

MiR-183 modulates multi-drug resistance in hepatocellular cancer (HCC) cells via miR-183-IDH2/SOCS6-HIF-1 α feedback loop

X.-J. WANG¹, D.-L. ZHANG², C. FU³, B.-Z. WEI⁴, G.-J. LI³

¹Clinical Laboratory, the Central Hospital of Yishui, Linyi, Shandong, China

²1st Department of Infectious Diseases, Binzhou People's Hospital, Binzhou, Shandong, China

³Department of Radiology, the People's Hospital of Gaomi, Gaomi, Shandong, China

⁴Department of Hepatobiliary Surgery, the Central Hospital of Zibo City, Zibo City, Shandong, China

Xiangjun Wang and Donglan Zhang contributed equally to this study

Abstract. – OBJECTIVE: MiR-183 acts as an oncomiR and is usually upregulated in hepatocellular cancer (HCC). This study aims to study the association between miR-183 dysregulation and multi-drug resistance (MDR). Also, how it is dysregulated in HCC cells was investigated.

MATERIALS AND METHODS: The association between miR-183 and HIF-1 α in HCC cell line BEL-7402 and the multidrug-resistant variant BEL-7402/5-fluorouracil (BEL-7402/5-FU) were studied using qRT-PCR and Western blot analysis. The mediators involved in feedback regulation between miR-183 and HIF-1 α were further studied. Then, the effect of the miR-183-SOCS6 axis on IC50 of BEL-7402/5-FU cells to 5-FU were investigated by MTT assay.

RESULTS: The expression of miR-183 and HIF-1 α are positively correlated in BEL-7402 and BEL-7402/5-FU cells. IDH2 knockdown resulted in significantly increased HIF-1 α expression in both BEL-7402 and BEL-7402/5-FU cells. Knockdown of SOCS6 had similar but stronger effect as miR-183 in promoting MRP2, P-gp, p-STAT3 and HIF-1 α expression in BEL-7402 cells, while SOCS6 overexpression also showed similar but stronger effect as miR-183 inhibition in reducing MRP2, P-gp, p-STAT3 and HIF-1 α levels in BEL-7402/5-FU cells. Both SOCS6 overexpression and miR-183 knockdown significantly increased the sensitivity of BEL-7402/5-FU cells to 5-FU. MiR-183 overexpression partly abrogated the effect of SOCS6 in enhancing 5-FU sensitivity.

CONCLUSIONS: Both HIF-1 α -miR-183-IDH2-HIF-1 α and HIF-1 α -miR-183-SOCS6-p-STAT3-HIF-1 α feedback loops are involved in miR-183 upregulation in HCC cells. MiR-183 can modulate MDR of HCC cells at least partly through suppressing SOCS6.

Key Words:

MiR-183, MDR, HCC, HIF-1 α .

Introduction

Hepatocellular carcinoma (HCC) is a common malignancy across the world and is the leading cause of malignancy-related death in China^{1,2}. Surgical resection and liver transplantation are the most effective therapies for HCC³. However, these strategies have limited application since only a small proportion of the patients are with resectable cancer and the liver donors are in a great shortage^{4,5}. Chemotherapy is an important therapeutic strategy for most of the HCC patients^{6,7}. Conventional chemotherapy with agents such as 5-fluorouracil (5-FU), cisplatin, epirubicin and doxorubicin has been administered for the patients³. However, multi-drug resistance (MDR) is one of the toughest obstacles to successful treatment. One well-recognized mechanism underlying MDR is the activation of the multi-drug resistance protein 1 (MDR1) gene and subsequently overexpression of P-glycoprotein (P-gp)⁸. However, the detailed mechanisms of MDR in HCC are complex and have not been fully revealed.

HCC is a type of solid tumor characterized as increasing hypoxia during tumor growth. One previous study reported that miR-183 is associated with dysregulated HIF-1 α expression in glioma cells via targeting isocitrate dehydrogenase 2 (IDH2)⁹. In fact, miR-183 acts as an oncomiR and is usually upregulated in HCC^{10,11}. It can inhibit apoptosis of HCC cells via repressing the PDCD4 expression¹⁰. MiR-183 upregulation is also associated with higher level cell invasion of HCC¹¹. Another recent study¹² reported that miR-183 can suppress suppressors of cytokine signaling 6 (SOCS6) expression and modulate growth and invasion of HCC cells. In addition, it

might be a miRNA related to chemosensitivity of HCC cells¹³. However, how it is dysregulated in HCC and the how it is involved in regulation of chemosensitivity of HCC cells have not been fully elucidated.

SOCS6 functions as a regulator of survival signaling due to its apoptosis-inducing effect via promoting ligand-dependent ubiquitination¹⁴. The low SOCS6 expression is associated with aggressive tumor progression and poor prognosis of HCC¹⁵. The loss of SOCS6 expression enhances cell resistance to programmed cell death¹⁶. Also, some recent studies¹⁷ suggest that low SOCS6 expression contributes to chemoresistance in breast cancer. Therefore, we hypothesized that this protein might be involved in MDR regulation in HCC. In this study, we investigated the mechanism of miR-183 dysregulation and further studied its role in the regulation of 5-FU resistance in HCC.

