LncRNA HCG11 inhibits adipocyte differentiation in human adipose-derived mesenchymal stem cells by sponging miR-204-5p to upregulate SIRT1

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Research

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Abstract

Background: LncRNAs have been discovered to play a key role in adipogenesis, vital in regulating adipose development. Numerous evidences show that adipogenesis is the leading cause of obesity, while the role of lncRNA HLA complex group 11 (HCG11) in adipocyte differentiation has not been elucidated.

Methods: hAdMSCs were used to establish a model of cell differentiation in vitro. Expression of lncRNA HCG11 was detected by RT-qPCR analysis. Transfection of the shRNA targeting HCG11 or pcDNA-HCG11 into hAdMSCs was also assessed. The adipogenic marker proteins C/EBP α , FABP4 and PPAR γ 2 and important inflammatory factors IL-6 and TNF- α were detected by Western blot. Bioinformatics analysis predicted the target genes of HCG11 and miR-204-5p, which was confirmed by luciferase reporter gene analysis and RNA pull-down analysis.

Results: Here we show that lncRNA HCG11 was decreased as the degree of adipogenesis. The expression of C/EBP α , FABP4 and PPAR γ 2 were significantly downregulated transfected with pcDNA-HCG11 in hAdMSCs at different stages, while knockdown lncRNA HCG11 can promote adipocyte differentiation. In addition, miR-204-5p was a potential target gene of HCG11 and SIRT1 could directly target miR-204-5p. Overexpression of SIRT1 or transfected with agonists of SIRT1 (Res) significantly inhibited adipogenic marker protein levels and inflammatory responses, and the proliferation of hAdMSCs were also inhibited. pcDNA-HCG11 and miR-204-5p mimic were co-transfected into hAdMSCs, we found that miR-204-5p mimic reversed the suppressor effect of pcDNA-HCG11.

Conclusion: Our findings showed that HCG11 negatively regulated cell proliferation, inflammatory responses and adipogenesis by miR-204-5p/SIRT1 axis. And our findings may provide a new target for the study of adipogenesis in hAdMSCs and obesity.

Introduction

Obesity was a chronic inflammatory state, accompanied by elevated levels of plasma inflammatory factors, such as tumor necrosis factor, IL-6 and C-reactive protein [1]. Obesity was caused by excessive hypertrophy and an increased number of fat cells [2]. Adipogenesis was the process by which fibroblast-like preadipocytes differentiate into mature, lipid-filled, and insulin-sensitive adipocytes. Adipose tissue cell differentiation went through two stages. The first stage was the directional transformation of mesenchymal cells into adipocytes, and the second stage was the differentiation of preadipocytes into mature adipocytes [3]. Induced by insulin, glucocorticoids, and intracellular cAMP, preadipocytes had four processes: the contact inhibition phase, the mitotic clone expansion phase, the middle differentiation phase, and the end differentiation phase.

With the continuous improvement of people's living standards, the occurrence of obesity and related metabolic diseases have become one of the important factors that endanger human health [4, 5]. Studies have shown that excessive accumulation of triglyceride content in fat cells may cause obesity, which increased the risk of many diseases such as insulin resistance [6, 7], type 2 diabetes [8], cardiovascular

disease [9] and hypertension [10]. The formation and maturation of adipocytes required the sequential expression of a large number of genes through various developmental stages, and a variety of biological processes were involved. At the same time, adipose tissue secreted a large number of adipokines through autocrine, paracrine, and endocrine methods to affect a series of biological activities of the entire body [11, 12].

Adipose tissue is a dynamic organ and its expansion is controlled by the process and adipogenesis. With high-throughput sequencing technology, recent studies showed that long noncoding RNAs (lncRNAs) were involved in adipogenesis, such as Blnc1 [13] and lncBATE10 [14], vital in regulating adipose development. Furthermore, based on lncRNAs location with respect to protein-coding genes, lncRNAs can be classified into subclasses including sense, antisense, bidirectional, intronic, and intergenic [15]. lncRNAs involved in various cellular processes such as proliferation [16], apoptosis [17], differentiation and inflammation [18].

