European Review for Medical and Pharmacological Sciences 2020; 24: 9932-9939

MiR-329-3p inhibits hepatocellular carcinoma cell proliferation and migration through USP22-Wnt/β-Catenin pathway

R.-Q. XIN[1], W.-B. LI[1], Z.-W. HU[1], Z.-X. WU[1], W. SUN[2]

[1]Department of Hepatobiliary Surgery, Inner Mongolia People's Hospital, Hohhot, Inner Mongolia, P.R. China
[2]The Postdoctoral Research Station, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, P.R. China

Ruiqiang Xin and Wubing Li contribute equally and should be regarded as co-first authors

Abstract. – OBJECTIVE: MicroRNA-329-3p (miR-329-3p) has been shown to be involved in tumor development. But its role in hepatocellular carcinoma has not been explored. Our study aims to explore the effect and mechanism of miR-329-3p on hepatocellular carcinoma development.

PATIENTS AND METHODS: Hepatocellular carcinoma tissues and paired paracancerous specimens from 31 hepatocellular carcinoma patients undergoing surgery were collected. Quantitative real-time polymerase chain reaction and Western blot were employed to measure genes expression at mRNA and protein level. CCK-8 and transwell assays were performed to evaluate hepatocellular carcinoma cells proliferation and migration. Dual-Luciferase reporter gene assay was designed to validate the target gene of miR-329-3p.

RESULTS: Our study showed miR-329-3p expression was significantly lower in hepatocellular carcinoma tissue. MiR-329-3p mimic inhibits proliferation and migration of HepG2 cells. By using Dual-Luciferase reporter gene assay, we proved that miR-329-3p inhibited HepG2 cell proliferation and migration by targeting USP22 directly. By up- and downregulation of USP22 expression, we also proved that USP22 can activate the Wnt/β-Catenin pathway, which in turn affected the proliferation and migration of HepG2 cells.

CONCLUSIONS: We demonstrated that miR-329-3p can inhibit HepG2 cell proliferation and migration by inhibiting USP22-Wnt/β-Catenin pathway. Our study provides novel insights into the aetiology and potential treatment of hepatocellular carcinoma.

Key Words:
MicroRNA-329-3p, USP22, Hepatocellular carcinoma, Proliferation, Migration, Wnt/β-Catenin.

Introduction

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. Globally, liver cancer is the fourth leading cause of cancer-related deaths[1]. Every year, a large number of patients with HCC die, which places a heavy burden on medical systems. The 5-year survival rate of liver cancer is only 18% and it is the second fatal tumor after the pancreatic cancer[2]. The poor prognosis of liver cancer is due to high recurrence and metastasis rates[3]. The cause of HCC is not fully understood. But its common causes include hepatitis virus infection, alcoholic or non-alcoholic cirrhosis, etc.[4]. Nowadays, increasing researchers are studying the pathogenesis of HCC in order to find a way to improve the treatment effect of HCC.

The role of microRNA (miRNA) in malignant tumors has attracted widespread attention from researchers all over the world[5,6]. MiRNA is a type of small RNA with a length of 20 to 26 nt. It often targets to the 3 'untranslated region (3'UTR) of the target mRNA, which makes the downstream target gene degraded and inactivated[7,8].

The discovery of miRNA has completely changed our understanding of cancer's complex gene networks. MiRNAs play an important role in various biological and pathological processes including proliferation and metastasis[9] and down-regulation of tumor suppressive miRNAs or overexpression of oncogene miRNAs plays a key role in tumorigenesis[10-12].

MiR-329-3p was found to play a role as a tumor suppressor[13-15]. However, there are few reports about miR-329-3p in HCC. This study

Corresponding Author: Wei Sun, MD; e-mail: sunwei85@pumch.cn

focused on the role of miR-329-3p in HCC and its mechanism.

Patients and Methods

Tissue Sample

From 2007 to 2019, HCC tissues and paired paracancerous specimens from 31 HCC patients undergoing surgery were collected. The patients signed an informed consent before the operation. The medical Ethics Committee of the Inner Mongolia People's Hospital approved the study.

Cell Culture

Human HCC cell lines HepG2 were all cultured in Dulbecco's Modified Eagle's Medium (DMEM; GIBCO, Carlsbad, CA, USA) with 10% (V / V) FBS (Hyclone, Chicago, IL, USA), 100 IU/ml penicillin and 100 μg/ml streptomycin (GIBCO, Carlsbad, CA, USA), cultured in an incubator at 37°C, 5% CO_2. PNU-74654 (Wnt/β-actin pathway inhibitor, Selleck Chemicals, Shanghai, China) is dissolved in dimethylsulfoxide (DMSO; Solarbio, Beijing, China) to make a solution with a concentration of 10 μM.

Transfections

MiR-329-3p mimic, miR-329-3p inhibitor, USP22 siRNA, USP22 overexpression plasmids (pcDNA3.1- USP22) were designed and synthesized by GenePharma (GenePharma, Shanghai, China). HepG2 cells were seeded on 12-well plate with a density of 5×10^5 cells/well. Lipofectamine 2000 (Invitrogen, Shanghai, China) was used to transfect miR-329-3p mimic, miR-329-3p inhibitor, USP22 siRNA, pcDNA3.1- USP22 into HepG2 according to the protocol. Proteins and genes were isolated from HepG2 cells for subsequent experiments.

