SAD-A kinase controls islet β-cell size and function as a mediator of mTORC1 signaling

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The mammalian target of rapamycin (mTOR) plays an important role in controlling islet β-cell function. However, the underlying molecular mechanisms remain poorly elucidated. Synapses of amphids defective kinase-A (SAD-A) is a 5′ adenosine monophosphate-activated protein kinase-related protein kinase that is exclusively expressed in pancreas and brain. In this study, we investigated a role of the kinase in regulating pancreatic β-cell morphology and function as a mediator of mTOR complex 1 (mTORC1) signaling. We show that global SAD-A deletion leads to defective glucose-stimulated insulin secretion and petite islets, which are reminiscent of the defects in mice with global deletion of ribosomal protein S6 kinase 1, a downstream target of mTORC1. Consistent with these findings, selective deletion of SAD-A in pancreas decreased islet β-cell size, whereas SAD-A overexpression significantly increased the size of mouse insulinomas cell lines β-cells. In direct support of SAD-A as a unique mediator of mTORC1 signaling in islet β-cells, we demonstrate that glucose dramatically stimulated SAD-A protein translation in isolated mouse islets, which was potently inhibited by rapamycin, an inhibitor of mTORC1. Moreover, the 5′-untranslated region of SAD-A mRNA is highly structured and requires mTORC1 signaling for its translation initiation. Together, these findings identified SAD-A as a unique pancreas-specific effector protein of mTORC1 signaling.

GLP1 | incretin | AMPK | LKB1

The AMPK (5′ adenosine monophosphate-activated protein kinase)-related family of kinases, which consists of 12 members, plays an important role in regulating glucose and energy homeostasis. AMPK is a key regulator of energy homeostasis and is activated in response to an increase in AMP/ATP ratio under low nutrient conditions (1). These functions are partly mediated by AMPK's regulatory role in nutrient sensing in hypothalamic neurons. However, it remains questionable whether AMPK regulates glucose sensing by pancreatic β-cells (2). Other members of the AMPK family are also implicated in energy homeostasis, including MARK kinases (MAP/microtubule affinity-regulating kinases) (3). In addition, targeted deletion of liver kinase B1 (LKB1), a master upstream kinase of AMPK and 11 other members of the AMPK-related kinase family (4), leads to increased pancreatic β-cell mass and insulin secretion (5, 6). The phenotype directly contradicts that of the AMPK knockout (KO) mice (7), indicating that other members in the AMPK-related family of kinases may also regulate islet function. Furthermore, AMPK signaling is intrinsically linked to the mammalian target of rapamycin (mTOR) pathways to coordinate nutritional status with protein synthesis in pancreatic β-cells (2). Thus, targeted deletion of LKB1 in mice leads to β-cell hypertrophy and mTOR activation (6, 7).

mTOR is an evolutionarily conserved serine/threonine kinase that functions in two complexes, mTORC1 and mTORC2. The mTORC1 complex functions as a sensor of nutritional status and responds by altering metabolic processes, whereas the mTORC2 complex is involved in the regulation of cytoskeletal organization (1, 8). Multiple lines of evidence suggest a critical role of mTORC1 in regulating pancreatic β-cell mass and function (9–11). Accordingly, targeted deletion of tuberous sclerosis 1 (TSC1) or TSC2, repressors of mTORC1, significantly increases islet β-cell mass and glucose-stimulated insulin secretion (GSIS) (9–11). Conversely, targeted deletion of S6K1, an effector of mTORC1 signaling, or ablation of S6K1 phosphorylation site in ribosomal protein S6 leads to hypoinsulinemia, defective GSIS, and reduction in islet β-cell size (12, 13). Furthermore, inhibition of mTORC1 with rapamycin also causes reduction in islet mass and insulin content, leading to the exacerbation of type 2 diabetes (14, 15). However, the downstream effector proteins that mediate the mTORC1 effects in pancreatic β-cells remain elusive.

Synapses of amphids defective kinase-A (SAD-A), also referred to as BR serine/threonine kinase 2 (BRSK2), is a member of the AMPK-related family of kinases that is most closely related to AMPK (4). SAD-A and its highly conserved SAD-B isoform were recently shown to regulate neuronal polarity and axon specification in the developing nervous system (16, 17). SAD-B, which is localized at synaptic vesicles, regulates neurotransmitter release, possibly through phosphorylation of regulating synaptic membrane exocytosis 1 (RIM1) (18). SAD-B kinase is also required for cell division by controlling centromere duplication via phosphorylation of γ-tubulin (19). In contrast, little is known about the function of SAD-A in pancreas, although SAD kinases are activated by stimuli that evoke GSIS, including activation by PKA (Protein Kinase A)- and CamKK1 (Calcium/calmodulin-dependent protein Kinase Kinase 1)-mediated signaling pathways (20, 21). In this study, we investigated the role of SAD-A in regulating islet β-cell function by gain and loss of SAD-A functional studies, using mice with targeted deletion of SAD-A and β-cell lines stably overexpressing the kinase. We identified a critical role of SAD-A in regulating multiple β-cell functions as a downstream target of mTORC1 signaling.