In recent years, more and more studies have found that lncRNAs can regulate the adipocyte differentiation [18, 19]. For example, lncRNA SRA affected adipogenesis by binding to the promoter region of PPAR γ and promoting its transcription [20]. Gene expression profiling analysis on undifferentiated preadipocytes and mature adipocytes were used to further study of lncRNAs. After comparison, the researchers obtained 175 lncRNAs that changed during differentiation, and some of these lncRNAs were highly expressed in adipose tissue. These 20 lncRNAs, which were important in the differentiation process, were obtained according to their upregulation levels and the application of siRNA technology, which were called lncRNAs regulated in adipogenesis (lncRAPs) [21]. These studies provided evidences for the key role of lncRNAs in adipocyte differentiation. lncRNA HCG11 has been demonstrated to participate in the regulation of progression and prognosis in glioma [22]. In addition, downregulation of lncRNA HCG11 in prostate cancer was associated with poor prognosis [23] and HCG11 could regulated its target protein insulin-like growth factor 2 mRNA-binding protein 1 (IGF2BP1). These suggested that HCG11 was involved in embryo development and cell differentiation. However, the detailed function and regulatory mechanism of HCG11 in cell progression of obesity have not been fully illustrated.

In our preliminary study, we induced adipocyte human adipose-derived mesenchymal stem cells (hAdMSCs) to differentiate into mature adipocytes, and detected relevant differentiation proteins at different stages of differentiation. The results showed that the expression of lncRNA HCG11 gradually decreased in cell differentiation. We have constructed hAdMSCs that overexpression or knockdown lncRNA HCG11 to induce differentiation. Experimental data showed that overexpression of lncRNA HCG11 can inhibit adipocyte differentiation and knockdown lncRNA HCG11 can promote its differentiation. These data also showed lncRNA HCG11 was a negative regulator of adipocyte differentiation.

Materials And Methods

Cell culture and differentiation of hAdMSCs

hAdMSCs (Procell, Wuhan, China) were resuspended in Dulbecco's modified Eagle medium (DMEM) (Thermo Fisher Scientific, Waltham, MA, USA) mixture supplemented with 10% fetal bovine serum (GIBCO, Carlsbad, CA, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin (Thermo Fisher Scientific, Waltham, MA, USA) in a humidified incubator containing 5% CO₂ at 37°C. hAdMSCs at passage five were grown for 2 days, followed by differentiation into adipocyte. For adipogenic differentiation, hAdMSCs were induced using the StemPro Adipogenesis differentiation kit media. The differentiation medium was subsequently changed every 3 days. After several days, Oil Red O staining was obtained for adipogenic differentiation of hAdMSCs.

Oil red O staining

The cellular lipid content was assessed by Oil Red O staining (Sigma, MO, USA). At 6 days and 12 days, cells were washed and fixed in 4% formalin for 1 h, stained with Oil Red O working solution and incubated for another 1 h at room temperature. After being washed twice with phosphate-buffered saline, the cells were photographed by a light microscope (Olympus, Japan).

Cell transfection

To overexpress HCG11, HCG11 genomic fragment was cloned by polymerase chain reaction and then inserted into the pcDNA3.1 empty vector. Cells were planted in 6-well plates, at about 70% confluence, and then transfected transiently with miR-204-3p mimic, miR-204-5p inhibitor and their negative control, following the manufacturer's instructions. pGPU6/GFP/Neo vector (GenePharma, Shanghai, China) was used to construct vector with short hairpin RNA (shRNA) against HCG11, and the non-targeting sequences (scramble shRNA).

RNA extraction and real-time quantitative polymerase chain reaction (RT-qPCR) analysis

Total RNA of hAdMSCs was extracted in accordance with the instruction of Trizol reagent (Thermo Fisher Scientific Inc., Waltham, MA, USA). The reverse transcription of mRNA was performed using the High-Capacity complementary DNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA). The mRNA level was quantified by RT-qPCR using a SYBR Premix Ex Taq (TaKaRa Biotech, Dalian, China), and GAPDH was used as the endogenous control. The experimental operation was repeated at least in three times independently. The experiment used a 20 µL reaction system: cDNA (1 µL), specific primers (1 µL), SYBR Green Mix (10 µL), and ddH₂O (7 µL). All PCR steps were performed on the ABI 7300 Real Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) under the following conditions: 95 °C for 1 min followed by 35 cycles of 95 °C for 20 s, then 56 °C for 10 s and 72 °C for 15 s and the data were analyzed using the relative quantification $2^{-\Delta\Delta CT}$ method.

