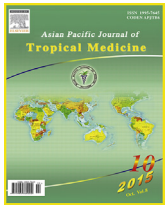
HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm



Original research http://dx.doi.org/10.1016/j.apjtm.2015.09.008

Effect of siRNA on *Wisp-1* gene expression, proliferation, migration and adhesion of mouse hepatocellular carcinoma cells

Jian Ge[1,#], Xiao-Hua Zhang[1,#], Fang Wang[2,#], Yu Wang[3], Qing-Yan Li[3], Wei Tao[5*], Guo-Hua Ren[4*]

[1]*Department of Gastroenterology, Affiliated Shandong Provincial Hospital of Shandong University, Ji'nan 250012, China*

[2]*School of Nursing, Binzhou Vocational College, Binzhou 256603, China*

[3]*Department of Gastroenterology, Zhangqiu People's Hospital, Zhangqiu 250200, China*

[4]*Department of Medical Oncology, Shandong Cancer Hospital and Institute, Ji'nan 250117, China*

[5]*Department of Gastroenterology, General Hospital of Ningxia Medical University, Yinchuan 750004, China*

ARTICLE INFO

Article history:
Received 15 Jul 2015
Received in revised form 20 Aug 2015
Accepted 15 Sep 2015
Available online 3 Oct 2015

Keywords:
Hepatocellular carcinoma cells
Wisp-1
RNA interference
AKT

ABSTRACT

Objective: To study the inhibition effect of siRNA on the expression of *Wisp-1* gene in Hca-F of mouse hepatocellular carcinoma cells strain and also its effect on the proliferation, migration and adhesion of hepatocellular carcinoma cells.

Methods: Three expression vectors of siRNA were constructed. Lipo2000 was employed to transfect Hca-F cells and Western blot was used to detect the inhibition effect of siRNA on the expression of *Wisp-1* gene. Afterward, CCK8 was adopted to detect the effect of *Wisp-1* siRNA on the proliferation of Hca-F cells; Annexin V-FITC/PI double staining flow cytometry was used to detect the effect of *Wisp-1* siRNA on the apoptosis of Hca-F cells; Transwell was used to detect the effect of *Wisp-1* siRNA on the migration of Hca-F cells. The *in vitro* cell adhesion kit was used to detect of *Wisp-1* siRNA on the change in the components of extracellular matrix to which Hca-F cells adhered. Western blot was used to detect the activation of protein kinase B (AKT)/glycogen synthase kinase-3β pathway and the expression of downstream target protein p53 and matrix metalloproteinases-2.

Results: The siRNA showed interference effect on the expression of *Wisp-1* gene. Compared with the control group, after being transfected to cells, *Wisp-1* siRNA could significantly inhibit the proliferation, migration and adhesion of Hca-F cells and also promote the cell apoptosis, which was related to the down-regulated phosphorylation of AKT and glycogen synthase kinase-3β and the expression of p53 and matrix metalloproteinases-2 ($P < 0.05$).

Conclusions: The inhibition of *Wisp-1* expression can reduce the proliferation, migration and adhesion of mouse hepatocellular carcinoma cells, which is related to the AKT/glycogen synthase kinase-3β pathway. *Wisp-1* gene may be the potential target to cure the hepatocellular carcinoma.

1. Introduction

The primary hepatocellular carcinoma is one of most common cancers in the clinical practice. A research reported that [1] there were about 750 000 new diagnosed patients with the hepatocellular carcinoma every year in the world and about 700 000 patients who died of the hepatocellular carcinoma, where 50% occurred in China. The most common histologic

*Corresponding author: Wei Tao, M.M., Deputy Attending Physician, Department of Gastroenterology, General Hospital of Ningxia Medical University, Yinchuan, China.
Tel: +86 13895475006
E-mail: nyfytaowei@163.com
Guo-Hua Ren, Attending Physician, Department of Medical Oncology, Shandong Cancer Hospital and Institute, Ji'nan 250117, China.
E-mail: rghhappiness@126.com

Peer review under responsibility of Hainan Medical College.
Foundation project: It was supported by Shandong Scientific and Technological Development Project Fund (No. 2013GSF11825).

# These authors contributed equally to this work.

subtype of primary hepatocellular carcinoma is the hepatocellular carcinoma, accounting for 70%–80% of the total. Most of hepatocellular carcinomas were the chronic infection caused by the hepatitis B virus and hepatitis C virus [2]. In recent years, with the advance in the techniques of molecular biology, there had been the great progress in the treatment of hepatocellular carcinoma; however, because of the high recurrence rate and metastasis rate, the overall prognosis for the hepatocellular carcinoma was still poor [3]. The biological characteristics of tumor cells are the most important factors to affect the prognosis and the recurrence and metastasis of tumor is the centralized reflection of malignant tumor [4–6]. Therefore, there will be of theoretical and practical significance to study the issues related to the proliferation, migration and adhesion of hepatocellular carcinoma.

Wisp-1 is the member of cysteine rich 61/connective tissue growth factor/nephroblastoma overexpressed gene family. Wisp-1 plays a critical role in the proliferation, adhesion and metastasis of cells, as well as the extracellular matrix accumulation and mitosis [7]. According to recent researches, it is indicated that the expression of Wisp-1 protein was related to the onset, development, metastasis, infiltration and prognosis of many malignant tumors. Besides, the distribution and biological function of Wisp-1 was different in different tumors [8]. It is reported that the expression of *Wisp-1* gene in the tumor cells of melanoma was negatively related to the infiltration and metastasis of malignant tumor cells [9]. Wisp-1 showed the high expression in the colon cancer and esophageal cancer [10,11]. Presently, there has been no research on the effect of Wisp-1 in the hepatocellular carcinoma. To explore the effect and mechanism of Wisp-1 in the hepatocellular carcinoma and seek the new target for the treatment of hepatocellular carcinoma, in this study, mouse hepatocellular carcinoma cells (Hca-F) were chosen as the subjects, while RNA interference was adopted to observe the change in the proliferation, migration and adhesion of Hca-F cells after the silencing of *Wisp-1* gene, in order to explain the effect of Wisp-1 on the biological behavior of hepatocellular carcinoma cells.

