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Antifungal drug resistance in *Candida glabrata*: role of cellular signaling and gene regulatory networks

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Abstract

Nakaseomyces glabratus (*Candida glabrata*) is an opportunistic human fungal pathogen of high priority that shares an ancestor with the non-pathogenic yeast *Saccharomyces cerevisiae*. *Candida glabrata* causes infections of the mucosal surfaces as well as fatal deep-seated tissue infections in immunocompromised individuals. The co-resistance to two commonly used antifungal drug classes, azoles and echinocandins, is increasingly being reported in clinical isolates of *C. glabrata* all over the world, which poses a significant threat to the successful treatment of *C. glabrata* infections. Acquisition of drug resistance in hospital settings is a complex multifaceted process that is governed by various factors including antimicrobial stewardship. This review summarizes both the key clinical antifungal resistance mechanisms, and the contribution of cellular stress signaling pathways to drug resistance acquisition in *C. glabrata*. Specifically, we discuss the emerging concepts regarding the role of mitochondrial functions, epigenetic modifications, and the host niche in the development of drug resistance. Lastly, we outline some potential areas for future research that will enable us to better understand the drug evolutionary dynamics of this important human fungal pathogen.

Keywords: antimicrobial resistance; human pathogenic fungi; azole and echinocandin drugs; calcineurin signaling and Hsp90; mitochondria; genetic and epigenetic modifications

Introduction

Invasive fungal infections are associated with high morbidity and mortality in hospitals worldwide, with ∼2.5 million people dying of these infections every year (Bongomin et al. 2017, Denning 2024). *Candida* species are a leading cause of opportunistic invasive mycoses, with *Candida albicans* and *C. auris* recently being categorized as fungal pathogens of critical priority by the World Health Organization [World Health Organization (WHO) 2022; Denning 2024]. *Candida glabrata*, recently renamed as *Nakaseomyces glabratus*, belongs to the class of high-priority fungal pathogens [Takashima and Sugita 2022, World Health Organization (WHO) 2022], and is a common causative agent of bloodstream, oral cavity, vaginal and urinary tract, and deep-seated infections including intra-abdominal abscess, in patients with compromised immune system (Fidel et al. 1999, Vazquez and Sobel 2002, Li et al. 2007, Achkar and Fries 2010, Lamoth et al. 2018, Gajdács et al. 2019, Soriano et al. 2023).

Candida glabrata is the second most frequently isolated *Candida* species in clinical settings in many parts of the world including the United States of America and northwestern Europe, and contributes up to 35% of total *Candida* bloodstream infections (Astvad et al. 2018, Lamoth et al. 2018, Pfaller et al. 2019, Soriano et al. 2023). *Candida glabrata* infections are associated with some of the worst rates of mortality amongst *Candida* species, typically between 25% and35% (Meyahnwi et al. 2022, Salmanton-García et al. 2024) and soaring up beyond 50%, as found in some studies (Gupta et al. 2015, Won et al. 2021).

Over the last two decades, *C. glabrata* has been exhibiting an ever-increasing rate of resistance towards azole and echinocandin drugs under clinical settings (Perlin et al. 2017, Astvad et al. 2018, Lamoth et al. 2018, Pfaller et al. 2019, Soriano et al. 2023). The current antifungal arsenal is mainly comprised of three antifungal classes, azoles, echinocandins, and polyenes, and the growing co-resistance of *C. glabrata* to azoles and echinocandins is a major medical concern (Perlin et al. 2017, Lamoth et al. 2018, Pfaller et al. 2019, Rasheed et al. 2020).

The current review provides an overview of prevalent antifungal resistance mechanisms in *C. glabrata*, with a particular emphasis on the contributions of cellular signaling pathways to drug resistance acquisition. Additionally, we summarize how epigenetic modifications may govern the cellular response to antifungal stress and aid in the development of drug resistance. Finally, while highlighting the gaps in our current knowledge, we discuss the importance of tracking emerging drug resistance trends and implementation of focussed all-encompassing strategies to better control multidrug-resistant *C. glabrata* infections.

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Nature and pathogenicity of *C. glabrata*

Candida glabrata is a haploid yeast, which is a part of the whole-genome duplication clade *Candida* species (Galocha et al. 2019, Rasheed et al. 2020). *Candida glabrata* and the budding yeast *Saccharomyces cerevisiae* are descendants of a common recent ancestor and display genome synteny (Dujon et al. 2004, Rasheed et al. 2020). *Candida glabrata* divides by budding, forms pseudohyphae under a very limited set of conditions, and largely lacks hyphae formation and true mating (Galocha et al. 2019, Rasheed et al. 2020). Of note, a 6-month continuous exposure of *C. glabrata* to macrophages led to a genetically stable, pseudohyphae-like growth morphology, which was attributed to a point mutation in the *CHS2* gene that codes for chitin synthase (Brunke et al. 2014). The genome of *C. glabrata* encodes 5272 ORFs (Open Reading Frames), including multigene families of adhesin proteins, drug transporters, hexose transporters, mannosyl transferases, and aspartyl proteases (*http://www.Candidagenome.org/cache/C_glabrata_CBS138_genomeSnapshot.html*). The characteristic features of *C. glabrata*, that promote its pathogenicity, are manyfold including a capability to stick to a wide variety of surfaces and host tissues, replicate in macrophages, impede phagolysosome maturation, suppress the host innate immune response, and display genome plasticity and proclivity to acquire a great degree of resistance towards oxidative, thermal and antifungal drug stresses (Seider et al. 2011, Galocha et al. 2019, Rasheed et al. 2020).

Antifungal drugs

Azoles, polyenes, and echinocandins are three major classes of antifungal drugs that are used to treat *C. glabrata* infections. Their modes of action are illustrated in Fig. 1, and briefly discussed below.

Azoles

Azoles belong to a family of five-membered heterocyclic compounds in which one of the constituent atoms needs to be nitrogen, along with at least one non-carbon atom *viz*., oxygen, sulfur, or nitrogen, in their ring structures (Robbins et al. 2016). The azole family of drugs includes two classes of antifungal compounds: imidazoles with two nitrogen atoms in the azole ring, such as ketoconazole, miconazole, etc., and triazoles with three nitrogen atoms in the azole ring such as fluconazole, voriconazole, etc. (Pappas et al. 2016, Robbins et al. 2016). Fluconazole is a widely used antifungal drug largely due to its cost-effectiveness, oral route of administration, efficaciousness, and low toxicity (Pappas et al. 2016, Rasheed et al. 2020). Azoles interfere with the biosynthesis of the predominant cell membrane sterol, ergosterol, by binding non-competitively to, and inhibiting cytochrome p450 enzyme lanosterol 14α-demethylase, encoded by the *CgERG11* gene in *C. glabrata* (Robbins et al. 2016, Perlin et al. 2017, Rasheed et al. 2020). Azole drug treatment leads to intracellular as well as cell membrane accumulation of toxic 14α-methylated sterols, while also causing a deficiency of ergosterol in the cell membrane, and hampers the growth of *C. glabrata* cells (Robbins et al. 2016, Perlin et al. 2017, Rasheed et al. 2020).

Polyenes

The chief target of the polyene class of fungicidal drugs, which consists of cyclic lactone rings of 25–38 polyunsaturated carbon atoms, and multiple hydroxyl groups, that impart hydrophilicity, is the plasma membrane ergosterol (Robbins et al. 2016). Two prominent examples of polyene antifungals used in clinical settings are amphotericin B and nystatin (Pappas et al. 2016, Rasheed et al. 2020). Ergosterol-binding of polyenes in the cell membrane results in altered membrane permeability and architecture leading to pore formation, extraction of sterols from the plasma membrane, osmotic cell lysis, and cell death (Rasheed et al. 2020, Brüggemann et al. 2022, Maertens et al. 2022). Polyenes are not a preferred choice for antifungal therapy, as their usage is often associated with nephrotoxicity (Pappas et al. 2016, Robbins et al. 2016, Brüggemann et al. 2022). However, liposomal formulations of amphotericin B have decreased toxicity and are used as a last-resort drug for treatment of fungal infections (Arendrup and Patterson 2017, Brüggemann et al. 2022, Maertens et al. 2022).

