Candida albicans Niche Specialization: Features That Distinguish Biofilm Cells from Commensal Cells

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Abstract The fungus *Candida albicans* is a frequent commensal colonizer of the human gastrointestinal (GI) tract, but is also an opportunistic pathogen. This review explores features that distinguish the colonizing and pathogenic forms of *C. albicans*. *Candida albicans* in a biofilm is used as an example of a pathogenic form of the organism, because biofilms are a common feature of device-associated *C. albicans* infections. Biofilms (complex, sessile communities of cells) have been the subject of several large-scale gene expression studies. Biofilms and commensal *C. albicans* colonizing the murine GI tract show a variety of differentially expressed genes. Cell surface proteins encoded by these differentially expressed genes are especially attractive as targets for new clinical prevention, diagnosis, or treatment tools that are specific for *C. albicans* in its pathogenic biofilm state.

Keywords *Candida albicans* · Biofilm · Pathogenesis · Cell surface · Gene expression · Commensal · Gastrointestinal

Introduction

The opportunistic fungal pathogen *Candida albicans* is a common commensal colonizer of humans. However, the commensal organisms can give rise to pathogenic, disease-causing cells. How then can disease-causing organisms be distinguished from commensal colonizers? In this review, we

discuss differences in the features of commensal cells and pathogenic cells. The focus of this discussion is on the fungal cell surface, as the surface represents the part of the cell that is most available for interaction with the host and for interrogation with probes that might determine the status of this fungal cell. As an example of *C. albicans* in a pathogenic state, we focus on cells in biofilms. Biofilms (sessile communities of microbial cells that are encased in exopolymeric matrix and exhibit high resistance to antimicrobial drugs [1•, 2•, 3•]) are key in device-associated infections. Because of their drug resistance, biofilm infections are refractory to treatment with antifungal drugs, and device removal is often required to treat the infection. Using cells in biofilms as an example of pathogenic cells, we compare features of pathogens and commensals and identify molecules that could be used to distinguish these forms of *C. albicans*.

Candidemia and Candidiasis

Candida albicans is a common commensal colonizer of the human gastrointestinal (GI) tract [4, 5]. In addition, the organism asymptotically colonizes the oral cavity of 20–75 % of the general population [6]. When the human host is immunocompromised, however, *C. albicans* can cause significant morbidity associated with infection of the skin and mucosal surfaces, or can cause life-threatening candidemia [7–11]. Known risk factors for invasive candidiasis include many modern medical techniques, such as the use of central venous catheters (CVCs) [7, 12], deep surgery [7], and broad-spectrum antibiotics [12]. Invasive candidiasis is also associated with neutropenia [7, 12]. Of note, multiple studies have implicated *Candida* colonization of the GI tract as the main source of *C. albicans* found in candidemia [13–15], and a high host burden of colonizing *Candida* in the GI tract has been viewed as a risk factor for invasive candidiasis [16].

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Candidemia is the fourth most common cause of nosocomial bloodstream infection in the US [17•, 18], and causes significant increases in length of stay and per-patient hospital charges [19]. Most importantly, candidemia was associated with a 14.5 % increase in mortality in adult patients (10.0 % in pediatric patients) compared to matched hospitalized controls [19]. The crude mortality among patients with candidemia is 46–75 % [20], and of the ten most common causative species of healthcare-related bloodstream infections, *Candida* species are associated with the highest overall crude mortality [17•, 18]. Further, the risk of mortality in hospital-associated invasive candidiasis did not decrease between the 1980s and the late 2000s [20].

Recently, a large prospective, population-based surveillance for candidemia in the US was conducted in Atlanta, Georgia and Baltimore City/County, Maryland, from 2008 to 2011 [21]. In Atlanta, the crude incidence rate of candidemia was 13.3 per 100,000 person-years (40 % due to *C. albicans*), and in Baltimore the rate was 26.2 per 100,000 person-years (33 % *C. albicans*). The results of this study showed that the overall incidence of candidemia in adult populations has not decreased over the past 20 years [21].

Therefore, much work remains to be done to prevent candidemia-associated mortality. There is currently no vaccine against *Candida* infection [22, 23]. Blood cultures used to detect disseminated candidiasis require multiple days of incubation, and are negative in 30–50 % of patients with disseminated candidiasis [24]. Food and Drug Administration-approved tests for diagnosis of invasive candidiasis are currently limited to either culture-based tests, or an assay to detect beta-1,3 glucan [24], a carbohydrate that is plentiful in the cell wall of *Candida* [23, 25, 26]. In clinical settings, beta-1,3 glucan tests have been found to have a sensitivity and specificity of 64–77 % and 83–92.4 % [27, 28], and there were multiple clinical factors that contributed to false-positive results, such as hemodialysis, administration of albumin, or recent surgery using surgical sponges or gauze [27]. The net result of these many challenges is that mortality associated with candidemia continues to be very high.

