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Liver X Receptor β and Peroxisome Proliferator-Activated Receptor δ regulate cholesterol transport in cholangiocytes

Xuefeng Xia[a,b,1], **Dongju Jung**[c,*], **Paul Webb**[a], **Aijun Zhang**[a], **Bin Zhang**[a], **Lifei Li**[d], **Stephen D. Ayers**[a], **Chiara Gabbi**[e,f], **Yoshiyuki Ueno**[g], **Jan-Åke Gustafsson**[e,f], **Gianfranco Alpini**[h,i,j], **David D. Moore**[c], and **Gene D. LeSage**[k,1]

[a]The Methodist Hospital Research Institute, Weill Cornell School of Medicine, 6670 Bertner Ave, Houston, TX 77030

[b]The Third Affiliated Hospital, Guangzhou Medical College, Guangzhou, Guangdong 510150, China

[c]Department of Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX, 77030

[d]The First Hospital, Lanzhou University, Lanzhou, Gansu 730000, China

[e]Center for Nuclear Receptor and Cell Signaling, University of Houston, Calhoun Road, Houston TX77004

[f]Karolinska Institutet, Department of Biosciences and Nutrition, NOVUM, 14186 Stockholm, Sweden

[g]Division of Gastroenterology, Tohoku University Graduate School of Medicine, Sendai, Japan

[h]Scott & White Digestive Diseases Research Center, Temple, TX 76504

[i]Division Research, Central Texas Veterans Health Care System, Temple, TX 76504

[j]Department of Medicine, Texas A & M Health Science Center College of Medicine, Temple, TX 76504

[k]Department of Internal Medicine at East Tennessee State University's James H. Quillen College of Medicine, Johnson City, TN 37614

Abstract

Nuclear receptors (NRs) play crucial roles in regulation of hepatic cholesterol synthesis, metabolism and conversion to bile acids, but their actions in cholangiocytes have not been examined. In this study, we investigated the roles of NRs in cholangiocyte physiology and cholesterol metabolism and flux. We examined the expression of NRs and other genes involved in cholesterol homeostasis in freshly isolated and cultured rodent cholangiocytes and found that these cells express a specific subset of NRs which includes Liver X Receptor β (LXRβ) and Peroxisome Proliferator-Activated Receptor δ (PPARδ). Activation of LXRβ and/or PPARδ in cholangiocytes induces ATP-binding cassette cholesterol transporter A1 (ABCA1) and increases cholesterol

[1]Correspondence to: Xuefeng Xia, The Methodist Hospital Research Institute, 6670 Bertner Ave, R8-117, Houston, TX 77030, Telephone: 713-441-6665, Fax: 713-793-7162, xxia2@tmhs.org and Gene LeSage, Department of Internal Medicine, East Tennessee State University, VA Bldg. 1, Box 70622, Johnson City, TN 37614, Telephone: 423-439-6282, Fax: 423-439-6387, lesage@mail.etsu.edu.
*Present address: Institute for Integrated Cell-Material Sciences (WPI-iCeMS), Kyoto University, Kyoto, Japan

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export at the basolateral compartment in polarized cultured cholangiocytes. In addition, PPARδ induces Niemann Pick C1 Like L1 (NPC1L1), which imports cholesterol into cholangiocytes and is expressed on the apical cholangiocyte membrane, via specific interaction with a PPRE within the NPC1L1 promoter. Based on these studies, we propose that (i) LXRβ and PPARδ coordinate NPC1L1/ABCA1 dependent vectorial cholesterol flux from bile through cholangiocytes and (ii) manipulation of these processes may influence bile composition with important applications in cholestatic liver disease and gallstone disease, serious health concerns for humans.

Keywords

Cholangiocyte; LXRβ; PPARδ; ABCA1; NPC1L1

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While effects of Nuclear Receptors (NRs) upon hepatic cholesterol synthesis and degradation and conversion to bile acids have been studied extensively, their effects on cholangiocytes are unclear [(1)]. Cholangiocytes are epithelial cells and actively regulate bile composition through a process of absorption and secretion; they modulate content of water, organic anions and cations, electrolytes in bile [(2)]. Cholangiocytes can also reabsorb bile acids, a process that is important in cholestatic liver disease [(3)]. While cholesterol is present at high levels in bile, it is not yet known whether cholangiocytes influence bile cholesterol content or whether this process is regulated by NRs. This issue is important in devising means to reduce cholesterol in bile to prevent/block cholestatic liver disease and gallstone formation.

NRs, including Liver X Receptors (LXRs), the Peroxisome Proliferator Activated Receptor (PPAR) subgroup α, γ and δ, Farnesoid X Receptor (FXR) and Short Heterodimer Partner (SHP) regulate genes with roles in cholesterol and bile acid metabolism in a variety of cell types such as hepatocytes, cholangiocytes and macrophages [(4)]. For example, LXRα regulates CYP7A1, Low Density Lipoprotein Receptor (LDLR), and Sterol Response Element Binding Proteins (SREBPs) that mediates fattyacid regulation in hepatocytes [(5)]. In macrophages, LXRβ and PPARs regulate ABCA1, an ATP-binding cassette cholesterol transporter that mediates cholesterol efflux and reverse cholesterol transport (RCT) from the periphery to the liver [(4)].

Niemann Pick C1 Like L1 (NPC1L1) is another key component of cholesterol metabolism [(6)]. The protein is the putative target for the cholesterol uptake inhibitor ezetimibe; it is expressed on the brush border of the small intestine, where it mediates dietary cholesterol absorption [(7)]. NPC1L1 is also expressed in human liver, but its precise distribution is controversial with some studies showing high hepatocyte expression [(8)] while others show higher expression in gallbladder epithelial cells [(9)]. Regulatory elements in the NPC1L11 promoter are partly characterized with suggestive evidence for direct roles of Hepatocyte Nuclear Factor 1α (HNF1α) and SREBP2 [(10)] and a possible indirect inhibitory role for PPARα [(11)].

