WAKMAR2, a Prognosis-related Enhancer RNA in Gastric Cancer

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Abstract

Purpose An increasing number of long non-coding RNAs (lncRNAs) are thought to be associated with gastric cancer (GC). A lncRNA subclass that promotes enhancer function is called enhancer RNA (eRNA). We aimed to identify an eRNA that can predict GC prognosis and response to immune checkpoint inhibitors (ICIs).

Methods Kaplan–Meier survival analysis was utilized to screen eRNA which can predict the prognosis of GC ($P < 0.05$). The method of Spearman correlation analysis was employed in the filtration of target genes related to eRNA ($r > 0.4$, $P < 0.001$). According to the median of WAKMAR2 expression, the patients were subdivided into low expression group and high expression group. Subsequently, differences of immune checkpoint-related genes and immune cell infiltration between the two groups were further explored. Furthermore, we analyzed the correlation of WAKMAR2 with tumor mutation burden (TMB) and microsatellite instability (MSI) in GC and other types of cancer.

Results WAKMAR2 and its target gene TNFAIP3 entered the subsequent analysis. Patients with high-WAKMAR2 expression had a favorable prognosis compared to patients with low-WAKMAR2 expression ($P = 0.048$). Immune checkpoint-related genes (PD-L1, CTLA4, PDCD1, LAG3) in the WAKMAR2 high-expression group were also highly expressed, except for B7-H3. In addition, infiltration levels of B cells naive, T cells CD8, T cells CD4 memory activated, as well as Macrophages M1 in high-WAKMAR2 group were greater than in low-WAKMAR2 group. Last, the expression of WAKMAR2 in GC was significantly correlated with TMB and MSI.

Conclusion WAKMAR2, a new eRNA, is a promising biomarker that can be used to predict the overall survival (OS) of GC patients, and WAKMAR2 expression can be utilized to identify ICB responders in GC, providing new insights for immunotherapy strategies.

Introduction

Gastric cancer (GC) is a kind of malignant tumor with poor therapeutic effect, whose incidence and mortality rate shows a gradual upward trend[1]. Over the last few years, there has been major developments in GC diagnosis and treatment, but its prognosis is still poor[2]. At present, the most commonly used methods to evaluate the prognosis of GC are still histological diagnosis and TNM staging[3]. It is well described that GC is a highly heterogeneous disease[4]. Clinically, it is difficult to predict the prognosis of GC patients accurately by using the existing evaluation methods. Therefore, biomarkers that can accurately predict the prognosis of GC would help improve the clinical outcome of these patients.

There is mounting evidence showing that aberrant expression of long non-coding RNA (lncRNA) is closely related to numerous diseases, including cancer[5, 6]. ERNA is a non-coding RNA transcribed from enhancer. Some recent studies have found that enhancer dysfunction is considered a key process of tumorigenesis[7, 8]. Up to the present time, there is no research on GC related eRNA.

Checkpoint inhibitors targeting cytotoxic T lymphocyte antigen 4 (CTLA4) and programmed cell death 1 (PD1) pave the way for the era of cancer immunotherapy, thus changing the way of cancer treatment[9, 10]. However, although anti-PD-1 mAb is a promising approach for the treatment of patients with advanced GC, its response rate is still limited, so it is necessary to develop new strategies to maximize the efficacy of ICIs. However, although advanced GC patients can choose anti-PD-1 mAb treatment, the response rate is disappointing.

There is evidence to indicate the importance of the tumor microenvironment (TME) in tumor progression[11]. Tumor-infiltrating immune cells (TIICs) are one of the components of the TME, and it was a potential indicator to evaluate the therapeutic effect[12]. Many people had explored the link between TMB and immunotherapy response[13, 14]. Higher TMB produce more neoantigens, the greater the possibility of being recognized by immune cells, so TMB is a good marker for predicting the response of immunotherapy[15, 16]. Microsatellites (MS) are composed of repeating sequences of 1–6 nucleotides[17]. Its loss or gain is called MSI, which is also a powerful biomarker for immunotherapy[18].

In this study, we found that the expression of WAKMAR2 was related to the prognosis of GC, and it may be an immune-related eRNA. Its expression was related to the expression of immune checkpoint genes and the abundance of immune infiltrating cells. In addition, we also studied the correlation between WAKMAR2 expression and TMB and MSI in pan-cancer. This may help to accurately immunotherapy GC patients.

Materials And Methods

Data extraction

The gene expression, mutation data, clinical and survival data of GC and other type cancers were downloaded from the UCSC-Xena browser. From previous literature[19], we obtained a list of enhancer RNA and its predicted target genes.

Screening and identifying eRNA

Patients were sorted into two groups according to the median level of eRNA expression. The Kaplan-Meier method was used to generate two groups of high and low expression survival curves. The difference of survival curve was determined by Log-rank test($p<0.05$). The spearman method was used to analyze the correlation between eRNA and predicted target genes (correlation coefficient $r > 0.4$, $P <0.001$). It was considered as a candidate eRNA only if the following conditions were met, which was related to the overall survival of patients with GC ($p <0.05$) and the correlation coefficient between eRNA and target genes meets the conditions ($r> 0.4$, $P <0.001$).

GO and KEGG enrichment analysis

In addition to the predicted targets, other transcripts that were significantly related to eRNA were obtained through correlation analysis. In order to study the possible molecular mechanism of eRNA related coding

genes, GO and KEGG pathway enrichment analysis was performed using the R package ("DOSE", "clusterProfiler", "enrichplot"). GO terms with both p- and q-value of <0.05 were considered as significant. KEGG terms with p < 0.05 were considered to be significantly enriched.

Relationship between eRNA expression and immune checkpoint-related genes

We extracted expression data of immune checkpoint-related genes (PD-L1, CTLA4, PDCD1, LAG3, B7-H3) from the expression matrix of GC. The Wilcoxon rank sum test was utilized to compare the expression difference of immune checkpoint-related genes between high and low expression eRNA groups.

CIBERSORT analysis

CIBERSORT is an analysis tool that can infer the expression matrix of 22 human leukocyte subtypes based on RNA-seq data. In order to quantify TIICs in GC samples, we use CIBERSORT algorithm. The Wilcoxon rank sum test was performed to compare the different abundance of immune infiltration between the two groups (eRNA high expression group and low expression group).

