The farnesoid X receptor induces very low density lipoprotein receptor gene expression

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Abstract The farnesoid X receptor (FXR) is a nuclear receptor activated by bile acids (BAs). In response to ligand-binding, FXR regulates many genes involved in BA, lipid, and lipoprotein metabolism. To identify new FXR target genes, microarray technology was used to profile total RNA extracted from HepG2 cells treated with the natural FXR agonist chenodeoxycholic acid (CDCA). Interestingly, a significant increase of transcript level of the very low density lipoprotein receptor (VLDLR) was observed. Our data, resulting from selective FXR activation, FXR RNA silencing and FXR-deficient mice, clearly demonstrate that BAs up-regulate VLDLR transcript levels via a FXR-dependent mechanism in vitro in human and in vivo in mouse liver cells.

Keywords: Farnesoid X receptor; Microarray analysis; VLDL receptor; Small interfering RNA

1. Introduction

The farnesoid X receptor (FXR; NR1H4) is a member of the nuclear receptor superfamily that is expressed in liver, kidney, intestine and the adrenal gland [1]. FXR is activated by bile acids (BAs), such as the primary BA chenodeoxycholic acid (CDCA), which is the most potent BA ligand for the human FXR [2,3]. In addition to BAs, synthetic FXR agonists have also been identified [4,5]. In response to ligand-binding, FXR regulates a variety of genes involved in BA, cholesterol, triglyceride (TG) and lipoprotein metabolism. Targeted disruption of the FXR gene in mice confirmed its critical role in BA and lipid metabolism [6]. FXR links cholesterol to BA metabolism by inhibiting transcription of the CYP7A1 gene [7], which encodes the rate-limiting enzyme catalyzing the conversion of cholesterol into BAs. FXR also regulates the expression of various genes involved in the uptake, intracellular transport and export of BAs such as intestinal BA-binding protein (I-BABP) [8], the bile salt export pump (BSEP) [9] and the Na^+-taurocholate cotransporting polypeptide (NTCP) [10]. Furthermore, FXR plays an important role in lipoprotein and high density lipoprotein (HDL) metabolism. FXR positively regulates the phospholipid transfer protein (PLTP) gene [11], which encodes a secreted protein that facilitates the transfer of phospholipids between lipoproteins and modulates plasma HDL metabolism. In addition, apo AI, the major apolipoprotein (apo) component of HDL, is down-regulated by FXR [12]. FXR also controls plasma TG levels by activating the peroxisome proliferator-activated receptor α (PPARα) gene, another nuclear receptor controlling TG metabolism [13], and the apo CII gene [14], an obligate cofactor for lipoprotein lipase (LPL) responsible for the hydrolysis of TGs in chylomycrons, and by inhibiting apo CIII [15], which plays an important role in the control of TG metabolism. More recently, it has been demonstrated that FXR activates transcription of the human multidrug resistance MDR3 gene [16], encoding a P-glycoprotein which is a critical translocator for phospholipids across canalicular membranes of hepatocytes. As part of a systematic effort to identify new FXR target genes implicated in lipid metabolism, we performed DNA microarray experiments on human hepatoblastoma HepG2 cells treated with the natural FXR agonist CDCA. We report here the identification of the very low density lipoprotein receptor (VLDLR) as a new target gene for FXR in human and mouse liver cells.

2. Materials and methods

2.1. Cell culture and treatments

Human hepatoblastoma HepG2 and HuH7 cells (American Type Culture Collection, Rockville, MD) were grown in Dulbecco's modified

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Abbreviations: apo, apolipoprotein; BA, bile acid; BSEP, bile salt export pump; CDCA, chenodeoxycholic acid; FA, fatty acid; FGF, fibroblast growth factor; FXR, farnesoid X receptor; FXRE, FXR response element; HDL, high density lipoprotein; I-BABP, intestinal bile acid binding protein; IR, inverted repeat; LPL, lipoprotein lipase; LRH, liver receptor homolog; PFIC, progressive familial intrahepatic cholestasis; PLTP, phospholipid transfer protein; PPAR, peroxisome proliferator-activated receptor; SHP, small heterodimer partner; TG, triglyceride; MDR, multidrug resistance gene; NTCP, Na^+-taurocholate transporting polypeptide; siRNA, small interfering RNA; TCA, taurocholic acid; VLDLR, very low density lipoprotein receptor

