ROLE OF ASPARTIC PROTEINASES IN CANDIDA ALBICANS VIRULENCE.

PART II: EXPRESSION OF SAP1-10 ASPARTIC PROTEINASE DURING CANDIDA ALBICANS INFECTIONS IN VIVO

ROLA PROTEAZY ASPARTYLOWEJ W WIRULENCJI CANDIDA ALBICANS
CZĘŚĆ II: EKSPRESJA SAP1-10 PROTEAZY ASPARTYLOWEJ PODCZAS ZAKAŻEŃ CANDIDA ALBICANS IN VIVO

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Wpłynęlo w maju 2012 r.

1. Ekspresja genów proteazy aspartylowej podczas zakażeń Candida albicans in vivo. 2. Ekspresja genów proteazy aspartylowej u innych gatunków z rodzaju Candida. 3. Inhibitory proteazy aspartylowej. 4. Podsumowanie

Abstract: Candida albicans is an opportunistic fungal pathogen known to produce several secreted hydrolytic enzymes, among which aspartic proteinases are considered to be a key virulence factor in pathogenesis. During last decade, Saps have been extensively studied in several in vivo studies based on human samples and animal models. It has been demonstrated that SAP5 and SAP9 are the most highly expressed proteinase genes in vivo. Despite many studies, very little is known about SAP7 and SAP8 role in C. albicans pathogenesis. Moreover, this review presents Sap regulation by nutritional supplementation and environmental factors, i.e. temperature, pH and the growth phase of C. albicans cells. In addition, Saps presence is discussed in Candida tropicalis as well as Candida parapsilosis and Candida guilliermondii as contribution of these non-albicans Candida strains in clinical infections is gradually increasing. Furthermore, the review underscores the need for studies using Sap enzymes as a potential drug-target due to their key role in virulence of Candida spp. The studies using the classical aspartic PI pepstatin A and HIV PIIs provided evidence for the contribution of Sap to C. albicans virulence. Therefore, more conclusive studies concerning the 10 SAP gene expression and their regulation during infective process, association of Saps production with other virulence processes of C. albicans and Saps immune response in animal and human infection still have to be conducted.

1. Aspartic proteinase genes expression during Candida albicans infections in vivo. 2. Other non-albicans species that produce aspartic proteinases. 3. Aspartic proteinase inhibitors. 4. Summary

Słowa kluczowe: Candida albicans, kandydoza, proteaza aspartylowa, wirulencja

Key words: Candida albicans, aspartic proteinases, candidiasis, virulence

1. Aspartic proteinase genes expression during Candida albicans infections in vivo

Enzymatic activities of C. albicans have received considerable attention in several in vivo studies [7, 15, 36, 42, 44]. Thus, the role of Saps in the development and progression of candidiasis has been studied for systemic and mucosal candidal infections [4, 23, 31, 37, 43, 53]. In addition, the research involving human samples and animal models are discussed in the manuscript (Table I). With regard to human mucosal infections, an early study by Schaller et al. [43] suggested pathogenetic role of the Sap1-3 during oral candidiasis. This notion was supported when a sensitive, RT-PCR technique was used that was able to detect SAP1 to SAP3 transcripts in patients with oral candidiasis [34]. On the contrary, Huber and Naglik [22] demonstrated the production of Sap1-8 proteinases during oral and vaginal candidiasis. The results indicated that SAP1, SAP3, SAP4, SAP7, SAP8 expression was correlated with oral disease, whereas SAP1, SAP3, and SAP6-8 expression was correlated with vaginal disease. It should be noted that although SAP1, SAP3, and SAP8 were expressed either in oral or vaginal infections, the SAP1-3 were preferentially expressed in vaginal, rather than oral, infections. Since then to more recent study [37], the results indicated either the differential expression of the SAP in humans or correlation these genes with active disease

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and anatomical location. Address to the above, the letter authors [37] found that SAP5 and SAP9 are the most highly expressed proteinase genes in vivo.