Correlation analysis of eRNA expression and TMB and MSI

We download the mutation data of 33 tumors from the UCSC website, and then calculate the TMB value of each sample in GC through a Perl script calculation. The MSI value of all tumor sample was obtained from the previous literature. Spearman correlation coefficient was calculated to assess the strength of the correlation between eRNA expression of GC TMB and MSI. In addition, we also conducted the above correlation analysis in other types of tumors.

Verification in pan-cancer

In order to determine whether eRNA expression can predict the OS of other types of tumors, we performed pan-cancer survival verification. In addition, we also verified the correlation between eRNA and target gene expression in pan-cancer.

Statistical analysis

Statistical analyses were conducted in R statistical package Version 3.6.3. Kaplan-Meier analysis was completed using R package ‘survival’. The Wilcoxon rank sum test was used for comparison between two groups of clinicopathological parameters, and Kruskal-Wallis (K-W) test was used for three or more groups. All correlation coefficients were calculated by Spearman correlation analysis. For all analyses, two-tailed P-value < 0.05 was thought significant.

Results

Identification of eRNA associated with prognosis of GC

We used a Perl script to convert the eRNA transcript ID into a gene symbol for subsequent analysis. Then, the expression of eRNA was extracted from the expression matrix of GC and combine it with the survival time. As showed in Table 1, 23 eRNAs significantly related to the OS of GC were filtered by Kaplan-Meier method ($p < 0.05$). Different from other eRNA, HAGLR has five predicted target genes. Surprisingly, levels of these 23 eRNA were significantly correlated with their predicted levels of target gene mRNAs ($r > 0.4$, $p < 0.001$; Table 1).

LncRNA WAKMAR2 is a Key eRNA in GC

LncRNA WAKMAR2 was selected as eRNA for further study, and its expression level was positively correlated with its predicted target gene TNFAIP3 level. Compared with patients in the WAKMAR2 high expression group, patients in the WAKMAR2 low expression group had a shorter OS (Figure 1A, $p < 0.05$). As showed in Figure 1B, WAKMAR2 and TNFAIP3 mRNA levels are moderately correlated ($r = 0.55$, $p < 0.001$). It is worth noting that we studied the prognostic effect of WAKMAR2 in other cancer types and its correlation with TNFAIP3 mRNA levels. The impact of WAKMAR2 on OS and TNFAIP3 was specific for 8 types of cancer only, which were GC, Adrenocortical carcinoma, Breast invasive carcinoma, Brain Lower Grade Glioma, Mesothelioma, Pancreatic adenocarcinoma, Pheochromocytoma and Paraganglioma and Thymoma (Table 2).

Association between WAKMAR2 expression and clinicopathological features

In order to verify the potential clinical utility of WAKMAR2 expression, the clinicopathological features of 371 GC patients were included in this study (Table 3). Figure 2 summarizes the associations between WAKMAR2 expression and clinicopathological features. Compared with Stage I and Stage II, WAKMAR2 expression level was higher in Stage III (Stage I vs III, $p < 0.05$; Stage II vs III, $p < 0.01$). However, compared with stage II and stage III, WAKMAR2 expression was lower in stage IV (Stage II vs IV, $p < 0.05$; Stage III vs IV, $p < 0.001$). The difference in WAKMAR2 expression level between Grade2 and Grade3 was statistically significant (G2 vs G3, $p < 0.001$). Furthermore, higher WAKMAR2 expression levels correlated with advanced T stage (T2 vs T3, $p < 0.01$; T2 vs 4, $p < 0.01$). However, no difference was observed between age, gender, N stage, M stage, family history, neoplasm status, pylori infection, and radiation therapy.

Functional enrichment analysis

To further elucidate WAKMAR2 function, we used correlation analysis to identify significantly co-expressed genes in GC. Including TNFAIP3, a total of 2335 transcripts were significantly associated with WAKMAR2 ($p<0.001$). Co-expressed genes underwent GO enrichment analysis to identify the functions (Figure 3A). In BP category, "T cell activation", "T cell differentiation" and "lymphocyte differentiation" has been enriched, which means that co-expressed genes affect the function of the immune system in tumor microenvironment. Enriched CC terms included “phagocytic cup”, “mast cell granule”, “immunological synapse”, and the enriched MF terms included “cytidine deaminase activity”, “G protein–coupled chemoattractant receptor activity”, “chemokine receptor activity”. In addition, to determine the co-expressed gene enrichment pathway, we conducted KEGG enrichment analysis, including " Purine

metabolism ", " Primary bile acid biosynthesis " and " Taurine and hypotaurine metabolism " (Figure 3B). We analyzed the correlation between genes which were enriched in the "immune response-activating cell surface receptor signaling pathway" and WAKMAR2 ($p < 0.001$). Immune genes with Spearman correlation coefficient > 0.40 were listed in Table 4.

To evaluate the clinical significance of immune checkpoint-related genes

The median value of WAKMAR2 expression was used as a cut-off value, patients were divided into high and low-expression groups. In this study, high-expression group patients exhibited higher gene expression of PDL1 ($p < 0.001$, Figure 4A), CTLA4 ($p < 0.001$, Figure 4B), PDCD1 ($p < 0.001$, Figure 4C) and LAG3 ($p < 0.001$, Figure 4D). However, the expression level of B7-H3 was higher in the low-expression group ($p < 0.001$, Figure 4e). The results of the study indicated that patients in the high-expression group were expected to be candidates for ICIs. Inhibitors against B7-H3 seem to have better therapeutic effects on patients in the low-expression group.

Differences in the abundance of immune cells between groups with high and low WAKMAR2 expression

Co-expressed genes have been shown to be involved in immune regulation, so we wanted to further analyze the differences in the immune fractions between the WAKMAR2 high-expression group and the WAKMAR2 low-expression group. The fractions of infiltrating immune cells were calculated by the CIBERSORT algorithm. Among the 22 leukocyte subtypes, B cells naive, T cells CD8, T cells CD4 memory activated and Macrophages M1 was positively correlated with WAKMAR2 expression (Figure 5). However, in the WAKMAR2 low-expression group, T cells CD4 memory resting, Macrophages M0, Macrophages M2, Mast cells activated, and Neutrophils accounted for a high proportion (Figure 5).

