Reconstruction and Differential Expression Profile Analysis of the CircRNA-MiRNA- MRNA Network Based on Competitive Endogenous RNA in Ulcerative Colitis

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Abstract

Ulcerative colitis (UC) is among the common autoimmune disease worldwide. Circular RNAs (CircRNAs) are members of the noncoding RNA family (NcRNAs), in addition to its role in numerous biological processes, they are also linked to a vast range of diseases, including UC. Although previous studies looking at many circRNAs, we are still unclear about the physiological and pathological roles of the circRNAs-associated competing endogenous RNA (ceRNA) network in UC. Based on this, we constructed a circRNA–miRNA–mRNA network based on the ceRNA theory by analyzing from the National Center for Biotechnology Information Gene Expression Omnibus (NCBI-GEO) database. Genes with higher degrees than others in the ceRNA network were selected as central nodes in constructing the corresponding core subnetworks. In order to fully comprehend the biological function of the ceRNA network, we entered all differentially expressed mRNAs (DEmRNAs) which from the ceRNA network into the Database for Annotation and Integrated Discovery (DAVID) for GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) analyses and the STRING for PPI: protein-protein interaction (PPI) network analysis, genes with the degree value > 5 were defined as the hub genes in the regulatory network. In a nutshell, the ceRNA network was composed of 403 circRNA nodes, 5 miRNA nodes, 138 mRNA nodes and 559 edges. 3 core ceRNA subnetworks centered on hsa-miR-342-3p, hsa-miR-199a-5p and hsa-miR-142-3p were reconstructed. The results elucidated that enrichment of 167 GO categories and 14 KEGG pathway terms. The core PPI network was made up of 15 core targets, of which CD44, HIF1A and MMP2 were the most significant core targets. Overall, this study offered a comprehensive and detailed analysis of the fundamental roles that the ceRNA network played in UC, the hub nodes derived from this research may also serve as diagnostic markers and therapeutic targets.

1. Introduction

Ulcerative colitis (UC), one of the two major types of inflammatory bowel disease (IBD), which having perceived risk of colorectal cancer (CRC), is among the common autoimmune disease worldwide [1-3]. The notably consistent features of UC are characterized by chronic uninterrupted inflammation of the intestinal mucosa, exhibiting persistent infections in the affected tissue, bleeding, diarrhea, urgency and abdominal pain [4]. The disease are frequently alternative cycles of exacerbation and remission [5]. The prevalence of UC varies considerably across different countries, the incidence is higher in western countries, particularly within Europe and North America [6]. According to epidemiological studies, the highest incidence of UC is 57.9/100,000 person-years in Northern Europe and 23.14/100,000 person-years in North America [7]. Since the end of the last century, a steep increase of UC in low-incidence areas such as Asia have been observed [8]. Indeed, a number of diseases are associated with UC such as rheumatoid arthritis and interstitial nephritis [9,10]. This disease is endemic and prevalent around the world, exerting a substantial healthy and economic burden in many countries due to lost labor and high cost to health care systems [11-13].

Mechanisms controlling pathophysiological processes of UC are extremely complicated, and the exact pathogeneses are not clear [14,15]. Currently, it has been suggested that the occurrence of UC is

associated with dysregulation of the host's mucosal immune system, environmental factors, changes in the intestinal microbiome and genetic susceptibilities [16-18]. Numerous studies have demonstrated that a number of molecular changes are implicated in the progression of UC, to identify therapeutic strategies for reducing the incidence of UC, it is imperative to investigate UC-specific molecular pathogenesis and look for a novel curative way to treat it.

Numerous public databases, such as GENCODE, have been identified and documented thousands of genes, but they are still in their infancy when it comes to functional characterization. Circular RNAs (CircRNAs) are members of the noncoding RNA family (NcRNAs) discovered by electron microscopy in 1979 [19]. A circRNA has a specific circular structure and is produced by 'back-splicing' mechanism as opposed to linear RNAs [20]. In humans, circRNAs play a significant role in regulating gene expression to participate in diseases, but their functions in UC remain controversial [21]. Identified as a class of small noncoding RNAs, microRNAs (miRNAs) are short (18-25 nucleotides in length), endogenous, noncoding single-stranded RNAs that highly conserved. Even though they have no ability to generate proteins, they regulate gene expression negatively [22]. MiRNAs have effect on several pathophysiological processes, such as cell division, apoptosis, immune response and tissue homeostasis [23]. Since they are part of an intricate network of regulatory molecules, miRNAs have the ability to modulate different molecular mechanisms involved in disease. According to the competing endogenous RNA (ceRNA) theory, circRNA, miRNA and mRNA form the interaction network, circular RNAs may play pivotal roles in gene regulation, for example, acting as microRNA's "sponges" to release microRNAs and target messenger mRNAs [24,25]. Nonetheless, there have been few studies on the interactions of circRNA-miRNA-mRNA network in UC. In light of the fact that genetic factors play a crucial role in the pathogenesis of UC, it will be beneficial to enhance the relationships between circRNAs, miRNAs and target genes in order to learn more about UC mechanisms as well as identify novel biomarkers.

In the present study, an extensive analysis of the circRNA, miRNA and mRNA expression profiles in UC patients and healthy people was performed. Based on the ceRNA theory, we constructed a circRNA-miRNA-mRNA triple network, and the core ceRNA subnetwork was reconstructed. After that, we examined pathways linked to significant differentially expressed genes (DEGs) based on Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG). Finally, PPI network analysis were also conducted on DEmRNAs. Through this research, we gained greater understanding of the role that the circRNA-associated ceRNA network played in UC pathogenesis and obtained valuable clues for further research. Workflow for circRNA-associated ceRNA network analysis in Ulcerative Colitis is illustrated in Figure 1.

2. Materials And Methods

2.1Raw data

MRNA (GSE36807, GSE48958), miRNA (GSE43009, GSE48957, GSE53867), circRNA (GSE131911, GSE178753) expression and corresponding clinical information of UC patients were freely accessible

from National Center for Biotechnology Information Gene Expression Omnibus (NCBI-GEO) (https://www.ncbi.nlm.nih.gov/geo/#), GEO is a public functional genomic data repository that accepts microarrays and sequencing data. It also provides tools to help users query and download experimental gene expression profiles. The data set of our study included 109 samples, of which 43 were human normal colonic mucosa samples and 66 were human UC colonic mucosa samples. Downloaded the matrix and platform files in the chip.

2.2 Screening of differentially expressed circRNAs, miRNAs and mRNAs

Performing background correction and matrix data normalization for chips downloaded from GEO database, the differentially expressed circRNAs (DEcircRNAs), differentially expressed miRNAs (DEmiRNAs) and differentially expressed mRNA (DEmRNAs) between UC patients and the healthy controls were further calculated. The DEGs from all of the data sets with |logFC, $\log_2$ fold change| $\geq$ 1.0 and a *P*-value < 0.05 after correction were considered as the selection criteria for subsequent analysis. Consequently, the intersection of DEmRNAs, DEmiRNAs and DEcircRNAs on the chip were obtained respectively, and logFC $\geq$ 1.0 indicated that the DEmRNAs, DEmiRNAs and DEcircRNAs were up-regulated in UC, and vice versa. Drew the heat map and volcano map of GSE36807 for visualizing.

2.3 Prediction of target circRNAs and mRNAs of differentially expressed miRNAs

First, it should be stated that combining with the DEmiRNAs, we predicted the integrated circRNA-miRNA pairs using starBase (http://starbase.sysu.edu.cn/). Second, the prediction of targeted mRNAs related to miRNAs were retrieved from three databases: TargetScanHuman (https://www.targetscan.org/vert_72/), mirTarBase (http://mirtarbase.cuhk.edu.cn/), miRDB (http://mirdb.org/). Finallly, we established the matched circRNA-miRNA pairs and miRNA-mRNA pairs.

2.4 Construction of the circRNA-miRNA-mRNA ceRNA network

The circRNA–miRNA–mRNA network was established by recombining all co-expression circRNA-miRNA pairs and miRNA-mRNA pairs and was visualized using Cytoscape 3.7.2 software. Simultaneously, all node degrees of the miRNA–circRNA–mRNA network were calculated, the subnetworks of the circRNA-associated ceRNA network were then estimated.

2.5 The key circRNA-miRNA-mRNA subnetworks reconstruction

We further reconstructed the key subnetworks by comparing genes (circRNAs, mRNAs and miRNAs) node degree along with its correlative circRNA-miRNA pairs and miRNA-mRNA pairs in the holistic ceRNA network. The reconstruction of key subnetworks was using Cytoscape 3.7.2 plug-in CytoHubba [26], this plug is used to discover the key targets and subnetworks of complex networks and calculate the information of each node in the network diagram. According to recent research that the top 25 % nodes in ceRNA networks can be selected [27], and merely, the complete circRNA-miRNA-mRNA axes were retained.

