Whole Organism High Content Screening Identifies Stimulators of Pancreatic Beta-Cell Proliferation

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Abstract

Inducing beta-cell mass expansion in diabetic patients with the aim to restore glucose homeostasis is a promising therapeutic strategy. Although several in vitro studies have been carried out to identify modulators of beta-cell mass expansion, restoring endogenous beta-cell mass in vivo has yet to be achieved. To identify potential stimulators of beta-cell replication in vivo, we established transgenic zebrafish lines that monitor and allow the quantification of cell proliferation by using the fluorescent ubiquitylation-based cell cycle indicator (FUCCI) technology. Using these new reagents, we performed an unbiased chemical screen, and identified 20 small molecules that markedly increased beta-cell proliferation in vivo. Importantly, these structurally distinct molecules, which include clinically-approved drugs, modulate three specific signaling pathways: serotonin, retinoic acid and glucocorticoids, showing the high sensitivity and robustness of our screen. Notably, two drug classes, retinoic acid and glucocorticoids, also promoted beta-cell regeneration after beta-cell ablation. Thus, this study establishes a proof of principle for a high-throughput small molecule-screen for beta-cell proliferation in vivo, and identified compounds that stimulate beta-cell proliferation and regeneration.

Introduction

Pancreatic beta-cells secrete insulin which is the only hormone known to directly lower blood glucose concentrations. Type 1 diabetes mellitus is an autoimmune disease characterized by the destruction of beta-cells in the pancreatic islets, leading to insulin deficiency and hyperglycemia. In contrast, type 2 diabetes has a multifactorial origin that commences with insulin resistance and increased serum insulin levels followed by beta-cell destruction, insulin deficiency and hyperglycemia [1,2]. Restoring functional beta-cell mass is recognized as a promising therapeutic strategy towards normalizing glucose levels in both type 1 and 2 diabetics. Potential strategies for beta-cell mass restoration can generally be categorized as ex vivo strategies involving the generation of beta-cells from stem cells, either embryonic stem (ES) cells or induced pluripotent stem (iPS) cells, and their subsequent transplantation, as well as in vivo regeneration approaches, including beta-cell mass expansion via reprogramming of other cell types and/or stimulation of proliferation of preexisting beta-cells. It remains unclear which approach will ultimately prove successful, and both approaches may even be synergistic [3].

Although human beta-cell proliferative capacity may decrease with age, replication can be clearly observed in response to metabolic demand, such as in obesity or during pregnancy [4]. Therefore, the identification of means to enhance beta-cell replication is of great interest. However, the regulation of beta-cell proliferation remains poorly understood, partly because of the lack of unbiased approaches to identify the underlying signaling mechanisms. Only recently, a screening platform based on freshly isolated rat islet preparations, which are thought to maintain the metabolic characteristics of primary beta-cells, was used to screen for small molecules that promoted beta-cell replication [5]. This approach identified adenosine kinase inhibitors that promoted the replication of cultured primary beta-cells from mice, rats and pigs. Notably, an independent screen also found an adenosine kinase inhibitor, as well as other positive modulators of adenosine signaling, as potent enhancers of beta-cell regeneration in vivo in zebrafish and mice [6]. It is of course important to note that in addition to the artifacts associated with ex vivo environments, in vitro screens will not identify compounds that stimulate beta-cell proliferation indirectly (e.g., by affecting other cell types in the pancreas or other organs). The zebrafish is thus an ideal model for testing small molecules in vivo.
system to carry out large-scale screens, including chemical screens, for beta-cell regeneration [6], beta-cell neogenesis [7] and gluconeogenesis [8]. In this study, we aimed to identify stimulators of beta-cell proliferation in vivo via direct quantification of proliferating beta-cells. To achieve this goal, we established an in vivo imaging approach utilizing the fluorescent ubiquitylation-based cell cycle indicator (Fucci) technology [9,10]. We performed a chemical screen using this approach and identified several small molecules that markedly increased beta-cell proliferation. Importantly, some of these compounds facilitated beta-cell regeneration as well.

