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Review

The multitude of targets for the immune system and drug therapy in the fungal cell wall

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Abstract

Recent studies on fungi revealed that several cytosolic and membrane components migrate to the cell wall together with secreted proteins and biosynthetic polysaccharides to build a dynamic immunoreactive structure. New aspects of fungal cell wall assembly and biosynthesis, focusing on the potential of glycolipids, melanin, heat-shock proteins, histone and surface antigens as targets of drugs and antifungal antibodies are discussed.

Keywords: Antifungal therapy; Antimicrobial antibodies; Fungal cell wall; Unfamiliar components

1. Introduction

The fungal cell wall is a compact albeit dynamic structure that plays important roles in several biological processes determining cell shape, morphogenesis, reproduction, cell–cell and cell-matrix interaction, osmotic and physical protection. The cell wall of *Saccharomyces cerevisiae* consists basically of a complex network of glucans and chitin. Chitin is found at the cell budding sites representing 1–2% of the cell mass that contains mainly (50%) β1–3 glucan. They both confer a high mechanical resistance to the cell wall. β1–6 Glucan-chains are directly attached to β1–3-glucan and both glucans can be linked to chitin [1]. *PHR1* and *PHR2* of *Candida albicans* encode putative glycosidases required for cross-linking of β-1,3- and β-1,6-glucans [2]. In *Aspergillus fumigatus* branching of β-1,3-glucan results in an increase of acceptor sites for chitin, galactomannan and a linear β-1,3/1,4-glucan which substitutes for β-1,6-glucan commonly expressed in other fungi [3]. β1–6 and β1–3 Glucans can also covalently bind to cell wall proteins (CWP). CWP are characteristically high-mannose *N*- and/or *O*-glycosylated proteins that account for 40–50% of the cell wall dry weight. Many of these molecules are attached to a glycosylphosphatidylinositol (GPI) anchor during their transport to the cell surface. At the cell wall, GPI-containing CWP are found linked to other components through a remnant of their GPI anchor, which no longer contains phosphatidylinositol and glucosamine. Various classes of cell wall assembly enzymes interconnect GPI-CWPs to β-1,6-glucan, β-1,6 glucan to β-1,3 glucan, chitin to β-1,3-glucan or β-1,6-glucan and CWPs directly to β-1,3-glucan through an alkali-sensitive linkage [4]. The cell wall complex structure makes it a most promising target for antifungal therapy.

Cell wall structures in pathogenic fungi may differ in architecture and dynamic function. In addition, several different components have been characterized such as specific enzymatic activities [5], heat-shock proteins [6], glycosphingolipids (GSL) [7], melanin [8] and histone [9]. Transient antigens and integrin-like proteins involved in binding to host extracellular matrix (ECM) are also considered. These components can be selectively targeted by antibodies or drugs controlling the growth of fungal pathogens in vitro and/or in vivo. This review focuses on the distribution and functional role of recently studied surface components of pathogenic fungi that have been clearly characterized as cell wall targets for drug and immunotherapy.

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2. Specific cell wall components of pathogenic fungi

2.1. Melanin

Fungal melanin is negatively-charged, hydrophobic and of high molecular weight, arising by the oxidative polymerization of phenolic and/or indolic precursors [8]. Melanization of fungal cells results in deposition of the polymeric complex in the cell wall. The sequential incubation with glycosidases, proteases, DNAses, RNAses, denaturing agents, detergents and hot acid results in the recovery of melanin 'ghosts' that retain the size and shape of the original fungal cells (Fig. 1). This evidence associated with immunofluorescence analysis [8,10] confirms that melanin is associated with the cell wall. As reviewed by Nosanchuk and Casadevall [8], melanin has been detected in pathogens such as *Cryptococcus neoformans*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, *Histoplasma capsulatum*, and *A. fumigatus*. More recently, it has been shown that *Blastomyces dermatitidis* conidia and yeast produce melanin in vitro [11]. The pigment, which is also synthesized by yeast cells in vivo, reduces the susceptibility of *B. dermatitidis* to amphotericin B [11].

