Original Article

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Knockdown expression of *MECR*, a novel gene of mitochondrial FAS II inhibits growth and colony-formation, promotes apoptosis of hepatocelluar carcinoma cells

Yulong Cai, Yixin Lin, Xianze Xiong, Jiong Lu, Rongxing Zhou, Yanwen Jin, Zhen You, Hui Ye, Fuyu Li, Nansheng Cheng*

Department of Biliary Surgery, West China Hospital, Sichuan University, Chengdu, Sichuan Province, China.

Summary **Mitochondrial trans-2-enoyl-CoA reductase (*MECR*) is a protein-coding gene, and the protein encoded by this gene is an oxidoreductase that catalyzes the last step in mitochondrial fatty acid synthesis (mtFASII). Numerous studies have shown disorder of lipid metabolism is closely related with malignance, especially in liver cancer. Through pre-experiment, we found that the expression of *MECR* gene was highly expressed in hepatocelluar carcinoma (HCC) cell lines *in vitro*. This suggests that the *MECR* gene may play a role of oncogene in HCC. Therefore, we conducted a preliminary experimental study on the role of *MECR* gene in HCC cells *in vitro*. Objective to explore whether the *MECR* gene can affect the malignant biological behavior of HCC. We selected HCC cell line BEL-7404 as experimental cell, which involves the highest expression of *MECR* in the pre-experiment. We constructed *MECR* knockdwon lentivirus vector, and then infected HCC cell lines to down-regulate *MECR* expression, and establish negative control group (NC). Through various experiments of cytology, our study showed that knockdown of *MECR* inhibited cell proliferation and colony formation, promoted apoptosis, and inhibited metastasis in HCC cell lines BEL-7404. *MECR* might serve as a novel gene therapeutic target for the treatment of HCC. Further study is needed to elucidate the signaling pathway through which *MECR* functions in HCC.**

Keywords: Hepatocellular carcinoma, *MECR*

1. Introduction

Hepatocellular carcinoma (HCC) arises from hepatocytes and represents the most frequent type of primary liver cancer, occuping more than 90% (*1*). Worldwide, there are more than 700,000 new HCC patients each year, ranking fifth and seventh in the incidence of malignant tumors in men and women, respectively (*2*). At present, the overall prognosis rate of liver malignant tumors is still low. According to statistics, the 5-year survival rate of liver cancer in the United States is only 16.6%. Due to the continuous improvement of HCC treatment methods, the prognosis of HCC is relatively improved (*3,4*). Poor prognosis means finding more accurate biomarkers to predict a patient's prognosis and finding a more effective therapeutic gene targets are top priority for current research (*5,6*). Although some carcinogenic mechanisms and molecular mechanisms of HCC have been studied and proved, including genomic instability (*7,8*), gene mutation (*9,10*), carcinogenic and tumor suppressor gene expression (*11*), signaling pathways and so on (*12-15*), the overall and exact molecular pathology of HCC remains unclear. Still need a lot of basic scientific research and studies.

Despite the presence of a cytosolic fatty acid synthesis pathway, mitochondria have retained their own means of creating fatty acids *via* the mitochondrial fatty acid synthesis (mtFASII) pathway. Mitochondrial enoyl-CoA reductase (*MECR*), the last enzyme in the mtFASII

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**Address correspondence to:*
Dr. Nansheng Cheng, Department of Bile Duct Surgery, West China Hospital, Sichuan University,No. 37 GuoXue Xiang, Chengdu 610041, Sichuan Province, China.
E-mail: nanshengcheng2012@163.com

pathway, is a 2-enoyl thioester reductase that acts as a dimer, with a pocket forming between the two monomers that can accommodate fatty acid chains up to 16 carbons in length (*16-18*). The initial study of the *MECR* gene was found to be associated with peroxisome proliferator-activated receptor alpha (*PPARα*). Recent studies further elucidate that overexpression of the *MECR* gene activates the *PPARα* transcriptional pathway, possibly as a coactivator, or by increasing mtFASII activity (*19,20*). *PPARα* is a transcription factor whose main role is to participate in liver fat metabolism and adipocyte differentiation. Studies have also shown that *PPARα* plays a role in promoting metabolic pathways in the development of liver cancer (*21*). There are few studies on the relationship between *MECR* gene and disease. According to the literature, mutations in *MECR* gene can cause dystonia and optic atrophy in children (*22*). Animal experiments show that overexpression of *MECR* leads to cardiac dysfunction in mouse (*23*). In addition, it may be related to small airway obstruction (*24*). In terms of tumors, the *MECR* gene has not yet been reported.

A large number of studies have shown that lipid metabolism disorders are closely related to tumor cells, especially liver malignancies (*25*). However, studies on mitochondrial fat metabolism and HCC are still lacking. Therefore, we conducted a preliminary experimental study on the role of the *MECR* gene (encoding a key protein for mtFASII) in HCC cells *in vitro*. Objective to explore whether *MECR* gene can affect the malignant biological function of HCC.

Through pre-experiment, we found that *MECR* gene was highly expressed in four HCC cell lines, BEL7404, BEL7402, SMMC7721, and HepG2. This suggests that the *MECR* gene may play a role as oncogene in the HCC cell lines. Therefore, we further explored the effects of *MECR* gene on the malignant biological behavior of HCC cells by knocking down the *MECR* gene and establishing a negative control group, laying a foundation for future molecular pathway research.

2. Materials and Methods

2.1. *Cell Culture*

Human HCC cell lines, BEL-7402, BEL-7404, SMMC-7721, HepG2 and human renal epithelial 293T cells were purchased from the Shanghai Cell Bank (Shanghai, China). Cell lines were cultured in RPMI-1640 medium, supplemented with 10% fetal bovine serum FBS (Gibco®, Shanghai, China), 1% penicillin and 1% streptomycin at 37°C in a 5% CO_2 incubator.