2. Materials and methods

2.1. Materials and reagents

The mouse Hca-F were stored in this laboratory; RPMI 1640 and fetal bovine serum were purchased from HyClone, Lipo2000 from Invitrogen, BCA kit and cell adhesion analytical reagent, Annexin V-FITC/PI double staining flow cytometry kit from Beyotime Biotechnology, CCK8 kit from Dojindo, Transwell cell plate from Corning, enzyme-labeled instrument from Bio-Rad; anti-human Wisp-1 antibody and anti-human GAPDH antibody from Abcam; rabbit anti p53, matrix metalloproteinases (MMP)-2, protein kinase B (AKT), p-AKT, glycogen synthase kinase-3β (GSK3β) and p-GSK3β antibodies from Cell Signaling Technology; horseradish peroxidase labeled secondary antibody from Beijing Zhongshan Jinqiao Biotechnology; ECL Chemiluminescent Substrate Reagent Kit from Life Technologies-WP20005; PVDF (polyvinylidene fluoride) film from Millpore. The optical microscope is Olympus BX53; CO_2 incubator is Thermo Scientific Series 8000.

2.2. Methods

2.2.1. Design of siRNA sequence

The human *Wisp-1* gene sequence was obtained from GeneBank. According to the design principle of siRNA, the siRNA target Designers (Ambion) was used to design 3 siRNAs targeting the specific *Wisp-1*. The related sequence and negative control were shown in Table 1. Each sequence was synthesized by Shanghai Biochemical Engineering Co., Ltd.

2.2.2. Transfection and screening of siRNA

On the day before the transfection, mouse hepatocellular carcinoma cells that were in the phase of logarithmic growth were seeded on 6-well plate according to the cell density of 6×10^4 cells/well. When the cell confluency reached to about 70%–80%, the Opti-MEM I that contained 0.5% fetal bovine serum was mixed with Lipo2000 and siRNA. The mixture of Lipo2000 and siRNA was added in cells. It was incubated in the incubator at 37 °C and with 5% CO_2 for 48 h. The number of transfected cells was observed under the fluorescence microscopy. Meanwhile, RT-PCR and Western Blot were employed to detect the protein expression of *Wisp-1*, in order to determine the best interference effect.

2.2.3. Detection of effect of Wisp-1 siRNA on cell proliferation

The cell density of mouse hepatocellular carcinoma cells in the phase of logarithmic digestion was adjusted to 1×10^4 cells/mL and they were added in 96-well plate by 100 μL/well. Cells were divided into three groups, namely *WisP-1* siRNA transfection group, control group (without treatment) and siRNA-NC transfection group. Each group had 5 repeated wells and cells were incubated in the incubator at 37 °C and with 5% CO_2. At 24 h, 28 h and 72 h of *in vitro* culture, each well was added with 10 μL CCK-8 to be cultured in the incubator for another 1 h. The enzyme-labeled instrument was employed to detect the optical density of samples in each group at 450 nm.

2.2.4. Detection of cell apoptosis by Annexin V-FITC/PI double staining flow cytometry

According to the above procedure, cells were seeded on 100 mm dish and then given the administration. At 24 h after the

Table 1
siRNA sequence of *Wisp-1* gene.

Sequence name	Sense (5′-3′)	Antisense (5′-3′)
siRNA-*Wisp-1*	GTACCTCAGAACGUGGACUATT	UAUCTCACCUCUGAAGACCTT
siRNA-*Wisp-1*	GACTAUCAGGACUUTATACGUA	UUCTGCAAATGCTATTCGGCTT
siRNA-*Wisp-1*	GACUTGAAUTTGACTTCTGAGG	ACCCAUGGCATCCCGGGAGTT
Negative control (siRNA-NC)	UUCTCCGAACGUGCUCACGUTT	ACCUGACACGUUCGGAGAATT

administration, according to the procedure mentioned in the instruction manual of Annexin V-FITC/propidium iodide (PI) cell apoptosis detection kit, cells were digested with 0.25% trypsin (without EDTA), washed with PBS, centrifuged at 2 000 rpm for 5 min and then collected. A total of 500 μL binding buffer was added to suspend the cells and then it was mixed with 5 μL Annexin V-FITC and 5 μL PI. It was reacted in a dark place and at the room temperature for 5–15 min and then measured by the flow cytometry within 1 h.

2.2.5. Detection of effect of Wisp-1 siRNA on cell migration

Cells were divided into three groups, namely *WisP-1* siRNA transfection group, control group and siRNA-NC transfection group. The cell density was adjusted to 3×10^5 cells/mL and then the starvation culture was performed on cells in the incubator for 24 h. The Transwell upper chamber was added by 100 μL starvation cultured cells, while the lower chamber was added by 500 μL culture medium that contained 10% fetal bovine serum and 10 μg/mL fibronectin. After being cultured in the incubator at 37 °C and with 5% CO_2 for 12 h, Transwell chamber was taken out. The residual culture medium and non-migrated cells were removed by the cotton swab carefully. Cells were dried at the room temperature for half an hour. Then the chamber was placed in the solution with 0.1% crystal violet for the staining and then it was washed with PBS several times. The number of migrated hepatocellular carcinoma cells in 10 visual fields was counted under the inverted microscopy and the mean values were collected for the statistical analysis.

