ISID Research Grant Report

Epigenetics regulates transcription and pathogenesis in the parasite *Trichomonas vaginalis*

Natalia de Miguel[1], Tomas Pachano[1]

[1] Laboratorio de Parásitos Anaerobios, Instituto de Investigaciones Biotecnológicas-Instituto Tecnológico Chascomús (IIB-INTECH), CONICET-UNSAM, Chascomús B7130IWA, Argentina

Natalia de Miguel

Dr. de Miguel obtained her bachelor in Biochemistry from Universidad de Buenos Aires in 2002 and her PhD in Molecular Biology and Biotechnology from the Universidad General San Martin in 2007 working on the parasite Toxoplasma gondii. She went to the University of California, Los Angeles (UCLA, USA) for post-doctoral training where she worked on identifying novel factors involved in Trichomonas vaginalis pathogenesis. She returned to Argentina in 2011 to work as an independent researcher at the IIB-INTECH (Chascomus). Current interest in her laboratory is focused on shedding light on the pathogenesis process of two anaerobic parasites for which little biological information is available: Trichomonas vaginalis, the most prevalent, non-viral, sexually-transmitted infection worldwide; and Tritrichomonas foetus; an important pathogen of the bovine reproductive tract.

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Introduction

Trichomonas vaginalis is the flagellated protozoan parasite etiologic agent of trichomoniasis, the most frequent non-viral sexually transmitted infection. Symptoms caused by infection of this parasite are highly different and they depend both on the host and the parasite strain. In this regard, different *T. vaginalis* strains are highly variable in their adherence, aggregation and cytolysis capacity (Coceres et al., 2015; Lustig, Ryan, Secor, & Johnson, 2013) even when they have almost identical amino acid sequences (de Miguel et al., 2010). These differences among strains could suggest that the proteins mediating these interactions vary greatly, either in abundance or type between strains (Lustig et al., 2013). Therefore, the contrasting phenotypes might be attributed to differentially expressed genes as a consequence of extra-genetic variation, such as epigenetic modifications. Within this context, in this study we explored the role of histone acetylation in regulating gene transcription and pathogenesis in *T. vaginalis*.

Materials and Methods

The presence and abundance of H3KAc was assessed in two different *T. vaginalis* strain (adherent strain B7268 and less-adherent strain G3) by immunofluorescence assay (IFA), Western blot and Chromatin Immunoprecipitation assay followed by qPCR (ChIP-qPCR) using an anti-H3KAc antibody (that specifically recognize histone H3 acetylated in K9 + K14 + K18 + K23 + K27).

Additionally, a Class I and II HDAC inhibitor, trichostatin A (TSA), was used to evaluate the role of histone acetylation in regulation of gene expression. G3 parasites were treated with 400 nM of TSA for 12 h and the level of transcription and abundance of H3KAc in candidate genes was evaluated by RT-qPCR and ChIP-qPCR, respectively.

Finally, parasite aggregation and adherence to host cells was analyzed in parasites treated with TSA using *in vitro* assays (Bastida-Corcuera, Okumura, Colocoussi, & Johnson, 2005).

Results and Discussion

Chromatin post-translational modifications have been broadly studied in several microbial pathogens; however, nothing is known about the role of these marks in *T. vaginalis*. Interestingly, we found a strict correlation between the abundance of H3KAc and the corresponding level of expression (Figure 1) in two genes (BAP1 and BAP2) differentially expressed and implicated in *T. vaginalis* adhesion to host cells (de Miguel et al., 2010).

