Meta Analysis

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Association between long non-coding RNA (lncRNA) GAS5 polymorphism rs145204276 and cancer risk

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Abstract

Objective: The long non-coding RNA (lncRNA) growth arrest–specific transcript 5 (GAS5) plays an important role in various tumors, and an increasing number of studies have explored the association of the GAS5 rs145204276 polymorphism with cancer risk with inconclusive results.

Methods: PubMed, Medline, EMBASE, Cochrane databases, and Web of Science were searched, and nine studies involving 6107 cases and 7909 controls were deemed eligible. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated to evaluate the relationship between rs145204276 and cancer risk in six genetic models.

Results: The pooled results suggest that the variant allele del was not associated with overall cancer risk. However, the subgroup analysis showed that allele del was significantly associated with a 22% decreased risk of gastrointestinal cancer (OR = 0.78, 95% CI: 0.72–0.85). Both sensitivity analyses and trial sequential analyses (TSA) demonstrated that the subgroup results were reliable and robust. Moreover, False-Positive Report Probability (FPRP) analysis indicated that the results had true significant correlations.

Conclusion: These findings provide evidence that the GAS5 rs145204276 polymorphism is associated with the susceptibility to gastrointestinal cancer. Further studies with different ethnicities and larger sample sizes are warranted to confirm these results.

Keywords

GAS5, rs145204276, cancer risk, gastrointestinal cancer, polymorphism, long non-coding RNA

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Introduction

Long non-coding RNAs (lncRNAs) are a group of functional RNAs that do not code for protein and are more than 200 nucleotides in length.[1] High throughput sequencing technology has helped identify various lncRNAs that play important roles in cell cycle progression, apoptosis, epigenetics, and regulation of gene expression.[2,3] LncRNAs can interact with DNA, other RNAs, and proteins, as well as regulate the expression and function of various genes on the epigenetic, transcriptional, post-transcriptional, and translational levels.[3,4] They are a current research hotspot, especially in cancer research because of their functions in tumorigenesis, cancer progression, and metastasis.[5]

The gene for lncRNA growth arrest-specific 5 (LncRNA GAS5) is located at chromosome 1q25, and the full RNA molecule is 630 nucleotides long. GAS5 was identified as a tumor suppressor gene in various tumor types, including breast cancer,[6] gastric cancer (GC),[7] bladder cancer,[8] pancreatic cancer,[9] prostate cancer,[10] colorectal cancer (CRC),[11] and others. GAS5 expression is significantly reduced in breast cancer samples relative to adjacent normal breast epithelial tissues, and its expression induces growth arrest and apoptosis of breast cancer cell lines.[6] GAS5 expression levels in GC are also lower compared with the normal counterparts, and can enhance G1 cell cycle arrest via the YBX1/p21 pathway.[7] Similarly, downregulation of GAS5 promotes bladder cancer cell proliferation,[8] which has also been observed in pancreatic cancer, prostate cancer, and CRC.[9–11] Upregulation of GAS5 in digestive tumors inhibits cancer cell proliferation, invasion, and migration by regulating related microRNAs (miRNAs), inhibiting epithelial-mesenchymal transition (EMT) processes, activating certain signaling pathways (PI3K/Akt, Wnt/β-cat, NF-κB), and inhibiting cell cycle progression.[12] Therefore, GAS5 plays important roles in various tumors.[9–12]

Single nucleotide polymorphisms (SNPs) are one of the main types of genetic variation, and account for more than 90% of all known polymorphisms.[13] A recent study showed that polymorphisms present in the promoter region of lncRNA genes can affect regulation of the RNA expression level.[14] There is a five base pair (bp) insertion/deletion (indel) polymorphism (rs145204276, AGGCA/-) in the GAS5 promoter region, and allele del increases luciferase activity and expression levels of GAS5.[15] Aminian et al. found that the del/del genotype showed protective effects on GC risk ($P = 0.01$) by modulating cyclin-dependent kinase inhibitor 1B ($p27^{Kip1}$) protein expression.[16] Li et al. also observed that allele del was associated with decreased risk of GC ($P = 0.005$), lymph node metastasis ($P = 0.01$), and distant metastasis of GC,[17] as well as with a higher patient survival rate ($P = 0.01$).[18] These characteristics of GAS5 rs145204276 were also observed in CRC,[19] lung cancer,[20] breast cancer,[21] and osteosarcoma.[22] However, other research groups found that rs145204276 del allele increased the risk of hepatocellular carcinoma (HCC) ($P < 0.01$)[15] and glioma ($P < 0.01$).[23] Because these controversies require further analysis on rs145204276, we conducted a meta-analysis to evaluate the role of this polymorphism in various tumors.

Materials and methods

Publication search

We performed this meta-analysis in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020 statement) guidelines,[24] and the protocol has been registered

in the INPLASY database (INPLASY2 02170036). Two independent investigators performed a literature search of the articles found on PubMed, Medline, Cochrane Library, EmBase, and Web of Science published before 9 February 2020 using the following keywords: "GAS5/growth arrest-specific 5," "polymorphisms/SNP/single nucleotide polymorphism," and "cancer/carcinoma/tumor/neoplasm." All included studies met the following criteria: (1) the study focused on the association between lncRNA SNPs and cancer risk; (2) the study was a clinical case–control study; (3) a study with the distribution of genotypes in controls was consistent with Hardy–Weinberg equilibrium (HWE); and (4) the study was published in the English language. The exclusion criteria were: (1) the study was a duplicate study; (2) the study was not relevant to cancer or lncRNA SNPs; or (3) the study had no available data and the authors could not be contacted. Two authors independently reviewed the titles and abstracts. Full texts of individual studies were then thoroughly reviewed according to the inclusion and exclusion criteria. A flowchart of the detailed screening process is shown in Figure 1.

Data extraction

Two investigators independently extracted the data and reached a consensus regarding all items. The following information was extracted from the included studies: first author's name, year of publication, country of origin, ethnicity, type of cancer, genotyping method, source of the control group (population- or hospital-based), total number of cases and controls, genotype distributions in the cases and controls, and adjusted factors. Additionally, we categorized ethnicity as Caucasian or Asian. If the data were not stated clearly in the paper, the corresponding author was contacted for further information.

