**Kbtbd11** gene expression in adipose tissue increases in response to feeding and affects adipocyte differentiation

Kazuhisa Watanabe*, Ken Yoshida, Sadahiko Iwamoto
Division of Human Genetics, Center for Molecular Medicine, Jichi Medical University, Shimotsuke, Japan

**Keywords**
3T3-L1 preadipocytes, Differentiation, Kbtbd11

*Correspondence
Kazuhisa Watanabe
Tel: +81-285-58-7341
Fax: +81-285-44-4902
Email address: kwatanabe@jichi.ac.jp

**ABSTRACT**

**Aims/Introduction:** The putative tumor suppressor gene, **KBTBD11**, might play a role in tumorigenesis, and is associated with cellular apoptosis and proliferation in colorectal cancer cells. However, the function of **Kbtbd11** during adipogenesis is unknown. The aim of the present study was to investigate the role of **Kbtbd11** in the differentiation of 3T3-L1 preadipocytes.

**Materials and Methods:** For the fasting–refeeding protocol, mice were subjected to fasting for 24 h, followed by a chow diet for 12 h. Adenovirus infection methods were used to examine the effect of **Kbtbd11**, and 3T3-L1 cells were analyzed with Oil Red O staining and real-time polymerase chain reaction.

**Results:** The white adipose tissue expression of **Kbtbd11** messenger ribonucleic acid (mRNA) was significantly higher in the re-fed state than in the fasted state. **Kbtbd11** mRNA levels were markedly increased in epididymal white adipose tissue of diet-induced obesity mice compared with those in the mice fed a chow diet. In addition, **Kbtbd11** mRNA expression was increased in a differentiation-dependent manner in 3T3-L1 cells. Knockdown of **Kbtbd11** mRNA through the infection with adenoviral vectors remarkably inhibited triglyceride accumulation and adipocyte differentiation in 3T3-L1 cells. In contrast, the overexpression of **Kbtbd11** promoted the differentiation of 3T3-L1 adipocytes.

**Conclusions:** The present findings show that **Kbtbd11** expression might be involved in nutritional regulation and is increased in obese adipose tissue. In addition, **Kbtbd11** appears to be required for the differentiation of adipocytes in 3T3-L1 cells. Collectively, these results show a novel link between the expression of **Kbtbd11** and fat accumulation, and suggest that **Kbtbd11** is a new therapeutic target for obesity.

**INTRODUCTION**
Being obese or overweight is associated with the risk of developing type 2 diabetes, atherosclerosis, hyperlipidemia, steatosis and various cancers1–3. Adipocyte differentiation represents a multistep process involving a cascade of transcription factors for key proteins that induce gene expression and lead to adipocyte development. The 3T3-L1 cell line is a well-established preadipose cell line, which is useful for investigating the mechanisms underlying adipocyte proliferation, differentiation and lipid metabolism, as well as the identification of genes that regulate adipocyte physiology4,5.

Kelch repeat and BTB domain containing 11 (KBTBD11) belongs to the BTB superfamily, which includes the KLHL and KLHDC subfamilies, and has BTB/POZ and Kelch domains. The BTB/POZ domain functions as the domain for protein–protein interaction to enable dimer formation and an interaction with non-BTB domain-containing proteins, including the scaffold protein of the E3 ubiquitin ligase complex6,7. The Kelch domain, an evolutionarily conserved structure, widely found in mammals and insects, usually comprises two to seven repeats of four-stranded β-sheet motif forming one blade of the β-propeller structure7,8.

A variant allele of **KBTBD11**, rs11777210, is significantly associated with colorectal cancer cell susceptibility. **KBTBD11**
expression is significantly decreased in tumor tissues compared with that in adjacent paired normal tissues. Additionally, in colorectal cancer cells, KBTBD11 knockdown inhibits apoptosis and promotes proliferation, whereas KBTBD11 overexpression promotes apoptosis and inhibits cell growth. In tumorigenesis, KBTBD11 is a putative tumor suppressor, the expression of which is regulated by MYC.

