Atherosclerosis 275 (2018) 246–255

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Overexpression of transforming growth factor β induced factor homeobox 1 represses *NPC1L1* and lowers markers of intestinal cholesterol absorption

Paolo Parini [a, b, 1], Tiffany A. Melhuish [c, 1], David Wotton [c], Lilian Larsson [a], Osman Ahmed [a, d], Mats Eriksson [b], Camilla Pramfalk [a, e, *]

[a] Division of Clinical Chemistry, Department of Laboratory Medicine Sweden
[b] Metabolism Unit, Department of Medicine, Karolinska Institutet, Stockholm, Sweden
[c] Department of Biochemistry and Molecular Genetics and Center for Cell Signaling, University of Virginia, Charlottesville, VA, USA
[d] Department of Biochemistry, Faculty of Medicine, Khartoum University, Khartoum, Sudan
[e] Department of Biosciences and Nutrition, NOVUM, Karolinska Institutet, Stockholm, Sweden

ARTICLE INFO

Article history:
Received 26 March 2018
Received in revised form
7 June 2018
Accepted 15 June 2018
Available online 19 June 2018

Keywords:
Cholesterol metabolism
Lipoproteins
Liver
Transcription
Dyslipidemia

ABSTRACT

Background and aims: Transforming growth factor β induced factor homeobox 1 (TGIF1) is a transcriptional repressor that limits the response to transforming growth factor ß signaling and also represses transcription independent of this pathway. Recently, we found higher serum cholesterol levels and more hepatic lipid accumulation in mice lacking *Tgif1*, and showed that TGIF1 can repress the expression of *Soat2,* the gene encoding the cholesterol esterifying enzyme acyl-Coenzyme A:cholesterol acyltransferase 2. Although there is evidence that TGIF1 plays a role in lipid metabolism, its role in this metabolic pathway is not fully characterized. Here we investigate whether overexpression of TGIF1 affects intestinal cholesterol absorption.

Methods and results: TGIF1 was found to repress human and mouse Niemann-Pick C1 like 1 (*Npc1l1*) promoter activity in intestinal Caco2 cells. We also found TGIF1 to be able to oppose the induction of the promoter activity by sterol regulatory element binding protein 2 and hepatocyte nuclear factor 1α and 4α. To validate these effects of TGIF1 *in vivo*, we generated transgenic mice specifically overexpressing TGIF1 in the intestine (*Villin-Tgif1*). We observed lower intestinal expression levels of *Npc1l1* that was associated with lower expression of ATP-binding cassette transporter (*Abc)* a1, *Abcg5*, and *Abcg8*. *Villin-Tgif1* mice fed regular chow or a high-fat diet had lower levels of markers of intestinal cholesterol absorption than wild types.

Conclusions: We suggest TGIF1 as a new player in intestinal cholesterol metabolism.

1. Introduction

Transforming growth factor β induced factor homeobox 1 (TGIF1) was named TG-interacting factor 1 when discovered. TGIF1 is commonly known as a transcriptional repressor which limits the transcriptional output of transforming growth factor β-signaling (TGFβ) [1–3]. TGIF1 can be recruited to DNA via interaction with TGFβ-activated Smad transcription factors, or can repress transcription independent of TGFβ. TGIF1 exerts repression of target genes by binding to its cognate binding site or to binding sites for retinoid X receptor α (RXRα) [4,5]. RXRα is a heterodimeric partner of several nuclear receptors (e.g. peroxisome proliferator-activated receptor α and liver X receptor α; LXRα). Hence, TGIF1 may

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ACAT2, acyl-Coenzyme A:cholesterol acyltransferase 2; apo, apolipoprotein; CYP7A1, cholesterol 7α-hydroxylase; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; HNF, hepatocyte nuclear factor; LDL, low density lipoprotein; LXR, liver X receptor; NEFA, nonesterified fatty acid; NPC1L1, Niemann-Pick C1 like 1; PCSK9, proprotein convertase subtilisin/kexin type 9; RXR, retinoid X receptor; SREBP, sterol regulatory element binding protein; TGFβ, transforming growth factor β; TGIF1, transforming growth factor β induced factor homeobox 1; TICE, transintestinal cholesterol efflux.

