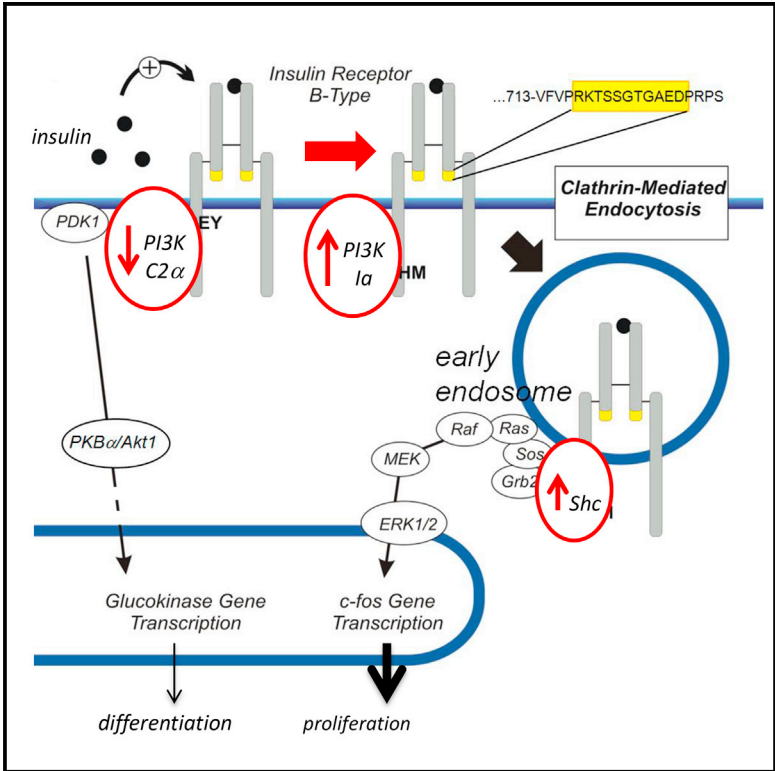# Cell Reports

PI3K-C2α Knockdown Results in Rerouting of Insulin Signaling and Pancreatic Beta Cell Proliferation

Graphical Abstract

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In Brief

Leibiger et al. provide evidence that reduced PI3K-C2α expression in pancreatic β cells results in rerouting from metabolic to mitogenic insulin signaling, allowing the β cell to switch from differentiation to proliferation.

Highlights

- Insulin-resistant pancreatic β cells have reduced PI3K-C2α levels

- Reduced PI3K-C2α levels result in rerouting of the insulin signal

- Reduced PI3K-C2α levels result in pancreatic β cell proliferation

- Insulin-resistant and insulin-sensitive signaling pathways coexist

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PI3K-C2α Knockdown Results in Rerouting of Insulin Signaling and Pancreatic Beta Cell Proliferation

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SUMMARY

Insulin resistance is a syndrome that affects multiple insulin target tissues, each having different biological functions regulated by insulin. A remaining question is to mechanistically explain how an insulin target cell/tissue can be insulin resistant in one biological function and insulin sensitive in another at the same time. Here, we provide evidence that in pancreatic β cells, knockdown of PI3K-C2α expression results in rerouting of the insulin signal from insulin receptor (IR)-B/PI3K-C2α/PKB-mediated metabolic signaling to IR-B/Shc/ERK-mediated mitogenic signaling, which allows the β cell to switch from a highly glucose-responsive, differentiated state to a proliferative state. Our data suggest the existence of IR-cascade-selective insulin resistance, which allows rerouting of the insulin signal within the same target cell. Hence, factors involved in the rerouting of the insulin signal represent tentative therapeutic targets in the treatment of insulin resistance.

INTRODUCTION

An unsolved paradox with regard to insulin receptor (IR) signaling is the coexistence of insulin resistance and insulin sensitivity in the same insulin target cell/tissue. With regard to the pancreatic β cell, it has been demonstrated that under conditions of insulin resistance provoked by a high-fat diet, insulin signaling in β cells is required for a compensatory increase in pancreatic β cell mass by proliferation (Okada et al., 2007). This observation raised the question of how the pancreatic β cell can switch from an insulin-dependent differentiated state that is glucose sensitive in terms of insulin secretion and biosynthesis to an insulin-dependent proliferative state under conditions of insulin resistance. Increasing pancreatic β cell mass is of vital importance in

response to relative insulin deficiency in physiology (e.g., pregnancy) or in the pre-state of type 2 diabetes mellitus (T2DM) (e.g., obesity) including insulin resistance. Several factors have been shown to stimulate β cell proliferation, including innervation by the autonomous nervous system (Imai et al., 2008) as well as humoral factors such as glucose, incretins (GLP-1, GIP), adipokines (adiponectin, leptin), growth hormones (GHs; prolactin, placental lactogen), and insulin (for review, see Leibiger et al., 2008; Liu et al., 2009; Kulkarni, 2005; Vasavada et al., 2006; Yesil and Lammert, 2008). Interestingly, among these factors, insulin has also been shown to contribute to the differentiated β cell phenotype by, for example, regulating expression and nuclear activity of transcription factors Pdx-1/Ipf-1, FoxO1, and FoxA2 (Leibiger et al., 2008).

