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MicroRNA-153-3p promotes hepatocellular carcinoma progression by modulating SPRY2 expression and the MAPK/ERK signalling pathway

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ABSTRACT: Hepatocellular carcinoma (HCC) is the most common primary liver cancer and among the most prevalent digestive system cancers. MicroRNAs (miRNAs) have been linked to multiple cellular processes, including cellular proliferation, differentiation, and cancer development. Although miRNA-153-3p participates in various malignancies, its role in HCC and its underlying mechanisms remain unclear. In this study, we investigated the expression and molecular mechanisms of miRNA-153-3p in HCC. miRNA-153-3p and SPRY2 expression was evaluated in human tissues and cell lines using quantitative PCR and western blotting. The cell proliferation, migration, and invasion capacities were assessed using Cell Counting Kit-8 and Transwell assays. The cell cycle and apoptosis were analysed using flow cytometry, and the relationship between miRNA-153-3p and SPRY2 was confirmed using a dual-luciferase reporter assay. miRNA-153-3p was upregulated, whereas SPRY2 was downregulated in HCC tissues compared with para-carcinoma tissues. Overexpression of miRNA-153-3p promotes HCC proliferation, migration, and invasion by targeting SPRY2 and mitogen-activated protein kinase/extracellular signal-regulated kinase signalling pathway-related proteins. It was also observed that SPRY2 overexpression partially rescued the effects of miRNA-153-3p. This study provides an experimental reference for identifying molecular targets for HCC therapy.

KEYWORDS: hepatocellular carcinoma, MAPK/ERK signalling pathway, MicroRNA, protein expression, SPRY2

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common digestive malignancy and the fourth leading cause of cancer-related death worldwide [1]. Despite improvements in curative treatment methods (including surgery [2], radiotherapy [3], chemotherapy [4], and immunotherapy [5]), the 5-year survival rate of patients with HCC remains lower than 15% due to the high malignancy, rapid progression, and high recurrence rates of HCC [6–8]. Therefore studying the molecular mechanisms underlying the disease progression of HCC is needed to identify more effective biomarkers for enhancing early diagnosis, therapy, and prognosis of HCC.

MicroRNAs (miRNAs) are a class of small non-coding RNAs comprising 20–25 nucleotides and functioning as endogenous regulators in eukaryotic organisms [9]. The main regulatory mechanism of these non-coding RNAs relies on their direct binding to the 3′-untranslated region (3′-UTR) of the target gene transcript, resulting in inhibition of translation or transcript degradation to negatively regulate gene expression [10]. miRNAs participate in various biological activities, including proliferation, migration, invasion, and apoptosis; and several lines of evidence suggest that abnormal expression of miRNAs plays a critical role in cancer progression [11–14]. Recent studies revealed that miRNA-153-3p is dysregulated in multiple human tumours and participates in the circular RNA (circRNA)/long intergenic non-coding RNA-miRNA-mRNA axis that promotes the development of several tumours, including breast cancer [15], cervical cancer [16], malignant melanoma [17], oesophageal squamous cell carcinoma [18], and papillary thyroid cancer [19]. However, the role and molecular mechanisms of miRNA-153-3p in HCC require further investigation.

The Sprouty (SPRY) gene, first discovered in fruit flies, is involved in regulating the development of bronchus [20] and eyes [21]. Subsequently, four subtypes of SPRY gene were found in mammals, namely SPRY1, 2, 3, and 4. SPRY2 was first identified as an intracellular inhibitor of the RTK and MAPK/ERK signalling pathways [22, 23] and plays important roles in normal cellular proliferation, differentiation, and organ development [24–27]. SPRY2 exhibits a high degree of evolutionary conservation, and its loss or dysregulation has been linked to various cancers, including HCC [28], gastric cancer [29], ovarian cancer [30], and pancreatic cancer [31]. Here, we explored the role of miRNA-153-3p in HCC and implemented bioinformatics predictions and experimental validation to confirm whether SPRY2 is a direct target of miRNA-153-3p. Our findings provide a theoretical basis and experimental reference for identifying molecular targets for HCC treatment.

MATERIALS AND METHODS

Tissue sample collection

We collected cancerous and para-cancerous tissue samples from 20 patients with HCC, along with complete pathological data from the Pathology Department of the Henan Oncology Hospital; the para-cancerous tissues were collected from at least 2 cm above the tumour edge. Patients did not receive treatment before the operation, and the specimens were placed in liquid nitrogen immediately after removal. We obtained informed consent from the patients and their families before the experiments.

Cell lines and cell culture

The human HCC cell line HepG2 was purchased from the Institute of Cell Research, Chinese Academy of Sciences (Shanghai, China). The human renal epithelial cell line 293T was provided by the Shanghai Chinese branch of the College Shanghai Cell Bank and cultured in Dulbecco's Modified Eagle Medium high-glucose (SH30022.01; HyClone, Logan, UT, USA) containing 10% foetal bovine serum (AusGeneX, Molendinar, Australia), at 37 °C in a humidified incubator with 5% CO_2.

