Pooled genetic screens to identify factors promoting aberrant EEC proliferation

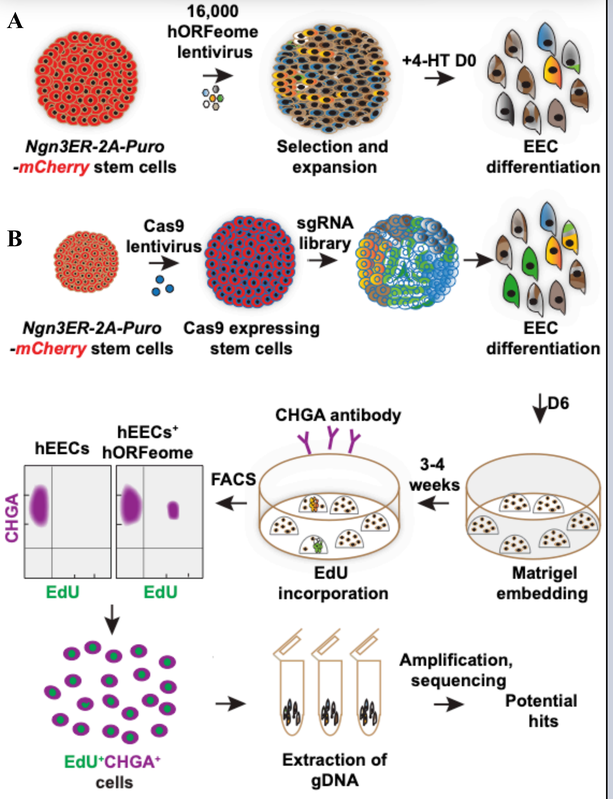
Sanlan Li[1], Xiaofeng Steve Huang[1], Wei Gu[1], Ramesh Shivdasani[2], Joe Q. Zhou[1]

[1]Department of Medicine, Weill Cornell Medicine; [2]Dana-Farber Cancer Institute, Harvard Medical School

INTRODUCTION

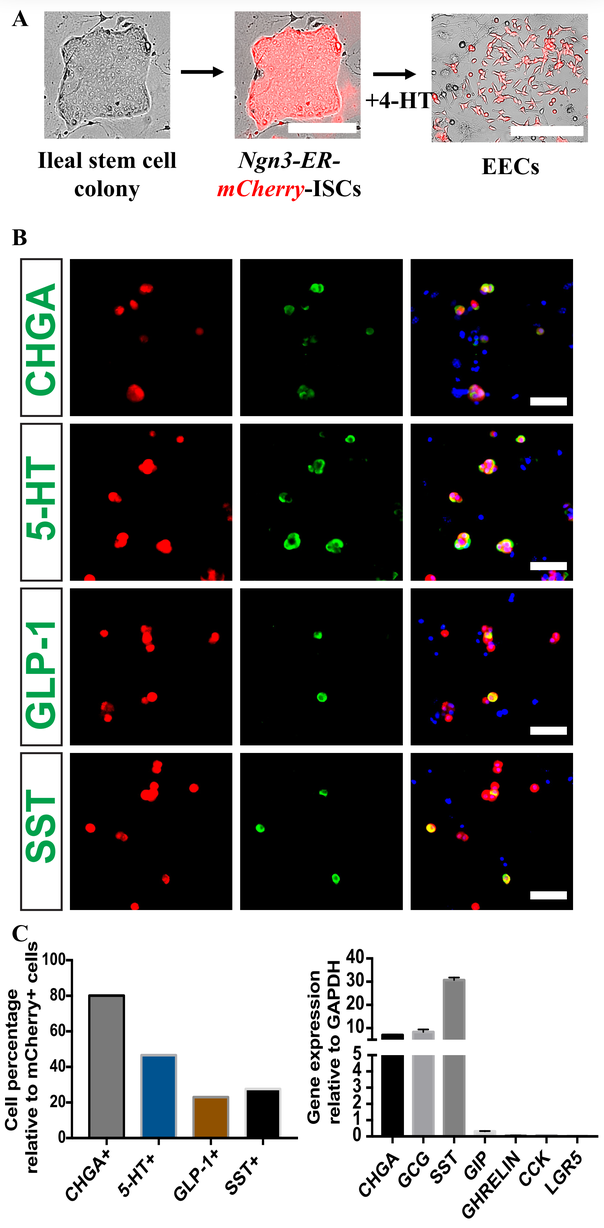
Carcinoid tumors exhibit features of advanced cytodifferentiation and our comparison of carcinoid tumors with normal human enteroendocrine cell (EEC) transcriptomes and epigenomes (Accelerator project – Zhou & Shivdasani) affirms that carcinoid tumors are indeed arrested late in the EEC differentiation trajectory. Normal mature enteroendocrine cells (EECs), however, do not proliferate. Thus, a simple hypothesis for carcinoid formation is that aberrant gene regulation during EEC differentiation prevent cell cycle exit and enables continued proliferation, sowing the seeds for carcinoid tumorigenesis. We designed gain- (cDNA) and loss-of-function (CRISPR) screens using normal human EECs to identify causal factors that promote aberrant, continued EEC proliferation.

