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Weaving βKlotho into bile acid metabolism

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Commentary

Bile acids are natural detergents that assist in the absorption and digestion of fats in the intestine. In liver, the synthesis of bile acids from cholesterol is regulated by multiple signaling cascades that repress transcription of the gene encoding cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in the classic bile acid synthesis pathway. In this issue of the *JCI*, Ito and coworkers demonstrate that mice lacking βKlotho, a membrane protein with 2 putative glycosidase domains, have increased *Cyp7a1* mRNA levels and bile acid concentrations. βKlotho-KO mice also have small gallbladders and are resistant to cholesterol gallstone formation. These findings highlight the central role of βKlotho in bile acid homeostasis and raise the possibility that this protein could be a pharmacologic target for the treatment of gallstones.

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Weaving βKlotho into bile acid metabolism

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Bile acids are natural detergents that assist in the absorption and digestion of fats in the intestine. In liver, the synthesis of bile acids from cholesterol is regulated by multiple signaling cascades that repress transcription of the gene encoding cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in the classic bile acid synthesis pathway. In this issue of the *JCI*, Ito and coworkers demonstrate that mice lacking βKlotho, a membrane protein with 2 putative glycosidase domains, have increased *Cyp7a1* mRNA levels and bile acid concentrations (see the related article beginning on page 2202). βKlotho-KO mice also have small gallbladders and are resistant to cholesterol gallstone formation. These findings highlight the central role of βKlotho in bile acid homeostasis and raise the possibility that this protein could be a pharmacologic target for the treatment of gallstones.

Bile acids are cholesterol metabolites that are synthesized in the liver, stored in the gallbladder, and released during a meal into the small intestine, where they are crucial for the absorption of lipophilic nutrients and vitamins (1). Despite their importance in normal physiology, bile acids are strong detergents whose concentrations must be tightly controlled. Dysregulation of bile acid homeostasis is associated with a range of pathophysiological disorders including cholestatic liver disease and cholesterol gallstone formation. In this issue of the *JCI*, Ito et al. make an interesting and unexpected link between the protein βKlotho and the regulation of bile acid synthesis (2).

Feedback repression of bile acid synthesis

Cholesterol 7α-hydroxylase (CYP7A1), which is encoded by the *CYP7A1* gene, catalyzes the rate-limiting step in the conversion of cholesterol to bile acids (Figure 1). Bile acids act via a feedback mechanism to repress *CYP7A1* transcription. Work from a number of laboratories has revealed the complexity of this regulation (1, 3). One pathway through which *CYP7A1* is

Nonstandard abbreviations used: CYP7A1, cholesterol 7α-hydroxylase; FGFR4, fibroblast growth factor receptor 4; FXR, farnesoid X receptor; SHP, small heterodimer partner.

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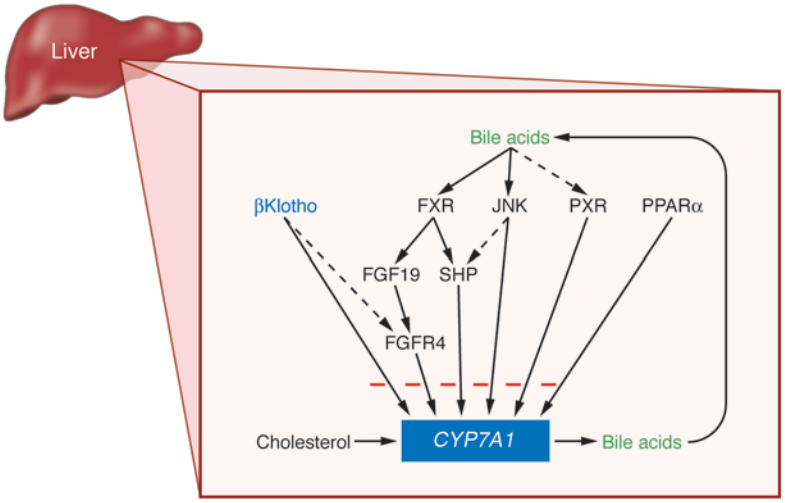