In this study, we investigated the roles of NRs in cholangiocyte physiology and cholesterol transport and metabolism. We examined the expression of NRs and other genes involved in cholesterol homeostasis in freshly isolated and cultured rodent cholangiocytes and found that cholangiocytes express a subset of NRs that are different from hepatocytes, and that agonists for two of these receptors (LXRβ and PPARδ) induce vectorial cholesterol transport through the cholangiocyte basolateral membrane via ABCA1 induction. We also found that NPC1L1 is expressed on the apical cholangiocyte membrane and is regulated by PPARδ via specific interaction with a PPRE located within the NPC1L1 promoter, the demonstration that PPARδ or any ligand-dependent NR plays a direct role in NPC1L1

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expression in cholangiocytes. We propose that LXRβ and PPARδ coordinate NPC1L1/ABCA1 dependent cholesterol flux from bile through cholangiocytes and that it will be possible to manipulate these processes to influence bile physiology in cholestatic liver disease and gallstones.

Materials and Methods

Cells and reagents

Cholangiocytes and hepatocytes were isolated from normal and bile duct ligation (BDL) rat liver as described by Alpini [(3)]. Normal Rat and Cholangiocyte (NRC) were isolated and established in culture as described [(12)]. AAV-293 cells were from Stratagene (La Jolla, CA). T0901317, GW501516, water-soluble cholesterol, 27-, 22-hydrocholesterol, fatty acid-free BSA, ApoAI, Flag and βactin monoclonal antibodies and other chemicals were from Sigma (St. Louis, MO). All cell-culture reagents were from Invitrogen (Carlsbad, CA). ABCA1 polyclonal antibody was from Novus (Littleton, CO). PPARδ and LXRβ polyclonal antibodies were purchased from Santa Cruz (Santa Cruz, CA). Anti-NPC1L1 polyclonal was from Cayman Chemical (Ann Arbor, MI).

RT-qPCR and Northern Blot

For RT-qPCR, total RNA was purified using RNeasy mini kit following manufacturer's instructions.

Luciferase assays

PCR fragments containing murine NPC1L1 5' flanking region (-1045 to –1, relative to translation start ATG) were cloned into pGL3zeocin at MluI-XhoI to generate pGL3-1.1NPC1L1-Luc. PPARδ coding region was amplified from pCMX.mPPARδ template and inserted into p3XFLAG-CMV-10 (Sigma) at Hind-III/BamH1 site to obtain pFLAG-PPARδ. Luciferase activity was measured using the Dual-Luciferase kit according to manufacturer's instruction (Promega, Fitchburg, WI) and reported as a ratio of firefly luciferase to Renilla luciferase.

RNA interference

The PPARδ target sequence (RNAi#1: AATCCGCATGAAGCTCGAGTA, encoding aa 630–650) was cloned into pSilencer-2.1-U6-Hygro according to the manufacturer's instructions. The construct was verified by sequencing the junctions and inserts before transfection. To knockdown PPARδ mRNA levels, PPARδ-RNAi was transfected separately using Lipofectamine 2000 (Invitrogen). pSilencer-2.1-U6-Hygro was utilized as a negative control.

Immunohistochemistry (IHC) and Immunofluorescence (IF)

For IHC, paraffin-embedded rat liver sections (4-μm) were dewaxed and antigens were retrieved. After blocking, slides were incubated with primary antibodies. EnVision (Dako) kit was used for avidin-biotin complex method to visualize the signal following manufacturer's instructions. For IF, cultured cholangiocytes were made permeable for 20 min at 20 °C with acetone, washed, and incubated with the appropriate antibodies. After washing, slides were reprobed with goat anti-rabbit Alexa 594 (Invitrogen), slides were mounted with Fluoromount G. Nuclei were counterstained with DAPI (4, 6-diamidino-2-phenylindole). Slides were analyzed with a Nikon A1 cofocal microscope system.

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Western blot analysis

Western blot analysis was performed by using protein extraction from cultured cholangiocytes. Samples were separated by electrophoresis on 12% (wt/vol) polyacrylamide gels and electrophoretically transferred to a PVDF membrane (Bio-Rad Laboratories). Blots were probed with primary antibodies, followed by HRP-conjugated secondary antibodies.

Cholesterol efflux and uptake

Cells were exposed to either 1 μM T0901317, GW501516 or vehicle (0.1% DMSO) and and incubated with 0.5 μCi/ml [^{3}H]-cholesterol (Amersham) and 50 μg/ml cholesterol for 24h in serum free medium (SFM) containing 0.2% BSA. Cells were washed twice with PBS and efflux of cholesterol was initiated in serum free medium in the presence or absence of 10 μg/ml apoA1. After 4 h, medium was removed, cell debris was pelleted and effluxed radioactivity was measured by scintillation counting. To obtain cell-associated [^{3}H]-cholesterol, cells were lysed in 0.2 M NaOH and radioactivity was measured by scintillation counting. Percent efflux was determined by dividing radioactivity in the culture media by the sum of radioactivity in the cell lysate and the media. ApoA1-dependent efflux was calculated by subtracting percent efflux without apoA1 from efflux with apoA1 present.

EMSA

Nuclear extracts were prepared from PPARδ expressing AAV293 cells using a Nuclear/ Cytosol Fractionation Kit according to manufacturer's instruction (BioVision, Mountain View, CA).