Materials and Methods

Zebrafish lines
This study was carried out in strict accordance with the NIH guidelines and was approved by the University of California San Francisco Committee on Animal Research. All embryonic dissociations were performed under tricaine anesthesia, and every effort was made to minimize suffering. Zebrafish were raised under standard conditions at 28’C. Phenylthiourea (PTU) was added at 12 hpf to prevent pigmentation. We used the following lines: Tg(ins:mCherry-zCdt1(1/190))s947, Tg(ins:mAG-zGeminin(1/100))s948, Tg(ins:mAG-zGeminin(1/100))s949 [12].

Chemical Screening
We bred homozygous Tg(ins:mAG-zGeminin(1/100))s947 with wild-type zebrafish to generate hemizygous Tg(ins:mAG-zGeminin(1/100))s947 animals for chemical screening in order to avoid the variability of fluorescent signal present in a mixture of homozygous and hemizygous transgenics. The eye-marker cassette, cryaa:RFP [8] was introduced during the generation of the Tg(ins:mAG-zGeminin(1/100)) lines in order to facilitate identification of transgenic carriers [12]. Larvae were kept in egg water supplemented with 0.2 mM 1-phenyl-2-thiourea (TCI America) from 1–3 dpf to inhibit pigment formation. Compounds were dissolved in 300 μl of egg water to a final concentration of 1% DMSO and added to the wells of a 96-well plate (Matriplate, 170 um glass bottom, Brooks Life Science Systems). Four larvae were pipetted in 200 μl of egg water and placed in each well, for a final volume of 500 μl for 1 day of chemical treatment. We screened the following chemical libraries (NIH Clinical Collection 1 and 2 (727 compounds, Evotec), The InhibitorSelect 96-Well Protein Kinase Inhibitor Library II (80 compounds, EMD Millipore), Nuclear receptor ligand library (76 compounds, Enzo Life Sciences)). Two wells, each containing four larvae, were used to evaluate each compound. Initially, we tested the compounds at 10 μM, a routinely used concentration for chemical screens in zebrafish [8]. Compounds that exhibited toxicity at 10 μM, such as those causing pericardial edema or lethality, were retested by gradual reduction of their concentration until a non-toxic dose was identified. At 4 dpf, the larvae were anesthetized with Tricaine, and the number of Tg(ins:mAG-zGeminin(1/100))s947 beta-cells was counted using a wide-field Zeiss Z1 inverted microscope with a 20x objective. We counted the number of proliferating beta-cells in each larva by adjusting the focus of the microscope in order to visualize the entire primary islet.

Immunohistochemistry
Antibody staining was performed as previously described [13], using insulin antibodies (1:1000, Sigma I8510) and Alexa Fluor conjugated secondary antibodies (1:500, Invitrogen). Cell nuclei were visualized with TOPRO3 (1:2000, Invitrogen T3605). Proliferation was assessed by EdU labeling using the Click-IT EdU imaging kit (Invitrogen). Larvae were incubated in 2.5 mM EdU. For confocal analysis, images were captured with a Zeiss LSM5 Pascal confocal microscope. Images were prepared for further examination using ImageJ.

Blood Glucose Levels
Free glucose was determined by grinding larvae in groups of four and using a glucose assay kit (BioVision) [8]. For blood glucose measurements, adult zebrafish were anesthetized in cold water then decapitated by cutting cleanly through the pectoral girdle with scissors. Whole blood was analyzed immediately with a glucometer test strip (Accu-Check Aviva, Roche Diagnostics) [14].

Statistical Analysis
Statistical analyses were carried out by two-tailed t-tests and displayed as ±SEM.

