Prolonged Efficiency of siRNA-Mediated Gene Silencing in Primary Cultures of Human Preadipocytes and Adipocytes

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Objective: Primary human preadipocytes and differentiated adipocytes in culture are valuable cell culture systems to study adipogenesis and adipose function in relation to human adipose biology. To use these systems for mechanistic studies, siRNA-mediated knockdown of genes for its effectiveness was studied.

Methods: Methods were developed to effectively deliver siRNA for gene silencing in primary preadipocytes isolated from human subcutaneous adipose tissue and newly differentiated adipocytes. Expression level of genes and proteins was measured using quantitative RT-PCR and western blotting. Lipid droplet morphology was observed using microscopy, and glycerol release was quantified as a measure of lipolysis.

Results: siRNA-mediated knockdown of genes in primary human preadipocytes resulted in prolonged silencing effects, suppressing genes throughout the process of their differentiation. In newly differentiated adipocytes, siRNA-mediated gene knockdown allowed proteins to stay depleted for at least 5 days. It was possible to re-express a protein after its siRNA-mediated depletion. Importantly, siRNA transfected human adipocytes remained metabolically active, responding to $\beta$-adrenergic stimulation to increase lipolysis.

Conclusions: Our study describes the methods of gene silencing in primary cultures of human preadipocytes and adipocytes and their prolonged effectiveness.

Introduction

Obesity, the excess accumulation of fat mass, is increasing worldwide. Expansion of adipose tissue depends on both new adipocyte formation (recruitment of progenitors and differentiation into adipocytes) and hypertrophy of existing adipocytes. In addition to their important metabolic function, adipocytes secrete a myriad of peptide hormones and cytokines that regulate systemic energy homeostasis and metabolism. Thus, mechanistic studies of how new-fat cells are formed and their metabolic and endocrine functions are altered are central to understanding the etiology of obesity and its metabolic complications. Widely used mouse 3T3-L1 and 3T3-F442A cell lines provide invaluable model systems, yet there is a growing need for human preadipocytes and adipocytes that have clear translational relevance. Although several human adipose cell lines are available, including Simpson-Golabi-Behmel syndrome (SGBS) and Human multipotent adipose-derived (hMAD) (1,2), primary preadipocytes isolated from adipose tissues can be differentiated into adipocytes in vitro and are useful for studies of donor or depot-dependent effects (3-12). In addition, newly differentiated human adipocytes in culture respond to physiologically relevant concentrations of hormones, insulin, and beta-adrenergic agonists (13-16). Thus, primary cultures of human preadipocytes and adipocytes are valuable tools for the studies of adipocyte development and function and their use is expected to increase.

Both loss and gain of function studies are utilized to test the roles of genes of interest. RNA interference (RNAi) is a robust gene-silencing mechanism with which knockdown of genes can be easily achieved for functional studies. Overexpression of genes in human adipocytes is readily achieved through adenovirus-mediated gene delivery (17). Although RNAi-mediated gene knockdown in primary human adipocytes and hMAD cells have been used in several studies (18-20), its efficacy in primary human preadipocytes or adipocytes has not been clearly described. Previously, we described an ex vivo method to deliver siRNA into primary mouse and human adipose tissue explants (21). In this study, we describe methods to effectively deliver siRNA to primary cultures of human preadipocytes and newly differentiated adipocytes for gene silencing. We demonstrate that RNAi-mediated knockdown of genes can easily be used for the studies to test their roles in adipogenesis and metabolic functions in human preadipocytes and adipocytes, respectively. In
addition, the expression of proteins can be repleted with lenti-virus delivery. The methods described are easily applicable to many genes, further increasing the use of primary cultures of human preadipocytes and adipocytes for mechanistic studies.

Methods

Materials
All chemicals were purchased from Sigma, except Rosiglitazone (Enzo) and recombinant human insulin (Lilly). Collagenase type I was purchased from Worthington Biochemical. Fetal bovine serum (FBS) and culture media were obtained from Life Technologies. siRNAs were purchased from Qiagen, Dharmacon, or Santa Cruz Biotechnologies. Transfection reagents were purchased from Qiagen (HiPerFect) and Life Technologies (Lipofectamine and PLUS reagents).

Human Subjects
Adipose tissues were obtained from five subjects (mean age 48 ± 4.1 years and BMI 34 ± 3.9 kg/m²) during panniculectomy in reduced obesity. All subjects provided informed consent. The protocol was approved by Institutional Review Board of Boston University Medical Center.

