Effects of leukemia inhibitory factor receptor on the adipogenic differentiation of human bone marrow mesenchymal stem cells

TAO WANG1, RUIQIAO YAN2, XIAOYUAN XU1, HUAN YU1, JIANFANG WU1, YAOFANG YANG1 and WEIDONG LI1

1Key Laboratory of System Bio-Medicine of Jiangxi Province, Jiujiang University; 2Clinical Skills Center, Affiliated Hospital of Jiujiang University, Jiujiang, Jiangxi 332000, P.R. China

Received June 25, 2018; Accepted April 1, 2019

DOl: 10.3892/mmr.2019.10140

Abstract. Leukemia inhibitory factor (LIF) modulates various biological processes. Although previous studies have described the effects of LIF on adipocyte differentiation, the role of LIF receptor (LIFR) on adipocyte differentiation remains unclear. Using reverse transcription-quantitative PCR (RT-qPCR), LIFR expression was demonstrated to increase during adipogenic differentiation of human bone marrow mesenchymal stem cells (hMSCs), indicating that LIFR may be involved in this process. To further evaluate the association between LIFR and adipogenic differentiation, lentivirus-mediated LIFR knockdown was performed in hMSCs. Cells were divided into two groups: Negative control group and LIFR-knockdown group. During the adipogenic differentiation process, intracellular lipid accumulation was assessed with oil red O staining at various time points (days 3, 6 and 9). Additionally, the mRNA and protein expression levels of LIF, LIFR and three molecular indicators of adipogenesis, peroxisome proliferator-activated receptor γ (PPARγ), CCAAT enhancer binding protein α (C/EBPα) and fatty acid binding protein 4 (FABP4/aP2), were assessed by RT-qPCR and western blotting. The culture supernatant was collected to evaluate the concentration of LIF using ELISA. The present results suggested that LIFR expression progressively increased during adipogenic differentiation of hMSCs. Conversely, LIFR knockdown significantly suppressed this process. Additionally, PPARγ, C/EBPα and aP2 were inhibited following LIFR knockdown. In contrast with LIFR, the expression levels of LIF were significantly decreased after the initiation of adipogenic differentiation. Therefore, the expression levels of LIF and LIFR exhibited opposite trends. Collectively, the present results suggested that LIFR promoted adipogenic differentiation, whereas LIF may negatively regulate this process.

Introduction

At present, obesity poses a major health problem worldwide. Obesity is associated with mortality and multiple comorbidities, including cancer and various cardiometabolic disorders (1,2). Modulation of adipogenesis may be used in the treatment of obesity (3). Additionally, elucidating the molecular mechanisms underlying adipogenesis may have important applications, in particular for the development of novel treatments for obesity and other related metabolic disorders.

Mesenchymal stem cells from various types of tissues, including bone marrow, are able to differentiate into adipocytes, similar to the pluripotent stem cells found in fat tissue (4). Bone marrow human mesenchymal stem cells (hMSCs) exhibit self-renewal capabilities and are multipotent (5). A previous in vitro study suggested that hMSCs may differentiate, not only into adipocytes (6), but also into other cell types, including chondrocytes (7) and osteoblasts (8). Therefore, hMSC cultures may represent an optimal model for analyzing the molecular mechanisms that regulate adipogenesis in humans (9).

The leukemia inhibitory factor (LIF) receptor (LIFR) consists of α and β subunits (10,11). LIFR can be activated by LIF, which induces heterodimerization of the two subunits of the receptor, thus activating protein phosphorylation and downstream signaling pathways involved in various biological processes (12-14). LIF has been reported to exhibit various additional functions in adipocytes (15,16). However, to the best of our knowledge, the number of studies investigating LIFR in the context of adipogenic differentiation remains limited. In the present study, the expression levels of LIF were revealed to progressively increase during adipogenic differentiation of hMSCs, suggesting that LIFR may be involved in this process.

In order to investigate the regulatory role of LIFR in adipogenesis, lentivirus-mediated LIFR knockdown was performed in hMSCs, and the expression levels of LIF and LIFR were analyzed during adipogenic differentiation. Silencing LIFR
expression significantly suppressed adipocyte differentiation. Furthermore, the expression levels of LIF and LIFR exhibited opposite trends. Collectively, the present results suggested that LIFR may promote adipogenic differentiation, whereas LIF may negatively regulate this process.