Materials and Methods

Cell Culture

Human hepatocellular carcinoma cell line BEL-7402 and the multidrug-resistant variant BEL-7402/5-fluorouracil (BEL-7402/5-FU) were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Both of the cell lines were cultured in RPMI-1640 medium supplemented with 10% FBS (fetal bovine serum), 100 U of penicillin/ml and 100 μ g of streptomycin/ml in a humidified atmosphere containing 5% CO₂ at 37°C. To maintain the MDR phenotype, the cultured medium for BEL-7402/5-FU was further supplemented with 20 μ g/ml 5-FU.

Cell Transfection

MiR-183 mimics, miR-183 inhibitors (IH), HIF-1 α siRNA, SOCS6 siRNA, IDH2 siRNA and the scramble negative controls were synthesized by GenaPharma (Shanghai, China). BEL-7402 were transfected with 50 nM miR-183 mimics, 75 nM IDH2 siRNA or 75 nM SOCS6 siRNA using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. BEL-7402/5-FU cells were transfected with 100 nM miR-183 inhibitors, 50 nM HIF-1 α siRNA or 50 nM IDH2 siRNA using Lipofectamine 2000 (Invitrogen).

pCMV-HIF-1 α and pCMV-SOCS6 expression vectors were purchased from GeneChem (Shanghai, China). BEL-7402 cells were transfected

with pCMV-HIF-1 α for HIF-1 α overexpression. BEL-7402/5-FU cells were transfected with pCMV-SOCS6 alone or in combination with 50 nM miR-183 mimics or miR-183 inhibitors using Lipofectamine 2000 (Invitrogen).

QRT-PCR analysis

Total RNA was isolated using the TRIZOL reagent (Invitrogen) according to the manufacturer's instruction. For the miRNA reverse transcription (RT), miRNA-specific cDNA was firstly synthesized using the stem-loop primers and the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Then, the mature miR-183 level was determined using the TaqMan MicroRNA Assays Kit (Applied Biosystems).

For RT of mRNA, the first strand cDNA was synthesized using the PrimeScript RT reagent kit (TaKaRa, Dalian, Liaoning, China). The primers for IDH2 and SOCS6 are synthesized and obtained from Qiagen (Suzhou, Jiangsu, China). Then, qRT-PCR was performed using SYBR Green PCR master mix (TaKaRa, Otsu, Shiga, Japan) on the ABI 7500HT System (Applied Biosystems). GAPDH was used as the internal control to normalize the expression of IDH2 and SOCS6. The 2^{- $\Delta\Delta$ Ct} method was used to calculate relative miRNA and mRNA expression.

Western Blot Analysis

Cells were firstly lysed using a RIPA lysis buffer (Beyotime, Shanghai, China) for protein extraction. The protein concentration was measured using the BCA method. Then, samples containing 20 μ g protein were loaded in 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) for separation. The first antibody include anti-HIF-1 α (1:1000, ab16066, Abcam, Cambridge, UK), anti-IDH2 (1:1000, ABC57, Merck Millipore, Darmstadt, Germany), anti-MRP2, anti-P-gp (1:2000, ab129450, Abcam), anti-SOCS6 (1:1000, ab157168, Abcam), anti-STAT3 (1:1000, ab137803, Abcam), anti-p-STAT3 (1:1000, sc-81523, Santa Cruz, CA, USA) and anti-actin (1:2000, ab8227, Abcam). The second HRP conjugated secondary antibodies were obtained from Abcam. The signal intensity of the protein bands was visualized using the ECL Western blotting substrate (Promega, Madison, WI, USA). The signal intensity was quantified using ImageQuant TL (GE Healthcare, Piscataway, NJ, USA).

Drug Sensitivity Assay

BEL-7402/5-FU cells transfected with miR-183 mimics, inhibitors or SOCS6 expression vectors alone or with combined transfection of SOCS6 vectors and miR-183 mimics or miR-183 inhibitors were plated in 96-well plates and incubated at 37°C in a humidified 5% CO₂ atmosphere for 24 hours. Then, 5-FU in various concentrations (0.2, 1, 5, 25, 125 µg/ml) were added. 48 hours later, cell viability was assessed using a MTT assay. Three independent experiments were performed in triplicate.

Statistical Analysis

Quantitative data were reported as mean ± SD. One-way ANOVA was performed to compare means of multiple group experiments. Between group difference was compared by using *t*-test (Mann-Whitney rank sum test). *p* < 0.05 was considered as statistically significant.

Results

The Expression of miR-183 and HIF-1α Are Positively Correlated in HCC Cells

MiR-183 has been reported as an oncomiR in HCC^{10,12}. However, its association with MDR is not clear. By performing qRT-PCR analysis, we

observed that the BEL-7402/5-FU cells had significantly higher miR-183 expression than the 5-FU sensitive BEL-7402 cells (Figure 1A). One recent study observed that the oncoprotein hepatitis B X-interacting protein (HBXIP) can block the degradation of HIF-1α, which binds to the promoter region of miR-183/96/182 cluster and results in enhanced transcription of miR-183/96/182¹⁸. Therefore, we hypothesized that miR-183 upregulation in BEL-7402/5-FU cells might be related to HIF-1α dysregulation. Through performing western blot analysis, we confirmed that BEL-7402/5-FU cells also had significantly higher level of HIF-1α expression than BEL-7402 cells (Figure 1B). Then, we overexpressed HIF-1α in BEL-7402 cells and inhibited HIF-1α in BEL-7402/5-FU cells (Figure 1C). In BEL-7402 cells, enforced HIF-1α expression directly led to miR-183 upregulation (Figure 1D). In contrast, knockdown of endogenous HIF-1α significantly suppressed miR-183 expression (Figure 1E). These results suggest that the expression of miR-183 and HIF-1α are positively correlated in HCC cells.

