TR4 Inhibits LXR-mediated Decrease of Lipid Accumulation in 3T3-L1 Adipocytes

Hojung Choi and Eungseok Kim*
Department of Biological Sciences, Chonnam National University,
Gwangju 500-757, Korea

Abstract

TR4 has been suggested to play an important role in lipid metabolism in adipocytes. Although TR4 facilitates lipid accumulation during adipogenesis, the regulatory effect of TR4 on lipid storage in mature adipocytes remains unclear. We showed that TR4 inhibited the LXR agonist GW3965-mediated decrease of lipid accumulation in 3T3-L1 adipocytes. A reporter gene analysis revealed that TR4 suppressed LXRα transcriptional activity, although LXRα was unable to affect TR4 transcriptional activity. Moreover, adding TR4 resulted in reduced LXRα binding to the LXR responsive element in a gel shift assay. Additionally, the suppressive effect of GW3965 on perilipin expression and lipid accumulation in 3T3-L1 adipocytes was abolished by TR4 overexpression. Taken together, our data demonstrate that TR4 plays an inhibitory role in LXRα-mediated suppression of lipid accumulation in 3T3-L1 adipocytes. This TR4 protective effect is mediated, in part, by blocking the suppressive effect of GW3965 on perilipin gene expression.

Key words: Testicular orphan nuclear receptor 4, liver X receptor, adipocyte, perilipin, and lipid accumulation

Introduction

Adipocytes are the cells critical for energy homeostasis. Adipocytes store the extra lipids as fat during energy excess and mobilize them at the period of the energy deprivation. Dysregulation of lipid homeostasis in adipocytes by altered expression of genes involved in lipid metabolism is a major risk factor for the obesity and diabetes, hypertension, and coronary artery disease (Reaven et al., 1988). Epidemiological studies indicate that diets containing high proportion of saturated fatty acids, are also considered as a risk factor for development and progression of these diseases since high levels of saturated fatty acids cause insulin resistance and consequently increase the risk of these diseases (Hunnicutt et al., 1994). Thus, fatty acid profile in dietary fat including milk fat is also considered important.

Testicular orphan nuclear receptor 4 (TR4; Nr2c2), a member of the nuclear receptor superfamily, is able to regulate the expression of target genes through binding to direct repeats (DRs) of AGGTCA core motifs with variable numbers of spacer nucleotides (Kim et al., 2003; Lee et al., 1998a; Lee et al., 1995; Lee et al., 1997; Lee et al., 1999). Several studies suggested that TR4 may play key roles in glucose and lipid metabolism (Liu et al., 2007). Recently, we found that knockdown of TR4 in 3T3-L1 adipocytes resulted in decrease of intracellular lipid accumulation via downregulation of fatty acid transport protein 1 and perilipin genes. In addition, we also reported that TR4-deficient mice showed reduced fat mass and lipid deposition in the liver with decreased expression of hepatic stearoyl-CoA desaturase 1 (SCD1) (Kim et al., 2011a). SCD1 is the rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids (Kim et al., 2011b). SCD1 transgene in goats alters milk fatty acid composition to less saturated fatty acid content (Zidi et al., 2010).

The liver X receptor (LXR) is initially identified in the liver and has a critical role in foam cell formation through regulation of genes involved in reverse cholesterol transport (Ory, 2004; Willy et al., 1995). In the liver, LXR facilitates conversion of cholesterol to bile acids by induction of cholesterol 7α-hydroxylase (Ory, 2004). LXR also regulates genes required for lipid metabolism such as sterol-regulatory element binding protein 1c, fatty acid synthase, lipoprotein lipase, and SCD1. Given the

