Letter to the Editor

Clinical Significance and Function of *MALAT1* Gene Expression and the rs619586 Polymorphism in Colorectal Cancer*

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Colorectal cancer (CRC) is a malignant tumor of the digestive system that poses a serious threat to human health. In 2018, around 1.8 million people were newly diagnosed with CRC, and 881,000 people died from the disease[1]. The identification of CRC-related genes and genetic polymorphisms will aid in the prevention and treatment of this disease.

Metastasis is associated with the lung adenocarcinoma transcript 1 (*MALAT1*) gene located on human chromosome 11q13.1, which encodes a lncRNA that participates in the malignant progression of multiple cancers, including CRC[2-4]. Several studies have found that the rs619586 A>G polymorphism in the *MALAT1* gene was associated with the risk of multiple cancers, suggesting that the polymorphism might serve as a potential indicator for cancer risk[5]. The current study aims to investigate the relationship of MALAT1 expression with survival prognosis and immune infiltrates in CRC patients and drug sensitivity, as well as the role of the rs619586 polymorphism in CRC risk, which will help search for new CRC biomarkers.

TIMER (cistrome.shinyapps.io/timer) was utilized to explore the relationship of MALAT1 expression with survival prognosis and immune infiltrates in CRC patients. GSCA (http://bioinfo.life.hust.edu.cn/GSCA/#/) was used to analyze the correlation between MALAT1 expression and drug IC50 by Pearson correlation analysis. A false discovery rate (FDR) < 0.05 and |r| > 0.1 were considered significant. LinkedOmics (http://www.linked omics.org/) was utilized to obtain the genes coexpressed with *MALAT1* in CRC. The co-expression conditions were as follows: |r| > 0.3 and FDR < 0.05. Functional enrichment analysis and protein–protein association networks for coexpressed genes were conducted by using the DAVID tool and STRING database. Protein–protein association networks were further analyzed by using Cytoscape software.

We collected peripheral blood samples from 300 CRC patients, 300 healthy individuals, and 27 pairs of CRC and normal paracancerous tissues (Supplementary Table S1, available in www.besjournal.com). The study protocol (No. 047-001) was approved by the Ethics Committee at Shanghai's Xuhui District Central Hospital. The TIANamp genomic DNA Kit was used to extract DNA from peripheral blood and tissue samples. The polymerase chain reaction (PCR) method was used to amplify the sequence containing the rs619586 polymorphism. The PCR reaction conditions and primer sequences were as follows: 95 °C 5 min; 35 cycles of 94 °C 30 sec, 57 °C 30 s, 72 °C 30 s; 72 °C 10 min;

F: 5′-GGGAGAAAGTCCGCCATTTTGCCAC-3′;

R: 5′-ACGGGTCATCAAACACCC-3′.

Genotyping was performed by Sanger sequencing.

PubMed, Embase, and the China National Knowledge Infrastructure databases were used to search for case-control studies on the association of the *MALAT1* rs619586 polymorphism with CRC risk in the Chinese population. The last search was conducted on February 10, 2022. Two researchers independently collected information from the included studies. Any differences were resolved through discussion.

TRIzol reagent was used to extract total RNA. A reverse transcription kit was used to convert mRNA into cDNA. The cDNA was then amplified using the Applied Biosystems 7500 Real-Time PCR System. Syber green was utilized to detect fluorescence

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signals. The sequences of the primers were as follows: MALAT1 forward: 5′-TGACGGAGGTTGAG-ATGAAGCT-3′ and reverse: 5′-TAATTCGGGG CTCTGTAGTCCT-3′; GAPDH forward: 5′-GTCTCCT-CTGACTTCAACA-3′ and reverse: 5′-TGAGGGTCTCTCT CTTCCT-3′. Relative expression of the *MALAT1* gene was calculated using the $2^{-\Delta\Delta Ct}$ method. All the experiments were repeated in triplicate.