Results
The FUCCI technology effectively labels proliferating beta-cells
In order to be able to quantitate pancreatic beta-cell proliferation in vivo, we utilized recently generated zebrafish transgenic lines expressing mCherry-zCdt1(1/190) (a G1 marker) and mAG-zGeminin(1/100) (an S/G2/M marker) under the control of the insulin regulatory elements [12] (Fig. 1A). Using live imaging and 5-ethyl-2‘-deoxyuridine (EdU) incorporation analyses, we found that the Tg(ins:mAG-zGeminin(1/100))s947 and Tg(ins:mcCherry-zCdt1(1/190))s948 lines mark the proliferating and quiescent beta-cells, respectively, and that Tg(ins:mAG-zGeminin(1/100))s947 expression disappears several minutes after mitosis [12]. In order to facilitate the counting of proliferating beta-cells using a fluorescence microscope, we generated Tg(ins:mcAG-zGeminin(1/100))s947, a new line that exhibits a much brighter fluorescent signal in beta-cells compared to the previous line (Fig. 1B). This effect is very likely due to a higher basal level of expression of the insulin promoter as a result of a more favorable genomic integration site and/or a higher number of transgenic concatemers. Importantly, using live imaging, we found that the dividing beta-cells maintained visible levels of Tg(ins:mcAG-zGeminin(1/100))s947 expression in their daughter cells for up to several hours after mitosis (352±175 minutes, n = 3 dividing cells in 2 movies) (Fig. 1C). We reasoned that for the purposes of a chemical screen, this line provided a more sensitive readout of beta-cell proliferation. It allows one to score not only the beta-cells in the S/G2/M phase of the cell cycle but also those that divided several hours before the end of the compound treatment period. In addition and consistent with our previous observations [12], Tg(ins:mAG-zGeminin(1/100))s947 single positive beta-cells readily incorporated EdU whereas the Tg(ins:mcAG-zGeminin(1/100))/Tg(ins:mcCherry-zCdt1(1/190)) double-positive beta-cells were either EdU- or weakly EdU+, indicating that they were in an early stage of S-phase (Fig. 1D).

High levels of beta-cell proliferation occur during two stages of early zebrafish development
The temporal regulation of beta-cell proliferation in zebrafish has not been studied in detail during the embryonic and early larval stages. To address this issue, we first analyzed Tg(ins:mcAG-zGeminin(1/100))s947 animals during narrow developmental
Figure 1. Development and characterization of fluorescent ubiquitylation-based cell cycle indicator (FUCCI) for pancreatic beta-cells in zebrafish.

(A) Schematic diagrams of FUCCI constructs for pancreatic beta-cells. The S/G2/M reporter mAG-zGeminin(1/100) and the G1 indicator mKO2-zCdt1(1/190) are expressed under the zebrafish insulin promoter. For efficient selection of transgenic animals, an eye-marker cassette, cryaa:RFP or cryaa:CFP, was introduced into Tg(ins:mAG-zGeminin(1/100)) and Tg(ins:mCherry-zCdt1(1/190)), respectively [8].

(B) Tg(ins:mCherry-zCdt1(1/190),cryaa:CFP)s948;Tg(ins:mAG-zGeminin(1/100),cryaa:RFP)s947 larvae were examined at 4.5 dpf using fluorescence microscopy. A close up of the islet is shown in the inset. A majority of the beta-cells are Tg(ins:mCherry-zCdt1(1/190))^+ indicating that they are in the G1 phase of the cell cycle. Only four beta-cells are Tg(ins:mAG-zGeminin(1/100))^+ indicating that they are in the S/G2/M phase of the cell cycle. Note that the animals are expressing the eye-marker, e.g., cryaa:CFP fluorescence can be observed through the GFP filter.

(C) Time-lapse imaging of Tg(ins:mCherry-zCdt1(1/190))^487,Tg(ins:mAG-zGeminin(1/100),cryaa:RFP)^487 larvae at 4 dpf. Arrowheads point to dividing Tg(ins:mAG-zGeminin(1/100))^487 + beta-cells.

(D) Tg(ins:mCherry-zCdt1(1/190))^487,Tg(ins:mAG-zGeminin(1/100))^487 larvae were incubated with EdU from 3 to 4 dpf. The white arrow points to a Tg(ins:mAG-zGeminin(1/100))^487 + single-positive beta-cell. This cell exhibits high levels of EdU incorporation. The yellow arrow points to a Tg(ins:mCherry-zCdt1(1/190))^487,Tg(ins:mAG-zGeminin(1/100))^487 double positive beta-cell which exhibits low levels of EdU incorporation indicating that this cell entered S phase at the end of the EdU labeling period.

