Candidate genes and potential mechanisms for chemoradiotherapy sensitivity in locally advanced rectal cancer

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Abstract. The aim of the present study was to investigate candidate genes for chemoradiotherapy (CRT) sensitivity in patients with locally advanced rectal cancer (LARC), and the potential mechanisms of their action. A microarray dataset (GSE98959) was obtained from the Gene Expression Omnibus database that included microRNA (miRNA, miR) expression profiling of 22 samples from patients with LARC who had received preoperative radiotherapy and chemotherapy. Of these patients, 10 responded to the treatment and 12 did not. Differentially expressed miRNAs (DEMs) were identified, followed by the construction of an miRNA-gene network. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) function analyses were performed on the target genes in the miRNA-gene network. Furthermore, a protein-protein interaction (PPI) network was constructed on the basis of the target genes, followed by GO function enrichment and KEGG pathway analysis. A total of 30 DEMs were identified between the responder and non-responder groups. Thiamine metabolism (including miR-371a-3p) was the pathway with the highest enrichment of DEMs. The pathway that was most markedly enriched in the target genes of upregulated miRNAs was the pluripotency of stem cells pathway, as indicated by phosphoinositide-4,5-bisphosphate 3-kinase γ (*PIK3CG*) and anaphase-promoting complex subunit 2 (*APC2*). Pathways in cancer exhibited the highest enrichment in the set of target genes of downregulated miRNAs. KEGG pathway and GO function analysis indicated that target genes in the PPI network were enriched in the glioma pathway and assembled in the intracellular signaling cascade function, as indicated by the proto-oncogene *NRAS*. miR-371a-3p may be a candidate miRNA for CRT sensitivity in LARC via the thiamine metabolism pathway. *PIK3CG* and *APC2* may contribute to CRT sensitivity via signaling pathways regulating the pluripotency of stem cells. Furthermore, *NRAS* may serve an important role in mediating CRT sensitivity via an intracellular signaling cascade.

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Introduction

Locally advanced rectal cancer (LARC) occurs in the distal large intestine (1) and is the fourth most common cause of mortality worldwide, with a ~30% incidence rate (2). Preoperative chemoradiotherapy (CRT) has been established as the standard treatment for LARC (3). Of patients with LARC, 12-15% undergo a complete response to long-term CRT (4). Despite CRT improving the efficiency of clinical treatment and decreasing toxicity, it does not result in an improved survival rate for patients with LARC (5). Furthermore, a number of patients with LARC do not respond well to specific CRT regimes (6). Thus, to understand the heterogeneity of patient response to CRT, investigation of predictive biomarkers in LARC is of considerable clinical interest.

MicroRNAs (miRNAs, miRs) have emerged as crucial factors in carcinogenesis (7). The translational control by miRNA expression patterns of rectal cancer can be used to predict responses to CRT (8). A previous study identified that miRNAs, including miR-215 and miR-450b-5p, are involved in the response of patients with LARC to preoperative CRT (9). Drebber *et al* (10) identified that high expression levels of certain miRNAs, including onco-miRNA-21, were associated with successful CRT, which further indicated an association between miRNA expression and radioresistance and chemoresistance in LARC (10). Furthermore, identifying the target genes of miRNAs is key to understanding the disease and identifying potential predictive biomarkers (11). In a study of colorectal cancer, miR-338-5p was identified to induce cancer cell migration by suppressing phosphoinositide 3-kinase subunit 3 (*PIK3C3*) expression and autophagy (12). Additionally, polymorphisms in miRNA-binding sites in nucleotide excision repair genes have been associated with increased risk of rectal cancer (13). However, the potential association between miRNA expression in LARC and sensitivity to CRT remains unclear.

The miRNA expression profile of LARC samples with preoperative CRT was generated and made available in the Gene Expression Omnibus (GEO) database (GSE98959) (14). This dataset was previously used to identify differentially expressed miRNAs (DEMs) between responders and non-responders to CRT, and an association between the DEMs and *c-MYC* was revealed. miRNA-375 and *c-MYC* were suggested to be promising predictive biomarkers of the response to neoadjuvant treatment in patients with LARC (14). The aim of the present study was to identify candidate genes and key mechanisms underlying CRT sensitivity in patients with LARC, using the available miRNA expression profile dataset to investigate DEMs between responders and non-responders to CRT, and to subsequently perform a comprehensive bioinformatics analysis, including function and pathway enrichment analysis, and to conduct miRNA-target gene regulation network and a protein-protein interaction (PPI) network analysis.

Materials and methods

Microarray data. The miRNA expression profile GSE98959 dataset was obtained from the GEO database (http://www.ncbi.nlm.nih.gov/geo). In total, samples from 22 patients with LARC who had received preoperative chemotherapy and radiotherapy were profiled using TaqMan OpenArray human microRNA plates (14). Each patient sample was profiled twice, initially in pool A, and replicated in pool B.

Data preprocessing. The preprocessing of expression profile data, including original data formation, background correction and expression quantile normalization was performed in pool A and pool B using linear models for microarray data package (limma; version 3.36.1; http://www.bioconductor.org/packages/release/bioc/html/limma.html) (15) for R software (version 3.5.2; https://www.r-project.org/). The probe ID was converted into the gene symbol based on the chip platform notes file.

Analysis of DEMs. DEMs between the responder and non-responder groups were revealed respectively in pool A and pool B using the limma package. The P-values of the DEMs were corrected using Benjamini-Hochberg method (16). $P<0.05$ was selected as the threshold for the identification of DEMs. Subsequently, the top 10 DEMs according to their P-values in pool A and pool B were used for further investigation.

miRNA-gene regulation network construction. Using the miRWalk 2.0 (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/index.html) (17) software, the potential target genes for the top 10 DEMs were investigated using on six databases including miRWalk (http://mirwalk.uni-hd.de) (18), miRanda (http://www.microrna.org/microrna/home.do) (19), miRDB (http://www.mirdb.org) (20), miRMap (http://mirmap.ezlab.org) (21), RNA22 (https://cm.jefferson.edu/rna22) (22) and Targetscan (http://www.targetscan.org) (23). The parameters for the miRNA information retrieval system were as follows: Minimum seed length, 7 and P-value <0.05. Subsequently, the common miRNA-target genes that were present in the six databases were submitted for network construction. The resulting miRNA-gene regulation network was visualized using cytoscape software (version 3.2.0; http://www.cytoscape.org) (24).

