Functional characterization of *Candida albicans* Hst3p histone deacetylase

ABSTRACT

The unicellular eukaryotic organism *Candida albicans* is one of the most important fungi in medicine, used as experimental model to study fungal pathologies and the underlying biology of dimorphic fungi. This fungus is a component of the human mucosal microbiota; it is normally found as a commensal in vaginal and oral mucosal districts, and in the gastrointestinal tract. However, at these sites it behaves as an opportunistic pathogen: following environmental changes in the host this fungus can become pathogenic, leading to invasive and lethal infections in susceptible individuals. Therefore, during the last decades, *Candida* has emerged as a major human fungal pathogen responsible for an extended variety of mucosal and systemic infections.

The ability of this opportunistic fungus to cause and propagate successfully infections is linked to the expression of different and alternative virulence factors. Its key virulence trait is its morphological plasticity: its ability to shift from oval budding yeasts to elongated cell structures (pseudohyphal and hyphal filaments) responding to diverse and numerous environmental cues.

Adaptive chromatin changes promote *Candida* variability and phenotypic plasticity. Therefore, epigenetic regulation of gene expression is considerably involved in the morphogenesis and virulence of this polymorphic fungus. Adaptation of *C. albicans* to drug pressure, yeast to hyphae transition, biofilm formation, white-opaque switch are important pathogenic mechanisms, in which posttranslational histone modifications play a prominent role. In particular, acetylation – deacetylation of histones modulates morphological switch in *C. albicans* and, consequently, this modification is correlated to fungal virulence.

Histone H3 Lys56 acetylation (H3K56ac) is an important post-translational modification in yeast, that contributes to fungal genome stability.

In *C. albicans,* acetylation levels of H3K56 are regulated by two enzymes with fungal-specific properties: the acetyl transferase Rtt109p and the NAD^+-dependent histone deacetylase (sirtuin) Hst3p, encoded, respectively, by *RTT109* and *HST3* genes. *HST3* is an essential gene for *C. albicans*: homozygous deletion mutants for this sequence are not viable. The essentiality of *HST3* gene for *C. albicans* viability, combined with fungal-specific properties of its enzyme Hst3p, make it an attractive potential target for antifungal therapy.

Focus of this study was to examine the molecular pathways regulated by Hst3p of *C. albicans.* Considering that deletion of this sirtuin is lethal for this fungus, it is intuitive to understand that it regulates vital process in the fungal cell. As histone deacetylase, Hst3p modulates gene expression, in particular induces the repressive state of chromatin, inhibiting transcriptional activation.

Consequently, deletion of this gene or repression of its protein induces dysregulation of gene expression, leading to fungal death. Based on these considerations I focused my interest on this fungal protein, in order to characterize its role in *C. albicans* biology and virulence and its downstream targets, as potential new targets for the treatment of fungal infections.

Substrate of Hst3p is the acetylated histone H3 Lysine 56. To analyse the effect of Hst3p inhibition on its substrate, I grew up *C. albicans* in the presence of nicotinamide (NAM), a non-specific sirtuin-inhibitor. Mass spectrometry analysis allowed me to evaluate, for the first time, acetylation levels of H3K56 during *Candida* growth and their variations upon NAM treatment. Interestingly, nicotinamide treatment induced the accumulation of H3K56 acetylation levels during *C. albicans* growth, demonstrating the inhibitory effect of NAM on Hst3p activity.

One important attribute of *Candida* is its morphological variability, which is the result of the adaptive response to environmental changes which in turn this morphological plasticity triggers infection.

To study the role of Hst3p in fungal virulence, I analyzed the potential involvement of this sirtuin in phenotypic switch. Morphological analyses were performed under NAM treatment to investigate the effect of Hst3p inhibition on cell duplication and filamentation. Hst3p inhibition resulted in a reduction of fungal growth rate and alteration of yeast-hyphae transition in *C. albicans*: NAM induced an abnormal filamentous growth, with formation of V-shaped hyphae under conditions that normally maintain the yeast shape of *Candida*. This phenotypic analysis was performed also on two azole-resistant strains of *C. albicans* to investigate the role of Hst3p in drug resistance. Hst3p inhibition had similar effect on the resistance strains compared to the control wild-type strain, inducing morphological alterations and reducing cell duplication rate.

These phenotypic assays highlighted the effect of Hst3p inhibition on regulation of *Candida* morphology. V-shaped hyphae formation in *Candida* in non-inducing filamentation conditions require the structural rearrangement of the whole cell, which is a result of alteration in gene expression, induced by NAM treatment. Based on these considerations, I analysed the entire transcriptome of *C. albicans* strain SC5314 by RNA-sequencing to investigate whether the inhibition of Hst3p by NAM was responsible for changes in the pattern of expression of Virulence-related Genes. This analysis showed that gene categories most dysregulated upon NAM treatment are those associated with hyphal growth, adherence, white-opaque switch, drug resistance and cell wall maintenance. RNA-Sequencing analysis allowed to identify some dysregulated genes upon Hst3p inhibition; considering that no alteration in gene expression was detected for up-stream members of pathways that control these dysregulated genes, to verify if the expression of these genes is regulated

epigenetically by H3K56 acetylation, future experiments of chromatin immunoprecipitation (ChIP) will be performed.

To select inhibitors of Hst3p to be used as potential fungicidal compounds, I expressed and purified both the full length and a short sequence of recombinant Hst3p. These proteins did not show enzymatic activity, due probably to denaturing conditions used during purification, that were necessary considering that both the full length and the short sequence of Hst3p were complexed to the bacterial molecular chaperon GroEL. To improve protein folding in bacterial host and avoid denaturing conditions for purification, I expressed and purified recombinant Hst3p from a bacterial system over-expressing some molecular chaperons. Once determined the enzymatic activity of recombinant protein, an enzymatic assay will be set up, useful to screen and select small molecules, potential inhibitor of the fungal sirtuin Hst3p, which could be used as antifungal compounds.