Original article

MOLECULAR METABOLISM

Autonomous interconversion between adult pancreatic α-cells and β-cells after differential metabolic challenges

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ABSTRACT

Background: Evidence hints at the ability of β-cells to emerge from non-β-cells upon genetic or pharmacological interventions. However, their quantitative contributions to the process of autonomous β-cell regeneration without genetic or pharmacological manipulations remain to be determined.

Methods & results: Using PANIC-ATTAC mice, a model of titratable, acute β-cell apoptosis capable of autonomous, and effective islet mass regeneration, we demonstrate that an extended washout of residual tamoxifen activity is crucial for β-cell lineage tracing studies using the tamoxifen-inducible Cre/loxP systems. We further establish a doxycycline-inducible system to label different cell types in the mouse pancreas and pursued a highly quantitative assessment to trace adult β-cells after various metabolic challenges. Beyond proliferation of pre-existing β-cells, non-β-cells contribute significantly to the post-challenge regenerated β-cell pool. α-cell trans-differentiation is the predominant mechanism upon post-apoptosis regeneration and multiparity. No contributions from exocrine acinar cells were observed. During diet-induced obesity, about 25% of α-cells arise *de novo* from β-cells. Ectopic expression of Nkx6.1 promotes α-to-β conversion and insulin production.

Conclusions: We identify the origins and fates of adult β-cells upon post-challenge upon autonomous regeneration of islet mass and establish the quantitative contributions of the different cell types using a lineage tracing system with high temporal resolution.

Keywords Lineage tracing; Adult β-cell origins; Nkx6.1; Tamoxifen artifacts

1. INTRODUCTION

Both type 1 and type 2 diabetes can be attributed to a failure of insulin-producing β-cells. For the development of regenerative therapies, the reconstitution of β-cell mass under metabolically challenging conditions has therefore attracted great research interest. Initial lineage tracing studies concluded that self-replication accounts for nearly all of the turnover and regeneration of adult β-cells [1,2]. Other investigators reported non-β-cell precursors for adult β-cells in specific contexts, such as pancreatic duct ligation [3–5], pregnancy [6], extreme β-cell ablation [7,8], or induced differentiation [9–15]. The concept of interconversion of different pancreatic cell types is emerging, but its quantitative contribution to the cell-autonomous process, i.e. without any genetic or pharmacological manipulations, of β-cells turnover upon metabolic challenges remains largely unknown. Furthermore, most of these studies used the tamoxifen-inducible CreER/loxP system to label specific pools of cells in a temporally controlled manner. However, recent observations have cast doubt on the ability of temporal control of gene expression/elimination with tamoxifen [16]. In adipocytes, the residual tamoxifen enables nuclear translocation of the CreER recombinase beyond two months of a washout period, along with significant metabolic side effects due to the pharmacological nature of tamoxifen as an estrogen receptor antagonist [17]. It is therefore essential to gain a better understanding of the actions of tamoxifen in the context of β-cell lineage tracing studies, compare it to other systems, and re-visit the data interpretation if necessary. As an alternative, the doxycycline-inducible system has proven to generate reliable lineage tracing results in adipose tissue, with fewer side effects and better temporal control on Cre recombinase activity [17,18]. As a common strategy for regenerative studies, β-cell ablation can be induced in adult rodents through a variety of methods, including pancreatectomy, pancreatic duct ligation, and administration of streptozotocin, alloxan, targeted delivery of diphtheria toxin, or targeted mutations in critical genes [19]. These approaches have

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disadvantages such as unspecific ablation [20], poor control over the extent of the ablation, unspecific toxicity, limited regenerative capacity post ablation [21], or irreversibly impaired functionality or viability of surviving β-cells. In contrast, the PANIC-ATTAC ("pancreatic islet β-cell apoptosis through targeted activation of caspase 8") transgenic mouse has been characterized as a model of inducible, titratable, acute β-cell apoptosis [22,23]. Administration of a chemical dimerizer, which is nontoxic and has a short half-life of around 5 h in mice, activates the intracellular apoptosis signaling in β-cells. In adult mice, the dimerizer-induced β-cell death peaks by 2 weeks post treatment, without any detectable inflammation (F4/80 or CD3 immunostaining, data not shown) or other side effects. More importantly, a moderate ablation, as indicated by a peak fed blood glucose level of 250–600 mg/dL accompanied by 50–75% β-cell loss, allows for autonomous recovery of β-cell mass and blood glucose by 10 weeks post treatment.

Here, we utilized the PANIC-ATTAC model to trace the regenerated β-cells with a tamoxifen-inducible labeling system. After a short washout of 1 week, the nuclear Cre was sustained by the residual tamoxifen activity and labeled most of the insulin$^+$ cells during the chase phase. In contrast, a much more extensive washout period of 4 months revealed ~20% β-cells with a non-β-cell origin. This was further confirmed independently with a doxycycline-inducible lineage tracing system. Such a significant contribution from non-β-cells was also evident after multiparity or high-fat diet-induced obesity. Labeling other pancreatic cells with the doxycycline system identified the glucagon-producing α-cell as a predominant source of β-cell trans-differentiation in the cases of post-apoptosis regeneration and multiparity. In contrast, in diet-induced obesity, β-cell conversion contributed to about one fourth of the expanded α-cell pool, explaining the disorganized distribution of α-cells post high-fat diet. In fact, *de novo* and/or trans-differentiation were active mechanisms to replenish α-cells. We failed to detect any acinar-to-β-cell trans-differentiation. Ectopic expression of Nkx6.1, a key transcription factor for β-cell differentiation [24] and identity [25], promotes α-cell trans-differentiation and systemic insulin production. Here, we provide comprehensive and highly quantitative measurements of the autonomous contributions from multiple pancreatic cell types to the adult β-cell pool upon different metabolic challenges. Our results suggest that adult β-cells preferentially originate from cells with relatively small developmental distance and high pre-existing abundance, and the relative contribution can be changed by metabolic insults or pharmacological interventions. We demonstrate the general usefulness of our lineage tracing system for the comprehensive and quantitative analysis of pancreatic cell fate and for the development of regenerative therapies.

