COMMENT

Candida vaginitis: the importance of mitochondria and type I interferon signalling

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The interaction between fungal pathogens and host epithelial barriers remains largely unexplored. In a recent issue of Nature Microbiology, Pekmezovic et al. (Nat. Microbiol. 2021) provides evidence for the pivotal role of mitochondria-associated type I interferon signalling in the pathophysiology of vulvovaginal candidiasis.

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Vulvovaginal candidiasis (VVC) is the second most common cause of vaginitis after bacterial vaginosis. These fungal infections of the lower female reproductive tract are diagnosed in the primary care setting in up to 40% of otherwise healthy women. Prominent risk factors for VVC include broad spectrum antibiotic treatment, high-oestrogen containing oral contraceptives, sexual activity, pregnancy and uncontrolled diabetes mellitus.[1] *Candida* microorganisms are yeasts that are widely distributed in nature and a common constituent of the human vaginal microflora. However, when there are alterations in the vulvovaginal microenvironment, *Candida* can proliferate and become pathogenic, resulting in symptomatic infection. Although *Candida albicans* remains the most frequently isolated aetiological agent of VVC, non-*C. albicans* species also cause VVC, most notably *C. glabrata*, *C. parapsilosis* and *C. tropicalis*.[2] Importantly, while sharing the same genus name, these four yeast species are distantly related at the phylogenetic level. For instance, the genetic distance between *C. albicans* and *C. glabrata* is larger than that between humans and some fishes.[3] Considering that dozens of other *Candida* species are not pathogenic for humans, the most reasonable conclusion is that *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis* have independently evolved pathogenic determinants that enable these yeasts to trigger symptomatic infections of the lower female reproductive tract.[4] Although the development of genomic resources and comparative approaches has helped identify species-specific pathogenic factors in *Candida*, the putative involvement of these factors in the specific context of VVC has remained elusive. The majority of information available regarding the *Candida*–host interaction during VVC has related to the host immune response to the prominent species *C. albicans*. This interaction involves the complex interplay between fungal attributes (e.g. cell wall components, toxins such as candidalysin) and immune cells and soluble factors (e.g. cytokines, alarmins).[5] However, while the vaginal epithelium represents the initial barrier encountered by *Candida* pathogens, the cellular and molecular interchange that occurs during this important host–fungal interaction is unclear in VVC. As such, the study led by the Bernhard Hube research group and recently published in *Nature Microbiology* provides an unprecedented insight into the host–pathogen crosstalk between *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis* and vaginal epithelial cells.[6]

In preliminary experiments using a well-defined in vitro vaginal epithelial infection model, Pekmezovic and colleagues first shed light on *Candida* species-specific pathogenicity factors when interacting with epithelial cells. These differential fungal factors relate to distinct morphologies, levels of adhesion and invasion, but also damaging capacity. While all *Candida* species displayed similar invasion capacity in vitro, *C. albicans* was the most pathogenic, exhibiting greater adhesion and damaging capacity compared with the other species. A key pathogenic trait of *C. albicans* is its ability to transform from a budding yeast to a hyphal filament (production of germ tubes). Over the years, this morphogenic transition has been tightly correlated with *C. albicans* pathogenicity in in vitro and in vivo models.[7] Thus, it is highly likely that the yeast-to-hypha transition in *C. albicans* represents the main damaging attribute that accounts for the marked increase in pathogenicity of this yeast compared to other *Candida* species. However, it is likely that additional cell programs may underlie the differences in pathogenic potential displayed by *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis* when interacting with vaginal epithelial cells. To decipher the complex and dynamic molecular processes that may orchestrate *Candida*–epithelial interactions, the investigators undertook a extensive dual RNA-Seq strategy using their in vitro infection model.

Pekmezovic and colleagues first studied the transcriptional reprogramming that operates in each *Candida* species during infection of epithelial cells. This showed a rapid transcriptional response within *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis* when interacting with vaginal epithelial cells and that the differentially expressed gene profile was largely *Candida* species-specific. This trend was detectable early during the infection process (3 h) and was amplified during latter infection stages (12–24 h). Therefore, *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis* may have evolved differential transcriptional