Cell Proliferation and Cell Viability Assay

HepG2 cells were seeded in 96-well plates at density of 2×10^3 cells / well, each well was loaded with 200 μL culture medium. 20 μL of CCK-8 solution (Dojindo Laboratories, Kumamoto, Japan) was added to each well, and after 24 h, 48 h, 72 h, 96 h incubation, the optical density value (OD value) of each well was measured by a microplate reader (Bio-Rad Laboratories, Hercules, CA, USA) at 450 nm, and the cell proliferation was calculated by the OD value.

Transwell Assay

2×10^4 HepG2 cells were seeded on the surface of phospholipid carbonate of 12-well transwell plates (Corning, Corning, NY, USA), the culture condition was 37°C, the cells on the membrane were fixed with 1% paraformaldehyde (Solarbio, Beijing, China) after 24 hours, and stained with 0.2% crystal violet solution (Solarbio, Beijing, China). Ten fields were randomly selected under the microscope, and the number of perforating cells was calculated. The experiment was repeated 3 times.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

TRIzol (Invitrogen, Carlsbad, CA, USA) was used to extract and isolate RNA. The Nano Drop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the RNA concentration. PrimeScript RT reagent kit (TaKaRa, Otsu, Shiga, Japan) and TB Green® Premix Ex Taq™ II kit (TaKaRa, Otsu, Shiga, Japan) were used for detection of genes according to the manufacturer's protocol on a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). U6 and GAPDH were used as the internal reference. All reactions were in triplicate. Relative expression levels were quantified with $2^{-\Delta\Delta CT}$ method.

The primer sequence is as follows: GAPDH, forward 5′-AATGGGCAGCCGTTAGG AAA-3′ and reverse 5′-TGAAGGGGTCATTGATGGCA-3′; USP22, forward 5′-GG CGGAAGATCACCACGTAT-3′ and reverse 5′-TTGTTGAGACTGTCCGTGGG-3′; U6: forward 5′-GCTTCGGCAGCACATATACTAAAAT-3′ and reverse 5′-CGCTTC ACGAATTTGCGTGTCAT-3′; miR-329-3p: forward 5′-GTGGAACAGACCTGGT AAAC-3′ and reverse 5′-CAAGTGCGAGTCGTGCAGT-3′ .

Western Blot

According to the manufacturer's protocols, the cells and tissues were lysed with radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China), and the total protein was extracted. Proteins were loaded on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The loading amount was 30 μg of protein per well. Then, the protein was transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Burlington, MA, USA). Then, the PVDF membrane was incubated

with primary USP22 (Thermo Fisher Scientific, Waltham, MA, USA; dilution rates of 1:5000), β-catenin (Abcam, Cambridge, UK; dilution rates of 1:5000), c-Myc (Abcam, Cambridge, United Kingdom; dilution rates of 1:5000), Wnt3a (Abcam, Cambridge, UK; dilution rates of 1:5000) and β-actin (Abcam, Cambridge, UK; dilution rates of 1:5000) antibodies with 5% fat-free milk at 4°C overnight. Then, the membrane was washed in PBS for three times and incubated with anti-mouse horseradish peroxidase binding secondary antibody (Abcam, Cambridge, UK; dilution rates of 1:10000) for one hour. Super ECL Detection Reagent (Thermo Fisher Scientific, Shanghai, China) was used to detect protein bands.

Dual-Luciferase Reporter Gene Assay

Wild and mutant plasmids of USP22 (pmirGLO- USP22-wt, pmirGLO- USP22-mut) containing a wild or mutant miR-329-3p binding sites were designed and synthesized by Genepharma (GenePharma, Shanghai, China). HepG2 cells were seeded at a confluence of 70% into a 96-well plate. Transfection of Luciferase reporter gene plasmid and RNA after 24h using Lipofectamine 2000. After co-transfection of the plasmid for 48 hours, discard the medium and wash it once. Then, the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) was used to evaluate fluorescence intensity changes according to the manufacturer's protocol.

Statistical Analysis

Student's *t*-test or analysis of variance (ANOVA) was used to analyze measurement data. All data are expressed as mean ± SD. $p<0.05$ was considered statistically significant. Statistical analysis was performed using SPSS 19 software (IBM Corp., Armonk, NY, USA).

