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RESEARCH

miR-182-5p promotes the proliferation and invasion of hilar cholangiocarcinoma cells by inhibiting FBXW7

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Abstract

Background Hilar cholangiocarcinoma (HCCA) is a common type of cholangiocarcinoma (CHOL) that originates from the right and/or left hepatic duct near the biliary tract confluence. The objective of this study is to investigate the impact of miR-182-5p on the proliferation and invasion of HCCA cells and identify a potential target for HCCA treatment.

Methods HCCA tissues were collected and HCCA cells were cultured. miR-182-5p and F-box and WD repeat domain containing 7 (FBXW7) were detected. After transfection of miR-182-5p inhibitor into HCCA cells, cell proliferation and invasion were detected by cell counting 8-kit and Transwell assay. FBXW7 expression was detected by Western blot. The targeted relationship between miR-182-5p and FBXW7 3'UTR was verified by dual-luciferase report assay. si-FBXW7 and miR-182-5p inhibitor were transfected into cells for combined experiments. HCCA cells with lowly-expressed miR-182-5p were injected into nude mice to establish the xenograft tumor model, and subsequent observations were made on tumor growth and gene expression changes.

Results miR-182-5p exhibited high expression levels in both HCCA tissues and cell lines. Inhibiting miR-182-5p effectively suppressed the proliferation and migration of HCCA cells. miR-182-5p bounded to FBXW7 3 'UTR and inhibited FBWX7 expression. Suppressing FBXW7 expression partially reversed the inhibitory effect of miR-182-5p inhibitor on HCCA cell proliferation and invasion. Silencing miR-182-5p could inhibit the HCCA growth in vivo.

Conclusion miR-182-5p promoted the proliferation and invasion of HCCA cells by targeting and inhibiting FBXW7 expression.

Keywords Hilar cholangiocarcinoma · miR-182-5p · FBXW7 · Cell proliferation · Cell invasion

Background

Cholangiocarcinoma (CHOL) arises from malignant growth of the bile duct epithelium and can be classified into intrahepatic, perihilar, and distal subtypes according to the location of its occurrence (Elvevi et al. 2022). Hilar cholangiocarcinoma (HCCA) is the most common type that accounts for 70% of all cases, which develops from the right and/or left hepatic duct at or near the confluence of the biliary tract (Poruk et al. 2015). HCCA accounts for about 2% of all cancers and can trigger infectious complications of liver failure and/or biliary obstruction, and few patients meet the criteria for surgery or transplantation (Inchingolo et al. 2021). Therefore, the prognosis of HCCA is extremely poor, with median survival after surgical resection ranging from 11 to 38 months and 5-year survival rates generally ranging from 20 to 40%, while most patients without surgery could live less than a year from diagnosis (Khan and Dageforde 2019). Therefore, there is an urgent need to find new therapeutic targets for HCCA.

MicroRNAs (miRNAs/miRs) are a class of highly conserved non-protein coding RNAs that maintain cell homeostasis through negative gene regulation (Mishra et al. 2016). More importantly, miRNAs play important roles in tumorigenesis, growth, and metastasis, and have the potential to be used as clinical therapeutic targets (Ali Syeda et al. 2020). Among them, miR-182-5p is widely involved in the development of various types of cancers. For example, silencing miR-182-5p can enhance the sensitivity of breast

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cancer cells to tamoxifen and inhibit tumor growth (Sang et al. 2019). Overexpression of miR-182-5p worsens prostate cancer progression and activates the epithelial-mesenchymal transition process in cancer cells, which leads to decreased overall survival of patients (Souza et al. 2022). Importantly, miR-182-5p is highly expressed in CHOL and induces angiogenesis (Wang et al. 2023). Nevertheless, the role of miR-182-5p in HCCA still needs further exploration.

F-box and WD repeat domain containing 7 (FBXW7), located on chromosome 4q32, is a member of the F-box protein family, a key tumor suppressor, and one of the most common deregulated ubiquitin-proteasome system proteins in human cancers (Yeh et al. 2018). Previous studies confirm that miR-182-5p can target and inhibit FBXW7 expression, while FBXW7 up-regulation can inhibit cell proliferation, migration, and tumor growth and migration in kidney cancer (Cao et al. 2022). Additionally, the miR-182-5p/FBXW7 axis is also involved in the regulation of cell cycle and invasion in cervical cancer (Zhang et al. 2020a). Notably, CHOL patients with low FBXW7 expression show a larger tumor volume and significantly shorter overall survival than patients with high FBXW7 expression (Ishii et al. 2017). Inhibition of FBXW7 also reduces the sensitivity of CHOL cells to chemotherapy, resulting in reduced apoptotic efficiency (Mori et al. 2018). However, the role of miR-182-5p/FBXW7 in CHOL is still unknown.

In this study, we explored the role of miR-182-5p and FBXW7 in HCCA tissues and cells and nude mice, so as to find new targets for the treatment of HCCA.

Materials and methods

Ethics statement

All tissues were obtained with informed consent from the patients. Our study followed the Declaration of Helsinki, and ethical approval was obtained from the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University. All animal experiments were carried out approval of the Animal Ethics Committee of The First Affiliated Hospital of Chongqing Medical University, and the experimental process followed the Guidelines for the Use of Laboratory Animals (National Research Council (US) 2011).

Clinical sample collection

A total of 52 pairs of tumor samples and corresponding adjacent non-tumoral tissues of HCCA patients, which were collected from tumor surgery in The First Affiliated Hospital of Chongqing Medical University, were included in this study. Fresh tissue was harvested immediately after surgery and immediately stored in liquid nitrogen after washing with frozen phosphate-buffered saline (PBS).

