Brief Communication

MOLECULAR METABOLISM

GPR142 prompts glucagon-like Peptide-1 release from islets to improve β cell function

Hua V. Lin [1,*], Jingru Wang [2], Jie Wang [2], Weiji Li [2], Xuesong Wang [2], James T. Alston [1], Melissa K. Thomas [1], Daniel A. Briere [1], Samreen K. Syed [1], Alexander M. Efanov [1]

ABSTRACT

Objective: GPR142 agonists are being pursued as novel diabetes therapies by virtue of their insulin secretagogue effects. But it is undetermined whether GPR142's functions in pancreatic islets are limited to regulating insulin secretion. The current study expands research on its action.
Methods and Results: We demonstrated by in situ hybridization and immunostaining that GPR142 is expressed not only in β cells but also in a subset of α cells. Stimulation of GPR142 by a selective agonist increased glucagon secretion in both human and mouse islets. More importantly, the GPR142 agonist also potentiated glucagon-like peptide-1 (GLP-1) production and its release from islets through a mechanism that involves upregulation of prohormone convertase 1/3 expression. Strikingly, stimulation of insulin secretion and increase in insulin content via GPR142 engagement requires intact GLP-1 receptor signaling. Furthermore, GPR142 agonist increased β cell proliferation and protected both mouse and human islets against stress-induced apoptosis.
Conclusions: Collectively, we provide here evidence that local GLP-1 release from α cells defines GPR142's beneficial effects on improving β cell function and mass, and we propose that GPR142 agonism may have translatable and durable efficacy for the treatment of type 2 diabetes.

Keywords GPR142; Intra-islet GLP-1; α cell; PC1/3

1. INTRODUCTION

GPR142 is a tryptophan-activated Gαq-coupled receptor with enriched expression in pancreatic islets. Both natural and synthetic ligands for this receptor were shown to enhance glucose-dependent insulin secretion and to improve in vivo glucose homeostasis in animals [1,2]. Several investigative reports have disclosed efforts at developing small-molecule GPR142 agonists for the treatment of type 2 diabetes [2–6]. However, which specific cell type (s) in the pancreatic islet expresses this receptor and what mechanisms mediate augmented insulin release remain poorly understood.

Post-translational processing of hormone precursor proglucagon into mature, metabolically-competent hormones happens in a tissue-specific manner. In islet α cells, prohormone convertase 2 (PC2, encoded by Pcsk2) plays a predominant role to release glucagon from its precursor as well as other peptides [7]. In intestinal L cells, proglucagon is cleaved by prohormone convertase 1/3 (PC1/3, encoded by Pcsk1) to primarily give rise to Glucagon-like peptide 1 (GLP-1). The prevailing view on its physiology is that, once secreted from its main tissue production source, intestinal L cells, GLP-1 travels via general circulation and acts on pancreatic β cells to stimulate insulin secretion and promote their proliferation and cell survival. However, there have also been reports of bioactive GLP-1 being secreted by α cells from both rodent and human islets [8,9]. Moreover, recent studies showed α cell-derived GLP-1 is required for normal glucose homeostasis in mice [10], and intra-islet GLP-1 production from α cells is induced by β cell ablation, insulin resistance, or inflammation [11–13]. Nevertheless, it is largely understudied if pharmacologic activation of local GLP-1 release from α cells is able to produce salutary metabolic effects via augmentation of insulin secretion and increases in β cell mass and if so whether those effects are translatable to treat diabetes.

In this study, we show that in addition to its localization in β cells, GPR142 is measurably expressed in α cells in both human and mouse pancreas. Using a potent and selective GPR142 agonist, a GLP-1 receptor (GLP1R) antagonist, and Glp1r knockout mice, we further demonstrate that GPR142 activation stimulates glucagon secretion as well as GLP-1 production and release from the islet. The latter determines GPR142's insulinotropic, β cell proliferative, and pro-survival effects, connecting together the biology of GPR142, GLP-1, and insulin at the local islet level.

2. MATERIALS AND METHODS

2.1. In situ hybridization and immunostaining of pancreas sections

In situ hybridization and immunostaining were performed on formalin-fixed paraffin-embedded human pancreas sections from two donors. Reagents used were Hs-GPR142 probe (ACD Bio, cat# 404611) and RNAscope 2.0 high definition red kit (ACD Bio), rabbit anti-glucagon antibody (Leica), guinea pig anti-insulin antibody (Dako), and Alexa488-labled secondary antibodies (Invitrogen). RNAscope assay was performed according to manufacturer's protocol, followed by standard immunostaining procedures. At least 5 islet images per

[1]Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN, USA [2]Lilly China Research and Development Center, Shanghai, China

*Corresponding author. Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285, USA. E-mail: lin_hua@lilly.com (H.V. Lin).

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sample were captured and analyzed. For mouse pancreas, pancreata from three male Gpr142 knockout mice were embedded in O.C.T (VWR), and frozen sections were cut and stained. Key reagents were mouse anti-glucagon antibody (Millipore), rabbit β-galactosidase antibody (Fisher) and Cy2-and Cy3-labeled secondary antibodies (Invitrogen).

