

The Dysregulated circRNAs in Hepatocellular Carcinoma Were Identified Through Circular RNA Sequencing and Bioinformatics Analysis

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Abstract: Background: The research evidence suggests that dysregulated circRNAs are closely associated with the progression of HCC and can serve as diagnostic markers and therapeutic targets for HCC. However, the status of differentially expressed circRNAs in HCC cancerous tissues and adjacent non-cancerous tissues remains unclear. Methods: Firstly, circRNA-Seq was employed to screen for dysregulated circRNAs in HCC tissues and adjacent non-cancerous tissues. Secondly, differential expression of circRNAs in the GSE97332 dataset was identified using bioinformatics methods. Lastly, the intersection of circRNAs that were upregulated or downregulated in both the circRNA-Seq data and the GSE97332 dataset was taken. Results: The results of the circRNA-Seq showed that there are a total of 2477 differentially expressed circRNAs between HCC tissues and adjacent non-cancerous tissues, with 1567 upregulated and 910 downregulated. We identified 869 differentially expressed circRNAs in the GSE97332 dataset, with 421 upregulated and 448 downregulated. Additionally, we took the intersection of circRNAs that were upregulated or downregulated in both the circRNA-seq dataset and the GSE97332 dataset, ultimately obtaining 13 and 6 circRNAs, respectively. Conclusion: This study identified dysregulated circRNAs in HCC through circRNA-seq and analysis of the dataset GSE97332, which offer new options for the discovery of diagnostic markers and therapeutic targets for HCC.

Keywords: HCC; circRNA; circRNA-Seq; GSE97332.

1. Introduction

As the most common type of primary liver cancer, hepatocellular carcinoma (HCC) has a high incidence and mortality rate[1]. Due to its insidious onset, rapid progression, high aggressiveness, and the limitations of early screening methods, patients are often diagnosed at advanced stages[2]. Current treatment methods for liver cancer mainly include hepatectomy, interventional therapy, targeted therapy, immunotherapy, and liver transplantation[3]. Despite advancements in treatment methods, patients often have a short survival rate and a poor prognosis due to the recurrence and metastasis of hepatic tumors[4]. Therefore, there is an urgent need to identify effective and sensitive early diagnostic markers for HCC, as well as to develop new treatment methods.

Circular RNAs (circRNAs) are derived from the reverse splicing of precursor mRNA and are widely distributed in cells and tissues[5]. Due to their closed loop structure, lack of 5' cap and 3' poly-tail, circRNAs exhibit higher stability than linear RNAs[6]. Furthermore, circRNAs are selectively expressed in different cells, tissues, or states and are highly conserved evolutionarily[7]. It is these excellent properties that give circRNAs great potential as biomarkers and therapeutic targets[8]. Chen G and colleagues conducted a study with 100 patients having pathologically confirmed

HCC from the First Affiliated Hospital of Jinan University, as well as 100 healthy volunteers as controls. They observed that the expression level of circ_0000437 was increased in HCC tissues. The area under the ROC curve (AUC) was 0.9281, suggesting a strong diagnostic potential for HCC[9]. Liu W et al. conducted whole-genome sequencing on HCC tissues and adjacent non-tumor tissues and found that circ-ZEB1 was highly expressed in the HCC samples. Further analysis elucidated the impact of circ-ZEB1 on the progression of HCC, leading to the conclusion that it can serve as a therapeutic target[10]. These studies indicate that dysregulated circRNAs play a significant role in the progression of HCC, providing new insights for the diagnosis and treatment of the disease and bringing good news to tumor patients. Therefore, screening for dysregulated circRNAs in HCC is of great importance.

In this study, we conducted circular RNA sequencing (circRNA-seq) on five pairs of HCC and adjacent non-tumor tissues, and analyzed the public dataset GSE97332. By comparing the results, we identified differentially expressed circRNAs in HCC tissues, with the goal of developing new strategies for the diagnosis and treatment of HCC.

