Transforming growth factor-β1 -509T reduces risk of colorectal cancer, but not adenoma in Koreans

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The proliferation of colorectal epithelial cells is regulated by various stimuli including cytokines and growth factors, thus the variants of those genes can modify the colorectal cancer risk. TGF-β1 can act as both a tumor suppressor and a stimulator of tumor progression. TGF-β1 C-509T polymorphism in the promoter sequence has been associated with increased levels of plasma TGF-β1 in individuals with T allele. To evaluate the potential influences of this polymorphism on colorectal adenoma and cancer risk, a case-control study was conducted in Korea. A total of 646 subjects were prospectively enrolled in Seoul National University Hospital. Risk of colorectal neoplasms was evaluated separately for 244 patients with colorectal adenoma, 152 patients with colorectal cancer relative to 250 healthy controls. Genotypes were determined by the PCR-RFLP method. ORs and 95% CIs were calculated by a multivariate logistic regression analysis. The TGF-β1 -509T allele containing genotypes posed a reduced risk of colorectal cancer (adjusted OR = 0.59, 95% CI = 0.28–0.92). But there was no association between this polymorphism and colorectal adenoma. Our results suggest that the TGF-β1 -509T allele may have a protective role in the development of colorectal cancer, possibly consistent with its role as an inhibitor of epithelial malignant transformation. (*Cancer Sci* 2007; 98: 401–404)

Colorectal cancer is one of the most common cancers in the world, accounting for nearly 10% of all incident cases of cancer.[1] Korea has experienced a rapid increase in mortality from colorectal cancer in the past 50 years.[2] As resources are increasingly directed towards disease prevention, strategies for identifying and targeting high-risk individuals are important. Most sporadic colorectal cancers derive from pre-existing adenomatous polyps through an adenoma-carcinoma sequence driven by altered expression of several genes that control cellular proliferation, apoptosis, and intercellular contacts.[3–5]

Although genetic and environmental risk factors for colorectal carcinogenesis have been described,[6,7] specific growth factors or cytokines that contribute to the development of colorectal neoplasms have not been identified. TGF-β has been identified as both a tumor suppressor during the early stages of carcinogenesis and a stimulator of tumor growth, invasion and metastasis during tumor progression.[8–12] Among three TGF-β isoforms (TGF-β1, TGF-β2 and TGF-β3), TGF-β1 demonstrates a growth-inhibiting effect on gastrointestinal epithelium.[13,14] Changes in the expression of TGF-β1, have been implicated in oncogenesis in transgenic mice; deletion of one copy of the TGF-β1 gene leads to increased cell turnover and susceptibility to liver and lung tumors when induced by carcinogens.[15] In addition, TGF-β is an important immunoregulatory cytokine within the gastrointestinal tract and this is shown in TGF-β knockout mice, which proceed to develop uncontrolled gastrointestinal inflammation.[16]

On the other hand, many tumor cells overexpress TGF-β, and are resistant to its growth-inhibiting effects.[9,10] It has been known that increased production of TGF-β inhibits local inflammatory responses, thereby allowing cancer cells to escape host immunosurveillance.[9,10] Increased levels of plasma TGF-β1 in cancer patients and overexpression of TGF-β1 in tumor cells have both been shown to correlate with increased tumor vascularity and a poor prognosis.[10,17,18] TGF-β1 can induce the expression of cyclooxygenase-2,[19] which has been implicated in colon carcinogenesis.[20]

Single nucleotide polymorphisms have emerged as one of the important tools for tracking down the genes responsible for conferring susceptibility to cancer. Previous published studies of TGF-β1 gene polymorphisms have not detected any relation between the distribution of these polymorphisms and the susceptibility of colorectal adenoma or cancer. An American case–control study reported that a reduced risk of colorectal hyperplastic polyp is associated with the C allele of TGF-β1 T869C (A10P) but there was no association between this polymorphism and colorectal adenomatous polyp.[21] A European population-based case-control study showed no significant association of TGF-β1 C-509T polymorphisms with colorectal cancer.[22]

The production of TGF-β1 is under genetic control,[23,24] and seven polymorphisms have been described in the TGF-β1 gene, including a C to T transition at −509 in the region encoding the promoter.[25] Because the T allele has been related to a higher plasma concentration of the cytokine and a higher level of transcriptional activity than the C allele,[23] it could be postulated that having the -509T allele is protective against colorectal neoplasms. The present study aimed to investigate the relationship between TGF-β1 C-509T polymorphism and the risk of colorectal adenoma or cancer occurrence in Korea.