Correlation of WAKMAR2 expression level with TMB and MSI in different cancer types

As depicted in Figure 6A, the expression of WAKMAR2 was negatively correlated with TMB in Stomach adenocarcinoma ($r = -0.288$, $p<0.001$), Esophageal carcinoma ($r = -0.222$,$p<0.01$), Head and Neck squamous cell carcinoma ($r = -0.154$,$p<0.001$), Kidney renal clear cell carcinoma($r = -0.206$,$p<0.001$), Liver hepatocellular carcinoma ($r = -0.222$,$p<0.001$), Lung adenocarcinoma ($r = -0.121$,$p<0.01$), Lung squamous cell carcinoma ($r = -0.130$,$p<0.01$), Pancreatic adenocarcinoma ($r = -0.417$,$p<0.001$), Pheochromocytoma and Paraganglioma ($r=-0.295$,$p<0.001$), Prostate adenocarcinoma ($r = -0.267$,$p<0.001$), Thyroid carcinoma ($r = -0.161$,$p<0.001$) and Thymoma ($r = -0.768$,$p<0.001$). On the contrary, the expression of WAKMAR2 was negatively correlated with TMB in Adrenocortical carcinoma ($r = 0.277$, $p<0.05$), Brian Lower Grade Glioma ($r= 0.298$, $p<0.001$), Ovarian serous cystadenocarcinoma ($r = 0.133$, $p<0.05$).

Similarly, the expression of WAKMAR2 was significantly correlated with MSI in Stomach adenocarcinoma ($r = -0.128$,$p<0.05$),Breast invasive carcinoma ($r = 0.089$,$p<0.01$), Lymphoid Neoplasm Diffuse Large B-cell Lymphoma ($r = -0.460$,$p<0.01$), Esophageal carcinoma ($r = -0.220$,$p<0.01$), Kidney renal clear cell carcinoma ($r = -0.117$,$p<0.05$), Kidney renal papillary cell carcinoma ($r = -0.119$,$p<0.05$), Rectum adenocarcinoma ($r = 0.164$,$p<0.05$), Thyroid carcinoma ($r = 0.123$,$p<0.01$) (Figure 6B).

Discussion

ERNA is a special type of lncRNAs, which originate in the region of gene enhancers and can affect the transcription of corresponding genes through cis-acting. According to our screening criteria, WAKMAR2 was selected to enter the follow-up study. The original name of WAKMAR2 is LOC100130476, its genomic locus is very special, its transcription direction is antisense, and there is an overlapping region with the TNFAIP3 gene body and promoter part[20, 21]. The expression of WAKMAR2 and TNFAIP3 has a strong correlation in various tumor types. A previous study confirmed that LOC100130476 was down-regulated in gastric cardiac adenocarcinoma tissue, suggesting that it had a tumor suppressive effect[22]. In addition, another study proved that LOC100130476 was significantly down-regulated in esophageal cancer cell lines and primary esophageal squamous cell carcinoma tissues, and it's up-regulation could inhibit the proliferation and invasion ability of cancer cells[23]. In other words, the survival rate of patients with cardia adenocarcinoma and esophageal cancer with low expression of LOC100130476 is poor, which is consistent with the results of our study.

As we all know, the higher the expression level of immune checkpoint-related genes in tumor tissues, the better the therapeutic effect of ICI. There is now accumulated evidence suggests that ICI have therapeutic potential in GC[24, 25]. Nevertheless, only a few patients benefit from ICI. In addition, tumor immune microenvironment plays a crucial role in tumor progression. It is particularly important to identify precisely those GC patients who will benefit most from ICI. Very coincidentally, the expression level of WAKMAR2 is a potential marker to accurately distinguish these patients. We explored the association between WAKMAR2 and immune checkpoint-related genes for expression. The expression levels of PDL1, CTLA4, PDCD1 and LAG3 were significantly increased in WAKMAR2 high expression group. However, the level of B7-H3 increased significantly in the low expression group. This result provides strong evidence for our hypothesis that eRNA expression levels can be used to screen which patients are more likely to respond to ICI.

Immune-cell infiltration is one of the characteristics of many cancers, including GC. Disorders of immune system function are related to the occurrence of malignant tumors[26]. Our study found that the abundance of B cells naive, T cells CD8, T cells CD4 memory activated and Macrophage M1 in WAKMAR2 high expression group were higher; T cells CD4 memory resting, Macrophages M0, Macrophages M2, Mast cells activated, and Neutrophils have high abundance in the low expression group of WAKMAR2.These results suggested that the expression of WAKMAR2 can affect the signatures of immune cell infiltration. Based on the results of previous studies, dysregulation of immune components might be the reason for the difference in survival between the two patient groups[27]. The results of this study were in agreement with the findings of previous reports. For example, with regard to the relationship between $CD8^+$ T cells infiltration in tumors and survival of patients with GC, previous studies have shown that high $CD8^+$ T cell expression levels are associated with a good prognosis[28]. The increase of M1 macrophages may be related to the better OS of GC patients[29, 30]. In another such report, M2 macrophages are involved in the development of gastric cancer peritoneal dissemination[31]. Mast cells not only to promote the metastasis of GC, but also regulate the tumor microenvironment by

releasing IL-17[32]. Neutrophils promote migration and invasion of GC cells by activating the ERK pathway and inducing epithelial-mesenchymal transition, suggesting that neutrophils may play a crucial role in GC metastasis[33]. Therefore, this study provides a new understanding of the role of WAKMAR2 in the regulation of TIICs.

With the development of high-throughput sequencing technology, detecting TMB and MSI from genome sequencing is a new method to predict the efficacy of ICB, and it has been proven to be effective in a variety of tumors[34]. A potential problem in daily clinical practice is that TMB and MSI detection was expensive and time-consuming. Therefore, it is particularly important to explore alternative biomarkers for TMB and MSI. In the study of GC, the expression of WAKMAR2 has a certain correlation with TMB and MSI, and may be used as a potential marker to replace them. In the process of pan-cancer verification, it was surprisingly found that TMB of thymoma had a negative correlation with WAKMAR2, and the correlation coefficient was as high as 0.768. This result will provide new insights for immunotherapy of thymoma.

Conclusions

In conclusion, we have identified a new prognostic marker for GC, the enhancer RNA WAKMAR2. Patients in the WAKMAR2 high expression group may have a better prognosis when receiving ICI therapy.

