Fibroblast Growth Factor 19 Treatment Ameliorates Disruption of Hepatic Lipid Metabolism in Farnesoid X Receptor (Fxr)-Null Mice

Masaaki MIYATA,* Yumi SAKAIDA, Hitomi MATSUZAWA, Kouichi YOSHINARI, and Yasushi YAMAZOE

Division of Drug Metabolism and Molecular Toxicology, Graduate School of Pharmaceutical Sciences, Tohoku University; 6-3 Aoba, Aramaki, Aoba-ku, Sendai 980-8578, Japan.

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Human fibroblast growth factor 19 (FGF19) is an enterohepatic hormone that is involved in the regulation of hepatic metabolism of bile acids, lipids, and glucose. *Farnesoid X receptor (Fxr)*-null mice exhibit steatosis-like symptoms, showing higher hepatic lipid levels than with the wild-type mice. We investigated the influence of FGF19 treatment on hepatic lipogenesis in *Fxr*-null mice. Recombinant FGF19 treatment (400 $\mu\text{g/kg/d}$) for 3 d prevented the accumulation of lipid droplets and decreased serum alanine aminotransferase activity and hepatic lipid levels, including those of triglycerides and free fatty acids. The treatment significantly decreased the hepatic mRNA levels of *acetyl-CoA carboxylase 1 (Acc1)*, *Cd36*, and *sterol regulatory element-binding protein-1c (Srebp-1c)* as well as those of *acetyl-CoA carboxylase 2 (Acc2)*, *stearoyl CoA desaturase 1 (Scd1)*, and *Cyp7a1*. FGF19 treatment (4 $\mu\text{g/kg/d}$) for 3 d also decreased the hepatic free fatty acid levels and mRNA levels of *Acc1*, *Cd36*, and *Srebp-1c*. These results indicate that FGF19-mediated signaling ameliorates disrupted hepatic lipogenesis in *Fxr*-null mice.

Key words fibroblast growth factor 19; farnesoid X receptor; lipogenesis; *Cd36*; acetyl-CoA carboxylase 1

Farnesoid X receptor (FXR) is a bile acid sensor that protects the liver from bile acid toxicity. Recently, the physiological role of FXR has been expanded to include the regulation of the homeostasis of lipids and glucose as well as of bile acid.^{1–4)} Over the past decade, many studies have indicated the presence of several FXR signaling pathways that regulate hepatic lipid metabolisms (lipogenesis). FXR directly regulates the expression of the genes involved in hepatic lipogenesis, such as acetyl-CoA carboxylase 1 (*Acc1*) and fatty acid synthase (FAS).⁵⁾ On the other hand, FXR down-regulates hepatic lipogenesis by the induction of small heterodimer partner (SHP), which is a negative regulator of sterol regulatory element-binding protein-1c (SREBP-1c)-mediated lipogenic signaling.⁶⁾ Moreover, recent research has highlighted the role of FXR-dependent fibroblast growth factor 19 (FGF19) signaling in the regulation of hepatic lipid metabolism.^{7–10)}

FGF19 and FGF15 (the mouse ortholog of FGF19) are members of the FGF family.^{11,12)} They are specifically expressed in the intestine and function as endocrine hormones, regulating various metabolic processes.^{13,14)} FGF15/19 acts on the liver through the activation of FGF receptor 4 (FGFR4).^{15,16)} FGF15/19 has been reported to down-regulate the hepatic expression of CYP7A1 to reduce bile acid synthesis.^{8,16,17)} Studies using FGF19-transgenic mice and FGF19-treated hyperlipidemic mice have indicated that FGF19 also regulates the hepatic levels of lipids, such as triglyceride (TG) and cholesterol.^{7,9)} However, how the FGF15/19-FGFR4 signal controls the hepatic lipid levels remains unclear.

Fxr-null mice have disrupted lipogenesis and their livers show elevated levels of not only hepatic bile acids but also TGs and free fatty acids (FFAs).¹⁸⁾ Furthermore, insulin resistance has been observed in these mice.^{19–21)} *Fxr*-null mice are now considered to be models of metabolic syndrome with hepatic steatosis. *Fxr*-null mice, lacking FXR signaling, show lower expression levels of intestinal *Fgf15* and hepatic *Shp* mRNA.^{17,18)} Therefore, not only the lack of direct FXR

signaling but also the attenuation of FGF15- and SHP-mediated signaling contributes to the elevation of the hepatic lipid levels in *Fxr*-null mice. However, whether FGF15/19 signaling compensates for the lack of FXR signaling that regulates hepatic lipogenesis remains unknown.