2.2.6. Effect of Wisp-1 siRNA-transfected cells on the adhesion of components of extracellular matrix

The cell adhesion was employed to analyze the components of each extracellular matrix in the kit. The culture medium that contained 0.5% fetal bovine serum was used to prepare the suspension cells. The cell density was adjusted to 1×10^6 cells/mL. 150 μL cell suspension was added in each well of cell adhesion plate. It was incubated for 1 h and then the supernatant was sucked from each well. It was washed with PBS several times. A total of 200 μL cell staining buffer was added in each well and it was incubated at the room temperature for 10 min. The cell staining buffer was then removed. It was washed with ddH_2O one time and then be sucked and dried. A total of 200 μL extraction solution was added in each well. It was incubated at the shaker for 10 min. A total of 100 μL solution was sucked from each well to 96-well plate. The enzyme-labeled instrument was used to measure OD value at 560 nm.

2.2.7. Detection of expression of transfected Wisp-1 protein and other related proteins by Western Blot

At 48 h after the transfection, cells in each group were washed with PBS twice. After being digested with pancreatin, the supernatant was removed. The precipitated cells were lysed with RIPA lysis buffer. Then the protease inhibitor cocktail was added for the blowing and mixing. After being put on the ice for 30–40 min, cells were lysed using the ultrasound. The probe-type ultrasound was used to produce the short impact with the appropriate frequency on the ice. The lysis mixture was centrifuged at 4 °C and 13 000 rpm/min for 20 min. The supernatant was transferred to the new centrifuge tube. BCA kit was employed to detect the protein concentration. SDS-PAGE electrophoresis was performed on 20 μg total protein samples. The gel was soaked in the transfer buffer for 10 min of equilibrium. It was installed with the transfer 'sandwich', 100 V and 45–60 min. After the transfer, PVDF film was washed with TBS for 10–15 min. The film was placed in TBS/T blocking buffer containing 5% (w/v) skimmed milk powder and shaken at the room temperature for 1 h. Then the primary antibody with the appropriate degree of dilution was added [diluted with TBST containing 1% (w/v) skimmed milk powder]. It was incubated at the room temperature for 2 h and then the film was washed with TBST for 3 times and 5–10 min each time. The film was incubated with the secondary antibody (1:10 000, horseradish peroxidase-labeled) that was diluted with TBST containing 0.05% (w/v) skimmed milk powder. It was incubated at the room temperature for 1 h and then the film was washed with TBST for 3 times and 5–10 min each time. It was exposed and then photographed to save the experimental results. Quantity one v4.62 was used to measure the gray value of molecular band.

2.3. Statistical analysis

Results were expressed by mean ± SD. Three repeats were performed on the data of each group at least. The *t* test was employed to detect the significant difference, where $P < 0.05$ referred to the statistically significant difference. The experimental data was processed using SPSS 17.0.

3. Results

3.1. Screening of siRNA interference fragment

Results of Western blot showed that the expression of Wisp-1 protein in cells of siRNA-2 groups was significantly lower

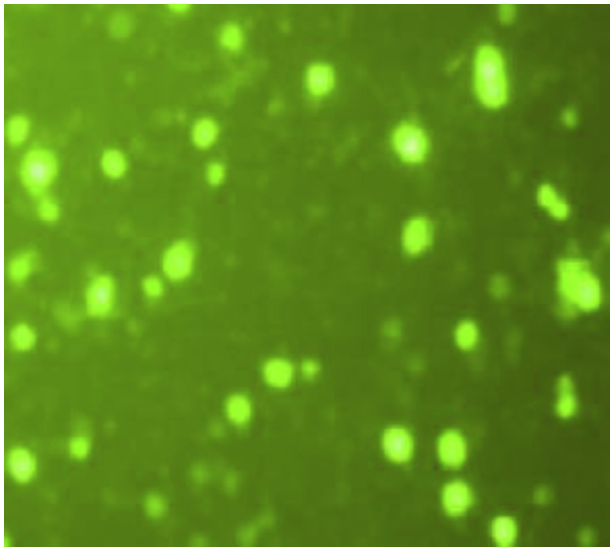


Figure 1. Fluorescence photograph of *Wisp-1* siRNA-transfected Lipo2000 of mouse hepatocellular carcinoma cells.

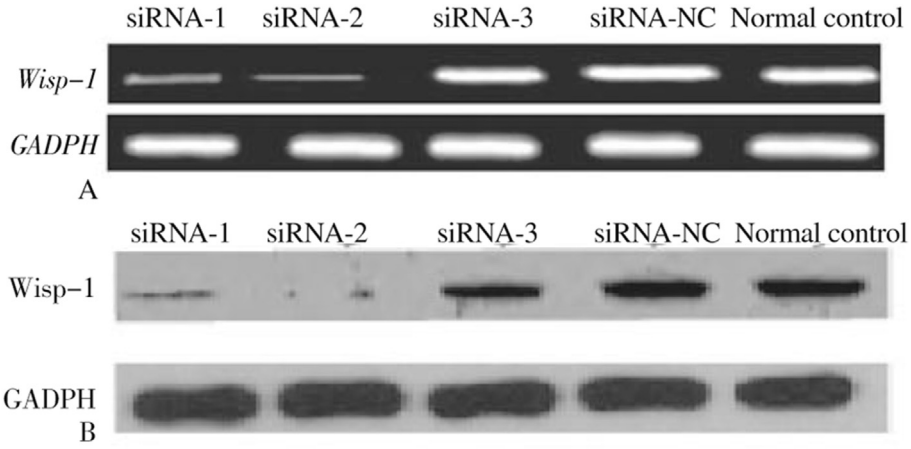


Figure 2. Expression of Wisp-1 protein.
A; RT-PCR; B: Western Blot.

than ones in the groups of siRNA-1, siRNA-3, siRNA negative control and normal control, which indicated that the interference effect of siRNA-2 was best. It was chosen as the interference fragment for the further experiments, as shown in Figures 1 and 2.