Results

MiR-329-3p Is Low in HCC Tissue, and MiR-329-3p Is Inversely Related to the USP22 Expression

We detected miR-329-3p in HCC tissue and surrounding paracancerous tissues by qRT-PCR. The results showed that the expression level of miR-329-3p in HCC tissue was significantly lower than that in normal adjacent cancer tissues (Figure 1A). Through StarBase 3.0 we found that miR-329-3p may affect USP22, and then inhibit its expression (Figure 1B). Therefore, we also examined the expression level of USP22 in HCC tissue and surrounding paracancerous tissue. We found that the expression level of USP22 in HCC tissue was significantly higher

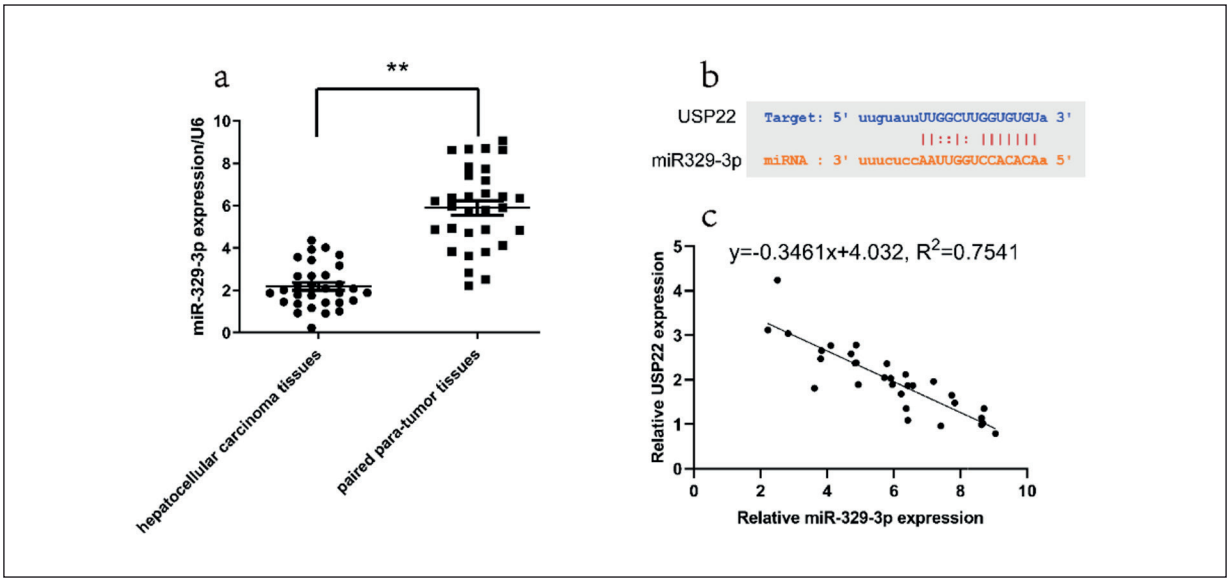

Figure 1. MiR-329-3p is low in HCC tissue, and miR-329-3p is inversely related to the USP22 expression. **a,** Relative expression level of miR-329-3p in 31 cases of HCC and surrounding paracancerous tissues. **b,** The miR-329-3p putative binding site in the 3'-UTR of USP22. **c,** Correlation between miR-329-3p and USP22 mRNA in HCC tissues using linear correlation. **: $p<0.01$.

than that in normal paracancerous tissues. We further conducted a correlation analysis and found that the two are negatively correlated, the R^2 is 0.7541 (Figure 1C).

MiR-329-3p Inhibits Proliferation and Migration of HCC Cells

We further examined the role of miR-329-3p in the HCC cell line HepG2. We transfected miR-329-3p mimic and miR-329-3p inhibitor into HepG2 cells and found that miR-329-3p mimic can increase miR-329-3p expression and miR-329-3p inhibitor can inhibit miR-329-3p expression (Figure 2A). Through further functional experiments, we found that miR-329-3p mimic can inhibit the proliferation and migration of HepG2 cells, miR-329-3p inhibitor promote the proliferation and migration of HepG2 cells (Figure 2B, 2C).

MiR-329-3p Inhibited USP22 Expression by Targeting it Directly

We have predicted that miR-329-3p may directly inhibit USP22 expression through StarBase 3.0. It suggested that miR-329-3p may directly inhibit the expression of USP22. To prove this, we first transfected miR-329-3p mimic and miR-329-3p inhibitor into HepG2 cells, and then we examined the expression of USP22. We found that miR-329-3p mimic inhibited USP22 expression (Figure 3A). To further demonstrate that miR-329-3p can directly inhibit the expression of USP22, we performed a Dual-Luciferase reporter gene assay. It turns out that pmirGLO- USP22-wt co-transfection significantly reduced the Luciferase activity (Figure 3B).

USP22 Promoted HCC Cell Proliferation and Migration

We have previously demonstrated that miR-329-3p directly inhibits USP22 expression. We need to prove whether miR-329-3p inhibits the proliferation and migration of HepG2 cells through USP22. We further study the function of USP22. We detected the proliferation and migration of HepG2 cells by up- or down-regulating of USP22 (Figure 4A, B). As a result, we found that pcDNA3.1- USP22 can promote the proliferation and migration of HepG2 cells. (Figure 4C, D).

