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Characterization of Tamoxifen as an Antifungal Agent Using the Yeast *Schizosaccharomyces Pombe* Model Organism

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Tamoxifen, a selective estrogen receptor modulator used for managing breast cancer, is known to have antifungal activity. However, its molecular mechanism remains unknown. Using the fission yeast *Schizosaccharomyces pombe* as a model organism, we have explored the mechanism involved in antifungal action of tamoxifen. Since tamoxifen was shown to inhibit the binding of calmodulin to calcineurin in fungi, we first examined involvement of these molecules and found that overexpression of a catalytic subunit of calcineurin and its constitutively active mutant as well as calmodulin increases tamoxifen sensitivity. Since terbinafine and azoles inhibit enzymes for ergosterol biosynthesis, Erg1 and Erg11, for their antifungal actions, we also examined involvement of these molecules. Overexpression of Erg1 and Erg11 reduced the sensitivity to terbinafine and azoles, respectively, but increased tamoxifen sensitivity, suggesting that ergosterol biosynthesis is differently related to the action of tamoxifen and those of terbinafine and azoles. To elucidate molecules involved in tamoxifen action, we performed a genome-wide screen for altered sensitivity to tamoxifen using a fission yeast gene deletion library, and identified various hypersensitive and resistant mutants to this drug. Notably, these mutants are rarely overlapped with those identified in similar genetic screens with currently used antifungals, suggesting a novel mode of antifungal action. Furthermore, tamoxifen augmented antifungal actions of terbinafine and azoles, suggesting synergetic actions between these drugs. Therefore, our findings suggest that calmodulin-calcineurin pathway and ergosterol biosynthesis are related to antifungal action of tamoxifen, and propose novel targets for antifungal development as well as combined therapy with tamoxifen for fungal diseases.

INTRODUCTION

Invasive fungal infections have become a life-threatening problem among immunocompromised patients and aging people, especially who have acquired immunodeficiency syndrome (AIDS) or those receiving organ transplantation and cancer chemotherapy. Pharmaceutical development for decades has discovered several antifungal drugs of distinct pharmacological actions. In clinics, terbinafine, azoles, and micafungin are commonly prescribed antifungal drugs. Terbinafine and azoles are known to inhibit enzymes responsible for ergosterol biosynthesis, squalene monooxygenase encoded by *erg1*$^+$ gene and lanosterol 14-α-demethylase encoded by *erg11*$^+$ gene, respectively (Fang et al. 2012). Micafungin is an inhibitor of 1, 3-β-D-glucan synthase critical for fungal cell wall synthesis. However, since both pathogenic fungi and their hosts are eukaryotes and have similar biological processes, antifungal drugs may cause serious side effects. Furthermore, there are pathogenic fungi resistant to these drugs, and chronic treatment with antifungal drugs increases the incidence of antifungal resistance. Since these growing problems limit their use in clinics (Georgiadou and Kontoyiannis 2012; Paul and Moye-Rowley 2014), there is a great need to develop novel antifungal drugs and those that augment antifungal activity of existing drugs.

To address this issue, we have been studying the mechanisms underlying the actions of antifungal drugs using the fission yeast *Schizosaccharomyces pombe* (*S. pombe*) as a model organism, given its similarity with some pathogenic fungi and its feasibility of genetic modification. We performed genome-wide screens in *S. pombe* for altered sensitivity to clotrimazole, terbinafine and micafungin, and identified a number of genes and pathways associated with the sensitivity and resistance to these antifungal drugs (Fang et al. 2012; Zhou et al. 2013). Recently, it was discovered that tamoxifen, a selective estrogen receptor (ER) modulator most widely used for anticancer drugs in managing breast cancer, has antifungal action (Dolan et al. 2009; Butts et al. 2014).

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This finding has gained attention, since it may be exploitable for identifying a novel target for pharmaceutical development of antifungal drugs. Since fungi do not express ER or its ortholog, this antifungal action should be ER-independent. It was shown that tamoxifen binds to and inhibits the binding of calmodulin to calcineurin in fungi. Indeed overexpression of calmodulin suppressed, and its deletion facilitated, the toxicity of tamoxifen in *S. cerevisiae* (Dolan et al. 2009), and reduced expression of calmodulin increased sensitivity to tamoxifen in *Cryptococcus neoformans* (Butts et al. 2014). Therefore, calmodulin is not required for, but is related to, antifungal actions of tamoxifen. However, the molecular mechanism underlying antifungal action of tamoxifen has not systematically analyzed. Furthermore, whether its mechanism is similar or distinct from those of existing antifungal drugs has not been explored.

In this study, using fission yeast as a model organism, we have examined and found involvement of calmodulin and its target calcineurin as well as enzymes for ergosterol biosynthesis, Erg1 and Erg11, in antifungal action of tamoxifen. Overexpression of Erg1 and Erg11 reduced the sensitivity to terbinafine and azoles, respectively, but increased the sensitivity to tamoxifen, suggesting that ergosterol biosynthesis is differently related to the actions of tamoxifen and existing antifungal drugs. Through a genome-wide screen, we have identified various mutants of altered sensitivity to tamoxifen, most of which are rarely overlapped with those identified with existing antifungal drugs in our previous reports (Fang et al. 2012), suggesting a novel mode of antifungal action. Furthermore, tamoxifen augmented antifungal actions of terbinafine and azoles, suggesting a potential benefit of combination therapy with tamoxifen for fungal diseases.