Figure 1
Shown are signaling pathways that repress transcription of the gene encoding CYP7A1, the rate-limiting enzyme in bile acid synthesis. Established pathways for the repression of *Cyp7a1* in mice are indicated by solid lines. These include JNK and the nuclear bile acid receptor FXR. Activation of FXR represses *CYP7A1* by induction of the orphan nuclear receptor SHP and FGF19, which acts through FGFR4. Activation of the nuclear receptors pregnane X receptor (PXR) and PPARα by xenobiotics and fatty acids, respectively, also represses *CYP7A1* through unknown mechanisms. Speculative pathways are shown as dotted lines. In this issue, Ito and colleagues show that mice lacking βKlotho have increased *Cyp7a1* expression and bile acid synthesis and are resistant to the formation of gallstones (2). The similar phenotypes of βKlotho-KO and FGFR4-KO mice (8), including increased bile acid synthesis and small gallbladders, suggest that these proteins may act through a common pathway.

repressed involves the nuclear bile acid receptor farnesoid X receptor (FXR). Activation of FXR represses *CYP7A1* indirectly through induction of the orphan nuclear receptor small heterodimer partner (SHP), which then binds to another orphan nuclear receptor, liver receptor homolog–1, in the *CYP7A1* gene promoter to repress gene transcription. Mice lacking FXR or SHP have increased *Cyp7a1* expression and a corresponding increase in bile acid synthesis (4–7). FXR also induces the expression of FGF19 in human hepatocytes, which represses *CYP7A1*, presumably in an autocrine or paracrine fashion (8). Mice lacking fibroblast growth factor receptor 4 (FGFR4), a receptor for FGF19, have increased *Cyp7a1* mRNA levels and a corresponding increase in bile acid synthesis (8). Thus, FXR contributes to the repression of *Cyp7a1* through multiple mechanisms.

However, FXR-mediated suppression does not constitute the whole story when it comes to feedback regulation of *CYP7A1*. Treatment of SHP-KO and FGFR4-KO mice with pharmacologic doses of bile acids still results in repression of *Cyp7a1*, which reveals the presence of additional regulatory mechanisms (5, 6, 9). One of the FXR/SHP-independent pathways involves activation of liver Kupffer cells, which secrete inflammatory cytokines such as TNF-α (10). These cytokines repress *Cyp7a1* in hepatocytes through a signaling pathway that involves JNK (11). The nuclear receptors pregnane X receptor and PPARα, which are activated by xenobiotics and fatty acids, respectively, also repress *Cyp7a1* through mechanisms that do not appear to involve FXR or SHP (3). Thus, multiple regulatory pathways converge to repress *Cyp7a1* (Figure 1).

βKlotho regulates bile acid homeostasis

Klotho made a dramatic entrance several years ago when it was shown that mice with reduced levels of this protein have a premature aging phenotype that includes dysregulation of calcium and phosphorus homeostasis (12). Recently, a close relative, termed βKlotho, was shown to be expressed in liver, pancreas, and fat (13). Both Klotho and βKlotho are membrane proteins containing 2 regions in the extracellular domain with homology to those in family 1 glycosidases, which hydrolyze glycosidic bonds. To gain insight into βKlotho's function, Ito and colleagues disrupted the gene encoding βKlotho in mice (2). Notably, βKlotho-KO mice have pronounced alterations in bile acid metabolism, including marked increases in *Cyp7a1* mRNA levels and a corresponding increase in bile acid excretion in the feces. These changes are consistent with disruption of the normal bile acid feedback regulatory loop (Figure 1).

It is not clear how βKlotho contributes to the repression of *Cyp7a1*. Although bile acid–mediated induction of hepatic *Shp* mRNA was reduced in the βKlotho-KO mice, there was no significant difference in basal *Shp* mRNA levels in βKlotho KO and wild-type mice (2). These data suggest that βKlotho-mediated repression of *Cyp7a1* does not involve an increase in SHP concentrations. Studies with selective FXR agonists in the βKlotho-KO mice are needed to clarify whether βKlotho is required for FXR to repress *Cyp7a1*.