Echinocandins

The main target of the echinocandin class of fungicidal drugs is the fungal cell wall (Gow et al. 2017). Echinocandins, which are comprised of cyclic non-ribosomal hexapeptides with N-linked acyl lipid side chains, do not cause cytotoxicity in the host (Robbins et al. 2016, Gow et al. 2017). Caspofungin, micafungin, and anidulafungin are common clinically used echinocandin antifungal drugs (Pappas et al. 2016, Rasheed et al. 2020). The cell wall in *C. glabrata* is a highly ordered dynamic structure consisting of chitin, β,1–3 glucan, β,1–6 glucan, and mannoproteins (de Groot et al. 2008). Echinocandins non-competitively target a key cell wall biogenesis, multi-subunit enzyme, β,1–3 glucan synthase (Douglas 2001, Perlin et al. 2017). The catalytic subunit of β,1–3 glucan synthase is encoded by three genes in *C. glabrata*, *CgFKS1*, *CgFKS2*, and *CgFKS3* (Garcia-Effron et al. 2009, Robbins et al. 2016). Echinocandin treatment results in impaired cell wall synthesis, due to reduced β,1–3 glucan polymer formation, and a weakened cell wall (Robbins et al. 2016, Perlin et al. 2017, Rasheed et al. 2020). Due to intrinsic low azole susceptibility of *C. glabrata*, echinocandins are the frontline therapy to manage *C. glabrata* infections (Pappas et al. 2016, Arendrup and Patterson 2017, Perlin et al. 2017, Rasheed et al. 2020, Droney et al. 2025).

Antifungal drug resistance trends in *C. glabrata*

Drug resistance is a dynamic trait, constantly evolving to impart evolutionary fitness benefits to pathogenic microbes in the context of survival and invasion of their hosts. On an average, 6.0%–11.0% and 2.0%–4.0% clinical isolates of *C. glabrata* have been found to exhibit fluconazole and echinocandin resistance, respectively, with *C. glabrata* also being intrinsically less susceptible to fluconazole (Pham et al. 2014, Astvad et al. 2018, Lamoth et al. 2018, Pfaller et al. 2019, Castanheira et al. 2022). Concernedly, echinocandin co-resistance has been reported in ∼9% of fluconazole-resistant *C. glabrata* blood isolates (Lamoth et al. 2018, Pfaller et al. 2019). Further, 1.5% of *C. glabrata* isolates displayed acquired resistance to amphotericin B in a study spanning a 12-year surveillance program (Astvad et al. 2018), highlighting the growing threat of multidrug resistance in *C. glabrata*. Consistent with this, fluconazole resistant-*C. glabrata* bloodstream infections were found to result in a 1.8-fold higher 90-day mortality rate in a multi-centric study conducted in South Korea over a period of 11 years from 2008 to 2018 (Won et al. 2021). Further, survival and persistence of *C. glabrata* in the presence of antifungal chemicals is often promoted by the formation of a biofilm, that is composed of densely packed yeast cells, and aids in resisting antifungal stress via several mechanisms including poor drug penetration, and transcriptional and metabolic reprogramming (Rodrigues et al. 2017). However, more studies are warranted to link biofilm formation with drug resistance acquisition in *C. glabrata* under clinical settings.

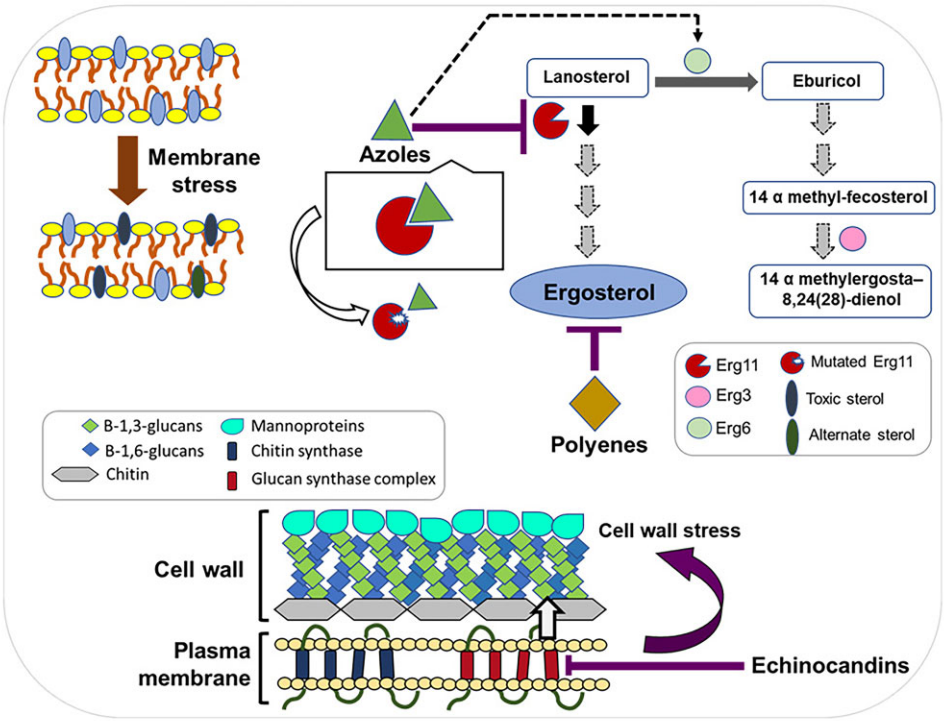

Figure 1. Pictorial representation of mechanisms of action of three common classes of antifungal drugs, azoles, echinocandins, and polyenes. The fungistatic azole antifungals inhibit ergosterol biosynthesis by binding to lanosterol 14-α-demethylase (Erg11) enzyme, which causes ergosterol deficiency, membrane stress, and methylated toxic sterol production via Erg3 and Erg6 enzyme actions. The fungicidal echinocandin drugs impair cell wall integrity by binding to β-1,3-glucan synthase complex subunits, Fks1 and Fks2, which leads to diminished β-glucan in the cell wall and a weakened cell wall. The fungicidal polyene drugs function by binding to ergosterol and scavenging ergosterol from the plasma membrane, which results in pore formation and membrane stress.

Antifungal drug resistance mechanisms in *C. glabrata*

The prevailing mechanisms of antifungal resistance in *C. glabrata* primarily involve efflux of the azole drugs and modifications of the echinocandin drug target (Perlin et al. 2017, Rasheed et al. 2020, Lee et al. 2021). At the molecular level, overexpression of the ATP (adenosine triphosphate)-binding cassette family of multidrug transporters, *viz.*, CgCdr1, CgSnq2, and CgCdr2, has been associated with azole drug resistance in clinical isolates of *C. glabrata* (Vermitsky and Edlind 2004, Sanguinetti et al. 2005, Ferrari et al. 2009, Arendrup and Patterson 2017, Perlin et al. 2017, Rasheed et al. 2020, Lee et al. 2021, Castanheira et al. 2022). This multidrug efflux pump overexpression is largely attributed to gain-of-function mutations in *Cg PDR1* gene whose protein product belongs to the zinc-finger family of transcriptional regulators (Ferrari et al. 2009, Rasheed et al. 2020). CgPdr1 contains four distinct domains, a DNA binding domain; a transcriptional inhibitory domain, a middle homology domain and a transactivation domain, and mutations have predominantly been mapped to the latter three domains, which elevate the transcriptional activation activity of CgPdr1 and result in *CgPDR1* and drug transporter gene overexpression (Ferrari et al. 2009, Pais et al. 2022, Salazar et al. 2022). A list of prevalent gain-of-function mutations in *CgPDR1*, identified in azole-resistant clinical isolates of *C. glabrata*, is presented in Table 1.

