The Construction and Analysis of ceRNA Network in H pylori infection atrophic gastritis

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Research Article

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Abstract

Background：Chronic atrophic gastritis (CAG) is an established pre-cancerous lesion of intestinal type gastric cancer(GC),H pylori infection is the main pathogenic cause,this study intends to study the pathogenesis of atrophic gastritis(Hp+) from the lncRNA-miRNA-mRNA ceRNA regulatory network, in order to provide the oretical basis and data support for the treatment of atrophic gastritis.

Results:GSE111762 downloaded from GEO database was used to analyze the differentially expressed lncRNAs and mRNAs(DEGs).A total of 395 differentially expressed lncRNA (225 upregulated,170 downregulated) and 1093 DEGs (674 upregulated, 419 downregulated) are obtained. Through the cross-mapping of miRcode, starBase, Sponescan,miRTarBase and miRBase databases,16 miRNAs were predicted,and the lncRNA-miRNA-mRNA ceRNA regulatory network consisting of 71 lncRNAs,16 miRNAs and 597 mRNAs was constructed.597 DEGs were analyzed by David database for functional enrichment. A total of 250 GO enrichment items were obtained, including 160 BP entries, 48 CC entries and 42 MF entries,29 signal pathways were obtained by enrichment analysis of KEGG pathways, mainly p53 signaling pathway, PI3K-Akt signaling pathway and MAPK signaling pathway. Using Cytoscape plug-in CytoHubba to filter 597 DEGs with "MCC, MNC, Degree" top20 as screening conditions, Eleven key hub targets are obtained from the intersection of jvenn.Protein interaction analysis of key hub targets through Cytoscape plug-in GeneMania, it was found that 87.65% displayed similar co-expression characteristics.Construct ceRNA regulatory network of the key hub targets,11 mRNAs(such as BRCA1, RAD54L),12 miRNAs(such as hsa-miR-340-5p,hsa-miR-182-5p) and 58 lncRNAs(such as PCGEM1,FTX) were predicted.

Conclusions:Clarify the complex reticular regulation of atrophicgastritis with multi-targets, multi-pathways and multi-pathways.Which provides a new idea for the study of the mechanism of action of atrophicgastritis (Hp+) and a potential target for its treatment,thus to further early diagnosis and reversal of gastric cancer.

Introduction

Chronic atrophic gastritis (CAG) is a complex syndrome with gastric atrophy as a common trait. It is slightly symptomatic, affects various aspects of general health, and remains a predisposing factor for gastric cancer[1].Chronic atrophic gastritis (CAG) is an established pre-cancerous lesion of intestinal type gastric cancer which is ranked fifth in the cancer incidence and is the third leading cause of cancer death reported in the global cancer statistics 2018.[2]At present,the clinical treatments of CAG are mainly relying on the synthetic medicine such as vitamin B12 and vitacoenzyme,[3]which remain unsatisfying due to its long course of application,recurrent episodes, invasive and adverse effects.[4]CAG is related to H pylori infection, environmental factors, and genetic factors, among which H pylori infection is the main pathogenic cause of the disease.[5]Hp infection increases oxidative stress by inducing apoptosis and then disrupts cellular integrity and produces inflammation-related tumors[6]. However, Gene expression and the multistage pathological process of Hp-related gastric diseases were not displayed enough formerly. Thus,

to reveal the development of gastric diseases, a systematic understanding of Hp-related gastric precancerous diseases and gene expression alternations in normal mucosa tissues is in urgent need.

Recent studies have verified that less than 2% of the total genome contains protein-coding genes, but non-coding genes exist in most of the human transcriptome [7].Non-coding RNAs (ncRNAs) show higher tissue specificity when compared to protein-coding mRNAs [8,9], thus, functionalizing the mechanism and role of non-coding RNA will undoubtedly lead to further insight into basic physiology and disease progression. Long non-coding RNA(lncRNA),circular RNA (circRNA),and microRNA(miRNA) are functional members of ncRNAs,which are involved in the regulation of a variety of biological behaviors. miRNA can inhibit the translation of target genes by binding to the microRNA response elements (MREs) of mRNAs and initiate their degradation. Recent studies have found that MREs also exist on lncRNAs and circRNAs [10]. It is likely that miRNAs can bind to multiple types of RNA. Hence, different types of RNA can bind to the same miRNA through the same MREs,forming lncRNA-miRNA-mRNA or circRNA-miRNA-mRNA competitive endogenous RNA(ceRNA) regulation networks to regulate the physiopathological processes.

This study intends to study the pathogenesis of atrophic gastritis from the lncRNA-miRNA-mRNA regulatory network, in order to provide theoretical basis and data support for the treatment of atrophic gastritis.

Results

Data Acquisition of Differentially Expressed lncRNA and Differentially Expressed mRNA(DEGs)

The actual test covers six atrophic gastritis (Hp+) tissues and three normal mucosa tissues. The differences between lncRNA and mRNA are analyzed according to the screening criteria of logFC > 1 or <-1, and Pvalue < 0.01. A total of 395 differentially expressed lncRNA (225 upregulated,170 downregulated) and 1093 DEGs (674 upregulated, 419 downregulated) are obtained. The volcano map and heat map of differentially expressed lncRNA and DEGs are made by using GraphPadPrism8 and omicshare (https://www.omicshare.com/tools/) online websites, as shown in figure 1-2,table 1-2.

Table 1.The 5 most significantly down-and upregulated lncRNAs in

normal vs.Hp-GA

Probe Set ID	Gene Symbol	logFC	P.Value	
ASHG19A3A024539	LINC01218	1.859076	0.0000147	UP
ASHG19A3A040889	AC136489.1	1.7846209	0.0000153	UP
ASHG19A3A049987	USP12-AS2	1.5254833	0.0000157	UP
ASHG19A3A040464	AL035443.1	1.6712951	0.0000164	UP
ASHG19A3A009689	AC009720.1	1.5716018	0.0000232	UP
ASHG19A3A014136	LINC01159	-3.0183493	0.000000366	DOWN
ASHG19A3A032524	AC005062.1	-5.580665	0.000145	DOWN
ASHG19A3A037148	AL442638.1	-2.3895796	0.00021	DOWN
ASHG19A3A038529	AL357874.2	-1.9609088	0.000364	DOWN
ASHG19A3A026044	AC093722.1	-3.315502	0.000374	DOWN

Table 2.The five most significantly down-and upregulated DEGs in

normal vs.Hp-GA

Probe Set ID	Gene Symbol	logFC	P.Value	
ASHG19A3A046646	C11orf86	4.3715708	1.71E-08	UP
ASHG19A3A045578	RAB3B	3.4929084	0.00000137	UP
ASHG19A3A024202	NMU	3.795363	0.00000266	UP
ASHG19A3A019518	SLC7A4	2.6607729	0.00000307	UP
ASHG19A3A019541	LCK	1.8638013	0.00000333	UP
ASHG19A3A027870	C5orf38	-5.9437832	0.000000626	DOWN
ASHG19A3A002235	IRX2	-4.9645162	0.00000689	DOWN
ASHG19A3A010352	RNF152	-2.5559105	0.0000117	DOWN
ASHG19A3A050007	MTUS2	-1.5396142	0.0000317	DOWN
ASHG19A3A029772	HLA-DPA1	-1.9953632	0.0000417	DOWN

Pre-test of miRNA bound by differentially expressed lncRNA and DEGs

Pre-test of miRNA bound by differentially expressed lncRNA

The intersection of miRNA predicted by miRcode, starBase and Sponescan databases was obtained by jvenn online software and Venn diagrams were drawn. MiRNA that existed in at least two databases were included in this study. Finally, 22 predicted miRNAs were obtained, as shown in Figure 3A.

Pre-test of miRNA bound by DEGs

The intersection of miRNA predicted by miRTarBase, starBase and miRBase databases was obtained by jvenn online software and Venn diagrams were drawn. MiRNA that existed in at least two databases were included in this study. Finally, 333 predicted miRNAs were obtained, as shown in Figure 3B.

miRNAs of lncRNA-miRNA-mRNA network

The pre-test of miRNAs bound by differentially expressed lncRNA and DEGs draw the venn diagram in jvenn software, and a total of 16 intersecting miRNA are obtained, which are used as the miRNA of the lncRNA-miRNA-mRNA network. They are hsa-miR-421, hsa-miR-423-5p, hsa-miR-485-5p, hsa-miR-491-5p, hsa-miR-542-3p, hsa-miR-543, hsa-miR-615-3p, hsa-miR-18a-5p, hsa-miR-182-5p, hsa-miR-185-5p, hsa-miR-296-3p, hsa-miR-324-5p, hsa-miR-340-5p, hsa-miR-342-3p, hsa-miR-346, hsa-miR-361-5p, as shown in figure 4.A total of 71 lncRNAs were obtained from miRNA of lncRNA-miRNA-mRNA network after de-weighting of differentially expressed lncRNA regulated by miRcode, starBase and Spongescan database, and 597 DEGs were obtained after de-weighting of DEGs regulated by starBase and miRTarBase and miRBase database. 71 differentially expressed lncRNAs and 597 DEGs and 16 miRNAs formed lncRNA-miRNA-mRNA network.