Kbtbd11 might play an important role in cellular differentiation and proliferation in tumor tissues. However, the expression profile and functional significance of Kbtbd11 in adipose tissue is unknown. In the present study, we investigated the expression profile of Kbtbd11 in adipose tissue and its effects in 3T3-L1 preadipocyte differentiation.

METHODS

Animal Experiments

For all experiments, 8-week-old male C57BL/6J mice from Japan SLC (Hamamatsu, Japan) were used. The mice were maintained on a standard chow diet. For the fasting protocol, the mice were subjected to fasting for 24 h and then fed a chow diet for 12 h. For obtaining diet-induced obesity (DIO) mice, C57BL/6J mice were fed a high-fat diet for 4 weeks. Feed ingredients content were as follows: the standard chow diet (CE-2) comprised 50.3% carbohydrates, 25.4% protein and 32.0% fat (CLEA Japan Inc., Tokyo, Japan). Animal experimental protocols were approved by the animal ethics committee of Jichi Medical University (permit number 17177), and were carried out in accordance with the Use and Care of Experimental Animals Guidelines of the Jichi Medical University Guide for Laboratory Animals and the Jichi Medical University Guide for Laboratory Animals.

Cells and Adipocyte Differentiation

3T3-L1 cells were maintained in high-glucose Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, and 100 units each of penicillin and streptomycin at 37°C in 5% CO2. For the adipocyte differentiation study, 3T3-L1 cells were cultured to confluence. After 2 days, the medium was replaced with high-glucose Dulbecco’s modified Eagle’s medium containing 5 μg/mL insulin, 1 μmol/L dexamethasone (DEX) and 0.5 mmol/L 3-isobutyl-1-methylxanthine (IBMX). After 48 h, the medium was changed to high-glucose Dulbecco’s modified Eagle’s medium containing 5 μg/mL insulin. The medium was renewed every other day. For the adenovirus infection study, 3T3-L1 cells at day 2 before the induction of differentiation were infected with adenovirus, and incubated for 0, 4 and 8 days.

Oil Red O Stain

3T3-L1 cells were fixed with 10% formalin in phosphate-buffered saline for 10 min at 37°C and stained with 60% Oil red O solution (Muto pure Chemicals Co., Tokyo, Japan) for 20 min at room temperature. The cells were washed with distilled water, and the retained dye was eluted by 60% isopropanol.

Adenoviral Expression Vectors

Adenoviruses were prepared and amplified with the Viral Power Adenoviral Expression System (Invitrogen, Carlsbad, CA, USA), as previously described. Polymerase chain reaction (PCR)-amplified, mouse Kbtbd11 complementary deoxyribonucleic acid was subcloned into the pENTR Directional TOPO vector (Invitrogen). The short hairpin ribonucleic acids (shRNAs) of Kbtbd11 and LacZ were cloned into BLOCK-iT U6 entry vector (Invitrogen). The sequence of the shRNA for Kbtbd11 shRNA#1 was as follows: 5’-cacc GGACATATTG-GAAATCTGTA tcaagaga TCAGATTTCACATATGTCC-3’, and Kbtbd11 shRNA#2 was as follows: cacc GCAAGTAAGT-GACATTTAA tcaagaga TTAATGTCACCTACTTGC. Inserts of pENTR vectors were transferred into the adenovirus vectors pAd/CMV-DEST or pAd/PL-DEST using the Gateway system (Invitrogen). Recombinant adenoviruses were purified by the Adenovirus Purification Miniprep Kit (Cell Biolabs, San Diego, CA, USA) according to the manufacturer’s protocol.

