TOP2 gene disruption reduces drug susceptibility by increasing intracellular ergosterol biosynthesis in *Candida albicans*

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In this study the role of the *TOP2* gene in fungal drug susceptibility was investigated by disrupting and overexpressing the gene in *Candida albicans*. MIC determination and a spot assay showed that a *top2Δ/Δ* null mutant (strain T2bc) was more resistant to the antifungals tested than the wild-type (strain CAI4). Real-time RT-PCR and rhodamine 6G efflux examination showed that *TOP2* did not influence the activity of drug efflux pumps. Sterol analysis with GC/high-resolution MS indicated that the intracellular ergosterol composition of the *top2Δ/Δ* mutant was significantly increased. Subsequently, fluorescence polarization measurements also revealed that Top2-deprived cells displayed a decrease in membrane fluidity, resulting in enhanced passive diffusion of the drugs. Quantitative real-time RT-PCR analysis further confirmed that the *ERG11* gene, an essential gene in ergosterol biosynthesis, was upregulated. These results demonstrate a close relationship between the *TOP2* gene and drug susceptibility in *C. albicans*.

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INTRODUCTION

Candida albicans is reported to be responsible for approximately 50% of all clinical fungal infections (Pappas *et al.*, 2003; Wisplinghoff *et al.*, 2004) and has become a prevalent opportunistic fungal pathogen (Silver *et al.*, 2004; White *et al.*, 2002). Following the long-term administration of the current antifungal drugs, *C. albicans* has developed resistance to these drugs, in particular to fluconazole (Perea *et al.*, 2001; Dassanayake *et al.*, 2002).

It is generally accepted that the most common mechanism underlying antifungal drug resistance is constitutive overproduction of multidrug transporters leading to drug efflux from the cells (Anderson, 2005; Shao *et al.*, 2007; White *et al.*, 2002). Two types of azole transporters have been defined: ATP-binding cassette transporters encoded by *CDR1* and *CDR2*, and the major facilitator superfamily transporters encoded by *MDR1* (Ghannoum & Rice, 1999). Less commonly, stable resistance can also result from alterations of lanosterol 14 α -demethylase (CYP51), targeted by fluconazole, and encoded by *ERG11* (Albertson *et al.*, 1996; Perea & Patterson, 2002). In recent years, there

has been documented evidence about the molecular basis of related genes in resistant strains and the transcription regulators that mediate their constitutive overexpression in drug resistance (Akins, 2005; Morschhauser, 2002).

Although the molecular basis of drug resistance in *C. albicans* is not clear, accumulated evidence indicates that multidrug resistance is a multifactorial phenomenon. Recent evidence also suggests that lipids could play an important role in drug susceptibility. Researchers have shown that some resistant *C. albicans* isolates exhibit changes in sterol composition and membrane phospholipids (Loffler *et al.*, 2000; Kohli *et al.*, 2002). It appears that the associated alterations in membrane lipid composition and its order (fluidity) could be significant determinants of drug susceptibility in yeast cells (Mukhopadhyay *et al.*, 2002).

Investigations in both prokaryotes and eukaryotes have demonstrated that topoisomerase II (Top2), a DNA unwinding enzyme, has a wide spectrum of functions in cellular processes, and the ensemble of topoisomerase II enzymes in mammalian cells appears to be more complex than previously thought (Wang, 2002; Zini *et al.*, 1992). The *C. albicans* *TOP2* gene has been analysed by heterologous expression in *Saccharomyces cerevisiae* to study Top2 inhibitors (Keller *et al.*, 1997). Recent studies in our

Abbreviation: HRMS, high-resolution MS.

Additional figures and tables are available as supplementary material with the online version of this paper.

laboratory have indicated that there may be a relationship between the *TOP2* gene and drug resistance in *C. albicans*, but the biological function of Top2 in relation to drug resistance in *C. albicans* remains ambiguous.

In this work, we studied the effect of the *TOP2* gene on drug susceptibility by disrupting both alleles of *TOP2* using the classical ura-blaster method and overexpressing the gene in the pCaEXP system in *C. albicans*. The results showed that deletion of *TOP2* attenuated the drug susceptibility to some extent. In addition, potential action mechanisms of the *TOP2* gene were investigated by measuring the uptake and efflux of drugs, and ergosterol biosynthesis. It was found that Top2 depletion introduced an increase in ergosterol content, which in turn led to attenuation of membrane fluidity and passive diffusion of drugs, thereby resulting in reduced drug susceptibility. In addition, the increased ergosterol level in a *top2* null mutant was probably the result of upregulation of *ERG11*.

