a. Background

Carcinoid tumors exhibit cellular and molecular features of an advanced differentiated state. Normal mature enteroendocrine cells (EECs), however, do not proliferate. Latest results from our Accelerator project (Zhou & Shivdasani), by comparing the transcriptomic and epigenetic state of normal human EECs with carcinoids, suggest that carcinoids indeed appear to be arrested at a late step along the EEC differentiation trajectory. Thus, a simple hypothesis for carcinoid formation is that aberrant gene expression events during EEC differentiation prevent normal cell cycle exit and enable continued proliferation, sowing the seed for carcinoids. We designed gain- (cDNA) and loss-of-function (CRISPR) screens using normal human EECs to identify causal factors that promote aberrant, continued EECs proliferation.

b. Experimental Approach and key findings

To obtain large numbers of human EECs for the screens, we established primary human ileal stem cell lines and engineered them for inducible expression of NGN3, the master regulator of EEC development. Upon NGN3 induction, ileal stem cells synchronously differentiate into EECs. We further optimized the culture condition to minimize persistence of proliferating stem/progenitors after EEC differentiation is complete.

We performed a test screen with a human Lentiviral ORFome library containing 16,000 cDNAs by transducing cultured ileal stem cells with the lentiviral library, followed by EEC differentiation. We harvested ~3.2 million EECs (~50% transduction efficiency, thus 100x coverage) and cultured them in Matrigel drops for 6 weeks. The majority of EECs persist in these long-term cultures, with formation of a small number of EEC clusters. EdU incorporation revealed rare EdU^+CHGA^+ cells, indicating the presence of proliferating EECs. We are currently developing methods to purify the EdU^+CHGA^+ cells by FACS and to recover and identify the cDNAs in these cells.

Complementary to the cDNA gain-of-function screen, we plan to screen a CRISPR library focused on ~500 epigenetic factors (~2,500 gRNA, 5 gRNA per gene) using the same approach discussed above. The focus on epigenetic factors is based on the observation that carcinoid tumors lack recurrent genetic mutations, suggesting epigenetic mechanisms as the leading cause for dysregulated gene expression and tumorigenesis. A whole-genome CRISPR screen is not feasible at the moment as it will require many tens of millions of pure EECs that we cannot easily scale up to produce.

c. Conclusions

We developed an experimental system to enable cDNA and CRISPR genetic screens in normal ileal EECs with the goal of uncovering causal factors able to promote aberrant EEC proliferation. If successful, this will provide candidate factors in elucidating the molecular mechanisms of carcinoid tumorigenesis.