An Investigation of 5-Fluorouracil Resistance in Kinetoplast Parasites

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Kinetoplastid parasites are a widespread group of flagellated protozoan pathogens and the defining feature of these parasites is the presence of a large mitochondrial DNA region known as the 'kinetoplast'. The most common human diseases caused by kinetoplastid parasites are: 1) African trypanosomes (African sleeping sickness), 2) *Leishmania* species (leishmaniasis) and 3) *Trypanosoma cruzi* (Chagas' disease). The two types of nucleotides in the cell are purine and pyrimidine which have a very significant role in nucleic acid synthesis (DNA and RNA) and the metabolism of prokaryotic and eukaryotic cells. Kinetoplastid parasites are capable of salvage as well as synthesis of pyrimidine nucleotides. Kinetoplastid protozoa express many such membrane transport proteins which enable them to take up nutrients, efflux metabolites, regulate physiological concentrations, translocate various molecules, and import or export drugs. Resistance to 5-fluorouracil (5-FU) was generated in both *T. b. brucei* BSF s427- wild type and *Leishmania mexicana* promastigotes, yielding clonal lines Tbb-5FURes and Lmex-5FURes, respectively. The gene family encoding pyrimidine nucleobase transporters in kinetoplast parasites has not yet been discovered. We try to identify these using the antimetabolite 5-FU as a probe. Previous work in our laboratories (RNA-seq and RIT-seq) analyses of 5-FU resistant cell lines has identified candidate genes for pyrimidine transporters, including genes annotated as cation transporters (*Tbb-CATs*), fatty acid desaturase (*Tbb-FAD* and *Lmex-FAD*) and glucose transporters (*2A*, *1B* and *1E*). Apart from some glucose transporters, none of these potential transport genes have been previously characterised in protozoa and as such they are of interest in their own right as well. The main aim of this study therefore is to identify the gene(s) encoding the protozoan transporters of pyrimidines, particularly uracil, and assess candidate genes that may be involved in transport of, or sensitivity to, 5-FU. For this we will use reverse genetics approaches such as knockout constructs, targeted RNAi, and overexpression of the target genes. We determined the sensitivity of the 5-FU and 6-Azauracil (6-AU) in a sKO of *Tbb-CATs* and *T. b. brucei 427 WT* with the use of alamar blue drug sensitivity assay, and found no significant difference. We were unable to make a full double knockout for the CATs, as this led to the death of the cells, showing that their function is essential for the growth of BSF *T. b. brucei* in vitro. Also, the effect of increased gene expression of *Tbb-FAD* in Tbb-5FURes and *Lmex-FAD* in Lmex-5FURes on 5-FU and 6-AU sensitivities were analysed using the alamar blue assay. Results showed no significant differences in the EC_{50} values of 5-FU and 6-AU between the overexpressing cell lines and the control lines. Efforts to identify the pyrimidine transporter genes are presently ongoing and identification of these genes will significantly improve our understanding of drug and nutrient transporters of kinetoplast parasites.