2.6 Functional enrichment analysis

To assess functional enrichment, Database for Annotation and Integrated Discovery (DAVID, https://david.ncifcrf.gov/) was utilized for analyzing the functional enrichment analysis of DEmRNAs. We entered all DEmRNAs which from the circRNA-associated ceRNA network into the DAVID database, questing and acquiring the biological processes in Gene Ontology (GO) as well as Kyoto Encyclopedia of Genes and Genomes pathway (KEGG) ($p < 0.05$) of DEmRNAs. And Bioinformatics software (http://www.bioinformatics.com.cn) was used ultimately for visualizing GO and KEGG functional enrichment analysis of DEmRNAs.

2.7 Building of protein-protein interaction (PPI) network

The protein-protein interaction network was constructed by using the STRING online database (STRING, https://string-db.org/). We input DEmRNAs within the circRNA-associated ceRNA network into the STRING database, the corresponding PPI network was obtained after operation. Afterwards, the constructed PPI network was visualized by using the CytoHubba plug in Cytoscape 3.7.2. According to a previous study, the nodes with degree value > 5 in the network are defined as hub genes in the PPI regulatory network [28], so we rebuilt a core PPI network.

3. Results

3.1Differentially expressed circRNA, miRNA and mRNA

The expression profiles of mRNA, miRNA and circRNA between 66 UC samples and 43 normal samples were calculated. The pre-processing of raw data revealed 1809 differentially expressed genes (DEGs), including 878 DEmRNAs, 881 DEcircRNAs and 50 DEmiRNAs ($|\log FC, \log_2$ fold change$| \geq 2.0$ and a P-value < 0.05). Of these, 499 DEmRNAs, 460 DEcircRNAs and 14 DEmiRNAs were up-regulated; A total of 379 DEmRNAs, 421 DEcircRNAs and 36 DEmiRNAs were down-regulated. The volcano map and heat map of the analyzed DEmRNAs (GSE36807) are shown in Figure 2 and Figure 3.

3.2 Establishment of miRNA-mRNA□circRNA-mRNA interactions

The TargetScan, miRDB as well as miRTarBase databases respectively predicted mRNAs by using 50 DEmiRNAs. The results obtained from the three databases were incorporated, a total of 5954 miRNA-mRNA pairs were acquired after removing duplicates. Then mRNAs in 5954 pairs were intersected with 878 DEmRNAs, successfully attained 285 pairs of miRNA-mRNA interactions, consisting of 13 DEmiRNAs and 209 DEmRNAs. Through the same procedure, starBase database successfully measured circRNAs by using 50 DEmiRNAs, harvesting 403 pairs of circRNA-mRNA interactions, which comprising 5 DEmiRNAs and 403 DEcircRNAs. The results received from the database were intersected with 881 DEcircRNAs, since no intersecting pairs of circRNA-mRNA were found, the ceRNA network was constructed using the 403 circRNA-mRNA pairs predicted by the starBase database.

3.3 Construction of Competing Endogenous RNAs Network

In order to better understand the role of DEcircRNAs in UC and to further elucidate the interaction between these DEcircRNAs and DEmiRNAs, we established a circRNA-miRNA-mRNA related competing endogenous RNAs network of UC. All circRNA-miRNA pairs and miRNA-mRNA pairs were integrated, and Cytoscape 3.7.2 was further adopted for constructing and visualizing the circRNA-miRNA-mRNA network. As shown in Figure 4, the circRNA-associated ceRNA network was composed of 403 circRNA nodes, 5 miRNA nodes, 138 mRNA nodes and 559 edges.

3.4 The key circRNA–miRNA–mRNA subnetwork

For further identification of hub gene as well as their relevanted networks, Cytoscape 3.7.2 plug-in CytoHubba was utilized for calculation and visualization of all node degree values in the circRNA-associated ceRNA network. We selected the top 10 nodes ranked by the degree value, nodes selected were far fewer than the top 25 % initial nodes in the ceRNA network, which represented more accuracy. The top 10 nodes including hsa-miR-342-3p, hsa-miR-199a-5p, hsa-miR-650, hsa-miR-142-3p, hsa-miR-483-3p, ZNF652, INO80D, ANK3, TSPAN6 and PCDH17. The key network formed by the top 10 nodes is shown in Figure 5. To be specific, hsa-miR-342-3p, hsa-miR-199a-5p, hsa-miR-650, hsa-miR-142-3p and hsa-miR-483-3p were five of the top-ranked nodes, it was regretful that hsa-miR-650 and hsa-miR-483-3p were not interconnected with the key subnetwork, therefore, we got rid of them, and 3 new key subnetworks were reconstructed. 3 miRNAs served as the center of subnetwork were significant in regulating transcription, 3 key subnetworks of circRNA-hsa-miR-342-3p-mRNA, circRNA-hsa-miR-199a-5p-mRNA, circRNA-hsa-miR-142-3p-mRNA were extracted and shown in Figure 6 (A-C). The key subnetwork of circRNA-hsa-miR-342-3p-mRNA comprised 1 miRNA node, 50 mRNA nodes as well as 89 circRNA nodes (Figure 6A), The key subnetwork of circRNA-hsa-miR-199a-5p-mRNA consisted of 1 miRNA node,

43 mRNA nodes and 81 circRNA nodes (Figure 6B), The key subnetwork of circRNA-hsa-miR-142-3p-mRNA was composed of 1 miRNA node, 44 mRNA nodes along with 62 circRNA nodes (Figure 6C).

3.5 Functional Enrichment Analysis of Differentially Expressed mRNAs

Analysis about the functions of 138 DEmRNAs which came from the circRNA-associated ceRNA network were carried out. The results elucidated that enrichment of 167 GO categories occurred in the biological process, including 115 biological processes (BP), 36 cell components (CC) and 16 molecular functions (MF) with a threshold value of P 0.05. The top 5 most significant GO terms in each section are shown in Table 1, Figure 7. On the biological process (BP) level, the DEmRNAs were principally participated in positive regulation of angiogenesis, cellular response to fluid shear stress, semaphorin-plexin signaling pathway involved in axon guidance, wound healing, spreading of cells, negative regulation of cell proliferation. Those in cellular component (CC), in general, were affiliated with the parts of adherens junction, postsynaptic density, filopodium, actin cytoskeleton, GABA-ergic synapse. The majority of DEmRNAs enriched in molecular function (MF) were associated with long-chain fatty acid-CoA ligase activity, xenobiotic-transporting ATPase activity, arylsulfatase activity, efflux transmembrane transporter activity, protein binding. Subsequently, KEGG pathway enrichment analysis of all DEmRNAs in the circRNA-associated ceRNA network were implemented. The total 14 KEGG pathway terms are demonstrated in Table2. The genes were significantly enriched in 7 pathways, comprising with pathways in cancer, cAMP signaling pathway, Metabolic pathways, PPAR signaling pathway, TNF signaling pathway, PI3K-Akt signaling pathway, AGE-RAGE signaling pathway in diabetic complications, as shown in the Figure 8. As mentioned above, these results proved that DEmRNAs played a crucial role in a number of biological processes and functions, such as inflammation response and immune response, which were crucial for the occurrence and development of UC.

Table1. The top 5 GO terms in each section are obtained by analyzing DEmRNAs.

CC	GO:0005912	adherens junction	4	3.636905	0.096518
CC	GO:0014069	postsynaptic density	5	2.892992	0.093555
CC	GO:0030175	filopodium	3	5.800633	0.093466
CC	GO:0015629	actin cytoskeleton	5	3.018775	0.083094
CC	GO:0098982	GABA-ergic synapse	3	6.277397	0.081715
MF	GO:0004467	long-chain fatty acid-CoA ligase activity	2	18.95455	0.099786
MF	GO:0008559	xenobiotic-transporting ATPase activity	2	18.95455	0.099786
MF	GO:0004065	arylsulfatase activity	2	20.30844	0.093453
MF	GO:0015562	efflux transmembrane transporter activity	2	20.30844	0.093453
MF	GO:0005515	protein binding	96	1.090299	0.091253

BP, biological process; CC, cellular component; MF, molecular function. Enrichment score: the enrichment score of GO, which is accounted by -log 10 (*P*-value).

Table2. The total 14 KEGG pathway terms are obtained by analyzing DEmRNAs. KEGG, Kyoto Encyclopedia of Genes and Genomes.

