miR-202 suppresses cell proliferation in human hepatocellular carcinoma by downregulating LRP6 post-transcriptionally

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A B S T R A C T

MicroRNAs have emerged as important regulators of carcinogenesis. In the current study, we observed that microRNA-202 (miR-202) is downregulated in hepatocellular carcinoma (HCC) cells and tissues, indicating a significant correlation between miR-202 expression and HCC progression. Overexpression of miR-202 in HCC cells suppressed cell proliferation and tumorigenicity, while downregulation of miR-202 enhanced the cells' proliferative capacity. Furthermore, we identified low-density lipoprotein receptor-related protein 6 (LRP6) as a direct target of miR-202. miR-202 suppresses the expression of LRP6 by binding to the 3′-untranslated region (UTR) of its mRNA. Finally, we found that silencing the expression of LRP6 is the essential biological function of miR-202 during HCC cell proliferation. Collectively, our findings reveal that miR-202 is a potential tumor suppressive miRNA that participates in carcinogenesis of human HCC by suppressing LRP6 expression.

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1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers and is the third most common cause of cancer deaths in the world. HCC is one of the most common types of cancer in Asian countries, where chronic hepatitis or liver diseases are prevalent [1]. Most patients with HCC are asymptomatic until the later stages and their prognosis is generally very poor, with a survival rates 20–65% for 1 year, 10–30% for 3 years and 10–20% for 5 years [2]. At present, surgical resection is the best curative treatment for HCC. Identification of early stage HCC, which potentially meets the criteria for resection, would improve the survival rate [3]. The prognosis of HCC has not been improved despite significant progress in the diagnostic and therapy of HCC. Many studies have identified factors associated with HCC survival, but the results are inconsistent [2]. Specific and effective diagnostic biomarkers for early stage HCC are needed urgently.

MicroRNAs (miRNAs), a class of endogenous non-coding small RNAs of 20–22 nucleotides, have been found to negatively regulate genes expression by targeting the 3′ untranslated region (UTR) of target mRNAs in a sequence-specific manner and are involved in multiple biological processes [4,5]. Increasing numbers of reports have shown that miRNAs have key roles in the progression of various human cancers, where some miRNAs are identified as onco-miRs and some as tumor suppressors [6–8]. Considering the modulation of miRNAs during tumorigenesis, miRNAs are currently considered as substantial novel biomarkers for tumor diagnosis and therapy. miR-202 is located within a chromosomal fragile site in 10q26, deletion of which is associated with endometrial and brain tumors [9–11]. Although miR-202 was observed to be deregulated in gastric cancer, breast cancer, cervical squamous cell carcinoma, colorectal cancer, follicular lymphoma and leukemia [12–16], the effects of miR-202 on HCC have not been documented.

The low-density lipoprotein receptor-related protein 6 (LRP6), a member of the expanding low-density lipoprotein (LDL) receptor

family, is widely expressed in many tissues, including heart, brain, placenta, lung, kidney, pancreas, spleen, testis, ovary, liver, skeletal muscle, prostate and the mucosal lining of the colon [17]. Li et al. found that LRP6 is significantly expressed in human colon cancer, breast cancer, lung cancer, fibrosarcoma, and kidney tumors. Overexpression of LRP6 promotes cell proliferation and tumorigenesis [18]. Liu et al. found that LRP6 expression is frequently upregulated in breast cancer, and might define a class of breast cancer subtype and may be considered as a putative target for therapy [19]. In HCC, the transcripts and protein levels of LRP6 are upregulated in cells and tissues, and overexpression of LRP6 enhanced HCC cells proliferation, migration and invasion [20]. However, modulation of LRP6 by miRNAs in HCC has not been reported.

In this study, we identified that microRNA-202 (miR-202) is downregulated in HCC and that ectopic expression of miR-202 in HCC cells leads to suppression of cell proliferation, tumorigenicity and cell cycle progression. By contrast, inhibition of miR-202 enhances cell proliferation. Furthermore, miR-202 targets LRP6 directly by post-transcriptional downregulation. LRP6 plays essential role in miR-202-mediated proliferation of HCC cells. Collectively, our present study demonstrated, for the first time, that miR-202 suppresses cell proliferation, tumorigenicity and cell cycle progression in HCC cells by suppressing the expression of the LRP6 oncogene. Thus, miR-202 might be considered a potential tumor suppressor in HCC.

2. Material and methods

2.1. Cell culture

Normal liver epithelial cells, THLE3, were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured according to the manufacturer's instruction. The HCC cell lines (BEL-7402, MHCC97L, MHCC97H, QGY-7703, HCCC-9810, Hep3B, HepG2, and Huh7) were maintained in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Invitrogen), 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen), within a humidified atmosphere containing 5% CO_2 at 37 °C.

2.2. Tissue specimens

Eight pairs of snap-frozen HCC tumor (Presented as T1–T8 in Figs. 1 and 4) and matched normal tissues from adjacent regions, which were diagnosed histopathologically at the Zhongnan Hospital of Wuhan University from 2011 to 2012, were immediately stored in liquid nitrogen after surgery until further use. For the

use of the clinical materials for research purposes, the Institutional Research Ethics Committee approved the study, and prior patient consent was obtained.

