

ISCC Research Project Abstracts



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CHROMATIN AND TRANSCRIPTIONAL CONTROL OF LGR5+ CRYPT BASE STEM CELLS

Disorders such as Inflammatory Bowel Disease and colorectal cancer reflect intestinal stem cell (ISC) dysfunction. One small pool of Bmi1hi ISC divides infrequently and occupies high crypt tiers, while a larger pool of Lgr5+ cells at the crypt base replicates briskly and serves the "workhorse" function of continuous epithelial renewal. The two ISC populations interconvert with surprising ease, but our understanding of fundamental aspects, such as epigenetic and transcriptional control of ISC identity and function, or determinants of cell location and convertibility, is incomplete. A better understanding of these aspects will improve treatment of intestinal disorders, while sparing normal digestive functions. We propose innovative approaches for chromatin and other molecular analyses of purified crypt populations, especially Lgr5+ ISC, and advanced 2-photon confocal microscopy to image ISC populations in situ in unprecedented detail. Thousands of gene-regulating enhancer elements are not separately primed in the Enterocyte (Ent) and Secretory (Sec) lineages that descend from ISC; many Ent- or Sec-active enhancers are already primed in Lgr5+ ISC. Enhancer priming is the purview of sequence-specific "pioneer" transcription factors (TFs), which identify relevant cis-regulatory elements and mark flanking nucleosomes with histone modifications such as Histone 3 Lysine 4 methylation (H3K4me). In Aim 1 we propose that the "pioneer" TF that primes the transcriptional program in Lgr5+ ISC is ASCL2, an intestine-restricted TF that is required for Lgr5+ ISC survival and function. We will test in cultured cells and transgenic mice whether ASCL2 fulfills "pioneer" attributes, i.e., if it binds at most primed enhancers in Lgr5+ ISC and other crypt cells, and if active histone marks at these enhancers are extinguished in its absence in vivo. Cell differentiation requires both gene activation and silencing, but mechanisms by which ISC repress inappropriate genes are unknown. One major repressive pathway in embryonic cells uses Polycomb Repressive Complex (PRC)2 to place the histone mark H3K27me3. Absence of PRC2 in the intestinal epithelium impairs replication of Lgr5+ ISC and other crypt cells, producing stunted, defective villi that are soon replaced by PRC2-competent units. In Aim 2 we propose an integrative analysis of H3K27me3 marks, PRC2-dependent gene expression, and mutant phenotypes in purified Lgr5+ ISC and their descendant crypt and villus cells. Our specific goals are to understand the significance of dynamic chromatin alterations and to define mechanisms of gene activation and silencing in ISC. Lastly, progress in understanding functional relationships among different ISC pools and their corresponding niches is hampered in part by difficult visualization of Bmi1hi +4 ISC; we have improved 2-photon confocal microscopy methods to detect these and surrounding cells in their native state. In Aim 3 we will further refine this technology, while specifically interrogating how Bmi1hi +4 ISC respond to crypt perturbations such as replicative deficiency in Lgr5+ ISC or ablation of Paneth cells.