Real-Time PCR

Total RNA was isolated using acid guanidinium thiocyanate-phenol reagent. Complementary deoxyribonucleic acid synthesis was carried out using the Verso cDNA Kit (Thermo Scientific, Waltham, MA, USA) with random hexamer primers. Quantitative PCR (qPCR) assays were carried out using the ViiA7 Real-Time PCR System and KAPA SYBER FAST ROX Low qPCR kit (Kapa Biosystems, Wilmington, MA, USA). Relative gene expression levels were quantified by qPCR followed by normalization to the internal control gene 36B4. The following primers were used for this analysis: Kbtbd11 Fwd, 5’-TCACGGTTTTTCGGCTACCAT-3’ and Kbtbd11 Rv, 5’-AACCA CAAACGAAGGGCTGGA-3’; Cebpa Fwd, 5’-GCGGATCGGAGA AACTCTCA-3’ and Cebpa Rv, 5’-GGGCTCTGGAGGTTG ACTGCT-3’; Cebpb Fwd, 5’-GACCCCGGACAGGGCAGAA-3’ and Cebpb Rv, 5’-GGCGTTTCTCGGCGTGTCAC-3’; Ppar Fwd, 5’-TTCCACTATGGAGITCATGCTTG-3’ and Ppar Rv, 5’-TCCGGCTAGTTAAGATCACCTA-3’; p27 Fwd, 5’-AGGAGACCGAGGTGTCAGC-3’ and p27 Rv, 5’-CAGA GTTGGCCTGAACCCCAA-3’; Cyclin D1 Fwd, 5’-GGTTCATTTTC CAACCCACCCTC-3’ and Cyclin D1 Rv, 5’-AGAAAGTTCGCC TGGCGCTAG-3’; Bcl2 Fwd, 5’-GACCAAGCCGAGAATGGATC-3’ and Bcl2 Rv, 5’-AGGACACGCGGAAATGACCAAC-3’; Tnfα Fwd, 5’-CAGGGC TGGGGTGCACCTT-3’ and Tnfα Rv, 5’-GGGCCGA TACCAGGGTTTGA-3’; Il6 Fwd, 5’-CTGGGATGCCTTTGTGGAAC-3’ and Il6 Rv, 5’-AAGTGCATCATCGTTTTC-3’; Pparg Fwd, 5’-GACTCGTGAGAGGACATATGTCC-3’, and Pparg Rv, 5’-cacc GGACATATGTCC-3’. Statistical Analysis

Experimental studies were carried out in triplicates or greater. Statistical significance was tested using the unpaired two-tailed Student’s t-test.
Student’s t-test. Data are shown as mean ± standard error of the mean. Differences were considered to be significant at P < 0.05.

RESULTS

Kbtbd11 mRNA Expression in Epididymal White Adipose Tissue

We first examined the expression pattern of Kbtbd11 in epididymal white adipose tissue (eWAT) of C57BL/6 mice in the fasted and re-fed states. In the fasted state, Kbtbd11 mRNA expression was low, but was profoundly promoted by re-feeding in the eWAT of C57BL/6 mice (Figure 1a). Next, we compared the expression of Kbtbd11 levels in the eWAT of obese mice. In DIO mice, Kbtbd11 mRNA was elevated >10-fold (Figure 1b). These results show that Kbtbd11 expression levels might be dependent on triglyceride accumulation in WAT.

Kbtbd11 Expression During 3T3-L1 Adipose Differentiation

To elucidate whether Kbtbd11 plays a role in adipose differentiation, we examined the expression of Kbtbd11 in 3T3-L1 cell differentiation using qPCR methods. 3T3-L1 cells were induced with insulin, DEX, and IBMX. Total RNA was then isolated from cells and subjected to qPCR to evaluate Kbtbd11 mRNA levels. Kbtbd11 mRNA levels increased rapidly by insulin, and were upregulated threefold at early times from 2 to 4 h after the addition of DEX (Figure 3a,b). After the addition of IBMX, Kbtbd11 mRNA gradually, but significantly, increased from 4 h to 8 h. The levels of Cebpα and Cebpβ, the inducers of adipocyte differentiation, also rapidly increased in preadipocytes by adding individual drugs. These results suggested that Kbtbd11 transcripts, increased rapidly by the adipogenic cocktail, were involved in 3T3-L1 adipocyte differentiation.