We investigated the influence of human FGF19 treatment on the hepatic lipid levels in *Fxr*-null mice to verify the role of FGF15/19 signaling in the regulation of hepatic lipogenesis. The results showed that FGF19 treatment significantly decreased the elevated hepatic lipid levels in *Fxr*-null mice.

MATERIALS AND METHODS

Animal Treatment, Sample Collection, and Histological Analysis *Fxr*-null mice¹⁸⁾ and wild-type mice were housed under a standard 12-h light–dark cycle (9 a.m.—9 p.m.). Before beginning the experiments, the mice were fed standard rodent chow (CE2; CLEA Japan, Tokyo, Japan) and water when needed. Age-matched groups of 8–10-week-old female mice were intravenously injected with recombinant FGF19 (400 μg or 4 $\mu\text{g/kg/d}$) or vehicle (saline) through the tail vein once a day for 3 d. They were killed 6 h after the final injection. Liver tissues were frozen in liquid nitrogen and stored at -80°C until use. For histological analysis, some of the frozen liver sections were stained with Oil Red O using standard procedures. All experiments were performed in accordance with the Guidelines for Animal Experiments of Tohoku University (Sendai, Japan). The protocol was approved by the Institutional Animal Care and Use Committee at Tohoku University (Permission No. 22-Pharm-Animal-6).

Expression and Purification of Recombinant Human FGF19 Human FGF19 DNA fragment containing the amino acid coding region (3–215) was amplified by polymerase chain reaction (PCR) with the primers, 5'-GCG-CATATGCTCGCCTTCTCGGACGCGGG-3' containing an *NdeI* site and 5'-GCGAAGCTTTTACTTCTCAAAGCTGGAC-3' containing a *HindIII* site and cDNA derived from

* To whom correspondence should be addressed. e-mail: miyata@m.tohoku.ac.jp

Caco-2 cells (Riken BioResource Center, Ibaraki, Japan) as the template by using KOD-FX (Toyobo, Osaka, Japan). After the initial denaturation at 94 °C for 2 min, DNA was amplified for 30 cycles at 98 °C for 10 s and 68 °C for 1 min. The PCR product was digested with *NdeI* and *HindIII* and ligated into *NdeI* and *HindIII* sites of the pET-28a(+) vector (Merck, Rahway, NJ, U.S.A.). The recombinant plasmid was transformed into Origami 2(DE3) (Merck). Recombinant FGF19 protein was purified on a Ni-NTA column (Qiagen, Valencia, CA, U.S.A.) as per the manufacturer's instructions. The purity of the isolated recombinant FGF19 protein was estimated to be >98% by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Protein concentration was determined using the Bradford assay.²²⁾

Determination of Hepatic Diagnostic Marker Levels and Bile Acid Levels Serum alanine amino transferase (ALT) activity was determined using a commercial kit, Transaminase CII-B-test Wako (Wako Pure Chemicals, Osaka, Japan). Serum alkaline phosphatase (ALP) activity was determined by the Bessey–Lowry method using the Alkali-phosphatase B-test Wako kit (Wako Pure Chemicals). The total hepatic bile acid levels were measured by high-performance liquid chromatography (HPLC) as previously described.^{23,24)}

Determination of Hepatic Lipid Levels A liver section of was homogenized in 2 volumes of 75 mM KCl and 75 mM KH₂PO₄–K₂HPO₄ buffer (pH 7.4). The homogenate was diluted with four times the amount of buffer containing 2% Triton X-100 and was incubated at 65 °C for 30 min. After centrifugation, the supernatants were assessed for the hepatic lipid levels.²⁵⁾ Hepatic TGs, FFAs and total cholesterol (TC) were determined using the Triglyceride E-test Wako, NEFA E-test Wako, and Cholesterol E-test Wako kits (Wako Pure Chemicals), respectively.

