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Regulation of the human lipoprotein lipase gene by the forkhead box transcription factor FOXA2/HNF-3β in hepatic cells

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ABSTRACT

Lipoprotein lipase (LPL) plays a major role in the hydrolysis of triglycerides (TG) from circulating TG-rich lipoproteins. The role of LPL in the liver has been controversial but recent studies in mice with liver LPL overexpression or deficiency have revealed important new roles of the enzyme in glucose and lipid metabolism. The objective of this study was to identify regulatory elements and factors that control the transcription of the human LPL gene in hepatocytes. Deletion analysis of the human LPL promoter revealed that the proximal region which harbors a binding site for the forkhead box transcription factor FOXA2/HNF-3β at position −47/−40 is important for its hepatic cell activity. Silencing of FOXA2 in HepG2 cells reduced the LPL mRNA and protein levels. Direct binding of FOXA2 to the novel binding site was established in vitro and ex vivo. Mutagenesis of the FOXA2 site reduced the basal activity and abolished the FOXA2-mediated transactivation of the LPL promoter. Insulin decreased LPL mRNA levels in HepG2 cells and this was associated with phosphorylation of AKT and nuclear export of FOXA2. In summary, the data of the present study combined with previous findings on the role of FOXA2 in HDL metabolism and gluconeogenesis, suggest that FOXA2 is a key regulator of lipid and glucose homeostasis in the adult liver. Understanding the mechanisms by which FOXA2 exerts its functions in hepatocytes may open the way to novel therapeutic strategies for patients with metabolic diseases such as dyslipidemia, diabetes and the metabolic syndrome.

1. Introduction

Lipoprotein lipase (LPL) plays an important role in lipoprotein metabolism by catalyzing the hydrolysis of triglycerides (TG) in plasma TG-rich lipoproteins including very low density lipoproteins (VLDL) and chylomicrons. Apolipoprotein CII (apoC-II) is a cofactor of this enzyme [1]. LPL is synthesized by various tissues such as the adipose tissue, skeletal muscle and heart but can be found at lower levels in many other tissues including macrophages, kidney, brain, adrenals, lung and embryonic liver [2–6]. Following its secretion, LPL is attached to endothelial cells via heparin sulfate proteoglycans (HSPGs) with the help of a protein called glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1 (GPIHBP1) [1,3]. It has been shown that LPL can act as a ligand of lipoprotein receptors such as the LDL receptor or the LDL receptor-related protein 1 (LRP1) suggesting that it could contribute to lipoprotein catabolism in the liver [7–9].

Mice with total LPL deficiency die immediately after birth from hypertriglyceridemia [10,11]. On the other hand, overexpression of LPL in mice was associated with higher LPL activity, decreased plasma TG and reduced atherosclerosis due to the reduction in lipoprotein remnants [12–14]. Previous studies have shown that deficiencies in LPL or apoC-II in humans are associated with hypertriglyceridemia, reduced levels of high density lipoprotein cholesterol (HDL), familial chylomicronemia or/and premature atherosclerosis [15–19]. Patients with familial LPL deficiency are characterized by very low or absence of LPL activity due to loss-of-function mutations in the LPL gene. Mutations in the homozygous or compound heterozygous form result in markedly decreased or absent LPL activity with consequent extremely low levels of HDL cholesterol, severe hypertriglyceridemia and pancreatitis. Heterozygous LPL deficiency usually results in normal to moderately elevated plasma triglyceride concentrations as a result of a decrease in the LPL activity but this can lead to more severe hypertriglyceridemia when there are other conditions like diabetes or high alcohol intake [20–26].

Members of the peroxisome proliferator activated receptor (PPAR) family of hormone nuclear receptors such as PPARα and PPARγ regulate the activity of the LPL promoter in response to fibrates, fatty acids or fasting [27–32]. The oxysterol receptor Liver X Receptor (LXR) was shown to bind to a responsive element in the first intron of the mouse LPL gene and to facilitate the induction of LPL gene in response to a

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high cholesterol diet or to a synthetic LXR agonist in the liver and macrophages, but not in muscle or adipose tissue where the expression of LPL is constitutively high [33].

In adult mice LPL gene expression in the liver is low compared with other tissues [34]. However, hepatic LPL was recently shown to play an important physiological role in plasma lipid homeostasis in adult mice because ablation of hepatic LPL decreased significantly plasma LPL content and elevated plasma TG and cholesterol levels [34].

Forkhead box A2 (FOXA2)/Hepatocyte Nuclear Factor 3β (HNF3β) belongs to the forkhead box family of liver transcription factors that includes also FOXA1 and FOXA3 (or HNF3α, HNF3γ) [35]. These factors contain a conserved DNA binding domain and bind as monomers to DNA elements having homology with the consensus sequence 5′-T(G/A)TTT(A/G)(C/T)T-3′ [36,37]. FOXA2 is expressed at high levels in the liver and at lower levels in other tissues [38]. Mice with total ablation of FOXA2 die early during embryogenesis due to developmental defects [39,40].

FOXA2 plays a critical role in glucose homeostasis in the liver, by controlling the expression of gluconeogenesis genes in response to insulin, including phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) [41–43]. During fasting, liver FOXA2 enhances the catabolism of fatty acids and the secretion of VLDL and HDL by activating genes involved in these pathways [44–47]. Furthermore, liver-specific FOXA2 ablation in mice revealed that FOXA2 plays an important role in bile acid homeostasis because it regulates the expression of genes of bile acid metabolism such as bile acid transporters [48]. Mice with FOXA2 haploinsufficiency or with hyperinsulinemia caused by mutations in leptin signaling exhibited decreased pre-β HDL levels in the plasma and this has been correlated with low levels of expression of the apolipoprotein M (apoM) gene which is a FOXA2 target [45,49]. In a previous study we had shown that FOXA2 inhibits the expression of the cholesterol and phospholipid transporter ATP binding cassette transporter 1 (ABCA1) in hepatic cells thus regulating HDL biogenesis [50].