2.2. Compounds

Compound A was synthesized as previously described [14]. Exendin (9-39) and somatostatin were obtained from Sigma—Aldrich.

2.3. Experimental animals

Male C57BL/6 mice were obtained from Shanghai Laboratory Animal Center and MARC of Nanjing University. Gpr142 knockout mice on C57BL/6 background were previously described [1] and maintained at HD Biosciences (Shanghai). Glp1r knockout [15] and Gipr knockout mice on C57BL/6 background were maintained at Taconic (Hudson, NY). Mice were allowed free access to standard chow diet and deionized water and maintained on a 12:12 h light:dark cycle. Starting from 6 weeks of age, diet-induced obese C57BL/6 mice were allowed free access to high-fat diet (D12492, Research Diets) for 16 weeks before the study began. All animal procedures were approved by Institutional Animal Care and Use Committees of Eli Lilly and Company, Covance Shanghai, and HD Biosciences.

2.4. Mouse pancreatic islet studies

Animals were euthanized by CO_2 inhalation followed by cervical dislocation. Pancreatic islets were isolated by injection of collagenase XI solution (0.5 mg/mL, Sigma—Aldrich) into the common bile duct, excision of the pancreas, digestion at 37 °C for 17 min, and Dextran density gradient separation. Isolated islets were cultured in RPMI-1640 medium containing 11 mM glucose, 10% FBS, and 2 mM glutamine and allowed to recover overnight. For acute glucagon secretion, islets were pre-incubated in KRB buffer with 11.1 mM glucose and 0.5% BSA for 60 min, transferred to a 96-well plate with 10 islets per well containing 200 μl of compound solutions prepared in appropriate glucose concentrations and 0.1% BSA, and incubated at 37 °C for 2 h. For acute insulin secretion, a similar procedure was used with the following modifications: KRB buffer 2.8 mM glucose during pre-incubation, 5 islets per well, Ex9-39 incubation for 1 h followed by addition of compound A and incubation for another hour. For chronic incubation experiments, after overnight recovery, islets were cultured (5 islets per well) for 96 h in complete media with appropriate glucose concentrations and compounds. Secretion was stopped by refrigerating the plates at 4 °C for 3 min. Supernatant was collected from each well, and islets were washed in KRB buffer twice then lysed in RIPA buffer supplemented with proteinase inhibitor (Thermo) and DPP-4 inhibitor (only for aGLP-1 secretion experiment) (Merck). Hormone levels in supernatant or cell lysate were measured using the Glucagon HTRF Assay Kit (Cisbio), Mouse/Rat Insulin Kit, Active GLP-1 kit, and Total GLP-1 kit (Meso Scale Discovery).

2.5. Human pancreatic islet studies

Human pancreatic islets were purchased from Prodo Labs at the Scharp-Lacy Research Institute (Irvine, CA, www.prodolabs.com) through the Integrated Islet Distribution Program in accordance with internal review board ethical guidelines for use of human tissue. Islets were isolated at Prodo Labs from human pancreata from listed cadaver organ donors that were refused for primary human pancreas transplantation or isolated islets transplantation into listed diabetic recipients. Hormone secretion studies were conducted as described above for mouse islets, with the modifications that 5 human islets per well were used for insulin secretion and 8 human islets per well were used for glucagon secretion.

2.6. Quantification of β cell proliferation

After overnight recovery, mouse or human islets were dispersed into single cells and seeded into 96-well plates with RPMI-1640 medium containing 5.6 mM glucose, 10% FBS, and 10 μM compound A or DMSO control. After 2.5 days, two thirds of the culture media were removed, and fresh media/compound mixtures containing EdU were added to the wells, with 4—6 replicate wells per treatment. After another 2.5 days, cells were washed and fixed. Click-iT EdU HCS assay (ThermoFisher), insulin immunostaining, and DAPI staining were performed. 20—25 images fields (500—1000 cells per image) were captured, and 10000—15000 insulin-positive cells were analyzed per treatment. The number of EdU- insulin-double positive cells was quantified as a percentage of all insulin-positive cells.

2.7. Islet cell apoptosis

After overnight recovery, mouse or human islets were plated (5 islets per well) in RPMI-1640 medium containing 11.1 mM glucose and 10% FBS, and pretreated with or without compound A for 3 h. Mouse islets were treated with a cytokine mixture containing 3.7 ng/ml interferon-γ, 119 ng/ml TNFα, and 45 pg/ml IL-1β (R&D Systems). Human islets were treated with 0.3 mM palmitate and 20 mM glucose. After 48-hour incubation, caspase activity was measured by Caspase-Glo 3/7 Assay (Promega) according to manufacturer's protocol.

