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The role of JAZF1 on lipid metabolism and related genes in vitro

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Abstract

JAZF1 is a novel gene that is associated with diabetes mellitus and prostate cancer according to genomewide association studies; however, little is known about the function of this gene in regulating metabolism. In the present study, we have shown the expression of JAZF1 in various mouse tissues. To elucidate its role in metabolism, we investigated the influence of an overexpression of JAZF1 on 3T3-L1 adipose cells and hepatoma carcinoma Hepa1-6 cells that represent target tissues for diabetes and insulin resistance. In both cells, JAZF1 overexpression led to a substantial reduction in the expression of acetyl–coenzyme A carboxylase, fatty acid synthetase, and sterol regulatory element–binding protein 1 messenger RNA (mRNA). The level of hormone-sensitive lipase mRNA significantly increased. The expression of JAZF1 in 3T3-L1 adipocyte exhibited suppressive effects on lipid accumulation and decreased droplet size. In addition, the transcription for glucose transport 1 was significantly higher than the control in the Hepa1-6 cell line; but it was not significantly different in 3T3-L1. These results showed that JAZF1 in adipocytes and liver cells reduces lipid synthesis and increases lipolysis mainly by down-regulating the levels of sterol regulatory element–binding protein 1, acetyl–coenzyme A carboxylase, and fatty acid synthetase mRNA expression and by increasing hormone-sensitive lipase mRNA expression. Because it had an effect on the decrease of the maturation of lipid droplets and fat storage, we speculate that JAZF1 might represent a potential target against diabetes and obesity.

1. Introduction

Epidemiologic studies have shown the association of diabetes mellitus (DM) with either increased or decreased risk of developing malignant tumors [1-4]. For instance, obesity and DM have been shown to increase the risk of prostate cancer; however, an inverse association of DM and prostate cancer is also reported [5-7]. A plausible explanation for these contradictions is that DM, which is associated with both obesity and a decreased risk of prostate cancer, could confound or distort observed associations of obesity with prostate cancer risk [8].

Adipose tissue, besides its role in energy storage, has been shown to play important endocrine functions by producing adipocytokines such as adiponectin, leptin, resistin, and plasminogen activator inhibitor-1 [9-11]. Chronic inflammation in adipose tissue is closely associated with the development of insulin resistance and DM [12]. Recently, genomewide association studies have suggested that there is an association of JAZF1 (juxtaposed with another zinc finger gene 1) with DM [13] and prostate cancer [14]. JAZF1 encodes a 27-kd nuclear protein containing 3 putative zinc finger motifs and is expressed in a variety of tissues in mice, with the highest expression in adipose tissue and testes [15]. To our knowledge, the role of JAZF1 in regulating glucose and lipid metabolism has not been reported. The current study demonstrates that overexpression of JAZF1 in 3T3-L1 results in decreased lipogenesis and increased lipolysis. We also transfected JAZF1 in hepatoma carcinoma Hepa1-6

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cells to research the function, as it also has important effects in metabolism.

2. Materials and methods

2.1. Expression of JAZF1 messenger RNA in mouse tissues

Age-matched adult male mice (C57BL/6J), weighing 25 to 30 g, were obtained from the animal center at the Chongqing Medical University. Mice were killed by Metofane (Sinopharm Chemical Reagent Co, Ltd, Shanghai, China) overdosing under the guidelines of the Animal Experimentation Ethics Committee (Chongqing Medical University) and in accordance with the National Health and Medical Research Council of China Guidelines on Animal Experimentation. Total RNA was extracted from various tissues using Trizol reagent (Takara Bio Co Ltd, Dalian, China). The expression of JAZF1 messenger RNA (mRNA) was determined using quantitative reverse transcription polymerase chain reaction (qRT-PCR) with the following primers: 5′-ACGCCGAGAACAGGAATC-3′ (forward) and 5′-GTGCTGCTGCGGAATGAA-3′ (reverse).

2.2. Construction of eukaryotic expression plasmid PIRES2-EGFP-JAZF1

The full-length (CDS domain) JAZF1 complementary DNA (cDNA) (540 base pairs [bp]) was amplified from mouse tissue using RT-PCR with primers 5′-CCGGAATTCACCATGACAGATGCTGCACGCCG-3′ (forward) and 5′-GCGGTCGACCTACTGCTGCATCTTCCTGATCATC-3′ (reverse). Purified PCR products and PIRES2-EGFP (5.3 kilobases) were digested with *Eco*RI and *Sal*I. The 540-bp (JAZF1 cDNA) and 5288-bp (PIRES2-EGFP) fragments were collected in low melting point agarose and then ligated using T4 ligase at 16 hours overnight. *Escherichia coli* (DH-5a) were transformed using ligation products. Recombinant plasmids (PIRES2-EGFP-JAZF1) were purified from transformed *E coli*, screened on kanamycin plates, and verified by restriction endonuclease digestion and DNA sequencing.