The HIF-1α-miR-183-IDH2-HIF-1α Loop is Involved in miR-183 Upregulation in HCC Cells

To further explore the association between miR-183 and HIF-1α in HCC cells, we overexpressed miR-183 in BEL-7402 cells and sup-

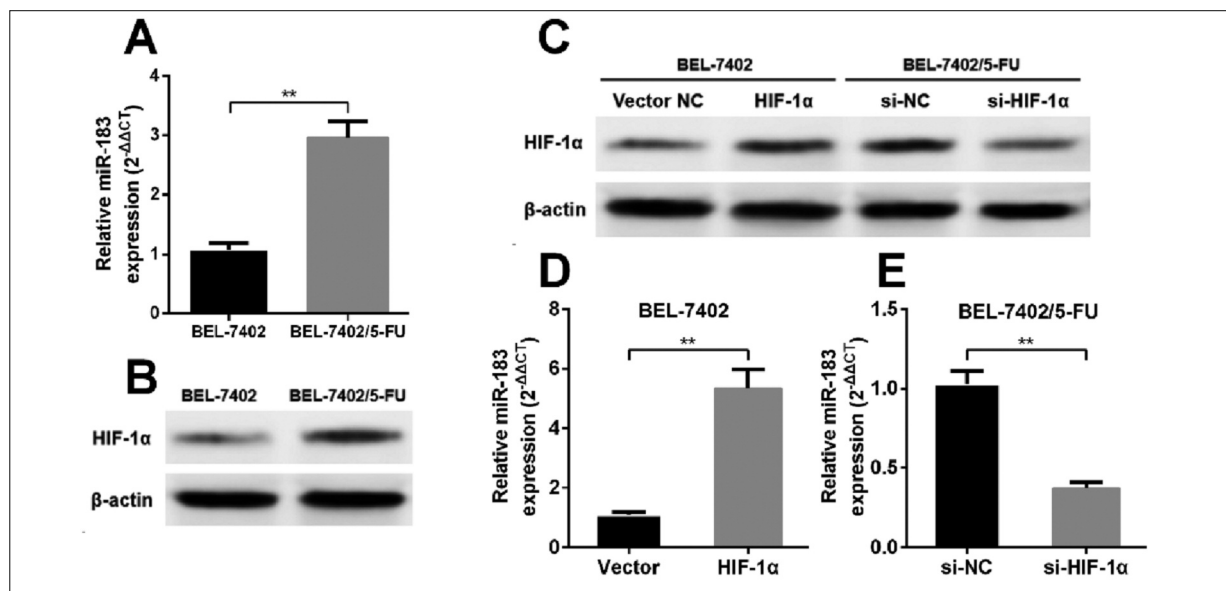


Figure 1. The expression of miR-183 and HIF-1α are positively correlated in HCC cells. A and B. QRT-PCR analysis of miR-183 expression (A) and Western blot analysis of HIF-1α expression (B) in BEL-7402 and BEL-7402/5-FU cells. C-E, Western blot analysis of HIF-1α expression (C) and QRT-PCR analysis of miR-183 expression (D-E) in BEL-7402 cells transfected with HIF-1α expression vector and in BEL-7402/5-FU cells transfected with HIF-1α si-RNA. **p* < 0.05, ***p* < 0.01.

pressed miR-183 in BEL-7402/5-FU cells (Figure 2 A and B). Interestingly, we found that BEL-7402 cells with miR-183 overexpression had significantly increased HIF-1 α expression (Figure 2 C and E), while the BEL-7402/5-FU cells with miR-183 knockdown had substantially suppressed HIF-1 α expression (Figure 2 D and E). One recent study⁹ suggests that miR-183 can directly target IDH2 and thereby increase HIF-1 α expression in glioma cells. Actually, IDH2 acts as a tumor suppressor of HCC and is usually downregulated in the cancer^{19,20}. In BEL-7402 cells, miR-183 overexpression significantly decreased IDH2 mRNA and protein (Figure 2 F and H). In comparison, BEL-7402/5-FU cells with miR-183 knockdown had significantly increased IDH2 mRNA and protein (Figure 2 G and H). Then, we further investigate how IDH2 affects HIF-1 α expression in the cells. BEL-7402 and BEL-7402/5-FU cells were firstly transfected with IDH2 siRNA (Figure 2 I-J). Knockdown of IDH2 significantly increased HIF-1 α expression (Figure 2K). Therefore, we infer that there is a HIF-1 α -miR-183-IDH2-HIF-1 α feedback loop involved in miR-183 upregulation in HCC cells.

