female patients with C/C, C/T and T/T genotypes were 48 (85.7%), 7 (12.5%) and 1 (1.8%), while in female controls, they were 53 (86.9%), 8 (13.1%) and 0 (0%), respectively. In male patients and controls, frequencies of the C genotype were 49 (94.2%) and 118 (93.7%), and the T genotype were 3 (5.8%) and 8 (6.3%), respectively. Genotype frequencies were not significantly different between controls and colorectal cancer patients. Distance metastasis was significantly associated with the FoxP3 polymorphism as calculated using Pearson's chi-squared test (P = 0.006 in men and P = 0.03 in women). While 50% of metastatic patients had T or C/T (or T/T) genotype, the percentage of this genotype in non-metastatic patients was 4% in men and 11.5% in women. However, P values did not remain significant if differences were calculated using two-sided Fisher's exact test (P = 0.11 in men, and P = 0.09 in women); this might be due to the low number of our metastatic patients. Other characteristics of patients including age, tumor grade and stage were not significantly associated with the polymorphism.
Conclusions: Our results supported an association between C-2383T FoxP3 polymorphism and metastatic colorectal cancer in southern Iran.

Keywords: Colorectal cancer; FoxP3; Polymorphism; Single Nucleotide Polymorphisms (SNP)

Article type: Research Article; Received: 02 Mar 2013, Accepted: 04 Apr 2013; DOI: 10.5812/acr.11478

Implication for health policy/practice/research/medical education:
An association between FoxP3 polymorphism and metastatic colorectal cancer warrant further investigated on the possible employment of this molecule as a prognostic marker.

Please cite this paper as:
Mojtahedi Z, Erfani N, Haghshenas MR, Hosseini SV, Ghaderi A. Association of FoxP3/Scurfin Germline Polymorphism (C-2383T/rs3761549) with Colorectal Cancer. Ann Colorectal Res: 2013;1(1): 12-6. DOI: 10.5812/acr.11478

1. Background

Colorectal carcinoma is one of the leading causes of cancer worldwide (1). In Iran, this disease is the sixth most frequent cancer in men and the fifth in women (2). Colorectal carcinoma develops in a special microenvironment enriched in the enterobacterial antigens, and numerous myeloid and lymphoid cells, including infiltrating T cells into intestinal mucosa (3, 4). Enteric bacteria are known to induce pro-inflammatory cytokines, which provoke pro-angiogenic and tumor-enhancing factors such as NF-κB. The excessive inflammatory reaction and maintenance of intestinal homeostasis are essentially controlled by a subset of T-cells, called T regulatory cells (Tregs). Mice deficient in T cells were shown to be highly susceptible to inflammatory bowel diseases and to the development of carcinomas, which were prevented by adoptive transfer of Tregs, particularly those being positive for FoxP3 marker (3). FoxP3, also called scurfin, belongs to the forkhead/winged-helix family of transcriptional factors. It acts as a main regulator in the development and function of Tregs whose main functions are attenuation of inflammation and suppression of effector T cells. FoxP3 is known as a reliable marker for Tregs, previously identified by non-specific markers such as CD25 (5, 6). Alterations in the numbers of FoxP3+ Tregs are found in a variety of diseases. For example, patients with an autoimmune disease such as systemic lupus erythematosus have a significant decrease in the suppressive function of Tregs and reduced levels of FoxP3 molecule (7). In contrast, these cells accumulate in tumors and the peripheral blood of cancer patients and promote tumor growth through inhibition of anti-tumor immune responses. However, evidence suggests that in certain cancers, such as colorectal carcinoma, Tregs are advantageous to the host where they suppress bacteria provoked chronic inflammation related to carcinogenesis (3). In fact, in colorectal carcinoma, the relationship between the FoxP3+ Tregs abundance and favorable prognosis has been demonstrated. Tregs appear to suppress destructive inflammation in early stages of carcinogenesis, while they might attenuate the immune response against colorectal carcinoma at advanced stages (3). Immune related genes have been associated with colorectal cancer risk and progression (8). The human *FoxP3* gene contains 11 coding exons, and maps to the p arm of the X chromosome. There are several studies in the case of *FoxP3* polymorphisms and autoimmunity and allergy (9-13). However, a few publications have investigated *FoxP3* polymorphisms in cancer (14, 15), and there is no published literature on *Foxp3* polymorphism and colorectal cancer. In the present study, we aimed to study single nucleotide polymorphisms (SNP) in the promoter region of *FoxP3* gene (C-2383T/rs3761549) in colorectal cancer patients. This SNP has been associated with endometriosis, a benign inflammatory lesion with malignant potential (11, 12, 16, 17). Moreover, the associations between this SNP and clinicopathological parameters of the patients were analyzed.

