Integrated Analysis of lncRNA-miRNA-mRNA ceRNA Network in Human Diarrhea Irritable Bowel Syndrome

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Research Article

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Abstract

Background: Many studies on long chain non-coding RNAs (lncRNAs) are published in recent years. But the roles of lncRNAs in diarrhea irritable bowel syndrome (IBS-D) are still unclear and should be further examined. The present work focused on determining the molecular mechanisms underlying lncRNAs regulation in IBS-D on the basis of the lncRNA-miRNA-mRNA competing endogenous RNA (ceRNA) network.

Methods: This study collected the mRNAs (GSE36701) expression data within human tissue samples with IBS-D group and normal group based on Gene Expression Omnibus (GEO) database and collected the differentially expressed lncRNAs (DELs) and differentially expressed miRNAs (DEmiRs) based on PubMed.Functional enrichment analysis of DEGs was performed on the DAVID database. Then the interaction network was constructed and visualized using STRING database and Cytoscape.

Results: This study identified 3192 DEmRNAs (1437 with up-regulation and 1755 with down-regulation),29 DEmiRs (18 upregulated and 11 downregulated)and 2 DELs(one upregulated and one downregulated) between IBS-D and control samples.Furthermore,we constructed a lncRNA-miRNA-mRNA network through two DELs (lncRNA TUG1 with up-regulation and lncRNA H19 with down-regulation), four DemiRs (hsa-miR-148a-3p,hsa-miR-342-3p,hsa-miR-149-5p with up-regulation and hsa-miR-219a-5p with down-regulation)and 24 DEGs (4 with up-regulation and 20 with down-regulation) with 42 axes. Simultaneously, we conducted functional enrichment and pathway analyses on genes within the as-constructed ceRNA network. According to our PPI/ceRNA network and functional enrichment analysis results, two critical genes were found (BCL2L11 and QKI).

Conclusion:In conclusion, the ceRNA interaction axis we identified is a potentially critical target for treating IBS-D.BCL2L11 axis(LncH19-hsa-miR-148a-3p-BCL2L11) may via interaction with PI3K/AKT pathways in IBS-D.Our results shed more lights on the possible pathogenic mechanism in IBS-D using a lncRNA-associated ceRNA network.

Introduction

Irritable bowel syndrome (IBS) is a common chronic functional bowel disease, characterized by no organic lesions, but recurrent abdominal pain, abdominal distension, and (or) changes in stool characteristics[1].It is the most commonly diagnosed gastroenterology disorder which affects over 10% of the population globally and the prevalence varies regionally[2],significantly affect quality of life.IBS patients can be categorized into four distinct subtypes according to the ROME criteria which are based on predominant stool pattern: IBS with diarrhoea (IBS-D), constipation (IBS-C), mixed (IBS-M) and an undefined pattern of abnormal stool (IBS-U)[3].As the most common clinical subtype of IBS,diarrhea-predominant irritable bowel syndrome (IBS-D) is accompanied by mental and emotional abnormalities that affect the living quality of patients, except the shared features[4].The pathophysiology of IBS has not been clearly understood yet, but everal factors have been involved such as alterations in gastrointestinal

motility , visceral hypersensitivity , small intestinal bacterial overgrowth (SIBO), environmental factors,low-grade inflammatory[5-6].The current treatment options for IBS, which predominantly target the individual symptoms, are limited and make adequate treatment of global IBS symptoms a significant challenge.

For decades, research has focused on the 2% of the human genome that codes for proteins[7-8]. 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) [9].CHAO et al.[10] found that the down-regulation of LncRNA H19 resulted in the expression changes of AQP1 and AQP3 may play an important role in the occurrence and development of IBS-D.ZHAO et al.[11] confirmed lncRNA TUG1 attenuated TNF-α-caused apoptosis and inflammatory response in ICC by down-regulating miR-127 and then inactivating NF-κB and Notch pathways in patients with IBS-D.Meanwhile,It has been proved that microRNAs (miRs) have been verified to be related with the progression and development of IBS-D[11-28].Such as hsa-miR-150 and hsa-miR-342-3p link to pain and inflammatory pathways both of which are thought to be dysregulated in IBS[12];The cxolonic mucosal microRNAs, microRNA-219a-5p and microRNA-338-3p are downregulated in Irritable Bowel Syndrome and are associated with barrier function and MAPK signaling[13];Serum exosomes derived from Irritable Bowel Syndrome patient increase cell permeability via regulating miR-148b-5p/RGS2 signaling in human colonic epithelium cells[14].To sum up, lncRNA-miRNA-mRNA regulatory network exerts a vital part in IBS-D genesis and progression.At the same time, there are few reports about the ceRNA regulation mechanism of lncRNA-miRNA related to IBS-D and the interaction between ncRNAs.

In this study, lncRNAs together with the corresponding mechanisms of action within human tissue from IBS-D cases were explored by means of bioinformatic analysis.Firstly, this study applied Gene Expression Omnibus (GEO) database and PubMed for obtaining the IBS-D-related differentially expressed genes (DEGs), differentially expressed miRNAs (DEmiRs), and differentially expressed lncRNAs (DELs). Cytoscape 3.7.2 was utilized to construct a lncRNA-miRNA-mRNA network, followed by the construction of a PPI network.

The present study aims to further screen the key lncRNA-miRNA-mRNA ceRNA axis within IBS-D with data collected from public databases and bioinformatics methods. Besides, our results shed more lights on the IBS-D molecular mechanism, thus providing a novel direction for targeted therapy of IBS-D. To further examine the key gene regulatory axis, we established a lncRNA-miRNA-mRNA regulatory subnetwork.The data flow mechanism diagram is displayed in (Figure1).

Results

Differential expression analysis

According to the cutoff criteria of GSE36701 (log|FoldChange|>0.176, P.Value<0.05), volcano map and heat map for differential expressed mRNAs were obtained(Figure2-3). In which 3192 DEmRNAs (1437 with up-regulation and 1755 with down-regulation) were discovered through the comparison of gene expression data between IBS-D and control samples.

Through PubMed ,altogether 29 DEmiRs (18 upregulated and 11 downregulated)and 2 DELs(one upregulated and one downregulated)were identified(Table1-2). The most significant differentially expressed genes were shown in(Table3).

Table 1.DEmiRs in normal vs. IBS-D through PubMed

miRNA	up/down-regulation	regulated gene	mechanism of action	The first author	published time
hsa-miR-29	up	HTR7	visceral hypersensitivity	He Zhu	2019[15]
hsa-miR-125b-5p,hsa-miR-16	down	CGN,CLDN2	intestinal epithelial barrier function	Cristina Martínez	2017[16]
miRNA-29a	up	ZO-1,CLDN1	intestinal barrier function	He Zhu	2020[17]
miR-199	down	TRPV1	visceral pain	QiQi Zhou	2016[18]
miR-219a-5p ,miR-338-3p	down	MAPK	barrier function,visceral hypersensitivity	Swapna Mahurkar-Joshi	2021[13]
miRNA-199	down	TRPV1	visceral hypersensitivity	Lixia Pei	2018[19]
miR-199b	down	not described	increase in the count of Coliform	Marwa A Mansour	2016[20]
miR-29a	up	GLUL	intestinal permeability	QiQi Zhou	2011[21]
miR-16, miR-103	down	HTR4	alterations in intestinal sensitivity and motility	Carolin Wohlfarth	2017[22]
miR-24	up	SERT	visceral hypersensitivity	Xiu-Jun Liao	2016[23]
hsa-miR-150,hs-a-miR-342-3p	up	AKT2	pain and inflammatory	Nicolaas H Fourie	2014[12]
miR-1305, miR-575, miR-149-5p, miR-190a-5p, miR-135a-5p, miR-148a-3p	up	not described	not described	Jing Guo	2021[24]
miR-194-5p, miR-127-5p	down	not described	not described	Jing Guo	2021[24]
miR-29a	up	GLUL	intestinal membrane permeability	QiQi Zhou	2010[25]
miR-127	down	NF-κB	inflammatory	K Zhao	2019[11]
miR-145, miR-148-5p and miR-592	up	IL-6, IL-10 and TNF-α	inflammatory	Damian Jacenik	2018[26]
miR-148b-5p	up	RGS2	intestinal barrier	Ying Xing	2021[14]

hsa-miR-106b, hsa-miR-26a, hsa-miR-29b	up	MAKP	not described	Wenhua Tao	2016[27]
miR-510	down	PRDX1	inflammatory	Yu Zhang	2019[28]

