Gene co-expression analysis identifies gene clusters associated with isotropic and polarized growth in *Aspergillus fumigatus* conidia

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Conidial germination inside human lungs

Conidia are microscopic reproductive structures with a diameter of 2 – 3 µm, this is small enough to reach the lower airways of the human lung¹. Conidial outgrowth inside human lungs is the first step of infection in immunocompromised individuals. Mortality rates are high and go up to 90% and more in specific host groups². Despite the significance of conidial outgrowth inside human lungs, molecular mechanisms fundamental to germination remain mostly unclear.

In this study, we analyzed gene expression during the first 8 h of germination and performed a weighted gene co-expression network analysis to identify genes with similar expression patterns.

Two distinct morphological phases: isotropic growth (2-4h) and polarized growth (6-8h)

Germination of *A. fumigatus* conidia as observed by fluorescence microscopy. Bar represents 5 µm.

Weighted Gene Co-expression Network Analysis

- WGCNA resulted in 22 highly co-expressed gene modules.
- Modules contain differentially expressed genes and non-differentially expressed genes.
- For each module the correlation with the growth phase was calculated (dormant, isotropic growth and polarized growth).
- Correlated modules (correlation $\geq |0.75|$ and p-value $\leq 0.01^*$) were selected for Gene Ontology (GO) analysis.
- Only modules with significant correlation values are shown in the figure below.

Module-growth phase relationships

Conidial growth & transcriptome analysis

- Two *A. fumigatus* isolates were inoculated in RPMI medium and incubated for 0h, 2h, 4h, 6h, and 8h at 37°C in a rotator.
- RNA was extracted using TRIzol reagent.
- TruSeq mRNA libraries were generated, sequencing was performed in a paired-end 2 x 75 bp mode on a NextSeq500 (Illumina).
- Reads were mapped to the genome sequence of *A. fumigatus* af293 CADRE 30 using STAR.
- DESeq2 (Bioconductor) was used for differential expression analysis (p-value ≤ 0.01 , adjusted p-value ≤ 0.05 , log2FC ≥ 2 or ≤ -2).
- A signed weighted gene co-expression network was built using the WGCNA R-package.

Validation of RNA-Seq data by qRT-PCR

GO analysis

- AspGD Term Finder* searches for significant shared GO terms in each module (only differentially expressed genes were used).
- Four modules had significant hits: *black*, *orange*, *bisque4*, and *sienna3*.

Gene expression patterns of each module

Conclusions

Transcript levels of genes encoding for secondary metabolites were high in dormant conidia. During isotropic growth, transcript levels of genes involved in cell wall modifications increased. Two modules encoding for growth and cell cycle/DNA processing were associated with polarized growth.

Baltussen, T.J.H. et al., 2018. Gene co-expression analysis identifies gene clusters associated with isotropic and polarized growth in *Aspergillus fumigatus* conidia. *Fungal Genet. Biol.* doi:10.1016/j.fgb.2018.04.013 *In press*.