

The Effect of miR122 and miR185 on Hepatic Differentiation of Human Umbilical Cord Mesenchymal Stem Cells

Qun YU

Kunming Medical University <https://orcid.org/0000-0001-9277-5046>

Sha LV

Kunming Municipal First People's Hospital

Wen Jun SU

Kunming Municipal First People's Hospital

Yan Feng YIN

Kunming Municipal First People's Hospital

Zheng GUAN

Kunming Municipal First People's Hospital

Lei ZHANG (✉ zlei01@hotmail.com)

<https://orcid.org/0000-0001-6514-0686>

Research

Keywords: human Umbilical Cord Mesenchymal Stem Cells (hUC-MSCs), hepatic inducing differentiation, miR122, miR185

Posted Date: June 29th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-37954/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Human umbilical cord mesenchymal stem cells (hUC-MSCs) have been considered as a promising cell source in liver diseases. miRNAs have been shown to play an important role in hepatic differentiation of hUC-MSCs. The study seeks to explore whether miR122 and miR185 could affect induction of hUC-MSCs into hepatic differentiation.

Methods: miR122 and miR185 stable overexpression by hUC-MSCs were firstly created, then hUC-MSCs were cultured by hepatic differentiation conditional medium. After 28 days culture in hepatic inducing conditional medium, hepatic markers expressed by these cells were detected by qRT-PCR and western-blot. The cell functions were also evaluated by PAS staining and ICG phagocytosis.

Results: Our results demonstrated that at the end of 28 days, hUC-MSCs overexpressing miR122 had increasing expression of hepatocyte markers including AFP, ALB, CK18, CK19 and HNF4a in both mRNA level and protein expression, while in the miR185 overexpression group, hUC-MSCs showed decreasing expression of hepatocyte markers. Moreover, there was also improvement of glycogen deposits as well as ICG phagocytosis ability in the hepatic inducing miR122 overexpression cells, while in the hepatic inducing miR185 overexpression group, hUC-MSCs showed decreasing glycogen deposits and ICG phagocytosis ability.

Conclusions: We thus speculate that it is possible to promote hepatic differentiation of hUC-MSCs by overexpression of miR122 and this effect may be inhibited by miR185 overexpression. It seems miR122 and miR185 have an antagonistic effect on hUC-MSCs hepatic differentiation. Overexpression of certain kind of miRNA in cells by transfection or other gene modification skills could be an effective way to modulate stem cell fate.

1. Background

The evolution from hepatitis to cirrhosis and end-stage liver failure is a trilogy of developmental process which is difficult to control in clinical treatment. Liver transplantation and cell therapy is considered as a promising solution to end-stage liver failure treatment^[1], which can effectively improve the quality of life and prolong the life of patients. However, liver donor and seeding cell shortage is an obstacle to the above two techniques. In order to solve the problem, researchers are trying to explore more effective treatment methods, such as immunotherapy^[2], stem cell therapy^[3] and artificial liver support^[4]. Induced hepatic differentiation of stem cells and the development of cell transplantation technology provide a potential prospect for liver replacement therapy.

MSC is one of the important members in the stem cell family. MSCs exist in almost all tissues and which can be isolated and successfully amplified in vitro from bone marrow^[5], adipose tissue^[6], umbilical cord^[7], placenta^[8], fetal liver, muscle tissue and lung tissue^[9, 10]. MSCs are a kind of cells with regenerative, secretory and immunomodulatory functions, which have the characteristics of self-

replication, multidirectional differentiation potential, hematopoietic support and promotion of stem cell implantation, immune regulation. MSCs have the potential for cellular therapy and have been shown to be beneficial for a variety of diseases^[11]. Human umbilical cord mesenchymal stem cells (hUC-MSCs) have the following characteristics, self-renewal, replicate, low immunogenicity and multi-directional differentiation potential^[12]. hUC-MSCs have been extensively studied in protective effect, immune regulation, disease prevention and disease treatment^[13-15]. In recent years, hUC-MSCs have become an ideal source of cells for cell therapy and tissue engineering research^[16].

miRNAs are involved in the regulation of multiple genes in embryonic development, such as differentiation, proliferation, and organ formation^[17]. In addition, in almost all types of diseases, miRNAs expression levels are significantly different from those in normal tissues^[18, 19]. Changes in miRNAs network are closely related to liver diseases, including steatohepatitis^[20], viral hepatitis^[21], liver fibrosis^[22], cirrhosis^[23] and hepatocellular carcinoma^[24, 25]. miRNAs play an important role in improving the efficiency of liver differentiation^[26]. miR122 is the most common and highly abundant miRNA in adult liver^[27]. miR122 has been shown to be an important host factor and antiviral target for hepatitis c virus infection^[24]. Lou et al^[28]. found that MSCs modified with miR122 were more conducive to the treatment of liver fibrosis. Reconstruction of miR122 expression is a promising therapeutic strategy in liver tumor diseases that simultaneously reduces tumor invasiveness and disease recurrence^[29]. Another miRNA associated with liver disease is miR185. In the study of Zhou et al.^[30], miR185 inhibits the activation of hepatic stellate cells by inhibiting the Ras homolog enriched in brain (RHEB) and rapamycin-insensitive companion of mammalian target of rapamycin (RICTOR) pathways, thus preventing the occurrence of liver fibrosis. miR185 has been found to be involved in the differentiation of myelogenic inhibitory cells^[31]. Inhibition of miR185 can promotes adipogenic differentiation^[32] and osteogenic differentiation^[33], but no study has been conducted on hepatic differentiation.

The purpose of this study is to explore the effect of miR122 and miR185 overexpression on the hepatic inducing differentiation of hUC-MSCs.

2. Methods

2.1 The culture and identification of hUC-MSCs

2.1.1 hUC-MSCs culture

Umbilical cord were obtained from health puerperant who delivered in our hospital and consented for the donation. The donation procedure and informed consent were approved by the ethics committee. The umbilical cords were firstly disinfected by immersion in 75% alcohol and rinsed in PBS to remove as much residual blood as possible. Then, the umbilical cords were cut into 3–5 cm segments and removed the arteries and veins. Wharton's jelly in tissues was cut into small pieces of 2 mm and evenly spread in a 6-well cell culture plates, and cultured in cell incubator (37°C, 5% CO₂) for one week in Dulbecco's

modified Eagle's medium (DMEM). The tissue blocks were removed after one week and the medium was replaced every three days.

2.1.2 Flow cytometry

Cells were treated with 0.5% trypsin, then being pipetted repeatedly to separate them into single cell suspension, and then being transferred into the flow tubes. After washing with PBS, cells were incubated respectively with antibodies as isoform control IgG-FITC/PE, CD34-FITC, CD45-FITC, CD90-PE, CD105-PE for 30 min. For Oct-4 analysis, cells were incubated and fixed in 1% paraformaldehyde at 4°C for 20 min after well suspension, Afterwards, they were centrifuged at 350 g for 10 min and washed twice with PBS. The cells were then re-suspended with PBS and labeled respectively with Oct-4-PE antibody and IgG-PE for 30 min. At the end of incubation, cells were washed thoroughly with PBS buffer and then tested by flow cytometry.

2.1.3 Multidirectional differentiation potential of hUC-MSCs

After 28 days of osteogenic and adipogenic differentiation culture, the cells were stained by Alizarin Red and Oil Red O, respectively, to identify calcium deposition and lipid droplets multidirectional differentiation potential of hUC-MSCs. The composition of osteogenic induction conditional medium is low glucose DMEM (Dulbecco's Modified Eagle Medium) with 10% fetal bovine serum (FBS), 10^{-7} mol/L dexamethasone, 10 mmol/L β -glycerophosphate, 50 mol/L L-ascorbic acid. While, that of the adipogenic induction conditional medium is high glucose DMEM with 10% FBS, 1 μ mol/L dexamethasone, 10 μ g/mL insulin, 0.5 mmol/L 3-isobutyl-1-methylxanthine (IBMX), 0.1 mmol/L indomethacin. The medium was replaced every three day.

