RESEARCH ARTICLE

Lack of Influence of the *SMAD7* Gene rs2337107 Polymorphism on Risk of Colorectal Cancer in an Iranian Population

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Abstract

***SMAD7* has been identified as a functional candidate gene for colorectal cancer (CRC). *SMAD7* protein is a known antagonist of the transforming growth factor beta (TGF-β) signaling pathway which is involved in tumorigenesis. Polymorphisms in *SMAD7* may thus alter cancer risk. The aim of this study was to investigate the influence of a *SMAD7* gene polymorphism (rs2337107) on risk of CRC and clinicopathological features in an Iranian population. In total, 210 subjects including 105 patients with colorectal cancer and 105 healthy controls were recruited in our study. All samples were genotyped by TaqMan assay via an ABI 7500 Real Time PCR System (Applied Biosystems) with DNA from peripheral blood. The polymorphism was statistically analyzed to investigate the relationship with the risk of colorectal cancer and clinicopathological properties. Logistic regression analysis revealed that there was no significant association between rs2337107and the risk of colorectal cancer. In addition, no significant association between genotypes and clinicopathological features was observed (p value>0.05). Although there was not any association between genotypes and disorder, CT was the most common genotype in this population. This genotype prevalence was also higher in the patients with well grade (54.9%) and colon (72.0%) tumors. Our results provide the first evidence that this polymorphism is not a potential contributor to the risk of colorectal cancer and clinicopathological features in an Iranian population, and suggests the need of a large-scale case-control study to validate our results.**

Keywords: Colorectal cancer - *SMAD7* - TNM stage - single nucleotide polymorphism

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Introduction

Colorectal cancer (CRC) as the third most common cancer brings about the fourth cause of worldwide cancer mortality (Jemal et al., 2011; Shemirani et al., 2011). According on World Health Organization (WHO) reports, CRC is one of the common diseases in an Asian population (Moghimi-Dehkordi et al., 2012; Haerian et al., 2014). It is noticeable that incidence of this cancer has been rising in an Iranian population during recent decades (Azadeh et al., 2007; Milanizadeh et al., 2013). Thereby, the importance of CRC as a major worldwide health issue is also increasing in our population (Haghighi et al., 2009). Although numerous risk factors and causes have been taken into account for CRC, genetic component has a great contribution on CRC development. To emphasize this fact, nearly 35% of total cases have been reflected by twin- and family based studies (Lichtenstein et al., 2000).

It has been established that the transforming growth factor beta (TGF-β) signaling pathway has strongly contributed to tumor initiation, invasion, and metastasis (Massagué, 2008). The point is that the TGF-β signaling pathway has been regarded as both a tumor suppressor pathway and a promoter of tumor progression and invasion (Derynck et al., 2001). The increased TGF-β1 expression correlation with tumor progression and recurrence in CRC has been reported (Xu et al., 2007). TGF-β signaling pathway is mediated by receptors and intracellular signal transducers known as the *SMADs* (Levy et al., 2006).

SMAD7 was initially identified as an inhibitor of TGF-β because of its ability to bind TGF-β receptor typeI and prevent TGF-β-associated SMAD signaling (Yan et al., 2011). *SMAD7* as a negative regulator of the TGF-β signaling pathway promotes the anti-inflammatory effects of TGF-β signaling, which performed by binding to TAB2 and TAB3 and inhibiting TAK1 (Hong et al., 2007). Two

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GWAS introduced a risk locus for CRC at 18q21 which maps to *SMAD7*, a functional candidate gene for CRC (Broderick et al., 2007; Tenesa et al., 2008). Despite of *SMAD7* role in hepatic metastasis in CRC (Halder et al., 2008), its role in cancer development, particularly colorectal cancer, has not been utterly investigated.

Although it has been shown that *SMAD7* has a compounding role as an intracellular mediator of TGF-β typeI receptor in cancer development, the relevance of several genetic variants within *SMAD7* with CRC has been proved by several studies (Thompson et al., 2009; Slattery et al., 2010; Li et al., 2011; Nassiri et al., 2013). Single-nucleotide polymorphisms (SNPs) were determined as the most frequent and subtle genetic variation in the human genome and has great potential for application to association studies of multifactorial disorder (Kirk et al., 2002).

In this end, we explored one polymorphism of *SMAD7*, rs2337107; based on hypothesis that there may be the association between that SNP and CRC in an Iranian population. Additionally, we used the clinical features to investigate the correlation between them and mentioned SNP. Factors evaluated including an age, sex, tumor site, tumor grade and stage.