2.2. *Quantitative RT-PCR*

Total RNA from the 4 cell lines, BEL-7402, BEL-7404, SMMC-7721, HepG2, was extracted using the TRIzol reagent (Invitrogen, Shanghai, China), according to the manufacturer's instructions and was then used for RT reaction. Briefly, 2 μg of total RNA from each sample was reverse transcribed to single-stranded cDNA. One microliter of cDNA was used as a template for the following PCR. The primers used were as follows: for MECR forward, 5'-GTTGGAGGGAACGAAGGTGT-3' and reverse, 5'-CGCTCTGAAGAGGGATGTCA-3'; for TP53 forward, 5'-GAG GTTGGCTCTGACTGTACC-3' and reverse, 5'-TCCGTCCCAGTAGATTACCAC-3'; and for GAPDH forward, 5'-TGACTTCAACAGCG ACACCCA-3', and reverse, 5'-CACCCTGTTGCTGTA GCCAAA-3'. The quantitative RT-PCR comprised an initial denaturation at 95°C for 15 sec, then 45 cycles at 95°C for 5 sec and 60°C for 30 sec. The PCR products of *MECR*, *TP53* and GAPDH were 175, 133 and 121 bp. All samples were examined in triplicates.

2.3. *Recombinant lentiviral vector production and cell infection*

The complementary DNA sequence (TCATAT CTTCAAAGCAGAT) of *MECR* was designed from the full-length *MECR* sequence (NIM: 608205, GenBank: NM_016011.3) by GeneChem Co. Ltd. (Shanghai, China). After testing knockdown effciencies, the stem-loop oligonucleotides were synthesized and inserted into the lentivirus-based PSCSI-GFP (GeneChem Co. Ltd.) with AgeI/EcoRI sites. Lentivirus particles were prepared as previously described (*26*).

For lentivirus infection, BEL-7404 cells were cultured into 6-well plates and then the *MECR*-shRNA-lentivirus or negative control (NC) lentivirus was added according to a multiplicity of infection (MOI). After 72 h of infection, the cells were observed under a fluorescence microscope (MicroPublisher 3.3RTV; Olympus, Tokyo, Japan). After 120 h of infection, the cells were harvested to determine knockdown effciency by quantitative RT-PCR.

2.4. *Western blot analysis*

After protein quantization by the Coomassic brilliant blue assay, 20 μg of protein was bloed in loading buffer, resolved on 10% SDS-polyacrylamide gels, electrotransferred to nitrocellulose membranes, and incubated overnight with antibodies against *MECR* (1:2000 dilution; Sigma), *TP53* (1:500 dilution; CST) and BAX (1:500 dilution; Abcam). Secondary antibody was applied, and the relative content of the target proteins was detected by chemiluminescence. GAPDH was used as loading control.

2.5. *Cell growth assay*

BEL-7404 cells at the logarithmic phase after infection with *MECR*-shRNA-lentivirus and scrambled shRNA lentivirus were digested, resuspended, counted and

inoculated in 96-well plates. From the second day, cells with green fluorescence were taken photos and counted by Cellomics ArrayScan VT1 Readers once a day. Cell growth was observed continuously for 5 days, and cell growth curves were drawn.

2.6. *Colony formation assay*

Cells at the logarithmic phase after infection were inoculated in 6-well plates at the density of 800 cells/well. Culture medium was changed every three days. Cells were allowed to grow for 11 days to form colonies. When the cell numbers in most single colonies were greater than 50, cells were washed with PBS once and fixed in paraformaldehyde (Sangon Biotech Shanghai Co. Ltd) for 30 min. Cells were then washed with PBS and stained with Giemsa dye (Chemicon) for 20 min. After washing with ddH_2O for several times, the number of colonies was counted under fluorescence microscope (Olympus, Japan).

2.7. *FACS (fluorescence assisted cell sorting) cell cycle analysis*

Cells were cultured in 6-well plates and were infected with *MECR*-shRNA-lentivirus or control. When the cells grew to 80% confluence, they were collected by trypsinization and centrifugation at 1500 rmp for 5 min, washed once with PBS, and fixed in 70% alcohol for 1 h. Then, cells were rehydrated and resuspended in PBS containing Rnase (100 mg/mL) on ice, stained with propidium iodide (PI), filtered through a 50- mm nylon mesh, and then analyzed using a BD FACS Calibur Flow Cytometer (BD Biosciences, San Diego, CA, USA). All experiments were performed in triplicate.

2.8. *Detection of apoptosis by FACS*

Cell apoptosis was assayed by staining with Annexin V-APC and detected by FACS. For analysis of apoptosis, the cells were harvested, washed with 1 × binding buffer and resuspended in 1 mL 1 × staining buffer. Then 5 mL Annexin V-APC was added into 100 mL of the above cell suspension (about $1 \times 10^5 – 1 \times 10^6$ cells), and incubated at room temperature in the dark for 10-15 min. After incubation, cells were analyzed using the BD FACS Calibur Flow Cytometer (BD Biosciences, San Diego, CA, USA). All experiments were performed in triplicate.

2.9. *Transwell assay*

The upper surface of Transwell invasion assay filters (Corning Costar, Shanghai, China) was coated by matrigel at 37℃ for 30 min. Then cell culture medium (600 mL) was added into the basolateral chamber, and the apical chamber was added with serum-free medium containing 5×10^4 transfected cells. After 24h culture at 37℃, the serum-free medium was absorbed, and the adherent cells in upper surface were removed. Next, the rest cells were fixed with 100% methanol and stained with crystal violet. The number of cells penetrating through matrigel was the standard to evaluate the invasive ability. Three high power fields were randomly chosen to calculate the cell number. Each experiment was independently performed in triplicate.

