Gene Signatures of NEUROGENIN3+ Endocrine Progenitor Cells in the Human Pancreas

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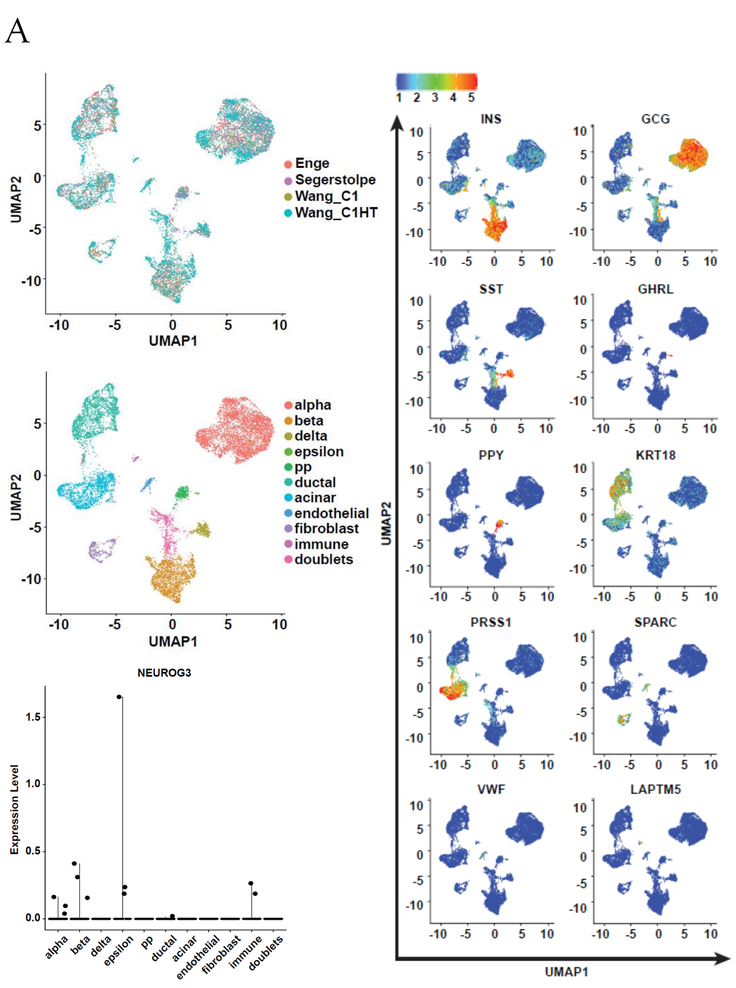
Hypothesis

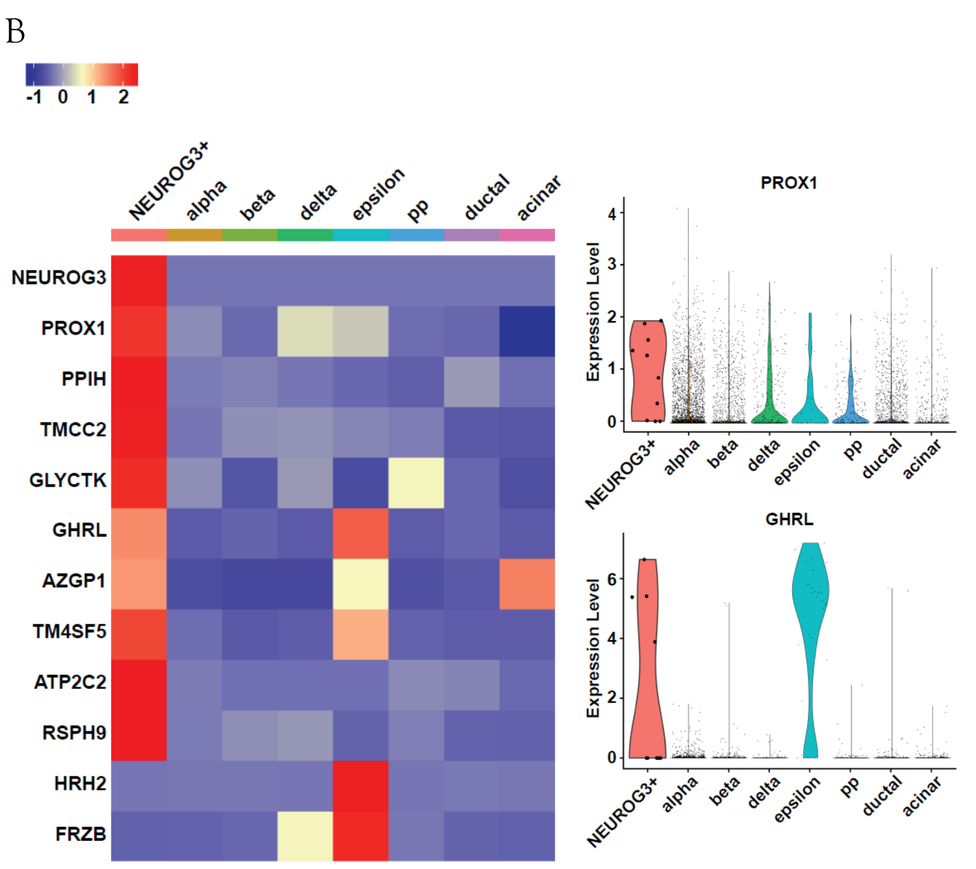
Neurogenin3 (*Neurog3*) encodes a transcription factor considered to be the master regulator of pancreatic endocrine differentiation in mice. In humans, patients carrying *NEUROG3* mutations often develop neonatal diabetes. However, due to the ultra-low abundance of NEUROG3+ cells in the postnatal human pancreas, our current knowledge on the molecular program of NEUROG3+ cells in humans is largely extrapolated from studies in mice. Because of the central role of NEUROG3 in endocrine differentiation, understanding the molecular characteristics of NEUROG3+ cells in humans has large implications for diabetes treatment. We hypothesized that single-cell RNA-sequencing enables in-depth exploration of the rare NEUROG3+ cells directly in humans.