3.2. CCK-8 cell proliferation

The optical density of each group after CCK8 detection was shown in Table 2. According to the statistical treatment, there was no significant difference at 24 h, 48 h and 72 h between the control group and siRNA-NC transfection group ($P > 0.05$), but the optical density of *Wisp-1* siRNA transfection group was significantly lower than ones of control group and siRNA-NC transfection group ($P < 0.05$).

3.3. Cell apoptosis

The apoptosis rate of *Wisp-1* siRNA transfection group was significantly higher than ones of control group and siRNA-NC transfection group ($P < 0.05$), but there was no significant difference between the control group and siRNA-NC transfection group ($P > 0.05$), which indicated that the apoptosis rate of mouse hepatocellular carcinoma cells after the silencing of *Wisp-1* gene was increased, as shown in Figure 3.

3.4. Transwell cell migration

The number of migrated cells in *Wisp-1* siRNA transfection group was significantly lower than ones in the control group and siRNA-NC transfection group ($P < 0.05$), but there was no significant difference between the control group and siRNA-NC transfection group ($P > 0.05$), which indicated that the migration capacity of mouse hepatocellular carcinoma cells after the silencing of *Wisp-1* gene was significantly reduced, as shown in Table 3 and Figure 4.

3.5. Cell adhesion

Compared with the siRNA-NC transfection group and control one, the adhesion capacity of collagen I and fribrictin in *Wisp-1* siRNA transfection group was significantly reduced ($P < 0.05$), but there was no significant difference in the adhesion of other components. There was no significant difference in the cell adhesion between siRNA-NC transfection group and control one, as shown in Table 4.

3.6. Expression of p53 and MMP-2

Compared with the siRNA-NC transfection group and control group, the expression of p53 and MMP-2 was significantly inhibited in *Wisp-1* siRNA transfection group ($P < 0.05$). There was no change in the expression of p53 and MMP-2 in siRNA-NC transfection group and control group, as shown in Figure 5.

3.7. Activation of AKT/GSK3β pathway

Compared with the siRNA-NC transfection group and control group, the phosphorylation of AKT and GSK3β was significantly inhibited in the *Wisp-1* siRNA transfection group ($P < 0.05$) and without any effect on its expression. AKT/GSK3β was not activated in the siRNA-NC transfection group and control group, as shown in Figure 6.

Table 2
CCK-8 results of hepatocellular carcinoma cells at different time points (mean ± SD).

Group	Optical density		
	24 h	48 h	72 h
Control group	0.166 3 ± 0.011 7*	0.210 0 ± 0.012 4*	0.391 3 ± 0.013 5v
siRNA-NC transfection group	0.167 6 ± 0.008 2*	0.202 5 ± 0.012 3*	0.459 9 ± 0.014 5*
Wisp-1 siRNA transfection group	0.132 3 ± 0.006 1	0.146 0 ± 0.009 6	0.247 3 ± 0.011 6

Note: * Compared with *Wisp-1* siRNA transfection group, $P < 0.05$.

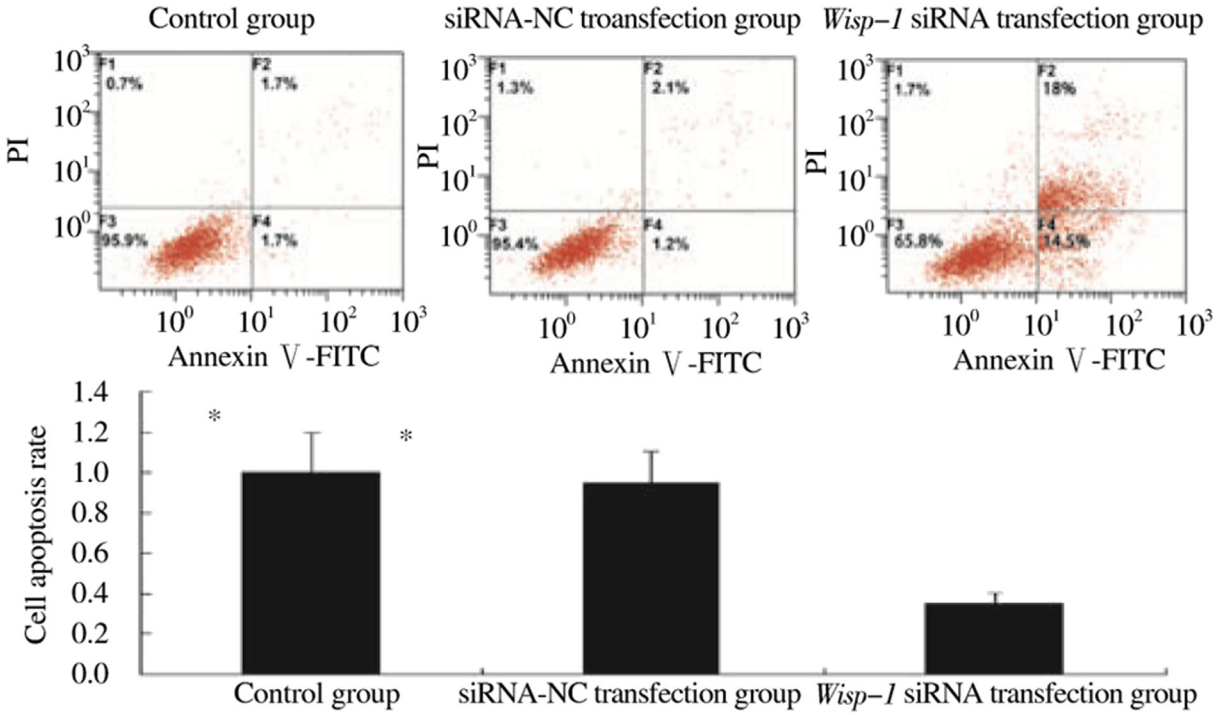


Figure 3. Cell apoptosis.
*: Compared with *Wisp-1* siRNA transfection group, $P < 0.05$.

