Differential Analysis of lncRNAs and mRNAs Expression in HCC and the Predictive Value of lncRNAs

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ABSTRACT: Background: Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer-related death. However, the molecular mechanism of its pathogenesis is still unclear. This study analyzed the pathophysiology of HCC based on the perspective of lncRNAs and mRNAs expression to find effective diagnostic strategies and prognostic markers.

Methods: Data for HCC patients and normal controls were downloaded from the GEO and TCGA databases. LncRNAs and mRNAs differentially expressed in HCC patients were screened by R language (3.6.0). The ROC curves of the ten lncRNAs with the most significant expression differences were drawn, and survival analysis was conducted. Then, the differentially expressed mRNAs were annotated by using GO and KEGG databases. The protein–protein interactions in the lncRNA–mRNA coexpression network were analyzed with the STRING database. Tissue and cell experiments were performed to verify the results of bioinformatics analysis.

Results: A large number of differentially expressed lncRNAs and mRNAs in HCC were correlated. Four lncRNAs were significantly associated with the prognosis of HCC patients. These differentially expressed lncRNAs mainly regulated the proliferation and lipid metabolism of HCC. Cell experiments confirmed that lnc-MMADHC-5 could inhibit the development of HCC.

Conclusion: lncRNAs regulated the proliferation and lipid metabolism of HCC. Lnc-APBB1-1, lnc-FBXO42-1, lnc-JAKMIP2-1, and lnc-MMADHC-5 might be prognostic markers for HCC. Lnc-MMADHC-5 could inhibit the development of HCC.

KEY WORDS: lncRNA, hepatocellular carcinoma, cell proliferation, lnc-MMADH-5

I. INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most frequently diagnosed cancers globally and the fourth most common cause of cancer-related death. In China, 466,100 people are diagnosed with liver cancer every year.[1] Currently, the main treatment methods for HCC include hepatectomy, liver transplantation, transarterial chemoembolization, ablation, and small-molecule targeted drug therapy.[2] In these options, only surgical resection and liver transplantation have been considered as potential cures. However, only 15% of patients are eligible for potentially curative treatment.[3] Clinical outcomes in patients with HCC need to be improved urgently, which requires us further to understand the mechanism of HCC occurrence and development. Since HCC is an inherited disease, it is essential to explore the pathological mechanism of HCC at the genetic level to discover new therapeutic targets.[4]

Long noncoding RNA (lncRNA) is RNA with more than 200 nucleotides in length and no protein-coding ability, characterized by conserved sequence and low expression level.[5] Dysregulation of lncRNAs

expression represents a common event in the pathogenesis of various human genetic diseases, including cancer.[6] For example, the differential expression of lncRNA HOTTIP in HCC affects the occurrence and development of HCC by regulating the self-renewal ability and other biological functions of HCC cells.[7] At present, a large number of studies on HCC focused on a specific lncRNA. There is no study to generalize the lncRNAs characteristic expressions in HCC. Therefore, we attempted to provide a holistic overview of how lncRNAs affect the compositions and functions of HCC cells using a bioinformatics approach.

LncRNAs perform a variety of functions at the transcriptional, post-transcriptional, and epigenetic levels.[8] According to the Noncode Database (http://www.noncode.org), humans currently have 96,308 lncRNAs. However, only a few lncRNAs have been well characterized in function at present.[9] LncRNAs can affect the transcription, processing, editing, translation, or degradation of target mRNA.[10] LncRNAs and mRNAs in exosomes play an essential role in the occurrence and development of HCC.[11] The abundance of mRNAs in human blood is a potential biomarker for cancer diagnosis.[12] LncRNAs usually perform their functions by binding to one or more proteins.[13] Therefore, in this study, we analyzed the mRNAs and proteins associated with the differentially expressed lncRNAs in HCC to predict the functions of these lncRNAs.

In this study, we downloaded genetic data from Gene Expression Omnibus (GEO) database and The Cancer Genome Atlas (TCGA) to analyze the differential expressions of lncRNAs and mRNAs in HCC patients and their related biological functions. We then took clinical samples and conducted cell experiments to verify them. This study is expected to further advance the understanding of differentially expressed lncRNAs in HCC and the molecular mechanisms involved in the pathology and provide valuable help for the diagnosis and prognostic strategies of HCC.

II. MATERIALS AND METHODS

A. Genetic Data

Data set GSE101728 was downloaded from the GEO database study cohort, and 7 cases of HCC patients and 7 cases of normal controls were selected. The data of 377 patients with HCC were downloaded from the TCGA database. There were two data types: lncRNAs and mRNAs microarray. R language (3.6.0), Cytoscape (3.6.1), and the STRING database (11.0) were used for further analysis.

B. Differential Expression Analysis

The original microarray expression data of GSE101728 was downloaded by using the R-package GeoQuery. Then the R-package limma was performed to analyze the differential expression of lncRNAs and mRNAs in HCC patients and normal controls. Data selection criteria were $|\log FC| > 2$ and $P < 0.05$. (FC: fold change). The results of the analysis were visualized in the form of volcano maps.

