# Immature colon carcinoma transcript-1 promotes cell growth of hepatocellular carcinoma via facilitating cell cycle progression and apoptosis resistance

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**Abstract.** Immature colon carcinoma transcript-1 (ICT1) is a newly identified oncogene, which regulates proliferation, cell cycle progression and apoptosis of cancer cells. However, the clinical significance, biological function and underlying mechanisms of ICT1 in hepatocellular carcinoma (HCC) remain poorly known. In the present study, we showed that the expression of ICT1 in HCC tissues were notably overexpressed compared to corresponding non-tumor tissues. Accordingly, the relative levels of ICT1 were upregulated in HCC cell lines compared with LO2 cells. The positive expression of ICT1 was correlated with large tumor size and advanced TNM tumor stage. Kaplan-Meier plots indicated that ICT1-positive expression in HCC patients showed a prominent shorter survival. In addition, ICT1 knockdown inhibited proliferation and cell cycle progression, and induced apoptosis in HepG2 cells. While, ICT1 overexpression showed opposite effects on these cellular processes of Hep3B cells. *In vivo* experiments demonstrated that ICT1 deficiency reduced the growth of subcutaneous HCC in nude mice. Notably, ICT1 knockdown reduced the levels of CDK1, cyclin B1 and Bcl-2 and increased the expression of Bax in HepG2 cells. ICT1 overexpression resulted in upregulation of CDK1, cyclin B1 and Bcl-2, and downregulation of Bax in Hep3B cells. Furthermore, microRNA-134 (miR-134) was recognized as a direct upstream regulator and inversely modulated ICT1 abundance in HCC cells. Altogether, our data support that miR-134 regulation of ICT1 facilitates malignant phenotype of HCC cells probably via cell cycle and apoptosis-associated proteins including CDK1, cyclin B1, Bcl-2 and Bax.

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## Introduction

Hepatocellular carcinoma (HCC), one of the leading causes for cancer-related deaths, is the most aggressive and lethal of liver tumors (1). During the last several decades, remarkable advances have been made regarding the diagnosis and treatment of HCC (2). However, the long-term survival of HCC patients is still poor (3). The exact mechanisms responsible for the malignant growth and metastatic behaviors of HCC cells remain largely uncovered. Thus, it is worth investigating the novel oncogene, which plays an essential role in the initiation and progression of HCC.

Immature colon carcinoma transcript-1 (ICT1), previously named DS-1, is identified as a regulator during colorectal cancer (CRC) cell differentiation in the study by van Belzen *et al* (4). Further studies report that ICT1 plays an important role in the progression of CRC, lung, prostate and breast cancer, and that it has been suggested as a potential biomarker (5-10). In prostate cancer, ICT1 knockdown suppresses cancer cell proliferation and viability, leading to cell cycle arrest at G2/M phase and apoptosis (7). ICT1 is overexpressed in CRC tissues and associates with poor prognosis of patients (8). Furthermore, ICT1 promotes CRC progression by regulating cell cycle, migration and apoptosis of cancer cells (8). Similarly, the study by Wang *et al* (9) report that ICT1 acts as a potential biomarker for diagnosis and treatment of lung cancer and facilitates non-small cell lung cancer cell proliferation via promoting cell cycle progression and inhibiting apoptosis. Recently, Wang *et al* (10) showed that ICT1 silencing restrains cell growth of breast cancer by inducing cell cycle arrest and apoptosis. These above studies suggest that ICT1 functions as an oncogene in different cancer types. The present study aimed to investigate the clinical significance, the biological function and the underlying mechanisms of ICT1 in HCC.

This study demonstrates that ICT1 overexpression correlates with malignant clinical features and poor prognosis of HCC patients. Functionally, ICT1 promotes proliferation, cell cycle progression and inhibits apoptosis of HCC cells. ICT1 regulates the expressions of CDK1, cyclin B1, Bcl-2 and Bax, and is directly targeted by microRNA-134 (miR-134) in HCC cells. In conclusion, the present study supports the first evidence that ICT1 is a potential prognostic biomarker and therapeutic target for HCC.

## Materials and methods

*Patients*. Sixty-eight HCC tissues and matched tumor-adjacent tissues were obtained from the The First Affiliated Hospital of Xi'an Medical University. Tissue specimens were conserved in 10% formalin for further investigation. No patients received immunotherapy, radiotherapy or chemotherapy before surgery. Tumor staging was based on the seventh Union for International Cancer Control/American Joint Committee on Cancer (UICC/AJCC) staging system (11). All samples were used after obtaining informed consent. The Ethics Committee of The First Affiliated Hospital of Xi'an Medical University approved the protocols according to the Declaration of Helsinki.

*Cell culture and transfection*. The human immortalized normal hepatocyte cell line (LO2) and human HCC cell lines including Hep3B, MHCC97L, MHCC97H and HCCLM3 were obtained from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). All cell lines were cultivated in Dulbecco's minimum essential medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) using 10% fetal calf serum (FCS; Gibco, Grand Island, NY, USA) at 37˚C in a humidified atmosphere of 5% $CO_2$.