Oligonucleotides and transfection

MiRNA-153-3p mimic, mimic NC, inhibitor-miRNA-153-3p, and inhibitor NC (GenePharma Co., Ltd., Shanghai, China) were transfected into HepG2 cells at a concentration of 100 nM per construct using Lipofectamine 3000 transfection reagent (Invitrogen, Carlsbad, CA, USA) and incubated for 48 h. Transfected cells were used in subsequent experiments.

RNA extraction and quantitative reverse transcription-PCR

Total RNA was extracted from tissues and cells using RNAiso Plus reagent (TaKaRa, Shiga, Japan), and the RNA concentration was measured. The extracted RNA was used to synthesise cDNA using a PrimeScript™ RT reagent Kit and a gDNA Eraser Kit (TakaRa). miRNA-153-3p cDNA was synthesised using an All-in-One™ First-Strand Gene Kit (GeneCopoeia, Guangzhou, China). Then, a quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed using the synthesised cDNA sample as a template. The mRNA expression of SPRY2 was determined using the TBGreen™ Premix Ex Taq™ II Kit (TakaRa), with *GAPDH* serving as an internal reference; whereas miRNA-153-3p was evaluated using the All-in-One™ miRNA RT-qPCR Detection System (GeneCopoeia), with *U48* as an internal reference. The expression levels of each gene were calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences were listed in Table S1.

Western blot assay

Total protein was extracted from the cells using RIPA buffer and quantified using the BCA assay. A total of 30 mg of total protein was separated using 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis and then transferred onto a polyvinylidene difluoride membrane (Millipore, Billerica, MA, USA). The following steps were performed on a shaker: the membrane was incubated with 5% fat-free milk at room temperature for 2 h and then incubated overnight with the appropriate primary antibody [SPRY2 (1:1000, Abcam, Cambridge, UK), p-c-Raf, p-MEK1/2, p-p90RSK, p-MSK1 (1:1000), and p-ERK (1:2000, Cell Signaling Technology, Danvers, MA, USA] at 4 °C; the membranes were washed three times with Tris-buffered saline containing Tween-20. The membrane was incubated with a horseradish peroxidase-labelled goat anti-rabbit secondary antibody at room temperature for 1 h. The blots were washed three times with Tris-buffered saline containing Tween-20 and visualised using an enhanced chemiluminescence kit (HRP, Novland, Shanghai, China), with β-actin acting as an internal reference. Expression was quantified using densitometry and ImageJ software (NIH, Bethesda, MD, USA); the results represent the ratio of the grey values relative to β-actin.

Plasmid construction and dual-luciferase reporter assay

To determine whether SPRY2 is a direct target of miRNA-153-3p, the wild-type (WT) and mutant (MUT) 3′-UTR sequences from SPRY2 were cloned into the PmiR-RB-Report vector (Guangzhou Ribobio Technology Co., Ltd., Guangzhou, China). The mimic-miRNA-153-3p/mimic NC was co-transfected with the WT SPRY2 3′-UTR or MUT SPRY2-3′-UTR plasmid in 293T cells and incubated for 48 h. Next, luciferase activity was measured using the dual-luciferase reporter assay. The *hLuc*$^+$ luciferase gene was used as an internal reference, and relative changes in hRluc luciferase activity were recorded.

CCK8 assay

Cells were collected 48 h post-transfection, and 100 µl cell suspensions with a density of 1×10^4 cells/ml were seeded into 96-well plates. The outside of the 96-well plate was filled with phosphate-buffered saline (PBS) to prevent evaporation, and the plates were cultured at 37 °C in a 5% CO_2 incubator. After adherence, 10 µl of CCK8 reagent (Dalian Meilun Biotech Co., Ltd., Dalian, China) was added to each well, and the plate was incubated for 4 h. The OD_{450} values at 0, 24, 48, 72, and 96 h were measured using an enzyme calibrator. The experiment was repeated three times, with three replicates per group per experiment.

Transwell assay

We used 8 µm Transwell chambers (Corning Costar Corp, Corning, NY, USA) to evaluate the migratory and invasive abilities of treated cells. Briefly, 1×10^4 cells were seeded into the top chamber containing 200 µl of serum-free medium, and 500 µl of complete culture medium (with 10% foetal bovine serum) was added to the lower chamber to act as a chemoattractant. After 24 h of incubation, the cells were fixed in 4% paraformaldehyde for 1 h and then stained with 0.1% crystal violet for 30 min. Cells in the upper layer of the chamber that had not migrated to the opposite surface were carefully removed with a cotton swab, and cells on the lower surface were imaged and counted under an inverted microscope at 200× magnification in five random fields of view for each membrane. To investigate the invasive capacity of the treated cells, 50 µl Matrigel (50 µg/ml, BD Biosciences, San Jose, CA, USA) was added to each well, followed by the addition of cells. The plate was incubated for 2 h before being evaluated, as described above.