Materials and Methods

Subjects. This study is a hospital-based case-control study. A total of 646 Korean subjects were consecutively enrolled; eligible subjects consisted of a series of histologically confirmed colorectal adenoma ($n = 244$) and sporadic colorectal cancer patients ($n = 152$) from the Division of Gastroenterology in Seoul National University Hospital and 250 healthy controls from the Seoul National University Hospital Healthcare System between November 2005 and June 2006. The study design was approved by the Institutional Review Board of Seoul National University Hospital. Informed consent was obtained at the time of blood sampling.

Genotyping. The TGF-β1 C-509T genotypes were determined by the PCR-RFLP method. Genomic DNA was extracted from the buffy coat by using a commercial kit (Gene All, General Biosystem, Seoul, Korea) and stored at −70°C until use. PCR was carried out in a reaction mixture of 25 μL containing 0.5 units of Taq DNA polymerase and template DNA 100 ng using a GeneAmp PCR System 9600 (Perkin Elmer Biosystems, Foster City, CA, USA). The primer sequences used in the

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Abbreviations: TGF-β, transforming growth factor-beta; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; OR, odds ratio; CI, confidence interval; NSAID, non-steroidal anti-inflammatory drug.

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Table 1. Characteristics of the study population

Variable	Controls (n = 250)	Colorectal neoplasm			
		Adenoma (n = 244)	P-value*	Cancer (n = 152)	P-value*
Location of largest tumor (%)					
Proximal		28.7		23.7	
Distal		54.9		39.5	
Rectum		16.4			36.8
Mean age (years)	56.9	58.6	0.164	60.0	0.332
Sex					
Male	51.5	71.7	0.037	62.5	0.101
Female	48.5	28.3		37.5	
Family history of colorectal cancer					
Yes	19 (7.6)	23 (9.4)	0.467	15 (9.9)	0.603
Smoking status					
Yes	16.8	32.4	0.019	23.0	0.040
Regular user of aspirin or NSAIDs					
Yes	17.6	20.9	0.107	25.0	0.211

*Compared with controls. NSAIDs, non-steroidal anti-inflammatory drugs.

present study are as follows: ATA AGG GCC TTA GGA CAC CA (sense), and GCT ACT TCT CCA GGC TCA CA (antisense). The amplification was conducted by 5 min of initial denaturation at 95°C, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 62°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 7 min. Aliquots of the 330 bp PCR product were digested with 4 IU of *Bsu*36 I (New England BioLABS, UK) at 37°C overnight and visualized on a 3% agarose gel containing 2 μL/100 mL of ethidium bromide. We carried out direct sequencing for selected samples in order to confirm our PCR-RFLP data. The results of specific nucleotide sequences for selected PCR products were all exactly matched with those of our PCR-RFLP data.

Statistical analysis. The χ^2 tests or t-tests were used for comparing characteristics of cases and controls. To assess the effect of each genotype on colorectal neoplasm risk, the genotype and allele frequencies for TGF-β1 C-509T were compared between the normal subjects and patients with colorectal adenoma or cancer using a χ^2 test. The association between TGF-β1 C-509T polymorphism and the risk of colorectal adenomas or cancers was assessed by means of adjusted and stratified ORs, and 95% CIs according to a multivariate logistic regression analysis. Statistical adjustment was made for age, sex, family history of colorectal cancer, smoking, and regular use of aspirin or NSAIDs (at least once per week). All statistical tests were two-sided and all analyses were performed using the Statistical Package for the Social Sciences (version 12.0; SPSS Inc. Chicago, IL, USA). A $P < 0.05$ was considered to be statistically significant.

