Investigating the impact of Leishmania mexicana NAT10 acetyltransferase in gene expression and chromatin structure regulation

Gabriela Gomes Alves[1]; Ariely Leite[1]; Suellen Maran[1]; Giovanna Panessa[2]; Heloisa Monteiro[3]; Carlos Eduardo G. Alves[1]; Ana V. Ibarra[4]; Luisa Antunes[5]; Tiago R. Ferreira[6]; José Renato Cussiol[2]; Katlin Massirer[3]; Christopher Fenandez-Prada[4]; Igor Cestari[5]; David Langlais[7]; Nilmar Moretti[1,4]

[1]Laboratory of Molecular Biology of Pathogens (LBMP), Paulista School of Medicine, Federal University of Sao Paulo, Brazil.
[2]Department of Biochemistry, Paulista School of Medicine, Federal University of Sao Paulo, Brazil.
[3]Center for Medicinal Chemistry, State University of Campinas, Brazil.
[4]Departament of Pathology and Microbiology, Université de Montréal, Canada.
[5]Institute of Parasitology, McGill University, Canada.
[6]Intracellular Parasite Biology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD 20892, USA.
[7]Departament of Human Genetics and Microbiology & Immunology, McGill University, Canada.

Leishmania spp. is a parasite that causes leishmaniasis and has a complex life cycle, requiring sophisticated gene regulation mechanisms to adapt to different environments. *Leishmania* primarily regulates gene expression at the post-transcriptional level, but epigenetic factors and chromatin organization may also influence this process. The N-acetyltransferase NAT10 is responsible for adding acetylation in citidine in RNA molecules (ac4C) and also has a role in processes like modulating DNA accessibility,influencing gene expression. In this study, we investigated the role of NAT10 in chromatin structure and cellular responses in *Leishmania mexicana* through functional and omics-based approaches. Functional complementation assays using the *Saccharomyces cerevisiae* model revealed partial phenotypic complementation. *In vitro* activity assays with recombinant wild-type and mutant proteins confirmed its acetyltransferase activity. Phenotypic analyses of *L. mexicana* strains overexpressing wild-type and mutant NAT10 showed that the presence of the mutated gene led to reduced proliferation and morphological alterations. Electron microscopy images revealed higher chromatin compaction in the wild-type strain compared to the NAT10 single knockout. Additionally, ATAC-seq and RNA-seq experiments were performed, showing that in strand switch regions, chromatin is more accessible in the wild-type strain (T7/Cas9) compared to the NAT10 single knockout. RNA-seq analysis comparing the wild-type and NAT10-mutant overexpressing strains revealed that the most affected genes are involved in post-transcriptional regulation and RNA processing, chromatin organization and epigenetics, DNA repair, autophagy and protein degradation, as well as reduced metabolism and transport. The results of this study will complement ongoing research on this parasite and contribute to characterizing the role of NAT10 in *Leishmania* biology, contributing to our understanding of how it regulates gene expression.

Keywords: *L. mexicana*; chromatin structure, gene expression.