After 4 weeks, the cells induced differentiation were washed with PBS twice and treated with 10% neutral formaldehyde for 30 min at room temperature. They were then rinsed with double distilled H₂O (ddH₂O) 5 min for 3 times. After that, cells were stained respectively with Alizarin Red and Oil Red O staining. For Alizarin Red staining, 0.5 mL of 0.5 mg/mL alizarin red dye solution was added into each well of 24-well culture plates and incubated for 20 min at room temperature. For Oil Red O staining, 0.5 mL of Oil Red O staining solution was added to each well of 24-well culture plates and treated at room temperature for one hour. Cells were then rinsed with ddH₂O until clean, and observed and took photos under the microscope.

2.2 miRNA overexpression by transfection and their influence on cell proliferation

hUC-MSCs were plated onto 6-well cell culture plates at a number of 5×10^4 cells followed by the 50 nM transfection concentration (The experiment was conducted according to the protocol suggested by RNA transfection kit (Ribobio, Guangzhou)). The experiment was divided into five groups: BC (blank control) group, NC (negative control) group (transfected with NC mimic), miR122 group (transfected with 50 nM miR122), miR185 group (transfected with 50 nM miR185), and miRAB group (transfected with both miR122 and miR185 50 nM each). After transfection of miRNA, hUC-MSCs were cultured in DMEM and

their expression of miR122 and miR185 in all groups were detected at day 1, 7, 14, 21 and 28 by qRT-PCR. For qPCR, total RNA, including miRNAs, was firstly extracted using Eastep Super total RNA extraction kit (Promega). Then, miRNA expression was performed using the miRNA qRT-PCR Starter Kit (Ribobio, Guangzhou) according to the manufacturer's instructions. Finally, miRNA relative expression levels were detected and normalized by housekeeping gene U6. Cell proliferations after transfection were also analyzed by CCK-8 method. And all of the above experiments were repeated at least four times.

2.3 Hepatic inducing differentiation of hUC-MSCs

hUC-MSCs hepatic inducing differentiation was performed by the following steps: hUC-MSC were pre-cultured firstly 2 days in low glucose DMEM with 20 ng/mL epidermal growth factor (EGF) and 10 ng/mL basic fibroblast growth factor (bFGF)). Then, they were cultured in Step1 conditional medium for 5 days, which is DMEM with 20 ng/mL hepatocyte growth factor (HGF), 10 ng/mL bFGF, and 0.61 g/L nicotine. Finally, cells were cultured in Step2 conditional medium (DMEM with 20 ng/mL oncostain M (OSM) + 1 m/L dexamethasone + 1% insulin transferrin sodium selenite supplement (ITS⁺) for 21 days.

hUC-MSCs were divided into six groups: BC group which are cells cultured in normal DMEM, BCin group which are normal hUC-MSCs cultured with hepatic inducing procedure, NCin group which are hUC-MSCs transferred with non-specific miRNA mimic and treated with hepatic inducing conditional medium, miR122in group which are hUC-MSCs overexpress miRNA122 and treated with hepatic inducing conditional medium, miR185in group which are hUC-MSCs overexpress miRNA185 and treated with hepatic inducing conditional medium, and miRABin group which are hUC-MSCs overexpress both miRNA122 and miRNA 185, and then treated with hepatic inducing conditional medium. Each experiment was repeated at least three times. After 28 days of culture, the expression level of ALB (albumin), AFP (alpha fetoprotein), CK18 (cytokeratin 18), CK19 (cytokeratin 19), and HNF4a (liver cell nucleus factor 4 a) in every group was detected by qRT-PCR for mRNA expression and by western-blot for protein expression. Cell function of glycogen storage and endocytosis were also tested by periodic acid-Schiff (PAS) staining and dilute indocyanine green (ICG).

2.3.1 RNA extraction and quantitative real time PCR (qRT-PCR)

Total RNA was extracted using Eastep Super total RNA extraction kit, Iscript cDNA synthesis kit (Bio-rad, USA) and PowerUp SYBR Green Master Mix (Thermo Fisher scientific, USA) were used for reverse transcription of cDNA amplification. Three-step procedure PCR was performed with the conditions listed in Table 1 and the primers used in this experiment was listed in Table 2. GAPDH was used to normalize the target gene expression as a reference gene.

Table 1
PCR amplification procedure

Cycles	Steps	Temperature°C	Time
1	Initial denaturation	95	10 min
40	Denaturation	95	15 s
	Annealing	59	60 s
	Extension	72	30 s
	Melt curve stage	60	60 s

Table 2
Primer sequences used for qRT-PCR

Target gene	Sequence (5'-3')	Length(bp)
AFP	F: GCTTGGTGGTGGATGAAACA R: TCCTCTGTTATTGTGGCTTTG	157
ALB	F:GCCTGCTGACTTGCCTTCATTAG R:TCAGCAGCAGCACGACAGAGTA	149
CK18	F:AATGGGAGGCATCCAGAACGAGAA R:GGGCATTGTCCACAGTATTGCGA	288
CK19	F:AGGAGATTGCCACCTACCG R:CCTTCCCATCCCTCTACCC	145
HNF4α	F: CTTCTTGACCCAGATGCCAAG R: GAGTCATACTGGCGGTCGTTG	111
GAPDH	F:AGCCACATCGCTCAGACAC R:GCCCAATACGACCAAATCC	66
miR122	F: UGGAGUGUGACAAUGGUGUUUG R: CAAACACCAUUGUCACACUCCA	22
miR185	F: UGGAGAGAAAGGCAGUUCUGA R: UCAGGAACUGCUUUCUCUCCA	22
NC	F:UCACAACCUCCUAGAAAGAGUAGA R:UCUACUCUUUCUAGGAGGUUGUGA	24

2.3.2 Protein extraction and Western-blot

The cells were collected at 28th day of cell culture in each group, and the total proteins of cells in each group were extracted by RIPA lysate. Western-blot was used to detect the expression of the hepatocyte specific proteins, ALB, AFP, CK18, CK19, and HNF4α (Proteintech, USA) in each group and the relative quantification was calculated referring to β-actin.

2.3.3 Cell function tests

Glycogen storage of hepatocyte like cells was evaluated with periodic acid-Schiff (PAS) staining. After 28 days of cell culture, the medium was washed with PBS twice, the cells were fixed with 4% neutral formaldehyde for 30 min, then iodine acid reagent were added and incubated for 10 min, then fixed cells were rinsed with ddH₂O for 3 times. Afterwards, Schiff's reagent (Sigma Aldrich, USA) were added and incubated with cells for 15 min. Cells were rinsed with ddH₂O again and stained in hematoxylin reagent for 90 seconds, rinsed once more with ddH₂O, dried naturally at room temperature, and observed.

0.5 mg/mL dilute indocyanine green (ICG) solution was prepared according to the production protocol in serum-free DMEM. After 28 days of cell culture, cells in each group were washed with PBS twice, 0.5 mg/mL ICG solution was added to the cell culture plates and cultured in incubator at 37°C for an hour. The ICG solution was removed, and cells were rinsed with PBS 3 times. Normal DMEM was added and ICG cells were observed the phagocytosis in each group. The culture medium was changed every 2 hours, and ICG in each group was observed after 6 hours.

2.4 Statistical analysis

Real-time PCR and western-blot data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) and post-hoc analysis were used to compare the differences between groups at the specified time points. P ≤ 0.05 was considered statistically significant. All statistical calculations were performed using the statistical package for social sciences (SPSS) for windows, version 16.

