

Gen 3-2019 ISCC Research Project Abstract



Calvin Kuo, M.D., Ph.D.

Principal Investigator; Voting SC Member
Stanford University

INVESTIGATION AND TRANSLATION OF THE INTESTINAL STEM CELL NICHE

The intestine is endowed with a remarkable ability to continually regenerate its epithelial layer, both to maintain tissue homeostasis and to heal injury, mediated by Lgr5-expressing active and injury/reserve intestinal stem cells (ISCs) and complex cellular and humoral niche. Disruption of these regenerative properties results in absorptive or barrier dysfunction or frank overgrowth in malabsorptive, infectious, inflammatory and neoplastic intestinal disorders. An improved understanding of ISCs and their niche is therefore crucial to the clinical translation of this biology as is the mission of the NIH Intestinal Stem Cell Consortium (ISCC). As a current ISCC member laboratory, we have extensively collaborated to discern the biology of homeostatic and injury/reserve ISCs relative to their niche. Here, we continue these studies in three aims.

In **Aim 1**, we continue our prior studies on the Lgr5⁺ ISC niche to explore crosstalk between Lgr5⁺ ISC and its closely associated PDGFRA⁺ mesenchyme via the concept that PDGFRA-expressing cells sense and respond to Lgr5⁺ ISC damage, while Lgr5⁺ ISC conversely elaborate stimulatory factors for PDGFRA⁺ cells. This extends preliminary data where primary manipulation of Lgr5⁺ ISC by radiation or R-spondin results in secondary effects on PDGFRA⁺ cell number, proliferation and expression of niche factors including R-spondins. This Lgr5⁺ ISC/PDGFRA⁺ crosstalk will be studied by deletion of R-spondins in PDGFRA⁺ populations and by genetic and pharmacologic manipulation of PDGF signaling. This is complemented by enteroid reconstitution studies and unbiased tandem single cell RNA-seq and ATAC-seq of PDGFRA⁺ cells.

Aim 2 furthers our studies of air-liquid interface (ALI) intestinal organoids containing epithelium and mesenchyme towards pre-clinical translation. First, we will extensively characterize stromal components by IF and perform unbiased single cell RNA-seq, to inform and optimize the ALI culture method and stromal preservation therein. Secondly, we develop in vivo orthotopic transplantation of human and mouse ALI intestinal organoids, leveraging biocompatible ECMs and leveraging second-generation bioengineered Wnt agonists having Fzd-subtype specificity. Thirdly, we apply mouse ALI organoids to achieve the first organoid transplantation correction of a disease phenotype in a Ferroportin (*Fpn*)-KO model of intestinal iron absorption deficiency anemia.

Lastly, **Aim 3** exploits our ability to convert bioengineered Wnt agonists into Fzd-subtype specific antagonists for biological probes and therapeutics. Fzd-subtype specific antagonists will be used to probe the requirement of specific Fzds during intestinal homeostasis in vivo, with parallel modeling in enteroid culture. Overall, each of these aims represent highly collaborative studies that would not be possible without the integral participation of fellow ISCC labs and the ISCC Coordinating Center, towards advancing the dual ISCC goals of defining niche biology and therapeutic translation.

Public Health Relevance Statement:

The intestine possesses remarkable regenerative capability that originates in its stem cells that are nourished by a supportive environment termed a “niche”. Here, we characterize the supportive cells and signals that comprise the intestinal stem cell niche towards generating improved methods to transplant intestinal tissue and derive novel intestinal therapeutics.