Proteomic mechanisms of drug resistance in *Candida* cell membrane fractions

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Abstract

Introduction: The African continent has the highest burden of HIV infection, accounting for approximately 65% of new infections globally. Oral candidiasis is a major cause of increased morbidity in HIV-infected individuals and is usually treated with fluconazole, an antifungal drug which interferes with ergosterol synthesis in the fungal cell membrane. The increased resistance of *Candida* species to antifungal drugs leads to treatment failure and an increase in untreatable *Candida* infections.

Objectives/Methods: Drug susceptibility patterns were determined for *Candida* species isolated from oral swabs of 254 patients from two HIV-positive African populations. Forty cell membrane fractions isolated from the clinical isolates were analyzed using High Performance Liquid Chromatography – Mass Spectrometry (HPLC-MS) and the results compared to the UniProt protein database for the identification of *Candida* peptides known to be involved in colonization and drug resistance strategies within the host.

Results: Fluconazole-susceptible and –resistant *C. albicans* isolates were found to express oxidoreductases that bind mammalian estrogens with high affinity. Peptides that confer resistance to formaldehyde were found in *C. albicans*, *C. glabrata* and *C. dubliniensis* in both susceptible and resistant cell fractions. Multidrug resistance proteins (CDR1 and CDR2) were seen in *C. albicans* cell fractions.

Conclusion: The combination of different drug resistance mechanisms and binding abilities to salivary histatins and estrogen seem to be instrumental in the colonisation and retention of *Candida* in these immunocompromised patients. The results also suggest the presence of differences in efflux transporter protein expression in fluconazole-susceptible and –resistant isolates within the HIV population.

Introduction

Sub-Saharan Africa has one of the highest rates of HIV infection worldwide. *Candida* species commonly cause oropharyngeal or systemic dissemination in the HIV-infected population, leading to an increase in patient morbidity and mortality.

Candidiasis is usually treated with fluconazole in the African continent. This drug acts by interfering with the synthesis of ergosterol in the fungal cell membrane. However, drug resistance is known to be on the increase [1], leaving patients with untreated infections.

The use of proteomic tools for the study of drug resistance in *Candida* isolates from the African continent has not been documented.

Objectives:

The objective of this study was to identify proteins from *Candida* species isolated from HIV-infected African individuals known to be involved in colonization and drug resistance strategies within the host.

Materials and Methods

254 oral *Candida* isolates were collected from HIV-infected South African and Cameroonian patients presenting with white pseudomembranous plaque on the tongue or other visible oral candidiasis. Approval from Ministry of Health Regional Hospital Institutional Review Board (IRB) in Cameroon and from the Ethics Committee at the University of the Western Cape was obtained.

Confirmation of *Candida* species was achieved using microscopy, Gram staining and the germ tube test. Growth on Oxoid and Fluka chromogenic media [2] allowed for *Candida* species identification. Presumptive *C. albicans* and *C. dubliniensis* isolates were differentiated by growth at 37˚C for 48 hours in Tomato (V8) agar [3]; at 28˚C for 48-72 hours in Tobacco agar [4] and at 45˚C for 24-48 hours in Sabouraud dextrose agar [5]. Drug susceptibility testing of the isolates was done using the TREK Sensititre YeastOne 9 (YO9) system (Thermo Scientific, USA), a CLSI-approved broth micro-dilution method.

Forty (40) crude cell membrane fractions isolated from the clinical isolates were prepared using a protein isolation method first described by Niimi *et al* [6]. The samples were prepared for mass spectrometry analysis using Filter Aided Sample Preparation (FASP). These were subsequently analyzed using High Performance Liquid Chromatography – Mass Spectrometry (HPLC-MS), using a Thermo Scientific EASY-nLC II connected to a LTQ Orbitrap Velos mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with a nano-electrospray source.

The HPLC-MS data results (acquired using the Xcaliber software package) were compared to the UniProt protein database for the identification of *Candida* peptides known to be involved in colonization and drug resistance strategies within the host.

Results

Identified proteins of interest included SSA1, SSA2 and 90 homolog salivary histatin-binding heat-shock proteins, which affect the fungicidal activity of these human antimicrobial proteins (seen across all tested isolates), estrogen-binding NADPH dehydrogenase oxidoreductases in *C. albicans* isolates, pH responsive protein 2 and multi drug resistance transporter proteins CDR1 and CDR2 in *C. albicans* isolates (table 1).

Table 1: Summary of drug resistance-related *Candida* proteins identified by HPLC-MS.

Candida spp.	Fluconazole resistance	Description of protein	Function
C. albicans, C. dubliniensis, C. glabrata, C. parapsilopsis/krusei, C. tropicalis	S, R	Heat shock protein SSA1	Binds human HTN3/histatin-3, a peptide from saliva, and mediates its fungicidal activity
C. albicans, C. dubliniensis, C. glabrata, C. parapsilopsis/krusei, C. tropicalis	S, R	Heat shock protein SSA2	Binds human HTN3/histatin-3, a peptide from saliva, and mediates its fungicidal activity
C. albicans, C. glabrata, C. parapsilopsis/krusei, C. krusei, C. tropicalis	S,R	Heat shock protein 90	Binds human HTN3/histatin-3, a peptide from saliva, and mediates its fungicidal activity
C. albicans	S, R	Probable NADPH dehydrogenase	Oxidoreductase that binds mammalian estrogens with high affinity
C. albicans, C. dubliniensis, C. glabrata, C. parapsilopsis/krusei	S, R	pH-responsive protein 2	May be integral to the pathogenic ability of the organism
C. albicans, C. dubliniensis, C. glabrata	S, R	S-(hydroxymethyl) glutathione dehydrogenase	Confers resistance to formaldehyde
C. albicans	S, R	Multidrug resistance protein CDR1	Confers resistance to the chemical cycloheximide
C. albicans	R	Multidrug resistance protein CDR2	Multidrug efflux transporter. Confers resistance to azole antifungal agents, to other antifungals (terbinafine, amorolfine) and to a variety of metabolic inhibitors

S=susceptible; R=resistant

Discussion

It is known that *Candida* species express estrogen-binding proteins, which result in a higher predisposition of females to *Candida* infection [7]. High performance liquid chromatography-mass spectrometry analysis of cell surface fractions obtained from African *Candida* isolates in this study revealed the presence of oxireductase (probable NADPH dehydrogenase) proteins that bind to mammalian estrogen with high affinity. These findings further elucidate the predisposition of females to *Candida* colonization.

The expression of *Candida* proteins that are related to colonization and pathogenicity mechanisms were found in different *Candida* species, in both drug-susceptible and –resistant isolates. The combination of different drug resistance mechanisms and binding abilities to salivary histatins and estrogen found in *Candida* species through HPLC-MS analysis seem to be instrumental in the ability of these organisms to colonize immunocompromised patients and resist the action of different chemicals and antifungal drugs.

The presence of a multidrug efflux transporter protein (CDR2) known to confer resistance to azole drugs, which was seen only on a fluconazole-resistant *C. albicans* cell fraction, appears to demonstrate differences in the protein expression of fluconazole-susceptible and –resistant isolates in the HIV population.

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