MATERIALS AND METHODS

Strains and media

Heterozygous diploid deletion strains used for the genetic screen were purchased from Bioneer Corporation and Korea Research Institute of Biotechnology and Bioscience (http://pombe.bioneer.co.kr/). These deletion strains were generated using the method of PCR-based targeted gene deletion with a genetic background of h^+ *leu1-32 ura4-D18 ade6-M210* or *M216* (Fang et al. 2012; Zhou et al. 2013). The deletion collection consists of 3004 mutants that represent around 84% of nonessential genes in *S. pombe* as described previously (Fang et al. 2012; Zhou et al. 2013). Another strain used in this study is HM123 (h^- *leu1-32*).

Standard yeast media, notation, and general experimental methods were used as described previously (Moreno et al. 1991; Fang et al. 2014) except where otherwise noted. All chemicals and reagents were purchased from commercial sources.

Plasmids

For a plasmid overexpressing Cam1, the *cam1*$^+$ gene was amplified by PCR (forward primer: 5'-CGG GAT CCG AGC AGA TTT ACA AAC ACT GTC ACG G-3'; reverse primer: 5'-CGG GAT CCT TAC ATG AAG TCA TCA AAG TCA TC-3') from the genomic DNA of *S. pombe* wild-type cells and subcloned into the BamHI site of pBluescript SK (+) (Stratagene) containing ARS1 and LEU2. The construct was validated by DNA sequencing. This construct is fully functional as expression of Cam1 with this plasmid complemented the phenotypes associated with the *cam1* mutation (data not shown). Plasmids overexpressing Ppb1, Cnb1 and C-terminal-deleted Ppb1 (CNΔC) were generated in our laboratory, as previously reported (Sio et al. 2005). Plasmids overexpressing Erg1, Erg11, Bfr1 and Prt1 are derived from an *S. pombe* genomic DNA library constructed in the vector pDB248 (Beach et al. 1982).

Genome-wide screen for tamoxifen-sensitive and tamoxifen-resistant deletion mutants

We used streak assay for a preliminary screen and spot assay for a secondary screen, as described in detail previously (Fang et al. 2012; Zhou et al. 2013). Briefly, as a preliminary screen, the log-phase cells of the deletion library were streaked onto YPDA plates with or without tamoxifen and incubated at 27°C for 5 days. Deletion mutants which showed significantly altered sensitivity in the preliminary screen were retested for the secondary screens using a dilution-series spot assay (Fang et al. 2012; Zhou et al. 2013). Three independent spot assays were performed for each mutant strain.

Miscellaneous Methods

In all experiments, we examined the effects of tamoxifen on the cell growth at several escalating doses, namely 10, 15, 20, 25, 30, 32, 35, 38, and 40μg/ml. In Figures 2-5, the images only at a single dose of tamoxifen were chosen to show representative figures. We noted that the wild-type cells transformed with the control vector in Figure 2 and Figure 3 grew with tamoxifen at 35μg/ml unlike the non-transformed wild-type cells used in the other figures. For this reason, we had to show images at 38μg/ml as representative images in Figure 2, in which the largest differences across comparison groups could be observed. The reduced sensitivity to tamoxifen

in Figures 2 and 3 may be because the cells were transformed with some vectors in these figures but not in the others, or because the auxotrophic wild-type cells used in these figures may have slightly different sensitivity to tamoxifen, compared with those in Figure 1.

Database searches were performed using the National Center for Biotechnology Information BLAST network service (www.ncbi.nlm.nih.gov) and the *S. pombe* database search service (www.pombase.org). In the table of genome-wide screens, the systematic name, standard gene name (if applicable), as well as a brief description of each gene product (obtained from http://www.pombase.org/) are indicated, as described previously (Fang et al. 2012). If the standard name of the *S. pombe* mutant genes is not available, for convenience, we named the genes based on their *S. cerevisiae* counterparts.

RESULTS AND DISCUSSION

Tamoxifen suppresses the cell growth of *S. pombe*.

In order to characterize the antifungal activity of tamoxifen in *S. pombe*, we first tested the effect of tamoxifen on the growth of *S. pombe* wild-type cells. Tamoxifen was tested at a range of concentrations from 10 μg/ml to 40 μg/ml. As shown in Figure 1, whereas the wild-type cells grew normally at YPD plates, tamoxifen at 35 μg/ml suppressed the cell growth and no colony was formed in the presence of 40 μg/ml tamoxifen. Therefore, tamoxifen inhibits the growth of *S. pombe* in a dose-dependent manner.

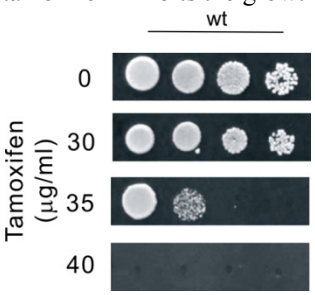

Figure 1. Effects of tamoxifen on fission yeast cell growth. The wild-type (wt) cells grown at log-phase were spotted onto each plate as indicated, and then incubated at 27°C for 4 days.

Calmodulin and calcineurin are related to antifungal action of tamoxifen.

