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Minireview

MINIREVIEW

Tipping the balance both ways: drug resistance and virulence in *Candida glabrata*

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One sentence summary: This review gives a short overview of the pathogenesis of the fungal pathogen *Candida glabrata* and summarizes in this pathogen the impact of drug resistance on virulence.

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ABSTRACT

Among existing fungal pathogens, *Candida glabrata* is outstanding in its capacity to rapidly develop resistance to currently used antifungal agents. Resistance to the class of azoles, which are still widely used agents, varies in proportion (from 5 to 20%) depending on geographical area. Moreover, resistance to the class of echinocandins, which was introduced in the late 1990s, is rising in several institutions. The recent emergence of isolates with acquired resistance to both classes of agents is a major concern since alternative therapeutic options are scarce. Although considered less pathogenic than *C. albicans*, *C. glabrata* has still evolved specific virulence traits enabling its survival and propagation in colonized and infected hosts. Development of drug resistance is usually associated with fitness costs, and this notion is documented across several microbial species. Interestingly, azole resistance in *C. glabrata* has revealed the opposite. Experimental models of infection showed enhanced virulence of azole-resistant isolates. Moreover, azole resistance could be associated with specific changes in adherence properties to epithelial cells or innate immunity cells (macrophages), both of which contribute to virulence changes. Here we will summarize the current knowledge on *C. glabrata* drug resistance and also discuss the consequences of drug resistance acquisition on the balance between *C. glabrata* and its hosts.

Keywords: *Candida glabrata*; antifungal drug resistance; virulence

INTRODUCTION

Candida glabrata is a member of the commensal microflora of humans, along with other *Candida* species. *Candida glabrata* can be isolated from different parts of the body of healthy individuals, including the oropharynx, the stool, the vagina and, most importantly, the urinary tract, where *C. glabrata* is often the most common *Candida* species causing infection (Sobel *et al.* 2011). The real proportion of asymptomatic colonization is not well known. Most studies addressing colonization by *Candida* species have been performed on hospitalized patients, with exogenous nosocomial transmission probably skewing the numbers upwards (Luque *et al.* 2009; Lau *et al.* 2015). In the general human population, percentages of colonization are estimated to be anywhere between 20 and 50%, sometimes even higher (Ghannoum *et al.* 2010).

Antifungal drug classes that are active against *C. glabrata* include polyenes, azoles, flucytosine and echinocandins. Azoles (fluconazole, voriconazole, posaconazole and isavuconazole) inhibit ergosterol biosynthesis, while polyenes (amphotericin B, AmB) bind to ergosterol in the plasma membrane where they form large pores that disrupt cell membrane function. Flucytosine (5-fluorocytosine, 5-FC) inhibits pyrimidine metabolism and DNA synthesis. Finally, the echinocandins (caspofungin, anidulafungin and micafungin) inhibit the biosynthesis of β−1,3-D-glucan, a major structural component of the fungal cell wall. The use of antifungal agents is coupled with the inevitable

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Table 1. MIC values of *C. albicans* and *C. glabrata*.[a)]

Antifungal agent	MIC breakpoint (mg/L) EUCAST				MIC breakpoint (mg/L) CLSI						ECV (mg/L) EUCAST		ECV (mg/L) CLSI	
	C. albicans		*C. glabrata*		*C. albicans*			*C. glabrata*			*C. albicans*	*C. glabrata*	*C. albicans*	*C. glabrata*
	S	R	S	R	S	SDD	R	S	SDD	R				
Ampho B	≤1	>1	≤1	>1	≤1		≥1	NA	NA	NA	1	2	2	2
Anidulafungin	≤0.03	>0.03	≤0.06	>0.06	≤0.25	0.5	≥1	≤0.12	0.25	≥0.5	0.03	0.12	0.12	0.25
Caspofungin	NA	NA	NA	NA	≤0.25	0.5	≥1	≤0.12	0.25	≥0.5	NA	NA	0.12	0.12
Micafungin	≤0.016	>0.016	≤0.03	0.03	≤0.25	0.5	≥1	≤0.06	0.12	≥0.25	0.03	0.03	0.03	0.03
Fluconazole	≤2	>4	≤0.002	>32	≤2	4	≥8		≤32	>32	1	32	0.5	32
Voriconazole	≤0.125	>0.125	NA	NA	≤0.12	0.25–0.5	≥1	NA	NA	NA	0.12	1	0.03	0.5

