miR-106b downregulates adenomatous polyposis coli and promotes cell proliferation in human hepatocellular carcinoma

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Abstract

We investigated the role of miR-106b in the regulation of adenomatous polyposis coli (APC). We found that miR-106b downregulated APC in human hepatocellular carcinoma (HCC) cells, which promoted cell proliferation and anchorage-independent growth. These effects were mediated by silencing APC and by reducing the activity of cyclin D1, a key player in cell cycle control. The findings indicate that miR-106b may be a potential therapeutic target for HCC.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant cancers worldwide (1,2). Although the genetic events responsible for either HCC initiation or HCC progression are not clear, they involve at least three carcinogenesis pathways: the p53, Rb and Wnt/β-catenin signaling pathways (3).

Wnt/β-catenin is involved in biological processes such as cell differentiation, proliferation, movement and cell death, and it is also essential for cell development and morphogenesis (4). β-catenin plays a central role in Wnt/β-catenin signaling in the canonical pathway (5). In the absence of Wnt/β-catenin signaling, cytoplasmic β-catenin exists in a complex with axin, adenomatous polyposis coli (APC) and GSK-3β is glycogen synthase kinase-3 (GSK-3) exists as two homologs, GSK-3α and GSK-3β, and glycogen synthase kinase-3 (GSK-3β), is encoded by independent genes, which share similar kinase domains but differ substantially in their termini (4,6,7). GSK-3β is constitutively phosphorylates β-catenin at Ser33, Ser37 and Thr41 (S33/37T41), thereby facilitating ubiquitylation by a cullin 1-containing E3 ligase (also known as the SCF complex), leading to proteasomal degradation (8–11). When stimulated by Wnt, frizzled and LDL-receptor-related protein (LRP), which are essential for efficiently transducing a signal from Wnt cell surface receptors inactivate the GSK-3β kinase and stabilize β-catenin, which translocates to the nucleus and functions as a transcription cofactor of the T-cell factor (TCF) to activate the transcription of target genes, such as cyclin D1 and c-myc (12–15). Increased β-catenin expression in the cytoplasm or nucleus occurs in 50–70% of HCC patients, and its elevated level is thought to play a role in HCC development (16). APC and axin serve as essential scaffolds for GSK-3β and β-catenin binding, and impaired association of APC, axin and β-catenin leads to constitutive activation of Wnt signaling (17–19).

Entry into the cell cycle requires activation of cyclin-dependent kinases (CDKs) in the G1 phase of the cell cycle. These kinases promote expression of cell cycle genes controlled by E2F transcription factors (20). CDK inhibitors, such as p21Cip1 and p27Kip1, prevent cell cycle entry through association with cyclin/CDK complexes and inhibition of their activity. In addition, members of the retinoblastoma protein (pRb) family inhibit cell cycle entry through repression of E2F-regulated cell cycle genes (21). When activated in the G1 phase, cyclin D/CDK4, 6 and cyclin E/CDK2 kinases phosphorylate pRb, thereby preventing its association with E2F and allowing E2F transcription factors to induce S-phase gene expression.

Micro RNAs (miRNAs) are short (19–25 nucleotides) RNA molecules that can modulate the expression of a wide range of target genes by pairing homologous sequences within the 3′-untranslated region (3′-UTR) of messenger RNAs (mRNAs), thus preventing or impairing their translation or promoting RNA degradation (22–24). miR-106b is overexpressed in multiple tumor types, including breast, colon, kidney, gastric and lung cancer. miR-106b gain of function promotes cell cycle progression by modulating checkpoint functions (25–27).

In this study, we discovered that miR-106b probably promoted hepatoma cell proliferation by directly targeting the 3′-UTR of the APC mRNA, consequently leading to activation of Wnt/β-catenin pathway, nuclear accumulation of β-catenin and upregulation of the cyclin D1 cell cycle regulator. Our results suggest that miR-106b plays an important role in the development and progression of HCC.

Materials and methods

Ethics statement

For the use of clinical materials for research purposes, prior patients’ consent and approval were obtained from the Second Affiliated Hospital of Guangzhou Medical University and Nanfang Hospital of Southern Medical University Board.