Declarations

Ethics approval and consent to participate

No permissions were required to use the repository data.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

This research was conducted in collaboration with all authors. Yankai Zhang and Yichao Yan performed the data curation and analysis. Yankai Zhang and Ning Ning analyzed and interpreted the results. Yankai

Zhang,Zhanlong Shen and Yingjiang Ye drafted and reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The raw data of this study was obtained from the UCSC Xena website portal, which is a publicly available database.

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Tables

Table 1
List of prognostic eRNAs and their target genes

Symbol	Log-Rank P-Value	Predicted Target	Correlation between lncRNA and the Target	
			P-Value	Correlation coefficient r
EMX2OS	0.001	EMX2	< 0.001	0.762
AL021937.3	0.001	SLC5A4	< 0.001	0.454
HAGLR	0.002	HOXD1	< 0.001	0.759
HAGLR	0.002	HOXD3	< 0.001	0.557
HAGLR	0.002	HOXD4	< 0.001	0.506
HAGLR	0.002	HOXD8	< 0.001	0.483
HAGLR	0.002	HOXD9	< 0.001	0.454
RASSF8-AS1	0.003	RASSF8	< 0.001	0.882
CASC16	0.005	TOX3	< 0.001	0.490
NR2F1-AS1	0.005	NR2F1	< 0.001	0.855
ILDR2	0.010	ILDR2	< 0.001	1.000
VLDLR-AS1	0.013	VLDLR	< 0.001	0.774
OTX2-AS1	0.016	OTX2	< 0.001	0.505
ZFHX4-AS1	0.017	ZFHX4	< 0.001	0.783
LINC02381	0.018	HOXC4	< 0.001	0.653
AL445426.1	0.025	WNT2B	< 0.001	0.605
SERPINB9P1	0.029	SERPINB9	< 0.001	0.436
HSD11B1-AS1	0.029	G0S2	< 0.001	0.430
AC002451.1	0.036	PDK4	< 0.001	0.663
AC109479.1	0.040	ADAMTS2	< 0.001	0.560
WDFY3-AS2	0.041	WDFY3	< 0.001	0.461
IGFBP7-AS1	0.041	IGFBP7	< 0.001	0.778
CCDC144NL-AS1	0.044	CCDC144NL	< 0.001	0.607
LINC02519	0.045	THBS2	< 0.001	0.450

Symbol	Log-Rank P-Value	Predicted Target	Correlation between lncRNA and the Target	
			P-Value	Correlation coefficient r
LINC01389	0.047	FOXD2	< 0.001	0.443
WAKMAR2	0.048	TNFAIP3	< 0.001	0.550
AL355916.1	0.049	HIF1A	< 0.001	0.427

Table 2
Correlation between survival analysis and gene expression of WAKMAR2 and TNFAIP3 in 33 cancer types

Tumor Type		WAKMAR2 and Overall Survival Log-Rank P-Value	WAKMAR2 and TNFAIP3	
Abbreviation	Detail		Correlation P-Value	Correlation Coefficient
ACC	Adrenocortical carcinoma	0.042	P<0.001	0.815
BLCA	Bladder Urothelial Carcinoma	0.491	P<0.001	0.732
BRCA	Breast invasive carcinoma	0.042	P<0.001	0.791
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma	0.983	P<0.001	0.575
CHOL	Cholangiocarcinoma	0.632	P<0.001	0.715
COAD	Colon adenocarcinoma	0.487	P<0.001	0.671
DLBC	Lymphoid Neoplasm Diffuse Large B-cell Lymphoma	0.248	P<0.001	0.724
ESCA	Esophageal carcinoma	0.871	P<0.001	0.676
GBM	Glioblastoma multiforme	0.918	P<0.001	0.428
HNSC	Head and Neck squamous cell carcinoma	0.112	P<0.001	0.423
KICH	Kidney Chromophobe	0.455	P<0.001	0.620
KIRC	Kidney renal clear cell carcinoma	0.873	P<0.001	0.613
KIRP	Kidney renal papillary cell carcinoma	0.747	P<0.001	0.600
LAML	Acute Myeloid Leukemia	0.353	P<0.001	0.370
LGG	Brain Lower Grade Glioma	0.000	P<0.001	0.608
LIHC	Liver hepatocellular carcinoma	0.661	P<0.001	0.684
LUAD	Lung adenocarcinoma	0.401	P<0.001	0.748
LUSC	Lung squamous cell carcinoma	0.659	P<0.001	0.698
MESO	Mesothelioma	0.030	P<0.001	0.714
OV	Ovarian serous cystadenocarcinoma	0.085	P<0.001	0.507
PAAD	Pancreatic adenocarcinoma	0.018	P<0.001	0.661
PCPG	Pheochromocytoma and Paraganglioma	0.036	P<0.001	0.585
PRAD	Prostate adenocarcinoma	0.458	P<0.001	0.643
READ	Rectum adenocarcinoma	0.349	P<0.001	0.643

SARC	Sarcoma	0.369	P<0.001	0.652
SKCM	Skin Cutaneous Melanoma	0.189	P<0.001	0.740
STAD	Stomach adenocarcinoma	0.048	P<0.001	0.550
TGCT	Testicular Germ Cell Tumors	0.793	P<0.001	0.620
THCA	Thyroid carcinoma	0.839	P<0.001	0.632
THYM	Thymoma	0.010	0.003	0.270
UCEC	Uterine Corpus Endometrial Carcinoma	0.933	P<0.001	0.489
UCS	Uterine Carcinosarcoma	0.624	0.002	0.409
UVM	Uveal Melanoma	0.654	P<0.001	0.775

Table 3
Clinicopathological parameters of GC patients.

Covariates	N (%)	Covariates	N (%)
Age		M_Stage	
<65	155(41.78%)	M0	327(88.14%)
>=65	216(58.22%)	M1	25(6.74%)
Gender		unknow	19(5.12%)
Female	132(35.58%)	Family_history	
Male	239(64.42%)	NO	268(72.24%)
Stage		unknow	87(23.45%)
Stage I	51(13.75%)	YES	16(4.31%)
Stage II	110(29.65%)	Pylori_infection	
Stage III	150(40.43%)	No	143(38.54%)
Stage IV	37(9.97%)	unknow	210(56.6%)
unknow	23(6.2%)	Yes	18(4.85%)
Grade		Neoplasm_status	
G1	10(2.7%)	TUMOR FREE	163(43.94%)
G2	133(35.85%)	unknow	136(36.66%)
G3	219(59.03%)	WITH TUMOR	72(19.41%)
unknow	9(2.43%)	Radiation_therapy	
T_Stage		NO	234(63.07%)
T1	18(4.85%)	unknow	92(24.8%)
T2	77(20.75%)	YES	45(12.13%)
T3	168(45.28%)		
T4	100(26.95%)		
unknow	8(2.16%)		
N_Stage			
N0	109(29.38%)		
N1	96(25.88%)		
N2	75(20.22%)		

N3	73(19.68%)
unknow	18(4.85%)

Table 4
List of immune genes associated with WAKMAR2 expression (r > 0.400, p < 0.001).

