Growth Hormone Stimulates Transcription of the Fibroblast Growth Factor 21 Gene in the Liver through the Signal Transducer and Activator of Transcription 5

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Fibroblast growth factor 21 (FGF21) is a recently discovered metabolic regulator. Interestingly, FGF21 is also known to inhibit Janus kinase 2 (JAK2)-signal transducer and activator of transcription 5 (STAT5) signaling from the GH receptor in the liver, where FGF21 mRNA is predominantly expressed. In this study, we tested the hypothesis that *FGF21* gene expression in the liver is controlled by GH through STAT5. We found that GH injection to cattle increased FGF21 mRNA expression in the liver. Mapped by a 5′-rapid amplification of cDNA ends assay, transcription of the *FGF21* gene in the bovine liver was mainly initiated from a nucleotide 24 bp downstream of a TATA box. The bovine FGF21 promoter contains three putative STAT5-binding sites. EMSA confirmed the ability of them to bind to liver STAT5 protein from GH-injected cattle. Chromatin immunoprecipitation assays demonstrated that GH administration increased the binding of STAT5 to the FGF21 promoter in the liver. Cotransfection analyses showed that GH induced reporter gene expression from the FGF21 promoter in a STAT5-dependent manner. GH also stimulated FGF21 mRNA expression in cultured mouse hepatocytes. These data together indicate that GH directly stimulates *FGF21* gene transcription in the liver, at least in part, through STAT5. This finding, together with the fact that FGF21 inhibits GH-induced JAK2-STAT5 signaling in the liver, suggests a novel negative feedback loop that prevents excessive JAK2-STAT5 signaling from the GH receptor in the liver. **(*Endocrinology* 153: 750–758, 2012)**

Growth hormone (GH) is a major regulator of animal growth, development, and metabolism (1, 2). At the cellular level, the action of GH is initiated by binding to the GH receptor (GHR) on the cell surface (3, 4). Binding of GH to GHR activates the receptor-associated tyrosine kinase Janus kinase 2 (JAK2), which phosphorylates various signaling molecules, including STAT1, STAT3, and STAT5. The phosphorylated STAT proteins translocate to the nucleus, where they bind to specific DNA sequences and regulate gene transcription (3–6).

Among the signaling pathways from the GHR, the JAK2-STAT5 pathway appears to be the major pathway that mediates the action of GH on gene transcription in the liver (4, 7, 8). This pathway has been shown to be responsible for the transcriptional action of GH on IGF-I and suppressor of cytokine signaling 2 (SOCS2) (9–12). IGF-I is a mitogenic factor for various cell types (13–15), and GH-induced production of IGF-I by the liver is the major source of circulating IGF-I (16, 17). SOCS2 is known as an inhibitor of JAK2-STAT5 signaling (18–21).

Fibroblast growth factor (FGF)21 is a recently discovered regulator of glucose homeostasis, lipid metabolism, and insulin sensitivity (22–24). In the body, FGF21 is expressed predominantly in the liver (25). In addition to

ISSN Print 0013-7227 ISSN Online 1945-7170
Printed in U.S.A.

doi: 10.1210/en.2011-1591 Received August 4, 2011. Accepted November 16, 2011.
First Published Online December 13, 2011

Abbreviations: ChIP, Chomatin immunoprecipitation; CHO, Chinese hamster ovary; FGF, fibroblast growth factor; GHR, GH receptor; JAK2, Janus kinase 2; RACE, 5′-rapid amplification of cDNA ends; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription.

regulating metabolism, FGF21 inhibits the GH-activated JAK2-STAT5 signaling, causing GH resistance in the liver (26, 27). This interesting effect of FGF21 prompted us to hypothesize that, like SOCS2, FGF21expression in the liver might be also stimulated by GH through the JAK2-STAT5 pathway. The objective of the study reported here was to test this hypothesis.

Materials and Methods

Animal experiment

Angus cows (3–5 yr old) were injected sc with 500 mg of recombinant bovine (b) GH formulated for 2-wk sustained release (Monsanto Co., St. Louis, MO) or an equal volume of saline. Liver biopsies were taken 1 wk after the injection as described previously (28, 29). The liver samples were either used immediately for chromatin and nuclear protein isolation or stored at −80 C until RNA extraction. Throughout this study, the cattle were kept outdoors and were fed 10 kg corn-based diet per animal per day in addition to free access to hay and water. All animal-related procedures were approved by the Institutional Animal Care and Use Committee of Virginia Tech.