2.10. *Immunoflorescence detection*

Cells of each group, which were incubated for 48 hours in 24-well plates, were fixed with 2.5% glutaraldehyde for 15 minutes after the upper residual liquid was removed. Then they were incubated with 0.25% Triton X-100 for 15 minutes at room temperature, followed by blocking with 4% goat serum for 30 minutes at room temperature. *TP53* antibody (1:100) was added and incubated for 12 hours at 4°C. After washing with PBS for three times, rhodamine-labeled florescent secondary antibody (1:400) was added and incubated in a 37°C incubator for 40 minutes. 4',6-Diamidino-2- phenylindole was added to stain for 5 minutes. At last, the cells were observed under a florescence microscope and the number of positive cells was counted. Cells that exhibited blue immunoflorescence were *TP53*-positive cells.

2.11. *Statistical analysis*

SPSS 21.0 statistical software was used to analysis. The data shown are presented as the mean ± standard deviation (SD) of three independent experiments. Statistical significance was determined with Student's *t* test. A *P* value of less than 0.05 was considered significant.

3. Results

3.1. *MECR mRNA detection in four HCC cell lines*

In order to find out whether there is a relationship between *MECR* and HCC, we detected the MECR mRNA in four HCC cell lines BEL-7402, BEL-7404, SMMC-7721, HepG2 by PCR. *GAPDH* gene was amplified as internal control. The relative expression of target gene was calculated with $2^{-\Delta\Delta Ct}$ method (*27*). The results showed that *MECR* mRNA was expressed in all four cell lines and the *MECR* gene expression abundance in these cell lines was high (Figure 1).

3.2. *Knockdown efficiency determined by western blot analysis*

Human embryonic kidney 293T cells were infected with *MECR*-shRNA lentivirus or NC lentivirus. Western blot was used to detect the *MECR* protein expression. As

shown in Figure 2, compared with NC group the content of *MECR* protein was greatly reduced in the *MECR*-shRNA lentivirus group. This result indicated effective knockdown of the target sequence.

3.3. *Lentivirus-mediated knockdown of MECR in the human HCC cell line BEL-7404*

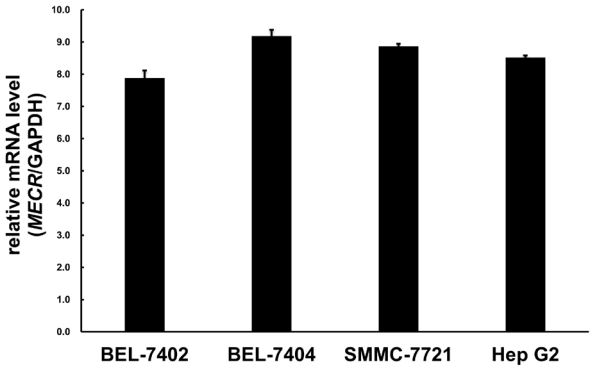

Figure 1. *MECR* mRNA levels in four HCC cell lines. Expression of *MECR* mRNA was measured by RT-PCR in the indicated cell lines. A constitutively expressed GAPDH gene was used as an internal control.

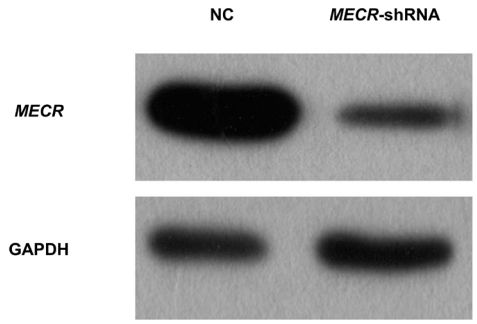

Figure 2. Knockdown of MECR protein expression in 293T cells. *MECR* protein expression was analyzed by western blotting in control-transfected (NC) and *MECR*-shRNA-transfected 293T cells. GAPDH was used as a loading control.

HCC cell line BEL-7404, which involved the highest relative expression of *MECR*, was adopted to explore the role of *MECR*. Knockdown of *MECR* gene BEL-7404 cell line group and NC group were established by infected with *MECR*-shRNA lentivirus and NC lentivirus. As shown in Figure 3A, by day 3 post infection, the proportion of infected cells was > 80% for both groups. And at day 5 post infection, *MECR* mRNA was assessed by real-time PCR. In the experiment group, the expression level of *MECR* mRNA was significantly lower than NC group (Figure 3B).

3.4. *Knockdown of MECR in BEL-7404 cells inhibited cell proliferation*

To investigate the effect of *MECR* on cell growth, BEL-7404 cells were infected by either the *MECR*-shRNA or NC lentivirus, and then were seeded into 96-well plates and analyzed by Cellomics Arrayscan with time-dependent manner for 5 days. As showed in Figure 4A, fluorescently stained cells were greatly increased in the NC group, and the cell-count comfirmed our observation (Figure 4B). By analysis, the growth rate was also higher in the NC group while the number of *MECR*-shRNA-transfected cells did not change much. The results showed *MECR* knockdown signifcantly inhibited proliferation of the BEL-7404 cells ($P < 0.05$).