Materials and Methods

Study population

Our sample study was comprised of one hundred and five controls and one hundred and five patients. All patients were diagnosed with CRC and histologically confirmed by positive colonoscopy and pathology results for colon or rectum malignant tumor. Patients for the present study were recruited from October 2007 until January 2009 in cancer registry unit of the Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. All participants signed an informed consent prior to participation in the study, and all healthy subjects and patients completed a self-administrated questionnaire. Besides, all procedures were approved by Ethic committee of the Gastroenterology and Liver Diseases Research Center. Clinical data, pathological grade and clinical stage, were abstracted from medical reports. The histological classification and pathological staging were determined on the criteria of the UICC Tumor-Node-Metastasis classification of Malignant Tumors (TNM) 6th edition, 2002, colon and rectum (ICD-O C18-C 20). The controls were randomly selected in the same time. Our control population was individuals who showed no colonoscopy report for malignancy inflammatory ulcers or polyps, and they also have no families with the history of gastrointestinal defects.

DNA isolation and genotyping

Genomic DNA was extracted from peripheral blood using a phenol-chloroform standard protocol (Green et al., 2012). Agarose gel-electrophoresis was used to assay the quality of genomic DNA and then DNA concentration determined by NanoDrop spectrophotometry 1000. Determination of samples genotyping for the one *SMAD7* SNP (rs2337107) was performed via predesigned TaqMan probes (C-26177715-20; Applied Biosystems, Foster City, CA) via an ABI 7500Real Time PCR System (Applied Biosystems). The polymerase chain reaction was done with these conditions: 95 °C for10 minutes and 40 cycles of 92 °C for 15 seconds and 60 °C for 1 minute. SDS software version 1.3 (Applied Biosystems) was our analytical tool to identify individual genotypes.

Statistical analysis

SPSS software version 13 was applied to calculate statistical analysis. Distribution of the allele and genotype frequencies and also clinicopathological characteristics were compared by using of χ^2 test. Logistic regression analysis which used for the adjustment of confounding factors such as age, gender and smoking, was carried out to calculate Odds ratio (OR) and its 95% confidence intervals (95% CI). T-test or χ^2 test were used to determine differences in demographic factors. Data were considered significant if they had p values less than 0.05 in all comparisons.

Results

Baseline characteristics

The frequency distributions of baseline characteristics and histological parameters in one hundred and five colorectal cancer cases and one hundred and five healthy subjects are presented in Table 1. Baseline characteristics of patients and controls were all well matched.

The mean age of CRC patients and controls were 51.27 (Standard deviation, SD±15.05) and 42.74 (SD±15.24) years respectively. Logistic regression method was applied to adjust the effect of age, gender and smoking status as confusing variations.

The prevalence of colon cancer in the patients was noticeably more than rectal cancer (70.5% and 29.5%, respectively). According to histological differentiation of tumor grades, 5.6%, 27.6%, and 46.7%, patients were classified in three grades, poor, moderate, or well grade,

Table 1. Characteristics of Patients and Controls

Characteristics		Total (n=210)		p value
		CRC (105) N (%)	C (105) N (%)	
Sex	Female	51 (48.6)	64 (61.0)	0.071
	Male	54 (51.4)	41 (39.0)	
Age (years), Mean (SD)		51.27±15.05	42.74±15.24	0.603
Smoking status	Never	90 (85.7)	97 (92.4)	0.122
	Current	15 (14.3)	8 (7.6)	
Tumor location	Colon	74 70.5)		
	Rectum	31 (29.5)		
Grade	Poor	9 (5.6)		
	Moderate	29 (27.6)		
	Well	49 (46.7)		
	Not determined	18 (17.1)		
TNM	I	15 (14.3)		
	II	35 (33.3)		
	III	47 (44.8)		
	IV	8 (7.6)		
Dukes stage	A	5 (4.8)		
	B	46 (43.8)		
	C	45 (42.9)		
	D	9 (8.6)		

*CRC: colorectal cancer patients; C: controls; TNM: Tumor Node Metastasis

respectively. Besides, patients were grouped into four classes from I to IV regarding tumor node metastasis (TNM) at the time of diagnosis, 14.3%, 33.3%, 44.8%, and 7.6%, respectively.

Genotypic and allelic frequencies

The genotypic and allelic frequencies of the one SNP of *SMAD7* in the whole study groups are summarized in Table 2. Furthermore, Figure 1, 2 illustrates the patterns of genotypes of *SMAD7* gene. Genotype frequencies of the SNP for each group separately were in accordance with Hardy–Weinberg equilibrium (for cases: p value =0.955 and for controls p value =0.145). Genotypes and allelic distributions of this genetic polymorphism of *SMAD7* were not significantly different between CRC patients and control groups.