Candidemia Associated with *C. albicans* Biofilms

Almost all device-associated *C. albicans* infections involve the growth of the infectious organism as a biofilm [29]. Biofilms are structured and complex communities of microorganisms that are attached to a surface and enveloped in a self-produced extracellular matrix (ECM) [3•, 17•, 30] which, in *C. albicans* biofilms, contains beta-1,3 glucan and other polymers [29, 31]. *Candida albicans* biofilms typically contain *C. albicans* cells in two different structural forms: a unicellular yeast form and a filamentous hyphal form [32, 33].

Biofilm-formation is important in *C. albicans* pathogenesis, and is considered to be a virulence trait of the organism [17•]. A recent experiment compared the mortality rates of wax moth larvae experimentally infected with clinical strains of *C. albicans* that had or lacked the propensity to form biofilms. Larvae infected with biofilm-producing strains showed significantly higher mortality [34]. Similarly, a mouse model of candidemia showed that *C. albicans* cells that had detached from a biofilm caused accelerated mortality, as compared to *C. albicans* cells grown in planktonic (nonbiofilm) form, despite the fact that both *C. albicans* populations were predominantly in the yeast morphology [35]. Furthermore, in patients with candidemia, biofilm-producing strains of *Candida* are associated with higher morbidity and mortality than strains that do not produce biofilms [17•].

Catheter-related candidemia, involving *Candida* biofilm formation on a catheter such as a CVC, has been reported to be present in 20–70 % of patients diagnosed with candidemia [24]. In such patients with catheter-associated infection, removal of the device is usually required in order to cure the infection [17•, 24]. Removal of the CVC in patients with candidemia is associated with decreased mortality and duration of the infection [36].

One of the traits that makes biofilm-associated candidemia so challenging to treat is the intrinsic drug resistance shown by *C. albicans* cells within a biofilm [3•, 17•, 29, 32, 37]. Compared to planktonic *C. albicans* cells, cells forming biofilms under laboratory conditions can show 1,000-fold greater resistance to certain antifungal drugs [38–40]. In an in vivo model of CVC-associated biofilm infection in rats, the *C. albicans* cells that were removed from the catheter-associated biofilm were shown to have >128-fold higher resistance to the antifungal drug fluconazole, compared to planktonically-grown cells of the same strain [41]. Multiple mechanisms contribute to this drug resistance, including limited penetration of the drug into the ECM, as well as increased expression of antifungal resistance genes (e.g., azole efflux carriers) by the cells within the biofilm [17•, 30, 32, 37]. Biofilm-associated *C. albicans* cells also exhibit increased resistance to killing by the host immune system [37, 41, 42].

When *C. albicans* cells form a biofilm or circulate in the bloodstream, how similar are they to cells that are colonizing the GI tract as commensals? To understand the specializations of *C. albicans* that adapt the cells to different niches within the host, we compare gene expression profiles of *C. albicans* cells growing under two conditions: as a commensal in the murine GI tract, and as a pathogen in a biofilm. We particularly focus on *C. albicans* genes that are not only expressed differentially during growth in different niches, but that also encode products that affect the surface of the cell, because surface molecules are accessible for potential use as targets for vaccines or diagnostic tests.

Distinguishing *C. albicans* Associated with Disease and *C. albicans* Associated with Commensal Colonization

The complete sequence of the *C. albicans* genome was released in 2004 [43, 44], and numerous studies have investigated the expression of large numbers of genes in *C. albicans* growing under different conditions. This section considers similarities and differences in gene expression between *C. albicans* cells growing as biofilms and those that are colonizing a mammalian host in the commensal state.

Several genome-wide microarray studies of gene expression in biofilms have been conducted. A recent comprehensive study was conducted by Nobile et al. using biofilm cells grown on plastic surfaces [45•]. A noteworthy earlier study, conducted by Andes et al., used biofilms formed on CVCs in rats as the source of cells for RNA extraction and microarray hybridization [41]. Additional studies employing various laboratory models of biofilm formation were conducted by Yeater et al. [46] and Garcia-Sanchez et al. [47]. For the discussion here, genes that were detected as upregulated in any of the biofilms studied (both in vivo and in vitro biofilms) relative to liquid-grown (planktonic) cells were considered part of the “biofilm gene set”. This set included 1,805 genes (out of over 6,000 total *C. albicans* genes) that were expressed at higher levels during biofilm growth as compared to standard laboratory growth in liquid culture.

To define the “commensal gene set”, studies of gene expression during commensal colonization of mice were examined [48, 49•]. In these studies, *C. albicans* cells were recovered from the cecal lumen of experimentally colonized mice and used for RNA extraction and microarray hybridization. The commensal gene set comprised 741 genes that in at least one of the studies were expressed at higher levels during colonization in comparison to standard laboratory growth in liquid culture.