Notably, overexpression of, or mutations in the *CgERG11* gene, are not a common mechanism of azole-resistance in clinical isolates of *C. glabrata* (Sanguinetti et al. 2005, Lee et al. 2021, Castanheira et al. 2022). However, a recent genome-wide study has identified a missense mutation in *UPC2A* (codes for a transcription factor that regulates the expression of ergosterol biosynthesis genes) and a nonsense mutation in *CgERG3* (codes for C-5 sterol desaturase, an enzyme of the ergosterol biosynthesis pathway) genes in azole-resistant clinical isolates (Pais et al. 2022), thereby raising the possibility of these mutations contributing to azole resistance under hospital settings. Importantly, nonsynonymous mutations in *CgRAD6* (codes for an E2 ubiquitin-conjugating enzyme) and *CgANB1* (codes for a translation elongation factor eIF-5A) genes were found to be solely present in azole-resistant clinical isolates, as compared to azole-susceptible isolates (Pais et al. 2022), highlighting the potential multifaceted clinical azole resistance mechanisms.

Echinocandin resistance in *C. glabrata* under clinical settings stems mostly from amino acid substitutions in two hotspot regions (HS1 and HS2) of two functionally redundant enzymes, CgFks1 and CgFks2 (catalytic subunits of the β-1,3,-D glucan synthase complex), that result in decreased drug-enzyme binding, diminished enzyme activity or varied expression, and associated with clinical failure of the echinocandin therapy (Garcia-Effron et al. 2009, Pham et al. 2014, Arendrup and Patterson 2017, Perlin et al. 2017, Astvad et al. 2018, Pfaller et al. 2019, Lee et al. 2021, Castanheira et al. 2022, Pais et al. 2022). Notably, a substantially higher number of mutations have been reported in the *CgFKS2* gene, compared to the *CgFKS1* gene (Perlin et al. 2017, Lee et al. 2021). The prevalent mutations in *CgFKS1* and *CgFKS2* genes, which are associated with reduced echinocandin susceptibility in clinical isolates, are summarized in Table 2.

Polyene resistance is very infrequent in clinical isolates of *C. glabrata*, and mutations at threonine-121 residue in CgErg2 and a

Table 1. A list of prevalent *CgPDR1* mutations[#] identified in clinical isolates of *C. glabrata*.

Mutations	Reference
S76P	(Salazar et al. 2022)
V91I	(Salazar et al. 2022)
L98S	(Salazar et al. 2022)
L139I	(Usher and Haynes 2019)
T143P	(Carreté et al. 2019)
D243N	(Arastehfar et al. 2019)
E259G	(Hou et al. 2018)
D261G	(Ferrari et al. 2009)
K274Q	(Salazar et al. 2018)
I280F	(Ferrari et al. 2009)
R293I	(Usher and Haynes 2019)
R293G	(Hou et al. 2018)
W297S	(Tsai et al. 2006)
S316I	(Usher and Haynes 2019)
L328F	(Ferrari et al. 2009)
Y336H	(Hou et al. 2018)
S343F	(Carreté et al. 2019)
L344S	(Pais et al. 2022)
G346D	(Ni et al. 2018)
L347F	(Pais et al. 2022)
C350R	(Pais et al. 2022)
Y372N	(Ferrari et al. 2009)
Y372C	(Cavalheiro et al. 2018)
R376W	(Vale-Silva et al. 2013)
R376Q	(Carreté et al. 2019)
R376G	(Usher and Haynes 2019)
I378T	(Hou et al. 2018)
G389V	(Hou et al. 2018)
I392M	(Salazar et al. 2022)
E555K	(Salazar et al. 2022)
G558C	(Salazar et al. 2022)
F575L	(Tsai et al. 2006)
H576Y	(Berila et al. 2009)
Y584C	(Usher and Haynes 2019)
T588A	(Carreté et al. 2019)
R592S	(Usher and Haynes 2019)
R592G	(Usher and Haynes 2019)
F612V	(Pais et al. 2022)
L669F	(Hou et al. 2018)
N691D	(Usher and Haynes 2019)
L732S	(Hou et al. 2018)
N768D	(Tantivitayakul et al. 2019)
V785D	(Usher and Haynes 2019)
I803T	(Salazar et al. 2022)
F817S	(Usher and Haynes 2019)
E818G	(Hou et al. 2018)
E818K	(Tantivitayakul et al. 2019)
P822L	(Ferrari et al. 2009)
F853Q	(Usher and Haynes 2019)
F859L	(Usher and Haynes 2019)
D876Y	(Usher and Haynes 2019)
P927L	(Vermitsky and Edlind 2004)
P927S	(Ni et al. 2018)
S942F	(Hou et al. 2018)
G943S	(Usher and Haynes 2019)
L946S	(Usher and Haynes 2019)
F948I	(Usher and Haynes 2019)
D1082G	(Ferrari et al. 2009)
E1083Q	(Ferrari et al. 2009)
D1089Y	(Usher and Haynes 2019)
L1093P	(Usher and Haynes 2019)
G1099D	(Ni et al. 2018)

[#]CgPdr1 contains four domains: DNA binding domain (25–69 aa), Inhibitory domain (312–88 aa), middle homology region domain (559–633 aa), and C-terminal transactivation domain (800–1107 aa) (Pais et al. 2022).

missense mutation (phenylalanine replacing cysteine) in CgErg6 enzymes of the ergosterol biosynthesis pathway have been reported in amphotericin-B-resistant clinical isolates of *C. glabrata* (Vandeputte et al. 2007, 2008, Hull et al. 2012, Perlin et al. 2017, Pais et al. 2022).

Stress response pathways in antifungal drug resistance

The canonical drug resistance acquisition mechanisms in different *Candida* species appear to be largely conserved, thereby raising the question of why *C. glabrata* is so efficient in developing resistance against mechanistically distinct antifungal drugs in hospitals worldwide. In this context, it is worth noting that *C. glabrata* is also unique in exhibiting a high level of resistance against hydrogen-peroxide-induced oxidative stress (Galocha et al. 2019). Robust cellular stress response signaling pathways are likely to confer an advantage during the initial adaptation phase while fungi encounter various stresses. Specifically, an increase in the cell wall chitin content upon caspofungin exposure, and activation of protein kinase C (PKC)-mediated cell wall integrity pathway, calcineurin signaling, endoplasmic reticulum (ER) stress-mediated unfolded protein response (UPR), upon azole as well as echinocandin exposure, have been reported under *in vitro* conditions that may aid *C. glabrata* resist antifungal drug stress, as detailed below.