ChIP coupled RT-qPCR

ChIP was performed using Chromatin Immunoprecipitation Assay Kit (Millipore, Billerica, MA) according to the manufacturer's protocol.

Statistics

Data represent mean ±SEMs. We used t test and ANOVA to calculate and determine statistical significance.

Results

Cholangiocytes and hepatocytes express different NRs and genes involved in cholesterol metabolism

To determine the expression of NRs in cholangiocytes and their possible roles in cholesterol transport and metabolism, we analyzed gene expression in freshly isolated hepatocytes, large and small cholangiocytes (Fig.1A, C, S3-6) and cultured cholangiocytes (Fig.1B, S1&2). Purity of cell preparations was verified by RT-qPCR with specific cholangiocyte and hepatocyte marker genes (Fig.S5).

We detected expression of multiple NRs in cholangiocytes, including LXRβ, PPARδ, TRα, Estrogen Receptors (ERs) α and β, Retinoid X Receptors β and γ and Vitamin D Receptor (VDR) (Figs.1A&B, S1, S3, S4). Of these, several transcripts were preferentially expressed in cholangiocytes versus hepatocytes, including PPARδ (Fig.1A), TRα and VDR (Fig.S4). Conversely, many NRs that are expressed in hepatocytes such as PPARα, PPARγ, HNF4α, TRβ, LXRα, RXRα, FXR, SHP, Pregnane X Receptor (PXR), Liver Receptor Homolog 1 (LRH-1) and Constitutive Androstane Receptor (CAR) were undetectable levels in cholangiocytes (Fig.S1, S3, S4). Both PPARδ and LXRβ were highly expressed in rat cholangiocytes as shown by Western blotting analysis and immunohistochemical analysis of rat liver sections (Fig.1B&C). Expression of many of these NRs, including PPARδ, PPARγ,

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LXRβ, HNF4α, FXR, TRβ and RXRα,β, was similar in both large and small cholangiocytes although expression of TRα, ERα,β, RXRγ and VDR are higher in small versus large cholangiocytes and the NR coregulators PGC1α and β were expressed at higher levels in large cholangiocytes implying that NR actions may not be completely identical.

Hepatocytes and cholangiocytes displayed unique expression profiles of genes associated with bile acid and cholesterol metabolism. CYP27A1, which catalyzes conversion of cholesterol to oxysterols, was the only significantly expressed gene product with a role in cholesterol metabolism that could be detected in large cholangiocytes, albeit at a much lower level than hepatocytes (Fig.1D, S1). Other enzymes typically involved in the conversion from cholesterol to bile acids (CYP7A1, CYP7B1 and CYP8B1) were undetectable (Fig.1D, S1).

Among cholesterol and bile acid receptors, transporters and apolipoprotein components, we detected high expression of ABCG1 and LDLR transcripts in cholangiocytes relative to hepatocytes (Fig.S6), whereas ABCA1, CD36, SR-BI, ABCG5, ABCG8, Apolipoprotein E (apo E), Fatty Acid Binding Protein (FABP) and hepatocyte bile acid transporters BSEP, NTCP and OATP were low or essentially undetectable (Fig.S2, S5-6). The expression of ABCA1, CD36, SR-B1, FABP was the same in both large and small cholangiocytes, whereas ABCG1 was higher and LDLR were lower in large cholangiocytes.

LXR-dependent basolateral cholesterol efflux in cholangiocytes

The pattern of LXR and PPAR subtype expression in cholangiocytes is more similar to macrophages, which function in reverse cholesterol transport, than hepatocytes. We therefore asked whether activation of LXRβ and PPARδ would mediate an analogous response in cultured cholangiocytes. Treatment of normal rat cholangiocytes (NRCs) with the LXR agonist T0901317 (TO) increased the expression of ABCA1 and ABCG1 whereas FXR and PPARγ agonists Chenodeoxycholic Acid (CD) and Rosiglitazone (RO) had no effect (Fig.2A). The PPARδ agonist GW501516 (GW) also induced ABCA1 in cultured cholangiocytes, although it had no effect on ABCG1 expression levels (Fig.2B). The combination of TO and GW elicited an additive effect on ABCA1 transcript and protein levels (Fig.2C, D). Confocal fluorescence micrograph (XZ sections) of NRC demonstrated ABCA1 was localized within the basolateral membrane region (Fig. 2E). Expression of LXRβ and PPARδ was not affected by any treatment (not shown).

Since ABCA1 mediates cholesterol efflux, we examined the effects of LXR and PPARδ ligands on cholesterol efflux from polarized NRCs cultured in a transwell plate assay (Fig. 2F). NRCs grown on polycarbonate membrane form monolayers with suitable polarization and retain the phenotypic and functional characteristics that define cholangiocytes *in vivo* [12]. After labeling with [^{3}H]-cholesterol for 24h, cells were treated with LXR ligand TO and/or PPARδ ligand GW for 24h. During the last 4h of ligand treatment, new media containing delipidated Apo-AI was added to apical and basolateral compartments as an acceptor for excreted cholesterol. Cholesterol release into the apical compartment was unaffected by either treatment (Fig. 2G). We observed an increased secretion of cholesterol into the basolateral compartment in response to increasing concentrations of TO, and the combination of TO and GW (Fig. 2G). Basolateral efflux was not increased by lower concentrations of TO and GW (1 μM) in the absence of the ApoA1 acceptor protein, but was modestly elevated by higher concentrations. Thus, activation of LXRβ and PPARδ promote ABCA1-dependent basolateral cholesterol efflux in cholangiocytes.

