The Potential circRNAs in Imatinib Resistance of GIST

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Research

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Abstract

Background: Circular RNAs are a recently (re-)discovered abundant RNA species, belongs to part of the competing endogenous RNA network(ceRNA), Which was proved to play a critical role in the development, diagnosis and progress of diseases.

Methods: We analyzed the expression of circular RNAs in paired normal gastric tissues(N), primary GIST (gastrointestinal stromal tumor) tissues (Y or YC) and imatinib mesylate secondary resistance GIST tissues(C) with microarray and predicted 8677 dysregulated circular RNAs.

Results: We identified 15 circRNAs was up-regulated and 8 circRNAs were down-regulated in C group. Gene ontology (GO)and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis identified that the host linear transcripts of these differentially expressed circRNAs were involved in many critical biological pathways and molecular functions, predicting the potential tumor-genesis and drug resistance mechanism was related with HIF-1 pathway, later we draw the cirRNA-miRNA-mRNA network involved in the HIF-1 pathway, found that several dysregulated circRNAs and the relationship between circRNA-miRNAs-mRNA, such as circRNA_06551, circRNA_14668, circRNA_04497, circRNA_08683, circRNA_09923(Green, down-regulation) and circRNA_23636, circRNA_15734(Red, up-regulation).

Conclusions: Taken together, we identified a panel of dysregulated circRNAs that may be potential biomarkers even therapy relevant to the GIST, especially imatinib secondary resistance GIST.

Background

Gastrointestinal stromal tumor (GIST) is the most common gastrointestinal mesenchymal tumors[1].The pathogenesis of GIST is mainly due to the protooncogene tyrosine kinase receptor KIT or platelet-derived growth factor receptor α(PDGFRA-α) gene activation mutation, as a result, abnormal activation of downstream signaling pathways, cell proliferation, apoptosis is inhibited and transformed into tumor cells[2, 3]. According to the type of gene mutation, GISTs were divided into KIT mutant (80%~85%), PDGFRA mutant (5%~10%) and wild type (10%)[4]. This provides a theoretical basis for the molecular targeted drug imatinib (IM) mesylate. IM is a drug that inhibits the activity of tyrosine kinase of KIT and PDGFRA gene, which is effective in the treatment of advanced GIST, and achieves satisfactory results[5]. However, more and more studies have found that IM occurs primary and secondary resistance in the treatment process of GIST, and the mechanism of drug resistance is complicated. Thus, targeting of KIT inhibitors alone does not benefit all GIST patients, especially in patients with wild-type GIST.

CircRNAs is a class of non coding RNA (ncRNAs) molecules usually composed of more than one exon, formed mainly by back-splicing and covalent binding, which misinterpreted as a rare event (splicing error)[6]. CircRNA functions as competitive endogenous RNA (ceRNA) efficiently targeting miRNA and inhibiting miRNA transcription like a molecular sponge, indirectly regulate mRNA expression[7]. Through targeting miRNA, circRNA regulates downstream gene expression and may play a crucial role in disease mechanisms. Inhibition of circ_0067934 could block cell proliferation, migration, invasion and EMT and induced apoptosis in NSCLC cells. via through miR-1182/KLF8 axis and activating Wnt/β-catenin pathway[8]. circ_0014130 could inhibit cell

apoptosis in NSCLC cells by sponging miR-136-5p and regulation of Bcl-2[9]. Over-expression of circ_0004015 could enhance resistance to gefitinib in NSCLC cells through miR1183- PDK1 axis[10].

In the present study, we investigated the differentially expressed circRNAs using human circRNAs array in GIST tissues. We firstly demonstrated circRNAs of imatinib mesylate secondary resistance GIST and primary GIST compared to normal tissues and discovered a correlation of circular RNA abundance and imatinib mesylate relapse resistance and make a function prediction.

Methods

Patients And Clinical Specimen

The tumor tissues (all the tumor are greater than 5 cm) and matched normal gastric tissues were collected from 9 patients who undergoing surgical resection at the First Affiliated Hospital of Wenzhou Medical University, china. All tissues samples were confirmed to be malignant GIST by Pathology and immunohistochemistry (CD117(+), CD34(+), mitotic phase greater than 5/50HPF) and then stored in liquid nitrogen for further use. The study was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University and written informed consent was given before operation.

RNA extraction, library construction and sequencing

Total RNA was extracted using the mirVana miRNA Isolation Kit (Cat. AM1561, Thermo Fisher Scientific, USA) following the manufacturer's protocol. RNA purity and quantification were evaluated using the NanoDrop2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). RNA integrity was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The samples with RNA Integrity Number (RIN) ≥ 7 were subjected to the subsequent library construction. After rRNA depleted and linear RNA digested by Ribonuclease R (Epicentre, Madison, WI, USA), libraries were constructed using TruSeq Stranded Total RNA with Ribo-Zero Gold (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Then these libraries were sequenced on the Illumina sequencing platform (HiSeq X Ten) and 150 bp paired-end reads were generated. The circRNA sequencing and analysis were conducted by OE Biotech Co., Ltd. (Shanghai, China).

Bioinformatic analysis

Raw data (raw reads) of fastq format were firstly processed using the Trimmomatic software[11]. Clean data (clean reads) were obtained by removing reads containing adapter, reads containing ploy-N and lower quality reads from raw data. Clean reads were aligned to the reference genome GRCh38 utilizing the MEM algorithm of Burrows-Wheeler aligner (BWA, version0.7.5a)[12]. Based on the junction reads and GT-AG splicing signals, circRNAs were verified using CIRI2 softwere[13]. Combined with annotation information in protein database, circRNAs were annotated for further analysis. The expression level of circRNAs was measured by RPM (Mapped back-splicing junction reads per million mapped reads). To identify differentially expressed circRNAs, statistical comparison between two different groups was determined by the DESeq (2012) R package[14], with setting the threshold of adjusted p-value < 0.05 and foldchange > 2 or foldchange < 0.5. FDR (false discovery rate) was used as the threshold of p-value in multiple test to judge the significance of gene expression difference. CircRNA-miRNA interaction was predicted by miRanda software with the threshold of score > 150, energy < -30 and strict paired in the seed region[15]. The target genes of miRNA were predicted based on intersection of the

results on miRWalk and miRDB database. Differentially expressed genes data of the group C vs YC were obtained from another research[16].

