ChinaRxiv

AI translation · View original & related papers at
chinarxiv.org/items/chinaxiv-201808.00177

Effects of MicroRNA-196a-5p on Proliferation and Differentiation of 3T3-L1 Preadipocytes (Postprint)

Authors: Yang Qiong, Wang Linghui, Gu Hao, Liu Jinyuan, Du Jingjing, Zhang Shunhua, Zhu Li

Date: 2018-08-29T17:32:20+00:00

Abstract

Objective: To investigate the effects of miR-196a-5p on the proliferation and differentiation of mouse preadipocytes and its underlying molecular mechanisms. Methods: Establish a mouse obesity model and detect miR-196a-5p expression levels in adipose tissue by RT-PCR; Induce differentiation of 3T3-L1 preadipocytes using the cocktail method and detect changes in miR-196a-5p expression during differentiation by RT-PCR; Synthesize miR-196a-5p mimics and inhibitors for transfection into 3T3-L1 cells, and assess the effects of miR-196a-5p on 3T3-L1 preadipocyte proliferation using CCK8 and EdU kits; Evaluate the effects of miR-196a-5p on 3T3-L1 cell differentiation using Oil Red O staining and triglyceride measurement; Detect the effects of miR-196a-5p on genes related to preadipocyte proliferation and differentiation by RT-PCR; Screen and validate target genes of miR-196a-5p regulating adipocyte differentiation using bioinformatics software and luciferase reporter systems, combined with previous literature. Results: miR-196a-5p was highly expressed in adipose tissue of obese mice and showed an initial increase followed by a decrease during 3T3-L1 preadipocyte differentiation; Compared with the negative control group, mimics transfection inhibited 3T3-L1 cell proliferation, while inhibitors transfection promoted 3T3-L1 cell proliferation; Compared with the negative control group, the mimics group accumulated numerous Oil Red O-stained lipid droplets with increased triglyceride content, whereas the inhibitors group showed fewer and smaller lipid droplets with relatively decreased triglyceride content; Compared with the negative control group, mimics transfection inhibited the expression of proliferation marker genes Cyclin D1, Cyclin E, CDK2, and CDK4, while promoting the expression of differentiation marker genes PPARγ, C/EBPα, LPL, aP2, etc.; inhibitors transfection exhibited opposite effects to mimics transfection; miR-196a-5p could significantly inhibit the luciferase activity of wild-type MAP4K3 and MAPK1 3'UTR, while mutation

of the binding sites abolished this inhibitory effect. Conclusion: miR-196a-5p can not only inhibit 3T3-L1 preadipocyte proliferation but also promote their induced differentiation and lipid droplet deposition; miR-196a-5p may mediate 3T3-L1 preadipocyte differentiation by targeting and regulating MAP4K3 and MAPK1.

Full Text

The Effect of MicroRNA-196a-5p on the Proliferation and Differentiation of 3T3-L1 Preadipocytes

Qiong Yang[1]**,, *Linghui Wang***[2]**,, Hao Gu**[2]**, Jinyuan Liu**[3]**, Jingjing Du**[2]**, Shunhua Zhang**[2]**, Li Zhu**[2] [1]Chengdu Agricultural Science and Technology Vocational College, Chengdu, 611130, China [2]College of Animal Science and Technology, Sichuan Agricultural University, Chengdu, 611130, China [3]Sichuan Animal Science Academy, Chengdu, 610066, China

*These authors contributed equally to this work.

Corresponding author: Li Zhu, Professor, Doctoral Supervisor. Research interests: pig genetics and breeding. E-mail: zhuli7508@163.com

---

Abstract

Objective: To investigate the effect of miR-196a-5p on the proliferation and differentiation of mouse adipocytes and explore its potential molecular mechanisms.