Results

Targeted Deletion of SAD-A Impaired GSIS in Vivo and in Vitro. SAD-A, a kinase under the control of LKB1, is exclusively expressed in pancreas and brain, but the physiological function of the kinase in pancreas has not been studied. Using mice with global deletion of SAD-A (16), we examined the effect of SAD-A deficiency on GSIS in vivo and in vitro from isolated islets. Although the SAD-A KO mice developed normally without gross abnormality, they exhibited growth retardation (Fig. S1). On further examination, we found that SAD-A deficiency caused hypoinsulinemia, as evidenced by a significantly lower serum insulin level after an overnight fast (Fig. 1*A*). The hypoinsulinemia was primarily caused by a defective GSIS, which is supported by significantly lower serum insulin levels at 30 and 60 min after glucose load in SAD-A KO mice during a glucose tolerance test (Fig. 1*A*). Consistent with results from the

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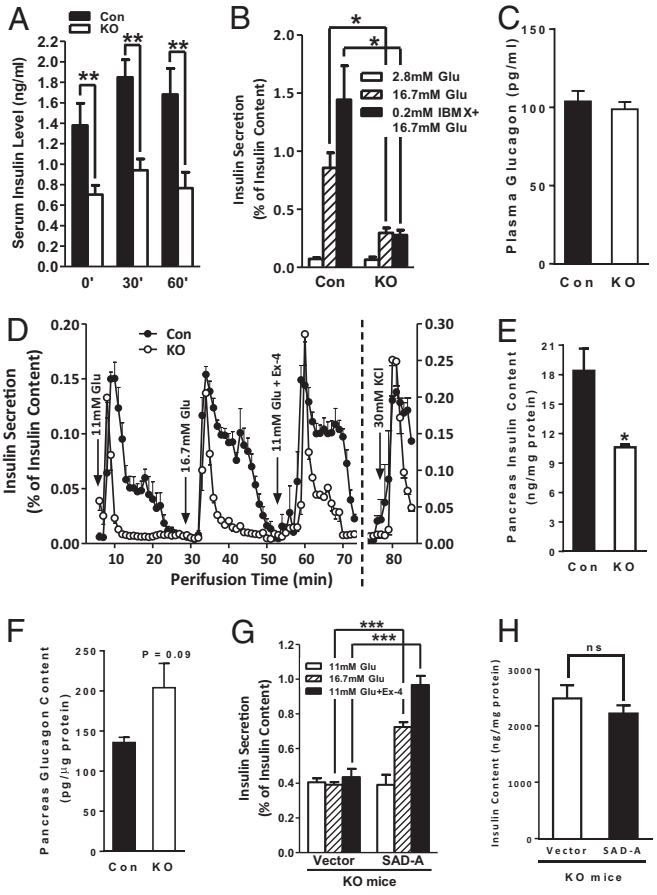

Fig. 1. SAD-A deficiency impaired GSIS in vivo and in vitro. (*A*) Male SAD-A knock out (KO) mice and WT controls (Con) ($n = 10$) at 2 mo old were fasted overnight and orally gavaged with glucose at 2.5 g/kg body weight, followed by measurement of serum insulin levels at the indicated times. (*B*) Isolated islets from SAD-A KO mice and WT controls were analyzed for insulin secretion by static incubation in response to stimulation with 16.7 mM glucose or glucose plus 0.2 mM 3-isobutyl-1methylxanthine (IBMX), a phosphodiesterase inhibitor that potentiates GSIS by raising cAMP level ($n = 5$). (*C*) Plasma glucagon levels were analyzed from SAD-A KO mice and WT controls by RIA ($n = 10$). (*D*) Analysis of insulin secretion from perifused islets isolated from SAD-A mice and WT controls in response to stimulation with 11 mM glucose (Glu), 16.7 mM Glu, 11 mM Glu plus 1 nM Exendin-4 (Ex-4), and 30 mM KCl, respectively ($n = 5$). (*E* and *F*) Analysis of pancreatic insulin and glucagon content from SAD-A KO mice and WT controls ($n = 10$). (*G*) isolated islets from SAD-A KO mice were infected with the recombinant adenoviruses overexpressing SAD-A or an empty vector (negative control), followed by analysis of insulin secretion in response to treatment with indicated secretagogues, after 48 h of infection ($n = 5$). (*H*) Analysis of insulin content in isolated islets used in *G*. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared with controls.

in vivo studies, insulin secretion was also impaired in isolated mouse islets from SAD-A KO mice in response to stimulation with glucose or glucose plus 3-isobutyl-1-methylxanthine, a phosphodiesterase inhibitor that raises cAMP levels and potentiates GSIS (Fig. 1*B*). In contrast, SAD-A deficiency did not affect circulating glucagon levels (Fig. 1*C*).

