Contents lists available at ScienceDirect

IF: 0.926

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi: 10.1016/S1995-7645(14)60350-3

Effects and mechanism of miR-214 on hepatocellular carcinoma

Li-Li Zhang, Yan-Jun Guo, Chun-Na Zhao, Jian-Yun Gao*

Department of Gastroenterology, Qiqihaer Medical College Affiliated training hospital Daqing Oil Field General Hospital, Daqing City, Heilongjiang Province, China

ARTICLE INFO

Article history:
Received 15 February 2015
Received in revised form 20 March 2015
Accepted 15 April 2015
Available online 20 May 2015

Keywords:
miR-214
Hepatocellular carcinoma
β-catenin pathway

ABSTRACT

Objective: To explore the role of miR-214 in the progression of hepatocellular carcinoma (HCC) and its inhibitory mechanisms in depressing the signaling pathway of β-catenin, this study was conducted. **Methods:** We ectopically expressed miR-214 in HepG2 cells to obtain cell lines Lv-miR-214-HepG2 and their control Lv-control-HepG2. Differences between the two cell lines were compared in cell growth, proliferation, colony forming ability and cell cycles. RT-PCR method was applied for the quantification of β-catenin mRNA expression. Western-blot method was applied for the determination of the protein level of β-catenin and their downstream targets (*ie.* Cyclin D1, c-Myc and TCF-1). The effect of miR-214 on cells was further explored through RNA interference and restoring miR-214 expression. **Results:** In comparison with negative (Lv-control-HepG2) and blank (HepG2) control, a significant inhibition of cell growth and proliferation caused by miR-214 was observed after 48~72h of cell culture experiments ($P<0.05$). The miR-214 treatment resulted in a colony forming efficiency of (23.28±3.26)%, which was significantly lower than that of negative control [(51.31±3.97)%] ($P<0.05$). According to FCM results, the experimental group, compared with control, showed a higher proportion of cells in G_0/G_1 phase [(70.32±3.12)%] but a lower proportion in S phase [(18.42±2.90)%] ($P<0.05$). The MTT assay demonstrated a significant inhibition of the proliferation and β-catenin expression of HCC cells compared with control ($P<0.05$), while no significant difference was observed after HCC cells being transfected with β-catenin overexpression plasmid ($P>0.05$). By comparing to the RT-PCR and Western-blot results of control, the miR-214 treatment led to a slightly decrease in the β-catenin mRNA expression ($P>0.05$), but an extremely inhibition in the protein level of β-catenin and its downstream targets Cyclin D1, c-Myc, and TCF-1 ($P<0.05$). **Conclusions:** miR-214 functions as a suppressor during the progression of HCC, and its inhibitory role was achieved by down-regulating β-catenin signaling pathway.

1. Introduction

Studies in past decades have shown the significant role of micro RNAs (miRNA) in hepatocellular cancinoma (HCC)[1,2]. The miRNA family plays an important role in the cell proliferation, differentiation, apoptosis and necrosis, *etc* and its dysfunction contributes to the tumor onset and progression[3-5]. It is reported that more than 50% of miRNA genes are located in fragile sites or cancer-associated genomic regions[6]. Thus, the alteration in the miRNA expression will activate the abnormal expression of cancer genes, influencing the progression and metastasis etc. of cancers[7]. Many miRNAs are involved in the hepatocarcinogenesis, including miR-214. The general down-regulation of miR-214 gene in HCC[8,9] can stimulate the development of this cancer by targeting on its downstream proteins[9]. To date, a number of studies on the relationship between miR-214 expression and HCC have been reported at home and abroad, while there is scarce information on its

*Corresponding author: Jian-Yun Guo, Department of Gastroenterology, Qiqihaer Medical College Affiliated training hospital Daqing Oil Field General Hospital, Daqing City, Heilongjiang Province, China.
Tel: +86 15145930002
E-mail: zhanglili020@126.com

mechanism in HCC development. In this study, the human hepatoma cells were used as the research object and their responses to miR-214 treatment were evaluated in the cell growth, proliferation, colony forming ability and cell cycles, as well as β-catenin pathway. The present study provided further information on the mechanism of miR-214 in HCC and may be a scientific reference for HCC therapy through modulating miRNA expression.

2. Materials and methods

2.1. Materials

Cell lines: Human hepatoma (HepG2), normal hepatic cells and 293T cells were obtained from the Institute of Biochemistry and Cell Biology of Chinese Academy of Sciences. The *Escherichia coli* (*E. coli*) strains (DH5α) were produced in our laboratory.

