

**Title: Transcriptional changes during pancreatic endocrine differentiation in real time at single-cell level**

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**a. Background/Significance to NETs:**

Although they constitute only 1-2% of the total pancreatic mass, dysfunction of pancreatic endocrine cells, caused by mutations, deregulated differentiation or hormone secretion, can result in diseases such as pancreatic neuroendocrine tumours (PNETs) and diabetes. Identification of factors driving differentiation of the various types of pancreatic endocrine cells will, in the long-term, aid the development of treatment strategies for diseases involving these cells.

Differentiation of pancreatic endocrine cells is initiated by a brief expression of the master transcription factor Neurogenin3 (Neurog3). This leads to both transient and constitutive expression of downstream factors (such as transcription factors, inhibitors and ubiquitin ligases), ultimately resulting in maturation of the various types of endocrine cells. This dynamic differentiation process requires distinct gene sets to be expressed at specific stages. Tight regulation of this process is mainly achieved through transient expression of factors regulating the expression of these gene sets. However, compared to constitutively expressed factors, which have been relatively easy to identify, transiently expressed factors have remained more elusive.

**b. Materials and Methods/Experimental Approach**

To identify transiently expressed factors along the endocrine cell differentiation trajectory we utilized the novel Neurog3Chrono reporter mouse. In this model, the knock-in of a bi-fluorescent reporter of the Neurog3 allele enables real time measurement of the onset of endocrine cell differentiation.

Reporter positive cells from the pancreata of Neurog3Chrono reporter mice at different embryonic days of development were isolated by flow cytometry and single cell transcriptional libraries were prepared. To identify coding and non-coding transcriptional changes, we used a novel full transcriptome single-cell library preparation method called VASA-seq.

**c. Results (key findings to report at this meeting)**

Combining the Neurog3Chrono reporter mouse and VASA-seq, we were able to generate a time-resolved map of transcriptional changes during pancreatic endocrine development. This transcriptional map not only provides a description of the developmental pancreatic endocrine hierarchy and its sub-lineages, it also identifies differential kinetics between sub-lineages and predicts multiple transiently expressed novel coding as well as non-coding molecular regulators.

#### **d. Conclusions/next steps**

Our time-resolved, single-cell transcriptional map provides an extensive resource to understand pancreatic endocrine differentiation during development. It allows for identification of novel constitutive and transiently expressed regulatory factors. Comparing the gene sets from different stages of our time-resolved transcriptional map of pancreatic endocrine differentiation to gene sets found to be enriched in PNETs, will allow us to determine whether certain phases (and gene sets) of differentiation are associated with certain types or stages of this disease. Indeed, re-activation of embryonically expressed genes in adult cells is often observed in cancer and understanding the commonalities between neuroendocrine differentiation and cancer at the molecular and cellular levels are likely to aid in the development of cell-based and/ or targeted treatment strategies.