HHS Public Access

Author manuscript

Lab Invest. Author manuscript; available in PMC 2016 May 23.

Published in final edited form as:

Lab Invest. 2016 February ; 96(2): 230–238. doi:10.1038/labinvest.2015.141.

Nuclear Hormone Receptor LXRα Inhibits Adipocyte Differentiation of Mesenchymal Stem Cells with Wnt/beta-catenin Signaling

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Abstract

Nuclear hormone receptor liver X receptor-alpha (LXRα) plays a vital role in cholesterol homeostasis and is reported to play a role in adipose function and obesity although this is controversial. Conversely, mesenchymal stem cells (MSCs) are suggested to be a major source of adipocyte generation. Accordingly, we examined the role of LXRα in adipogenesis of MSCs. Adult murine MSCs (mMSCs) were isolated from wild type (WT) and LXR-null mice. Using WT mMSCs, we further generated cell lines stably overexpressing GFP-LXRα (mMSC/LXRα/GFP) or GFP alone (mMSC/GFP) by retroviral infection. Confluent mMSCs were differentiated into adipocytes by the established protocol. Compared with MSCs isolated from WT mice, MSCs from LXR-null mice showed significantly increased adipogenesis, as determined by lipid droplet accumulation and adipogenesis-related gene expression. Moreover, mMSCs stably overexpressing GFP-LXRα (mMSC/LXRα/GFP) exhibited significantly decreased adipogenesis compared with mMSCs overexpressing GFP alone (mMSC/GFP). Since Wnt/beta-catenin signaling is reported to inhibit adipogenesis, we further examined it. The LXR-null group showed significantly decreased

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Conflict of interest

None declared.

Wnt expression accompanied by a decrease of cellular beta-catenin (vs. WT). The mMSC/LXRα/GFP group exhibited significantly increased Wnt expression accompanied by an increase of cellular beta-catenin (vs. mMSC/GFP). These data demonstrate that LXRα has an inhibitory effect on adipogenic differentiation in murine mesenchymal stem cells with Wnt/beta-catenin signaling. These results provide important insights into the pathophysiology of obesity and obesity related consequences such as metabolic syndrome and may identify potential therapeutic targets.

Keywords

adipocyte; mesenchymal stem cell; LXRα; Wnt/beta-catenin signaling; differentiation

Liver X receptor-alpha (LXRα) is an oxysterol-regulated nuclear hormone receptor and plays a vital role in cholesterol and lipid homeostasis[1–9]. The expression of LXRα is restricted to tissues known to play important roles in lipid metabolism, such as the liver, adipose tissue, kidney, small intestine, skeletal muscle, and adrenal gland, whereas LXRβ is expressed ubiquitously. The functions of LXRs in liver have been studied extensively. A recent study demonstrated that an LXR agonist attenuated endotoxin-induced liver injury in high-fat diet-induced hepatic steatosis in mice[11]. New evidence also points to an obesity-related effect of LXRs. Gao et al reported that an LXR agonist protected mice against the development of high-fat diet-induced obesity[12]. In addition, recent studies imply a role for LXRα in adipose tissue because of the following evidence. First of all, expression of LXRα is high in adipocytes[13]. Second, expression of LXRα is increased during adipogenesis[14–16]. Finally, expression levels of many LXRα target genes are also high in adipocytes[17]. However, the role of LXRα in adipose tissue is not well defined. Interestingly, the role of LXRα in adipogenic differentiation is suggested by the studies using preadipocytes although those studies are limited and inconsistent[14, 15].

Obesity is one of the most important risk factors of metabolic syndrome. Severe obesity is associated with both an increase in adipose cell size and increased adipose cell number[18–20]. With respect to an increase in adipose cell number, mesenchymal stem cells (MSCs) are of note. Recent studies revealed that adult adipose tissue contains stem cells and they are much like bone marrow derived mesenchymal stem cells[21–23]. In addition, Liechty et al showed that human MSCs transplanted into fetal sheep marrow differentiated and incorporated into normal adult adipose tissue[24]. Moreover, Crossno et al reported that adipocyte progenitor cells originating from bone marrow contribute to an increase in adipocyte number [25]. Taken together, mesenchymal stem cells (MSCs) are suggested to be a major source of adipocyte generation; however, the effect of LXRα on MSC differentiation to adipocyte is unknown.

Wnt/beta-catenin signaling is one of the most important regulators of mesenchymal stem cell fate [26, 27]. This signaling is also known to play a vital role in adipogenic differentiation of preadipocytes [28, 29]. Activation of Wnt/beta-catenin signaling blocks adipogenesis [28–30], whereas inhibition of endogenous Wnt signaling promotes adipogenesis [29, 31]. Thus Wnt/beta-catenin signaling is considered as a brake to adipogenic differentiation. Especially, Ross et al reported that Wnt 10b is the most important endogenous regulator of adipogenesis [29].

In this study, we test our hypothesis that LXRα plays an important role in adipogenic differentiation of mesenchymal stem cells. Our data demonstrate that the deletion of LXR accelerates adipogenesis and the overexpression of LXRα inhibits adipogenic differentiation. Furthermore, this inhibitory effect of LXRα on adipogenesis is associated with Wnt/beta-catenin signaling which plays a vital role to inhibit adipogenesis.