Expression of Kbtbd11 During Early 3T3-L1 Adipose Differentiation

3T3-L1 cells undergo differentiation to adipocytes in response to a cocktail of adipogenic stimuli containing insulin; the synthetic glucocorticoid, DEX; and the phosphodiesterase inhibitor, IBMX. As Kbtbd11 mRNA expression increased in the re-fed eWAT and during 3T3-L1 adipocyte differentiation, the presence of individual adipogenic stimuli in the short-term might have any influence on Kbtbd11 mRNA expression in 3T3-L1 preadipocytes. To elucidate the Kbtbd11 expression pattern in individual adipogenic factors, 3T3-L1 preadipocytes were collected at 0, 1, 4 and 8 h after the addition of one of the following: insulin, DEX and IBMX. Total RNA was then isolated from cells and subjected to qPCR to evaluate Kbtbd11 mRNA levels. Kbtbd11 mRNA levels increased rapidly by insulin, and were upregulated threefold at early times from 2 to 4 h after the addition of DEX (Figure 3a,b). After the addition of IBMX, Kbtbd11 mRNA gradually, but significantly, increased from 4 h to 8 h. The levels of Cebpα and Cebpβ, the inducers of adipocyte differentiation, also rapidly increased in preadipocytes by adding individual drugs. These results suggested that Kbtbd11 transcripts, increased rapidly by the adipogenic cocktail, were involved in 3T3-L1 adipocyte differentiation.

Knockdown and overexpression of Kbtbd11 mRNA are reciprocally involved in mitotic clonal expansion (MCE) and triglyceride accumulation during 3T3-L1 adipocyte differentiation

To investigate the role of Kbtbd11 in adipocyte differentiation, including in the early stage, we examined the effects of Kbtbd11 knockdown on 3T3-L1 differentiation. Adenoviral infection of either of the two independent Kbtbd11 shRNA constructs decreased Kbtbd11 mRNA expression levels by up to approximately 60% compared with that of the LacZ-specific short hairpin ribonucleic acid (shLacZ) control (Figure 4a). On day 8, after inducing differentiation, the shLacZ control cells showed abundant lipid droplets (observed with Oil Red O staining) (Figure 4b), and a significant increase in the expression of Cebpα, Cebpβ and Pparg during the middle (day 4) and late stages (day 8) of 3T3-L1 adipogenesis. In addition, in the early stage, the mRNA expression of Cebpβ was upregulated, reaching a peak at 2 h after the induction of differentiation. Furthermore, the expression of Cyclin D1, acting on MCE, rapidly increased in preadipocytes after the addition of the adipogenic cocktail. Meanwhile, the expression of p27, a key regulator of cell cycle progression, decreased (Figure 4c). In contrast, lipid accumulation and adipocyte differentiation markers (Cebpα, Cebpβ and Pparg) were significantly decreased in Kbtbd11-knockdown cells during the middle and late stages of 3T3-L1 adipogenesis (Figure 4b,c). In the early stage, Cebpβ and Cyclin...
Kbtbd11 were significantly decreased and p27 was increased in Kbtbd11-knockdown cells (Figure 4c). In Kbtbd11-overexpressing 3T3-L1 cells, Kbtbd11 mRNA expression increased by 40-fold (Figure 4d), which promoted lipid accumulation (Figure 4e). Adipocyte differentiation markers were significantly increased in the Kbtbd11-overexpressing 3T3-L1 cells on days 4 and 8 after the induction of differentiation. In the early stage, Cebpb and Cyclin D1 were significantly increased, and p27 was decreased in Kbtbd11-overexpressing cells (Figure 4f). These results suggest that Kbtbd11 plays an important role in MCE, and is involved in triglyceride accumulation and adipocyte differentiation.