2. MATERIALS AND METHODS

2.1. Mice

The transgenic mouse strains *MIP-CreER^{T2}*, *MIP-rtTA*, *PPG-rtTA* [26], and *TRE-Nkx6.1* were generated and recently characterized by our laboratory. The transgene constructs were generated by subcloning the coding DNA sequence (CDS) into a plasmid containing the promoter: *MIP-CreER^{T2}*, the 1983-bp *CreER^{T2}* CDS following a 8.3-kb mouse insulin 1 promoter; MIP-rtTA, the 747-bp *rtTA.M2* CDS following a 8.3-kb mouse insulin 1 promoter; PPG-rtTA, the 747-bp *rtTA.M2* CDS following a 1.7-kb mouse preproglucagon promoter; TRE-Nkx6.1, the 1098-bp golden hamster *Nkx6.1* CDS following a 0.3-kb TRE-tight promoter. The PANIC-ATTAC transgenic mouse was generated by our laboratory as previously described [22]. The mouse strains *TRE-Cre* (#006234), *RIP-rtTA* (#008250), *Rosa26-LacZ* (*Rosa26-loxP-STOP-loxP-LacZ*, #003474), *Rosa26-Tomato* (*Rosa26-loxP-STOP-loxP-tdTomato Ai9*, #007909), and *Ptf1a-rtTA* (#018070) were purchased from the Jackson Laboratory. All mice were bred in the C57BL/6 genetic background. Mice were fed on regular (LabDiet #5058), high-fat (60%, Research #D12492), or doxycycline chow diet (600 mg/kg, Bio-Serv #S4107). Mice were maintained in 12-h dark/light cycles, with *ad libitum* access to diet and water. All protocols for mouse use and euthanasia were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center.

2.2. Genotyping PCR

Approximately 3 mm of mouse tail tip was incubated in 80 μL 50 mM NaOH at 95 °C for 1.5 h. 8 μL 1 M Tris–HCl (pH 8.0) was added for neutralization. After vortexing and a short spin down, 0.5–1 μL of supernatant was used as PCR template. Primer sequences for genotyping PCR are listed in Table S1. The PCR program was: 95 °C for 5 min, followed by 35 cycles of 95 °C for 15 s, 62 °C for 30 s, and 72 °C for 30 s, and ended with 72 °C for 3 min.

2.3. Tamoxifen administration

A 25-mg tamoxifen citrate pellet with a release time of 21 days (Innovative Research #E-351) was implanted subcutaneously, and the mice were housed individually during the release period.

2.4. Dimerizer administration

Mice were subjected to one intraperitoneal injection of the dimerizer AP20187 (Clontech #635059) at the dose of 0.3–0.5 μg/g body weight/day. The dimerizer stock solution was stored at −80 °C, and freshly diluted in 2% Tween-20 with 10% polyethylene glycol 400 before injection.

2.5. Multiparity

Adult female mice were mated to be pregnant at least three times and sacrificed for pancreas paraffin sections during the last pregnancy, at around 15.5 days post-coitus.

2.6. β-gal staining

Mice were subjected to isoflurane anesthesia and cardiac perfusion of 0.2% glutaraldehyde in PBS (10–15 mL per mouse). Tissues were immediately dissected, transferred to 20-mL scintillation vials with 0.2% glutaraldehyde in PBS, and minced into 1–3 mm wide slices. Tissue slices were washed with rinse buffer (0.1 M sodium phosphate, 2 mM $MgCl_2$, 0.01% sodium deoxycholate, and 0.02% NP-40, pH 7.3) three times for 30 min and incubated with X-gal staining buffer (1 g/L X-galactoside, 5 mM $K_3[Fe(CN)_6]$, and 5 mM $K_4[Fe(CN)_6]$ in rinse buffer) in the dark, at room temperature, with shaking at 100 rpm, for 24 h. Tissues slices were then fixed in 10% formalin overnight and briefly rinsed three times with 50% ethanol. In the University of Texas Southwestern Medical Center Molecular Pathology Core, tissue slices were embedded in paraffin blocks, and the sections were counterstained with Nuclear Fast Red. Bright field images were acquired on a Nikon Coolscope digital microscope or an Olympus FSX100 all-in-one microscope.

2.7. Immunohistochemistry

Mouse tissues were collected and processed for paraffin sections as previously described [23]. Primary antibodies and dilution for immunostaining or immunofluorescence were: β-galactosidase (1:50, Abcam #ab9361), insulin (1:500, Dako #A0564), Cre (1:100, Millipore # 69050-3), RFP for tdTomato (rabbit polyclonal, 1:100, Abcam

