Reports of Biochemistry & Molecular Biology
Vol.13, No.2, Jul 2024

Original article

www.RBMB.net

Modelling of miRNA-mRNA Network to Identify Gene Signatures with Diagnostic and Prognostic Value in Gastric Cancer: Evidence from *In-Silico* and *In-Vitro* Studies

Mohammad Bagher Jahantab[1], Mohammad Salehi[2], Mehdi Koushki[3,4], Reyhaneh Farrokhi Yekta[5], Nasrin Amiri-Dashatan*[5,6], Mostafa Rezaei-Tavirani*[5]

Abstract

Background: Gastric cancer (GC) is a prevalent malignancy with high recurrence. Advances in systems biology have identified molecular pathways and biomarkers. This study focuses on discovering gene and miRNA biomarkers for diagnosing and predicting survival in GC patients.

Methods: Three sets of genes (GSE19826, GSE81948, and GSE112369) and two sets of miRNA expression (GSE26595, GSE78775) were obtained from the Gene Expression Omnibus (GEO), and subsequently, differentially expressed genes (DEGs) and miRNAs (DEMs) were identified. Functional pathway enrichment, DEG-miR-TF-protein–protein interaction network, DEM-mRNA network, ROC curve, and survival analyses were performed. Finally, qRT-PCR was applied to validate our results.

Results: From the high-throughput profiling studies of GC, we investigated 10 candidate mRNA and 7 candidate miRNAs as potential biomarkers. Expression analysis of these hubs revealed that 5 miRNAs (including miR-141-3p, miR-204-5p, miR-338-3p, miR-609, and miR-369-5p) were significantly upregulated compared to the controls. The genes with the highest degree included 6 upregulated and 4 downregulated genes in tumor samples compared to controls. The expression of miR-141-3p, miR-204-5p, SESTD1, and ANTXR1 were verified in vitro from these hub DEMs and DEGs. The findings indicated a decrease in the expression of miR-141-3p and miR-204-5p and increased expression of SESTD1 and ANTXR1 in GC cell lines compared to the GES-1 cell line.

Conclusions: The current investigation successfully recognized a set of prospective miRNAs and genes that may serve as potential biomarkers for GC's early diagnosis and prognosis.

Keywords: Biomarker, Gastric cancer, GEO, MicroRNA, PPI-network, Stomach neoplasms.

Introduction

Gastric cancer (GC) (which is called as stomach cancer) was the 5th most common and the 4th lethal cancer worldwide in 2022 (1). Gastric cancer is usually diagnosed at the middle-advanced stages due to the lack of specificity in the current clinical manifestations that is why its early diagnosis and treatment be very crucial (2). Despite the extensive advances in endoscopic screening methods, the 5-year survival rate for GC has

1: Clinical Research Development Unit, Shahid Jalil Hospital, Yasuj University of Medical Sciences, Yasuj, Iran.
2: Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
3: Cancer Gene Therapy Research Center, Zanjan University of Medical Sciences, Zanjan, Iran.
4: Department of Clinical Biochemistry, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran.
5: Proteomics Research Center, School of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
6: Zanjan Metabolic Diseases Research Center, Health and Metabolic Diseases Research Institute, Zanjan University of Medical Sciences, Zanjan, Iran.
***Corresponding author: Nasrin Amiri-Dashatan; Tel: +98 24-33739021; E-mail: nasrinamiri91@gmail.com & Mostafa Rezaei-Tavirani; Tel: +98 9122650447; E-mail: tavirany@yahoo.com.**
Received: 9 Jul, 2024; Accepted: 8 Dec, 2024

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]

remained extremely poor (3). In this regard, the development of reliable biomarkers, especially with less invasive screening tests (i.e., biomarkers in biofluids) for the diagnosis and prognosis of GC are in progress. Currently, the most frequently used markers for detecting GC are carcinoembryonic antigen (CEA), carbohydrate antigens (CA19-9, CA72-4, CA125, CA24-2, and CA50), pepsinogen, and α-fetoprotein. However, these markers are not very accurate or specific. The GC initiation and progression is influenced by several genetic modifications, including large chromosome gain or loss, single nucleotide polymorphisms, mutations, and epigenetic alterations such as DNA methylation, histone modifications, and dysregulation of microRNAs (miRs) and long non-coding RNAs (lncRNAs) (4). Among the above-mentioned alterations, miRs have great importance as they are associated with the regulation of tumor cell growth, invasion, metastatic potential, and apoptosis. Furthermore, the aberrant expression of miRs is potentially useful for GC diagnosis, prognosis, and disease monitoring (5). For instance, high expression of miR-150, miR-214, miR-20b, and miR-375 and low expression of miR-433 and miR-125-5p are associated with shorter survival rates in GC. Altered levels of miR-126, miR-148, miRNA-218, miRNA-27a and miR-650 have also been shown to be associated with lymph node metastasis (6). The miR-155, miR-16, and miR-146a were found to be up-regulated in the gastric epithelial cells of patients who were infected with Helicobacter pylori (7). Moreover, the alteration of multiple miRNAs including miR-181a-5p, miR-223, and miR-181b, exhibited a range of impacts on the biological processes such as Epithelial-to-Mesenchymal Transition (EMT), as well as on the proliferation and migration of tumor cells in Gastric Cancer (8). In addition, in a meta-analysis, the miR-125 family was reported as a potential biomarker for the prognosis of gastric cancer (9). Systems biology approaches (such as genomics, transcriptomics, proteomics and metabolomics) have developed into a new research platform over the past decade in the field of biomedical research and biomarker discovery in different human diseases, especially cancer (10-14). In the current study, by using omics platforms of transcript and miR microarrays with bioinformatic methods, we have focused on the discovery of novel and precise panel of genes and miRNA biomarkers both for diagnosis and predicting overall survival in GC patients (Fig. 1). The most authentic results were then validated by real time RT-PCR.

Materials and Methods

Microarray data analysis

This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences (Ethical code: IR.SBMU.RETECH.REC.1400.1005). After searching for the NCBI/GEO series (http://www.ncbi.nlm.nih.gov/geo/) using the keywords [“microRNA OR miR OR miRNA OR gene OR transcript” AND “gastric cancer OR stomach cancer”], 4020 results were obtained. By restricting the results to “Homo sapiens” and “Tissue”, 1231 results remained, from which, 192 results belonged to data series. From the 192 series, 29 were selected and analyzed with GEO2R platform and finally 5 datasets with the highest number of meaningful results were picked up. These included 3 gene expression datasets (GSE19826, GSE81948, GSE112369), and 2 miR expression datasets (GSE26595, GSE78775). From the miRs datasets, 4 outliers were removed from GSE78775. GEO2R was used to normalize all expression datasets, and the statistical differentiation of genes (DEGs) and miRs (DEMs) was achieved using the cutoff criteria of p-value< 0.05 and Fold-change> 1.5. The details of each dataset are summarized in Table 1. Normalized datasets and the volcano plots show the significant miRs and genes. Finally, the result of the dataset analysis was integrated using the Venny online platform to identify common differentially expressed genes and miRs between datasets.

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]

Table 1. Details of the selected microarray platforms.

GEO series	Platform	#Tumor samples	#Control samples	Ref.
GSE19826	GPL570 [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array	12	15	Wang et al. (41)
GSE81948	GPL6244 [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version]	15	5	Canu et al. (42)
GSE112369	GPL15207 [Prime View] Affymetrix Human Gene Expression Array	36	6	Nanki et al. (43)
GSE26595	GPL8179 Illumina Human v2 MicroRNA expression beadchip	60	8	Lim et al. (44)
GSE78775	GPL10850 Agilent-021827 Human miRNA Microarray (V3) (miRBase release 12.0 miRNA ID version)	26	26	Yu et al. (45)

Construction of mRNA-miRNA networks

Common genes between datasets were searched in mirDIP (microRNA Data Integration Portal), miRTarBase and innateDB to find all the possible interactions of the DEGs with miRs, transcription factors and protein-protein interactions. Common miRs were also searched in mirDIP, TaeBase, miRTarBase, and miRecords to find all the possible target genes of the 29 DEMs. The interactions were used to construct a DEM-mRNA network for the common miRs between datasets and a DEG-miR-TF-PPI network for the common genes in Cytoscape software.

Gene ontology enrichment and Pathway analysis

ClueGO, a plug-in in Cytoscape software, was used to find the significant enriched biological processes (BP), molecular functions (MF), cellular components (CC), and KEGG pathways in the DEG-miR-TF-PPI network. The Bonferroni-corrected p-val<0.05 was considered as statistically significant.

Hub selection

The constructed networks were analyzed in Cytoscape and the nodes with the highest numbers of interactions were selected as the hub genes and hub miRs. Hub nodes usually play key roles in the regulation of network-related pathways. These nodes might also serve as potential biomarkers of the diseases. The top-ranked selected hub nodes included ten genes and 7 miRs from both networks, respectively.

ROC analysis

After the selection of the hub genes and hub miRs, the ROC curves were plotted in GraphPad prism 8.0, according to the nodes normalized expression data (Figs. 4 & 5). The p-value< 0.05 and the area under the curve (AUC)> 0.90 was set as the cutoff for selection of potential biomarkers.

Survival analysis

Survival analysis was performed only for the hub genes and hub miRs with AUC> 0.90. To check the possibility of the DEGs correlation with overall survival, the Human Protein Atlas database was used. The Human Protein Atlas (HPA) is an open-source online platform for mapping of human proteins in tissues, cells, and organs by using the data derived from omics studies and antibody-based imaging. In the "pathology" section of HPA, we looked for the impact of the expression levels of DEG on the survival of patients with gastric cancer. The proteins with meaningful correlation with overall survival were selected according to the Kaplan-Meier survival curves. The cutoff p-value was set 0.05. For the DEMs, survival data were searched in Oncomir and CancerMIRNome databases.

***In-vitro* analysis**

Cell culture

The human gastric cancer cell lines (including SNU-NCC-19 and MKN-74) and human normal gastric cell line (GES-1) were

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]

purchased from iCell Bioscience Inc., (Shanghai, China). Cell lines were cultured in DMEM medium (Gibco, Germany) supplemented with 1% glutamine, 10% fetal bovine serum (FBS) (Gibco) and 1% penicillin/streptomycin. Then, the cells were incubated in a 5% CO2 incubator at a temperature of 37 °C for 24h.

