The bile acid sensor FXR regulates insulin transcription and secretion

Barbara Renga a,⁎, Andrea Mencarelli a, Piero Vavassori a,1, Vincenzo Brancaleone b,2, Stefano Fiorucci a

a Department of Experimental and Clinical Medicine, University of Perugia, Perugia, Italy
b Department of Experimental Pharmacology, University of Naples "Federico II", Naples, Italy

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A B S T R A C T

Farnesoid X Receptor plays an important role in maintaining bile acid, cholesterol homeostasis and glucose metabolism. Here we investigated whether FXR is expressed by pancreatic β-cells and regulates insulin signaling in pancreatic β-cell line and human islets. We found that FXR activation induces positive regulatory effects on glucose-induced insulin transcription and secretion by genomic and non-genomic activities. Genomic effects of FXR activation relay on the induction of the glucose regulated transcription factor KLF11. Indeed, results from silencing experiments of KLF11 demonstrate that this transcription factor is essential for FXR activity on glucose-induced insulin gene transcription. In addition FXR regulates insulin secretion by non-genomic effects. Thus, activation of FXR in βTC6 cells increases Akt phosphorylation and translocation of the glucose transporter GLUT2 at plasma membrane, increasing the glucose uptake by these cells. In vivo experiments on Non Obese Diabetic (NOD) mice demonstrated that FXR activation delays development of signs of diabetes, hyperglycemia and glycosuria, by enhancing insulin secretion and by stimulating glucose uptake by the liver. These data established that an FXR-KLF11 regulated pathway has an essential role in the regulation of insulin transcription and secretion induced by glucose.

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1. Introduction

Insulin is secreted uniquely from the islet β-cells of the pancreas and plays a non dispensable role in the maintenance of glucose and energy homeostasis. Insulin secretion is tightly regulated to maintain blood glucose levels within a narrow physiological range and an insufficient secretion of insulin from β-cells contributes to the chronic hyperglycemia characteristic of diabetes [1]. Glucose is the major nutrient regulator of pancreatic β-cell function and coordinately regulates insulin gene expression, insulin biosynthesis and secretion [1]. A highly conserved region lying ~350 bp immediately upstream of the transcription initiation start, referred to as the insulin promoter, confers both tissue-specific expression and metabolic regulation of the insulin gene [2,3]. Several transcription factors: PDX1, MafA, BET2, E47 and KLF11 act on this region, generating a highly sophisticated transcriptional network that ensures precise regulation of insulin release [4–8]. Glucose controls all steps of insulin gene expression, including transcription, pre-RNA splicing, and mRNA stability. Thus, glucone promotes the binding of PDX-1 and KLF11 transcription factors to the insulin promoter and the recruitment of co-activators, such as p300, that modulate chromatin structure through post-translational modifications of histones such as methylation and/or acetylation [9–11]. In addition to its major effects on the rate of insulin gene transcription, glucose stabilizes pre-proinsulin mRNA through the direct binding to pyrimidine-rich sequence located in the 3′-untranslated region of the insulin mRNA [12–14].

The Farnesoid X Receptor (FXR) is a member of the nuclear receptor superfamily of ligand regulated receptors that function as a bile acid sensor [15–17]. A major physiological role of FXR is the regulation of the conversion of cholesterol into bile acids. FXR protects liver cells from bile acid overload by decreasing their uptake, endogenous production and by accelerating their biotransformation and excretion [18,19]. The generation and phenotype characterization of FXR-deficient (FXR−/−) mice has allowed to establish that FXR has an important role in lipid metabolism and glucose homeostasis. Thus, not only do FXR−/− mice display elevated serum levels of free fatty acids (FFAs), triglycerides and high density lipoprotein cholesterol (HDL-C) [20,21] but also develop signs of insulin resistance as shown by hyperglycemia, impaired glucose tolerance, and severely blunted insulin signaling in both liver and muscle [22,23]. Activation of FXR by synthetic agonists or hepatic overexpression of constitutively active FXR by adenovirus-mediated gene transfer reduces blood glucose levels in obese fa/fa rats, diabetic, leptin deficient, db/db and wild-type mice [23,24]. This decrease in plasma glucose levels in db/db mice was associated with decreased glucose-6-phosphatase expression, increased glycogen levels and synthesis in the liver providing...
2. Material and methods