Previous studies showed that overexpression of calmodulin suppressed the sensitivity to tamoxifen in *S. cerevisiae* (Dolan et al. 2009) and in *Cryptococcus neoformans* (Butts et al. 2014). It was also shown that tamoxifen inhibits the binding of calmodulin to calcineurin in vitro and suppresses calcineurin-mediated transcription in *C. neoformans*. Therefore, we examined the effects of overexpression of calmodulin encoded by the *cam1*$^+$ gene and the catalytic and regulatory subunits of calcineurin, Ppb1 and Cnb1, as well as a constitutively active mutant of Ppb1 (CNΔC). Overexpression of Ppb1 and its constitutively active mutant (CNΔC) as well as calmodulin increased the sensitivity to tamoxifen, though the effect of CNΔC was larger than the others (Figure 2). On the other hand, overexpression of Cnb1 appeared not to increase the sensitivity to tamoxifen. These results suggest that calmodulin-calcineurin pathway is related to antifungal action of tamoxifen, though its mechanism is still under investigation.

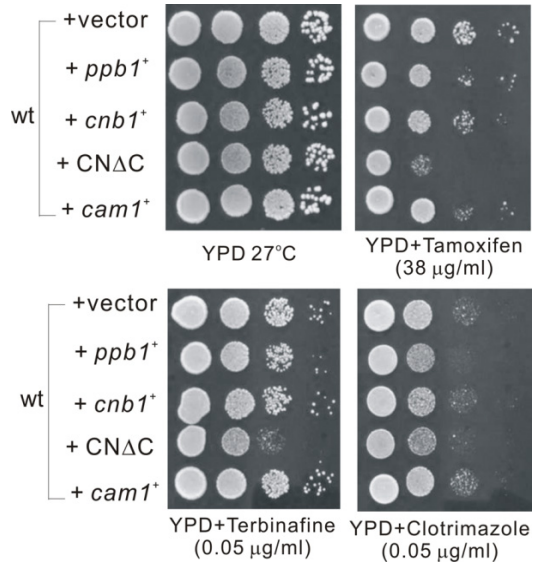

Figure 2. Calmodulin and calcineurin are related to antifungal action of tamoxifen in fission yeast. The wild-type cells were transformed with either a control vector, or the vector containing *cam1*$^+$, *ppb1*$^+$, *cnb1*$^+$, or the constitutively active truncated calcineurin gene (CNΔC). Cells were then spotted onto each plate as indicated, and then incubated for 4 days at 27°C.

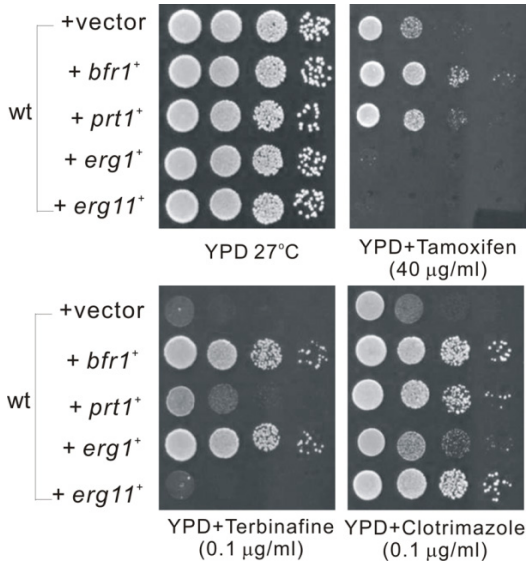

Figure 3. Ergosterol biosynthesis is related to antifungal action of tamoxifen in fission yeast. The wild-type cells were transformed with either a control vector, or the vector containing *bfr1*$^+$, *prt1*$^+$, *erg1*$^+$, or *erg11*$^+$ genes. Cells were then spotted onto each plate as indicated, and then incubated for 4 days at 27°C.

We also examined the effects of overexpression of these genes on terbinafine, clotrimazole and micafungin. Overexpression of the constitutively active Ppb1 slightly increased the sensitivity of terbinafine, similarly to that of tamoxifen, whereas overexpression of the others did not (Figure 2). On the other hand, the sensitivity to clotrimazole (Figure 2) and micafungin (data not shown) was not affected by overexpression of any of these genes. Therefore, calmodulin-calcineurin pathway is differently related to antifungal actions of tamoxifen and existing antifungal drugs.

Ergosterol biosynthesis is related to antifungal action of tamoxifen.

Erg1 and Erg11 are targets of terbinafine and azoles, respectively, and overexpression of either of these genes reduces the sensitivity to the respective antifungal drugs (Shingu-Vazquez and Traven 2011; Leber et al. 2003). We investigated whether Erg1 and Erg11 are involved in antifungal action of tamoxifen. As expected, overexpression of *erg1*$^+$ gene, but not *erg11*$^+$ gene, reduced the sensitivity to terbinafine, and overexpression of *erg11*$^+$ and *erg1*$^+$ genes reduced the sensitivity to clotrimazole, though the effect was much weaker for *erg1*$^+$ gene (Figure 3). Surprisingly, overexpression of *erg1*$^+$ and *erg11*$^+$ genes increased the sensitivity to tamoxifen (Figure 3). These findings suggest that ergosterol biosynthesis mediated by Erg1 and Erg11 is related to antifungal action of tamoxifen. Although the mechanism by which ergosterol biosynthesis augments the action of tamoxifen remains unknown, tamoxifen, cholesterol and ergosterol have similar structures, and biochemical characterizations of all these molecules displayed the ability of inhibiting lipid peroxidation in vitro (Wiseman et al. 1990). Therefore, ergosterol, cholesterol and tamoxifen may share the same targets related to cell growth and/or survival.