3.5. *Knockdown of MECR inhibited BEL-7404 cells colony formation*

We then studied the colony-formation ability of knockdown *MECR* in BEL-7404 cells. BEL-7404 cells treated with *MECR*-shRNA or control lentivirus were allowed to grow for 11 days to form colonies. As shown in Figure 5, *MECR* knockdown resulted in significant decrease in the number of colonies in BEL-7404 cells ($P < 0.01$), compared with the control groups. These results

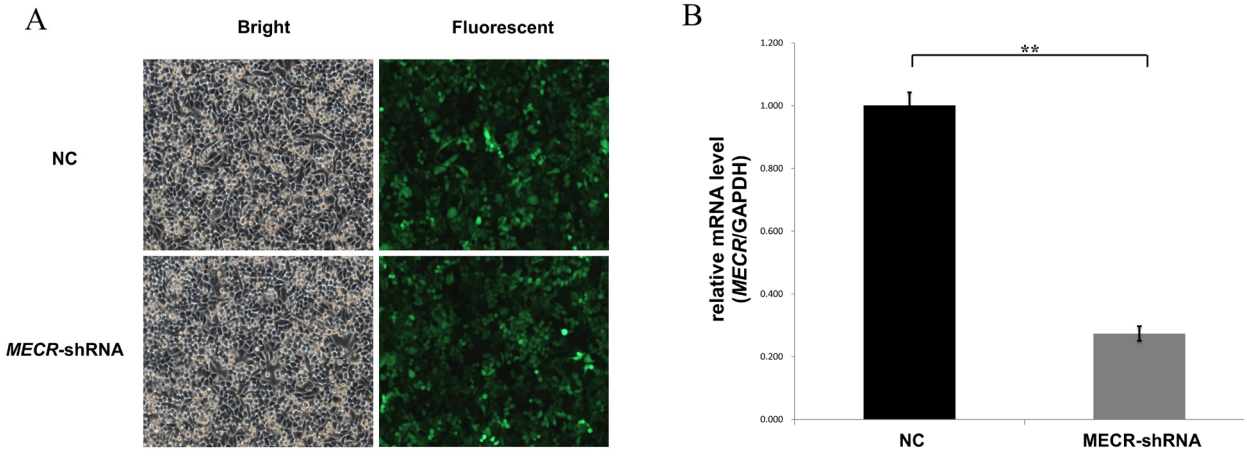

Figure 3. *MECR* silencing efficiency by shRNA lentivirus. (A) Lentivirus infection in BEL-7404 HCC cell line. Bright and fluorescent photomicrographs of BEL-7404 cells were taken 72 h after lentivirus infection at a magnification of × 100; **(B)** Identification of *MECR* knockdown efficiency using shRNA lentivirus by real-time PCR in BEL-7404 cells (**$P < 0.01$).

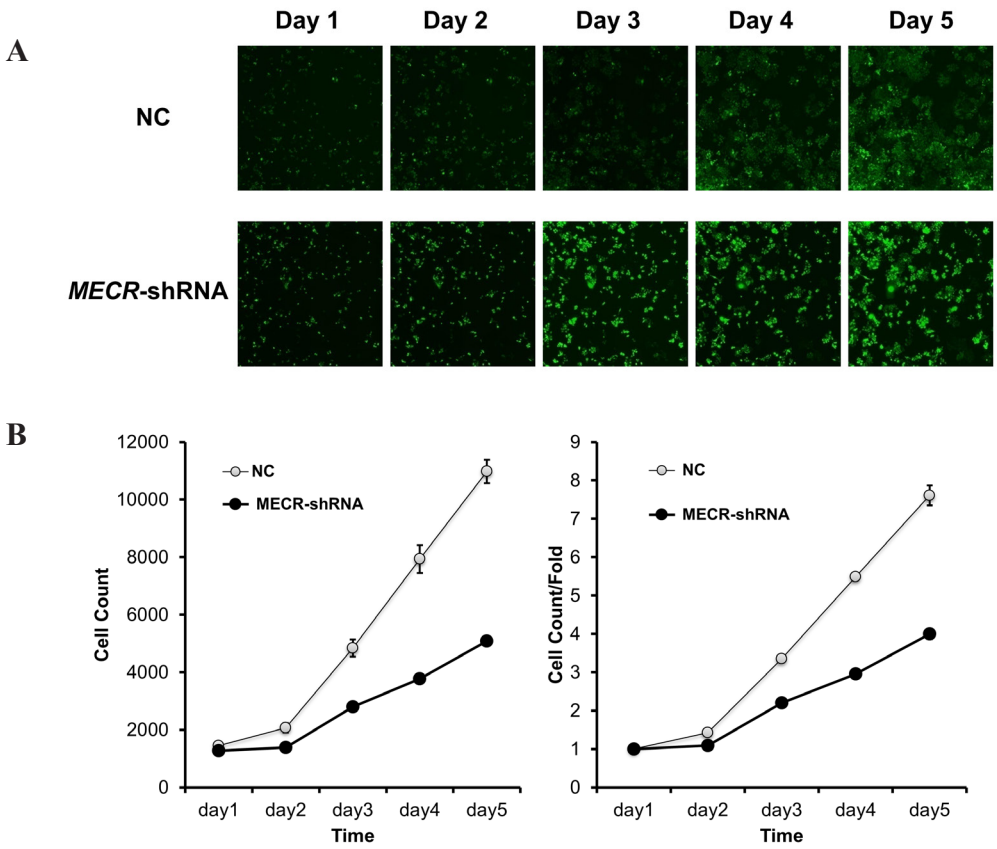

Figure 4. *MECR* silencing inhibited HCC cell proliferation. (A) Cells were infected with the control or *MECR*-shRNA lentivirus and high content cell imaging was applied every day as indicated to acquire raw imagesof cell growth. **(B)** *MECR* silencing by shRNA lentivirus significantly inhibited the growth rate of BEL-7404 cells, as shown by cell count for 5 days ($P < 0.05$).

