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TITLE: The Role of the Noncanonical NF-KappaB Pathway in Colon Cancer

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14. ABSTRACT Inflammation is an essential mechanism leading to progression of colon cancer. We have found that in mouse models of colonic intestinal inflammation and in inflammatory bowel disease samples that the non-canonical NF-κB2 signaling cascade is highly activated in intestinal epithelial cells compared to normal control. Mice disrupted for NIK in intestinal epithelial cells were highly susceptible to several mouse models of colitis as assessed by histology, colon length and body weight loss. These mice demonstrated a significant increase in apoptosis, with a robust regenerative proliferative response compared to wild-type mice. NIK knockout mice also exhibited increased the colonic tumor number and tumor burden following AOM + DSS model of colon cancer. Interestingly, mice which overexpress NIK in intestinal epithelial cells were also susceptible to mouse models of inflammation and cancer. Conclusions: Together these data suggest an important rheostatic role of NF-κB2 signaling in intestine. Future work is focusing on the precise mechanism by which NF-κB2 signaling can modulate intestinal inflammation.					
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INTRODUCTION:

There is a great need for mechanistic studies in colon cancer as this may provide for the development of alternative treatment strategies for this disease. The canonical NF- κ B1 pathway is well documented as a critical signaling pathway in intestinal inflammation and colon cancer (1). Our data has uncovered an essential role for the non-canonical NF- κ B2 pathway in mediating intestinal inflammation. The NF- κ B2 pathway demonstrates distinct regulatory and signaling components to the canonical pathway. The non-canonical pathway is specifically activated by NF- κ B-inducing kinase (NIK) leading to activation of the p52/RelB dimer (2). The long-term goals of this study are to determine the molecular mechanisms by which NF- κ B2 regulates inflammation and the implication of this regulation in colon cancer progression. The experimental focus of this proposal is on the regulatory role of NF- κ B2 pathway in the pathogenesis of colon cancer through the following Specific Aims:

Aim 1: Determine mechanisms by which intestinal NF- κ B2 pathway is activated in colon cancer. Our preliminary data demonstrates that hypoxia can robustly stimulate NIK activity. The goal of this Aim will be to assess how hypoxic signaling interacts with the NF- κ B2 pathway, and understand the importance of this crosstalk in colon cancer.

Aim 1.1: Assess the requirement of HIF-1 α and/or HIF-2 α in hypoxia induced NF- κ B2 activation.

Aim 1.2: Assess if HIF signaling is essential for NF- κ B2 activation in colon tumors.

Aim 1.3: Characterize the role of caveolin-1 in HIF-mediated NIK stabilization.

Specific Aim 2: Determine the requirement for the non-canonical NF- κ B2 pathway in colon cancer. This Aim will assess the role of NF- κ B2 activation in cell proliferation and survival in colon cancer-derived cell lines. In addition, this Aim will utilize two mouse models we have developed, the intestine-specific NIK overexpressing and the intestine-specific NIK knockout mice. We will assess the role of NIK in the progression of colitis-associated colon cancer and sporadic colon cancer.

Aim 2.1: Characterize the role of Stat3 in **NIK-mediated** increase in cell proliferation and survival.

Aim 2.2: Test the effects of intestine-specific NIK disruption or overexpression on the progression of sporadic colon cancer.

Aim 2.3: Test the effects of intestine-specific NIK disruption or overexpression on the progression of colitis-associated colon cancer.

KEYWORDS:

NIK, NF- κ B2, colon cancer, ulcerative colitis, Crohn's Disease, hypoxia, HIF1 α , HIF2 α , EPAS1.

ACCOMPLISHMENTS:

Overall we demonstrated that NIK expression has a rheostatic mechanism in regulating colitis-associated colon cancer. Our major tasks have been fulfilled as outlined in our previous progress reports. For Aim 1 we have shown in year 1 and 2 progress reports that HIF-2 α signaling is a critical mechanism leading to NIK activation. This is mediated by

the HIF-2 α target gene caveolin-1. We further show in year 1 progress report that caveolin-1 interaction with Traf2 is critical for this regulation.

For Aim 2 we reported in year 1 progress report that STAT3 did not mediate growth following NIK activation. We are currently still assessing other pathways, but have fulfilled the tasks for Aim 2.1. For the in vivo data we clearly that show that increase in NIK signaling leads to and robust increase in tumorigenesis in a CAC model (progress report year 1). However, disruption of NIK signaling also leads to increase tumors in a CAC model (progress report year 1). Suggesting that NIK signaling is a rheostatic mechanism in the colon and if dysregulate is essential in tumorigenesis which is preceded by inflammation. Also we assessed the role of NIK in a sporadic colon cancer model. Increased expression of NIK in sporadic colon cancer model also demonstrated an increase tumorigenesis similar to the CAC. Disruption of NIK decreased colon tumorigenesis (Year 2 progress report). This a major difference in NIK's role in colon carcinogenesis.

Following Year 2 we asked for an 8-month no cost extension to provide additional data to understand the mechanism by which NIK regulates colon tumorigenesis. We were able to clearly show that NIK was essential in M-cell differentiation. M-cells are important epithelial cells that directly sample bacterial changes in the gut and educate the mucosal immune response. Intestinal epithelial cell differentiate to M-cells via the RANK ligand. Using intestinal enteroid models we show that RANK ligand-induced differentiation was completely attenuated following NIK deletion as assessed by qPCR of canonical M-cells gene markers (SpiB, GP2, CCL20 and Anxa5) (Figure 1). Future studies are ongoing to deplete M-cells and assess if this recapitulates what is observed following NIK disruption.