2.1. Cell culture

Mouse βTC6 cells, an insulinoma cell line, were grow at 37 °C in Dulbecco’s modified Eagle medium with a low glucose concentration (final: 5 mM) and supplemented with 10% FBS, l-glutamine, penicillin, and streptomycin. Cells were stimulated with 6E-CDCA (1 µM) or high glucose (final: 25 mM) or both. Human HepG2 cells, a hepatocarcinoma cell line, were grown at 37 °C in Minimum Essential Medium with Ear’s salts containing 10% FBS, l-glutamine, penicillin, and streptomycin. Cells were regularly passaged to maintain exponential growth. Human islets were kindly provided by Dr. Giuseppe Basta, Department of Internal Medicine, Section of Internal Medicine, Endocrine and Metabolic Sciences, University of Perugia. The use of human islets was approved by the Ethics Committee of the University of Perugia (Italy).

2.2. Animals

The animal studies were approved by the Animal Study Committee of the University of Naples (Italy). Non Obese Diabetic (NODIII) mice with severe glyceria were housed at the animal facility of the University of Naples “Federico II”, Naples, Italy. Animals from 14 to 24 weeks of age were administered three times a week by gavage with 6E-CDCA 5 mg/kg. Control animals, naïve NOD, were administered vehicle alone (carboxymethylcellulose 0.2%). Blood glucose levels and urinary glucose excretion were measured every week, while plasmatic insulin concentrations have been assessed at the end of the experimental treatment. Glyceria was monitored by the Accucheck system (Roche, Milan, Italy), glycosuria was detected with the Glucose trinder kit 100 (Sigma Chemical Co. Milano, Italy) and insulin plasmatic levels were assayed by Mercodia insulin ELISA immunoassay (cat. 10-113-01, Mercodia, Bologna, Italy).

2.3. Insulin release

Insulin release from βTC6 cultures and human islets was assayed by Mercodia mouse or human insulin ELISA immunoassay (cat. 10-1150-01 and cat. 10-113-01, respectively).

2.4. Glucose uptake

For this assay, βTC6 cells were cultured on 10 cm² Petri culture dishes at the concentration of 2 × 10⁶ and then starved overnight. Next day cells were incubated for 2h at 37 °C in glucose uptake buffer (8.1 mM Na₂HPO₄, 1.4 mM KH₂PO₄, 2.6 mM KCl, 136 mM NaCl, 0.5 mM MgCl₂, 0.9 mM CaCl₂, pH 7.4), stimulated with 1 mM of 6E-CDCA for 45 min and subsequently incubated with 1 µC of [3H]2-deoxyglucose (New England Nuclear, Boston, MA) in glucose uptake buffer for 15 min at 37 °C. Cells were then lysated with ice cold 1 mM NaOH and the radioactivity was measured using a scintillation counter.

2.5. Qualitative PCR

Total RNA was isolated from HepG2, βTC6 and human pancreatic islets using the TRIzol reagent according to the manufacturer’s specifications (Invitrogen, Milan, Italy). One µg RNA was purified of the genomic DNA by DNase I treatment (Invitrogen) and random reverse-transcribed with Superscript II (Invitrogen) in 20µl reaction volume. The amplification of cDNA (50 ng) was achieved in 50 µl mixture containing 200nM dNTPs, 1.5 mM MgCl₂, 200nM of gene specific sense and antisense primers and 1U of Platinum Taq DNA Polymerase (Invitrogen, Milan, Italy). PCR was conducted as follows: after an initial denaturation at 94 °C for 5 min, 35 cycles of amplification (94 °C for 30s, 58 °C for 30s, and 72 °C for 30s) were performed followed by 5 min of final extension at 72 °C. The quality of RNA samples was evaluated using GAPDH specific primers. PCR products were separated by electrophoresis on 2% agarose gel and stained with ethidium bromide. The band of each target transcript was visualized and photographed by ultraviolet transillumination (Bio-Rad, Gel Doc 2000). All PCR primers were designed with PRIMER3-OUTPUT software using published sequence data from the NCBI database (Table 1).

