A Genetic Variant of rs145204276 in the Promoter Region of Long Noncoding RNA *GAS5* Is Associated With a Reduced Risk of Breast Cancer

Yiyin Tang, Yishan Wang, Xi Wang, Yang Liu, Kai Zheng

Abstract

The growth arrest-specific 5 (*GAS5*) rs145204276 AGGCA/- polymorphism was analyzed in 575 patients with sporadic breast cancer (BC) and 602 controls to test the association between the polymorphism and BC risk. The rs145204276 del allele might protect against the development of BC via inducing the promoter activity by binding to transcriptional factor specificity protein 1, and finally resulting in higher levels of *GAS5*.

Introduction: Growth arrest-specific 5 (*GAS5*), downregulated in breast cancer (BC), functions as a tumor suppressor by affecting tumor growth and cell apoptosis in vivo and in vitro. This study was designed to determine whether an insertion (ins)/deletion (del) polymorphism (rs145204276 AGGCA/-) in the promoter region of *GAS5* was a susceptibility gene to the occurrence of BC. **Patients and Methods:** A hospital-based case-control study was conducted and the *GAS5* rs145204276 genotype was analyzed in 575 sporadic BC patients and 602 controls to test the association between the polymorphism and BC risk. Further functional analysis was performed to evaluate the effect of the polymorphism on the promoter activity and *GAS5* expression levels using quantitative polymerase chain reaction and dual luciferase reporter assay. **Results:** The prevalence of BC was lower in carriers with rs145204276 ins/del and del/del genotypes compared with those with ins/ins genotype (adjusted odds ratio [OR], 0.74; 95% confidence interval [CI], 0.58-0.92; $P = .009$). An allelic test for association with BC was also significant (del vs. ins: adjusted OR, 0.78; 95% CI, 0.65-0.93; $P = .007$). Genotype-phenotype analysis revealed that individuals with rs145204276 ins/del and del/del genotypes expressed significantly higher levels of *GAS5*. Luciferase reporter analysis revealed that rs145204276 del allele enhanced the promoter activity of *GAS5*. **Conclusion:** The rs145204276 del allele might protect against the development of BC via inducing the promoter activity by binding to transcriptional factor specificity protein 1, and finally resulting in higher levels of *GAS5*.

Clinical Breast Cancer, Vol. 19, No. 3, e415-21

Keywords: Growth arrest-specific 5, Polymerase chain reaction (PCR)-polyacrylamide gel electrophoresis, Polymorphism, Quantitative PCR, Transcriptional activity

Introduction

Breast cancer (BC) is the most common cancer, accounting for an expected 30% of all new cancer diagnoses in 2018.[1] It is also the leading cause of cancer death from ages 20 to 59 years in women who are still raising and supporting families, making it a public health problem worldwide.[1] Risk factors for BC included smoking consumption,[2] high alcohol drinking,[3] high-fat diet,[4] and high levels of blood cholesterol.[5] In addition to these environment factors, genetic factors have been shown to be involved in the occurrence and development of BC. Yan et al reported that a minor allele of C677T in methylenetetrahydrofolate reductase reduced activity of the enzyme, increased the level of homocysteine, and finally resulted in an increased risk of BC.[6] Sawyer et al reported that BC patients with -161CC genotype in uridine glucuronosyltransferase 2B7 had a decreased epirubicin clearance and an increased risk of Grade 3 to 4 leukopenia, suggesting that the single nucleotide polymorphism

Y.T. and Y.W. contributed equally to this work.

Department of Breast Surgery, the Third Affiliated Hospital of Kunming Medical University, Kunming Yunnan, P.R. China

Submitted: Jun 29, 2018; Revised: Oct 21, 2018; Accepted: Nov 6, 2018; Epub: Nov 14, 2018

Address for correspondence: Kai Zheng, MS, Department of Breast Surgery, Third Affiliated Hospital of Kunming Medical University, No 519 Kunzhou Rd, Kunming 650118, China
Fax: +86 871 68 185730; e-mail contact: zhengkai1003@yeah.net

1526-8209/$ - see frontmatter
https://doi.org/10.1016/j.clbc.2018.11.006

(SNP) might be a predictor for drug metabolism, toxicity, and efficacy in BC patients receiving chemotherapy.[7] To date, a series of susceptibility genes of BC have been discovered. The precise etiology, however, remains poorly understood.