Cell proliferation assay

Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) method was used to measure the cell proliferation efficiency after transfected 48 h. First, cells were grown at 2×10^4 cells in 6-well plates containing 8 μ L CCK-8 plus 100 μ L FBS-free medium. Then measured the cell proliferation efficiency at 24, 48, 72 and 96 h and the absorbance were read at 450 nm. Cells were incubated in a humidified incubator containing 5% CO₂ at 37°C.

Luciferase reporter gene assay

Target gene analysis was performed using the online database of StarBase biological prediction website (<http://starbase.sysu.edu.cn/>). The full length of the 3' untranslated region (3'-UTR) region of the SIRT1 or HCG11 were cloned and amplified, then the PCR product was cloned into the polyclonal loci downstream of the pmir-GLO luciferase gene (Promega, Madison, WI, USA). Cells co-transfected were in HEK293T cells (Shanghai, china), and the luciferase activity was measured using a Synergy 2 Multi-detection Microplate Reader (BioTek Instruments, Inc.). The experiment was independently repeated three times. Data were normalized for transfection efficiency by dividing firefly luciferase activity by *Renilla* luciferase activity.

Western blot analysis

PBS was used to wash the hAdMSCs twice and cell lysed using RIPA lysis buffer (CW Biotech, Beijing, China). Protein concentration was measured by BCA kit (KeyGEN, Nanjing, China) and adjusted to 3 μ g/ μ L prior to conducting Western blot. 10 μ L boiled proteins per well with 5 $\times$ loading buffer (Beyotime Biotechnology, China) were separated in 12% SDS-PAGE at 70 V for 20 min and 100 V for 100 min. Samples were then transferred to PVDF membranes (Millipore, Bedford, MA, USA) at 350 mA for 105 min. Then the membranes were blocked with 5% nonfat milk 2 h incubated with primary antibodies. The following antibodies were respectively used to incubate with the membrane at 4°C overnight: rabbit fatty acid binding protein (FABP4) antibody (1:600 dilution, ab92501, Abcam, Cambridge, UK), rabbit peroxisome proliferator-activated receptor gamma (PPAR γ) antibody (1:500 dilution, ab45036, Abcam, Cambridge, UK), rabbit Adiponectin (AdipoQ) antibody (1:400 dilution, ab3455, Abcam, Cambridge, UK), rabbit CCAAT-enhancer-binding protein α (C/EBP α) antibody (1:500 dilution, ab40764, Abcam, Cambridge, UK), rabbit lipoprotein lipase (LPL) antibody (1:300 dilution, ab247525, Abcam, Cambridge, UK), rabbit interleukin-6 (IL-6) antibody (1:400 dilution, ab6672, Abcam, Cambridge, UK), rabbit tumor necrosis factor- α (TNF- α) antibody (1:400 dilution, ab6671, Abcam, Cambridge, UK). D-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was set as the endogenous control. After 3 times washing with TBS-T, the secondary antibody (goat Anti-Rabbit IgG, ab205718, Abcam, Cambridge, UK) was respectively used to incubate with the membrane for 1 h at room temperature. The ECL system was used to show the banding of proteins and the optical density of the protein bands were quantified using Image J software (National Institutes of Health, Bethesda, Md, USA). The experiment was performed three times to obtain a mean value. The relative expression level of the target gene was displayed as the fold change versus control group.