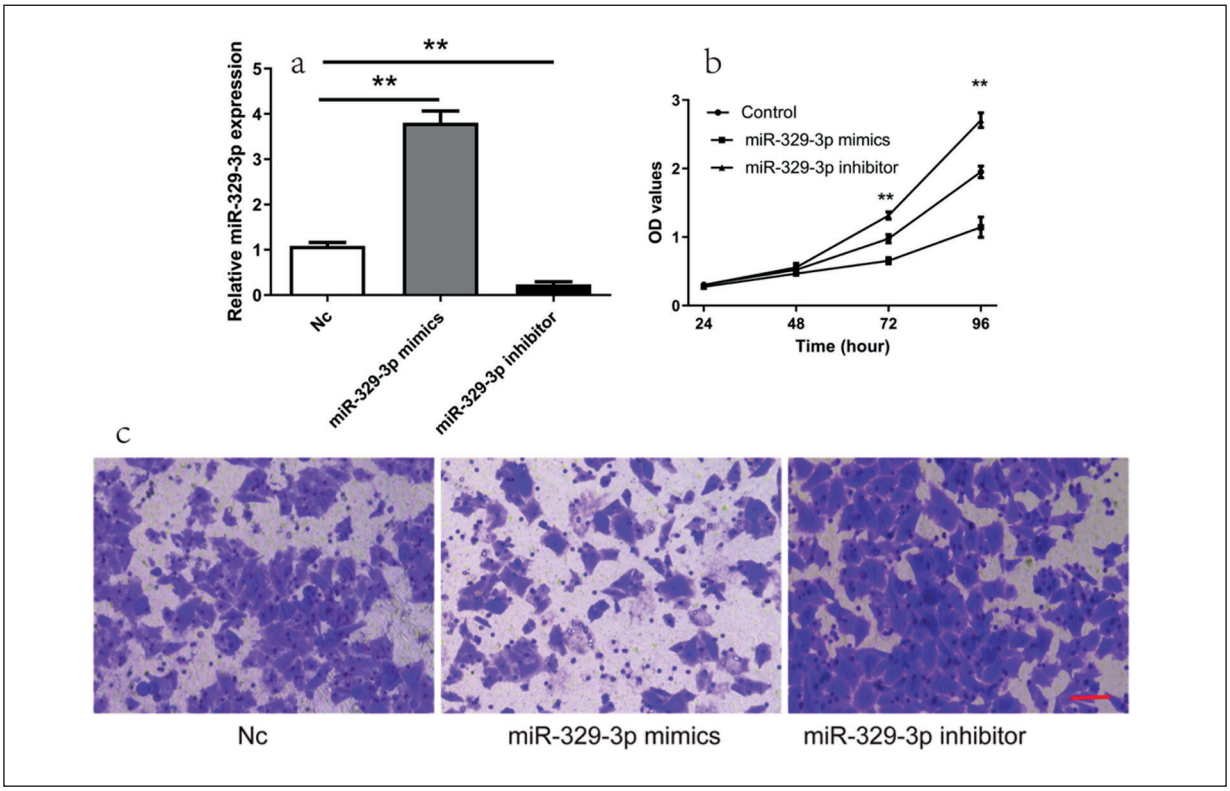

Figure 2. Effects of miR-329-3p on cell proliferation and migration in HepG2 cells. **a,** MiR-329-3p expression in HepG2 cells treated with miR-329-3p mimic and miR-329-3p inhibitor. **b,** Proliferation of HepG2 cells treated with miR-329-3p mimic and miR-329 inhibitor. **c,** Migration of HepG2 treated with miR-329-3p mimic and miR-329-3p inhibitor, scale bar, 50 μm. **: $p<0.01$.

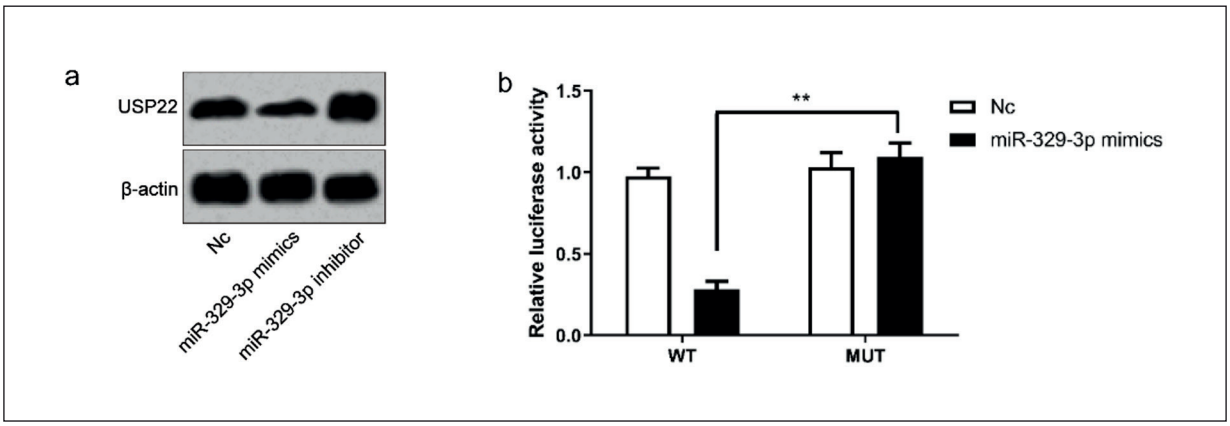

Figure 3. USP22 is a direct target of miR-329-3p in HepG2 cells. **a,** USP22 expression in HepG2 cells treated with miR-329-3p mimic and miR-329-3p inhibitor. **b,** Fluorescence of co-transfection of miR-329-3p mimic and pmirGLO-USP22-mut or pmirGLO-USP22-wt. **: $p<0.01$.