2.3. Cell culture, differentiation of 3T3-L1 cells, and transient-transfection assay

Hepatoma carcinoma HEPA1-6 cells were cultured in Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (Gibco-Invitrogen, Grand Island, NY) in 6-well plates.

3T3-L1 preadipocytes were cultured and induced to differentiate as previously described [10]. In short, 3T3-L1 preadipocytes were seeded in 6-well plates in DMEM containing 25 mmol/L glucose and 10% calf serum in 5% CO_2 at 37°C. After cells reached 100% confluence for 48 hours (day 0), differentiation was induced by changing the medium to DMEM containing 25 mmol/L glucose, 0.5 mmol/L 3-isobutyl-1-methylxanthine, 1 μmol/L dexamethasone, 10 μg/mL insulin, and 10% fetal bovine serum (FBS). After 48 hours, the medium was replaced with DMEM containing 25 mmol/L glucose, 10 μg/mL insulin, and 10% FBS. On day 4, the medium was replaced with DMEM containing 25 mmol/L glucose and 10% FBS; and then the medium was changed every 2 days. In differentiated adipocytes, various experiments involved stimulation with insulin (10 μg/mL) for 30 minutes at 37°C.

Transient transfection assays were carried out using Hepa1-6 cells and differentiated 3T3-L1 adipocytes. When Hepa1-6 cells reached 90% confluence and 90% of 3T3-L1 cells exhibited the adipocyte phenotype on day9 of induction of differentiation, they were transfected with 4 μg of the pIRES2-EGFP or pIRES2-EGFP-JAZF1 expression

Table 1
Characteristics of the specific primer used for real-time qPCR analysis

Gene	Forward and reverse primers	Base pairs	Annealing temperature (°C)
JAZF1	5′-ACGCCGAGAACAGGAATC-3′ 5′-GTGCTGCTGCGGAATGAA-3′	171 bp	57
FAS	5′-TGGTGGGTTTGGTGAATTGTC-3′ 5′-GCTTGTCCTGCTCTAACTGGAAGT-3′	213 bp	55
SREBP1	5′-GATCAAAGAGGAGCCAGTGC-3′ 5′-TAGATGGTGGCTGCTGAGTG-3′	191 bp	55
ACC	5′-CTGTGAGGTGGATCAGAGAT-3′ 5′-TTCAGCTCTAACTGGAAAGC-3′	129 bp	55
ATGL	5′-TGCTACCCGTCTGCTCTTTC-3′ 5′-GACCTGATGACCACCCTTTC-3′	169 bp	55
HSL	5′-AAGACCACATCGCCCACA-3′ 5′-CTGAAGGCTCTGAGTTGCT-3′	154 bp	55
GLUT1	5′-ATCCTGTTGCCCTTCTGC-3′ 5′-AGGTCTCGGGTCACATCG-3′	119 bp	57
GLUT4	5′-GATTCTGCTGCCCTTCTGTC-3′ 5′-ATTGGACGCTCTCTCTCCAA-3′	168 bp	57
β-Actin	5′-GTTGCCAATAGTGATGACCT-3 5′-GGACCTGACAGACTACCTCA-3′	208 bp	55-57

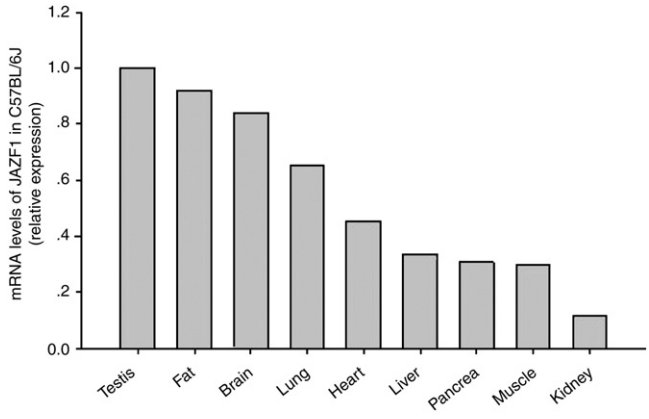

Fig. 1. Distribution of JAZF1 mRNA in C57BL/6J. The mRNA levels of JAZF1 from different tissues of C57BL/6J were measured by real-time PCR and normalized to β-actin expression. Data were analyzed by the $2^{-\Delta\Delta Ct}$ method and are representative of 3 independent experiments.

plasmids. This was accomplished by using 10 μL of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) and a ration Opti-MEM I Reduced-serum Medium (GIBCO) per well for 48 hours, respectively. The transfection was performed according to standard protocol. The experimental groups include negative controls (Lipofectamine 2000 and phosphate-buffered saline [PBS] negative controls), a blank control (transfected with PIRES2-EGFP), and the PIRES2-EGFP-JAZF1 group (transfection group). All experiments were done in triplicate.

