Elevated Cholesterol Metabolism and Bile Acid Synthesis in Mice Lacking Membrane Tyrosine Kinase Receptor FGFR4*

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The fibroblast growth factor (FGF)† tyrosine kinase signaling complex is an intrinsic mediator of cell to cell communication in tissue remodeling in development and cellular homeostasis in adult organs (1, 2). The FGF receptor kinase family consists of an extensive repertoire of alternately spliced products of four genes (fgfr1 to fgfr4), which are expressed in development in a temporal- and spatially specific mode (3) and in adult tissues in a cell type-specific mode (2). Disruption of the fgfr1 and fgfr2 genes in mice disrupt development, exhibit global proliferation defects, and are embryonic lethal (4, 5). Mice in which fgfr3 was disrupted are viable, but exhibit severe skeletal dysplasias due to overgrowth of long bones, which is a consequence of loss of restraints on growth of chondrocytes during endochondral ossification (6, 7).

FGF-1, FGF-2, and FGF-8 have been implicated in development of the liver from foregut endoderm where FGFR4 is expressed (8). However, disruption of fgfr4 in the mouse germline resulted in no overt abnormalities (9). All four fgfr genes are expressed in adult liver (10), but only FGFR4 is expressed in mature hepatocytes (11). External administration of FGF-7 and FGF-18, or expression of FGF-18 under control of liver specific-promoters, elicits hyperplasia in the liver (12, 13). FGF-1 and FGF-7 elicit DNA synthesis in primary liver cell cultures enriched in hepatocytes (14, 15).

To determine whether FGFR4 plays a role in liver in vivo, we examined the morphology of the liver and associated organs in fgfr4/fgfr4 mice, including the compensatory growth response after partial hepatectomy. Here we report no differences in liver architecture and kinetics or extent of restoration of liver mass between wild-type and fgfr4 mice (9). All four fgfr genes are expressed in adult liver (10), but only FGFR4 is expressed in mature hepatocytes (11). External administration of FGF-7 and FGF-18, or expression of FGF-18 under control of liver specific-promoters, elicits hyperplasia in the liver (12, 13). FGF-1 and FGF-7 elicit DNA synthesis in primary liver cell cultures enriched in hepatocytes (14, 15).

The FGFR4 deficiency caused a significant elevation of the excreted and total bile acid pools. Elevation of bile acid pools were coincident with constitutively elevated expression of Cyp7a, the limiting enzyme in the classical pathway for bile acid synthesis (16), and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, the rate-limiting step in cholesterol synthesis (17). The FGFR4 knockout mice exhibited a cholate-dependent, cholesterol-induced hepatomegaly. Analysis of gene expression in the hepatomegalic livers of the mutant mice suggested points where both FGFR4 and bile acids exert negative controls on liver bile acid synthesis. These results implicates the pericellular matrix-controlled FGFR4 kinase complex in hepatocytes in control of cholesterol metabolism and bile acid synthesis in a physiological setting.

EXPERIMENTAL PROCEDURES

Animals and Diets—Disruption of the mouse fgfr4 locus was carried out in 129 Sv strain-derived ES cells as described (9). Wild-type 129 Sv mice were obtained from the Jackson Laboratory. FGFR4 (+/−) mice were generated by crossing FGFR4 (+/−) mice with wild-type 129 Sv mice, or by further crossing FGFR4 (+/−) with FGFR4 (+/−) mice. Only

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† The abbreviations used are: FGF, fibroblast growth factor; FGFR, FGF receptor; Cyp7a, cholesterol 7α-hydroxylase; RIPA, receptor interacting protein 140; LXR, liver X receptor; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; PH, partial hepatectomy; RT-PCR, reverse transcription-polymerase chain reaction; ISBT, ileal sodium-dependent bile acid transporter; IBADF, intestinal bile acid binding protein; RPA, ribonuclease protection; BrdU, bromodeoxyuridine; Cyp7a, oxysterol 7α-hydroxylase; RXRα, retinoid X receptor; nt, nucleotide(s); PBS, phosphate-buffered saline.