Using animal models by Stringaro et al. [53] and RHE by Schaller et al. [46] both groups demonstrated that Sap1 and Sap2 are favoured during experimental rat vaginitis and actively secreted by C. albicans hyphal forms. Other studies [15, 41, 50, 51] showed that Sap4 and Sap6 are constitutively expressed during intraperitoneal infection of mouse models, while Sap2, Sap3, and Sap5 are only Occasionally detected. A more recent study by Correia et al. [11] suggested that Sap1 to Sap6 do not play a significant role in C. albicans virulence in a murine model of hematogenously disseminated candidiasis and that Sap1 to Sap3 are not necessary for successful C. albicans infection. Finally, it was emphasized that Sap4-6 proteins play role during the initial phase of organ invasion, while Sap1-3 proteins play part in the later phases of the pathogenesis process. A more recent study by Jackson et al. [23] found that morphogenic conversion of C. albicans is closely associated with Sap6 production during corneal infection. Moreover, Jackson et al. [23] using Sap mutants suggested that SAP6 is involved in the pathogenesis of C. albicans keratitis as SAP6-altered strains did not establish persistent infection and failed either to invade or to trigger inflammation. Furthermore, reintroduction of the SAP6 gene into the fungal genome reconstituted corneal pathogenicity [23].

However, virulence differences in human data and previous mouse data (mentioned above) depend on dif-

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Table 1

Expression of secreted aspartyl proteinases in human and in animal models
different isolates of *C. albicans* and different infection models or molecular techniques employed. Animal models are powerful tools to study the pathogenesis of diverse types of candidiasis [9]. Moreover, murine models are particularly attractive because of cost, easy of handling, and experience with their use [9]. Despite of the above, there are differences in the interaction of *C. albicans* with these two mammalian species because of *C. albicans* is a natural commensal/pathogen of human but not of mice [37].

In general, Sap expression is regulated by nutritional supplementation and environmental factors, i.e., temperature, pH and the growth phase of *C. albicans* cells [35]. Chen et al. [8] determined that change of pH and temperature in the culture medium affects Sap4-6 expression. According to some authors [1, 22, 28, 46], Sap2 and Sap3 expression were optimal at pH 3.5 while Sap6 was most efficiently expressed in a less acidic (pH 5.0) medium as determined by tests conducted at 37°C in media with nitrogen source. Furthermore, according to Wu et al. [57] low levels of extracellular Sap may also stimulate the transcription and translation of Sap genes. Human serum provides proteins such as albumins and globulins generally considered to be cleaved by Sap enzymes which provide the essential nitrogen for cell growth [51, 55]. Early work [6] claimed that among substrates hydrolyzed by Sap are: salivary lactoferrin, lactoperoxidase, mucin, secretory immunoglobulins, including secretory IgA. Moreover, three reports [6, 35, 46] noted that Sap2 exhibits broad substrate specificity. Their findings showed that collagen, stratum corneum, laminin and fibronectin are efficiently degraded by Sap2. The first experimental evidence [9, 10] suggests that Sap2 may be involved in the progression extracellular digestion of the intestinal mucus barrier observed after oral-intra gastric inoculation of *C. albicans* in the infect mouse model. In addition, L e r m a n n and M o r s c h h ä u s e r [29] found that only SAP1 is required for *C. albicans* growth in YCB-BSA medium (yeast carbon base – bovine serum albumin). The authors examined SAP4 Δsap5Δ Δsap6Δ triple mutant and demonstrated that SAP2 expression does not depend on any of those other proteinases. In summary, the studies [1, 6, 20, 21, 35, 38, 46] noted that Sap2 is the most abundant secreted protein in vitro under growth in presence of exogenous protein. Thus, in these conditions high levels of aspartic proteinases must be secreted to support growth [20].

Very little is known about SAP7 and SAP8 role in *C. albicans* pathogenesis, however according to Hornbach et al. [19] the expressions of Sap7 and Sap8 do not correlate with virulence. Furthermore, A l b r e c h t et al. [4] note that mutants lacking SAP9 and/or SAP10 had altered adhesion properties and were mitigated in inducing tissue damage. According to Hornbach et al. [19] expression of SAP9 is possibly independent of pH and morphotype. Studies of *C. albicans* gene expression during experimental infection reveal that different stress responses are mounted during different types of infections, presumably because different environments present different challenges [27]. In the future the search for Saps involvement in virulence and the molecules that determine the differences in Sap expression by quantification of experimental data, with using real-time PCR ought to be conducted.

