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The biological effects of hsa-miR-1908 in human adipocytes

Lei Yang · Chun-mei Shi · Ling Chen · Ling-xia Pang · Guang-feng Xu · Nan Gu · Li-jun Zhu · Xi-rong Guo · Yu-hui Ni · Chen-bo Ji

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Abstract MicroRNAs (miRNAs) are small non-coding RNAs involved in the regulation of gene expression. MiR-1908 is a recently identified miRNA that is highly expressed in human adipocytes. However, it is not known what role of miR-1908 is involved in the regulation of human adipocytes. In this study, we demonstrate that the level of miR-1908 increases during the adipogenesis of human multipotent adipose-derived stem (hMADS) cells and human preadipocytes-visceral. Overexpression of miR-1908 in hMADS cells inhibited adipogenic differentiation and increased cell proliferation, suggesting that miR-1908 is involved in the regulation of adipocyte cell differentiation and metabolism, and, thus, may have an effect on human obesity.

Keywords Obesity · Hsa-miR-1908 · Human adipocytes · Adipogenisis

L. Yang · C. Shi · L. Chen · L. Pang · X. Guo · Y. Ni (✉) · C. Ji (✉)
Institute of Pediatrics, Nanjing Medical University, No. 140 Hanzhong Road, Nanjing 210029, China
e-mail: niyuhui@hotmail.com

C. Ji
e-mail: chenboji@njmu.edu.cn

L. Yang · C. Shi · L. Chen · L. Pang · N. Gu · L. Zhu · X. Guo · Y. Ni · C. Ji
Department of Children Health Care, Nanjing Maternity and Child Health Care Hospital Affiliated to Nanjing Medical University, No. 123 Tianfei Road, Nanjing 210004, China

G. Xu
Department of Laboratory Medicine, The 82nd Hospital of the People's Liberation Army, No. 100 Jiankang East Road, Huaian 223001, China

Introduction

The obesity pandemic has become a significant health threat, and has resulted in considerably higher morbidity and mortality rates and increasing healthcare costs [1]. In humans, a pool of multipotent progenitor cells (such as hMADS cells) persists in adipose tissue throughout life, and is able to differentiate to form adipocytes [2–4]. Obesity is an excessive accumulation of adipose tissue, so we can tell the importance of hMADS cells on human obesity.

MicroRNAs (miRNAs) are endogenous, highly conserved small non-coding RNA molecules of about 22 nucleotides in length that regulate gene expression by binding to the 3′ untranslated region (3′-UTR) of the complementary target mRNA sequence, resulting in translational repression and/or gene silencing [5–7]. Through their influence on target mRNAs, microRNAs are involved in numerous metabolic processes, including as tissue development, cell proliferation, and lipid metabolism [8–10]. Since the discovery of the first miRNA, lin-4, in *Caenorhabditis elegans* [11], thousands of miRNAs have been experimentally or computationally identified in different species [12]. MiR-103, miR-143, miR-17–92, miR-21, miR-30c, and miR-204/211 have been reported to promote adipogenesis [13–18]; while miR-130 and the miR-27 family inhibits adipogenesis [19, 20]. The functions of miR-30c and miR-27b on human adipocytes differentiation have been analyzed using hMADS cells; these cells represent a novel and unique tool for the study of human adipose cell development.

Hsa-miR-1908 was first discovered in human embryonic stem cells in 2008 [21], but there was no functional study of this miRNA until 2012 [22]. To date, the function of miR-1908 in adipocytes is unknown. In this study, we

investigated the functional significance of miR-1908 in hMADS cells.

Materials and methods

Cell culture and induction of differentiation

hMADS cells and HPA-v (Sciencell Research Laboratories, San Diego, CA, USA) were both maintained in preadipocyte medium (PAM; Sciencell Research Laboratories) supplemented with 5 % fetal bovine serum (FBS), 1 % preadipocyte growth supplement (PAGS) and 1 % penicillin/streptomycin solution (P/S) at 37 °C in 5 % CO_2.

To induce differentiation, confluent hMADS cells and HPA-v (day 0) were cultured in serum-free PAM containing 5 μg/ml insulin, 1 μM dexamethasone, 0.5 mM 3-isobutyl- 1-methylxanthine and 1 μM rosiglitazone. The medium was changed every 2 days for the first 4 days. Thereafter, the medium was replaced with serum-free PAM containing 5 μg/ml insulin, which was changed every 2 days until accumulation of lipid droplets was observed (day 15).

Human tissue sample

The human adipose tissue (subcutaneous and visceral) of obese ($n = 16$, BMI ≥ 28) and matched normal ($n = 12$, $18.5 \leq$ BMI < 24) as well as clinical data were obtained from Nanjing Maternity and Child Health Care Hospital Affiliated to Nanjing Medical University with the approval of institutional review boards and the ethics committee of Nanjing Medical University [23]. Informed consent was obtained from all subjects.