We have recently shown that signaling via insulin receptor B (IR-B) and phosphatidylinositol 3-kinase (PI3K) class II member PI3K-C2α is required for glucose-stimulated insulin secretion (Leibiger et al., 2010a), thus keeping the β cell in a glucose-sensitive, differentiated state. PI3K-C2α has been shown to be involved in various steps of signal transduction and membrane trafficking (Campa et al., 2015). We now demonstrate an increase in proliferation of primary rodent and human β cells when knocking down PI3K-C2α. This was unexpected, because knockdown of PI3K-C2α has been shown to increase cell death in other cell systems (Elis et al., 2008; Kang et al., 2005; Ng et al., 2009). Our published data demonstrate that different insulin signaling cascades coexist in β cells, thus allowing different selective biological effects (Leibiger et al., 2008, 2010b). In this context, our new data suggest the existence of IR-cascade-selective insulin resistance, which allows rerouting of the insulin signal in the same cell. We propose that insulin resistance in one signaling cascade, here via IR-B/PI3K-C2α, leads to a partial loss of the differentiated, glucose-responsive state of the β cell but at the same time promotes β cell proliferation by a different signaling cascade. Thus, PI3K-C2α represents a key regulatory factor that allows the β cell to turn from differentiation (PI3K-C2α available) to proliferation (PI3K-C2α unavailable) in response to insulin.
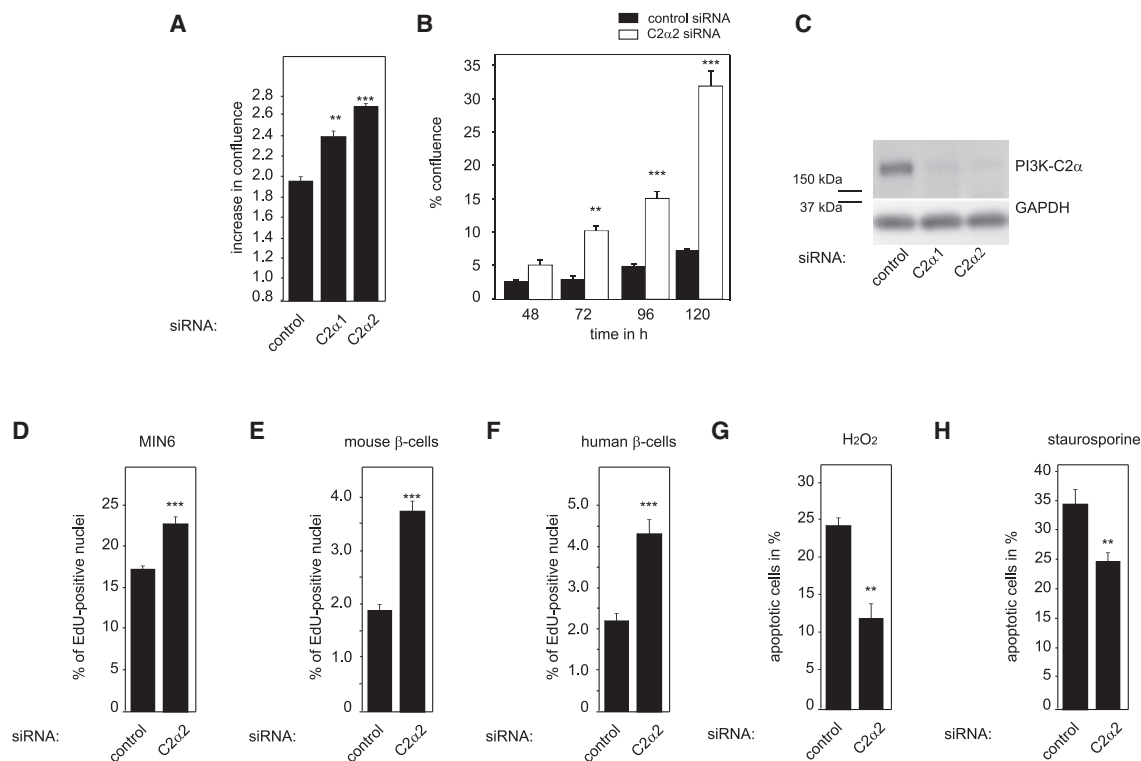
Figure 1. Knockdown of PI3K-C2α Leads to an Increase in Pancreatic β Cell Proliferation

(A and B) Knockdown of PI3K-C2α in MIN6m9 cells leads to an increase in cell confluence. Increase in cell confluence 96 hr versus 48 hr after start of transfection (A) and over time (B).

(C) Representative western blot demonstrating knockdown of PI3K-C2α with two different siRNAs.

(D–F) Knockdown of PI3K-C2α leads to increased proliferation of MIN6m9 cells (D), primary mouse β cells (E), and primary human β cells (F) measured by EdU incorporation.

(G and H) Knockdown of PI3K-C2α leads to protection against apoptosis induced by H_2O_2 (G) and staurosporine (H).