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| Plasmid, small interfering RNA and transfection | The TOP-flash luciferase (plasmid 16558) and FOP-flash luciferase (plasmid 16559; Addgene, Cambridge, MA) reporters were used to measure β-catenin transcriptional activity. The T cell factor 4 dominant-negative mutant (TCF4-dn) (plasmid 16513; Addgene) was used to block Wnt/β-catenin signaling. Cyclin D1 promoter-Wt (plasmid 32727; Addgene) and cyclin D1 promoter-Mut (plasmid 32733; Addgene) were used to examine the Wnt/β-catenin-dependent regulation of cyclin D1 expression. A region of the human APC 3′-UTR from 1171 to 1507 was generated by PCR amplification from HepG2 cell genomic DNA and cloned |
into the pEGFP-C1 (Clontech, Mountain View, CA) and pGL3 (Promega, Madison, WI) vectors. The primers selected were as follows: APC-3′-UTR-FGP-up, 5′-GCCCTGACCTGAGAACAGCGCTGACA GAC-3′; APC-3′-UTR-GFP-dn, 5′-GCCCTGACCTGAGAACAGCGCTGACA GAC-3′; APC-3′-UTR-GFP-dn, 5′-GCCCTGACCTGAGAACAGCGCTGACA GAC-3′. Expression data were normalized to the geometric mean of housekeeping gene GAPDH to control the variability in expression levels.

Forward primer 5′-AGTTTGGACGCGTTCGAG-3′ and reverse 5′-CTCTTCGATATCGTGGAGCGG-3′ were synthesized from 5 ng total RNA using the TaqMan miRNA reverse transcription kit (Applied Biosystems). The expression of miRNA was defined based on expression in cultured cells and fresh surgical HCC tissues was extracted using the mirVana miRNA isolation kit (Ambion, Austin, TX) as a loading control.

Western blot analysis was performed according to standard methods as described previously (28), using anti-β-catenin (#8480, 1:2000; Cell Signaling), anti-cyclin D1 (#2926, 1:1000; Cell Signaling), anti-pRb (#9309, 1:1000; Cell Signaling) and antiphospho-α-ethylamine (pERK, #16591, 1:1000; Cell Signaling), anti-APC (#2504, 1:500, Cell Signaling), anti-cyclin D1 (anti-APC-siRNA#1, 5′-GCAACAG- AAGCAGAGAGGUU-3′ and APC-siRNA#2, 5′-GACGUUGCAAGAAGUGUUGU-3′). The membranes were stripped and re-probed with an anti-α-tubulin antibody (Sigma, St Louis, MI) as a loading control.

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RNA extraction and real-time quantitative PCR

Total miRNA from cultured cells and fresh surgical HCC tissues was extracted using the mirVana miRNA isolation kit (Ambion, Austin, TX) according to the manufacturer’s instructions. Complementary DNA was synthesized from 5 ng of total RNA using the TaqMan miRNA reverse transcription kit (Applied Biosystems). The expression of miRNA was defined based on expression in cultured cells and fresh surgical HCC tissues was extracted using the mirVana miRNA isolation kit (Ambion, Austin, TX) as a loading control.

Transfection of negative control (NC), miR-106b and miR-486 inhibitor was performed using the lipofectamine 2000 reagent (Invitrogen) according to the manufacturer’s instructions.

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miR-106b in human HCC cell lines and tissues. (A) Real-time PCR analysis of miR-106b expression in THLE3-immortalized normal liver epithelial cells and HCC cell lines, including HepG2, QGY-7703, BEL-7402, MHCC97H, HCCC9810, Hep3B, MHCC97L and Huh7. Transcript levels were normalized to U6 expression. (B) miR-106b expression was examined in paired primary HCC tissues (T) with paired adjacent normal tissues (ANT) of eight individual patients. Transcript levels were normalized to U6 expression. (C) miR-106b is elevated in HCC tissues compared with that in matched non-cancerous liver tissues (n = 166; NCBI/GEO/GSE31384). Experiments were repeated at least three times (A and B). Each bar represents the mean of three independent experiments. *P < 0.05.
miR-106b promotes hepatocellular carcinoma cell proliferation

(A) MTT assays revealed that upregulation of miR-106b induced growth of QGY-7703 and HepG2 HCC cell lines. (B) Representative micrographs (left) and quantification (right) of crystal violet-stained cell colonies. (C) Upregulation of miR-106b promoted the anchorage-independent growth of HCC cells. Representative micrographs (left) and quantification of colonies that were >0.1 mm (right). (D) Representative micrographs (left) and quantification of BrdU-incorporating cells (right) following transfection with miR-106b or NC. (E) Flow cytometric analysis of the indicated HCC cells transfected with NC or miR-106b. Each bar represents the mean of three independent experiments. *P < 0.05.