Several studies have used genome wide analyses (ChIP-Seq, ChIP-chip) in liver-derived cell lines and liver samples from wild type or hepatocyte-specific FOXA2 deficient mice and identified many FOXA2 binding sites within the promoters or introns of genes involved in lipid and triglyceride metabolism [37,48,51–53]. Furthermore, gene chip expression analysis of FOXA2-induced genes in livers from mice expressing constitutively active FOXA2 showed increased expression levels of genes involved in β-oxidation, ketogenesis, and TG metabolism including the LPL gene [44].

The objective of this study was to identify cis-acting DNA elements and transcription factors that control the expression of the human LPL gene in hepatocytes.

2. Materials and methods

Materials and a more detailed description of methodologies used in this study can be found in the Supplement.

2.1. Plasmid constructions

The human LPL promoter plasmids (−883/+39) LPL-Luc, (−669/+39) LPL-Luc, (−466/+39) LPL-Luc, (−262/+39) LPL-Luc, (−109/+39) LPL-Luc and (−28/+39) LPL-luc were generated by PCR amplification and cloned at the *Xho*I-*Hin*dIII sites of pBluescript vector bearing the luciferase gene. The (−883/+39) mut LPL-luc and (−109/+39) mut LPL-luc constructs which bear mutations in the FOXA2 binding site of the LPL promoter were generated by site-directed mutagenesis using the QuickChange Site-Directed Mutagenesis Kit. All oligonucleotides used as primers in PCR amplification or in mutagenesis were synthesized at the microchemical facility of the Institute of Molecular Biology and Biotechnology (IMBB) (Heraklion, Greece), and their sequence is shown in Table 1. The expression vector CMV-rFOXA2 was described previously [50].

2.2. Cell culture and treatments

Human hepatoma HepG2 cells and human embryonic kidney cells (HEK293T) were cultured in Dulbecco's modified Eagle's medium (DMEM high glucose) supplemented with 10% fetal bovine serum (FBS), L-glutamine, and penicillin/streptomycin at 37 °C in a 5% CO_2 atmosphere. For the treatment of HepG2 cells with insulin, cells were plated in DMEM low glucose + Glutamax supplemented with 10% fetal bovine serum and penicillin/streptomycin, serum-starved for 18 h with DMEM low glucose + Glutamax supplemented with penicillin/streptomycin and stimulated with insulin for 24 h at a final concentration of 500 nM. Mouse 3T3-L1 pre-adipocytes were grown in DMEM (high glucose) supplemented with 10% newborn calf serum (NBCS), L-glutamine, 1% sodium pyruvate and penicillin/streptomycin (Normal Growth Medium) at 37 °C in a 5% CO_2 atmosphere. For adipocyte differentiation, 3T3-L1 cells were maintained for 1 day in normal growth medium and then switched for 2 days to differentiation medium, a mixture of DMEM supplemented with 10% FBS, 1% sodium pyruvate, penicillin/streptomycin, 0.17 μM insulin, 0.25 μM dexamethasone and 0.5 mM isobutylmethylxanthine (IBMX). After this time cells were cultivated in differentiation medium without the inducers dexamethasone and IBMX for 2–3 days. About 2 days after removal of dexamethasone and IBMX, adipocyte colonies began to be visible as regions containing rounded cells with numerous intracellular lipid droplets. Then the medium of cells was switched again to the differentiation medium supplemented with the inducers (dexamethasone and IBMX) for 2 more days. The cells were maintained for 1 day in differentiation medium without the inducers and the next day cells were harvested.

2.3. Transient transfections, siRNA silencing and reporter assays

Transient transfections in HepG2 and HEK293T cells were performed using the calcium phosphate [$Ca_3(PO_4)_2$] co-precipitation method. Lipofectamine 2000 reagent was used in HepG2 cells that were transfected with FOXA2 expression vector for protein and RNA extraction, according to the manufacturer's instructions. Transient transfections in 3T3-L1 cells and insulin treated HepG2 cells were performed using Lipofectamine 2000. HepG2 and HEK293T cells were treated with scrambled siRNA or siRNA against FOXA2 for 40 h using Lipofectamine RNAiMAX according to the manufacturer's instructions. The silencing efficiency of FOXA2 was confirmed by western blotting or/and qPCR. Luciferase assays were performed 40 h post transfections using the luciferase assay kit from Promega Corp. Normalization for transfection efficiency was performed by β-galactosidase assays.

2.4. RNA isolation, reverse transcription, PCR and quantitative PCR (qPCR)

Total RNA was prepared from HepG2, HEK293T or 3T3-L1 cells using Trizol reagent according to the manufacturer's instructions. All oligonucleotide sequences used as primers in qPCR or PCR experiments are shown in Table 1.

2.5. Chromatin Immunoprecipitation (ChIP) and DNA Affinity Precipitation (DNAP) assays

Chromatin Immunoprecipitation and DNA Affinity Precipitation Assays were performed as described previously [50]. The oligonucleotides used as primers in these assays are shown in Table 1.

2.6. Statistical analysis

Results are expressed as mean ± S.D. Statistical significance was determined using two-tailed Student's *t*-test. Differences with $p < 0.05$