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Fungal Hyphal Growth – Spitzenkörper versus Apical Vesicle Crescent

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Abstract

Pseudallescheria/Scedosporium species are filamentous fungi widely distributed in nature. Some are considered emerging pathogen due to its clinical importance, especially in immunocompromised patients. During the last few years, many virulence factors have been described, some of them being molecules localized on the fungal cell wall, such as glycoproteins, polysaccharides and glycolipids. In this context, one glycosphingolipid specifically, glucosylceramide (GlcCer), has been chemically identified and its biological roles have been described. GlcCer is a conserved structure in the Pseudallescheria/Scedosporium complex and also in other fungal species. In addition, it plays important roles in fungal growth and differentiation, host-pathogen interaction, immune response modulation and it has been considering a potential target for new antifungal drugs. The use of monoclonal antibodies has shown a possible synergism with current antifungal drugs use in the clinical settings. Therefore, the study of this class of lipids is promising in order to clarify the Pseudallescheria/Scedosporium growth, pathogenesis and fungal treatment.

Keywords: Glucosylceramides; Scedosporium; Pseudallescheria; Sphingolipids

Introduction

The complex Pseudallescheria/Scedosporium is composed by filamentous fungi world widely distributed in nature, especially associated to human impacted areas, such as soil, sewage and polluted water [1,2]. Among the Pseudallescheria/Scedosporium species, some are clinically relevant, such as *Scedosporium aurantiacum*, *Scedosporium* (Lomentospora) *prolificans*, *Scedosporium apiospermum* and *Pseudallescheria boydii* [3-5]. Infections caused by them present a wide spectrum pattern and the disease ranges from superficial (cutaneous and subcutaneous) infections, such as the mycetoma, to disseminated colonization reaching the central nervous system, especially in immunocompromised patients [1]. Pseudallescheria/Scedosporium species are the second most frequent filamentous fungi colonizing respiratory tract, including in cystic fibrosis (CF) patients [6-9].

The cell wall of Pseudallescheria/Scedosporium complex possesses different glycoconjugates, including peptidorhamnomannans, rhamnomannans, α-glucans and also glucosylceramides [10-12]. These molecules have been extensively studied in order to identify their structures and elucidate their biological functions (Figure 1). Glucosylceramides (GlcCer) or cerebrosides (CMH) are the main neutral glycosphingolipids expressed in fungal pathogens, composed by a sugar unit covalently linked to a ceramide, and its structure is highly conserved among different fungal species [10,11]. GlcCer are bioactive molecules in fungal cells with several distinct roles. They are associated with fungal growth, morphological transitions and pathogenesis in *Cryptococcus neoformans*, *P. boydii*, *Candida albicans*, *Aspergillus fumigatus* and *Collectotrichum gloeosporioides* [13-17]. These glycosylated molecules are structurally different from most mammalian cells, being excellent targets for the design of new agents that inhibit fungal growth and the differentiation of pathogens [18].

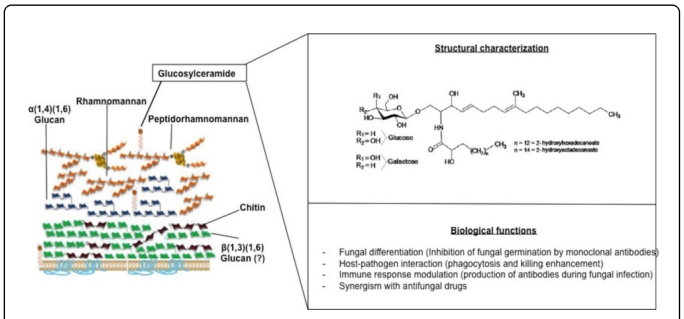

Figure 1: Schematic representation of the major cell wall components of fungi from the Scedosporium/Pseudallescheria complex. Localization of glucosylceramide (GlcCer) on the cell wall, structural characterization and biological functions. Adapted from Barreto-Bergter and Figueiredo [23] and Barreto-Bergter et al. [11].

Considering the GlcCer relevance for fungal physiology and pathogenesis, this review aims to summarize the most recent and important contributions in the literature regarding fungal GlcCer structures and its roles in fungal cells, focusing on the Pseudallescheria/Scedosporium complex.

Structural Characterization of Glucosylceramides

Isolation and purification of glucosylceramides (GlcCer)

The methodology described in Figure 2 follows the steps of purification routinely used in our laboratory for GlcCer extraction and purification [10,19]. Using mixtures of chloroform/methanol followed by chromatographic steps of purification, GlcCer can be purified for further structural analysis.

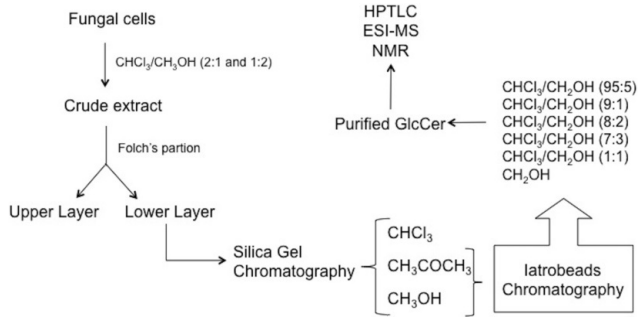

Figure 2: Overview of the strategy used for purification of glucosylceramides (GlcCer) from fungal cells, according to Barreto-Bergter et al. [11].