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Eagle's medium supplemented with 10% fetal calf serum, streptomycin/penicillin, sodium pyruvate, glutamine and non-essential amino-acids (Life Technologies, Cergy-Pontoise, France) at 37 °C, in a humidified 5% CO_2 atmosphere. Human primary hepatocytes were isolated as described [17] and incubated for 48 h with chenodeoxycholic acid (CDCA; 75 μM) (Sigma, Saint-Quentin, France) or GW4064 (1 μM). GW4064 was synthesized at GENFIT (Loos, France), according to Maloney et al. [4]. For mRNA analysis, HepG2 cells were treated in 6 well plates at 60% confluence with either CDCA or GW4064 at the indicated concentration. For the actinomycin D and cycloheximide experiments, cells were pre-treated during 1 h before adding FXR agonists for 36 h.

2.2. Animals and treatments

FXR-deficient mice, obtained as described previously [6], were backcrossed for five generations in the C57BL/6 genetic background. Male C57BL/6 wild-type mice (IFFA-CREDO, L'Arbresle, France) and FXR-deficient mice were divided into two groups of animals ($n = 4$ per group). One group received a standard rodent chow diet (control), whereas the other group received the same diet containing 0.5% (wt/wt) taurocholic acid (TCA) (Sigma, Saint-Quentin, France) for 5 days. Animals were killed at the end of the treatment, livers were collected and frozen until further analysis.

2.3. RNA analysis

Total RNA was isolated from cells and tissues using TRI-Reagent according to the manufacturer's instructions (Sigma, Saint-Quentin, France). For gene expression analysis, total RNA was reverse transcribed as described previously [18]. Real-time Quantitative PCR analysis was performed using the LightCycler Technology (Roche Diagnostic) according to the manufacturer's instructions. The PCR program consisted of a denaturing step at 95 °C for 8 min followed by 40 cycles of 10 s at 55 °C, 10 s at 95 °C and 15 s at 72 °C. Primers used were as follows : 36B4 forward 5′-CATGCTCAACATCTCCCCCTTCTCC-3′ and reverse 5′-GGGAAGGTGTAATCCGTCTCCACAG-3′; GAPDH forward 5′-GACATCAAGAAGGTGGTGAA-3′ and reverse 5′-CCACATACCAGGAAATGAGC-3′; human VLDLR forward 5′-TGGTCGCTGTATTACGCTGTTG-3′ and reverse 5′-GGCACTGTTCTGGGCTTTCA-3′; human FXR forward 5′-CCGTGAATGAAGACAGTGAAGGTCG-3′ and reverse 5′-ACCCTTTCAGCAAAGCAATCTGGTC-3′; human small heterodimer partner (SHP) forward 5′-GGCTGGCAGTGCTGATTCAG-3′ and reverse 5′-TGGGGTGTGGCTGAGTGAAG-3′; mouse VLDLR forward 5′-CTTGTAGAGCAACTAAGGAACACGG-3′ and reverse 5′-CAGTCTGAC-CAGTAAACAAAGCCC-3′. Transcript levels were normalized to 36B4 RNA levels.

2.4. Gene silencing with small interfering RNA (siRNA)

The siRNA targeting human FXR with the cDNA sequence 5′-GTCGTGACTTGCGACAAG-3′ was chemically synthesized by Eurogentec (Seraing, Belgium) and annealed according to the manufacturer's instructions. The siRNA negative control from Ambion (Austin, US) was used to test non-specific effects on gene expression. HepG2 cells were transfected using oligofectamine (Invitrogen, UK) according to the manufacturer's instructions in 12-well plates containing 150×10^3 cells/well with 125 nM siRNA/well. 12 h after transfection, cells were treated for 24 h with FXR agonists at the indicated concentrations before RNA analysis.

