

Long Noncoding RNA *MAGI2-AS3* in the Tumorigenesis of Stomach Adenocarcinoma



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ABSTRACT

Background: Gastric cancer is among the most prevalent and fatal malignancies worldwide. Although radical surgical resection, radiotherapy, and chemotherapy are standard treatment strategies, the accurate diagnosis and effective management of gastric cancer remain challenging. Therefore, identifying reliable biomarkers is of great importance. Increasing evidence has highlighted the critical role of long noncoding RNAs (lncRNAs) in the pathogenesis of gastric cancer. In this study, we explored interactions among lncRNAs, miRNAs, and mRNAs using the cancer genome atlas (TCGA) data and identified novel candidate biomarkers with significant diagnostic and prognostic potential.

Materials and Methods: RNAseq, miRNAseq, and corresponding clinical data were obtained from the TCGA database. Differential expression analysis was performed using the limma package in R. A competing endogenous RNA (ceRNA) network was constructed based on the STAR database. Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway analyses were conducted to explore functional enrichment. The diagnostic and prognostic significance of candidate biomarkers was evaluated using the Kaplan–Meier survival analysis with the log-rank test and receiver operating characteristic (ROC) curve analysis.

Results: Differential expression analysis identified 2127 differentially expressed mRNAs in gastric cancer, of which 935 were upregulated, and 1192 were downregulated. GO and KEGG pathway analyses revealed that these mRNAs were significantly enriched in key biological processes and cancer-related signaling pathways. Construction of the ceRNA network demonstrated that the lncRNA *MAGI2AS3* plays a pivotal role in gastric cancer initiation and progression. Moreover, survival and ROC analyses indicated that *MAGI2AS3* has strong potential as a diagnostic and prognostic biomarker for patients with gastric cancer.

Conclusion: In summary, this study elucidates the interactions among lncRNAs, miRNAs, and mRNAs and identifies regulatory networks that may serve as promising therapeutic targets and biomarkers in gastric cancer.

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Introduction

As a third leading cause of cancer-related death, stomach cancer remains one of the most prevalent and deadly cancers globally, with a high recurrence rate [1]. According to statistics from the global cancer observatory (GLOBOCAN) 2020, more than 1089103 new diagnoses were made in 2020, and an estimated 768793 patients died from stomach cancer in this year [2]. Depending on whether the tumor is located near the gastroesophageal junction (cardia) or away from it, stomach cancer is subdivided anatomically into cardia and non-cardia (true stomach adenocarcinomas [STAD]). STAD, the focus of this gene expression landscape analysis, comprises more than 90% of all cancers originating in the stomach [3]. Other types of stomach cancer include mesenchymal, lymphoproliferative, and neuroendocrine neoplasms [4]. Despite the significant decline in incidence during the past half-century, STAD continues to be associated with a high mortality rate, and the absence of early pathognomonic symptoms unfortunately leads to delayed diagnosis [5]. Therefore, early diagnosis and timely treatment are essential for improving prognosis and reducing mortality in patients with STAD [6]. Shedding light on the gene-regulatory network and, consequently, identifying key regulatory molecules in STAD can provide valuable insights into biomarkers for early diagnosis and management of the disease [7, 8].

Long noncoding RNAs (lncRNAs), the focus of this study, are a large and diverse class of ncRNA transcripts with lengths around 200 nucleotides [9]. To date, analysis of encyclopedia of DNA elements (ENCODE) data has led to the annotation of more than 120000 lncRNA transcripts in the human genome [10, 11]. A great diversity of physiological cellular processes, ranging from the development and control of the cell cycle to apoptosis and malignancy, have contributed to the expression of lncRNAs with diverse and versatile functional mechanisms such as chromatin remodeling, regulation of post-transcriptional mRNA decay, and regulating the mRNA splicing through miRNA sponging [8, 12-15].

At present, the definitive diagnosis of STAD is primarily established using invasive techniques, including contrast-enhanced CT scanning and preoperative endoscopy. However, these diagnostic methods are quite expensive and difficult to use as a first-line examination method [16]. Therefore, a novel diagnostic strategy to overcome the aforementioned problems is urgently needed. Interestingly, numerous studies have focused on establishing multigene expression signatures for STAD diagnosis, patient stratification, and disease prognosis [17-19]. Among the vari-

ous biomarkers, lncRNAs have great potential as valuable targets for investigating multigene expression signatures. Given that lncRNA expression is more tissue-specific, numerous studies have recently focused on the roles of several dysregulated functional lncRNAs in cancers, especially STAD [20-23]. A strategy for constructing biomarker-driven models is to leverage pathway-based biomarkers alongside extensive multi-omics data generated by large initiatives, including the cancer genome atlas (TCGA) [24]. In this study, we demonstrated that lncRNA *MAGI2-AS3* is an important player in STAD.