Function enrichment analysis of DEGs in the lncRNA-miRNA-mRNA Network

597 DEGs were analyzed by David database for functional enrichment. A total of 250 GO enrichment items were obtained, including 160 BP entries, 48 CC entries and 42 MF entries. BP is mainly enriched in positive regulation of transcription,DNA-templated,positive regulation of transcription from RNA polymerase II promoter,response to unfolded protein,negative regulation of epithelial cell proliferation;CC is mainly enriched in nucleoplasm, cytosol, nucleus, membrane;MF is mainly enriched in protein binding,transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding,RNA polymerase II core promoter proximal region sequence-specific DNA binding. Take the top 10 positions of Pvalue to draw column chart and bubble chart, as shown in figure 5. 29 signal pathways were obtained by enrichment analysis of KEGG pathways, mainly p53 signaling pathway, PI3K-Akt signaling pathway and MAPK signaling pathway. As shown in figure 6A and table 3. In order to show a smaller subset of high-dimensional data, use sangerbox software to draw the chord diagram of the first five bits of count, as shown in figure 6B, draw PI3K-Akt signaling pathway diagram, as shown in figure 7.

Table 3. KEGG Pathway enrichment analysis of 597 targets in lncRNA-miRNA-mRNA regulatory network for Hp-GA.

Term	Pathway	Count	PValue	Genes
hsa05200	Pathways in cancer	27	0.001305	CEBPA, SPI1, RASSF1, SUFU, RAC3, IKBKG, HRAS, EGLN3, HSP90AA1, SMAD3, PRKCB, PLEKHG5, STAT3, PGF, TGFBR2, AR, CDK6, SMO, CCNE1, TRAF5, CDK2, AGTR1, FAS, PLCB2, FGFR2, FGFR1, PPARD
hsa04151	PI3K-Akt signaling pathway	19	0.059907	PHLPP2, HSP90AA1, FLT1, ITGB5, BRCA1, YWHAZ, EFNA4, PGF, CDK6, CCNE1, CDK2, ITGA8, SPP1, IKBKG, SGK1, IL7R, HRAS, FGFR2, FGFR1
hsa04010	MAPK signaling pathway	17	0.016255	DUSP4, MEF2C, PRKCB, CACNA2D1, HSPA2, ARRB2, ELK1, CDC25B, MAPK8IP1, TGFBR2, IL1B, FAS, RAC3, IKBKG, HRAS, FGFR2, FGFR1
hsa05168	Herpes simplex infection	16	0.001902	CD74, HLA-C, HCFC2, HNRNPK, UBE2R2, IL1B, TRAF5, CDK2, FAS, TAF6, IKBKG, HLA-DOA, TAF3, MYD88, HLA-DPA1, NECTIN1
hsa05166	HTLV-I infection	15	0.063443	IL15RA, SPI1, SMAD3, HLA-C, NFATC2, ELK1, TGFBR2, FOSL1, CDC23, LCK, CHEK2, IKBKG, HLA-DOA, HRAS, HLA-DPA1
hsa05203	Viral carcinogenesis	14	0.028368	HPN, STAT3, HLA-C, GTF2H1, HDAC9, YWHAZ, HNRNPK, CDK6, CCNE1, DNAJA3, TRAF5, CDK2, IKBKG, HRAS
hsa04110	Cell cycle	13	0.001323	SMAD3, CDC7, YWHAZ, CDC25B, CDC23, CDK6, ESPL1, CCNE1, CHEK2, CDK2, SFN, MCM6, E2F5
hsa04550	Signaling pathways regulating pluripotency of stem cells	12	0.010126	MEIS1, SMAD3, ACVR1C, RIF1, STAT3, SMARCAD1, PAX6, LIFR, HRAS, SMAD5, FGFR2, FGFR1
hsa05161	Hepatitis B	12	0.012968	CDK6, CCNE1, PRKCB, STAT3, CDK2, FAS, NFATC2, IKBKG, HRAS, ELK1, YWHAZ, MYD88
hsa04514	Cell adhesion molecules (CAMs)	11	0.027688	CD86, CDH3, PTPRC, ITGA8, HLA-C, CTLA4, HLA-DOA, CD34, CLDN1, HLA-DPA1, NECTIN1

Key target screening for DEGs in lncRNA-miRNA-mRNA network

Using Cytoscape plug-in CytoHubba to filter 597 DEGs in lncRNA-miRNA-mRNA network with "MCC, MNC, Degree" top20 as screening conditions, as shown in figure 8.Eleven key hub targets are obtained from the intersection of jvenn, namely BRCA1, RAD54L, MKI67, ESPL1, KIF23, MCM6, CDK2, RFC4, RACGAP1, PRC1, UBE2C, as shown in figure 9 and Table 4.

Table 4. The key mRNAs in Hp-GA.

mRNA	description	logFC
BRCA1	BRCA1 DNA repair associated	-0.4446247
RAD54L	RAD54 like	-1.2749238
MKI67	marker of proliferation Ki-67	1.7765538
ESPL1	extra spindle pole bodies like 1, separase	1.067037
KIF23	kinesin family member 23	1.1965313
MCM6	minichromosome maintenance complex component 6	1.1970757
CDK2	cyclin dependent kinase 2	-3.3605487
RFC4	replication factor C subunit 4	1.1740276
RACGAP1	Rac GTPase activating protein 1	1.5779255
PRC1	protein regulator of cytokinesis 1	0.6779732
UBE2C	ubiquitin conjugating enzyme E2 C	2.044657

Construct ceRNA regulatory network of the key hub targets

The lncRNA-miRNA-mRNA network predicted 11 mRNAs, 12 miRNAs, and 58 lncRNAs after removing duplicates.The above lncRNAs, miRNAs and mRNAs were visualized in Cytoscape software, as shown in the figure 10 and table 5.

Construction of key hub targets PPI protein interaction network

Protein interaction analysis of key hub targets through Cytoscape plug-in GeneMania. Among the 11 targets and their interacting proteins, it was found that 87.65% displayed similar co-expression characteristics,Others as shown in figure 11.

Discussion

Nonatrophic gastritis, atrophic gastritis (GA), intestinal metaplasia (IM), and dysplasia were included in the pathological process which led to the GC ultimately [11]. In the process above, the risk of Hp-positive GA patients to develop GC is 6.4-11.8 times as high as the noninfected ones [12].Therefore, searching for the GA molecular markers associated with Hp infection is of great significance to the early diagnosis and reversal of GC.

For decades, research has focused on the 2% of the human genome that codes for proteins [13,14]. In recent years,researchers have found that the remaining 98% of the genome that was once considered as

nonfunctional “junk” includes noncoding RNAs (ncRNAs) that play important roles in a wide range of biological processes such as growth,development, and organ function. Non-coding RNAs have been classified as small non-coding RNAs, including microRNAs,and long non-coding RNAs (lncRNAs) [15]. The lncRNAs are more than 200 nucleotide size transcripts,which have been found in nearly all organisms, suggesting that they are a fundamental component of gene expression regulation [16]. Genomic studies have revealed a huge diversity of lncRNAs with different mechanisms of action [17].Studies have reported that the differentially expressed lncRNAs, identified in Hp-infected tissue of GC, could be involved in the development of Hp-related gastric diseases [18]. However, the research on Hp-related transcription and noncoding regulation is still in its infancy.A total of 395 differentially expressed lncRNA were obtained in this study(225 upregulated ,such as PCGEM1,DSCR8,LINC01218,AC136489.1,USP12-AS2,AL035443.1,AC009720.1，170 downregulated such as FTX,LINC01159,AC005062.1,AL442638.1,AL357874.2,AC093722.1).PIAO et al.[19]found that lncRNA PCGEM1 was overexpressed in GC cells.CHEN et al.[20]indicated that DSCR8 could adsorb miR-137 to reduce its inhibitory effect on Cdc42 expression, thereby promoting the progression of GC cells and regulating the cell cycle.CHEN et al.’s [21]meta-analysis found FTX may be a potential oncogene, with high FTX expression being associated with a poorer prognosis in patients with colorectal cancer (CRC), hepatocellular carcinoma (HCC), osteosarcoma (OSC), and glioma.

miRNAs are small noncoding RNA with a length of 21–22 nucleotides. More than 2000 miRNAs have been discovered in humans, many of which have been associated with different physiological and pathological conditions. miRNAs act as repressors of target genes at the posttranscriptional level. Therefore, they can modulate the expression of their target genes in the regulatory networks[22].miRNAs have been widely reported for their role in various human disorders[23].Alexander Link at el.[24]found the expression pattern of miRNAs in the gastric mucosa is gradually increased with progression of Correa’s cascade and H.pyloriinfection, suggesting miRNAs as potential biomarkers for preneoplastic precursor conditions.In this study, there are 16 miRNA in lncRNA-miRNA-mRNA regulatory network,such as hsa-miR-421,hsa-miR-423-5p,hsa-miR-543,hsa-miR-182-5p,hsa-miR-346.Kim Yu Jin et al.’s[25] study suggests that increased CREBZF by hsa-miR-421/hsa-miR-29b-1-5p inhibition may be important to prevent the progression of gastric cancer in its early stage.SHI et al.’s[26] results suggest that in the progression of H. pylori-associated gastric cancer, CagA induces overexpression of miR-543, which subsequently targets SIRT1 to suppress autophagy.

