Investigating the Transcriptome-Level Response of Differentially-Polarized Macrophages to *Cryptococcus neoformans* Infection

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Introduction

Cryptococcus neoformans (C. neoformans; Figure 1) is a fungal pathogen found in soils and bird excrement in urban environments worldwide. When inhaled into the lungs and deposited into the alveoli, it can cause pneumonia, but then develop into meningoencephalitis, an infection that causes inflammation of the meninges of the central nervous system. This specific encephalitis can lead to death if not treated (Coelho *et al*., 2014; Liu *et al*., 2008). Individuals who are immunocompromised (AIDs patients, etc.) are more likely to contract this disease compared to individuals with healthy immune systems (Coelho *et al*., 2014; Liu *et al*., 2008; McClelland *et al*., 2007).

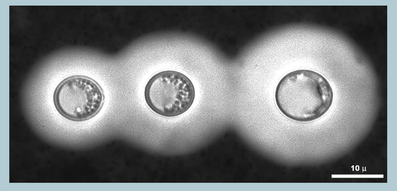

Figure 1. *C. neoformans with stained polysaccharide capsule. (image by E. McClelland)*

The immune system is a vital part of the body's defense system towards pathogens such as *C. neoformans* by using specific cells, like macrophages, to monitor, protect, defend, and repair the body.

Macrophage functions include:

- Phagocytosis of harmful agents
- Display of antigen specific molecules for target and attack
- Production of cytokines to regulate inflammation.

Macrophages are classified into three categories:

- M0, the initial state
- M1, the defense state, which generates a major inflammatory response (pro-inflammatory)
- M2, the healing state that involves the recovery following an active immune response (anti-inflammatory).

Objectives

The objectives of this research are
(1) to determine which genes are differentially expressed between fungal-infected or mock-infected mouse macrophages based on polarization state
(2) to identify biological pathways that are affected by *C. neoformans* in each polarization state.

Materials

Transcriptome data used for analysis of the differentially expressed genes were produced in collaboration with Dr. David Nelson and Dr. Erin McClelland. The experimental conditions were performed in triplicate. RNA sequencing of each library sample was performed at Novogene (Sacramento, CA) to produce transcriptome paired-end reads of 150 bp.

- **M0** • Mock-treated to remain in naïve state
- **M1** • Mock-infected with PBS for 2 hrs. M1 cells activated with IFN-γ. 24 h incubation. Washed cells along with media replacement every 6 hrs. Polarization maintained with cytokines.
- **M1+*Cn*** • Infected with *C. neoformans* for 2 hrs. M1 cells activated with IFN-γ. 24 h incubation. Washed cells along with media replacement every 6 hrs. Polarization maintained with cytokines.
- **M2** • Mock-infected with PBS for 2 hrs. M1 cells activated with IFN-γ. M1 cells repolarized to M2 with IL-4. 24 h incubation. Washed cells along with media replacement every 6 hrs. Polarization maintained with cytokines.
- **M2+*Cn*** • Infected with *C. neoformans* for 2 hrs. M1 cells activated with IFN-γ. M1 cells repolarized to M2 with IL-4. 24 h incubation. Washed cells along with media replacement every 6 hrs. Polarization maintained with cytokines.

Methodology

RNA sequencing data generated from *in vitro* infected and mock-infected macrophages in the three polarization states were analyzed using data tools within the CyVerse Discovery Environment and custom PERL scripts, as well as other bioinformatics tools. Differentially expressed genes (DEG) were identified through pairwise comparisons: M0-M1, M0-M2, M1-M2, M1-M1+*Cn*, M2-M2+*Cn*, M1+*Cn*-M2+*Cn*.

- **Inputs** • RNA Sequencing Data
- **FastQC** • Quality Check
- **STAR** • Assemble Reads on Genome
- **StringTie** • Assemble Transcriptome
- **CuffDiff2** • Differentially Expressed Genes