* Corresponding author. Division of Clinical Chemistry, Department of Laboratory Medicine, C1-74, Karolinska Institutet at Karolinska University Hospital Huddinge, S-141 86, Stockholm, Sweden.
E-mail address: camilla.pramfalk@ki.se (C. Pramfalk).

[1] P Parini and TA Melhuish contributed equally to this study.

https://doi.org/10.1016/j.atherosclerosis.2018.06.867
0021-9150/

modulate other nuclear receptor responses, and we have previously shown that TGIF1 can repress some LXRα-regulated genes [6].

Although we have previously shown strong evidence that TGIF1 plays a role in regulating lipid metabolism, its roles is not fully characterized. We previously showed that knock-down of TGIF1 in human hepatoma HepG2 cells increased the expression of the two LXRα target genes, the ATP-binding cassette transporter A1 (*ABCA1*) and sterol regulatory element binding protein 1c (*SREBF1*) genes [6]. We also found mice lacking *Tgif1* to have higher hepatic apolipoprotein (apo) c2 and a4 (*Apoc2* and *Apoa4*) expression levels, two other *Lxrα* target genes, compared to wild type mice [6]. In a second study, we identified TGIF1 to function as a transcriptional repressor of the gene Sterol O-acyltransferase 2 (*Soat2)* that encodes for the cholesterol esterifying enzyme acyl-Coenzyme A:cholesterol acyltransferase 2 (ACAT2) [7]. TGIF1 was also found to be able to oppose the induction of the promoter activity of *SOAT2* by hepatocyte nuclear factor (HNF) 1α and 4α in hepatic and intestinal cells. Moreover, *Tgif1−/−* mice have more hepatic lipid accumulation and higher serum cholesterol levels [7].

Niemann-Pick C1 like 1 (NPC1L1) localizes to the brush border membrane of enterocytes and mediates intestinal cholesterol absorption [8]. Sterols transported by NPC1L1 are selectively esterified by ACAT2 to generate cholesteryl esters, which can be packed into chylomicrons and secreted to lymph. Mice lacking *Npc1l1* have more than 70% lower intestinal cholesterol absorption but serum cholesterol levels do not differ compared to controls due to a compensatory higher cholesterol synthesis [8–10]. In humans, heterozygous carriers of *NPC1L1* inactivating mutations were recently reported to have ~0.3 mmol/L lower low density lipoprotein (LDL) cholesterol concentrations, which was associated with a more than 50% relative reduced risk of coronary heart disease compared to noncarriers [11]. NPC1L1 is a target for the drug ezetimibe, which is used to treat patients with elevated serum cholesterol levels [8,9]. Treatment with ezetimibe as monotherapy lowers serum LDL cholesterol levels by ~17% [12,13], and ezetimibe in combination therapy with cholesterol synthesis inhibitors (i.e., statins) leads to additional reductions of LDL cholesterol [14,15] and decreased mortality in patients with acute coronary syndrome [16].

As mentioned above, disruption of *Tgif1* in mice is associated with dyslipidemia, and here we investigate the effects of overexpression of TGIF1 on expression of the *Npc1l1* gene and markers of intestinal cholesterol absorption.