Materials and Methods

Murine mesenchymal stem cell isolation and culture

All animal procedures were approved by the Duke University Institutional Animal Care and Use Committees. Murine mesenchymal stem cells (mMSCs) were isolated by their adherence to plastic as previously described [32_38]. Bone marrow was collected from 12-week-old male LXRα/LXRβ double knock out (LXRKO)[39] and wild type C57BL/6 mice by flushing femurs and tibias with the mMSC growth medium constituted of Minimum Essential Medium α (MEMα) with GlutaMAX (Invitrogen, Carlsbad, CA), 20 % fetal bovine serum (FBS), and penicillin/streptomycin (100 U/ml and 100 μg/ml, respectively). The bone marrow cells were then filtered through a 40 μm nylon mesh filter. Mononuclear cells were separated by gradient density using Ficoll-Paque Plus (Amersham Bioscience, Uppsala, Sweden). Cells were then washed twice with phosphate-buffered saline (PBS) and plated in plastic dishes. After 3 days, non-adherent cells were removed by two washes with PBS and adherent cells were further cultured in the mMSC growth medium. Cells were then propagated in culture. Medium was changed every 3 days.

Confluent cells were incubated in adipogenic medium (Alpha-MEM-GlutaMax medium supplemented with 10 μg/ml insulin, 0.1 μmol/l dexamethasone, 50 μmol/l 3-isobutyl-1-methyl-xanthine (IBMX), 20 μmol/l indomethacin, 20% FBS, 100 U/ml penicilline, and 100 μg/ml streptomycin) for 9 days. Medium was changed every 3 days. Cells were harvested at day 9 after the initiation of differentiation.

To quantitate adipocyte differentiation, AdipoRed Assay Reagent (Cambrex, Walkersville, MD) was employed following the manufacturer's protocol. AdipoRed is fluorescent dye which binds to lipid droplets. Total fluorescent signal was quantitated by the fluorimeter.

Quantitative RT-PCR

Total RNA was isolated by the Tri Reagent (Sigma) and further purified using RNeasy columns (Qiagen, Valencia, CA). The concentration of RNA was determined using spectrophotometry. First-strand cDNA was synthesized from total RNA using High Capacity cDNA Archive Kit (Applied Biosystem, Foster City, CA) following the manufacturer's protocol. The taqman probe primer system (Applied Biosystem) was used for quantitative RT-PCR. The primer and probe sets for murine peroxisome proliferator-activated receptor-gamma (PPAR-gamma), fatty acid synthase (FAS), Wnt1, Wnt3a, Wnt5a, Wnt10b, and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Applied Biosystems. The primer and probe sets for human Wnt10b, LXRα, and GAPDH were also purchased from Applied Biosystems. TaqMan PCR was performed using ABI Prism 7700 Sequence Detection System as instructed by manufacturer (Applied

Biosystem). Target gene mRNA expression was normalized to GAPDH mRNA expression and the relative amounts of all mRNAs were calculated using the comparative CT method [40].

Cell lines stably overexpressing GFP or GFP-LXRα

For the generation of murine mesenchymal stem cell lines stably expressing GFP or GFP-LXRα, LXRα cDNA was cloned into an MSCV-IRES-GFP plasmid backbone. Retroviral particles were then obtained by tripartite transfection in HEK 293 T cells and concentrated by ultracentrifugation. Wild type C57BL/6 murine MSCs were infected with retroviral particles in the presence of Polybrene (Sigma). Each infection was repeated twice. Pools of infected cells were then subcultured.

Immunoblotting

Cells were lysed at 4 °C with RIPA Lysis Buffer (Upstate, Lake Placid, NY). Equal amounts of proteins (20 μg per lane) were separated by NuPAGE 4–12 % Bis-Tris Gel (Invitrogen, Carlsbad, CA) electrophoresis. Protein fractions were then electrophoretically transferred onto a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked with Blocker/Diluent solution (Western Blot Kit, Invitrogen). Then the membrane was incubated with rabbit polyclonal antibody to human beta-catenin (Cell Signaling Technology, Inc., Danvers, MA) for 1 hour at room temperature. After washing in wash buffer (Western Blot Kit, Invitrogen), the membrane was incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit immunoglobulin G (Western Blot Kit, Invitrogen) for 1 hour at room temperature. The antigen antibody-peroxidase complex was visualized using the ECL chemiluminescence solution (Western Blot Kit, Invitrogen). The blot was subsequently stripped with Re-Blot Plus Western Blot Recycling Kit (Chemicon International, Inc., Temecula, CA) and rehybridized with an anti-GAPDH antibody (Santa Cruz Biotechnology, INC.) as a control for protein loading. Densitometric quantitation was performed using the ImageJ software (NIH, Bethesda, MD). Band intensity values of beta-catenin were standardized to those of GAPDH.

Analysis of cellular responses of human mesenchymal stem cells to LXR or Wnt ligand

Human mesenchymal stem cells (hMSCs) were obtained from Cambrex (Walkersville, MD). Cells were positive for CD105, CD166, CD29, and CD44 and negative for CD14, CD34, and CD45. Human MSCs were grown in hMSC growth medium (Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 4 mmol/L L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin). The growth medium was changed every 3 days. The natural LXR agonist 22-hydroxycholesterol (22OH-C) was purchased from Sigma. Recombinant human Wnt1 protein was purchased from Abcam (Cambridge, MA). Cells were subjected to serum depletion for 16 hours. Then, cells were exposed to 22OH-C (0.01 μmol/L) or vehicle for 6 hours, after which Wnt10b mRNA expression was determined using quantitative RT-PCR. For analysis of Wnt ligand, cells were serum depleted for 16 hours, as above, before being exposed to human recombinant Wnt1 protein (final concentration 20 ng/mL) or vehicle for 6 hours. Cells were then harvested and total RNA was analyzed by quantitative RT-PCR to determine LXRα mRNA expression.

Statistical analysis

All statistical procedures were done using the Statgraphics Plus version 5.0 software (StatPoint, Inc., Herndon, VA). Comparisons between two groups were made using Student's t test and the differences were considered to be significant when $P < 0.05$.