Cell culture

Human intrahepatic biliary epithelial cells (HIBEpiC) and cholangiocarcinoma cell lines (KKU-100, QBC939, HUCCT1, RBE, HCC-9810) were purchased from the Shanghai Institute of Biological Sciences, Chinese Academy of Sciences. Cells were cultured in RPMI-1640 medium (Invitrogen, Waltham, MA, USA) with 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 mg/ mL) (Invitrogen) in a humidified incubator at 37 °C and 5% CO_2.

Cell treatment

Cells were transfected with miR-182-5p inhibitors (200 nM/well) (RiboBio, Guangzhou, Guangdong, China), si-FBXW7 (200 nM/well) (RiboBio), and the negative control (NC) (RiboBio) using Lipofectamine 3000 (L3000015, Invitrogen) according to the manufacturer's instructions. Next, the cells were cultured in a constant temperature incubator for 48 h for subsequent experiments. To obtain cells with stable low expression of miR-182-5p, we packaged antagomiR-182-5p (RiboBio) with adenovirus (RiboBio), which was then infected with KKU-100 cells. We chose stably expressing cells by adding puromycin (2 μg/mL) and used them for the in vivo experiments.

Cell counting kit-8 (CCK-8) assay

The transfected cells were seeded into 96-well plates with 3×10^3 cells/well and incubated at 37°C for 48 h. After that, 10 μL CCK-8 reagent was added to each well and incubated at 37°C for 1 h. The absorbance at 450 nm was detected using a microplate reader.

Colony formation assay

Cells were seeded in 6-well plates (500 cells/well) and incubated in RPMI-1640 medium supplemented with 10% FBS for 14 days. The colonies were fixed with methanol and stained with 0.5% crystal violet. Colonies were then counted by two investigators blinded to the study.

Transwell assay

Transwell assay was performed using a 24-well Transwell coated with Matrigel (Corning, New York, USA). In brief, 100 μL of serum-free RPMI 1640 medium containing 10^6 cells was added to the upper chamber, and 500 μL of RPMI

1640 medium containing 10% FBS was added to the lower chamber. The cells were incubated at 37 °C for 48 h and stained with crystal violet. The number of invasive cells was counted.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from cells using a TRIzol reagent (Invitrogen). After assessing the quality and concentration of RNA, reverse transcription of RNA into complementary DNA (cDNA) was performed using RevertAid™ (Thermo Fisher Scientific, Waltham, MA, USA). qPCR was performed using Power SYBR Green PCR MasterMix (Thermo Fisher Scientific) on an Applied Biosystems StepOnePlus Real-time PCR system (Thermo Fisher Scientific). The relative expression levels of genes or miRNAs were analyzed by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal reference for FBXW7, and U6 as the internal reference for miR-182-5p (Zhang et al. 2020b). Primer sequences are shown in Table 1.

Western blot assay

The cells were treated with radioimmunoprecipitation assay lysis buffer (Beyotime, Shanghai, China), and the total protein concentration was determined using a bicinchoninic acid kit (Pierce Biotechnology, Rockford, IL, USA). The target protein was isolated by 8–15% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene fluoride membranes. After being blocked with 5% bovine serum albumin for 1 h, the membranes were incubated with primary antibodies against FBXW7 (1:1000, ab192328, Abcam, Cambridge, MA, USA) and β-actin (1:1000, ab8227, Abcam) at 4°C overnight. After washing with Tris-buffered saline with Tween, the membranes were incubated with the secondary antibodies for 1 h. The signals were detected with an enhanced chemiluminescence detection reagent (Beyotime).

Table 1 PCR primer sequences

Gene	Sequence (5'-3')
FBXW7	F: GGCCAAAATGATTCCCAGCAA
	R: ACTGGAGTTCGTGACACTGTTA
miR-182-5p	F: GCCGAGTTTGGCAATGGTAGA
	R: CTCAACTGGTGTCGTGGA
GAPDH	F: GGAGCGAGATCCCTCCAAAAT
	R: GGCTGTTGTCATACTTCTCATGG
U6	F: AACGCTTCACGAATTTGCGT
	R: GTGACGTTTGGGTCAGGTGC

Bioinformatics

StarBase (https://rnasysu.com/encori/) (Li et al. 2014) database was used to predict the miR-182-5p expression in CHOL. StarBase, TargetScan (http://www.targetscan.org/) (Agarwal et al. 2015), miRWalk (http://mirwalk.umm.uni-heidelberg.de/) (Sticht et al. 2018), miRDB (http://mirdb.org/) (Chen and Wang 2020), and miRTarBase (http://mirtarbase.cuhk.edu.cn/php/index.php) (Huang et al. 2022) were used to predict downstream target genes of miR-182-5p.

Dual-luciferase report assay

The FBXW7 3'UTR sequence binding to miR-182-5p was cloned into the pmirGlo dual-luciferase miRNA target expression vector (Promega, Madison, WI, USA) to construct a luciferase reporter vector (FBXW7-WT), and the sequence containing the binding site with miR-182-5p was mutated to construct a mutant luciferase reporter vector (FBXW7-MUT). The cells were seeded into 96-well plates and transfected with miR-182-5p mimic or mimic NC when they reached 50-70% confluence. The luciferase activity was measured at 48 h after transfection by dual-luciferase reporter kits (Promega).