MiR-183 is Involved in Regulation of MDR-related Proteins

Since we confirmed different miR-183 expression in BEL-7402 and BEL-7402/5-FU cells, we then detected how its expression is associated with MDR-related proteins, including MRP2 and P-gp. By performing Western blot analysis, we confirmed that BEL-7402/5-FU cells had significantly higher basal levels of MRP2 and P-gp than BEL-7402 cells (Figure 3A). MiR-183 overexpression significantly enhanced MRP2 and P-gp expression in BEL-7402 cells, while miR-183 suppression significantly reduced the level of MRP2 and P-gp in BEL-7402/5-FU cells (Figure 3B).

MiR-183 Results in 5-FU-resistance and HIF-1 α Upregulation Partly Via Suppressing SOCS6

Then, we further investigated the downstream regulation of miR-183 in HCC. One previous study¹² reported that miR-183 can modulate growth and invasion of hepatocellular carcinoma (HCC) cells via targeting SOCS6. SOCS6 is an important regulator of survival signaling due to its initiating effect on ubiquitination of receptors

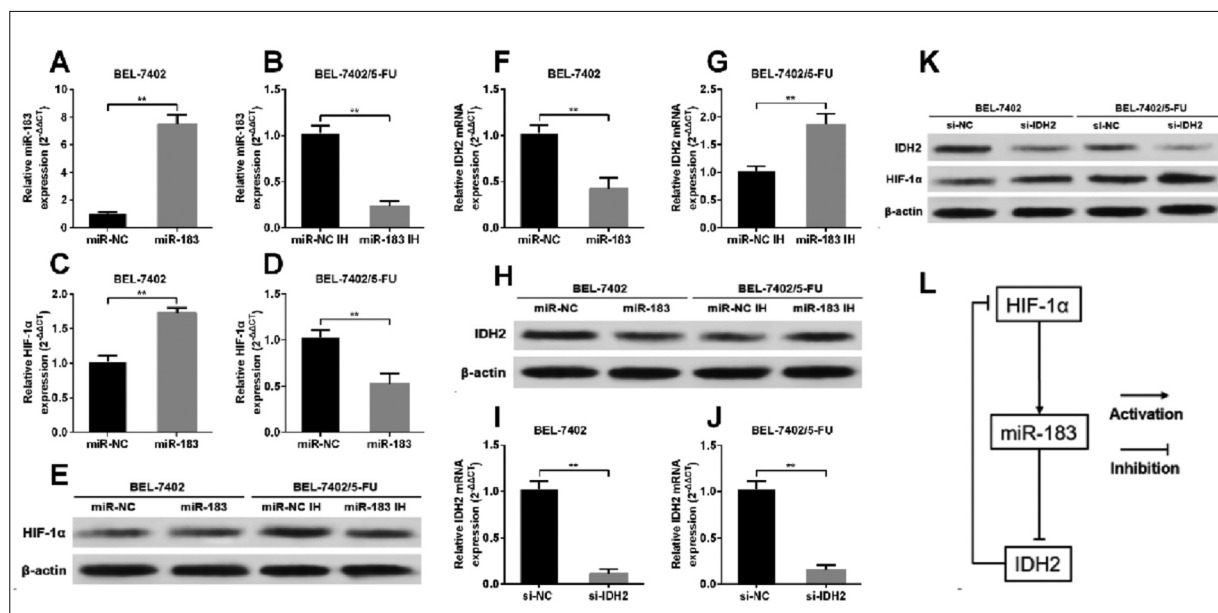


Figure 2. The HIF-1 α -miR-183-IDH2-HIF-1 α loop is involved in miR-183 upregulation in HCC cells. A-B. QRT-PCR analysis of miR-183 expression in BEL-7402 cells (A) transfected with miR-183 mimics and in BEL-7402/5-FU cells (B) transfected with miR-183 inhibitors (IH). C-E. Western blot analysis of HIF-1 α expression in BEL-7402 cells transfected with miR-183 mimics (C and E) and in BEL-7402/5-FU cells transfected with miR-183 inhibitors (D and E). F-H. QRT-PCR analysis (F-G) and Western blot analysis (H) of IDH2 expression in BEL-7402 cells transfected with miR-183 mimics and in BEL-7402/5-FU cells transfected with miR-183 inhibitors. I-J. QRT-PCR analysis of IDH2 expression in BEL-7402 and BEL-7402/5-FU cells transfected with IDH2 siRNA. K. Western blot analysis of IDH2 and HIF-1 α expression in BEL-7402 and BEL-7402/5-FU cells transfected with IDH2 siRNA. L. Schematic representation of the proposed model of HIF-1 α -miR-183-IDH2-HIF-1 α loop in HCC cells. * $p < 0.05$, ** $p < 0.01$.