Results

Deletion of LXR promotes adipocyte differentiation of murine MSCs

Murine MSCs (mMSCs) were isolated from the bone marrow of wild type (WT) and LXRα/LXRβ double knock out (LXRKO) mice. Cells were incubated in adipogenic medium (Alpha-MEM-GlutaMax medium supplemented with 10 μg/ml insulin, 0.1 μmol/l dexamethasone, 50 μmol/l 3-isobutyl-1-methyl-xanthine (IBMX), 20 μmol/l indomethacin, 20% FBS, 100 U/ml penicilline, and 100 μg/ml streptomycin) for 9 days. As shown in Figure 1A, differentiated adipocytes exhibited lipid droplets in cytoplasm. AdipoRed fluorescent dye (Cambrex, Walkersville, MD) was employed to stain lipid droplets (Fig. 1B). Adipogenesis was quantitated by measuring fluorescence using AdipoRed fluorescent dye. As shown in Figure 1C–E, lipid droplet accumulation was significantly greater in differentiated adipocytes from LXRKO mMSCs than those from the WT group (147.1 ± 24.9 %; $n = 12$; $P < 0.05$), suggesting an anti-adipogenic effect of LXR on mMSCs.

Deletion of LXR increases the expression of adipocyte related genes

We also examined the expression of adipocyte related genes by quantitative RT-PCR. Murine MSCs from WT and LXRKO mice were incubated in adipogenic medium for 9 days. Total RNA was analyzed by real-time RT-PCR for mRNA expression of PPAR-gamma and Fatty acid synthase on day 9. Consistent with the results regarding lipid droplet accumulation, PPAR-gamma and fatty acid synthase expression was greater in the LXRKO compared with WT group (2.33 ± 0.45- and 1.60 ± 0.24-fold increases, respectively; $n = 4$; $P < 0.05$; Fig. 2A and B).

Overexpression of LXRα inhibits adipocyte differentiation of murine MSCs

We next generated murine MSC cell lines stably expressing GFP-LXRα (mMSC/LXRα/GFP) by retroviral infection. Cell lines expressing GFP alone (mMSC/GFP) were also generated as control. Cells were incubated in adipogenic medium for 9 days. Adipogenesis was quantitated by assessing lipid droplet accumulation using AdipoRed fluorescent dye. As shown in Figure 3A–C, lipid droplet accumulation was significantly lower in differentiated adipocytes from the mMSC/LXRα/GFP group compared with the control mMSC/GFP group (59.2 ± 8.2%; $n = 12$; $P < 0.05$), suggesting an anti-adipogenic effect of LXRα on mMSCs.

Overexpression of LXRα decreases the expression of adipocyte related genes

Murine MSCs overexpressing GFP alone (mMSC/GFP) and mMSCs overexpressing GFP-LXRα (mMSC/LXRα/GFP) were incubated in adipogenic medium for 9 days. Total RNA was analyzed by quantitative RT-PCR for mRNA expression of PPAR-gamma and Fatty acid synthase on day 9. Consistent with the results regarding lipid droplet accumulation, PPAR-

gamma and fatty acid synthase expression was decreased in the mMSC/LXRα/GFP group compared with the control mMSC/GFP group (0.75 ± 0.04- and 0.36 ± 0.02-fold changes vs. the mMSC/GFP group, respectively; n = 4; $P < 0.05$; Fig. 2C and D).

Deletion of LXR decreases Wnt expression

Since Wnt/beta-catenin signaling, especially Wnt 10b, is reported to inhibit adipogenesis, we examined the expression of Wnt genes by quantitative RT-PCR. Murine MSCs from WT and LXRKO mice were incubated in adipogenic medium for 9 days. Total RNA was analyzed by real-time RT-PCR for mRNA expression of Wnt1, Wnt3a, Wnt5a, and Wnt10b on day 9. As shown in Figure 4A–C, the LXRKO group showed a decreased expression of Wnt1, 5a, and 10b compared with the WT group (0.63 ± 0.14-, 0.54 ± 0.04-, and 0.25 ± 0.03-fold changes vs. the WT group, respectively; n = 4; $P < 0.05$). Especially, Wnt10b expression was remarkably decreased in the LXRKO group (Fig. 4C). Wnt 3a was not expressed in any samples (data not shown).

Deletion of LXR decreases cellular beta-catenin

To confirm the suppression of Wnt/beta-catenin signaling in the LXRKO group, we further examined cellular beta-catenin protein expression by immunoblotting. Murine MSCs from WT and LXRKO mice were incubated in adipogenic medium for 9 days. Cell lysates were isolated and subjected to immunoblotting analysis using antibodies for beta-catenin and GAPDH. As shown in Figure 4D, cellular beta-catenin protein expression was decreased in the LXRKO group compared with WT group. Densitometric quantification from three independent experiments revealed that cellular beta-catenin protein expression was significantly suppressed in the LXRKO compared with WT group (Fig 4E), suggesting that the inhibitory effect of LXR on the adipogenesis of mMSCs is associated with Wnt/beta-catenin signaling.

Overexpression of LXRα increases Wnt expression

We also examined the expression of Wnt genes in the mMSC/LXRα/GFP group and the mMSC/GFP group by quantitative RT-PCR. Murine MSC/GFP and mMSC/LXRα/GFP were incubated in adipogenic medium for 9 days. Total RNA was analyzed by real-time RT-PCR for mRNA expression of Wnt1, Wnt3a, Wnt5a, and Wnt10b on day 9. As shown in Figure 5A–C, the mMSC/LXRα/GFP group showed an increased expression of Wnt1, 5a, and 10b compared with the control mMSC/GFP group (3.45 ± 1.05-, 1.75 ± 0.14-, and 7.35 ± 0.90-fold increase, respectively; n = 4; $P < 0.05$). Especially, Wnt10b expression was remarkably increased in the mMSC/LXRα/GFP group (Fig. 5C). Wnt3a was not expressed in any samples (data not shown).