3. Results

3.1 Identification of hUC-MSCs

Cells isolated from Wharton's jelly of umbilical cord showed fibroblast like growth pattern and they were observed a uniform shape of long spindles, closely arranged in irregular shape and showed a vortex growth under microscopy (Fig. 1). Flow cytometry results showed a high positive expression of CD90 (99.16% ± 0.85%) and CD105 (99.26% ± 0.78%), but no expression of CD34 (0.59% ± 0.16%) and CD45 (1.50% ± 2.04%) by these cells and an average of 66.57% ± 4.61% cells expressed positively Oct-4, an embryonic stem cell marker (Fig. 1). After 28 days of osteogenic induction, calcium salt deposition in hUC-MSCs were detected by Alizarin red staining which became dense red nodules (Fig. 1). After 28 days of adipogenic induction, small lipid droplets in hUC-MSCs were observed after red oil O staining (Fig. 1).

The cells obtained from umbilical cord expressing specific MSC markers and had potential to be differentiated into osteoblast and adipocyte.

3.2 hUC-MSCs transfection with miRNA

hUC-MSCs were transfected by the 50 nM miRNA during 24 hours according to the protocol suggested by RNA transfection kit (Ribobio, Guangzhou). There was a plateau of miR122 and miR185 overexpression in all three miR122, miR185 and miRAB groups from day 1 to day 28 after transfection (Table 3, Table 4). Transfection of miRNA NC mimic had no effect on the expression level of miR122 and miR185 by hUC-MSC (Fig. 2). The number of cells were counted and used to draw cell growth curve which showed that the cells proliferated stably and grew well in all 5 groups (Fig. 2), which means the overexpression of miR122 and miR185 didn't have any impact on cell proliferation.

Table 3
The relative expression of miR122 in NC group, miR122 group and miRAB group (S)

Groups	D1	D7	D14	D21	D28
NC	1.03 ± 0.64	1.16 ± 0.59	0.50 ± 0.19	0.65 ± 0.35	0.45 ± 0.14
miR122	10.88 ± 2.91	11.16 ± 2.18	13.10 ± 0.47	14.21 ± 2.08	13.38 ± 3.26
miRAB	9.76 ± 3.21	10.23 ± 1.51	12.68 ± 0.42	13.34 ± 1.02	11.98 ± 2.68

Table 4
The relative expression of miR185 in NC group, miR185 group and miRAB group (S)

Groups	D1	D7	D14	D21	D28
NC	1.04 ± 1.05	0.55 ± 0.39	0.70 ± 0.47	1.02 ± 0.12	0.36 ± 0.37
miR185	10.88 ± 0.49	10.17 ± 0.41	10.43 ± 0.61	11.36 ± 1.47	11.70 ± 0.32
miRAB	9.32 ± 0.42	8.85 ± 0.56	8.68 ± 0.06	9.81 ± 1.12	9.43 ± 0.53

3.3 The effect of miR122 and miR185 overexpression on hepatic differentiation of hUC-MSCs

To study the effects of miR122 and miR185 on hUC-MSCs hepatic differentiation, hUC-MSCs firstly overexpressed miR122, miR185 or both by transfection, then were induced into hepatic differentiation using conditional culture medium. After 28 days of induction, q-RT-PCR results showed that the ALB expression level in miR122 group was the highest (1.72 ± 0.43) and the lowest in miR185 induction group (0.47 ± 0.17). The expression level of miR185 and the miRAB induction group (0.36 ± 0.13) was significantly lower than that in the BC induction group (1.10 ± 0.19) and NC induction group (1.41 ± 0.14). Although the ALB mRNA expression was the highest in miR122 group, there was no statistical significance between miR122 induction group and BC induction group ($P > 0.05$). As for the AFP, the expression level is the lowest in miR185 induction group (0.64 ± 0.27), which was significantly lower than

that in the BC induction group (2.29 ± 0.89). No statistical significance was observed among miR122 induction group (2.04 ± 0.96), BC induction group (2.29 ± 0.89) and NC induction group (2.39 ± 0.94) in AFP expression level ($P > 0.05$). The expression of CK18 and CK19 mRNA were the highest in miR122 induction group (1.93 ± 0.21 and 1.50 ± 0.27). The same is true for HNF4 α mRNA expression with an expression rate of 19.03 ± 2.13 . The expressions of CK18 (0.21 ± 0.07) and CK19 (0.29 ± 0.28) were decreased in the miR185 induction group, which were significantly lower ($P < 0.05$) than that in the BC induction group (0.84 ± 0.14 and 1.13 ± 0.25). And the expressions of CK19 (0.23 ± 0.15) and HNF4 α (0.58 ± 0.33) were decreased in the miRAB induction group, which were significantly lower ($P < 0.05$) than that in the BC induction group (1.13 ± 0.25 and 9.36 ± 1.81) (Fig. 3).

Western-blot results showed that the relative protein expressions of ALB (1.00 ± 0.05), AFP (0.85 ± 0.03), CK18 (1.13 ± 0.01), CK19 (1.09 ± 0.03), HNF4 α (1.41 ± 0.03) were increased in the miR122 hepatic induction group. While that of ALB (0.49 ± 0.03), CK18 (0.72 ± 0.03), CK19 (0.45 ± 0.02) and HNF4 α (0.24 ± 0.02) were decreased in the miR185 induction group. The expression level of ALB (0.62 ± 0.04) and AFP (0.19 ± 0.02) in the miRAB induction group was higher than that in the BC induction group and lower than miR122 induction group ($P < 0.05$). The expression level of CK19 (0.47 ± 0.03) and HNF4 α (0.36 ± 0.01) in the miRAB induction group was lower than that in the BC induction group ($P < 0.05$). And there was no statistical significance between the miRAB induction group (0.85 ± 0.05) and BC induction group (0.84 ± 0.03) in the expression of CK18 ($P > 0.05$) (Fig. 4).

Cell function test was performed after 28 days of hepatic differentiation induction. The PAS staining showed no glycogen deposition in BC group. Comparing the five induction groups, we found that the amount of glycogen deposition in BC induction group and NC induction group is almost the same, while this amount in miR122 induction group is significantly higher than that of BC induction group and NC induction group. The glycogen content in miR185 induction group was significantly lower than that of the BC induction group and the NC induction group. The glycogen concentration of the miRAB induction group was similar to that of BC induction group and NC induction group (Fig. 5).

In ICG phagocytosis and exocytosis experiment, after 1 hour incubation with ICG, no ICG was observed in BC group cells and the quantity of ICG in BC induction group and NC induction group were similar, while a large number of ICG accumulation was found in the miR122 induction group. ICG accumulation was significantly lower in miR185 induction group cells than that of BC and NC induction group. ICG in miRAB induction group cells was slightly less than that of BC and NC induction group, but a little bit more than that in miR185 induction group. ICG was seen to be almost completely exocytosis after 6 hours of culture in the ICG-free medium (Fig. 6).

4. Discussion

hUC-MSCs positive express MSCs' surface markers, such as CD44, CD90, CD105, EphA2. hUC-MSCs do not express CD11b, CD19, CD34, CD45 and so on the hematopoietic stem cell surface markers^[34, 35]. The pluripotent stem cells surface markers express in hUC-MSCs including Oct-4, Sox-2^[36]. hUC-MSCs have

the potential of multidirectional differentiation, and which can differentiate into chondrocytes^[37], hepatocytes^[38], and adipocytes^[39]. The cells we obtained from umbilical cord express CD90 (99.16% ± 0.85%) and CD105 (99.26% ± 0.78%), but no expression of CD34 (0.59% ± 0.16%) and CD45 (1.50% ± 2.04%) by these cells and 66.57% ± 4.61% cells expressed positively Oct-4 analyzed by flow cytometry. And they have as well the potential to be induced into osteoblast and adipocyte. Which shows that they have potential of multidirectional differentiation. We can conclude that the cells we isolated from Wharton's jelly are hUC-MSCs.

