Role of FKS Gene in the Susceptibility of Pathogenic Fungi to Echinocandins

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ABSTRACT

Echinocandins are antifungal agents that specifically inhibit the biosynthesis of 1,3-β-D-glucan, a major structural component of fungal cell walls. Echinocandins are recommended as first-line or alternative/salvage therapy for candidiasis and aspergillosis in antifungal guidelines of various countries. Resistance to echinocandins has been reported in recent years. The mechanism of echinocandin resistance involves amino acid substitutions in hot spot regions of the FKS gene product, the catalytic subunit of 1,3-β-D-glucan synthase. This resistance mechanism contributes to not only acquired resistance in Candida spp., but also inherent resistance in some pathogenic fungi. An understanding of the echinocandin resistance mechanism is important to develop both effective diagnosis and treatment options for echinocandin-resistant fungal diseases.

Key words: 1,3-β-D-glucan synthase, echinocandins, FKS, hot spot, resistance

Introduction

The three echinocandins, namely, anidulafungin, caspofungin, and micafungin, are in widespread clinical use. Of these, caspofungin and micafungin are available in Japan. Echinocandins are exclusively indicated for treatment of Candida and Aspergillus infections because they show excellent antifungal activity against Candida spp. and Aspergillus spp. without cross-resistance to existing antifungal agents. They are less active, however, against Cryptococcus spp., Trichosporon spp., Fusarium spp., Scedosporium/Lomentospora spp., and Mucorales. Echinocandins have generally favorable safety and tolerability profiles with adequate pharmacokinetics and few drug interactions. Moreover, in recent years, a large amount of clinical evidence for echinocandins have been accumulated. In consequence, the Infectious Diseases Society of America (IDSA), the European Society for Clinical Microbiology and Infectious Diseases (ESCMID), the Japanese Society for Medical Mycology (JSM), and the Japanese Mycoses Forum (JMF) recommend echinocandins as initial therapy for invasive candidiasis in their clinical guidelines. In Japan, caspofungin and micafungin are also recommended as first-line therapy for chronic progressive pulmonary aspergillosis. For the reasons mentioned above, the use of echinocandins for prophylaxis and treatment has been expanding, and more than 60% of candidemia patients are now reported to receive an echinocandin. The incidence of echinocandin resistance has remained relatively low for a decade. However, as the application of echinocandins increased, clinical failures due to resistant organisms, especially Candida spp., are becoming more frequent. Echinocandins exhibit their antifungal activity by inhibition of the fungal-specific enzyme 1,3-β-D-glucan synthase, a catalytic subunit of which is encoded by the FKS gene. In this review, we will focus on the role of the FKS gene in various pathogenic fungi to explain their susceptibility or resistance (reduced susceptibility) to echinocandins.

Target of echinocandins: 1,3-β-D-glucan synthase

Echinocandins specifically and non-competitively inhibit the biosynthesis of 1,3-β-D-glucan, a major structural component of fungal cell walls. Echinocandins substantially show fungicidal activity against susceptible Candida spp., and have killing activity against actively growing hyphae of Aspergillus spp., even though they are not fungicidal against the entire fungal burden. The 1,3-β-D-glucan synthase
Aspergillus fumigatus and glabrata expression level is very low relative to single Candida albicans protein, localizing within the plasma membrane with at most reduced the sensitivity of 1,3-β-D-glucan synthase to echinocandins. The modification of the target site while Fks3 shares 56% identity with Fks1 and Fks2 GTP-binding protein, Rho1 resulted in mutants with remarkable decrease in sensitivity of 1,3-β-D-glucan synthase and increase in MICs 1. Candida spp.  