GO and KEGG enrichment analysis of 597 DEGs of lncRNA-miRNA-mRNA regulatory network, a total of 250 GO enrichment items were obtained, including 160 BP entries, 48 CC entries and 42 MF entries. BP is mainly enriched in positive regulation of transcription,DNA-templated,positive regulation of transcription from RNA polymerase II promoter,response to unfolded protein,negative regulation of epithelial cell proliferation;CC is mainly enriched in nucleoplasm, cytosol, nucleus, membrane;MF is mainly enriched in protein binding,transcriptional activator activity, RNA polymerase II core promoter proximal region sequence-specific binding,RNA polymerase II core promoter proximal region sequence-specific DNA

binding.29 signal pathways were obtained by enrichment analysis of KEGG pathways, mainly p53 signaling pathway, PI3K-Akt signaling pathway and MAPK signaling pathway.

PI3K/AKT pathway is the main signal pathway of downstream of many growth factor receptors and the most active signal pathway in human tumors. It also promotes the proliferation and malignant transformation of tumor cells through phosphorylation of PI3K and AKT protein and inhibits the apoptosis of tumor cells. More and more studies have reported that PI3K/AKT signaling pathway regulates the growth, apoptosis and invasion of tumor cells in malignant tumors of digestive system[27].Previous studies p-PI3K can activate Akt to form p-Akt, and then participate in the process of gastric cancer development and development[28,29,30,31,32].ZHANG et al.[33] validated that YARS exerted its malignant roles in GC through activated PI3K-Akt signaling.KONG et al.'s[34] experiments showed that NOLC1 overexpression accelerated proliferation, migration, invasion, and cyclin B1 expression and inhibited the apoptosis and cleaved-caspase-3 expression of Esophageal carcinoma (ESCA) cells via activating PI3K/AKT pathway. CHENG et al.[35]found blockage of Osteopontin (OPN) downregulates the activation of the PI3K-AKT-GSK/3b-b/catenin pathway, accompanied by the inhibition of CRC stem cell proportion. LI et al.'s[36] findings indicate that scoparone inhibits pancreatic cancer cell proliferation in vitro and in vivo, inhibits migration and invasion, and induces cycle arrest and apoptosis in vitro through the PI3K/Akt signaling pathway.As a digestive system disease, we speculate that PI3K/AKT signaling pathway is also involved in the pathogenesis of atrophic gastritis.

The 597 DEGs in the lncRNA-miRNA-mRNA regulatory network were screened by "degree, MNC, MCC" top20 in cytoHubba software, and then intersected to obtain 11 key hub targets, namely BRCA1, RAD54L, MKI67, ESPL1, KIF23, MCM6, CDK2, RFC4, RACGAP1, PRC1, UBE2C.BRCA mutations are defined as pathogenic variants in either the BRCA1 or BRCA2 gene: These two tumour suppressor genes are involved in different crucial pathways including DNA repair, cell proliferation control and apoptosis. In particular, BRCA1 and BRCA2 genes are mainly involved in the homologous recombination (HR) process, responsible for maintenance of genome integrity through an error-free repair pathway for DNA double-strand breaks in response to DNA damage[37]. Loss-of-function mutations in BRCA1/2 may lead to accumulation of DNA double-strand breaks and result in genomic instability and tumour development.BRCA1/2mutation carriers are known to have a higher risk of developing breast and ovarian cancer[38].Over the last decade, awareness of the BRCA1 and BRCA2 genes in the oncological community has led to more aggressive patient screening and the subsequent discovery of other BRCA-related alignancies. Cancer of the prostate, pancreas, gallbladder, bile duct and stomach, along with malignant melanoma, have all been described at increased incidences in BRCA mutation carriers.BRCA mutations are the most common germline genetic alterations known to occur in pancreatic cancer (PC), inherited in an autosomal dominant pattern with incomplete penetrance[39].Recently,Hännimen et al. [40] and Quaas et al.[41] highlighted a possible role of BRCA1 and BRCA2 mutations in small bowel cancer pathogenesis.DU et al.'s[42] study highlights BRCA1 as an independent rognosticator of early-stage colon cancer.Mauri et al[43] suggested that BRCA1/2 mutations might determine an increase in colorectal cancer(CRC) diagnoses, particularly in young patients.More and more reseachs indicate a positive correlation of

germline BRCA1/2 mutations and an increased GI cancer risk[37].BRCA1/2 variant assessment will soon become one of the most promising fields of research in these kinds of diseases.KEGG enrichment analysis showed that BRCA1 was enriched in PI3K/Akt signaling pathway. Combined with ceRNA network, we deduced that the expression of lncRNA PCGEM1-miRNA hsa-miR-340-5p-mRNA BRCA1 may regulated by PI3K/Akt signaling pathway induced atrophic gastritis.

By integrating and analyzing the expression differences of atrophic gastritis (Hp+) mucosa tissues lncRNA and mRNA, and successfully constructing the ceRNA regulatory network related to lncRNA through prediction, it is revealed that 58 lncRNAs may participate in the pathogenesis of atrophic gastritis by indirectly regulating 11 key differentially expressed mRNA and related pathways through 12 miRNA, to clarify the complex reticular regulation of atrophicgastritis with multi-targets, multi-pathways and multi-pathways.Which provides a new idea for the study of the mechanism of action of atrophicgastritis (Hp+) and a potential target for its treatment,thus to further early diagnosis and reversal of gastric cancer.

Nevertheless, some limitations to our study should be acknowledged. First of all, we cannot avoid that our research results are based on retrospective analysis, and further experimental studies are needed to verify this regulatory effect in the future. In the future, more studies such as dual luciferase reporting experiments will be designed to verify the biological function of the ceRNA regulatory network model in vivo and in vitro.

Conclusion

In summary, we successfully construc the lncRNA-miRNA-mRNA ceRNA regulatory network,Which provides a new idea for the study of the mechanism of action of atrophicgastritis (Hp+) and a potential target for its treatment,thus to further early diagnosis and reversal of gastric cancer.

Data And Methods

Data Source and Selection

In the high-throughput gene expression(Gene Expression Omnibus,GEO) database(https://www.ncbi.nlm.nih.gov/geo/)),the key words are "Atrophic gastritis" and "Homosapiens".Download the chip GSE111762(platform GPL15314,Arraystar Human LncRNA microarray V2.0 (Agilent-033010 Probe Name version)) from the GEO database according to the search results. Four normal mucosa tissues,six atrophic gastritis (Hp+) tissues and six gastric cancer (Hp+) tissues is included in the data set, and finally three normal mucosa tissues,six atrophic gastritis (Hp+) tissues are selected.

Methods (The research process of this research is shown in figure 11.)

Data Acquisition of Differentially Expressed lncRNA and Differentially Expressed Genes(DEGs)

The chip downloaded from GEO database was used to analyze the differentially expressed lncRNA and DEGs, and the screening standard was set to p.Value <0.01, |log2foldchange (FC)|>1.The differentially expressed lncRNA and mRNA of atrophicgastritis (Hp+) mucosa tissues and normal mucosa tissues were screened, and the heat map and volcano map of differentially expressed lncRNA and mRNA were drawn. LogFC greater than 0 means up-regulation, otherwise it is down-regulation.

Pre-test of miRNA bound by lncRNAs and mRNAs

According to miRcode database(http://www.mircode.org/),starBase database (https://starbase.sysu.edu.cn/)and Spongescan database (http://spongescan.rc.ufl.edu/), the pre-measured miRNA from at least 2 of the above three databases were included in the study as the miRNA for regulating differentially expressed lncRNA.According to starBase database(https://starbase.sysu.edu.cn/),miRTarBase database(https://mirtarbase.cuhk.edu.cn/)and miRBase database (https://www.mirbase.org/)the pre-measured miRNA from at least 2 of the above three databases were included in the study as the miRNA for regulating DEGs. The miRNA predicted by the differentially expressed lncRNA and mRNA take the intersection as the miRNA in the lncRNA-miRNA-mRNA network, and the differentially expressed lncRNA and DEGs regulated by the intersection miRNA in the previous databases are used as the lncRNA and mRNA in the network.