Determination of mRNA Levels Total hepatic RNA was isolated using the acid guanidine–phenol–chloroform method. Single-strand cDNA was synthesized using an oligo(dT) primer and Ready-to-Go You-Prime First-stand Beads (GE Healthcare, Chalfont St. Giles, Buckinghamshire, U.K.) and was used for quantitative real-time PCR (qRT-PCR) using the Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, U.S.A.) with the ABI PRISM 7000 Sequence Detection System (Applied Biosystems). The Relative mRNA levels were calculated using the comparative threshold cycle method. The following specific forward and reverse primers were used for qRT-PCR: *β-actin*: sense, 5'-ACCCTGTGCTGCTCACCAG-3' and antisense, 5'-CTGGATGGCTACGTACATGGCT-3'; *Cyp7a1*:

sense, 5'-AGCAACTAAACAACCTGCCAGTACTA-3' and antisense, 5'-GTCCGGATATTCAAGGATGCA-3'; *Cpt1a*: sense, 5'-TGCAGACTCGGTCAACCACTCAAGAT-3' and antisense, 5'-CTCGCGGGGAACACACCAGTG-3'; *Scd1*: sense, 5'-AGTTAACCCTGAATGCGAGG-3' and antisense, 5'-GAGGTCCATTAGCACTTGCC-3'; *Acc1*: sense, 5'-ACAGTGAAGGCTTACGTCTG-3' and antisense, 5'-TGTGGACGATGGAGTCCATG-3'; *Acc2*: sense, 5'-CATA-CACAGAGCTGGTGTGGACT-3' and antisense, 5'-CATGCCCCACCTCGTTAC-3'; *Cd36*: sense, 5'-GCAC-CACTGTGTACAGACAG-3' and antisense, 5'-GTGCAGCTGCTACAGCCAG-3'; *Shp*: sense, 5'-CGATC-CTCTTCAACCCAGATG-3' and antisense, 5'-AGGGCTC-CAAGACTTCACACA-3'; *Srebp-1c*, sense, 5'-GCTGTTG-GCATCCTGCTATC-3' and antisense, 5'-TAGCTGGAAGT-GACGGTGGT-3'; *Fas*: sense, 5'-CGGTCTGGAAAGCT-GAAGGATC-3' and antisense, 5'-CGGAGTGAGGCTG-GGTTGA-3'.

Statistical Analyses Values are presented as mean ± S.D. Data were analyzed by the unpaired Student's *t*-test or by analysis of variance followed by the Dunnett's method using Prism 4.0 software (GraphPad Software Inc., San Diego, CA, U.S.A.) for evaluating differences between the mean values of each group. Significance was established at *p* < 0.05.

RESULTS

Influence of FGF19 Treatment on Liver Diagnostic Markers of Liver Disorders *Fxr*-null mice were intravenously injected with FGF19 for 3 d and killed 6 h after the final injection. FGF19 treatment (400 μg/kg/d) decreased serum ALT and ALP activities to 29% and 61% of those in vehicle-treated mice, respectively (Table 1). In particular, ALT activity in *Fxr*-null mice treated with FGF19 (400 μg/kg/d) was decreased to that in vehicle-treated wild-type mice. No significant decreases in ALT activity were observed in *Fxr*-null mice treated with FGF19 (4 μg/kg/d). FGF19 treatments (4, 400 μg/kg/d) of *Fxr*-null mice decreased the hepatic bile acid levels in a dose-dependent manner but had no effect on liver weight and body weights.

Influence of FGF19 Treatment on Hepatic Lipid Levels in *Fxr*-Null Mice *Fxr*-null mice showed high hepatic TG and FFA levels compared with wild-type mice. FGF19 treatment (400 μg/kg/d) reduced the hepatic TG and FFA levels in *Fxr*-null mice to levels similar to those in vehicle-treated wild-type mice (Fig. 1). FGF19 treatment (4 μg/kg/d) significantly reduced the FFA levels to those in vehicle-treated

Table 1. Influence of FGF19 Treatment on Relative Liver Weight and Liver Diagnostic Markers

	<i>Fxr</i> -null			Wild-type
	0 (μg/kg/d)	4 (μg/kg/d)	400 (μg/kg/d)	0 (μg/kg/d)
Liver weight (g)	1.27 ± 0.24	1.26 ± 0.12	1.33 ± 0.19	1.05 ± 0.06
Body weight (g)	19.7 ± 0.86	19.3 ± 0.44	19.3 ± 0.42	20.6 ± 0.66
Liver/body weight ratio (%)	6.46 ± 1.19	6.50 ± 0.53	6.84 ± 0.92	5.12 ± 0.21
ALT (IU/l)	82.7 ± 48.7	81.5 ± 31.8	24.2 ± 7.3*	19.1 ± 0.3 ^{###}
ALP (IU/l)	151.4 ± 67.1	95.8 ± 20.9	90.6 ± 21.1	68.7 ± 7.7 ^{###}
Hepatic bile acid (μmol/g liver)	1.43 ± 0.91	1.24 ± 0.38	0.91 ± 0.44	0.51 ± 0.11 ^{###}

