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ORIGINAL RESEARCH

RETRACTED ARTICLE: Circular RNA Gprc5a Promotes HCC Progression by Activating YAP1/TEAD1 Signalling Pathway by Sponging miR-1283

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Background: Circular RNA (circRNA) plays a critical role in tumorigenesis and tumor progression. Many studies indicate that circRNA Gprc5a is significantly upregulated and functions as an oncogene in a variety of cancers. However, the molecular mechanism of circGprc5a in liver cancer remains unclear.

Methods: qRT-PCR was used to measure the expression levels of circGprc5a, miR-1283, YAP1 and TEAD1 mRNA in hepatocellular carcinoma (HCC) tissues or cells. YAP1 and TEAD1 protein levels were detected by Western blot. CCK-8 assay, cell colony formation, BrdU incorporation and Annexin V-FITC/PI assays were performed to analyze the effects of circGprc5a and miR-1283 on cell proliferation and apoptosis. The relationship between circGprc5a, miR-1283, YAP1 and TEAD1 was analyzed using bioinformatic analysis and luciferase. The tumor changes in mice were detected by in vivo experiments.

Results: CircGprc5a was highly expressed in liver cancer, and closely related poor survival of patients with liver cancer. Knockout of circGprc5a inhibited proliferation of HCC and induced apoptosis. CircGprc5a activated the YAP1/TEAD1 signaling pathway by acting as a sponge for miR-1283. Furthermore, overexpression of miR-1283 abolished the promotion of circGprc5a on HCC cells. Therefore, miR-1283 expression correlated negatively with circGprc5a expression yet positively with the expression of YAP1/TEAD1 in liver cancer.

Conclusion: CircGprc5a promoted the development of HCC by inhibiting the expression of miR-1283 and activating the YAP1/TEAD1 signaling pathway.

Keywords: circGprc5a, miR-1283, YAP1/TEAD1, HCC, proliferation

Introduction

Hepatocellular carcinoma (HCC) ranks fifth in the prevalence of cancer.[1,2] In recent years, hepatectomy, chemotherapy, radiation therapy, topical therapy (such as interventional embolization) and liver transplantation are still the main treatment of HCC. However, patients with HCC are prone to recurrence and tolerance to chemotherapy drugs.[3,4] Due to the lack of effective target genes, the development of HCC targeted therapy is limited. Although many research centers worldwide have devoted lots of efforts to study the mechanism of the occurrence, development and metastasis of HCC, the pathogenesis of HCC has not been clarified. Therefore, further researches on the pathogenesis of HCC are urgently needed to provide new ideas for the clinical treatment of liver cancer.

Circular RNAs (circ RNAs) are closed-loop, single-stranded RNAs that were previously considered to be a rare RNA.[5,6] In recent years, high-throughput sequencing has revealed a large number of circs in eukaryotic cells, including humans.[7] For

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example, in all tested cells or tissues, more than 10% of the genes are capable of producing circ RNAs. Moreover, many circular RNAs are highly abundant, with cell-specificity or tissue-specificity.[8] In addition, the expression levels of hundreds of circular RNAs have altered during epithelial-mesenchymal transition in human cells.[9,10] The above findings indicate that these overexpressed circular RNAs are not redundant but functional during the shearing process. In fact, many studies have shown that circular RNA can adsorb microRNAs or bind proteins.[11] The expression changes of circular RNA induce the expression of tumor-related genes, which will affect the occurrence and development of tumors.[12] Studies have explored the role of circular RNA in liver cancer. CircGprc5a (hsa_circ_0025508) is a recently discovered new circ RNAs that have been found to be abnormally expressed in a variety of malignancies.[13] However, the mechanism of circGprc5a in liver cancer is still unclear.

Among epigenetic factors, microRNA (miRNA) plays an important role.[14,15] miRNA is involved in the regulation of multiple cellular biological processes, including cell cycle, migration.[16,17] Researches indicate that the imbalance of miRNA is not only involved in the progression of HCC, but also closely related to the occurrence of HCC.[18,19] Studies have found that miR-339 inhibits the proliferation of HCC by targeting ZNF689.[20] miR-1283 has been confirmed to be abnormally expressed in various tumor tissues, suggesting that it may play a part in tumorigenesis, but its function in liver cancer is still unclear.[21]

The IFNT gene predominantly expresses in ruminant preimplantation embryonic trophoblast cells.[22,23] In addition, studies have shown that most embryonic and extraembryonic tissues during mammalian development express at least one TEAD protein.[24] The TEAD family of proteins binds to the YAP (Yes-associated protein) and participates in the Hippo signaling pathway, regulating cell contact inhibition, controlling organ volume and cancer.[25] Previous studies have confirmed that YAP1/TEAD1 also plays a part in the expanded of cancer.[26] Therefore, it was hypothesized that the circular RNA Gprc5a activated the YAP1/TEAD1 signaling pathway by mutagenizing miR-1283 to promote the progression of liver cancer. The main purpose of this study was to explore the mechanism of action of circular RNA Gprc5a in the regulation of liver cancer.

Materials and Methods

Tissue Sample

A total of 64 HCC samples were obtained from the clinical sample bank of Guangdong Provincial People's Hospital. The collection of human specimens was approved by the Biomedical Ethics Committee of Guangdong Provincial People's Hospital, and all patients signed written consent. Patients enrolled in the radical hepatectomy between August 2015 and August 2017 were pathologically diagnosed as HCC by two senior pathologists. Written informed consents were obtained from all the participants.

Cell Culture, Vector Construction and Transfection

Human HCC cell lines (MHCC97, PLC, Hep1, Huh7, Hep3B and HepG2) and human LO2 normal liver cell lines were obtained from the Cell Center of the Shanghai Institute of Biological Sciences. The human HCC cell line HepG2 was cultured in DMEM supplemented with 10% FBS (Invitrogen), and the remaining cells were cultured in RPMI 1640 medium supplemented with 10% FBS (Invitrogen).

The si-circGprc5a, miR-1283 inhibitor, miR-1283 mimetic and the corresponding control (GenePharma, Shanghai, China) were transfected into cells using Lipofectamine 2000 (Invitrogen, Waltham, MA, USA). The sequence of si-circGprc5a was as follows: 5′-AATGAAAGCTGTGTGCAAATA-3′. The pBLV-CMV-IRES-ZsGreen circGprc5a cDNA lentiviral plasmid was obtained from Genelily BioTech Co., Ltd. (Shanghai, China). Forty-eight hours after transfection, cells were treated with puromycin for 2 weeks. A cell line with stable circGprc5a overexpression was then constructed.

