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FTF and LRH-1, two related but different transcription factors in human Caco-2 cells: Their different roles in the regulation of bile acid transport

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Abstract

The apical sodium dependent bile acid transporter (ASBT) mediates ileal bile acid reabsorption. The transcription factors, liver receptor homologue-1 (LRH-1:mouse) and fetoprotein transcription factor (FTF:human), are presumably orthologues. Bile-acid induced negative feedback regulation of mouse (m) and human (h) ASBT occurs via LRH-1 and RAR/RXR, respectively. hASBT has a potential FTF cis-element, although its functional role is unknown. hASBT and mASBT promoter constructs and an FTF cis-element mutated hASBT (hASBT/FTFμ) were assessed in human Caco-2 cells treated with chenodeoxycholic acid (CDCA) and/or co-transfected with hFTF, mLRH-1, or specific small interfering FTF or LRH-1 RNA (siFTF or siLRH). Basal promoter activity was reduced in hASBT/FTFμ, although bile acid response persisted. hFTF activated hASBT but not mASBT, while mLRH-1 activated mASBT but not hASBT. siFTF reduced hASBT but not mASBT activity; siLRH reduced mASBT but not hASBT activity. siLRH but not siFTF abrogated bile acid responsiveness. Electrophoretic mobility shift assays demonstrated distinct and specific binding of the mLRH-1 or hFTF cis-elements. In conclusion, FTF and LRH-1 are two related but different transcription factors in human Caco-2 cells, suggesting that they may be homologues and not orthologues. FTF is not involved directly in bile acid mediated negative feedback regulation of the ASBT.

Keywords: Ileum; Liver; Nuclear receptor; Transcription; Enterohepatic

1. Introduction

The enterohepatic circulation of bile acids is an important physiological process that facilitates intestinal lipid digestion/absorption and maintains both lipid and bile acid homeostasis in the body. Bile acids are synthesized from cholesterol in the liver and secreted into the small intestine. More than 95% of secreted bile acids are reabsorbed in the terminal ileum. Ileal reabsorption of bile acids is mediated primarily by the apical sodium-dependent bile acid transporter (ASBT).

In the early 1990s, a number of nuclear receptors (NR) were identified as orphan receptors, since their ligands had not been identified [1]. NRs are one of the largest families of nuclear transcription factors. The NR superfamily has been divided into seven subfamilies (NR0-NR6). Mouse LRH-1 belongs to the NR5A, or Fushi tarazu factor 1 (Ftz-F1) subfamily [2]. LRH-1 (GenBank accession number M81385) was initially identified in the mouse because of its homology to the *Drosophila* Ftz-F1 (NR5A3), the first NR5A member cloned [3,4]. LRH-1 is expressed in tissues derived from endoderm including intestine, liver, exocrine pancreas and ovary. It plays a predominant role in development, reverse cholesterol transport, bile acid homeostasis and steroidogenesis [2]. LRH-1 knock out is embryonic lethal in mice [5]. Since mouse LRH-1 was initially identified, LRH-1 orthologues were subsequently found in several species. Human LRH-1 was isolated by different groups, each providing different names for it, such as fetoprotein transcription factor (FTF) [6–10]. hFTF and mLRH-1 have a high degree of identity in their nucleotide and amino acid sequences (Fig. 1a) [2]. The general assumption has been that mLRH-1 and hFTF are orthologues.

Mouse LRH-1 mediates species- and cell line-specific bile acid dependent negative feedback regulation of the ASBT