All data are expressed as mean ± SEM (n = 3). **p < 0.01; ***p < 0.001. See also Figures S1 and S2.

RESULTS

Knockdown of PI3K-C2α Leads to an Increase in Pancreatic β Cell Proliferation

Our previous study showed that insulin signaling via IR-B and PI3K-C2α keeps the pancreatic β cell in a differentiated, glucose-responsive state by, among other things, regulating the expression of the β cell glucose sensor glucokinase (Leibiger et al., 2010a). To our surprise, small interfering RNA (siRNA)-mediated knockdown of PI3K-C2α expression led to a significant increase in the confluence of insulin producing MIN6 cells by two independent siRNAs against PI3K-C2α already 96 hr after transfection with the siRNA. This effect was even more pronounced 120 hr after start of transfection (Figures 1A–1C). To test whether this increase in cell confluence was due to increased cell proliferation, we studied the incorporation of labeled DNA precursors by 5-ethynyl-2′-deoxyuridine (EdU) treatment. Four days after transfection with siRNA against PI3K-C2α, MIN6 cells were treated with EdU for 1 hr, while mouse and human pancreatic islet cells were treated with EdU for 24 hr. Primary β cells were identified by immunostaining against insulin C-peptide. Knockdown of PI3K-C2α led to a 1.5-fold increase in proliferation in MIN6 cells and to a more

than 2-fold increase in proliferation in primary β cells (Figures 1D–1F and S1). Because knockdown of PI3K-C2α has been shown to lead to increased cell death in other cell systems (Elis et al., 2008; Kang et al., 2005; Ng et al., 2009) and the now observed increase in cell proliferation could reflect a compensatory response to β cell apoptosis, we next analyzed the effect of PI3K-C2α knockdown on stimulus-induced apoptosis in MIN6 cells. Four days after start of transfection with siRNA against PI3K-C2α, MIN6 cells were treated with either 20 μM H_2O_2 or 6 μM staurosporine for 16 hr. The cell apoptosis rate was determined by triple staining with Hoechst 33342, propidium iodide, and Alexa 488/annexin V, where Alexa 488/annexin V-positive/propidium-iodide-negative stained cells were considered apoptotic. As shown in Figures 1G, 1H, and S2, knockdown of PI3K-C2α expression led to protection against stimulus-induced apoptosis rather than an increase in the apoptosis rate.

Knockdown of PI3K-C2α Leads to Decreased Metabolic and Increased Mitogenic Insulin Signaling

We have previously shown that knockdown of PI3K-C2α in pancreatic β cells abolishes insulin-stimulated activation of PKBα, which affected the phosphorylation of PKB substrate

TBC1D4, a Rab GTPase-activating protein (Leibiger et al., 2010a). It is noteworthy that knockdown of PI3K-C2α did not affect insulin-stimulated activation of PKBβ or of PKBγ in β cells. Consequently, we wanted to know whether knockdown of PI3K-C2α would affect other PKB substrates that potentially could be involved in β cell proliferation, such as FoxO1 and TSC2. As shown in Figure S3A, knockdown of PI3K-C2α led to an increase in FoxO1 activity as indicated by its decrease in phosphorylation. This, together with the observed decrease in phosphorylation of TSC2 (Figure S3B), is not in line with an increase in β cell proliferation (Bartolomé et al., 2010; Okamoto et al., 2006). Because knockdown of PI3K-C2α leads to a decrease in the expression of glucokinase (GK) and GK has been suggested to play a role in β cell proliferation (Terauchi et al., 2007), we next analyzed whether knockdown of GK affects β cell proliferation and survival. siRNA-mediated knockdown of GK protein levels to 35% in MIN6 cells did not affect cell confluence or stimulus-induced apoptosis in these cells (Figures S3C–S3E). These data, together with our earlier published data (Leibiger et al., 2010a), demonstrate that PI3K-C2α knockdown results in decreased insulin signaling via the metabolic branch of signal transduction. To test whether the knockdown leads to an increase in mitogenic signaling, we analyzed the effect of PI3K-C2α knockdown in MIN6 cells on the activity of ERK1/2 as well as on the expression of the proto-oncogene c-fos. As shown in Figures 2A–2C, the reduced expression of PI3K-C2α led to a 1.3-fold increase in ERK1/2 phosphorylation as well as an increase in c-fos promoter activity and c-fos protein expression.