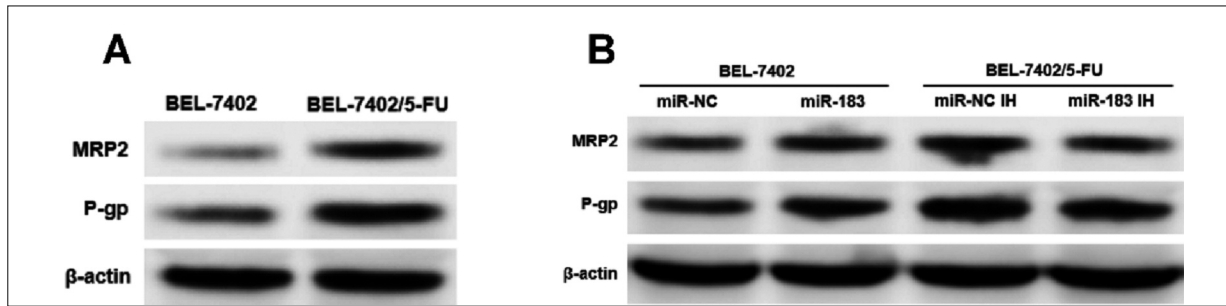


Figure 3. MiR-183 is involved in regulation of multi-drug resistance related proteins. **A**, Western blot analysis of MRP2 and P-gp proteins in BEL-7402 and BEL-7402/5-FU cells. **C-E**, Western blot analysis of MRP2 and P-gp proteins in BEL-7402 cells transfected with miR-183 mimics and in BEL-7402/5-FU cells transfected with miR-183 inhibitors.

or substrate proteins¹⁴. Some recent studies also found it is related to chemoresistance in breast cancer¹⁷. Therefore, we decided to further study their role in 5-FU-resistance of HCC. Firstly, BEL-7402 cells were transfected with SOCS6

siRNA (Figure 4A), while BEL-7402/5-FU cells were transfected with SOCS6 expression vectors (Figure 4B). By performing Western blot analysis, we observed that si-SOCS6 showed similar but stronger effects as miR-183 in enhancing

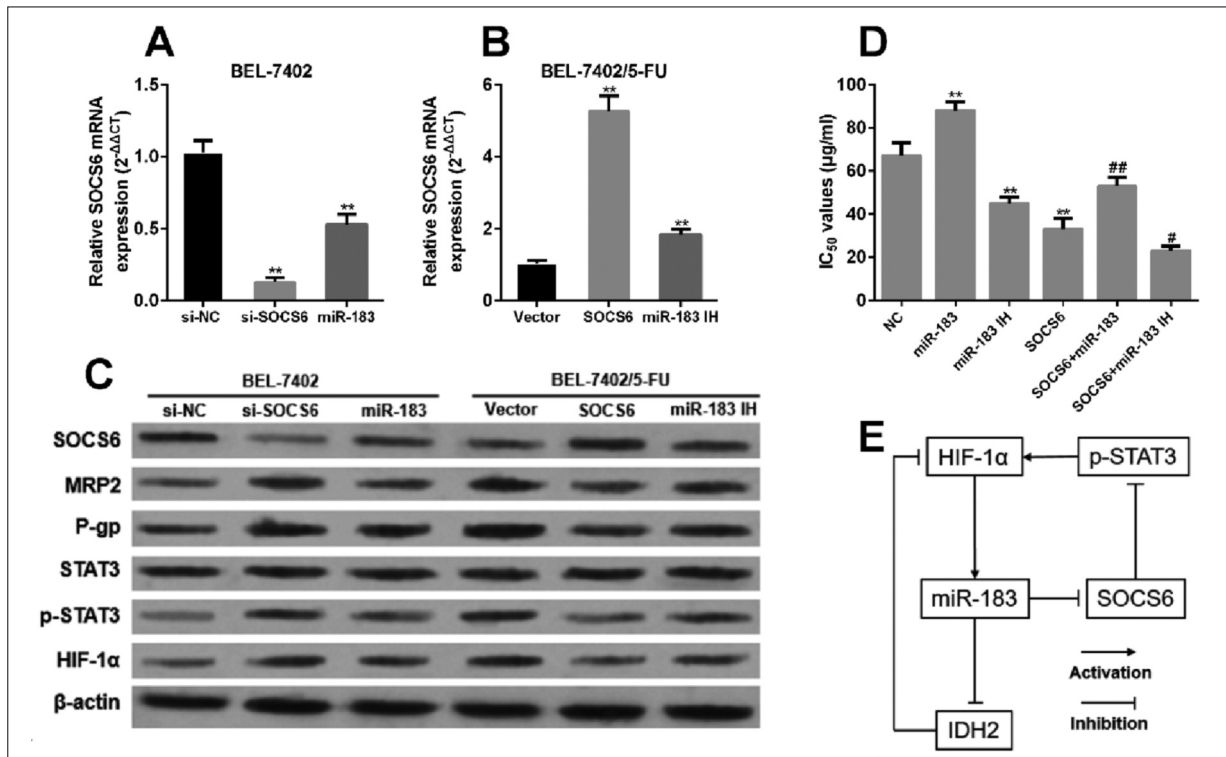


Figure 4. MiR-183 results in 5-FU-resistance and HIF-1 α upregulation partly via suppressing SOCS6. **A-B**, QRT-PCR analysis of SOCS6 expression mRNA in BEL-7402 cells (**A**) transfected with SOCS6 siRNA or miR-183 mimics and in BEL-7402/5-FU cells (**B**) transfected with SOCS6 expression vector or miR-183 inhibitors. **C**, Western blot analysis of SOCS6, MRP2, P-gp, STAT3, p-STAT3, HIF-1 α expression in BEL-7402 cells transfected with SOCS6 siRNA or miR-183 mimics and in BEL-7402/5-FU cells transfected with SOCS6 expression vector or miR-183 inhibitors. **D**, The sensitivities BEL-7402/5-FU cells to different doses of 5-FU after transfection with miR-183 mimics, miR-183 inhibitors or SOCS6 expression vector alone or in combination with miR-183 mimics or miR-183 inhibitors. **E**, Schematic representation of the proposed model of HIF-1 α -miR-183-SOCS6-p-STAT3-HIF-1 α feedback loop in HCC cells. *comparison with si-NC, vector or NC, #comparison with SOCS6. * and # $p < 0.05$, ** and ## $p < 0.01$.