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PPARδ induces NPC1L1 expression in cholangiocytes

NPC1L1 mediates intestinal cholesterol uptake but is also expressed in liver [7–9]. We detected NPC1L1 transcripts in both the large and small cholangiocytes (Fig.3A). Confocal fluorescence micrographs revealed that NPC1L1 protein was localized to the apical membrane (Fig.3B&C) in NRC. In addition, immunohischemical analysis of rat liver sections revealed NPC1L1 protein throughout the bile duct cells (Fig.4C).

PPARδ specifically induced NPC1L1. The PPARδ agonist (GW) induced NPC1L1 mRNA and protein within the cultured cholangiocytes (Fig.3D&E). LXRβ agonist (TO) and PPARγ agonist (RO) did not alter the expression level of NPC1L1. NPC1L1 expression was inhibited by specific depletion of PPARδ expression via RNAi, indicating that PPARδ is required for optimal expression in unstimulated conditions (Fig.3F left); Western Bolt analysis confirmed that RNAi eliminated PPARδ protein expression in cultured cholangiocytes (Fig.3G right).

To evaluate the role of NPC1L1 in cholesterol uptake in cholangiocytes, we examined effects of GW and the NPC1L1 inhibitor ezetimibe on cholesterol uptake in polarized NRC. The treatment of cholangiocytes with GW leads to an increase in apical cholesterol uptake, while the addition of ezetimibe inhibited this effect (Fig.3H). This suggests that PPARδ may regulate NPC1L1 expression and NPC1L1-dependent cholangiocyte apical cholesterol uptake.

NPC1L1 regulates the absorption of cholesterol in the small intestine[7] so we speculated that dramatic increases in cholesterol as observed during cholestasis would have a significant impact on the expression of NPC1L1 in the cholangiocytes. Accordingly, we observed an increase in both PPARδ and NPC1L1 expression in both large and small cholangiocytes after bile duct ligation (Fig.4A-C).

Since cholangiocytes express CYP27A1 which converts cholesterol to oxysterols that act as LXR ligands; we also investigated whether cholesterol influx into cholangiocytes would elicit LXR dependent changes in gene expression in NRC. Multiple LXR target genes including ABCA1, ABCG1, Sult2b1, ABCC1 and CYP46A1 were increased in NRC in response to cholesterol loading (Fig.4D). Similar changes in gene expression were observed after the treatment of NRC with either oxysterols 27- and 22-hydroxysterols (not shown). This data is consistent with the idea that oxysterol production is increased in cholangiocytes in response to elevations of cholesterol.

PPARδ regulated NPC1L1 expression in cultured cholangiocytes

Three lines of evidence suggest that PPARδ directly regulates the NPC1L1 promoter. First, GW increased the activity of an NPC1L1 promoter (-1045/-1) driven luciferase reporter in cultured cholangiocytes (Fig.5A top). Second, PPARδ overexpression activated the NPC1L1 promoter, whereas a PPARδ with a dominant negative (PPARδ –DN) in which the mutation impairs DNA binding had no effect (Fig.5A middle). Finally, PPARδ RNAi reduced basal NPC1L1 promoter activity (Fig.5A, bottom).

We searched the NPC1L1 5’ flanking region for putative PPREs and identified one possible PPRE at -142bp from the transcription start site (Fig.5B). Mutation of this site eliminated GW-dependent increases in NPC1L1 promoter activity this specific RXR-PPAR association with the PPRE was confirmed by gel shifts using nuclear extracts of transfected cells and *in vitro* translated NMCs (Fig.5C-E). Finally, chromatin immunoprecipitation (ChIP) assays revealed PPARδ association with the fragment that contains the PPRE in cholangiocytes (Fig.5F).Thus, PPARδ regulates NPC1L1 expression by associating with a PPRE which lies 5’ of the translational start site.

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Discussion

We profiled cholangiocyte expression of NRs and key enzymes and lipid transporters implicated in cholesterol and bile acid metabolism. Whereas hepatocytes express LXRα, LRH1, PPARα and other NRs with established roles in liver cholesterol metabolism, cholangiocytes express LXRβ and PPARδ. Further, cholangiocytes expressed high levels of CYP27 which converts cholesterol to oxysterols that can serve either as precursors for bile acids or as LXR ligands. Thus, cholangiocytes and hepatocytes exhibit marked differences in the expression of NRs that modulate bile acid and cholesterol metabolism and gene products that regulate key steps in these processes. While many NRs and lipid transporters are expressed at similar levels in large and small cholangiocyte, there are some differences in expression pattern and further studies of differences in gene expression and protein function between small and large cholangiocytes will likely be of great interest.

Agonists for both LXRβ and PPARδ induced expression of the cholesterol pump ABCA1 in cholangiocytes. This is accompanied by increased cholesterol export at the cholangiocyte basolateral membrane. This action of LXRβ and PPARδ agonists resembles regulation of ABCA1 dependent cholesterol transport mechanisms in peripheral macrophages, where LXRβ and PPARs cooperate to promote reverse cholesterol transport from the periphery back to the liver [(13)]. The LXRβ agonist also induces the cholesterol transporter in ABCG1 in cultured cholangiocytes. ABCG1 cooperates with ABCA1 by addition of additional lipids to the apolipoprotein ApoAI, resulting in maturation of HDL particles [(14)]. Thus, our data suggest that ABCA1 and ABCG1 could cooperate in basolateral extrusion of cholesterol from cholangiocytes.