Mice received vehicle or FGF19 (4, 400 μg/kg/d) for 3 consecutive days through tail vein injection. Livers were dissected 6 h after the last injection. Data are shown as the mean ± S.D. (*n* = 5–8). The significant differences were assessed by Dunnett's test (**p* < 0.05 vs. corresponding control mice) or Student's *t*-test (^{##}*p* < 0.01, ^{###}*p* < 0.001 vs. vehicle-treated *Fxr*-null mice).

Fig. 1. Influence of FGF19 Treatment on Hepatic Lipid Levels

Fxr-null mice and wild-type mice were treated with vehicle or FGF19 (4, 400 µg/kg/d) for 3 d through tail vein injection and killed 6 h after the final injection. Lipid levels in the liver were determined as described in Materials and Methods. Values are presented as mean ± S.D. ($n=5-8$). Significant differences were assessed by Dunnett's test (** $p<0.01$ vs. corresponding control mice) or the Student's *t*-test (## $p<0.01$, ### $p<0.001$ vs. vehicle-treated *Fxr*-null mice). Null, *Fxr*-null mice; Wild, wild-type mice.

Fig. 2. Oil Red O Staining of Liver Sections from *Fxr*-Null Mice Treated with FGF19

Fxr-null mice were treated with vehicle or FGF19 (400 µg/kg/d) for 3 d through tail vein injection and killed 6 h after the final injection. Liver sections were stained with oil red O and hematoxylin. Representative liver sections are shown. Original magnification: 400×.

wild-type mice. However, FGF19 treatments (4, 400 µg/kg/d) had no effect on the hepatic TC levels. To confirm that the reduction of the hepatic TG levels was FGF19-mediated, we performed Oil Red O staining of liver sections. Histological analysis revealed many Oil Red O-positive lipid droplets in the livers of vehicle-treated *Fxr*-null mice (Fig. 2). The number of lipid droplets was markedly decreased in the livers of *Fxr*-null mice treated with FGF19 (400 µg/kg/d). These results indicate that FGF19 has a preventive effect on the hepatic accumulation of lipid droplets in *Fxr*-null mice.

Influence of FGF19 Treatment on Hepatic mRNA Levels of Genes Involved in Lipogenesis We determined the hepatic mRNA levels of genes involved in lipogenesis in order to investigate the mechanisms of the FGF19-mediated changes in the lipid levels in *Fxr*-null mice (Fig. 3). The hepatic mRNA levels of *Cyp7a1*, the target gene of FGF15 signaling, were considerably decreased in mice by FGF19 treatment (4 µg/kg/d or 400 µg/kg/d). Thus, intravenously injected FGF19 probably acted on the livers of mice. The hepatic mRNA levels of *Shp*, a negative regulator of *Srebp-1c* expression, were significantly increased in the mice treated

with FGF19 (400 µg/kg/d), whereas the mRNA levels of *Srebp-1c* and *Acc1*, but not *Fas*, were decreased in the mice treated with FGF19 (4, 400 µg/kg/d). Consistent with previous reports using mice treated with FGF19 (1 mg/kg/d),⁹ the hepatic mRNA levels of *Acc2*, a negative regulator of fatty acid β -oxidation, and *Scd1* were significantly decreased in *Fxr*-null mice by treatment with FGF19 (400 µg/kg/d). The hepatic mRNA levels of fatty acid β -oxidation related genes, *carnitine palmitoyltransferase 1a* (*Cpt1a*), *carnitine palmitoyltransferase 2* (*Cpt2*), *long chain acyl-CoA dehydrogenase* (*Acadl*), *medium chain acyl-CoA dehydrogenase* (*Acadm*), and *short chain acyl-CoA dehydrogenase* (*Acads*), were significantly decreased in *Fxr*-null mice by treatment with FGF19 (400 µg/kg/d). The ratios of *Cpt1a*, *Cpt2*, *Acadl*, *Acadm* and *Acads* mRNA levels in mice treated with FGF19 (400 µg/kg/d) to those in vehicle-treated mice were 0.45, 0.65, 0.70, 0.58 and 0.75 respectively. We also determined the hepatic mRNA levels of genes involved in lipid transport. FGF19 treatments (4, 400 µg/kg/d) significantly decreased the hepatic mRNA levels of *Cd36*, which encodes a fatty acid uptake transporter in *Fxr*-null mice.