**Effects of Kbtbd11 Knockdown on Mature 3T3-L1 Adipocytes**

To examine the role of Kbtbd11 in mature adipocytes, we investigated effects of Kbtbd11 knockdown on mature 3T3-L1 adipocytes. On day 8 after the induction of differentiation, mature 3T3-L1 adipocytes were infected with adenoviral vectors for expressing shLacZ control or Kbtbd11 shRNA. Kbtbd11 adenoviral shRNA decreased Kbtbd11 mRNA expression levels by up to approximately 60% compared with the shLacZ control (Figure 5b). The number of lipid droplets of mature 3T3-L1 adipocytes was not different between the shLacZ control and Kbtbd11 knockdown (Figure 5a). mRNA expression analyses of adipocytes (Pparg and aP2) and inflammation markers (Tnfa and Il6), as well as lipogenic (Fasn) and proapoptotic genes (Bax and Bcl2), were carried out using qPCR. mRNA levels of these markers and genes in adipocytes were not different compared with those in shLacZ control, suggesting that Kbtbd11 might only play a role in the early stage of adipogenesis.

**DISCUSSION**

In the present study, we identified Kbtbd11 as a newly discovered adipogenesis-related gene. At first, we examined the expression of Kbtbd11 mRNA in nutritional regulation and obese adipose tissue using qPCR analysis. Kbtbd11 was significantly increased in re-fed eWAT compared with that in fasted eWAT, and was strongly induced in eWAT of DIO mice compared with that in the mice fed a chow diet (Figure 1b). We hypothesized that the elevation of Kbtbd11 in eWAT of obese mice might be associated with the accumulation of triglyceride in adipocytes. Next, we investigated whether Kbtbd11 was induced in 3T3-L1 cells during adipose differentiation. The present results showed that Kbtbd11 expression was induced during 3T3-L1 adipocyte differentiation (Figure 2). These findings, demonstrating that Kbtbd11 mRNA expression levels in 3T3-L1 adipocytes show differentiation-dependent expression, support our hypothesis.

As Kbtbd11 mRNA expression increased in the re-fed eWAT, and during 3T3-L1 adipose differentiation, we further examined whether Kbtbd11 transcription is regulated by insulin, DEX and IBMX individually. The present results showed that the expression of Kbtbd11 was rapidly increased by insulin (Figure 3a), suggesting that Kbtbd11 is indeed regulated by nutritional regulation. The differentiation of 3T3-L1 preadipocytes into adipocytes was accompanied by a transient induction of Cebpb expression in response to treatment with IBMX and DEX, respectively. In addition, Cebpb led to the upregulation of the expression of adipogenic factors, such as Cebpa and Pparg, which appears to perform an important function in adipogenesis by controlling the late-stage differentiated phenotype. After the addition of DEX and IBMX, respectively, in 3T3-L1 preadipocytes, Kbtbd11 was upregulated later than the expression of
Cebpα and Cebpβ (Figure 3b,c). These data suggested that Kbtbd11 is regulated by adipogenic factors and related to adipocyte differentiation process.

Additional convincing evidence came from our results showing a close relationship between Kbtbd11 and adipocyte differentiation in 3T3-L1 cells. In Kbtbd11 knockdown cells, differentiation of 3T3-L1 adipocytes was markedly inhibited, with an accompanying decrease in the expression of Cebpα and Pparg (Figure 4b,c). Conversely, Kbtbd11-overexpressing 3T3-L1 cells upregulated the expression of Cebpα, Cebpβ and Pparg. Together, these results suggested that Kbtbd11 regulates the differentiation of 3T3-L1 adipocytes.

After hormonal induction, growth-arrested 3T3-L1 preadipocytes synchronously re-enter the cell cycle for one to two rounds of cell division, a phenomenon known as MCE, which is one of the important events occurring at the early stage during 3T3-L1 adipocyte differentiation. Cyclin D1 functions as a key sensor for mitogenic stimuli and promotes cell cycle progression from the G1 to S phase. In contrast, p27, a cyclin-dependent kinase inhibitor, is a key negative regulator of the cell cycle during progression from the G1 to S phase. The balance of the cyclin D1-p27 control system might play an important role in MCE. After MCE, CEBPB activates Cebpα and Pparg, which then transcriptionally activate genes that give rise to the adipocyte phenotype. These are accompanied by the early events of the cell cycle and initiation of a transcriptional cascade, which results in terminal adipogenic differentiation. Because KBTBD11 is associated with cellular differentiation and proliferation in tumor tissues, Kbtbd11 might play an important role in MCE of 3T3-L1 differentiating adipocytes. Although KBTBD11 knockdown promotes proliferation in colorectal cancer cells, Kbtbd11 knockdown inhibits MCE and 3T3-L1 differentiating adipocytes. The role of Kbtbd11 in 3T3-L1 adipogenesis might be

