Nuclear Receptor

BioMed Central

Research



# Establishment of a monoclonal antibody for human LXRα: Detection of LXRα protein expression in human macrophages

Yuichiro Watanabe[1,2], Toshiya Tanaka[1], Yasutoshi Uchiyama[1], Tetsu Takeno[2], Akashi Izumi[1], Hisahiko Yamashita[3], Junko Kumakura[3], Hiroko Iwanari[3], Jiang Shu-Ying[4], Makoto Naito[4], David J Mangelsdorf[5], Takao Hamakubo[1] and Tatsuhiko Kodama*[1]

Address: [1]Department of Life Sciences, The University of Tokyo Research Center for Advanced Science and Technology (RCAST), Tokyo, Japan, [2]Immunology Research Dept, Tokyo New Drug Research Laboratories II, Pharmaceutical division, Kowa Co, Ltd, Tokyo, Japan, [3]Institute of Immunology Co., Ltd, Tokyo, Japan, [4]Department of Cellular Function, Division of Cellular and Molecular Pathology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan and [5]Department of Pharmacology, Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Texas, USA

Email: Yuichiro Watanabe - watanabe@med.rcast.u-tokyo.ac.jp; Toshiya Tanaka - tanaka@med.rcast.u-tokyo.ac.jp; Yasutoshi Uchiyama - uchiyama@med.rcast.u-tokyo.ac.jp; Tetsu Takeno - t-takeno@kowa.co.jp; Akashi Izumi - kodama@lsbm.org; Hisahiko Yamashita - kodama@lsbm.org; Junko Kumakura - kodama@lsbm.org; Hiroko Iwanari - iwanari@tokumen.co.jp; Jiang Shu-Ying - s-jiang@med.niigata-u.ac.jp; Makoto Naito - mnaito@med.niigata-u.ac.jp; David J Mangelsdorf - dmange@biochem.swmed.edu; Takao Hamakubo - hamakubo@lsbm.org; Tatsuhiko Kodama* - kodama@lsbm.org

* Corresponding author

Published: 9 May 2003

*Nuclear Receptor* 2003, **1**:1

Received: 1 April 2003
Accepted: 9 May 2003

This article is available from: http://www.nuclear-receptor.com/content/1/1/1



## Abstract

Liver X activated receptor alpha (LXRα) forms a functional dimeric nuclear receptor with RXR that regulates the metabolism of several important lipids, including cholesterol and bile acids. As compared with RXR, the LXRα protein level in the cell is low and the LXRα protein itself is very hard to detect. We have previously reported that the mRNA for LXRα is highly expressed in human cultured macrophages. In order to confirm the presence of the LXRα protein in the human macrophage, we have established a monoclonal antibody against LXRα, K-8607. The binding of mAb K-8607 to the human LXRα protein was confirmed by a wide variety of different techniques, including immunoblotting, immunohistochemistry, and electrophoretic mobility shift assay (EMSA). By immunoblotting with this antibody, the presence of native LXR protein in primary cultured human macrophage was demonstrated, as was its absence in human monocytes. This monoclonal anti-LXRα antibody should prove to be a useful tool in the analysis of the human LXRα protein.

## Background

Liver X activated receptor alpha (LXRα) was first identified as an orphan member of the nuclear receptors expressed mainly in the liver [1,2]. LXRα is highly expressed in liver, intestine, kidney, spleen, Lung, and adipose tissue. LXRα requires retinoid X receptors (RXRs) as a partner to recognize and bind to its hormone response elements (HREs) called LXRE, and regulates LXRE target gene expression in a ligand dependent manner. LXRα has been shown to be activated by a specific class of oxidized derivatives of cholesterol [3,4].

Previously, we compared LXRα mRNA expression in various internal organs using a DNA micro array and reported

the highest level of mRNA expression in human macrophages differentiated from human monocytes in the presence of GM-CSF [5]. LXRα regulates the expression of various genes in macrophages such as the ATP binding cassette transporters (ABCA1, G1 / G4 / G8) [6–10], apolipoproteins (ApoE / C-I / C-IV / C-II) [11,12], and lipoprotein lipase (LPL) [13] in macrophages. LXRα also regulates LXRα gene expression in macrophages [14–16].