Construction of a miR-1908 overexpressing lentiviral vector: pGLV-H1-miR-1908-GFP/Puro

Using polymerase chain reaction (PCR), the miR-1908 minigene fragments were amplified from genomic DNA prepared from hMADS cells, then cloned into a lentivirus expression vector (pGLV-H1-GFP/Puro; GenePharma, Shanghai, China) according to the manufacturer's instructions. After sequencing to validate that the cloning had occurred correctly, the expression plasmid and the packaging plasmids were cotransfected into HEK-293T cells to produce the pGLV-H1-miR-1908-GFP/Puro lentivirus expression vector; the supernatant containing the lentiviral vector particles was used to infect the hMADS cells. The lentiviral vector with a non-targeting control was also constructed in the same way.

Transfection of hMADS cells with the pGLV-H1-miR-1908-GFP/Puro expression vector

hMADS cells were seeded onto 6 well plates (Corning, New York, USA) at a density of 10^5 cells/well and 24 h later were infected with the miR-1908 expression vector and non-targeting control vector, using supernatant containing lentiviral particles and 5 μg/ml hexadimethrine bromide (polybrene; Sigma, St. Louis, MO, USA), according to the manufacturer's instructions.

Oil red O staining of miR-1908 overexpressing hMADS cells

Mature adipocytes (day 15) were rinsed three times in phosphate-buffered saline (PBS), fixed in 4 % (m/v) paraformaldehyde for 10 min, and then rinsed again with PBS. The fat droplets in the cells were stained with 0.2 % (m/v) oil red O (Sigma, St. Louis, MO, USA) in isopropanol for 30 min and then examined microscopically (Olympus, Tokyo, Japan).

Quantification of triglyceride content

Mature adipocytes (day 15) were harvested in lysis buffer and the cells homogenized. Triglyceride content was measured using a tissue triglyceride assay kit (Applygen, Beijing) using a DNM-9602 microplate reader (Perlong, Beijing), according to the manufacturer's instructions.

Quantitative real-time polymerase chain reaction (q-RT-PCR) to determine miR-1908 expression levels

To assess miRNA expression levels, total RNA was isolated from hMADS cells, HPA-v, adipocytes, the cells during the adipogenesis and the human adipose tissue with TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions, and reverse-transcribed into cDNA using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) in 15 μl RT reactions containing 200 ng of total RNA, 50 nM stem-loop RT primer, RT buffer, 0.25 mM of each dNTP, 3.33 U/μl Multiscribe reverse transcriptase (RT) and 0.25 U/μl RNase inhibitor. Reactions conditions were as follows: 30 min at 16 °C, 30 min at 42 °C, and 5 min at 85 °C.

For real-time PCR, 1 μl (1:20 dilution) of cDNA, 0.1 μM TaqMan probe, 0.2 μM forward primer, 0.2 μM reverse primer and TaqMan Universal PCR Master Mix II (Applied Biosystems, Foster City, CA, USA) were included in 20 μl reactions, using the following reaction conditions: 10 min at 95 °C then 40 cycles of 15 s at 95 °C and 1 min at 60 °C. All

real-time PCR experiments were carried out using the ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). The RT primer, hsa-miR-1908 and miR-let-7 PCR primers, and the TaqMan probe for hsa-miR-1908 and miR-let-7 were purchased from Applied Biosystems. The real-time PCR results were analyzed and expressed relative to the miRNA expression of CT (threshold cycle) value. Small nucleolar RNA U6 (snRU6) and miR-103 are both stably expressed in cells, so they were used as the normalizing control [24, 25]. The miR-let-7 played an important role in adipocyte differentiation and was highly expressed in adipocytes [26].

Quantitative real-time polymerase chain reaction (q-RT-PCR) to determine expression levels of PPARγ (peroxisome proliferator-activated receptor γ) and C/EBPα (CCAAT enhancer binding protein α) mRNA.

To assess the mRNA expression level of PPARγ and C/EBPα, total RNA was reverse-transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) in 20 μl RT reactions containing 480 ng of total RNA, 50 nM RT primer, RT buffer, 0.25 mM each dNTP, 5 U/μl MultiScribe RT and 2.5 U/μl RNase inhibitor. Reactions conditions were as follows: 10 min at 25 °C, 120 min at 37 °C, and 5 min at 85 °C.

Real-time PCR was performed as described above. The PCR primer sets used were as follows: PPARγ forward primer:

5′-AAATATCAGTGTGAATTACAGCAAACC-3′, reverse primer: 5′-GGAATCGCTTTCTGGGTCAA-3′, probe: 5′FAM-TGCTGTTATGGGTGAAACTCTGGGAGATTCT-3′TAMRA;

C/EBPα forward primer: 5′-TGTGCCTTGGAAATGCAAAC-3′, reverse primer: 5′-CGGGAAGGAGGCAGGAA-3′, probe: 5′ FAM-CACCGCTCCAATGCCTACTGAGTAGGG-3′ TAMRA;

18S forward primer: 5′-CGCCGCTAGAGGTGAAATTC-3′, reverse primer: 5′-CATTCTTGGCAAATGCTTTCG-3′, probe: 5′ FAM-ACCGGCGCAAGACGGACCAGA-3′ TAMRA. 18S is stably expressed in cells, so it was used as the normalizing control.