The miRNASNP-v3 database was utilized to investigate whether the rs619586 polymorphism affected the binding of miRNA to MALAT1. The psiCHECK2 vector was used to create recombinant dual-luciferase reporters. A 200-bp sequence containing the rs619586 A or G allele was synthesized and inserted into the psiCHECK2 vector to generate the wild-type vector (psiCHECK2-WT) containing the A allele and the mutant vector (psiCHECK2-MT) containing the G allele. The 293 T cell line was grown in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 at 37 °C. In the logarithmic growth phase, 293 T cells were seeded into 24-well plates at a density of 10^5 cells/well. According to the protocol, 16 hours after plating, the recombinant dual-luciferase vectors were cotransfected with miR-214-3p mimics or miRNA-NC into 293 T cells using Lipofectamine 2000. The transfected cells were collected 48 hours after transfection, and their luciferase activity was evaluated using a dual-luciferase assay system. All experiments were performed independently in triplicate.

Zheng et al. found that the expression level of MALAT1 was higher in CRC tissues than in noncancerous tissues[6]. A higher expression of MALAT1 might act as a negative prognostic marker in patients with stage II/III CRC. Yang et al. revealed that MALAT1 overexpression in primary CRC could increase cell proliferation, invasion, and migration *via* PRKA kinase anchor protein 9[7]. In addition, our research found that high MALAT1 expression was associated with poor survival of patients with colon cancer (Supplementary Figure S1, available in www.besjournal.com). These findings indicated that the *MALAT1* gene is capable of acting as an oncogene in CRC. The presence of tumor-infiltrating B cells is associated with poor outcomes in several cancers[8]. Our research showed that MALAT1 expression was positively correlated with B-cell infiltration in colon cancer (Supplementary Figure S2, available in www.besjournal.com). Furthermore, MALAT1 expression was also correlated with the sensitivity of multiple anti-CRC drugs, such as camptothecin and cetuximab (Supplementary Table S2, available in www.besjournal.com).

There were 1,270 genes coexpressed with MALAT1, including 106 negatively related genes and 1164 positively related genes. These coexpressed genes were significantly enriched in multiple biological processes, molecular functions, and cellular components, such as GO:0003676~nucleic acid binding, GO:0005634~nucleus, and GO:0005622~intracellular (Supplementary Table S3, available in www.besjournal.com). Protein–protein association networks of the coexpressed genes showed that node CDC42, with the most edges, was the hub gene (Supplementary Figure S3, available in www.besjournal.com). CDC42 was a small GTPase of the Rho subfamily, which regulated signaling pathways that controlled diverse cellular functions, including cell morphology, migration, endocytosis, and cell cycle progression. *CDC42* gene expression dysregulation involved several pathogenic processes of CRC[9]. Therefore, MALAT1 may be involved in the progression of CRC by regulating the expression of the *CDC42* gene.

The current case-control study showed that the *MALAT1* rs619586 polymorphism was significantly associated with CRC risk [AG *vs.* AA: *OR* = 0.64, 95% *CI* = 0.43–0.96, *P* = 0.03; (AG + GG) *vs*. AA: *OR* = 0.62, 95% *CI* = 0.42–0.91, *P* = 0.02; G *vs*. A: *OR* = 0.62, 95% *CI* = 0.44–0.89, *P* = 0.01] (Table 1). A similar result

Table 1. Association of the *MALAT1* rs619586 polymorphism with CRC risk in a case-control study

Genotype	Cases (*n* = 300)	Controls (*n* = 300)	[a]*OR* (95% *CI*)	[a]*P* value
AA	244 (81.3%)	220 (73.3%)	Reference	
AG	52 (17.3%)	72 (24.0%)	0.64 (0.43–0.96)	0.03
GG	4 (1.3%)	8 (2.7%)	0.34 (0.09–1.29)	0.10
AG + GG	56 (18.7%)	80 (26.7%)	0.62 (0.42–0.91)	0.02
AA + AG	296 (98.6%)	292 (97.3%)	Reference	
GG	4 (1.3%)	8 (2.7%)	0.37 (0.10–1.41)	0.13
A	540 (90%)	512 (85.3%)	Reference	
G	60 (10%)	88 (14.7%)	0.62 (0.44–0.89)	0.01

Note. [a]Adjusted for age and gender.