were sedimented in a chilled centrifuge and resuspended in cold PBS. Bovine pancreatic RNase (Sigma–Aldrich) was added to a final concentration of 2 µg/ml, and cells were incubated at 37°C for 30 min, followed by incubation with 20 µg/ml of propidium iodide (Sigma–Aldrich) for 20 min at room temperature. Cell cycle profiles of 5 × 10^6 cells were analyzed using a FACSCalibur flow cytometer (BD Biosciences).
Nuclear/cytoplasmic fractionation

Cells were washed with cold PBS and resuspended in buffer containing 10 mM Hepes (pH 7.8), 10 mM KCl, 0.1 mM ethylenediaminetetraacetic acid, 1 mM Na3VO4, 1 mM dithiothreitol, 1:500 protease inhibitors (Sigma) and 0.2 mM phenylmethylsulfonyl fluoride, and the cells were incubated on ice for 15 min. Detergent was added, and cells were vortexed for 10 s at highest setting. Nuclei and supernatant (‘cytoplasm’) were separated by centrifugation at 4°C. Nuclei were resuspended in buffer containing 20 mM Hepes (pH 7.8), 0.4 M NaCl, 1 mM ethylenediaminetetraacetic acid, 1 mM Na3VO4, 1 mM dithiothreitol and 1:500 protease inhibitors, and nuclei were incubated on ice for 15 min. Nuclear extracts were collected by centrifugation at 14 000 g for 10 min at 4°C.

**Fig. 3.** Inhibition of miR-106b suppresses HCC cell proliferation. (A) MTT assays revealed that inhibition of miR-106b suppressed the growth of QGY-7703 and HepG2 HCC cell lines. (B) Representative micrographs (left) and quantification (right) of crystal violet-stained cell colonies. (C) Inhibition of miR-106b impaired the anchorage-independent growth of HCC cells. Representative micrographs (left) and quantification of colonies that were >0.1 mm (right). (D) Representative micrographs (left) and quantification of BrdU-incorporating cells transfected with miR-106b inhibitor or NC. (E) Flow cytometric analysis of the indicated HCC cells transfected with NC or miR-106b inhibitor. Each bar represents the mean of three independent experiments. *P < 0.05.
miR-106b activates the Wnt/β-catenin pathway. (A) Left panel, western blotting analysis of expression of cyclin D1, phosphorylated pRb (p-pRb) and total pRb protein in indicated HCC cells. α-Tubulin served as the loading control. Right panel: Real-time PCR analysis of expression of cyclin D1 in indicated HCC cells. GAPDH was used as a loading control. (B) Cyclin D1-luciferase reporter gene assays with wild-type promoter and TCF-binding site-mutated promoter were performed in indicated cells. (C) TOP/FOP luciferase ratio reported Wnt/β-catenin pathway activity in the indicated cells. (D) Relative mRNA expression of Wnt/β-catenin-regulated genes in the indicated cells was assessed by real-time PCR. GAPDH was used as a loading control. (E) Western blotting analysis of β-catenin expression in the cytoplasm (C) and nucleus (N) of the indicated cells. Nuclear protein p84 was used as a nuclear protein marker and EF-1α as a loading control. (F) Flow cytometric analysis of the indicated HCC cells transfected with miR-106b or miR-106 combined with TCF4-dn. Each bar represents the mean of three independent experiments. *P < 0.05.
Statistical analysis
The Student’s t-test was used to evaluate the statistical significance of differences between two groups of data in all pertinent experiments. A \( P < 0.05 \) (using a two-tailed paired t-test) was thought to be significantly different for two groups of data.

Results

miR-106b is overexpressed in HCC cell lines and tissues

Real-time PCR analyses showed that expression of miR-106b was markedly upregulated in all eight analyzed HCC cell lines, including HepG2, QGY-7703, BEL-7402, MHCC97H, HCC9810, Hep3B, MHCC97L and Huh7, as compared with that in immortalized normal liver epithelial cells THLE3 (Figure 1A). To determine whether the upregulation of miR-106b in HCC cell lines is clinically relevant, we further examined the miR-106b expression in eight pairs of HCC tissues and matched tumor-adjacent tissues. As shown in Figure 1B, the comparative analysis indicated that miR-106b was differentially overexpressed in all eight examined tumor tissues paired with adjacent non-cancerous tissues from the same patient. Importantly, consistent with our results, a published microarray-based high-throughput assessment has shown that miR-106b was elevated in HCC tissues compared with that in matched non-cancerous liver tissue (\( n = 166; P < 0.001; \) NCBI/GOE/GSE31384) (Figure 1C). Taken together, these results indicate that miR-106b is upregulated in HCC.

