Journal of Microbiology, Immunology and Infection (2016) xx, 1–6

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

Fluconazole induces rapid high-frequency *MTL* homozygosis with microbiological polymorphism in *Candida albicans*

Tsong-Yih Ou [a,g], Fang-Mo Chang [b,g], Wei-Ning Cheng [c], Adela Lara [b], Ming-Li Chou [d], Wei-Fang Lee [e], Kai-Cheng Lee [d], Che-Tong Lin [b], Wen-Sen Lee [a], Fang-Lan Yu [f], Ching-Hua Su [c,*]

[a] *Division of Infectious Disease, Department of Internal Medicine, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan*
[b] *School of Dentistry, College of Oral Medicine, Taipei Medical University, Taipei, Taiwan*
[c] *Department of Microbiology and Immunology, Taipei Medical University, Taipei, Taiwan*
[d] *Graduate Institute of Medical Sciences, Taipei Medical University, Taipei, Taiwan*
[e] *School of Dental Technology, Taipei Medical University, Taipei, Taiwan*
[f] *Division of Microbiology, Department of Clinical Laboratory, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan*

Received 25 June 2015; received in revised form 15 October 2015; accepted 14 December 2015
Available online ■ ■ ■

KEYWORDS
Candida albicans;
fluconazole;
loss of heterozygosity;
mating type-like gene

Abstract *Background: Candida albicans*, a common fungal pathogen that can cause opportunistic infections, is regarded as an apparently asexual, diploid fungus. A parasexual cycle was previously found between homozygotes with opposite mating type-like loci (*MTL*a/α). Fluconazole-resistant strains had a higher proportion of *MTL* homozygotes, whereas *MTL* homozygous *C. albicans* was found in only about 3.2% of clinical strains. *MTL* heterozygotes had a low frequency (1.4×10^{-4}) of white–opaque switching to *MTL* homozygotes in nature.
Methods: Here, a reference *C. albicans* strain (SC5314) was used in a fluconazole-induced assay to obtain standard opaque *MTL* homozygous strains and first-generation daughter strains from the fluconazole inhibition zone. Further separation methods were employed to produce second- and third-generation daughter strains. Polymerase chain reaction analysis based on *MTL* genes was used to define *MTL* genotypes, and microscopic observations, a flow-cytometric assay, and an antifungal E-test were used to compare microbiological characteristics.
Results: *MTL* homozygotes were found at a high frequency (17 of 35; 48.6%) in fluconazole-induced first-generation daughter strains, as were morphological polymorphisms, decreased DNA content, and modified antifungal drug susceptibility. High-frequency *MTL* homozygosity

* Corresponding author. Department of Microbiology and Immunology, Taipei Medical University, 250, Wu-Hsing Street, Taipei 110, Taiwan.
E-mail address: curator@tmu.edu.tw (C.-H. Su).
[g] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jmii.2015.12.009

Please cite this article in press as: Ou T-Y, et al., Fluconazole induces rapid high-frequency *MTL* homozygosis with microbiological polymorphism in *Candida albicans*, Journal of Microbiology, Immunology and Infection (2016), http://dx.doi.org/10.1016/j.jmii.2015.12.009

was identified inside the fluconazole inhibition zone within 24 hours. The DNA content of fluconazole-induced daughter strains was reduced compared with their progenitor SC5314 and standard *MTL* homozygous strains.
Conclusion: Treatment with fluconazole, commonly used to treat invasive candidiasis, inhibited the growth of *C. albicans* and altered its microbiological characteristics. Our results suggest that fluconazole treatment induces the high frequency of loss of heterozygosity and microbiological polymorphism in *C. albicans*.