Calcineurin signaling pathway in antifungal drug tolerance

Calcineurin is a highly conserved, stress-responsive, Ca^{2+}/calmodulin-requiring serine/threonine protein phosphatase, that is a heterodimer of a catalytic and a regulatory subunit, encoded by *CgCNA1* and *CgCNB1* genes, respectively (Miyazaki et al. 2010b, Yu et al. 2015). The role of calcineurin in antifungal tolerance in *C. glabrata* was first uncovered when calcineurin signaling inhibitors, *viz.*, cyclosporine A and FK-506, imparted a fungicidal activity to the fungistatic drug fluconazole, and FK506 led to a reversal of *CgFKS2* mutation-associated echinocandin resistance (Onyewu et al. 2003, Kaur et al. 2004, Katiyar et al. 2012). Ca^{2+}-bound calmodulin-mediated activation of calcineurin is known to result in the dephosphorylation of its major substrate CgCrz1 (Calcineurin responsive zinc finger 1), which in turn translocates to the nucleus and drives the expression of stress-responsive genes (Yu et al. 2015). It has been reported that while *CgCRZ1* deletion, had no appreciable effect on fluconazole susceptibility, *CgCNA1* and *CgCNB1* deletion led to elevated susceptibility towards fluconazole (Miyazaki et al. 2010b, Schwarzmüller et al. 2014). However, all three mutants *viz.*, *Cgcrz1*Δ, *Cgcna1*Δ, and *Cgcnb1*Δ, were found to be more sensitive to echinocandins, compared to their parental (wild-type) strains, thereby highlighting the role of calcineurin signaling in regulating antifungal tolerance *in vitro* (Miyazaki et al. 2010b, Schwarzmüller et al. 2014, Rosenwald et al. 2016). Further, *CgCRZ1* deletion in two echinocandin-resistant clinical isolates, that carried a deletion in the *CgFKS2* gene (*F659del*), rendered cells susceptible to echinocandins (Ceballos-Garzon et al. 2022). However, studies with additional clinical isolates containing other prevalent echinocandin resistance-conferring mutations are required to demonstrate the importance of CgCrz1 in echinocandin resistance acquisition. Intriguingly, increased chitin accumulation was observed upon ER stress caused by *CgCNE1* (codes for the ER chaperone calnexin/calreticulin) loss, with *Cgcne1*Δ mutant also exhibiting increased sensitivity to

Table 2. A list of prevalent *CgFKS1* and *CgFKS2* mutations identified in clinical isolates of *C. glabrata*.

Protein	Hotspot region carrying mutation	Mutation	Reduced susceptibility to echinocandins	Reference
CgFks1	HS1	F625S	CSP, ANF, and MCF[#]	(Alexander et al. 2013), (Dudiuk et al. 2014), and (Pham et al. 2014)
	HS1	S629P	CSP, ANF, and MCF	(Katiyar et al. 2012), (Beyda et al. 2014), and (Biswas et al. 2017)
	HS1	D632G	ANF and CSP	(Dudiuk et al. 2014)
	HS1	D632E	CSP, ANF, and MCF	(Dudiuk et al. 2014)
	HS1	D632H	CSP	(Memon et al. 2023)
	HS1	D632Y	ANF	(Garcia-Effron et al. 2009)
	HS1	R631G	MCF	(Zimbeck et al. 2010)
	HS1	D632V	CSP, ANF, and MCF	(Hou et al. 2019)
	Outside HS1	I634V	MCF	(Pham et al. 2014)
	HS1	P633T	CSP	(Katiyar et al. 2012)
	HS1	F625C	CSP and ANF	(Katiyar et al. 2012)
	Outside HS1	W508STOP	CSP, ANF, and MCF	(Hou et al. 2019)
	HS1	F625del	CSP	(Katiyar et al. 2012)
CgFks2	HS1	F659V	CSP, ANF, and MCF	(Thompson et al. 2008)
CgFks2				
	HS1	R665G	MCF	(Zimbeck et al. 2010)
	HS1	F659Y	ANF and CSP	(Zimbeck et al. 2010) and (Pham et al. 2014)
	HS1	F659S	CSP, ANF, and MCF	(Castanheira et al. 2014a)
	HS1	F659del	CSP, ANF, and MCF	(Costa-de-Oliveira et al. 2011) and (Sasso et al. 2017)
	HS1	S663Y	ANF and MCF	(Bordallo-Cardona et al. 2019)
	HS1	S663P	CSP, ANF, and MCF	(Castanheira et al. 2010), (Garcia-Effron et al. 2010), (Naicker et al. 2016), and (Biswas et al. 2017)
	HS1	D666G	CSP and ANF	(Arendrup and Perlin 2014)
	HS1	D666V	ANF, CSP, and MCF	(Sig et al. 2021)
	HS1	D666N	ANF	(Bordallo-Cardona et al. 2019)
	HS1	D666E	CSP and MCF	(Pfaller et al. 2017)
	HS1	P667T	CSP and ANF	(Alexander et al. 2013)
	HS1	S663F	CSP and ANF	(Zimbeck et al. 2010)
	HS1	L664R	ANF and CSP	(Castanheira et al. 2014b)
	HS2	W1375L	CSP	(Garcia-Effron et al. 2009)
	HS2	R1377STOP	ANF and CSP	(Garcia-Effron et al. 2009)
	HS2	R1377K	ANF and MCF	(Naicker et al. 2016)
	HS1	F658del	ANF, CSP, and MCF	(Pham et al. 2014) and (Bordallo-Cardona et al. 2019)
	HS1	P667H	ANF and CSP	(Zimbeck et al. 2010)
	HS1	L662W	ANF and MCF	(Szymankiewicz et al. 2021) and (Rivero-Menendez et al. 2019)
	HS1	D666H	ANF	(Rivero-Menendez et al. 2019)
	Outside HS1	E655K	CSP, ANF, and MCF	(Xiao et al. 2018)
	Outside HS1	W715L	MCF and ANF	(Bordallo-Cardona et al. 2019)
	Outside HS1	E655A	ANF	(Bordallo-Cardona et al. 2018)
CgFks1 & CgFks2	HS1 of CgFks1 + HS2 of CgFks2	D632Y + R1377STOP	CSP, ANF, and MCF	(Pham et al. 2014)

[#]ANF: Anidulafungin, CSP: Caspofungin, and MCF: Micafungin.

micafungin and azole drugs (Tanaka et al. 2018). Importantly, this increase in chitin content was negatively regulated by the calcineurin pathway, thereby pointing towards a cross-talk between calcineurin signaling and ER stress survival mechanisms (Tanaka et al. 2018). Of note, unlike other *Candida* pathogens, caspofungin treatment in *C. glabrata* neither increased the cell surface exposure of β,1–3 glucan and chitin nor altered its ingestion by murine macrophages (Walker and Munro 2020), pointing towards its distinct cell wall stress response and host interaction mechanisms.

Further, *C. glabrata* calcineurin signaling mutants have been reported to uniquely exhibit thermal stress susceptibility (Chen et al. 2012, Yu et al. 2015). Notably, a recent study has identified an essential requirement for calcineurin in azole resistance that was imparted upon expression of the two hyperactive alleles of CgPdr1 (Vu et al. 2023). Further, phosphatase activity of the calcineurin was found to be required for the transcriptional activation of *CgPDR1* and *CgCDR1* genes, in response to fluconazole exposure (Vu et al. 2023). This result underscores the role of calcineurin as an upstream regulator of the clinically prevalent CgPdr1-dependent azole resistance mechanisms. Of note, while a hyperactive calcineurin resulted in reduced caspofungin susceptibility, the simultaneous treatment with FK506 and caspofungin led to substantially attenuated cell growth (Vu et al. 2023). Furthermore, calcineurin signaling was activated upon treatment with a new experimental drug, manogepix, that im-

pedes the maturation of glycosylphosphatidylinositol-anchored proteins by targeting the Gwt1 enzyme (Pavesic et al. 2024). Collectively, these results suggest that calcineurin signaling may be pivotal to adaptation to, and survival of antifungal drug stress, due to its role in driving various cellular stress response pathways. In this context, it is worth noting that a major heat shock protein CgHsp90 (Heat Shock Protein 90) governs echinocandin tolerance in *C. glabrata* via its client protein, calcineurin, with Hsp90 inhibitors (geldanamycin and radicicol) diminishing the resistance of echinocandin-resistant clinical isolates (Singh-Babak et al. 2012). Moreover, fungal Hsp90 has been demonstrated to be an integral part of a tripartite antifungal resistance-regulatory system of Mkc1 [mitogen-activated protein kinase of PKC signaling pathway], calcineurin and Hsp90, with Hsp90 positively regulating the stability of Mkc1 (Lafayette et al. 2010). Of note, it has also been reported that PKC inhibitors abate fluconazole resistance in azole-resistant *C. albicans* isolates, and act synergistically with fluconazole and micafungin (Lafayette et al. 2010). Further, abrogation of calcineurin or CgHsp90 functions led to an impairment in caspofungin-induced activation of *CgFKS2* gene expression (Singh-Babak et al. 2012), thereby shedding light on the molecular basis of CgHsp90-calcineurin axis-dependent echinocandin tolerance regulation in *C. glabrata*. Of note, structural analysis of the nucleotide-binding domain of *C. albicans* Hsp90, followed by compound synthesis, identified a new fungal-selective Hsp90 inhibitor, which also exhibited less toxicity towards mammalian cells (Whitesell et al. 2019). This study has paved the path for developing fungal-specific Hsp90 inhibitors that do not compromise host Hsp90 functions. Importantly, a recent study has implicated CgHsp90 and calcineurin in governing both tolerance and resistance towards fluconazole (Zheng et al. 2024), highlighting the role that CgHsp90-calcineurin nexus may play in the acquisition of antifungal drug resistance in *C. glabrata*.