was also observed in the pooled analysis of 1266 CRC cases and 1288 healthy controls [GG *vs*. AA: *OR* = 0.46, 95% *CI* = 0.25–0.84, *P* = 0.01; AG *vs*. AA: *OR* = 0.73, 95% *CI* = 0.60–0.89, *P* = 0.002; (AG + GG) *vs*. *AA*: *OR* = 0.71, 95% *CI* = 0.58–0.85, *P* = 0.0003; GG) *vs*. (AG + AA): *OR* = 0.49, 95% *CI* = 0.27–0.89, *P* = 0.02; G *vs*. A: *OR* = 0.71, 95% *CI* = 0.60–0.84, *P* < 0.0001] (Supplementary Table S4 and Supplementary Figure S4, available in www.besjournal.com). Further genotype-tissue expression analysis showed that the expression level of MALAT1 was significantly lower in the AG + GG genotype than in the AA genotype in CRC and normal paracancerous tissues (Figure 1). Bioinformatics analysis showed that the rs619586 G allele contributed to the binding of several miRNAs, such as miR-214-3p, miR-3619-5p, and miR-761, to MALAT1 (Supplementary Table S5 available in www.besjournal.com). Among these miRNAs, miR-214-3p could inhibit tumor proliferation and metastasis in CRC by targeting the PLAGL2-MYH9 axis. The dual-luciferase assay showed that the rs619586 G allele facilitated the binding of miR-214-3p to MALAT1 (Figure 2), which was consistent with previous research findings[10]. Thus, the rs619586 G allele might reduce CRC risk by facilitating the binding of miR-214-3p to MALAT1 and thus reducing the expression of the cancer-promoting molecule MALAT1. Additionally, the rs619586 polymorphism might also affect the survival prognosis and anti-cancer drug sensitivity of CRC patients; however, this hypothesis awaits confirmation by future studies.

Although the current study has yielded some

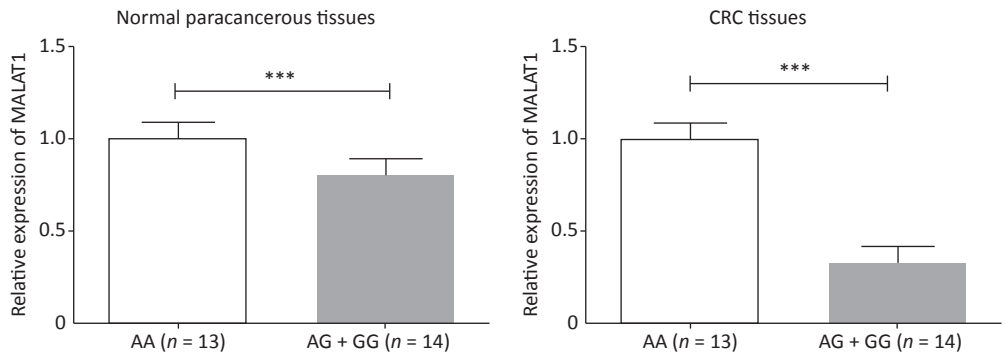

Figure 1. Association of the rs619586 polymorphism with MALAT1 expression (Error bars indicate Standard Deviation). ***$P < 0.001$.

A

miRNA	Binding site	TargetScan score	ΔG Duplex	ΔG Binding	ΔG Open energy	AU content	Exact probability	Alignment
hsa-miR-214-3p	23-30	23.16267	−14.60000	−14.95483	7.21502	0.66267	0.00459	Query: 5′ CUUCAAAAGGUGGUAAACUAUACCUGCUGUCCCUCAAGAG miRNA: 3′ UGACGGACAGACACGGACGACA

B

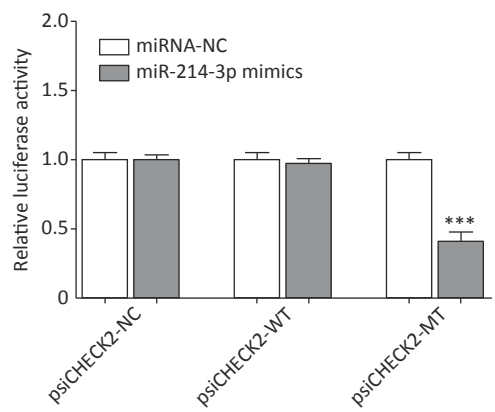

Figure 2. Influence of the rs619586 polymorphism on the binding of miR-214-3p to MALAT1 (A: Bioinformatics analysis; B: Dual-luciferase assay; Error bars indicate Standard Deviation). ***$P < 0.001$.

interesting findings, there remain some shortcomings. For example, the specific molecular mechanisms by which MALAT1 expression is correlated with B-cell infiltration and anti-CRC drug sensitivity were not revealed. The risk analysis did not correct for several confounding factors, including smoking, alcohol consumption, red meat intake, etc.