Reagents: hsa-miR-214 plasmid (Beijing OriGene Techologies Co., Ltd, China); RPMI-1640 and DMEM medium (Gibco, USA); fetal bovine serum and propidium iodide (PI) (Beijing solarbio science & technology co., ltd., China); methyl thiazolyl tetrazolium (MTT) (System Biosciences, USA); pLenR-GPH and Lv-control vectors and Lentivirus plasmid (Shanghai Innovation Biotechnology Co.,Ltd., China); Lipofectamine2000 and TRIzol commercial kits (Invitrogen, USA); Opti-MEM (Hyclone, USA); endonucleases including *Bam*H I, *Eco*R I, *Kpn* I and *Xba* I (NEB, USA); Taqman microRNA RT kit, Taqman miRNA Assays, Taqman Universal PCR Master Mix (Appliedbiosysrems, USA); T4DNA ligase (Thermo, USA); Agarose, PCR kit, BAC kit (Sangon Biotech (Shanghai) Co.,Ltd., China); Antibody and second antibody of β-catenin, Cyclin D1, c-Myc, TCF-1 and β-actin (Beijing Chinese fir golden bridge biotechnology co., Ltd., China).

Instruments: Synergy 2 multimode reader (Biotek, USA); SHELLAB CO_2 incubator (SHELLAB, USA); Stratedigmflow cytometry (Stratedigm, USA); Biometra gradient PCR amplifier (Biometra, Germany); DYY-2C electrophoresis apparatus (Beijing Liuyi Instrument Plant, China); BioSpectrum- UVP Gel Imaging System (UVP, USA).

2.2. Preparation of miR-214 cell lines

The pre-miR-214 primer was designed using the software Primer 5.0. After PCR amplification, the AGE and following recovery and purification of PCR products were conducted using SanPrep gel extraction kit (Sangon Biotech (Shanghai) Co.,Ltd., China). The obtained target gene and pCDH-CMV-MCS-EF1-copGFP lentiviral vector were treated with double enzymes restriction in presence of *Eco*R I and *Bam*H I according to protocols of commercial kits (Axygen) respectively. Target genes were cloned into the vector according to the manufacture's protocol.

The miR-214 lentiviral vector was prepared with the connection product and *E. coli* strain DH5 α. The final bacterial suspensions transfected with miR-214 was used for PCR amplification and followed by enzyme digestion and sequencing if bacterial colonies were determined to be positive. The plasmids were extracted from the bacterial suspensions. The packaged lentiviral vector was named Lv-miR-214, using the empty lentiviral vector as a control (Lv-control).

Exponential 293T cells were cultured in serum-free medium in a CO_2 incubator (37 ℃, 5% CO_2). After a fusion of 90%-95%, cells were transfected with Lv-miR-214 or Lv-control using Lipofectamine 2000 Transfection Kit (Invitrogen) and further cultured in medium with 10% FBS. HepG2 cells were infected with the packaged lentiviruses to obtain the HepG2 transfectant stably expressing miR-214 (Lv-miR-214-HepG2) and its control Lv-control-HepG2.

All reaction systems and conditions were listed as following:

PCR system (50 μL): 10×buffer 5 μL, sense (10 μM) and anti-sense primers (10 μM) 1 μL respectively, has-miR-214 plasmid (100 ng/μL) 1 μL, dNTPs (10 mM) 0.5 μL, Pfu enzymes1 μL, ddH_2O 40.5 μL.

PCR amplifying conditions: 95 ℃ 2 min; 95 ℃ 20 s, 60 ℃ 20 s, 72 ℃ 15 s (30 cycles); 72 ℃ 3 min.

Enzyme digestion system (20 μL): 10×buffer 2 μL, 10×BSA 2 μL, *Eco*R I (10 U/μL) and *Bam*H I (10 U/μL) 1 μL respectively, vectors or purified PCR product (500 ng/μL) 10 μL, ddH_2O 4 μL, 37 ℃ 2 h.

Connection system (10 μL): vectors 2 μL, target genes 6 μL, 10×T4 buffer 1 μL, T4DNA ligase 1 μL, 22 ℃ 1 h.

2.3. Cell growth

Exponential Lv-miR-214-HepG2 (experiment group), Lv-control-HepG2 (negative control group) and HepG2 (blank control group) cells at the concentration of 4×10^4 cells/mL were inoculated into 96-well plate and culture at 37 ℃, 5% CO_2 and humidity of 60% for 5 d. Each group was triplicated. Over culture period, cells in each group were counted every 24 h.

2.4. Cell proliferation

Exponential Lv-miR-214-HepG2 and Lv-control-HepG2 cells were cultured at 37 ℃ and 5% CO_2 for 24 h. Then cells were cultured at 2×10^4 cells/ml in a fresh culture medium. Each group was quintuplicated. During the culture period of 4 d, inhibition of cell proliferation was determined at 0, 24, 48, 72 and 96 h by the MTT method. Briefly, 20 μL MTT (5 g/L) was added into each well and the medium was replaced with 150 μL DMSO. The absorbance was read at 570 nm after a continuous shake of 10 min. The inhibitory rate (%) = $(OD_{control} - OD_{experiment})/ OD_{control}\times100$

2.5. Cell colony forming ability

Lv-miR-214-HepG2 and Lv-control-HepG2 of 2×10^2 cells were respectively inoculated into soft agar whose diameter was 60 mm and concentration was 4 g/L at the top and 5 g/L at the bottom. After two weeks of culture at 37 ℃ and 5% CO_2, the colonies were observed using an inverted phase contrast microscope. The colony diameter >50 μm was used as standard to calculate the colony forming ability according to the formula: colony forming ability (%) = colony number /seeded cells × 100 with.