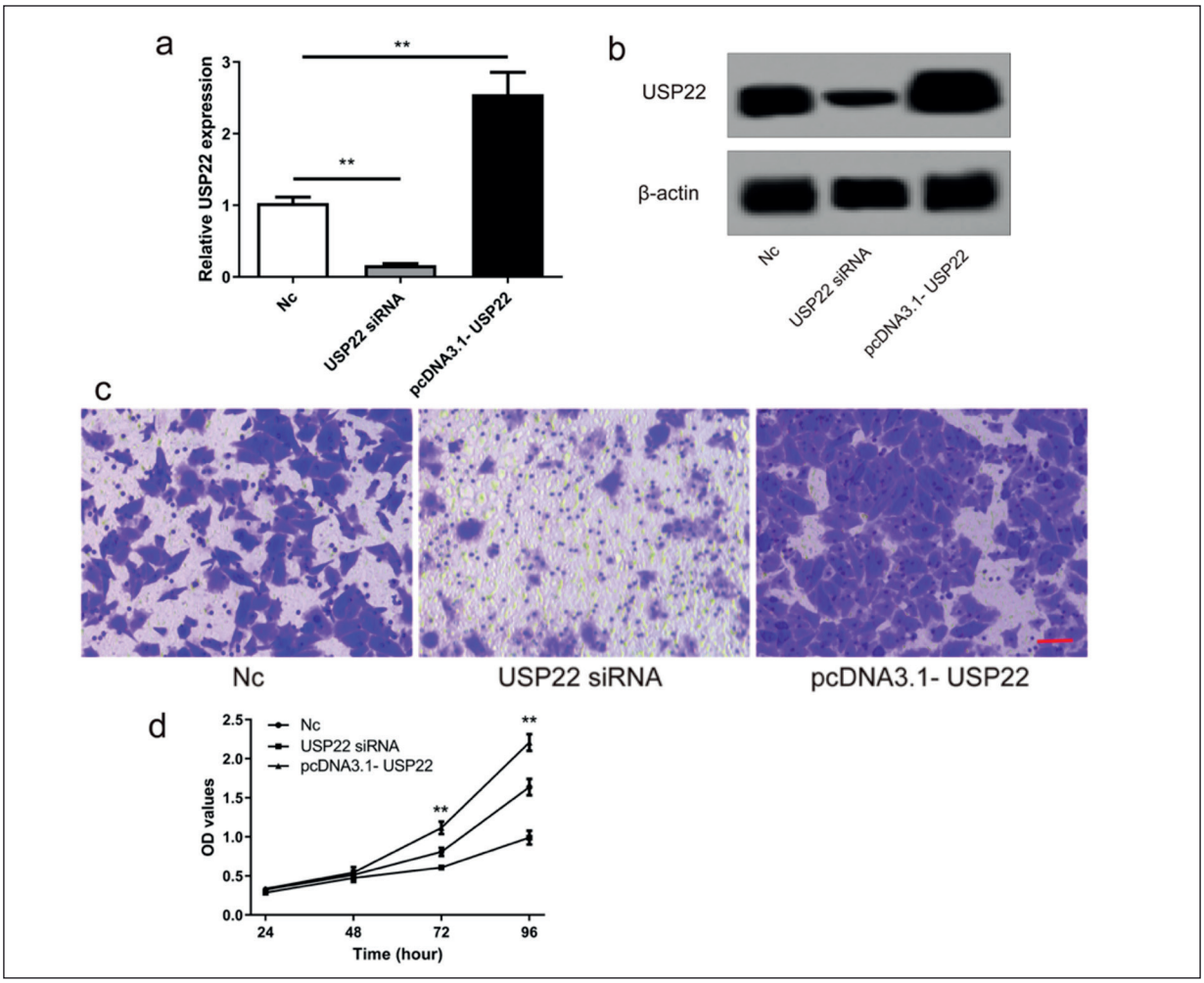

Figure 4. USP22 promoted HepG2a cell proliferation and migration. **a,** The USP22 gene expression in HepG2 cells treated with USP22 siRNA and pcDNA3.1- USP22. **b,** The USP22 protein level in HepG2 cells treated with USP22 siRNA and pcDNA3.1- USP22. **c,** The migration of HepG2 treated with USP22 siRNA and pcDNA3.1- USP22, scale bar, 50 μm. **d,** The proliferation of HepG2 treated with USP22 siRNA and pcDNA3.1- USP22. **: $p<0.01$.

USP22 Promoted HepG2 Proliferation and Migration Through Wnt/β-Catenin Pathway

USP22 siRNA inhibited Wnt/β-catenin pathway related protein, β-catenin, c-Myc, and Wnt3a expression and pcDNA3.1-USP22 promoted β-catenin, c-Myc, and Wnt3a expression (Figure 5A). According to functional experiments, pcDNA3.1-USP22 promoted HepG2 cells proliferation and this effect was inhibited by PNU-74654 (Wnt/β-catenin pathway inhibitor) (Figure 5B). pcDNA3.1-USP22 promoted HepG2 cells migration and this effect was also inhibited by PNU-74654 (Figure 5C).