Gene Function Analysis

The functions of circRNAs were predicated using (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis[17, 18]. KEGG analysis was performed to determine the involvement of target genes in different biological pathways(Cell Component, Molecular Function and Biological Process) using KOBAS software (KEGG Orthology-Based Annotation System)[19]. The threshold for GO terms and KEGG pathway enrichment analysis was $P<0.05$.

Construction Of Circrna-mirna-mrna Interaction Networks

miRanda software was used to predict the circRNA-miRNA interaction with the threshold of score > 150, energy < -30 and strict paired in the seed region. The target genes of miRNA were predicted based on intersection of the results on miRWalk and miRDB database. CircRNA-miRNA-mRNA network was visualized by the Cytoscape software.

Results

C-KIT two mutation sites in patients with imatinib mesylate secondary resistance

All specimens were analyzed by RT-PCR amplification, DNA sequencing and analysis and then comparison with wild type c-KIT gene, C-KIT/PDGFR-α mutation were detection, secondary mutation were found in 3 GIST specimens with drug resistance. one case locus in the v654a of exon 13, one case locus in the T760I of exon 14, the other locus in the v654a of exon 17(Figure 1), which confirm the resistance of imatinib mesylate and offer a potential theoretical basic of the application probability of circRNAs.

Identification And Characteristics Of Circrnas

We performed ribosomal RNA-depleted RNA sequencing to explore circRNA expression profiles in three normal gastric tissue samples (N), three primary GIST samples (Y or YC) and three GIST samples secondarily resistant to IM (C). The number of circRNAs identified in each sample is shown in Figure 2A. Venn analysis for comparison of predicted circRNAs with the data published in the circBase, showing that 11935 circRNAs were found between predicted circRNAs and circBase (Figure 2B). According to the circular RNAs array, a total of 30,550 were detected in 9 samples, and the length mostly distribute in 201-400bp and >2000bp (Figure 2C). These circRNAs originated from all chromosomes, chr1, chr2, and chr3 were the three chromosomes to which the most circRNAs were mapped (Figure 2D). The number of exons per circRNA was less than six for most circRNAs (Figure 2E). Similarly, most of the identified circRNAs (27050,88.54%) were generated from sense-overlapping regions, indicating that the formation of circRNAs is closely associated with pre-mRNA splicing mechanism (Figure 1F). Approximately 3.82% (1167) and 5.41% (1652) of circRNAs arose from exons (exonic

circRNA) and the intergenic regions. A small proportion of circRNAs were antisense circRNAs (383, 1.25%) and intronic circRNAs (298, 0.98%). General features of the circRNA sequencing data were list in Table 1.

Table 1
General features of the circRNA sequencing data

Sample	raw reads	raw bases	clean reads	clean bases	valid bases	Q30	GC
Sample_N1	84587074	10573384250	81651670	10201851969	96.48%	93.56%	55.50%
Sample_N2	91507150	11438393750	88469786	11053620694	96.63%	93.73%	56.00%
Sample_N3	91320298	11415037250	88647372	11076539842	97.03%	94.29%	57.00%
Sample_C1	84193988	10524248500	81676720	10204694315	96.96%	94.20%	58.00%
Sample_C2	82799646	10349955750	80243548	10025345565	96.86%	93.93%	58.00%
Sample_C3	85145886	10643235750	82621864	10322885971	96.99%	94.11%	55.50%
Sample_YC_1	84743526	10592940750	81602896	10186655571	96.16%	93.57%	57.00%
Sample_YC_2	83362440	10420305000	80906514	10108276460	97.00%	94.37%	50.50%
Sample_YC_3	82891684	10361460500	80622518	10073190258	97.21%	94.35%	55.50%

The Potential Functions Identification

It has been confirmed that circRNAs possess tissue-specific expression characteristics. To screen dysregulated circRNAs in three different GIST samples, we used DEseq software to analyze circRNA expression profiles RPM (mapped back-splicing junction reads per million mapped reads), found that that no abnormal expression was observed in three different GIST samples (Figure 3A). PCA (Principal Component Analysis) was performed to analyze the circRNA expression profiles of the three groups samples. The distance between points represented the similarity between the two samples, and the repeatability of the three groups of samples was ideal in Figure 3B. Differential analysis was conducted among the three comparison groups by Volcano plots. The circRNA differentially expressed was screened using the criteria of "adjusted pvalue < 0.05 and absolute value of log2Foldchange >1". The red dot on the volcano map was significantly up-regulated circRNA, the green dot was significantly down-regulated circRNA, and the gray dot showed no obvious difference (Figure 3C).These were 159, 98, and 37 circRNAs up-regulated, 277, 284, and 23 circRNAs down-regulated in comparison C-vs-N, YC-vs-N and C-vs-YC, respectively(Figure 3D). Venn analysis of the three comparison groups was shown in Figure 3E. In general, the same kind of samples can be clustered in the same cluster, and the genes in the same cluster may have similar biological functions, our results show that all samples in paired groups have the co-regulated (up or down) genes (Figure 3F). The top ten different expression circRNA in the three comparison groups was shown in Table 2.

Table 2
Top 10 differentially expressed circRNAs in the three comparison groups

circRNA_id	circBase_id	log2FoldChange	P-value	Adjusted P-value	Regulation	Host genes
C-vs-N						
circRNA_09533	hsa_circ_0006867	-8.62	9.25E-90	2.61E-85	Down	LRBA
circRNA_17427	hsa_circ_0018064	-inf	3.05E-64	4.30E-60	Down	SVIL
circRNA_20776	hsa_circ_0026782	-9.86	7.21E-53	6.78E-49	Down	ITGA7
circRNA_09530	-	-inf	2.72E-46	1.92E-42	Down	LRBA
circRNA_13055	hsa_circ_0079284	-5.41	2.84E-43	1.60E-39	Down	RNF216
circRNA_11884	hsa_circ_0004119	-5.40	1.63E-41	7.69E-38	Down	RAB23
circRNA_01991	hsa_circ_0005230	5.26	1.57E-35	6.32E-32	Up	DNM3
circRNA_13811	hsa_circ_0004365	-inf	7.44E-35	2.62E-31	Down	SEMA3C
circRNA_03576	hsa_circ_0000994	-4.03	2.99E-30	8.43E-27	Down	SLC8A1
circRNA_26953	hsa_circ_0000825	-5.70	2.70E-30	8.43E-27	Down	MTCL1
C-vs-YC						
circRNA_01991	hsa_circ_0005230	3.92	1.33E-19	2.49E-15	Up	DNM3
circRNA_03862	hsa_circ_0004435	-2.74	1.07E-12	6.67E-09	Down	FANCL
circRNA_15734	-	Inf	9.93E-13	6.67E-09	Up	-
circRNA_11269	hsa_circ_0003718	-4.00	6.83E-12	3.20E-08	Down	RANBP17
circRNA_02957	hsa_circ_0002922	-2.17	2.05E-11	7.68E-08	Down	ZNF124
circRNA_30179	-	-inf	3.18E-11	9.95E-08	Down	DIAPH2
circRNA_30540	hsa_circ_0009024	-inf	7.27E-11	1.95E-07	Down	-

