Mutations in *A. fumigatus hmg1* which confer triazole resistance also alter sterol composition and increase multi-drug efflux pump expression

**Background**

- *Aspergillus fumigatus* is the most common *Aspergillus* species isolated from invasive aspergillosis patients, a disease implicated in over 200,000 life-threatening infections annually, and associated with mortality rates of 35–90%.1
- While clinical rates of resistance to itraconazole and voriconazole among *A. fumigatus* in the United States are estimated to be 5 and <1%, respectively, reports of resistant isolates are increasing globally and triazole resistance has exceeded 10–15% in some regions.2,3,4
- HMG-CoA reductase, encoded by the *A. fumigatus* gene *hmg1*, catalyzes the first committed step in ergosterol biosynthesis and is believed to regulate this biosynthetic pathway through direct interactions between a predicted sterol sensing domain and sterols.5
- Mutations in the *A. fumigatus hmg1* gene which are unique to triazole-resistant clinical isolates and encode amino acid alterations in the predicted sterol sensing domain have been described. These mutations were subsequently shown to directly contribute to high-level triazole resistance, but the mechanism by which this novel genetic determinant impacts triazole susceptibility remains unknown.6

**Methods**

- **Strains used in these studies**
  - 4 previously characterized strains of *A. fumigatus*, constructed in the *ohbPR1Δ background* and harboring wildtype or triazole resistance conferring *hmg1* alleles (*F262del, 3105P, I142S*) were included.
- **RNAseq derived transcriptional profiling**
  - Each strain was grown at 35°C for 48 hours in RPMI supplemented with 0.2% glucose buffered to pH 7.0 with MOPS for 48 hours in an orbital shaker at 180 RPM and 35°C.
  - For voriconazole treated conditions, the RPMI media was supplemented with voriconazole at half the respective strain voriconazole MIC (0.125µg/mL for the *hmg1 WT* strain and 0.5µg/mL for the *hmg1 mutant strains*).
  - RNA was isolated from liquid nitrogen crushed, mature hyphal cells using TRIzol based extraction.
  - Libraries were sequenced on an Illumina HiSeq 4000 sequencer. RNAseq transcripts were aligned to the A293 reference genome. Reads per kilobase exon per million reads were then compared to determine relative expression of genes of interest. Log, fold-change shown are relative to *hmg1 WT* strain grown under untreated conditions.
- **Comprehensive sterol profiling**
  - Fresh conidial suspensions of each strain were prepared in saline with tween 80 from AMM agar plates. Conidial were grown in 25µl RPMI supplemented with 0.2% glucose buffered to pH 7.0 with MOPS for 48 hours in an orbital shaker at 180 RPM and 35°C.
  - For voriconazole treated conditions, the RPMI media was supplemented with voriconazole at half the respective strain voriconazole MIC (0.125µg/mL for the *hmg1 WT* strain and 0.5µg/mL for the *hmg1 mutant strains*).
  - Cells were flash frozen using liquid nitrogen, dry weights obtained, and alcoholic KOH was utilized to extract nonaponifiable lipids.
  - A vacuum centrifuge was then used to dry samples, prior to being derivatized by the addition of anhydrous pyridine, N.O-bis(trimethylsilyl)trifluoroacetamide- 10% trimethylsilyl), and two hours of heating at 80°C.
  - Gas chromatography-mass spectrometry was then used to analyze and identify TMS- derivatized sterols.
- 6 independent biological replicates were measured and included in analysis.

**Results**

**Mutations in *hmg1* alter gene expression**

**Putative ergosterol biosynthesis pathway**

**Mutations in *hmg1* alter cellular sterol composition**

**Conclusions**

- Mutations in *A. fumigatus hmg1* which are known to confer increased resistance to the triazole class of antifungals, were found to alter the expression of efflux pump and ergosterol biosynthesis protein encoding genes under both untreated and voriconazole-treated conditions.7
- The expression of efflux pump encoding genes *abcA*, *abcC*, *mdr1* and *mdrA* were notably increased (~2 to 86-fold relative to *hmg1 WT* untreated) under voriconazole-treated conditions.
- The expression of ergosterol biosynthesis protein encoding genes *erg6*, *smt1*, *erg2A*, and *erg2B* also notably increased (6 to 57-fold relative to *hmg1 WT* untreated) under voriconazole-treated conditions.
- Sterol profiling revealed significantly increased accumulation of C24 and C28 methylated sterols following voriconazole treatment among *hmg1* mutant strains.

**Future Directions**

- Investigate the role of altered expression of efflux pump encoding genes such as *abcA*, *abcC*, *mdr1* and *mdrA* on the increased triazole resistance conferred by mutations in *hmg1*.
- Characterize the role of altered expression of sterol biosynthesis protein encoding genes such as *erg6*, *erg2A*, *erg2B*, and *erg24B* on the increased triazole resistance conferred by mutations in *hmg1*.
- Investigate the impact of altered cellular sterols, such as C24 and C28 methylated sterols, on the triazole resistance conferred by mutations in *hmg1*.
- Delineate the impact of *hmg1* mutations when present in combination with other previously characterized mechanisms of triazole resistance such as mutations in *erg5*.

**References**


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