2.5. DNA microarray analysis

HepG2 cells were cultured as described above and treated with 75 μM CDCA for 36 h. Total RNA (50 μg), isolated using TRI-reagent (Sigma) and purified with RNeasy total RNA isolation kit (Qiagen), was reverse transcribed by using a T7-(dT)24 primer (Genset Corp.) and the superscript Choice system (Life-technologies). The resulting cDNA was used to generate biotin-labelled cRNA with a bio-array high-yield transcript labelling kit (Enzo). Fragmentation of cRNA was performed at 94 °C using 40 mM Tris–acetate (pH 8.1), 100 mM potassium acetate, and 30 mM magnesium acetate. Samples were hybridized according to the manufacturer's instructions to Affymetrix human U95Av2 microarrays in triplicate for each treatment. The results of the microarrays were analyzed with Microarray Analysis Suite 4.0 software (Affymetrix).

3. Results

3.1. FXR agonists induce VLDLR transcript levels in human liver cells

To identify new FXR target genes, we used a global genomic approach by performing a DNA microarray experiment. HepG2 cells were treated with vehicle or the natural FXR ligand CDCA (75 μM) for 36 h. Total RNA was submitted to microarray analysis on human U95Av2 Affymetrix arrays ($n = 3$). Analysis of the results (data not shown) revealed regulation of several known FXR target genes by CDCA treatment, including SHP (2.7-fold), apoAI (3-fold) and MRP3 (5.4-fold). In addition, the microarrays showed a 2.1-fold induction of VLDL receptor RNA. In line with this observation, a significant dose-dependent increase of VLDLR RNA levels was observed in HuH7 cells treated with various doses of CDCA for 36 h (Fig. 1A). Since BAs may exert FXR-independent effects by activating other signal transduction pathways [19,20], the influence of the synthetic non-steroidal FXR specific agonist GW4064 on VLDLR transcript expression was tested. Treatment of HepG2 cells with GW4064, as well as CDCA, resulted in an increase of VLDLR transcript levels compared to control vehicle-treated cells (Fig. 1B). Moreover, up-regulation of VLDLR RNA levels also occurred in primary human hepatocytes treated with CDCA and GW4064 (Fig. 1C). Taken together these data demonstrate that FXR agonists up-regulate VLDLR RNA levels in human liver cells.

3.2. Induction of VLDLR transcript levels by FXR agonists requires ongoing transcription and protein synthesis

In order to investigate in more detail the action mechanism of FXR agonists on VLDLR transcript levels, a time course experiment was performed in HepG2 cells (Fig. 2). The induction of VLDLR transcript expression by CDCA occurred very early, similar to the response of SHP expression to FXR agonists [18]. Furthermore, pretreatment with either actinomycin D, an inhibitor of transcription (Fig. 3A), or cycloheximide, a translation inhibitor (Fig. 3B), completely abolished the induction of VLDLR RNA levels in HepG2 cells by CDCA. These results indicate that FXR agonists induce the expression of the VLDLR gene at the transcriptional level by a mechanism requiring de novo protein synthesis.

3.3. CDCA- and GW4064-mediated up-regulation of VLDLR transcript levels occurs by an FXR-dependent mechanism

To determine whether the regulation of VLDLR gene expression by FXR agonists observed in human liver cells is dependent on FXR activation, a FXR gene silencing experiment was performed using specific siRNA duplexes to reduce expression of FXR transcript prior to treatment with FXR agonists. A negative siRNA was used to control non-specific effects of the transfection on gene expression. Compared to control cells, transfection of HepG2 cells with the FXR-specific siRNA reduced the level of FXR RNA by about 80% (Fig. 4A), whereas there was no effect on GAPDH RNA levels (Fig. 4B). As a positive control, the levels of SHP transcript were quantified in the same samples (Fig. 4C), and as expected, FXR ligand treatment increased SHP levels in control cells treated with FXR agonists, but not in FXR knockdown cells. Interestingly, FXR silencing abolished the CDCA- and GW4064-mediated up-regulation of VLDLR RNA (Fig. 4D). Taken together these results demonstrate that FXR mediates