circRNA_id	circBase_id	log2FoldChange	P-value	Adjusted P-value	Regulation	Host genes
circRNA_03489	hsa_circ_0000992	3.91	4.07E-10	9.55E-07	Up	PRKD3
circRNA_04497	-	-2.60	5.75E-10	1.20E-06	Down	DPP10
circRNA_25651	-	Inf	9.34E-10	1.75E-06	Up	ZC3H18
YC-vs-N						
circRNA_09533	hsa_circ_0006867	-8.15	1.75E-86	4.41E-82	Down	LRBA
circRNA_17427	hsa_circ_0018064	-inf	1.81E-67	2.28E-63	Down	SVIL
circRNA_13055	hsa_circ_0079284	-7.80	8.02E-60	6.75E-56	Down	RNF216
circRNA_04497	-	Inf	5.19E-58	3.27E-54	Up	DPP10
circRNA_09530	-	-inf	4.50E-47	2.27E-43	Down	LRBA
circRNA_11884	hsa_circ_0004119	-4.77	1.01E-39	4.26E-36	Down	RAB23
circRNA_20776	hsa_circ_0026782	-6.94	1.03E-33	3.70E-30	Down	ITGA7
circRNA_26999	hsa_circ_0008821	-inf	5.42E-32	1.71E-28	Down	RAB31
circRNA_13811	hsa_circ_0004365	-6.68	1.69E-29	4.74E-26	Down	SEMA3C
circRNA_18913	hsa_circ_0000277	6.50	1.12E-27	2.82E-24	Up	PDE3B

GO enrichment analysis for the host genes of differentially expressed circRNAs

After get the differentially expressed genes, we selected the top10 functional enrichment analysis. The enriched functional terms were used as the predicted functional term of given circRNAs. Analysis the difference gene expression with GO analysis, to describe its function (with GO annotation). GO analyses covered three subgroups: biological process (BP), cellular component (CC), and Molecular function (MF). The GO analysis with the most significant enrichment in the BP, CC, and MF subgroups by C-vs-N comparison groups is regulation of transcription, DNA-templated, cytosol and metal ion binding, respectively. In C-vs-YC group, the GO analysis with the most significant enrichment in the BP, CC, and MF subgroups is regulation of transcription, DNA-templated, nucleoplasm and double-stranded DNA binding. In YC-vs-N group, the GO analysis with the most significant enrichment in the BP, CC, and MF subgroups is regulation of transcription from RNA polymerase II promoter, cytosol and metal ion binding (Figure 4A-C).

Construction Of The Circrna-mirna Interaction Network In Drug Resistance/hif-1

We combined the chip data (OE2016Q1031Y) from another of our published articles to draw the cirRNA-miRNA-mRNA network in the group C vs YC, and found that, 15 cirRNA were up-regulated (Red), 8 cirRNA was down-regulated (Green) (Figure 5A). GO enrichment analysis of the cirRNA-miRNA-mRNA network, the bubble diagram shows the top 20 enriched GO terms (P<0.05) (Figure 5B). KEGG pathway enrichment analysis of the cirRNA-miRNA-mRNA network, and found out potential relationship between differential expression genes with changes of cell pathways, such as HIF-1 pathway, Central carbon metabolism in cancer, AMPK signaling pathway, Autophagy-animal and so on (Figure 5C). Later, we analyzed the cirRNA-miRNA-mRNA network involved in the HIF-1 pathway, found that the correlation between each dysregulated circRNA-miRNAs-mRNA, circRNA_06551, circRNA_14668, circRNA_04497, circRNA_08683, circRNA_09923(Green, down-regulation) and circRNA_23636, circRNA_15734(Red, up-regulation) (Figure 5D).

Discussion

CircRNAs, featured with a covalently closed loop structure without 5'and 3'termini, mainly caused by back-splicing and covalently binding, which was detected 20 years ago but considered as a rare event for so long[20, 21]. Recent studies have proved that circRNAs plays critical roles in the development and prognosis of many diseases, such as Alzheimer, cardiac hypertrophy and heart failure, cancers[22]. Now a greater appreciation rose that circRNAs which thought to be a promising direction in diverse diseases. More and more studies have shown that circRNA is deregulated in different types of human cancers[6, 23–25]. CircRNA plays an important role in the biological processes involved in tumor progression and drug resistance[26]. It also acts as a microRNA (miRNA) sponge and RNA binding protein sponge, gene transcription. For instance, over-expression of circRNA_0025202 could regulate tamoxifen sensitivity through regulation of the miR-182-5p/FOXO3a axis in breast cancer[27]. Circular RNA AKT3 could enhance cisplatin resistance in gastric cancer cells via inhibition of miR-198 and upregulation PIK3R1[28]. Up-regulation of Circular RNA MCTP2 could inhibit resistance to cisplatin in gastric cancer by regulation of miR-99a-5p/ MTMR3 axis[29]. Inhibition of circCELSR1 could enhance sensitivity to paclitaxel in ovarian cancer cells vis FOXR2 /miR-1252 axis[30].However, the expression profiles and functions of circRNA in IM resistance in GIST are poorly understood.

Supporting the theory that circRNAs may be a new and stable biomarker and breakthrough Therapeutic direction for now known intractable diseases. In the present study, we characterized the circRNA expression patterns in 9 patients (three normal gastric tissue samples (N), three primary GIST samples (Y or YC) and three GIST samples secondarily resistant to IM (C)) using high-throughput RNA sequencing. Obtain microarray specimen results as shown that the length of most circRNA was around 201-400bp, which consistent with previous reports showing the median length of circRNAs is around 500 nt [31]. Circular RNA is mainly produced by the exons or introns of its host linear transcript and participates in the regulation of host gene expression[32, 33]. Therefore, after we screened out the differentially expressed circRNAs between Y and C tissue samples and predicted the biological functions of their host linear transcripts using GO and KEGG pathway analyses, indicating that it was involved in HIF-1 signaling pathway.