[a)]Data obtained from selected publications (Rodriguez-Tudela *et al.* 2010; Orasch *et al.* 2014; Pfaller *et al.* 2014) and the EUCAST MIC Database (http://mic.eucast.org/Eucast2/SearchController/).

emergence of drug resistance. The emergence of acquired drug resistance is problematic since it restricts treatment options and then patient management. In *C. glabrata*, azoles are intrinsically less active than in *C. albicans*. For example, the epidemiological cutoff value (ECV) for fluconazole, which indicates the MIC value identifying the upper limit of the wild-type population, is 32 μg ml^{-1} in *C. glabrata* while it is only 0.5 μg ml^{-1} for *C. albicans* (Pfaller *et al.* 2010). With regard to other agents, they exhibit similar ECV values in both pathogenic fungal species (Table 1). Clinical breakpoints established by either EUCAST or CLSI reflect similar differences in activities of azoles in *C. glabrata* (Table 1).

Acquisition of resistance to antifungal agents is greatly helped by the establishment of ECVs. In *C. glabrata*, the incidence of azole resistance is variable but higher than for other pathogens. Resistance to fluconazole among *C. glabrata* isolates varies from 13.0% in the Asia-Pacific region to 19.5% in North America (Pfaller and Diekema 2010). In the USA, resistance to fluconazole in *C. glabrata* has increased from 9% in 1992–2001 to 14% in 2001–07 (Pfaller *et al.* 2012a). An increase can also be detected in Europe, as exemplified by a comprehensive study performed in Denmark (Arendrup *et al.* 2013). Resistance to echinocandins has also risen significantly in *C. glabrata* with the increased use of these agents in therapy. Susceptibility testing on 1380 isolates of *C. glabrata* collected between 2008 and 2013 showed that 3.1, 3.3 and 3.6% of the isolates were resistant to anidulafungin, caspofungin and micafungin, respectively (Pham *et al.* 2014). Intriguingly from this study was that, of the isolates resistant to at least one echinocandin, 36% were also resistant to fluconazole. The recent emergence of this type of multidrug resistance is of concern, since it reduces possible therapeutic alternatives (Pham *et al.* 2014). Resistance of *C. glabrata* to other agents such as AmB and 5-FC is occurring more rarely (Krogh-Madsen *et al.* 2006; Edlind and Katiyar 2010).

It is generally accepted that drug resistance can have some fitness costs in several microorganisms (Melnyk, Wong and Kassen 2014). The incidence of antifungal drug resistance being much higher for *C. glabrata* than it is for *C. albicans*, it is important to address the impact of antifungal drug resistance on *C. glabrata* fitness. In this review, we will summarize the current knowledge on this aspect.

Host–pathogen interactions, a well-kept balance

Like other *Candida* species, *C. glabrata* is an opportunistic pathogen and can cause invasive infection in susceptible patients. As opportunistic pathogens, *Candida* species depend on host susceptibility to initiate active infection. Microbial pathogenesis is a relative attribute that results from the balance between both pathogen and host-associated factors (Casadevall and Pirofski 2001) and, in accordance, the emergence of opportunistic candidiasis over the last half century is mostly due to the expansion of the human population at risk (Edwards 1991). Major contributors to this trend are the increased number of immunosuppressed patients, due to cancer, organ transplants and the AIDS pandemic, as well as the use of broad-spectrum antibiotic therapy and the growth of the elderly population. Studies tracking the origin of etiological agents of candidiasis generally find them to be of endogenous origin (Eggimann and Pittet 2014), meaning that preexisting colonization, either hospital acquired or preceding hospitalization, is a requirement for infection.