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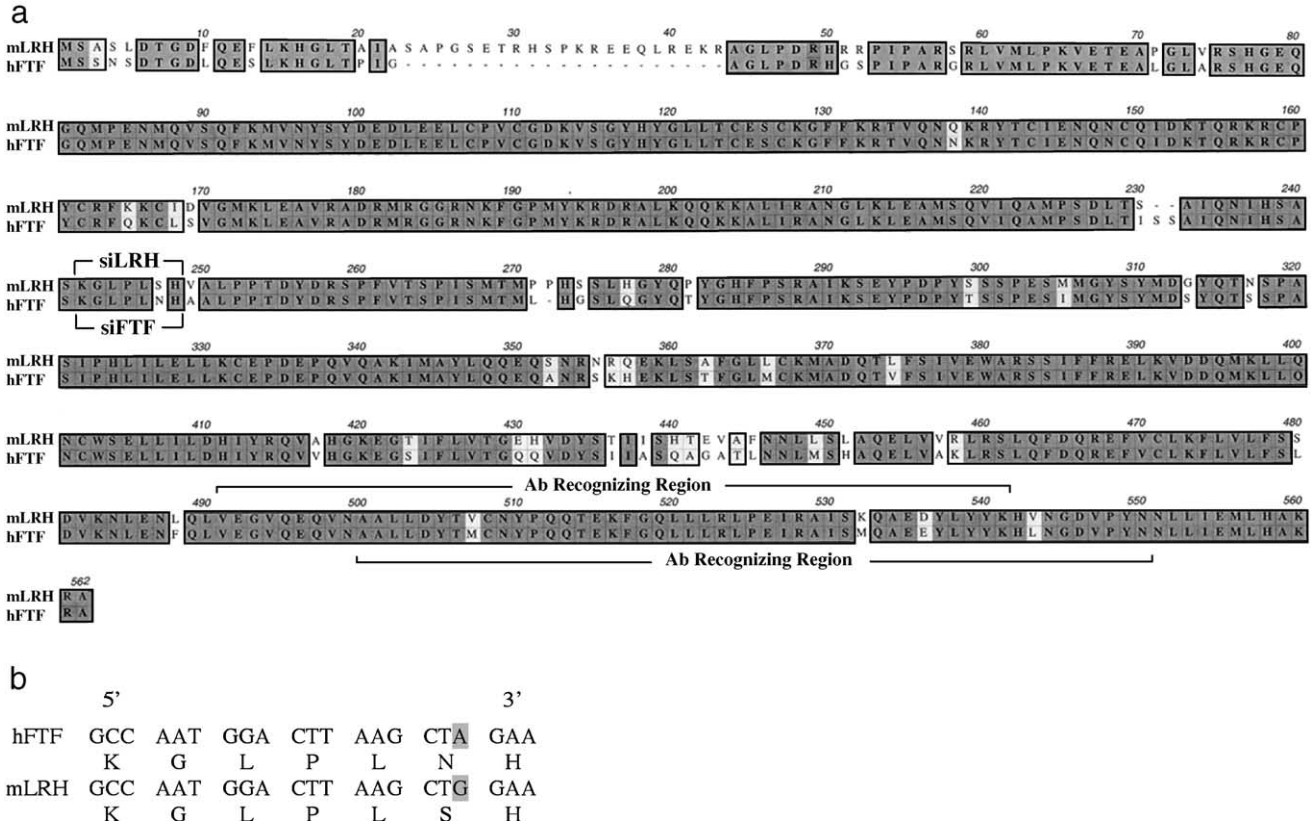

Fig. 1. Comparison of Mouse LRH-1 and Human FTF. (a) Alignment of the mLRH-1 and hFTF primary amino acid sequences. The sequences represent the full length isoforms. The sites for the designed anti-sense constructs are indicated. The general region of the anti-peptide antibodies are shown. Note the exact peptide sequence used in the genesis of the antibody is proprietary and is not available from the manufacturer. (b) Diagram of the LRH-1 and FTF anti-sense constructs. The specific nucleotide sequences targeted by the anti-sense constructs, siLRH and siFTF, are shown along with their corresponding amino acids.

[11]. The expression of the ASBT gene is unaffected by bile acids in rat, however it is under negative-feedback regulation in both human and mouse [11,12]. Multiple steps have been found in the bile acid mediated negative feedback regulation of ASBT (Fig. 2). In both mouse and human, bile acids bind to their receptor farnesoid X-receptor (FXR) leading to up-regulation of short heterodimer partner (SHP) expression. In the mouse, SHP in turn represses LRH-1-dependent activation of the ASBT gene, while in the human, SHP represses retinoic acid receptor (RAR/RXR) mediated activation of ASBT.

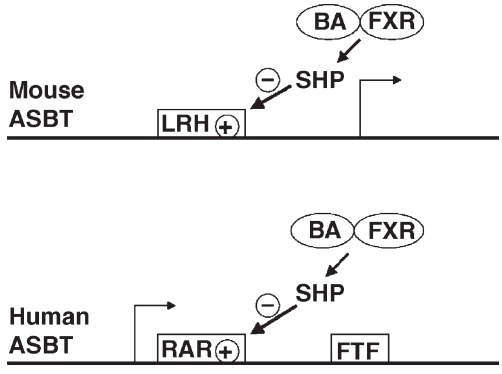

Fig. 2. Models of mouse and human ASBT Bile acid response. Diagrams of the mouse and human apical sodium dependent bile acid transporter (ASBT) promoters are shown [11,12]. The bent arrow indicates the transcription initiation start site.

The regulation of ASBT expression is of fundamental importance to both cholesterol metabolism and cholestatic liver disease [13,14]. Sequence analysis revealed a putative FTF binding site in the human ASBT promoter, however, its role in the transcriptional regulation of ASBT promoter has not been studied. The objective of this study was to investigate the role that presumed mLRH-1 orthologue, hFTF, plays in regulation of human ASBT, in particular, the bile acid mediated negative feedback regulation of the promoter. This study also sought to determine whether hFTF and mLRH-1 are orthologues.

2. Materials and methods

2.1. Cell culture

The human colon epithelial cell line Caco-2 (HTB-37, American Type Culture Collection, Rockville, MD) was used in all experiments except one study where the intestinal epithelial cell line IEC-6 was utilized. Caco-2 cells were maintained at 37 °C in Dulbecco's Modified Eagle Medium (Mediatech, Inc, Herndon, VA) supplemented with 10% fetal calf serum. IEC-6 cells were maintained in the Dulbecco's Modified Eagle Medium (ATCC, Manassas, VA) supplemented with fetal calf serum and insulin (1 μunits/ 10 ml). The plasmid-transfected cells were cultured for 40 h before harvest in the medium containing 0.5% charcoal-treated fetal calf serum to minimize the effect of bile salts found in calf serum.