HIF is often overexpressed in various cancer cells and is associated with the progression and poor clinical outcomes of many different tumor entities[34, 35]. HIF genes regulate the expression of many genes related to angiogenesis, tumor growth, metastasis, and therapeutic resistance[36]. Hypoxia inducible transcription factor 1α (HIF-1α), as the main regulator of hypoxia-induced drug resistance, is considered to be an attractive target for tumor therapy[37]. The present study constructed the cirRNA-miRNA-mRNA network involved in the HIF-1 pathway through bioinformatic prediction and clarified cirRNA-miRNA-mRNA axis participation in this regulatory network. We found several circRNA were up-regulated or down-regulated, such as circRNA_23636, circRNA_15734(up-regulation); circRNA_06551, circRNA_14668, circRNA_04497, circRNA_08683, circRNA_09923(down-regulation); and there are many cirRNA-miRNA-mRNA axis, such as, circRNA_23636-has-miR-6077-SLC2A1, circRNA_15734-hsa-miR-6893-5p-PFKFB3, circRNA_15734-hsa-miR-8485-VEGFA); circRNA_06551-hsa-miR-1915-3p- PFKFB3), circRNA_14668-hsa-miR-8485- VEGFA), circRNA_08683-hsa-miR-6728-5p-ENO2), circRNA_04497-hsa-miR-6808-5p-PFKFB3), circRNA_04497-hsa-miR-8485- VEGFA), circRNA_09923-hsa-miR-4755-3p- PFKFB3), circRNA_09923-hsa-miR-3155p-HK2), circRNA_09923-hsa-miR-3155a- HK2). Although the interaction between cirRNA-miRNA-mRNA axis has not been completely verified, we speculate that differentially expressed circRNAs during IM resistance may exert their biological functions through interaction with miRNA-mRNA.

Conclusion

This study serves as the first, to our knowledge, circRNAs sequencing and functional analysis in primary GIST and imatinib mesylate secondary resistance GIST. After IM failure, few therapeutic options remain, so it is urgent to identify the mechanism of drug resistance, HIF-1 seems to be crucial in the future research.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University and written informed consent was given before operation.

Consent for publication

Not applicable

Availability of data and materials

We declare that all data supporting the conclusions of the study.

Competing interests

The authors declare that they have no competing f interests.

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Authors' contributions

XS and JY conceived the idea; XC, QD and JL analyzed the data; XSwrote the manuscript. All authors have read and approved the final version of the manuscript.

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Not applications.

References

1. Kelly CM, Gutierrez Sainz L, Chi P. The management of metastatic GIST: current standard and investigational therapeutics. J Hematol Oncol. 2021;14:2.
2. Jia N, Tong H, Zhang Y, Katayama H, Wang Y, Lu W, Zhang S, Wang J. CeRNA Expression Profiling Identifies KIT-Related circRNA-miRNA-mRNA Networks in Gastrointestinal Stromal Tumour. Front Genet. 2019;10:825.
3. Heinrich MC, Jones RL, von Mehren M, Schoffski P, Serrano C, Kang YK, Cassier PA, Mir O, Eskens F, Tap WD, et al. Avapritinib in advanced PDGFRA D842V-mutant gastrointestinal stromal tumour (NAVIGATOR): a multicentre, open-label, phase 1 trial. Lancet Oncol. 2020;21:935–46.
4. Joensuu H, Hohenberger P, Corless CL. Gastrointestinal stromal tumour. Lancet. 2013;382:973–83.
5. Mohammadi M, Gelderblom H. Systemic therapy of advanced/metastatic gastrointestinal stromal tumors: an update on progress beyond imatinib, sunitinib, and regorafenib. Expert Opin Investig Drugs. 2021;30:143–52.
6. Li J, Sun D, Pu W, Wang J, Peng Y. Circular RNAs in Cancer: Biogenesis, Function, and Clinical Significance. Trends Cancer. 2020;6:319–36.
7. Bai S, Wu Y, Yan Y, Shao S, Zhang J, Liu J, Hui B, Liu R, Ma H, Zhang X, Ren J. Construct a circRNA/miRNA/mRNA regulatory network to explore potential pathogenesis and therapy options of clear cell renal cell carcinoma. Sci Rep. 2020;10:13659.
8. Zhao M, Ma W, Ma C. Circ_0067934 promotes non-small cell lung cancer development by regulating miR-1182/KLF8 axis and activating Wnt/beta-catenin pathway. Biomed Pharmacother. 2020;129:110461.
9. Geng Y, Bao Y, Zhang W, Deng L, Su D, Zheng H. Circular RNA hsa_circ_0014130 Inhibits Apoptosis in Non-Small Cell Lung Cancer by Sponging miR-136-5p and Upregulating BCL2. Mol Cancer Res. 2020;18:748–56.
10. Zhou Y, Zheng X, Xu B, Chen L, Wang Q, Deng H, Jiang J. Circular RNA hsa_circ_0004015 regulates the proliferation, invasion, and TKI drug resistance of non-small cell lung cancer by miR-1183/PDPK1 signaling pathway. Biochem Biophys Res Commun. 2019;508:527–35.
11. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30:2114–20.
12. Li H, Durbin R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics. 2010;26:589–95.
13. Gao Y, Wang J, Zhao F. CIRI: an efficient and unbiased algorithm for de novo circular RNA identification. Genome Biol. 2015;16:4.
14. Anders S, McCarthy DJ, Chen Y, Okoniewski M, Smyth GK, Huber W, Robinson MD. Count-based differential expression analysis of RNA sequencing data using R and Bioconductor. Nat Protoc. 2013;8:1765–86.