Materials and Methods

Sample and data collection

STAD patient data were obtained from TCGA database. The inclusion criteria comprised a confirmed histopathological diagnosis of STAD and availability of complete demographic and clinical information, including age, vital status, race, ethnicity, pathological stage, TNM (tumor, nodes, metastasis) classification, and overall survival time.

A total of 356 primary STAD tumor samples and 87 normal gastric tissue samples were included in the RNAseq analysis. Genes with consistently low expression across samples were filtered using standard DESeq2 preprocessing criteria before normalization. Among enrolled patients, 211 were 67 years or older, and 232 were younger than 67. The cohort consisted of 158 males and 285 females. Of the 443 patients, 5 were identified as Hispanic or Latino and 318 as non-Hispanic or non-Latino. The racial distribution included 278 White patients, 13 Black or African American patients, 89 Asian patients, and 1 Native Hawaiian or other Pacific Islander. Pathological staging revealed 59 patients at stage I, 130 at stage II, 183 at stage III, and 44 at stage IV. The detailed clinicopathological characteristics are presented in Table 1.

RNA-seq and miR-seq DATA ANALYSIS

RNA-seq and miR-seq level 3 molecular profiles of STAD were downloaded from the TCGA database. Raw read counts were normalized using the Voom transformation in conjunction with the TMM normalization method. Differentially expressed mRNAs, lncRNAs, and miRNAs between normal solid tissue samples and primary tumor tissues were identified using the limma package in R. The resulting datasets were filtered using a threshold of $|\log_2FC| > 1$ for all RNA types. A $P < 0.05$ and an $FDR < 0.05$ were applied as criteria for statistical significance. All computational analyses were performed in R.

Table 1. Clinicopathological characteristics of STAD patients

Characteristics		No. (%)
Age (y)	Age (y)	65.68(10.74)
	>67	211(47.63)
	≤67	232(52.37)
Sex	Male	158(35.67)
	Female	285(64.33)
Ethnicity	Hispanic or Latino	5(1.13)
	Not Hispanic or Latino	318(71.78)
	NA	120(27.09)
Race	Hawaiian/other pacific islander natives	1(0.23)
	Asian	89(20.09)
	Black or African American	13(2.93)
	White	278(62.75)
	NA	62(14)
Vital status	Alive	268(60.5)
	Dead	175(39.5)
Pathologic (stage)	I	59(13.32)
	II	130(29.35)
	III	183(41.31)
	IV	44(9.93)
	NA	27(6.09)
Pathologic (tumor)	T1	23(5.19)
	T2	93(20.99)
	T3	198(44.7)
	T4	119(26.86)
	TX	10(2.26)
Pathologic (metastasis)	M0	391(88.26)
	M1	30(6.77)
	MX	22(4.97)

Characteristics		No. (%)
Pathologic (nodes)	N0	132(29.8)
	N1	119(26.86)
	N2	85(19.19)
	N3	88(19.86)
	NX	17(3.84)
	NA	2(0.45)

NA: Not available.



LncRNA-miRNA-mRNA competing endogenous (ce)RNA network construction

A lncRNA-miRNA-mRNA competing endogenous (ce) RNA network was established in R using the GDCRNA-Tools package, incorporating interaction information from the starBase database [9]. The resulting network was visualized and analyzed by Cytoscape software, version 3.7.2.

In silico functional enrichment analysis and protein-protein interaction (PPI) network

GO enrichment analysis was conducted in the biological process, cellular component, and molecular function categories, together with Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. Visualization of enrichment results was performed using the ggplot2 package in R. Protein-protein interaction (PPI) networks were generated based on the STRING database and visualized in Cytoscape software, version 3.7.2. Significant network modules were identified using the molecular complex detection (MCODE) algorithm, with a confidence score >0.4.

Statistical analysis

All the differentially expressed data were analyzed by using R software, version 3.5.2 through the GDCRNA-Tools package software, version 1.16.1. Data visualization was performed using ggplot2 software, version 3.1.0. Kaplan-Meier survival analysis with the log-rank test was performed to evaluate the association between RNA expression levels (classified as high or low based on the median) and patient overall survival. Receiver operating characteristic (ROC) curve analysis was conducted using SPSS software, version 21. A $P < 0.05$ was considered statistically significant. Potential batch effects were assessed during exploratory data analysis. Since the TCGA Level 3 data are uniformly normalized, no significant batch effects were observed. Samples with missing clinicopathological information (including race, ethnicity, or pathological stage) were

excluded only from analyses that required those variables. No imputation was performed, and such samples were retained for all gene expression-based analyses.

Results

Differentially expressed genes

Differential expression analysis revealed a total of 2127 mRNAs that were differentially expressed in STAD, comprising 935 upregulated and 1192 downregulated transcripts. Additionally, 170 lncRNAs were identified as differentially expressed, with 119 upregulated and 51 downregulated. Among miRNAs, 125 showed differential expression, consisting of 119 upregulated and 51 downregulated miRNAs. These results are presented in Figure 1 and Tables 2 and 3. Upregulated and down-regulated genes are demonstrated in red and green, respectively.