The structure and function of the LXRα protein has been studied in genetically engineered proteins or mammalian cell expression systems, but little information is available thus far on the physiologically expressed native protein. Rat liver LXRα protein has been studied by means of an antibody via immunoblotting [17,18] and electrophoretic mobility supershift assay [3,19,20], but analysis of the native human LXRα protein has not been carried out due to the lack of a sensitive anti-human LXRα monoclonal antibody.

Recently we have initiated a project designed to carry out a comprehensive analysis of the nuclear hormone receptors using a cluster of anti-nuclear hormone receptor monoclonal antibodies. Sensitive monoclonal antibodies against PPAR proteins and the RXRα protein helped complete an analysis of the native human PPAR proteins. We have also established a monoclonal antibody against the human LXRα protein, K-8607. Here we report the establishment and characterization of an anti-human LXRα monoclonal antibody. By means of this monoclonal antibody, native human LXRα protein in human monocyte-derived macrophage can be detected by immunoblotting. This antibody can be used for electrophoretic mobility supershift assay and immunostaining of COS-7 cells transfected with a human LXRα expression vector.

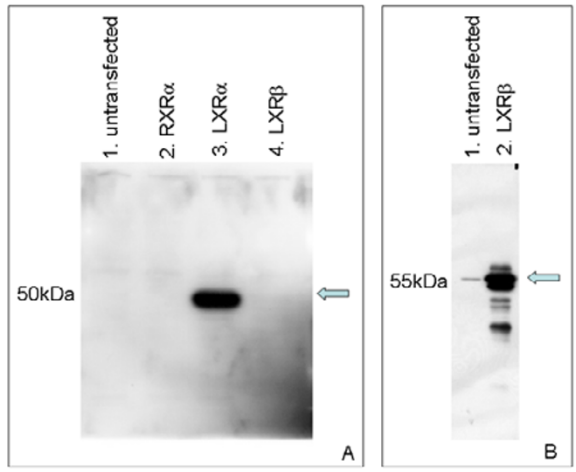


**Figure 1**
**Immunoblot detection of LXRα in transfected COS-7 cells using anti-LXRα mAb and anti-LXRβ mAb**
Nuclear extracts of RXRα, LXRα and LXRβ transfected COS-7 cells (20 μg per lane) were separated on a SDS-polyacrylamide gel (10%), subjected to electrophoresis and transferred to PVDF membrane. (A) Anti-LXRα mAb was used as first antibody. Lanes: 1, untransfected; 2, RXRα; 3, LXRα; 4, LXRβ. (B) Anti-LXRβ mAb was used. Lanes: 1, untransfected; 2, LXRβ.

## Results

### *Specificity of the anti-human LXRα mAb, K-8607*

Figure 1(A) indicates the immunoblot analysis of the specificity of the mAb K-8607. Nuclear extracts were obtained form COS-7 cell transfected with human RXRα, LXRα or LXRβ expression vectors. Nuclear extract from untransfected COS-7 cells were used as a control. In each lane, nuclear extracts containing 20 ug of protein were electrophoresed. The immunoblotting study indicated that K-8607 bound specifically to a 50 kDa protein expressed in COS-7 cells transfected with an LXRα expression vector. The apparent molecular weight of this protein is closely related to the calculated molecular weight of the human LXRα protein. The previously reported antibodies against LXRα have usually cross-reacted with LXRβ due to the high similarity of the primary amino acid sequence of both LXRs. In order to confirm the expression of LXRβ protein in COS-7 cells, immunoblotting analysis using mAb for human LXRβ K-8917 was performed. Figure 1(B) indicates that a 55 kDa protein abundantly expressed in COS-7 cells transfected with an LXRβ expression vector and a small quantity of LXRβ protein was expressed inherently in COS-7 cells. These results indicate that K-8607 is specific to LXRα protein and does not recognize LXRβ.

### *K-8607 supershift electrophoretic mobility of the DR4 oligonucleotide-nuclear extract protein complex*

Figure 2 lanes 1 to 3 indicate the results of EMSA for synthetic DR4 oligonucleotide and nuclear extracts from COS-7 cells transfected with a human LXRα expression vector. DR4, which is the consensus binding sequence for human LXRα/RXR heterodimer complex, makes a complex with protein(s) in nuclear extract from COS-7 cells transfected with the LXRα expression vector (arrow I). This complex was not detected when nuclear extract from untransfected cells was used (data not shown). The band disappeared upon addition of a 200-fold excess of unlabeled DR4 oligonucleotide, indicating that the binding is saturable (lane 2). The complex band did not change after the addition of a 200-fold excess of mutated LXRE oligonucleotide, which cannot bind to the LXRα / RXR heterodimer (lane 3).