Nude mice xenograft model

Male BALB/c thymic nude mice (aged 6–8 weeks) purchased from the National Laboratory Animal Center (Beijing, China) were kept under specific pathogen-free conditions for 1 week before the experiment. Cells (4×10^6) with stable low miR-182-5p expression or negative control were injected subcutaneously into the right side of each mouse, and tumor volume was measured every 3 days after the tumor was identified. The formula was calculated: volume (mm^3) = length × width^2/2. On day 21 after injection, all mice were euthanized by intraperitoneal injection of pentobarbital (200 mg/kg). The tumors were isolated and weighed, and samples of 6 mice in each group were randomly selected for rapid freeze in liquid nitrogen, while the tumors of other 6 mice were used for paraffin embedding and immunohistochemical detection.

Immunohistochemistry (IHC)

Paraffin embedded tissue samples were cut into 5 μm sections. Sections were incubated with Ki67 (1:200, ab16667, Abcam) overnight at 4°C. After washing with PBS, the sections were incubated with secondary antibody immunoglobulin G (1:2000, ab150077, Abcam) at 25°C for 20 min and then developed by diaminobenzidine. The images were photographed under a microscope (Olympus, Tokyo, Japan).

Statistical analysis

SPSS21.0 statistical software (IBM, Armonk, NY, USA) and GraphPad Prism 8.0 software (GraphPad Software Inc., San Diego, USA, USA) were used for data analyses and data plotting. First, normality and homogeneity of variance tests were conducted, which verified that the data were in normal distribution and homogeneity of variance. Data comparisons between two groups were analyzed by *t* test; data comparisons among multiple groups were analyzed by one-way or two-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons for post hoc test. $p < 0.05$ was considered statistically significant.

Results

miR-182-5p is highly expressed in HCCA

StarBase database predicted that miR-182-5p was highly expressed in CHOL (Fig. 1A). qRT-PCR showed that miR-182-5p was highly expressed in HCCA tissues compared to paired adjacent tissues ($p < 0.01$, Fig. 1B). CHOL cell lines were cultured in vitro. Compared with HIBEpiC, miR-182-5p was also highly expressed in CHOL cell lines ($p < 0.01$, Fig. 1C). We chose KKU-100 and QBC939 with the highest relative expression for subsequent experiments.

Inhibition of mir-182-5p inhibits the proliferation and invasion of HCCA cells

We reduced the expression of miR-182-5p in KKU-100 and QBC939 cells using miR-182-5p inhibitor ($p < 0.01$, Fig. 2A). It was observed that after inhibition of miR-182-5p, cell viability was significantly decreased ($p < 0.01$, Fig. 2B), the number of cell clone formation was reduced ($p < 0.01$, Fig. 2C), and cell invasion ability was also reduced ($p < 0.01$, Fig. 2D). These results indicated that inhibition of miR-182-5p expression inhibited the proliferation and invasion of HCCA cells.

miR-182-5p targets and inhibits the expression of FBXW7

Through online prediction of StarBase, TargetScan, miRWalk, miRDB, and miRTarBase databases, the downstream target genes of miR-182-5p were obtained and the intersection was taken (Fig. 3A), in which we focused on FBXW7. It has been reported that CHOL patients with low expression of FBXW7 have a poor prognosis (Ishii 2017). Dual-luciferase reporter assay confirmed that miR-182-5p could bind to FBXW7 3'UTR ($p < 0.01$, Fig. 3B), and FBXW7 was poorly expressed in tissues of HCCA patients and HCCA cells ($p < 0.01$, Fig. 3C), while FBXW7 was increased after inhibition of miR-182-5p ($p < 0.01$, Fig. 3D). The above results indicated that miR-182-5p targeted and inhibited FBXW7 expression in HCCA cells.