Analysis of miR-202 expression in HCC tissues compared with that in matched non-cancerous hepatic tissues was using a published microarray-based high-throughput assessment ($n = 166$, $P < 0.001$; NCBI/GEO/GSE31384) [21]. The data were downloaded from NCBI and analyzed using SPSS13.0 software.

2.3. Generation of stably engineered cell lines

The miR-202 expression plasmid, pMSCV-miR-202, was generated by cloning the genomic pre-miR-202 gene into plasmid pMSCV-puro (Clontech Laboratories Inc., Mountain View, CA, USA). pMSCV-puro-miR-202 was then cotransfected with plasmid pIK into 293FT cells using the calcium phosphate transfection method, as previously described [22]. Supernatants were collected 36 h after cotransfection and incubated with HCC cells to be infected for 24 h. Puromycin (1.5 μg/ml, Sigma, Saint Louis, MO, USA) was used to select stably transduced cells over 10 days.

2.4. RNA extraction and real-time quantitative PCR

Total cellular RNA was extracted using the TRIzol solution (Invitrogen), according to the manufacturer's protocol. Reverse transcription and Real-time PCR were performed using RT Real-Time™ SYBR Green (Bio-Rad Laboratories, Berkeley, CA, USA). The housekeeping gene glyceraldehyde phosphate dehydrogenase (*GAPDH*) and the small nuclear RNA U6 were used as internal controls for mRNAs and microRNA, respectively. The primers used were as follows: *MYC* forward: 5′-TCAAGA GG CGAACACACAAC-3′, *MYC* reverse: 5′-GGCCTTTTCATTGTTTTCCA-3′; *Cyclin D1* forward: 5′-AACTACCTGG ACCGCTTCCT-3′, *Cyclin D1* reverse: 5′-CCACTTGAGC TT GTTCAC-CA-3′; *LEF1* forward: 5′-CACTGTAAGTGATGAGGGGG-3′, *LEF1* reverse: 5′-TGGATCTC TTTCTCCACCCA-3′; *GAPDH* forward: 5′-GACT CATGACCACAGTCCAT GC-3′, *GAPDH* reverse: 3′-AGAGGCAGGGATG ATGTTCTG-5′.

Expression levels of genes were calculated as $2^{-[(C_t\ of\ p21,\ CyclinD1)-(C_t\ of\ GAPDH)]}$ (C_t represents the threshold cycle for each transcript). The relative expression of the miRNA was calculated as $2^{-[(C_t\ of\ miR-202)-(C_t\ of\ U6)]}$.

2.5. Western blotting

Cellular proteins were prepared in sample buffer [62.5 mM Tris–HCl (pH 6.8), 10% glycerol, 2% SDS] and heated for 10 min at 100 °C. Equal quantities of protein were electrophoresed through
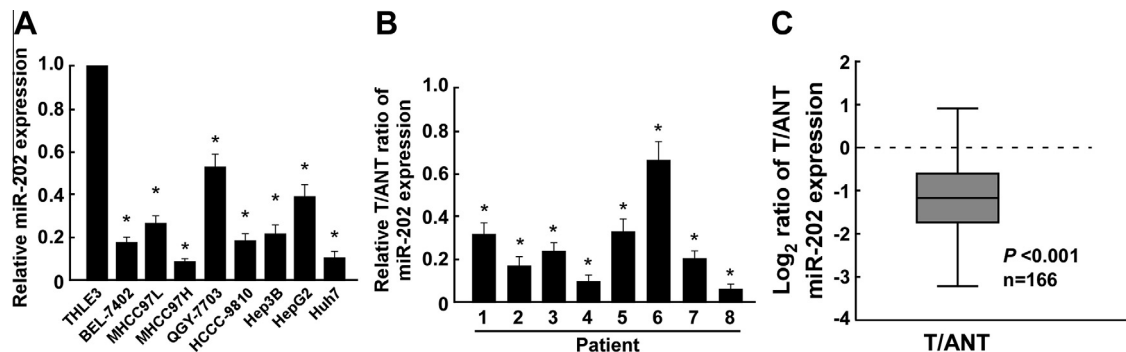

Fig. 1. Expression of miR-202 is downregulated in HCC. (A) Real-time PCR analysis of miR-202 expression in normal liver epithelial cells THLE3 and in hepatocellular carcinoma cell lines. (B) Comparison of miR-202 levels in HCC tissues and normal control, including eight paired cancerous tissues (T) and their adjacent non-cancerous hepatic tissues (ANT). The average miR-202 expression was normalized using U6 expression. (C) The expression of miR-202 in HCC tissues compared with that in matched non-cancerous hepatic tissues, as measured using a published micro-array-based high-throughput assessment ($n = 166$, $P < 0.001$; NCBI/GEO/GSE31384). Each bar represents the mean ± S.D. of three independent experiments. *$P < 0.05$.

a 10% SDS/polyacrylamide gel and transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, MA, USA). The membranes were incubated with anti-LRP6, anti-c-Myc, anti-Cyclin D1, anti-β-catenin, anti-Rb, anti-phosphorylated Rb and anti-α-Tubulin antibodies (1:1000; Cell Signaling Technology, Danvers, MA, USA), respectively.

2.6. Oligonucleotides, siRNA and transfection

A miR-202 mimic, a miR-202 inhibitor and a negative control (NC) were purchased from RiboBio (RiboBio Co. Ltd, Guangzhou, Guangdong, China). For depletion of LRP6, an siRNA was synthesized and purified by RiboBio (The *LRP6* siRNA sequence was CCGATGCAATGGAGATGCAAA). Transfection of oligonucleotides and siRNAs were performed using Lipofectamine 2000 (Invitrogen), according to the manufacturer's protocol.