2. Materials and Methods

2.1. HCC Tissues and Adjacent Non-tumor Tissues

The Ethical Committee of the Affiliated Hospital of Youjiang Medical University for Nationalities approved this study (Approval Number: 2019012601). Our research group collected five pairs of fresh HCC tissues and matched adjacent non-tumor tissues from patients who underwent radical resection at the Affiliated Hospital of Youjiang Medical University for Nationalities between June 2020 and September 2020. The inclusion criteria for the cases were as follows: all patients provided informed consent; no preoperative radiotherapy or chemotherapy; no other malignancies or significant underlying diseases. The postoperative pathological diagnosis was confirmed by two pathologists and was identified as HCC.

2.2. CircRNA-seq Analysis

We sent five pairs of HCC tissues and their corresponding normal tissues to Shenzhen BGI Genomics Co., Ltd. for high-throughput sequencing. They utilized the DNBSEQ-T7 platform for circRNA-seq, followed by quality control, data filtering, and annotation of the raw sequencing data. By applying the screening criteria of adjusted P Val < 0.05 and $|\text{LogFC}(\text{fold change})| > 1.0$, we utilized the limma R software package to identify differentially expressed circRNAs.

2.3. GSE97332 Microarray Analysis

We logged into the GEO database website (<https://www.ncbi.nlm.nih.gov/geo/>), searched for keywords “circRNA” and “HCC,” and selected the GSE97332 microarray datasets. We used the limma package to perform differential analysis on the GSE97332 datasets, setting the screening criteria to $\text{adj.}P$ Val < 0.05 and $|\text{LogFC}(\text{fold change})| > 1.0$.

2.4. Bioinformatics Analysis

After intersecting the circRNAs that are either upregulated or downregulated in both the GSE97332 dataset and circRNA-seq data, we utilized the Venn tool within the TBtools software to create a Venn diagram, and then performed clustering analysis on differentially expressed circRNAs using the pheatmap R package, generating heat maps to visualize the results.

3. Results

3.1. Identification of Dysregulated circRNAs in the circRNA-seq Data

The circRNA-seq results showed that there was a total of 2477 differentially expressed circRNAs between hepatocellular carcinoma tissues and adjacent non-tumor tissues, with 1567 circRNAs upregulated and 910 circRNAs downregulated.

3.2. Identification of Dysregulated circRNAs in the GSE97332 Dataset

Through analysis of the GSE97332 dataset, 869 differentially expressed circRNAs were identified, with 421 upregulated and 448 downregulated.

3.3. Identification of Upregulated or Downregulated circRNAs in Both circRNA-seq Data and the GSE97332 Dataset

To further narrow down the screening range and improve the reliability of the analysis, we performed an intersection of the upregulated circRNAs present in both the high-throughput sequencing data and the GSE97332 dataset, and found that 13 circRNAs were overlapping (Figure 1). Additionally, we performed an intersection of the downregulated circRNAs present in both the high-throughput sequencing data and the GSE97332 dataset, and found that 6 circRNAs were overlapping (Figure 2). The differentially expressed genes (DEGs) are listed in Table 1. We then performed clustering analysis on the differentially expressed circRNAs obtained from circRNA-seq (Figure 3) and the GSE97332 dataset (Figure 4), generating heat maps to visualize the results.

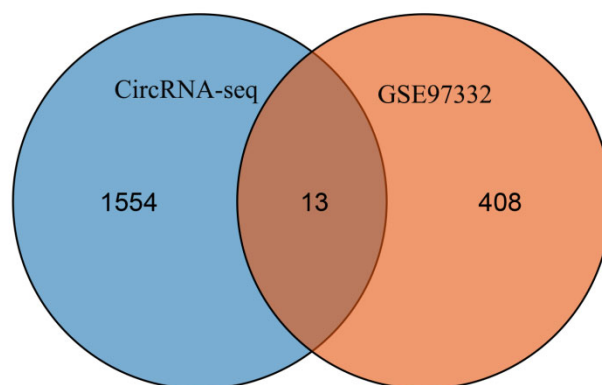


Figure 1. Venn diagram showing upregulated circRNAs in both the circRNA-seq data and the GSE97332 dataset