Hepatocyte isolation and culture

Hepatocytes were isolated from 8-wk-old C56BL/6 mice using the two-step perfusion procedure (30). In brief, the liver was perfused first with Krebs Ringer buffer supplemented with 0.1 mM EGTA and then with 1 mg/ml collagenase D (Roche Applied Science, Indianapolis, IN) through the inferior vena cava. The isolated hepatocytes were plated in six-well plates at a density of 1×10^6 cells per well in DMEM supplemented with 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA). The cells were cultured in this medium for 24 h and then in serum-free DMEM for 8 h, followed by treatment with recombinant bGH (National Hormone and Peptide Program, Torrance, CA) at 500 ng/ml or an equal volume of PBS for 4 h. This experiment was repeated four times, each time using hepatocytes isolated from a different mouse.

RNA isolation and real-time RT-PCR

Total RNA from frozen liver samples or cultured hepatocytes was extracted using TRI reagent (Molecular Research Center, Cincinnati, OH). Concentration and integrity of RNA were determined with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). Total RNA was reverse transcribed using random primers. Real-time quantitative PCR was performed using the Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). The primers for bovine and mouse FGF21 mRNA and 18S rRNA and bIGF-I mRNA are shown in Table 1. These primers were validated for specificity and efficiency. The PCR primers for mouse IGF-I mRNA were reported in a previous study (31). The PCR data were analyzed using 18S rRNA as the internal control. Based on the Ct values, expression of 18S rRNA was not affected by GH ($P > 0.1$).

5′-Rapid amplification of cDNA ends (RACE)

A 5′-RACE kit was used to amplify the 5′-end region of the bovine liver FGF21 mRNA (Invitrogen, Carlsbad, CA). Bovine liver poly(A) RNA was isolated from bovine liver total RNA using a Oligotex mRNA Mini Kit (QIAGEN, Valencia, CA). First-strand cDNA was synthesized from bovine liver poly(A) RNA using oligo d(T) as the primer. A homopolymeric cytosine tail was added to the 3′-terminus of the cDNA using dCTP and terminal deoxyribonucleotide transferase. The dCTP-tailed cDNA was amplified in PCR using the abridged anchor primer from the kit as the forward primer and a bFGF21-specific primer named "bFGF21R1" as the reverse primer (Table 1). The product of this PCR was diluted at 1:1000, and 1 μl of the diluted product was amplified in a second PCR using the abridged universal amplification primer from the kit as the forward primer

TABLE 1. Oligonucleotides used in this study

Name	Sequence (5′-3′)	Application
bFGF21R1	CTACACAGCGGACGTCTTCA	5′-RACE
bFGF21F2	AGGCGTCATTCAGATCTTGG	RT-PCR
bFGF21R2	TCTCCGACTGGTAGACGTTG	RT-PCR, 5′-RACE
bFGF21F3-*Kpn*I	ATGGTACCCTCCGAGAATCACAGCCTTC	PCR cloning
bFGF21R3-*Xho*I	ATCTCGAGATCTCTTGGGATGGATGCAG	PCR cloning
bFGF21F4	CACCTGGGAGGTGGTCTTTA	ChIP-PCR
bFGF21R4	CAGACAGGTGCCCTCAAAAT	ChIP-PCR
bIGF1F1	GTTGGTGGATGCTCTCCAGT	RT-PCR
bIGF1R1	CTCCAGCCTCCTCAGATCAC	RT-PCR
b18SqPCRF2	GTAACCCGTTGAACCCCATT	RT-PCR
b18SqPCRR2	CCATCCAATCGGTAGTAGCG	RT-PCR
mFGF21F1	ACCTGGAGATCAGGGAGGAT	RT-PCR
mFGF21R1	GTCCTCCAGCAGCAGTTCTC	RT-PCR
m18SqF1	TTAAGAGGGACGGCCGGGGG	RT-PCR
m18SqR1	CTCTGGTCCGTCTTGCGCCG	RT-PCR
STAT5-binding site 1	CCTCTTCAAGGAACAGA	EMSA
STAT5-binding site 2	TTGGTTCCAGGAAATTG	EMSA
STAT5-binding site 3	ACTTTTCCCTGAATAGC	EMSA
Non-STAT5 oligo	TTGTAGATAAATGTGA	EMSA

mFGF, Murine FGF; m18S, murine 18S.

and a second bFGF21-specific primer named "bFGF21R2" as the reverse primer (Table 1). The product of this PCR was resolved on an agarose gel, and major DNA bands were extracted from the gel and cloned into the pGEM-T Easy vector (Promega Corp., Madison, WI). The sequences of the cDNA inserts were determined by standard DNA sequencing.

Plasmid construction

A 1997-bp bFGF21 promoter, from nucleotide 1990 upstream to nucleotide 7 downstream of the mapped transcription start site, was amplified from bovine genomic DNA by PCR using the primers bFGF21F3-*Kpn*I and bFGF21R3-*Xho*I (Table 1). The PCR product was cloned into the promoterless luciferase reporter vector pGL2-basic (Promega) to generate the plasmid pGL2B-bFGF21P. The insert of this plasmid was verified by DNA sequencing.

