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ORIGINAL ARTICLE

Basic Study

MicroRNA-548a-5p promotes proliferation and inhibits apoptosis in hepatocellular carcinoma cells by targeting Tg737

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Abstract

AIM: To investigate whether Tg737 is regulated by microRNA-548a-5p (miR-548a-5p), and correlates with hepatocellular carcinoma (HCC) cell proliferation and apoptosis.

METHODS: Assays of loss of function of Tg737 were performed by the colony formation assay, CCK assay and cell cycle assay in HCC cell lines. The interaction between miR-548a-5p and its downstream target, Tg737, was evaluated by a dual-luciferase reporter assay and quantitative real-time polymerase chain reaction. Tg737 was then up-regulated in HCC cells to evaluate its effect on miR-548a-5p regulation. HepG2

cells stably overexpressing miR-548a-5p or miR-control were also subcutaneously inoculated into nude mice to evaluate the effect of miR-548a-5p up-regulation on *in vivo* tumor growth. As the final step, the effect of miR-548a-5p on the apoptosis induced by cisplatin was evaluated by flow cytometry.

RESULTS: Down-regulation of Tg737, which is a target gene of miR-548a-5p, accelerated HCC cell proliferation, and miR-548a-5p promoted HCC cell proliferation *in vitro* and *in vivo*. Like the down-regulation of Tg737, overexpression of miR-548a-5p in HCC cell lines promoted cell proliferation, increased colony forming ability and hampered cell apoptosis. In addition, miR-548a-5p overexpression increased HCC cell growth *in vivo*. MiR-548a-5p down-regulated Tg737 expression through direct contact with its 3′ untranslated region (UTR), and miR-548a-5p expression was negatively correlated with Tg737 levels in HCC specimens. Restoring Tg737 (without the 3′UTR) significantly hampered miR-548a-5p induced cell proliferation, and rescued the miR-548a-5p induced cell proliferation inhibition and apoptosis induced by cisplatin.

CONCLUSION: MiR-548a-5p negatively regulates the tumor inhibitor gene Tg737 and promotes tumorigenesis *in vitro* and *in vivo*, indicating its potential as a novel therapeutic target for HCC.

Key words: microRNA-548a-5p; Tg737; Proliferation; Apoptosis; Hepatocellular carcinoma cells

Core tip: Tg737 gene functions as a tumor suppresser in hepatocellular carcinoma (HCC). However, studies based on regulation of Tg737 were rare. MiR-548a-5p is a novel miRNA which negatively regulates Tg737 and promotes tumorigenesis *in vitro* and *in vivo*, indicating its potential as a novel therapeutic target for HCC.

Zhao G, Wang T, Huang QK, Pu M, Sun W, Zhang ZC, Ling R, Tao KS. MicroRNA-548a-5p promotes proliferation and inhibits apoptosis in hepatocellular carcinoma cells by targeting Tg737. *World J Gastroenterol* 2016; 22(23): 5364-5373 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i23/5364.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i23.5364

INTRODUCTION

Primary hepatocellular carcinoma (HCC) is currently one of the most common and lethal malignancies, and the leading cause of death among patients with cirrhosis[1]. Despite advances in surgical therapies, the reason for frequent recurrence remains mostly obscure. Several studies showed that some genes may play a major role in cell proliferation and migration in HCC cells[2]. However, little information is available about the regulation of these genes.

The Tg737 gene, first found in algae[3], is now identified as a tumor suppressor gene in multiple cancers, including cancers of the liver, kidney and pancreas[4-5]. In liver tissues from HCC patients, a 59% down-regulation of the Tg737 was observed[6]. This gene may participate in alterations in a series of human or rodent liver tumors and tumorigenic cell lines[7]. In the previous investigation, we found that Tg737 contributed to hypoxia-induced invasion and migration in HepG2 and MHCC97H cells[8], and that Tg737 inhibition resulted in malignant transformation in fetal liver stem/progenitor cells by promoting cell-cycle progression and differentiation arrest[9]. However, the regulatory factor for Tg737 is not yet clear.

In recent years, microRNAs (miRNAs) emerged as a group of important endogenous modulators of gene function at the posttranscriptional level. It has been revealed that many miRNAs are underscored by the promotion of tumorigenesis and cancer progression[10-11]. More importantly, in HCC, several miRNAs have been demonstrated to contribute to the tumor regulation, including, but not limited to, migration, invasion and proliferation[12-13]. Multiple miRNAs have been identified as down-regulated tumor-suppressing genes involved in cellular processes, including miR-34a[14], miR-122[15], miR-199a[16] and miR-200[17]. Contrarily, miR-21[18], miR-148a[19] and miR-221[20], as oncogenic miRNAs, showed up-regulated expression which potentially targeted many tumor-suppressive genes. These results suggest the involvement of miRNAs in HCC.