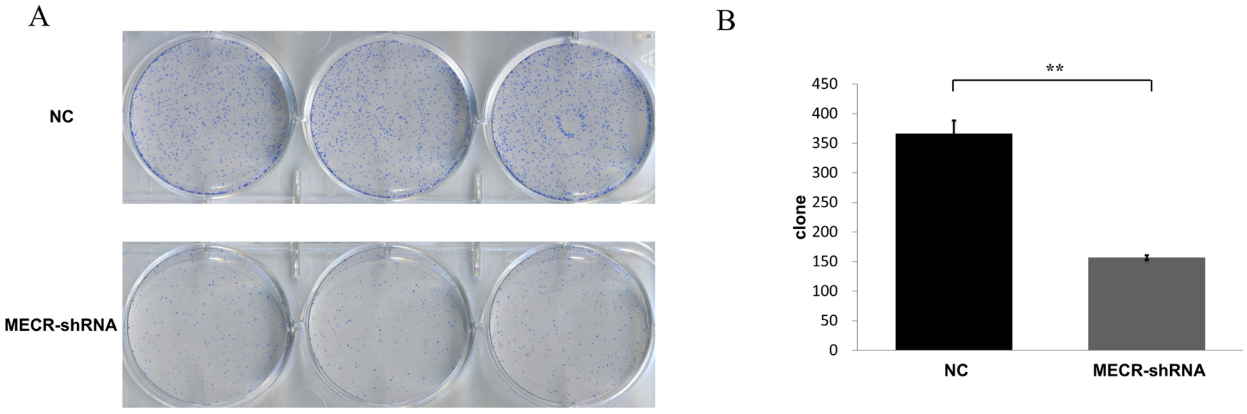

Figure 5. *MECR* silencing inhibited HCC cell colony formation. (A) Photomicrographs of Giemsa-stained colonies of BEL-7404 cells growing in 6-well plates for 11 days after infection; **(B)** The number of cells in colony of BEL-7404 cells was counted. Cell number in *MECR* shRNA group was significantly reduced, as compared with the control group (**$P < 0.01$).

suggested that the *MECR* gene is associated with colony forming ability of HCC cells.

3.6. *Knockdown of MECR in BEL-7404 cells do not affect cell cycle*

In this experiment, it was detected that *MECR* can inhibit the proliferation and colony-formation of HCC cells after knockdown. To explore whether *MECR* can affect cell cycle progression in BEL-7404 cells, the experimental group and control group cells were assessed the cell cycle phase by flow cytometry after 5 days of transfection. The results showed that the proportion of cells in the G1 phase after *MECR* knockdown was 46.3%, which was lower than that in the NC group (48.76%), and the difference was statistically significant ($P < 0.01$). However, in the S phase (17.77% *vs* 17.79%) and the G2/M phase (35.93% *vs* 33.45%), there was no

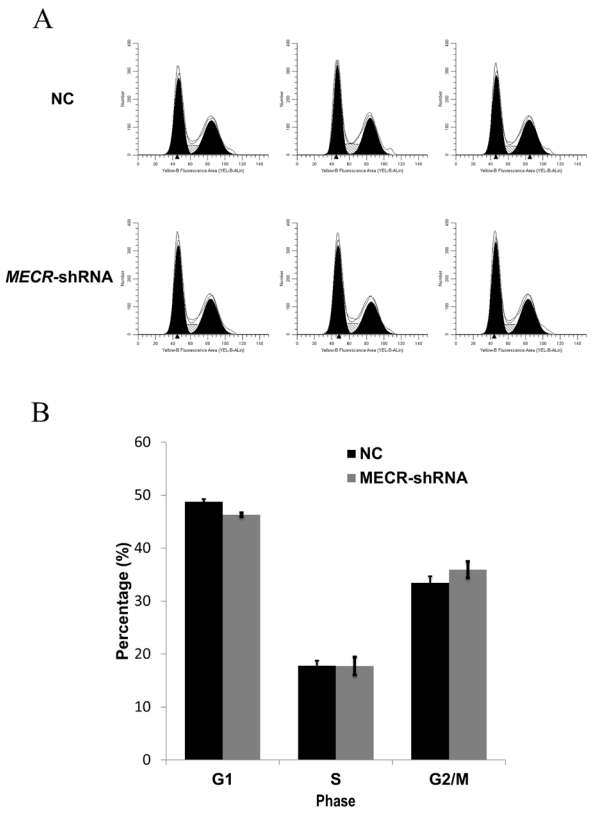

Figure 6. The effects of MECR on cell cycle. (A) The result of flow cytometry; **(B)** The proportion of cells in the G1 phase after *MECR* knockdown was decreased, but there were no significant difference between two groups in S phase and G2/M phase.

significant difference in cell proportion (Figure 6). From the results, it was shown that although knockdown of the *MECR* gene significantly inhibited the proliferation of HCC BEL-7404 cells, it did not affect the cell cycle.

3.7. *Knockdown of MECR in BEL-7404 cells promoted cell apoptosis*

We first used Annexin V-APV to stain and culture the experimental group and the control group for 5 days, and then used flow cytometry to detect apoptosis of the two groups. After repeated three experiments, the results showed that the apoptosis rate of BEL-7404 cells in the experimental group after *MECR* knockdown was 9.38%, 9.66%, and 9.68%, and the mean value was 9.57%, while the apoptosis rate of BEL-7404 cells in NC group was 3.80 %, 3.56%, 3.69%, with an average of 3.68%. It can be seen that after *MECR* gene knockdown, the apoptosis rate of cells increased significantly, and the difference was statistically significant ($P < 0.01$, Figures 7A and 7B). In addition, we further detect and verify apoptosis by measuring the activity of Caspase 3-7. The results showed that compared with the NC group, the activity of Caspase 3-7 in the experimental group increased, suggesting that apoptotic cells increased, and the difference was statistically significant ($P < 0.01$ Figure 7C). The results showed that *MECR* knockdown signifcantly promoted apoptosis of the BEL-7404 cells.