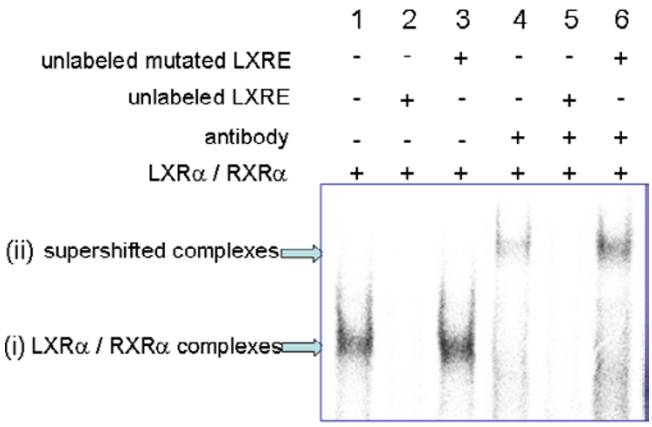


**Figure 2**
**Electrophoretic Mobility Shift Assay (EMSA)** EMSA were performed with 10 μg whole cell extracts and a 10 fmol [gamma-$^{32}$P] labeled probe. The specificity of binding to the respective probes was determined by using a 200-fold molar excess of unlabeled oligonucleotide as a competitor, which completely removed all the binding, indicating that these bound complexes resulted from sequence specific DNA-protein interactions. The arrows indicate the LXRα / RXRα complexes (i) and supershifted complexes (ii), respectively. Lanes: 1, LXRα / RXRα; 2, with the addition of unlabeled DR4 oligonucleotide; 3, with the addition of unlabeled mutated LXRE oligonucleotide; 4, supershifted complex; 5, with the addition of unlabeled DR4 oligonucleotide; 6, with the addition of unlabeled mutated LXRE oligonucleotide.

Lanes 4 to 6 indicate the results of supershift assay with mAb K-8607. A higher molecular weight band (arrow II) appeared upon the addition of 10 μg of mAb K-8607. This higher molecular weight band disappeared with the addition of a 200-fold excess of unlabelled DR4 oligonucleotide. This band was not affected by the addition of a 200-fold excess of mutated LXRE oligonucleotide. The original DR4-nuclear extract complex (arrow I) did disappear with the addition of mAb K-8607. These results indicate that mAb K-8607 is able to recognize the complex formed by DR4 and a component of nuclear extract from COS-7 cells transfected with LXRα expression vector. These results strongly suggest that mAb K-8607 is able to bind to the DR4/LXRα complex.

### *Nuclear localization of mAb K-8607 antigen in COS-7 cells transfected with human LXRα expression vector*

Figure 3 depicts the result of mAB K-8607 immunostaining of COS-7 cells transfected with human LXRα expression vector. About one third of the transfected COS-7 cells exhibited pronounced staining (left panel). At higher magnification, the staining is clearly detectable in the nucleus. COS-7 cells transfected with other expression vectors did not indicate this nuclear staining (data not shown). These results suggest that the antigen for mAb K-8607 is located within the nucleus. These results suggest the nuclear localization of human LXRα expressed in COS-7 cells.

### *Detection of native human LXRα protein in human monocyte-derived macrophages by mAb K-8607*

Figure 4 depicts the results of the immunoblotting study of human monocytes and macrophages using mAb K-8607. Previously our investigation had indicated that mRNA for LXRα is most abundantly expressed in human monocyte-derived macrophages. MAb K-8607 is able to detect human LXRα protein expressed in COS-7 cells. An identical molecular weight protein can be detected in human monocyte-derived macrophages. This protein was not detected in human monocytes, which express only a very small amount of LXRα mRNA. These results suggest that the 51 kDa protein detected by mAb K-8607 is native human LXRα protein. The amount of native protein in macrophages is significantly lower than in COS-7 cells expressing human LXRα protein.

## Discussion

Analysis of the structure and function of the human LXRα protein to date has been performed mainly with genetically engineered proteins, since the analysis of native human LXRα protein has been hampered by the lack of a sensitive monoclonal antibody against the LXRα protein. The mAb K-8607 was established with an N-terminal 94 amino acid sequence in a baculovirus expression system. This monoclonal antibody recognizes a 51 kDa protein in nuclear extract from COS-7 cells, which is consistent with the calculated molecular mass (49,000) of the 447 amino-acid human LXRα protein.

