

Knocking-out TcNTPDase-1 Gene in *Trypanosoma cruzi*

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Introduction: The Ecto-Nucleoside Triphosphate Diphosphohydrolases are enzymes that hydrolyze tri- and/or di-phosphate nucleotides. Evidences pointed out to their participation in *Trypanosoma cruzi* virulence, infectivity and purine acquisition. **Objectives:** In this work, we develop and evaluated the viability of a recombinant parasite knockout for the NTPDase-1 gene, and confirm the gene deletion stability and survival of the parasite. **Material/Methods:** For this purpose, intergenic regions upstream and downstream of the TcNTPDase-1 were amplified by PCR and these regions were inserted in vectors for knockout pNEO2 and pHYG2 thus, epimastigote forms of the recombinant parasites Dm28c clone (Hygro and Neo - #1 to #4) were cultivated at 28°C in 10% FCS supplemented LIT medium. RNA was extracted using TRIzol®, followed by the quantification and treatment with DNase before the cDNA synthesis, by using the SuperScript IV kit. The RT-qPCR assay were performed using TcGAPDH and TcCalmoduline as housekeeping genes. For assays of western blotting and ELISA, we used anti-apyrase immune serum, and the protein of the clones was extracted by the freezing and thawing method. **Results:** We observed that the Hygro and Neo hemi-knockout showed a lower cell growth when compared to the wild type clone. In addition, we also observed that the Hygro clones showed low expression of TcNTPDase-1, while the Neo clones had an overexpression of this gene, when compared to wild clone Dm28c. These data observed in the mRNA levels corroborate with the data obtained in western blot and ELISA experiments. **Conclusions:** When *T.cruzi* was knocked-out in both alleles of the NTPDase-1 gene, it was not possible to observe the parasite survival. But, the hemi-knockout parasites were viable and presented an altered expression level of the NTPDase-1 gene. We are now evaluating the infectivity and virulence of the recombinant parasite, in order to investigate the role of NTPDase-1 in *T.cruzi*.

Keyword: *Trypanosoma cruzi*; Chagas Disease, TcNTPDase-1.