miR-361-3p Regulates Liver Tumor-initiating Cells Expansion and Chemo-resistance

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Research

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Abstract

**Background:** Increasing evidence shows that liver tumor-initiating cells (T-ICs) closely associated with the progression, metastasis, recurrence and chemo-resistance of hepatocellular carcinoma (HCC). However, the underlying mechanism for the propagation of liver T-ICs remains unclear. The purpose of our study was to explore the role of miR-361-3p in the expansion of liver T-ICs and the potential molecular mechanism.

**Results:** Flow cytometry analysis was performed to isolate CD24\(^+\), CD133\(^+\) or EpCAM\(^+\) cells from primary HCC cells or HCC cell lines. Real-time PCR was used to detect the expression of miR-361-3p in liver T-ICs. The impact of miR-361-3p on liver T-ICs expansion was investigated both *in vivo* and *in vitro*. The correlation between miR-361-3p expression and TACE (transcatheter arterial chemoembolization) or sorafenib benefits in HCC was evaluated in patient cohorts. miR-361-3p expression is upregulated in liver T-ICs. Knockdown of miR-361-3p impairs the self-renewal and tumorigenicity liver T-ICs. Conversely, forced miR-361-3p expression enhances the self-renewal and tumorigenicity liver T-ICs. Mechanistically, miR-361-3p directly targets SOX1 via binding its 3’-UTR in liver T-ICs. Moreover, miR-361-3p knockdown hepatoma cells are more sensitive to cisplatin or sorafenib treatment. Clinical cohort analysis demonstrates that miR-361-3p low HCC patients are benefited from TACE or sorafenib treatment.

**Conclusions:** Our findings revealed the crucial role of the miR-361-3p in liver T-IC expansion and TACE or sorafenib response, rendering miR-361-3p an optimal target for the prevention and intervention in HCC.

Background

With nearly 700,000 new cases per year, hepatocellular carcinoma (HCC) ranks as the sixth most common cancer in the world (1). However, the early symptoms of HCC are not obvious. Most HCC patients are diagnosed at advanced stage (2). Due to high grade invasive HCC can develop to life threatening metastases and less than 20% patients with Portal vein thrombus-only have 2-year overall survival (3). Currently, most of drugs which used for clinical HCC patients were proved to be disappointed. So, the underlying mechanism of HCC initiation and progression needs to be deeply explored.

Tumor-initiating cells (T-ICs) or cancer stem cells (CSCs) are a subgroup of cancer cells, which have the self-renewal ability and tumorigenesis capacity (4, 5). T-ICs or CSCs were first discovered in blood system diseases (6). Later studies also confirmed the existence of T-ICs or CSCs in solid tumors, including breast cancer, liver cancer and glioma (7–9). Currently researches suggest that T-ICs or CSCs are responsible for the progression, metastasis, recurrence and chemo-resistance of cancers (10–12). Therefore, identification of the underlying mechanisms governing T-ICs or CSCs propagation may lead to the discovery of promising therapeutic strategies for cancer patients.

MicroRNAs (miRNAs) are a class of small noncoding RNA molecules that contain approximately 22 nucleotides (13). It was reported to be essential for many physiological processes, such as cellular homeostasis, development, differentiation, cell survival and death, tumor initiation and progression (14,
miR-361-3p is a newly discovered miRNA, and its function and mechanism of action in biological processes and diseases are not completely understood. Previous studies found that miR-361-3p promotes human breast cancer cell viability by inhibiting the E2F1/P73 signaling pathway (16). Moreover, miR-361-3p regulates ERK1/2-induced EMT via DUSP2 mRNA degradation in pancreatic ductal adenocarcinoma (17). However, the function of miR-361-3p in liver T-ICs is unclear.

In this study, we for first find that miR-361-3p is highly expressed in liver T-ICs. Next, by using loss-of-function analysis and gain-of-function analysis in liver T-ICs, we demonstrate that miR-361-3p promotes the self-renewal capacity and tumorigenicity of liver T-ICs. Further mechanism study reveals that miR-361-3p directly targets SOX1 in liver T-ICs. Interestingly, miR-361-3p knockdown hepatoma cells are more sensitive to cisplatin or sorafenib treatment. Clinical cohort analysis demonstrates that miR-361-3p low HCC patients are benefited from TACE or sorafenib treatment. Altogether, we discover that miR-361-3p promotes the expansion of liver T-ICs via interacting with SOX1.