The lentiviral particles of sh-circGprc5a were designed and purchased from GenePharma Co., Ltd. To generate the lenti-viruses, shRNA plasmids were cotransfected into 293T cells along with envelope (VSVG) and packaging (pGag/Pol, pRev) plasmids using lipofectamine 2000 (Invitrogen). Seventy-two hours after transfection, the lenti-viruses were collected and applied to infecting Hep3B. Following infection for 48 h, Hep3B cells were selected with 2.0 μg/mL puromycin (Sigma). Knockdown efficiencies were examined by qRT-PCR.

Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR)

Total RNA in tissues and cells was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA). qRT-PCR was performed using a ViiATM 7 real-time PCR system (Life Technologies, Grand Island, NY). The expression levels of circGprc5a and miR-1283 were calculated by the 2-ΔΔCT method. The expression level of circGprc5a was

normalized to GADPH, while the level of miR-1283 was normalized to U6. qRT-PCR methods were performed with reference.[27] The primer sequences were as follows:

circGprc5a (divergent primer): forward: 5′- CTTTTC TGGGCCAAATCGG -3′;

circGprc5a (divergent primer) reverse: 5′- ACGGG TACCGACGGGTC-3′;

Gprc5a (convergent primer): forward: 5′-ACGTTGT GAGAATCAGGGG-3′;

Gprc5a (convergent primer) reverse: 5′-TTCCAGCTC TTCGTGGTTG-3′.

miR-1283: forward: 5′- GGGAGAUCAGGUUCGG UCAGAG-3′, miR-1283: reverse: 5′- CTGCCTGCATTC CTCTCAGA-3′ YAP1: forward: 5′- CAAAGTGCTTCGT TGGGAAA -3′, YAP1: reverse: 5′- GTTTGCGCGCGCG AC CAAA -3′.

TEAD1: forward: 5′- ATCCAGGGCCACGAAAG GTGGCAATCGG GGTG -3′, TEAD1: reverse: 5′- GGGA AGATCTCATTGTCACTCCTCAGTCGACAA-3′,
GAPDH: forward: 5′-CGCGATGGAGAACCCAGAT-3′, GAPDH: reverse: 5′-GGGCTTGTACCATAGATGAC-3′.

U6: forward: 5′-ATCCGGCAGATGGCTGTTGAC-3′.

U6: reverse: 5′-GGCCGGTACACCATTCCGATTC-3′.

Cell Viability Assay

Cells were seeded in 96-well plates at a density of 50, 0 cells per well. One hundred microliter CCK solution (Liji, Shanghai, China) was added. After incubation 4 h the absorbance at 450 nm was measured a microplate reader (Peiou Instruments, Shanghai, China).

Colony Formation Assay

Cells were plated in 6-well plates and incubated in dmem containing 10% fetal bovine serum. Two weeks later, the cells were fixed in methanol for 30 minutes and stained with 1% crystal violet dye.

BrdU Incorporation Assay

Transfected cells were seeded in 96-well plates at a density of 2000 cells per well. Forty-eight hours after transfection, cell proliferation was analyzed using the BrdU Cell Proliferation Assay Kit (#5213S, Cell Signaling).

Apoptosis Assay

The cells were plated in a 6-well plate at a density of 5 x 10^5 cells/well, and cells were harvested and counted when the cells were grown to logarithmic growth phase. After centrifugation, cells were resuspended by adding 195 μL of Annexin V-FITC binding solution. Five microliter of Annexin V-FITC and ten microliter of propidium iodide staining solution were added to mix. The cells were incubated in the dark for 10–20 min, and then subjected to flow cytometric analysis.

Immunohistochemical Analysis

Immunohistochemical staining was performed on 4-mm thick sections of the paraffin-embedded tissues, according to the streptavi-din-biotinperoxidase complex (SABC) method. After deparaffinization and rehydration, tissue specimens were treated with 3% hydrogen peroxide to block endogenous peroxidase. Normal goat serum was used for blocking non-specific binding for 30 min at 37°C, then the specimens were incubated with rabbit anti-TEAD1 (CST, #12292S; 1:200) antibody, or rabbit anti-YAP1 (CST, #14074S, 1:200) antibody overnight at 4°C. After washing with PBS, the specimens were incubated with a horseradish peroxidase-labeled polymer conjugated anti-mouse secondary antibody (Zymed) for 1h. Subsequently, the specimens were stained with 3, 3-diaminobenzi- dine tetrahydrochloride, and then nuclei were counterstained with 0.1% (w/v) hematoxylin. Finally, the specimens were mounted with neutral balsam.

Xenograft Mouse Model

Male athymic BALB/c nude mice were purchased from the National Experimental Animal Center (Beijing, China). Each mouse was injected subcutaneously with 10^6 Hep3B cells to establish a mouse xenograft model. On day 9, tumor was injected with sh-circGprc5a, miR-1283 inhibitor and negative control (GenePharma). Five weeks later, the mice were euthanized. The method of IHC was previously described. Frozen sections (5 μm) from mouse xenografts were incubated with primary antibody against Ki67. The respective proteins were visualized using NexES automated stainers and the I-View Detection Chemistry system (Ventana Medical Systems, Tucson, AZ). All animal experiments were conducted at the Shanghai Oriental Hospital Animal Experiment Center and followed the Guide to Nursing and Use of Laboratory Animals (Bethesda National Institute of Health, Maryland, USA). All animal protocols were approved by the Shanghai Oriental Hospital Animal Protection and Use Committee.

Luciferase Reporter Gene Assay

The wild type or mutant sequence of circGprc5a and YAP1 or TEAD1 3′ untranslated region (3′-UTR) was cloned into the pmirGLO vector. After 48 h of transfection, luciferase activity was tested by a dual luciferase assay system (Promega Corporation, Fitchburg, WI, USA).

RNA Immunoprecipitation

RNA immunoprecipitation assays were performed by the EZMagna RIP kit (Millipore). Cells were lysed using complete RIP lysis buffer. Cell extracts were incubated with magnetic beads conjugated to anti-Argonaute 2 (AGO2) or control anti-immunoglobulin G (IgG) antibodies for 6 hours. Then, as the protein beads were removed, the RNA was purified by RT-qPCR analysis.

