PPARγ Regulates Expression of Carbohydrate Sulfotransferase 11 (CHST11/C4ST1), a Regulator of LPL Cell Surface Binding

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Abstract

The transcription factor PPARγ is the key regulator of adipocyte differentiation, function and maintenance, and the cellular target of the insulin-sensitizing thiazolidinediones. Identification and functional characterization of genes regulated by PPARγ will therefore lead to a better understanding of adipocyte biology and may also contribute to the development of new anti-diabetic drugs. Here, we report carbohydrate sulfotransferase 11 (CHST11/C4ST1) as a novel PPARγ target gene. CHST11 can sulphate chondroitin, a major glycosaminoglycan involved in development and disease. The CHST11 gene contains two functional intronic PPARγ binding sites, and is up-regulated at the mRNA and protein level during 3T3-L1 adipogenesis. CHST11 knockdown reduced intracellular lipid accumulation in mature adipocytes, which is due to a lowered activity of lipoprotein lipase, which may associate with the adipocyte cell surface through CHST11-mediated sulfation of chondroitin, rather than impaired adipogenesis. Besides directly inducing Lpl expression, PPARγ may therefore control lipid accumulation by elevating the levels of CHST11-mediated proteoglycan sulfation and thereby increasing the binding capacity for Lpl on the adipocyte cell surface.

Introduction

The close connections between obesity and its complications, such as type 2 diabetes and cardiovascular diseases, has firmly established white adipose tissue as a key regulator of whole body glucose and lipid metabolism [1]. White adipose tissue mainly regulates metabolism through storage of lipids (as triglycerides) and the secretion of so-called adipokines, which function in an endocrine or paracrine fashion. Several independent lines of research have firmly established the transcription factor peroxisome proliferator activator γ (PPARγ) as the master regulator of adipocyte differentiation, maintenance and function. For example, in vitro differentiation of fibroblasts into mature white adipocytes can be induced by introduction of PPARγ [2]. In addition, this protein directly regulates a large set of “adipocyte genes” involved in lipid and glucose metabolism [3,4]. Furthermore, PPARγ-/- mice are severely lipodystrophic, while PPAR γ-/- mice have reduced amounts of adipose tissue [5,6,7,8]. PPARγ is also essential for the maintenance of adipose tissue, since conditional knockout of the Pparγ gene resulted in reduced in vivo survival of mature adipocytes [9]. Finally, human Familial partial lipodystrophy subtype 3 (FPLD3, MIM 604367) patients, harbouring heterozygous mutations in the PPARγ gene, are characterized by aberrant fat distribution and metabolic disturbances, including insulin resistance and dyslipidemia [10].

PPARγ activity can be stimulated by thiazolidinediones (TZDs), a class of anti-diabetic drugs that includes rosiglitazone [11]. Since elevated levels of serum free fatty acids promote insulin resistance [12], an important potential mechanism for the beneficial effects of TZDs is therefore the net partitioning of lipids in adipose tissue. This may partly be explained by the PPARγ-mediated stimulatory effect of TZDs on adipocyte differentiation, resulting in increased lipid storage capacity in adipose tissue. In addition, PPARγ also directly regulates genes involved in all different aspects of lipid handling, such as lipid uptake (e.g. lipoprotein lipase [Lpl] [13]), intracellular lipid transport (e.g. fatty acid binding protein 4 [Fabp4] [14]) and lipid storage (e.g. perilipin [15]), as well as anti-lipolytic genes (e.g. GPR81 [16]). While PPARγ is clearly a suitable pharmacological target, TZD use has unfortunately been linked to adverse side effects such as undesired weight gain, fluid retention, peripheral oedema, and potential increased risk of cardiac failure [11,17]. Interestingly, recent findings indicate that a more restricted modulation of PPARγ activity may provide a new way of improving insulin sensitivity. A clear example of this is the recently identified phosphorylation site at serine 273 in PPARγ, CDK5-mediated phosphorylation of serine 273 in PPARγ leads to deregulation of a subset of genes whose expression is altered in obesity including the insulin-sensitizing adipokine, adiponectin [18]. Interestingly, S273 phosphorylation is blocked in vivo and in vitro by TZDs, but also by certain antidiabetic drugs that are weak PPARγ agonists or non-agonists [18,19]. These findings indicate that a comprehensive view on the mechanisms regulating
PPARγ activity as well as its downstream target genes is required to develop the next generation of PPARγ-based antidiabetic drugs. In the past few years, several genome wide PPARγ binding profiles have been generated in adipocytes, using either ChIP-ChIP [4,20,21], ChIP-seq [3,22] or ChIP-PET technology [23]. These global approaches have provided important new concepts, like the extensive crosstalk between PPARγ and C/EBPs as deduced from the overlap in their cistromes [3,4]. The binding profiles have also given important information on the single gene level, i.e. the identification of novel PPARγ target genes involved in lipid and glucose metabolism (e.g. Agpat2 and Hk2, respectively, [3]). Furthermore, genome wide binding profiles have helped to elucidate complex gene regulatory mechanisms, as exemplified by the genomic GPR31-GPR109A and UCP3-UCP2 regions, where single PPREs regulate multiple genes [16,24].

