RNA-binding proteins ALBA3 and DRBD3 characterization on *Trypanosoma cruzi* under gamma irradiation stress

Daniela Ferreira Chame[1], Daniela De Laet Souza[1], Eddie Luidy Imada[1], Helaine Graziele Santos Vieira[2], Andrea Mara Macedo[1], Carlos Renato Machado[1], Dominik Kaczorowski[2], Glória Regina Franco[1]

[1]*Departamento de Bioquímica e Imunologia, UFMG BH/Brazil,* [2]*Garvan Institute of Medical Research, Sydney/Australia*

Trypanosoma cruzi is highly resistant to gamma irradiation. After 500 Gy of ionizing radiation dose the chromosomal bands are fragmented, but after 48 hours the parasite is able to restore its initial chromosomal patterns. RNA binding proteins (RBPs) are important modulators of gene expression in normal and stress conditions and are involved in processing, decay, stability and transport of mRNAs. In order to investigate the involvement of RBPs in the response to radiation stress, we aimed to characterize the *T. cruzi* RBPs TcALBA and TcDRBD3 and their associated RNAs in irradiated and non-irradiated parasites. To explore the role of these RBPs, epimastigotes of the CL Brener clone were transfected with the vector pRock/Neo to obtain *T. cruzi* cell lines overexpressing the proteins TcALBA and TcDRBD3, containing a C-terminal 6His tail. The recombinant proteins were successfully detected by western blot in whole protein extracts from transfected epimastigotes. The phenotype of RBPs overexpressing cell lines was evaluated through growth curves in the presence and absence of stress induction. Under gamma radiation stress, epimastigotes overexpressing TcALBA showed a faster growth recovery while TcDRBD3 showed a slower growth recovery in comparison with control cells. Immunofluorescence assays were performed to visualize the distribution pattern of these proteins throughout the cell. TcALBA3 was mainly located in the cytoplasm, 4h and 24h after irradiation. On the other hand, TcDRBD3 exhibited a perinuclear localization 4h after irradiation and a cytoplasmic granular distribution, 24h after irradiation. Given that TcALBA3 and TcDRBD3 genes are annotated in the *T. cruzi* genome as hypothetical proteins, structural analyses of these proteins were performed to predict conserved domains and secondary structures using the softwares InterProScan and PSSpred. *In silico* analyses revealed the presence of ALBA and RRM domains on TcALBA3 and TcDRBD3 and a secondary structure similar to *T. brucei* ALBA3 and DRBD3, respectively. *TcALBA3* and *TcDRBD3* gene expression levels were also analyzed by RNAseq (package DESeq2 from R/Bioconductor) in non-transfected cells on 4, 24 and 96 hours after exposure to 500 Gy of gamma radiation. *TcALBA3* and *TcDRBD3* are among the most expressed genes in their genomic vicinity. It was observed a two-fold change reduction in TcDRBD3 and TcALBA after gamma irradiation, suggesting their role in the parasite stress response. In order to continue our investigation on the function of RBPs in response to gamma radiation, we intend to perform RIP assays and identify RNAs present in these RBPs complexes.