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Fungal cell wall components are unique antifungal targets

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ABSTRACT. Fungal cell wall is absent in mammalian cell. In fungal the cell wall is fused to the cell physiology playing a crucial role in the entire cell functionality. The cell wall components are synthesized and modified in the cell wall space by the actions of cell wall proteins through a range of signaling pathways that are all unique to fungi, thus making the cell wall and its components suitable drug targets.

Key words: Polysaccharide; Cell lysis; Mannoproteins; Antifungal

INTRODUCTION

The fungal cell wall is an essential organelle. It is made up of three major components: chitin, mannoproteins, and glucans (β-1,3-orβ-1,6-). Chitin and β-1,3-glucan form the structural polysaccharides of the cell wall. Any molecule that can block the biosynthesis of the cell components, thereby leading to cell lysis and death is considered fungicidal; till date, the echinocandins are the only licensed cell wall active antifungal agents. The discovery and development of other cell wall active antifungal agents will be significant in the management of mycoses

CELL WALL ARCHITECTURE

The fungal cell wall comprises an outer layer of mannosylated proteins and a polysaccharide-rich inner layer composed of chitin and glucans. Chitin is a linear homopolymer containing β-1,4-linked N-acetylglucosamine (NAG) residues and are synthesized by membrane-bound chitin synthases proteins. β-1,3-glucan is a glucose polymer and synthsised by a membrane-bound β-1,3-glucan synthase proteins. β-1,3-glucan is elastic in nature and has tensile strength making it the main structural polysaccharide in most fungal cell wall. β-1,6-glucan does not have any known biosynthetic proteins responsible for its synthesis and it is crucial for cell wall organization by interconnecting mannosylated proteins to the cell wall matrix. These components are vital in maintaining the integrity of the fungal cell, and thus have become targets for antifungals (1).

ANTIFUNGAL AGENTS TARGETING CELL WALL COMPONENT

Due to the ever-rising mortality rate of invasive mycotic infections, there has been an urgent need to develop specific antifungal agents capable of targeting the fungal cell wall (Figure 1) [1]. The echinocandins were developed and has since been the only licensed antifungal agent that targets the fungal cell wall. A critical understanding of the synthesis and organization of the cell wall including stress its adaptation mechanisms could help in the development of prophylactics, more effective therapeutics, and rapid and robust diagnostics.

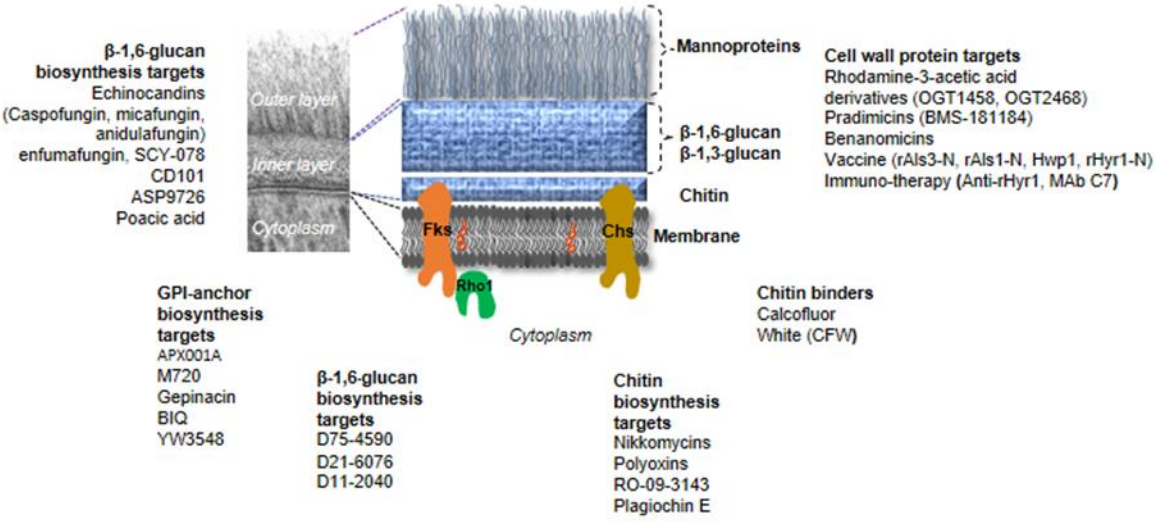

Figure 1: A model of fungal cell wall with the different unique components for antifungal agents.