C. albicans has three FKS genes, FKS1, FKS2, and FKS3. C. glabrata has also three FKS genes. FKS1 is essential in C. albicans and most other Candida spp., while simultaneous disruption of FKS1 and FKS2 is lethal in C. glabrata, meaning FKS1 and FKS2 are functionally redundant in C. glabrata. FKS genes have been cloned and sequenced from various Candida spp., and amino acid sequences of HS1 in Fks1 have been predicted from them (Table 1). The HS1 regions of Fks1 in C. glabrata, Candida tropicalis, Candida kefyr, Candida dubliniensis, Candida lusitaniae share high homology with those of C. albicans. All of them are susceptible to echinocandins like C. albicans.

The Candida parapsilosis complex (Candida parapsilosis sensu strict, Candida orthopsilosis, and Candida metapsilosis) have higher MIC values for echinocandins compared with other susceptible Candida spp. The 1,3-β-D-glucan synthase prepared from C. parapsilosis is 10- to 50-fold less sensitive to echinocandins relative to that from C. albicans. C. parapsilosis complex has a naturally occurring polymorphism in HS1 of Fks1. In comparison with amino acid sequence of HS1 in C. albicans Fks1, Pro660 of HS1 in C. parapsilosis is substituted with alanine (P660A, equivalent to P649A in C. albicans). Engineering the P647A mutation into Fks1 of S. cerevisiae (equivalent to P660A in C. parapsilosis and P649A in C. albicans) resulted in mutants with remarkable decrease in sensitivity of 1,3-β-D-glucan synthase and increase in MICs for echinocandins. It appears that one amino acid substitution in Fks1 of C. parapsilosis complex accounts for their intrinsically reduced susceptibility to echinocandins. Candida guilliermondii also has higher echinocandin MIC value like C. parapsilosis. C. guilliermondii harbors two amino acid polymorphisms in HS1 of Fks1, where Leu633 is substituted with methionine (L633M, equivalent to L642M in C. albicans) and Thr634 is substituted with alanine (T634A, equivalent to T643A in C. albicans). The engineered strains of S. cerevisiae, in which a region of FKS1 gene was replaced with that of C. guilliermondii, showed 32-fold increase in echinocandin MIC values. The reduced echinocandin susceptibility of C. guilliermondii appears to be due to these naturally occurring substitutions.

FKS gene and susceptibility to echinocandins

Genetic studies with echinocandin analogs in S. cerevisiae and C. albicans indicated that Fks1 is the target of echinocandins. The modification of the target site reduced the sensitivity of 1,3-β-D-glucan synthase to echinocandins, causing decreased susceptibility of these organisms to echinocandins. Mutations conferring reduced susceptibility in S. cerevisiae and C. albicans were mapped to FKS1. Inversely, mutations in FKS1 were confirmed to confer resistance following their introduction into a susceptible strain. Amino acid substitutions associated with resistance defined two limited but highly conserved regions termed “hot spot 1 (HS1)” and “hot spot 2 (HS2)” of Fks1 in S. cerevisiae and various pathogenic fungi. HS1 encompasses Phe639-Pro647 in S. cerevisiae and Phe641-Pro649 in C. albicans (Table 1), and HS2 includes Asp1353-Leu1360 in S. cerevisiae and Asp1357-Leu1364 in C. albicans. Strains with acquired echinocandin resistance have mutations in the hot spot regions of FKS genes, while some of the inherently echinocandin-resistant fungal species, such as Candida parapsilosis and Fusarium solani, have naturally occurring polymorphisms in these regions.

FKS genes in pathogenic fungi

Comprises a catalytic subunit embedded in the plasma membrane and a regulatory subunit localized in the cytoplasm. In the baker’s yeast Saccharomyces cerevisiae, the catalytic subunit is an approximately 215-kDa integral protein with 16 transmembrane helices. The regulatory subunit is a small GTP-binding protein, Rho1.