Table 2.DELs in normal vs. IBS-D through PubMed

lncRNA	up/down-regulation	regulated gene	mechanism of action	The first author	published time
TUG1	up	NF-κB	inflammatory	K Zhao	2019[11]
H19	down	AQP1 and AQP3	Intestinal Barrier	Guanqun Chao	2021[10]

Table 3. The five most significantly down-and upregulated DEGs in normal vs.IBS-D

Gene.symbol	Gene.title	P.Value	logFC	
HBB	hemoglobin subunit beta	0.00157	1.08	up
CD177	CD177 molecule	0.00173	0.951	up
MST1L	macrophage stimulating 1 like	0.00992	0.83	up
HLA-DQB1	major histocompatibility complex, class II, DQ beta 1	0.0149	0.801	up
FAM187B	family with sequence similarity 187 member B	0.000209	0.772	up
CAPN8	calpain 8	0.000000096	-0.966	down
KLF2	Kruppel like factor 2	0.00195	-0.805	down
SERPINB5	serpin family B member 5	0.00919	-0.801	down
PNLIPRP2	pancreatic lipase related protein 2 (gene/pseudogene)	0.0101	-0.781	down
LINC00960	long intergenic non-protein coding RNA 960	0.0248	-0.765	down

Forecast of miRNA-mRNA and lncRNA-miRNA pairs

Interactive relationships between DELs and miRNAs were predicted with lncRNA StarBase,then we found 275 miRNAs and 304 lncRNA-miRNA pairs.We acquired the common miRNAs through the intersection of estimated DELs with DemiRs,finally,a total of 6 miRNAs(hsa-miR-148a-3p,hsa-miR-149-5p,hsa-miR-190a-5p□hsa-miR-194-5p□hsa-miR-219a-5p□hsa-miR-342-3p) were found(Figure4A).According to the negative

regulatory relationship between miRNAs and lncRNAs, hsa-miR-190a-5p was deleted,because it was regulated in the same direction as lncRNA TUG1 ,so 5 miRNAs were acquired.

Compared with lncRNA prediction,mRNAs combined with the above 5miRNAs were predicted by miRTarBase, TargetScan, StarBase and miRDB databases, and 214 mRNAs were obtained by intersection of the above 4 databases(Figure4B).We acquired the common mRNAs through the intersection of 214mRNAs with 3192 DemiRs of GSE36702,a total of 29 mRNAs were found(Figure4C).

lncRNA-miRNA-mRNA ceRNA network construction

According to the negative regulatory relationship between miRNAs and lncRNAs,miRNAs and mRNAs,lncRNA-miRNA-mRNA ceRNA network was constructed.Cytoscape (version 3.7.2) was employed to visualize the interaction between two DELs (one with up-regulation and one with down-regulation), four DemiRs (three with up-regulation and one with down-regulation)and 24 DEGs (4 with up-regulation and 20 with down-regulation). Therefore, the lncRNA-miRNA-mRNA ceRNA network was built, there are 42 axes(Figure5,Table4),such as LncRNA TUG1 can be linked by one DEmiR and 4 DEGs, LncRNA H19 can be linked by 3 DEmiRs and 20 DEGs.It includes LncRNA TUG1-hsa-miR-219a-5p-NEURL1B (neuralized E3 ubiquitin protein ligase 1B)/FZD5 (frizzled class receptor 5); LncRNA H19- hsa-miR-148a-3p-BCL2L11 (BCL2 like 11)/QKI (QKI, KH domain containing RNA binding)/AKT2(AKT serine/threonine kinase 2), LncRNA H19- hsa-miR-342-3p-PPARD(peroxisome proliferator activated receptor delta)/SOCS2(suppressor of cytokine signaling 2) and LncRNA H19-hsa-miR-149-5p-AAK1 (AP2 associated kinase 1)/MET (MET proto-oncogene, receptor tyrosine kinase),et al.

Table 4.lncRNA-miRNA-mRNA ceRNA network of IBS-D

lncRNA		miRNA		mRNA	logFC
H19	down	hsa-miR-148a-3p	up	EGR3	-0.403
H19	down	hsa-miR-342-3p	up	EGR3	-0.403
H19	down	hsa-miR-149-5p	up	AAK1	-0.38
H19	down	hsa-miR-342-3p	up	SOCS2	-0.363
H19	down	hsa-miR-148a-3p	up	MDM4	-0.331
H19	down	hsa-miR-342-3p	up	MDM4	-0.331
H19	down	hsa-miR-148a-3p	up	NEURL4	-0.321
H19	down	hsa-miR-342-3p	up	NEURL4	-0.321
H19	down	hsa-miR-148a-3p	up	SLC2A1	-0.298
H19	down	hsa-miR-149-5p	up	SLC2A1	-0.298
H19	down	hsa-miR-342-3p	up	OTUD3	-0.288
TUG1	up	hsa-miR-219a-5p	down	NEURL1B	0.272
H19	down	hsa-miR-149-5p	up	AKAP13	-0.27
H19	down	hsa-miR-148a-3p	up	AKAP13	-0.27
H19	down	hsa-miR-342-3p	up	AKAP13	-0.27
TUG1	up	hsa-miR-219a-5p	down	FZD5	0.256
H19	down	hsa-miR-148a-3p	up	ITGB8	-0.245
H19	down	hsa-miR-342-3p	up	ITGB8	-0.245
H19	down	hsa-miR-148a-3p	up	MET	-0.236
H19	down	hsa-miR-149-5p	up	MET	-0.236
TUG1	up	hsa-miR-219a-5p	down	DDX6	0.23
H19	down	hsa-miR-148a-3p	up	PPARD	-0.229
H19	down	hsa-miR-149-5p	up	PPARD	-0.229
H19	down	hsa-miR-342-3p	up	PPARD	-0.229
H19	down	hsa-miR-148a-3p	up	BCL2L11	-0.225
H19	down	hsa-miR-149-5p	up	BCL2L11	-0.225
H19	down	hsa-miR-342-3p	up	BCL2L11	-0.225
H19	down	hsa-miR-148a-3p	up	QKI	-0.223

TUG1	up	hsa-miR-219a-5p	down	MBNL3	0.205
H19	down	hsa-miR-148a-3p	up	AKT2	-0.199
H19	down	hsa-miR-149-5p	up	AKT2	-0.199
H19	down	hsa-miR-342-3p	up	AKT2	-0.199
H19	down	hsa-miR-148a-3p	up	KIAA1549	-0.192
H19	down	hsa-miR-149-5p	up	KIAA1549	-0.192
H19	down	hsa-miR-148a-3p	up	EOGT	-0.182
H19	down	hsa-miR-342-3p	up	TJAP1	-0.181
H19	down	hsa-miR-148a-3p	up	MTMR9	-0.133
H19	down	hsa-miR-149-5p	up	MTMR9	-0.133
H19	down	hsa-miR-342-3p	up	MTMR9	-0.133
H19	down	hsa-miR-148a-3p	up	ALCAM	-0.122
H19	down	hsa-miR-149-5p	up	SETD5	-0.094
H19	down	hsa-miR-342-3p	up	SETD5	-0.094

PPI network analysis and key gene prediction

When uploading the DEGs identified in ceRNA network to STRING website, 8 edges were found to be present in 24 DEGs incorporated in constructing a PPI network(Figure6). In the as constructed network, QKI could interact with KIAA1549,DDX6(DEAD-box helicase 6) and BCL2L11. BCL2L11 could interact with AKT2,KIAA1549 and QKI. QKI and BCL2L11 have high degree values and have more than 3 direct connections with other nodes, so they are of great significance in PPI network,we predicted QKI and BCL2L11 were key genes.

Functional enrichment and differential expression analysis

In the present study, the 24 DEGs of ceRNA network used to analyze GO/KEGG were imported into DAVID website. A total of 7 GO enrichment items were obtained, including four BP entries,one CC entry and two MF entries(Figure7A,7B,Table5). Four signal pathways were obtained by enrichment analysis of KEGG pathways(Figure7C,7D,Table6).In order to show a smaller subset of high-dimensional data, use sangerbox software to draw the chord diagram of GO and KEGG,as shown in figure 8.To accurately explore the differential expression of key genes in the dataset, two key genes were visualized in(Figure 9).In the GSE36701 dataset, QKI and BCL2L11 were all down-regulated, further revealing the underlying mechanisms for key genes.