miR-106b overexpression increases HCC cell proliferation

To explore the role of miR-106b upregulation in the development and progression of HCC, we next examined its effect on cellular proliferation. An MTT assay showed that miR-106b upregulation significantly increased the proliferation rate of QGY-7703 and HepG2 HCC cells (Figure 2A), and this was further confirmed by a colony formation assay (Figure 2B). Strikingly, we found that enforced expression of miR-106b in QGY-7703 and HepG2 HCC cells drastically enhanced their anchorage-independent growth ability (Figure 2C), as shown by increased colony numbers and sizes, thus suggesting that miR-106b upregulation enhances HCC cell tumorigenicity in vitro.

Using a BrdU incorporation assay, we found that the percentage of cells in S phase was dramatically increased in miR-106b-overexpressing QGY-7703 (53.25%) and miR-106b (50.83%) cells compared with control cells (QGY-7703 cells, 30.54%; HepG2 cells, 28.93%; Figure 2D). Similarly, flow cytometry showed that miR-106b overexpression increased the percentage of cells in S phase and significantly decreased the percentage of cells in G1/G0 (Figure 2E). Collectively, our results suggest that miR-106b may mediate HCC cell proliferation through regulation of G1/S transition.

miR-106b inhibition reduces HCC cell proliferation

We further examined the effect of miR-106b inhibition on HCC cell proliferation. Consistent with the above-mentioned results, MTT and colony formation assays showed that miR-106b suppression dramatically inhibited the growth rate of both QGY-7703 and HepG2 HCC cells as compared with that of control cells transfected with NC (Figure 3A and 3B). In addition, the anchorage-independent growth ability of QGY-7703 and HepG2 HCC cells was significantly decreased in response to miR-106b inhibitor (Figure 3C). Furthermore, we found that transfection of the miR-106b inhibitor drastically decreased the percentage of cells in S phase but increased that in the G0/G1 peak (Figure 3D and 3E), suggesting that the antiproliferative effect of miR-106b inhibition in HCC cells occurs through induction of G0/S arrest.

miR-106b activates the Wnt/\( \beta \)-catenin pathway

Consistent with the above-mentioned results, pRb phosphorylation was increased in miR-106b-overexpressing cells and decreased in the miR-106b-inhibited cells, thus providing further evidence that miR-106b plays an important role in HCC cell proliferation (Figure 4A). Interestingly, both protein and mRNA levels of cyclin D1, a CDK regulator important for regulating the G0/S transition (29–31), were upregulated in QGY-7703 and HepG2 HCC cells transfected with miR-106b mimic, but decreased in the cells transfected with miR-106b inhibitor, relative to control cells (Figure 4A).

Cyclin D1 is reported to be regulated by the Wnt/\( \beta \)-catenin pathway (14, 32, 33), which is hyperactivated in HCC (34–36). Indeed, the stimulatory effect of miR-106b on the cyclin D1 expression was abolished by the inhibition of Wnt/\( \beta \)-catenin signaling via TCF dominant-negative mutant transfection (Figure 4A, right panel). Moreover, overexpression of miR-106b increased the luciferase expression of cyclin D1-luciferase reporter with wild-type promoter to about 3.11-fold and 2.89-fold in QGY-7703 and HepG2 cells, respectively. However, this effect could be robustly impaired using the cyclin D1-luciferase reporter with TCF-binding site-mutated promoter (Figure 4B). Altogether, our results indicated that miR-106b might activate Wnt/\( \beta \)-catenin signaling. As expected, the \( \beta \)-catenin/TCF activity and the expression levels of six classically recognized Wnt/\( \beta \)-catenin target genes significantly increased in the miR-106b-overexpressing HCC cells but decreased in the miR-106b-inhibited cells (Figure 4B and 4C). Furthermore, cellular fractionation showed that miR-106b overexpression promoted nuclear accumulation of \( \beta \)-catenin (Figure 4D), indicating that miR-106b activates Wnt/\( \beta \)-catenin pathway through promoting nuclear \( \beta \)-catenin accumulation. Moreover, we observed that inhibition of Wnt/\( \beta \)-catenin signaling by transfection of a TCF dominant-negative mutant led to a dramatic decrease in the S peak but increase in the G0/G1 peak population in the miR-106b-overexpressing cells (Figure 4E), suggesting that activation of Wnt/\( \beta \)-catenin pathway is functionally relevant to miR-106b-mediated proliferation.