Table 3
Number of migrated cells (*n*).

Group	Repeated well 1	Repeated well 2	Repeated well 3	Average (mean ± SD)
Control group	215.3	208.9	214.7	213.0 ± 23.5*
siRNA-NC transfection group	207.4	210.5	212.2	210.0 ± 22.7*
Wisp-1 siRNA transfection group	145.7	140.6	129.8	138.7 ± 20.6

Note: * Compared with *Wisp-1* siRNA transfection group, $P < 0.05$.

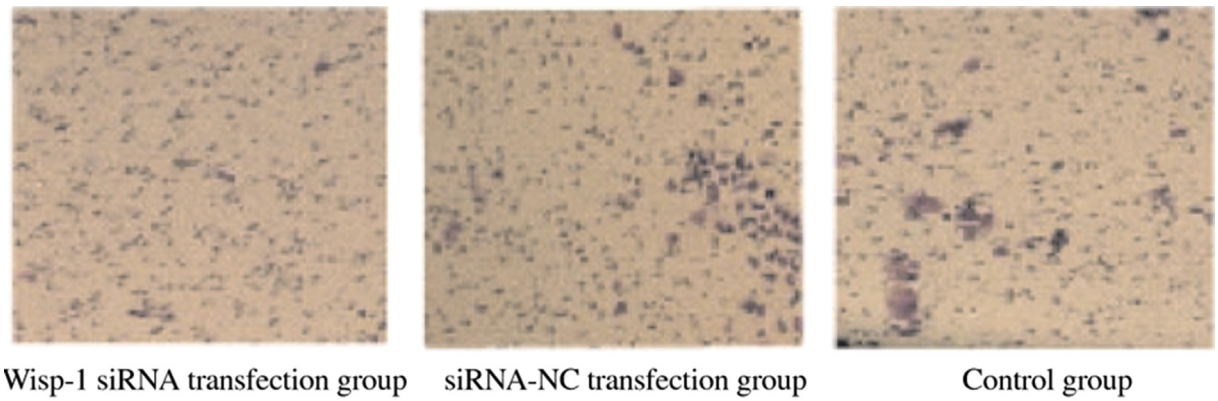


Figure 4. *In vitro* migration capacity of mouse hepatocellular carcinoma cells after down-regulated expression of *Wisp-1* gene.

Table 4
Effect of *Wisp-1* gene silencing on adhesion capacity of mouse hepatocellular carcinoma cells (mean ± SD).

Group	Fribrinogen	Ln	Collagen I	Collagen V	Fribrictin
Control group	0.95 ± 0.12	0.85 ± 0.15	1.25 ± 0.20*	0.91 ± 0.25	1.48 ± 0.27*
siRNA-NC transfection group	0.88 ± 0.10	0.79 ± 0.08	1.19 ± 0.17*	0.89 ± 0.20	1.46 ± 0.25*
Wisp-1 siRNA transfection group	0.75 ± 0.09	0.70 ± 0.05	0.87 ± 0.11	0.77 ± 0.18	1.01 ± 0.23

Note: * Compared with *Wisp-1* siRNA transfection group, $P < 0.05$.

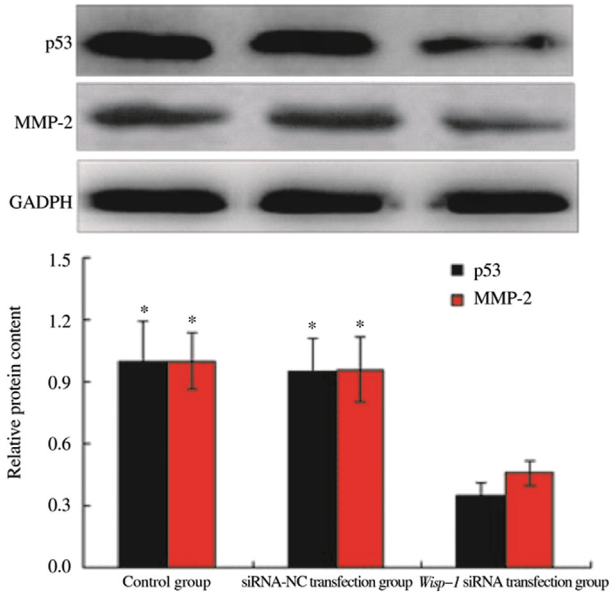


Figure 5. Effect of *Wisp-1* gene silencing on expression of p53 and MMP-2 in mouse hepatocellular carcinoma cells.
* Compared with *Wisp-1* siRNA transfection group, $P < 0.05$.

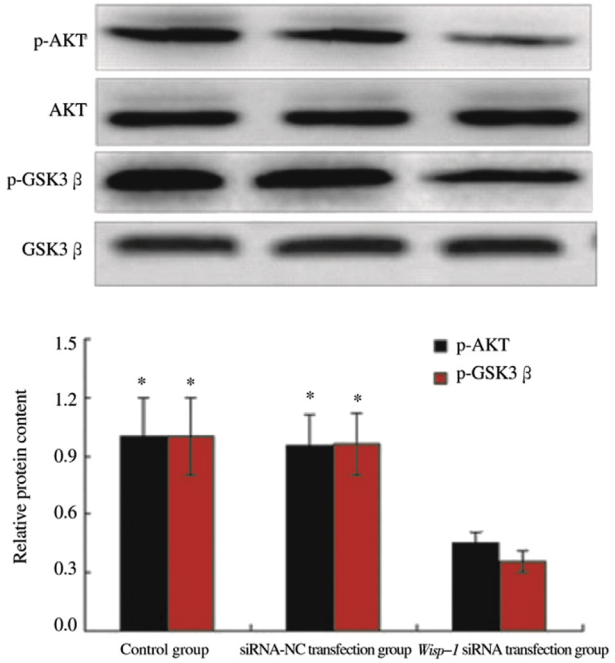


Figure 6. Effect of *Wisp-1* gene silencing on activation of AKT/GSK3β pathway of mouse hepatocellular carcinoma cells.
* Compared with *Wisp-1* siRNA transfection group, $P < 0.05$.