Potential diagnostic and prognostic lncRNA

Diagnostic accuracy, assessed by area under the curve (AUC) values, was combined with prognostic relevance, evaluated using hazard ratios, to identify potential lncRNA biomarkers in STAD patients. The integrated analysis, summarized in Table 4, identified *MAGI2-AS3*, *NR2F1-AS1*, and *LINC01235* as promising lncRNAs with both diagnostic and prognostic value in STAD.

LncRNA-miRNA-mRNA ceRNA network construction

Based on the competing endogenous RNA (ceRNA) hypothesis, which posits that lncRNAs regulate mRNA expression by competing for shared miRNAs, a ceRNA network was constructed in R using differentially expressed genes from the starBase database. Network nodes and edges were visualized in Cytoscape software, version 3.7.2. Analysis of the ceRNA network identified *MAGI2-AS3* as

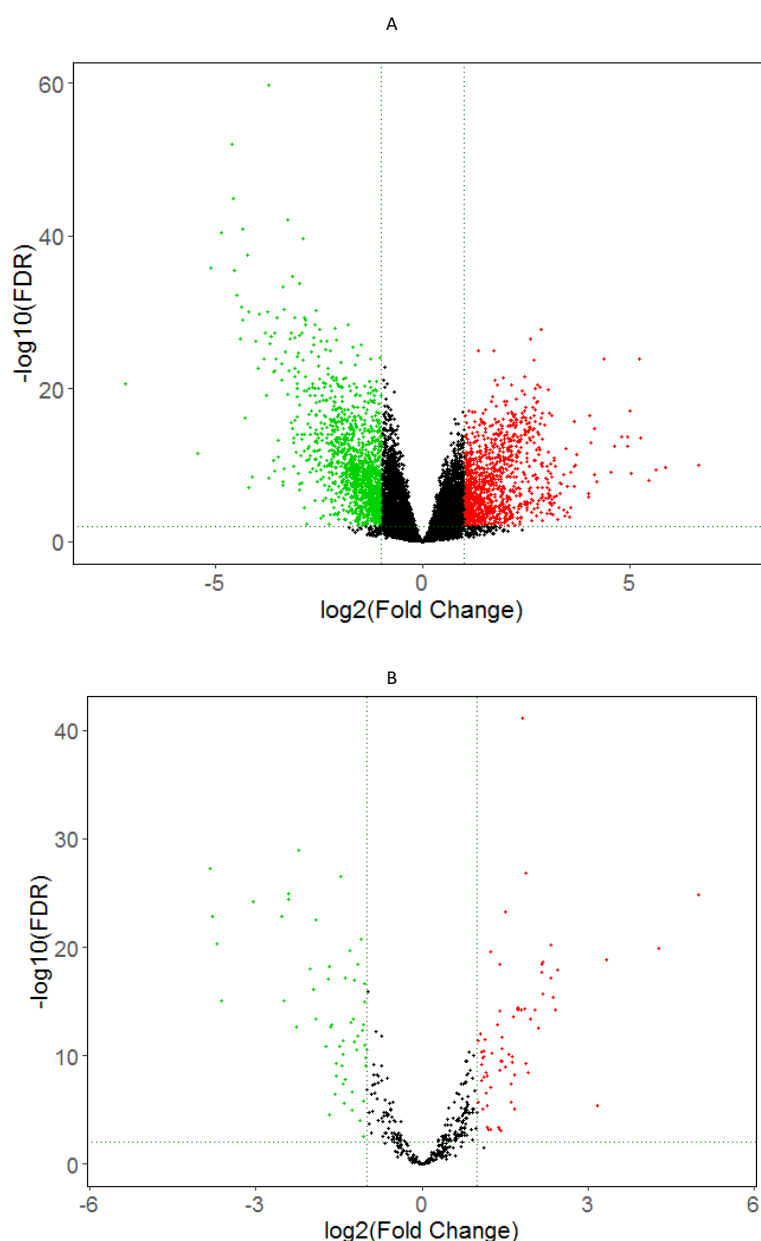


Figure 1. Volcano plot of differentially expressed genes and miRNAs; A, DE lncRNAs and DE mRNAs. B, DE miRNA

a key lncRNA with a critical role in STAD development (Figure 2).

GO enrichment and KEGG pathway analysis

GO enrichment analysis identified key biological functions associated with DE mRNAs. Biological process analysis highlighted enrichment in extracellular matrix organization, organelle fission, mitotic nuclear division, chromosome segregation, and urogenital system development. Cellular component terms showed significant localization to collagen-containing extracellular matrix, contractile fi-

ber structures, condensed chromosomes and centromeric regions, and collagen trimers. Molecular function analysis further indicated enrichment in extracellular matrix structural constituents, glycosaminoglycan and heparin binding, sulfur compound binding, and RNA polymerase II-specific DNA-binding transcription activator activity (Figure 3). KEGG pathway enrichment analysis showed that the DE mRNAs were mainly involved in cell cycle regulation, p53 signaling, protein digestion and absorption, cytokine-cytokine receptor interaction, and ECM-receptor interaction pathways (Figure 4).

