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Review

Fungal morphogenetic changes inside the mammalian host

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ABSTRACT

One of the main features of the majority of pathogenic fungi is the ability to switch between different types of morphological forms. These changes include the transition between cells of different shapes (such as the formation of pseudohyphae and hyphae), or the massive growth of the blastoconidia and formation of titan cells. Morphological changes occur during infection, and there is extensive evidence that they play a key role in processes required for disease, such as adhesion, invasion and dissemination, immune recognition evasion, and phagocytosis avoidance. In the present review, we will provide an overview of how morphological transitions contribute to the development of fungal disease, with special emphasis in two cases: *Candida albicans* as an example of yeast that switches between blastoconidia and filaments, and *Cryptococcus neoformans* as an example of a fungus that changes the size without modifying the shape of the cell.

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1. Introduction

Fungal pathogens are a frequent cause of superficial and disseminated diseases. Topical fungal infections, such as colonization of the skin and mucosae, affect millions of people. However, in the last decades, there has also been an increase in the incidence of invasive fungal diseases (IFD), associated with the rise in the proportion of immunocompromised patients [1–5]. Opportunistic IFDs are a problem of concern because they significantly increase the

Abbreviations: IFDs, Invasive Fungal Disease; PAMPs, Pathogen-Associated Molecular Patterns; PRRs, Pattern Recognition Receptors; NET, Neutrophil Extracellular Trap; ROS, Reactive Oxygen Species.

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morbidity, mortality and the management cost of patients [6]. The main opportunistic human fungal pathogens are divided into yeasts (mainly *Candida* and *Cryptococcus*) and filamentous fungi or molds (mainly *Aspergillus, Fusarium, Scedosporium* and *Mucorales*).

The development of IFD is a multifactorial process that involves elements from both the host and the pathogen [7,8]. One of the main factors that determines the incidence of opportunistic IFDs is the alteration of the immune response. In addition, fungi also express proteases, lipases and other lytic enzymes that can cause damage in the host. But another important factor that contributes to disease is the ability of fungi to adapt to the host environment and proliferate in cases of defective immunity. The niches of fungi during infection are quite variable, so virulence requires adaptation to different temperatures, pH and nutrient sources and to the attacks of the immune system. Furthermore, fungi have developed other mechanisms to cause disease, which mainly rely in their ability to develop morphological transitions. These changes occur in the environment, but also take place inside the mammalian host. Fungal morphogenesis is involved in processes required for adaptation and host invasion, and for this reason, the characterization of the different morphologies is required to understand the diseases caused by these microorganisms. In the present review, we will summarize how morphological transitions can favour the adaptation and development of fungal diseases in mammalian hosts.

2. Types of fungal morphologies

During their life cycle, fungi can develop several morphological structures (or morphotypes). Most fungi can be found as spores or conidia, which are single cells of rounded or oval shape. In the case of budding yeast, these cells are denominated blastoconidia, or more commonly, "yeasts" [9,10]. Conidia or blastoconidia can switch to different morphologies depending on the fungal species and on the environmental conditions. In general, morphological transitions can be divided in two classes. Some fungi can change the shape of the cells, being the most widely characterized the formation of filaments in *Candida* spp. and molds as *Aspergillus* spp. Since microscopic fungi lack motile structures (such as cilia or flagella), the formation of filaments provides a mechanism to reach more favourable niches.

Other fungi can undergo another transition that results in a change in the size of the cell without changing the shape (which is most particular in *Cryptococcus* spp.). This change results in the formation of cells of an abnormal enlarged size (titan cells) that pose a problem for the immune system.

In this context, it is appropriate to differentiate between fungi that exhibit several morphotypes and true dimorphic fungi, which are those found as filaments in the environment and as yeasts during infection in the host. This is the case of *Histoplasma*, *Paracoccidioides*, *Blastomyces* and *Sporothrix* [11,12].

In the following sections, we will review the main phenotypic characteristics of these changes and how they contribute to the development of fungal diseases.