With a given opportunity in patients, *C. glabrata* makes use of several different virulence factors to establish active infection. *Candida glabrata* is less pathogenic than *C. albicans*, which probably explains the predominance of *C. albicans* as the most common etiological agent of invasive candidiasis. It is a helpful exercise to think of *C. glabrata* pathogenesis as being of an intermediate degree between that of baker's yeast *Saccharomyces cerevisiae*, only relatively rarely isolated as an infectious agent (Enache-Angoulvant and Hennequin 2005), and that of *C. albicans*, the most common pathogenic *Candida* species. Genetically, *C. glabrata* is more closely related to baker's yeast than to other *Candida* species (Dujon *et al.* 2004). Compared to baker's yeast, however, it has evolved specific adaptations to the commensal lifestyle. These adaptations include virulence factors that, together with the ability to readily develop drug resistance, allow it to cause infection. Recognized virulence factors of *C. glabrata* are at least the decoration of the cell surface with a variety of adhesins, the ability to undergo rapid adaptive transcriptional reprogramming when facing changing environmental conditions and a robust resistance to both nutrient starvation and oxidative stress (Roetzer, Gabaldon and Schuller 2011a). The *C. glabrata* strategy for pathogenicity seems to be based on a 'stealth-like' approach. This is illustrated by the fact that it can persist in immunocompetent mice for weeks following systemic infection, eliciting only mild immune responses and pathology (Westwater *et al.* 2007; Jacobsen *et al.* 2010). This is additionally corroborated by its ability to cross epithelial cell barriers without significant damage to host cells. Besides making use of the occasional accidental and medical-induced trauma to epithelial barriers (in wounds and catheters), *C. glabrata* can cross intact cell barriers such as confluent human enterocyte monolayers *in vitro* with close to no damage induced to host cells (Perez-Torrado *et al.* 2012). Also, it readily disseminates in a chicken embryo infection model without causing mortality (Jacobsen *et al.* 2011). This

is consistent with the fact that, unlike *C. albicans*, *C. glabrata* does not seem to rely on secreted hydrolases or filamentation for host invasion (Kaur *et al.* 2005). The mechanisms underlying this host cell-friendly transmigration across epithelial cell barriers remain cryptic, however.

A central virulence attribute of *C. glabrata*, and one that clearly separates it from baker's yeast, is its avid adherence to host tissues. The genome of *C. glabrata* contains an extensive number of genes encoding predicted glycosyphosphatidylinositol (GPI)-anchored adhesin-like cell wall proteins (de Groot *et al.* 2013; Gabaldón *et al.* 2013). The number of adhesin genes exhibits a wide intraspecies variability, with between 60 and 80 adhesins in different strains (L. Vale-Silva and D. Sanglard, unpublished). In addition, adhesins and other genes encoding cell wall proteins often include mini- and megasatellites (Thierry *et al.* 2008; Thierry, Dujon and Richard 2010), DNA tandem repeats whose size shows high intraspecies variability. Adhesin gene number variations combined with size variability of intragenic tandem repeat regions reflects a large genetic plasticity of this gene family (de Groot *et al.* 2013). Adhesins of *C. glabrata* are divided in several subfamilies, among which the *EPA* (EPithelial Adhesion) subfamily is the most important for interaction with host cells. The first member of this family, *EPA1*, was identified by Cormack, Ghori and Falkow (1999) as the main mediator of adherence to epithelial cells, with its deletion reducing adherence of *C. glabrata* to human epithelial cells by 95%. Most *C. glabrata* adhesin genes are found in subtelomeric chromosomal loci, where they are subject to transcriptional silencing by chromatin-based regulation mechanisms (Domergue *et al.* 2005). The subtelomeric gene silencing protein machinery depends on nicotinamide adenine dinucleotide (NAD+) as a cofactor, synthesized from nicotinic acid. *Candida glabrata* is a nicotinic acid auxotroph, thus relying on environmental availability of this precursor to synthesize NAD+. This way limitation of nicotinic acid availability in the urine releases subtelomeric adhesin genes from transcriptional repression (Domergue *et al.* 2005). This indirectly might mediate selective expression of adhesins in the host's urine, and thus configures a host environment sensing mechanism likely underlying *C. glabrata*'s tropism to the host's urinary tract. Nicotinic acid limitation-based transcriptional activation may also mediate biofilm formation *in vivo*, with important clinical consequences for catheter-associated infections. In this case, the phenotype relies on an additional member of the *EPA* adhesin subfamily, *EPA6* (Iraqui *et al.* 2005).