2. Objectives

The present study was conducted to investigate whether the C-2383T (rs3761549) polymorphism in the promoter region of the *FoxP3* gene is associated with colorectal cancer.

3. Patients and Methods

The study was approved by the Ethics Committee of Shiraz University of Medical Sciences. All the participants were informed that blood samples would be used for genotyping, and their consent was obtained. One-hundred and eight patients with colorectal cancer were enrolled in this study. Histologically, adenocarcima was confirmed in all patients. Data on clinicopathological characteristics of patients were obtained from the patients' medical files (Table 1). Fifty-two patients were men with a mean age of 62.5 ± 10.5 and age range of 39-82 years, and 56 were women with a mean age of 54.9 ± 12.7 and age range of 23-83 years. The control group consisted of 187 healthy individuals with no history of autoimmunity and malignancy. They comprised 126 men with a mean age of 62.0 ± 10.2 and age range of 39-82 years, and 61 women with a mean age of 54.9 ± 12.0 and age range of 23-83 years.

Table 1. Characteristics of 108 Colorectal Cancer Patients at Diagnosis

Characteristics	Men (No. = 52)	Women (No. = 56)
Age at diagnosis, y, Mean ± SD	62.58 ± 10.5	54.9 ± 12.7
Median age, y	60.5	54.0
Tumor location, No. (%)		
Colon	44 (84.6)	51 (91.1)
Rectum	8 (15.4)	5 (8.9)
Tumor stage[a], No. (%)		
I/II	19 (63.3)	15 (53.6)
III/IV	11 (36.7)	13 (46.4)
Histological grade, No. (%)		
Well differentiated	39 (75.0)	27 (48.2)
Moderately or poorly differentiated	13 (25.0)	29 (51.8)
Distant metastasis, No. (%)		
Yes	2 (3.8)	4 (7.1)
No	50 (96.2)	52 (92.9)

[a] Data on tumor stage were not available for all patients.

3.1. DNA analysis

Genomic DNA was extracted using conventional Proteinase K protocol. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used for determination of *C*/T *FoxP3* polymorphism at position -2383 using the following primer pair: forward, 5'-CTGAGACTTTGGGACCGTAG-3' and reverse, 5'-TGCGCCGGGCTTCATCGACA-3'. PCR was performed in a volume of 15 μL, containing 0.6 μL (20 ng) of genomic DNA, 0.6 μL of each primer, 1.2 μL of Taq DNA polymerase, 0.45 μL dNTP, 1.5 μL buffer, 0.50 μL MgCl2 and 9.6 μL of ddH2O. Our PCR condition was initial denaturation at 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 sec, 60 °C for 30 sec, and 72 °C for 30 sec, with the final extension of 72 °C for 10 min. The PCR products then underwent RFLP reaction with restriction enzyme BseNI (Fermentas, Lithuania) and incubated at 65 °C for 16 hours. Finally, the products were separated on 3% agarose gel and visualized by GerRed (Biotium-US) staining. The resulted bands were two fragments of 261 and 127-bp (*T/T* in women and *T* in men), three fragments of 184, 127 and 77-bp (*C/C* in women and *C* in men) or four fragments of 261,184, 127, and 77-bp (*C/T* in women).