Table 2. Genotype and allele frequency of transforming growth factor-β-1 C-509T polymorphism in normal subjects and colorectal adenoma or cancer patients

	Controls (n = 250)		Colorectal neoplasm					
			Adenoma (n = 244)		P-value*	Cancer (n = 152)		P-value*
	n	%	n	%		n	%	
Genotypes								
CC	60	24.0	69	28.3	0.385	53	34.9	0.107
CT	137	54.8	123	50.4		69	45.4	
TT	53	21.2	52	21.3		30	19.7	
Alleles								
C	257	51.4	261	53.5	0.780	175	57.6	0.347
T	243	48.6	227	46.5		129	42.4	

*Compared with controls.

Results

Demographic and other selected characteristics of cases and controls are presented in Table 1. Individuals with colorectal adenoma or cancer were more likely to smoke compared with controls. Cases and controls did not show statistically significant differences with regard to age, family history of colorectal cancer and regular use of aspirin or NSAID. The genotype distribution of TGF-β1 -509 polymorphism was not significantly different between the controls and cases (Table 2). No evidence of a departure from the Hardy–Weinberg equilibrium was apparent in all groups, with an allele frequency of 0.439 for the T allele in healthy controls.

The -509 CT and TT TGF-β1 genotypes showed weakly inverse relationship with the risk of colorectal adenomas, relative to the CC (wild type) genotype without statistical significance (Table 3). There were no significant differences in the ORs of colorectal adenomas when stratified by location, size, or number of the largest tumors (data not shown). On the other hand, the risk of colorectal cancer was significantly lower in patients with TGF-β1 -509T allele containing genotypes (CT or TT) compared with those with the CC genotype (adjusted OR = 0.59, 95% CI = 0.28–0.92). The risk of colorectal cancer did not appear to vary largely within strata of putative risk factors; age, sex, smoking and regular use of aspirin or NSAID (Table 4). There were no statistically significant associations of striking patterns or differences in the ORs when stratified by the tumor location, size, stage, differentiation or metastasis (data not shown).

Discussion

To our best knowledge, this is the first report of a strong association between polymorphisms in the TGF-β1 gene and the

Table 3. Association of transforming growth factor-β1 C-509T polymorphism with risk of colorectal neoplasms

Genotypes	Colorectal neoplasm			
	Adenoma (n = 244)		Cancer (n = 152)	
	Crude OR (95% CI)	Adjusted OR (95% CI)†	Crude OR (95% CI)	Adjusted OR (95% CI)†
CC	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
CT	0.78 (0.29–1.29)	0.78 (0.28–1.29)	0.57 (0.25–0.90)	0.56 (0.24–0.90)
TT	0.85 (0.34–1.35)	0.85 (0.34–1.36)	0.64 (0.29–0.98)	0.62 (0.28–0.97)
CT + TT	0.80 (0.30–1.32)	0.81 (0.29–1.30)	0.59 (0.30–0.93)	0.59 (0.28–0.92)

†Adjusted for age, sex, family history of colon cancer, smoking, and regular use of aspirin or non-steroidal anti-inflammatory drugs (NSAIDs). CI, confidence interval; OR, odds ratio.

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Table 4. Association between transforming growth factor-β-1 C-509T polymorphism with risk of colorectal cancer, stratified by factors associated with cancer development

Controls (n = 250) (%)		Cases (n = 152) (%)	Genotypes		
			CC OR (95% CI)†	CT OR (95% CI)†	TT OR (95% CI)†
Age (years)					
<60	58.8	46.1	1.00 (reference)	0.98 (0.62–1.59)	0.69 (0.32–1.33)
≥60	41.2	53.9	1.05 (0.47–1.80)	0.87 (0.38–1.51)	0.72 (0.40–1.45)
Sex					
Male	51.5	62.5	1.00 (reference)	0.82 (0.45–1.59)	0.92 (0.29–2.12)
Female	48.5	37.5	0.96 (0.18–1.92)	1.19 (0.47–2.95)	0.76 (0.28–1.75)
Family history of colorectal cancer					
No	92.4	90.1	1.00 (reference)	0.93 (0.33–1.21)	0.78 (0.26–1.30)
Yes	7.6	9.9	1.05 (0.40–1.39)	0.69 (0.25–1.40)	0.88 (0.31–1.60)
Smoking					
No	64.0	42.8	1.00 (reference)	2.31 (0.44–4.52)	1.59 (0.55–2.82)
Yes	36.0	57.2	0.93 (0.26–1.62)	1.65 (0.36–2.48)	1.81 (0.55–3.01)
Regular use of aspirin or NSAIDs					
No	82.4	79.1	1.00 (reference)	0.63 (0.32–1.27)	0.77 (0.26–1.59)
Yes	17.6	20.9	1.50 (0.38–2.15)	0.83 (0.32–2.02)	0.63 (0.21–1.44)