2.6. Cell cycles

Exponential Lv-miR-214-HepG2 and Lv-control-HepG2 cells were digested with trypsin and rinsed by PBS. Cells were fixed for 24 h at 4 ℃ after an addition of ice-cold ethanol (75%) and then rinsed by PBS again. RNA enzyme A was added to stop the catalytic reaction and removed by PBS. Following the addition of PI, samples were placed in the darkness for 30 min to form color. Cell cycles were analyzed with a flow cytometry.

2.7. β-catenin and downstream proteins

The mRNA level of β-*catenin* was determined with the RT-PCR method. A proper quantity of cell samples was mixed with Trizol reagent. Total RNA was extracted using a commercial kit (Invitrogen, USA) and its content (A_{260}) and purity (A_{260}/A_{280}) was detected. Total RNA of 1 μg was reverse transcribed into the first strand of cDNA and the product was PCR amplified after addition of β-*catenin* primers. β-*catenin* was used as the reference gene. Primer sequences were listed in Table 1. The amplification results were analyzed using the $2^{-\Delta\Delta Ct}$ method.

PCR system (20 μL): 2×master 10 μL, sense (10 μM) and anti-sense primers (10 μM) 1 μL respectively, cDNA 1 μL, DEPC H_2O 7 μL.

PCR amplifying conditions: 95 ℃ 10 min; 95 ℃ 20 s, 60 ℃ 1 min (40 cycles); cooling at 4 ℃.

The protein levels of β-catenin and its downstream proteins (*ie*. Cyclin D1, c-Myc and TCF-1) were determined with Western-blot analysis. Collected cells were lysed with RIPA lysis for 20 min on ice and supernatants were collected after centrifugation for 10 min at 4 ℃ and 12 000 rpm. Protein extracts were separated by 10% SDS polyacrylamide gel electrophoresis and then transferred onto a polyvinylidenefluoride (PVDF) membrane. After blocking with 5% non-fat milk, the blots were incubated with primary antibodies against β-catenin, CyclinD1, c-Myc, TCF-1 and β-actin at 4 ℃ overnight, followed by incubation with horseradish peroxidase-conjugated secondary antibody (1: 3 000) for 1 h at room temperature. The membrane was rinsed by TBST and color was developed in presence of chemoluminescent ECL-plus reagent. The intensity of protein fragments was quantified using Image-Pro Plus software.

2.8. sh β-catenin effect

According to the method described in 2.2, HepG2 cells transfected with sh β-catenin, named Lv-shRNA β-catenin-HepG2, were established to evaluate their cell proliferation capability (see 2.4) and β-catenin protein levels (see 2.6).

The primer sequence of target gene (*sh β-catenin*) was listed in Table 1. All reaction systems and conditions were listed as following:

PCR system (20 μL): 10×buffer 2 μL, sense (10 μM) and anti-sense primers (10 μM) 1 μL respectively, ddH_2O 16 μL.

PCR amplifying conditions: 95 ℃ 10 min; 75 ℃ 10 min, 55 ℃ 10 min, 35 ℃ 10 min, 15 ℃ 10 min.

Enzyme digestion system (20 μL): 10×buffer 2 μL, *Kpn* I (10 U/μL) and *Bam*H I (10 U/μL) 1 μL respectively, DNA (500 ng/μL) 8 μL, ddH_2O 8 μL, 37 ℃ 4 h.

Connection system (10 μL): vectors 1 μL, target genes 7 μL, 10×T4 buffer 1 μL, T4DNA ligase 1 μL, 16 ℃ overnight.

2.9. Over expressing β-catenin effect

According to the method described in 2.2, HepG2 cells over expressing β-catenin, named Lv-β-catenin-HepG2, were established to evaluate their cell proliferation capability (see 2.4) and β-catenin protein levels (see 2.6).

The primer sequence of target gene (CTNNB 1) was listed in Table 1. All reaction systems and conditions were listed as following:

PCR system (50 μL): 10×buffer 5 μL, sense (10 μM) and anti-sense primers (10 μM) 1 μL respectively, CTNNB1 plasmid (100 ng/μL) 1 μL, dNTPs (10 mM) 5 μL, Pfu polymerase 1 μL, ddH_2O 36 μL. PCR amplifying conditions: 94 ℃ 5 min; 95 ℃ 30 s, 58 ℃ 30 s, 68 ℃ 2 min 30 s (30 cycles); 68 ℃ 5 min.

Enzyme digestion system (23 μL): 10×buffer 4 μL, *Xba* I (10 U/μL) and *Bam*H I (10 U/μL) 1 μL respectively, DNA (500 ng/μL) 8 μL, ddH_2O 9 μL, 37 ℃ 4 h.