Using islet perifusion analysis, we next examined the effect of SAD-A deficiency on biphasic insulin secretion from isolated islets in response to stimulation with various insulin secretagogues, including glucose, KCl, and exendin-4 (Ex-4), a long-acting therapeutic glucagon-like peptide 1 (GLP-1) analog that potentiates GSIS by activating the exchange protein directly activated by cAMP 2 (Epac2) and PKA signaling pathways (22). KCl was used here to identify a role of SAD-A in regulating stimulus–secretion coupling by bypassing glucose metabolism. In further support of the results from the in vivo studies, SAD-A deficiency dramatically impaired the second phase of GSIS (Fig. 1*D*). In addition, SAD-A deficiency abolished the potentiating effect of Ex-4 on GSIS. In contrast, SAD-A depletion did not affect first-phase insulin secretion or the effect of KCl on insulin exocytosis after being normalized with insulin content. The results are consistent with our recent report that SAD-A promotes GSIS through activation of the p21-associated kinase (23), which was reported to primarily regulate second-phase insulin (24). A global SAD-A deficiency also decreased pancreatic insulin content (Fig. 1*E*) without significantly affecting pancreatic glucagon content (Fig. 1*F*).

Overexpression of SAD-A Partially Restored GSIS in Isolated Mouse Islets from SAD-A KO Mice. In addition to impaired GSIS, global SAD-A deletion also decreased islet mass (see following), raising the question of whether or not the impaired GSIS in SAD-A KO mice was primarily caused by a developmental defect of the islets. Using recombinant adenoviruses overexpressing the human SAD-A protein, we next examined whether SAD-A overexpression would rescue GSIS in isolated islets from SAD-A KO mice. As shown in Fig. 1*G*, adenovirus-mediated overexpression of SAD-A partially restored the glucose responsiveness of isolated islets from SAD-A KO mice compared with vector control. The restoration in GSIS was unlikely to be caused by increased insulin content, as SAD-A overexpression did not increase insulin biogenesis in isolated islets from SAD-A KO mice (Fig. 1*H*), further confirming a role of SAD-A in promoting GSIS.

SAD-A Depletion Led to a Reduction in Islet Size and β-Cell Mass. To identify mechanisms underlying the observed defects in GSIS, we next carried out systemic biochemical, morphometric, and immunohistological analysis of pancreatic islets from SAD-A KO mice and the WT controls. As shown in Fig. 2*A*, SAD-A deficiency significantly reduced islet size in SAD-A KO mice. The reduction was likely caused by a loss of β-cell mass, as suggested by decreased β-cell number (Fig. 2*B*) and insulin content (Fig. 1*E*) in SAD-A KO mice. In contrast, SAD-A deficiency did not significantly affect pancreatic islet density (Fig. 2*C*), implicating an islet β-cell–specific defect.

A key role of SAD-A in regulating islet size was further confirmed by results from H&E staining of pancreatic samples and from immunohistochemical analysis of β-cells and α-cells. In contrast to islets from WT control mice (Fig. 2 *D* and *F*), the islets from SAD-A KO mice exhibited smaller size and a higher proportion of glucagon-positive α-cells (Fig. 2 *E* and *G*). In addition, in comparison with peri-islet distribution of pancreatic α-cells in WT control mice (Fig. 2*F*), SAD-A-depleted islets exhibited scattered distribution of α-cells (Fig. 2*G*), suggesting a potential role of SAD-A in regulating islet–β-cell biogenesis or survival.

Targeted Deletion of SAD-A in Pancreas Decreased β-Cell Size and Islet Mass. In addition to pancreas, the SAD-A gene is also expressed in brain, raising the question of whether the decreased islet size in KO mice is caused by a feedback response from the brain. To clarify the issue, we next determined a role of SAD-A in regulating islet morphology, using mice with selective deletion of SAD-A in pancreas (pSADKO). The pSADKO mice were generated by crossing a newly generated mouse line with floxed *SAD-A* allele ($SAD^{loxP/loxP}$) with Pdx-Cre transgenic mice (21). Consistent with findings in global SAD-A KO mice, SAD-A deficiency in pancreas significantly decreased islet size and mass, as evidenced by results from islet size distribution analysis (Fig. 3*A*, quantified in Fig. 3*B*). The decreased islet mass in pSADKO mice was primarily caused by decreased β-cell size (Fig. 3*C*), which is supported by normal pancreatic α-cell size, β-cell number, and islet insulin content (Fig. 3 *D*–*F*). The petite islet phenotype was further confirmed by results from H&E staining (Fig. 3*G*) and by immunohistochemical analysis of islet β-cells and α-cells (Fig. 3*H*), further supporting a key role of SAD-A in regulating islet β-cell size.

