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Differential activation of the human farnesoid X receptor depends on the pattern of expressed isoforms and the bile acid pool composition

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ABSTRACT

The farnesoid X receptor (FXR) is a key sensor in bile acid homeostasis. Although four human FXR isoforms have been identified, the physiological role of this diversity is poorly understood. Here we investigated their subcellular localization, agonist sensitivity and response of target genes. Measurement of mRNA revealed that liver predominantly expressed FXRα1(+/−), whereas FXRα2(+/−) were the most abundant isoforms in kidney and intestine. In all cases, the proportion of FXRα(1/2)(+) and FXRα(1/2)(−) isoforms, i.e., with and without a 12 bp insert, respectively, was approximately 50%. When FXR was expressed in liver and intestinal cells the magnitude of the response to GW4064 and bile acids differs among FXR isoforms. In both cell types the strongest response was that of FXRα1(−). Different efficacy of bile acids species to activate FXR was found. The four FXR isoforms shared the order of sensitivity to bile acids species. When in FXR-deficient cells FXR was transfected, unconjugated, but not taurine- and glycine-amidated bile acids, were able to activate FXR. In contrast, human hepatocytes and cell lines showing an endogenous expression of FXR were sensitive to both unconjugated and conjugated bile acids. This suggests that to activate FXR conjugated, but not unconjugated, bile acids require additional component(s) of the intracellular machinery not related with uptake processes, which are missing in some tumor cells. In conclusion, cell-specific pattern of FXR isoforms determine the overall tissue sensitivity to FXR agonists and may be involved in the differential response of FXR target genes to FXR activation.

1. Introduction

Bile acids (BAs) are amphipathic molecules with a steroid backbone that are synthesized from cholesterol exclusively in hepatocytes. These compounds are secreted into bile and released into the intestinal lumen upon meal ingestion to facilitate the digestion and intestinal absorption of dietary lipids and fat-soluble vitamins. In these biological functions, a key role is played by the specific physicochemical characteristics of these detergents, which are determined by the number and the orientation of the substituents on the steroid nucleus, and influenced by the amidation of the side chain with glycine or taurine.

In addition to their role in digestion, BAs have been associated to a large variety of body functions [1]. In the late 90s these compounds were identified as the natural ligands of the until then orphan nuclear receptor farnesoid X receptor (FXR, gen symbol *NR1H4*) [2–4]. A few years later, four isoforms of murine [5] and human [6] FXR were identified. They are derived from a single gene as a result of alternative promoter usage and alternative splicing of the mRNA. These isoforms have been classified using different nomenclatures, including this used here, i.e., FXRα1(+), FXRα1(−), FXRα2(+) and FXRα2(−) (Fig. 1A and 1B). FXRα1 and FXRα2 differ

Abbreviations: ACA, allocholic acid; BA, bile acid; BSEP, bile salt export pump; CDCA, chenodeoxycholic acid; CA, cholic acid; DCA, deoxycholic acid; FGF, fibroblast growth factor; FXR, farnesoid X receptor; GCDCA, glycochenodeoxycholic acid; IBABP, ileal bile acid binding protein; LCA, lithocholic acid; OATP, organic anion transporter polipeptide; OST, organic solute transporter; RXR, retinoid X receptor; SHP, small heterodimer partner; TCDCA, taurochenodeoxycholic acid; UDCA, ursodeoxycholic acid.

