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Virulence factors of *Candida* species

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Candida albicans is an opportunistic human pathogen, which colonizes at several anatomically distinct sites including skin, oral, gastrointestinal tract, and vagina. How harmless commensal *C. albicans* become a human pathogen when opportunity comes is not clear. This review will focus on the molecular dissection of virulence factors of *C. albicans*, including adhesion, proteinases secretion, hyphal formation, and phenotypic switching. This review will also describe briefly the virulence factors in non-albicans *Candid*a spp.

Key words: *Candida*, virulence factors

Cellular immunity is the primary host defense against microbial infections. In the healthy host, opportunistic fungal pathogens are commensal fungi commonly colonizing human mucosal surfaces. Fungal pathogens cause from minor infections in immunocompetent individuals, such as thrush in babies and vaginal infections in women, to fatal, systemic infections in immunocompromised patients. *Candid*a species now rank as the fourth most common cause of nosocomial bloodstream infections in the United States and the attributable mortality rate is 35% [1]. Approximately 70% of women experienced vaginal infections caused by *Candida* spp. and 20% of them suffered from recurrence [2]. The increase in the proportion of bloodstream infections due to opportunistic fungal pathogens is likely associated with increasing number of critically ill patients, surgical procedures, cytotoxic therapy with prolonged neutropenia, other immunosuppressive therapies, use of broad-spectrum antibiotics, indwelling invasive devices, and intensive care support [3,4]. Many of the currently available drugs have several problems, namely, having undesirable side effects, being ineffective against new or reemerging fungi, and leading to the rapid development of resistance, which have profound effects on human health. The rise in the prevalence of fungal infections has exacerbated the need for the new effective antifungal agents. One way to develop more effective antifungal agents is to understand the mechanism of pathogenicity.

Virulence Factors

The proteins encoded by genes on the pathogenicity islands found in many enteric bacteria are defined as virulence factors for primary pathogens. There are several lines of argument for what real virulence factors are in *C. albicans*, an opportunistic pathogen. They are as follow: "all traits required to establish disease" [3], "factors that interact directly with mammalian host cells" [5], and "a component of a pathogen that damages the host" [6]. In order to establish an infection, opportunistic pathogens have to evade the immune system, survive and divide in the host environment and spread to new tissues. *Candida* spp. colonize and/or cause diseases at several anatomically distinct sites with unique physiological environment including skin, oral cavity and esophagus, gastrointestinal tract, vagina, and vascular system. *C. albicans* expresses PHR1 in bloodstream or in tissues to adapt the neutral pH, while it expresses RPH2 in vaginal canal to survive at acid pH [7,8]. The homologues of *PHR1* and *PHR2* in *Candida dubliniensis* have been cloned and their functions are similar to that in *C. albicans* [9]. *EPD1*, *2* of *Candida maltosa* are similar in sequence to *PHR1*, *2* of *C. albicans*. Epdlp could be involved in cell wall maintenance and is essential for pseudohyphal growth. The transcription of *EPD2* is induced strongly when cells are grown in SD medium of higher pH (pH 7), but not in SD medium of lower pH (pH 4) [10]. Although the infections caused by non-albicans *Candida* species have increased, *C. albicans* is still the most common pathogen among fungal pathogens and has been established for the molecular study. This review focuses on genes for adhesion, proteinases secretion, hyphal formation, and phenotypic switching in *C. albicans* (Table 1 and Fig. 1).

Adhesion

Adherence of *C. albicans* to host cells is seen as an essential early step in the establishment of disease. In

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Table 1. Genes involved in virulence of *Candida* species

Function	*C. albicans*	Non-*albicans*	Ref
Adhesion	*ALS1*		[14,15]
	ALS5 (*ALA1*)		[14]
	HWP1		[17]
	INT1		*[18]*
	MMT1		[21]
	PMT1		[23]
	PMT6		[22]
		ALS	[24]
		EPA1	[25]
Proteinase and phospholipase			
	SAP1-9		[29]
	CaPLB1		[44]
	CaPLB2		[44]
	CaPLC		[44]
	CaPLD		[44]
		SAP1-4	[31,41]
		SAP1-7	[42,43]
		PLB	[47,48]
Hyphal formation			
	CPH1		[49]
	EFG1		[49]
	INT1		[18]
	TUP1		[61]
		CLS12	[59]
		CPH1	[43]
Phenotypic switching			
	SAP1		[29]
	SAP3		[34]
	WH11		[65]
Environment adaptation			
	PHR1		[7]
	PHR2		[66]
		PHR1	[9]
		PHR2	[9]
		EPD1	[10]
		EPD2	[10]

addition, *Candida* spp. can also adhere on the surfaces of medical devices and form biofilms, which results in an increase in candidemia and antifungal resistance related to catheter insertion [11,12]. There is a positive association between the degree of virulence and the ability to form biofilms [12].