Isolation of Adipose Stromal Vascular Cells
Stromal vascular cells were isolated with collagenase digestion (type 1, 1 mg/ml in HBSS) for 2 hours (22,23). After digestion, the mixture was filtered with a 250 μm mesh and centrifuged at 500 g for 10 minutes. Cell pellets were treated with erythrocyte lysis buffer (0.154 mM NH₄Cl, 10 mM K₂HPO₄, and 0.1 mM EDTA, pH 7.3) and repelleted with centrifugation. Cells were resuspended in growth media (x-MEM with 10% FBS) and then plated for culturing. Cells were subcultured up to six passages. Cells from individual subjects were used without pooling. Experiments were repeated in cells derived from at least three independent donors.

Differentiation of Human Preadipocytes into Adipocytes
Preadipocytes plated in 12-well plates (5,000-15,000 cells/cm²) were cultured and differentiated as previously described (23). Briefly, 2d-post confluent cells were induced to differentiate in the complete differentiation media (DMEM/F12 with 500 μM IBMX, 100 nM insulin, 100 nM dexamethasone, 2 nM T₃, 10 μg/ml transferrin, 1 μM Rosiglitazone, 33 μM biotin and 17 μM pantothenic acid) for 7 days followed by maintenance in DMEM/F12 supplemented with 33 μM biotin, 17 μM pantothenic acid, 10 nM insulin, and 10 nM dexamethasone.

Gene Silencing in Preadipocytes
1. In the morning, preadipocytes were trypsinized and seeded at 15,000-00 cells/cm² in 12-well cell culture plates.
2. At late afternoon, cells were transfected with siRNA using HiPerFect reagent (Qiagen). For 1-well of a 12-well plate, siRNA (5-40 nM final concentration) was mixed with 6 μl HiPerFect reagent in total 200 μl serum-free x-MEM (without antibiotics) and incubated for 15 minutes at room temperature.
3. During the incubation, cells were re-fed with 200 μl growth media (x-MEM with 10% FBS without antibiotics). To prevent cells from drying out, the plate was swirled intermittently during refeeding.
4. The pre-incubated siRNA–HiPerFect mix was added dropwise to the wells, resulting in total 400 μl of x-MEM with 5% FBS. After gently rocking to mix, the plate was transferred to cell culture incubator.
5. After overnight transfection, cells were re-fed with regular growth media and allowed to proliferate until they were ready for differentiation. Cells generally reach post-confluent state between 4 and 5 days after transfection.

Gene Silencing in Differentiated Adipocytes
Between days 9 and 14 of differentiation, adipocytes were transfected with siRNA using Lipofectamine and PLUS reagents (Life Technologies). Serum and antibiotic-free DMEM/F12 were used for transfection.

1. For 1-well of a 12-well plate, siRNA (5-40 nM final concentration) was diluted in 50 μl DMEM/F12 and mixed with 5 μl PLUS reagent. The mixture was incubated at room temperature for 15 minutes.
2. In a separate tube, 3 μl Lipofectamine reagent was diluted with 50 μl DMEM/F12.
3. Preincubated siRNA–PLUS and diluted Lipofectamine were combined and incubated for 15 minutes at room temperature.
4. Meanwhile, cells were re-fed with 300 μl maintenance media. The siRNA–PLUS–Lipofectamine mixture was added dropwise to the cells and plate was gently rocked to mix the solutions.
5. After overnight transfection, cells were re-fed with regular maintenance media and maintained for additional 4-5 days with refeeding every 2-3 days.

Lentivirus production and Transduction
293T cells were seeded in 10-cm plates. Recombinant lentiviruses were produced by a five-plasmid transfection procedure as described (24). The packaged recombinant lentiviruses were harvested from the supernatant of cell cultures 48 hours after transfection and filtered through 0.45-μm filters. About 500 μl supernatant and 10 μg/ml polybrene were added to each well of the 12-well plates containing differentiated human adipocytes for overnight infection. Cells were re-fed with the maintenance medium on the following day and protein expression was measured with immunoblotting 4 days after transduction.

Lipolysis
Glycerol accumulation in culture media during the final day of culture was used as a measure of lipolysis. To provide a better assessment of changes in lipolytic rates, we also performed a 2-hour acute incubation in Krebs–Ringer bicarbonate buffer with 5 mM glucose and 4% bovine serum albumin (KRB + 4% BSA) under basal and β-adrenergically stimulated (isoproterenol, 1 μM) conditions as previously described (23). Glycerol concentration in the incubation media was measured fluorometrically (14).

