MiR-27a-3p enhances the cisplatin sensitivity in hepatocellular carcinoma cells through inhibiting PI3K/Akt pathway

Ying Yang, Zhifang Yang, Ruili Zhang, Chunli Jia, Rui Mao, Sha Ya, Yuefen Zhang, Ge Wu, Yan na Sun, Xiao yan Jia, Ainiwaer Aimudula, Hua Zhang§ and Yongxing Bao§

Department of Cancer Center, the First Affiliated Hospital of Xinjiang Medical University, Urumqi, China

§Corresponding author

Email addresses:
Ying Yang: 297215484@qq.com
Yongxing Bao: baoyx@vip.sina.com
Hua Zhang: 657015630@qq.com
Abstract

MicroRNAs (miRNAs) play an important role in drug-resistance, and it’s reported that MiR-27a-3p regulated the sensitivity of cisplatin in breast cancer, lung cancer and ovarian cancer. However, the relationship between miR-27a-3p and chemosensitivity of cisplatin in HCC was unclear, especially the underlying mechanism was unknown. In present study, we analyzed miR-27a-3p expression levels in 372 tumor tissues and 49 adjacent tissues in HCC samples from TCGA database, and found that the miR-27a-3p was downregulated in HCC tissues. The level of miR-27a-3p was associated with metastasis, Child-Pugh grade and race. MiR-27a-3p was regarded as a favorable prognosis indicator for HCC patients. Then, miR-27a-3p was overexpressed in HepG2 cell, and was knockdown in PLC cell. Next, we conducted a series of vitro assays, including MTT, apoptosis and cell cycle assays to observe the biological changes. Further, inhibitor rate and apoptosis rate were detected with pre- and post-cisplatin treatment in HCC. The results showed that overexpression of miR-27a-3p repressed the cell viability, promoted apoptosis and increased the percentage of cells in phase G0/G1 phase. Importantly, overexpression of miR-27a-3p significantly increased the inhibitor rate and apoptosis rate with cisplatin intervention. Besides, we found that miR-27a-3p added cisplatin sensitivity potentially through regulating PI3K/Akt signaling pathway. Taken together, MiR-27a-3p acted as a tumor suppressor gene in HCC cells, and it could be useful for modulating cisplatin sensitivity in chemotherapy therapy.
1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common and highly lethal malignant tumors of digestive system worldwide [1]. The 5-year overall survival rate is merely 12% for most of patients who are already in the advanced stage of HCC at the time of diagnosis [2]. Therefore, systematic treatments are recommended by experts of in the HCC guidelines. These options including targeted drugs of Sorafenib and Lenvima, as well as systematic chemotherapy. However, the treatment effect of the chemotherapy was generally unsatisfactory [3-4]. Several cytotoxic agents, including cisplatin, doxorubicin and 5-florouracil (5-FU) have showed multiple drug resistance which limit their therapeutic efficacy [5]. Therefore, it is urgent to explore the molecular targets to improve the sensitivity of these cytotoxic drugs in HCC.

MicroRNAs (miRNAs) refer to a group of small and non-coding RNAs, which are 22 nucleotides in length. Its main function is to regulate gene expression at translation level. Recently, it is reported aberrant expression of miRNAs can modulate cell growth, apoptosis, as well as tumorigenesis [6]. Besides, MiRNAs can also make contributions to the chemosensitivity in HCC [7-8]. However, the underlying molecular mechanisms of chemosensitivity have not been clarified.

MiR-27a-3p is located on chromosome 19 (19p13.1), which is expressed in multiple malignant tumors, such as renal carcinoma, oral squamous cell carcinoma and pancreatic cancer and so on [9-12]. In addition, it is reported that miR-27a-3p acts as a vital role in invasion, metastasis, and epithelial-mesenchymal transition in HCC [13]. Furthermore, MiR-27a-3p also regulated the sensitivity of cisplatin in breast cancer, lung cancer and ovarian cancer [14-16]. Nevertheless, the relationship between miR-27a-3p and chemosensitivity of cisplatin in HCC is unknown, and its underlying mechanism needs to be explored.