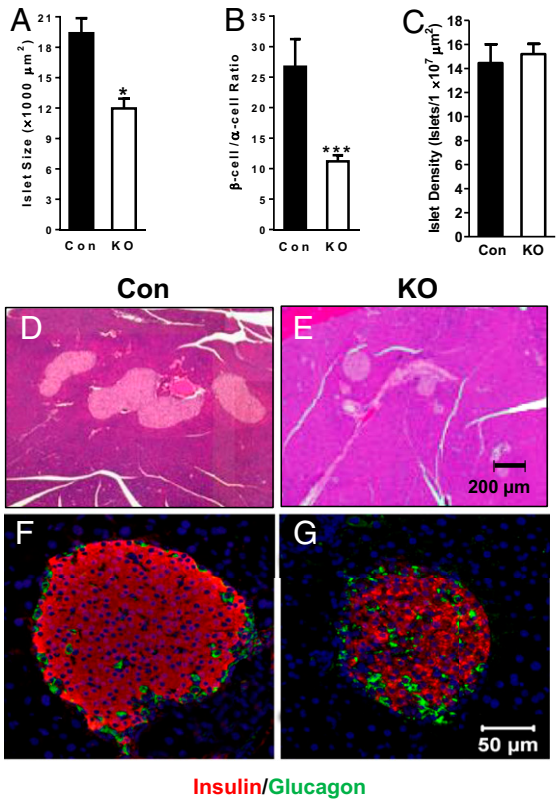

Fig. 2. SAD-A KO mice exhibited a reduction in islet size, β-cell mass, and islet density. Isolated pancreas from SAD-A KO mice and WT controls were analyzed for changes: (*A*) islet size, (*B*) ratio of β-cell number to α-cell number, and (*C*) islet density, expressed as the average number of islets per 1×10^7 μM^2 of pancreas area ($n = 5$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with WT controls. (*D* and *E*) Analysis of pancreatic islet morphology from WT control (*D*) and SAD-A KO (*E*) mice by H&E staining. (*F* and *G*) Immunohistochemical analysis of pancreatic islets from WT control (*F*) and SAD-A KO (*G*) mice, using anti-insulin (red) and antiglucagon (green) antibodies.

Overexpression of SAD-A Caused Hypertrophy of MIN6 Islet β-Cells. As a complementary approach to SAD-A depletion in mice, we next examined the role of SAD-A overexpression in regulating the size of MIN6 β-cells. MIN6 cells were stably transfected with expression vector for SAD-A or vector control and were stained for F-actin, followed by confocal imaging analysis of MIN6 cell size and morphology. As a result, we show that SAD-A overexpression significantly increased MIN6 β-cell size (Fig. 4*C*, enlarged in Fig. 4*D*) relative to the vector control (Fig. 4 *A* and *B*, quantified in Fig. 4*E*). The results provide direct support for the findings from global and pancreas-specific SAD-A KO mice, further confirming a key role of SAD-A in regulating islet β-cell size.

Glucose-Stimulated SAD-A Protein Expression in Islet β-Cells Through Activation of mTORC1 Signaling. Glucose activates mTORC1 signaling in pancreatic β-cells (2), leading to increased insulin content and GSIS (9, 12, 14). Strikingly, mice with global S6K1 deletion exhibit a similar phenotype to that caused by SAD-A depletion, including growth retardation, petite islets, insulin deficiency, and defective GSIS (12). These similarities prompted us to investigate a role of mTORC1 signaling in regulating expression of the SAD-A kinase in pancreatic β-cells. We first investigated a role of glucose on translational control of SAD-A protein expression in isolated mouse islets. Our results show that SAD-A protein expression was potently upregulated after 30 min of stimulation with high glucose or glucose plus Ex-4 (Fig. 5*A*), suggesting a translational regulation. The upregulated SAD-A protein expression was significantly attenuated by rapamycin (Fig. 5*B*). It should be noted that rapamycin only partially inhibited SAD-A expression and mTOR signaling, which is likely caused by poor permeability of the compound to intact islets (25), as evidenced by partial inhibition of mTOR phosphorylation (p-mTOR). Likewise, rapamycin dramatically inhibited SAD-A protein expression in insulin secreting cell lines-1 (INS-1) β-cells concurrent with a decreased level of S6K1 phosphorylation at Thr389, a key indicator of mTORC1 activation (Fig. 5*C*, quantified in Fig. 5*D*). Together, these findings suggest that glucose stimulates SAD-A protein expression in islet β-cells, which is regulated by mTORC1 signaling.