RNA pull-down assay

HEK293 cells were transfected with biotinylated miRNA (200 nM) for 24 h. The cells were gently washed twice with PBS and were added ice-cold RNA pull-down lysis buffer on ice for 10 min. Centrifuge the lysate obtained in the previous step and aliquot the obtained supernatant into 50 μ L for subsequent input research. Streptavidin magnetic beads (Thermo Scientific Fisher Scientific, Waltham, MA, USA) were added to the remaining lysate and incubated at room temperature. Then RNase-free bovine serum albumin (Sigma, St. Louis, MO, USA) and yeast tRNA (Sigma, St. Louis, MO, USA) were added. The mixture was incubated at 4 °C for 3 h. In order to obtain a pure sample, ice-cold lysis buffer and low-salt buffer were used to wash the sample three times, and finally with high-salt buffer. At last, TRIzol (Sigma, St. Louis, MO, USA) was used to purify the bound RNAs and RT-qPCR was used to analysis the expression of HCG11.

Statistical analysis

Data analysis was performed with SPSS version 22.0 software. Each measurement was obtained from at least triple experiments and expressed as the mean $\pm$ standard error of mean (SEM). Statistical significance difference was set at $P < 0.05$ using one-way variation analysis followed by student's *t*-test.

Results

Establishment of hAdMSCs induced differentiation model in vitro

After hAdMSCs were cultured to 100%, the cells were continued to culture and then made the cells draw from the growth cycle and be in contact-inhibited state for 2 days. Follow the steps described in the experimental methods to induce differentiation and establish a model of hAdMSC differentiation *in vitro*. The cells on days 0 and 12 of differentiation were collected for oil red O staining, and lipid droplets were observed under a light microscope to identify the degree of adipocyte differentiation. At the same time, cells on days 0, 3, 6, 9, and 12 of the differentiation processes were collected adipocytes. Compared with preadipocytes (day 0), differentiation of hAdMSCs showed lipid droplets in the cytoplasm on day 12, and the adipocyte differentiation rate was close to 80%. Moreover, with the induction and differentiation of adipocytes, the expression of transcription factor C/EBP α (Figure 1A) increased sharply on the third day of the test, while transcription factor PPAR γ 2 (Figure 1B) continued to increase. Expression of AdipoQ (Figure 1C) was also first increased and then decreased, while expression of FABP4 (Figure 1D) and LPL (Figure 1E) continued to increase. These results proved that adipocytes induced differentiation successfully. The expression of HCG11 was significantly decreased in hAdMSCs (Figure 1F) during different test days, and gradually decreased as the expression of test days increased. Western blot was used to detect the expression of adipogenic marker proteins (Figure 1G). These results demonstrated that downregulation of HCG11 might associate with adipocyte differentiation in hAdMSCs.

HCG11 overexpression inhibited adipocyte differentiation in hAdMSCs

To investigate the function of HCG11 in adipocyte differentiation, HCG11 were overexpressed in hAdMSCs. The cells transfected with pcDNA-HCG11 (3 µg/mL) and its control on days 0 and 6, 12 of differentiation were collected for oil red O staining, and lipid droplets were observed under a light microscope to identify the degree of adipocyte differentiation. Moreover, with the induction and differentiation of adipocytes, the expression of transcription factor C/EBPα (Figure 2A) and PPARγ2 (Figure 2B) were decreased in hAdMSCs transfected with pcDNA-HCG11 (3 µg/mL), and expression of AdipoQ (Figure 2C), FABP4 (Figure 2D) and LPL (Figure 2E) were also decreased in hAdMSCs transfected with pcDNA-HCG11 (3 µg/mL) compared with transfected with control or vector. HCG11 overexpression was achieved by transfecting the pcDNA-HCG11 (3 µg/mL) into hAdMSCs and its transfection efficiency was detected (Fig. 2F). Western blot was used to detect the expression of adipogenic marker proteins (Figure 2G). Especially, expression of C/EBPα, FABP4 and PPARγ2 significantly decreased on the third day, which proved that HCG11 overexpression affected adipocyte differentiation successfully in hAdMSCs.