Lipid Droplet Staining
Cells plated on glass cover slips were washed twice with PBS, fixed in 4% formaldehyde for 20 minutes, and quenched with 0.1 M glycine. Cells were then incubated with 0.5 μg/ml of Nile Red for 30
minutes and washed with PBS. The cover slips were mounted on glass slides with VectaShield (Vector Labs, CA) mounting medium.

**Microscopy**
Microscopy was performed using a Zeiss LSM 710-Live Duo scan (Carl Zeiss, Oberkochen, Germany) with a 100× oil immersion objective. Images were processed using Metamorph imaging software, version 6.1 (Universal Imaging, Downingtown, PA).

**RNA Extraction and Gene Expression**
Total RNA was extracted using Qiazol (Qiagen). After assessing quantity and quality with a Nano-Drop, 0.5–1 μg total RNA was reverse transcribed using Transcriptor First Strand cDNA synthesis kit (Roche). qPCR was performed on Light Cycler 480 (Roche) using Taqman probes (Applied Biosystems). 18S rRNA was used as a reference gene.

**Immunoblotting**
Cells were washed with ice-cold PBS and scraped into cell–lysis buffer (Cell Signaling) supplemented with 5% SDS and protease inhibitor cocktails (Pierce). Cell lysates were processed and 5-10 μg proteins was resolved in 10% or 15% Tris-HCl gels (Biorad) and transferred to PVDF membranes. After blocking in 5% milk, blots were probed for VDR (D-6, Santa Cruz), CIDEC (25), and loading controls (HSP90, α-tubulin, total AKT, Santa Cruz). Chemiluminescent images were captured using a Luminescent Image Analyzer (LAS4000, Fuji).

**Statistics**
Data were expressed as mean ± SE. Differences between groups were determined by analysis of variance (ANOVA) with repeated measures and Student t-test using GraphPad Prism.

**Results**
**Efficient Knockdown of Genes in Primary Human Preadipocytes**
siRNA-mediated knockdown is a useful tool to study the role of various proteins in cells. We describe methodologies of depleting proteins effectively in primary cultures of human preadipocytes and adipocytes using commercially available transfection reagents. Initially, we silenced COUP-TFII (a known adipogenic inhibitor (26,27)) in human subcutaneous preadipocytes and studied the effects of its knockdown on adipogenesis. As described in the methods, preadipocytes were transfected with control (scrambled siRNA) or COUP-TFII-siRNA using HiperFect reagents. After overnight transfection, cells were grown for additional 4-5 days till they were ready for differentiation. About 60-90% knockdown of COUP-TFII was achieved in preadipocytes derived from four independent donors (Figure 1A) (P < 0.02, n = 4).

To test whether knockdown of COUP-TFII-affected adipogenesis, preadipocytes were induced to differentiate following our published protocol (23) and expression markers of adipogenesis were measured during adipogenesis. As expected, silencing of COUP-TFII enhanced the degree of differentiation as demonstrated by higher expression levels of adipocyte markers, PPARγ, and FABP4 mRNA (Figure 1B). These data show that we can effectively silence genes of interest in primary human preadipocytes and study its down-stream effects, that is, adipogenesis.

We performed siRNA-mediated depletion of additional ten randomly picked genes in preadipocytes (5-40 nM final concentration of siRNA). About 50-95% reduction in the mRNA and protein levels was generally achieved (data for all the genes are not shown). siRNA transfection caused about 65% and 75% reduction of VDR at mRNA (Figure 2A) and protein levels, respectively (Figure 2B). These data show that our methodology of siRNA-mediated gene knockdown is efficient and applicable to a wide variety of genes.