Statistical analysis

The HWE test was conducted on the allele frequency of the control group by using the chi-square test, and $P < 0.05$ was considered as statistically significant disequilibrium. The risk of cancer associated with each polymorphism was summarized as odds ratios (ORs) and the 95% confidence intervals (95% CI; $P < 0.05$ was considered statistically significant) for each study. Both the chi-square test and I^2 statistics were used to examine the heterogeneity across the included studies. When significant heterogeneity existed across the studies ($I^2 > 50\%$ or $P < 0.10$), the random-effect model was used for the meta-analysis. Otherwise, the fixed-effect model was implemented.[25,26] For genotype comparisons, the risk of six genetic models including the dominant model, recessive model, additive model, homozygous model, heterozygous model, and allele model was estimated respectively. Subgroup analysis was performed by type of cancer. Potential publication bias was assessed by funnel plot and the Harbord test.[27] All analyses were performed using STATA version 12.0 (Stata Corporation, College Station, TX, USA), and $P < 0.05$ of the two-tailed probability was considered statistically significant.

Trial sequential analysis (TSA)

Because of systematic errors and random errors caused by sparse data and repetitive testing, conventional meta-analyses of cumulative trails may include false positive results (type I errors) and false negative results (type II errors).[28] Trial sequential analysis (TSA) is a tool for quantifying the statistical reliability of data to overcome these limitations of traditional

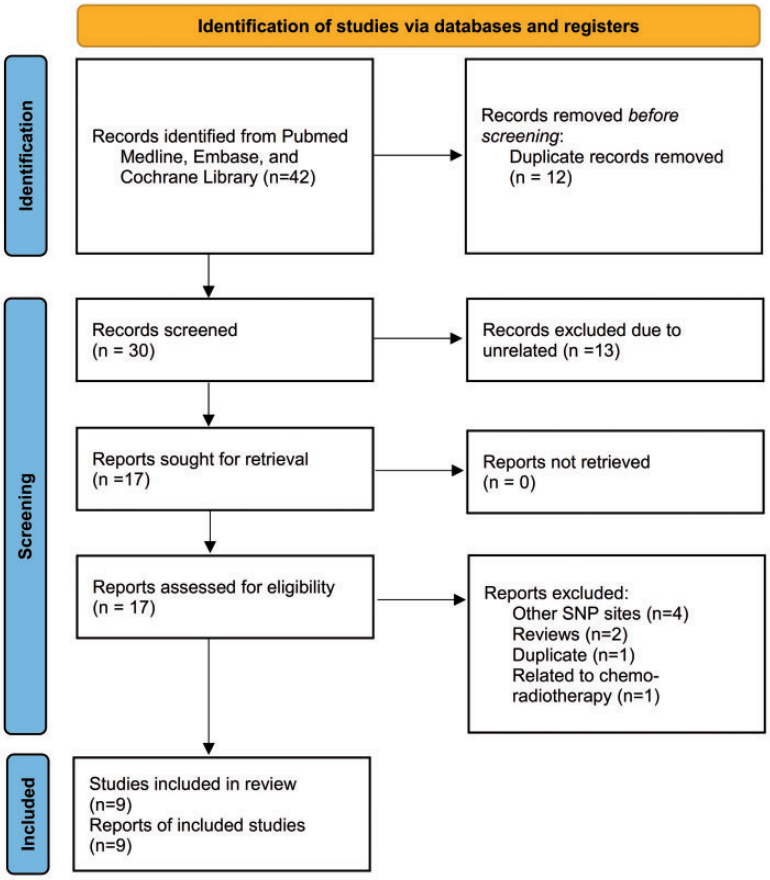


Figure 1. Flow diagram of the study selection process.

meta-analyses. Therefore, TSA was performed to control for random errors and to assess the required sample information.[29] In TSA, we generated the cumulative Z-curve of each study and assessed their crossing Z-value as 1.96 ($P = 0.05$), as well as the trial sequential monitoring boundaries. To calculate the optimal information size, type I error was set at 5% and type II error was set at 20%. The TSA was conducted by TSA program (TSA version 0.9 beta software, Copenhagen Trial Unit 2011, http://www.ctu.dk/tsa).

False-Positive Report Probability Analysis (FPRP) and Single-tissue expression quantitative trait loci (eQTL) in the Genotype-Tissue Expression (GTEx) database

FPRP analyses were performed to assess the significant results observed in the current study. We set an FPRP cutoff value of 0.2 and a prior probability level of 0.01 to detect an OR of 1.5 (for risk factor) or 0.65 (for protective factor) for an association with genotypes. Only a significant result with an FPRP < 0.20 was considered noteworthy.

To evaluate the influence of the rs145204276 polymorphism on GAS5, we searched the GTEx database to explore the association between this polymorphism and GAS5 expression levels (dbGaP Accession phs000424.v8.p2).

Results

Characteristics of the eligible studies

Seventeen articles met our inclusion criteria, but eight studies were excluded for the following reasons: studies that focused on other SNP sites in GAS5,[30–33] review articles,[12,34] a study related to chemoradiotherapy,[35] and a duplicate study.[17] The remaining nine case-control studies were included in this meta-analysis.[15–23] The selection process is summarized in Figure 1, and the characteristics of the included studies are shown in Table 1. There were a total of 6107 cases and 7909 controls included in our meta-analysis. Among these, there were two studies on GC[16–18] and one study each on CRC,[19] lung cancer,[20] breast cancer,[21] HCC,[15] osteosarcoma,[22] glioma,[23] and prostate cancer.[36] For genotyping methods, amplification-refractory mutation system–polymerase chain reaction (ARMS-PCR) was used in one study,[16] MassArray was applied in another study,[23] and real-time PCR was used in the other studies. The *P*-value of the HWE test was more than 0.05 in all the studies. The genotype frequency distribution of rs145204276 involved in the nine included studies are also presented in Table 1.