Western Blot

The transfected cells were collected, total protein was extracted, and the protein concentration was quantified using the BCA Protein Assay Kit. It was incubated with anti-YAP1 (1:1000, Amyjet, Wuhan, China), or TEAD1 (1:1000, Amyjet, Wuhan, China) and anti-GAPDH antibodies (1:1000, Amyjet, Wuhan, China) overnight at 4 °C. Then it was incubated with rabbit anti-rabbit secondary antibody (1:1000, Cell Signaling Technology, Boston, MA, USA) for 1 h. Western blot analysis was carried out with reference.[28]

Statistical Methods

The monitoring data were analyzed by SPSS19.0 statistical software. The results of data analysis were represented as mean ± standard deviation (mean ±SD). Multigroup data analysis was founded on one-way ANOVA. LSD test was used for subsequent analysis. $P < 0.05$ indicated the difference was significant.

Results

CircGprc5a Was Up-Expressed in HCC Tissues and Cell Lines

To explore the function of circGprc5a in the progression of liver cancer, the expression level of circGprc5a in liver cancer was first measured by qRT-PCR. As shown in (Figure 1A), the expression level of circGprc5a in HCC tissues (n = 64) was significantly increased contrasted with normal tissues ($P < 0.05$). However, there was no significant difference in Gprc5a mRNA in HCC tissues as compared with the control (Figure 1B). The expression level of circGprc5a in HCC cell lines (MHCC97, PLC, SK-Hep1, Huh7, Hep3B and HepG2) was also significantly increased ($P < 0.05$) contrasted with LO2 normal liver cell lines (Figure 1C). The best performing HepG2 and Hep3B cells were selected for further testing. To analyze whether circGprc5a can be a potential target for HCC patients, HCC tissues were divided into two groups based on the average expression level of circGprc5a. As shown in (Figure 1D), the overall survival rate of patients in the circGprc5a high expression group was significantly reduced. As shown in (Table 1), high expression of circGprc5a was associated with tumor size ($P<0.01$) and differentiation stage ($P<0.05$). These data indicated the potential carcinogenic effects of circGprc5a in liver cancer.

Knockdown of CircGprc5a Inhibited HCC Growth

Next the biological function of circGprc5a in HCC was analyzed. As shown in (Figure 2A), siRNA-circGprc5a can significantly reduce the expression level of circGprc5a without affecting its linear isomer. In addition, as shown in (Figure 2B–D), si-circGprc5a was able to effectively inhibit cell proliferation and decrease the number of cell colonies in HepG2 and Hep3B cells as compared with the si-NC group ($P < 0.05$). And si-circGprc5a significantly induced apoptosis (Figure 2E). In summary, silencing circGprc5a inhibited HCC cell growth and induced apoptosis.

CircGprc5a Serves as a Sponge of miR-1283

It was found that miR-1283 had a sequence complementary to circGprc5a by searching the network tool Circular RNA Interactome (Figure 3A). Luciferase reporter gene assay showed that luciferase activity was significantly decreased in cells co-transfected with miR-1283 and circGprc5a-WT ($P < 0.05$), but luciferase activity of circGprc5a-MUT did not change significantly (Figure 3B). RIP analysis was performed by anti-Ago2 in cell extracts, as shown in (Figure 3C), circGprc5a and miR-1283 were preferentially enriched in miRNPs containing Ago2 contrasted with anti-IgG immunoprecipitation. As shown in (Figure 3D), si-circGprc5a significantly raised the expression level of miR-1283 contrasted with the si-NC group ($P < 0.05$). Furthermore, miR-1283 expression levels were significantly reduced in liver cancer tissues contrasted with normal tissues ($P < 0.01$, Figure 3E). In addition, there was a negative correlation ($p < 0.01$) between circGprc5a and miR-1283 in liver cancer tissues (Figure 3F). In summary, circGprc5a may directly target miR-1283 in HCC.

miR-1283 Knockdown Effectively Reversed Si-circGprc5a-Induced Inhibition of HCC Progression

We next investigated whether circGprc5a regulated HCC progression through miR-1283. The results were shown in (Figure 4). Contrasted with the si-NC group, si-circGprc5a inhibited the proliferation of HCC cells, but the miR-1283

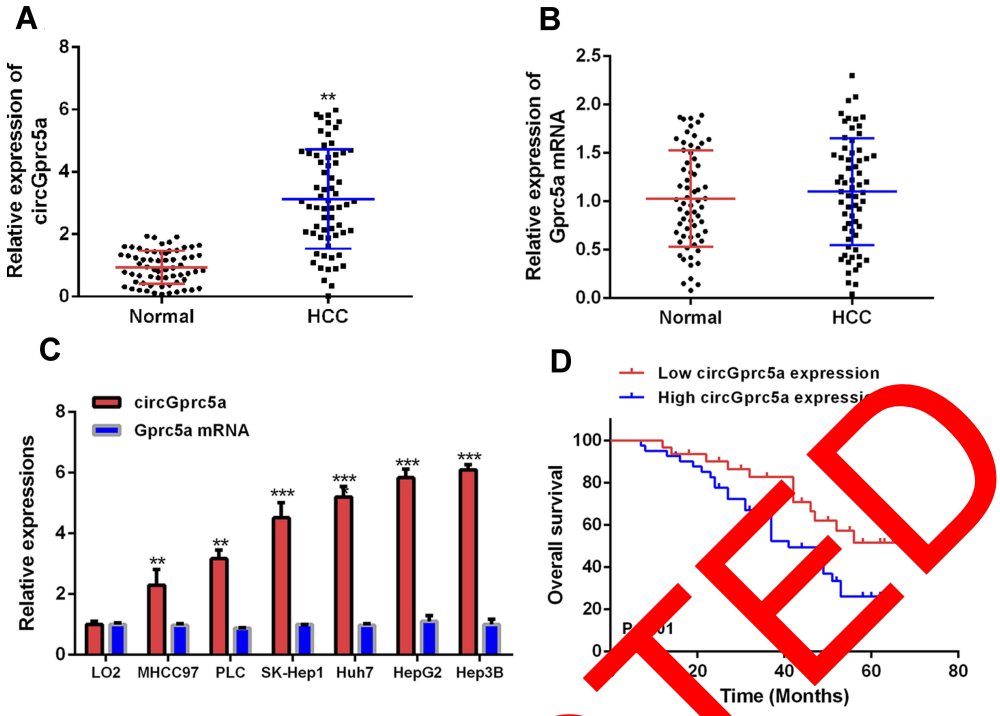

Figure 1 CircGprc5a expression was up-regulated in HCC tissues and cell line. (**A**) The expression level of circGprc5a was detected in HCC tissues and paired normal tissues. (**B**) The expression level of Gprc5a mRNA was detected in liver cancer tissues and paired normal tissues. (**C**) CircRNA Gprc5a mRNA expression levels in HCC cell lines and L02 cells. (**D**) Kaplan–Meier survival analysis of circGprc5a expression.**P <0.01, ***P <0.001.

inhibitor group increased cell proliferation (P < 0.05). Co-transfection of si-circGprc5a with miR-1283 inhibitor eliminated the effect of si-circGprc5a on cell proliferation (P < 0.05). As shown in (Figure 2D), si-circGprc5a induced apoptosis of HCC cells, however miR-1283 inhibitor group inhibited apoptosis (P < 0.05). What's more, Co-transfection of si-circGprc5a with miR-1283 inhibitor eliminated apoptosis of si-circGprc5a (P < 0.05). In summary, circGprc5a exerted a biological effect on HCC cells through miR-1283.

