MIR502 Acts As a Tumor Suppressor Gene to Accelerate Apoptosis and Suppress Proliferation by Targeting Hippo Signaling Pathway in Ovarian Cancer

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Research

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Abstract

Background Ovarian cancer (OC) is the major cause of death among women due to the lack of early screening methods and complex pathological progression. Increasing evidence indicated that microRNAs were considered as gene expression regulators in tumors by interacting with mRNAs. Although researches regarding with OC and microRNAs are extensive, the vital role of *MIR502* in OC still remains unclear.

Methods We integrated two microRNA expression arrays from GEO to identify differentially expressed genes. Kaplan–Meier method was used to screen miRNAs that had influence on survival outcome. Upstream regulator of *MIR502* was predicted by JASPAR and verified by ChIP-seq data. The LinkedOmics database was used to study correlated genes with *MIR502*. Gene Set Enrichment Analysis (GSEA) was conducted to reveal the functional annotation of GO and KEGG pathway enrichment analysis by using the open access WebGestalt tool. We constructed PPI network by using the STRING to further explore core protein.

Results We found that expression level of *MIR502* was significantly down regulated in OC, which was related to poor overall survival outcome. NRF1 as the upstream regulator of *MIR502* was predicted by JASPAR and verified by ChIP-seq data. In addition, anti-apoptosis and pro-proliferation genes attending in Hippo signaling pathway, including CCND1, MYC, FGF1 and GLI2 were negatively regulated by *MIR502* in the analysis of GO and KEGG pathway enrichment results. PPI network further demonstrated that CCND1 and MYCN were at core position in the development of ovarian cancer.

Conclusions *MIR502*, which was regulated by NRF1, acted as a tumor suppressor gene to accelerate apoptosis and suppress proliferation by targeting Hippo signaling pathway in ovarian cancer.

Introduction

Ovarian cancer (OC) is a common gynecological malignancy with the highest mortality rate[1].Primary cytoreductive surgery following chemotherapy is the conventional therapy of OC .Tumor often occurs proliferation, invasion, and lymph node metastasis at the time of diagnosis due to the lack of typical symptoms at the early stage ,which leads to delay in initiating appropriate treatment and poor outcome in the meanwhile[2]. The pathogenesis of OC is complicated because it is regulated by a variety of oncogenes and tumor suppressor genes[3]. Currently, multiple ovarian cancer oncogenes have been elucidated, whereas relatively few studies have focused on antioncogenes, and the molecular mechanisms regulating the progression of OC still remains largely unclear. Therefore, it is of great significance to explore new molecular markers for regulating proliferation and apoptosis of ovarian cancer cells, and provide potential signaling pathway for clinical treatment.

microRNAs (miRNAs) are small RNA molecules with length of approximately 20 nucleotides, whose function is negatively regulating gene expression through binding target mRNAs on 3'-untranslated regions (3’-UTRs) in post-transcriptional level [4, 5].A substantial amount of researches have confirmed that multiple miRNAs played pivotal role in the process of tumor development such as apoptosis,

proliferation, invasion, migration and recurrence by overexpression or low expression[6, 7]. In particular, various miRNAs have been shown to serve different roles in ovarian cancer. However, the regulatory mechanism and target genes of miRNAs are still in their infancy, and the relationship between miRNAs and tumor, especially ovarian cancer, is not fully understood. Nowadays, the effect of *MIR*502 in cancer has been researched widely. The results from our study indicated that *MIR*502 produced a marked effect on suppressing ovarian cancer proliferation.

Nuclear respiratory factor 1(NRF-1)is an important transcription factor in human genome. It was estimated that 6% of human promoter region genes contained NRF-1 response element in a systematic bioinformatics study [8]. NRF-1,also known as a-pal, was originally identified as a mitochondrial gene involved in the regulation of energy conduction[9].NRF1 encodes a protein, which has the function of homologous dimer and transcription factor, activates the expression of some key metabolic genes regulating cell growth[10].

Hippo signaling pathway is known as a crucial role in regulating cell proliferation, regeneration and controlling organ growth[11]. This pathway is composed by a large number of proteins. It has the function of controlling cell fate not only in the process of development and differentiation, but also in the pathological process including cancer [12]. The main Hippo core kinase cascades are Yes-associated transcriptional regulator (YAP) and transcriptional coactivator with the PDZ-binding motif (TAZ). Observations have demonstrated that there was a strong relationship between Yap activation and cancer. In many tumors, including brain ,lung, breast, pancreatic, liver, colon, skin and ovarian cancer, YAP and TAZ promote cell proliferation and anti-apoptosis in cooperation with transcription factors via translocating into the nucleus to regulate many well-known oncogenes [13–17]. A study about podocytes found that YAP overexpression lead CCND1 significantly up-regulated, which confirmed CCND1 as a downstream target gene of YAP[18]. Previous research of gastric tumor has identified MYC as a key downstream molecular target of YAP. The positive correlation between MYC and YAP in human gastric cancers also supported regulation of MYC by YAP, which was an important molecular mediator of gastric tumorigenesis[19]. Shan Xu has verified that YAP promoted VEGFA by targeting GLI2 in renal cancer[20].Some studies have shown that FGF promoted Hippo/Yap signal transduction in the proliferation and differentiation process of lens epithelial cell, and FGF induced nuclear Yap expression played an important role in promoting lens epithelial cell proliferation[21]. Accordingly, it has been reported that YAP, acting as an oncogene, was associated with poor prognosis in ovarian cancer [22–24].

The big data generated by high-throughput research is generally characterized by large amount, a wide range of data types, deep value mining and fast processing response. The big data provides opportunities for the discovery of tumor molecular targets, but also brings great challenges to the full mining, integration and utilization of it. It’s certainly needed to investigate the complex genetic mechanisms by applying appropriate statistical method[25]. In this study, we found that the expression level of *MIR*502 is significantly down regulated in ovarian cancer by using bioinformatics analysis of two public databases. We also analyzed the association of *MIR*502 expression with clinical overall survival (OS) outcome, and correlated pathways were explored to improve prognostic and therapeutic value in

preventing ovarian cancer progression. Gene Ontology (GO) and Kyoto Encyclopedia of Gene and Genomes (KEGG) pathway analysis showed that Hippo signaling pathway was correlated with *MIR*502. And transcription factor NRF1 was predicted as the upstream regulator of *MIR502*.The authors believe that it may provide more effective and scientific guidance to clinicians for the early diagnosis of patients with ovarian cancer, along with the individual treatment and improve prognosis to the patients and achieve benefit ultimately.

Materials & Methods

Access of public database

The microRNA expression datasets used in this study (GEO: GSE83693 and GSE119055) were acquired from the National Center for Biotechnology Information (NCBI) Gene expression (http://www.ncbi.nlm.nih.gov/geo/) .

Analysis Of Public Database

GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r/) is an analysis tool which is used to compare two sets of data coming from the GEO database. We used GEO2R to screen miRNAs of differential expression between normal tissue and ovarian cancer tissue in the GSE83693 and GSE119055 dataset. We selected genes whose |log2FC (fold change)| > 2 and adjust *p*-value < 0.05 as differential expression genes.

Survival Analysis

According to the lower quartile expression level of *MIR502*, OC patients were divided into high expression group and low expression group. The overall survival was analyzed by using Kaplan–Meier plotter (http://kmplot.com/analysis/index.php?p=background). The hazard ratio with 95% confidence intervals and log rank P value were calculated and displayed.

Gene Correlation Expression Analysis

The LinkedOmics database (http://www.linkedomics.org/admin.php) contains 32 TCGA cancer-associated multi-dimensional datasets, including ovarian cancer. The website was used to study correlation between *MIR*502 and interested genes expression in the TCGA OC cohort. Results were analyzed statistically using Pearson's correlation coefficient.

Prediction And Verify Of Transcription Factors

We developed JASPAR (http://jaspar.genereg.net) to predict the transcription factors of CLCN5, and Cistrome Data Browser (http://cistrome.org/db/), which provided resource of human cis-regulatory information obtained from the chromatin analysis from ChIP-seq, DNase-seq and ATAC-seq was used to verify the prediction results.

