The liver X receptor-alpha, LXRα, is expressed in the liver of the little skate, *Raja erinacea*

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The liver X receptors (LXRα and LXRβ) are nuclear receptor transcription factors that are activated by certain oxysterol derivatives of cholesterol, and play a central role in the regulation of lipid metabolism. These transcription factors belong to the NR1 family of nuclear receptors, whose members share a common heterodimerization partner, retinoid X-receptor (RXR). These nuclear receptors are subject to cross-talk interactions with other nuclear receptors and with a broad range of other intracellular signaling pathways, including those activated by certain cytokines and growth factors[1]. LXRα and LXRβ stimulate the catabolic degradation of cholesterol by activating the gene encoding P450 cholesterol 7 alpha-hydroxylase (CYP7A1), the rate-limiting enzyme in the formation of bile acids. In addition, LXRs play a critical role in regulating the transport of sterols between tissues: LXRs upregulate the ATP-binding cassette (ABC) transporters ABCG5 and ABCG8, that function to limit intestinal absorption and promote biliary excretion of sterols[5-7]. LXRs also induce expression of ABCA1 and ABCG1, which function as cholesterol efflux transporters in macrophages[2,6]. Identification of LXR ligands and target genes has been crucial to helping understand the function of these receptors in the regulation of lipid metabolism, and much of this analysis has been carried out in LXR-deficient mice[4]. These studies establish that LXRs function as sensors of dietary cholesterol, and that LXRalpha-deficiency is associated with the accumulation of large amounts of cholesterol in the liver, and with impaired hepatic function.

In contrast to mammalian liver, the livers of the little skate *Raja erinacea* secrete only small quantities of cholesterol and phospholipids into hepatic bile[3], indicating that skate liver lacks either the lipid transporters or the intracellular regulatory pathways. The goal of the present study was to assess whether skate liver expresses LXRalpha or LXRbeta, two key regulators of cholesterol transport into bile.

Total RNA from skate liver tissue was isolated by guanidinium thiocyanate extraction followed by cesium chloride gradient separation. The RNA was treated with DNase I, quantified by UV spectroscopy and used as template in degenerate RT-PCR employing Invitrogen's One-Step Superscript with Platinum Taq. Degenerate primers for skate LXR were designed to conserved regions of the corresponding mammalian receptors using the CODE-HOP degenerate primer design site located online at: http://blocks.fhcrc.org/blocks/codehop.html. Primers were purchased from Integrated DNA Technologies, Coralville, IA. Following RT-PCR, reaction products were separated by agarose gel electrophoresis and bands of predicted size were excised and sequenced. Sequencing reactions were completed on site at the MDIBL sequencing center. DNA and amino acid sequence comparisons were made using the DNA and protein sequence analysis program Lasergene from DNAStar Inc (Madison, WI).

A 714 base pair sequence for LXRalpha was successfully identified from skate liver RNA. This sequence is predicted to encode 239 amino acids that exhibit high homology with amino acids 142-379 of human LXRalpha (Figure 1). The extent of amino acid identity is 73% with human LXRalpha in the sequenced region. The skate LXRalpha sequence includes the end of the DNA-binding region, the

intervening region, and the majority of the ligand-binding domain (Figure 1). The amino acid identity between the skate and human sequences is higher in the DNA binding and ligand binding domains (83% and 79%, respectively) than in the intervening region (63%).

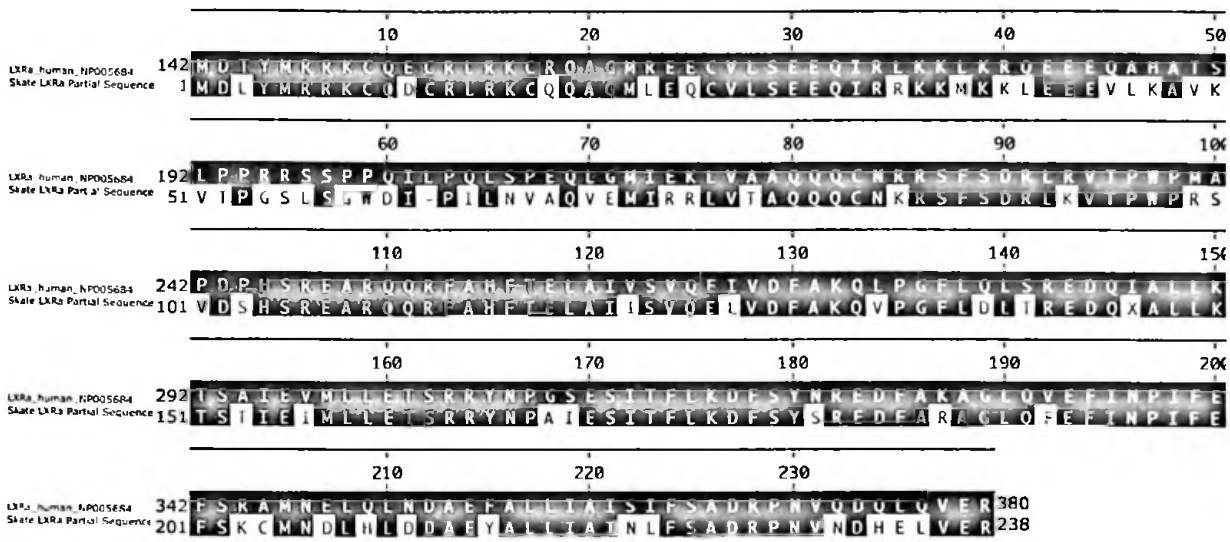

Figure 1. Amino acid alignment of predicted skate LXRalpha partial sequence with the human LXRalpha sequence (accession number NP005684).

The high predicted amino acid identity between the skate and human LXRalpha supports the evolutionary importance of this transcription factor and suggests that the skate model may be used to further study LXR ligands and target genes. In addition, the presence of LXR mRNA in skate liver indicates that the impaired ability of the skate to secrete cholesterol into bile is not related to the absence of LXR, but is likely attributed to absence or inefficiency of plasma membrane cholesterol transport mechanisms (Supported by ES03828, ES01247, DK34989, DK25636, DK48823, and by NSF-REU DBI0139190).

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