In conclusion, our study suggests that MALAT1 expression is associated with survival prognosis and B-cell infiltration in patients with colon cancer and anti-CRC drug sensitivity, and the rs619586 polymorphism is associated with CRC risk. Thus, MALAT1 expression and the rs619586 polymorphism may act as biomarkers for assessing CRC risk, prognosis, and anti-CRC drug sensitivity.

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Supplementary Table S1. The demographic characteristics between CRC patients and healthy controls

Variables	CRC patients (*N* = 300)	Lealthy controls (*N* = 300)	*P*-value
Age (Mean ± SD)	59.1 ± 7.6	59.8 ± 8.1	0.28
Gender, *n* (%)			
Male	184 (61.3)	179 (59.7)	0.68
Female	116 (38.7)	121 (40.3)	
Tumor site, *n* (%)			
Colon	153 (51.0)		
Rectum	147 (49.0)		
Tumor stage, *n* (%)			
I + II	176 (58.7)		
III + IV	124 (41.3)		

Note. CRC: Colorectal cancer.

Supplementary Table S2. The correlation between *MALAT1* gene expression and drug sensitivity

GDSC			CTRP		
Drug	Correlation	FDR	Drug	Correlation	FDR
Lapatinib	−0.165	0.006	afatinib	−0.127	0.004
Afatinib	−0.164	< 0.001	VAF-347	−0.118	0.044
Cetuximab	−0.158	< 0.001	SB-743921	0.102	0.007
Gefitinib	−0.127	0.001	ouabain	0.102	0.008
AKT inhibitor VIII	−0.119	0.004	KW-2449	0.102	0.008
CCT007093	−0.101	0.012	COL-3	0.102	0.026
GSK1904529A	−0.101	0.018	marinopyrrole A	0.102	0.044
Gemcitabine	0.101	0.022	doxorubicin	0.103	0.006
PI-103	0.102	0.004	leptomycin B	0.105	0.005
QL-X-138	0.103	0.004	AT7867	0.106	0.009
KIN001-260	0.103	0.004	GSK-3 inhibitor IX	0.106	0.011
CX-5461	0.106	0.003	LY-2183240	0.106	0.005
NG-25	0.108	0.002	topotecan	0.106	0.005
XMD13-2	0.108	0.002	chlorambucil	0.107	0.006
ZSTK474	0.109	0.002	entinostat	0.107	0.006
Cytarabine	0.109	0.013	necrosulfonamide	0.108	0.024
Vinblastine	0.109	0.007	etoposide	0.108	0.005
Y-39983	0.110	0.002	SB-225002	0.108	0.004
PLX4720	0.110	0.004	SNX-2112	0.109	0.004
AICAR	0.111	0.004	BRD-K70511574	0.109	0.004
Phenformin	0.115	0.002	PIK-93	0.111	0.007
Dabrafenib	0.115	0.003	BMS-345541	0.111	0.005
Belinostat	0.116	0.001	daporinad	0.113	0.010
NU-7441	0.116	0.036	pazopanib	0.113	0.004
Foretinib	0.116	0.002	MK-1775	0.114	0.003