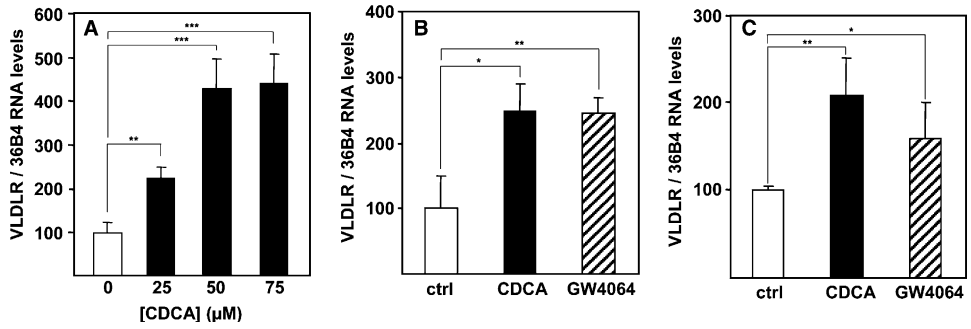

Fig. 1. Natural and synthetic FXR agonists enhance VLDLR transcript levels in human liver cells. (A) HuH7 cells were treated for 36 h with increasing concentrations of CDCA (as indicated in the figure). HepG2 cells (B) and primary human hepatocytes (C) were treated with DMSO (ctrl), CDCA (75 μM) or GW4064 (1 μM) for 36 and 48 h, respectively. VLDLR transcript levels were measured by real-time quantitative PCR. Values are means ± S.D. ($n = 3$). Statistically significant differences between control and FXR agonists-treated cells are indicated by asterisks (Student's t-test: ***, $P < 0.001$; **, $0.001 < P < 0.01$; and *, $0.01 < P < 0.05$).

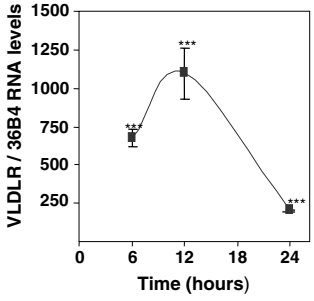

Fig. 2. CDCA induces VLDLR transcript levels in HepG2 cells in a time-dependent manner. HepG2 cells were treated with DMSO (ctrl) or CDCA (75 μM) for 6, 12, 24 and 48 h. VLDLR transcript levels were measured by real-time quantitative PCR. Values are means ± S.D. ($n = 3$) and each time point was normalized to control set as 100%. Statistically significant differences between control and CDCA-treated cells are indicated by asterisks (Student's t-test: ***, $P < 0.001$).

the up-regulation of VLDLR transcript levels by CDCA and GW4064 in HepG2 cells.

3.4. Bile acids induce VLDLR RNA levels in vivo in the liver of wild type but not FXR-deficient mice

To determine if the FXR-dependent regulation of VLDLR gene expression observed in vitro in liver cells also occurs in vivo, wild type and FXR-deficient mice were treated for 5 days with TCA, and VLDLR RNA levels were analyzed in the liver. VLDLR transcript levels were not significantly different in livers of wild type versus FXR-deficient mice under normal feeding conditions (100% versus 89%, student's t-test: $P = 0.832$). However, in wild-type mice BA treatment induced the expression of VLDLR transcript, whereas in FXR-deficient mice VLDLR RNA levels did not change significantly (Fig. 5). Taken together these data indicate that BAs regulate VLDLR gene expression in mouse liver in vivo via activation of FXR.

4. Discussion

The results from this study indicate that VLDLR gene expression is regulated by an FXR-dependent mechanism both in vitro in human and in vivo in mouse liver cells. A DNA microarray gene expression profiling approach was used to identify new target genes of FXR agonists and the VLDL receptor was identified as a FXR target gene in the liver. This was unexpected since VLDLR is expressed at high concentrations in skeletal muscle, heart, adipose tissue, brain and macrophages, but is found only in trace amounts in the liver [21,22]. The VLDLR has been described to bind apo E-rich VLDL and LPL [23]. Recent reports have proposed several possible functions of the VLDLR in lipoprotein metabolism [24], atherosclerosis [25], obesity and insulin resistance [26],