Since ABCA1 mediates basolateral extrusion of cholesterol from cholangiocytes, we also studied mechanisms that could promote transport of cholesterol across the apical membrane from bile. Several studies indicated that the NPC1L1 cholesterol transporter may be expressed in biliary system [(9, 15)] andwe found that NPC1L1 transcripts and protein are expressed in cultured cholangiocytes and detected NPC1L1 protein in bile ducts in rat. We find that NPC1L1 is induced by PPARδ agonist (GW) and this effect is accompanied by GW-dependent increases in cholesterol uptake across the apical membrane of polarized cultured cholangiocytes. Moreover, these actions are blocked by ezetimibe, which targets NPC1L1. Evidence from NPC1L1 knockout mice is consistent with the idea that NPC1L1 mediates reabsorption of cholesterol from bile; these mice have a 41% increase in biliary cholesterol compared to wild type mice[(16)].

Together, our findings suggest a model for coordinated NR-dependent cholesterol flux through cholangiocytes (Fig. 6). First, PPARδ-dependent increases in NPC1L1 expression promote apical cholesterol import from bile into the cholangiocyte. Second, LXRβ/PPARδ-dependent increases in ABCA1 expression and, possibly, LXRβ-dependent increases in ABCG1 expression promote cholesterol efflux from the basolateral membrane [(17)]. Since cholangiocytes express CYP27 it is likely that elevated levels of cholesterol cholangiocytes result in increased production of oxysterol LXRβ ligands, which would induce ABCA1/ABCG1 dependent cholesterol export from cells[(18)] and the fact that our experiments with cholesterol-loading into cultured cholangiocytes mimic effects of LXR agonist treatment supports this idea.

To our knowledge, our data represents the demonstration that PPARδ or any ligand-dependent NR plays a direct role in NPC1L1 expression in cholangiocytes. There is evidence that PPARα activation results in decreased NPC1L1 expression in intestine[(11)], but this effect appears to be indirect since it is delayed and there is no identifiable PPARα effect on the NPC1L1 promoter. In this regard it will be interesting to ask whether similar effects

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of PPARδ regulation of NPC1L1 with effects on cholesterol excretion occur in other cell types, including enterocytes.

We do not know why cholangiocytes exhibit the capacity for NR-dependent cholesterol flux, one possibility is that cholangiocytes prevent excess cholesterol accumulation in the bile and that coordinated cholesterol flux protects the cholangiocyte from high levels of intracellular cholesterol and its oxidized metabolites[19], which are toxic to cells[20] due to induction of unfolded protein response [21] and FAS-dependent apoptosis[22]. We further recognize that strategies to manipulate vectorial cholesterol transport may be useful in cholestatic liver disease, in which local abnormalities in lipid metabolism promote cholangiocyte injury with severe toxic effects on local hepatocytes and increased levels of bile acids in the circulation, and possibly in treatment and prevention of gallstone disease, where increased concentrations of biliary cholesterol promote stone formation. Since NRs are major targets for pharmaceutical development, it is important to consider possible applications of NR ligands in these conditions. LXR ligand treatment alone is not likely to be useful because LXR agonists increase cholesterol saturation index in the bile, leading to formation of cholesterol crystals in the gallbladder [23]; this lithogenic effect is caused by increased biliary efflux of cholesterol and phospholipids, a result of increased expression of hepatic cholesterol transporters Abcg5, Abcg8 and Abca1. However, it may be possible to use PPARδ ligands or combinations of PPARδ and LXRβ ligands to manipulate bile cholesterol. Since other NRs are expressed in cholangiocytes, it will also be interesting to understand effects of these receptors on cholesterol transport and reabsorption from bile.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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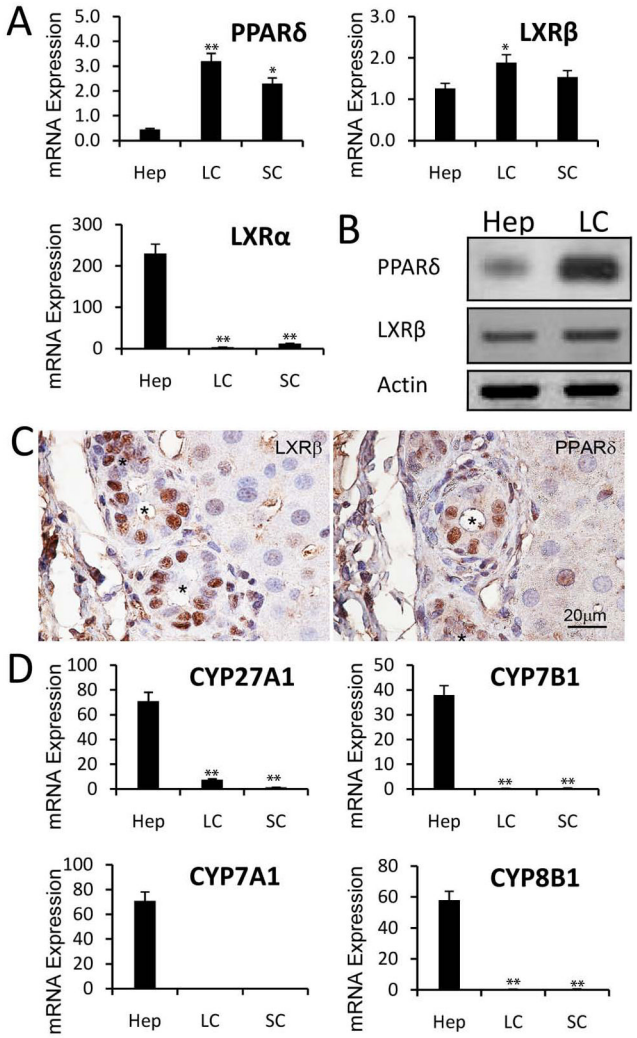

Figure 1.