Western blots to determine the protein expression levels of PPARγ and C/EBPα

Whole-cell lysates from hMADS cells were prepared using radioimmunoprecipitation assay (RIPA) buffer (Beyotime, Shanghai, China). The Whole-cell lysates were boiled in 5 × Sodium Dodecyl Sulfonate (SDS) sample buffer (Beyotime, Shanghai, China) for 5 min and subsequently centrifuged at 12,000*g* for 15 min at 4 °C. The protein content was measured using a Pierce BCA Protein Assay Kit (Thermo Scientific, New York, USA), according to the manufacturer's instructions. The extracted protein (30 μg) was separated on a 10 % sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel under reducing conditions and then electroblotted onto nitrocellulose membranes (Millipore, Bedford, MA, USA). After blocking with 5 % skimmed milk, the membranes were incubated overnight at 4 °C with the appropriate antibodies—PPARγ antibody (Cell Signaling Technology, Danvers, MA, USA), C/EBPα antibody (Cell Signaling Technology, Danvers, MA, USA) and anti-β-actin antibody (Abcam, Cambridge, MA, USA). Subsequently, the membranes were hybridized with a secondary antibody conjugated with peroxidase (Santa Cruz Biotechnology, Dallas, USA). The protein levels were quantitated using the ChemiDoc system (Bio-Rad).

Cell proliferation assay

Cell growth was analyzed using a WST-8 Cell Counting Kit-8 (CCK-8; Dojindo, Japan). Isolated cells were seeded in a 96-well microplate at a density of 400 cells per well to a final volume of 100 μl, and incubated for 4 h. Before an additional 1 h incubation period at 37 °C, 10 μl of CCK-8 was added to each well. Cell growth was measured at $OD_{450\ nm}$ using a DNM-9602 microplate reader (Perlong, Beijing, China). This process was performed every 24 h until the wells were full.

Analysis of apoptosis in miR-1908 overexpressing hMADS cells

Cells were stained with Annexin V-FITC and propidium iodide (PI) and analyzed for apoptosis according to the manufacturer's instructions (BD FACSCalibur, Mountain View, CA, USA). Briefly, after being serum-deprived for 12 h, cells were cultured in PAM supplemented with 5 % fetal bovine serum (FBS), 1 % preadipocyte growth supplement (PAGS) and 1 % penicillin/streptomycin solution (P/S) at 37 °C in 5 % CO_2 again for 24 h. After that, cells were trypsinized and washed with Dulbecco's PBS, and then resuspended in 400 μl of PBS. Subsequently, 100 μl of this cell suspension was incubated in the dark with 5 μl of PI (50 μg/ml) and 10 μl Annexin V-FITC for 15 min at room temperature. Cells were then analyzed by flow cytometry.

Analysis of cell cycle distribution of miR-1908 overexpressing hMADS cells

The distribution of cells at specific cell cycle stages was evaluated by assessment of DNA content using flow

cytometry. To synchronize cell cycle, cells were serum-deprived for 12 h, and then were cultured in PAM supplemented with 5 % fetal bovine serum (FBS), 1 % preadipocyte growth supplement (PAGS) and 1 % penicillin/streptomycin solution (P/S) at 37 °C in 5 % CO_2 again for 24 h. After that, the cells were trypsinized and washed in PBS, and fixed in 70 % ethanol at 4 °C overnight. Then cells were treated with RNase A (100 μg/ml) and PI (50 μg/ml) at 37 °C for 30 min. Subsequently, cells were subjected to FACS analysis based on DNA content. Samples were analyzed using the FACStar cell sorter and CellQUEST Pro (BD FACSCalibur, Mountain View, CA, USA).

Statistical analyses

All transfection experiments were repeated six times. The qRT-PCR assays were performed in triplicate and each experiment was repeated at least three times. Data are presented as the mean ± SD of three or more independent experiments. Statistical analyses were performed using one-way ANOVA or the paired Student's t tests. The differences between groups were considered to be statistically significant when $P < 0.05$.

Results

MiR-1908 is highly expressed in human adipocytes

To examine the expression of miR-1908 in hMADS cells, HPA-v and adipocytes, we cultured the hMADS cells and HPA-v, and induced both cell types to mature to adipocytes. During the differentiation, we extracted total RNA at day 0 and day 15. The expression of miR-1908 was assessed by PCR; there was no difference in the expression of miR-1908 in hMADS cells and HPA-v, and the relative level of miR-1908 expression was greater in the differentiation of day 15 (Fig. 1; $P < 0.05$).

The expression of miR-1908 during the differentiation of hMADS cells decreased first but increased later

As hMADS cells will eventually differentiate to become mature adipocytes, we examined the level of miR-1908 expression using PCR during the differentiation process to verify the effect of miR-1908. Using small nucleolar RNA U6 and miR-103 as expression references, we demonstrated that the level of miR-1908 expression was decreased by day 4 of the differentiation process, but was increased at day 15 (Fig. 2; $P < 0.05$).

