

MiRNA-200b-3p represses LRH-1 expression and is associated with reduced serum bile acid in human obstructive cholestasis

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Abstract

Background

MicroRNAs are closely associated with liver diseases including cholestasis, but the roles of miR-200 family in cholestasis has not been determined. Liver receptor homolog-1 (LRH-1/NR5A2) plays an important role in maintaining bile acid homeostasis by regulating some genes, including CYP7A1 and CYP8B1, but the mechanistic details remain to be elucidated in human cholestasis.

Methods

The mRNA and or protein expressions of miR-200b-3p, LRH-1, CYP7A1 and CYP8B1 in human liver tissues (11 controls and 24 cholestasis) were analyzed using RT-PCR or Western blot. HepG2 cells was transfected with miR-200b-3p mimic to determine gene expressions. We used luciferase gene reporter assay to identify the potential response element of miR-200b-3p in LRH-1 gene.

Results

MiR-200b-3p was increased for about 2 folds in cholestatic liver tissues, which were divided into low and high expression group. Serum TBA was negatively correlated with miR-200b-3p in miR-200b-3p high expression group. Levels of LRH-1, CYP8B1 and CYP7A1 were decreased, LRH-1 and CYP8B1 were negatively correlated with miR-200b-3p in miR-200b-3p high expression group. MiR-200b-3p mimic was transfected into HepG2 cells, the levels of LRH-1, CYP7A1 and CYP8B1 were significantly repressed by 45%-55%. Bioinformatics analysis revealed the potential binding site of LRH-1 and miR-200b-3p and luciferase reporter gene assay showed that miR-200b-3p might directly bind with LRH-1.

Conclusions

Our findings indicate that miR-200b-3p represses liver LRH-1, which in turn decreases bile acid synthesis, suggesting that miR-200b-3p may be a potential therapeutic target for cholestasis.

Background

Cholestasis is a condition that bile flow is impaired and result in bile acids accumulated in the liver and systemic circulation. Many genes are involved in maintaining bile acid homeostasis, including membrane transporters, nuclear receptors, as well as bile acid synthesis enzymes such as cytochrome P450 family 7 subfamily A member 1 (CYP7A1). CYP7A1 is the rate-limiting enzyme in converting cholesterol to bile acid in the liver[1, 2], which is tightly regulated in response to bile acid homeostasis. CYP7A1 was found to be reduced not only in patients with cholestasis and alcoholic hepatitis, but also in bile duct ligated (BDL) mice[3, 4].

The expression of CYP7A1 is positively regulated by hepatic nuclear factor 4 alpha (HNF4α) and liver receptor homolog-1 (LRH-1)[5, 6], whereas it is negatively regulated by farnesoid X receptor (FXR)/small

heterodimer (SHP) signaling pathway[6, 7]. This negative regulation is mediated through LRH-1. When the bile acid nuclear receptor FXR is activated, its target SHP expression is increased. SHP then binds to LRH-1 and represses its transcriptional activity, leading to decreased CYP7A1 expression[8, 9]. LRH-1 is encoded by the NR5A2 gene. It is mainly expressed in liver, intestine and pancreas and it regulates the expressions of CYP7A1 and CYP8B1, which are critical in maintaining the homeostasis of bile acid and cholesterol[10–13].

MicroRNAs (miRNAs) are a kind of small non-coding RNA, which contain about 22 nucleotides and play an important role in regulating the expression of genes by RNA silencing. They participate in both physiological and pathological processes. Altered expression of miRNAs has been associated with cholestatic liver diseases[14–17] both in the serum and liver tissue, including primary biliary cholangitis (PBC) and primary sclerosing cholangitis[16, 18].

MiR-200b-3p is a member of miR-200 family, which is initially found associated with cancer metastasis, chemotherapeutic resistance and epithelial-mesenchymal transition (EMT)[19–22]. Recent studies revealed that miR-200b was also related with cholestatic liver injury. Xiao, et al reported that miR-200b significantly affected the development and progression of liver fibrosis[23]. Upregulation of miR-200b promoted hepatic stellate cell proliferation and migration through PI3K/Akt signaling pathway in cholestatic patients[24]. Furthermore, upregulation of miR-200 and downregulation of miR-124 contributed to cholangiocyte proliferation in the liver of patients with biliary atresia (BA)[25]. However, it is not known whether miR-200b plays any roles in obstructive cholestasis.

In this study, we sought to investigate the functional role of miR-200b-3p in cholestatic liver diseases. We examined the expression of miR-200b-3p in human obstructive cholestatic patients and the relationship between miR-200b-3p and serum total bile acid (TBA). We found that increased expression of miR-200b-3p is associated with lower expression of LRH-1 and CYP7A1 in obstructive cholestatic patient livers. Further mechanistic studies indicate that miR-200b-3p repressed LRH-1 mRNA expression and resulting in decreased CYP7A1 expression. These findings not only reveal the functional role of miR-200b-3p in cholestatic liver diseases but also provide a therapeutic target for treating cholestatic liver injury.

Methods

Patients and liver samples collection

24 cholestatic liver samples were obtained from patients with pancreatic carcinoma, intrahepatic bile duct calculi and gallbladder cancer, and 11 control liver samples were obtained from patients suffering the resection of liver metastasis that without cholestasis. All patients had the typical symptoms of human obstructive cholestasis, including itchiness, jaundice, dark urine and so on; the level of ALP was 1.5 times higher than the upper limit of normal (ULN), and the level of GGT was 3 times higher than ULN. All patients provided written informed consent. Individuals were excluded if they were suffering with liver diseases, including autoimmune liver disease, virus hepatitis, hepatic cancers, heart dysfunction, renal

dysfunction, etc. Liver samples were obtained and were immediately cut into small pieces and then stored in liquid nitrogen until usage. The general clinical characteristics and features of the control group and the patient group were shown in Table 1 and Table 2. No significant differences were found in age and sex between the two groups, however, significant differences were found in AST, ALT, ALP, GGT, TBIL, DBIL, IBIL and TBA between the two groups ($p < 0.05$). This research was approved by the Faculty of Medicine's Ethics Committee of Southwest Hospital, in agreement with the Helsinki declaration.