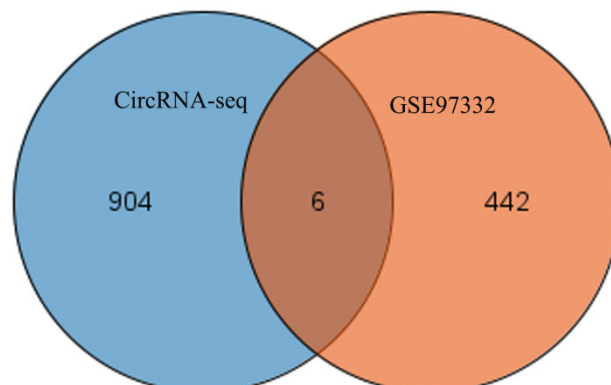


Figure 2. Venn diagram showing downregulated circRNAs in both the circRNA-seq data and the GSE97332 dataset

Table 1. The differentially expressed genes

DEGs	CircRNAs
Up-regulated	hsa_circ_0046555, hsa_circ_0044556, hsa_circ_0004053, hsa_circ_0051042, hsa_circ_0005273, hsa_circ_0004071, hsa_circ_0004599, hsa_circ_0006421, hsa_circ_0004788, hsa_circ_0084429, hsa_circ_0004315, hsa_circ_0003644, hsa_circ_0072758
Down-regulated	hsa_circ_0001861, hsa_circ_0003757, hsa_circ_0030045, hsa_circ_0028123, hsa_circ_0002372, hsa_circ_0069249

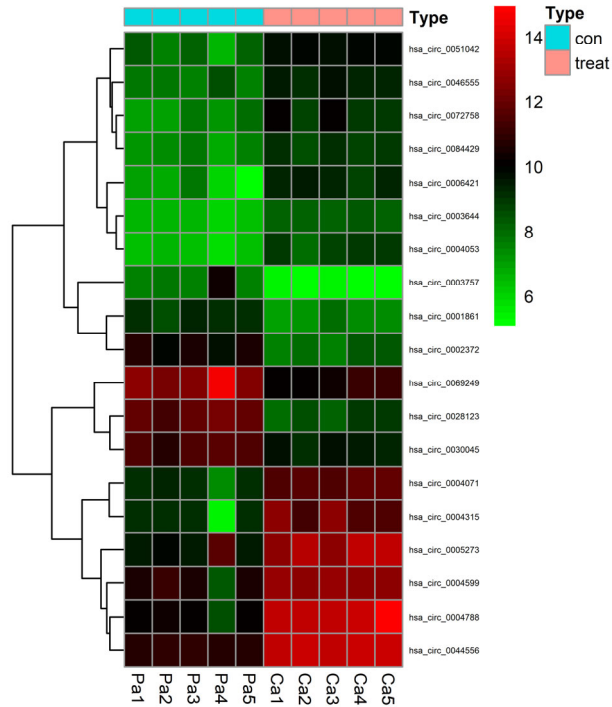


Figure 3. Clustering heatmap of differentially expressed circRNAs obtained from circRNA-seq

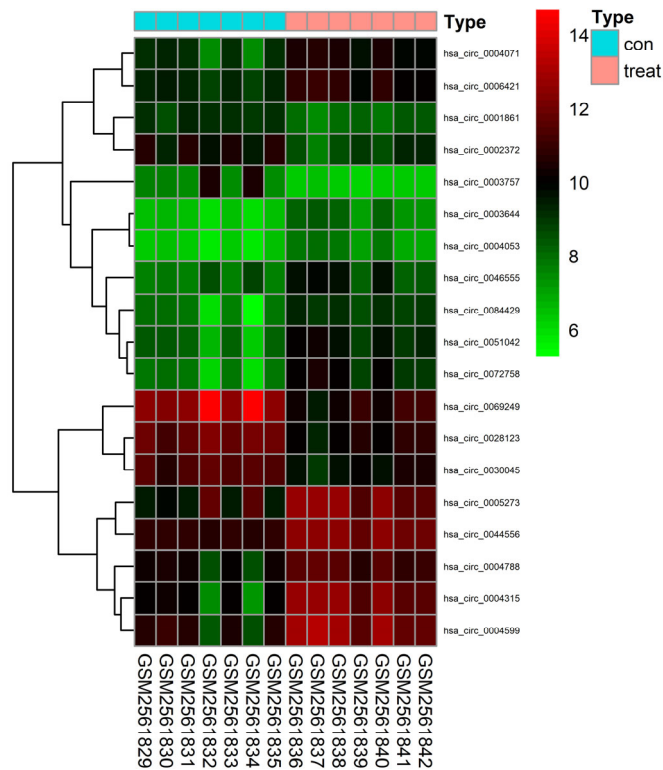


Figure 4. Clustering heatmap of differentially expressed circRNAs obtained from the GSE97332 dataset