C. Receiver Operating Characteristic (ROC) Curve and Survival Analysis

The ROC curve was drawn by using R-package pROC for the differential lncRNAs in the clustering heat map. LncRNAs with an area under the curve (AUC) more than 0.75 were selected for subsequent validation. According to the cluster analysis results, the data of HCC patients downloaded from the TCGA database were divided into two groups: the high expression group and the low expression group of lncRNAs. The grouping was based on the median of logFC. The difference in survival curves between the two groups was compared to determine whether the difference was significant.

D. Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and (Protein–Protein Interaction) PPI Network

Biological functions related to differentially expressed mRNAs were annotated with GO and KEGG by R-package ClusterProfiler. The significantly enriched pathways were plotted. PPI associated with lncRNA–mRNA coexpression network was analyzed using the online STRING database (https://string-db.org/). It was devised as a PPI network graph.

E. Tissues and Cells

Ten cases of HCC and ten cases of paracancerous tissues were taken from Hunan Cancer Hospital, and all procedures were carried out in strict accordance with the Helsinki Declaration. All experiments had been approved by Ethics Committee for Human Studies at Hunan Cancer Hospital (No. KYJJ-2020-095; Hunan, China). Clinical tissue was divided into two groups: normal group and HCC group. HepG2 cell line (a type of HCC cell) was purchased from Nanjing Cobioer Gene Technology Co. Ltd. (#CBP60199; Nanjing, China). The cells were cultured in DMEM (D5796; Sigma Aldrich, St. Louis, MO, USA) containing 10% fetal bovine serum (FBS, 0099141; Gibco, Waltham, MA, USA) and 1% penicillin–streptomycin double antibody (SV30010; Beyotime, Jiangsu, China) at 37°C, 5% CO_2, and saturated humidity. The cells were divided into three groups: control group, NC group, and sh-MMADHC-5 group. sh-MMADHC-5 sequence (GCAAATTGAGCAGAAGTTTGC) was provided by Ribobio (Guangzhou, China). A scrambled siRNA was used as the transfection control. Scrambled siRNA and sh-MMADHC-5 plasmids were transfected into cells of the NC and the sh-MMADHC-5 groups by using LIP2000 (11668019; Invitrogen, Waltham, MA, USA), respectively. The cells in the control group were not treated. The cells were further analyzed 48 h after transfection.[14]

F. Real-time Quantitative Polymerase Chain Reaction (RT-qPCR)

Trizol lysates were used to extract total RNA from tissues and cells. mRNA reverse transcription kit (CW2569) was purchased from Cwbio (Beijing, China). cDNA was obtained by reverse transcription by using the total mRNA of tissue or cell as templates. By querying NCBI, the primers of the target gene were designed by Primer5 software and synthesized by Shanghai Sangon Biotech (Table 1). A PCR reaction system containing UltraSYBR Mixture (CW2601; Cwbio) was prepared. Then, the reaction system was put into an RT-qPCR instrument (Quantstudio1; Thermo Fisher Scientific, Waltham, MA, USA) for PCR reaction and fluorescence signals were monitored in real-time. Finally, by using β-actin as an internal reference, the relative expression levels of the target genes in each tissue or cell were calculated by $2^{-\Delta\Delta CT}$ method.

G. Western Blot (WB)

The cells were lysed with RIPA lysate (P0013B, Beyotime) to obtain protein supernatant. Ten percent and 4.8% polyacrylamide gels were prepared. The protein supernatant was mixed with 5× loading buffer and boiled together in boiling water for 5 min. 10-μl protein samples were added to each well of the gel. Electrophoresis was performed at a constant pressure of 75 V for 130 min. The target proteins were cut out according to the indicator of the marker. Then the proteins were transferred to nitrocellulose carbon film at a constant flow of 300 mA. Five percent skim milk powder was used to seal the films. The films were incubated with the primary antibody Bcl2 (12789-1-AP, 1:2000; Proteintech, Rosemont, IL, USA), Proliferating Cell Nuclear Antigen (PCNA) (10205-2-AP, 1:1500; Proteintech), Bax (ab32503, 1:1000; Abcam, Cambridge, UK), caspase3 (#9664, 1:1000; Cell Signaling Technology, Danvers, MA, USA), Ki67 (ab16667, 1:1500; Abcam), β-actin (66009-1-Ig, 1:5000; Proteintech) at 4°C overnight. On the second day, the films were incubated with the secondary antibody HRP goat anti-mouse IgG (SA00001-1, 1:5000; Proteintech), HRP goat anti-rabbit IgG (SA00001-2, 1:6000; Proteintech) for 90 min. Finally, the protein bands were visualized with a chemiluminescence solution (K-12045-D50; Advansta, San Jose, CA, USA). The expression level of target protein in each group was calculated by using β-actin as an internal reference.