Table 1
Clinical characteristics of control group and patient group

Parameters	Patient group (n = 24)	Control group(n = 11)	χ^2/t	p
Age(years)	53.73 ± 11.59	56.63 ± 11.24	0.702	0.488
M/F (cases)	15/9	7/4	0.004	0.949
AST(IU/L)	137.38 ± 84.05	22.25 ± 7.13	6.658	<0.01
ALT(IU/L)	180.93 ± 152.49	25.45 ± 20.77	4.897	<0.01
ALP(IU/L)	667.33 ± 557.99	89.09 ± 18.39	5.071	<0.01
GGT(IU/L)	772.04 ± 692.18	31.73 ± 25.86	5.232	<0.01
TBIL($\mu\text{mol/L}$)	215.20 ± 122.44	19.19 ± 18.68	7.651	<0.01
DBIL($\mu\text{mol/L}$)	106.66 ± 65.47	6.02 ± 10.22	7.338	<0.01
IBIL($\mu\text{mol/L}$)	95.14 ± 63.44	13.17 ± 8.80	6.201	<0.01
TBA($\mu\text{mol/L}$)	116.05 ± 94.16	6.70 ± 3.87	5.678	<0.01

Abbreviations: M, male; F, female; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, γ -glutamyl transpeptidase; TBIL, total bilirubin; DBIL, direct bilirubin; IBIL, indirect bilirubin; TBA, total bile acids.

Table 2
Clinical features of patient group

Clinical feature	patients(n = 24)
Pancreatic head carcinoma	15
Duodenal papilla carcinoma	4
Cholangiocarcinoma invades the pancreas	2
Intrahepatic bile duct calculi	2
Gallbladder cancer	1

Cell culture and transfection

HepG2 cell line was obtained from American Type Culture Collection (ATCC; Manassas, VA, USA) and the cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Rockville, MD, USA) with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) at 37 °C and 5% CO₂ in a culture incubator. The human miR-200b-3p mimic, mimics negative control (NC), miR-200b inhibitor and inhibitor NC were purchased from RiBo Biotech (GuangZhou RiBo Biotech, GuangZhou, China). HepG2 cells were transfected with miR-200b-3p mimic and NC by using X-tremeGENE HP DNA Transfection Reagent (Roche, IN, USA) according to its instructions.

RNA extraction and quantitative real-time PCR

Total RNA of tissue samples and cultured cells was extracted using Eastep® Super Total RNA Extraction Kit (Promega, Shanghai, China). Reverse transcription was performed to generate cDNA using PrimeScript™ RT reagent Kit (Takara Biotech, Tokyo, Japan). MiR-200b primers and U6 primers were purchased from RiBo Biotech (GuangZhou, China). The TaqMan probes of CYP7A1 (Hs00167982_ml) and GAPDH (Hs02758991_gl) were purchased from Applied Biosystems, ABI. Real-time quantitative PCR was performed in a Bio-Rad CFX96 real time system machine. Oligo DNA primers for PCR were synthesized by Gene Pharma (TsingKe Biological Technology, Beijing, China) and sequences were listed in Table 3. SYBR® premix Ex Taq™ II kit (Takara Biotech, Tokyo, Japan) was used to detect the mRNA levels of miR-200b and U6, and a LightCycler® 480 Probes Master (Roche, IN, USA) was used to detect the mRNA levels of CYP7A1 and GAPDH. The mRNA expressions were normalized to U6 or GAPDH and 2- $\Delta\Delta$ CT method was used to detect the relative gene expression.

Table 3
Sequences of primers for RT-PCR

Gene Names	Forward Primer (5'→3')	Reverse Primer (5'→3')
CYP27A1	CTCACTCTATGCCACCTTCCTC	CATTCCAACCATCCAGGTATC
CYP7B1	GGAGCACATCATTTAGGCTTTC	CCCTTTCTTTTGACCTGTTGAC
CYP8B1	CCCTCTTTCCCTACCTCTCAGT	AAGTGTGTGACCATAAGCAGGA
CYP3A4	AGTGGAAAACCTCAAGGAGATGG	CGATGTTCACTCCAAATGATGT
FXR	CCTCCTCACCTCATTGTCTC	ACCTGCCACTTGTTCTGTTA
HNF1 α	GATGAGCTACCAACCAAGAAGG	CCTATTGCACTCCTCCACTAGC
HNF4 α	ACCTCAAAGCCATCATCTTCTT	GTCATACTGGCGGTCGTTG
SHP	AGAATATGCCTGCCTGAA	TGGTCGGAATGGACTTGA
LRH-1	CCACTCTCCATCTTTTCGGGG	GGACTTGCTTAGGCCACACT
GSTM1	CTACTTGATTGATGGGGCTCAC	ATGGTCTGGTTCTCCAAAATGT
GSTM2	ACCTTCCTTCCTGTTAGTG	TCAATGCTGCTCCTTCAT
GSTM3	GCTCACCTTTGTGGATTTTCTC	TGTAAGTAGGCAGCGATTTTCTC
GSTM4	ACTTTCCCAATCTGCCCTACTT	TAGCCTGGTTCTCCAAAATGTC
GSTA1	AATGGTTGAGATTGATGGGATG	GGGCTCTCTCCTTTATGTCTTTC
GSTA2	CCTTCTTCTGCCCTTTAGTCAA	GTAGTCTTGTCCGTGGCTCTTT
GSTA3	TGAAAACCAGAATCAGCAACCT	ACAACAGGCACAATCAACACTT
GSTA4	GAGTCCGTGAGATGGGTTTTAG	TTGGAACAGCAGGTGGTTAC
UGT2B4	CCTATGTGCCTGTTGTTATGTCA	AACATTTGGTAAGAGTGGGTGAG
UGT2B7	CTCTGGGGTCAATGGTCAGTA	CCACTTGTAGAGCCGAGTATTG
SULT2A1	AACAGGACACAGGAAGAACCAT	CAGTCCCCAGATACACCTTTTC
MRP2	CATTCTGCATGGCAGGACCTA	GGTGAGGAAGGCCCAAAACT
MRP3	AAAAGCAGACGGCACGACA	GCAGGCACTGATGAGGAAGC
OST α	AACACCCTTTGCCCCATCAA	ACGAGGGATCCAGAGACCAA
OST β	CTCTCTGTGTCGTAGGCGTG	CATGTGTGGAGGAGCACTGT
GAPDH	GGAGTCCACTGGTGTCTTCA	GGGAACTGAGCAATTGGTGG