Function enrichment analysis of DEGs in the lncRNA-miRNA-mRNA Network

GO and KEGG Pathway Enrichment Analysis.Gene Ontology (GO) analysis is the primary bioinformatics tool to unify the characterization of genes and gene products. GO contains three categories of terms, including cellular component(CC), molecular function(MF), and biological process(BP).KEGG is a set of databases containing information about genomes, biological pathways, diseases, and chemicals.DAVID (https://david.ncifcrf.gov/) is a bioinformatics data resource with an integrative bioinformation database and analysis tools and benefits to discover the biological meaning behind genes. DEGs were enriched and analyzed by DAVID for GO and KEGG pathways, respectively.P<0.05 was considered statistically significant.After that, the online drawing software Sangerbox3.0 (http://vip.sangerbox.com/home.html) should be used for visualization.

Key target screening for DEGs in lncRNA-miRNA-mRNA network

Using Cytoscape(3.7.2) plug-in CytoHubba to filter DEGs in lncRNA-miRNA-mRNA network with "MCC, MNC, Degree" top 20 as screening conditions, 20 corresponding core targets are obtained. Venn diagram is made on jvenn(http://jvenn.toulouse.inra.fr/app/example.html) and intersection is taken as the key target.

Construct ceRNA regulatory network of the key hub targets

The ceRNA network of the core targets are constructed by using Cytoscape software, and the connection represents the regulatory relationship between the nodes.

Construction of key hub targets PPI protein interaction network

Protein interaction analysis of key hub targets through Cytoscape plug-in GeneMania.Given a query list, GeneMANIA can list the genes that have shared properties, or function similarly with the original query. The potential candidate target genes were entered into the search bar after selecting Homo sapiens from the organism option, and the results were further collated.After that, the visualization was carried out by using Cytoscape software.

Abbreviations

	description
CAG	Chronic atrophic gastritis
Hp+	H pylori infection
DEGs	differentially expressed mRNA
BP	biological process
CC	cellular component
MF	molecular function
lncRNA	Long non-coding RNA
miRNA	microRNA
MREs	microRNA response elements
ceRNA	competitive endogenous RNA
GO	Gene Ontology
GC	gastric cancer

Declarations

Ethics approval and consent to participate

Not necessary.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Wen-jun BAI designed the research topic, drafted the manuscript,and analyzed the data. Jun-wei LIANG participated in the revision of the manuscript and figures. Xiao-yan WANG and Jun-wei LIANG were involved in the work instruction and financial support.All authors contributed to the article and approved the submitted version.

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Availability of data and materials

The raw data of this study are derived from the GEO data portal (https:// www. ncbi. nlm. nih. gov/ geo/), which is publicly available database.

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Tables

Due to technical limitations, Table 5 is only available as a download in the Supplemental Files section.

Figures

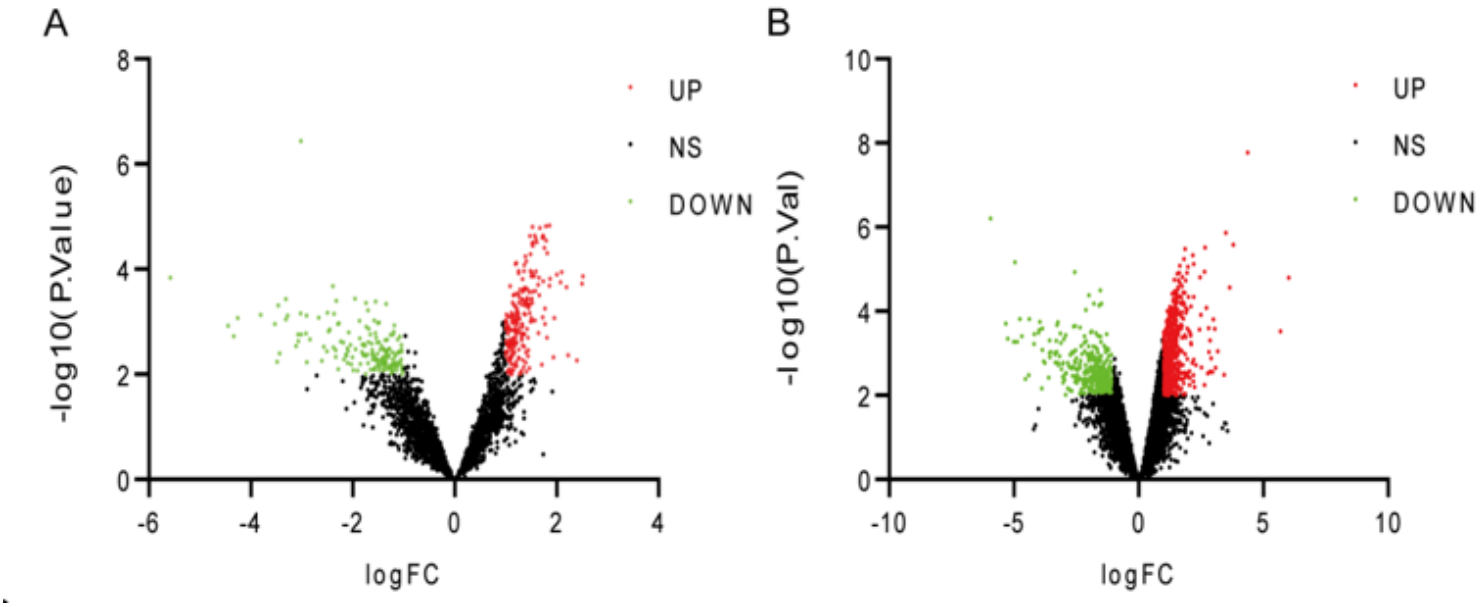

Figure 1

Volcano map of differentially expressed lncRNA and DEGs A Differentially Expressed lncRNA B DEGs Red color is indicative of upregulated genes and green color of downregulated genes,black color indicates genes that are not differentially expressed in a statistically significant manner.

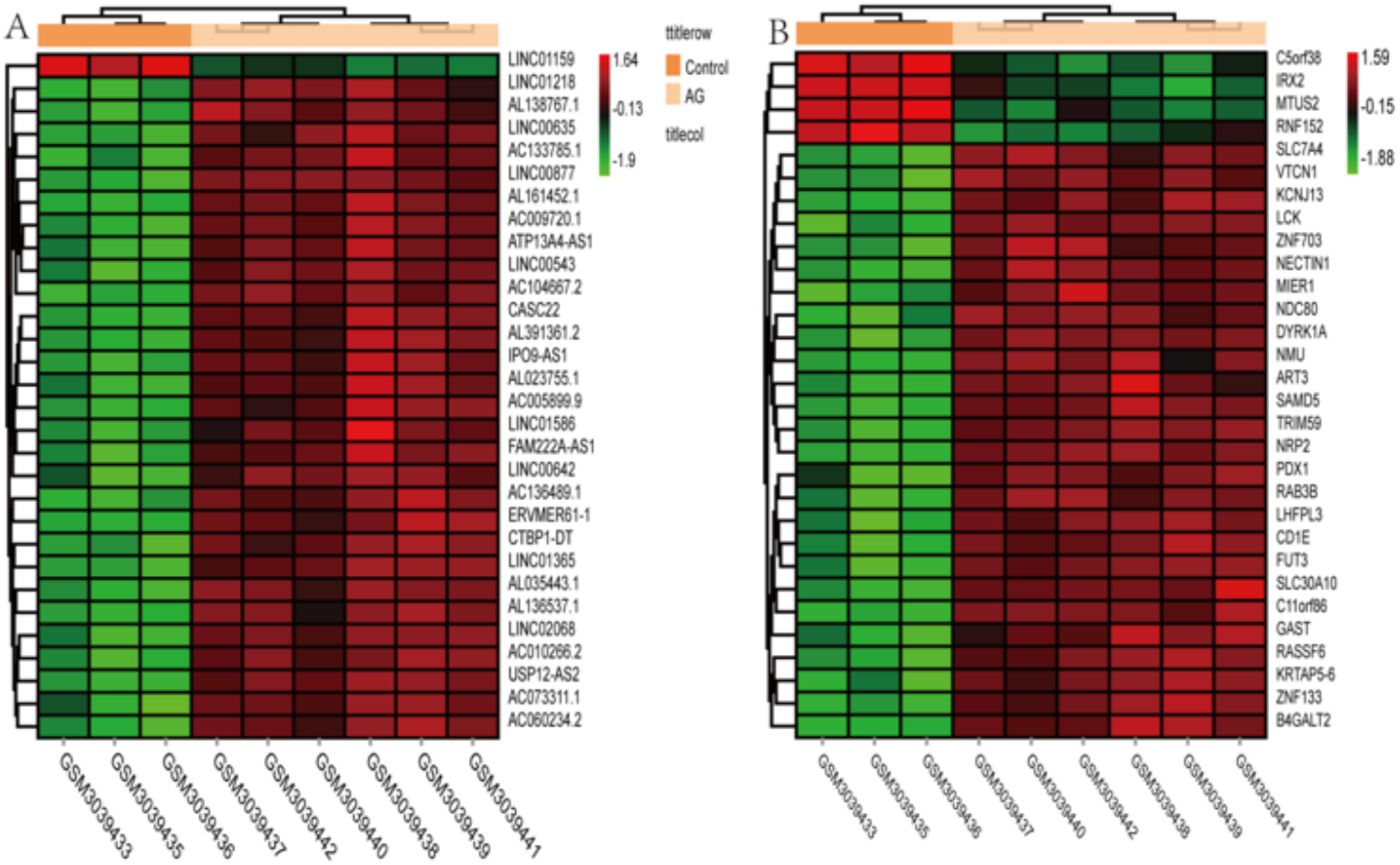

Figure 2

heat map of differentially expressed lncRNA and DEGs A Differentially Expressed lncRNA B DEGs Red color is indicative of upregulated genes and green color of downregulated genes,black color indicates genes that are not differentially expressed in a statistically significant manner .