Connection system was similar to that in 2.8.

Table 1
Primers sequence in this experiment.

Primers		Sequence
pre-miR-214	F	5'- ATAGAATTCTTTCTCCCTTTCCCCTTACTCTCC-3'
	R	5'- CCAGGATCCTTTCATAGGCACCACTCACTTTAC-3'
β-*catenin*	F	5'-AAAATGGCAGTGCGTTTAG-3'
	R	5'-TTTGAAGGCAGTCTGTCGTA-3'
β-*actin*	F	5'-CTCCATCCTGGCCTCGCTGT-3'
	R	5'-GCTGTCACCTTCACCGTTCC-3'
shRNA β-*catenin*	F	5'-TTGTTATCAGAGGACTAAATA-3'
	R	5'-TATTTAGTCCTCTGATAACAA-3'
CTNNB 1	F	5'-CGCTCTAGAATGGCTACTCAAGCTGATTTGATGG-3'
	R	5'-CCAGGATCCTTACAGGTCAGTATCAAACCAGGCC-3'

2.10. Statistical analysis

Data in the present study were analyzed with SPSS 13.0 software and expressed as mean±SD. Differences between groups were determined using Student's *t*-test. *P*-values<0.05 were considered statistically significant for all variance tests.

3. Results

3.1. Effects of miR-214 on HCC cell growth

Figure 1 showed the cell growth of Lv-miR-214-HepG2, Lv-control-HepG2 and HepG2 over the 5 days of culture. Ectopic expression of miR-214 reduced cell growth compared to the negative and blank control groups, being significantly different after 72 h (*P*<0.05).

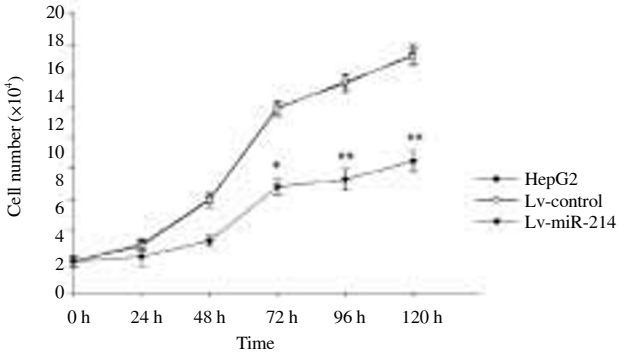

Figure 1. Effect of miR-214 on the growth of HCC cells.
Note: "*" indicates a significant difference compared to blank control (*P*<0.05), while "**" indicates an extremely significant difference (*P*<0.01).

3.2. Effects of miR-214 on HCC cell proliferation

The cell proliferation of cell lines (*ie.* Lv-miR-214-HepG2, Lv-control-HepG2 and HepG2) was recorded in Figure 2. Ectopic expression of miR-214 inhibited cell proliferation by 21.7%, 38.8% and 49.7% at 48, 72 and 96 h respectively, which was statistically different to that of the negative and blank control group (*P*<0.05).

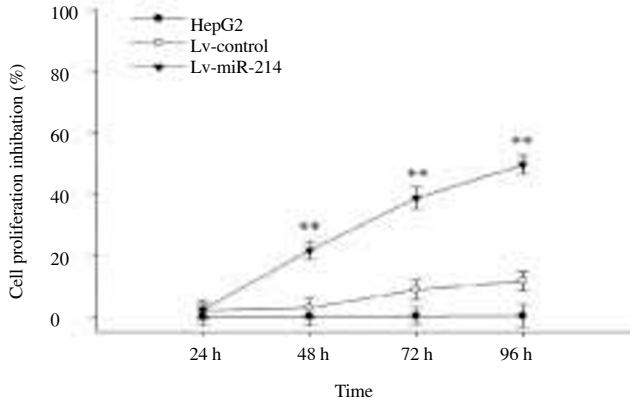

Figure 2. Effect of miR-214 on the proliferation of HCC cells.
Note: "*" indicates a significant difference compared to blank control (*P*<0.05), while "**" indicates an extremely significant difference (*P*<0.01).

3.3. Effects of miR-214 on HCC cell colony forming ability

The colonies in HCC cells with ectopic expression of miR-214 were smaller in size and fewer compared to the control group (Figure 3). The effects on cell colony forming efficiency were less pronounced in cells transduced with miR-214 [(23.28±3.26)]% than in control cells [(51.31±3.97)%] (*P*=0.021).

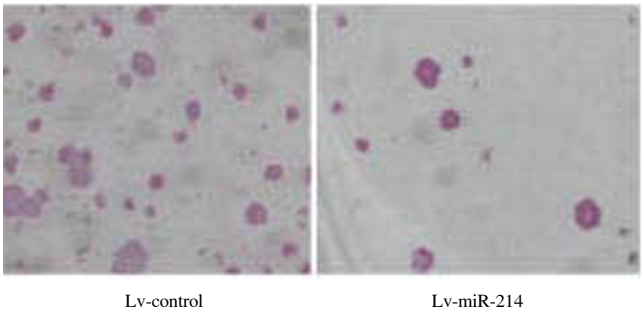

Figure 3. Effect of miR-214 on the colony formation of HCC cells.

