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The effects of EBOV sGP on macrophages via RNA sequencing

Miriam Brown, OMSII; Chelsea Weidman, MS; Jillian Bradley, Ph.D.
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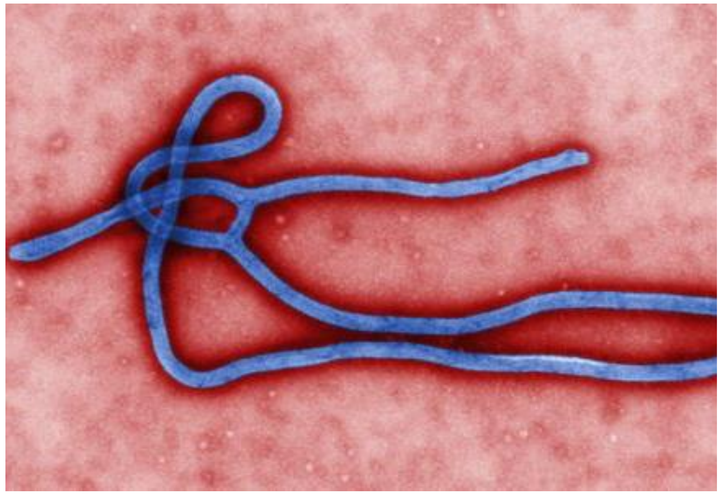
TAP TO GO BACK TO KIOSK MENU

Introduction | Methods | Results | Discussion

Currently, the second largest outbreak of Ebola Virus in history is taking place in the Democratic Republic of Congo. While the 2014-2016 outbreak in West Africa sparked research for a cure, there is still relatively little known about the pathogenesis of an Ebola Virus (EBOV) infection. When a person is infected, if they are able to live long enough to develop antibodies, they will most likely recover. Therefore, understanding how Ebola affects cells of the early immune response, such as macrophages, could have implications for developing a targeted treatment for acute infection. During infection, large quantities of the secreted form of the glycoprotein (sGP) are found in the serum, suggesting a role in the pathogenesis of the virus. Previously, this lab found that when macrophages were exposed to sGP, production of the pro-inflammatory cytokines was inhibited. To uncover the mechanism behind this inhibition, the experiment was repeated and samples were sent for RNA-sequencing.

Click Below to Learn More!

- CDC: Ebola for Clinicians
- WHO: Current Ebola Outbreak
- Read about RNA sequencing



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1. THP-1 monocytes are added to a 96-well, flat bottom plate at 200k/mL stimulated into macrophages with a three day incubation period with PMA (200uM), followed by a five day rest. After the three day incubation period, the PMA is washed and the media refreshed. During the five day rest, the media is refreshed every other day.
2. In order to polarize the macrophages to the M1 phenotype, Interferon gamma (20ng/mL) is added for 24 hours.
3. After 24 hours, the cells are washed and the media refreshed. LPS (1ug/mL), a TLR4 agonist, is added to activate the M1s for 24 hours.
4. After 24 hours, the cells are washed of the LPS and the media is refreshed. EBOV sGP (8ug/mL) is added to the cells for 24 hours.
5. The cells were collected via trypsonization
6. Media was removed, and pellet at bottom of conical was disrupted
7. Buffer RLT (350 μL) added and vortexed
8. Samples snap frozen in liquid nitrogen
9. Shipped on dry ice for processing

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On average, 80 thousand isoforms were identified per sample. All samples passed quality control metrics. The radar plot (Fig. 1) shows consistency in the number of genes identified, showing the samples are all of similar quality. The principal component analysis (Fig. 2) reveals how well the sample groups cluster based on the genes that have the largest coefficient of variation, showing that clustering is based on biological differences between sample groups. The volcano plot (Fig. 3) provides a visualization of changes in gene expression that are statistically significant. The heat map (Fig. 4) demonstrates the upregulation and downregulation of genes, and shows which samples have the most similar gene expression.