Overexpression of LXRα increases cellular beta-catenin

To confirm the activation of Wnt/beta-catenin signaling in the mMSC/LXRα/GFP group, we examined cellular beta-catenin protein expression by immunoblotting. Murine MSC/GFP and mMSC/LXRα/GFP were incubated in adipogenic medium for 9 days. Cell lysates were isolated and subjected to immunoblotting analysis using antibodies for beta-catenin and GAPDH. As shown in Figure 5D, cellular beta-catenin protein expression was increased in

the mMSC/LXRα/GFP group compared with the control mMSC/GFP group. Densitometric quantification from three independent experiments revealed that cellular beta-catenin protein expression was increased significantly in the mMSC/LXRα/GFP compared with the control mMSC/GFP group (Fig 5E). These results suggest that LXRα inhibits the adipogenesis of mMSCs in association with Wnt/beta-catenin signaling.

LXR agonist 22-hydroxycholesterol upregulates Wnt10b expression in human MSCs

Because an association between LXRα and Wnt/beta-catenin signaling was suggested in murine genetic models, we further examined cellular responses to LXR or Wnt ligand in human MSCs. After 16 hours of serum depletion, hMSCs were exposed to 0.01 μmol/L 22OH-C for 6 hours and Wnt10b mRNA expression evaluated using quantitative RT-PCR. As shown in Fig. 6A, Wnt10b expression increased significantly in 22OH-C- compared with vehicle-treated hMSCs (1.6 ± 0.2-fold increase; $n = 3$; $P < 0.05$). Next, the effects of human recombinant Wnt1 protein (final concentration 20 ng/mL) on LXRα expression in hMSCs were evaluated. After 16 hours of serum depletion and 6 hours of exposure to 20 ng/mL human recombinant Wnt1 protein, there was no significant change in LXRα expression in hMSCs compared with vehicle treatment (Fig. 6B; 1.0 ± 0.1-fold change vs vehicle treatment; $n = 3$).

Discussion

In this study, we have shown that the deletion of LXR accelerates adipogenesis and the overexpression of LXRα inhibits adipogenesis of murine mesenchymal stem cells (MSCs), demonstrating that LXRα has an inhibitory effect of adipogenesis of murine MSCs. Furthermore, we have demonstrated that the inhibitory effect of LXRα on adipogenesis is associated with Wnt/beta-catenin signaling which plays a vital role to inhibit adipocyte differentiation. Our data would suggest that the high level of expression of LXRα in adipose tissue[13] and the increase in LXRα mRNA levels during adipogenesis[14–16] contribute to the modulation of adipogenesis and obesity.

Increased adiposity predisposes individuals to both type 2 diabetes and metabolic syndrome. Severe obesity is associated with both an increase in adipose cell size and increased adipose cell number [18–20]. Recent studies demonstrate that adipose tissue contains stem cells, much like bone marrow stromal mesenchymal stem cells [21–23]. New fat cells arise from a pre-existing pool of adipose stem cells irrespective of age [23, 41]. In addition, Crossno et al reported that adipocyte progenitor cells originating from bone marrow contribute to development of new adipocytes in adult animals [25]. Taken together, MSCs are suggested to play an important role in the maintenance of the mass and function of adult adipose tissue. Accordingly, in vitro differentiation of MSCs towards the adipogenic lineage provides a useful means for studying in tissue culture the regulation of adipogenesis[42, 43].

Nuclear hormone receptor liver X receptor-alpha (LXRα) plays a vital role to control lipid and cholesterol homeostasis[1–9]. The roles of LXRα in the liver and in macrophages have been studied extensively. In a recent study, an LXR agonist attenuated endotoxin-induced liver injury in high-fat diet-induced hepatic steatosis in mice[11]. In addition, Gao et al reported that an LXR agonist protected mice against the development of high-fat diet-

induced obesity[12]. With regard to the relationship between adipocytes and cholesterol, adipocytes contain the largest pool of free cholesterol in the body [44] and recent studies have demonstrated high levels of LXRα expression in adipocytes [13], increased LXRα mRNA levels during adipogenesis[14–16], and high levels of expression of many LXRα target genes in adipocytes [17]. However, the role of LXRα in adipose tissue is not well defined. Despite the fact that the expression of LXRα increases during adipogenesis, the role of LXRα in adipose differentiation has remained conflicting. The studies using preadipocytes reported the function of LXRα as both positive and negative regulator of adipocyte differentiation [14, 15].

Ross et al have reported that LXR activity inhibits adipocyte differentiation and lipid accumulation in cultured preadipocytes [15]. They showed that ectopic expression of a constitutively active VP16-LXR-alpha fusion protein inhibited the differentiation of preadipocytes 3T3-L1 cells. In contrast to Ross et al, Juvet et al reported that LXR agonist treatment of differentiating adipocytes led to increased lipid accumulation [14]. Our observation that LXRα has inhibitory effects on adipogenesis is consistent with the former report. These inconsistent data may reflect differences in cell types, cell line variations, and culture conditions. In this study, we used MSCs instead of preadipocytes. Developmentally, MSCs are at the step between the undifferentiated multipotent embryonic stem cells and the preadipocytes[45–48]. Our data could suggest the effect of LXRα on the first step of adipogenesis.