Acquire overexpression genes of ovarian cancer from the Cancer genome atlas database

The Gene Expression Profiling Interactive Analysis (GEPIA) website (http://gepia.cancer-pku.cn) can provide varied functions based on TCGA data, including gene expression, gene correlation analysis, survival analysis and so on. GEPIA was used to find overexpression genes in ovarian cancer. $P < 0.05$ was considered statistically significant.

Go And Kegg Pathway Analysis

Gene Set Enrichment Analysis (GSEA) was conducted to reveal the functional annotation of GO and KEGG pathway enrichment analysis by using the open access WebGestalt tool (http://www.webgestalt.org). GO analysis included biological process (BP), cellular component (CC) and molecular functions (MF). Results with the false discovery rate (FDR) ≤ 0. 05 were considered noteworthy.

Target genes prediction of MIR502

miRWalk(http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk/micrornapredictedtarget.html) was applied to forecast the genes targeted by *MIR*502. In total, five servers with DIANA-mT, miRanda, miRWalk, PICTAR5 and Targetscan were used. Only those genes projected by all of five servers were selected as target genes.

Protein-protein Interaction Network Construction

Protein-protein interaction (PPI) network was constructed based on the overlaps genes that appeared both in predicted genes in miRWalk and overexpression genes in GEPIA by using the Search Tool for the Retrieval of Interacting Genes (STRING, version 11.0, https://string-db.org/) database.

Statistical Analysis

Statistical analysis was performed by using Prism software (GraphPad, CA, USA). Statistical significance of differences between and among groups was assessed using the t-test. Significant differences are indicated as follows: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Results

The expression level of MIR502 was lower in ovarian cancer tissue comparing with normal ovary tissue

In order to explore the difference of microRNAs expression in human ovarian cancer tissue, we obtained two microarray gene profiling datasets (GSE83693 and GSE119055) from public GEO datasets of NCBI. The detailed information about two datasets were showed in Table 1. After analyzing the expression of microRNAs, we screened out 39 and 25 differentially expressed genes (DEGs) from GSE83693 and GSE119055 datasets respectively, which were showed in volcano plots (Fig. 1A,1B). Seven common DEGs were screened out with Bioinformatics and Evolutionary Genimocs (http://bioinformatics.psb.ugent.be/webtools/Venn/) (Fig. 1C) and listed on Fig. 1D.

Table 1
Features of the enrolled datasets

Accession	GPL	Year	Samples		Source
			Control	OC	
GSE83693	GPL22079	2017	4	8	tissue
GSE119055	GPL21572	2019	3	6	tissue
OC Ovarian cancer					

Expression of MIR502 affected the overall survival in OC patients

After analyzing overall survival in OC patients by using Kaplan-Meier plotter, we found that only the different expression of *MIR*502 ($p < 0.01$) and *MIR*532($p = 0.013$) affected the overall survival outcome among seven microRNAs (Fig. 2A-2G). The researches about the interaction between *MIR*532 and ovarian cancer have made headway, but relatively few studies demonstrated the mechanism of *MIR*502 in OC, so our object of the study was *MIR*502.For obvious view, we presented box-plot to show the expression of *MIR*502 in each database (Fig. 2H,2I).

Correlated genes with MIR502 in ovarian cancer

The volcano plot showed the positive and negative correlation genes with *MIR*502 (Fig. 3A). The top 50 significant gene sets with positive and negative correlation to *MIR*502 were shown in the heat map (Fig. 3B,3C). This result demonstrated a widespread influence of *MIR*502 on the transcriptome.

MIR502 was closely related to CLCN5

CLCN5 showed the strongest positive correlation with *MIR*502 in Fig. 3B and Fig. 4B (Pearson-correlation = 0.6512, $P < 0.01$). For further exploration, we found *MIR*502 hosting in the third intron of the CLCN5 gene by searching NCBI (Fig. 4A). The expression level of CLCN5 in OC was lower than that in normal ovary tissue (Fig. 4C), what was consistent with *MIR*502. JASPAR (http://jaspar.genereg.net/) database were used to analyze and predict the transcription factors that potentially regulated the expression of CLCN5.

By matching the 2000 bp region of nucleotide sequence upstream of the promoter of CLCN5 gene, we found transcription factor NRF1 was the highest matched (Fig. 4D).

Nrf1 Acted As Transcription Factor Of Clcn5

Match score and binding site of NRF1 were showed in Fig. 5A. Expression of NRF1 is positively correlated with CLCN5(Pearson-correlation = 0.33, $P < 0.01$) (Fig. 5B). We used Cistrome Data Browser (http://cistrome.org/db) database to analyze the ChIP-seq data of tumor cells, we found that NRF1 emerged DNA binding peak in the promoter region of CLCN5 (Fig. 5C). It is further confirmed that NRF1 bound and regulated CLCN5 expression as a transcription factor.

GO and KEGG pathway analysis of correlated genes with MIR502 **in ovarian cancer**

GO term analysis was given a broad overview by using Go Slim (Fig. 6). Results indicated that these genes could be categorized into several important biological processes, including biological regulation, metabolic process, membrane, nucleus, protein binding and ion binding. Significant GO terms were performed more detailedly by GSEA, showing that genes correlated with *MIR*502 were located mainly in protein localization to endoplasmic reticulum (GO:0070972) and translational initiation (GO:0006413)for BP, ribosome(GO:0005840) and tertiary granule(GO:0070820) for CC, and structural constituent of ribosome(GO:0003735) and pattern recognition receptor activity(GO:0038187)for MF (Table 2). The KEGG pathway analysis showed that correlated genes enriched in various pathways (Figuer7A), including ribosome, allograft rejection pathways, systemic lupus erythematosus and so on. It should be noted that Hippo signaling pathway also appeared in the enrichment results. The detailed signaling pathway diagram is showed in Fig. 7B. The correlated genes with *MIR*502 is marked red. The significant enrichment results were showed in Fig. 8.

Table 2
Enriched GO and KEGG items

Enriched Category	Description	Count	NES	P-Value	FDR
Biological process					
GO:0070972	protein localization to endoplasmic reticulum	135	-2.628	0	0
GO:0006413	translational initiation	179	-2.581	0	0
GO:0034341	response to interferon-gamma	187	2.395	0	0
Cellular components					
GO:0005840	ribosome	216	-2.309	0	0
GO:0070820	tertiary granule	155	2.238	0	0
GO:0042581	specific granule	152	2.129	0	0
Molecular function					
GO:0003735	structural constituent of ribosome	152	-2.666	0	0
GO:0038187	pattern recognition receptor activity	20	1.992	0	0.009
GO:0019843	rRNA binding	58	-1.971	0	0.004
KEGG pathway					
hsa03010	Ribosome	129	-2.728	0	0
hsa05322	Systemic lupus erythematosus	122	2.255	0	0
hsa04390	Hippo signaling pathway	148	-1.778	0	0.035
Table shows three items each from GO-BP, GO-CC, GO-MF and KEGG					

MIR502 **regulated CCND1, FGF1, MYC and GLI2**

Our study showed that six popular genes with the function of anti-apoptosis and pro-proliferation participated in the Hippo signaling pathway, including CCND1, FGF1, MYC, GLI2, AFP and AXIN2(Fig. 7B). The LinkedOmics database was used to confirm the correlation between six genes and *MIR*502.The results indicated that *MIR*502 regulated CCND1(Pearson-correlation=-0.2092,$p < 0.01$), FGF1(Pearson-correlation=-0.1955, $p < 0.01$), MYC(Pearson-correlation=-0.1448, $p < 0.05$) and GLI2(Pearson-correlation=-0.1395, $p < 0.05$) negatively(Fig. 9).

CCND1 and MYCN were at core position in PPI network

860 common genes were selected as predicted target genes of *MIR502*(Fig. 10A). A total of 44 genes were selected in the overlaps area of 860 predicted target genes and 1501 overexpression genes in GEPIA of ovarian cancer (Fig. 10B). PPI network revealed CCND1 and MYCN at core position (Fig. 10C). NRAS, PMAIP1 and MYBL2 showed interaction relationship with both CCND1 and MYCN.