The Proliferative Effect of PI3K-C2α Knockdown Is IR-B Dependent

The pancreatic β cell expresses both isoforms of the insulin receptor, i.e., IR-A and IR-B (Leibiger et al., 2001), and both isoforms signal simultaneously by utilizing different signaling cascades (Leibiger et al., 2001; Uhles et al., 2003, 2007). Because mitogenic insulin signaling has been shown to be more often associated with IR-A (reviewed in Belfiore et al., 2009), we wanted to know whether PI3K-C2α knockdown results in a switch from metabolic signaling via IR-B to mitogenic signaling involving IR-A. To study which IR isoform contributes to β cell proliferation, we first made use of antibodies that block signaling via IR. A pan-antibody that abolishes signaling through both IR isoforms suppressed the proliferative effect of PI3K-C2α knockdown in both mouse and human β cells (Figures 2D and 2E). Interestingly, employing an IR-B-selective blocking antibody also abolished β cell proliferation (Figures 2F and 2G). Because there is no IR-A-selective blocking antibody available, we used aptamers that selectively block signaling via each IR isoform. In Figure S3F, we demonstrate that aptamer-A abolishes insulin-induced activation (i.e., auto-phosphorylation) of IR-A, but not IR-B, and vice versa that aptamer-B abolishes insulin-induced activation of IR-B, but not IR-A. Employing these aptamers on cells that were treated with either control siRNA or siRNA against PI3K-C2α, we observed that blocking insulin signaling via IR-A did not abolish the proliferative effect of PI3K-C2α knockdown, while blocking signaling via IR-B did (Figure 2H). This suggested that PI3K-C2α knockdown does not lead to a switch from IR-B- to IR-A-mediated signaling but results in a

rerouting from metabolic IR-B signaling to mitogenic IR-B signaling. To test this, we employed reporter gene constructs that report on the activity of these two pathways. While β cell GK promoter-driven GFP (βGK.GFP) expression serves as a readout for IR-B-mediated metabolic signaling via IR-B/PI3K-C2α/PKBα (Leibiger et al., 2010a), c-fos promoter-driven DsRed (cfos.DsRed) expression serves as a readout for IR-B-mediated mitogenic signaling via IR-B/Shc/ERK (Uhles et al., 2007). To ensure expression of both reporters in the same cell, we transfected cells with plasmids that contain both expression cassettes. We co-transfected cells with the reporter plasmid and either control siRNA or C2α2-siRNA and combined this with or without insulin stimulation. Indeed, knockdown of PI3K-C2α led to a more pronounced activation of the mitogenic signaling cascade, resulting in increased c-fos promoter-activated DsRed expression, while the metabolic signaling leading to GK promoter-driven GFP expression was suppressed (Figure 2I).

Knockdown of PI3K-C2α Leads to Increased IR-B/Shc Interaction

To mechanistically explain how the knockdown of PI3K-C2α expression results in pronounced IR-B-mediated mitogenic signaling, we studied the association of IR-B with the adaptor protein Shc in two independent ways. First, we studied this association in cells overexpressing myc-tagged IR-B in co-immunoprecipitates using anti-myc antibodies. PI3K-C2α knockdown led to a 1.8-fold higher association of IR-B with Shc compared to control (Figures 3A and 3B). Second, we studied the interaction between IR-B and Shc by fluorescence resonance energy transfer (FRET). MIN6 cells were co-tranfected with pIR-B-mVenus, pShc-mCFP, and either control siRNA or C2α2-siRNA. PI3K-C2α knockdown led to a 2.5-fold higher FRET efficiency between IR-B and Shc compared to control siRNA-transfected cells (Figure 3C). Next, we wanted to know whether this shift in interaction between IR-B with PI3K-C2α and IR-B with Shc also occurs under conditions that mimic a diabetic situation, e.g., prolonged hyperglycemia. Therefore we incubated cells co-expressing either IR-B-mVenus with PI3K-C2α-mCerulean or IR-B-mVenus with Shc-mCFP at different glucose concentrations and measured FRET efficiency between IR-B and either PI3K-C2α or Shc. As shown in Figure 3D, an increase in glucose concentration resulted in a higher association of IR-B with Shc. The same culture conditions also led to an increase in cell proliferation (Figure 3E).