Downregulation of HCG11 promoted adipocyte differentiation in hAdMSCs

To further determine the potential role of HCG11 in adipocyte differentiation, we transfected with short hairpin RNA (shRNA) against HCG11 in hAdMSC. The cells on days 0 and 6, 12 of differentiation were collected for oil red O staining, and lipid droplets were observed under a light microscope to identify the degree of adipocyte differentiation. At the same time, cells on days 0, 3, 6, 9, and 12 of the differentiation processes were collected adipocytes. The result was as expected, expression of transcription factor C/EBPα (Figure 3A) and PPARγ2 (Figure 3B) were decreased, and expression of AdipoQ (Figure 3C), FABP4 (Figure 3D) and LPL (Figure 3E) were also increased in hAdMSCs transfected with shRNA against HCG11 compared with transfected with control or vector. The expression of HCG11 was downregulated on days 0, 3, 6, 9, and 12 (Fig. 3F). Western blot was used to detect the expression of adipogenic marker proteins (Figure 3G). It was revealed that cells transfected with shRNA against HCG11 effectively promoted the differentiation of adipocytes and accelerated the rate of adipocyte differentiation in hAdMSCs.

Mir-204-5p was a target of HCG11 in hAdMSCs

As is known to all, one of the most typical regulatory mechanisms of lncRNAs is served as ceRNAs or molecular sponges for miRNAs to negatively regulate the expression and activities of miRNAs. Bioinformatic analysis with online database of StarBase (<http://starbase.sysu.edu.cn/>) showed HCG11 directly bind to miR-204-5p (Figure 4A). To test whether HCG11 bind to miR-204-5p, we used the luciferase reporter gene assay to validate the binding of them. The results indicated HCG11 directly bind to miR-204-5p (Figure 4B). HEK293 cells were transfected with biotinylated miR-204-5p or its mutant form, and then a biotin-based pull-down assay was performed to detect HCG11 expression and normalize to a biotinylated mimic control by RT-qPCR (Figure 4C). Moreover, we detected expression of miR-204-

5p under different processing conditions by RT-qPCR analysis. It was found that miR-204-5p was significantly downregulated when HCG11 was overexpressed, while miR-204-5p was upregulated when cells were transfected with shRNA against HCG11 (Figure 4D). In addition, expression of miR-204-5p was significantly upregulated when cells were transfected with miR-204-5p mimic, while miR-204-5p was downregulated when cells were transfected with miR-204-5p inhibitor in hAdMSCs (Figure 4E). These results demonstrated that HCG11 negatively modulated miR-204-5p expression by binding to its 3'-UTR and might serve as a molecular sponge.

MiR-204-5p promoted the cell proliferation and induced inflammatory factor production in hAdMSCs

To study the effect of miR-204-5p on the biological function of adipocyte differentiation in hAdMSCs, we transfected miR-204-5p mimic, inhibitor and corresponding negative control oligonucleotides respectively in hAdMSCs. First, we tested expression of miR-204-5p after transfected with different oligonucleotides to detect the expression of adipogenic marker proteins. As the results showed that expression of different adipogenic marker proteins were significantly upregulated transfected with miR-204-5p mimic, while expression of different adipogenic marker proteins were significantly downregulated transfected with miR-204-5p inhibitor by Western blot in hAdMSCs (Figure 5A-5E). Next, we examined the expression of important inflammatory factors IL-6 and TNF- α . The expression of IL-6 and TNF- α were also significantly upregulated transfected with miR-204-5p mimic, while the expression of IL-6 and TNF- α were significantly downregulated transfected with miR-204-5p inhibitor by Western blot in hAdMSCs (Figure 5F and 5G). Moreover, we examined the effect of transfected with miR-204-5p mimic or miR-204-5p inhibitor on the proliferation in hAdMSCs. The CCK-8 detected that upregulation of miR-204 significantly promoted cell growth in hAdMSCs, while downregulation of miR-204-5p decreased the cell proliferation (Figure 5H). Western blot was used to detect the expression of adipogenic marker proteins (Figure 5I). These results indicated that miR-204-5p was also significantly promoted the cell proliferation and induced inflammatory factor production in hAdMSCs.