Long noncoding RNAs (lncRNAs), a set of noncoding RNAs more than 200 nucleotides in length, were recently identified to contribute important roles in a variety of biological processes.[8-10] In BC, hundreds of lncRNAs were reported to be deregulated, and the deregulation led to aberrant gene expression that were key events in the progression of BC.[11-13] Current evidence showed that an lncRNA growth arrest-specific 5 (*GAS5*) was downregulated in BC, and the downregulation was not only associated with cell proliferation and apoptosis, but also associated with clinical features of BC such as clinical stage, lymph node metastasis, and overall survival.[14-16] These findings indicate that lncRNA *GAS5* might be a critical player in BC tumorigenesis.

Within the promoter region of lncRNA *GAS5*, a polymorphism rs145204276 AGGCA/- was found to be functional, with the deletion (del) allele increasing luciferase activity and expression levels of GAS5.[17] Previously, the association of *GAS5* rs145204276 polymorphism with cancer risk has been studied with conflicting results.[17-21] Tao et al reported that the rs145204276 del allele increased the risk of hepatocellular carcinoma.[17] In contrast, a reduced risk of the rs145204276 del allele was associated with gastric cancer,[18,19] lung cancer,[20] colorectal cancer,[21] cervical squamous cell carcinoma,[22] and osteosarcoma.[23] To date, no report investigated the relationship between *GAS5* rs145204276 and BC risk. In this study, we aimed to examine whether the rs145204276 in the promoter of lncRNA *GAS5* contributes to the susceptibility of BC in a Chinese Han population. Furthermore, whether the SNP affected *GAS5* expression level was also analyzed.

Patients and Methods

Study Population

The study population consisted of 575 sporadic BC patients obtained from the Third Affiliated Hospital of Kunming Medical University between April 2013 and August 2017. All patients were breast adenocarcinoma with pathological confirmation of malignancy on resection. None of the patients received preoperative systemic chemotherapy or radiotherapy. The exclusion criteria for patients were as follows: (1) a history or family history of cancer; (2) recurrent BC; and (3) patients with breast hyperplasia. Clinical information relevant to the disease status was obtained from patients' medical charts, including positive or negative estrogen receptor (ER) and progesterone receptor (PR), size of the primary tumor (T), presence and extent of regional lymph node metastasis (N), and absence or presence of distant metastasis (M). We also recruited 602 female controls visiting the same hospital for comprehensive medical checkup and matched them with cases according to age, ethnicity, age at menarche, and living area. We excluded controls who had a personal history or family history of any malignancy, breast hyperplasia, and/or breast mastitis. This study was reviewed and approved by the Ethical Committee of the Kunming Medical University. After informed constant was provided by each participant, blood and paraffin wax-embedded BC tissues were collected from the same patient.

Genotyping

Genomic DNA was extracted from blood and paraffin wax-embedded BC samples using commercial kits (BioTeke, Beijing, China; Qiagen, Hilden, Germany). Polymerase chain reaction (PCR)-polyacrylamide gel electrophoresis was performed for genotyping rs145204276. The primer sequences used were described previously[17]: 5′-TCCCGACTGAGGAGGAAGAGCA-3′ (forward) and 5′-AACACCGTCCCGGAAGTG AAA-3′ (reverse). PCR products were run on a polyacrylamide gel and stained with 1.0 g/L argent nitrate. For quality control, the rs145204276 was genotyped in a double-blinded way. Moreover, approximately 5% of samples were randomly selected for Sanger sequencing, yielding a 100% concordance rate between the 2 methods.

Quantitative Real-Time PCR

Total RNA was extracted from BC tissues and corresponding nontumor normal tissues using Trizol reagent (Takara, Dalian, China). Equal amounts of total RNA (1 μg) were reverse-transcribed to generate cDNA using the First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions. The expression level of lncRNA *GAS5* was determined via quantitative real-time PCR (qPCR) using the SYBR green method. The primers for amplifying lncRNA *GAS5* were described previously[17]: 5′-AGCTTACTGCTT-GAAAGGGTC-3′ (forward) and 5′-TCTTCTTGTGCCATGA-GACTC-3′ (reverse). Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as in internal control and the primer sequences were as follows: 5′-CTCTCTGCTCCTCCTGTTCGAC-3′ (forward) and 5′-TGAGCGATGTGGCTCGGCT-3′ (reverse). The assay was performed using the ABI 7500 system (Applied Biosystems, Foster City, CA). The $2^{-\Delta\Delta Ct}$ algorithm was applied to calculate the relative expression of lncRNA *GAS5* after normalization to *GAPDH*.