Together with the transcriptional control of *EPA* genes, the dynamic processing of cell wall proteins also plays a role in the interaction with the host. A family of 11 GPI-anchored cell wall aspartyl proteases (yapsins, encoded by genes *YPS1–YPS11*), 8 of which are found in a characteristic gene cluster in *C. glabrata*, are required for virulence in murine models of systemic candidiasis (Kaur, Ma and Cormack 2007; Rai *et al.* 2012). Yapsins are known to be involved in proteolytic processing of Epa1 (Kaur, Ma and Cormack 2007) and many other GPI-anchored cell wall proteins, which implicates them in several cellular processes. For example, yapsins have been shown to participate in regulation of pH homeostasis under acidic conditions and vacuole homeostasis (Bairwa and Kaur 2011; Bairwa *et al.* 2014). Yapsins are also known to directly affect interaction with cells of the host innate immune defense, since they are required for survival of *C. glabrata* within macrophages following phagocytosis (Kaur, Ma and Cormack 2007).

Candida glabrata has long been known to be able to survive phagocytosis and replicate within macrophages *in vitro* (Otto and Howard 1976). In the phagolysosome, it faces environmental stresses like the drop in pH, oxidative stress and nutrient deprivation. In order to cope with such stress, *C. glabrata* undergoes drastic transcriptional remodeling (Kaur, Ma and Cormack 2007; Seider *et al.* 2011), relying on chromatin organization control mechanisms (Rai *et al.* 2012). The response mounted by *C. glabrata* involves switching its metabolism to the utilization of alternative carbon sources (Kaur, Ma and Cormack 2007), as well as resorting to autophagy to recycle intracellular resources and deal with nutrient starvation (Roetzer *et al.* 2010). *Candida glabrata* may not need much adaptation to oxidative conditions, since it is intrinsically tolerant of oxidative stress (Cuellar-Cruz *et al.* 2008), but it does seem to induce antioxidant strategies (Brunke *et al.* 2010; Roetzer *et al.* 2011b). In addition to these defensive strategies, *C. glabrata* actively inhibits phagosome maturation by different mechanisms, thus restricting the environmental stresses (Seider *et al.* 2011; Rai *et al.* 2015).

In spite of its lower pathogenicity compared to *C. albicans*, emergence of *C. glabrata* has been observed in recent years in many regions of the world (Arendrup 2014). This trend is associated with the emergence of antifungal drug-resistant *C. glabrata* isolates. *Candida glabrata* is intrinsically less susceptible to the widely used azole antifungals and, upon exposure, it readily develops full resistance. Resistance to more recently available antifungal alternatives is emerging as well, with multidrug resistance becoming an important problem in this pathogen (Pfaller 2012; Pfaller *et al.* 2012b; Arendrup and Perlin 2014). The extensive use of antifungal therapy and prophylaxis programs in modern medicine may be establishing selective pressures for *C. glabrata* against *C. albicans*.

Antifungal drug resistance mechanisms of *C. glabrata*

Resistance mechanisms to azoles occurring in *C. glabrata* have been elucidated by several laboratories. The vast majority of azole-resistant isolates upregulate ATP-binding cassette (ABC) transporter genes, among which *CgCDR1* and *CgCDR2* play a major role. This upregulation is mediated by a transcriptional activator, *CgPDR1*, that belongs to the family of zinc finger transcription factors with Zn(2)Cys(6) domains. Mutations in *CgPDR1*, so-called gain-of-function (GOF) mutations, are responsible for the high expression of ABC transporters in azole-resistant isolates. The GOF mutations are diverse and are located at different functional domains of the protein (Tsai *et al.* 2006, 2010; Ferrari *et al.* 2009; Paul, Schmidt and Moye-Rowley 2011). It has been shown that *CgPDR1* interacts with the Mediator complex, thus creating a link between the activator and the transcriptional machinery in *C. glabrata* (Thakur *et al.* 2008). *CgPDR1* regulates other genes in *C. glabrata* due to the presence of a PDRE in target genes. It was shown recently that *CgPDR1* directly binds to these targets genes via the PDRE (Paul, Bair and Moye-Rowley 2014). Thus, GOF mutations in *CgPDR1* have not only an effect on ABC transporter genes, but they also affect several other target genes involved in other cellular processes. Upregulation of ABC transporter genes can also occur without GOF mutations but in mutants with mitochondrial defects. These mutants, so-called petite-mutants exhibit respiratory defects. Even if their growth rate is compromised in laboratory-grown conditions, these mutants can still be obtained from patient samples, thus suggesting that their growth deficit can still be compensated by other yet-unknown factors in the host (Ferrari *et al.* 2011a,b). Upregulation of *CgCDR1* and *CgCDR2* also seems to be behind the notable azole resistance of *C. glabrata* biofilms, at least during the intermediate phases of biofilm development (Song *et al.* 2009).