The association between GAS5 rs145204276 and overall cancer susceptibility

There were nine studies involving 6107 cancer patients and 7909 controls that investigated the association between rs145204276 and cancer risk. As shown in Table 2, the pooled results indicated that GAS5 polymorphism rs145204276 was not associated with overall cancer risk in any of the five genetic models (Figure 2): dominant model: OR = 0.86, 95% CI: 0.69–1.07; recessive model: OR = 0.93, 95% CI: 0.68–1.28; additive model: OR = 1.13, 95% CI: 1.00–1.28; heterozygote model: OR = 0.86, 95% CI: 0.71–1.04; and homozygote model: OR = 0.87, 95% CI: 0.59–1.29. When pooled together, the GAS5 allele del was not associated with the overall cancer susceptibility (OR = 0.89, 95% CI: 0.74–1.08). Significant heterogeneity existed across the studies ($I^2 > 50\%$, shown in Table 2), so the random-effect model was used for the meta-analysis here.

The association between GAS5 rs145204276 and gastrointestinal cancer susceptibility

Because of significant heterogeneity, subgroup analyses were conducted based on cancer type. In the gastrointestinal cancer subgroup, 2783 patients and 2984 controls were included in three studies estimating the association between rs145204276 and gastrointestinal cancer risk.[16,18,19] The pooled OR suggested that rs145204276 was significantly associated with a decreased risk of gastrointestinal cancer (Figure 2) in the dominant model (OR = 0.74, 95% CI: 0.67–0.82), recessive model (OR = 0.72, 95% CI: 0.60–0.87), additive model (OR = 1.23, 95% CI: 1.11–1.37), heterozygote model (OR = 0.76, 95% CI: 0.68–0.85), and homozygote model (OR = 0.64, 95% CI: 0.53–0.78). The pooled OR indicated that the variant GAS5 allele del was significantly associated with a 22% decreased risk of gastrointestinal cancer (OR = 0.78, 95% CI: 0.72–0.85). For this analysis, the I^2 was 0% and $P > 0.1$ (shown in Table 2), so no obvious

Table 1. Characteristics and rs145204276 genotype frequency distributions of eligible studies.

								Genotyping distribution						Allele					
								Case			Control			Case		Control			
Author	Year	Region	Ethnicity	Source of control group	Cancer	Genotyping method	Case	Control	ins/ins	ins/del	del/del	ins/ ins	ins/del	del/del	ins	del	ins	del	HWE	Adjusted factors
Aminian, K.	2018	Iran	Caucasian	PB	Gastric cancer	T-ARMS-PCR	130	230	88	36	6	126	84	20	212	48	336	124	0.27	age and sex
Li, Q. J.	2018	China	Asian	PB	Gastric cancer	Real-time PCR	1253	1354	682	483	88	638	593	123	1847	659	1869	839	0.38	NM
Li, W.	2017	China	Asian	PB	Lung cancer	Real-time PCR	600	600	287	270	43	246	292	62	844	356	784	416	0.07	age, sex, and smoking status
Lin, C. Y.	2019	China	Asian	NM	Prostate cancer	Real-time PCR	579	579	263	252	64	237	270	72	778	380	744	414	0.72	NM
Tang, Y.	2018	China	Asian	HB	Breast cancer	Real-time PCR	575	602	310	220	45	279	261	62	840	310	819	385	0.93	age and age at menarche
Tao, R.	2015	China	Asian	PB	Hepatocellular cancer	Real-time PCR	1034	1054	414	480	140	504	468	82	1308	760	1476	632	0.06	sex, age, smoking, drinking, tumor stage and HBV infection
Xu, L.	2018	China	Asian	HB	Osteosarcoma	Real-time PCR	132	1270	80	42	10	616	543	111	202	62	1775	765	0.58	NM
Yuan, J.	2018	China	Asian	HB	Glioma	MassArray	404	820	154	198	52	419	346	55	506	302	1184	456	0.14	age and sex
Zheng, Y.	2016	China	Asian	HB	Colorectal cancer	Real-time PCR	1400	1400	738	550	112	639	610	151	2026	774	1888	912	0.76	age, sex, alcohol and smoking status

HB, hospital based; PB, population based; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; CRS-RFLP, create restriction site-restriction fragment length polymorphism; NM, not mentioned; HBV, hepatitis B virus; ARMS-PCR, amplification-refractory mutation system – polymerase chain reaction.

Table 2. Meta-analysis of the association between rs145204276 and cancer risk.

	Overall (N = 9)								Gastrointestinal cancer (N = 3)							
Models	OR	95% *CI*	*P*	Phet *	I^2 (%)	*Begg*	*Egger*	*Harbord*	OR	95% *CI*	*P*	Phet *	I^2 (%)	*Begg*	*Egger*	*Harbord*
Allele (del vs. ins)	0.89	0.74, 1.08	0.24	<0.001	91.4	0.6	0.71	0.75	0.78	0.72,0.85	**<0.001**	0.42	0	1	0.06	0.07
Dominant (ins/del+del/del vs. ins/ins)	0.86	0.69, 1.07	0.17	<0.001	89.1	0.6	0.79	0.78	0.74	0.67, 0.82	**<0.001**	0.54	0	0.3	0.25	0.26
Recessive (del/del vs. ins/ins+ins/del)	0.93	0.68, 1.28	0.67	<0.001	83.5	0.92	0.77	0.81	0.72	0.60, 0.87	**0.001**	0.73	0	1	0.27	0.3
Additive (ins/ins+del/del vs. ins/del)	1.13	1.00, 1.28	0.06	0.003	65.3	0.92	0.58	0.61	1.23	1.11, 1.37	**<0.001**	0.5	0	0.3	0.23	0.23
Heterozygote (ins/del vs. ins/ins)	0.86	0.71, 1.04	0.12	<0.001	83.1	0.92	0.74	0.75	0.76	0.68, 0.85	**<0.001**	0.64	0	0.3	0.12	0.13
Homozygote (del/del vs. ins/ins)	0.87	0.59, 1.29	0.49	<0.001	89	0.6	0.79	0.86	0.64	0.53, 0.78	**<0.001**	0.64	0	1	0.21	0.24

Note: The results are in bold if $P < 0.05$, *P-value of the heterogeneity test.
OR, odds ratio; CI, confidence interval.

