Peroxisome Proliferator-activated Receptor γ (PPARγ) and Its Target Genes Are Downstream Effectors of FoxO1 Protein in Islet β-Cells

MECHANISM OF β-CELL COMPENSATION AND FAILURE*

Received for publication, May 19, 2013, and in revised form, June 14, 2013. Published, JBC Papers in Press, June 20, 2013, DOI 10.1074/jbc.M113.486852

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Background: The molecular mechanisms for islet β-cell compensation and failure are not fully known. We used in vitro and in vivo systems to show that FoxO1, an integrator of metabolic stimuli, inhibits PPARγ expression in β-cells, thus transcription of its target genes (Pdx1, glucose-dependent insulinotropic polypeptide (GIP), receptor, and pyruvate carboxylase) that are important regulators of β-cell function, survival, and compensation. FoxO1 inhibition of target gene transcription is normally relieved when upstream activation induces its translocation from the nucleus to the cytoplasm. Attesting to the central importance of this pathway, islet expression of PPARγ and its target genes was enhanced in nondiabetic insulin-resistant rats and markedly reduced with diabetes induction. Insight into the impaired PPARγ signaling with hyperglycemia was obtained with confocal microscopy of pancreas sections that showed an intense nuclear FoxO1 immunostaining pattern in the β-cells of diabetic rats in contrast to the nuclear and cytoplasmic FoxO1 in nondiabetic rats. These findings suggest a FoxO1/PPARγ-mediated network acting as a core component of β-cell adaptation to metabolic stress, with failure of this response from impaired FoxO1 activation causing or exacerbating diabetes.

Results: FoxO1/PPARγ signaling regulates key β-cell genes, with this network being up-regulated in nondiabetic insulin-resistant rats and impaired in rodents with diabetes. We examine the potential for the FoxO1/PPARγ network as a feature of β-cell compensation and failure. We identify targets for prevention of type 2 diabetes.

Conclusion: We identify targets for prevention of type 2 diabetes.

Significance: We identify targets for prevention of type 2 diabetes.

The molecular mechanisms and signaling pathways that drive islet β-cell compensation and failure are not fully resolved. We have used in vitro and in vivo systems to show that FoxO1, an integrator of metabolic stimuli, inhibits PPARγ expression in β-cells, thus transcription of its target genes (Pdx1, glucose-dependent insulinotropic polypeptide (GIP), receptor, and pyruvate carboxylase) that are important regulators of β-cell function, survival, and compensation. FoxO1 inhibition of target gene transcription is normally relieved when upstream activation induces its translocation from the nucleus to the cytoplasm. Attesting to the central importance of this pathway, islet expression of PPARγ and its target genes was enhanced in nondiabetic insulin-resistant rats and markedly reduced with diabetes induction. Insight into the impaired PPARγ signaling with hyperglycemia was obtained with confocal microscopy of pancreas sections that showed an intense nuclear FoxO1 immunostaining pattern in the β-cells of diabetic rats in contrast to the nuclear and cytoplasmic FoxO1 in nondiabetic rats. These findings suggest a FoxO1/PPARγ-mediated network acting as a core component of β-cell adaptation to metabolic stress, with failure of this response from impaired FoxO1 activation causing or exacerbating diabetes.

Peroxisome proliferator-activated receptor γ (PPARγ)2 is a member of the nuclear hormone receptor family of ligand-inducible transcription factors and plays a pivotal role in diverse biological processes such as glucose and lipid homeostasis, inflammation, and cellular proliferation and differentiation (1, 2). In addition to adipocytes and several other cell types, it is expressed in islet β-cells (3). We have shown that PPARγ in β-cells regulates transcription of the prodiabetogenic transcription factor Pdx1 (4, 5) and the receptor for the incretin hormone glucose-dependent insulinotropic polypeptide (GIP) (6). Others have reported that PPARγ regulates expression of the anaplerotic enzyme pyruvate carboxylase in adipose tissue (7), but whether this occurs in β-cells is unknown. Collectively, these genes are key controllers of β-cell mass, mitochondrial fuel metabolism, and insulin secretion, suggesting the potential for a central role of PPARγ in β-cell compensation and/or failure. Consistent with this proposal, we have shown increased expression of PPARγ and its target genes in islets from rodents with successful β-cell adaption to an experimental reduction of β-cell mass (4, 6) and to obesity and insulin resistance (6, 8) and decreased expression in a diabetic rodent model (6, 8). Also, clinical trials with thiazolidinedione PPARγ agonists (9) have shown a high success rate at stabilizing β-cell function and preventing type 2 diabetes (10).