Discussion

The aim of our study was to distinguish miRNAs that were obviously different expression in OC comparing with normal tissue, and to improve ovarian cancer patients' clinical survival outcome by exploring the mechanism attending in particular pathway. We selected *MIR*502 as our interested miRNA after screening miRNAs through strict selection process. Our survival analysis showed that *MIR*502 conferred a protective phenotype to OC patients in which higher expression of *MIR*502 predicted longer overall survival. *MIR*502 located in the third intron of the CLCN5 gene, and it showed strong positive correlation with CLCN5 in ovarian cancer, we predicted NRF1 as a transcription factor of CLCN5, and ChIP-seq data of various tumor cells verified the binding peak between NRF1 and CLCN5. We demonstrated NRF1 as transcription factor of CLCN5 regulated the expression of *MIR502* indirectly, which expounded the upstream regulatory mechanism of *MIR*502 deeply.

In order to explore downstream regulatory mechanism of *MIR*502 in the process of ovarian cancer, we further predicted and analyzed genes correlated with *MIR*502. We identified a set of biological functions and related signaling pathways which *MIR*502 might regulate in ovarian cancer. Furthermore, GSEA annotation analysis results showed that *MIR*502 regulated anti-apoptosis and pro-proliferation genes such as CCND1, FGF1, MYC, GLI2 negatively in Hippo signaling pathway. All the results demonstrated strongly that the expression of *MIR502* was down regulated in OC, which increased the expression level of oncogene CCND1, FGF1, MYC and GLI2, they served an important function of anti-apoptosis and promote development of OC. PPI network also suggested that CCND1 and MYCN were both target genes regulated by *MIR502*, and they were at the center position interaction with other proteins.

CCND1,also known as cyclin D1, is a member of cell cycle family protein [26]. CCND1 regulates cell cycle progression by promoting the cell cycle transition from the G1 to S phase [27–29]. The abnormal expression of CCND1 promotes cell proliferation by regulating cell cycle.[30]. Previous researches have demonstrated that CCND1, identified as a proto-oncogene, served as an essential role in the development of many kinds of tumors, including lung adenocarcinoma, glioma and renal cell cancer[31–33]. In addition, some studies have shown that overexpression of CCND1 promoted tumor cell invasion and metastasis in breast cancer gastric cancer, leading to a poor prognosis[34, 35]. Compared with normal tissues, the expression of CCND1 were obviously higher in bladder cancer, reproductive system tumors, gastric cancer and lung cancer, which were correlated with the pathological type and clinical stage of the tumor[36–38] .CCND1 expression was closely related with cell proliferation ability and apoptosis in epithelial ovarian cancer cells. A study of epithelial ovarian cancer observed that overexpression of CCND1 leads to stronger cell growth ability and fewer apoptosis[39]. In our study, *MIR*502 is down regulated in ovarian cancer, and CCND1 was negatively correlated with *MIR502*, which means the

expression of CCND1 is overexpressed in OC. In addition, PPI network showed that CCND1 played a core function in interacting with other proteins, further verified the important role of CCND1 in regulating progress of OC. The development of OC may be slowed down by up regulating MIR502, which decreases expression of CCND1 and restrains cell cycle.

The MYC family proto-oncogenes is comprised of *c*-MYC, MYCN and MYCL [40].c-MYC as an oncogene in numerous cancer cells, plays an important role in a myriad of biological processes including cell growth, cell cycle progression and proliferation [41, 42] by cooperating with YAP and activating a great number of target genes[43]. In fact, amplification of *c*- MYC has been reported in ovarian cancer[44].And previous studies showed that higher levels of *c*-MYC expression led faster recurrence and worse overall survival rate of patients with high grade serous ovarian cancer, and was related to cisplatin resistance in ovarian cancer cells. Silencing of *c*-MYC inhibited cell growth of cisplatin-resistant ovarian cancer. Thus, *c*-MYC targeted therapy is a potential treatment for ovarian cancer patients with high expression of *c*-MYC, including those who are resistant to cisplatin. It means that *c*-MYC may act as a new biomarker and therapy target of chemotherapy response. Another member of the MYC family, MYCN, controls the basic process of embryonic development. MYCN signaling disorder leads to a variety of tumors, including neuroblastoma, medulloblastoma, rhabdomyosarcoma, Wilms tumor, prostate cancer and lung cancer. In neuroblastoma, genetic aberration of MYCN amplification is the most related to poor prognosis and failure of therapy. MYCN targeted therapy has been proposed as a new strategy for cancer treatment, and a lot of efforts have been made to develop direct and indirect MYCN inhibitors with potential clinical applications[45].

FGF1 belongs to fibroblast growth factors (FGFs) family, whose function is regulating many cellular processes including cell proliferation, differentiation and survival as an oncogene [46–48].FGF1 is associated with tumor development, as it is upregulated in various cancers, including breast cancer, gliomas and ovarian cancer. The expression of FGF-1 has strong relationship with severity of prognosis and chemoresistance of tumor[49–52]. FGF1 has been considered as a potential prognostic marker for OC[53]. Compared with other family members, FGF1 genetic variation has the most significant correlation with the increased risk of ovarian cancer[54]. In addition, FGF1 expression is also an important determinant of survival and response to platinum chemotherapy. Therefore, the regulation of FGF1 by different mechanisms may play an important role in the development of ovarian cancer[55].Our study suggested that *MIR502* played counter-regulation expression effect on FGF1, *MIR502* low level of expression increases FGF1expression in ovarian cancer, which may lead OC development and platinum chemotherapy resistance.

GLI family zinc finger proteins mediate Sonic hedgehog (Shh) signaling and they exist in embryonic tumor cells as effective oncogenes. The proteins encoded by GLI2 belong to the C2H2-type zinc finger protein subclass of the GLI family. Researchers have found that the expression of GLI2 was regulated by Yap/TAZ, which activated the downstream regulatory factors of Shh signaling and promote proliferation[56]. A large number of evidences implicate that GLI2 regulates the key link of cell cycle. Nagao et al. reported that silencing the expression of GLI2 made the cell cycle stop in G1 phase, which

prevented the growth of osteosarcoma [57]. Similar mechanisms have been reported in human vascular smooth muscle cells[58] and myofibroblasts[59]. Same cases occurred in cervical cancer that overexpression of GLI2 increased proliferation. All researches demonstrated that GLI2 promoted cell proliferation and exerted a tumor-promoting role in cancer. In our study, GLI2 as a downstream molecular of Hippo signaling pathway, highly expressed due to the negatively regulated by *MIR*502, resulting in accelerating the pathological process of ovarian cancer. And GLI2 may be targeted as a novel therapeutic strategy in the future.

In summary, we have discovered that *MIR*502 expression in ovarian cancer was lower than that in normal tissue, which means *MIR*502 acting as a significant tumor-suppressor in ovarian cancer and through K-M analysis, *MIR*502 expression is connected to ovarian cancer patient overall survival outcomes. Additionally, analysis showed that the expression of *MIR*502 was regulated by NRF1 and further exerted apoptosis and inhibiting proliferation by regulating genes downstream of the Hippo signaling pathway including, CCND1, FGF1, MYC and GLI2. In our study, we propose novel mechanisms between *MIR*502 and ovarian cancer that have not been elucidated previously. The immediate application of our findings is that *MIR*502 can be used as prognostic tools in ovarian cancer. The better result is that our research on *MIR*502 in ovarian cancer will promote the more extensive research on the molecular mechanism of *MIR*502 and provide reference for improving the clinical treatment of ovarian cancer.

Conclusion

Our study suggested that *MIR*502 might be modulated by NRF1 and play function as a potential tumor suppressor by regulating Hippo signaling pathway downstream anti-apoptosis and pro-proliferation genes, therefore provided a novel candidate for developing *MIR*502-based therapeutic strategies.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The microRNA expression datasets used in this study (GEO: GSE83693 and GSE119055) were acquired from the National Center for Biotechnology Information (NCBI) Gene expression (http://www.ncbi.nlm.nih.gov/geo/) .

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

W.Y. helped in conception and design and the development of the methodology. L.Y. conducted all analysis, and interpretation of data, and written the manuscript. W.Q. helped in the acquisition of data. N.N. and T.F.L. provided critical revision of final manuscript. All authors read and approved the final manuscript.