2.7. Luciferase assay

A region of the human *LRP6* 3′-UTR (500–1500 bp) containing two miR-202 binding sites was amplified by PCR and cloned into vector pGL3 (Promega, Madison, WI, USA). The primers used were as follows: *LRP6*-3′UTR-up: 5′-GCCCCGCGGCTCTGGTAAGATGCCAT GAAGCTGT-3′; *LRP6*-3′UTR-dn: 3′-GCCCTGCAGTGTCGAAGTACCGT AGAATGGTCTC-5′. One hundred nanograms of pGL3-*LRP6*-3′UTR-luciferase plasmid was transfected into HCC cells using the Lipofectamine 2000 (Invitrogen). Luciferase and control signals were measured at 48 h after transfection using the Dual Luciferase Reporter Assay Kit (Promega), according to manufacturer's instruction. Three independent experiments were performed and the data were presented as the mean ± standard deviation (S.D.).

2.8. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay

Cells were seeded in 96-well plates. 100 μl of sterile MTT dye (0.5 mg/ml, Invitrogen) was added to the cells at the indicated time points, and the plate was incubated for another 4 h at 37 °C. 150 μl of dimethyl sulfoxide (DMSO) (Sigma) was then added and the absorbance was measured at 570 nm, with 655 nm as the reference wavelength. Three independent experiments were performed and the data were presented as the mean ± S.D.

2.9. Colony formation assay

Cells were seeded in a 6-well plate (1×10^3 cells per well) and cultured for 10 days. The colonies were then fixed with 10% formaldehyde for 15 min and stained with 1.0% crystal violet for 5 min. Three independent experiments were performed and the data were presented as the mean ± S.D.

2.10. Anchorage-independent growth ability assay

The bottom layer comprising 1% complete medium agar (Sigma) mixture was poured into a well of the 6-well plate. After solidification, cells (1×10^3) were trypsinized and suspended in 2 ml culture medium plus 0.3% agar, and then plated on top of the bottom layer. After 10 days incubation, colony sizes were measured with an ocular micrometer, and colonies greater than 0.1 mm in diameter were counted. The experiment was performed for indepedently three times for each cell line.

2.11. Flow cytometry analysis

All cells in a culture dish were harvested by trypsinization, washed in ice-cold PBS, and fixed in 80% ice-cold ethanol. Before staining, the cells were pelleted in a cooled centrifuge (4 °C, 1000 rpm, 5 min) and resuspended in the cold PBS. RNAase (2 μg/ml, Sigma) was added, and the cells were incubated at 37 °C for 30 min, followed by incubation in propidium iodide (20 μg/ml, Sigma) for 20 min. 20000 cells were analyzed on a flow cytometer (FACSCalibur; BD Biosciences, Bedford, MA, USA).

2.12. Statistical analysis

All data were expressed as the mean ± S.D. Student's *t* test was used to evaluate the significance of the differences between two groups of data in all the pertinent experiments. The *P* value reported was two-sided, and a value of *P* < 0.05 was considered statistically significant.

3. Results

3.1. miR-202 is downregulated in HCC

The basal expression of miR-202 in HCC cell lines was assessed by the real-time PCR analysis. Compared with the normal liver epithelial cells THLE3, all eight HCC cell lines used in our study (BEL-7402, MHCC97L, MHCC97H, QGY-7703, HCCC-9810, Hep3B, HepG2 and Huh7) expressed a low level of miR-202 (Fig. 1A). The comparative analysis showed that miR-202 was markedly downregulated in eight pairs of cancerous tissues compared with the adjacent non-cancerous hepatic tissues (Fig. 1B). Moreover, analysis using a published microarray-based high-throughput assessment showed that miR-202 expression is significantly downregulated in HCC tissues compared with that in matched non-cancerous hepatic tissues (*n* = 166, *P* < 0.001; NCBI/GEO/GSE31384) (Fig. 1C). Collectively, these results suggested that miR-202 was downregulated in HCC.

3.2. Ectopic expression of miR-202 inhibits HCC cell proliferation in vitro

To investigate the function of miR-202 in HCC progression, HCC cell lines QGY-7703 and HepG2 stably expressing miR-202 were established (Fig. 2A). The result of MTT assay showed that ectopic expression of miR-202 significantly inhibited the growth rate of both HCC cell lines compared with control cells (Fig. 2B). A colony formation assay further revealed that ectopic expression of miR-202 resulted in decreased cell proliferation of HCC cells (Fig. 2C). Additionally, an anchorage-independent growth assay showed a consistent result: QGY-7703-miR-202 and HepG2-miR-202 showed fewer and smaller-sized colonies (Fig. 2D), indicating that cells overexpressing miR-202 had a significantly reduced ability to form colonies in soft agarose compared with control cells. The same experiments were performed in another two HCC cell lines, BEL-7402 and MHCC97H, and the similar results were obtained (Supplemental Fig. 1A–C). Furthermore, flow cytometry was carried out to identify the specific phases of the cell cycle. As shown in Fig. 2E, the percentage of cells at G0/G1 phase in the miR-202 overexpressing group was significantly higher, and the percentage of cells at S phase was much lower, than that of the control group. These results suggested that upregulation of miR-202 inhibited the proliferative capacity and tumorigenicity of HCC cells in vitro.