Results

miR-361-3p is upregulated in liver T-ICs.

It was well accepted that CD24, CD133 and EpCAM were liver T-ICs markers (18–20). Therefore, we isolated CD24, CD133 and EpCAM positive HCC cells by flow cytometry. As expected, the expression of miR-361-3p was dramatically increased in CD24, CD133 and EpCAM positive HCC cells compared with their negative HCC cells (Figure. 1A-C). Spheroid culture of cancer cells is a routine approach to enrich T-ICs. We observed that expression of miR-361-3p was significantly upregulated in the self-renewing spheroids compared with the attached cells (Figure. 1D). In serial passages of Huh7 or HepG2 spheroids, miR-361-3p expression was gradually increased (Figure. 1E). Consistently, miR-361-3p expression was also upregulated in CD24, CD133 and EpCAM positive primary HCC cells compared with negative primary HCC cells (Figure. 1F-H). The level of miR-361-3p was increased in primary HCC spheroids compared with the attached cells (Figure. 1I). Taken together, our data demonstrated that miR-361-3p was upregulated in liver T-ICs.

miR-361-3p promotes liver T-ICs self-renew and tumorigenesis.

To evaluate the potential role of miR-361-3p in liver T-ICs, Huh7 and HepG2 cells were infected with miR-361-3p overexpression virus. The overexpression effect of miR-361-3p was determined by real-time PCR (Figure. 2A). Spheroids formation is a routine approach to assess self-renewal ability. We found that Huh7/HepG2 miR-361-3p mimic cells formed much more spheres than control cells (Figure. 2B). Moreover, the expression of liver T-ICs markers was upregulated in miR-361-3p mimic HCC cells compared with control cells (Figure. 2C). Consistently, the expression of stemness associated transcription factors was increased in miR-361-3p mimic HCC cells compared with control cells (Figure. 2D). Furthermore, the *in vitro* and *in vivo* limiting dilution assay indicated that the proportion of liver T-ICs and tumorigenic ability were enhanced in miR-361-3p mimic HCC cells (Figure. 2E-F).
miR-361-3p knockdown inhibits liver T-ICs self-renew and tumorigenesis.

To further explore the role of miR-361-3p in liver T-ICs, Huh7 and HepG2 cells were infected with miR-361-3p interference virus. The knockdown effect of miR-361-3p was determined by real-time PCR (Figure. 3A). Spheroids formation is a routine approach to assess self-renewal ability. We found that Huh7/HepG2 miR-361-3p knockdown cells formed much less spheres than control cells (Figure. 3B). Moreover, the expression of liver T-ICs markers was downregulated in miR-361-3p knockdown HCC cells compared with control cells (Figure. 3C). Consistently, the expression of stemness associated transcription factors was decreased in miR-361-3p knockdown HCC cells compared with control cells (Figure. 3D). Furthermore, the in vitro and in vivo limiting dilution assay indicated that the proportion of liver T-ICs and tumorigenic ability were impaired in miR-361-3p knockdown HCC cells (Figure. 3E–F). Collectively, our results demonstrated that miR-361-3p promoted liver T-ICs expansion.

SOX1 is required in miR-361-3p-mediated liver T-ICs expansion.

To elucidate mechanism underlying miR-361-3p-mediated liver T-ICs expansion, TargetScan and miRbase were used to predicted the potential targets of miR-361-3p in liver T-ICs. Bioinformatics analysis found that miR-361-3p has a putative binding site in SOX1 mRNA 3’-UTR (Figure. 4A). As expected, both the mRNA and protein expression of SOX1 were upregulated in miR-361-3p knockdown HCC cells (Figure. 4B–C). Luciferase reporter assays with a vector that included the wild-type (WT) 3’-UTR or mutant-type (MUT) 3’-UTR of SOX1 were then performed to determine whether miR-361-3p could directly regulate SOX1. The results showed that miR-361-3p knockdown significantly enhanced the relative luciferase activity compared with control group. However, such effects were diminished when the predicted binding site was mutated (Figure. 4D). These results indicated that miR-361-3p directly suppressed SOX1 expression by binding to its 3’-UTR. In addition, a significant negative correlation was identified between SOX1 and miR-361-3p expression in clinical samples of HCC ($R^2 = 0.734$, $P < 0.05$, $n = 50$) (Figure. 4E). To further confirm whether SOX1 was required for miR-361-3p mediated liver T-ICs expansion, the special SOX1 siRNA was used (Figure. 4F). SOX1 siRNA abrogated the self-renewal ability and liver T-ICs frequency between miR-361-3p knockdown HCC cells and control cells (Figure. 4G–H).

miR-361-3p knockdown HCC cells are sensitive to cisplatin and sorafenib treatment.

