Original Article

The association between survivin −31G>C polymorphism and susceptibility to sporadic colorectal cancer in a Southern Chinese population

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ABSTRACT

Background: The case–control study aimed to investigate the association between the –31G>C polymorphism in the promoter of survivin gene and the susceptibility to sporadic colorectal cancer (CRC) in a Southern Chinese population.

Materials and Methods: The study was carried out on 711 healthy controls and 702 CRC cases of a Southern Chinese population. Survivin gene –31G>C genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism. The association between CRC risk and –31G>C genetic polymorphism was estimated using an unconditional logistic regression model.

Results: The number of CC genotype carried in CRC patients was much higher than those of controls ($P < 0.001$). Compared with CC genotypes, GC, GG genotypes and –31G wild-type genotypes (i.e., GC + GG) had a significantly decreased risk of CRC ($P < 0.001$). In addition, survivin –31G wild-type genotypes were not associated with decreased risk of sporadic CRC patients with body mass index (BMI) ≥28.0 kg/m^2, family cancer history, and premenopausal.

Conclusion: Survivin –31G>C polymorphism is associated with sporadic CRC risk in the Southern Chinese population. The –31G wild-type genotypes and GC, GG genotypes are the independent protective factors against sporadic CRC excluding those with a BMI ≥28.0 kg/m^2, family cancer history, and premenopausal.

KEY WORDS: Clinical research, colorectal cancer, genetic susceptibility, single nucleotide polymorphism, survivin

INTRODUCTION

Colorectal cancer (CRC) is the fourth most common cancer worldwide for both of incidence and mortality.[1] It is the complex interaction between genetic and environmental factors that lead to the pathogenesis of CRC.[1-3]

Survivin is 16.5 kDa large and the smallest member of the inhibitor of apoptosis family of antiapoptotic proteins. It is encoded by human survivin gene, which is located on the 17q25 chromosome. Survivin is shown to be regulated at the transcriptional level according to the presence of cell cycle-dependent element/cell cycle gene homology region (CDE/CHR) boxes located in the survivin promoter region. The expression of survivin is known to be highly upregulated in most cancer cells including CRC, but it is rarely present in normal nonmalignant adult cells. It was demonstrated by Xiaoyuan *et al*. that the expression of survivin was an independent prognostic indicator of CRC.[2] However, the mechanism underlying this upregulation remains unclear. In the past, several studies have indicated that the increased expression of survivin might be closely related to the following mechanisms: (1) Proliferation of survivin gene;[4] (2) DNA demethylation or hypomethylation of its promoter (i.e., CpG islands);[5-7] (3) loss of wild-type p53;[8] (4) mutation or deletion of APC gene;[9] (5) a single nucleotide polymorphism (SNP) in the promoter element (i.e., core promoters, upstream promoters or enhancers), which might affect gene transcription by changing binding sites of transcription factors or affecting their binding kinetics.[10] Xu *et al*. first disclosed the significant

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association of the high expression of survivin with −31G>C polymorphism of CDE/CHR boxes in the promoter of survivin gene.[11] This polymorphism may affect the susceptibility to CRC through its influence on the expression of survivin. In the study, we conducted a multi-centered trial to investigate putative associations of −31G>C polymorphism with the susceptibility to develop sporadic CRC in a Southern Chinese population.

MATERIALS AND METHODS

Study participants

From July 2002 to December 2008, 702 patients with CRC were recruited from six professional clinical centers located in the South China. All CRC patients were confirmed by histopathology. Criteria for CRC patients' eligibility were as follows: (1) Han Chinese; (2) permanent residents in Guangzhou and its surrounding areas; (3) no blood relationship with each other. Patients with familial adenomatous polyposis or hereditary nonpolyposis CRC were excluded. Over the same period, 711 matched healthy controls of Han Chinese by age (±5 years) and gender were enrolled from a subject pool who participated in health checkup programs in the same district. Baseline characteristics of enrolled participants were recorded including age, gender, height, weight, smoking habits, alcohol intake, family history of cancer, and menstrual history (female). Subjects were considered smoking only if they smoked at least 100 cigarettes/year. Those who drank alcohol at least once a week for more than 1 year were defined as consumers of alcohol. Besides, subjects were classified as low or normal weight if their body mass index (BMI) was ≤3.9 kg/m^2, overweight if their BMI was 24.0–27.9 kg/m^2, and obesity if the BMI was ≥28 kg/m^2. Subjects were recorded with a family history of cancer if it was diagnosed in one first- or second-degree relative at least. After the one-on-one interview, 5-mL samples of venous blood were collected from each subject. This study was approved by the Institutional Review Board, and all participants gave written informed consent.