Previous studies also showed several genes involved in multidrug resistance (MDR), such as *bfr1*$^+$ gene encoding a transmembrane transporter belonging to the ATP-binding cassette (ABC) family and the *prt1*$^+$ gene encoding a transcription factor responsible for drug-induced expression of ABC transporters. Overexpression of these genes exhibited resistance to tamoxifen, terbinafine, and clotrimazole (Figure 3), but not to micafungin (data not shown).

Since overexpression of any of these genes did not affect the sensitivity of micafungin, the mechanism of action of tamoxifen should be different from that of micafungin, which suppresses the cell wall integrity.

Identification of tamoxifen-resistant and tamoxifen-sensitive mutants from genome-wide library screen

To identify novel molecules related to antifungal action of tamoxifen, we screened a genome-wide library containing 3,004 haploid deletion strains to identify nonessential genes whose disruption increased and decreased the sensitivity to tamoxifen compared with wild-type cells. Then tamoxifen-sensitive mutants and tamoxifen-resistant mutants were confirmed with spot assay, in which the cells were spotted at serial dilution on agar plates with or without tamoxifen.

First, the tamoxifen-sensitive strains were classified by the level of sensitivity with the spot assay with tamoxifen at 10μg/ml. In this condition, tamoxifen did not affect the growth of wild-type cells, such that the cells grew at all the spots (Figure 4A). In this classification, severe sensitivity (+++) indicates that the cells did not

grow at any spots, moderate sensitivity (++) indicates that the cells grew at the second spot, but not at the third spot, if to the lesser extent than wild-type cells, and mild sensitivity (+) indicates that the cells grew at the third spot, but not at the fourth spot, if to the lesser extent than wild-type cells (Figure 4A). Through this procedure, we isolated 54 deletion strains that displayed tamoxifen sensitivity at various degrees. Among these mutants, 2 mutants exhibited severe sensitivity (+++), 34 mutants exhibited moderate sensitivity (++), and 18 mutants exhibited mild sensitivity (+) (Table I). These genes were classified into 10 functional categories including those relating to gene expression and nucleic acid metabolism (7 genes), membrane trafficking (7 genes), mitochondrial function (5 genes), lipid metabolism (4 genes), ubiquitination (3 genes), amino acid synthesis and metabolism (3 genes), signal transduction (3 genes), transmembrane transporter (2 genes), other known functions (17 genes), and other unknown functions (3 genes) (Table I). Among the 54 genes, the genes *pal*$^{+}$, *rud3*$^{+}$, *caf5*$^{+}$, *psh3*$^{+}$, *nht1*$^{+}$, *pzh1*$^{+}$, *ppr2*$^{+}$, *dad1*$^{+}$, *aim2*$^{+}$, and *SPAC4A8.10* are conserved only in fungi, whereas 41 genes are conserved from yeast to human. The genes identified here could influence the antifungal activity of tamoxifen in yeast. In our previous studies, we identified 109 terbinafine- and clotrimazole-sensitive mutants and 159 micafungin-sensitive mutants using the same *S. pombe* deletion collection (Fang et al. 2012; Zhou et al. 2013). Notably, among these sensitive mutants, only three mutants, namely *ent3*, *rrd1*, and *mug14* deletion mutants, showed hypersensitivity to both tamoxifen and the existing antifungal drugs. Therefore, the mutants of hypersensitivity to tamoxifen showed minimal overlap with those to other drugs, suggesting that the actions of tamoxifen and other drugs are differentially regulated.

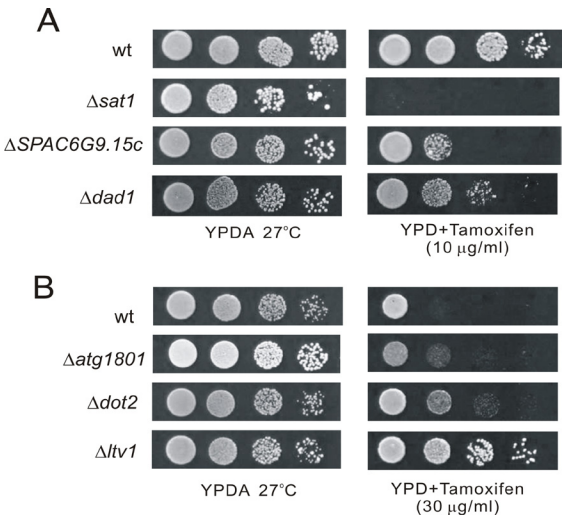

Figure 4. Representative examples of the *S. pombe* deletion mutants screened for altered sensitivity to tamoxifen. (A) The wild-type and sensitive mutant cells grown at log-phase were spotted onto each plate as indicated, and then incubated at 27°C for 5 days. (B) The wild-type and resistant mutant cells grown at log-phase were spotted onto each plate as indicated, and then incubated at 27°C for 5 days.

