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Greasing the machinery toward maturation of stem cell-derived β cells

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Getting mature and functional stem cell-derived, insulin-producing β cells is an important step for disease modeling, drug screening, and cell replacement therapy. In this issue, Hua et al.[1] used single-cell multiomics analysis coupled with chemical screening to identify a crucial role for ceramides in generating mature stem cell-derived β cells.

Loss of the mature and functional insulin-producing β cell mass is a critical event in the development and progression of diabetes. Replacing the lost cells with β cells derived from pluripotent stem cells (PSCs) therefore holds great promise as a therapeutic option. A potentially unlimited β cell source like this could be used in cell replacement therapy for the management of diabetes, and initial results from clinical trials implanting encapsulated stem cell-derived β cells (SC-β cells) into patients with type 1 diabetes are indeed promising.[2,3] Yet, the final SC-β cell product, produced using current state-of-the-art protocols, cannot achieve the same maturation level as the *in vivo* cell population.[4] The work published in the current issue of *Cell Stem Cell* by Hua et al.[1] aimed to address this knowledge gap, advance our basic understanding of functional SC-β cell differentiation, and improve current protocols.

It is established that *in vitro* differentiated SC-β cells can further mature following transplantation in mice.[5] In the current work,[1] the researchers profiled the maturation process leading to improved functionality of SC-β cells after transplantation *in vivo* to better understand the drivers and extrapolate the knowledge to improve current *in vitro* differentiation protocols. To address this, they used current state-of-the-art multiomics methodologies, single-cell RNA sequencing (scRNA-seq) combined with single-cell assay for transposase-accessible chromatin (ATAC) sequencing (scATAC-seq), to profile gene expression and chromatin accessibility of SC-β cells at several time points following transplantation (0, 3, 7, 14, 30, 42, 56, and 98 days post transplantation). The 8-week post-transplantation timeline was carefully curated as the peak of *in vivo* SC-β cell maturation using a wide array of biochemical and molecular approaches. In parallel, the authors used available scRNA-seq datasets of primary human β cells, the *in vivo* counterpart, comparing them with the SC-β cell data to understand the molecular differences and drivers of SC-β cell maturation *in vivo*. The integrated computational analysis across all modalities and samples suggested that β cells found in the SC-β cell clusters, which contain all cell types of the pancreatic islets together with their developmentally related progenitors, quickly resolved into more mature states as early as 1 week after transplantation. Comparing the SC-β cells to the primary human β cells using pseudotime trajectory inference clearly showed that the SC-β cells are closely related to the juvenile/adult human β cells at the end of the transplantation period. Further integration of scRNA-seq and scATAC-seq datasets suggested that the maturation process was characterized by extensive gene expression reduction and chromatin closure. The authors identified four distinct ways for the observed global gene expression reduction during maturation acquisition of SC-β cells to proceed from the initial steps of metabolic rewiring to a maturation transcriptional signature, leading to the final phase of the establishment of the distinct molecular β cell

signature. In conclusion, this work provides an extensive temporal molecular profile of the *in vivo* maturation process of transplanted SC-β cells that highlights an important role for metabolic rewiring, especially of the mitochondrial and lipid metabolisms.

Intrigued by the prominent role of metabolic processes in SC-β cell maturation, the authors carefully designed a chemical screen to target what they refer to as "maturation dominators." Of note, maturation dominators were deduced from the integrated scRNA-seq and scATAC-seq modalities comparing immature to mature SC-β cell states and their correlation with domains of regulatory chromatins and genes with predictive chromatin status. The drug candidates were chosen using the Enrichr computational pipeline based on their predicted ability to modulate expression of immature and mature genes. The chemical screen was performed *in vitro.* SC-β cells were treated for 7 days with the compounds, and glucose-stimulated insulin release was used as a readout for SC-β cell functional maturation. The chemical screen identified four primary hits (all belonging to the histone deacetylase [HDAC] inhibitor class of chemicals) that could improve functionality of SC-β cells, with the most prominent being the small molecule TH34, an HDAC6/8/10 inhibitor. scRNA-seq of SC-β cells before and after treatment with TH34 confirmed the maturation of SC-β cells on the molecular level, as defined by upregulation of the mature β cell marker genes *MAFA*, *IAPP*, and *NFIC*.

The authors used computational pipelines coupled with metabolite measurements to identify the mechanism of action of TH34-induced SC-β cell maturation and link the drug-induced phenotype to the observed metabolic rewiring identified in the first part of their study. This analysis showed that TH34 altered the sphingolipid metabolism and, specifically, ceramide accumulation in SC-β cells. Reducing ceramide accumulation in SC-β cells improved their insulin secretion capacity, whereas supplementation of the media with exogenous ceramide could reverse the TH34-induced maturation phenotype. These experiments provided a link connecting TH34 treatment, ceramide metabolism, and SC-β cell maturation.

Overall, the work by Hua et al.[1] advances our understanding of the molecular pathways involved in functional maturation of SC-β cells after transplantation. This study has the advantage of a detailed temporal molecular profiling of this process using multimodal sequencing-based technologies. Combining this with work from other laboratories,[6–9] which utilized human embryonic and induced PSCs as starting material, we now have a comprehensive resource to understand the functional maturation of SC-β cells following transplantation. A direct comparison of all multiomics studies would benefit the community and identify common drivers of SC-β cell maturation across modalities, differentiation protocols, and starting stem cell materials. Moreover, the finding that the lipid metabolism is a targetable pathway to increase SC-β cell maturation *in vitro* can improve current protocols through the addition of the TH34 molecule in the last differentiation step. This observation builds on recent reports showing a link between glucose and mitochondrial metabolism and SC-β cell maturation status[8] and a role of ceramide synthase 2 in insulin processing[10] and reports a previously unappreciated role for sphingolipid metabolism in the maturation status of SC-β cells *in vitro*. Given the strong effects of the TH34 treatment, it would be interesting to follow up on the mechanism of action of this drug and identify the main HDAC that is inhibited as well as how it connects to ceramide metabolism and is responsible for the phenotype. Overall, this work offers innovative insights into the maturation process of SC-β cells, implicating ceramide metabolism in the process with the eventual aim of improving the SC-β cell product to be used for cell therapy in diabetes but also for drug screening and disease modeling.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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