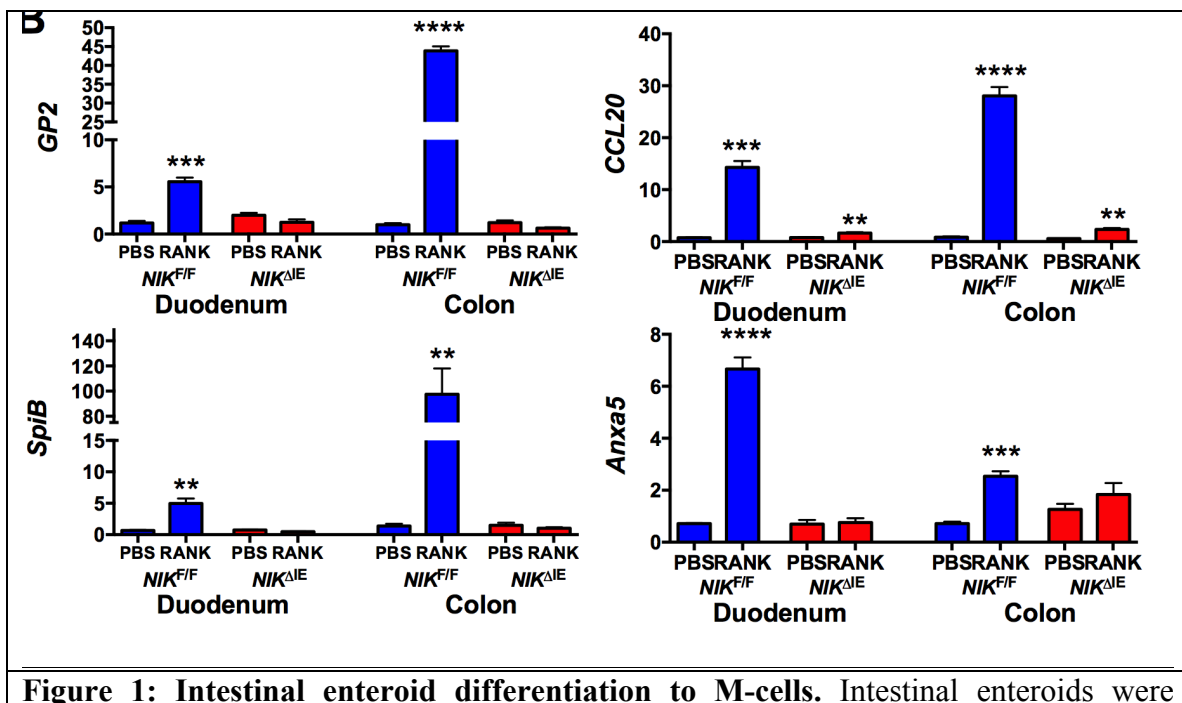


Figure 1: Intestinal enteroid differentiation to M-cells. Intestinal enteroids were

derived from NIK knockout mice (NIK^{ΔIE}) or wild-type littermates (NIK^{F/F}) and treated for 24-hours with RANL ligand. QPCR analysis was performed for M-cells markers.

Key research accomplishments:

- Identified mechanism of NIK regulation in colon tumors.
- NIK has an essential role in sporadic tumorigenesis.
- NIK disruption in intestinal epithelial cells leads to decrease M-cells.

IMPACT:

Our data shows that in mouse models of colitis and colitis-associated colon cancer, and in patients with ulcerative colitis and Crohn's disease, the expression of NIK and p52 were highly induced in intestinal epithelial cells suggesting a potential role for NF-κB2 pathway in intestinal inflammation and cancer. Intestinal epithelial specific disruption of NIK resulted in increased susceptibility to mouse models of colitis. Mechanistically, disruption of NIK in the epithelial cells resulted in loss of follicle associated microfold (M)-cells and decrease in lymphoid associated B-cells. The defect in M-cell differentiation following NIK disruption results in increased inflammatory response to microbiota upon DSS treatment. Consistent with this data, antibiotic treatment significantly attenuated DSS-induced colitis in mice with epithelial disruption of NIK. Further work in colon cancer models demonstrated that NIK signaling has a dual function in colorectal tumorigenesis. NIK protects intestinal epithelial cells against colitis-associated colon cancer. Epithelial disruption of NIK results in enhanced colitis-induced epithelial damage and inflammation, which potentiates colon tumor formation. Interestingly, disruption of NIK in established colon tumors reduces tumor burden. Thus, epithelial NIK suppresses inflammation-associated epithelial damage and tumorigenesis but contributes to the proliferation and survival of tumor cells. Further analysis is underway to determine if non-canonical NF-κB2 pathway regulation of M-cell differentiation is critical in colon cancer progression.

CHANGES/PROBLEMS:

Nothing to report

PRODUCTS:

Huabing Zhang presented an abstract at the 2014 University Michigan Cancer Center Symposium, GI Peptide Symposium, Cancer Center Retreat, and Molecular & Integrative Physiology Research Symposium. **Title: NIK Modulates Intestinal Homeostasis and Inflammation.**

Xiaoya Ma presented the work at a Gut group meeting. **Title: NIK an essential link between epithelial cells and mucosal immune response in colitis and cancer.**

We are presently preparing two manuscripts for publication. The colitis aspect will be submitted to *Immunity* in the next 2-3 months, whereas the colon cancer data will be submitted to *Cancer Cell* in the next 5-6 months.

PARTICIPANTS:

Name:	<i>Yatrik Shah</i>
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Nearest person month worked:	<i>0.6</i>
Contribution to Project:	<i>Was involved in the planning of experiments and data interpretation.</i>

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Project Role:	<i>Postdoctoral Fellow</i>
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Contribution to Project:	<i>Was involved in the planning of experiments and data interpretation.</i>

APPENDIX:

none