Genotyping

Genome DNA was extracted from peripheral blood of objects, using the QIAGEN DNA Isolation Kit (Qiagen, Valencia, CA, USA). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods were performed for genotyping survivin −31G>C (RefSNP ID: Rs9904341) in the promoter region of the human survivin gene. PCR was performed with 12 μL of reaction mixture that was initially predenatured at 94°C for 5 min, then denatured at 94°C for 30 s, followed by 35 cycles of 45 s at annealing temperature and 15 s for extension at 72°C, and a final extension of 10 min at 72°C. Suitable primers were used to amplify the corresponding PCR products, and restriction products were digested by Msp I (New England BioLabs). Restriction DNA products were separated by 2% agarose gel electrophoresis and visualized by ultraviolet light. In addition, 10% of randomly selected samples were further sequenced to verify the accuracy of the genotyping results of PCR-RFLP.

Statistical analysis

All statistical analyses were performed using SPSS 13.0 (SPSS Inc, Chicago, IL, USA) software. Pearson's Chi-square test was used to compare the differences in gender, age, smoking, alcohol intake, BMI, family history of cancer, menstrual history, genotype, and allele frequency at survivin −31G>C between CRC patients and control groups. A goodness-of-fit Chi-square test was used for testing the Hardy–Weinberg genetic equilibrium. Besides, the correlation between survivin −31G>C and CRC susceptibility was analyzed using a nonconditional logistic regression model. Gender, age, smoking, alcohol intake, BMI, family history of cancer, and other factors were also included in a multivariate logistic regression model to correct and estimate relative risks of the SNPs tested on the incidence of CRC by adjusted odds ratio (OR) and a 95% confidence interval (CI). All statistical tests were two-tailed, and $P < 0.05$ was considered statistically significant.

RESULTS

Demographics of the enrolled subjects

The distribution of demographic parameters between CRC patients and controls is shown in Table 1. No significant differences were found between patients and control subjects in terms of age, gender, and menstrual history ($P > 0.05$), suggesting that the frequency matching was adequate. However, the frequencies of smoking, alcohol intake, high BMI, and family history of cancer were found to be significantly higher in the CRC group than that of the control group ($P < 0.05$) [Table 1]. Nonconditional logistic regression analysis showed that smoking was not associated with CRC, while the associations of both alcohol intake and family history of cancer with CRC were significant ($P < 0.05$) [Table 1].

Association of single nucleotide polymorphism with susceptibility to sporadic colorectal cancer

From the analyzed results of genome DNA, Msp I restriction map of survivin gene −31G>C polymorphism was shown [Supplementary Figure 1a]. The SNPs derived from PCR-RFLP were further validated by sequence in 10% of randomly selected samples [Supplementary Figure 1b]. Distributions of the genotypes' frequencies of the polymorphism among patients and controls are shown in Table 2. In the control group, the genotype distribution frequency of −31G>C in the survivin gene promoter was found to be consistent with a Hardy-Weinberg genetic equilibrium ($\chi^2 = 0.62$, $P = 0.43$), indicating that there were no differences in genetic background between the CRC patient and controls. Besides, the frequency rates of −31C variant alleles and CC genotype in CRC group were significantly higher than those of control group (58.0% vs. 50.4%, $\chi^2 = 16.24$, $P < 0.001$; 36.5% vs. 26.2%, $\chi^2 = 17.89$, $P < 0.001$). Nonconditional logistic regression analysis showed that compared to individuals carrying the variant homozygous genotype CC, those with GC, GG, and wild-type −31G (GC + GG) exhibited a 39% (95% CI = 0.46–0.80, $P < 0.001$), 48% (95% CI = 0.38–0.71, $P < 0.001$), and 42% (95% CI = 0.45–0.74, $P < 0.001$) lower risk of developing CRC,