15. Enright AJ, John B, Gaul U, Tuschl T, Sander C, Marks DS. MicroRNA targets in Drosophila. Genome Biol. 2003;5:R1.
16. Yan J, Chen D, Chen X, Sun X, Dong Q, Du Z, Wang T. Identification of imatinib-resistant long non-coding RNAs in gastrointestinal stromal tumors. Oncol Lett. 2019;17:2283–95.
17. Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lempicki RA. DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biol. 2003;4:P3.
18. The Gene Ontology C. The Gene Ontology Resource: 20 years and still GOing strong. Nucleic Acids Res. 2019;47:D330–8.
19. Wen C, Xu G, He S, Huang Y, Shi J, Wu L, Zhou H. Screening Circular RNAs Related to Acquired Gefitinib Resistance in Non-small Cell Lung Cancer Cell Lines. J Cancer. 2020;11:3816–26.
20. Zhou WY, Cai ZR, Liu J, Wang DS, Ju HQ, Xu RH. Circular RNA: metabolism, functions and interactions with proteins. Mol Cancer. 2020;19:172.
21. Huang A, Zheng H, Wu Z, Chen M, Huang Y. Circular RNA-protein interactions: functions, mechanisms, and identification. Theranostics. 2020;10:3503–17.
22. Kristensen LS, Andersen MS, Stagsted LVW, Ebbesen KK, Hansen TB, Kjems J. The biogenesis, biology and characterization of circular RNAs. Nat Rev Genet. 2019;20:675–91.
23. Zhao Y, Zheng R, Chen J, Ning D. CircRNA CDR1as/miR-641/HOXA9 pathway regulated stemness contributes to cisplatin resistance in non-small cell lung cancer (NSCLC). Cancer Cell Int. 2020;20:289.
24. Xu T, Wang M, Jiang L, Ma L, Wan L, Chen Q, Wei C, Wang Z. CircRNAs in anticancer drug resistance: recent advances and future potential. Mol Cancer. 2020;19:127.
25. Hong W, Xue M, Jiang J, Zhang Y, Gao X. Circular RNA circ-CPA4/ let-7 miRNA/PD-L1 axis regulates cell growth, stemness, drug resistance and immune evasion in non-small cell lung cancer (NSCLC). J Exp Clin Cancer Res. 2020;39:149.
26. Hua X, Sun Y, Chen J, Wu Y, Sha J, Han S, Zhu X. Circular RNAs in drug resistant tumors. Biomed Pharmacother. 2019;118:109233.
27. Sang Y, Chen B, Song X, Li Y, Liang Y, Han D, Zhang N, Zhang H, Liu Y, Chen T, et al. circRNA_0025202 Regulates Tamoxifen Sensitivity and Tumor Progression via Regulating the miR-182-5p/FOXO3a Axis in Breast Cancer. Mol Ther. 2019;27:1638–52.
28. Huang X, Li Z, Zhang Q, Wang W, Li B, Wang L, Xu Z, Zeng A, Zhang X, Zhang X, et al. Circular RNA AKT3 upregulates PIK3R1 to enhance cisplatin resistance in gastric cancer via miR-198 suppression. Mol Cancer. 2019;18:71.
29. Sun G, Li Z, He Z, Wang W, Wang S, Zhang X, Cao J, Xu P, Wang H, Huang X, et al. Circular RNA MCTP2 inhibits cisplatin resistance in gastric cancer by miR-99a-5p-mediated induction of MTMR3 expression. J Exp Clin Cancer Res. 2020;39:246.
30. Zhang S, Cheng J, Quan C, Wen H, Feng Z, Hu Q, Zhu J, Huang Y, Wu X. circCELSR1 (hsa_circ_0063809) Contributes to Paclitaxel Resistance of Ovarian Cancer Cells by Regulating FOXR2 Expression via miR-1252. Mol Ther Nucleic Acids. 2020;19:718–30.
31. Zheng Q, Bao C, Guo W, Li S, Chen J, Chen B, Luo Y, Lyu D, Li Y, Shi G, et al. Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs. Nat Commun. 2016;7:11215.

32. Li Z, Huang C, Bao C, Chen L, Lin M, Wang X, Zhong G, Yu B, Hu W, Dai L, et al. Exon-intron circular RNAs regulate transcription in the nucleus. Nat Struct Mol Biol. 2015;22:256–64.
33. Zhang Y, Zhang XO, Chen T, Xiang JF, Yin QF, Xing YH, Zhu S, Yang L, Chen LL. Circular intronic long noncoding RNAs. Mol Cell. 2013;51:792–806.
34. Balamurugan K. HIF-1 at the crossroads of hypoxia, inflammation, and cancer. Int J Cancer. 2016;138:1058–66.
35. Albadari N, Deng S, Li W. The transcriptional factors HIF-1 and HIF-2 and their novel inhibitors in cancer therapy. Expert Opin Drug Discov. 2019;14:667–82.
36. Wigerup C, Pahlman S, Bexell D. Therapeutic targeting of hypoxia and hypoxia-inducible factors in cancer. Pharmacol Ther. 2016;164:152–69.
37. Xu K, Zhan Y, Yuan Z, Qiu Y, Wang H, Fan G, Wang J, Li W, Cao Y, Shen X, et al. Hypoxia Induces Drug Resistance in Colorectal Cancer through the HIF-1alpha/miR-338-5p/IL-6 Feedback Loop. Mol Ther. 2019;27:1810–24.