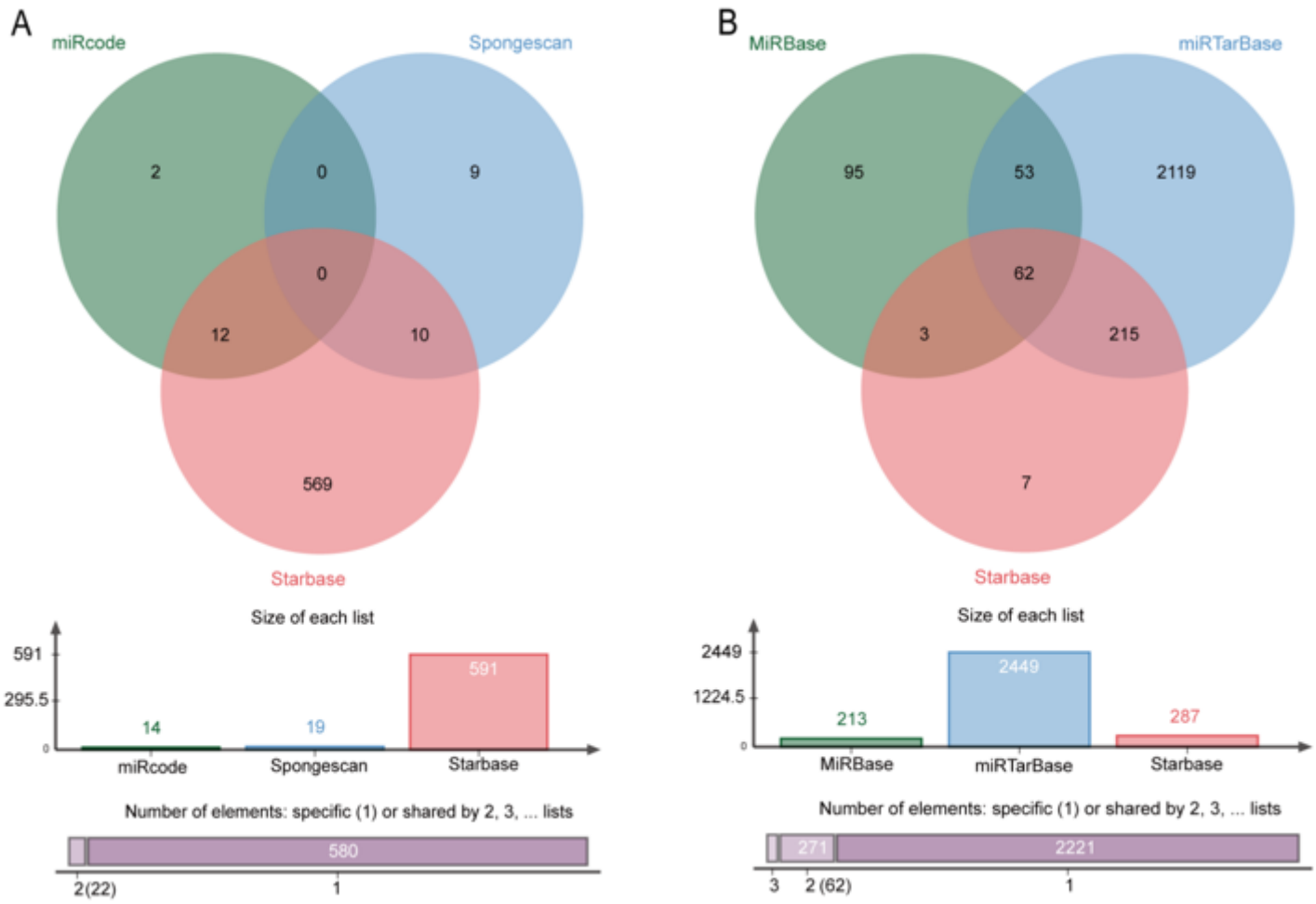

Figure 3

Pre-test of miRNA bound by differentially expressed lncRNA and DEGs A. Pre-test of miRNA bound by differentially expressed lncRNA;B. Pre-test of miRNA bound by DEGs

