ALTERED GOBLET CELL FUNCTION IN HIRSCHSPRUNG’S DISEASE

Tristan Lim, Class of 2018, Department of Chemical and Biological Engineering
National Children’s Research Centre at Our Lady’s Children’s Hospital, Crumlin, Dublin, Ireland
Funded by the Princeton Internships in Civic Service and the Center for Health and Wellbeing

Introduction
Hirschsprung’s disease (HD) is the most common congenital gut motility disorder
- Common cause of intestinal obstruction
- Occurs in about 1 in 5000 live births in US
- Treatment is complicated by Hirschsprung’s disease associated enterocolitis (HAEC)
- Can occur before or after optimal treatment
- Affects 77%-50% of HD patients

HAEC is the leading cause of HD-related morbidity and mortality
- Cause is poorly understood
- Etiology is critical in improving HD outcomes
- Hypothesized defect in gut mucosal barrier

Objective of the Study
To better the understanding of HAEC etiology, the expression of genes related to mucus-producing goblet cell development (MUC2, TFF3, SPDEF, and KLF4) in the intestine was examined

Methods
Study Design: Prospective Study
- Hospital study sites in two Dublin hospitals
- Recruited over 100 patients
Patient Selection: Pediatric Patients
- Cases: Pediatric HD Patients
- Controls: Pediatric Colostomy Patients
Lab Methods: Genetic/Protein Analyses
- Tissue specimens fixed in formalin at RT
- Each sample will undergo RNA isolation, cDNA library synthesis, and staining
- PCR, Western Blot, Immunohistochemistry
Data Analysis: Performed in Excel
- Student’s t test for comparison of groups
- Values presented as mean with SEM

Results
Figure 1. qRT-PCR revealed significantly decreased relative mRNA expression levels of SPDEF, KLF4 and TFF3 in the aganglionic and ganglionic HSCR specimens (n = 10) compared to normal control tissue (n = 10). Results are presented as mean ± SEM (p = .003 by one-way ANOVA). Western blotting revealed significantly decreased protein expression levels SPDEF, KLF4 and TFF3 in the aganglionic and ganglionic HSCR specimens (n = 10) compared to normal control tissue (n = 10). Results are presented as mean ± SEM (*p = .003 by one-way ANOVA).

Figure 2. SPDEF, KLF4 and TFF3 expression (green) in the colonic epithelium (red) of HSCR compared to healthy controls. A μm (scale bar 25 μm, original magnification × 63). SPDEF, KLF4 and TFF3 expression (green) in the colonic epithelium (red) of HSCR compared to healthy controls. A total of 8 specimens were analyzed; only the most representative stainings are shown. Compared with the control group there were significantly decreased expression of SPDEF, KLF4 and TFF3 in colonic epithelium of patients with HSCR.

Figure 3. Alcian blue staining revealed significantly decreased number of goblet cell in the aganglionic and ganglionic HSCR specimens compared to normal control tissue. Results are presented as mean ± SEM (p = .04 by one-way ANOVA).

Discussion
Insignificant change in MUC2 expression
- No difference between aganglionic/ganglionic sections of patients with HD
- Functionally secreted levels may be different

Decreased expression of TFF3, SPDEF, KLF4 in both ganglionic and aganglionic sections
- TFF3 – less protective barrier properties
- SPDEF – less development of goblet cells
- KLF4 – less goblet cell differentiation

Decreased population of goblet cells
- Decreased in both aganglionic/ganglionic
- Possibly explains why HD patients suffer even after pull-through treatment

Conclusion
- Decreased goblet cell function, development, and population in cases compared to normal controls
- That ganglionic and aganglionic sections of cases have decreased gene expression of target genes compared to controls may explain why surgical treatment does not prevent HAEC
- Results suggest altered goblet cell function in HD may lead to intestinal barrier dysfunction

Future Plans
- Examine MUC2 secretion levels
- Examine knockout models for candidate genes

Acknowledgements
I would like to thank:
- Professor Prem Puri and Dr. Hiroki Nakamura for being fantastic project advisors
- NCRC for making my summer wonderful and giving me insight into pediatric research
- CHW and PICS for funding my summer and providing me this amazing opportunity

Example References