Table 2
Effect of miR-214 on the colony forming efficiency of HCC cells.

	Lv-miR-214	Lv-control	*t*	*P*
Colony number	46.00±5.23	125.00±11.80	-	-
colony forming efficiency (%)	23.28±3.26	51.31±3.97	12.351	0.021

3.4. Effects of miR-214 on HCC cell cycles

The rate of cells at G_0/G_1, S and G_2 phase were shown in Figure 4. Cells transduced with miR-214 were significantly higher in the G_0/G_1 phase fraction [(70.72±3.12)%] than the control group [(54.61±2.10)%] (*P*<0.05), but lower in the S phase fraction [(18.63±2.90)%] than the control group [(35.26±3.35)%] (*P*<0.05). No significant difference was observed in G_2 phase fraction between cells with or without ectopic expression of miR-214 (*P*>0.05).

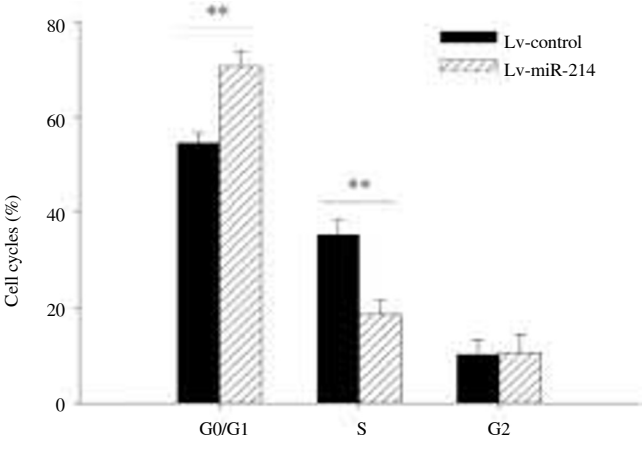

Figure 4. Effect of miR-214 on HCC cell cycles.
There is a significant difference in G_0/G_1 phase and S phase between experimental and control group (t is 7.92 and 8.56 respectively, and *P*=0.000). "**" indicates an extremely significant difference (*P*<0.01).

3.5. Effects of miR-214 on β-catenin pathway

RT-PCR results showed that there was a reduction in the β-*catenin* mRNA expression in cells transduced with miR-214 compared to the control cells (Figure 5), while no significant difference was recorded (P>0.05). According to Western-blot results, ectopic expression of miR-214 reduced the protein level of β-catenin and its downstream proteins Cyclin D1, c-Myc and TCF-1 compared to the control group (Figure 6).

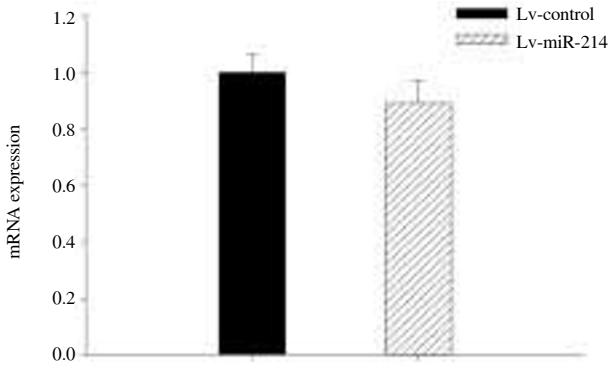

Figure 5. Effect of miR-214 on the β-*catenin* mRNA expression of HCC cells.

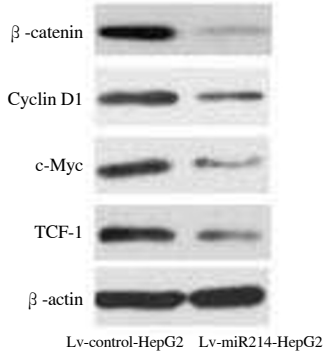

Figure 6. Effect of miR-214 on the protein expression level of β-catenin and its downstream proteins.

3.6. Effects of RNA interference and restoring β-catenin expression on cell proliferation and β-catenin expression

Figure 7 showed the effects of RNA interference and restoring β-*catenin* expression on HCC cell proliferation. No significant difference was observed in the cell proliferation between cells treated with miR-214 and those treated with shRNA β-catenin (P>0.05), while both of them were significantly lower than the control cells and cells over expressing β-catenin (P<0.05).

Figure 8 showed the effects of RNA interference and restoring β-catenin expression on the protein level of β-catenin. Western-blot results showed a similar β-catenin protein level of cells transduced with shRNA β-catenin to that transduced with miR-214 and both of them were lower compared to the control group. A higher β-catenin protein level was observed in cells over expressing β-*catenin* than in control cells.