2.4. Quantitative RT-PCR analysis

Total RNA was isolated from cells using Trizol reagent (Takara Bio) and was reverse transcribed into cDNA using PrimeScript RT reagent Kit (Takara Bio). Quantitative PCR (qPCR) was performed using Lightcycler (Roche Applied Bioscience, Indianapolis, IN) and SYBR Premix EX Taq (Takara Bio). The expression of all genes were normalized by β-actin as well as experimental controls, and the results were expressed as $2^{-\Delta\Delta Ct}$. The primer sequences, product lengths, and annealing temperatures are shown in Table 1.

2.5. Western blot analysis

Proteins were isolated in a lysis buffer containing 20 mmol/L Tris, 0.5% sodium dodecyl sulfate, and a protease inhibitor cocktail tablet. Anti-JAZF1 immunoglobulin G (1:1000 dilution) was obtained from ABONVA (Taipei, China). Immunoblot were visualized using the ECL detection system (Amersham Biosciences, Piscataway, NJ).

2.6. Statistical analysis

All data are presented as means ± SD. Statistical analyses were performed using 1-way analysis of variance. Differences were considered statistically significant at values of $P < .05$.

3. Results

3.1. Expression of JAZF1 mRNA in mouse tissue

The expression of JAZF1 gene in various mouse tissues has been previously reported [15]. We confirmed and further examined the expression pattern of JAZF1 in mice using qRT-PCR. As shown in Fig. 1, JAZF1 is expressed in all tissues examined, with the highest levels detected in testes and adipose tissues and the lowest levels in muscles and kidneys.

3.2. Overexpression of JAZF1 in vitro

To study the role of JAZF1 in vitro, we constructed a JAZF1 recombinant plasmid and transfected it into 3T3L1

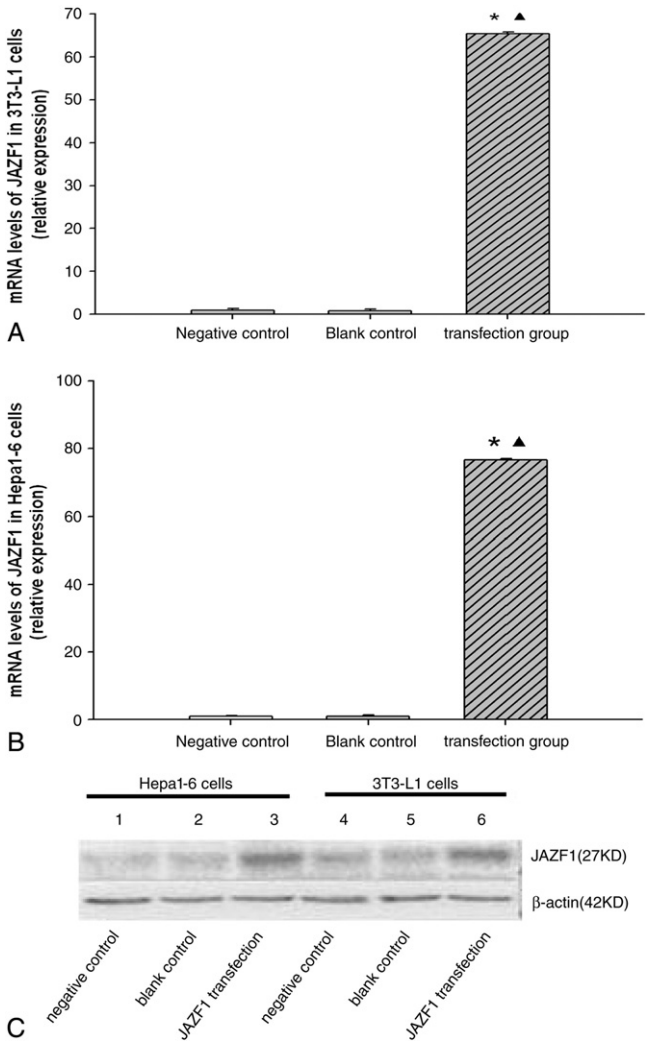

Fig. 2. Effect of overexpression JAZF1 in 3T3-L1 cells and Hepa1-6 cells. Negative control: cells were treated with the mixture of Lipofectamine 2000 and PBS; Blank control: transfected with the pIRES2-EGFP. JAZF1 transfection group: infection of JAZF1 in 3T3-L1 cells or Hepa1-6 cells, respectively, for 2 days. Data are presented as the increase or decrease(n-fold) compared with control groups and are mean ± SD. For 3 samples per group and in triplicate (n = 9, per group). *Compared with negative control group, $P < .001$. ▲Compared with blank control group, $P < .001$.

and Hepa1-6 cells. Expression of JAZF1 was evaluated by RT-PCR and Western blot (Fig. 2). The mRNA levels of JAZF1 were significantly up-regulated by 65-fold ($P < .001$, Fig. 2A) in 3T3-L1 cells and by 77-fold ($P < .001$, Fig. 2B) in Hepa1-6 cells as compared with the respective controls. Overexpression of JAZF1 led to an increase in protein levels in both cells as demonstrated by immunoblot analysis (Fig. 2C).