H. Flow Cytometry (FCM)

The cells were isolated with trypsin digestion solution (C20201; Beyotime) and washed twice with PBS. The cell suspension was made by adding 500 μl of binding buffer per 5×10^5 cells. The cell suspension was incubated with 5 μl Annexin V-FITC (KGA108; Keygen Biotech, Nanjing, China) and 5 μl propidium iodide in the dark at room temperature for 15 min. Cell apoptosis was detected by flow

TABLE 1: RT-qPCR primer sequences

Gene	Sequences (5'–3')	Product length (bp)
lnc-FBXO42-1	F: CTGCACACCAAACATGAGCCT	99
	R: CACCTGGGCAATGACAACGAC	
lnc-JAKMIP2-1	F: ATGCACCAAGATATATGACCCT	98
	R:TAGAAGTCTGGCGTTTCCGAT	
lnc-MMADHC-5	F: ATCGAGCCTTTGTTTCATTCCC	161
	R: GCTCCAGAAAATTCACCTACCTT	
TPX2	F: GGGCCTTTCTGGTTCTCTAGT	141
	R: TCCTGTAGTCTGGCCTCCTC	
PI3K	F: TGCGTCTACTAAAATGCATGG	122
	R: AACTGAAGGTTAATGGGTCA	
AKT	F: AGCCCTGGACTACCTGCACTCG	98
	R: CTGTGATCTTAATGTGCCCGTCCT	
β-actin	F: ACAGCCTCAAGATCATCAGC	224
	R: GGTCATGAGTCCTTCCACGAT	

cytometry (A00-1-1102; Beckman Coulter Life Sciences, Indianapolis, IN, USA) within 1 h.

I. Cell Counting Kit-8 (CCK-8)

CCK8 was purchased from our Japanese Dojindo (NU679) to test cell viability. Cells were digested with trypsin digestion solution and inoculated in 96-well plates. Each well contains about 1×10^4 cells. Each group was provided with three duplicate holes. After cells were cultured for 24 h, 10 μl of CCK8 solution was added to each well. The absorbance of the cells at 450 nm was analyzed by Bio-Tek enzyme plate (MB-530; Heales) after the cells were cultured at 37°C and 5% CO_2 for 4 h.

J. EDU Assay

The cells were incubated overnight with 50 μM EDU (C10310; Ribobio) medium and labeled with EDU. The cells were then fixed with 4% paraformaldehyde and glycine solution. The cells were incubated with the osmotic in a shaker for 10 minutes. Finally, the cells were stained with 1 × Apollo® and 1 × Hoechst33342 solution for 30 min in the dark at room temperature, respectively. Immediately after staining, the cells were observed using an inverted biological microscope (DSZ2000; Cnmicro, Beijing, China).

K. Statistical Analysis

Student's *t*-test analyzed the differential expression of GSE101728 in HCC tissues and paracancerous tissues. The Benjamini–Hochberg method was used to calculate each mRNA and lncRNA's false discovery rate and FC. The correlation matrix (correlation coefficient) was calculated by the Cor function of the R language, and then cluster analysis was implemented by hierarchical clustering. The difference in survival among HCC patients in TCGA was assessed using Kaplan–Meier curves. Student's *t*-test was performed to compare the differences between paracancerous tissues and HCC tissues. One-way ANOVA analyzed the differences among different groups of cells. $P < 0.05$ represented a significant difference.

III. RESULTS

A. Screening of Differentially Expressed lncRNAs

The change of lncRNAs expression is related to the occurrence of a variety of diseases.[15] First, we

analyzed the differential expression of lncRNAs and mRNAs in HCC patients. Seven-hundred-ninety-three differentially expressed lncRNAs were screened, of which 549 were downregulated, and 244 were upregulated. In contrast, a total of 1175 differentially expressed mRNAs were screened, of which 713 were downregulated, and 462 were upregulated (Fig. 1A). Then, we selected 10 lncRNAs with the highest expression difference and analyzed their expression differences between the HCC and normal groups. As shown in Fig. 1B, compared with the normal group, the expression levels of lnc-MMADHC-5 and lnc-JAKMIP2-1 in the HCC group significantly increased. In contrast, the expression levels of 8 lncRNAs, such as lnc-APBB1-1, were significantly decreased. Moreover, we found that the top 10 lncRNAs with the most significant expression difference were significantly correlated with the expressions of the top 100 mRNAs (Fig. 1C). These results indicated that the expression of lncRNA and mRNA in HCC patients was changed considerably.

B. ROC Diagnosis and Prognosis Assessment of lncRNAs

To explore whether the screened lncRNAs can be used as prognostic markers for HCC, we performed ROC diagnosis and survival analysis on these 10 lncRNAs. The greater the value of AUC, the stronger the correlation between this lncRNA and HCC. As shown in Fig. 2A, there were 8 lncRNAs whose AUC value was more than 0.75. They were lnc-MMMADHC-5, lnc-AC009237.1-4, lnc-DAB1-1, lnc-CDS1-3, lnc-FBXO42-1, lnc-AMBN-1, lnc-APBB1-1, and lnc-JAKMIP2-1. Survival analysis was then performed in HCC patients with high or low lncRNA expression. The significant difference