Continued

GDSC			CTRP		
Drug	Correlation	FDR	Drug	Correlation	FDR
T0901317	0.119	0.002	AT13387	0.116	0.048
PAC-1	0.119	0.002	BI-2536	0.120	0.001
KIN001-244	0.120	0.001	BRD-K11533227	0.120	0.005
KIN001-102	0.120	0.001	narciclasine	0.122	0.001
ATRA	0.120	0.004	GSK461364	0.122	0.001
BHG712	0.120	0.001	parbendazole	0.124	0.001
DMOG	0.123	0.002	N9-isopropylolomoucine	0.124	0.002
VNLG/124	0.123	0.001	KX2-391	0.125	0.001
KU-55933	0.124	0.009	STF-31	0.125	0.002
FK866	0.124	< 0.001	rigosertib	0.126	0.001
THZ-2-102-1	0.124	< 0.001	momelotinib	0.127	0.002
CUDC-101	0.125	0.001	CD-437	0.129	0.001
AZD8055	0.130	0.001	lovastatin	0.129	0.003
OSI-027	0.130	< 0.001	obatoclax	0.134	< 0.001
JW-7-24-1	0.133	< 0.001	alvocidib	0.136	0.018
Tubastatin A	0.133	< 0.001	GW-843682X	0.137	0.001
Camptothecin	0.134	0.001	cytarabine hydrochloride	0.141	< 0.001
WZ3105	0.134	< 0.001	tivantinib	0.143	0.011
SNX-2112	0.134	< 0.001	cucurbitacin I	0.145	< 0.001
UNC0638	0.134	< 0.001	clofarabine	0.148	< 0.001
TL-1-85	0.135	< 0.001	vincristine	0.149	< 0.001
ZM-447439	0.136	0.001	omacetaxine mepesuccinate	0.150	0.001
AT-7519	0.138	< 0.001	dinaciclib	0.154	0.006
Genentech Cpd 10	0.139	< 0.001	triazolothiadiazine	0.163	< 0.001
CAY10603	0.142	< 0.001	PF-3758309	0.175	0.002
THZ-2-49	0.142	< 0.001	docetaxel	0.179	0.002
QL-XII-61	0.144	0.013			
BIX02189	0.144	< 0.001			
AR-42	0.147	< 0.001			
BX-795	0.149	< 0.001			
Salubrinal	0.150	0.044			
Navitoclax	0.152	< 0.001			
PHA-793887	0.153	< 0.001			
GSK1070916	0.156	< 0.001			
NPK76-II-72-1	0.157	< 0.001			
I-BET-762	0.158	< 0.001			
CP466722	0.160	< 0.001			
Sunitinib	0.164	0.010			
TPCA-1	0.166	< 0.001			
PIK-93	0.167	< 0.001			

Continued

GDSC			CTRP		
Drug	Correlation	FDR	Drug	Correlation	FDR
MS-275	0.174	0.012			
BX-912	0.176	< 0.001			
TG101348	0.178	< 0.001			
BMS345541	0.181	< 0.001			
Vorinostat	0.209	< 0.001			
Methotrexate	0.218	< 0.001			
AZD7762	0.221	< 0.001			
CEP-701	0.226	< 0.001			

Note. GDSC: genomics of drug sensitivity in cancer; CTRP: cancer therapeutics response portal; FDR: false discovery rate.

Supplementary Table S3. Enrichment analysis for the genes co-expressed with MALAT1

Category	Term	FDR
GOTERM_MF_DIRECT	GO:0003676~nucleic acid binding	2.93E-13
GOTERM_CC_DIRECT	GO:0005634~nucleus	4.88E-07
GOTERM_BP_DIRECT	GO:0006351~transcription, DNA-templated	1.24E-05
GOTERM_BP_DIRECT	GO:0006355~regulation of transcription, DNA-templated	2.84E-05
GOTERM_CC_DIRECT	GO:0005622~intracellular	4.93E-05
GOTERM_MF_DIRECT	GO:0003677~DNA binding	1.10E-04
GOTERM_MF_DIRECT	GO:0046872~metal ion binding	5.08E-04
GOTERM_CC_DIRECT	GO:0005814~centriole	0.002

Note. FDR: false discovery rate.

