Liver-specific Activities of FGF19 Require Klotho beta*\$t

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Hepatocyte function is regulated by members of the fibroblast growth factor (FGF) family of proteins, but little is known about the specific molecular mechanisms of this endothocrine pathway. FGF19 regulates bile acid homeostasis and gall bladder filling; FGF19 binds only to FGFR4 (FGFR4), but its liver-specific activity cannot be explained solely by the distribution of this receptor. Although it has been suggested that Klotho beta (KLB) may have a role in mediating FGF19 activity, we have provided for the first time definitive evidence that KLB is required for FGF19 binding to FGFR4, intracellular signaling, and downstream modulation of gene expression. We have shown that FGFR4 is widely distributed in mouse, whereas KLB distribution is more restricted. Liver was the only organ in which both genes were abundantly expressed. We show that in mice, FGF19 injection triggers liver-specific induction of c-Fos and repression of CYP7A1. The tissue-specific activity of FGF19 supports the unique intersection of KLB and FGFR4 distribution in liver. These studies define KLB as a novel FGFR4 co-receptor required for FGF19 liver specific functions.

Bile acids are amphipathic cholesterol metabolites essential for the absorption of lipophilic nutrients and cholesterol homeostasis (1). They are synthesized by the liver and stored in the gall bladder (2). The cycle of gallbladder filling and emptying controls the flow of bile into the intestine for digestion. Because bile acid accumulation can lead to hepatotoxicity and cholestasis, this process is tightly regulated (3) by a negative feedback mechanism. Bile acids bind to the farnesoid X receptor, a member of the nuclear receptor family of ligand-regulated transcription factors, which is highly expressed in liver and intestine. In the liver, farnesoid X receptor activation represses the transcription of cholesterol 7α-hydroxylase (CYP7A1),2 a rate-limiting enzyme in the biosynthesis of bile acids (4, 5). In the small intestine, bile acid activation of farnesoid X receptor induces the expression of fibroblast growth factor (FGF) 15 (FGF15; mouse orthologue of human FGF19). The secreted FGF15 signals from the intestine to the liver to repress CYP7A1 expression through a FGF receptor 4 (FGFR4) mediated mechanism (6). In addition, the FGF15/FGF19 feedback loop prevents bile excretion into the digestive tract by promoting gallbladder filling (7).

The role of FGF15/FGF19 in bile acid homeostasis is supported by the increased liver CYP7A1 expression and decreased gallbladder volume that occurs in FGF15-deficient mice (FGF15−/− mice) and in FGF4-deficient mice (FGFR4−/− mice) (6, 7). FGF19 is mainly expressed in the gall bladder and the small intestine but is also found in circulation (8, 9). Transgenic mice, which express FGF19, have reduced fat mass and an increased metabolic rate, and they do not become obese or diabetic on a high fat diet (10, 11). These metabolic phenotypes suggested that the biological activity of FGF19 occurs primarily in the liver (12). Although FGF19 binds only to FGFR4, the liver-specific activity of FGF19 cannot be explained solely by the distribution of this receptor because of its wide tissue expression.

Klotho beta (KLB) encodes a 130-kDa type 1 transmembrane protein with a short (29 amino acids) intracellular domain that has no predicted kinase activity (13). KLB has two extracellular glycosidase domains that lack a characteristic glutamic acid residue essential for enzymatic activity. KLB-deficient mice (Klb−/−) have increased CYP7A1 expression and decreased gallbladder size, indicating that Klb−/− mice can no longer suppress bile acid synthesis (6, 14). Because FGF15−/− mice develop several similar phenotypes, we proposed that KLB participates in FGF15/FGF19 functions. In this study, we demonstrate that KLB acts as an FGFR4 co-receptor required for FGF19 binding, intracellular signaling, and downstream modulation of gene expression. KLB and FGFR4 are co-expressed in the liver where they mediate the tissue-specific activity of FGF19.