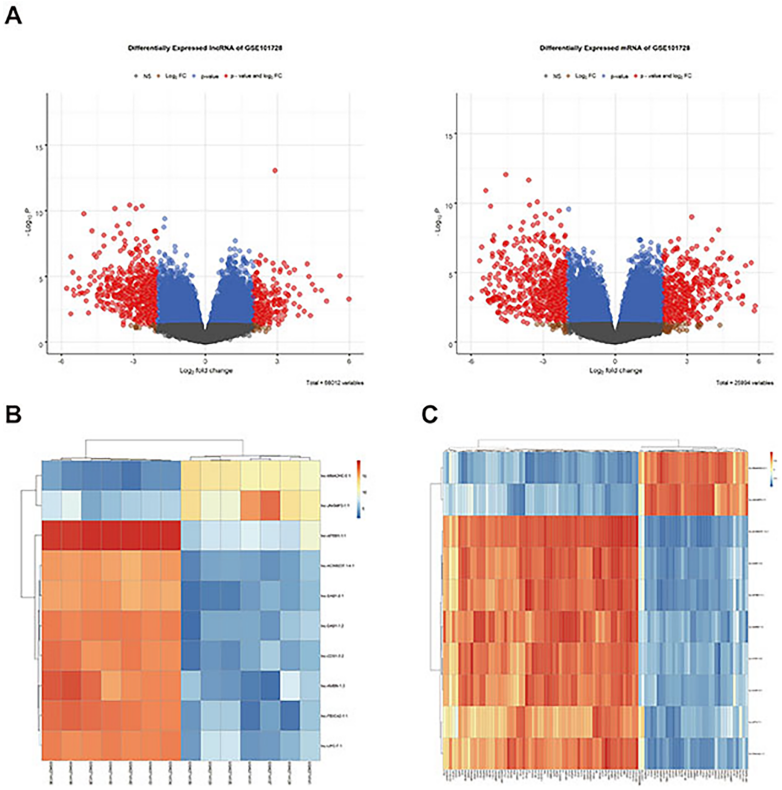

FIG. 1: Screening of differentially expressed lncRNAs. (A) Difference of lncRNAs and mRNAs expression between HCC patients and normal controls. (B) The 10 lncRNAs with the highest differential expression in HCC patients. (C) Correlation between differentially expressed lncRNAs and mRNAs in HCC patients.

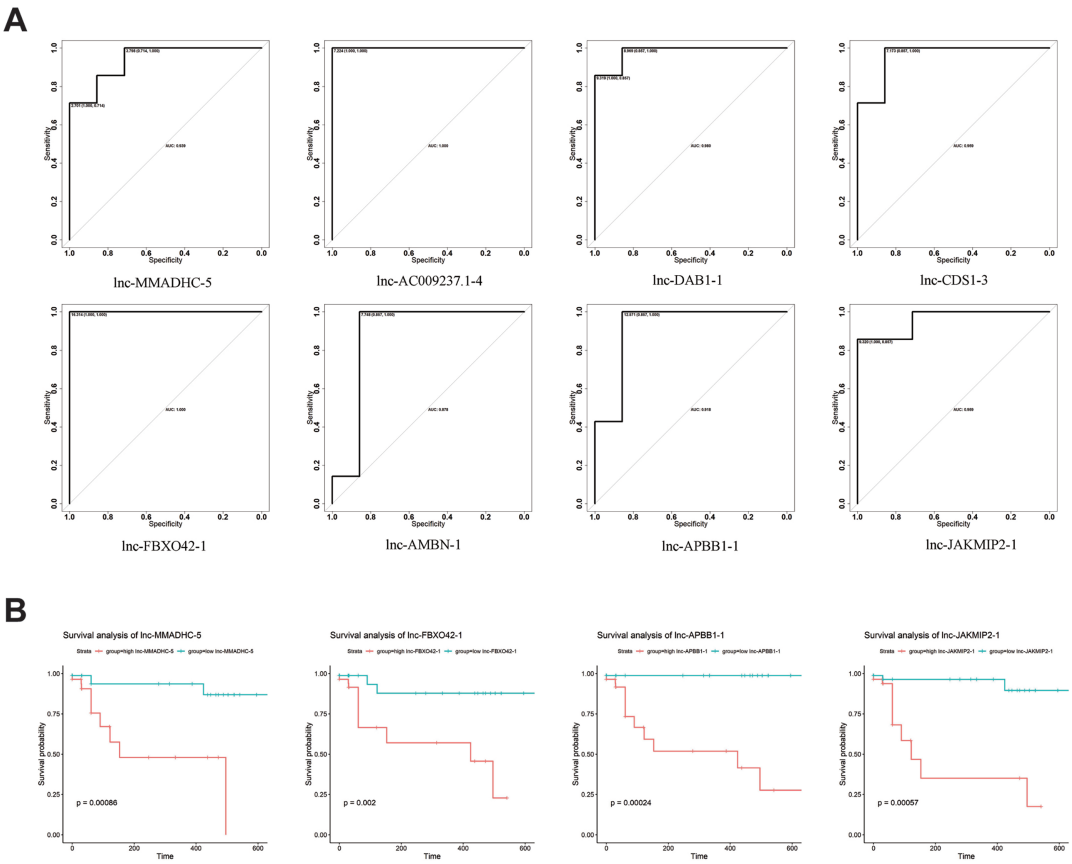

FIG. 2: ROC diagnosis and prognosis assessment of lncRNAs. (A) The ROC curves of 8 lncRNAs with AUC > 0.75. (B) The survival analysis of the 4 lncRNAs.

between the high and low expression groups indicated that lncRNA was a potential marker for HCC. There were substantial differences in the survival curves of four lncRNAs, namely lnc-APBB1-1, lnc-FBXO42-1, lnc-JAKMIP2-1, and lnc-MMADHC-5 (Fig. 2B).

