Candida albicans and virulence factors that increases its pathogenicity

Cristiane Aparecida Pereira, **Anna Carolina Borges Pereira Costa**, **Michelle Peneluppi Silva**, **Graziella Nuernberg Back-Brito** and **Antonio Olavo Cardoso Jorge**

Department of Oral Bioscience and Diagnosis, Institute of Science and Technology, UNESP- Univ Estadual Paulista, Av. Engenheiro Francisco José Longo, 777, 12240000 São José dos Campos, SP, Brazil

Candida albicans are yeasts frequently human commensal and it is the main cause of different forms of oral, superficial or systemic candidiasis. The transformation from a harmless commensal to a virulent pathogen under the conditions of a dysfunctional host defense system is attributable to an extensive repertoire of selectively expressed virulence determinants. Several virulence factors contribute to the pathogenicity of *C. albicans*, including the ability to adhere to epithelial cells through production of hydrolytic enzymes, hemolytic factors and germ tubes formation. Cell surface hydrophobicity, also is considered important virulence factor, which increased the ability of mycelia-phase *Candida* cells to adhere to host tissues and for resistance to phagocytic killing. Additionally biofilm formation, which consists of a complex community of cells embedded in a matrix of extracellular polysaccharides that also promotes resistance to antimicrobial agents. Therefore, the aim of this chapter is a comprehensive analysis of virulence factors that increases the pathogenicity of *C. albicans.*

Keywords: *Candida albicans*; virulence factors, pathogenicity

1. Introduction

The most common fungal pathogen in humans is *Candida albicans*, causing infections both in immunocompromised and immunocompetent individuals [1]. This yeast is responsible for 90-100% of mucosal infections and for 50-70% of cases of candidemia [2,3]. *Candida albicans* is a commensal pathogen that lives on the skin and mucosal surfaces of the genital and intestinal tracts as well as the oral cavity [4]. An imbalance between the host immunity and this opportunistic fungus, as occurs in immunocompromised individuals, may trigger infection of the mucosal epithelia followed by dissemination via the bloodstream and infection of internal organs [5]. Candidiasis is the most common cause of fungal infections in humans and systemic involvement is associated with high mortality rates [6,7]. Several virulence factors contribute to the transformation from a harmless commensal to a virulent pathogen, under the conditions of a dysfunctional host defense system. These virulence factors determinants include the ability to adhere to host tissue, to produce extracellular enzymes and to undergo dimorphic transition [8]. Adherence to the host tissue, which is required for colonization and subsequent infection, is cited as the first stage of the infection process for the members of the genus *Candida* [9]. Extracellular hydrolytic enzymes, especially proteinases and phospholipases, are believed to play an important role in *Candida o*vergrowth because these facilitate adherence, tissue penetration and the subsequent invasion of the host. The ability of *Candida* to acquire elemental iron through hemolysin production is pivotal to its survival and ability to establish infections within humans, particularly disseminated candidiasis [10]. Another virulence factor is cell surface hydrophobicity, which is considered important for the ability of mycelia-phase *Candida* cells to adhere to host tissues and for resistance to phagocytic killing [11]. Additionally, *Candida* yeasts can also be organized into biofilms, which consist of a complex community of cells embedded in a matrix of extracellular polysaccharides. Cells in a biofilm exhibit phenotypic properties that are distinct from those of planktonic cells and demonstrate increased resistance to antimicrobial agents, primarily due to insufficient penetration of antifungals [12,13].

2. Extracellular Enzymes

C. albicans is a facultative pathogenic microorganism that has developed several virulence traits enabling invasion of host tissues and avoidance of host defence mechanisms. Virulence factors that contribute to this process are the extracellular enzymes [14]. Apart from facilitating the nutrient supply, these enzymes are involved in candidal: (1) invasion by digesting host cell membranes, (2) adhesion by degrading host cell surface molecules, and (3) resistance to host immunity by attacking the immune system [15].

Extracellular enzymes are important determinants for the development of diseases caused by opportunistic fungi, especially *Candida* spp. Enzymes may promote the fungus access to host tissues to drag nutrients, even penetrating the cells through haustorium-like structures [16]. When hydrolysis of substrate affects the function and viability of host's cells, the enzymes involved may be considered as virulence factors that contribute for the establishment of infection.

Extracellular hydrolytic enzymes such as proteinase and phospholipase are major facilitators of host tissue invasion and of the disease process that ensues [17].

The term phospholipase describes a heterogeneous group of enzymes with the ability to hydrolyse one or more ester linkages in glycerophospholipids. Besides *C. albicans*, extracellular phospholipases are considered as virulence factors for other fungi, many pathogenic bacteria and protozoa [14]. In *C. albicans*, four types are classified into phospholipase A, B, C, D, lysophospholipase, and lysophospholipase-transacylase according to their mode of action and the target within the phospholipid molecule, which is a major component of biological membranes [18]. Phospholipases act by cleaving the phospholipids, affecting the stability of the membrane and causing cell lysis. They seem to be connected to production of germ tubes, transition into hyphal forms, and tissue injury. Phospholipase production is concentrated on the tips of the hyphae, and the productive activity is greater when the hypha is in direct contact with the membrane, what shows that the extra-cell phospholipases are relevant in tissue invasion from *C. albicans* [18,19].