Category	id	terms	Gene count	Enrichment score	*P* value
KEGG-PATHWAY	hsa05200	Pathways in cancer	10	1.916667	0.070678
KEGG-ATHWAY	hsa00071	Fatty acid degradation	3	7.100581	0.06501
KEGG-PATHWAY	hsa04024	cAMP signaling pathway	6	2.763122	0.063043
KEGG-PATHWAY	hsa04216	Ferroptosis	3	7.446951	0.059764
KEGG-PATHWAY	hsa01100	Metabolic pathways	22	1.453929	0.058577
KEGG-PATHWAY	hsa04610	Complement and coagulation cascades	4	4.789412	0.049268
KEGG-PATHWAY	hsa05418	Fluid shear stress and atherosclerosis	5	3.660971	0.045751
KEGG-PATHWAY	hsa05133	Pertussis	4	5.356579	0.037282
KEGG-PATHWAY	hsa03320	PPAR signaling pathway	4	5.428	0.036056
KEGG-PATHWAY	hsa04668	TNF signaling pathway	5	4.543527	0.023162
KEGG-PATHWAY	hsa04151	PI3K-Akt signaling pathway	9	2.5875	0.020934
KEGG-PATHWAY	hsa01212	Fatty acid metabolism	4	7.142105	0.017674
KEGG-PATHWAY	hsa04933	AGE-RAGE signaling pathway in diabetic complications	5	5.08875	0.015947
KEGG-PATHWAY	hsa05205	Proteoglycans in cancer	9	4.468171	7.94E-04

3.6 Building of protein-protein interaction (PPI) Network

We input DEmRNAs within the circRNA-associated ceRNA network into the STRING database for building a PPI network, which including 71 nodes and 150 edges, shown in Figure 9. Thereafter, this PPI network was analyzed by the CytoHubba plug in Cytoscape 3.7.2. According to the degree value obtained by topological analysis, nodes whose degree value >5 in the PPI network were further selected as the core targets, genes totaling 15 were selected, including CD44, HIF1A, MMP2, LOX, PTGS2, CAV1, VCAM1, EDN1, IL1A, HGF, COL1A2, PCSK9, CYR61, ACSL1, ADAMTS5. We further reconstructed a PPI network graphic of the 15 core targets based on the ceRNA network, and the results are shown in Figure 10. The core PPI network consisted of 15 nodes and 69 edges, nodes were represented by circular, nodes from small to large represented the degree value from low to high and color from light to dark, the higher the value, the more important the target. Among them, CD44 was the highest degree value (degree=19), followed by HIF1A (degree= 16) and MMP2 (degree=15). Thus, these 3 targets were worthy to be the most

signifcant core targets in the ceRNA network associated with UC and considered conducting further research.

4. Discussion

In the present study, we integrated microarray data on circRNA, miRNA and mRNA expression profiles in human colon tissue. With |logFC| ≥2.0 and a *P*-value < 0.05 thresholds, the expression of 460 up-regulated and 421 down-regulated circRNAs, 14 up-regulated and 36 down-regulated miRNAs, along with 499 up-regulated and 379 down-regulated mRNAs showed significant differences between the UC patients and control groups. As genes enumerated above were abnormally expressed in UC, speculation is warranted that these genes are involved in UC pathogenesis and development. For instance, RNU2-1 is considered as a potential diagnostic biomarker for pancreatic and colorectal adenocarcinoma [29], in this research RNU2-1 was up-regulated in UC, which has indicated that UC patients are more prone to colon cancer. However, alterations in DPP10 levels can modify inflammatory responses of lung epithelial cells [30], in this research DPP10 was down-regulated in UC, suggesting a role in the inflammatory process in the disease.

A new principle underlying RNAs interactions has been revealed by the ceRNA hypothesis: circRNAs in complex ceRNA networks of regulatory are competitively bound to miRNAs to affect gene silencing caused by miRNAs, participate in gene regulation and regulate mRNAs expression. Molecules in the ceRNA network are in a state of equilibrium during normal physiology, once the balance is disturbed, disease will occur [31]. As a consequence, the role of the circRNA-related ceRNAs networks and its regulatory mechanisms are of immense significance. In study after study, the ceRNA networks have built corresponding to atrial fibrillation, heart failure and colon cancer [32-34], little information is available on the UC-linked ceRNA network. In this research, we innovatively generated a characteristic circRNA-miRNA-mRNA network related to UC, which was composed of 403 circRNA nodes, 5 miRNA nodes, 138 mRNA nodes and 559 edges. We also calculated the nodes known as hub nodes, which have previously been shown to be characterized by their high degree of interconnection with other nodes, can be used as topological properties of the network to determine the momentous genes [35,36]. As a result of extracting the subnetworks in the circRNA-associated ceRNA network, we identified 3 key genes, including: hsa-miR-342-3p, hsa-miR-199a-5p, hsa-miR-142-3p. CircRNA-miRNA pairs associated with key genes can be candidated as diagnostic biomarkers of UC and indicated irreplaceable implications for UC.

As hub elements of the ceRNA network, miRNAs appear to be crucial to RNA transcription crosstalk. MiR-342-3p, miR-199a-5p and miR-142-3p are three substantial miRNAs implicate in diverse disease pathways. MiR-342-3p takes part in the development of inflammation which is associated with chronic diseases, report has indicated that the over expression of miR-342-3p following the inhibition of NEAT1 decreases inflammatory cytokines IL-6, IL-1β, TNF-α and cyclooxygenase-2 released in type 1 diabetes mellitus [37]. MiR-342-3p expression has been observed to decrease in the rectosigmoid area of UC

patients and implicate in mediating inflammation and cancer processes [38]. MiR-342 is also demonstrated to act as a tumor suppressor gene that inhibits the growth of colorectal carcinoma by regulating aberrant DNA hypermethylation [39]. Currently, miR-199a-5p is primarily investigated in oncology, including epithelial ovarian cancer and lung cancer, etc [40,41]. Pathways linked to miR-199a-5p have also been implicated in the inflammatory response to certain diseases. MiR-199a-5p levels are elevated in both crohn's disease (CD) and UC when compared to healthy controls [42], these observations supported that miR-199-5a is involved in the inflammation process that is common to IBD and other diseases. MiR-142-3p, a novel inflammatory regulator that controls pro-inflammatory mediators, inhibits apoptosis and may be associated with inflammation process in multiple diseases [43]. According to a previous study, miR-142-3p levels in mixed saliva samples are significantly higher in UC cases compared to controls, but no noticeable changes in CD [44]. MiR-142-3p is therefore involved in the pathological process of UC and is also a good diagnostic indicator of UC and CD. Additionally, miR-142-3p acts as an epigenetic regulator, playing a different role in tumorigenesis: it is not just a tumor suppressor in gastric cancer [45], it is a tumor promoter that exacerbates the development of colorectal cancer [46] as well.

MiR-342-3p, miR-199a-5p and miR-142-3p are both interconnected and unique, all three affect different diseases and cancer, they also implicate in the inflammation and pathogenesis of UC. As of right now, each of the three miRNAs has been the subject of an abundance of studies, but the circRNA-associated ceRNA network shaped by each miRNA has received little attention. In present study, we established a circRNA-associated ceRNA network of UC and 3 core subnetworks of circRNA-hsa-miR-342-3p-mRNA network, circRNA-hsa-miR-199a-5p-mRNA network and circRNA-hsa-miR-142-3p-mRNA network. This study for the first time shown that miR-342-3p, miR-199a-5p and miR-142-3p coexisted in a circRNA-related ceRNA network, and each miRNA comprised a corresponding core subnetwork. Since all three miRNAs participate in the regulation of UC inflammation, it is speculated that whether the entire ceRNA network or three core subnetworks have direct correlations with the occurrence and development of UC, particularly link with inflammation and carcinogenesis. Owing to differences in specific miRNAs' expressions are among the factors that can bring about increasing risk of colorectal cancer at the rectosigmoidal area [38], we should plan to further understand the pathological mechanism of UC by focusing on genes in the core ceRNA networks, striving to find a regulatory pathway that is inextricably linked to UC, then an intervention treatment will be administered in order to prevent cancerization and further spread of UC.

Analyses of GO and KEGG terms allowed us to better understand the biological functions of downstream mRNAs. As a result of GO enrichment analysis, we observed a large number of genes that were involved in proliferation and apoptosis of cells, immune response, intercellular communication and signal transmission. Furthermore, the KEGG pathway enrichment analysis revealed that 7 pathways were enriched and predominantly involved: Pathways in cancer, cAMP signaling pathway, Metabolic pathways, PPAR signaling pathway, TNF signaling pathway, PI3K-Akt signaling pathway and AGE-RAGE signaling pathway in diabetic complications.