3.8. *Knockdown of MECR inhibited BEL-7404 cells migration*

In this experiment, the *MECR* gene is a mitochondrial metabolism-related gene, and the mitochondria is a cell "energy plant". *MECR* knockdown may have an effect on cancer cells athletic ability. Therefore, in this experiment, the migrating ability of BEL-7404 cells knocking down the *MECR* gene was explored. This experiment was performed using the Transwell method to detect the migration ability of HCC cells. After 24 hours incubation in a transwell chamber, the transfected cells were stained and counted. As shown in Figure 8, the results showed that compared with the NC group, the number of metastatic cells in the experimental group knocking down the *MECR* was significantly decreased, and the difference was statistically significant ($P < 0.01$). The results indicated that knockdown of *MECR* gene can inhibit the migration of HCC BEL-7404 cells *in vitro*.

3.9. *Preliminary study on the regulation mechanism of MECR on HCC apoptosis*

This experiment has initially confirmed that knockdown of *MECR* gene can effectively promote apoptosis of HCC cells. Since this experiment is the first to report that the *MECR* gene is associated with tumors, there is currently no available reference to analyze its specific molecular biological mechanisms. Therefore, we have a preliminary exploration of the mechanism of apoptosis induced by *MECR* in HCC cells. Whether *MECR* gene knockdown can affect the expression of proteins associated with HCC apoptosis has been confirmed. We tested BAX and *TP53* proteins by means of wstern blot assay. As shown in Figure 9, the BAX protein showed no significant difference between the two groups, while the *TP53* protein showed a downward trend in the experimental group. Therefore, *TP53* gene and protein expression were further verified by qRT-PCR and immunofluorescence staining. The results showed that there was no significant difference in mRNA expression of *TP53* gene between the experimental group and the NC group, and there was no difference in expression between the two groups of *TP53* proteins by immunofluorescence detection. The results showed that knockdown of the *MECR* gene did not affect the expression of BAX and *TP53* proteins.

4. Discussion

According to WTO data, HCC is currently the fifth most common malignant tumor and is the second tumor-killing disease worldwide (*28*). Although

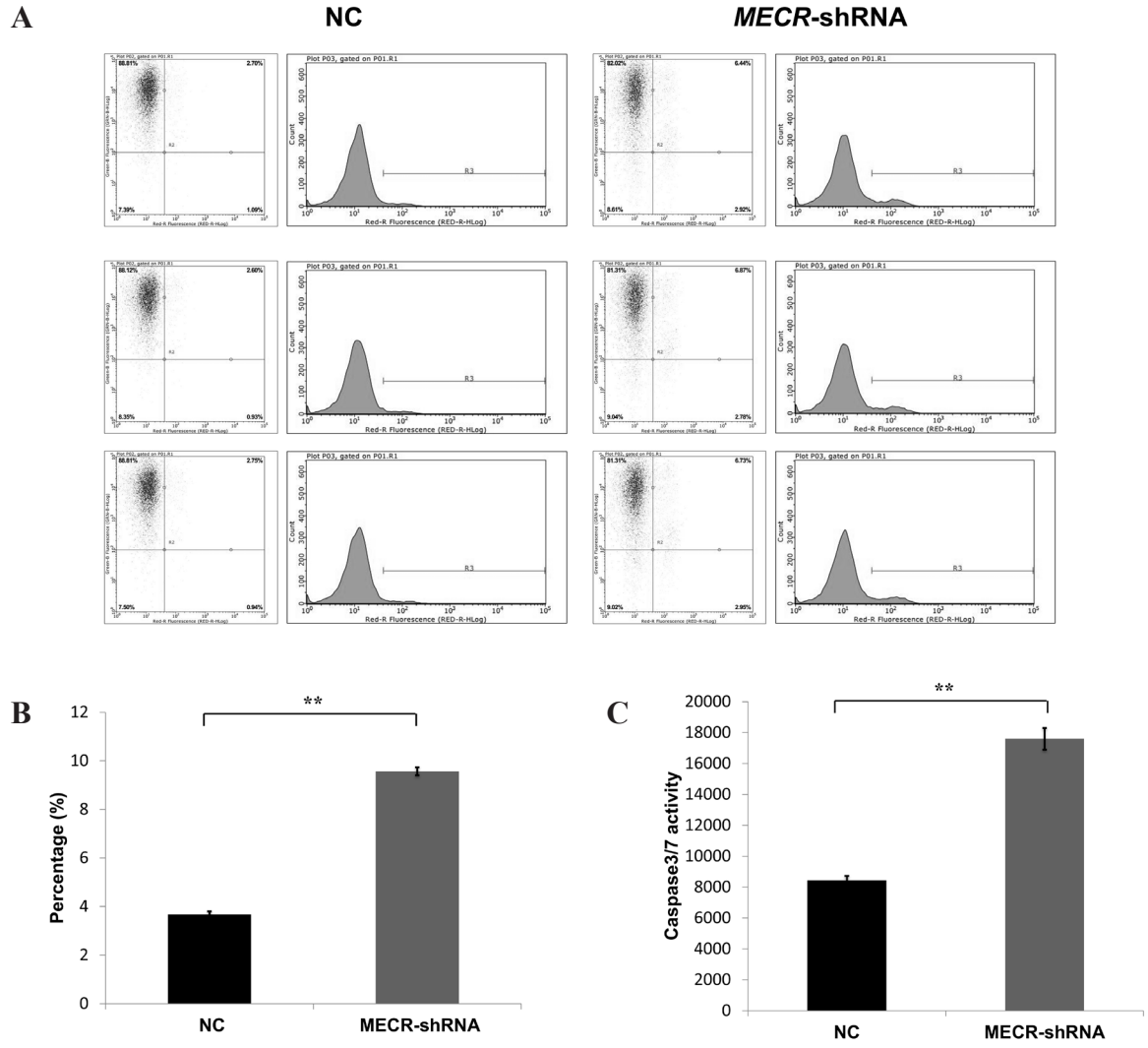

Figure 7. *MECR* silencing promoted HCC cell apoptosis. (A) Cell death was determined by Annexin V staining and flow cytometry; **(B)** Quantifcation of results showd the signifcant increase in apoptosis in the *MECR*-sihRNA cultures compared with the NC (**$P < 0.01$); **(C)** The results showed that the activity of Caspase 3-7 in the experimental group increased significantly (**$P < 0.01$).