Using the PPARγ-RXRα ChIPseq profile by Nielsen et al. [3] as a starting point, we identified the mouse chondroitin-4-sulfate-transferase 1 gene (GAST1/Chst11) as a novel target of PPARγ. Chst11 is a Golgi-bound enzyme that catalyses the transfer of sulphate groups to the 4-O position of chondroitin sulphate (CS) and dermatan sulphate (DS). Membrane-bound sulphated proteoglycans are necessary for lipid accumulation in adipocytes, possibly because of their ability to interact with lipases like Lpl [25,26]. We found Chst11 mRNA and protein expression to be upregulated by PPARγ during 3T3-L1 adipogenesis and identified two functional intronic PPARγ binding sites in the Chst11 gene. In common with disruption of Lpl function [27], siRNA-mediated knock down of Chst11 resulted in reduced intracellular lipid accumulation in mature 3T3-L1 adipocytes. This effect is probably not due to inhibition of adipogenesis, as the expression of typical adipogenic genes such as C/EBPα, Fabp4, Lpl and adiponectin (Adipoq) was unaffected. Rather, knockdown of Chst11 inhibited the activity of Lpl. These findings suggest that PPARγ may regulate Lpl-mediated lipid accumulation by two different mechanisms: it increases Lpl transcription directly [13], but can also indirectly regulate activity of the Lpl protein by elevating Chst11 expression and thereby increasing the number of docking sites for Lpl on the adipocyte cell surface.

Cell Culture, Differentiation and Reporter Assays

The human osteosarcoma cell line U2OS (ATCC, Manassas, VA) was maintained in DMEM Glutamax (Dulbecco) containing 10% foetal calf serum (Life Technologies, Inc., Rockville, MD), penicillin and streptomycin (both 100 μg/ml; Life Technologies). The murine 3T3-L1 cell line (ATCC, Manassas, VA) was cultured in the same media but with 10% bovine serum (Life Technologies) and penicillin and streptomycin (both 100 μl/ml; Life Technologies). For differentiation, 3T3-L1 cells were grown to confluence and after 2 days incubated with culture medium containing dexamethasone (250 nM), 3-isobutyl-1-methylxanthine (500 μM) and insulin (170 nM) for 2 days. On day 3, medium was changed for culture medium supplemented with insulin (170 nM) and left for a week. Subsequently, cells were stained with Oil-red-O, or lysed and subjected to Western blot analysis as described before at day 5 after differentiation [16,29,30]. Western blot analyses, differentiated 3T3-L1 cells were lysed in RIPA lysis buffer (200 mM Tris-HCl, pH 8.0; 0.1% SDS, 1% Triton X-100; 10 mM EDTA; 150 mM NaCl; 1% sodium deoxycholate containing protease inhibitors). Cell lysates were subjected to SDS-PAGE, and transferred to Immobilon membranes (Millipore). ECL Plus (PerkinElmer Life Sciences) was used for detection on an ImageQuant LAS 4000 (GE Lifesciences).