The result of hUC-MSCs miRNA transfection showed no effects on cell proliferation in all groups. qRT-PCR result showed that NC mimic had no effect on the expression of miR122 and miR185 of hUC-MSCs. The corresponding miRNA levels of miR122 group, miR185 group and miRAB group at days 1, 7, 14, 21 and 28 were stable and sustained at a high level, which are satisfied with the requirement of subsequent experiments. The stem cells transfected in our experiment have a strong self-amplification ability and can withstand a long screening process.

miR122 is a hepatic specific microRNA that plays an important role in liver self-regulation and hepatic differentiation^[40]. Studies have shown that miR122 promotes hepatic differentiation while miR122 in MSCs gradually increases during the process of induction. miR122 has been shown to promote hepatic differentiation by down-regulating the expression of cellular interstitial marker genes SOX11 and VIM^[26]. Down-regulation of miR122 transcription inhibits the hepatocyte differentiation potential of liver progenitor cells^[41]. Chien et al. found that miR122 can shorten the culture time of iPSC to hepatocyte differentiation. miR122-iPSC-Heps may be a viable cell source, which providing an effective alternative strategy for liver regeneration in acute liver failure^[40]. In our study, it was found that the expressions of ALB, AFP, CK18, CK19 and HNF4a in miR122 induction group were increased different degree compared with BC induction group. miR122 induction group also had stronger glycogen storage and ICG phagocytosis and exocytosis function than that of other groups. These two functions are two of the most important hepatocytic functions. Therefore, we speculate that miR122 had a certain promotion effect on hepatic differentiation of hUC-MSCs. We also transfected miR185 in hUC-MSCs because it is reported associating with cell induced differentiation and has been found to be involved in the differentiation of myelogenic inhibitory cells^[31]. It has been shown that overexpression of miR185 can lead to a significant reduction in lipid accumulation, while the low level of miR185 can promote adipogenic differentiation^[32]. Studies have demonstrated that the inhibition of miR185 could promote bone formation and osteogenic differentiation^[33, 42, 43]. In this study, it was found that the expressions of hepatic relative genes and proteins by cells overexpression of miR185 induction group were decreased compared with normal hUC-MSCs after hepatic induction differentiation, which means, differentiation of hUC-MSCs was delayed by overexpression of miR185. Glycogen storage and ICG phagocytosis and exocytosis function were inhibited in miR185 overexpression cells comparing with normal hUC-MSCs differentiation induction group. Therefore, we hypothesize that miR185 could inhibit the hepatic differentiation of hUC-MSCs. There was no significant change in hepatic specific protein expression and functions in cells overexpressed both miR122 and miR185 induction group compared with normal hUC-MSCs

differentiation induction group. We thus surmise that the effect of miR122 and miR185 are contradicting and they can be neutralized by each other. The limitation of this study is that we have not been able to conduct animal experiments to further prove our findings.

5. Conclusions

In conclusion of the above, overexpression of miR122 can promote hUC-MSCs differentiating into hepatocytes. While, overexpression of miR185 inhibits hepatic differentiation of hUC-MSCs. It seems that miR122 and miR185 have an antagonistic effect on hUC-MSCs hepatic differentiation. Overexpression of certain kind of miRNA in cells by transfection or other gene modification skills could be an effective way to modulate stem cell fate.

Abbreviations

Abbreviations	
AFP	Alpha fetoprotein
ALB	Albu min
BC	Blank control
bFGF	Basic fibroblast growth factor
bp	Base pair
CD	Clusters of differentiation
CK	Cytokeratin
DMEM	Dulbecco's modified eagle medium
EGF	Epidermal growth factor
FITC	Fluorescein isothiocyanate
GAPDH	Glyceraldehydes-3-phosphate dehydrogenase
HGF	Hepatocyte growth factor
HNF4α	Hepatocyte nuclear factor 4α
hUC-MSCs	human Umbilical cord Mesenchymal Stem Cells
IBMX	3-isobutyl-1-methylxanthine
ICG	Indocyanine Green
ITS ⁺	Insulin transferrin sodium selenite supplement
mRNA	Messenger ribonucleic acid
miRNA	microRNA
MSCs	Mesenchymal Stem Cells
NC	Negative control
Oct-4	Octamer-binding transcription factor 4
OSM	Oncostain M
PAS	Periodic Acid-Schiff Stain
PBS	Phosphate buffer saline
q-RT-PCR	Quantitative real-time polymerase chain reaction

Declarations

Ethics approval and consent to participate:

Approved by the ethics committee, the informed consent of healthy full-term pregnant women was obtained, and the donation consent was signed.

Consent for publication:

Not applicable.

Availability of data and materials:

The datasets generated and/or analysed during the current study are not publicly available due [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

Competing interests:

The Authors declared that they have no conflict of interests.

Funding:

National Nature Science Foundation of China (81660303) and Nature Science Foundation of Yunnan Province – United with Kunming Medical University (2017FE67).

Authors' contributions:

Each author is expected to have made substantial contributions to the conception OR design of the work; OR the acquisition, analysis, OR interpretation of data; OR the creation of new software used in the work; OR have drafted the work or substantively revised it.

Acknowledgements:

The authors would like to acknowledge support from National Nature Science Foundation of China (81660303) and Nature Science Foundation of Yunnan Province – United with Kunming Medical University (2017FE67).

References

1. Sarin S K, Kedarisetty C K, Abbas Z, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the Study of the Liver (APASL) 2014 [J]. *Hepatol Int*, 2014, 8(4): 453-471.
2. Niu Y H, Yin D L, Liu H L, et al. Restoring the Treg cell to Th17 cell ratio may alleviate HBV-related acute-on-chronic liver failure [J]. *World journal of gastroenterology*, 2013, 19(26): 4146-4154.
3. Lin B L, Chen J F, Qiu W H, et al. Allogeneic bone marrow-derived mesenchymal stromal cells for hepatitis B virus-related acute-on-chronic liver failure: A randomized controlled trial [J]. *Hepatology*, 2017, 66(1): 209-219.
4. Wan Y M, Li Y H, Xu Z Y, et al. Therapeutic plasma exchange versus double plasma molecular absorption system in hepatitis B virus-infected acute-on-chronic liver failure treated by entercavir: A prospective study [J]. *J Clin Apher*, 2017, 32(6): 453-461.
5. Anjos-Afonso F, Bonnet D. Nonhematopoietic/endothelial SSEA-1+ cells define the most primitive progenitors in the adult murine bone marrow mesenchymal compartment [J]. *Blood*, 2007, 109(3): 1298-1306.
6. Zuk P A, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies [J]. *Tissue engineering*, 2001, 7(2): 211-228.
7. Fu Y S, Lu C H, Chu K A, et al. Xenograft of Human Umbilical Mesenchymal Stem Cells from Wharton's Jelly Differentiating into Osteocytes and Reducing Osteoclast Activity Reverses Osteoporosis in Ovariectomized Rats [J]. *Cell transplantation*, 2018, 27(1): 194-208.
8. Luan X, Li G, Wang G, et al. Human placenta-derived mesenchymal stem cells suppress T cell proliferation and support the culture expansion of cord blood CD34(+) cells: a comparison with human bone marrow-derived mesenchymal stem cells [J]. *Tissue & cell*, 2013, 45(1): 32-38.
9. In 't Anker P S, Scherjon S A, Kleijburg-van der Keur C, et al. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta [J]. *Stem Cells*, 2004, 22(7): 1338-1345.
10. Bianco P, Robey P G, Simmons P J. Mesenchymal stem cells: revisiting history, concepts, and assays [J]. *Cell Stem Cell*, 2008, 2(4): 313-319.
11. Wei X, Yang X, Han Z P, et al. Mesenchymal stem cells: a new trend for cell therapy [J]. *Acta Pharmacol Sin*, 2013, 34(6): 747-754.
12. Weiss M L, Anderson C, Medicetty S, et al. Immune properties of human umbilical cord Wharton's jelly-derived cells [J]. *Stem Cells*, 2008, 26(11): 2865-2874.
13. Zhang G Z, Sun H C, Zheng L B, et al. In vivo hepatic differentiation potential of human umbilical cord-derived mesenchymal stem cells: Therapeutic effect on liver fibrosis/cirrhosis [J]. *World journal of gastroenterology*, 2017, 23(46): 8152-8168.
14. Yao J, Zheng J, Cai J, et al. Extracellular vesicles derived from human umbilical cord mesenchymal stem cells alleviate rat hepatic ischemia-reperfusion injury by suppressing oxidative stress and neutrophil inflammatory response [J]. *FASEB J*, 2019, 33(2): 1695-1710.
15. Quaranta P, Focosi D, Di Iesu M, et al. Human Wharton's jelly-derived mesenchymal stromal cells engineered to secrete Epstein-Barr virus interleukin-10 show enhanced immunosuppressive