Mir-204-5p targeted SIRT1 in hAdMSCs

To investigate the mechanisms of miR-204-5p in hAdMSCs, we used online database of StarBase (<http://starbase.sysu.edu.cn/>) to bioinformatic analysis. It showed that miR-204-5p directly bind to SIRT1 3'-UTR (Figure 6A). To test whether miR-204-5p bind to SIRT1 3'-UTR, we used luciferase reporter gene assay to validate. As the results showed, miR-204-5p directly bind to SIRT1 3'-UTR (Figure 6B). Then we detected expression of SIRT1 in hAdMSCs by Western blot analysis. These results showed that expression of SIRT1 was significantly decreased transfected with miR-204-5p mimic and upregulated transfected with miR-204-5p inhibitor (Figure 6C). Then we transfected resveratrol (Res), which was one of the famous agonists of SIRT1 in hAdMSCs. The cell transfected with agonists of SIRT1 (Res) was downregulated the expression of C/EBP α (Figure 6D), PPAR γ 2 (Figure 6E), AdipoQ (Figure 6F), FABP4 (Figure 6G) and LPL (Figure 6H). It showed us that Res might effectively reduce the secretion of fat-related cells, thereby reducing the differentiation of fat cells. Similar results also occurred when SIRT1 was overexpressed, leading to the expression of adipogenesis-related proteins decreased and affected fat

differentiation. Moreover, overexpression of SIRT1 also attenuated the effect of the miR-204-5p mimic decreased the expression of adipogenic marker proteins C/EBP α , PPAR γ 2, AdipoQ, FABP4 and LPL (Figure 6D–6H). Next, we tested the expression of IL-6 and TNF- α . After transfected with Res, the expression of IL-6 (Figure 6I) or TNF- α (Figure 6J) were both decreased, but their expression were significantly increased transfected with miR-204-5p mimic. And overexpressed of SIRT1 also attenuated the effect of miR-204-5p mimic decrease expression of IL-6 (Figure 6I) or TNF- α (Figure 6J). The CCK-8 detected that upregulation of miR-204 significantly promoted cell growth in hAdMSCs, while transfected with Res decreased the cell proliferation (Fig. 6K). Western blot was used to detect the expression of adipogenic marker proteins (Figure 6L). It has demonstrated that overexpression of SIRT1 or transfected with Res was also significantly inhibited the cell proliferation and reduced inflammatory factor production in hAdMSCs.

MiR-204-5p overexpression reversed the effect of HCG11 on inhibiting cell proliferation in hAdMSCs

In order to further validate the correlation between HCG11 and miR-204-5p, we co-transfected with pcDNA-HCG11 and miR-204-5p mimic in hAdMSCs. As shown in figures, pcDNA-HCG11 decreased the expression of adipogenic marker proteins C/EBP α , PPAR γ 2, AdipoQ, FABP4 and LPL, while mir-204-5p significantly attenuated the pcDNA-HCG11, while mir-204-5p significantly reversed the effect of pcDNA-HCG11 on the expression of adipogenic marker proteins (Figure 7A–7E). Next, we investigated the expression of inflammatory factor IL-6 and TNF- α under the same treatment with pcDNA-HCG11 and miR-204-5p mimic. pcDNA-HCG11 also decrease the expression of IL-6 and TNF- α , while mir-204-5p significantly attenuated the effect of pcDNA-HCG11. Next, mir-204-5p significantly reduced the inhibitory effect of pcDNA-HCG11 on cell proliferation (Fig. 7H). Western blot was used to detect the expression of adipogenic marker proteins (Figure 7I). Collectively, these results suggested that HCG11 overexpression inhibited cell proliferation, adipogenic differentiation and production of inflammatory factors in hAdMSCs by targeting miR-204-5p.

Discussion

Adipogenesis is a tightly ordered, multi-stage process that requires the continuous activation of multiple transcription factors, including the C/EBP family and PPAR [24]. Our studies suggested HCG11 downregulated transcription factors C/EBP and PPAR γ 2 expression during adipogenesis. Many studies have shown that several lncRNAs fulfill their roles by "sponging" miRNAs and competitively inhibiting their biological functions [18]. Our studies focused on the involvement of HCG11 in hAdMSCs and its possible molecular mechanism in adiposis. As the results showed that HCG11 was low expressed and regulated cell proliferation in hAdMSCs. Moreover, HCG11 was partially attributed to its ability to serve as a molecular sponge of miR-204-5p, leading to upregulation of SIRT1. Therefore, this study elucidated the important role of the HCG11/miR-204-5p/ SIRT1 axis in hAdMSCs, and also provided therapeutic targets on adipocyte differentiation.