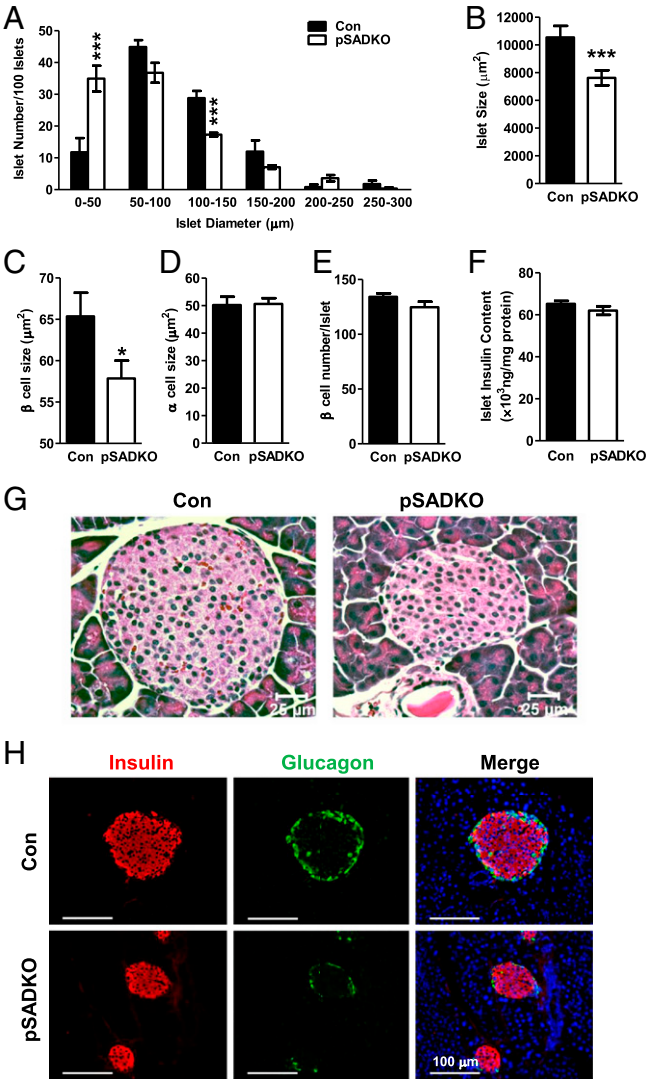

Fig. 3. Targeted deletion of SAD-A in pancreas decreased islet size and β-cell mass. (*A*) Profiling of islet size distribution in pSADKO mice and WT controls by morphometric analysis. Images of islets were traced manually from H&E-stained pancreatic sections, which were evenly sectioned throughout each pancreas, separated by 200 μm between two sections. Islet diameters and areas were determined by SPOTcam software. About 25% of each pancreas was assessed ($n = 3$). (*B*) Average islet size from *A*, determined by dividing total islet area by the total number of islets. (*C*–*F*) Analysis of β-cell size, α-cell size, β-cell number, and islet insulin content from pSADKO mice and the WT controls. (*G*) Representative morphology of pancreatic islets from pSADKO and WT controls analyzed by H&E staining. (*H*) Immunohistochemical analysis of insulin (red) and glucagon (green) positive cells from islets of the pSADKO mice and WT controls, using anti-insulin and antiglucagon antibodies.

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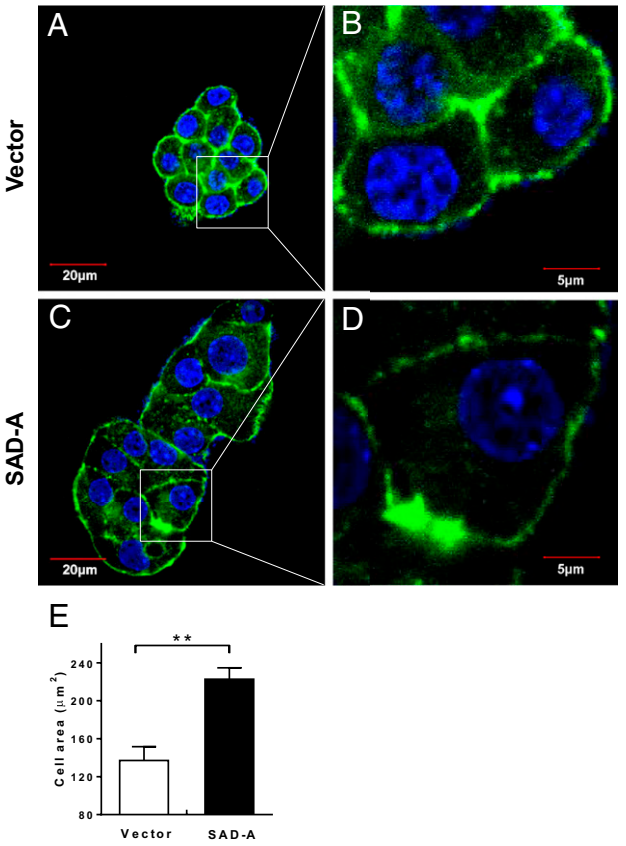

Fig. 4. SAD-A overexpression led to hypertrophy of MIN6 β-cells. (*A*–*D*) Confocal imaging analysis of MIN6 β-cells stably overexpressing SAD-A (*A*, highlighted in *B*) or vector control (*C*, highlighted in *D*) stained for F-actin by fluorescent phallotoxin. (*E*) Quantitative analysis of MIN6 β-cell size by morphometric analysis, using FV10-ASW2.1 software ($n = 200$). $^{**}P < 0.01$.