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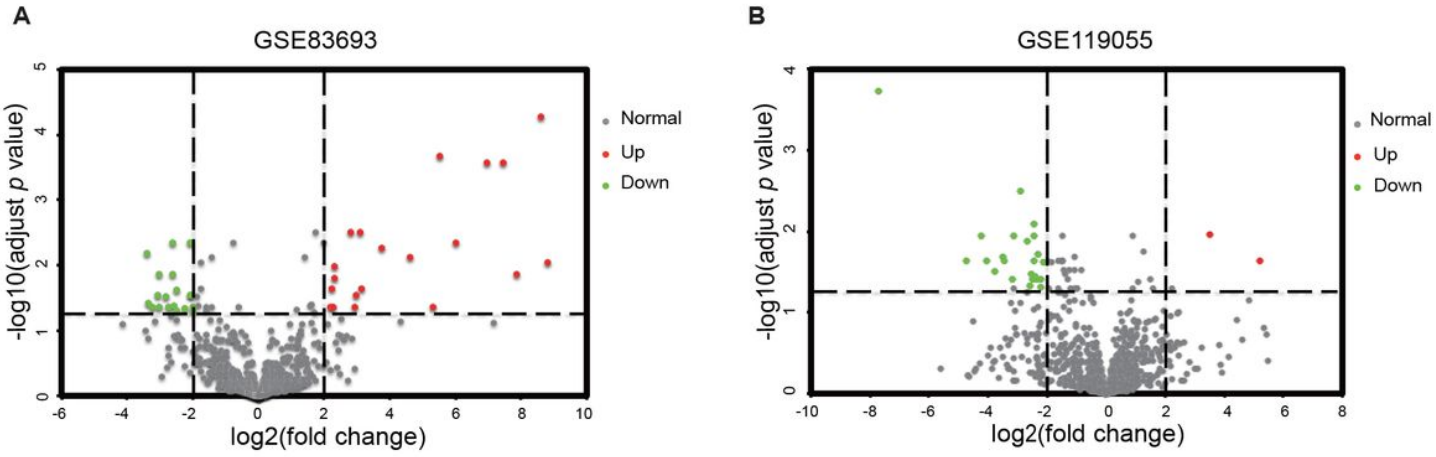
Figures



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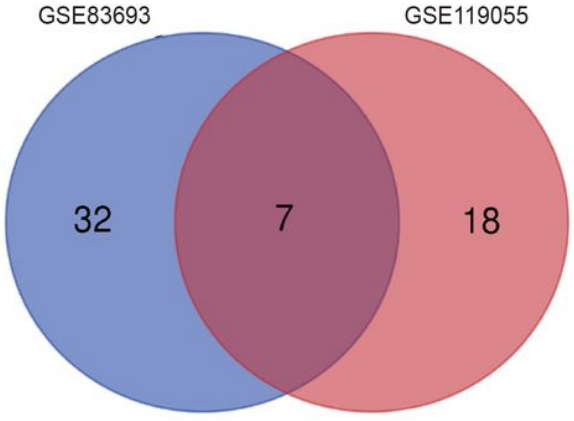


D

Gene Name	GSE83693		GSE119055	
	log_2(FC)	adj.*p*	log_2(FC)	adj.*p*
MIR21	3.090	0.003	3.524	0.011
MIR29C	-2.599	0.005	-3.127	0.011
MIR99A	-2.746	0.043	-2.128	0.024
MIR101	-2.062	0.005	-2.887	0.003
MIR502	-2.585	0.042	-2.235	0.048
MIR532	-2.810	0.030	-2.568	0.046
MIR4324	-3.047	0.029	-4.726	0.023

Figure 1

The expression level of MIR502 is lower in ovarian cancer tissue comparing with normal ovary tissue. A-B Volcano plots of detectable genome-wide miRNA profiles in ovarian cancer tissue and normal ovarian tissue samples from GSE83693 and GSE119055, respectively. Green and red plots represent aberrantly expressed miRNAs with P<0.05 and |log2(FC)|>2. Green plots indicate down-regulated genes, red plots indicate up-regulated genes and grey plots indicate normally expressed miRNAs. C Venn diagram of GSE83693 and GSE119055D Detailed information of seven common different expression miRNAs were listed.

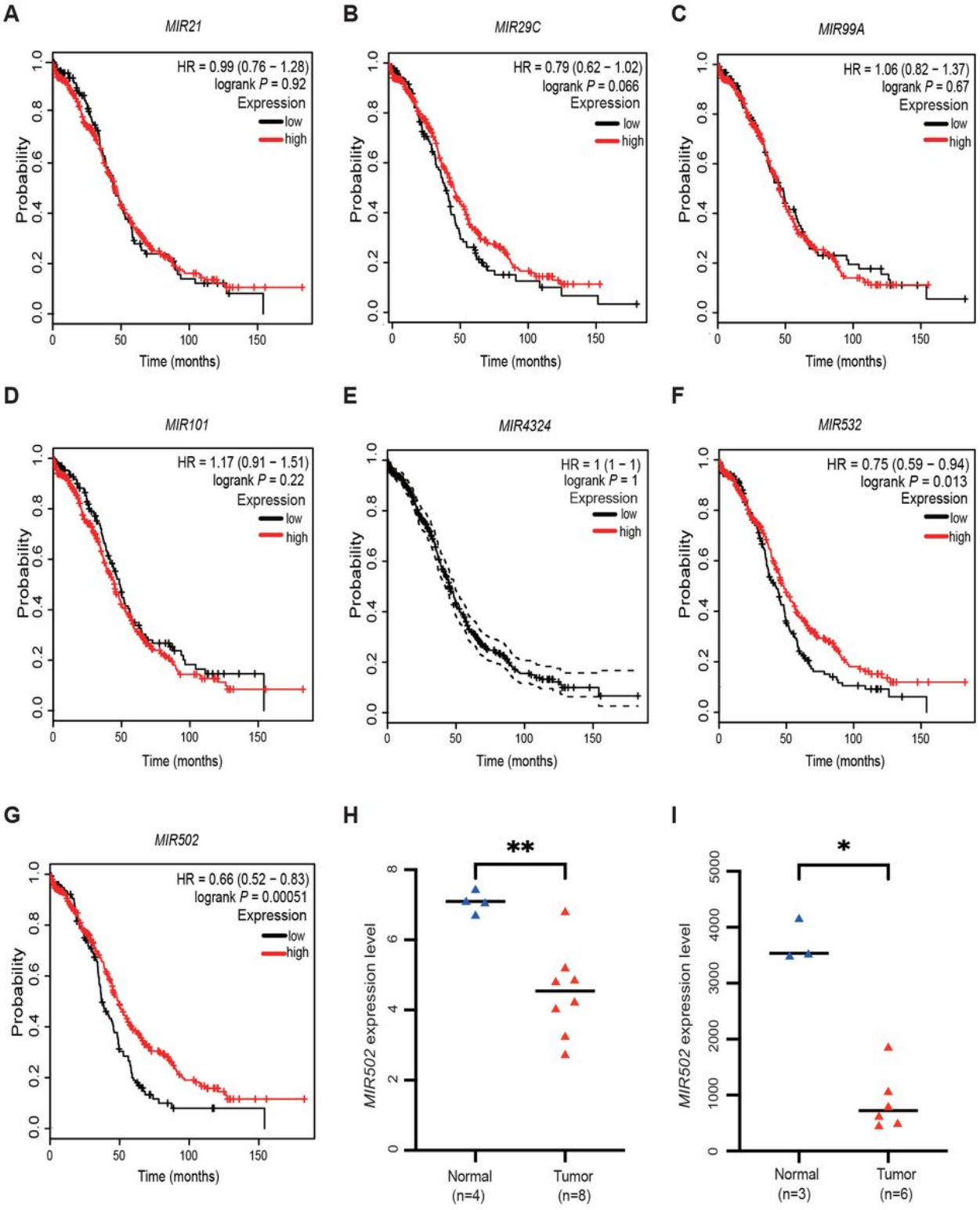


Figure 2

Expression of MIR502 affected the overall survival in OC patients. Kaplan-Meier analysis of overall survival (OS) in OC patients based on K-M plotter dataset. A-E MIR21, MIR29c, MIR99a and MIR4324 expression is not correlated with OS in OC patients. F MIR532 is positive correlated with OS in OC patients, P<0.05.G MIR502 is positive correlated with OS in OC patients, P<0.01.H The expression level of MIR502 in normal and ovarian cancer tissues from GSE83693 (normal tissues, n=4; OC tissues, n=8,P<0.01).I The expression level of MIR502 in normal and ovarian cancer tissues from GSE119055 (normal tissues, n=3; OC tissues, n=6,P<0.05).**P<0.01,*P<0.05.