ER stress response and cell wall integrity pathway in antifungal drug resistance

ER is an important organelle for proteostasis wherein the UPR is activated in response to accumulation of unfolded/misfolded proteins (Miyazaki et al. 2013). An ER-resident transmembrane kinase/endoribonuclease Ire1 is an important constituent of the ER protein quality control system which mediates an unconventional splicing of the mRNA, encoding a bZIP family transcription factor Hac1, resulting in Hac1 activation, nuclear translocation and subsequent upregulation of UPR target genes (Miyazaki et al. 2013). However, *C. glabrata* lacks this CgIre1-CgHac1-dependent UPR, and abates ER stress, via CgIre1, by degrading ER-located mRNAs through regulated Ire1-dependent decay (Miyazaki et al. 2013). Of note, activation of calcineurin signaling and CgSlt2-dependent cell wall integrity pathway also contribute to UPR gene upregulation and ER stress survival (Miyazaki et al. 2013). Notably, CgIre1 loss had no effect on the susceptibility of *C. glabrata* towards azole and echinocandin drugs, unlike other fungal pathogens (Miyazaki et al. 2013), suggesting a diversification of UPR functions in antifungal tolerance modulation in *C. glabrata*. Further, although the azole susceptibility of the *Cgcnb1*Δ*ire1*Δ double mutant points toward a possible functional redundancy between calcineurin and ER stress signaling in regulating antifungal stress survival (Miyazaki et al. 2013), the underlying molecular basis remains to be deciphered.

CgSlt2 is the terminal mitogen-activated protein kinase of the PKC-mediated call integrity pathway, and its loss is known to result in hypersusceptibility to ER stressors, and azole and

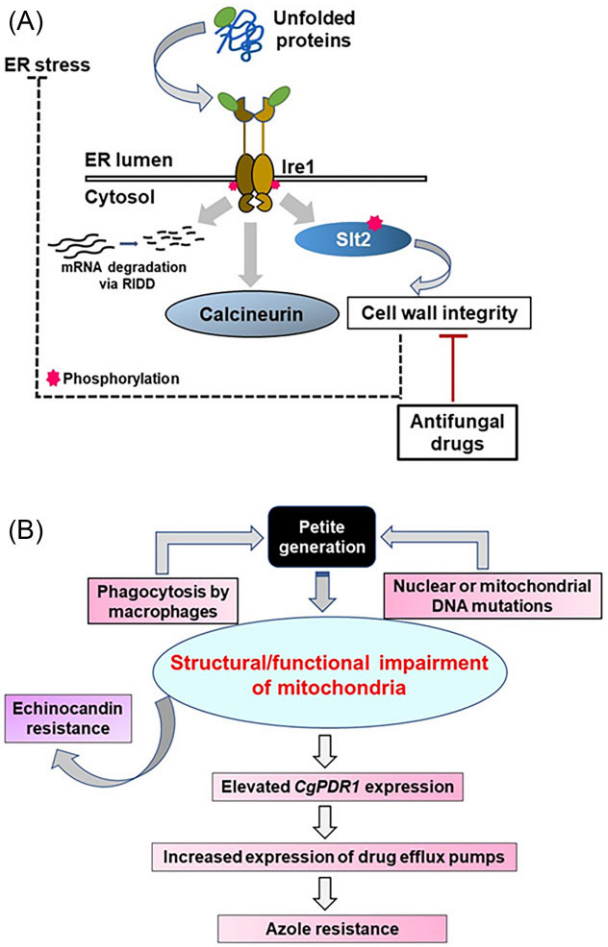

Figure 2. Graphic illustration of the link between endoplasmic reticulum (ER) stress and antifungal stress (A), and between dysfunctional mitochondria and antifungal drug resistance (B) in *C. glabrata*.

echinocandin drugs (Miyazaki et al. 2010a, 2013, Borah et al. 2011). Fluconazole treatment activated CgSlt2 pathway, as reflected in elevated levels of total and phosphorylated CgSlt2 in azole-treated *C. glabrata* cells (Borah et al. 2011). In accordance, the ER-localized, UDP-glucose:glycoprotein glucosyltransferase CgKre5 was found to be crucial for maintaining ER homeostasis and cell wall integrity, with *CgKRE5* depletion leading to CgSlt2 activation, diminished cell wall β-1,6-glucan, increased chitin levels, and micafungin susceptibility (Tanaka et al. 2016). Since increased chitin levels have been reported to protect *C. glabrata* cells from caspofungin-mediated killing (Cota et al. 2008), the increased micafungin sensitivity associated with *CgKRE5* deficiency (Tanaka et al. 2016) is intriguing and merits further investigation. The nexus among ER stress, calcineurin, and CgSlt2 is pictorially represented in Fig. 2A.

Importantly, an essential requirement for CgSlt2-mediated cell wall integrity pathway in governing antifungal tolerance has been reinforced by systematic gene knockout and mutant screens which revealed that disruption of many genes, belonging to CgSlt2 pathway, led to an increased susceptibility to azole and echinocandin drugs (Borah et al. 2011, Schwarzmüller et al. 2014, Rosenwald et al. 2016). Consistent with this, caspofungin treatment led to a better clearance of *Cgslt2*Δ cells in a gastrointestinal colonization mouse model, as well as reduced the emergence of echinocandin-resistant colonies (Garcia-Rubio et al. 2021a). Further, CgSwi4 (DNA binding component of the Swi4–Swi6 cell cy-

cle box-binding factor) transcription factor, which is regulated by CgSlt2, has been implicated in governing micafungin tolerance (Nagayoshi et al. 2014). Similarly, CgSlt2 pathway inhibition has been reported to block fluconazole-induced CgPdr1-regulon activation (Borah et al. 2011). In light of these findings, further studies are needed to fully delineate how CgSlt2 pathway activation could contribute to azole and echinocandin resistance acquisition in *C. glabrata* under hospital settings.

Role of mitochondria in antifungal drug resistance

Candida glabrata is a petite-positive yeast that ferments and utilizes only glucose and trehalose as carbon sources (Fidel et al. 1999). Importantly, a reciprocal correlation between mitochondrial function and tolerance to azole antifungals has been reported, with mutants containing dysfunctional mitochondria and/or lacking mitochondrial DNA exhibiting a high degree of fluconazole resistance, which in part has been attributed to an elevated expression of *CgPDR1* and/or *CgCDR1* genes (Sanglard et al. 2001, Brun et al. 2004, Kaur et al. 2004, Paul et al. 2014, Rodrigues et al. 2017, Gale et al. 2023, Chow et al. 2024). Similarly, deletion of the phosphatidylglycerolphosphate synthase-encoding *CgPGS1* gene led to a deficiency of two anionic phospholipids, phosphatidylglycerol, and cardiolipin, and reduced susceptibility to azole drugs (Batova et al. 2008). Of note, phosphatidylglycerol and cardiolipin are pivotal to mitochondrial biogenesis (Batova et al. 2008). Consistently, *Cgpgs1*Δ mutant exhibited diminished respiratory activity, reduced amounts of cytochrome a and b, and attenuated growth in the medium containing non-fermentable carbon sources (Batova et al. 2008). Further, azole resistance in the *Cgpgs1*Δ mutant was attributed to an increased expression of *CgPDR1*, and *CgCDR1*, *CgCDR2*, and *CgSNQ2* drug transporter genes, arising likely from a perturbed mitochondrial phospholipid homeostasis (Batova et al. 2008).