Research has shown several molecules and compounds at various levels with animal and human models to possess antifungal activities but so far, only one antifungal agent, the echinocandins has been licensed to target the cell wall of fungi [1].

Chitin

Chitin is an attractive drug target, since it is covalently bonded to β-1,3-glucan to form the cell wall structural layer and also compensate the function of β-1,3-glucan. It is synthesized by a class of chitin synthase proteins, which vary in copy number across fungal genomes. Polyoxins and nikkomycins are known chitin synthesis inhibitors. Due to the structural similarities between them and the chitin synthase substrate, NAG, they are able to compete with NAG for chitin synthase binding, thus making them chitin synthase prtoein inhibitors. The nikkomycins showed high *in vitro* activity and, evidence of *in vivo* activity against Candida species, but research on them was discontinued due to poor uptake and poor efficacy on fungal species [2]. The polyoxins also possess a similar activity to that of the nikkomycins, and have been shown to be active against C. neoformans, A. flavus, and C. albicans [3].

Currently molecules/compounds such as the derivatives of 3-substituted amino-4-hydrocycoumarin, compound 6b [4], IMB-D10 and IMB-F4 [5], and plagiochin E [6], have been shown to have moderate to excellent inhibitory activity against Chs proteins thus blocking a range of fungal species. Research on this molecules are ongoing for a potential breakthrough chitin synthesis inhibitor.

β-1,3-glucan

β-1,3-glucan plays a major role in cell wall architecture and aids cell wall matrix formation. It is biosynthesised by β-1,3-glucan synthase complexes, which has Fks as its catalytic subunit and a *Rho1* as a regulatory subunit. Through Fks inhibition, the echinicandins non-competitively block the synthesis of β-1,3-glucan [7], and are effective against *Candida* and *Aspergillus* species but not C. neoformans. Resistance

in fungal species to the echinicandins class has been recently shown to be due to the acquisition of single nucleotide polymorphisms in the gene encoding *Fks1* [8]. Tolerance to the echinicandins has also been shown to be due to cell wall remodelling activities in compensatory response
to β-1,3-glucan inhibition [1].
However novel molecules/compounds/drug derivatives have been shown to inhibit β-1,3-glucan synthesis by binding on a different *Fks1* site, thus blocking fungal growth and they include: SCY-078 (active against *Candida* and *Aspergillus* speices. *in vivo*), piperazinyl-pyridazinone, CD101 (currently in its phase II clinical trial and active against *Candida* and *Aspergillus* speceis), and ASP9726 (active against *Candida and Aspergillus species, C. neoformans, Fusarium moniliforme, and Trochophyton* species.) [9,10].
Poacic acid a plant derived antifungal agent has been shown to be active against fungal growth. Poacic acid binds to and blocks the biosynthesis of β-1,3-glucan causing cell death. It is active against Sclerotinia sclerotiorum, Alternaria solani, and Phytophthora sojae. Interestingly poacic acid has synergistic activity with caspofungin indicating a different binding on the *Fks1* protein [11].

β-1,6-glucan

β-1,6-glucan has a vital role in cell wall organization and structure. The synthetic proteins directly linked to the biosynthesis of β-1,6-glucan has not been identified making it difficult to develop molecules that can block its function and synthesis. However, a gene has been identified whose protein product, Kre9 is essential in the synthesis of β-1,6-glucan. Inhibition of Kre9 by a pyridobenzimidazole derivative, D75-4590 affected the levels of β-1,6-glucan in the cell wall [12]. Other derivatives of pyridobenzimidazole such as D21-6076 has been developed and exhibits *in vivo* activity against *Candida* species. D11-2040 was developed and shown to have synergistic interaction with caspofungin against C. albicans [13].