4. Discussion

More and more researches have shown that Wisp-1 is widely expressed in many human tissues and organs. Because of its biological functions to mediate the cell migration, promote the cell proliferation and extracellular matrix accumulation and stimulate the cell adhesion, it has been highly emphasized and focused in the field of biology at present [12]. The research showed that the coding *Wisp-1* gene is at 8q24.1-8q24.3 of human chromosome. It is a secretory protein and peptide with 367 amino acids that is rich in cysteine, which can be synthesized and secreted by vascular smooth muscle cells, vascular endothelial cells and fibroblasts [13,14]. There was the great difference in the expression of *Wisp-1* gene in different tissues, without the tissue specificity. The expression of Wisp-1 could promote the pulmonary fibrosis, be involved in the development and repairing of bones and myocardial infarction, and also promote the cancer progression [15–19].

In recent years, it is reported that relying on the interaction with the extracellular matrix, Wisp-1 could regulate the onset, development, metastasis and infiltration of malignant tumors. Besides, the expression of Wisp-1 was closely related to the prognosis and recurrence of tumors [20–22]. However, the distribution and biological function of Wisp-1 was different in different tumor tissues, which could negatively or positively regulate the onset and development of tumors. Pennica *et al.* [23] reported that, according to RT-PCR analysis, the expression of Wisp-1 was significantly increased in most colon cancer cells, about 8 times more than the normal one. The mRNA in about 84% colon cancer tissues was increased by 2–15 times higher than the related neighboring tissues. Based on the autocrine or paracrine, Wisp-1 could stimulate the abnormal proliferation of mammary epithelial cells and then cause the formation of breast cancer. Besides, its protein expression was significantly related to the size of breast cancer, metastasis and course of lymph nodes [24]; however, the expression of Wisp-1 in the lung cancer tissue was significantly lower than the one in the neighboring normal lung tissue and the metastasis rate for the lung tumor with the high expression of Wisp-1 was significantly lower than the one for the tumor with the low expression of Wisp-1 [25]. There has been no specific research on the function of Wisp-1 in the hepatocellular carcinoma tissue. This study was to explore the role of Wisp-1 in mouse hepatocellular carcinoma cells. The siRNA was employed to inhibit the expression of *Wisp-1* gene, while Western Blot was used to detect the expression of Wisp-1 in mouse Hca-F stains that was transfected with *Wisp-1* siRNA-2 and results showed that the expression was significantly reduced. Afterward, according to the experiments of cell proliferation, cell migration and cell adhesion, it is to study the effect of inhibition of Wisp-1 expression on the proliferation, migration and adhesion of cells. Results showed that after the inhibition of Wisp-1 expression, the proliferation, migration and adhesion of cells in *Wisp-1* siRNA group was significantly lower than ones in the control group and siRNA-NC group ($P < 0.05$). It is indicated that Wisp-1 could promote the proliferation, migration and adhesion of hepatocellular carcinoma cells. The extracellular matrix and extracellular attachments played a key role in the Wisp-1-induced tumor. It is recognized that the adhesion molecule is the only known receptor of Wisp-1. Wisp-1 may cause the downstream reaction through the adhesion molecule pathway and thus result in the tumor. The proliferation, migration and adhesion are the basic characteristics of biological behavior of tumor cells and also the key cause for the metastasis and recurrence of malignant tumors.

The specific mechanism of Wisp-1 in the tumors has not been clear. Wnt-1 and β-catenin can activate the Wisp-1 to affect the transcriptional activity of Wisp-1; while Wisp-1 can induce the phosphorylation of AKT to cause the phosphorylation of GSK3β and reduce the activity of GSK3β to inhibit the cell apoptosis as the result of DNA injury [26,27]. Wisp-1 could regulate the activity of AKT, increase the expression of surviving and inhibit the release of Bax and cytochrome C [28]. Colston *et al.* [29] reported the function of Wisp-1/AKT signal in the cardiac hypertrophy and indicated that Wisp-1

could enhance the cell proliferation and survival. AKT is involved in many basic cell processes of proliferation, apoptosis, migration and invasion. AKT can cause the loss of cell adhesion, change of morphology and disappear of polarity, which thus reduce the cell adhesion and enhance the movement. The abnormal activation of AKT in the hepatocellular carcinoma tissue might be closely related to the prognosis of patients [30]. The activated AKT could rely on the phosphorylation to inhibit the expression of downstream target proteins such as GSK-3β, p53 and MMP 2 and thus regulate the proliferation, apoptosis and invasion of cells [31,32]. GSK-3β is a key factor to regulate the cell apoptosis and the important target protein in the downstream pathway of PI3K/AKT, which was involved in the growth, development and proliferation of cells [33,34]. By down-regulating the phosphorylation of GSK-3β, it could significantly inhibit the proliferation of hepatocellular carcinoma cells [35]. Wisp-1 could also inhibit p53 to regulate the cell apoptosis and thus promote the tumor formation [36]. Wisp-1 could include the expression and secretion of MMP-2 in human chondrosarcoma cells and thus the interference against the expression of MMP-2 could significantly inhibit the Wisp-1-induced cell migration [20]. The knockdown of mouse MMP-2 might affect the secretion of MMPs, which was realized through PI3K/Akt pathway [37]. The high expression of MMP-2 and p53 was positively correlated to the size of hepatocellular carcinoma and metastasis of lymph node. In hepatocellular carcinoma cells, it could down-regulate the expression of p53 and MMP-2 through PI3K/AKT pathway and thus inhibit the cell growth and invasion [38]. In this study, Western blot was employed to detect the activation of AKT/GSK-3β and the expression of p53 and MMP-2 in cells of each group. Results showed that the interference against the expression of Wisp-1 in hepatocellular carcinoma cells could inactivate the AKT/GSK-3β pathway and reduce the expression of p53 and MMP-2 ($P < 0.05$). It can be seen that taking Wisp-1 as the treatment target can improve the prognosis of malignant tumors such as the hepatocellular carcinoma. However, as this study was performed based on the *in vitro* experiment, it still requires the further *in vivo* experiment for the validation.