3.3. Inhibition of miR-202 promotes proliferation of HCC cells

To further test whether inhibition of miR-202 results in promotion of HCC cell proliferation, a miR-202 inhibitor (miR-202-in) was used in a loss-of-function study (Fig. 3A). The results showed that suppression of miR-202 significantly enhanced the growth
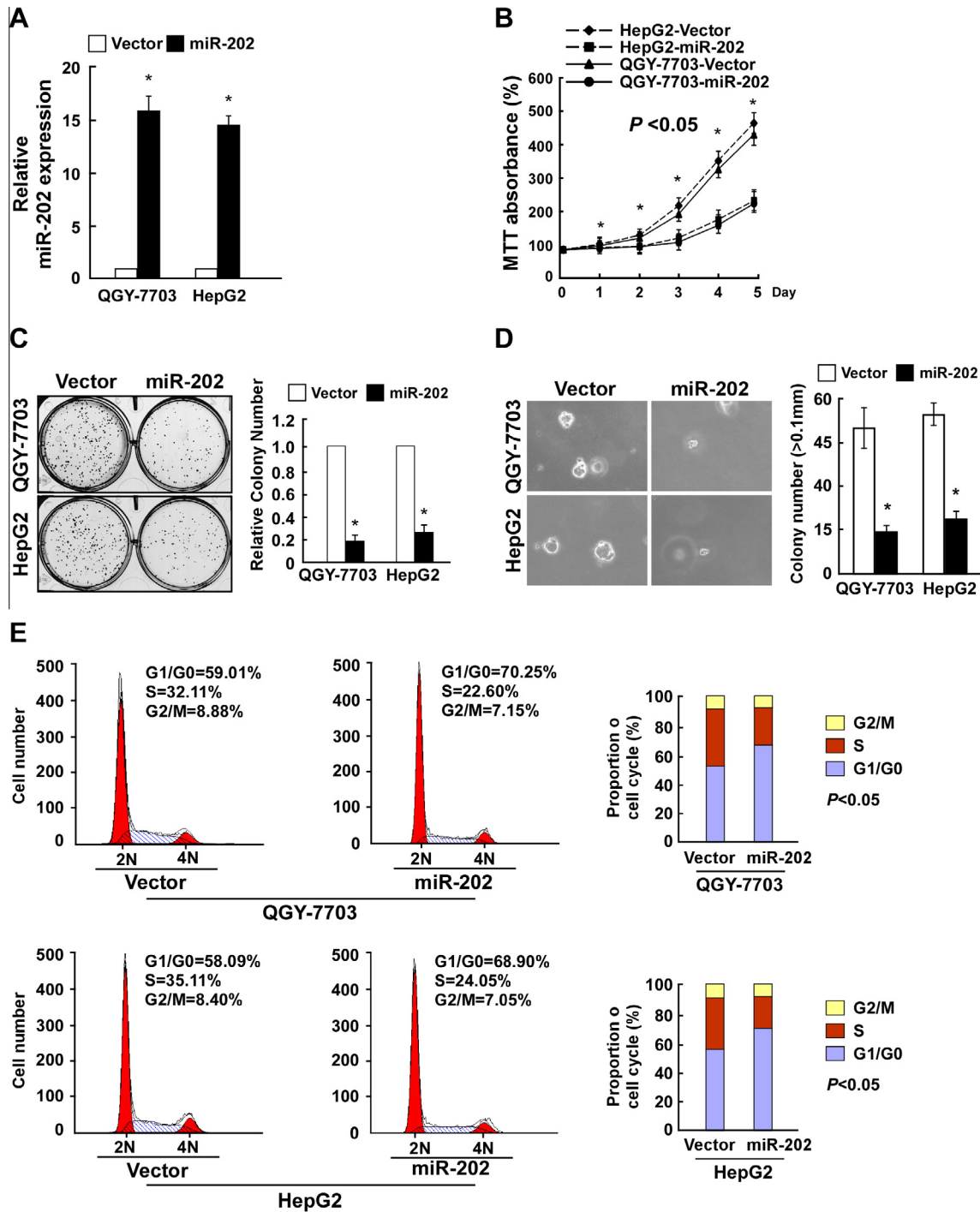
Fig. 2. Ectopic expression of miR-202 inhibits HCC cell proliferation. (A) Real-time PCR analysis of miR-202 expression in QGY-7703 and HepG2 cells stably expressing miR-202. (B) MTT-analysis of cell growth rates for different stable cell lines at indicated times after seeding. (C) Representative micrographs (left panel) and quantification (right panel) of cell colonies in the indicated HCC cell lines, as determined by a colony formation assay. (D) Representative micrographs (left panel) and quantification (right panel) of colony formation, as determined by anchorage-independent growth ability assay. Colonies larger than 0.1 mm were scored. (E) Flow cytometry analysis of indicated HCC cells. Each bar represents the mean ± S.D. of three independent experiments. *$^*P < 0.05$.

rate of both QGY-7703 and HepG2 cells transfected with the miR-202 inhibitor compared with that of NC transfected cells (Fig. 3B and C). An anchorage-independent growth assay also revealed that both cells transfected with miR-202 inhibitor produced more and larger-colonies than the negative control (NC) cells (Fig. 3D). Inhibiton of miR-202 was also performed in another two HCC cell lines, BEL-7402 and MHCC97H, and the similar results were obtained (Supplemental Fig. 2A–C). In addition, flow cytometry revealed a dramatic decrease in the percentage of cells at G1/G0 phase and

an increase in the percentage of cells at S phase in miR-202 inhibitor-transfected cells compared with NC transfected cells (Fig. 3E). These results suggested that downregulation of miR-202 promoted the proliferation and tumorigenicity of HCC cells.