Chromatin immunoprecipitation assay (ChIP)

Chromatin from fresh liver biopsies was isolated and sheared to 500-1000 bp by sonication as previously described (31, 32). Chromatin immunoprecipitation was performed using a ChIP-IT kit (Active Motif, Carlsbad, CA). Briefly, the sheared chromatin from 200 mg liver tissue was mixed with 25 μl of protein G-Dynal magnetic beads and 3 μg of a STAT5 antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) in a final volume of 200 μl of kit-provided ChIP buffer 1. The mixture was incubated overnight at 4 C. The immunocomplexes binding to the magnetic beads were collected using a magnetic stand. The magnetic beads were washed, and the chromatin was eluted using the reagents from the ChIP-IT kit. The immunoprecipitated chromatin was incubated overnight at 65 C to reverse the DNA-protein cross-linking. A small amount (10% of that used for immunoprecipitation) of the original sheared chromatin was incubated the same way to serve as input chromatin. Abundance of the FGF21 promoter DNA in STAT5 antibody-precipitated chromatin or input chromatin was measured by real-time PCR using the primers bFGF21F4 and bFGF21R4 (Table 1). The abundance of an 18S rDNA region was quantified as a loading control by using the primers b18SqPCRF2 and b18SqPCRR2 (Table 1).

EMSA

Nuclear proteins from fresh liver samples were isolated as previously described (31, 32). Double-stranded oligonucleotides corresponding to the putative STAT5-binding sites (Table 1) in the bFGF21 promoter were end labeled using T4 polynucleotide kinase (Promega) and [γ-^{32}P]ATP (PerkinElmer, Waltham, MA). Bovine liver nuclear proteins (20 μg) were incubated with 1×10^5 disintegrations per minute (dpm) of ^{32}P-labeled oligonucleotide in a reaction buffer containing 20% glycerol, 20 mM Tris-HCl (pH 7.5), 100 mM KCl, 1 mM dithiothreitol, 1 mM EDTA, and 2 μg poly (deoxyinosine-deoxycytosine) for 90 min at 4 C. The DNA-protein mixtures were resolved on native 6% polyacrylamide gels. The gels were dried, and the radioactive signals were detected by phosphorimaging. For supershift assays, the mixtures were added with 2 μg of an anti-STAT5 antibody (Santa Cruz Biotechnology) or rabbit preimmune serum and incubated for an additional 4 h at 4 C before gel electrophoresis. For competitive gel-shift assays, the ^{32}P-labeled oligonucleotide was incubated with the nuclear protein extracts in the presence of 1×, 10×, and 100× molar excess of the same oligonucleotide without ^{32}P label or an unlabeled nonspecific oligonucleotide (Table 1) before gel electrophoresis.

Transfection and luciferase assay

The Chinese hamster ovary (CHO) cells were seeded in 24-well plates at a density of 5×10^4 per well. The cell culture medium was MEM supplemented with 10% fetal bovine serum. The cells were cultured for 24 h, and then transfected with the STAT5b expression plasmid pMX-STAT5b or the corresponding empty pMX vector (kindly provided by Dr. Toshio Kitamura, The University of Tokyo) (33), the bGHR expression plasmid pcDNA3.1-bGHR (34), and the transfection efficiency control plasmid pRL-CMV (Promega), using FuGENE6 as the transfection reagent (Roche Applied Science). The medium was replaced 24 h later by serum-free MEM, and the cells were cultured for 8 more hours. The cells were then treated with recombinant bGH for 16 h. Cell lysis and dual-luciferase assays were carried out using the Dual-Luciferase Reporter Assay System (Promega), according to the manufacturer's instructions. The luciferase activity from the promoter construct was divided by that from pRL-CMV to normalize the potential variation in transfection efficiency.

Statistical analysis

All statistical analyses were performed using the General Linear Model of SAS (SAS Institute, Inc., Cary, NC). Two-group comparisons were analyzed using *t* test. Multiple comparisons were analyzed using ANOVA followed by Tukey's test. All data are expressed as the mean ± SE of the mean (SEM). Differences at $P < 0.05$ are considered significant.