Table 5. GO analysis of 24 DEGs of ceRNA

GO	Count	PValue	Genes
BP:negative regulation of apoptotic process	4	0.015991644	SOCS2, EGR3, MDM4, PPARD
BP:negative regulation of myoblast differentiation	2	0.027055566	MBNL3, PPARD
BP:positive regulation of gene expression	3	0.038306981	ITGB8, QKI, PPARD
BP:glucose transport	2	0.03860065	SLC2A1, PPARD
CC:cortical actin cytoskeleton	2	0.048424139	AKAP13, SLC2A1
MF:protein binding	18	0.006112241	DDX6, FZD5, SLC2A1, MTMR9, QKI, TJAP1, SOCS2, AKAP13, NEURL1B, NEURL4, BCL2L11, ALCAM, AKT2, AAK1, MDM4, MBNL3, MET, PPARD
MF:protein kinase activity	3	0.072632515	AKT2, AAK1, MET

Table 6. KEGG analysis of 24 DEGs of ceRNA

KEGG	Count	PValue	Genes
Pathways in cancer	5	0.004964312	FZD5, AKT2, SLC2A1, MET, PPARD
Central carbon metabolism in cancer	3	0.006221886	AKT2, SLC2A1, MET
Renal cell carcinoma	3	0.006605925	AKT2, SLC2A1, MET
PI3K-Akt signaling pathway	4	0.024573105	BCL2L11, AKT2, ITGB8, MET

Construction of regulatory model diagram:

According to the negative regulatory relationship between miRNA and mRNA,miRNA and lncRNA, 9 nodes in the above PPI network were selected to construct the core lncRNA-miRNA-mRNA regulatory model diagram related to the progress of IBS-D, as shown in Figure 10.

Discussion

IBS is one of the most commonly diagnosed gastrointestinal (GI) conditions. Although plenty of protein-coding genes have been identified as IBS-D-related genes, these genes couldn't perfectly explain how IBS-

D occurs and develops. Recently, a growing body of research has focused on the epigenetic regulation and the roles of ncRNAs in IBS-D pathogenesis. In this study, we constructed the ceRNA network based on the expression profiles of whole rectal mucosa of healthy controls and IBS-D patients.

In this study,we discovered 3192 DEmRNAs (1437 with up-regulation and 1755 with down-regulation) of GSE36701.Through PubMed ,nineteen articles were included altogether 29 DEmiRs (18 upregulated and 11 downregulated)and 2 DELs(one upregulated and one downregulated)were identified.According to the negative regulatory relationship between miRNAs and lncRNAs,miRNAs and mRNAs,lncRNA-miRNA-mRNA ceRNA network was constructed.There are two DELs (lncRNA TUG1 with up-regulation and lncRNA H19 with down-regulation), four DemiRs (hsa-miR-148a-3p,hsa-miR-342-3p,hsa-miR-149-5p with up-regulation and hsa-miR-219a-5p with down-regulation)and 24 DEGs (4 with up-regulation and 20 with down-regulation) with 42 axes.

Then we performed the functional analyses of GO enrichment and KEGG pathways based on the genes in the ceRNA network.A total of 7 GO enrichment items were obtained, including four BP entries,one CC entry and two MF entries.BP is enriched in negative regulation of apoptotic process,negative regulation of myoblast differentiation,positive regulation of gene expression,glucose transport;CC is enriched in cortical actin cytoskeleton;MF is enriched in protein binding,protein kinase activity. Four signal pathways were obtained by enrichment analysis of KEGG pathways,that are Pathways in cancer,Central carbon metabolism in cancer,Renal cell carcinoma,PI3K-Akt signaling pathway.

The Phosphatidylinositol 3-kinase (PI3K)/phosphorylated protein kinase B (AKT) pathway is an important pathway in the regulation of cell proliferation and apoptosis[29]. Akt is a serine/threonine kinase and an important target of PI3K.Fei and WANG's[30] study revealed that miR-495 upregulation can reduce visceral sensitivity in IBS-D via inhibition of PI3K/AKT signaling pathway by targeting PKIB.SONG et al.[31] found overexpression of miR-340 or the silencing of SPP1 inhibited GC cell proliferation, invasion, migration, and EMT process through inhibition of the PI3K/AKT signaling pathway, but promoted apoptosis of GC cells.

When uploading the 24 DEGs identified in ceRNA network to STRING website, 8 edges were found to be present in 9 DEGs incorporated in constructing a PPI network. QKI and BCL2L11 have high degree values and have more than 3 direct connections with other nodes, so they are of great significance in PPI network,we predicted QKI and BCL2L11 were key genes.

Quaking (QKI) is a member of the signal transduction and activator of RNA metabolism (STAR) and hnRNP Khomology-type family of RNA-binding proteins[32].Substantial research implicated QKI RNA-binding protein function in many more cell types than initially anticipated.Like many mRNA regulators, quaking-related proteins regulate the expression of diverse mRNA targets by various mechanisms and have essential roles in cell cycle and differentiation regulation[33-35].Among the four known splice variants of the human QKI gene (QKI-5, QKI-6, QKI-7, and QKI-7b)[32], QKI-5 is the variant most abundantly expressed in normal human colon tissues, and preferentially down regulated in human colorectal

cancer(CRCs)[36].In this reseach,QKI was also down-regulated in IBS-D.QKI has been demonstrated in the regulation of cellular processes such as cell cycle, apoptosis, and differentiation[37].CHU et al.[38] found QKI could promote the proliferation, metastasis, and invasion of pancreatic cancer through activating fibroblasts surrounding pancreatic cancer and accelerating EMT and increasing the autophagy in pancreatic cancer.Mukohyama Junko et al.[39] found that overexpression of QKI-5 suppressed organoid-forming capacity and tumorigenic capacity of colorectal carcinoma PDX cells .In addition to this studies[40-41] found that QKI could inhibit the proliferation and tumorigenesis in multiple cancer types such as lung adenocarcinoma, and renal cell carcinoma.

BCL2L11 (also known as BIM) is a member of BCL-2 family, inducing apop-tosis and inhibiting autophagy by inactivating BCL2 or by activating BAX-BAK1 and by bridging BECN1 or DYNLL1, respectively[42].According to previous reports, BCL2L11 is involved in biological pro-cesses in a variety of solid tumors such as ovarian cancer, endometrial adenocarcinoma, prostate tumor and gastric cancer[43-45].PAN et al.'[46] data demonstrated that lncRNA ACTA2-AS1 could suppress colon adenocarcinoma progression via sponging miR-4428 to regulate BCL2L11 expression.ZHANG et al.'[47] studies from both in vitro and in vivo shown that miR-24 regulates BCL2L11 expression by directly binding with 3'UTR of mRNA, thus promoting gastric cancer cell growth, migration while inhibiting cell apoptosis.YUAN et al.'[48] results showed that downregulation of LncRNA H19 could promote cell proliferation, inhibit cell apoptosis, and suppress multiple inflammatory cytokine expressions in HK-2 cells by modulating the miR-130a/BCL2L11 pathway.As a disease of digestive system,IBS-D was speculated to be closely related to the above key genes QKI and BCL2L11 and PI3K/AKT signaling pathway.

According to the difference significance, PPI interaction correlation and regulation relationship, BCL2L11 axis (LncH19-hsa-miR-148a-3p-BCL2L11) may play a key role in the treatment of IBS-D. KEGG enrichment revealed BCL2L11 was enriched in the PI3K/AKT signaling pathway,we speculate that the BCL2L11 axis(LncH19-hsa-miR-148a-3p-BCL2L11) may via interaction with PI3K/AKT pathways in IBS-D.

Certain limitations should be noted in the present work. The way of action has been predicted based on the measured RNA network, however, which has not been confirmed (dual luciferase reporter gene analysis, gene overexpression or gene knockout). Although several related genes have been screened out in the present study, further in vitro clinical research and in vivo experiments should be carried out to confirm its expression and functional mechanism in terms of IBS-D.