†Adjusted for age, sex, family history of colorectal cancer, smoking, and regular use of aspirin or NSAIDs where appropriate. CI, confidence interval; NSAIDs, non-steroidal anti-inflammatory drugs; OR, odds ratio.

risk of colorectal cancer. The present study had methodological advantages in that comparative analyses were carried out in both colorectal adenoma and cancer patients with normal controls simultaneously. We had hypothesized that individuals with TGF-β1 -509T allele could be at a reduced risk of colorectal neoplasms given that this allele has been associated with increased levels of TGF-β1 mRNA and protein, and overexpression of TGF-β1 in animal models has been shown to reduce the occurrence of benign epithelial growth.[8]

There is a strong rationale for investigating the role of this genetic variant as a possible contributor to cancer susceptibility. It has been estimated that more than 80% of colorectal tumors have inhibitory mutations in the TGF-β pathway.[26] Laboratory studies have shown that TGF-β is an important regulator of various cellular process in the normal or malignant colorectal epithelium.[13,27] Acting through its downstream elements, Smad proteins and its receptors, TGF-β inhibits cell cycle progression, and then acts as a tumor suppressor in early stages of tumor development.[28,29] In later stages, as a result of changes in tumor cell responsiveness, TGF-β acts as a promoter by enhancing tumor cell motility and invasiveness.[30,31] Furthermore, high levels of TGF-β1 in patients with colorectal cancer has been shown to correlate with disease progression and metastasis.[32–34] This dual role has been clearly demonstrated in several transgenic mouse models, some highly relevant to human colorectal carcinogenesis.[35,36]

The present study showed that the possession of the -509T allele (T/T or C/T) is associated with a decreased risk of colorectal cancer as compared with individuals having the C allele only (C/C). These findings suggest that TGF-β1-induced suppression of colorectal cancer could be augmented by a slight increase of the TGF-β1 level by these genetic factors. Our data are consistent with the results of studies in transgenic mice and in hepatoma cell lines.[15,37] Transgenic mice with a single TGF-β1 gene deletion are more susceptible to liver and lung tumors induced by carcinogens,[15] while the increased expression of TGF-β1 under the control of a murine mammary tumor virus promoter reduces the risk of mammary carcinoma.[38] Whereas, our results demonstrated no association between the TGF-β1 C-509T polymorphism and the risk of colorectal adenomas. An interpretation of this finding is that TGF-β may play an important role in the prevention of colorectal carcinogenesis by inhibiting progression from adenoma to carcinoma or initiation of *de novo* carcinoma.

An early event in the molecular evolution of many malignancies, the loss of response to TGF-β as a growth inhibitor is frequently caused by mutation or decreased expression of type I or type II TGF-β receptor.[39,40] There are only sporadic reports of mutations or deletions in type I TGF-β receptor.[41] Most human colon cancers with microsatellite instability have frameshift mutations in polynucleotide repeats within the type II TGF-β receptor coding region; these mutations truncate the receptor protein and disable the serine/threonine kinase to produce TGF-β resistance.[42,43] It is also reported that the lack of functional type I and type II TGF-β receptors expression was found in colon cancer cells.[44] Further investigations are needed to examine the mutation status of TGF-β receptors in patients' cancer tissue.

Our study has some limitations. The sample size was relatively small and thus the power to exclude an association of low-to-moderate relative risk might be low and the risk of a false–positive association might be increased. In addition, we enrolled hospital-based controls, which could lead to a potential selection bias. In conclusion, our results suggest that TGF-β1 -509T allele may have a protective role in the development of colorectal cancer, possibly consistent with its role as an inhibitor of epithelial malignant transformation.

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