3. RESULTS

3.1. GPR142 expression in pancreatic α cells and β cells

In order to determine which cell type(s) expresses GPR142 in the human pancreas, we obtained a human GPR142 probe. This reagent generated a specific signal in an AV-12 cell line overexpressing human GPR142 but not in untransfected control cells (data not shown). We performed GPR142 in situ hybridization and immunostaining for either glucagon (Figure 1A) or insulin (Figure 1B) in sections of human pancreata. The GPR142 signal was intense and abundant in areas representative of pancreatic islets and barely detectable in exocrine pancreas. Within islets, GPR142 expression was mostly apparent in a subset of glucagon-positive α cells (Figure 1A, arrows) as well as a subset of insulin-positive β cells (Figure 1B, arrows). The negative control DapB probe did not generate any signal in the adjacent sections (data not shown), confirming the specificity of the GPR142 signal. To determine whether Gpr142 is also expressed in mouse pancreatic α cells, we took advantage of a LacZ reporter inserted into exon 3 of the Gpr142 gene in the Gpr142 knockout (KO) mouse line. Co-immunostaining of LacZ with glucagon in pancreatic sections of Gpr142 KO mice indicated the LacZ reporter is localized in α cells (Figure 1C, arrows), while the LacZ signal seen in glucagon-negative cells likely reflects the subset of β cells that also express Gpr142. These data suggested GPR142 is present in both human and murine α and β cells.

3.2. GPR142 agonism increases glucagon secretion

To examine the function of GPR142 in α cells, we probed the effect of receptor activation on glucagon secretion using the synthetic agonist N-[(3-methylimidazol-4-yl)methyl]-1-[5-methyl-4-(2-thienyl)pyrimidin-2-yl]-5-propyl-pyrazole-4-carboxamide [14] (example 49), hereafter referred to as compound A. We've previously shown that compound A triggered GPR142-specific signaling in vitro [16],