Materials and methods

Cell culture and adipocyte differentiation. Human bone marrow mesenchymal stem cells (hMSCs; HUXMA-01001) were purchased from Cyagen Biosciences, Inc. Cells were evaluated for specific surface protein expression using flow cytometry. Flow cytometry was performed using FACSCalibur (BD Biosciences). The following fluorescent-conjugated monoclonal antibodies were used in this study: Rat allophycocyanin (APC) anti-human/mouse CD44 (1:50; cat. no. 17-0441-82; clone IM7; eBioscience; Thermo Fisher Scientific, Inc.), APC mouse anti-human CD29 (1:50; cat. no. 303008; clone TS2/16, BioLegend, Inc.), FITC mouse anti-human CD105 (1:50; cat. no. 561443; Endoglin; clone 266; BD Biosciences), APC mouse anti-human CD45 (1:50; cat. no. 555485; clone HI10; BD Biosciences) and APC mouse antihuman CD14 (1:50; cat. no. 17-0149-42; clone 61D3; eBioscience; Thermo Fisher Scientific, Inc.). The cells were washed with PBS with 3% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) after detachment with 0.05% trypsin-EDTA (Gibco; Thermo Fisher Scientific, Inc.) and re-suspended in 50 µl of PBS with 3% FBS, and re-suspended in 500 µl of PBS with 3% FBS for flow cytometry. The fluorescence intensity of the cells was evaluated using a FACS aria instrument, and data were analyzed with the FlowJo10.0.7 software (FlowJo LLC). hMSCs were confirmed to be positive for CD44, CD29 and CD105 (>70%) and negative for CD45 and CD14 (<5%).

Cells were grown to a cell density of 5x10^4 cells/cm^2 in OriCell hMSCs growth medium (cat. no. HUXMA-9001c; Cyagen Biosciences, Inc.), supplemented with 10% FBS, 100 IU/ml penicillin (Gibco; Thermo Fisher Scientific, Inc.), 100 µg/ml streptomycin (Gibco; Thermo Fisher Scientific, Inc.) and 0.4% glutamine (Gibco; Thermo Fisher Scientific, Inc.). Cells were maintained at 37˚C in an atmosphere containing 5% CO₂ and 95% humidity. Cells were passaged according to the manufacturer's protocol. First-strand cDNA was obtained using the Reverse Transcription System and oligo(dT), according to the manufacturer's protocol (Thermo Fisher Scientific, Inc.). qPCR was performed using a SYBR Premix Ex Taq kit (Takara Bio Inc.) with a 7300 real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The PCR thermocycling conditions consisted of an initial denaturation at 95°C for 1 min, followed by 40 cycles at 95°C for 15 sec and at 60°C for 34 sec. Relative expression levels were quantified using the 2^(-ΔΔCq) method (17). The expression levels of the target genes were normalized to the expression levels of β-actin. The sequences of the primers used are listed in Table I.
Western blotting. Cells were lysed using RIPA buffer [50 mM Tris (pH 7.4), 1% Triton X-100, 150 mM NaCl, 0.1% SDS, 1% sodium deoxycholate, 1 mmol/l sodium orthovanadate, 50 mmol/l sodium fluoride, 1 mM EDTA and 2 µg/ml leupeptin]. The amount of protein was measured using a Micro bicinchoninic acid Protein assay kit following the protocol provided by the manufacturer (Thermo Fisher Scientific, Inc.). Cell samples were diluted 10, 20 and 40 times in NaCl 0.9% and after 2 h incubation at 37˚C, absorbance was measured using the Bio-rad iMark microplate reader (Bio-Rad Laboratories, Inc.). The extracted proteins were boiled for 5 min in 5x SDS sample buffer. Electrophoresis was performed on 15 µg of protein loaded onto 10% SDS-Page, followed by transfer to PVDF membranes (EMD Millipore). Membranes were blocked for 90 min with 5% skim milk for 120 min at room temperature, followed by incubation at 4˚C overnight with primary antibodies. The primary antibodies used were: Mouse anti-lIFR (1:1,000; cat. no. ab89792), rabbit anti-PParγ (1:1,000; cat. no. ab91407), rabbit anti-C/EBPα (1:1,000; cat. no. ab92501) and mouse anti-β-actin (1:2,000; cat. no. ab173838; all Abcam). Subsequently, membranes were incubated for 60 min with the appropriate secondary antibody at room temperature: Anti-mouse horseradish peroxidase (HRP)-conjugated IgG (1:5,000; cat. no. 7076P2; Cell Signaling Technology, Inc.) or anti-rabbit HRP-conjugated IgG (1:5,000; cat. no. 704P2; Cell Signaling Technology, Inc.). Finally, enhanced chemiluminescence (cat. no. P0018; BeyoECL Plus; Beyotime Institute of Biotechnology) was performed to evaluate the intensity of the protein bands.

