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C0459 PRE-ADAPTION TO ANTIMONIALS IS GENERAL IN LEISHMANIA DONOVANI FROM THE INDIAN SUBCONTINENT ()

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1 Background

Antimonials (SSG) have been used for many decades in the Indian Sub-Continent (ISC) to treat patients with visceral leishmaniasis (VL). We previously sequenced the whole genome of 204 *L. donovani* isolates and found out that all isolates originating from the lowland hyper-endemic region (93% of the sample), possessed an exceptional, intra-chromosomal amplification of the gene encoding MRPA (involved in efflux of trivalent antimony, Sb^{III}); this amplicon was absent in 'Yeti' strains originating from Nepalese highlands. This unique feature could represent a pre-adaptation of lowland strains to antimonials. To verify this, we experimentally selected Sb^{III}-Resistance in lowland and Yeti strains and characterized the time-to- and the type-of-resistance.

2 Methods

Promastigotes of 3 cloned strains, BPK282 and BPK275 (lowlands) and BPK026 (Yeti) were selected *in vitro* using progressively increasing doses of Sb^{III}. Genomic and metabolomic changes were followed by high-throughput sequencing and mass-spectrometry, respectively. We also performed a 'flash-selection', by directly exposing parasites to the single highest drug concentration.

3 Results

Baseline Sb^{III} sensitivity of BPK282 and BPK275 was comparable and 10 times lower than BPK026. Time-to-resistance was shorter for the 2 lowland strains (4 cycles) than for BPK026 (7 cycles). All quadruplicates of BPK275 and BPK282 reached the last cycle but only 1 replicate of BPK026 was recovered at the final concentration. Intracellular amastigotes of the selected Sb^{III}-R strains were also resistant to SSG. At genomic level there were no local CNVs, no indels and very few SNPs (only in BPK026); in contrast, chromosomal copy number increased (noteworthy chr23 carrying MRPA), even more in BPK026. From a metabolomic point of view, lowland and Yeti isolates were very different. During SbIII-R selection, the number of changed metabolites was much higher for BPK026: among them, arginine was unchanged during selection of lowland isolates, but increased and reached an equivalent level in Yeti strains. Pre-adaptation of lowland strains was further confirmed by flash-selection: in two weeks, both strains were growing in highest Sb^{III} concentration, while Yeti was killed.

4 Conclusions

Lowland strains are pre-adapted to Sb^{III}, among others because of multiple copies of MRPA present on chr23 and Yeti strains need much more time and molecular changes to become resistant. Results will be discussed in the context of treatment outcome in patients.