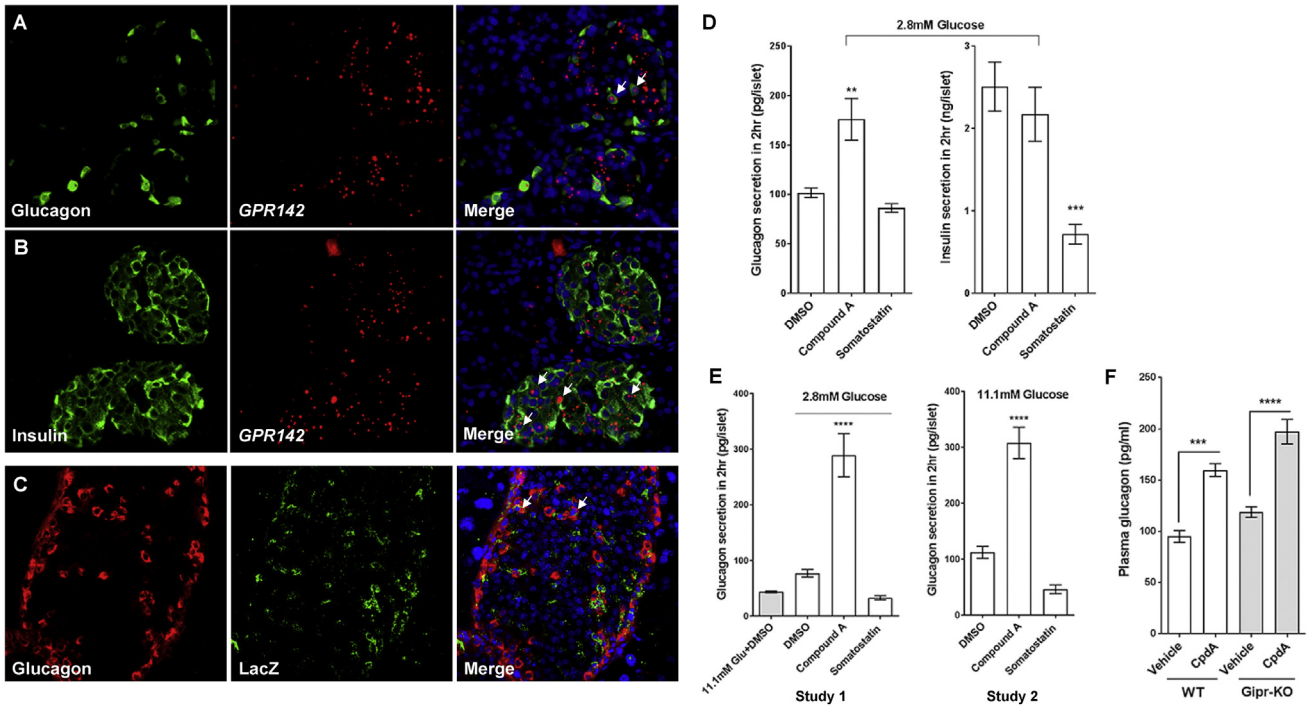

Figure 1: GPR142 is expressed by α cells and regulates glucagon secretion in human and murine islets. (A) Glucagon immunostaining and GPR142 in situ hybridization in human pancreas. Arrows in the merge panel indicate individual α cells with GPR142 expression. (B) Insulin immunostaining and GPR142 in situ hybridization in human pancreas. Arrows in the merge panel indicate individual β cells with GPR142 expression. (C) Glucagon and LacZ immunostaining in Gpr142 knockout mouse pancreas. Arrows in the merge panel indicate individual α cells with LacZ expression. Representative images are shown. (D) Primary human islets were incubated in low glucose (2.8 mM), in the presence of GPR142 agonist compound A (10 μM), somatostatin (100 nM), or control condition with matched DMSO concentration (1%). Compound A significantly increased glucagon secretion but not insulin secretion, while somatostatin led to a non-significant decrease in glucagon secretion and significantly decreased insulin secretion. Islets from two non-diabetic donors were tested in independent experiments, which showed similar results. Representative data are shown. N = 8 replicates per treatment condition. (E) Primary mouse islets were incubated in both low (2.8 mM) and high glucose (11.1 mM) conditions, in the presence of compound A (10 μM), somatostatin (100 nM), or DMSO. Compound A significantly increased glucagon secretion independent of ambient glucose, while somatostatin tended to decrease glucagon secretion. (F) Vehicle (1% w/v HEC, 0.25% v/v Tween80, 0.05 v/v Antifoam in DI water) or compound A (30 mg/kg) was dosed orally to overnight fasted male wild type (WT) and Gipr knockout (KO) mice. 30 min later, cardiac blood was collected, and plasma glucagon level was measured. N = 5 replicates per treatment condition. Data are mean ± SEM. **, ***, ****: $p < 0.01$, 0.001, 0.0001 by one-way ANOVA and Dunnett's multiple comparisons test.

improved glucose tolerance, and increased secretion of insulin and incretins in vivo, all in a GPR142-dependent manner [1]. Furthermore, in vitro counter-screening of compound A in assays encompassing 17 other GPCRs, 18 enzymes, and 3 ion channels did not reveal meaningful activities at concentrations ≥10 μM (data not shown), confirming that compound A is a highly selective GPR142 agonist. As such, compound A increased glucagon secretion in human islets by 73% in the presence of low ambient glucose (2.8 mM), (Figure 1D). Insulin secretion under this condition was not affected (Figure 1D), consistent with our previous report that this GPR142 action was strictly glucose-dependent [16]. In primary mouse islets, compound A increased glucagon secretion under both low (2.8 mM) and high (11.1 mM) glucose conditions (Figure 1E) to 2.7 ∼ 3.8-fold of control treatment, indicative that the GPR142 effect on glucagon is glucose-independent. Somatostatin (SST) tended to decrease both glucagon and insulin secretion under all conditions tested, confirming the validity of the experimental system. To study if these findings translate in vivo, we dosed compound A (30 mg/kg) or vehicle to overnight fasted normal mice and found plasma glucagon levels to be significantly higher (by 68%) in the compound A treated group 30 min after dosing (Figure 1F). Since GPR142 agonists are also known to increase glucose-dependent insulinotropic peptide (GIP) in vivo [1], which can, in turn, stimulate glucagon secretion [17], we used GIP receptor (Gipr) knockout mice to determine whether the effects of compound A on glucagon is mediated by GIP. However, compound A increased plasma glucagon in Gipr knockout mice (Figure 1F) and lowered glucose (Figure S1) to a similar extent as in wild type controls, indicating that the regulation of glucagon secretion by GPR142 in vivo is independent of GIP and likely to be α cell-autonomous.

3.3. GPR142 agonism triggers GLP-1 release and increases its content in islets

It has been previously reported that GPR142 agonists increased plasma levels of GLP-1 in mice [1]. In addition to gut L cells, pancreatic α cells can secrete GLP-1, we next examined the effect of GPR142 activation on GLP-1 secretion in primary pancreatic islets ex vivo, in a condition lacking any gut-derived GLP-1. To note, the levels of GLP-1 in the conditioned media from primary mouse islets were elevated after 96-hour treatment with compound A as measured by a sandwich immunoassay in both low and high ambient glucose to a similar extent (Figure 2A). We hypothesized compound A may stimulate GLP-1 secretion via increasing its production. Indeed, treatment with compound A more than tripled GLP-1 content in islet cell lysates (Figure 2B), while the effect on glucagon content did not reach significance (Figure 2C). These data indicate that GPR142 agonism in pancreatic α cells is perhaps a signal for preferential processing of