Precursor miR-134, miR-205 and miR-340, and miR-134 inhibitor as well as corresponding negative control vectors were purchased from GeneCopoeia (Guangzhou, China). ICT1 shRNA, non-targeting (NT) shRNA, ICT1 overexpression vector and empty vector (EV) were synthesized and obtained from Shanghai Genechem Co., Ltd. (Shanghai, China). Vectors were transferred into cells using Lipofectamine 2000 (Thermo Fisher Scientific) in accordance with the manufacturer's protocols.

*Immunohistochemistry (IHC)*. The HCC tissues that were previously formalin-fixed and paraffin-embedded were sliced into 4 $\mu$m sections, and underwent deparaffination and then rehydration. Antigen retrieval, suppression of endogenous peroxidase activity and 10% skim milk blocking were performed before primary antibody incubation. ICT1 (AP20382b; Abgent, Inc., San Diego, CA, USA) and Ki-67 primary antibody (ab15580; Abcam, Cambridge, MA, USA) was used for incubation overnight at 4˚C. The slides were subsequently incubated with peroxidase conjugated secondary antibody (ZSGB Bio, Beijing, China) for 90 min, and a peroxidase-labeled polymer, DAB solution was used for signal development for 5 min. The sections were counterstained with hematoxylin followed by dehydrating and mounting. Staining intensity was scored as no staining, 0, weak staining, 1, moderate staining, 2, and strong staining, 3. Staining quantity was graded as <25%, 1, 25-75%, 2, and >75%, 3. IHC score was manually confirmed by two independent experienced pathologists using the formula: IHC score = staining intensity x staining quantity. IHC score ≥3 was considered as positive expression of ICT1.

*Quantitative real-time PCR*. RNA was isolated using TRIzol (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocols. The first strand cDNA was compounded using a Tianscript RT kit (Tiangen Biotech, Co., Ltd., Beijing, China). PCR amplification for ICT1 mRNA was performed with the SYBR Premix Ex Taq™ kit (Takara Bio, Shiga, Japan) in ABI 7300 system (Applied Biosystems, Foster City, CA, USA). GAPDH was employed as a reference gene to normalize the expression of ICT1 mRNA. The primers used for ICT1 and GAPDH were purchased from Sangon Biotech Co., Ltd. (Shanghai, China).

*Colony formation assay*. For cell proliferation, colony formation assay was performed. HCC cells transfected with corresponding vectors were seeded in 6-well plates and maintained in cell incubators for 14 days. The formed cell colonies were stained with crystal violet solution. The number of cell colonies was counted to represent the cell proliferation ability of HCC cells.

*Cell cycle and apoptosis analysis*. For cell cycle assay, HCC cells were collected 72 h after transfection. These cells were fixed with 80% ethanol overnight, and then were stained with propidium iodide (50 $\mu$g/ml; BD Biosciences, Franklin Lakes, NJ, USA) at room temperature for 30 min. The percentage of cells in each cell cycle was measured with FACSCalibur system (BD Biosciences). For apoptosis assay, HCC cells after transfection were subjected to an apoptosis assay. The percentage of apoptotic HCC cells were measured using Annexin V/propidium iodide kit (BD Biosciences, San Diego, CA, USA) based on the protocol provided by manufactures.

*In vivo tumor growth assay*. For tumor growth studies, nude mice were injected subcutaneously with $1x10^6$ HepG2 cells transfected with ICT1 shRNA or NT shRNA. Tumor sizes were measured every 4 days after subcutaneous injection. Three weeks later, the mice were sacrificed by cervical dislocation under anesthesia, and tumors were removed for the volume measurement. The protocols for animal experiments were approved by the Ethics Committee of The First Affiliated Hospital of Xi'an Medical University.

*Luciferase reporter assay*. Wild-type (wt) or mutant (mt) 3'-UTR of ICT1 was amplified and cloned into pmiR-RB-REPORT™ Luciferase. Then, the 3'-UTR of ICT1 and corresponding miRNA vectors were co-transfected into HepG2 cells, respectively. Forty-eight hours after the co-transfection, the cells were lysed and detected using a Dual-Luciferase® reporter assay kit (Promega, Madison, WI, USA) based on the manufacturer's protocols.

*Western blotting*. Total proteins were collected with RIPA lysis buffer (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), and 40 $\mu$g protein were subjected to 4-20% SDS gel electrophoresis (Sigma-Aldrich) and were then transferred to PVDF membranes (Roche, Indianapolis, IN, USA). Then, 5% milk blocked membranes were incubated with ICT1 (Abgent), CDK1 (ab18; Abcam), cyclin B1 (#12231; Cell Signaling Technology, Beverly, MA, USA), Bax (#5023; Cell Signaling Technology) or Bcl-2 (#15071; Cell Signaling Technology) primary antibody and subsequently incubated with matched secondary antibodies (Cell Signaling Technology). Then, signals for each protein expression were detected with the Bio-Rad Gel imaging system (Bio-Rad Laboratories, Hercules,