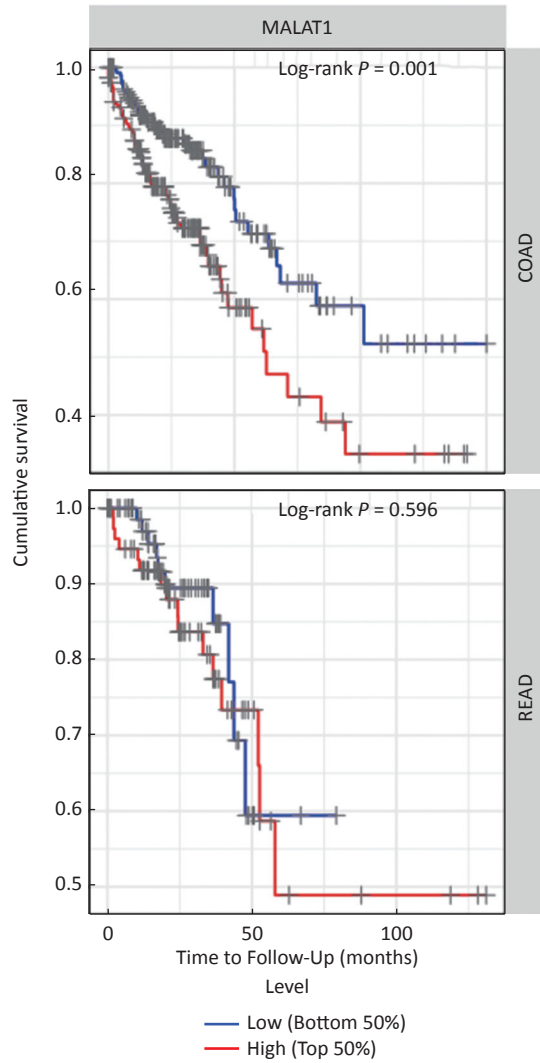
Supplementary Table S4. Main characteristics of case-control studies included in the pooled analysis

First author's name	PMID	Countries	Genotyping methods	Cases					Controls					P_{HWE}
				AA	AG	GG	A	G	AA	AG	GG	A	G	
Zhao KX	30538572	China	TaqMan	784	170	12	1738	194	750	213	25	1713	263	0.04
Gao XR	–	China	Sanger sequencing	244	52	4	540	60	220	72	8	512	88	0.48

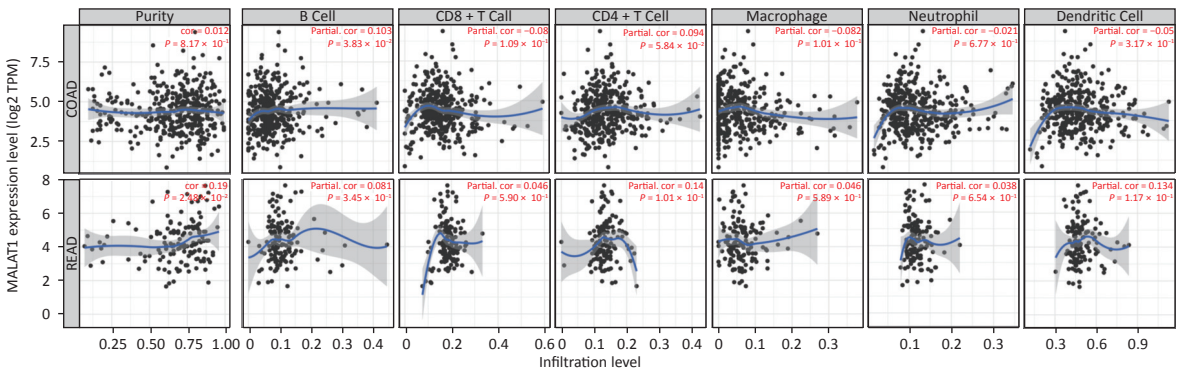
Note. PMID: PubMed Unique Identifier.