3. Transitions that involve changes in shape: the role of filaments during infection of *C. albicans*

Most of the information about the role of fungal morphogenesis during pathogenesis is derived from studies focused on the different behaviour of filaments and blastoconidia or spores. Molds, such as *Aspergillus fumigatus*, grow mainly as filaments, with spores or conidia emerging as a result of asexual reproduction. These cells can remain latent or germinate into new filaments (see Supplemental Video 1). In contrast, yeasts can grow as blastoconidia or filaments, depending on the environmental conditions. In the case of *Candida* spp., blastoconidia divide by budding in regular rich media, originating a progeny of similar size and morphology. But in certain conditions, blastoconidia can undergo a morphogenetic switch that results in the formation of filaments. Pseudohyphae are formed when budding blastoconidia do not fully separate, so a chain of bound cells is formed (see Supplemental Video 2). In contrast, true hyphae are characterized by the appearance of a tube from a blastoconidium (which is denominated as germ tube), which after continuous growth results in a long filament were individual cells are not visible (see Supplemental Video 3). Hyphae can contain septa that separate cells with individual nuclei, or be continuous with multiple nuclei. Some *Candida* spp., such as *C. albicans*, can form both pseudohypha and true hyphae [13], while others, such as *C. parapsilosis* or *C. tropicalis*, mainly develop pseudohyphae. It is noteworthy that *C. glabrata*, which is the second or third most common cause of candidemia worldwide, is found mainly as small blastoconidia and rarely produces filaments. However, a recent report indicates that mutations in the *CHS2* gene (which encodes chitin synthase) result in a pseudofilamentous strain that has increased virulence [14].

Hyphae formation has been mainly characterized in *C. albicans*. This yeast induces the switch to filamentous growth in response to multiple stimuli *in vitro*, such as growth at 37 °C, mammalian serum, *N*-acetyl glucosamine and neutral pH [15–18]. The formation of hyphae depends on signal transduction pathways and on transcription factors (such as Efg1, Cph1, Nrg1 and Ume6 [19,20]) that regulate the expression of hypha-specific genes. Interestingly, the induction of hyphae or pseudohyphae share common signalling pathways and effectors, but the type of transition depends on the concentration of the Ume6 transcription factor. When this protein is present at low levels, the cells develop pseudohyphae, and when its concentration is high, the yeasts transform mainly into true hyphae [21].

3.1. Structural changes associated with filamentation: cell wall rearrangements

Hyphae development involves important cellular modifications (such as cell cycle changes, polarized growth and increase of turgor pressure at the tip of the filament, and expression of hypha-specific genes, see Ref. [15]). Many of them affect the composition of the cell wall, which is present in all fungi and confers a rigid physical layer that protects the cell from environmental stress. The cell wall is organized in two main layers. The major components of the inner part of the cell wall are carbohydrates (mainly β-1,3- and β-1,6-glucans and a small proportion of chitin), and the outer layer is composed mainly of mannans and proteins. The cell wall plays a key role during the interaction with the host because it is recognized by immune cells and has immunogenic properties. In consequence, understanding the changes and exposure of the components of this structure during hypha formation is a key aspect to understand the virulence of *C. albicans*.

Hyphae and blastoconidia present differences in their cell wall composition [22,23]. Chitin is around 3–5 times more abundant in the cell wall of the hyphae than in blastoconidia [24–26]. In addition, in blastoconidia, chitin is concentrated in the ring that surrounds the bud scars [27], meanwhile in hyphae the polysaccharide is distributed around the lateral cell wall (see review in Ref. [28]). Concerning β-glucans, there are small differences related to its distribution over the cell wall of hyphae and blastoconidia, although the proportion of β-1,3/β-1,6 glucan seems to be different in both cell types [22]. Recent studies have shown that hyphae contain β-glucan structures and linkages that are not present in glucans from blastoconidia [29]. This study also suggests that the content of β-1,3-glucans and β-1,6-glucans in hyphae is higher compared to yeast. An important aspect of the biological function of the cell