The Aspergillus fumigatus Spindle Assembly Checkpoint components, sldA and sldB, play roles in maintenance of triazole susceptibility

Ashley V. Nywening1,2, Adela Martin-Vicente1, Xavier Guruceaga1, Harrison I. Thorn1, Jinhong Xie1, Wenbo Ge1, Jarrod R. Fortwendel1

1Department of Clinical Pharmacy, University of Tennessee Health Science Center, Memphis, Tennessee, 38163, USA.  
2College of Graduate Health Sciences, Integrated Biomedical Sciences Program, The University of Tennessee Health Science Center, Memphis, TN, 38163, USA.

INTRODUCTION
The rise of triazole resistance in Aspergillus fumigatus is of increasing concern as infection with resistant isolates is associated with increased treatment failure. Much remains unknown concerning adaptation to antifungal stress and development of antifungal resistance, threatening the future of triazoles for medical use. Reversible protein phosphorylation through protein kinase activity mediates many cellular processes in eukaryotic organisms, including fungi. A. fumigatus is predicted to encode 147 protein kinases, the majority of which are uncharacterized, and the influence of these kinases on triazole susceptibility and adaptation to triazole drugs remains largely unknown. Here, we sought to reveal the impact of each of the predicted protein kinases on susceptibility to medical, mold-active triazoles.

METHODS
CRISPR/Cas9 gene editing was used to generate a library of 118 protein kinase disruption mutants in the wild type strain. Voriconazole minimum inhibitory concentrations (MIC) for the disruption strains were determined using CLSI broth microdilution assays and E-test. Screening revealed that disruption of the uncharacterized gene encoding the putative sldA kinase resulted in reduced susceptibility to the triazole antifungal voriconazole. This kinase is a direct ortholog of proteins involved in the cell cycle spindle assembly checkpoint of other eukaryotic organisms. We sought to generate deletion strains lacking the entire gene coding sequence for sldA or that of a putative non-kinase binding partner, sldB. Targeted gene deletions and recombinations were performed by CRISPR/Cas9-mediated gene editing approaches. These mutants were re-examined for changes in growth or germination and for altered susceptibilities to a panel of mold-active triazoles, as well as the spindle poison benomyl. Culture for growth and stress analyses were carried out in standard Aspergillus minimal media.

CONCLUSIONS
Loss of A. fumigatus genes sldA and sldB does not result in defective hyphal growth or conidial germination. Loss of A. fumigatus genes sldA and sldB generates increased susceptibility to the spindle poison benomyl. Loss of A. fumigatus genes sldA and sldB generates reduced susceptibility to triazole antifungals. The previously uncharacterized A. fumigatus SIAA kinase and its binding partner SIB likely play conserved roles in regulation of the SAC.

ACKNOWLEDGMENTS