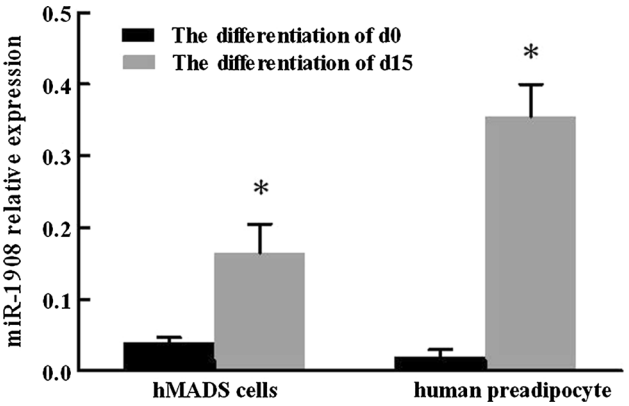

Fig. 1 The miR-1908 relative expression in hMADS cells and HPA-v at the differentiation of day 0 and day 15 The expression levels of miR-1908 in hMADS cells and HPA-v at the differentiation of day 0 were lower than the expression levels of miR-1908 in cells at the differentiation of day 15. Data are representative of three independent experiments; data are expressed as the mean ± SD. *$P < 0.05$

MiR-1908 expression is different in human adipose tissue

We analyzed the expression of miR-1908 in human adipose tissue (subcutaneous and visceral) of obese and matched normal. The results revealed that the expression level of miR-1908 of obese was lower than normal in human adipose tissue-subcutaneous. On the other hand, the expression level of miR-1908 of obese was higher than normal in human adipose tissue- visceral (Fig. 3).

We construct a miR-1908 overexpressing lentiviral vector: pGLV-H1-miR-1908-GFP/Puro successfully

Lentiviral vectors, particularly those derived from human immunodeficiency virus (HIV), are effective DNA delivery systems [27]. To investigate the effects of miR-1908, we constructed a lentiviral vector to overexpress miR-1908 in hMADS cells. After the vector sequence was confirmed, we introduced the pGLV-H1-miR-1908-GFP/Puro vector into hMADS cells, and after 72 h, tested the level of miR-1908 expression. In these cells, the level of miR-1908 expression was approximately fourfold higher than the expression level in the negative control cells (Fig. 4a). Additionally, we demonstrated that the expression level of let-7 in the cells transfected with the miR-1908 overexpression vector was no different to the level of let-7 expression in the negative control cells (Fig. 4b), and therefore we concluded that the overexpression of miR-1908 has no effect on the expression of other endogenous miRNAs.

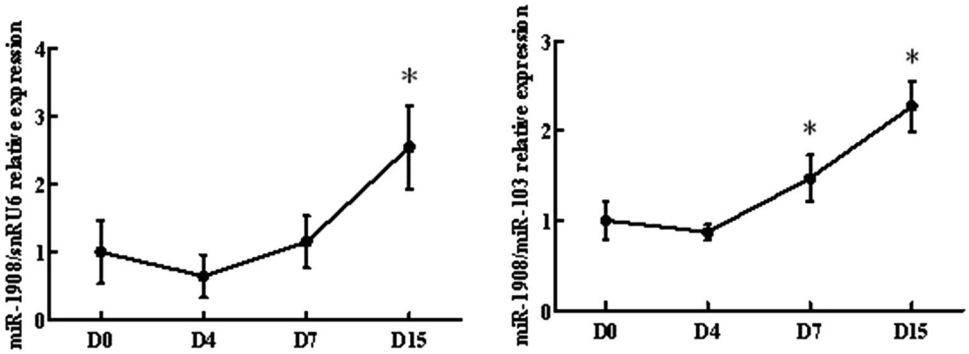

Fig. 2 The miR-1908 relative expression during hMADS cells differentiation The expression of miR-1908 decreased between day 0 to day 4 during hMADS cells differentiation, but by day 15 its expression had increased. Data are representative of three independent experiments; data are expressed as the mean ± SD (The data were different from the above ones because they were from different experiments). *P < 0.05

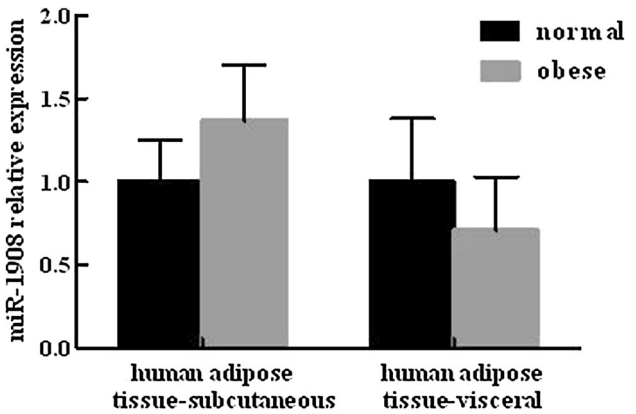

Fig. 3 The miR-1908 relative expression in human adipose tissue The expression level of miR-1908 in of obese was lower than normal in human adipose tissue-subcutaneous. However, the expression level of miR-1908 of obese was higher than normal in human adipose tissue- visceral. Data are representative of three independent experiments; data are expressed as the mean ± SD

Overexpression of miR-1908 inhibits differentiation of hMADS cells into adipocytes

To explore the effect of miR-1908 on adipocyte differentiation, we used oil red O to investigate the effect of miR-1908 on the forming of lipid droplets in the miR-1908 overexpression hMADS cells (Fig. 5a); we also measured the triglyceride content of these cells (Fig. 5b). There were no differences in number of lipid droplets or the triglyceride content of transfected hMADS cells or negative control cell.