We next explored whether miR-361-3p was involved in the regulation of chemo-resistance of HCC. As expected, miR-361-3p expression was markedly upregulated in cisplatin-resistance or sorafenib-resistance xenograft (Figure. 5A). Consistently, miR-361-3p expression was also significantly increased in cisplatin-resistance or sorafenib-resistance HCC cell lines (Figure. 5B–C). Moreover, the sensitivity of cisplatin or sorafenib was increased in miR-361-3p knockdown HCC cells compared with control HCC cells (Figure. 5D). We also observed that interference of miR-361-3p sensitized HCC cells to undergo cisplatin-induced or sorafenib-induced cell apoptosis (Figure. 5E–H). Furthermore, Kaplan-Meier analysis revealed the survival benefits in adjuvant TACE-treated or sorafenib-treated HCC patients with low miR-361-3p levels (Figure. 5I–J).
Discussion

Hepatocellular carcinoma (HCC) is the sixth most common cancer in the world, accounting for 90% of human liver cancer (21). The incidence of liver cancer is rising due to various factors such as hepatitis, alcoholic fatty liver, nonalcoholic fatty liver and aflatoxin (22, 23). Hepatectomy and liver transplantation are commonly used in patients with early stage HCC (24). However, most patients with advanced HCC are contradicting for surgery, and the survival benefit of TACE or targeted drug sorafenib is limited (25). Therefore, it is necessary to further clarify the development of HCC. In this study, we confirmed for the first time that miR-361-3p was highly expressed in liver T-ICs, which enhanced liver T-ICs self-renewal and tumorigenesis ability. Our clinical data showed that miR-361-3p could be used to predict TACE and sorafenib response in HCC patients.

MiRNAs, a small non-coding RNA molecule (containing about 22 nucleotides), regulates RNA silencing and post-transcriptional of gene expression (26). miRNAs act as oncogenes or tumor suppressors in tumors dependent on special conditions. miR-361-3p has been reported upregulated in various human tumors, including breast cancer, oral squamous cancer and pancreatic cancer (16, 17, 27). However, miR-361-3p functions in liver T-ICs have never been investigated before. In current studies, we found that miR-361-3p was upregulated in liver T-ICs. Moreover, we found that miR-361-3p knockdown inhibited liver T-ICs self-renew and tumorigenesis. Conversely, forced miR-361-3p expression promoted liver T-ICs self-renew and tumorigenesis.

SOX1, belonging to SRY (sex determining region Y)-box (SOX) family proteins, is evolutionarily conserved in many species and participated as a key regulator of neural cell fate determination and differentiation (28, 29). Previous studies have demonstrated that SOX1 plays an essential role in liver cancer progression (30). In addition, SOX1 was also reported to be involved in the regulation of cancer stemness (31). We hereby found that miR-361-3p knockdown increased SOX1 mRNA and protein expression in liver T-ICs. Moreover, we found that miR-361-3p directly regulates SOX1 expression via interaction with its 3’ UTR. In addition, a significant negative correlation was identified between SOX1 and miR-361-3p expression in clinical samples of HCC. SOX1 siRNA could diminish the self-renewal ability and liver T-ICs frequency between miR-361-3p knockdown HCC cells and control cells.

For advanced HCC patients, TACE (transcatheter arterial chemoembolization) and the targeted drug sorafenib are the major means of treatment for a long time. However, only a small proportion of patients respond to sorafenib or TACE, and the majority of patients not only have no curative effect, but also have serious side effects (32, 33). Therefore, elucidating the mechanism of TACE and sorafenib resistance has become an important link to prolong the survival time of HCC patients. Due to the different responses to sorafenib and TACE in different HCC patients, how to select biomarkers to predict drug reactivity has become a key issue to improve the clinical efficacy of sorafenib and TACE. In the present study, our finding revealed that miR-361-3p knockdown of HCC cells is more sensitive to cisplatin or sorafenib treatment. The TACE or sorafenib cohort analysis further indicated that a low miR-361-3p level in HCC patients can serve as a reliable predictor for TACE or sorafenib response.
Conclusion

We demonstrate that miR-361-3p is upregulated in liver T-ICs, which in turn promotes the self-renewal and tumorigenicity of liver T-ICs. In addition, miR-361-3p promotes liver T-ICs expansion through directly regulating SOX-1. Moreover, miR-361-3p knockdown HCC cells are more sensitive to cisplatin or sorafenib treatment. These data provide insight into the miR-361-3p as a potential therapeutic target against liver T-ICs and a potential predictor for TACE or sorafenib treatment of HCC patients.

