**A Single-cell Atlas of the Developing Human Pancreas**

Z. Liu,1 D.M. Wong,1 S. de la O,1 J.O. Bouza,1 R. McMullen,1 J.B. Sneddon.1,2,3 *1Diabetes Center;2 Department of Anatomy; 3Broad Center of Regeneration Medicine and Stem Cell Research, University of California, San Francisco, CA.*

**Background:** The mammalian pancreas arises through a series of coordinated events, including specification, proliferation, differentiation, and maturation. Despite substantial progress in understanding the signaling events underlying these processes in rodent models, a global view of the timing and dynamics of these processes in the developing human pancreas has not previously been possible.

**Methods:** We have used a combination of single-cell RNA-sequencing, pseudo-temporal ordering analysis, immunohistochemistry, and *in situ* hybridization to construct a transcriptional roadmap of the human prenatal pancreas, across developmental time and at single-cell resolution.

**Results:** This roadmap serves as a guidebook for human pancreatic development, predicting the existence of novel intermediate progenitor states and lineage relationships, and characterizing cellular dynamics across developmental time. In the exocrine lineage, we have identified key cellular stages through which cells transit as they differentiate to acinar or ductal fates.

Within the endocrine lineage, we have characterized a distinct progenitor population defined by differential expression of the transcription factor *FEV* and reconstructed lineage relationships among *NGN3*+, *FEV*+, and differentiated, hormone+ cells. We have further identified candidate transcriptional regulators along the differentiation trajectory of the *FEV*+ progenitor population towards the beta cell lineage. Lastly, we have integrated genome editing techniques into a stepwise differentiation platform for generating beta cells from human embryonic stem cells (hESCs), thereby establishing a platform for interrogating the function of putative transcriptional regulators of human endocrine cell fate identified in our study.

**Conclusion:** In summary, these studies have revealed heretofore unknown cellular heterogeneity in the developing human pancreas. In addition to contributing to our knowledge about the basic developmental biology of this organ, this work will also provide important clues as to which cellular states should be recapitulated *in vitro* in directed differentiation experiments aimed at generating truly bona fide beta cells from hESCs for patients with diabetes.