Fig. 3. Influence of FGF19 Treatment on Hepatic mRNA Levels of Genes Involved in Lipogenesis

Fxr-null mice and wild-type mice were treated with vehicle or FGF19 (4, 400 µg/kg/d) for 3 d through tail vein injection and killed 6 h after the final injection. Quantitative reverse transcription-PCR analyses were performed on total RNA from individual mouse livers. The mRNA levels were normalized to those of β -actin, and the mRNA levels of vehicle-treated *Fxr*-null mice were set at 1. Values are presented as means $\pm$ S.D. ($n=5-8$). Significant differences were assessed by Dunnett's test (* $p<0.05$, ** $p<0.01$ vs. corresponding control mice) or the Student's *t*-test (# $p<0.05$, ## $p<0.01$, ### $p<0.001$ vs. vehicle-treated *Fxr*-null mice). Null, *Fxr*-null mice; Wild, wild-type mice.

DISCUSSION

We demonstrated that the FGF19 treatment of *Fxr*-null mice significantly decreased their hepatic levels of TGs and FFAs to those observed in wild-type vehicle-treated mice. This suggests that FGF19 signaling compensates for the disruption of hepatic lipid metabolism due to the absence of FXR signaling. Furthermore, our data suggest that the attenuation of enterohepatic FGF15 signaling contributes, at least in part, to the elevation of the hepatic lipid levels in *Fxr*-null mice.

Significant decreases were observed in the hepatic FFA levels, but not in the TG levels in *Fxr*-null mice treated with a low dose of FGF19 (4 µg/kg/d). Regulation of the hepatic FFA levels might be more sensitive to FGF15/19 signaling than that of the hepatic TG levels. Significant decreases in the hepatic mRNA levels of *Cd36*, a fatty acid uptake transporter, and *Acc1*, a lipogenic enzyme, were also observed in *Fxr*-null mice treated with FGF19 (4 µg/kg/d). These results suggest that FGF19-mediated reduction of hepatic *Cd36* and *Acc1* expression is possibly involved in the reduction of the hepatic FFA levels in *Fxr*-null mice treated with a low dose of FGF19 (4 µg/kg/d).

FGF19 treatment increased the hepatic *Shp* mRNA levels in *Fxr*-null mice treated with FGF19 (400 µg/kg/d), whereas it decreased the *Srebp-1c*, *Acc1*, and *Scd1* mRNA levels. We propose that decreases in SREBP-1c-mediated lipogenesis are possibly, at least in part, involved in the reduction of the hepatic TG and FFA levels in *Fxr*-null mice treated with FGF19 (400 µg/kg/d). Decreased expression of many fatty acid β -oxidation-related genes was observed in *Fxr*-null mice by treatment with FGF19 (400 µg/kg/d). It is unlikely

that fatty acid β -oxidation contributes to the decrease in the hepatic TG and FFA levels in *Fxr*-null mice treated with FGF19. Recently, it has been reported that fatty acid β -oxidation was decreased in perfused livers from mice infected with FGF15-expressing adenovirus for 3 d.²⁶⁾ FGF19 may act on the down-regulation of hepatic lipogenesis and hepatic fatty acid uptake, thereby reducing the hepatic TG and FFA levels in *Fxr*-null mice.

Although recent reports suggest an important role of FGF15/19 signaling in the regulation of hepatic lipogenesis, the underlying mechanism remains largely unknown. In primary hepatocytes, FGF19 suppresses the insulin-mediated elevation of hepatic lipid synthesis.¹⁰⁾ Thus, FGF15/19 signaling may be involved in the regulation of hepatic lipogenesis through an insulin signaling pathway. Conversely, it has been reported that FGF19 activates the insulin-independent endocrine pathway that regulates hepatic protein and glycogen metabolism.²⁷⁾ FGF19 treatment has been clearly shown to decrease the hepatic TG and FFA levels in *Fxr*-null mice in which the hepatic insulin signaling pathway was markedly blunted.^{20,21)} The relationship between FGF19 and insulin signaling in hepatic lipogenesis remains unclear.