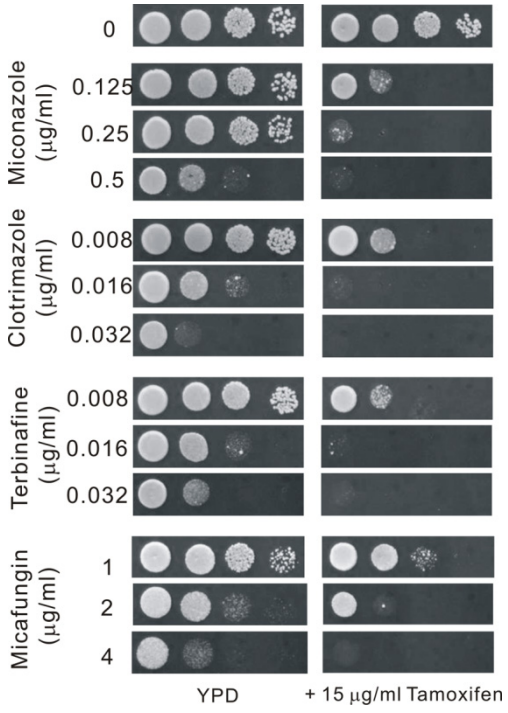

Figure 5. Tamoxifen augments antifungal actions of terbinafine and azoles. Wild-type cells were spotted onto each plate as indicated and incubated at 27°C for 4 days.

Second, the tamoxifen-resistant mutants were classified by the level of resistance with the spot assay with tamoxifen at 30μg/ml. In this condition, wild-type cells can grow only at the first spot. In this classification, strong resistance (+++) indicates that the cells grew at all the spots, moderate resistance (++) indicates that the cells grew at the third, but not the fourth, spot, and mild resistance (+) indicates that the cells grew at the second, but not the third, spot (Figure 4B and Table II). As shown in Table II, we identified 59 genes whose deletions confer resistance to tamoxifen. Among these mutants, 19 mutants exhibited strong resistance (+++), 8 mutants exhibited moderate resistance (++), and 32 mutants exhibited mild resistance (+) (Table II). The genes as

mentioned are categorized into the following groups including those relating to ribosome biogenesis and assembly (15 genes), membrane trafficking (9 genes), nucleic acid metabolism (8 genes), signal transduction (6 genes), histone acetylation and deacetylation (4 genes), meiosis (2 genes), transmembrane transporter (2 genes), other functions (10 genes), and unknown functions (3 genes). Among them, 49 genes (or 83 %) are conserved from yeast to human, suggesting that human cells may use similar mechanisms to provide resistance to tamoxifen as fission yeast. These results pave the way for identifying molecules involved in antifungal action of tamoxifen, which may be novel targets for pharmaceutical development of antifungal drugs.

This genetic screen for tamoxifen resistance has identified the mutations of many genes associated with ribosome biogenesis and assembly including 10 proteins of small (40s) ribosomal subunits, 2 proteins of large (60s) ribosomal subunits, and several other proteins implicated in the assembly of ribosomal subunits (Wilson and Doudna Cate 2012). Two other proteins found in this screen are bud23 and cpd1, which are involved in nuclear export of pre-40S subunits (White et al. 2008) and post-transcriptional tRNA maturation (Guy and Phizicky 2014), respectively. These findings suggest that ribosomal proteins are involved in the antifungal action of tamoxifen in fission yeast. Interestingly, it was reported that tamoxifen inhibits protein synthesis in reticulocyte lysates (Guille and Arnstein 1986). Therefore, it is plausible tamoxifen directly binds to ribosomes and suppresses their functions, although this possibility remains to be tested. Another large group of genes identified in the genetic screens for both tamoxifen sensitivity and resistance is related to membrane trafficking, especially those involved in vacuolar formation (e.g. ESCRT proteins). Although how these genes are related to the antifungal action of tamoxifen also remains unknown, one of these genes, Ubp3, was reported to be essential for the removal of 60S subunits of mature ribosomes called “ribophagy” upon cell starvation in *S. cerevisiae* (Kraft et al. 2008). However, this genetic screen has identified many genes implicated in other cellular functions, suggesting that various biological processes affect tamoxifen sensitivity directly and indirectly.

Tamoxifen augments antifungal actions of terbinafine and azoles.

Drug combination therapy is an emerging promising strategy to enhance the efficacy of current antifungal drugs and to overcome drug resistance. It has been reported that the combination of tamoxifen and fluconazole, another azole antifungal drug with different structure, is more effective against *Cryptococcus neoformans* and *Candida albicans* than fluconazole alone (Butts et al. 2014; Bulatova and Darwish 2008). To evaluate the effect of a combination of tamoxifen with other existing antifungal drugs, we compared the growth of wild-type cells on agar plates containing miconazole, clotrimazole, terbinafine, and micafungin, respectively, with or without tamoxifen. Simultaneous treatment with tamoxifen increased the sensitivity to miconazole, clotrimazole and terbinafine (Figure 5). Notably, the combination of tamoxifen and any one of the latter three drugs suppressed the cell growth at the doses not sufficient to suppress the cell growth if each of these drugs were used alone (Figure 5). Tamoxifen also increased the sensitivity to micafungin, though to the much lesser extent than to other existing antifungals. These further suggest that tamoxifen and existing antifungal drugs act on distinct biological processes, which may cooperatively regulate the cell growth and/or survival. Consistent with this notion, our genetic screen described above have implicated inhibition of protein synthesis as a potential mechanism underlying the antifungal action of tamoxifen, whereas azoles, terbinafine and micafungin exert antifungal actions through interfering with ergosterol biosynthesis or disturbing the cell wall integrity. According to our findings, tamoxifen may be used in combination therapy with existing antifungal drugs to enhance their therapeutic effects and to reduce their side effects.

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Table I. *S. pombe* genes identified in Tamoxifen- sensitive screen.