CircGprc5a Sponged and Sequestered miR-1283 to Upregulate YAP1/TEAD1 Expression

Targetscan was used to search for potential targets for miR-1283 (Table S1), both YAP1 and TEAD1 were predicted to be potential targets for miR-1283 (Figure 5A). The total potential targets were shown in the table of supplementary material. Luciferase reporter gene assay showed that miR-1283 overexpression significantly reduced luciferase activity of YAP1 and TEAD1 wild-type vectors, but there was no significant change in luciferase activity of YAP1-MUT and TEAD1-MUT (Figure 5B). In addition, the expression levels of YAP1 and TEAD1 were significantly reduced in the miR-1283 overexpression group, while the expression levels of YAP1 and TEAD1 were significantly raised in the circGprc5a group (P < 0.05). Co-transfection of miR-1283 with circGprc5a reversed this change (P < 0.05, Figure 5C). And the expression levels of YAP1 and TEAD1 in HCC tissues were significantly down-regulated as compared with normal tissues (P < 0.01, Figure 5D). In the liver cancer tissues, the expression levels of YAP1 and TEAD1 were negatively correlated with miR-1283 expression (P < 0.001, Figure 5E). These data indicated that circGprc5a enhanced YAP1/TEAD1 expression levels by acting as a cavern of miR-1283 in HCC.

In vivo Verification of Influence of CircGprc5a/miR-1283/YAP1/TEAD1 on HCC Cell Growth

Finally, the effect of circGprc5a on HCC progression in vivo was analyzed. The results showed that sh-circGprc5a inhibited tumor weight and tumor volume contrasted with si-NC group, miR-1283 inhibitor group increased tumor weight and tumor volume (P < 0.05). Co-transfection of sh-circGprc5a with miR-1283 inhibitor reversed the effect of si-circGprc5a on tumor weight and volume (P < 0.05, Figure 6A–C). Furthermore, as shown in (Figure 6D), sh-circGprc5a inhibited the protein

Table 1 Correlation Between CircGprc5a Expression and Clinical Pathological Characteristic in HCC (N= 64)

Parameters	Group	n	CircGprc5a Expression		P value
			High (n=32)	Low (n=32)	
Age (years)	≤60 >60	24 40	13 19	11 21	0.520
Gender	Female Male	18 46	8 24	10 22	0.344
Cirrhosis	Positive Negative	21 43	13 19	8 24	0.126
AFP (ng/mL)	≤400 >400	31 33	14 18	17 15	0.381
Tumor size (cm)	≥5 <5	36 28	27 5	9 23	0.003**
Intrahepatic metastasis	Positive Negative	17 47	12 20	5 27	0.082
Extrahepatic metastasis	Positive Negative	15 49	9 23	6 26	0.104
BCLC stage	A B C	10 35 19	4 16 12	6 19 7	0.065
Differentiation	Well-moderate Moderate to low	44 20	12 15	27 5	0.013*

Notes: Pearson's χ^2 tests were used for analysis of associat between circGprc5a expression and clinical pathological characteristic. *Indicates significant difference. *P<0.05, **P<0.01.

expression levels of YAP1 and TEAD1 ($P < 0.01$), but the miR-1283 inhibitor group reversed the protein expression levels of YAP1 and TEAD1 ($P < 0.01$). Co-transfection of sh-circGprc5a with miR-1283 inhibitor reversed the effect of si-circGprc5a on the protein expression levels of YAP1 and TEAD1 ($P < 0.05$). Figure 6E showed the expression level of YAP1 and TEAD1 demonstrated by representative immunohistochemical staining of each group's tissues, which was in line with the result of (Figure 6D). The quantitative score of immunohistochemical staining was shown in (Figure 6). These data indicated that overexpression of circGprc5a promoted HCC progression by miR-1283/YAP1/TEAD1 axis.

Discussion

Liver cancer can be divided into primary and secondary types, of which primary is more common.[29] Primary liver cancer can be divided into hepatocellular carcinoma (HCC), cholangiocarcinoma (ICC) and mixed cells cancer according to cell type.[30,31] Studies have shown that in the comprehensive statistics of 36 common cancers in 185 countries around the world, 4.7% of the incidence of liver cancer ranks the sixth, and 8.2% of the death rate ranks the fourth.[32] Characterized by high incidence and poor prognosis, liver cancer is related to various factors such as hepatitis B virus alcohol, aflatoxin, liver cirrhosis, parasitic diseases, and other viral hepatitis. Among these factors, hepatitis B virus is the vital one.[33] Although the current treatment for liver cancer is not limited to radiotherapy, chemotherapy and traditional surgery, there are many treatments such as radiofrequency ablation, alcohol ablation, and intervention. In recent years, with the rise of treatments such as targeting and immunotherapy, a series of studies has been carried out on the treatment of cancer.[34] However, for liver cancer, there is still no effective therapeutic drug. This suggests that the research on the biomolecular mechanism of liver cancer is more important, and it can be opened from the mechanism and molecular direction to open up the understanding of liver cancer. Moreover, the molecular mechanism of studying primary liver cancer will provide a basis for revealing its various biological behaviors, and it can provide important information and support for its diagnosis, clinical treatment and prognosis evaluation.