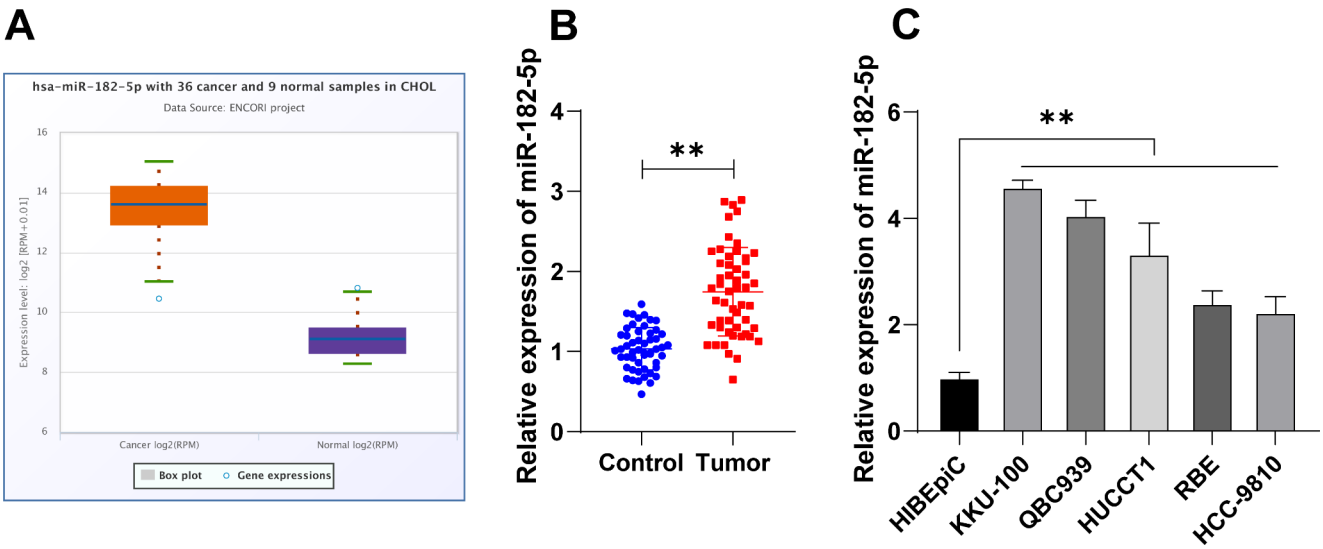

Fig. 1 miR-182-5p is highly expressed in HCCA. **A**: StarBase database predicted the expression of miR-182-5p in CHOL. **B**: The expression of miR-182-5p in HCCA tissue (Tumor) and tumor adjacent tissue (Control) was detected by qRT-PCR, $n = 52$. **C**: The expression of miR-182-5p in (HIBEpiC) and bile duct cancer cell lines (KKU-100, QBC939, HUCCT1, RBE, and HCC-9810) was detected by qRT-PCR. Cell experiment was repeated three times independently. ** $p < 0.01$. Data in panel B were analyzed by *t* test; data in panel C were analyzed by one-way ANOVA, followed by Tukey's multiple comparisons post hoc test

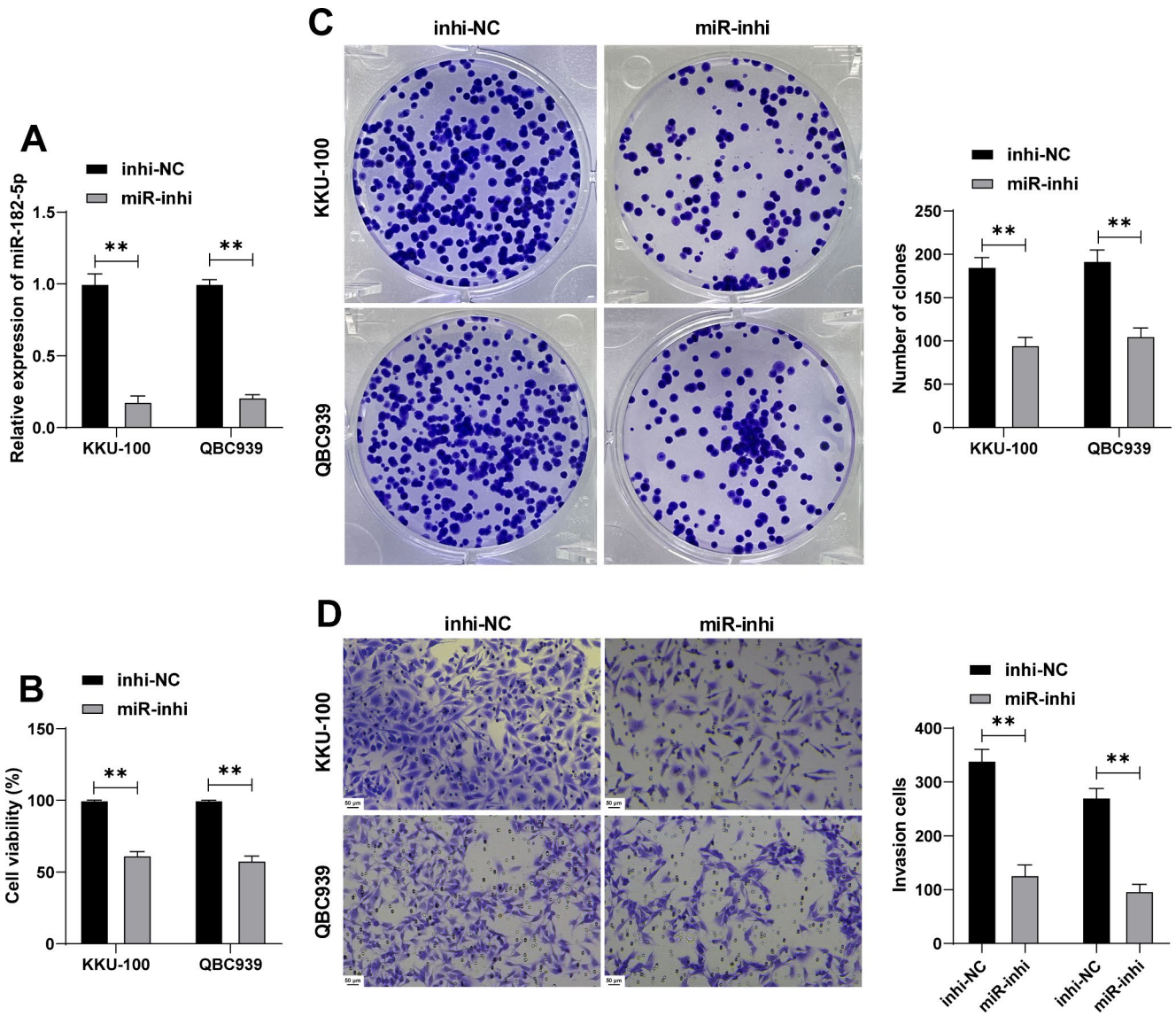

Fig. 2 Inhibition of miR-182-5p inhibits the proliferation and invasion of HCCA cells. miR-182-5p inhibitor (miR-inhi) was transfected into KKU-100 and QBC939 cells, and inhi-NC was used as control. **A**: The expression of miR-182-5p was detected by qRT-PCR. **B**: Cell viability was detected by CCK-8. **C**: Cell proliferation was detected by colony formation assay. **D**: Cell invasion was detected by Transwell assay. Cell experiments were repeated three times independently, ** $p < 0.01$. Data were analyzed by two-way ANOVA, followed by Tukey's multiple comparisons post hoc test