Long-term UC patients have a much great risk of developing colitis-associated cancer [47], TLR4/NF-κB, TNF-α and IL-6 related pathways are crucial in the transformation of UC into colon cancer [48]. One of the most universal comorbidities of UC is diabetes mellitus, the interaction of AGE-RAGE has been demonstrated to affect the morphology and function of the gut in diabetics, and the RAGE signaling pathway is relevant to intestinal inflammation and permeability in CD and UC [49]. Evidence suggested that gut microbiota is also likely to affect UC by altering metabolism by generating specific enzymes and/or metabolites [50]. Because of this, UC has possessed a strong connection to metabolic diseases and pathways. An increasing body of evidence support cAMP's role in triggering key features of inflammation resolution: induction of pro-resolving mediators, apoptosis, efferocytosis and phagocytosis, nonphlogistic recruitment of macrophage, macrophage polarization and tissue homeostasis [51]. PPARs (peroxisome proliferator-activated receptors) are nuclear receptor transcription factors that are ligand-inducible, they can regulate UC. Oleoylethanolamide restores mRNA transcription of PPAR-α blocked by dextran sodium sulfate (DSS) in mice colitis [52], activating the PPAR-γ pathway in the intestinal epithelium by 5-Aminosalicylic acid ameliorates colitis in mice treated by DSS [53]. TNF is a key proinflammatory cytokine, and myricetin derivative M10 inhibits necroptosis in inflamed colonic mucosal cells in mice via down-regulating the TNF-α pathway [54]. PI3K/Akt signaling pathways have been shown to regulate and release pro-inflammatory cytokines, such as TNF-α [55], additional studies have documented that PI3K/Akt/mTOR signaling pathway exerts a vital regulatory effecting on inflammation and apoptosis of UC cells [56]. Collectively, based on the findings of this study, multiple targets and pathways such as inflammation, immunity, cell proliferation and apoptosisand as well as cancer participate in the pathogenesis and progression of UC.

A subsequent analysis of the PPI results revealed that CD44, HIF1A and MMP2 ranked as the top 3 DEmRNAs based on the circRNA-associated ceRNA network. CD44 and HIF1A are ferroptosis-related genes in ulcerative colitis [57]. CD44 is a glycoprotein presented on the surface of cells that participates in cell-cell interactions, adhesions and migrations [58], not only is CD44 involved in the pathogenesis of UC [57], but can also serve as a predictor of tumor development and a therapeutic target [59]. Hypoxia leads to the hypoxia-inducible factor (HIF)-signaling pathway being activated during inflammatory diseases [60], research has proven that in severe UC, Cyclosporine A modulates neutrophil functions directly through the SIRT6-HIF-1α-glycolysis axis, ameliorating intestinal mucosal damage and alleviating clinical symptoms [61]. The novel H3NT protease MMP-2 is required for myogenic gene activation and myoblast differentiation [62], the data have indicated that MMP-2 and MMP-9 levels in UC patients' serum are markedly elevated, which has further exacerbated occurrence and progression of UC [63]. Likewise, PPI network analysis shows that mRNAs based on the ceRNA network are closely connected to UC, especially in inflammation, cell proliferation and apoptosis as well as cancer. The GO and KEGG enrichment analyses and PPI network analysis moved forward to confirming our previous hypothesis.

5. Conclusions

On the basis of the ceRNA theory, we innovatively constructed a circRNA-miRNA-mRNA network, systematically analyzed and observed this network-mediated the roles of genes in occurrence and

development with UC for the first time. CircRNAs and mRNAs are both regulated and co-expressed negatively with certain particular miRNAs, so circRNAs' functions correspond to that of the linked mRNAs. We identified 3 miRNAs (hsa-miR-342-3p, hsa-miR-199a-5p, hsa-miR-142-3p) as core miRNAs in the cricRNA-associated ceRNA network. Additionally, the functions and potential pathways of mRNAs in the cricRNA-associated ceRNA network connected to UC were further illustrated using GO and KEGG enrichment analyses as well as PPI network analysis. As a result of the study, it was appeared that circRNA-associated ceRNA network was engaged in the inflammatory response, immune response, cell proliferation and apoptosis as well as carcinogenesis of UC.

According to analyzing circRNA-associated ceRNA network, this study is to provide effective evidence and bases for refining our understanding of UC from a molecular perspective. However, this study also has certain limitations. As the fact that each miRNA corresponds to multiple circRNAs or mRNAs, we describe the core miRNAs and mRNAs in the ceRNA network rather than circRNAs. For this reason, it is necessary to conduct the corresponding circRNAs intervention analysis, carrying out stricter scientific verification and research strategies to determine the corresponding circRNAs, ultimately, finding a specific pathway within this circRNA-associated ceRNA network.

Abbreviations

UC
Ulcerative colitis
CircRNAs
Circular RNAs
MiRNAs
microRNAs
NcRNAs
the noncoding RNA family
ceRNA
competing endogenous RNA
NCBI-GEO
National Center for Biotechnology Information Gene Expression Omnibus
DAVID
Database for Annotation and Integrated Discovery
GO
Gene Ontology
KEGG
Kyoto Encyclopedia of Genes and Genomes
PPI
protein-protein interaction
IBD
inflammatory bowel disease

CRC
colorectal cancer
DEcircRNAs
differentially expressed circRNAs
DEmiRNAs
differentially expressed miRNAs
DEmRNAs
differentially expressed mRNAs
DEGs
differentially expressed genes
BP
biological processes
CC
cell components
MF
molecular functions
CD
crohn's disease
PPARs
peroxisome proliferator-activated receptors
DSS
dextran sodium sulfate

Declarations

Ethics approval and consent to participate

All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated and/or analysed during the current study are available in the GEO database (https://www.ncbi.nlm.nih.gov/geo/).

Conflict of Interest

The authors declare no conflict of Interest.

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Authors' Contributions

Yunsheng Xu participated in the design of the study and revised the manuscript. Sai Xu drafted the manuscript. Sai Xu and Shouqiang Chen conducted construction of the ceRNA network. Menghe Zhang and Wenrong An compiled GO and KEGG enrichment analyses. Jie Li and Zhenhai Sun performed the PPI network analysis. The authors all approved the final edited version of the manuscript.

