Virulence 6:6, 535–536; August/September 2015; © 2015 Taylor & Francis Group, LLC

Histone deacetylases: Targets for antifungal drug development

Livia Kmetzsch[1,2,*]

[1]Programa de Pós-Graduação em Biologia Celular e Molecular; Centro de Biotecnologia; UFRGS; Porto Alegre, Brazil; [2]Departamento de Biologia Molecular e Biotecnologia; Instituto de Biociências; UFRGS; Porto Alegre, Brazil

Keywords: *Cryptococcus neoformans*, fungal pathogens, histone deacetylases, histone deacetylases inhibitors, trichostatin A, sodium butyrate

The interaction of pathogens and its hosts causes a drastic change in the transcriptional landscape in both cells. Among the several mechanisms of gene regulation, transcriptional initiation is probably the main point. In such scenario, the access of transcriptional machinery to promoter is highly regulated by post-translational modification of histones, such as acetylation, phosphorylation and others. Inhibition of histone deacetylases is able to reduce fungal pathogens fitness during infection and, therefore, is currently being considered for the development of new antifungal therapy strategies.

Post-translational modification of histones represents an important mechanism of gene expression regulation in eukaryotic cells. The impact of such modifications, either singly or in combination, is to form a language that could be deciphered by a set of proteins that regulate downstream functions in chromatin. Among such modifications, acetylation of lysine lying at N- and C-terminal domains that protrude from the nucleosome core particle plays an important role in gene expression regulation. This occurs mainly at transcriptional level by altering DNA-histone and histone-histone interactions and by the function of other proteins that can alter chromatin dynamics and functions.[1] Histone acetylation is a dynamic process regulated by the activity of 2 groups of enzymes conserved from yeast to humans: histone acetyltransferases (HAT), generally associated with the positive regulation of transcription, and histone deacetylases (HDAC), whose function is linked to negative regulation of transcription.[2] HDACs constitute a family of enzymes that are able to remove the acetyl group from histones and other cellular proteins. These enzymes can be classified by sequence homology into the Rpd3/Hda1 (classical HDAC) and into the sirtuin family.[3] A recent genomic analysis revealed that the number of genes that codes for HDACs in fungal genomes varies from 2 to 11[2]. In Ascomycota, classical HDACs gene numbers range from 2 to 5, and the number of sirtuin family genes ranges from 2 to 9[2]. A similar number of genes of both families are found in Basidiomycota (2 to 7 for classical HDACs and 3 to 8 for sirtuin HDACs, respectively) and other Phyla (Blastocladiomycota, Chytridiomycota, Microsporidia, and Zygomycota).[2]

The pathogenic yeast *Cryptococcus neoformans*, together with its sibling species *Cryptococcus gattii*, causes cryptococcosis, a life-threatening disease with over 1 million new cases and 600.000 deaths every year.[4] This disease is normally characterized by an initial pneumonia that could evolve to meningitis, which is normally the death cause. Cryptococcosis is generally treated with antifungal drugs, as fluconazole, flucytosine and amphotericin B.[5] However, resistance to fluconazole was already observed in this pathogenic yeast.[6] In this way, new targets for the development of antifungals are needed. In this issue of *Virulence*, Brandão and coworkers described the effects of pharmacological inhibition of HDACs in the human fungal pathogen *C. neoformans*.[7] Employing the HDAC inhibitors sodium butyrate (SB) and Trichostatin A (TSA), the authors found that pivotal virulence factors, such as growth at 37°C, melanin synthesis, phospholipase and capsule polysaccharide production are affected in a dose dependent fashion. In addition, they found alterations in morphogenetic traits (filamentation and mating) and in cell cycle (leading to arrest at G2/M). However, they could not found differences in the infectious potential of pre-treated fungal cells in a non-mammalian model of cryptococcosis. The results presented by Brandão and coworkers show that the effects of HDAC inhibition by SB were more pronounced, if not unique, than those obtained with inhibition by TSA. HDACs are currently considered as targets for the development of new antifungal drugs, since such enzymes have been described as regulators of key virulence aspects in important pathogenic fungal