Overexpression of miR-1908 inhibits the expression of PPARγ and C/EBPα

The expression of PPARγ and C/EBPα, which are key adipogenic differentiation transcription factors, was also examined. Using qRT-PCR to analyze mRNA expression, we found that miR-1908 inhibited the transcriptional induction of PPARγ and C/EBPα at day 15 of differentiation (Fig. 5c). Using western blot analysis, the protein expression levels of PPARγ and C/EBPα were determined in human adipocytes on days 4, 7, 10 and 15 of differentiation. On day 4, both PPARγ and C/EBPα were expressed at a low level. At day 7, the levels of both PPARγ and C/EBPα were strongly increased. After 15 days of differentiation, PPARγ and C/EBPα expression was uncompletely blocked in the miR-1908-transfected cells (Fig. 5d). These data suggest that miR-1908 inhibits adipocyte differentiation by blocking the transcription of the adipogenesis determination genes PPARγ and C/EBPα.

Overexpression of miR-1908 promotes the growth of hMADS cells

The growth of adipose tissue is the result of adipocyte hypertrophy and hyperplasia. We analyzed the proliferation of the transfected hMADS cells in 144 h to evaluate the effect of miR-1908 on adipocyte hyperplasia. The hMADS cells transfected with miR-1908 grew faster than the negative control cells (Fig. 6). Therefore, miR-1908 promotes the proliferation of hMADS cells.

Overexpression of miR-1908 does not influence apoptosis of hMADS cells

As apoptosis is a process that is closely related to cell proliferation, we examined the effect of miR-1908 on the apoptosis of hMADS cells (Fig. 7a). The overexpression of miR-1908 slightly inhibited the apoptosis of hMADS cells, but this effect was not statistically significant (Fig. 7b).

Overexpression of miR-1908 induces S phase during the cell cycle of hMADS cells

To further explore the function of miR-1908 in the promotion of cell proliferation, we examined the distribution

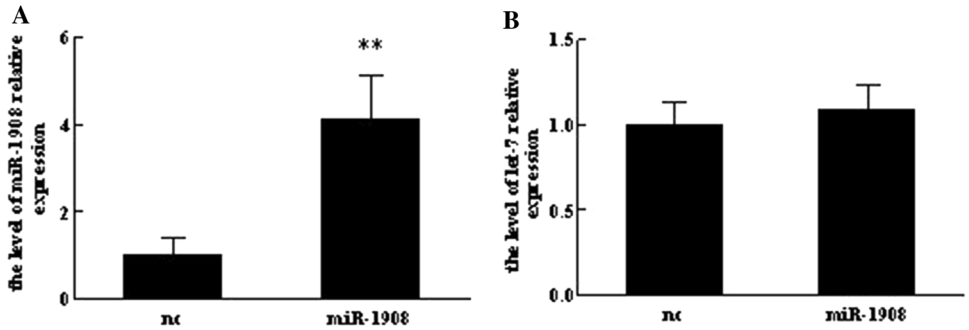

Fig. 4 The successful construction of the lentiviral miR-1908 expression vector A lentiviral miR-1908 expression vector was constructed and the level of miR-1908 expression was tested. **a** The miR-1908 expression level in transfected hMADS cells 72 h post-transfection. The expression level of miR-1908 was higher than in the negative control cells. **b** The expression level of let-7 in transfected hMADS cells 72 h post-transfection. There was no difference between the expression levels between the transfected cells and the negative control cells. Data are representative of three independent experiments; data are expressed as the mean ± SD. **P < 0.01