Thus, in this study, we intended to assess the effect of miR-27a-3p in cisplatin treatment of HCC, and try to identify its mechanism. We found that miR-27a-3p was an indicator of favorable prognosis in HCC patients. Besides, in vitro assays, up regulation of miR-27a-3p decreased the cell viability, promoted the apoptosis, and
blocked cells in G0/G1 phase. Importantly, overexpression of miR-27a-3p markedly increased the inhibitor rate and apoptosis rate when cisplatin was added in HCC cells.

In contrast, knockdown of miR-27a-3p significantly showed an opposite trend. In addition, western blot revealed that miR-27a-3p plus cisplatin revealed weaker expressions of PI3K and p-Akt and stronger level of C-caspase-3. Thus, PI3K/Akt pathway probably mediated this process. Hence, miR-27a-3p added cisplatin sensitivity potentially through regulating PI3K/Akt signaling pathway.

2. Materials and methods

2.1. TCGA data analysis

The online accessible TCGA data portal (https://tcga-data.nci.nih.gov/tcga/) was used. We mainly focused on miR-27a-3p expression and clinical data of HCC patients, including age, sex, race, TNM stage, grade, and Child-Pugh stage. All values were collected and analyzed from 372 HCC patients. MiR-27a-3p expression was quantified using RSEM based on the TCGA methods. The upper quartile data is normalized according to the TCGA normalization protocol.

2.2. Cell culture

Hepatoma cell lines (HepG2, Huh-7, PLC) and the human normal liver cell line LO2 were purchased from the Cell Bank of the Chinese Academy of Science (Shanghai, China). All cell lines were cultured in RPMI 1640 medium (Life Technologies, USA), supplemented with 10% fetal bovine serum (Life Technologies, USA) and cultured in a humidified atmosphere containing 5% CO2 at 37 °C.

2.3. Reagents

The miExpress™ Precursor miRNA Expression (Lot No.21895-1), inhibitor Expression (Lot No.B302) Clone of miR-27a-3p and control were purchased from GenePharma Company (Shanghai, China). The following antibodies used in the study: anti-PI3K, anti-Akt, anti-p-Akt, anti-C-caspase were obtained from Cell Signaling Technology (Beverly, MA). The PI3K/p-Akt signaling inhibitor LY49002 was purchased from Apicent Biological Technology Company (Shanghai, China). β-actin was purchased from Bioworld Technology (CA, USA).

2.4. Cell transfection
Before transfection, a total of $1.5 \times 10^5$ HCC cells were seeded into 6-well plates 24h. Lipofectamine 3000 (Invitrogen, USA) was used for the transient transfection according to the manufacturer’s instructions. HepG2 cells were chosen for miR-27a-3p overexpression by plasmid transfection and PLC cells for miR-27a-3p knockdown using siRNA transient transfection. Cells were divided into four groups, miR-27a-3p overexpression and control were referred to miR-27a and miR-Con respectively; miR-27a-3p inhibitor and inhibitor control were named as miR-inhibitor-27a and miR-inhibitor-Con respectively. The expression levels of miRNAs were confirmed by qRT-PCR assay.

2.5. Cell viability and proliferation assay

Twenty-four hours after cell transfection, cell viability was identified by 3-(4,5-Dimethylthiazol-2-yl)-2,5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl) -2H-tetrazolium assay (MTS, Promega, USA). HepG2 cells and PLC cells were triplicate plated in a 96-well plate at the density of $5 \times 10^3$/well and incubated overnight. Then, the cells were treated with different concentrations of cisplatin 0 µg/ml, 3 µg/ml, 6 µg/ml, 9 µg/ml, 12 µg/ml for 48h, respectively. Subsequently, the MTS reagent (20 µl) was added to each well, followed by incubation at 37°C in 5% CO₂ atmosphere for 4h. Lastly, the absorbance was read by using a Synergy 2 (BioTek, USA) plate reader.