MiR-548 is a big, poorly conserved primate-specific miRNA family. There are 68 members of the hsa-miR-548 family recorded in the miRBase database[21]. The products of miR-548c-5p and miR-548c-3p show discrepant evolutionary patterns, which brings about great genetic distances between pre-miRNAs to some extent and might contribute to dynamic expression profiles and regulatory network[22]. MiR-548c-3p was identified as a source of functional biomarkers for the primary prostate cancer progression[23]. It also significantly increased in *Helicobacter pylori*-negative cancer tissues[24]. In addition, our previous studies found the high expression of miR-548c-3p and miR-548c-5p in side population cells from HCC[25]. However, like the homologous gene, the exact effect of miR-548a-5p on HCC is still unknown. The direct aim of this study was to investigate whether Tg737 is regulated by miR-548a-5p, and correlates with HCC cell proliferation and apoptosis.

MATERIALS AND METHODS

Cell lines and culture

HCC cell lines HepG2 and MHCC97-H were maintained in DMEM medium (Invitrogen, United States) sup-

plemented with 10% fetal bovine serum (FBS, Invitrogen, United States), 100 IU/mL penicillin, and 100 μg/mL streptomycin. Cells were cultured in a humidified cellular incubator (Thermo Fisher, United States) supplemented with 5% CO_2 at 37 ℃.

RNA isolation, reverse transcription and quantitative real-time polymerase chain reaction

Total RNA was extracted using RNAiso Plus (Takara, Japan) according to the manufacturer's instructions. Reverse transcription was carried out using the PrimeScript RT Reagent Kit Perfect Real Time (Takara, Dalian, China) or the miScript II RT Kit (Qiagen, Germany). Quantitative polymerase chain reaction (qPCR) was used to detect the miRNA expression levels. The SYBR Green dye (Takara, Dalian, China) was used according to the manufacturer's protocol. The following primers were used for analysis: TG737: forward primer, 5'-GTGCCAGTAGTAAAGGTG-3' and reverse primer, 5'-GGTCGTTCTATTTGAGGG-3'; β-actin: forward primer, 5'-TCACCAACTGGGACGACA-3' and reverse primer, 5'-ACAGCACCGCCTGGATAG-3'; miR-548a-5p: forward primer, 5'-AAAAGTAATTGCGAGTTTACC-3' and reverse primer, Universal Primer (Qiagen, Germany). The CFX96 real-time PCR detection system (Bio-Rad, United States) was used to perform real-time PCR and the $2^{-\Delta\Delta CT}$ method was used to determine the relative gene expression. Mature miRNA was normalized to U6-snRNA.

Plasmid construction

MiR-548a-5p was obtained from Gene Pharma (Shanghai, China). The miR-548a-5p and 150 bp of flanking sequence was amplified with forward primer, 5'-CAGCTGGGTGCTCAGCCAGG-3', reverse primer, 5'-GGCAACTTAATGTTTCTTGC-3'. A PCR fragment was inserted into the pENTRTM3C vector (Invitrogen, United States) using EcoR I and Xho I sites. The pLenti6.3-miR-548a-5p vector was constructed using Gateway LR Clonase II Enzyme Mix (Invitrogen, Carlsbad, CA, United States) according to the manufacturer's instructions. The constructs were sequenced for verification. Overexpression and RNAi vectors were determined by Gene Pharma (Shanghai, China). The target sequence of sh-Tg737 was 5-'GTTACATACTTGAGACAAA-3'. Tg737 and Tg737-3'UTR overexpression vectors were also designed.

Western blot

Proteins were extracted from cells with a RIPA buffer purchased from Beyotime with 1 mmol/L PMSF and a cocktail of protease inhibitors. The proper amount of loading buffer was added into cell lysis, followed by a BCA protein determination. SDS-PAGE was performed according to Genshare's instructions (Genshare, Xi'an, China). The results were analyzed using Image J 5.0 software. Anti-Tg737 antibody (13967-1-AP) and anti-β-actin antibody (14395-1-AP) were purchased from Proteintech (Proteintech, Wuhan, China). Goat anti-rabbit IgG HL (HRP) (ab6721) was supplied by Abcam (United States).

Colony formation assay

Two hundred cells were seeded into a 6 cm dish and cultured in a complete medium for 10 d. Cells were fixed in methanol and stained with crystal violet, washed with PBS three times before fixing and staining. Colonies were washed with ddH_2O after 30-min staining. The number and area of the colonies were calculated with Image J 5.0 software.

Cell viability assay

Cells were seeded in 96-well plates (2.0×10^3 cells/well). The cells were incubated for 0, 1, 2, 3 and 4 d using three replicates. Cell Counting Kit-8 (CCK-8) (7Sea Pharmatech, China) was used for cell proliferation analysis according to the manufacturer's protocol. The Bio-Rad iMARK™ microplate reader was used to detect the optical density at 450 nm.

Cell cycle and apoptosis assay

Cells were seeded in 6-well plates with 2.0×10^5 cells in each well. After 48 h, flow cytometry was performed to detect cell cycle analysis and the percentage of dying cells. The annexin V-FITC/PI apoptosis detection kit (Biovision, Palo Alto, CA, United States) was used according to the manufacturer's protocol. Flow cytometry was performed with the Epics XL-MCL (BECKMAN coulter, United States). The results were analyzed using ModFit LT V3.1 (BECKMAN coulter).