Discussion

MiRNAs play an important role in the development of tumors[8]. MiR-329-3p is a newly discovered miRNA. At present, there are relatively few researches on his function, mostly focusing on tumor research. Chang et al[16] found that the low expression of miR-329-3p is related to the poor prognosis of cervical cancer. Li et al[17] on cervical cancer found that miR-329-3p could inhibit cervical cancer by directly inhibiting MAPK1 expression.

MiRNAs can target genes and regulate downstream signaling pathways, thereby affecting tumor biological behavior. The regulation of miRNAs on target genes is generally achieved through the complementary base pairing of the 5′ end of the target gene. If the target gene is a tumor suppressor or oncogenic factor, then miRNA is likely to affect tumor progress[18]. To explore how miR-329-3p inhibits HepG2 cell proliferation and migration, we predict its downstream mRNA through StarBase 3.0. We found that USP22 mRNA is one of the possible targets of miR-329-3p.

USP22 is a histone-modifying enzyme and is known to function as a histone deubiquitinating component[19]. USP22 is found to be highly expressed in a variety of tumors. This suggests that USP22 may work as an oncogene. McCann et al[20] found that USP22 acts as an oncogene in prostate cancer to regulate cell proliferation and DNA repair. Liu et al[21] related to gastric cancer proved

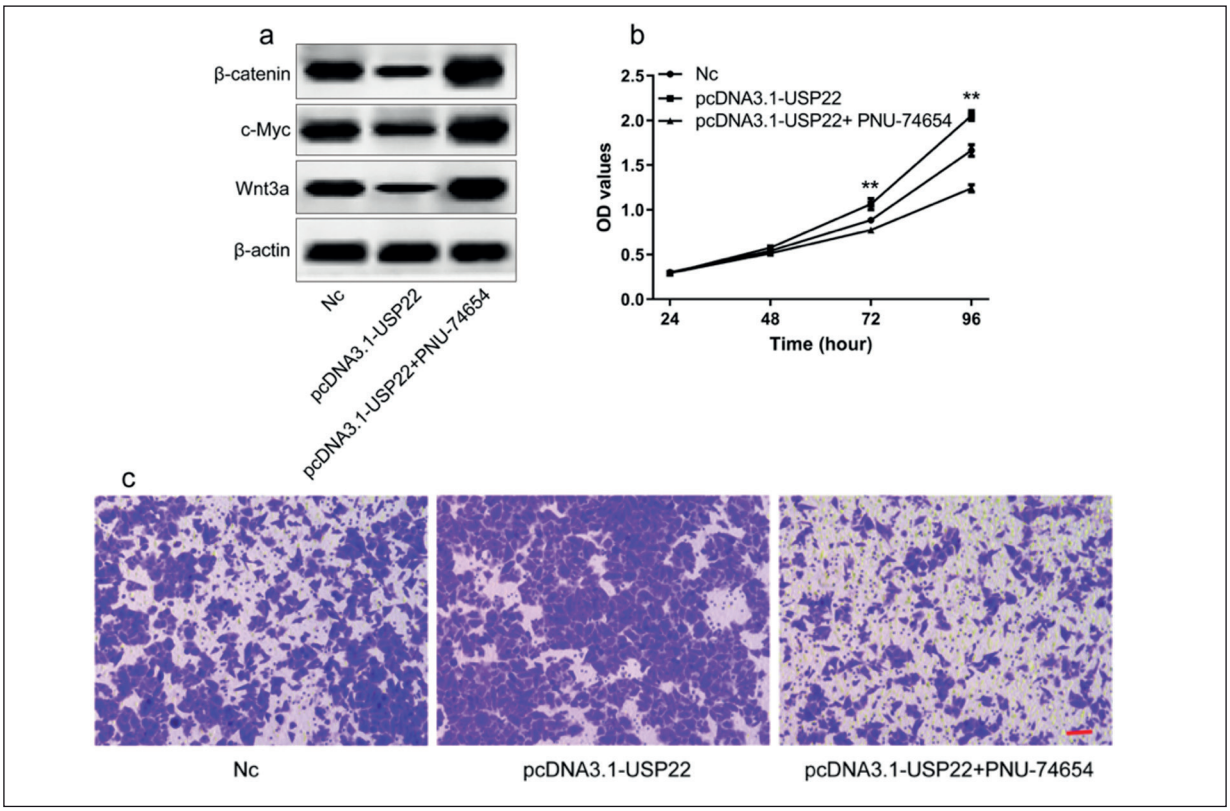

Figure 5. USP22 promoted Wnt/β-catenin signaling pathway. **a,** USP22 siRNA inhibited β-catenin, c-Myc, and Wnt3a expression and pcDNA3.1-USP22 promoted β-catenin, c-Myc, and Wnt3a expression. **b,** pcDNA3.1-USP22 promoted HepG2 cells proliferation and this effect was inhibited by PNU-74654 (Wnt/β-actin pathway inhibitor). **c,** pcDNA3.1-USP22 promoted HepG2 cells migration and this effect was also inhibited by PNU-74654, scale bar, 100 μm. **: $p<0.01$.