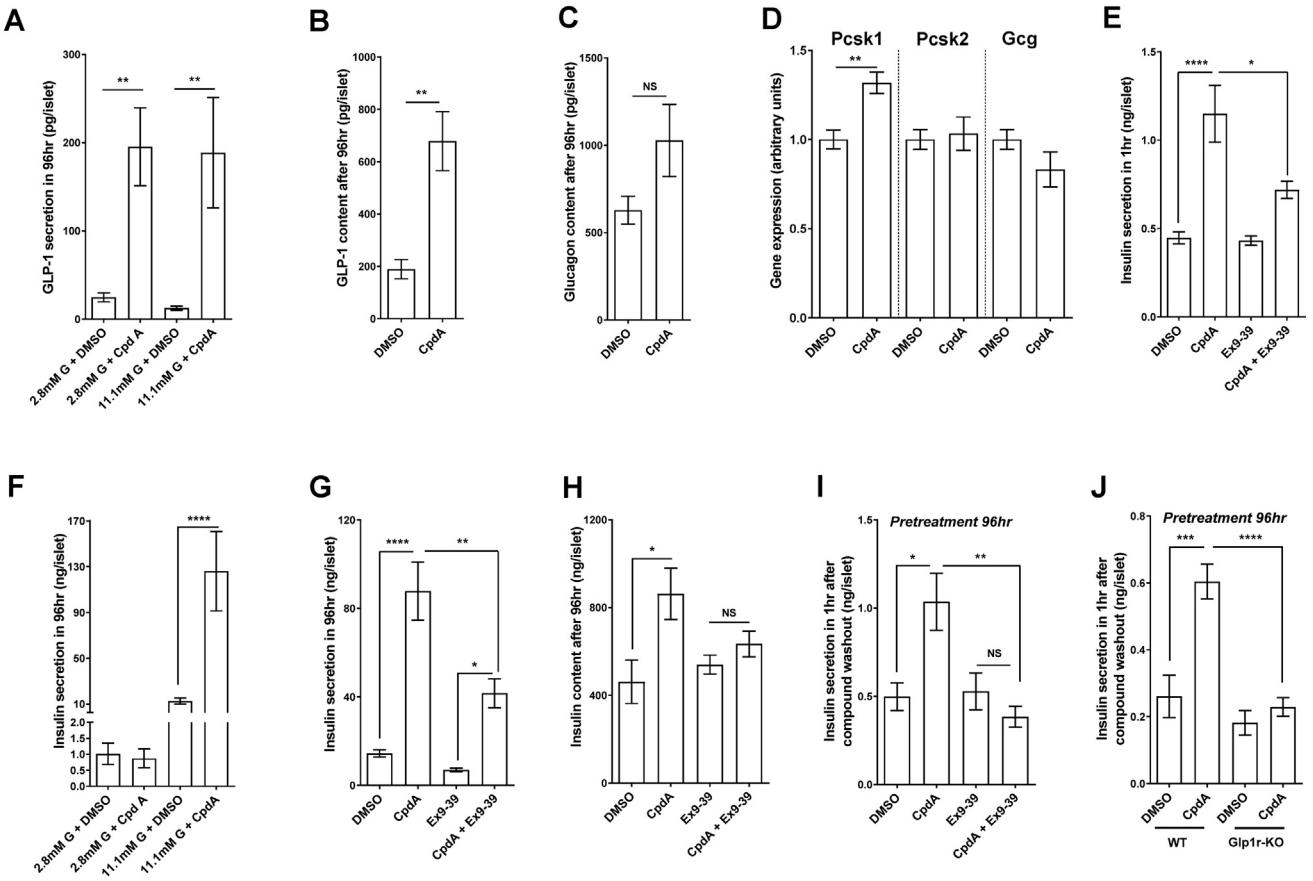

Figure 2: Intra-islet GLP-1 secretion and GLP-1R signaling contribute to GPR142 agonist's insulinotropic effects. (A) Mouse islets were incubated in media containing DPP-4 inhibitor and either low (2.8 mM) or high glucose (11.1 mM), in the presence of compound A (10 μM) or control condition with matched DMSO concentration (1%) for 96 h. The level of active GLP-1 secreted into media was measured. (B–C) Mouse islets were incubated in high glucose with DMSO (1%) or compound A (10 μM) for 96 h, and total GLP-1 content (C) and glucagon content (D) in cell lysates were measured. (D) Mouse islets were incubated in high glucose with compound A (10 μM) or DMSO (1%) for 24 h, and mRNA levels of Pcsk1, Pcsk2, and Gcg in cell lysates were determined by quantitative RT-PCR. Data were normalized to the geometric mean of housekeeping genes Tbp and Arbp, and the mean of the DMSO control treatment for each gene was set to 1.0. (E) Mouse islets were incubated in high glucose with DMSO (1%), compound A (10 μM), Exendin (9-39) (40 nM), or compound A + Exendin (9-39) for 1 h. The level of insulin secreted into media was measured. (F) Mouse islets were incubated in low or high glucose with DMSO (1%) or compound A (10 μM) for 96 h. The level of insulin secreted into media was measured. (G–H) Mouse islets were incubated in high glucose with DMSO (1%), compound A (10 μM), Exendin (9-39) (40 nM), or compound A + Exendin (9-39) for 96 h. The level of insulin secreted into media (G) and insulin content in cell lysates (H) were measured. (I) Mouse islets were incubated in high glucose with DMSO (1%), compound A (10 μM), Exendin (9-39) (40 nM), or compound A + Exendin (9-39) for 96 h. Islets were then thoroughly washed and plated in fresh KRB buffer containing 11.1 mM glucose (with no other compound added) for 1 h. The level of insulin secreted into media was measured. (J) Islets isolated from wild type mice or Glp1r knockout mice were incubated in high glucose with DMSO (1%) or compound A (10 μM) for 96 h. Islets were then thoroughly washed and plated in fresh KRB buffer containing 11.1 mM glucose for 1 h. The level of insulin secreted into media was measured. Data are mean ± SEM. N = 5 replicates per treatment condition. *, **, ***, ****: $p < 0.05$, 0.01, 0.001, 0.0001 by one-way ANOVA and Dunnett's multiple comparisons test (for >2 treatment groups) or by unpaired t test (for 2 treatment groups). NS: not significant.

