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Genome plasticity: an essential adaptive mechanism that drives drug resistance in *Leishmania* spp.

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Background

Given the expanding global prevalence of leishmaniasis, a Neglected Tropical Disease, and the limited efficacy of existing therapeutics due to issues related to resistance, cost, toxicity, and administration, the identification and validation of new drug targets in *Leishmania* spp. [1], and the mechanisms that drive drug resistance in this protozoan parasite are crucial [2-3]. Genetic validation of novel targets primarily relied on targeted gene knockout through homologous recombination and more recently on knockouts generated with CRISPR Cas9 [3-6]. A significant proportion of targeted genes (approximately 70%) were classified as non-essential. In this study, we used whole genome sequencing to evaluate a *Leishmania major* knockout which lacks sphingolipid biosynthesis (LCB2KO) while remaining viable and retaining its infectivity [3,7]. Here, we discuss genomic changes observed in several *Leishmania* spp. resistant to amphotericin B (AmB) and miltefosine [3] and the application of our drug discovery pipeline (genomics, metabolomics, lipidomics, gene editing, drug screening assays, *in vivo* infection model) to deconvolute the mode of action and resistance (MoAR) to these and new compounds with antileishmanial activity including orphan drug candidates.

Material and methods

Whole genome sequencing (WGS) with short (Illumina) and long reads using Oxford nanopore (ONT) reads were applied to identify SNPs, CNVs [2,3,8] and structural variants of a series of mutants generated using forward and reverse genetics, as follows: (A) drug-resistant cell lines (n=15) to amphotericin B [3], the orphan drug candidate [8], clemastine (n=4) and eleven new compounds (n>30) [unpublished] were generated *in vitro*. (B) Sphingolipid-deficient mutants were generated using homologous recombination (*L. major*) [7] and CRISPR-Cas9 (*L. mexicana*) [2,4]. Further phenotypical characterisation was performed using metabolomics and lipidomics, drug assays (resazurin) and infection in macrophages and using a murine model [2,4,7,8].

Results

WGS led to the identification of genomic changes and the MoAR in multiple *Leishmania* cell lines (n= ~50) resistant to AmB, miltefosine and other structurally distinct antileishmanial candidates whose mechanism in *Leishmania* spp. was unknown. Similarly, we validated IPC synthase as a target of the orphan drug clemastine fumarate in two *Leishmania* spp [8, unpublished]. WGS showed in a historic *L. major* knockout devoid of sphingolipid biosynthesis, LCB2, which is viable and infective in mice, genomic lesions such as the deletion of two genes, the ABC3A sterol- and the miltefosine transporter. In our previous study, the latter was also deleted in multiple cell lines in addition to SNPs in two genes of the sterol biosynthetic pathway which led to AmB resistance [3]. Importantly, simultaneous deletion of this ABC3A gene using CRISPR-Cas9 in *L. mexicana*, facilitated LCB2-targeted knockout, suggesting a compensatory effect. Furthermore, ONT long-read sequencing revealed the presence of an additional deleted gene that remained undetected by short-read sequencing. This discrepancy can be attributed to the gene's location within a highly repetitive region. Currently, we are employing WGS alongside other tools within our pipeline to deconvoluting the MoA of new compounds from the GSK Leishbox with activity against *Leishmania* and *T. cruzi* and newly identified drug targets. Following SNP calling and identification of coding mutations, four potential targets were selected for genetic validation, including two hypothetical proteins, one encoding a predicted amino acid transporter (AATP11), and one encoding a folate biopterin transporter (FBT), which MoA was validated using null mutants.

Conclusions

Target deconvolution methods use multiple assays to identify high-confidence targets. These compounds are now employed in cellular thermal shift proteomics and untargeted metabolomics to enhance the understanding of their modes of action. Additionally, it's crucial to re-evaluate historical *Leishmania spp* knockout lines, including genes like LCB2, previously considered non-essential.

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References:

1. Norcliffe, J., Alvarez-Ruiz, E., Martin-Plaza, J. et al. The utility of yeast as a tool for cell-based, target-directed high-throughput screening. Parasitology. 2014, 141(1), 8–16.
2. Alpizar-Sosa EA, Kumordzi Y, Wei W, et al. Genome deletions to overcome the directed loss of gene function in *Leishmania*. Frontiers in cellular and infection microbiology. 2022, 23;12:988688.
3. Alpizar-Sosa E, Ithnin N, Wei W, et al. Amphotericin B resistance in Leishmania mexicana: Alterations to sterol metabolism and oxidative stress response. PLOS Neglected Tropical Diseases. 2022;16(9):e0010779.
4. Beneke T, Madden R, Makin L, et al. A CRISPR Cas9 high-throughput genome editing toolkit for kinetoplastids. Royal Society open science. 2017, 3;4(5):170095.
5. Sollelis L, Ghorbal M, MacPherson CR, et al. First efficient CRISPR-C as9-mediated genome editing in *Leishmania* parasites. Cellular microbiology. 2015;17(10):1405-12.
6. Zhang WW, Matlashewski G. CRISPR-Cas9-mediated genome editing in *Leishmania donovani*. MBio. 2015;6(4):10-128.
7. Denny, P., Goulding, D., Ferguson, M., Smith, D. Sphingolipid-free leishmania are defective in membrane trafficking, differentiation, and infectivity. Mol. Microbiol. 2004, 52 (2), 313–327.
8. Mina J, Charlton R, Alpizar-Sosa E, et al. Antileishmanial chemotherapy through clemastine fumarate mediated inhibition of the leishmania inositol phosphorylceramide synthase. ACS infectious diseases. 2020 Dec 8;7(1):47-63.