---

**Figure 3 | Kbtbd11 messenger ribonucleic acid (mRNA) expression in response to adipogenic stimuli at early time points.** 3T3-L1 preadipocytes were cultured to confluence, serum-starved for 12 h and treated with (a) 5 μg/mL insulin, (b) 2.5 μmol/L dexamethasone (DEX) or (c) 200 μmol/L 3-isobutyl-1-methylxanthine (IBMX) for the indicated times; n = 3 per group, *P < 0.01 versus 0 h.

---
Figure 4 | The effects of knockdown and overexpression of Kbtbd11 on 3T3-L1 cellular differentiation. (a) The expression of Kbtbd11 messenger ribonucleic acid (mRNA) in Kbtbd11 knockdown 3T3-L1 cells at day 8. Kbtbd11 knockdown adenovirus particles were used with either of the two independent Kbtbd11 short hairpin ribonucleic acid (shRNA) constructs (shRNA#1 and shRNA#2); n = 3 per group, *P < 0.01 versus LacZ-specific short hairpin ribonucleic acid (shLacZ); (b) triglyceride accumulation in 3T3-L1 cells on day 8, visualized using Oil Red O staining; (c) the mRNA levels in 3T3-L1 cells expressing each shRNA at various time points after inducing differentiation; n = 3 per group, *P < 0.01 versus shLacZ; (d) the expression of Kbtbd11 mRNA in Kbtbd11-overexpressing 3T3-L1 cells at day 8. Cells were infected with adenoviral vectors for expressing green fluorescent protein (GFP) or mouse Kbtbd11; n = 3 per group, *P < 0.01 versus LacZ-specific short hairpin ribonucleic acid (shLacZ); (e) triglyceride accumulation in 3T3-L1 cells on day 6 was detected using Oil Red O staining. (f) Relative mRNA levels in each group of 3T3-L1 cells at various time points after inducing differentiation; n = 3 per group, *P < 0.01 versus GFP.

Figure 5 | Effects of knockdown of Kbtbd11 on mature 3T3-L1 adipocytes. (a) Triglyceride accumulation in Kbtbd11-knockdown mature 3T3-L1 adipocytes at 48 h after either of the two independent Kbtbd11 short hairpin ribonucleic acid (shRNA) adenoviral infections (shRNA#1 and shRNA#2) visualized using Oil Red O staining; (b) RNA was harvested at 48 h after adenoviral infection, and expression levels of Kbtbd11, adipocytes (Pparg and aP2) and inflammation markers (Tnfa and Il6), and lipogenic (Fasn) and proapoptotic genes (Bax and Bcl2) were measured using quantitative polymerase chain reaction; n = 3 per group, *P < 0.01 versus shLacZ.
different from that in cellular differentiation and proliferation during tumorigenesis. Furthermore, Kbtbd11 knockdown inhibited adipogenesis, but only before MCE (not mature 3T3-L1 adipocytes), and Kbtbd11 overexpression induced MCE, leading to the expression of Cebpa and Pparg (Figure 4c,f).

In conclusion, the present study shows that Kbtbd11 expression is involved in nutritional regulation and is increased in obese adipose tissue. Kbtbd11 is a regulator of 3T3-L1 adipose differentiation that acts in the early stages of adipogenesis. These data showed a novel link between the expression of Kbtbd11 and fat accumulation, suggesting that Kbtbd11 could represent a new therapeutic target in obesity. However, further research is required to elucidate the physiological functions of Kbtbd11. KBTBD11 transgenic mice have not yet been reported, but would be an important biological tool in understanding the molecular mechanism(s) of adipogenesis, including adipocyte differentiation.