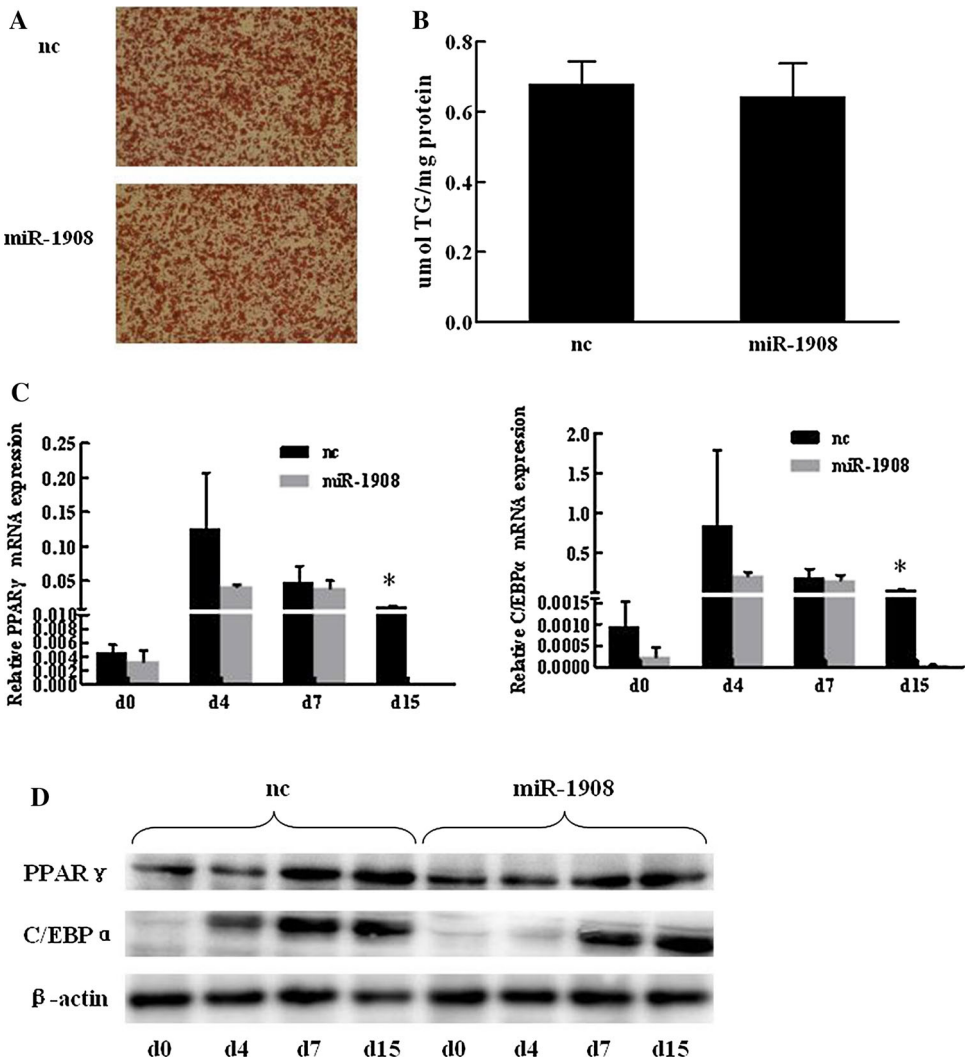

Fig. 5 The effect of miR-1908 on hMADS cells differentiation. **a** Lipid droplets stained with oil red O in transfected cells and control cells (×100). There were no differences in the number of lipid droplets between miR-1908-transfected cells and the negative control cells. **b** Triglyceride accumulation was quantified and normalized to protein amount. There were no differences between the triglyceride content of miR-1908-transfected cells and negative control cells. **b** The analysis of PPARγ and C/EBPα mRNA expression using qRT-PCR. The level of PPARγ and C/EBPα mRNA expression in miR-1908-transfected cells was lower than in the negative control cells. **d** Western blot analysis of PPARγ and C/EBPα protein expression. MiR-1908 transfected cells had a low level of PPARγ and C/EBPα protein compared with negative control cells. Data are representative of three independent experiments; data are expressed as the mean ± SD. *P < 0.05

of cells at different cell cycle phases in miR-1908 transfected hMADS cells using flow cytometry (Fig. 8a). The miR-1908 transfected cell population showed a substantial increase of cells in S phase and a decrease in the number of cells in G1 phase, compared with negative control cells (Fig. 8b). This data suggests that miR-1908 promotion of

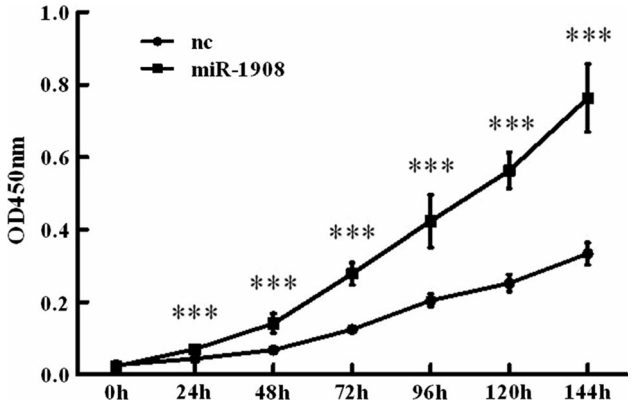

Fig. 6 The effect of miR-1908 on hMADS cells proliferation. We used CCK-8 to analyze the proliferation of transfected hMADS cells and negative control hMADS cells. The miR-1908 transfected cells grew faster than the negative control cells. Data are representative of three independent experiments; data are expressed as the mean ± SD. ***$P < 0.001$