Apoptosis assay

Flow cytometry was performed to evaluate the ratio of apoptotic cells after the aforementioned treatments. The cells were harvested via trypsinisation, washed in ice-cold PBS, and treated with 5 µl of Annexin V-FITC (50 µg/ml) for 15 min in the dark, followed by the addition of 5 µl of propidium iodide (10 µg/ml). The stained cells were subjected to flow cytometry within 1 h of staining.

Cell cycle assay

After transfection, cells were harvested using 0.25% trypsin, washed with PBS, and fixed in 70% ethanol at 4 °C overnight. The cells were then washed and incubated with 100 µl of RNaseA (50 mg/ml) for 30 min at 37 °C, then incubated with 400 µl of propidium iodide (5 mg/ml) for 30 min before being subjected to flow cytometry.

Statistical analysis

We used SPSS23.0 statistical software (SPSS, Inc., Chicago, IL, USA) for statistical analyses; the results are expressed as the mean±SEM. Differences between two paired samples were analysed using a paired-samples *t*-test. Correlation analysis was conducted using Spearman's correlation coefficient, and statistical significance was set at $p < 0.05$.

RESULTS

miRNA-153-3p overexpression inversely correlated with SPRY2 expression in HCC

Twenty pairs of human HCC tissues and their para-cancerous counterparts were used to determine the relative expression of miRNA-153-3p using qRT-PCR. miRNA-153-3p expression was significantly upregulated in HCC tissues relative to paired para-cancerous samples (Fig. 1A). Western blotting and qRT-PCR analyses also revealed that SPRY2 was downregulated at both the mRNA and protein levels in HCC tissues comparing with the para-cancerous counterparts (Fig. 1B and 1C). Furthermore, the expression of miRNA-153-3p and SPRY2 showed a negative correlation (Fig. 1D and 1E), and SPRY2 mRNA and protein levels showed a positive correlation (Fig. 1F). These results indicate that SPRY2 is a target of miRNA-153-3p in HCC, and this interaction may play a key role in inhibiting tumour development.

MiRNA-153-3p directly targets SPRY2

We evaluated the relationship between SPRY2 and miRNA-153-3p. First, we used TargetScan and miRbase to predict the targets of miRNA-153-3p and identified SPRY2 as a potential target (Fig. 2A). This relationship was validated using a dual-luciferase assay. We constructed WT and mutant (MUT) SPRY2-3′-UTR (Fig. 2B) constructs and evaluated the luciferase activity in response to miRNA-153-3p or its mimics. Luciferase activity was reduced when the mimic-miRNA-153-3p and WT SPRY2-3′-UTR were co-transfected into 293T cells. However, there was no obvious change in luciferase activity when mimic-miRNA-153-3p and MUT SPRY2-3′-UTR were co-transfected into the same cells (Fig. 2C).

miRNA-153-3p upregulation enhances HepG2 proliferation, migration, and invasion

We hypothesised that increased expression of miRNA-153-3p plays an important role in the development of HCC and evaluated this hypothesis using the HepG2 cell line. We transfected HepG2 cells with mimic-miRNA-153-3p, a mimic NC, inhibitor-miRNA-153-3p, or an inhibitor NC construct; and evaluated their effects using qRT-PCR and various cellular assays. Initial evaluation revealed that miRNA-153-3p and SPRY2 mRNA expression expectedly increased and decreased in response to the miRNA-153-3p mimic, respectively (Fig. 2D and 2E).

The CCK8 assay was then performed to determine the influence of miRNA-153-3p on HCC cell proliferation; the results showed that miRNA-153-3p overexpression promoted HepG2 cell proliferation compared with the control cells, while the opposite effect was observed in the presence of a miRNA-153-3p inhibitor (Fig. 3A and 3B). Hence, the results indicate that HCC cellular proliferation is associated with miRNA-153-3p expression.