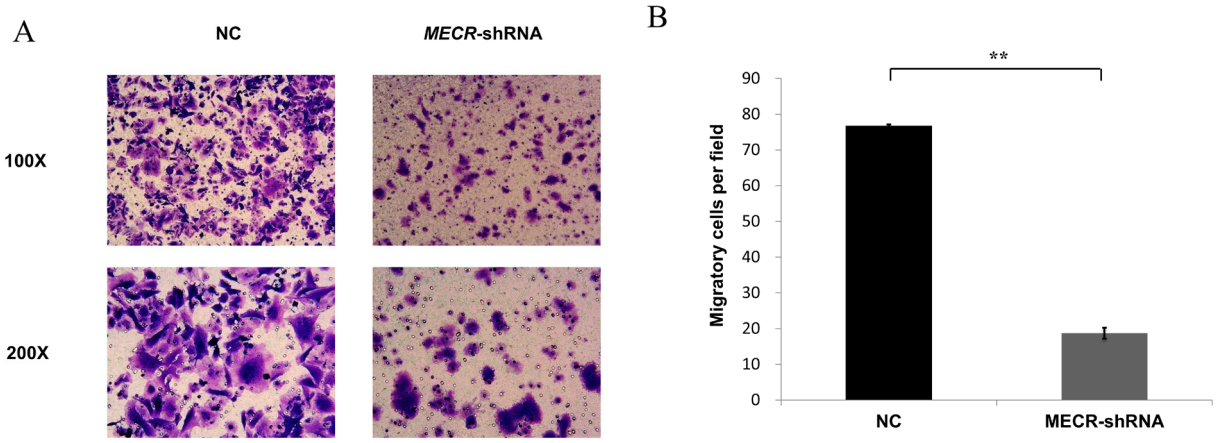

Figure 8. *MECR* silencing inhibited HCC cell migration. (A) Representative images of migration assays of BEL-7404 HCC cells transfected with *MECR*-shRNA or control lentivirus; **(B)** Quantification of results showed the number of metastatic cells in the experimental group knocking down the MECR was significantly decreased, and the difference was statistically significant (**$P < 0.01$).

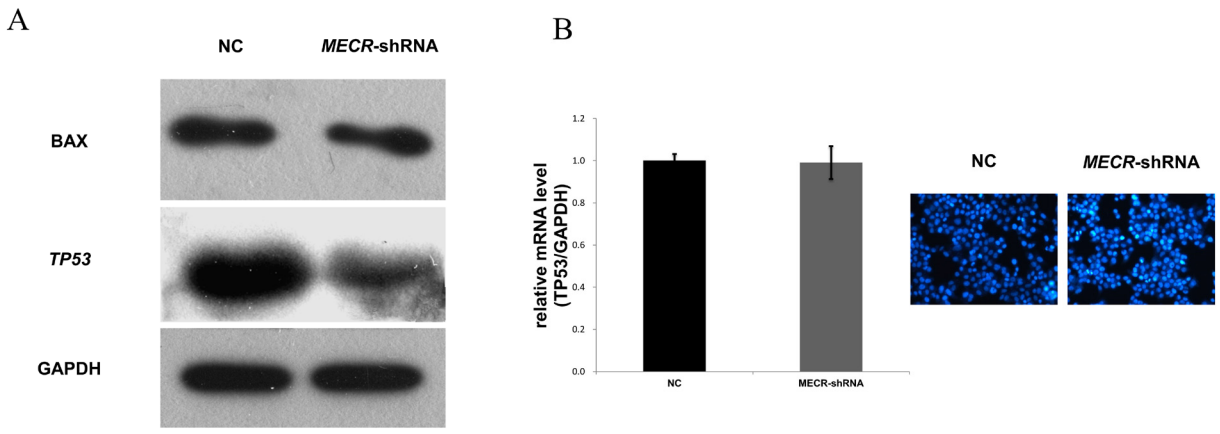

Figure 9. The expression of *BAX* and *TP53* proteins after knockdown *MECR*. (A) The protein levels of BAX and *TP53* were determined by western blot assays; **(B)** RT-PCR was used to assess the mRNA levels of TP53 and immunofluorescence was applied to verify the protein levels of *TP53*.

the current treatment of surgery, TACE, RFS and chemotherapy has improved the patient's 5 year survive rate, its high recurrence and metastasis rate make the overall therapeutic effect of HCC still unsatisfactory (*6,29*). With the expectation of more effective and thorough treatment, gene therapy is considered as a possible treatment (*30*). Therefore, it is particularly important to identify key genes and corresponding molecular mechanisms that affect tumor growth and recurrence (*31*).

The *MECR* gene is a protein-coding gene, and its expressed protein is a reductase that regulates the final step of mitochondrial lipid metabolism. It has not been reported in other tumors. This experiment found that the expression abundance of *MECR* gene in four HCC *in vitro* cell lines was high, suggesting that there is a link between high expression of *MECR* gene and HCC.

Lentivirus is a highly efficient gene vector (*32*). This experiment uses shRNA as an interference vector to transfect BEL-7404 cell line knockdown *MECR* gene with lentivirus as vector, and transfected with negative lentiviral shRNA as NC Control, a series of cytological tests to study the effect of *MECR* gene on the malignant behavior of HCC cell lines.

