CHARACTERIZATION OF A GENE ENCODING A HYPOTHETICAL PROTEIN IN *TRYPANOSOMA CRUZI* POPULATIONS RESISTANT TO BENZNIDAZOLE

Araújo, S.S.[1], Campos, F.M.F.[1], Nogueira, F.B.[1], Romanha, A.J.[1] & Murta, S.M.F.[1]

[1]Centro de Pesquisa René Rachou, Fundação Oswaldo Cruz, Minas Gerais, Brazil

Murta *et al.* (2002) investigated the differential gene expression in *T. cruzi* populations with *in vitro*-induced (17LER) and *in vivo*-selected (BZR) resistance to benznidazole (BZ). Using the microarray methodology, the authors selected the *Tc*Hipo gene encoding a hypothetical protein (Tc00.1047053511807.220). This gene was overexpressed in the *T. cruzi* populations resistant to BZ. Many sequences from *T. cruzi* genome are apparently non-identified genes that correspond to hypothetical proteins. In this study, we investigate differences in the levels of *Tc*Hipo mRNA in *T. cruzi* populations susceptible and drug-resistant to BZ. The northern blot profile of total RNA from *T. cruzi* samples hybridized with *Tc*Hipo probe revealed one transcript of 980bp. Quantitative analysis revealed that the *T. cruzi* drug-resistant populations 17LER and BZR expressed 4 and 2-fold more *Tc*Hipo mRNA than drug-susceptible 17WTS and BZS, respectively. In addition, the *Tc*Hipo encoding region was cloned into pGEX expression vector. The GST-fusion recombinant TcHipo protein (~65KDa) expressed in *Escherichia coli* BL21 was used as an antigen for producing rabbit anti-rTcHipo polyclonal antibodies. Western blot analysis of *T. cruzi* protein extracts probed with anti-rTcHipo polyclonal antibody revealed a unique polypeptide of 50KDa for all strains analyzed. Further studies will focus the TcHipo protein level analysis in the *T. cruzi* strains susceptible and resistant to BZ. Keywords: *Trypanosoma cruzi*, drug resistance, DNA microarray, hypothetical protein. Supported by FAPEMIG, CNPq and CPqRR/FIOCRUZ.