Azole resistance mechanisms excluding efflux-mediated mechanisms are very uncommon. To our knowledge, there is a single report documenting that the azole target (*ERG11*) was rendered non-functional by an *ERG11* mutation in a clinical isolate (Hull *et al.* 2012b). This is in sharp contrast with the high incidence of this type of resistance mechanism in *C. albicans* (Sanglard and Odds 2002), however with the difference that mutations are affecting azole affinity in *C. albicans*.

Resistance to echinocandins in *C. glabrata* is explained by the occurrence of mutations in genes encoding glucan synthases (*FKS1*, *FKS2*). Elevated echinocandin MICs have been associated with a number of single amino acid substitutions caused by mutations in specific 'hot spot' regions of the well-conserved target genes *FKS1* and *FKS2* in *C. glabrata*. The positions, as well as the specific amino acid substitutions, determine the degree of MIC elevation in the individual isolate to specific echinocandins (Arendrup and Perlin 2014).

Resistance to AmB has been rarely reported in *C. glabrata*. AmB resistance in clinical isolates is caused by mutations in the genes participating to sterol biosynthesis. Among these genes, *ERG6* and *ERG2* were shown to harbor loss of function mutations with the consequence of eliminating ergosterol from mutant cells (Vandeputte *et al.* 2007, 2008; Hull *et al.* 2012a). *Candida glabrata* has the surprising ability to be able to capture sterols from the host and therefore to compensate for the loss of ergosterol by these mutations (Nagi *et al.* 2013).

Whole genome alterations in *C. glabrata* and drug resistance

In contrast to *C. albicans*, a few studies have addressed whole genome changes in *C. glabrata* following acquisition of drug resistance. One study revealed a 4-fold increase in the copy number of *ERG11* in an azole-resistant isolate that corroborated with a 8-fold increase in the corresponding mRNA. The authors showed copy number increase of the chromosome containing *ERG11* (most probably in Chromosome E as deduced from CBS138 genome data). Interestingly, azole resistance could be reverted in the isolate and also correlated with decreased *ERG11* copy number (Marichal *et al.* 1997). A number of other studies have shown chromosomal pattern alterations associated with drug resistance, however without precise mapping of drug resistance genes (Chauhan, Kruppa and Calderone 2007). Recently, Ahmad *et al.* (2013) investigated 192 *C. glabrata* isolates from patients and demonstrated that these strains exhibited large karyotype polymorphisms. Nine of these strains contained small chromosomes, which were smaller than 0.5 Mb. Apparently, *C. glabrata* chromosomes are frequently reshuffled resulting in new genetic configurations (Muller *et al.* 2009). These large-scale genetic changes may be a common mechanism in *C. glabrata* of adaptation to the host environment, and thus virulence, as well as development of drug resistance (Poláková *et al.* 2009). Ahmad *et al.* (2013) suggested that small chromosomes were containing drug resistance genes (among which *CgCDR2*), thus contributing to resistance development. An earlier report had already implicated chromosome duplications in the development of drug resistance, mainly through *CgCDR1* (Shin *et al.* 2007).