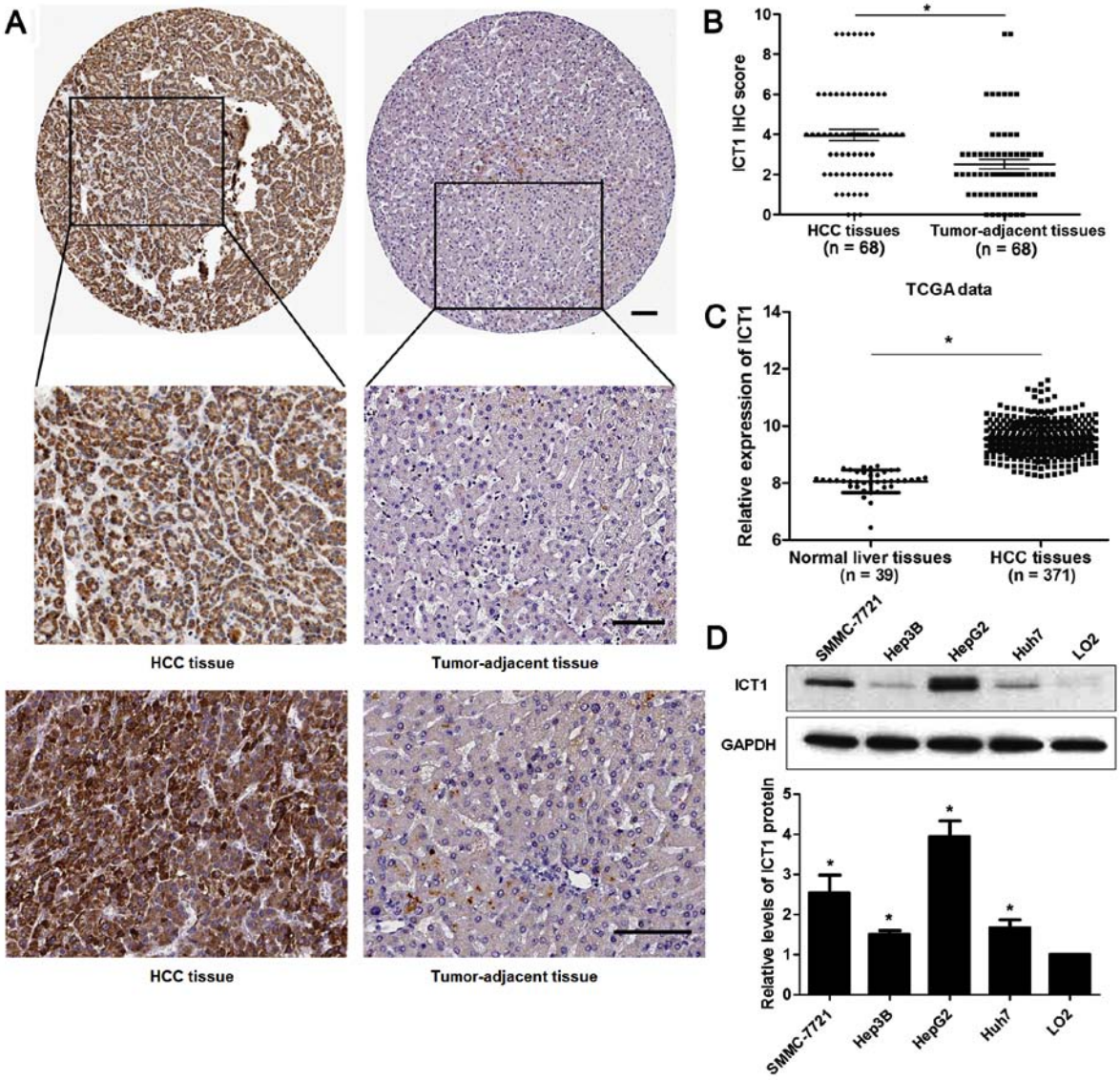


Figure 1. The expression of ICT1 in HCC. (A) Representative IHC staining showed positive expression of ICT1 in HCC tissue and negative expression of ICT1 in tumor-adjacent tissue. Scale bar, 100 $\mu$m. (B) The expression differences of ICT1 between HCC tissues and matched tumor-adjacent tissues. n=68, $^{*}$P<0.05. (C) TCGA data from R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl) showed that the expression of ICT1 is significantly increased in HCC tissues (n=371) compared to normal liver tissues (n=39). $^{*}$P<0.05. (D) Relative expression of ICT1 in HCC cell lines (SMMC-7721, HepG2, Hep3B and Huh7) and the human immortalized normal hepatocyte cell line (LO2) detected by immunoblotting. n=3, $^{*}$P<0.05.

CA, USA). GAPDH (G8140; US Biological, Swampscott, MA, USA) was used as a loading control.

*Statistical analysis*. Data are presented as mean ± SD and analyzed by GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, CA, USA). Chi-squared test was employed to explore the association between two variables. The Student's t-test and analysis of variance (ANOVA) were carried out to analyze continuous variables. Survival curves were constructed and differences among groups were calculated using the Kaplan-Meier method and log-rank test. The value of P<0.05 was considered to have statistical significance.