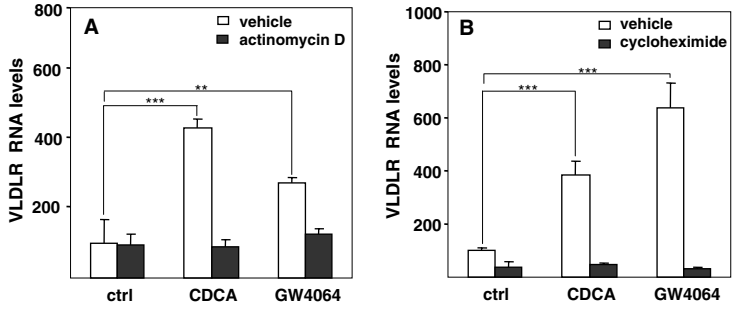

Fig. 3. CDCA- and GW4064-mediated up-regulation of VLDLR transcript levels requires ongoing transcription and protein synthesis. HepG2 cells were incubated with CDCA (75 μM) or GW4064 (1 μM) for 24 h, in the presence of vehicle or actinomycin D 5 (μg/μl) (A) or cycloheximide 10 (μg/μl) (B). VLDLR transcript levels were measured by real-time quantitative PCR. Values are means ± S.D. ($n = 3$). Statistically significant differences between control and FXR agonists-treated cells are indicated by asterisks (Student's t-test: ***, $P < 0.001$; **, $0.001 < P < 0.01$).

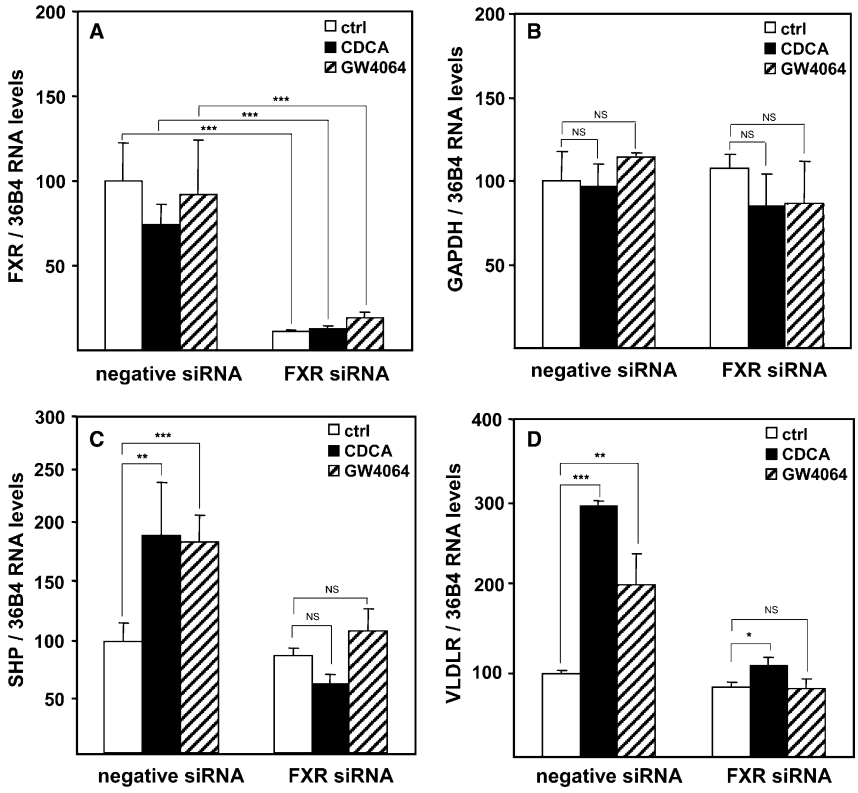

Fig. 4. Knockdown of FXR RNA levels abolishes the up-regulation of VLDLR transcript levels by FXR agonists in HepG2 cells. HepG2 cells were transfected with siRNA duplexes specific for FXR or a siRNA negative control, and treated with DMSO (ctrl), CDCA (75 μM) or GW4064 (1 μM) for 24 h. FXR (A), GAPDH (B), SHP (C) and VLDLR (D) transcript levels were measured by real-time quantitative PCR. Values are means ± S.D. ($n = 3$). Statistically significant differences are indicated by asterisks (Student's t-test: ***, $P < 0.001$; **, $0.001 < P < 0.01$; and *, $0.01 < P < 0.05$; and NS: not significant).

cardiac fatty acid (FA) metabolism [27] and neuronal migration [28]. Although, relatively little is known about VLDLR function in the liver, adenovirus-mediated overexpression of the VLDLR into LDLR-deficient mice liver showed a beneficial effect on lipoprotein metabolism and atherosclerosis [29].