The 5′-Untranslated Region of SAD-A mRNA Mediated Its Translational Activation by mTORC1 Signaling. The mTORC1 complex controls mammalian cell size through activation of S6K1, rather than through phosphorylation of eukaryotic initiation factor 4E binding proteins (4E-BPs) (26). In addition, S6K1 is required for translation of mRNAs with highly structured 5′-untranslated regions (5′-UTRs), including genes encoding *ODC1* (ornithine decarboxylase 1), *MYC* (myelocytomatosis oncogene), *VEGF* (vascular endothelial growth factor), and *HIF1α* (hypoxia inducible factor 1, alpha subunit) (27). As shown in Fig. S2*B*, the 5′-UTR of *SAD-A* mRNA is extremely guanine-cytosine<-rich, which predicts an extensive secondary structure (Fig. S2*C*) and suggests a possible regulation by S6K1. To identify molecular mechanisms by which mTORC1 controls the translational initiation of *SAD-A* mRNA, we next determined a role of the 5′-UTR in mediating the mTORC1 response in islet β-cells, using a dual-luciferase reporter assay. A luciferase reporter plasmid construct was engineered by subcloning the 5′-UTR of *SAD-A* upstream of a firefly luciferase reporter (Fig. 6*A*). INS-1 islet β-cells were cotransfected with the 5′-UTR reporter plasmid, along with an expression vector for Renilla luciferase, which was used as an internal control for the assay. As shown in Fig. 6*B*, the luciferase reporter was minimally active when INS-1 β-cells were cultured in a medium that contained low glucose. In contrast, high glucose significantly stimulated the reporter activity. The stimulation was largely mediated by mTORC1 activation, as treatment of INS-1 islet β-cells with rapamycin significantly attenuated the reporter activity.

We next examined a role of mTORC1 activation in regulating *SAD-A* mRNA translation by coexpressing the *SAD-A* 5′-UTR luciferase reporter with an expression vector for gain-of-function Rheb-S16H (protein Ras homolog enriched in brain with serine 16 mutate to histidine) or EGFP, which was used as a negative control. Rheb is a key player downstream of TSC1/2 in activating mTORC1 effectors of cell growth such as S6K1 and 4E-BP1. Overexpression of the mutant S16H Rheb constitutively stimulates mTORC1 activation (28), which was also confirmed by our studies, as supported by increased Thr389 phosphorylation of S6K1 in INS-1 β-cells cultured under normal conditions (Fig. S2*D*). In further support for a key role of mTORC1 activation in regulating *SAD-A* mRNA translation, overexpression of Rheb-S16H significantly stimulated *SAD-A* translation initiation, which was completely ablated in response to treatment with rapamycin (Fig. 6*C*).

Discussion

mTORC1 signaling is implicated in multiple functions in pancreatic islets, including GSIS, β-cell size, and islet mass (9–11). However, the downstream targeting protein or proteins that mediate these effects remain to be elucidated. In this study, we identified a key of SAD-A in controlling multiple islet β-cell functions as a pancreas-specific mediator of mTORC1 signaling, which is supported by multiple lines of evidence. First, we demonstrated that global SAD-A deletion caused multiple defects in islet β-cell function that are highly reminiscent of defects observed in mice with global deletion of S6K1, including growth retardation, hypoinsulinemia, insulin deficiency, petite islets, and diminished β-cell mass (12). Second, in support of the phenotype in global SAD-A KO mice, we show that mice with selective deletion of SAD-A in pancreas significantly decreased islet size and β-cell mass. Third, in direct support of SAD-A as a downstream