proglucagon into GLP-1. To this end, we measured in mouse islets the mRNA expression of the prohormone convertases Pcsk1 and Pcsk2, and Gcg (encoding proglucagon) 24 h post compound A treatment, and found the expression of Pcsk1 to be increased by 32% whereas no significant change in other transcripts was observed (Figure 2D). Together, this data suggests that GPR142 agonism in a Pcsk1-dependent manner mechanistically couples to increases in GLP-1 production and secretion specifically from α cells in the islet.

3.4. GLP-1 receptor signaling is required for GPR142's insulinotropic actions in islets

To expand on this striking finding, we next set out to determine if intra-islet GLP-1 secretion and subsequent GLP-1 receptor (GLP-1R) signaling participate in GPR142's insulinotropic actions. After 1-hour acute treatment of mouse islets, compound A led to increased insulin secretion in high ambient glucose condition (11.1 mM) but this effect was blunted when the GLP-1R antagonist Exendin (9-39) (Ex9-39) [18] was co-administered (Figure 2E). Importantly, Ex9-39 alone at the concentration tested did not affect basal insulin secretion. Similar effects were observed under a chronic 96-hour treatment paradigm. Again, compound A in a high glucose-dependent fashion increased insulin levels in the conditioned media from mouse islets (Figure 2F); under the high glucose condition, co-treatment of Ex9-39 substantially inhibited, yet did not fully eliminate, the effect of compound A on insulin secretion (Figure 2G). However, the compound A-driven increase in insulin content in islet cell lysates was completely blocked by Ex9-39 co-incubation (Figure 2H). Furthermore, even after a washout following 96-hour pre-treatment with compound A, the capacity of mouse islets to secrete insulin in high glucose condition was substantially higher than after control pre-treatment, and this effect again was blocked

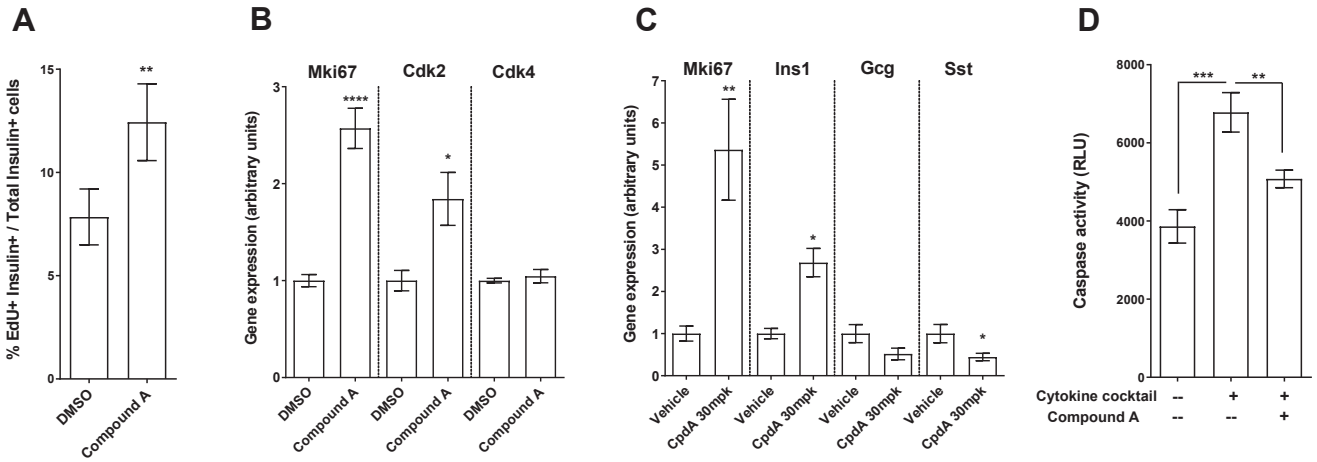

Figure 3: GPR142 agonist promotes β cell proliferation and protects from stress-induced apoptosis in mouse islets. (A) Mouse islets were dispersed into single cells and incubated in the presence of compound A (10 μM) or DMSO (1%) for 5 days, with EdU added for the last 60 h of incubation. The number of insulin- and EdU-double positive cells was quantified as a percentage of all insulin-positive cells. N = 5 independent experiments. **: $p < 0.01$ by paired t test. (B) Mouse islets were incubated in 5.6 mM glucose with compound A (10 μM) or DMSO (1%) for 24 h, and mRNA levels of Mki67, Cdk2, and Cdk4 were determined by quantitative RT-PCR. Data were normalized to the geometric mean of housekeeping genes Tbp and Arbp, and the mean of the DMSO control treatment for each gene was set to 1. N = 5 replicate wells. *, ****: $p < 0.05$, 0.0001 by unpaired t test. (C) Diet-induced obese mice were randomized by body weight to two groups and received daily oral gavage of vehicle (1% w/v HEC, 0.25% v/v Tween80, 0.05% v/v Antifoam in DI water) or compound A (30 mg/kg) for 3 days. After the third dose, mice were fasted for 6 h and euthanized, and islets were isolated. mRNA levels of Mki67, Ins1, Gcg, and Sst were determined by quantitative RT-PCR. Data were normalized to the geometric mean of Tbp and Arbp, and the mean of vehicle group for each gene was set to 1. N = 10 mice per group. (D) Mouse islets were incubated in the presence of compound A (10 μM) or DMSO (1%), followed by addition of a cytokine cocktail and incubation for 48 h. Caspase activity in cell lysates was measured by a luminescence assay. N = 5 replicate wells. Data are mean ± SEM. **, ***: $p < 0.01$, 0.001 by one-way ANOVA and Dunnett's multiple comparisons test.