Reporter assays were performed essentially as described before [29,30]. In short, cells were transfected in 24-well plates with 1 μg reporter plasmid, 10 ng PPARγ expression construct, and 2 ng pCMV-Remila reporter plasmid (Promega). The next day, cells were washed twice with PBS and subsequently maintained in medium in absence or presence of rosiglitazone for 24 h. After incubation, cells were washed twice with PBS and harvested in passive lysis buffer (Promega) and assayed for luciferase activity according to the manufacturer’s protocol (Promega Dual-Luciferase Reporter Assay System) and for Remila luciferase activity to correct for transfection efficiency. The relative light units were measured by a CentroLB 960 luminometer (Berthold Technologies, Bad Wildbad, Germany).

Chromatin Immunoprecipitation (ChIP)

Chromatin immunoprecipitation (ChIP) was performed exactly as described before [30]. Quantitative PCR was performed with primers against mouse Fabp4 PPRE (5′-GAGGACGCAAATGGTGTTCTCCAGA-3′; 5′-TTGGGCTGACTGACCTCCAC-3′), Chst11 peak 1 (5′-ACAGGCGTGGCTTGGCAC-3′; 5′-ACAGTCATCTACCTGGCATC-3′), Chst11 peak 2 (5′-CTCATCCAACTGTTTGTGG-3′; 5′-GATTTCTGAAGCTTGAAGACCTATG-3′) and the mouse beta globin gene as control (5′-GCTGCCCTCCTCTATCCGTGTC-3′; 5′-GCAAATGTTGGTGGCAGAAG-3′).

siRNA Transfection

3T3-L1 cells were transfected with siRNA oligonucleotides as described before [30] using Lipofectamine RNAiMax (Invitrogen) according to the manufacturer’s protocol. The siRNA oligonucleotides used were siControl (D-0010610-10, Dharmacon), siPPARγ (L-040712-00-0010, Dharmacon), siChst11 (J-040396-11-0020, Dharmacon), siLpl (L-042649-01-0005, Dharmacon).

RNA Isolation and qPCR

3T3-L1 fibroblasts were differentiated as described above. Four independent samples of total RNA were extracted at different time points using TRIzol reagent (Invitrogen). cDNA was synthesized using the superscript first strand synthesis system (Invitrogen) according to manufacturer’s protocol. Gene expression levels were
Figure 1. Chst11 is a novel PPARγ target gene. (A) Chip-Seq data of PPARγ and RXRα occupancy on the Chst11 gene according to Nielsen et al. [3]. UCSC Genome Browser tracks at day 0, 1, 2, 4 and 6 of differentiation are shown. Please note differences in y-axes. Two intronic PPARγ-RXRα binding sites were designated site 1 and 2. (B) ChIP-PCR on 3T3-L1 preadipocytes and adipocytes. Chromatin was prepared on day 0 and day 6 of differentiation and subjected to immunoprecipitation using antibodies against PPARγ. Enriched DNA was analysed using quantitative PCR with primers located at site 1 and the Chst11 gene (dark grey and black bars, respectively). As a positive control, primers located at the Fabp4 PPREs (~5500 bp from transcription start site; light gray bars) were used, primers located in the globin locus were used as a negative control (white bars). Results are shown relative to normalized ChIP recovery data of day 0 and results are representative of at least 3 independent experiments. (C and D) U2OS cells were cotransfected with a reporter construct (Tk-pGL3) containing Chst11 site 1 or site 2 sequences, or the parental reporter construct, together with empty (pCDNA) or PPARγ encoding expression vectors. Activation of the luciferase reporter in the absence or presence of 1 μM rosiglitazone is expressed as fold induction over that with empty reporter cotransfected with pCDNA in the absence of rosiglitazone after normalisation for Renilla luciferase activity. The error bars display SEM and significance is shown by the asterisks (p<0.05), n = 3 (E) Chst11 mRNA expression in 3T3-L1 adipocytes that had been treated with or without PPARγ siRNA from the start of differentiation and analyzed at day 5. Relative mRNA expression levels were related to control siRNA treated cells and normalized for the TFIIb reference gene. The error bars display SEM and significance is shown by the asterisks (p<0.05), n = 3 (F) Chst11 mRNA expression in 3T3-L1 adipocytes and the effect of rosiglitazone treatment (1 μM, 24 h). Relative mRNA expression levels were normalized for the TFIIb reference gene. (G) As in panel F, but after treatment with the PPARγ antagonist GW9662 (10 μM, 24 h).