*Corresponding author: Eungseok Kim, Department of Biological Sciences, Chonnam National University, Gwangju 500-757, Korea. Tel: 82-62-530-3402, Fax: 82-62-530-3409, E-mail: ekim@chonnam.ac.kr
established role of LXR in lipid metabolism in the liver. LXR is considered to play a role in adipogenesis. However, conflicting results about the role of LXR in adipogenesis have been reported. Several studies have reported that LXR is significantly induced in the early phase of differentiation and participates in adipogenesis (Seo et al., 2004; Ulven et al., 2005). In contrast, a recent study shows that LXR decreases fat storage in the mature adipocytes in part by increase of basal lipolysis (Stenson et al., 2011). LXR forms a heterodimer with retinoid X receptor (RXR) and regulates expression of target genes via direct binding to DR4 response elements located in the target gene promoters (Apfel et al., 1994). Recently, LXR and thyroid receptor have shown to regulate carbohydrate responsive element-binding protein gene expression through competition for DR4 located in the promoter of carbohydrate responsive element-binding protein gene (Hashimoto et al., 2009). TR4 also participates in other nuclear receptor signaling pathways through DNA binding competitions to their target genes or protein-protein interaction (Lee et al., 1998b; Shyr et al., 2002), strongly suggesting that TR4 and other nuclear receptors such as LXR may construct the regulatory network for lipid homeostasis in adipocytes. Since both TR4 and LXR activate perilipin expression and have binding affinity for DR4 response elements, it will be interesting to study the possible cross-talk between TR4 and LXR to understand the regulatory network for lipid homeostasis in adipocytes. Here, we demonstrate that TR4 suppresses LXRα-mediated inhibition of lipid accumulation in 3T3-L1 adipocytes through competitive regulation of perilipin gene expression. Taken together, cross-talk between TR4 and LXR forms a regulatory network to maintain lipid homeostasis which is important for adipocyte biology.

Materials and Methods

Cell cultures and adipocyte differentiation

Cells were maintained in DMEM medium supplemented with 10% fetal bovine serum (HEK293T) or 10% newborn calf serum (3T3-L1 and NIH-3T3). 3T3-L1 cells stably transfected with pcDNA3, pcDNA3-TR4, pcDNA3TR4α, pcDNA3TR4α, or pcDNA3 TR4α were maintained in DMEM containing 800 μg/mL Geneticin or 10 μg/mL Blastidacin. Two days after confluence (designated day 0), 3T3-L1 cells were differentiated using 10% FBS DMEM containing 5 μg/mL insulin, 0.25 mM 3-isobutyl-1-methylxanthine (IBMX), and 0.25 μM dexamethasone. Seventy-two hour later, the medium was replaced by adipocyte medium containing 5 μg/mL insulin and 0.25 μM dexamethasone. Oil Red O staining was performed as previously described (Kim et al., 2010).

Plasmids and reporter gene assay

Plasmids pCMX-TR4, pCMX-LXRα, pCMX-RXRα, pCMX-VP16-RXRα, pCMX-VP16-TR4, pG5-Luc, TR4RE-Luc, and LXRE-Luc were described previously (Kim et al., 2003; Willy et al., 1995). pCMX-Gal4-LXRα was constructed by cloning of ligand binding domain amplified by PCR from pCMX-LXRα. Transfections were performed by using SuperFect (Qiagen). Relative luciferase activity (fold) was expressed based on the induction relative to the transfection of empty vector (set as 1-fold) without agonist; the results are mean±standard deviation (SD) of three separate experiments.

Reverse transcriptase PCR (RT-PCR) and real-time PCR

For RT-PCR and real-time PCR quantification analysis of perilipin and SCD1 mRNA expression, total RNA was isolated from 3T3-L1 cells using RiboEX (Geneall), and cDNA was synthesized using MMLV-RTase and oligo (dT) primer (Promega). RT-PCR was performed in 3 individual samples per group and relative mRNA levels of each gene were expressed as the mean±SD of three individual experiments. Real-time PCR was performed using a Corbett Rotor-Gene 6000 (Qiagen) and relative quantification of PCR product was based on value differences between the target and 36B4 control using the delta-delta CT comparative method. Each group was analyzed in triplicate using total RNA pooled from 3 individual cells. Primer sequences for genes were: perilipin sense; 5'-CTCTGG-GAAACATCGAGAAA-3' and antisense; 5'-GATCCA- CATGGCCAGAGTG-3', SCD1 sense; 5'-CCTACGACA- AGAACATTCAAT-3' and antisense; 5'-CAGGAAACT- CAGAAGCCAGAG-3', SCD1 sense; 5'-GCCGCAAT- GCAGATGAG-3'.

Gel shift assay

Gel shift assay was performed as previously described (Kim et al., 2003). Briefly, TR4, LXRα, and RXRα were in vitro synthesized using a T7-quick coupled TNT reticulocyte lysate system (Promega). The following oligomers were used for gel shift assays: consensus DR1 (cDR1) (5'-GATCTCCCTAGGTCCAAAGGTCATTTG-3') and consensus DR4 (cDR4) (5'-GATCACCTCAGGTCATTTG-3').