Dual Luciferase Reporter Assay

The lncRNA *GAS5* promoter fragment of 350 base pairs containing the rs145204276 insertion (ins) allele was synthesized and cloned into pGL3-basic vector (Promega, Madison, WI). Its counterpart with the rs145204276 del allele was generated using the QuikChange Lightening Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA). The insert fragments were confirmed using Sanger sequencing. These vectors were transiently transfected into cultured Human embryonic kidney 293 (HEK293), Michigan cancer foundation-7 (MCF7), human breast carcinoma (MDA-MB-231 and T-47D), and Human lung adenocarcinoma (A549) cells using the Lipofectamine 3000 kit (Thermo Fisher Scientific). Promega's Renilla luciferase vector was used as in internal control and cotransfected. At 24 hours after transfection, transcriptional activities were determined using the Dual-Luciferase Reporter Assay System (Promega) and measurements were presented as the ratio of the firefly and renilla luciferase activities.

Statistical Analysis

Statistical analyses were performed using SPSS software version 19.0 (IBM Corp, Armonk, NY). A comparison of population characteristics was carried out using Student *t* test. The

Table 1 Characteristics of the Study Population

Variables	Controls (n = 602)	Patients With BC (n = 575)	P
Age, y	50.8 ± 13.8	51.7 ± 9.9	.14
Age at Menarche, y	13.8 ± 1.5	13.9 ± 1.5	.80
Estrogen Receptor			
Positive		327 (56.9)	
Negative		248 (43.1)	
Progesterone Receptor			
Positive		286 (49.7)	
Negative		289 (50.3)	
Primary Tumor			
T1-2		392 (68.2)	
T3-4		183 (31.8)	
Regional Lymph Nodes			
N0		245 (42.6)	
N1-3		330 (57.4)	
Distant Metastasis			
M0		567 (98.6)	
M1		8 (1.4)	

Data are presented as mean ± SD or n (%).
Abbreviation: BC = breast cancer.

rs145204276 genotype distribution was tested for Hardy–Weinberg equilibrium (HWE) using the Pearson χ^2 test. Differences of rs145204276 genotype frequencies between cases and controls were assessed using the χ^2 test. Results are presented as odds ratios (ORs) with 95% confidence intervals (CIs) and *P* values after adjustment for age and age at menarche. Multiple testing was performed by adjusting the significance level of 0.0125 (0.05/4) using Bonferroni correction. The difference of lncRNA *GAS5* expression between the 2 groups was compared using the Mann–Whitney *U* test. The relative level of luciferase activity among groups was compared using 1-way analysis of variance. TRANSFAC 7.0 (geneXplain GmbH, Wolfenbüttel, Germany) was used to predict the binding site of rs145204276 to transcriptional factor. $P < .05$ was considered as significant.

Compliance With Ethical Standards

All procedures performed in studies involving human participants were in accordance with the institutional review board of the Kunming Medical University, and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Results

