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MEASURING C. ALBICANS AND HOST GENE EXPRESSION DURING INTRA-ABDOMINAL CANDIDIASIS OF HUMANS AND MICE WITH RNA-SEQ

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Objectives: Our objective was to perform transcriptome analysis during invasive candidiasis (IC) of humans and mice. Such studies may provide insights into pathogenesis, but they are technically challenging during disseminated or oral candidiasis. Intra-abdominal candidiasis (IAC) is an under-studied manifestation of IC that is suited to studies of *in vivo* gene expression.

Methods: We performed RNA-Seq on peritoneal fluid (PF) from a patient with *C. albicans* IAC. Expression of 145 *C. albicans* genes was compared to nanoString data from peritoneal fluid (PF) of 3 mice with IAC. The role of the highly-expressed gene *ALS1* in virulence during IAC was tested in mice.

Results: PF was collected from intra-abdominal sites (right and left upper quadrants) in a 63 year old man with *C. albicans* IAC 48 hrs after biliary leak. PF from mice was collected 48 hrs after IP injection of *C. albicans* SC5314+sterile stool. pH of all samples was 8.0. Illumina MiSeq run on human samples generated ~6 million reads; ~3.8 million reads (64%) were mapped to *C. albicans* coding sequences, representing 93% of ORFs. The 181 most highly expressed genes (>1000 mean RPKM) were assessed for GO term enrichment. Cell wall ($p=6e^{-25}$) and host interaction ($p=4e^{-8}$), mitochondrion ($p=4e^{-4}$) and energy derivation ($p=2e^{-3}$) were over-represented. RNA-Seq data correlated well with nanoString data from mice ($R^2=0.72$). Among the most strongly expressed genes in both patient and mice were *ALS1*, *RIM101*, *ENA2*, *ENA21*, *GPD2, SSB1*, *CAT1*, *TRR1* and *TRX1,* involved in processes like adhesion and alkaline pH, osmotic and oxidative stress/neutrophil (PMN) responses. Hyphal genes *HWP1* and *HYR1* were poorly expressed. Disruption of *C. albicans ALS1*, which encodes an adhesin/invasin, did not impact peritonitis or tissue invasion during mouse IAC, but significantly attenuated persistence in abscesses compared to wild-type and complemented strains ($p<0.001$). The *als1* mutant was more susceptible to phagocytosis and killing by PMNs *in vitro* ($p<0.001$). Over-expression of *C. albicans ALS1* in *C. glabrata* BG2 resulted in greater persistence within abscesses ($p<0.001$), and resistance to PMN phagocytosis and killing *in vitro* ($p<0.001$). RNA-Seq reads during mouse IAC mapped exclusively to mouse genes. The most highly expressed genes were over-represented for antigen presentation/immunoglobulin ($p=3e^{-4}$), chemotaxis ($p=3e^{-4}$), and regulation of adaptive immunity ($p=5e^{-3}$).

Conclusions: RNA-Seq is a powerful tool for studying *C. albicans* and host gene expression *in vivo*. Transcriptome data from PF afford insights into *C. albicans* biologic processes involved in pathogenesis of IAC, and host immune responses. While a number of highly-expressed *C. albicans* genes in PF encode known virulence determinants, their specific contributions to IAC may be unanticipated. For example, *C. albicans* Als1 does not increase adhesion to abdominal organs, but facilitates resistance to phagocytosis and survival within abscesses.