Systematic name	Common name	Gene description	TAM sensitive
Gene expression and nucleic acid metabolism			
SPAC4G9.06c	chz1	histone chaperone Chz1	++
SPAC10F6.08c	nht1[c]	HMG box protein	++
SPCC74.02c	ppn1	PNUTS homologue, mRNA cleavage and polyadenylation specificity factor complex associated protein	++
SPBC25D12.05	trm1	tRNA (guanine-N2-)-methyltransferase	+
SPAC16A10.05c	dad1[c]	DASH complex subunit Dad1	+
SPBC29A3.14c	trt1	telomerase reverse transcriptase 1 protein Trt1	+
SPAC19G12.02c	pms1	MutL family mismatch-repair protein Pms1	+
Membrane trafficking			
SPBC23E6.08	sat1	Golgi membrane exchange factor subunit Sat1	+++
SPAC9E9.14	vps24	ESCRT III complex subunit Vps24	++
SPCC794.11c	ent3	ENTH domain protein Ent3	++
SPAC823.05c	tlg2	SNARE Tlg2	++
SPBC409.20c	psh3[c]	ER chaperone SHR3 homologue Psh3	++
SPAC824.09c	age1[b]	GTPase activating protein	++
SPBC119.12	rud3[c]	Golgi matrix protein Rud3	+
Mitochondrial function			
SPBC1709.09	rrf1	mitochondrial translation termination factor Rrf1	+++
SPBC30D10.14	aim2[bc]	dienelactone hydrolase family	++
SPBC30B4.06c	NA	mitochondrial GIDA family tRNA uridine 5-carboxymethylaminomethyl modification enzyme	++
SPBC16A3.16	cwc27	mitochondrial inner membrane protein involved in cytochrome c oxidase assembly Pet191	++
SPBC18H10.11c	ppr2[c]	mitochondrial PPR repeat protein Ppr2	++
Lipid metabolism			
SPAC19G12.08	scs7	sphingosine hydroxylase Scs7	++
SPBC31F10.02	NA	acyl-CoA hydrolase	++
SPAC4A8.10	NA[c]	Lipase	+
SPBP4H10.11c	lcf2	long-chain-fatty-acid-CoA ligase	+
Ubiquitination			
SPAC343.18	rfp2	SUMO-targeted ubiquitin-protein ligase subunit Rfp2	++
SPBC887.04c	lub1	WD repeat protein Lub1	++
SPBC6B1.06c	ubp14/ucp2	ubiquitin C-terminal hydrolase Ubp14	+
Amino acid synthesis and metabolism			
SPBC3B8.03	lys9[b]	saccharopine dehydrogenase	++
SPBC29A3.02c	his7[c]	phosphoribosyl-AMP cyclohydrolase/phosphoribosyl-ATP pyrophosphohydrolase His7	+
SPBC4F6.11c	NA	asparagine synthase	+
Signal transduction			
SPAC57A7.08	pzh1[c]	serine/threonine protein phosphatase Pzh1	++
SPAC4F10.04	ypa1/rrd1	protein phosphatase type 2A regulator, PTPA family Ypa1	++
SPBC11B10.07c	ivn1	CDC50 domain protein, implicated in signal transduction	++
Transmembrane transporter			
SPBC609.04	caf5[c]	spermine family transmembrane transporter Caf5	++
SPAC22F8.04	pet1	phosphoenolpyruvate transporter Pet1	+
Other functions			
SPBC1604.08c	imp1	importin alpha	++
SPBC660.11	tcg1	single-stranded telomeric binding protein Tgc1	++
SPCC4G3.04c	coq5	C-methytransferase	++
SPAC12G12.03	cip2	RNA-binding protein Cip2	++

SPAC27E2.07	pvg2[d]	galactose residue biosynthesis protein Pvg2	++
SPBC21B10.03c	ath1	ataxin-2 homolog	++
SPAC1D4.01	tls1	splicing factor Tls1	++
SPBC29A10.16c	cyb5[b]	cytochrome b5	++
SPBC359.06	mug14	adducin (Has a role in meiosis)	++
SPCP1E11.04c	pal1[c]	membrane associated protein Pal1	++
SPAC227.01c	erd1	Erd1 homolog	++
SPCC417.07c	mto1[b]	MT organizer Mto1	+
SPBC16G5.02c	rbk1[b]	Ribokinase	+
SPAC2H10.02c	nas2[b]	26S proteasome regulatory particle assembly protein	+
SPAC823.16c	atg1802	WD repeat protein involved in autophagy Atg18b	+
SPBC36.07	elp1/iki3	elongator subunit Elp1	+
SPBC1734.12c	alg12	dolichyl pyrophosphate Man7GlcNAc2 alpha-1,3-glucosyltransferase Alg12	+
Unknown functions			
SPAC6G9.15c	NA[d]	Schizosaccharomyces specific protein	++
SPCC14G10.04	NA[d]	Schizosaccharomyces specific protein	++
SPBC106.07c	nat2[b]	N alpha-acetylation related protein Nat2	+

NOTE:
+++, severely sensitive; ++moderately sensitive; +, mildly sensitive.
[a] Gene description as indicated in the *S. pombe* database.
[b] means that the common name is taken from the orthology of *S. cerevisae*
[c] means that the gene is conserved in fungi only.
[d] means that the gene is identified in *S. pombe* only.
N/A indicates that common gene name is not applicable.

Table II. *S. pombe* genes identified in Tamoxifen- resistant screen.

