Project Number: U01DK85547-01

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Title: Collaborative Approaches to the Study of Intestinal Epithelial Stem Cells

Abstract Text:
DESCRIPTION (provided by applicant): This proposal is submitted in response to an RFA for an intestinal stem cell (ISC) Consortium. As noted in the RFA, although the existence of stem cells in the small intestinal epithelium has been known for decades, there are currently no published methods to isolate pure preparations of these cells. In 2005 our group (1) used flow cytometric side population (SP) sorting to isolate for the first time a fraction enriched in ISCs as judged by enrichment for Musashi-1 mRNA (at that time the only known marker of ISCs). Subsequent microarray, in situ hybridization and resection (2; 3) studies have strengthened the evidence that SP cells from mouse small intestine include the ISCs. Although we empahsized from the outset (1) that the SP is not a pure ISC prepartion, better approaches to isolate ISCs from normal mouse or human small intestine have not yet been reported. In addition, to date proof of stemness with SP cells (or any other isolated cell fraction from the small intestine) has been hampered by lack of functional assays to demonstrate self-renewal and multi-lineage differentiation. Recent reports on new markers for ISC suggest that there may be active and quiescent subpopulations. However these have not yet been isolated and little is known about the the behavior of these two cell populations and cells expressing other ISC markers during intestinal development. To address these significant gaps we propose a collaborative approach which combines experts in intestinal development, stem cell isolation, surgical models of intestinal growth, genetics of stem cells and mouse models with altered signaling of key intestinal growth factors. Specifically, we propose the following aims: 1. To improve isolation methods for ISC by the identification of suitable membrane antigens for antibody-based sorting and to use functional properties to separate active and quiescent ISC; 2. To develop in vivo assays to functionally validate self-renewal and multi-lineage differentiation of putative ISC preparations using three novel approaches; and 3. To characterize ISC behavior during intestinal development and to create a developmental tissue bank of small intestine for localization of known or novel ISC markers by other Consortium members. We believe that achievement of these goals is feasible.

PUBLIC HEALTH RELEVANCE: Understanding the biology of intestinal stem cells is central to the development of regenerative medicine approaches to various disorders in which intestinal function is compromised. For example expanding the pool of residual stem cells would be an ideal way to prevent or treat intestinal failure and short gut syndrome. Moreover, in situations where the stem cells themselves are damaged (e.g. after radiation or chemotherapy), transplantation of healthy stem cells may afford a novel and effective therapy.