FUNCTIONAL CHARACTERIZATION OF THE ENZYME DNA TOPOISOMERASE 2 FROM THE MOSQUITO Aedes aegypti (Diptera, Culicidae) AND IN AaG2 CELLS

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Introduction: The mosquito Aedes aegypti is a major public health problem, being the vector of dengue and urban yellow fever. DNA topoisomerases are involved in cell cycle events and are targets for the development of new compounds for cancer and other diseases. Abnormal division of Drosophila melanogaster S2 cells was observed through the silencing of DNA topoisomerase 2. Objective: Characterize the function of the enzyme DNA Topoisomerase 2 (AeTop2) in the mosquito Aedes aegypti and Aag2 cell line. Material and methods: Total RNA of Aag2 cells and organs (fat body, intestine and ovary) from mosquito were extracted using Trizol reagent and analysis of the abundance of mRNAs encoding the enzyme AeTop2 by gPCR (PCR-real time). The inhibition assay of AeTop2 was performed by incubation with specific inhibitor (etoposide) at different concentrations for 24 hours. Cell viability was evaluated by MTT method. Treated Aag2 cells were fixed with 4% paraformaldehyde (PFA) and conjugated with DAPI and phalloidin for microscopical analysis. Results and Discussion: Etoposide reduces cell viability in low concentration 50uM and affect the proliferation of cells, confirmed by microscopy analysis. qPCR experiment showed that treatment with the inhibitor in concentrations of 50uM and 100uM decreased the expression of gene Aetop2 in larvae (L2). Larvae exposed to inhibitor 50uM and 100uM exhibited a delay in the development. Conclusion: Our results suggest that Top2 is a good molecular target as the inhibitor affected the proliferation and viability of cells as well as reduced gene expression in larvae.

Keywords: Topoisomerase 2, *Aedes aegypti*, Aag2 cells. Supported by: CAPES, FAPERJ, CNPq, INCT-EM.