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mice 7–8 weeks old were used in the study. Male mice were used for partial hepatectomy (PH)-induced liver regeneration experiments, and female mice were used in all other protocols. Mice were maintained in 12-h light/12-h dark cycles and were given free access to food and water. Standard rodent chow containing 0.02% (w/w) cholesterol and the standard diet was solubilized in saline and administered intraperitoneally at 25% (w/v) cholesterol and 2% (w/w) sodium cholate was obtained from Alief Purina Feed Store, Inc. (Alief, TX). Two to four mice were employed for each experimental group, as described in the specific figure legends. After the mice were weighed, anesthetized, and exsanguinated, the livers or other tissues were harvested at 10:00 a.m., except that of the mice used in the PH-induced liver regeneration, which were harvested at 120 min after a 25% saline challenge. The bile acid concentration was measured enzymatically. The daily feces output (g/day per 100 g body weight) and fecal bile acid content (μmol/g) were used to calculate the rate of bile acid excretion (μmol/day/100 g body weight).

The total bile acid pool size was determined as bile acid content of the small intestine, the gallbladder, the liver, and their contents. After the mice were weighed, anesthetized, and exsanguinated, the fresh organ contents were homogenized and total cholesterol was determined enzymatically, and the pool size was expressed as micro-moles of bile acid/100 g of body weight.

Measurement of Hepatic Cholesterol—To measure hepatic cholesterol level, 50 mg of fresh liver was minced, hydrolyzed, and extracted in 4 ml of 1% KOH/ethanol (diluted aqueous KOH (10 x) with 9 volumes of methanol) at 66 °C for 2 h. The extract was centrifuged, 1 ml samples of supernatant for assay were diluted to 4 ml with 75% ethanol, and then 1-ml samples were diluted to 4 ml with 25% PBS. Bile acids were determined enzymatically, and the pool size was expressed as milligrams of cholesterol/g of liver weight.

Histological Procedures—Liver tissues were fixed overnight in Histochoice Tissue Fixative MB (no. H120–4L, Amresco), dehydrated through a series of ethanol treatments, and embedded in paraffin according to standard procedure. Sections were prepared and stained with hematoxylin and eosin.

Partial Hepatectomy and DNA Synthesis—A 70% hepatectomy, consisting of removal of the anterior and left lateral hepatic lobes, was performed on male mice at 10:00 a.m. Two hours prior to sacrifice of the animals for analysis, 50 μg/l of body weight of bromodeoxyuridine (BrdUrd) was administered intraperitoneally. Remnant livers were removed and weighed at different times. BrdUrd incorporation in fixed liver sections was visualized with an anti-BrdUrd monoclonal antibody (no. 2531, Sigma) and an alkaline phosphatase-conjugated second antibody. Positive hepatocytes were counted, and BrdUrd incorporation was expressed as the percentage of the number of labeled hepatocytes in four or five visual fields.

Statistical Analyses—Values are expressed as the mean ± standard deviation (S.D.) with the number of replicates described in the legends to figures. The statistical significance of differences between mean values (p < 0.05) was evaluated using the two-tailed Student’s t test.

RESULTS

Normal Liver Architecture and Regeneration after Partial Hepatectomy in FGFR4-deficient Mice—A histological examination of the liver revealed no apparent abnormalities in the overall morphology, cellular content and arrangement of different compartments in wild-type (+/+) and FGFR4-deficient (−/−) mice. The knockout animals exhibited normal blood chemistry including glucose, protein, and aspartate aminotransferase and alanine aminotransferase levels (data not shown). Partial hepatectomy was performed on both FGFR4 (+/−) mice and FGFR4 (+/+) mice, and both DNA synthesis and restoration of liver mass was measured periodically for up to 168 h after the operation. DNA synthesis peaked at the expected 38 h time point, and both the rate and extent of restoration of liver mass were identical in both wild-type and mutant mice (Fig. 1, A and B). Thus, FGFR4 is either not directly involved in compensatory growth of the liver in response to loss of 67% of the liver mass, or it is fully compensated for by other proliferative regulators.