Importantly, Beaven et al recently reported that LXRKO mice, when bred onto the OB background (ob/ob LXRα$\beta^{-/-}$ mice), exhibited increased total body adiposity compared with OB mice[49]. Conversely, LXRKO mice are likely to be protected against diet-induced obesity through increased energy expenditure[50]. However, adipose tissue-specific LXR-null mice have not yet been generated. Therefore, it is difficult to identify the precise roles of LXR in adipose tissue. Future studies are needed to elucidate the involvement of LXR in adipocyte differentiation and adipose biology.

We also studied the mechanism of the inhibitory effect of LXRα on adipogenesis of MSCs. Wnt/beta-catenin signaling is reported to be an important regulator of mesenchymal stem cell fate [26, 27]. This signaling is also known to play a vital role in adipogenic differentiation of preadipocytes [28, 29]. Activation of Wnt/beta-catenin signaling blocks adipogenesis [28–30]. On the other hand, inhibition of endogenous Wnt signaling is reported to promote adipogenesis [29, 31]. Therefore, Wnts are considered as a brake to adipogenic differentiation. In particular, Ross et al reported that Wnt 10b is the most important endogenous regulator of adipogenesis [29]. Longo et al created transgenic mice in which Wnt10b is expressed from the FABP4 promotor (FABP4-Wnt10b mice) and found that these mice had reduced adiposity and were resistant to diet-induced obesity[30]. In addition, Wright et al reported that expression of Wnt10b in adipose tissue reduces adiposity in the *ob/ob* obesity model[51]. Those authors also reported that Wnt10b protected against genetic obesity in mice due to ectopic expression of agouti (A^{y})[51]. In this study, the deletion of LXR decreases Wnt1, 5a, and 10b expression. Especially, Wnt10b expression is remarkably decreased. Moreover, the deletion of LXR decreases cellular beta-catenin protein expression, confirming that Wnt/beta-catenin signaling is suppressed. Consistent with the data of LXR deletion, overexpression of LXRα increases mRNA expression of Wnts, especially Wnt10b.

Overexpression of LXRα also increases cellular beta-catenin protein expression. The results of the present study suggest that the mechanism underlying the inhibitory effect of LXRα on the adipogenesis of MSCs is associated with Wnt/beta-catenin signaling. Furthermore, the data suggest that the action may be LXR ligand dependent. However, the precise mechanisms underlying the association between LXRα and Wnt/beta-catenin signaling remain unclear. Whether LXRα acts as a heterodimer or not and the role of LXRβ are unknown. In addition, there are a number of molecules that regulate adipogenesis, even though Wnt/beta-catenin signaling has been suggested as one of the most important pathways. Therefore, further experiments are needed to elucidate the mechanism(s) underlying the anti-adipogenic effect of LXRα more clearly.

In conclusion, the present study demonstrates that LXRα inhibits adipocyte differentiation of murine mesenchymal stem cells with Wnt/beta-catenin signaling. Such a role of LXRα might be physiologically important in the maintenance of the mass and function of adult adipose tissue. Our results support a role for LXRα in adipose tissue and further characterization of the role of LXRα in adipocyte biology is important for future research on obesity, with possible therapeutic implications for treatment of obesity and obesity related consequences, such as metabolic syndrome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of Funding: This work was supported, in whole or in part, by grants from the National Heart, Lung, and Blood Institute (RO1 HL35610, HL58516, HL72010, and HL73219 to Victor. J. Dzau), the Edna Mandel Foundation (to Victor. J. Dzau), the Leducq Foundation (to Victor. J. Dzau), the Ministry of Education, Culture, Sports, Science, and Technology of Japan (KAKENHI 21790745 to Kenichi Matsushita), the Japan Society for the Promotion of Science (KAKENHI 26461086 to Kenichi Matsushita), the Uehara Memorial Foundation (to Kenichi Matsushita), the Inoue Foundation for Science (to Kenichi Matsushita), and the Swedish Science Council (to Jan-Åke Gustafsson).

The authors thank Hui Mu for her technical assistance.

Abbreviations

GFP	green fluorescent protein
LXR	liver X receptor
MSCs	mesenchymal stem cells
WT	wild type

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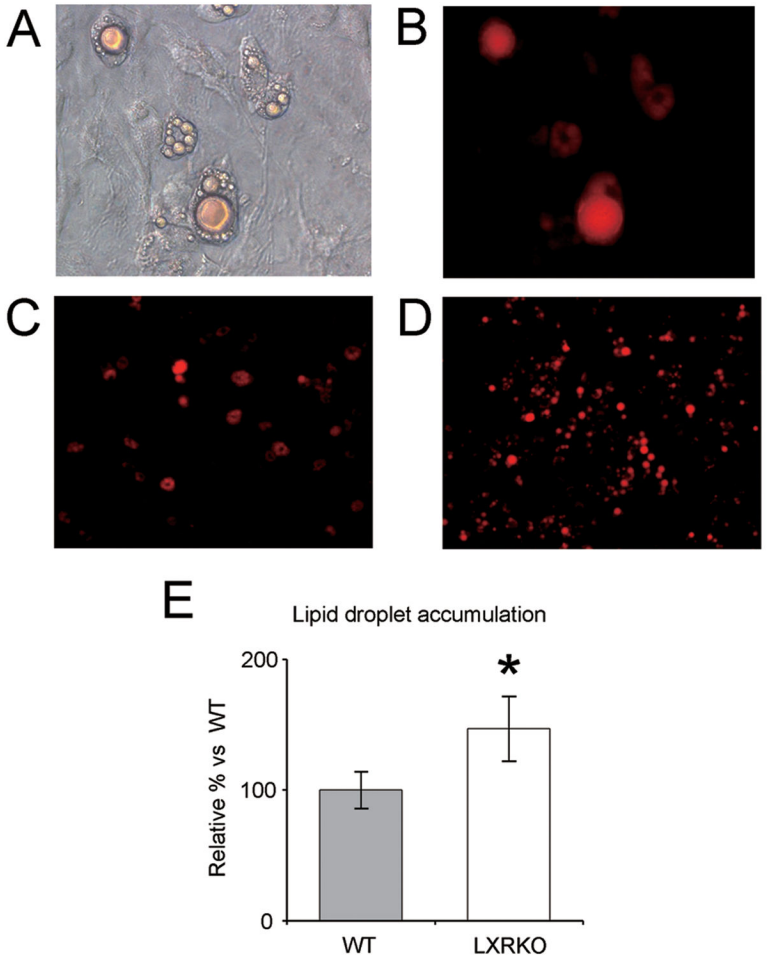