METHODS

Strains and culturing. *C. albicans* strain CAI4 was kindly provided by Dr William A. Fonzi (Department of Microbiology and Immunology, Georgetown University, Washington, DC, USA). Details of the other strains constructed during this study are listed in Supplementary Table S1 (available with the online journal).

The *ura3/ura3* strains (CAI4, T1bc and T2bc) were routinely grown on YPD medium (1% yeast extract, 2% peptone, 2% glucose) supplemented with 0.005% uridine (Sigma) at 30 °C. Ura⁺ transformants (OET4, OET5, PECA and PET) were cultured in SC (synthetic complete) medium (2% glucose, 0.67% yeast nitrogen base with various amino acids as required) lacking methionine, cysteine and uridine.

Escherichia coli DH5 α , used as a host for plasmid construction and amplification, was grown in Luria–Bertani medium with 50 μ g ampicillin ml⁻¹ at 37 °C.

Drugs. The antifungal drugs used in this study were fluconazole (Pfizer); ketoconazole, miconazole, amphotericin B and caspofungin (Sigma-Aldrich); and itraconazole, voriconazole and terbinafine (Department of Organic Chemistry, Second Military Medical University, Shanghai, PR China). Dilutions of the drugs were prepared in DMSO and stored in plastic bags at 4 °C until use.

Disruption of the *C. albicans* *TOP2* gene. Disruption of both *C. albicans* *TOP2* alleles was performed using the ura-blaster approach (Fonzi & Irwin, 1993). A 472 bp *KpnI*–*BglII* fragment containing the 3' coding region of the ORF and the 3' non-coding region of the *TOP2* gene was amplified with oligonucleotide primers TOP2-D-F and TOP2-D-R (Supplementary Table S2 available with the online journal) and cloned into the p5921 vector upstream of the *hisG*–*URA3*–*hisG* cassette, yielding plasmid pTU1. In the same way, a 489 bp *BamHI*–*HindIII* fragment homologous to the 5' region of the *TOP2* gene was amplified with primers TOP2-U-F and TOP2-U-R (Supplementary Table S2 available with the online journal) and cloned into the downstream region of the *hisG*–*URA3*–*hisG* cassette of pTU1 to generate the resulting *TOP2* knockout plasmid named pTU2. The orientation of the inserted PCR products of the *TOP2* 5' and 3' regions was verified at each step by PCR. In addition, DNA sequencing of plasmid pTU2 confirmed that the cloned *TOP2* gene was identical to the candidate *TOP2* sequence in the *Candida*

database (CandidaDB: <http://genolist.pasteur.fr/CandidaDB/>). pTU2 was then linearized with *KpnI* and used in transformation of *C. albicans* CAI4 using the lithium acetate method according to Sanglard *et al.* (1996). The same procedure was repeated to delete the remaining functional allele of the *TOP2* gene.

DNA extraction and Southern blotting. *TOP2* disruption in heterozygous and homozygous strains was verified by Southern blotting. Genomic DNA was isolated from all the strains and digested with *BglII*. The 811 bp hybridization probe was amplified by PCR using the primers TZ-F and TZ-R (Supplementary Table S2 available with the online journal). Labelling of the DNA probe and subsequent hybridization were carried out using a random-primed digoxigenin DNA-labelling detection kit (Roche).

Reintegration of the *C. albicans* *TOP2* gene. The sequence of the *C. albicans* *TOP2* gene was retrieved from the annotated CandidaDB database. The plasmid pTWD containing the complete ORF of the *C. albicans* *TOP2* gene was amplified from the genomic DNA of strain CAI4 by PCR and sequenced. The vector pCaEXP was digested with *BamHI* and *PstI* to enable integration of the complete ORF of *TOP2* into the *MET3* promoter, producing the overexpressing vector pEXP-*TOP2*. After sequencing, pEXP-*TOP2* was linearized at the unique *StuI* site and transformed into *C. albicans* strain T2bc using the lithium acetate method to obtain *TOP2* overexpression strains, designated OET. Simultaneously, the empty pCaEXP plasmid was digested with *StuI* and was used to transform separately strains CAI4 and T2bc to serve as negative controls, designated PECA and PET2, respectively. Transformed cells were selected as Ura⁺ in SC medium without methionine, cysteine or uridine (Care *et al.*, 1999).