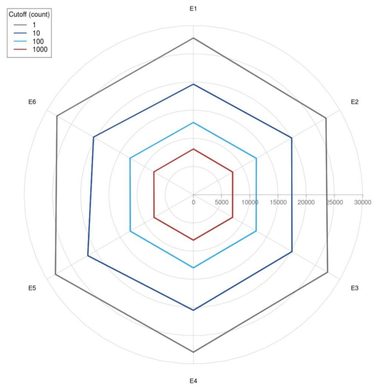

Figure 1. Radar plot showing number of genes identified for each sample at different fragment count cut-off values. FPKM is a unit of measuring gene expression used for NGS experiments. The number of reads corresponding to the particular gene is normalized to the length of the gene and the total number of mapped reads (Fragments Per Kilobase of transcript per Million mapped reads).

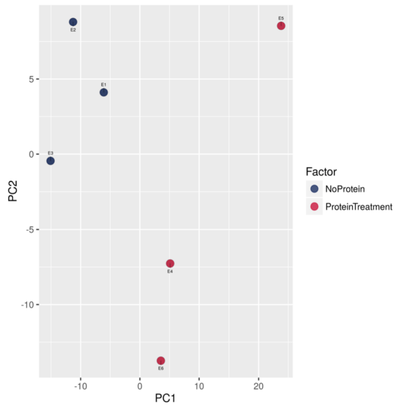

Figure 2. Principal component analysis (PCA). The PCA was performed on all samples using the 500 genes that have the largest coefficient of variation based on FPKM counts. Each circle represents a sample.

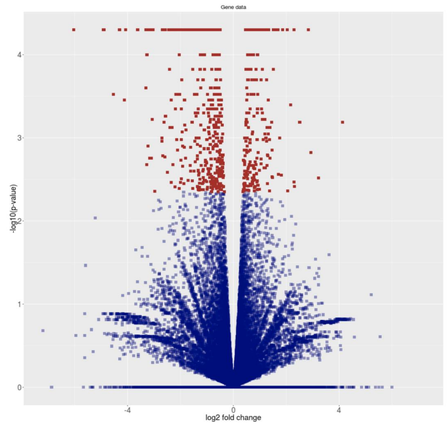

Figure 3. Volcano plot showing the relationship between the raw p-values and the log2 fold change in normalized expression (FRKM) between the Protein Treatment and No Protein Treatment groups. There are two regions of interest in the plot: the points near the top of the plot signify high statistical significance, and the points at the extreme left or right signify strongly down and up-regulated, respectively.

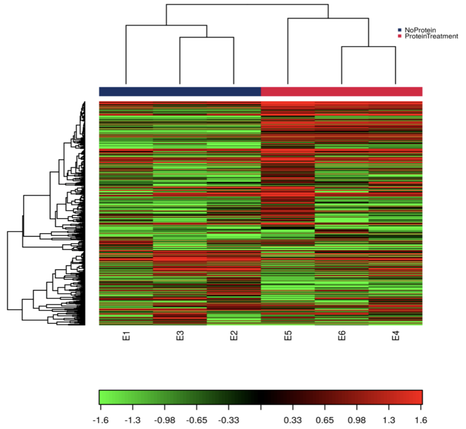

Figure 4. Heat map and unsupervised hierarchical clustering by sample and genes were performed using the 500 genes that have the largest coefficient of variation based on FPKM counts.