(E) Confocal stacks of Tg(ins:mAG-zGeminin(1/100))^487 + (green) beta-cells stained for Insulin (blue). The animals were fixed at 12 h intervals until 5 dpf. (Scale bar = 20 μm.)

(F) The graph shows a quantification of the number of Tg(ins:mAG-zGeminin(1/100))^487 + beta-cells. Error bars represent SEM; n = 13–15 larvae for each time point. B is a lateral view, anterior to the left and dorsal to the top. C–E show lateral views, anterior to the top and dorsal to the left.

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intervals (12 h) from 24 to 144 hpf in live and fixed preparations (Fig. 1E). Interestingly, we found that during early development, there are two peaks of proliferation in pancreatic beta-cells, one around 36 hpf and the other around 120 hpf (Fig. 1F). As a temporal reference point, the heart starts beating at around 24 hpf [15]. These two peaks coincide with the two periods when the animals exhibit an increase in glucose levels [8,16], suggesting that high glucose levels stimulate a compensatory increase in beta-cell number. Whether glucose exerts a direct effect on beta-cell proliferation or whether the increase in proliferation is the result of transient insulin resistance during these two periods, as shown in mouse models [5,16], remains to be determined. Importantly, these analyses also revealed that from 72 to 96 hpf, a majority of beta-cells enter a period of quiescence.

In vivo high content screening identifies stimulators of pancreatic beta-cell proliferation

With this in vivo platform based on the FUCCI reporter, we designed a screening protocol of low molecular weight compounds to find ones that enhance beta-cell proliferation. A typical time series of Tg(ins:mAG-zGeminin(1/100))\textsuperscript{s947} larvae is shown in Figs. 1E and F. For chemical screening, we chose the developmental period during which most beta-cells are in a quiescent state, in order to identify compounds that stimulate their proliferation. We arrayed hemizygous Tg(ins:mAG-zGeminin(1/100))\textsuperscript{s947} larvae in 96-well plates, and exposed them to chemicals from selected libraries of bioactive compounds as shown in Fig. S1. After 24 hours of chemical exposure, the number of proliferating beta-cells in the primary islet was counted. A typical example of Tg(ins:mAG-zGeminin(1/100))\textsuperscript{s947} + beta-cell counting using an inverted fluorescence microscope is shown in Fig. S1. At this developmental stage, the basal in vivo beta-cell proliferation count showed moderate inter-experiment variability.
Table 1. List of compounds that increase the number of proliferating beta-cells in Tg(ins:mAG-Geminin(1/100))s947 larvae.

| Classification | Chemical Name | # mAG⁻ cells | Pharmacological Action | Dose (µM) | Library |
|----------------|---------------|--------------|------------------------|-----------|---------|
| Control        | DMSO          | 0.5±0.06     | Retinoid receptor agonist | 10 NRL    |         |
| Retinoic acid  | Retinoic acid, all trans | 5.0±0.7 | RAR agonist | 10 NRL    |         |
|                | 4-Hydroxyretinoic acid | 3.7±0.9 | Retinoid metabolite | 10 NRL    |         |
|                | 9-cis Retinoic acid | 2.5±0.5 | RXR agonist | 10 NRL    |         |
| Serotonin      | Trazodone     | 1.3±0.3      | Serotonin uptake inhibitor | 10 NIH I  |         |
|                | Lofepramine   | 1.0±0.3      | Serotonin and noradrenalin re-uptake inhibitor | 10 NIH I  |         |
| Glucocorticoids| Fluticasone   | 2.7±0.6      | Glucocorticoid receptor ligands | 10 NIH I  |         |
|                | Hydrocortisone acetate | 2.8±0.7 |             | 10 NIH II |         |
|                | Prednisolone acetate | 2.9±0.7 |             | 10 NIH II |         |
|                | Clobetasol propionate | 2.7±0.5 |             | 10 NIH II |         |
|                | Triamcinolone acetonide | 2.9±0.5 |             | 10 NIH II |         |
|                | Westcort      | 2.6±0.4      |             | 10 NIH II |         |
|                | Budesonide    | 3.2±0.4      |             | 10 NIH II |         |
|                | Methylprednisolone acetate | 2.0±0.4 |             | 10 NIH II |         |
|                | Fluocinolone acetonide 21-acetate | 2.3±0.4 |             | 10 NIH II |         |
|                | Dexamethasone | 1.4±0.3      |             | 10 NIH II |         |
|                | Cortisone acetate | 1.2±0.3 |             | 10 NIH II |         |
|                | Amincinoide   | 2.1±0.4      |             | 10 NIH II |         |
|                | Fluocinolone acetonide | 1.7±0.3 |             | 10 NIH II |         |