2. Other non-*Candida albicans* species that produce aspartic proteinase

Many pathogenic non-*C. albicans* fungi of the genus *Candida* including such species as *Candida glabrata*, *C. dubliniensis*, *C. tropicalis* and *C. parapsilosis* possess SAP genes [24, 36]. For example, experiments held with using SAP1 DNA as a probe allowed identifying cross-reacting bands in the genomic DNA of *C. tropicalis*, *C. parapsilosis* and *C. guilliermondii* [18, 32].

The yapsins of *S. cerevisiae* are a family of five glycosphatidylinositol (GPI)-linked aspartyl proteinases (Yps1-3, Yps6, and Yps7) that have been shown to cleave peptides C-terminal to basic residues both in vitro and in vivo [24, 26]. The *S. cerevisiae* YPS genes are induced during cell wall remodelling; furthermore they have homologues in other fungi such as *C. albicans* as well as *C. glabrata* [26]. In *C. albicans*, the Yps-related proteinases are Sap9 and Sap10 [24]. Kaur et al. [24] using mouse macrophage-like cell line J774A.1 showed that macrophage-internalized *C. glabrata* exhibit transcriptional induction of a specific for this species cluster of genes encoding a family of putative aspartyl proteinases. Furthermore, 11 GPI-linked aspartyl proteinases are encoded by *C. glabrata* are closely related to YPS genes of *S. cerevisiae* [24]. Kaur et al. [24] noted that these genes are also required for survival within macrophages and for virulence in a murine model of disseminated candidiasis caused by *C. glabrata*. However, no typical SAP genes were described in *C. glabrata* [39].

Previously, the existence of the SAP1 gene family in *C. tropicalis* was suggested [32]. Furthermore the study of Z a u g g et al. [58] demonstrated that this gene family is likely to contain only four members (SAPT1-4). During *in vitro* studies it was observed that *C. tropicalis* show Sap activity in a medium containing bovine serum albumin as the sole source of nitrogen [57]. These authors [57] suggested that Sap2, Sap3, and Sap4 could be produced under conditions yet to be described *in vitro* or during infection. However, it appears that Sap1 plays a little role in *C. tropicalis* pathogenesis [18] as studies of
Togni et al. [55] showed that Sap1 did not contribute significantly to fungal virulence in inoculation candidiasis of the normal mouse. Although mortality rate was slightly lower in groups infected with the Δsapt1 strain, the mutant still possessed the ability to invade and grow in the kidneys, which eventually led to the death of the infected mice [54]. Effect of disturbing Sapt2p, Sapt3p, and Sapt4p has yet to be studied [18]. Based on phylogenetic analyses, Parra-Ortega et al. [39] demonstrated that C. tropicalis SAPT4 is orthologous to C. albicans SAP1 and C. dubliniensis SAPD1.

It has been described that C. dublinensis is species that is most closely related to C. albicans [33]. Furthermore, C. dublinensis strains show highly proteolytic activity by producing greater amounts of protease than reference C. albicans strains [17]. Existence of SAP family in C. dublinensis was confirmed by the study of Gilfillan et al. [17]. The latter authors [17] demonstrated that all the C. dublinensis isolates examined possessed homologues to each of the seven C. albicans SAP genes tested. In total, eight SAP genes (Sapcd1-Sapcd4; Sapcd7-Sapcd10) in C. dublinensis were described [39]. Furthermore, SAPD4 is only member of the Sap4-6 family found in the C. dublinensis genome and the other non-albicans Candida species have no Sap4-6 homologues [39]. However role of Sap isoenzymes in C. dublinensis pathogenesis requires further studies [18].