Whole genome analysis of drug-resistant *C. glabrata* isolates is still rare. In one remarkable study, Singh-Babak *et al.* (2012) provided the first global analysis of mutations accompanying drug resistance in a human host utilizing a series of *C. glabrata* isolates that evolved echinocandin resistance. The whole genome sequencing identified a mutation in the drug target, *FKS2*, but in addition eight non-synonymous mutations. While the *FKS2* mutation was responsible for echinocandin resistance, it was associated with a fitness cost that was probably compensated by the other additional mutations. The study could however not detect chromosomal changes due to the used sequencing technology (Singh-Babak *et al.* 2012).

Our laboratory recently compared the genome sequences of two sequential isolates, one of which developed azole resistance within 50 days of therapy in a patient. The sequencing technology allowed the assembly of large fragments that almost reconstituted entire chromosomes. While the results showed no significant chromosomal rearrangements between the two isolates, they exhibited a relatively small number of nucleotide variations (18 non-synonymous SNPs difference). One of the SNPs was located in *CgPDR1* (L280F) and is known to be associated with azole resistance. The other non-synonymous changes could reflect compensatory mechanisms necessary for host adaptations (L. Vale-Silva and D. Sanglard, unpublished).

Evolution of antifungal drug resistance

Resistance to specific antifungal agents is mediated by several mechanisms and some of them have been resolved as above mentioned by the alteration of specific genes. The occurrence of these mechanisms in clinical isolates and their selection by drug exposure can have a cost (or fitness cost) for the overall cellular metabolism. Fitness can be defined as the ability of an organism to propagate and evolve within a given environment. Antifungal resistance may have a negative impact on fitness and therefore may reduce the competitiveness of strains as compared to wild-type isolates, especially when the drug selection is removed. The decrease in fitness of resistant strains may indeed compromise their virulence. Some studies have addressed this question by testing the virulence of azole-resistant *C. albicans* isolates as compared to their azole-susceptible parents; however, no direct relationship could be established between the development of azole resistance and virulence. The trajectory of azole resistance and the associated genome changes had rather an unpredictable effect on fitness (Graybill *et al.* 1998).

In vitro studies have also addressed the relationship between the development of azole resistance and changes in fitness. Individual *C. albicans* colonies were subcultured in fluconazole-containing medium and each developed individual trajectories in their development of azole resistance (Cowen, Kohn and Anderson 2001). These *in vitro* studies showed no direct relationship between the cost of developing azole resistance and changes in fitness as measured by competition assays between susceptible and resistant isolates. Remarkably, in-depth analysis of genome changes of azole-resistant isolates produced by Cowen, Kohn and Anderson (2001) has revealed several alterations, among which aneuploidies (for example, the formation of an isochromosome from the chromosome 5 left arm, so-called 5iL). These alterations were however not affecting *in vitro* fitness of *C. albicans* (Selmecki *et al.* 2009). The notion of neutral fitness cost upon azole exposure *in vitro* has been confirmed by others (Huang *et al.* 2011).

With regard to the cost of resistance to other antifungal agents, few studies exist. Candin resistance in *C. albicans* has been associated with reduced virulence in animal models (Kurtz *et al.* 1996). Recent work has demonstrated that *FKS1* mutations in *C. albicans* resulted, in addition to candin resistance, to thicker cell walls attributable to increased cell wall chitin content. Fks1 mutants were hypovirulent in fly and mouse models of candidiasis and exhibited reduced fitness in competitive mixed infection models, which may limit their epidemiological and clinical

impact (Ben-Ami *et al.* 2011). Other studies have confirmed that candin resistance results in decreased virulence as compared to parent wild types. Fungal factor(s) critical for this negative interaction are starting to be firmly identified (Slater *et al.* 2011; Lee *et al.* 2012). Due to the cell wall remodeling action mediated by candin resistance, recognition of fungal elements from the host immune system probably favor host responses and therefore negatively impact fungal virulence (Lewis, Viale and Kontoyiannis 2012).

