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SUSCEPTIBILITY

Sensitivity of White and Opaque *Candida albicans* Cells to Antifungal Drugs

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ABSTRACT White and opaque cells of *Candida albicans* have the same genome but differ in gene expression patterns, metabolic profiles, and host niche preferences. We tested whether these differences, which include the differential expression of drug transporters, resulted in different sensitivities to 27 antifungal agents. The analysis was performed in two different strain backgrounds; although there was strain-to-strain variation, only terbinafine hydrochloride and caspofungin showed consistent, 2-fold differences between white and opaque cells across both strains.

KEYWORDS antifungal drugs, antifungal sensitivity, *Candida albicans*, white-opaque switching

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The human fungal pathogen *Candida albicans* switches between two cell types named white and opaque (1–6). Both cell types are heritable; under standard laboratory conditions, stochastic switching events between the two cell types occur approximately once in every 10,000 cell divisions (7). Roughly 15% of the *C. albicans* genome is differentially expressed between the two cell types (476 genes are 2-fold upregulated in opaque cells, 487 genes are 2-fold upregulated in white cells [8]). White and opaque cells differ in their ability to mate (9), their metabolic preferences (10), their responses to environmental signals (11–16), and their interactions with the innate immune system (17–21). In addition to noticeable morphological differences between the cell walls of white and opaque cells (22, 23), transcripts of several putative drug pumps are differentially regulated between the two cell types: *CDR3* and *NAG4* are upregulated in opaque cells, and *QDR1*, *CDR4*, *TPO3*, *TPO4*, *FLU1*, and *MDR1* are upregulated in white cells (8, 10, 24). Given these differences in expression, we tested whether they translated into differential drug sensitivities (≥2-fold) by determining the sensitivity of white and opaque cells from two independent strain backgrounds to a panel of 27 antifungal drugs.

We tested white and opaque isolates of the WO-1 strain: a naturally occurring α mating-type strain isolated from the blood and lungs of a patient in 1984 (1) and an **a** mating-type derivative of the commonly used SC5314 strain isolated from a patient with disseminated candidiasis before 1968 (25–28) (see Table S1 in the supplemental material). Although the patient details pertaining to drug treatment before isolation of these strains are not available, note that both strains were isolated before the development of most current antifungal drugs. Strains were grown at 25°C in synthetic complete media supplemented with 2% glucose, amino acids, and 100 μg/ml uridine (SD+aa+Uri) (29). We determined the 50% reduction in turbidity compared with that of the growth control well (MIC-2) using a 96-well MIC assay modified to avoid environmentally induced opaque-to-white switching (30–32). Specifically, MIC assay plates were incubated for 2 days at 25°C in SD+aa+Uri with 2-fold drug titration gradients, because opaque cells are stable under this condition. After the 2-day incubation, cell density (optical density at 600 nm) was measured on a Tecan Infinite M1000 Pro plate reader, taking the average of five reads from distinct locations across

TABLE 1 MIC-2 for white and opaque cells from two strain backgrounds exposed to 27 antifungal agents in SD+aa+Uri at 25°C

Class and drug	SC5314			WO-1		
	White MIC-2 (μg/ml [μM])	Opaque MIC-2 (μg/ml [μM])	Opaque MIC-2/ white MIC-2	White MIC-2 (μg/ml [μM])	Opaque MIC-2 (μg/ml [μM])	Opaque MIC-2/ white MIC-2
Echinocandin						
Anidulafungin	0.74 (0.65)	0.45 (0.39)	0.60	0.06 (0.05)	0.03 (0.02)	0.50
Caspofungin	0.50 (0.41)	1.00 (0.82)	2.00	0.13 (0.11)	0.25 (0.21)	2.00
Micafungin	0.50 (0.39)	0.25 (0.20)	0.50	0.12 (0.10)	0.12 (0.10)	1.00
Imidazole						
Bifonazole	15.52 (50.00)	7.76 (25.00)	0.50	5.17 (16.67)	7.76 (25.00)	1.50
Butoconazole	0.02 (0.05)	0.02 (0.05)	1.00	0.01 (0.03)	0.01 (0.03)	1.00
Clotrimazole	0.13 (0.39)	0.13 (0.39)	1.00	0.07 (0.20)	0.07 (0.20)	1.00
Econazole nitrate	0.17 (0.39)	0.17 (0.39)	1.00	0.07 (0.16)	0.06 (0.13)	0.80
Ketoconazole	0.21 (0.39)	0.21 (0.39)	1.00	0.10 (0.20)	0.10 (0.20)	1.00
Miconazole nitrate	0.10 (0.20)	0.05 (0.10)	0.50	0.02 (0.05)	0.02 (0.05)	1.00
Oxiconazole Nitrate	0.19 (0.39)	0.19 (0.39)	1.00	0.10 (0.20)	0.10 (0.20)	1.00
Sulconazole nitrate	0.18 (0.39)	0.18 (0.39)	1.00	0.18 (0.39)	0.18 (0.39)	1.00
Tioconazole	0.03 (0.08)	0.02 (0.05)	0.60	0.02 (0.04)	0.02 (0.05)	1.20
Thiazole						
Abafungin	4.73 (12.50)	9.46 (25.00)	2.00	0.30 (0.78)	0.20 (0.52)	0.67
Triazole						
Fluconazole	1.56 (5.09)	1.56 (5.09)	1.00	0.78 (2.55)	0.78 (2.55)	1.00
Itraconazole	0.07 (0.10)	0.07 (0.10)	1.00	0.04 (0.05)	0.04 (0.05)	1.00
Terconazole	1.66 (3.13)	1.66 (3.13)	1.00	1.66 (3.13)	1.66 (3.13)	1.00
Voriconazole	0.02 (0.05)	0.01 (0.03)	0.50	0.01 (0.03)	0.01 (0.03)	1.00
Polyene						
Amphotericin B	16.00 (17.31)	10.67 (11.54)	0.67	8.00 (8.66)	8.00 (8.66)	1.00
Candicidin	2.31 (2.08)	3.47 (3.13)	1.50	0.87 (0.78)	0.87 (0.78)	1.00
Natamycin	5.55 (8.33)	4.16 (6.25)	0.75	2.77 (4.17)	2.08 (3.13)	0.75
Nystatin	3.86 (4.17)	2.89 (3.13)	0.75	1.45 (1.56)	2.41 (2.60)	1.67
Squalene epoxidase inhibitor						
Terbinafine hydrochloride	21.86 (66.67)	8.20 (25.00)	0.38	21.86 (66.67)	5.47 (16.67)	0.25
Amorolfine hydrochloride	0.55 (1.56)	0.55 (1.56)	1.00	0.03 (0.10)	0.03 (0.10)	1.00
Other						
Ciclopirox olamine	1.30 (6.25)	1.30 (6.25)	1.00	1.30 (6.25)	1.30 (6.25)	1.00
Flucytosine	>12.91 (100)	>12.91 (100)	NA[a]	>12.91 (100)	>12.91 (100)	NA
Griseofulvin	>35.28 (100)	>35.28 (100)	NA	>35.28 (100)	>35.28 (100)	NA
Tolnaftate	>30.74 (100)	>30.74 (100)	NA	>30.74 (100)	>30.74 (100)	NA