The hits are organized into three categories based on their pharmacological action. All hits showed a statistically significant increase in beta-cell proliferation compared to DMSO controls (n = 12 to 20 animals for each compound); p < 0.05.

(0.53±0.04 cells per larva, n = 788). A compound that showed a statistically significant increase in the number of Tg(ins:mAG-Geminin(1/100))s947 beta-cells compared to DMSO-treated control was regarded as a “hit”. We screened 883 compounds for their ability to induce beta-cell proliferation. The primary screen and the subsequent secondary validation identified 20 hit compounds (2.27% hit rate) (Table 1). Notably, all hits could be grouped into three common pharmacological classes, namely retinoid receptor agonists, enhancers of serotonin signaling, and glucocorticoids (Table 1). We further tested representative compounds for each pathway; retinoic acid, trazodone (serotonin uptake inhibitor), and prednisolone acetate (glucocorticoid) (Fig. 2A). All of these compounds induced replication of beta-cells in a dose-dependent manner with the following effective concentrations to achieve a 2-fold increase in proliferation (EC₂₅): ~0.67, 1.11, and 10.3 µM, respectively (Fig. 2B). Moreover, these compounds increased the number of beta-cells in S-phase, as assessed by EdU incorporation (Fig. S2).

Next, we tested the effect of the hits on glucose levels because high glucose levels are known to increase beta-cell mass in vivo [17] and in vivo [18]. By measuring free glucose, i.e., glucose that has not been phosphorylated intracellularly by hexokinases, we estimated glycemia [6,8]. Using this assay, we found that free glucose levels were not elevated by the treatment with retinoic acid, or trazodone, but prednisolone did significantly increase glucose levels (Fig. 2C). To further analyze the effect of prednisolone on glucose levels, we treated adult fish. We found that plasma glucose levels were two folds higher after a 24 hour exposure to prednisolone compared to controls (Fig. 2D), suggesting that the induction of beta-cell proliferation by prednisolone was caused by hyperglycemia. Thus, the proliferation effect of glucocorticoids is likely indirect, i.e., via increased glucose levels, in agreement with previous studies [19].

The hit compounds promote beta-cell proliferation under feeding metabolism

The chemical screening was carried out with animals during the early larval stage. During this stage, the animals rely on yolk consumption for energy production and enter a fasting state by 4–5 dpf [8]. To test the capability of the hit compounds to promote beta-cell proliferation under a more active metabolism, we evaluated the effect of representative hits of two of the more potent classes (prednisolone and retinoic acid) during the late larval stages (Fig. 3A), when the animals are feeding externally. The number of Tg(ins:mAG-Geminin(1/100))s947 beta-cells was significantly higher in animals treated with the compounds compared to those treated with DMSO (Figs. 3B and 3C), indicating that they can stimulate proliferation at later developmental stages and under feeding metabolism.