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determined by quantitative real time PCR with the MvIq cycler (Bio-Rad) using SYBR-green (Bio-Rad) and normalized to TFIIb expression.

The primers used were as follows: murine TFIIb forward primer, 5’-TCTCTCTGCAGCGGCTTTC-3’, and reverse primer, 5’-CCCTGGTCGATGCCTGAT-3’, murine PPARγ forward primer, 5’-CCGTGATGCTGCTCTATGA-3’, and reverse primer, 5’-AGAGGCTCCACAGGCTTGATCC-3’, murine Chst11 forward primer, 5’-GTCCTCCTGCAAGCTCTA-3’, and reverse primer, 5’-CTCATCTGGTTGAGATGG-3’, murine Fapb4 forward primer, 5’-GGGAGACGAGGAGGT-3’, and reverse primer, 5’-TTCCATCCTCCATCGA-3’, murine Adipoq forward primer, 5’-GGGACTTGGTCAGGTGAT-3’, and reverse primer, 5’-TCTCCATGCTCTCTTCT-3’, murine Lpl forward primer, 5’-TTTGATGAAATGCCATGACAAG-3’, and reverse primer, 5’-TCAACCAACACAGGAGGT-3’, murine C/EBPα forward primer, 5’-AAAACACGCAACGTGAGA-3’, and reverse primer, 5’-GGGGCTATTTGTCATCTGGC-3’.

Immunofluorescence

For immunofluorescence staining, 3T3-L1 cells were plated on glass coverslips and differentiated for 5 days. Subsequently, cells were fixed with 4% paraformaldehyde (20’, RT) and permeabilized in PBS supplemented with 0.2% Triton for 5 minutes. After 30’ incubation in blocking buffer (2% BSA in PBS), cells were stained with primary antibodies for 2 h at room temperature, and then incubated with secondary fluorochrome-conjugated antibodies. After several washes, coverslips were incubated Nile-Red and Hoechst, washed with PBS, mounted in Mowiol and analysed with an LSM710 Met confoal microscope (Carl Zeiss, Jena, Germany).

Lpl Activity Assays

Lpl activity was measured according to the manufacturers instructions. In short, cells were grown and differentiated in 6-well plates. Media were removed and cells were incubated with 50 units of heparin in 500 ul of PBS (30’, 37°C). Supernatants were collected and debris was removed by centrifugation (12,000 rpm, 10’). Supernatants (25 μl) were removed by cassette buffer mix (175 μsassy buffer + z00 analysed on a Victor3 (Perkin Elmer) at wavelengths 355/460.

Results

Chst11 is a Novel PPARγ Target Gene

To identify novel PPARγ target genes we thoroughly analysed ChIPseq data generated by Nielsen et al. [3]. We focused on loci that displayed PPARγ/RXRα binding at day 6 with little or no binding of these transcription factors at day 0 of 3T3-L1 adipogenesis. Using these criteria we identified the carbohydrate sulfotransferase 11 Chst11, also known as Chst1, as a potential direct PPARγ target gene. Chst11 can sulphate chondroitin sulphate-proteoglycans (CSPG), which plays an important role in development and disease ([31,32]; see also Discussion). Two intronic binding sites for PPARγ were observed on day 2 of adipogenesis, with increased binding during later stages of adipogenesis (Fig. 1G). Taken together, these data classify Chst11 as a novel direct PPARγ target gene.