Table I. Differentially expressed miRNAs between rectal cancer chemoradiotherapy responders and non-responders.

miRNA	P-value
hsa-miR-381	6.05×10^{-4}
hsa-miR-371-3p	2.47×10^{-3}
hsa-miR-644	3.68×10^{-3}
hsa-miR-375	6.27×10^{-3}
hsa-miR-221	6.66×10^{-3}
hsa-miR-561	9.07×10^{-3}
hsa-miR-1291	1.00×10^{-2}
hsa-miR-98	1.22×10^{-2}
hsa-miR-452#	1.46×10^{-2}
hsa-miR-148a	1.59×10^{-2}
hsa-miR-370	1.76×10^{-2}
hsa-miR-149#	1.77×10^{-2}
hsa-miR-502	2.05×10^{-2}
hsa-miR-361	2.30×10^{-2}
hsa-miR-1272	2.41×10^{-2}
hsa-miR-372	2.55×10^{-2}
hsa-miR-605	2.70×10^{-2}
hsa-miR-672	2.80×10^{-2}
hsa-miR-888	2.80×10^{-2}
hsa-miR-142-5p	3.65×10^{-2}
hsa-miR-155	3.69×10^{-2}
hsa-let-7f	3.70×10^{-2}
hsa-miR-146a#	4.09×10^{-2}
hsa-miR-129	4.17×10^{-2}
hsa-miR-650	4.29×10^{-2}
hsa-miR-635	4.41×10^{-2}
hsa-miR-548K	4.43×10^{-2}
hsa-miR-617	4.64×10^{-2}
hsa-miR-503	4.81×10^{-2}
hsa-miR-1256	4.97×10^{-2}

miRNA/miR, microRNA; hsa, *Homo sapiens*.

Functional annotation and pathway analysis. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of miRNAs in the miRNA-target gene regulation network was performed based on the clusterProfiler package (25) for R software. The Gene Ontology-Biological Process (GO-BP) function analysis and KEGG pathway analysis of target genes in the miRNA-target gene regulation network were performed by using the multifaceted analysis tool for human transcriptome (http://www.biocloudservice.com) (26). The present enrichment analyses for target genes were based on Fisher's method. $P<0.01$ was considered to indicate a statistically significant difference.

PPI network construction. The search tool for the retrieval of interacting genes/proteins (STRING) database (version 10.0;

Table II. GO function and KEGG pathway analysis for target genes associated with differentially expressed miRNAs.

Analysis	Identifier	Name	Count	P-value	Genes
GO	GO:0007242	Intracellular signaling cascade	7	$4.16x10^{-5}$	*NRAS*, *TP53*, *IGF1*, *SHC1*, *PAK1*, *PIK3R1*
	GO:0007169	Transmembrane receptor protein tyrosine kinase signaling pathway	4	$3.50x10^{-4}$	*CBL*, *SHC1*, *PIK3R1*, *KDR*
	GO:0006275	Regulation of DNA replication	3	$7.29x10^{-4}$	*TP53*, *IGF1*, *SHC1*
	GO:0042127	Regulation of cell proliferation	5	$1.13x10^{-3}$	*NRAS*, *TP53*, *IGF1*, *SHC1*, *KDR*
	GO:0007167	Enzyme-linked receptor protein signaling pathway	4	$1.20x10^{-3}$	*CBL*, *SHC1*, *PIK3R1*, *KDR*
KEGG	hsa05214	Glioma	6	$3.01x10^{-8}$	*PIK3CG*, *NRAS*, *TP53*, *IGF1*, *SHC1*, *PIK3R1*
	hsa05220	Chronic myeloid leukemia	6	$7.34x10^{-8}$	*PIK3CG*, *NRAS*, *CBL*, *TP53*, *SHC1*, *PIK3R1*
	hsa04012	ErbB signaling pathway	6	$1.56x10^{-7}$	*PIK3CG*, *NRAS*, *CBL*, *SHC1*, *PAK1*, *PIK3R1*
	hsa05218	Melanoma	5	$4.17x10^{-6}$	*PIK3CG*, *NRAS*, *TP53*, *IGF1*, *PIK3R1*
	hsa04510	Focal adhesion	6	$1.02x10^{-5}$	*PIK3CG*, *IGF1*, *SHC1*, *PAK1*, *PIK3R1*, *KDR*

GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; miRNA, microRNA; hsa, *Homo sapiens*.

http://www.string-db.org) is a biological database of known and predicted PPIs (27). In the present study, STRING was used to predict interactions between the target genes of the DEMs. PPIs were selected according to the STRING database with a high confidence score of 0.7. The centrality degree was defined as the number of connections for each target protein. The PPI network was then constructed using cytoscape software. The sub-networks (modules) with a score of >5 were identified using the molecular complex detection plugin (version 1.4.2; http://apps.cytoscape.org/apps/MCODE) for cytoscape. KEGG and GO-BP analysis were performed on the top 10 genes in the PPI network according to centrality degree, as well as on genes in modules with $P<0.05$.

Results

Identification of DEMs. Following data preprocessing of the gene set, there were 361 and 371 miRNAs in pool A and pool B, respectively. A total of 18 DEMs and 12 DEMs with $P<0.05$ were identified in pool A and pool B, respectively. The DEMs identified in pool A and pool B were combined, with a total of 10 upregulated and 20 downregulated DEMs. These DEMs are listed in Table I.

miRNA-target gene regulation network analysis. To investigate the function of DEMs, the association between miRNAs and their target genes were identified using the following six databases: miRWalk, miRanda, miRDB, miRMap, RNA22 and Targetscan. A total of 935 interactions and 849 nodes were revealed in the miRNA-target gene interaction network. Furthermore, five upregulated miRNAs, miR-561-3p, miR-148a-5p, miR-148a-3p, miR-1291 and miR-375, and six downregulated miRNAs, miR-644a, miR-98-5p, miR-221-5p, miR-221-3p, miR-381-3p and miR-371a-3p, were prominent in the miRNA-gene regulation network (Fig. 1). Notably, the target genes of miR-371a-3p were those encoding C-terminal domain small phosphatase-like 2, thiamin pyrophosphokinase 1 and exocyst complex component 8.