In the study of these new molecular mechanisms, the role of circular RNA has attracted widespread attention.[35] As an important part of non-coding RNA, Circular RNA has attracted more and more attention from scholars in recent years due to its unique structure and potential function. CircRNA may play a role in sponge adsorption of miRNAs that prevent translation of target genes by regulating splice sites to affect gene expression transcription or interaction with RNA-binding proteins.[36,37] Studies have shown that circular RNA plays a role in biological processes. Importantly, certain circular RNAs are expressed in tumor tissues with specificity.[38] The role of circular RNA in the development of HCC has also been reported.[39] For example, the circ-MTO1 is significantly down-regulated in hepatocellular carcinoma, and its low-expression patients have significantly shorter survival and can adsorb miRNA-9. It inhibits important physiological functions such as proliferation and invasion of hepatocellular carcinoma.[40] circGprc5a is a recently discovered circular RNA, and it is found that circGprc5a is abnormally expressed in various cancers. For example, studies have found that circGprc5a can inhibit the development of bladder cancer.[41] There is currently no research on circGprc5a in liver cancer. This study found

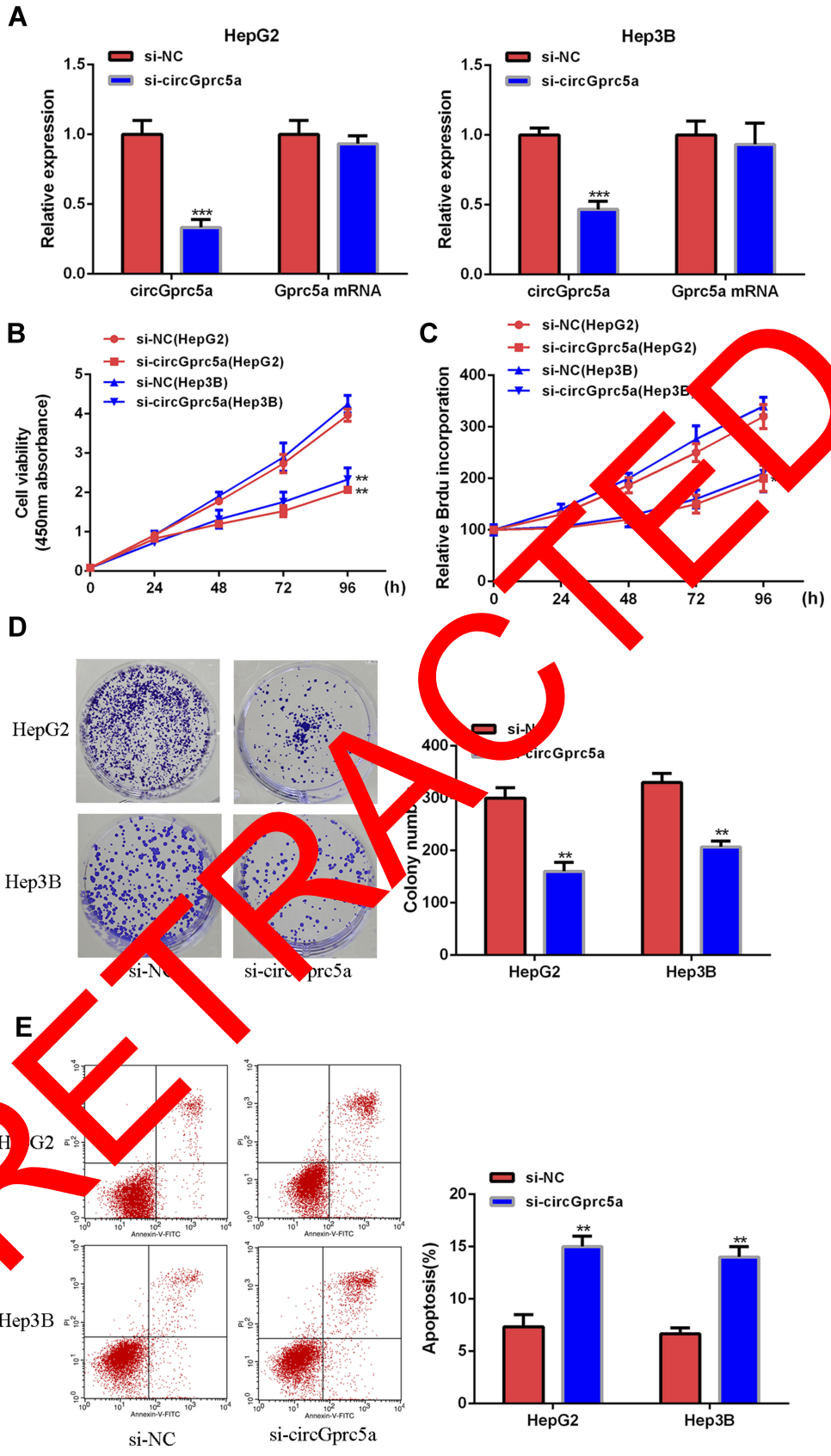

Figure 2 CircGprc5a knockdown inhibited the growth of HCC cells. (**A**) CircGprc5a mRNA expression levels in HepG2 and Hep3B cells. (**B, C**) CCK8 assay, BrdU incorporation assay to analyze the effect of circGprc5a on cell proliferation. (**D**) Colony formation test. (**E**) The effect of circGprc5a on apoptosis was determined by flow cytometry.**p <0.01 and ***p <0.001.