miR-106b directly targets APC in HCC cells

Mammals carry two APC genes: APC (APC1/APC1) and APC2, and both APC1 and APC2 can inhibit the Wnt/\( \beta \)-catenin signaling (37, 38). By analysis with the use of two publicly available algorithms (TargetScan and miRanda), we found that APC1/APC1 mRNA, but not APC2 mRNA, is theoretically the target gene of miR-106b (Figure 5A). Importantly, western blotting analysis showed that ectopic expression of miR-106b dramatically decreased, but inhibition of miR-106b increased, APC protein expression in both QGY-7703 and HepG2 HCC cells (Figure 5B). To examine whether miR-106b downregulation of APC was mediated by the 3′-UTR of APC, we subcloned the APC 3′-UTR fragment, containing the miR-106b binding site, into pEGFP-C1 and pGL3 dual luciferase reporter vectors. As shown in Figure 5C, overexpressing miR-106b only decreased expression of a green fluorescent protein (GFP) vector containing the APC 3′-UTR, but it had no effect on GFP-\( \gamma \)-tubulin expression, suggesting that miR-106b specifically affected the 3′-UTR of APC. Furthermore, a consistent and dose-dependent reduction of luciferase activity was observed following miR-106b transfection in both HCC cells, whereas the repressive effect of miR-106b on the luciferase activity of APC 3′-UTR was abolished by miR-106b inhibitor (Figure 5D). Meanwhile, overexpressing miR-106b had no effect on the luciferase activity of APC 3′-UTR-mut, containing point mutations in the miR-106b-binding seed region of APC 3′-UTR (Figure 5E). Collectively, these results demonstrate that APC is a bona fide target of miR-106b.

APC downregulation is required for miR-106b-induced proliferation of HCC cells

To further investigate the role of APC repression in miR-106b-induced HCC proliferation, we first examined the effects of APC downregulation on HCC cell proliferation. As predicted, APC silencing by two specific APC siRNAs significantly increased the percentage of cells in S phase (Figure 5F). However, further overexpression of miR-106b in the APC-silenced cells did not have an additive effect on cells proliferation, as analyzed by the BrdU incorporation assay (Figure 5F). Importantly, G0/S arrest induced by miR-106b inhibition was abrogated by APC siRNAs (Figure 5G). Taken together, these results demonstrate that direct APC downregulation is required for miR-106b-induced HCC cell proliferation.
The key finding of this study is that miR-106b expression was markedly upregulated in HCC cells and HCC tissues as compared with that in immortalized normal liver epithelial cells and normal hepatic tissues. Furthermore, ectopic expression of miR-106b enhanced the proliferation and anchorage-independent growth of HCC cells, whereas miR-106b inhibition had the opposite effect. Moreover, we demonstrated that miR-106b upregulation in HCC cancer cells led to the upregulation of cyclin D1 cell cycle regulator through downregulation of APC via directly targeting the 3′-UTR of APC. These findings suggest that deregulation of miR-106b may play an important role in promoting carcinogenesis and progression of HCC.

HCC is the most frequent primary malignancy of the liver and accounts for >5% of all cancers worldwide. The worldwide incidence is estimated to be between 250 000 and 1.2 million new cases each year, and the disease causes 500 000 to 1 million deaths annually (39,40). Additionally, HCC mortality displays the fastest rate of increase in the USA, whereas the overall cancer-related mortality has been declining (41).

Wnt/β-catenin signaling controls fundamental cellular processes during tissue homeostasis, including proliferation, and aberrant activation of this pathway is implicated in a wide range of human cancers (4). β-Catenin is the major cellular effector of the Wnt signaling and is normally captured by a complex of axin, APC, glycogen synthase kinase-3β and casein kinase 1α and targeted for proteasome-mediated degradation following phosphorylation and ubiquitination (5–7).