- properties [J]. *Cyotherapy*, 2016, 18(2): 205-218.
16. Yang J-F, Cao H-C, Pan Q-L, et al. Mesenchymal stem cells from the human umbilical cord ameliorate fulminant hepatic failure and increase survival in mice [J]. *Hepatobiliary & Pancreatic Diseases International*, 2015, 14(2): 186-193.
17. Cui L, Zhou X, Li J, et al. Dynamic microRNA profiles of hepatic differentiated human umbilical cord lining-derived mesenchymal stem cells [J]. *PLoS One*, 2012, 7(9): e44737.
18. Volinia S, Calin G A, Liu C G, et al. A microRNA expression signature of human solid tumors defines cancer gene targets [J]. *Proc Natl Acad Sci U S A*, 2006, 103(7): 2257-2261.
19. Lu J, Getz G, Miska E A, et al. MicroRNA expression profiles classify human cancers [J]. *Nature*, 2005, 435(7043): 834-838.
20. Cai C, Lin Y, Yu C. Circulating miRNAs as Novel Diagnostic Biomarkers in Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis [J]. *Can J Gastroenterol Hepatol*, 2019, 2019(2096161).
21. Luna J M, Scheel T K, Danino T, et al. Hepatitis C virus RNA functionally sequesters miR-122 [J]. *Cell*, 2015, 160(6): 1099-1110.
22. Hyun J, Wang S, Kim J, et al. MicroRNA-378 limits activation of hepatic stellate cells and liver fibrosis by suppressing Gli3 expression [J]. *Nat Commun*, 2016, 7(10993).
23. El-Araby R E, Khalifa M A, Zoheiry M M, et al. The interaction between microRNA-152 and DNA methyltransferase-1 as an epigenetic prognostic biomarker in HCV-induced liver cirrhosis and HCC patients [J]. *Cancer Gene Ther*, 2019,
24. Bandiera S, Pfeffer S, Baumert T F, et al. miR-122—a key factor and therapeutic target in liver disease [J]. *J Hepatol*, 2015, 62(2): 448-457.
25. Lowey B, Hertz L, Chiu S, et al. Hepatitis C Virus Infection Induces Hepatic Expression of NF-kappaB-Inducing Kinase and Lipogenesis by Downregulating miR-122 [J]. *mBio*, 2019, 10(4):
26. Raut A, Khanna A. Enhanced expression of hepatocyte-specific microRNAs in valproic acid mediated hepatic trans-differentiation of human umbilical cord derived mesenchymal stem cells [J]. *Exp Cell Res*, 2016, 343(2): 237-247.
27. Davoodian N, Lotfi A S, Soleimani M, et al. The combination of miR-122 overexpression and Let-7f silencing induces hepatic differentiation of adipose tissue-derived stem cells [J]. *Cell biology international*, 2017, 41(10): 1083-1092.
28. Lou G, Yang Y, Liu F, et al. MiR-122 modification enhances the therapeutic efficacy of adipose tissue-derived mesenchymal stem cells against liver fibrosis [J]. *Journal of cellular and molecular medicine*, 2017, 21(11): 2963-2973.
29. Boix L, Lopez-Oliva J M, Rhodes A C, et al. Restoring miR122 in human stem-like hepatocarcinoma cells, prompts tumor dormancy through Smad-independent TGF-beta pathway [J]. *Oncotarget*, 2016, 7(44): 71309-71329.

30. Zhou L, Liu S, Han M, et al. miR-185 Inhibits Fibrogenic Activation of Hepatic Stellate Cells and Prevents Liver Fibrosis [J]. *Molecular therapy Nucleic acids*, 2018, 10(91-102).
31. Shang W, Tang Z, Gao Y, et al. LncRNA RNCR3 promotes Chop expression by sponging miR-185-5p during MDSC differentiation [J]. *Oncotarget*, 2017, 8(67): 111754-111769.
32. Ning C, Li G, You L, et al. MiR-185 inhibits 3T3-L1 cell differentiation by targeting SREBP-1 [J]. *Bioscience, biotechnology, and biochemistry*, 2017, 81(9): 1747-1754.
33. Chang H, Wang Y, Liu H, et al. Mutant Runx2 regulates amelogenesis and osteogenesis through a miR-185-5p-Dlx2 axis [J]. *Cell Death Dis*, 2017, 8(12): 3221.
34. Hao S C, Ma H, Niu Z F, et al. hUC-MSCs secreted exosomes inhibit the glioma cell progression through PTENP1/miR-10a-5p/PTEN pathway [J]. *European review for medical and pharmacological sciences*, 2019, 23(22): 10013-10023.
35. Wen Y C, Du M K, Li M W, et al. EphA2-positive human umbilical cord-derived mesenchymal stem cells exert anti-fibrosis and immunomodulatory activities via secretion of prostaglandin E2 [J]. *Taiwanese journal of obstetrics & gynecology*, 2018, 57(5): 722-725.
36. Borys-Wojcik S, Brazert M, Jankowski M, et al. Human Wharton's jelly mesenchymal stem cells: properties, isolation and clinical applications [J]. *Journal of biological regulators and homeostatic agents*, 2019, 33(1): 119-123.
37. Li X, Chang H, Luo H, et al. Poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) scaffolds coated with PhaP-RGD fusion protein promotes the proliferation and chondrogenic differentiation of human umbilical cord mesenchymal stem cells in vitro [J]. *Journal of biomedical materials research Part A*, 2015, 103(3): 1169-1175.
38. Bharti D, Shivakumar S B, Park J K, et al. Comparative analysis of human Wharton's jelly mesenchymal stem cells derived from different parts of the same umbilical cord [J]. *Cell Tissue Res*, 2018, 372(1): 51-65.
39. Ozkan S, Isildar B, Oncul M, et al. Ultrastructural analysis of human umbilical cord derived MSCs at undifferentiated stage and during osteogenic and adipogenic differentiation [J]. *Ultrastructural pathology*, 2018, 42(3): 199-210.
40. Chien Y, Chang Y L, Li H Y, et al. Synergistic effects of carboxymethyl-hexanoyl chitosan, cationic polyurethane-short branch PEI in miR122 gene delivery: accelerated differentiation of iPSCs into mature hepatocyte-like cells and improved stem cell therapy in a hepatic failure model [J]. *Acta Biomater*, 2015, 13(228-244).
41. Tanimizu N, Kobayashi S, Ichinohe N, et al. Downregulation of miR122 by grainyhead-like 2 restricts the hepatocytic differentiation potential of adult liver progenitor cells [J]. *Development*, 2014, 141(23): 4448-4456.
42. Cui Q, Xing J, Yu M, et al. Mmu-miR-185 depletion promotes osteogenic differentiation and suppresses bone loss in osteoporosis through the Bgn-mediated BMP/Smad pathway [J]. *Cell Death Dis*, 2019, 10(3): 172.