Understanding why β-cell PPARγ expression is defective under hyperglycemic conditions requires characterizing its upstream regulation. In adipocytes, PPARγ gene expression is under inhibitory control by FoxO1 (11, 12). FoxO1 is a member of the family of winged-helix/forkhead transcription factors that serve important roles in cellular differentiation, proliferation, apoptosis, and the response to cellular stress in many tissues. FoxO1 is highly expressed in β-cells and is a key regulator of β-cell development, mass, and function (13–16). The best described FoxO1 target gene in β-cells is Pdx1 (17). The mechanism is reported to be competition for the FoxA2-binding element on the Pdx1 promoter, with phosphorylation of FoxO1 resulting in nuclear export and release of its blockade of FoxA2-dependent Pdx1 expression. However, the evidence is mostly correlative: EMSA and competition assays for FoxO1 and FoxA2 on the Pdx1 promoter, and imaging studies showing
FoxO1 activity is modulated by post-translational modifications and protein-protein interactions that impact its intracellular trafficking and function (18). In the β-cell, insulin or growth factor PI3K/Akt-induced FoxO1 phosphorylation leads to its translocation from the nucleus to cytoplasm, relieving its inhibition of target gene transcription (14). The incretin hormones GIP and glucagon-like peptide 1 (GLP-1) also activate this sequence (19, 20). Another is the balance between oxidative stress-induced FoxO1 acetylation and deacetylation (21). Thus, FoxO1 orchestrates β-cell transcriptional regulatory pathways for adaptive responses to many hormones, growth factors, and metabolic stresses. Whereas much is known about FoxO1 activity in β-cells, much less is known regarding its downstream target genes.

In this study we provide evidence for a central role for PPARγ in FoxO1-dependent regulation of Pdx1 expression and other key β-cell genes. Also, we show strong nuclear localization of FoxO1 in β-cells along with reduced expression of PPARγ and its target genes in diabetic rats, suggesting a novel mechanism for β-cell failure.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and In Vitro Assays—**INS-1 (832/13) cells and βTC6 cells were maintained as described previously (5). PPARγ and FoxO1 siRNA were carried out in INS-1 cells using pooled SMART selection-designed siRNA duplexes with UU 3′-overhang and 5′-phosphate on the antisense strand that targeted rat FoxO1 (NM_012560) and rat PPARγ (NM_013124) (Dharmacon RNA Technology). Transfections were carried out with DharmaFect 4 transfection reagent for 24 h at final siRNA concentrations of 100 pm. The protocol was two siRNA transfections at 0 h and 48 h; control cells were transected with scrambled siRNA.

EMSA used nuclear extracts from rat-derived INS-1 cells and mouse-derived βTC6 cells with PAGE-purified oligonucleotides for the mouse PPARγ promoter containing the putative FoxO1 binding site (forward, 5′-TCTCAGATAGATACAAATTT-3′ and reverse, 5′-CATCTATAGTTAATAATCG-3′) labeled by end filling with [α-32P]dCTP (PerkinElmer Life Sciences) as described previously (5). PPARγ specificity was determined with rabbit polyclonal FoxO1 antibody (Chemicon, AB4130) and competition studies that added increasing amounts of unlabeled dsDNA oligonucleotide sequences.

ChIP was performed with βTC6 cells using ChIP-IT kit (Active Motif) and a previously described method (5). Rabbit polyclonal FoxO1 antibody (Chemicon) was added to aliquots of precleared 300–500-bp chromatin fragments and incubated overnight, with parallel samples incubated with the negative-control IgG provided with the kit. Protein G-agarose beads were added to the mixture for 1.5 h at 4°C. After reversing cross-links, DNA was isolated and PCR performed using primers for the mouse PPARγ promoter. Primer sequences were: forward, 5′-AATTCATCAACTTGGGATACATAGTC-3′ and reverse, 5′-GGTTGGACTCAATCAGGCAGTGGTGG-3′ with an expected 281-bp PCR product. PCR conditions were 1 cycle at 94°C for 3 min, 40 cycles at 94°C for 30 s, 62°C for 1 min, 72°C for 1 min. A mouse-specific positive control was performed using a kit based on the binding of the transcription factor EFL-α by anti-RNA polymerase II (Active Motif).