Decrease in Weight/Volume of the Gallbladder Suggested Abnormal Bile Acid Metabolism in the FGFR4-deficient Mice—During surgical manipulation of livers for partial hepatectomy, we noted that the gallbladders of FGFR4 (−/−) mice, inclusive of contents, were smaller and weighed on average about 30% of those from FGFR4 (+/+) mice (Fig. 1C). The differential was maintained in mice fed a high cholesterol/cholate diet, although...
 FGFR4 and Bile Acid Synthesis

Fig. 1. Liver regeneration and gallbladder size in FGFR4 knockout versus wild-type mice. A and B, similar restoration of liver in response to PH in wild-type (+/+ ) and knockout (−/−) mice. In B, analysis of BrdUrd incorporation was monitored in three animals per time point (mean ± S.D.) after PH as a measure of proliferating hepatocytes as described under “Experimental Procedures.” C and D, abnormally small gallbladders in specifically FGFR4-deficient mice. Gallbladders were removed and weighed from wild-type (+/+ ) and FGFR4 knockout (−/−) mice on standard chow (−) or chow containing 2% cholate (Chol) and 2% cholic (Chtc) (+).

Data are expressed as the mean ± S.D., n = 4 animals. Significance of difference between wild-type (+/+ ) and (−/−) on both diets was p < 0.002.

gallbladders exhibited an expected 4-fold increase in total weight (Fig. 1D). Histological analysis revealed no apparent difference in gallbladder morphology and structure between mutant and wild-type mice (data not shown).

Analysis of combined liver, gallbladder, and small intestine, as well as feces from both male and female FGFR4 (−/−), FGFR4 (+/+ ), and FGFR4 (+/+ ) mice, revealed that bile acids were elevated 2–3-fold in FGFR4-deficient mice (Fig. 2, A and B). Surgical ablation of the gallbladders from wild-type and FGFR4-deficient mice had no effect on the fecal bile acid excretion rate (Fig. 2A). This suggested that an abnormality in bile acid metabolism and flow was the cause of the smaller gallbladders in mutant mice, rather than a defect in architecture and function of the gallbladders. It has been shown previously that acceleration of bile acid synthesis, by blocking bile acid feedback inhibition by blocking intestinal uptake, can accelerate gallbladder emptying and a decrease in gallbladder volume (18, 19). Although the bile acid pool increased by an expected 60% in FGFR4 (+/+ ) mice on a high cholesterol diet (20), the diet had little effect on the already elevated pool observed in the FGFR4-deficient animals (Fig. 2C). Both newborn FGFR4 (+/+ ) and FGFR4 (−/−) animals exhibited a pool size of 20 μmol/100 g of body weight, which rose to 30 μmol/100 g on day 3 (Fig. 2D). By day 6, the pool in FGFR4 (−/−) mice was 2 times that of normal at 60 μmol/100 g, and continued to increase through day 12, while pools were static in normal mice. During weaning, which causes an increase in the bile acid pools of both normal (21) and mutant mice, the pool in FGFR4 (−/−) mice rose to 3 times (250 μmol/100 g of body weight) that of wild-type adult levels (about 80 μmol/100 g of body weight) at 21 days. Levels in mutant mice dropped to about 160 μmol/100 g after 1 month and remained static thereafter. This indicated that an elevation of bile acid pools in the FGFR-deficient mice occurs prior to maturation of mechanisms for reabsorption of bile acids in the ileum and liver (22, 23) and the secondary acid pathway for synthesis of bile acids (24). Expression of mRNA for IBABP and ISBT, whose transcription is activated and repressed by bile acids, respectively (25, 26), was increased and depressed, respectively, in mature FGFR4-deficient mice (Fig. 2, E and F). These observations suggested a role of FGFR4 in bile acid metabolism at the site of synthesis in the liver.