Figure 1. Deletion of LXR promotes adipocyte differentiation of murine MSCs

(A) and (B) are representative micrographs of differentiated adipocytes (day 9) from wild type murine MSCs (A: phase contrast, B: AdipoRed fluorescence. Original magnification, x 400). (C) and (D) are representative AdipoRed fluorescent micrographs of differentiated adipocytes (day 9) from WT (C) and LXRKO (D). Original magnification, x 200.

(E) Quantification of fluorescent lipid droplet accumulation. Relative % vs. WT group. Data are mean ± S.D. (n = 12). *P<0.05 vs. WT group.

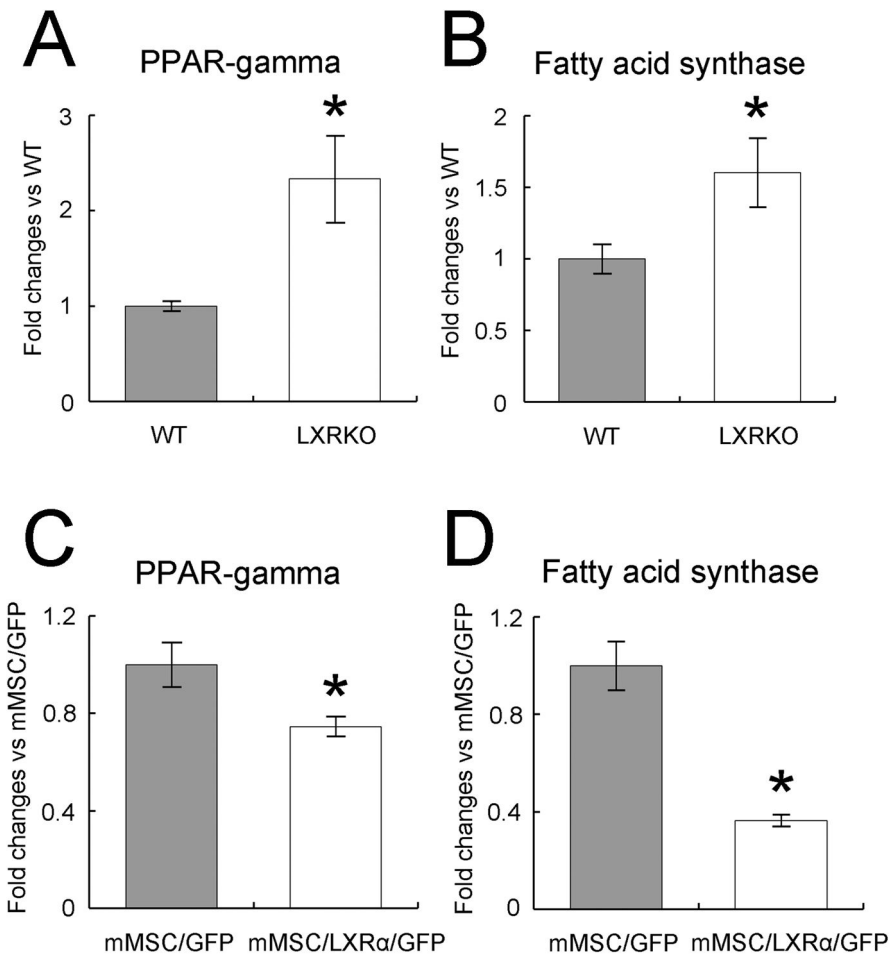

Figure 2. Expression of adipocyte related genes

(A) and (B): Murine MSCs from wild type (WT) and LXR-null (LXRKO) mice were incubated in adipogenic medium for 9 days. Total RNA was analyzed by quantitative RT-PCR for mRNA expression of PPAR-gamma (A) and Fatty acid synthase (B) on day 9. Fold changes vs. WT group. Data are mean ± S.E.M. (n = 4). *P<0.05 vs. WT group.

(C) and (D): Murine MSCs overexpressing GFP alone (mMSC/GFP) and mMSCs overexpressing GFP and LXRα (mMSC/LXRα/GFP) were incubated in adipogenic medium for 9 days. Total RNA was analyzed by quantitative RT-PCR for mRNA expression of PPAR-gamma (C) and Fatty acid synthase (D) on day 9. Fold changes vs. mMSC/GFP group. Data are mean ± S.E.M. (n = 4). *P<0.05 vs. mMSC/GFP group.

