Identification of mRNA-miRNA-lncRNA Competing Endogenous RNA Network in Adrenocortical Carcinoma Using Bioinformatics Analysis

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Abstract

Background: Adrenocortical carcinoma (ACC) is a rare malignant tumor originating from the adrenal cortex. However, there are no effective therapies to treat patients with ACC. LncRNA participates in a variety of biological processes of cancers. We constructed ceRNA network and identify key competing endogenous RNAs (ceRNAs) in adrenocortical carcinoma (ACC) using bioinformatic processing tools.

Methods: Firstly, the differentially expressed genes (DEGs) were identified by analyzing GSE12368 and GSE19750 datasets. SangerBox was used to generate volcano maps. DAVID database was used for functional enrichment analysis. STRING database was used to conduct Protein-protein interaction (PPI) network, and hub genes were identified by Cytoscape plug-in CytoHubba. UCSC database was used to construct hierarchical clustering of hub genes. Upstream miRNAs of mRNAs were predicted by miRTarBase and upstream lncRNAs of miRNA by miRNet. Expression analysis for lncRNAs were performed via GEPIA. Prognostic analysis for genes, miRNAs and lncRNAs were performed via cBioPortal, OncomiR and GEPIA, respectively.

Results: In this study, 49 and 276 upregulated and downregulated significant DEGs were identified. KEGG pathway enrichment analysis showed that they were significantly enriched in cancer-associated pathways. According to node degree, the top 10 upregulated genes and downregulated genes were classfied as hub genes. However, only 9 hub genes were defined as key genes because alteration was significantly associated with worse prognosis and all the 9 key genes were upregulated hub genes. Then, 15 miRNAs were predicted to target the 7 out of 9 key genes. But only 4 miRNAs were defined as key miRNAs because alteration significantly influenced prognosis in cancer. 185 lncRNAs were predicted to potentially interaction with the 4 miRNAs. Only 3 lncRNAs(XIST, HOXA11-AS and TMPO-AS1) were up-regulated and only 1 lncRNA (HOXA11-AS) indicated alteration was significantly associated with worse prognosis in adrenocortical carcinoma. HOXA11-AS were finally identified as key lncRNA. Finally, RRM2-miR-24-3p/let-7a-5p-HOXA11-AS, CDK1/MCM4-miR-24-3P-HOXA11-AS competing endogenous RNA (ceRNA) sub-networks were constructed in adrenocortical carcinoma.

Conclution This study has constructed RRM2-miR-24-3p/let-7a-5p-HOXA11-AS, CDK1/MCM4-miR-24-3p-HOXA11-AS competing endogenous RNA (ceRNA) sub-networks. Our results suggested that these sub-networks might be potential therapeutic targets or prognostic biomarkers in ACC.

Background

Adrenocortical carcinoma (ACC) is a rare, strong invasiveness and poor prognosis malignant tumor that occurs in the adrenal cortex(1, 2). According to a report based on the Surveillance, Epidemiology, and End Results (SEER) database, the annual incidence of ACC is approximately 0.72 per million cases, causing to 0.2% of all cancer deaths in the United States(3). Its 5-year overall survival rate is still less than 40%(4). In addition, adult ACC patients own higher malignancy than children(5). Currently, there are no effective therapies to treat patients with adrenocortical carcinoma. The first-line treatment for ACC is still surgical

removal(6). But, nearly 50% patients have recurrence 6–24 months after surgery(7). Therefore, it is crucial to explore molecular mechanisms and develop effective therapeutic targets and discover prognostic biomarkers for ACC.

LncRNAs are recognized as RNA transcripts with more than 200 nucleotides and no protein-coding capacity(8, 9). Some studies showed that lncRNA participates in a variety of biological processes. Changes in the function or expression of lncRNA are associated with many diseases, including cancer(10–12). In addition, aberrant expression of long noncoding RNAs (lncRNAs) have been found in pathological specimens of ACC(13). Therefore, identifying lncRNAs associated with ACC and exploring their interactions with protein-coding genes are essential to explore molecular mechanisms and develop effective therapeutic targets for ACC.

MiRNAs are also a type of non-coding RNAs with the length of 20–22nt(14). MiRNAs negatively regulate the gene expression by binding to the 3'-untranslated region (UTR) of the target mRNA transcripts, and subsequently triggers mRNA degradation or protein translation inhibition(15, 16). Aberrantly expressed miRNAs acted as oncogenes or tumor suppressors to regulate the tumor progression by changing the expression of proteins(17–19). Similarly, aberrant expression of miRNAs have been found in pathological specimens of ACC(20). Therefore, identifying miRNAs associated with ACC is also of great significance.

In 2011, a new regulatory mechanism between non-coding RNA (ncRNA) and messenger RNA (mRNA) was proposed, namely the hypothesis of competing endogenous RNA (ceRNA)(21). In this hypothesis, lncRNA competes with mRNA to bind miRNA to form a sponge-like structure, buffering and reducing the ability of miRNA to interfere with the protein encoded by the target gene (22). Increasing evidences has proved that the lncRNA-miRNA-mRNA ceRNA network plays a key role in a variety of human cancers, such as breast cancer(23), gastric cancer(24), liver cancer(25) and pancreatic cancer(26).

In this study, we first acquired common differentially expressed genes (DEGs) by analyzing two GEO datasets. Subsequently, we performed functional enrichment analysis for these DEGs. Then, protein-protein interaction analysis was also performed, and hub genes were identified. By evaluating prognostic roles of hub genes in adrenocortical carcinoma, the key genes were selected for subsequent analysis. Next, upstream miRNAs of the key genes were predicted. In addition, we also further evaluated the prognostic roles of these miRNAs in adrenocortical cancer and the key miRNAs were identified. Then upstream lncRNAs of the key miRNA were predicted. we also further evaluated the prognostic roles and expresstion of these lncRNAs in adrenocortical carcinoma. Finally, some new ceRNA regulatory sub-networks related to the prognosis of adrenocortical carcinoma patients were successfully established. They may serve as promising diagnostic biomarkers or therapeutic targets for adrenocortical carcinoma in the future.

Materials And Methods

Microarray data

Firstly, we searched the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) to compare gene expression datasets between adrenocortical carcinoma tissues and normal tissues. The titles and abstracts of these datasets were screened and full information of the datasets of interest were further evaluated. Finally, only two datasets (GSE12368 and GSE19750), based on the platform of Affymetrix Human Genome U133 Plus 2.0 Array (GPL570), were selected for subsequent analyses. GSE12368 dataset contained 12 adrenocortical carcinoma samples, 16 adrenocortical adenomas samples and 6 normal samples, and GSE19750 dataset contained 44 adrenocortical carcinoma samples and 4 normal samples.The volcano maps of two datasets (GSE12368 and GSE19750) is generated by the volcano drawing tool of SangerBox.

Differential expression analysis

The online analysis tool GEO2R (https://www.ncbi. nlm.nih.gov/geo/geo2r), provided by the GEO database, was utilized to obtain DEGs from the two datasets. |log2FC| > 1 and adjusted p-value (adj. p-value) < 0.05 were set as the cut-off criteria for differential expression analysis. In addition, we used the online tool, Bioinformatics Evolutionary Genomics (http://bioinformatics.psb.ugent.be/webtools/Venn) to generate the Venn diagrams. The DEGs that were commonly appeared in both GSE12368 and GSE19750 datasets were redefined as the significant DEGs, including upregulated significant DEGs and downregulated significant DEGs.

Gene ontology and KEGG pathway enrichment analysis

We used the online tool, Database for Annotation, Visualization, and Integrated Discovery (https://david.ncifcrf.gov) to conduct Gene Ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. p-value < 0.05 was considered as statistically significant. Then, the top 3 enriched GO terms and KEGG pathways were displayed.

Protein-protein interaction (PPI) network

The PPI interaction networks between the DEGs were constructed by Search Tool for the Retrieval of Interacting Genes (STRING) database (http://stringdb.org/)(27). Only these interactors with combined confidence score > = 0.4 were shown in the bitmap.

Identification and analysis of hub genes

The hub genes in the PPI networks were identified using CytoHubba, a plugin in Cytoscape software (Version 3.7.2). According to node degree, the top 10 hub genes were displayed in the Cytoscape software (Version 3.7.2). Hierarchical clustering of hub genes was constructed using UCSC Cancer Genomics Browser (http:// genome-cancer.ucsc.edu)

Prognostic analysis

In terms of prognostic values in adrenocortical carcinoma, genes were analyzed using cBioPortal database, miRNAs were analyzed using OncomiR database and lncRNAs were analyzed using GEPIA database. The hazard ratio (HR) with 95% confidence interval and logrank p-value were automatically

calculated and directly displayed on the webpage. Logrank p-value < 0.05 was regarded as statistically significant. Those statistically significant genes, miRNAs and lncRNAs were used for following analyses

Prediction of miRNA

Upstream miRNAs of key genes were predicted using miRTarbase database(28). In miRTarbase database, the collected microRNA-target interactions are experimentally validated by reporter assay, western blot, qPCR, microarray and next-generation sequencing experiments. To obtain more accurate prediction results, in this study, we only included microRNA-target interactions that were validated by strong evidence (reporter assay, western blot, qPCR).

Prediction and expression analysis of lncRNA

Then, we predicted the upstream lncRNAs of miRNAs using miRNet database(29, 30).“Organism-H.sapiens”, “ID type-miRBase ID”,“Tissue-Not specified” and “target type-lncRNAs” were set as selection criteria. The expression of lncRNA in adrenocortical carcinoma were analyzed using Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancerpku.cn/detail.php). Genes with |log2FC| > 1 and p-value < 0.05 were considered as statistically significant.

Results

Significant DEGs in Adrenocortical Carcinoma

By searching gene expression microarrays regarding adrenocortical carcinoma in the GEO database, two datasets (GSE12368 and GSE19750) were finally included. Subsequently, GEO2R was utilized to obtain the differentially expressed genes (DEGs) from the two datasets (|log2FC| > 1 and adj. pvalue < 0.05). These DEGs from GSE12368 and GSE19750 datasets were shown in Fig. 1A and Fig. 1B, respectively. Then, we redefined some DEGs which were commonly appeared in the two datasets as the significant DEGs. As shown in Fig. 1C and Fig. 1D, a total of 49 and 276 upregulated and downregulated significant DEGs in adrenocortical carcinoma were identified. These upregulated and downregulated significant DEGs were also listed in Table S1 and Table S2, respectively. These significant DEGs were selected for subsequent analyses.

Functional enrichment analysis for the significant DEGs

Subsequently, we used DAVID database to perform functional enrichment analysis, including three GO terms (BP: biological process; CC: cellular component; MF: molecular function) and KEGG pathway. For upregulated significant DEGs, as showed in Table 1, the enriched GO functions included cell division,G1/S transition of mitotic cell cycle and regulation of signal transduction by p53 class mediator in the BP category; protein binding, protein kinase binding and histone kinase activity in the MF category; and nucleus, nucleoplasm and cytoplasm in the CC category. Besides, Table 1 revealed that these upregulated significant DEGs were significantly enriched in some cancer-associated pathways, such as pathways in Cell cycle, p53 signaling pathway and Progesterone-mediated oocyte maturation.

Table 1
GO and KEGG pathway enrichment analysis of DEGs in ACC samples.

Term	Description	Count in gene set	P-value
Upregulated			
GO:0051301	cell division	10	4.98E-08
GO:0000082	G1/S transition of mitotic cell cycle	6	2.80E-06
GO:1901796	regulation of signal transduction by p53 class mediator	6	7.30E-06
GO:0005515	protein binding	31	0.013849
GO:0019901	protein kinase binding	6	0.002563
GO:003517	histone kinase activity	2	0.010151
GO:0005634	nucleus	31	2.84E-08
GO:0005654	nucleoplasm	24	3.60E-09
GO:0005737	cytoplasm	22	0.002791
hsa04110	Cell cycle	6	4.56E-06
hsa04115	p53 signaling pathway	4	3.70E-04
hsa04914	Progesterone-mediated oocyte maturation	4	7.97E-04
Downregulated			
GO:0007165	signal transduction	27	0.008208
GO:0007155	cell adhesion	16	0.001567
GO:0030198	extracellular matrix organization	13	1.56E-05
GO:0005515	protein binding	130	0.046226
GO:0005509	calcium ion binding	22	5.55E-04
GO:0005102	receptor binding	10	0.045281
GO:0005886	plasma membrane	77	6.34E-04
GO:0070062	extracellular exosome	53	0.005591
GO:0005576	extracellular region	46	8.94E-07
hsa05200	Pathways in cancer	13	0.00959

GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes; ACC, adrenocortical carcinoma.