4. Discussion

HCC is one of the leading causes of cancer-related deaths, imposing significant physical and emotional burdens on patients and a substantial disease burden on the global population[11]. The specificity and sensitivity of the early diagnostic marker α -Fetoprotein (AFP) are not high. Moreover, the cost-intensive MRI and CT scans are not feasible for screening hepatocellular carcinoma. These factors often lead to patients being diagnosed at advanced stages, missing optimal treatment opportunities[12]. Treatment options for advanced liver cancer are limited, yielding suboptimal outcomes and low patient survival rates[13]. Increasing evidence indicates that dysregulated circRNAs participate in the initiation and progression of HCC, functioning as either tumor suppressors or oncogenes, and can act as diagnostic markers and therapeutic targets for HCC[14]. Therefore, delving into circRNAs associated with HCC promises a promising future for hepatocellular carcinoma research.

CircRNA-Seq, a type of high-throughput sequencing technique, not only enables the discovery of new sequences but also provides continuously improving throughput and accuracy. It has now been widely applied in transcriptomic studies of various cancers[15]. Liu Z and colleagues performed RNA-seq on three pairs of HCC cell lines (HepG2, SMMC-7721, and Huh7) treated with and without sorafenib (SF). They detected a total of 3963 circRNAs reported in circBase. Of these, 2506 were excluded due to their low abundance, 1320 showed no significant changes in levels following SF treatment, and 137 were differentially expressed[16]. We sent five pairs of HCC tumor tissues and adjacent non-tumor tissues to BGI Genomics for circRNA-seq. The results showed that there were 2477 differentially expressed circRNAs between the tumor and adjacent non-tumor tissues, with 1567 upregulated and 910 downregulated. Microarray technology, based on the hybridization principle, typically uses optical methods to detect the hybridization signals of known sequence probes with target molecules, thereby obtaining gene detection data[17]. Its limitation lies in the need to depend on known sequence probes for microarray preparation, making it difficult to complete the detection of some unknown RNAs. Yang C and colleagues used circRNA microarrays to detect sorafenib-resistant cell lines (SR-HepG2) and HepG2 cells, identifying 748 differentially expressed circRNAs, with 416 upregulated and 332 downregulated[18]. We utilized the GEO database to download the dataset GSE97332 and analyzed the expression profile of circRNAs, obtaining 869 differentially expressed circRNAs, with 421 upregulated and 448 downregulated. To further refine the research scope and improve the reliability of the study, we took the intersection of the circRNAs that were upregulated or downregulated in both datasets, ultimately identifying 13 and 15 candidate circRNAs, respectively. In this study, we combined circRNA-Seq with microarray technology and applied bioinformatics analysis to the identification of dysregulated molecules in HCC. This integration deepened the transcriptomic analysis and provided new directions for the discovery of diagnostic markers and therapeutic targets for HCC.

The limitations of this study are that we have not yet uncovered the role and potential molecular mechanisms of circRNAs in the progression of HCC. In the next step, we will

continue to explore the correlation between circRNAs and HCC and how they regulate the progression of HCC.

5. Conclusion

In conclusion, the dysregulated circRNAs identified through circRNA-seq and the dataset GSE97332 analysis may be involved in the progression of hepatocellular carcinoma, and are promising to become diagnostic markers and therapeutic targets for HCC.

Institutional Review Board Statement

Not applicable.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

The authors declare no conflict of interest.

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Author Contributions: Conceptualization, J.S1., J.P., L.Z., Y.D.; methodology, J.S2.; software, L.Z.; investigation, J.S2. and L.Z.; writing—original draft preparation, J.S1., J.P., L.Z.; writing—review and editing, Y.D.; supervision, Y.D.; funding acquisition, Y.D. All authors have read and agreed to the published version of the manuscript.

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