Elevation of Liver HMG-CoA Reductase and Cyp7a Expression in FGFR4-deficient Mice—Elevation of bile acids may result from accelerated conversion from cholesterol, or indirectly through increased availability of cholesterol substrate through its synthesis or deposition in the liver. Enzymes whose levels are regulated at transcription by substrates and products regulate both liver pathways (27). We first measured mRNA levels of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis in animals on standard rodent chow (−0.02% cholesterol w/w). HMG-CoA reductase mRNA, which is repressed by sterols and activated when they are deficient (28), was elevated by 7-fold in the FGFR4 (−/−) animals, but down-regulated to near equal levels in both FGFR4 (+/+ ) and FGFR4 (−/−) animals fed a cholesterol-rich diet (Fig. 3A).

Cyp7a, which converts cholesterol into 7α-hydroxycholesterol, is the rate-limiting enzyme in the classical route of bile acid synthesis (16). Cyp7a is positively regulated at transcription in a feed-forward mode by oxysterol metabolites of cholesterol (29), and negatively regulated in feedback mode by bile acids. The expression of liver Cyp7a mRNA was elevated by 2.5-fold in FGFR4 (−/−) mice compared with wild-type animals (Fig. 3B). Immunoblot analysis revealed that the elevation of the level of CYP7A protein was similar to the rise in mRNA (Fig. 3C). Although the high cholesterol diet increased Cyp7a expression by 1.5-fold in wild-type mice, the increase failed to reach the elevated level of Cyp7a expression observed in FGFR4 (−/−) mice (Fig. 3B). No increase over the elevated Cyp7a mRNA levels in the FGFR4-deficient animals was observed as a consequence of the high cholesterol chow. Cholate is
a strong repressor of Cyp7a transcription (30). The addition of cholate (2%, w/w) to the high cholesterol chow revealed that mechanisms for bile acid-mediated repression of Cyp7a expression were intact and similar to wild type. These combined results suggest that FGFR4 negatively regulates bile acid synthesis through repression of Cyp7a expression and that Cyp7a

**FIG. 2. Elevated bile acid excretion and pool size in FGFR4 knockout mice.** A, fecal bile acid excretion. Feces was collected, extracted and analyzed as described under “Experimental Procedures.” Values are the mean ± S.D. (n = 4 animals). Significance of differences between (-/-) and wild-type (+/+ or (+/-)) animals was p < 0.001. The two bars at right are from mice in which the gallbladders were surgically removed. B, total bile acid pool size (small intestine, gallbladder and liver) in FGFR4 (+/+), (+/-), and (-/-) littermates. Values are the mean ± S.D. (n = 4 animals). Significance of differences between (-/-) and wild-type (+/+ or (+/-)) animals was p < 0.001. C, comparison of the bile acid pool in FGFR4 (+/+ and (-/-) mice fed standard chow and chow containing 2% cholesterol. Values are mean ± S.D. (n = 4 animals). Significance of differences between (+/+ or animals) fed standard chow and the diet containing 2% cholesterol (Chol), and (-/-) animals fed normal chow or the 2% cholesterol diet was significant at p < 0.02. D, comparison of postnatal changes in the bile acid pool in FGFR4 (+/+ and FGFR4 (-/-) mice on standard chow. Values are the mean ± S.D. All time points after day 3 between mutant (-/-) and wild-type (+/+ or (+/-)) animals were significantly different (p < 0.03). E and F, expression of IBABP and ISBT genes in the ileum of FGFR4 (+/+ and FGFR4 (-/-) mice. mRNA levels in 50 μg of total RNA isolated from the ileum of three mice were determined by RNase protection. P, probes. Individual band density was standardized relative to the internal β-actin control and expressed in units (-fold change) relative to wild-type (+/+ values assigned a unit of 1 as described under “Experimental Procedures.” The indicated analysis is one of two reproductions.