Term	Description	Count in gene set	P-value
hsa04015	Rap1 signaling pathway	11	7.62E-04
hsa05205	Proteoglycans in cancer	10	0.002085

GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes; ACC, adrenocortical carcinoma.

In addition, for downregulated significant DEGs, the enriched GO functions included signal transduction, cell adhesion and extracellular matrix organization in the BP category; protein binding, calcium ion binding and receptor binding in the MF category; and plasma membrane, extracellular exosome and extracellular region in the CC category. Similarly, these downregulated significant DEGs were significantly enriched in some cancer-associated pathways, such as Pathways in cancer, Rap1 signaling pathway and Proteoglycans in cancer(Table 1).

Construction and analysis of PPI network

As shown in Fig. 2A and Fig. 2C, PPI networks of the upregulated significant DEGs and downregulated significant DEGs were constructed using STRING database, respectively. According to node degree, the top 10 hub genes were displayed in the Cytoscape software (Fig. 2B,D). Additionally, the top 10 hub genes and their corresponding node degrees were listed in Table 2. The top 10 upregulated hub genes were CDK1,CCNB1,MCM4、MAD2L1、AURKA、NCAPG、RRM2、TYMS、TPX2 and SMC4 and top 10 downregulated hub genes were IGF1、DCN、CXCL12、TLR4、HGF、LUM、ADCY2、FMOD、CAT and MC2R. The 20 hub genes were chosen for following analyses.

Table 2
The top 10 hub genes in PPI networks.

Upregulated gene		**Downregulated gene**	
Gene symbol	Degree	Gene symbol	Degree
CDK1	24	IGF1	24
CCNB1	23	DCN	18
MCM4	22	CXCL12	16
MAD2L1	21	TLR4	13
AURKA	20	HGF	12
NCAPG	20	LUM	12
RRM2	19	ADCY2	12
TYMS	18	FMOD	11
TPX2	18	CAT	11
SMC4	18	MC2R	10

Hub gene clustering and functional analysis

Hierarchical clustering of hub genes was constructed using UCSC Cancer Genomics Browser. As showed in the cluster heat map(Fig. 2E), the hub genes have significant differences in pathologic stage. In addition, the gene MC2R of top10 downregulated hub genes in this study is shown as upregulated. The names, abbreviations and functions for these hub genes are shown in Table 3.

Table 3
Functional roles of 20 hub genes.

No.	Gene symbol	Full name	Function
1	CDK1	Cyclin-dependent kinase 1	CDK1 can regulate the cell cycle progression, apoptosis and carcinogenesis of tumor cells
2	CCNB1	Cyclin-B1	Essential for the control of the cell cycle at the G2/M (mitosis) transition.
3	MCM4	Minichromosome maintenance complex component 4	MCM4 involve in DNA replication initiation and elongation
4	MAD2L1	Mitotic arrest deficient 2 like 1	MAD2L1 promotes the proliferation of gastric cancer cells AGS and BGC-823
5	AURKA	Aurora kinase A	AURKA promotes the proliferation of various cancer cells
6	NCAPG	Non-SMC condensin I complex subunit G	NCAPG promotes the growth and metastasis of liver cancer
7	RRM2	Ribonucleotide reductase regulatory subunit M2	RRM2 promotes the proliferation and invasion of various cancer cells
8	TYMS	Thymidylate synthetase	TYMS expression promotes drug resistance in colorectal cancer
9	TPX2	Targeting protein for Xklp2	The expression of TPX2 promotes the proliferation, migration and tumor growth of a variety of cancer cells
10	SMC4	Structural maintenance of chromosomes 4	The expression of SMC4 promotes the proliferation and migration of a variety of cancer cells and is closely related to the prognosis of cancer
11	IGF1	Insulin like growth factor 1	The expression of IGF1 promotes the proliferation and metastasis of ovarian cancer and rectal cancer
12	DCN	decorin	DCN deficiency promotes renal cell carcinoma growth and metastasis through downregulation of P21 and E-cadherin
13	CXCL12	C-X-C motif chemokine ligand 12	The CXCL12/CXCR4 axis is related to tumor progression, angiogenesis, metastasis and survival.
14	TLR4	Toll like receptor 4	TLR4 promotes the invasion and migration of various cancers
15	HGF	Hepatocyte growth factor	HGF promotes the invasion, migration and angiogenesis of various cancers
16	LUM	Lumican	Lumican may regulate collagen fibril organization and circumferential growth, corneal transparency, and epithelial cell migration and tissue repair

No.	Gene symbol	Full name	Function
17	ADCY2	Adenylate cyclase 2	ADCY2 catalyzes the formation of the secondary messenger cyclic adenosine monophosphate (cAMP)
18	FMOD	Fibromodulin	FMOD affects the rate of fibrils formation and may have a primary role in collagen fibrillogenesis
19	CAT	Catalase	CAT is a key antioxidant enzyme in the bodies defense against oxidative stress.
20	MC2R	Melanocortin 2 receptor	MC2R can increase the proliferation of prostate cancer

Hub gene overall survival and disease-free analysis

The overall survival and disease-free analysis of the hub genes was performed using cBioPortal online platform. ACC patients with CDK1, CCNB1, MCM4, AURKA, NCAPG, RRM2, TYMS and TPX2 alteration showed worse overall survival (Figure. 3A). Nonetheless, ACC patients with CDK1, CCNB1, MCM4, MAD2L1, AURKA, NCAPG, RRM2, TYMS and TPX2 alteration showed worse disease-free survival (Figure. 3B).In addition, the MAD2L1 alteration was significantly associated with worse disease-free survival but not overall survival (Figure. 3B). The 9 genes were redefined as key genes for following analyses. Besides, we noticed that all the 9 key genes were upregulated hub genes.

Prediction and validation of upstream key miRNAs of key genes

Subsequently, we predicted upstream miRNAs of the 9 key genes by using miRTarBase. In this study, we only included microRNA-target gene interactions that were validated by strong evidence (reporter assay, western blot, qPCR). Finally, we identified a total of 15 miRNAs that could potentially regulate 7 key genes (CDK1, TYMS, RRM2, CCNB1, MAD2L1, MCM4 and NCAPG) expression as presented in Fig. 4A.

Upstream potential miRNAs of two other key genes (AURKA and TPX2) were not observed. In addition, we assessed prognostic values of the 15 predicted miRNAs using oncomiR database. The results of survival analysis showed that 4 (miR-212-3p, miR-24-3p, let-7a-5p and miR-196a-5p) out of 15 miRNAs alteration significantly influenced prognosis in cancer as presented in Fig. 4B. The 4 miRNAs were defined as the key miRNAs.

Prediction and validation of upstream key lncRNAs of key miRNAs

Increasing studies have reported that lncRNA functions as ceRNA to interact with mRNA by competing for shared miRNA. So we further predicted upstream lncRNAs of the 4 key miRNAs (miR-212-3p, miR-24-3p, let-7a-5p and miR-196a-5p) using an online database miRNet. A total of 185 lncRNAs were discovered (including duplicate lncRNAs,Table S3). There is a negative correlation between lncRNA and miRNA

based on the ceRNA hypothesis. So, we further analyzed these lncRNAs expression in drenocortical carcinoma using GEPIA database. Only 3 (XIST HOXA11-AS and TMPO-AS1) out of 185 lncRNAs were significantly upregulated in adrenocortical carcinoma samples. Survival analysis for the 3 upregulated lncRNAs demonstrated that patients with high expression of HOXA11-AS had unfavorable prognosis. Combined the results of expression analysis and survival analysis for these predicted lncRNAs, we re-defined HOXA11-AS as key lncRNAs(Fig. 5).

Construction of key mRNA-miRNA -lncRNA triple sub-network in adrenocortical carcinoma

A key mRNA-miRNA-lncRNA competitive endogenous RNA triple regulatory network in adrenocortical carcinoma were constructed. The network totally contained 8 mRNA-miRNA pairs (MCM4-miR-24-3p, RRM2-miR-24-3p, RRM2-miR-211-5p, RRM2-let-7a-5p, CDK1-miR-24-3p, CDK1-miR-663a, CDK1-miR-31-5p and CDK1-miRNA-302a-3p), 2 miRNA-lncRNA pairs (miR-24-3p-HOXA11-AS and let-7a-5p-HOXA11-AS) and 3 mRNA-lncRNA pairs (MCM4-HOXA11-AS, CDK1-HOXA11-AS and RRM2-HOXA11-AS). This network was depicted in Fig. 6. Taken all the three levels into consideration, we constructed some novel mRNA-miRNA-lncRNA triple sub-networks, RRM2-miR-24-3p/let-7a-5p-HOXA11-AS, CDK1/MCM4-miR-24-3P-HOXA11-AS. They are significantly associated with prognosis of adrenocortical carcinoma. The sub-networks may also be developed as promising diagnostic biomarkers or therapeutic targets for adrenocortical carcinoma in the future.

Disscusion

ACC is a rare aggressive type of endocrine cancer that originates in adrenal cortical cells, whose prognosis is poor(3). At present, the molecular mechanism of ACC development has not yet been elucidated, which brings great challenges to the clinical diagnosis and treatment of ACC. Recent studies have reported that ncRNAs, including miRNAs and lncRNAs, play important roles in cancer initiation and progression(31–35). After the first proposal of ceRNA hypothesis by Salmena et al(22), in which lncRNA competes with mRNA to bind miRNA to form a sponge-like structure, buffering and reducing the ability of miRNA to interfere with the protein encoded by the target gene. Increasing studies about ceRNAs in human cancers have been reported. For example, Long Noncoding RNA (lncRNA)-Mediated Competing Endogenous RNA Networks Provide Novel Potential Biomarkers and Therapeutic Targets for Colorectal Cancer(36). LncRNA-CDC6 promotes breast cancer progression and function as ceRNA to target CDC6 by sponging microRNA-215(37). LncRNA XLOC_006390 facilitates cervical cancer tumorigenesis and metastasis as a ceRNA against miR-331-3p and miR-338-3p(38). LncRNA HOTAIR participates in the development and progression of adrenocortical carcinoma via regulating cell cycle(39). However, some analysis for ceRNAs in adrenocortical carcinoma is still not enough. In this study, a novel mRNA-miRNA-lncRNA triple regulatory network was constructed and each RNA in this network significantly associated with prognosis in adrenocortical carcinoma.

In this study, we identified a total of 325 significant DEGs, consisting of 49 upregulated and 276 downregulated DEGs by searching GEO database, and analyzing two datasets, GSE12368 and GSE19750. GO is widely used as functional enrichment analysis for a large number of genes(40). The results of these significant DEGs related GO analysis showed that they were significantly enriched in some GO terms that were associated with cancer biological behaviors, including cell division, cell adhesion, regulation of signal transduction by p53 class mediator. KEGG pathway enrichment analysis revealed that the significant DEGs were significantly enriched in some cancer-associated pathways, such as pathways in Cell cycle, p53 signaling pathway and Progesterone-mediated oocyte maturation, Proteoglycans in cancer.

To further analyze the relationships and functions of significant DEGs in adrenocortical carcinoma, we used STRING database and obtained PPI networks. It has been widely acknowledged that genes with more node degree in the PPI network usually play more roles. Therefore, we screened the hub genes in the two PPI networks according to node degree. The top ten upregulated and downregulated hub genes were selected for further expression and survival analyses to identify key genes in adrenocortical carcinoma. The analytic results showed that 9 upregulated (CDK1, CCNB1, MCM4, AURKA, NCAPG, RRM2, TYMS and TPX2) hub genes may act as the key genes in adrenocortical carcinoma because alteration was significantly associated with worse prognosis.