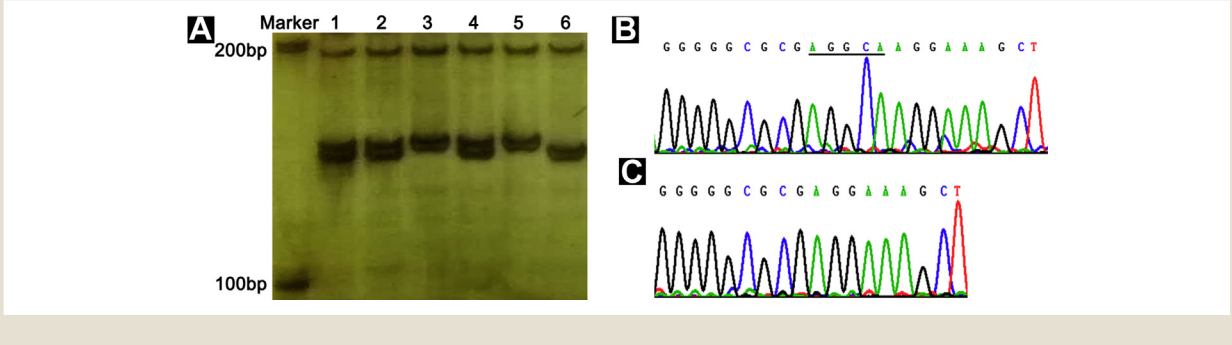
Characteristics of the Study Population

The characteristics of the study population are summarized in Table 1. BC cases were diagnosed at a mean age of 51.7 (±9.9) years, and the mean age of controls was 50.8 (±13.8) years. The mean age at menarche was 13.9 (±1.5) years in BC patients and 13.8 (±1.5) years in controls. No significant difference of age and age at menarche was found between cases and controls ($P = .14$ and .80, respectively). Approximately half of the patients were positive for ER and PR (327 patients [56.9%] and 286 patients [49.7%], respectively). Most tumors were T1 to T2 (392 patients [68.2%]), nodal-positive (330 patients [57.4%]), and with absence of distant metastasis (567 patients [98.6 %]).

The Association of rs145204276 in the Promoter of lncRNA GAS5 With BC Risk

Results of PAGE analysis and subsequent Sanger sequencing are presented in Figure 1. The genotype distribution of rs145204276 in the promoter of lncRNA *GAS5* was in line with HWE in cases and controls ($P = .50$ and .93, respectively). The association between rs145204276 and BC risk is shown in Table 2. The prevalence of BC was lower in carriers with rs145204276 ins/del and del/del genotypes compared with those with ins/ins genotype (adjusted OR, 0.74; 95% CI, 0.58-0.92; $P = .009$). An allelic test for association with BC was also significant (del vs. ins: adjusted OR, 0.78; 95% CI, 0.65-0.93; $P = .007$). Genetic variants are often different

Figure 1 Polyacrylamide Gel Electrophoresis Analysis of the rs145204276 AGGCA/- Insertion (Ins)/Deletion (Del) Polymorphism (A). Lines 1, 2, and 4: Ins/Del; Lines 3 and 5: Ins/Ins; Line 6: Del/Del. Sanger Sequencing of the rs145204276 AGGCA/- Ins/Del Polymorphism: Ins Allele (B) and Del Allele (C)



Abbreviation: bp = base pairs.

Table 2 Association Between rs145204276 in the Promoter of *GAS5* and Risk of Breast Cancer (DNA From Blood Samples)

Polymorphism	Controls (n = 602), n (%)	Breast Cancer (n = 575), n (%)	Adjusted OR (95 % CI)[a]	*P*
Ins/Ins	279 (46.3)	310 (53.9)	1.00	
Ins/Del	261 (43.4)	220 (38.3)	0.76 (0.59-0.96)	.02
Del/Del	62 (10.3)	45 (7.8)	0.64 (0.42-0.98)	.04
Dominant Model	323 (53.7)	265 (46.1)	0.74 (0.58-0.92)	.009
Ins allele	819 (68.0)	840 (73.0)	1.00	
Del allele	385 (32.0)	310 (27.0)	0.78 (0.65-0.93)	.007

Abbreviations: Del = deletion; Ins = insertion; OR = odds ratio.
[a]Odds ratios were adjusted for age and age at menarche.

between blood and tissues; we therefore genotyped the rs145204276 in paraffin wax-embedded tumor tissues from 575 BC patients. Because of DNA quality, only 512 samples were successfully genotyped. As shown in Table 3, similar to the results of blood samples, the rs145204276 ins/del and del/del genotypes and the del allele were associated with reduced risk of BC in tumor tissues (adjusted OR, 0.72; 95% CI, 0.56-0.91; $P = .006$; adjusted OR, 0.76; 95% CI, 0.63-0.91; $P = .003$, respectively).

After subgroup analyses on the basis of ER, PR, T, and N status, we found that the rs145204276 ins/del and del/del genotypes were associated with a reduced risk of PR-positive (PR^+) patients (adjusted OR, 0.70; 95% CI, 0.52-0.93, $P = .01$) but not PR^- patients. A reduced risk of the del/del genotype was also observed in T3 to T4 patients (adjusted OR, 0.44; 95% CI, 0.22-0.89; $P = .01$) but not in T1 to T2 patients. Moreover, the presence of the rs145204276 ins/del genotype was associated with a reduced risk of N1 to N3 patients in heterozygote comparison and dominant genetic model (ins/del vs. ins/ins: adjusted OR, 0.66; 95% CI, 0.50-0.88; $P = .005$; dominant model: adjusted OR, 0.66; 95% CI, 0.50-0.86; $P = .002$). However, neither ER^+ nor ER^- was associated with the risk of rs145204276 in BC patients (Table 4).