**FIG. 3. Differential expression of liver HMG-CoA reductase and Cyp7a in wild-type and FGFR4-deficient mice on standard, high cholesterol, and high cholesterol/cholate Diets.** mRNA levels of HMG-CoA reductase (A) and Cyp7a (B) were determined by RNase protection using 50 μg of total RNA isolated from the liver of three mice fed standard chow (Chow), chow containing 2% cholesterol (Chol), or 2% cholesterol and 2% cholate (Chol + Chol). P, labeled riboprobes. Quantitation of bands relative to β-actin controls, and the -fold change indicated was performed as described under “Experimental Procedures.” C, immunoblot analysis of liver CYP7A protein from wild-type and FGFR4-deficient mice on standard diet. Data are representative of one of three independent reproducions.

a strong repressor of Cyp7a transcription (30). The addition of cholate (2%, w/w) to the high cholesterol chow revealed that mechanisms for bile acid-mediated repression of Cyp7a expression were intact and similar to wild type. These combined results suggest that FGFR4 negatively regulates bile acid synthesis through repression of Cyp7a expression and that Cyp7a
is constitutively elevated in its absence.

**Hepatomegaly in FGFR4-deficient Mice on High Cholesterol and Cholate—** FGFR4 (+/+) and FGFR4 (−/−) mice on a high cholesterol diet (Fig. 4, A and B) exhibited no significant difference in liver size or liver cholesterol concentration. When challenged with a diet containing both 2% cholesterol and cholate (Chol and Cholate), the liver weight in the FGFR4-deficient animals doubled within 2 weeks on the combined high cholesterol/cholate diet, and was 1.8 times larger than wild-type livers after 1 month (Figs. 4D and 5A). Administration of cholate alone had no effect (data not shown). The cholesterol/cholate-induced hepatomegaly was confirmed by the 32% fewer hepatocytes per visual field in sections of livers from FGFR4 (−/−) mice (Fig. 5B). A separate analysis of DNA synthesis by incorporation of BrdUrd (data not shown) confirmed that the hepatomegaly in FGFR4 (−/−) mice was due to hepatic hypertrophy rather than an increase in hepatocyte number.

Why is cholesterol-induced hepatomegaly dependent on dietary cholate, and why does it occur specifically in the FGFR4-deficient mice, which appear to be more capable of disposal of cholesterol? When Cyp7a is deficient (31), the secondary acid pathway of bile acid synthesis compensates by generation and disposal of potentially hepatotoxic oxysterols from cholesterol (24, 32). Moreover, the pathway is less stringently feedback-inhibited by bile acids than the classical pathway (33). We examined expression of cholesterol 27α-hydroxylase (Cyp27a) and oxysterol 7α-hydroxylase (Cyp7b), rate-limiting enzymes of the alternative acid pathway. Nuclease protection analysis revealed no difference between expression levels of Cyp27a or Cyp7b mRNA between FGFR4 (+/+) and FGFR4 (−/−) mice on standard chow. However, Cyp27a was repressed twice as effectively in mutants as in wild-type mice on the high cholesterol/cholate combination (Fig. 6A). We also observed a decrease in Cyp27a in the mutant mice fed high cholesterol without cholate. No difference in Cyp7b expression was detected under any of the conditions (data not shown). The exaggerated depression of Cyp27a in absence of FGFR4 under conditions where Cyp7a is severely repressed may contribute to the selective hepatomegaly in the FGFR4-deficient mice.