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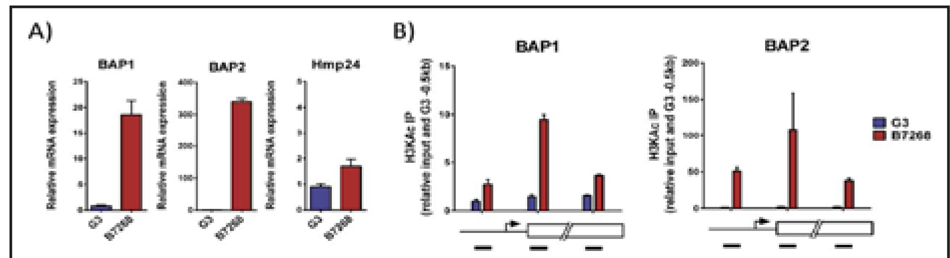

Figure 1. A) mRNA expression levels of BAP1 and BAP2 genes normalized using alpha-tubulin gene in G3 (blue) and B7268 (red) strains. As control, the expression of a constitutively expressed gene (Hmp24) was used. B) Abundance and distribution of histone acetylation within BAP1 and BAP2 genes in G3 and B7268 strain.

Histone acetylation is strictly controlled by the activity of HATs (add acetyl groups) and HDACs (remove acetyl groups) activities (Kuo & Allis, 1998). Hence, we used an HDAC inhibitor (TSA) to evaluate the role of histone acetylation in regulation of gene transcription. To this end, we first demonsratae by IFA and Western blot assays that TSA increases acetylation levels in *T. vaginalis* G3 strain. Then, ChIP-qPCR

assays showed that TSA, in G3, increases H3KAc levels in the promoters of BAP1 and BAP2 genes, which are normally hypoacetylated in this strain. Most significantly, RT-qPCR expression analysis of TSA treated G3 parasites demonstrated that HDAC inhibition upregulates genes that are poorly-expressed in this strain (de Miguel et al., 2010). For the five genes analyzed, mRNA levels of TSA treated G3 resembled the high-expression levels of B7268 strain.

We have previously noticed that highly adherent strains generally aggregate in cell culture, in contrast to less-adherent strains, such as G3, and this observation has been correlated with the ability of the strain to adhere and be cytotoxic to host cells (Coceres et al., 2015). Interestingly, when we treated G3 strain with TSA, we observed a dose-dependent increase in clumps formation (Figure 2a and b). Parasite aggregation favors adhering parasites to carry others to the surface of the host cell. Consequently, we performed an adherence assay with host cells and demonstrated that 400 nM TSA treatment resulted in an increase in attachment to host cells (~2.5 fold), compared to nontreated G3 parasites (Figure 2c).

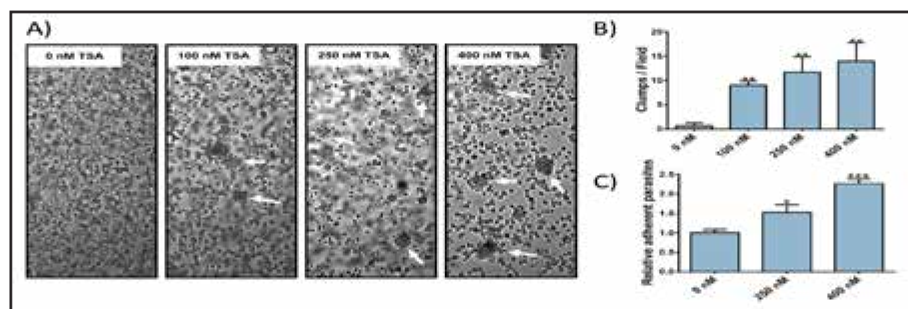

Figure 2. A) Ability of G3 strain to form clumps after treatment with increasing concentrations of HDAC inhibitor TSA. White arrows indicate a clump. B) Quantification of clump formation of TSA treated parasites. A clump is defined as an aggregate of more than 5 cells. B) Attachment of G3 parasites treated with TSA. Data are expressed as –fold increase compared to untreated G3 cells ± the standard deviation of the mean. * $p < .05$, ** $p < .001$, *** $p < .0001$.

Conclusions

We demonstrated that histone acetylation can regulate gene expression and pathogenesis in *T. vaginalis* by the use of the HDAC inhibitor TSA. Although TSA treatment might be

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modifying the levels of histone acetylation and transcription in numerous genes, we specifically demonstrated that this drug increases transcription of genes that are downregulated in non-pathogenic G3 strain and that are possibly implicated in parasite pathogenesis. Interestingly, this upregulation caused by TSA is accompanied by an increase in parasite aggregation and adherence to host cell.

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