MiRNAs and lncRNAs, are involved in regulation of gene expression and function by ceRNA mechanism as previously described. So the upstream miRNAs of the key genes were first predicted. Survival analysis revealed that 4 miRNAs (miR-212-3p, miR-24-3p, let-7a-5p and miR-196a-5p) significantly influenced prognosis in cancer. The tumor suppressive roles of the 4 miRNAs have been reported. For example, Long Noncoding RNA KCNQ1OT1 Accelerates the Progression of Ovarian Cancer via MicroRNA-212-3/LCN2 Axis(41). Consistently, ectopic expression of miR-24-3p suppressed the cell migration, invasion, and proliferation of MCF7, Hep3B, B16F10, SK-Hep1, and PC-3 cells by directly targeting p130Cas(42). Moreover, let-7a-5p inhibited BCL2L1 expression and suppressed lung cancer cell proliferation, migration, and invasion(43). LncRNA NEAT1 promotes colorectal cancer cell proliferation and migration via regulating glial cell-derived neurotrophic factor by sponging miR-196a-5p(44). Then, we further predicted 185 upstream lncRNAs of these key miRNAs. By combining expression analysis and survival analysis for these lncRNAs in adrenocortical carcinoma using GEPIA, only 1 lncRNAs (HOXA11-AS) were defined as the key lncRNAs. The tumor promoted roles of the HOXA11-AS have been reported. For example, LncRNA HOXA11-AS Promotes Proliferation and Invasion of Gastric Cancer by Scaffolding the Chromatin Modification Factors PRC2, LSD1, and DNMT1(45). LncRNA HOXA11-AS promotes proliferation and invasion by targeting miR-124 in human non-small cell lung cancer cells(46). HOXA11-AS promotes the growth and invasion of renal cancer by sponging miR-146b-5p to upregulate MMP16 expression(47). Thus, a mRNA-miRNA-lncRNA network in adrenocortical cancer was successfully established. In this network, some pairs have been identified in some cancers. For example, RRM2-let-7a-5p-SNHG16/MAL2 as key ceRNA subnetwork associated with prognosis of breast cancer(48). MiR-31-5p acts as a tumor suppressor in renal cell carcinoma by targeting cyclin-dependent kinase 1 (CDK1)(49). These reports further indicate the accuracy of our current analytic results. Of course, although attractive findings have

been obtained by a series of bioinformatic analyses in our current study, more laboratory experiments need to be performed in the future.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors Consent for publication

Author contributions

SY, LY participated in the conception and design of the study. SY, HW, WL conducted the data collection and drafted the manuscript. All authors approved the final manuscript.

Confilict of interest

The authors declare that they have no conflicts of interest.

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Data availability statement

All data used during the study are available from the corresponding author by request.

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Figures

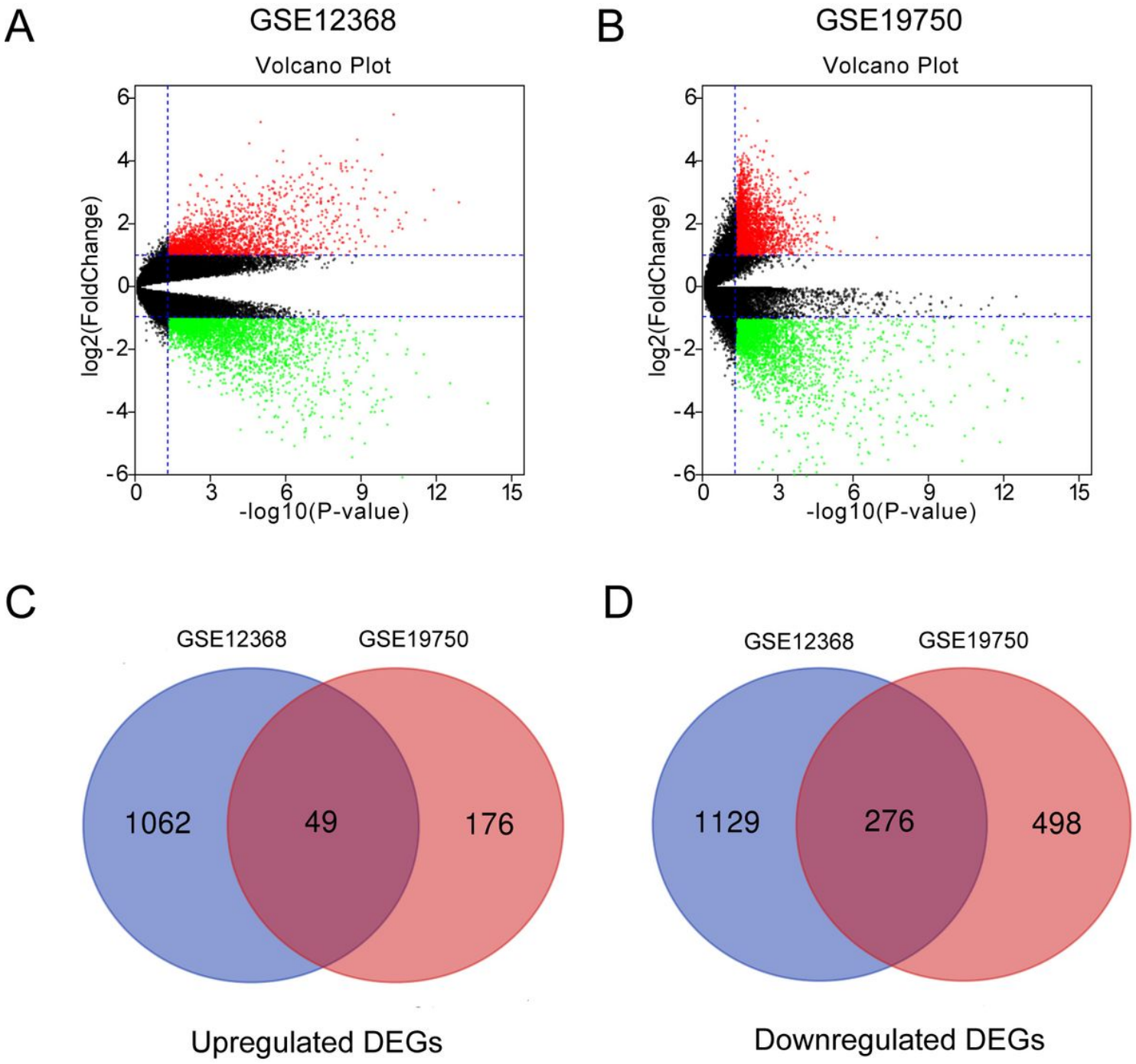
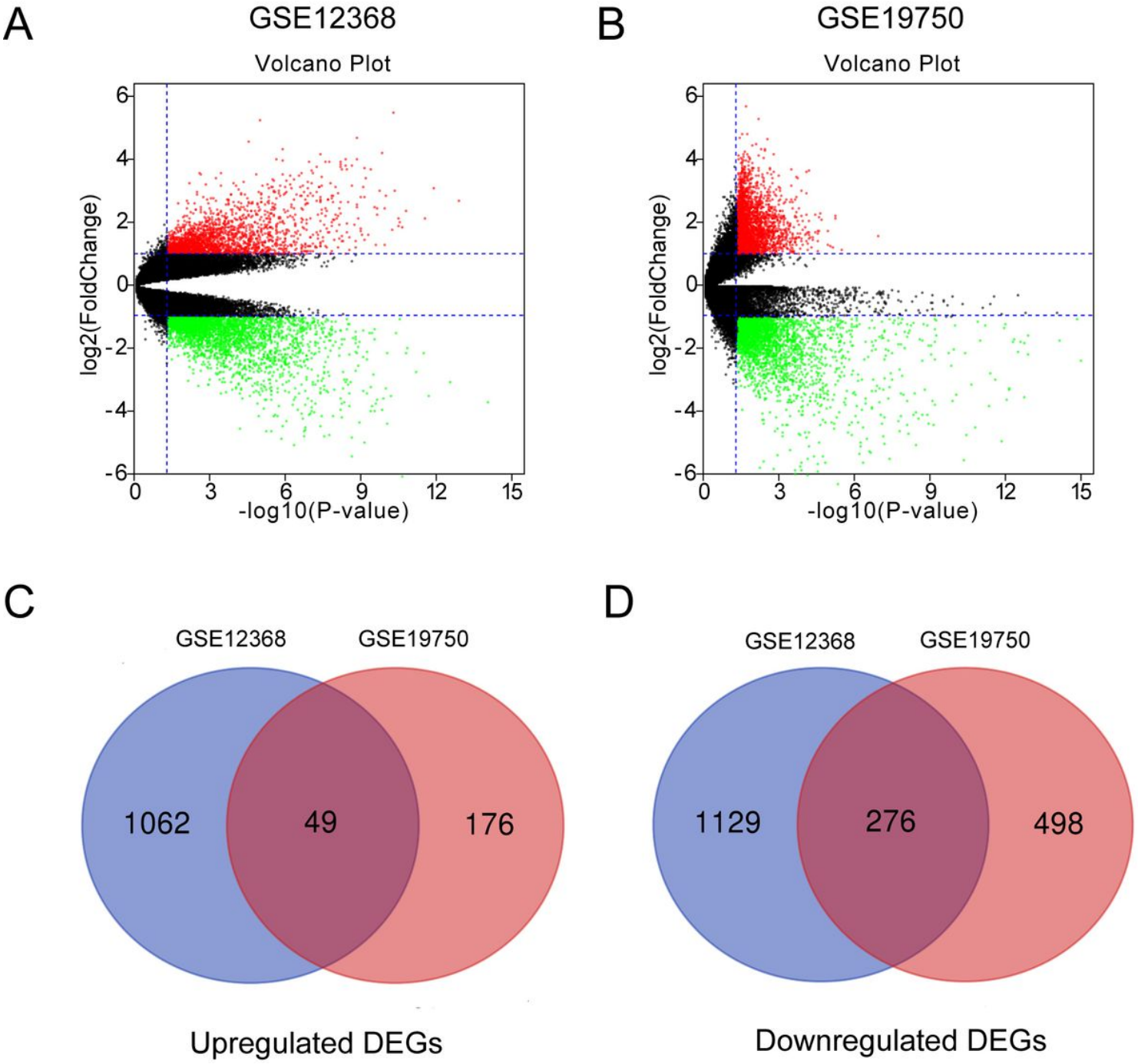


Figure 1

Identification of significant differentially expressed genes (DEGs) in adrenocortical carcinoma. (A) Volcano plot showing the DEGs identified from GSE12368. (B) Volcano plot showing the DEGs identified from GSE19750. (C) The intersection of upregulated DEGs of GSE12368 and GSE19750 datasets. (D) The intersection of downregulated DEGs of GSE12368 and GSE19750 datasets. The intersected DEGs were redefined as the significant DEGs.