Initial studies on VLDLR-deficient mice revealed no alterations in lipoprotein profile, total plasma cholesterol, TG and free FA levels as compared to wild-type mice [30]. However, when VLDLR transgenic and deficient mice were studied under conditions of LDLR deficiency, fed a high fat-diet or subjected to prolonged fasting [31], VLDLR overexpression was associated with a decrease, whereas VLDLR deficiency was associated with an increase in plasma TG levels. In the absence of the VLDLR, the lipid composition of nascent VLDL was unaffected, but circulating VLDL displayed a high TG content suggesting that the VLDLR modulates peripheral TG metabolism. BA-sequestering resins, that are used for the treatment of hypercholesterolemia, also increase plasma TG due to accumulation of VLDL particles enriched in TG [32,33]. Because these resins interrupt the enterohepatic circulation of BAs, treatment with these drugs is likely to decrease the exposure of hepatocytes to BAs, thus potentially dampening FXR activity and subsequently decreasing activation of FXR target genes, such as the VLDLR. It is tempting to speculate that such a decrease in VLDLR expression would contribute to the observed changes in lipid metabolism after resin treatment. Of more pathophysiological relevance, induction of VLDLR by FXR may occur under conditions of high intrahepatic BA concentrations, such as in progressive familial intrahepatic cholestasis (PFIC) [34].

In this study, we have clearly demonstrated, by using a non-steroidal FXR-selective agonist, FXR knockdown and FXR-deficient mice, that BAs up-regulate human VLDLR RNA level via a FXR-dependent mechanism. However, in silico

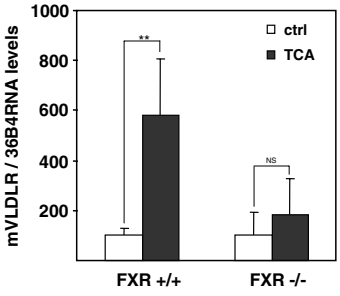

Fig. 5. Bile acid treatment induces VLDL receptor transcript levels in livers of wild-type but not FXR deficient mice. Mouse VLDLR (mVLDLR) transcript levels were measured by real-time quantitative PCR in livers of C57BL/6 wild type (FXR +/+) and FXR deficient (FXR −/−) mice fed with a standard rodent chow diet containing or not TCA (0.5% TCA wt/wt) for 5 days. Values are means ± S.D. ($n = 4$). Statistically significant differences between control and TCA-treated samples are indicated by asterisks (Student's t-test: $^{**}P < 0.001$; and NS: not significant).

DNA sequence analysis and transient transfection assays with the putative inverted repeat 1 (IR1) sites did not reveal the existence of a functional FXR response element (FXRE) in the human VLDLR promoter. Although, FXR may also bind in vitro to various atypical FXREs [35] these observations suggest that FXR does not directly regulate the VLDLR. In line with this, the regulation of VLDLR by BA requires de novo protein synthesis suggesting the existence of an indirect mechanism, or a direct mechanism that requires the synthesis of a very labile and essential coactivator. Several mechanisms for indirect transcriptional regulation of FXR target genes by BAs have been proposed. BAs can down-regulate CYP7A1 via FXR-mediated induction of the SHP [7,36], which represses expression of CYP7A1 by inhibiting the activity of liver receptor homolog (LRH-1). FXR directly regulates expression of fibroblast growth factor-19 (FGF-19), and in turn FGF-19 strongly suppresses expression of CYP7A1 in primary cultures of human hepatocytes and mouse liver through a c-Jun N-terminal kinase pathway [37].

In conclusion, this study identifies VLDLR as a novel FXR target gene in the liver. These results suggest a novel mechanism by which BAs may modulate lipid and lipoprotein metabolism.