Supplementary Table S5. The effect of rs619586 polymorphism on the binding of MALAT1 to miRNA

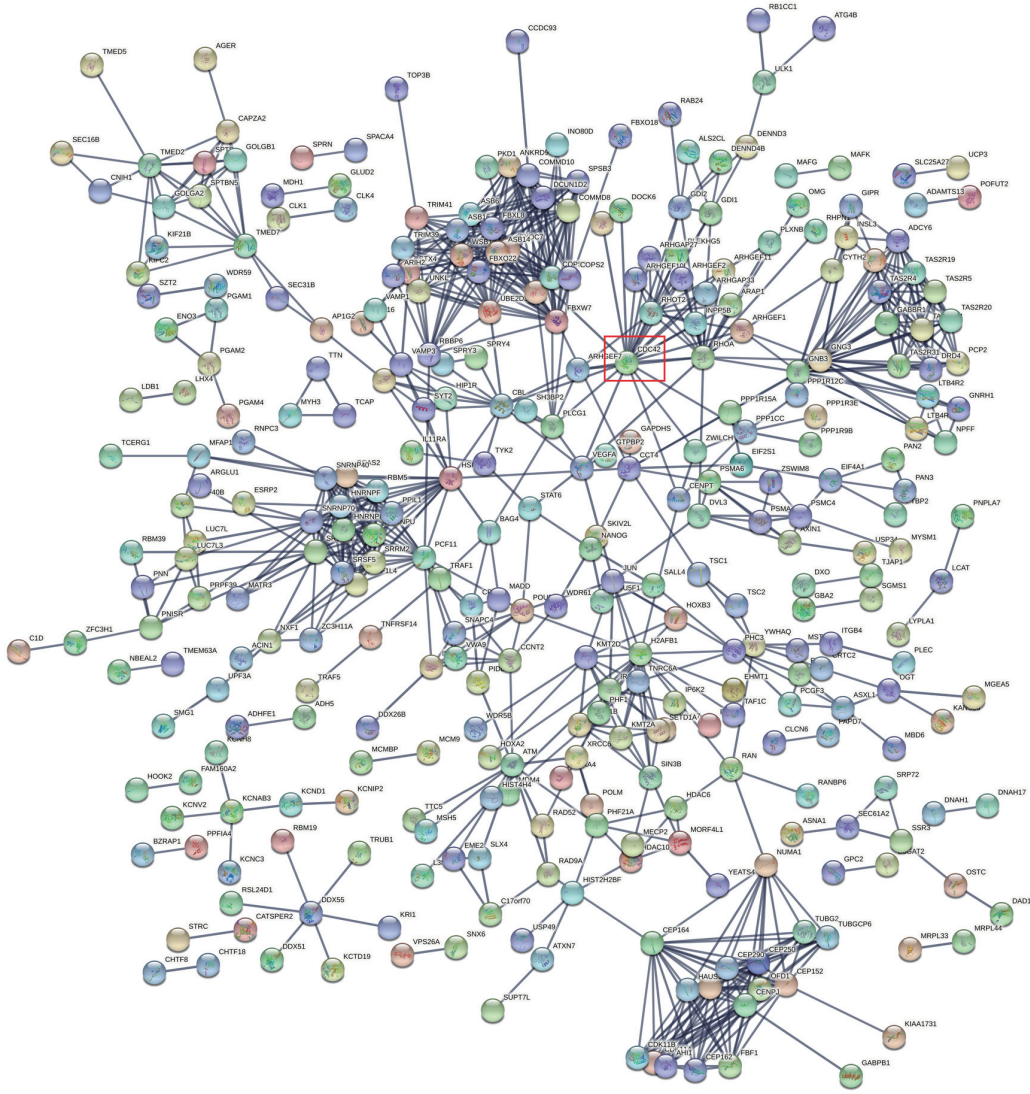
	miRNA target gain	miRNA target loss
The effect of rs619586 (A > G)	**hsa-miR-214-3p**, hsa-miR-3619-5p, hsa-miR-761, hsa-miR-2277-3p, hsa-miR-922, hsa-miR-3665, hsa-miR-657, hsa-miR-3120-3p, hsa-miR-4690-5p, hsa-miR-6165, hsa-miR-6510-5p	hsa-miR-101-3p, hsa-miR-144-3p, hsa-miR-199a-3p, hsa-miR-199b-3p, hsa-miR-3129-5p, hsa-miR-331-5p, hsa-miR-4645-3p, hsa-miR-936