Results

GH administration increased FGF21 mRNA expression in bovine liver

To determine the effect of GH on liver expression of FGF21 mRNA, we injected adult cattle with 500 mg of recombinant bGH formulated for 2-wk sustained release. This injection was expected to elevate serum concentration of GH to approximately 15 ng/ml by d 7 after the injection (29). Liver expression of FGF21 mRNA in GH-

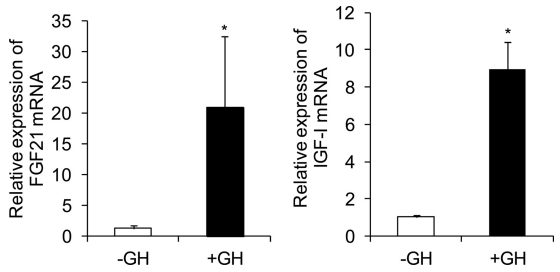

FIG. 1. Administration of GH increased expression of FGF21 and IGF-I mRNA in bovine liver. Cattle were injected with recombinant bGH formulated for 2-wk sustained release (indicated as +GH in the *graph*) or saline (−GH). Liver biopsies were taken 1 wk after the administration. Expression levels of mRNA were quantified by real-time RT-PCR using the 18S rRNA as the internal control. *, $P < 0.05$ (n = 5 animals) compared with −GH.

injected cattle was more than 16 times that in control cattle (Fig. 1). This GH-induced change in FGF21 mRNA expression was even greater than that in IGF-I mRNA (Fig. 1), a widely known GH target gene in the liver.

bFGF21 promoter contains three putative STAT5-binding sites

We subsequently tested the possibility that GH stimulates *FGF21* gene expression in the bovine liver through STAT5. To this end, we first performed a 5′-RACE to map the transcription start site in the *bFGF21* gene. The 5′-RACE generated four major DNA fragments from bovine liver mRNA (Fig. 2A). DNA sequencing revealed that the longest, also the most abundant band, was amplified from a FGF21 transcript the 5′-end nucleotide of which matches a nucleotide 24 bp downstream of a putative TATA box in the *FGF21* gene (Fig. 2B). Because it is located immediately downstream of a putative TATA box, this nucleotide is likely a true transcription start site. The second longest DNA band did not match the FGF21 mRNA sequence and was likely the result of nonspecific binding of primers during the 5′-RACE. The third and fourth bands matched the FGF21 mRNA sequence 237 and 256 nucleotides, respectively, downstream of the predicted ATG codon (Fig. 2B). These two bands were generated probably due to incomplete reverse transcription during the 5′-RACE.

The consensus sequence of the STAT5-binding site is TTCNNNGAA, where N stands for A, C, G, or T (35, 36). Sequence analysis of the proximal 2-kb bFGF21 promoter revealed three such consensus sequences, located at 1283 bp, 798 bp, and 727 bp 5′ from the mapped transcription start site (Fig. 2C).

The putative STAT5-binding sites bound to GH-activated STAT5 protein *in vitro*

We next performed EMSA to determine whether the putative STAT5 binding sites in the bFGF21 promoter can bind STAT5 protein *in vitro*. As shown in Fig. 3A, the oligonucleotides corresponding to the three putative STAT5-binding sites each formed multiple DNA-protein complexes with liver nuclear proteins from both GH-injected cattle and control cattle. One DNA-protein complex (marked by *arrow B* in the figure), however, appeared to be specific to GH-injected cattle (Fig. 3A), suggesting that the DNA-binding activity of the protein (or proteins) in this complex was induced by GH. To determine whether this protein is STAT5, the oligonucleotide corresponding

