

## Ph.D. DISSERTATION DEFENSE

**Candidate:** Kylee Zgeib  
**Degree:** Doctor of Philosophy  
**School/Department:** Charles V. Schaefer, Jr. School of Engineering and Science /  
Chemistry and Chemical Biology  
**Date:** Monday, May 5<sup>th</sup>, 2025  
**Time/Location:** 3:00 p.m. / McLean 510  
**Title:** Molecular Characterization of Dedifferentiation-Induced Oncogenic Stemness in the Intestinal Epithelium

**Chairperson:** Dr. Ansu Perekatt, Department of Chemistry and Chemical Biology,  
School of Engineering & Science

**Committee Members:** Dr. Paola DiMarzio, Department of Chemistry and Chemical Biology,  
School of Engineering & Science  
Dr. Patricia Muisener, Department of Chemistry and Chemical  
Biology, School of Engineering & Science  
Dr. Xiaojun Yu, Department of Biomedical Engineering, School of  
Engineering & Science

## ABSTRACT

The molecular features distinguishing tissue-specific stem cells from cancer-associated stemness remain poorly characterized. Here we are investigating the molecular events associated with dedifferentiation-induced oncogenic stemness within the intestinal epithelium, utilizing a Smad4 knockout: $\beta$ -catenin gain-of-function (Smad4<sup>KO</sup>: $\beta$ -catenin<sup>GOF</sup>) conditional mutant mouse model. The intestinal epithelium is spatially organized, delineating the proliferative crypt compartment from the differentiated villus compartment. The crypt compartment houses the stem cells which have been shown to be the cell-of-origin of colon cancer. However, cancer has also been shown to arise from the differentiated cells of the villus compartment. Thus, there are two models for the origin of colon cancer: the top-down model, wherein tumors arise from differentiated cells, and the bottom-up model, wherein tumorigenesis occurs from stem cells. The mouse model employed here mimics the top-down model of tumorigenesis and allows for the molecular characterization of cellular changes during cellular reprogramming from differentiated cells to oncogenic stem cells. Upon induction of this mutation, we observe cell fate reversal to oncogenic stemness within the differentiated compartment. This cellular event is characterized by the aberrant activation of Notch signaling, a pathway normally restricted to the crypt compartment in healthy epithelium. Furthermore, single-cell RNA sequencing (scRNA-seq) analyses reveal a heterogenous stem cell population, along with aberrant Notch activity and oncogenic markers during the dedifferentiation process. Compartment specific metabolic differences are also observed. Ultimately, this study provides valuable insights into the molecular changes underlying dedifferentiation-induced oncogenic stemness in the intestinal epithelium and advances our understanding of colorectal cancer pathogenesis, providing a foundation for further studies to evaluate the therapeutic vulnerabilities of oncogenic stem cells.