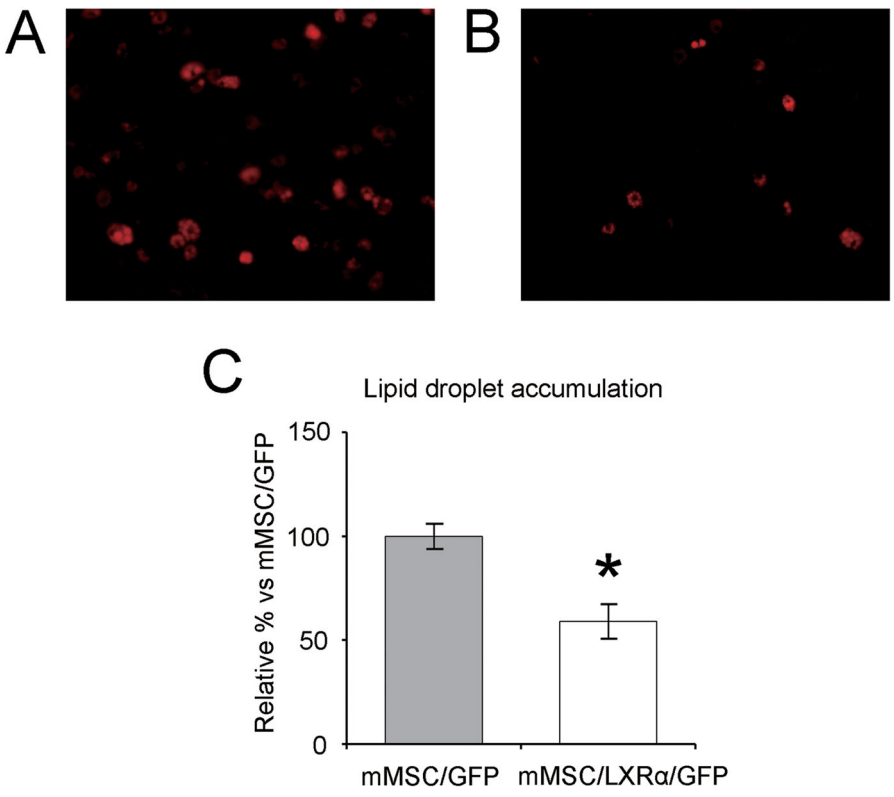

Figure 3. Overexpression of LXRα inhibits adipocyte differentiation of murine MSCs

(A) and (B) are representative AdipoRed fluorescent micrographs of differentiated adipocytes (day 9) from mMSCs overexpressing GFP alone (A: mMSC/GFP) and mMSCs overexpressing GFP and LXRα (B: mMSC/LXRα/GFP). Original magnification, x 200. (C) Quantification of fluorescent lipid droplet accumulation. Relative % vs. mMSC/GFP group. Data are mean ± S.D. (n = 12). *P<0.05 vs. mMSC/GFP group.

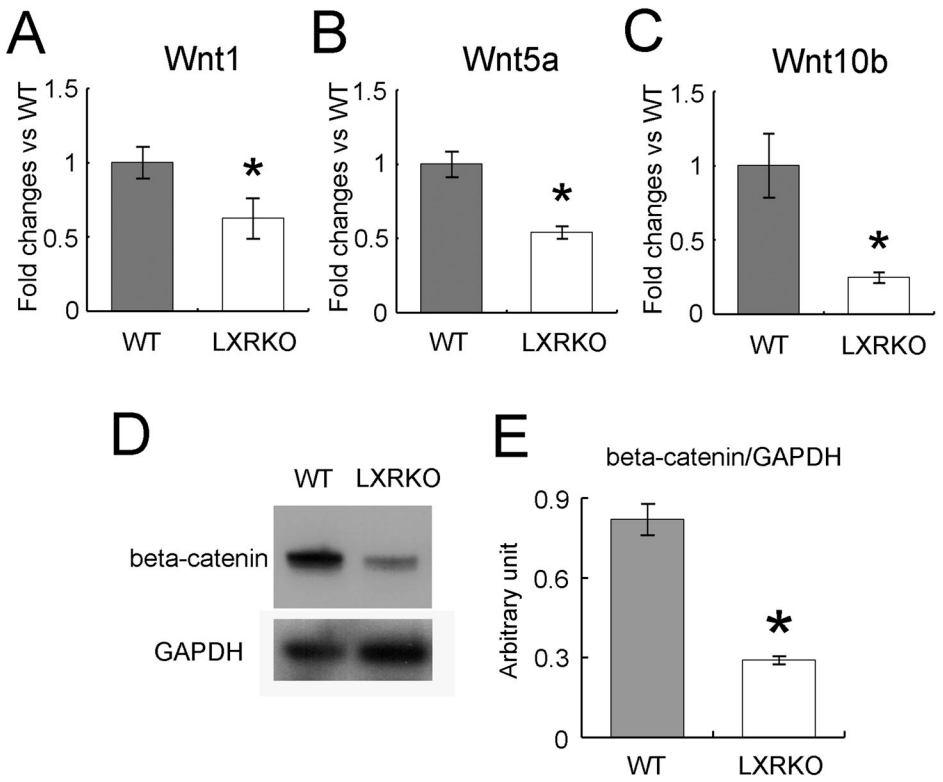

Figure 4. Deletion of LXR decreases Wnt expression and cellular beta-catenin

Murine MSCs from wild type (WT) and LXR-null (LXRKO) mice were incubated in adipogenic medium for 9 days. (A)–(C): Total RNA was analyzed by quantitative RT-PCR for mRNA expression of Wnt1 (A), Wnt5a (B), and Wnt10b (C) on day 9. Fold changes vs. WT group. Data are mean ± S.E.M. (n = 4). *P<0.05 vs. WT group. (D) and (E): Cell lysates were isolated and subjected to immunoblotting analysis using antibodies for beta-catenin and GAPDH. (D) is a representative immunoblot from three independent experiments. Densitometric quantitation from three independent experiments is shown in the bar graph (E). Data are mean ± S.E.M.; *P<0.05 vs. WT group.

