

Project Number: U01DK85527-01

Principal Investigator: Calvin J. Kuo

Awardee Organization: Stanford University

Title: In Vivo and In Vitro Characterization of BMI1 + Intestinal Stem Cells

Abstract Text:

DESCRIPTION (provided by applicant): Intestinal homeostasis is maintained by the robust activity of intestinal stem cells (ISC) which allow complete regeneration of the intestinal epithelium every 5-7 days. Recent functional studies have established the existence of two types of ISC, a crypt base columnar (CBC) cell expressing LGR5 and prominin-1, and a distinct ISC population expressing Bmi1, located higher in the crypt at approximately the +4 position and restricted to the small intestine. Despite potent stem cell attributes of the Bmi1+ cells in lineage tracing studies, their regulation, relationship to LGR5+ cells and transcriptome have remained poorly defined. The overall goal of this application is the analysis of the Bmi1+ lineage in vivo and in vitro, using recently developed Bmi1-CreER knock-in mice, our robust methodology for small intestinal culture, and R-Spondin1 and Dkk1 adenoviruses allowing gain- and loss-of-function Wnt manipulation in vivo. Accordingly, Aim 1 will investigate the regulation and functional relevance of the Bmi1+ lineage during intestinal regeneration. The number and fate of Bmi1+ cells will be examined during regeneration in response to radiation or R-spondin1, both in vivo and in vivo using the Bmi1-CreER mouse or cultures derived thereof. Importantly, the functional contribution of the Bmi1+ ISC to intestinal regeneration after radiation or R-spondin treatment will be assessed by diphtheria toxin-mediated lineage ablation. Aim 2 will address the important question of relationships between the Bmi1+ and LGR5+ ISC lineages. Fate mapping of the Bmi1 lineage will be performed in vivo and in vitro to formally demonstrate if Bmi1+ cells or their progeny can express LGR5. Culture of isolated Bmi1+ cells from Bmi1-CreER mice will be performed both within and without an ISC niche to explore if Bmi1+ cells can give rise to LGR5+ cells in vitro. In Aim 3, transcriptional profiling of Bmi1+ cells will be performed and compared to the published LGR5+ transcriptome and target validation performed exploiting in vitro intestinal culture. Finally, Aim 4 will explore the ex vivo expansion and transplantation of Bmi1+ ISC. Our ISC niche-dependent intestinal culture system, as well as niche-free systems will be used to expand Bmi1+ cells ex vivo, followed by single cell or population transplantation in vivo. Questions of plasticity will also be addressed with introduction of small intestine Bmi1+ cells into the colon both in vitro and in vivo.

PUBLIC HEALTH RELEVANCE: The intestine possesses highly active stem cell populations with therapeutic relevance to diverse conditions including inflammatory bowel diseases, metabolic disorders and cancer. Here, the Bmi1+ intestinal stem cell population will be investigated with regards to regenerative potential both in vivo and in vitro.