RNA extraction and quantitative RT-PCR

Total RNA was extracted by RiboEx reagent (GeneAll Biotech, Korea), and reverse-transcribed to cDNA using 3 µg RNA, and 1λ oligo-dT (Thermofisher, USA) using a RevertAid™ First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). The quality and quantity of isolated RNA were performed by electrophoresis and Nanodrop 1000, respectively. The gene expression analysis was conducted by qRT-PCR using SYBR Green 2x Master Mix Green (Ampliqon). RT-qPCR was performed using StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA) to assess the variations in the RNA expression level of SESTD1 and ANTXR1 genes. PCR was performed in 20 µl reactions containing 1 µl cDNA target, 100 nM forward and reverse primers, and 1x SYBR Green RealQ Plus Master Mix (Ampliqon, DK-5230 Odense M, Denmark). Experiments were carried out in duplicate for target genes. The PCR condition was as follows: activation at 95 °C for 3 min, amplification at 95 °C for 10 s, 60 °C for 1 min for 40 cycles. All examinations were conducted in triplicate for each cell sheet.

miRNA extraction, and quantitative RT-PCR

Total miRNA was isolated from MKN-74, SNU-NCC-19 and GES-1 cell lines using the Quick-RNA miniprep kit according to the manufacturer's protocol. BioFuture MD2000 spectrophotometer was utilized to measure the concentration of extracted miRNAs. The extracted miRNA (10 ng) was converted to cDNA using cDNA synthesis kit (Applied Biosystems, Foster city, CA, USA) following the manufacturer's protocol. The miRNA expression was analyzed by quantitative reverse transcription-PCR using TaqMan Fast Advanced Master Mix (Applied Biosystems) on a StepOnePlus Real-Time PCR System. The following conditions were used to perform quantitative RT-PCR: an enzyme activation step at 95 °C for 20 s, 40 cycles of denaturation at 95 °C for 1 s, and a final annealing extension at 60 °C for 20 s. Sequences of primers used in this study are listed in Table 2.

Table 2. Sequences of primers used in this study.

Gene name	"F" sequence (5′-3′)	"R" sequence (5′-3′)
SESTD1	CAACATCCCTAATAAGCCATCCA	GCAAAATCTCTTACAAGCAGCTCTT
ANTXR1	CGCCTCTTACTACGGTGGAC	TAGCTTCGCCCCTTCTTCTG
GAPDH	CAGCCGCATCTTCTTGTGC	CAGCCGCATCTTCTTGTGC
miR-141-3P	CGCAGTAACACTGTCTGGT	GTCCAGTTTTTTTTTTTTTTCCATCT
miR-204-5P	UUCCCUUUGUCAUCCUAUGCCU	CTCAACTGGTGTCGTGGA
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT

"F": Forward primer; **"R":** Reverse primer.

Statistical Analysis

The relative expression levels of genes and miRNAs were compared with Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene and U6 control transcripts, respectively. The $2^{-\Delta\Delta ct}$ method was used to calculate the fold changes. Statistical analyses were performed with GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA).

Results

Differentially expressed miRs and genes

To find the differentially expressed genes and miRs, the intersection between datasets was

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]

extracted from the Venn diagrams. According to the cutoff criteria, GSE81948, GSE19826, and GSE112369 had 2463, 944, and 3179 significantly altered genes, respectively. The intersection between these 3 datasets yielded in 71 common genes (Fig. 2a). The miR dataset GSE26595 resulted in 138 and GSE78775 resulted in 93 significantly altered miRs, respectively. The intersection between the two miR datasets led to the identification of 29 miRs that were common between them. (Fig. 2b). The common DEGs and DEMs were used for further analysis (Table 3).

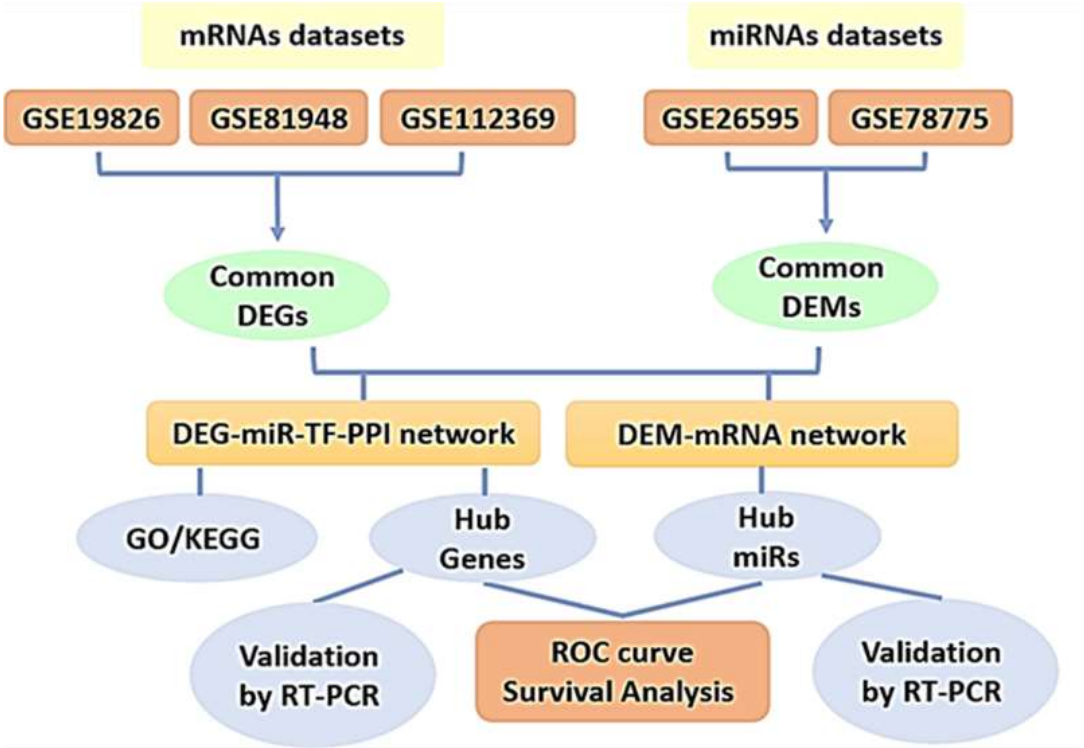

Fig. 1. The flowchart of this study. **DEG:** Differentially Expressed Gene; **DEM:** Differentially Expressed miRNA; **GO:** Gene Ontology; **KEGG:** Kyoto Encyclopedia of Genes and Genomes; **PPI:** Protein-Protein Interaction.

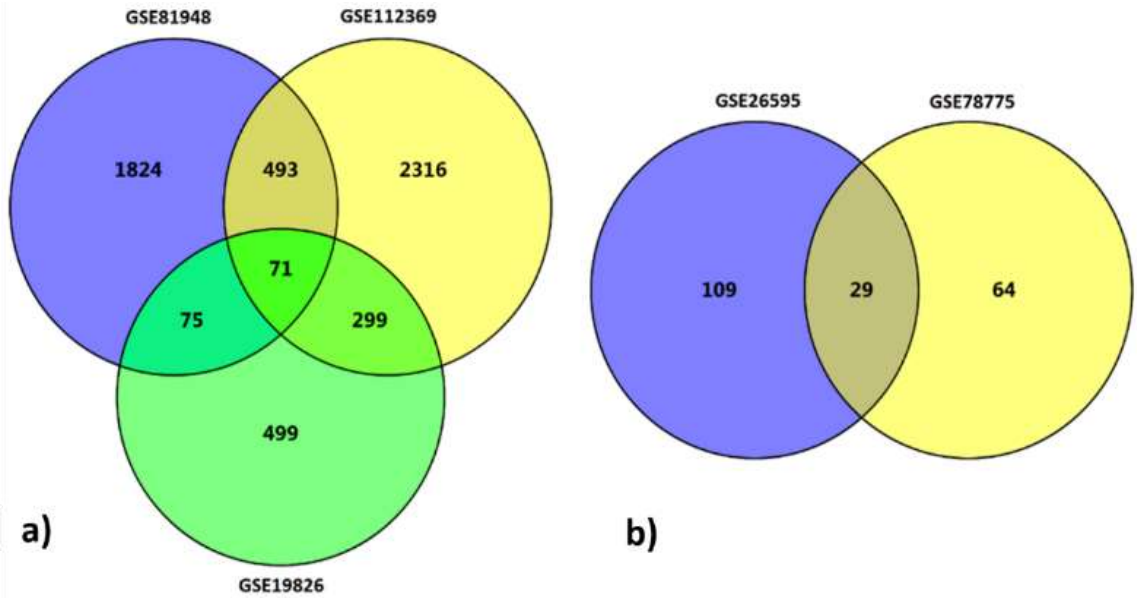

Fig. 2. Venn diagrams showing common genes (a) and common miRs (b) between the selected GEO datasets.

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]

Table 3. Common DEGs and DEMs between GEO datasets extracted from the Venn diagrams.

29 common miRs	hsa-miR-204, hsa-miR-196b, hsa-miR-148a, hsa-miR-29c, hsa-miR-92a, hsa-miR-200a, hsa-miR-10a, hsa-miR-98, hsa-miR-224, hsa-miR-23a, hsa-miR-1249, hsa-miR-18a, hsa-miR-31, hsa-miR-93, hsa-let-7i, hsa-miR-574-5p, hsa-miR-214, hsa-miR-30e, hsa-miR-1228, hsa-miR-369-5p, hsa-miR-181a, hsa-miR-146b-5p, hsa-miR-130a, hsa-miR-338-3p, hsa-miR-1234, hsa-let-7e, hsa-miR-609, hsa-miR-141, hsa-miR-185
71 common Genes	ARHGAP24, ACER2, ANTXR1,VSIG2, ADGRG2, ADAM28, CYSTM1, MAST4, TCOF1, PPFIA3, KAT2B, ARHGAP18, PTS, CDCA5, C20orf27, FAM189A2, UGCG, SLC26A9, RAB31, B4GALNT3, GLUL, OGFOD1, OSR2, PTPRE, GEMIN5, ADAM17, PGC, NUP188, TMEM161B, L3MBTL4, B3GNT6, BANF1, GDPD5, PAK1IP1, FBXO8, EPN3, PDGFD, EP400, ACOX1, MAGI1, PPP2R3A, PLCG1, TRIM50, CAP9, APOBEC2, KAT2A, METTL7A, ZFAS1, SESTD1, MARVELD3, ZMIZ2, TNFRSF10B, COLCA1, PGF, RASSF6, MYRF, NFE2L2, MYRIP, FBXO45, GPRC5C, EPHB2, TRMT1, ARHGEF37, CENPJ, UBL3, GRIA3, NRP1, HADH, HOMER2, FRMD8, SMARCA4

DEGs: Differentially Expressed Genes; **DEMs:** Differentially Expressed MicroRNAs.