NRs and lipid metabolic gene expression in cholangiocytes. A: The expression of PPARδ, LXRβ and LXRα in rat hepatocytes (Hep), large cholangioacytes (LC) and small cholangiocytes (SC) as detected by RT-qPCR. Data presented as means of three assays performed in duplicate ± SD and normalized to cyclophilin. *Significant differences by Student's t-test (* P< 0.05, ** P<0.01 versus hepatocytes). B: Comparison of PPARδ and LXRβ proteins levels from isolated hepatocytes and large cholangiocytes by determined by Western blot analysis. C: LXRβ and PPARδ localization in rat liver. LXRβ- and PPARδ-positive immunoreactivity is observed in bile ducts (star) of rat liver sections (Scale bars: 20 μm). D: The expression of bile acid metabolic genes in isolated rat hepatocytes, large cholangiocytes and small cholangiocytes. Data presented as means of three assays performed in duplicate ± SD and normalized to cyclophilin. *Significant differences by Student's t-test (* P< 0.05, ** P<0.01 versus hepatocytes).

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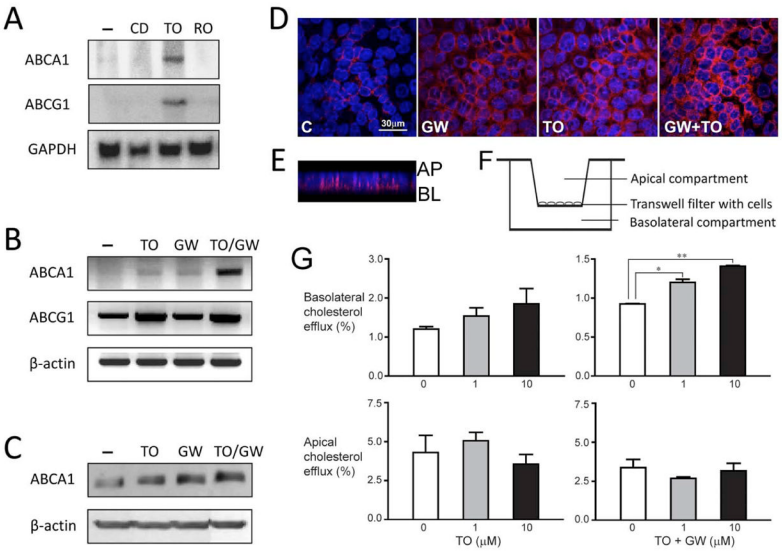

Figure 2.

LXRβ and PPARδ dependent gene expression in cholangiocytes. A. Northern blot of total RNA prepared from NRCs grown for 24hr in serum-free medium and treated +/− DMSO vehicle or indicated ligands: LXR ligand: T0901317 (TO); FXR ligand: CDCA (CD); and PPARγ ligand: rosiglitazone (RO). B. RT-PCR analysis on the effects of treatment of cholangiocytes with TO and PPARδ ligand GW501516 (GW). C. The expression of ABCA1 protein in cholangiocyte treated with TO, GW and a combination of TO and GW by Western blot analysis. D. Confocal fluorescence micrograph (x-y plane) of NRCs cultured stained with ABCA1 antibody (Red) and DAPI to visualize the nucleus (Blue) (Scale bars: 30 μm). E. Confocal fluorescence micrograph(x-z plane) showed the distribution of ABCA1 molecules alone the basolateral membrane (AP: apical membrane;BL: basolateral membrane, Blue: nuclei. F. A schematic of the transwell experimental design. G. Total radiolabeled cholesterol extruded into apical or basolateral compartments from NRCs. The results are shown as percentage efflux [(counts in apical or basolateral media/total counts in cells and in media from both compartments) × 100]. Values are mean+ S.E.M. from duplicate determinations, representative of three experiments. * P< 0.05, ** P<0.01 versus no ligand.

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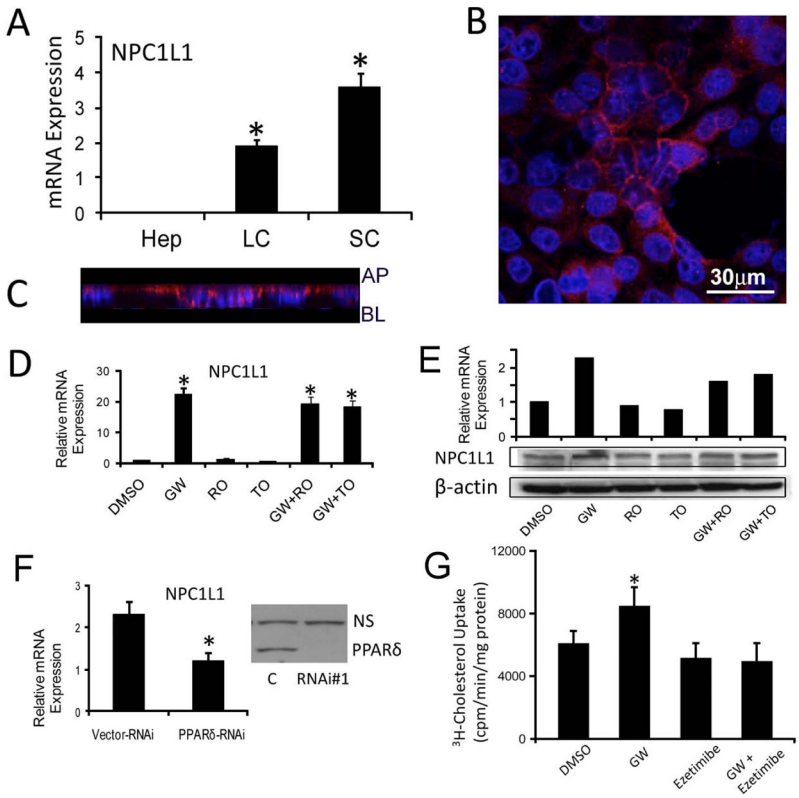

Figure 3.