EXPERIMENTAL PROCEDURES

DNA Constructs—Total RNA from HepG2 cells was extracted using the RNeasy kit (Qiagen). The KLB gene was cloned using the SuperScript III One-step RT-PCR kit (Invitrogen) and the following primers: forward, 5′-CGGGCGCTAGCATGAAGC-CAGGCTGTGCGGCAGG-3′; reverse, 5′-CAGTGATCC-TACTTATCGTCGTCATCCTTGTAATCGCTAACAACT-3′. The resulting KLB PCR product was digested with EcoRI and AscI and ligated into pCMV-Tag4A (Stratagene) to obtain the full-length KLB with a FLAG tag at the C-terminal end (pCMV-KLB-FLAG). The secreted KLB extracellular domain (KLBΔTM) was obtained by PCR using pCMV-KLB-FLAG as the template and the following primers: forward, 5′-GAGCTTCATGCTGAGAGG-CCTGTCAGCAGGTCGCGC-3′; reverse, 5′-CCCTGGCGGACAACT-CTCTGGCTTCTTCCTTCTTCC-3′. The resulting KLB PCR product was digested with Nhel and BamHI and ligated into pCMV-Tag4A (Stratagene). The resulting 12-kDa KLB cDNA was cloned into the pCDH vector (Clontech) to obtain the pCDH-KLB plasmid. The resulting plasmid was used to transfect 293T cells to obtain the KLB-FLAG protein. The KLB-FLAG protein was purified using an anti-FLAG antibody resin (Sigma) and analyzed by Western blotting.

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1. The abbreviations used are: CYP7A1, cholesterol 7α-hydroxylase; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; FRS, FGFR substrate; KLB, Klotho beta; ERK, extracellular signal-regulated kinase; pERK, phosphorylated ERK; PBS, phosphate-buffered saline; RT, reverse transcription; siRNA, small interfering RNA.

2. The on-line version of this article (available at http://www.jbc.org) contains supplemental Materials and Figs. S1 and S2.
The human FGFR4 cDNA was cloned from total HepG2 cell RNA using the SuperScript III One-step RT-PCR kit and the following primers: forward, 5'-CCGCCGGATATCATGCGGCTGCTGCTGGCCCTGTTGG-3'; reverse, 5'-CCGCCGGGAATTCCTCTGTCGACCCCGACCCGAGGGG-3'. The resulting PCR product was digested with EcoRV and EcoRI and ligated into pIRESpuro3 (Clontech).

KLBΔTM-conditioned Medium—HEK 293 cells were transfected with the KLBΔTM or the corresponding empty vector and maintained in serum-free PS25 medium for 72–96 h. The resulting media were filtered, supplemented with HEPES, pH 7.2 (final concentration 40 mM), concentrated, and evaluated for KLBΔTM content by immunoblotting using a KLB-specific antibody (MAB3738 antibody; R&D Systems).

Co-immunoprecipitation Assay—The control or KLBΔTM-conditioned medium was supplemented with Triton X-100 (Calbiochem) to a final concentration of 0.5% and incubated with or without 0.5 μg/ml FGFR-Fc (R&D Systems), 0.5 μg/ml heparin (Sigma), 1 μg/ml FGF19 (R&D Systems), and 10 μl of protein A-agarose gel (Sigma) at 4 °C for 18 h. The affinity matrix was centrifuged and washed three times with phosphate-buffered saline (PBS)/0.5% Triton X-100 and once with PBS. Immunoblot analysis was performed using antibodies against KLB (R&D Systems), FGF19 (clone 1A6; Genentech Inc.), or FGFR4 (clone 8G11; Genentech Inc.) and a horse-radish peroxidase-conjugated antibody against human IgG (Jackson Immunochemical).

Cell Culture and Stable Cell Lines—HEK 293, HepG2, and Hep3B cells were obtained from American Type Culture Collection (Manassas, VA) and maintained in an F-12/Dulbecco’s modified Eagle’s medium mix (50:50) supplemented with 10% fetal bovine serum and 2 mM L-glutamine. HEK 293 cells stably expressing empty vector, hFGFR4, KLB-FLAG, or hFGFR4 and KLB-FLAG were created and grown in selective medium containing 500 μg/ml Geneticin and 2.5 μg/ml puromycin.