Currently, little research is conducted to explore the lncRNA mechanism in IBS-D. The present work has its certain strengths. For instance,for the first time construct the lncRNA-miRNA-mRNA network based on GEO database and PubMed. Nonetheless, our findings were just obtained from bioinformatics analysis. Therefore, it is of necessity to conduct a thorough study for verifying the potential effects of those 42 axes within IBS-D. In summary,we confirmed that the ceRNA networks, including the regulated networks,such as lncRNA TUG1-hsa-miR-219a-5p-DDX6, lncRNA H19-hsa-miR-149-5p/hsa-miR-148a-3p-KIAA1549, and lncRNA H19-hsa-miR-148a-3p-AKT2/PPARD/QKI/MDM4, might be associated with the

pathogenesis of and development of IBS-D.Nevertheless, ceRNA networks and their associations with IBS-D should be validated.

Conclusion

In summary, the ceRNA interaction axis we identified is a potentially critical target for treating IBS-D.We speculate that the BCL2L11 axis(LncH19-hsa-miR-148a-3p-BCL2L11) may via interaction with PI3K/AKT pathways in IBS-D.Our results shed more lights on the possible pathogenic mechanism in IBS-D using a lncRNA-associated ceRNA network.

Materials And Methods

Microarray data and data processing One dataset was obtained by setting the screening criteria for the species type as“Homo sapiens”, from GEO database of National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/geo/), with such keywords being searched as“Diarrhea irritable bowel syndrome”(IBS-D). Then select the study type as " Expression profiling by array", so that we get the series matrix files. One dataset was included in this study, namely GSE36701. As for mRNA expression data , relative to normal rectal mucosa in control group, the experimental group collected diarrhoea predominant IBS subjects to identify differentially expressed genes. Simultaneously, we applied GEO database for downloading series matrix files and expressive data.

DEGs,DEmiRs,DELs analysis The microarray data of GSE36701 was downloaded from the GEO database (www.ncbi.nlm.nih.gov/geo/) with its microarray platform as GPL570 (Affymetrix Human Genome U133 Plus 2.0 Array). In this microarray,53 samples of rectal mucosa from 27 IBS-D patients with obvious abdominal pain were obtained and 40 samples from 21 healthy volunteers as controls. Subjects completed a Talley IBS symptom questionnaire modified to include days with pain. IBS was diagnosed according to the Rome II criteria. Those with IBS also completed IBS quality of life questionnaire and the IBS Symptom Severity Score Questionnaire including severity of visceral pain. Those agreeing to take part underwent sigmoidoscopy without bowel preparation. Two biopsies from each donor were obtained using endoscopic biopsy forceps and the mRNA were extracted for further analysis.After RNA extraction and Microarray processing,samples without visible 18S and 28S rRNA peaks were excluded. There was one donor in IBS-D group and two donors in HV group only obtained one microarray data. Details of clinical data collection, RNA extraction and microarray processing can be found in GSE36701 dataset citation(https ://www.ncbi.nlm.nih.gov/geo/query /acc.cgi?acc=GSE36 701).The selection criteria for GSE36701 dataset including |FoldChange|>1.5 and P.Value<0.05 were regarded as threshold values. There existed statistical significance for the selection of this threshold, and those genes that were up- and down-regulated can also be selected for performing the subsequent analysis. This indicates statistical significance. Heml and GraphPad Prism 8 was also adopted as a heat map and volcano map drawing of DEGs. Log in PubMed website(https://pubmed.ncbi.nlm.nih.gov/30968977/) and search DEmiRs and DELs that have been experimentally verified and related to IBS-D patients since the establishment of database with the keywords "miRNA+IBS" and "lncRNA+IBS".Subsequently, these

DEGs,DEmiRs,DELs were classified as the up-regulation or down-regulation group.These data would be used in the following ceRNA network construction and protein interaction network construction.

Prediction of miRNA-mRNA and lncRNA-miRNA pairs Interaction relationships between lncRNAs and miRNAs were predicted with Starbase database.By using jveen(http://jvenn.toulouse.inra.fr/app/example.html) to output all the predicted results of DElncRNAs, and intersecting the output results with DEmiRs, the interaction pair of lncRNAs and miRNAs can be obtained. Similarly, we use datebases to predict the miRNAs in the lncRNA-miRNA interaction pair, and intersect the prediction result with the DEmRNAs. As a result,the miRNA-mRNA interaction pair can be obtained.The difference is that we use four databases when predicting mRNAs, we take the intersection of four databases as our prediction result.Interactions between lncRNAs and miRNAs were analyzed on the basis of lncRNA target prediction databases shown below:

StarBase(https://starbase.sysu.edu.cn/).

Interactions between miRNAs and mRNAs were analyzed in line with 4 miRNA target prediction databases:

miRTarBase (https://maayanlab.cloud/Harmonizome/resource/MiRTarBase),

TargetScan (http://www.targetscan.org/vert_72/),

StarBase(https://starbase.sysu.edu.cn/),

miRDB (http://mirdb.org/).

Establishment of the ceRNA network of lncRNA-miRNA-mRNA According to the negative regulatory relationship between miRNAs and lncRNAs,miRNAs and mRNAs,this study established a ceRNA network with related DELs by using commonly interactive miRNAs with DELs and DEmRNAs. Cytoscape (version 3.7.2) was later utilized to visualize the ceRNA network of lncRNA/miRNA/mRNA.

PPI network analysis and key gene identification To further identify the relationship between target genes in the lncRNA-miRNA-mRNA ceRNA regulatory network, Using the STRING(Search Tool for the Retrieval of Genes)(http://strin- g-db.org/) database for protein-protein Interaction (PPI) analysis, Set medium confidence >0.4. The PPI network was visualized using Cytoscape software. Each node in PPI network is evaluated by Degree to screen hub-gene. The higher the Degree of a node is, the greater its significance in PPI network is.

Functional enrichment (DEGs of ceRNA network) and differential expression analysis (key genes) Gene ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were done for analyzing DEGs by using DAVID online tool (version 6.8)(https ://david -d.ncifc rf.gov/), $P<0.05$ indicated that the pathways or GO biological process terms were significantly enriched.The present work adopted sangerbox(http://www.sangerbox.com/Signin) to visualize key DEGs in GSE36701.

Abbreviations

IBS-D:diarrhea irritable bowel syndrome;LncRNA: Long non-coding RNA; GO: Gene ontology;hsa:Homo sapiens; KEGG: Kyoto encyclopedia of genes and genomes;mRNA: Messenger RNA

Declarations

Acknowledgements

Not Applicable.

Authors' contributions

Jun-wei LIANG and 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,Jun-wei LIANG and Li-li CHI 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 datasets generated and analyzed during the current study are available in the Gene Expression Omnibus (GEO,https://www.ncbi.nlm.nih.gov/geo/)database (Accession Number: GSE36701) and PubMed(https://pubmed.ncbi.nlm.nih.gov/30968977/).

Ethics approval and consent to participate

Not Applicable.