C. Enrichment Analysis and PPI Network Construction

We wondered how these differentially expressed lncRNAs affected cell compositions and functions, but there were insufficient resources for annotating lncRNAs. As can be seen from Fig. 1C, the top 10 lncRNAs with the most significant expression difference were significantly correlated with the 100 mRNAs expressions. Therefore, these 100 mRNAs were annotated with GO and KEGG functions. 100 mRNAs were enriched in biological process (BP), cell component (CC), and molecular function (MF) using GO database. As Fig. 3A, 100 mRNAs were mainly related to mitotic nuclear division in BP, the collagen-containing extracellular matrix in CC, monooxygenase activity in MF. The enrichment results of KEGG database showed that 100 mRNAs were involved in 18 biological processes, such as neuroactive ligand receptor interaction, cell cycle, and chemical carcinogenesis (Fig. 3B). To sum up, the differentially expressed mRNAs in HCC were closely related to mitosis, cell cycle, arachidonic acid metabolism, cytochrome P450, and P53 signaling pathways. Figure 3C showed the PPI in the lncRNA–mRNA coexpression network. One ball represented one protein, and the lines between the balls indicated that the two proteins interacted. The network had a concentrated region, and the main

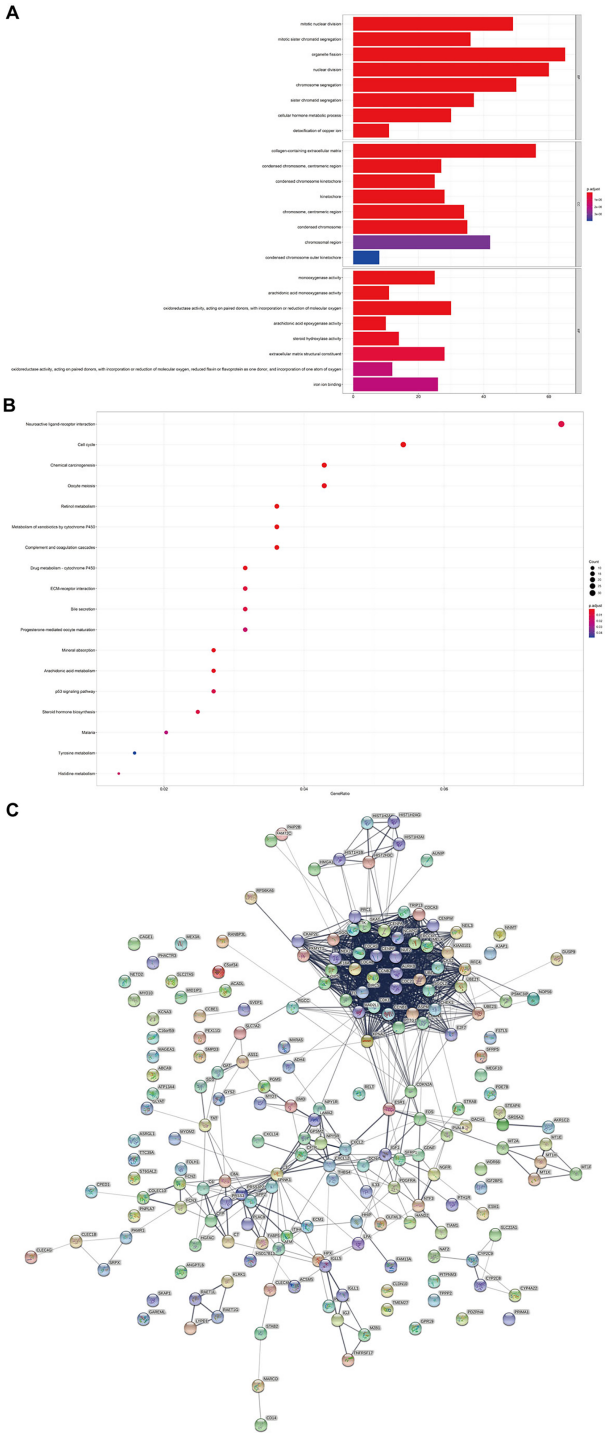

FIG. 3: Enrichment analysis and PPI network construction. (A) The GO database enrichment analysis of the differentially expressed mRNAs. (B) The KEGG database enrichment analysis of the differentially mRNAs. (C) PPI in the lncRNA–mRNA coexpression network.

proteins in the focused area include CDK1, CCNB2, CDCA8, AURKB, CENPF, etc. (Fig. 3C).

D. Lnc-MMADHC-5 Regulated the Expression of Genes and Proteins Related to Apoptosis and Proliferation in HepG2 Cells

We collected clinical tissues to verify the reliability of the results of bioinformatics analysis. First, the expression levels of three lncRNAs in paracancerous tissues and HCC tissues were analyzed by RT-qPCR. We found that the expression levels of lnc-FBXO42-1, lnc-JAKMIP2, and lnc-MMADHC in the HCC group were significantly higher than those in the normal group (Fig. 4A). Then, we constructed HepG2 cells that interfered with the expression of lnc-MMADHC-5 for further verification. Figure 4B showed that lnc-MMADHC-5 was successfully knocked down in the sh-MMADHC-5 group, and the expression levels of its downstream genes TPX2, PI3K, and AKT were also significantly reduced. The gene expression was consistent with the correlation shown in Fig. 1C. WB was utilized to detect the expression of proteins in HepG2 cells. Compared with the NC group, the expression levels of Bax and caspase3 in the sh-MMADHC-5 group were increased, while the expression levels of Bcl2, PCNA, and Ki67 were decreased (Fig. 4C and 4D). These results indicated that Lnc-MMADHC-5 could regulate the expression of genes and proteins related to proliferation and apoptosis in HepG2 cells.