Methods: (1) RT-PCR was used to measure miR-196a-5p expression levels in adipose tissues from obese and normal mice; (2) After inducing 3T3-L1 cell differentiation using the cocktail method, RT-PCR was performed to detect miR-196a-5p expression changes during the differentiation process; (3) Synthetic miR-196a-5p mimics and inhibitors were transfected into 3T3-L1 cells, and CCK8 and EdU kits were used to assess the effect of miR-196a-5p on preadipocyte proliferation; (4) Oil red O staining and triglyceride measurement were employed to evaluate the effect of miR-196a-5p on 3T3-L1 cell differentiation; (5) RT-PCR was conducted to examine the impact of miR-196a-5p on genes related to preadipocyte proliferation and differentiation; (6) Based on previous literature, bioinformatics software and luciferase reporter systems were used to screen and validate target genes through which miR-196a-5p regulates adipocyte differentiation.

Results: (1) miR-196a-5p was highly expressed in adipose tissues of obese mice and showed dynamic expression during 3T3-L1 preadipocyte differentiation; (2) Compared with the negative control group, mimics transfection inhibited 3T3-L1 cell proliferation while inhibitors transfection promoted it; (3) Compared

with the negative control, mimics transfection increased lipid accumulation and triglyceride content, whereas inhibitors transfection decreased them; (4) Compared with the negative control, mimics transfection suppressed expression of proliferation markers (Cyclin D1, Cyclin E, CDK2, and CDK4) and promoted expression of differentiation markers (PPARγ, C/EBPα, LPL, aP2, etc.), while inhibitors transfection showed opposite effects; (5) miR-196a-5p significantly suppressed luciferase activity of wild-type MAP4K3 and MAPK1 3'UTRs, and mutation of the binding sites abolished this inhibitory effect.

Conclusion: miR-196a-5p not only inhibits 3T3-L1 preadipocyte proliferation but also promotes induced differentiation and lipid droplet deposition. miR-196a-5p may mediate 3T3-L1 preadipocyte differentiation by targeting MAP4K3 and MAPK1.

Keywords: miR-196a-5p; proliferation; differentiation; MAP4K3; MAPK1

---

1. Introduction

Obesity has become a global health concern. Numerous studies have demonstrated that obesity or overweight directly threatens human health by significantly increasing the risk of type II diabetes, insulin resistance, cardiovascular disease, chronic inflammation, fatty liver disease, and other conditions [1-3]. Excessive accumulation of adipose tissue caused by excessive daily energy intake or metabolic disorders is considered the primary cause of obesity or overweight [4], and in-depth investigation of the molecular mechanisms underlying adipogenesis and lipid metabolism will facilitate the prevention and treatment of obesity-related diseases.

MicroRNAs (miRNAs) are a class of single-stranded non-coding small RNA molecules approximately 18-24 nt in length that can specifically bind to the 3'-untranslated region (3'UTR) of target mRNAs through their seed sequences, thereby regulating target genes at the post-transcriptional level and mediating complex biological processes such as cell proliferation, apoptosis, and differentiation [5-7]. In recent years, high-throughput sequencing technology has identified numerous miRNAs associated with adipose development [8-10]. Functional analyses have revealed that miRNAs play important roles in adipogenesis and lipid metabolism. For example, Karbiener et al. [11] found that miR-27b inhibits adipocyte differentiation by directly targeting PPARγ; Peng et al. [12] reported that miR-224 not only negatively regulates early adipocyte differentiation but also participates in fatty acid metabolism; and Lv et al. [13] demonstrated that miR-129-5p inhibits 3T3-L1 preadipocyte proliferation by negatively regulating G3BP1. Existing evidence indicates that miR-196a-5p is widely involved in biological process regulation, such as mediating bladder cancer cell apoptosis, promoting glioma progression, and regulating embryonic stem cell self-renewal [14-16]. However, no studies have reported whether miR-196a-5p mediates adipogenesis. Mouse 3T3-L1 preadipocytes are a classic cellular model for studying

adipose development. Using 3T3-L1 cells as a research vehicle, this study aims to explore the role of miR-196a-5p in adipocyte proliferation and differentiation and to investigate its underlying molecular mechanisms.