Figures

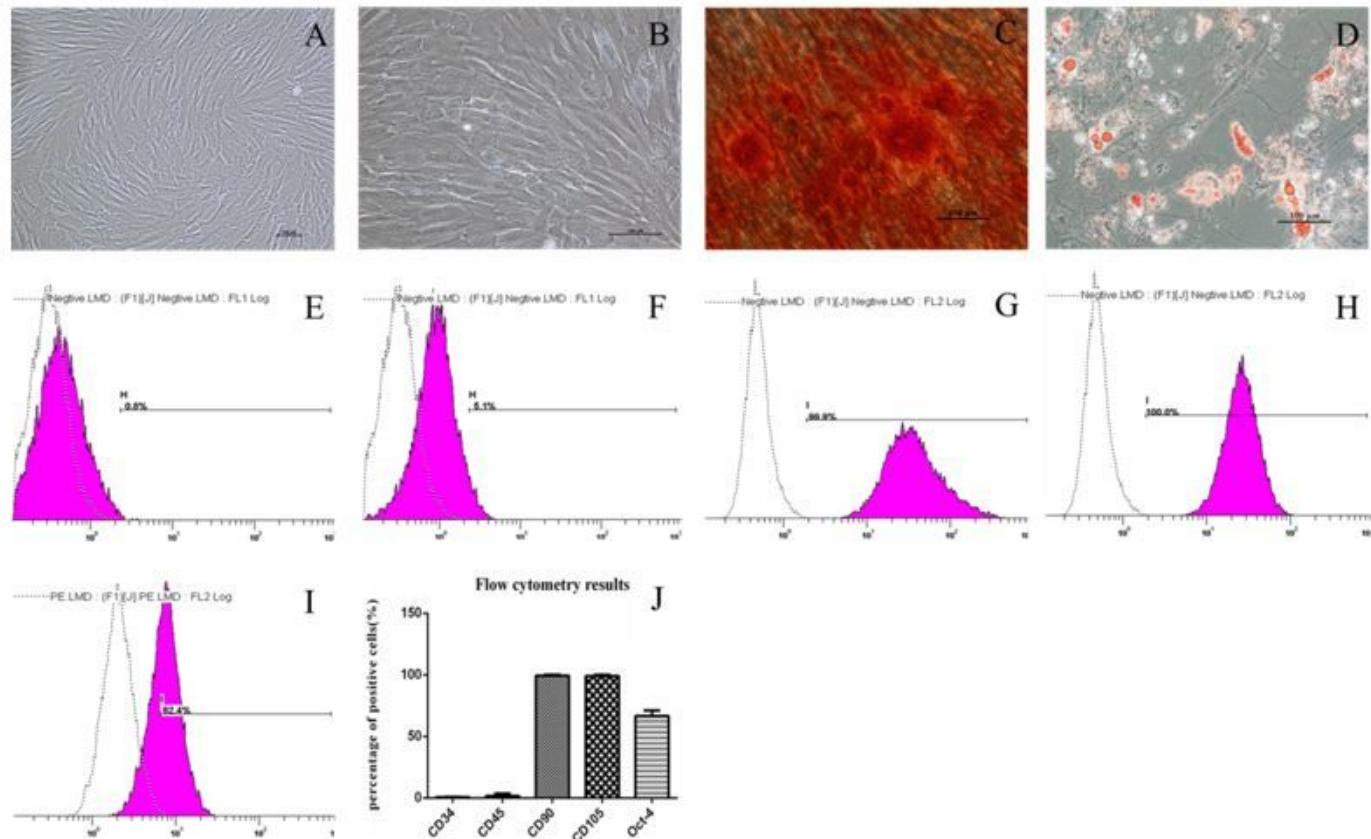


Figure 1

hUC-MSCs culture and identification. A: The hUC-MSCs are spindle-shaped and densely packed, with a few showing a swirl (Original magnification, 100×). B: Cell morphology at (Original magnification, 200×). Scale bars, 100 μm. C and D are the picture of identification of the differentiation potential of hUC-MSCs. C: After 28 days of osteogenic induction culture, hUC-MSC showed calcium nodule deposition stained on red by Alizarin Red. D: After 28 days of adipogenic induction culture, lipid droplets in hUC-MSCs were stained on red by Oil Red O and observed under microscope. (Original magnification, 200×). Scale bars, 100 μm. E, F, G, H and I represent the expression of CD34, CD45, CD90, CD105 and Oct-4 of hUC-MSCs detecting by flow cytometry (n=9), respectively. J: Histogram of molecular expressions on hUC-MSCs.

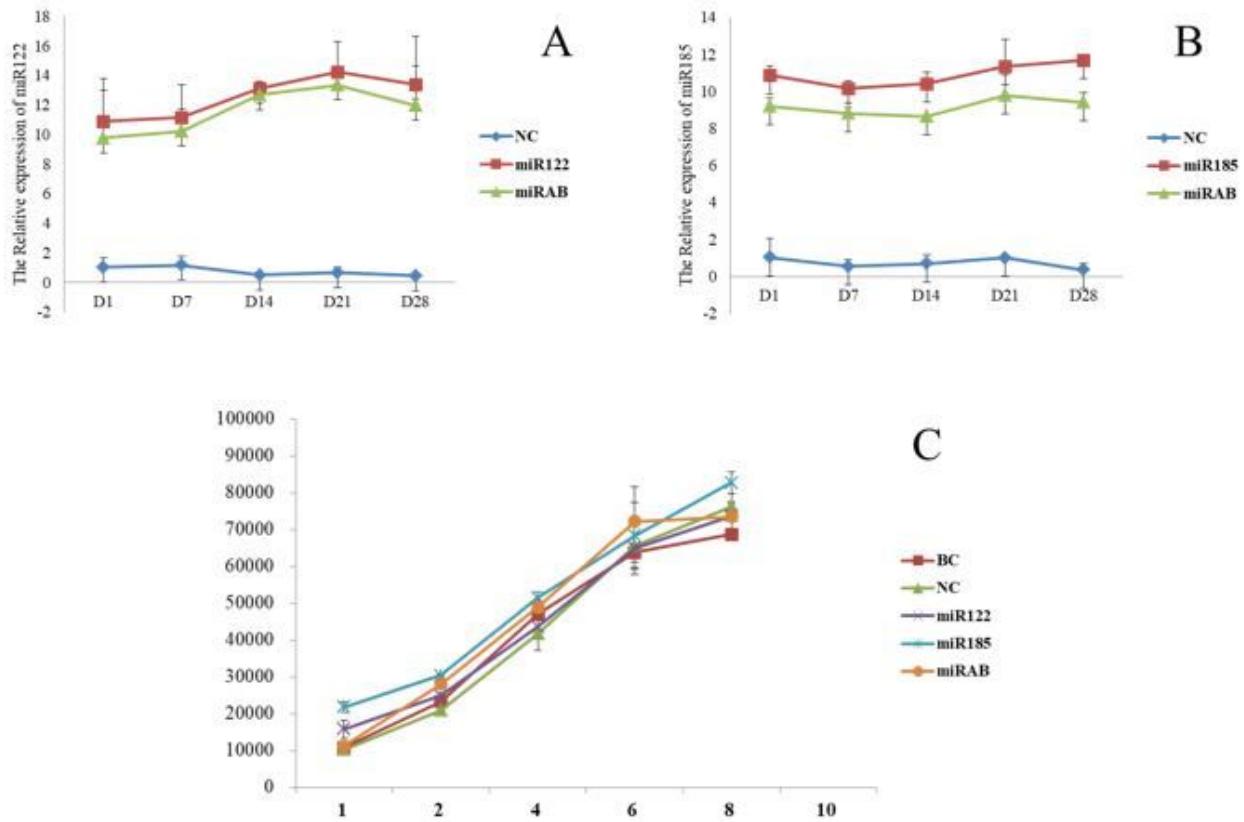


Figure 2

A and B represent the relative expression of miR122 and miR185, respectively, in NC group, miR122 group and miRAB group (n=3). C: The proliferation of hUC-MSCs after the over-expression of miRNA (n=3)