3.2. Statistical Analysis

Statistical analysis was performed using SPSS 11.5 software packages. The differences in the distribution of genotypes in different groups were calculated using Pearson's chi-squared test except where stated. The difference between the ages of the patients according to the *FoxP3* polymorphism was calculated using one-way analysis of variance (ANOVA) in women and *t*-test in men.

4. Results

Here, *FoxP3C*-2383T SNP was studied in 108 colorectal cancer patients and 187 healthy controls. The frequencies of the gene variants were analyzed separately in men and women because *FoxP3* is an X-linked gene. The frequency of the genotypes in the patient group did not differ from that in healthy controls in both male and female subgroups ($P > 0.05$), (Table 2). Moreover, the mean age of colorectal cancer onset did not differ among patients by the *FoxP3* polymorphism in men and women, as well. It was 62.2 ± 10.3 and 68.6 ± 14.5 years in carriers of the *C* and *T* genotypes in men, respectively. In women carrying the *C/C* and *C/T* (or *T/T*) genotype, it was 54.9 ± 13.5 and 55.1 ± 11.5 years, respectively.

Table 3 indicates the distributions of the genotypes in the studied groups by tumor grade, tumor stage and the presence of distant metastasis at diagnosis. The genotype frequencies were not significantly associated with tumor stage and grade, though a higher percentage of patients with *T/T* or *C/T* genotype was diagnosed as moderately/poorly adenocarcinoma than patients with the *C/C* genotype in female subgroup (5/8; 62.5% *vs*. 24/48; 50%). The only individual carrying the *T/T* genotype was a female patient whose disease, as poorly differentiated adenocarcinoma in the rectum, was diagnosed at stage IV with distant metastasis. Distance metastasis was significantly associated with the disease as calculated through Pearson's chi-squared test with $P = 0.006$ in men, and $P = 0.03$ in women. While 50% of metastatic patients (1 out of 2 men and 2 out of 4 women) had *T* or *C/T* (or *T/T*) genotype, the percentage of this genotype in non-metastatic patients was 4% in men (2/50) and 11.5% in women (6/52). However, the Pvalues did not remain significant when the differences were calculated using the two-sided Fisher's exact test ($P = 0.11$ in men, and $P = 0.09$ in women).

Table 2. GenotypeFrequencies of FoxP3 Polymorphism in Colorectal Cancer Patients and Healthy Individuals

Genotypes[a]	Case (n=108)	Control (n=187)
Men, No. (%)		
C	49 (94.2)	118 (93.7)
T	3 (5.8)	8 (6.3)
Women, No. (%)		
CC	48 (85.7)	53 (86.9)
CT	7 (12.5)	8 (13.1)
TT	1 (1.8)	0 (0)

[a] Comparing the patient groups with control subjects, P values (calculated using the chi-square test) were not significant.

5. Discussion

A master molecule in the development and function of Tregs is the transcriptional factor FoxP3. Tregs control both adaptive and innate immune responses through several pathways, notably the suppression of $CD4^+$ T-cells through competition for interleukin-2, inactivation of $CD8^+$ T-cells via cell contact and transforming growth factor-β, and attenuation of inflammation through secretion of interleukin-10 (5, 6, 18). Colorectal cancer develops in the presence of huge numbers of organisms and foreign antigens that can provoke a destructive inflammatory reaction if they were not controlled by local regulatory mechanisms, particularly Tregs (3). The chronic inflammation can promote different aspects of tumorigenesis, and has contributed to colorectal cancer pathogenesis (3).

Several polymorphisms exist within the *FoxP3* gene, of which some have contributed to a number of diseases including allergy and autoimmune disease (4, 9-15). For example, Fodor *et al*. analyzed genotype distribution of the rs3761548 *FoxP3* polymorphism in allergic rhinitis among the Hungarians. They found a protective effect of the rare *FoxP3* rs3761548 genotype (*A/A*) against allergic