To establish the relevance of our rerouting model in vivo, we chose the leptin-deficient ob/ob mouse. Three-month-old ob/ob mice are hyperinsulinemic and hyperglycemic and have an increased β cell mass due to β cell proliferation, and in addition to being overall insulin resistant, they show insulin resistance at the islet/β cell level in the metabolic branch of insulin signaling (Åvall et al., 2015; Ilegems et al., 2013; Lindström, 2007). Western blot analysis of islet lysates show a decreased expression of GK and an increased expression of c-fos in ob/ob islets compared to islets from control mice (Figures 4A–4C), thus being in agreement with a rerouting of insulin signaling. An increased mitogenic signaling was further supported by a higher ERK1/2 activity in ob/ob islets compared to controls (Figures 4D and 4E). Both western blot analysis and immunohistochemistry
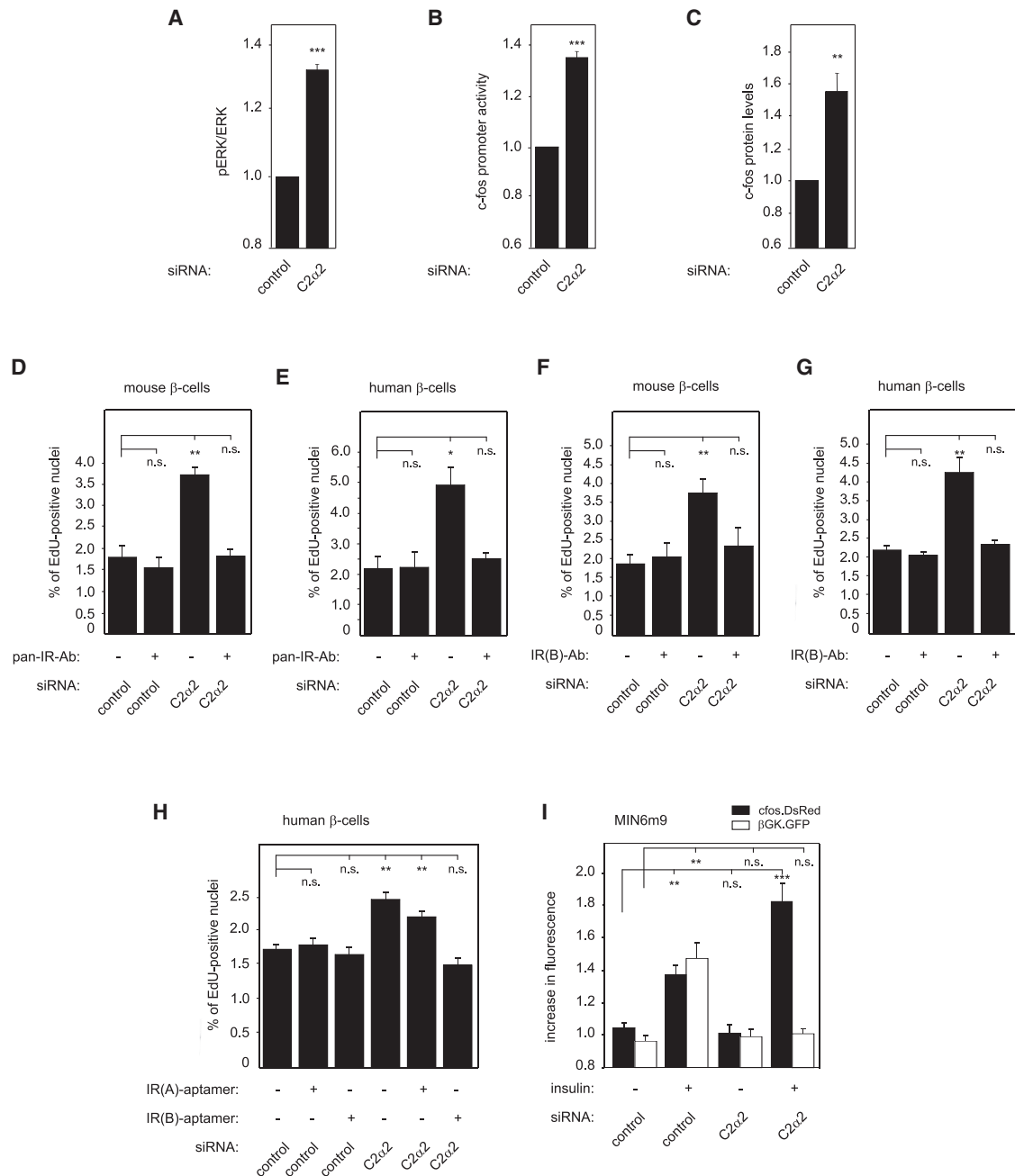
Figure 2. Knockdown of PI3K-C2α Leads to Decreased Metabolic and Increased Mitogenic Insulin Signaling, and the Proliferative Effect of PI3K-C2α Knockdown Is IR-B Dependent

(A) Knockdown of PI3K-C2α leads to increased ERK1/2 phosphorylation at Thr202 and Tyr204 evaluated by western blotting.

(B) Knockdown of PI3K-C2α leads to increased c-*fos* promoter activity evaluated by luciferase assay.

(C) Knockdown of PI3K-C2α leads to increased c-*fos* protein levels evaluated by western blot.

(D and E) The proliferative effect of PI3K-C2α knockdown is insulin/IR dependent and can be blocked by antibodies that block signaling via both IRs. Primary mouse β cells (D) and primary human β cells (E) were treated with antibodies during EdU incorporation.

(F and G) The proliferative effect of PI3K-C2α knockdown is IR-B dependent. Primary mouse β cells (F) and primary human β cells (G) were treated with antibodies blocking signaling through IR-B during EdU incorporation.

(H) The proliferative effect of PI3K-C2α knockdown can be blocked by aptamers blocking signaling via IR-B. Primary human β cells were treated with blocking aptamers during EdU incorporation.

(I) Knockdown of PI3K-C2α results in a more pronounced activation of the mitogenic signaling cascade leading to c-*fos* promoter-activated DsRed expression (cfos.DsRed, solid bars), while metabolic signaling leading to glucokinase promoter-driven GFP expression (βGK.GFP, open bars) is diminished.