Agglutinin-like sequence (ALS) family is consisted of several glycosylated proteins with 3 common features, a conserved 5' domain, a central domain containing 108 bps tandem repeats, and a serine-threonine-rich 3' domain. These proteins have homology to α-agglutinin, required for cell-cell recognition during mating in *Saccharomyces cerevisiae* [13]. Als1p and Als5p (Ala1p) have an adhesin function to human buccal epithelial cells (HBEC) and fibronectin, respectively [14]. Als1p is important for the adherence of the organism to the oral mucosa during the early stage of the infection [15].

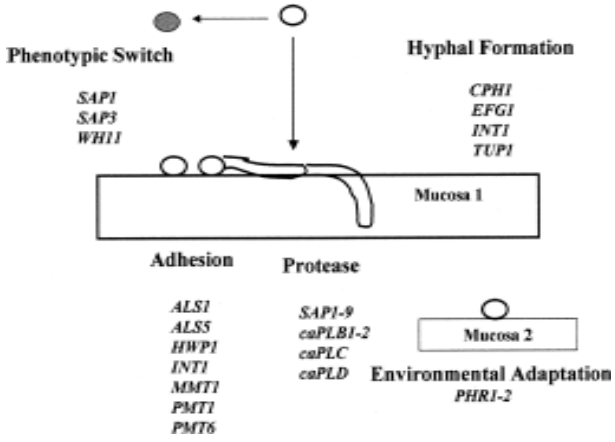

Fig. 1. The genes involved in the virulence of *Candida albicans*. Rectangles with mucosa indicate host cells under different environments. Genes discussed are listed under each category of virulence factors (in bold).

Germ tubes are the initial projections observed when *Candida* switches from yeast form to hyphal growth. *C. albicans* germ tubes form stable complexes with HBEC. The amino acid sequence of the N-terminal domain of Hwp1p resembles mammalian TGase substrates [16] suggesting that Hwp1p is involved in the formation of stable complexes with BEC. Hwp1p contains a cell surface-exposed N-terminal domain and C-terminal features conferring covalent integration into cell wall [16]. It has been reported recently that the *hwp1/hwp1* mutant strain was greatly impaired in the ability to form stable attachments to HBEC even though total adherence to BEC was not changed. This mutant was less virulent than the HWP1/HWP1 strain in a mouse model of systemic candidiasis [17].

Int1p is similar to vertebrate leukocyte adhesions, which bind extracellular matrix proteins and induce morphologic changes in response to extracellular signals [18,19]. Cells of the budding yeast *S. cerevisiae* expressing the *INT1* gene switch to filamentous growth. Disruption of *INT*1 in *C. albicans* reduces 40% adhesion to human epithelial cells. The mutation in *INT1* reduced the virulence in *C. albicans* in a mouse model [18]. A cytoskeleton protein Sla2p may interact with Int1p to mediate morphogenesis by modulating the actin cytoskeleton [20].

The adhesion and virulence were affected by a deletion of α-1,2-mannosyltransferase gene (*MNT1*) [21]. An O-glycosylation mannosyltransferase (*PMT1* and *PMT6*) is also required for adherence to an epithelial cell line [22,23]. All 3 genes are involved in mannan synthesis and mannan is a major constituent of the fungal cell wall. Thus, Mnt1p, Pmt1p, and Pmt6p may be involved in host recognition [13].

At least 3 ALS genes in both *Candida tropicalis* and *C. dubliniensis* have been identified through southern analysis and western blotting with an anti-ALS antibody [24]. A mutation in *EPA1*, encoding a lectin in *Candida glabrat*a, reduced 95% of ability to bind epithelial cells *in vitro*. However, this mutant had a very significant difference from wild-type cells in mucosal candidiasis [25]. Finally, there are also interesting proteins that have been assigned an adhesin function even though their encoding genes have not been identified, such as a mannan adhesin [26] and a fimbrial adhesin [27] in *C. albicans* and an integrin in *C. tropicalis* [28].

Proteinases secretion

Secreted aspartyl proteinases (SAPs) degrade many human proteins at lesion sites, such as albumin, hemoglobin, keratin, and secretory immunoglobulin A [29]. To date, 9 different *SAP* (*SAP1-9*) genes have been identified in *C. albicans*. Their proteolytic activity has been associated with tissue invasion [30]. The expression of *SAP*s has also been observed in murine macrophages after phagocytosis of *C. albicans* cells [31]. The observations described above suggest that proteolytic activity of Saps is important for the virulence of *C. albicans*. A specific inhibitor of acid proteinases, pepstatin, blocks *C. albican*s the events occurring in early invasion of murine skin and modulates the course of experimental candidasis in mice [32]. The *SAP* gene in *C. albicans* is regulated at the transcriptional level and processed by a signal peptidase and a Kex2-like proteinase [33]. *In vitro* studies show that *SAP1*, *2*, and *3* are expressed by yeast cells only, whereas *SAP4-6* expression is confined to hyphae. The expression of *SAP4*, *5*, and *6* has been detected in *C. albicans* undergoing a transition from yeast to hyphae at neutral pH [29,34]. The expression of *SAP7* has never been detected under any laboratory growth conditions. *SAP8* transcripts have been detected in yeast cells grown at 25^{o}C in a defined medium and *SAP9* is preferentially expressed in later growth phases [35].