We then used a Transwell assay to verify the oncogenic role of miRNA-153-3p in HCC cells. The results showed that miRNA-153-3p upregulation increased the migration rate, whereas its downregulation reduced migration (Fig. 3C). Addition of Matrigel

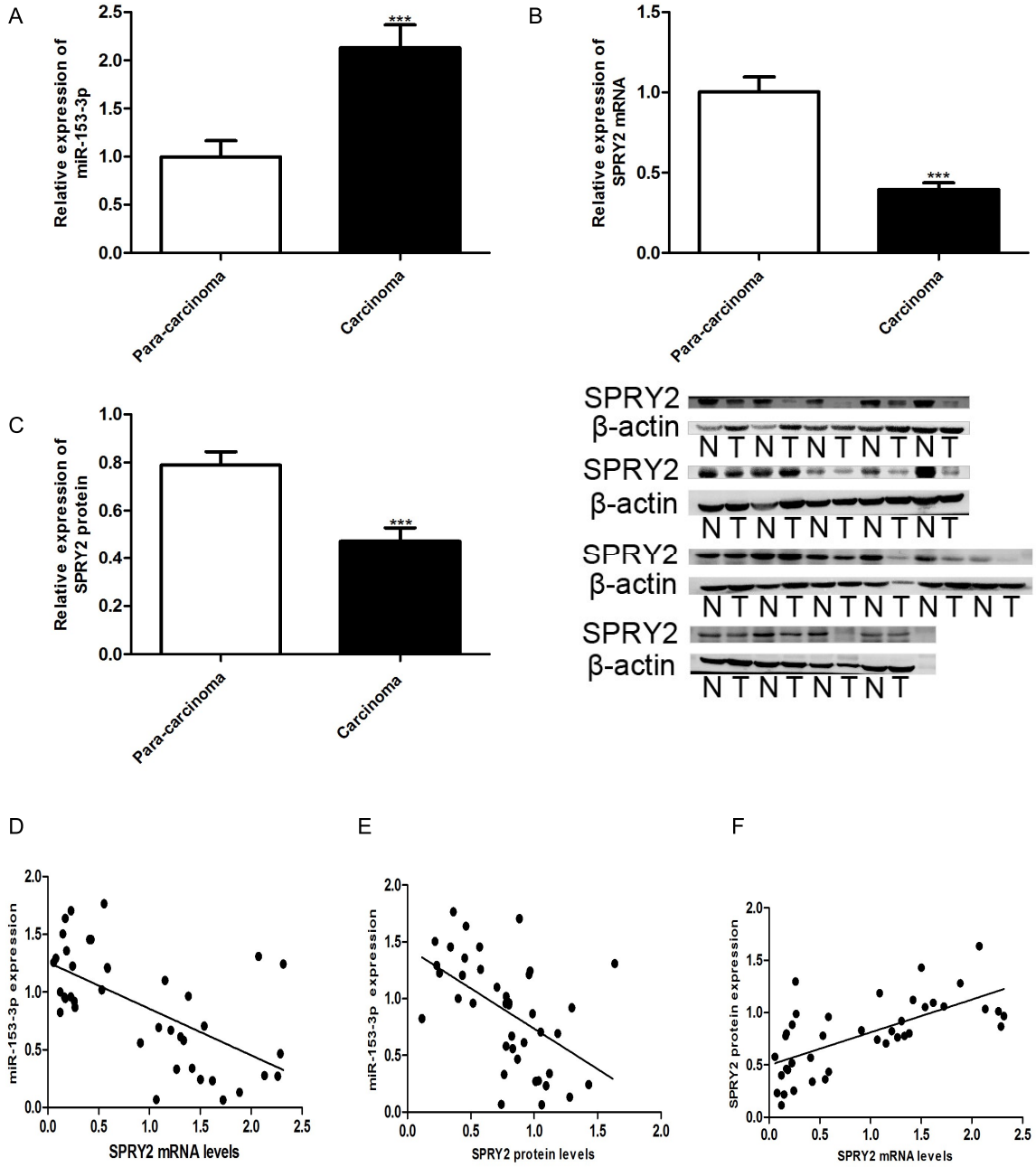


Fig. 1 The expression of miRNA-153-3p and SPRY2 in 20 pairs of HCC and their para-cancerous tissue counterparts. A and B, qRT-PCR; C, Western blot analysis the level of miRNA-153-3p and SPRY2 in human HCC tissues and para-cancerous tissues; D and E, correlation between miRNA-153-3p and SPRY2 expression; F, correlation between SPRY2 mRNA and SPRY2 protein expression. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, $n = 20$). Data are shown as the mean±SEM.

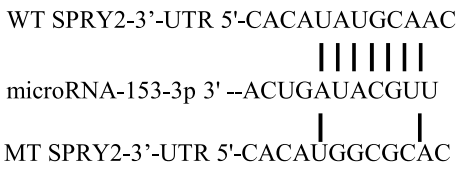
enabled the evaluation of the invasion capacity of these cells in response to miRNA-153-3p. Invasion increased with increasing miRNA expression compared with the control, and the opposite effect was observed when miRNA-153-3p was inhibited (Fig. 3D).