Western blot analysis

Total protein was extracted from HepG2 cells using a RIPA lysis buffer (Sigma, Saint Louis, Missouri, USA) with protease inhibitor (Roche, Palo Alto, CA, USA) and phosphatase inhibitor (Roche, Palo Alto, CA, USA). Proteins were separated using 10% SDS-PAGE and then transferred to PVDF membrane at room temperature. The membranes were incubated with primary antibody overnight at 4 °C and subsequently incubated with matched secondary antibodies. The dilutions of primary antibodies are: CYP7A1 1:2000 (Santa Cruz Biotechnology, Santa Cruz, CA, USA); GAPDH 1:5000 (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Protein bands were detected through enhanced chemiluminescence (ECL) by utilizing a Pierce ECL Western blot substrate (Thermo Fisher Scientific, Shanghai, China) and exposed to an X-ray film using an ECL detection system (Thermo Fisher Scientific, Shanghai, China).

Luciferase gene reporter assays

The potential binding sequences of miR-200b-3p and LRH-1 was predicted using software from www.targetscan.org, which was shown in Fig. 4A. The pmirGLO-basic vectors containing the 3'UTR region of LRH-1 and its truncated mutants were produced by TSINGKE Biological Technology (TSINGKE Biological Technology, Beijing, China), which were named as pmirGLO-LRH-1-wt and pmirGLO-LRH-1-mut. pmirGLO vectors or miR-200b-3p mimic were transfected into HepG2 cells. Twenty-four hours post-transfection, luciferase activities were assayed using dual-luciferase reporter assay kit from Promega (Madison, WI, USA).

Statistical analysis

All data were analyzed by SPSS version 21.0 (SPSS Inc., Chicago IL, USA). Data were expressed as means \pm SD. The methods of independent-sample t-test and one-way ANOVA were used to analyze these data, and multiple comparison between these groups was performed by using S-N-K method. The value of $p < 0.05$ was statistically significant.

Results

MiR-200b-3p was upregulated in human cholestatic liver tissues.

To determine whether miRNAs play any roles in human obstructive cholestasis, we initially performed microarray analysis using liver samples from 3 cholestatic patients and 3 control patients. We found that miR-200b-3p expression was increased in the cholestatic patients along with miR-363-5p and miR-3609, while miR-4665-5p was downregulated. To verify these observations, we performed RT-PCR to measure the levels of these four miRNAs using RNA extracted from twenty-four cases of human obstructive cholestatic liver tissues and eleven cases of control liver tissues. We found that miR-200b-3p was significantly upregulated for 2 folds in cholestatic liver tissues when compared with the healthy controls ($p < 0.05$) (Fig. 1A). In contrast, the expressions of miR-363-5p, miR-4665-5p and miR-3609 were not changed (Fig. 1B-D). Of note, previous reports indicated that the expression of these three microRNAs were significantly altered in hepatocellular carcinoma, colorectal cancer, pancreatic cancer, etc[26–29].

Together, these findings indicate that the altered expression of miR-200b-3p is specific to obstructive cholestasis in humans.

Elevated levels of miR-200b-3p is associated with lower serum TBA in cholestatic patients.

To understand the functional role of miR-200b-3p in human obstructive cholestasis, we first performed correlation analysis of miR-200b-3p expression with liver function indexes in all patients. We found that miR-200b-3p was negatively correlated with serum total bile acids (TBA) in some extent, especially in patients with high expression of miR-200b-3p. In order to further determine whether miR-200b-3p was correlated with TBA, cholestatic patients were divided into two groups, i.e. miR-200b-3p low expression group and miR-200b-3p high expression group, where miR-200b-3p high expression group were significantly higher than the low expression group and the health controls ($p < 0.05$) (Fig. 2A). Results showed that serum TBA was significantly lower in miR-200b-3p high expression group ($p < 0.05$) than miR-200b-3p low group (Fig. 2B), whereas there were no significant differences in serum bilirubin between these two groups (Fig. 2C). To explore whether miR-200b-3p was correlated with liver function indexes, correlation analysis was performed. Results showed that serum TBA was negatively correlated with miR-200b-3p levels in miR-200b-3p high expression group ($p < 0.05$) (Fig. 2D), while no significant correlation had been found with TBA in miR-200b-3p low expression group and TBIL in both groups (Fig. 2E-G). These findings indicated that miR-200b-3p was associated with lower serum TBA in cholestatic patients, which indicated that miR-200b-3p might play some roles in regulating bile acid homeostasis in human obstructive cholestatic patients. However, the mechanism remained unknown.

LRH-1 and CYP8B1 was upregulated, while CYP7A1 was downregulated in human cholestatic liver tissues.

To investigate how elevated miR-200b-3p could lead to lower serum TBA in our patients, we asked whether miR-200-3p regulated the expression of genes involved in bile acid synthesis and transport as well as nuclear receptors that regulate the expression of these enzymes and transporters. Computer software analysis (from www.targetscan.org) indicates that LRH-1 may be one of the downstream target genes of miR-200-3p. To determine whether miR-200-3p regulates LRH-1 expression in the liver, we first measured the mRNA expression of LRH-1 and its targets CYP7A1 and CYP8B1 in these cholestatic livers using RT-PCR. As shown in Fig. 3, LRH-1 and CYP8B1 mRNA levels were significantly upregulated in cholestatic liver tissues, while the levels of LRH-1 and CYP8B1 mRNA in miR-200b-3p low expression group were much higher than these in control group and miR-200b-3p high expression group ($p < 0.05$) (Fig. 3A,B). Furthermore, CYP7A1 mRNA levels in miR-200b-3p high expression group were much lower than these in control group ($p < 0.05$) (Fig. 3C). These results indicated that miR-200b-3p might repress the expressions of LRH-1, CYP8B1 and CYP7A1. However, whether these gene expressions were correlated with miR-200b-3p remained unknown.

MiR-200b-3p was negatively correlated with LRH-1 and CYP8B1 in cholestatic patients with miR-200b-3p high expression.