**FKS gene encoding 1,3-β-D-glucan synthase catalytic subunit**

In S. cerevisiae, mutations in FKS1 (for FK506 hypersensitive 1) conferred hypersensitivity to the immunosuppressants FK506 and cyclosporine A, both of which intrinsically show weak antifungal activity. On the other hand, mutations in the S. cerevisiae gene named ETG1 (for echinocandin target gene 1) gave resistance to echinocandins. The FKS1 and ETG1 genes were found to be identical and to encode a catalytic subunit of 1,3-β-D-glucan synthase. In S. cerevisiae, three related FKS genes, FKS1, FKS2, and FKS3 have been cloned. Fks1 and Fks2 are highly homologous (88%) while Fks3 shares 56% identity with Fks1 and Fks2. FKS1 is primarily expressed during vegetative growth, while FKS2 is mostly expressed during sporulation. S. cerevisiae with disruption of either FKS1 or FKS2 is viable, but simultaneous disruption of FKS1 and FKS2 is lethal, suggesting that Fks1 and Fks2 are alternative subunits with essential overlapping functions. FKS3 is also expressed during sporulation, but its expression level is very low relative to FKS1 and FKS2. The orthologs of S. cerevisiae FKS genes have been cloned in various pathogenic fungi. Candida albicans and Candida glabrata have three FKS genes like S. cerevisiae, while Aspergillus fumigatus and Cryptococcus neoformans have a single FKS gene. The FKS gene family encodes a ~210 kDa protein, localizing within the plasma membrane with at most 16 transmembrane helices predicted by hydropathy analysis.
2. *Aspergillus* spp.

*Aspergillus nidulans*, *Aspergillus fumigatus*, and *Aspergillus lentulus* (a cryptic species of *A. fumigatus*) have been reported to have a single FKS gene (*FKS1*) to date. In comparison with amino acid sequence of HS1 in *C. albicans* Fks1, both *A. nidulans* and *A. fumigatus* have two amino acid substitutions in their HS1 (Table 1). In HS1 of *A. nidulans* Fks1, Leu675 is substituted with isoleucine (L675I, equivalent to L646I in *C. albicans*), and Arg676 is substituted with lysine (R676K, equivalent to R647K in *C. albicans*). Likewise, in HS1 of *A. fumigatus*, Leu680 is substituted with phenylalanine (L680F, equivalent to L646F in *C. albicans*), and Arg681 is substituted with lysine (R681K, equivalent to R647K in *C. albicans*). Amino acid sequence of HS1 in *A. lentulus* is the same as that in *A. fumigatus* (Table 1). *A. fumigatus*, *A. nidulans*, and most other *Aspergillus* spp. are susceptible to echinocandins. However, *A. lentulus* isolates overall are less susceptible to caspofungin, although they are susceptible to anidulafungin and micafungin. These differential echinocandin susceptibilities in *A. lentulus* are considered to be independent of the polymorphisms of its Fks1.


*Cryptococcus neoformans* and *Cryptococcus gattii* are inherently resistant to echinocandins. Nevertheless, *C. neoformans* has a single FKS gene (*FKS1*) that is essential for viability despite a lower content of glucan in its cell wall. In comparison with amino acid sequence of HS1 in *C. albicans* Fks1, Leu538 of HS1 in *C. neoformans* is substituted with phenylalanine (L538F, equivalent to L646F in *C. albicans*). The leucine-to-phenylalanine substitution at the homologous position (L644F) in *S. cerevisiae* Fks1 had no

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**Table 1. Amino acid (AA) alignment in hot spot 1 (HS1) of Fks1 for various pathogenic fungi and *Saccharomyces cerevisiae***

