LC-MS/MS HELPS CHARACTERIZING THP-1 CELLS PROTEOME AFTER INFECTION WITH DRUG RESISTANT LEISHMANIA STRAINS

Lorenzo Tagliazucchi [1], Ana Perea-Martinez [2], Greta Fiorini [1], José Ignacio Manzano [2], Raquel García-Hernández [2], Filippo Genovese [3], Diego Pinetti [3], Francisco Gamarro [2], Maria Paola Costi [1]

[1.] Department of Life Science, University of Modena and Reggio Emilia, Via Campi 103, 41125-Modena, Italy;

[2.] Instituto de Parasitología y Biomedicina ''López-Neyra'' (IPBLN-CSIC), Avda. del Conocimiento 17, 18016- Armilla (Granada), Spain;

[3.] Centro Interdipartimentale Grandi Strumenti (CIGS), Via Campi 213/A, University of Modena and Reggio Emilia, Modena, Italy;

ABSTRACT

Leishmaniasis is a vector-borne neglected tropical disease (NTD) which directly affects 98 countries, being responsible for 12 million infections worldwide. Its diffusion is intricately linked to environmental changes and socioeconomic risk factors, advocating the importance of the One Health approach. Moreover, as the world is currently facing increasing difficulties to treat this disease with available therapies, deeper investigation into the molecular mechanisms responsible for both drug resistance (DR) and treatment failure (TF) is essential in drug discovery. Indeed, the few available chemotherapeutics cause severe side effects and have developed several resistance mechanisms [1].

Thus, our aim is to identify the human monocytic biochemical pathways that are differentially expressed when they are infected by different *Leishmania infantum* clinical isolates, from patients with either DR or with TF outcome. This helps understand the mechanisms of drug inactivation and identify outstanding proteins that can be druggable to contain resistance. For this purpose, we have employed a whole-cell differential Mass Spectrometry (MS) bottom-up approach, which was already reported as exhaustive for guest-host interaction studies [2]. Miltefosine, Sb(V), and paromomycin resistant lines were used to represent DR, along with four samples non-DR from HIV-immunocompromised patients who failed their LV treatment. We have infected THP-1 monocytes with the *L. infantum* strains along with a non-treated control, and a positive control obtained with heat-inactivated parasites. Tryptic peptides were analysed by high resolution LC-MS/MS, and a first differential analysis between positive and negative controls identified THP-1 proteins non-specifically related to parasitic infection (Δc group). Then, One-way ANOVA tests were applied between the resulting Δc group and each sample, which identified a total of 44 differentially expressed proteins (DEP). We have adopted network enrichment analysis (STRING environment) to integrate the DEP from proteomics studies with the corresponding RT-qPRC quantitation of the transcripts from the same infected cells. Transferrin Receptor C (TFRC) and Nucleoside Diphosphate Kinase 3 (NDK3) were identified as overexpressed proteins in THP-1 cells infected with paromomycin, Sb(V), and miltefosine resistant *L. infantum* lines, along with their transcripts [3].

The overall achievements represent founding concepts to confirm new targets involved in the parasitic drug resistance and TF mechanisms, and to consider in perspective the importance of a dual host- guest pharmacological approach to treat the acute stage of the disease.

REFERENCES

[1] Kaye, P., Scott, P. Leishmaniasis: complexity at the host–pathogen interface. *Nat Rev Microbiol* **2011**, 9;604–615

[2] Denny PW, Kalesh K. How can proteomics overhaul our understanding of *Leishmania* biology? *Expert Rev Proteomics*. **2020**,17(11-12):789-792.

[3] Perea-Martínez A, García-Hernández R, Manzano JI, Gamarro F. Transcriptomic Analysis in Human Macrophages Infected with Therapeutic Failure Clinical Isolates of *Leishmania infantum*. *ACS Infect Dis*. **2022** Apr 8;8(4):800-810.