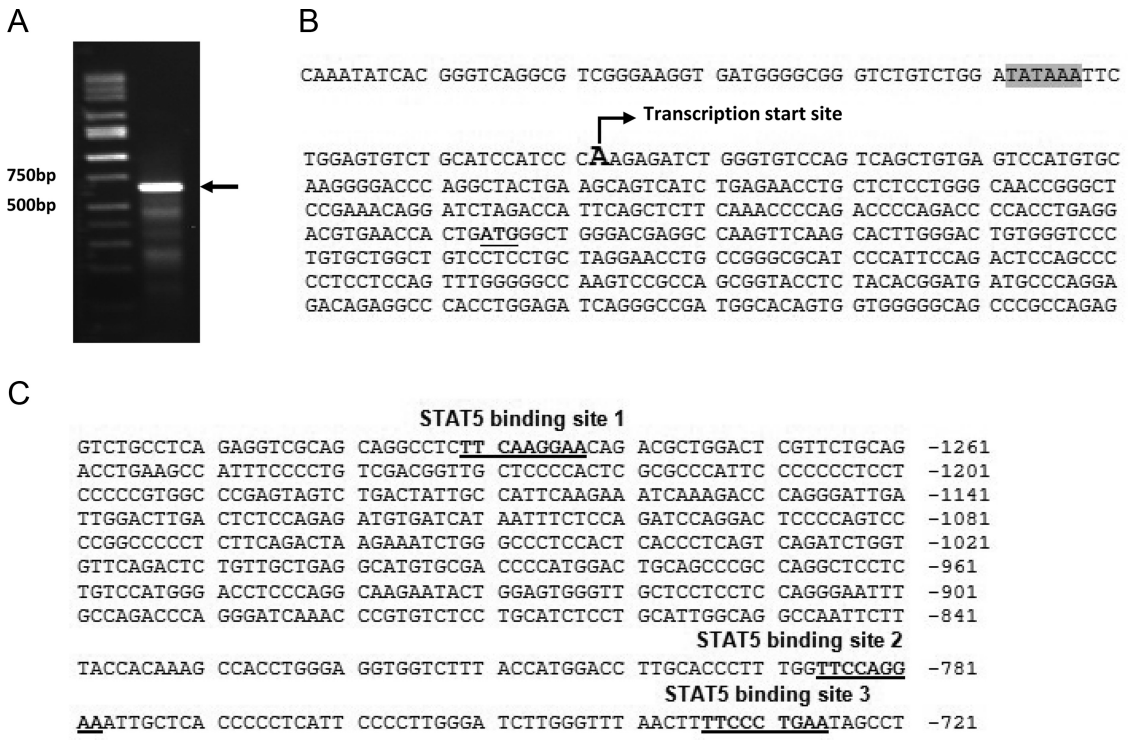

FIG. 2. Transcription start site and putative STAT5-binding sites in the bFGF21 promoter. A, Mapping the transcription start site by 5′-RACE. Shown is a gel image of the 5′-RACE products. B, Location of the FGF21 transcription start site. Sequencing the most abundant 5′-RACE product (indicated by an *arrow* in panel A) indicated that transcription of the *FGF21* gene in the liver of cattle was mainly initiated from an adenine base (indicated by a *forward arrow*) located 24 bp downstream of a putative TATA box. C, Presence of three putative STAT5-binding sites in the bFGF21 promoter. Shown is a partial sequence of the bFGF21 promoter. Nucleotides of the sequence are numbered relative to the mapped transcription start site, which is numbered +1.

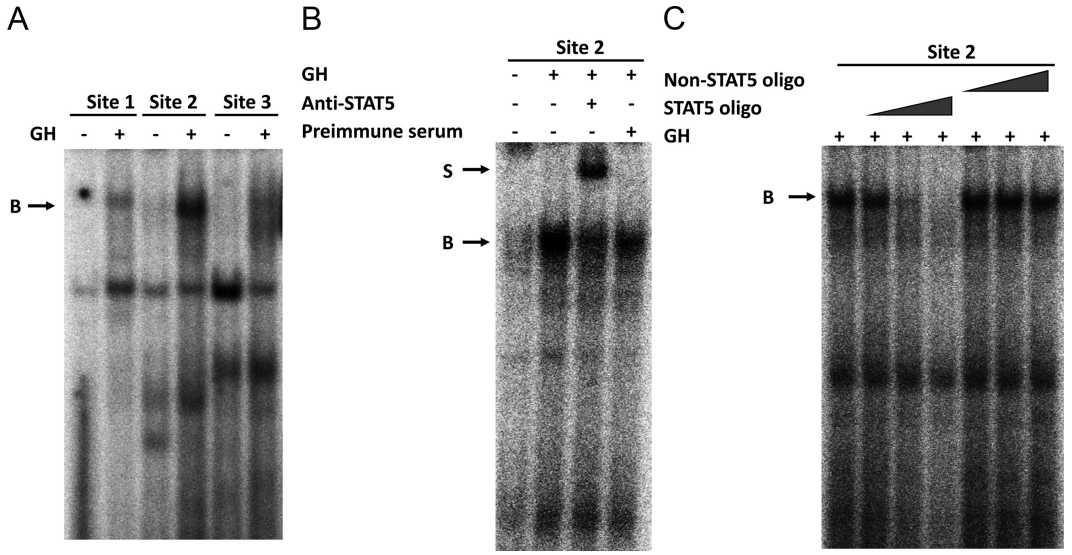

FIG. 3. Binding of the putative STAT5-binding sites in the FGF21 promoter to liver STAT5 protein. A, EMSA of the three putative STAT5-binding sites. The ^{32}P-labeled oligonucleotides corresponding to the three putative STAT5-binding sites were incubated with liver nuclear protein extracts from cattle injected with GH (indicated as +GH in the *graph*) or saline (−GH), followed by gel electrophoresis. The *arrow B* marks a DNA-protein complex the formation of which appeared to be induced by GH. B, Supershift assay of the putative STAT5-binding site 2. In this assay, the ^{32}P-labeled oligonucleotide corresponding to this STAT5-binding site was incubated with liver nuclear protein extracts from GH-injected cattle in the presence of an anti-STAT5 antibody or an equal protein amount of rabbit preimmune serum. The *arrow S* indicates a supershift of the DNA-protein complex marked by the *arrow B*. C, Competitive gel-shift assay of the putative STAT5-binding site 2. In this assay, the ^{32}P-labeled oligonucleotide corresponding to this STAT5-binding site was incubated with GH-treated liver nuclear protein extracts in the presence of 1×, 10×, and 100× molar excess of the same unlabeled oligonucleotide or an unlabeled oligonucleotide that bore no resemblance to a STAT5-binding site.

