FID-ISPAD Diabetes Research Grant

Project: EXPLORING THE ROLE OF IRON IN ENHANCING THE MATURATION, FUNCTION, AND SURVIVAL OF PANCREATIC BETA CELLS

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Progress Report

Planned aim

We aimed to investigate the role of iron availability during the differentiation and maturation of stem cell-derived (SC) beta cells by modulating iron supply at distinct developmental stages. Iron deficiency would be induced through exposure to 100 µM deferoxamine (DFO) and iron supplementation by exposure to 500 µM Ferric Citrate (FeCitr). The effects of iron deprivation are ideally assessed by evaluating cell morphology and cell death, transcript and protein expression (including transcriptomic profiling), glucose-stimulated insulin secretion, and cellular metabolism using Seahorse extracellular flux analysis.

Balance and use of the funds

- Expenditures: 15.226,57 EUR
- Unexpended, requested for no cost extension: 8.250,77 EUR

Explanation expenditures

The grant funding was used to support the purchase of essential research materials and laboratory reagents required for the project. This included antibodies, ELISA kits, and reagents for flow cytometry (FACS), as well as other consumables (i.e., culture medium and supplements) and core facility costes necessary to perform the planned experiments. These expenditures directly contributed to the successful execution of the research plan, the generation of key results, and the publication of a research article. The unexpended funds are currently being used to build on our existing dataset and assess iron metabolism in the context of inflammatory cytokines, more specifically the role of the Divalent Metal Transporter (DMT)-1 in pancreatic beta cells.

Research progress

We were able to complete the planned experiments for the study “Exploring the role of iron in enhancing the maturation, function, and survival of pancreatic beta cells”. Using SC-beta cells, we modulated iron supply by chelating iron with deferoxamine (DFO) at distinct stages of SC-beta differentiation. The effects of altered iron availability were assessed using complementary approaches, including cell viability, immunofluorescence, ELISA-based glucose-stimulated insulin secretion assays, gene expression analyses, and transcriptomics.

Our findings demonstrated that iron is a critical metabolic regulator during beta cell maturation. Iron deprivation selectively impaired oxidative metabolism, reduced beta cell function, and induced beta cell death in immature (SC-)beta cells, whereas mature (SC-)beta cells were largely resistant to iron deficiency. This identifies a previously unrecognized maturation-dependent switch in iron dependency of the pancreatic beta cells. Mechanistic studies using murine models demonstrated that iron regulates mitochondrial function and metabolic maturation, providing new insight into the requirements for generating functional SC-beta cells. These findings culminated in the publication "Iron deficiency induces maturation-dependent loss of pancreatic β-cells" in Nature Communications (2026), highlighting iron as a key determinant of beta cell maturation and survival, thereby providing a framework for further improving SC-beta cell generation for diabetes research and cell replacement therapies.

Results

(Adapted from "Van Mulders, A., Willems, L., Coenen, S. et al. Iron deficiency induces maturation-dependent loss of pancreatic β-cells. Nat Commun 17, 2826 (2026). https://doi.org/10.1038/s41467-026-69574-y)")

We used human iPSC to model developing β-cells using a 7-stage protocol followed by long-term (LC) culture29,30. Six-day DFO exposure at the endocrine progenitor stage 5 (S5) severely disrupted aggregate morphology and viability, increasing cell death ~20-fold (Figure 1a-b). Sensitivity to iron depletion decreased with maturation: immature stage 7 (S7) and LC iPSC-β-cells aggregates exhibited reduced and delayed cell death following DFO treatment (Figure 1a-b). Consistent with our prior mouse and human models, iron deprivation upregulated TFRC expression in both S7 and LC aggregates (Figure 1c).

To further dissect responses in immature β-cells, we performed RNA sequencing on magnetic-activated cell sorting (MACS)-purified S7 β-cells exposed to DFO (with a mean ± s.e.m. composition of 75.4 % ± 5.2 INS+, 4.4% ± 1.9 GCG+, 1.7% ± 0.7 SST+, and 3.9% ± 0.1 polyhormonal cells, n = 4 independent differentiations). Differential expression analysis identified 38 significantly altered genes (FDR < 0.05, |log2fc | >0.58), including upregulation of *TFRC*, *LDHA*, *SLC11A2*, *SLC40A1*, *HK2*, *SLC2A1*, *NDRG1*, and *IGFBP3* (Figure 1d). Upregulation of iron import (*TFRC* and *SLC11A2*) and glycolysis (*HK2*, *LDHA*, *SLC2A1*) genes suggest a metabolic shift from oxidative phosphorylation toward glycolysis under iron-limited conditions (Figure 1d). Gene set enrichment analysis confirmed increased glycolysis and iron transport activity, along with downregulation of respiratory chain components (Figure 1e).

Finally, mature LC iPSC-β-cell function remained intact under iron deprivation, with preserved insulin content and glucose- and forskolin-stimulated insulin secretion (Figure 1f-i).

Collectively, these findings mirror our observations in mice, demonstrating that TFRC is consistently upregulated under iron deprivation and that TFRC-dependent iron uptake is critical for the survival of immature human β-cells but becomes dispensable with β-cell maturation.