Gene Symbol	Spearman Correlation Coefficient r	Gene Symbol	Spearman Correlation Coefficient r
ITK	0.617	MEF2C	0.489
ZAP70	0.606	PRKCH	0.484
CD226	0.603	PSMA8	0.484
KLRK1	0.593	NCKAP1L	0.478
LAT	0.578	PRKCQ	0.474
ELMO1	0.577	CD28	0.471
GRAP2	0.577	NFKBID	0.466
CD3G	0.563	GPLD1	0.464
PRKCB	0.559	TRBC1	0.456
FYB1	0.556	CD79B	0.451
PIK3CD	0.553	WAS	0.450
TRAT1	0.541	BTK	0.450
TESPA1	0.537	CD22	0.449
LAX1	0.537	PVRIG	0.448
CCR7	0.535	NCR3	0.447
CTLA4	0.530	PLCG2	0.447
WIPF1	0.530	RFTN1	0.445
LPXN	0.529	CD247	0.445
PTPN22	0.526	TXK	0.442
KLHL6	0.526	BTN2A2	0.442
CD160	0.525	MALT1	0.440
CD19	0.523	PLCG1	0.439
THEMIS	0.521	GATA3	0.438
CD3D	0.519	NFATC2	0.436
SLA2	0.518	LAT2	0.436
CD38	0.515	BCL2	0.435

TRAC	0.514	CYLD	0.433
FCRL3	0.511	LCP2	0.431
FYN	0.509	CD79A	0.429
TRBC2	0.507	VAV1	0.427
PTPRC	0.505	FOXP3	0.426
UBASH3A	0.502	LCK	0.425
MS4A1	0.496	CR2	0.419
CD3E	0.493	BLK	0.416
MYO1G	0.493	CLEC10A	0.411
STAP1	0.491	THEMIS2	0.410

Figures

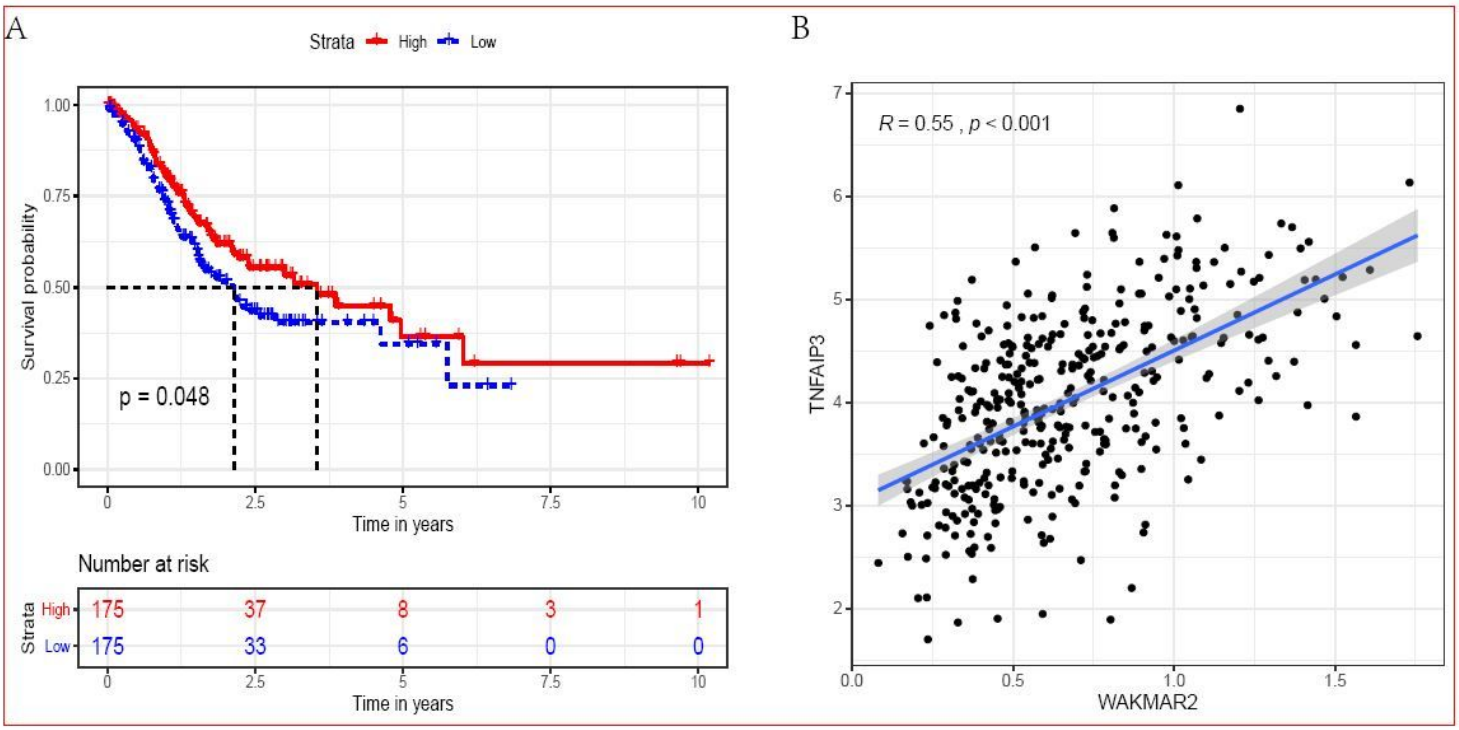

Figure 1

Effect of lncRNA WAKMAR2 on gastric cancer (GC). A. Kaplan-Meier survival analysis of the relationship between WAKMAR2 expression and overall survival (OS) in the GC patients; B. Scatterplot showing correlation between WAKMAR2 and TNFAIP3 expression.