Co-immunoprecipitation—HEK 293 cells that transiently (24–48 h transfection) or stably expressed empty vector, hFGFR4, KLB-FLAG, or hFGFR4 and KLB-FLAG were lysed with radioligand binding assays lysis buffer (PBS containing 1% Triton X-100 and 1% Nonidet P-40) supplemented with Complete EDTA-free protease inhibitor mixture (Roche Applied Science). Total protein concentrations were determined by BCA protein assay (Pierce). Equal amounts of total protein for each sample lysate were incubated in the absence or presence of 0.5 μg/ml heparin along with control- or FGF19-conditioned medium and immunoprecipitated with an anti-FLAG (Sigma) or anti-FGFR4 (1G7; Genentech, Inc.) agarose affinity matrix at 4 °C for 18 h. Immunoprecipitated proteins were washed twice with PBS, 0.5% Triton X-100 and twice with PBS and then analyzed by immunoblotting using antibodies against KLB, FGFR4, and FGF19.

FGF Pathway Activation—HEK 293 cells transiently or stably expressing empty vector, hFGFR4, KLB-FLAG, or hFGFR4 and KLB-FLAG were starved of serum for 24 h before they were exposed to 0–500 ng/ml FGF19 (R&D Systems) for 10 min. Hep3B or HepG2 cells were transfected with control or KLB siRNA for 72 h (starved of serum for the final 24 h) before exposure to 0 or 100 ng/ml FGF19 (Genentech, Inc.) for 10 min. In each case, cells were treated with 20 ng/ml FGF1 (FGF acidic, R&D Systems) or 20 ng/ml epidermal growth factor (Roche Applied Science) as positive controls. Cells were lysed with radioimmunoprecipitation assay lysis buffer (Upstate Biotechnology) supplemented with Complete EDTA-free protease inhibitor mixture (Roche Applied Science) and phosphatase inhibitor mixtures 1 and 2 (Sigma). Equal amounts of protein were analyzed by immunoblotting using antibodies against phospho-ERK1/2 (pERK1/2), phospho-FRS2 (pFRS2), ERK1/2 (Cell Signaling Technology), FRS2 (Upstate Biotechnology), KLB (R&D Systems), or β-actin (Sigma). Quantitation of FGFR substrate 2 (FRS2) and extracellular signal-regulated kinase-1 and -2 (ERK1/2) phosphorylation was performed as described previously, with modifications (15). Briefly, the densitometric analysis of the pFRS2, pERK1/2, total FRS2, and total ERK1/2 protein bands was done using NIH ImageJ software. The normalized relative density values (RDV) were determined as follows: RDV pFRS2 = (DV pFRS2 − DV background)/(DV total FRS2 − DV background). Normalized RDV pERK1/2 = [ΣRDV pFRS2]/n for a given treatment − [ΣRDV pFRS2]/n at no treatment ± S.E. (n = 3). The same method was used to determine the normalized RDV for pERK1/2. Values were plotted as normalized RDV.

Semiquantitative RT-PCR—Total RNA was extracted using the RNaseasy kit (Qiagen). Specific primers and fluorogenic probes were used to amplify and quantitate gene expression (see the supplemental data for sequences) (16). The gene-specific signals were normalized to the RPL19 housekeeping gene. All TaqMan RT-PCR reagents were purchased from Applied Biosystems (Foster City, CA). A minimum of a triplicate set of data was analyzed for each condition. Data are presented as the mean ± S.E. (SD).

siRNA Transfection—KLB and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) siRNA oligos were from Dharmacon (see supplemental data for sequences). The various siRNA duplexes were transfected using the DharmaFECT transfection kit (Dharmacon) following the manufacturer’s recommended protocol.

