PH.D. THESIS

THE ROLE OF BIOACTIVE LIPIDS AND FATTY ACID BIOSYNTHESIS IN *CANDIDA PARAPSILOSIS* VIRULENCE

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Introduction

Candida parapsilosis is an emerging opportunistic human fungal pathogen, and it is currently one of the leading causes of invasive candidiasis. *C. parapsilosis* can colonize and cause disease in immunocompromised patients (with AIDS, organ transplantation etc.) or in particular patient groups such as neonates or elders. *C. parapsilosis* is the second most commonly isolated *Candida* species from blood cultures, and it even outranks *C. albicans* in some European, Asian and South American hospitals. Unlike *Candida albicans*, *C. parapsilosis* is frequently transmitted horizontally, and it is able to cause invasive disease without colonizing the host before dissemination. The use of prosthetic devices and indwelling catheters also increases the risk for *C. parapsilosis* infection. Despite the increasing clinical prevalence, little is known about the pathogenesis and virulence factors of *C. parapsilosis*. Thus, in our recent study, we investigated the biosynthetic pathway of fungal prostaglandins (PGs), a putative virulence factor of *C. parapsilosis*. Prostaglandins are biologically active polyunsaturated fatty acids (PUFAs) build up of 20 carbon atoms. PGs have important signaling and immunomodulatory functions. Mammalians produce immune response regulator prostaglandins *de novo* from membrane-derived arachidonic acid (AA) via cyclooxigenases COX1 and COX2. Although fungi do not possess cyclooxygenase homologs, several pathogenic species, such as *C. albicans* and *Cryptococcus neoformans* are able to produce prostaglandins from host originated arachidonic acids. Although little information is available about the biosynthesis of prostaglandins in *Candida* spp., one study has described that the multicopper oxidase gene FET3 and the stearyl-CoA desaturase gene OLE2 are involved in PGE2 synthesis in *C. albicans*. This finding

indicates a direct link between the production of fatty acids and bioactive lipid mediators, underscoring the role of *de novo* fatty acid biosynthesis in pathogenic yeasts. In this recent work we demonstrated that *C. parapsilosis* produces fungal prostaglandins, similarly to *C. albicans*, from the exogenic AA. In order to investigate this pathway we generated a deletion mutant strain of the *CpOLE2*, a putative Δ9 fatty acid desaturase. We revealed that the *CpOLE2* gene does not participate in the fungal prostaglandin production. On the other hand the fatty acid profile analysis may suggest a Δ12-, rather than a Δ9-desaturase activity of the CpOle2. In order to identify other genes that can participate in the prostaglandin biosynthetic pathway, we carried out a microarray analysis after AA treatment. We identified 5 over-expressed genes, one with a transcription factor activity (*CpUGA3*). In order to determine whether these genes participate in the prostaglandin biosynthetic pathway, we knocked out these genes using the fusion PCR technique. By the analysis of the prostaglandin production in the *CpUGA3* deletion mutant, we found a strongly reduced prostaglandin production in comparison with that of the wild type strain. These results suggest that the *CpUGA3* transcription factor plays significant role in the *C. parapsilosis* prostaglandin biosynthetic pathway.

Methods

Cultivation, co-incubation and transformation: isolation and cultivation of primary phagocytes, cultivation of bacterial and yeast strains, transformation of bacterial and yeast strains, growth assays of yeast strains, *in vitro* co-incubation of yeast cells and phagocytes, yeast elimination assay

Molecular techniques: PCR, gel electrophoresis, DNA isolation from yeast, plasmid isolation from *E. coli*, molecular cloning, directed gene deletion from yeast, Southern hybridisation, RNA isolation from yeast, cDNA synthesis, microarray analysis, qRT-PCR, flow cytometry

Microscopic techniques: acridin orange/crystal violet fluorescent staining and fluorescent microscopy

Protein works: Enzyme-linked immunosorbent assay (ELISA)

In vivo virulence assays: In vivo infection of *Galleria mellonella* larvae

Analytical assays (kollaboration): fatty acid measurement with gas chromatography, determination of prostaglandin profile and prostaglandin E_2 measurement from supernatants with HPLC-MS technique, determination of prostaglandin profile and prostaglandin E_2 measurement from supernatants with HPLC-FLD technique

Results

Investigation of *C. parapsilosis* prostaglandin production: We characterized the prostaglandin profile of *C. parapsilosis* with HPLC-MS analysis and compared to that of *C. albicans*. We revealed that *C. parapsilosis* can produce fungal prostaglandins, similarly to *C. albicans*, from supplemented arachidonic acid (AA).