Consent for publication

Not Applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

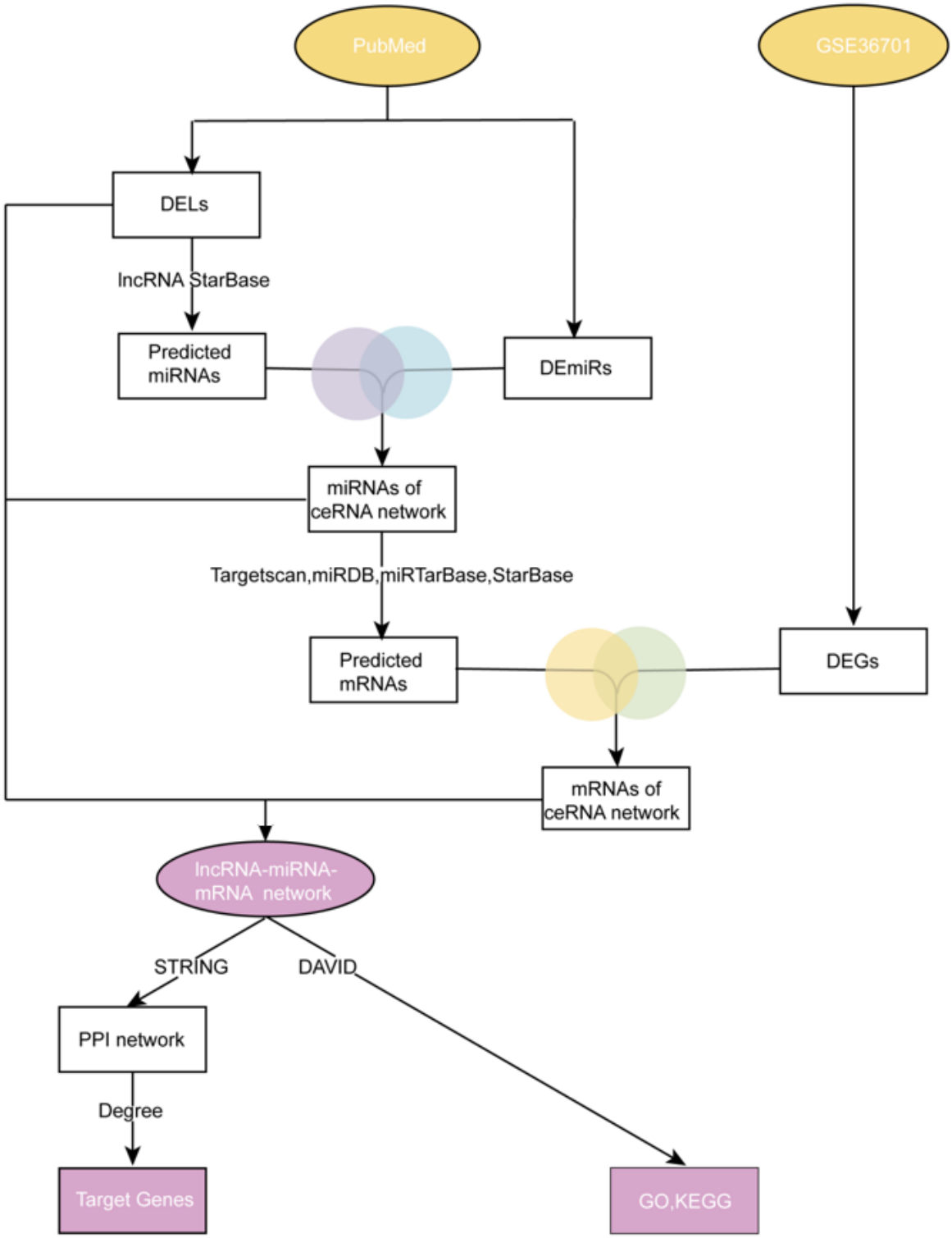

Figure 1

Flow diagram of data processing. The difference expression of mRNAs of GSE36701 and lncRNAs,miRNAs of PubMed were analyzed, and then the intersection was selected; Construction of CeRNA network, protein-protein interaction network and functional enrichment analysis, and finally determine the key genes

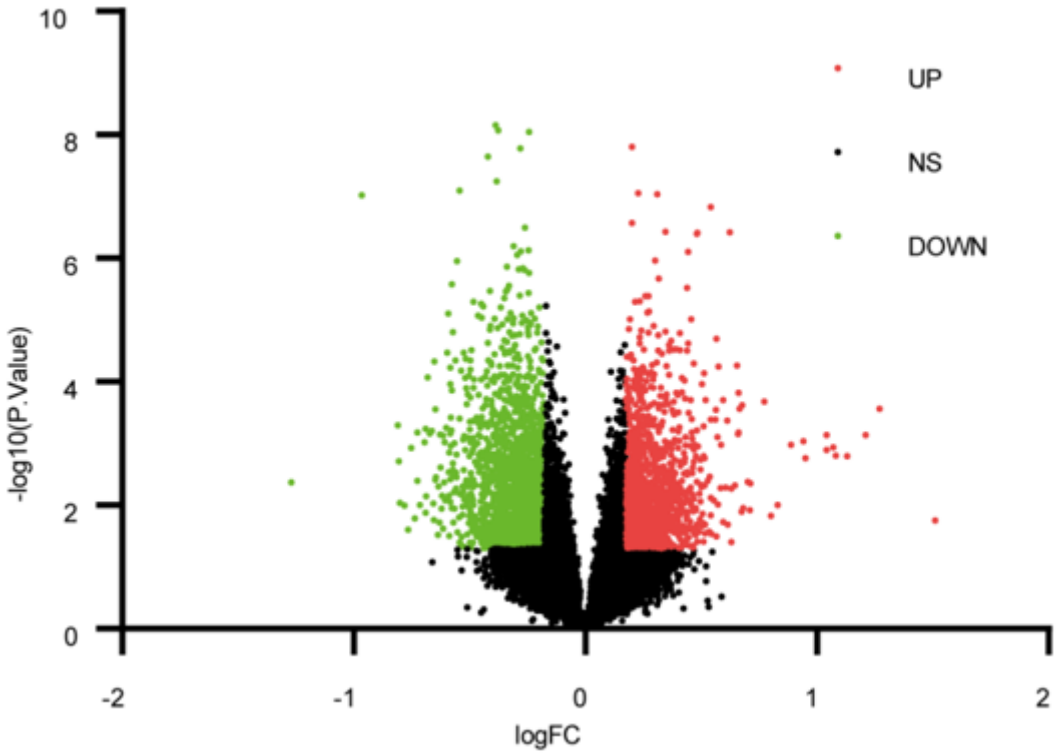

Figure 2

Volcano map of differentially expressed analysis of mRNAs of GSE36701. 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 .

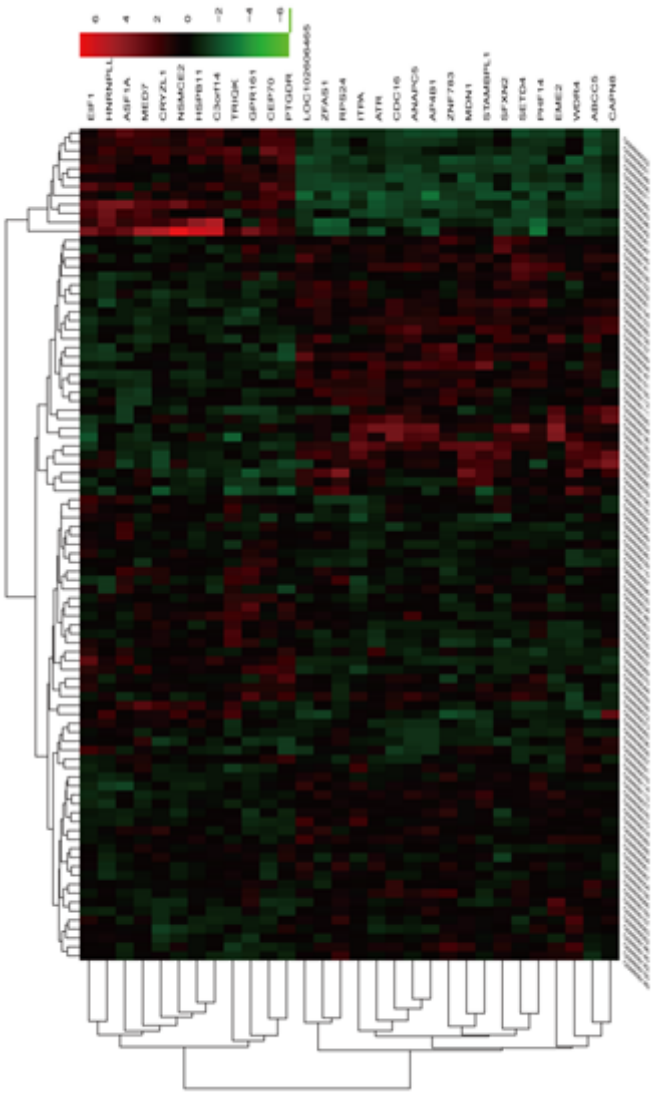

Figure 3

Heatmap of differentially expressed analysis of mRNAs of GSE36701.