In Silico Expression Analysis—For expression analysis, box- and whisker-plots were generated with the normalized gene expression data extracted from the BioExpress™ database (Gene Logic, Inc., Gaithersburg, MD). The distribution of KLB and FGFR4 expression was evaluated using the signals associated to the probes 244276_at and 204579_at, respectively.

In Vivo Experiments—All animal protocols were approved by an Institutional Animal Care and Use Committee. Female FVB mice at 5 or 6 weeks old were obtained from Charles River Laboratories. The mice were provided standard feed and water ad libitum until 12 h before injection, at which time feed was removed. Mice were given intravenous injections of vehicle (PBS) or 1 mg/kg FGF19. After 30 min, mice from all groups were sacrificed, and tissue samples were collected, frozen in liquid nitrogen, and stored at −70 °C. Total RNA from frozen tissue samples was prepared using the RNAeasy kit (Qiagen). Groups of 3–5 animals were analyzed for each condition. Data are presented as the mean ± S.E. and were analyzed by the Student’s t test.

RESULTS

KLB Forms a Complex with FGFR4, FGF19, and Heparin—We first tested the binding of KLB to FGF19 and FGFRs using a co-precipitation assay. FGF19, heparin and different FGFR-Fc fusion proteins were incubated in conditioned medium con-
FGF19 Activity Requires Klotho beta

FIGURE 1. KLB forms a complex with FGF19, FGFR4, and heparin. A, FGF19 (0.5 μg), heparin (0.5 μg), and different FGFR-Fc fusion proteins (0.5 μg) were incubated in KLBΔTM-conditioned medium for 18 h at 4 °C. The protein interactions were determined by protein A-agarose precipitation and immunoblot analyses. B, KLBΔTM- or control-conditioned medium was incubated in the presence or absence of FGFR4-Fc, FGF19, and FGFR4 readily pulled down FGF19. This interaction was further stabilized in the presence of heparin (Fig. 1, C and D). E, the FGFR4-KLB interaction in HepG2 cell lysates was co-precipitated from lysates of cells transfected with empty vector, FGFR4, KLB-FLAG, or a combination of FGFR4 and KLB expression vectors. The transfected cells were incubated with vehicle (PBS) or FGF19 (0–500 ng/ml) for 10 min and then lysed, and FRS2 and ERK1/2 phosphorylation was analyzed by immunoblot.

We used the same assay to evaluate the contribution of each component to the complex formation. Control or KLBΔTM containing conditioned medium was incubated in the presence or absence of FGFR4-Fc, FGF19, and/or heparin. In the absence of heparin and FGF19, no interaction was detected between KLB and FGFR4-Fc (Fig. 1B). Heparin was a weak promoter, whereas FGF19 was a strong promoter of the KLB-FGFR4 interaction. The maximal level of stabilization of the KLB-FGFR4-Fc interaction occurred in the presence of both heparin and FGF19. Conversely, FGF19 binding to FGFR4-Fc required the presence of heparin or KLB. The maximal level of FGF19 binding to FGFR4 occurred when both heparin and KLB were included in the reaction. These data demonstrate that KLB is sufficient to support FGF19 binding to FGFR4. They also show that KLB promotes the previously demonstrated heparin-dependent interaction of FGF19 with FGFR4 (9). Therefore, each individual component contributes to the stability of the FGF19-FGFR4-KLB-heparin complex.

To test whether KLB and FGFR4 also participate in the formation of a complex with FGF19 and heparin at the cell surface, we evaluated the ability of FGFR4 and KLB to immunoprecipitate FGF19 from lysates of transiently or stably transfected cells in the presence or the absence of heparin. No detectable FGF19 was co-precipitated from lysates of cells transfected with only a control or an FGFR4 expression vector (Fig. 1, C and D). KLB pulled down FGF19 from KLB-transfected cell lysate only in the presence of heparin, indicating that KLB expression promotes heparin-dependent FGF19 binding to the endogenous HEK 293 FGFR4.