2.6. Quantitative real-time PCR

Fifty ng template was added to the PCR mixture (final volume 25 µl) containing the following reagents: 0.2 µM of each primer and 12.5 µl of 2X SYBR Green qPCR master mix (Invitrogen, Milan, Italy). All reactions were performed in triplicate and the thermal cycling conditions were: 2 min at 95 °C, followed by 40 cycles of 95 °C for 20s, 55 °C for 20s and 72 °C for 30s in iCycler iQ instrument (Bio-Rad, Hercules, CA). The mean value of the replicates for each sample was calculated and expressed as cycle threshold (Cₜ: cycle number at which each PCR reaction reaches a predetermined fluorescence threshold, set within the linear range of all reactions). The amount of gene expression was then calculated as the difference (∆Cₜ) between the Cₜ value of the sample for the target gene and the mean Cₜ value of that sample for the endogenous control (GAPDH). Relative expression was calculated as the difference (∆∆Cₜ) between the ΔCₜ values of the test sample and of the control sample (not treated) for each target gene. The relative quantitation value was expressed and shown as 2⁻^ΔΔCₜ. All PCR primers were designed with PRIMER3-OUTPUT software using published sequence data from the NCBI database (Table 1).

2.7. Western blotting anti-FXR

Cell cultures of HepG2 and βTC6 and in vivo isolated human pancreatic islets were lysated in NuPAGE sample buffer 1X (Invitrogen) containing Sample Reducing agent (Invitrogen) and 2 × 10⁵ cells were separated by polyacrylamide gel electrophoresis (PAGE). The proteins were then transferred to nitrocellulose membrane (Bio-Rad) and probed with primary antibodies FXR (Santa Cruz H-130) and tubulin (Sigma). The anti-immunoglobulin G horse eradish peroxidase conjugate (Bio-Rad) was used as the secondary antibody and specific protein bands were visualized using Super Signal West Dura (Pierce, Euroclone, Milan, Italy), following the manufacturer’s suggested protocol.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Primers used for qualitative and quantitative PCR.</th>
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<tr>
<td></td>
<td>Forward</td>
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<tr>
<td>hGAPDH</td>
<td>gagatgtgcttggtgaatgagt</td>
</tr>
<tr>
<td>hFXR</td>
<td>tacagggagagatggctgaaga</td>
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<tr>
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<tr>
<td>mGLUT4</td>
<td>gcttgaagggaccacaa</td>
</tr>
<tr>
<td>mKLF11</td>
<td>gctgaaacagcagaaaggt</td>
</tr>
<tr>
<td>NOTE: h = human, m = mouse.</td>
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</table>
2.8. Western blotting anti Akt and anti-phospho-Akt

Cell cultures of βTC6 were serum starved for 24 h and then incubated for 0, 5, 15, 30 and 60 min with 1 μM 6E-CDCA. Total lysates were prepared by solubilization of cells in E1A lysis buffer (250 mM NaCl, 50 mM hepes pH 7, 0.1% NP40, 5 mM EDTA) containing both protease and phosphatase inhibitors (Roche) and separated by polyacrylamide gel electrophoresis (PAGE). The proteins were then transferred to nitrocellulose membrane (Bio-Rad) and probed with primary antibodies phospho-Akt (threonin 308) and total Akt (Cell Signaling, Milan, Italy). The anti-immunoglobulin G horseradish peroxidase conjugate (Bio-Rad) was used as the secondary antibody and specific protein bands were visualized using Super Signal West Dura (Pierce), following the manufacturer’s suggested protocol.