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Figures

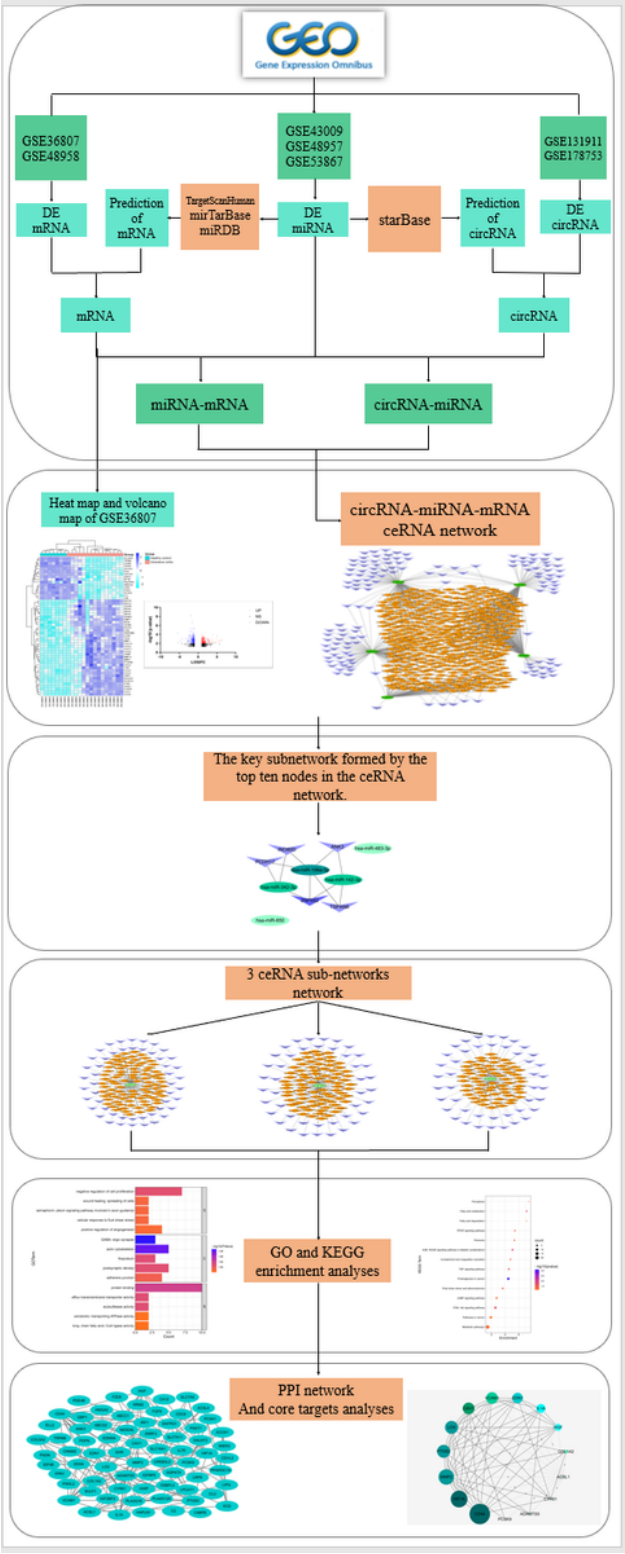

Figure 1

Workflow for circRNA-associated ceRNA network analysis in Ulcerative Colitis

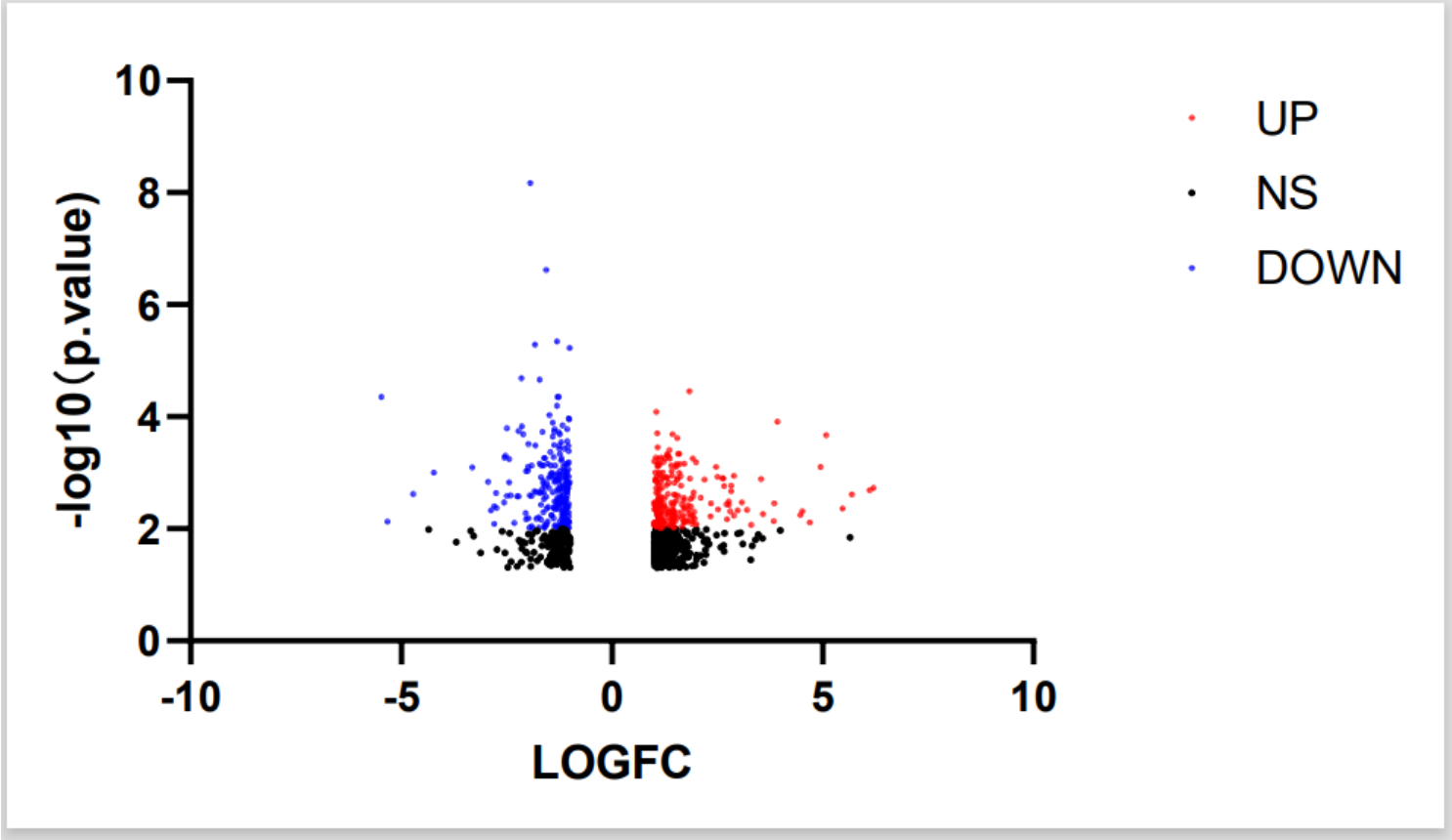

Figure 2

Volcano map of DEmRNAs from GSE36807 of UC. Red represents up-regulated genes; blue represents down-regulated genes.

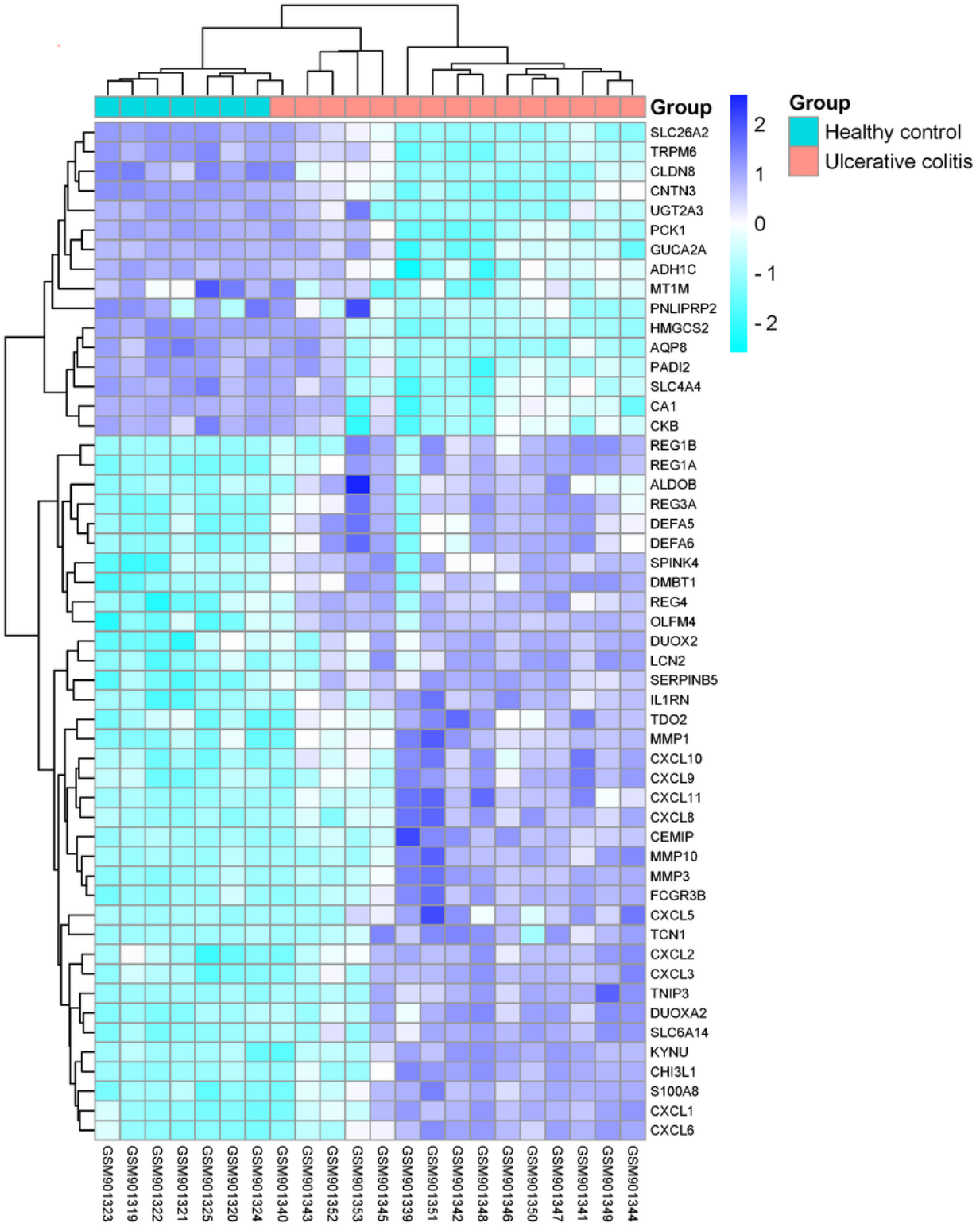

Figure 3

Heat map of DEmRNAs from GSE36807 of UC. Purple boxes represent up-regulated genes and blue boxes represent down-regulated genes. Pink and blue represent UC and healthy normal, respectively.