HCG11 is a member of long non-coding family, and HCG11 had played a role in tumor suppression in many studies, such as hepatocellular carcinoma [25] and gastric cancer [26]. In the present study, the regulation of IGF2BP1 by HCG11 can inhibit the apoptosis of liver cancer cells through MAPK signal transduction. Moreover, HCG11 promoted the proliferation and migration of gastric cancer by contracting miR-1276/CTNNB1 and activating the Wnt signaling pathway. However, in our study, HCG11 could effectively inhibit adipocyte differentiation and inhibited cell growth. And HCG11 was partially attributed to its ability to serve as a molecular sponge of miR-204-5p. To further understand the mechanism of miR-204-5p in hAdMSCs, we used bioinformatic tools to predict the downstream targets of miR-204-5p. As expected, luciferase reporter gene assays showed the combination of miR-204-5p and SIRT1. The research showed that activation of SIRT1 led to deacetylation of Re1A/p65, thereby leading to transcriptional repression of various inflammation-related genes [27].

Clearly, the balance between pro-inflammatory and anti-inflammatory signals was critical to disease progression, and it had been recognized that many adipocyte-derived factors played a key role in maintaining inflammatory homeostasis. Related studies have described important cytokines associated with local pro-inflammatory reactions in adipocytes, leading to increased ectopic lipid accumulation and insulin resistance [28], while anti-inflammatory adipokines such as adiponectin strongly oppose inflammation, hyperglycemia or lipotoxicity damage [29]. Our studies showed that HCG11 regulated SIRT1 by targeting miR-204-5p to reduce the secretion of inflammatory factors IL-6 and TNF- α . As other studies have shown, the expression of miR-204-5p was downregulated in response to exogenous pro-inflammatory stimulus TNF- α in HK-2 cells, while that of IL-6 receptor α (IL-6 R) was upregulated [30]. Moreover, inflammation is related to the pathogenesis of insulin resistance and can predict the development of type 2 diabetes [31]. That meant that HCG11 can partially inhibit the inflammatory response caused by obesity, thereby reducing the risk of other related diseases.

Conclusion

In conclusion, we demonstrated that HCG11 plays an important role in inflammation, cell proliferation and adipose tissue cell differentiation. Inhibition of miR-204-5p could eventually alleviate inflammation and adipose tissue cell differentiation by upregulating the expression of SIRT1. Therefore, HCG11/miR-204-5p/SIRT1 axis may play a crucial role in hAdMSCs of the adiposis, and it also was a negative regulator of adipocyte differentiation.

Declarations

Conflict of interest statement

No conflict of interests.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

The research was conceived and designed by Dandan Li, Yang Liu and Wei Gao. The experiments was carried out by Dandan Li and Jiakai Han. The data was analyzed by Rongrong Yuan and Mengdi Zhang. The manuscript was wrote by Dandan Li and Zhenying Ge

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Figures

Figure 1

Identification of hAdMSCs adipocytes induce differentiation. (A) Expression of C/EBPα, (B) PPARγ2, (C) AdipoQ, (D) FABP4 and (E) LPL and in different stages (D0, D3, D6, D9, and D12). (F) Expression of HCG11 in different stages (D0, D3, D6, D9, and D12). (G) The protein was detected by Western blot. GAPDH was used as a loading control in each sample. Statistical significance was determined using an independent sample t-test. Values were expressed as mean ± SEM, n = 3. *P < 0.05 and **P < 0.01 vs. normal or control.

Figure 2

Differentiation of adipocytes overexpressing lncRNA HCG11. (A) Expression of C/EBPα, (B) PPARγ2, (C) FABP4, (D) C/EBPα, (E) LPL and (F) HCG11 in different stages (D0, D6 and D12) transfected with pcDNA-HCG11 and control. (G) The protein was detected by Western blot. GAPDH was used as a loading control in each sample. Statistical significance was determined using an independent sample t-test. Values were expressed as mean ± SEM, n = 3. *P < 0.05 and **P < 0.01 vs. normal or control.