3.4. LRP6 is a direct target of miR-202

To explore the mechanism underlying the growth inhibition by miR-202 in HCC cells, we used publicly available algorithms
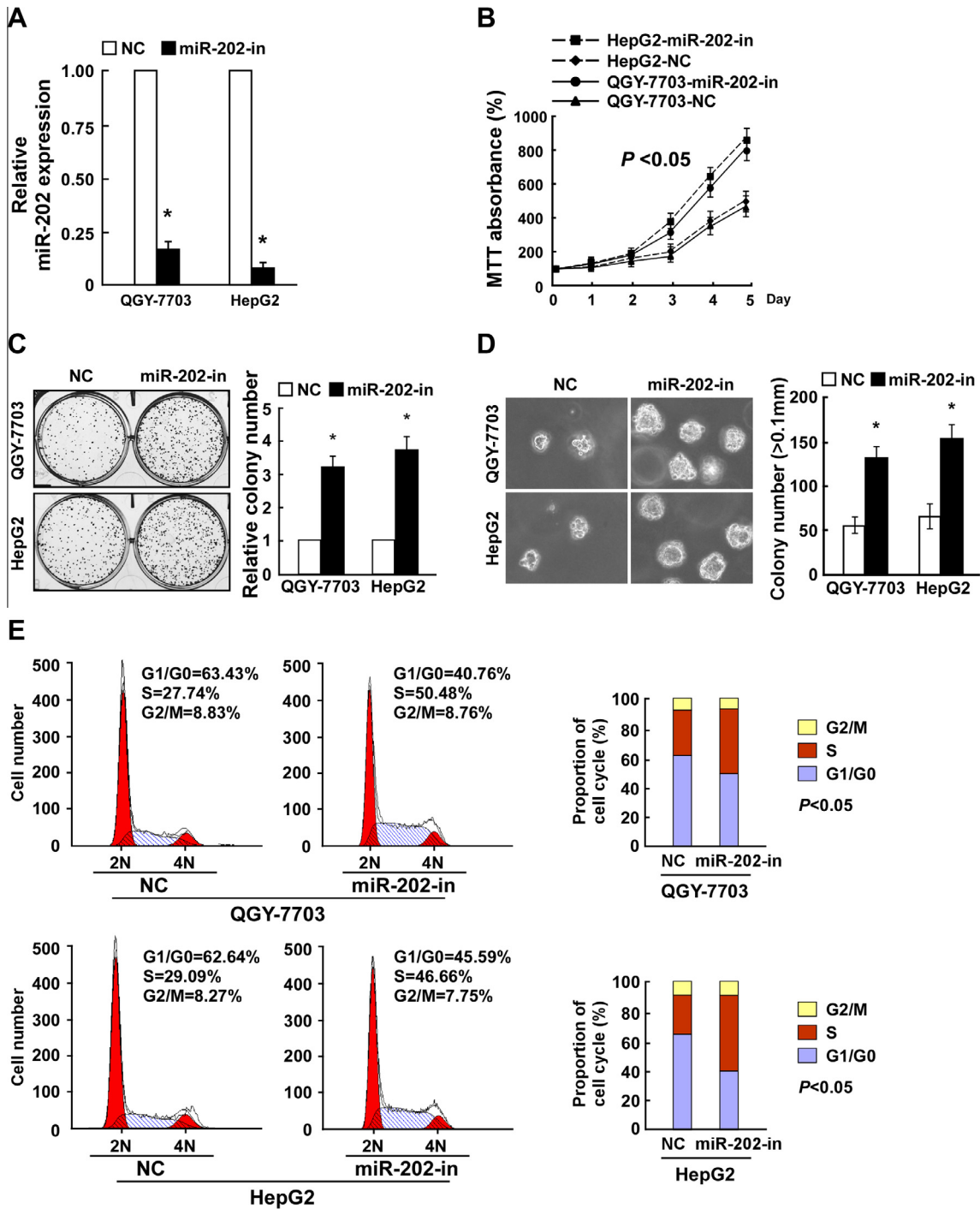
Fig. 3. Inhibition of miR-202 enhances HCC cell proliferation. (A) Real-time PCR analysis of miR-202 expression in QGY-7703 and HepG2 cells transfected with miR-202 inhibitor or NC. (B) MTT-analysis of the proliferation ability of HCC cells transfected with miR-202 inhibitor (miR-202-in) or NC analyzed. (C) Representative micrographs (left) and quantification (right) of cell colonies in indicated HCC cell lines, as determined by a colony formation assay. (D) The ability of tumorigenicity of HCC cells transfected with miR-202 inhibitor or NC, as measured by an anchorage-independent growth ability assay. Colonies larger than 0.1 mm were scored. (E) Flow cytometry analysis of indicated HCC cells transfected with miR-202 inhibitor or NC. Each bar represents the mean ± S.D. of three independent experiments. $^*P < 0.05$.