Function and pathway investigation. KEGG pathway analysis was performed on the top 10 DEMs to evaluate their functions in the miRNA-gene regulation network. A total of 59 pathways, including thiamine metabolism [*Homo sapiens* (hsa) 00730; $P=2.20x10^{-3}$], signaling pathways regulating pluripotency of stem cells (hsa04550; $P=6.21x10^{-2}$) and neuroactive ligand-receptor interaction (hsa04080; $P=6.71x10^{-2}$) were enriched by DEMs. Notably, miR-371a-3p was significantly enriched in thiamine metabolism. The main pathways enriched by the top 10 DEMs are presented in Fig. 2.

GO function analysis and KEGG pathway investigation were performed on miRNA-target genes. The results of the KEGG pathway analysis indicated that genes associated with upregulated miRNAs were primarily enriched in certain pathways, including signaling pathways regulating the pluripotency of stem cells, insulin signaling pathway and Type II diabetes mellitus (Fig. 3A). The regulation of the pluripotency of stem cells pathway (hsa04550; $P=2.39\ x10^{-4}$) was identified by the following genes: Phosphoinositide-4,5-bisphosphate 3-kinase γ (*PIK3CG*), SMAD family member 9, anaphase-promoting complex subunit 2 (*APC2*), SMAD family member 5 (*SMAD5*), sex-determining region Y box 2 (*SOX2*) and others. The insulin signaling pathway (hsa04910; $P=1.06x10^{-3}$) was identified by the genes *PIK3CG*, insulin receptor substrate 2 (IRS2), hexokinase domain-containing 1 (*HKDC1*), protein kinase AMP-activated non-catalytic subunit β2, suppressor of cytokine signaling 1 (*SOCS1*) and others. The Type II diabetes mellitus pathway (hsa04930; $P=5.67x10^{-3}$) was identified by the following genes: *PIK3CG*, *IRS2*, *HKDC1*, *SOCS1* and calcium voltage-gated channel α1 B. GO-BP function analysis indicated that the target genes that were associated with upregulated miRNAs were primarily enriched in the functions presented in Fig. 3B, including negative regulation of transcription by RNA polymerase II (GO, 0000122; $P=8.07x10^{-4}$) as indicated by the following genes: EP300-interacting inhibitor of differentiation 1, hepatocyte nuclear factor 1 homeobox B (*HNF1B*), SATB homeo box 2, zinc finger protein 280C, metadherin and others.

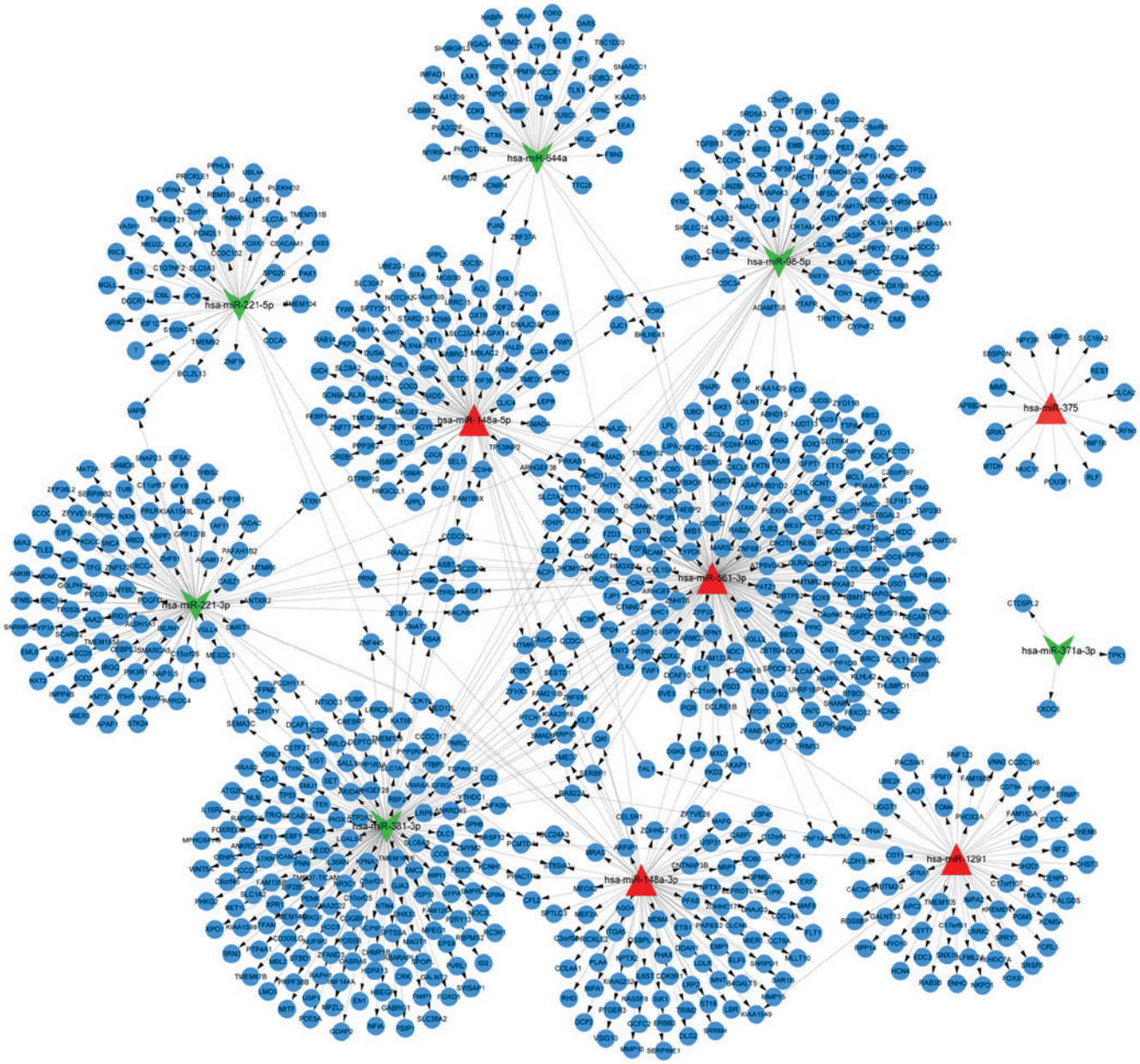

Figure 1. MicroRNA-target gene regulation network. Red triangles represent upregulated miRNAs, green arrowheads represent downregulated miRNAs, blue circles represent target genes and arrows represent the regulatory association.