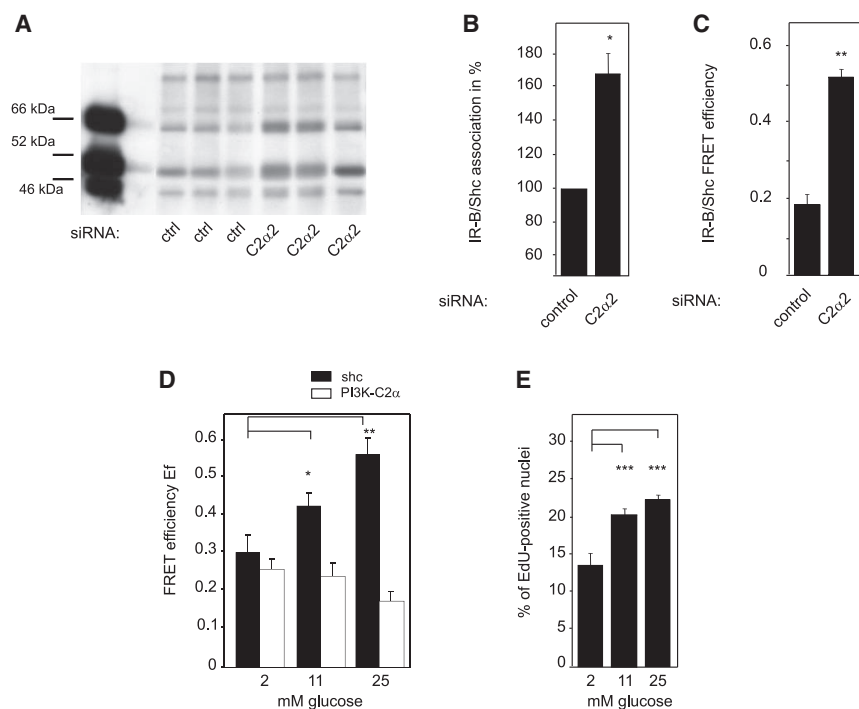All data are expressed as mean ± SEM (n = 3). *p < 0.05; **p < 0.01; ***p < 0.001. See also Figure S3.

Figure 3. Knockdown of PI3K-C2α Leads to Increased IR-B/Shc Interaction

(A and B) Knockdown of PI3K-C2α leads to increased interaction between overexpressed myc-tagged IR-B and endogenous Shc. The interaction was evaluated by western blot after immunoprecipitation with myc-antibodies (A). Data from three individual experiments are shown. The left-most lane shows Shc bands from a MIN6 cell lysate. (B) Quantification of IR-B/Shc interaction from (A). Data are expressed as mean ± SEM (n = 3).

(C and D) Knockdown of PI3K-C2α (C) or hyperglycemic culture conditions (D) lead to increased interaction between IR-B and Shc in MIN6m9 cells analyzed by fluorescence resonance energy transfer (FRET). Data are expressed as mean ± SEM (n = 10).

(E) Hyperglycemic culture conditions of MIN6m9 cells lead to an increase in cell proliferation. Data are expressed as mean ± SEM (n = 24).

$*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

revealed decreased expression levels of PI3K-C2α in ob/ob islet/β cells compared to controls (Figures 4D, 4F, and S4). Moreover, western blot analysis showed an increase in Shc expression in ob/ob islets (Figures 4D and 4G). It is noteworthy, that proliferating β cells (cells positive for Ki67 and insulin) in ob/ob islets showed even lower levels of PI3K-C2α (Figure S4). Finally, we analyzed the association of PI3K-C2α, class Ia PI3K adaptor protein p85 and Shc with insulin receptors in ob/ob and control islet lysates. Although we failed to detect PI3K-C2α in IR co-immunoprecipitates, we observed an increased association of IR with p85 and Shc in ob/ob islets compared to controls (Figures 4H–4J).

DISCUSSION

A remaining question in the insulin-signaling field is to mechanistically explain how an insulin target cell/tissue can be insulin resistant in one biological function and insulin sensitive in another at the same time. A classical example is the paradox of selective insulin resistance in the liver resulting in insulin's inability to suppress glucose output while at the same time showing increased insulin-stimulated lipogenesis (discussed in Brown and Goldstein, 2008). Similar phenotypes have been observed for other cell/tissue systems (Book and Dunaif, 1999; Jiang et al., 1999; Li et al., 2010; Pandolfi et al., 2005), including the pancreatic β cell. With regard to the latter, data by Kulkarni's group demonstrated that under conditions of insulin resistance provoked by a high-fat diet, β cell IR-mediated signaling is required for compensatory increase in pancreatic β cell mass by β cell proliferation (Okada et al., 2007). This observation raised the question of how the pancreatic β cell can switch from an insulin-dependent differentiated state to an insulin-dependent