Network analysis, hub genes and hub miRs

The DEG-miR-TF-PPI network consisted of 1569 nodes and 2319 edges. The DEM-mRNA network also consisted of 2431 nodes and 3104 edges (Figs. 3a & b). The networks were analyzed and the hub nodes with the highest connectivity degrees for each network were selected as the potential biomarkers for ROC analysis. The top hub DEGs were included SESTD1, ANTXR1, NRP1, EPHB2, FBXO45, ACOX1, UBL3, TMEM161B, KAT2B, and PTPRE (Table 4a). The top hub miRs in the DEM-mRNA network were included hsa-mir-141-3p, hsa-mir-204-5p, has-miR-146b-5p, hsa-miR-338-3p, hsa-miR-574-5p, hsa-miR-609, and hsa-miR-369-5p (Table 4b).

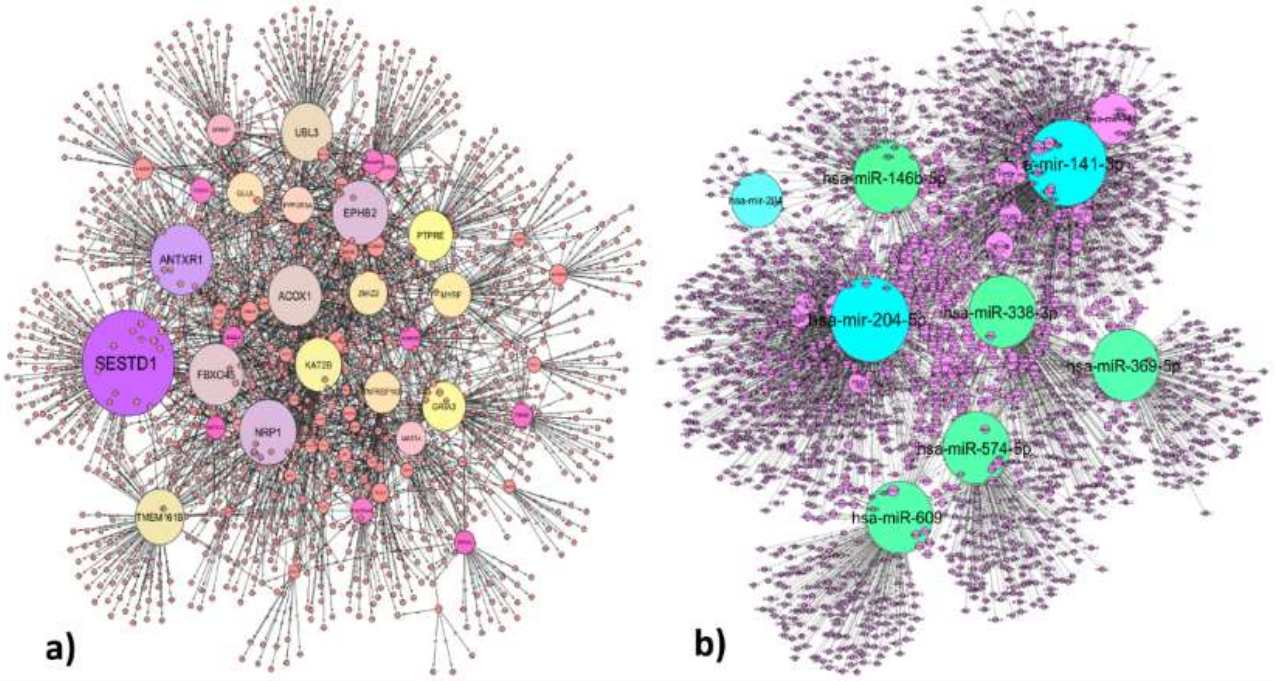

Fig. 3. (a) The DEG-miR-TF-PPI network, and (b) The DEM-mRNA network, constructed in Cytoscape based on all possible interactions of DEGs and DEMs with their targets in mirDIP, TarBase, miRTarBase, miRecords and innateDB. Larger circles denote higher degree hub genes and miRs.

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]

Table 4. a. Top 10 genes with the highest degrees in the DEG-miR-TF-PPI network. b. Top miRs with the highest degrees in the DEM-mRNA network.

No.	ID_REF	Gene ID	P-val	Fold-change	Tumor/control	Hub Degree
a) Top 10 genes with highest degrees in DEG-miR-TF-PPI network						
1	SESTD1	91404	0.015	7.33	↑	160
2	ANTXR1	84168	0.002	4.69	↑	107
3	NRP1	8829	0.005	6.28	↑	99
4	EPHB2	2048	0.04	4.28	↑	97
5	FBXO45	200933	0.037	6.02	↑	94
6	ACOX1	51	0.016	6.69	↓	92
7	UBL3	5412	0.044	8.34	↓	88
8	TMEM161B	153396	0.01	6.59	↓	84
9	KAT2B	8850	0.0006	2.04	↓	80
10	PTPRE	5791	0.0065	4.03	↑	76
b) Top miRs with the highest degrees in the DEM-mRNA network						
1	hsa-mir-141-3p	406933	4.16E-13	2.62	↓	1069
2	hsa-mir-204-5p	406987	0.008	6.6	↓	984
3	hsa-miR-146b-5p	574447	0.015	18.6	↑	226
4	has-miR-338-3p	442906	0.041	7.73	↓	210
5	hsa-miR-574-5p	693159	0.016	9.67	↑	198
6	hsa-miR-609	693194	0.0019	14.4	↓	194
7	hsa-miR-369-5p	442914	0.005	1.51	↓	187

Gene ontology

Based on ClueGO platform, the meaningful enriched gene ontology (GO) terms and KEGG pathways were determined for the DEG-miR-TF-PPI network (Table 5). The most significant biological processes included the regulation of the cellular ketone metabolic process by positive regulation of transcription from RNA polymerase II promoter, regulation of interleukin-12 biosynthetic process, histone H3-K14 acetylation, positive regulation of gluconeogenesis, negative regulation of cofactor metabolic process, replicative senescence, rRNA transcription, sprouting angiogenesis, positive regulation of endothelial cell migration, and fatty acid beta-oxidation. The top enriched molecular functions included histone acetyltransferase binding, repressing transcription factor binding, RNA polymerase II transcription factor binding, and p53 binding. The most significant cellular components included I-kappaB/NF-kappaB complex, PcG protein complex, RNA polymerase II transcription factor complex, and replication fork. KEGG pathways enrichment results also showed that Notch signalling pathway, Apoptosis, Adipocytokine signalling pathway, and p53 signalling pathway were the most meaningful pathways related to gastric cancer.

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]

Table 5. Gene ontology and KEGG pathway enrichment results resulted from ClueGO.

	GO ID	GO Term	Bonferroni-corrected P-value	% Associated Genes	Associated Genes Found
Biological Process	**GO:0072366**	regulation of cellular ketone metabolic process by positive regulation of transcription from RNA polymerase II promoter	0.000092	60	KAT2A, KAT2B, PPAR
	GO:0045075	regulation of interleukin-12 biosynthetic process	0.0000088	40	MAST4, NFKB1, REL, RELA
	GO:0044154	histone H3-K14 acetylation	0.00066	33.33	KAT2A, KAT2B, SIRT1
	GO:0045722	positive regulation of gluconeogenesis	0.002	23.07	KAT2A, KAT2B, PPARA
	GO:0051195	negative regulation of cofactor metabolic process	0.0025	21.42	PPARA, STAT3, TP53
	GO:0090399	replicative senescence	0.0042	17.64	ANTXR1, ATM, TP53
	GO:0009303	rRNA transcription	0.019	8.82	SMARCA4, TCOF1, TP53
	GO:0002040	sprouting angiogenesis	0.02	4.28	HDAC9, NRP1, PGF
	GO:0010595	positive regulation of endothelial cell migration	0.013	4.16	HDAC9, NRP1, PLCG1
	GO:0006635	fatty acid beta-oxidation	0.0048	4	ACOX1, HADH, PPARA
Molecular Function	**GO:0035035**	histone acetyltransferase binding	0.0011	12.5	KAT2B, MTF1, SP1, TP53
	GO:0070491	repressing transcription factor binding	0.000039	9.67	HDAC9, MYC, PPARA, RELA, SP1, STAT3
	GO:0001085	RNA polymerase II transcription factor binding	0.00012	5.83	ATF4, ELK1, NFE2L2, PPARA, SP1, STAT3, TP53
	GO:0002039	p53 binding	0.013	4.16	SIRT1, SMARCA4, TP53
Cellular Component	**GO:0033256**	I-kappaB/NF-kappaB complex	0.00045	37.5	NFKB1, REL, RELA
	GO:0031519	PcG protein complex	0.025	6.66	MAGI1, SIRT1, YY1
	GO:0090575	RNA polymerase II transcription factor complex	0.0084	4.58	ATF4, DDIT3, KAT2A, STAT3, TP53
	GO:0005657	replication fork	0.025	4.41	DNMT1, TP53, ZMIZ2
KEGG Pathway	**GO:0004330**	Notch signalling pathway	0.027	6.25	ADAM17, KAT2A, KAT2B
	GO:0004210	Apoptosis	0.0031	5.88	ATM, NFKB1, RELA, TNFRSF10B, TP53
	GO:0004920	Adipocytokine signalling pathway	0.013	5.71	NFKB1, PPARA, RELA, STAT3
	GO:0004115	p53 signalling pathway	0.023	4.34	ATM, TNFRSF10B, TP53

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]

ROC Curves

All Receiver-Operating Characteristic (ROC) curves were plotted using Graphpad prism software (Figs. 4 & 5). We performed ROC curve analysis for top hub genes and miRs in both DEG-miR and DEM-mRNA networks. AUC cutoff for selection of putative biomarkers was 0.90. According to the results, SESTD1, ANTXR1, NRP1, ACOX1, PTPRE, hsa-mir-141-3p, hsa-mir-204-5p, and hsa-miR-609, showed highest accuracy and had AUC values more than 90%. These nodes were proposed as potential diagnostic markers for gastric cancer (Table 6). They might also have important biological roles in gastric cancer pathogenesis.