Figure 3

Interfering with adipocytes differentiation of sh-HCG11. (A) Expression of C/EBPα, (B) PPARγ2, (C) AdipoQ, (D) FABP4, (E) LPL and (F) HCG11 in different stages (D0, D6 and D12) transfected with sh-HCG11 and control. (G) The protein was detected by Western blot. Statistical significance was determined using an independent sample t-test. Values were expressed as mean ± SEM, n = 3. *P < 0.05 and **P < 0.01 vs. normal or control.

Figure 4

HCG11 directly targeted mir-204-5p. (A) Online database StarBase showed sequence alignment between HCG11 and miR-204-5p. (B) The luciferase reporter gene assay was validated the binding of HCG11 and miR-204-5p. Firefly and Renilla luciferase activities were determined. (C) HEK293 cells were transfected with biotinylated miR-204-5p (Bio-204-5p-wt) or its mutant form (Bio-204-5p-mut), and then a biotin-based pull-down assay was performed to detect HCG11 expression and normalized to a biotinylated mimic control (Bio-NC) by RT-qPCR. (D) Expression of mir-204-5p transfected with pcDNA-HCG11 or sh-HCG11. (E) Expression of mir-204-5p transfected with miR-204-5p mimic or inhibitor. Statistical significance was

determined using an independent sample t-test. Values were expressed as mean $\pm$ SEM, $n = 3$. * $P < 0.05$ and ** $P < 0.01$ vs. normal or control.

Figure 5

MiR-204-5p overexpression promoted the cell proliferation and adipocytes differentiation in hAdMSCs. The hAdMSCs were transfected with NC mimic (20 nM), miR-204-5p mimic (20 nM), NC inhibitor (20 nM), miR-204-5p inhibitor (20 nM) for 48 h. (A) Expression of C/EBP α , (B) PPAR γ 2, (C) AdipoQ, (D) FABP4 and (E) LPL in hAdMSCs transfected with miR-204-5p mimic, miR-204-5p inhibitor and their control. Expression of (F) IL-6 and (G) TNF- α in hAdMSCs transfected with miR-204-5p mimic, miR-204-5p inhibitor and their control. (H) Cell proliferation ability was tested by CCK-8. (I) The protein was detected by Western blot. Statistical significance was determined using an independent sample t-test. Values were expressed as mean $\pm$ SEM, $n = 3$. * $P < 0.05$ and ** $P < 0.01$ vs. normal or control.

Figure 6

MiR-204-5p directly targeted SIRT1. (A) Online database StarBase showed sequence alignment between miR-204-5p and SIRT1. (B) The luciferase reporter gene assay was validated the binding of miR-204-5p and SIRT1. Firefly and Renilla luciferase activities were determined. (C) Expression of SIRT1 in hAdMSCs transfected with miR-204-5p mimic, miR-204-5p inhibitor and their control. Expression of (D) C/EBPα, (E) PPARγ2, (F) AdipoQ, (G) FABP4 and (H) LPL in hAdMSCs transfected with miR-204-5p mimic, Res and pcDNA-SIRT1. Expression of (I) IL-6 and (J) TNF-α in hAdMSCs transfected with miR-204-5p mimic, Res and pcDNA-SIRT1. (K) Cell proliferation ability was tested by CCK-8. (L) The protein was detected by Western blot. Statistical significance was determined using an independent sample t-test. Values were expressed as mean ± SEM, n = 3. *P < 0.05 and **P < 0.01 vs. normal or control.

Figure 7

MiR-204-5p overexpression reversed the effect of HCG11 in hAdMSCs. (A) Expression of C/EBP α , (B) PPAR γ 2, (C) AdipoQ, (D) FABP4 and (E) LPL in hAdMSCs transfected with miR-204-5p mimic, pcDNA-HCG11 and their control. Expression of (F) IL-6 and (G) TNF- α in hAdMSCs transfected with miR-204-5p mimic, pcDNA-HCG11 and their control. (H) Cell proliferation ability was tested by CCK-8. (I) The protein was detected by Western blot. Statistical significance was determined using an independent sample t-test. Values were expressed as mean $\pm$ SEM, n = 3. *P < 0.05 and **P < 0.01 vs. normal or control.