MRP2 and P-gp expression in BEL-7402 cells (Figure 4C), while SOCS6 overexpression significantly decreased the expression of MRP2 and P-gp in BEL-7402/5-FU cells (Figure 4C). By performing drug sensitivity assay, we observed that miR-183 overexpression further enhanced the 5-FU resistance of BEL-7402/5-FU cells (Figure 4D). Both SOCS6 overexpression and miR-183 knockdown significantly increased the sensitivity of BEL-7402/5-FU cells to 5-FU. MiR-183 overexpression partly abrogated the effect of SOCS6 in enhancing 5-FU sensitivity (Figure 4D). Previous studies^{17,21} suggested that SOCS6 can suppress phosphorylation of STAT3. The phosphorylation of STAT3 is an upstream signaling of HIF-1 α upregulation^{22,23}. Therefore, we decided to investigate whether this mechanism is involved in HIF-1 α upregulation in HCC cells. SOCS6 knockdown showed a similar effect as miR-183 in enhancing the expression of p-STAT3 and HIF-1 α in BEL-7402 cells (Figure 4D). However, SOCS6 overexpression and miR-183 knockdown significantly decreased the expression of p-STAT3 and HIF-1 α in BEL-7402/5-FU cells (Figure 4D). Therefore, we infer that the HIF-1 α -miR-183-SOCS6-p-STAT3-HIF-1 α feedback loop is also involved in the miR-183 upregulation and the regulation of 5-FU resistance in HCC cells (Figure 4E).

Discussion

HCC is a type of solid tumor characterized as increasing hypoxia during tumor growth. Previous studies suggest that HIF-1 α induced under hypoxia can promote MDR and lead to significantly upregulated expression of MDR-related genes, such as mDR1, MRP1 and LRP at mRNA and protein level²⁴. In fact, HIF-1 α is a transcription factor that is involved in a wide range of signaling pathways related to tumorigenesis and drug sensitivity of the cancer cells. For example, HIF-inducible miR-191 can enhance migration of breast cancer through a complex regulation of TGF- β signaling pathway²⁵. HIF-1 α can also enhance the transcriptional activity of the miR-27a via binding to the upstream promoter region of miR-27a²⁶. Increased miR-27a expression results in upregulated MDR1/P-gp, LRP and Bcl-2 expression, thereby enhancing MDR²⁶.

MiR-183 has been reported as an oncomiR in HCC due to its inhibiting effect on SOCS6 and PDCD4, two inducers of apoptosis^{10,12,14}. It is al-

so a miRNA related to paclitaxel resistance of HCC cells¹³. One recent study¹⁸ observed that HIF-1 α can bind to the promoter region of the miR-183/96/182 cluster and result in enhanced transcription of miR-183/96/182. Also, another recent study⁹ reported that miR-183 can directly target IDH2 and thereby increase HIF-1 α expression in glioma cells. This triggered our interest to investigate whether there is a feedback regulation between miR-183 and HIF-1 α in HCC cells and the involvement of miR-183 in MDR of HCC.

By using 5-FU sensitive BEL-7402 and the 5-FU resistant BEL-7402/5-FU cells, we observed that BEL-7402/5-FU cells had significantly higher level of HIF-1 α and miR-183. HIF-1 α overexpression resulted in increased miR-183 expression, while HIF-1 α knockdown decreased miR-183 level. Previous studies reported that IDH2, a mitochondrial NADP-dependent isocitrate dehydrogenase, acts as a tumor suppressor in HCC^{19,20} and also may suppress HIF-1 α expression⁹. In this study, we confirmed that miR-183 overexpression decreased IDH2 in BEL-7402 cells, while miR-183 knockdown increased IDH2 in BEL-7402/5-FU cells. IDH2 knockdown resulted in significantly increased HIF-1 α expression in both BEL-7402 and BEL-7402/5-FU cells. Therefore, we confirmed that the HIF-1 α -miR-183-IDH2-HIF-1 α feedback loop is involved in miR-183 upregulation in HCC cells.

Then, we investigated how miR-183 influence two MDR-related proteins, including MRP2 and P-gp expression, two ATP-binding cassettes (ABC) transporters²⁷. Suppressing ABCB1 transcription and subsequently P-gp expression can significantly enhance the sensitivity of multiple HCC cell lines to anticancer drugs²⁸. Increased MRP2 expression was associated with a poorer response to neoadjuvant chemotherapy with cisplatin in HCC²⁹. In this study, we confirmed that BEL-7402/5-FU cells had significantly higher expression of MRP2 and P-gp. MiR-183 overexpression increased MRP2 and P-gp expression in BEL-7402 cells, while miR-183 knockdown reduced MRP2 and P-gp levels in BEL-7402/5-FU cells. Therefore, we decided to further investigate its downstream regulation. SOCS6, as a direct target of miR-183 in HCC cells, plays an important role in regulating survival signaling due to its initiating effect on ubiquitination of receptors or substrate proteins¹⁴. It is also related to chemosensitivity in breast cancer¹⁷. In addition, SOCS6 can suppress phosphorylation of STAT3^{17,21}, which is an upstream signaling of HIF-1 α upregulation^{22,23}.

