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EDITORIAL

# Gray phenotype: Enhanced fitness strategy for *Candida dubliniensis?*

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*Candida albicans* is an opportunistic human pathogen that has been defined as a "skilled transformer" for its elevated plasticity in developing distinct forms of growth associated to a virulence program that is fully committed to escape from host immunity.[1] A major component of the above plasticity is the white-opaque phenomenon, first described by David Soll's research group in a classic paper published in 1987.[2] The white-opaque switch is the spontaneous generation of opaque colonies, consisting of slightly elongated cells, from the well-known white colonies of typic yeast cells. This switch is essential for fungal mating and is closely linked to hyphal transition, biofilm formation and secreted aspartyl proteinases (Sap) production, 3 significant interrelated and critical features of *C. albicans* virulence program. Recently, Tao L. et al [3] have discovered an independent and stable colony variant in *C. albicans*, called the gray phenotype, consisting in cells elongated but smaller than white and opaque cells, thus collectively forming a tristable white-gray-opaque phenotype. Gray cells of *C. albicans* are more virulent than white and opaque cells in models of mucosal infections.[3] Many studies have demonstrated that different cell types evolved to adapt to different host niches.[3-5]

No surprise, the high genetic similarity between *C. albicans* and *C. dubliniensis* anticipated a similar phenotypic switch phenomenon in the latter, that in fact has now been described by Yue H. et al in this issue of Virulence.[6]

In this Editorial, we will focus on the possible role of gray phenotype of *C. dubliniensis* as a new strategy of this fungus to survive, grow and manifest its virulence in selected host niche, comparing it with the gray cells of *C. albicans*. The possible role of gray cells of *C. dubliniensis* as a mechanism evolved by this fungus to escape immune response at mucosal surfaces and grow undisturbed in the oral cavity of HIV-positive patients is discussed.

Although *C. dubliniensis* is much less virulent and less prevalent than *C. albicans* [7], its importance as a pathogen is linked to its primary association with oral colonization and infection in human immunodeficiency virus (HIV)-positive patients, causing in these patients recurrent infections.[8]

Gray cells of *C. dubliniensis* are similar to their counterparts in *C. albicans* in terms of several biological aspects including cellular morphology, mating competence and genetic regulatory mechanisms.[6] However, the gray phenotypes of the 2 species have some distinguishing features which may contribute to explain the colonization of a specific oral niche by *C. dubliniensis* and conversely the easier adaptation of *C. albicans* to most host tissues.

Yue H. et al [6] show that while the gray phenotype of *C. dubliniensis* is fostered by the combined use of N-Acetylglucosammine (GlcNAc) and $CO_2$ while the opaque phenotype is favored in *C. albicans* under the above conditions. Given that commensal bacteria release in the oral cavity GlcNAc and $CO_2$, the switch to the gray phenotype could help *C. dubliniensis* to compete with bacterial members and *C. albicans* itself, for colonizing this preferred biological niche. This could explain why *C. dubliniensis* is primarily associated with oral colonization and infection in HIV-positive patients.

Yue H. et al [6] also pinpoint a perhaps major difference between the switching phenomena in the 2 *Candida* species i.e. the differential expression profile and activity of Sap, a family of enzymes with increasing evidence for a master pathogenicity role in mucosal, particularly vaginal, candidiasis. These authors show that Sap activity is induced by the protein bovine serum albumin (BSA) in gray cells of *C. albicans* but not in the gray cells of *C. dubliniensis*. Moreover, the expression level of *SAP2* gene, a dominant member of Sap family in mucosal

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infection, is increased thousand times in the presence of BSA in gray cells of *C. albicans* but not in the gray cells of *C. dubliniensis*. Finally, in a model of mucosal infection, gray cells grow faster than white and opaque cells of *C. albicans* but not in *C. dubliniensis*.[3] Overall, the above differences support the notion of a higher virulence of *C. albicans* as compared to *C. dubliniensis*.

Interestingly, Sap, in particular Sap2, has recently been shown to be responsible for the pathogenic inflammation typically associated to vaginal candidiasis in a mouse model.[9] NLRP3 inflammasome activation with high production of inflammasome-dependent IL-1$\beta$ and neutrophils recruitment are a landmark of this experimental vaginitis. Interestingly, *C. dubliniensis* is rarely if ever found in the human vagina. In contrast, NLRP3 inflammasome activity appears to be a protective mechanism against oral candidiasis [10,11], hence the observation that the gray cells of *C. dubliniensis* are less inflammatory at mucosal levels as compared to *C. albicans* would suggest that the development of the *gray phenotype* help *C. dubliniensis* avoid recognition by host protective inflammation. These speculations should be taken cautiously, however, given the complexity of functions exerted by the members of Sap family, their redundancy and relation with other virulence traits of both *C. albicans* and *C. dubliniensis*. Overall, why *C. dubliniensis* is so fit for oral cavity of HIV subjects remains a subject to be further investigated.

Yue H. et al [6] also report that at least 9 genes involved in ergosterol biosynthesis and 3 mannanbiosyntesis-related genes were up-regulated in gray cells of *C. dubliniensis*. These genes play a critical role in the regulation of antifungal resistance and other stresses. It has been suggested that *C. dubliniensis* is more capable of developing antifungal resistance (e.g. to azoles) than *C. albicans*.[7,12,13] Again, this feature of *C. dubliniensis* may be associated with its prevalence in AIDS patients, who are often subjected to antifungal treatments with resistance outbreaks.

Yue H. et al [6] through their innovative findings have attempted to rise the attention to a new phenotype of the pathogenic fungus *C. dubliniensis*: the gray cells. This adds new knowledge on pathogenicity of this fungus and may help to explain its prevalence in HIV-patients subjected to antifungal treatments.

While in-depth mechanisms relating the gray phenotype to the biology and pathogenicity of *C. dubliniensis* are awaiting further studies, this report by Yue H. et al [6] recalls our attention to *C. dubliniensis* as a special pathogen in search for a specific place in the biology and pathogenicity of *Candida* species and its separation from *C. albicans*.[14]

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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