Candida parapsilosis exhibit protease activity in medium where BSA is the sole nitrogen source [13]. In the study of De Viragh et al. [13] two genes encoding putative secreted aspartic proteases were identified, though only one product was identified as extracellular aspartic protease [40]. Among them ritonavir was found to be the most potent inhibitor of fungal adhesion to epithelial cells [14]. Indeed, the drug capable of blocking adhesion could prove to be attractive anti-fungal. However, later it was reviewed [14] that future derivatives designed to treat mucosal candidiasis in humans may require improvements. For more information on remain HIV PIs efficacy at inhibition of C. albicans protease activity, the reader is guided to reference by Mardegan et al. [30] and Fear et al. [14]. Another promising approach was the development of antibodies against Sap. In 2007 De Bernardis et al. [12] showed that human domain antibodies against Sap2 inhibit the adherence of C. albicans to epithelial cells of rat vagina and that they exert a protective activity against experimental vaginal candidiasis. As reviewed Gauwerk et al. [16], today the obvious need for completely new antymycotic agents is clear. Thus, drug combination that target not only essential genes but also important virulence factors that are essential for steps in infection could be attractive in the treatment of candidasis.

3. Aspartic proteinase inhibitors

The role of Candida proteinases in pathogenesis and their potential as antifungal targets – have driven the use of aspartyl proteinase inhibitors (PIs) in Candida research. The studies using the classical aspartyl PI pepstatin A and HIV PIs provided evidence for the contribution of Sap to C. albicans virulence. Schaller et al. [44] showed that pepstatin A was able to influence adhesion and invasion of C. albicans in vitro. Their findings indicated that pepstatin A can reduce histological damage during C. albicans infection in the model of human oral candidiasis. Furthermore, this would indicate that Sap activity contributes to the virulence in this in vitro model. Unfortunately, it was shown [42] that classical ligand pepstatin A cannot be used clinically, at least not systematically, because of its metabolism in the liver and rapid clearance from blood. Moreover, the results obtained by Lerman and Morschhäuser [29] cannot unequivocally include the possibility that pepstatin A inhibit Sap activity under the conditions used in the RHE model. Similar results were obtained by Schill et al. [50], who demonstrated that Sap9/Sap10 exhibiting a limited inhibition potential by pepstatin A. Candida albicans aspartic proteinase belongs to the same family as abundant HIV proteinase, the effect of three HIV proteinase inhibitors (ritonavir, indinavir and saquinavir) was studied on Candida adhesion to epithelial cells [14]. Among them ritonavir was found to be the most potent inhibitor of fungal adhesion [5, 10]. The latter authors [5] concluded that although the HIV protease inhibitors were found to attenuate adhesion of C. albicans to epithelial cells in vitro, but were not able to modulate phagocytosis of cells by PMNs. Indeed, the drug capable of blocking adhesion could prove to be attractive anti-fungal. However, later it was reviewed [14] that future derivatives designed to treat mucosal candidiasis in humans may require improvements. For more information on remain HIV PIs efficacy at inhibition of C. albicans proteinase activity, the reader is guided to reference by Mardegan et al. [30] and Fear et al. [14]. Another promising approach was the development of antibodies against Sap. In 2007 De Bernardis et al. [12] showed that human domain antibodies against Sap2 inhibit the adherence of C. albicans to epithelial cells of rat vagina and that they exert a protective activity against experimental vaginal candidiasis.

4. Summary

The role of Sap in the development and progression of candidiasis has been studied in vivo for systemic and mucosal candidal infections. In addition, the research
involving both human samples and animal models were discussed (Table I). Those studies indicate that there are differences in the interaction of *C. albicans* with *in vivo* human and animal model. Moreover, it has been demonstrated that Sap mutants and specific aspartic proteinases inhibitors (i.e. pepstatin A) reduce the ability of *C. albicans* to damage host tissues [3, 6, 9, 22, 36, 55].

Although other non-*albicans* *Candida* species possess SAP genes, *C. albicans* aspartyl proteinases by far remain best characterised [36].

The knowledge on how the pathogen regulates the production of different virulence factors contributes to our better understanding of the pathogenesis [2]. Therefore further studies on SAP production and expression are required, which will not only help to develop more effective treatment of candidiasis but could also offer therapeutic options in the treatment of other inflammatory conditions.

Acknowledgements
Our own work was supported by the research project NN404 113639 founded by the National Science Centre of Poland.

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