A number of experimental results support the hypothesis that the mAb K-8607 antigen is human LXRα. The sequence similarity of the human LXRα and LXRβ proteins causes a specificity problem for establishing antibodies. In order to overcome this problem, we selected the N-terminal 94 amino acids. Within this region the similarity of the amino acid sequences is relatively low (35% identity). The results of immunoblotting indicated that mAb K-8607 is able to specifically recognize LXRα. Additional evidences further supports the specific recognition of the LXRα protein by mAb K-8607.

Immunohistochemical studies with COS-7 cells transfected with a human LXRα expression vector indicated that the antigen for mAb K-8607 is located in the nucleus. The intracellular localization of human LXRα has not been reported previously. Among the nuclear hormone receptors, several proteins are known to be expressed in the nucleus,

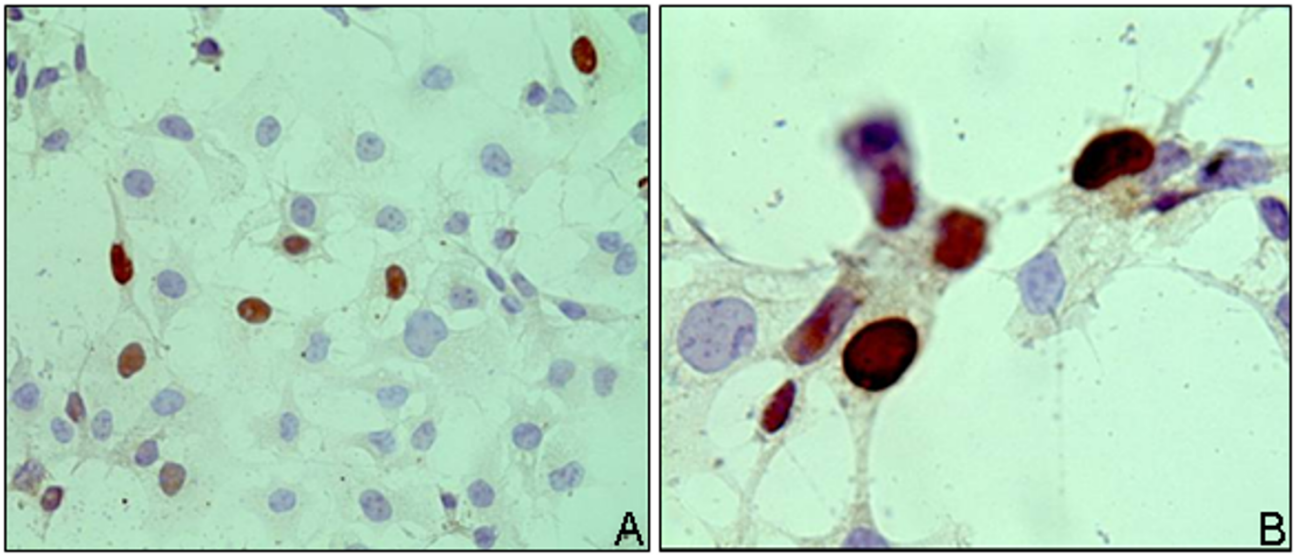


**Figure 3**
**Immunohistochemistry of LXRα protein in LXRα transiently transfected COS-7 cells** Human LXRα transfected COS-7 cells were fixed in 4% paraformaldehyde in PBS for 10 min at room temperature. After fixation, the sections were treated as described previously.

but some of the receptors, including the glucocorticoid receptor are expressed in the cytoplasm and ligand binding causes receptor translocation to the nucleus. In addition, the EGFP fused to N-terminal portion of human LXRα can be detected in the nucleus (Y. Watanabe et al manuscript in preparation). These results support LXRα protein localization in the nucleus.

Results of EMSA and supershift assay indicate that mAb K-8607 can cause supershift of the complex formed by the DR4 oligonucleotide binding sequence and nuclear extract protein from COS7-cells transfected with human LXRα expression vector.