## Results

*ICT1 is overexpressed in HCC*. IHC staining was performed to detect the expression of ICT1 in 68 pairs of HCC and matched tumor-adjacent tissues. Forty-seven of 68 (69.12%) HCC tissues showed positive expression of ICT1, while ICT1 signal was observed in only 27 of 68 (39.71%) tumor-adjacent tissues (P<0.05; Fig. 1A). Our data indicated that the levels of ICT1 in HCC tissues were significantly higher than those in matched non-cancerous tissues (P<0.05; Fig. 1B). TCGA data from R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl) showed that the expression of ICT1 is significantly upregulated in HCC tissues compared to normal liver tissues (P<0.05; Fig. 1C), which is consistent with our results. In addition, the expression of ICT1 in HCC cell lines (SMMC-7721, Hep3B, HepG2 and Huh7) were notably upregulated compared to LO2 cells (P<0.05, respectively; Fig. 1D). Thus, the expression of ICT1 is restrained in HCC.

*Clinical significance of ICT1 in HCC patients*. Next, we investigated the clinical significance of ICT1 in HCC

Table I. Correlation between the clinicopathological features and ICT1 expression in hepatocellular carcinoma (n=68).

| Characteristics | Total no. of patients | ICT1 expression | | P-value |
|---|---|---|---|---|
| | | Positive (n=47) | Negative (n=21) | |
| Age (years) | | | | 0.723 |
| <50 | 27 | 18 | 9 | |
| ≥50 | 41 | 29 | 12 | |
| Sex | | | | 0.612 |
| Male | 49 | 33 | 16 | |
| Female | 19 | 14 | 5 | |
| HBV | | | | 0.635 |
| Absent | 20 | 13 | 7 | |
| Present | 48 | 34 | 14 | |
| Serum AFP level (ng/ml) | | | | 0.155 |
| <400 | 24 | 14 | 10 | |
| ≥400 | 44 | 33 | 11 | |
| Tumor size (cm) | | | | 0.020[a] |
| <5 | 25 | 13 | 12 | |
| ≥5 | 43 | 34 | 9 | |
| No. of tumor nodules | | | | 0.445 |
| 1 | 54 | 39 | 15 | |
| ≥2 | 14 | 8 | 6 | |
| Cirrhosis | | | | 0.373 |
| Absent | 27 | 17 | 10 | |
| Present | 41 | 30 | 11 | |
| Venous infiltration | | | | 0.880 |
| Absent | 51 | 35 | 16 | |
| Present | 17 | 12 | 5 | |
| Edmondson-Steiner grading | | | | 0.067 |
| I+II | 48 | 30 | 18 | |
| III+IV | 20 | 17 | 3 | |
| TNM tumor stage | | | | 0.004[a] |
| I+II | 49 | 29 | 20 | |
| III+IV | 19 | 18 | 1 | |

HBV, hepatitis B virus; AFP, α-fetoprotein; TNM, tumor-node-metastasis. [a]Statistically significant.

patients. Sixty-eight HCC patients were divided into ICT1 positive expression group (IHC score ≥3) and ICT1 negative expression group (IHC score <3). Clinical association analysis found that the ICT1 positive expression was correlated with larger tumor size and advanced TNM tumor stage (P<0.05, respectively; Table I). Notably, Kaplan-Meier plots indicated that ICT1-positive expression in HCC patients showed a significant reduced overall survival and recurrence-free survival compared to ICT1 negative expression cases (P<0.05, respectively; Fig. 2). These results suggest that ICT1 may act as a promising prognostic marker for HCC patients.

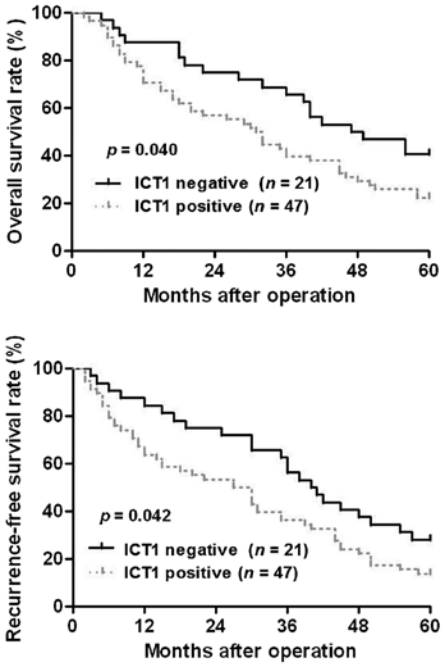


Figure 2. The prognostic significance of ICT1 in HCC. HCC patients were divided into ICT1 positive expression group (n =47, IHC score ≥3) and ICT1 negative expression group (n=21, IHC score <3). ICT1-positive expression of HCC patients showed a significant shorter overall survival and recurrence-free survival compared to ICT1 negatively expressing cases.

*ICT1 regulates proliferation, cell cycle progression and apoptosis of HCC cells*. Next, we investigated the biological function of ICT1 in HCC cells. HepG2 cells showed the highest level of ICT1, while Hep3B cells showed the lowest level of ICT1. Thus, HepG2 and Hep3B were used for loss- and gain-of-function experiments, respectively. Loss-of-function experiments were performed in HepG2 cells after ICT1 knockdown (P<0.05; Fig. 3A). Colony formation assays revealed that ICT1 knockdown prevented proliferation of HepG2 cells (P<0.05; Fig. 3B). In addition, ICT1 silencing resulted in cell cycle arrest at G2/M phase and increased apoptosis in HepG2 cells (P<0.05, respectively; Fig. 3C and D). Then, ICT1 overexpression was confirmed by immunoblotting in Hep3B cells (P<0.05; Fig. 4A). Further experiments disclosed that ICT1 restoration contributed to proliferation and cell cycle progression, and reduced apoptosis of Hep3B cells (P<0.05, respectively; Fig. 4B-D).