Discussion

The key finding of this study is that miR-106b expression was markedly upregulated in HCC cells and HCC tissues as compared with that in immortalized normal liver epithelial cells and normal hepatic tissues. Furthermore, ectopic expression of miR-106b enhanced the proliferation and anchorage-independent growth of HCC cells, whereas miR-106b inhibition had the opposite effect. Moreover, we demonstrated that miR-106b upregulation in HCC cancer cells led to the upregulation of cyclin D1 cell cycle regulator through downregulation of APC via directly targeting the 3′-UTR of APC. These findings suggest that deregulation of miR-106b may play an important role in promoting carcinogenesis and progression of HCC.

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Ablerrant activation of Wnt/β-catenin signaling results in enhanced cell growth and malignant cellular transformation. Although Wnt/β-catenin signaling is frequently activated in HCC, causes of its activation are not well understood. Oncogenic β-catenin mutations, inactivating APC mutations, upregulation of frizzled-type receptors and/or other
alterations in Wnt signaling pathway play important roles in more than 33–67% of HCCs (42–44). Somatic mutations of β-catenin have been observed in only 12–26% of HCCs (16,45), but cytoplasmic and nuclear accumulation of β-catenin are more common, occurring in 40–70% of HCCs (46). APC is crucial for β-catenin capture in the degradation complex and binds β-catenin directly through its central armadillo repeats (47). Upon Wnt activation, accumulated β-catenin enters the nucleus and binds to TCF/lymphoid-enhancer-factor family transcriptional factors to induce target gene expression (12,13). A key event in both Wnt signaling transduction and cancer cell proliferation is the regulation of β-catenin stability and activity.

miRNAs, a class of small regulatory RNA molecules that negatively regulate target mRNAs in a sequence-specific manner, have been demonstrated to play important roles in multiple biological processes, such as cellular differentiation, proliferation, oncogenesis, angiogenesis, invasion and metastasis, and can function as either tumor suppressors or oncogenes (48). Recent evidences indicate that miR-106b participates in the development and progression of human cancers, such as hepatocellular cancer, prostate cancer, gastric cancers, laryngeal carcinoma and renal cell carcinoma (25–27,49,50). In this study, we found that miR-106b was significantly upregulated in HCC cells as compared with that in immortalized normal liver epithelial cells THLE3. Meanwhile, miR-106b was shown to be upregulated in HCC tissues in comparison to that in adjacent non-cancerous tissues from the same patient. Furthermore, we demonstrated that ectopic miR-106b expression drastically increased the growth rate of QGY-7703 and HepG2 cells as compared with that of controls, whereas suppression of miR-106b inhibited cell proliferation and colony formation on soft agar, indicating that upregulation of miR-106b may correlate with clinical HCC progression and that miR-106b may function as an onco-miRNA.

miR-106b is a member of the miR-106b-25 cluster. Several genes have been identified as miR-106b targets, including p21/CDKN1A and the transforming growth factor-β type II receptor. Ivanovska et al. reported that miR-106b gain of function promotes cell cycle progression, whereas loss of function reverses this phenotype. In addition, p21/CDKN1A is a direct target of miR-106b and its silencing plays a key role in miR-106b-induced cell cycle phenotypes (51). In the pathogenesis of Alzheimer’s diseases, miR-106b regulates transforming growth factor-β type II receptor expression via binding to the 3’-UTR of transforming growth factor-β type II receptor mRNA, thereby impairing TGF-β signaling (52). Through bioinformatics analysis, the APC tumor suppressor gene was indicated as a theoretical miR-106b target gene. We were able to demonstrate APC as a bona fide target of miR-106b using different methods. Western blotting analysis showed that ectopic miR-106b expression reduces the level of APC protein. Real-time PCR analysis showed that β-catenin downstream targets, including MYC, CYR61, CD44, Snail, TCF4, RUNX2 and cyclin D1, were significantly upregulated in miR-106b-transfected HCC cells. Additionally, a luciferase reporter and point mutation analysis demonstrated that APC downregulation is mediated by miR-106b through the APC 3’-UTR. Furthermore, overexpression of miR-106b in the APC-silenced cells did not have an additive effect on cells proliferation, whereas G1/S arrest induced by miR-106b inhibition was abrogated by APC siRNAs, suggesting that direct APC downregulation is required for miR-106b-induced HCC cell proliferation. The biological function of miR-106b in protection against apoptosis and in cell survival, as related to APC function, is currently under investigation in our laboratory.

In summary, we have showed that miR-106b is an oncogenic miRNA in HCCs and that APC is a novel and critical miR-106b target. Therefore, miR-106b might be a potential therapeutic target for HCC that requires more in-depth analysis.

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**References**

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