The rs145204276 Ins/Del and Del/Del Genotypes Were Associated With Increased Levels of lncRNA GAS5

We performed qPCR on RNA isolated from tissues of 69 BC patients and corresponding nontumor normal tissues. As shown in Figure 2A, lower levels of lncRNA *GAS5* were observed in BC patients compared with normal controls ($P < .05$). The expression data of lncRNA *GAS5* were also analyzed with regard to the rs145204276 ins/del polymorphism. Compared with rs145204276 ins/ins genotype carriers, individuals with rs145204276 ins/del and del/del genotypes expressed significantly higher levels of lncRNA *GAS5* in BC patients (Figure 2B) and in normal controls (Figure 2C; $P < .05$).

The rs145204276 Del Allele Increased Transcriptional Activity

Investigation of putative transcriptional factor for rs145204276 was performed using TRANSFAC software. As shown in Figure 3A, in silico analysis showed that the rs145204276 del allele but not ins allele contains the binding sequence (GCGCGAGGAA) of transcriptional factor specificity protein 1 (SP1). To determine whether rs145204276 results in the change of transcriptional activity, we cloned the promoter region of lncRNA *GAS5* containing rs145204276 del or ins allele and transfected into HEK293, MCF7, MDA-MB-231, T-47D, and A549 cells. As shown in Figure 3B, a significant increase of luciferase activity was observed in cells transfected with rs145204276 del construct compared with cells transfected with rs145204276 ins construct ($P < .01$).

Discussion

In this study, we designed a hospital-based case-control study to explore the relationship between rs145204276 in the promoter of lncRNA *GAS5* and BC risk in a Chinese Han population. We presented, to our knowledge, the first evidence that the rs145204276 del allele is associated with a reduced risk of BC whether the samples came from blood or tumor tissues. The expression levels of *GAS5* were significantly increased in carriers with rs145204276 ins/del and del/del genotypes. Through functional analysis using dual luciferase reporter assay, we confirmed

Table 3 Association Between rs145204276 in the Promoter of *GAS5* and Risk of Breast Cancer (DNA From Tissues of Breast Cancer)

Polymorphism	Controls (n = 602), n (%)	Breast Cancer (n = 512), n (%)	Adjusted OR (95 % CI)[a]	*P*
Ins/Ins	279 (46.3)	280 (54.7)	1.00	
Ins/Del	261 (43.4)	194 (37.9)	0.74 (0.58-0.95)	.02
Del/Del	62 (10.3)	38 (7.4)	0.60 (0.39-0.93)	.02
Dominant Model	323 (53.7)	232 (45.3)	0.72 (0.56-0.91)	.006
Ins allele	819 (68.0)	754 (73.6)	1.00	
Del allele	385 (32.0)	270 (26.4)	0.76 (0.63-0.91)	.004

Abbreviations: Del = deletion; Ins = insertion; OR = odds ratio.
[a]Odds ratios were adjusted for age and age at menarche.

Table 4 Subgroup Analysis Between rs145204276 in the Promoter of *GAS5* and Risk of Breast Cancer

Genotype	Controls, n (%)	Case I		Case I vs. Controls		Case II vs. Controls	
		ER^+	ER^-	Adjusted OR (95% CI)[a]	*P*	Adjusted OR (95% CI)[a]	*P*
Ins/Ins	279 (46.3)	175 (53.5)	135 (54.4)	1.00		1.00	
Ins/Del	261 (43.4)	126 (38.5)	94 (37.9)	0.77 (0.58-1.02)	.07	0.75 (0.55-1.02)	.07
Del/Del	62 (10.3)	26 (8.0)	19 (7.7)	0.67 (0.41-1.10)	.11	0.63 (0.36-1.10)	.09
Dominant Model	323 (53.7)	152 (46.5)	113 (45.6)	0.75 (0.57-0.98)	.03	0.72 (0.54-0.97)	.03
		PR^+	PR^-				
Ins/Ins	279 (46.3)	158 (55.2)	152 (52.6)	1.00		1.00	
Ins/Del	261 (43.4)	108 (37.8)	112 (38.8)	0.73 (0.54-0.98)	.04	0.79 (0.59-1.06)	.12
Del/Del	62 (10.3)	20 (7.0)	25 (8.7)	0.57 (0.33-0.98)	.04	0.73 (0.44-1.20)	.21
Dominant Model	323 (53.7)	128 (44.8)	137 (47.4)	0.70 (0.52-0.93)	.01	0.78 (0.59-1.03)	.08
		T1-2	T3-4				
Ins/Ins	279 (46.3)	207 (52.8)	103 (56.3)	1.00		1.00	
Ins/Del	261 (43.4)	150 (38.3)	70 (38.2)	0.77 (0.59-1.01)	.06	0.73 (0.51-1.03)	.07
Del/Del	62 (10.3)	35 (8.9)	10 (5.5)	0.74 (0.47-1.17)	.19	0.44 (0.22-0.89)	.01
Dominant Model	323 (53.7)	185 (47.2)	80 (43.7)	0.77 (0.59-0.99)	.04	0.67 (0.48-0.94)	.02
		N0	N1 to N3				
Ins/Ins	279 (46.3)	123 (50.2)	187 (56.7)	1.00		1.00	
Ins/Del	261 (43.4)	103 (42.0)	117 (35.5)	0.90 (0.66-1.22)	.49	0.66 (0.50-0.88)	.005
Del/Del	62 (10.3)	19 (7.8)	26 (7.9)	0.68 (0.39-1.19)	.17	0.62 (0.38-1.02)	.05
Dominant Model	323 (53.7)	122 (49.8)	143 (43.3)	0.86 (0.64-1.15)	.31	0.66 (0.50-0.86)	.002