Luciferase reporter gene assays were carried out in 60–70% confluent INS-1 cells in 6-well plates incubated overnight in antibiotic-free medium. Co-transfections were performed with the FoxO1 overexpression cassette (pcDNA3-FLAG-FKHR from Dr. Kun-Liang Guan, University of Michigan) and the Pdx1 promoter pTAL-PPRE-Pdx-1 luciferase plasmid (Dr. Roland Stein, Vanderbilt University) or 3× PPRE-luciferase reporter vector (Dr. Speigelman laboratory, Addgene) with Lipofectamine 2000 transfection reagent (Invitrogen). Renilla luciferase reporter plasmid (pRL-TK, Promega) was included (0.05 µg) in all transfections as internal control. Cells were lysed 48 h after transfection, and luciferase assay performed in a TD 20/20 luminometer (Turners Design) using a dual luciferase assay kit (Promega). Firefly luciferase activity was normalized to Renilla luciferase and expressed as relative luciferase activity of the reporter constructs with and without transfecion of the FoxO1 overexpression cassette.

**Animal Studies—**All protocols were approved by the University of Vermont Institutional Animal Care and Use Committee. FoxO1-haplodeficient mice (17) and mice that overexpress a constitutively nuclear FoxO1 transgene (FoxO1S253A) in β-cells and liver (13, 22) were obtained from D. Accili (Columbia University) on a mixed background and were backcrossed for >10 generations to the C57BL/6 background. Mice with PPARγ deficiency restricted to pancreatic epithelium (PANC PPARγ−/−) were generated by crossing Pdx1-Cre mice (original source, D. Melton, Harvard University) and mice with two floxed PPARγ alleles as detailed previously (5). Some animals underwent glucose tolerance testing after an overnight fast that consisted of 2 g/kg intraperitoneal glucose with serum glucose measured at 0, 30, 60, 90, and 120 min (Freestyle glucose meter). Zucker lean (ZL, fa/+ or +/+) and Zucker fatty (ZF, fa/fa) male rats (Harlan) underwent 60% pancreatectomy (Px) or sham Px surgery at 6 weeks of age as described previously (8). Islets were isolated by pancreas duct perfusion with collagenase, Histopaque gradient separation, and hand picking.

**Islet Expression Studies—**Immunoblot and quantitative PCR analyses were performed as described previously (5). Immunoblot antibodies were rabbit polyclonal anti-Pdx1 (1:2000, Upstate/Millipore), mouse monoclonal anti-PPARγ (1:1000, Chemicon/Millipore), and rabbit polyclonal anti-FoxO1 (1:1000, Chemicon/Millipore), followed by goat anti-mouse/rabbit-HRP-conjugated antibody (Bio-Rad). Pyruvate carboxylase affinity blotting was performed using HRP-conjugated streptavidin. Detection was by chemiluminescence using Hyperfilm ECL (Amersham Biosciences). Membranes were stripped and reprobed to establish equivalent loading using anti-β-actin (Sigma). Islet quantitative PCR was carried out in a PTC-200 Peltier Thermal Cycler (MJ Research) using cDNAs, Taq polymerase (Promega), and primer combinations (sequences available on request). The thermal cycle program was denaturing step at 95°C for 2 min followed by 35 cycles for PPARγ, 25 cycles for Pdx1, 30 cycles for FoxO1, at 94°C for 15 s, 56°C for 30 s, and 72°C for 60 s, with an extension step of 5 min at 72°C.
FoxO1 and PPARγ Signaling in β-Cells

Results are expressed relative to control gene expression (cyclophilin B).

GLP-1 Induction of Pdx1 Gene Expression—Isolated islets from PANC PPARγ−/− and WT mice were cultured overnight in RPMI 1640 medium, 10% FBS, 11.2 mM glucose, 100 units/ml penicillin, 100 μg/ml streptomycin, followed by overnight culture in serum-free medium with 3% BSA. Islets were incubated in serum-free medium with 3% BSA and 11.2 mM glucose plus freshly dissolved 100 μM mouse GLP-1 (Phoenix Pharmaceutical) or vehicle for 8 h followed by quantitative PCR analysis.