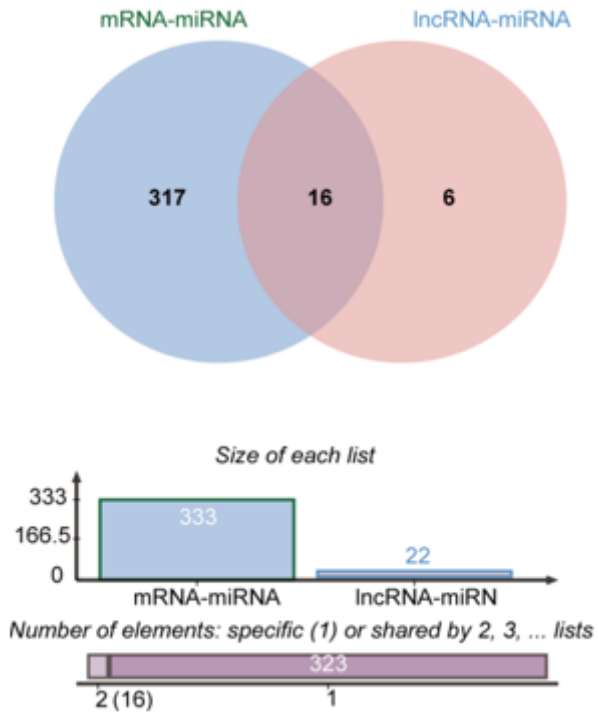

Figure 4

miRNA of lncRNA-miRNA-mRNA network

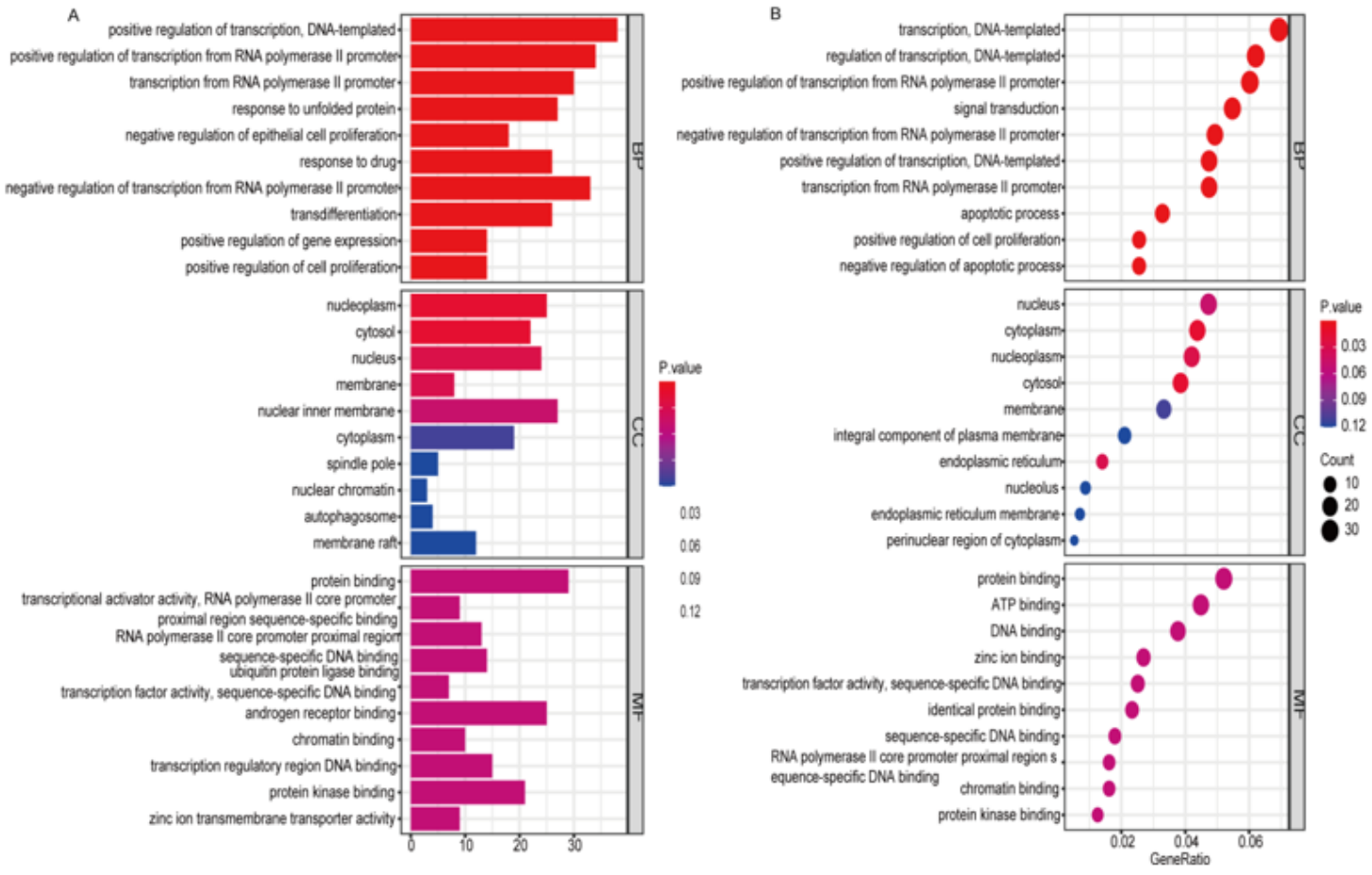

Figure 5

GO enrichment of 597 DEGs A. Histogram for GO enrichment of DEGs in normal vs.Hp-GA ;B.Bubble plot. The gene ratio is assigned to the x -axis and the description of GO to the y-axis. The area of the displayed graphic is proportional to the number of genes assigned to the term,and the color corresponds to the P.value.

