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**“New insights into drug adaptation and clinical
resistance in fungi”**

by

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ABSTRACT

Invasive fungal infections are a serious threat to human health, causing at least 1.2 million deaths worldwide each year. Such infections are common in immunocompromised patients and are associated with high mortality: 30–40% for invasive candidiasis and 30–90% for invasive aspergillosis. Patients with invasive or chronic pulmonary aspergillosis are typically treated with azoles as a first-line therapy. However, resistance to azoles is on the rise and therefore second-line therapy with echinocandins is becoming prominent. Echinocandins target fungal cell membrane enzyme β -(1,3)-glucan synthase, required for the synthesis of an important cell wall component β -(1,3)-D-glucan. Echinocandins are primarily used in the treatment of *Candida* infections and are second-line therapy for treatment of *Aspergillus* infections. Here we identified a novel mechanism of echinocandin resistance in *A. fumigatus*. We show that caspofungin induces mitochondrial reactive oxygen species (ROS) production as an off-target effect inside cells. Further, upon caspofungin exposure, some strains of *A. fumigatus* modify glucan synthase enzyme making it insensitive (>4 log orders) to all echinocandins *in vitro*. We also observe changed lipid composition [increased dihydrosphingosine (DhSph) and phytosphingosine (PhSph)] in the micro-environment of the enzyme in cells treated with caspofungin. Collectively, we hypothesize that caspofungin induced ROS alter the lipid composition around glucan synthase, changing its conformation and making it insensitive to echinocandins, eventually leading to resistance. Further, we provide new insights into drug adaptation and paradoxical growth behavior in *Candida* and *Aspergillus* spp. Paradoxical effect is a phenomenon characterized by the ability of fungal strains to grow in the presence of high concentrations of echinocandins (mainly caspofungin) while showing susceptible phenotype at lower concentrations. In our study, kinetic inhibition assay measuring the activity of glucan synthase isolated from *A. fumigatus* strain grown in paradoxical growth conditions was insensitive to caspofungin compared to the enzyme grown in the absence of the drug. Additionally, lipid composition around the micro-environment of the enzyme revealed an increase in lipids DhSph and PhSph under paradoxical growth. Analysis of lipid profile from membrane preparations of *Candida* spp. showed consistent increase in lipid PhSph in cells growing under paradoxical growth conditions. Lastly, previously identified off-target effect of increased ROS production by CAS was observed under paradoxical conditions in different *Candida* spp. Thus, we concluded that ROS-induced changes in glucan synthase and its lipid micro-environment cause paradoxical growth in *Candida* and *Aspergillus* species.