to the putative STAT5-binding site 2, which appeared to have the greatest binding ability to the protein, was further analyzed in supershift and competitive gel-shift assays. As shown in Fig. 3B, this oligonucleotide formed a specific DNA-protein complex with nuclear proteins from GH-injected cattle liver, and that part of the complex (marked by *arrow S* in the figure) was supershifted by a STAT5 antibody but not by preimmune serum, indicating that this complex was formed between the oligonucleotide and the STAT5 protein. The presence of STAT5 in this DNA-protein complex was further validated by a competitive gel-shift assay. As shown in Fig 3C, this DNA-protein complex (marked by *arrow B*) was increasingly diminished by an increasing molar excess (1×, 10×, and 100×) of the same oligonucleotide without ^{32}P labeling, but it was not affected by a molar excess of an oligonucleotide that did not contain a STAT5-binding site (Table 1).

GH-induced binding of STAT5 to the FGF21 promoter in the liver

We next performed ChIP assays to determine whether GH induces STAT5 binding to the FGF21 promoter *in vivo*. As shown in Fig. 4, STAT5 antibody enriched the FGF21 promoter DNA from the liver of both GH-injected cattle and control cattle ($P < 0.05$), suggesting that there was STAT5 binding to the FGF21 promoter in the liver even when the cattle were not injected with GH. However, the STAT5 antibody enriched more FGF21 promoter DNA from the liver of GH-injected cattle than that of control cattle (Fig. 4, $P < 0.05$). This difference indicated that GH injection increased the binding of STAT5 to the FGF21 promoter in the liver.

GH activated the FGF21 promoter in a STAT5-dependent manner in cells

We determined whether GH can activate the FGF21 promoter by cotransfection analysis. As shown in Fig. 5,

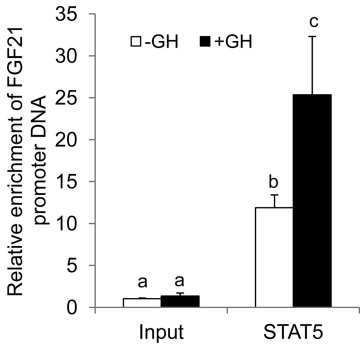

FIG. 4. GH administration induced binding of STAT5 to the FGF21 promoter in the liver. Liver chromatin from GH-injected (indicated as +GH in the *graph*) or saline-injected (−GH) cattle was precipitated by a STAT5 antibody, followed by real-time PCR of the FGF21 promoter region spanning the second and third STAT5-binding sites (shown in Fig. 2) and an 18S rDNA region that does not contain putative STAT5-binding sites. Chromatin before immunoprecipitation served as input chromatin. The y-axis represented the enrichment of the FGF21 DNA over 18S rDNA in the immunoprecipitates or input chromatin. *Bars* labeled with *different letters* differ ($P < 0.05$, n = 4 animals).

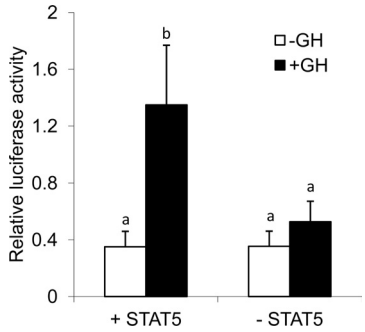

FIG. 5. GH induced reporter gene expression from the bFGF21 promoter in a STAT5-dependent manner. A STAT5 expression plasmid or the corresponding empty vector (indicated by + STAT5 and −STAT5, respectively) was cotransfected with a firefly luciferase reporter plasmid inserted with a 2-kb bFGF21 promoter into CHO cells. A *Renilla* luciferase expression plasmid was cotransfected to control for transfection efficiency. The cells were serum-starved 24 h after the transfection for 8 h and then treated with 500 ng/ml bGH or an equal volume of PBS (indicated as −GH) for 16 h before the dual-luciferase assay. *Bars* labeled with *different letters* differ ($P < 0.05$, n = 5 transfection experiments).