RNA extraction and quantitative real-time PCR. RNA was isolated from each sample using a fungal RNAout kit (Tiandz) and samples were treated with DNase (Takara) and then reverse transcribed using avian myoblastosis virus reverse transcriptase and random sixmers (Takara). Real-time RT-PCR used to quantify gene expression levels was performed subsequently using a SYBR Green real-time PCR master mix (Fermentas) following the manufacturer's recommended protocols. Fluorescence signals were monitored using a Bio-Rad system. The threshold cycle (*C_t*) for target mRNA was normalized with transcripts of 18S rRNA and the mean value was determined. Gene expression levels were calculated from normalized *C_t* results, and the mean results were used to represent relative fold changes between the experimental groups. The results were obtained from three independent experiments. Statistical significance was estimated using Student's *t*-test ($P \leq 0.05$). Primer pairs specific for *TOP2* (RT-FWD and RT-RVE), *MDR1* (MDR1-F and MDR1-R), *CDR1* (CDR1-F and CDR1-R), *CDR2* (CDR2-F and CDR2-R) and *ERG* (ERG-F and ERG-R) are listed in Supplementary Table S2 (available with the online journal).

MIC determination. Drug sensitivity testing was performed by the broth microdilution technique as described by Espinel-Ingroff *et al.* (1995). The MIC values of the *top2Δ/Δ* mutant towards the antifungal drugs described above were determined as optical density values at 630 nm using a microplate reader after 24 h incubation at 30 °C (see Table 3). DMSO, which was used to dissolve the antifungals in the experiments, was also used as a negative control. The results shown are typical of one determination, which was confirmed by three independent experiments.

Spot assay. To further assess the resistant genotype of *top2* mutants to the drugs, the mutant strains of *C. albicans* were studied using a spot assay. The *C. albicans* strains were grown overnight and then suspended in medium to an OD₆₀₀ of 0.1. Serial tenfold dilutions of each strain were prepared. Five microlitres of each dilution of each *Candida* culture was spotted onto the appropriate medium. The

wild-type strain (CAI4) and *top2* heterozygous and homozygous mutant strains (T1bc and T2bc) were spotted onto YPD plates with uridine and the indicated antifungal agents. The *TOP2* overexpression mutants (OET4 and OET5) and the control strains (PET2 and PECA) were spotted onto SC medium plates (without methionine, cysteine or uridine) with a specific antifungal compound as indicated. The differences between the strains were observed after incubation for 2 days at 30 °C.

Rhodamine 6G efflux assay. *C. albicans* cells were grown in appropriate medium at 30 °C for 16 h, subcultured into the same medium and incubated at 30 °C for 5 h. Cells were then washed three times in PBS, and resuspended in PBS at an OD₆₀₀ of 2.0 to exhaust their energy. Rhodamine 6G (10 µM) was added to the de-energized cells and incubation was carried out for a further 45 min. The cells were washed three times and the final concentration of cells was kept at 5×10^7 cells ml⁻¹. Aliquots of cells were pre-incubated at 30 °C for 5 min, and rhodamine 6G efflux from the cells was then initiated by the addition of 2 mM glucose to stimulate efflux, with continuous incubation at 30 °C. At specified time points after the addition of glucose, cells were collected from duplicate assays by centrifugation, and 100 µl aliquots of the cell supernatant were transferred to the wells of 96-well flat-bottomed microtitre plates (BMG Labtech). The fluorescence signal of rhodamine 6G in the samples was measured using a Scientific Varioskan Flash plate reader (Thermo) using excitation and emission wavelengths of 515 and 555 nm, respectively.

Quantitative sterol analysis. *C. albicans* cells (strains CAI4, T2bc and OET4 in the absence or presence of 2 µg fluconazole ml⁻¹) incubated for 24 h were harvested by centrifugation at 5000 r.p.m. (Biofuge Stratos superspeed refrigerated centrifuge; Heraeus) for 5 min, washed with PBS three times and 0.5 mg thallus was measured separately. The cell pellets were saponified using saponification agent at 80 °C for 1 h. Non-saponifiable lipids (ergosterols and ergosterol precursors) were extracted with petroleum ether (at 30–60 °C) three times, dried at 60 °C and then dissolved in cyclohexane. Sterols were analysed by GC/high-resolution MS (GC/HRMS) using split injections with a split ratio of 10:1. The sterol composition was quantified based on comparing relative retention times to that of an internal standard (cholesterol). The operating conditions used for GC/HRMS were as described by Howell *et al.* (1990).

