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Fungal echinocandin resistance

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Abstract

Echinocandins are the most recent introduction to the antifungal armamentarium and target the synthesis of β-(1,3)-glucan, the major structural polysaccharide of the fungal cell wall. Mechanisms have been identified that reduce the efficacy of the echinocandins: mutations of the Fks subunit of the target enzyme complex or a compensatory increase in the production of chitin, the second structural cell wall polysaccharide.

Introduction and context

Caspofungin was the first echinocandin approved for clinical use, followed by anidulafungin and micafungin [1]. Echinocandins are cyclic lipopeptides that target the fungal cell wall by inhibiting β-(1,3)-glucan synthesis [2]. β-(1,3)-glucan is synthesized by a protein complex composed of an integral membrane protein catalytic subunit, Fks, and a regulatory subunit, the Rho1 GTPase, which also regulates protein kinase C (PKC) [3,4]. Most fungi have a number of alternative Fks subunits. Echinocandins are effective against a range of fungal human pathogens and are fungicidal against a number of *Candida* species, including *Candida albicans*. In the case of the filamentous mould *Aspergillus fumigatus*, echinocandins are fungicidal against actively growing hyphal tips but are less effective against non-growing subapical cells [5].

Major recent advances

Although echinocandins are highly efficacious in the treatment of invasive fungal infections and now are used as first-line therapies in many hospitals, several examples of clinical failures due to breakthrough infections have been reported over the last few years [6]. Lab-based studies with *C. albicans* identified dominant mutations in the target protein Fks1, the catalytic subunit of β-(1,3)-glucan synthase which conferred acquired echinocandin resistance [7]. The *C. albicans* Fks1 mutations mapped onto two hotspot regions at amino acids 641-649 and 1345-1365. The same hotspot mutations were identified in clinical isolates from patients who failed or responded poorly to echinocandin therapy, and the *in vivo* echinocandin resistance of these isolates was validated in a systemic candidiasis murine model [7]. Acquired mutations in *FKS1* and *FKS2* genes have now been identified in a wide range of *Candida* species and *A. fumigatus* [8-10]. Sequencing the *FKS* genes from fungi cultured from echinocandin-treated patients with clinical failure due to breakthrough infections has identified mutations in some but not all of the isolates [6,11]. In general, the prevalence of Fks mutations in geographically diverse clinical isolates of several *Candida* species remains low [12]. β-(1,3)-glucan synthase kinetic assays have shown that the sensitivity of the mutated glucan synthase to caspofungin is reduced, resulting in an increased inhibition constant (K_i) [13,14].

Candida parapsilosis and *Candida guilliermondii* have a reduced susceptibility to echinocandins, and this susceptibility is thought to result from naturally occurring polymorphisms in the Fks1p hotspot region which match the acquired mutations identified in echinocandin-resistant isolates of other species [15-17]. Hotspot mutations are more likely to confer resistance to caspofungin than to anidulafungin and micafungin and in many cases result in higher minimum inhibitory concentrations

(MICs) for caspofungin than for the other two [12,14]. However, differences in the potency of the three echinocandin drugs observed *in vitro* diminish in the presence of 50% serum and therefore cross-resistance would occur *in vivo* [18].

Another mechanism that results in reduced echinocandin susceptibility *in vitro* is the activation of cell wall salvage or compensatory pathways (the PKC cell integrity pathway in particular) [19,20], which result in elevated chitin levels. Treatment of *C. albicans in vitro* with sub-MIC caspofungin activates chitin synthesis, and reciprocally cells that have higher cell wall chitin are less susceptible to caspofungin [19,21]. Elevated chitin appears to be an adaptive response to growth in the presence of echinocandins in an attempt to maintain cell wall integrity, and subsequent growth in the absence of drug restores chitin to wild-type levels. Therefore, this is an example of a drug tolerance mechanism rather than resistance. In addition to the importance of the PKC pathway in the response to echinocandins, the Ca^{2+}/calcineurin signaling pathway plays a role as genetic or pharmaceutical blockade of that pathway renders *C. albicans* and *A. fumigatus* hypersensitive to echinocandins [19,21]. The chaperone protein Hsp90, acting through its client protein calcineurin, has also been implicated in the regulation of echinocandin resistance [22]. As Ca^{2+}/calcineurin in turn regulates chitin synthesis [23] and cell wall biogenesis, there are intriguing connections between Hsp90, cell wall and membrane stress, and drug resistance and tolerance.

Future directions

To date, fungal echinocandin resistance does not seem to be a major cause for concern in the treatment of patients with invasive mycoses [24]. However, there is increasing evidence of natural and acquired resistance resulting in recalcitrant life-threatening infections and clinical failure. The reduced susceptibility of fungal cells with elevated chitin requires further investigation to determine whether this phenomenon, observed *in vitro*, also occurs when infected patients are exposed to sub-MIC echinocandin doses. Within a population, there is a subset of cells with higher-than-average chitin levels [19] and it has yet to be determined whether these can persist in the presence of echinocandin treatment and out grow, resulting in a drug-tolerant population. Fungal azole antifungal resistance is dependent on a number of different mechanisms that include upregulation of drug efflux pumps as well as mutation of the drug target [25]. Chromosomal rearrangements and specifically the generation of an isochromosome of the left arm of chromosome 5 also result in azole resistance in *C. albicans* [26,27]. Genome-wide population studies have been used to map the evolution of azole resistance in *Saccharomyces cerevisiae* [28] and *C. albicans* [29]. Similar population studies to look at the evolution of echinocandin resistance would be informative to assess the relative contributions of acquisition of Fks point mutations and activation of chitin biosynthesis as resistance and tolerance mechanisms and to identify alternative factors and pathways that play a role in decreased echinocandin susceptibility.

Abbreviations

MIC, minimum inhibitory concentration; PKC, protein kinase C.

Competing interests

The author declares that she has no competing interests.

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