2.6. Cell apoptosis and cell cycle analysis by FACS

Annexin V/propidium iodide (Av/PI) staining (Beyotime Biotechnology, China) was analyzed by flow cytometry. Cells were collected and washed twice with phosphate-buffered saline (PBS), followed by resuspension in 250 µl binding buffer. 5 µl FITC–annexin V and 10 µl PI (20 µg/ml) were added to each 100 µl cell suspension, and then the cells were incubated at room temperature for 15 min. Subsequently, 400 µl PBS was added to the cell suspensions, and the samples were detected by flow cytometry (Becton-Dickinson, USA). The percentage of the cells in different phases was counted and compared.

For cell cycle analysis, cells were cultured in serum-free medium for 24h to induce cell cycle synchronization. Cells were harvested at different time points. For DNA content analysis, cells were fixed in 70 % ethanol, rehydrated in PBS, treated
with RNase A (10 mg/ml) for 30 min, then stained with propidium iodide (10 µg/ml) for 5 min. The percentage of cells in the S, G0/G1, and G2/M phases were counted and compared.

2.7. Western blot

Cells were harvested and washed twice with phosphate-buffered saline (PBS, HyClone, Logan, UT). Total protein was extracted using RIPA cell lysis buffer (Beyotime Biotechnology, China). Protein concentration was determined by the bicinchoninic acid protein assay (Pierce, USA), and proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blotted onto polyvinylidene difluoride membranes (PVDF, Millipore, MA). The membranes were incubated with primary antibodies in blocking buffer overnight at 4 °C. The membrane was washed three times for 5 min each time with washing buffer and incubated with secondary antibodies (Invitrogen, USA) for 1.5 h at room temperature. The proteins were visualized with the Western Breeze Kit (WB7105, Invitrogen, USA) and analyzed with Quantity One software (Bio-Rad Laboratories, USA).

2.8. Statistical analysis

All statistical analysis was performed using SPSS software, version 17.0 (SPSS, Chicago, USA). The results are expressed as the mean ± standard deviation (SD). The data was compared among groups by one-way analysis of variance followed by Bonferroni correction. Each experiment was done independently at least three times. P value<0.05 was considered as statistically significant difference.

3. Results

3.1. MiR-27a-3p was downregulated in tumor tissue in HCC patients.

To explore the clinical significance of miR-27a-3p in HCC patients, we downloaded the clinical data and miR-27a-3p expression in TCGA data set. A total of 372 tumor tissues and 49 adjacent normal tissues were involved. Results showed that miR-27a-3p was significantly low expressed in tumor tissue (Fig. 1A). And Kaplan-Meier survival curve revealed that high level of miR-27a-3p significantly
correlated with better overall survival in HCC patients (Fig. 1B). Moreover, miR-27a-3p expression was associated with metastasis, Child-Pugh grade and race (Fig. 1C-E), but not with the T stage, N stage, and differentiation grade. The correlation between miR-27a-3p level and clinicopathological features in HCC patients was shown in Table 1 and 2. Taken together, these results reflected that miR-27a-3p level was an indicator for favorable prognosis of HCC patients.

3.2. MiR-27a-3p acted as a tumor suppressor gene in HCC

As miR-27a-3p was low expressed in HCC tumor tissue, we speculated that miR-27a-3p may function as a tumor suppressor gene. Thus, we conducted a series of vitro assays to explore its biological function. First, we detected the expression of miR-27a-3p in several hepatoma cell lines (HepG2, Huh-7 and PLC) and one normal liver cell line (L02). RT-qPCR results showed that miR-27a-3p expressions were differed in these cells. HepG2 cells had a relative low level whereas PLC cells had a relative high expression (Fig 1F). Therefore, HepG2 cells were chosen for miR-27a-3p overexpression and PLC cells for miR-27a-3p knockdown (Fig 1G-I).