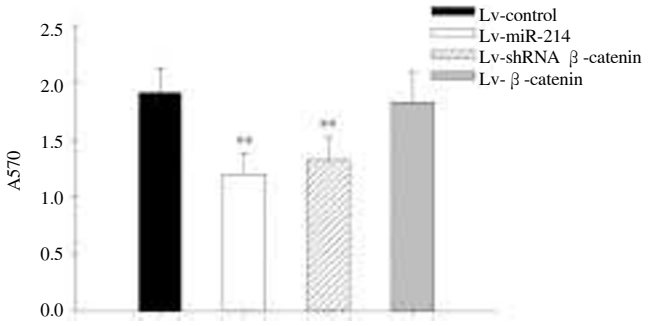

Figure 7. Effect of β-*catenin* RNA interference and overexpression on HCC proliferation.

There is a significant difference between the treatments of Lv-miR-214 and Lv-shRNA β-catenin and control (t is 0.352 and 0.398 respectively, P=0.000). "**" indicates an extremely significant difference (P<0.01).

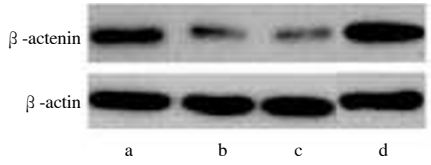

Figure 8. Effect of β-catenin RNA interference and overexpression on the protein level of β-catenin of HCC.

a, b, c and d means Lv-control, Lv-miR-214, Lv-shRNA β-catenin and Lv-β-catenin, respectively.

4. Discussion

HCC is one of the fatal cancers in clinical practice and studies on its pathogenesis have long been the focus for researchers at home and abroad to improve treatment technology for this disease. In past decades, the miRNA discovery opened a new door to the cancer therapy and many miRNA-related treatment methods developed. These small noncoding RNAs bind to the 3' untranslated region (UTR) or the open reading frame (ORF) of the target mRNA, resulting in the degradation of target mRNA and therefore a translational repression[1,10]. The miRNA has been pathologically proved to be a new target of drug action during cancer onset and progression[10,11]. Due to this potential value, miRNA has been used as a marker of cancer diagnosis and prognosis. In the present study, the effect of miR-214 on HCC was explored according to the cell growth, proliferation, colony forming ability and cell cycles determind in cells transduced with miR-214. At the same time, the expression of β-catenin and its downstream proteins in cells transduced with miR-214 was determined and compared with that in cells treated with RNA interference and restoring β-catenin expression to elucidate the mechanism of miR-214 influencing HCC by targeting β-catenin pathway.

4.1. Effects of miR-214 on HCC cell growth, proliferation, colony forming ability and cell cycles

miR-214 is reported to have a significant role in tumor inhibition. For example, the expression of miR-214 can promote apoptosis and reduce cell growth, proliferation, migration and invasion etc. in cancers such as gastric carcinoma, cervical carcinoma and nasopharyngeal carcinoma[12–14]. In the present study, we ectopically expressed miR-214 in HepG2 cells and a significant reduction in cell growth after culture of 48-72 h and colony forming ability was obtained. Meanwhile, treatment of miR-214 resulted in an increase in the G_0/G_1 phase fraction, but a decrease in S phase fraction. This indicates that miR-214 expression makes cancer cells arrest in G_0/G_1 phase and inhibits their proliferation by interfering with their progress through to the S phase where DNA is synthesized. Similarly, other miRNAs such as miR-7 and miR-126 are also reported to arrest cells in G_0/G_1 phase[15,16]. Thus, the inhibitory effect of miR-214 on HCC cells is confirmed and its abnormal variation is expected to significantly affect the development of HCC. However, the expression of miR-214 is tumor-specific. As reported, miR-214 was up-regulated in pancreatic carcinoma, leading to the poor response of pancreatic cancer cells to chemotherapy[17]. For gastric carcinoma, the up-regulation of miR-214 expression could promote the cancer cell proliferation and migration, which may be related with its negative role in regulation of PTEN protein[12]. While in HCC[8,18], ovarian carcinoma[19] and cervical carcinoma[13], miR-214 expression was up-regulated. In addition to miR-214, many other miRNAs contribute to the HCC onset and progression and they showed different expression patterns: there is a down-regulation in the expression of some miRNAs such as miR-30a, miR-125b and miR-185[20–22], but an up-regulation in the expression of other miRNAs such as miR-224, miR-517a and miR-520c[22–24].