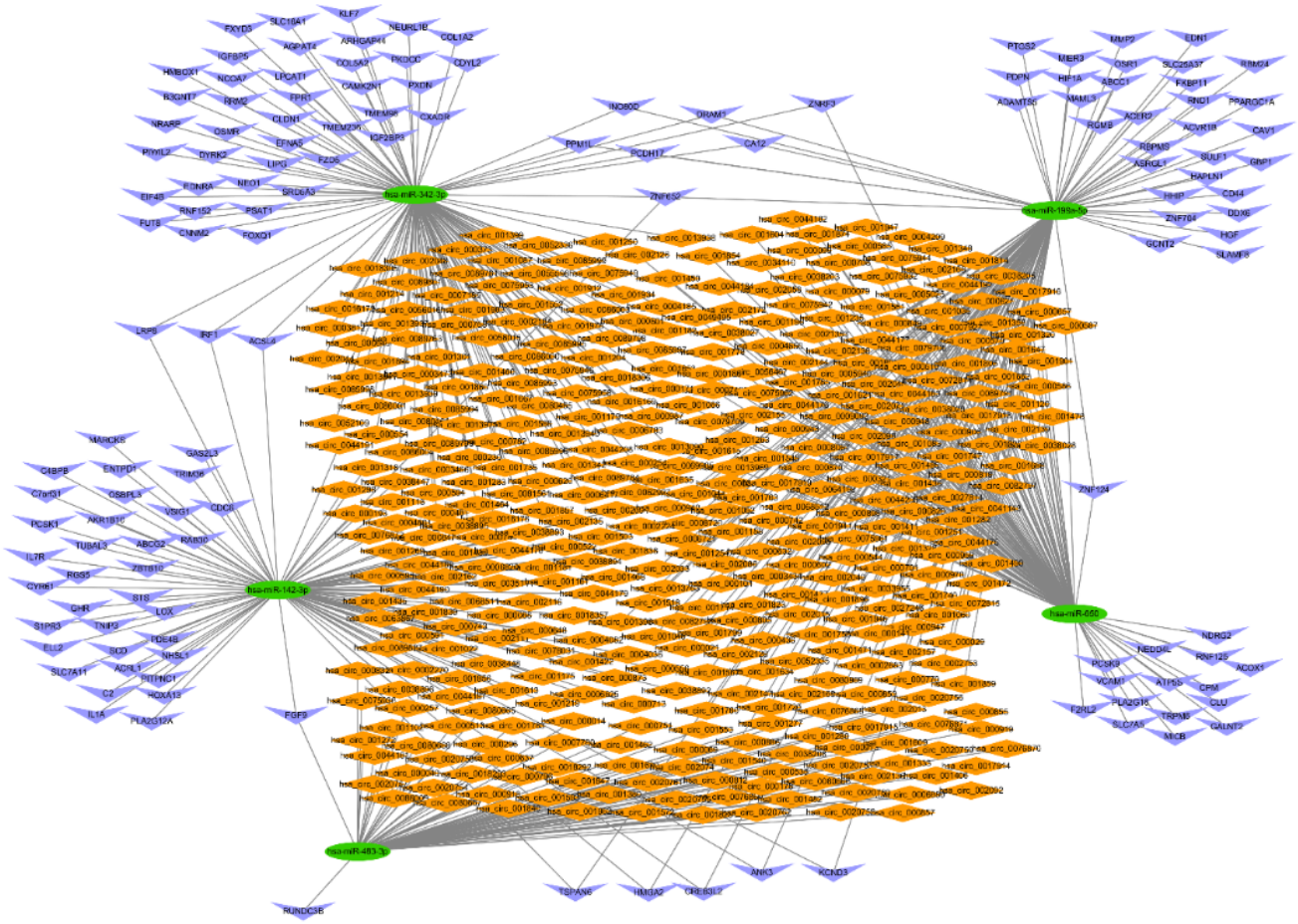

Figure 4

The UC-related circRNA-mRNA-miRNA ceRNA regulatory network. Orange diamond for circRNAs, green oval for miRNAs, purple arrow for mRNAs.

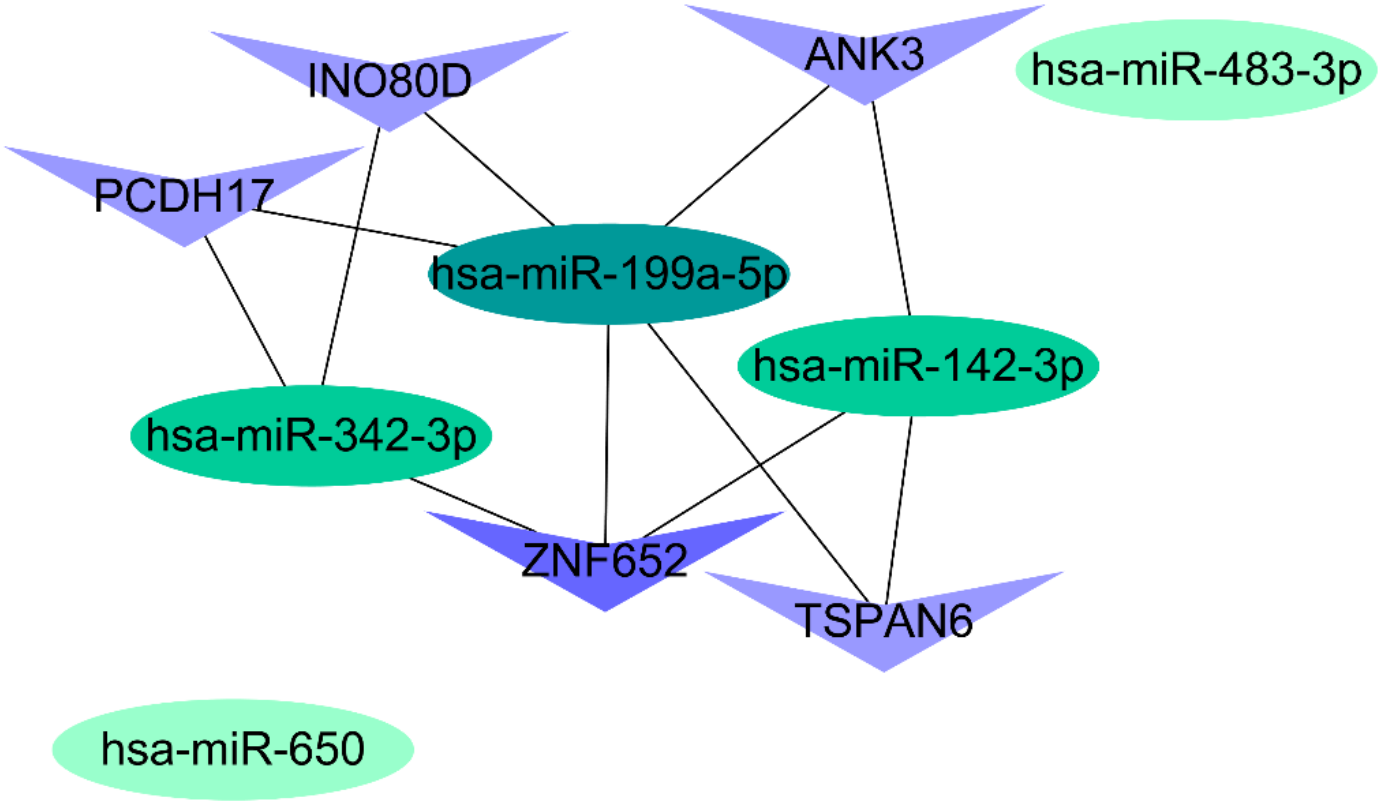

Figure 5

The top ten nodes in the circRNA-associated ceRNA network. Oval for miRNAs, arrow for mRNAs, Nodes with darker colors are more important.