Materials And Methods

Cell Culture.

Huh7 and HepG2 cells were maintained at 37 °C in 5% CO\textsubscript{2} incubator with Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 25 µg/ml of gentamicin. FBS was purchased from Nova-Tech (Grand Island, NE). miR-361-3p mimic virus or miR-361-3p sponge virus was purchased from RIBOBIO (Guangzhou, China). siRNA SOX1 was purchased from Genechem (Shanghai, China).

Patients and specimens

HCC tissues were obtained from patients who underwent liver resection in the Eastern Hepatobiliary Surgery Hospital (EHBH). Samples were frozen in liquid nitrogen immediately after surgical resection for further RNA extraction. A total of 70 patients received TACE therapy after surgery for primary HCC at EHBH from 2010–2015 were included in Cohort 1. Detailed clinicopathological features of these patients are described in supplementary Table 1. A total of 68 patients received adjuvant sorafenib therapy after surgery for primary HCC at EHBH from 2011–2015 were included in Cohort 2. Detailed clinicopathological features of these patients are described in supplementary Table 2. Another group of 50 HCC specimens were used for analyzing the correlation between SOX1 and miR-361-3p expression. Patient informed consent was obtained and the procedure of human sample collection was approved by the Ethics Committee of EHBH.

Reverse transcription polymerase chain reaction (RT-PCR).

Total RNA was extracted from the cells using Trizol reagent (Invitrogen, 15596-018). Total cDNAs were synthesized by ThermoScript TM RT-PCR system (Invitrogen, 11146-057). The mRNA amount presented in the cells was measured by semi quantitative RT-PCR. PCR conditions included 1 cycle at 94 °C for 5 minutes, followed by up to 40 cycles of 94 °C for 15 seconds (denaturation), 60 °C for 30 seconds (annealing) and 72 °C for 30 seconds (extension). The sequences of primers used was listed in Supplementary Table 3.

Luciferase reporter assay.
A 300-bp fragment of the SOX1 3’UTR containing the conserved miR-361-3p-binding sites was inserted into a luciferase reporter plasmid. The SOX1 3’UTR mutant luciferase plasmid contained changes in potential miR-361-3p-binding base sequence “CUGGGGG” to “GACUCUA”. HCC cells were transfected with SOX1 3’UTR luciferase reporter in combination with the pRL-TK vector (Promega, E2241) as an internal control. The dual luciferase assay kit was purchased from Promega (0000060417). The luciferase activities were determined using a luminometer (Wallac 1420 Victor 2 multilabel counter system) as described in previous studies (34).

Western Blotting Assay.

The cells collected by cell lysis buffer, then disposed like we described before (35). Equal aliquots of cell extracts were separated on SDS-polyacrylamide gels. The proteins were then transferred to PVDF membranes (Bio-Rad), blocked, and probed with the antibodies. Primary antibody-bound proteins were detected by using an alkaline phosphatase-linked secondary antibody and an ECF Western Blotting system (Amersham). The densitometric analyses of the protein bands vs. the individual loading controls were performed using the ImageQuant 5.2 software (GE Healthcare). The primary antibodies used were listed in supplementary Table 4. The results shown were representative one of three independent experiments.

Spheroid formation assay

HCC cells were digested and then cultured in a 96-well ultra-low attachment (300 cells per well) and cultured in DMEM/F12 (Gibco) media, supplemented with 1% FBS, 20 ng/mL bFGF and 20 ng/mL EGF for 7 days. The total number of spheres was counted under the microscope (Olympus).

In vitro limiting dilution assay

Various numbers of Huh7/HepG2 miR-361-3p mimic or miR-361-3p sponge and their control cells were digested and seeded into 96-well ultra-low attachment (2, 4, 8, 16, 32, 64 cells per well, n = 8) and cultured in DMEM/F12 (Gibco) supplemented with 1% FBS, 20 ng/mL bFGF and 20 ng/mL EGF for seven days. The CSC proportions were analyzed using Poisson distribution statistics and the L-Calc Version 1.1 software program (Stem Cell Technologies, Inc., Vancouver, Canada) as previously described (36).