2.9. Immunocytochemistry analysis

Immunocytochemistry analysis of FXR was performed in βTC6, human islets and HepG2. Briefly, cells were fixed in acetone 95% for 5 min and endogenous peroxidase were blocked using Dako Peroxide Blocking (DAKO) for 10 min. Antibody anti-FXR purchased from Santa Cruz Biotechnology was used at a dilution of 1:50 for 1 h at room temperature and biotin–streptavidin-HRP detection system was used using DAB substrate chromogen.

2.10. Design and transfection of FXR and KLF11 siRNA

Synthetic and validated pre-designed siRNA for mouse FXR and KLF11 were synthesized and purified by Dharmacon Research, Inc (Dallas, TX). Transfections of siRNA (at 13 nM) were performed in βTC6 in the presence of high glucose concentrations using the Transit TKO Transfection reagent (Mirus, Madison, WI). 48 h post transfection cells were stimulated with 1 μM 6E-CDCA for 18 h and then lysated in Trizol Reagent (Invitrogen) to extract RNA and to check the expression of FXR, KLF11 and insulin by quantitative real-time PCR. Cellular supernatant from βTC6 was used to test the quantity of insulin released in the culture medium.

2.11. Immunofluorescence assay

Immunofluorescence analysis of glucose transporter GLUT2 was performed in βTC6 cells. After treatment with 1 μM 6E-CDCA for 60 min cells were washed with ice-cold PBS, fixed with 4% paraformaldehyde in PBS for 10 min on ice and then permeabilized with 0.2% Tween-20/PBS for 15 min. After washing, cells were stained with primary antibody GLUT2 (Santa Cruz) at a dilution of 1:50 in PBS followed by FITC-conjugated goat anti-Rabbit IgG (Invitrogen) incubation. Nuclei were stained with DAPI. The images were acquired with a fluorescence microscope (Olympus) using the ProgResC14 camera and the IAS 2000 image software (Delta Sistemi, Rome, Italy). Negative controls included the omission of the primary or secondary antibody. No staining was observed under the negative control conditions.

Fig. 1. Farnesoid X Receptor is expressed in human islets and in βTC6 cell line. Quantitative real-time PCR (A), Qualitative PCR (B), Western blotting (C) and Immunocytochemistry analysis (original magnification 40×) (D), showing FXR expression in human islets and βTC6 cell line.
2.12. Statistical analysis

All values are expressed as mean±S.D. of n values per group. Comparisons of more than two groups were made with a one-way ANOVA with post-hoc Tukey’s test. Comparison of two groups was made using Student’s t-test for unpaired data when appropriate. Differences were considered statistically significant if p was <0.05.

3. Results

3.1. FXR is expressed in human islets and in βTC6

We first investigated whether human islets and βTC6 cells express a functionally active FXR [30]. As illustrated in Fig. 1, the FXR mRNA was detected in primary cultures of human islets and in βTC6 by quantitative (Fig. 1A) and qualitative PCR (Fig. 1B). In these experiments HepG2 cells

Fig. 2. Farnesoid X Receptor function on glucose-induced insulin secretion and transcription. βTC6 cells were incubated 18 h with 6E-CDCA 1 μM in conditions of low and high glucose (5 mM or 25 mM). Insulin secretion (A), insulin-1 mRNA expression (C) and FXR mRNA expression (D) were measured. Human pancreatic islets were incubated with 6E-CDCA 1 μM in conditions of high glucose (25 mM) and insulin release was measured (B). Values are mean ± S.D. of 3 experiments. *p<0.05 versus high glucose not stimulated cells.

Fig. 3. siRNA targeting FXR reduces both insulin secretion and transcription. βTC6 cells were transiently transfected with a small interfering RNA targeting FXR and then stimulated with 6E-CDCA 1 μM for 18 h in the presence of 25 mM glucose. Quantitative PCR of insulin-1 mRNA expression (A), FXR mRNA expression (B) and insulin release (C) were measured. Values are mean ± S.D. of 3 experiments. *p<0.05 versus wild-type cells. #p<0.05 versus wild-type stimulated cells.
were used as a positive control. FXR mRNA measured by qualitative PCR was found as a 148 bp band in either HepG2 or human islets, while in murine βTC6 cells FXR mRNA was detected as a 153 bp band. FXR protein (∼55 kDa) was detected by Western blot analysis in HepG2, βTC6 cells and, although at lower levels, in human islets (Fig. 1C). In human islets, the FXR antibody recognizes two bands likely representative of two FXR isoforms. By immunocytochemistry analysis expression of FXR (Fig. 1D) was found predominantly in the nucleus with weak staining throughout the cytoplasm.

