NIH SPECIFIC AIMS PAGE

1st Paragraph

Hook

- Why should we care?
- Establish a sense of importance or urgency.

What is Known

- Provide background info necessary to understand why you are proposing the work.
- KEY DETAILS ONLY.

Gap in Knowledge

- Piece of information that is not known.

Critical Need

- What is the knowledge that you propose to develop?
- Why is this the logical next step for advancing the field?

2nd Paragraph

This paragraph should answer these questions:

- *What do you want to do?*
- *Why are you doing it?*
- *How do you want to do it?*

Long-Term Goal

- Must align with funder's objectives
    - Research
    - Should remain general

Hypothesis and Proposal Objectives

- You want to demonstrate to the reviewers that you have a hypothesis-driven proposal that is testable.
- Describe how your project addresses the critical need.
- Clearly state the proposed solution.

Rationale

- Explain how you arrived at your central hypothesis (past studies, published literature, etc.)
- State what your project's completion would make possible and TIE IT TO THE FUNDING AGENCY'S MISSION

Qualifications

Specific Aims

3rd Paragraph

Innovation

Expected Outcomes/Deliverables

Impact

HYPOTHESIS AND SPECIFIC AIMS:

The transcription factor FOXP3 is critical to the regulation of numerous debilitating human immune-mediated diseases, the prevalence of which together affect over 8.5 million people (1 in 31 U.S. residents). In Inflammatory Bowel Disease (IBD) chronic intestinal inflammation indicates aberrant *in vivo* FOXP3+ T regulatory (Treg) cell function (1). Similarly, proinflammatory signals *in vitro* impair Treg function (2). Our lab was the first to characterize the essential role for the histone methyltransferase (HMT) EZH2 in the epigenetic regulation of FOXP3 (3). Recent published work extended our observations indicating a key role for EZH2 in FOXP3 repressor function (4); however the regulation and biological impact of the FOXP3-EZH2 pathway to IBD is unknown. This knowledge is important given the apparent loss of function of Treg cells in inflammation.

Our *long-term goal* is to dissect epigenetic mechanisms regulating Treg cellular differentiation and function, particularly within the setting of GI inflammatory diseases; as these discoveries will facilitate design of human cell therapy trials for IBD. Consequently, the *objective* of this grant is to characterize the role for the epigenetic regulator EZH2 in Treg suppressive function. These investigations are strongly supported by preliminary data demonstrating that: 1) EZH2 is required for Treg suppressive function; 2) IL6 signaling leads to phosphorylation and inhibition of EZH2; 3) lymphocytes isolated from the intestine of IBD patients demonstrate activation of IL6-induced gene networks and loss of EZH2 HMT function; and 4) conditional knockout of EZH2 in FOXP3+ T cells leads to *in vivo* immune dysfunction. Based upon these compelling data we propose the **CENTRAL HYPOTHESIS that EZH2 plays a critical role in the homeostasis of Treg cells, and the disruption of EZH2 function by inflammatory signaling pathways contributes to IBD.** Our rationale is that identification of the mechanism(s) to restore Treg suppressive function in the setting of intestinal inflammation will offer new therapeutic opportunities within the field of IBD. Our specific aims will test the following hypotheses:

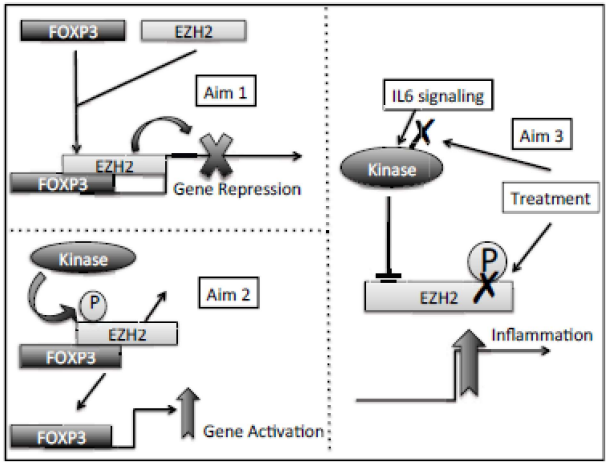

Figure 1: Conceptual framework. Through the mechanistic experiments designed in the following aims we will identify the role for FOXP3 in the recruitment of EZH2 to core target genes required for Treg function (Aim 1). We will define the signaling network responsible for phosphorylation of EZH2 and disrupted HMT function (Aim 2). Finally, we will perform pre-clinical studies of innovative therapy designed to generate Treg cells resistant to disruptive modifications in the setting of inflammation (Aim 3).

Aim 1: Repression of immunoregulatory gene networks by FOXP3 requires the formation of a complex between this transcription factor and EZH2.

Aim 2: Inflammatory stimuli, such as IL6 lead to EZH2 phosphorylation and thereby disrupt the enzymatic activity of this epigenomic regulator.

Aim 3: Inhibition of the IL6 to EZH2 signaling pathway permits sustained Treg suppressive function in the setting of intestinal inflammation.

Upon conclusion, we will understand the role for EZH2 in Treg loss of function in the setting of active inflammation. This discovery will stimulate new areas for experimental therapeutics in human chronic inflammatory diseases. Our environment in the Epigenetic and Chromatin Dynamics Laboratory combined with the Department of Immunology at the Mayo Clinic makes us uniquely qualified to pursue this objective given the extensive collective experience of histone methyltransferase biology, proinflammatory signaling networks, and *FOXP3* gene regulation.