---

2. Materials and Methods

2.1 Animal Experiments

Twelve male Kunming mice (6 weeks old) purchased from Dasuo Biotechnology Company (Chengdu, China) were divided into two groups and fed either a high-fat diet (HFD) or normal chow (NCW) under identical housing conditions. After 16 weeks, the mice were euthanized by cervical dislocation, and gonadal, inguinal, and perirenal adipose tissues were rapidly collected and placed in liquid nitrogen. Samples were subsequently transferred to -80°C for storage. All animal experiments were approved by the Experimental Animal Care and Use Committee of the College of Animal Science and Technology, Sichuan Agricultural University.

2.2 Cell Culture

Mouse 3T3-L1 preadipocytes (Stem Cell Bank, Chinese Academy of Sciences, Shanghai, China) were cultured in DMEM high-glucose medium (Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) at 37°C in a 5% CO_2 incubator. When cells reached 80% confluence in culture flasks, they were digested with trypsin and seeded in 12-well plates. When cell density in the 12-well plates reached 70-80%, the proliferation medium (DMEM + 10% FBS) was replaced with induction medium (DMEM + 10% FBS + 1.7 mol/L insulin + 1 mol/L dexamethasone + 0.5 mol/L IBMX). After 3 days, the induction medium was replaced with maintenance medium (DMEM + 10% FBS + 1.7 mol/L insulin). Three days later, the maintenance medium was replaced with proliferation medium until the end of the experiment. Insulin, dexamethasone, and IBMX were all purchased from Sigma (Shanghai, China).

2.3 Cell Transfection

According to the reagent instructions, 3T3-L1 cells in 96-well or 12-well plates at the required densities (30-40% for proliferation experiments, 70-80% for differentiation experiments) were transfected with miR-196a-5p mimics (50 nM, product number miR20000518-1-5; 5 -UAGGUAGUUUCAUGUUGUUGGG-3), inhibitors (100 nM, product number miR20000518-1-5; 5 -CCCAACAACAUGAAACUACCUA-3), or negative control (NC, 50 nM, product numbers miR01201-1-5 and miR02201-1-5; 5 -UUUGUACUACACAAAAGUACUG-3 and 5 -CAGUACUUUUGUGUAGUACAAA-3) designed and synthesized by Guangzhou RiboBio Company, using Lipofectamine 2000 (Invitrogen, USA). After 12 hours, the cell culture medium was

replaced with fresh medium. To ensure transfection efficiency, miR-196a-5p mimics and inhibitors were transfected every 2 days.

2.4 CCK8 and EdU Assays

3T3-L1 cells in proliferation medium at 30-40% confluence in 96-well plates were transfected with miR-196a-5p mimics (50 nM), inhibitors (100 nM), or negative control (50 nM). Cell proliferation was subsequently measured at 0, 12, 24, 48, and 72 hours post-transfection using the CCK8 Proliferation Detection Kit (Beyotime, Shanghai, China) according to the manufacturer's instructions. Specifically, 10 L of CCK8 solution was added to each well of treated cells, followed by incubation for 1.5 hours in the cell culture incubator, and absorbance was measured at 450 nm using a microplate reader. For EdU proliferation analysis, 3T3-L1 cells transfected for 24 hours were incubated with 10 M EdU solution for 10 hours, and newly generated cells were detected using the EdU Kit (product number C10310-3) according to the manufacturer's protocol. In these experiments, each treatment group at each time point had 6 replicates, and final EdU images were captured using an Olympus IX53 fluorescence microscope (Olympus; Tokyo, Japan).

2.5 Oil Red O Staining

Oil red O staining was performed as follows: (1) 3T3-L1 cells transfected with miR-196a-5p mimics, inhibitors, or negative control and induced to differentiate for 8 days were first washed with PBS, then fixed with 4% paraformaldehyde at room temperature for 30 minutes; (2) After removing paraformaldehyde, cells were washed 3 times with PBS (5 minutes each), then incubated with oil red O working solution at room temperature; (3) After 1 hour, cells were washed 3 times with PBS (5 minutes each), and finally 300 L of PBS was added to each well to cover the stained cells for microscopic observation and photography; (4) To indirectly measure triglyceride content, 200 L of isopropanol was added to each well after photography to extract oil red O bound to lipid droplets, and absorbance was measured at 510 nm using a microplate reader.