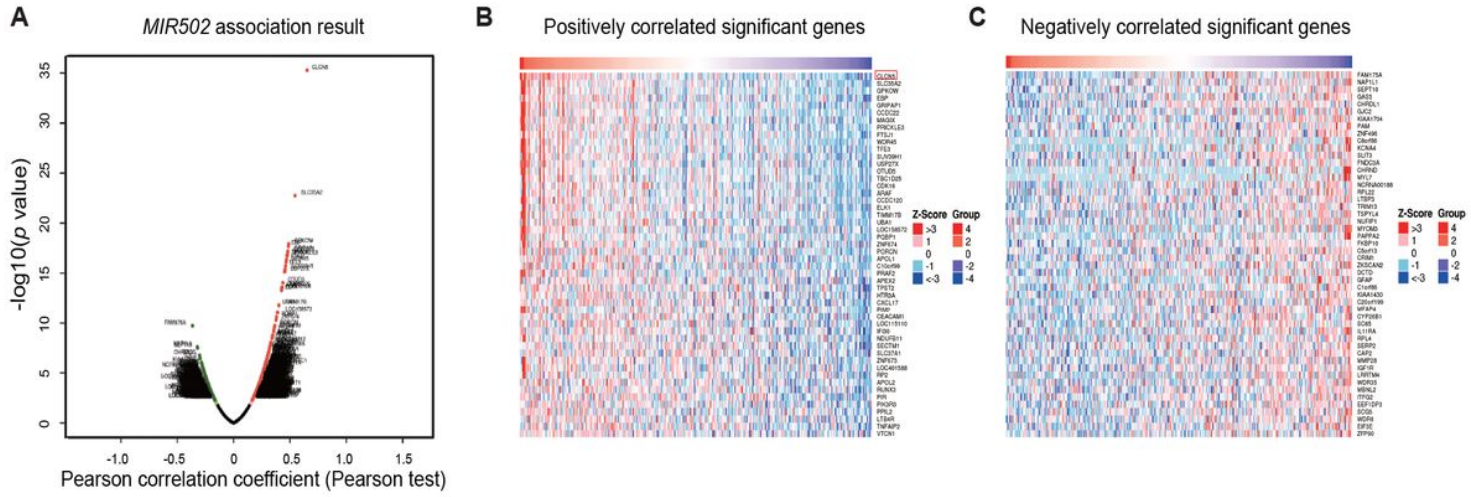
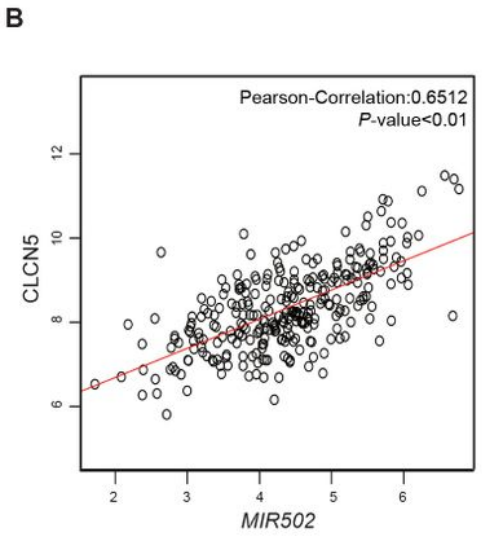
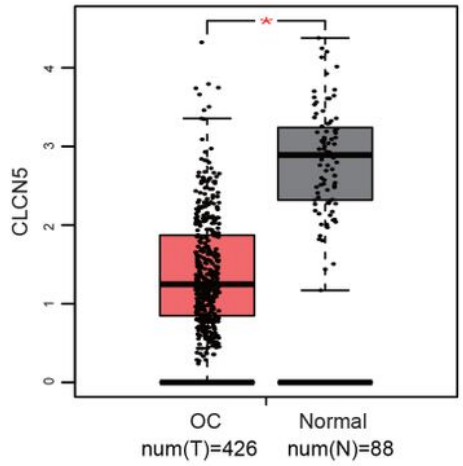


Figure 3

Correlated genes with MIR502 in ovarian cancer. A Pearson test was used to analyze correlations between MIR502 and genes differentially expressed in ovarian cancer. B-C Heat maps showing genes positively and negatively correlated with MIR502 in ovarian cancer (TOP 50). Red indicates positively correlated genes and blue indicates negatively correlated genes.





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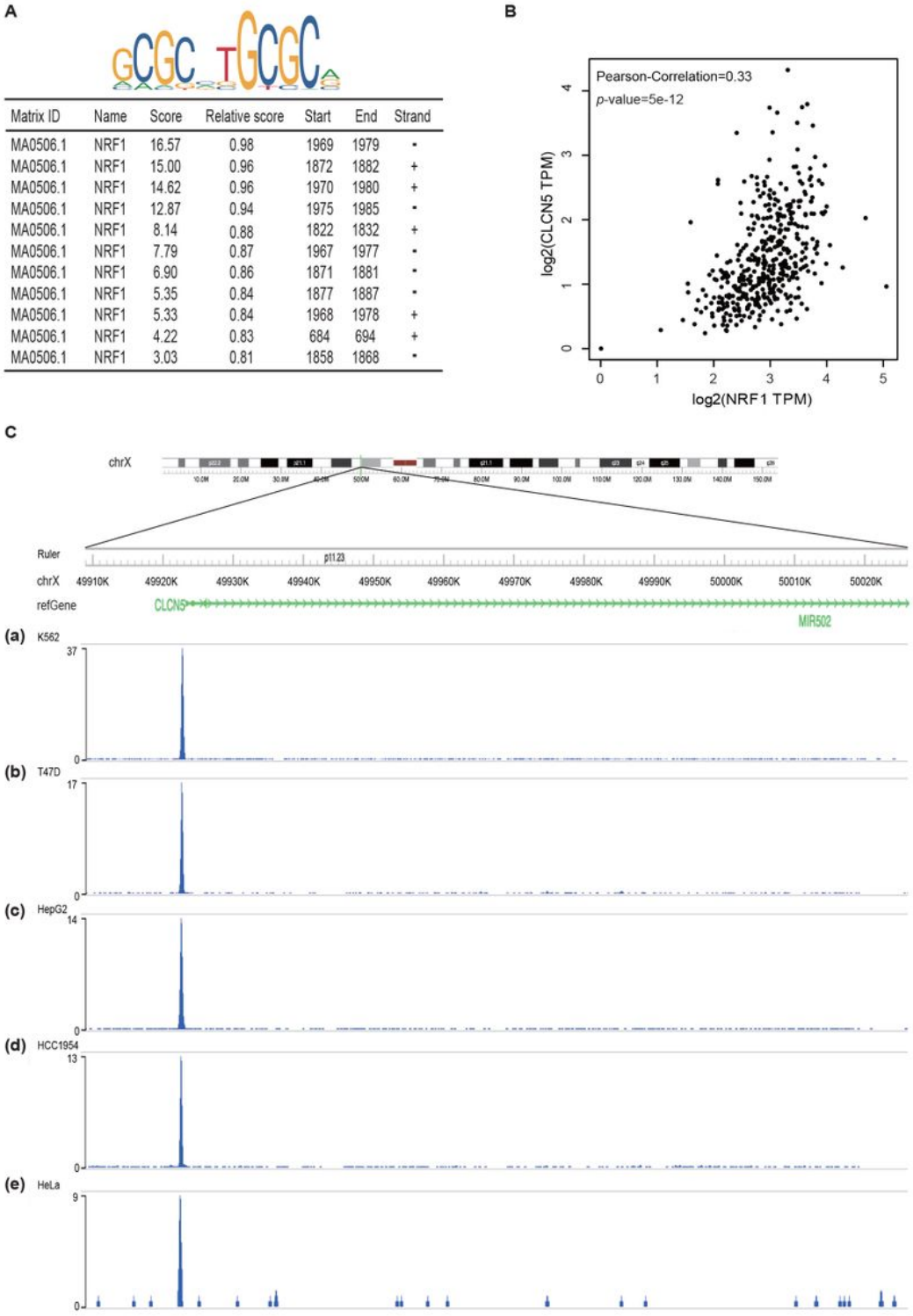
Matrix ID	Name	Score	Relative score	Start	End	Predicted sequence	Sequence logo
MA0506.1	NRF1	15.00	0.96	1872	1882	GCGGCTGCGCA	
MA0658.1	LHX6	14.69	1.00	1157	1166	ACTAATTAGC	
MA0506.1	NRF1	14.62	0.96	1970	1980	GCGCACGCGCA	
MA0684.2	RUNX3	14.35	0.99	47	58	GAAACCTCAATT	
MA0809.2	TEAD4	14.16	0.96	722	733	CAACATTCCACA	
MA0912.2	HOXD3	14.14	1.00	1664	1671	CTAATTAC	
MA0889.1	GBX1	14.07	1.00	1157	1166	ACTAATTAGC	
MA0490.1	JUNB	13.85	0.97	1178	1188	AAGTGAGTCAG	
MA0598.1	EHF	13.79	1.00	224	231	ACTTCCTG	
MA0700.1	LHX2	13.75	0.99	1157	1166	ACTAATTAGC	