Furthermore, the KEGG pathway analysis of the target genes of the downregulated miRNAs were enriched in certain pathways, including pathways in cancer (hsa05200; $P=2.31x10^{-3}$), proteoglycans in cancer (hsa05205; $P=2.77x10^{-5}$) and human T-cell lymphotropic virus-I infection (hsa05166; $P=4.41x10^{-3}$) (Fig. 3C). These three pathways were identified by the following genes: Wnt family member 5A, collagen type IV α1 chain, prostaglandin E receptor 3 (*PTGER3*), transforming growth factor β receptor 1, melanogenesis-associated transcription factor and others. The GO-BP function analysis indicated that the target genes associated with downregulated miRNAs were primarily involved in functions including positive regulation of transcription, DNA-templated (GO, 045893; $P=1.39x10^{-3}$), as indicated by the following genes: ENY2 transcription and export complex 2 subunit, *HNF1B*, *SOX11*, *SMAD5*, *SOX2* and others (Fig. 3D).

PPI network and module analysis. To identify the potential interactions of the target genes associated with DEMs, a PPI network and associated modules were constructed on the basis of the protein interactions of the target genes. The results indicated that a total of 406 nodes and 830 interactions were included in the present PPI network (Fig. 4). According to the degree of centrality, the top 10 nodes included seven downregulated and three upregulated genes. The downregulated genes were tumor protein p53 (*TP53*), phosphoinositide-3-kinase regulatory subunit 1 (*PIK3R1*), *CBL* proto-oncogene, insulin-like growth factor 1 (*IGF1*), *NRAS*, kinase insert domain receptor (*KDR*) and p21-activated kinase 1 (*PAK1*). The three upregulated genes that were in the top 10 nodes were *PIK3CG*, SHC adaptor protein 1 (*SHC1*) and nuclear cap-binding protein subunit 1. The GO functional analysis indicated that these genes were primarily assembled

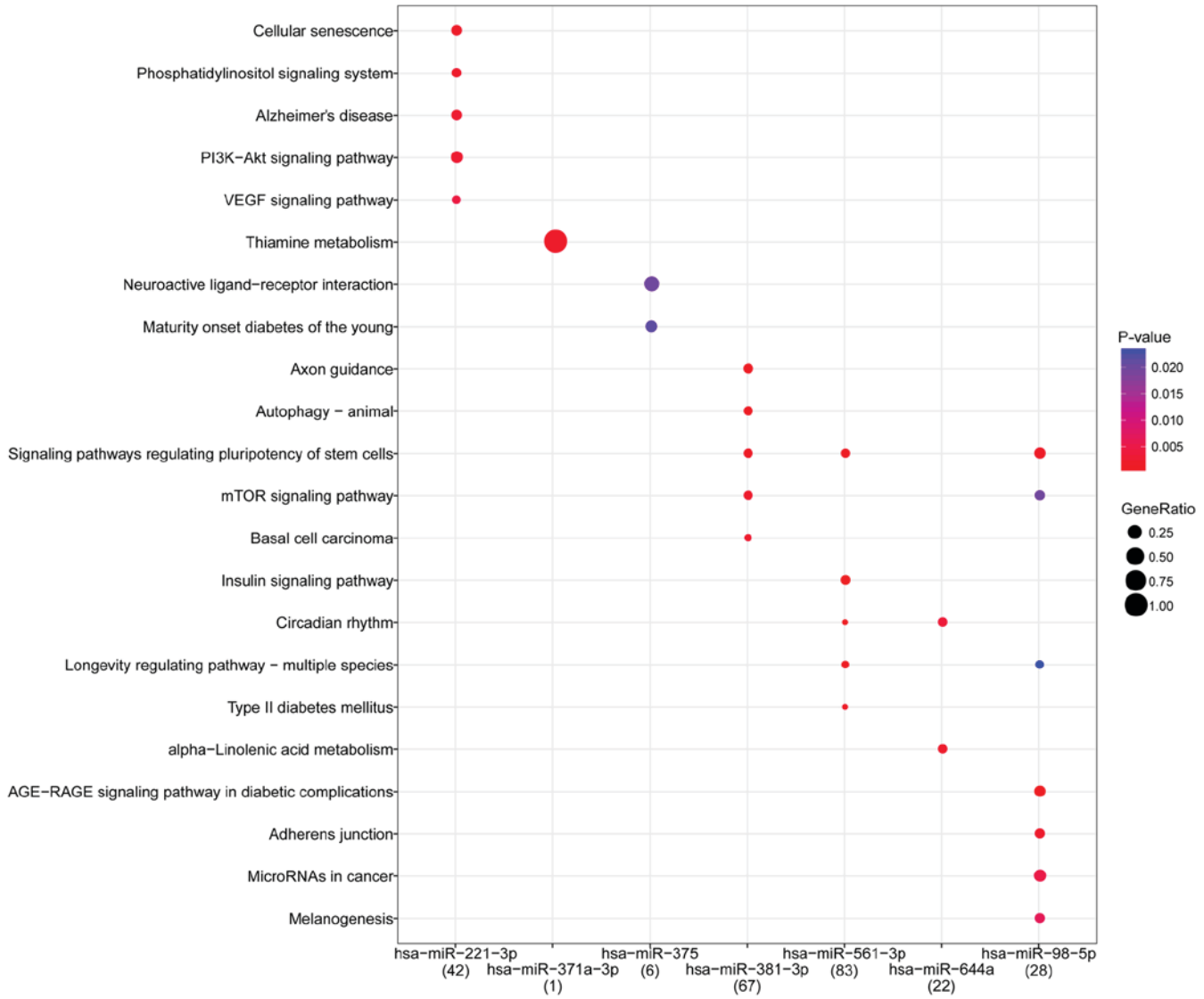

Figure 2. KEGG pathway enrichment analysis on the differentially expressed miRNAs. Darker shades of red indicate lower P-values and larger circles indicate larger gene ratios. Gene ratios are defined as the number of differentially expressed miRNAs enriched in each KEGG pathway/the total number of miRNAs enriched in all KEGG pathways. hsa, *Homo sapiens*; miR/miRNA, microRNA; KEGG, Kyoto Encyclopedia of Genes and Genomes.