In vivo limiting dilution.

For the in vivo limiting dilution assay, Huh7 miR-361-3p mimic or Huh7 miR-361-3p sponge and their control HCC cells digested and then mixed with Matrigel (BD) at a ratio of 1:1 and injected subcutaneously at indicated cell doses (1 × 10^3, 5 × 10^3, 1 × 10^4, 5 × 10^4) per NOD-SICD mouse (n = 6). After two months, tumors formation was evaluated.

Apoptosis Assay.

Huh7 miR-361-3p sponge and control cells were treated with cisplatin (10 µg/ml) or sorafenib (10 µM) for 48 hours, followed by staining with Annexin V and 7-AAD for 15 minutes at room temperature in the dark.
Apoptotic cells were determined by an Annexin V FITC Apoptosis Detection Kit I (BD Pharmingen, San Diego, CA) and detected by flow cytometry according to the manufacturer’s instructions.

**Statistical Analysis.**

GraphPad Prism (GraphPad Software, Inc. La Jolla, USA) was used for all statistical analyses. Statistical analysis was carried out using t test or Bonferroni Multiple Comparisons Test: *p < 0.05. A p value of less than 0.05 was considered significant.

**Abbreviations**

HCC
Hepatocellular carcinoma; miRNA: microRNAs; DMEM: Dulbecco's modified Eagle's medium; FBS: fetal bovine serum; T-ICs: tumor-initiating cells; CSCs: cancer stem cells; EpCAM: epithelial cell adhesion molecule; CD24: cluster of differentiation 24; CD133: cluster of differentiation 133; SOX: SRY (sex determining region Y)-box; TACE: transcatheter arterial chemoembolization.

**Declarations**

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no.

**Authors’ contributions**

DW, JZ and SPQ conceived and designed the experiments. SPQ, XBZ and HWF analyzed and interpreted the results of the experiments. SPQ, XBZ and YW performed the experiments. All authors read and approved the final manuscript.

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**Availability of data and materials**

All data generated or analyzed during this study are included in this published article.

**Ethics approval and consent to participate**

This study protocol was approved by the Ethics Committee of the Eastern Hepatobiliary Surgery Hospital.

**Consent for publication**

Not applicable.

**Competing interests**
The authors declare that they have no competing interests, and all authors should confirm its accuracy.

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Figures
miR-361-3p was upregulated in liver T-ICs. A. The expression of miR-361-3p in CD24+ and CD24- HCC cells was assessed by real-time PCR assay. B. The expression of miR-361-3p in CD133+ and CD133- HCC cells was detected by real-time PCR assay. C. The expression of miR-361-3p in EpCAM+ and EpCAM- HCC cells was checked by real-time PCR assay. D. The expression of miR-361-3p in hepatoma spheroids was examined by real-time PCR assay. E. miR-361-3p expression in serial passages of hepatoma spheroids
was analyzed by real-time PCR. F. The expression of miR-361-3p in CD24+ and CD24- primary HCC cells was assessed by real-time PCR assay. G. The expression of miR-361-3p in CD133+ and CD133- primary HCC cells was detected by real-time PCR assay. H. The expression of miR-361-3p in EpCAM+ and EpCAM- primary HCC cells was checked by real-time PCR assay. I. The expression of miR-361-3p in primary hepatoma spheroids was examined by real-time PCR assay.

**Figure 2**

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| cells Injected | Huh7 Control | Huh7 miR-361-3p mimic |
|----------------|--------------|-----------------------|
| $1 \times 10^6$ | 2/6          | 5/6                   |
| $5 \times 10^6$ | 5/6          | 3/6                   |
| $1 \times 10^7$ | 3/6          | 5/6                   |
| $5 \times 10^7$ | 4/6          | 6/6                   |
| Tumor incidence | 18/24        | 18/24                 |