proliferative state under conditions of insulin resistance. Although it is well documented that insulin can activate two different branches in signal transduction, namely the metabolic branch involving PKB and PKC isoforms and the mitogenic branch involving the MAP kinase cascade, it remains unclear whether and mechanistically how the same insulin target cell can switch between these two modes. Here, we provide evidence that creating insulin resistance in IR-B/PI3K-C2α/PKBα-mediated metabolic signaling, a pathway that maintains the β cell glucose-responsive state, leads to rerouting of the insulin signal to IR-B/Shc/ERK-mediated mitogenic signaling, resulting in increased c-fos expression and pancreatic β cell proliferation. This was unexpected, because mitogenic insulin signaling has been mostly associated with the IR-A isoform (Belfiore et al., 2009). Moreover, data obtained in engineered β cell lines expressing only one or the other IR isoform (Rec A and Rec B) showed a higher proliferative activity for the IR-A-expressing cells (Bartolomé et al., 2010). Data on insulin-dependent β cell proliferation from different laboratories demonstrate the involvement of signaling components such as IRS2, PKB, TSC2, p70s6k, and FoxO1 (Bartolomé et al., 2010; Bernal-Mizrachi et al., 2001; Cantley et al., 2007; Fatrai et al., 2006; Hashimoto et al., 2006; Kubota et al., 2004). It is noteworthy that knockdown of PI3K-C2α still allows insulin-stimulated action of PKBβ and PKBγ and basal activity of PKBα. Within this scenario, we observed an increased activity of FoxO1, which is in disagreement with increased proliferative activity. Under these conditions, knockdown of PI3K-C2α leads to a decrease in TSC2 activity, which makes the involvement of the downstream components mTORC1/p70s6k in β cell proliferation unlikely.

While the siRNA-mediated knockdown of PI3K-C2α expression serves as an example for rerouting of β cell IR-mediated signaling, this model bears relevance to T2DM. Data provided by Dominguez and co-authors (Dominguez et al., 2011) show a decreased pancreatic islet expression/availability of PI3K-C2α
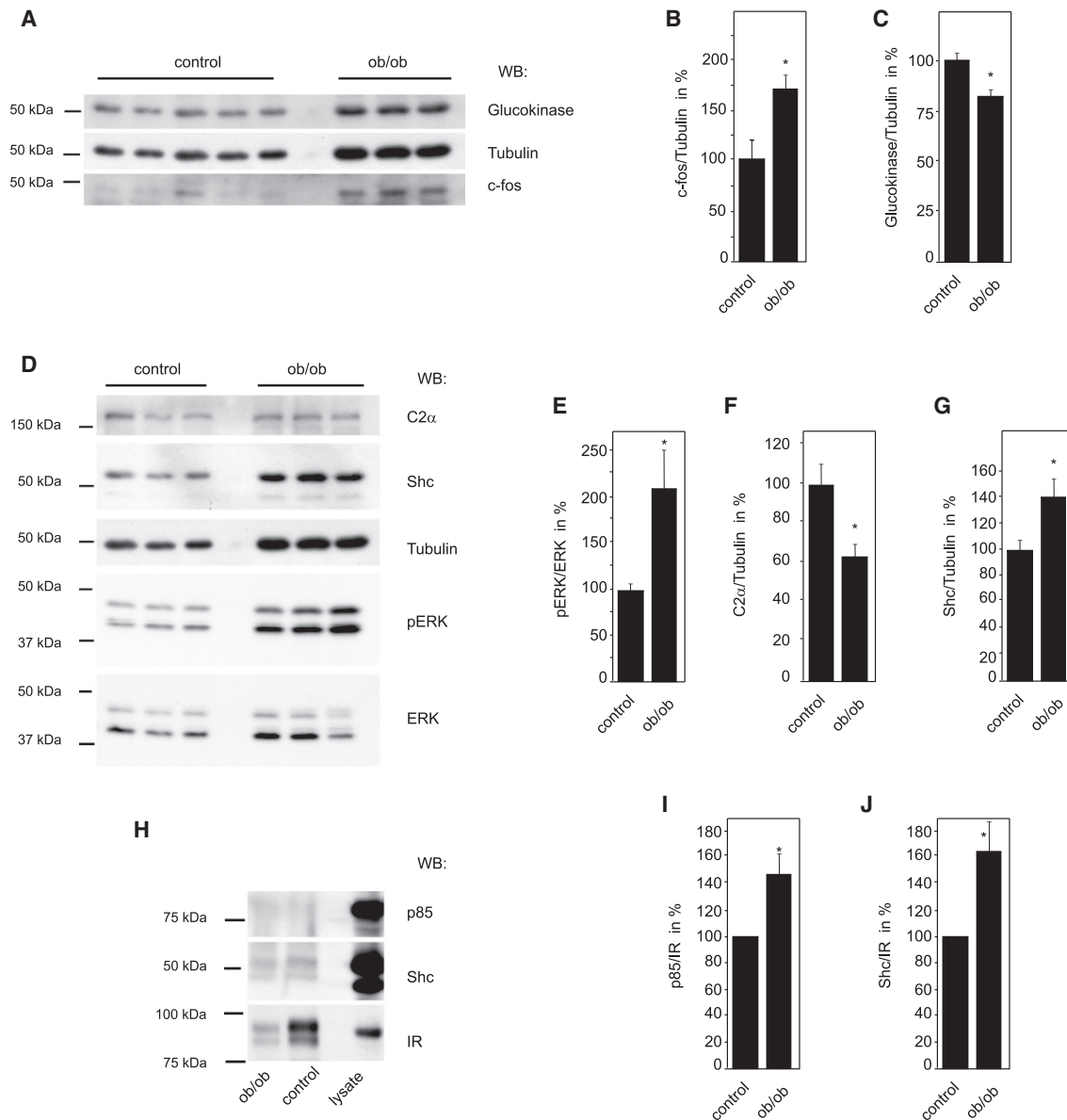
Figure 4. Decreased PI3K-C2α and Increased Shc Protein Levels in ob/ob Mice
(A–C) GK protein levels are decreased and c-*fos* protein levels are increased in islets of ob/ob mice compared to age-matched control mice. (A) Western blot. (B and C) Quantification of c-*fos* (B) and GK (C) protein levels normalized to α-tubulin levels.
(D–G) PI3K-C2α protein levels are decreased and Shc protein levels and ERK1/2 phosphorylation levels are increased in ob/ob islets compared to controls. (D) Western blot. (E) Quantification of ERK1/2 phosphorylation levels. (F and G) Quantification of PI3K-C2α (F) and Shc (G) protein levels normalized to α-tubulin levels.
(A–G) Data are expressed as mean ± SEM (n = 3–5). *$p < 0.05$.
(H–J) Increased association of IR with p85 and Shc in co-immunoprecipitation analysis from ob/ob islet lysates. (H) Western blot. (I and J) Quantification of p85 (I) and Shc (J) protein levels in IR immunoprecipitates. Data are expressed as mean ± SEM (n = 2). *$p < 0.05$.
See also Figure S4.