Figure 2. Forest plots of the relationship between the GAS5 rs145204276 polymorphism and cancer risk and the subgroup analysis. (a) Allele contrast (del vs. ins). (b) Dominant model (del/del + del/ins vs. ins/ins). (c) Recessive model (del/del vs. del/ins + ins/ins). (d) Additive model (del/del + ins/ins vs. del/ins). (e) Heterozygote model (del/ins vs. ins/ins). (f) Homozygote model (del/del vs. ins/ins).

heterogeneity was observed across the studies and the fixed-effect model was used.

Sensitivity analyses were conducted by omitting a single study in each turn to substantiate the stability of significant results, which showed that all the pooled ORs were essentially unchanged (Figure 3). Additionally, in TSA, the Z-curve crossed the trial sequential monitoring boundary and reached the required information size

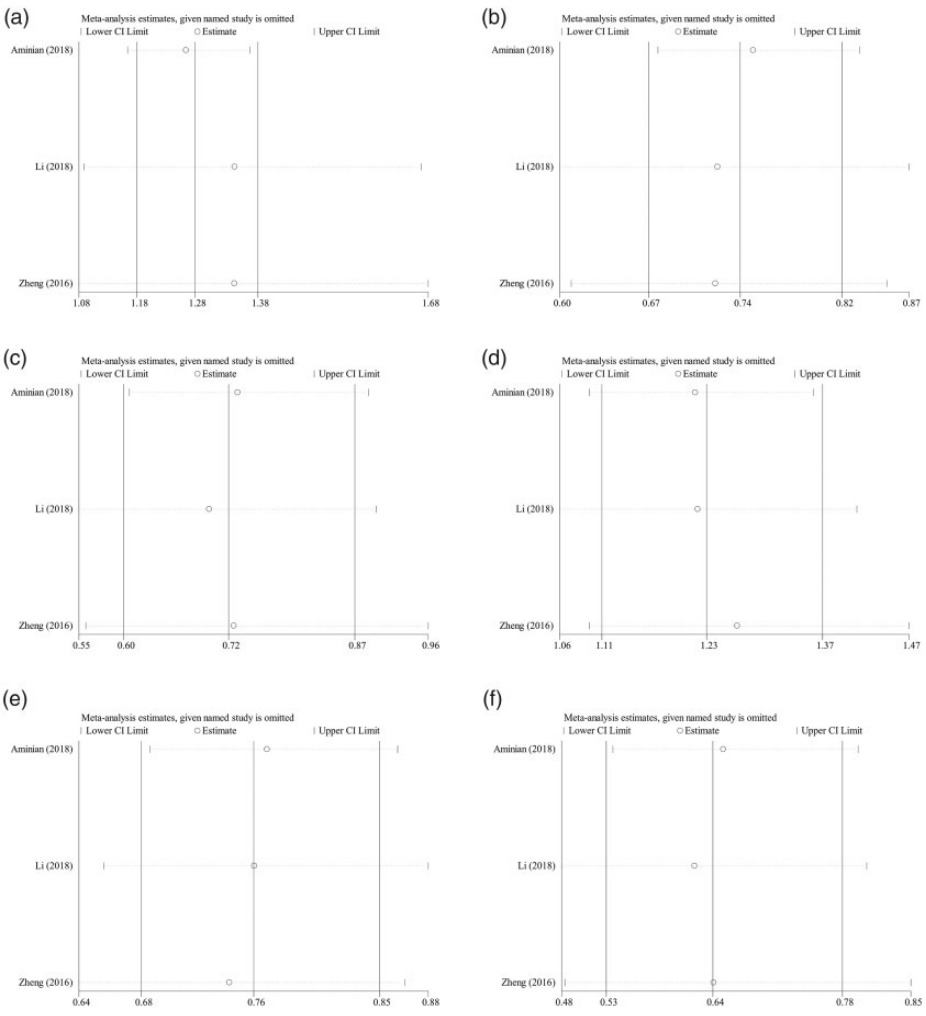


Figure 3. Sensitivity analyses of the relationship between the GAS5 rs145204276 polymorphism and the risk of gastrointestinal cancer. (a) Allele contrast (del vs. ins). (b) Dominant model (del/del+del/ins vs. ins/ins). (c) Recessive model (del/del vs. del/ins + ins/ins). (d) Additive model (del/del+ins/ins vs. del/ins). (e) Heterozygote model (del/ins vs. ins/ins). (f) Homozygote model (del/del vs. ins/ins).

in all models, which demonstrated the subgroup results were reliable (Figure 4). Moreover, the significant results were also further assessed by the FPRP test. As demonstrated in Table 3, for a prior probability setting at 0.01, the FPRP values were all less than the cut-off value of 0.20 in those significant findings, indicating that these were truly significant correlations.

Publication bias

No statistically significant publication bias was found in any of the genetic models in the present study (Table 2). Taking the allele model data as an example, the modified Harbord test and Begg's funnel plot showed no evidence of publication bias ($P > 0.05$, Figure 5).