Figure 4

MIR502 was closely related to CLCN5. A MIR502 hosted in the CLCN5 gene. B Correlation of the expression levels of MIR502 and CLCN5.C Expression level of CLCN5 in ovarian cancer(number=426) and normal ovary tissues(number=88), $P < 0.05$. D Predicted transcription factors of CLCN5.



Matrix ID	Name	Score	Relative score	Start	End	Strand
MA0506.1	NRF1	16.57	0.98	1969	1979	-
MA0506.1	NRF1	15.00	0.96	1872	1882	+
MA0506.1	NRF1	14.62	0.96	1970	1980	+
MA0506.1	NRF1	12.87	0.94	1975	1985	-
MA0506.1	NRF1	8.14	0.88	1822	1832	+
MA0506.1	NRF1	7.79	0.87	1967	1977	-
MA0506.1	NRF1	6.90	0.86	1871	1881	-
MA0506.1	NRF1	5.35	0.84	1877	1887	-
MA0506.1	NRF1	5.33	0.84	1968	1978	+
MA0506.1	NRF1	4.22	0.83	684	694	+
MA0506.1	NRF1	3.03	0.81	1858	1868	-

Figure 5

NRF1 acted as transcription factor of CLCN5. A The upper part of the picture showed the NRF1 binding sequence, and the lower table showed the prediction of NRF1 binding sites within the promoter region of CLCN5 provided by the JASPAR database. B Positive correlation of the expression levels of CLCN5 and NRF1. C Analysis of CLCN5 ChIP-seq data from K562, T47D, HepG2, HCC1954 and HeLa cells at the CLCN5 promoter from Cistrome Data Browser databases.

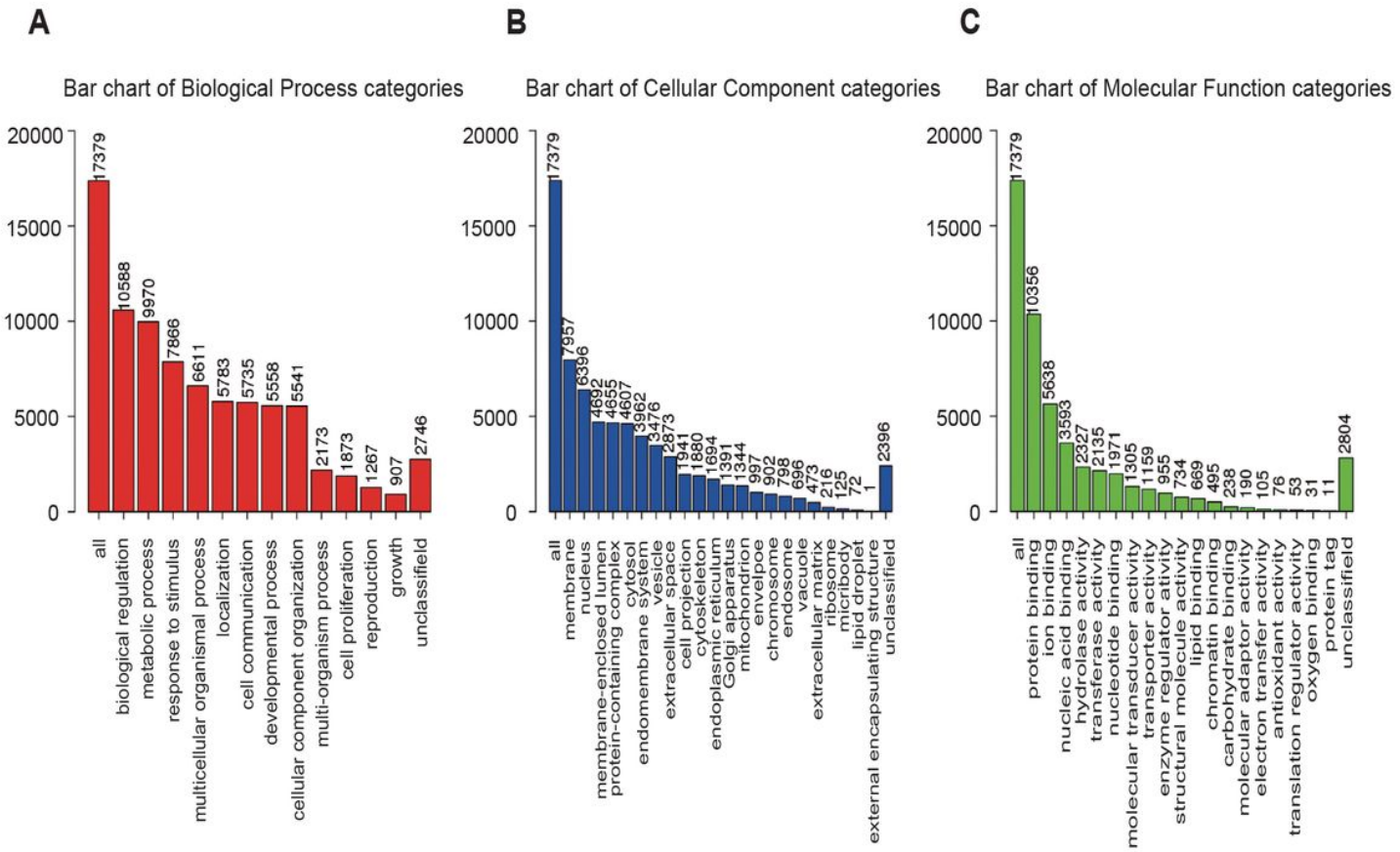


Figure 6

GO term analysis of correlated genes with MIR502 in ovarian cancer. A For biological process categories. B For cellular component categories. C For molecular function categories.

