Commentary

High-throughput whole genome analysis provides insight into how the major drugs against African sleeping sickness operate

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Invited Commentary on 'High-throughput decoding of antitrypanosomal drug efficacy and resistance', Alsford et al., Nature Genetics 2012

A recent paper by Alsford *et al.* in *Nature* presents an experimental *tour de force*, where the molecular mechanisms behind the uptake and metabolism of the five major drugs against human African trypanosomiasis (HAT) are elucidated using cutting edge molecular methods.¹ The African trypanosome *Trypanosoma brucei* causes one of the neglected tropical diseases, African sleeping sickness in humans and *nagana* in livestock, both endemic to sub-Saharan Africa.² *T. brucei* sp. are transmitted by tsetse fly insect vectors to a wide range of mammalian hosts. Here, these flagellated protozoa multiply extracellularly in the bloodstream and tissues, forming chronic infections despite being faced with continuous immune attack. Trypanosomiasis is normally fatal if left untreated. Available treatments for HAT are limited, and include very old drugs like suramin and pentamidine, which were developed in the 1920s and 1930s.³ During the later stages of disease, trypanosomes invade the central nervous system, restricting treatment options to drugs that include the highly toxic arsenical melarsoprol or the more recently developed drug, eflornithine, which is only effective against West African trypanosomiasis.³ Unsatisfactorily, the mode of action of most of these antitrypanosomal drugs has not yet been elucidated.

Recently, Alsford and Horn, at the London School of Hygiene and Tropical Medicine developed a method for whole genome phenotypic analysis of *T. brucei* using a high-throughput phenotyping approach which they referred to as RNAi interference (RNAi) target sequencing or RIT-seq.⁴ Here, RNAi

libraries containing the entire genome of *T. brucei* were transfected into trypanosomes. Whole genome Illumina sequencing was then performed on genomic DNA isolated from *T. brucei* cultures grown in the presence or absence of induction of RNAi. This method allowed determination of the essentiality of the estimated 7000–8000 *T. brucei* coding sequences.

This RIT-seq method has now been used to obtain insight into how *T. brucei* takes up and metabolises the major drugs used against it.¹ *T. brucei* transfected with an RNAi library was simultaneously treated with different antitrypanosomal drugs. Induction of RNAi revealed the necessity of different *T. brucei* genes for drug sensitivity. This approach has revealed the *T. brucei* transporters, enzymes, and both metabolic and cellular pathways that mediate sensitivity of these parasites to a variety of antitrypanosomals. As a proof of principle, the known drug transporters were identified, as well as more than fifty additional genes linked to drug action. The authors have also identified the importance of aquaglyceroporins in pentamidine and melarsoprol cross-resistance. Knowledge about the *T. brucei* molecular pathways behind the efficacy of these drugs will facilitate the search for new and improved compounds with reduced toxicity to humans. In addition, a deeper understanding of the molecular mechanisms behind *T. brucei* drug metabolism will facilitate screening for drug resistant parasites, thereby allowing more targeted therapeutic approaches.

References

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