Second, MTT assay showed that high expression of miR-27a-3p impaired the cell viability in HepG2, whereas low level of miR-27a-3p added viability in PLC (Fig. 2A-B). Besides, flow cytometry showed that overexpression of miR-27a-3p increased the apoptosis rate compared with miR-Con group in HepG2. In contrast, knockdown of miR-27a-3p significantly reduced the apoptosis rate in PLC (Fig. 2C-D). In addition, cell cycle analysis indicated that the overexpression of miR-27a-3p led to an increase in G0/G1 phase and a decrease in S phase in HepG2, while knockdown of miR-27a-3p resulted in a reverse trend in PLC (Fig. 2E-F).

For miR-27a-3p has the function of inhibiting cell viability, inducing cell apoptosis, as well as affecting the cell cycle progression, it suggests that miR-27a-3p play a key role in tumor suppression in the development of HCC.

3.3 MiR-27a-3p enhanced the cisplatin sensitivity of HCC cells.

To explore whether miR-27a-3p could affect the chemosensitivity of cisplatin in HCC, we treated HepG2 and PLC cells with different concentrations of cisplatin (0, 3,
6, 9 and 12 µg/ml) and examined the effects of miR-27a-3p on cisplatin treatment. Firstly, we observed that the cell inhibition rates were gradually increased with the elevated concentration of cisplatin in blank group in both HepG2 and PLC. It indicated that the killing effect of cisplatin (Fig.3A-3B). Interestingly, with cisplatin stimulation, MTT assay showed that overexpression of miR-27a-3p had the higher inhibition rate than that in miR-Con group in HepG2. On the contrary, knockdown of miR-27a-3p significantly decreased the inhibition rate compared with miR-inhibitor-Con group in PLC (Fig.3A-3B).

Subsequently, we selected a moderate concentration of cisplatin (3 µg/ml DDP) for further intervention. As revealed in Fig.3C-3D, cisplatin had the effects of inducing apoptosis in blank +DDP group in both HepG2 and PLC. When miR-27a-3p was overexpressed, the apoptosis rate in miR-27a +DDP group (31.8%) was higher than miR-Con +DDP group (13.3%, \(P<0.05\)). When miR-27a-3p was knockdown, the apoptosis rate in miR-inhibitor-27a+DDP group (6.3%) was lower than miR-inhibitor-Con+DDP group (16.3%, \(P<0.05\)). Collectively, we found that more inhibition rate and more apoptosis rate exhibited when miR-27a-3p was overexpressed in treatment of cisplatin in HCC cells. Thus, it implies that high level of miR-27a-3p obviously enhances cisplatin sensitivity in HCC cells.

### 3.4 MiR-27a-3p added the cisplatin sensitivity potentially by inhibiting PI3K/Akt pathway

In order to show the potential mechanism by which miR-27a-3p exerted anti-cancer effects in HCC cells, the signaling pathway proteins were assessed. Firstly, as revealed in Fig.4A, compared with miR-Con group, overexpression of miR-27a-3p inhibited PI3K/p-Akt signaling and elevated C-caspase-3 in HepG2. Nevertheless, PLC cells exhibited an opposite trend, compared with miR-inhibitor-Con group, silencing of miR-27a-3p activated the PI3K/p-Akt signaling and resulted in reduced C-caspase-3 level (Fig.4B). In addition, it is known that cisplatin has anti-cancer effect. And DDP group exhibited the suppression of PI3K/p-Akt pathway and up regulation of C-caspase-3. Interestingly, miR-27a+DDP group showed the identical
effect, and which was more obvious than that in DDP group in HepG2. It indicated that overexpression of miR-27a-3p facilitated the anti-cancer effect of cisplatin. However, the miR-inhibitor-27a+DDP group revealed stronger expressions of PI3K and p-Akt protein and weaker level of C-caspase-3, compared with DDP group in PLC. Silencing of miR-27a-3p attenuated the cisplatin effect.