Intriguingly, we found a prolonged effect of siRNA-mediated knockdown in primary human preadipocytes. siRNA against CIDEC, a differentiation-regulated lipid droplet associated protein, which regulates lipid droplet morphology and lipolysis in adipocytes (28-30), was transfected in preadipocytes. As expected, CIDEC levels increased during adipogenesis. Its expression, however, remained at reduced levels throughout differentiation process in CIDEC knockdown cells (Figure 2C). Even at day 14 of differentiation, CIDEC protein levels were lower: about 20% of its level in control cells (Figure 2D). Similar data were observed for other genes we tested (data not shown).
Knockdown of Genes in Newly-Differentiated Human Adipocytes

We next developed a method to silence genes in differentiated adipocytes. On day 9 of differentiation, newly differentiated human adipocytes were transfected with siRNA using Lipofectamine and PLUS reagents as described in the methods. About 85% reduction of VDR protein was observed after 5 days of transfection (Figure 3A). Knockdown of at least ten other genes was also tested where 60-95% reduction in protein expression was achieved (data not shown). We also tested our method of gene silencing in fully differentiated adipocytes (day 14) and found that it was less efficient and 15-20% lower levels of knockdown was achieved compared to the transfection on day 9 of differentiation (data not shown).

We next tested the functional outcomes of gene knockdown in differentiated human adipocytes. Human adipocytes were transfected with control or CIDEC siRNA on day 9 of differentiation, and maintained for up to an additional 10 days. CIDEC protein was reduced by >90% after 5 days of transfection (Figure 3B) and remained about 80% depleted even after 10 days post siRNA transfection, showing that the gene-silencing effects were maintained for at least 5 days. As expected, lipid droplets were smaller when CIDEC was silenced compared to the control cells (Figure 3C).

To study the metabolic consequences of CIDEC knockdown in human adipocytes, we measured lipolytic rates. Glycerol accumulation in culture media over 24-hour period was increased by 3.2-fold in CIDEC-silenced cells (P < 0.01, n = 5) (Figure 3D) compared with the control cells. To better assess the lipolytic capacity, cells were incubated in the KRB buffer with 4% albumin in both basal and beta-adrenergically-stimulated (isoproterenol, 1 μM) conditions for 2 hours. Similar to the data in culture media, CIDEC knockdown increased basal lipolytic rates by 2.4-fold (Figure 3E). In addition, β-adrenergically stimulated rates were also increased when CIDEC levels were reduced. These data show that CIDEC knockdown increases overall lipolytic rates without altering responsiveness to β-adrenergic stimuli.

CIDEC Repletion in CIDEC-Depleted Human Adipocytes

Since siRNA-mediated knockdown showed prolonged effectiveness, we wanted to investigate whether we could restore the expression of a protein after its depletion. CIDEC levels were reduced with RNAi in preadipocytes and cells were differentiated. On day 12 of differentiation, CIDEC lentivirus was transduced and cells were harvested on d17. As shown in Figure 4, CIDEC expression was restored with CIDEC-siRNA targets the 3'-untranslated region (UTR) of the CIDEC mRNA and the lentivirus contains cDNA sequence, which does not have the 3'-UTR. Therefore, the lentivirus-mediated CIDEC expression vector was not targeted by the CIDEC siRNA.

Discussion

Primary preadipocytes isolated from human adipose tissue and the adipocytes differentiated from them are important tools to study adipose biology pertaining to human physiology. The main purpose of this study was to design and study the effectiveness of siRNA-mediated gene silencing in human primary preadipocytes and differentiated adipocytes. We first demonstrated that gene silencing can be effectively achieved in primary human preadipocytes and adipocytes, and we then used it for testing its functional effects by silencing genes involved in adipogenesis and lipolysis. In addition, we present data showing repletion of a protein after silencing, thus...
demonstrating that expression levels of proteins can be easily modulated in human adipocytes.

Unexpectedly, we found that the silencing effects in preadipocytes are maintained throughout the differentiation at least till day 14 of differentiation (>20 days after transfection). We were also able to achieve an efficient and prolonged knockdown of genes in mature adipocytes. Gene and protein knockdown were observed after about 5 days of transfecting siRNA in differentiated adipocytes and remained effective at least for another 5 days. We did not test the effect after 5 days because we believe that this much time is enough to perform most of the biochemical studies in these cells. Gene silencing via transient transfection of siRNA is short lived in various cell types including 3T3-L1 (28,31,32). In fact, the siRNA effect starts fading away after about 3 days or so, thus limiting the time to perform various functional studies. We believe the prolonged silencing effects are at least in part due to the fact that siRNA is not diluted through extensive cell divisions during the differentiation.
process since human primary preadipocytes do not undergo cell division to enter differentiation (23), and cell numbers remain same during differentiation (23). In addition, another possibility could be that siRNA is stable in primary preadipocytes and adipocytes. Further studies are needed to determine the reason of prolonged effect of siRNA in these cells.