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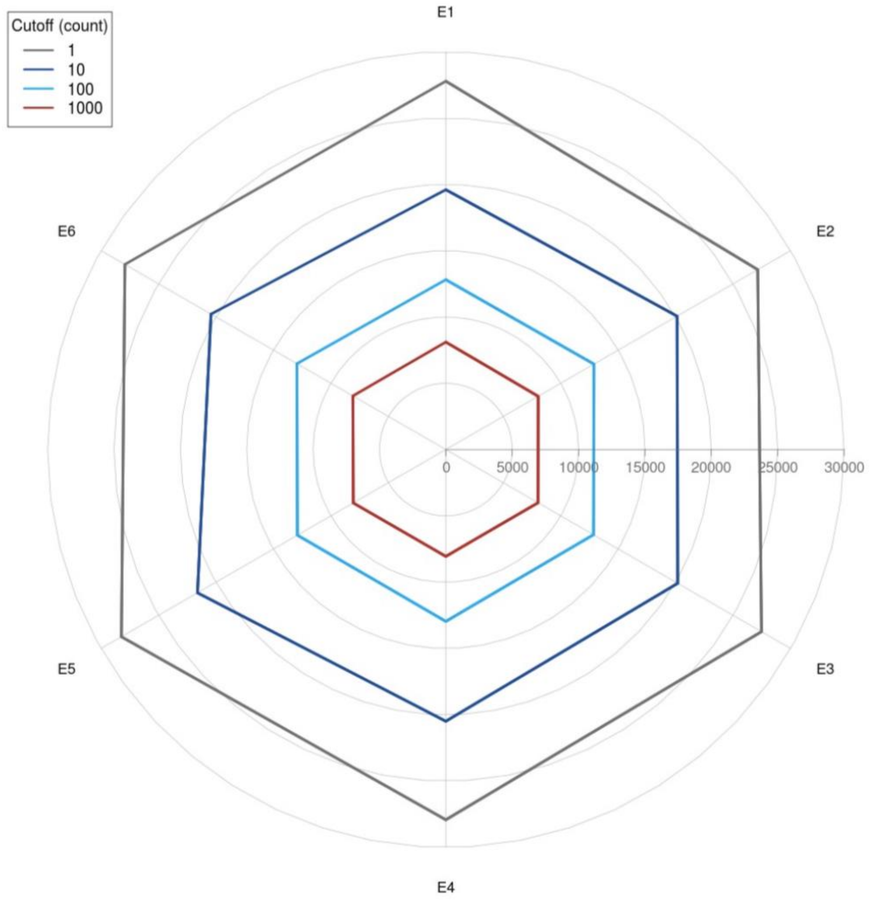
- **Data Analysis:** 85% of the sample's data was mapped using gene ontology (GO) analysis. The most significant GO term was modulation by a virus of host morphology. Other significant GO terms included inflammatory response, regulation of transcription, intracellular protein transport, and regulation of small GTPase mediated signal transduction.

- **Future Direction:** The results of the mRNA-sequencing data show significant differences between the samples of LPS activated macrophages exposed to sGP and LPS activated macrophages not exposed to sGP. Continued research is underway to combine the lists of the most significantly differentially expressed genes and isoforms, the GO terms and network, and our current knowledge of Ebola Virus and the human body to uncover the mechanism of sGP inhibition of pro-inflammatory cytokine production by macrophages.

- **Limitations:** It is important to recognize that, while this mRNA-sequencing experiment provides a wealth of information, there are limitations. mRNA-sequencing only provides a single snapshot of the content of the transcriptome, while a living cell has an ever changing environment. This limitation was addressed by keeping data collection times consistent across experiments, as well as submitting multiple samples with the same exposure and conditions for analysis. Additionally, mRNA-sequencing does not reflect changes that occur with protein translation and post-translational processing of proteins. While this is simply the nature of mRNA-sequencing, it is something to keep in mind for further inquiry to Ebola Virus pathogenesis.

- **Acknowledgements:** We would like to thank many people for their contributions. mRNA ngs Data Analysis report performed by QIAGEN Genomic Services. Dr. Randal Gregg assisted with mechanism guidance. This projected was funded by the Research Eureka Accelerator Program, generously provided by The Edward Via College of Osteopathic Medicine.
- References: Click to view