To corroborate these findings, we investigated whether modulation of endogenous PPARγ protein affected Chst11 expression. First, siRNA-mediated knock down of PPARγ was performed. PPARγ knock down resulted in a significant reduction of Chst11 mRNA expression in mature adipocytes (Fig. 1E). Furthermore, treatment of mature adipocytes with the PPARγ agonist rosiglitazone resulted in increased Chst11 levels (Fig. 1F), while incubation with the antagonist GW9662 lowered Chst11 expression (Fig. 1G). Taken together, these data classify Chst11 as a novel direct PPARγ target gene.
Chst11 is Expressed in Mature Adipocytes

Next, we examined Chst11 mRNA and protein expression in adipogenesis. Using quantitative RT-PCR, Chst11 mRNA levels were found to increase 8 fold and reached its climax at day 2 of differentiation after which the levels were stable (Fig. 2A). As a control, expression of PPARγ was analysed, which increased steadily over the course of differentiation (Fig. 2B). To investigate Chst11 protein expression and localization, immunofluorescent staining of Chst11 was combined with fluorescent staining for intracellular lipid droplets (Nile red) to mark differentiated cells. As shown in Fig. 2C, mature adipocytes (Nile Red positive) also stained positive for Chst11 protein, while Chst11 could not be detected in preadipocytes (Nile Red negative). Chst11 displayed cytoplasmic localization in mature adipocytes, in agreement with

Figure 2. Chst11 is expressed in mature 3T3-L1 adipocytes. (A) and (B): mRNA expression profiles of Chst11 and PPARγ in adipogenesis of 3T3-L1 cells during different days. Data are represented as described in Figure 1E. (C) Representative confocal microscopy images of 3T3-L1 (pre)adipocytes. Endogenous Chst11 (red) was visualized utilizing specific antibodies, differentiated cells were identified with Nile Red (green), Hoechst was used to visualize the nuclei (blue).

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PPARγ Regulates CHST11/C4ST1

A

Chst11

siControl
siChst11

Relative mRNA expression

B

siControl
siChst11

C

siControl
siChst11
siPPARγ

Chst11
Tubulin

C/EBPα

Lpl

Adipoq

Fabp4

Relative expression

PPARγ

n.s.

n.s.

n.s.

n.s.

n.s.

n.s.

n.s.

n.s.

n.s.

n.s.
Knockdown of Chst11 Leads to Decreased Intracellular Lipid Accumulation, but not Adipogenesis

Having established that PPARγ regulates Chst11 levels (Fig. 1) and that Chst11 is expressed on the mRNA and protein level in mature adipocytes (Fig. 2), we wished to investigate the role of Chst11 in adipocytes. To address this, siRNA-mediated knockdown was performed in 3T3-L1 cells, and cells were subsequently subjected to adipogenic culture conditions. siRNA-mediated knockdown reduced Chst11 mRNA and protein (Fig 3A). Chst11 knockdown led to decreased lipid accumulation, as assessed by Oil-red-O staining of intracellular lipids, a phenomenon also observed upon Lpl knock down (Fig. 3B) [27]. Reduced lipid staining may be the result of reduced adipogenesis or only reduced lipid accumulation. To distinguish between these possibilities the effect of Chst11 knock down on the expression of C/EBPζ and PPARγ, two key regulators of adipogenesis, was examined. Only marginal differences were observed between cells that had been treated with Chst11 siRNA oligonucleotides or scrambled siRNA oligonucleotides (Fig. 3C). PPARγ protein levels were also not affected dramatically by Chst11 knockdown. Expression of the genes encoding Lpl, adiponectin (Adipoq) and Fabp4, which are all up-regulated in adipogenesis, was also unaffected by Chst11 knockdown. Knock down of PPARγ, which has repeatedly been reported to block adipogenesis (e.g. [8] [34]), however significantly inhibited the expression of all these genes. These findings therefore indicate that Chst11 specifically regulates lipid accumulation rather than adipogenesis.