In lysate from KLB- and FGFR4-co-transfected cells, KLB and FGFR4 readily pulled down FGF19. This interaction was further stabilized in the presence of heparin (Fig. 1, C and D). These data show that KLB is required for FGF19 binding to...
FGF19 Activity Requires Klotho beta

A

KLb

0 h 1 h 2 h 4 h 6 h 24 h

HepG2  Hep3B  HEK293  SW620  Colo205

Relative KLb Expression (Folds)

B

c-Fos

0 h 1 h 2 h 4 h 6 h 24 h

HepG2  Hep3B  HEK293  SW620  Colo205

Relative c-Fos Expression (Folds)

C

JunB

0 h 1 h 2 h 4 h 6 h 24 h

HepG2  Hep3B  HEK293  SW620  Colo205

Relative JunB Expression (Folds)

D

c-Jun

0 h 1 h 2 h 4 h 6 h 24 h

HepG2  Hep3B  HEK293  SW620  Colo205

Relative c-Jun Expression (Folds)

E

HEP3B cells

Control KLb siRNA

Transfection: 150 kDa -

KLb siRNA

Control #1 #2 #3 #4

KLb

β-Actin

F

HEP3B cells

KLb siRNA

Transfection: Control FGF19:

FGF19: 75 kDa - + - + - + + FGF1

75 kDa - + - + - + + pFRS2

50 kDa - + - + - + + pFRS2

50 kDa - + - + - + + pERK1/2

pERK1/2

G

HEP3B cells

KLb c-Fos

Relative Expression

#1 #2 #3 #4 Control siRNA

H

HEK293

- FGF19 - FGF19

Relative c-Fos Expression

Empty Vec. KLb FGFR4 KLb/FGFR4

p=0.016

HEK 293 Cell Lines
the cell surface FGFR4 and that heparin promotes this interaction. In addition, FGFR4 and KLB readily interacted in a heparin- and ligand-independent manner in co-transfected cells. This result contrasts with the heparin- and ligand-dependent complex formation observed with the secreted chimeric FGFR4 and KLB proteins. This discrepancy indicates a role for the KLB and FGFR4 transmembrane domains in the complex formation.

To test the hypothesis that KLB and FGFR4 form a constitutive complex at the cell surface, we evaluated whether KLB co-immunoprecipitated with FGFR4 from HepG2 cell lysates in the absence of FGF19 or heparin. Incubation of HepG2 cell lysates with an antibody against FGFR4-immunoprecipitated FGFR4 and KLB, whereas no protein was immunoprecipitated with the control antibody (Fig. 1E), showing that the endogenous transmembrane KLB and FGFR4 form a constitutive heparin- and ligand-independent complex.

**KLB Is Required for FGF19 Signaling**—To test whether KLB contributes to the activation of the FGF19 signaling pathway, we evaluated the effects of FGF19 on FR52 and ERK1/2 phosphorylation in KLB- and/or FGFR4-transfected HEK 293 cells as well as in controls. FGF19 did not promote FR52 or ERK1/2 phosphorylation in cells transfected with an empty expression vector (Fig. 2 and supplemental Fig. S2). HEK 293 cells transfected with KLB or FGFR4 showed only a weak, dose-dependent increase in ERK1/2 phosphorylation but no detectable FR52 phosphorylation following exposure to FGF19. The co-transfection of FGFR4 with KLB promoted FGF19 signaling in HEK 293 cells, indicated by the robust, dose-dependent increase of both FR52 and ERK1/2 phosphorylation. One possible explanation for this effect is that local, high concentrations of FGF19 and FGFR4 allow for weak signaling in the absence of KLB. However, because FGF19 has an endocrine function and its average circulating concentration is 193 ± 36 pg/ml (a range of 49 to 590 pg/ml), this explanation is unlikely (8). Therefore the robust induction of FGF19 signaling by KLB is likely to occur at physiological concentrations of FGF19.