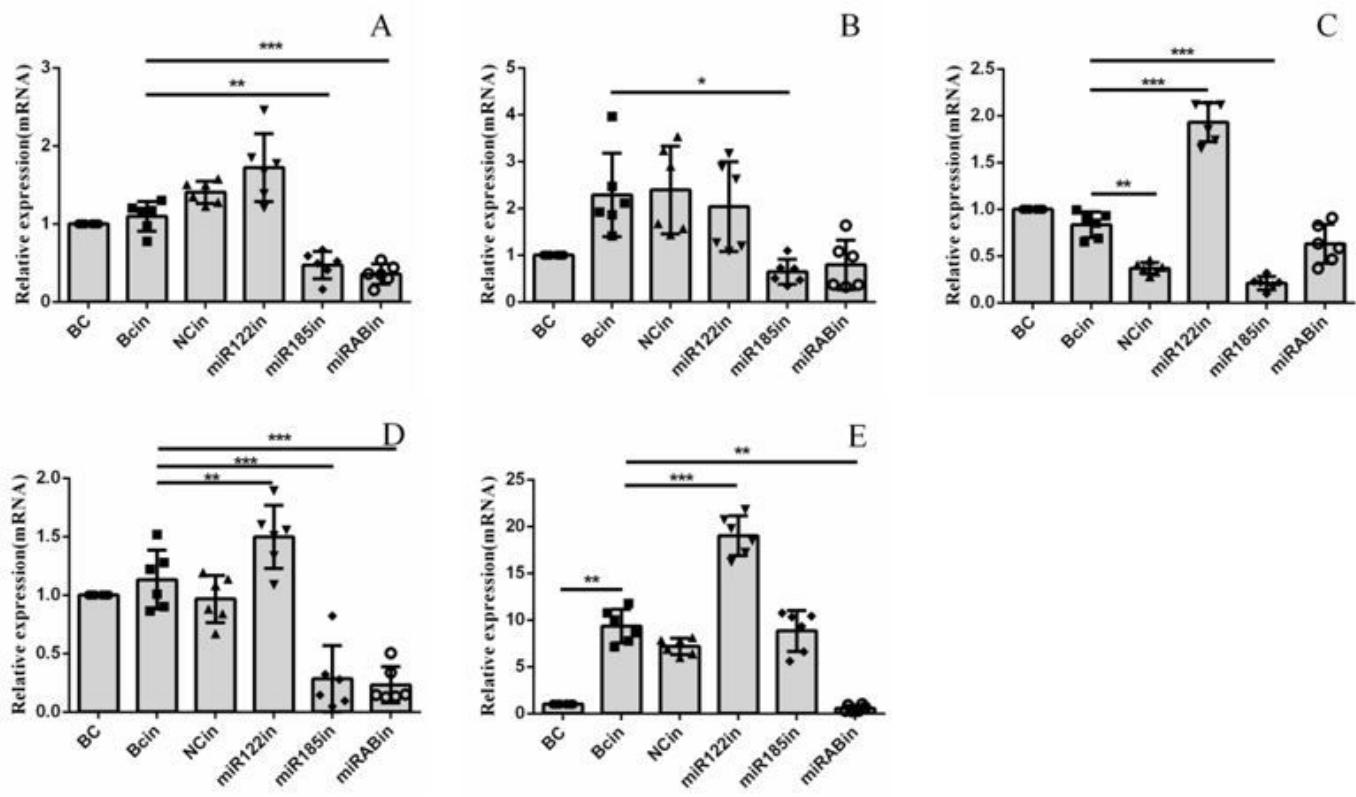


Figure 3

mRNA expression of hepatocyte-specific protein by hUC-MSC analyzed by qRT-PCR ($n=6$). A, B, C, D and E represent the relative mRNA expression of ALB, AFP, CK18, CK19 and HNF4 α in each group, respectively ($n=6$). “*” represents $P \geq 0.05$ compared with the BC group, “**” and “***” represent $P \leq 0.025$ and $P \leq 0.001$ compared with BC group, respectively. The semiquantitative analysis was performed with help of ABI 7500 and statistic analysis was performed with GraphPad prism 6.0 Software.

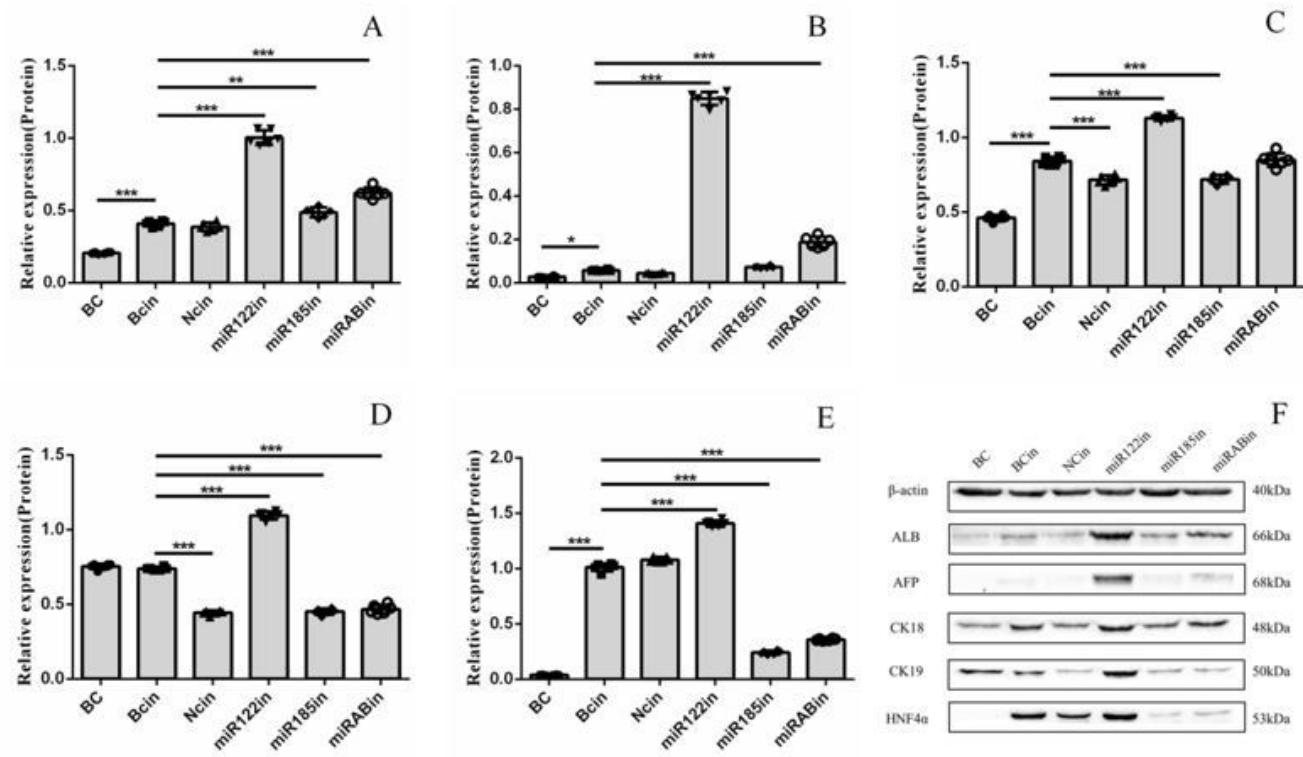


Figure 4

Expression of hepatocyte-specific proteins detected by western-blot ($n=6$). A, B, C, D and E represent the proteins expression of ALB, AFP, CK18, CK19 and HNF4 α in each group, respectively. “*” represents $P \geq 0.05$ compared with the BC group, “**” and “***” represent $P \leq 0.025$ and $P \leq 0.001$ compared with BC group, respectively. F-Chemiluminescence imaging of each experimental group. The imaging analysis software (Bio-Rad, USA) was used for analyzing the images and statistic analysis was performed with GraphPad prism 6.0 Software.