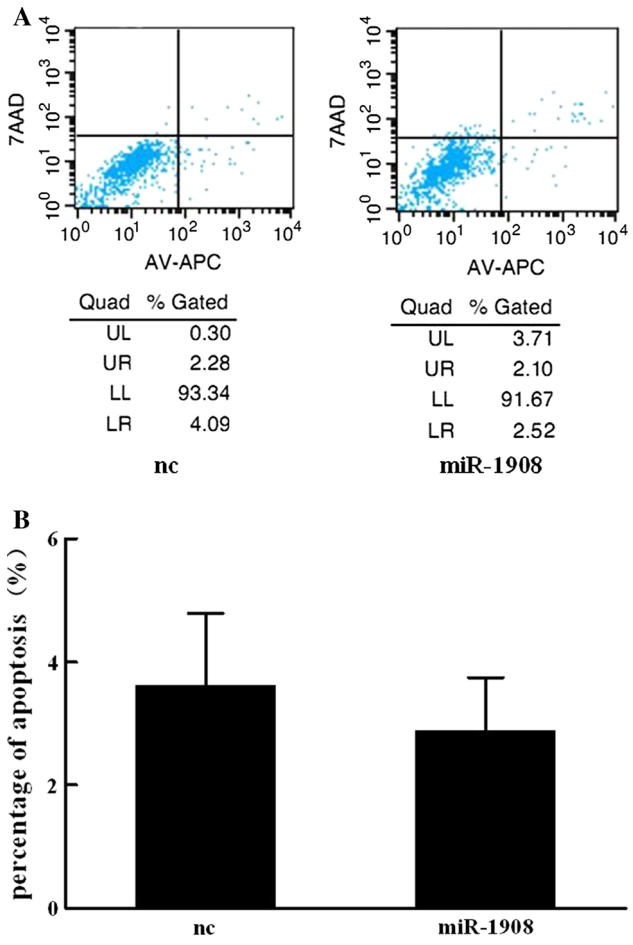

Fig. 7 The effect of miR-1908 on hMADS cells apoptosis. **a** Apoptosis in miR-1908 transfected hMADS cells was analyzed by flow cytometry. **b** The overexpression of miR-1908 inhibited the apoptosis of hMADS cells, but this was not statistically significant. Data are representative of three independent experiments; data are expressed as the mean ± SD

hMADS cell growth by may be mediated through an induction of S arrest.

Discussion

Obesity is an excessive accumulation of adipose tissue due to energy imbalance, characterized by an increase in cell number and volume, and is associated with health problems, including insulin resistance, type 2 diabetes, hypertension, cardiovascular disease, and some cancers [28]. In our previously research, we found that the expression of many microRNAs in human adipocytes was higher than that in human preadipocytes and hMADS cells. The other members of our research group have studied the function of several microRNAs [29, 30]. MiR-1908 is a recently identified miRNA that is located in the first intron of FADS1 on human chromosome 11 [21]. The functions of its putative target genes were enriched in Wnt receptor signaling pathways through beta-catenin, cell cycle, cell proliferation and other biological processes. GnRH signaling, MAPK signaling, insulin signaling, cell cycle signal transduction pathways and pancreatic cancer were significantly enriched. Both the MAPK signaling and the insulin signaling pathways are known to be involved in adipocyte differentiation and obesity [31, 32]. All of the signaling pathways and biological processes that the putative target genes are involved in are concerned with adipocyte differentiation and metabolism, so we hypothesized that miR-1908 would have an effect on the differentiation and metabolism of human adipocytes. Additionally, miR-1908 has been identified as having a close relationship with the processes of metastatic invasion, angiogenesis, and colonization of melanomas [22]; it may be involved in the malignant progression of chordoma [33]; and may participate in the formation of hepatoma cells [34].

The process of adipogenesis can be divided into early, intermediate and late stages. PPARγ and C/EBPα are both adipogenic transcription factors that are involved at the early stage of adipocyte differentiation. Early stage adipocytes have the potential to influence future adipocyte biology, and influence the formation of adipose tissue; consequently, the analysis of early adipogenic transcription factors has been a primary target for anti-obesity strategies.

In this study, we identified that miR-1908 was upregulated during differentiation of hMADS cells and HPA-v to form adipocytes. Subsequent functional analyses demonstrated that miR-1908 promotes the proliferation of hMADS cells and inhibits their differentiation to adipocytes, as evidenced by the downregulation of the key adipogenic transcription factors PPARγ and C/EBPα. This downregulation ultimately resulted in decreased expression of adipocyte marker genes (PPARγ and C/EBPα) and an

