Team MINIGUT: Disruption of E-cadherin promotes intestinal stem cell proliferation in colonoids

Aaliyah Arpon, Michaela Bell, Katie Brown, Alisa Forsberg, Ananya Krishnan, Ryan Margolis, Laura Marhefka, Jerry Yang, Isha Yardi
Team Mentor: Dr. Younggeon Jin Team Librarian: Jodie Coulter

UNIVERSITY OF MARYLAND HONORS COLLEGE

GEMSTONE Honors College University of Maryland

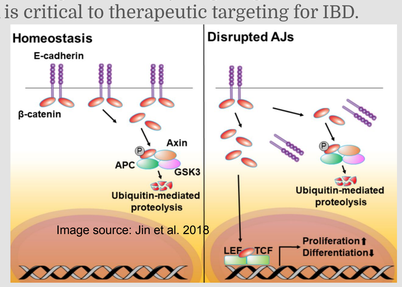
Abstract

Reconstitution of the wounded epithelium is integral to achieve the full healing of the gut mucosa in treating Inflammatory Bowel Disease (IBD). The ability of intestinal stem cells (ISCs) to indefinitely self-renew while generating new functional epithelia makes them a potential therapeutic tool for IBD. Transmembrane protein E-cadherin, a major constituent of AJs, regulate the Wnt signaling pathway. This pathway is vital for the ISC homeostasis and regeneration. However, the role of E-cadherin in ISCs is an important yet notably understudied phenomenon. Disruption of E-cadherin increases unbound cytosolic β-catenin levels, which go to the nucleus and increase Wnt transcription. We hypothesize that down-regulating E-cadherin will increase proliferation of ISCs. In our experiments, we disrupt E-cadherin with different concentrations of EGTA, a calcium chelator, and see the effect it has on colonoid growth and development. Our experiments showed that with EGTA there was greater proliferation; 1 mM EGTA experimental groups had larger colonoids than vehicle control colonoids on day 6. This indicates that EGTA treatment may induce more growing proliferation of the organoid with E-cadherin disruption. For future study, we will check and confirm Wnt signaling levels by qPCR and immunofluorescence studies. Ultimately our study has clinical applications down the line for patients living with IBD through personalized medicine.

Introduction

Pathogenesis of IBD is related to the dysregulation of the epithelial barrier. Intestinal renewal and regeneration upon injury is mediated by intestinal epithelial stem cells (ISCs), a cluster of undifferentiated cells located in the intestinal crypt. The Wnt signaling pathway is responsible for stem cell proliferation, and differentiation. It is regulated by β-catenin protein degradation and activated to regenerate during inflammation (Garcia et al., 2018).

Adherens Junctions (AJs) are cell-cell adhesion complexes with critical functions in stem cells and their niches. AJ components β-catenin, coupled with E-cadherin, play essential roles in the Wnt-signaling pathway (Vancamelbeke et al., 2017). E-cadherin acts as an anchor for β-catenin and is important in cell-cell adhesion and maintaining homeostasis. Reduced E-cadherin during injury (in IBD) disrupts the AJ compromising the complex. EGTA can mimic this disruption of E-cadherin binding to β-catenin. With more unbound β-catenin in the cytosol, the β-catenin goes to the nucleus and increases transcriptional activity which results in increased ISCs proliferation. The activation of the Wnt/β-catenin signaling is essential during intestinal homeostasis and regeneration. Additionally, disruption of E-cadherin is expected to disrupt and damage adherens junctions in colonoids, resulting in morphological changes that can be monitored. There is a poor understanding of the function of E-cadherin in the stem cells and their niche. Understanding the impact of AJs on ISCs populations and their contributions to epithelial regeneration by targeting E-cadherin disruption with EGTA is critical to therapeutic targeting for IBD.



Research Aim & Hypothesis

Determine the role of E-cadherin during the development of mouse colonoids.

Our Hypothesis: E-cadherin disruption will release of β-catenin from AJs, resulting in increased Wnt signaling pathway activity, which will increase proliferation of ISC.

Methodology

Creating L-WRN Conditioned Media

- Prior to the experiment, the supply of L-WRN conditioned media was being continually replenished to sustain the colonoids culture models. L-WRN CM consists of Wnt-3a, R-spondin 3, and Noggin and is required for the intestinal stem cell niche.

Establish the colonoids from mouse colonic crypts

- 2D colonoids were established from thawed mouse colonic crypts.
- Cell suspension from crypts were plated onto collagen hydrogel.
- 50% L-WRN CM with supplements was replaced every two days to maintain the colonoids.