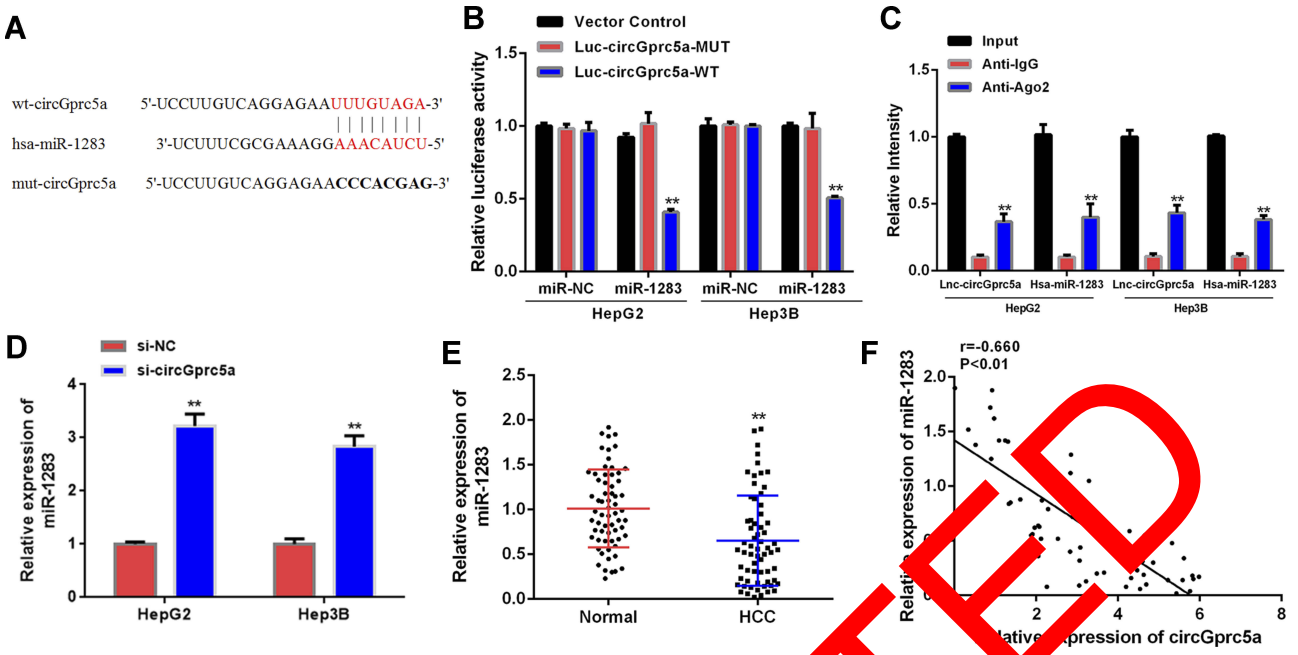

Figure 3 CircGprc5a directly targeted miR-1283 in HCC. (**A**) Putative targeting sites for circGprc5a and miR-1283. (**B**) Analysis of luciferase activity in HCC cells co-transfected with circGprc5a-WT or circGprc5a-Mut vector. (**C**) Immunoprecipitation (RIP) assay for the degree of enrichment of circGprc5a and miR-1283 RNA. Anti-immunoglobulin G (IgG) was used as a control.**P <0.01. (**D**) Expression level of miR-1283 in HCC cells knocked down with circGprc5a. (**E**) Expression levels of miR-1283 in HCC tissues and adjacent normal tissues (n = 64). (**F**) Pearson correlation analysis of circGprc5a and miR-1283 in liver cancer tissues (n = 64) (r = −0.660, P <0.01). **P <0.01.

that the expression level of circGprc5a was increased in HCC. Moreover, the high expression of circGprc5a was significantly associated with tumor size and differentiation, stage and overall survival rate. Si-circGprc5a was effective in inhibiting cell proliferation. In vivo experiments showed that si-circGprc5a effectively inhibited the weight and volume of tumors in mice. Therefore, circGprc5a can be used as an oncogene to control the development of liver cancer by inhibiting its expression.

Circular RNA regulates protein translation and cellular activity by modulating miRNA.[42] At present, miRNA can play a role as a protooncogene or a tumor suppressor gene in tumors.[43] miRNA can also participate in a variety of biological functions such as tumor growth, apoptosis, invasion, migration, and differentiation. Therefore, the study of mi RNA is more helpful in clarifying the mechanism of tumor development and development, and even provides a basis for judging the prognosis and the choice of treatment options for tumors.[44] These are of great significance for tumor-related research and clinicians. For HCC, there have been many reports on miRNA and HCC in recent years. Abnormal expression of miRNA can promote or inhibit the development of HCC. These studies also demonstrate that miRNA is involved in the mechanism and regulation of HCC.[45] For example, studies have found that miR-543 is highly expressed in hepatocarcinoma tissues.[46] The expression level of miR-181-5p in liver cancer tissues is lower than that in normal liver tissues.[47] miR-1283 is a miRNA with tumor growth inhibition found in recent years. For example, studies have found that miR-1283 can inhibit the proliferation of glioma.[21] We screened the target gene of miR-1283 as circGprc5a by database. Down-regulation of circGprc5a may result in an increase in miR-1283 expression. In addition, the expression of miR-1283 in liver cancer tissues was reduced. In liver cancer tissues, there was a negative correlation between circGprc5a and miR-1283. The miR-1283 inhibitor group can increase cell proliferation, inhibit apoptosis, and increase tumor volume and weight. Co-transfection of si-circGprc5a with miR-1283 inhibitor reversed the effect of si-circGprc5a on proliferation, apoptosis and tumor weight volume. These results indicated that circGprc5a can promote the growth of liver cancer by modulating miR-1283.

Researches indicate that miRNA participates in the process of tumor formation and progression during tumor formation, and participates in a regulatory factor.[48] As an critical regulator of cell apoptosis,[49] Hippo pathway is closely related to the occurrence of tumors.[50] YAP1 is one of the key proteins of the Hippo pathway, and YAP1 phosphorylation in the cytoplasm undergoes ubiquitin-dependent degradation, which reduces the interaction with the Sd homologous protein and the transcription enhancer TEF-1 (TEAD1). Mutations in related