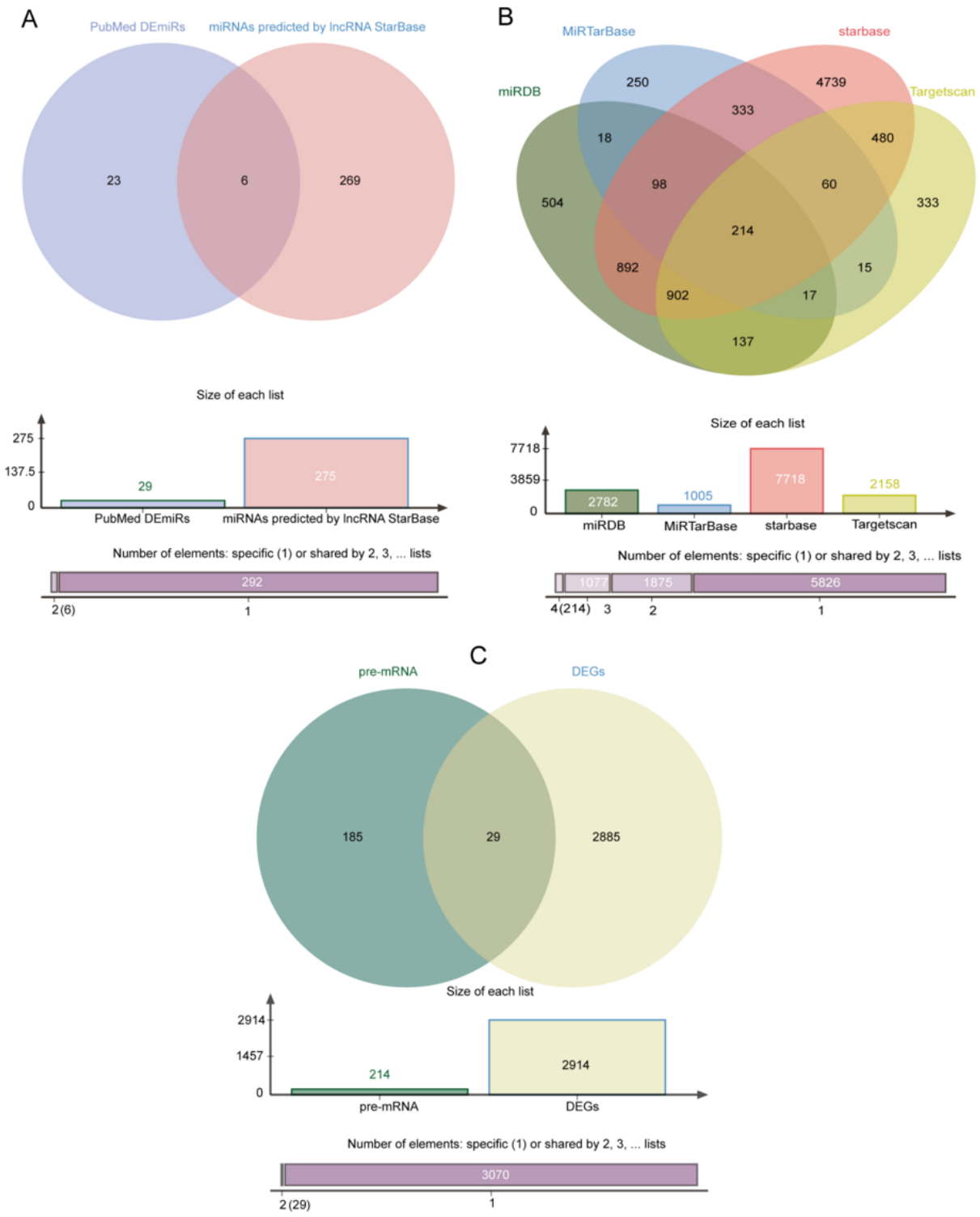

Figure 4

Forecast of lncRNA-miRNA and miRNA-mRNA pairs.(A)The common miRNAs through the intersection of estimated DELs with DemiRs.(B)Pre-mRNAs binding pre-miRNAs.(C)The venn diagram in jvenn of pre-mRNAs and DEGs of GSE36701.

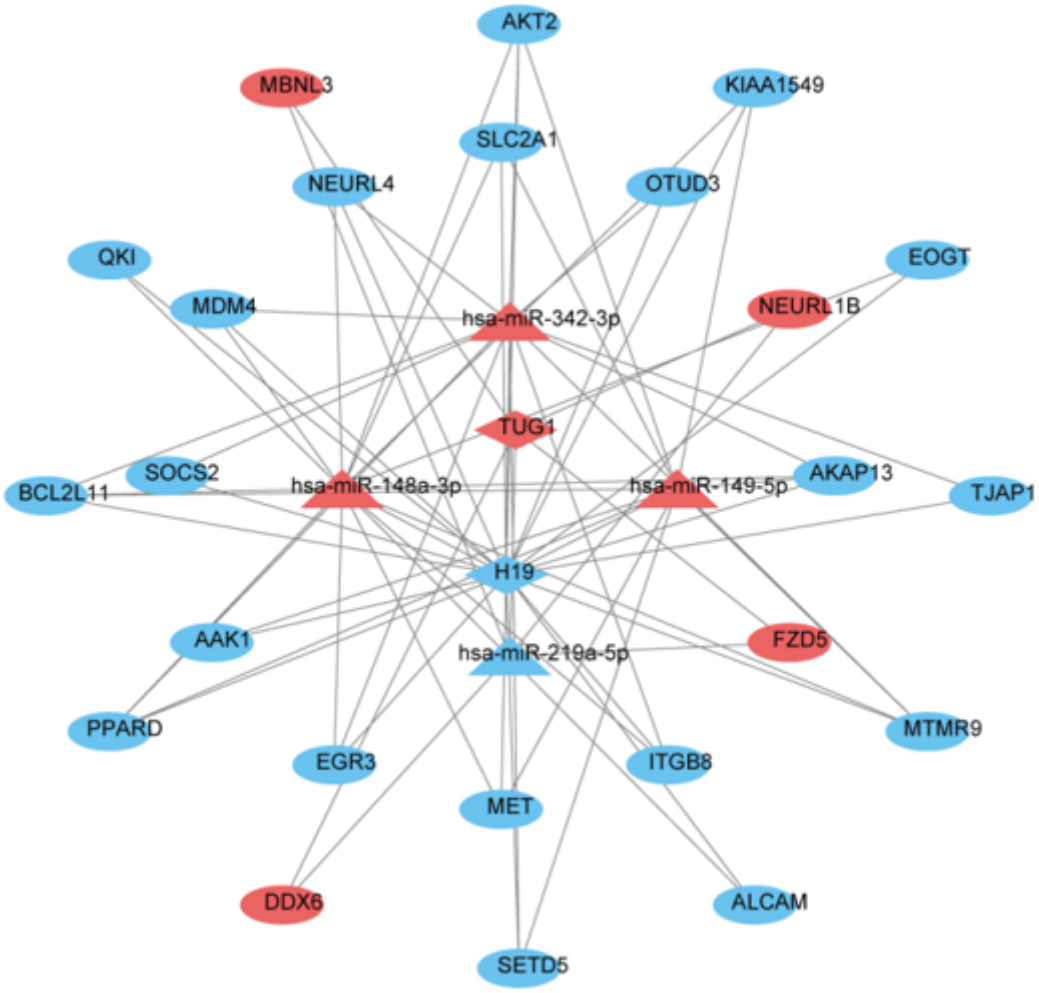

Figure 5

The lncRNA-miRNA-mRNA ceRNA network.The diamond: lncRNA, the triangle: miRNA, and the ellipse: mRNA, Red: up-regulation, blue: down-regulation