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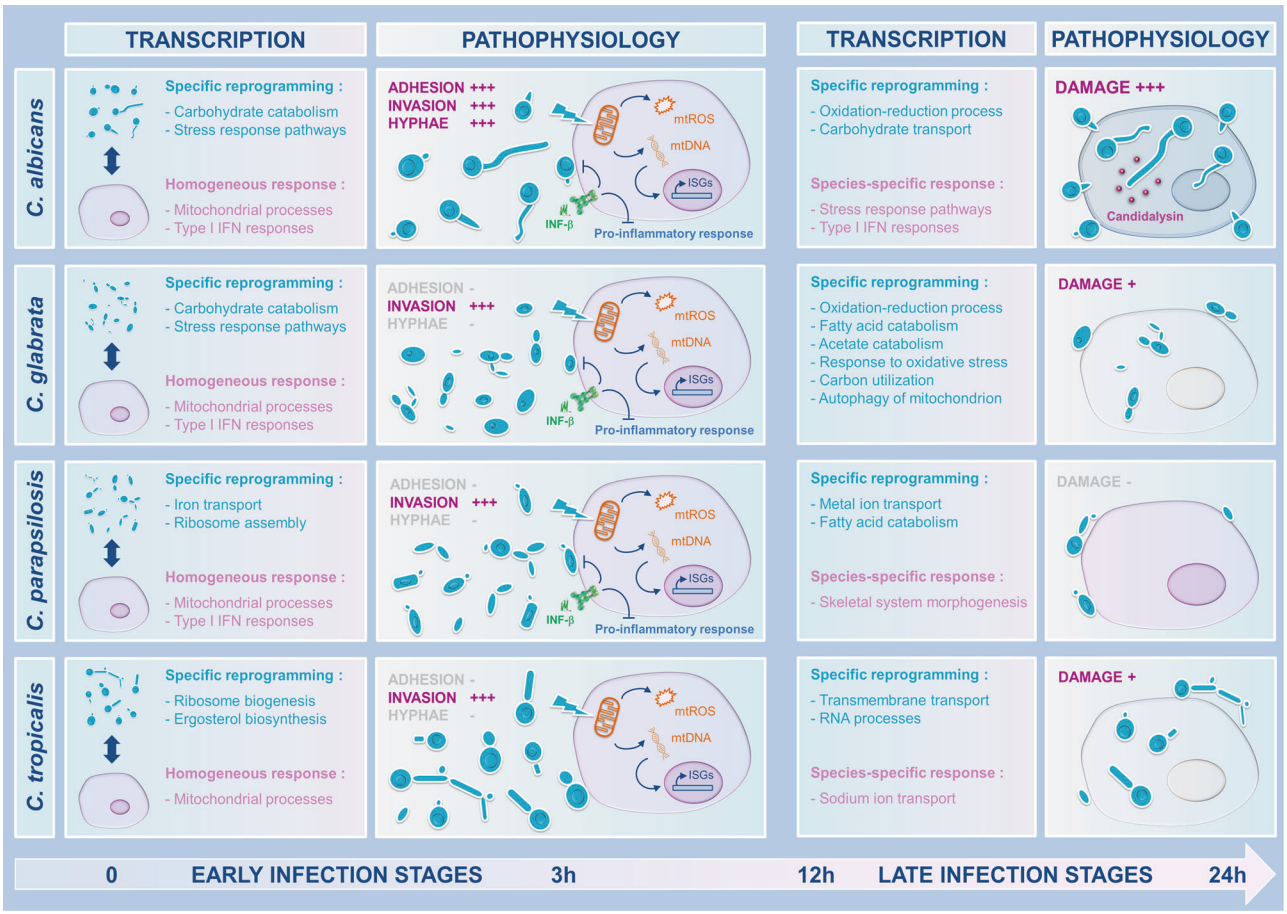

Fig. 1 Deciphering the complex and dynamic molecular processes that orchestrate *Candida*–epithelial interactions. *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis* exhibit distinct pathogenicity patterns with marked species-specific transcriptional reprogramming during infection of vaginal epithelial cells. On the contrary, host cells exhibit a homogeneous response to all *Candida* species at the early stages of infection (3 h) involving the induction of mitochondrial signalling and a protective type I interferon response. At the later stages of infection (12–24 h), the transcriptional response of the host diverges in a species-dependent manner. In *C. albicans*, this divergence is primarily driven by the extent of epithelial damage elicited by the toxin candidalysin.

programs to deal with an encounter with the female genital mucosa (Fig. 1). Attention then turned to identifying infection-specific genes that were differentially expressed in each *Candida* species throughout infection. Interestingly, most of the genes induced were also found to be essential for normal fungal growth in culture media devoid of epithelial cells. Therefore, although *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis* possess species-specific strategies to infect epithelial cells, the data suggested that these strategies may not be critically important during infection of the female genital tract.