Luciferase reporter assays

HepG2 and MHCC97H cells were seeded in 48 well plates and allowed to be adherent for 24 h. The cells were then co-transfected with a mixture of 100 ng pGL3-Tg737-3'UTR, 20 μmol miR-548a-5p mimic or antisense-miR-548a-5p, and 5 ng pRL-TK using Lipofectamine 2000 reagent and performed in three independent experiments. Firefly and Renilla luciferase activities were detected using the Dual-Glo luciferase assay system (Promega, United States) following the manufacturer's instructions.

Tumor xenograft

HepG2 cells (2×10^6) stably overexpressing miR-548a-5p or miR-control were injected subcutaneously into the flank site of male Balb/c nude mice (Fourth Military Medical University, Xi'an, China). Tumor volume was measured with calipers every 5 d[26]. On day 30, mice were killed by cervical dislocation. All animal procedures were performed in accordance with a protocol approved by the Fourth Military Medical University Animal Care and Usage Committee.

Statistical analysis

Sample capacity for each experiment was adjusted according to the variance obtained. All experiments were performed in triplicate. In graphs, all data are

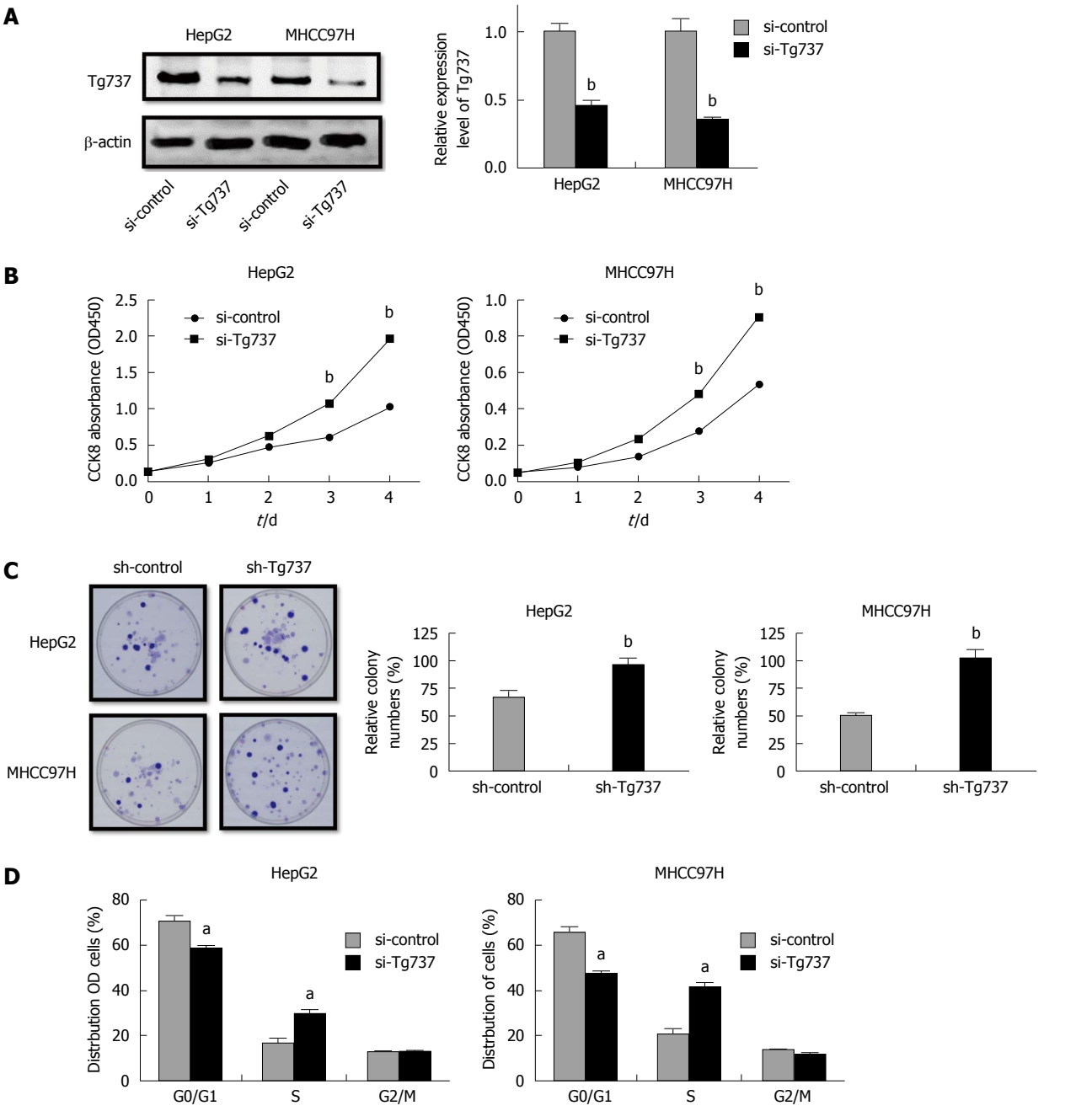

Figure 1 Down-regulation of Tg737 accelerates hepatocellular carcinoma cell proliferation. A: Western blot analysis of Tg737 expression in HepG2 and MHCC97H cells transfected with si-Tg737 or negative control (si-control); B: Impact of Tg737 down-regulation on HCC cell proliferation detected by CCK-8 assay after si-Tg737 and si-control transfection in HepG2 and MHCC97H cell lines; C: Impact of Tg737 down-regulation on colony forming ability of HepG2 and MHCC97H cell lines. The relative percentage of colony numbers from the si-Tg737 group is given as 100%; D: Cell cycle assay of HepG2 and MHCC97H cells transfected with si-Tg737 and si-control. The charts were illustrated by the percentage of cells in the G0/G1, S, and G2/M phase of cell cycle, respectively. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control.

presented as mean ± SD and were evaluated by the Student's *t*-test. Statistical analyses were carried out using SPSS 16.0 software. Differences were considered significant at $P < 0.05$.