Table 2. Top 20 upregulated mRNAs, lncRNAs, and miRNAs

	Symbol		logFC	AveExpr	t	P	FDR	B
mRNA	ENSG00000170373	CST1	6.66	3.63	7.09	0.00	0.00	16.69
	ENSG00000123500	COL10A1	5.86	3.41	6.92	0.00	0.00	15.67
	ENSG00000180818	HOXC10	5.62	1.39	6.83	0.00	0.00	15.14
	ENSG00000106031	HOXA13	5.46	2.58	6.26	0.00	0.00	11.81
	ENSG00000180806	HOXC9	5.22	0.40	11.57	0.00	0.00	50.02
	ENSG00000060718	COL11A1	5.02	2.48	6.60	0.00	0.00	13.79
	ENSG00000123388	HOXC11	4.93	1.24	8.40	0.00	0.00	25.31
	ENSG00000005073	HOXA11	4.93	0.11	7.96	0.00	0.00	22.30
	ENSG00000128610	FEZF1	4.79	-0.61	8.42	0.00	0.00	25.43
	ENSG00000170689	HOXB9	4.55	2.50	6.71	0.00	0.00	14.40
Long non-coding	ENSG00000230316	FEZF1-AS1	5.26	0.51	8.39	0.00	0.00	25.23
	ENSG00000228630	HOTAIR	5.01	-0.44	9.58	0.00	0.00	33.96
	ENSG00000240990	HOXA11-AS	4.64	-0.44	7.97	0.00	0.00	22.37
	ENSG00000243766	HOTTIP	4.13	0.45	6.53	0.00	0.00	13.34
	ENSG00000275216	AL161431.1	4.00	-0.06	5.23	0.00	0.00	6.47
	ENSG00000230061	TRPM2-AS	3.69	0.62	7.05	0.00	0.00	16.43
	ENSG00000272763	AC103702.2	3.66	2.36	5.89	0.00	0.00	9.80
	ENSG00000244649	LINC02086	3.56	1.22	6.69	0.00	0.00	14.29
	ENSG00000254290	AC124067.4	3.17	0.34	5.25	0.00	0.00	6.55
	ENSG00000281406	BLACAT1	3.17	1.13	7.43	0.00	0.00	18.82
MiRNA	hsa-miR-196a-5p		4.99	6.14	11.53	0.00	0.00	51.24
	hsa-miR-196b-5p		4.27	7.19	10.14	0.00	0.00	39.15
	hsa-miR-135b-5p		3.32	4.85	9.84	0.00	0.00	36.77
	hsa-miR-552-5p		3.16	1.78	4.88	0.00	0.00	4.5
	hsa-miR-194-5p		2.44	13.10	9.53	0.00	0.00	34.13
	hsa-miR-194-3p		2.40	6.33	8.37	0.00	0.00	25.33
	hsa-miR-192-5p		2.36	14.27	8.74	0.00	0.00	28.01
	hsa-miR-135b-3p		2.33	0.21	9.32	0.00	0.00	32.61
	hsa-miR-200a-5p		2.32	9.81	10.22	0.00	0.00	39.78
	hsa-miR-141-5p		2.19	8.71	9.76	0.00	0.00	35.99