*ICT1 knockdown restrains growth of HCC in mice*. HepG2 cells that were transfected with NT shRNA or ICT1 shRNA were implanted into nude mice via subcutaneous injection. Tumor growth curves showed that ICT1 knockdown reduced the volume of subcutaneous tumor nodules in nude mice with HCC (P<0.05; Fig. 5A). Furthermore, Ki-67 staining was performed to detect the proliferative index of HCC *in vivo*. The percentage of Ki-67 positive cancer cells in ICT1 knockdown group was notably reduced compared to control group (P<0.05; Fig. 5B). These results indicate that ICT1 inhibits

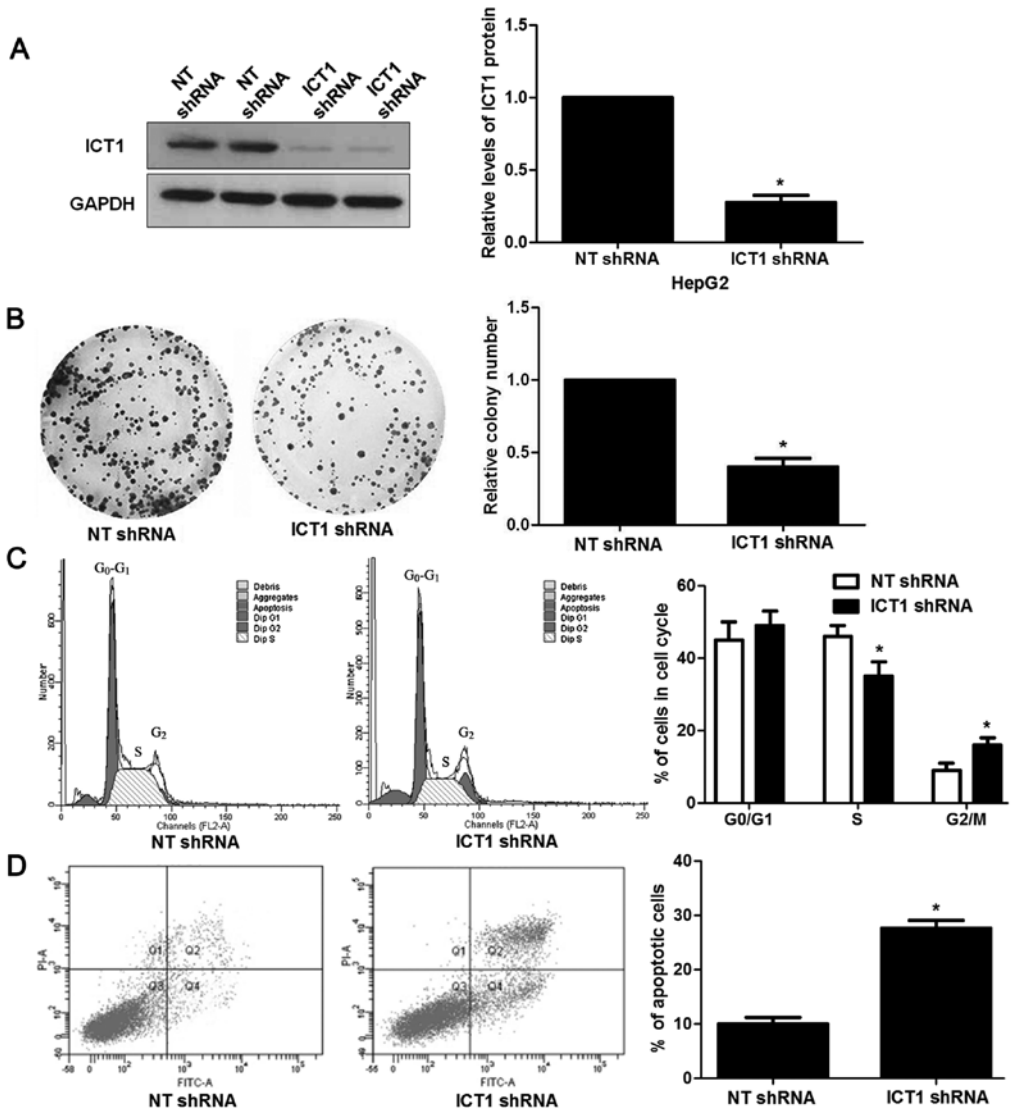


Figure 3. ICT1 knockdown inhibits proliferation and cell cycle progression, and induces apoptosis of HepG2 cells. (A) HepG2 cells that were transfected with ICT1 shRNA and non-targeting (NT) shRNA, respectively, were subjected to immunoblotting for ICT1 expression. n=3, *P<0.05. (B) Colony formation assays indicated that ICT1 knockdown inhibited proliferation of HepG2 cells. n=3, *P<0.05. (C) ICT1 silencing led to cell cycle arrest at G2/M phase in HepG2 cells. n=3, *P<0.05. (D) The percentage of apoptotic cells was notably increased after ICT knockdown in HepG2 cells. n=3, *P<0.05.

the progression of HCC via regulating proliferation, cell cycle progression and apoptosis.

