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FTO promotes SREBP1c maturation and enhances CIDEC transcription during lipid accumulation in HepG2 cells

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ABSTRACT

The fat mass and obesity-associated (FTO) gene is tightly related to body weight and fat mass, and plays a pivotal role in regulating lipid accumulation in hepatocytes. However, the mechanisms underlying its function are poorly understood. Sterol regulatory element binding protein-1c (SREBP1c) is a transcription factor that regulates lipogenesis. Cell death-inducing DFFA (DNA fragmentation factor-α)-like effector c (CIDEC) plays a crucial role in lipid droplets (LDs) size controlling and lipid accumulation. In this report, we first observed that FTO overexpression in HepG2 cells resulted in an increase of lipogenesis and up-regulation of SREBP1c and CIDEC, two key regulatory factors in lipogenesis. In contrast, FTO knockdown in HepG2 cells resulted in a decrease of lipogenesis and down-regulation of SREBP1c and CIDEC expression. Moreover, SREBP1c knockdown resulted in a decrease of lipogenesis in HepG2 cells with FTO overexpression. In addition, FTO demethylation defect mutant presented less transcription of the key genes, and less nuclear translocation and maturation of SREBP1c. Further investigation demonstrated that overexpression of SREBP1c in HepG2 cells also promoted high CIDEC expression. Luciferase reporter assays showed that SREBP1c significantly stimulated CIDEC gene promoter activity. Finally, CIDEC knockdown reduced SREBP1c-induced lipogenesis. In conclusion, our studies suggest that FTO increased the lipid accumulation in hepatocytes by increasing nuclear translocation of SREBP1c and SREBP1c maturation, thus improving the transcriptional activity of LD-associated protein CIDEC. Our studies may provide new mechanistic insight into nonalcoholic fatty liver disease (NAFLD) mediated by FTO.

1. Introduction

Genome-wide association studies (GWAS) have identified that the fat mass and obesity-associated (FTO) gene is associated with obesity in humans [1,2]. The association has been further confirmed in different human populations [3–7]. In mice, overexpression of FTO leads to obesity [8]. Conversely, mice with FTO knockout [9–11] or with loss-of-function mutants of FTO [12] have reduced body weight. FTO is an iron and 2-oxoglutarate-dependent dioxygenase and belongs to the AlkB family [13,14]. Previous studies also indicate that FTO-dependent N6-methyladenosine (m6A) demethylation plays a critical role in mRNA processing and adipogenesis [15].

Sterol regulatory element binding protein-1c (SREBP1c) is a transcription factor and regulates genes required for de novo lipogenesis [16]. The precursor form of SREBP1c (125 kDa) is synthesized and attached to endoplasmic reticulum (ER) membrane. To carry out its transcriptional activities, SREBP1c precursor is escorted from ER to the Golgi apparatus by SREBP cleavage-activating protein (SCAP) [17,18]. In the Golgi apparatus, the precursor form of SREBP1c (125 kDa) undergoes proteolytic cleavage by Site 1 and Site 2 proteases (S1P and S2P). Then the mature form of SREBP1c (68 kDa) is released from the Golgi apparatus and translocated into nucleus [19]. As a transcription

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factor, the matured SREBP1c binds to the promoter of target genes, including fatty acid synthase (FAS) [20], stearoyl-CoA desaturase (SCD) [21,22], and diacylglycerol acyltransferase 2 (DGAT2) [23]. These enzymes are involved in fatty acid and triglyceride (TG) synthesis and their gene regulation by SREBP1c depends on the nutritional conditions for lipid storage [24].

Lipid droplets (LDs) as principal subcellular organelles are used to store cellular TG. LDs are multilocular and small in hepatocytes [25]. LD-associated protein cell death-inducing DFFA (DNA fragmentation factor-α)-like effector c (CIDEC), also called fat-specific protein of 27 kDa (FSP27), is dramatically upregulated in fatty liver [26]. CIDEC is an important factor for controlling LD size [27] and TG storage [28,29] in adipocytes. CIDEC is rich in LD-LD contacting sites and promotes lipid storage by transforming smaller LDs into unilocular larger LDs [30,31]. The mechanisms that regulate CIDEC expression in hepatocytes remains unknown. It is unclear whether CIDEC plays a role in hepatic lipid accumulation.

Nonalcoholic fatty liver disease (NAFLD) is closely associated with obesity [32]. Excess fat accumulation is the common feature of the disease. The expression of FTO is significantly increased in the livers of non-alcoholic steatohepatitis (NASH) patients and in a rodent model of NASH [33]. However, the molecular mechanisms of lipid accumulation remain unclear in NAFLD. Therefore, we explored the role of FTO in lipid accumulation of hepatocytes. Our studies suggest that the FTO promotes SREBP1c maturation and subsequently induces CIDEC expression for lipid accumulation in hepatocytes.

2. Results

2.1. FTO promotes lipogenesis in HepG2 cells

To understand the role of FTO in hepatic de novo lipogenesis, HepG2 cells with or without overexpression of FTO were cultured for 24 h with free fatty acids (FFAs, mixture of oleic acid and palmitic acid). The results of Oil Red O staining showed more lipid accumulation in cells with FTO overexpression (Fig. 1A). Measurement of TGs and glycerol contents indicated that FTO overexpression also increased TG levels and decreased glycerol levels (Fig. 1B, C). The expression of genes, tightly related to fatty acid de novo synthesis, esterification, and desaturation, was evaluated by real-time PCR in HepG2 cells. The results showed that overexpression of FTO significantly upregulated the transcription levels of FAS and SCD1 (Fig. 1D). Further analysis showed that FTO overexpression caused an increase in the mRNA levels of PLIN and S3-12 (Fig. 1E), two important factors in lipid accumulation.