in functions including intracellular signaling cascade (GO, 0007242; P=4.16x10^{-5}), as indicated by the genes *PIK3CG*, *NRAS*, *TP53*, *IGF1*, *SHC1*, *PAK1* and *PIK3R1*. The KEGG pathway analysis indicated that these genes were primarily enriched in pathways such as glioma (hsa05214; P=3.01x10^{-8}), as indicated by *PIK3CG*, *NRAS*, *TP53*, *IGF1*, *SHC1* and *PIK3R1* (Table II).

In addition, three modules with a score of >5 were identified (Fig. 5). There were 19 nodes and 82 interactions in module A (score=9.11), eight nodes and 28 interactions in module B (score=8), and 12 nodes and 29 interactions in module C (score=5.27). The GO functions in which the target genes of DEMs in module A, module B and module C were highly enriched were cellular macromolecule catabolic process, G-protein-coupled receptor protein signaling pathway and cell division, respectively. Cellular macromolecule catabolic process (GO, 0044265; P=2.54x10^{-9}), was identified by the genes E3 ubiquitin-protein ligase Praja-2, E3 ubiquitin-protein ligase RNF123, low-density lipoprotein receptor, BTB domain-containing 1, ubiquitin-conjugating enzyme E2K (*UBE2K*), and others. G-protein-coupled receptor protein signaling pathway (GO, 0007186; P=2.67x10^{-8}) was identified by the genes P2Y purinoceptor 13 (*P2RY13*), sphingosine 1-phosphate receptor 1 (*S1PR1*), *PTGER3*, proenkephalin A, C-X-C motif chemokine 5 and others. Cell division (GO, 0051301; P=2.56x10^{-5}) was identified by the genes centromere protein O, PDS5 cohesion-associated factor B, AT-hook-containing transcription factor 1, zwilch kinetochore protein and cell division cycle-associated 5. The KEGG pathways with the highest enrichment of target genes of DEMs in module A, module B and module C were ubiquitin-mediated proteolysis, neuroactive ligand-receptor and focal adhesion, respectively (Table III). The ubiquitin mediated proteolysis pathway (hsa04120; P=9.34x10^{-5}) was identified by the genes *UBE2K*, ubiquitin-conjugating enzyme E2 G1, *SOCS1*, *CBL* and cell division cycle 34. The neuroactive ligand-receptor pathway (hsa04080; P=8.69x10^{-5}) was indicated by *P2RY13*, *S1PR1*, *PTGER3*, neuropeptide Y receptor Y2 and γ-aminobutyric