The ability of pathogenic fungi to produce melanin is associated with virulence. In *C. neoformans*, melanin protects fungal cells against oxidative agents and also by inhibiting cell-mediated responses. Melanin can interfere with complement activation and reduce the susceptibility to antifungal agents [reviewed in 8]. In *A. fumigatus*, *H. capsulatum*, *P. brasiliensis* and *S. schenckii*, melanin was detected in vitro and in vivo, but its role in fungal infections is still unclear.

F. pedrosoi, the principal agent of chromoblastomycosis, produces melanin constitutively and forms typical melanin ghosts [10]. Cell-associated melanin protects it against destruction by host immune cells in vitro. Ingestion of *F. pedrosoi* conidia by mouse macrophages results in the release of melanin granules into the cytosol [12]. The ability of *F. pedrosoi* to produce extracellular melanin is involved in the activation of human neutrophils. Human neutrophils activated by soluble melanin ingested *F. pedrosoi* more efficiently, and this was followed by enhanced oxidative burst associated with fungal killing [10].

Sera from patients with chromoblastomycosis reacted with *F. pedrosoi* melanin. Purified melanin-binding antibodies reacted with melanin ghosts, conidia, mycelium and sclerotic cells from in vitro and in vivo sources. *F. pedrosoi* conidia were opsonized in presence of these antibodies, resulting in increased phagocytosis and killing by human and animal phagocytes. An interesting finding was the fact that antibodies to melanin inhibited in vitro growth of conidial and sclerotic cells. Antibodies to melanin were highly toxic for *F. pedrosoi*; after antibody treatment, only 2.8% of conidia remained viable [10]. The above results agree with previous data demonstrating that, in *C. neoformans*, administration to lethally infected mice of monoclonal antibodies (mAbs) to melanin significantly improved survival [13]. It was also shown that melanin-binding mAbs completely abrogated yeast growth, whereas there was no effect on the replication of non-melanized cells. The growth of *C. neoformans* was also inhibited by melanin-binding peptides [13] and glyphosate [14], which inhibited autopolymerization of L-dopa and oxidation of L-epinephrine by cryptococcal cells. Taken together, these results demonstrate that melanin is a key cell wall polymer that could be targeted by antifungal agents.

To be considered as a target of therapeutic potential, melanin should be produced during fungal infection. In several pathogens, this has been clearly demonstrated and, as stated above, blocking melanin biosynthesis or expression seems to modify the course of infection in favor of the host, making melanin an attractive structure for the design of new therapeutic agents against fungal infections.

2.2. Histone-like proteins

Histones are key proteins of chromatin, acting as spools around which DNA winds. Apart from this classical definition histones can be found at the cell surface of different organisms. For instance, H2B was described at the cell surface of mammalian cells [15]. In addition, a highly conserved 21-kDa histone-like protein is present in several species of mycobacteria. In *Mycobacterium leprae*, this protein mediates bacterial adhesion to host cells via attachment to the α2-chain of laminin [16].

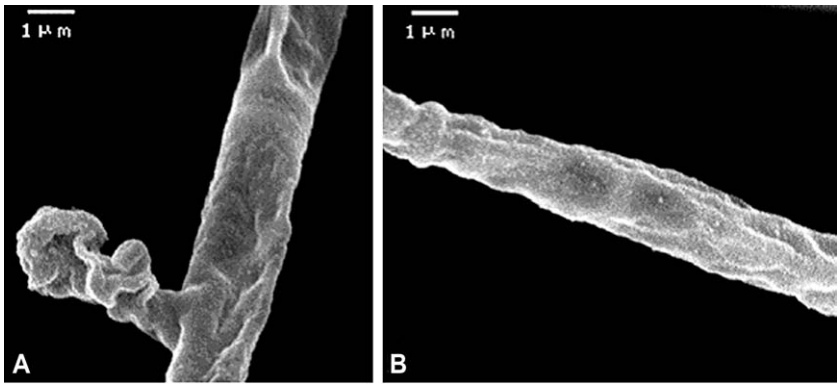

Fig. 1. Melanin "ghosts" of *F. pedrosoi* hyphae. Melanin-rich residues are obtained after several steps of chemical and enzymatic hydrolyses and treatment with denaturing agents (for experimental details, see Ref. [10]). Analysis of these residues (A) or intact hyphae (B) by scanning electron microscopy reveals that these preparations have extremely similar morphologies, consistent with the cell wall distribution of melanin. Scale bars, 1 µm. Courtesy of Daniela S. Alviano.