Inhibition of FBXW7 partially reverses the inhibitory effect of miR-182-5p silencing on the proliferation and invasion of HCCA cells

To verify the above mechanism, we transfected two si-FBXW7 to inhibit FBXW7 expression ($p < 0.01$, Fig. 4A-B), and si-FBXW7-1 with a better transfection effect was chosen for the combined experiments with miR-182-5p inhibitor. Compared with miR-182-5p silencing alone, cell viability in the combined group was promoted ($p < 0.01$, Fig. 4C), and the proliferation and invasion abilities were also increased ($p < 0.01$, Fig. 4D-E), indicating that inhibition of FBXW7 partially reversed the inhibitory effect of miR-182-5p silencing on cell proliferation and invasion.

Inhibition of miR-182-5p reduces tumor growth of HCCA *in vivo*

The *in vivo* tumor model was established by subcutaneous injection of lowly-expressed miR-182-5p and antagomiR-NC. The tumor growth of KKU-100 cells with low-expressed miR-182-5p was significantly slower than that of the control group ($p < 0.01$, Fig. 5A). The tumor weight was also significantly lower than that of the control group

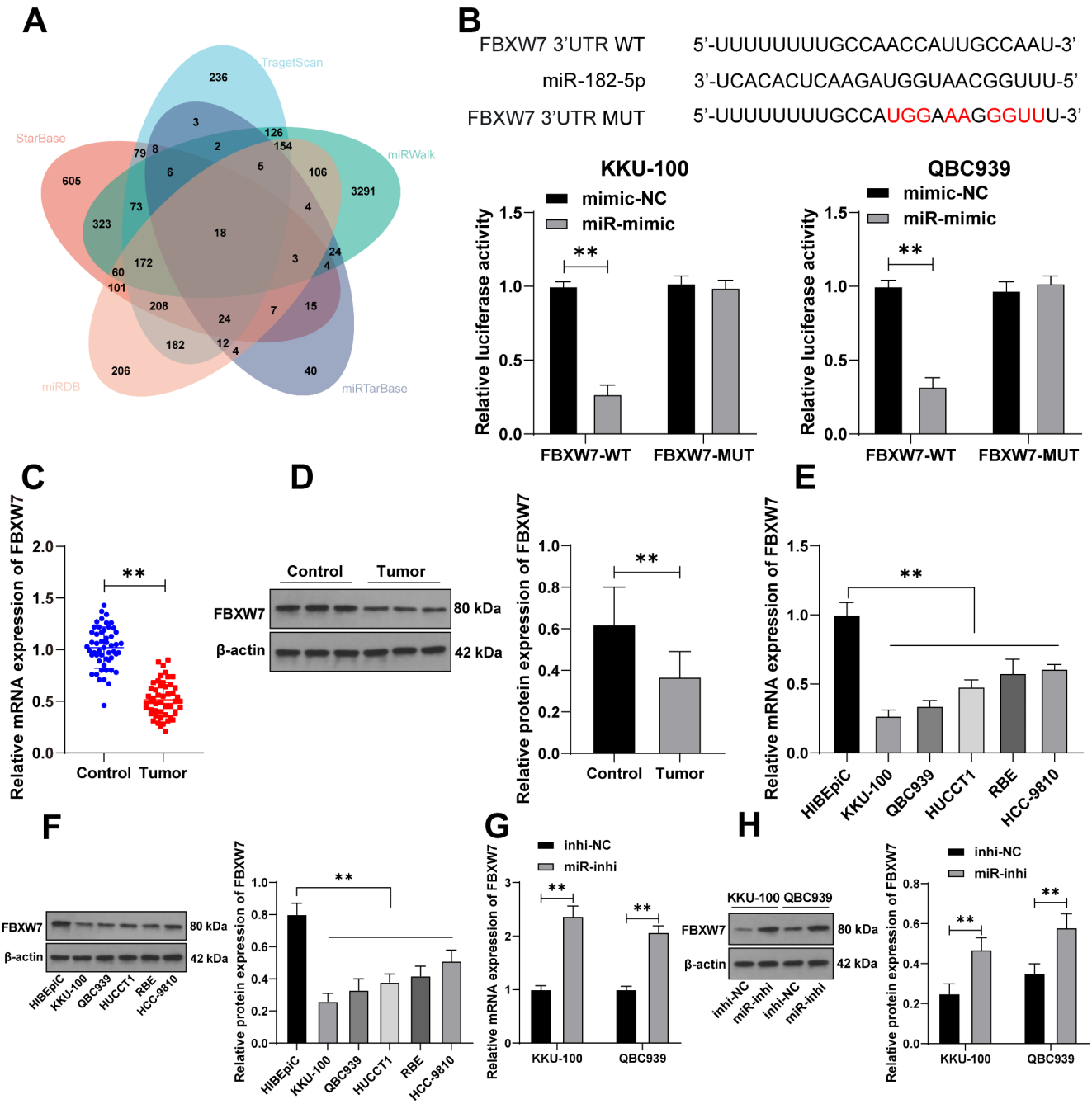

Fig. 3 miR-182-5p targets and inhibits the expression of FBXW7. **A**: The downstream target genes of miR-182-5p were predicted by StarBase, TargetScan, miRWalk, miRDB, and miRTarBase databases, and the intersection was taken. **B**: The binding of miR-182-5p to FBXW7 3'UTR sequence was analyzed by dual-luciferase reporter assay. **C-H**: The expression of FBXW7 in tissues ($n=52$) and cells was analyzed by qRT-PCR and Western blot. Cell experiments were repeated three times independently, ** $p<0.01$. Data in panels C and D were analyzed by t test; data in panels B, G, and H data were analyzed by two-way ANOVA; data in panels E and F were analyzed by one-way ANOVA, followed by Tukey's multiple comparisons post hoc tests