Despite a strong association between azole resistance and mitochondrial dysfunction, azole hyper-susceptibility has been reported in one respiration-deficient clinical isolate of *C. glabrata* that contained normal sterol levels and exhibited increased *CgPDR1* and *CgCDR1* expression (Vandeputte et al. 2009), pointing towards the multi-faceted azole stress survival mechanisms. Consistent with this, *Hermes* transposon insertions in ∼130 mitochondrial protein-encoding nuclear genes led to fluconazole resistance, and α-ketoglutarate synthesis in the mitochondria negatively regulated the innate fluconazole resistance of *C. glabrata* by restricting CgPdr1 functions (Gale et al. 2020, 2023). In this regard, it is worth noting that CgPdr1 has also been shown to be activated upon direct binding to ketoconazole (Thakur et al. 2008). Further, *CgPDR1* expression has recently been reported to be positively regulated by another Zn(2)Cys(6) cluster-containing transcriptional activator, CgUpc2, in an azole-dependent manner, with CgUpc2A also being a master transcriptional activator of *CgERG* (ergosterol biosynthesis genes) genes, thereby unveiling a link between ergosterol biosynthesis and CgPdr1-mediated azole resistance (Vu et al. 2019, 2021).

The role of mitochondria in governing cellular response to echinocandin drugs has also been established in *C. glabrata*. Echinocandin treatment has been reported to increase the production of reactive oxygen species in *C. glabrata* (Garcia-Rubio et al. 2021b). Further, although the respiratory-deficient mutants with impaired mitochondrial functions did not display reduced caspofungin susceptibility, these were found to be unresponsive to the synergistic effect of caspofungin and inhibitors of Hsp90 or calcineurin (Singh-Babak et al. 2012). Interestingly, while chemical inhibition of the mitochondrial respiratory chain complexes I and IV led to better survival under echinocandin stress, deletion of genes, that code for mitochondrial components, resulted in diminished echinocandin tolerance at sub-minimal inhibitory concentrations *in vitro* (Garcia-Rubio et al. 2021b). Further, macrophage-ingested *C. glabrata* cells survived better in the presence of the echinocandin drug, micafungin (Arastehfar et al. 2023a). Of note, a transient impairment of mitochondrial functions has been reported to provide protection against fluconazole, and during early stages of macrophage infection, with both fluconazole and macrophage internal milieu elevating the frequency of petite formation in *C. glabrata* (Siscar-Lewin et al. 2021). This study also reported the emergence of petite mutants under hospital settings, despite mitochondrial dysfunction-associated fitness defects (Siscar-Lewin et al. 2021). The petite prevalence in clinical isolates of *C. glabrata* was further strengthened by an analysis of 1000 international blood isolates, which revealed a petite prevalence rate of up to 3.5% (Arastehfar et al. 2023b). This study also found *C. glabrata* petite mutants to exhibit higher echinocandin tolerance, invoke a pro-inflammatory response in macrophages, and display diminished fitness in mice (Arastehfar et al. 2023b). Importantly, macrophages have been demonstrated to be a reservoir for echinocandin-tolerant *C. glabrata* cells, which eventually increases the emergence of echinocandin-resistant *C. glabrata* cells (Arastehfar et al. 2023a). Altogether, these reports highlight the complex nature of clinical antifungal resistance. Consistent with this, the genotypic and phenotypic diversity was observed in *C. glabrata* colonies with indistinguishable morphologies, recovered from the same candidemic patient (Badrane et al. 2023). This study also underscored the importance of monitoring *Candida* growth in a clinical laboratory for smaller, petite (azole-resistant) colonies, that emerged after 84 h incubation and were associated with relapsing or persistent infections due to fluconazole-resistant *C. glabrata* strains (Badrane et al. 2023). Figure 2B schematically depicts the association between perturbed mitochondrial functions and antifungal resistance.

Further, disruption of the genes, that regulate ribosome assembly, led to elevated fluconazole tolerance in *C. glabrata* (Kaur et al. 2004, Gale et al. 2023), while deletion of the genes, that code for components of the phosphatidylinositol 3,5-bisphosphate synthesis complex, resulted in an increased susceptibility to both azole and echinocandin drugs and perturbed vacuolar homeostasis (Bhakt et al. 2018, Choudhary et al. 2019). Although these findings indicate roles of other cellular organelles in governing antifungal resistance in *C. glabrata*, more detailed studies are required to uncover the underlying molecular underpinnings.

Of note, how cellular stress signaling cascades could facilitate the clinical drug resistance development is yet to be elucidated, however, it is possible that these global stress-adaptive strategies in *C. glabrata* may provide adequate time and a framework necessary to rewire its genetic, transcriptomic, or proteomic circuitry, upon antifungal drug treatment, which subsequently result in breakthrough infections and/or antifungal resistance development.

Genetic alterations in antifungal drug resistance

Candida glabrata possesses a dynamic genome, and chromosome reconfigurations have been reported in clinical isolates as well as laboratory reference strains (Poláková et al. 2009, Bader et al. 2012, Carreté et al. 2019, Pais et al. 2022). Particularly, DNA damage repair mechanisms, heteroresistance, new chromosome formation,

aneuploidy, and epigenetic modifications have been shown to play crucial roles in antifungal resistance in *C. glabrata* (Poláková et al. 2009, Ben-Ami et al. 2016, Pais et al. 2022, Patra et al. 2022). Notably, *C. glabrata* has been reported to possess few features that are unique to its DNA repair mechanisms, and distinct from those of its close cousin, *S. cerevisiae* (Shor et al. 2020). For example, the exposure of *C. glabrata* to the DNA damage-causing agent methyl methanesulfonate did not result in hyperphosphorylation of a conserved DNA damage response kinase CgRad53, which probably contributed to muted DNA damage checkpoint signaling, non-arrest in S-phase and subsequent aberrant cell division and death (Shor et al. 2020). However, how these distinct DNA damage response mechanisms in *C. glabrata* promote genetic/epigenetic alterations that lead to antifungal resistance development under hospital settings, remains to be investigated.

In this regard, it is worth noting that Poláková *et al.* had identified two extra chromosomes, respectively, of 120 and 200 kb length carrying duplications of Chromosome E and F, in two clinical isolates of *C. glabrata* (Poláková et al. 2009). The presence of the new mini chromosome F, carrying the multidrug transporter *CgCDR1* gene, exhibited a good correlation with the azole resistance phenotype of the isolate (Poláková et al. 2009). Similarly, an increase in the copy number of *CYP51* (*CgERG11*) gene, via chromosome duplication, has been reported to account for the azole resistance phenotype of a clinical isolate (Marichal et al. 1997).

Further, mutations in the mismatch repair gene, *CgMSH2*, have been reported to be associated with accelerated multidrug resistance under clinical settings in some studies, but not all (Dellière et al. 2016, Healey et al. 2016, Hou et al. 2018, Singh et al. 2018). Specifically, the *Cgmsh2*Δ mutant, upon drug selection, revealed the emergence of ∼ 18-, 82-, and 9-fold higher number of fluconazole, caspofungin and amphotericin B-resistant colonies, respectively, with all highly echinocandin-resistant *Cgmsh2*Δ colonies carrying mutations in the hot spot regions of CgFks1 or CgFks2 (Healey et al. 2016), underscoring the clinical relevance of *CgMSH2* mutations. Notably, non-synonymous mutations in the *CgRAD6* gene have also been found to be exclusively present in azole-resistant clinical isolates of *C. glabrata* (Pais et al. 2022). Moreover, the proclivity of *C. glabrata* to acquire fluconazole resistance, upon azole exposure, has been attributed to heteroresistance (variable drug response in a clonal cell population), with heteroresistance also contributing to the failure of fluconazole therapy in eradicating *C. glabrata* infection in a mouse model (Ben-Ami et al. 2016).