Knockdown of Chst11 Leads to Decreased Lpl Activity

Since Chst11 knock down reduced lipid accumulation (Fig. 3B) and given that Lpl is required for lipid accumulation in 3T3-L1 adipocytes (Fig. 3B) [27] we hypothesized that Chst11 activity may regulate Lpl activity in mature adipocytes. In favour of this hypothesis is the finding that Lpl requires a stable negatively charged docking site on HS PGs for binding to the cell surface of endothelial cells [35], which can be provided by Chst11-mediated sulfation of CS in adipocytes. To test this possibility, 3T3-L1 cells were differentiated and subjected to scrambled, PPARγ and Chst11 siRNA-mediated knock down, after which Lpl was released from the adipocyte cell surface by heparin treatment [36], and enzymatic activity was determined as a measure for cell-surface-associated Lpl. Upon knock down of Chst11 a significant reduction in Lpl activity was observed (Fig. 4; p<0.05). As a control, knock down of PPARγ, which regulates the expression of both Chst11 (current study) and Lpl [13], was performed. As expected, reduced PPARγ expression also resulted in impaired Lpl activity (Fig. 4). These data support a model in which PPARγ regulates Chst11 expression, leading to increased sulfated CS chains which can then form docking sites for Lpl, ultimately contributing to lipid accumulation in mature adipocytes.

Discussion

Multiple extracellular signalling pathways have been implicated in adipogenesis, ultimately leading to upregulation of the transcription factor PPARγ, the master regulator of adipogenesis [37]. PPARγ directly regulates the expression of genes involved in various aspects of lipid handling, including Lpl [38]. Here we have identified the enzyme Chst11, which harbours sulfotransferase activity, as a novel PPARγ target gene. Knockdown of Chst11 does not affect the adipogenic gene program, but rather inhibits lipid accumulation, possibly through inhibition of the cell surface binding of Lpl. These findings suggest that the central role of PPARγ in lipid metabolism in adipocytes extends beyond the regulation of genes directly involved in lipid handling, and includes genes like Chst11, which play a more indirect role. Chst11 can sulphate the 4-O position of GalNac residues in chondroitin sulphate (CS), one of the major classes of glycosaminoglycans (GAG) [39,40]. GAGs are sulphated repeating disaccharide units, which together with core proteins can form proteoglycans like heparan- and chondroitin sulphate proteoglycan (HSPG/CSPG), and function in cell-cell communication, adhesion, and protein presentation [41]. In addition, CSPG/HSPG are necessary for lipid accumulation in adipocytes [25].
Sulfation of GAGs by sulfotransferases like Chst11 adds negative charge to proteoglycans, which is important for interactions with various other proteins like growth factors, apolipoproteins, extracellular matrix, and plasma proteins [41]. Importantly, sulphated and thus negatively charged HSPG can function as a docking site for Lpl in endothelial cells [35,42]. Furthermore, macromolecules produce an oversulfated CSPG that can also bind Lpl [43], and CSPG is the dominant proteoglycan on the adipocyte cell surface [25,44]. When combined with our current data, these findings suggest that the main role of Chst11 in adipocytes is to increase the amount of sulphated, negatively charged CS chains that could then act as binding sites for Lpl activity on the adipocyte cell surface. Additional experiments are required to establish the sulphation status of CSPG, and its role in Lpl binding in adipocytes.