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>First AA No.</th>
<th>AA sequences</th>
<th>References</th>
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</thead>
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<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>639</td>
<td>F L V L S L R D P</td>
<td>24, 34</td>
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<td><em>Candida albicans</em></td>
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<td>37</td>
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<tr>
<td><em>Candida krusei</em></td>
<td>655</td>
<td>F L I L S I R D P</td>
<td>38</td>
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<tr>
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<td><em>Pneumocystis jirovecii</em></td>
<td>711</td>
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*The *C. lusitaniae*, *C. metapsilosis* and *L. prolificans* Fks1 sequences are incomplete, and hence are not numbered.
effect on the echinocandin sensitivity of either whole cells or enzyme activity. Moreover, 1,3-β-D-glucan synthase activity from C. neoformans was very sensitive to caspofungin. These results indicate that the mechanism of inherent resistance of C. neoformans to echinocandins is not related to target enzyme insensitivity. Recently, the CDC50 gene encoding the β-subunit of lipid flippase, a protein that regulates the asymmetrical orientation of membrane lipids, was found to be responsible for C. neoformans resistance to caspofungin. On the other hand, C. neoformans was sensitized to micafungin by introducing a mutation in some of the ERG genes encoding late ergosterol biosynthetic pathway enzymes. Addition of ergosterol to the culture of C. albicans F641Y (C. albicans equivalent) substitution in HS1 (F639Y) resulted in decreased its susceptibility to echinocandins. Besides, the W695F (S. cerevisiae equivalent) substitution in L. prolificans Fks1 has been reported to impart reduced echinocandin susceptibility, in the same way that this mutation in S. cerevisiae decreased its susceptibility to echinocandins. Alternaria infectoria exhibits reduced susceptibility to caspofungin. A. infectoria has a single FKS gene (FKS1). Amino acid sequence of HS1 in A. infectoria Fks1 is the same as that of A. nidulans (Table 1). The mechanism of the inherently reduced susceptibility of A. infectoria to echinocandins is unclear.

4. Trichosporon spp.

Trichosporon spp. also show inherent resistance to echinocandins, although the plasma β-D-glucan concentration is elevated in patients infected with them. The amino acid sequence of HS1 in Trichosporon asahii Fks1 (GenBank database Accession No. JH925095) is the same as that of C. neoformans (Table 1). Unfortunately, there is no available literature concerning Trichosporon spp. FKS gene. The mechanism of inherent resistance of Trichosporon spp. to echinocandins remains to be clarified.

5. Fusarium spp.

Fusarium solani has a single essential FKS gene (FKS1). In comparison with amino acid sequence of HS1 in C. albicans Fks1, F. solani has three amino acid substitutions in its HS1 (Table 1). Phe683 is substituted with tyrosine (equivalent to C. glabrata and A. fumigatus, respectively, whereas the equivalent substitution of F683Y was implicated in the acquired echinocandin resistance of a C. albicans clinical isolate. Replacement of A. fumigatus Fks1 with F. solani Fks1 caused a modest increase in the tolerance of A. fumigatus for caspofungin. In addition, replacement of S. cerevisiae Fks1 with hybrid S. cerevisiae Fks1 incorporating F. solani HS1 (F639Y) resulted in significantly reduced echinocandin susceptibility. These results indicate that the mechanism of inherent resistance of F. solani to echinocandins can be attributed to the amino acid substitution in HS1 of its Fks1.

6. Dematiaceous fungi

Lomentospora prolificans is intrinsically resistant to echinocandins. L. prolificans has a single FKS gene (FKS1). In comparison with amino acid sequence of HS1 in C. albicans Fks1, L. prolificans has two amino acid substitutions in its HS1 (Table 1). Phenylalanine and leucine of HS1 in L. prolificans Fks1 (equivalent to Phe641 and Leu646 of HS1 in C. albicans Fks1, respectively) are substituted with tyrosine and phenylalanine, respectively. Of these amino acid substitutions, F641Y (C. albicans equivalent), as mentioned regarding F. solani Fks1, is believed to contribute to the inherent resistance of L. prolificans to echinocandins. Besides, the W695F (S. cerevisiae equivalent) substitution in L. prolificans Fks1 has been reported to impart reduced echinocandin susceptibility, in the same way that this mutation in S. cerevisiae decreased its susceptibility to echinocandins. Alternaria infectoria exhibits reduced susceptibility to caspofungin. A. infectoria has a single FKS gene (FKS1). Amino acid sequence of HS1 in A. infectoria Fks1 is the same as that of A. nidulans (Table 1). The mechanism of the inherently reduced susceptibility of A. infectoria to echinocandins is unclear.