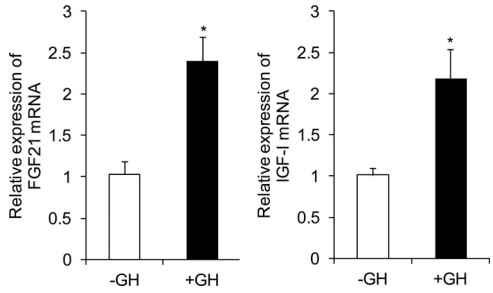

FIG. 6. GH stimulated FGF21 mRNA expression in cultured hepatocytes. Primary mouse hepatocytes were cultured in serum-free medium for 8 h and then treated with 500 ng/ml bGH or an equal volume of PBS (indicated as −GH) for 4 h before RNA extraction. Expression of FGF21 and IGF-I mRNAs was measured by real-time RT-PCR and was normalized to 18S rRNA. *, $P < 0.05$ (n = 4 cell culture experiments) compared with −GH.

GH increased luciferase expression from the bFGF21 promoter cotransfected with GHR expression and STAT5 expression plasmids into the CHO cells ($P < 0.05$). These data indicated that GH can activate transcription from the *FGF21* promoter. To determine whether this activation is dependent on STAT5, we replaced the STAT5 expression plasmid with an empty vector in the cotransfection analysis. As shown in Fig. 5, this replacement completely abolished the response of the FGF21 promoter to GH ($P < 0.05$), indicating that GH activated the FGF21 promoter through STAT5.

GH stimulated FGF21 mRNA expression in cultured mouse hepatocytes

There is a possibility that GH stimulates FGF21 mRNA expression in the liver through a GH-regulated endocrine factor. To rule out this possibility, we determined whether GH can directly stimulate FGF21 mRNA expression in hepatocyte culture. Because bovine hepatocytes are difficult to isolate and culture, we used mouse hepatocytes instead. As shown in Fig. 6A, mouse hepatocytes treated with 500 ng/ml recombinant bGH for 4 h expressed 1.5 times more FGF21 mRNA than control hepatocytes ($P < 0.05$). This GH-induced increase in FGF21 mRNA expression was comparable to that in IGF-I mRNA expression (Fig. 6B).

Discussion

In this study, we observed that GH administration increased FGF21 mRNA expression and STAT5 binding to the FGF21 promoter in the liver of cattle, that the bFGF21 promoter has three putative STAT5-binding sites, that the putative STAT5-binding sites bound to STAT5 protein from the liver of GH-injected cattle, and that GH activated reporter gene expression from the FGF21 promoter in a STAT5-dependent manner in cell culture. These observations together indicate that GH stimulates *FGF21* gene transcription in the liver of cattle and that this stimulation is mediated, at least in part, through STAT5. This GH, STAT5, and FGF21 link may be not limited to cattle, because FGF21 mRNA expression in mouse liver (data not shown) and cultured mouse hepatocytes was also stimulated by GH. In addition, liver expression of FGF21 mRNA was lower in STAT5-null and STAT5 signaling-defective GHR mice than in their wild-type counterparts (37). Based on computational analyses, the FGF21 promoters contain many other potential transcription factor-binding sites (data not shown); therefore, GH regulation of FGF21 expression likely involves transcription factors in addition to STAT5.

What is the physiological significance of this GH-STAT5-FGF21 link? GH not only has direct mitogenic effects on bone and muscle cells but also stimulates the production of IGF-I, a potent mitogen for many cell types (13–15). IGF-I is present at high concentrations in the blood, and most of the blood IGF-I is produced by the liver under the stimulation of GH (2). The effect of GH on IGF-I production in the liver is mediated through the JAK2-STAT5 signaling pathway (10, 38–40). As such, prolonged JAK2-STAT5 signaling will lead to excessive production of IGF-I and increase the risk of malignant growth. The body, however, seems to have developed multiple mechanisms to attenuate or terminate excessive JAK2-STAT5 signaling from the GHR (18, 41). These mechanisms include GHR internalization followed by ubiquitin-mediated proteolytic cleavage of GHR; dephosphorylation of GHR, JAK2, and STAT5 by protein tyrosine phosphatases; inhibition of JAK2