The results of this experiment show that *MECR*-shRNA lentivirus effectively knocks down the mRNA and protein expression of the *MECR* gene. On the basis of this, using cell-count by Cellomics Arrayscan with time-dependent manner, it was found that the knockdown of the *MECR* revealed that the growth of the HCC cell line BEL-7404 was inhibited.

In order to explore the reasons for knockdown of the *MECR* gene to induce cell growth inhibition, the cells were further assayed for apoptosis, cell cycle and cell clone formation. One of the important features of early apoptosis is the activation of the cysteine-specific protease Caspase family of proteins. This type of enzyme can participate in a series of biochemical reactions, respond to early signs of apoptosis and cause the corresponding protein substrate to cleave, which in turn triggers cell disintegration. Activation of the Caspase family can effectively promote apoptosis (*33,34*). Apoptosis-related experiments found that knockdown of the *MECR* gene effectively activated apoptosis, and the number of apoptotic cells in the experimental group increased and the caspase 3/7 activity was significantly increased. Cell cycle refers to the activity of cells from the end of the previous division to the end of the next division, divided into G0/G1 phase (diploid, DNA content is 2N), S phase (DNA content between G1 and G2) And G2/M phase (tetraploid, DNA content 4N). The results of cell cycle experiments in this experiment showed that although the number of cells in the G1 phase was small, the number of cells in the S phase was the same, and the number of cells in the G2/M phase was increased, only the G1 phase was statistically significant. The results indicate that knockdown of the *MECR* gene did not affect the HCC cell cycle. In addition, the results of colony formation experiments showed that after knockdown of MECR gene, the viability of individual cells decreased significantly, and the ability to form clones in experiment group was significantly inhibited. The above experiments show that *MECR* knockdown may increase the apoptosis by activating the apoptotic system, and inhibit the cell cloning ability, thereby inhibiting cell proliferation.

Although the 5-year overall survival rate of HCC patients can be as high as about 50% after surgery, its extremely high metastasis and recurrence is the main obstacle to further improve the survival of patients with HCC, and the recurrence rate can reach more than 60% after 5 years (*35*). In order to investigate whether the *MECR* gene affects the metastatic ability of HCC cells after affecting the metabolism of mitochondria, this experiment used transwell assay to test cell migration ability. The results showed that the migration ability of BEL-7404 cells in the experimental group knocking

down the *MECR* gene was significantly decreased, indicating that silencing the *MECR* gene can effectively inhibit the metastatic ability of HCC cells.

It is the next experimental research direction of this experiment to determine which signal pathway of *MECR* gene affects HCC cells. We underwent a preliminary exploration of proteins that have been closely related to tumor cell apoptosis. *TP53* has been widely studied and found to be an extremely important tumor suppressor gene. *TP53* gene mutation is found in many human cancer patients (*36,37*). Several studies have also shown that *TP53* protein plays an important role in HCC, regulating the apoptotic process of cells (*38,39*). BAX protein is another important protein in the regulation of tumor apoptosis, which can promote the release of cytochrome C and activate Caspase 9 and Caspase 3, which has been confirmed as one of the major proteins regulating apoptosis in HCC (*40,41*). This experiment explored the expression of these two important proteins of apoptosis exploratoryly. Unfortunately, there was no difference in the expression of *TP53* and BAX between the experimental group and the NC group. The mechanism of *MECR* gene in apoptosis needs further exploration. In addition, according to the current literature reports, the only clearer is that the high expression of the *MECR* gene can significantly activate *PPARα*. *PPARα* is a ligand of *PPARs*, which is mainly expressed in tissues with strong fatty acid metabolism such as liver, muscle and heart. It participates in lipid breakdown and oxidation to regulate lipid metabolism balance (*42*). Chang *et al*. found that miRNA-33a can promote Huh7 cell proliferation and inhibit apoptosis *in vitro*. The main target of this gene is *PPARα* (*43*). In addition, the Shah team found that activation of *PPARα* promoted HCC cell proliferation and induced hepatocarcinogenesis, and was associated with miRNA-let7C. In addition, mice with congenital deficiency of *PPARα* fed *PPARα* agonist Wy-14,643 did not induce liver cancer (*44*). In addition, there are a large number of studies in recent years that clarify that *PPARα* plays an important regulatory role in the development of HCC (*45-47*). The effect of *MECR* gene knockdown on HCC cells may be related to the *PPARα* pathway and have corresponding effects, which needs further experiments in this experimental group to verify.

5. Conclusion

The Warburg effect states that tumor cells provide function primarily through glycolysis even under aerobic conditions (*48*). In addition, due to the low glycolysis efficiency of tumor cells under aerobic conditions, it is a very important way for tumor cells to activate and strengthen lipid metabolism during the process of maintaining high-speed replication (*49*). Whether the metabolic abnormality of mitochondrial mtFASII dominated by *MECR* gene-coding protein is related to tumor diseases has not been reported yet. This experiment has confirmed the close relationship between *MECR* gene and HCC proliferation, apoptosis and metastasis through a series of cytological experiments. In conclusion, the results of this experiment show that the knockdown of the *MECR* gene can be effectively silenced by lentiviral transfection of the HCC cell line BEL-7404 *in vitro*. Knockdown of *MECR* can inhibited cell proliferation and colony formation, promoted apoptosis, and inhibited metastasis in HCC cell lines BEL-7404. Therefore, knockdown of *MECR* gene-targeted therapy can be a possible therapeutic approach in patients with high expression of the *MECR* gene. How the specific *MECR* gene affects the molecular pathway of the signaling pathway of HCC cell lines needs further experimental research.

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