3.2. FXR regulates glucose-induced insulin secretion and transcription

To investigate the functional role of FXR in pancreatic β-cells, βTC6 and human islets were incubated 18 h with the synthetic ligand 6E-CDCA at 1 μM in conditions of low and high glucose (5 or 25 mM). As shown in Fig. 2, 6E-CDCA increases the release of insulin in both βTC6 and human islets exposed to 25 mM glucose but not in βTC6 cells incubated with low glucose concentrations (Figs. 2A and B, n = 3; *p<0.05). Similarly, βTC6 exposed to high glucose and 6E-CDCA showed a 20 fold increase in the expression of insulin-1 mRNA in comparison to cells exposed to high glucose only (Fig. 2C, n = 3; *p<0.05). We have also assessed the relative mRNA expression of FXR in cells exposed to the FXR ligand and found that receptor ligation caused a robust induction of the FXR gene expression. Again this effect was documented only in βTC6 cells exposed to high glucose (Fig. 2D, n = 3; *p<0.05). In aggregate, these data suggest that FXR plays a role in regulating insulin release and transcription induced by high glucose.

![Image](image-url)
3.4. FXR regulates GLUT2 translocation, glucose uptake and Akt phosphorylation in βTC6 cells

To test whether FXR directly regulates the synthesis and release of insulin, a small interfering RNA targeting FXR was transfected into βTC6 stimulated with or without 6E-CDCA, 1μM for 18h, in the presence of 25 mM glucose. As illustrated in Fig. 3, transfection of βTC6 with an FXR siRNA resulted in a profound downregulation of FXR gene expression as well as a downregulation of insulin-1 mRNA (Figs. 3A and B, n = 3; *p<0.05 versus wild-type cells; #p<0.05 versus wild-type stimulated cells). In addition, insulin transcription and secretion failed to respond to stimulation by 6E-CDCA in cells transfected with the FXR siRNA, further confirming the specificity of this synthetic ligand (Figs. 3A and C, n = 3; *p<0.05 versus wild-type cells; #p<0.05 versus wild-type stimulated cells).

3.5. FXR induces KLF11 transcription factor

Since KLF11 is a glucose-inducible regulator of the insulin gene, we have investigated whether FXR modulates the expression of this transcription factor in βTC6 cells. For this purpose, cells were incubated 18h with 6E-CDCA 1μM, in conditions of low and high glucose (5 and 25 mM). Results from these experiments demonstrate that KLF11 mRNA expression was robustly induced by high glucose concentrations (Fig. 5, columns 1 and 3, n = 3; *p<0.05 versus low glucose alone). This effect was amplified by FXR activation in condition of both low and high glucose (Fig. 5, n = 3; *p<0.05 versus low glucose alone; #p<0.05 versus high glucose alone).

3.6. Small interfering RNA targeting KLF11 reduces both insulin release and expression by βTC6

To investigate the role of KLF11 in mediating insulin gene transcription and secretion in response to FXR, βTC6 cells were transiently transfected with a siRNA for KLF11 and then stimulated with 6E-CDCA, 1μM for 18h, in the presence of high glucose. As shown in Fig. 6, KLF11 silencing strongly downregulated the relative mRNA expression of insulin-1 as well as the insulin secretion. In this experimental setting exposure to 6E-CDCA failed to stimulate insulin secretion induced by glucose (Figs. 6A and B, n = 3; *p<0.05 versus wild-type cells; #p<0.05 versus wild-type stimulated cells). Thus, KLF11 is required for regulation of insulin transcription and secretion by FXR.