Table 1: Frequencies of confounding factors and distribution of demographic characteristics in CRC patients and controls

	CRC, *n* (%)	Controls, *n* (%)	χ^2	*P*[a]	Crude OR (95%CI)	*P*	Adjusted OR (95%CI)[b]	*P*[b]
Age (year)								
≤49	156 (22.2)	156 (21.9)	0.032	0.984				
50-60	184 (26.2)	189 (26.6)						
>60	366 (51.6)	366 (51.5)						
Gender								
Male	435 (62.0)	442 (62.6)	0.006	0.938				
Female	267 (38.0)	269 (37.8)						
Smoking status								
No	309 (44.0)	394 (55.4)	32.289	<0.001	1.00		1.00	
Yes (Past)	78 (11.1)	102 (14.3)			0.98 (0.70-1.36)	0.88	0.75 (0.47-1.19)	0.220
Yes (Present)	315 (44.9)	215 (30.3)			1.87 (1.49-2.35)	<0.001	0.99 (0.68-1.46)	0.970
Alcohol consumption status								
No	305 (43.4)	540 (75.9)	175.175	<0.001	1.00		1.00	
Yes (Past)	25 (3.6)	34 (4.8)			1.30 (0.76-2.22)	0.33	2.34 (1.27-4.30)	0.006
Yes (Present)	372 (53.0)	137 (19.3)			4.81 (3.78-6.12)	<0.001	8.83 (6.29-12.40)	<0.001
BMI (kg/m^2)								
≤23.9	388 (55.3)	457 (64.3)	12.082	0.002	1.00		1.00	
≥24.0	314 (44.7)	254 (35.7)			1.46 (1.18-1.80)	0.001	1.39 (1.10-1.76)	0.006
Family history of cancer								
No	612 (87.2)	647 (91.0)	5.305	0.021	1.00		1.00	
Yes	90 (12.8)	64 (9.0)			1.49 (1.06-2.09)	0.022	1.61 (1.11-2.33)	0.012
Menopause								
No	53 (19.9)	52 (19.3)	0.032	0.880				
Yes	214 (80.1)	217 (80.7)						

[a]*P* values for a two-sided χ^2 test. [b]adjusted for age, gender, tobacco smoking, alcohol consumption status, family history of cancer and BMI. CRC=Colorectal cancer

Table 2: Distribution of genotypes and alleles of surviving -31G>C between CRC patients and controls

	CRC, *n* (%)	Controls, *n* (%)[a]	Crude OR (95%CI)[b]	Adjusted OR (95%CI)[b]	*P*[b]
Genotypes					
CC	256 (36.5)	186 (26.2)	1.00	1.00	
CG	302 (43.0)	345 (48.5)	0.64 (0.50-0.81)	0.61 (0.46-0.80)	<0.001
GG	144 (20.5)	180 (25.3)	0.58 (0.44-0.78)	0.52 (0.38-0.71)	<0.001
CG + GG	446 (63.5)	525 (73.8)	0.62 (0.49-0.78)	0.58 (0.45-0.74)	<0.001
Alleles					
C	814 (58.0)	717 (50.4)	1.00	1.00	
G	590 (42.0)	705 (49.6)	0.74 (0.64-0.86)	0.69 (0.59-0.82)	<0.001

[a]The observed genotype frequency of -31G>C among the control subjects was in agreement with the Hardy-Weinberg equilibrium (χ^2=0.62, *P*=0.43); [b]OR and *P* values were adjusted in a non-conditional logistic regression model that included age, gender, smoking, alcohol consumption, BMI, and family history ofcancer. CRC=Colorectal cancer

respectively. Compared to those carrying the variant allele C, individuals with allele G exhibited a 31% (95% CI = 0.59–0.82, $P < 0.001$) lower risk for developing CRC [Table 2].