Proteins

The surface proteins consist of glucosylphosphatidylinositol, GPI modified proteins functioning as adhesins and invasins, such as Als1 and Als3, and *Hwp1*, and those with carbohydrate-modifying activities, such as Phr and Crh families. The GPI modified proteins make up 88% of surface proteins and have been the major target for developing immunotherapies and diagnostics.
However cell surface proteins have also been attractive targets for antifungal development. Their synthesis and localisation to the cell wall have been a major target. The pramicidins are broad spectrum antifungal agents that disrupt the fungal cell wall through blocking cell wall proteins and BMS-181184, a new pramicidin derivative, has been shown to possess *in vitro* activity against most yeasts and moulds [14]. A pyridine-2-amine-based antifungal agents APX001A, active against *Candida*, *Aspergillus*, *Fusarium*, and *Scedosporium* species, and also against caspofungin-resistant *C. albicans* [15-17] has been shown to be capable of interfering with GPI molecule synthesis, therefore affecting GPI anchor attachment and cell wall protein localization. Another molecule, 1-[4-butylbenzyl] isoquinoline (BIQ), has been shown to block the localisation of GPI modified proteins and reduce C. albicans adherence to epithelial cells [15]. Another enzyme, YW3548, inhibits the GPI modified protein posttranslational modification during their passage through the secretory pathway [18].
Successful development of antifungal agents that block the function of the cell wall polysaccharides and interfere with cell wall protein function including their synthesis and localisation will make a major impact in managing the challenging situation possessed by invasive fungal infections.

CELL WALL PROTEINS AS DIAGNOSTIC AND PROPHYLACTIC TOOLS

Als1 and Als3, Hyr1 and *Hwp1* are cell surface GPI modified proteins that have been used with great success in the development of vaccines and immunotherapies. Als3 has been shown to confer protection against recurrent vaginal candidiasis in women [19]. Hyr1 has been shown to induce enough anti-*Candida* IgG antibody with potential for use as diagnostic and can confer protection against candidosis

in mice [20]. *Hwp1* has been used in a vaccine construct that was found to confer protection on mice against systemic candidosis [21].
Data have shown that rat or mice treated with *Sap2* (*Candida proteinase*) with cholera toxin as an adjuvant [22-23], or *Sap2* assembled with virosomes [24] can protect against vaginal or oral candidiasis. *Sap2* has shown cross protection within *Candida* species. Currently, vaccination of mice with *Sap2* from C. parapsilosis can confer protection in mice model of *C. tropicalis candidosis* [25] Furthermore, passive transfer of anti-*Sap2* immune serum to mice reduce fungal burdens. *Sap2* has also been shown to have immunomodulatory property [25].

Studies with biomarkers have shown that serum anti-*Candida* cell wall antibody expression patterns can be used to improve the diagnosis of systemic candidosis. For example, a significant difference was seen in serum anti-*Bgl2*-antibody level in candidosis patients and healthy control persons. Measuring the serum anti-*Bgl2* antibodies differences may be used as a diagnostic biomarker for systemic candidosis. *Bgl2* is also an attractive candidate for future development of vaccine.

CONCLUSION AND PERSPECTIVES

Fungal cell wall remains an avenue for tackling invasive fungal infections that has not been fully exploited. The highly anticipated cell wall active antifungal agents are those that can execute
their activities in the cell wall space without crossing the cell membrane. These novel agents though yet to be discovered and developed will target and block the function of carbohydrate active enzymes and inhibit the crosslinking activity of structural proteins in the cell wall. Agents that block the function of these proteins are believed to be excellent candidates for the development of new and effective therapeutics and diagnostics.