A recent study involving an analysis of 97 *C. glabrata* isolates, varying in their geographical origins, host niches, and antifungal resistance profiles, identified various polymorphisms and insertions and deletions in several genes (Pais et al. 2022). It will be intriguing to compare these variations with genomes of other sequenced *C. glabrata* clinical isolates to unveil their association with specific drug resistance profiles. Furthermore, a comparative genomic analysis of 151 *C. glabrata* isolates underscored the nuclear and mitochondrial genome diversity, while also reporting microevolution in the serial isolates from patients, and mutation accumulation in *CgFKS1* and *CgFKS2* genes (Helmstetter et al. 2022). Interestingly, through an *in vitro* evolution analysis, it has recently been reported that anidulafungin resistance is more stable than fluconazole resistance in *C. glabrata*, with point mutations in the antifungal resistance genes expectedly exhibiting more stability than aneuploidies (Ksiezopolska et al. 2024). Notably, a genomic evolution study reported that exposure of azole-susceptible isolates to azole resulted in the accumulation of azole resistance-conferring mutations in the hexose-transporter gene *CgHXT4/6/7*, thereby implicating glucose transporters in the uptake of azole drugs and azole resistance modulation (Galocha et al. 2022).

Altogether, more genome-wide, systematic analyses are required to uncover and establish the role that the genetic circuitry of *C. glabrata* plays in drug resistance acquisition.

Epigenetic modifications driving antifungal resistance acquisition

A central role of epigenetic changes including histone modifications and nucleosome reconfiguration in drug tolerance is beginning to be uncovered in *C. glabrata* (Patra et al. 2022). Genes and mechanisms involved in epigenetic modifications, that impact antifungal resistance, are presented in Table 3 and Fig. 3, respectively.

Recently, enzymes carrying out lysine methylation of histone H3 have been implicated in governing antifungal tolerance, *albeit* conversely, in *C. glabrata* (Moirangthem et al. 2021, Baker et al. 2022). For example, CgSet1 and CgSet2, respectively, which represent H3K4 methyl transferase and H3K36 methyl transferase enzymes, have been reported to positively and negatively regulate fluconazole tolerance in *C. glabrata*, with their disruption abolishing H3K4 mono, di and trimethylation, and H3K36 trimethylation (Moirangthem et al. 2021, Baker et al. 2022). Further, azole sensitivity of the *Cgset1*Δ mutant was attributed to a deficient activation of *CgERG* genes including *CgERG3* and *CgERG11*, in response to azole treatment (Baker et al. 2022). More importantly, chromatin immunoprecipitation analysis, using the anti-histone H3K4 trimethylation antibody, revealed a significant enrichment of H3K4 trimethylation at the 5′ ends of the *CgERG3* and *CgERG11* genes, under regular-growth conditions, which was further elevated upon azole exposure (Baker et al. 2022), thereby directly linking H3K4 trimethylation with azole-induced *CgERG* gene activation. Interestingly, although the *CgPDR1* regulon was found to be positively regulated by a putative histone H3 lysine-36 demethylase CgRph1, with *Cgrph1*Δ mutant exhibiting fluconazole sensitivity and impaired basal expression of *CgCDR1* and *CgPDR1* genes (Moirangthem et al. 2021), the abundance of differentially methylated histone H3 on *CgPDR1* gene and/or promoter is yet to be determined. Nonetheless, since H3K4 and H3K36 methylation are associated with active transcription (Freitag 2017), their enrichment at the 5' ends of *ERG* and other antifungal tolerance-conferring genes will result in increased gene expression, which may lead to better survival of antifungal stress.

Further, the loss of two putative, functionally redundant histone chaperones, CgFpr3 and CgFpr4, that regulate histone protein homeostasis, imparted azole tolerance (Moirangthem et al. 2021). The *Cgfpr3*Δ*fpr4*Δ double mutant exhibited increased histone H3 and H4 levels and reduced fluconazole susceptibility (Moirangthem et al. 2021). This diminished azole susceptibility was attributed to an increased expression of *CgPDR1* and its target genes (Moirangthem et al. 2021). Moreover, a mutant lacking the histone chaperone CgRtt106 showed increased susceptibility to the azole drugs, with CgRtt106 binding to the *CgCDR1* promoter and positively regulating *CgCDR1* gene expression (Nikolov et al. 2022). Further, the SWI/SNF chromatin remodeling complex that determines nucleosome positioning, has been reported to be important for controlling azole-induced *CgPDR1* gene regulon activation, with disruption of the complex catalytic subunit CgSnf2 resulting in azole sensitivity (Nikolov et al. 2022). Altogether, these findings accentuate the importance of CgPdr1 as a promising target for abating the development of azole resistance in clinical settings. In accordance with this, a small-molecule inhibitor iKIX1, that interfered with the interaction between the ac-

Table 3. A list of enzymes involved in antifungal resistance-associated epigenetic modifications in *C. glabrata*.

Enzyme	Gene name	Histone modification/Function	Drug susceptibility profiles associated with gene disruption		Reference
			Azoles	Echinocandins	
Histone acetyl transferases (HATs)	*CgRTT109*	H3K56 acetylation	ND[#]	Susceptibility	(Rosenwald et al. 2016)
	CgGCN5	H3K9 and H3K14 acetylation	Susceptibility	Susceptibility	(Yu et al. 2022)
	CgADA2	H3K9 acetylation	Susceptibility	Susceptibility	(Yu et al. 2018)
Histone deacetylases (HDACs)	*CgRPD3*	Histone deacetylation	ND	Susceptibility	(Filler et al. 2021)
	CgHST1	H3K14 deacetylation	Resistance	ND	(Orta-Zavalza et al. 2013)
Histone methyl transferases	*CgSET1*	H3K4 methylation	Susceptibility	Susceptibility	(Baker et al. 2022, Bhakt et al. 2022)
	CgSET2	H3K36 methylation	Resistance	Susceptibility	(Moirangthem et al. 2021, Bhakt et al. 2022)
Histone demethylases	*CgRPH1*	Putative H3K36 and H3K9 demethylation	Susceptibility	ND	(Moirangthem et al. 2021)
Chromatin remodeling complex	*CgSNF2*	Nucleosome dynamics	Susceptibility	ND	(Nikolov et al. 2022)
Histone chaperones	*CgRTT106*	Histone homeostasis	Susceptibility	ND	(Nikolov et al. 2022)
	CgFPR3 & CgFPR4	Histone protein homeostasis	Resistance	No change	(Moirangthem et al. 2021)

[#]Not determined.