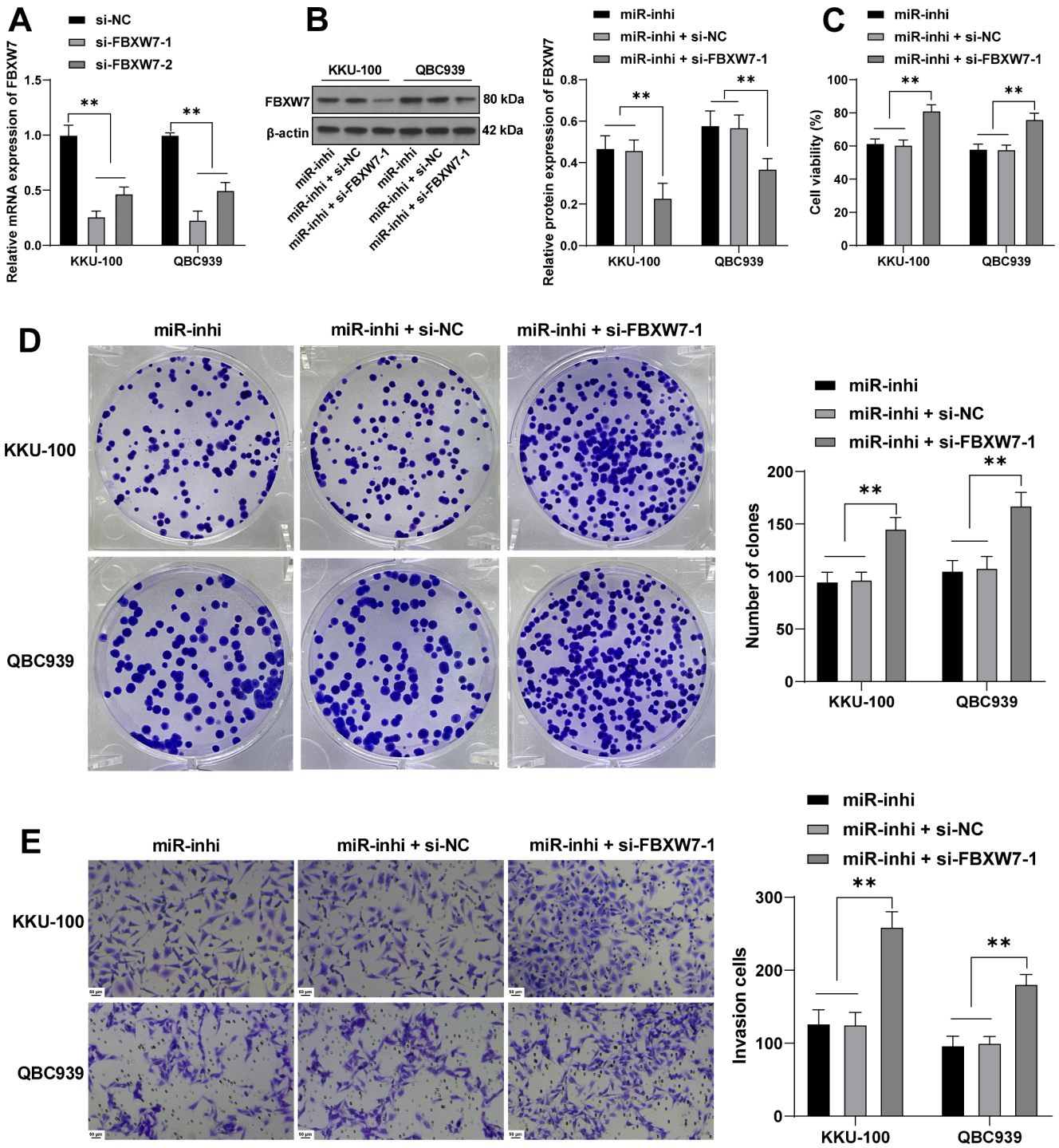

Fig. 4 Inhibition of FBXW7 partially reverses the inhibitory effect of miR-182-5p silencing on the proliferation and invasion of HCCA cells. Two siRNAs targeting FBXW7 (si-FBXW7-1 and si-FBXW7-2) were transfected into KKU-100 and QBC939 cells, respectively, with si-NC as control. **A**: The transfection efficiency was detected by qRT-PCR. si-FBXW7-1 with better transfection effect and miR-inhi were selected for combined experiment. **B**: The expression of FBXW7 in cells was detected by Western blot. **C**: Cell viability was detected by CCK-8. **D**: Cell proliferation was measured by clone formation test. **E**: Cell invasion was detected by Transwell assay. Cell experiments were repeated three times independently, ** $p < 0.01$. data were analyzed by two-way ANOVA, followed by Tukey's multiple comparisons post hoc test