The expression of NPC1L1 in cholangiocytes. A. NPC1L1 mRNA expression levels in isolated rat hepatocytes (Hep), large and small cholangiocytes (LC and SC). Data presented as means of three assays performed in duplicate ± SD and normalized to cyclophilin. * indicates a significant differences as determined by Student's t-test ($P < 0.05$). B. NPC1L1 protein localization in NRCs by confocal fluorescence micrograph(x-y plane); NPC1L1 antibody (Red) and DAPI was utilized to visualize the nucleus (Blue) (Scale bars: 30 μm). C. The distribution of NPC1L1 protein to the apical membrane (AP: apical membrane; BL: basolateral membrane, Blue: nuclei) by confocal fluorescence microscopy. D. The expression of NPC1L1 mRNA by RT-qPCR analysis of NRC +/− indicated ligands. E. The expression of NPC1L1 protein by Western blot analysis of NRC +/− indicated ligands. Graphs show the relative levels of NPC1L1 protein under the indicated conditions determined by densitometry. F. RT-qPCR analysis of NPC1L1 transcripts in NMC transfected with pSilencer 2.1-U6-hygro negative control or similar vector expressing PPARδ-RNAi; an immunoblot confirming PPARδ knockdown is shown on the right with a non-specific band indicated as NS. G. The effects of GW and Ezetimide on apical cholesterol absorption in cholangiocytes. Results are shown as percentage efflux [(counts in apical media/total counts in cells and in media from both compartments) × 100]. Values are mean+ S.E.M. from duplicate determinations, representative of three experiments. * $P< 0.05$ versus no ligand.

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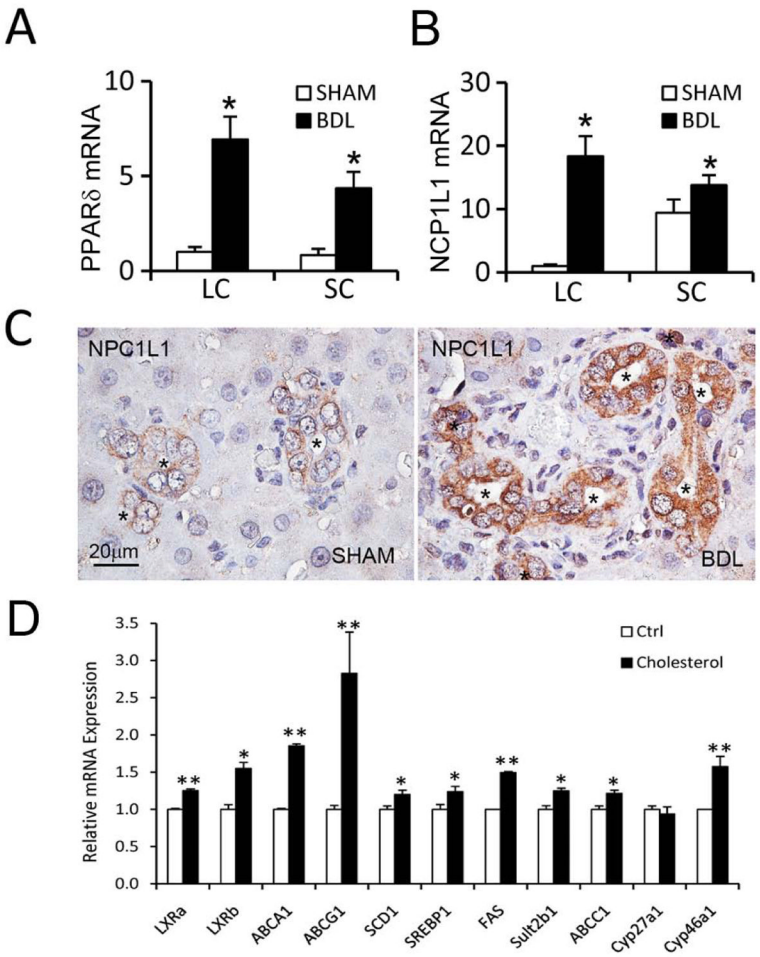

Figure 4.

Lipid metabolism in BDL mice. A&B. The expression of PPARδ and NPC1L1 mRNAs in large cholangiocytes (LC) and small cholangiocytes (SC) isolated from sham and BDL rats. C. The expression of NPC1L1 protein in bile ducts of sham and BDL rats by immunohistochemistry (Scale bars: 30 μm). D. The expression of LXR target genes in NRCs treated with water-soluble cholesterol for 24 hours. Data presented as means of three assays performed in duplicate ± SD and was normalized to cyclophilin. *Significant differences by Student's t-test (* P< 0.05, ** P<0.01 versus no cholesterol).

