

Bioinformatics Analysis of hsa_circ_0036683 Reveals its Potential Role in Hepatocellular Carcinoma

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Abstract: Background: Hepatocellular carcinoma (HCC) is an aggressive malignancy with poor prognosis. Circular RNAs (circRNAs) exert crucial regulatory effects on HCC progression via the competing endogenous RNA (ceRNA) mechanism. As the potential role of hsa_circ_0036683 in HCC remains unclear, this study aimed to explore its expression characteristics and ceRNA regulatory mechanism in HCC through bioinformatics analysis. Methods: Differentially expressed circRNAs were identified from publicly available Gene Expression Omnibus (GEO) datasets. Furthermore, CircAtlas 3.0 was utilized to analyze the tissue expression, host gene expression pattern and cross-species conservation of hsa_circ_0036683. CircBank and CircInteractome were used to predict circRNA-miRNA interactions, and core miRNAs were screened via comprehensive evaluation of multiple binding indicators. TargetScan 8.0, miRTarBase and miRDB were applied to predict and screen overlapping target genes of the candidate miRNAs. Functional enrichment analyses, protein-protein interaction (PPI) networks were performed. Results: Bioinformatics analysis verified that hsa_circ_0036683 was significantly downregulated in HCC, with its expression positively correlated with the host gene, and presented high cross-species conservation. Eleven common miRNAs were obtained from two circRNA databases, and three core miRNAs (hsa-miR-591, hsa-miR-626, hsa-miR-630) were selected. Further target gene prediction yielded 7 overlapping genes for hsa-miR-591 and 9 for hsa-miR-630, while no common target was found for hsa-miR-626. GO and KEGG enrichment indicated involvement in transcriptional regulation, protein interaction, PI3K-Akt, HIF-1 and cancer-related signaling pathways. The PPI network analysis identified YAP1, ZEB1, H3-3B, and IGF1R as core hub genes, forming a central regulatory module involved in Hippo signaling, EMT, and PI3K-Akt pathways. Conclusion: This study clarified the expression and conservation characteristics of hsa_circ_0036683 in HCC and constructed a potential hsa_circ_0036683-miRNA-mRNA ceRNA regulatory network. The results provide novel bioinformatics insights for exploring the molecular mechanism of HCC, indicating that hsa_circ_0036683 has the potential to be a candidate biomarker and therapeutic target for HCC.

Keywords: Hepatocellular Carcinoma; Circular RNA; hsa_circ_0036683; ceRNA; Biomarker.

1. Introduction

Hepatocellular carcinoma (HCC) is among the most prevalent malignancies worldwide and a leading cause of cancer-related death[1, 2]. Despite surgical resection, targeted therapy, and immunotherapy, prognosis remains poor due to high recurrence and metastasis rates[3, 4]. Identifying reliable biomarkers is critical for improving patient outcomes[5].

Circular RNAs (circRNAs) are a class of endogenous noncoding RNAs with covalently closed loop structures, providing high stability and resistance to exonuclease degradation[6-8]. CircRNAs can act as miRNA sponges, regulate transcription, and interact with RNA-binding proteins, thus influencing cancer progression[9-11].

Recent studies have identified multiple circRNAs as dysregulated in HCC[12-15]. Among them, hsa_circ_0036683 has been reported as significantly downregulated in tumor tissues[16]. However, the molecular mechanisms and regulatory networks of hsa_circ_0036683 in HCC remain largely unexplored.

In this study, we performed a comprehensive bioinformatics analysis to investigate the regulatory mechanisms of hsa_circ_0036683 in HCC. A ceRNA network was constructed. This study aims to provide novel insights into the role of hsa_circ_0036683 in HCC progression.

2. Materials and Methods

2.1. Data Acquisition

CircRNA expression profiles were obtained from publicly available Gene Expression Omnibus datasets. Differentially expressed circRNAs were identified using the limma package in R software. Genes with $|\log_2FC| > 1$ and adjusted P-value < 0.05 were considered differentially expressed for subsequent analysis.

2.2. Prediction of miRNA Targets

Potential miRNAs interacting with hsa_circ_0036683 were predicted using CircBank, CircInteractome. Only overlapping miRNAs were considered for downstream analysis.

2.3. Prediction of Downstream mRNAs

The predicted miRNAs were used to identify potential mRNA targets via TargetScan, miRDB, and miRTarBase. Overlapping targets were considered as candidate downstream genes.