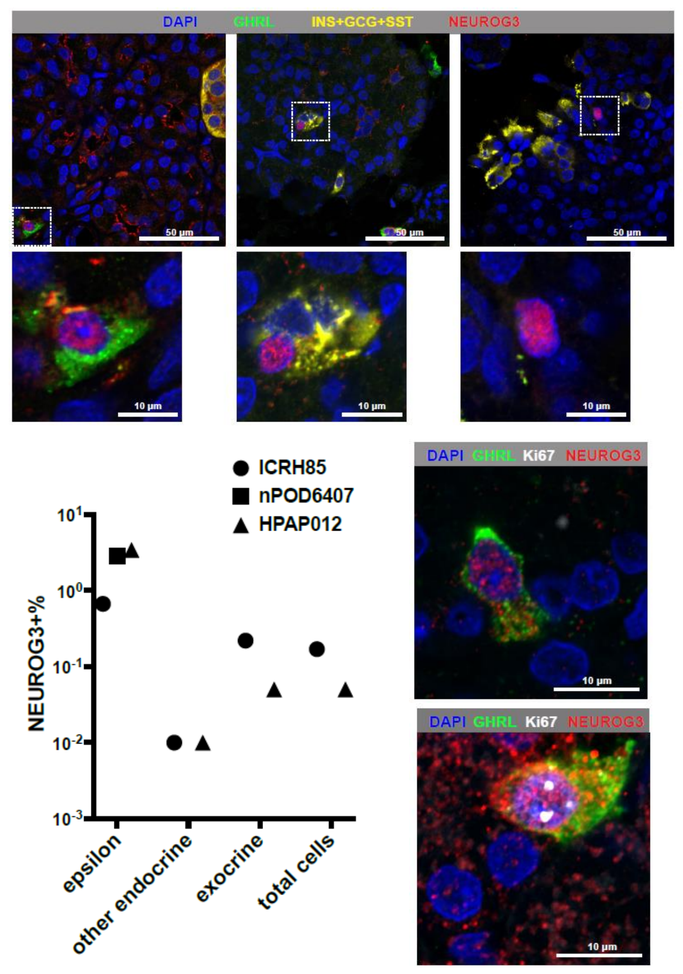
Methods

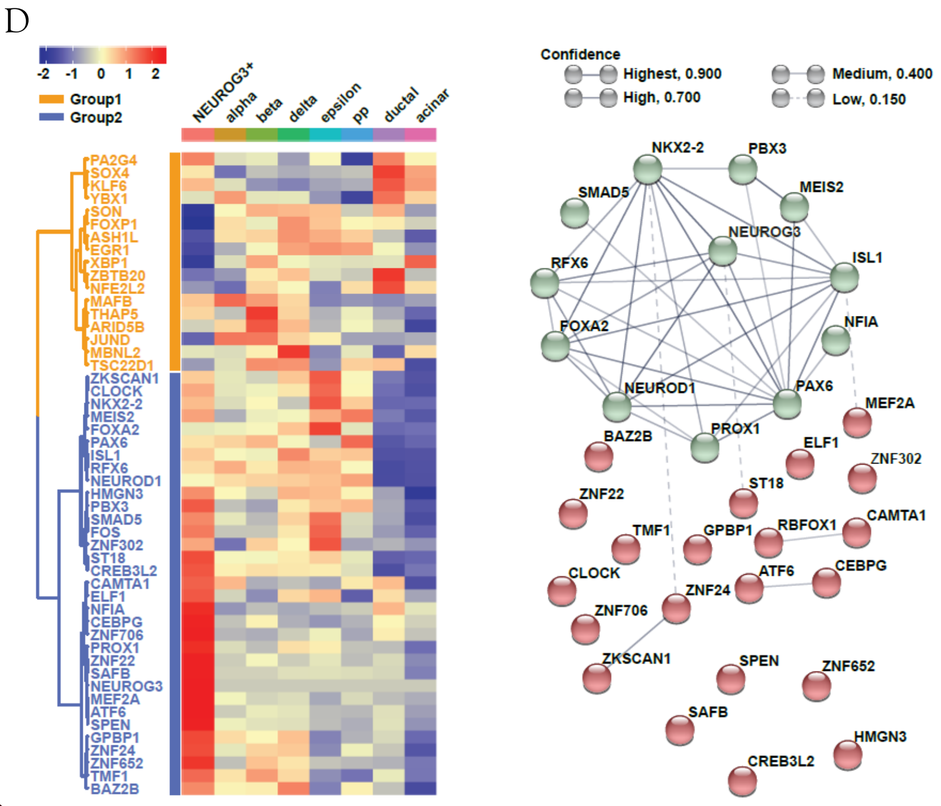
We aligned four large single-cell RNA-sequencing datasets from postnatal human pancreas. Our integrated analysis revealed 10 NEUROG3+ epithelial cells. We confirmed the co-expression of NEUROG3 with endocrine markers based on immunostaining on pancreatic tissue sections. Then, we further identified unique genetic signatures of the NEUROG3+ cells by differential expression and transcriptional network analysis.

