Characterization of colonic apoptosis following DSS induced colitis in the absence of Lrig3

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Introduction

Abstract

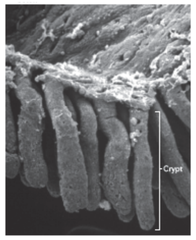
Research in the Powell lab focuses on the epithelium of the small intestine and colon during homeostasis and disease. Homeostasis within the gut requires intricate control of stem cell proliferation, differentiation, migration and controlled cell death. Previous research in the Powell lab has shown the transmembrane protein and intestinal stem cell marker Lrig1, may play both a protective and restorative role in the colon of injury induced mice. Another member of the family, Lrig3, is also expressed in the intestines and localized to the stem cell compartment. In my project, I examine how a structurally similar protein, Lrig3, may play a role in the protection of the colonic epithelium of injury induced mice. My experiments examined the intestinal pathology of ulcerative colitis, that is characterized by inflammation of the colon, by using Dextran Sulfate Sodium (DSS) to induce colitis in an Lrig3 transgenic mouse model. Apoptosis in the cells of the colonic epithelium is a normal result of DDS-induced ulcerative colitis. Understanding if the loss of the Lrig3 affects cell death in the presence of an inflammatory response, is an important step towards potential recovery and/or protective methods for colitis and other inflammatory bowel conditions. Thus, we wanted to investigate if the loss of Lrig3 prior to induced injury would influence this response.

To accomplish this, I used a transgenic mouse model with a tamoxifen inducible cre-lox recombination system controlled by an Lrig1 promoter, to allow for specific spatial and temporal excision of Lrig3, only in Lrig1 expressing cells. The experimental cohort was injected with tamoxifen for 3 days and then treated with 3% DSS for 7 days, allowing us to examine molecular and morphological changes in the absence of Lrig3 during DSS-induced colitis. The tissue was co-stained with a TUNEL assay and Beta-catenin antibody to visualize apoptotic cells and adheren junctions, respectively, in the colonic epithelium. The amount of apoptosis present in the colonic epithelium of mice with and without Lrig3 was quantified and analyzed. Although a two tailed t-test revealed no biological statistical significance, we did observe a slight decrease (p=0.0655) in the number of apoptotic cells per mm of colon analyzed in the Lrig3 mutants when compared to the DSS treated control animals. From these results, we will next analyze changes in proliferation, inflammation, and morphology to determine if Lrig3 participates in the protection or recovery of the colonic epithelium following induced injury.

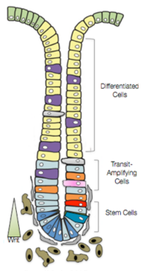
Hypothesis: Loss of Lrig3 causes a decrease in apoptosis after DSS induced colitis

Background

The Colonic Epithelium



Nick Barker, 2014.

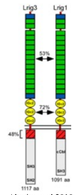


Powell Lab, 2017.

Electron micrograph showing crypt structures in the colon and a diagram showing the location of different cell types in the epithelium. Stem cells at the bottom of the crypt proliferate and differentiate as they migrate up the crypt walls and then undergo apoptosis before they are shed at the top of the crypt. The entire intestinal epithelium is renewed every 3-5 days.

Lrig and Ulcerative Colitis

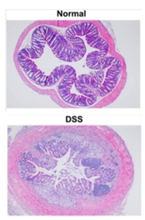
Leucine-rich repeats and immunoglobulin-like domains



Abraira et al 2010

- Family of transmembrane proteins
- Lrig1 is a known stem cell marker, tumor suppressor and EGFR inhibitor
- Lrig3 has a similar structure to Lrig1 but it's unknown whether they have analogous functions
  - Some research has shown LRIG3 to also be an EGFR inhibitor

- Ulcerative Colitis is a type of irritable bowel disease
- Characterized by inflammation and ulceration of the colon and rectum.
- Able to be induced in mouse models with the use of Dextran Sodium Sulfate (DSS)
- 200,000+ cases per year in the US alone
- Unknown etiology



