RESEARCH HIGHLIGHT

Pancreatic endocrinogenesis revisited: “I have all the answers, who has the questions?”

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The endocrine pancreas is composed of islets of Langerhans that are scattered throughout the gland and contain multiple hormone-producing cell types (α , β , δ , PP and ϵ cells); endocrine progenitors (EPs) can differentiate into these distinct lineages, but the regulation of their fate choice — especially for the low-abundance δ , PP and ϵ cells — remains incompletely understood. In a paper published in *Cell Research*, Yu et al. provide a notable resource to decipher the differentiation pathways of murine and human EPs.

Pancreatic endocrine cells develop during prenatal life from endocrine progenitors (EPs). This process of endocrinogenesis is complex and currently incompletely understood. It is important to better understand this developmental process for the sake of gaining knowledge, but also because it serves as our guide to make hormone-producing cells from stem cells which may in the future be used as a curative cell therapy in people with diabetes. In a study published in *Cell Research*, Yu et al.¹ resolved an important temporal dimension of the fate choices of pancreatic EP. First, the authors set out to transcriptionally profile all mouse endocrine lineages using single-cell RNA sequencing (scRNA-seq). Yu et al. did so by using both a high-quality Smart-seq2 approach on sorted cells of reporter mice and an unbiased high-throughput 10 $\times$ Genomics approach on dissociated cells from dorsal and ventral pancreas at different embryonic and postnatal ages during endocrinogenesis. Moreover, to generate comprehensive Smart-seq2 datasets, they complemented pre-existing data from reporter mice for pancreatic progenitor (PP), EP, α and β cells, with new data of ϵ , δ and PP cells which they sorted from newly generated reporter mouse lines. Both approaches independently led to the identification of 10 major endocrine cell types — 5 endocrine progenitor clusters and 5 hormone-producing cell clusters — of which they next determined the developmental trajectory. They were able to unravel the temporal allocation pathways of all pancreatic endocrine lineages and verified the different branch nodes on the trajectory using genetic tracing methods, advanced bioinformatic analyses and validation at the protein level by immunostaining. Remarkably, after having done so comprehensively for mice, the authors turn to human endocrinogenesis and demonstrate both overlap and some interesting interspecies differences in the developmental trajectory of the pancreatic endocrine lineages.

The study by Yu et al. is impressive both in comprehensiveness and thoroughness. It offers a new and important resource with single-cell transcriptomic data that covers endocrinogenesis

better than any previous work. However, this study brings to mind an image recurrently presented by the senior author (RS) to challenge junior author (WS) during scientific discussions: *An old rabbi walks along the Wailing Wall in Jerusalem and acclaims loudly: “I have all the answers, who has the questions?”* (see ‘[I have all the answers, who has the questions?](#)’). Indeed, this work is a technological tour de force that possibly provides us with all transcriptomic answers to pancreatic endocrinogenesis, but its full biological importance will reveal itself in how the data will be used.

Illustration by Willem Staels

One intriguing observation of the authors that requires further study is the nonlinearity of endocrinogenesis. Yu et al. demonstrate for example that early α and PP cells are quite heterogeneous and they attribute this heterogeneity to different progenitors from which these cell types can differentiate. The authors conclude that “various states of EP cells create different permissive windows for the generation of certain endocrine lineages”. These findings are reminiscent of how single-cell technologies improved our understanding of the dynamics of hematopoietic stem cell differentiation and revealed that this process is far less hierarchical and limited than assumed in past Waddingtonian views.²

Some more outstanding questions are approached in this study: What is the origin of β cell heterogeneity, is it important, and how is it maintained? Yu et al. describe in detail two developmental branches that generate β cells: a β^{early} branch primarily consisting of E13.5–E15.5 cells and a β^{late} branch mainly composed of E16.5–P3 cells. This resonates with discoveries made nearly 50

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years ago by Pictet and Rutter describing based on ultrastructural analysis of pancreas development two waves of pancreatic endocrinogenesis³ and with more recent data describing the in vitro differentiation of multipotent stem cells into β cells resembling β^{early} and β^{late} cells.^{4,5}

Dissecting the distinct origin of different subtypes of β cells is innovative and may allow us to generate those subtypes that are of interest to a regenerative therapy in diabetes, for example, the Fltp⁺ β cells with enhanced proliferative capacity,⁶ the Ucn3⁺ virgin β cells that characterize a neogenic niche in the adult islet,⁷ and the “hub” or “leader” β cells that coordinate the insulin secretion response.⁸

The observed differences between mouse and human also merit further study especially regarding the less abundant cell types. Yu et al. confirmed the observation that *Pax6* expression in ϵ cells is restricted to mouse and showed that the same holds true for *Irx2*. On the contrary, *ERO1B* is specifically expressed in human δ cells, whereas *MEF2C* is restricted to mouse δ cells. The genetic networks that regulate endocrinogenesis in humans and mice might thus differ, but the extent to which this is the case and whether this translates into the acquisition of distinct functional characteristics remains to be investigated.

As a last example, the authors could not identify Procr as an EP marker, which contrasts with its recent identification as a surface marker of progenitor cells that persists in adult mouse islets,⁹ prompting the need for further studies to validate Procr.

There are still ample opportunities to learn more about pancreatic endocrinogenesis, for example by integrating dimensions that are currently understudied in this field: (i) proteomics on sorted subsets or at the single-cell level — proteins are the main

drivers of cellular function and recent technical progress now makes such studies feasible,^{10,11} (ii) spatial information by expanding the distinction made by the authors between dorsal and ventral pancreas (e.g., head, body and tail regions could be separated, followed by a distinction between periductal, truncal and tip tissues) or more sophisticated by 3D analyses of cell interactions,¹² and finally (iii) integrating the environmental cues that assist cell fate choices.

The blueprint of pancreatic endocrinogenesis offered by Yu et al. is likely to push the field forward as it offers great clarity and support for future work that is aimed at answering some of the above outstanding questions or at tackling the integration of multiple biological dimensions. It will be interesting to see whether this study that is based on frontier technologies will also improve our understanding of monogenic diabetes syndromes.

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