The hit compounds promote beta-cell regeneration

We also investigated whether the hit compounds could compensate for beta-cell loss in a diabetic model [11,20]. In this model, beta-cells are ablated via cell-specific transgenic expression of nitroreductase (NTR), an enzyme that converts the chemical metronidazole (MTZ) to a cell lethal product. We exposed Tg(ins:CFP-NTR);Tg(ins:H2BGFP) larvae to MTZ from 50 to 80 hpf, followed by a wash out of the drug (Fig. 4A). Typically,
MTZ-treated larvae exhibit only rare beta-cells after 24 hours in MTZ (Fig. 4B). After allowing beta-cells to regenerate for 2 days in the presence of each hit compound, we analyzed beta-cell regeneration by counting the number of H2BGFP+ beta-cells. In DMSO-treated larvae, 8.7 ± 1.0 beta-cells were present 2 days after ablation (Figs. 4B and 4C). Strikingly, retinoic acid and prednisolone significantly increased the number of beta-cells to 12.3 and 13.2, respectively (Figs. 4B and 4C), indicating that the hit compounds can also promote beta-cell regeneration. Interestingly, trazodone, which only mildly increased beta-cell proliferation in the absence of beta-cell ablation, failed to promote beta-cell regeneration (Figs. 4B and 4C). We conclude that the compounds that induce a potent increase in beta-cell proliferation under physiological conditions can also stimulate beta-cell regeneration consistent with the notion that enhancing beta-cell proliferation...
can increase regeneration as was shown for the nonselective adenosine agonist NECA [6].

Discussion

In this study we identified 20 compounds that increased beta-cell proliferation in zebrafish. Interestingly, despite using an unbiased screening approach of over 800 compounds, we found that several compounds converge on common pharmacological classes, namely retinoid receptor agonists, enhancers of serotonin signaling, and glucocorticoids.

Among the retinoid receptor agonists, retinoic acid is well known to play a crucial role shortly after gastrulation in endoderm patterning and pancreatic development, a role which is conserved from zebrafish to humans [21–23]. This role in pancreatic induction has also been examined during mouse and human ES cell differentiation in vitro, and has been shown to be relevant for the induction of the pancreatic endoderm marker, PDX1 [24–26]. Although little is known about the proliferation effect of retinoic acid, recent studies have shown that retinoic acid can increase the proliferation of cultured human pancreatic progenitor cells [27] and the number of pancreatic beta-cells in zebrafish [28].

Serotonin has been widely studied as a brain neurotransmitter for its effects on appetite and mood, especially depression. A recent analysis of pregnant and non-pregnant mice revealed that serotonin acts downstream of lactogen signaling to stimulate beta-cell proliferation during pregnancy [29]. Moreover, serotonin is highly expressed in mammalian beta-cells [30]. Identification of several serotonin signaling enhancers from our screen, including trazodone (a serotonin uptake inhibitor) is consistent with this role. Interestingly, trazodone did not promote beta-cell regeneration in our beta-cell ablation model. This lack of effect on regeneration might be due to a greatly reduced serotonin pool after the ablation of the serotonin-expressing beta-cells or to its lower capacity to...
induce beta-cell proliferation compared to retinoic acid and prednisolone.

The consistency between our results and previous data (e.g., beta-cell proliferation induced by serotonin and glucocorticoid) establishes the validity of our **in vivo** screen based on the FUCCI technology to identify relevant pathways in beta-cell proliferation. In the past decade, the zebrafish has emerged as a viable model organism for small-molecule discovery. Using zebrafish, it is now possible to assess the specificity, efficacy, and toxicity of small molecules in the context of live animals [31]. By using zebrafish for chemical screening, we identified candidates that induced beta-cell proliferation. The next step is to implement a system for automated image analysis in order to achieve a high throughput screening of beta-cell proliferation **in vivo**. This strategy could make a significant contribution for a primary drug screening for inducers of beta-cell proliferation, identifying not only direct inducers but also indirect ones, i.e., compounds that would affect beta-cell proliferation through cell-cell interaction and/or inter-organ crosstalk. Since current **in vitro** screening approaches cannot identify such indirect inducers of beta-cell proliferation [32–34], our platform can be expected to find novel drug candidates. It must be noted of course, that tests with human cells and tissues will be necessary to further evaluate the ability of the hits from such screens in zebrafish to induce the formation of functional beta-cells.