RESULTS

Down-regulation of Tg737 promotes HCC cell proliferation in vitro

To illuminate the role of Tg737 in HCC cell proliferation, HCC cell lines HepG2 and MHCC97H were transfected with a si-Tg737 sequence or negative control (Figure 1A). Down-regulation of Tg737 significantly promoted the proliferation of HepG2 and MHCC97H cells and enhanced colony forming capability (Figure 1B and C). The distribution of HepG2 and MHCC97H cell cycles showed that the percentage of cells in G0/G1 phase significantly decreased in Tg737 down-regulated cells compared with their counterparts, while the cells in S phase increased sharply (Figure 1D). In all cases, down-regulation of Tg737 promoted cell proliferation and inhibited G0/G1 phase arrest in HCC cells.

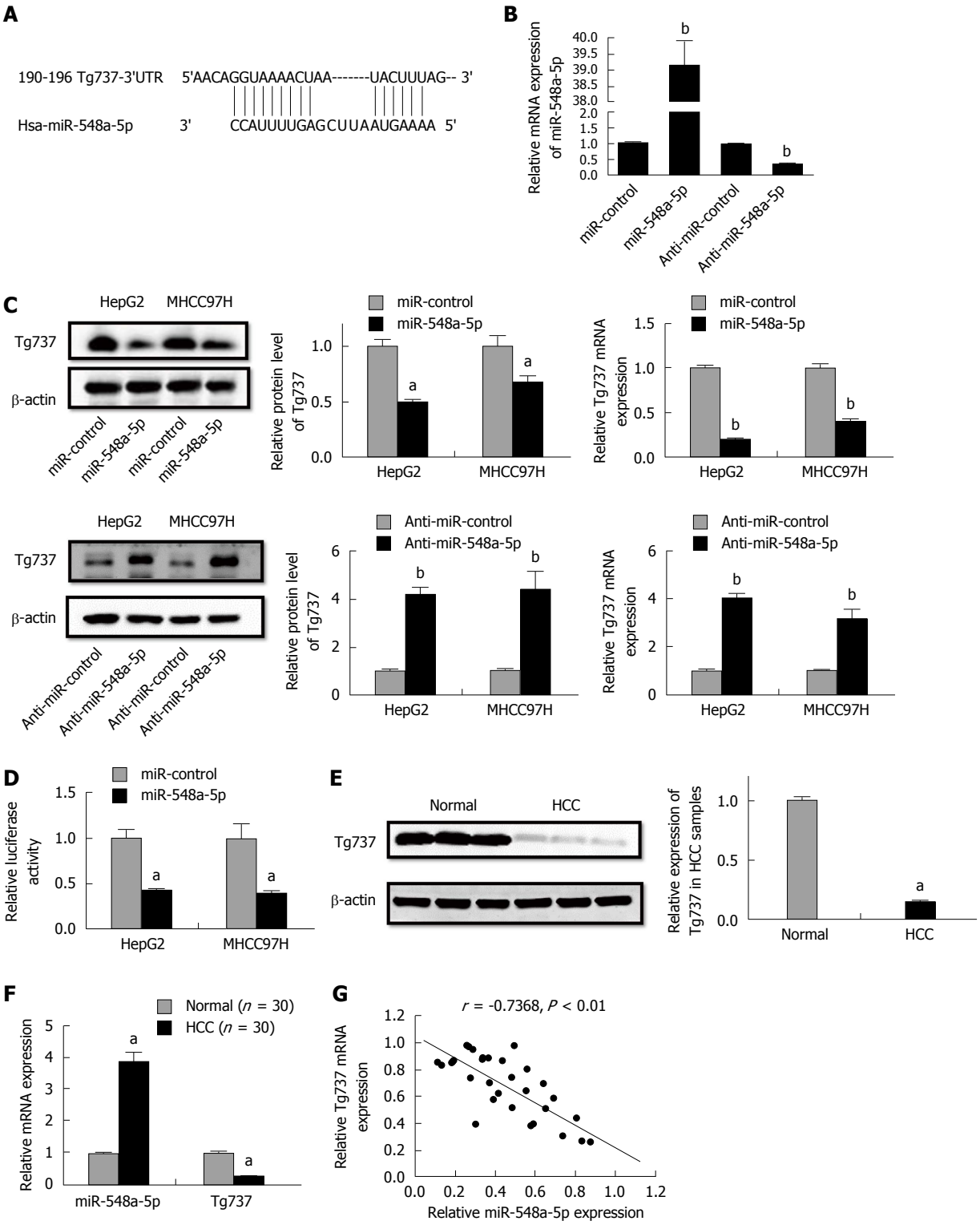

Figure 2 Tg737 is a target gene of miR-548a-5p. A: MiR-548a-5p and its deductive binding domain in the 3'UTR of Tg737; B: Relative expression of miR-548a-5p; C: qRT-PCR and Western blot analysis demonstrated that miR-548a-5p overexpression significantly decreased Tg737 mRNA expression and miR-548a-5p knockdown significantly increased Tg737 mRNA expression in HepG2 and MHCC97H cells; D: Luciferase reporter assay of the connection between the 3'UTR of Tg737 and miR-548a-5p in HepG2 and MHCC97H cells; E: Western blot analysis demonstrated higher miR-548a-5p and lower Tg737 expression in human HCC specimens compared to normal liver tissue; F: qRT-PCR demonstrated higher miR-548a-5p and lower Tg737 expression in human HCC specimens compared to normal liver tissue; G: Spearman's correlation assay showed that miR-548a-5p was negatively correlated with Tg737 mRNA expression in HCC specimens. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control.

MiR-548a-5p down-regulates Tg737 by interacting with its 3'UTR

A bio-informatics assay with TargetScan demonstrated that Tg737 is a potential target of miR-548a-5p (Figure 2A). To illuminate whether miR-548a-5p acts on Tg737, we transfected miR-548a-5p as well as anti-miR-548a-5p to HCC cells. Relative miR-548a-5p levels are shown in Figure 2B. MiR-548a-5p overexpression decreased

mRNA and protein levels of Tg737, while miR-548a-5p inhibition increased mRNA and protein levels of Tg737 in HepG2 and MHCC97H cells (Figure 2C). To confirm whether Tg737 acts as a molecular target regulated by miR-548a-5p, we constructed luciferase reporter vectors containing Tg737-3'UTR. The reporter vectors were co-transfected into HepG2 and MHCC97H cells. Up-regulation of miR-548a-5p expression significantly decreased the luciferase activity of Tg737 containing 3' UTR (Figure 2D). Further, to illuminate whether miR-548a-5p inhibits Tg737 in patients diagnosed with HCC, we detected the expression of miR-548a-5p and Tg737 in 30 HCC specimens as well as 30 normal ones. Compared with normal liver tissues, the HCC specimens showed higher miR-548a-5p and lower Tg737 expression (Figure 2E and F). In addition, a statistically significant correlation was revealed by Spearman's correlation analysis between mRNA levels of miR-548a-5p and Tg737 (r = -0.7368, P < 0.01; Figure 2G). Together, these data suggest that Tg737 is a target of miR-548a-5p in HCC.