Finally, we evaluated both the cell cycle and apoptosis in cells with and without miRNA-153-3p to reveal whether overexpression of this miRNA influences the percentage of cells in each phase of the cell cycle or alters the apoptosis ratios in these cells. The results

A

	Predicted consequential pairing of target region (top) and miRNA (bottom)
Position 60-66 of SPRY2 3' UTR	5‘ ---UUUUUUUAAUACACAUAUGCAAC
hsa-microRNA-153-3p	3‘ CUAGUGAAAACACUG----AUACGUU

B



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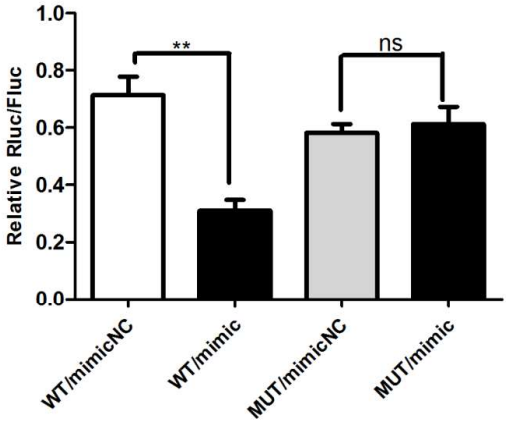

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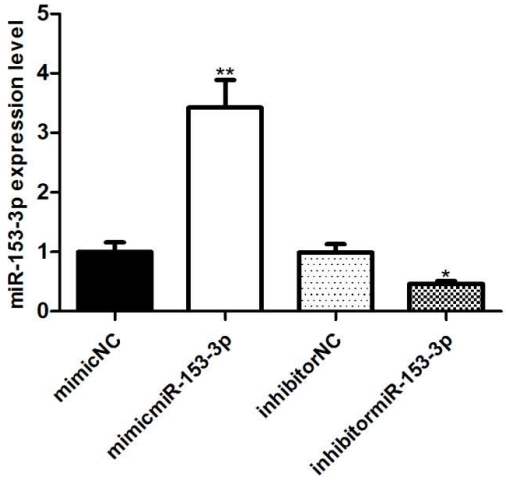

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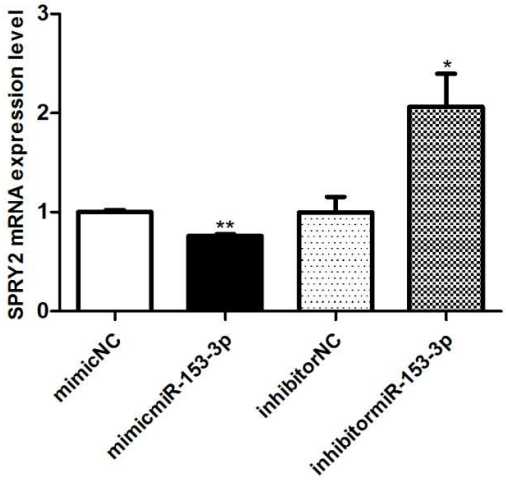

Fig. 2 SPRY2 expression is regulated by miRNA-153-3p *in vitro*. A, SPRY2 – a potential target of miRNA-153-3p; B, luciferase reporter gene plasmids containing wild-type (WT) and mutant (MUT) SPRY2-3′-UTR; C, luciferase activity reduced when mimic-miRNA-153-3p co-transfected with the WT-SPRY2-3′-UTR plasmid in 293T cells and no expression change observed in cells co-transfected with the mimic-miRNA-153-3p and MUT SPRY2-3′-UTR plasmid. (** $p < 0.01$ vs. control, $n = 3$); D, relative expression of miRNA-153-3p; E, relative expression of SPRY2 mRNA in HepG2 cells transfected with mimic-miRNA-153-3p or inhibitor-miRNA-153-3p.