Stratification analysis of single nucleotide polymorphism and colorectal cancer risk

To evaluate the effects of survivin −31G>C genotypes on the risk of CRC, patients and controls were stratified based on age, sex, smoking status, drinking status, family history of cancer, and BMI [Table 3]. According to the correction analysis for confounding factors, it was found that those with wild-type −31G (GC + GG) exhibited a significantly decreased risk of CRC for all ages (adjusted OR = 0.58, 95% CI = 0.34–1.00; adjusted OR = 0.47, 95% CI = 0.28–0.78; adjusted OR = 0.61, 95% CI = 0.43–0.87), both for men (95% CI = 0.43–0.85) and women (95% CI = 0.43–0.87) compared to carriers of variant homozygous CC alleles. Besides, either drinking or not, those with wild-type −31G (GC + GG) exhibited a significantly decreased risk of CRC compared with carriers of CC alleles (adjusted OR = 0.61, 95% CI = 0.42–0.81; adjusted OR = 0.59, 95% CI = 0.39–0.90). However, the wild-type −31G (GC + GG) was not significantly protective against CRC in those with family history of cancer (adjusted OR = 0.69, 95% CI = 0.36–1.31, $P = 0.765$), high BMI (≥28.0 kg/m^2) (adjusted OR = 0.68, 95% CI = 0.24–1.96, $P = 0.67$), and nonmenopause (adjusted OR = 0.55, 95% CI = 0.23–1.34, $P = 0.187$) [Table 3]. In addition, the results of stratified analyses of colon cancer and rectal cancer were in accordance with those of CRC except that wild-type −31G (GC + GG) was not significantly associated with the decreased risk of colon cancer (adjusted OR = 0.65, 95% CI = 0.41–1.03, $P = 0.066$) when compared with the variant homozygous CC alleles [Supplementary Tables 1-4].

DISCUSSION

The −31G>C polymorphism of CDE/CHR boxes in the promoter was found to be significantly associated with the high expression of survivin gene, and this regulation was proved to be at the transcriptional level. Thus, we deduced that the regulation of the expression of survivin gene by this SNP might affect the susceptibility to sporadic CRC. In our study, 702 CRC patients and 711 matched controls in South China were enrolled

Table 3: Stratified analysis of the genotype frequencies of -31G>C between CRC patients and controls

	CRC (*n*=702)		Controls (*n*=711)		Crude OR (95%CI)	Adjusted OR (95%CI)[a]	*P*[a]
	CC *n* (%)	GC+GG *n* (%)	CC *n* (%)	GC + GG *n* (%)	GC + GG vs CC	GC + GG vs CC	
Age (year)							
≤49	57 (36.5)	99 (63.5)	42 (26.9)	114 (73.1)	0.64 (0.40-1.04)	0.58 (0.34-1.00)	0.049
50-60	73 (39.7)	111 (60.3)	49 (25.9)	140 (74.1)	0.53 (0.34-0.83)	0.47 (0.28-0.78)	0.003
>60	126 (34.8)	236 (65.2)	95 (26.0)	271 (74.0)	0.66 (0.48-0.90)	0.61 (0.43-0.87)	0.006
Gender							
Male	155 (35.6)	280 (64.4)	118 (26.7)	324 (73.3)	0.66 (0.49-0.88)	0.60 (0.43-0.85)	0.003
Female	101 (37.8)	166 (62.2)	68 (25.3)	201 (74.7)	0.56 (0.38-0.81)	0.55 (0.38-0.81)	0.003
Smoking status							
No	117 (37.9)	192 (62.1)	105 (26.6)	289 (73.4)	0.60 (0.43-0.82)	0.58 (0.41-0.80)	0.001
Yes	139 (35.4)	254 (64.6)	81 (25.6)	236 (74.4)	0.63 (0.45-0.87)	0.63 (0.42-0.93)	0.020
Alcohol consumption status							
No	113 (37.0)	192 (63.0)	141 (26.1)	399 (73.9)	0.60 (0.44-0.81)	0.61 (0.42-0.81)	<0.001
Yes	143 (36.0)	254 (64.0)	45 (26.3)	126 (73.7)	0.63 (0.43-0.94)	0.59 (0.39-0.90)	0.013
BMI (kg/m^2)							
≤23.9	160 (41.2)	228 (58.8)	130 (28.4)	327 (71.6)	0.57 (0.43-0.76)	0.54 (0.40-0.75)	<0.001
24.0-27.9	83 (34.0)	161 (66.0)	47 (23.4)	154 (76.6)	0.59 (0.39-0.90)	0.61 (0.38-0.97)	0.035
≥28.0	13 (18.6)	57 (81.4)	9 (17.0)	44 (83.0)	0.90 (0.35-2.29)	0.68 (0.24-1.96)	0.670
Family history of cancer							
No	233 (38.1)	379 (61.9)	169 (26.1)	478 (73.9)	0.58 (0.45-0.73)	0.55 (0.42-0.71)	<0.001
Yes	23 (25.6)	67 (74.4)	17 (26.6)	47 (73.4)	1.05 (0.51-2.19)	0.69 (0.36-1.31)	0.765
Menopause							
No	19 (35.8)	34 (64.2)	12 (23.1)	40 (76.9)	0.54 (0.23-1.26)	0.55 (0.23-1.34)	0.187
Yes	82 (38.3)	132 (61.7)	56 (25.8)	161 (74.2)	0.56 (0.37-0.84)	0.57 (0.38-0.87)	0.010