The hepatomagic phenotype in FGFR4 (−/−) mice induced by the high cholesterol/cholate combination was similar to that induced by cholesterol alone in mice devoid of the gene for the nuclear oxysterol receptor and transcription factor LXRα (20). We observed no change in the expression of LXRα or LXRβ.
mRNAs in all described conditions in wild-type and FGFR4-deficient mice (data not shown). However, a screen for diet-dependent differences in expression of candidate co-activator/co-repressors of LXRα in the wild-type and FGFR4 knockout mice revealed that expression of the multi-functional co-activator and co-repressor RIP140 was responsive to the dietary manipulation (Fig. 6A). Expression of RIP140 was depressed by 40% in wild-type mice fed high cholesterol, but elevated 1.8-fold in mice fed both cholesterol and cholate. RIP140 mRNA in the wild-type and FGFR4 knockout mice revealed the exquisite metabolite-controlled transcriptional networks that balance the concentration of harmful, but essential, cholesterol and its metabolites in a physiological context (Fig. 7).

**Negative Regulation of Bile Acid Synthesis by Liver Transmembrane Kinase FGFR4**—Here we implicate the transmembrane tyrosine kinase FGFR4 in control of cholesterol metabolism to bile acids by targeted gene disruption in mice. In contrast to the Cyp7a and Cyp27a knockout mice, both the excreted and total bile acid pools are elevated. This indicates that FGFR4 normally exerts a negative control on cholesterol metabolism to bile acids, which is abrogated by disruption of the *fgfr4* gene. A defect in uptake and recycling underlying the elevated fecal bile acid levels in the FGFR4-deficient mice was unlikely, since 1) the elevation of bile acid pools was significant prior to developmental maturation of intestinal uptake and recycling mechanisms (Fig. 2D); and 2) induced malabsorption of ileal bile acids accompanied by elevation of fecal bile acid content, Cyp7a, and HMG-CoA reductase, causes a decrease in the total bile acid pool size (40, 41). The normal sensitivity of Cyp7a expression in the FGFR4-deficient mice to dietary bile acids argued against an alteration in the bile acid-mediated feedback regulation in which the bile acid receptor FXR has...
recently been implicated. Although the anticipated phenotype of FXR knockout mice is similar to that which we describe here for FGFR4-deficient mice, the mechanism of negative control exerted by FGFR4 and FXR appears to be different, *e.g.* one does not mediate or compensate for the other.

On low cholesterol chow (<0.02%), *de novo* synthesis determined by the level of rate-limiting HMG-CoA reductase provides sufficient cholesterol to compensate for metabolism and the 5% of the bile acid pool that normally escapes uptake and recycling (42). In the FGFR4-deficient mice, we also observed an elevation of HMG-CoA reductase concurrent with the elevation of bile acid pools and Cyp7a. On the one hand, elevation of HMG-CoA reductase and cholesterol biosynthesis can elevate Cyp7a by feed-forward activation. On the other hand, elevation of Cyp7a and flux to bile acids can cause elevated HMG-CoA reductase through depletion of cholesterol substrate. If FGFR4 is normally a repressor of HMG-CoA reductase, then repression occurs independent of cholesterol-mediated regulation, since there was no defect in the dietary cholesterol-induced repression of HMG-CoA reductase in the FGFR4-deficient animals. Since Cyp7a remains elevated under the same dietary conditions that repressed HMG-CoA reductase, the elevation of Cyp7a expression as solely a consequence of increased cholesterol synthesis was ruled out. The elevation of HMG-CoA reductase observed in the FGFR4-deficient mice is more likely a consequence of cholesterol depletion due to accelerated bile acid synthesis.

Finally, a negative role of FGFR4 on the secondary acid pathway of bile acid synthesis was ruled out since the elevated bile acid pools in FGFR4 (−/−) mice was evident in neonatal mice at a time when Cyp7b and activity of the pathway is absent (24). Taken together, these findings demonstrate that FGFR4 exerts a negative control on cholesterol metabolism and bile acid synthesis in liver at the level of expression of Cyp7a that cannot be compensated for in its absence.