2.6 Real-Time Fluorescent Quantitative PCR

Total RNA was extracted from cell or tissue samples after experimental treatment using the Trizol method (cell samples were first washed 3 times with PBS). One microgram of RNA was reverse-transcribed to obtain cDNA corresponding to miRNA and mRNA using the Mir-X™ miRNA First-Strand Synthesis Kit (TaKaRa, Dalian, product number 1604342A) and PrimeScript® RT reagent Kit with gDNA Eraser (TaKaRa, product number AK4002), respectively, and diluted to 50 ng/ L. Subsequently, miR-196a-5p and related genes were detected using the SYBR Premix Ex Taq Kit on a CFX96 fluorescence quantification instrument (Bio-Rad, USA). Finally, β-actin and U6 were used as internal references for mRNA and miRNA, respectively, and relative expression levels were

calculated using the $2^{(-\Delta\Delta Ct)}$ method. Primer sequences are listed in Table 1 .

2.7 Luciferase Reporter Gene Assay

Wild-type (WT-MAPK1, WT-MAP4K3) or mutant (Mut-MAPK1, Mut-MAP4K3) 3'UTRs of MAPK1 and MAP4K3 were designed and constructed by Chengdu Tsingke Biotechnology. Using Lipofectamine 3000, miR-196a-5p mimics or negative control were co-transfected with either (1) WT-MAPK1 or Mut-MAPK1, or (2) WT-MAP4K3 or Mut-MAP4K3 into 3T3-L1 cells at 80% confluence. After 24 hours, luciferase activity was measured according to the Dual-Luciferase Reporter Assay System kit (Promega, USA) instructions. Relative luciferase activity was calculated as firefly luciferase/renilla luciferase, with 5 replicates per treatment.

2.8 Statistical Analysis

Experimental data were analyzed using SPSS 22.0 software with Student's t-test; $P < 0.05$ was considered statistically significant. All results are expressed as mean ± standard deviation (Mean ± SD).

---

3. Results

3.1 miR-196a-5p Is Highly Expressed in Adipose Tissues of Obese Mice

To examine differential expression of miR-196a-5p in adipose tissues from obese versus normal mice, RT-PCR was performed. The results showed that miR-196a-5p expression levels were significantly higher in gonadal, subcutaneous inguinal, and perirenal adipose tissues of obese mice compared with normal mice. Notably, the expression difference was most pronounced in inguinal adipose tissue, reaching a 7-fold increase [Figure 1: see original paper]. These results suggest that miR-196a-5p may be associated with lipid formation.

3.2 Expression Changes of miR-196a-5p During 3T3-L1 Preadipocyte Differentiation

3T3-L1 preadipocytes were induced to differentiate using the cocktail method, and miR-196a-5p expression changes were detected on days 0, 2, 4, 6, and 8 of differentiation. The results showed that miR-196a-5p expression gradually increased from day 0 to day 4, peaking on day 4. Specifically, compared with day 0, miR-196a-5p expression was upregulated 1.9-fold on day 2 and 10.3-fold on day 4. After day 4, miR-196a-5p expression rapidly declined, eventually returning to levels similar to day 0 by day 8 [Figure 2: see original paper].