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References

[1] Seol, W., Choi, H.S. and Moore, D.D. (1995) Mol. Endocrinol. 9, 72–85.
[2] Parks, D.J., Blanchard, S.G., Bledsoe, R.K., Chandra, G., Consler, T.G., Kliewer, S.A., Stimmel, J.B., Willson, T.M., Zavacki, A.M., Moore, D.D. and Lehmann, J.M. (1999) Science 284, 1365–1368.
[3] Makishima, M., Okamoto, A.Y., Repa, J.J., Tu, H., Learned, R.M., Luk, A., Hull, M.V., Lustig, K.D., Mangelsdorf, D.J. and Shan, B. (1999) Science 284, 1362–1365.
[4] Maloney, P.R., Parks, D.J., Haffner, C.D., Fivush, A.M., Chandra, G., Plunket, K.D., Creech, K.L., Moore, L.B., Wilson, J.G, Lewis, M.C., Jones, S.A. and Willson, T.M. (2000) J. Med. Chem. 43, 2971–2974.
[5] Pellicciari, R., Fiorucci, S., Camaioni, E., Clerici, C., Costantino, G., Maloney, P.R., Morelli, A., Parks, D.J. and Willson, T.M. (2002) J. Med. Chem. 45, 3569–3572.
[6] Sinal, C.J., Tohkin, M., Miyata, M., Ward, J.M., Lambert, G. and Gonzalez, F.J. (2000) Cell 102, 731–744.
[7] Davis, R.A., Miyake, J.H., Hui, T.Y. and Spann, N.J. (2002) J. Lipid Res. 43, 533–543.
[8] Hwang, S.T., Urizar, N.L., Moore, D.D. and Henning, S.J. (2002) Gastroenterology 122, 1483–1492.
[9] Plass, J.R., Mol, O., Heegsma, J., Geuken, M., Faber, K.N., Jansen, P.L. and Muller, M. (2002) Hepatology 35, 589–596.
[10] Denson, L.A., Sturm, E., Echevarria, W., Zimmerman, T.L., Makishima, M., Mangelsdorf, D.J. and Karpen, S.J. (2001) Gastroenterology 121, 140–147.
[11] Urizar, N.L., Dowhan, D.H. and Moore, D.D. (2000) J. Biol. Chem. 275, 39313–39317.
[12] Claudel, T., Sturm, E., Duez, H., Torra, I.P., Sirvent, A., Kosykh, V., Fruchart, J.C., Dallongeville, J., Hum, D.W., Kuipers, F. and Staels, B. (2002) J. Clin. Invest. 109, 961–971.
[13] Pineda Torra, I., Claudel, T., Duval, C., Kosykh, V., Fruchart, J.C. and Staels, B. (2003) Mol. Endocrinol. 17, 259–272.
[14] Kast, H.R., Nguyen, C.M., Sinal, C.J., Jones, S.A., Laffitte, B.A., Reue, K., Gonzalez, F.J., Willson, T.M. and Edwards, P.A. (2001) Mol. Endocrinol. 15, 1720–1728.
[15] Claudel, T., Inoue, Y., Barbier, O., Duran-Sandoval, D., Kosykh, V., Fruchart, J., Fruchart, J.C., Gonzalez, F.J. and Staels, B. (2003) Gastroenterology 125, 544–555.
[16] Huang, L., Zhao, A., Lew, J.L., Zhang, T., Hywna, Y., Thompson, J.R., Pedro Nd, N., Royo, I., Blevins, R.A., Pelaez, F., Wright, S.D. and Cui, J. (2003) J. Biol. Chem. 278, 51085–51090.
[17] Chippers, I.J., Moshage, H., Roelofsen, H., Muller, M., Heymans, H.S., Ruiters, M. and Kuipers, F. (1997) Cell. Biol. Toxicol. 13, 375–386.
[18] Barbier, O., Torra, I.P., Sirvent, A., Claudel, T., Blanquart, C., Duran-Sandoval, D., Kuipers, F., Kosykh, V., Fruchart, J.C. and Staels, B. (2003) Gastroenterology 124, 1926–1940.