Pyruvate Carboxylase (PC) Activity—Fresly isolated islets from 3 week postsurgery sham and 60% PxFZ and ZL rats were frozen in liquid nitrogen and shipped to Dr. Ye Q. Liu at the University of Louisville. PC activity was measured as described previously (23).

Pancreas Immunofluorescence and Islet Morphometry—Excised pancreata were immersion-fixed overnight in 4.0% paraformaldehyde in 0.1 M phosphate buffer at 4 °C followed by embedding in paraffin. β-Cell mass was quantified using a previously detailed computer planimetric method (24). For multiple-labeling immunofluorescence studies of FoxO1 and Pdx1, sections were stained with guinea pig anti-insulin (Linco/Millipore) and rabbit anti-FoxO1 (Cell Signaling, clone C29H4). Secondary antibodies were multiple-labeling grade anti-species-specific IgG conjugated to either CY2, CY3, or Alexa Fluor 647, respectively (Jackson ImmunoResearch or Molecular Probes/Invitrogen). Sections were imaged confocally using an LSM 510 META (University of Vermont Microscopy Imaging Center).

Statistical Analysis—Data are presented as mean ± S.E. or S.D., as indicated. Each data point from the animal studies represents an individual animal. Statistical significance was determined by the unpaired Student’s t test or two-way ANOVA.

RESULTS

Identification and Characterization of FoxO1 Binding Site on Mouse PPARγ Promoter—The initial studies sought to identify a biological basis for FoxO1 regulation of PPARγ expression. The consensus binding sequence for FoxO1 is GTAAACA (25). We searched GenBank and identified a putative FoxO1 binding site within the mouse PPARγ promoter at positions −825 to −831 from the transcription start site (GenBank AY236530) with one nucleotide mismatch from the consensus sequence (Fig. 1A). MatInspector software identified homologous sequences in the rat and human PPARγ promoters. We confirmed that this sequence binds FoxO1 by performing EMSA and FoxO1 antibody inhibition studies using α-32P-labeled complementary strand oligomers of the identified sequence and nuclear extracts from mouse-derived βTC6 and rat-derived INS-1 cells (Fig. 1B). Also, the specificity of binding was established with a competition assay using excess unlabeled probe.

ChIP assay was performed in βTC6 cells to confirm FoxO1 binds to this sequence in an intact insulin-containing cell line, using FoxO1 antibody-precipitated DNA fragments as the PCR template and a primer pair that spanned the region containing the FoxO1 binding site (Fig. 2A). Input DNA and the FoxO1 antibody-precipitated chromatin fragments both contained the correct 281-bp product in contrast to the nonimmune IgG negative control (Fig. 2B). Also shown is the positive control based on the binding of RNA polymerase II to the transcription factor EFl-α (Fig. 2C).

Expression studies confirmed FoxO1 inhibits PPARγ expression in an insulin-containing cell line. A FoxO1 siRNA protocol was established in INS-1 cells that resulted in a 60% knockdown of FoxO1 mRNA level and 47% reduction in FoxO1 protein by 96 h (Fig. 3A). PCR analysis showed a 2.0 ± 0.1-fold increase in PPARγ mRNA level compared with the scrambled siRNA control, and a similar increase in Pdx1 mRNA level: 2.2 ± 0.2-fold (Fig. 3B). Also, FoxO1 was overexpressed by transient transfection in INS-1 cells and assayed for PPARγ promoter activity using two PPRE luciferase reporters, Pdx1-PPRE and 3×PPRE. A 48-h overexpression of FoxO1 lowered Pdx1-PPRE driven luciferase activity by 41% compared with no FoxO1 overexpression (Fig. 3C). In addition, 3×PPARγ-PPRE reporter activity was decreased 44% with the FoxO1 overexpression (Fig. 3D).

In Vivo Studies—FoxO1 inhibition of PPARγ expression in native β-cells was investigated using isolated islets from genetically modified mice. We first tested global FoxO1-haploinsufficient mice. Null mice are embryonically lethal, but the haploinsufficient mice are normoglycemic with relatively normal
pancreas morphology and β-cell mass, insulin content, and insulin secretion (17, 22). Islet lysates from 8-week-old FoxO1+/− mice had twice the PPARγ mRNA and protein levels of WT mice (Fig. 4). Pdx1 mRNA and protein levels also were increased 2-fold in the FoxO1+/− islets.