To examine the relationship between miR-200b-3p and these bile acid-synthetic genes, we performed correlation analysis between the levels of miR-200b-3p and LRH-1, CYP8B1 and CYP7A1 in cholestatic patients. As shown in Fig. 4B,D, LRH-1 and CYP8B1 were negatively correlated with miR-200b-3p in patients with miR-200b-3p high expression ($p < 0.05$) whereas the mRNA expression of CYP7A1 was not correlated with miR-200b-3p level (Fig. 4A,C), despite their levels were lower in these patients. These results indicated that MiR-200b-3p was negatively correlated with LRH-1 and CYP8B1 in cholestatic patients with miR-200b-3p high expression, suggesting that miR-200b-3p may directly regulates LRH-1 expression.

LRH-1, CYP7A1 and CYP8B1 were significantly repressed after miR-200b-3p mimic transfection in HepG2 cells.

To determine whether miR-200b-3p directly control the expression of LRH-1 and its targets of bile acid-synthetic enzyme, we transfected miR-200b-3p mimic into HepG2 cells. As shown in Fig. 5A, the expression of MiR-200b-3p was significantly increased compared to the un-transfected cells and negative control ($p < 0.05$). The mRNA expressions of CYP7A1 and CYP8B1 were significantly repressed after miR-200b-3p mimic transfection ($p < 0.05$), while no significant difference had been found in CYP7B1, CYP27A1 and CYP3A4, compared to mock and miR-NC (Fig. 5B). Furthermore, we found that LRH-1 was also significantly repressed ($p < 0.05$), while there were no significant differences in other nuclear receptors, such as FXR, HNF4 α , HNF1 α , SHP (Fig. 5C). To further explore the functions of miR-200b-3p, detoxification enzymes, such as GSTM1-4, GSTA1-4, CYP3A4, UGT2B4, UGT2B7 and SULT2A1 were also detected using RT-PCR. However, no significant differences had been found in these enzymes (Fig. 5D-F). Membrane transport protein MRP3 was significantly repressed ($p < 0.05$), while no significant differences had been found in MRP2, OST α and OST β (Fig. 5G). Western blot results showed the expressions of LRH-1, CYP7A1, CYP8B1 and MRP3 were significantly repressed after miR-200b-3p mimics transfection ($p < 0.05$) (Fig. 5H,I). These results indicated that miR-200b-3p repressed the expressions of LRH-1, CYP7A1, CYP8B1 and MRP3. However, the detail mechanism remained unclear. Further studies showed that LRH-1 promoted MRP3 expression[30] and facilitated the expressions of CYP7A1 and CYP8B1[10–13]. Therefore, we assumed that miR-200b-3p repressed expressions of CYP7A1, CYP8B1 and MRP3 through repressing LRH-1.

MiR-200b-3p repressed CYP7A1 and CYP8B1 levels through binding with LRH-1.

LRH-1 plays an important role in regulating bile acid homeostasis through promoting CYP7A1 and CYP8B1 expression[10–13], to further study the mechanism, luciferase reporter gene assay was carried out to examine whether LRH-1 was the direct target gene of miR-200b-3p. The potential binding sequences of miR-200b-3p and LRH-1 was predicted from www.targetscan.org (Fig. 6A). And pmirGLO-basic vectors containing the sequences in 3'UTR region of LRH-1 and its truncated mutants were generated, which were named as pmirGLO-LRH-1-wt and pmirGLO-LRH-1-mut. After post-transfection with pmirGLO vectors for 24 h and miR-200b-3p mimic transfection for 24 h, relative luciferase activities were analyzed. Results showed that cotransfection of miR-200b-3p mimic and pmirGLO-LRH-1-wt luciferase

construct into HepG2 cells led to a marked inhibition of luciferase activity by 40–45% compared with controls, which was reversed after the mutation of pmirGLO-LRH-1-wt sequence in pmirGLO-LRH-1-mut ($p < 0.01$) (Fig. 6B). Together, these results indicated that miR-200b-3p could directly bind with LRH-1, therefore, repressing the expressions of CYP7A1 and CYP8B1.

Discussion

In this study, we assessed the expressions and functions of miR-200b-3p in human obstructive cholestasis due to blockage of bile ducts. We had obtained the following findings. (1) Hepatic miR-200b-3p was significantly increased in cholestatic patients when compared to control patients. (2) Hepatic level of miR-200b-3p was negatively correlated with levels of serum TBA and hepatic mRNA of LRH-1 and CYP8B1. (3) Transfection of miR-200b-3p mimic resulted in decreased expression of LRH-1, CYP8B1 and CYP7A1 in HepG2 cells. (4) Luciferase gene reporter assay identified the binding site of miR-200b-3p in LRH-1 gene, and mutation of this binding site abolished miR-200b-3p effect in LRH-1 expression. Therefore, we conclude that miR-200b-3p regulates bile acid homeostasis by modulating LRH-1 expression and function in obstructive cholestasis, a novel role of miR-200b-3p in adaptive response to cholestasis.

Bile acid homeostasis is critical to liver health. It is tightly regulated by a number of genes involved in bile acid synthesis and transport, including CYP7A1, the rate-limiting enzyme in bile acid synthesis[10–13]. Previous studies indicate that CYP7A1 expression is repressed in cholestasis and this adaptive response is mediated through the bile acid nuclear receptor FXR by up-regulating the expressions of both hepatic SHP and ileal FGF19[4, 31–33]. Here we found that miR-200b-3p can also repress CYP7A1 expression through down-regulating LRH-1 expression and function, revealing a novel functional role of miR-200b-3p in maintaining bile acid homeostasis. However, it is not known whether miR-200b-3p is a target of FXR. Future studies may address this question.

LRH-1 expression is increased in cholestatic rodent liver, which may stimulate the expression of its target genes such as MRP3/Mrp3 and CYP7A1[30, 34, 35]. It is conceivable that up-regulation of MRP3 would protect liver by excreting conjugates of bile acids and bilirubin from hepatocytes. However, this would also mean stimulating CYP7A1 expression, which consequently leads to increased level of bile acid in hepatocytes, an event that could potentially hurt the liver. Therefore, it is important not to over increase LRH-1 expression and function. To do so, hepatocytes may have evolved a feedback mechanism by stimulating miR-200b-3p expression to counter-act LRH-1 expression and function in cholestasis to protect the liver.