The transcripts of *SAP2*, *SAP4*, *SAP5*, and *SAP6* can be detected in both asymptomatic *Candida* carriers and patients with oral candidiasis. Interestingly, the expression of *SAP1* and *SAP3* transcripts is in oral candidiasis patients but not in *Candida* carriers. *SAP1-3*, *SAP 6*, and *SAP8* are detected in oral and skin infections but not in vaginal infections by reverse transcriptase-polymerase chain reaction (RT-PCR) and immune microscopy. In the human epidermis model, *SAP1-2* expressed first for early invasion, then *SAP8* for extensive penetration, and finally *SAP*6 for extensive hyphal growth [36]. The disruption of *SAP1*, *SAP2*, or *SAP3* showed attenuated virulence in a mouse model [37]. The triple disruption of the *SAP4-6* genes was less virulent than the *sap1/sap1*, *sap2/sap2*, and *sap3/sap3* single mutants. The roles of Sap1p in skin colonization are both cellular, leading to increased adhesion, and extracellular, leading to tissue penetration [38]. The product of *SAP2* is required for disease development in a vaginitis model [39].

SAP1-4 genes were identified in *C. tropicalis* and Sap1p is the predominant enzyme produced *in vitro*. The transcript level of the remaining 3 *SAP* genes is low and can only be detected by RT-PCR [40]. However, mutation in *SAP1* had little effect on virulence in *C. tropicalis* [41]. At least 7 proteases have been identified in *C. dubliniensis* [42]. Although there are some proteinases detected in *C. glabrata*, *Candida parapsilosis*, *Candida lusitaniae*, *Candida krusei*, and *Candida guillermondii* [13,43], the roles of these proteinases have not been well defined.

Phospholipases hydrolyze one or more ester linkages of glycerophospholipids. According to the different and specific ester bond cleaved, these enzymes have been classified into phospholipases A, B, C, and D [44]. Genes encoded for phospholipases B, C, and D have been identified. Phospholipase B, the major activity of phospholipases in *Candida* species, has both hydrolase (fatty acid release) and lysophospholipase-transacylase activities [44]. Extracellular phospholipases secreted from *C. albicans* was first identified by growing the cells on solid media containing egg yolk or lecithin and analyzing the lipid breakdown products [45]. The activity of phospholipids was highest where the hyphae were in direct contact with the object [46]. *C. albicans* strains isolated from blood produce higher level of phospholipases than commensal strains. Cells producing less phospholipases are less virulent than strains producing high phospholipases in a murine model suggesting that phospholipases may be virulence factors. The activity of phospholipases has been identified in many fungal pathogens including *Candida* species, *Cryptococcus neofomans*, and *Aspergillus fumigatus* [44,47,48].

Hyphal formation

The ability to switch between the yeast form and the filamentous form is thought to be important for the virulence in *C. albicans* (Fig. 2) [49]. A filamentous cycle was discovered in a laboratory strain of *S. cerevisiae* [50], opening new doors for research into signal transduction pathways that regulate morphogenesis in *C. albicans*. It has been reported that

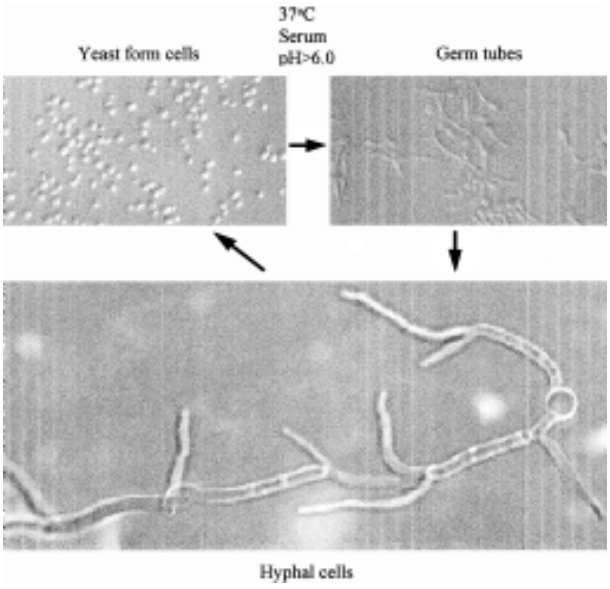

Fig. 2. The switch between the yeast form and the filamentous form of *Candida albicans*. Arrow indicates the direction of morphological transformation. The condition for induction of germ tube formation is indicated at the top of the arrow.