E. Lnc-MMADHC-5 Regulated the Apoptosis and Proliferation of HepG2 Cells

Next, we measured the cellular function of HepG2 cells. FCM was performed to analyze the proportion of apoptotic cells in each group. As shown in Fig. 5A and 5B, compared with the NC group, the proportion of cell apoptosis was increased in the sh-MMADHC-5 group. This reflected that Lnc-MMADHC-5 could inhibit the apoptosis of HepG2 cells. The test results of CCK-8 showed that compared with the NC group, cell activity of the sh-MMADHC-5 group was decreased (Fig. 5C). Finally, EDU assay was used to detect the cell proliferation rate. EDU emitted red fluorescence, and cells with red fluorescence were considered proliferative cells. According to Fig. 5D and 5E, the proliferation rate of the sh-MMADHC-5 group was lower than that of the NC group. That was to say, the loss of Lnc-MMADHC-5 promoted HepG2 cell apoptosis, weakened cell viability, and inhibited cell proliferation.

IV. DISCUSSION

Many studies have demonstrated that lncRNAs play a crucial role in the occurrence and development of HCC.[16] Through differential expression analysis, 10 lncRNAs with the highest differential expression were screened (Fig. 1C). Except for lnc-MMADHC-5, lnc-DAB1-1, and lnc-DAB1-2, the other 7 lncRNAs have been reported to be associated with cancer. For example, the lncRNA JAKMIP2-AS1 promotes the growth of colorectal cancer and suggests a poor prognosis.[17] Amyloid β precursor protein-binding family member 1 (APBB1) enhances cancer stem cells and epithelial–mesenchymal transformation in non–small cell lung cancer cells.[18] CDS1, a cell cycle checkpoint, has been well known as a tumor suppressor.[19] In melanoma, mutations in FBXO42 (a ubiquitin ligase) lead to increased mitotic activity and a high rate of lymph node metastasis.[20] Disabled1 (DAB1) can promote cell proliferation, osteogenic differentiation, and angiogenesis of BMSCs.[21,22] The growth and survival of solid tumors depend on angiogenesis. Neovascularization is an important channel of tumor cells to distant metastasis.[23] Therefore, DAB1 may be closely related to the development and metastasis of HCC. Among these 10 lncRNAs, only FBXO42 has been reported to be associated with the development of HCC.[24] The remaining 9 lncRNAs might be novel therapeutic targets for HCC. Then, eight potential biomarkers and four potential prognostic markers were identified by ROC diagnosis and survival analysis (Fig. 2).

LncRNAs exert physiological functions by regulating mRNAs or directly interacting with proteins.[25] In our study, the mRNAs expressed differentially in HCC were closely related to