*ICT1 modulates the levels of cell cycle and apoptosis-associated proteins in HCC cells*. Previous studies report that ICT1 regulate cell growth of breast, CRC, prostate and lung cancer by modulating cell cycle and apoptosis-associated proteins including CDK1, cyclin B1, Bax and Bcl-2 (7-10). Thus, western blot analysis was performed to detect the levels of CDK1, cyclin B1, Bax and Bcl-2 after modulating ICT1 expression in HCC cells. ICT1 knockdown reduced the levels of CDK1, cyclin B1 and Bcl-2 and increased Bax expression in HepG2 cells (Fig. 6). In turn, ICT1 overexpression led to upregulation of CDK1, cyclin B1 and Bcl-2 and suppression of Bax in Hep3B cells. Thus, ICT1 regulates the levels of CDK1, cyclin B1, Bax and Bcl-2 in HCC cells.

*ICT1 is a direct target of miR-134 in HCC cells*. The mechanism involved in ICT1 overexpresion in HCC has rarely been investigated. Increasing evidence indicates that miRNAs play a critical role in regulating the expression of oncogenes and tumor suppressors in human cancers (12-14). Thus, we used two publicly available databases (TargetScan and miRanda) to search for the potential miRNAs involved in regulation of ICT1 in HCC. Five miRNAs including miR-134-5p, miR-340-5p, miR-205-5p, miR-543 and miR-758-3p were recognized as candidates. miR-758-3p is rarely investigated and miR-543 is reported to be overexpressed in HCC (15). Thus, we checked the regulatory effects of miR-134, miR-340, miR-205 on ICT1 mRNA expression. Our data indicated that only miR-134 overexpression significantly reduced the level of ICT1 mRNA in HepG2 cells (P<0.05; Fig. 7A). As suggested by Fig. 7B, the complementary sequence of miR-134 was found in the 3'-UTR of ICT1 mRNA. Notably, luciferase reporter assays demonstrated that miR-134 overexpression reduced the luciferase activity of wt but not mt 3'-UTR of ICT1 (P<0.05; Fig. 7C). Next, we found that miR-134 inversely regulated the levels of ICT1 protein in HepG2 and Hep3B cells (Fig. 7D). Hence,