Besides, the PI3K/Akt pathway inhibitors LY49002 was used, we observed a significant suppression of PI3K/ Akt signaling and an increase expression of C-caspase-3 (Fig.4C). Whereas knockdown of miR-27a-3p, the above trend was attenuated in miR-inhibitor-27a group, compared with miR-inhibitor-Con group. This suggests that PI3K/Akt signaling is the main pathway affecting cisplatin sensitivity. And miR-27a-3p adds its sensitivity potentially through the inhibition of PI3K/Akt signaling.

4. Discussion

In this study, clinically we mainly found that miR-27a-3p was downregulated in HCC tissue and could be regarded as an indicator for favorable prognosis of HCC patients. And in vitro assays, consistent with current studies, we noticed that miR-27a-3p acted as a tumor suppressor gene in HCC. For it had the functions of inhibiting cell viability, inducing the apoptosis and affecting the cell cycle. However, whether miR-27a-3p contributed to the sensitivity of cisplatin was unclear. Importantly, for the first time, we reported that overexpression of miR-27a-3p significantly increased the inhibition rate and apoptosis rate with cisplatin treatment in HCC cells. It suggests that miR-27a-3p play a vital role in regulating cisplatin sensitivity. Besides, we preliminarily found that miR-27a-3p added cisplatin sensitivity potentially through regulating PI3K/Akt signaling pathway. Our data highlights a novel molecular target for chemotherapy of HCC.

In clinical samples, the TCGA analysis showed that miR-27a-3p was downregulated in HCC tissues, and was associated with metastasis, Child-Pugh Grade and race. HCC patients with a high level of miR-27a-3p had better prognosis than those with a low level. This was consistent with Zhao N’s findings [13]. He detected
the level of miR-27a by qRT-PCR in 42 cases of HCC tissues and 35 cases of adjacent tissues. Identically, they also found that miR-27a was significantly lower in HCC tissues and it could be considered as an indicator for favorable prognosis in HCC patients.

In vitro assays, we firstly verified its basic biological function in HCC cells. We demonstrated that overexpression of miR-27a-3p inhibited cell viability, promoted apoptosis and blocked the cell cycle in G0/G1 phase. It means that miR-27a-3p plays a key role in tumorigenesis of HCC. However, in other types of cancer, miR-27a-3p exerted different functions. In gastric cancer, Liu T et al reported that miR-27a worked as an oncogene of promoting cell growth [17]. And in nasopharyngeal carcinoma, Li L et al found that miR-27a-3p had the function of promoting cell proliferation, facilitating migration and invasion by targeting Mapk10 protein[18]. Besides, Mertens-Talcott SU et al observed that the oncogenic role of miR-27a ,which increased the percentage of breast cancer cells in G2-M phase by inducing target gene Myt-1[19]. The above evidence reflects that miR-27a-3p acts different roles in different types of cancer.

Current researches have reported that some miRNAs could regulate the sensitivity of chemotherapy drug cisplatin in multiple types of cancer, including HCC. For examples, Let-7 affected the sensitivity of cisplatin by IL-6/STAT3 pathway in esophageal cancer [20]. And miR-214 led to the cell survival and cisplatin resistance by targeting PTEN in ovarian cancer [21]. What’s more? Li J et al observed that up regulation of miR-27a contributed to the chemoresistance of cisplatin by suppressing RKIP expression in lung cancer cells [15]. And in HCC, some studies have confirmed that several specific miRNAs affect the chemosensitivity of cisplatin in HCC, which includes miR-33a, miR-34a-5p, miR-133a, miR-193b and so on [22-25]. However, the relationship between miR-27a-3p and cisplatin sensitivity in HCC was unknown.