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miR-361-3p promoted liver T-ICs self-renew and tumorigenesis. A. Huh7 and HepG2 cells were transfected with miR-361-3p mimic virus. The RNA was extracted and identified by real-time PCR. B. Representative images of hepatoma spheroids generated from Huh7/HepG2 miR-361-3p mimic and control cells. The number of spheroids was counted and compared. C. Flow cytometry analysis of CD24+ populations in spheroids derived from Huh7/HepG2 miR-361-3p mimic and control cells. Representative results from three independent experiments were shown. D. The expression of transcription factors (SOX2, OCT4 and c-Myc) in Huh7/HepG2 miR-361-3p mimic and control cells were detected by real-time PCR. E. The in vitro limiting dilution assay was used to check the population of liver T-ICs in Huh7/HepG2 miR-361-3p mimic and control cells. F. The in vivo limiting dilution assay was used to determine the tumorigenicity and liver T-ICs frequency.
miR-361-3p knockdown inhibited liver T-ICs self-renew and tumorigenesis. A. Huh7 and HepG2 cells were transfected with miR-361-3p sponge virus. The RNA was extracted and identified by real-time PCR. B. Representative images of hepatoma spheroids generated from Huh7/HepG2 miR-361-3p sponge and control cells. The number of spheroids was counted and compared. C. Flow cytometry analysis of CD24+ populations in spheroids derived from Huh7/HepG2 miR-361-3p sponge and control cells. Representative
results from three independent experiments were shown. D. The expression of transcription factors (SOX2, OCT4 and c-Myc) in Huh7/HepG2 miR-361-3p sponge and control cells were detected by real-time PCR. E. The in vitro limiting dilution assay was used to check the population of liver T-ICs in Huh7/HepG2 miR-361-3p sponge and control cells. F. The in vivo limiting dilution assay was used to determine the tumorigenicity and liver T-ICs frequency.

Figure 4

A

B

C

D

E

F

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miR-361-3p directly targeted SOX1 in liver T-ICs. A. Schematic diagram of luciferase plasmids of SOX1 3’-UTR wild-type (WT) and SXO1 3’-UTR mutant (MUT). B. The SOX1 mRNA expression in Huh7/HepG2 miR-361-3p sponge and control cells were detected by real-time PCR. C. The SOX1 protein expression in Huh7/HepG2 miR-361-3p sponge and control cells were detected by western blot. D. HCC cells were stably transfected with SOX1 3’-UTR wild-type (WT) and SXO1 3’-UTR mutant (MUT) luciferase reporter for 24 hours. The luciferase activity was measured as described in “materials and methods”. E. A negative correlation was observed between miR-361-3p and SOX1 expression in 50 clinical samples of HCC (R² = 0.734, P < 0.05, by Spearman’s correlation analysis.) F. Huh7/HepG2 miR-361-3p sponge and control cells were transfected with siRNA SOX1 or its vector control. The stable transfectants were identified by Western Blotting. G. Huh7/HepG2 miR-361-3p sponge and control cells transfected with shRNA SOX1 and then subjected to spheroids formation assay. H. Huh7/HepG2 miR-361-3p sponge and control cells transfected with shRNA SOX1 and then subjected to in vitro limiting dilution assay.
miR-361-3p knockdown HCC cells were sensitive to cisplatin and sorafenib treatment. A. The expression of miR-361-3p in cisplatin-resistant or sorafenib-resistant xenograft was measured by real-time PCR. B. The expression of miR-361-3p in cisplatin-resistant HCC cell lines was measured by real-time PCR. C. The expression of miR-361-3p in sorafenib-resistant HCC cell lines was measured by real-time PCR. D. Cell survival curves of Huh7 miR-361-3p sponge and control cells treated with cisplatin or sorafenib. E. Huh7
miR-361-3p sponge and control cells were treated with cisplatin (4 μg/ml) for 48 hours. The apoptotic cells were detected by flow cytometry. F. Huh7 miR-361-3p sponge and control cells were treated with sorafenib (10 μM) for 48 hours. The apoptotic cells were detected by flow cytometry. G. Huh7 miR-361-3p sponge and control cells were treated with cisplatin (4 μg/ml) for 48 hours. The cell extracts were subjected to western blotting with special antibody against PARP. H. Huh7 miR-361-3p sponge and control cells were treated with sorafenib (10 μM) for 48 hours. The cell extracts were subjected to western blotting with special antibody against PARP. I. The total of 70 HCC patients (Cohort 1) were divided into low miR-361-3p group (n = 35) and high miR-361-3p group (n = 35), and the overall survival of the patients in the two groups were compared. J. The total of 68 HCC patients (Cohort 2) were divided into low miR-361-3p group (n = 34) and high miR-361-3p group (n = 34), and the overall survival of the patients in the two groups were compared.

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