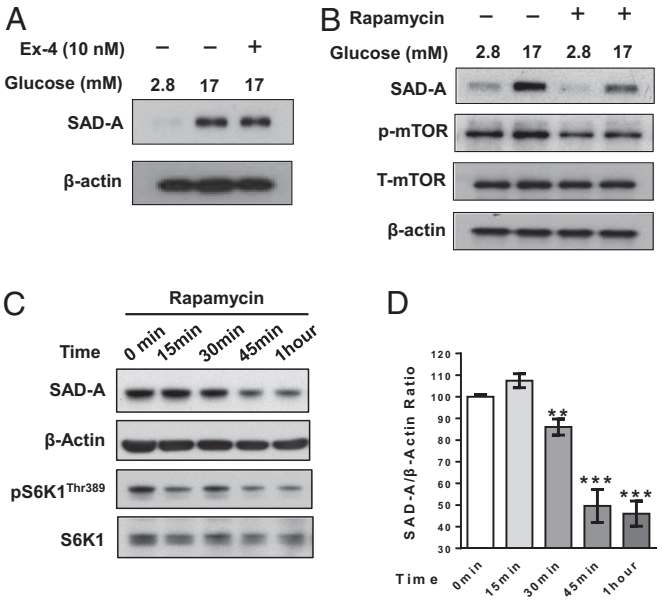

Fig. 5. SAD-A protein expression was controlled by mTOR signaling. (*A*) Isolated mouse islets were cultured in a medium that contains 2.8 mM glucose and were then stimulated with 17 mM glucose for 30 min, followed by Western blot analysis of SAD-A expression, using β-actin as an internal control. (*B*) Isolated mouse islets were cultured in a medium that contained 11 mM glucose and 100 nM rapamycin for 3 d and then switched to a medium that contained 17 mM glucose and 100 nM rapamycin for 30 min, followed by Western blot analysis of SAD-A, phosphor-mTOR (S2481), mTOR, and β-actin. (*C*) INS-1 β-cells were cultured in a medium that contains 11 mM glucose and then treated with 100 nM rapamycin for the indicated period, followed by Western blot analysis of SAD-A, phosphorylation of S6K1 at Thr389 ($p70S6K1^{Thr389}$), S6K1, and β-actin, which was used as internal control for protein loading. (*D*) Quantitative analysis of SAD-A protein expression level relative to that of β-actin in *A* from three repeated experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with 0 min.

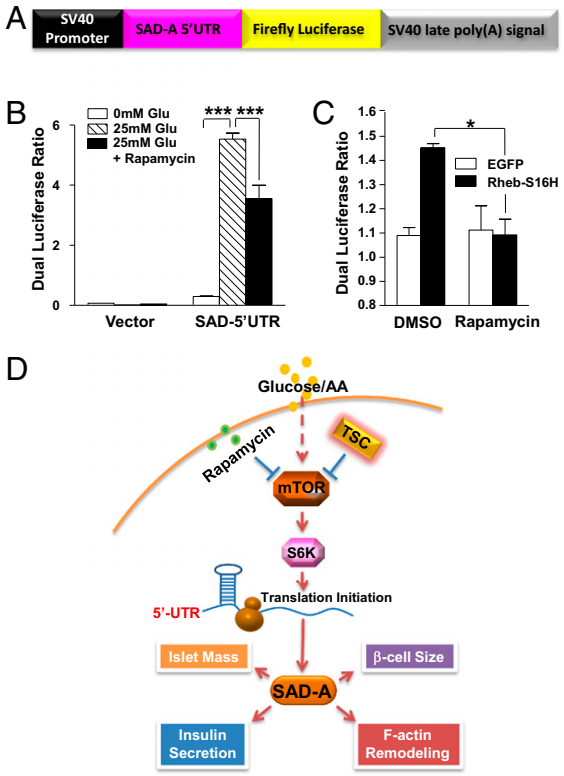

Fig. 6. mTORC1 regulated *SAD-A* mRNA translation in INS-1 β-cells through 5′-UTR. (*A*) Diagram depicting a luciferase reporter DNA construct used for the dual luciferase assay of *SAD-A* 5′-UTR activity in an INS-1 β-cell. (*B*) Analysis of *SAD-A* 5′-UTR reporter activity in response to treatment with 25 mM glucose and 100 nM rapamycin. INS-1 β-cells were cotransfected with *SAD-A* 5′-UTR luciferase reporter plasmid or vector control with Renilla luciferase plasmid, which is used as the internal control for transfection efficiency. The transfected cells were cultured in a medium that contains 0 mM glucose (Glu), 25 mM Glu, or 25 mM Glu plus 100 nM rapamycin, respectively, followed by analysis of *SAD-A* 5′-UTR reporter activity by measuring dual luciferase activity ratio. (*C*) INS-1 β-cells were transiently cotransfected with 5′-UTR luciferase reporter, expression vector for Renilla luciferase, and expression vector for Rheb-S16H, a constitutively active Rheb, followed by treatment with DMSO (vehicle) or 100 nM rapamycin and analyzed for dual luciferase activity ratio as a measurement of *SAD-A* 5′-UTR activity ($n = 3$). $^*P < 0.05$, $^{***}P < 0.001$ compared with vector control. (*D*) Diagram depicting the role of SAD-A in regulating multiple pancreatic β-cell functions as an effector protein of mTORC1 signaling.

target of mTORC1 signaling, we showed that SAD-A protein expression in isolated mouse islets was potently induced by glucose, which was significantly attenuated by rapamycin treatment. Likewise, rapamycin treatment also significantly repressed expression of SAD-A protein in INS-1 islet β-cells. These findings are consistent with a previous report that SAD-A protein expression in isolated primary neurons, the only other tissue that expresses SAD-A, was potently repressed by rapamycin and stimulated by mTORC1 activation (29). Consistent with a key role of SAD-A in regulating insulin exocytosis, SAD kinase is associated with synaptic vesicles, in which it regulates neural transmitter release (18).