[a]OR and *P* values were adjusted in a non-conditional logistic regression model that included age, gender, smoking, alcohol consumption, BMI, and family history ofcancer.CRC=Colorectal cancer

over the same period. The frequency rates of −31C variant alleles and CC genotype in CRC patients were significantly higher than those control group. Compared to individuals carrying the variant homozygous genotype CC, those with GC, GG, and wild-type −31G (GC + GG) exhibited decreased risk of developing CRC. Individuals carrying allele G also exhibited lower risk for developing CRC when compared with those carrying the variant allele C. Wild-type −31G (GC + GG), GC and GG genotypes might promote the binding of the repressor with CDE/CHR boxes and inhibited the transcription of survivin promoter. These findings confirm with the previous researches of the previous studies[12-14] that documented −31G/C polymorphism, −31CC and −31C were associated with increased risk of CRC. Besides, Xu *et al.*[11] have reported that the presence of the −31G/C polymorphism was more frequent in malignant cell lines with increased survivin expression at both mRNA and protein levels. In addition, in a Gaozouli *et al.*'s[14] study, they have observed that in cancer cases, the survivin mRNA in the samples homozygous for −31CC genotype was approximately 1.6-fold higher than the carriers of the −31GG and −31GC genotypes. Survivin plays a role as the antagonist of apoptotic cell death and functions at the regulation of mitosis.[15,16] Thus, the overexpression of survivin relates closely to the increase of invasion and metastasis of CRC.[17] However, the mechanism underlying the overexpression of survivin in cases with −31CC genotype remains unknown.[14] As a result, the expression of survivin was downregulated and apoptosis increased. Therefore, the susceptibility to CRC of these genotypes decreased.

In the study, a stratified analysis of subgroups showed that when in those with BMI ≥28.0 kg/m^2, family history of cancer, and nonmenopause, wild-type −31G (GC + GG), GC and GG genotypes were not significantly associated with the decreased risk of CRC. A variety of previous studies suggested that obesity, as an independent risk factor of CRC, could significantly increase the susceptibility to CRC.[17-19] Usually, obesity is accompanied by certain metabolism disorders such as insulin resistance, hyperinsulinism, increased IGF-1, decreased adiponectin, and IGFBP-3. These issues were proved to be closely related to the incidence of CRC.[20-23] Besides, wild-type −31G was no longer protective in those with a family history of cancer, which might be due to the change of whole genetic background. Epidemiological surveys validated that estrogen and progesterone could decrease the risk of CRC,[24-26] which might result from the inhibition of the CRC differentiation by estrogen and its receptors and led to cell cycle arrest and apoptosis.[27,28] Hence, we suspect that obesity, family history of cancer, and estrogen could resist the protection effect of wild-type −31G (GC + GG) against CRC in the Southern Chinese population.

In the stratified analysis of colon cancer, there were no significant associations between wild-type survivin −31G and decreased susceptibility to sporadic CRC. As is known, cigarette produces more than sixty types of carcinogens, including polycyclic aromatic hydrocarbons, heterocyclic amines, nitrosamine, and benzopyrene.[29] These carcinogens can reach colorectal tissues through the circulatory system without a direct effect on colon and rectum.[30] It has been shown that benzopyrene-DNA adducts could be detected more frequently in colorectal mucosa cells of smokers than that of nonsmokers, thus increasing the risk of CRC.[31] Therefore, the synergistic effects of smoking and survivin −31G>C

polymorphism may weaken the protection of wild-type −31G genotypes against sporadic CRC. Several limitations of this case–control study need addressing. The sample size was not determined by power calculations, which might affect the accuracy of the results. Besides, an SNP may only have a modest effect. More studies focusing on the combined effects of multiple variants will help estimate the genetic factors of sporadic CRC comprehensively.