3.3 miR-196a-5p Inhibits 3T3-L1 Preadipocyte Proliferation

3T3-L1 cells in the proliferation phase were transfected with miR-196a-5p mimics, inhibitors, or negative control using Lipofectamine 2000. After 48 hours, miR-196a-5p expression was measured in each group. Compared with the negative control group, the mimics group showed a 5.3-fold increase in miR-196a-5p expression, while the inhibitors group showed a 62.4% decrease, confirming that miR-196a-5p mimics and inhibitors effectively increased or suppressed intracellular miR-196a-5p levels, respectively [FIGURE:3(a)]. The effects of miR-196a-5p on 3T3-L1 cell proliferation were then evaluated using CCK8 and EdU assays. CCK8 detection revealed that, compared with the negative control, miR-196a-5p overexpression significantly reduced 3T3-L1 cell proliferation at 24 h and 72 h post-treatment and extremely significantly reduced proliferation at 48 h. Conversely, miR-196a-5p inhibition significantly promoted 3T3-L1 cell proliferation at 24 h and extremely significantly promoted it at 48 h and 72 h [FIGURE:3(b)]. EdU assays showed that, compared with the negative control, miR-196a-5p overexpression significantly decreased the ratio of EdU-positive cells, while inhibition significantly increased this ratio, indicating that overexpression or inhibition of miR-196a-5p could decrease or increase the number of newly generated 3T3-L1 cells, respectively [FIGURE:3(c-d)]. To further confirm these results, expression changes in cell proliferation marker genes were examined. Compared with the negative control, miR-196a-5p overexpression significantly suppressed expression levels of Cyclin D1, Cyclin E, CDK2, and CDK4, while inhibition significantly increased their expression levels [FIGURE:3(e)]. These results demonstrate that miR-196a-5p can inhibit 3T3-L1 cell proliferation.

3.4 miR-196a-5p Promotes 3T3-L1 Preadipocyte Differentiation

We further investigated the effect of miR-196a-5p on the differentiation of 3T3-L1 preadipocytes into mature adipocytes. Transfection efficiency of miR-196a-5p mimics and inhibitors is shown in [FIGURE:4(a)]. The results indicated that, compared with the negative control, mimics and inhibitors transfection could increase or suppress miR-196a-5p expression in differentiating 3T3-L1 cells, respectively. Oil red O staining of transfected cells on differentiation day 8 revealed that, compared with the negative control, the mimics group showed obvious differentiation signs with numerous large lipid droplets, whereas the inhibitors group showed attenuated differentiation with few small lipid droplets [FIGURE:4(b)]. Triglyceride content measurement showed similar results: compared with the negative control, the mimics group had significantly increased triglyceride content, while the inhibitors group had extremely significantly decreased triglyceride content [FIGURE:4(c)]. To further verify these results, expression levels of adipose-specific genes PPARγ, C/EBPα, LPL, aP2, SCD, ELOVL6, SREBP1, and FAS were measured. The results showed that, compared with the control group, the mimics group exhibited increased expression of PPARγ, C/EBPα, LPL, aP2, SCD, ELOVL6, SREBP1, and FAS by 4.6-fold, 4.2-fold, 2.1-fold, 3.6-fold, 2.4-fold, 2.8-fold, 2.45-fold, and 2.89-fold, re-

spectively. In contrast, the inhibitors group showed decreased expression of these genes by 71.6%, 64.8%, 35.2%, 46.5%, 40.3%, 33.7%, 39.1%, and 46.8%, respectively, indicating that miR-196a-5p overexpression promotes while inhibition reduces expression of adipose-specific genes [FIGURE:4(d)].

3.5 Identification of Target Genes Regulating 3T3-L1 Preadipocyte Differentiation by miR-196a-5p

TargetScan 7.1 online software was used to predict potential target genes of miR-196a-5p. The analysis revealed that nucleotides 1026-1032 of the MAP4K3 3'UTR [FIGURE:5(a)] and nucleotides 435-442 of the MAPK1 3'UTR [FIGURE:5(b)] were perfectly complementary to the miR-196a-5p seed sequence (positions 2-8 of the mature sequence). Furthermore, qRT-PCR analysis showed that miR-196a-5p overexpression in differentiating 3T3-L1 cells significantly suppressed MAP4K3 and MAPK1 expression, while inhibition significantly promoted their expression [FIGURE:5(c-d)]. Previous studies by Huang et al. [17] and Huang [18] have demonstrated that MAPK1 can inhibit 3T3-L1 preadipocyte differentiation. These results suggested that MAP4K3 and MAPK1 are potential target genes through which miR-196a-5p regulates 3T3-L1 preadipocyte differentiation. To verify the direct targeting relationship between miR-196a-5p and MAP4K3/MAPK1, dual-luciferase reporter assays were performed. As shown in [FIGURE:5(e-f)], luciferase activity detection revealed that miR-196a-5p significantly suppressed the luciferase activity of MAP4K3 and MAPK1 3'UTRs, while this inhibitory effect disappeared when the binding sites were mutated.