Radar Plot



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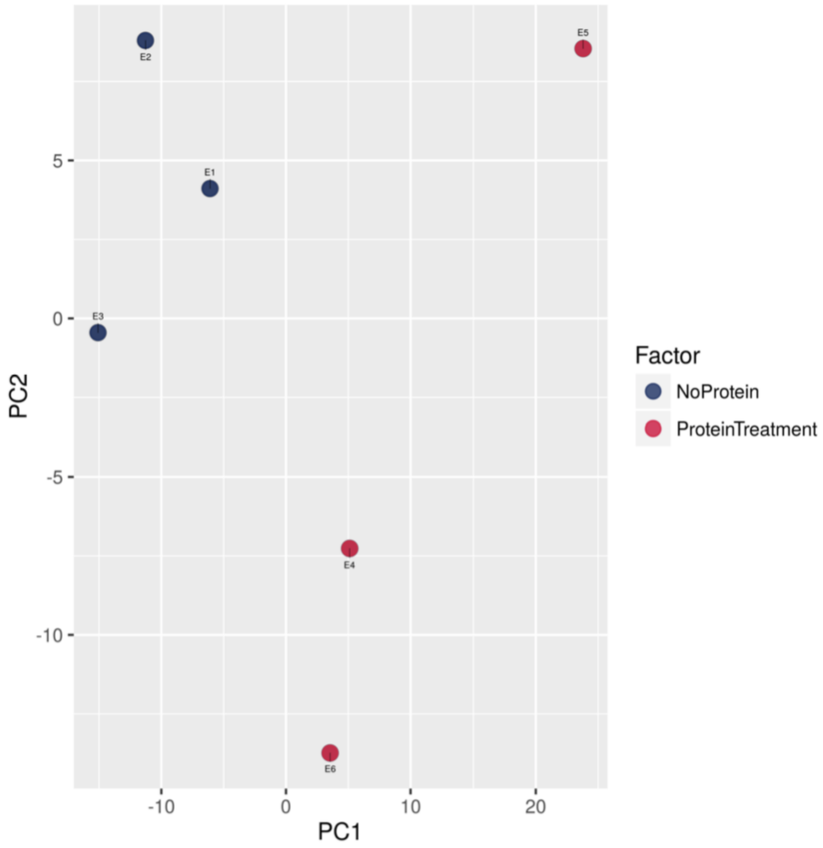
Principal Component Analysis



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Volcano Plot

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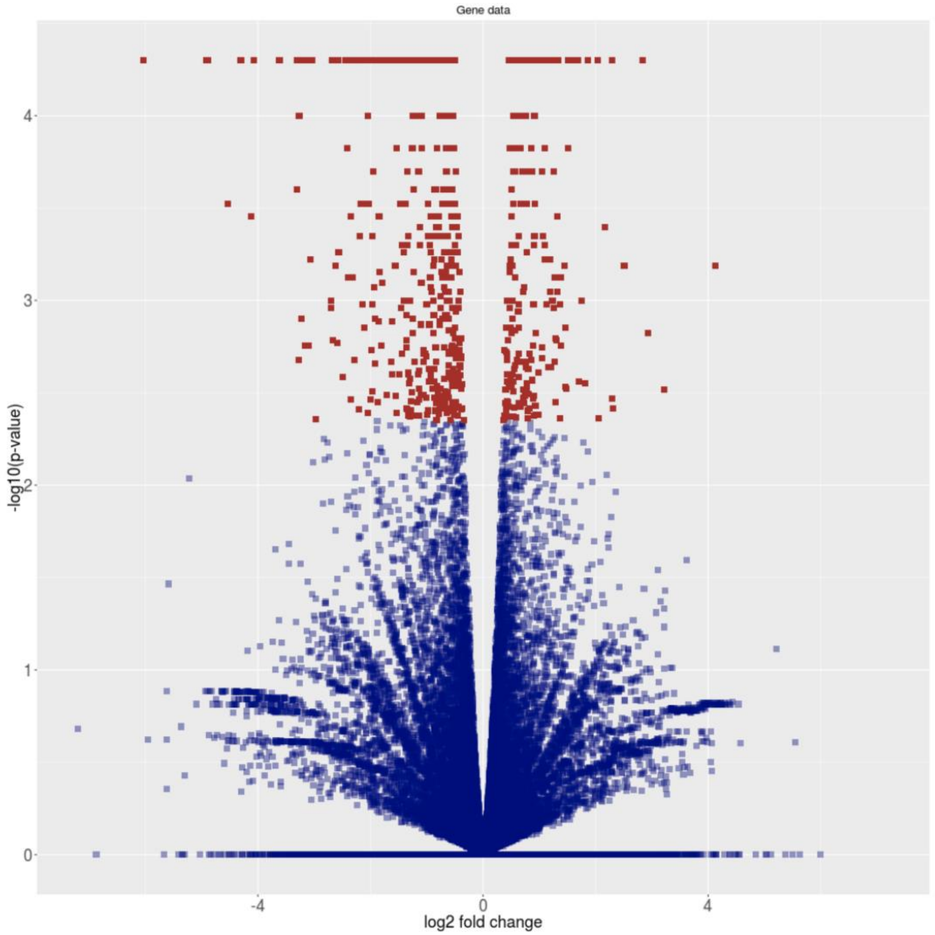