**Supporting Information**

**Figure S1** Schematic outline of the screening protocol used to identify compounds that promote beta-cell proliferation. The images show typical examples from the screen. **Tg(ins:mAG-zGeminin(1/100))** larvae were arrayed in 96-well plates and exposed to 10 μM of a compound in 1% DMSO from 3 to 4 dpf (i.e., when most beta-cells are in a resting phase) **Figure 1F).** Larvae were incubated in 1% DMSO as a negative control. **Tg(ins:mAG-zGeminin(1/100))** beta-cells in 4 dpf anesthetized larvae were counted by eye under an inverted fluorescence microscope. Beta-cell proliferation can be easily quantified because mAG-zGeminin(1/100) labels the nuclei of proliferating beta-cells with bright fluorescence. Fluorescent image at the bottom panel is a lateral view, anterior to the left and dorsal to the top (Scale bar = 100 μm). **(TIF)**

**Figure S2** The hit compounds increase the number of beta-cells undergoing S-phase. **(A) Tg(ins:Kaede) larvae** were treated from 3 to 5 dpf with 1% DMSO, 1 μM retinoic acid, 10 μM trazodone, or 10 μM prednisolone in the presence of 2.5 mM EdU. The numbers of **Tg(ins:Kaede)+** and Edu+ (red) beta-cells were increased in the animals treated with the hit compounds as compared to DMSO-controls. **(B)** Quantification of the number of **Tg(ins:Kaede)+** and EdU+ beta-cells. Retinoic acid (**n = 18 animals**) and prednisolone (**n = 16 animals**) significantly increased the number of Edu+ beta-cells compared to DMSO controls (**n = 15 animals**). Trazodone (**n = 17 animals**) only mildly increased the number of Edu+ beta-cell compared to DMSO controls consistent with its less potent effect on beta-cell proliferation (see Table 1); this effect was not statistically significant (N.S.) (p = 0.46). *p<0.05 and **p<0.01. Error bars represent SEM. **(TIF)**