It is intriguing that during contact with cells of the innate immune system, some genes involved in antifungal resistance are upregulated. For example, ABC transporter genes such as *CDR1* from *C. albicans* and *CgCDR2/PHD1* from *C. glabrata* are upregulated during their phagocytosis by neutrophils and by macrophages (Fradin *et al.* 2007; Kaur, Ma and Cormack 2007). Likewise, *CDR1* of *C. albicans* is upregulated in tissues (kidneys) during infections (Walker *et al.* 2009). Moreover, strains of *Cryptococcus neoformans* overexpressing the ABC transporter *AFR1* that is responsible for fluconazole resistance exhibit enhanced virulence in mice and alter macrophage function in an *AFR1*-dependent manner to modify their survival in macrophages (Sanguinetti *et al.* 2006; Orsi *et al.* 2009). Interestingly, azole resistance involving *AFR1* upregulation is mediated by aneuploidy on a specific chromosome carrying *ERG11* (Sionov *et al.* 2010).

In *C. glabrata*, a few studies have addressed the relationship between acquisition of drug resistance and changes in virulence traits. As referred above, Poláková *et al.* (2009) and Ahmad *et al.* (2013) showed that *C. glabrata* is capable in the host to form minichromosomes containing genes involved in drug resistance, which may contribute to selective advantages *in vivo*. Unfortunately, these studies did not address this hypothesis using animal models. As mentioned above, a study (Singh-Babak *et al.* 2012) evaluated the acquisition of candin resistance by whole genome sequence analysis of sequential isolates. The authors showed that genomic mutations in addition to the expected *FKS2* mutation were probably the result of host pressure. As suggested by the authors, these mutations may play a compensatory role for the presence of the *FKS2* mutation (Singh-Babak *et al.* 2012). *CgPDR1* GOF mutations and their impact on fitness and virulence have been addressed in *C. glabrata*. It was found that *CgPDR1* GOF mutations, besides their role in the upregulation of ABC transporters and mediators of azole resistance, could enhance virulence of *C. glabrata* as compared to wild-type isogenic isolates in an intravenous infection model in mice (Ferrari *et al.* 2009). Enhanced virulence was accompanied by higher fungal loads in infected organs and treatment failure with azoles. Enhanced virulence could be associated with gain of fitness of strains carrying *CgPDR1* GOF. Therefore, the results obtained by Ferrari *et al.* (2009) have so far challenged the dogma existing between development of drug resistance and fitness costs *in vivo*. These results have motivated the understanding of this phenomenon at the molecular level using whole transcriptome analysis. Enhanced virulence could be due to specific genes commonly regulated by all *CgPDR1* GOFs. A detailed analysis revealed that only two genes were commonly upregulated by at least 2-fold by all GOFs, i.e. *CgCDR1*, the well-known ABC transporter involved in azole resistance, and the ORF CAGL0M12947g, which was named *PUP1* (for *PDR1* UPregulated gene). This gene is highly similar to YIL077c, a gene encoding a protein of unknown function thought be located in the mitochondria. These two genes were inactivated in *C. glabrata* as single mutants or combined mutants. Moreover, the two genes were overexpressed in *C. glabrata* in a *CgPDR1*-independent manner using a strong constitutive promoter (*THD3*). The results showed that *CgCDR1* and *PUP1* deletion decreased virulence of *C. glabrata* in a mice model of infection (Ferrari *et al.* 2011b). Overexpression of these genes showed however intermediate phenotypes, thus suggesting that other *C. glabrata* factors are involved in the gain of virulence observed in drug-resistant isolates.

A follow-up study investigated the role of *CgPDR1* mutations (Vale-Silva *et al.* 2013) in the interaction with murine bone marrow-derived macrophages and human acute monocytic leukemia cell line (THP-1)-derived macrophages, as well as different epithelial cell lines. *CgPDR1* GOF mutations led to decreased adherence to and uptake by macrophages. The interaction with epithelial cells revealed an opposite trend, suggesting that *CgPDR1* GOF mutations may favor epithelial colonization of the host by *C. glabrata* through increased adherence to epithelial cell layers. These data reveal that *CgPDR1* GOF mutations modulate the interaction with host cells in ways that may contribute to increased virulence (Fig. 1), which is consistent with previously published results (Ferrari *et al.* 2009).