**KLB Is Required for FGF19 Downstream Modulation of Gene Expression**—Because KLB is required for FGF19 signaling, we evaluated the effect of FGF19 on KLB expression in various cell lines. We detected high KLB expression in liver cell lines (HepG2 and Hep3B) but only traces in kidney (HEK 293) or colon cell lines (Fig. 3A, SW620 and Colo205). Upon exposure to FGF19, KLB expression in HepG2 and Hep3B cells was gradually repressed to 50–60% the level of unexposed cells after 6 h, and it remained at this level for at least 24 h. Exposure to FGF19 did not affect KLB expression levels in the other cell lines. The repression of KLB expression by FGF19 might be a regulatory negative feedback mechanism in liver cells.

Because a plethora of physiological and pathological stimuli induce the genes of the Fos and Jun family in a wide variety of cell types, we tested whether FGF19 modulates c-Fos, JunB, and c-Jun expression in various cell lines (17–19). FGF19 up-regulated c-Fos and JunB expression, as well as c-Jun expression to a lesser extent, in KLB-expressing cells (Fig. 3, B–D, HepG2 and Hep3B). The induction of c-Fos, JunB, and c-Jun expression occurred within 30 min of exposure to FGF19, and in most cases, expression returned to basal levels after 6 h. JunB expression remained elevated for at least 24 h in Hep3B cells (Fig. 3C).

To test whether KLB promotes FGF19 signaling and c-Fos induction in Hep3B and HepG2 cells, we inhibited KLB expression using specific siRNAs. KLB siRNA transfection significantly reduced KLB mRNA and protein expression in Hep3B (Fig. 3, E and G) and HepG2 cells (supplemental Fig. S1). The individual transfection of four different KLB siRNAs significantly attenuated FGF19-mediated FR52 and ERK1/2 phosphorylation (Fig. 3F). In addition, transfection of Hep3B cells with KLB siRNA inhibited FGF19-mediated c-Fos induction by 62–80% compared with the control cells (Fig. 3G). Similarly, transfection of HepG2 cells with KLB siRNA reduced the levels of FGF19-dependent FR52 and ERK1/2 phosphorylation as well as c-Fos induction as compared with the control cells (supplemental Fig. S1). These results indicate that KLB expression is required for FGF19-dependent pathway activation and c-Fos induction.

To further assess the participation of KLB in FGF19-mediated c-Fos induction, we transfected HEK 293 cells with empty, KLB, or FGFR4 vector or a combination of KLB and FGFR4 expression vectors and exposed the cells to FGF19. Only cells transfected with both KLB and FGFR4 expression vectors induced c-Fos in response to FGF19 (Fig. 3H). These data indicate that KLB is required for FGF19 pathway activation and modulation of gene regulation.

**KLB and FGFR4 Distribution Dictate FGF19 Tissue-specific Activity**—We evaluated KLB and FGFR4 expression in a variety of human tissues by analyzing the BioExpress data base (Gene Logic, Inc.). In decreasing order of signal intensity, KLB was expressed in adipose, liver, pancreas, and breast tissues (Fig. 4A). In a decreasing order of signal intensity, FGFR4 was expressed in liver, lung, gall bladder, small intestine, pancreas, colon, lymphoid, ovary, and breast tissues (Fig. 4B). These data