Thus, we aimed to explore whether miR-27a-3p could affect the sensitivity of cisplatin. We treated HCC cells with different concentrations of cisplatin. An interesting phenomenon emerged, the cell viability was obviously inhibited and the
apoptosis rate was significantly added when miR-27a-3p was overexpressed with cisplatin intervention in HepG2. Yet, the reverse trend was noticed in PLC cells when miR-27a-3p was knocked down. Collectively, it proved that high level of miR-27a-3p sensitized the effect of cisplatin by suppressing cell viability and inducing cell apoptosis. As we know that multi-drug resistance (MDR) is the main problem that limits the therapeutic efficiency of chemotherapy drug. And P-gp protein and its encoded gene MDR1 have been verified to play a vital role in drug resistance. Li Z et al found that miR-27a may be involved in drug resistance by regulating MDR1/P-gp protein expression in ovarian cancer cells [16].

It is reported that a variety of extracellular stimuli such as insulin and chemotherapy drugs can activate signal pathways, apart from the Smad-dependent signaling, Smad-independent signaling, including the PI3K/Akt, JAK/STAT3 and MAPK pathways [26]. These pathways exert their functions by signal cascade amplification[27]. And among them, PI3K/Akt signaling plays a critical role in affecting the sensitivity of chemotherapy drug[28]. The apoptosis rate detection is one of important methods to determine the drug sensitivity. The apoptosis pathway is mainly mediated by the caspase family protein, which determines whether the cell continues to survive or die through the internal or mitochondrial apoptosis pathway[29].

We all know that cisplatin has anti-cancer effect. In our study, DDP resulted in down regulation of PI3K, p-Akt proteins and up regulation of C-caspase-3 level. Notably, miR-27a+DDP showed more obvious trend than that in DDP alone, it implies that cisplatin inhibits the PI3K/Akt signaling and induces cell apoptosis, and miR-27a-3p increases the cytotoxicity of cisplatin. In contrast, when miR-27a-3p was knockdowned, it showed the activation of PI3K/Akt signaling and suppression of C-caspase-3. It indicates that silencing of miR-27a-3p attenuates the cisplatin effect by affecting PI3K/Akt signaling.

To negatively validate whether miR-27a-3p affects cisplatin sensitivity by PI3K/Akt signaling, we used the pathway inhibitors LY49002. As shown in Fig.4C,
when LY49002 was added, it caused a significant suppression of the PI3K/AKT signaling and an obvious increase expression of C-caspase-3. This suggests that PI3K/Akt signaling is the main pathway increasing cisplatin sensitivity. Whereas knockdown of miR-27a-3p, the above trend was attenuated in miR-inhibitor-27a group, compared with miR-inhibitor-Con group. Thus, we infer that miR-27a-3p adds cisplatin sensitivity potentially through regulating of PI3K/Akt signaling. This is supported by Zhu B’s result (30), he found that miR-27a-3p might be associated with resistance of breast cancer cells to adriamycin treatments, by targeting BTG2 and promoting the PI3K/Akt pathway in breast cancer cells. And the shortcoming of this paper is that we haven’t determined the target gene of miR-27a-3p that involved in cisplatin intervention, and we will continue to explore in future study.

In conclusion, miR-27a-3p acts as a tumor suppressor gene in HCC. Importantly, Overexpression of miR-27a-3p adds cisplatin sensitivity potentially through PI3K/Akt signaling in hepatoma cell lines. Our data highlights a molecular target in chemotherapy strategy of HCC.

5. Data Availability

All data generated or analyzed during the present study are included in this published article and its Supplementary Material files.