REFERENCES

1. Becker JM, Marcus S and Tallock J. Use of the chitin-synthesis inhibitor nikkomycin to treat disseminated candidiasis in mice.
2. Mehta RJ, Kingsbury WD and Valenta J. Anti-*Candida* activity of polyoxin: Example of peptide transport in yeasts.
3. Ge Z, Ji Q and Chen C. Synthesis and biological evaluation of novel 3-substituted amino-4-hydroxylcoumarin derivatives as chitin synthase inhibitors and antifungal agents.
4. Li Y, Sun H and Zhu X. Identification of new antifungal agents targeting chitin synthesis by a chemical-genetic method.
5. Wu XZ, Cheng AX and Sun LM. Effect of plagiochin E, an antifungal macrocyclic bis(bibenzyl), on cell wall chitin synthesis in *Candida albicans*
6. Douglas CM, D'Ippolito JA and Shei GJ. Identification of the *FKS1* gene of *Candida albicans* as the essential target of 1,3-beta-D-glucan synthase inhibitors
7. Balashov SV, Park S and Perlin DS. Assessing resistance to the echinocandin antifungal drug caspofungin in *Candida albicans* by profiling mutations in *FKS1*.
8. Walker SS, Xu Y and Triantafyllou I. Discovery of a novel class of orally active antifungal beta-1,3-D-glucan synthase inhibitors.
9. Ong V, Hough G and Schlosser M. Preclinical evaluation of the stability, safety, and efficacy of CD101, a novel echinocandin.
10. Piotrowski JS, Okada H and Lu F. Plant-derived antifungal agent poacic acid targets beta-1,3-glucan. *Proc Natl Acad Sci*
11. Kitamura A, Someya K and Hata M. Discovery of a small-molecule inhibitor of beta-1,6-glucan synthesis.
12. Kitamura A, Someya K. *In vitro* antifungal activities of D11-2040, a beta-1,6-glucan inhibitor, with or without currently available antifungal drugs.
13. Hasim S and Coleman JJ. Targeting the fungal cell wall: current therapies and implications for development of alternative antifungal agents. *Future Med Chem*.
14. Tsukahara K, Hata K and Nakamoto K. Medicinal genetics approach towards identifying the molecular target of a

novel inhibitor of fungal cell wall assembly. *Mol Microbiol*.
15. Hata K, Horii T and Miyazaki M. Efficacy of oral E1210, a new broad-spectrum antifungal with a novel mechanism of action, in murine models of candidiasis, aspergillosis, and fusariosis
16. Wiederhold NP, Najvar LK and Fothergill AW. The investigational agent E1210 is effective in treatment of experimental invasive candidiasis caused by resistant *Candida albicans*.
17. Sutterlin C, Horvath A and Gerold P et al. Identification of a species-specific inhibitor of glycosylphosphatidylinositol synthesis. *EMBO J*
18. Alqarihi A, Singh S and Edwards JE. NDV-3A vaccination prevents *C. albicans* colonization of jugular vein catheters in mice. *Sci Rep*.
19. Rudkin FM, Raziunaite I and Workman. Single human B cell-derived monoclonal anti-Candida antibodies enhance phagocytosis and protect against disseminated candidiasis.
20. Xin H, Dziadek S and Bundle DR. Synthetic glycopeptide vaccines combining beta-mannan and peptide epitopes induce protection against candidiasis.
21. De Bernardis F, Amacker M and Arancia S. A virosomal vaccine against candidal vaginitis: immunogenicity, efficacy and safety profile in animal models.
22. Sandini S, La Valle R and Deaglio S. A highly immunogenic recombinant and truncated protein of the secreted aspartic proteases family (r*Sap2*t) of *Candida albicans* as a mucosal anticandidal vaccine. *FEMS Immunol Med Microbiol.*
23. De Bernardis F, Arancia S and Sandini S. Studies of immune responses in *Candida* vaginitis. *Pathogens*. 2015
24. Shukla M and Rohatgi S. Vaccination with Secreted Aspartyl Proteinase 2 Protein from *Candida parapsilosis* can enhance survival of mice during *C. tropicalis*-mediated systemic candidiasis. *Infect Immun.* 2020
25. Pitarch A, Jimenez A and Nombela C. Decoding serological response to *Candida* cell wall immunome into novel diagnostic, prognostic, and therapeutic candidates for systemic candidiasis by proteomic and bioinformatic analyses. *Mol Cell Proteomics.*