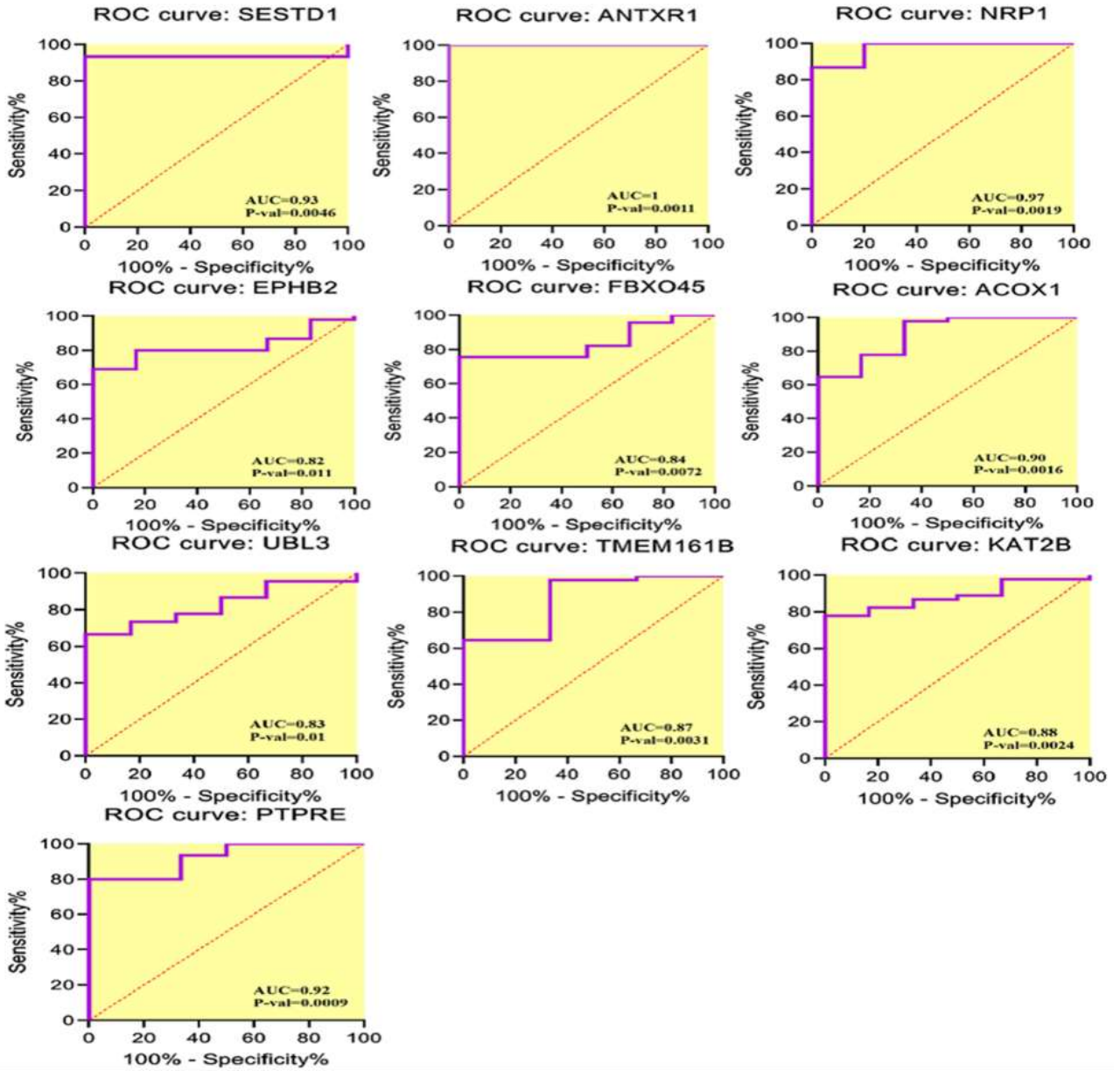

Fig. 4. ROC curve analysis of the top 10 hub genes in the DEG-miR network. Genes with area under the curve (AUC) ≥ 0.9 (including SESTD1, ANTXR1, NRP1, ACOX1, and PTPRE) were selected as potential markers for gastric cancer.

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]

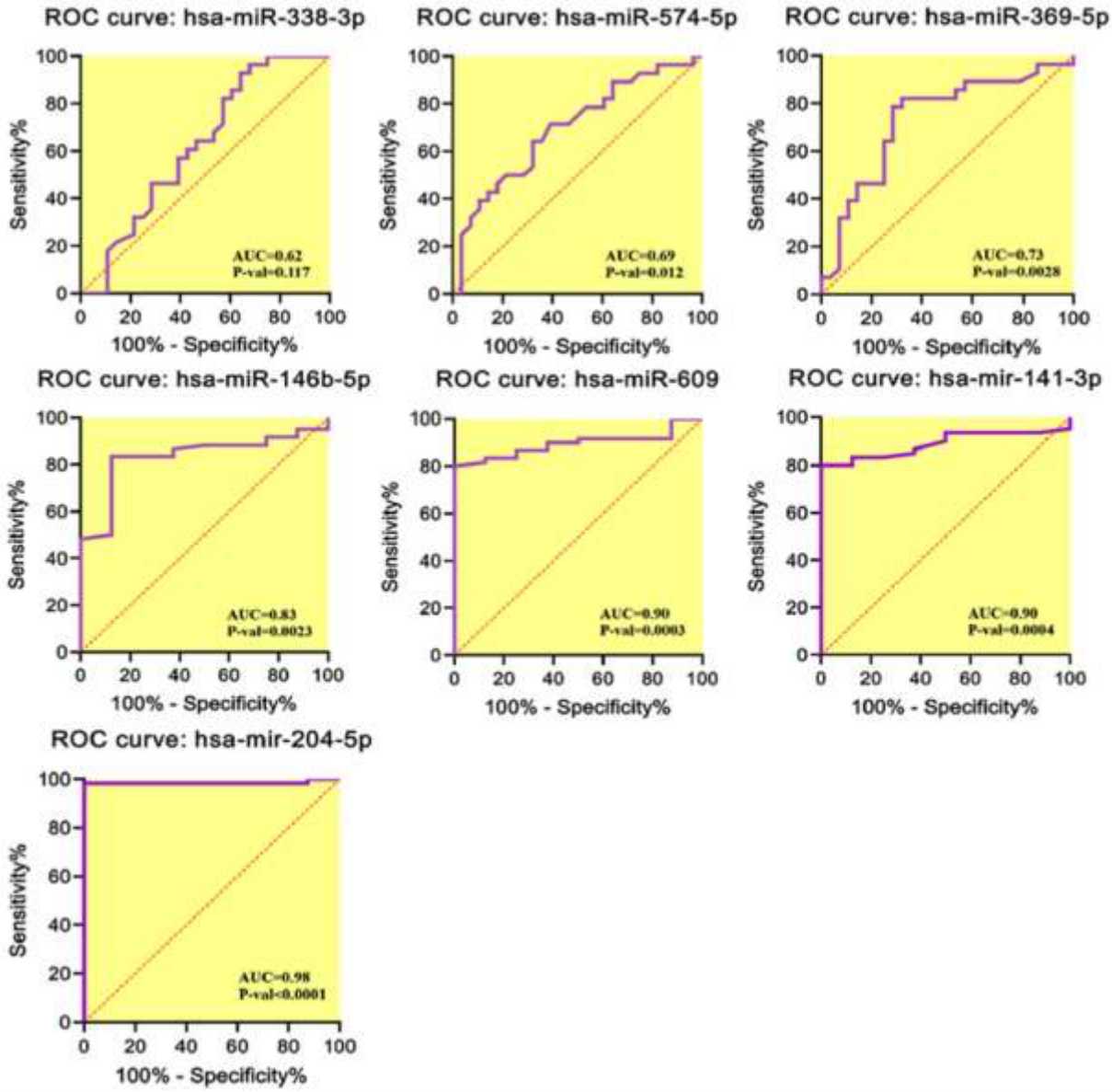

Fig. 5. ROC curve analysis of the top hub miRs in the DEM-mRNA network. miRs with area under the curve (AUC) ≥ 0.9 (including hsa-mir-141-3p, hsa-mir-204-5p, and hsa-miR-609) were selected as potential markers for gastric cancer.

Table 6. A panel of potential biomarkers for gastric cancer diagnosis, based on ROC curve analysis results. Markers with AUC ≥ 0.90 are selected.

Gene Biomarkers			miR Biomarkers		
Gene symbol	**AUC**	**P-value**	**miR ID**	**AUC**	**P-value**
SESTD1	0.93	0.0046	hsa-mir-141-3p	0.90	0.0004
ANTXR1	1	0.0011	hsa-mir-204-5p	0.98	<0.0001
NRP1	0.97	0.0019	hsa-miR-609	0.90	0.0003
ACOX1	0.90	0.0016			
PTPRE	0.92	0.0009			

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]

Survival analysis

The prognostic markers related to overall survival (OS) in gastric cancer are shown in Table 7. To assess the relationship between the proposed biomarkers and OS of gastric cancer patients, survival data was extracted from the main databases including Human Protein Atlas, CancerMIRNome, and Oncomir. Among the putative gene/protein markers, SESTD1 and NRP1 showed a correlation with OS according to Human Protein Atlas (Fig. 6). High expression of both SESTD1 and NRP1 is unfavorable in stomach cancer. This means the OS of patients with stomach cancer decreases as the expression of these two genes goes up. No survival data were found for hsa-mir-141-3p, hsa-mir-204-5p, and hsa-miR-609. Then, we constructed a network of these 3 miRs with their highest- ranked gene targets extracted from miRDB and TarBase databases (Fig. 7). TarBase results were restricted to Homo sapience, gastric and stomach tissues. All the 107 extracted target genes were searched in HPA database to find the target genes with possible correlation with overall survival in gastric cancer. Among the results, 4 genes showed a significant relationship with the survival of gastric cancer patients in HPA, including SPRED1, AM126A, WDFY3, and CDK8 (Fig. 8). These nodes are highlighted yellow in the network. CDK8 shows a desirable correlation, while SPRED1, AM126A, and WDFY3 have an undesirable correlation with survival. Therefore, we proposed a putative panel of prognostic markers related to OS in gastric cancer that is composed of SESTD1, NRP1, SPRED1, AM126A, WDFY3, and CDK8.