that USP22 can promote gastric cancer growth and metastasis by activating c-Myc/NAMPT/SIRT1-dependent FOXO1 and YAP signaling. In addition, other studies have found the role of USP22 in HCC. Ling et al[22] demonstrated that when TP53 is inactivated, USP22 promotes hypoxia-induced HCC stemness through a HIF1α/USP22 positive feedback loop. Of course, in some tumors, USP22 is also found to be highly expressed and can exist as a tumor suppressor gene. Kosinsky et al[23] found that USP22 acts as a tumor suppressor gene in colon cancer by inhibiting mTOR activity.

According to qRT-PCR and Luciferase experiments, we found that miR-329-3p can inhibit USP22 expression by directly targeting USP22. From previous studies[20,24], we also know that USP22 can affect the proliferation, metastasis, DNA repair, and stemness of tumor cells. We also found that USP22 can promote the proliferation and migration of HepG2 cells.

USP22 can regulate tumor progression by affecting Wnt/β-Catenin Pathway in colon cancer[25,26]. And Wnt/β-Catenin Pathway had been shown to affect the proliferation and migration of tumor cells[27,28]. In this research, we verified whether USP22 also affects Wnt/β-Catenin pathway in HepG2 cells. As a result, we found that USP22 can activate Wnt/β-Catenin Pathway, and the activation of Wnt/β-Catenin pathway promoted the proliferation and migration of HepG2 cells. This showed that miR-329-3p can affect the proliferation and migration of HepG2 cells through USP22-Wnt/β-catenin pathway.

Conclusions

In summary, we demonstrated that miR-329-3p can inhibit HCC cell proliferation and migration by inhibiting USP22-Wnt/β-Catenin pathway. Our study provides novel insights into the aetiology and potential treatment of HCC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

Not applicable.

Funding

No funding was received.

References

1) Villanueva A. Hepatocellular carcinoma. N Engl J Med 2019; 380: 1450-1462.
2) Henley SJ, Ward EM, Scott S, Ma J, Anderson RN, Firth AU, Thomas CC, Islami F, Weir HK, Lewis DR, Sherman RL, Wu M, Benard VB, Richardson LC, Jemal A, Cronin K, Kohler BA. Annual report to the nation on the status of cancer, part I: National cancer statistics. Cancer 2020; 126: 2225-2249.
3) Raoul JL, Edeline J. Systemic treatment of hepatocellular carcinoma: standard of care in China and elsewhere. Lancet Oncol 2020; 21: 479-481.
4) Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. Nat Rev Gastroenterol Hepatol 2019; 16: 589-604.
5) Yang J, Chen S, Dou W, Xie R. MiR-654-3p predicts the prognosis of hepatocellular carcinoma and inhibits the proliferation, migration, and invasion of cancer cells. Cancer Biomark 2020; 28: 1-7.
6) Wang H, Jiang F, Liu W, Tian W. miR-595 suppresses cell proliferation and metastasis in hepatocellular carcinoma by inhibiting NF-κB signalling pathway. Pathol Res Pract 2020; 216: 344-338.
7) Prabhu KS, Raza A, Karedath T, Raza SS, Fathima H, Ahmed EI, Kuttikrishnan S, Therachiyil L, Kulinski M, Dermime S, Junejo K, Steinhoff M, Uddin S. Non-coding RNAs as regulators and markers for targeting of breast cancer and cancer stem cells. Cancers (Basel) 2020; 12: 351-380.
8) Rupaimoole R, Slack FJ. MicroRNA therapeutics: towards a new era for the management of cancer and other diseases. Nat Rev Drug Discov 2017; 16: 203-222.
9) Nishibeppu K, Komatsu S, Imamura T, Kiuchi J, Kishimoto T, Arita T, Kosuga T, Konishi H, Kubota T, Shiozaki A, Fujiwara H, Okamoto K, Otsuji E. Plasma microRNA profiles: identification of miR-1229-3p as a novel chemoresistant and prognostic biomarker in gastric cancer. Sci Rep 2020; 10: 3161.
10) Zheng J, Zhang Y, Cai S, Dong L, Hu X, Chen MB, Zhu YH. MicroRNA-4651 targets bromodomain-containing protein 4 to inhibit non-small cell lung cancer cell progression. Cancer Lett 2020; 476: 129-139.
11) Hsu WC, Li WM, Lee YC, Huang AM, Chang LL, Lin HH, Wu WJ, Li CC, Liang PI, Ke HL. MicroRNA-145 suppresses cell migration and invasion in upper tract urothelial carcinoma by targeting ARF6. FASEB J 2020; 34: 5975-5992.
12) Xu F, Ye ML, Zhang YP, Li WJ, Li MT, Wang HZ, Qiu X, Xu Y, Yin JW, Hu Q, Wei WH, Chang Y, Liu L, Zhao Q. MicroRNA-375-3p enhances chemosensitivity to 5-fluorouracil by targeting thymidylate synthase in colorectal cancer. Cancer Sci 2020; 111: 1528-1541.
13) Liao CH, Liu Y, Wu YF, Zhu SW, Cai RY, Zhou L, Yin XM. MicroRNA-329 suppresses epithelial-to-mesenchymal transition and lymph node

metastasis in bile duct cancer by inhibiting laminin subunit beta 3. J Cell Physiol 2019; 234: 17786-17799.