3.3. The effect of JAZF1 in lipid accumulation in 3T3-L1 adipocytes

The representative images of oil red O staining demonstrated that overexpression of JAZF1 resulted in decreased lipid accumulation in differentiated 3T3-L1 cells (Fig. 3A). Quantitative analysis showed that the cells overexpressing JAZF1 had a 30% lower intracellular neutral lipid content than the controls ($P< .05$, Fig. 3B). These results suggest an antilipogenic role of JAZF1 in differentiating adipocytes.

3.4. JAZF1 triggers gene expression changes

To further investigate the role of JAZF1 in regulating metabolism, we examined the expression of genes involved in glucose and lipid metabolism in 3T3-L1 cells and Hepa1-6 cells. The expressions of several genes implicated in the metabolism of cells were measured by using qPCR. This line of research was focused on adipocyte (3T3-L1 cells) and hepatoma carcinoma lines (Hepa1-6 cells), which represent target tissues for diabetes and obesity.

The expression of transcription factors involved in lipid synthesis was analyzed. As shown in Fig. 4 and Fig. 5, the mRNA levels for acetyl–coenzyme A (CoA) carboxylase

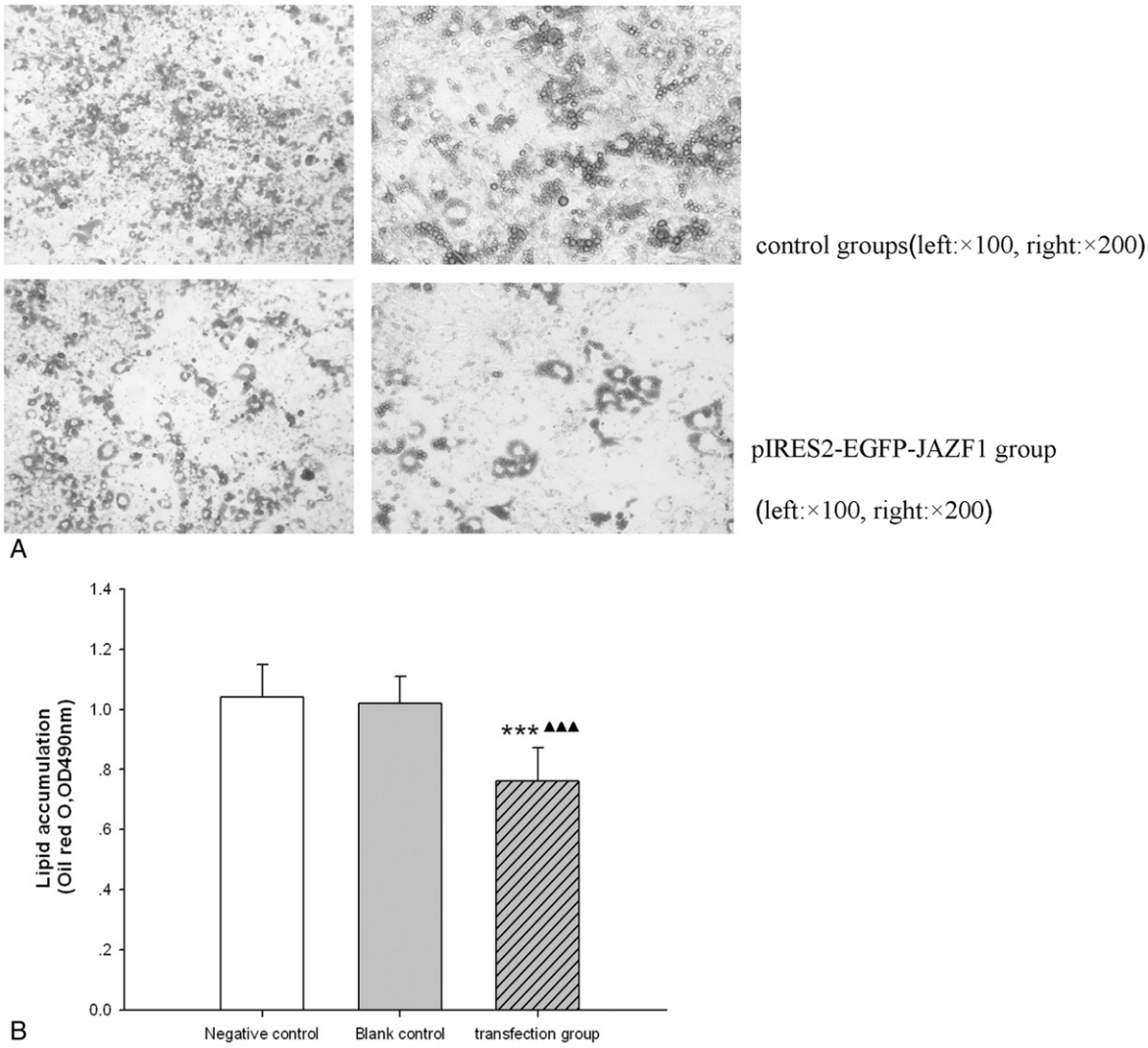

Fig. 3. Lipid accumulation of overexpression JAZF1 in 3T3-L1 adipocytes. A, Control groups transfected cells and JAZF1 transfected cells were fixed, stained with oil red O, and photographed after transfection for 2 days. B, The stained lipid was extracted and measured as described in "Materials and methods." The data represent the mean ± SD from 3 independent experiments. Negative control: cells were treated with the mixture of Lipofectamine 2000 and PBS; Blank control: transfected with the pIRES2-EGFP. JAZF1 transfection group: infection of JAZF1 in 3T3-L1 cells or Hepa1-6 cells, respectively, for 2 days. ***Compared with negative control group, $P < .05$. ▲▲▲Compared with blank control group, $P < .05$.