Table 7. A panel of potential prognostic biomarkers related to overall survival for gastric cancer, based on HPA results.

Gene symbol	5-year survival (In high expression group)	5-year survival (In low expression group)	P-score
SESTD1	19%	45%	0.00027
NRP1	0%	43%	0.000012
SPRED1	20%	55%	0.000083
AM126A	26%	54%	0.0006
WDFY3	9%	44%	0.00094
CDK8	39%	0%	0.00092

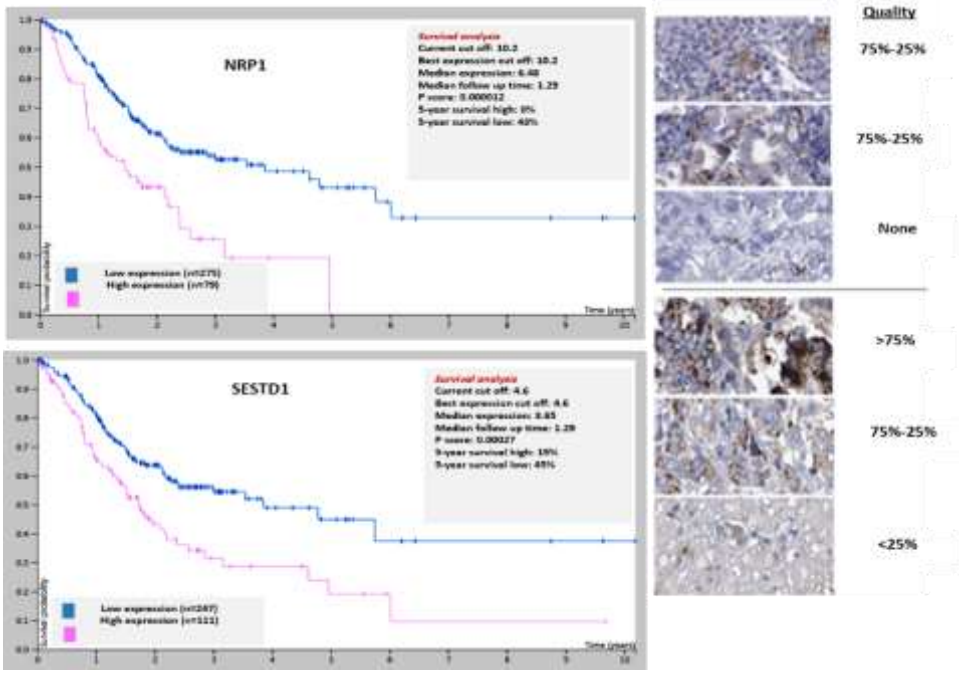

Fig. 6. Kaplan-Meier survival analysis of SESTD1 and NRP1 from the Human Protein Atlas. Expression of both genes shows an unfavorable correlation with survival.

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]

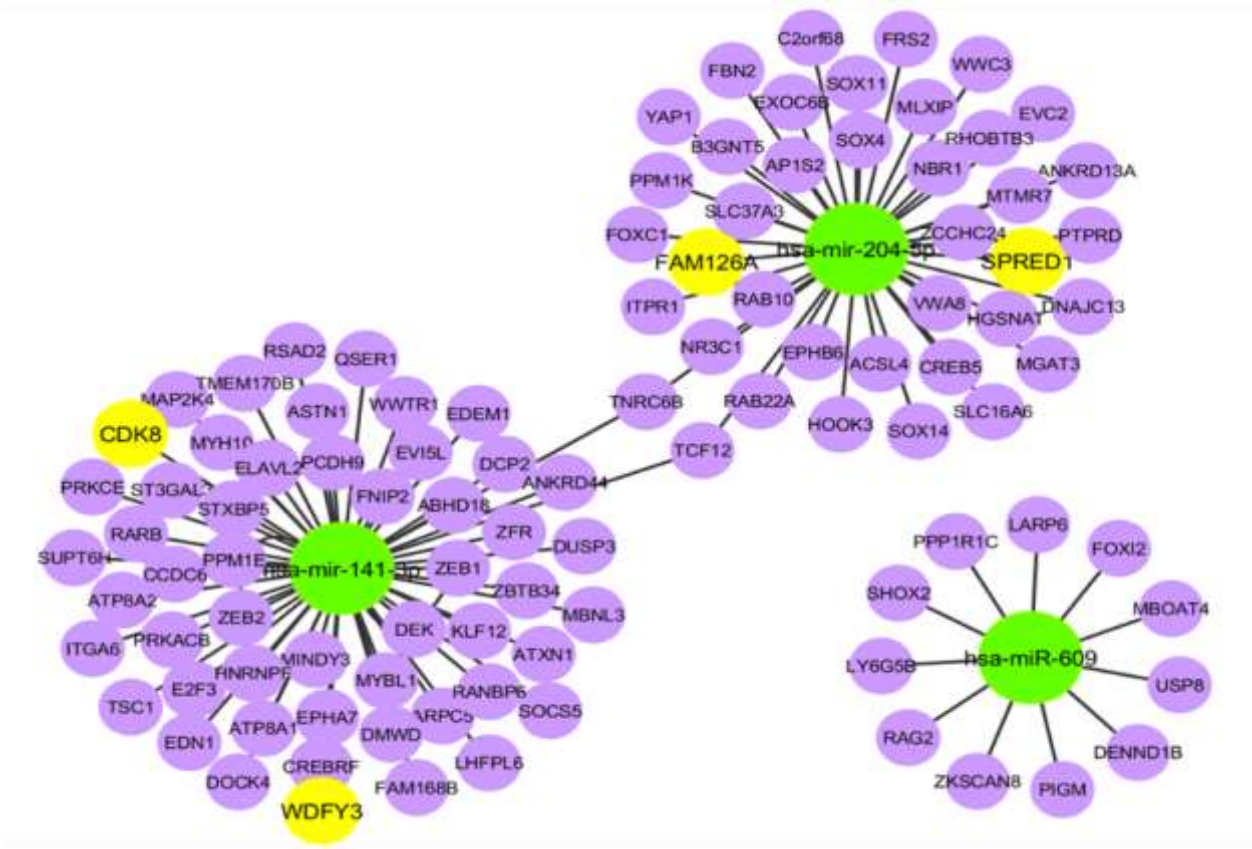

Fig. 7. Target genes of the most significant miRs selected from TarBase and miRDB. Only the top ranked targets are presented. Yellow target nodes show correlation with overall survival in stomach cancer according to Human Protein Atlas.

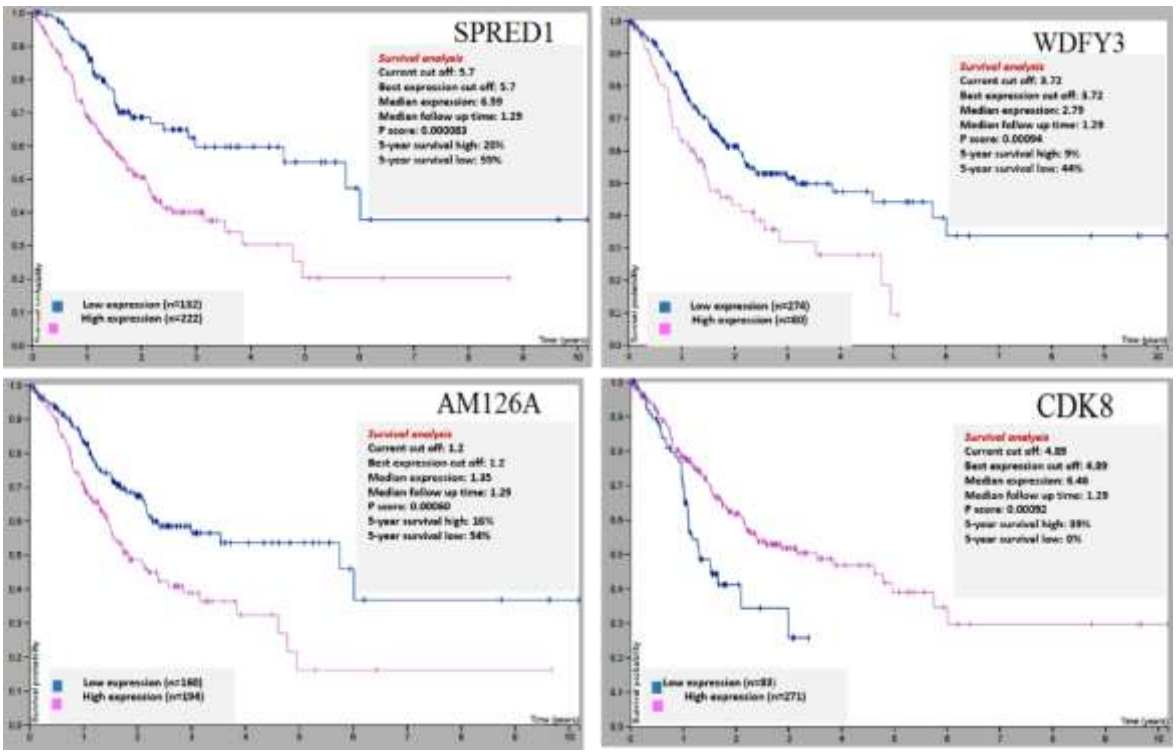

Fig. 8. Kaplan-Meier survival analysis of SPRED1, AM126A, WDFY3, and CDK8 from the Human Protein Atlas. The expression of CDK8 shows a favorable correlation, whereas the expression of SPRED1, AM126A, and WDFY3 shows an unfavourable correlation with survival.