2.4. Functional Enrichment Analysis (GO and KEGG)

GO and KEGG enrichment analyses were performed using DAVID (version 6.8) to explore the potential functions of the candidate target genes. Homo sapiens was selected as the

reference species. GO analysis included biological processes (BP), cellular components (CC), and molecular functions (MF), while KEGG analysis was used to identify enriched pathways. A P value < 0.05 was considered statistically significant.

2.5. Protein–Protein Interaction Network Construction

A protein–protein interaction (PPI) network was constructed using the STRING database (version 12.0). Candidate target genes were uploaded to STRING with Homo sapiens selected as the reference species. The network was visualized to explore interactions among the candidate genes and identify potential key nodes in the hsa_circ_0036683-mediated regulatory network.

3. Results

3.1. Screening of Differentially Expressed circRNAs in HCC Tissues

Two GEO datasets (GSE121714 and GSE242797) were analyzed to screen differentially expressed circRNAs. A total of 44 differentially expressed circRNAs were obtained after intersection, including 16 upregulated and 28 downregulated circRNAs. Volcano plots and heatmaps were plotted to visualize the differential expression of circRNAs between HCC tissues and adjacent normal tissues (Figure 1A, 1B). Based on fold change and P-value, hsa_circ_0036683 with the most significant downregulation was selected for subsequent research.

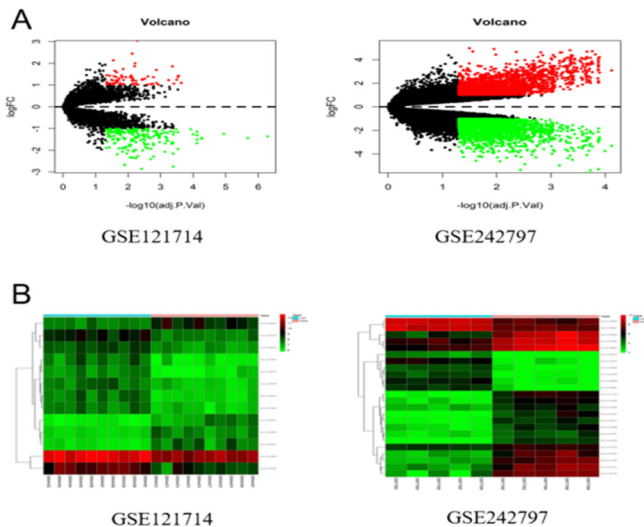


Figure 1. Expression profiles of differentially expressed circRNAs in HCC. (A) Volcano plots of differentially expressed circRNAs in GSE121714 and GSE242797; (B) Heatmaps of differentially expressed circRNAs in GSE121714 and GSE242797.

3.2. Structural Characteristics of hsa_circ_0036683

The structural characteristics of hsa_circ_0036683 were analyzed using circRNA annotation databases. As shown in Figure 2A, hsa_circ_0036683 is derived from its host gene through a back-splicing event involving exonic regions. Figure 2B illustrates the formation of the circular RNA structure, which lacks free 5' and 3' ends and may therefore exhibit enhanced stability.

To investigate its potential regulatory function, putative

miRNA-binding sites were predicted. Top three candidate miRNAs selected after comprehensive evaluation, including hsa-miR-591, hsa-miR-626, and hsa-miR-630, were identified as potential targets of hsa_circ_0036683 (Figure 2C). These results suggest that hsa_circ_0036683 may contribute to the progression of hepatocellular carcinoma through a ceRNA-mediated regulatory mechanism.

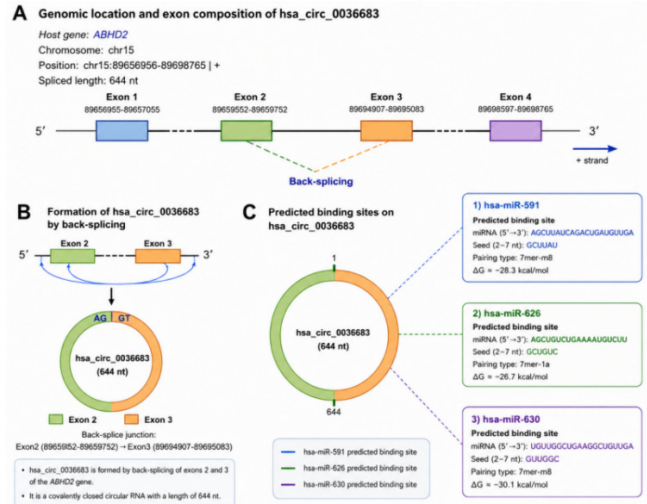


Figure 2. Structural characteristics and predicted miRNA-binding sites of hsa_circ_0036683. (A) Genomic organization and exon composition of hsa_circ_0036683 derived from its host gene. (B) Schematic illustration of circRNA formation through back-splicing. (C) Predicted binding sites of hsa-miR-591, hsa-miR-626 and hsa-miR-630 within hsa_circ_0036683.