Figures

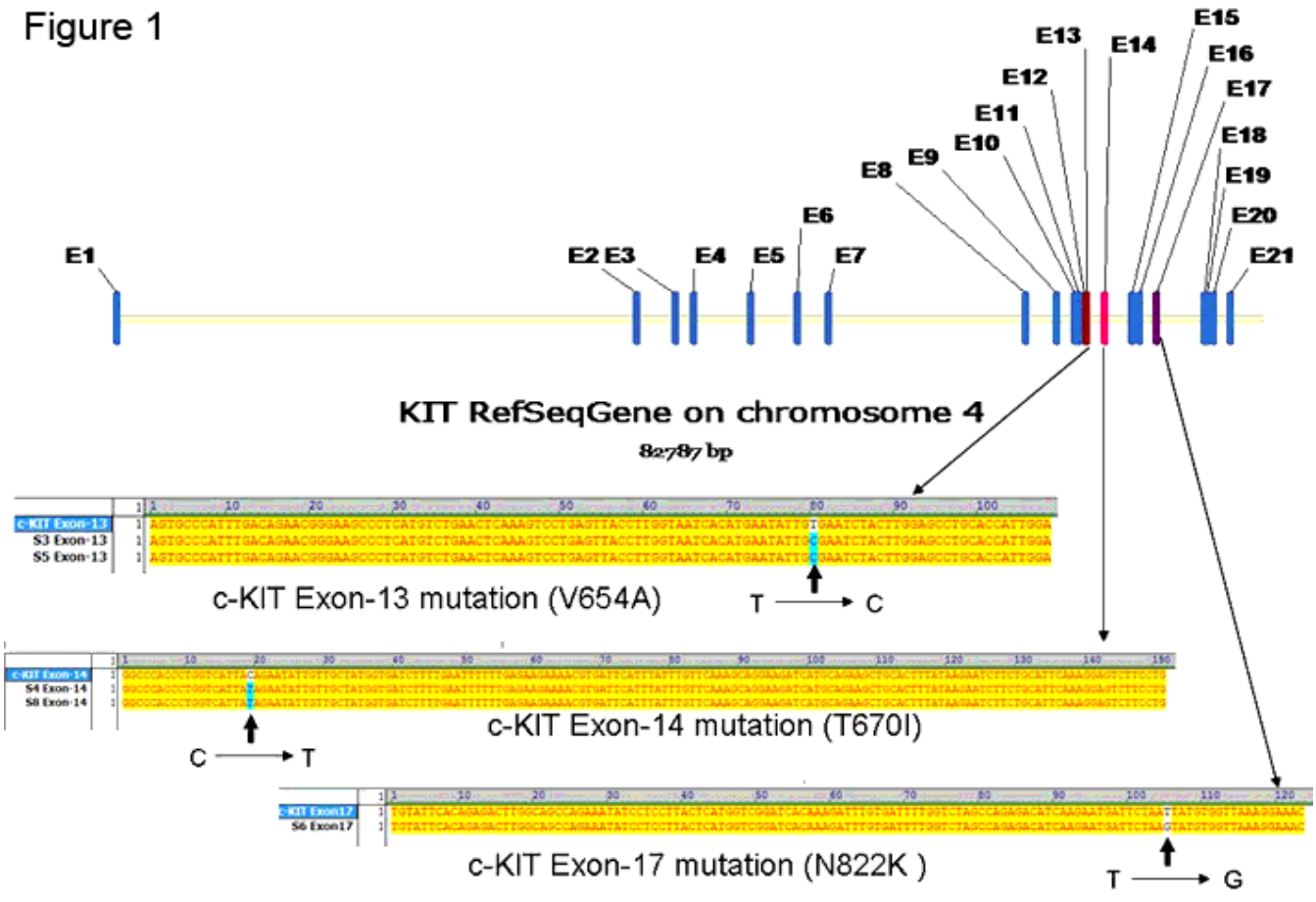

Figure 1

C-KIT two mutation sites in patients with imatinib mesylate secondary resistance C-KIT secondary mutation sites in patients with imatinib mesylate secondary resistance GIST (one case locus in the v654a of exon 13, one

case locus in the T760I of exon 14, another one locus in the v654a of exon 17).

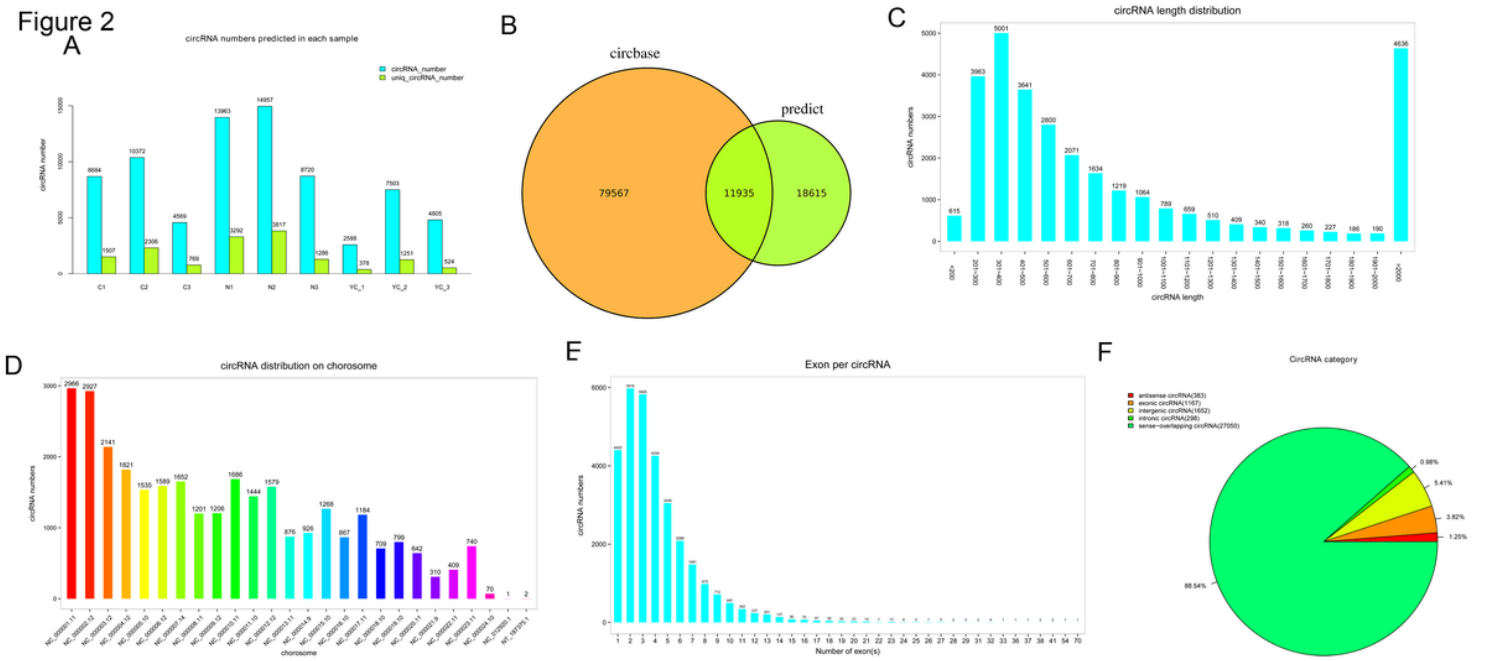

Figure 2

Identification and characteristics of circRNAs A. Identification of circRNAs in different samples. B. Venn analysis for comparison of predicted circRNAs with the data published in the circBase. C. The length distribution of circRNAs. D. Chromosome distribution of circRNAs. E. Distribution of the exon numbers of circRNAs. F. Category of circRNAs based on genomic origin.