MiR-548a-5p promotes HCC cell proliferation in vitro and in vivo

Continuing our analysis, we detected the impact of miR-548a-5p on HCC cell proliferation. Similar to the effect of Tg737 knockdown, miR-548a-5p overexpression in HepG2 and MHCC97H cells significantly accelerated cell proliferation, promoted colony forming capacity, and inhibited cell arrest in G0/G1 phase (Figure 3A-C). To investigate the relationship between miR-548a-5p and its tumor promoting characteristics *in vivo*, HepG2 cells exhibiting sustained expression of miR-548a-5p or control cells were injected into the subcutaneous skin layer of nude mice. Tumor size was detected every 5 d from the injection. The mice were killed 30 d after implantation. As shown in Figure 3D, tumors in those specimens with miR-548a-5p overexpression were larger significantly than those in the control group. The average size and weight of miR-548a-5p overexpressing tumors were increased significantly (Figure 3D and E). Collectively, miR-548a-5p may have a vigorous ability to enhance HCC cell proliferation *in vivo* and *in vitro*.

Restoration of Tg737 inhibits miR-548a-5p-mediated HCC cell proliferation inhibition and protects against cisplatin induced apoptosis

We further traced whether the restoration of Tg737 could arrest the HCC cell proliferation induced by miR-548a-5p overexpression in HepG2 and MHCC97H cells. A Western blot technique was performed 48 h after transfection of control, Tg737 and Tg737-3' UTR overexpression vectors into miR-548a-5p overexpressing HepG2 and MHCC97H cells (Figure 4A). Tg737 up-regulation significantly decreased the capacity of cell proliferation and colony formation (Figure 4B). Up-regulation of Tg737 rescued miR-548a-5p induced inhibition in cell cycle arrest (Figure 4B-D). However, overexpression of Tg737-3'UTR in miR-548a-5p overexpressing HepG2 and MHCC97H cells had no significance (Figure 4B-D). These results again illustrate that Tg737 is a functional target of miR-548a-5p. We evaluated the impact of miR-548a-5p on apoptosis by flow cytometry. Cell apoptosis increased in anti-miR-548a-5p transfected HCC cells (Figure 4E). Meanwhile, the cell apoptosis ratio decreased in miR-548a-5p transfected HCC cells (Figure 4F).

DISCUSSION

To our knowledge, this is the first report describing the effects of miR-548a-5p on HCC cell proliferation and apoptosis and the relationship with the Tg737 protein. In this study, we showed that Tg737 was the key regulator of the proliferation of human HCC cell lines. Overexpression of miR-548a-5p observably increased oncogenesis in nude mice. Moreover, when the expression of Tg737 was inhibited through interaction with its 3'UTR, HCC cells grew quickly when induced by miR-548a-5p. These findings suggest that miR-548a-5p acts as a novel tumor promoter in HCC cells and contributes to the tumor behavior by targeting the Tg737 gene.