Structural characterization of GlcCer

Using a combination of thin-layer chromatography (HPTLC), mass spectrometry (ESI-MS) and NMR (1H and 13C) spectroscopy, a complete structural elucidation of glucosylceramides (GlcCer) has been done from pathogenic and opportunistic fungi (Figure 3).

In contrast with mammalian glycosphingolipids, fungal GlcCer shows some differences in the ceramide portion such as the presence of C-8 unsaturation and a methyl group in the C-9 of the sphingosine (Figure 3).

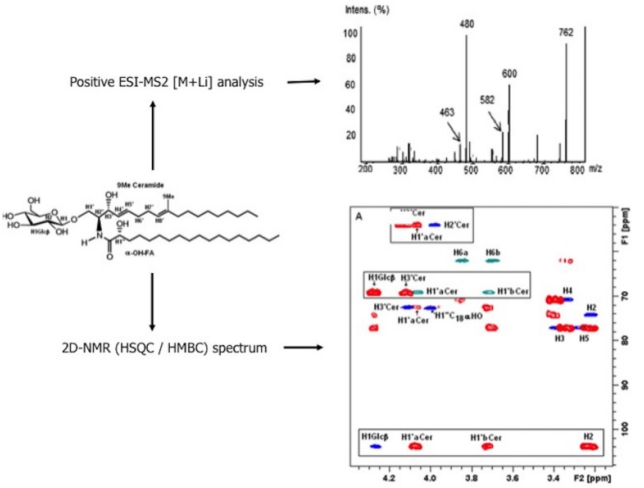

Figure 3: Spectrometric and spectroscopic analysis used to identify the glucosylceramide (GlcCer) structure. Adapted from Barreto-Bergter et al. [11] and Calixto et al. [24].

Biological functions of glucosylceramides

The roles of GlcCer on fungal growth have been studying for the last twenty years. In Pseudallescheria/Scedosporium complex, the first description about the presence of this molecule was in 2002 with a study identifying the main GlcCer structures in *P. boydii* [15]. Monoclonal antibodies (mAbs) against GlcCer have been shown to be a useful tool to study the lipid functions for fungal cells and have been used in the main studies to indirectly demonstrate the biological roles of GlcCer. In *P. boydii*, the mAbs were able to bind to the cell surface, suggesting that GlcCer is exposed on the fungal cell wall [15]. Moreover, incubating the mAbs with *P. boydii* conidia, the cells were not able to germinate properly, indicating that GlcCer is a crucial molecule for fungal germination [15]. Similar results have been obtained for other species, such as *Fonsecaea pedrosoi*, *C. gloesporioides* and *C. neoformans*, the last one presenting *in vivo* protection effects in an animal model [16,20,21]. More recently, mAbs anti-GlcCer have been shown to recognize the sphingolipid on the surface of *S. apiospermum* conidia and hyphae, indicating that GlcCer are exposed on the cell wall of different fungal growth stages. Besides, *S. apiospermum* germination was also blocked by mAbs anti-GlcCer, corroborating with previous works [19].

GlcCer has also been reported to influence the host-fungi interaction and modulate the immune response. In *S. apiospermum*, the use of mAbs anti-GlcCer revealed that it can increase phagocytosis and killing of conidia by host macrophages, suggesting an opsonizing effect of these antibodies and an enhanced antimicrobial activity of host immune cells [19]. A study with *C. neoformans* has demonstrated that GlcCer is not able to cause an increase of cytokines, such as IL-4, IL-6, IFN-γ, TNF-α, IL-1β and IL-12 produced by liver mononuclear cells, but it elicits a modest antibody response with the production of IgM [22]. However, a deeper study with Pseudallescheria/Scedosporium complex evaluating how GlcCer modulates the immune response has never been done.

Recently, GlcCer has been shown to display in vitro cytotoxicity with a dose-dependent inhibitory effect on two different cell lines, L. 929 (mouse fibroblasts) and RAW (macrophages-like cells), suggesting that GlcCer can directly injure host cells [19], and not only modulate the immune response.

Sphingolipids are potential targets for the development of new antifungal drugs, since its fungal structures present some important differences from the mammalian ones [19]. Interestingly, mAbs anti-GlcCer has shown a synergistic effect with itraconazole in treatment with *S. apiospermum*, indicating its potential use in therapy. However, no effect was observed with amphotericin B and more studies are needed to elucidate this interaction [19]. In addition, all the effects and relevancies of GlcCer for fungal growth highlight the potential use of these lipids as a new therapeutic target [18]. For these reasons, the study of GlcCer and other sphingolipids should be intensified in order to clarify and increase the acknowledge of Pseudallescheria/Scedosporium complex growth, pathogenesis and treatments.

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