(TargetScan, Pictar, miRANDA) to predict the potential targets of miR-202 in humans. The results showed that there are two miR-202 binding sites in *LRP6* mRNA 3′UTR (Fig. 4A). As predicted, western blotting showed that LRP6 expression was downregulated by ectopic miR-202 and upregulated by miR-202 inhibitor in both QGY-7703 and HepG2 cells (Fig. 4B). To further confirm whether LRP6 is a direct target of miR-202, the pGL3-LRP6-3′-UTR-luciferase reporter was constructed, which contains two putative miR-202 binding sites. The luciferase reporter assay showed that ecto-

pic expression of miR-202 decreased the luciferase activity of the pGL3-LRP6-3′-UTR-luciferase reporter, and suppression of miR-202 increased luciferase production. By contrast, a miR-202 mutant containing three altered nucleotides in the seed sequence failed to show an inhibitory effect on the luciferase activity (Fig. 4C). It was reported previously that LRP6 is closely correlated with Wnt/β-catenin signaling activity; therefore, we examined the mRNA expression levels of downstream genes in the Wnt/β-catenin signaling pathway. The downstream genes regulated by Wnt/
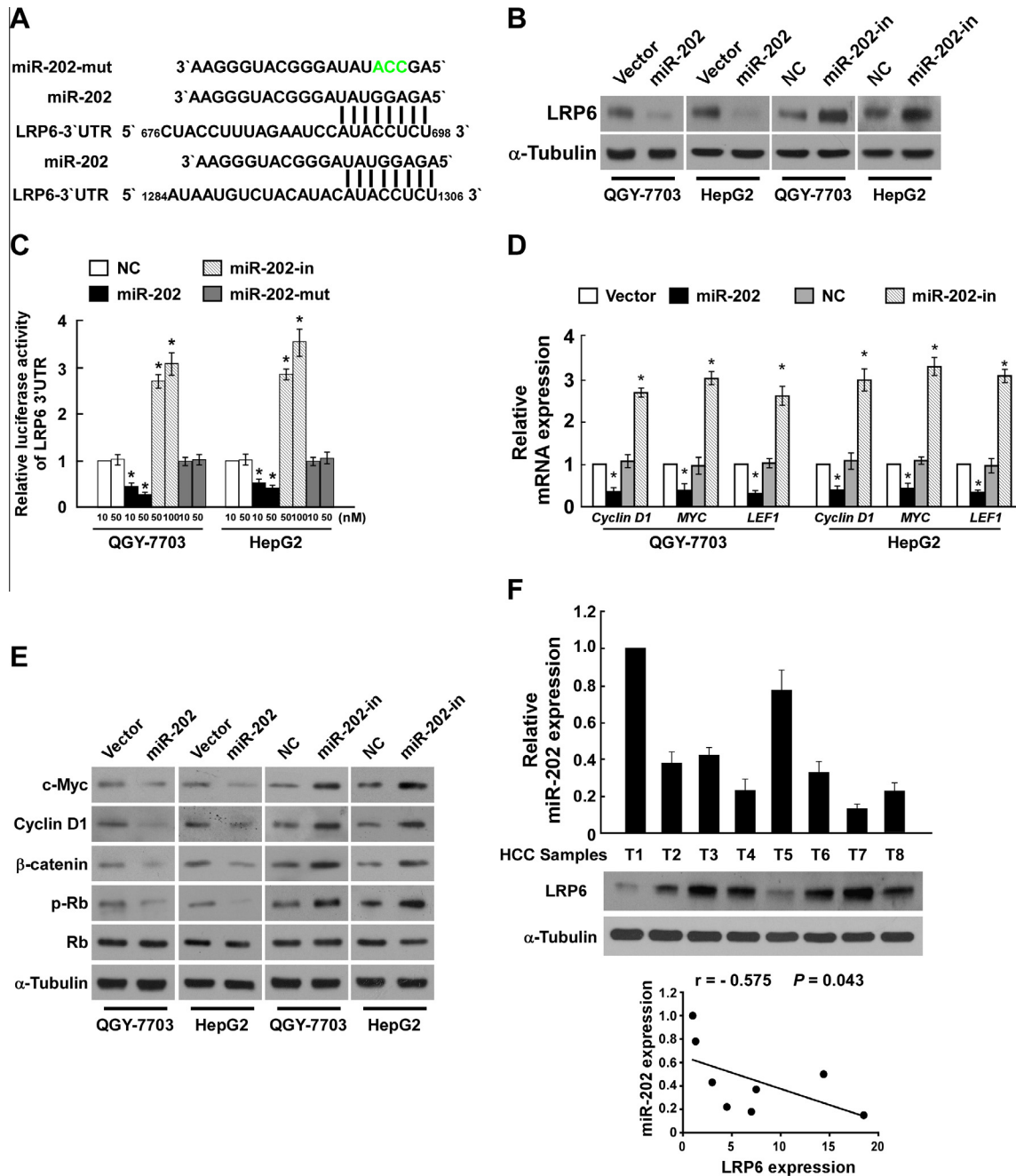
Fig. 4. *LRP6* is a direct target of miR-202. (A) Schematic representation of the mature miR-202 sequence, a miR-202 mutant containing three altered nucleotides in the seed sequence (miR-202-mut), and miR-202 target sites in the 3'-UTR of *LRP6* mRNA. (B) Western blotting analysis of the expression levels of LRP6 protein in HCC cells overexpressing miR-202 or transfected with miR-202 inhibitor, compared with NC; α-Tubulin served as a loading control. (C) Luciferase assay of pGL3-LRP6-3'UTR reporter cotransfected the miR-202 mimic or miR-202-mut (10, 50 nM) in indicated cells, or miR-202 inhibitor (50, 100 nM). (D) Real-time PCR analysis of mRNA expression of genes, *Cyclin D1, MYC* and *LEF1*, in indicated HCC cells. (E) Expression of c-Myc, Cyclin D1, phosphorylated Rb, and Rb protein, as measured by western blotting, in indicated HCC cells; α-Tubulin served as a loading control. (F) The expression of miR-202 and LRP6 protein in eight HCC tissues (Presented as T1–T8, previously used in Fig. 1B), measured by real-time PCR analysis and western blotting, respectively; The correlation between miR-202 and LRP6 expression level was analyzed by SPSS software (lower panel). Each bar represents the mean ± S.D. of three independent experiments. *$P < 0.05$.