Conclusion

Taken together, this study revealed that miR-200b-3p was upregulated in human cholestatic liver tissues, and it could repress the expressions of bile acid-synthetic enzymes via binding with LRH-1, such as CYP7A1 and CYP8B1. These results suggested the functional significance of reducing bile acid synthesis

and protecting hepatocytes from cholestasis, which might be a potential therapeutic target for cholestasis.

Abbreviations

LRH-1: liver receptor homolog-1; miRNAs/miRs: microRNAs; BDL: bile duct ligated; CYP7A1: cytochrome P450 family 7 subfamily A member 1; CYP8B1: cytochrome P450 family 8 subfamily B member 1; HNF4 α : hepatic nuclear factor 4 alpha; FXR: farnesoid X receptor; SHP: small heterodimer; TBA: total bile acid; ULN: upper limit of normal; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: γ -glutamyl transpeptidase; TBIL: total bilirubin; DBIL: direct bilirubin; IBIL: indirect bilirubin.

Declarations

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Authors' contributions

Wen Qiu performed the main experiment and was the major contributor in writing the manuscript. Xueqian Zhou collected the human tissue samples for experiment and contributed to write the manuscript. Chunwei Song and Xinchan Feng gave the experimental technical support and advice. Huaizhi Wang provided the human tissue samples. Shi-Ying Cai analyzed the experimental designment, revised the manuscript. Wensheng Chen designed and interpreted for the experiment, revised the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figures

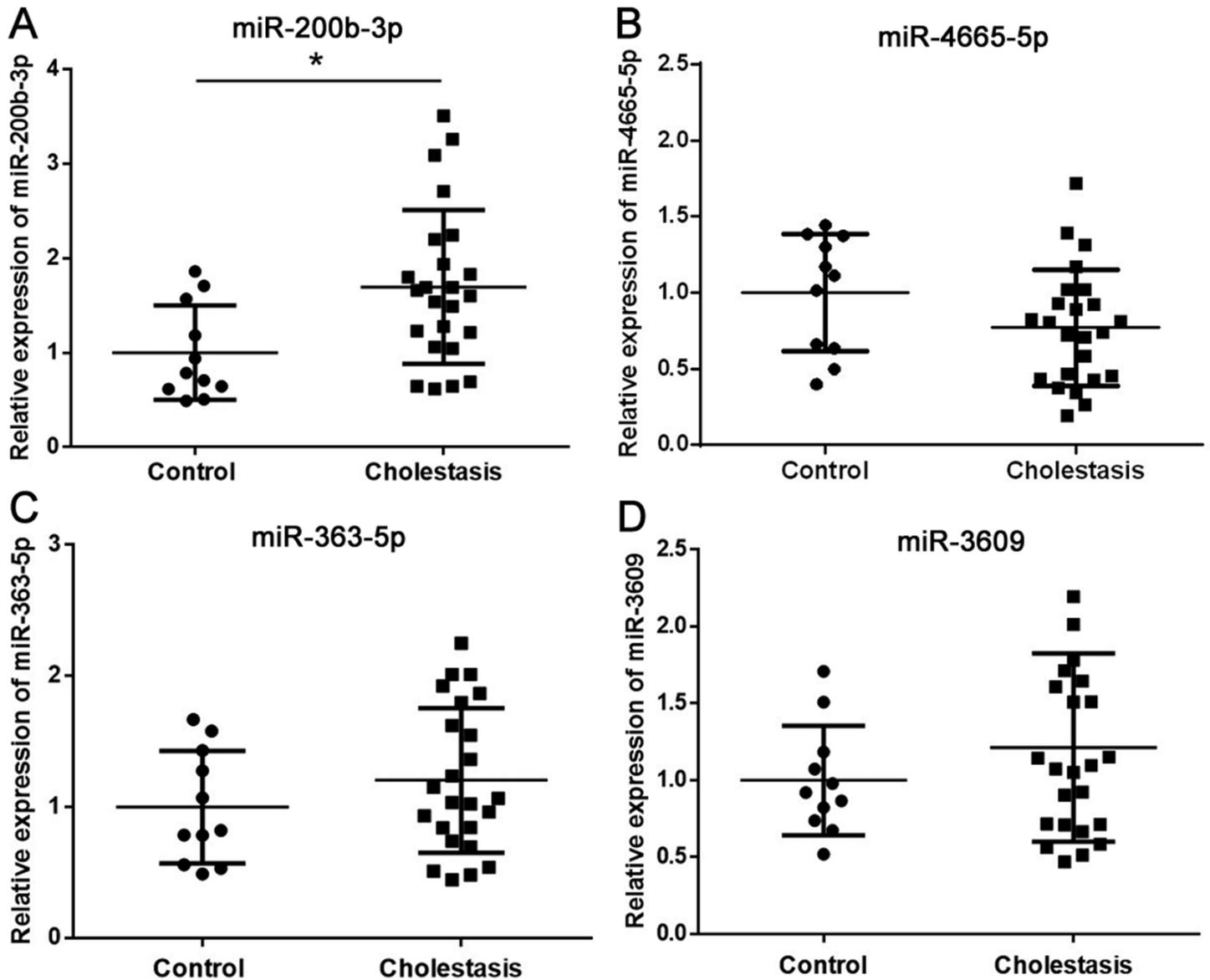


Figure 1

MiR-200b-3p was upregulated in human cholestatic liver tissues. (A) MiR-200b-3p was significantly upregulated in cholestatic liver tissues. (B-D) It was reported that miR-363-5p, miR-4665-5p and miR-3609 were differentially expressed and played some roles in some cancers, so these three miRNAs were also detected, there were no significant differences of another three dysregulated miRNAs in control and cholestatic liver tissues. Data are shown as mean \pm SD based on at least three independent experiments, * $p < 0.05$, *** $p < 0.001$.