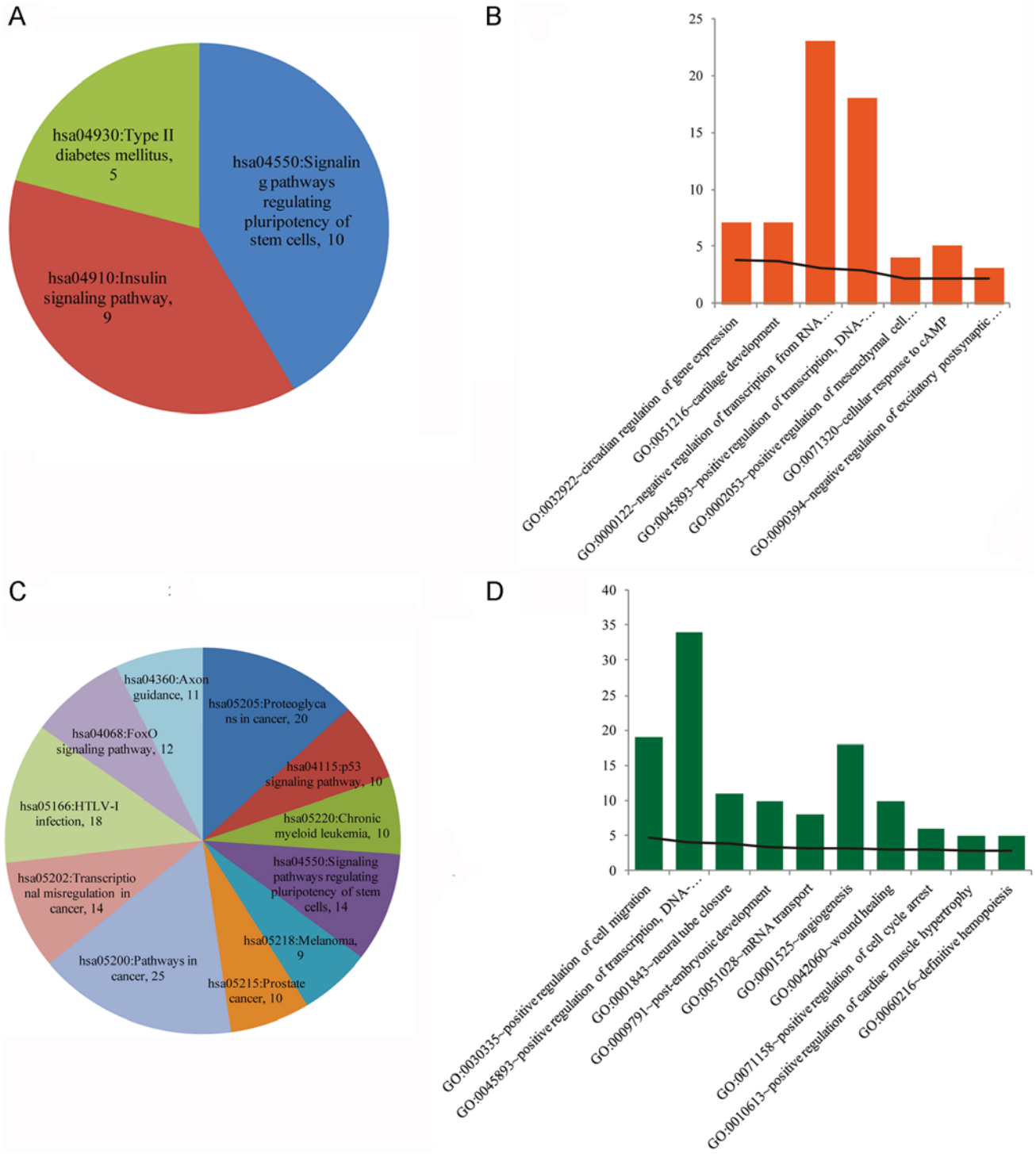

Figure 3. KEGG pathway enrichment and GO functional analyses on the target genes of differentially expressed miRNAs. (A) KEGG pathway results for target genes of upregulated miRNAs; (B) GO function results for target genes of upregulated miRNAs; (C) KEGG pathway results for target genes of downregulated miRNAs; and (D) GO function results for target genes of downregulated miRNAs. KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology.

acid type B receptor subunit 2, and the genes identifying the focal adhesion pathway (hsa04510; $P=5.74x10^{-4}$) were *PIK3CG*, FMS-related tyrosine kinase 1, *IGF1* and *KDR*.

Discussion

Although CRT has been established as a gold standard for LARC, the differences in CRT sensitivity between patients with LARC remain a challenge in clinical treatment (3). In the present study, a total of 30 DEMs were identified between responders and non-responders in terms of sensitivity to CRT. Consistent with previous results (14), it was also identified that miR-375 and miR-148a were differentially expressed. In addition, a total of 11 miRNAs, including miR-371a-3p, exhibited significantly altered expressed in the miRNA-target gene network. These miRNAs were enriched in pathways including thiamine metabolism, signaling pathways regulating pluripotency of

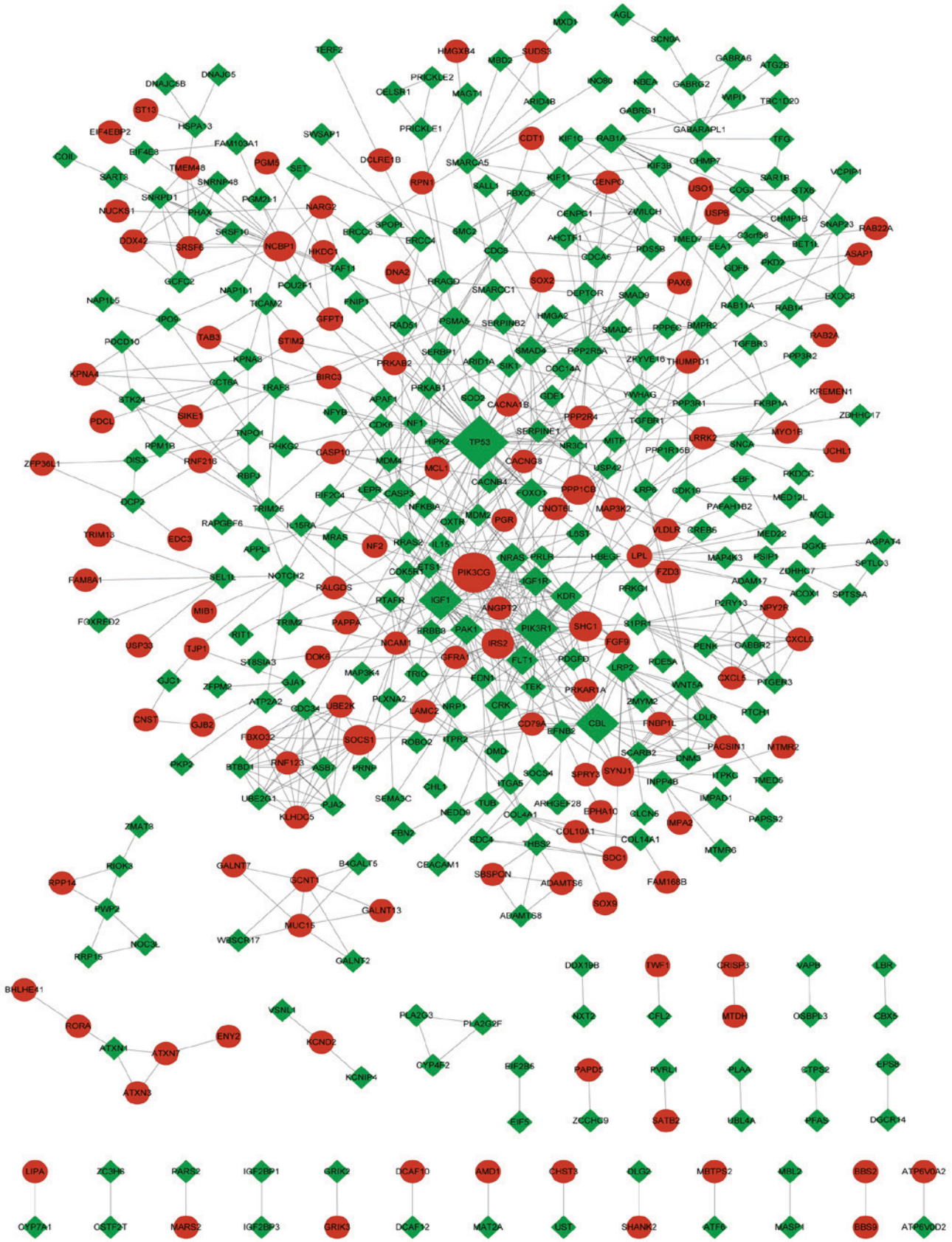

Figure 4. Protein-protein interaction network of target genes of differentially expressed microRNAs. Red circles represent upregulated genes and green diamonds represent downregulated genes. A larger node represents a higher degree of centrality.

stem cells and neuroactive ligand-receptor interaction. The target genes of these miRNAs were enriched in signaling pathways regulating pluripotency of stem cells and functions such as negative regulation of transcription from the