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]

Real-time qPCR Validation

The expression of miR-141-3p, miR-204-5p, SESTD1, and ANTXR1 were evaluated in GC cell lines compared to normal cell lines. The results indicated that the expression levels of miR-141-3p and miR-204-5p were lower in GC cell lines compared with the GES-1 cell line. Both miRs exhibited a greater decrease in SNU-NCC-9 than the MKN74 cell line (Fig. 9). On the other hand, the mRNA of SESTD1 and ANTXR1 were markedly up regulated in GC compared to the normal cell line (Fig. 10).

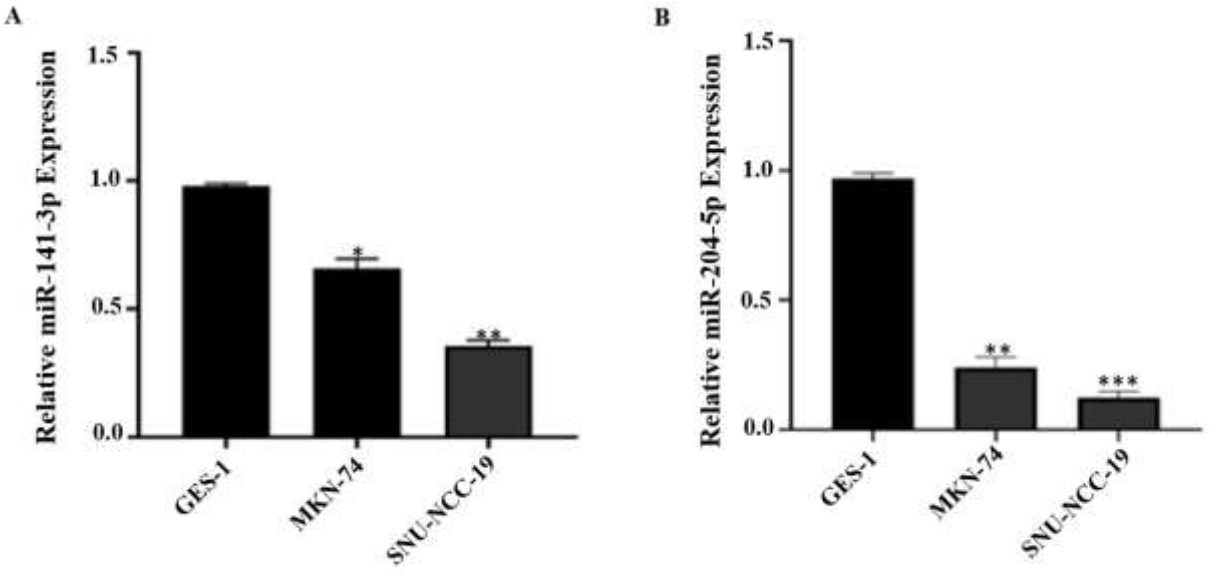

Fig. 9. Expression of miR-141-3p and miR-204-5p in cell lines. A) miR-141-3p expression was lower in GC cell lines compared with the GES-1 normal cell line. B) In the cell lines, miR-204-5p expression was significantly reduced in GC cell lines compared with the GES-1 normal cell line. * p< 0.05, **p< 0.01 and ***p< 0.001 vs. the GES-1 normal cell line. GC: Gastric cancer.

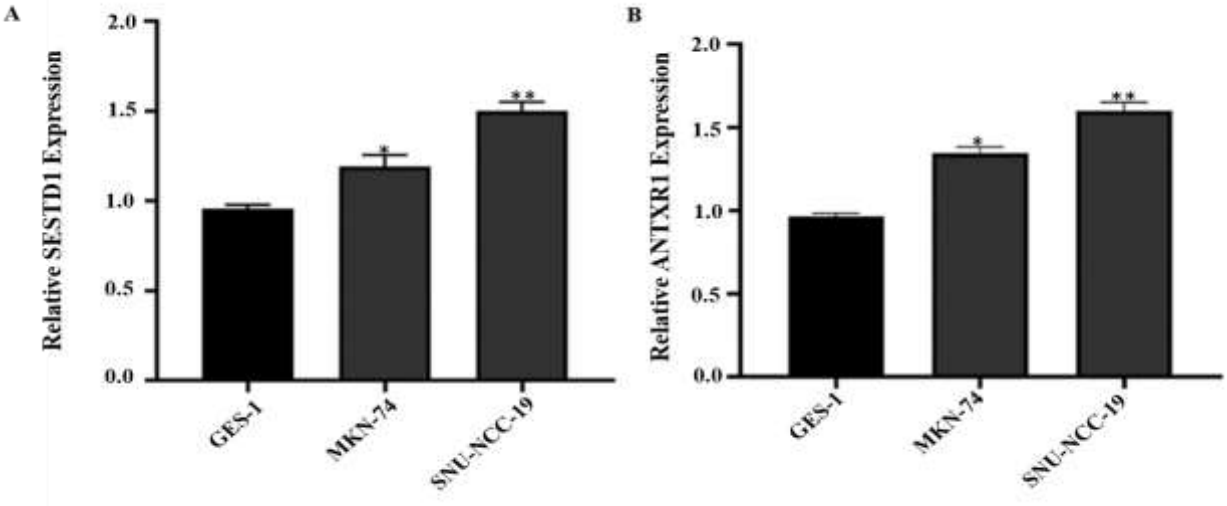

Fig. 10. The expression levels of A) SESTD1 and B) ANTXR1 in gastric cancer cell lines of MKN-74 and SNU-NCC-19 in comparison with the GES-1 gastric normal cell line analyzed by RT-qPCR normalized to β-actin. *p<0.05, **p<0.01.

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]

Discussion

The development and progression of gastric cancer is a multifaceted phenomenon, due to the disruption of proper regulation of gene expression networks in these pathological conditions. Recently, noncoding RNAs (mainly miRNAs) in conjunction with mRNAs, have been recognized as molecular markers for clinical management due to their altered expression patterns in cancer. However, the exact relationship between mRNAs and miRNAs is still elusive because it hasn't been completely elucidated. In this regard, we conducted a bioinformatics analysis to ascertain disease-associated hub DEGs and DEMs, as well as to predict the miRNA-mRNA network in individuals with gastric cancer. Interestingly, GO and KEGG enrichment analysis showed that the most significant pathways were the Notch signalling pathway (p-value: 0.027), apoptosis (p-value: 0.0031), adipocytokines signalling pathway (p-value: 0.013), and p53 signalling pathway (p-value: 0.023). The Notch pathway is a regulator of cell proliferation, differentiation and survival in various tissues and cells. In addition, it is defined as one of the pathways that are frequently activated in the process of cancer development and functions as an oncogenic factor in different types of cancers. Numerous previous studies have demonstrated the importance of the Notch signalling pathway in determining the fate of cells afflicted with gastric cancer. Furthermore, it has been proven that a significant number of the components in this pathway are significantly increased in tissue samples of gastric cancer. In contrast, crosstalk signalling that relates to the Notch pathway and other pathways, such as Wnt, Ras, and NF- B, has been found to promote carcinogenesis in gastric tissue. As a consequence, this leads to an augmentation in cell proliferation and impedes programmed cell death in gastric cancer cells, accordingly promoting the stimulation of blood vessel formation and expediting the process of transitioning from an epithelial to a mesenchymal state (15).

The GO enrichment analysis revealed that the primary biological processes of the genes were involved in the regulation of cellular ketone metabolic process by positive regulation of transcription from RNA polymerase II promoter, regulation of interleukin-12 biosynthetic process, and histone H3-K14 acetylation. Investigations have revealed that the advancement and metastasis of tumors are facilitated by the production and utilization of ketone bodies. Ketone inhibitors have the potential to be innovative therapeutic agents for treating patients with advanced malignancies, as well as those with recurrent and metastatic tumors. In a study carried out by Martinez-Outschoorn, ketone bodies, identified as oncometabolites, were observed to have a significant influence. The authors propose that the enzymes HMGCS2, ACAT1/2, and OXCT1/2 may act as metabolic oncogenes (16). It has been established that dysregulation of ketone metabolism is linked to poor prognosis in certain carcinomas, such as clear cell renal cell carcinoma (17). Our findings confirm that the IL-12 family cytokines play a significant role in regulating the tumor immune contexture. Murakami and his colleagues conducted an assessment of the immunological condition of individuals diagnosed with gastric cancer prior to undergoing surgery, with the aim of investigating the potential correlation between serum IL-12 levels and various clinicopathological factors. They concluded that serum IL-12 levels in patients with far-advanced gastric cancer were significantly lower than patients with less-advanced gastric cancer. This is due to macrophages in patients with far-advanced cancer being chaotic and unable to produce enough IL-12 (18). Histone H3-K14 acetylation was another biological process related to our results. Accumulative data show that epigenetic changes play an important role in the development and progression of various cancers, including gastric cancer (19). Epigenetics refers to changes in the process of gene expression