Lpl is best known for its role in endothelium, where secreted Lpl travels through the capillary walls towards the luminal space of the endothelium, and binds to the proteoglycans that are anchored into vascular endothelial cells [42,45,46,47]. The transport of Lpl from adipocytes and myocytes, which produce high Lpl levels, to the capillary walls has just recently been unravelled. The GPI-linked protein, which is present on both apical and basolateral surface of endothelial cells, is capable of transporting Lpl from basolateral to the apical surface of the cells [48]. At the luminal side, Lpl can help to cleave lipids off chylomicrons and VLDL particles, so that the fatty acids can enter through the capillaries towards the adipocytes where it can be stored. Lpl molecules that are internalized by endothelial cells are recycled back to the cell surface [26]. Although Lpl binds to the surface of adipocytes with 5–10 fold higher efficiency compared to endothelial cells [49], its role in adipocytes is less well-defined. Multiple studies have provided evidence that lipolysis of lipoproteins at the endothelium by Lpl can loosen the junctions between endothelial cells so that large lipid enriched lipoproteins can pass through them [45,50,51]. In this event, Lpl should also be present at the cell surface of adipocytes to deplete the lipoproteins of their lipids. Surface-bound Lpl activity, which may be positively regulated by sulfotransferases like Chst11, can therefore be regarded as a back-up system for efficient take-up of lipids from lipoproteins. As genetic inactivation of Chst11 is associated with severe developmental abnormalities [31], adipose tissue-specific Chst11 knock-out mice will need to be generated to establish the precise role for Chst11 in lipid accumulation in adipocytes in vivo.

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Author Contributions
Conceived and designed the experiments: IT RB EK. Performed the experiments: IT. Analyzed the data: IT RB EK. Wrote the paper: IT EK.