The results of association between the *SMAD7* genotypes and clinicopathological characteristics in colorectal cancer patients are shown in Table 3. There was no significant association between *SMAD7* genotypes of rs12337107 and these features. Although there was not any significant association between genotypes and disorder, CT was the most common genotype in this population.

Table 2. Distribution of *SMAD7* Genotypes among Colorectal Cancer Patients and Controls

	Patients[a] (n=105)	Controls[a] (n=105)	p value	OR (95% CI) Crude	Adj.[c]
Genotype-wise comparison, n (%)					
CC	35 (33.3)	33 (31.4)	0.533	1.0 (Reference)	1.0 (Reference)
CT[b]	51 (48.6)	58 (55.2)	0.545	0.829 (0.452-1.520)	0.943 (0.494-1.798)
TT[b]	19 (18.1)	14 (13.3)	0.564	1.280 (0.553-2.959)	1.301 (0.541-3.132)
CT or TT	70 (66.7)	72 (68.6)	0.957	0.917 (0.514-1.634)	1.017(.0549-1.885)
Allele-wise comparison, n (%)					
C	121 (57.6)	124 (59.0)	0.767	1.0 (Reference)	-
T	89 (42.4)	86 (41.0)		1.601 (0.719-1.563)	-

[a]The observed genotype distribution of patients and controls were in agreement with the Hardy-Weinberg equilibrium; [b]Additive Genetic model; [c]Adjusted for age, gender and smoking status

Table 3. Association between *SMAD7* Genotypes and Clinic Pathological Characteristics

Characteristics		Genotype CC	CT	TT	p value
Tumor grade	Well	11	28	10	0.398
	Moderate	12	12	5	
	Poor	3	5	1	
	Not determined	9	6	3	
Location	Colon	24	37	13	0.903
	Rectum	11	14	6	
TNM Stage	0+I+II	16	22	12	0.317
	III+IV	19	29	7	
T	T1	1	1	1	0.495
	T2	7	10	2	
	T3	23	33	16	
	T4	4	4	0	
	Unknown	0	3	0	
N	N0	16	23	12	0.469
	N1	11	20	7	
	N2	5	5	0	
	Unknown	3	3	0	
M	M0	32	46	19	0.376
	M1	3	5	0	
Dukes stage	A	0	4	1	0.478
	B	15	24	7	
	C	18	19	8	
	D	2	4	3	

*TNM, Tumor Node Metastasis

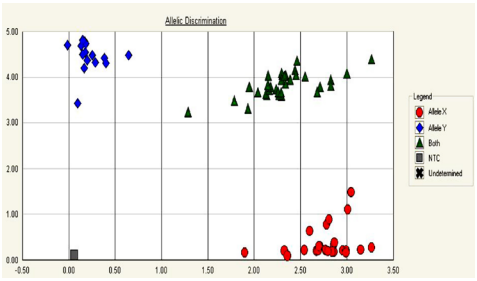

Figure 1. Allelic Discrimination Plot Represents Genotypes with Four Symbols (Squares, Diamonds, Triangles and Circles) for NTC Sample, CC, CT and TT Genotypes Respectively. The x-axis is amount of emission for flourophore channels (FAM) and on the y-axis represents emission for flourophore channels (VIC)

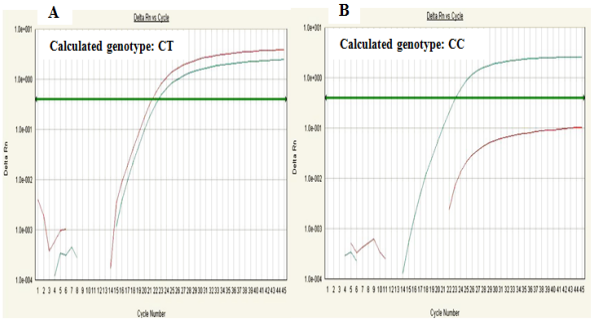

Figure 2. Allelic Discrimination Curves Produced by the SDS Analysis Software. The x-axis is the amplification cycle number and on the y-axis represents raw fluorescent value. A) Example of a true heterozygote (CT) with the amplification curve for both flourophore channels (VIC, FAM); B) Example of a true homozygote (CC) with the amplification curve of the VIC channel

This genotype prevalence was also higher in the patients with well grade and colon tumors.