We next studied mice that hyperexpress a constitutively nuclear FoxO1 transgene in β-cells and liver; the transgene encodes an amino acid substitution at the Ser253 phosphorylation site that prevents insulin-stimulated translocation of the mutant FoxO1 to the cytoplasm and is under control of the FoxO1 transcription site that prevents insulin-stimulated translocation of the nuclear FoxO1 transgene in WT mice (20), to probe the FoxO1/PPARγ signaling in vitro. Normal mouse islets were incubated for 8 h with or without 100 nM GLP-1(7–36 amide) at 11.1 mM glucose followed by real time PCR analysis. PPARγ and Pdx1 signals were increased 2–2.5-fold whereas MafA and FoxA2 were unchanged, showing the selectivity of the effect (Fig. 6A). We used this protocol with islets from PANC PPARγ−/− mice. In agreement with the prior results, GLP-1 stimulation nearly doubled PPARγ and Pdx1 mRNA expression in the floxed control mice. In contrast, in the PANC PPARγ−/− islets a GLP-1-induced increase in Pdx1 expression was absent (Fig. 6B), demonstrating that PPARγ is essential for in vitro GLP-1 activation of Pdx1 expression.

PPARγ Regulates PC Expression in Islet β-Cells—We have shown that PPARγ transcriptionally regulates Pdx1 (4, 5) and GIP receptor genes (6) in β-cells. Others have shown PPARγ regulation of PC expression in white and brown adipose tissue (7). We previously studied ZF rats that are obese, hyperlipidemic, and insulin-resistant secondary to a mutated leptin receptor, but are virtually normoglycemic because of compensatory increases in β-cell mass and function (26, 27). Those studies showed that PC plays a critical role in their β-cell adaptive response by preventing dysmetabolism-induced defects in β-cell mitochondrial fuel metabolism (27). Also, reduced islet PC expression is found in rodents (28, 29) and humans with type 2 diabetes (30).

We investigated whether β-cell PC expression is PPARγ-regulated using an established siRNA for PPARγ in INS-1 cells that lowers PPARγ mRNA and protein 75% (4). Cells were treated 72 h with or without the PPARγ agonist troglitazone. PPARγ siRNA reduced PC mRNA band intensity >60% (Fig. 7A, first and fourth lanes) whereas troglitazone enhanced it 3-fold (second lane); that effect was lost when the PPARγ siRNA and troglitazone were used together (third lane).

We previously developed and characterized a diabetic rat model by performing a 60% Px in ZF rats (8). Px ZF rats became hyperglycemic (~15 mm) by 3 weeks after surgery with the
same free fatty acid levels and obesity as normoglycemic ZF shams. Additional controls were sham and 60% Px ZL rats that were euglycemic and metabolically indistinguishable. Islet immunoblots from 3 week postsurgery sham ZF rats had a doubling of PPARγ protein and its transcriptional target GIP receptor expression compared with both groups of ZL rats, whereas the diabetic Px ZF rats displayed 20–30% reductions in nuclear PPARγ expression and parallel findings for its target gene FoxO1 expression. Also, transgenic mice with altered FoxO1 expression or defective islet FoxO1 activation and/or translocation caused loss of the adaptive response to hyperglycemia in both ZL and ZF rats. As expected, the band intensity in the sham ZF islets was nearly twice that of the ZL rats (171 ± 10%) whereas it was subnormal in the diabetic Px ZF rats (67 ± 12%, Fig. 7B). Comparable results were obtained for PC activity of islet extracts (Fig. 7C), with a 60% increase in sham ZF islets versus ZL rats (p < 0.001), but no increase in the Px ZF islets (p < 0.002 sham ZF versus Px ZF).