---

4. Discussion

It has been established that miRNAs can extensively regulate gene expression at the post-transcriptional level, thereby mediating complex and orderly biological processes such as embryonic stem cell self-renewal, tumor development and suppression, cell proliferation, differentiation, and apoptosis [19-21]. With the rapid development of high-throughput sequencing technology, numerous miRNAs differentially expressed during adipose development have been identified. Functional analyses have demonstrated that these differentially expressed miRNAs play important roles in adipose development. For example, miR-155 knockout mice can upregulate brown fat marker genes and resist high-fat diet-induced white fat deposition [22]; miR-145 can promote mouse preadipocyte proliferation and inhibit their differentiation into mature adipocytes [23]; and miR-199a-3p can regulate adipocyte differentiation and fatty acid composition by negatively regulating SCD expression [24].

In this study, we constructed an obese mouse model to detect miR-196a-5p expression changes during rapid fat deposition. As shown in [Figure 1: see original paper], miR-196a-5p was significantly highly expressed in adipose tissues of

high-fat diet-induced obese mice compared with normal chow-fed mice. Mouse 3T3-L1 preadipocytes are a classic cellular model for studying adipose development. Numerous studies have shown that miRNAs dynamically expressed during 3T3-L1 cell differentiation often participate in regulating adipocyte proliferation and differentiation [23, 25]. Therefore, we detected miR-196a-5p expression changes after inducing 3T3-L1 preadipocyte differentiation. The results showed that miR-196a-5p expression first increased and then gradually decreased during differentiation, suggesting that miR-196a-5p also plays an important role in adipocyte proliferation and differentiation. Overexpression and inhibition experiments demonstrated that miR-196a-5p inhibits 3T3-L1 preadipocyte proliferation and promotes their differentiation and lipid accumulation.

Adipocyte proliferation and differentiation are regulated by many factors. For instance, Cyclin D1 and Cyclin E play key roles in cell cycle progression from G1 to S phase, primarily by regulating cyclin-dependent kinases CDK2 and CDK4 to drive the cell cycle from G1 to S phase, thereby promoting cell proliferation [26-29]. Meanwhile, PPARγ and C/EBPα are key transcription factors regulating adipocyte differentiation that can promote adipocyte differentiation and lipid deposition by targeting fatty acid synthesis and transport-related factors such as LPL, SCD, ELOVL6, SREBP1, and FAS [30-33]. Consistent with our functional studies on miR-196a-5p, RT-PCR detection in this study revealed that miR-196a-5p suppressed cell proliferation regulators Cyclin D1, Cyclin E, CDK2, and CDK4 while promoting expression of adipocyte differentiation marker genes including PPARγ, C/EBPα, LPL, SCD, ELOVL6, and SREBP1. These results indicate that miR-196a-5p can regulate adipocyte proliferation and differentiation by directly or indirectly modulating key regulators of these processes.

Previous studies have shown that miRNAs can regulate complex biological processes by complementing specific sites in target gene 3'UTRs through their seed sequences to inhibit transcription [34]. To deeply explore the molecular mechanism by which miR-196a-5p regulates adipocyte differentiation, we further used bioinformatics software TargetScan to predict potential target genes of miR-196a-5p. As shown in [Figure 5: see original paper], the 3'UTRs of MAPK1 and MAP4K3 contain reverse complementary sequences to the miR-196a-5p seed sequence. Overexpression or inhibition of miR-196a-5p in 3T3-L1 cells significantly decreased or increased MAPK1 and MAP4K3 mRNA levels, indicating that miR-196a-5p negatively regulates MAPK1 and MAP4K3 expression. Subsequent dual-luciferase experiments confirmed that MAPK1 and MAP4K3 are direct target genes of miR-196a-5p.