Table 3. Top 20 down-regulated mRNAs, lncRNAs, and miRNAs

	Symbol		logFC	AveExpr	t	P	FDR	B
mRNA	ENSG00000169605	GKN1	-7.17	1.20	-10.66	0.00	0.00	42.74
	ENSG00000182333	LIPF	-5.44	1.77	-7.64	0.00	0.00	20.01
	ENSG00000119147	C2orf40	-5.13	0.63	-14.73	0.00	0.00	79.42
	ENSG00000164530	PI16	-4.88	0.68	-15.89	0.00	0.00	90.62
	ENSG00000143196	DPT	-4.61	2.06	-18.65	0.00	0.00	117.97
	ENSG00000168079	SCARA5	-4.58	1.29	-17.00	0.00	0.00	101.53
	ENSG00000162706	CADM3	-4.56	0.04	-14.67	0.00	0.00	78.75
	ENSG0000018625	ATP1A2	-4.50	1.02	-13.83	0.00	0.00	70.92
	ENSG00000196616	ADH1B	-4.41	2.58	-12.30	0.00	0.00	56.87
	ENSG00000175785	PRIMA1	-4.38	1.19	-13.45	0.00	0.00	67.41
LncRNA	ENSG00000152931	PART1	-3.62	0.34	-11.08	0.00	0.00	46.31
	ENSG00000268388	FENDRR	-2.04	3.44	-7.92	0.00	0.00	21.87
	ENSG00000224078	SNHG14	-2.00	3.17	-8.29	0.00	0.00	24.50
	ENSG00000249669	CARMN	-2.00	2.59	-6.43	0.00	0.00	12.57
	ENSG00000166770	ZNF667-AS1	-1.89	1.55	-8.43	0.00	0.00	25.60
	ENSG00000232229	LINC00865	-1.89	0.33	-7.40	0.00	0.00	18.64
	ENSG00000255248	MIR100HG	-1.83	2.90	-6.19	0.00	0.00	11.13
	ENSG00000229619	MBNL1-AS1	-1.81	1.93	-6.81	0.00	0.00	14.91
	ENSG00000281181	FP236383.3	-1.78	0.22	-4.27	0.00	0.00	2.10
	ENSG00000167912	AC090152.1	-1.78	0.20	-6.94	0.00	0.00	15.76
	Symbol		logFC	AveExpr	t	P	FDR	B
miRNA	hsa-miR-133b		-3.82	1.78	-12.24	0.00	0.00	57.73
	hsa-miR-133a-3p		-3.78	4.49	-10.97	0.00	0.00	46.22
	hsa-miR-1-3p		-3.71	5.03	-10.27	0.00	0.00	40.22
	hsa-miR-490-3p		-3.62	1.26	-8.65	0.00	0.00	27.36
	hsa-miR-204-5p		-3.05	1.31	-11.34	0.00	0.00	49.56
	hsa-miR-145-5p		-2.54	11.57	-10.95	0.00	0.00	46.06
	hsa-miR-129-5p		-2.50	1.82	-8.67	0.00	0.00	27.51
	hsa-miR-139-3p		-2.41	2.93	-11.56	0.00	0.00	51.48
	hsa-miR-145-3p		-2.40	5.95	-11.40	0.00	0.00	50.03
	hsa-miR-5683		-2.26	0.39	-7.83	0.00	0.00	21.58

Table 4. Merge diagnostic and prognostic data of the LncRNAs (top 10)

Symbol	logFC	FDR	AUC	SE	P	Expres- sion	HR	Lower 95	Upper 95	P
MAGI2-AS3	-1.18	0.00	0.71	0.05	0.00	Low	1.64	1.18	2.27	0.00
NR2F1-AS1	-1.47	0.00	0.76	0.04	0.00	Low	1.61	1.16	2.23	0.00
LINC01235	2.74	0.00	0.89	0.03	0.00	High	1.55	1.12	2.15	0.01
MIR99AHG	-1.61	0.00	0.72	0.05	0.00	Low	1.50	1.08	2.08	0.01
RAP2C-AS1	-1.10	0.00	0.77	0.04	0.00	Low	1.42	1.03	1.97	0.03
AC067750.1	-1.15	0.00	0.72	0.05	0.00	Low	1.40	1.01	1.95	0.04
LINC02381	-1.41	0.00	0.71	0.04	0.00	Low	1.40	1.01	1.94	0.04
AC010478.1	-1.23	0.00	0.71	0.06	0.00	Low	1.39	1.00	1.93	0.05
MIR194-2HG	2.33	0.00	0.67	0.07	0.00	High	0.72	0.52	1.00	0.05
AL121839.2	1.02	0.00	0.77	0.03	0.00	High	0.72	0.52	1.00	0.05



LncRNA *MAGI2-AS3* as a potential prognostic biomarker

Prognostic (HR) values of the LncRNA *MAGI2-AS3* have been demonstrated to be a potential prognostic biomarker in STAD patients (Figure 5).

Discussion

Stomach cancer is one of the most common and deadly malignancies, with a poor prognosis in affected patients [25]. Early diagnosis and the absence of distant metastasis are associated with better survival [26]. Thus, there is an urgent need to understand the underlying mecha-

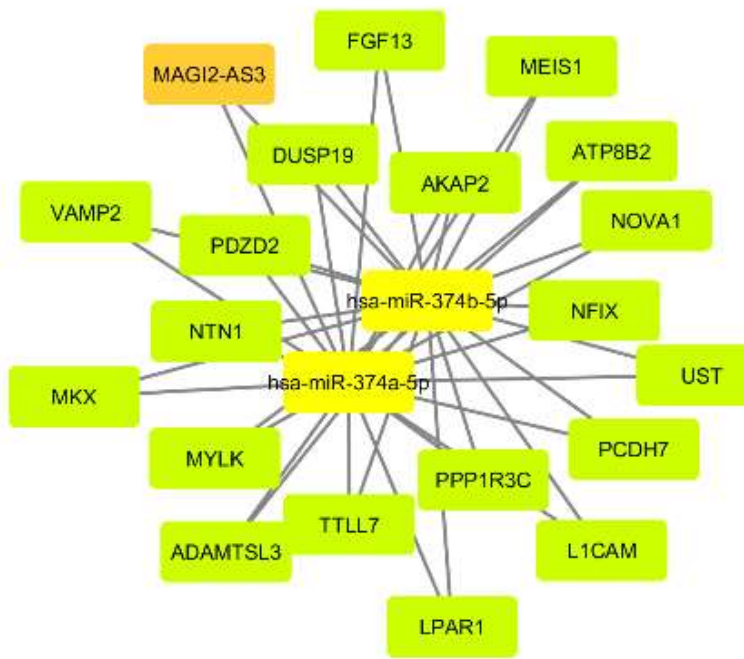


Figure 2. LncRNA-miRNA-mRNA ceRNA network construction of *MAGI2-AS3* in STAD (orange: LncRNA, yellow: miRNA, and green: mRNA)