Passive diffusion assay. Passive diffusion of rhodamine 6G was determined by a published protocol (Mukhopadhyay *et al.*, 2002). Briefly, *Candida* cells were collected and washed in PBS without glucose and then resuspended in PBS (without glucose) supplemented with 5 mM dinitrophenol and 5 mM 2-deoxy-D-glucose. Rhodamine 6G (10 µM) was added to the de-energized cell suspension and incubated for 40 min. The cells were pelleted and the absorbance of the supernatant was measured spectrophotometrically at 527 nm.

Detection of fluorescence polarization. Steady-state fluorescence polarization measurements of *C. albicans* cells were carried out with the fluorescent probe 1,6-diphenyl-1,3,5-hexatriene at excitation and

emission wavelengths of 360 and 426 nm, respectively (Prasad *et al.*, 2005).

RESULTS AND DISCUSSION

Generation and identification of *top2Δ/Δ* mutants and reintegration mutants

The role of the *TOP2* gene in *C. albicans* was studied by deleting the two *top2* alleles in strain CAI4. The ura-blaster strategy is shown in Supplementary Fig. S1(a) (available with the online journal). The strains produced in this process are listed in Supplementary Table S1. Southern blotting showed that both *top2* alleles were disrupted successfully (Supplementary Fig. S1b available with the online journal).

The *TOP2* ORF was placed under the control of the *MET3* promoter in pCaEXP and transformed into the *top2* null mutant (strain T2bc). Relative quantification by real-time RT-PCR was used to confirm overexpression of the *TOP2* gene in OET (T2bc harbouring pEXP-TOP2) compared with its expression in PECA (CAI4 harbouring pCaEXP). The *TOP2* expression level in OET4 and OET5 was 15.7-fold and 8.7-fold higher, respectively, than that in PECA (Supplementary Fig. S2 available with the online journal).

top2Δ/Δ mutant shows a reduction in drug susceptibility

To identify the role of the *TOP2* gene in drug susceptibility, the phenotype of the parental strain (CAI4) and the *top2* null mutant (T2bc) were studied. No significant difference in the growth rate of CAI4 and T2bc was observed (Supplementary Fig. S3, available with the online journal). As shown in Table 1, T2bc had a higher MIC value and was less susceptible to azoles compared with CAI4. The MIC value of T2bc increased 16-fold in the case of fluconazole and ketoconazole, eightfold for voriconazole, and fourfold for miconazole and itraconazole, compared with that for CAI4.

Spot assay analysis also showed that the null mutant T2bc was less susceptible to fluconazole, miconazole, ketoconazole, itraconazole and voriconazole relative to native CAI4 (Fig. 1a), which was consistent with the MIC results, whilst the *TOP2* overexpression strains (OET4 and OET5) became more susceptible (Fig. 1b). To see whether the decreased susceptibility of *C. albicans* as a result of Top2

Table 1. MIC values of the wild-type strain CAI4 and *top2* mutants T1bc and T2bc, tested by the broth microdilution method

Strain	MIC (µg ml ⁻¹)				
	Fluconazole	Miconazole	Ketoconazole	Itraconazole	Voriconazole
CAI4	0.250	0.250	0.015	0.050	0.001
T1bc	2.000	0.500	0.125	0.100	0.001
T2bc	4.000	1.000	0.250	0.200	0.008

deprivation was limited to azoles, the spot assay was also performed with amphotericin B, terbinafine and caspofungin. T2bc also displayed attenuated susceptibility to these drugs, indicating that the *TOP2* expression levels did not only affect azole susceptibility.

***TOP2* gene does not influence efflux activity**

Resistance of *C. albicans* to azole antifungals is often caused by increased expression of genes encoding multidrug efflux pumps, such as *MDR1*, *CDR1* and *CDR2*. Real-time RT-PCR determination of the expression levels of *MDR1*, *CDR1* and *CDR2* revealed that there was no significant alteration between the wild-type (CAI4) and the *top2* mutant strains (T2bc and OET4) (see Supplementary Fig. S4 available with the online journal).