Introduction

Candida albicans, a common fungal pathogen that can cause opportunistic infections, is increasingly being recognized as a human pathogen in immunocompromised hosts, such as premature infants, cancer chemotherapy patients, solid-organ transplant recipients, and patients coinfected with human immunodeficiency virus or undergoing immunosuppressive treatment. *C. albicans* accounts for 50—60% of invasive fungal infections in humans, and the mortality rate of candidemia is higher (up to 61%).[1,2] *C. albicans* is regarded as a diploid, apparently asexual fungus. The frequency of spontaneous white-to-opaque cell switching is about 1.4×10^{-4} for *C. albicans* and about 10^{-2} for the specific WO-1 strain, as revealed by phloxine B-containing Lee's medium. Mating type-like loci *MTL*(a/α) identified in *C. albicans* are orthologous genes to mating-type loci *MAT*(a/α) in *Saccharomyces cerevisiae*. Only *MTL* homozygotes undergo white-to-opaque cell switching, and all individuals of strain WO-1 and 3.2% of 220 clinical isolates were found to be *MTL* homozygotes.[3—6] Opaque cells are 10^6 times more mating competent than white cells. The tetraploid parasexual cycle of *C. albicans* consisting of mating followed by chromosome loss has been described previously.[7—9]

Fluconazole is among the most common antifungal drugs to treat invasive fungal diseases caused by *C. albicans*; thus, much attention has been paid to fluconazole-resistant *C. albicans* strains.[10,11] A significantly higher proportion of *MTL* homozygosity was found in the fluconazole-resistant group than in the fluconazole-susceptible group among clinical strains.[12,13] However, fluconazole resistance is not directly affected by *MTL* homozygosity. One study of the evolution of fluconazole resistance was conducted to obtain a series of 330-generation daughter strains by treating one clinical strain with and without fluconazole. Further studies of the 330-generation series of *C. albicans* strains show that they appeared to have gradually developed adaptations against fluconazole through genomic evolution with corresponding fluconazole-resistant genes, which has indirectly correlated with *MTL* homozygosity.[14,15] However, recent analyses of these evolved strains indicated that rapid acquisition of aneuploidy and fluconazole resistance occurred within the first few generations.[16] Fluconazole resistance in *C. albicans* is due to both accumulated point mutations and loss of heterozygosity (LOH); therefore, *MTL* homozygosity is coincident with LOH on the left arm of chromosome 5, where two genes corresponding to fluconazole resistance, *ERG*11 and *TAC*1, are located. Recent analyses of these evolved strains indicate that rapid acquisition of aneuploidy and fluconazole resistance occurred within the first few generations.[17,18]

In a recent study, we found that fluconazole induced random chromosome loss and LOH in *C. albicans*, but the frequency of LOH is unclear.[19] Therefore, we collected isolates and offspring that were treated by fluconazole for a short term, calculated the frequency of LOH, and analyzed microbiological polymorphism.

Methods

C. albicans strains

The strains used in this study are listed in Table 1. The *C. albicans* reference strain SC5314 was used for its complete genome sequence.[6] A fluconazole-induced assay was used to obtain 35 fluconazole-induced first-generation daughter strains (FI-FGDSs) of SC5314. FI-FGDSs were analyzed MTL locus, and the heterozygotes were further separated and isolated by plating culture and micromanipulation to selected 87 fluconazole-induced second-generation daughter strains (FI-SGDSs) and 141 fluconazole-induced third-generation daughter strains (FI-TGDSs).

Fluconazole-induced assay and strain purification

The strain SC5314 was treated with the fluconazole-induced assay as described previously.[19] Phloxine B distinguishes opaque sectors and colonies by differentially staining them red. The strain SC5314 was treated with a fluconazole-containing disc (100 mg fluconazole per disc) or a fluconazole E-test strip (AB BIODISK, Solna, Sweden) on PB—YPD agar (YPD plus 5 μg/mL phloxine B) for 12—16 hours at 30°C. In this period, an inhibition zone was formed, and cells in the inhibition zone were observed using a microscope. The 35 FI-FGDSs were isolated from the inhibition zone with needle and each colony was spread on YPD agar for 6—8 hours. The initial cell morphology was observed to ensure that the strain was pure; if the morphology was different, we would isolate each different cell by a micromanipulator to be an FI-SGDS. In the 87 FI-SGDSs also, each colony was spread on YPD agar for 6—8 hours and the initial cell morphology was observed; each different cell was isolated to be an FI-TGDS.

Please cite this article in press as: Ou T-Y, et al., Fluconazole induces rapid high-frequency *MTL* homozygosis with microbiological polymorphism in *Candida albicans*, Journal of Microbiology, Immunology and Infection (2016), http://dx.doi.org/10.1016/j.jmii.2015.12.009