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**FIGURE 3. KLB is required for FGF19 downstream modulation of gene expression.** A, FGF19 represses KLB expression. Cell lines were incubated with FGF19 (100 ng/ml; 0–24 h), and KLB expression levels were analyzed by RT-PCR. All values were compared with KLB expression levels in Hep3B cells at time 0. B–D, FGF19 promoted expression of c-Fos, JunB, and c-Jun. Cell lines were incubated with FGF19 (100 ng/ml; 0–24 h), and c-Fos (B), JunB (C), and c-Jun (D) expression was analyzed by RT-PCR. The values represent the relative -fold increase in the expression of a particular gene compared with its expression before exposure to FGF19. E, KLB siRNA transfection represses KLB synthesis. Hep3B cells transfected with each of four different KLB siRNAs were analyzed for KLB expression by immunoblot. F, KLB siRNA transfection inhibits FGF19 signaling. Hep3B cells transfected with each of four different KLB siRNAs were incubated with vehicle (PBS) or FGF19 (100 ng/ml) for 10 min and analyzed for FR52 and ERK1/2 phosphorylation by immunoblot. G, KLB siRNA transfection inhibits FGF19-mediated c-Fos induction. Hep3B cells transfected with each of four different KLB siRNAs were incubated with FGF19 (100 ng/ml) for 90 min, and KLB and c-Fos expression levels were analyzed by RT-PCR. The values represent the relative expression of each particular gene compared with that of cells transfected with control siRNA. H, HEK 293 cells transfected with empty vector, KLB, FGFR4, or a combination of FGFR4 and KLB expression vectors were incubated with PBS or FGF19 (100 ng/ml) for 90 min; c-Fos expression was analyzed by RT-PCR. The values represent the -fold increase in c-Fos expression compared with the expression levels before cells were exposed to FGF19. A–D, G, and H, a triplicate set of data was analyzed for each condition. Data are presented as the mean ± S.E.
show that KLB expression is restricted to only a few tissues, whereas FGFR4 expression is more widely distributed. A high level of co-expression of KLB and FGFR4 was observed only in liver and pancreas. Because the expression of KLB and FGFR4 are required for FGF19 activity, these findings suggest that liver and pancreas are the major organs in which they are active. Marginal levels of KLB and FGFR4 expression were also observed in breast tissues. KLB was highly expressed in adipose tissues, but the absence of FGFR4 precludes the function of FGF19 in this tissue.

To test the hypothesis that FGF19 acts only on tissues that express both FGFR4 and KLB, we first surveyed Klb and FGFR4 distribution in various mouse organs using semiquantitative RT-PCR. The relative mRNA levels represent the relative -fold expression compared with brain (organ with the lowest expression surveyed). Klb expression was predominantly expressed in liver (Fig. 4C). Lower levels of Klb expression were also found in adipose and colon tissues. Additional organs tested showed marginal expression of Klb. FGFR4 was highly expressed in liver, lung, adrenals, kidney, and colon (Fig. 4D). Lower levels of FGFR4
expression were also observed in intestine, ovaries, muscle, and pancreas. The overall Klb and FGFR4 distribution in mouse tissues was similar to that of human tissues. However, contrary to the findings in human tissues, no consistent Klb or FGFR4 expression could be detected in mouse pancreas. In addition, a low level of Klb expression was detected in mouse colon, whereas no expression was found in the corresponding human tissues. These differences might be attributable to species- and/or strain-specific tissue distribution. These data indicate that liver is the only mouse organ in which Klb and FGFR4 are highly co-expressed.

To determine the FGF19-specific site of action, we compared the levels of c-Fos expression in organs of mice injected with FGF19 with those of mice injected with PBS (controls). We chose to monitor the c-Fos response to FGF19 because c-Fos expression is ubiquitous and its induction is sensitive to FGF19 stimulation. C-Fos expression was 1300-fold higher in the liver of mice injected with FGF19 compared with the livers of mice injected with PBS (Fig. 4E). The FGF19-dependent c-Fos induction was at least 150-fold lower in all other organs tested. The activity of FGF19 in liver was confirmed by a 98% inhibition of CYP7A1 expression (Fig. 4F). These data demonstrate that FGF19 acts specifically in liver, the only mouse organ that expresses high levels of both Klb and FGFR4.

DISCUSSION

In this study we have provided evidence that FGF19 requires KLB for binding to FGFR4, intracellular signaling, and downstream gene modulation. However, the reason for such a requirement is still unclear. Compared with the paracrine FGF family members, FGF19 has a low heparin-binding affinity that restricts the endocrine activity of FGF19 to tissues that express FGFR2 (21, 25). The liver-specific activity of FGF19 is supported by this molecular mechanism.

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