Nishimura et al.,2010

Apoptosis

Cell receives a signal to initiate an apoptotic protein cascade

Condensation and fragmentation of DNA

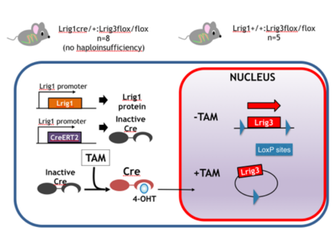
Blebbing off of apoptotic bodies

Apoptotic bodies engulfed and digested by phagocytes

Programmed cell death (apoptosis) is an necessary process within all types of tissue. The colonic epithelium requires intricate control of proliferation at the bottom of the crypts and apoptosis as the cells shed at the top of the crypts. Studying the factors that influence these homeostatic mechanisms may be an important step towards understanding the etiology of ulcerative colitis.

http://humanbody-literaturesubject.blogspot.com/2015/03/programmed-cell-death-apoptosis.html

Cre-lox recombination



Transgenic mice were created to utilize a Tamoxifen inducible Cre-lox recombination method. In one of the transgenic cohorts a cre recombinase gene was inserted after the promoter of one of the LRIG1 loci, causing those mice to express cre instead of Lrig1. This causes the inducible excision of Lrig3 in Lrig1 positive cells when the mice are administered tamoxifen. Previous literature has shown no haploinsufficiency in heterozygous Lrig1 individuals.

Methods

1 Does Lrig3 play a role in colonic tissue repair after DSS induced colitis?

- The Powell lab is investigating this question by examining a**poptosis**, Leukocyte infiltration, proliferation, and the morphology of a DSS induced colitis colon in models with and without Lrig3.

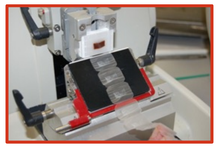
Transgenic mice

All transgenic mice were treated with tamoxifen for 3 days, to activate Cre recombinase in one of the cohorts. This caused the excision of Lrig3 from one of the cohorts whereas the other cohort still had Lrig3 when DSS treatment began. DSS was administered to the drinking water of the mice for 7 days causing acute inflammation in the colon before they were sacrificed on the 10th day.

2

colon

Colons from all 13 animals were removed, dissected and embedded in frozen and paraffin blocks. These blocks were cut using a microtome creating 5um slices of tissue that were applied to slides and ready to be stained.

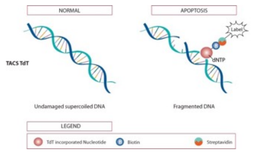


http://www.leicabiosystems.com/pathologyleaders/an-introduction-to-specimen-preparation/

3

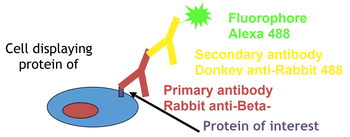
Stained samples

TUNEL Assay



Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL). Enzymatic stain that labels fragmented DNA.

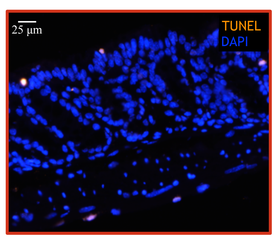
Beta-catenin Immunohistochemistry



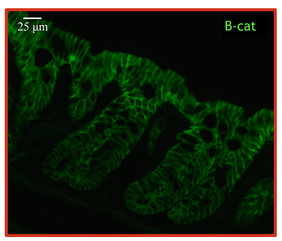
Antibody stain marking Beta-catenin, a transmembrane protein that labels epithelial cell junctions.

4 Staining visualized using fluorescence microscopy

Microscopy



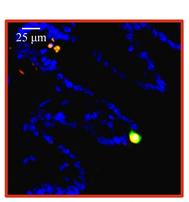
The blue DAPI staining locates cell nuclei. Cells were only counted as TUNEL positive if they were co-expressing DAPI and TUNEL.



Green Beta-catenin staining allows for differentiation between epithelial cells and other cell types (e.g. leukocytes, muscle cells etc.)

Future Directions

- Further investigation of intrinsic vs extrinsic apoptotic pathways
- EGFR activity
- Leukocyte infiltration, proliferation, morphology of colon after DSS induced colitis with and without LRIG3



Co-stain with Caspase 3 and TUNEL. Caspase 3 is a cytoplasmic protein involved in the apoptotic protein cascade.