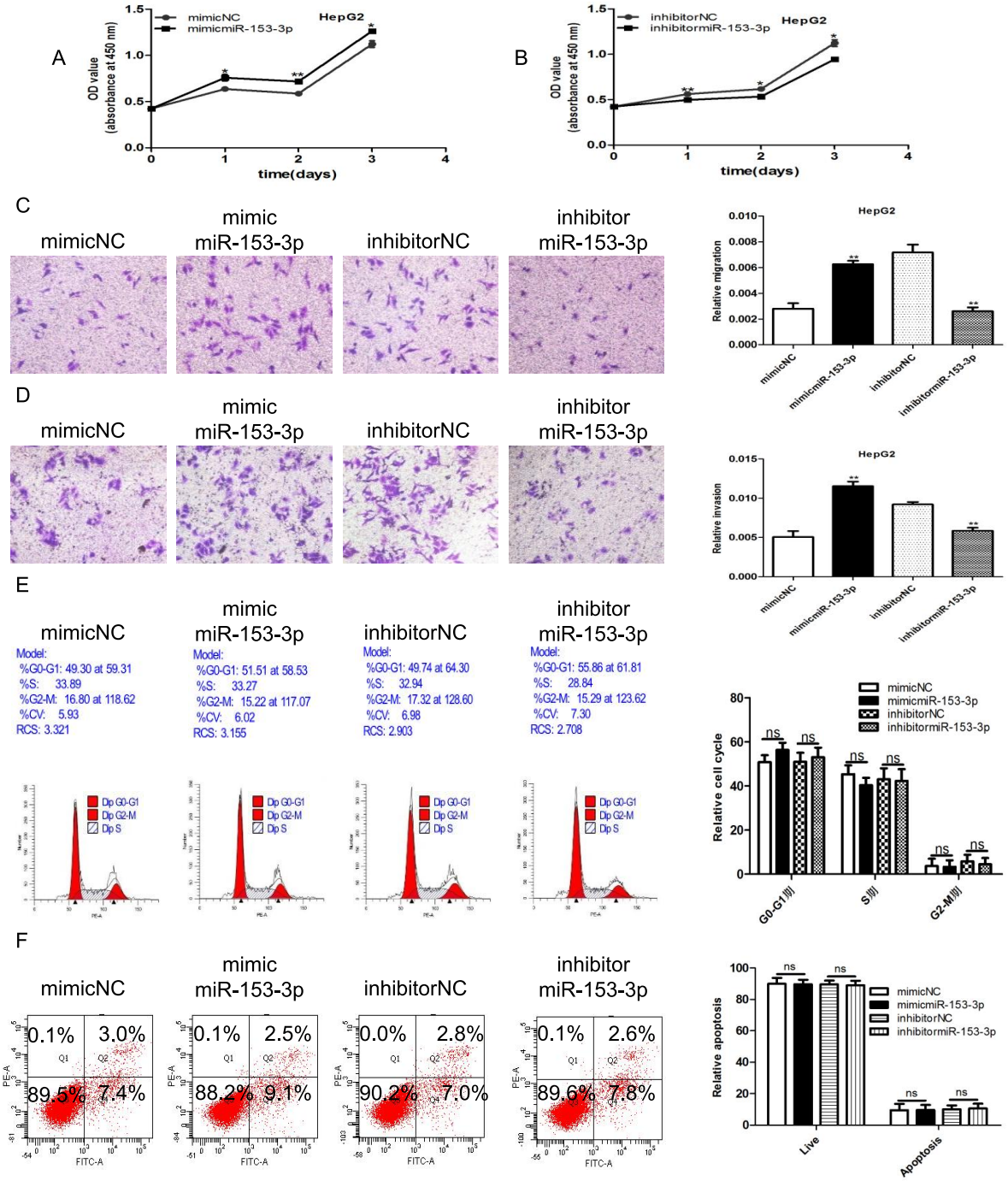

Fig. 3 miRNA-153-3p promotes HepG2 cell proliferation, migration, and invasion. A and B, cell proliferation assessed by CCK-8 assays; C and D, migratory and invasive abilities assessed by Transwell assays; E, cell cycle assessed by flow cytometry and propidium iodide staining; F, cellular apoptosis assessed by Annexin V-FITC/PI staining and flow cytometry. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, $n = 3$). Data are shown as the mean±SEM.

showed no obvious changes in either the cell cycle or apoptosis ratio in these cells in response to changes in the expression levels of miRNA-153-3p (Fig. 3E and 3F).

miRNA-153-3p promotes HCC development by targeting SPRY2 and MAPK/ERK signalling pathway

Our results showed that miRNA-153-3p promoted HCC development by inhibiting SPRY2 *in vitro*. Previous studies also showed that SPRY2 is an intracellular inhibitor of the MAPK/ERK signalling pathway. Based on previous results, we explored the molecular mechanisms underlying the effects. We performed a western blot assay to evaluate changes in the expression of SPRY2 and several critical components of the MAPK/ERK pathway, including p-c-Raf, p-MEK1/2, p-ERK1/2, p-p90RSK, and p-MSK1, following transfection of HepG2 cells with mimic-miRNA-153-3p/mimic NC, inhibitor-miRNA-153-3p or inhibitor NC. Our data showed that SPRY2, p-MEK1/2, p-ERK1/2, p-p90RSK, and p-MSK1 expression were reduced in response to miRNA-153-3p overexpression, whereas the expression of p-c-Raf increased with decreasing SPRY2 expression (Fig. 4A) relatively to the control. Of note, the downregulation of miRNA-153-3p had the opposite effect (Fig. 4B).