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]

regulation without mutations and alterations in DNA sequence (19). One of the most common epigenetic events in cancer is histone modification and subsequently chromatin remodelling. Histone modifications regulate the structure of chromatin and thereby alter the transcriptional level of genes (20). Meanwhile, histone acetylation plays critical roles in transcriptional regulation. For example, inactivation of chromatin by histone deacetylation is involved in the transcriptional repression of several tumor suppressor genes such as p21. Mitani et al. carried out a study in order to identify the in vivo characteristics of histone acetylation in gastric carcinoma. Their findings led them to the conclusion that modifications in histone acetylation take place in human cancer tissue samples, including those derived from gastric carcinoma. (21). In the present investigation, we have chosen 10 mRNA and 7 miRNA candidates as plausible biomarkers from the high-throughput profiling investigations of GC, correspondingly. Analysis of the aforementioned hubs indicated that there was a notable upregulation in the expression of five microRNAs, namely miR-141-3p, miR-204-5p, miR-338-3p, miR-609, and miR-369-5p, in comparison to the control group. The highest degree genes included 6 upregulated and 4 downregulated genes in tumors compared to control samples. However, in the validation phase, only SESTD1, ANTXR1, NRP1, ACOX1, PTPRE, hsa-mir-141-3p, hsa-mir-204-5p, and hsa-miR-609 with AUC≥ 0.9 were suggested as potentially diagnostic markers in GC. SESTD1, also known as SEC14 and Spectrin Domain Containing 1, is classified as a Protein Coding gene. Its crucial role lies in potentially serving as the principal docking protein responsible for the regulation of membrane turnover and the assembly of the transient receptor potential channels TRPC4 and TRPC5. In a study, Xia et al., determined the tumorigenic properties of SESTD1 in H1299 cells via CRISPR-Cas9 (22). In the context of gastric cancer, SESTD1 was identified as a gene associated with gastric cancer through the utilization of bioinformatics analysis by Gu et al (23). anthrax toxin receptor 1 (ANTXR1) is a type I transmembrane protein that is encoded by conserved tumor endothelial marker TEM8 gene. ANTXR1 is selectively expressed in tumor vasculature and promotes tumor angiogenesis (24). Several in-vitro and in-vivo analyses clarified that knockdown of ANTXR1 in GC cells significantly inhibited cell proliferation, cell cycle progression, invasion and migration, and tumorigenesis by causing apoptosis. Cai et al found that ANTXR1 promotes gastric cancer progression by activation of the PI3K/AKT/mTOR signalling pathway (25). However, literature findings offered ANTXR1 as a novel oncogene in GC that could be a new diagnostic and therapeutic target, potentially. Neuropilin (NRP)-1 is a transmembrane glycoprotein and co-receptor for vascular endothelial growth factor (VEGF), which is involved in the growth and metastasis of cancer cells via targeting signalling pathways. In the context of gastric cancer (GC), Li et al. conducted a study to evaluate the level of NRP-1 in GC tissue compared to normal gastric tissue (26). The findings demonstrated a higher level of NRP-1 in gastric cancer tissues. Remarkably, the expression of NRP-1 was found to be associated with the stage of cancer. Furthermore, the inhibition of NRP-1 resulted in the suppression of cell proliferation by inducing cell cycle arrest in the G1/S phase and reducing cell migration. Recently, a study has evaluated the correlation between the expression of NRP1 protein and clinicopathological features of gastric cancer by meta-analysis. The authors have concluded that the expression of NRP1 protein in GC is highly correlated with clinical stage, tumor size, TNM stage, differentiation, and lymph node metastasis (27). The findings of the conducted studies have conclusively validated NRP as a potent target for inhibiting angiogenesis and suppressing tumor growth. The ACOX1 gene encodes the enzyme peroxisomal acyl-coenzyme A oxidase 1,

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]

which plays a crucial role in the desaturation of acyl-CoAs to 2-trans-enoyl-CoAs in the fatty acid beta-oxidation pathway in humans. Recently, there has been a significant emphasis towards modified lipid metabolism-associated enzymes as a potentially encouraging approach to anticancer therapy. Li et al. reported lipid metabolism alterations based on the integration of clinical and transcriptomics analysis in gastric cancer (28). PTPRE was the other detected hub gene, which was detected. Proteins encoded by this gene are known as protein tyrosine phosphatases (PTPs). PTPs were considered as tumor suppressors and there is a large volume of evidence that misregulation of PTP activities plays a main role in the progression and metastasis of cancers. In recent years, investigations have elucidated the workings of PTPs in the context of cancer, shedding light on their potential to either promote or suppress tumor growth. These diverse PTPs exert their influence on critical cancer-signalling pathways such as PI3K-AKT and Ras, thus underscoring their significance in cancer research (29). It has been proved that epithelial-mesenchymal transition (EMT) is an important process associated with the invasion, metastasis, and prognosis of malignant cancers (30). On the other hand, miR-141 is one of the miRNAs that have significant variations in expression levels in different types of cancers (31). In this regard, Xiao and colleagues demonstrated that the miR-141-3p ($p<0.05$) possesses the capability to effectively differentiate between high-risk and low-risk collectives, while also serving as a reliable prognostic biomarker for patients diagnosed with gastric cancer (32). Another study has explored the role and possible mechanism of miR-141 in gastric cancer prognosis. The results indicated the potential roles of miR-141 as a prognostic marker and as a novel therapeutic alternative for GC and cleared that this microRNA is associated with Insulin-like growth factor 1 receptor (IGF1R) (33).

miR-204-5p belongs to the miRNA group that exhibits down-regulation and serves as a prospective modulator in a multitude of gastrointestinal malignancies, including gastric and colorectal cancers (34). Based on the expression profiling data derived from gastric tumor tissues and adjacent noncancerous tissues, it can be observed that miR-204-5p is among the highly dysregulated miRNAs, with a notable impact on inhibiting the proliferation of gastric cancer cells. Conversely, it is found that the inhibition of mir-204-5p improves cell proliferation in gastric cancer (35). Zhang and colleagues unveiled that there was a notable reduction in the levels of miR-204-5p in both the tissue and serum samples obtained from patients diagnosed with gastric cancer, particularly those with lymphatic metastasis. Furthermore, utilizing TargetScan analysis, dual luciferase assay, and western blotting analysis, CXCL12 and CXCR4 were predicted and subsequently confirmed as the functional targets of miR-204-5p. Additionally, they ascertained that miR-204-5p inhibits the movement and infiltration in gastric cancer. All of these findings imply novel anti-cancer roles and mechanisms for miR-204-5p in the progression of gastric cancer and offer a fresh prospective diagnostic element and therapeutic objectives for the management of gastric cancer. As an integral component of the microRNAs family, miRNA-338 has been discerned as a significant contributor in a multitude of cellular mechanisms, including but not limited to tumorigenesis. To illustrate, there have been documented instances of miRNA-338–3p downregulation in gastric cancer tissues, which exhibits an inverse correlation with advanced clinical stage and lymph node infiltration (36). In a separate investigation, Guo et al. additionally discovered that miRNA-338-3p exhibits suppressive properties on cellular proliferation in an in vitro environment and tumorigenicity in an in-vivo setting through a PTEN-AKT axis by specifically targeting P-REX2a (37). Furthermore, it has been demonstrated that the proliferation, infiltration, and spreading of

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]

gastric cancer are suppressed by miRNA-338–3p through its interaction with key signalling cascades such as PTP1B, NRP1, EphA2, and the Wnt/β-catenin pathway (38). However, miRNA-338 exhibits a precise function of inhibiting tumor growth during the onset and advancement of gastric cancer, thereby presenting itself as a prospective therapeutic target. Our research aligns with previous investigations on cancer, which have identified miR-609 as a biomarker. Pertaining to this matter, miR-609 has been discerned as a circular miRNA that is downregulated in colon cancer patients when compared to healthy control subjects, as revealed by a microarray study (39). Another miRNA that has been identified as having a significant function in gastric cancer is miR-369-5p. In a study, Hu et al. reported that miR-369 involves in cellular reprogramming and regulation of malignant phenotypes of colorectal cancer (40).

In conclusion, gastric cancer biomarkers play a crucial role in early detection, as they can help identify the presence of cancer at an early stage when it is more treatable. Furthermore, these biomarkers can assist in predicting the aggressiveness of the cancer, determining the most effective treatment methods, and monitoring the progression of the disease. With the aid of biomarker analysis, healthcare providers can tailor personalized treatment plans for patients, leading to improved outcomes and enhanced quality of life. The intricate interplay between various biomarkers and their correlation with gastric cancer progression is a captivating area of research. The present study has identified a panel of potential miRNAs and genes/proteins that could be offered as potential biomarkers for the diagnosis of GC in the early stages besides predicting survival in these patients. The analysis of the expression of the central nodes revealed that five miRNAs, namely, miR-141-3p, miR-204-5p, miR-338-3p, miR-609, and miR-369-5p, were significantly upregulated in comparison to the controls. In vitro evaluation of the expression of miR-141-3p, miR-204-5p, SESTD1, and ANTXR1 was performed using these central differentially expressed miRNAs and genes. The results indicated a decrease in the expression of miR-141-3p and miR-204-5p in gastric cancer cell lines when compared to the GES-1 cell line. Conversely, the mRNA expression of SESTD1 and ANTXR1 was significantly up-regulated in GC in comparison to the normal cell line. The results of our study offer a novel perspective that has the potential to enhance the utilization of miRs and genes/proteins as prognostic and early diagnostic biomarkers in GC within a clinical setting. However, it is important to note that further validation using larger sample sizes is necessary. From aiding in early detection to guiding treatment decisions, biomarkers significantly impact patient care and outcomes. As research in this field continues to evolve, the outlook for gastric cancer patients is increasingly optimistic, emphasizing the critical importance of biomarkers in combating this complex and challenging condition.

Acknowledgment

We thank the Proteomics Research Center of Shahid Beheshti University of Medical Sciences that supported this study.

Conflict of interest

The authors declare that they have no conflict of interest.

Financial support

This study was financially supported by grant (No: 31872) from Shahid Beheshti University of Medical Sciences.