Systematic name	Common name	Gene description	TAM resistant
Ribosome biogenesis and assembly			
SPAC3F10.17	ltv1[b]	ribosome biogenesis protein Ltv1	+++
SPAC1952.02	tma23	ribosome biogenesis protein	+++
SPAC25G10.06	rps2801	40S ribosomal protein S28	+++
SPAC26A3.07c	rpl1101	60S ribosomal protein L11	++
SPCC24B10.09	rps1702/rps17	40S ribosomal protein S17	+
SPAC328.10c	rps502/rps5-2	40S ribosomal protein S5	+
SPAC17G6.06	rps2401/rps24	40S ribosomal protein S24	+
SPCC962.04	rps1201/rps12	40S ribosomal protein S12	+
SPAC144.11	rps1102/rps11	40S ribosomal protein S11	+
SPAC664.04c	rps1602/rps16	40S ribosomal protein S16	+
SPAPJ698.02c	rps002	40S ribosomal protein S0B	+
SPAC23C11.02c	rps23	40S ribosomal protein S23	+
SPBC19G7.03c	rps3002	40S ribosomal protein S30	+
SPAC3A12.10	rpl2001	60S ribosomal protein L20a	+
SPBC16C6.03c	rsa1[b]	ribosome assembly protein	+
Membrane trafficking			
SPAC17A2.06c	vps8	WD repeat protein Vps8	+++
SPAC1142.07c	vps32/snf7	ESCRT III complex subunit Vps32	+++
SPAC4F8.01	did4/vps2	vacuolar sorting protein Did4	++
SPBC651.05c	dot2/vps22	ESCRT II complex subunit Dot2	++
SPAC19A8.05c	sst4/vps27	sorting receptor for ubiquitinated membrane proteins, ESCRT 0 complex subunit Sst4	+
SPAC17G6.05c	bro1	BRO1 domain protein Bro1	+
SPAC20G8.10c	atg6	beclin family protein, involved in autophagy	+
SPBC354.09c	tre1[b]	Tre1 family protein, involved in vacuolar protein degradation	+
SPBP8B7.21	ubp3	ubiquitin C-terminal hydrolase Ubp3	+

Nucleic acid metabolism			
SPBC28F2.02	mep33	mRNA export protein Mep33	+++
SPCC162.11c	urk1[b]	uridine kinase /uracil phosphoribosyltransferase	+++
SPAC23C4.06c	NA	methyltransferase, human Fam119 ortholog	+++
SPAC4G9.11c	cmb1	cytosine-mismatch binding protein 1	+++
SPAC30D11.07	nth1	DNA endonuclease III	++
SPAC26A3.06	bud23	rRNA (guanine) methyltransferase Bud23	+
SPAC17C9.05c	pmc3/med27	mediator complex subunit Pmc3/Med27	+
SPAC9G1.12	cpd1	tRNA (m1A) methyltransferase complex subunit Cpd1	+
Signal transduction			
SPCC31H12.05c	sds21	serine/threonine protein phosphatase Sds21	+++
SPBC30B4.01c	wsc1	transmembrane receptor Wsc1	+++
SPAC3G9.05	spa2[c]	GTPase activating protein Spa2	+++
SPBC609.02	ptn1	phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase	++
SPAC1B9.02c	sck1	serine/threonine protein kinase Sck1	+
SPCC162.10	ppk33	serine/threonine protein kinase Ppk33	+
Histone acetylation and deacetylation			
SPBC14C8.17c	spt8/sep9[c]	SAGA complex subunit Spt8	+++
SPAC57A10.14	sgf11	SAGA complex subunit Sgf11	+
SPBC31F10.13c	hip1/hir1	hira protein , histone chaperone Hip1	+
SPAC1952.05	gcn5/kat2	SAGA complex histone acetyltransferase catalytic subunit Gcn5	+
Meiosis			
SPACUNK4.12c	iph1/mug138	insulinase pombe homologue 1	+++
SPCC1840.03	sal3/pse1	karyopherin Sal3	+
Transmembrane transporter			
SPBPB10D8.07c	ssu1[bc]	membrane transporter	+++
SPCC1235.13	ght6/meu12	hexose transporter Ght6	++
Other functions			
SPBC609.03	iqw1/iqwd1	WD repeat protein, Iqw1	+++
SPCC1682.01	qcr9	ubiquinol-cytochrome-c reductase complex subunit 9	++
SPAP8A3.07c	aro3[bc]	phospho-2-dehydro-3-deoxyheptonate aldolase	++
SPBC2D10.06	rep1/rec16	MBF transcription factor activator Rep1	+
SPAC589.07c	atg1801/atg18	WD repeat protein involved in autophagy Atg18a	+
SPAC11D3.15	oxp1[b]	5-oxoprolinase (ATP-hydrolizing)	+
SPBC365.14c	uge1/gal10	UDP-glucose 4-epimerase Uge1	+
SPBC530.08	NA[c]	membrane-tethered transcription factor	+
SPBC24C6.04	put2[b]	delta-1-pyrroline-5-carboxylate dehydrogenase	+
SPBC1105.04c	cbp1/abp1	CENP-B homolog	+
Unknown functions			
SPAP27G11.16	NA[d]	Schizosaccharomyces pombe specific protein	+++
SPBC19G7.04	NA[c]	HMG box protein	+++
SPAC29B12.11c	NA	human WW domain binding protein-2 ortholog	+++

NOTE:

+++, strongly resistant; ++, moderately resistant; +, mildly resistant.

[a] Gene description as indicated in the *S. pombe* database.

[b] means that the common name is taken from the orthology of *S. cerevisae*

[c] means that the gene is conserved in fungi only.