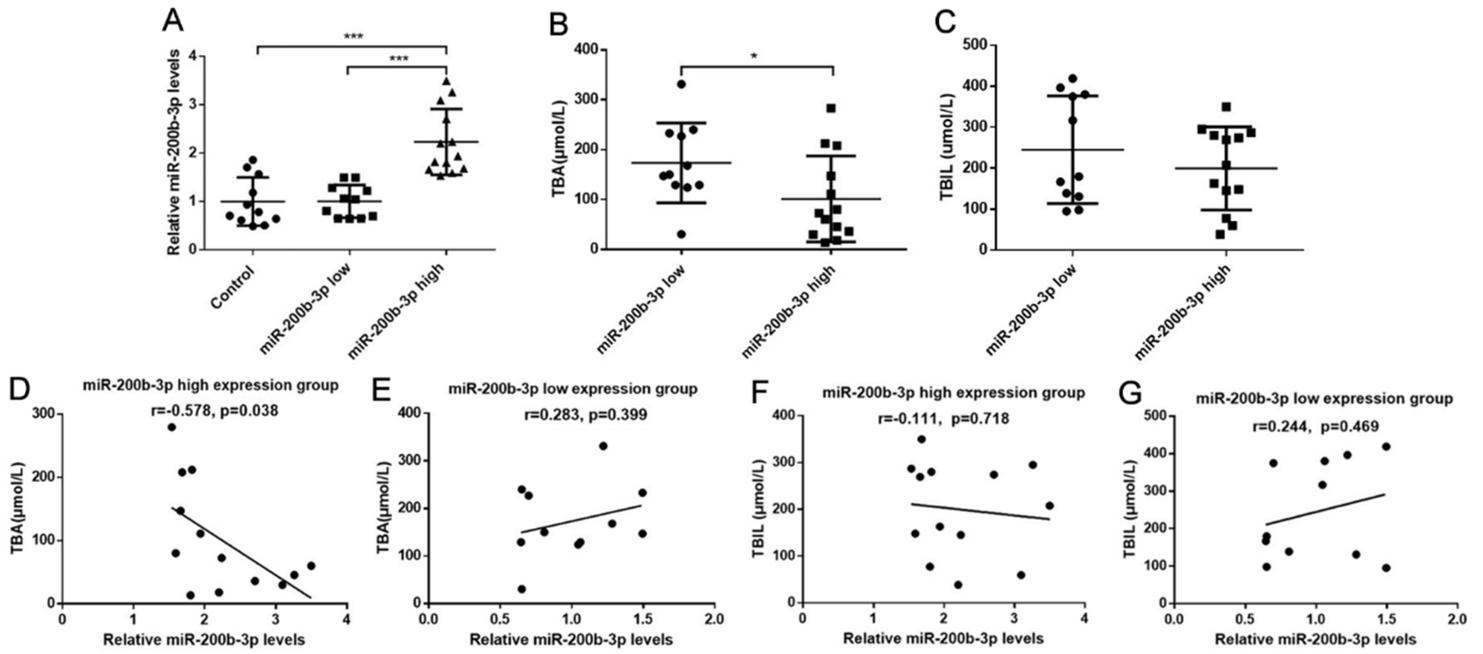


Figure 2

Elevated levels of miR-200b-3p is associated with lower serum TBA in cholestatic patients. (A) Cholestatic liver tissues were divided into miR-200b-3p low expression group and miR-200b-3p high expression group, the expressions of miR-200b-3p in the last group were much higher than other two groups. (B) Serum TBA was downregulated in miR-200b-3p high expression group. (C) No significant difference had been found in serum TBIL. Correlation analysis of serum liver function indexes and miR-200b-3p in cholestatic patients was performed. (D) Serum TBA was negatively correlated with miR-200b-3p levels in patients with miR-200b-3p high expression. (E) There was no significant difference between miR-200b-3p and TBA in miR-200b-3p low expression group. (F,G) There were no significant differences between miR-200b-3p and TBIL in both groups. * $p < 0.05$.

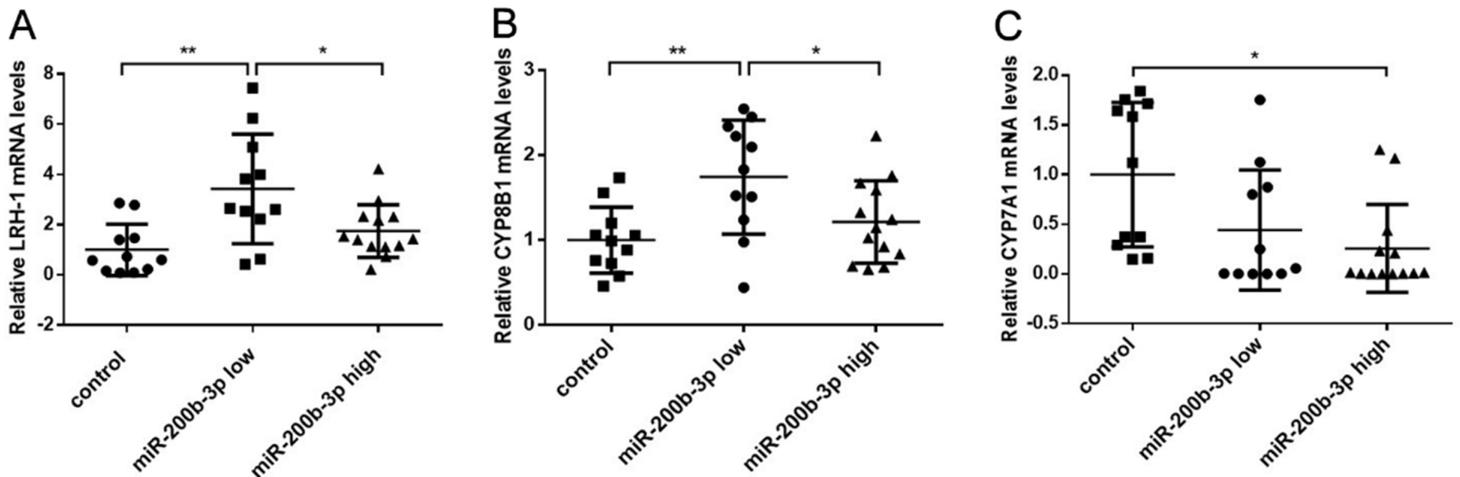


Figure 3

LRH-1 and CYP8B1 was upregulated, while CYP7A1 was downregulated in human cholestatic liver tissues. (A) LRH-1 levels in miR-200b-3p low expression group were significantly upregulated than those of control and miR-200b-3p high expression group. (B) CYP8B1 levels in miR-200b-3p low expression group were significantly upregulated than those of control and miR-200b-3p high expression group. (C) CYP7A1 levels in miR-200b-3p high expression group were significantly lower than those of control group. Data are shown as mean \pm SD based on at least three independent experiments, * $p < 0.05$, ** $p < 0.01$.