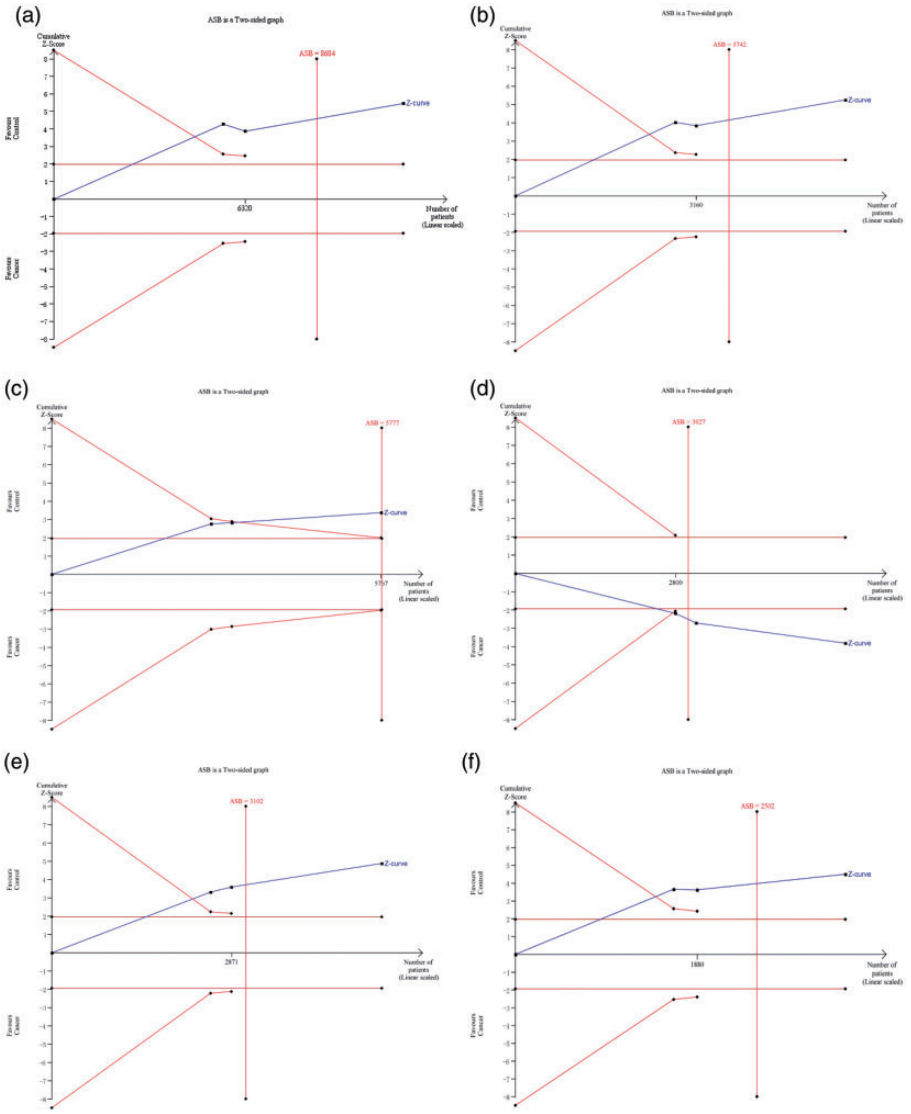


Figure 4. Trial sequential analyses of the relationship between the GAS5 rs145204276 polymorphism and the risk of gastrointestinal cancer. (a) Allele contrast (del vs. ins). (b) Dominant model (del/del+del/ins vs. ins/ins). (c) Recessive model (del/del vs. del/ins + ins/ins). (d) Additive model (del/del+ins/ins vs. del/ins). (e) Heterozygote model (del/ins vs. ins/ins). (f) Homozygote model (del/del vs. ins/ins).

Single-tissue eQTLs in the GTEx database

According to the GTEx portal data, as illustrated in Figure 6, the mutant allele of rs145204276 led to a dose-dependent upregulated expression of GAS5 in different tissues and cell lines.

Discussion

Recently, an increasing number of studies have explored the associations of GAS5 polymorphisms with cancer risk, but had inconclusive results. In the current analysis, we statistically summarized the association

Table 3. False-positive report probability values for associations between rs145204276 polymorphisms and gastrointestinal cancer risk.

						Prior probability			
Site	Gene models	OR	95% CI	*P*	Power	0.25	0.1	0.01	0.001
rs145204276	Allele (del vs. ins)	0.78	0.72–0.85	**<0.001**	0.87	<0.001	<0.001	0.01	0.11
	Dominant (ins/del+del/del vs. ins/ins)	0.74	0.67–0.82	**<0.001**	0.99	<0.001	<0.001	<0.001	<0.001
	Recessive (del/del vs. ins/ins+ins/del)	0.72	0.60–.87	**0.001**	0.86	0.002	0.007	0.07	0.44
	Additive (ins/ins+del/del vs. ins/del)	1.23	1.11–1.37	**<0.001**	1.00	<0.001	0.002	0.02	0.14
	Heterozygote (ins/del vs. ins/ins)	0.76	0.68–0.85	**<0.001**	1.00	<0.001	<0.001	<0.001	0.002
	Homozygote (del/del vs. ins/ins)	0.64	0.53–0.78	**<0.001**	0.44	<0.001	<0.001	0.002	0.02

OR, odds ratio; CI, confidence interval.

between lncRNA GAS5 polymorphism rs145204276 and cancer risk based on the currently published data. After strict screening of the current literature, we included eight studies for this quantitative analysis. The results indicated that the variant allele del of GAS5 was not associated with overall cancer risk. However, the subgroup analysis showed that allele del was significantly associated with a 22% decreased risk of gastrointestinal cancer.

GAS5 is a newly discovered lncRNA that has attracted recent attention and plays an important role in the development of tumors. The mechanism behind these observations is still unclear, and various tumors possibly have different mechanisms. In prostate cancer, GAS5 may bind directly to transcription factor E2F1 and then activate the $p27^{Kip1}$ promoter, which mainly inhibits the Cdk2-Cyclin E complex and thus induces a cell cycle arrest in the G0-G1 phase.[37] GAS5 in GC may enhance G1 cell cycle arrest via the YBX1/p21 pathway.[7] GAS5 may also inhibit miRNAs and then regulate their target genes, as well as tumor functions. Examples include miR-103/PTEN in endometrial cancer,[38] miR-196a and miR-205 in cervical cancer,[39] and miR-21/PTEN in non-small cell lung cancer (NSCLC).[40] Studies have shown that the presence of rs145204276 del/del significantly activated GAS5 promoter activity.[15,19] Consistent with previous studies,[41] our GTEx portal database analysis showed that the mutant allele of rs145204276 led to a dose-dependent upregulation of GAS5 expression, which plays an important role in cancer development.