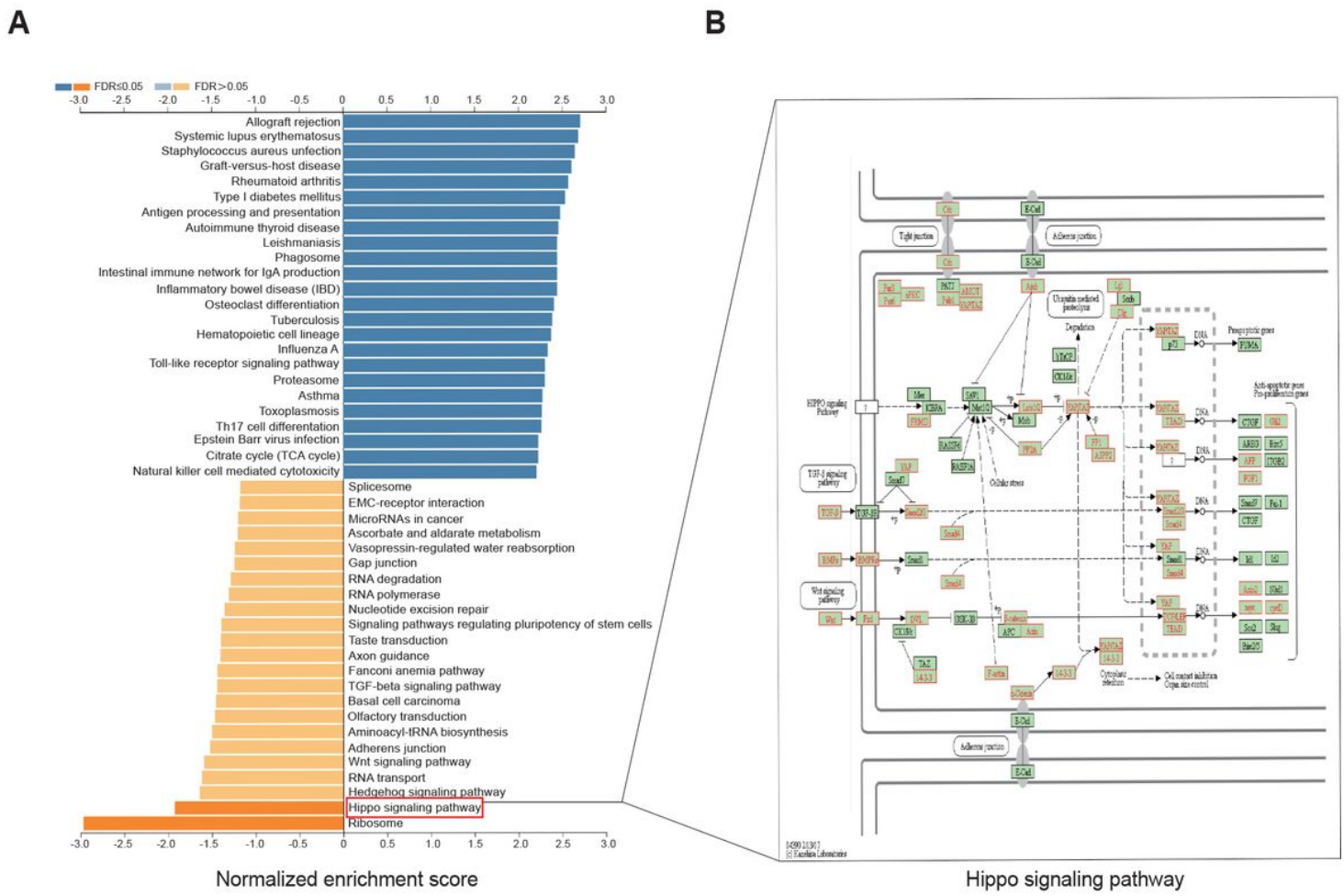


Figure 7

KEGG pathway analysis and Hippo signaling pathway. A Bar of KEGG analysis of MIR502 correlated genes-associated pathways. B Hippo signaling pathway diagram.

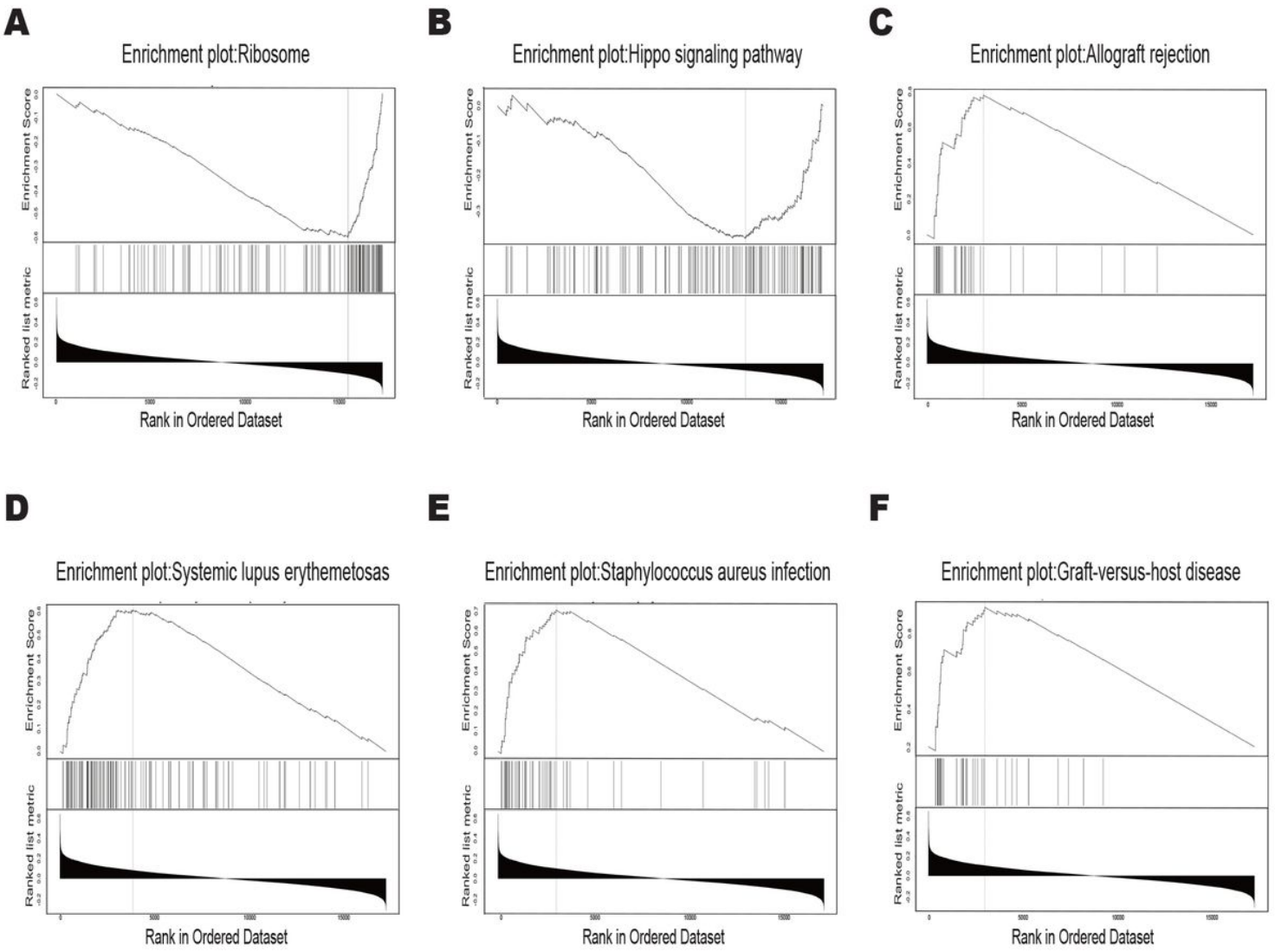


Figure 8

GSEA analysis of MIR502 correlated expressed genes. A Ribosome, NSE=-2.728, P=0. B Hippo signaling pathway, NSE=-1.7788, P=0. C Allograft rejection, NSE=2.188, P=0. D Systemic lupus erythemetosas, NSE=2.255, P=0. E Staphylococcus aureus infection, NSE=2.208, P=0. F Graft-versus-host disease, NSE=2.149, P=0.