4.2. Mechanism of miR-214 inhibiting HCC development by targeting β-catenin pathway

β-catenin is reported to be significant for the development of cancers such as adrenal carcinoma[25], colorectal carcinoma[26] and gastroenteric carcinomas[27] because this gene silence can activate apoptosis and consequently inhibit cancer cell proliferation. According to results in the present study, the protein level of β-catenin was significantly inhibited by miR-214, while its mRNA level showed no significant difference compared to the negative control group. This suggested that miR-214 mainly affect the expression of β-catenin on the level of translation. For HCC, the Wnt/β-catenin signaling pathway is an important biomarker due to its comprehensive function in cell differentiation, proliferation, and maintenance of homeostasis[28,29]. As deregulation of the Wnt pathway can affect the expression of target genes including Cyclin D1 and c-Myc[28], it is considered as an early event in hepatocarcinogenesis[30]. We determined the protein level of β-catenin and its downstream proteins (*ie.* Cyclin D1, c-Myc and TCF-1) with Western-blot method and confirmed the inhibitory effect of miR-214 on the expression of downstream proteins in the β-catenin pathway. Nevertheless, our experiment results showed that the inhibited HCC cell proliferation and β-catenin expression could recover due to the restoring β-catenin expression. Therefore, miR-214 is suggested to play an inhibit role in the β-catenin pathway, consequently reducing HCC cell proliferation.

This study demonstrates that miR-214 is a suppressor for the development of HCC. This can provide a better understanding of the mechanism of HCC onset and progression, and may also used as a reference for HCC therapy. Previous reports have shown that the function of miR-214 in tumor suppression is correlated with its inhibition on β-catenin. However, it must be taken into consideration that the type and number of target gene or pathway of miR-214 is tumor-specific. In cervical carcinoma, miR-214 can negatively regulate the expression of Bcl212, though this situation can partially be reversed by over-expression of Bcl2l2[13]. In myeloma, miR-214 is associated with the up-regulation of p53 expression and the down-regulation of PSMD10 expression with the latter encoding the oncoprotein gankyrin[31]. While the restoring expression of Leucine zipper putative tumor suppressor 1 can reverse the promotion effect of up-regulated miR-214 expression on osteosarcoma cell proliferation[32]. Thus, a further study is needed to elucidate the effect of miR-214 on other target genes and pathways, and the synergistic effect of target genes and pathways on HCC onset and progression.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Giordano S, Columbano A. MicroRNAs: new tools for diagnosis, prognosis, and therapy in hepatocellular carcinoma? *Hepatology* 2013; **57**(2): 840-847.

[2] Rossi L, Zoratto F, Papa A, Iodice F, Minozzi M, Frati L, et al. Current approach in the treatment of hepatocellular carcinoma. *World J Gastrointest Oncol* 2010; **2**(9): 348-359.

[3] Babashah S, Soleimani M. The oncogenic and tumour suppressive roles of microRNAs in cancer and apoptosis. *Eur J Cancer* 2011; **47**(8): 1127-1137.

[4] Xia H, Ooi LLPJ, Hui KM. MiR-214 targets β-catenin pathway to suppress invasion, stem-like traits and recurrence of human hepatocellular carcinoma. *PLoS One* 2012; **7**(9): e44206.

[5] Karakatsanis A, Papaconstantinou I, Gazouli M, Lyberopoulou A,

Polymeneas G, Voros D. Expression of microRNAs, miR-21, miR-31, miR-122, miR-145, miR-146a, miR-200c, miR-221, miR-222, and miR-223 in patients with hepatocellular carcinoma or intrahepatic cholangiocarcinoma and its prognostic significance. *Mol Carcinog* 2013; **52**(4): 297-303.

[6] Sonia AM, Manel E. Dysregulation of microRNAs in cancer: Playing with fire. *Epigenetics* 2011; **585**(13): 2087-2099.

[7] Croce CM. Causes and consequences of microRNA dysregulation in cancer. *Nat Rev Genetics* 2009; **10**(10): 704-714.

[8] Duan Q, Wang X, Gong W, Ni L, Chen C, He X, et al. ER stress negatively modulates the expression of the miR-199a/214 cluster to regulates tumor survival and progression in human hepatocellular cancer. *PLoS One* 2012; **7**(2): e31518.

[9] Shih TC, Tien YJ, Wen CJ, Yeh TS, Yu MC, Huang CH, et al. MicroRNA-214 downregulation contributes to tumor angiogenesis by inducing secretion of the hepatoma-derived growth factor in human hepatoma. *J Hepatol* 2012; **57**(3): 584-591.

[10]Kong YW, Ferland-McCollough D, Jackson TJ, Bushell M. microRNAs in cancer management. *Lancet Oncol* 2012; **13**(6): 249-258.

[11]Pereira DM, Rodrigues PM, Borralho PM, Rodrigues CM. Delivering the promise of miRNA cancer therapeutics. *Drug Discov Today* 2013; **18**(5): 282-289.

[12]Yang TS, Yang XH, Wang XD, Wang YL, Zhou B, Song ZS. MiR-214 regulate gastric cancer cell proliferation, migration and invasion by targeting PTEN. *Cancer Cell Int* 2013; **13**(1): 68.

[13]Wang F, Liu M, Li X, Tang H. MiR-214 reduces cell survival and enhances cisplatin-induced cytotoxicity via down-regulation of Bcl2l2 in cervical cancer cells. *FEBS Lett* 2013; **587**(5): 488-495.