**References**

1. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, et al. (2003) Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetologia 46: 102–110.
2. Wajchenberg BL (2007) beta-cell failure in diabetes and preservation by clinical treatment. Endocrine Rev 28: 187–218.
3. Borowiak M, Melton DA (2009) How to make beta cells? Current Opinion in Cell Biology 21: 727–732.
4. Bovens L, Rooman I (2005) Regulation of pancreatic beta-cell mass. Physiological Reviews 85: 1255–1270.
5. Amos JP, Ryu JH, Lam K, Carolan PJ, Utz K, et al. (2012) Adenosine kinase inhibition selectively promotes rodent and porcine islet beta-cell replication. Proceedings of the National Academy of Sciences of the United States of America 109: 3915–3920.
6. Andersson O, Adams BA, Yoo D, Ellis GC, Gut P, et al. (2012) Adenosine Signaling Promotes Regeneration of Pancreatic beta Cells In Vivo. Cell Metabolism 15: 805–814.
7. Rovira M, Huang W, Yusuff S, Shrim JS, Ferrante AA, et al. (2011) Chemical screen identifies FDA-approved drugs and target pathways that induce precocious pancreatic endocrine differentiation. Proceedings of the National Academy of Sciences of the United States of America 108: 19264–19269.
8. Gut P, Baeza-Raja B, Andersson O, Hasekamp L, Hsiao J, et al. (2013) Whole-organism screening for glucogenesisis identifies activators of fasting metabolism. Nature Chemical Biology 9: 97–104.
9. Sakauke-Sawano A, Kurokawa H, Morimura T, Hanyu A, Hama H, et al. (2008) Visualizing spatiotemporal dynamics of multicellular cell-cycle progression. Cell 132: 487–498.
10. Sugiyama M, Sakauke-Sawano A, Imura T, Fukami K, Kitaguchi T, et al. (2009) Illuminating cell-cycle progression in the developing zebrafish embryo. Proceedings of the National Academy of Sciences of the United States of America 106: 20812–20817.
11. Curado S, Anderson RM, Jungblut B, Mumm J, Schroeter E, et al. (2007) Conditional targeted cell ablation in zebrafish: A new tool for regeneration studies. Developmental Dynamics 236: 1025–1035.
12. Ninov N, Hesselson D, Gut P, Zhou A, Fidelin K, et al. (2013) Metabolic Regulation of Cellular Plasticity in the Pancreas. Current Biology 23: 1242–1250.
13. Ninov N, Borius M, Stainier DYR. (2012) Different levels of Notch signaling regulate quiescence, renewal and differentiation in pancreatic endocrine progenitors. Development 139: 1557–1567.
14. Eames SC, Philipson LH, Prince VE, Kinkel MD (2010) Blood sugar measurement in zebrafish reveals dynamics of glucose homeostasis. Zebrafish 7: 203–213.
15. Stainier DY, Lee RK, Fishman MC (1995) Cardiovascular development in the zebrafish. I. Myocardial fate map and heart tube formation. Development 119: 31–40.
16. Jurczyk A, Roy N, Bajwa R, Gut P, Lipson K, et al. (2011) Dynamic glucocregulation and mammalian-like responses to metabolic and developmental disruption in zebrafish. General and Comparative Endocrinology 170: 334–345.
17. Swenne I (1982) The Role of Glucose in the Invitro Regulation of Cell-Cycle Kinetics and Proliferation of Fetal Pancreatic B-Cells. Diabetes 31: 754–760.
18. Bernard G, Berthault MP, Saudnier G, Kiseva A, Neogenaesis vs. apoptosis as main components of pancreatic beta cell mass changes in glucose-infused normal and mildly diabetic adult rats. Faseb. Journal 13: 1195–1205.
19. Rabalch A, Cestari TM, Taboas SR, Bosciero AG, Bosqueiro JR (2009) High doses of desamethasone induce increased beta-cell proliferation in pancreatic rat islets. American Journal of Physiology-Endocrinology and Metabolism 296: E661–E669.
20. Fisharath H, Rhee JM, Swanson MA, Leach SD, Parsons MJ (2007) Targeted ablation of beta cells in the embryonic zebrafish pancreas using E-coli nitroreductase. Mechanisms of Development 124: 216–229.
21. Stafford D, Hornbruch A, Mueller PR, Prince VE (2004) A conserved role for retinoid signaling in vertebrate pancreas development. Development Genes and Evolution 214: 432–441.
22. Stafford D, White RJ, Kinkel MD, Linville A, Schilling TF, et al. (2006) Retinoids signal directly to zebrafish endoderm to specify insulin-expressing beta-cells (vol 133, pg 949, 2006). Development 133: 5001–5001.
23. Ostrom M, Loffler KA, Edfalk S, Selander L, Dahl U, et al. (2006) Retinoic Acid Promotes the Generation of Pancreatic Endocrine Progenitor Cells and Their Further Differentiation into beta-Cells. Plos One 3.
24. Cai J, Yu C, Liu YX, Chen S, Guo YX, et al. (2010) Generation of Homogeneous PDX1(+) Pancreatic Progenitors from Human ES Cell-derived Endoderm Cells. Journal of Molecular Cell Biology 2: 50–60.
25. Shi Y, Hou LL, Tang FC, Jiang W, Wang PG, et al. (2005) Inducing embryonic stem cells to differentiate into pancreatic beta cells by a novel three-step approach with activin A and all-trans retinoic acid. Stem Cells 23: 656–662.
26. Micallef SJ, Janes ME, Knezevic K, Davis RP, Elefanty AG, et al. (2005) Retinoic acid induces Pdx1-positive endoderm in differentiating mouse embryonic stem cells. Diabetes 54: 301–305.
27. Ng KY, Ma MT, Leung KK, Leung PS (2011) Vitamin D and Vitamin A Receptor Expression and the Proliferative Effects of Ligand Activation of These Receptors on the Development of Pancreatic Progenitor Cells Derived from Human Fetal Pancreas. Stem Cell Reviews and Reports 7: 53–63.