upon Ex9-39 co-administration (Figure 2I). Finally, in stark contrast to the result obtained on islet cultures from wild type animals, compound A was ineffective at inducing higher insulin secretion when applied to islets from Glp1r knockout mice (Figure 2J), providing genetic confirmation to our pharmacologic data. Collectively, our results indicate that increased insulin production and secretion via GPR142 activation requires intra-islet GLP-1 release and GLP-1R signaling. Interestingly however, compound A lowered glucose to a similar degree in both wild type and Glp1r knockout animals (Figure S2), indicating GLP-1R signaling is not the sole mediator of GPR142 pharmacology.

3.5. GPR142 agonism promotes β cell proliferation and survival

As GLP-1 is a well-known growth factor that stimulates proliferation of β cells, we went on to examine whether activation of GPR142 can promote β cell expansion and survival. We thus incubated dispersed mouse islet cells with or without compound A for 1 week, used insulin immunostaining to measure β cells population, labeled proliferating cells with EdU, and quantified the total number of insulin- and EdU/insulin-double positive cells. Strikingly, compound A treatment led to substantial enrichment in actively proliferating β cells (Figure 3A). Consistent with this finding, increased mRNA expression levels of the proliferation marker Ki-67 (Mki67) as well as cyclin-dependent kinase 2 (Cdk2) were detected in mouse islets even after only 24 h of stimulation with compound A (Figure 3B) but not that of cyclin-dependent kinase 4 (Cdk4). Moreover, oral dosing of compound A (30 mg/kg/day) for 3 days to diet-induced obese mice led to a significant increase in islet Mki67 transcript (Figure 3C), indicating accelerated islet cell proliferation in vivo. Accordingly, Ins1, but not Gcg or Sst mRNAs were elevated in islets isolated from compound A-treated mice (Figure 3C), consistent with a β cell-specific proliferative effect. Noteworthy, mouse islets pretreated with compound A revealed reduced caspase activity upon cytokine challenge suggestive of improved cell survival by GPR142 engagement (Figure 3D).

In human islets, GPR142 agonist induced similar effects. Compound A significantly increased the proportions of actively proliferating β cells (Figure 4A, EdU/insulin-double positive cells, arrows) in islets from three adult non-diabetic human donors to 203 ± 25% of control treatment ($p < 0.05$), while there was no significant increase in the total number of EdU-positive cells. Furthermore, GPR142 agonism led to marked attenuation of glucolipotoxicity-induced apoptosis (Figure 4B), altogether indicative of the protective effects of GPR142 agonism being translatable to human islets.

4. DISCUSSION

Activation of GPR142 impacts glucose homeostasis via augmentation of insulin secretion in β cells. In our previous report, we successfully deorphanized GPR142, identified tryptophan as its endogenous ligand [1], and described highly potent and selective GPR142 agonists that mimic tryptophan's action on intracellular signaling and insulin secretion [1,16]. To expand on GPR142 biology, in this study, we characterized GPR142 cell type distribution in the pancreas and used a synthetic agonist to probe its mechanism of insulin secretion in the pancreas. This led us to the identification of novel functions of GPR142 in α cells.

The primary function of pancreatic α cells is to secrete glucagon. Tryptophan is known to stimulate glucagon secretion but the mechanism of this action remains poorly understood. When delivered into the stomach or the duodenum, this amino acid consistently raised glucagon levels in rat, dog, and man [19–21]. This effect may in part be mediated by stimulation of GIP secretion via targeting K cells in the gut, which we previously showed to be GPR142-dependent [1]. In contrast, intravenous delivery of tryptophan does not seem to increase glucagon plasma levels in most studies [20,22]. Tryptophan may also be utilized by β cells as a limiting substrate to synthesize serotonin,