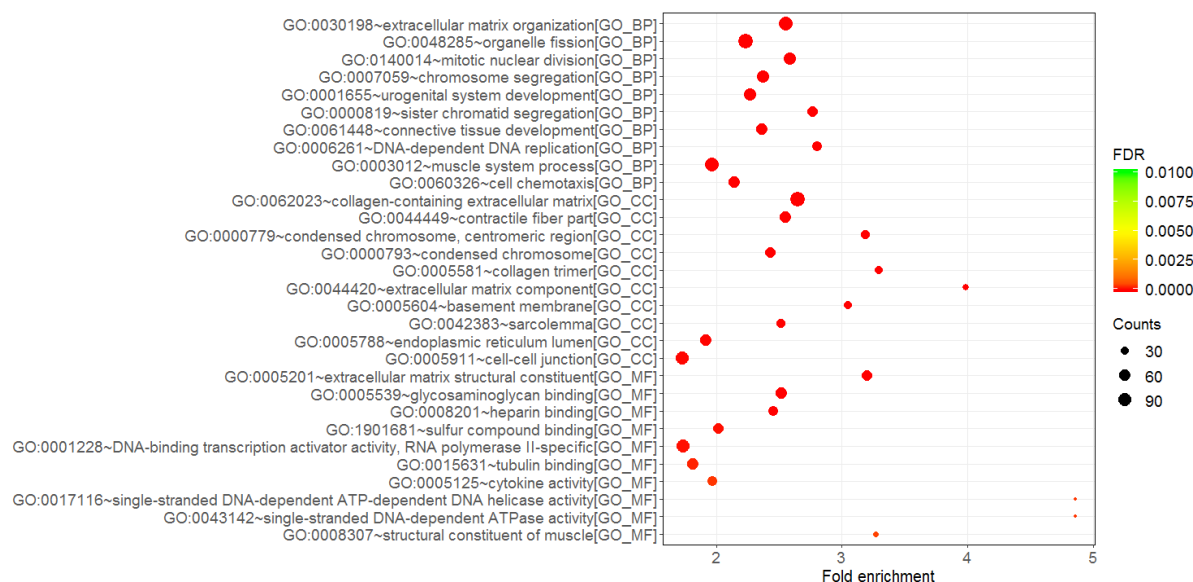


Figure 3. GO enrichment analysis of differentially expressed mRNAs in STAD

Note: It shows the top 10 GO terms that are significantly enriched.

nisms to develop novel, non-invasive, and easy-to-use biomarkers for early detection of STAD.

Accumulating evidence suggests that lncRNAs are key regulators of tumor development, progression, invasion, and chemoresistance across multiple cancer types [27]. In our recent endeavor, we have fully investigated the mechanisms of noncoding RNAs in gastric cancer

chemoresistance [8]. Recently, based on lncRNA bio-availability, numerous studies have demonstrated that lncRNAs are potential biomarkers in various cancers [28-30]. In the present study, we thoroughly investigated lncRNAs from the TCGA database, particularly focusing on those that may play a role in STAD. The data showed that lncRNAs are differentially expressed together with mRNAs and miRNAs.