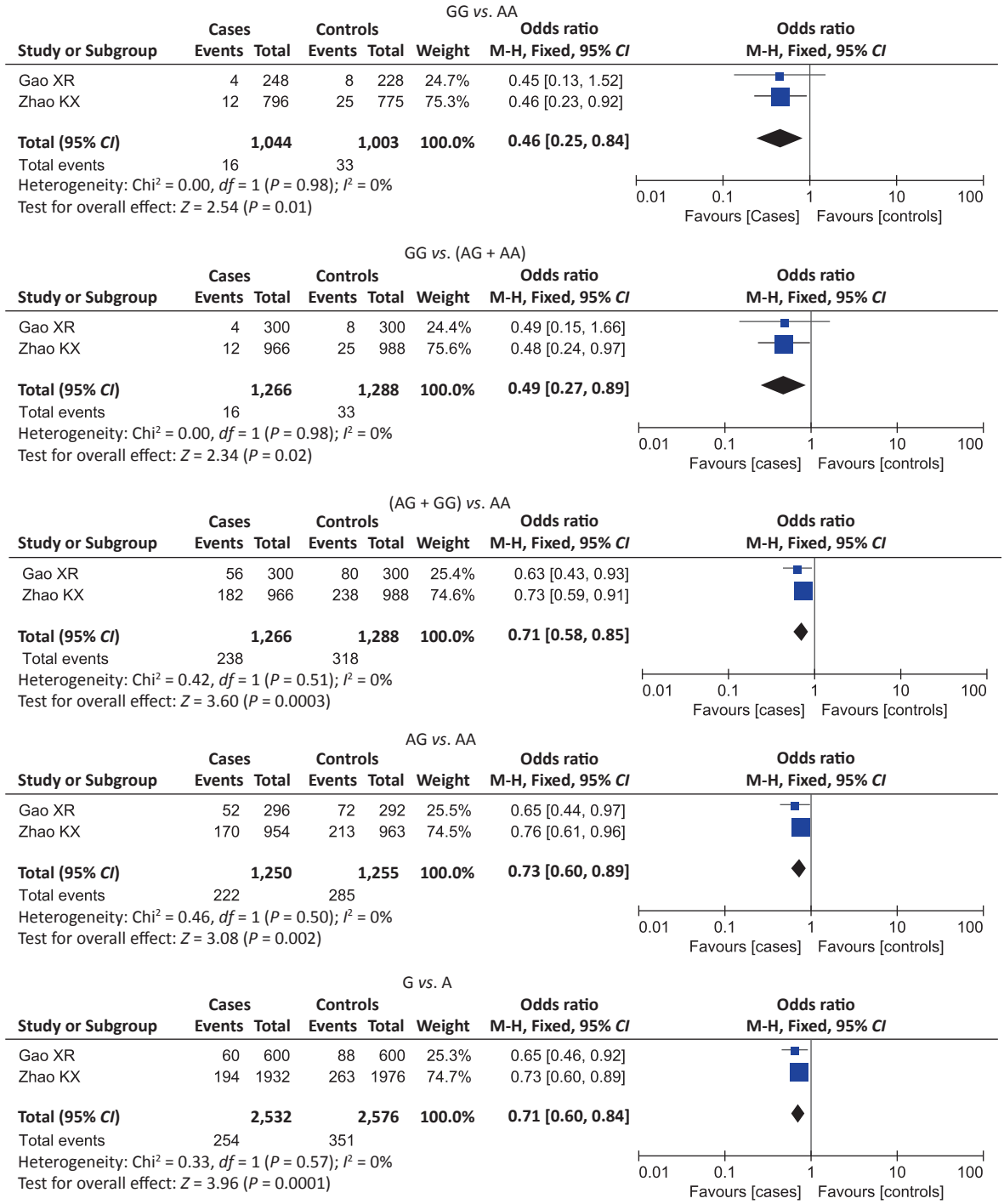
Supplementary Figure S1. The association of *MALAT1* gene expression with the survival of CRC patients. CRC: colorectal cancer.



Supplementary Figure S2. The correlation of *MALAT1* gene expression with immune infiltration in CRC. CRC: colorectal cancer.



Supplementary Figure S3. Protein-protein association networks of the genes co-expressed with MALAT1.



Supplementary Figure S4. Meta-analysis of the association of *MALAT1* rs619586 polymorphism with CRC risk in the Chinese population.