By performing Western blot analysis, we observed miR-183 overexpression inhibited SOCS6 expression. Knockdown of SOCS6 had similar but stronger effect as miR-183 in promoting MRP2, P-gp, p-STAT3 and HIF-1 α expression in BEL-7402 cells, while SOCS6 overexpression also showed similar but stronger effect as miR-183 inhibition in reducing MRP2, P-gp, p-STAT3 and HIF-1 α levels in BEL-7402/5-FU cells. Therefore, we infer that SOCS6 is an important downstream regulator of miR-183 in regulating the 5-FU sensitivity of HCC cells and the HIF-1 α -miR-183-SOCS6-p-STAT3-HIF-1 α feedback loop is also involved in miR-183 upregulation in the cells.

Conclusions

Both HIF-1 α -miR-183-IDH2-HIF-1 α and HIF-1 α -miR-183-SOCS6-p-STAT3-HIF-1 α feedback loops are involved in miR-183 upregulation in HCC cells. MiR-183 can modulate MDR of HCC cells at least partly through suppressing SOCS6.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

- 1) NGUYEN LH, NGUYEN MH. Systematic review: Asian patients with chronic hepatitis C infection. *Aliment Pharmacol Ther* 2013; 37: 921-936.
- 2) SIEGEL R, MA J, ZOU J, JEMAL A. Cancer statistics, 2014. *CA Cancer J Clin* 2014; 64: 9-29.
- 3) RAHBARI NN, MEHRABI A, MOLLBERG NM, MULLER SA, KOCH M, BUCHLER MW, WEITZ J. Hepatocellular carcinoma: current management and perspectives for the future. *Ann Surg* 2011; 253: 453-469.
- 4) OSAKI Y, NISHIKAWA H. Treatment for hepatocellular carcinoma in Japan over the last three decades: Our experience and published work review. *Hepatol Res* 2015; 45: 59-74.
- 5) BERRETTA M, DI FRANZIA R, TIRELLI U. Sorafenib combined with percutaneous radiofrequency ablation for the treatment of medium-sized hepatocellular carcinoma. *Eur Rev Med Pharmacol Sci* 2015; 19: 2521-2522.
- 6) HU MD, JIA LH, LIU HB, ZHANG KH, GUO GH. Sorafenib in combination with transarterial chemoembolization for hepatocellular carcinoma: a meta-analysis. *Eur Rev Med Pharmacol Sci* 2016; 20: 64-74.
- 7) ZOU Y, GUO CG, ZHANG MM. Inhibition of human hepatocellular carcinoma tumor angiogenesis by siRNA silencing of VEGF via hepatic artery perfusion. *Eur Rev Med Pharmacol Sci* 2015; 19: 4751-4761.
- 8) ZHUO L, LIU J, WANG B, GAO M, HUANG A. Differential miRNA expression profiles in hepatocellular carcinoma cells and drug-resistant sublines. *Oncol Rep* 2013; 29: 555-562.
- 9) TANAKA H, SASAYAMA T, TANAKA K, NAKAMIZO S, NISHIHARA M, MIZUKAWA K, KOHTA M, KOYAMA J, MIYAKE S, TANIGUCHI M, HOSODA K, KOHMURA E. MicroRNA-183 upregulates HIF-1alpha by targeting isocitrate dehydrogenase 2 (IDH2) in glioma cells. *J Neurooncol* 2013; 111: 273-283.
- 10) LI J, FU H, XU C, TIE Y, XING R, ZHU J, QIN Y, SUN Z, ZHENG X. miR-183 inhibits TGF-beta1-induced apoptosis by downregulation of PDCD4 expression in human hepatocellular carcinoma cells. *BMC Cancer* 2010; 10: 354.
- 11) LEUNG WK, HE M, CHAN AW, LAW PT, WONG N. Wnt/beta-Catenin activates MiR-183/96/182 expression in hepatocellular carcinoma that promotes cell invasion. *Cancer Lett* 2015; 362: 97-105.
- 12) LI ZB, LI ZZ, LI L, CHU HT, JIA M. MiR-21 and miR-183 can simultaneously target SOCS6 and modulate growth and invasion of hepatocellular carcinoma (HCC) cells. *Eur Rev Med Pharmacol Sci* 2015; 19: 3208-3217.
- 13) YAN H, WANG S, YU H, ZHU J, CHEN C. Molecular pathways and functional analysis of miRNA expression associated with paclitaxel-induced apoptosis in hepatocellular carcinoma cells. *Pharmacology* 2013; 92: 167-174.
- 14) KABIR NN, SUN J, RONNSTRAND L, KAZI JU. SOCS6 is a selective suppressor of receptor tyrosine kinase signaling. *Tumour Biol* 2014; 35: 10581-10589.
- 15) QIU X, ZHENG J, GUO X, GAO X, LIU H, TU Y, ZHANG Y. Reduced expression of SOCS2 and SOCS6 in hepatocellular carcinoma correlates with aggressive tumor progression and poor prognosis. *Mol Cell Biochem* 2013; 378: 99-106.
- 16) LIN HY, LAI RH, LIN ST, LIN RC, WANG MJ, LIN CC, LEE HC, WANG FF, CHEN JY. Suppressor of cytokine signaling 6 (SOCS6) promotes mitochondrial fission via regulating DRP1 translocation. *Cell Death Differ* 2013; 20: 139-153.
- 17) SHEN R, WANG Y, WANG CX, YIN M, LIU HL, CHEN JP, HAN JQ, WANG WB. MiRNA-155 mediates TAM resistance by modulating SOCS6-STAT3 signalling pathway in breast cancer. *Am J Transl Res* 2015; 7: 2115-2126.
- 18) LIU F, ZHANG W, YOU X, LIU Y, LI Y, WANG Z, WANG Y, ZHANG X, YE L. The oncoprotein HBXIP promotes glucose metabolism reprogramming via downregulating SCO2 and PDHA1 in breast cancer. *Oncotarget* 2015; 6: 27199-27213.
- 19) LIU WR, TIAN MX, JIN L, YANG LX, DING ZB, SHEN YH, PENG YF, ZHOU J, QIU SJ, DAI Z, FAN J, SHI YH. High expression of 5-hydroxymethylcytosine and isocitrate dehydrogenase 2 is associated with favor-