β-catenin (*Cyclin D1, MYC* and *LEF1*) were all significantly downregulated by miR-202, and upregulated by the miR-202 inhibitor (Fig. 4D). Furthermore, the expression of c-Myc, Cyclin D1, β-catenin and phosphorylated Rb protein were downregulated in miR-202 overexpressing cells, and upregulated in cells transfected with the miR-202 inhibitor (Fig. 4E). In addition, we also examined the expression of miR-202 and LRP6 in eight HCC tissues and analyzed the correlation between them. The results showed an obviously negative correlation between the expression of

miR-202 and the LRP6 protein levels (Fig. 4F). Taken together, our results suggested that LRP6 mRNA is a direct target of miR-202 in HCC cells.

3.5. LRP6 suppression is essential for miR-202-inhibited cell proliferation in HCC

To examine the effect of LRP6 suppression on miR-202-inhibited cell proliferation in HCC, we suppressed endogenous

LRP6 expression with a LRP6-specific siRNA (Fig. 5A). Silencing LRP6 in miR-202 inhibitor or NC transfected cells decreased the growth rate and proliferation of cells, as assessed by MTT and colony formation assays (Fig. 5B and C). The anchorage-independent growth ability of miR-202-inhibited cells was also significantly suppressed in response to LRP6 inhibition (Fig. 5D). These results revealed that further silencing of LRP6 expression in miR-202-inhibited cells reversed the promoting effect of the miR-202 inhibitor on the proliferative capacity of HCC cells. It further confirmed that depletion of LRP6 is essential for miR-202-mediated inhibition of proliferation of HCC cells.

4. Discussion

Dysregulation of miRNAs frequently occurs in various human cancers, and aberrant expression of miRNAs might contribute to human carcinogenesis by affecting the expression of multiple target genes [6,8,23]. Therefore, a comprehensive understanding of the relationship between specific miRNAs and tumor development is valuable for the diagnosis and therapy of tumors.

miR-202 has been reported to function as a novel tumor suppressor in gastric cancer, and its anti-tumor activity may be attributed the direct targeting and inhibition of glioma-associated oncogene homolog 1 (Gli1) [12]. Another study showed that miR-202 directly targets proto-oncogene MYCN, resulting in the inhibition of neuroblastoma cell proliferation [24]. These previous studies indicated a possible role of miR-202 during tumor development. However, the function of miR-202 in HCC development had not been elucidated. Our study revealed that miR-202 is downregulated in HCC cell lines and tissues, indicating a potential function of miR-202 in HCC progression. Ectopic expression of miR-202 suppressed the proliferation and tumorigenicity of HCC cells in vitro, while inhibition of miR-202 led to the promotion of cell proliferation. The mechanism of the tumor-suppressive function of miR-202 might operate via downregulation of LPR6 by targeting its 3′-UTR. We demonstrated that miR-202 might have important role during HCC progression through a LRP6-mediated signal pathway.