6. Grant support

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7. Author Contribution
Ying Yang drafted the manuscript and gave final approval of the version to be published. Zhifang Yang and Ruili Zhang participated in PCR, Western blot analysis. Chunli Jia and Rui Mao reviewed and edited the manuscript. Sha Ya and Ge Wu performed the statistical analyses. Yuefen Zhang completed the Western blot supplement experiment. Yan na Sun and Xiao yan Jia participated in flow cytometry, Ainiwaer Aimudula interpreted the significance of the results. Yongxing Bao and Hua Zhang conceived and designed the study. All authors read and approved the final manuscript.

8. Conflicts of interest

The authors declare no conflicts of interest.

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10. Figure legends

**Figure 1. MiR-27a-3p was downregulated in tumor tissue in HCC patients**

(A) The scatter diagram represented the expression of miR-27a-3p in 49 paired HCC tissues and adjacent non-tumor tissues, it showed that miR-27a-3p was significantly lower expressed in tumor tissue. (B) The Kaplan–Meier curve revealed that high level of miR-27a-3p significantly correlated with better overall survival in HCC patients. (C-E) The correlation analysis reflected that miR-27a-3p expressions were associated with metastasis, Child-Pugh grade and race in HCC patients. (F) The miR-27a-3p expressions in different cell lines were measured by RT-qPCR. (G) Expressions of miR-27a-3p were determined by RT-qPCR after 48h transfection (H-I). The transfection efficiency was visualized by fluorescence microscope. *P<0.05.

**Figure 2. MiR-27a-3p acted as a tumor suppressor gene in HCC**

HepG2 cells were chosen for miR-27a-3p overexpression and PLC cells were selected for miR-27a-3p knockdown. (A-B) MTT assays showed that high level of miR-27a-3p impaired the cell viability in HepG2, whereas low level of it added viability in PLC. (C-D) Upregulation of miR-27a-3p increased the apoptosis rate in HepG2. In contrast, knockdown of miR-27a-3p significantly reduced the apoptosis rate in PLC. (E-F) The cell cycle assays revealed that overexpression of miR-27a-3p led to an increase in G0/G1 phase and a decrease in S phase in HepG2, while knockdown of it resulted in a reverse trend in PLC. All experiments were performed in triplicate. *P<0.05.

**Figure 3. MiR-27a-3p enhanced the cisplatin sensitivity of HCC cells.**

(A-B) MTT assays showed that overexpression of miR-27a-3p increased the inhibition rate in HepG2. On the contrary, knockdown of miR-27a-3p significantly decreased the inhibition rate in PLC. (C-D) MiR-27a+DDP group had higher apoptosis rate than that in miR-Con+DDP group in HepG2 (P<0.05). MiR-inhibitor-27a+DDP group had lower apoptosis rate than that in miR-inhibitor-Con+DDP group in PLC (P<0.05).
Figure 4. MiR-27a-3p added the cisplatin sensitivity potentially by inhibiting PI3K/Akt pathway

(A-B) MiR-27a group inhibited PI3K/p-Akt signaling and elevated C-caspase-3 in HepG2. MiR-inhibitor-27a group exhibited the opposite trend in PLC cells. And DDP group showed suppression of PI3K/p-Akt pathway and up regulated of C-caspase-3. MiR-27a+DDP group showed more obvious trend than that in DDP group in HepG2. MiR-inhibitor-27a+DDP group revealed stronger expression in PI3K protein and weaker level of C-caspase-3, compared with DDP group in PLC. Silencing of miR-27a-3p attenuated the cisplatin effect. (C) The PI3K/Akt pathway inhibitors LY49002 was used, a significant suppression of PI3K/ Akt signaling and an increase expression of C-caspase-3 were observed. When knockdown of miR-27a-3p, the above trend was attenuated in miR-inhibitor-27a group, compared with miR-inhibitor-Con group. β-actin was used as a loading control. All of the experiments were performed in triplicate.
Table1. The correlation between clinicopathological features and miR-27a-3p expression in HCC patients