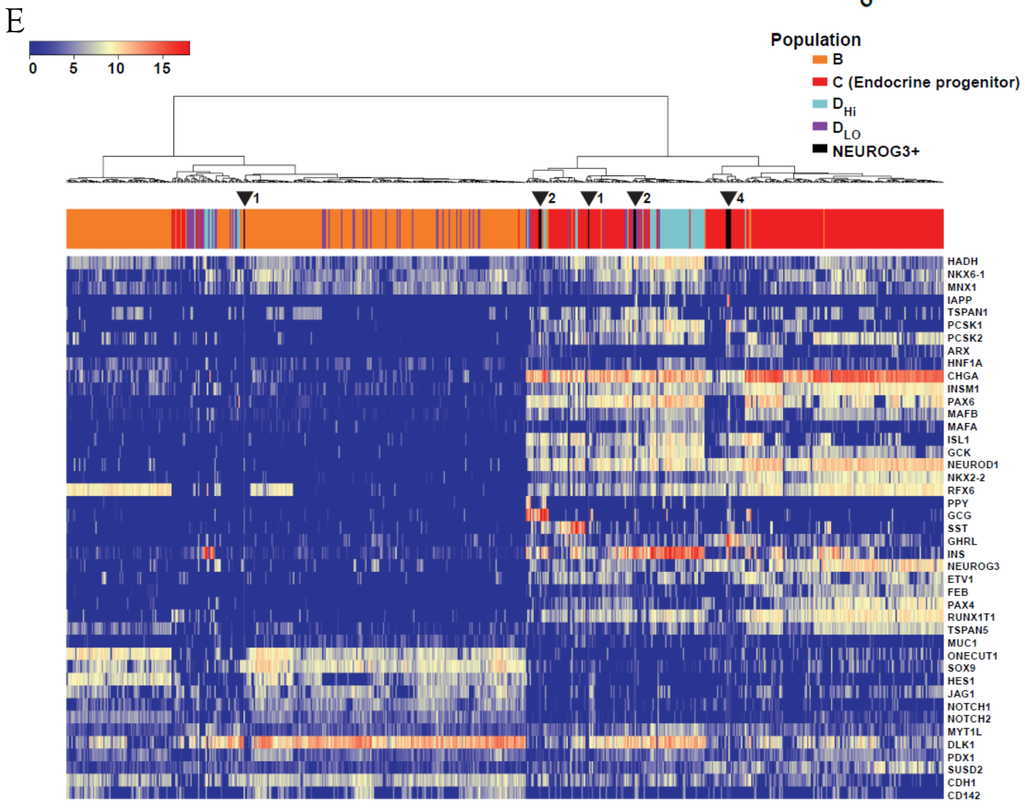
Results and Discussion











A. The identification of NEUROG3+ cells from 11,174 postnatal human pancreatic cells. **B.** NEUROG3+ cells have unique gene expression signatures. **C.** NEUROG3 is highly expressed in epsilon cells. Co-expression of Ki67 and NEUROG3 were observed in rare instances. **D.** NEUROG3+ cells contain an organized transcriptional network. **E.** NEUROG3+ cells in the postnatal human pancreas cluster with NEUROG3+ cells from the fetal pancreas.

Conclusions

We identified the existence of NEUROG3+ cells in postnatal human pancreas by utilizing single-cell RNA-sequencing technology. Noticeably, epsilon cells displayed the highest frequency of NEUROG3 positivity. Regulatory network analysis revealed that novel transcription factors including Prospero homeobox protein 1 (PROX1) may act with NEUROG3. Also, we confirmed NEUROG3+ cells in the postnatal human pancreas shared characteristics with NEUROG3+ cells during development. Taken together, knowledge gained from our study will provide insights on NEUROG3+ cells in humans.

Future Directions

Deep profiling single-cell RNA-sequencing and continued improvement in algorithms for data harmonization are required. Moreover, whether the NEUROG3+ cells play a significant role in the pancreatic turnover during normal homeostasis and in pathological conditions needs further study.

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