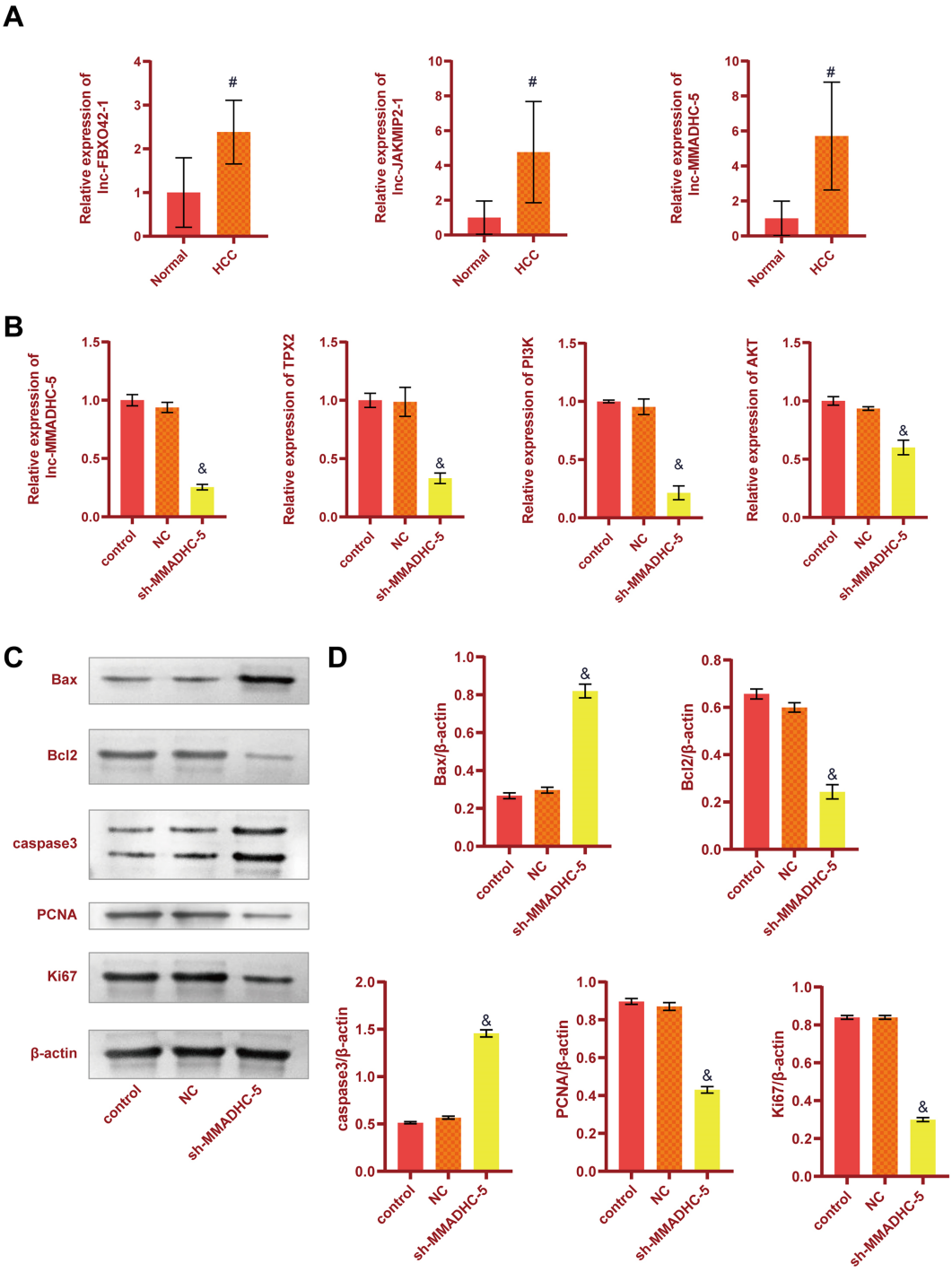

FIG. 4: Lnc-MMADHC-5 regulated the expression of genes and proteins related to apoptosis and proliferation in HepG2 cells. (A) RT-qPCR detected the relative expressions of lnc-FBXO42-1, lnc-JAKMIP2-1, and lnc-MMADHC-5. (B) RT-qPCR was performed to measure the relative expressions of lnc-MMADHC-5, TPX2, PI3K, and AKT. (C) WB was used to calculate the expressions of Bax, Bcl2, caspase3, PCNA, and Ki67. (D) Statistical analysis of (C). $^{\#}P < 0.05$ compared with the normal group. $^{\&}P < 0.05$ compared with the NC group.