Quantification of LIF concentration. LIF protein concentration was assessed in the cell culture supernatant using an ELISA kit (cat. no. SX01091; Shanghai Senxiong Biotech Industry Co., Ltd.), according to the manufacturer's protocol. Absorbance was measured at 450 nm following background correction. LIF concentration was calculated using a standard curve.

Statistical analysis. All experiments were performed in triplicate. Data are presented as the mean ± standard deviation. SPSS (version 16.0; SPSS, Inc.) was used to perform statistical analyses. Statistical analysis was performed using one-way analysis of variance (ANOVA) and Tukey's post-hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

LIFR expression during adipogenic differentiation. The expression levels of LIFR progressively increased during adipogenic differentiation (Fig. 1), suggesting that LIFR may be involved in the adipogenic differentiation of hMSCs.

Identification of stably infected hMSCs. hMSCs with stable infection were identified by treating cells with 0.5 µg/ml puromycin for 6 days. Inverted fluorescence microscopy was used to identify GFP-positive cells in both the nc group and in cells with knockdown of LIFR, indicating stable infection (Fig. 2A).

The efficiency of lentiviral infection was confirmed by RT-qPCR analysis. The expression levels of LIFR were reduced ~3-fold following LIFR knockdown compared with cells in the NC group (Fig. 2B).

Oil Red O staining. Adipogenesis was investigated in hMSCs at various time points (3, 6 and 9) following incubation in adipogenic medium. The majority of cells exhibited cytoplasmic lipid vesicles, as assessed by Oil Red O staining (Fig. 3A). LIFR knockdown inhibited adipogenic differentiation. Furthermore, the intracellular lipid content and the number of lipid droplets were lower in the LIFR-knockdown group compared with in the NC group (Fig. 3B).

mRNA expression levels of LIF, LIFR and adipogenic markers during adipogenic differentiation. The expression

| Gene symbol | Primer sequence (5'-3') | Amplicon length (bp) |
|-------------|------------------------|----------------------|
| PPARγ       | F: GGGATGTCTCATATGGCCATCAG R: GCCCTGCCTTTTGCTTTG | 97 |
| C/EBPα      | F: CCAAGAAGTCGGTTGCAAGAAGAC R: CACTTTCTGCCTGCTTCCCA | 122 |
| aP2         | F: GGTGATGAAACTGGTGCTGGAAATG R: CAGATGTGGTAGATGTTCAATGCCA | 123 |
| LIFR        | F: AGCCTCAAGCAAAAACCCAGAA R: TTGCCCCAGAGCTGTGAACC | 144 |
| LIF         | F: CTGTGATGTTCTGCACTGGAA R: CCCCTGGGCTGTAATAGA | 154 |
| β-actin     | F: GGAGAAGATGAACCCAGATCATGT R: TACCCCTCGTAGATGGCACA | 160 |

aP2, fatty acid binding protein 4; C/EBPα, CCAAT enhancer binding protein α; LIF, leukemia inhibitory factor; LIFR, LIF receptor; PPARγ, peroxisome proliferator-activated receptor γ; F, forward; R, reverse.