4. Discussion

Pancreatic β-cell contains a range of nuclear receptors implicated in the regulation of insulin secretion [30]. As an example, LXRβ activation in β-cells increases insulin secretion along with genes regulating β-cell differentiation [31–34]. Moreover, glucose stimulates LXR transcriptional activity in the liver and induces nuclear localization and activation of LXR in β-cells [35,36]. Similarly, peroxisome proliferator-activated receptor (PPAR) isoforms α and γ are expressed in pancreatic islets as well as in insulin-producing cell lines and ligands for PPARγ enhance glucose-induced insulin secretion in rat pancreatic cells [37–41]. Many of these effects are mediated by the transcriptional modulation of glucose transporters (GLUT1, 2, 3, and 4), a family of membrane proteins that transport glucose along a gradient concentration [42,43]. GLUT2 is the main glucose transporter expressed by pancreatic β-cells [43]. Substantial data support the notion that activation of the PI3-kinase (PI3K) by glucose and subsequent phosphorylation of Akt are essential steps for the stimulation of the translocation of glucose transporters at plasma membrane [44,45], thus regulating its own uptake. Furthermore, glucose coordinately recruits a highly sophisticated network of transcription factors and co-
Fig. 6. 6E-CDCA failed to regulate insulin release and expression in cells transfected with a siRNA targeting KLF11. βTC6 cells were transiently transfected with a small interfering RNA targeting KLF11 and then stimulated with 6E-CDCA 1 μM for 18 h in the presence of 25 mM glucose. Quantitative PCR of insulin-1 mRNA expression (A) and insulin release (B) were measured. Values shown are mean ± S.D. of 3 experiments. *p<0.05 versus wild-type cells. #p<0.05 versus wild-type stimulated cells.

Fig. 7. FXR activation stimulates insulin secretion in vivo. Non Obese Diabetic (NOD) mice were treated three times a week with 6E-CDCA 5 mg/kg by gavage from 14 to 24 weeks. (A) Time course of glycosuria. (B) Time course of glycemia. (C) Blood glucose and (D) plasma insulin levels after 24 weeks. (E) Ratio of the plasma insulin level to blood glucose level after 24 weeks. Values are mean ± S.D. of 6 experiments. *p<0.05 versus naïve NOD mice.
activators to the insulin promoter, and prolongs the half-life of insulin mRNA [3]. The repertoire of insulin gene transcription factors activated by glucose includes KLF11. In pancreatic β-cell KLF11 is regulated by TGF-β, a pathway playing a critical role in the development and homeostasis of both the exocrine and endocrine pancreas. In addition, KLF11 regulates genes encoding for scavengers of oxidative stress, such as SOD2 and catalase [8,47]. Sequencing of the KLF11 gene in families enriched for early onset of type 2 diabetes has uncovered two missense mutations (A347S and T220M) which segregated with diabetes. Other sequencing efforts have led to identification of 19 common polymorphisms several of which were associated with type 2 diabetes in an initial case-control study totaling 626 French individuals. Further genotyping studies have only partially replicated these data [8,47]. In light of these evidences, it has been speculated that KLF11 could be a target for the therapy in a subset of diabetic patients [8].

In this study we have shown that FXR regulates insulin transcription and secretion by genomic and non-genomic effects. Our in vitro results indicate that genomic effects of FXR activation on insulin lead to induction of insulin gene expression. Regulation of insulin mRNA expression by FXR is glucose dependent and requires induction of the transcription factor KLF11. We have shown that KLF11 is an essential modulator for the FXR activity on insulin gene expression and results from KLF11 silencing experiments have shown that FXR activation failed to regulate the transcription of the insulin gene in the absence of KLF11. Supporting this view, feeding NOD mice with an FXR agonist significantly increased insulin-1 gene expression in the pancreas. Despite the mechanistic relevance of KLF11 in regulating insulin secretion induced by FXR, we have been unable to find an FXR responsive element in promoter of this transcription factor, suggesting that regulation of KLF11 gene expression by FXR is due to a non-genomic effect. In aggregate, these results demonstrate that FXR acts by a KLF11 mediated pathway responsible for the ability of this nuclear receptor to modulate the glucose-induced insulin gene transcription and provide evidence that FXR could be considered a new activator of this transcription factor.