MiR-548 was identified as a large human gene family with 69 members, which are mainly expressed in primates and play a key role in multiple biological processes[22]. The miR-548 family possesses the most abundant primate-specific miRNA common seed (5'-AAAGUA-3') detected by computational characterization[27] and the signatures were identified to be down-regulated in peripheral blood mononuclear cells from heart failure patients. This information suggests that miR-548 level could be used as a potential biomarker specific for cardiac dysfunction[28]. In addition, endogenous miR-548 levels were suppressed during viral infections. MiR-548 shows significant identity to three viruses (HCV, HBV and HIV-1)[29], and regulated the host antiviral response *via* direct targeting interferon[30]. The marked up-regulation of miR-548ah-5p suggests that it might be related to the transition from the immune tolerance phase to the immune activation phase in chronic hepatitis B[31]. Such evidence makes miRNA a valuable therapeutic agent for patients diagnosed with multiple virus infection. Regarding the relationship of miR-548 with tumors, an analysis of the expression profiles and functional affinities of 3500 putative target genes of miR-548 suggested its cancer-related regulatory role[32]. A recent study found that miR-548d-3p, another miR-548 family member, is usually overexpressed in numerous types of cancer and affects tumorigenesis[33]. Overexpression of the miR-548 family, especially miR-548a-5p and miRNA-548d-5p, could play a complementary role in supporting the oncogenicity in cervical carcinogenesis[34]. Similar results were obtained in our study. MiR-548a-5p