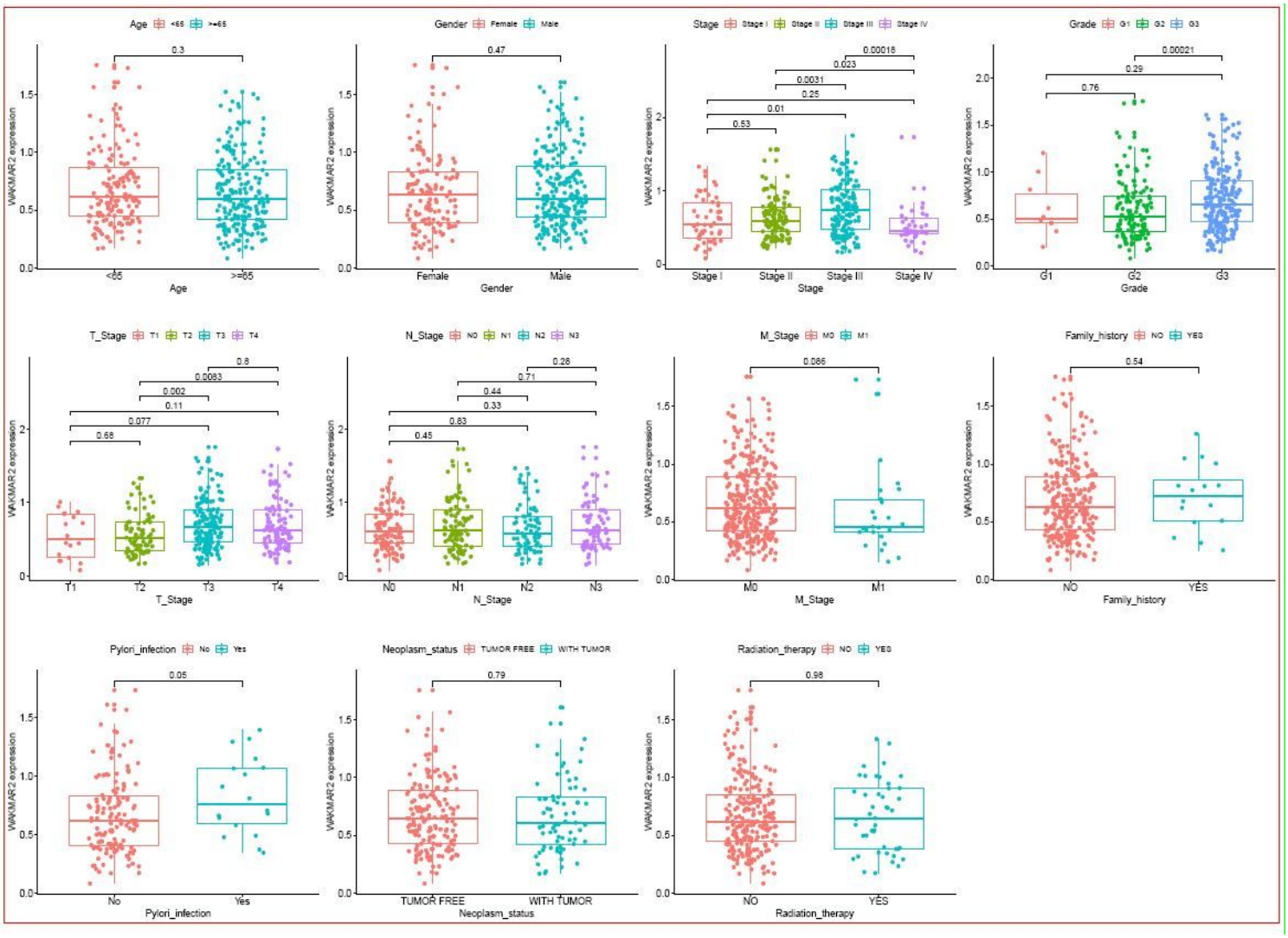

Figure 2

Association of WAKMAR2 expression with clinicopathological parameters.