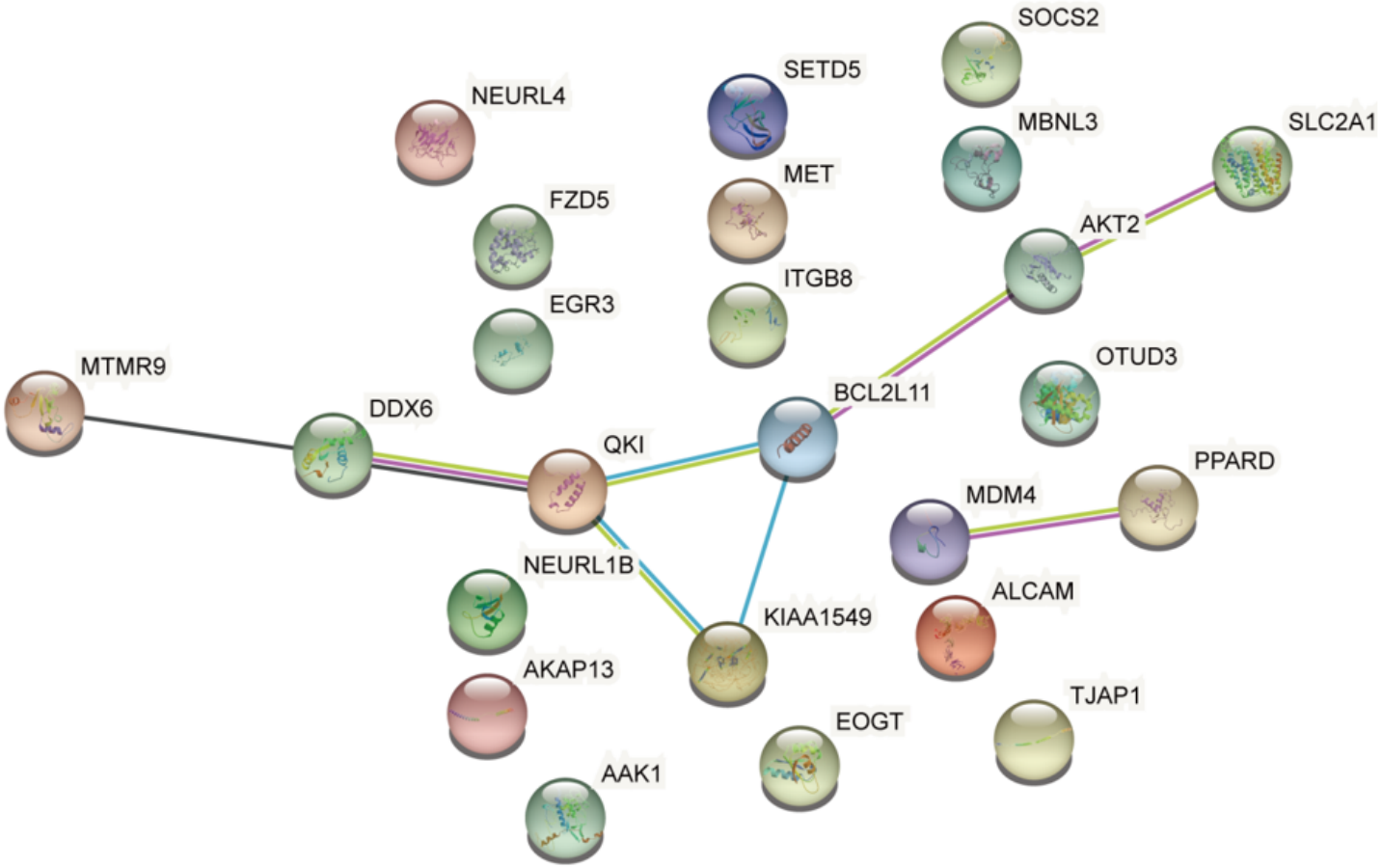

Figure 6

Protein-protein interaction analysis of 24 DEGs of ceRNA network.

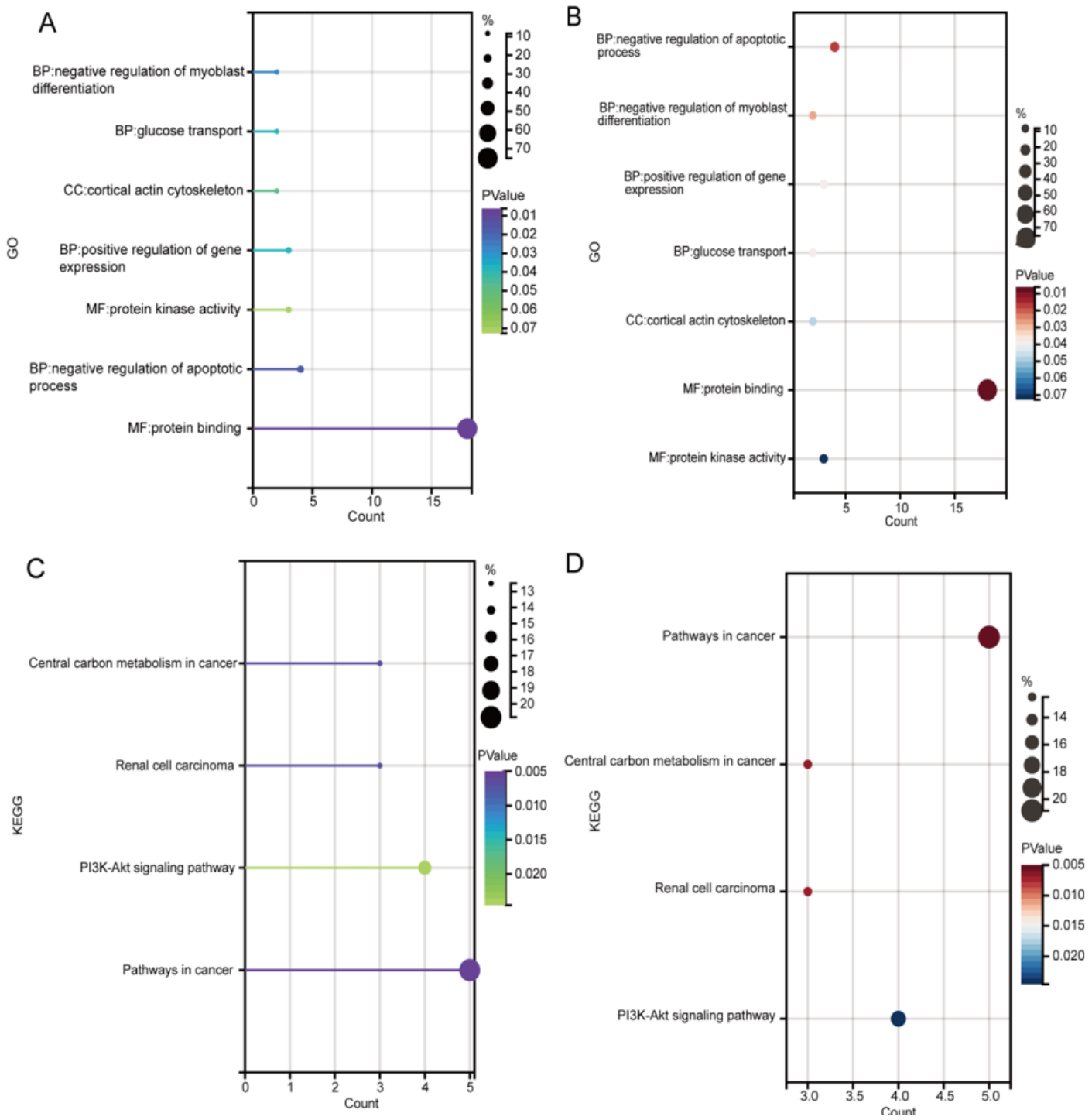

Figure 7

GO/KEGG analysis of 24 DEGs of ceRNA network. (A)Lollipop chart of GO;(B) Bubble chart of GO; (C)Lollipop chart of KEGG;(D) Bubble chart of KEGG.