3.3. hsa_circ_0036683 is Downregulated in HCC

CircAtlas 3.0 analysis indicated that hsa_circ_0036683 was significantly downregulated in HCC (Figure 3A). Its host gene exhibited synergistic expression changes with this circRNA (Figure 3B). Meanwhile, high CMP value suggested strong evolutionary conservation of hsa_circ_0036683 (Figure 3C).

3.4. Identification of hsa_circ_0036683-related miRNAs and mRNAs

Three overlapping miRNAs were identified: hsa-miR-1184, hsa-miR-1225-3p, hsa-miR-1231, hsa-miR-1265, hsa-miR-338-3p, hsa-miR-361-3p, hsa-miR-409-3p, hsa-miR-591, hsa-miR-626, hsa-miR-630 and hsa-miR-876-3p. A total of 11 common miRNAs were predicted by CircBank and CircInteractome (Figure 4). Since the two databases use different evaluation systems, we integrated all available parameters to evaluate their binding capacity. After comprehensive scoring and screening according to predefined criteria, three candidate miRNAs with the best binding performance were determined as key downstream molecules of hsa_circ_0036683: hsa-miR-591, hsa-miR-626 and hsa-miR-630. To further explore the regulatory mechanism of the three core miRNAs, we predicted their downstream target genes using three databases, including TargetScan 8.0, miRTarBase and miRDB. After intersecting the prediction results from the above databases, we obtained 7 overlapping target genes for hsa-miR-591 (Figure 5A). No common target gene was identified for hsa-miR-626 across the three databases (Figure 5B). Meanwhile, a total of 9 intersecting target genes were selected for hsa-miR-630 (Figure 5).

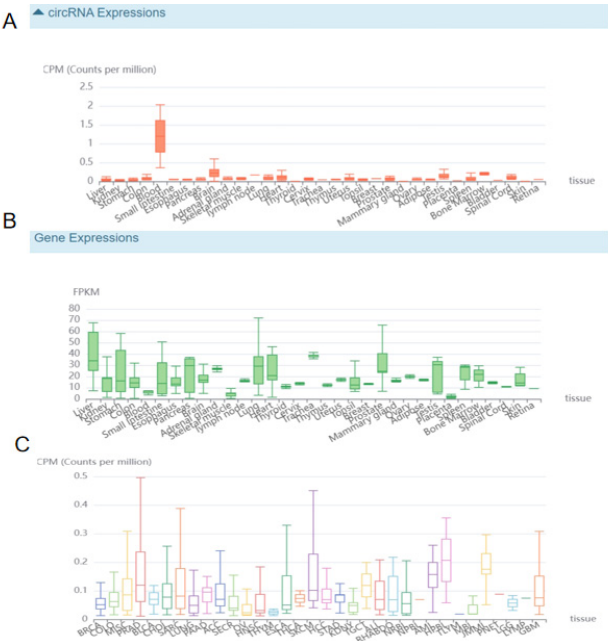


Figure 3. Expression and conservation analysis of hsa_circ_0036683 using CircAtlas 3.0. (A) circRNA expression levels (CPM) across different human tissues. (B) Expression of the host gene (FPKM) in corresponding tissues. (C) Cross-species conservation (CPM) of the circRNA across vertebrate species.

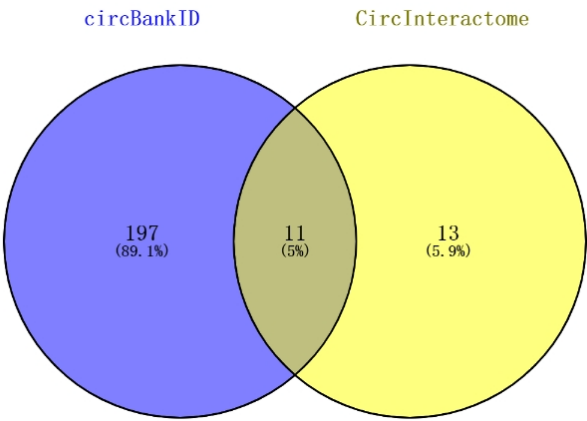


Figure 4. Venn diagram showing the overlapping miRNAs predicted by CircBank and CircInteractome. A total of 11 common miRNAs were obtained for subsequent screening.