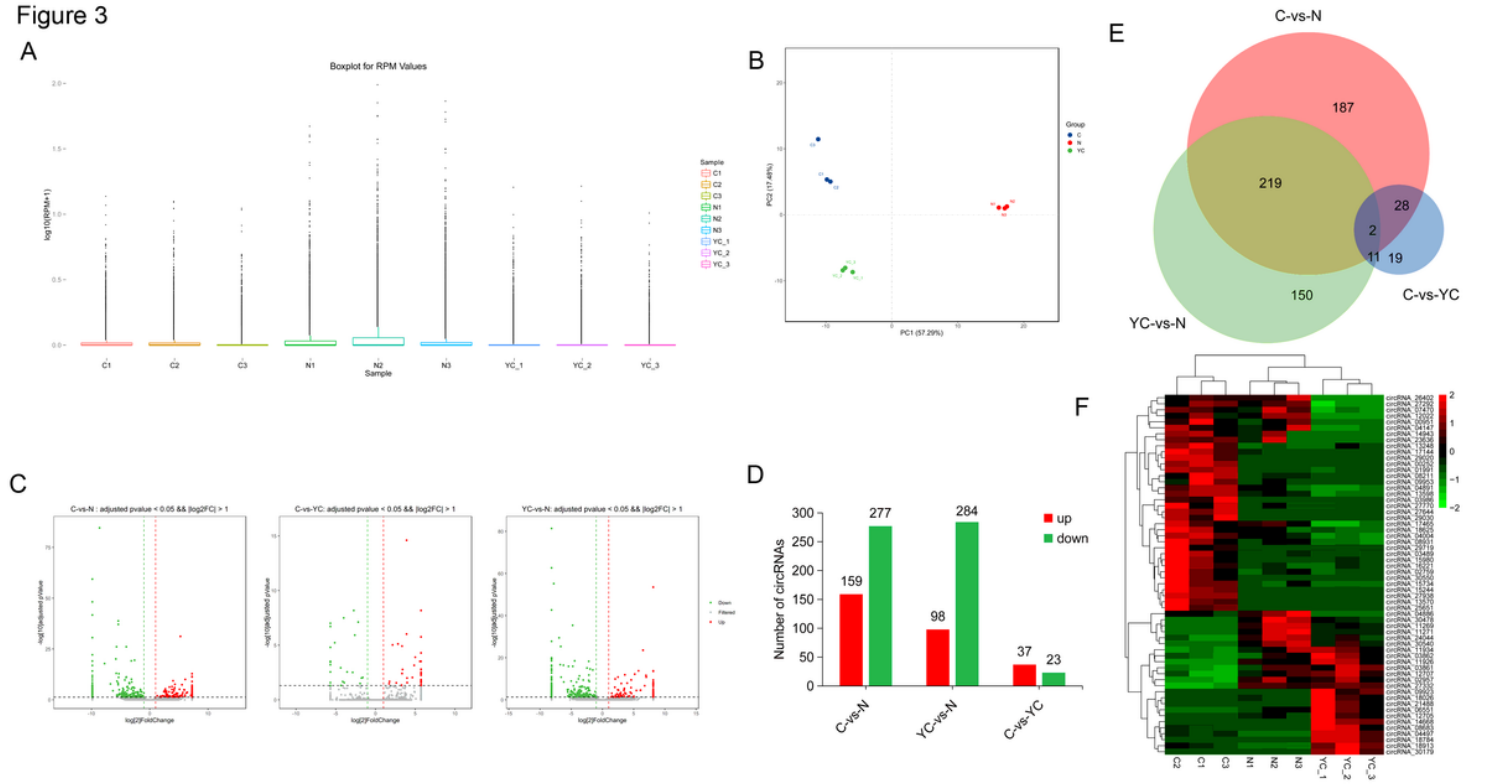

Figure 3

Differentially expressed circRNAs in the N, C and YC groups A. Box plots of reads per million (RPM) values of circRNAs in each sample. B. PCA plot of all the samples in N, C and YC groups. C. Volcano plots indicated the variation of circRNA expression in different comparison groups C vs N, YC vs N, C-vs-YC. D. Column chart of differentially expressed circRNAs in each comparison. The numbers on column show the numbers of up-regulated (red) and down-regulated (green) circRNAs. E. Venn analysis of the three comparison groups. F. Heatmap of differentially expressed circRNAs between YC and C groups.

