Trypanosoma cruzi glycosylation: the role of oligosaccharyltransferase enzyme on the N-glycosylation machinery

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The survival of the parasite *Trypanosoma cruzi* during its cell cycle and establishment of infection depends on specific processes that ensure its adaptation in the insect vector and mammalian hosts. In this context, post-translational modifications, such as glycosylation, have been studied to understand better the molecular mechanisms involved in host-pathogen interaction. Glycans decorate proteins, lipids, and RNA conferring different physicochemical and biological properties. N-linked glycans are characterized by the addition of oligosaccharides to the side chain of asparagine within a specific consensus sequence, NxT/S sequon. A series of enzymes located in the endoplasmic reticulum and Golgi apparatus are responsible for adding, removing and remodeling protein N-glycosylation. In trypanosomatids, the single and functional oligosaccharyltransferase enzyme (STT3) is responsible for transferring a pre-assembled high mannose oligosaccharide to asparagine residues of nascent polypeptides entering the lumen of the rough endoplasmic reticulum (RER). In *T. brucei*, the OST complex was described as consisting of three complete paralogous genes, and after silencing by RNA interference of TbSTT3A and TbSTT3B genes, the parasites showed significant reduction of N-glycans. However, in *T. cruzi*, the biological function of this enzyme remains uncharacterized. The data presented here show the functional and biological characterization of TcSTT3 using the CRISPR/Cas9 system. Based on the Y C6 strain genome assembly version 52 available at TritrypDB, we identified seven TcSTT3 gene sequences *in tandem* on chromosome 30, and analysis by gene expression showed the presence of two paralogues: six copies of paralogue 1 and one copy of paralogue 3. We designed two single-guide RNAs (sgRNA) to recognize the 5’ and 3’ UTR, and homologous recombination was done using a DNA donor containing a puromycin gene and a 3-c-myc tag sequence. The selected parasites were cloned by serial dilution, and total DNA was extracted to confirm TcSTT3 knockout. Four clones confirmed the insertion of DNA donor by PCR and decreased TcSTT3 gene expression. Flow cytometry and lectin immunoblots showed a reduction in Concanavalin A (ConA). ConA is a lectin that recognizes mannose, and this reduction suggests alterations in the glycome repertoire. The growth of the parasites compared to control cell. Ultimately, proteomics analysis was performed to uncover the molecular pathways differentially modulated after TcSTT3 knockout. A total of 2,106 proteins were quantified, and 159 regulated proteins were selected using ANOVA with Benjamini-Hochberg correction (FDR < 0.05). Principal component analysis (PCA) showed a clear separation between Cas9 (control) and the four clones. The analysis of biological processes showed proteins associated with cytoskeletal, mitochondrial, and metabolic processes. Further studies will be performed on the role of STT3 function during the life cycle of *T. cruzi*, metacyclogenesis, adhesion and infection in host cells. These results will provide novel information about TcSTT3 paralogue specificity and the role of N-linked glycosylation in *T. cruzi* biology

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