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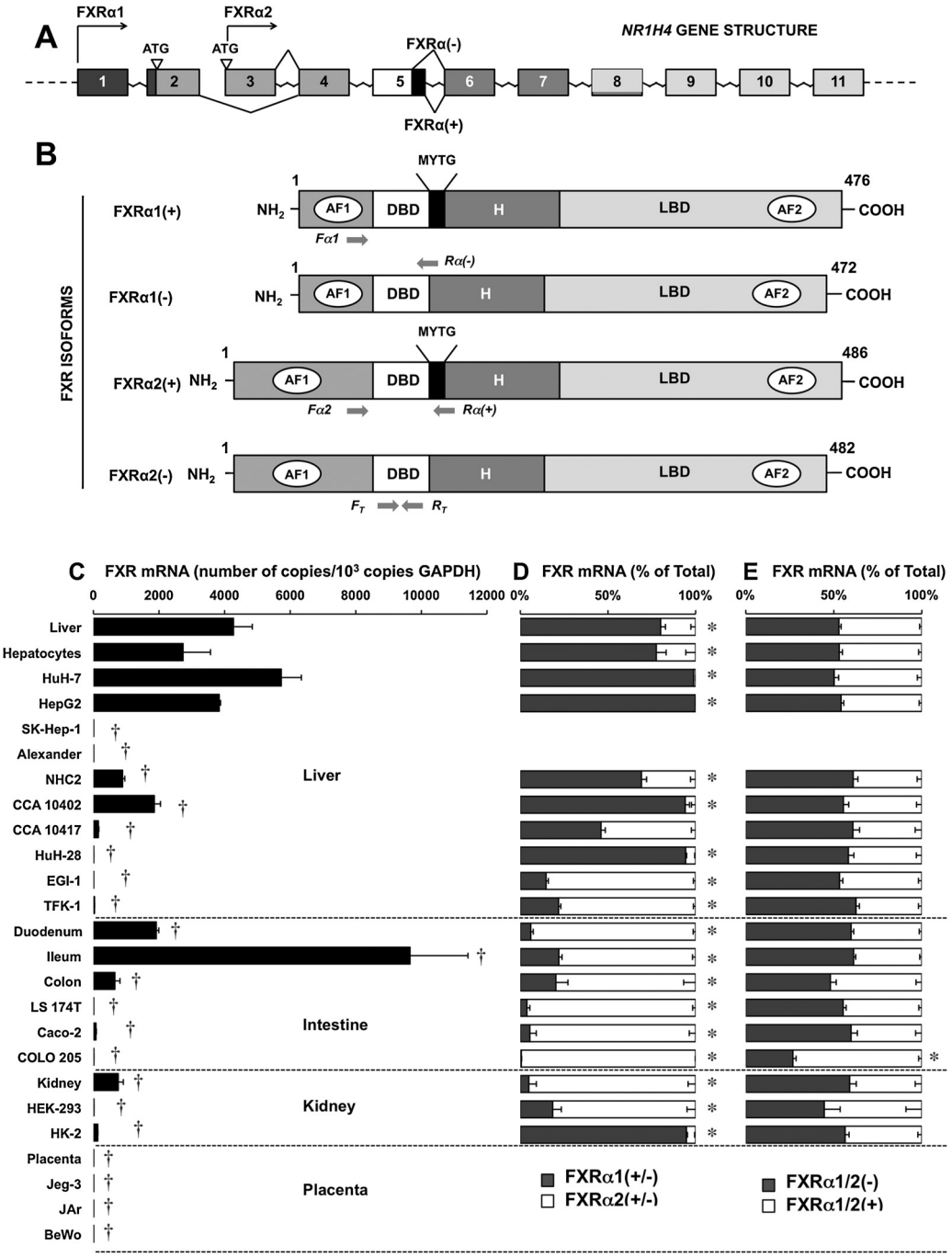

Fig. 1. Schematic representation of *NR1H4* gene (A) and FXR isoforms classified according to the difference in the initial region (exons 1–3) of mRNA (α1 and α2) and the presence (+) or absence (−) of a 12-bp insert in exon 5 (B). Absolute abundance of FXR mRNA (C) and relative proportions of FXRα(1/2) (D) and FXRα(+/−) isoforms (E) was determined by RT-QPCR using the following pairs of primers: F_T/R_T for all isoforms, $F\alpha1/R_T$ for FXRα1(+/−), $F\alpha2/R_T$ for FXRα2(+/−), $F_T/R\alpha(+)$ for FXRα(1/2)(+) and $F_T/R\alpha(-)$ for FXRα(1/2)(−). Samples were collected from healthy liver ($n = 13$), duodenum ($n = 3$), ileum ($n = 3$), colon ($n = 18$), kidney ($n = 3$) and placenta ($n = 3$). Samples from at least 3 different cultures of the indicated cell lines were determined. Values are means ± SD. $^{\dagger}p < 0.05$ as compared with human liver; $^{*}p < 0.05$ as compared with the opposite isoform. AF1 transactivation independent domain; AF2 transactivation dependent domain; DBD, DNA binding domain; LBD, ligand binding domain; H, hinge region, MYTG, four-amino acids insert.

in the start point of mRNA transcription, i.e., exon 1 or 3, respectively. This results in different amino acid sequence at the initial region of the protein. Moreover, both FXRα1 and FXRα2 can include (+) or lack (−) a 12-bp insert (amino acid sequence: MYTG) at the end of exon 5. Although some interesting data concerning the existence of human *NR1H4* variants have been reported [6], this question has been better studied in mice where the expression of FXR has been reported at high levels in the liver, intestine, kidney, and adrenal glands [5]. In the same study, differential expression of FXRα1 and FXRα2 isoforms among these tissues was reported.

FXR heterodimerizes with the retinoid X receptor α (RXRα) and binds to FXR response elements (FXREs), mainly IR-1 (inverted repeats separated by 1 nucleotide) [7] to activate the transcription of its target genes upon activation by specific agonists. Among them are these encoding most of the proteins involved in BA metabolism and transport. However, additional target genes have recently been described to be involved in FXR-mediated regulation of several body functions, such as prevention of hepatic and intestinal carcinogenesis [8,9], liver regeneration [10], intestinal barrier [11], attenuation of adverse effects of cholestasis [12],