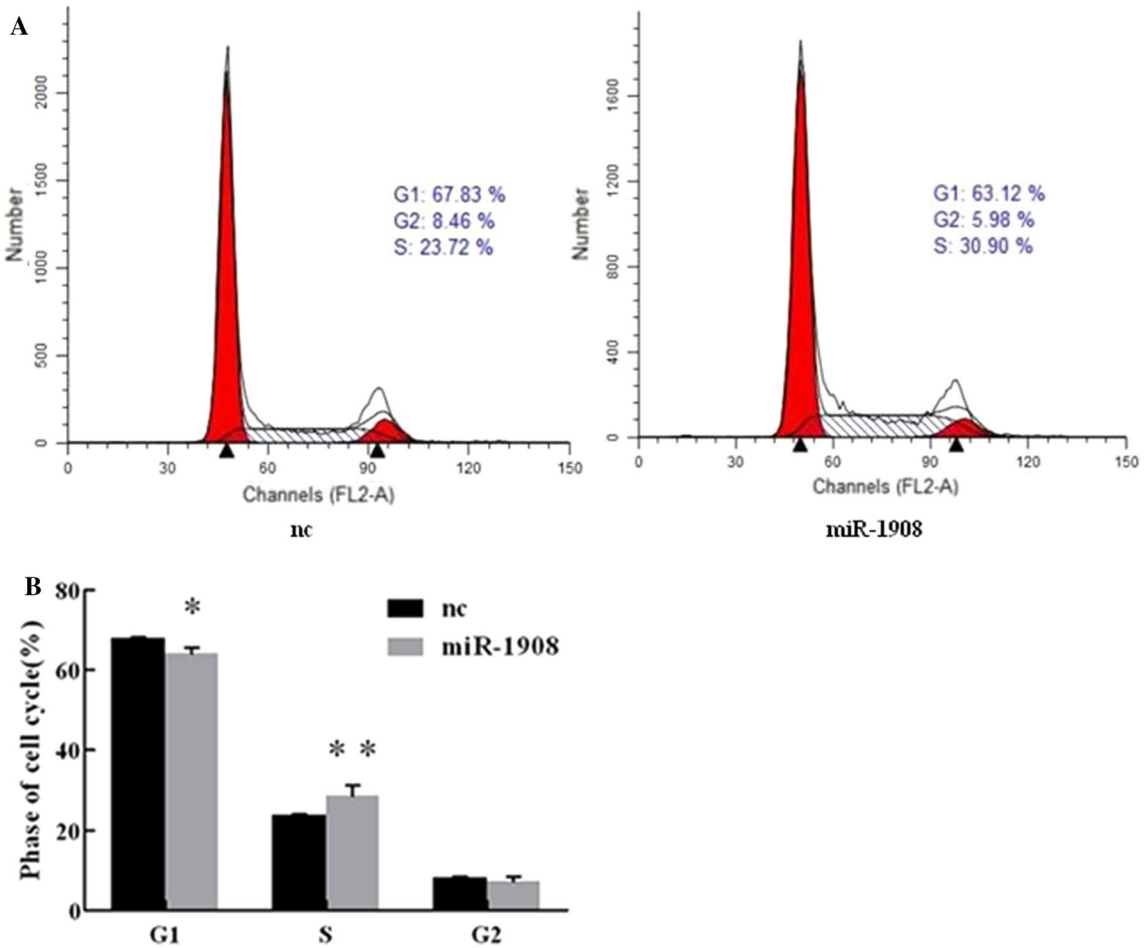

Fig. 8 The effect of miR-1908 on the cell cycle in hMADS cells. **a** FACS assay of hMADS cells transfected with miRNA-control and miR-1908 mimics. **b** Flow cytometry analysis. In miR-1908 transfected cells there are more cells in S phase and fewer cells in G1 phase, compared with the negative control cells. Data are representative of three independent experiments; data are expressed as mean ± SD. *P < 0.05; **P < 0.01

increase in hMADS cell proliferation. Previously, it was demonstrated that the antisense inhibition of specific miRNAs (miR-10b, 15, 26a, 34c, 98, 99a, 101, 101b, 143, 152, 183, 185, 224, and let-7b) that are upregulated during adipogenesis, did not affect adipocyte differentiation, as determined by marker gene expression and the accumulation of lipid droplets [35]. As there are many miRNAs and genes that can regulate the adipocyte differentiation during every stage, the specific mechanism of miR-1908 affecting the adipocyte differentiation remains unknown and demands further study.

The expression of miR-1908 differed between subcutaneous and visceral adipose tissue, suggesting that the molecular mechanism of adipogenesis is site dependent [6]. Further study is needed.

Growth-arrested hMADS cells initiate clonal expansion with adipogenic signals to re-enter the cell cycle progression. The cells become spherical, accumulate fat droplets and are then converted into fully differentiated adipocytes [36]. It has been suggested that miR-17-92 may modulate adipocyte differentiation by regulating the re-entry and exit of cells into the cell cycle [15]. In our study, miR-1908 inhibits adipocyte differentiation by promoting the proliferation of hMADS cells and influencing the cell cycle through expanding S phase and inhibiting G1 phase. However, how miR-1908 results in a rate decrease in G1 phase and increase in S phase remains to be further researched.

In summary, we found that the miRNA miR-1908 is highly expressed in human adipocytes. It inhibits the differentiation of hMADS cells to form mature adipocytes and promotes hMADS cell proliferation. Obesity is an excessive accumulation of adipose tissue, so these findings outline the importance of miR-1908 in human obesity. Further experiments are required to elucidate the mechanisms of miR-1908 in hMADS cells.

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Conflict of interest No competing financial interests exist.

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