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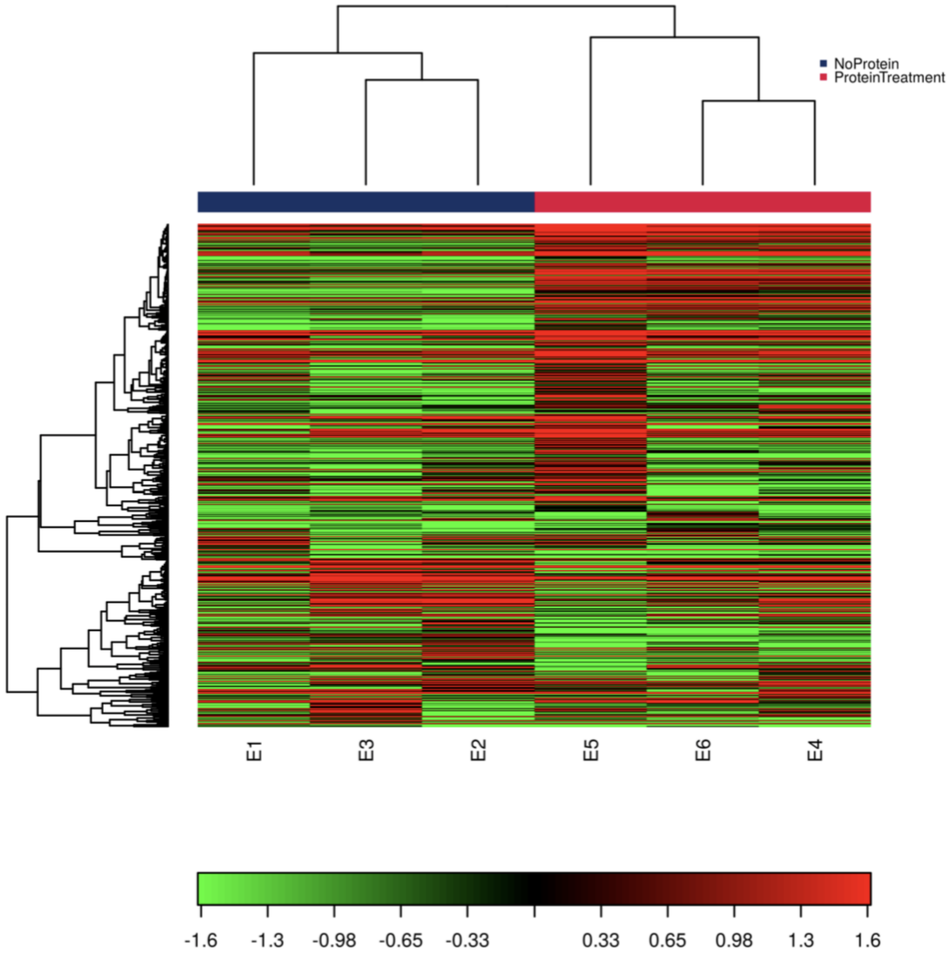
Heat Map



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References

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1. Lee JE, Saphire EO. Ebolavirus glycoprotein structure and mechanism of entry. *Future Virol*. 2009;4(6):621–635. doi:10.2217/fvl.09.56
2. Bradley, J., A. Harrison, A. Corey, N. Gentry, and R. Gregg. 2017. Ebola virus secreted glycoprotein decreases the anti-viral immunity of macrophages in early inflammatory responses. *Cellular Immunology.* ISSN 0008-8749
3. CDC. Ebola (Ebola Virus Disease). Accessed 03/31/2019. https://www.cdc.gov/vhf/ebola/index.html
4. https://www.who.int/ebola/situation-reports/drc-2018/en/
5. https://genohub.com/rna-seq-library-preparation/