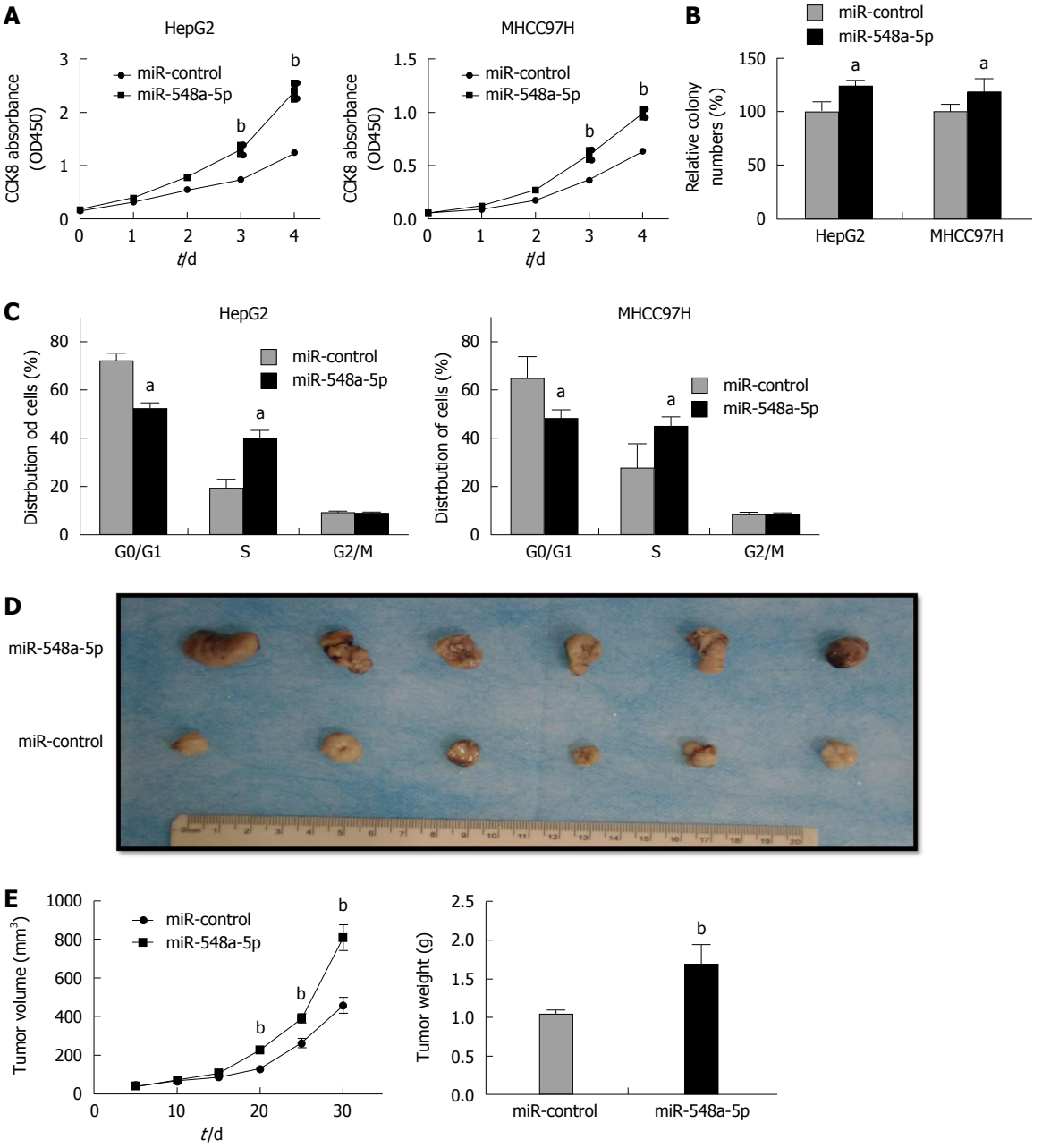

Figure 3 miR-548a-5p promotes hepatocellular carcinoma cell proliferation *in vitro* and *in vivo*. A: CCK-8 assays of HepG2 and MHCC97H cells constantly expressing miR-548a-5p or miR-control; B: Colony forming assays of HepG2 and MHCC97H cells constantly expressing miR-548a-5p or miR-control; C: Cell cycle assays of HepG2 and MHCC97H cells constantly expressing miR-548a-5p or miR-control; D: MiR-548a-5p or miR-control overexpressing HepG2 cells were injected into the subcutaneous layer of nude mice. The mice were killed 30 d after injection; E: Tumor volumes and weight. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control.

overexpression accelerated HepG2 and MHCC97H cell proliferation *in vitro* and increased the tumor size and weight *in vivo*, which indicates that miR-548a-5p promotes hepatic neoplasia.

Tg737 has attracted much attention because its functional inactivation by regulation of its expression is relevant to the formation of many solid tumors, such as those in the liver, kidney, and pancreas[4-5]. As a liver tumor suppressor gene, it has been implicated to be important for cell proliferation and apoptosis[35], because it is found deficient in HCC samples from chemical-induced rats and clinical patients[7,36]. In our previous research, Tg737 was found to be remarkably decreased in gene expression in side population cells of HCC, and the loss of heterozygosity of Tg737 was markedly associated with a poor prognosis in patients with HCC after curative liver resection[37]. In the present study, we confirmed these results and found that a loss of Tg737 function showed growth promotion in HepG2 and MHCC97H cells, which corresponds to the results of miR-548a-5p overexpression. Furthermore, miR-548a-5p knockdown rescued the effects caused by Tg737. There was a significant inverse correlation between miR-548a-5p expression and Tg737 mRNA and protein levels in hepatocellular carcinoma tissue and cell specimens. Thus, all these