Fxr-null mice show increased hepatic oxidative stress as well as increased the TG and FFA levels.²⁸⁾ The FGF19 treatment in *Fxr*-null mice decreased the serum ALT and ALP activities as well as the hepatic TG and FFA levels. Recent studies have indicated the protective role of FXR signaling in chemical-induced liver injury.²⁹⁻³¹⁾ Because FGF15/19 signaling is up-regulated by FXR activation, it probably participates in the protection of hepatic function. The present study raises the possibility of application of the FGF19 signaling pathway to liver disorders, such as non-alcoholic fatty liver

disease with insulin resistance, since *Fxr*-null mice seem to be models of the metabolic syndrome with hepatic steatosis. Conversely, *Fgfr4*-null mice exhibited features of the metabolic syndrome that include hyperlipidemia, glucose intolerance, and insulin resistance, whereas *Fgfr4*-null mice were resistant to hepatic steatosis induced by a high-fat diet.³² In a tetracycline-induced fatty liver model, FGFR4 inhibition reduced the accumulation of hepatic lipids.³³ Further studies are necessary to understand the relationship between FGF19/FGFR4 signaling and protection against hepatic steatosis.

In summary, we demonstrated that FGF19 treatment decreased the hepatic TG and FFA levels and the serum ALT activities in *Fxr*-null mice. We thereby conclude that FGF19 signaling ameliorates disrupted hepatic lipogenesis caused due to the absence of FXR signaling.

