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FoxO1 represses LXRα-mediated transcriptional activity of SREBP-1c promoter in HepG2 cells

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ABSTRACT

Recent studies have demonstrated that FoxO1 modulates the expression of SREBP-1c, but the exact mechanism remains unknown. Our results demonstrate that FoxO1 suppresses the SREBP-1c promoter transcriptional activity in HepG2 cells. This repression was independent of FoxO1 binding to the SREBP-1c promoter, but LXR responsive elements (LXREs) were crucial to this phenomenon. Moreover, FoxO1 also strongly inhibited the LXRα-mediated elevated transcription by SREBP-1c promoter. Electrophoretic mobility shift assay and chromatin immuno-precipitation further suggested the ability of FoxO1 to inhibit LXRα binding with the LXRE in the SREBP-c promoter. FoxO1-mediated suppression of SREBP-1c promoter activity could be partially alleviated by insulin.

1. Introduction

SREBP-1c is a transcription factor involving in fatty acid synthesis in differentiated tissues and organs. In addition, SREBP-1c also appears to be a mediator of insulin/glucose signaling to lipogenesis, and is linked with the insulin resistance, remnant lipoprotein and fatty livers [1].

Forkhead box-"Other" (FoxO) transcription factors critically control fundamental cellular processes, including metabolism, cell differentiation and other reactions to cellular stress. FoxO transcription factors bind as monomers to a consensus DNA-binding sequence, TTGTTTAC [2]. In the absence of any cellular stimulus, FoxOs are localized in the nucleus, where they regulate transcription of their target genes. Upon activation of protein kinase B (PKB) by growth or survival factors, FoxOs are phosphorylated at their highly conserved residues (corresponding to Thr-24, Ser-256 and Ser-319 in human FoxO1), relocalizing from the nucleus to the cytosol, and no longer function as transcriptional activators [3]. Consequently, insulin can activate PKB and attenuate FoxO-mediated expression of regulated proteins.

FoxO1 has been demonstrated to have the dual role in controlling hepatic insulin sensitivity and lipid metabolism. In conditions of impaired insulin signaling, FoxO1 activity increases, leading to excessive glucose production. At the same time, it increases Akt signaling and suppresses Peroxisome proliferator-activated receptor-α expression, leading to increased TG synthesis through SREBP-1 and decreased fatty acid oxidation [4]. In contrast, gene-array studies demonstrated that FoxO1 modulates the expression of multiple genes involved in glucose metabolism and lipogenesis in vivo, including glucokinase and SREBP-1c [5]. Recently, Kamei's study indicated that retinoid X receptor (RXR)/liver X receptor (LXR) up-regulated SREBP1c gene expression, and that FoxO1 antagonized this effect of RXR/LXR in skeletal muscle [6].

This study further investigated the modulatory effect of FoxO1 on mouse SREBP-1c promoter in HepG2 cells, by which FoxO1 inhibits the transcription of SREBP-1c.

2. Materials and methods

Materials and methods are shown as Supplementary material.

Abbreviations: SREBPs, sterol regulatory element binding proteins; FoxO, forkhead box-"Other"; Akt/PKB, protein kinase B; PPARα, peroxisome proliferator-activated receptor-α; RXR, retinoid X receptor; LXR, liver X receptor; FASN, fatty acid synthase; LXREs, LXR responsive elements; DR4-type, direct repeat 4 type; PI3K, phosphatidylinositol 3-kinase; PGC-1α, Peroxisome proliferator-activated receptor-γ coactivator-1α; EMSA, electrophoretic mobility shift assay; ChIP, chromatin Immuno-precipitation; IR, insulin receptor; RLA, relative luciferase activity

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3. Results and discussion

3.1. FoxO1 inhibits the promoter activity of mouse SREBP-1c

To study the effect of FoxO1 on SREBP-1c gene expression, FoxO1-3A expression plasmids were transfected into HepG2 cells. FoxO1-3A is a constitutive activity mutant of three principal Akt-catalyzed phosphorylation sites are substituted by alanine conferring it resistance to growth factor-induced phosphorylation and inactivation by nuclear exclusion [3]. Over-expression of FoxO1-3A down-regulates the expression of SREBP-1c and its target gene, fatty acid synthase (FASN), but not SREBP-1a (Fig. 1).

To investigate the molecular mechanism of FoxO1-inflicted suppression of SREBP-1c, promoter-luciferase construct, pBP1c2600-Luc, was co-transfected in HepG2 cells with plasmids expressing wild-type of FoxO1(FoxO1-wt), or mutants (FoxO1-3A or FoxO1-3A(H215R)), respectively. All of three forms of FoxO1 evidently decreased the reporter gene transcription driven by SREBP-1c promoter (Fig. 2). FoxO1-3A(H215R), a mutant whose His-215 residue has been replaced by arginine markedly impairing its DNA binding [7], inhibiting the SREBP-1c promoter activity suggests that the down-modulation of SREBP-1c promoter by FoxO1 does not require its physical binding to the DNA. As control, FoxO1-wt and FoxO1-3A enhanced the transcription activity of 3IRES-promoter, but not FoxO1-3A(H215R) (Fig. S1).