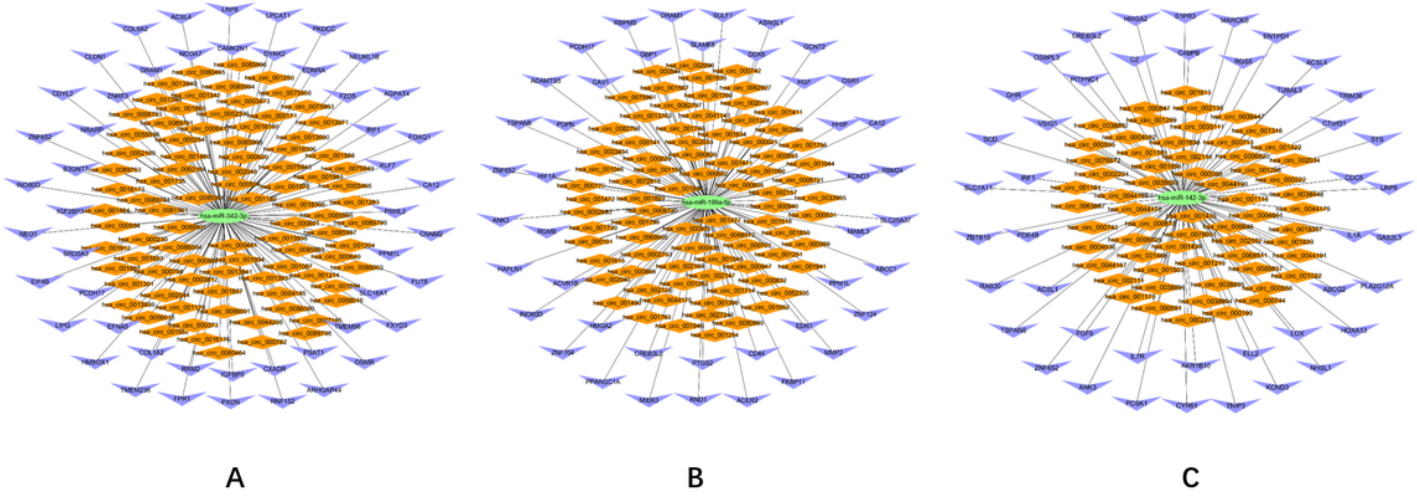

Figure 6

Three key circRNA-associated subnetworks. (A) The key subnetwork of circRNA-hsa-miR-342-3p-mRNA; (B) The key subnetwork of circRNA-hsa-miR-199a-5p-mRNA; (C) The key subnetwork of circRNA-hsa-miR-142-3p-mRNA.

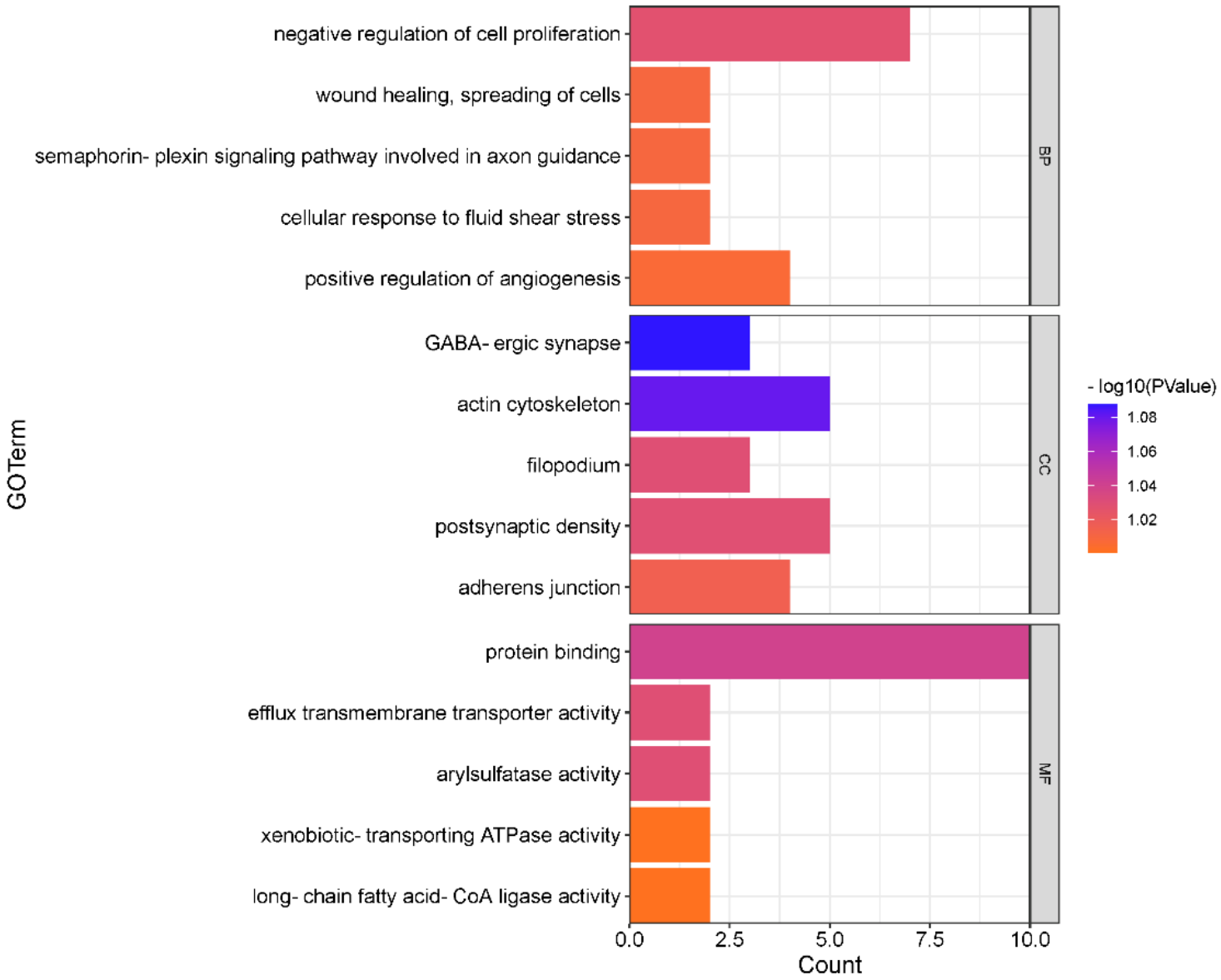

Figure 7

The top 5 GO terms in each section (BP, CC and MF) are displayed as a bar diagram by analysing DEmRNAs. The colors indicate different -log10 (*P* value).

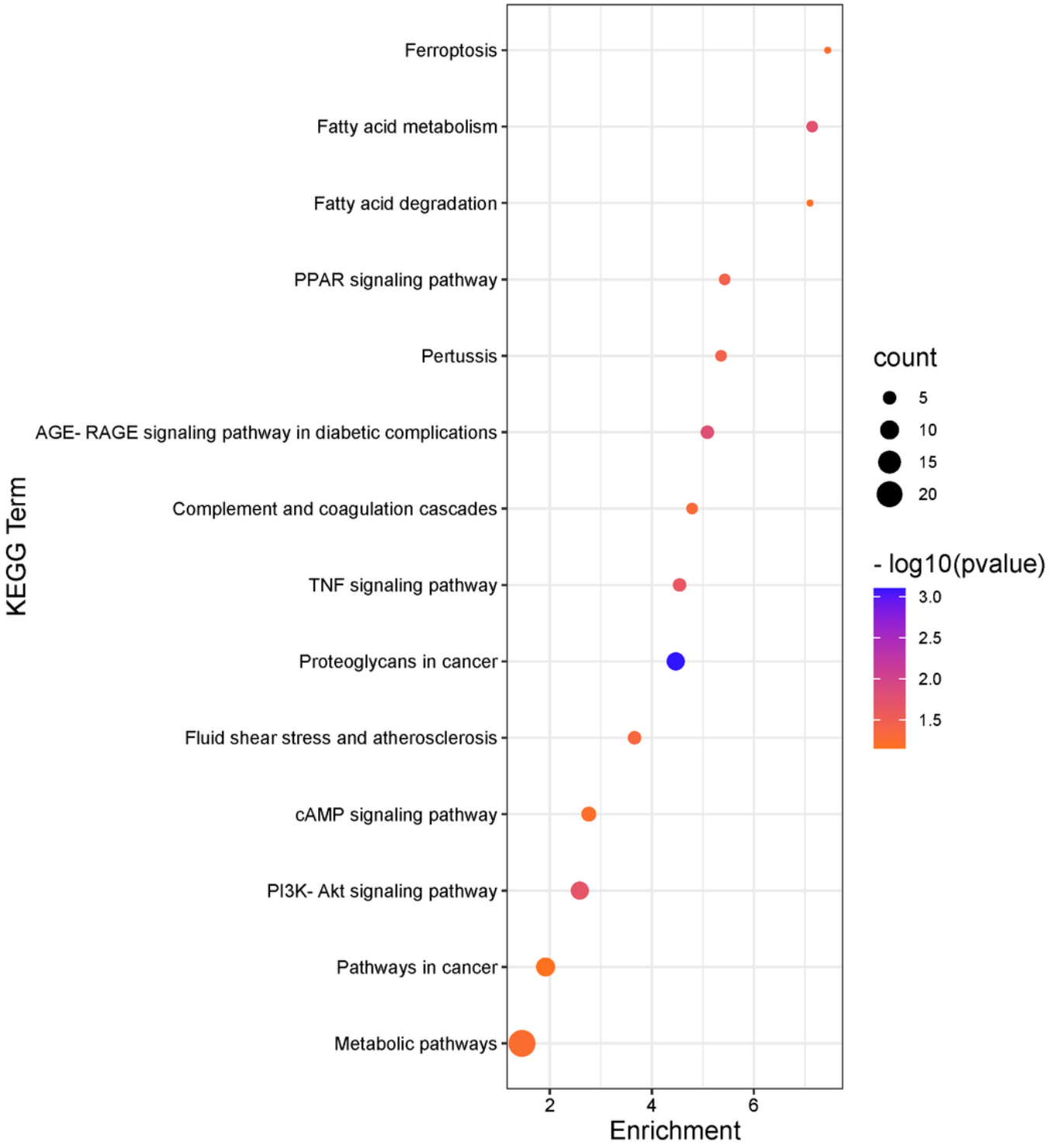

Figure 8

The total 14 KEGG pathway terms are displayed as a bubble diagram by analysing DEmRNAs. The colors indicate different -log10 (*P* value), and the sizes of the circle represent counts.