[14]Zhang ZC, Li YY, Wang HY, Fu S, Wang XP, Zeng MS, et al. Knockdown of miR-214 promotes apoptosis and inhibits cell proliferation in nasopharyngeal carcinoma. *PloS One* 2014; **9**(1): e86149.

[15]Zhang X, Hu S, Zhang X, Wang L, Zhang X, Yan B, et al. MicroRNA-7 arrests cell cycle in G_1 phase by directly targeting CCNE1 in human hepatocellular carcinoma cells. *Biochem Biophys Res Commun* 2014; **443**(3): 1078-1084.

[16]Feng R, Chen X, Yu Y, Su L, Yu B, Li J, et al. miR-126 functions as a tumour suppressor in human gastric cancer. *Cancer Lett* 2010; **298**(1): 50-63.

[17]Zhang XJ, Ye H, Zeng CW, He B, Zhang H, Chen YQ. Dysregulation of miR-15a and miR-214 in human pancreatic cancer. *J Hematol Oncol* 2010; **3**(1): 46.

[18]Li B, Han Q, Zhu Y, Yu Y, Wang J, Jiang X. Down-regulation of miR-214 contributes to intrahepatic cholangiocarcinoma metastasis by targeting Twist. *FEBS J* 2012; **279**(13): 2393-2398.

[19]Chou J, Werb Z. MicroRNAs play a big role in regulating ovarian cancer–associated fibroblasts and the tumor microenvironment. *Cancer Discov* 2012; **2**(12): 1078-1080.

[20]Liu Z, Tu K, Liu Q. Effects of microRNA-30a on migration, invasion and prognosis of hepatocellular carcinoma. *FEBS Lett* 2014; **588**(17): 3089-3097.

[21]Alpini G, Glaser SS, Zhang JP, Francis H, Han Y, Gong J, et al. Regulation of placenta growth factor by microRNA-125b in hepatocellular cancer. *J Hepatol* 2011; **55**(6): 1339-1345.

[22]Borel F, Konstantinova P, Jansen PLM. Diagnostic and therapeutic potential of miRNA signatures in patients with hepatocellular carcinoma. *J Hepatol* 2012; **56**(6): 1371-1383.

[23]Toffanin S, Hoshida Y, Lachenmayer A, Villanueva A, Cabellos L, Minguez B, et al. MicroRNA-based classification of hepatocellular carcinoma and oncogenic role of miR-517a. *Gastroenterology* 2011; **140** (5):1618-1628.

[24]Yu L, Zhang J, Guo X, Li Z, Zhang P. MicroRNA-224 upregulation and AKT activation synergistically predict poor prognosis in patients with hepatocellular carcinoma. *Cancer Epidemiol* 2014; **33**(4): 408-413.

[25]Gaujoux S, Hantel C, Launay P, Bonnet S, Perlemoine K, Lefèvre L, et al. Silencing mutated β-catenin inhibits cell proliferation and stimulates apoptosis in the adrenocortical cancer cell line H295R. *PloS One* 2013; **8**(2): e55743.

[26]Morikawa T, Kuchiba A, Lochhead P, Bonnet S, Perlemoine K, Lefèvre L, et al. Prospective analysis of body mass index, physical activity, and colorectal cancer risk associated with β-catenin (CTNNB1) status. *Cancer Res* 2013; **73**(5): 1600-1610.

[27]White BD, Chien AJ, Dawson DW. Dysregulation of Wnt/β-catenin signaling in gastrointestinal cancers. *Gastroenterology* 2012; **142**(2): 219-232.

[28]Tsao CM, Yan MD, Shih YL, Yu PN, Kuo CC, Lin WC, et al. SOX1 functions as a tumor suppressor by antagonizing the WNT/β-catenin signaling pathway in hepatocellular carcinoma. *Hepatology* 2012; **56**(6): 2277-2287.

[29]Mathew S, Ali A, Abdel-Hafiz H, Fatima K, Suhail M, Archunan G, et al. Biomarkers for virus-induced hepatocellular carcinoma (HCC). *Infect Genet Evol* 2014; **26**: 327-339.

[30]Pez F, Lopez A, Kim M, Wands JR, de Fromentel CC, Merle P. Wnt signaling and hepatocarcinogenesis: molecular targets for the development of innovative anticancer drugs. *J Hepatol* 2013; **59**(5): 1107-1117.

[31]Misiewicz-Krzeminska I, Sarasquete ME, Quwaider D, Krzeminski P, Ticona FV, Paíno T, et al. Restoration of miR-214 expression reduces growth of myeloma cells through a positive regulation of P53 and inhibition of DNA replication. *Haematologica* 2012. 2012.070011.

[32]Xu Z, Wang T. miR-214 promotes the proliferation and invasion of osteosarcoma cells through direct suppression of LZTS1. *Biochem Biophys Res Commun* 2014; **449**(2): 190-195.