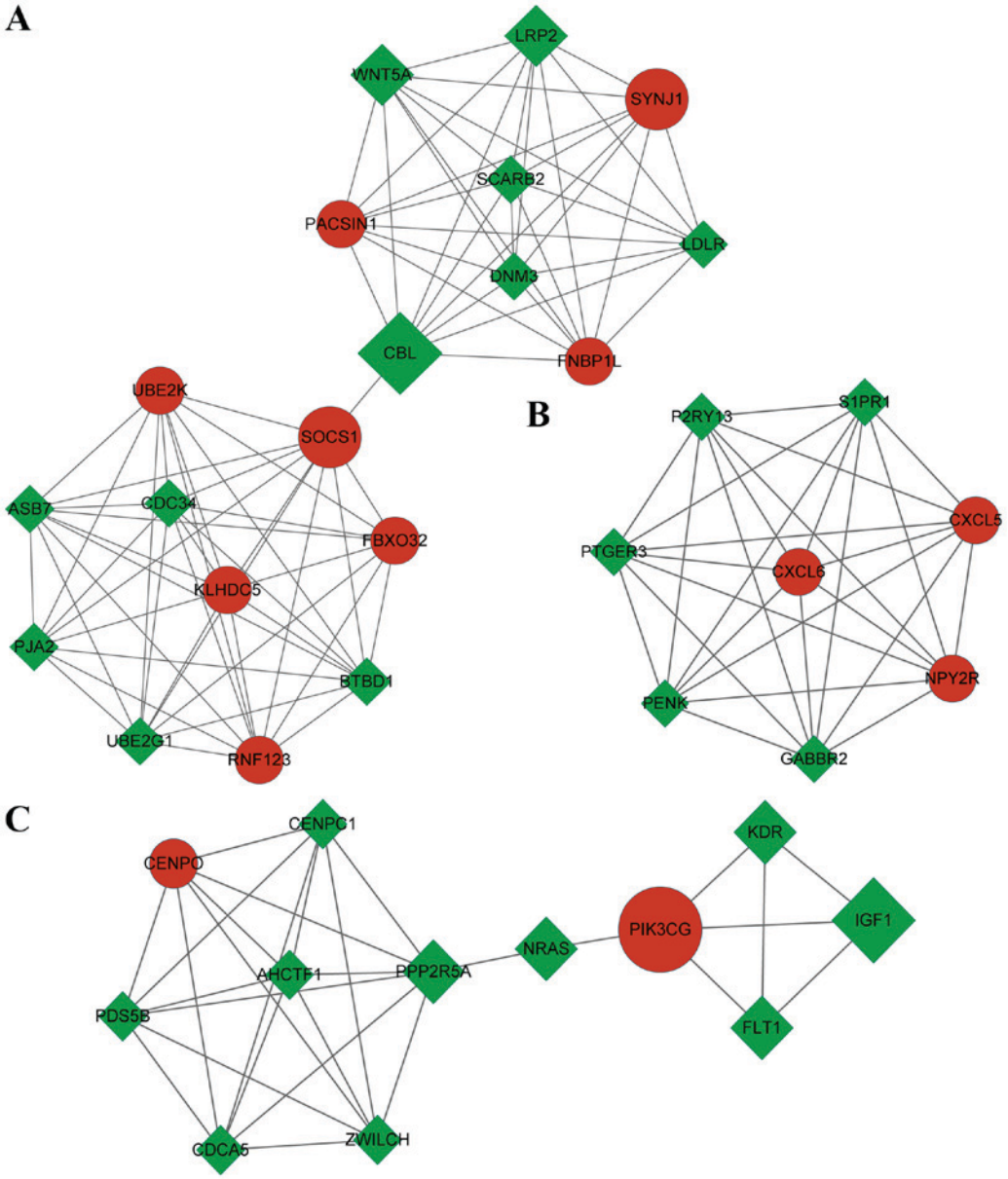

Figure 5. Three modules (A-C) extracted from the protein-protein interaction network. Red circles represent upregulated genes and green diamonds represent downregulated genes. A larger node represents a higher degree of centrality.

RNA polymerase II promoter. Furthermore, *NRAS* was revealed to be a key target gene of DEMs based on the PPI network, which was mainly enriched in functions such as intracellular signaling cascade.

miRNAs are involved in post-transcriptional gene regulation, and thus serve an essential role in numerous biological processes, including cancer cell proliferation (28,29). miR-371a-3p has been associated with tumor cell proliferation (30). Although previous studies have indicated that serum miR-371a-3p may be a novel biomarker for tumor cells (31,32), the association between miR-371a-3p and CRT sensitivity in LARC remains unknown. In the present study, the miRNA-target gene interaction analysis revealed that miR-371a-3p was downregulated. This result may indicate that miR-371a-3p is a potential factor for predicting CRT sensitivity in LARC. However, investigation of the mechanism of the effect of miR-371a-3p on CRT sensitivity is lacking. Notably, pathway analysis in the current study showed that thiamine metabolism was the pathway with the highest enrichment by miR-371a-3p. The role of thiamine in cancer is controversial according to previous studies. In one study, thiamine intake was inversely correlated with colon cancer risk (33). However, another study indicated that increased thiamine levels were beneficial for tumor cell survival, proliferation and chemotherapy resistance (34). Therefore, downregulated miR-371a-3p may affect CRT sensitivity in patients with LARC via the thiamine metabolism pathway.

Stem cells are responsible for maintaining differentiated cell numbers during normal physiology and at times of tissue stress (35). In CRT-treated patients with colorectal cancer, the upregulation of circulating cancer stem cell markers is vital for clinical parameters including CRT sensitivity (36). Hiroishi *et al* (37) indicated that prominin-1 and signal transducer CD24 contributed to the sensitivity of preoperative

Table III. GO function and Kyoto Encyclopedia of Genes and Genomes pathway analysis for target genes associated with differentially expressed miRNAs in modules.