In addition, the function of Cdr1p was studied by determining rhodamine 6G efflux. The effect of the expression of the *TOP2* gene on energy-dependent drug efflux was quantified by comparably pre-loading the *top2Δ/Δ* mutant (T2bc) and wild-type strain (CAI4) with the pump substrate rhodamine 6G. As shown in Supplementary Fig. S5 (available with the online journal), the function of efflux transporters was weak without a glucose supply, but was enhanced significantly after the addition of glucose to both strains, although the difference between them was negligible. In conclusion, the study on drug transporter genes and the function of Cdr1p indicated that the resistant phenotype of the *top2Δ/Δ* null mutant was not associated with drug efflux activity.

TOP2* gene affects ergosterol biosynthesis in *C. albicans

It is known that abnormalities of ergosterol biosynthesis are another cause of decreased drug availability in cells; therefore, the amount of intracellular ergosterol present in *top2* mutants (T2bc and OET4) and the isogenic wild-type strain (CAI4) was quantified by GC/HRMS. The results in

Table 2 showed that the ability of the *top2Δ/Δ* null mutant (T2bc) to biosynthesize ergosterol was enhanced significantly, with the greatest amount of ergosterol (81.80 %), whilst there was a reproducible decrease in ergosterol in OET4 (44.50 %). Simultaneously, when treated with 2 µg fluconazole ml⁻¹, the strains accumulated large amounts of 14α-methylated sterol in the form of 24,25-dihydrolanosterol. The ergosterol percentage of T2bc was markedly reduced from 81.80 to 16.36 %. In strains CAI4 and OET4, ergosterol levels decreased sharply to an undetectable level. This result is consistent with upregulation of the sterol biosynthetic pathway.

***ERG11* gene expression is upregulated in the *top2Δ/Δ* mutant**

Determination of sterol composition by GC/HRMS analysis showed that expression of the *TOP2* gene altered the drug sensitivity of *C. albicans* by contributing to ergosterol biosynthesis. Accordingly, gene expression levels of *ERG2*, *ERG3*, *ERG11*, *ERG24* and *ERG25* were measured. As expected, the expression level of the *ERG11* gene was increased in T2bc by more than twofold compared with CAI4. In contrast, overexpression of *TOP2* resulted in downregulation of *ERG11* in OET4 by approximately twofold compared with CAI4 (Fig. 2). Statistical analysis showed that there was a significant difference in *ERG11* gene expression among the experimental groups ($P \leq 0.05$). No obvious differences in the expression of *ERG2*, *ERG3*, *ERG24* and *ERG25* were observed among the experimental groups.

Top2 depletion leads to a decrease in diffusion of drugs and membrane fluidity

It is known that there is an intricate relationship between membrane fluidity and ergosterol content. In view of the increased ergosterol level in the *top2Δ/Δ* null mutant, passive diffusion and membrane fluidity were explored.

Fig. 1. Drug resistance profiles of *C. albicans* wild-type and *top2* mutant cells determined by spot assay as described in Methods. The names of the strains are indicated on the left.

Table 2. Quantification of sterol content in *C. albicans* strains CAI4, T2bc and OET4 with or without fluconazole by GC/HRMS

Treatment	Strain	Sterol composition* (%) (<i>n</i> =3)			
		Squalene	Ergosterol	Lanosterol	24,25-Dihydrolanosterol
Control†	CAI4	5.48 ± 0.86	64.49 ± 4.55	30.01 ± 1.86	ND
	T2bc	1.94 ± 0.76	81.80 ± 1.01	16.26 ± 1.78	ND
	OET4	ND	44.50 ± 2.76	55.50 ± 2.76	ND
Fluconazole (2 µg ml ⁻¹)	CAI4	ND	ND	17.20 ± 0.72	82.80 ± 0.72
	T2bc	ND	16.36 ± 4.65	26.63 ± 5.82	57.00 ± 1.17
	OET4	ND	ND	38.97 ± 2.22	61.03 ± 2.22

ND, Not detected.

*All samples were taken from triple independent experiments and are represented as mean ± SD.

†Control (in the absence of fluconazole).