Table I. Primer sequences used for reverse transcription-quantitative PCR analysis.
levels of LIF, LIFR, PPARγ, C/EBPα and aP2 were measured by RT-qPCR in LIFR-knockdown and NC groups (Fig. 4). LIFR downregulation significantly suppressed adipocyte differentiation, alongside inhibition of PPARγ, C/EBPα and aP2 expression (Fig. 4A-C). Furthermore, the mRNA expression levels of LIF were significantly decreased following induction of adipogenic differentiation in the negative control and LIFR knockdown groups, respectively (Fig. 4E). Conversely, the mRNA expression levels of LIFR exhibited an opposite trend (Fig. 4D).

Protein expression levels of LIF, LIFR and markers during adipogenesis. LIFR, PPARγ, C/EBPα and aP2 mRNA expression was elevated in each group, as aforementioned. Consistently, western blotting revealed that the protein expression levels of PPARγ, C/EBPα and aP2 appeared to be decreased during adipogenesis following LIFR knockdown (Fig. 5A). Additionally, ELISA was performed to measure the concentration of LIF in the medium, and LIF was decreased during adipogenesis in the negative control and LIFR knockdown groups (Fig. 5B).

Discussion

Adipogenic differentiation serves an important role in the development of obesity. The adipogenic differentiation of hMSCs through preadipocytes may represent an important source of adipose tissue during obesity (3,4,18). In the present

Figure 1. Expression levels of LIFR during adipogenesis at different time points, as assessed by reverse transcription-quantitative PCR analysis. **P<0.01 vs. day 0. LIFR, leukemia inhibitory factor receptor.

Figure 2. Identification of stably infected hMSCs. (A) Infected cells visualized under light and fluorescence microscopy. (B) Expression levels of LIFR were quantified by reverse transcription-quantitative PCR analysis. **P<0.01 vs. negative control. LIFR, leukemia inhibitory factor receptor.

Figure 3. Leukemia inhibitory factor receptor knockdown significantly impairs adipogenic differentiation. (A) Oil Red O-stained adipocytes at various time points during adipogenic differentiation. (B) Cytoplasmic lipid droplets in adipocytes at various time points during adipogenic differentiation. **P<0.01 vs. negative control.
study, LIFR expression was increased during the adipogenic differentiation of hMSCs, suggesting that this protein may be involved in the regulation of adipogenesis. To investigate this hypothesis, stable lentivirus-mediated infection was performed to efficiently downregulate the expression of LIFR in hMSCs. Subsequently, adipogenic differentiation of hMSCs was examined at different time points. LIFR downregulation significantly inhibited adipogenesis and the accumulation of intracellular lipids. The present findings indicated that LIFR may promote adipogenesis in these cells.

A previous study confirmed that microRNA (miR)-377-3p targeted LIFR and regulates adipogenic differentiation (19); however, the function of LIFR in adipogenesis had not been investigated. Therefore, the present study aimed to investigate the role of LIFR in adipogenic differentiation at various time points. In addition, the mRNA and protein expression levels of various molecular markers of adipogenesis, including PPARγ, C/EBPα, and aP2, were examined. LIFR downregulation in hMSCs caused a significant reduction in the mRNA and protein expression levels of these markers during differentiation. PPARγ and C/EBPα are transcription factors that promote adipogenesis, which may act synergistically (20,21). In addition, aP2 modulates lipid storage and metabolism, acting downstream of PPARγ and C/EBPα signaling (22,23). The
present results suggested that LIFR downregulation may be associated with a decrease in the expression levels of these three molecules, thus impairing adipogenic differentiation. Previous studies have demonstrated that these adipogenic markers are important transcription factors involved in adipogenic regulation, and in the pathophysiology of obesity and several endocrine-metabolic disorders (24-26). The present results indicated that LIFR may also be associated with these diseases.

In investigating LIFR function, it is important to examine LIF. LIF is a glycoprotein secreted by hMSCs (27) that is able to interact with LIFR, activating its downstream signaling pathway. Although previous studies have investigated the impact of LIF on adipogenic differentiation, the previous results were inconsistent or contradictory. For example, a previous study reported that LIF inhibits differentiation of 3T3-L1 adipocytes in vitro (28); however, in Ob1771 cells, LIF was revealed to promote differentiation (16). To the best of our knowledge, the pre-adipocyte cell lines, including 3T3-L1 and Ob1771, are immortalized and may not fully mimic human primary cells due to their murine origin. Since primary hMSCs extracted from the bone marrow may more closely mirror the physiological context of adipogenic differentiation in humans, these cells were selected and investigated in the present study.

