Original Article

MOLECULAR METABOLISM

# Modeling human pancreatic beta cell dedifferentiation

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## ABSTRACT

**Objective:** Dedifferentiation could explain reduced functional pancreatic β-cell mass in type 2 diabetes (T2D).
**Methods:** Here we model human β-cell dedifferentiation using growth factor stimulation in the human β-cell line, EndoC-βH1, and human pancreatic islets.
**Results:** Fibroblast growth factor 2 (FGF2) treatment reduced expression of β-cell markers, (*INS, MAFB, SLC2A2, SLC30A8*, and *GCK*) and activated ectopic expression of *MYC, HES1, SOX9*, and *NEUROG3*. FGF2-induced dedifferentiation was time- and dose-dependent and reversible upon wash-out. Furthermore, FGF2 treatment induced expression of *TNFRSF11B,* a decoy receptor for RANKL and protected β-cells against RANKL signaling. Finally, analyses of transcriptomic data revealed increased FGF2 expression in ductal, endothelial, and stellate cells in pancreas from T2D patients, whereas FGFR1, SOX,9 and HES1 expression increased in islets from T2D patients.
**Conclusions:** We thus developed an FGF2-induced model of human β-cell dedifferentiation, identified new markers of dedifferentiation, and found evidence for increased pancreatic FGF2, FGFR1, and β-cell dedifferentiation in T2D.


**Keywords** Beta-cell; Dedifferentiation; Type 2 diabetes; Human

## 1. INTRODUCTION

Type 2 diabetes develops as a consequence of a combination of insulin resistance and insufficient β cell mass. The decline in β cell mass has been attributed to a decrease in both β cell number and function [1]. It has been suggested that decreased β cell mass is the result of premature programmed cell death and research efforts focused on increasing β cell survival [2]. However, recent data challenge the β cell death hypothesis. These data suggest that β cell dedifferentiation represents an alternative mechanism to explain loss of functioning β cells [3]. During dedifferentiation, β cells do not undergo apoptosis. Rather, they lose identity and function with a decrease in expression of key β cell markers such as genes encoding major transcription factors, e.g. MafA, and proteins implicated in glucose-stimulated insulin secretion, e.g. Slc2a2 (Glut2) [4].
This concept of β cell dedifferentiation is now strongly supported by experiments performed in several murine models and based on lineage-tracing of β cells [3,5]. On the other hand, data on human β cell dedifferentiation remain scarce and mainly based on experiments performed on static models such as human pancreatic tissue sections. For example, studies at the electron microscopy level showed that pancreata from type 2 diabetic donors contain degranulated β cells that are depleted of insulin [6]. Pancreatic sections of type 2 diabetic patients demonstrated a 3-fold increase in the number of endocrine cells (Chromogranin A-positive) that stain negative for insulin, glucagon, somatostatin, or pancreatic polypeptide [7]. Such dedifferentiated human β cells, however, expressed the endocrine progenitor cell marker aldehyde dehydrogenase 1A3 (ALDH1A3) [7].
Our study aimed at developing a dynamic model of human β cell dedifferentiation. Access to preparations of primary human β cells is limited and human-translatable models to study their dedifferentiation are lacking. Here, we used EndoC-βH1, a recently developed functional human β cell line that was generated by lentiviral transduction of human fetal pancreas with oncogenes expression driven by the insulin promoter [8]. This line represents an efficient tool to study human β cells in pathophysiological conditions [8–10].
Our work specifically aimed at: i). Discovering molecules and pathways that induce human β cell dedifferentiation ii). Discovering new positive markers of human β cell dedifferentiation iii). Defining whether human β cell dedifferentiation is a reversible process and iv). Identifying new evidences of dedifferentiation in human islets from type 2 diabetic individuals. Here, we discovered that two members of the fibroblast

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growth factor family, fibroblast growth factor 1 (FGF1) and fibroblast growth factor 2 (FGF2), induce human β cell dedifferentiation, in a reversible manner. We also identified several transcription factors, SOX9, HES1, MYC, and TNFRSF11B, as new positive markers of human β cell dedifferentiation and showed that pancreata from type 2 diabetic patients display sustained expression of both FGF2/FGFR1 and our novel markers of dedifferentiation.

## 2. METHODS

### 2.1. Culture of human β cell lines and treatments

EndoC-βH1 cells were cultured in low-glucose (5.6 mM) Dulbecco's modified Eagle's medium (DMEM; Sigma—Aldrich) containing 2% BSA fraction V (Roche), 50 μM 2-mercaptoethanol (Sigma—Aldrich), 10 mM nicotinamide (Calbiochem), 5.5 μg/ml human transferrin (Sigma—Aldrich), 6.7 ng/ml sodium selenite (Sigma—Aldrich), and 100 units/mL Penicillin and 100 μg/mL Streptomycin (ThermoFischer Scientific) as previously described [8]. Cells were seeded at a density of $9.10^4$ cells/$cm^2$ on Matrigel (1.2%; Sigma—Aldrich) and Fibronectin (3 μg/ml; Sigma—Aldrich) -coated plates and cultured at 37 °C in 5% $CO_2$. EndoC-βH1 cells were treated with either recombinant human FGF1 (100 ng/mL) plus heparin sodium salt (2 μg/mL) (both from Sigma—Aldrich), recombinant human FGF2 (100 ng/mL, Peprotech), recombinant human Soluble RANK Ligand Protein (Merck Millipore), recombinant Human Osteoprotegerin/TNFRSF11B Protein (R&D Systems), or U0126 (Calbiochem).