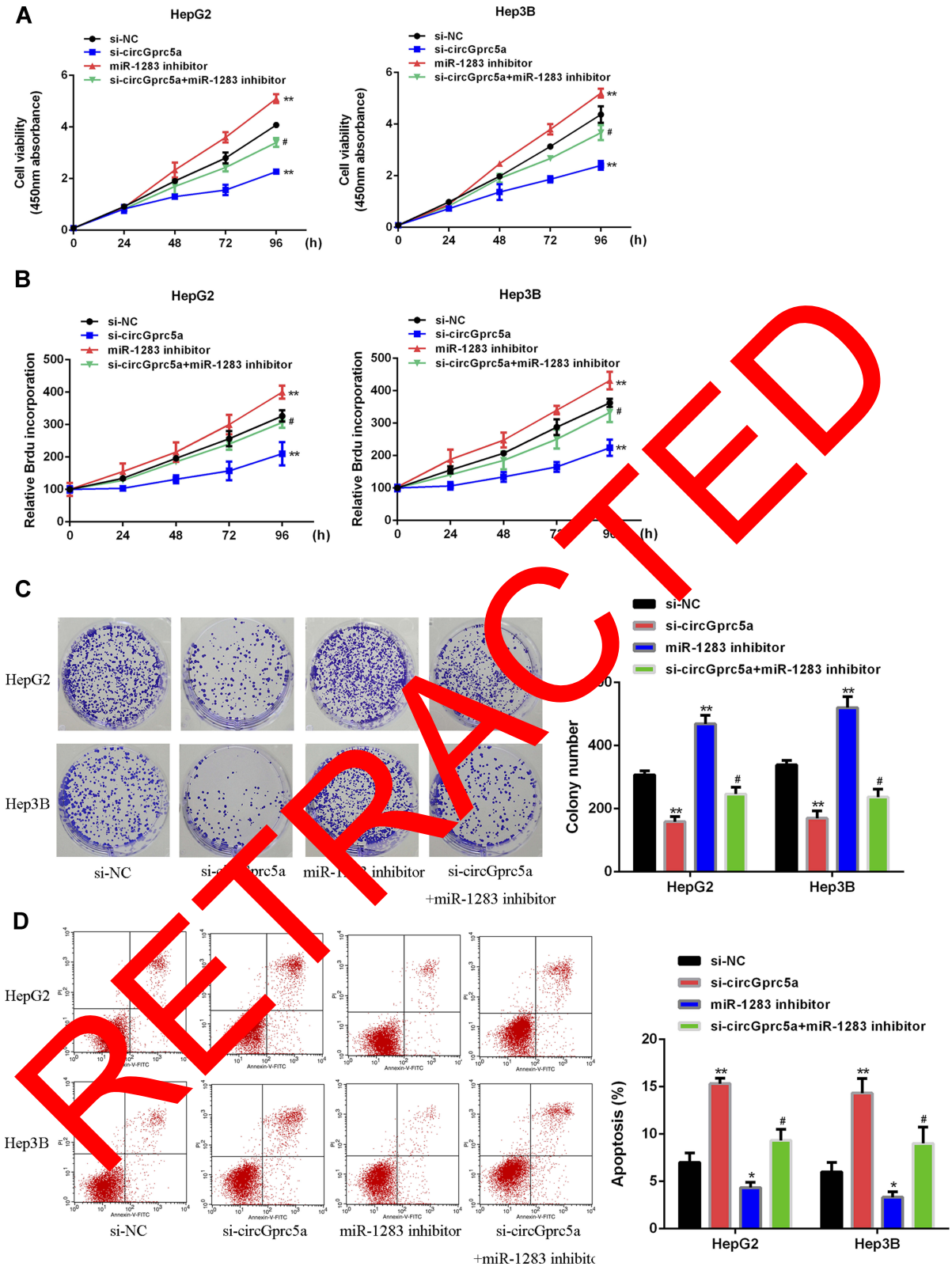

Figure 4 miR-1283 knockdown reversed the enhanced HCC progression of circGprc5a. (**A**) CCK-8 analysis of HepG2 and Hep3B cells co-transfected with circGprc5a siRNA or miR-1283 inhibitor. (**B**) BrdU incorporation assay of HepG2 and Hep3B cells co-transfected with circGprc5a siRNA or miR-1283 inhibitor. (**C**) Colony formation assay of HepG2 and Hep3B cells co-transfected with circGprc5a siRNA or miR-1283 inhibitor. (**D**) Flow cytometric analysis of HepG2 and Hep3B cells co-transfected with circGprc5a siRNA or miR-1283 inhibitor. Scale bar = 20 μm. * vs-NC group (P<0.05),** vs si-NC group (P <0.01), #vs si-circGprc5a group (P <0.05).

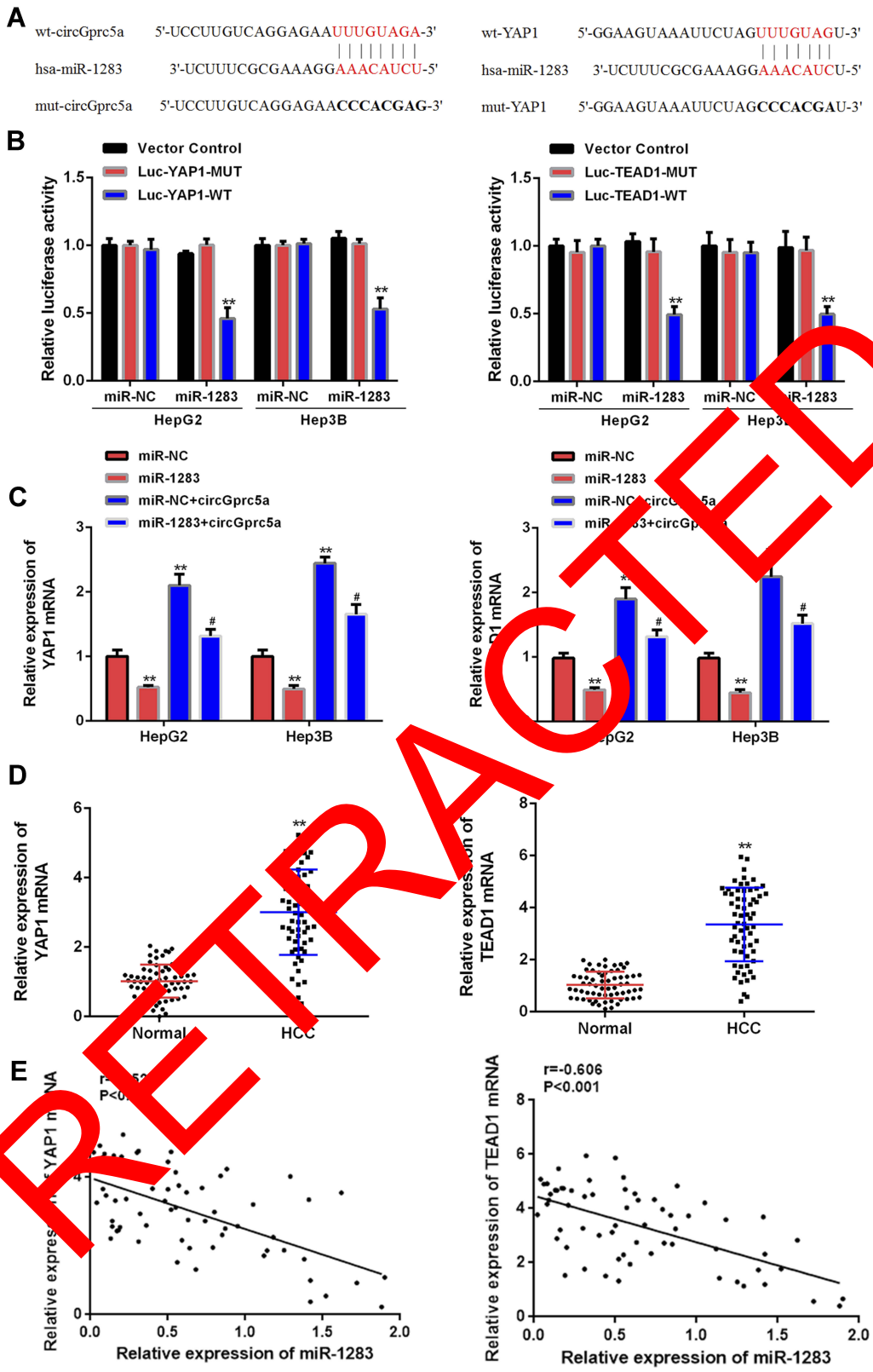

Figure 5 CircGprc5a up-regulated YAP1 and TEAD1 expression levels by muting miR-1283. (**A**) Putative binding sites for miR-1283 and YAP1 or TEAD1 3′-UTR. (**B**) Analysis of luciferase activity in cells co-transfected with miR-1283 mimetic and YAP1/TEAD1 3′-UTR-WT or YAP1/TEAD1 3′-UTR-Mut vector. (**C**) YAP1/TEAD1 mRNA expression levels in cells co-transfected with miR-1283 mimetic and circGprc5a vector. (**D**) Expression levels of YAP1 and TEAD1 in liver cancer tissues. (**E**) Pearson correlation analysis of miR-1283 and YAP1/TEAD1 mRNA in liver cancer tissues (n = 64). ** vs control group (P <0.01), #vs circGprc5a group (P <0.05).