Table 3. Genotype frequencies of FoXP3 polymorphisms in colorectal cancer

Characteristics	C in Men or CC in Women, No.	T in Men or CT/TT in Women, No.	P Value[a]
Men			
Histological grade			0.73
Well differentiated	37	2	
Moderately or poorly differentiated	12	1	
Tumor stage[b]			Not determined
I/II	19	0	
III/IIV	11	0	
Distant metastasis			0.006
No	48	2	
Yes	1	1	
Women			
Histological grade			0.51
Well differentiated	24	3	
Moderately or poorly differentiated	24	5	
Tumor stage			0.87
I/II	13	2	
III/IIV	11	2	
Distant metastasis			0.03
No	46	6	
Yes	2	2	

[a] P values calculated using the chi-squared test.
[b] The sum is less than the total number of patients because some data were not available.

rhinitis in homozygous women, and susceptibility to the disease in women being either wild-type (*C/C*) or heterozygous (*C/A*) for the rare allele (10). Our group has recently shown the association of an immune response-related gene, named programmed death-1 (PD-1), with colon cancer (8). We, therefore, hypothesized that another immune-related gene *FoxP3* is associated with colorectal cancer. We studied C-2383T (rs3761549) polymorphism in the promoter region of the *FoxP3* gene, because this SNP (*C/T* genotype) was associated with endometriosis (12, 13), a benign inflammatory lesion whose malignant transformation has been reported (16, 17). A few publications are available regarding cancer and *FoxP3* polymorphisms. They essentially failed to demonstrate a significant association between the studied cancer and *FoxP3* polymorphism (14, 15). We found that the genotype frequencies were not significantly different between controls and colorectal cancer patients. Moreover, the SNP was not significantly associated with clinicopathological characteristics of the patients including age, tumor grade and stage in both men and women. However, a significant association of metastatic colorectal cancer was observed with *T* genotype in men and *C/T* (*T/T*) genotype in women.

It has been shown that, in addition to lymphocytes, FoxP3 is aberrantly expressed in several tumor cells, including colorectal cancer cells. Although, the consequence of FoxP3 expression by tumor cells remains controversial, studies conducted on human samples have generally reported its association with metastasis (19). Whether -2383 *C* to *T* polymorphism of *FoxP3* gene through direct effects on FoxP3 molecule and disturbance of a balanced immune response in T lymphocytes, and/or its aberrant expression by tumor cells, or indirectly through linkage disequilibrium with other genes contributes to colorectal metastasis needs more investigation. The limitation of our study was the low number of our metastatic patients. The difference between our metastatic and non-metastatic groups regarding the distribution of *FoxP3* polymorphism did not remain significant when the differences were calculated using the two-sided Fisher's exact test, a likely more appropriate test when the data were unequally distributed among the cells of the table (20). In summary, for the first time, we investigated the association of *FoxP3* polymorphism with colorectal carcinoma. Our results support a significant association between metastasis and *FoxP3* polymorphism; this should

be confirmed in a larger number of patients. Further research is also required to disclose the functional consequence of *C/T (T/T)* genotype of C-2383T polymorphism on *FoxP3* molecule.

Acknowledgements

This work was financially supported by grants from Shiraz Institute for Cancer Research, and Shiraz University of Medical Sciences (91-01-16-4762). The authors declare no conflict of interest.

Authors' Contribution

Dr Mojtahedi, Erfani and Ghaderi have done Design, data analysis, revising the article draft, and final approval of the version to be published. Dr. Hosseini provided the conditions for the Referral of patients and prepared the article draft. Dr. Haghshenas collected the Data and prepared the article draft.

Financial Disclosure

The authors declare no conflict of interest.

Funding/Support

This work was financially supported by grants from Shiraz Institute for Cancer Research , and Shiraz University of medical sciences.