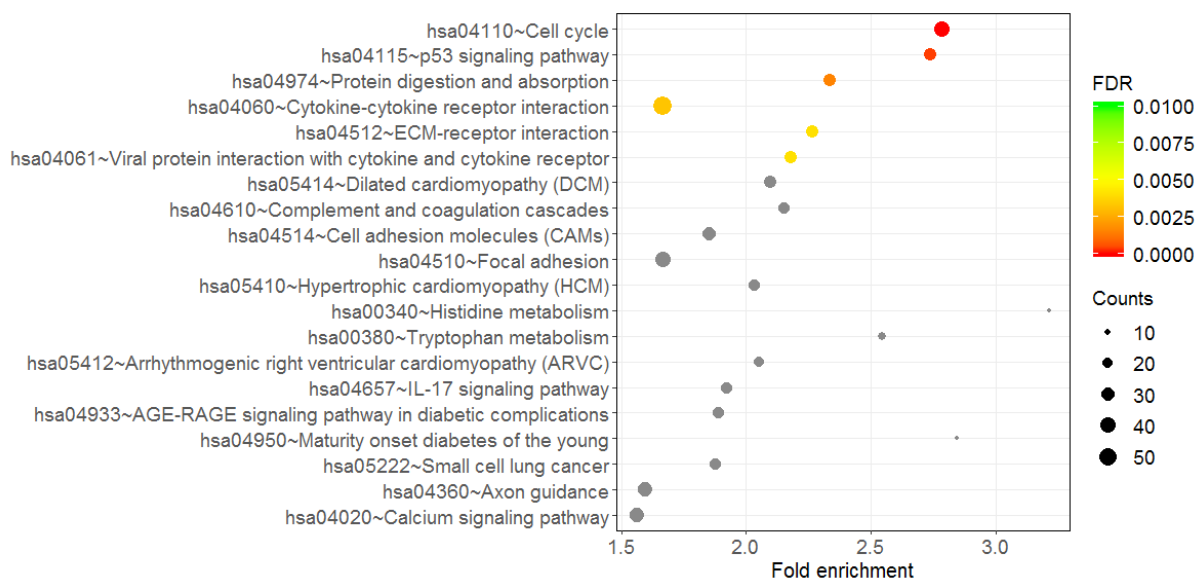


Figure 4. KEGG signaling pathway analysis of differentially expressed mRNAs in STAD

Note: The top 20 KEGG terms are presented.

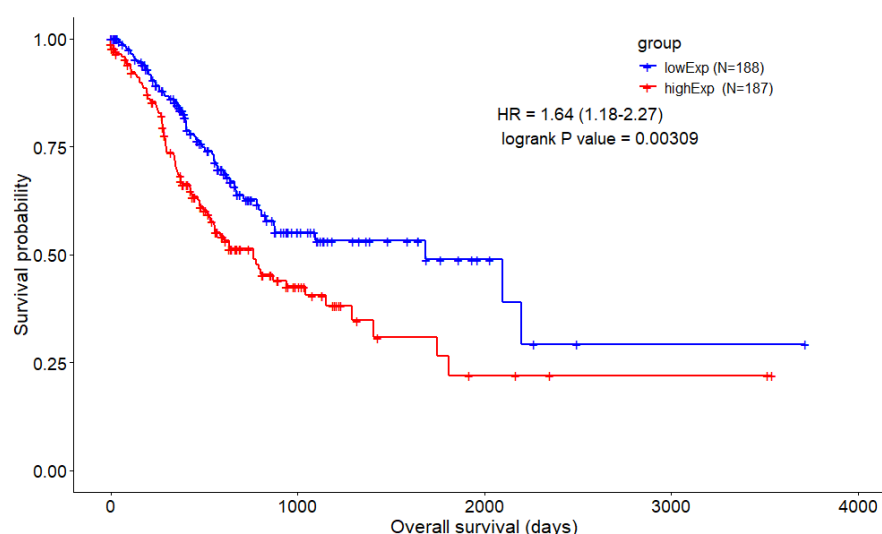


Figure 5. Kaplan-Meier analysis of the *MAGI2-AS3*



Numerous studies have shown that lncRNAs regulate gene expression by competing with miRNAs, forming a CE (competing endogenous) network in cells [9]. To draw a comprehensive picture of the lncRNA CE network in STAD, we constructed a CE network using our data. The survival analysis data and the construction of the lncRNA-miRNA-mRNA ceRNA network indicated that *MAGI2-AS3* might play a crucial role in the development and tumorigenesis of STAD. The data showed that the lncRNA *MAGI2-AS3* regulates various miRNAs and protein-coding genes by competing with miRNAs for binding in cells. *MAGI2-AS3*, an identified lncRNA associated with epithelial-mesenchymal transition (EMT), was found to be highly co-expressed with *ZEB1/2* in gastric cancer and normal stomach tissues. Loss/gain-of-function investigation revealed that *MAGI2-AS3* positively regulates *ZEB1* expression and enhances cell migration and invasion in gastric cancer.

Additionally, *MAGI2-AS3* is predominantly localized to the cytoplasm of gastric cancer cells. Moreover, *MAGI2-AS3* was found to be inversely correlated with miR-141/200a expression and to act as a negative regulator of miR-141/200a-3p in gastric cancer. *MAGI2-AS3* promotes tumor progression by sequestering miR-141/200a and sustaining increased *ZEB1* levels in gastric cancer [31].

In bladder cancer (Bca), *MAGI2-AS3* expression was found to be reduced compared to normal tissue, and this downregulation was associated with advanced tumor stage and poorer prognosis. Furthermore, *MAGI2-AS3* was positively correlated with its sense RNA, *MAGI2*.

When *MAGI2-AS3* is overexpressed in Bca cells, *MAGI2* and its downstream target, *PTEN*, are upregulated, leading to inhibition of cell migration and invasion. Conversely, the inhibitory effect of *MAGI2-AS3* overexpression on cell progression was reversed by disrupting *MAGI2* expression. These results suggest that *MAGI2-AS3* acts as a tumor suppressor in Bca by regulating the *MAGI2/PTEN/EMT* pathway [32].