Workflow

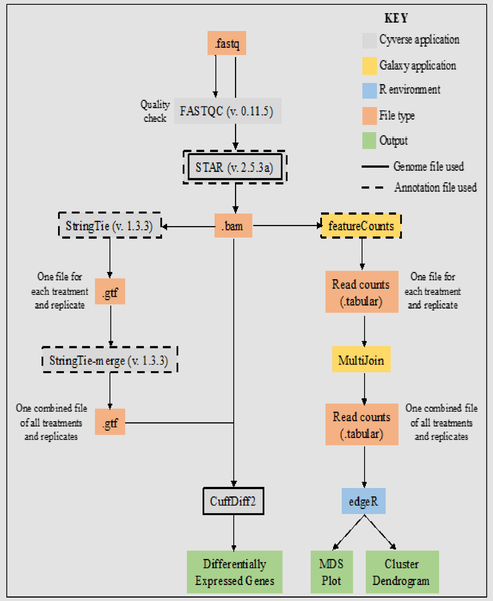

Figure 2. Representation of the Bioinformatics Workflow (Sircy *et al.*, 2018) that was used for the analysis of differentially expressed genes between fungal-infected and mock-infected mouse macrophages at different polarization states (M0, M1, and M2).

Results

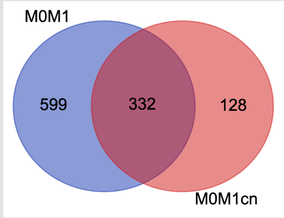

Figure 3. Unique and Common Genes are Differentially Expressed in M1 Polarized Macrophages based on Infection. Of the 931 DEG identified by comparing M0-M1 transcriptome differences, 332 are common to the 460 DEG identified by comparing the M0-M1+*Cn* transcriptome. This allowed identification of 599 genes that become more M0-like in response to infection and 128 non-M0 genes impacted by infection.

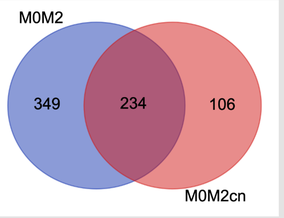

Figure 4. Unique and Common Genes are differentially expressed in M2 Polarized Macrophages based on Infection. Of the 583 DEG identified by comparing M0-M2 transcriptome differences, 234 are common to the 340 DEG identified by comparing the M0-M2+Cn transcriptome. This allowed identification of 349 genes that become more M0-like in response to infection and 106 non-M0 genes impacted by infection.

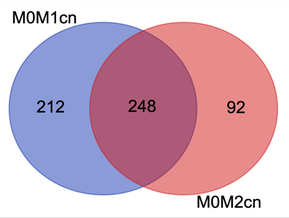

Figure 5. Unique and Common Genes are differentially expressed in fungal-infected M1 and M2 Polarized Macrophages. Of the 460 DEG identified by comparing M0-M1+*Cn* transcriptome differences, 248 are common to the 340 DEG identified by comparing the M0-M2+*Cn*. This allowed identification of 212 genes that become more M0-M1-like in response to infection and 92 non-M0-M1 genes impacted by polarization.

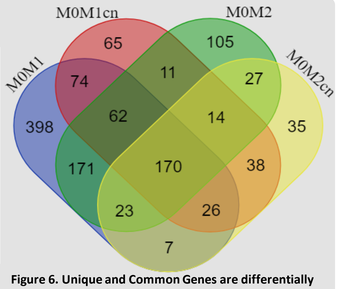

Figure 6. Unique and Common Genes are differentially expressed in the fungal-infected and mock-infected Polarization States of Macrophages (M0, M1, M2). Of the 1,226 total genes found in the four, pairwise comparisons, 398 are unique to M0-M1, 65 are unique to M0-M1+Cn, 105 are unique to M0-M2, and 35 are unique to M0-M2+*Cn*. This allowed identification among the groups of 533 common genes from M0-M1, 395 common genes from M0-M1+*Cn*, 478 common genes from M0-M2, and 305 common genes from M0-M2+*Cn*.

Discussion

This work shows that intracellular infection with *Cryptococcus neoformans* affects mouse macrophages at the transcriptome level and has both common and distinct effects based on macrophage polarization state (M0, M1, and M2).

Future Directions

The Database for Annotation, Visualization, and Integrated Discovery (DAVID) functional bioinformatics tool is being used to identify specific pathways and functions for each differentially expressed gene set.

STRING analyses will also be performed to identify functional protein association networks among the gene groups.

Current work includes:

- Analyzing the clustering of gene replicates in the R Environment
- Analyzing the DAVID output and identifying specific target genes to begin examining their unique roles
- Creating STRING analyses of specific gene groups for visual examination of associated networks between differentially expressed genes

Acknowledgements

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References

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