3.5. Functional Enrichment

GO and KEGG enrichment analyses were performed on 16 target genes of hsa_circ_0036683. These genes were mainly distributed in cytoplasm, nucleus and plasma membrane. Their biological functions focused on transcriptional regulation and ERK pathway modulation, and they also participated in apoptosis and immune response. Protein binding was the predominant molecular function, accompanied by multiple transcription-related activities. KEGG analysis revealed enrichment in HCC-related pathways including PI3K-Akt and HIF-1 signaling. Collectively, the ceRNA network regulates hepatocellular carcinoma progression via transcriptional control, protein interaction and oncogenic signaling pathways.

3.6. Protein–Protein Interaction Network Analysis

The PPI network of target genes revealed YAP1, ZEB1,

IGF1R, and H3-3B as core hub genes with extensive interactions. These central nodes form a regulatory module closely associated with Hippo signaling, EMT, and PI3K-Akt pathways, indicating their potential roles in driving tumor progression.

4. Discussion

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide and remains associated with poor prognosis despite advances in diagnosis and treatment[17, 18]. Increasing evidence suggests that circular RNAs (circRNAs) participate in tumor progression by acting as competing endogenous RNAs (ceRNAs) that regulate microRNA (miRNA) activity and downstream gene expression[19, 20].

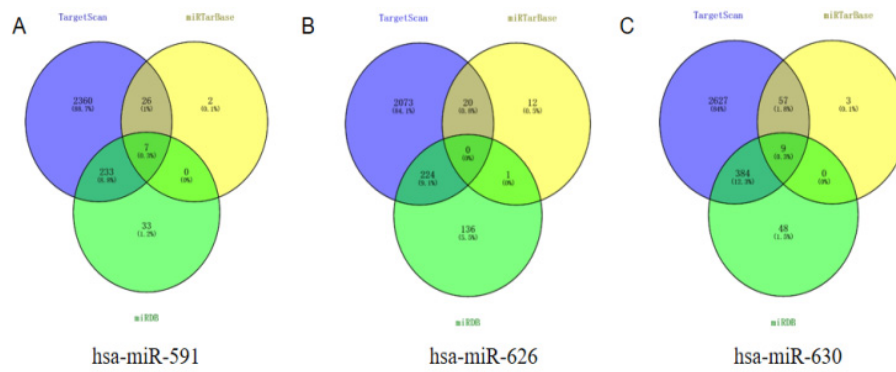


Figure 5. Venn diagrams showing target genes of hsa-miR-591, hsa-miR-626 and hsa-miR-630 predicted by TargetScan 8.0, miRTarBase and miRDB. The number of overlapping genes is indicated in the intersection area.