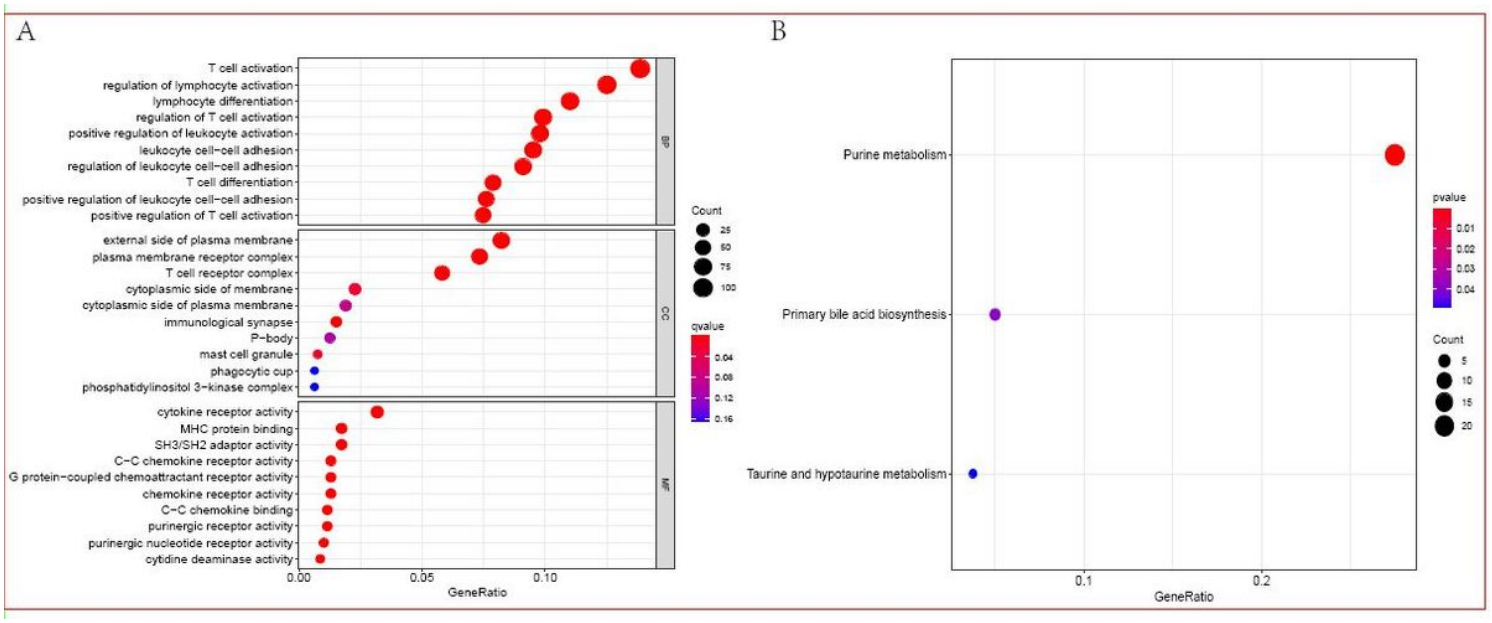

Figure 3

GO and KEGG pathway enrichment of WAKMAR2 co-expressed genes. A. GO enrichment; B. KEGG pathway enrichment.

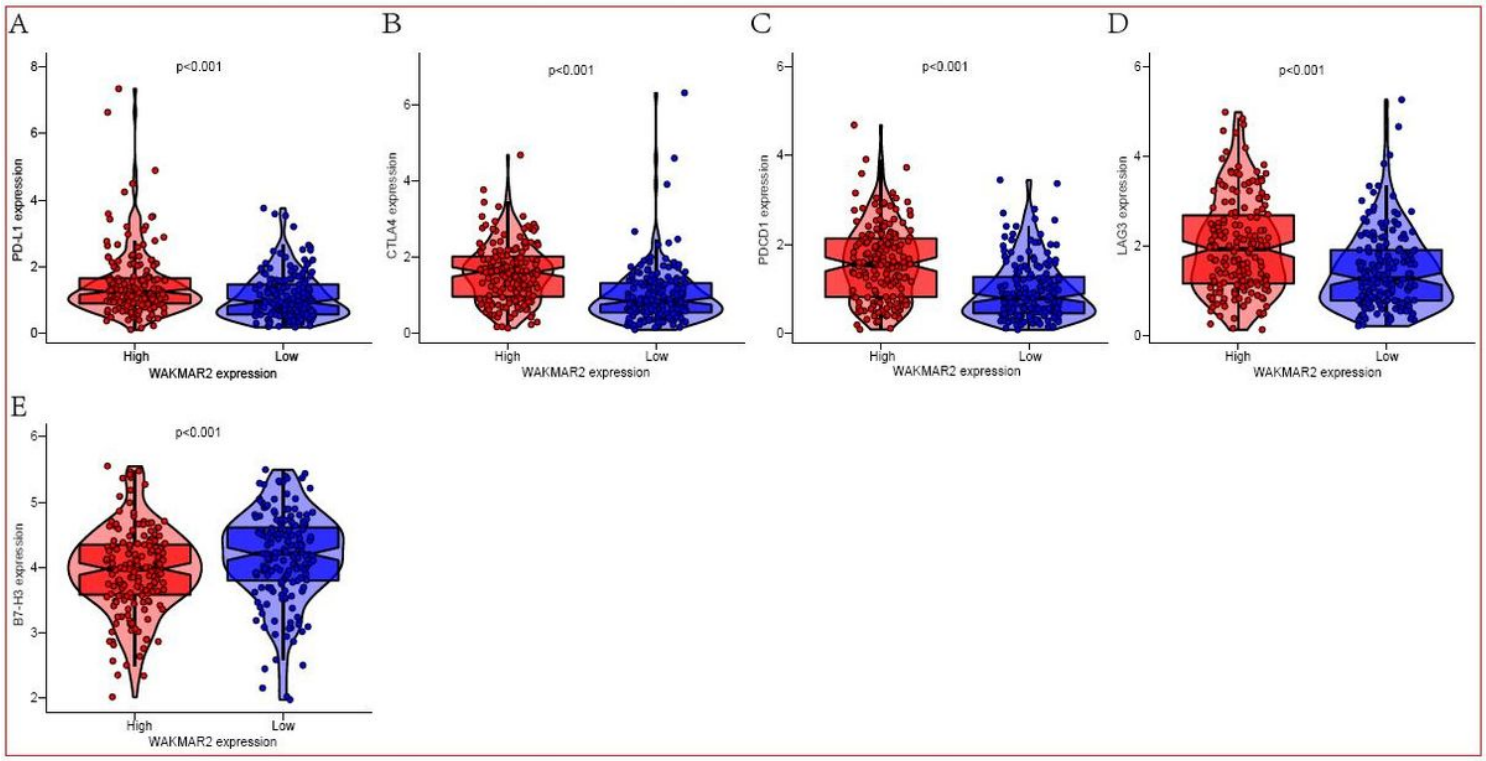

Figure 4

The expression of immune checkpoint-related genes in WAKMAR2 high- and low- expression groups. A. PD-L1 (p<0.001); B. CTLA4 (p<0.001); C. PDCD1 (p<0.001); D. LAG3 (p<0.001); E. B7-H3 (p<0.001).