**DISCUSSION**

The current results have established PPARγ expression in β-cells is under transcriptional restraint by FoxO1. That conclusion is supported by our having identified a FoxO1 binding site in the mouse PPARγ promoter with homologous sequences in rats and humans, and *in vitro* studies demonstrating FoxO1 binding and inhibition of PPARγ expression. Also, transgenic mice with altered FoxO1 expression or defective islet FoxO1 nuclear translocation exhibited changes in islet PPARγ expression and parallel findings for its target gene Pdx1. Of particular importance was our demonstrating that PPARγ is necessary for GLP-1 mediated augmentation of Pdx1 expression in vitro, as GLP-1 is an established FoxO1 activator (20). We have also shown that PPARγ regulates PC gene expression in β-cells, as has previously been characterized using molecular and *in vivo* techniques in white and brown adipose (7), making it the third PPARγ target gene we have identified in β-cells along with the Pdx1 (4, 5) and GIP receptor (6) genes.
These results are especially notable because of the importance of the identified PPARγ/H9253 target genes for regulating β-cell development and postnatal β-cell mass and function. Pdx1 is an essential transcription factor for pancreas morphogenesis and postnatally is a key regulator of β-cell differentiation, maturation, and survival (31). It is required for the normal adaptive increase in β-cell mass and function to insulin resistance (32, 33). Distal from Pdx1 is a network of genes that impact diverse aspects of β-cell signaling, mass, and function (34) including glucokinase with its well known regulatory effects on glycolysis, insulin secretion, and β-cell proliferation (35).

PC catalyzes mitochondrial pyruvate conversion to malate. In most cells, the normal pathway for pyruvate metabolism is through pyruvate dehydrogenase with entry into the citric acid cycle for ATP production. However in β-cells there is unusually high PC expression so that 50% of pyruvate undergoes carboxylation (36) with entry into the anaplerotic pathways of insulin secretion (37). Obese, insulin-resistant, hyperlipidemic ZF rats normally do not develop diabetes because of compensatory increases in β-cell mass and function (27). We previously studied ZF rats made hyperglycemic by a 60% pancreatectomy and observed subnormal islet expression of PPARγ/H9253 and its target gene GIP receptor versus the hyperexpression in nondiabetic ZF rats (6). The current study has shown the same pattern for PC along with reduced nuclear Pdx1 immunostaining in Px ZF rats, which are in accord with our previous report showing subnormal PC and Pdx1 mRNA expression in Px ZF islets (8).

Insight into the impaired PPARγ expression with hyperglycemia came with our finding a distinct change in the subcellular localization of FoxO1 in β-cells of ZF diabetic rats, specifically, instead of the nuclear and cytoplasmic pattern seen in the con-
control rats, there was an intense nuclear distribution. This finding is not unique to the Px ZF model, as nuclear FoxO1 and reduced overall islet FoxO1 expression were found in insulin-resistant diabetic mice created by deletion of insulin receptors in New Zealand obese mice that became diabetic when fed a carbohydrate-rich diet; reductions in islet FoxO1 phosphorylation were noted (47). Furthermore, islets from these mice, and diabetic mice created by deletion of insulin receptors in individual mouse.

The mechanism of the defective nuclear to cytoplasmic translocation of FoxO1 with diabetes is not known, as the regulation of FoxO1 activity related to compartmentalization in β-cells is multifactorial related to several post-translational modifications and protein interactions (14, 18, 21). Best known for the β-cell is insulin or growth factor PI3K/Akt pathway-induced phosphorylation causing nuclear export that removes its inhibition of target gene transcription (14). Supporting a role in diabetes, FoxO1 is required for GLP-1 induction of Pdx1 expression in β-cells is mediated by a responsive element (R1) that regulates PC gene expression in β-cells. Alternatively, FoxO1 activity and its nuclear location (49). Depletion of FoxO1 in insulinoma cells has been controversial. Another study reported reduced insulin receptor and insulin receptor substrate 2 expression in islets from humans with type 2 diabetes, and it noted nuclear retention of FoxO1 impairing PPARγ-mediated defenses against metabolic stresses is an unrecognized feature of failed β-cell adaptation.

FIGURE 6. PPARγ is required for GLP-1 induction of Pdx1 expression in mouse islets. A, normal mouse islets were incubated 8 h with or without 100 nM GLP-1(7–36 amide) followed by real time PCR analysis. Expression of PPARγ and Pdx1 was doubled compared with no observable effect on MafA or Foxa2 expression. B and C, that protocol was used in isolated islets from 8-week-old floxed control mice (B) and PANC PPARγ−/− mice (C) with the normal GLP-1 induction of Pdx1 expression absent in the PANC PPARγ−/− islets. Left, representative gels with each lane representing islets from an individual mouse. Right, mean ± S.D. (error bars) results of three mice in each group.