A number of previous studies have investigated the role of either LIF or LIFR (15,16,27,28). Similarly, our previous study investigated the function of LIFR as a miR-377 target gene involved in adipogenic differentiation (19). To the best of our knowledge, the present study is the first to investigate the relationship between the expression levels of LIF and LIFR during adipogenic differentiation. Previous studies have reported that the expression patterns of LIF and LIFR are similar in certain tissues and cells (29,30). In the present study, the expression levels of LIF and LIFR exhibited opposite trends during adipogenic differentiation of hMSCs. After initiation of adipogenesis, the expression levels of LIF were decreased, whereas the expression levels of LIFR were increased. LIFR knockdown was also associated with an increased expression of LIF compared with in the NC group, thus suggesting that LIFR may be a negative regulator of adipogenic differentiation. LIF is an inhibitor of cell differentiation in mouse embryonic stem cells (31,32). Although LIF overexpression and/or knockout were not performed in the present study, results from previous studies support our hypothesis of the role of LIF in cell differentiation. The present results suggested that LIFR and LIF may serve opposite roles during adipogenic differentiation of hMSCs. Previous studies investigated the use of hMSCs for the treatment of aplastic anemia (33,34). Notably, the relationship between the expression levels of LIF and LIFR, and hematopoietic and bone marrow differentiation is an interesting aspect to address in future studies.

In addition to LIF, LIFR may interact with other ligands, including oncostatin M and ciliary neurotrophic factor, which may promote the activation of multiple downstream signaling pathways involved in adipogenic differentiation (12,35,36), including the mitogen-activated protein kinase and Janus kinase/signal transducer and activator of transcription signaling pathways (12-14,37). Collectively, LIFR may be associated with various ligands and signaling pathways involved in adipogenic differentiation.

In conclusion, the present study suggested that LIFR may be a novel positive regulator of adipogenic differentiation. Conversely, LIF may negatively regulate adipogenic differentiation. The present findings suggested that LIFR-targeting treatments may represent novel potential strategies to treat obesity and other associated disorders.

Acknowledgements
Not applicable.

Funding
The present study was supported by The National Natural Science Foundation of China (grant no. 81460221), the Jiangxi Province Natural Science Foundation of China (grant nos. 20161BAB205197 and 20132BAB205012) and the Development Plan of Young and Middle-aged Teachers in General Universities of Jiangxi Province (grant no. 2012-132).

Availability of data and materials
All data generated or analyzed during this study are included in this published article.
Authors' contributions