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REFERENCES

- 1) Kalaany N. Y., Mangelsdorf D. J., *Annu. Rev. Physiol.*, **68**, 159—191 (2006).
- 2) Wang Y. D., Chen W. D., Moore D. D., Huang W., *Cell Res.*, **18**, 1087—1095 (2008).
- 3) Lefebvre P., Cariou B., Lien F., Kuipers F., Staels B., *Physiol. Rev.*, **89**, 147—191 (2009).
- 4) Zhang Y., Edwards P. A., *FEBS Lett.*, **582**, 10—18 (2008).
- 5) Duran-Sandoval D., Cariou B., Percevault F., Hennuyer N., Grefhorst A., van Dijk T. H., Gonzalez F. J., Fruchart J. C., Kuipers F., Staels B., *J. Biol. Chem.*, **280**, 29971—29979 (2005).
- 6) Watanabe M., Houten S. M., Wang L., Moschetta A., Mangelsdorf D. J., Heyman R. A., Moore D. D., Auwerx J., *J. Clin. Invest.*, **113**, 1408—1418 (2004).
- 7) Tomlinson E., Fu L., John L., Hultgren B., Huang X., Renz M., Stephan J. P., Tsai S. P., Powell-Braxton L., French D., Stewart T. A., *Endocrinology*, **143**, 1741—1747 (2002).
- 8) Holt J. A., Luo G., Billin A. N., Bisi J., McNeill Y. Y., Kozarsky K. F., Donahee M., Wang D. Y., Mansfield T. A., Kliewer S. A., Goodwin B., Jones S. A., *Genes Dev.*, **17**, 1581—1591 (2003).
- 9) Fu L., John L. M., Adams S. H., Yu X. X., Tomlinson E., Renz M., Williams P. M., Soriano R., Corpuz R., Moffat B., Vandlen R., Simmons L., Foster J., Stephan J. P., Tsai S. P., Stewart T. A., *Endocrinology*, **145**, 2594—2603 (2004).
- 10) Bhatnagar S., Damron H. A., Hillgartner F. B., *J. Biol. Chem.*, **284**, 10023—10033 (2009).
- 11) Wright T. J., Ladher R., McWhirter J., Murre C., Schoenwolf G. C., Mansour S. L., *Dev. Biol.*, **269**, 264—275 (2004).
- 12) Itoh N., *Cell Tissue Res.*, **342**, 1—11 (2010).
- 13) Jones S., *Mol. Pharm.*, **5**, 42—48 (2008).
- 14) Beenken A., Mohammadi M., *Nat. Rev. Drug Discov.*, **8**, 235—253 (2009).
- 15) Xie M. H., Holcomb I., Deuel B., Dowd P., Huang A., Vagts A., Foster J., Liang J., Brush J., Gu Q., Hillan K., Goddard A., Gurney A. L., *Cytokine*, **11**, 729—735 (1999).
- 16) Inagaki T., Choi M., Moschetta A., Peng L., Cummins C. L., McDonald J. G., Luo G., Jones S. A., Goodwin B., Richardson J. A., Gerard R. D., Repa J. J., Mangelsdorf D. J., Kliewer S. A., *Cell Metab.*, **2**, 217—225 (2005).
- 17) Miyata M., Takamatsu Y., Kuribayashi H., Yamazoe Y., *J. Pharmacol. Exp. Ther.*, **331**, 1079—1085 (2009).
- 18) Sinal C. J., Tohkin M., Miyata M., Ward J. M., Lambert G., Gonzalez F. J., *Cell*, **102**, 731—744 (2000).
- 19) Cariou B., van Harmelen K., Duran-Sandoval D., van Dijk T. H., Grefhorst A., Abdelkarim M., Caron S., Torpier G., Fruchart J. C., Gonzalez F. J., Kuipers F., Staels B., *J. Biol. Chem.*, **281**, 11039—11049 (2006).
- 20) Zhang Y., Lee F. Y., Barrera G., Lee H., Vales C., Gonzalez F. J., Willson T. M., Edwards P. A., *Proc. Natl. Acad. Sci. U.S.A.*, **103**, 1006—1011 (2006).
- 21) Ma K., Saha P. K., Chan L., Moore D. D., *J. Clin. Invest.*, **116**, 1102—1109 (2006).
- 22) Bradford M. M., *Anal. Biochem.*, **72**, 248—254 (1976).
- 23) Kitada H., Miyata M., Nakamura T., Tozawa A., Honma W., Shimada M., Nagata K., Sinal C. J., Guo G. L., Gonzalez F. J., Yamazoe Y., *J. Biol. Chem.*, **278**, 17838—17844 (2003).
- 24) Miyata M., Watase H., Hori W., Shimada M., Nagata K., Gonzalez F. J., Yamazoe Y., *Xenobiotica*, **36**, 631—644 (2006).
- 25) Miyata M., Nomoto M., Sotodate F., Mizuki T., Hori W., Nagayasu M., Yokokawa S., Ninomiya S., Yamazoe Y., *Eur. J. Pharmacol.*, **636**, 145—154 (2010).
- 26) Potthoff M. J., Boney-Montoya J., Choi M., He T., Sunny N. E., Satapati S., Suino-Powell K., Xu H. E., Gerard R. D., Finck B. N., Burgess S. C., Mangelsdorf D. J., Kliewer S. A., *Cell Metab.*, **13**, 729—738 (2011).
- 27) Kir S., Beddow S. A., Samuel V. T., Miller P., Previs S. F., Suino-Powell K., Xu H. E., Shulman G. I., Kliewer S. A., Mangelsdorf D. J., *Science*, **331**, 1621—1624 (2010).
- 28) Nomoto M., Miyata M., Yin S., Kurata Y., Shimada M., Yoshinari K., Gonzalez F. J., Suzuki K., Shibasaki S., Kurosawa T., Yamazoe Y., *Biol. Pharm. Bull.*, **32**, 172—178 (2009).
- 29) Cui Y. J., Aleksunes L. M., Tanaka Y., Goedken M. J., Klaassen C. D., *Toxicol. Sci.*, **110**, 47—60 (2009).
- 30) Meng Z., Wang Y., Wang L., Jin W., Liu N., Pan H., Liu L., Wagman L., Forman B. M., Huang W., *Mol. Endocrinol.*, **24**, 886—897 (2010).
- 31) Lee F. Y., de Aguiar Vallim T. Q., Chong H. K., Zhang Y., Liu Y., Jones S. A., Osborne T. F., Edwards P. A., *Mol. Endocrinol.*, **24**, 1626—1636 (2010).
- 32) Huang X., Yang C., Luo Y., Jin C., Wang F., McKeehan W. L., *Diabetes*, **56**, 2501—2510 (2007).
- 33) Chen Q., Jiang Y., An Y., Zhao N., Zhao Y., Yu C., *Biochem. Biophys. Res. Commun.*, **409**, 651—656 (2011).