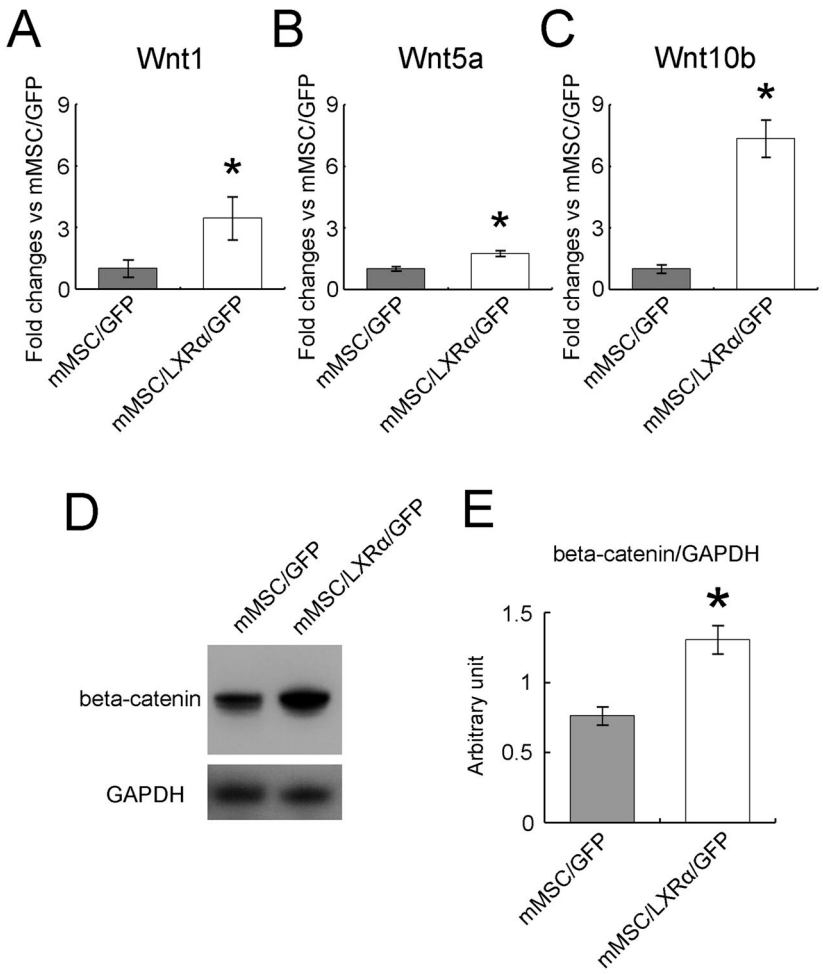

Figure 5. Overexpression of LXRα increases Wnt expression and cellular beta-catenin

Murine MSCs overexpressing GFP alone (mMSC/GFP) and mMSCs overexpressing GFP and LXRα (mMSC/LXRα/GFP) were incubated in adipogenic medium for 9 days. (A)–(C): Total RNA was analyzed by quantitative RT-PCR for mRNA expression of Wnt1 (A), Wnt5a (B), and Wnt10b (C) on day 9. Fold changes vs. WT group. Data are mean ± S.E.M. (n = 4). *P<0.05 vs. mMSC/GFP group. (D) and (E): Cell lysates were isolated and subjected to immunoblotting analysis using antibodies for beta-catenin and GAPDH. (D) is a representative immunoblot from three independent experiments. Densitometric quantitation from three independent experiments is shown in the bar graph (E). Data are mean ± S.E.M.; *P<0.05 vs. mMSC/GFP group.

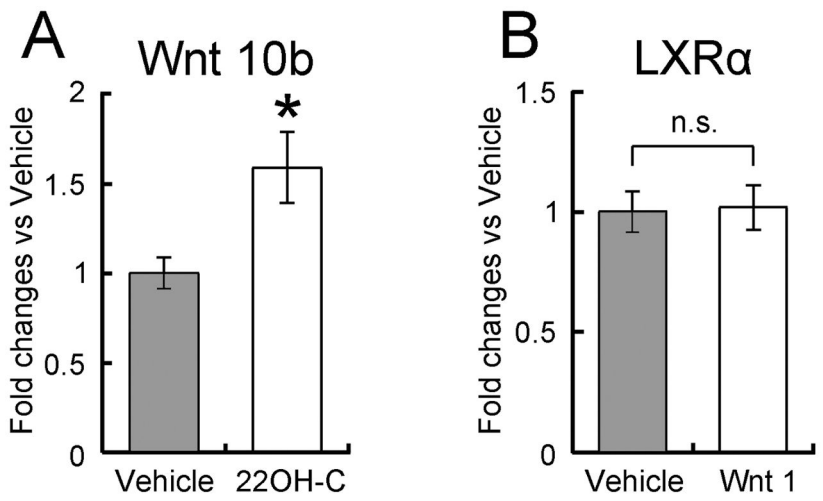

Figure 6. Cellular responses to LXR or Wnt ligand in human MSCs

After human MSCs had been serum starved for 16 hours, LXR ligand 22OH-C (final concentration 0.01 μmol/L) or human recombinant Wnt1 protein (final concentration 20 ng/mL) was added to medium and cells were cultured for a further 6 hours before being harvested. Total RNA was analyzed by quantitative RT-PCR to determine Wnt10b (A) or LXRα (B) mRNA expression. Data show fold changes vs. the vehicle-treated group (Vehicle). Data are mean ± S.E.M. (n = 3). *P<0.05 vs. vehicle-treated group. n.s., statistically non-significant.