- able prognosis after curative resection of hepatocellular carcinoma. *J Exp Clin Cancer Res* 2014; 33: 32.
- 20) TIAN GY, ZANG SF, WANG L, LUO Y, SHI JP, LOU GQ. Isocitrate Dehydrogenase 2 Suppresses the Invasion of Hepatocellular Carcinoma Cells via Matrix Metalloproteinase 9. *Cell Physiol Biochem* 2015; 37: 2405-2414.
- 21) PIAO L, PARK J, LI Y, SHIN S, SHIN S, KONG G, SHRESTHA R, TRAN O, HUR GM, KIM JL, PARK J. SOCS3 and SOCS6 are required for the risperidone-mediated inhibition of insulin and leptin signaling in neuroblastoma cells. *Int J Mol Med* 2014; 33: 1364-1370.
- 22) XU Q, BRIGGS J, PARK S, NIU G, KORTYLEWSKI M, ZHANG S, GRITSKO T, TURKSON J, KAY H, SEMENZA GL, CHENG JO, JOVE R, YU H. Targeting Stat3 blocks both HIF-1 and VEGF expression induced by multiple oncogenic growth signaling pathways. *Oncogene* 2005; 24: 5552-5560.
- 23) CHO KH, CHOI MJ, JEONG KJ, KIM JJ, HWANG MH, SHIN SC, PARK CG, LEE HY. A ROS/STAT3/HIF-1 α signaling cascade mediates EGF-induced TWIST1 expression and prostate cancer cell invasion. *Prostate* 2014; 74: 528-536.
- 24) ZHU H, CHEN XP, LUO SF, GUAN J, ZHANG WG, ZHANG BX. Involvement of hypoxia-inducible factor-1 α in multidrug resistance induced by hypoxia in HepG2 cells. *J Exp Clin Cancer Res* 2005; 24: 565-574.
- 25) NAGPAL N, AHMAD HM, CHAMEETTACHAL S, SUNDAR D, GHOSH S, KULSHRESHTHA R. HIF-inducible miR-191 promotes migration in breast cancer through complex regulation of TGF β -signaling in hypoxic microenvironment. *Sci Rep* 2015; 5: 9650.
- 26) ZHAO Q, LI Y, TAN BB, FAN LO, YANG PG, TIAN Y. HIF-1 α Induces Multidrug Resistance in Gastric Cancer Cells by Inducing MiR-27a. *PLoS One* 2015; 10: e0132746.
- 27) DIESTRA JE, CONDOM E, DEL MURO XG, SCHEFFER GL, PEREZ J, ZURITA AJ, MUNOZ-SEGUI J, VIGUES F, SCHEPER RJ, CAPELLA G, GERMA-LLUCH JR, IZQUIERDO MA. Expression of multidrug resistance proteins P-glycoprotein, multidrug resistance protein 1, breast cancer resistance protein and lung resistance related protein in locally advanced bladder cancer treated with neoadjuvant chemotherapy: biological and clinical implications. *J Urol* 2003; 170: 1383-1387.
- 28) YANG T, ZHENG ZM, LI XN, LI ZF, WANG Y, GENG YF, BAI L, ZHANG XB. MiR-223 modulates multidrug resistance via downregulation of ABCB1 in hepatocellular carcinoma cells. *Exp Biol Med (Maywood)* 2013; 238: 1024-1032.
- 29) KORITA PV, WAKAI T, SHIRAI Y, MATSUDA Y, SAKATA J, TAKAMURA M, YANO M, SANPEI A, AOYAGI Y, HATAKEYAMA K, AJIOKA Y. Multidrug resistance-associated protein 2 determines the efficacy of cisplatin in patients with hepatocellular carcinoma. *Oncol Rep* 2010; 23: 965-972.