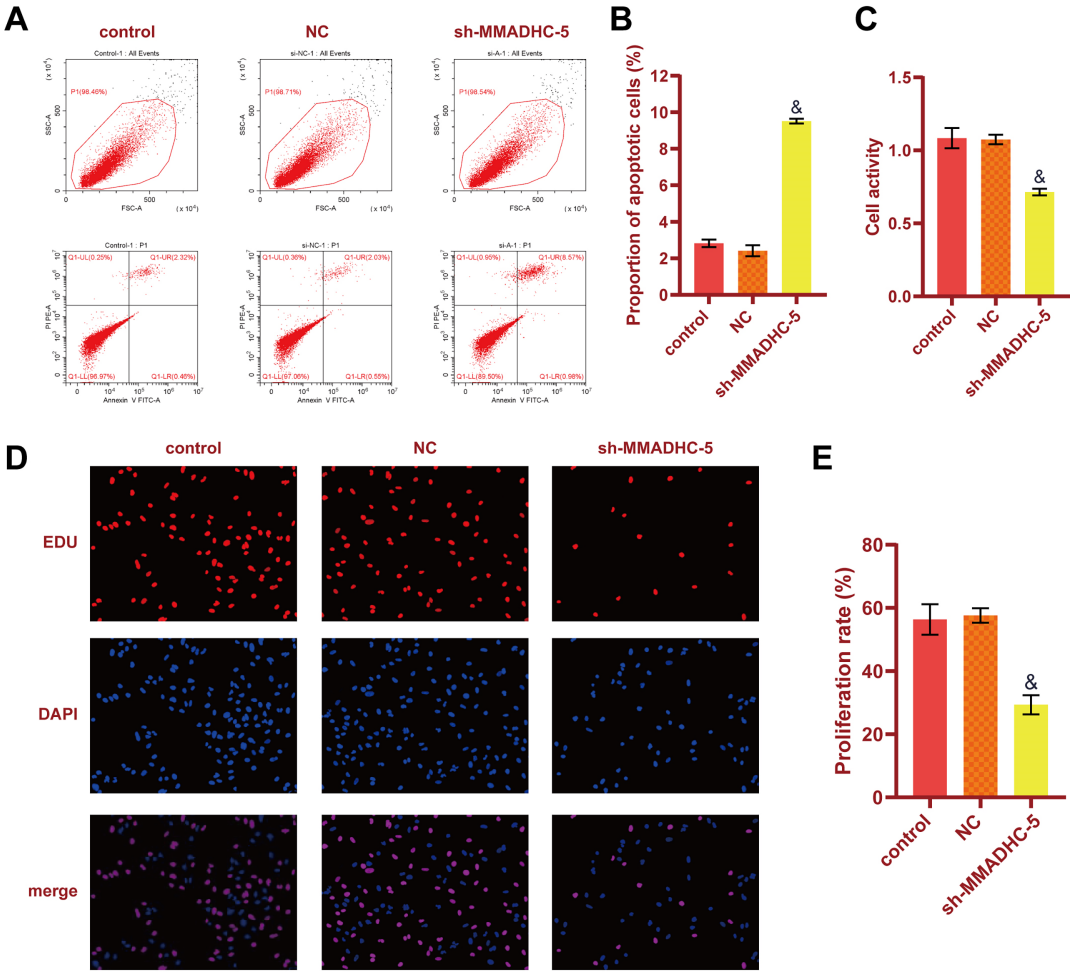

FIG. 5: Lnc-MMADHC-5 regulated the apoptosis and proliferation of HepG2 cells. (A) FCM analyzed the proportion of apoptotic cells. (B) Statistical analysis of (A). (C) CCK-8 measured cell activity. (D) The proliferation rate was detected by EDU assay. (E) Statistical analysis of (D). The magnification is 200 times, scale bar = 50 μm; $^{\&}P < 0.05$ compared with the NC group.

mitosis, cell cycle, arachidonic acid metabolism, cytochrome P450, and P53 signaling pathways. Mutations in cancer cells often cause changes in the cell cycle, leading to unrestricted growth compared to normal cells. Many drugs have been developed to treat cancer by inhibiting different cell cycle phases.[26] Antitumor drugs that target mitosis, such as microtubule poisons, are widely and often successfully used against various cancers.[27] Membrane-bound arachidonic acid was converted to eicosanoid acids in many precancerous lesions, promoting cancer survival, growth, and spread.[28] Cytochrome P450 is a membrane-bound hemoprotein that plays a crucial role in exogenous detoxifying substances, cell metabolism, and homeostasis and plays a leading role in the metabolism of carcinogens and drugs that slow cancer growth.[29] P53 is a tumor suppressor protein that strictly regulates cell growth by promoting apoptosis and DNA repair; mutation can lead to abnormal cell proliferation and tumor progression.[30] As shown in Fig. 3C, we found that most of the proteins in the densest region of the PPI network were related to the cell cycle, such as cyclin-dependent kinase 1 (CDK1) and cyclin B (CCNB). Recent studies have shown that CDK1, CCNB1,

and CCNB2 are prognostic biomarkers associated with immune infiltration in HCC.[31] In other words, lncRNAs differentially expressed in HCC affect HCC cell proliferation, apoptosis, lipid metabolism, and other biological processes by modifying mRNAs and interacting with proteins.

We found that the expression levels of lnc-JAKMIP2-1 and lnc-MMADHC-5 were significantly increased in HCC patients through the analysis of clinical samples, which was consistent with our results bioinformatics analysis (Fig. 1B). A positive correlation between the expression of lnc-MMADHC-5 and TPX2 (Fig. 1C), and TPX2 had an antihepatocellular carcinoma effect by regulating PI3K/AKT signaling pathway.[32] When we knocked down the expression of lnc-MMADHC-5 in HepG2 cells, we observed that the expression levels of TPX2, PI3K, and AKT were also decreased. This result was consistent with the results obtained by our bioinformatics analysis. WB showed that lnc-MMADHC-5 knockdown resulted in increased expression of Bax and caspase3, and decreased expression of Bcl2, PCNA, and Ki67 (Fig. 4C). Tumor cells may use several molecular mechanisms to inhibit apoptosis and acquire resistance to apoptotic agents, for example, through the expression of anti-apoptotic proteins (Bcl2) or the downregulation or mutation of pro-apoptotic proteins (Bax).[33] Matrine can induce apoptosis of various colorectal cancer cell lines by regulating the expression of Bcl2, Bax, and caspase3.[34] PCNA is involved in multiple DNA metabolic processes, including DNA replication and repair, chromatin organization and transcription, and sister chromatid aggregation.[35] Targeting PCNA is an effective strategy to inhibit tumor cell proliferation. The expression of Ki67 is closely related to the proliferation and growth of tumor cells, and it is a promising molecular target in cancer diagnosis.[36] After knockdown of lnc-MMADHC-5, cell function detection revealed increased apoptosis, decreased proliferation, and decreased cell viability of HepG2 cells. In general, lnc-MMADHC-5 can inhibit the growth of HepG2 cells through the TPX2-regulated PI3K/AKT signaling pathway. This result coincided with the results of our previous bioinformatics analysis.

Interestingly, the variation trend of lnc-FBXO42-1 in clinical samples was not the same as that predicted by bioinformatics methods. The lesser samples may cause this difference. This situation also indicates those lncRNAs, the prognostic marker screened by the bioinformatics method in this study, still need many experiments to confirm its universality.

V. CONCLUSION

In this study, the differential expression of lncRNAs in HCC and their related biological processes were revealed through the bioinformatics method, which was helpful to promote people's understanding of the pathogenesis of HCC. At the same time, we screened four lncRNAs, which may become new prognostic markers of HCC. Through tissue and cell experiments, we demonstrated that lnc-MMADHC-5 inhibited the development of HCC. It is the first time lnc-MMADHC-5 is involved in the development of cancer.

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All experiments had been approved by Ethics Committee for Human Studies at Hunan Cancer Hospital (No. KYJJ-2020-095).

The data that support the findings of this study are available from the corresponding author.

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