in patients with T2DM. This is in agreement with our data, mimicking T2DM-like conditions in terms of hyperglycemia, demonstrating a shift from an IR-B/PI3K-C2α-mediated metabolic signaling to an IR-B/Shc-mediated mitogenic signaling cascade leading to increased β cell proliferation. Furthermore, pancreatic islets obtained from 3-month-old ob/ob mice, which are insulin resistant in the metabolic branch of insulin signaling, demonstrate a decrease in GK expression (reflecting a decreased metabolic signaling), an increase in c-*fos* expression and ERK activity (reflecting an increased mitogenic signaling), a decreased expression of PI3K-C2α, an increased expression of Shc and an increased association of insulin receptors with PI3K

class Ia adaptor protein p85 as well as with Shc, all data being in agreement with the above proposed model.

Our data thus provide evidence that insulin resistance within one signaling cascade and increased insulin responsiveness within another can indeed coexist within the same insulin target tissue/cell and give support to the concept of signaling-cascade-selective insulin resistance. Hence, factors involved in the rerouting of the insulin signal represent tentative therapeutic targets in the treatment of insulin resistance.

EXPERIMENTAL PROCEDURES

Aptamers and siRNA-Mediated Knockdown
DNA-based aptamers that selectively recognize and block insulin receptors, i.e., IR-A-aptamer IR-A55 and IR-B-aptamer IRB-N11, were synthesized and screened at POSTECH. siRNAs against mouse PI3K-C2α (Pik3c2a: siRNA1ID 68525, siRNA2ID 68710), siRNA against human PI3K-C2α (PIK3C2A: s10508), and validated non-targeting negative control (AM4613) were purchased from Ambion (Applied Biosystems/Ambion). siRNA against mouse glucokinase (SI01010359) was purchased from QIAGEN.

EdU Incorporation
Cell proliferation measurements were performed utilizing the Click-iT EdU Alexa 488 HCS Assay kit (Invitrogen) following the manufacturer's instructions.

Western Blotting
The following primary antibodies were used: rabbit monoclonal phospho-tuberin/TSC2 (Thr1462), rabbit polyclonal tuberin/TSC2, rabbit polyclonal phospho-FoxO1 (Ser256), rabbit monoclonal FoxO1, rabbit monoclonal phospho-p44/42 MAPK (Erk1/2 Thr202/Tyr204), rabbit polyclonal p44/42 MAPK (Erk1/2), rabbit polyclonal c-fos, mouse monoclonal and rabbit monoclonal insulin receptor β, rabbit monoclonal PI3K class II α, rabbit monoclonal Shc, and rabbit monoclonal PI3K p85 (Cell Signaling Technology); rabbit polyclonal glucokinase and mouse monoclonal GAPDH (Abcam); rabbit polyclonal p-insulin Rβ (Tyr 1162/1163) (Santa Cruz Biotechnology); mouse monoclonal Shc (BD Transduction Laboratories); and mouse monoclonal α-tubulin (Sigma).

Immunoprecipitation
Immunoprecipitation was performed using protein A/G Plus agarose (Santa Cruz Biotechnology) and the following antibodies: mouse monoclonal antibody against c-myc (Santa Cruz Biotechnology), mouse monoclonal IR β antibody, and rabbit monoclonal IR β antibodies (both from Cell Signaling Technology).

FRET Analysis by Acceptor Photobleaching
FRET analysis by acceptor photobleaching was performed using a Leica TCS-SP2 confocal microscope as described in Leibiger et al. (2010a).

All mouse and human islet experiments were approved by the regional ethical review boards in Uppsala and in Stockholm (Sweden). Additional experimental procedures are described in Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.08.058.

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