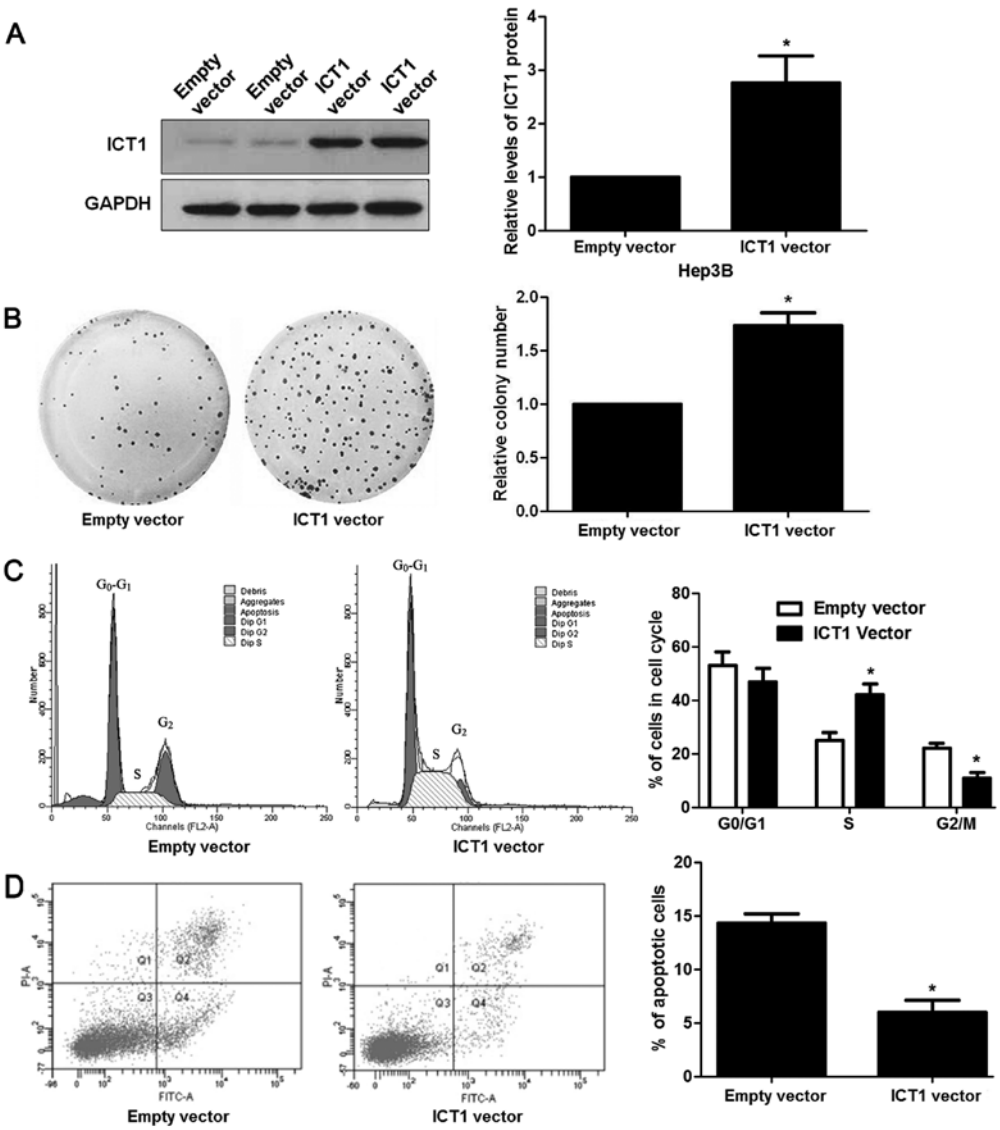


Figure 4. ICT1 restoration results in enhanced proliferation, cell cycle progression and decreased apoptosis of Hep3B cells. (A) Hep3B cells that were transfected with ICT1 vector and empty vector, respectively, were subjected to western blotting for ICT1 expression. n=3, *P<0.05. (B) Colony formation assays indicated that ICT1 overexpression promoted proliferation of Hep3B cells. n=3, *P<0.05. (C) ICT1 restoration facilitated cell cycle progression in Hep3B cells. n=3, *P<0.05. (D) The percentage of apoptotic cells was notably decreased after ICT1 overexpression in Hep3B cells. n=3, *P<0.05.

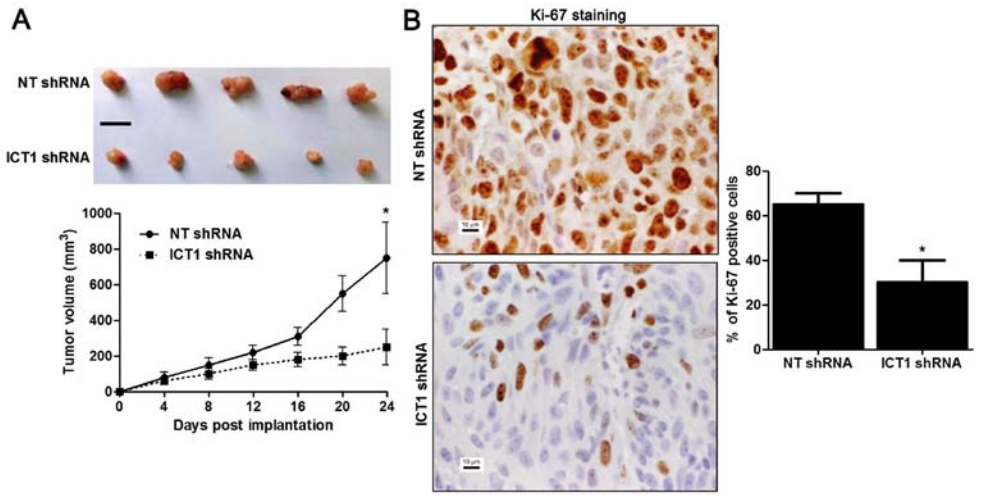


Figure 5. ICT1 knockdown suppresses tumor growth of HCC in nude mice. (A) HepG2 cells that were transfected with ICT1 shRNA or non-targeting (NT) shRNA were subcutaneously injected to the flank of nude mice. Tumor growth curves revealed that ICT1 knockdown significantly reduced HepG2 cell growth *in vivo*. n=5, *P<0.05. (B) The percentage of Ki-67-positive cells in ICT1 knockdown group was notably lower than that in control group. n=5, *P<0.05.

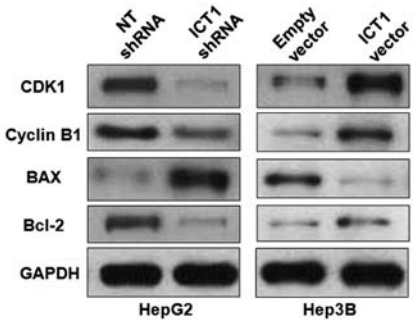


Figure 6. ICT1 modulates the levels of CDK1, cyclin B1, Bcl-2 and Bax in HCC cells. HepG2 cells that were transfected with ICT1 shRNA and non-targeting (NT) shRNA, respectively, were subjected to immunoblotting. ICT1 knockdown reduced the levels of CDK1, cyclin B1 and Bcl-2, and increased Bax expression in HepG2 cells. Furthermore, Hep3B cells were transfected with ICT1 vector and empty vector, respectively. ICT1 overexpression increased the levels of CDK1, cyclin B1 and Bcl-2, and reduced Bax expression in Hep3B cells.

