Confocal Microscopy to Evaluate the Angiogenic Response to Small Bowel Resection

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Introduction: Intestinal adaptation is a normal response after massive intestinal loss that results in increased mucosal surface area to maximize nutrient absorption. In the mouse model after small bowel resection (SBR), the mucosal changes associated with adaptation are increased villus height and crypt depth. Although intestinal adaptation is an important response to SBR, its mechanisms are not fully understood. Angiogenesis and endothelial cell proliferation after massive intestinal loss are not fully characterized. The purpose of this study was to identify the changes in capillary proliferation in a well-established SBR murine model.
Methods: Male C57BL/6 mice underwent a 50% SBR or sham operation (transection and reanastomosis). The small intestine was harvested on post-operative day 7. Before harvest, mice received retro-orbital injections of high molecular weight FITC dextran, a fluorescent dye that is maintained in the vasculature. The tissue was histologically fixed and stained with a red fluorescent dye (Syto 61). Tissue samples were visualized by confocal microscopy. Capillary density was assessed by counting the number of nuclei bounded by green capillary windows, with fewer nuclei per window corresponding to higher density. Tissue was also harvested for further protein and mRNA analysis.
Results: Much of this research period was spent developing and optimizing the above protocol. Presently we demonstrate confocal microscopy as a viable method to evaluate capillary proliferation in our SBR model.
Conclusion: Ongoing studies employing this technique will help to further characterize the role of angiogenesis in intestinal adaptation in our murine SBR model relative to sham operated mice. Tissue levels of various pro-angiogenic factors are also being analyzed for changes after resection.