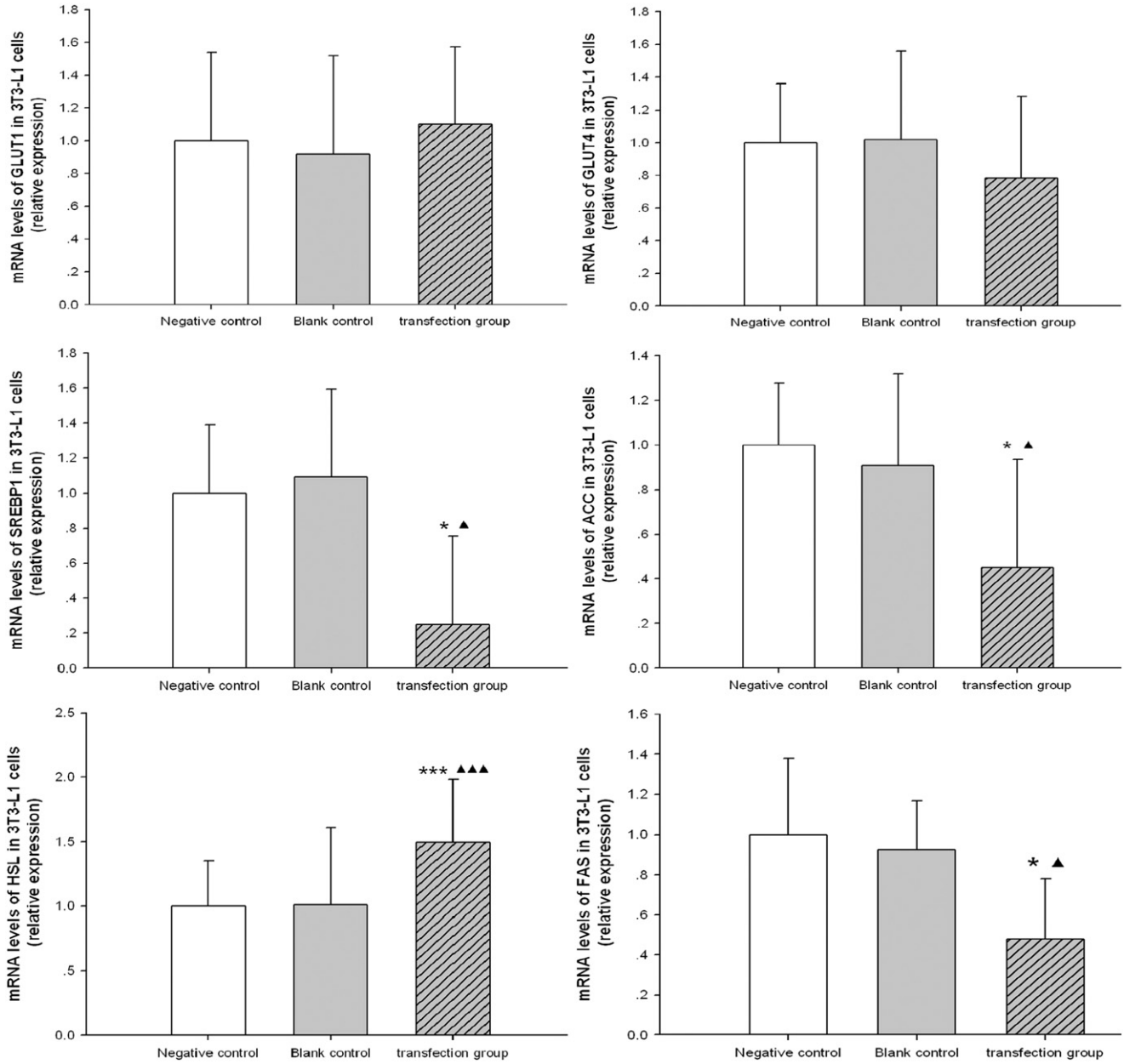

Fig. 4. Effect of JAZF1 on expression of various genes implicated in 3T3-L1 cells metabolism. 3T3-L1 adipocytes were treated with the mixture of Lipofectamine 2000 and PBS (negative control) and transfected with the pIRES2-EGFP empty vector (blank control) or pIRES2-EGFP-JAZF1 for 2 days. Total RNA was isolated from each group cells and subjected to qRT-PCR to determine JAZF1 and several genes expression normalized to β-actin levels as described in "Materials and methods." Data are presented as the increase or decrease (n-fold) compared with control groups and are mean ± SD. For 3 samples per group and in triplicate(n = 9, per group). *Compared with negative control group, $P < .001$. ▲Compared with blank control group, $P < .001$; ***Compared with negative control group, $P < .05$. ▲▲▲Compared with blank control group, $P < .05$.

(ACC), fatty acid synthetase (FAS), and sterol regulatory element–binding protein 1(SREBP1) were decreased by 55%, 52%, and 75%, respectively, in 3T3-L1(Fig. 4, $P < .001$) and by 43%, 52%, and 69%, respectively, in Hepa1-6 cells (Fig. 5, $P < .001$). The mRNA levels of hormone-sensitive lipase (HSL) was increased by 49% in 3T3-L1 (Fig. 4, $P < .05$) and by 65% in Hepa1-6 cells (Fig. 5, $P < .001$). However, the adipose triglyceride lipase (ATGL) was not changed. In addition, the expression of GLUT1 and GLUT4 mRNA was not statistically different ($P > .05$, data not shown) in adipocyte 3T3-L1 cells. In Hepa1-6 cells, the transcriptions for GLUT1 was significantly higher than basal (2.2-fold, $P < .001$, Fig. 5), whereas GLUT4 mRNA was not significantly different.

4. Discussion

JAZF1, a protein with an unknown function, is an mRNA of 3.1 kilobases and a basic protein with a molecular mass of