CONCLUSION

Survivin −31G>C polymorphism is associated with genetic susceptibility to sporadic CRC in a Southern Chinese population. Wild-type survivin −31G (GC + GG) and GC and GG genotypes are independent protective factors against sporadic CRC. However, this protective effect is not significant in those with BMI ≥28.0 kg/m^2, family history of cancer, and nonmenopause.

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Conflicts of interest

There are no conflicts of interest.

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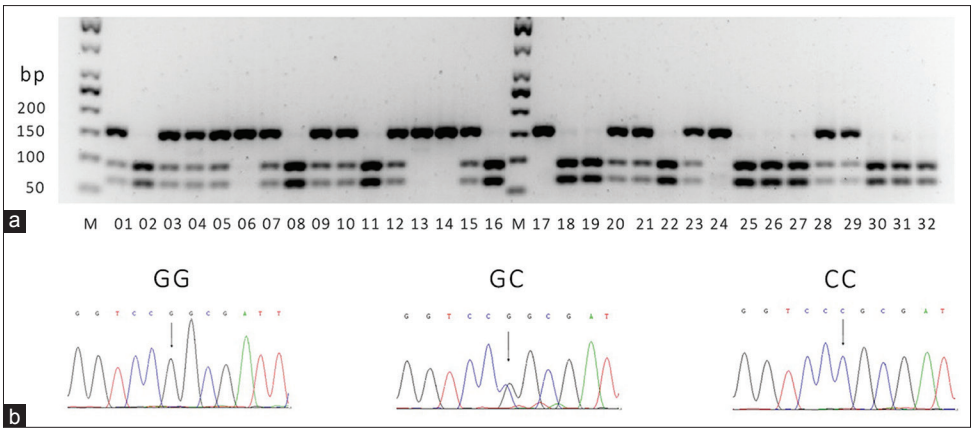
SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Restriction DNA map and sequence verification of survivin –31G>C polymorphism. (a) Msp I restriction map of survivin –31G>C polymorphism (1, 3–5, 7, 9, 10, 12, 15, 20, 21, 23, 28, and 29 are GC genotypes with 151 bp, 61 bp, and 90 bp fragments; 2, 8, 11, 16, 18, 19, 22, 25–27, and 30–32 are variant homozygous CC genotype with 61 bp and 90 bp fragments; 6, 13, 14, 17, and 24 are wild-type GG with only 151 bp fragment). (b) The single nucleotide polymorphisms derived from polymerase chain reaction-restriction fragment length polymorphism were further validated by sequence in 10% of randomly selected samples

Supplementary Table 1: Distribution of genotypes and alleles of surviving-31G>C between patients with colon cancer and controls

	Colon cancer, *n* (%)	Controls, *n* (%)[a]	Crude OR (95%CI)[b]	Adjusted OR (95%CI)[b]	*P*[b]
Genotypes					
CC	127 (36.5)	186 (26.2)	1.00	1.00	
CG	141 (40.5)	345 (48.5)	0.60 (0.44-0.81)	0.60 (0.43-0.83)	0.002
GG	80 (23.0)	180 (25.3)	0.65 (0.46-0.92)	0.59 (0.40-0.86)	0.006
CG + GG	221 (63.5)	525 (73.8)	0.62 (0.47-0.81)	0.60 (0.44-0.81)	0.001
Alleles					
C	395 (56.8)	717 (50.4)	1.00	1.00	
G	301 (43.2)	705 (49.6)	0.78 (0.65-0.93)	0.74 (0.60-0.90)	0.003

[a]The observed genotype frequency of -31G>C among the control subjects was in agreement with the Hardy-Weinberg equilibrium (χ^2=0.62, *P*=0.43); [b]OR and *P* values were adjusted in a non-conditional logistic regression model that included age, gender, smoking, alcohol consumption, BMI, and family history of cancer. CRC=Colorectal cancer