DISCUSSION

miRNAs play both tumour suppressive and oncogenic roles in tumour development [13]. Recent studies showed that miRNA-153-3p is dysregulated in various human tumours. Two studies show that miRNA-153-3p is upregulated in sport-related concussions [32] and human umbilical cord mesenchymal stem cells. Of note, miRNA-153-3p upregulation attenuated methylglyoxal-induced peritoneal fibrosis in rats [33]. Furthermore, in a specific context, miRNA-153-3p can be downregulated; for instance, Li et al [34] found that the overexpression of long noncoding RNA (lncRNA) HIF1A-AS2 promotes HIF-1α expression by downregulating miR-153-3p, fuelling human umbilical vein endothelial cell angiogenesis during hypoxia. In addition, Wang et al [35] found that increased expression of LINC00641 regulates autophagy and intervertebral disc degeneration through miR-153-3p/ATG5 during nutritional deprivation stress, providing a basis for developing treatments for intervertebral disc degeneration. CircRNAs and lncRNAs act as competitive endogenous RNAs that regulate miRNAs via circRNA/lncRNA-miRNA-mRNA axes, many of which promote tumorigenesis and disease development in several cancers, including breast cancer [15], cervical cancer [16], malignant melanoma [17], oesophageal squamous cell carcinoma [18], papillary thyroid carcinoma [19], and human oral carcinoma [40].

We first evaluated miRNA-153-3p expression in 20 pairs of HCC and para-cancerous tissues. The results showed that miRNA-153-3p was significantly upregulated in HCC tissues comparing with the para-cancerous controls. Thus, we explored the potential target genes of miRNA-153-3p in HCC using bioinformatics analysis. This evaluation identified several targets, including SPRY2, SPRED1, AKT3, and MAPKLIP1L. A recent study showed that SPRY2 is an intracellular inhibitor of the RAS/MAPK signalling pathway [23], playing an important role in normal cell proliferation, differentiation, angiogenesis, and tumour inhibition [36–39]. Its loss or dysregulation is associated with tumorigenesis and the development of various types of tumours, including HCC [28], gastric cancer [29], ovarian cancer [30], and pancreatic cancer [31]. We then showed that the mRNA expression of SPRY2 was significantly downregulated in the HCC tissues comparing with the para-cancerous tissues, consistent with the protein expression of SPRY2 in these tissues. Finally, we confirmed that SPRY2 is a target gene of miRNA-153-3p.

Besides, this analysis revealed that miRNA-153-3p expression was patient-specific, with tissues exhibiting highly heterogeneous levels of miRNA-153-3p. This finding confirmed the association between miRNA-153-3p and HCC and the heterogeneity in miRNA-153-3p expression. We further evaluated the underlying mechanism of these outcomes. SPRY2 acts as a tumour suppressor gene and is downregulated in HCC, suggesting that miRNA-153-3p acts as an oncogene in these tissues, ultimately promoting HCC development.

To further investigate the role of miRNA-153-3p in SPRY2 expression and its impact on the occurrence and development of HCC, we transfected HepG2 cells with mimic-miRNA-153-3p/mimic NC and inhibitor-miRNA-153-3p/inhibitor NC to examine their influence on the proliferation, migration, and invasion capacity of HepG2 cells. The results revealed that miRNA-153-3p upregulation promoted the proliferation, migration, and invasion of HepG2 cells, whereas miRNA-153-3p downregulation had the opposite effect; and both the upregulation and the downregulation of miRNA-153-3p did not significantly affect the cell cycle or apoptosis of HepG2 cells.

These findings suggest that miRNA-153-3p inhibited SPRY2, promoting cell proliferation, migration, and invasion in HCC, as hypothesised.

To explore the molecular mechanisms underlying these effects, we evaluated the effect of miRNA-153-3p on the MAPK/ERK signalling pathway. We assessed the expression of SPRY2 and MAPK/ERK pathway-related proteins (p-c-Raf, p-MEK1/2, p-ERK1/2, p-p90RSK, and p-MSK1) after transfection with mimic-miRNA-153-3p/mimic NC or inhibitor-miRNA-153-3p/inhibitor NC. The protein expression of SPRY2, p-MEK1/2, p-ERK1/2, p-p90RSK, and p-MSK1 decreased in response to increased miRNA-153-3p expression