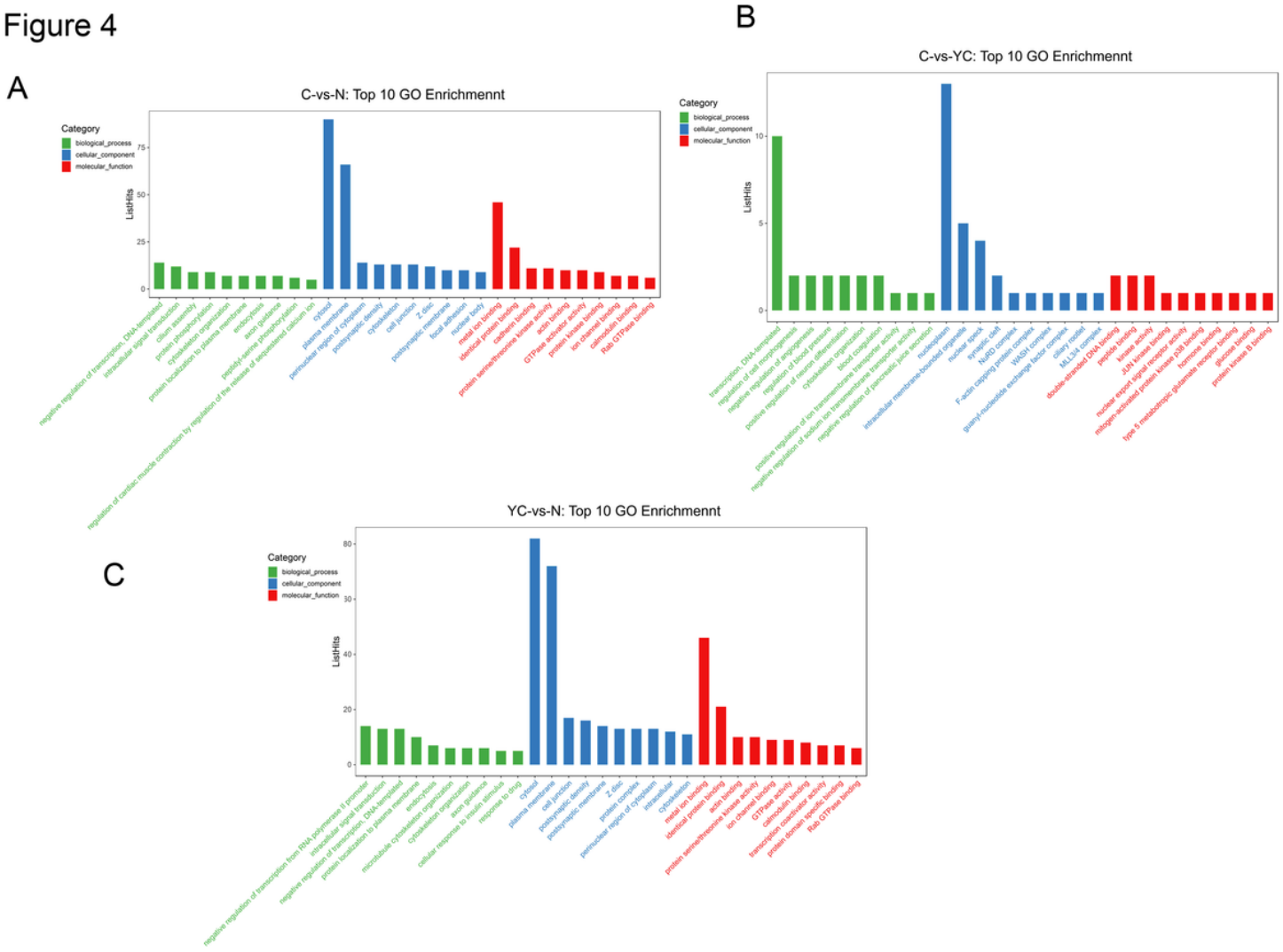

Figure 4

GO enrichment analysis for the host genes of differentially expressed circRNAs A-C. GO enrichment analysis was conducted based on the host genes of differentially expressed circRNAs. The bar diagrams show the top10 enriched GO terms (P<0.05) in the group C vs N(A), YC vs N(B), C-vs-YC(C), respectively

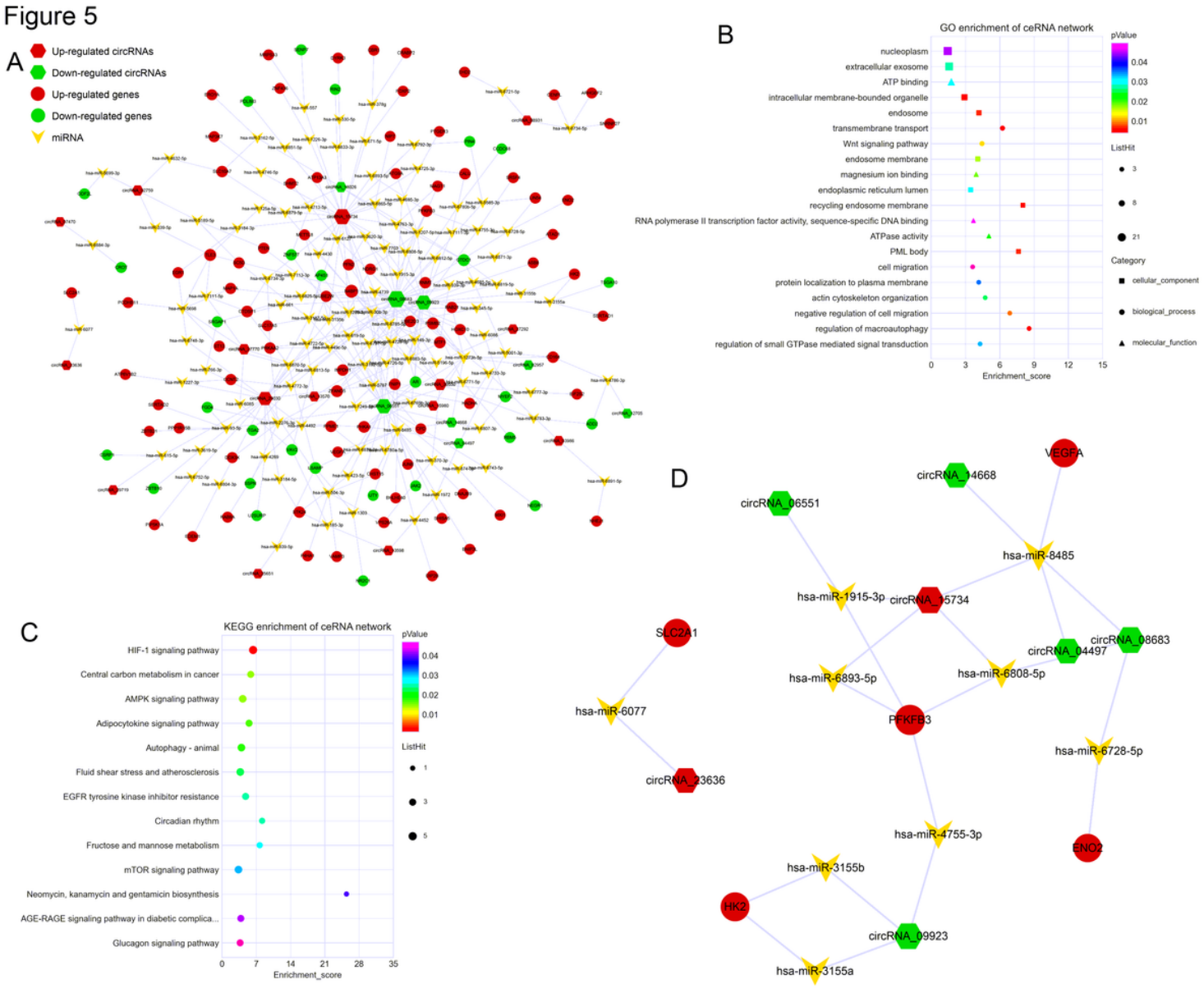

Figure 5

the ceRNA regulatory network in the group C vs YC A. the cirRNA-miRNA-mRNA network in the group C vs YC. Red, up-regulated. Green, down-regulated. Hexagon, circRNAs. Circle, mRNA. Arrowhead, miRNA. B. GO enrichment analysis of the cirRNA-miRNA-mRNA network. The bubble diagram shows the top 20 enriched GO terms (P<0.05). C. KEGG pathway enrichment analysis of the cirRNA-miRNA-mRNA network. The bubble diagram shows the enriched pathways (P<0.05). D. The cirRNA-miRNA-mRNA network involved in the HIF-1 pathway.