We next studied the recognition of native human LXRα protein by mAb K-8607. We speculated that the difficulty of native LXRα protein detection may be caused by the relatively low level of native LXRα protein. We selected human monocyte-derived macrophages treated with GM-CSF for 7 days as the source of native protein in order to detect the native LXRα protein because this cell expresses the highest level of LXRα mRNA according to our previous investigations. The mRNA level in human monocyte-derived macrophages is highest among the 8 tissues and cells studied and the level is several fold higher than that in the liver. The result of immunoblotting indicated that even in monocyte-derived macrophages the level of the LXRα protein is very low as judged from the intensity of the immunoblot staining. In the case of the experiment shown in Figure 4, we applied 2 ug of protein from COS-7 cells transfected with human LXRα expression vector but we needed to apply 75 ug of monocyte-derived macrophage nuclear extract protein in order to detect the presence of the 50 kDa protein. In the case of the RXRα protein, ordinarily it is possible to detect the native RXRα protein with nearly the same amount of nuclear extract protein from COS-7 cells transfected with human RXRα. This result suggests that the level of native immunoreactive LXRα protein may be extremely low in human cells or tissues. We were not able to detect the native LXRα protein in human liver. This is not surprising because the mRNA level for LXRα in human liver is several fold lower than that in monocyte-derived macrophage. We were able to apply up to 100 ug of liver nuclear extract protein for the immunoblotting assay, but this may not be sufficient for the detection of native protein from the result described here.

The reason the immunoreactive LXRα protein level is so low remains an open question, but previous difficulties with the study of the native LXRα protein are explainable based on this low protein level. LXRα is known to function as a heterodimer. In order to understand the delicate function of the LXRα protein in actual human cells, careful consideration and some novel technique will be needed to precisely assess the amount of the LXRα protein.

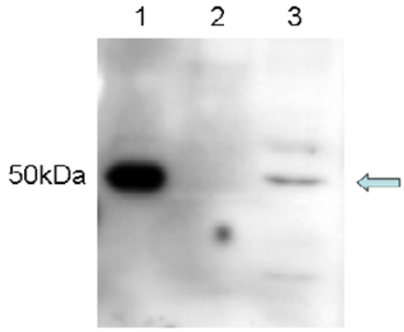


**Figure 4**
**Immunoblot detection of LXRα in human monocytes and macrophages using anti-LXRα mAb** Nuclear extracts of monocytes and macrophages (75 μg per lane) were separated on a SDS-polyacrylamide gel (10%), subjected to electrophoresis and transferred to PVDF membrane. Lanes: 1, Control; 2, monocytes; 3, macrophages.

Studies on the intracellular processing and/or degradation of the LXRα protein will be important future studies. The mAb K-8607 will be a critical tool for any such investigation.

## Conclusions

In summary, a mAb K-8607 was established which specifically detects human LXRα protein expressed in COS-7 cells or native human LXRα protein in monocyte-derived macrophages. The native human LXRα protein detected had an apparent MW of 50,000, which is close to the calculated 447 amino acids in the predicted LXRα protein. The preponderance of human LXRα protein in COS-7 cells is located in the nucleus. The expression level of native human LXRα protein is very low as compared with its heterodimeric partner, RXRα.

## Methods

### *Cell culture*

Human primary monocytes / macrophages were obtained as previously described [5] and maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS).

### *Establishment of antibody*

Monoclonal antibody for human LXRα K-8607 was established as described previously [21]. Briefly, the N-terminal sequence of the human LXRα cDNA encoding amino acids 4–97 was inserted into a baculoviral transfer vector. Recombinant virus was produced and was purified and then immunized. After ELISA screening mAB K-8607 was obtained. Monoclonal antibody for human LXRβ K-8917 was obtained by same method using the transfer vector inserted human LXRβ cDNA encoding amino acids 2–86.

### *Transient transfection*

COS-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS. Cells were plated in a 100 mm dish at $2.0 \times 10^6$ cells/dish for 16 hours prior to transfection. Transfections were performed by Effectene transfection reagent (QIAGEN) using 2 μg of the pcDNA3-hLXRα expression vector.

### *Immunoblotting*

Nuclear extracts were obtained as previously described [22]. Aliquots of each sample were separated on a 10% SDS-polyacrylamide gel and transferred to PVDF membrane. As a control for the correct LXRα protein band, we used a nuclear extract (2 μg protein) of pcDNA3-LXRα transfected COS-7 cells.

LXRα proteins were immunochemically detected using mAb K-8607 (1 μg/ml), and signal detection was achieved with a Super Signal West Dura Extended Duration Substrate.