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]

References

1. Morgan E, Arnold M, Camargo MC, Gini A, Kunzmann AT, Matsuda T, et al. The current and future incidence and mortality of gastric cancer in 185 countries, 2020-40: A population-based modelling study. EClinicalMedicine. 2022;47:101404.
2. Nooh M, Hakemi-Vala M, Nowroozi J, Fatemi S-R, Dezfulian M. Prediction of blood miRNA-mRNA regulatory network in gastric cancer. Rep Biochem Mol Biol. 2021;10(2):243-256.
3. Sousa JF, Ham AJ, Whitwell C, Nam KT, Lee HJ, Yang HK, et al. Proteomic profiling of paraffin-embedded samples identifies metaplasia-specific and early-stage gastric cancer biomarkers. Am J Pathol. 2012;181(5):1560-72.
4. Necula L, Matei L, Dragu D, Neagu AI, Mambet C, Nedeianu S, et al. Recent advances in gastric cancer early diagnosis. World J Gastroenterol. 2019 7;25(17):2029-2044.
5. Mahmoudivar S, Zarredar H, Asadi M, Zafari V, Hashemzadeh S, Farzaneh R, Asvadi Kermani T. Serum miR-23 and miR-150 Profiles as Biomarkers for Predicting Recurrence following Surgical Intervention in Colorectal Cancer Patients. Rep Biochem Mol Biol. 2024;12(4):540-549.
6. Jelski W, Mroczko B. Molecular and Circulating Biomarkers of Gastric Cancer. Int J Mol Sci. 2022;23(14):7588.
7. Xiao B, Liu Z, Li BS, Tang B, Li W, Guo G, et al. Induction of microRNA-155 during Helicobacter pylori infection and its negative regulatory role in the inflammatory response. J Infect Dis. 2009;200(6):916-25.
8. Liu X, Ma R, Yi B, Riker AI, Xi Y. MicroRNAs are involved in the development and progression of gastric cancer. Acta Pharmacol Sin. 2021;42(7):1018-1026.
9. Amiri-Dashatan N, Koushki M, Naghi-Zadeh M, Razzaghi MR, Mohaghegh Shalmani H. Prognostic value of microRNA-125a/b family in patients with gastric cancer: a meta-analysis. Gastroenterol Hepatol Bed Bench. 2021;14(Suppl1):S1-S9.
10. Amiri-Dashatan N, Koushki M, Jalilian A, Ahmadi NA, Rezaei-Tavirani M. Integrated Bioinformatics Analysis of mRNAs and miRNAs Identified Potential Biomarkers of Oral Squamous Cell Carcinoma. Asian Pac J Cancer Prev. 2020;21(6):1841-1848.
11. Amiri-Dashatan N, Koushki M, Rezaei Tavirani M, Ahmadi NA. Protein-Protein Interaction Network Analysis of Salivary Proteomic Data in Oral Cancer Cases. Asian Pac J Cancer Prev. 2018;19(6):1639-1645.
12. Amiri-Dashatan N, Yekta Farrokhi R, Rostami Nejad M, Nikzamir A. Pathway and network analysis in primary open angle glaucoma. Arc Adv Biosci. 2014;5(3): 92-101.
13. Amiri-Dashatan N, Koushki M, Abbaszadeh HA, Rostami-Nejad M, Rezaei-Tavirani M. Proteomics Applications in Health: Biomarker and Drug Discovery and Food Industry. Iran J Pharm Res. 2018;17(4):1523-1536.
14. Amiri-Dashatan N, Yekta RF, Koushki M, Arefi Oskouie A, Esfahani H, Taheri S, Kazemian E. Metabolomic study of serum in patients with invasive ductal breast carcinoma with LC-MS/MS approach. Int J Biol Markers. 2022;37(4):349-359.
15. Yao Y, Ni Y, Zhang J, Wang H, Shao S. The role of Notch signalling in gastric carcinoma: molecular pathogenesis and novel therapeutic targets. Oncotarget. 2017;8(32):53839-53853.
16. Martinez-Outschoorn UE, Lin Z, Whitaker-Menezes D, Howell A, Sotgia F, Lisanti MP. Ketone body utilization drives tumor growth and metastasis. Cell Cycle. 2012;11(21):3964-71.
17. Cui W, Luo W, Zhou X, Lu Y, Xu W, Zhong S, et al. Dysregulation of Ketone Body Metabolism Is Associated With Poor Prognosis for Clear Cell Renal Cell Carcinoma Patients. Front Oncol. 2019;9:1422.
18. Murakami S, Okubo K, Tsuji Y, Sakata H, Hamada S, Hirayama R. Serum interleukin-12 levels in patients with gastric cancer. Surg Today. 2004;34(12):1014-9.
19. Puneet, Kazmi HR, Kumari S, Tiwari S, Khanna A, Narayan G. Epigenetic Mechanisms and Events in Gastric Cancer-Emerging Novel Biomarkers. Pathol Oncol Res. 2018;24(4):757-770.
20. Michalak EM, Burr ML, Bannister AJ, Dawson MA. The roles of DNA, RNA and histone

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]

methylation in ageing and cancer. Nat Rev Mol Cell Biol. 2019;20(10):573-589.
21. Mitani Y, Oue N, Hamai Y, Aung PP, Matsumura S, Nakayama H, et al. Histone H3 acetylation is associated with reduced p21(WAF1/CIP1) expression by gastric carcinoma. J Pathol. 2005;205(1):65-73.
22. Xia L, Wang Z, Wu X, Zeng T, Luo W, Hu X, et al. Multiplatform discovery and regulatory function analysis of structural variations in non-small cell lung carcinoma. Cell Rep. 2021;36(10):109660.
23. Gu W, Sun Y, Zheng X, Ma J, Hu XY, Gao T, Hu MJ. Identification of Gastric Cancer-Related Circular RNA through Microarray Analysis and Bioinformatics Analysis. Biomed Res Int. 2018;2018:2381680.
24. Chaudhary A, Hilton MB, Seaman S, Haines DC, Stevenson S, Lemotte PK, et al. TEM8/ANTXR1 blockade inhibits pathological angiogenesis and potentiates tumoricidal responses against multiple cancer types. Cancer Cell. 2012;21(2):212-26.
25. Cai C, Dang W, Liu S, Huang L, Li Y, Li G, et al. Anthrax toxin receptor 1/tumor endothelial marker 8 promotes gastric cancer progression through activation of the PI3K/AKT/mTOR signalling pathway. Cancer Sci. 2020;111(4):1132-1145.
26. Li L, Jiang X, Zhang Q, Dong X, Gao Y, He Y, et al. Neuropilin-1 is associated with clinicopathology of gastric cancer and contributes to cell proliferation and migration as multifunctional co-receptors. J Exp Clin Cancer Res. 2016;35:16.
27. Cao H, Li Y, Huang L, Bai B, Xu Z. Clinicopathological Significance of Neuropilin 1 Expression in Gastric Cancer: A Meta-Analysis. Dis Markers. 2020;2020:4763492.
28. Li Y, Zhao J, Chen R, Chen S, Xu Y, Cai W. Integration of clinical and transcriptomics reveals programming of the lipid metabolism in gastric cancer. BMC Cancer. 2022;22(1):955.
29. Sivaganesh V, Sivaganesh V, Scanlon C, Iskander A, Maher S, Lê T, Peethambaran B. Protein Tyrosine Phosphatases: Mechanisms in Cancer. Int J Mol Sci. 2021;22(23):12865.
30. Javdani H, Mollaei H, Karimi F, Mahmoudi S, Farahi A, Mirzaei-Parsa MJ, Shahabi A. Review article epithelial to mesenchymal transition-associated microRNAs in breast cancer. Mol Biol Rep. 2022;49(10):9963-9973.
31. Ardila HJ, Sanabria-Salas MC, Meneses X, Rios R, Huertas-Salgado A, Serrano ML. Circulating miR-141-3p, miR-143-3p and miR-200c-3p are differentially expressed in colorectal cancer and advanced adenomas. Mol Clin Oncol. 2019;11(2):201-207.
32. Xiao J, Zhang F, Liu W, Zang W. Construction of epithelial-mesenchymal transition related miRNAs signatures as prognostic biomarkers in gastric cancer patients. bioRxiv. 2022:2022.09. 02.506322.
33. Huang M, Wu L, Qin Y, Li Z, Luo S, Qin H, et al. Anti-proliferative role and prognostic implication of miR-141 in gastric cancer. Am J Transl Res. 2016;8(8):3549-57.
34. Zhang B, Yin Y, Hu Y, Zhang J, Bian Z, Song M, et al. MicroRNA-204-5p inhibits gastric cancer cell proliferation by downregulating USP47 and RAB22A. Med Oncol. 2015;32(1):331.
35. Liu X, Pu K, Wang Y, Chen Y, Zhou Y. Gastric cancer-associated microRNA expression signatures: integrated bioinformatics analysis, validation, and clinical significance. Ann Transl Med. 2021;9(9):797.
36. Liu S, Suo J, Wang C, Sun X, Wang D, He L, et al. Downregulation of tissue miR-338-3p predicts unfavorable prognosis of gastric cancer. Cancer Biomark. 2017;21(1):117-122.
37. Guo B, Liu L, Yao J, Ma R, Chang D, Li Z, et al. miR-338-3p suppresses gastric cancer progression through a PTEN-AKT axis by targeting P-REX2a. Mol Cancer Res. 2014;12(3):313-21.
38. Sun F, Yu M, Yu J, Liu Z, Zhou X, Liu Y, et al. miR-338-3p functions as a tumor suppressor in gastric cancer by targeting PTP1B. Cell Death Dis. 2018;9(5):522.
39. Wang YN, Chen ZH, Chen WC. Novel circulating microRNAs expression profile in colon cancer: a pilot study. Eur J Med Res. 2017;22(1):51.
40. Hu YJ, Zhang JY, Luo Q, Xu JR, Yan Y, Mu LM, et al. Nanostructured Dihydroartemisinin Plus Epirubicin Liposomes Enhance Treatment Efficacy of Breast Cancer by Inducing Autophagy

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]

and Apoptosis. Nanomaterials (Basel). 2018;8(10):804.
41. Wang Q, Wen YG, Li DP, Xia J, Zhou CZ, Yan DW, et al. Upregulated INHBA expression is associated with poor survival in gastric cancer. Med Oncol. 2012;29(1):77-83.
42. Canu V, Sacconi A, Lorenzon L, Biagioni F, Lo Sardo F, Diodoro MG, et al. MiR-204 down-regulation elicited perturbation of a gene target signature common to human cholangiocarcinoma and gastric cancer. Oncotarget. 2017;8(18):29540-29557.
43. Nanki K, Toshimitsu K, Takano A, Fujii M, Shimokawa M, Ohta Y, et al. Divergent Routes toward Wnt and R-spondin Niche Independency during Human Gastric Carcinogenesis. Cell. 2018;174(4):856-869.e17.
44. Lim JY, Yoon SO, Seol SY, Hong SW, Kim JW, Choi SH, et al. Overexpression of miR-196b and HOXA10 characterize a poor-prognosis gastric cancer subtype. World J Gastroenterol. 2013;19(41):7078-88.
45. Yu B, Lv X, Su L, Li J, Yu Y, Gu Q, et al. MiR-148a Functions as a Tumor Suppressor by Targeting CCK-BR via Inactivating STAT3 and Akt in Human Gastric Cancer. PLoS One. 2016;11(8):e0158961.

[Downloaded from old.rbmb.net on 2026-06-21]

[DOI: 10.61186/rbmb.13.2.281]