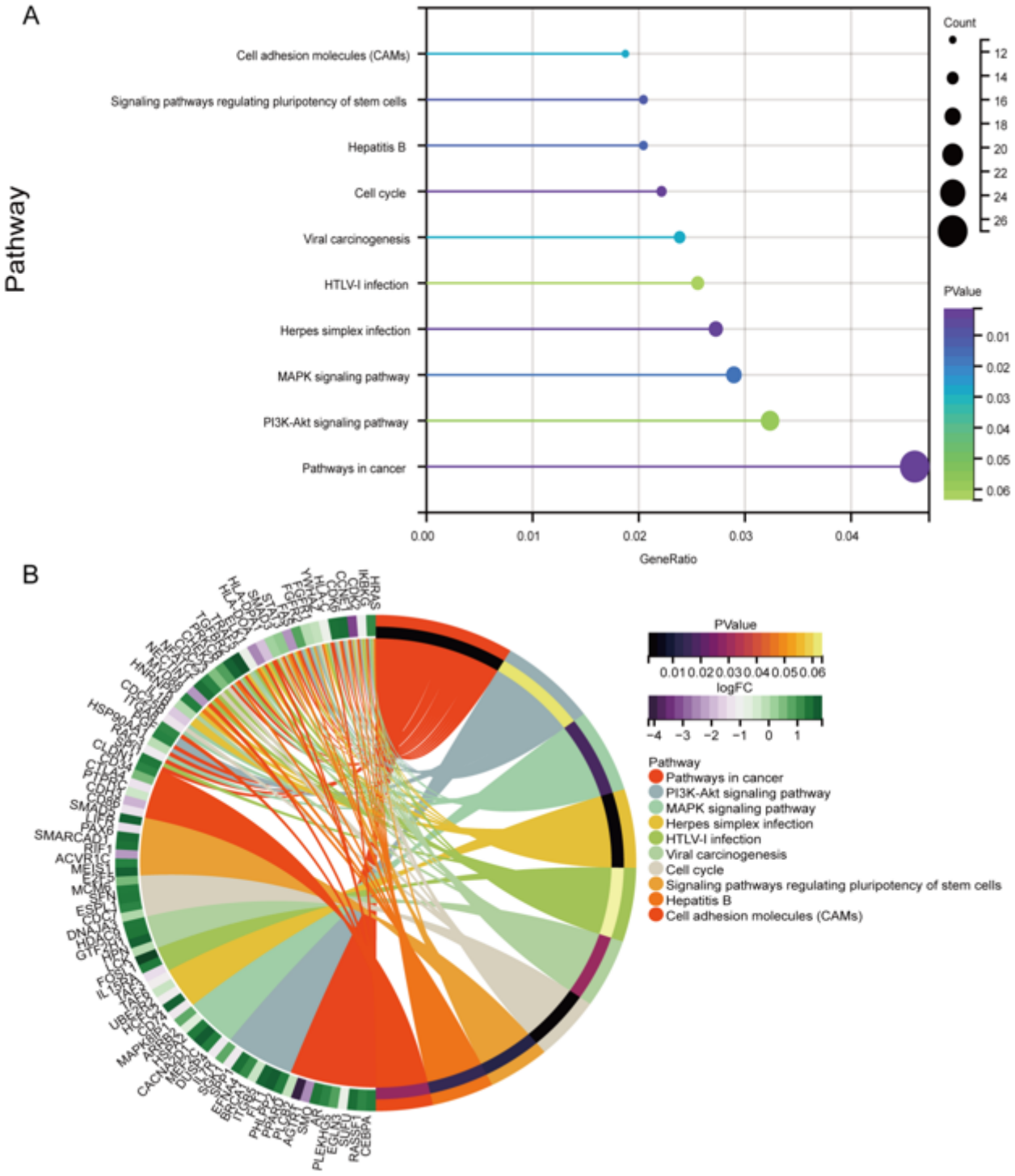

Figure 6

KEGG enrichment of 597 DEGs

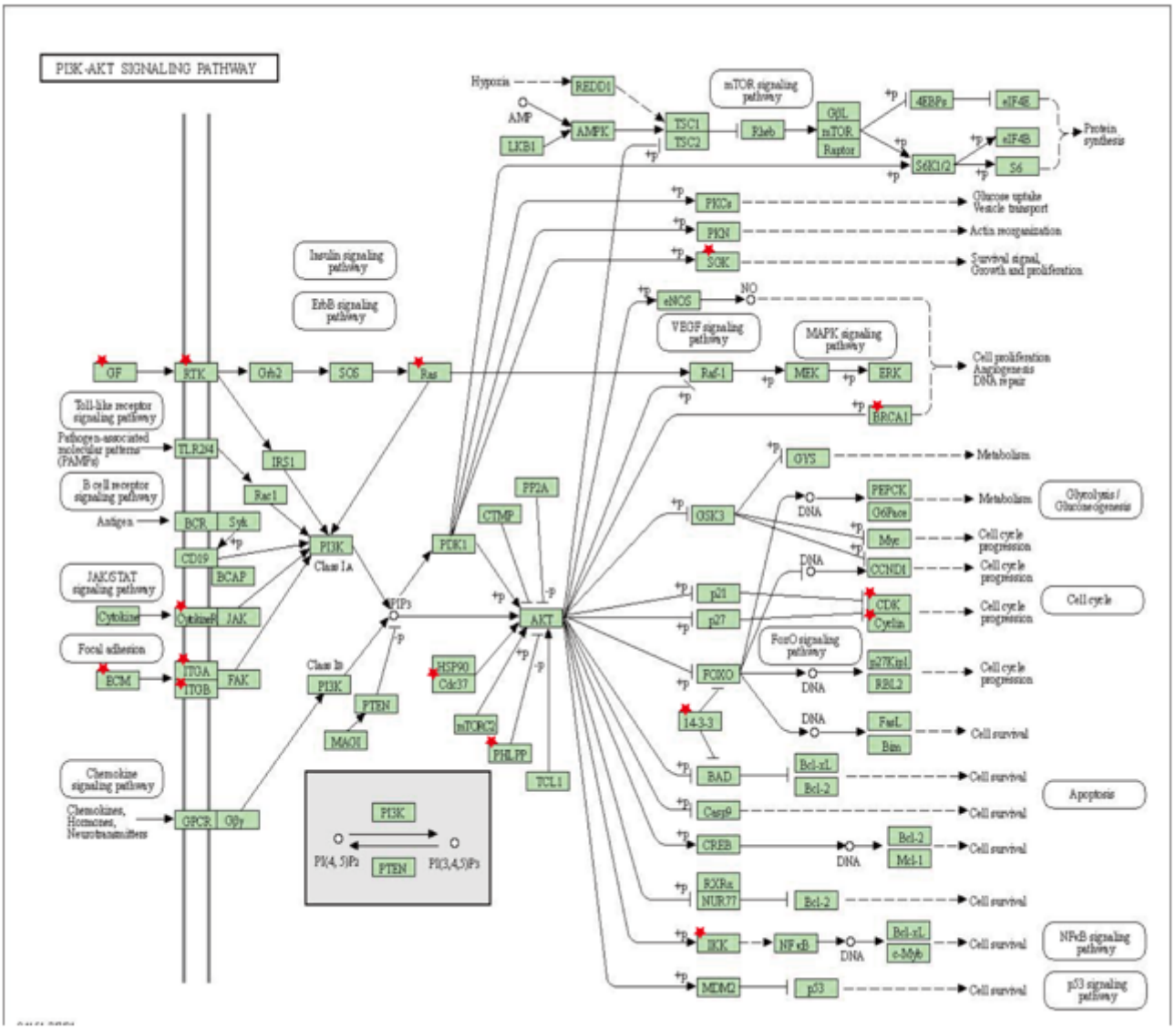

Figure 7

PI3K-Akt signaling pathway

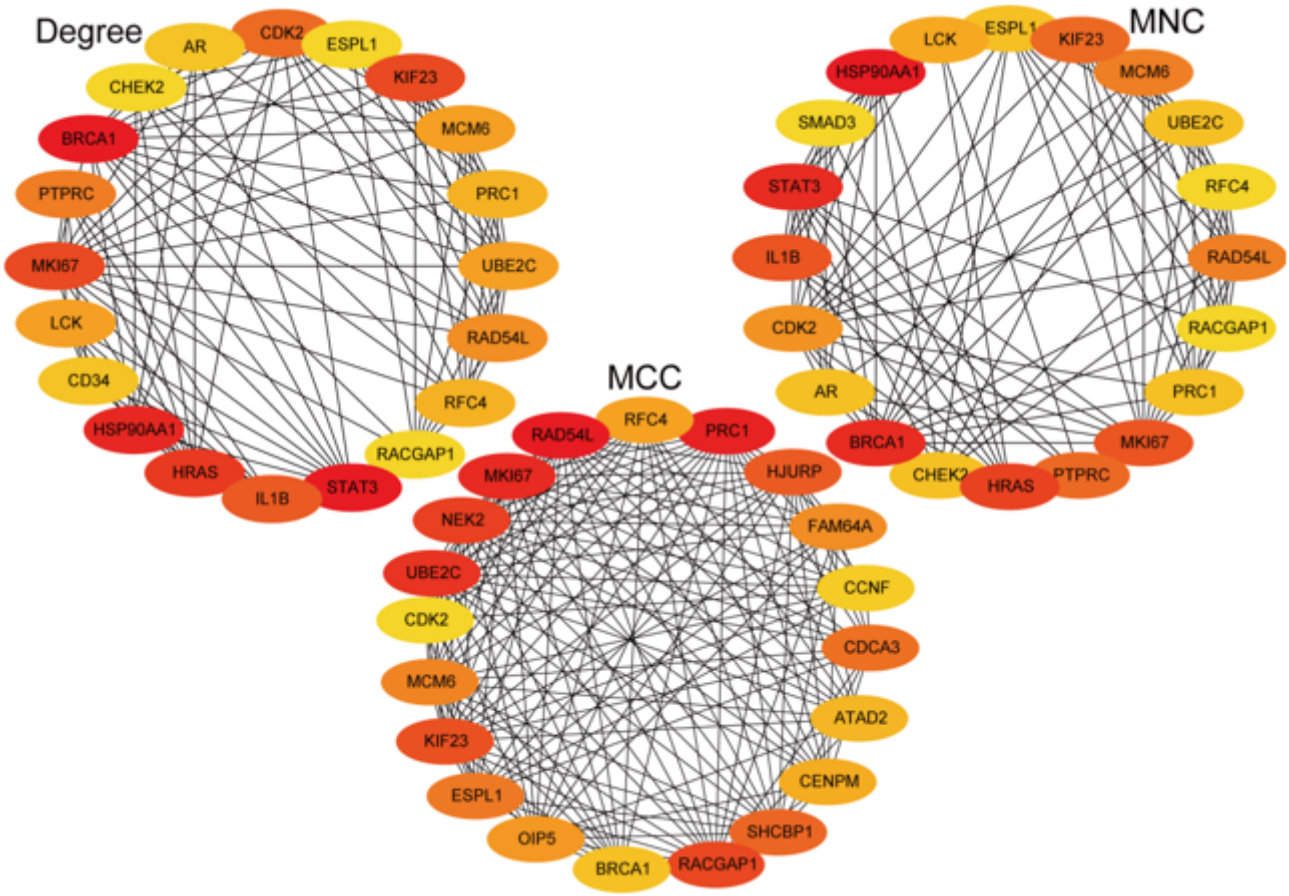

Figure 8

"MCC, MNC, Degree" top 20 genes

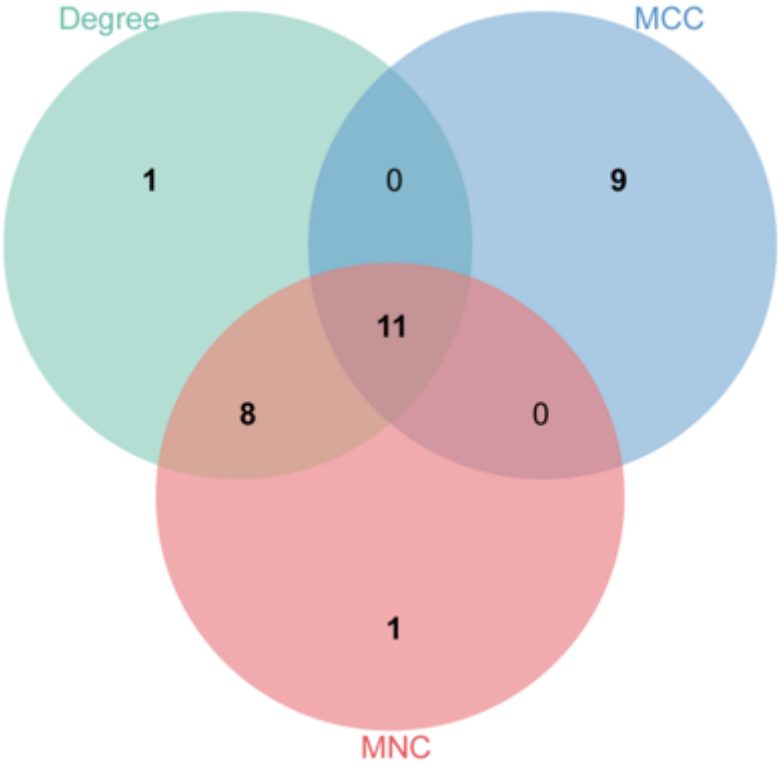

Figure 9

key hub targets

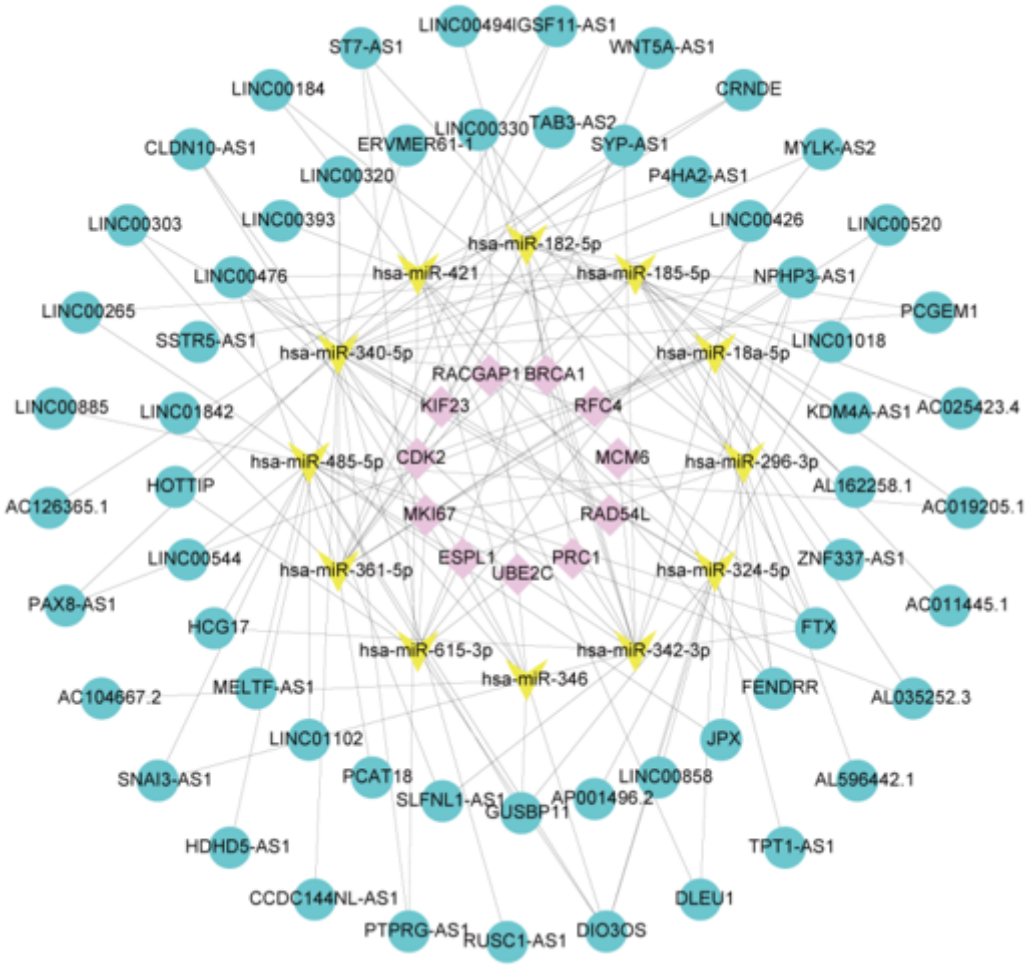

Figure 10

CeRNA regulatory network of the key hub targets. Rose diamond for mRNA, yellow inverted V for miRNA, green circle for lncRNA