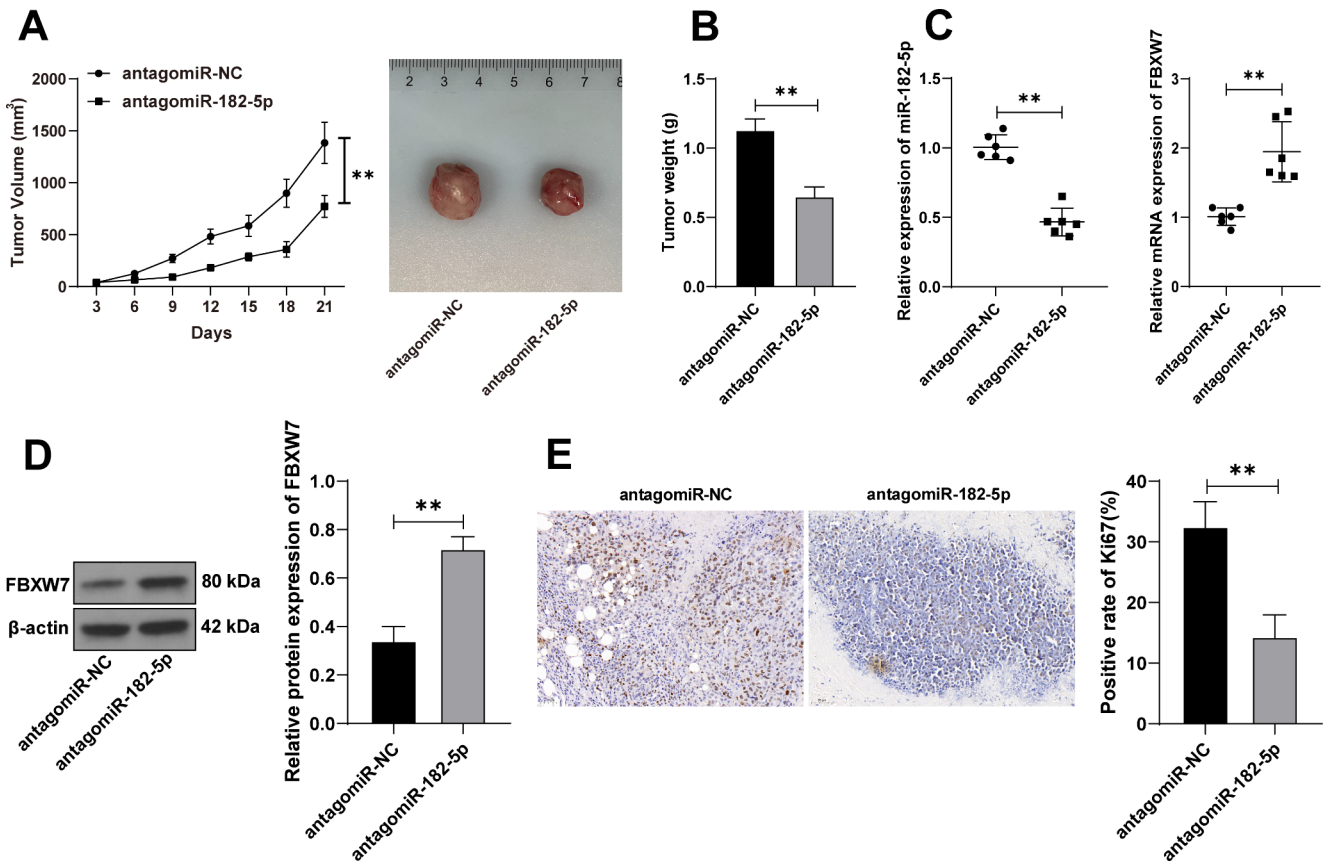

Fig. 5 Inhibition of miR-182-5p reduces tumor growth of HCCA in vivo. KKU-100 cells were infected with adenovirus containing antagomiR-182-5p to obtain cells with stable low miR-182-5p expression, and antagomiR-NC was used as control. The above cells were subcutaneously injected into nude mice. **A**: The volume of the tumors was monitored every 3 days, and representative images of the tumors were harvested after 21 days. **B**: Tumor weight. **C**: The expression of miR-182-5p and FBXW7 in tumors was detected by qRT-PCR. **D**: The expression of FBXW7 in the tumors was detected by Western blot. **E**: The positive expression of ki67 in the tumor was detected by IHC. $n = 6$, ** $p < 0.01$. Data in panel A were analyzed by two-way ANOVA, followed by Tukey's multiple comparisons post hoc test; data in panels B-E were analyzed by *t* test

($p < 0.01$, Fig. 5B). FBWX7 expression was increased in tumors with lowly-expressed miR-182-5p ($p < 0.01$, Fig. 5C-D). IHC detection showed that Ki67 expression was reduced in tumors with lowly-expressed miR-182-5p ($p < 0.01$, Fig. 5E). These results suggested that inhibition of miR-182-5p expression could effectively inhibit the growth of HCCA tumors in vivo.

Discussion

In published studies, miR-182-5p showed different characteristics in different cancers. In nasopharyngeal carcinoma (He et al. 2021) and liver cancer (Zheng et al. 2021), miR-182-5p functions as a pro-carcinogenic factor, whereas it acts as a tumor suppressor in thyroid carcinoma (Wang et al. 2022a) and colorectal cancer (Jin et al. 2019). While the involvement of miR-182-5p in tumorigenesis, invasion, and metastasis through gene expression regulation has been demonstrated in various cancers, our study specifically revealed significant upregulation of miR-182-5p in HCCA. Inhibition of miR-182-5p was closely associated with reduced activity, proliferation, and invasion ability of HCCA cells, highlighting that miR-182-5p could serve as a potential therapeutic target for HCCA.