In conclusion, through the direct expression in the tumor cells, Wisp-1 can affect the proliferation, migration and invasion of tumors, which may be related to the AKT/GSK-3β pathway. It is found that the silencing of *Wisp-1* gene in the primary hepatocellular carcinoma can reduce the proliferation, migration and adhesion of cells. Therefore, it will be necessary to study Wisp-1 as the prognosis evaluation factor of malignant tumor and choose it as the treatment target of tumors.

Conflict of interest statement

The authors declare that they have no conflict of interest.

References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**(2): 69-90.

[2] Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary hepatocellular carcinoma worldwide. *J Hepatol* 2006; **45**(4): 529-538.

[3] Li H, Wu K, Tao K, Chen L, Zheng Q, Lu X, et al. Tim-3/galectin-9 signaling pathway mediates T-cell dysfunction and predicts poor prognosis in patients with hepatitis B virus-associated hepatocellular carcinoma cells. *Hepatology* 2012; **56**(4): 1342-1351.

[4] Yun UJ, Park SE, Jo YS, Kim J, Shin DY. DNA damage induces the IL-6/STAT3 signaling pathway, which has anti-senescence and growth-promoting functions in human tumors. *Cancer Lett* 2012; **323**(2): 155-160.

[5] Yang X, Liang L, Zhang XF, Jia HL, Qin Y, Zhu XC, et al. MicroRNA-26a suppresses tumor growth and metastasis of human hepatocellular carcinoma cells by targeting interleukin-6-Stat3 pathway. *Hepatology* 2013; **58**(1): 158-170.

[6] Li S, Wang N, Brodt P. Metastatic cells can escape the proapoptotic effects of TNF-alpha through increased autocrine IL-6/STAT3 signaling. *Cancer Res* 2012; **72**(4): 865-875.

[7] Liu ZJ, Li Y, Tan Y, Xiao M, Zhang J, Radtke F, et al. Inhibition of fibroblast growth by Notch1 signaling is mediated by induction of Wnt11-dependent WISP-1. *PLoS One* 2012; **7**(6): e38811.

[8] Perbal B. CCN proteins: a centralized communication network. *J Cell Commun Signal* 2013; **7**(3): 169-177.

[9] Shao H, Cai L, Grichnik JM, Livingstone AS, Velazquez OC, Liu ZJ. Activation of Notch1 signaling in stromal fibroblasts inhibits melanoma growth by upregulating WISP-1. *Oncogene* 2011; **30**(42): 4316-4326.

[10] Davies SR, Davies ML, Sanders A, Parr C, Torkington J, Jiang WG. Differential expression of the CCN family member WISP-1, WISP-2 and WISP-3 in human colorectal cancer and the prognostic implications. *Int J Oncol* 2010; **36**(5): 1129-1136.

[11] Li WF, Zhang L, Li HY, Zheng SS, Zhao L. WISP-1 contributes to fractionated irradiation-induced radioresistance in esophageal carcinoma cell lines and mice. *PLoS One* 2014; **9**(4): e94751.

[12] Tang Q, Jiang X, Li H, Lin Z, Zhou X, Luo X, et al. Expression and prognostic value of WISP-1 in patients with endometrial endometrioid adenocarcinoma. *J Obstet Gynaecol Res* 2011; **37**(6): 606-612.

[13] Jian YC, Wang JJ, Dong S, Hu JW, Hu LJ, Yang GM, et al. Wnt-induced secreted protein 1/CCN4 in liver fibrosis both *in vitro* and *in vivo. Clin Lab 2014; 60(1): 29-35.*

[14] Mill C, Monk BA, Williams H, Simmonds SJ, Jeremy JY, Johnson JL, et al. Wnt5a-induced Wnt1-inducible secreted protein-1 suppresses vascular smooth muscle cell apoptosis induced by oxidative stress. *Arterioscler Thromb Vasc Biol* 2014; **34**(11): 2449-2456.

[15] Ono M, Inkson CA, Kilts TM, Young MF. WISP-1/CCN4 regulates osteogenesis by enhancing BMP-2 activity. *J Bone Min Res* 2011; **26**(1): 193-208.

[16] Blom AB, van Lent PL, van der Kraan PM, van den Berg WB. To seek shelter from the WNT in osteoarthritis? WNT-signaling as a target for osteoarthritis therapy. *Curr Drug Targets* 2010; **11**(5): 620-629.

[17] Zemans RL, Mcclendon J, Aschner Y, Briones N, Young SK, Lau LF, et al. Role of beta-catenin-regulated CCN matricellular proteins in epithelial repair after inflammatory lung injury. *Am J Physiol Lung Cell Mol Physiol* 2013; **304**(6): L415-L427.

[18] Sklepkiewicz P, Shiomi T, Kaur R, Sun J, Kwon S, Mercer B, et al. Loss of secreted frizzled-related protein-1 leads to deterioration of cardiac function in mice and plays a role in human cardiomyopathy. *Circ Heart Fail* 2015; **8**(2): 362-372.

