ePoster Viewing

Antifungal drug susceptibility and resistance

Identification of novel transcription factors associated with azole resistance in Aspergillus fumigatus

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Objective

Antifungal resistance is an increasingly common phenomenon in *A.fumigatus*, especially for the azole drug itraconazole, yet outside of mutations in the drug target (erg11A) relatively little is known about the mechanism behind this. Genome sequencing of drug resistant isolates provides limited information on the effects of point mutations on drug resistance but these mutations cannot be directly attributed to phenotype. Targeted single gene disruption can be used to directly attribute drug resistance to genetic mutations.

Methods

Transposon mediated mutagenesis was utilised to create a 500,000 member *A.fumigatus* mutant library for further investigation. The library was screened for resistance to itraconazole and a number of genes associated with azole resistance were identified including two cbf/nf-y transcription factors AFUB_029870 and AFUB_045980. Differential transcriptomics (RNAseq) was performed on null and wild-type isolates to identify genes under the control of these transcription factors. To determine proteins associated with these transcription factors we replaced the native genes with S-tagged alleles. The S-tagged transcription factors and their associated proteins were purified from the total cellular protein by immunoprecipitation with S-protein agarose before sequencing.

Results

A total 257 transposon mobilisation events gave isolates able to grown in the presence of ≥8mg/l itraconazole, 100 were randomly chosen for further characterisation. Of these 43 unique transposon insertion sites were sequenced, 8 were within a coding region with a further 12 within a likely promoter region. Directed mutagenesis confirmed a role for two transcription factors *AFUB_029870* and *AFUB_045980* in azole resistance (itraconazole MIC ≥8mg/l). The overlapping transcriptional profiles generated in the null mutants as determined by RNAseq analysis suggested the transcription factors may interact. This was confirmed in co-immunoprecipitation studies. An interaction was also identified between the transcription factors and the TBP associated factor Mot1.

Conclusion

It appears AFUB_029870, AFUB_045980 and Mot1 form a complex which is required for susceptibility to itraconazole in wild type *A.fumigatus.* The loss of one component of this complex appears to be sufficient for development of itraconazole resistance suggesting this could be relevant in resistant clinical isolates.