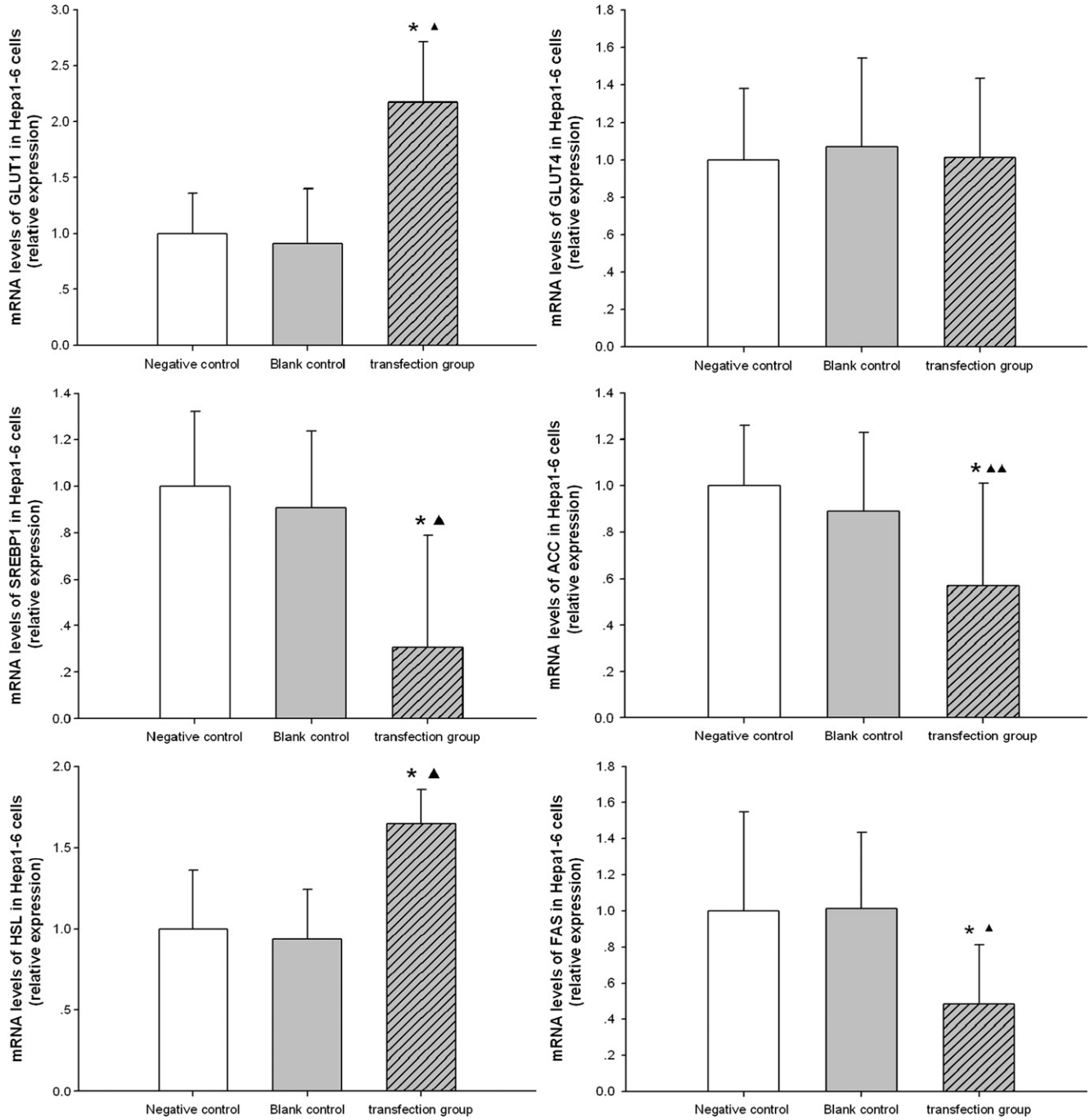

Fig. 5. Effect of JAZF1 on expression of various genes implicated in Hepa1-6 cells metabolism. Hepa1-6 cells were treated with the mixture of Lipofectamine 2000 and PBS (negative control) and transfected with the pIRES2-EGFP (blank control) and pIRES2-EGFP-JAZF1 for 2 days. Total RNA was isolated from each group cells and subjected to qRT-PCR to determine JAZF1 and several genes expression normalized to β-actin levels as described in "Materials and methods." Data are presented as the increase or decrease (n-fold) compared with control groups and are mean ± SD. For 3 samples per group and in triplicate (n = 9, per group). *Compared with negative control group, $P < .001$. ▲Compared with blank control group, $P < .001$. ▲▲Compared with blank control group, $P < .01$.

27.1 kd containing 3 putative zinc finger motifs. MotifScan and ScanProsite identified 2 putative zinc finger motifs with the consensus sequence CX4CX12HX4H, one at the N-terminus between Cys14 and His37 (ZF1) and one at the C-terminus between Cys175 and His198 (ZF2). A third putative zinc finger motif (ZF3; CXCX12HX4H) was observed between Cys210 and His230. JAZF1 was found to be identical to the recently described TIP27 that acts as a strong repressor of DR1-dependent transcriptional activation by TAK1 [11]. In addition, the zinc finger motifs of JAZF1 did not show homology with other zinc finger proteins. TAK1 (or TR4, NR2C2) belongs to a subclass of orphan