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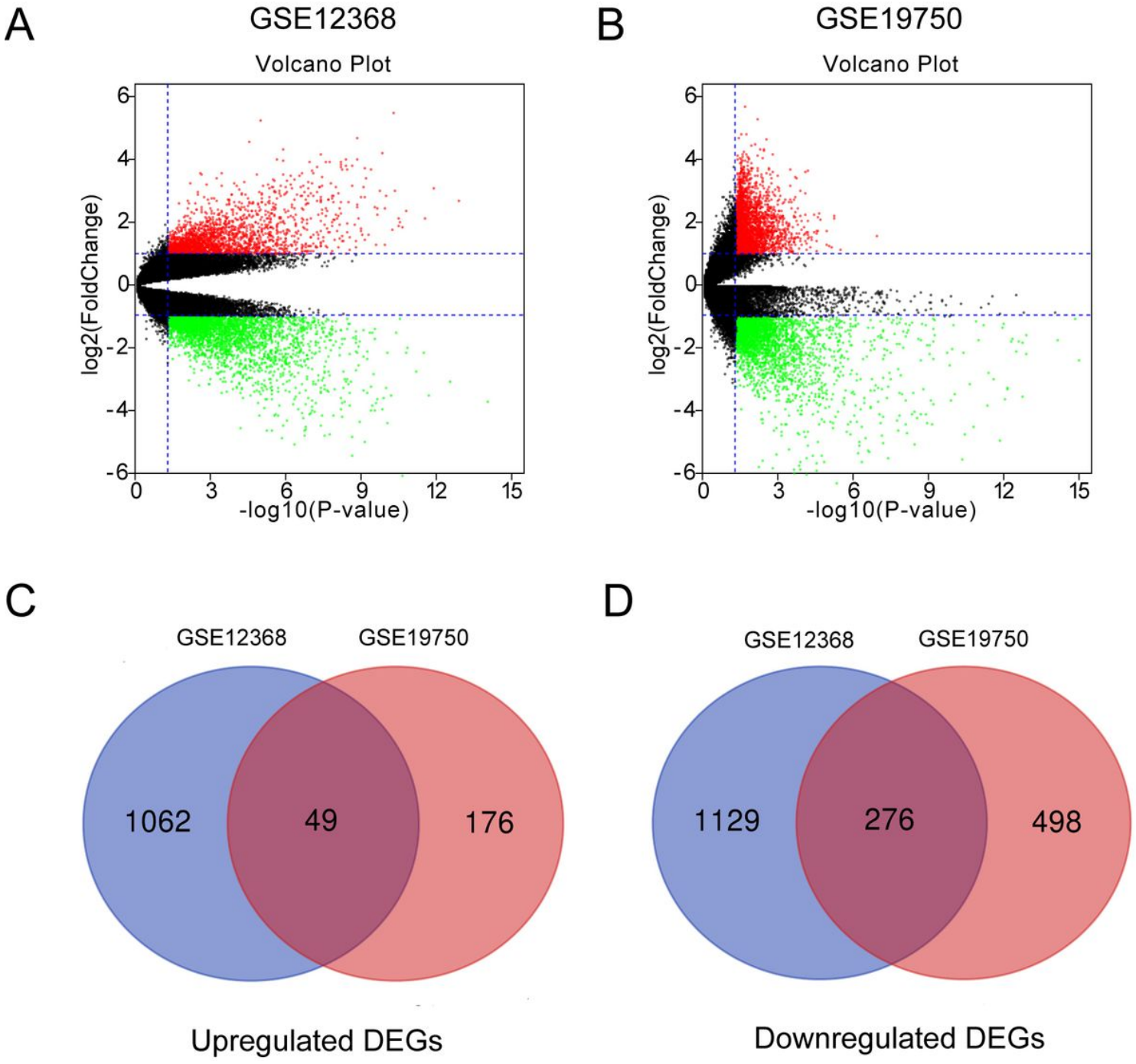

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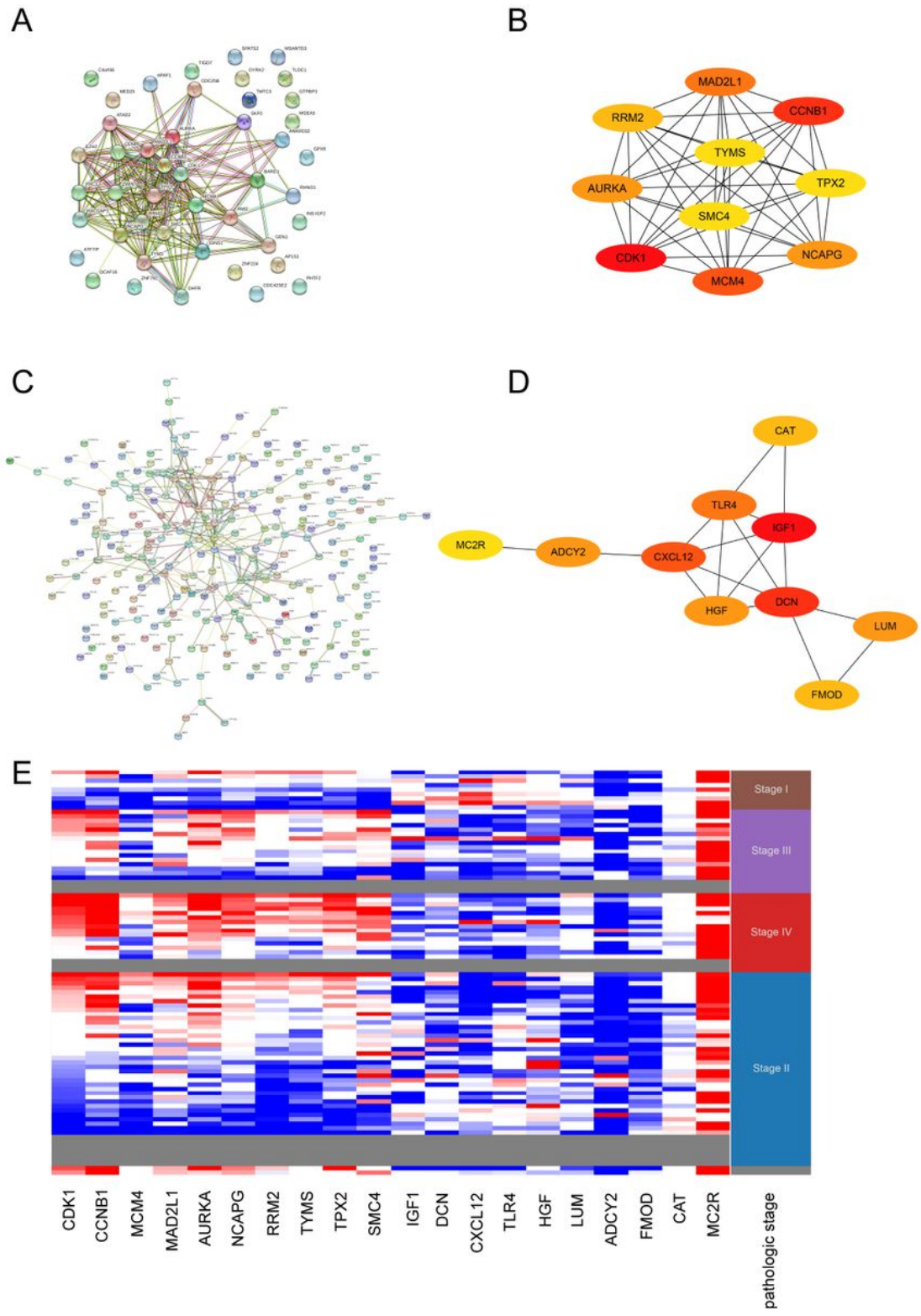
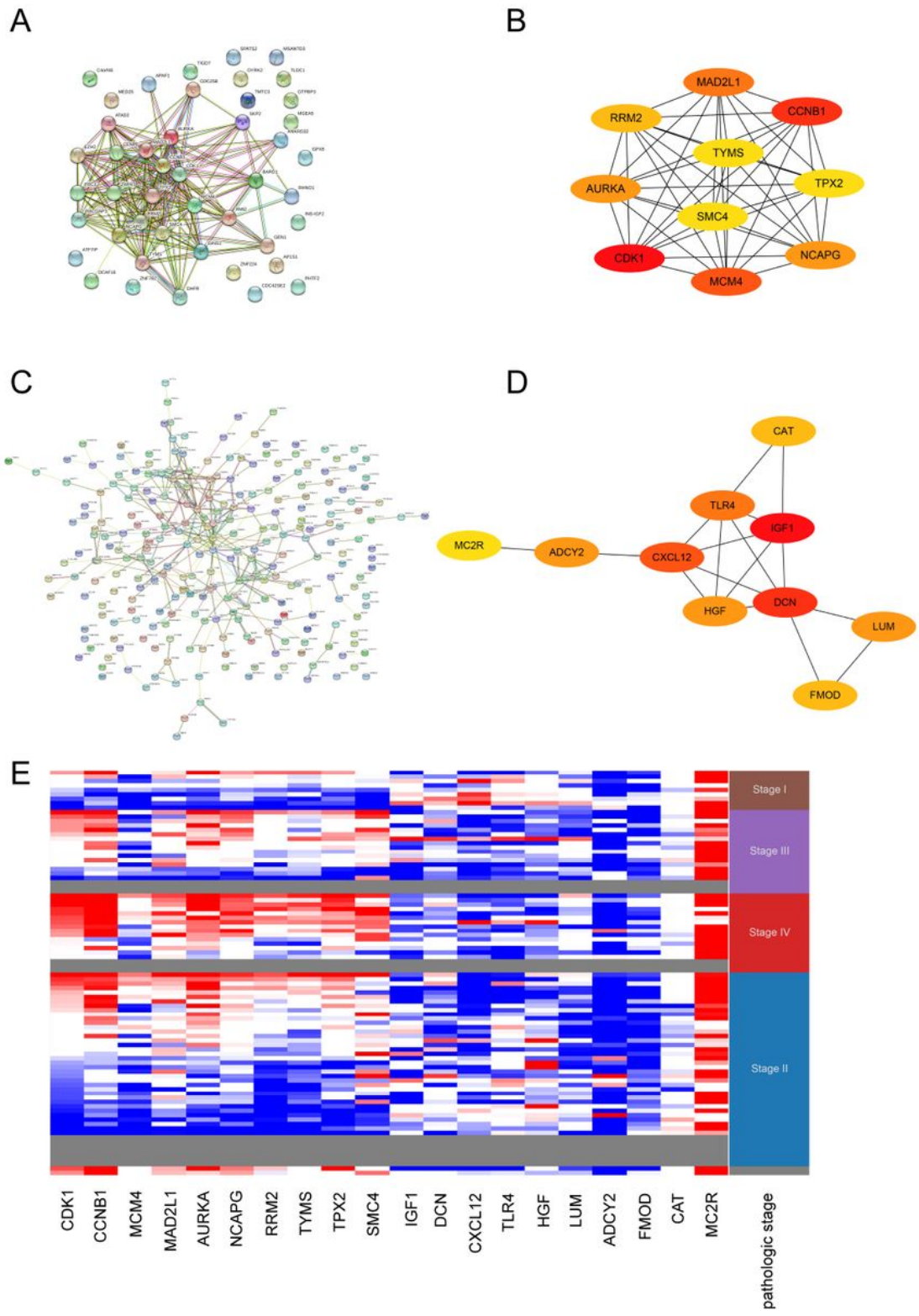


Figure 2

The top 10 hub genes identified in protein-protein interaction (PPI) networks. (A) The PPI network of the significant upregulated DEGs. (B) The top 10 hub genes of the significant upregulated DEGs. (C) The PPI network of the significant downregulated DEGs. (D) The 10 hub genes of the significant downregulated DEGs. (E) Hierarchical clustering of hub genes was constructed using UCSC. Upregulation of genes is marked in red; downregulation of genes is marked in blue.