Module	Identifier	Name	Count	P-value	Genes
A	GO:0044265	Cellular macromolecule catabolic process	11	2.54×10^{-9}	*PJA2*, *RNF123*, *LDLR*, *BTBD1*, *UBE2K*
	GO:0009057	Macromolecule catabolic process	11	5.22×10^{-9}	*PJA2*, *RNF123*, *LDLR*, *BTBD1*, *UBE2K*
	GO:0019941	Modification-dependent protein catabolic process	10	7.51×10^{-9}	*PJA2*, *RNF123*, *BTBD1*, *UBE2K*, *UBE2G1*
	GO:0043632	Modification-dependent macromolecule catabolic process	10	7.51×10^{-9}	*PJA2*, *RNF123*, *BTBD1*, *UBE2K*, *UBE2G1*
	GO:0051603	Proteolysis involved in cellular protein catabolic process	10	1.11×10^{-8}	*PJA2*, *RNF123*, *BTBD1*, *UBE2K*, *UBE2G1*
	hsa04120	Ubiquitin-mediated proteolysis	5	9.34×10^{-5}	*UBE2K*, *UBE2G1*, *SOCS1*, *CBL*, *CDC34*
	hsa04144	Endocytosis	3	4.84×10^{-2}	*DNM3*, *LDLR*, *CBL*
B	GO:0007186	G-protein-coupled receptor protein signaling pathway	8	2.67×10^{-8}	*P2RY13*, *S1PR1*, *PTGER3*, *PENK*, *CXCL5*
	GO:0007166	Cell-surface receptor-linked signal transduction	8	9.06×10^{-7}	*P2RY13*, *S1PR1*, *PTGER3*, *PENK*, *CXCL5*
	GO:0051350	Negative regulation of lyase activity	3	3.36×10^{-4}	*S1PR1*, *NPY2R*, *GABBR2*
	GO:0031280	Negative regulation of cyclase activity	3	3.36×10^{-4}	*S1PR1*, *NPY2R*, *GABBR2*
	GO:0007194	Negative regulation of adenylate cyclase activity	3	3.36×10^{-4}	*S1PR1*, *NPY2R*, *GABBR2*
	hsa04080	Neuroactive ligand-receptor interaction	5	8.69×10^{-5}	*P2RY13*, *S1PR1*, *PTGER3*, *NPY2R*, *GABBR2*
C	GO:0051301	Cell division	5	2.56×10^{-5}	*CENPO*, *PDS5B*, *AHCTF1*, *ZWILCH*, *CDCA5*
	GO:0048754	Branching morphogenesis of a tube	3	8.01×10^{-4}	*FLT1*, *IGF1*, *KDR*
	GO:0001763	Morphogenesis of a branching structure	3	1.04×10^{-3}	*FLT1*, *IGF1*, *KDR*
	GO:0042127	Regulation of cell proliferation	5	1.13×10^{-3}	*NRAS*, *FLT1*, *PDS5B*, *IGF1*, *KDR*
	GO:0007059	Chromosome segregation	3	1.24×10^{-3}	*CENPO*, *PDS5B*, *CDCA5*
	hsa04510	Focal adhesion	4	5.74×10^{-4}	*PIK3CG*, *FLT1*, *IGF1*, *KDR*
	hsa05214	Glioma	3	1.47×10^{-3}	*PIK3CG*, *NRAS*, *IGF1*
	hsa05218	Melanoma	3	1.87×10^{-3}	*PIK3CG*, *NRAS*, IGF1
	hsa04370	Vascular endothelial growth factor signaling pathway	3	2.09×10^{-3}	*PIK3CG*, *NRAS*, *KDR*
	hsa05215	Prostate cancer	3	2.93×10^{-3}	*PIK3CG*, *NRAS*, *IGF1*

GO, Gene Ontology; miRNAs, microRNAs; hsa, *Homo sapiens*.

CRT for LARC. In the present study, the GO functional analysis indicated that signaling pathways regulating the pluripotency of stem cells had the highest enrichment of upregulated miRNA-target genes, including *PIK3CG* and *APC2*. The product of *PIK3CG* is an modulator of extracellular signals and is vital for the maintenance of epithelia. A previous study indicated that downregulation of *PIK3CG* led to inhibition of the phosphoinositide 3-kinase/protein kinase B signaling pathway, and was associated with tumorigenesis and progression of colorectal cancer (38). Downregulation of *APC2* may result in cancerous tumors, as *APC2* regulates the uncontrolled growth of cells (39). The most common mutation in colon cancer is inactivation of *APC* (40). Thus, on the basis of the results of the present study, miRNA-target genes *APC2* and *PIK3CG* may contribute to CRT sensitivity through signaling pathways regulating the pluripotency of stem cells.

NRAS is an intracellular signal cascade mediator that initiates the mitogen-activated protein kinase signaling pathway (41). Mutation of *NRAS* is associated with progression of colorectal cancer (42). Although *NRAS* is an intracellular signal cascade mediator (41), the role of *NRAS* in CRT sensitivity in patients with LARC remains unknown. In the present study, target genes in the PPI network, including *NRAS* were enriched the intracellular signaling cascade pathway. This indicates that the differential expression of *NRAS* may serve

an important role in determining CRT sensitivity in LARC via the intracellular signaling cascade.

The present study has a number of limitations. First, the sample size was small. Secondly, the present study used bioinformatic tools to filter and predict potential target genes which could affect the CRT response in LARC, but did not investigate the potential mechanism *in vitro* or *in vivo*. No experiments using clinical samples were performed. Lastly, previous studies have confirmed that mutations in genes for potential biomarkers, including *KRAS* proto-oncogene, *BRAF* proto-oncogene and *NRAS*, are associated with tumorigenesis and response to anti-epidermal growth factor receptor therapy in colorectal cancer (43-45). Next-generation sequencing to identify mutations in these potential biomarkers may elucidate the key mechanisms mediating CRT sensitivity in patients with LARC. Therefore, further clinical investigation based on a larger sample size and using high-throughput sequencing data is required to confirm the results of the present study.

In conclusion, downregulated miR-371a-3p may affect CRT sensitivity in patients with LARC via the thiamine metabolism pathway. Target genes including *PIK3CG* and *APC2* may contribute to CRT sensitivity through signaling pathways regulating the pluripotency of stem cells. Furthermore, *NRAS* may serve an important role in determining CRT sensitivity in LARC via the intracellular signaling cascade.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

CL and CE were responsible for the conception and design of the research, drafting of the manuscript and performing the statistical analysis. YZ performed the data acquisition. WY performed the data analysis and interpretation. All authors read and approved the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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