nuclear receptors [16,17]. Mammalian 2-hybrid and pull-down analyses indicate that TIP27 interacts specifically with the ligand-binding domain of the nuclear orphan receptor TAK1 and functions as a TAK1-selective cofactor that may play an important role in mediating transcriptional repression by TAK1 [15]. Additional research found that JAZF1 may have a possible role as a tumor suppressor because a loss of expression for normal versions of JAZF1 was noted in multiple tumors [18].

A linkage and association analysis found JAZF1 region affecting human height. Because this gene also has a key function in the metabolism of growth, JAZF1 represents one of the strongest candidates influencing human height identified so far [19]. It has also been associated with susceptibility to prostate cancer in a genomewide association study of European origin-nested [14]. In addition, genome-wide association studies have identified that there is strong statistical evidence that JAZF1 is associated with DM [20]. To identify whether JAZF1 is involved in the metabolic disease, a eukaryotic expression plasmid of JAZF1 gene was constructed and transfected into 3T3-L1 cells and Hepa1-6 cells. Its function in metabolism regulation was investigated after confirming the JAZF1 protein in the cells.

Although the expression of JAZF1 gene in various mouse tissues has been previously reported [15]. We further examined the expression pattern of JAZF1 in mice by using qRT-PCR. In fact, JAZF1 was expressed in almost all the tissues of mice, with the highest levels detected in testes and adipose tissues, which are often connected to lipid metabolism.