Recently, Borah *et al.* (2014) discovered an indirect connection between *CgPDR1*-mediated drug tolerance and the interaction with the host. They showed that a tail subunit of the RNA polymerase II mediator protein complex is essential for azole drug tolerance, on one hand, and on the other hand its deletion leads to hyperadherence to epithelial cells. The first observation can be explained with the disruption of the recruitment of the transcriptional machinery by CgPdr1 and, consequently, of efflux pump regulation. The adherence phenotype reflects transcriptional regulation of the *EPA1* and *EPA7* genes through an unknown mediator (Borah *et al.* 2014). An earlier report had

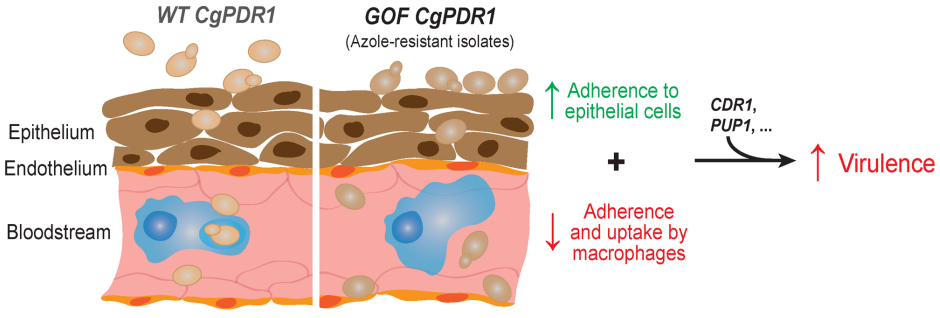

Figure 1. CgPdr1 hyperactivity modulates the interaction of *C. glabrata* with host cells. GOF mutations on *CgPDR1* not only allow resistance to azole drugs, but also enhance virulence of *C. glabrata* in murine models of disseminated candidiasis (partially mediated by *CgCDR1* and *PUP1* overexpression). This may be due at least in part to an increased adherence to epithelial cells and a decreased adherence to and phagocytosis by macrophages, mediated by GOF *CgPDR1* alleles in models of interaction of *C. glabrata* with murine and human cells *in vitro*.

already established a relationship between deletion of the sirtuin gene *HST1* and increased expression of *CgPDR1* and its target *CgCDR1* (Orta-Zavalza *et al.* 2013). This work suggests a connection between histone deacetylases, mediators of subtelomeric transcriptional silencing mechanisms, and *CgPDR1*, with possible implications for both drug resistance and the interaction with the host.

Mitochondrial dysfunction is one of the possible mechanisms by which azole resistance can occur in *C. glabrata*. Cells with mitochondrial DNA deficiency (so-called petite mutants) upregulate ABC transporter genes and thus display increased resistance to azoles. Interestingly, we recovered a mitochondrial clinical mutant (MIC fluconazole $> 256\ \mu g\ ml^{-1}$) from clinical origin and its respiratory-competent parent (MIC fluconazole $= 4\ \mu g\ ml^{-1}$), which enabled to address whether mitochondrial dysfunctions conferred a selective advantage during host infection. Surprisingly, even if the mitochondrial mutant was impaired in growth *in vitro* as compared to wild type, it was more virulent (as judged by mortality and fungal tissue burden) in systemic and vaginal murine infection models. The increased virulence of the petite mutant correlated with a drastic gain of fitness in mice as compared to its parental isolate (Ferrari *et al.* 2011a). Thus, this case highlighted that antifungal resistance in *C. glabrata* could result in a benefit for the fungal–host interaction, even if *in vitro* growth is severely affected (Ferrari *et al.* 2011a).

CONCLUSIONS AND PERSPECTIVES

The development of drug resistance without counterbalancing fitness costs, may be even showing a gain in fitness, represents a growing problem with potentially alarming consequences. Patients afflicted by systemic candidiasis are typically already severely ill and therapy is difficult, often not successful, even when the *C. glabrata* strain is not resistant to the drug. Consequently, it will be important to prevent the emergence of resistance in *C. glabrata*. Several measures can be taken targeting either the patient or the fungus. Therapeutic options aiming to modulate the host immune system can be envisaged; however, they will be confronted with the fact that a majority of infected patients have already partially compromised immune defenses. Therapeutic options targeting the fungus to limit resistance emergence can also be developed. Among them, drug combinations could be proposed and recent experimental infection models have demonstrated their efficacy in *C. glabrata* (Silva *et al.* 2013).

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