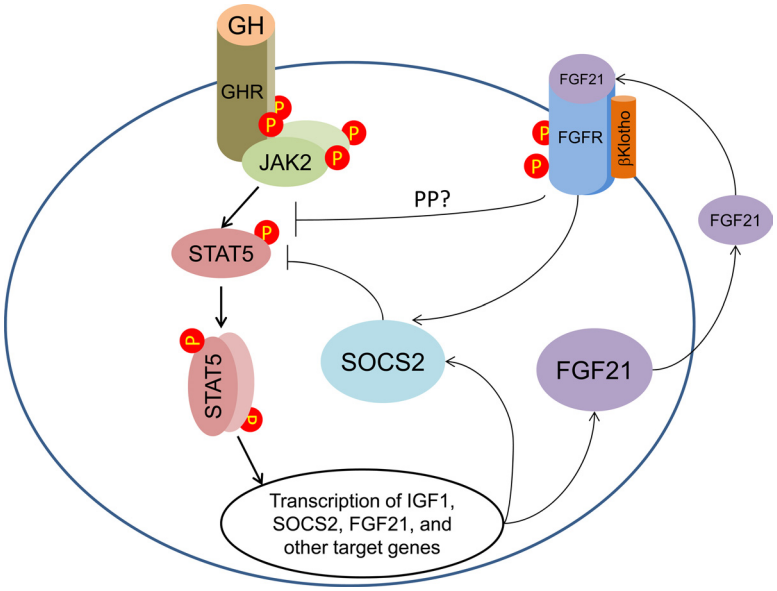

FIG. 7. Proposed negative feedback loops controlling GH-activated JAK2-STAT5 signaling in the liver. Binding of GH to the GHR activates JAK2, which subsequently phosphorylates the GHR and STAT5 proteins on tyrosine residues. The phosphorylated STAT5 proteins dimerize, translocate to the nucleus, and activate the transcription of genes such as *IGF-I*, *SOCS2*, and *FGF21*. This JAK2-STAT5 signaling is inhibited by two feedback loops: one involves SOCS2, which inhibits the signaling from within the cell, and the other loop involves FGF21, which inhibits the signaling from outside of the cell through the FGF receptor and its cofactor βKlotho. FGF21 may inhibit GH-induced STAT5 phosphorylation through SOCS2 (27) or a unknown phosphatase (PP).

and STAT5 phosphorylation by members of the SOCS family; and inhibition of the DNA-binding ability of STAT5 by protein inhibitors of activated STAT (18, 41). More recently, it was shown that GH-induced phosphorylation of STAT5 in the liver is inhibited by FGF21 (27, 42). Among the *SOCS* genes, expression of SOCS2 in the liver is stimulated by GH through the JAK2-STAT5 pathway (9–12), and this means that SOCS2 inhibits the JAK2-STAT5 signaling in the hepatocytes through an intracellular negative feedback loop (Fig. 7). The finding of the present study that GH stimulates FGF21 expression in the liver through STAT5 suggests that FGF21 also inhibits the JAK2-STAT5 signaling through a negative feedback loop (Fig. 7). FGF21 is a secreted protein (22–24). At the cellular level, FGF21 exerts its effects by binding to a cell membrane complex between FGF receptor 1, 2, 3, or 4 and coreceptor βKlotho and by subsequently activating the PI3K- and Erk-signaling pathways (22, 24, 43). It was proposed that FGF21 inhibits STAT5 phosphorylation in the liver through SOCS2, because FGF21 induces expression of SOCS2 in the liver (26, 27). In addition to SOCS2, expression of several dual specificity phosphatases has been shown to be induced by signaling from the FGF receptor (44), and this raises the possibility that FGF21 might inhibit STAT5 phosphorylation through dual-specificity phosphatases.

In addition to stimulating growth, GH plays major roles in regulating lipid and glucose metabolism (45, 46). Because FGF21 is a metabolic regulator and is present in the blood, the GH-FGF21 link in the liver raises the possibility that FGF21 mediates some of the metabolic effects of GH in the body. This possibility, however, is not supported by the fact that GH and FGF21 have more opposite than similar effects on lipid and glucose metabolism. Adipose tissue and liver are the major target tissues of FGF21 action in the body (24). In the adipose tissue, FGF21 inhibits lipolysis and stimulates lipogenesis (24), but GH is widely known to induce lipolysis and inhibit lipogenesis (45, 46). In the liver, although FGF21 and GH can both stimulate lipid oxidation and inhibit lipogenesis (24, 43, 45, 46), they have different effects on glucose metabolism, with FGF21 inhibiting and GH stimulating glucose output (46–48). Infusion of FGF21 improves insulin sensitivity in the body, whereas that of GH is believed to cause insulin resistance (24, 47, 48). These largely opposing effects of FGF21 and GH on lipid and glucose metabolism seem to favor the hypothesis that GH-induced FGF21 not only inhibits JAK2-STAT5 signaling, thereby preventing excessive production of IGF-I, but also counter-regulates the effects of GH on lipid and glucose metabolism.

In summary, this study shows that GH stimulates *FGF21* gene transcription in the liver and that this stimulation is mediated, at least in part, by the transcription factor STAT5. These findings, together with the fact that FGF21 inhibits JAK2-STAT5 signaling in the liver (26, 27), suggest a novel negative feedback loop that prevents excessive JAK2-STAT5 signaling from the GHR in the liver.

Acknowledgments

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This work was supported by National Research Initiative Competitive Grant 2009-35205-05221 from the US Department of Agriculture National Institute of Food and Agriculture. J.Y.

was supported by a scholarship from the China Scholarship Council.

Disclosure Summary: The authors have nothing to disclose.

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