*Correspondence to: Livia Kmetzsch; Email: liviak@cbiot.ufrgs.br
Submitted: 04/30/2015; Revised: 05/01/2015; Accepted: 05/04/2015
http://dx.doi.org/10.1080/21505594.2015.1049807
Comment on: Brandao FAS, et al. Histone Deacetylases inhibitors effects on *Cryptococcus neoformans* major virulence phenotypes. Virulence 2015; 6; http://dx.doi.org/10.1080/21505594.2015.1038014

species. Gene inactivation experiments led to association of individual HDAC genes with morphological transitions, virulence and expression and regulation of important drug resistance associated proteins, as the chaperone Hsp90 protein and drug efflux pumps.[8-11] Interestingly, inactivation of *C. albicans* HDAC coding genes *HDA1* and *RPD3* led to reduced trailing growth and reduced capability to evolve azole resistance, possibly due to a effect of these HDACs on the expression regulation of efflux pumps.[12] In the pathogenic mold *Aspergillus fumigatus*, HDAC gene inactivation led to defects in germination and in secondary metabolite production.[13] HDACs coding genes from plant fungal pathogens have also been characterized and associated with key events in virulence of *Fusarium graminearum* and *Magnaporthe oryzae*.[2] Recent evidences show that HDACs inhibition in pathogenic fungi constitutes a promising therapeutic strategy, resulting in altered expression of genes necessary for virulence or drug resistance. In line with this, treatment of *C. albicans* and other pathogenic *Candida* species with TSA lowered the expression of *ERG* genes (the products are targets of the azole drugs) and *CDR/MDR1* genes (code for multidrug transporters).[14] One of the pivotal work that supports this employment of HDACs inhibitors as antifungal drugs describes the synergistic effect of MGCD290, a HDAC inhibitor, with different azoles (fluconazole, posaconazole or voriconazole) in opportunistic fungal isolates from genera *Candida, Cryptococcus, Aspergillus, Rhodotorula, Fusarium, Trichosporum*, and others.[15] Also, TSA has been proposed for the treatment of invasive aspergillosis.[16] In *A. fumigatus*, the proposed mechanism of action of TSA refers to modulation of acetylation of Hsp90, which led to defects in growth and conidiation, as well as hypersensitivity to geldanamycin (an inhibitor of Hsp90).[17] In addition, TSA appears to potentiate the activity of caspofungin in *A. fumigatus*,[16] which would broaden the antifungal strategies for treatment of aspergillosis. More recently, the HDAC inhibitor MGCD290, in combination with echinocandins, was show to impair the growth of echinocandin-resistant *Candida* spp. isolates.[18] In *C. albicans*, pharmacological inhibition by TSA also led to alterations in the development of azole resistance.[12] Pharmacological inhibition of HDACs was also employed to characterize alterations of virulence traits in fungal pathogens. Treatment of *C. neoformans* and *C. albicans* with SB led to reduced biofilm formation and increased azole sensitivity. In addition, reduced germ tube formation was observed in *C. albicans* cells treated with SB[19] and reduced adhesion to pneumocytes were found in these fungal cells treated with different HDACs inhibitors.[20] Despite the classical use of HDACs inhibitors in the treatment of cancer,[3] HDACs inhibition appear to benefit immunological control of yeasts during host-pathogen interaction. At least *in vitro, C. neoformans* and *C. albicans* were found to be more sensitive to a SB-treated macrophage cell line (J774.16), and this effect was due to a raise in the macrophage cells levels of reactive nitrogen species.[19] Altogether, the results presented summarize the potential of HDACs inhibition as a new venue for antifungal drug development.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Rothbart SB, Strahl BD. Interpreting the language of histone and DNA modifications. Biochim Biophys Acta 2014; 1839:627-43; PMID:24631868; http://dx.doi.org/10.1016/j.bbagrm.2014.03.001
2. Jeon J, Kwon S, Lee YH. Histone acetylation in fungal pathogens of plants. Plant Pathol J 2014; 30:1-9; PMID:25288980; http://dx.doi.org/10.5423/PPJ.RW.01.2014.0003
3. Yang XJ, Seto E. The Rpd3/Hda1 family of lysine deacetylases: from bacteria and yeast to mice and men. Nat Rev Mol Cell Biol 2008; 9:206-18; PMID:18292778; http://dx.doi.org/10.1038/nrm2346
4. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS 2009; 23:525-30; PMID:19182676; http://dx.doi.org/10.1097/QAD.0b013e328322ffac
5. Kwon-Chung KJ, Fraser JA, Doering TL, Wang Z, Janbon G, Idnurm A, Bahn YS. *Cryptococcus neoformans* and *Cryptococcus gattii*, the etiologic agents of cryptococcosis. Cold Spring Harb Perspect Med 2014; 4:a019760; PMID:24985132; http://dx.doi.org/10.1101/cshperspect.a019760
6. Gast CE, Basso LR, Jr., Bruzual I, Wong B. Azole resistance in *Cryptococcus gattii* from the Pacific Northwest: Investigation of the role of *ERG11*. Antimicrob Agents Chemother 2013; 57:5478-85; PMID:23979758; http://dx.doi.org/10.1128/AAC.02287-12
7. Brandão FAS, Derengowski LS, Albuquerque P, Nicola AM, Silva-Pedreira IS, Poças-Fonseca MJ. Histone Deacetylases inhibitors effects on *Cryptococcus neoformans* major virulence phenotypes. Virulence 2015
8. Hnisz D, Majer O, Frohner IE, Komnenovic V, Kuchler K. The Set3/Hos2 histone deacetylase complex attenuates cAMP/PKA signaling to regulate morphogenesis and virulence of *Candida albicans*. PLoS Pathog 2010; 6:e1000889; PMID:20485517; http://dx.doi.org/10.1371/journal.ppat.1000889
9. Zacchi LF, Schulz WL, Davis DA. HOS2 and HDA1 encode histone deacetylases with opposing roles in *Candida albicans* morphogenesis. PLoS One 2010; 5:e12171; PMID:20730094; http://dx.doi.org/10.1371/journal.pone.0012171
10. Robbins N, Leach MD, Cowen LE. Lysine deacetylases Hda1 and Rpd3 regulate Hsp90 function thereby governing fungal drug resistance. Cell Rep 2012; 2:878-88; PMID:23041319; http://dx.doi.org/10.1016/j.celrep.2012.08.035
11. Wurtele H, Tsao S, Lepine G, Mullick A, Tremblay J, Drogaris P, Lee EH, Thibault P, Verreault A, Raymond M. Modulation of histone H3 lysine 56 acetylation as an antifungal therapeutic strategy. Nat Med 2010; 16:774-80; PMID:20601951; http://dx.doi.org/10.1038/nm.2175
12. Li X, Cai Q, Mei H, Zhou X, Shen Y, Li D, Liu W. The Rpd3/Hda1 family of histone deacetylases regulates azole resistance in *Candida albicans*. J Antimicrob Chemother 2015; 70:1993-2003; PMID:25825380; http://dx.doi.org/10.1093/jac/dkv070
13. Lee I, Oh JH, Shwab EK, Dagenais TR, Andes D, Keller NP. HdaA, a class 2 histone deacetylase of *Aspergillus fumigatus*, affects germination and secondary metabolite production. Fungal Genet Biol 2009; 46:782-90; PMID:19563902; http://dx.doi.org/10.1016/j.fgb.2009.06.007
14. Smith WL, Edlind TD. Histone deacetylase inhibitors enhance *Candida albicans* sensitivity to azoles and related antifungals: correlation with reduction in CDR and ERG upregulation. Antimicrob Agents Chemother 2002; 46:3532-9; PMID:12384361; http://dx.doi.org/10.1128/AAC.46.11.3532-3539.2002
15. Pfaller MA, Messer SA, Georgopapadakou N, Martell LA, Besterman JM, Diekema DJ. Activity of MGCD290, a Hos2 histone deacetylase inhibitor, in combination with azole antifungals against opportunistic fungal pathogens. J Clin Microbiol 2009; 47:3797-804; PMID:19794038; http://dx.doi.org/10.1128/JCM.00618-09
16. Lamoth F, Juvvadi PR, Steinbach WJ. Histone deacetylase inhibition as an alternative strategy against invasive aspergillosis. Front Microbiol 2015; 6:96; PMID:25762988; http://dx.doi.org/10.3389/fmicb.2015.00096
17. Lamoth F, Juvvadi PR, Soderblom EJ, Moseley MA, Asfaw YG, Steinbach WJ. Identification of a key lysine residue in heat shock protein 90 required for azole and echinocandin resistance in *Aspergillus fumigatus*. Antimicrob Agents Chemother 2014; 58:1889-96; PMID:24395240; http://dx.doi.org/10.1128/AAC.02286-13
18. Pfaller MA, Rhomberg PR, Messer SA, Castanheira M. In vitro activity of a Hos2 deacetylase inhibitor, MGCD290, in combination with echinocandins against echinocandin-resistant *Candida* species. Diagn Microbiol Infect Dis 2015; 81:259-63; PMID:25600842; http://dx.doi.org/10.1016/j.diagmicrobio.2014.11.008
19. Nguyen LN, Lopes LC, Cordero RJ, Nosanchuk JD. Sodium butyrate inhibits pathogenic yeast growth and enhances the functions of macrophages. J Antimicrob Chemother 2011; 66:2573-80; PMID:21911344; http://dx.doi.org/10.1093/jac/dkr358
20. Simonetti G, Passariello C, Rotili D, Mai A, Garaci E, Palamara AT. Histone deacetylase inhibitors may reduce pathogenicity and virulence in *Candida albicans*. FEMS Yeast Res 2007; 7:1371-80; PMID:17627775; http://dx.doi.org/10.1111/j.1567-1364.2007.00276.x