TW, YY and WL designed the present study. RY, XX and HY performed the experiments. JW analyzed the data. TW wrote the paper.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Kopelman PG: Obesity as a medical problem. Nature 404: 635-643, 2000.
2. Kahn BB and Flier JS: Obesity and insulin resistance. J Clin Invest 106: 473-481, 2000.
3. Keller M, Hopp l, Liu X, Wohland T, Rohde K, Canedo R, Mosca JD, Moorman MA, Simontetti DW, Craig S and Marshall DR: Multilineage potential of adult human mesenchymal stem cells. Science 284: 143-147, 1999.
4. Helander MN, Knippenberg M, Klein-Nulend J and Wuisman PJ: Stem cells from adipose tissue allow challenging new concepts for regenerative medicine. Tissue Eng 13: 1799-1808, 2007.
5. Barry F, Boynton RE, Liu B and Murphy JM: Chondrogenic differentiation of mesenchymal stem cells from bone marrow: Differentiation-dependent gene expression of matrix components. Exp Cell Res 268: 189-200, 2001.
6. Arinzeh Tc and Lane MD: Adipose development: From stem cell to adipocyte. Crit Rev Biochem Mol Biol 40: 229-242, 2005.
7. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simontetti DW, Craig S and Marshak DR: Multilineage potential of adult human mesenchymal stem cells. Science 284: 143-147, 1999.
8. Subash-Babu P and Alshatwi AA: Inhibition of activin A and TGF-beta in human adipose tissue unMASKS novel candidate genes for obesity. Mol Metab 6: 86-100, 2016.
9. Otto TC and Lane MD: Adipose development: From stem cell to adipocyte. Crit Rev Biochem Mol Biol 40: 229-242, 2005.
10. Subash-Babu P and Alshatwi AA: Aloe-emodin inhibits adipocyte differentiation and maturation during in vitro human mesenchymal stem cell adipogenesis. J Biochem Mol Toxicol 26: 529-300, 2012.
11. del Valle I, Rudloff S, Carles A, Li Y, Liszewska E, Vogt R and Klemm R: C-adherin is required for the proper activation of the LiFr/Gpl30 signaling pathway in mouse embryonic stem cells. Development 140: 1684-1692, 2013.
12.Pan W, Cao C, Yu Y and Kastin AJ: Receptor-mediated transport of LiF across blood-spinal cord barrier is upregulated after spinal cord injury. J Neuroimmunol 174: 119-125, 2006.
13. Plun-Favreau H, Perret D, Diveu C, Froger J, Chevalier S, Lerille E, Gascan H and Chabbert B: Leukemia inhibitory factor (LiF), cardiotrophin-1, and oncostatin M share structural and functional properties. J Immunol 167: 5797-5805, 2001.
14. Morton SD, Cadamuro M, Brivio S, Vismara M, Stecca T, Massani M, Bassi N, Furlanetto A, Joplin RE, Floreani A, et al: Leukemia inhibitory factor protects cholangiocytes from drug-induced apoptosis via a PI3K/AKT-dependent Mcl-1 activation. Oncotarget 6: 26052-26064, 2015.
15. Ikeda S, Itoh S, Yamamoto Y, Matsushita K, Naruse H and Hayashi M: Dexamethasone-redundant effects of leukemia inhibitory factor on adipocyte differentiation of murine bone marrow stromal cells. Cell Biochem Biophys 74: 11-17, 2016.
16. Aubert J, Dessolin S, Belmonte N, Li M, McKenzie FR, Staccini L, Villageois P, Banhain B, Vernallis A, Smith AG, et al: Leukemia inhibitory factor and its receptor promote adipocyte differentiation via the mitogen-activated protein kinase cascade. J Biol Chem 274: 24965-24972, 1999.
17. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
18. Zamboni MC, Auclair SL, Ouellette BW, Flier JS and Itoh S: Obese Mice: An Experimental Model for the Study of Adipose Tissue Growth and Metabolism. Int J Obes 32: 730-738, 2008.
19. Li X, Yang Y, Yan R, Xu X, Gao L, Mei J, Liu J, Wang X, Zhang J, Wu P, et al: mir-377-5p regulates adipose differentiation of murine bone marrow mesenchymal stem cells by regulating LIFR. Mol Cell Biochem 449: 295-303, 2018.
20. Farmer SR: Transcriptional control of adipocyte formation. Cell Metab 4: 263-273, 2006.
21. Kudo M, Sugawara A, Urubo A, Takeuchi K and Ito S: Transcription suppression of peroxisome proliferator-activated receptor gamma2 gene expression by tumor necrosis factor alpha via an inhibition of CCAAT/enhancer-binding protein delta during the early stage of adipocyte differentiation. Endocrinology 145: 4948-4956, 2004.