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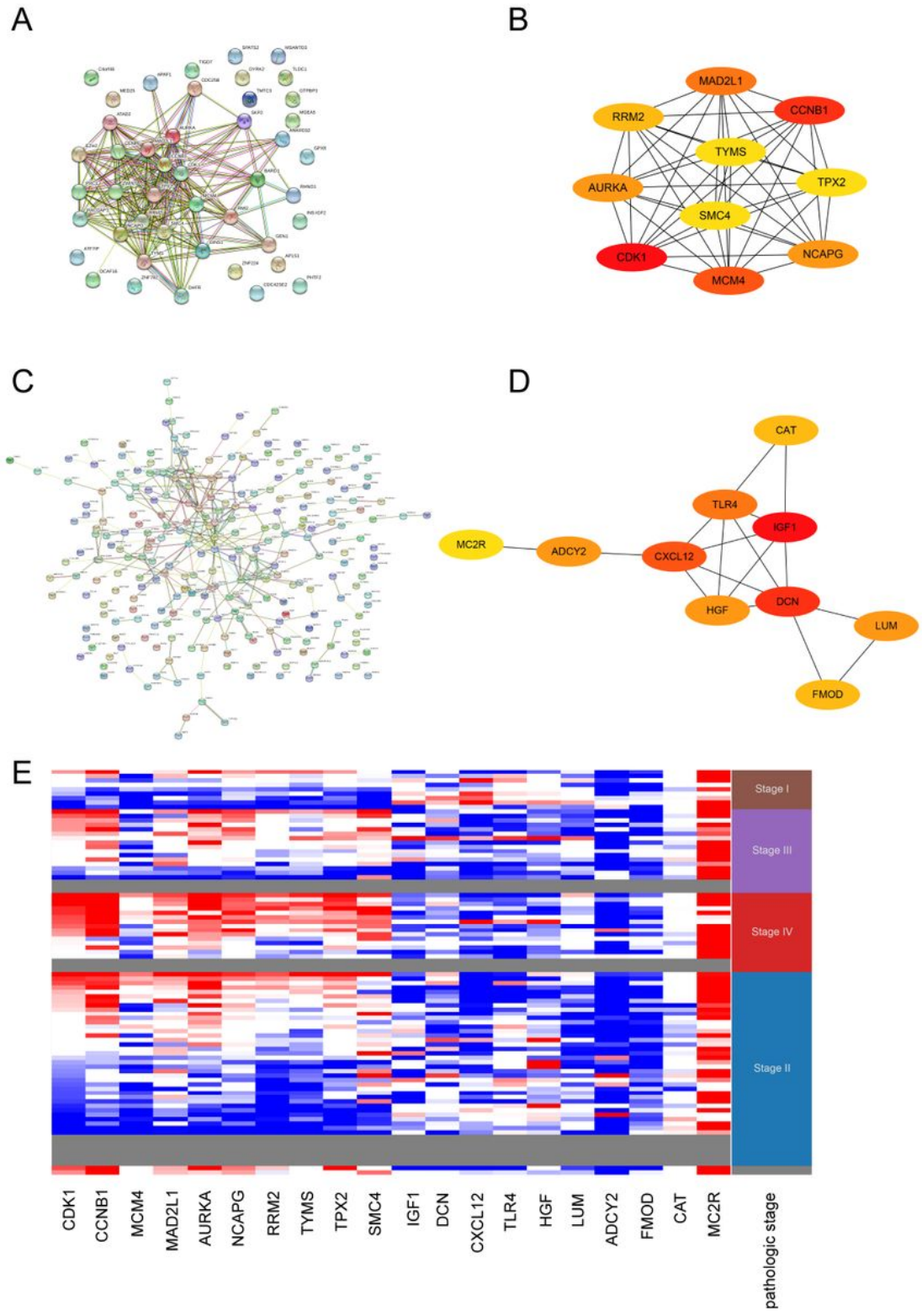

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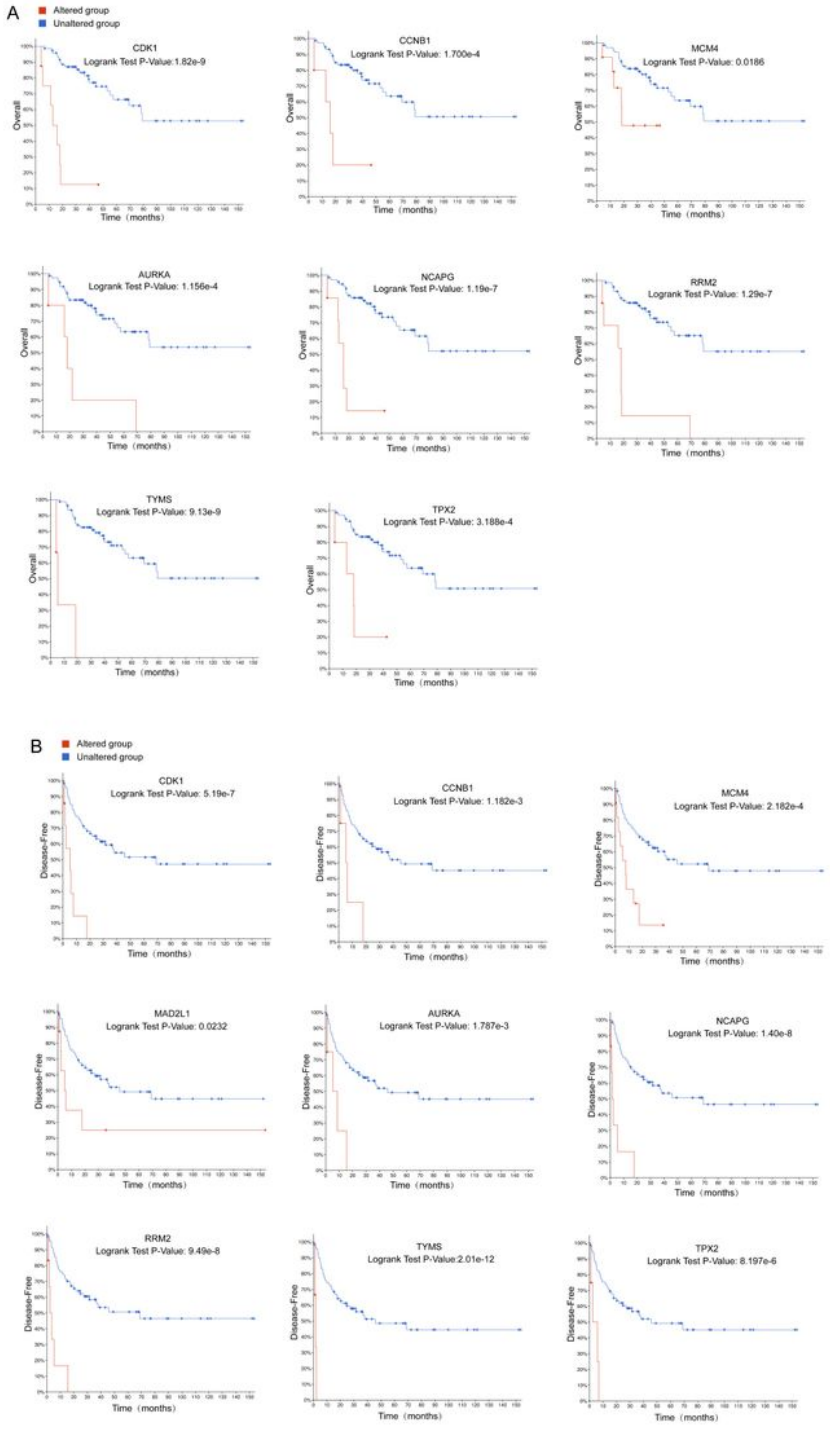
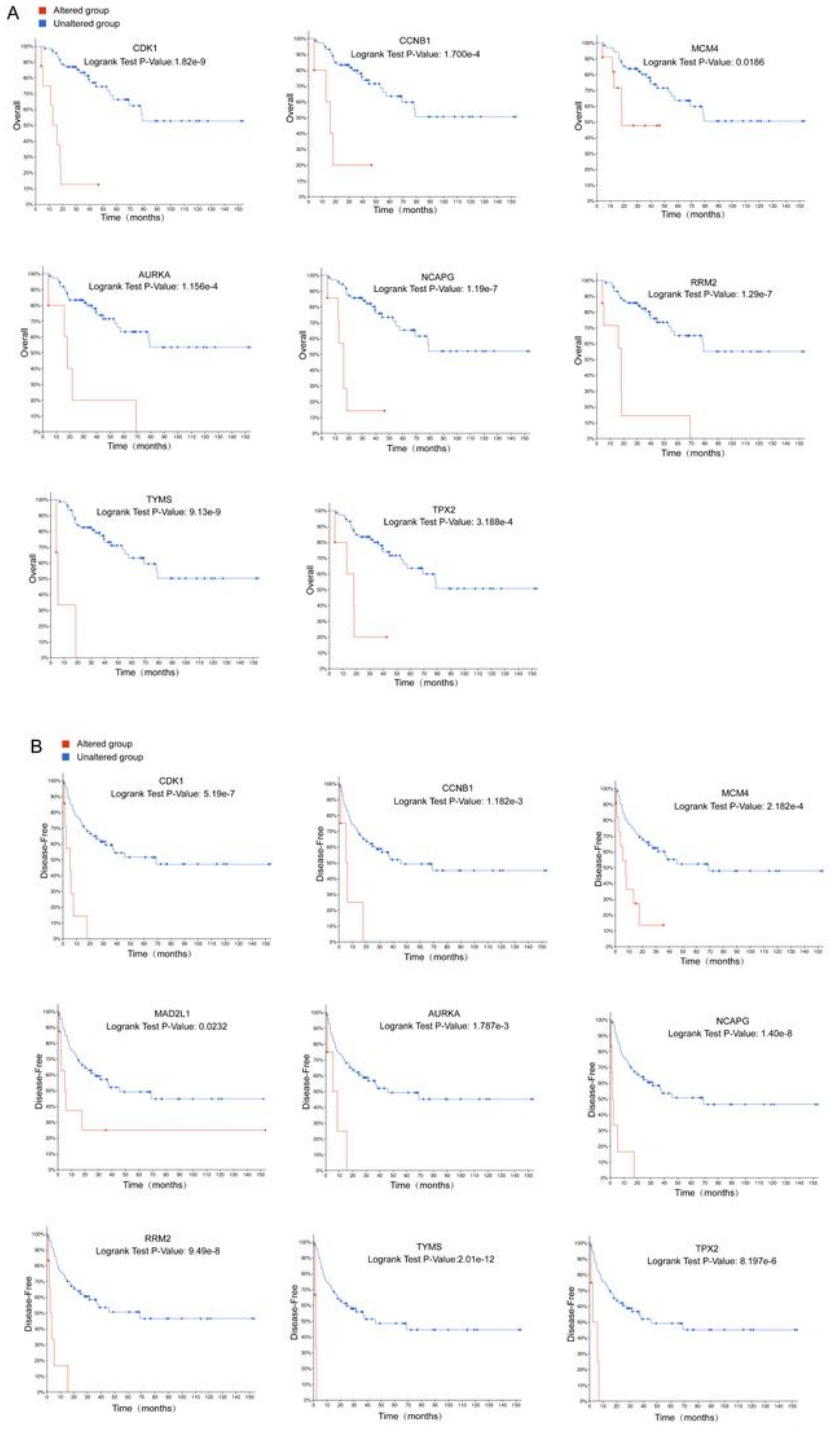


Figure 3

Prognostic analysis for genes. (A) Overall survival and (B) disease-free survival analyses of hub genes were performed using cBioPortal online platform. P<0.05 was considered statistically significant.



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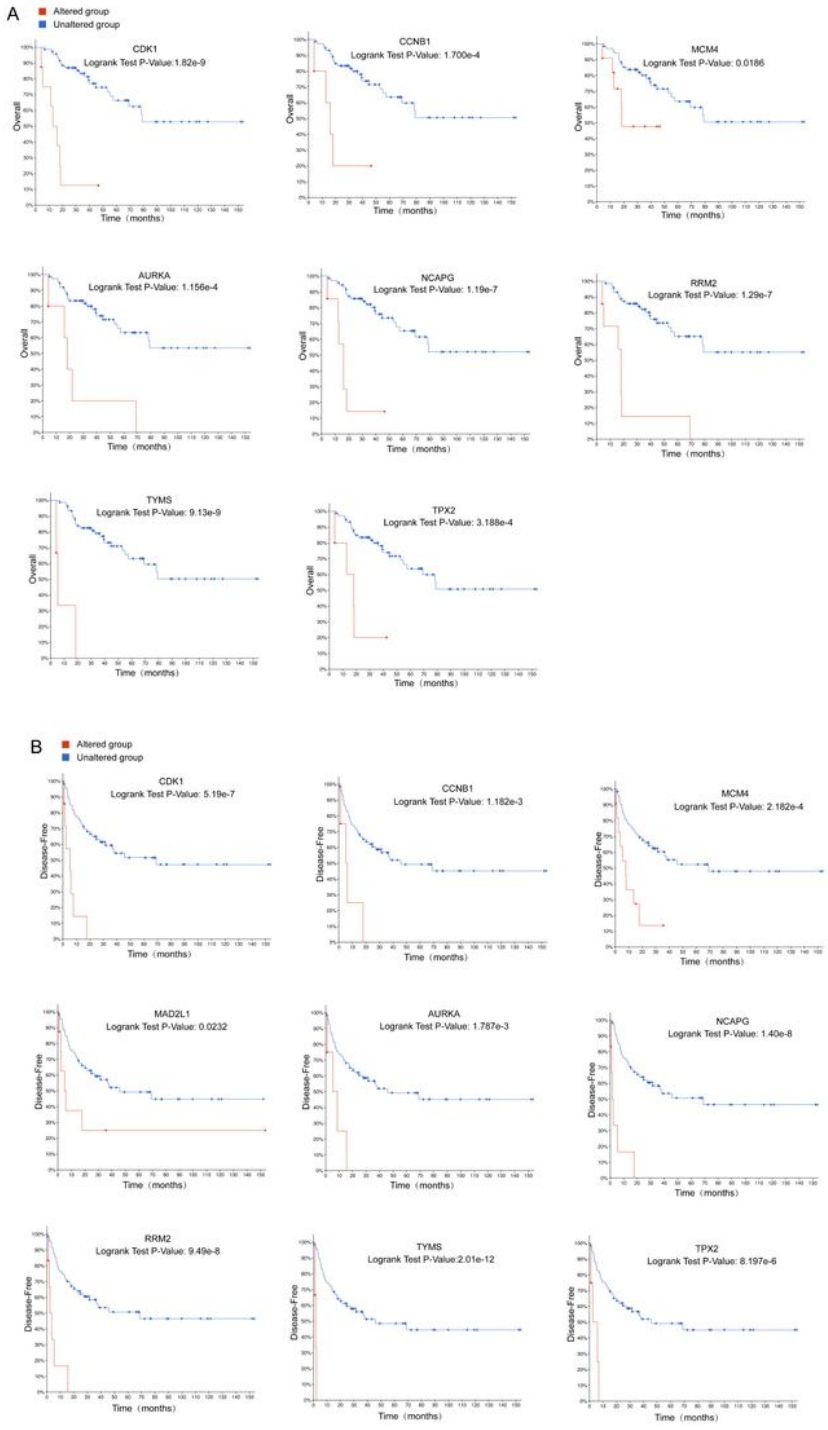