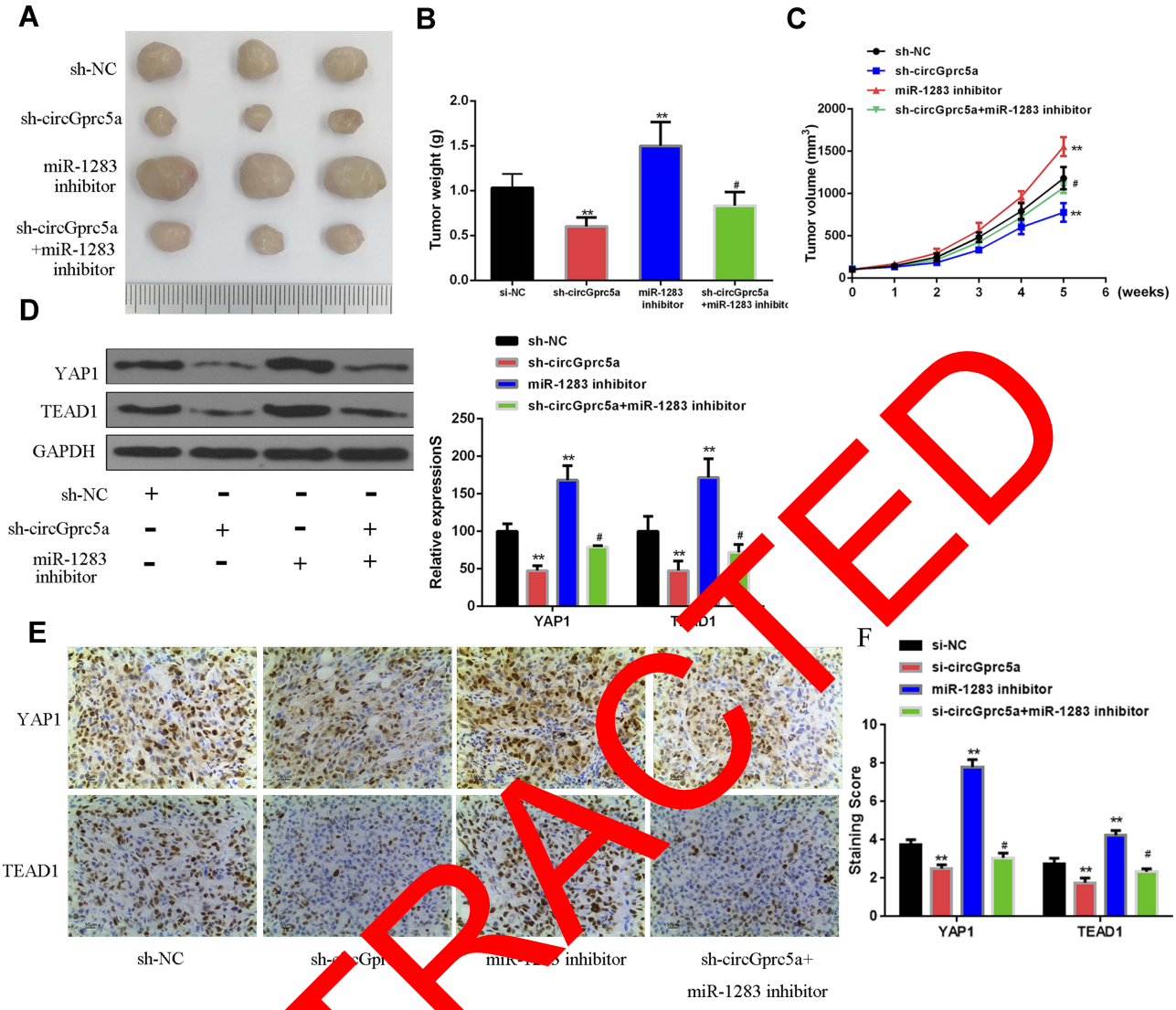

Figure 6 CircGprc5a overexpression inhibited HCC growth by modulating the miR-1283/YAP1/TEAD1 axis. (**A**) Representative images of three groups of subcutaneous tumors. (**B**) The tumor volume of nude mice was measured weekly. (**C**) The tumor weight of nude mice was measured on the last day. (**D**) Protein expression levels of YAP1/TEAD1. (**E**) The expression level of YAP1 and TEAD1 demonstrated by representative immunohistochemical staining of each group's tissues. (**F**) The quantitative score of immunohistochemical staining. Relative to sh-circGprc5a #P <0.05, relative to si-NC **P <0.01.

proteins in the Hippo pathway result in a high activation of YAP1 and TEAD1, which causes abnormal cell proliferation.[51] This study found that YAP1 and TEAD1 were target genes of miR-1283. Overexpression of miR-1283 reduced the expression levels of YAP1 and TEAD1, while ectopic expression of circGprc5a reversed this change. The expression of YAP1 and TEAD1 in HCC tissues was significantly down-regulated. In addition, the expression of YAP1 and TEAD1 mRNA was negatively correlated with miR-1283 expression in HCC tissues. Furthermore, knockdown of circGprc5a inhibited the expression of YAP1/TEAD1. The miR-1283 inhibitor upregulated the protein expression levels of YAP1 and TEAD1, and the co-transfection of si-circGprc5a with miR-1283 inhibitor reversed the effect of si-circGprc5a on the protein expression levels of YAP1 and TEAD1. These data indicated that overexpression of circGprc5a inhibited HCC progression by miR-1283/YAP1/TEAD1 axis.

Conclusion

Overexpression of circGprc5a promoted HCC progression by miR-1283/YAP1/TEAD1 axis, suggesting that circGprc5a can be a potential oncogene for liver cancer.

Ethical Approval

This study was approved by Biomedical Ethics Committee of Guangdong Provincial People's Hospital. All procedures

performed in participants were in accordance with the ethical standards of the Declaration of Helsinki. Written informed consents were obtained from all participants.

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Disclosure

The authors declare that they have no competing interests.

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