References

1. Potter JD. Colorectal cancer: molecules and populations. *J Natl Cancer Inst.* 1999;**91**(11):916-32.
2. Masoompour SM, Yarmohammadi H, Rezaianzadeh A, Lankarani KB. Cancer incidence in southern Iran, 1998-2002: results of population-based cancer registry. *Cancer Epidemiol.*2011;**35**(5):e42-7.
3. Whiteside TL. What are regulatory T cells (Treg) regulating in cancer and why? *Semin Cancer Biol.* 2012;**22**(4):327-34.
4. Park O, Grishina I, Leung PS, Gershwin ME, Prindiville T. Analysis of the Foxp3/scurfin gene in Crohn's disease. *Ann N Y Acad Sci.* 2005;**1051**:218-28.
5. Bilate AM, Lafaille JJ. Induced CD4+Foxp3+ regulatory T cells in immune tolerance. *Annu Rev Immunol.* 2012;**30**:733-58.
6. Rowe JH, Ertelt JM, Way SS. Foxp3(+) regulatory T cells, immune stimulation and host defence against infection. *Immunology.* 2012;**136**(1):1-10.
7. Valencia X, Yarboro C, Illei G, Lipsky PE. Deficient CD4+CD25high T regulatory cell function in patients with active systemic lupus erythematosus. *J Immunol.* 2007;**178**(4):2579-88.
8. Mojtahedi Z, Mohmedi M, Rahimifar S, Erfani N, Hosseini SV, Ghaderi A. Programmed death-1 gene polymorphism (PD-1.5 C/T) is associated with colon cancer. *Gene.* 2012;**508**(2):229-32.
9. Zhang Y, Duan S, Wei X, Zhao Y, Zhao L, Zhang L. Association between polymorphisms in FOXP3 and EBI3 genes and the risk for development of allergic rhinitis in Chinese subjects. *Hum Immunol.* 2012;**73**(9):939-45.
10. Fodor E, Garaczi E, Polyanka H, Koreck A, Kemeny L, Szell M. The rs3761548 polymorphism of FOXP3 is a protective genetic factor against allergic rhinitis in the Hungarian female population. *Hum Immunol.* 2011;**72**(10):926-9.
11. Ban Y, Tozaki T, Tobe T, Ban Y, Jacobson EM, Concepcion ES, et al. The regulatory T cell gene FOXP3 and genetic susceptibility to thyroid autoimmunity: an association analysis in Caucasian and Japanese cohorts. *J Autoimmun.* 2007;**28**(4):201-7.
12. Andre GM, Barbosa CP, Teles JS, Vilarino FL, Christofolini DM, Bianco B. Analysis of FOXP3 polymorphisms in infertile women with and without endometriosis. *Fertil Steril.* 2011;**95**(7):2223-7.
13. Barbosa CP, Teles JS, Lerner TG, Peluso C, Mafra FA, Vilarino FL, et al. Genetic association study of polymorphisms FOXP3 and FCRL3 in women with endometriosis. *Fertil Steril.* 2012;**97**(5):1124-8.
14. Kim MS, Yoo NJ, Lee SH. Absence of tumour suppressor FOXP3 gene mutation in prostate cancers of Korean patients. *Pathology.* 2011;**43**(3):287.
15. Raskin L, Rennert G, Gruber SB. FOXP3 germline polymorphisms are not associated with risk of breast cancer. *Cancer Genet Cytogenet.* 2009;**190**(1):40-2.
16. Gonzalez-Ramos R, Defrere S, Devoto L. Nuclear factor-kappaB: a main regulator of inflammation and cell survival in endometriosis pathophysiology. *Fertil Steril.* 2012;**98**(3):520-8.
17. Mann S, Patel P, Matthews CM, Pinto K, O'Connor J. Malignant transformation of endometriosis within the urinary bladder. *Proc (Bayl Univ Med Cent).* 2012;**25**(3):293-5.
18. Gounaris E, Blatner NR, Dennis K, Magnusson F, Gurish MF, Strom TB, et al. T-regulatory cells shift from a protective anti-inflammatory to a cancer-promoting proinflammatory phenotype in polyposis. *Cancer Res.* 2009;**69**(13):5490-7.
19. Triulzi T, Tagliabue E, Balsari A, Casalini P. FOXP3 expression in tumor cells and implications for cancer progression. *J Cell Physiol.* 2013;**228**(1):30-5.
20. Fisher's exact test. 2012; Available from: www.wikipedia.org