Barratt-Been et al. [20] examined phospholipase activity of yeasts including *C. albicans* and *C. parapsilosis* and assessed their virulence attributes such as adhesion to buccal epithelial cells and lethality in mice after intravenous inoculation. These experiments demonstrated positive correlations between phospholipase activity and fungal virulence proving it as an important factor in candidal virulence.

The secreted aspartyl proteinase (SAPs) degrades many human proteins on the lesion site, as albumin, hemoglobin and secretory immunoglobulin A (IgA-s) and skin proteins. The proteolytic activity has been associated with tissue invasion, contributing to tissue penetration by *Candida* spp. [19]. The proteolytic activity is attributed to a multigene Sap family of *C. albicans* with at least 10 members. Naglik et al. [21] reported that the expression of Sap-family genes is related to the co-regulation of other virulence factors, such as the biofilm formation, adherence, filamentation, in addition to pervasion, tissue invasion, nutrition and interaction with the host's immune system functions.

C. albicans strains from different group with various clinical infections have been isolated, and the level of Sap and phospholipase activity produced in vitro has been correlated with virulence. In oral candidiasis, the increased Sap and phospholipase activity occurred in *C. albicans* strains isolated from HIV-positive individuals [22, 23], diabetic patients with chronic periodontitis [24], patients with pulmonary tuberculosis [25], and patients with vaginitis [26]. *C. albicans* strains isolated from the oral cavities of breastfeeding infants and in their mother's mouths and nipples [27], and others *C. albicans* strains isolated from the oral cavities of smokers individuals [28] have also shown high levels of secretion of Sap and phospholipase.

Others extracellular enzymes are also involved in the *C. albicans* pathogenicity, such as hemolysin, chondroitinase, hyaluronidase and lipase.

The ability of pathogenic organisms to acquire iron in the mammalian host has been shown to be of critical importance in establishing infection. In humans, iron is primarily intracellular in the form of ferritin or heme-containing compounds. The small amount of extracellular iron is attached to the iron-binding and transport proteins transferrin and lactoferrin [29]. Therefore, microorganisms require iron acquisition mechanisms for their development.

Yeast destroys erythrocytes to obtain iron by producing substances known as hemolysins [30]. Hemolysin is another putative virulence factor thought to contribute to *Candida* pathogenesis. In particular, the secretion of hemolysins followed by iron acquisition facilitates hyphal invasion and the development of disseminated candidiasis [10]. It has been demonstrated that the hemolytic factor secreted by *C. albicans* causes the release of hemoglobin [31], which was further characterized as a mannoprotein that promotes the disruption of human erythrocytes by binding to their band three protein [32]. According to these authors, although systemic hemolysis may not be caused by the mannoprotein, hemolysis around infected sites may enhance fungal growth. Manns et al. [29] were the first to describe that *C. albicans* exhibits a hemolytic activity when grown on glucose-enriched blood agar. According to these authors, the hemolytic factor allows *C. albicans* to acquire iron from host erythrocytes.

The secretion of chondroitinase, together with hyaluronidase, is involved in fungal virulence, and the substrates of these enzymes are among the major constituents of the ground substances of connective tissue and the gingival epithelium. Chondroitinase and hyaluronidase enzymes can affect the permeability of the epithelium at the intercellular spaces by attacking the intercellular cementing substances of the tissue. As a consequence of this destruction, fungal microbial invasion of the tissue may occur [33]. Chondroitinase has been shown to be an important factor in the ability of *C. albicans* to adhere to host tissue during the mycelial phase of yeast cells and in determining its resistance to phagocytic killing. The secretion of lipases may also increase the pathogenicity of *C. albicans*, allowing their growth in environments in which lipids are the sole carbon source [34].

3. Cell surface hydrophobicity

The adhesion of microorganisms to host mucosal surfaces is a vital prerequisite for successful microbial colonization and infection, and its critical role in the pathogenesis of *Candida* infection is well recognized. Candidal adhesion has been implicated as the initial step in the pathogenesis of candidiasis and cell surface hydrophobicity (CSH) has been implicated in adhesion to mucosal surfaces [35].

Cell surface hydrophobicity (CSH) is connected with adhesion and pathogenic processes of *C. albicans*. Hydrophobic cells are more adherent than hydrophilic ones to epithelial and endothelial tissues as well as to abiotic surfaces [36,37]. CSH is an important virulence property conferred by the mannosylated surface proteins that coat fungal cells. Some of these proteins confer to the fungal cells the capacity to adhere to host cells or to inanimate

substrata and increase resistance to macrophages and germination competence, which are essential for the establishment of chronic lesions. Hydrophobicity of *C. albicans* cells alters in response to changes in environmental conditions (e.g. temperature, composition of medium) and growth phases [38] and can be switched between hydrophilic and hydrophobic phenotypes [39]. Hydrophilic cells have an elongated acid-labile mannan fraction in the cell wall and the length of this structure affects the folding of cell wall fibrils [40].