LRP6 has been reported to function as an onco-protein and to promote the progression of various tumor s through regulation of the Wnt/β-catenin signaling pathway. The Wnt/β-catenin signaling
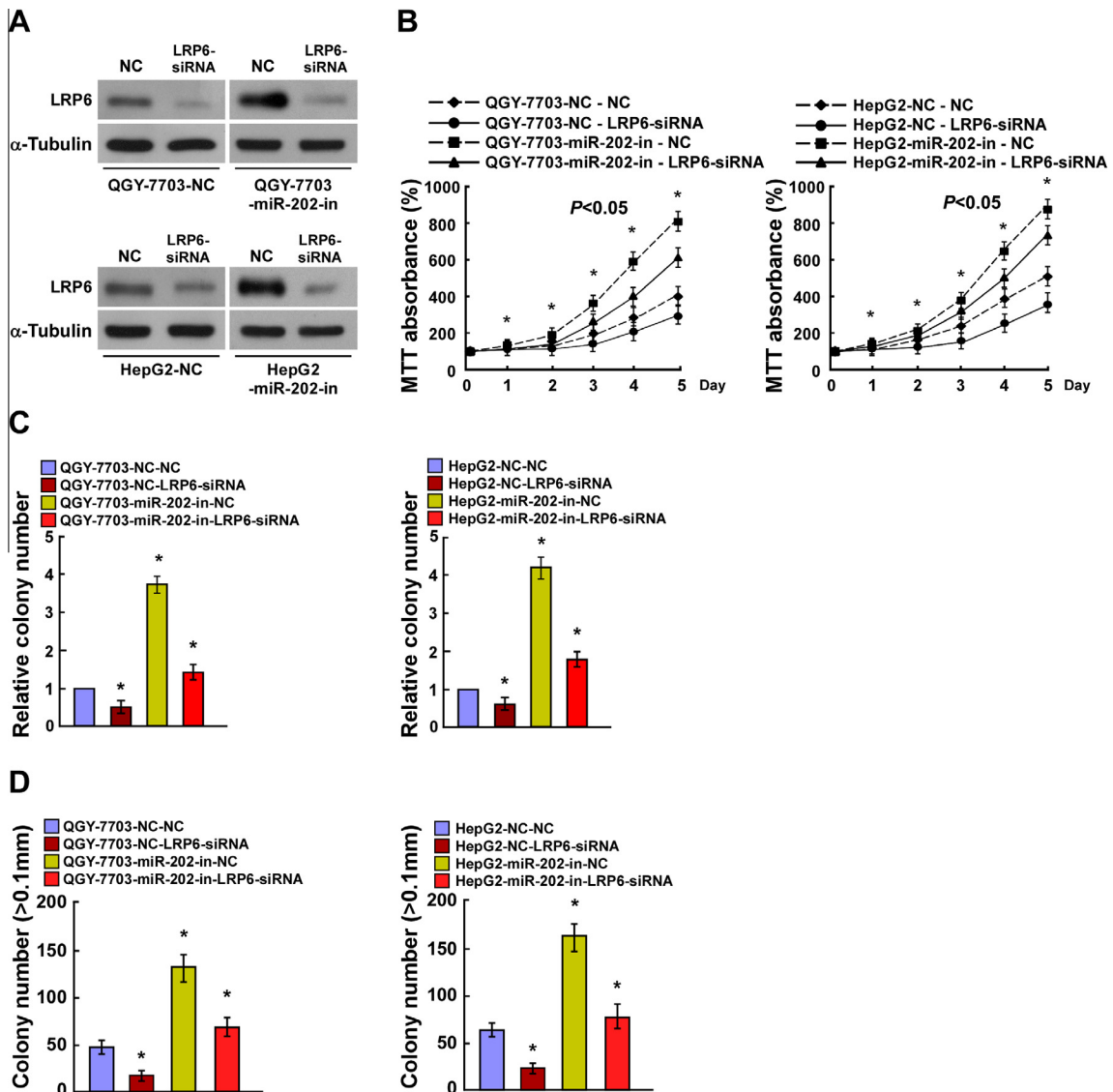

Fig. 5. miR-202 promotes proliferation of HCC cells via LRP6 inhibition. (A) Western blotting analysis of the expression levels of LRP6 in miR-202 inhibitor or NC transfected HCC cells that were transfected with a LRP6-siRNA; α-Tubulin served as a loading control. (B) MTT-analysis of the growth rates in LRP6-silenced cells. (C) Quantification of cell colonies formed by the indicated HCC cell lines 10 days after inoculation. (D) Quantification of colony numbers of indicated cells determined by an anchorage-independent growth assay; colonies larger than 0.1 mm in diameter were scored. Error bars represent the mean ± S.D. from three independent experiments. *$P < 0.05$.

Y. Zhang et al./FEBS Letters 588 (2014) 1913–1920

pathway is activated in various tumors and might play essential roles during tumor progression [25,26]. LRP6 has been reported to function as an indispensable co-receptor for the Wnt signaling pathway by interacting with several components of the pathway [18,27,28]. Overexpression of LRP6 contributes to the hyperactivation of the Wnt/β-catenin signaling pathway in human HCCs and may play a role in hepato-carcinogenesis [20]. Another report suggested that LRP6 expression increases cytosolic β-catenin levels and TCF/LEF transcriptional activity, and enhances Wnt/β-catenin signaling activity, to promote cancer cell proliferation both in vitro and in vivo [18]. In our study, we found that inhibition of LRP6 by miR-202 resulted in downregulation of the *Cyclin D1*, *MYC* and *LEF1* genes, which are the downstream target genes of the Wnt/β-catenin signaling pathway. These results indicated that the tumor suppressive function of miR-202 might be correlated with modulation of the Wnt/β-catenin signaling pathway. Considering the importance of the Wnt/β-catenin signaling pathway in tumorigenesis, including HCC, and the regulatory role of LRP6 in this signaling pathway, further study is needed to clarify the correlation between miR-202 and Wnt/β-catenin signaling.

In conclusion, the current study revealed that miR-202 is downregulated in HCC cells and tissues, and that ectopic expression of miR-202 inhibits, while anti-miR-202 enhances, the proliferation, tumorigenicity and cell cycle progression of HCC cells. Furthermore, we found that the putative oncogene *LRP6* is a direct target of miR-202, and miR-202 suppresses *LRP6* expression transcriptionally by binding to the 3′-UTR of its mRNA. Finally, we revealed that inhibition of LRP6 expression is essential for the tumor suppressive function of miR-202 during HCC progression. All the results indicated that miR-202 plays an important role during HCC carcinogenesis and may serve as a putative target for HCC diagnosis and therapy.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2014.03.030.

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