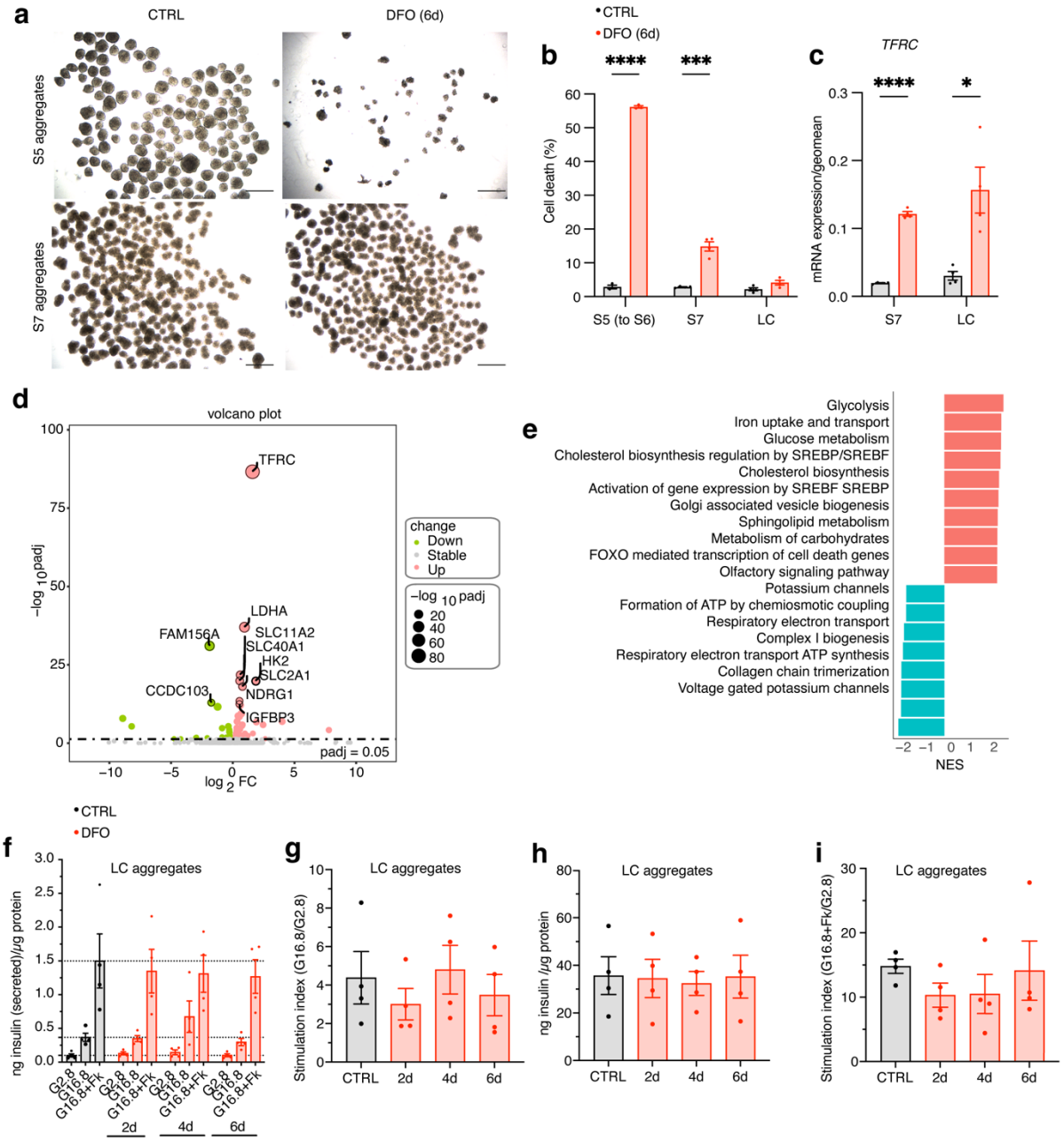

Figure 1. Iron requirements of pancreatic beta cells are maturation-stage dependent. ***(a)*** *Brightfield images of stage 5 (S5) and stage 7 (S7) iPSC-β-cell aggregates upon exposure to DFO for 6 days. Scale bar = 400 µm.* ***(b)*** *Cell death (%) in S5, S7, and long-cultured (LC) iPSC-β-cell aggregates upon exposure to DFO for 6 days (*** = 0.0003, **** <0.0001). S5 n = 3, S7 and LC n = 4 biologically independent replicates per group.* ***(c)*** *qRT-PCR of TFRC expression in S5, S7, and LC iPSC-β-cell aggregates upon exposure to DFO for 6 days (* = 0.0105, **** <0.0001). Expression levels normalized to the geometric mean of ACTB and VAPA. n = 4 biologically independent replicates per group.* ***(d)*** *Volcano plot showing the differential gene expression between CTRL and S7 MACS-purified iPSC-β-cell aggregates exposed to DFO, identified using Wald tests with Benjamini-Hochberg false discovery rate correction (FDR < 0.05 & |log2fc | >0.58).* ***(e)*** *Gene set enrichment analysis of S7 MACS-purified iPSC-β-cell aggregates exposed to DFO.* ***(f)*** *Insulin (ng) secreted in response to 2.8 mmol/L and 16.8 mmol/L glucose, and 16.8 mmol/L glucose with 10 µmol/L forskolin (Fk) by LC iPSC-β-cell aggregates exposed or not (gray bars) to DFO (red bars) for 2, 4, and 6 days.* ***(g)*** *Stimulation index (insulin release at G16.8/G2.8).* ***(h)*** *Insulin (ng) content of LC aggregates exposed or not (gray bars) to DFO (red bars) for 2, 4, and 6 days.* ***(i)*** *Stimulation index (insulin release at G16.8+Fk/G2.8). n = 4 biologically independent replicates per group and analyzed by one-way ANOVA and Dunnett's multiple comparisons test.*