The database predicted a high expression of miR-182-5p in CHOL. In addition, miR-182-5p was highly expressed in cancer tissues of HCCA patients. miR-182-5p can function as a cancer promoting factor. Overexpression of miR-182-5p promotes invasion and migration of non-small cell lung cancer (Yang et al. 2021) and is significantly correlated with pathological stage, grade, and lymphatic metastasis of bladder cancer cells (Zhang et al. 2022). It has been verified that miR-182-5p plays a carcinogenic role in CHOL. A previous study has found that miR-182-5p is ectopically up-regulated in bile exosomes of CHOL, thereby promoting the proliferation, invasion, epithelial-mesenchymal transition, and angiogenesis of CHOL by targeting

hydroxyprostaglandin dehydrogenase in CHOL cells and mast cells (Shu et al. 2023). Moreover, exosome-derived miR-182-5p can activate the PI3K/AKT/mTOR signaling pathway in CHOL, thus promoting vascular endothelial cell proliferation and migration and leading to gemcitabine and cisplatin resistance (Wang 2023). In line with previous studies, our study found that inhibiting miR-182-5p expression reduced the proliferation and invasion of HCCA cells, as well as the tumor weight and Ki67 expression in vivo, effectively limiting tumor growth.

Further database analysis and prediction showed that FBXW7 was the downstream target gene of miR-182-5p. The role of FBXW7 as a tumor suppressor gene has been widely established (Shen et al. 2022). FBXW7 recruitment, ubiquitination, dimerization, epigenetic mechanisms, and post-transcriptional modifications are major processes involved in cancer development (Fan et al. 2022). For instance, the overexpression of FBXW7 promotes ACTL6A ubiquitination and reduces the protein level of ACTL6A to inhibit the malignancy of hepatocellular carcinoma (Wang et al. 2022b). FBXW7 inhibits the migration and invasion of gastric cancer cells by negatively regulating the epigenetic regulator MECP2 (Zhao et al. 2022). Accordingly, FBXW7 also plays as a tumor suppressor in the development of CHOL, which can inhibit the migration and invasion of CC cells, and its insufficient expression is significantly related to the metastasis, TNM stage, and differentiation of HCCA (Yang et al. 2015). Meanwhile, in intrahepatic HCCA, the prognosis of the FBXW7-negative group is significantly worse than that of the FBXW7-positive group (3-year survival rate was 29.4% and 72.7%, respectively) (Enkhbold et al. 2014). Our study further confirmed that inhibiting FBXW7 increases HCCA cell viability and invasion. In addition, FBXW7 was inhibited by miR-182-5p, consistent with a previous report (Wu et al. 2020). Of note, miR-182-5p directly binds to the FBXW7 3'UTR, and weakening the expression of miR-182-5p can increase the level of FBXW7, inhibit the proliferation and invasion of cervical cancer cells, and slow down tumor progression (Zhang 2020a). Therefore, we conducted combined experiments of si-FBXW7-1 and miR-182-5p inhibitor and found that inhibiting FBXW7 partly counteracted the inhibitory effect of miR-182-5p knockdown on HCCA by promoting cell proliferation and invasion.

Our study still has some limitations. First, there are many downstream target genes of miR-182-5p, and we only chose FBXW7 for this study since low expression of FBXW7 has been reported to indicate poor prognosis in CHOL patients. Second, our investigation only focused on the impact of the miR-182-5p/FBXW7 axis on tumor growth in vivo and did not explore other pathological characteristics such as histology, lymph node metastasis, and lymphatic invasion. Third, the downstream mechanism of FBXW7 remains to be explored. Finally, it is unclear whether upregulation of miR-182-5p would promote cell proliferation. In the future, we will conduct related studies on other downstream target genes of miR-182-5p from intersections predicted in databases, explore the downstream regulation mechanism of FBXW7, improve the specific mechanism of miR-182-5p/FBXW7 in vivo and in vitro, and collect survival data from HCCA patient for analysis of the prognostic value of miR-182-5p and FBXW7.

Conclusion

In conclusion, we explored the role of miR-182-5p/FBXW7 in the proliferation and invasion of HCCA for the first time and found that miR-182-5p targeted and inhibited the expression of FBXW7, while inhibition of miR-182-5p could effectively inhibit the cell proliferation and tumor growth of HCCA, providing a theoretical basis for the treatment of HCCA.

Author contributions Hang Zhou: Conceptualization, Data curation, Formal Analysis, Methodology, Writing – original draft, Writing – review & editing; Yong Jiang: Data curation, Formal Analysis, Investigation, Validation; Yang Zhou: Conceptualization, Data curation, Investigation; Zhao Zhang: Data curation, Methodology, Validation; Shaoyin Li: Formal Analysis, Visualization, Writing – review & editing.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval All tissues were obtained with informed consent from the patients. Our study followed the Declaration of Helsinki, and ethical approval was obtained from the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University. All animal experiments were carried out approval of the Animal Ethics Committee of The First Affiliated Hospital of Chongqing Medical University, and the experimental process followed the Guidelines for the Use of Laboratory Animals (National Research Council (US) 2011).

Competing interests The authors declare no competing interests.

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