TITLE: UNCOVERING CODING AND NONCODING RNAS INVOLVED IN GLOBAL RESPONSE TO AZOLE ANTIFUNGALS IN *ASPERGILLUS FUMIGATUS*

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ABSTRACT

Aspergillus fumigatus is the major causative agent of invasive pulmonary aspergillosis (IPA) in human populations worldwide. The main treatment for IPA comprises azole antifungals, such as itraconazole (ITC) and voriconazole (VCZ). Transcriptomic analyses improve our understanding of the mechanisms involved in antifungal response, but previous studies with *A. fumigatus* have not integrated available datasets to study common responses to more than one azole. Moreover, they have not considered the expression of long noncoding RNAs (lncRNAs), which might have important regulatory roles in antifungal response and resistance. Here, we have integrated public RNA-seq datasets of *A. fumigatus* strains in different conditions to uncover the intergenic lncRNAs, which have not been annotated in this fungus. In summary, discovery of lncRNAs was carried out using alignment and transcript assembly in hisat2 and stringtie2, respectively, filtering out transcripts mapping coding gene *loci* (including UTRs). To discriminate between coding and noncoding RNAs, we trained RNAsamba software with fungal ncRNAs from Ensembl Fungi and classified *A. fumigatus* RNAs based on coding potential score. Additionally, we used mmseqs2 to identify and exclude RNAs matching Uniprot proteins. In total, we discovered novel 924 lncRNAs, which were used in downstream analyses. In order to raise novel candidates involved in a global response to azoles, we used three RNA-seq datasets of different genotypes and two azole drugs, ITC and VCZ. Differential gene expression was carried out with kallisto and sleuth, using as reference the *A. fumigatus* Af293 genome annotation available on FungiDB and the novel lncRNAs. We identified 699 and 807 genes that were down and upregulated in all azoles, respectively. Among them, we found 23 genes that were previously studied in the context of azole response and resistance. Based on gene expression, we identified a few coding genes that were either turned on and off in azole. We also identified one and 21 lncRNAs that were down and upregulated, respectively. To identify putative relationships between genes, we used the clust software with TMM expression values to identify clusters of co-expressed genes. As conclusions, we have raised novel possible coding and noncoding transcripts in azole response in *A. fumigatus* and further inspection of co-expressed genes is promising for identifying possible regulatory relationships (e.g. pairs of lncRNAs and coding RNAs).

Keywords: azole antifungals; *Aspergillus fumigatus*; long noncoding RNAs; RNA-Seq; gene co-expression

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