Pekmezovic and colleagues then investigated the transcriptional changes occurring in vaginal epithelial cells when exposed independently to the four *Candida* species. Notably, a globally uniform transcriptional response was observed during the early infection stages of all yeast species, and further enrichment analysis identified that this involved mitochondrial processes and type I interferon (IFN) responses (Fig. 1).[8] The interesting exception was *C. tropicalis*, which induced mitochondrial processes but not type I IFN responses. Why this is the case is unclear. Irrespective, at the later infection stages, the host response appeared less conserved since the epithelial cell transcriptome patterns diverged significantly in a *Candida* species-specific manner (Fig. 1). As a consequence, while *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis* display distinct pathogenicity and transcriptional profiles during early infection, this was not reciprocated in epithelial cells, which showed a common transcriptional profile. Notably, the epithelial transcriptional responses at the later stages of infection were specific to the respective *Candida* species.

To decipher the pathophysiological relevance of the mitochondria-associated processes and type I IFN signalling pathways that were triggered in epithelial cells upon infection, complementary cell biology and biochemical experiments were undertaken. These indicated that *Candida* cells in direct contact with epithelial cells induce mitochondrial dysfunction as revealed by membrane depolarization and the production of mitochondrial reactive oxygen species (Fig. 1). Release of host mitochondrial DNA (mtDNA) into the cytosol induced the expression of interferon-stimulated genes,[9] which in turn prevented epithelial cell damage, suggesting that type I IFN signalling increases epithelial resistance to *Candida* infection. Concurrently, type I IFN signalling in epithelial cells restricted the release of IL-8 and the subsequent neutrophil activation in the early stages of infection with *Candida* yeasts (Fig. 1).

Since host-cell damage is a major determinant of *Candida* pathogenicity, the investigators then assessed the transcriptional pattern that accompanies the late stages of epithelial infection. Globally, host transcriptome profiles in response to fungal damage were found to be species-specific, suggesting that *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis* possess distinct attributes that drive the differential damaging capacity of these yeasts. To gain more insight into this, the role of candidalysin, a cytosolic peptide toxin encoded by the *ECE1* gene in *C. albicans*

that mediates host-cell damage, was investigated.[10] Interestingly, a similar transcriptional profile was obtained in epithelial cells infected with the non-damaging *C. albicans ece1* mutant and the *C. parapsilosis* wild-type strain (non-damaging). This provides definitive evidence that epithelial cell damage shapes the host response at the transcription level. Finally, epithelial cells infected with the damaging *C. albicans* wild-type (candidalysin-producing) strain were found to up-regulate the expression of several genes encoding pro-inflammatory cytokines and chemokines, confirming the major role of candidalysin in driving host-cell damage and pro-inflammatory signals for neutrophil recruitment (Fig. 1).

In conclusion, this enlightening article provides a greater understanding of the transcriptional reprogramming that arises during epithelial cell infection with four prominent *Candida* species that cause VVC. The study supports the hypothesis that these four phylogenetically distant yeasts, *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis*, have independently acquired distinct transcriptional profiles and pathogenic factors to infect vaginal epithelial cells. By contrast, the vaginal epithelium does not seem to discriminate between the *Candida* spp and utilises a common transcriptional response in the early stage of fungal infection. However, the differential ability of the four *Candida* species to damage epithelial cells induces a far more robust response in the later stages of infection and drives a far more specific transcriptional response resulting in pro-inflammatory mediator release from epithelial cells. Since *Candida* spp are commensals of humans, the data suggest that human epithelial cells have evolved to tolerize the presence of fungi through the mitochondria-associated type I IFN pathway, which may play a pivotal role in ensuring the delicate balance between commensalism, pathogenicity and antifungal immunity. Indeed, activation of the mitochondrial-associated type I IFN pathway in response to commensal microbes is likely to be a fundamentally important process in most host cells (hematopoietic and non-hematopoietic) to restrain inflammation via tolerance. Finally, it must be highlighted that this study provides an elegant demonstration of coupling in vitro models and dual RNA sequencing to monitor the host–pathogen interaction, which can be employed for future discoveries in medical mycology.

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AUTHOR CONTRIBUTIONS

N.P. and J.R.N. wrote the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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