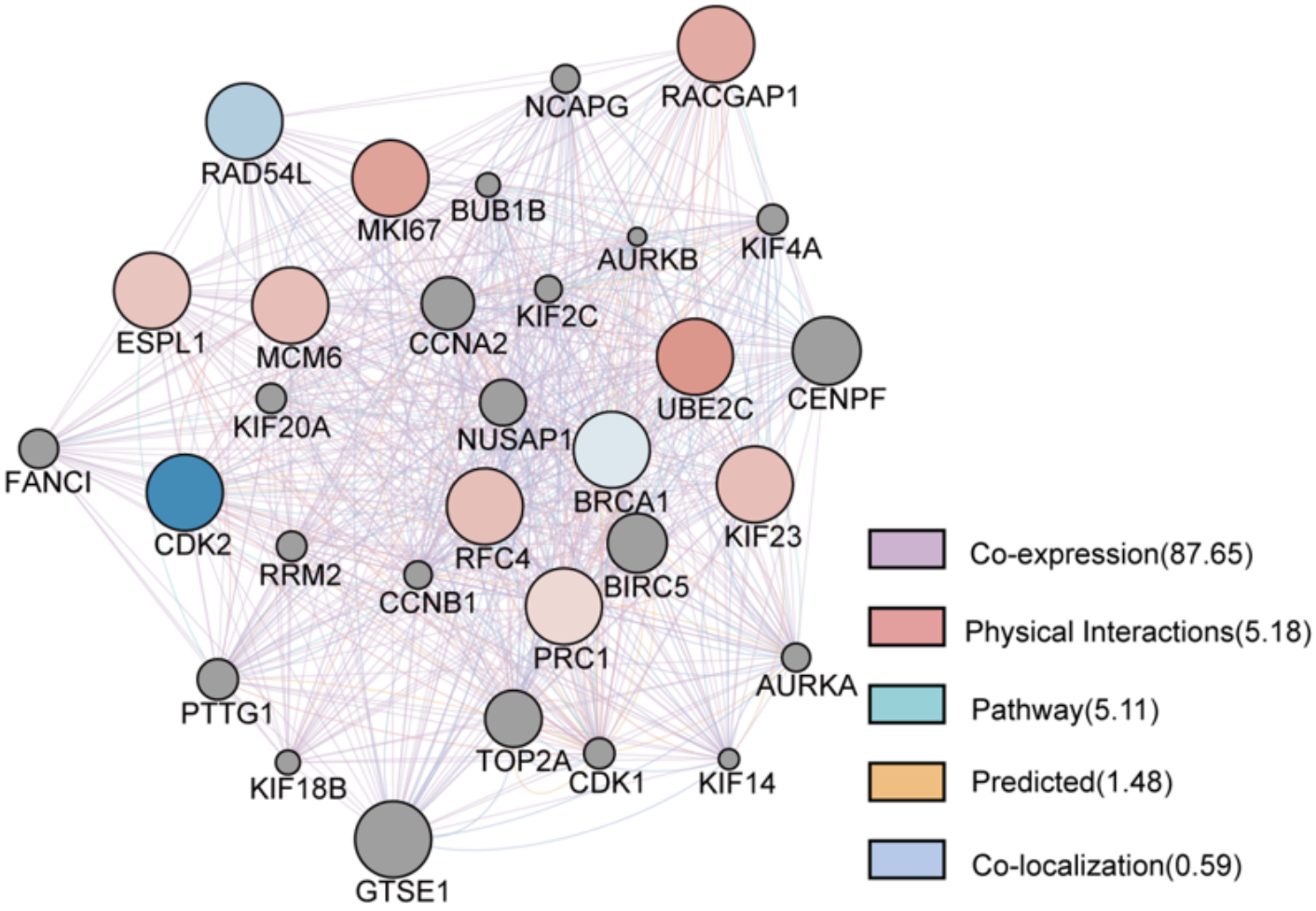

Figure 11

Protein network of key key hub targets.

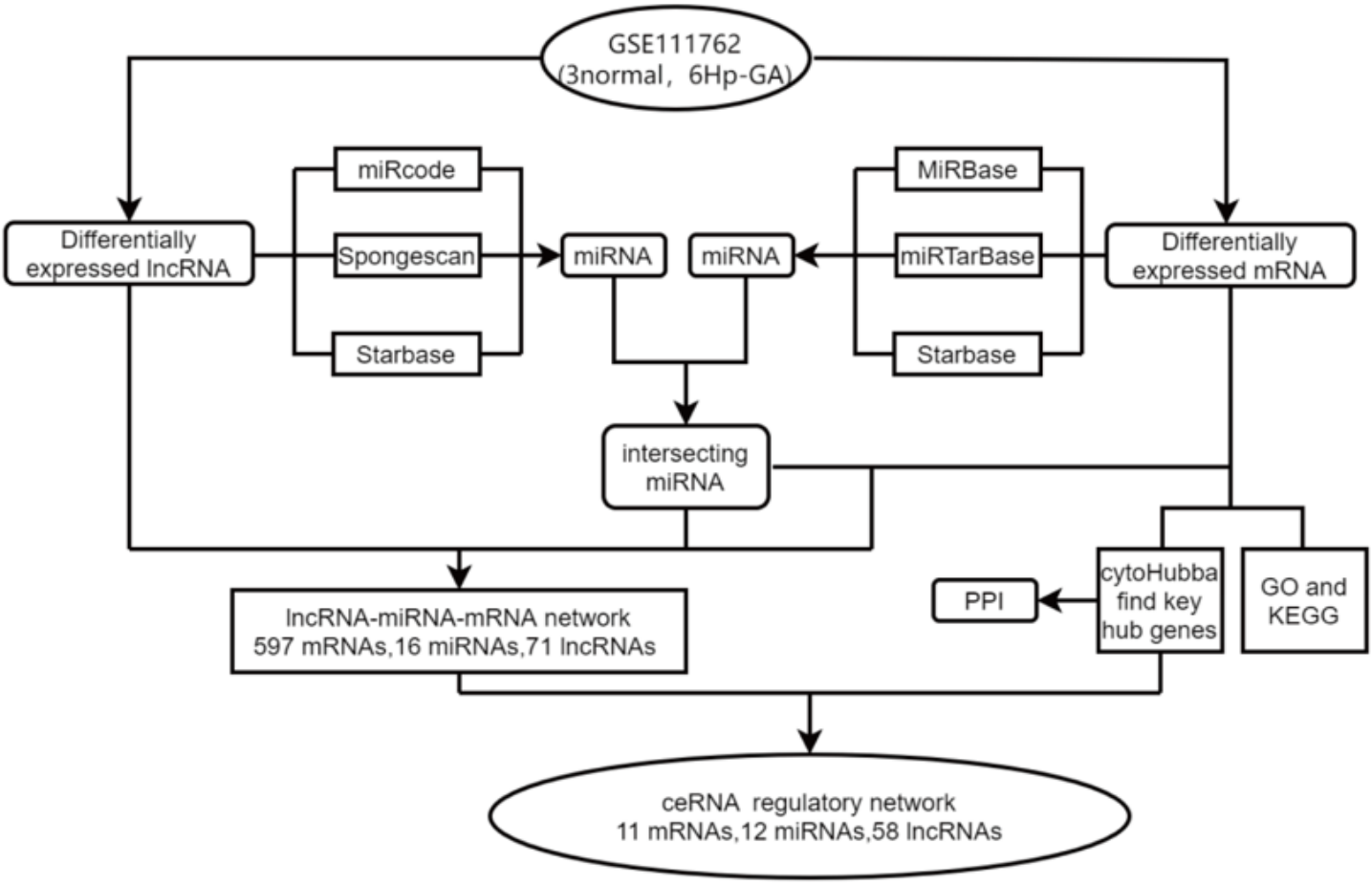

Figure 12

Flowchart of the research procedure in this study

Supplementary Files

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