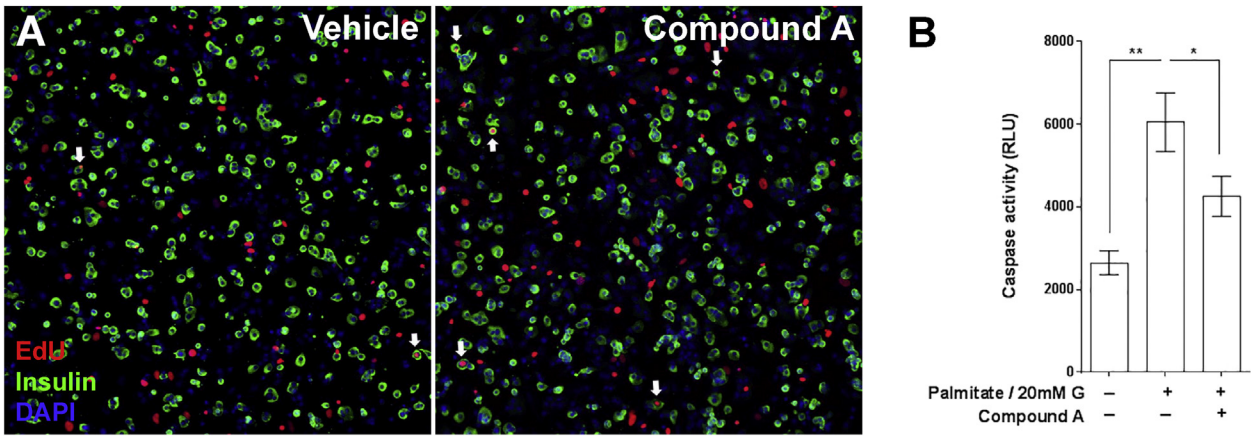

Figure 4: GPR142 agonist promotes β cell proliferation and protects from stress-induced apoptosis in human islets. (A) Dispersed human islets from two non-diabetic human donors were treated using the same procedure as in Figure 3A. Representative images of insulin and EdU staining are shown. (B) Islets from a non-diabetic human donor were incubated with compound A (10 μM) or DMSO (1%), followed by addition of palmitate and high glucose and incubation for 48 h. Caspase activity in cell lysates was measured. N = 5 replicate wells. Data are mean ± SEM. *, **: $p < 0.05$, 0.01 by one-way ANOVA and Dunnett's multiple comparisons test.

which, in turn, suppresses glucagon secretion from α cells [23], further complicating the mechanism of its action. In this study, we identify a novel cell-autonomous GPR142-dependent pathway that tryptophan engages in α cells to promote glucagon secretion. Therefore, the net effect of tryptophan on glucagon levels will be a composite response dependent upon this amino acid's availability and sensitivity to it in multiple cell types, namely, islet α cells and β cells, as well as intestinal K cells.

Here we present that GPR142 agonism leads to GLP-1 secretion from α cells. Mechanistically, stimulation of GLP-1 release from islets by GPR142 appears to be independent of ambient glucose. Moreover, GPR142 activation increased intracellular GLP-1 content in islets, while intracellular glucagon content was not elevated. Together, our data suggest that this intra-islet GLP-1 increase is primarily regulated through accelerated processing of proglucagon into GLP-1. Indeed, PC1/3 expression, which is a primary driver of proglucagon conversion into GLP-1 [24], is substantially elevated in islets upon GPR142 activation. As PC1/3 is also known to be expressed in β cells [25], future cell-sorting studies are needed to determine whether the increase in PC1/3 expression in response to GPR142 is α cell-specific.

The contribution of islet-derived GLP-1 on GPR142's efficacy to stimulate insulin secretion and its content in islets appears to be surprisingly large in our study. Nevertheless, this result is corroborated by both pharmacological and genetic tools, with the use of either GLP-1 antagonist Ex (9-39) or islets derived from Glp1r knockout mouse. However, GPR142 agonists still had a robust glucose lowering effect in Glp1r knockout animals. We therefore propose that intra-islet GLP-1 is not the sole mediator of GPR142's in vivo effects, and other mechanisms such as this receptor cell-autonomous action on β cells and/or induction of GIP secretion from gut K cells also contribute.

Collectively, we show in this report that: i) GPR142 is expressed in both α cells and β cells; ii) GPR142 activation leads to glucagon and GLP-1 release from islets and increases GLP-1 content in islets; iii) GPR142 requires intra-islet GLP-1 and GLP-1R signaling to stimulate insulin secretion and increase insulin content; iv) GPR142 agonism promotes β cell proliferation and islet cell survival in both rodent and adult human islets. The latter is of utmost importance as all forms of diabetes are associated with a decrease in β cell mass. With the exception of certain types of bariatric surgeries, no pharmacological interventions for type 2 diabetes to date have demonstrated the ability to cause regression/remission or even to slow disease progression. This is reflective of the dearth of robust studies assessing the effects of pharmacological mechanisms on cell death prevention or regeneration/proliferation of human β cells, despite plenty of studies in rodent β cells implicating many possible targets [26]. The translatability of GPR142's effects on β cell regeneration from the mouse to an ex vivo human system sparks the hope that this target could potentially produce durable glycemic efficacy and slow down or even reverse β cell failure in type 2 diabetes patients.

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CONFLICT OF INTEREST

All authors are current or former employees of Eli Lilly and Company and may hold stocks of Eli Lilly and Company.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at https://doi.org/10.1016/j.molmet.2018.02.008

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