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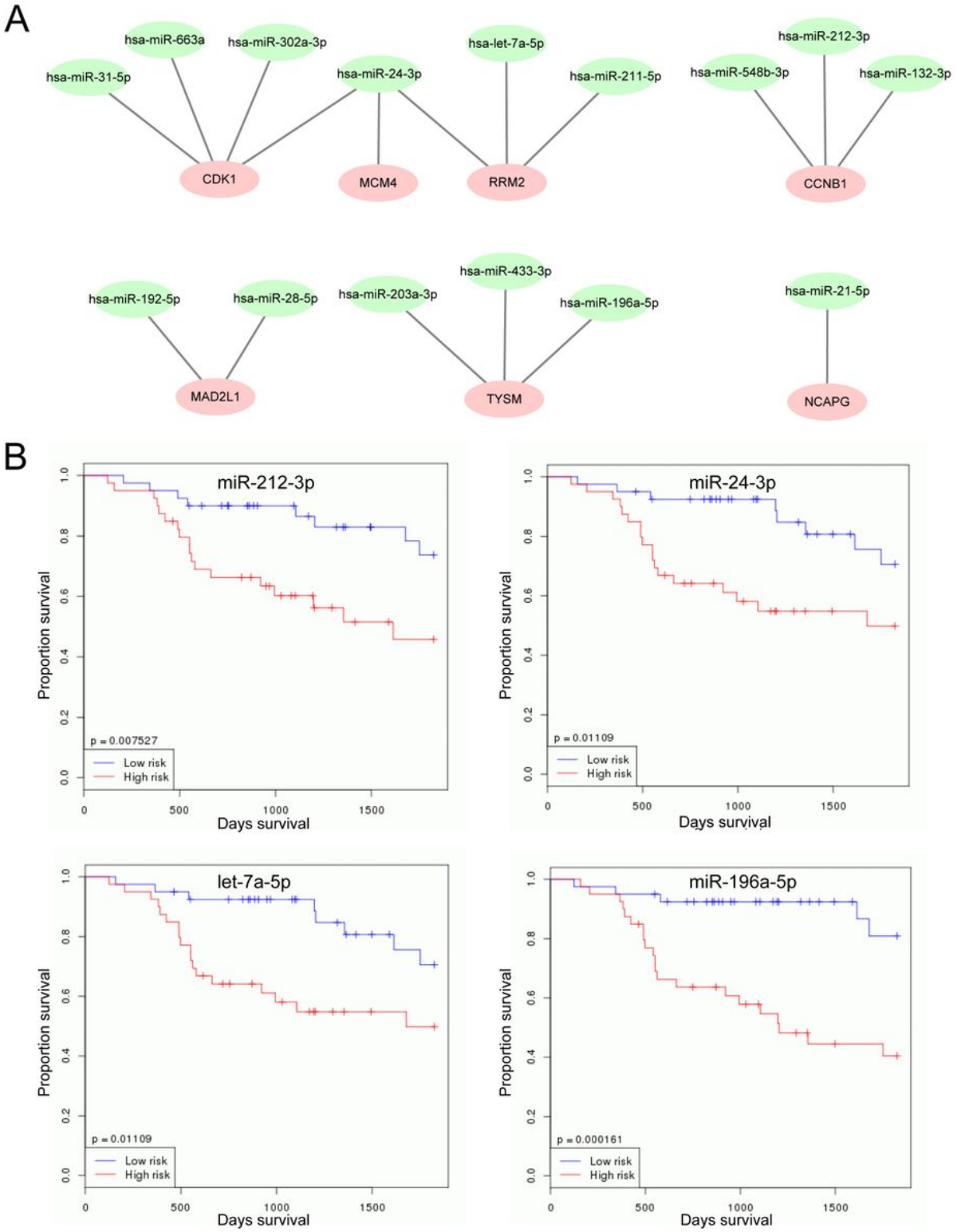
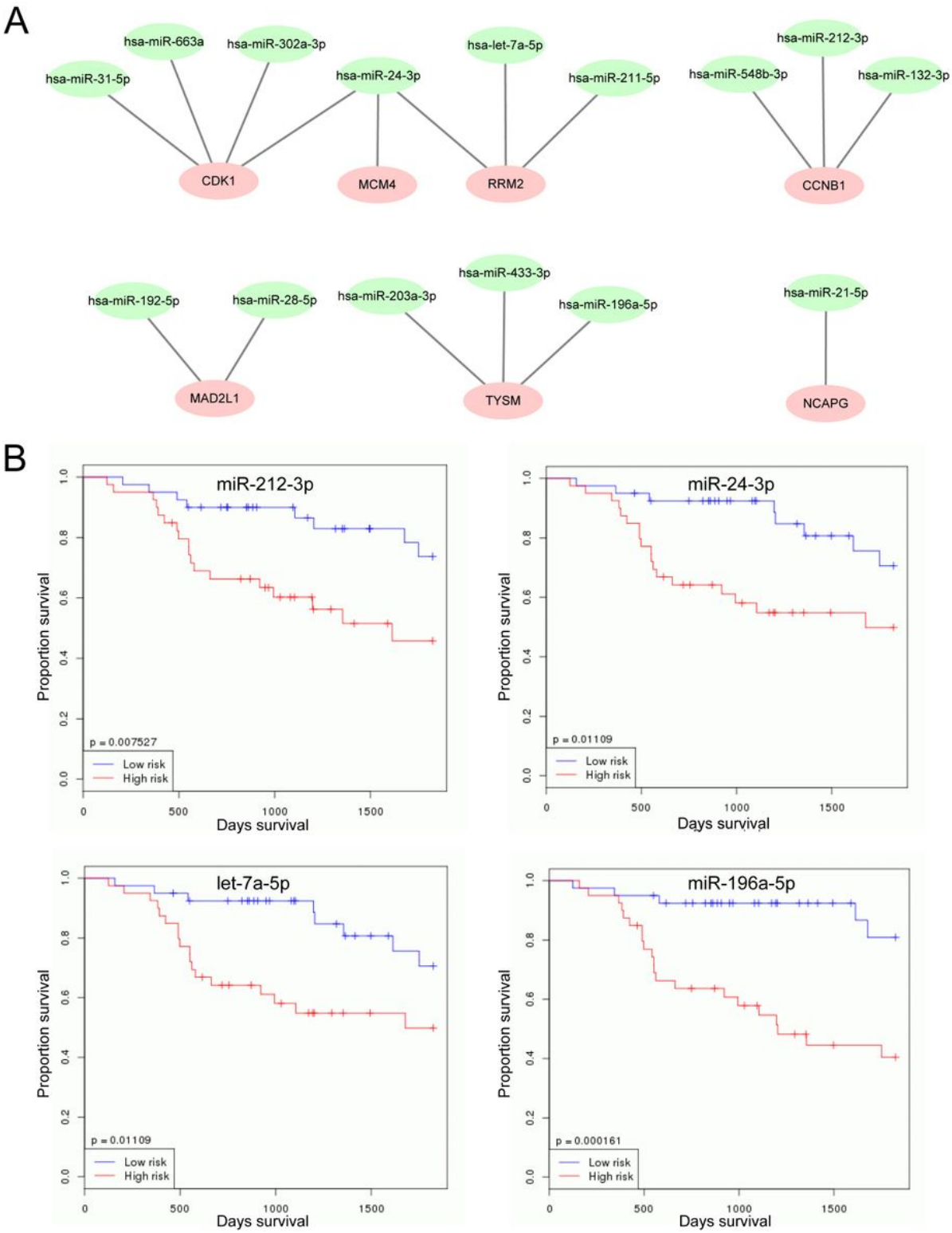


Figure 4

Screening the key miRNAs in adrenocortical carcinoma (A) Construction of miRNA-gene network using Cytoscape software (B) Prognostic value of has-miR-212-3p, has-miR-24-3p, has-let-7a-5p, has-miR-196a-5p in cancer.



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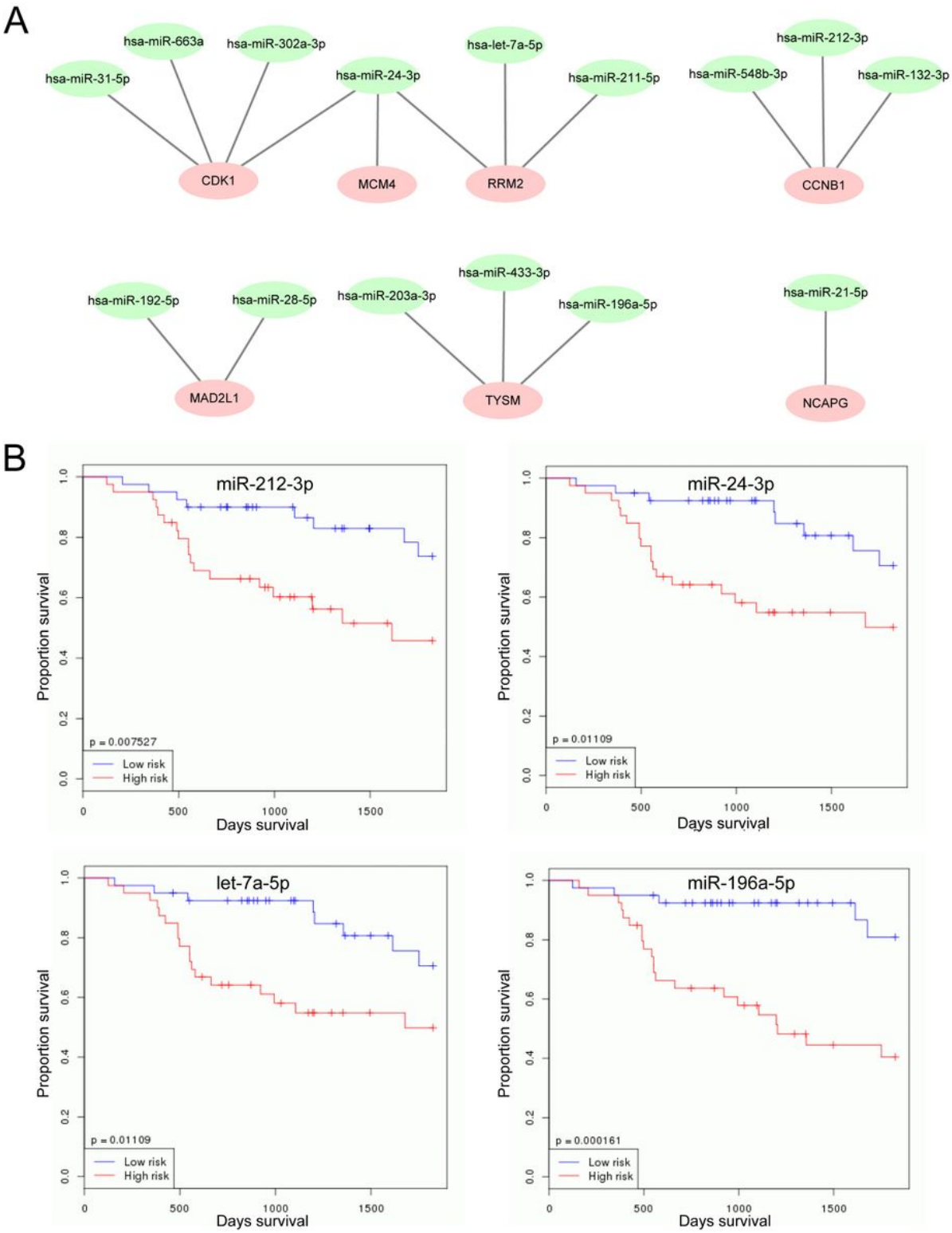