[19] Staudinger, J.L., Goodwin, B., Jones, S.A., Hawkins-Brown, D., MacKenzie, K.I., LaTour, A., Liu, Y., Klaassen, C.D., Brown, K.K., Reinhard, J., Willson, T.M., Koller, B.H. and Kliewer, S.A. (2001) Proc. Natl. Acad. Sci. USA 98, 3369–3374.
[20] Gupta, S., Stravitz, R.T., Dent, P. and Hylemon, P.B. (2001) J. Biol. Chem. 276, 15816–15822.
[21] Webb, J.C., Patel, D.D., Jones, M.D., Knight, B.L. and Soutar, A.K. (1994) Hum. Mol. Genet. 3, 531–537.
[22] Hiltunen, T.P., Luoma, J.S., Nikkari, T. and Yla-Herttuala, S. (1998) Circulation 97, 1079–1086.
[23] Takahashi, S., Suzuki, J., Kohno, M., Oida, K., Tamai, T., Miyabo, S., Yamamoto, T. and Nakai, T. (1995) J. Biol. Chem. 270, 15747–15754.
[24] Tacken, P.J., Hofker, M.H., Havekes, L.M. and van Dijk, K.W. (2001) Curr. Opin. Lipidol. 12, 275–279.
[25] Tacken, P.J., Delsing, D.J., Gijbels, M.J., Quax, P.H., Havekes, L.M., Hofker, M.H. and van Dijk, K.W. (2002) Atherosclerosis 162, 103–110.
[26] Goudriaan, J.R., Tacken, P.J., Dahlmans, V.E., Gijbels, M.J., van Dijk, K.W., Havekes, L.M. and Jong, M.C. (2001) Arterioscler. Thomb. Vasc. 21, 1488–1493.
[27] Kamataki, A., Takahashi, S., Masamura, K., Iwasaki, T., Hattori, H., Naiki, H., Yamada, K., Suzuki, J., Miyamori, I., Sakai, J., Fujino, T. and Yamamoto, T.T. (2002) Biochem. Biophys. Res. Commun. 293, 1007–1013.
[28] Trommsdorff, M., Gotthardt, M., Hiesberger, T., Shelton, J., Stockinger, W., Nimpf, J., Hammer, R.E., Richardson, J.A. and Herz, J. (1999) Cell 97, 689–701.
[29] Oka, K., Pastore, L., Kim, I.H., Merched, A., Nomura, S., Lee, H.J., Merched-Sauvage, M., Arden-Riley, C., Lee, B., Finegold, M., Beaudet, A. and Chan, L. (2001) Circulation 103, 1274–1281.
[30] Frykman, P.K., Brown, M.S. and Yamamoto, T. (1995) Proc. Natl. Acad. Sci. USA 92, 8453–8457.
[31] Tacken, P.J., Teusink, B., Jong, M.C., Harats, D., Havekes, L.M., van Dijk, K.W. and Hofker, M.H. (2000) J. Lipid Res. 41, 2055–2062.
[32] Witztum, J.L., Schonfeld, G. and Weidman, S.W. (1976) J. Lab. Clin. Med. 88, 1008–1018.
[33] Beil, U., Crouse, J.R., Einarsson, K. and Grundy, S.M. (1982) Metabolism 31, 438–444.
[34] Jansen, P.L. and Sturm, E. (2003) Liver Int. 23, 315–322.
[35] Laffitte, B.A., Kast, H.R., Nguyen, C.M., Zavacki, A.M., Moore, D.D. and Edwards, P.A. (2000) J. Biol. Chem. 275, 10638–10647.
[36] Goodwin, B., Jones, S.A., Price, R.R., Watson, M.A., McKee, D.D., Moore, L.B., Galardi, C., Wilson, J.G., Lewis, M.C., Roth, M.E., Maloney, P.R., Willson, T.M. and Kliewer, S.A. (2000) Mol. Cell. 6, 517–526.
[37] Holt, J.A., Luo, G., Billin, A.N., Bisi, J., McNeill, Y.Y., Kozarsky, K.F., Donahee, M., Wang da, Y., Mansfield, T.A., Kliewer, S.A., Goodwin, B. and Jones, S.A. (2003) Genes Dev. 17, 1581–1591.