**Targets for Negative Regulation by FGFR4**—At least eight transcriptional activators and their corresponding sequence response elements have been identified for Cyp7a (43). Positive transcriptional activators of Cyp7a expression are potential targets for negative modulation by the FGFR4 membrane kinase-signaling complex. Among these are the oysterol receptor LXRα (29), which works in partnership with RXR, which has been demonstrated to be indispensable in the induction of Cyp7a and tolerance of a high cholesterol challenge in the diet (20). Although both FGFR4 knockout and normal mice tolerated high dietary cholesterol, the addition of 2% (w/w) sodium cholate to the high cholesterol chow reproduced the hepatomegalic phenotype described in LXRA (−/−) mice administered only the high cholesterol (20). This paradoxical phenotype in mice with elevated bile acid synthesis in absence of dietary cholate yielded clues to the mechanism of both FGFR4- and the bile acid-mediated repression of bile acid synthesis. The cholate-dependent, cholesterol-induced hepatomegaly in the FGFR4 knockout mice was coincident with over a 10-fold increase in expression of RIP140, a repressor of LXRα/RXR-mediated transcription (34). The cholate-induced increase was over a depressed level of RIP140 expression in the knockout mice relative to levels in normal mice on both the low and high cholesterol diets. This suggests that FGFR4 may repress bile acid synthesis through induced expression of the co-repressor RIP140. The marked elevation of RIP140 expression induced by dietary cholate also suggested a bile acid/FXR-mediated feedback regulation at the feed-forward stage of cholesterol disposal, in addition to direct repression of Cyp7a through cis-acting elements in the cyp7a gene (37).

**Why does the expression of RIP140 overshoot wild-type levels in the presence of dietary cholate?** This paradoxical effect can be explained by the phenomena of co-factor sharing (44) between bile acid-activated FXR and a putative FGFR4-activated transcription factor, both of which activate RIP140. In wild-type mice, bile acid-dependent FXR must compete with an FGFR4-activated transcription factor for a shared co-factor, which limits its maximum activation potential. The absence of FGFR4 allows maximum activation by FXR, which now has a monopoly on available co-factor. Overall, our results illustrate a novel and elegant multilevel network of regulation of Cyp7a transcription and bile acid synthesis that will be the subject of future study.

**Integration of Transmembrane Signaling with Metabolite-controlled Transcriptional Networks**—Our results show that a transmembrane signaling complex, which mediates cell to cell communication and monitors changes in the tissue environment, is coupled to the metabolite-controlled transcriptional network that maintains cholesterol and bile acid homeostasis. It is conceivable that the FGFR complex directly senses cholesterol, bile acids, and intermediates through yet undefined co-factors. It is more likely that the pericellular matrix-controlled FGFR kinase complex in hepatocytes transmits changes in the tissue microenvironment that call for a rise in liver cholesterol and lipid metabolism in its anabolic roles for liver cells locally or for the organism (45). The liver response to acute infection triggered by endotoxins and cytokines causes transient cholesterol accumulation and hyperlipidemia coincident with depression of Cyp7a (45). Liver Cyp7a and serum 7a-hydroxy-cholesterol levels are also depressed after partial hepatectomy and prior to the regenerative response (46). In recent years, cholesterol has been implicated in an increasing number of membrane signaling functions, including caveolar function (47), covalent modification of hedgehog signaling proteins (48), and assembly of integrin-G protein complexes (49). Inherited autosomal dominant mutations in fgf4 genes other than fgsf4 result in constitutively active signaling complexes, which cause a variety of developmental abnormalities (50). Chronic deficiency or constitutive activity of FGFR4 may underlie defects in cholesterol and bile acid homeostasis. Our findings suggest the FGF-heparan sulfate-FGFR4 signaling complex as a target for prevention or therapy in maladies of cholesterol and bile acid metabolism. Both FGFR4 knockout mice and mice overexpressing FGFR4 in the liver provide new models for study of cholesterol and bile acid abnormalities.

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