3.2. LXR elements are crucial for FoxO1-inflicted inhibition of mSREBP-1c promoter

To elucidate the mode of FoxO1-suppression of the SREBP-1c promoter, we designed 5′-deletion experiments constructing multiple reporters with varying segments of the promoter. Luciferase reporter inhibition by ectopically expressed FoxO1 was estimated

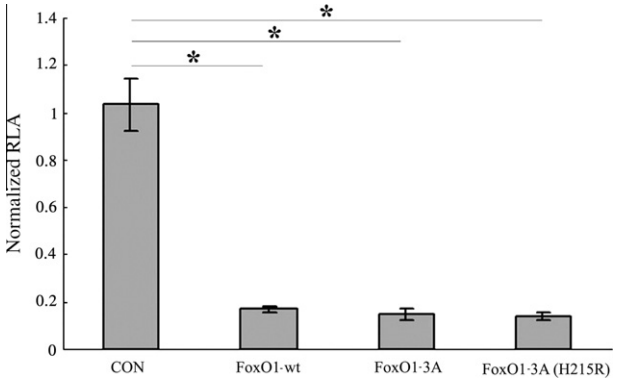

Fig. 2. Repression of mSREBP-1c promoter activity by over-expression of FoxO1. HepG2 cells were co-transfected with the luciferase reporter plasmids pBP1c2600-Luc (200 ng) and equivalent amounts of plasmids expressing FoxO1-wt, FoxO1-3A, FoxO1-3A(H215R) or control vectors. $^{*}P < 0.05$.

for all samples and normalized using pBP1c500-Luc and empty vector as the reference. In HepG2 cells harboring pBP1c500-Luc and pBP1c250-Luc constructs, FoxO1-3A-mediated inhibition of luciferase expression ranged from 6.4 to 4.7 fold, respectively. This FoxO1-repression of the luciferase activity reduced to only twofold in assays using pBP1c148-Luc. Likewise, no repression of the reporter gene that was governed by −94 bp region of promoter was observed in HepG2 cells (Fig. 3).

3.3. FoxO1 suppresses LXRα-mediated activation of mSREBP-1c promoter activity

By analysis the promoter of SREBP-1c, there are two LXR responsive elements (LXREs) between −250 and −148 bp. We

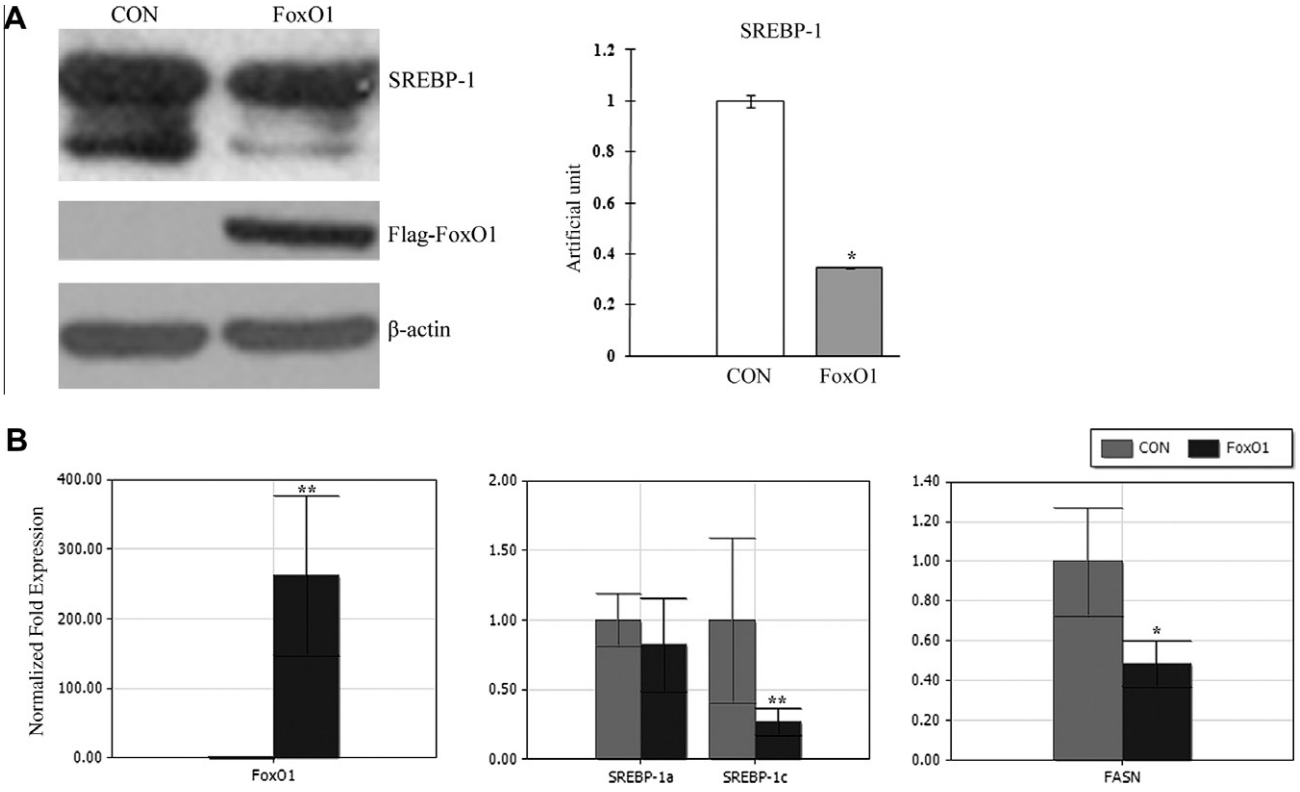

Fig. 1. FoxO1 down-regulates the expression of SREBP-1c and its target gene. HepG2 cells were transfected with pcDNA3-Flag-FoxO1-3A or control vector. 48 h later, total proteins were extracted and total RNA was isolated. (A) The protein level of SREBP-1, Flag-FoxO1-3A and actin was determined by Western blotting. (B) The mRNA level of SREBP-1a, 1c, FoxO1, and FASN was determined by real-time PCR and normalized to GAPDH level.$^{*}P < 0.05$, $^{**}P < 0.01$.