in *S. cerevisiae* this switch is controlled by 2 regulatory proteins, Ste12p and Phd1p [49]. Ste12p is a transcription factors and involved in mating and pseudohyphal growth pathways [51]. Phd1p belongs to the family of basic helix-loop-helix transcription factors [52]. Single mutant strains, *ste12/ste12* or *phd1/phd1*, are partially defective, whereas the *ste12/ste12 phd1/phd1* double mutant is completely defective in filamentous growth and is non-invasive. The equivalent *cph1/cph1 efg1/efg1* double mutant in *C. albicans* (Cph1p is the Ste12p homolog and Efg1p is the Phd1p homologue) is also defective in filamentous growth, unable to form filaments in response to many stimuli including serum or macrophages. This *Candida cph1/cph1 efg1/efg1* double mutant is avirulent in a mouse model [49]. The *cph1/cph1 efg1/efg1* double mutant is not completely defective in many functions associated with virulence. Riggle *et al* have showed that this *cph1/cph1 efg1/efg1* double mutant forms filaments in a matrix or rough surface. Immunosuppressed gnotobiotic newborn piglets infected with this mutant developed mild thrush lesion and superficial lesions of eyes [53]. This study has also showed that the genetic pathway controlling filamentous growth is a common scheme among fungal pathogens. However, it is still unclear whether it is the yeast or the hyphal form responsible for pathogenicity. It is a possibility that genes unrelated to cell shape but regulated by transcription factors Cph1p or/and Efp1p are required for the virulence.

The phosphorylation of Cph1p is regulated through a conserved mitogen-activated protein kinase pathway including *CST20*, *HST7*, and *CEK1*, which are *Saccharomyces* homologues of *STE20*, *STE7*, and *KSS1*, respectively [54,55]. *RAS1*, adenyl cyclase, protein kinase A (*TPK2*) are involved in the Efg1p pathway [13,49,54,55]. It has been proposed that *RIM101/PRR2* may regulate *EFG1* [56]. The *CRK1* gene, belonging to the Cdc2 kinase subfamily, is required for conversion of filamentous growth and *crk1/crk1* cells are avirulent in the systemic murine model [57]. Gale *et al* showed that Int1p in *C. albicans* is involved in adhesion as well as filamentous growth. Disruption of this gene in *C. albicans* reduced the virulence in a mouse model of systemic candidiasis [58].

The homologue of *CPH1* in *C. lusitaniae* (CLS12) has been cloned and its function in mating has been confirmed [59]. The homologues of *CPH1* in *C. glabrata* has also been cloned and it regulates cell wall biosynthesis and architecture in addition to its involvement in virulence [43]. Tup1p of *S. cerevisiae* is a global repressor that is recruited to promoter regions by interaction with sequence-specific DNA binding proteins [60]. Tup1p in *C. albicans* is a negative regulator of filamentation since the *tup1/tup1* mutant displays constitutive filamentation. Surprisingly, *tup1/tup1* hyperfilamentous strain has diminished virulence [61]. These findings suggest that only strains that can both form filament and produce yeast form cells are capable of penetrating vital organs and proliferating sufficiently to kill the host.

Phenotypic switching

The colonies of *C. albicans* can switch among different phenotypes including smooth, rough, star, stippled, hat, irregular wrinkle, and fuzzy at high frequency (10^{-4} to 10^{-1}) [62]. However, the basic mechanism of phenotypic switching and the involvement of this switching in the virulence of *C. albicans* are not clear. Smooth and white colonies with round-ovoid cells (white) can switch to flat and gray colonies with elongated or bean-shaped cells (opaque). *OPA1* (*SAP*1) and *SAP3* are expressed specifically in opaque cells, whereas *SAP2*, *WH1*1, and *EFG1* are expressed specifically in white cells. Opaque-phase cells have higher capability to colonize the skin in a cutaneous model and higher frequency for mating than do white-phase cells [63,64]. Interestingly, opaque cells are less virulent than white cells in a systemic animal model [38].

Conclusions

Changes in the host are generally required for

opportunistic pathogens to change from harmless commensal microorganisms to fatal human pathogens. The opportunistic pathogens utilize several genes whose functions in adhesion, proteinases secretion, hyphal formation, and phenotypic switching are required for virulence. Several virulence factors identified in *C. albicans* have homologues in other *Candida* species. It would be interesting to determine if these homologues in other *Candida* species also encode virulence factors.

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