22. Rosen ED, Walkey CJ, Puigserver P and Spiegelman BM: Transcriptional regulation of adipogenesis. Genes Dev 14: 1293-1307, 2000.
23. Zhang J, Huang Y, Shao H, Bi Q, Chen J and Ye Z: Grape seed procyanidin B2 inhibits adipogenesis of 3T3-L1 cells by targeting peroxisome proliferator-activated receptor γ with miR-183-5p involved mechanism. Biomed Pharmacother 86: 292-296, 2017.
24. Floyd ZE and Stephens JM: Controlling a master switch of adipocyte development and insulin sensitivity: Covalent modifications of PPARγ. Biochim Biophys Acta 1822: 1090-1095, 2012.
25. Furuhashi M, Tuncman G, Gorgun C, Makowski L, Atsumi G, Vaillancourt E, Kono K, Babaev VR, Fazio S, Linton MF, et al: Treatment of diabetes and atherosclerosis by inhibiting fatty-acid-binding protein FABP4. Nature 447: 89-96, 2007.
26. Hwang CS, Mandrup S, MacDougall OA, Geiman DE and Lane MD: Transcriptional activation of the mouse obese (ob) gene by CCAAT/enhancer binding protein alpha. Proc Natl Acad Sci USA 93: 873-877, 1996.
27. Oskowitz AZ, Lu J, Penfornis P, Ylostalo J, McBride J, Flemmington EK, Prockop DJ and Pochampally R: Human multipotent stromal cells from bone marrow and microRNA: Regulation of differentiation and leukemia inhibitory factor expression. Proc Natl Acad Sci USA 105: 18372-18377, 2008.
28. Hogan JC and Stephens JM: Effects of leukemia inhibitory factor on 3T3-L1 adipocytes. J Endocrinol 185: 485-496, 2000.
29. Margioula-Siarkou C, Prapas Y, Petousis S, Milias S, Ravanos K, Kalogiannidis I, Mavromatidis G, Haitoglou C, Prapas N and Rousson D: LiF and LeIF-R expression in the endometrium of fertile and infertile women: A prospective observational case-control study. Mol Med Rep 13: 4721-4728, 2016.
30. Li Y, Sun L, Zhao D, Ouyang J and Xiang M: Aberrant expression of leukemia inhibitory factor receptor (LiFR) and leukemia inhibitory factor (LiF) is associated with tubal pregnancy occurrence. Turk J Med Sci 45: 214-220, 2015.
31. Saito M, Asai Y, Imai K, Hiratoko S and Tanaka K: Connexin30.3 is expressed in mouse embryonic stem cells and is responsive to leukemia inhibitory factor. Sci Rep 7: 42403, 2017.
32. Cherепкова MY, Sineva GS and Pospelov VA: Leukemia inhibitory factor (LiF) withdrawal activates mTOR signaling pathway in mouse embryonic stem cells through the MEK/ERK/TSC2 pathway. Cell Death Dis 7: e2050, 2016.
33. Cheng HC, Liu SW, Li W, Zhao XF, Zhao X, Cheng M, Qi L and Ma J: Arsenic trioxide regulates adipogenic and osteogenic differentiation in bone marrow MSCs of aplastic anemia patients through BMP4 gene. Acta Biochim Biophys Sin (Shanghai) 47: 673-679, 2015.
34. Lecourt S, Vanneaux V, Leblanc T, Leroux G, Ternaux B, Truchard D, Biechler C, Barulich A, Marolleau JP, Gluckman E, et al: Bone marrow microenvironment in Fanconi anemia: A prospective functional study in a cohort of Fanconi anemia patients. Cell Dev 19: 203-208, 2010.
35. Natesh K, Bhosale D, Desai A, Chandrika G, Pujari R, Jagtap J, Chugh A, Ranade D and Shastry P: Oncostatin-M differentially regulates mesenchymal and proneural signature genes in gliomas via STAT3 signaling. Neoplasia 17: 225-237, 2015.

36. Wagener EM, Aurich M, Aparicio-Siegmund S, Floss DM, Garbers C, Breusing K, Rabe B, Schwanbeck R, Grötzinger J, Rose-John S and Scheller J: The amino acid exchange R28E in ciliaryneurotrophic factor (CNTF) abrogates interleukin-6 receptor-dependent but retains CNTF receptor-dependent signaling via glycoprotein 130 (gp130)/leukemia inhibitory factor receptor (LIFR). J Biol Chem 289: 18442-184450, 2014.

37. Liu GX, Zhu JC, Chen XY, Zhu AZ, Liu CC, Lai Q and Chen ST: Inhibition of adipogenic differentiation of bone marrow mesenchymal stem cells by erythropoietin via activating ERK and P38 MAPK. Genet Mol Res 14: 6968-6977, 2015.