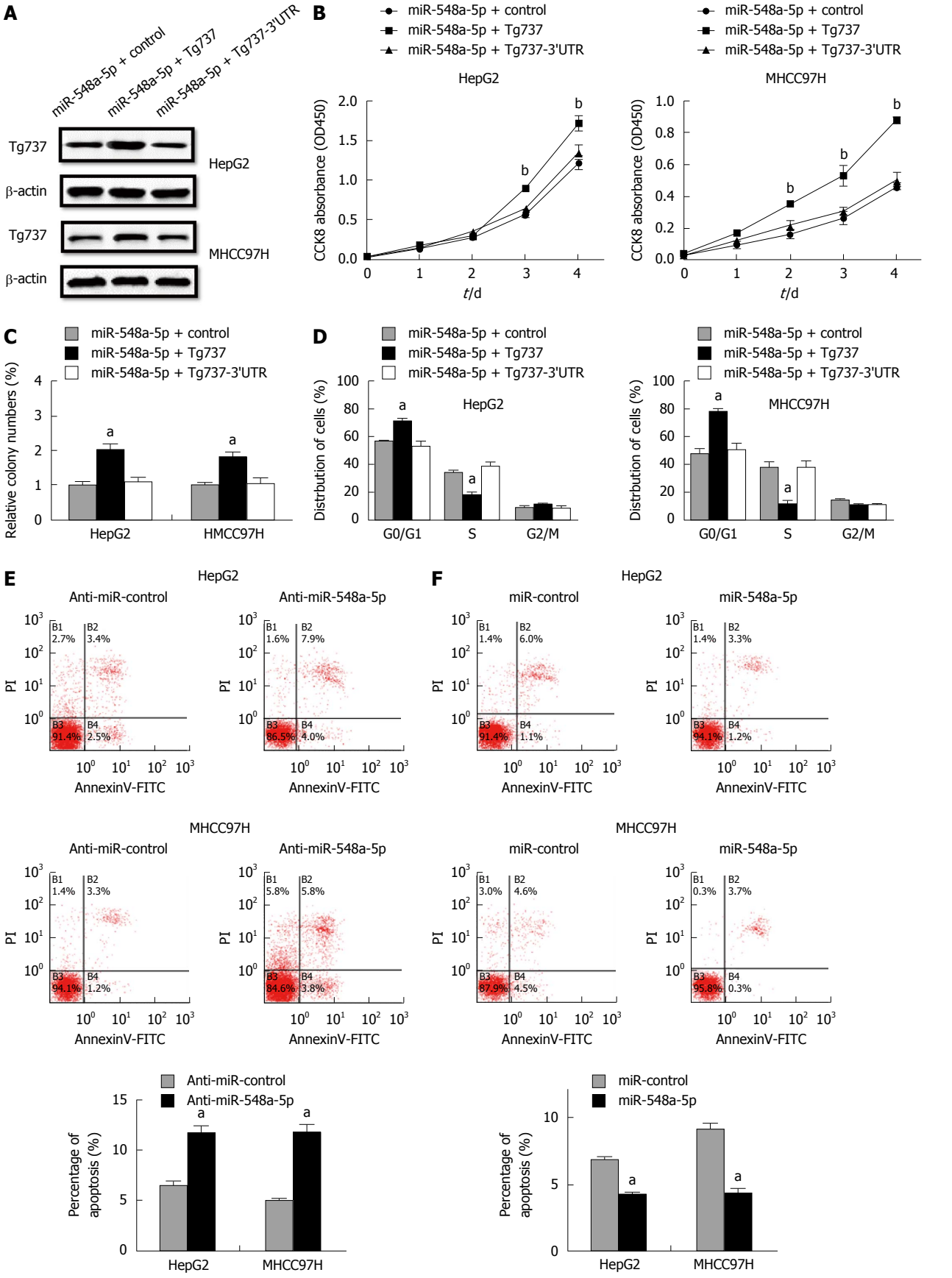

Figure 4 Restoration of Tg737 rescues the miR-548a-5p induced cell proliferation inhibition and apoptosis induced by cisplatin. A: Western blot was performed 48 h after transfection of pc CON, pc Tg737 and plasmid with 3'UTR of Tg737 into miR-548a-5p overexpressing HepG2 and MHCC97H cells; B: CCK-8 assay; C: Colony forming assay; D: Cell cycle assay; E: Cell apoptosis increased in anti-miR-548a-5p transfected HepG2 and MHCC97H cells; F: Cell apoptosis decreased in miR-548a-5p transfected HepG2 and MHCC97H cells. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control.

results support the hypothesis that miR-548a-5p functions in HCC by targeting the 3'UTR of Tg737.

In summary, our observations provide novel evidence that miR-548a-5p negatively regulates the tumor inhibitor gene Tg737 and promotes tumorigenesis *in vitro* and *in vivo*, adding to our understanding of the biological function of miR-548a-5p, as well as its relationship with Tg737. Therapeutic strategies to inhibit miR-548a-5p therefore potentially may be useful in limiting HCC growth and metastasis. A practical application of our findings could be the use of miR-548a-5p expression as a potential predictor of tumors that may be more likely to respond to Tg737-targeting therapies.

COMMENTS

Background

Primary hepatocellular carcinoma (HCC) is one of the most common and lethal malignancies. Despite advances in the surgical therapies, the reason of frequent recurrence remains mostly unclear. Several studies show that some genes may have good roles in cell proliferation and migration in HCC cells. The Tg737 gene is now identified as a tumor suppressor gene in multiple cancers, including those of the liver, kidney, and pancreas. In liver tissues from HCC patients, a 59% down-regulation of the Tg737 was observed. This gene may participate in alterations in a series of human or rodent liver tumors and tumorigenic cell lines. Nevertheless, the regulatory factor for Tg737 is not fully clear.

Research frontiers

In recent years, microRNAs (miRNAs) emerged as a group of important endogenous modulators of gene function at the posttranscriptional level. It has been revealed that many miRNAs are underscored by the promotion of tumorigenesis and cancer progression. More importantly, in HCC, several miRNAs have been demonstrated to contribute to the tumor regulation, including, but not limited to, migration, invasion, and proliferation. Multiple miRNAs have been identified as down-regulated tumor-suppressing genes involved in cellular processes, as oncogenic miRNAs, showed up-regulated expressions which potentially targeted many tumor-suppressive genes. MiR-548 is a large and poorly conserved primate-specific miRNA family. The authors previous studies have found high expression of miR-548c-3p and miR-548c-5p in side population cells from HCC. However, like the homologous gene, the exact effect of miR-548a-5p on HCC is still unknown. The direct aim of this study was to investigate whether Tg737 is regulated by miR-548a-5p, and correlates with HCC cell proliferation and apoptosis.

Innovations and breakthroughs

MiR-548a-5p negatively regulates the tumor inhibitor gene Tg737 and promotes tumorigenesis *in vitro* and *in vivo*. The novelty of the research represents the idea that the inhibition of miR-548a-5p may limit HCC growth, and miR-548a-5p expression may be the potential predictor of tumors that respond to Tg737-targeting therapies.

Applications

In summary, this observations provide novel evidence that miR-548a-5p negatively regulates the tumor inhibitor gene Tg737, and promotes tumorigenesis *in vitro* and *in vivo*, which adds to the authors understanding of the biological function of miR-548a-5p, as well as the relationship with Tg737. Therapeutic strategies to inhibit miR-548a-5p therefore potentially may be useful to limit HCC growth and metastasis. A practical application of the authors findings could be the use of miR-548a-5p expression as a potential predictor of tumors that may be more likely to respond to Tg737-targeting therapies.

Peer-review

The study by Zhao Ge *et al* on microRNA-548a-5p and its role in hepatocellular carcinoma *via* Tg737 is very interesting. The authors show that miR-548a-5p regulates negatively the tumor suppressor gene Tg737 and promotes tumorigenesis *in vitro* and *in vivo*. The authors suggest that therapeutic strategies to inhibit miR-548a-5p in the future may prove useful in limiting HCC growth and metastasis.

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