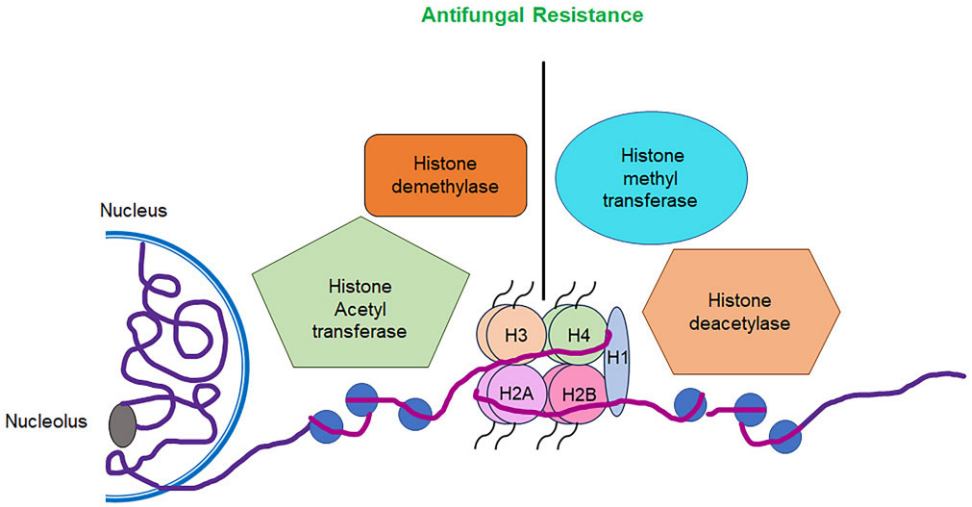

Figure 3. Schematic illustration of chromatin-regulated mechanisms that govern antifungal resistance in *C. glabrata*.

tivation domain of CgPdr1 and the three-helix bundle KIX (kinase-inducible domain interacting) domain of CgGal11A/Med15, led to re-sensitization of the azole-resistant clinical isolates (Nishikawa et al. 2016).

Compared to histone methylation, the role of histone acetylation in antifungal drug resistance regulation has been examined for a long time. The pharmacological modulation of histone H3 acetylation at lysine-56 residue has been proposed as an effective antifungal therapeutic option (Wurtele et al. 2010). Consistent with this, MGCD290, a Hos2 fungal histone deacetylase inhibitor, exhibited a synergistic effect with azole drugs in *C. glabrata* isolates (Pfaller et al. 2009). Intriguingly, deletion of the histone deacetylase *CgRPD3* rendered *C. glabrata* cells susceptible to caspofungin (Filler et al. 2021). Further, CgHst1, a NAD^+-dependent histone deacetylase, has been identified as a repressor of *CgPDR1* and *CgCDR1* expression, with *CgHST1* deletion conferring azole resistance to *C. glabrata* (Orta-Zavalza et al. 2013). Contrarily, CgGcn5, a catalytic subunit of the Spt-Ada-Gcn5-acetyltransferase (SAGA) complex, positively regulated azole and echinocandin tolerance, with *CgGCN5* deletion leading to reduced acetylation of histone H3 at lysine-9 and 14 residues (Yu et al. 2022). Moreover, *CgGCN5* loss also led to a decrease in the echinocandin resistance of *C. glabrata* strains carrying clinically relevant *CgFKS* mutations (Yu et al. 2022), suggesting that CgGcn5 impacts the echinocandin minimum inhibitory concentration (Yu et al. 2022). Similarly, *CgADA2*, which codes for a SAGA complex transcription adaptor and mediates histone H3 acetylation at lysine-9 residue, has also been shown to be a positive regulator of drug tolerance in *C. glabrata* (Yu et al. 2018). While underlining the complex interplay between histone acetylation/deacetylation and

methylation, these findings, collectively, raise the possibility that histone levels and histone posttranslational modifications may render the chromatin more accessible for the recruitment of transcriptional factors that may aid in activation of the genes required to counteract antifungal stress. This notwithstanding, the molecular underpinnings of chromatin/nucleosome dynamics-driven transcriptional control of antifungal resistance genes in *C. glabrata* are yet to be fully deciphered.

Host niches contributing to *C. glabrata* persistence and drug resistance

The field of host factors and niches, that promote drug resistance emergence in *C. glabrata*, is still in its infancy. Further, although clinical reservoirs driving azole resistance remain undefined, recent studies have implicated the host gastrointestinal tract as a potential source of *C. glabrata* persistence and drug resistance (Healey et al. 2017). In a gastrointestinal colonization model, upon caspofungin therapy, *C. glabrata* was found to display a drug resistance rate of up to 10%, which in part could be due to poor caspofungin penetration in the gastrointestinal tract lumen of *C. glabrata*-infected and drug-treated mice. Alarmingly, caspofungin-resistant *C. glabrata* cells were able to widely spread in the body under select conditions, upon immunosuppression (Healey et al. 2017), indicating a possible mode of echinocandin resistance acquisition by the endogenous/exogenous gut-resident *C. glabrata* cells. The contribution of *C. glabrata* mouse gut colonizers to caspofungin resistance was further highlighted by a recent study which showed the emergence of mutations in *CgFKS2* (codes for the caspofungin target enzyme) and *CgFEN1* (codes for a fatty acid elongase, that is involved in sphingolipid biosynthesis) genes during caspofungin treatment (Hassoun et al. 2024). Notably, the loss-of-function mutations in the *CgFEN1* gene in clinical isolates of *C. glabrata*, which were associated with reduced caspofungin susceptibility (Hassoun et al. 2024), suggest that the host gut is likely to be a clinical echinocandin resistance reservoir for *C. glabrata*.

Further, *C. glabrata* has also been reported to exhibit prolonged persistence in mouse models of systemic and intra-abdominal candidiasis (Rasheed et al. 2020). *C. glabrata* infections in the intra-abdominal candidiasis model progressed from peritonitis to formation of abscesses and were found to be associated with diminished neutrophil infiltration and higher fungal burden in abscesses, as compared to *C. albicans* (Cheng et al. 2014). However, a link, if any, between this long-term persistence of *C. glabrata*, without provoking a strong host immune response (Rasheed et al. 2020), and the development of antifungal resistance under clinical settings, is yet to be systematically investigated.

Recently, a link between macrophage ingestion, petite formation, and antifungal resistance acquisition has been reported in *C. glabrata* (Siscar-Lewin et al. 2021, Arastehfar et al. 2023a) raising a possibility of macrophages serving as an intracellular reservoir for multidrug-tolerant *C. glabrata* persister cells. Of note, azole-resistant *C. glabrata* isolates were found to fare poorly in macrophages, in mouse kidneys during systemic infections, and during colonization of the murine gut (Arastehfar et al. 2024). This study suggested that the spleen could be a permissive organ for the emergence of drug resistance in *C. glabrata* (Arastehfar et al. 2024). Overall, studies focussing on the evolution of azole and echinocandin drug resistance in different murine models of candidiasis will yield the much-needed molecular insights into the strategies that *C. glabrata* relies upon to adapt, survive, or develop resistance against antifungals in hospital settings.

Future perspectives

In view of the rising multidrug resistance in *C. glabrata*, it is vitally important to identify both primary drivers of antifungal resistance and secondary players that boost the emergence of drug resistance by thwarting the growth-inhibitory and growth-killing action of antifungal drugs via global stress response pathway activation. These molecular analyses need to be bolstered by the concerted global surveillance studies to closely monitor antifungal resistance trends in *C. glabrata*, along with the development of economical diagnostic tools to rapidly identify drug-resistant *C. glabrata* isolates and timely implement the optimal singular/combinatorial therapy. In parallel, how the commensal form of *C. glabrata* responds to antifungal stress and contributes to the fitness and/or selection of drug-resistant isolates via rewiring of the genetic/epigenetic circuitry may be examined. Further, identification of the determinants, that drive the interplay between *C. glabrata* and the mammalian host, during antifungal drug pressure, and promote the success or failure of antifungal therapy, should be prioritized by undertaking interdisciplinary approaches including mathematical modeling, evolutionary, therapeutic drug monitoring, and immunological analyses. Lastly, whether the vastly different incidence rates of *C. glabrata* infections and variability in drug resistance patterns, depending upon the geographical region, are due to variations in the strains/clades, and host genetic/metabolic makeup, warrants a closer systematic inspection. Similarly, how these incidence rates and resistance patterns are impacted by the type of antifungals prescribed, practices of healthcare workers, and hospital environments should be investigated to meet the ultimate goal of providing affordable antifungal therapy across the world.

Author contributions

S.N. and R.K. conceived and designed the content of the review. S.N., A.P., and R.K. prepared tables and figures. S.N., A.P., and R.K. wrote the manuscript.

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