Supplementary Table 2: Stratified analysis of the genotype frequencies of -31G>C between patients with colon cancer and controls

	Colon cancer (*n*=348)		Controls (*n*=711)		Crude OR (95%CI)	Adjusted OR (95%CI)[a]	*P*[a]
	CC *n* (%)	GC + GG *n* (%)	CC *n* (%)	GC + GG *n* (%)	GC + GG vs CC	GC + GG vs CC	
Age (year)							
≤49	30 (40.5)	44 (59.5)	42 (26.9)	114 (73.1)	0.54 (0.30-0.97)	0.52 (0.27-1.00)	0.05
50-60	32 (35.6)	58 (64.4)	49 (25.9)	140 (74.1)	0.63 (0.37-1.09)	0.58 (0.31-1.11)	0.10
>60	65 (35.3)	119 (64.7)	95 (26.0)	271 (74.0)	0.64 (0.44-0.94)	0.58 (0.38-0.90)	0.014
Gender							
Male	76 (35.3)	139 (64.7)	118 (26.7)	324 (73.3)	0.67 (0.47-0.95)	0.65 (0.42-0.99)	0.047
Female	51 (38.3)	82 (61.7)	68 (25.3)	201 (74.7)	0.54 (0.35-0.85)	0.56 (0.35-0.88)	0.01
Smoking status							
No	58 (37.9)	95 (62.1)	105 (26.6)	289 (73.4)	0.60 (0.40-0.88)	0.60 (0.40-0.91)	0.015
Yes	69 (35.4)	126 (64.6)	81 (25.6)	236 (74.4)	0.63 (0.43-0.92)	0.65 (0.41-1.03)	0.066
Alcohol consumption status							
No	54 (35.5)	98 (64.5)	141 (26.1)	399 (73.9)	0.64 (0.44-0.94)	0.65 (0.43-0.97)	0.037
Yes	73 (37.2)	123 (62.8)	45 (26.3)	126 (73.7)	0.60 (0.39-0.94)	0.57 (0.36-0.92)	0.02
BMI (kg/m^2)							
≤23.9	79 (41.4)	112 (58.6)	130 (28.4)	327 (71.6)	0.56 (0.40-0.80)	0.54 (0.37-0.80)	0.002
24.0-27.9	42 (33.1)	85 (66.9)	47 (23.4)	154 (76.6)	0.62 (0.38-1.01)	0.65 (0.37-1.14)	0.131
≥28.0	6 (20.0)	24 (80.0)	9 (17.0)	44 (83.0)	0.82 (0.26-2.58)	0.82 (0.21-3.15)	0.771
Family history of cancer							
No	114 (37.7)	188 (62.3)	169 (26.1)	478 (73.9)	0.58 (0.44-0.78)	0.56 (0.41-0.77)	<0.001
Yes	13 (28.3)	33 (71.7)	17 (26.6)	47 (73.4)	0.92 (0.39-2.15)	0.84 (0.31-2.27)	0.736
Menopause							
No	6 (23.1)	20 (76.9)	12 (23.1)	40 (76.9)	1.00 (0.33-3.06)	0.87 (0.26-2.93)	0.818
Yes	45 (42.1)	62 (57.9)	56 (25.8)	161 (74.2)	0.48 (0.29-0.78)	0.49 (0.29-0.81)	0.006

[a]OR and *P* values were adjusted in a non-conditional logistic regression model that included age, gender, smoking, alcohol consumption, BMI, and family history of cancer. CRC=Colorectal cancer

Supplementary Table 3: Distribution of genotypes and alleles of surviving-31G>C between patients with rectal cancer and controls

	Rectal cancer, *n* (%)	Controls, *n* (%)[a]	Crude OR (95%CI)[b]	Adjusted OR (95%CI)[b]	*P*[b]
Genotypes					
CC	129 (36.4)	186 (26.2)	1.00	1.00	
CG	161 (45.5)	345 (48.5)	0.67 (0.50-0.90)	0.62 (0.45-0.85)	0.003
GG	64 (18.1)	180 (25.3)	0.51 (0.36-0.74)	0.46 (0.31-0.68)	<0.001
CG + GG	225 (63.6)	525 (73.8)	0.62 (0.47-0.81)	0.56 (0.41-0.76)	<0.001
Alleles					
C	419 (59.2)	717 (50.4)	1.00	1.00	
G	289 (40.8)	705 (49.6)	0.70 (0.59-0.84)	0.66 (0.54-0.81)	<0.001