7. Mucorales

Mucor spp., Rhizopus spp., Cunninghamella spp., and Absidia spp. are intrinsically resistant to echinocandins. Rhizopus oryzae has a single FKS gene (FKS1). Nevertheless, caspofungin inhibited the activity of 1,3-β-D-glucan synthase from R. oryzae only at a high concentration and did not exhibit antifungal activity against this strain. There is no data available for amino acid sequence of HS1 in R. oryzae Fks1. Considering the inhibitory activity of caspofungin against 1,3-β-D-glucan synthase, it is probable, but not certain, that amino acid substitution(s) in HS1 may be related to the inherent resistance of R. oryzae to echinocandins.

8. Dimorphic fungi

Micafungin exhibited potent antifungal activity against the mycelial form of Histoplasma capsulatum, Blastomyces dermatitidis, and Coccidioides immitis, while it was very weakly active against the yeast-like form of H. capsulatum and B. dermatitidis (C. immitis: not tested). Micafungin also showed moderate antifungal activity against the mycelial form of Paracoccidioides brasiliensis, Penicillium marneffei, and Sporothrix schenckii, but little activity against their yeast-like form. The MICs of caspofungin against the mycelial form of H. capsulatum and B. dermatitidis (C. immitis: not tested) were also lower than those against its yeast-like form. In P. brasiliensis, one FKS gene (FKS1) and two DNA fragments related to FKS gene have been cloned. The amino acid sequence of HS1 in P. brasiliensis Fks1 is the same as that of C. neoformans (Table 1), wherein there is no polymorphism contributing to echinocandin resistance. It has been reported that the cell wall of the yeast-like form of P. brasiliensis contains predominantly α-glucan and only small amounts of β-glucan, whereas β-glucan is abundant in the mycelial form. Such is the case with H. capsulatum and B. dermatitidis. It thus appears reasonable that echinocandins...
are active against the mycelial form but inactive against the yeast-like form of these dimorphic fungi. *Coccidioides posadasii* has a single essential FKS gene (*FKSI*)\(^55\). The amino acid sequence of HS1 in *P. jirovecii* Fks1 is the same as that of *A. nidulans* (Table 1), i.e., *C. posadasii* has no mutations to induce echinocandin resistance\(^55\). In *C. posadasii*, *FKSI* is expressed during spherule development at levels similar to or slightly less than its levels in mycelia, but its expression levels decrease as spherules mature\(^55\). This may explain why the antifungal effect of cilofungin, an echinocandin analog, on *C. immitis* dramatically decreased with the development of spherules\(^77\).