In addition FXR regulates insulin secretion by non-genomic effects. Thus, FXR increases Akt phosphorylation induced by glucose favouring the Akt-dependent translocation of GLUT2 to plasma membrane. Because this event results in an increased glucose uptake by pancreatic β-cells these pathways might also contribute to insulin secretion. Support of this concept comes from the results of FXR silencing experiments demonstrating that insulin release triggered by high glucose was significantly reduced by FXR gene ablation. The effects of FXR activation on insulin secretion and transcription were observed only at high glucose, but the regulation of KLF11 mRNA was observed both at low and high glucose. Despite the fact that results shown in Fig. 6A demonstrates that KLF11 is essential for insulin release induced by FXR in condition of high glucose, these findings seems to establish a hierarchy among different regulatory mediators in insulin secretion. Thus, activation of KLF11 per se is unable to promote insulin secretion and transcription in condition of low glucose. This is likely related to the fact that exposure to high glucose recruits a number of regulatory factors to the insulin promoter (PDX1, MAF-A, etc) [4-8] that are not recruited in condition of low glucose.

An important observation made in this study was that in vivo activation of FXR delays the onset of autoimmune type I diabetes in NOD mice. Compared with other animal models of diabetes characterized by high levels of plasmatic insulin (fa/fa rats, db/db and ob/ob mice) NOD mice develop an insulin-dependent diabetes that is characterized in its first stage by a defective insulin secretion.
related to an impaired insulin signaling [48–50]. This is followed by a second stage of islets destruction, insulitis and leukocytic infiltration of the pancreatic islets, characterized by polydipsia, weight loss, glycosuria and persistent hyperglycemia [48–50]. This second phase has been partially ascribed to increased production of inflammatory mediators such as TNFα, IL-1β and IFNγ in the pancreas [41–52]. Despite the NOD model is characterized by progressive insulin deficiency, we felt that this could have been an important model to examine the effect of FXR on regulation of insulin signaling in pancreatic β-cells. Indeed, because FXR might function by increasing peripheral insulin sensitivity [20–24], this insulin-deficient model appears to be more relevant for the study of central activity of FXR than other models characterized by peripheral insulin resistance.

Present results demonstrate that blood glucose levels and glycosuria were significantly ameliorated by FXR activation in NOD mice. Furthermore, FXR activation ameliorated the ratio of plasma insulin level to blood glucose level, suggesting a stimulation of insulin secretion in vivo. The ability to modulate insulin release was not linked to prevention of β-cell destruction by the FXR agonist. Thus, despite we have not performed a morphometric examination of NOD pancreatic islets, we observed that not only mRNA expression of insulin-1, GLUT2 and FXR in pancreatic beta cells of mice administered with the FXR ligand were similar to that of naïve NOD mice, but also absolute plasma insulin levels were similar in both mice groups. Collectively, these data suggest that the two groups underwent a similar extent of pancreatic injury excluding that beta-cell protection ground the mechanism of action described in this paper.

In addition to these effects on pancreatic β-cells, FXR activation induces the expression of GLUT4 in the liver, confirming previous data indicating that the GLUT4 promoter contains a FXR responsive element [29]. Because the liver is an important tissue for glucose storage into glycogen, induction of GLUT4 and regulation of insulin receptor sensitivity [24] is also likely to contribute to the metabolic control we documented in this study and is consistent with previous reports demonstrating that FXR gene ablation impairs insulin signaling [20–24].

In summary, we have shown that the bile acid sensor FXR is expressed by pancreatic β-cells and human islets and regulates the insulin signaling by genomic and non-genomic effects. Genomic effects include KLF11 mediated stimulation of insulin gene expression. Non-genomic effects include an Akt mediated stimulation of glucose induced relocation of GLUT2 in β-cells. Finally these effects are reproduced in vivo in a rodent model of insulin-deficient diabetes developing in NOD mice. The present study provides further support for the potential role of the FXR agonists in regulation of glucose homeostasis.

References