2. Materials and methods

2.1. Materials

Caco2 cells were purchased from American Type Culture Collection (Manassas, Virginia). The human *NPC1L1* (a ~1700 bp fragment, ranging from −1570 to +137 bp, cloned into pGL3 vector) [17] and the mouse *Npc1l1* promoter (a ~1400 bp fragment, ranging from −1405 to +24 bp, cloned into pGL3 vector) were generous gifts from Dr Charlotte Murphy and Dr Mats Gåfvels, Karolinska Institutet, Sweden. The human pCMV5 Flag TGIF1 vector was purchased from Addgene (www.addgene.org) and the human pCMV SREBP2 vector was from American Type Culture Collection (Manassas, Virginia). The HNF4α expression vector [18] was kindly donated by Dr Theodore C. Simon, Washington University School of Medicine, S:t Louis, MO and the HNF1α expression vector [19] was kindly donated by Professor Pal R. Njølstad and Dr Lise Bjørkhaug Gundersen, Haukeland University Hospital, Norway. Cell culturing and transfection reagents, as well as Trizol and SYBRGreen master mix were purchased from Thermo Fisher Scientific (Gothenburg, Sweden). pGL3 basic vector, reporter lysis buffer, β-galactosidase, and luciferase assay kits were purchased from Promega (Madison, WI, US). Omniscript reverse transcriptase kit was purchased from QIAGEN (Hilden, Germany). Specific primers were designed to span exon junctions using NCBI Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/); primer sequences are available upon request. Cholesterol and triglyceride reagents were purchased from Roche Diagnostics GmbH (Mannheim, Germany). Nonesterified fatty acid (NEFA) reagents were purchased from Wako Chemicals GmbH (Neuss, Germany) and the proprotein convertase subtilisin/kexin type 9 (PCSK9) ELISA kit was purchased from R&D systems (Minneapolis, MN, US). The NPC1L1 antibody was a generous gift from Dr Lawrence L. Rudel, Wake Forest School of Medicine, US and antibodies against TGIF1, ABCA1, ABCG5, ABCG8, and NPC1L1 were from Santa Cruz Biotechnology Inc. (Heidelberg, Germany) and Abcam (Cambridge, UK). All other chemicals were purchased from Sigma Aldrich (St. Louis, MO, US).

2.2. In vitro experiments

Caco2 cells were grown in DMEM supplemented with 20% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin; the media was replaced every second day. Cells were plated out on six-well tissue culture plates so that they reached ~70% confluence on the day of transfection. The AliBaba 2.1 and JASPAR Core programs were used to search for putative binding sites in the mouse and human *NPC1L1* promoter sequences and specific mutations were generated [20]. Transfections were performed according to the manufacturer's instructions (ratio of 1.5:1, Lipofectamine LTX reagent:DNA). pGL3 basic vector was used to adjust for differences in amount of DNA added to the cells. Twenty-four hours post-transfection, cell lysates were prepared in reporter lysis buffer. β-galactosidase and luciferase activities were determined using β-galactosidase and luciferase assay kits, respectively, according to the manufacturer's instructions. Each experiment was performed in three replicates and repeated at least twice.

2.3. Chromatin immunoprecipitation (ChIP assay) in Caco2 cells

ChIP assay was performed using Caco2 essentially as previously described [20]. In brief, chromatin was immunoprecipitated with anti-TGIF1 (sc-17800×) or anti-IgG (sc-2027). Primers were designed to target the region −850 to −500 bp upstream of the ATG transcription site in the human *NPC1L1* promoter and relative binding was determined.

2.4. Animals

To generate intestine-specific TGIF1 overexpression, we used the well characterized mouse *Villin* promoter to drive expression of a human *TGIF1* transgene. The *TGIF1* coding sequence was amplified by PCR, and inserted into the 12.4 kb Villin-deltaATG vector (in a pUC18 backbone) (Addgene plasmid number 19358), a gift from Dr Deborah Gumucio [21], using XhoI and Asp718 sites. Following verification by sequencing, the transgene fragment was released by digestion with PmeI, and transgenics were generated by standard procedures at the University of Virginia GEMM core using B6SJL mice. Germ-line transmission was verified by PCR and expression of the transgene was verified by Western blot. Male mice were either maintained on a regular chow diet or fed a high-fat diet (21% fatty acids, 0.05% cholesterol (w/w), and 0.05% β-sitosterol (w/w)) for eight weeks, starting at 12 weeks of age. All mice were sacrificed at ~20 weeks of age, and tissues and serum collected as described [7]. The study was approved by the Animal Care and Use Committee of the University of Virginia.