[a]The observed genotype frequency of -31G>C among the control subjects was in agreement with the Hardy-Weinberg equilibrium (χ^2=0.62, *P*=0.43); [b]OR and *P* values were adjusted in a non-conditional logistic regression model that included age, gender, smoking, alcohol consumption, BMI, and family history of cancer. CRC=Colorectal cancer

Supplementary Table 4: Stratified analysis of the genotype frequencies of -31G>C between patients with rectal cancer and controls

	Rectal cancer (*n*=354)		Controls (*n*=711)		Crude OR (95%CI)	Adjusted OR (95%CI)[a]	*P*[a]
	CC *n* (%)	GC + GG *n* (%)	CC *n* (%)	GC + GG *n* (%)	GC + GG vs CC	GC + GG vs CC	
Age (year)							
≤49	27 (32.9)	55 (67.1)	42 (26.9)	114 (73.1)	0.75 (0.42-1.34)	0.56 (0.28-1.10)	0.09
50-60	41 (43.6)	53 (56.4)	49 (25.9)	140 (74.1)	0.45 (0.27-0.76)	0.39 (0.22-0.70)	0.002
>60	61 (34.3)	117 (65.7)	95 (26.0)	271 (74.0)	0.67 (0.46-0.99)	0.63 (0.41-0.97)	0.037
Gender							
Male	79 (35.9)	141 (64.1)	118 (26.7)	324 (73.3)	0.65 (0.46-0.92)	0.51 (0.34-0.78)	0.002
Female	50 (37.3)	84 (62.7)	68 (25.3)	201 (74.7)	0.57 (0.36-0.89)	0.59 (0.37-0.93)	0.022
Smoking status							
No	59 (37.8)	97 (62.2)	105 (26.6)	289 (73.4)	0.60 (0.40-0.89)	0.56 (0.38-0.85)	0.006
Yes	70 (35.4)	128 (64.6)	81 (25.6)	236 (74.4)	0.63 (0.43-0.92)	0.58 (0.37-0.92)	0.021
Alcohol consumption status							
No	59 (38.6)	94 (61.4)	141 (26.1)	399 (73.9)	0.56 (0.39-0.82)	0.53 (0.35-0.78)	0.002
Yes	70 (34.8)	131 (65.2)	45 (26.3)	126 (73.7)	0.67 (0.43-1.05)	0.61 (0.39-0.97)	0.038
BMI (kg/m^2)							
≤23.9	81 (41.1)	116 (58.9)	130 (28.4)	327 (71.6)	0.57 (0.40-0.81)	0.50 (0.34-0.74)	<0.001
24.0-27.9	41 (35.0)	76 (65.0)	47 (23.4)	154 (76.6)	0.57 (0.34-0.93)	0.60 (0.34-1.05)	0.074
≥28.0	7 (17.5)	33 (82.5)	9 (17.0)	44 (83.0)	0.96 (0.33-2.86)	0.63 (0.17-2.39)	0.497
Family history of cancer							
No	119 (38.4)	191 (61.6)	169 (26.1)	478 (73.9)	0.57 (0.43-0.76)	0.52 (0.38-0.72)	<0.001
Yes	10 (22.7)	34 (77.3)	17 (26.6)	47 (73.4)	1.23 (0.50-3.02)	0.70 (0.21-2.33)	0.558
Menopause							
No	13 (48.1)	14 (51.9)	12 (23.1)	40 (76.9)	0.32 (0.12-0.87)	0.26 (0.08-0.82)	0.021
Yes	37 (34.6)	70 (65.4)	56 (25.8)	161 (74.2)	0.66 (0.40-1.09)	0.71 (0.42-1.20)	0.199

[a]OR and *P* values were adjusted in a non-conditional logistic regression model that included age, gender, smoking, alcohol consumption, BMI, and family history of cancer. CRC, colorectal cancer