The MAPK family plays important roles in preadipocyte differentiation [35, 36]. Recently, Huang et al. [17] found that inhibiting MAPK1 expression in 3T3-L1 preadipocytes significantly promoted their differentiation into mature adipocytes, and miR-378a-3p could also promote lipogenesis by directly targeting and suppressing MAPK1. Additionally, shRNA interference of MAP4K3 expression in 3T3-L1 cells significantly reduced the proportion of preadipocytes

differentiating into adipocytes. Combined with previous dual-luciferase reporter and RT-PCR results, this study demonstrates that miR-196a-5p promotes 3T3-L1 preadipocyte differentiation into mature adipocytes by targeting MAPK1 and MAP4K3.

References

[1] Sun B, Karin M. Obesity, Inflammation and Liver Cancer. Journal of Hepatology, 2012, 56(3): 704-713.

[2] Kahn SE, Hull RL, Utzschneider KM, et al. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature, 2007, 444(7121): 840-846.

[3] Sarma S, Devlin RA, Gilliland J, et al. The Effect of Leisure-time Physical Activity on Obesity, Diabetes, High BP and Heart Disease among Canadians: Evidence from 2000/01 to 2005/06. Working Papers. 2013, 01:1-62.

[4] Mayer J, Thomas DW. Regulation of food intake and obesity. Science, 1967, 156(3773): 328-337.

[5] Li J. MicroRNAs: Target Recognition and Regulatory Functions. Cell, 2009, 136 (2) :215-233.

[6] Ambros V. The function of animal MicroRNAs. Nature, 2004, 431(7006): 350-355.

[7] Du T, Zamore PD. microPrimer: the biogenesis and function of microRNA. Development, 2005, 132(21): 4645-4652.

[8] Perri R, Nares S, Zhang S, et al. MicroRNA modulation in obesity and periodontitis. Journal of Dental Research, 2012, 91(1): 33-38.

[9] Kajimoto K, Naraba H, Iwai N. MicroRNA and 3T3-L1 pre-adipocyte differentiation. Rna-a Publication of the Rna Society, 2006, 12(9): 1626-1632.

[10] Berthold S, Kovacs P, Fasshauer M, et al. MicroRNA expression in human omental and subcutaneous adipose tissue. Plos One, 2009, 4(3): e4699.

[11] Karbiener M, Fischer C, Nowitsch S, et al. microRNA miR-27b impairs human adipocyte differentiation and targets PPARγ. Biochemical and Biophysical Research Communications , 2009, 390(2): 247-251.

[12] Peng Y, Xiang H, Chen C, et al. MiR-224 impairs adipocyte early differentiation and regulates fatty acid metabolism. International journal of biochemistry & cell biology, 2013, 45(8): 1585-1593.

[13] Lv S, Ma M, Sun Y et al. MicroRNA-129-5p inhibits 3T3-L1 preadipocyte proliferation by targeting G3BP. Animal Cells & Systems the Official Publication of the Zoological Society of Korea, 2017, 21(4): 269-277.

[14] Pan J, Li X, Wu W, et al. Long non-coding RNA UCA1 promotes cisplatin/gemcitabine resistance through CREB modulating miR-196a-5p in bladder cancer cells. Cancer Letters, 2016, 382(1): 64-76.

[15] Zhao X, Liu Y, Zheng J, et al. GAS5 suppresses malignancy of human glioma stem cells via a miR-196a-5p/FOXO1 feedback loop. Biochimica Et Biophysica Acta. 2017, 1864 (10): 1605-1617.

[16] Zhao HL, Yao N, Wei XJ, et al. miR-196a-5p suppresses self-renewal of mouse embryonic stem cells. Basic & Clinical Medicine, 2014, 34(12): 1645-1649.