| Clinical characteristics | Expression | χ²  | P value |
|--------------------------|------------|-----|---------|
|                          | Low  | High |       |         |
| Age                      | 186  | 186  | 0.269 | 0.604  |
| ≤60                      | 86   | 91   | 0.605 | 0.437  |
| >60                      | 100  | 95   | 0.012 | 0.914  |
| Gender                   |      |      |       |         |
| female                   | 56   | 63   | 0.013 | 0.013  |
| male                     | 130  | 123  |       |         |
| Race                     |      |      |       |         |
| white                    | 79   | 103  | 6.196 | 0.013  |
| no-white                 | 107  | 83   |       |         |
| T stage                  |      |      |       |         |
| T1-2                     | 147  | 132  | 3.226 | 0.072  |
| T3-4                     | 39   | 54   |       |         |
| N stage                  |      |      |       |         |
| N0                       | 161  | 170  | 2.220 | 0.136  |
| N1                       | 25   | 16   |       |         |
| M stage                  |      |      |       |         |
| M0                       | 159  | 172  | 4.633 | 0.031  |
| M1                       | 27   | 14   |       |         |
| Differentiation grade    |      |      |       |         |
| G1-2                     | 117  | 118  | 0.012 | 0.914  |
| G3-4                     | 69   | 68   |       |         |
Table 2. Univariate and multivariate analysis of overall survival in patients with HCC

| Covariate     | Univariate analysis |                      | Multivariate analysis |                      |
|---------------|---------------------|----------------------|-----------------------|----------------------|
|               | \( \chi^2 \)       | \( P \text{ value} \) | HR                    | ( 95% CI )           | \( P \text{ value} \) |
| Age           | 1.600               | 0.206                | 1.321                 | 0.384                | 3.989 | 0.721 |
| Gender        | 1.165               | 0.280                | 1.645                 | 0.381                | 5.542 | 0.285 |
| Race          | 0.990               | 0.320                | 1.725                 | 0.426                | 5.892 | 0.361 |
| AFP           | 0.093               | 0.761                | 0.895                 | 0.325                | 2.462 | 0.829 |
| Cirrhosis     | 1.080               | 0.299                | 1.895                 | 0.578                | 6.200 | 0.291 |
| Feature               | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 | Value 6 |
|----------------------|---------|---------|---------|---------|---------|---------|
| Tumor size           | 28.902  | < 0.001 | 0.135   | 0.042   | 0.052   | < 0.001 |
| Lymph node metastasis| 0.163   | 0.686   | 0.285   | 0.426   | 1.892   | 0.648   |
| Distant metastasis   | 4.031   | 0.045*  | 0.047   | 0.232   | 0.067   | 0.011*  |
| Differentiation grade| 0.192   | 0.661   | 0.296   | 0.431   | 1.910   | 0.653   |
| Child-Pugh Grade     | 8.018   | 0.005*  | 1.103   | 0.061   | 0.461   | 0.015*  |
| Tumor stage          | 24.084  | < 0.001 | 0.723   | 0.057   | 1.201   | 0.061   |
| miR-27a-3p           | 6.088   | 0.014*  | 1.117   | 0.013   | 0.291   | 0.010*  |

*Significance level is 0.05.
High expression of miR-27a

Low expression of miR-27a

Relative expression of miR-27a

P=0.011

Tumor

Non-tumor

Relative expression of miR-27a

P=0.031

M0

M1

Metastasis

Relative expression of miR-27a

P=0.043

White

No-white

Race

Relative expression of miR-27a

P=0.013

Overall Survival

Time (months)

High expression of miR-27a

Low expression of miR-27a

Child-Pugh

P<0.001

ChildA

ChildB-C

clinical features of miR-27a-3p in TCGA data of HCC

expression

clinical features

survival curve

biological functions

viability

apoptosis

cell cycle

overexpression and knockdown miR-27a-3p in cisplatin treatment

cisplatin effect

inhibition rate

apoptosis

miR-27a-3p adds cisplatin sensitivity potentially through PI3K/Akt signaling