A subset of genes in the biofilm gene set was also found in the commensal gene set (361 genes). To understand the nature of these genes, the Candida Genome Database Gene Ontology Term Finder (<http://www.candidagenome.org/cgi-bin/GO/goTermFinder>) was used to analyze the common genes. This method of analyzing gene sets searches for characteristics that are shared by several genes within the gene set. In the case of the genes present in both the biofilm and the commensal gene sets, genes involved in metabolic processes (especially monosaccharide metabolism), interaction with a host (especially adhesion to host), ion transport and oxidation–reduction processes were enriched. Therefore, these types of activities were relatively highly expressed in both cells in biofilms and cells colonizing the gut. Most likely, these genes represent activities that promote growth within the host, either as a commensal or as a pathogen.

The majority of genes in the biofilm gene set (1,444 genes) were not found in the commensal gene set. These genes may

represent adaptations that are specific to the biofilm lifestyle. The Gene Ontology terms that were characteristic of the biofilm-specific gene set were all related to protein translation (for example, ribosome biogenesis and ribosomal RNA processing). This observation is consistent with the previous demonstration that genes involved in the process of translation are upregulated in biofilms [47], but are expressed at lower levels during commensal colonization [48].

The importance of translation-related genes for biofilm development was demonstrated by Bonhomme et al. [50]. This group studied orf19.6090, a *C. albicans* gene that is homologous to *NSR1*, a gene required for the processing of pre-rRNA in the model eukaryote *Saccharomyces cerevisiae*. *NSR1* has been found to be upregulated in *C. albicans* biofilms in several studies [41, 46, 50], but not during commensal growth in the GI tract [48, 49•]. A *C. albicans* *NSR1* mutant shows reduced biofilm biomass relative to wild-type cells, indicative of defective biofilm growth [50]. These findings support the conclusion that optimal translation promotes biofilm development. Therefore, there are physiological differences between commensal and pathogenic cells.

Cell Surface Proteins Expressed Differentially in Biofilms and Commensal Colonization

To enable specific detection of pathogenic cells as opposed to commensal cells, cell surface proteins expressed in pathogenic cells but not in commensal cells could be used as probe targets. Therefore, to identify cell surface features that distinguish cells in biofilms from commensal cells colonizing the gut, the biofilm gene set and the commensal gene set were analyzed for cell surface genes.

Among the common genes, i.e., genes upregulated during both biofilm growth and commensal colonization, 26 genes encoding cell surface proteins were identified (Fig. 1a). Several of these genes are well studied genes whose products play a role in binding *C. albicans* to host cells (i.e., *ALS1*, *ALS3*, *HYR1*, *HWP1*, *PHR1*, *SAP9*, and *SUN41*; Fig. 1a). In addition, a subset of the 26 genes encoding cell surface proteins are also expressed during formation of *C. albicans* hyphae [51, 52], long filaments composed of highly elongated cells that remain attached after cell division. Expression of these 26 genes does not distinguish pathogenic cells from commensal cells.

In a search for surface proteins that are upregulated in pathogenic cells but not in commensal cells, an analysis of biofilm-specific genes found 45 genes encoding cell surface proteins, of which 10 are involved in biological adhesion and 8 are upregulated during hyphal growth (Fig. 1b) [51, 52]. Most of the 45 genes in this group are less well studied than the 26 common cell surface genes described above. Continued study of the biofilm-specific gene set is likely to lead to new insights into the mechanisms of pathogenesis.

a Genes annotated to cell surface compartment
Up-regulated in biofilms and in gut colonization

ADHESION TO HOST	HYPHAL	NON-HYPHAL
	ALS1 ALS3 HYR1 HWP1 PHR1	SAP9 SUN41
	HSP70 IHD1 INO1 PGA10 PGA7 RBT4 SOD5	CDR1 DDR48 ENO1 FBA1 HSP21 MP65 PGA34 PGA37 PGA56 PGA62 PLB3 TOS1

b Genes annotated to cell surface compartment
Up-regulated in biofilms, not in gut colonization

ADHESION	HYPHAL	NON-HYPHAL
	ALS2 ALS4 CSH1 HWP2 XOG1	ALS6 BGL2 IFF4 PRA1 SAP10
	PDC11 PGA55 SIT1	CHS3 DOT4 EBP1 EGD2 FET99 FRE10 GPD2 IFF3 IFF5 IFF9 KRE1 PGA11 PGA12 PGA13 PGA14 PGA16 PGA17 PGA22 PGA25 PGA27 PGA31 PGA39 PGA46 PGA50 PGA6 PHR2 RBR1 RBR2 SIM1 SOC4 SOD6 SPR1