References
1. Rosen ED, Spiegelman BM (2006) Adipocytes as regulators of energy balance and glucose homeostasis. Nature 444: 417–433.
2. Tontonoz P, Hu E, Spiegelman BM (1994) Stimulation of adipogenesis in fibroblasts by PPAR γ2, a lipid-activated transcription factor. Cell 79: 1147–1156.
3. Nielsen R, Pyderen TA, Hagenheek D, Moules P, Siersbaek R, et al. (2008) Genome-wide profiling of PPAR[gamma]: RXR and RNA polymerase II occupancy reveals temporal activation of distinct metabolic pathways and changes in RXR dimer composition during adipogenesis. Genes Dev 22: 2953–2967.
4. Lefevere MI, Zhang Y, Steger DJ, Schupp M, Schag J, et al. (2008) PPAR[gamma] and C/EBP factors orchestrate adipocyte biology via adjacent binding on a genome-wide scale. Genes Dev 22: 2941–2952.
5. Barak Y, Nelson MC, Ong ES, Jones YZ, Ruiz-Lozano P, et al. (1999) PPARγ is required for placental, cardiac, and adipose tissue development. Mol Cell 4: 385–395.
6. Kubota Y, Terauchi Y, Miki H, Tamemoto H, Yamauchi T, et al. (1999) PPARγ gene is required for adipogenesis in adipocytes and macrophages. Mol Cell Biol 30: 2078–2089.
7. Nadra K, Quignodon L, Sardella C, Joye E, Mucciolo A, et al. (2010) Sulfation of GAGs by sulfotransferases like Chst11 in lipid accumulation in adipocytes genetic inactivation of Chst11 is associated with severe development abnormalities. Genes Dev 24: 2967.
8. Rosen ED, Spiegelman BM (2006) Adipocytes as regulators of energy balance and glucose homeostasis. Nature 444: 417–433.
29. Jeninga EH, van Beckum O, van Dijk AD, Hamers N, Hendriks-Steegman RL, et al. (2007) Impaired peroxisome proliferator-activated receptor gamma function through mutation of a conserved salt bridge (R425C) in familial partial lipodystrophy. Mol Endocrinol 21: 1049-1065.
30. van Beckum O, Breukman AB, Grontved L, Hamers N, van den Broek NJ, et al. (2008) The adipogenic acetyltransferase Tip60 targets activation function 1 of PPARgamma. Endocrinology 149: 1840-1849.
31. Kluppel M, Wight TN, Chan C, Hinek A, Wrana JL (2005) Maintenance of chondroitin sulfation balance by chondroitin-4-sulfotransferase 1 is required for chondrocyte development and growth factor signaling during cartilage morphogenesis. Development 132: 3989-4003.
32. Kluppel M (2010) The roles of chondroitin-4-sulfotransferase-1 in development and disease. Prog Mol Biol Transl Sci 93: 113–132.
33. Willis CM, Wrana JL, Kluppel M (2009) Identification and characterization of TGFbeta-dependent and -independent cis-regulatory modules in the C4ST-1/CHST11 locus. Genet Mol Res 8: 1231–1243.
34. Koppen A, Houtman R, Pijnenburg D, Jeninga EH, Ruijtenbeek R, et al. (2009) Nuclear receptor-coregulator interaction profiling identifies TRIP5 as a novel PPARgamma cofactor. Mol Cell Proteomics 8: 2212–2226.
35. Lookene A, Chevreuil O, Ostergaard P, Olivercona G (1996) Interaction of lipoprotein lipase with heparin fragments and with heparan sulfate: stoichiometry, stabilization, and kinetics. Biochemistry 35: 12153–12163.
36. Weinstein MM, Yin L, Beigneux AP, Davies BS, Gin P, et al. (2000) Abnormal patterns of lipoprotein lipase release into the plasma in GPPIHBP1-deficient mice. J Biol Chem 275: 34511–34518.
37. Rosen ED, MacDougald OA (2006) Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol 7: 885–896.
38. Lehrke M, Lazar MA (2005) The many faces of PPARgamma. Cell 123: 993–999.
39. Yamauchi S, Hirahara Y, Usui H, Takada Y, Hoshino M, et al. (1999) Purification and characterization of chondroitin 4-sulfotransferase from the culture medium of a rat chondrosarcoma cell line. J Biol Chem 274: 2456–2463.
40. Hirase A, Nakagawa H, Ong E, Akama TO, Fukuda MN, et al. (2000) Molecular cloning and expression of two distinct human chondroitin 4-O-sulfotransferases that belong to the HNK-1 sulfotransferase gene family. J Biol Chem 275: 20188–20196.
41. Bishop JR, Schuksz M, Eko JD (2007) Heparan sulphate proteoglycans fine-tune mammalian physiology. Nature 446: 1050–1057.
42. Mead JR, Irvine SA, Ramji DP (2002) Lipoprotein lipase: structure, function, regulation, and role in disease. J Mol Med (Berl) 80: 753–769.
43. Edwards IJ, Xu H, Obukuro JC, Goldberg IJ, Wagner WD (1995) Differentiated macrophages synthesize a heparan sulfate proteoglycan and an oversulfated chondroitin sulfate proteoglycan that bind lipoprotein lipase. Arterioscler Thromb Vasc Biol 15: 400–409.
44. Calvo JC, Rodbard D, Katki A, Chernick S, Yanagishita M (1991) Differentiation of 3T3-L1 preadipocytes with 3-isobutyl-1-methylxanthine and dexamethasone stimulates cell-associated and soluble chondroitin 4-sulfate proteoglycans. J Biol Chem 266: 11237–11244.
45. Saxena U, Klein MG, Goldberg IJ (1993) Transport of lipoprotein lipase across endothelial cells. Proc Natl Acad Sci U S A 90: 2254–2258.
46. Obukuro JC, Lutz EP, Li Z, Paka I, Katozoda T, et al. (2001) Transcytosis of lipoprotein lipase across cultured endothelial cells requires both heparan sulfate proteoglycans and the very low density lipoprotein receptor. J Biol Chem 276: 8934–8941.
47. Goldberg IJ (1996) Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. J Lipid Res 37: 693–707.
48. Davies BS, Beigneux AP, Barnes RH, 2nd, Tu Y, Gin P, et al. (2010) GPPIHBP1 is responsible for the entry of lipoprotein lipase into capillaries. Cell Metab 12: 42–52.
49. Saxena U, Klein MG, Goldberg IJ (1991) Identification and characterization of the endothelial cell surface lipoprotein lipase receptor. J Biol Chem 266: 17516–17521.
50. Rutledge JC, Woo MM, Rezaei AA, Curtis LS, Goldberg IJ (1997) Lipoprotein lipase increases lipoprotein binding to the artery wall and increases endothelial layer permeability by formation of lipoprotein products. Circ Res 80: 819–828.