Although two studies have reported that the GAS5 ins/del polymorphism was associated with a predisposition to GC, the findings were not robust.[42,43] In the present study, we conducted meta-analyses, in addition to TSA analyses, FPRP tests, and single-tissue eQTLs. These results strongly demonstrated that GAS5 polymorphism rs145204276 is associated with the susceptibility to gastrointestinal cancer. Additionally, several meta-analyses

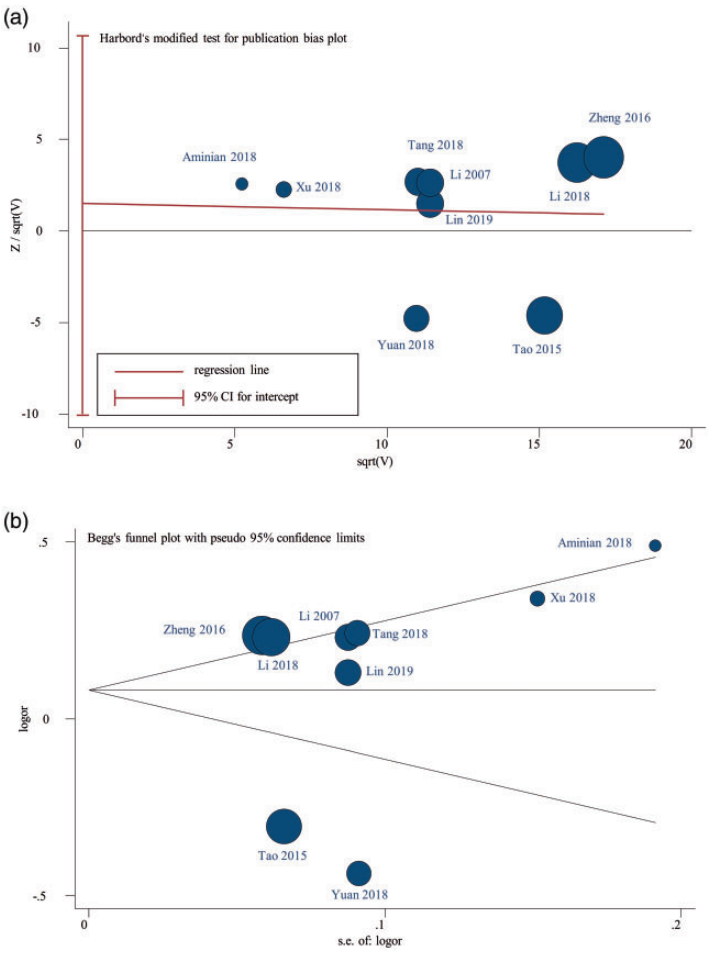
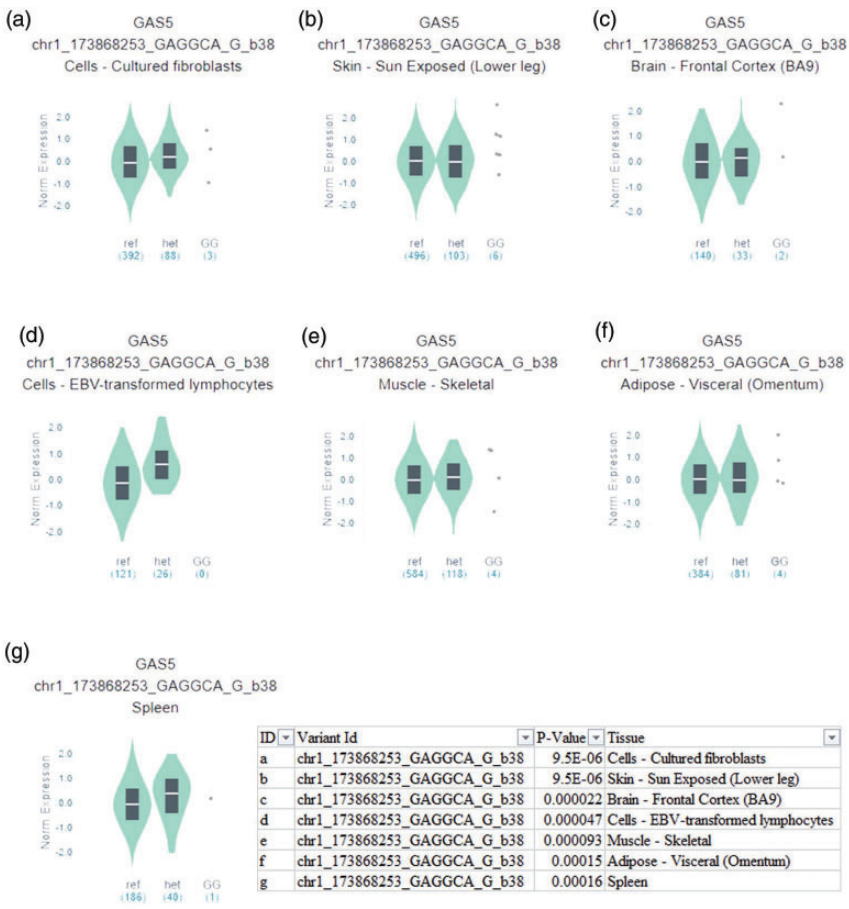


Figure 5. Harbord test and Begg's funnel plot of the relationship between the GAS5 rs145204276 polymorphism allele model and cancer risk.

evaluated the diagnostic and prognostic values of GAS5. Li et al. conducted a meta-analysis, which suggested that decreased GAS5 expression was associated with unfavorable overall survival (OS) (HR = 2.50, 95% CI: 1.85–3.38, $P < 0.001$) and disease-free survival (DFS) (HR = 2.24, 95% CI: 1.58–3.18, $P < 0.001$) in several tumor types.[32] Similar conclusions were drawn in other meta-analyses in bladder cancer,[44] lung cancer,[45] and other cancer types.[46,47] The 5 bp indel polymorphism rs145204276 in the promoter region of GAS5 can regulate the expression of GAS5 and thus its multiple biological functions.

In our current meta-analysis, we did not identify a significant relationship between the rs145204276 polymorphism and overall cancer risk. This is possibly caused by the variety of cancer types and the heterogeneity among the different tumors. Tao et al.[15] found that rs145204276 could regulate the expression of GAS5 and thus significantly increase the risk of HCC. Similarly, Yuan et al.[23] reported that rs145204276 was



ID	Variant Id	P-Value	Tissue
a	chr1_173868253_GAGGCA_G_b38	9.5E-06	Cells - Cultured fibroblasts
b	chr1_173868253_GAGGCA_G_b38	9.5E-06	Skin - Sun Exposed (Lower leg)
c	chr1_173868253_GAGGCA_G_b38	0.000022	Brain - Frontal Cortex (BA9)
d	chr1_173868253_GAGGCA_G_b38	0.000047	Cells - EBV-transformed lymphocytes
e	chr1_173868253_GAGGCA_G_b38	0.000093	Muscle - Skeletal
f	chr1_173868253_GAGGCA_G_b38	0.00015	Adipose - Visceral (Omentum)
g	chr1_173868253_GAGGCA_G_b38	0.00016	Spleen