### *Electrophoretic Mobility Shift Assay (EMSA)*

EMSA was performed with nuclear or whole cell extracts from transiently transfected COS-7 cells and T4 polynucleotide kinase end-labeled oligonucleotides. 10 μg nuclear extracts were incubated with 10 fmol of [gamma-$^{32}$P] labeled DR4 with or without a 200-fold molar excess of cold competitor oligonucleotide in a 15 μL reaction in EMSA binding buffer (10 mM Tris-HCl pH 7.5, 50 mM KCl, 10 mM EDTA, 1 mM DTT, 1% Glycerol) for 30 min on ice. Supershift assays were performed by adding antibodies 30 min before or after incubation with an oligonucleotide probe. Protein-DNA complexes were resolved by electrophoresis on 4% polyacrylamide gel in 0.5 × TBE. Following electrophoresis, gels were fixed with 10% methanol / 10% acetic acid, transferred to moistened filter paper, dried by heating at 80 C under vacuum, and exposed to the imaging plate. The following double-stranded oligonucleotides were synthesized and used in the EMSA (sense strand shown): DR4, GATCTTAGTTCACTCAAGTTCA-AGGATC; mutated LXRE, GATCTTGGTCCAGGCAAGTTCTAGGATC.

### *Immunohistochemistry*

COS-7 and human LXRα transfected cells were fixed in 4% paraformaldehyde in PBS for 10 min at room temperature. After fixation, the sections were treated as described previously [21].

***Competing interests***

none declared.

## Authors' contributions

Y.Watanabe carried out the molecular genetic studies and drafted the manuscript. T Tanaka, Y Uchiyama and T Takeno participated in the construction of the baculoviral transfer vector. A Izumi carried out the preparation of human monocytes. H Yamashita, J Kumakura and H Iwanari participated in the immunization. Jiang SY and M Naito participated in the immunohistochemical study. DJ Mangelsdorf provided the human LXRα expression vector for positive control. T Hamakubo participated in the study design of the study. Tatsuhiko Kodama conceived of the study and participated in its design and coordination.

## Acknowledgements

The authors would like to acknowledge C. Nagao, M. Kato, S. Yushina, J. Ishida, W. Kamiya, and M. Yoshikawa for their excellent technical assistance and Dr. Kevin Boru of Advanced Clinical Trials, Inc. for review of the manuscript. This work is supported by a grant from Joint Research & Development Projects with Academic Institutes and Private Companies.

