

# Stress-induced changes in the lipid microenvironment of $\beta$ -(1,3)-d-glucan synthase cause clinically important Echinocandin resistance in *Aspergillus fumigatus*.

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## Abstract

*Aspergillus fumigatus* is a leading cause of invasive fungal infections. Resistance to first-line triazole antifungals has led to therapy with echinocandin drugs. Recently, we identified several high-minimum-effective-concentration (MEC) *A. fumigatus* clinical isolates from patients failing echinocandin therapy. Echinocandin resistance is known to arise from amino acid substitutions in  $\beta$ -(1,3)-d-glucan synthase encoded by the *fks1* gene. Yet these clinical isolates did not contain mutations in *fks1*, indicating an undefined resistance mechanism. To explore this new mechanism, we used a laboratory-derived strain, RG101, with a nearly identical caspofungin (CAS) susceptibility phenotype that also does not contain *fks1* mutations. Glucan synthase isolated from

RG101 was fully sensitive to echinocandins. Yet exposure of RG101 to CAS during growth yielded a modified enzyme that was drug insensitive (4 log orders) in kinetic inhibition assays, and this insensitivity was also observed for enzymes isolated from clinical isolates. To understand this alteration, we analyzed whole-enzyme posttranslational modifications (PTMs) but found none linked to resistance. However, analysis of the lipid microenvironment of the enzyme with resistance induced by CAS revealed a prominent increase in the abundances of dihydrosphingosine (DhSph) and phytosphingosine (PhSph). Exogenous addition of DhSph and PhSph to the sensitive enzyme recapitulated the drug insensitivity of the CAS-derived enzyme. Further analysis demonstrated that CAS induces mitochondrion-derived reactive oxygen species (ROS) and that dampening ROS formation by antimycin A or thiourea eliminated drug-induced resistance. We conclude that CAS induces cellular stress, promoting formation of ROS and triggering an alteration in the composition of plasma membrane lipids surrounding glucan synthase, rendering it insensitive to echinocandins.

**IMPORTANCE** Resistance to first-line triazole antifungal agents among *Aspergillus* species has prompted the use of second-line therapy with echinocandins. As the number of *Aspergillus*-infected patients treated with echinocandins is rising, clinical observations of drug resistance are also increasing, indicating an emerging global health threat. Our knowledge regarding the development of clinical echinocandin resistance is largely derived from *Candida spp.*, while little is known about resistance in *Aspergillus*. Therefore, it is important to understand the specific cellular responses raised by *A. fumigatus* against echinocandins. We discovered a new mechanism of resistance in *A. fumigatus* that is independent of the well-characterized FKS mutation mechanism observed in *Candida*. This study identified an off-target effect of CAS, i.e., ROS production, and integrated oxidative stress and sphingolipid alterations into a novel mechanism of resistance. This stress-induced response has implications for drug resistance and/or tolerance mechanisms in other fungal pathogens.

## Involved units

[Molecular and Applied Microbiology](#) [Axel Brakhage](#) [Read more](#)



**Axel A. Brakhage**

[Details](#)



**Olaf Kniemeyer**

[Details](#)



Thomas Krüger

[Details](#)

**Identifier**

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