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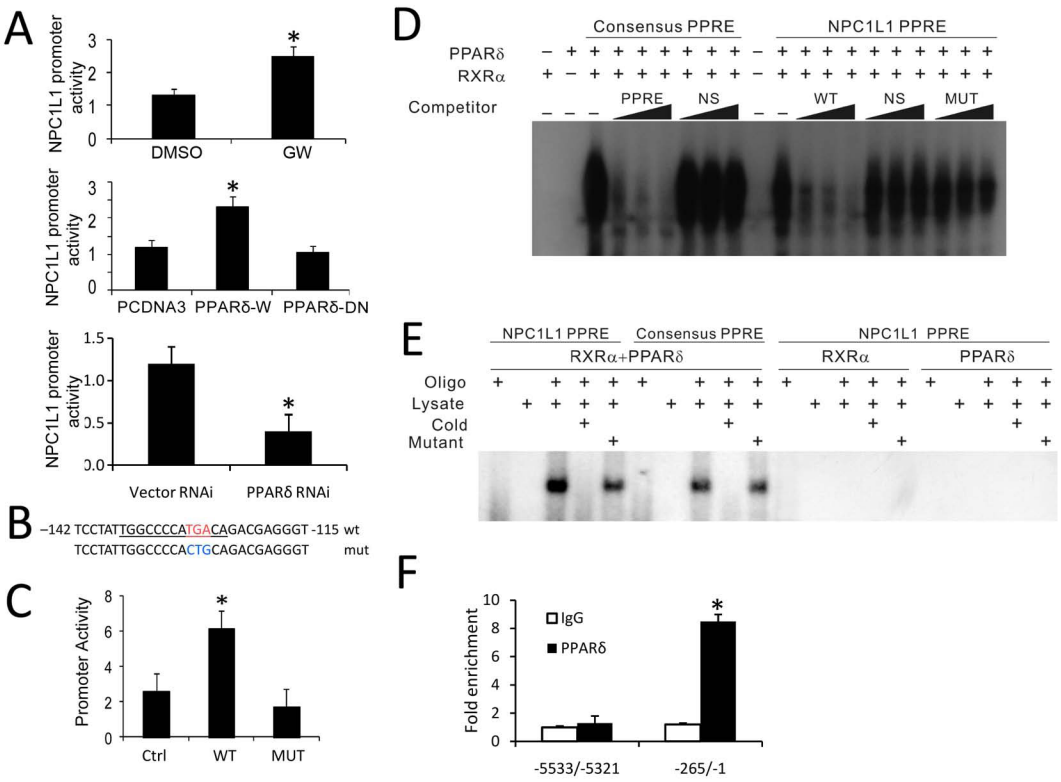

Figure 5.

PPARδ regulation of NPC1L1 promoter activity. A. Analysis of NMC transfected with a NPC1L1 promoter-luciferase construct. The panels represent cells treated +/− GW (top), transfected with vector control, or expression vectors for wild type (PPARδ –W) or a dominant negative (PPARδ –DN) PPARδ (middle) or treated with PPARδ RNAi (bottom) for 24h. *p<0.05 versus vector control; n=6. B. The sequence of the putative PPRE in the NPC1L1 promoter and mutant. C. Mutations in the PPRE abolished induction by GW. D. EMSA using nuclear extracts of AAV293 cells that co-transfected with PPARδ and RXRα and incubated with radiolabeled NPC1L1 PPRE probe. E. EMSA using *in vitro* translated NRs +/− 100-fold excess of wild type or mutant oligos. F. ChIP assays from NMC cells transfected with flag-PPARδ and treated with GW. Immunoprecipitated (IP) DNA analyzed by RT-qPCR with primers for designated promoter fragments (-265 to -1) or control region (−5533 to −5321).

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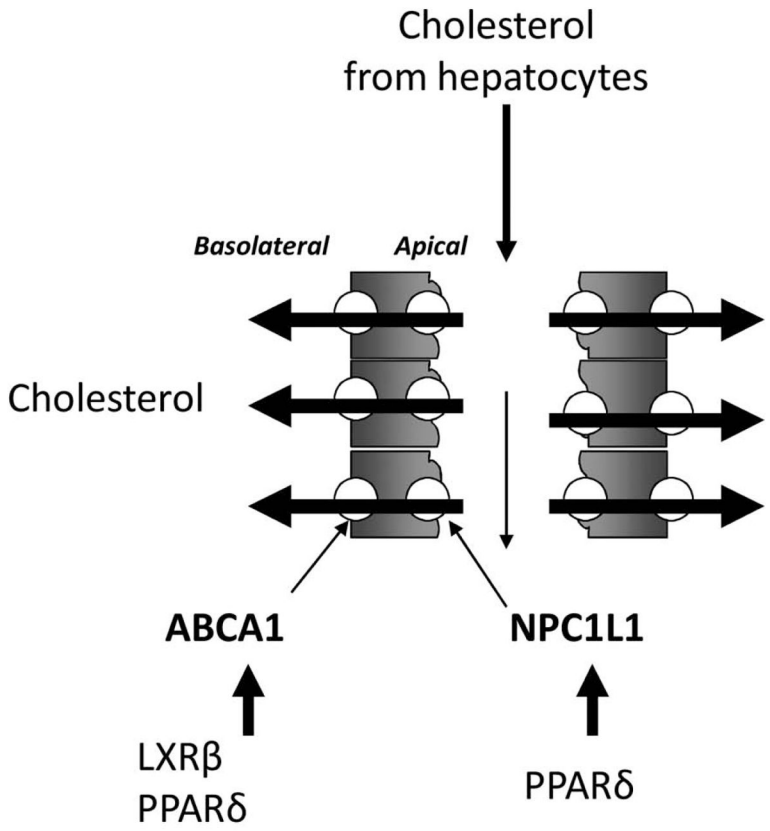

Figure 6.
A model for the role of NRs in the transport of cholesterol in cholangiocytes. Bile containing cholesterol and other components enters the bile ducts; the cholangiocytes reabsorb cholesterol through NPC1L1 on the apical membrane and efflux cholesterol into the circulation through ABCA1 on the basolateral membrane in response to combined activation of PPARδ and LXRβ.