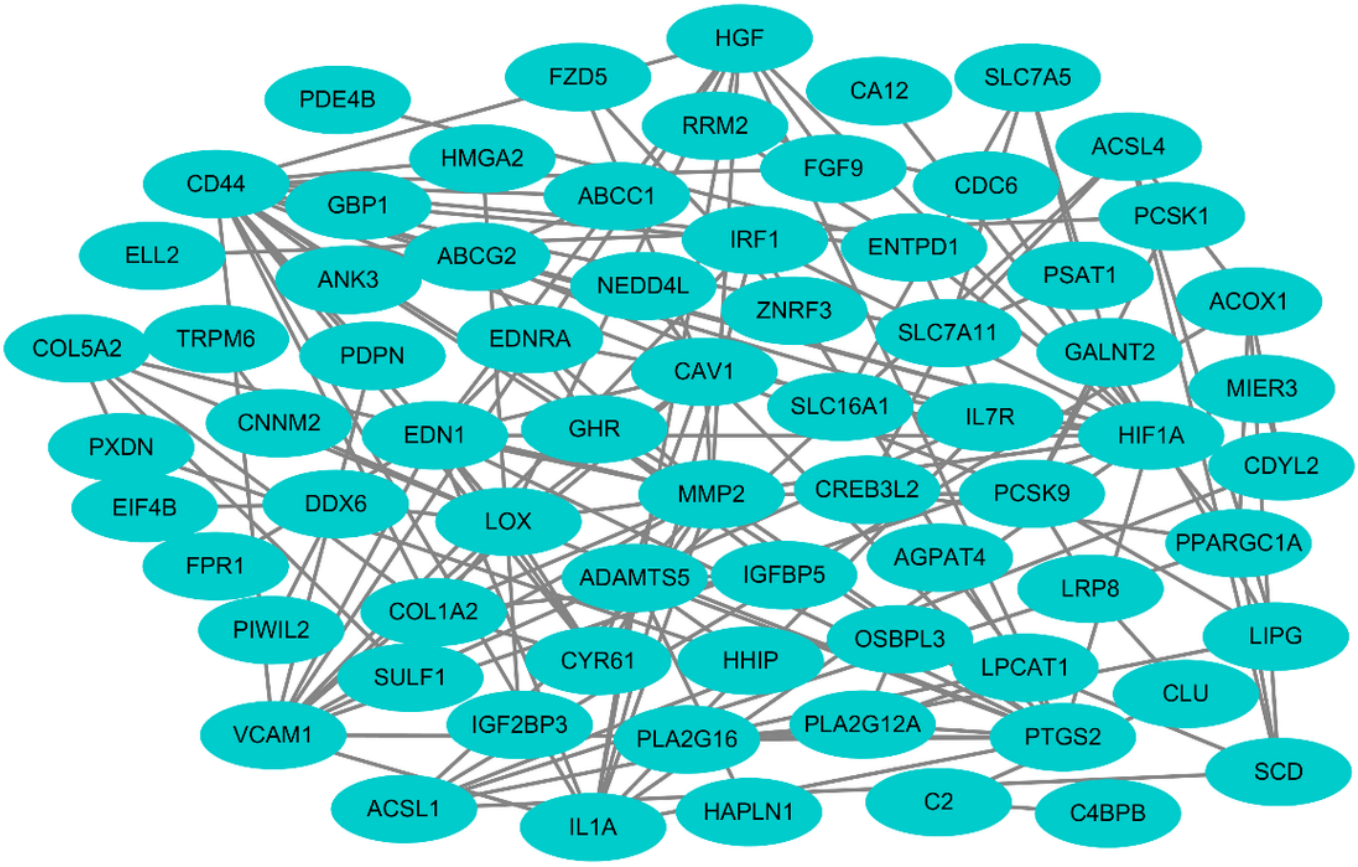

Figure 9

The PPI network of 71 targets of DEmRNAs. Edges represent protein–protein associations.

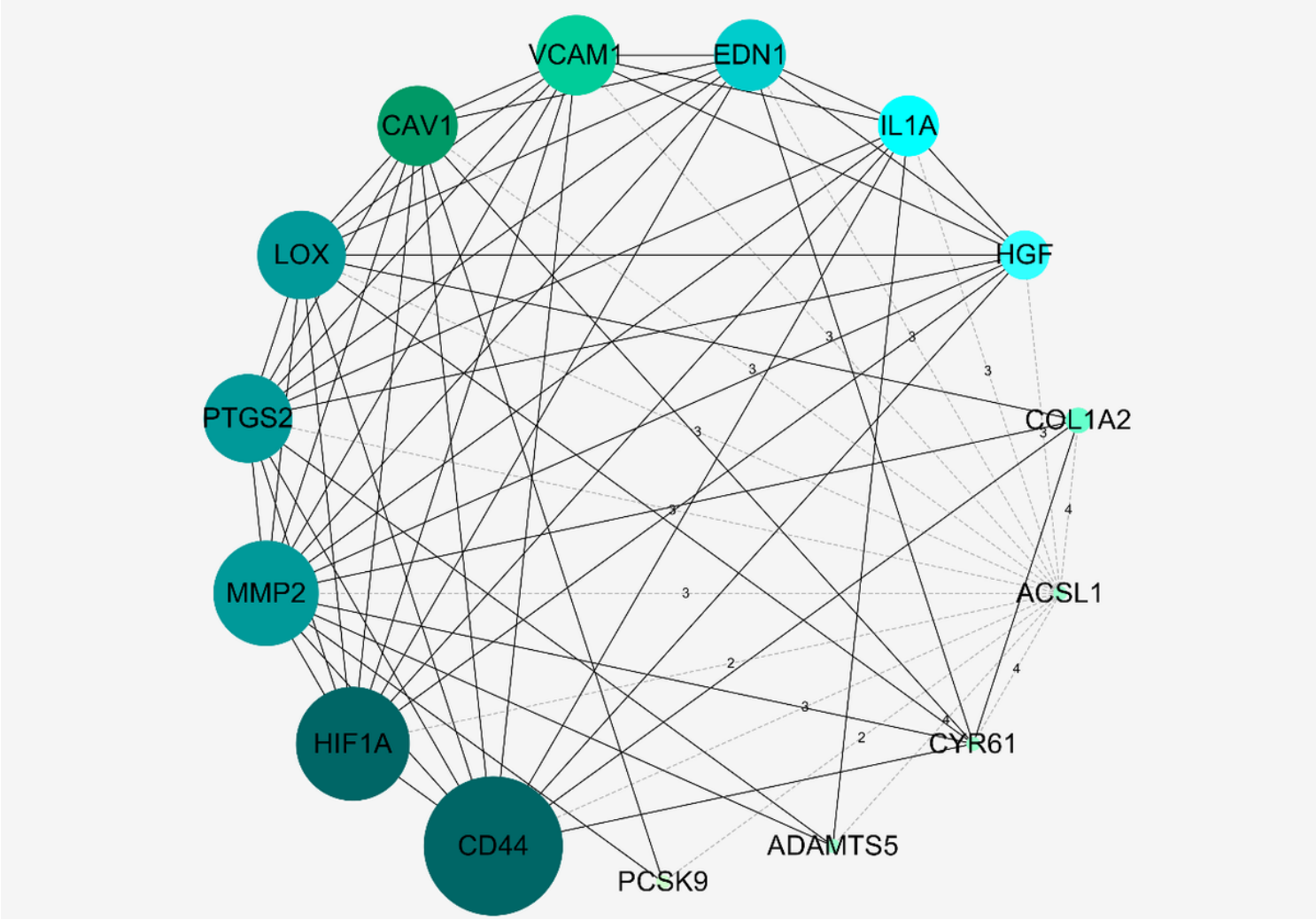

Figure 10

PPI network of 15 core targets. The degree value > 5. Nodes from small to large represent the degree value from low to high and color from light green to dark green.