To determine the potential mechanism in glucose metabolism, mRNA expressions of GLUT1 and GLUT4 in these cells were examined. It is well known that GLUT1 is thought to be responsible for basal glucose transportation in cells. Insulin promotes glucose transporter by stimulating the translocation of the major insulin-responsive glucose transporter, GLUT4, from intracellular storage vesicles to the plasma membrane [21]. The results indicate that overexpression JAZF1 in Hepa1-6 cells results in an increase in GLUT1. It also affected 3T3-L1 adipocytes in glucose metabolism, but it was not statistically significant.

To explore the molecular mechanisms leading to the down-regulation of fatty acid synthesis, the contribution of the transcription factors SREBP1 in this process was investigated. SREBP1 is a transcription factor that plays important roles in the controls of fatty acid metabolism and adipogenesis [22]. In human skeletal muscle, SREBP1 expression was significantly reduced in type 2 DM subjects but not in obese subjects. Within the diabetic group, the extent of SREBP1 suppression was inversely related to the level of the patient's metabolic control, as it was restored to normal level in the muscles of diabetic patients after being exposed to 3 hours of hyperinsulinemia. Transgenic mice overexpressing SREBP1 in vivo have elevated levels of lipogenesis and enhancement of lipogenic enzyme gene expression. Further studies have explored the role of SREBP1c by examining the regulation of lipogenic gene promoters in cultured cell lines such as in the Chinese hamster ovary or 293 cells [23,24]. Transformed cell lines have been substantially modified in how the cell manages its energy formation and storage. Because expression of the gene was reduced in cells in this study, the decrease of lipid content, reduction of the lipogenesis, and lipogenic enzyme gene expression may be factors.

Triglyceride stored within lipid droplets is catabolized into free fatty acids and glycerol in a process known as *lipolysis*. Hormone-sensitive lipase has been considered for a long time as the key enzyme catalyzing the rate-limiting step of adipose tissue lipolysis. It exhibits broad substrate specificity capable of hydrolyzing cholesteryl ester; tri- (TG), di- (DG), and monoacylglycerol; retinyl ester; and numerous water-soluble ester substrates [25,26]. The enzyme is most active against DG that is hydrolyzed 10-fold faster than TG. The ATGL is highly expressed in adipose tissue, and its expression markedly increases during 3T3-L1 adipocyte differentiation [27,28]. It selectively performs the first step in TG hydrolysis, resulting in the formation of DG and free fatty acids. The specific activity against TG is more than 10-fold higher than that against DG; and the enzyme shows essentially no hydrolytic activity when other lipid substrates are used, such as cholesteryl esters or retinyl esters. The current study showed that JAZF1 treatment significantly up-regulated the mRNA expression of HSL in Hepa1-6 cells and 3T3-L1 cells. Oil red O staining demonstrated that JAZF1 led to a decrease in the maturation of lipid droplets and fat storage in differentiating adipocytes. The glycerol and free fatty acids liberated from adipocyte lipid droplets enter the circulation and are metabolized. These results showed that JAZF1 might depress fat accumulation and increase lipolysis mainly through an increase of HSL expression.

In the fatty acid (FA) de novo synthesis, ACC and FAS catalyze the first 2 committed steps. We investigated the processes dependent on the ACC (that produces malonyl-CoA from acetyl-CoA) and FAS (that catalyzes the conversion of acetyl-CoA and malonyl-CoA into long-chain FA) [29]. Acetyl-CoA carboxylase is the committed step for fat biosynthesis with malonyl-CoA as its product, which is the substrate of synthesis long-chain FA. Fatty acid synthetase and ACC constitute master enzymes in lipid synthesis that may be linked to the development of DM and obesity in humans [30,31]. Fatty acid synthetase is transcriptionally regulated and a key enzyme in fatty acid synthesis [32]. Sterol regulatory element–binding protein 1c has been shown to be the principal regulatory transcription factor for fatty acid synthesis in animals [33]. Thus, FAS and SREBP1 have been shown to be critical to lipogenesis. Adipose tissue mRNA, protein, and activity levels of lipogenic genes such as FAS and ACC were lower on DM, overweight, and obese subjects [34,35]. Our results showed a 52% decrease in mRNA abundance of FAS as well as decreases in the enzyme activity of FAS and the expression of SREBP1 gene. This suggested that the lipid synthesis in

cells was repressed by JAZF1 in the expression of genes involved in de novo synthesis of FAs.

In conclusion, to our knowledge, this is the first report for JAZF1 in metabolism. Furthermore, we confirmed that overexpression of JAZF1 in adipocytes and liver cells led to reduced lipid synthesis and increased lipolysis by up-regulating HSL and reducing the expression of SREBP1, ACC, and FAS. Because it decreases the maturation of lipid droplets and fat storage, reduces lipid synthesis, and increases lipolysis, we speculate that JAZF1 may represent a potential target for regulating metabolism and obesity. In addition, its effect on glucose metabolism has also been implicated.

Acknowledgment

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