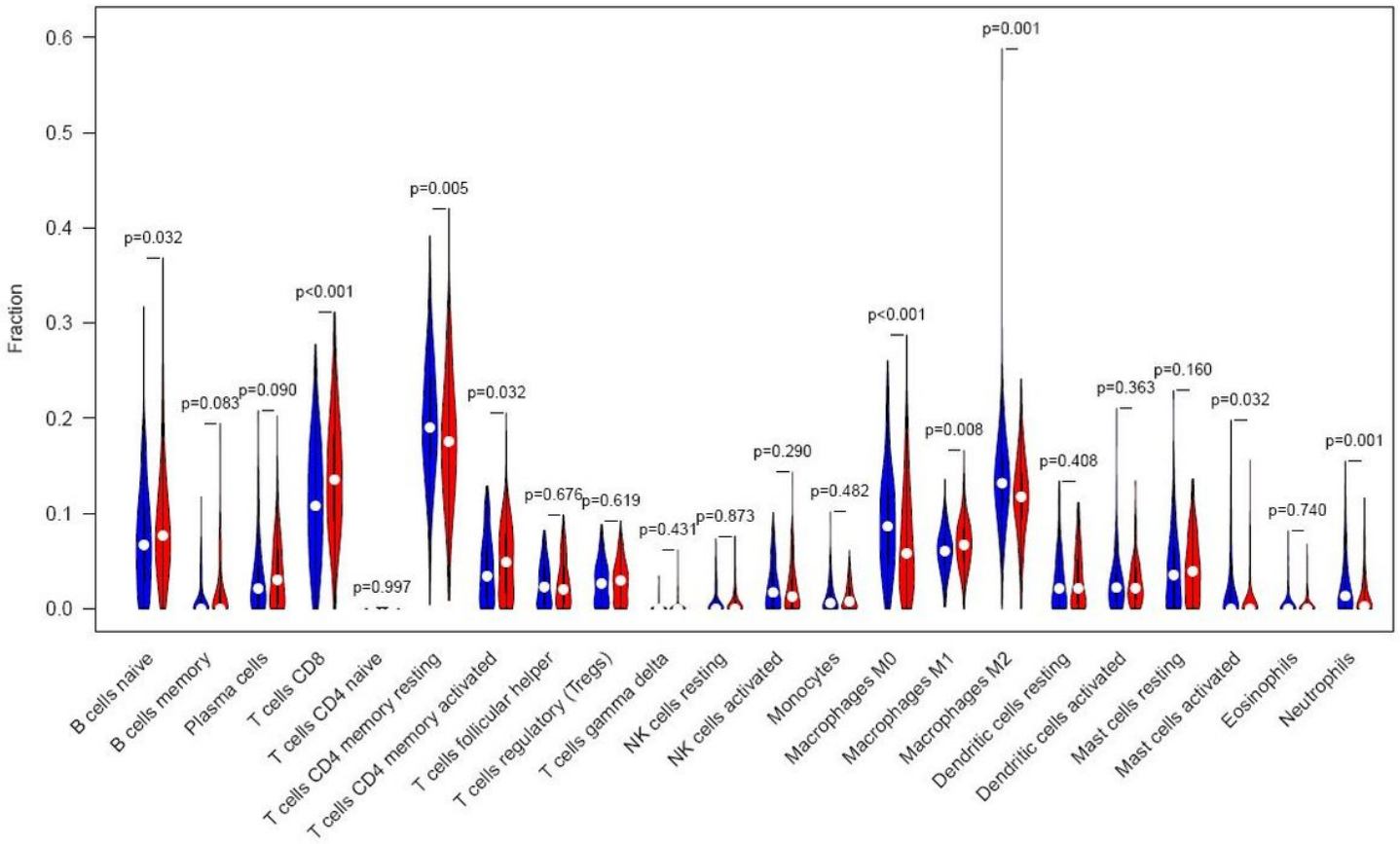

Figure 5

The relationship between immune cells infiltration and WAKMAR2 expression in gastric cancer (GC). A red violin represents the WAKMAR2 high expression group. A blue violin represents the WAKMAR2 low expression group. The white points inside the violin represent median values.

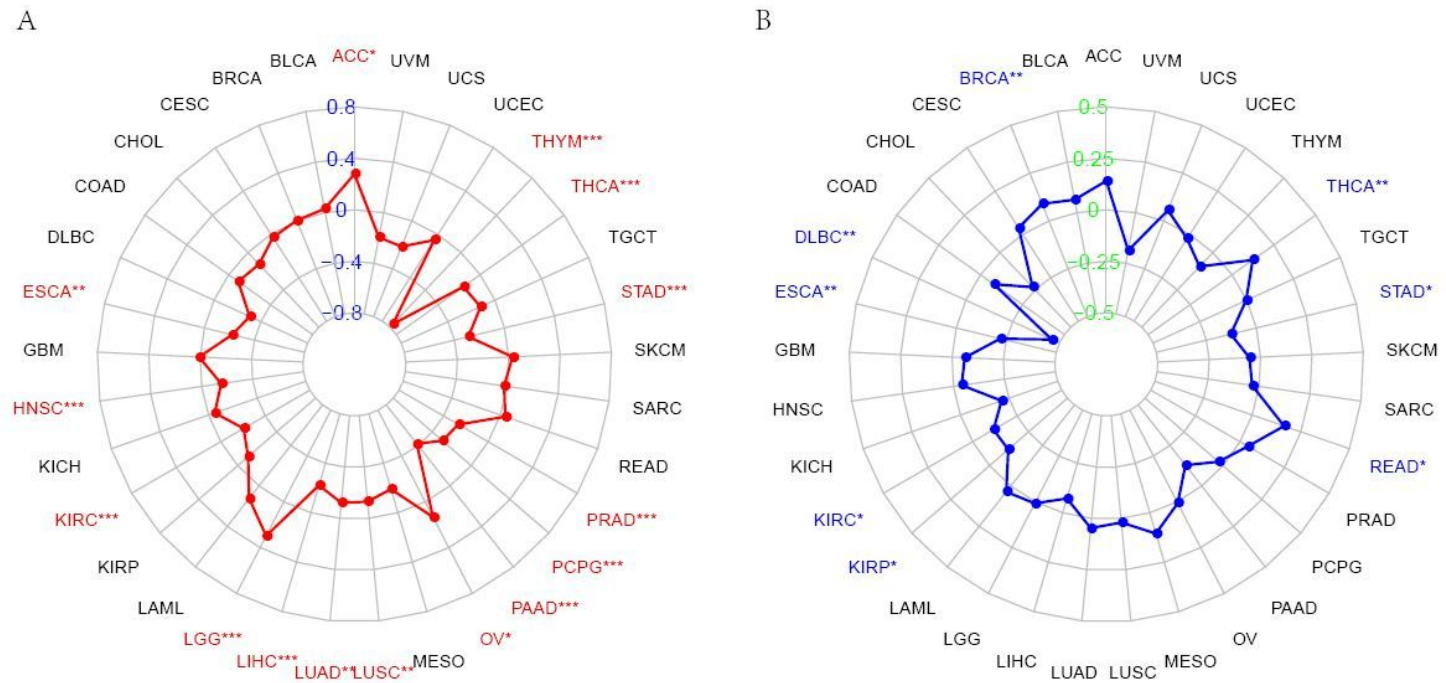

Figure 6

Radar chart of correlation between WAKMAR2 expression and tumor mutation burden (TMB) and microsatellite instability (MSI) in pan-cancer. A. TMB; B. MSI (*P<0.05, **P<0.01, *** P<0.001).