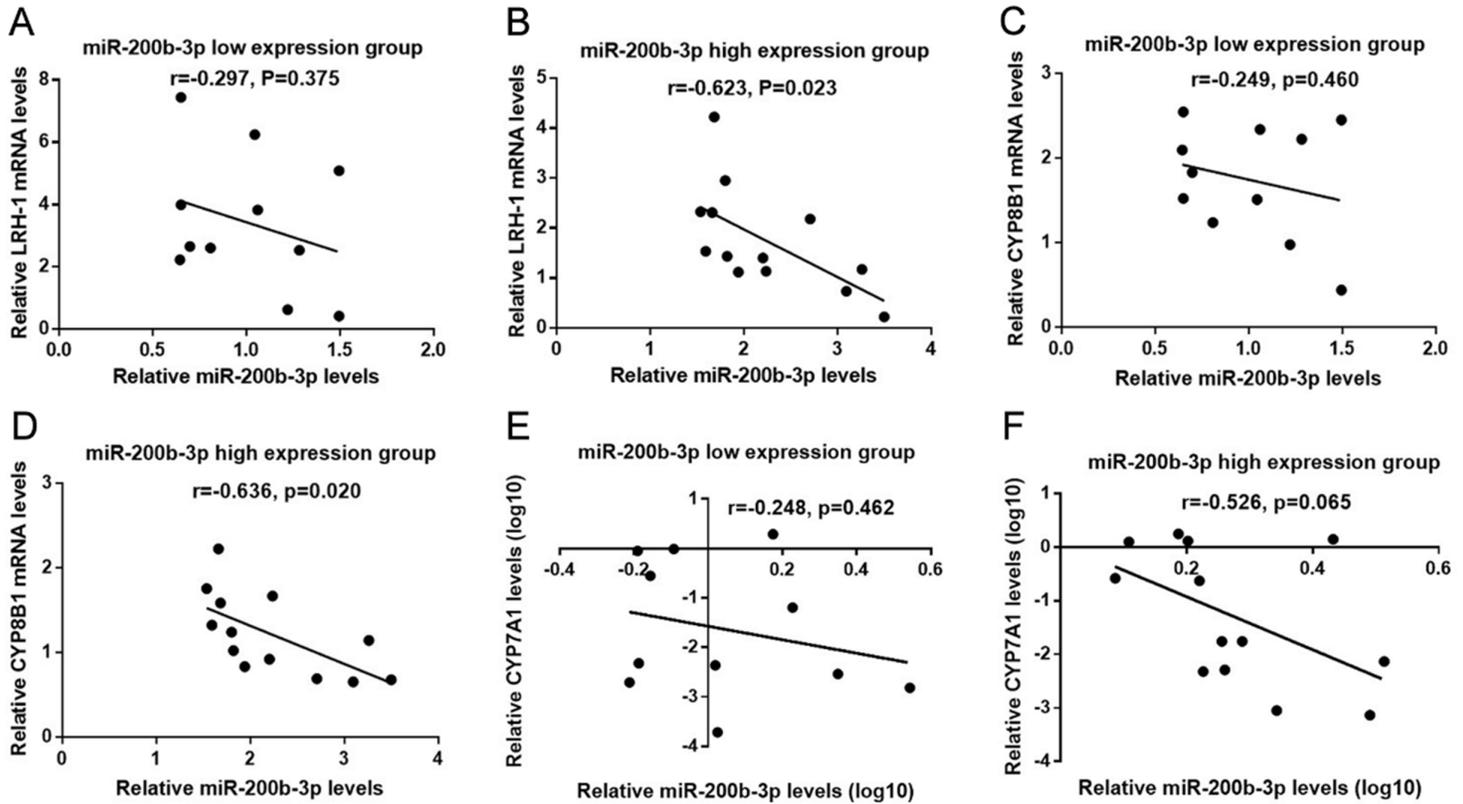


Figure 4

miR-200b-3p was negatively correlated with LRH-1 and CYP8B1 in cholestatic patients with miR-200b-3p high expression. Correlation analysis of miR-200b-3p and LRH-1, CYP8B1 and CYP7A1 in cholestatic patients was performed. (A) There was no significant difference between miR-200b-3p and LRH-1 in patients with miR-200b-3p low expression. (B) LRH-1 was negatively correlated with miR-200b-3p in patients with miR-200b-3p high expression. (C) There was no significant difference between miR-200b-3p and CYP8B1 in patients with miR-200b-3p low expression. (D) CYP8B1 was negatively correlated with miR-200b-3p in patients with miR-200b-3p high expression. (E,F) There were no significant differences between miR-200b-3p and CYP7A1 in cholestatic patients.