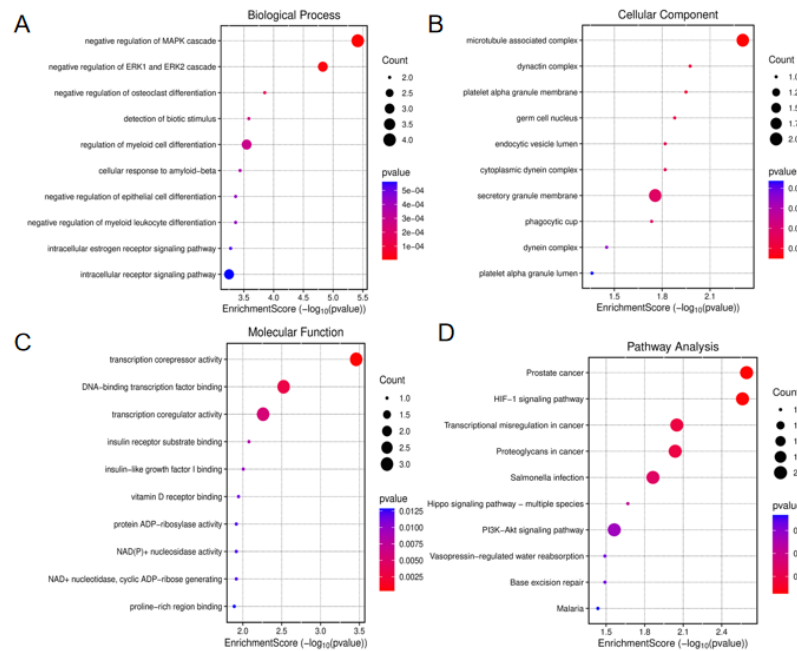


Figure 6. GO/KEGG enrichment analysis (A) Biological Process (BP) enrichment; (B) Cellular Component (CC) enrichment; (C) Molecular Function (MF) enrichment; (D) KEGG pathway analysis. The horizontal axis represents the enrichment score ($-\log_{10}(\text{p-value})$), the vertical axis represents the enriched terms/pathways, the size of each dot indicates the number of enriched genes, and the color gradient indicates the p-value (red represents lower p-value, blue represents higher p-value)

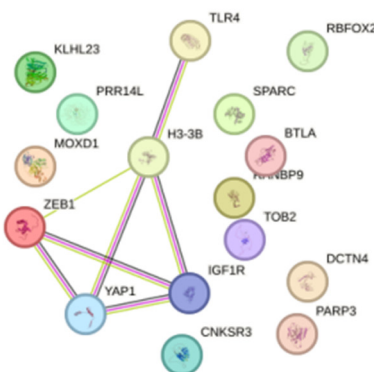


Figure 7. Protein-protein interaction (PPI) network

In the present study, bioinformatics analyses revealed that hsa_circ_0036683 was downregulated in HCC tissues. CircAtlas analysis further showed that its host gene exhibited a similar expression pattern and that hsa_circ_0036683 displayed relatively high evolutionary conservation,

suggesting a potential biological role in HCC development.

To explore the underlying regulatory mechanism, CircBank and CircInteractome were used to predict downstream miRNAs. Eleven overlapping miRNAs were identified, among which hsa-miR-591, hsa-miR-626, and hsa-

miR-630 were selected as candidate target miRNAs. Further analysis using TargetScan, miRTarBase, and miRDB identified several potential downstream target genes, suggesting that hsa_circ_0036683 may participate in HCC progression through a ceRNA regulatory network.

Previous studies have demonstrated that the candidate miRNAs identified in this study are involved in multiple cancer-related biological processes. Among them, miR-591 has been reported to suppress tumor cell proliferation and migration in several malignancies, suggesting a potential tumor-suppressive role[21]. The hsa-miR-626 has also been implicated in the regulation of cancer cell growth and apoptosis through diverse molecular pathways[22]. In addition, miR-630 has been shown to participate in tumor progression, metastasis, and treatment response in various cancers[23]. Therefore, dysregulation of these miRNAs may contribute to HCC development, supporting the potential involvement of the hsa_circ_0036683-miRNA-mRNA regulatory axis in hepatocarcinogenesis.

GO and KEGG enrichment analyses indicated that the target genes of hsa_circ_0036683 are mainly involved in transcriptional regulation, ERK signaling, apoptosis, and immune response. Enrichment of the PI3K-Akt and HIF-1 pathways further suggests a potential role in HCC progression. PPI network analysis identified YAP1, ZEB1, IGF1R, and H3-3B as core hub genes. Notably, YAP1, ZEB1, and IGF1R are key regulators of tumor growth, EMT, and cell survival, indicating that they may serve as important downstream effectors of the hsa_circ_0036683-mediated ceRNA network in hepatocellular carcinoma.

Several limitations should be acknowledged. First, all findings were based on bioinformatics analyses without experimental validation. Second, the expression and regulatory relationships of hsa_circ_0036683 and its downstream molecules require verification in clinical samples and HCC cell lines. Third, the biological functions of hsa_circ_0036683 in HCC progression remain unclear and warrant further investigation.

In conclusion, this study suggests that hsa_circ_0036683 may be involved in HCC development through a potential ceRNA regulatory network. These findings provide a basis for future experimental studies and indicate that hsa_circ_0036683 may serve as a potential biomarker and therapeutic target for HCC.

5. Conclusion

The hsa_circ_0036683 may represent a potential diagnostic biomarker and therapeutic target for HCC. This study lays a foundation for future experimental validation and potential therapeutic applications.

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