[d] means that the gene is identified in *S. pombe* only.

N/A indicates that common gene name is not applicable.

REFERENCE

1. **Beach, D., Piper, M, and Nurse, P.** 1982. Construction of a *Schizosaccharomyces pombe* gene bank in a yeast bacterial shuttle vector and its use to isolate genes by complementation. *Mol Gen Genet* **187** (2):326-329.
2. **Bulatova, N. R., and Darwish, R. M.** 2008. Effect of chemosensitizers on minimum inhibitory concentrations of fluconazole in *Candida albicans*. *Med Princ Pract* **17** (2):117-121.
3. **Butts, A., Koselny, K., Chabrier-Rosello, Y., Semighini, C. P., Brown, J. C., Wang, X., Annadurai, S., DiDone, L., Tabroff, J., Childers, W. E., Jr., Abou-Gharbia, M., Wellington, M., Cardenas, M. E., Madhani, H. D., Heitman, J., and Krysan, D. J.** 2014. Estrogen receptor antagonists are anti-cryptococcal agents that directly bind EF hand proteins and synergize with fluconazole in vivo. *MBio* **5** (1):e00765-00713.
4. **Dolan, K., Montgomery, S., Buchheit, B., Didone, L., Wellington, M., and Krysan D. J.** 2009. Antifungal activity of tamoxifen: in vitro and in vivo activities and mechanistic characterization. *Antimicrob Agents Chemother* **53** (8):3337-3346.
5. **Fang, Y., Hu, L., Zhou, X., Jaiseng, W., Zhang, B., Takami, T., and Kuno, T.** 2012. A genomewide screen in *Schizosaccharomyces pombe* for genes affecting the sensitivity of antifungal drugs that target ergosterol biosynthesis. *Antimicrob Agents Chemother* **56** (4):1949-1959.
6. **Fang, Y., Jaiseng, W., Ma, Y., Hu, L., Yamazaki, S., Zhang, X., Hayafuji, T., Shi, L., and Kuno, T.** 2014. E3 ubiquitin ligase Pub1 is implicated in endocytosis of a GPI-anchored protein Ecm33 in fission yeast. *PLoS One* **9** (1):e85238.
7. **Georgiadou, S. P., and Kontoyiannis, D. P.** 2012. The impact of azole resistance on aspergillosis guidelines. *Ann N Y Acad Sci* **1272**:15-22.
8. **Guille, M. J., and Arnstein, H. R.** 1986. The anti-oestrogen drug tamoxifen is an elongation inhibitor of eukaryotic protein biosynthesis. *FEBS Lett* **207** (1):121-126.
9. **Guy, M. P., and Phizicky, E. M.** 2014. Two-subunit enzymes involved in eukaryotic post-transcriptional tRNA modification. *RNA Biol* **11** (12):1608-1618.
10. **Kraft, C., Deplazes, A., Sohrmann, M., and Peter, M.** 2008. Mature ribosomes are selectively degraded upon starvation by an autophagy pathway requiring the Ubp3p/Bre5p ubiquitin protease. *Nat Cell Biol* **10** (5):602-610.
11. **Leber, R., Fuchsbichler, S., Klobucnikova, V., Schweighofer, N., Pitters, E., Wohlfarter, K., Lederer, M., Landl, K., Ruckenstuhl, C., Hapala, I., and Turnowsky, F.** 2003. Molecular mechanism of terbinafine resistance in *Saccharomyces cerevisiae*. *Antimicrob Agents Chemother* **47** (12):3890-3900.
12. **Moreno, S., Klar, A., and Nurse, P.** 1991. Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. *Methods Enzymol* **194**:795-823.
13. **Paul, S., and Moye-Rowley, W. S.** 2014. Multidrug resistance in fungi: regulation of transporter-encoding gene expression. *Front Physiol* **5**:143.
14. **Shingu-Vazquez, M., and Traven, A.** 2011. Mitochondria and fungal pathogenesis: drug tolerance, virulence, and potential for antifungal therapy. *Eukaryot Cell* **10** (11):1376-1383.
15. **Sio, S. O., Suehiro, T., Sugiura, R., Takeuchi, M., Mukai, H., and Kuno, T.** 2005. The role of the regulatory subunit of fission yeast calcineurin for in vivo activity and its relevance to FK506 sensitivity. *J Biol Chem* **280** (13):12231-12238.
16. **White, J., Li, Z., Sardana, R., Bujnicki, J. M., Marcotte, E. M., and Johnson, A. W.** 2008. Bud23 methylates G1575 of 18S rRNA and is required for efficient nuclear export of pre-40S subunits. *Mol Cell Biol* **28** (10):3151-3161.
17. **Wilson, D. N., and Doudna Cate, J. H..** 2012. The structure and function of the eukaryotic ribosome. *Cold Spring Harb Perspect Biol* **4** (5).
18. **Wiseman, H., Cannon, M., Arnstein, H. R., and Halliwell, B.** 1990. Mechanism of inhibition of lipid peroxidation by tamoxifen and 4-hydroxytamoxifen introduced into liposomes. Similarity to cholesterol and ergosterol. *FEBS Lett* **274** (1-2):107-110.
19. **Zhou, X., Ma, Y., Fang, Y., gerile, W., Jaiseng, W., Yamada, Y., and Kuno, T.** 2013. A genome-wide screening of potential target genes to enhance the antifungal activity of micafungin in *Schizosaccharomyces pombe*. *PLoS One* **8** (5):e65904.