

Gen 3-2019 ISCC Research Project Abstract



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ANATOMIC AND FUNCTIONAL CHARACTERIZATION OF THE INTESTINAL CRYPT-VILLUS NICHE

Intestinal epithelial integrity, turnover, and function require signals from the underlying mesenchyme. The new focus of the Intestinal Stem Cell Consortium (ISCC) of the NIDDK and NIAID is to investigate in depth the mesenchymal cells that provide crucial support, their organization within the tissue, and their molecular signatures, including essential secreted factors. The PI has been a member of the Consortium for the last 4 years and this application is made in response to RFA-DK-18-507, a Limited Competition to renew the ISCC Research Centers. Although recent progress in the field implicates various cell populations (CD34⁺, Foxl1⁺, PDGFRA⁺, Gli1⁺) as key niche elements, their precise identities, overlap, and contributions remain uncertain. By integrated consideration of high-resolution whole-mount confocal microscopy and ensemble and single-cell (sc) RNA-seq analysis of well-defined cell populations isolated by flow cytometry, we have identified three distinct mesenchymal cell types as leading candidates for essential niche functions. Over the last 2 years, our sharing of data on these telocytes and two distinct (anatomic and molecular) PDGFRA^{lo} cell populations has stimulated one important stream of investigation in several ISCC laboratories. Building on this experience and community, and following productive completion of all Aims from the current award period, we propose three lines of investigation. Specific Aim 1 will generate detailed molecular, anatomic, and functional maps of mouse stromal cell populations, with three purposes: (a) to identify the sources of Wnt, BMP, EGF, and other key mesenchymal signals at high spatial resolution, (b) to establish robust and reproducible culture conditions for selected important mesenchymal cell types, and (c) to inform identification and validation of the corresponding functional human cell populations. These studies consider the whole small intestine as a unit, and Aim 2 will advance purpose (c) further by considering each gut region (duodenum, jejunum, ileum, and ascending and descending colon) separately. Both Aims will integrate high-resolution anatomic and molecular analyses to generate accurate 3D maps, superimposed with assessments of mesenchymal cell functions conducted in collaboration with other ISCC groups. Aim 3 addresses an urgent need in the field and the specific RFA call to generate new Cre-driver and fluorescent reporter mice. Based on extensive molecular profiling in Aims 1 and 2, we will identify novel cell type-specific loci and use CRISPR/Cas9 editing to insert reporter constructs in up to 3 such loci. Each Aim involves extensive collaboration with ISCC laboratories under the umbrella of a comprehensive Collaborative Management Plan. In addition, we will contribute and adopt standardized protocols and share data, protocols, reagents, and mouse strains with ISCC investigators.

Public Health Relevance Statement:

Steady replenishment and repair of our intestinal lining requires signals from the adjacent 'sub-epithelial' tissue, where we have identified distinct cell types serving different supportive functions. Working collaboratively with other Consortium laboratories, we propose studies to advance understanding of these cells and signals. The overarching goal is to define cellular elements and drugs that can accelerate intestinal repair and contribute toward engineering intestinal tissue in the laboratory.