Of note, we found subject-dependent variations in the degree of knockdown with siRNA (data not shown). Although we do not completely understand the sources of these variations, we speculate the differences in extracellular matrix composition may affect the transfection efficiency. Nonetheless, the effects of gene silencing were observed in all of the subjects we used.

We present data showing siRNA-mediated gene silencing can be easily utilized for the functional studies, adipogenesis and lipolysis. COUP-TFI is an inhibitor of adipogenesis (26,27). As expected, we found that siRNA-mediated knockdown of COUP-TFI had a significant effect on differentiation of human primary preadipocytes. CIDEK knockdown in 3T3-L1 adipocytes caused lipid droplet fragmentation and increased lipolysis (28-30). As anticipated, siRNA-mediated CIDEK depletion in mature human adipocytes showed fragmentation of lipid droplets and an increased rate of lipolysis. These data showed that siRNA-transfected cells are metabolically active and respond to the β-adrenergic stimulation to increase lipolysis.

Overall, in this study, we describe methods to effectively silence genes of interest in primary preadipocytes and adipocytes. We believe these gene-silencing methods can be readily used for the functions studies. Primary cultures of adipose derived preadipocytes and adipocytes differentiated from them provide an additional cell culture system and can be used to study the potential donor- or depot-specific cell autonomous effects in human preadipocytes and adipocytes (3,5,10-12). When combined with modifications in proliferation and differentiation protocols (23,34), our gene-silencing methods are expected to increase the use of the primary human adipose cells.