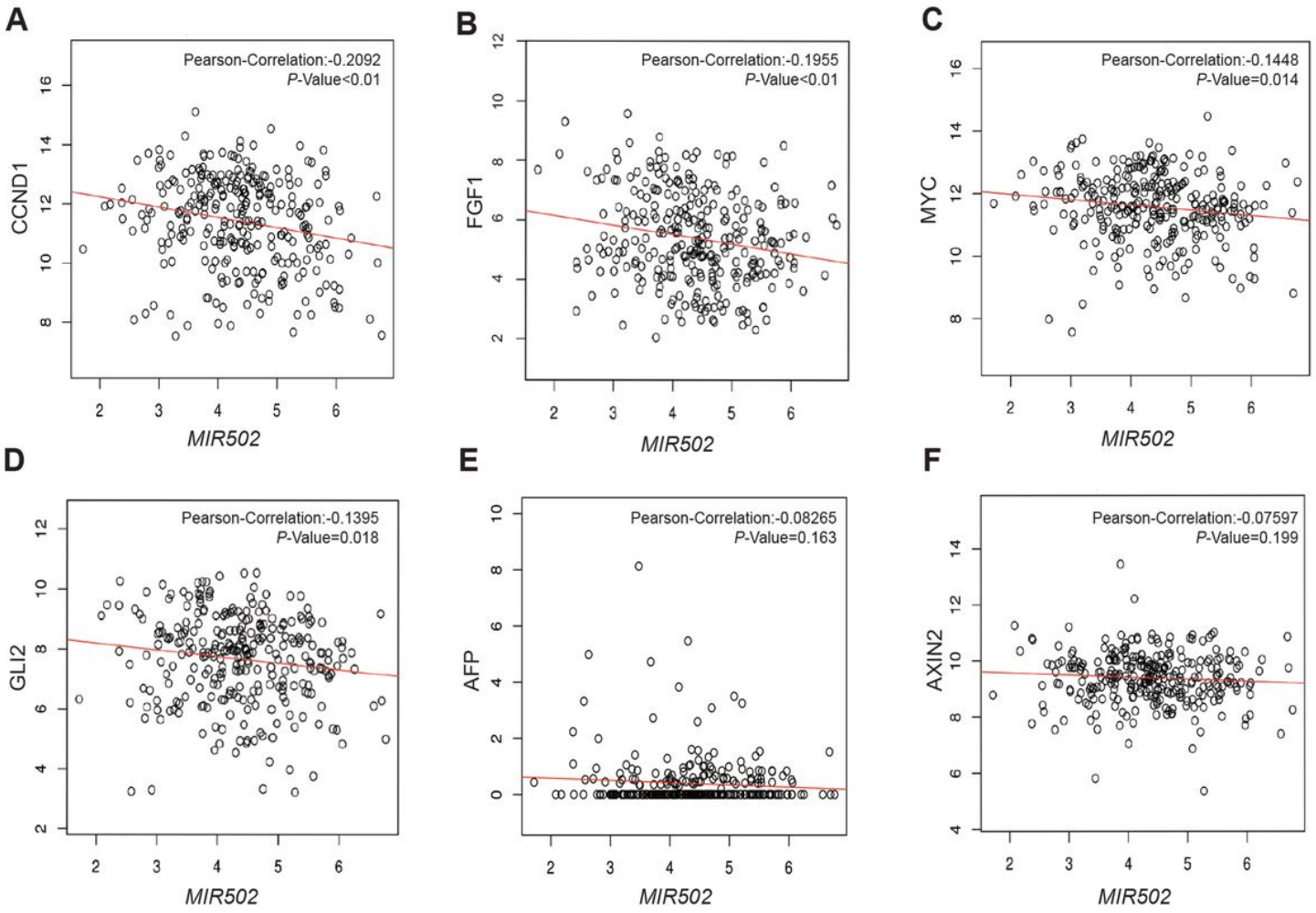


Figure 9

MIR502 regulated CCND1, FGF1, MYC and GLI2.Correlation of the expression levels of MIR502 and Hippo signaling pathway downstream genes, including CCND1, FGF1, MYC, GLI2, AFP and AXIN2.Data were analyzed using Pearson's R correlation. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

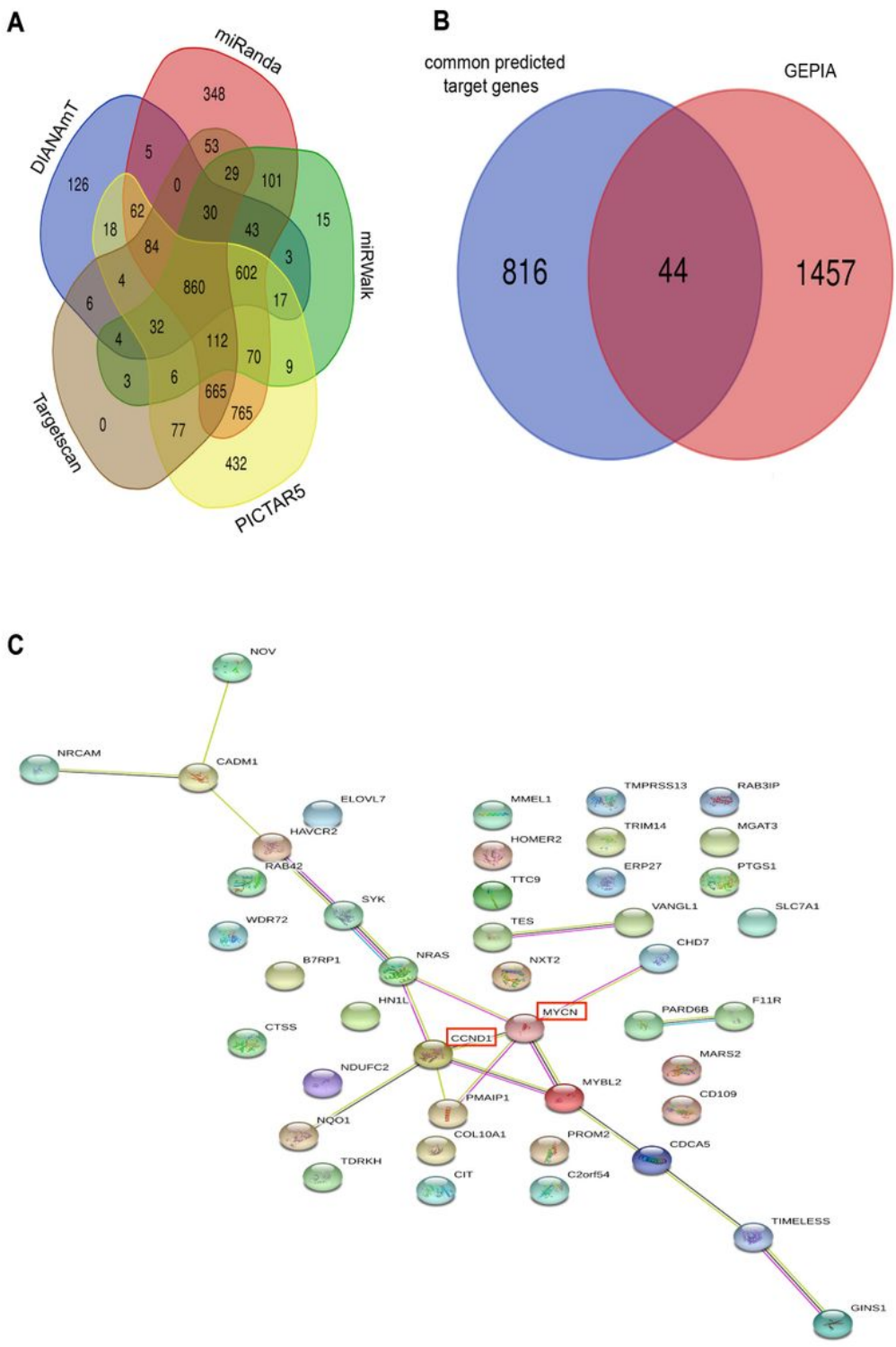


Figure 10

CCND1 and MYCN were at core position in PPI network. A Venn diagram of predicted target genes of MIR502 by using miRanda, miRWalk, PICTAR5, Targetscan and DIANAmT, 860 common genes were selected. B Venn diagram of 860 common predicted target genes and 1501 overexpression genes in ovarian cancer obtained from GEPIA,44 common genes were selected as hub genes. C The protein–protein interaction networks of 44 hub genes of MIR502 in ovarian cancer. Nodes represent gene-encoded

proteins. Connections between nodes represent the relationship between proteins. A bolder line implies a higher confidence level.