Investigation the role of *CpOLE2* gene in *de novo* fatty acid- and prostaglandin biosynthesis: In our recent study we investigated the role of the putative Δ9 fatty acid desaturase CpOle2 in the *de novo* fatty acid and prostaglandin biosynthesis of the emerging human pathogen *C. parapsilosis*. We generated a homozygous *CpOLE2* deletion mutant through repeated application of a caSAT1 flipper KO cassette. We characterized the pseudohyphae formation, FBS utilization ability and growth ability on different pH and temperature of the *CpOLE2* deletion mutant compared to that of the wild type strain. The mutant strain was found to show the same characteristics as the wild type. According to the *in vitro* virulence assays the *CpOLE2* deletion mutant showed slightly reduced virulence compared to the wild type strain. We characterized the prostaglandin profile of *C. parapsilosis* with HPLC-MS analysis and compared to that of *C. albicans*. We revealed that *C. parapsilosis* can produce fungal prostaglandins, similarly to *C. albicans*, from supplemented arachidonic acid (AA). Wild type-like PGE2 production was detected in *CpOLE2* deletion mutant, which indicates that the *CpOLE2* gene does not participate in the *C. parapsilosis* prostaglandin biosynthesis. Overall fatty acid production study by gas chromatography (GC) highlighted the accumulation of mono-unsaturated fatty acids in the *CpOLE2* deletion

mutant, which indicates the Δ12-desaturase activity of CpOle2. This finding correlates with the results in *C. albicans*, where *CaOLE2* gene was not able to complement the absence of S. cerevisiae Δ9-desaturase gene, but the desaturase activity was confirmed.

Identification and characterisation of further genes that may participate in the prostaglandin biosynthesis of *C. parapsilosis*: In order to identify genes that can participate in the *C. parapsilosis* prostaglandin biosynthesis, we carried out a microarray analysis on the wild type strain after AA treatment. According to the microarray data we found 5 genes that showed upregulation in response to the AA treatment. Notably, one of the up-regulated genes was the homologue of *UGA3*, a putative transcription factor with zinc cluster DNA-binding motif, which has been shown to be required for utilization of gamma-aminobutyrate (GABA) as a nitrogen source. We also found *SOU2* gene, which has ortologues with stereo-specific carbonyl-reductase activity and *OLE1*, the fatty acid desaturase gene as a potential member of the *C. parapsilosis* specific prostaglandin biosynthetic pathway. A previous work on *CpOLE1* gene proved its essential role in the *de novo* fatty acid synthesis of *C. parapsilosis*. The remaining two genes we have identified were putative genes, CPAR2_108490 and CPAR2_703920. The latter one has orthologues with cytochrome-c activity. In order to determine whether these genes participate in the prostaglandin biosynthetic pathway, we used reverse genetics and knocked out these genes using the fusion PCR technique. After obtaining mutant strains, we characterized their FBS utilization ability and growth ability on different pH and temperature. We found no difference in comparison to that of the wild type strain. We carried out different virulence assays and found that the deletion mutant strains have slightly different

virulence abilities than the wild type strain. Interestingly, the *CpUGA3* and *CpSOU2* deletion mutant strains showed hyper-virulent phenotype in *in vitro* killing and *in vivo Galleria mellonella* survival assays. As the *CpUGA3* is a transcription factor gene, it might be the first step of the prostaglandin biosynthetic pathway, therefore we analyzed the prostaglandin production of the *CpUGA3* deletion mutant strain by HPLC-FLD and compared it to that of the wild type. Notably, we found an explicit reduction in the prostaglandin production of the mutant strain, which suggests the role of the Uga3 protein in the prostaglandin biosynthetic pathway.

Summary

- *C. parapsilosis* cells are able to produce fungal prostaglandins from exogenus AA
- We generated a homozygous *CpOLE2* deletion mutant
- According to the physiological properties there were no differences between the wild type and the mutant strains, but the deletion of the *CpOLE2* gene reduced the mutant's virulence
- Wild type-like PGE_2 production was detected in *CpOLE2* deletion mutant, which indicates that the *CpOLE2* gene does not participate in the *C. parapsilosis* prostaglandin biosynthesis
- Overall fatty acid production study by gas chromatography indicates the Δ12-desaturase activity of CpOle2
- According to the microarray data we found 5 genes that showed upregulation in response to the AA treatment indicating their role in *C. parapsilosis* prostaglandin biosynthesis
- After obtaining mutant strains, we characterized their FBS utilization ability and growth ability on different pH and temperature. We found no difference in comparison to that of the wild type strain
- By the analysis of the prostaglandin production in the *CpUGA3* deletion mutant, we found a strongly reduced prostaglandin production in comparison with that of the wild type strain. These

results suggest that the *CpUGA3* transcription factor plays significant role in the *C. parapsilosis* prostaglandin biosynthetic pathway

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