Fig. 1 Genes expressed during biofilm growth. All of the genes shown in the figure are annotated to the cell surface compartment based on Gene Ontology annotations in the *Candida* Genome Database (<http://www.candidagenome.org/cgi-bin/GO/goTermFinder>), and upregulated in biofilms relative to cells grown in standard liquid cultures. Genes upregulated and not upregulated in *C. albicans* colonizing the murine gut relative to cells grown in standard liquid cultures are shown in **a** and **b**, respectively. Genes on the left of the vertical lines were found by Goyard et al. [51] or by Kadosh and Johnson [52] to be upregulated during hyphal growth relative to yeast cell growth. Genes above the horizontal lines are involved in adhesion, based on Gene Ontology annotations

In summary, genes encoding adhesion-related proteins are prominent among genes upregulated in biofilms. Also, some of the genes that are upregulated during biofilm growth are not upregulated in commensal colonization, making them attractive candidates for biofilm-specific cell surface markers that could be used as targets for diagnostic or therapeutic agents.

Beta-1,3 Glucan Production in Biofilms

In addition to a distinctive cell surface, cells in biofilms differ from commensals in that they are surrounded by an exopolymeric matrix that contributes to their characteristic drug resistance [29, 53, 54]. The polymer beta-1,3 glucan is a major constituent of this matrix, but it is not specific to

biofilm matrix and is also a major constituent of the fungal cell wall [23, 25, 26]. The regulatory events that lead to production of an ECM during biofilm growth are not completely understood.

Cells in biofilms also produce higher levels of soluble beta-1,3 glucan, which can be detected in culture supernatants and in the serum of rats carrying a *C. albicans*-infected CVC [24]. The production of soluble beta-1,3 glucan suggests that there are differences in the processing of beta-1,3 glucan in biofilm cells versus planktonic cells. Therefore, differences in the enzymes that produce or modify beta-1,3 glucan are expected in biofilms as compared to planktonic cells.

Synthesis of beta-1,3 glucan is accomplished by glucan synthase enzymes. There are three *C. albicans* genes that encode proteins predicted to have glucan synthase activity: *GSC1* (orf19.2929), *GSL1* (orf19.2495) and *GSL2* (orf19.3269). The *GSC1* gene product has been shown to synthesize beta-1,3 glucan [55], while the functions of *GSL1* and *GSL2* are not well understood. In addition, when the gene encoding one of the matrix-modifying enzymes Xog1, Phr1, or Bgl2 is deleted, the resulting mutant cells produce below-normal amounts of ECM and form biofilms with reduced resistance to fluconazole [56••].

All the genes *GSC1*, *GSL1*, *XOG1*, *PHR1* and *BGL2* have been shown to be upregulated during growth in biofilms both in a laboratory study [45••] and (except for *GSL1*) in the rat catheter model [41]. In contrast, of these five genes, only *PHR1* is upregulated during growth in the GI tract [48, 49••]. These results are consistent with observations that biofilm cells produce an extensive ECM but commensal cells do not.

Because of its important role in the enhanced drug resistance of cells in a biofilm [1••, 30, 37], the ECM represents a potentially useful target for therapeutic intervention. For example, reducing the production of ECM could enhance the activity of antifungal drugs against biofilms. A detailed understanding of the function of the various glucan synthesizing and modifying enzymes may enable the development of additional therapeutic agents directed against these proteins.

Conclusions

As noted above, candidemia is associated with high mortality, and diagnosis remains a challenge. As a result, empirical and prophylactic antifungal treatment strategies have been adopted. Antifungal prophylaxis against candidemia is effective in certain high-risk patient groups [12]. Currently, the Infectious Diseases Society of America recommends that empirical antifungal treatment be considered for critically ill patients at high risk of candidiasis [12]. However, the candidiasis risk factors that would determine antifungal prophylaxis usage are not well defined.

A recent study compared five different prediction rules for candidemia and invasive candidiasis published between 2003 and 2009 [36]. Among these five, only one prediction rule included a specifically *Candida*-related criterion, which was multifocal *Candida* colonization [36, 57]. All five prediction scores showed a sensitivity and/or a specificity level below 90 % [36, 58–60]. Thus, while these approaches have had some success, improvement is clearly needed.

It is an attractive possibility that a better understanding of the physiology and structure of *C. albicans* in biofilms might allow the development of new means of protection against, detection of, and treatment for candidemia. The identification of unique features of the cells in biofilms, including particular cell surface proteins and the expression of ECM, suggests that new armaments based on these molecules can be developed for medical interventions against candidiasis.

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Compliance with Ethics Guidelines

Conflict of Interest S.E. Herwald declares no conflicts of interest. C.A. Kumamoto declares no conflicts of interest.

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