[a]NA, not applicable

each well. Each assay was performed in triplicate, and the average MIC-2 values are reported for each strain-drug combination in Table 1. As the MIC-2 value is the accepted endpoint for most of the drugs tested (23 of 27), we chose to use this endpoint for all of the drugs (including the 4 polyenes) so that all of the drugs in this study were tested in a consistent manner. In addition to applying a consistent endpoint for all of the drugs, we wanted to avoid using MIC_{90} and/or MIC-0 endpoints for opaque cells because we have found them problematic, within an experiment and between different experiments, due to the lower final cell density achieved (relative to white cells) and the resulting decrease in dynamic range. Details of the antifungal stock solutions are provided in Table S2 in the supplemental material. To determine whether cell type switching had occurred, we plated single cells from the 2-day MIC assay on the SC5314 strain background and examined the morphology of the resulting colonies. None of the drugs tested induced *en masse* white-to-opaque or opaque-to-white switching; therefore, the observed MIC-2 values were representative of each of the two starting cell types.

We determined the MIC-2 for white and opaque *C. albicans* cells from each strain background exposed to 27 antifungal agents (2 squalene epoxidase inhibitors, 3 echinocandins, 9 imidazoles, 1 thiazole, 4 triazoles, 4 polyenes, and 4 others) (Table 1).

Opaque cells from at least one strain background were 2-fold more sensitive to 6 drugs (anidulafungin, bifonazole, micafungin, miconazole nitrate, terbinafine hydrochloride, and voriconazole), whereas white cells from at least one strain background were 2-fold more sensitive to 2 drugs (abafungin and caspofungin) (Table 1). However, only 2 of these 8 drugs had detectable differences between cell types in both strain backgrounds (opaque cells from both were 2- to 4-fold more sensitive to terbinafine hydrochloride, and white cells from both were 2-fold more sensitive to caspofungin). We did not observe a difference in sensitivity (≥2-fold) between the two cell types for 16 drugs. The 3 remaining drugs did not affect either cell type at concentrations of 100 μM (the highest concentration tested, equivalent to 12.91 μg/ml for flucytosine, 35.28 μg/ml for griseofulvin, and 30.74 μg/ml for tolnaftate). No obvious correlation emerged between specific classes of antifungal drugs and specific cell type sensitivities.

Despite the numerous differences between white and opaque cells, the cell types had similar sensitivities to the full spectrum of antifungal agents. Given that the 2-fold differences observed fell within the potential variability of the assay used, we do not consider our results to indicate any significant difference in drug sensitivity between the two cell types. Furthermore, note that the differences in white-opaque sensitivities were smaller than the differences observed between the two strain backgrounds (Table 1) or between the same strain in different media conditions (data not shown). No class of antifungal drugs tested in this study showed consistent selective efficacy against either cell type. For example, white and opaque cells varied in their sensitivities to different echinocandins. These results suggest that the processes and genes that are commonly affected by antifungal drugs do not change in a meaningful way between the two cell types. Thus, the large gene expression differences between white and opaque cells (~1,000 genes at least 2-fold and 350 genes at least 4-fold differentially regulated [8]) do not appear to affect antifungal sensitivity.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00166-17.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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