In order to further study the role of FTO in hepatic de novo lipogenesis, siRNA against FTO was transfected into HepG2 cells for 24 h. The cells exhibited reduced lipogenesis capacity, as measured with Oil Red-O staining for TGs (Fig. 2A). FTO knockdown efficiency was determined by real-time PCR and Western blotting, the mRNA and protein levels of FTO were significantly downregulated (Fig. 2B, C). Moreover, the expression of FAS and SCD1, the two genes tightly related to fatty acid de novo synthesis, esterification, and desaturation, were significantly downregulated the mRNA levels (Fig. 2D). The expression levels of ACOX1 (acyl-CoA oxidase A) and CPT1α (carnitine palmitoyltrasnferase 1) genes, which are related to lipolysis, showed no significant change (Fig. 2E). The reduced level of TGs was likely due to reduced lipogenesis. Downregualtion of PLIN and S3-12 at mRNA level were also observed (Fig. 2F). These results suggested that FTO could regulate lipogenesis in hepatocytes.

2.2. FTO might exert its effects on CIDEC by acting through SREBP1c

To further understand FTO function in lipid accumulation, the effects of FTO overexpression on the expression of SREBP1c and CIDEC were examined. SREBP1c is a key transcriptional factor to start transcription of genes involved in fatty acid synthesis. CIDEC is located on the lipid droplet surface and stimulates lipid accumulation. Both mRNA and protein levels of mature SREBP1c and CIDEC were significantly

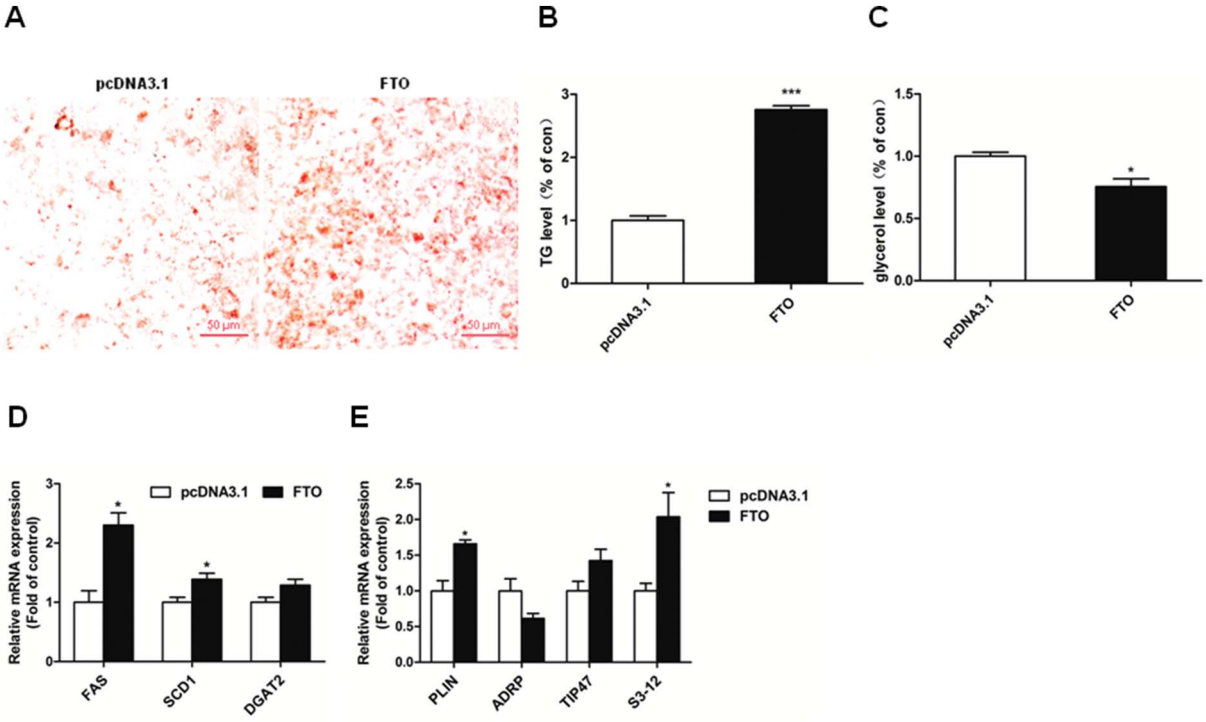

Fig. 1. FTO overexpression promotes TG synthesis and lipid accumulation in HepG2 cells. HepG2 cells were transfected with FTO or empty pcDNA3.1 for 24 h, and the mRNA levels of the indicated genes were measured by real-time PCR. (A) TG accumulation was visualized by Oil Red O staining (bar, 50um). Cellular TGs (B) and glycerol (C) contents were measured as described in the Methods and Materials. (D) The transcription levels of genes related to fatty acid de novo synthesis, esterification, and desaturation. (E) The transcription levels of genes playing a critical role in lipid accumulation. Data were represented as mean ± SEM (n = 3). *$p < 0.05$, ***$p < 0.001$.