Acknowledgements

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NIDDK NATIONAL INSTITUTE OF DIABETES AND DIGESTIVE AND KIDNEY DISEASES

SFURK

AGA THE AGA RESEARCH FOUNDATION

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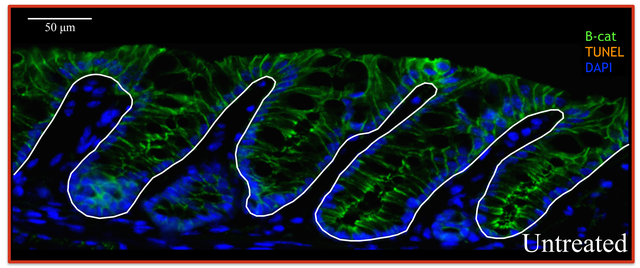
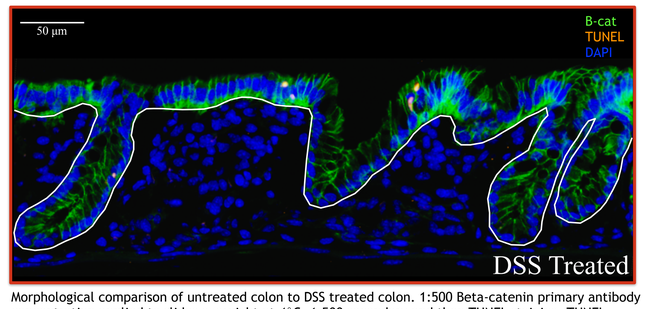
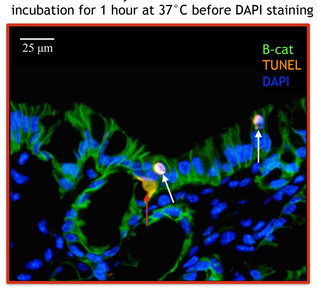
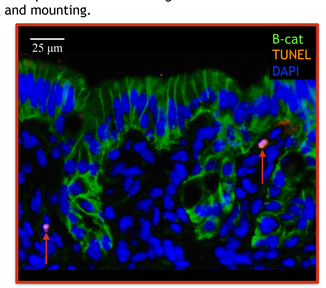
Results, Analysis & Conclusions





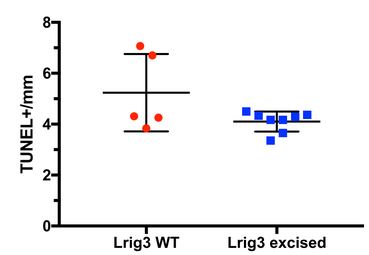
Morphological comparison of untreated colon to DSS treated colon. 1:500 Beta-catenin primary antibody concentration applied to slides overnight at 4˚C. 1:500 secondary and then TUNEL staining. TUNEL utilized a 1:20 enzyme to label solution and a 2 minute permeabilization using 0.1% Triton. TUNEL incubation for 1 hour at 37˚C before DAPI staining and mounting.





Types of stains observed. The analysis only included epithelial cels that were undergoing apoptosis. Cells were counted if they were co-stained with DAPI and TUNEL and were also within the epithelial boundary. White arrows show cells that were counted in the analysis, red arrows show cells that were not counted.

TUNEL Positive Cells per mm in Both Cohorts



TUNEL positive epithelial cells per millimeter of tissue in each of the 13 mouse models. A cross section of the entire distal colon of each animal was analyzed. Slides and pictures were blinded before counting. A two-tailed t-test revealed no statistically significant difference between the two cohorts (p=0.0655).

Conclusion

No significant difference in the amount of apoptosis that occurs during DSS induced colitis was observed between models that had Lrig3 versus models that did not have Lrig3. That being said, there was a slight decrease in the amount of apoptosis that occured in the cohort with Lrig3 excised compared to the cohort with two functional Lrig3 alleles. Additional research should be conducted to further explore the potential role of Lrig3 in DSS induced ulcerative colitis recovery.