ACKNOWLEDGMENTS
This study was supported by grants-in-aid (15K19523, 17K16153 and 17K09864) from Japan Society for the Promotion of Science (JSPS); a MEXT-supported program for the strategic research foundation at private universities (2013–2017); and Jichi Medical University young investigator award. Ms Kayo Nagashima and Ms Yukiko Ohashi for their excellent technical assistance.

DISCLOSURE
The authors declare no conflict of interest.

REFERENCES
1. Gallagher EJ, LeRoith D. Obesity and diabetes: the increased risk of cancer and cancer-related mortality. Physiol Rev 2015; 95: 727–748.
2. Hui E, Xu A, Bo Yang H, et al. Obesity as the common soil of non-alcoholic fatty liver disease and diabetes: role of adipokines. J Diabetes Investig 2013; 4: 413–425.
3. Vucenik I, Stains JP. Obesity and cancer risk: evidence, mechanisms, and recommendations. Ann N Y Acad Sci 2012; 1271: 37–43.
4. Ruiz-Ojeda FJ, Ruperez AI, Gomez-Llorente C, et al. Cell models and their application for studying adipogenic differentiation in relation to obesity: a review. Int J Mol Sci 2016; 17: 1040.
5. Zhu HJ, Ding HH, Deng JY, et al. Inhibition of preadipocyte differentiation and adipogenesis by zinc-alpha2-glycoprotein treatment in 3T3-L1 cells. J Diabetes Investig 2013; 4: 252–260.
6. Canning P, Cooper CD, Krojer T, et al. Structural basis for Cul3 protein assembly with the BTB-Kelch family of E3 ubiquitin ligases. J Biol Chem 2013; 288: 7803–7814.
7. Gong W, Gohla RM, Bowlin KM, et al. Kelch repeat and BTB domain containing protein 5 (Kbtbd5) regulates skeletal muscle myogenesis through the e2f1-dp1 complex. J Biol Chem 2015; 290: 15350–15361.
8. Dhanoa BS, Cogliati T, Satish AG, et al. Update on the Kelch-like (KLHL) gene family. Hum Genomics 2013; 7: 13.
9. Gong J, Tian J, Lou J, et al. A polymorphic MYC response element in KBTBD11 influences colorectal cancer risk, especially in interaction with a MYC regulated SNP rs6983267. Ann Oncol 2017; 29: 632–639.
10. Inoue N, Yahagi N, Yamamoto T, et al. Cyclin-dependent kinase inhibitor, p21WAF1/CIP1, is involved in adipocyte differentiation and hypertrophy, linking to obesity, and insulin resistance. J Biol Chem 2008; 283: 21220–21229.
11. Watanabe K, Watson E, Cremona ML, et al. ILDR2: an endoplasmic reticulum resident molecule mediating hepatic lipid homeostasis. PLoS One 2013; 8: e67234.
12. Watanabe K, Nakayama K, Ohta S, et al. Zinc finger protein ZNF70, a novel ILDR2-interacting protein, contributes to the regulation of HES1 gene expression. Biochem Biophys Res Commun 2016; 477: 712–716.
13. Pantoja C, Huff JT, Yamamoto KR. Glucocorticoid signaling defines a novel commitment state during adipogenesis in vitro. Mol Biol Cell 2008; 19: 4032–4041.
14. Wu Z, Bucher NL, Farmer SR. Induction of peroxisome proliferator-activated receptor gamma during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBPbeta, C/EBPdelta, and glucocorticoids. Mol Cell Biol 1996; 16: 4128–4136.
15. Hishida T, Naito K, Osada S, et al. Crucial roles of d-type cyclins in the early stage of adipocyte differentiation. Biochem Biophys Res Commun 2008; 370: 289–294.
16. Ferguson BS, Nam H, Morrison RF. Curcumin inhibits 3t3-l1 preadipocyte proliferation by mechanisms involving post-transcriptional p27 regulation. Biochem Biophysics Rep 2016; 5: 16–21.
17. Merkstein M, Laber S, McMurray F, et al. Fto influences adipogenesis by regulating mitotic clonal expansion. Nature Commun 2015; 6: 6792.