NSBB 552 Final Project Proposal
Erika Altamirano
2021-04-29

Background: Necrotizing enterocolitis (NEC) is a gastrointestinal disease that is the leading cause of premature infant death in the NICU, with a mortality rate of 20–50%. NEC is difficult to diagnose due to late stage signs such as abdominal distension, blood in stool, microbial imbalance, and gas in intestinal walls (pneumatosis intestinalis) which quickly progresses to local and systemic inflammation, multi-organ failure, and death. Even if the premature infant survives NEC, they may lead a decreased quality of life due to permanent bowel issues and neurodevelopmental delays from NEC exposure. The NEC profile is characterized by up-regulation of pro-inflammatory markers such as IL-6, IL-8, IL-1B, and TNF alpha.

Hypothesis: **We hypothesize that the development and severity of NEC can be attenuated by through inhibition of pro-inflammation pathways.**

Further understanding of how NEC affects pro-inflammatory markers is necessary in order to understand how to develop a treatment for it.

Tx: HB-EGF has been shown to have a protective effect against NEC by decreasing the expression of pro-inflammatory markers. The proposed mechanism that I believe my treatment (HB-EGF) functions is through attenuation of the TLR4 pathway. There are only proposed mechanisms of how HB-EGF fits into this schema, and I think further understanding of the affected inflammatory markers is necessary in order to understand where HB-EGF fits in.

Toolset:
The toolset that I believe I'll be using is a combination of R tools such as Deseq2, ggplot, ggplot2 and other genomic feature/libraries in order to clean raw fastq sequencing files into usable text files. I will be keeping an R notebook of relevant bash scripts as well as using the Salmon quantification tool to pull the data, align, and map the raw fastq files. Deseq2, ggplot, ggplot2 and other genomic feature/libraries will be very important for basic statistical summaries of what the data looks like. I would like to look at Pearson correlation heatmaps, these will be created Bioconductor tools such as pheatmap and ggplot2.. I would also like to use ggplot2 to generate volcano plots to visualize log fold change. Lastly I will use relevant genomic libraries in order to do a pathway analysis of the most up-regulated genes.

Visual Aides:
These tools will allow me to analyze RNA sequenced data of NEC samples in order to produce appropriate summary statistics, observe the log fold change, and probability distribution across base pairs. I'm really interested in observing the up-regulation of genes encoding for inflammatory cytokines in the Nec model in comparison to the control (non-NEC) group. Due to the increased presence of cytokines in patients with NEC, I think it would be interesting to observe the differential expression of healthy and NEC tissue with PCA plots and Pearson's correlation heat maps. The visualization of log fold changes across inflammatory-related genes of interest as well as their associated pathways could give the field a better understanding of how treatments such as HB-EGF fit into the attenuation of this disease.