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Title: Isolation and Characterization of Intestinal Stem Cells Using Novel Markers (RP)

Abstract Text:

DESCRIPTION (provided by applicant): In the intestine, epithelial renewal is reliant on stem cells and fueled by rapidly proliferating transit-amplifying cells (TA). Identification of intestinal stem cells is paramount to fully understanding this renewal process. The broad goals of the studies planned in this project are to isolate (ISCs) in order to elucidate the molecular features that distinguish them from their immediate descendents, the progenitor /TA cells and the more differentiated cell within the gut. One major obstacle in ISC biology has been the lack of definitive markers that identify ISCs. The recently described novel putative ISC marker DCAMKL-1 (doublecortin and CaM kinase-like-1) is predominantly observed in a unique quiescent cell population in the lower third of the intestinal crypt. Furthermore, cell surface expression of DCAMKL-1 allows for FAQs based sorting from intestinal tissues. Moreover the recent identification of another novel putative ISC marker LGR5 (leucine-rich-repeat-containing G-protein-coupled receptor) that is expressed in rapidly cycling cells within the crypt has allowed for examination of the effects of genotoxic injury on intestinal stem quiescence and proliferation in situ and in real time. The central hypothesis of the current proposal is that DCAMKL-1 marks quiescent ISCs and can be used to isolate, characterize, and distinguish quiescent stem cells from rapidly cycling stem/progenitor (LGR5+) cells. This hypothesis will be tested with the following specific aims: 1) To isolate stem cells from the normal adult mouse small intestine by FACS and develop in vitro and in vivo assays to assess clonogenicity, and lineage determination 2) To delineate the molecular signatures and signaling pathways that regulate intestinal stem cell fate and 3) To determine the molecular mechanisms that control intestinal epithelial stem quiescence and proliferation during the restitution phase following genotoxic injury. Collectively, these studies should provide new insights into the key cellular mechanisms and identify novel candidate genes and pathways that regulate the homeostatic interplay between stem and progenitor cells in the normal intestine and following genotoxic/cytotoxic injury during the primary regenerative response.

PUBLIC HEALTH RELEVANCE: The experimental studies proposed may have far reaching consequences in the study of ISC biology and in other gut stem cells and perhaps other solid tissues as well. These studies should help in the development of novel regenerative medicine strategies to treat inflammatory gastrointestinal diseases, and perhaps in targeting the stem cell in gut neoplasia.