EXPERIMENTAL DESIGN



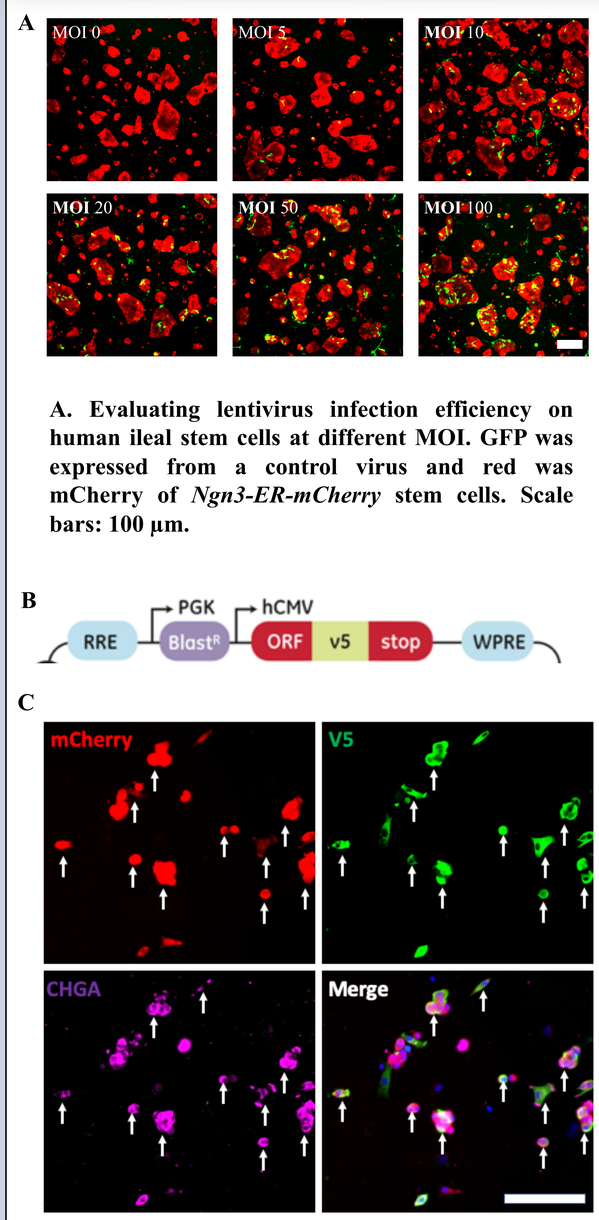
Schematic illustration of gain-of-function screen using hORFeome library (A) or loss-of-function screen using epigenetic sgRNA CRISPR library (B). Human ileal stem cells were engineered for inducible expression of NGN3, puromycin, and mCherry for EEC induction and labeling.

1. Efficient differentiation of EECs from engineered *Ngn3-ER-mCherry* human ileal stem cells



A. Representative images showing 2D cultured ileal stem cell colonies with inducible NGN3 expression and EEC cells generated after 4-hydroxy tamoxifen (4-HT) application; B, C. Immunostaining of EECs and quantification. Scale bars : 100 μm in A and 25 μm in B. Note the lack of stem cell marker LGR5 and rostral intestinal EECs including GIP, Ghrelin, and CCK.

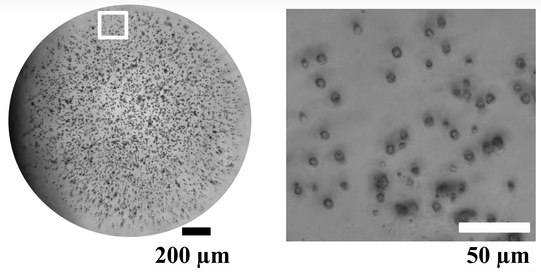
2. Efficient delivery of hORFeome into human ileal stem cells by lentiviral transduction



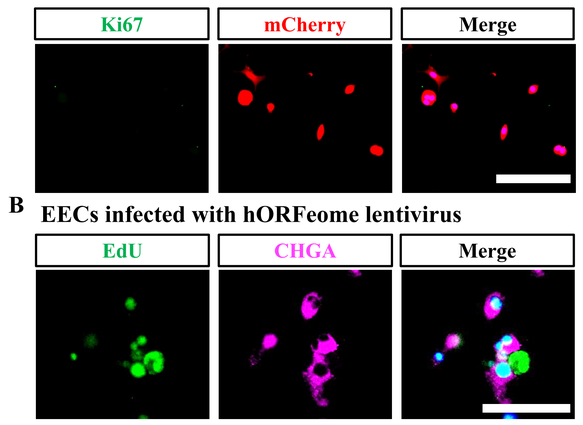
A. Evaluating lentivirus infection efficiency on human ileal stem cells at different MOI. GFP was expressed from a control virus and red was mCherry of *Ngn3-ER-mCherry* stem cells. Scale bars: 100 μm.

B. Diagram showing hORFeome plasmid; C. Immunostaining showing V5 epitope-tag expression in hEECs that integrated hORFeome lentivirus. hORFs are tagged with V5. Arrows indicate $mCherry^+V5^+CHGA^+$ cells. The percentage of $V5^+mCherry^+$ cells relative to $mCherry^+$ cells was approximately 60%. Scale bars: 50 μm .

3. Transduced ileal EECs persist for over 4 weeks in 3D matrigel drops



4. EdU^+CHGA^+ cells were detected in hORFeome transduced EEC cultures



A. Immunostaining showing negative Ki67 staining in $mCherry^+$ hEECs cells. B. Double staining showing EdU^+CHGA^+ cells emerged after hORFeome lentivirus infection. Scale bars: 50 μm in A and 25 μm in B.

CONCLUSIONS

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

FUTURE PLANS

- We will continue to optimize this system, especially FACS purification of EdU^+CHGA^+ cells
- At the same time, we will perform a CRISPR/Cas9 screen with a epigenetic target library (~500 genes).