References

1. Fischer-Posovszky P, Newell FS, Wabitsch M, Tornqvist HE. Human SGBS cells—a unique tool for studies of human fat cell biology. Obes Facts 2008;1:184-189.
2. Bordichia M, Liu D, Amri EZ, et al. Cardiac natuureptide act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. J Clin Invest 2012;122:1022-1036.
3. Hauner H, Wabitsch M, Pfeiffer EF. Differentiation of adipocyte precursor cells from obese and nonobese adult women and from different adipose tissue sites. Horm Metab Res Suppl 1988;19:35-39.
4. Tchkonia T, Giorgadze N, Pirtskhalava T, et al. Fat depot-specific characteristics are retained in strains derived from single human preadipocytes. Diabetes 2006;55:2571-2578.
5. Tchkonia T, Giorgadze N, Pirtskhalava T, et al. Fat depot origin affects adipogenesis in primary cultured and cloned human preadipocytes. Am J Physiol Regul Integr Comp Physiol 2002;282:R1286-R1296.
6. Tchkonia T, Giorgadze N, Pirtskhalava T, et al. Fat depot origin affects adipogenesis in primary cultured and cloned human preadipocytes. Am J Physiol 2005;288:E267-E277.
7. Tchkonia T, Giorgadze N, Pirtskhalava T, et al. Fat depot-specific characteristics are retained in strains derived from single human preadipocytes. Diabetes 2006;55:2571-2578.
8. Hauner H, Enterman G. Regional variation of adipose differentiation in cultured stromal-vascular cells from the abdominal and femoral adipose tissue of obese women. Int J Obes 1991;15:121-126.
9. Tchkakalova YD, Koutsari C, Votruba SB, et al. Sex- and depot-dependent differences in adipogenesis in normal-weight humans. Obesity 2010;18:1875-1880.
10. Isakson P, Hammarstedt A, Gustafson B, Smith U. Impaired preadipocyte differentiation in human abdominal obesity: role of Wnt, tumor necrosis factor-alpha, and inflammation. Diabetes 2009;58:1550-1557.
11. Perez LM, Bernal A, San MN, Lorenzo M, Fernandez-Veredolo S, Galvez BG. Metabolic rescue of obese adipose-derived stems cells by Lin28/LET7 pathway. Diabetes 2013;62:2368-2379.
12. Sepe A, Tchkonia T, Thomou T, Zamboni M, Kirkland JL. Aging and regional differences in fat cell progenitors—a mini-review. Gerontologist 2011;57:66-75.
13. Lysecki E, Westergren H, Brynhildson J, et al. Subcutaneous adipocytes from obese hyperinsulinemic women with polycystic ovary syndrome exhibit normal insulin sensitivity but reduced maximal insulin responsiveness. Eur J Endocrinol 2009;155:831-835.
14. Fried SK, Tittelbach T, Blumenthal J, et al. Resistance to the antilipolytic effect of insulin in adipocytes of African-American compared to Caucasian postmenopausal women. J Lipid Res 2010;51:1193-1200.
15. Lee MJ, Wu Y, Fried SK. A modified protocol to maximize differentiation of human preadipocytes and improve metabolic phenotypes. Obesity 2012;20:2334-2340.
16. Lee MJ, Fried SK. Glucocorticoids antagonize tumor necrosis factor-alpha-mediated lipolysis and resistance to the antilipolytic effect of insulin in human adipocytes. Am J Physiol Endocrinol Metab 2012;303:E1126-E1133.
17. Tiraby C, Tavernier G, Lefort C, et al. Acquorment of brown fat cell features by human white adipocytes. J Biol Chem 2003;278:33370-33376.
18. Pettersson AT, Laurencikiene J, Mejhet N, et al. A possible inflammatory role of twist1 in human white adipocytes. Diabetes 2010;59:564-571.
19. Bezaire V, Maial A, Ribet C, et al. Contribution of adipose triglyceride lipase and hormone-sensitive lipase to lipolysis in hMADS adipocytes. J Biol Chem 2009;284:18282-18291.
20. Lee YJ, Takahashi N, Yasubuchi M, et al. Triiodothyronine induces UCPI expression and mitochondrial biogenesis in human adipocytes. Am J Physiol Cell Physiol 2012;302:C563-C574.
21. Puri V, Chaklada A, Virbusiv JV, et al. RNAi-based gene silencing in primary mouse and human adipose tissues. J Lipid Res 2007;48:465-471.
22. Hauner H, Skurk T, Wabitsch M. Cultures of human adipocyte precursor cells. Methods Mol Biol 2001;155:239-247.
23. Lee MJ, Yu Y, Fried SK. A modified protocol to maximize differentiation of human preadipocytes and improve metabolic phenotypes. 426. Obesity 2012;20:2334-2340.
24. Mostoslavsky G, Kotton DN, Fabian AJ, Gray JT, Lee JS, Mulligan RC. Efficiency of transduction of highly purified murine hematopoietic stem cells by lentiviral and oncoretroviral vectors under conditions of minimal in vitro manipulation. Mol Ther 2005;11:932-940.
25. Jambunathan S, Yin J, Khan W, Tamori Y, Puri V. FSP27 Promotes lipodroplet clustering and then fusion to regulate triglyceride accumulation. PLoS One 2011;6:e28614.
26. Xu Z, Yu S, Hsu CH, Eguchi J, Rosen ED. The orphan nuclear receptor chicken ovalbumin upstream promoter-transcription factor II is a critical regulator of adipogenesis. Proc Natl Acad Sci U S A 2008;105:2421-2426.
27. Okamura M, Kudo H, Wakabayashi K, et al. COUP-TFI acts downstream of Wnt/beta-catenin signal to silence PPARgamma gene expression and repress adipogenesis. Proc Natl Acad Sci U S A 2009;106:5819-5824.
28. Puri V, Konda S, Ranjit S, et al. Fat-specific protein 27, a novel lipid droplet protein that enhances triglyceride storage. 375. J Biol Chem 2007;282:34213-34218.
29. Keller P, Petrie IT, De RP, et al. Fat-specific protein 27 regulates storage of triglyceride. J Biol Chem 2008;283:14355-14365.
30. Nishino T, Tamiro Y, Tayaet S, et al. FSP27 contributes to efficient energy storage in murine white adipocytes by promoting the formation of unilocular lipid droplets. J Clin Invest 2008;118:2808-2821.
31. Caprio M, Feve B, Claes A, Viengechareun S, Lombs M, Zennaro MC. Pivotal role of the mineralocorticoid receptor in corticosteroid-induced adipogenesis. FASEB J 2007;21:2185-2194.
32. Tang X, Guilherme A, Chaklada A, et al. An RNA interference-based screen identifies MAP4K4/NIK as a negative regulator of PPARgamma, adipogenesis, and insulin-responsive hormone transport. Proc Natl Acad Sci U S A 2006;103:2087-2092.
33. Enterman G, Hauner H. Relationship between replication and differentiation in cultured human adipocyte precursor cells. Am J Physiol 1996;270:C1011-C1016.
34. Skurk T, Ecklele S, Hauner H. A novel technique to propagate primary human preadipocytes without loss of differentiation capacity. Obesity 2007;15:2925-2931.