Adhesion studies show, either directly or by inference from the stated growth conditions, that hydrophobic *C. albicans* cells adhere better and with greater site diversity than hydrophilic cells to endothelial cells, epithelial cells, extracellular matrix proteins, and other host tissues [41-45]. Thus, surface antigenic changes related to hydrophobicity may provide a fungal virulence strategy for evasion of immune responses and for selective adhesion interactions with host cells [36].

The relative CSH of *C. albicans* is considered a non-biological factor of critical importance pertaining to candida adhesion. For instance Hazen and Hazen [46] have demonstrated that hydrophobic yeasts are more virulent than their hydrophilic counterparts. Hydrophobic cells also exhibited greater adherence to epithelial cells and extracellular matrix proteins and decreased susceptibility to phagocytic killing. Additionally, it has been stated that enhanced virulence of hydrophobic cells over hydrophilic cells may be due, in part, to the potential of hydrophobic cells to bind throughout various organs following clearance from the bloodstream [45].

4. Germ Tube

C. albicans is a pleomorphic opportunistic pathogen that takes different morphological forms under different environmental conditions, including budding yeast cells, germ tubes, and true hyphae and pseudohyphae [47]. Germ tubes, which mark the onset of hyphal growth, are a phenotypic characteristic of *Candida* that has been implicated in the pathogenesis of candidiasis [48].

Germ tubes are cylindrical extrusions known to facilitate yeast adherence to epithelial cells and impart resistance to phagocytic killing compared with the blastoconidia form [49]. The cell wall of the germ tube features some characteristics that facilitate the establishment of infection. The presence of specific surface antigens and the expression of surface proteins promote the interaction of the germ tube with tissue or host proteins, allowing the formation of pathogenic biofilms [50,51]. Furthermore, germ tubes tend to promote the aggregation of yeast cells and the bridging of adjacent hyphal elements, thereby bringing a large number of organisms into intimate contact with the oral epithelium [52].

The development of germ tube was reported to be inhibited as a response to stress, including oxidative stress, caused by exposure of *C. albicans* to immune system cells [53]. An oxidative-tolerant mutant developed by exposure of *C. albicans* to an oxidant agent also caused reduction of germ tube and pseudohypha formation, less extracellular phospholipase production, and less pathogenicity in mice, as an adaptative response to oxidative stress [54].

Ibrahim et al. [55] assessed the virulence of isolates of *C. albicans* from blood and commensal sources, and reported that the blood isolates were capable of producing longer germ tubes, in greater frequency, than the commensal ones. Those data reinforce the idea that the ability of *C. albicans* to change its cellular morphology from blastoconidia to hyphae contributes to the pathogenicity of the fungus [56]. The elevated presence of germ tubes in *C. albicans* strains isolated from individuals with denture stomatitis lead to the establishment of a correlation between the germ tube production as a factor of virulence of *C. albicans*, and its capacity to cause infection[57]. In the study of Kato et al., [58], mice infected with *C. albicans* that had been preexposed to antimicrobial photodynamic inactivation (APDI) developed a less aggressive infection, with increased mouse survival. This finding correlated with the inhibition of cell growth and germ tube formation caused by APDI.

5. Biofilm

C. albicans and a small number of related *Candida* species are known to be important agents of hospital-acquired infections. Many of these are implant-associated infections in which the microorganisms form adherent biofilms on the surfaces of catheres, joint replacements, prosthetic heart valves and other medical devices [59,60].

Biofilms are complex surface-associated cell populations embedded in an extracellular matrix that possess distinct phenotypes compared to their planktonic cell counterparts. Nutrients, quorum-sensing molecules, and surface contact are contributory factors. *C. albicans* biofilms are comprised primarily of yeast-form and hyphal cells, both of which are required for biofilm formation. Formation is a sequential process involving adherence to a substrate (either abiotic or mucosal surface), proliferation of yeast cells over the surface, and induction of hyphal formation [61]. Extracellular matrix, accumulates as the biofilm matures, and seems to contribute to cohesion [62]. *C. albicans* biofilms form on numerous abiotic [63] and biotic surfaces [64-66]. This can be considered an important virulence factor since it implies a greater propensity to develop drug resistance and to evade the host immune response [67-69].

Biofilm cell communities are more resistant to antifungal drugs than planktonic cells. Contributing factors include biofilm structural complexity, presence of extracellular matrix, metabolic heterogeneity intrinsic to biofilms, and

biofilm-associated up-regulation of efflux pump genes. The actual fold increase in resistance varies with both the drug and species. *C. albicans* biofilms are relatively resistant to fluconazole, amphotericin B, nystatin, voriconazole, and others [70].

Biofilm formation is a major virulence factor in the pathogenicity of *C. albicans*, especially because of their very high antifungal resistance. Consequently, research into the pathogenicity of *Candida* has focused on the strategies for preventing biofilm formation and dispersing established biofilms. Among the alternative therapies, the use of natural medicines obtained from the plants [71,72], and APDI [73-75] has received highlights due to promising results in the control of *C. albicans* biofilms.

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