Figure 6. Genotype-tissue expression analysis of rs145204276 in the GTEx database.

significantly associated with elevated risk of glioma. However, the other studies included showed that rs145204276 was associated with decreased risk of cancers. Taken together, the pooled meta-analysis results are negative for overall cancer susceptibility. Second, these studies contained a small sample size for overall cancer risk and may not be sufficiently large to reach a solid conclusion. According to the TSA results, the cumulative Z-curve did not cross any of the sequential monitoring boundaries or enter the futility area in any of the five models (data not shown), which indicated that additional well-designed studies are needed for stronger conclusions. Three studies[16,17,19] included gastrointestinal cancer cases, so we conducted a subgroup analysis stratified by cancer site. The subgroup results revealed that the variant GAS5 allele del was significantly associated with a 22% decreased risk of gastrointestinal cancer, indicating a tumor-suppressive role of the GAS5 allele del in gastrointestinal cancer. Furthermore, the TSA results showed that the Z-curve crossed the trial sequential monitoring boundary and reached the required information size in all models, while the FPRP values were all less than the cut-off value. These findings

both demonstrated that the results were reliable.

The current quantitative analysis explored the effect of the rs145204276 polymorphism on cancer risk for the first time. However, there are several limitations in our study. First, significant heterogeneity was observed among the included studies. In addition, studies not published in English were excluded, which may lead to potential publication bias. However, no statistically significant publication bias was shown in any of the genetic models. Furthermore, the sample size of the eligible reports was not large, which could result in decreased statistical power and increase the probability of random errors. More importantly, the majority of the subjects were Asian, so our results should be cautiously interpreted and implied when it comes to other ethnicities. Thus, well-conducted studies with larger sample sizes are needed to further explore the cancer risks associated with the GAS5 rs145204276 polymorphism, especially in Caucasians.

Conclusions

Despite these limitations, this meta-analysis indicates that the GAS5 rs145204276 allele del polymorphism was significantly associated with a decreased risk of gastrointestinal cancer in all five genetic models examined. Our study provides a theoretical basis and research direction for future studies. More rigorous studies in patients of different ethnicities and with a larger sample size are warranted to confirm our results.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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Author contributions

S. Zhao and LT designed the systematic review and performed the search and study selection. PL and ZR extracted and analyzed the data. JL, S. Zeng, and MZ drafted the manuscript. LT took responsibility for the whole process.

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References

1. Mercer TR, Dinger ME and Mattick JS. Long non-coding RNAs: insights into functions. *Nat Rev Genet* 2009; 10: 155–159.
2. Bhan A and Mandal SS. Long noncoding RNAs: emerging stars in gene regulation, epigenetics and human disease. *ChemMedChem* 2014; 9: 1932–1956.
3. Ponting CP, Oliver PL and Reik W. Evolution and functions of long noncoding RNAs. *Cell* 2009; 136: 629–641.
4. Lee JT. Epigenetic regulation by long noncoding RNAs. *Science* 2012; 338: 1435–1439.
5. Bhan A, Soleimani M and Mandal SS. Long Noncoding RNA and Cancer: A New Paradigm. *Cancer Res* 2017; 77: 3965–3981.
6. Mourtada-Maarabouni M, Pickard MR, Hedge VL, et al. GAS5, a non-protein-coding RNA, controls apoptosis and is downregulated in breast cancer. *Oncogene* 2009; 28: 195–208.
7. Liu Y, Zhao J, Zhang W, et al. lncRNA GAS5 enhances G1 cell cycle arrest via binding to YBX1 to regulate p21 expression in stomach cancer. *Sci Rep* 2015; 5: 10159.
8. Liu Z, Wang W, Jiang J, et al. Downregulation of GAS5 promotes bladder cancer cell proliferation, partly by regulating CDK6. *PLoS One* 2013; 8: e73991.

