Análisis del proteoma de *C. albicans* en respuesta a estrés
Analysis of the proteome in response to stress in *C. albicans*

Candida albicans is a dimorphic opportunistic pathogenic fungus. It is a commensal resident on the mucosal surfaces and can cause host damage (candidiasis) by mechanisms mediated both by host (predisposing factors) and by fungus (virulence factors). Depending on the underlying host defect, *C. albicans* can cause different types of infections ranging from superficial to invasive candidiasis (IC). This last one remains a leading infectious cause of morbidity and mortality in critically ill and/or severely immunocompromised patients. Clinical outcomes might be improved by early initiation of antifungal therapy. SC diagnosis, however, proves extremely difficult due to the lack both of specific signs and symptoms of invasive disease and of rapid and accurate diagnostic tests.

Proteins are the engines of most of the physiological and pathological processes of living cells and proteomics is a dynamic multidimensional platform that offers an overview of the complex world of proteins within a cell under different expression conditions at a given time point. Proteomics could, therefore, substantially complement the existing molecular understanding of this pathogen and appears to be a promising technology to tack the search for new diagnostic and therapeutic strategies for these infections as well as to study the host fungus interaction (Pitarch *et al*., 2006a).

We are studying the *C. albicans*- macrophages interaction, beacuse is the initial step in the development of host immune defences. An in vitro model of phagocytosis that includes a differential staining procedure to discriminate between internalized and non-internalized yeast was developed. Upon optimization of a protocol to obtain an enriched population of ingested yeasts, a thorough genomic and proteomic analysis was carried out on these cells. Both proteins and mRNA were obtained from the same sample and analyzed in parallel. We provide evidence of a rapid protein response of the fungus to adapt to the new environment inside the phagosome by changing the expression of proteins belonging to different pathways. The clear down-regulation of the C-compound metabolism, plus the up-regulation of lipid, fatty acid, glyoxylate and tricarboxylic acid cycles, indicates that yeast shifts to a starvation mode. There is an important activation of the degradation and detoxification protein machinery. The complementary genomic approach led us to detect specific pathways related to *Candida'*s virulence. Network analyses allowed us generate a hypothetical model of *Candida* cell death after macrophage interaction, highlighting the interconnection between actin cytoskeleton, mitochondria and autophagy in the regulation of apoptosis (Fernández-Arenas *et al.,* 2007). To demonstrate this hypothesis an apoptotic marker like double-stranded DNA breaks is being analysed using TUNEL and Electron Microscopy assays.

On the other hand, we are studying the *Candida* immunome (the subset of the proteome targeted by the immune system) during different host-fungus interactions by immunoproteomics (the combination of proteomics with serology). This study might allow the discovering of potential diagnostic, prognostic, predictive and monitoring biomarkers for IC. To address this hypothesis, different immunoproteomic studies were undertaken to individually screen and compare the serum profiles of anti-*Candida* IgG antibodies (*i*) in IC patients and non-IC subjects, (*ii*) in IC survivors and non-survivors, (*iii*) in IC responders and non-responders to a specific antifungal therapy (amphotericin B and/or fluconazole), and (*iv*) along the course of IC. Seven cell wall-associated proteins (including β-1,3-glucosyltransferase and glycolytic enzymes) and 42 different housekeeping intracellular proteins (diverse chaperones, heat shock proteins, glycolytic enzymes, fermentative proteins, other metabolic enzymes, elongation factors, ribosomal proteins, porins and redox enzymes, among others) of *Candida albicans* were characterized as specific targets of the human IgG antibody response to IC at an early stage. Serum IgG antibodies to a large panel of *C. albicans* proteins, particularly to Bgl2p, Eno1p, Pgk1p, Pcd11p, Tkl1p and Met6p , were found to be among the most important candidates for biomarkers predicting prognosis and treatment outcomes in IC patients. Furthermore, a remarkable rise in anti-Eno1p IgG antibody levels along the course of IC correlated with good prognosis, whereas a strong increase in expression of IgG antibodies to Ssb1p, Pgk1p and Met6p was associated with fatal outcomes (Pitarch *et al*., 2006; Pitarch *et al.,* 2008). In conclusion, our immunoproteomic approaches have led to the identification of a relatively high number of clinical biomarkers for diagnosis, prognosis, selecting appropriate antifungal therapy, and monitoring of IC. These findings are being validated in a multicenter study.

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This work was supported by Comisión Interministerial de Ciencia y Tecnología (BIO 2006-198), Fundación Ramón Areces, Comunidad de Madrid (DeRemicrobiana) and Instituto de Salud Carlos III (REIPI).