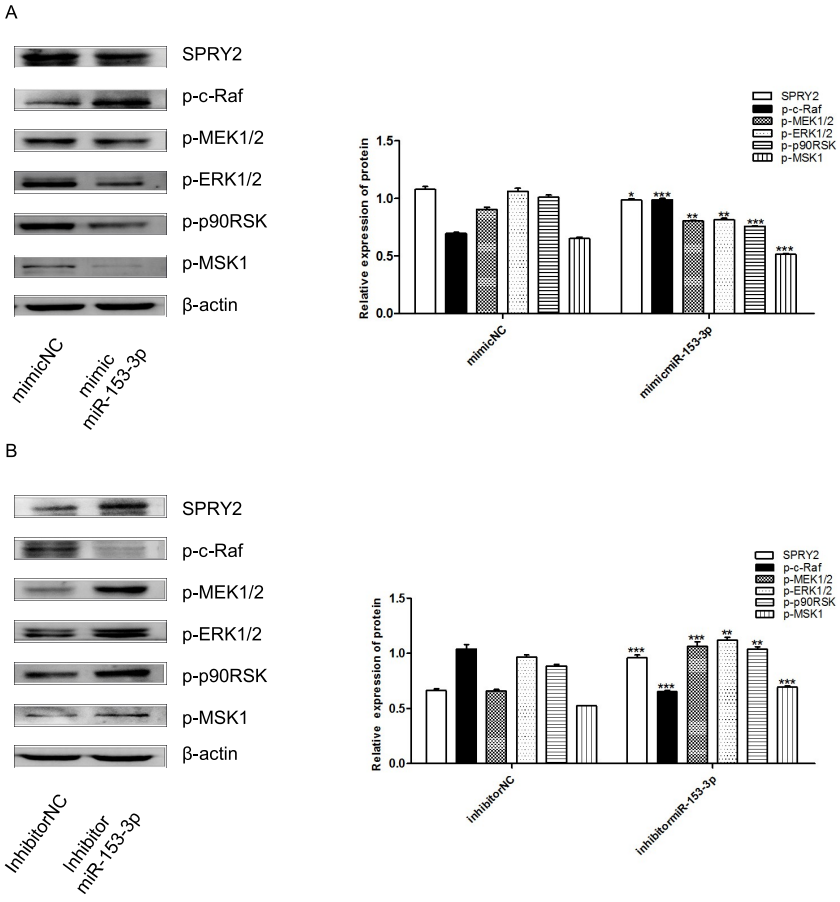

Fig. 4 miRNA-153-3p regulates the MAPK/ERK signalling pathway in HCC cells via downregulating SPRY2. Expression of SPRY2, p-c-Raf, p-MEK1/2, p-ERK1/2, p-p90RSK, and p-MSK1 in transfected HepG2 cells detected by Western blot: A, mimic-NC or mimic miRNA-153-3p or control assessed; B, inhibitor NC or inhibitor-miRNA-153. ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $n = 3$). Data are shown as the mean±SEM.

compared with the negative control (mimic NC). In contrast, the expression of p-c-Raf increased as the expression of its inhibitor, SPRY2, decreased. Transfection with inhibitor-miRNA-153-3p had the opposite effect.

The expression of p-c-Raf, p-MEK1/2, p-ERK1/2, p-p90RSK, and p-MSK1 was upregulated following transfection with mimic-miRNA-153-3p. However, these results were unexpected. Xiao et al [28] reported that miR-330-5p targeting SPRY2 promoted the progression of HCC through MAPK/ERK signalling, and miR-330-5p overexpression was associated with high expression of p-ERK. On the other hand, miR-330-5p inhibition was associated with low p-ERK expression, although the total expression of ERK did not change. Similarly, He et al [29] found that miR-592-targeted inhibition of SPRY2 promoted the proliferation, migration, and invasion of gastric cancer cells via the PI3K/AKT and MAPK/ERK signalling pathways and that the expression of SPRY2 decreased after transfection with miR-592 mimics. In contrast, p-ERK expression increased in cells transfected with a miR-592 inhibitor; however, the total level of ERK expression did not change. Therefore, we explored why miRNAs targeting one or more genes may yield different results.

CONCLUSION

Our biological assays showed results consistent with targeted gene regulation, and further evaluation revealed that the inhibitory effect of miRNA-153-3p was much more significant towards SPRY2 than towards MAPK1IP1L and SPRED1. However, the protein expression of SPRY2 and SPRED1 decreased in response to the upregulation of miRNA-153-3p, and both increased when abrogated. The decreased protein expression led to the increases of proliferation, migration, and invasion of HCC cells; and likely played important roles in the occurrence and development of tumours. Similarly, the downregulation of miRNA-153-3p had the opposite effects. Nevertheless, the relationship between miRNA-153-3p and SPRED1, MEK, and ERK must be further explored to determine why

the protein expression profiles of these pathways contradicting our expectations.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2024.021.

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Appendix A. Supplementary data

Table S1 The primer sequences of the genes.

Primer	Forward (5′–3′)	Reverse (5′–3′)
HA-SPRY2	GGACTGTGGCAAGTGCAAATGTA	AAGGCACTGCTTGTCGCAGA
HA-GAPDH	GCACCGTCAAGGCTGAGAAC	TGGTGAAGACGCCAGTGGA
U48	HmiRQP9021	Universal Adaptor PCR Primer, GeneCopoeia, USA
microRNA-153-3p	HmiRQP0214	GeneCopoeia, USA