9. Lu X, Fang Y, Wang Z, et al. Downregulation of gas5 increases pancreatic cancer cell proliferation by regulating CDK6. *Cell Tissue Res* 2013; 354: 891–896.
10. Pickard MR, Mourtada-Maarabouni M and Williams GT. Long non-coding RNA GAS5 regulates apoptosis in prostate cancer cell lines. *Biochim Biophys Acta* 2013; 1832: 1613–1623.
11. Yang Y, Shen Z, Yan Y, et al. Long non-coding RNA GAS5 inhibits cell proliferation, induces G0/G1 arrest and apoptosis, and functions as a prognostic marker in colorectal cancer. *Oncol Lett* 2017; 13: 3151–3158.
12. Lu S, Su Z, Fu W, et al. Altered expression of long non-coding RNA GAS5 in digestive tumors. *Biosci Rep* 2019; 39: BSR20180789.
13. Jiang Z, Wang H, Michal JJ, et al. Genome Wide Sampling Sequencing for SNP Genotyping: Methods, Challenges and Future Development. *Int J Biol Sci* 2016; 12: 100–108.
14. Sasamoto H, Nagasaka T, Notohara K, et al. Allele-specific methylation analysis on upstream promoter region of H19 by methylation-specific PCR with confronting two-pair primers. *Int J Oncol* 2004; 25: 1273–1278.
15. Tao R, Hu S, Wang S, et al. Association between indel polymorphism in the promoter region of lncRNA GAS5 and the risk of hepatocellular carcinoma. *Carcinogenesis* 2015; 36: 1136–1143.
16. Aminian K, Mashayekhi F, Mirzanejad L, et al. A functional genetic variant in GAS5 lncRNA (rs145204276) modulates p27 (Kip1) expression and confers risk for gastric cancer. *Br J Biomed Sci* 2018: 1–3.
17. Li Q, Ma G, Sun S, et al. Polymorphism in the promoter region of lncRNA GAS5 is functionally associated with the risk of gastric cancer. *Clin Res Hepatol Gastroenterol* 2018; 42: 478–482.
18. Li QJ, Ma G, Guo HM, et al. The Variant rs145204276 of GAS5 is Associated with the Development and Prognosis of Gastric Cancer. *J Gastrointestin Liver Dis* 2018; 27: 19–24.
19. Zheng Y, Song D, Xiao K, et al. LncRNA GAS5 contributes to lymphatic metastasis in colorectal cancer. *Oncotarget* 2016; 7: 83727–83734.
20. Li W, Huang K, Wen F, et al. Genetic variation of lncRNA GAS5 contributes to the development of lung cancer. *Oncotarget* 2017; 8: 91025–91029.
21. Tang Y, Wang Y, Wang X, et al. A Genetic Variant of rs145204276 in the Promoter Region of Long Noncoding RNA GAS5 Is Associated With a Reduced Risk of Breast Cancer. *Clin Breast Cancer* 2018.
22. Xu L, Xia C, Xue B, et al. A promoter variant of lncRNA GAS5 is functionally associated with the development of osteosarcoma. *J Bone Oncol* 2018; 12: 23–26.
23. Yuan J, Zhang N, Zheng Y, et al. LncRNA GAS5 Indel Genetic Polymorphism Contributes to Glioma Risk Through Interfering Binding of Transcriptional Factor TFAP2A. *DNA Cell Biol* 2018; 37: 750–757.
24. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 2021; 372: n71.
25. Ioannidis JP, Patsopoulos NA and Evangelou E. Uncertainty in heterogeneity estimates in meta-analyses. *BMJ* 2007; 335: 914–916.
26. Higgins JP and Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; 21: 1539–1558.
27. Harbord RM, Egger M and Sterne JA. A modified test for small-study effects in meta-analyses of controlled trials with binary endpoints. *Stat Med* 2006; 25: 3443–3457.
28. Pereira TV and Ioannidis JP. Statistically significant meta-analyses of clinical trials have modest credibility and inflated effects. *J Clin Epidemiol* 2011; 64: 1060–1069.
29. Wetterslev J, Jakobsen JC and Gluud C. Trial Sequential Analysis in systematic reviews with meta-analysis. *BMC Med Res Methodol* 2017; 17: 39.
30. Zhu L, Zhu Q, Wen H, et al. Mutations in GAS5 affect the transformation from benign prostate proliferation to aggressive prostate cancer by affecting the transcription efficiency of GAS5. *J Cell Physiol* 2019; 234: 8928–8940.

31. Wang Y, Wu S, Yang X, et al. Association between polymorphism in the promoter region of lncRNA GAS5 and the risk of colorectal cancer. *Biosci Rep* 2019; 39: BSR20190091.
32. Yan H, Zhang DY, Li X, et al. Long noncoding RNA GAS5 polymorphism predicts a poor prognosis of acute myeloid leukemia in Chinese patients via affecting hematopoietic reconstitution. *Leuk Lymphoma* 2017; 58: 1948–1957.
33. Rakhshan A, Esmaeili MH, Kahaei MS, et al. A Single Nucleotide Polymorphism in GAS5 lncRNA is Associated with Risk of Bladder Cancer in Iranian Population. *Pathol Oncol Res* 2019.
34. Ghaforui-Fard S and Taheri M. Growth arrest specific transcript 5 in tumorigenesis process: An update on the expression pattern and genomic variants. *Biomed Pharmacother* 2019; 112: 108723.
35. Guo Z, Wang Y, Zhao Y, et al. Genetic polymorphisms of long non-coding RNA GAS5 predict platinum-based concurrent chemoradiotherapy response in nasopharyngeal carcinoma patients. *Oncotarget* 2017; 8: 62286–62297.
36. Lin CY, Wang SS, Yang CK, et al. Impact of GAS5 genetic polymorphism on prostate cancer susceptibility and clinicopathologic characteristics. *Int J Med Sci* 2019; 16: 1424–1429.
37. Luo G, Liu D, Huang C, et al. LncRNA GAS5 Inhibits Cellular Proliferation by Targeting P27(Kip1). *Mol Cancer Res* 2017; 15: 789–799.
38. Guo C, Song WQ, Sun P, et al. LncRNA-GAS5 induces PTEN expression through inhibiting miR-103 in endometrial cancer cells. *J Biomed Sci* 2015; 22: 100.
39. Yang W, Hong L, Xu X, et al. LncRNA GAS5 suppresses the tumorigenesis of cervical cancer by downregulating miR-196a and miR-205. *Tumour Biol* 2017; 39: 1010428317711315.
40. Cao L, Chen J, Ou B, et al. GAS5 knockdown reduces the chemo-sensitivity of non-small cell lung cancer (NSCLC) cell to cisplatin (DDP) through regulating miR-21/PTEN axis. *Biomed Pharmacother* 2017; 93: 570–579.
41. Fan S, Meng J, Zhang L, et al. CAV1 polymorphisms rs1049334, rs1049337, rs7804372 might be the potential risk in tumorigenicity of urinary cancer: A systematic review and meta-analysis. *Pathol Res Pract* 2019; 215: 151–158.
42. Dong, X, Gao W, Lv X, et al. Association between lncRNA GAS5, MEG3, and PCAT-1 Polymorphisms and Cancer Risk: A Meta-Analysis. *Dis Markers* 2020; 2020: 6723487.
43. Cai H, Xu W and Zhang X. LncRNA growth arrest-special 5 polymorphisms and predisposition to cancer: A meta-analysis. *Int J Biol Markers* 2020; 35: 28–34.
44. Quan J, Pan X, Zhao L, et al. LncRNA as a diagnostic and prognostic biomarker in bladder cancer: a systematic review and meta-analysis. *Onco Targets Ther* 2018; 11: 6415–6424.
45. Dai SP, Jin J and Li WM. Diagnostic efficacy of long non-coding RNA in lung cancer: a systematic review and meta-analysis. *Postgrad Med J* 2018; 94: 578–587.
46. Song W, Wang K, Zhang RJ, et al. Long noncoding RNA GAS5 can predict metastasis and poor prognosis: a meta-analysis. *Minerva Med* 2016; 107: 70–76.
47. Gao Q, Xie H, Zhan H, et al. Prognostic Values of Long Noncoding RNA GAS5 in Various Carcinomas: An Updated Systematic Review and Meta-Analysis. *Front Physiol* 2017; 8: 814.