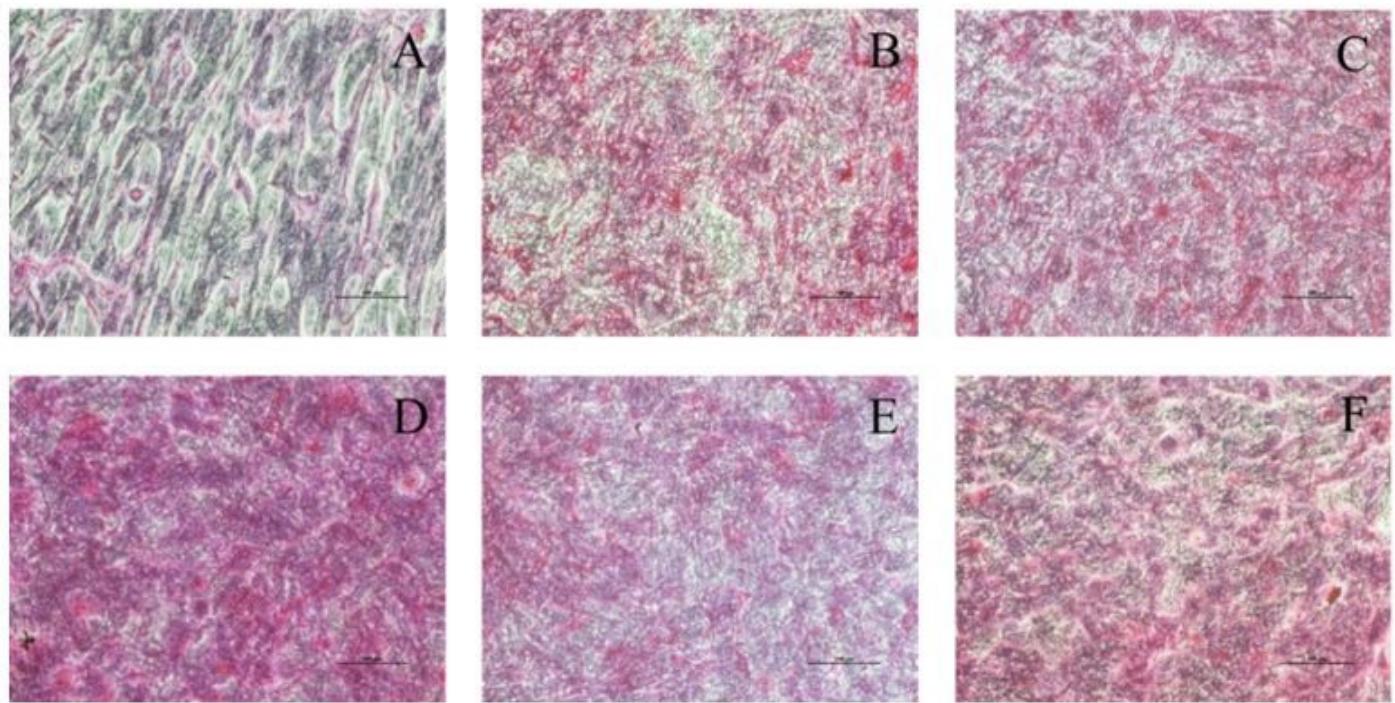


Figure 5

Photos of periodic acid-Schiff (PAS) stain in different groups. A, B, C, D, E and F represent the BC group, BC induction group, NC induction group, miR122 induction group, miR185 induction group and miRAB induction group, respectively. (Original magnification, 200 \times). Scale bars, 100 μ m.

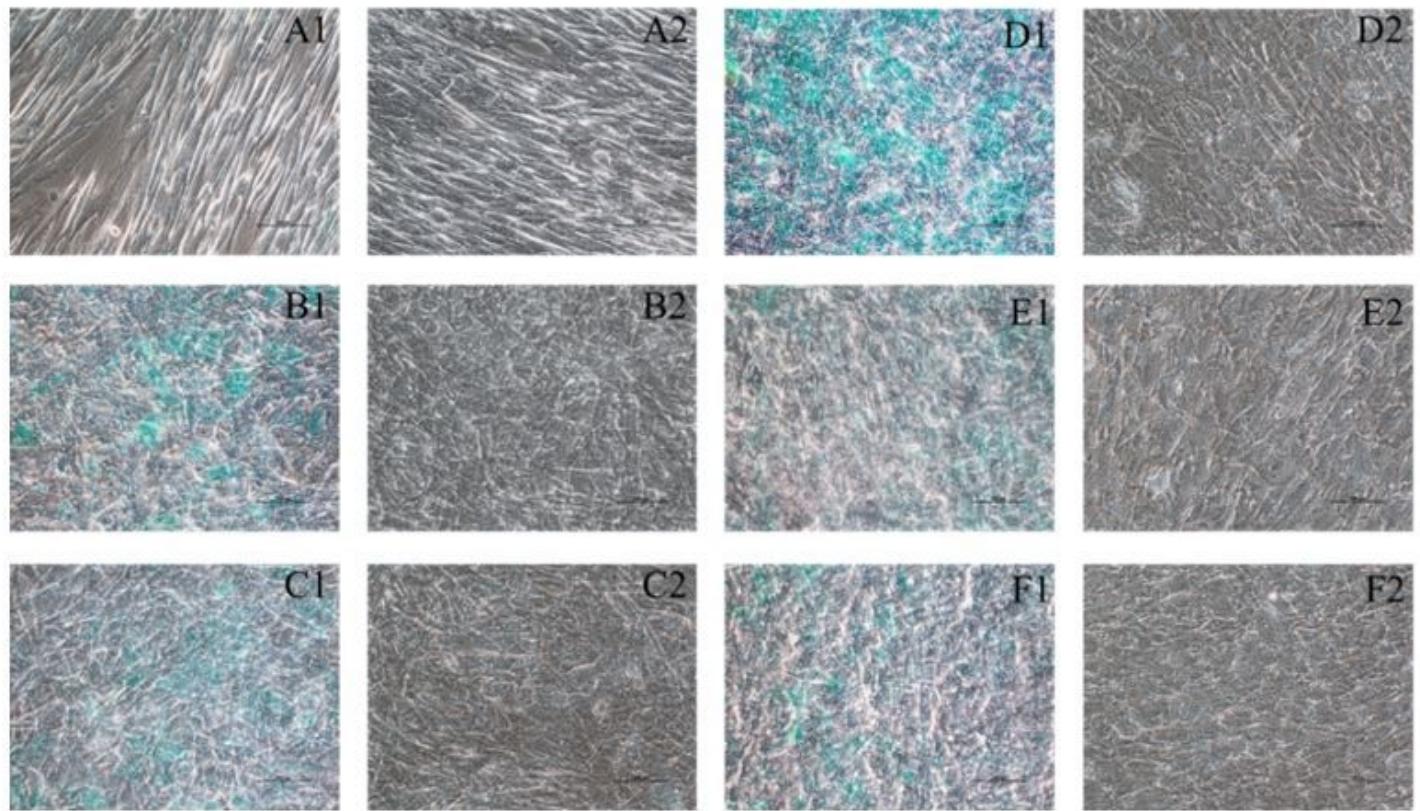


Figure 6

Indocyanine green phagocytic assay. A, B, C, D, E and F represent the BC group, BC induction group, NC induction group, miR122 induction group, miR185 induction group and miRAB induction group, respectively. A1, B1, C1, D1, E1 and F1 represent after 1 h incubation with ICG. A2, B2, C2, D2, E2 and F2 represent ICG was observed after 6 hours. (Original magnification, 200 \times). Scale bars, 100 μ m