FIGURE 7. PPARγ regulates PC gene expression in β-cells. A, representative results of PPARγ siRNA in INS-1 cells with or without the PPARγ agonist troglitazone, showing a 60% reduction in PC mRNA with the PPARγ siRNA (first and fourth lanes) whereas troglitazone enhanced it 3-fold (second lane); the latter was lost when the PPARγ siRNA and troglitazone were used together (third lane). B, PC expression assessed with streptavidin A affinity labeling of PAGE-resolved islet extracts from 3 weeks after surgery 60% Px and sham ZL and ZF rats. Sham (Sh) ZF rats are obese and insulin-resistant, but normoglycemic because of compensatory increases in β-cell mass and function. In contrast, Px ZF rats become hyperglycemic (~15 mmol/L) by 3 weeks after surgery with the same obesity and free fatty acid levels as the normoglycemic ZF shams. As we have seen with other PPARγ-regulated genes in β-cells, the PC band intensity in the sham ZF islets was nearly twice that of the ZL rats and below normal in the diabetic Px ZF rats. Top, representative gel. Bottom, mean ± S.D. (error bars) results of four rats in each group. C, PC enzyme activity measured in islet extracts from 3 weeks after surgery 60% Px and sham ZL and ZF rats. Results are mean ± S.D. of three rats in each group.
be normoglycemic with a standard chow diet and also after fat feeding (50). Another publication from these authors (51) using a tamoxifen-inducible Pdx1 Cre/loxP system that lowered islet PPARγ mRNA by 90% found no β-cell phenotype after 7 months of normal chow; glucose tolerance, insulin secretion, and β-cell mass were unchanged from WT mice as was expression of multiple islet genes including Pdx1, pyruvate carboxylase, and GIP receptor. In addition, 11 weeks of a high fat diet induced the same hyperglycemia, obesity, and insulin resistance in the PPARγ knock-out and WT mice. Also, there was no difference in β-cell mass or islet gene expression profiles between the fat-fed WT and PPARγ knock-out mice although the atypical finding of no effect of fat feeding on these parameters in WT mice makes these latter findings difficult to interpret. The authors concluded that PPARγ in β-cells is inconsequential. In contrast, we studied noninducible Pdx-1 Cre/loxP PPARγ-null mice (52) and found hyperglycemia at 8 weeks of age with standard chow along with near total absence of glucose-induced and GIP-induced insulin secretion in isolated islets, and a marked reduction in islet expression of Pdx-1 and GIP receptor (5, 6). There was no difference in β-cell mass or pancreas histology from control mice (5). As such, this mouse model is a vital intermediate. FoxO1 is well poised to be a master transcriptional regulator over β-cell adaptive responses, as its expression and activity are influenced by numerous stimuli such as nutrient overload, growth factors, incretin hormones, and oxidative stress (13, 14). Downstream from PPARγ is a network of genes that exert important regulatory control over prodifferentiation processes, incretin effects, glucose and mitochondrial fuel metabolism, and β-cell compensation to obesity and insulin resistance (13, 27, 32, 33). We have shown that islet PPARγ and its target genes are hyperexpressed in nondiabetic insulin-resistant rats (present study and Refs. 6, 8) and rats during the β-cell adaptation after a partial pancreatectomy (4, 6), plus we and others have shown impaired expression of these same genes in diabetic animals (6, 29, 44) and humans with type 2 diabetes (30, 45, 46). Collectively, these findings support, but do not prove, a FoxO1/PPARγ-mediated transcriptional network functioning as a core element of how β-cells adapt to metabolic stress, with failure of this response causing or exacerbating diabetes.

Acknowledgments—We thank Dr. Domenico Accili (Columbia University) for the FoxO1 mouse models, Drs. Richard Mortenson and Sheng Duan (University of Michigan) for the PANC PPARγ−/− mouse model, Dr. Ye Q. Liu (Louisville University) for performing the pyruvate carboxylase activity measurements, Dr. Kun-Liang Guan (University of Michigan) for the FoxO1 expression plasmid, Dr. Roland Stein (Vanderbilt University) for the Pdx-1 promoter plasmid, and Dr. Chris Newgard (Duke University) for the INS-1 cells. The 3X PPRE-luciferase reporter vector developed by Dr. Bruce Spiegelman (Dana-Farber Cancer Institute) was obtained from Addgene. The Pdx1 monoclonal antibody developed by Dr. O. Madsen was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD, National Institutes of Health, and maintained by The University of Iowa, Department of Biology.

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