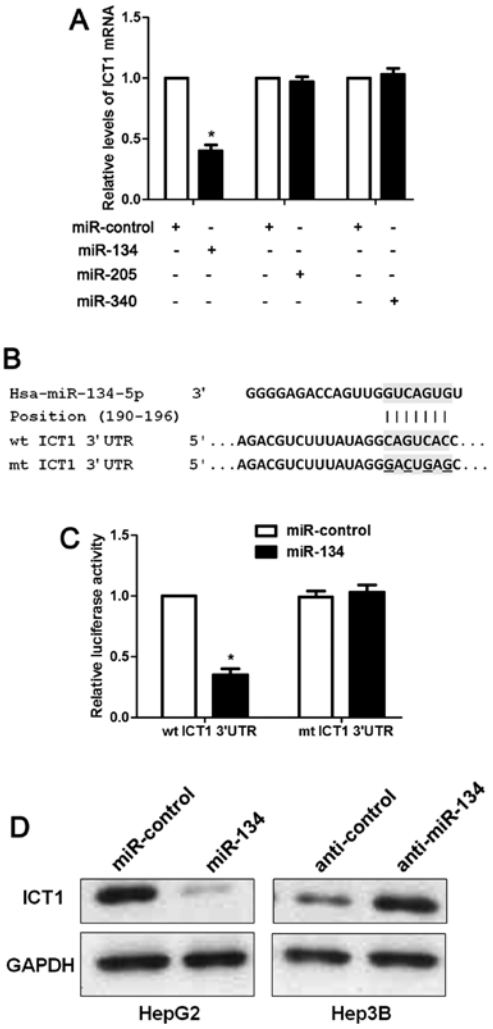


Figure 7. ICT1 is a direct target of miR-134 in HCC cells. (A) HepG2 cells that were transfected with miR-134, miR-205 and miR-340, respectively, were subjected to qRT-PCR for ICT1 mRNA expression. n=3, $^{*}P<0.05$. (B) The potential miR-134 binding site in wild-type (wt) 3'-UTR sequence of ICT1. The underlined part is the mutant site designed for mutant (mt) 3'-UTR sequence of ICT1. (C) miR-134 overexpression decreased the luciferase activity of wt 3'-UTR of ICT1. In contrast, no changes in relative luciferase activity were observed when the miR-134 binding site was mutated. n=3, $^{*}P<0.05$. (D) Western blot analysis indicated that miR-134 inversely regulated ICT1 abundance in HCC cells.

these results disclose that miR-134 inversely regulates ICT1 abundance in HCC cells.

## Discussion

The expression status and role of ICT1 in human cancers is limited. Thus, further study is necessary to investigate the clinical significance and role of ICT1 in HCC. In the present study, the expressions of ICT1 in the HCC tissues were prominently higher than those in the matched tumor-adjacent tissues. Furthermore, our data suggested that positive expression of ICT1 was prominently associated with large tumor size and advanced TNM tumor stage. Additionally, we supported the first evidence that ICT1-positive expression conferred a obvious poor prognosis of HCC patients. Thus, ICT1 potentially functions as a prognostic marker in HCC.

ICT1 has been reported to promote proliferation, cell cycle progression and inhibit apoptosis in CRC, lung, prostate and breast cancer (5-10). Then, we disclosed the biological function of ICT1 in HCC. We demonstrated that ICT1 knockdown inhibited proliferation and cell cycle progression and induced apoptosis of HepG2 cells. While, ICT1 restoration showed opposite effects on these cellular behaviors of Hep3B cells *in vitro*. In addition, ICT1 knockdown reduced the tumor growth of HCC in nude mice. These data reveal that ICT1 functions as an oncogene by regulating proliferation, cell cycle progression and apoptosis in HCC cells.

Previous studies report that ICT1 regulate cell growth of breast, CRC, prostate and lung cancer by modulating cell cycle and apoptosis-associated proteins including CDK1, cyclin B1, Bax and Bcl-2 (7-10). CDK1 and cyclin B1 are key regulators of cell cycle in HCC cells and restrained activation of cyclin B1-CDK1 kinase leads to G2/M arrest (16,17). Otherwise, Bcl-2/Bax axis plays an essential role in modulating apoptosis of HCC cells (18,19). In the present study, we revealed that ICT1 regulates the levels of CDK1, cyclin B1, Bax and Bcl-2 in HCC cells. These data indicate that ICT1 promotes growth of HCC probably by regulating these cell cycle and apoptosis-associated proteins. Furthermore, ICT1 was identified as a direct target of miR-134, which was previously reported to exert a dramatically suppressive effect on HCC malignancy (20,21). Thus, miR-134 regulation of ICT1 promotes malignant phenotypes of HCC cells probably via modulating CDK1, cyclin B1, Bax and Bcl-2 expression.

In conclusion, we show that ICT1 acts as an oncogene in HCC. First, our results demonstrate that ICT1 expression was upregulated in HCC tissues and cell lines. Then, our clinical data suggest that ICT1 may be used as a novel prognostic marker for HCC patients. Moreover, miR-134 regulation of ICT1 facilitates proliferation, cell cycle progression and suppresses apoptosis probably via modulating CDK1, cyclin B1, Bax and Bcl-2 expression in HCC cells. Taken together, our results verify that ICT1 may serve as a potential target for cancer therapeutics in HCC.

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