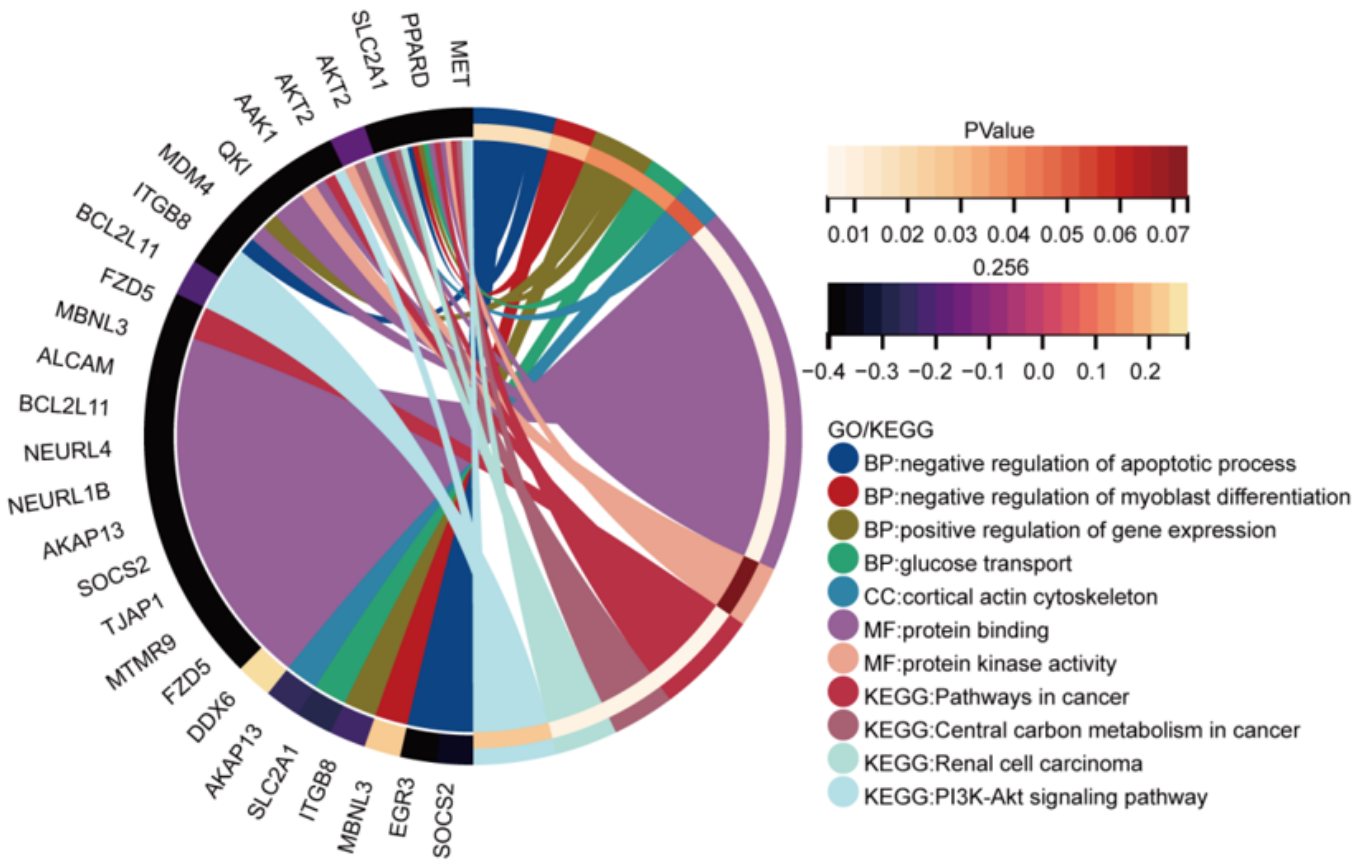

Figure 8

GO/KEGG chord diagram.

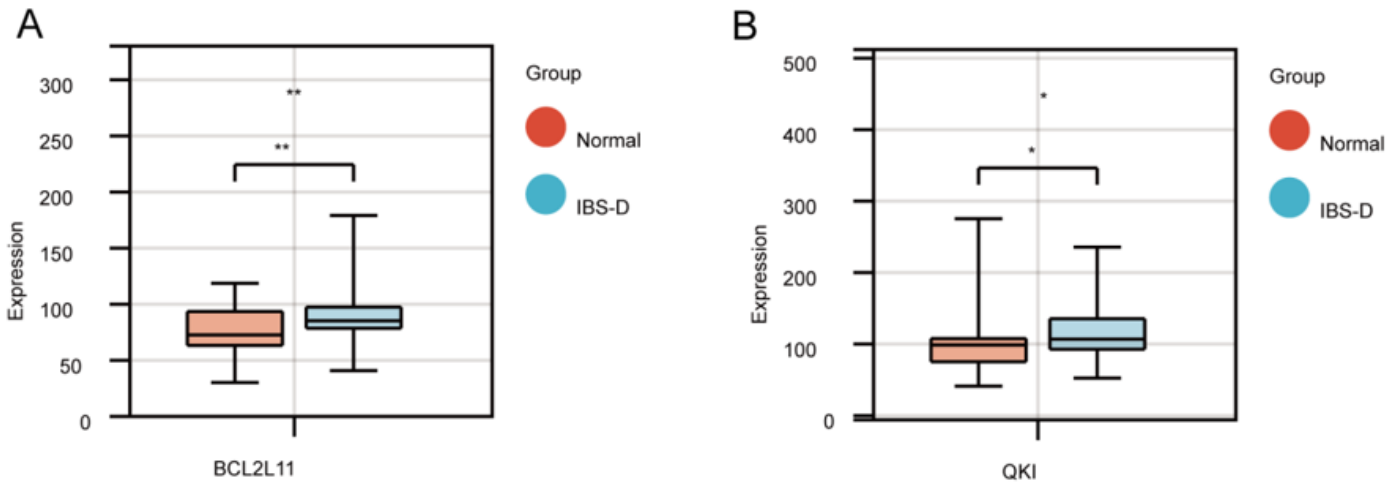

Figure 9

Difference Analysis Box Plot of key genes. (A) Expression of BCL2L11 between normal rectal mucosa tissues and IBS-D rectal mucosa tissues; (B) Expression of QKI between normal rectal mucosa tissues and IBS-D rectal mucosa tissues. Red represented normal samples, and blue represented IBS-D samples.

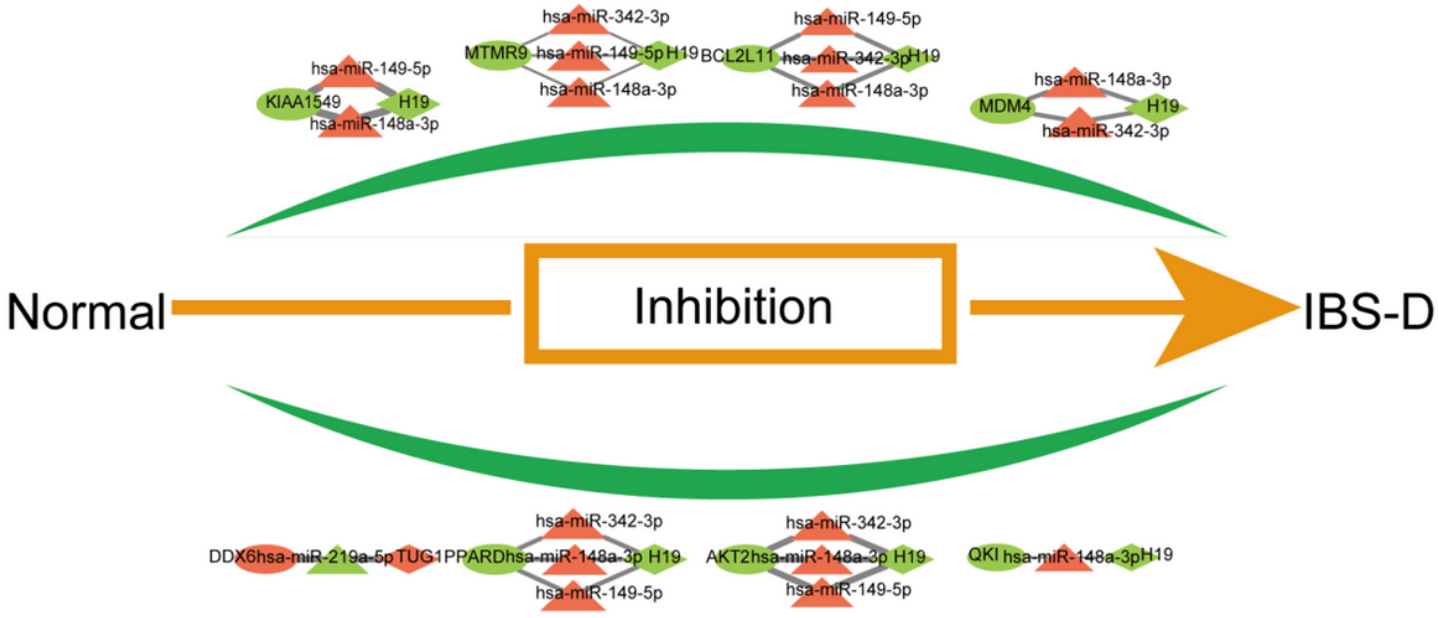

Figure 10

The potential candidate lncRNA-miRNA-mRNA regulatory schema chart in IBS-D. The diamond: lncRNA, the triangle: miRNA, and the ellipse: mRNA, Red: up-regulation,green: down-regulation