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cDNA microarray analysis of differential gene expression and regulation in clinically drug-resistant isolates of *Candida albicans* from bone marrow transplanted patients

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Abstract

Fungi have emerged as the fourth most common pathogens isolated in nosocomial bloodstream infections, and *Candida albicans* is the most common human fungal pathogen. Only a few antibiotics are effective in the treatment of fungal infections. In addition, the repetition and lengthy duration of fluconazole therapy has led to an increased incidence of azole resistance and treatment failure associated with *C. albicans*. To investigate the mechanism of drug resistance and explore new targets to treat clinically resistant fungal pathogens, we examined the large-scale gene expression profile of two sets of matched fluconazole-susceptible and -resistant bloodstream *C. albicans* isolates from bone marrow transplanted (BMT) patients for the first time by microarray analysis. More than 198 differentially expressed genes were identified and they were confirmed and validated by RT-PCR independently. Not surprisingly, the resistant phenotype is associated with increased expression of CDR mRNA, as well as some common genes involved in drug resistance such as *CaIFU5*, *CaRTA2* and *CaIFD6*. Meanwhile, some special functional groups of genes, including ATP binding cassette (ABC) transporter genes (*IPF7530*, *CaYOR1*, *CaPXA1*), oxidative stress response genes (*CaALD5*, *CaGRP1*, *CaSOD2*, *IPF10565*), copper transport and iron mobilization-related genes (*CaCRD1/2*, *CaCTR1/2*, *CaCCC2*, *CaFET3*) were found to be differentially expressed in the resistant isolates. Furthermore, among these differentially expressed genes, some co-regulated with *CaCDR1*, *CaCDR2* and *CaIFU5*, such as *CaPDR16* and *CaIFD6*, have a DRE-like element and may interact with *TAC1* in the promoter region. These findings may shed light on mechanisms of azole resistance in *C. albicans* and clinical antifungal therapy.

Keywords: *Candida albicans*; Microarray; Drug resistance; Bone marrow transplant; Differential gene expression

Introduction

Candida albicans is an important opportunistic fungal pathogen of humans and the major cause of

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oropharyngeal candidiasis (OPC) in AIDS patients (Franz et al., 1998; Jones et al., 2004). Fluconazole, an azole antifungal agent, is widely used to treat OPC (Demain and Zhang, 2005). Due to prolonged and repeated use of this agent, emergence of azole-resistant *C. albicans* strains leading to treatment failures were observed (Morschhäuser, 2002; Rex et al., 1995; White et al., 2002). Thus it is desirable to explore and identify new targets for the treatment of clinically resistant fungal pathogens (Quan et al., 2006; Zhang, 2005; Zhang et al., 2005). Phenotypically stable resistance to azole antifungal agents in *C. albicans* can result from mutations or increased expression of genes involved in the ergosterol synthesis pathway (including the target enzyme 14-α demethylase), and increased expression of ATP-binding cassette (ABC) transporter and major facilitator efflux pumps (White et al., 1998). Moreover, the resistance can be rapidly developed in *C. albicans* after short exposures to the drug, both in vitro (Calvet et al., 1997) and in vivo (Marr et al., 1997; Nolte et al., 1997), notably in a strain of *C. albicans* that caused disseminated infection in a patient after bone marrow transplantation (BMT) (Marr et al., 1997).

Characterizing whole-genome expression using DNA microarrays provides a snapshot of an organism's genome in action by revealing the relative transcript levels of thousands of genes at a time (Liu et al., 2005). Previous studies on fluconazole resistance in *C. albicans* by microarray analysis have used either laboratory-derived azole-resistant strains, or clinical isolates obtained in the setting of OPC in patients with AIDS (Barker et al., 2004; Cowen et al., 2002; Rogers and Barker, 2002, 2003). There are important differences between the development of resistance in the setting of OPC as compared to that in candidemia in BMT patients. Resistance in OPC usually follows multiple treatment failures and dose escalations with a prolonged period of time. Azole-resistant isolates obtained from BMT patients showed a more rapid development of resistance and it occurred under conditions of much higher azole concentrations (Marr et al., 1998, 2001).

To investigate the mechanism of drug resistance, we examined the changes in a large-scale gene expression profile of two sets of matched clinical fluconazole susceptible and resistant *C. albicans* isolates from BMT patients.

Materials and methods

C. albicans isolates and growth conditions

The azole-susceptible strain SC5314 and two matched sets of susceptible and resistant isolates of *C. albicans* used in this study are listed in Table 1. FH1/FH5 and TL1/TL3 were colonizing and bloodstream isolates obtained from two different patients who underwent bone marrow transplant (BMT) operation, respectively. The two sets of isogenic *C. albicans* isolates were obtained from Theodore C. White and have been identified at Fred Hutchinson Cancer Research Center and described previously (Marr et al., 1997, 1998, 2001). FH1/FH5 and TL1/TL3 represent the same strain by the identical banding patterns of RFLP analysis, respectively. An aliquot of glycerol stock from each isolate was diluted in YPD broth (1% yeast extract, 2% peptone, 1% dextrose) and grown overnight at 30 °C in a shaking incubator.

Antifungal agents and IC80 determinations

Powder formulations of fluconazole (Roerig-Pfizer, New York, N.Y.) suspended in distilled water were made to a final concentration of 0.125 μg/ml, filter sterilized, and stored at −70 °C. Media utilized in these studies included YPD broth and RPMI 1640 with 0.165 M MOPS (morpholinopropanesulfonic acid) buffered at pH 7.0. Antifungal susceptibility testing was performed by the standardized microdilution method from NCCLS document M27-A (National Committee for Clinical Laboratory Standards, Wayne, PA). To ensure consistent results, 80% inhibitory concentrations

Table 1. Compilation of previously reported and verified IC80s and described resistance mechanisms for *C. albicans* isolates used in this study

Isolate	IC80 (μg/ml)		Previously described resistance mechanism(s)[a]
	Previously determined[a]	This study	
SC5314	<1	0.25	Laboratory control strain
TL1	1	1	
TL3	32	64	Overexpression of CDR genes
FH1	4	4	
FH5	>64	32	Overexpression of *CDR1* and *CDR2*

[a]As reported in references Marr et al. (1997, 1998, 2001).