Discussion

SMAD7 acts as an intracellular antagonist of TGF-β signaling by binding stably to the receptor complex and blocking activation of downstream signaling events (Tenesa et al., 2009). Perturbation of *SMAD7* expression has been documented to influence CRC progression (Levy et al., 2006), and loss of chromosome 18q21 including *SMAD7* is common in CRC (Gaasenbeek et al., 2006). In this study, one polymorphism of this gene, rs2337107, was investigated to determine the correlation between *SMAD7* gene and CRC in an Iranian population. We also pursued the association of this polymorphism with clinicopathological factors such as tumor location, TNM stage and tumor grade. Our analysis demonstrated that variant genotypes of this polymorphism had no association with the risk of colorectal cancer in our population.

The noticeable point is that data emerging from experimental studies indicate that *SMAD7* may be differently regulated in various tumors depending on the context analyzed (Stolfi et al., 2013). Since *SMAD7* is considered as an inhibitor of TGF-β signaling, it's opposite (pro- and anti-tumorigenic) effects could originate from the different functionality of TGF-β1 pathway among distinct cancer types (Stolfi et al., 2013).

Although the potential importance of *SMAD7* in the etiology of CRC is supported by several researches and the GWAS (Tomlinson et al., 2008; Slattery et al., 2010; Song et al., 2012), underlying mechanism has not been fully elucidated. Boulay et al. (2003) found that CRC patients with deletion of *SMAD7* had a favorable clinical outcome compared with patients with *SMAD7* amplification (Boulay et al., 2003), and Halder et al reported that *SMAD7*-overexpressing FET cells show aggressive colony formation on soft agar and increased tumorigenicity in vivo compared with control FET cells (Halder et al., 2005). In contrast above finding, the opposing role of *SMAD7* in the control of sporadic and colitis-associated CRC has been reported by one study in which they showed that over-expression of *SMAD7* in T cells associates with severe colitis and reduces the growth of colitis-associated CRC (Rizzo et al., 2011). Therefore, further research on the functionality of *SMAD7* variants would be needed to better understand the observed associations.

Our results showed no significant genetic association between this intronic SNP, rs2337107 C>T, with CRC. Although genotype and allele distributions of this polymorphism did not show any significant disease association, our results confirmed that the heterozygote genotype of this SNP (CT) is the most common ones like Asian, European and some of Sub-Saharan African populations, according on NCBI information (www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi.rs=2337107). Furthermore, there were also no significant differences between this polymorphism and clinicopathological factors. Despite more frequency of CT genotype in patients with colon location and well grade tumors than others, the results indicated that we cannot recommend this polymorphism as a biomarker for CRC in an Iranian population.

There have been some earlier studies on the association *SMAD7* gene variants and CRC. Alemn et al. interrogated all polymorphisms within the 17-kb region of the 18q21 locus, which on the basis of LD (linkage disequilibrium), protects the disease-causing variants responsible for the *SMAD7*–18q21 association with CRC. Their results showed that rs2337107, not alone but together with 24 another SNPs, had an association with the developing of CRC at the 5% statistical threshold (Pittman et al., 2009). There is two another studies which implemented by Martah et al. and Xuejuan et al. separately, that their results demonstrated no association between rs2337107 and CRC in their population (Slattery et al., 2010; Jiang et al., 2013). Our result also appears consistent with these results.

This study was conducted in a well-defined homogenous sample with detailed clinical data. However, one of our limitations was sample size. Since we were in sanction and the price of probes were so high, we could only provide the small size of predesigned probes (S: 188uL; 40X). It seems logical that the genotype differences may be strictly attributed to chance due to the modest sample size. Therefore, larger population is needed to elucidate the exact conclusion of this SNP frequency in our population. Another point is that only one variant of the *SMAD7* gene was genotyped, and it was insufficient to conclude about the effect of whole gene. Since it has been documented that this SNP has a high linkage disequilibrium with three common genetic variations of *SMAD7* which strongly associated to CRC, it may be more informative to study the more number of *SMAD7* polymorphisms along with this SNP to throw light on the role of CRC progression in an Iranian population.

In conclusion, there is the first case-control study which has explored the influence of rs2337107 of *SMAD7* gene on clinicopathological features and CRC risk in Iranian population. Furthermore, the results showed no evidence of association between this SNP and the risk of initiation and development of CRC, also there was no significant effect of this SNP on clinicopathological features. To regard this point that this study had some inherent limitations such as small sample size; further studies in various populations should be implemented to clarify the association of this SNP with colorectal cancer.

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