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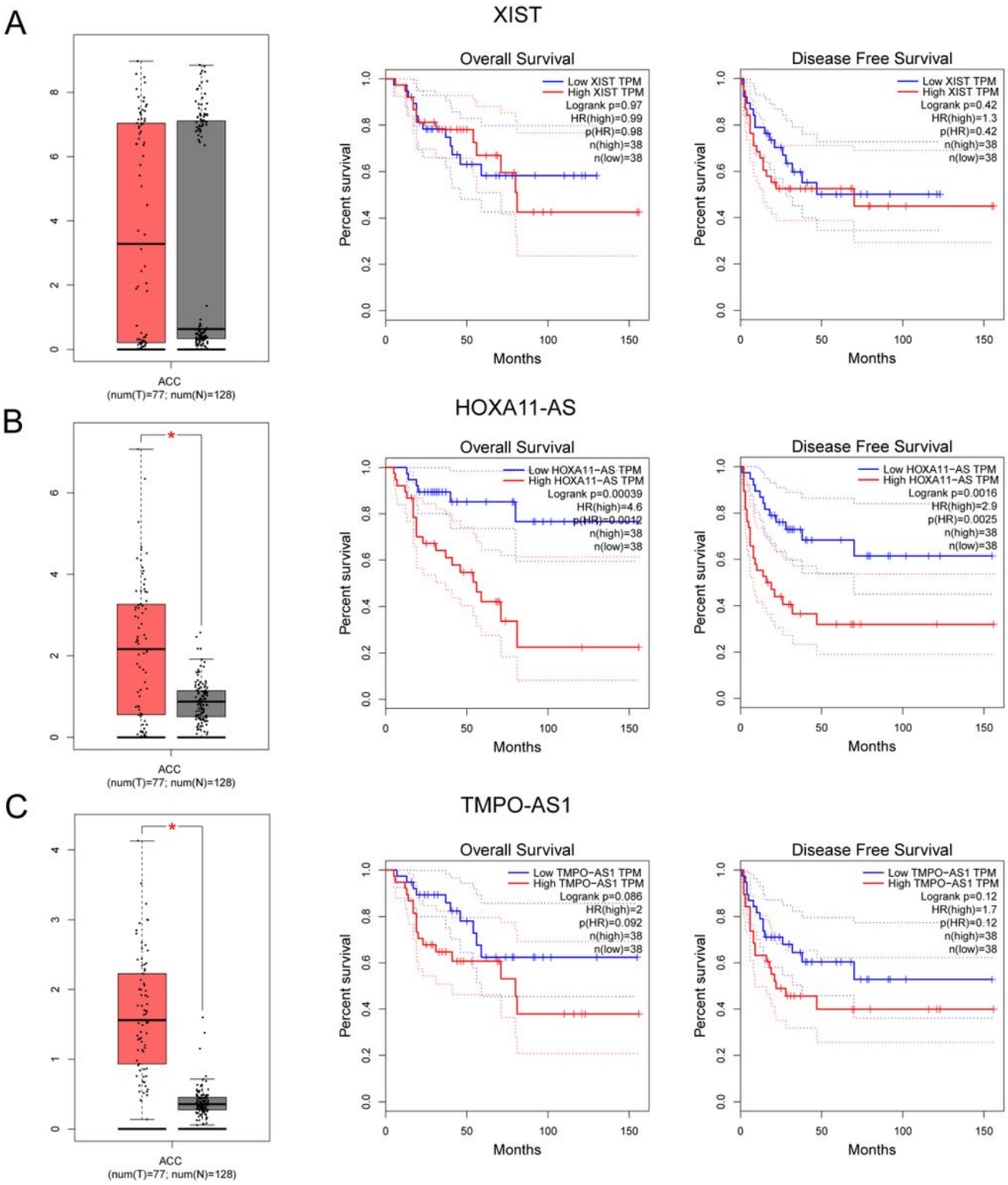
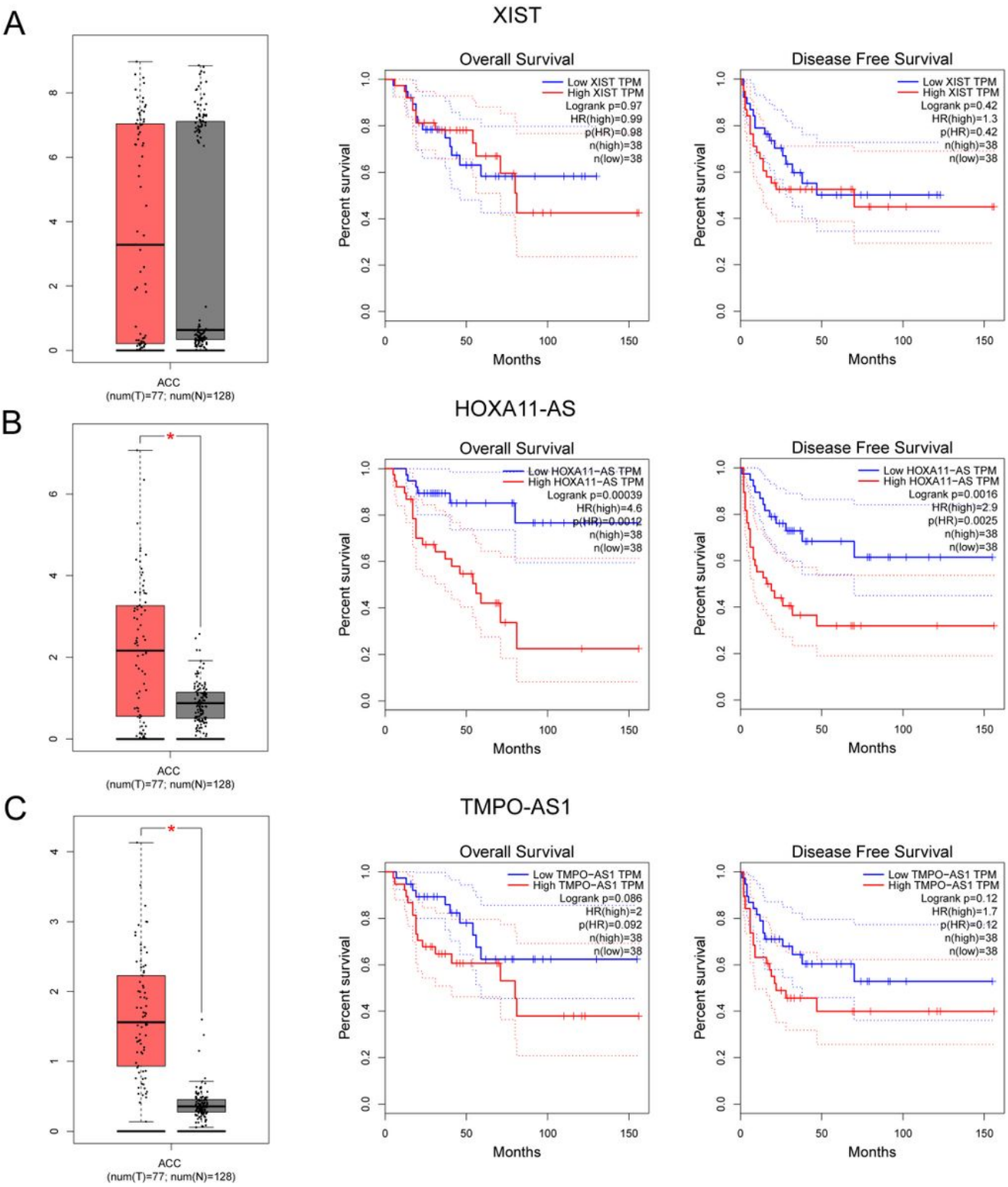


Figure 5

Screening the key lncRNAs in adrenocortical carcinoma. (A) Expression and prognostic value of XIST in adrenocortical carcinoma. (B) Expression and prognostic value of HOXA11-AS in adrenocortical carcinoma. (C) Expression and prognostic value of TMPO-AS1 in pancreatic cancer.



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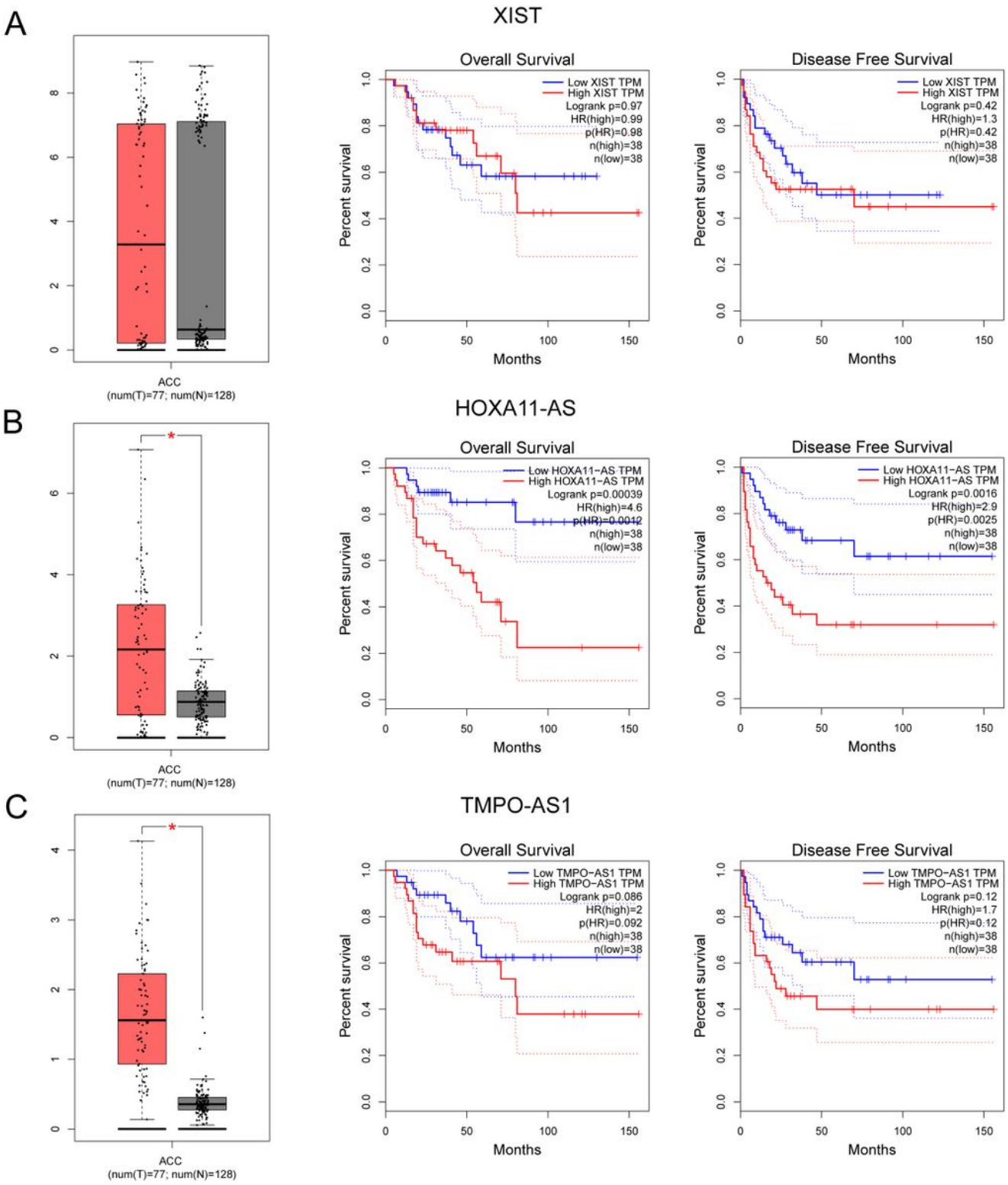