[17] Huang N, Wang J, Xie W, et al. MiR-378a-3p enhances adipogenesis by targeting mitogen-activated protein kinase 1. Biochemical & Biophysical Research Communications, 2015, 457(1): 37-42.

[18] Huang JY. The effect of Map4k3 on adipocytes differentiation. Dissertation of institute of life science, tzu chi university, 2012.

[19] Chen JF, Mandel EM, Thomson JM, et al. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. Nature Genetics, 2006, 38(2): 228-233.

[20] Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecologic Oncology, 2008, 110(1): 13-21.

[21] Delaloy C, Liu L, Lee JA, et al. MicroRNA-9 Coordinates Proliferation and Migration of Human Embryonic Stem Cell-Derived Neural Progenitors. Cell Stem Cell, 2010, 6(4): 323-335.

[22] Gaudet AD, Fonken LK, Gushchina LV, et al. miR-155 Deletion in Female Mice Prevents Diet-Induced Obesity. Scientific Reports, 2016, 6(22862): 1-10.

[23] Du J, Cheng X, Shen L, et al. Methylation of miR-145a-5p promoter mediates adipocytes differentiation. Biochemical and Biophysical Research Communications, 2016, 475(1): 140-148.

[24] Tan Z, Du J, Shen L, et al. miR-199a-3p affects adipocytes differentiation and fatty acid composition through targeting SCD. Biochemical and Biophysical Research Communications, 2017, 492(1): 82-88.

[25] Du J, Xu Y, Zhang P. et al. MicroRNA-125a-5p Affects Adipocytes Proliferation, Differentiation and Fatty Acid Composition of Porcine Intramuscular Fat. International Journal of Molecular Sciences, 2018, 19(2): 1-3.

[26] Baldin V, Lukas J, Marcote MJ, et al. Cyclin D1 is a nuclear protein required for cell cycle progression in G1. Genes & Development. 1993, 7(5): 812-821.

[27] Stacey DW. Cyclin D1 serves as a cell cycle regulatory switch in actively proliferating cells. Current Opinion in Cell Biology. 2003, 15(2): 158-163.

[28] Koff A, Dulic V, Lees E, et al. Formation and activation of a cyclin E-cdk2 complex during the G1 phase of the human cell cycle. Science, 1992, 257(5072): 1689-1694.

[29] Meyer CA, Jacobs HW, Lehner CF. Cyclin D-cdk4 is not a master regulator of cell multiplication in Drosophila embryos. Current Biology, 2002, 12(8): 661-666.

[30] Siersbæk R, Nielsen R, Mandrup S. PPARγ in adipocyte differentiation and metabolism – Novel insights from genome-wide studies. Febs Letters, 2010, 584(15): 3242-3249.

[31] Choi SK, Park S, Jang S, et al. Cascade regulation of PPARγ(2) and C/EBPα signaling pathways by celastrol impairs adipocyte differentiation and stimulates lipolysis in 3T3-L1 adipocytes. Metabolism Clinical & Experimental, 2016, 65(5): 646-654.

[32] J Z, F G, G W, et al. miR-20a regulates adipocyte differentiation by targeting lysine-specific demethylase 6b and transforming growth factor-β signaling. International Journal of Obesity, 2015, 39(8): 1282-1291.

[33] Rosen ED, Hsu CH, Wang X, et al. C/EBPα induces adipogenesis through PPARγ: a unified pathway. Genes & Development, 2002, 16(1): 22-26.

[34] Bushati N, Cohen SM. microRNA functions. Annual Review of Cell & Developmental Biology, 2007, 23(23): 175-205.

[35] Bost F, Aouadi M, Caron L, et al. The role of MAPKs in adipocyte differentiation and obesity. Biochimie, 2005, 87(1): 51-56.

[36] Machinalquélin F, Dieudonné MN, Leneveu MC, et al. Proadipogenic effect of leptin on rat preadipocytes in vitro: activation of MAPK and STAT3 signaling pathways. American Journal of Physiology-Cell Physiology, 2002, 282(4): C853-C863.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv — Machine translation. Verify with original.