### 2.2. Human islets

Human islets were obtained from 8 donors (from M.A., Paris; LM and PM, Pisa; and commercially from Prodo Laboratories Inc.). They were cultured during three days in medium supplemented with either FGF2 or with the FGF2 carrier (PBS-BSA 0.2%).

### 2.3. RNA isolation, reverse transcription, and real-time PCR

RNeasy Micro kit (Qiagen) was used to extract total RNA from EndoC-βH1 cells and from human islets. Maxima First Strand cDNA synthesis (ThermoFischer) was used to synthesize cDNA. Quantitative RT-qPCR was performed using Power SYBR Green mix (Applied Biosystems) with a QuantStudio analyzer (Applied Biosystems). Custom primers were designed with NCBI Primer-BLAST [11] and their efficiency was determined following serial dilutions of cDNA samples from EndoC-βH1-cells or human islets. Cyclophilin-A transcript levels were used for normalization. The list of primers is presented in Table S1.

### 2.4. Transcriptome analysis

Total RNA was isolated using RNeasy Micro kit (Qiagen). The quality of the RNA was assessed by a Fragment Analyzer (Advanced Analytical Technologies). One microgram of total RNA was used for each library. The RNA samples were processed with Illumina TruSeq Stranded mRNA Library prep kit following the manufacturer's recommendations. Libraries were quantified with Qubit HS (ThermoFisher) and Fragment Analyzer (Advanced Analytical Technologies). Indexed libraries were pooled and sequenced on an Illumina NextSeq 500 high-output run using paired-end chemistry with 75 bp read length.

### 2.5. Processing of RNA-Seq data and differential expression analysis

Paired-end reads were generated using an Illumina NextSeq 500 and processed using the RNA-seq pipeline implemented in the bcbio-nextgen project [https://bcbio-nextgen.readthedocs.org/en/latest/]. Reads were aligned to the human genome version hg38 using the Hisat2 aligner [12].

Counts of reads aligning to known genes are generated by featureCounts [13]. Differential expression analysis was performed using DESeq2 [14]. All data are MIAME compliant, and the raw data have been deposited in a MIAME compliant database (GEO, accession number GSE103383).

### 2.6. Analysis of published transcriptomes

Raw data from GSE25724 microarray study was downloaded from the GEO database. Differential gene expression was assessed as previously described [15,16]. The moderated t-statistics within the limma package were used to obtain p-values for differential expression of genes under investigation [17]. If multiple probes mapped to the same gene, only the probe with smallest p-value was retained. Given the small number of individual hypothesis tests, we did not further adjust p-values for multiple testing.

For the single-cell RNA sequencing studies (E-MTAB-5061), we downloaded fastq files from the GEO database and aligned them to human genome version hg38 using the hisat2 aligner [12], followed by quantitation using the Salmon algorithm [18] and cpm (counts per million) normalization. Differential expression analysis was carried out using the MAST (model-based analysis of single-cell transcriptomics) R-package [19]. In addition to the number of detected genes per cell and disease condition, we included ethnicity and gender as covariates in the model to prevent erroneous differential expression calls due to different ethnicity or gender distributions in the control and case group. P-values were then derived from a likelihood ratio test carried out with the generalized linear model parameters of the case and control group.

### 2.7. Immunostaining of EndoC-βH1 cells and primary pancreatic islets

EndoC-βH1 cells were cultured on 12-mm Matrigel/fibronectin-coated glass coverslips. They were treated with FGF2 for 3 days, fixed for 1 h with 4% paraformaldehyde, permeabilized, and stained with a rabbit anti-SOX9 antibody (1/500; Millipore) that was revealed with a Goat anti-Rabbit IgG Alexa Fluor 488 conjugate (Life Technologies). Human pancreatic islets were treated for 72 h with FGF2 and fixed in 3.7% formaldehyde prior to embedding in paraffin. Sections (4-μm thick) were prepared and processed, as described [20]. The sections were stained with the following antibodies: mouse anti—chromogranin A (1/50; Dako Cytomation); rabbit anti-SOX9 antibody (1/500; Millipore). The secondary antibodies were Goat anti-Rabbit IgG Alexa Fluor 488 conjugate (Life Technologies) and Goat anti-mouse IgG Alexa Fluor 594 conjugate (Jackson ImmunoResearch). Digital images of EndoC-βH1 cells were captured using a cooled 3-chip charge-coupled device camera (Hamamatsu C5810; Hamamatsu) that was attached to a fluorescent microscope (Leica; Leitz).

### 2.8. Western blot analyses

Protein lysates prepared in RIPA buffer were subjected to immunoblotting. The following antibodies were used: MAFA (1/500; a gift from Dr. Rezania, BetaLogics Venture, New Jersey, USA), SOX9 (1/500; Millipore), HES1 (1/1,000, Cell-Signaling), ZNT8 (1/1,200, clone 17H2.4 generously provided by Dr. Davidson, Barbara Davis Center, Denver, Colorado), PDX1 (1/2,000; [20]), phospho-P38 MAPK (1/1,000; Cell Signaling), P38 MAPK (1/1,000, Cell Signaling), and TUBULIN (1/2,000; Sigma—Aldrich). Species-specific HRP-linked secondary antibodies (Cell Signaling) were added on the membrane and visualization was performed on an ImageQuant LAS 4000

 