One intriguing possibility is that βKlotho works in concert with FGFR4. Both are highly expressed in liver, and the βKlotho-KO and FGFR4-KO mice have remarkably similar phenotypes, including elevated *Cyp7a1* mRNA levels and bile acid synthesis as well as a small gallbladder (2, 8). Interestingly, heparin/heparan sulphate–like glycosaminoglycans serve as cofactors for FGF signaling through FGFRs, which raises the possibility that βKlotho, with its 2 putative glycosidase domains, might regulate the concentrations or activity of a cofactor required for FGFR4 signaling.

Absence of βKlotho protects against gallstones

What are the potential clinical implications of this work? Ito et al. (2) show that βKlotho-KO mice are resistant to the formation of gallstones, which develop when the ratio of cholesterol to bile acids and phospholipids in the bile increases to the point that cholesterol precipitates (14). In mice, cholesterol gallstone formation can be induced by a lithogenic diet that includes high cholesterol and cholic acid concentrations (15). The authors suggest that the lack of gallstone formation in βKlotho-KO mice could be due to the increase in hepatic bile acid synthesis, which may alter the cholesterol/bile acid ratio in bile to reduce gallstone formation. However, the lipid composition of the bile in the βKlotho-KO mice was not tested directly nor were the hepatic protein and mRNA levels determined for bile salt export pump (BSEP; also known as ABCB11) and multidrug-resistance protein 2 (also known as ABCB4), which transport bile acids and phosphatidylcholine into the bile (16, 17). It is noteworthy that an increase in biliary bile acid secretion was not sufficient to prevent cholesterol gallstone formation in transgenic mice overexpressing the bile acid trans-

porter BSEP (18). Thus, the resistance of βKlotho-KO mice to gallstones is likely to be a consequence of more than just increased bile acid concentrations in the bile.

One possibility is that the small size of the gallbladder in the βKlotho-KO mice reflects changes that contribute to the prevention of gallstone formation. An increase in gallbladder contraction and emptying would be expected to reduce the time that cholesterol has to precipitate and form stones in the bile (19). Indeed, gallbladder stasis is associated with gallstone formation in both humans and rodents (20, 21). It will be interesting to learn whether βKlotho is present in the gallbladder and how it affects gallbladder function. Moreover, it will be interesting to see whether FGFR4-KO mice, which also have small gallbladders, are resistant to gallstones.

In summary, the work of Ito and colleagues (2) adds βKlotho to the complex tapestry of signaling networks that regulate bile acid homeostasis. Precisely how βKlotho integrates with these other regulatory pathways remains to be determined.

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A molecule's right to choose: how diabetogenic class II MHC products bind peptides

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The distinction between peptides that bind to class II MHC products under laboratory conditions and those that do so physiologically is important for the prediction of antigens recognized by autoreactive T cells. In this issue of the *JCI*, Suri et al., using antigen-presenting cells, compared the peptides that bound to human HLA-DQ8 and those that bound to mouse I-A^{g7}, both class II MHC products that predispose their carriers to type 1 diabetes (see the related article beginning on page 2268). The rules of engagement for the peptide ligands of the DQ8 and I-A^{g7} molecules involve similarities in their anchor residues, which mediate stable interaction with class II MHC products. The peptides identified derive from overlapping sets of self proteins.

The single most important genetic factor that predisposes to autoimmune disease is the MHC. In the case of type 1 diabetes, individuals who carry the MHC class II HLA-DQ8 allele are more likely to contract the disease than those who do not, and other alleles at the class II loci (HLA-DR3, HLA-DR4) can predispose to this disease as well. The predominant contribution of the MHC to disease susceptibility is mirrored in the available animal models of autoimmune disease, including diabetes.

In the absence of experiments conducted on humans, the appropriateness of various animal models as stand-ins for human disease continues to be called into question. In mice and humans, the MHC products are very similar in their 3-dimensional structure, although they are no more than 70% identical in amino acid sequence. Different residues constitute the peptide-bind-

Nonstandard abbreviations used: P9, position 9.

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