Fluorescence polarization measurements revealed that *TOP2* disruption led to a 36.5% decrease in membrane fluidity, resulting in decreased passive diffusion of rhodamine 6G by 30.3% (Table 3). These results implied that the intake of rhodamine 6G was less in the *top2Δ/Δ* mutant than in the wild type. Taken together, the decreased passive diffusion of rhodamine 6G by the *top2Δ/Δ* mutant indicated that the *top2Δ/Δ* mutant membrane was less permeable, a property that could explain the reduced sensitivity to the drugs.

As overexpression of drug efflux pump-encoding genes is the predominant mechanism responsible for drug resistance, we first hypothesized that the observed phenotype of *top2* mutants in drug susceptibility might be the result of changes in drug efflux. However, *TOP2* disruption was not able to modify the expression levels of the multidrug transporter genes. Thus, the effect of *TOP2* expression on drug sensitivity appeared to be independent of the efflux pump proteins. However, the ergosterol content of the *top2* null mutation strain was higher than that of the parental strain, which could account for the lowered membrane fluidity and passive diffusion of drugs observed. Our results demonstrate for the first time that mutations in the *TOP2* gene could play a role in the mechanism of *Candida*

resistance to antifungal drugs. *TOP2* expression appeared to have a relationship with *ERG11* expression, leading to the question of why and how *TOP2* negatively affects *ERG11* expression. An attempt to further understand the potentially insidious functions and mechanisms of action of Top2 is under way in our laboratory.

It has now been established that the Top2 protein maintains a favourable nucleic acid topology for replication, transcription, control of recombination and chromosome segregation, which are indispensable for normal growth of the organism (Nitiss, 1998; Cline & Hanawalt, 2006). However, *C. albicans* (CAI4) with both *TOP2* alleles disrupted was able to survive, and *TOP2* deletion did not affect the growth rate of the strains. Therefore, the *TOP2* gene does not seem

Table 3. Measurements of membrane fluidity and passive diffusion of *top2* mutants and the wild-type strain

Strain	P value*	OD ₅₂₇ †
CAI4	0.202 ± 0.013	0.344 ± 0.022
T2bc	0.275 ± 0.012	0.449 ± 0.023
OET4	0.145 ± 0.008	0.256 ± 0.013

*The fluorescence polarization (P) values of cells. P values are the means ± SD of three independent experiments.

†Extracellular rhodamine 6G concentrations. The values are represented as mean ± SD of three sets of experiments.

Fig. 2. Expression levels of *C. albicans* *ERG* genes in strains CAI4 (white bars), T2bc (black bars) and OET4 (grey bars) determined by RT-PCR. RNA levels (normalized to 18S rRNA levels) are represented as fold increase or decrease relative to the level for the control strain (CAI4). Error bars represent SEM. Asterisks denote statistical significance by Student's *t*-test ($P \leq 0.05$).

essential for growth in *C. albicans*, probably because Top2 could be substituted by the roles of some functional complementation genes. The current view assumes that supercoiling generated by helix unwinding is mainly relaxed by type IB enzymes (Schvartzman & Stasiak, 2004). The results of Kim & Wang (1989) showed that synthesis of short DNA chains can initiate in the absence of both topoisomerases in *S. cerevisiae*, but their further elongation requires at least one of the two topoisomerases. Inactivation of Top2 does not significantly alter the patterns of nascent DNA chain synthesis (Kim & Wang, 1989). Yeast cells can replicate DNA in *top1* or *top2* mutants, but not when both Top1 and Top2 functions are defective, suggesting that TOP1 might play an important role in assisting chromosome replication in the absence of Top2. Although extensive biochemical and structural studies in the past have determined the cellular roles of the enzymes, their exact roles in the process of DNA synthesis within the eukaryotic chromatin context remain unclear. Moreover, Top2 may possess other critical and unexpected biological functions in *C. albicans*, and this needs further study.

In summary, the results of the present study demonstrated that mutations in the *TOP2* gene correlated with the alteration of drug susceptibility in *C. albicans*. It is possible that the increased transcription level of *ERG11* in the *TOP2*-deleted strain augmented ergosterol biosynthesis, thus eliciting the decreased membrane fluidity and passive diffusion of drugs, leading ultimately to reduced susceptibility to the antifungal drugs. The influence of Top2 on the drug susceptibility of *Candida* suggests that Top2 might be another novel determinant of multidrug resistance.

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