Abbreviations: Del = deletion; ER = estrogen receptor; Ins = insertion; OR = odds ratio; PR = progesterone receptor.
[a]Odds ratios were adjusted for age and age at menarche.

that the transcriptional activity can be affected by rs145204276. Taken together, these findings suggest that rs145204276 del allele might protect against the occurrence of BC.

It is evident that SNP not only in protein-coding genes but also in noncoding RNAs might contribute to an individual's susceptibility to BC. For example, the risk-associated allele of rs661204 and rs78540526 in 11q13 reduced chromatin looping between a distal transcriptional enhancer (proteasome core particle subunit beta 4) and the promoter of lncRNAs (Cyclin D1 -upstream intergenic DNA repair 1 and 2).[24] Carriers with the AG genotype of rs619586 in lncRNA metastasis associated lung adenocarcinoma transcript 1 (*MALAT1*) had a decreased risk of BC in a codominant model, dominant mode, and overdominant model, possibly by reducing the expression level of *MALAT1*.[25] These data suggest that the lncRNA-related SNP might affect its expression and eventually influence the risk of BC development.

Growth arrest-specific 5, a downregulated lncRNA in BC, functions as a tumor suppressor by serving as a molecular sponge for micro-(miR)-196a-5p or miR-21.[14,15,26] Upregulation of *GAS5* can not only attenuate proliferation and promote apoptosis in vivo and in vitro but also increase the chemotherapeutic effect of dendrosomal curcumin in BC cells,[14,15,26,27] whereas reduced *GAS5*

Figure 2 (A) Relative Expression of Long Noncoding RNA (lncRNA) *GAS5* in Tissues With Breast Cancer (BC) and Corresponding Nontumor Normal Tissues. (B) Relative Expression of lncRNA *GAS5* in BC Patients Carrying rs145204276 Insertion (Ins)/Ins and Ins/Deletion (Del) and Del/Del Genotypes. (C) Relative Expression of lncRNA *GAS5* in Normal Controls Carrying rs145204276 Ins/Ins and Ins/Del and Del/Del Genotypes. *GAPDH* Was Used as an Internal Control. Data Are Presented as Median With Interquartile Range (*$P < .05$)