Last investigation revealed that both *CDK6* and *MAGI2-AS3* were upregulated in cervical squamous cell carcinoma (CSCC) and showed a positive correlation. High levels of *MAGI2-AS3* were associated with poor survival in CSCC patients. In CSCC cells, overexpression or downregulation of *MAGI2-AS3* increased or decreased *CDK6* levels, respectively. Overexpression of *CDK6* counteracted the effects of *MAGI2-AS3* silencing. *MAGI2-AS3* promoted the proliferation of CSCC cells by upregulating *CDK6* [33].

In a breast cancer study, overexpression of *MAGI2-AS3* was shown to inhibit the proliferation, migration, and invasion of breast cancer cells and to suppress breast cancer growth in vivo. *MAGI2-AS3* may act as a ceRNA for miR-15b-5p, which directly targets *CCDC19*, a tumor suppressor in breast cancer. Knockdown of *CCDC19* rescued the proliferation, migration, and invasion of breast cancer cells that were suppressed by *MAGI2-AS3* overexpression [34]. *MAGI2-AS3* and *TMEM106B* were significantly upregulated, while miR-3163 was downregulated in colorectal cancer (CRC) cells. Deficiency of *MAGI2-AS3* led to increased cell apoptosis but decreased cell proliferation and migration. *MAGI2-AS3*

acted in combination with miR-3163 to negatively regulate its expression. *MAGI2-AS3* drives CRC progression by regulating the miR-3163/TMEM106B axis in the CRC cells [35]. It was discovered that the expression of *MAGI2-AS3* is decreased in liver cancer tissues and is associated with various clinical characteristics and poor survival rates. Increased levels of *MAGI2-AS3* hindered the growth and spread of liver cancer cells.

Additionally, *MAGI2-AS3* can act as a sponge for miR-374b-5p, a microRNA that promotes cancer cell activity. By binding miR-374b-5p, *MAGI2-AS3* indirectly increases SMG1 levels, a protein that suppresses cancer progression. Knocking down SMG1 reversed the beneficial effects of *MAGI2-AS3* in liver cancer cells [36]. *MAGI2-AS3* was found to play a significant role in epithelial ovarian cancer (EOC), specifically in high grade serous ovarian carcinoma (HGSC). *MAGI2-AS3* could act as a tumor suppressor in HGSC by attenuating miR-15-5p, miR-374a-5p, and miR-374b-5p, and altering downstream signaling via a ceRNA network [37]. *MAGI2-AS3* was found to inhibit the growth, movement, and invasiveness of lung squamous cell carcinoma cells by increasing its expression levels. It also led to increased cell death. *MAGI2-AS3* was identified in the cytoplasm and was found to target *miR-374a/b-5p*. This miRNA, in turn, targeted *CADM2*. By reintroducing *CADM2* into cells, the effects of miR-374a/b-5p on cell activity were reversed, indicating a regulatory pathway in lung squamous cell carcinoma [38].

It has been shown that *MAGI2-AS3* inhibits the migration and invasion of breast cancer cells and suppresses miR-374a expression. Bioinformatics analysis suggested a link between *MAGI2-AS3* and *miR-374a*, with PTEN identified as a novel target of miR-374a. By upregulating *MAGI2-AS3*, breast cancer metastasis was suppressed by reducing miR-374a and increasing PTEN levels [39]. *MAGI2-AS3* showed strong expression correlation with *MAGI2* in breast cancer cells. Overexpression of *MAGI2-AS3* and *MAGI2* could suppress the Wnt/ β -catenin pathway, leading to reduced cell proliferation and migration, suggesting that *MAGI2-AS3* may function as a cis-acting regulator by decreasing DNA methylation levels in the *MAGI2* promoter. Inhibition of the DNA demethylase TET1 can reverse the effects of *MAGI2-AS3* overexpression on *MAGI2* and cell behavior [40].

A limitation of this study is the lack of multivariate survival analysis adjusting for clinicopathological variables, which should be addressed in future validation studies.

Conclusion

In summary, the present study comprehensively characterized lncRNA-miRNA-mRNA interaction networks and identified regulatory mechanisms that may represent potential therapeutic strategies in stomach cancer. Furthermore, *MAGI2-AS3* was proposed as a novel diagnostic and prognostic biomarker for patients with gastric cancer. Collectively, our findings lay the groundwork for future investigations focused on the discovery and clinical translation of novel biomarkers in STAD. Despite their high diagnostic potential, clinical translation of lncRNA biomarkers faces challenges, including tumor heterogeneity, RNA stability, and the need for large, independent validation cohorts.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors contribution's

Conceptualization, software, validation, visualization, supervision, and project administration: Arash Poursheikhani; Methodology, formal analysis, investigation, and data curation: Arash Poursheikhani and Naser Ajami; Writing the original draft: Negin Nokhandani and Mohammad Mofidi; Resources, review and editing: All authors.

Conflict of interest

The authors declared no conflict of interest.

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