### Acquired resistance to echinocandins by *fks* mutations

Resistance to echinocandins, since first reported in 2005\(^72\), remains relatively low (< 3%) in *C. albicans* and other *Candida* spp.\(^82\), but is rapidly increasing in *C. glabrata*\(^53, 84\). The SENTRY surveillance program (2006-2010) reported that 8.0-9.3% were resistant to echinocandins among 1,669 bloodstream isolates of *C. glabrata*\(^55\). Moreover, in a 10-year survey at Duke University Hospital, among 293 episodes (313 isolates) of *C. glabrata* bloodstream infection, resistance to echinocandins increased from 4.9% to 12.3% between 2001 and 2010\(^83\). Rapid emergence of resistance in *C. glabrata* has historically been ascribed to its haploid genome. Recently, it is reported that defects in mismatch repair represent a key underlying cellular mechanism that facilitates acquisition of resistance in *C. glabrata*\(^79\). Besides *C. albicans* and *C. glabrata*, acquired resistance has been reported in many *Candida* spp., such as *C. tropicalis*\(^86, 87\), *C. krusei*\(^82, 84\), *C. dubliniensis*\(^81, 82\), *C. kefyr*\(^42, 80\), and *C. lusitaniae*\(^80\). The most frequent amino acid substitutions in Fks1 of *C. albicans* are S645F/P/Y (46%), followed by F641S/L (33%), R1361H (6%), multiple mutations (6%), L644Stop (3%), D648Y (3%), and P649H (3%)\(^42, 84\). Of these, S645F/P/Y and F641S/L cause the most pronounced resistance\(^2, 55\). In *C. glabrata* clinical isolates, resistance-related mutations occur in both *FKSI* and *FKS2*, but the frequency of amino acid substitutions in Fks2 is twice that of Fks1\(^85, 86\). The most prominent amino acid substitutions in *C. glabrata* were Ser663 in Fks2, Ser629 in Fks1, and Phe659 in Fks2\(^89\). In the study of patients with invasive candidiasis due to *C. glabrata*, the presence of an fks mutation was the only independent risk factor for failure of echinocandin therapy, and detection of *C. glabrata* fks mutations was superior to MICs in predicting echinocandin therapeutic responses among these patients\(^89\). Recently, an echinocandin-resistant *A. fumigatus* isolate (F675S in HS1 of Fks1) was recovered from a patient with chronic pulmonary aspergillosis\(^96\).

### Conclusions and perspective

Pathogenic fungi can be divided into six types based on HS1 of their Fks and their susceptibility to echinocandins: (1) no mutations and susceptible, such as *C. albicans* and *A. fumigatus*, (2) with mutations and reduced susceptibility, such as *C. parapsilosis* and *C. guilliermondii*, (3) no mutations and susceptible in only one of two morphological forms, such as dimorphic fungi (mycelial-form) and *P. jirovecii* (cyst), (4) no information about HS1 and highly resistant, such as *Rhizopus oryzae*, (5) no mutations and highly resistant, such as *C. neoformans* and *T. asahii*, and (6) with mutations and highly resistant, such as *F. solani*, *L. prolificans*, and *Candida* spp. with acquired resistance.

For fungal infections by most of *Candida* spp. and *Aspergillus* spp. (type 1), echinocandins have been recommended as first-line or alternative/salvage therapy in various clinical guidelines\(^8, 15, 97, 98\). Echinocandins demonstrate higher MIC levels against *C. parapsilosis* and *C. guilliermondii* (type 2) than most other *Candida* spp., which raises the concern that they may be less responsive to echinocandins. However, there have been no clinical studies that have demonstrated the inferiority of echinocandins compared to other antifungals for the treatment of *C. parapsilosis* infections\(^80\). For treatment of blastomycesis\(^80\), *coccidioidomycosis*\(^81\), and *Pneumocystis pneumonia*\(^78, 80\) (type 3), though not approved nor recommended, it has been reported that echinocandins, alone or in combination, was effective. For therapy of mucormycosis (type 4), combination of lipid formulations of amphotericin B and echinocandins, though its mechanism is unknown, has often been used\(^102-104\) and recommended as first-line or salvage therapy in some guidelines\(^97, 105\). *Cryptococcus* spp. and *Trichosporon* spp. (type 5) show inherent resistance to
echinocandins by other mechanisms than point mutations of Fks. This indicates the possibility of combination therapy with echinocandins and an inhibitor of this unknown echinocandin-resistant mechanism. For F. solani, L. prolificans, and fungi with acquired resistance (type 6), novel echinocandins or chemical entities that inhibit mutant Fks/1,3-β-D-glucan synthase are desirable since the fungal-specific 1,3-β-D-glucan synthase is an ideal molecular target for antifungal agents. A better understanding of the echinocandin resistance mechanism is critical to develop both effective diagnosis and treatment options for echinocandin-resistant fungal diseases.

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Conflicts of interest

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