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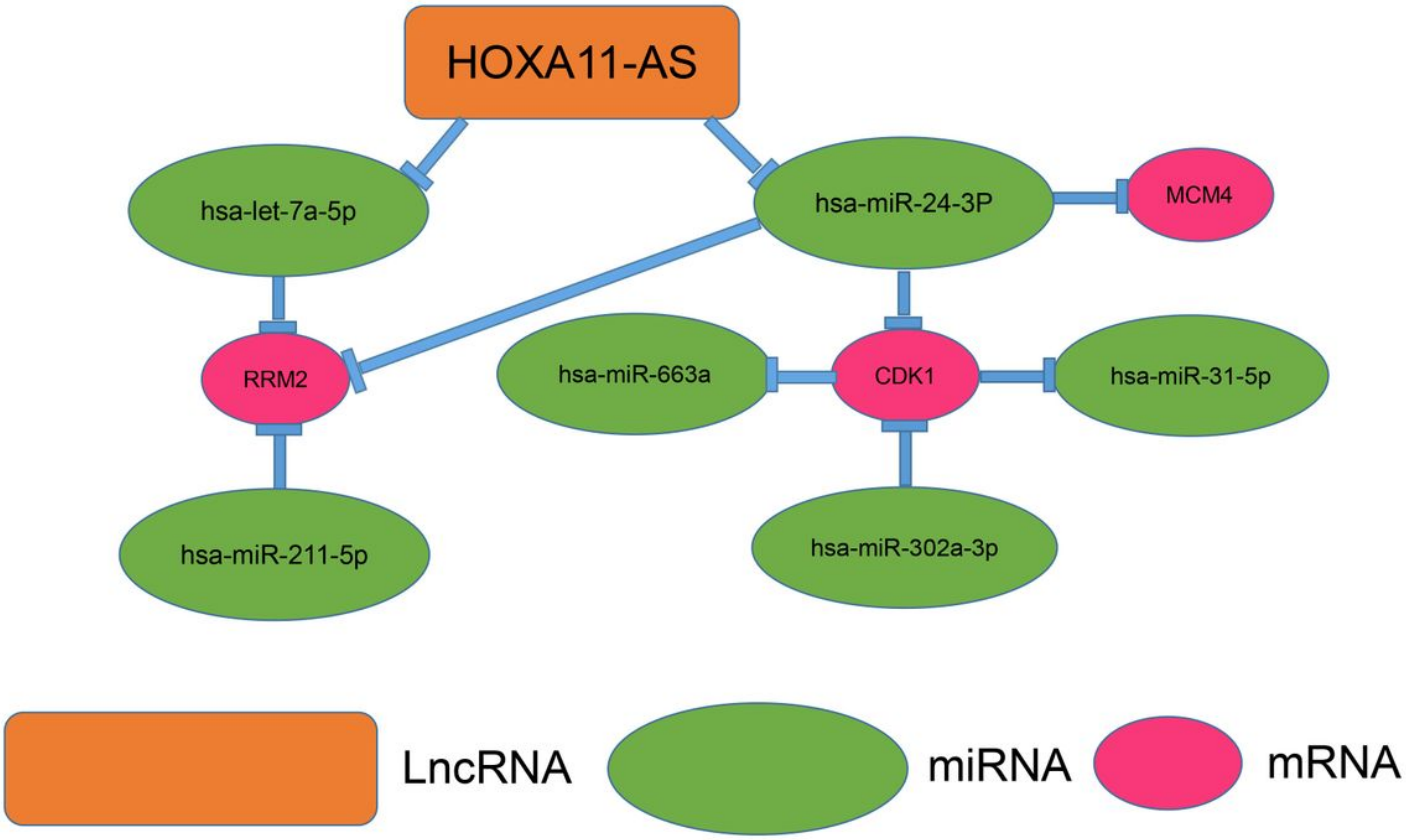
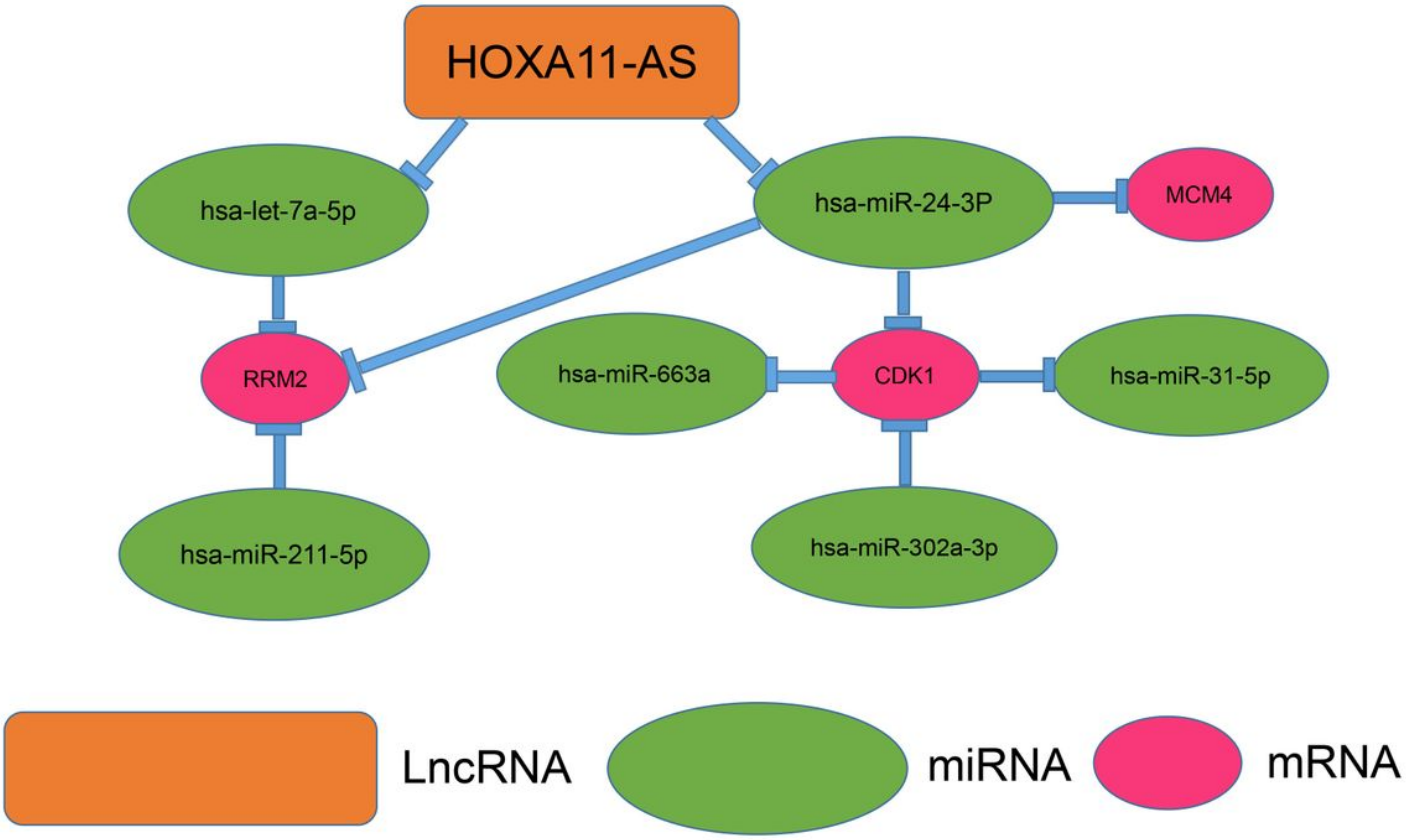


Figure 6

The novel mRNA-miRNA-lncRNA competing endogenous RNA (ceRNA) triple regulatory network associated with prognosis of adrenocortical carcinoma.



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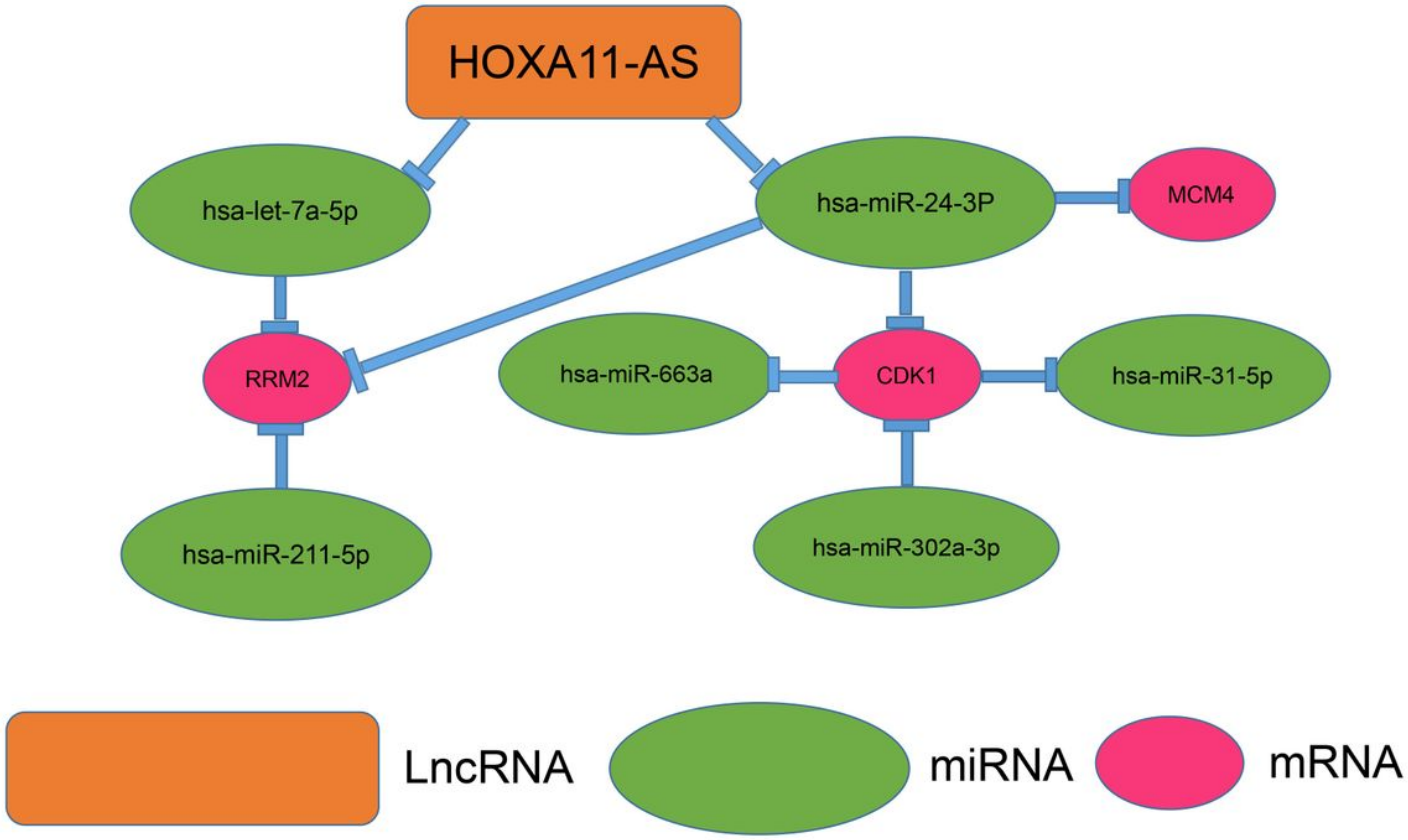

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Supplementary Files

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