The mTORC1 signaling pathways integrate mitogen and nutrient signals to control cell proliferation and cell size. Although 4E-BPs and S6K1 are major effectors of mTORC1 signaling, they mediate different effects on cell proliferation and cell size. In contrast to 4E-BPs which control cell proliferation (26), S6K1 and its downstream targets regulate cell size (13). Cumulative studies have also identified a key role of mTORC1 signaling in regulating islet β-cell proliferation and size. Thus, constitutive activation of mTORC1 leads to increased β-cell size and mass (9, 10), whereas targeted inactivation of S6K1 or inhibition of mTORC1 signaling by rapamycin impairs β-cell survival, leading to β-cell depletion (15, 30). In support of SAD-A as an effector protein of mTORC1 signaling in islet β-cells, we identified a key role of SAD-A in regulating islet β-cell size in this study. Accordingly, we demonstrated that global SAD-A depletion or targeted deletion of SAD-A in pancreas significantly reduced β-cell size and islet mass. Conversely, overexpression of SAD-A in islet MIN6 cells significantly increased β-cell size. Our findings are corroborated by a recent report indicating that targeted deletion of LKB1 leads to increased β-cell size through activation of mTOR signaling (5, 7).

mTORC1 is implicated in a number of human diseases, including diabetes and obesity (1, 8). Hence, inhibition of mTORC1 by rapamycin exacerbates the metabolic state in type 2 diabetes by preventing β-cell adaptation to hyperglycemia (14). In support of a regulatory role of mTORC1 in diabetes, activation of mTORC1 in islet β-cells is required for GLP-1's effect on pancreatic islet viability (31). GLP-1 is an incretin hormone that improves glucose responsive of islet β-cells. A number of long-acting GLP-1 analogs have been developed in recent years as an effective treatment of type 2 diabetes by improving multiple β-cell functions (22). In further support of SAD-A as a islet β-cell–specific effector protein of mTOR signaling, our recent studies show that SAD-A is activated in response to stimulation with GLP-1, and SAD-A activation is required for GLP-1's effect on GSIS in pancreatic β-cells (21). Consistent with the reported effect of GLP-1 on islet β-cell survival, we show in this study that SAD-A depletion abolished GLP-1 effect on GSIS concurrent with β-cell deficiency in global SAD-A KO mice.

The mTORC1 complex plays an important role in regulating the translation of mRNAs with highly structured 5′-UTR, including genes encoding MYC, HIF1, ODC1, cyclin D1, and VEGF. It does so through S6K1-mediated activation of eukaryotic translation initiation factor 4A1 (eIF4A) helicase activity, which is essential in unwinding a structured 5′-UTR for the initiation of translation (27). Consistent with this notion, we found that the 5′-UTR of the *SAD-A* mRNA is highly structured because of its extremely high GC content. Using a dual-luciferase reporter assay, we demonstrated that the 5′-UTR plays an essential role in mediating the effect of mTORC1 on *SAD-A* mRNA translation. Accordingly, we showed that the onset of GSIS greatly stimulated *SAD-A* 5′-UTR luciferase reporter activity in pancreatic β-cells, which was inhibited by rapamycin. Likewise, activation of mTORC1 through overexpression of constitutively active Rheb led to a great enhancement in the *SAD-A* 5′-UTR luciferase reporter activity, which was completely abolished by rapamycin treatment. Together, these results identified SAD-A as a unique pancreatic β-cell–specific mediator of mTORC1 signaling, as depicted in Fig. 6*D*. More important, our work has provided key insights on the targeting of SAD-A for the treatment of type 2 diabetes, as defective GSIS and islet β-cell deficiency play major roles in the etiology of the disease.

Materials and Methods

SAD-A KO mice were generated as previously reported (16), and insulin secretion analysis in vivo was carried out in mice killed and injected with D-glucose (2.5g/kg body weight). All experiments used littermate control of matched age and sex and were in accordance with approval of institutional animal care and use protocols according to National Institutes of Health guidelines (NIH publication 86-23, 1985). Dual luciferase assay, measurement of insulin and glucagon content, histological and morphometric analysis of pancreatic islets, islet isolation and perifusion assay, generation of recombinant adenoviruses, and all reagents used in this study are described in detail in *SI Materials and Methods*.

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