Seeding 3D Colonoids and EGTA Treatment

- On Day 0, 3D colonoids in the Matrigel on a 96-well plate were prepared with cells from 2D colonoids allow to grow for 1 days.
- Starting on Day 1, the plate was treated and replaced every other day with EGTA (1.4 mM, 1.2 mM, 1.0 mM, 0.8 mM, 0;6 mM) or its vehicle (1.4 mM and 1.0 mM NaOH) contained media.
- Organoid growth in each well was assessed daily by taking photos.

Morphological Analysis

- ImageJ was used to quantitatively analyze the area of colonoids to evaluate the organoid growth and proliferation.

Statistical Analysis

- One way ANOVA was run on area versus treatment with a null hypothesis of equal means and a 95% confidence interval while utilizing a tukey, fisher, and dunnett test.

Results

3D colonoids successfully developed using L-WRN CM

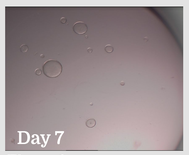


Figure 1a.

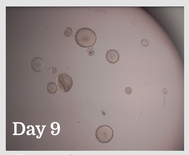


Figure 1b.

Figure 1c.

Figure 1a., 1b., 1c.: Colonoids at Day 7 vs. Day 9 vs. Day 11. Colonoid growth, differentiation, and death is visible between these days.

Results-Continued

EGTA-induced E-cadherin disruption resulted in increased growth of the colonoids

Figure 2a.

Figure 2b.

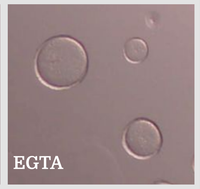


Figure 2c.

Figure 2a., 2b., 2c.: Representative images of colonoid growth 6 days post-seeding.

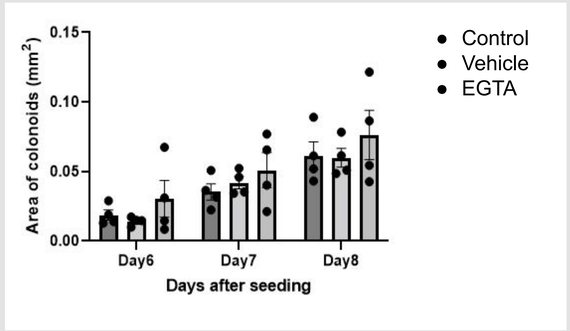


Figure 2d.

Figure 2d.: Graphed colonoid area on day 6, 7, and 8 post=seeding for each treatment group. Colonoid area for each treatment group was analyzed and averaged over four wells for each day. EGTA treated colonoids had the largest average colonoid size from days 6-8.

Conclusion

The colonoids treated with EGTA tended to have slightly larger colonoids than those of the vehicle control. Although this difference is not statistically significant, we suggest EGTA may induce increased proliferation of colonoids via WNT up regulation. A repeat validation experiment is necessary in which we will assess Wnt signaling levels and characterize the intestinal cell population by qPCR and immunofluorescence.

Our study has clinical applications for patients living with IBD. Using regenerative medicine, one can harvest cells of IBD patients and culture in vitro organoids, temporarily downregulate E-cadherin, and transplant them back into the patients. These organoids once implanted would rejuvenate and regenerate the inflamed intestinal tract, making it a viable clinical therapeutic for those who are resistant to traditional treatments.

Future Research

1. For future studies, we will study proliferation using Ki67 staining and PI staining methods with or without treatment of EGTA.
2. Additionally, we will aim to demonstrate the EGTA-induced disruption of E-cadherin and increased cytosolic/nuclear β-catenin using IF microscope analysis to further confirm our current data and hypotheses.
3. We would also like to explore the significance of the Wnt signaling pathways and different cellular population using qPCR and IF microscope analysis.
4. Ultimately, we can apply this to a clinical approach to address IBD in patients resistant to traditional treatments.

Other Progress: Review Paper

Our team began our data collection and lab work during 2021 while navigating campus covid restrictions. With global supply chain issues, there were delays to our experimental schedule and data collection took more time than expected. In the meantime some of our team members, with guidance from our mentor Dr. Jin, have been writing a comprehensive review paper on our topic. Our review paper covers topics including: Disease Models, Function & Evaluation, and Organoids. This paper has been the majority of the work our writing sub team has been doing since Fall of 2021. We have made great process and are preparing to submit for publication sometime in 2022.

Works Cited

Garcia, M. A., Nelson, W. J., & Chavez, N. (2018). Cell-Cell Junctions Organize Structural and Signaling Networks. Cold Spring Harbor perspectives in biology, 10(4), a029181. https://doi.org/10.1101/cshperspect.a029181

Vancamelbeke, M., & Vermeire, S. (2017). The intestinal barrier: A fundamental role in health and disease. Expert Review of Gastroenterology & Hepatology, 11(9), 821-834. doi:10.1080/17474124.2017.1343143

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