[19] Chang CC, Lin BR, Wu TS, Jeng YM, Kuo ML. Input of microenvironmental regulation on colorectal cancer: role of the CCN family. *World J Gastroenterol* 2014; **20**(22): 6826-6831.

[20] Hou CH, Chiang YC, Fong YC, Tang CH. WISP-1 increases MMP-2 expression and cell motility in human chondrosarcoma cells. *Biochem Pharmacol* 2011; **81**(11): 1286-1295.

[21] Li Z, Dang J, Chang KY, Rana TM. MicroRNA-mediated regulation of extracellular matrix formation modulates somatic cell reprogramming. *RNA* 2014; **20**(12): 1900-1915.

[22] Tai HC, Chang AC, Yu HJ, Huang CY, Tsai YC, Lai YW, et al. Osteoblast-derived Wnt-1-induced secreted protein 1 increases VCAM-1 expression and enhances prostate cancer metastasis by down-regulating miR-126. *Oncotarget* 2014; **5**(17): 7589-7598.

[23] Pennica D, Swanson TA, Welsh JW, Roy MA, Lawrence DA, Lee J, et al. WISP genes are members of the connective tissue

growth factor family that are up-regulated in wnt-1-transformed cells and aberrantly expressed in human colon tumors. *Proc Natl Acad Sci U.S.A* 1998; **95**(25): 14717-14722.

[24] Hu R, Tian C, Meng WJ, Zhang JH, Li L, Zhang PR, et al. The expression and clinical significance of Wnt-1 induced secreted protein-1 in breast carcinoma. *Sichuan Da Xue Xue Bao Yi Xue Ban* 2010; **41**(2): 231-234.

[25] Chen PP, Li WJ, Wang Y, Zhao S, Li DY, Feng LY, et al. Expression of Cyr61, CTGF, and WISP-1 correlates with clinical features of lung cancer. *PLoS One* 2007; **2**(6): e534.

[26] Stephens S, Palmer J, Konstantinova I, Pearce A, Jarai G, Day E. A functional analysis of Wnt inducible signalling pathway protein -1 (WISP-1/CCN4). *J Cell Commun Signal* 2015; **9**(1): 63-72.

[27] Alapati D, Rong M, Chen S, Lin C, Li Y, Wu S. Inhibition of LRP5/6-mediated Wnt/beta-catenin signaling by Mesd attenuates hyperoxia-induced pulmonary hypertension in neonatal rats. *Pediatr Res* 2013; **73**(6): 719-725.

[28] Khor TO, Gul YA, Ithnin H, Seow HF. A comparative study of the expression of Wnt-1, WISP-1, survivin and cyclin-D1 in colorectal carcinoma. *Int J Colorectal Dis* 2006; **21**(4): 291-300.

[29] Colston JT, de la Rosa SD, Koehler M, Gonzales K, Mestril R, Freeman GL, et al. Wnt-induced secreted protein-1 is a prohypertrophic and profibrotic growth factor. *Am J Physiol Heart Circ Physiol* 2007; **293**(3): H1839-H1846.

[30] Wang RY, Chen L, Chen HY, Hu L, Li L, Sun HY, et al. MUC15 inhibits dimerization of EGFR and PI3K-AKT signaling and is associated with aggressive hepatocellular carcinoma cellss in patients. *Gastroenterology* 2013; **145**(6): 1436-1448.

[31] Saiprasad G, Chitra P, Manikandan R, Sudhandiran G. Hesperidin induces apoptosis and triggers autophagic markers through inhibition of Aurora-A mediated phosphoinositide-3-kinase/Akt/mammalian target of rapamycin and glycogen synthase kinase-3 beta signalling cascades in experimental colon carcinogenesis. *Eur J Cancer* 2014; **50**(14): 2489-2507.

[32] Gwak HS, Park MJ, Park IC, Woo SH, Jin HO, Rhee CH, et al. Tetraarsenic oxide-induced inhibition of malignant glioma cell invasion *in vitro* via a decrease in matrix metalloproteinase secretion and protein kinase B phosphorylation. *J Neurosurg* 2014; **121**(6): 1483-1491.

[33] Yang Y, Li W, Li Y, Wang Q, Gao L, Zhao J. Dietary Lycium barbarum polysaccharide induces Nrf2/ARE pathway and ameliorates insulin resistance induced by high-fat via activation of PI3K/AKT signaling. *Oxid Med Cell Longev* 2014; **2014**: 145641.

[34] Sun Y, Gao C, Luo M, Wang W, Gu C, Zu Y, et al. Aspidin PB, a phloroglucinol derivative, induces apoptosis in human hepatocarcinoma HepG2 cells by modulating PI3K/Akt/GSK3beta pathway. *Chem Biol Interact* 2013; **201**(1–3): 1-8.

[35] Cao J, Feng XX, Yao L, Ning B, Yang ZX, Fang DL, et al. Saturated free fatty acid sodium palmitate-induced lipoapoptosis by targeting glycogen synthase kinase-3beta activation in human liver cells. *Dig Dis Sci* 2014; **59**(2): 346-357.

[36] Chuang JY, Chang AC, Chiang IP, Tsai MH, Tang CH. Apoptosis signal-regulating kinase 1 is involved in WISP-1-promoted cell motility in human oral squamous cell carcinoma cells. *PLoS One* 2013; **8**(10): e78022.

[37] Chetty C, Lakka SS, Bhoopathi P, Rao JS. MMP-2 alters VEGF expression via alphaVbeta3 integrin-mediated PI3K/AKT signaling in A549 lung cancer cells. *Int J Cancer* 2010; **127**(5): 1081-1095.

[38] Shan RF, Zhou YF, Peng AF, Jie ZG. Inhibition of Aurora-B suppresses HepG2 cell invasion and migration via the PI3K/Akt/NF-kappaB signaling pathway. *Exp Ther Med* 2014; **8**(3): 1005-1009.