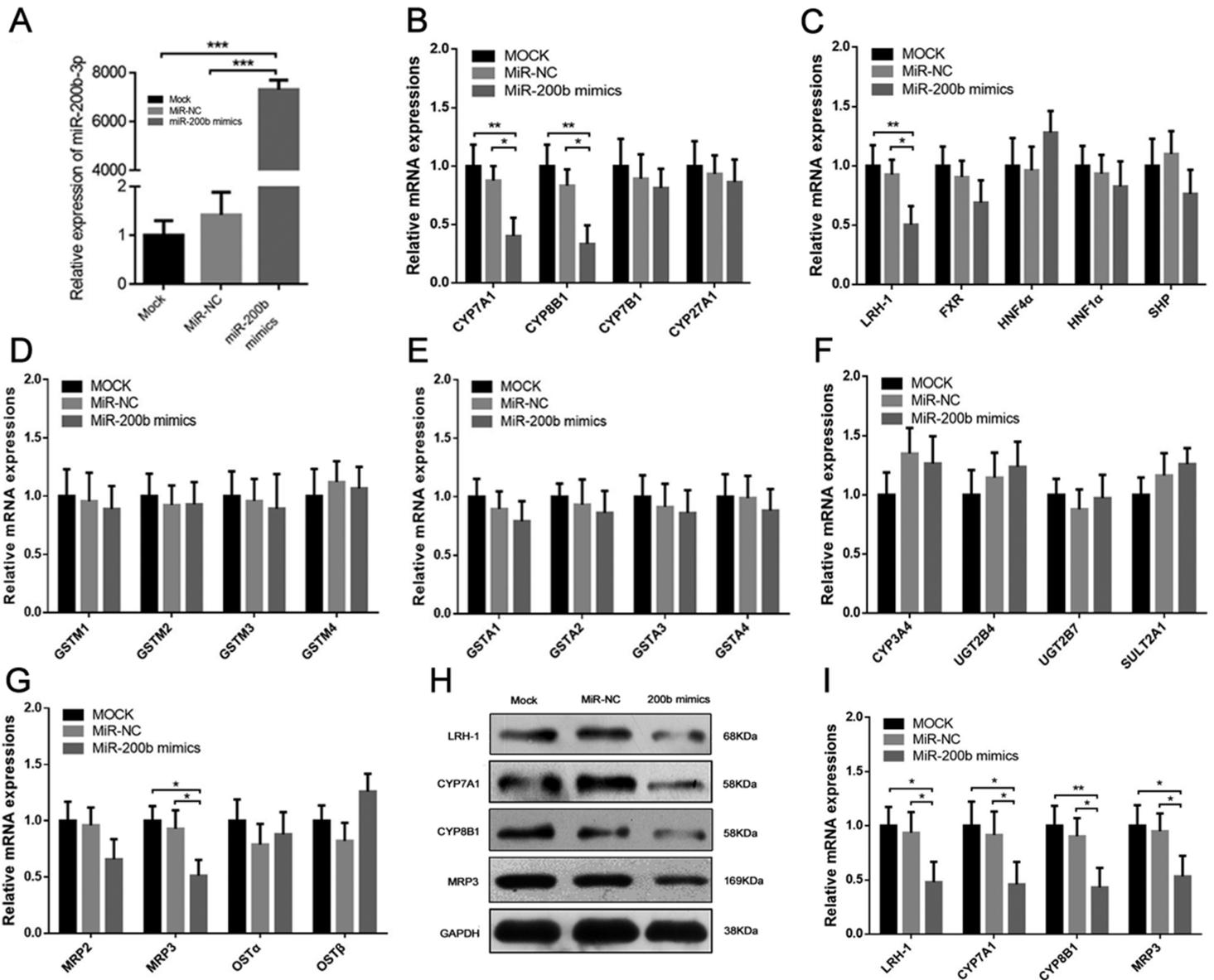


Figure 5

MiR-200b-3p was significantly increased and LRH-1, CYP7A1, CYP8B1 were significantly repressed after miR-200b-3p mimic transfection in HepG2 cells. (A) MiR-200b mimics was transfected into HepG2 cells and miR-200b-3p expression was detected by RT-PCR. (B) The expressions of CYP7A1, CYP8B1, CYP7B1, CYP27A1, CYP3A4 in HepG2 cells was detected by RT-PCR. (C) The nuclear receptors of FXR, HNF4 α , HNF1 α , SHP and LRH-1 were detected by RT-PCR. (D-F) The detoxification enzymes of GSTM1-4, GSTA1-4, CYP3A4, UGT2B4, UGT2B7 and SULT2A1 were detected by RT-PCR. (G) Membrane transport proteins MRP2, MRP3, OST α and OST β were detected by RT-PCR. (H,I) Protein levels of LRH-1, CYP7A1, CYP8B1 and MRP3 were detected by western blot. Data are shown as mean \pm SD based on at least three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

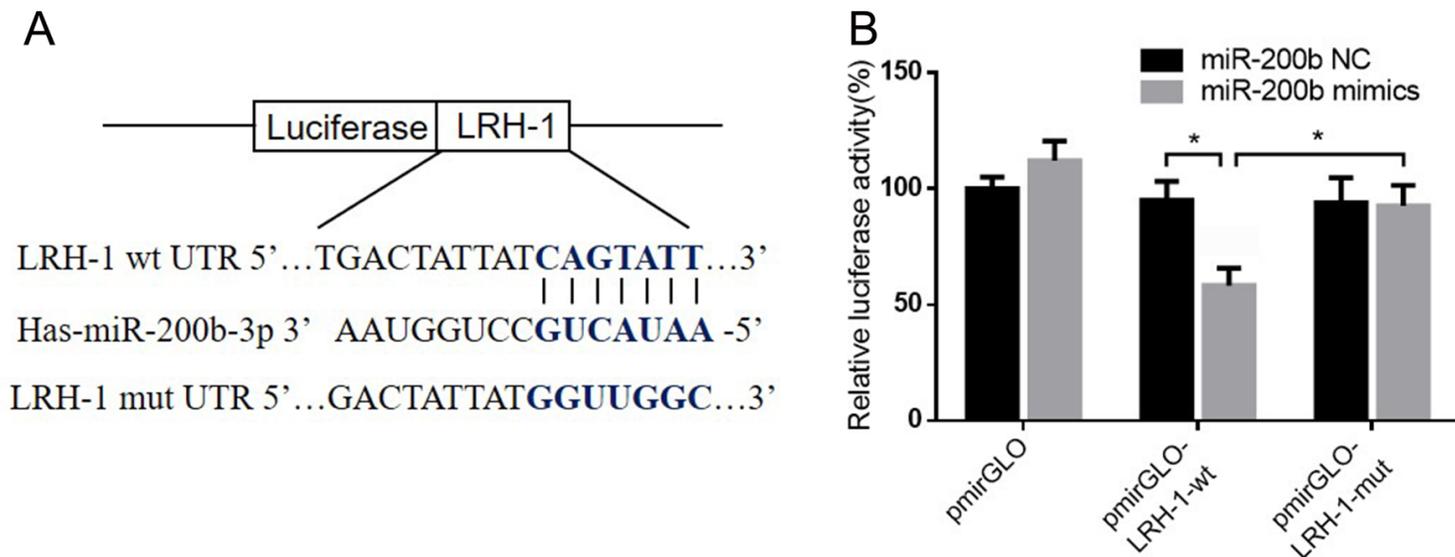


Figure 6

MiR-200b-3p repressed CYP7A1 and CYP8B1 levels through binding with LRH-1. (A) The potential binding sequences of miR-200b-3p and LRH-1 was predicted. (B) Luciferase activities were detected after post-transfection of pmirGLO vectors and miR-200b-3p mimic transfection. Data are shown as mean \pm SD based on at least three independent experiments, * $p < 0.05$.