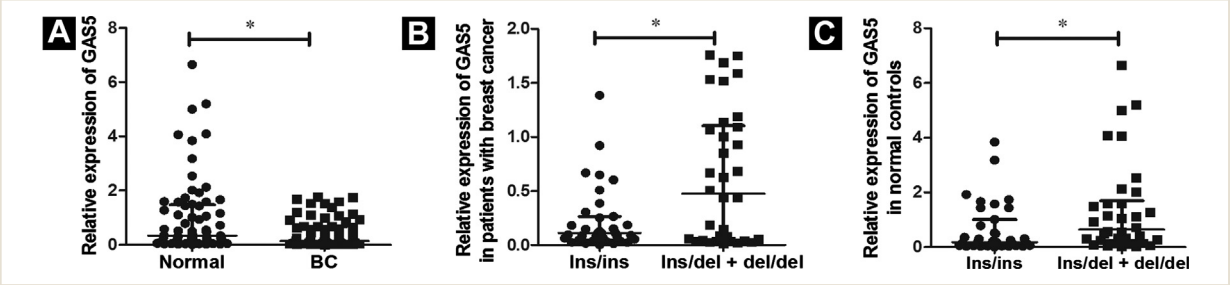
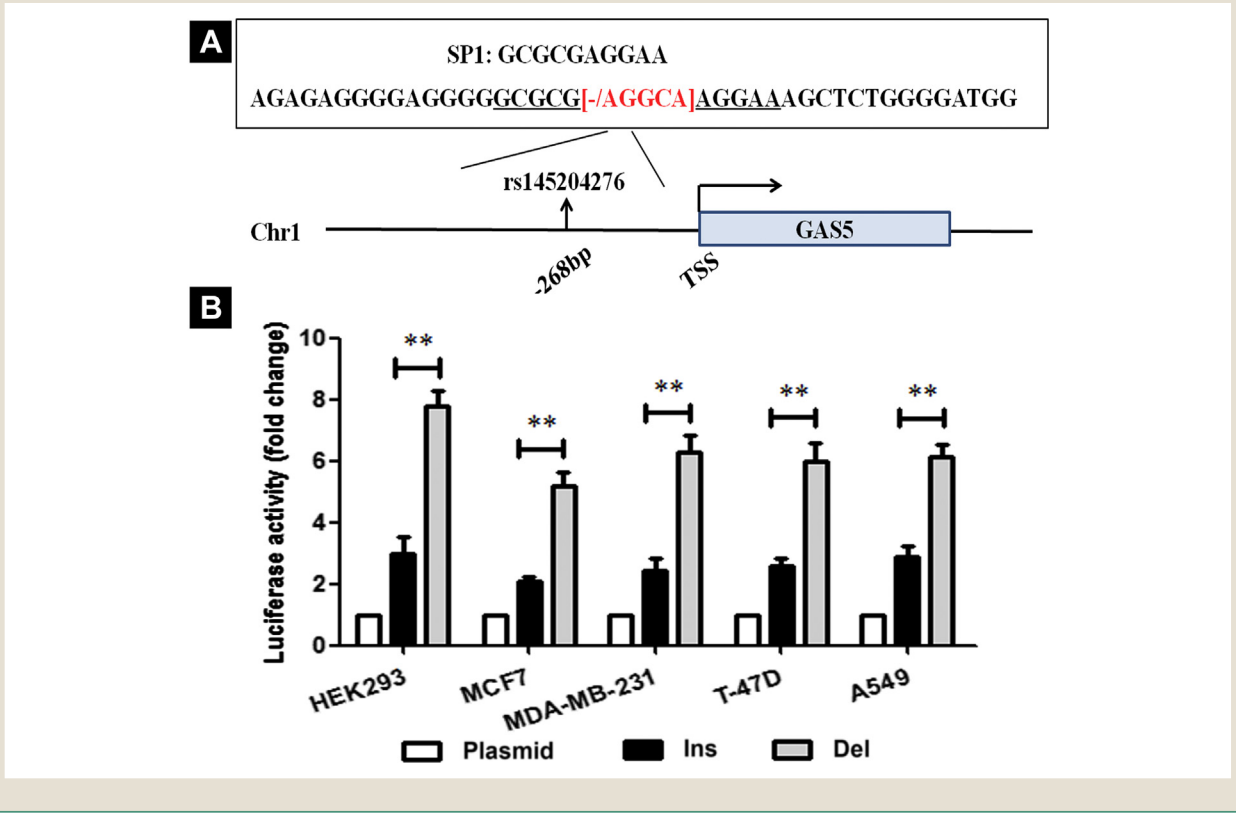


Figure 3 (A) Structure and Location of rs145204276 (-/AGGCA) in the Promoter Region of Long Noncoding RNA (lncRNA) *GAS5*. The rs145204276 Located in the -268 bp Upstream From the Transcriptional Start Site (TSS) of lncRNA *GAS5*. Underlined Sequence (GCGCGAGGAA) Indicates SP1-Binding Site. The rs145204276 Deletion (Del) But Not Insertion (Ins) Allele Can Bind to SP1. (B) The Fragments Containing rs145204276 Ins or Del Allele Were Inserted Into pGL3-Basic Vector. Empty Plasmid and Recombinant Plasmids Were Transfected Into HEK293, MCF7, MDA-MB-231, T-47D, and A549 Cells. At 24 Hours After Transfection, Relative Luciferase Activity Was Measured Using the Dual Luciferase Reporter Assay. Data Are Presented as Mean ± Standard Error (***P* < .01)