14) Wu L, Pei F, Men X, Wang K, Ma D. MiR-329 inhibits papillary thyroid cancer progression via direct targeting WNT1. Oncol Lett 2018; 16: 3561-3568.

15) Cai L, Chen Q, Fang S, Lian M, Cai M. MicroRNA-329 inhibits cell proliferation and tumor growth while facilitates apoptosis via negative regulation of KDM1A in gastric cancer. J Cell Biochem 2018; 119: 3338-3351.

16) Chang YH, Yin F, Fan GF, Zhao M. Down-regulation of miR-329-3p is associated with worse prognosis in patients with cervical cancer. Eur Rev Med Pharmacol Sci 2017; 21: 4045-4049.

17) Li W, Liang J, Zhang Z, Lou H, Zhao L, Xu Y, Ou R. MicroRNA-329-3p targets MAPK1 to suppress cell proliferation, migration and invasion in cervical cancer. Oncol Rep 2017; 37: 2743-2750.

18) Wang JY, Yang Y, Ma Y, Wang F, Xue A, Zhu J, Yang H, Chen Q, Chen M, Ye L, Wu H, Zhang Q. Potential regulatory role of lncRNA-miRNA-mRNA axis in osteosarcoma. Biomed Pharmacother 2020; 121: 109627.

19) Jeusset LM, McManus KJ. Ubiquitin specific peptidase 22 regulates histone H2B mono-ubiquitination and exhibits both oncogenic and tumor suppressor roles in cancer. Cancers (Basel) 2017; 9: 167.

20) McCann JJ, Vasilevskaya IA, Poudel Neupane N, Shafi AA, McNair C, Dylgjeri E, Mandigo AC, Schiewer MJ, Schrecengost RS, Gallagher P, Stanek TJ, McMahon SB, Berman-Booty LD, Ostrander WF, Knudsen KE. USP22 functions as an oncogenic driver in prostate cancer by regulating cell proliferation and DNA repair. Cancer Res 2020; 80: 430-443.

21) Liu H, Liu N, Zhao Y, Zhu X, Wang C, Liu Q, Gao C, Zhao X, Li J. Oncogenic USP22 supports gastric cancer growth and metastasis by activating c-Myc/NAMPT/SIRT1-dependent FOXO1 and YAP signaling. Aging 2019; 11: 9643-9660.

22) Ling S, Shan Q, Zhan Q, Ye Q, Liu P, Xu S, He X, Ma J, Xiang J, Jiang G, Wen X, Feng Z, Wu Y, Feng T, Xu L, Chen K, Zhang X, Wei R, Zhang C, Cen B, Xie H, Song P, Liu J, Zheng S, Xu X. USP22 promotes hypoxia-induced hepatocellular carcinoma stemness by a HIF1α/USP22 positive feedback loop upon TP53 inactivation. Gut 2019; 0: 1-13.

23) Kosinsky RL, Zerche M, Saul D, Wang X, Wohn L, Wegwitz F, Begus-Nahrmann Y, Johnsen SA. USP22 exerts tumor-suppressive functions in colorectal cancer by decreasing mTOR activity. Cell Death Differ 2020; 27: 1328-1340.

24) Cai Z, Zhang MX, Tang Z, Zhang Q, Ye J, Xiong TC, Zhang ZD, Zhong B. USP22 promotes IRF3 nuclear translocation and antiviral responses by deubiquitinating the importin protein KPNA2. J Exp Med 2020; 217.

25) Jiang S, Miao D, Wang M, Lv J, Wang Y, Tong J. MiR-30-5p suppresses cell chemoresistance and stemness in colorectal cancer through USP22/Wnt/β-catenin signaling axis. J Cell Mol Med 2019; 23: 630-640.

26) Jiang S, Song C, Gu X, Wang M, Miao D, Lv J, Liu Y. Ubiquitin-specific peptidase 22 contributes to colorectal cancer stemness and chemoresistance via Wnt/β-catenin pathway. Cell Physiol Biochem 2018; 46: 1412-1422.

27) Xu X, Dai Y, Feng L, Zhang H, Hu Y, Xu L, Zhu X, Jiang Y. Knockdown of Nav1.5 inhibits cell proliferation, migration and invasion via Wnt/β-catenin signaling pathway in oral squamous cell carcinoma. Acta Biochim Biophys Sin (Shanghai) 2020; 52: 527-535.

28) Zheng TL, Cen K. MiR-92a inhibits proliferation and promotes apoptosis of OSCC cells through Wnt/β-catenin signaling pathway. Eur Rev Med Pharmacol Sci 2020; 24: 4803-4809.