## References

1. Apfel R, Benbrook D, Lernhardt E, Ortiz MA, Salbert G and Pfahl M **A novel orphan receptor specific for a subset of thyroid hormone-responsive elements and its interaction with the retinoid/thyroid hormone receptor subfamily** *Mol Cell Biol* 1994, **14:**7025-7035
2. Willy PJ, Umesono K, Ong ES, Evans RM, Heyman RA and Mangelsdorf DJ **LXR, a nuclear receptor that defines a distinct retinoid response pathway** *Genes Dev* 1995, **9:**1033-1045
3. Lehmann JM, Kliewer SA, Moore LB, Smith-Oliver TA, Oliver BB, Su JL, Sundseth SS, Winegar DA, Blanchard DE, Spencer TA and Willson TM **Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway** *J Biol Chem* 1997, **272:**3137-3140
4. Fu X, Menke JG, Chen Y, Zhou G, MacNaul KL, Wright SD, Sparrow CP and Lund EG **27-hydroxycholesterol is an endogenous ligand for liver X receptor in cholesterol-loaded cells** *J Biol Chem* 2001, **276:**38378-38387
5. Kohro T, Nakajima T, Wada Y, Sugiyama A, Ishii M, Tsutsumi S, Aburatani H, Imoto I, Inazawa J, Hamakubo T, Kodama T and Emi M **Genomic structure and mapping of human orphan receptor LXR alpha : Upregulation of LXRα mRNA during monocyte to macrophage differentiation** *J Atheroscler Thromb* 2000, **7:**145-151
6. Schwartz K, Lawn RM and Wade DP **ABC1 gene expression and ApoA-I-mediated cholesterol efflux are regulated by LXR** *Biochem Biophys Res Commun* 2000, **274:**794-802
7. Costet P, Luo Y, Wang N and Tall AR **Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor** *J Biol Chem* 2000, **275:**28240-28245
8. Kennedy MA, Venkateswaran A, Tarr PT, Xenarios I, Kudoh J, Shimizu N and Edwards PA **276:** *J Biol Chem* 2001, **276:**239438-39447
9. Engel T, Lorkowski S, Lueken A, Rust S, Schluter B, Berger G, Cullen P and Assmann G **The human ABCG4 gene is regulated by oxysterols and retinoids in monocyte-derived macrophages** *Biochem Biophys Res Commun* 2001, **288:**483-488
10. Venkateswaran A, Repa JJ, Lobaccaro JM, Bronson A, Mangelsdorf DJ and Edwards PA **Human white/murine ABC8 mRNA levels are highly induced in lipid-loaded macrophages. A transcriptional role for specific oxysterols** *J Biol Chem* 2000, **275:**14700-14707
11. Laffitte BA, Repa JJ, Joseph SB, Wilpitz DC, Kast HR, Mangelsdorf DJ and Tontonoz P **LXRs control lipid-inducible expression of the apolipoprotein E gene in macrophages and adipocytes** *Proc Natl Acad Sci* 2001, **98:**507-512
12. Mak PA, Laffitte BA, Desrumaux C, Joseph SB, Curtiss LK, Mangelsdorf DJ, Tontonoz P and Edwards PA **Regulated expression of the ApoE/C-I/C-IV/C-II gene cluster in murine and human macrophages; A critical role for the nuclear receptors LXRalpha and LXRbeta** *J Biol Chem* 2002, **277:**31900-31908
13. Zhang Y, Repa JJ, Gauthier K and Mangelsdorf DJ **Regulation of lipoprotein lipase by the oxysterol receptors, LXRalpha and LXRbeta** *J Biol Chem* 2001, **276:**43018-43024
14. Laffitte BA, Joseph SB, Walczak R, Pei L, Wilpitz DC, Collins JL and Tontonoz P **Autoregulation of the human liver X receptor a promoter** *Mol Cell Biol* 2001, **21:**7558-7568
15. Whitney KD, Watson MA, Goodwin B, Galardi CM, Maglich JM, Wilson JG, Willson TM, Collins JL and Kliewer SA **Liver X receptor (LXR) regulation of the LXRa gene in human macrophages** *J Biol Chem* 2001, **276:**43509-43515
16. Li Y, Bolten C, Bhat BG, Woodring-Dietz J, Li S, Prayaga SK, Xia C and Lala DS **Induction of human liver X receptor alpha gene expression via an autoregulatory loop mechanism** *Mol Endocrinol* 2002, **16:**506-514
17. Tobin KA, Steineger HH, Alberti S, Spydevold O, Auwerx J, Gustafsson JA and Nebb HI **Cross-talk between fatty acid and cholesterol metabolism mediated by liver X receptor-alpha** *Mol Endocrinol* 2000, **14:**741-752
18. Tobin KA, Ulven SM, Schuster GU, Steineger HH, Andresen SM, Gustafsson JA and Nebb HI **Liver X receptors as insulin-mediating factors in fatty acid and cholesterol biosynthesis** *J Biol Chem* 2002, **277:**10691-10697
19. Zhang Y, Yin L and Hillgartner FB **Thyroid hormone stimulates acetyl-coA carboxylase-alpha transcription in hepatocytes by modulating the composition of nuclear receptor complexes bound to a thyroid hormone response element** *J Biol Chem* 2001, **276:**974-983
20. Yoshikawa T, Shimano H, Amemiya-Kudo M, Yahagi N, Hasty AH, Matsuzaka T, Okazaki H, Tamura Y, Iizuka Y, Ohashi K, Osuga J, Harada K, Gotoda T, Kimura S, Ishibashi S and Yamada N **Identification of liver X receptor-retinoid X receptor as an activator of the sterol regulatory element-binding protein 1c gene promoter** *Mol Cell Biol* 2001, **21:**2991-3000
21. Tanaka T, Takeno T, Watanabe Y, Uchiyama Y, Murakami T, Yamashita Y, Suzuki A, Aoi R, Iwanari H, Jiang SY, Naito M, Tachibana K, Doi T, Shulman AI, Mangelsdorf DJ, Reiter R, Auwerx J, Hamakubo T and Kodama T **The generation of monoclonal antibodies against human peroxisome proliferator-activated receptors (PPARs)** *J Atheroscler Thromb* 2002, **9:**233-242
22. Caruccio L and Banerjee R **An efficient method for simultaneous isolation of biologically active transcription factors and DNA** *J Immunol Methods* 1999, **230:**1-10