Abbreviations: bp = base pairs; HEK293 = human embryonic kidney 293; MCF7 = michigan cancer foundation-7; MDA-MB-231 = human breast carcinoma; pGL3 = promega's luciferase reporter vector; SP1 = specificity protein 1.

expression suppresses apoptosis induction by chemotherapeutic agents in BC cells.[28] In addition to *GAS5*, the *GAS5* hormone response element mimic sequence can also promote BC cell apoptosis.[29] Taken together, these findings indicate that *GAS5* might be a target for the development of BC therapy.

On the basis of the research background described herein, we hypothesized that lncRNA *GAS5*-related SNP might be a reason for explaining individual differences to develop BC. Previously, an ins/del polymorphism rs145204276 in the promoter region of lncRNA *GAS5* was shown to be functional and the del allele was associated with an increased risk of hepatocellular carcinoma[17] and cervical squamous cell carcinoma.[22] In contrast to these results, we found that the rs145204276 del allele was associated with a reduced risk of BC. Our results were consistent with the findings in gastric cancer,[18,19] lung cancer,[20] colorectal cancer,[21] and osteosarcoma.[23] The conflicting results might be explained by the possibility that the susceptibility loci are different in different cancer types. Additionally, the rs145204276 and environment factors might interact with each other in the pathogenesis of BC.

We then explored the potential mechanism of the rs145204276 in reducing BC risk, and we found that the rs145204276 del allele exhibited higher promoter activity and expression levels of lncRNA *GAS5*, further supporting the fact that the elevated levels of lncRNA *GAS5* were observed in cancer patients carrying the rs145204276 del/del genotype.[17-20,23] To recognize the triggers that caused the rs145204276 del allele to increase promoter activity of lncRNA *GAS5*, in silico analysis was used to predict the binding affinity of the rs145204276 with transcriptional factor. As expected, we found that rs145204276 ins allele disrupts the binding to transcriptional factor SP1. Taken together, we might conclude that rs145204276 del allele induced the promoter activity by binding to SP1, resulting in enhanced levels of lncRNA *GAS5* and a higher risk to develop BC in the Han Chinese population.

This study is not without limitation. The expected study power examining the observed effect of rs145204276 on the risk of BC was limited because of moderate sample sizes, and thus it should be noted that the results in this study required confirmation from other studies with larger sample sizes. BC is not a disease with a

single gene or single cause, but rather a collection with multiple genes and diverse risk factors. Gene—gene and gene—environment interactions are worthwhile to explore how these SNPs affect the risk of BC in people exposed to the natural environment. Moreover, follow-up data were not available for the patients, making it difficult to determine whether rs145204276 might serve as a prognosis biomarker for BC. Further studies, therefore, will require survival analysis to provide more comprehensive understanding of rs145204276 in the development and progression of BC.

Conclusion

We showed that the SNP rs145204276 is functional. The rs145204276 del allele increased the transcriptional activity and expression levels of lncRNA GAS5, which might ultimately exhibit a protective effect on the development of BC. Further studies on the mechanism of the functional rs145204276 should be conducted to determine the pathogenesis of BC.

Clinical Practice Points

- Long noncoding RNAs were recently identified to contribute important roles in a variety of biological processes, including the development of BC.
- Growth arrest-specific 5, a kind of lncRNA, functions as a tumor suppressor by affecting tumor growth and cell apoptosis in vivo and in vitro.
- The establishment of an ins/del polymorphism (rs145204276 AGGCA/-) in the promoter region of *GAS5* as a susceptibility gene to the occurrence of BC is of great value for personal treatment.
- In this study we found that the rs145204276 ins/del and del/del genotypes were associated with a reduced risk of BC.
- Moreover, individuals with rs145204276 ins/del and del/del genotypes expressed significantly higher levels of *GAS5* and rs145204276 del allele enhanced the promoter activity of *GAS5*.
- These findings suggest that the rs145204276 might be a biomarker for the risk of BC.

Acknowledgments

This work was supported by the project of the basic research on the application of science and technology department of Yunnan province and technology Kunming Medical University in 2017 (2017FE468 [-074]).

Disclosure

The authors have stated that they have no conflicts of interest.

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