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SCHISTOSOMA MANSONI HISTONE-MODIFYING ENZYMES AS DRUG TARGETS

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Enzymes that modify histones (HME) are under increasing scrutiny as therapeutic targets in a number of pathologies, ranging from cancer to parasitic diseases. In particular, inhibitors of histone deacetylases (HDACi) induce cell cycle arrest and/or apoptosis in cancer cells. Treatment of schistosomula or adult worms with HDACi induces the death of both larval (schistosomula) and adult worms and this is preceded in the larvae by the induction of apoptosis as measured by TUNEL staining and the increase in the activity of caspase 3/7. Moreover, such treatments induce a rapid increase in the general level of histone acetylation, particularly of H4. This in turn correlates with the overexpression of certain genes, including those encoding caspases 3 and 7. Finally, qChIP analysis shows that the proximal promoters of both these genes show hyperacetylation of histone H4 after HDACi treatment. These results led us to consider schistosome HDACs, as well as other HMEs, as promising targets for the development of new drugs against schistosomiasis. To this end, a project (SEtTReND) supported by funding from the European Commission has been initiated with the aim of developing specific inhibitors against selected schistosome HMEs that could be candidates as lead compounds for drug development. All HMEs encoded in the *S. mansoni* genome involved in histone acetylation/deacetylation and methylation/demethylation have been identified. Using a phenotypic screen we have shown that inhibitors of all these enzyme classes induced apoptosis and death in schistosomula. Among the schistosome HMEs chosen for further study, SmHDAC8 is particularly promising. Its catalytic domain is more divergent from the human orthologue than are those of other schistosome HDACs and its validity as a therapeutic target was confirmed by RNAi. A combination of high-throughput and in silico screening is being applied to identify potential specific inhibitors of SmHDAC8. In parallel, other HMEs are also being investigated as potential targets.

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CHARACTERIZATION OF FARNESYL DIPHOSPHATE SYNTHASE AND GERANYLGERANYL DIPHOSPHATE SYNTHASE IN *SCHISTOSOMA MANSONI* AND THEIR ROLE AS POTENTIAL DRUG TARGETS

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Schistosomiasis affects over 260 million people worldwide with over 200,000 deaths annually. There is currently only one drug available for disease treatment, praziquantel. We report here that *Schistosoma mansoni* farnesyl diphosphate (FPP) synthase (SmFPPS) and geranylgeranyl diphosphate synthase (SmGGPPS), essential enzymes in many eukaryotes involved in protein prenylation and the generation of sterols and non-sterol products of mevalonate, could serve as drug targets for the treatment of schistosomiasis. In humans, FPPS is a target for the bisphosphonate drugs widely used in bone resorption therapy. Validation of FPPS and GGPPS as drug targets may allow the repositioning of bisphosphonates for schistosomiasis treatment. SmFPPS and SmGGPPS have 35% identity to human FPPS and 53% identity to human GGPPS, respectively. We successfully expressed active, recombinant SmFPPS and SmGGPPS. Recombinant SmFPPS was found to be a soluble 44.2 kDa protein while SmGGPPS was a 38.3 kDa soluble protein. Characterization of the substrate utilization of the two enzymes showed that, unlike the human enzymes, which display strict substrate specificity, both worm enzymes were able to couple isopentenyl PP with three allylic acceptors (dimethylallyl diphosphate, geranyl diphosphate, and FPP). This indicates that the schistosome enzymes have overlapping substrate specificities, making their actions appear to be redundant. Against SmFPPS, several bisphosphonates had IC50s in the low nanomolar range; these inhibitors had significantly less activity against SmGGPPS. While hydrophilic bisphosphonates had no activity against cultured adult parasites, a lipophilic bisphosphonate at 50 µM was active against ex vivo adult worms with worm death occurring over 4-7 days.These results indicate that FPPS and GGPPS could be important targets in the search for new drugs for the treatment for schistosomiasis.

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SINGLE NUCLEOTIDE POLYMORPHISM-BASED SELECTIONS IN THE ß-TUBULIN GENE OF *ONCHOCERCA VOLVULUS*: A NEW STEP IN FILLING THE GAP OF THE POSSIBLE IVERMECTIN FAILURE

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The control of onchocerciasis or river blindness with ivermectin (IVM) has been a great success until now, so that in certain foci its elimination was found to be feasible. However, after more than 21 years of IVM repeated treatment, the disease still persists in many endemic countries. Though sub-optimal responses and genetic changes have been reported in *Onchocerca volvulus* populations under high IVM pressure, unequivocal evidence of resistance has yet to be established. This situation must therefore be urgently clarified to preserve the achievements of onchocerciasis control programs. In this study, *O. volvulus* adult worms were collected from the same patients, before IVM exposure and following three years of annual or three-monthly treatment at 150 µg/kg or 800 µg/kg. Four single nucleotide polymorphisms (SNPs) occurring in the β-tubulin gene of these parasites were investigated. We found multiple nucleotide changes in *O. volvulus* β-tubulin gene associated with the dose and the annual frequency of IVM. Among the SNPs investigated, three showed a high level of selection and nonrandom allelic associations after treatment. Therefore, they may be relevant markers to follow selection for IVM resistance in the field. These results strengthen the warning that selection for IVM resistance could emerge in some *O. volvulus* populations.

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A LATERALLY TRANSFERRED FERROCHELATASE GENE IS FUNCTIONAL AND ESSENTIAL IN FILARIAL NEMATODE PARASITES

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Species in the phylum Nematoda lack a heme biosynthetic pathway and require extraneous heme. Many filarial nematodes contain an obligate endosymbiont, *Wolbachia,* which has a functional heme biosynthesis pathway. Sequencing of the human filarial nematode *Brugia malayi* revealed a genomic ferrochelatase (BmFc) gene, the terminal step in heme biosynthesis. The BmFc gene contains 9 exons spanning ~ 4.5

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