A dual-Omics approach to identify the modulated proteins/genes in THP-1 cells infected with different drug resistant *L. infantum* clinical isolates.

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 Sciences, 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;

Leishmaniases are vector borne diseases that affects more than 12M patients every year. The few available chemotherapeutics available on the market cause severe side effects and have selected several resistant strains [1]. Therefore, the aim of our study is to identify the proteins from THP-1 cell lysates associated with drug resistance mechanisms, when monocytes are infected with different Leishmania infantum strains from clinical isolates, from patients with drug resistance, with a therapeutic failure (TF) outcome. For this purpose, we have developed a whole cell differential Mass Spectrometry (MS) approach coupled with UHPLC, which was already reported as exhaustive for guest-host interaction studies [2]. Miltefosine, Sb^V and paromomycin resistant strains were used to represent drug resistance (DR), along with four samples non-DR from HIV-immunocompromised patients, who failed their LV treatment (TF strains). Samples were collected from THP-1 infected colonies and the whole lysates were treated with a typical FASP protocol. The obtained tryptic peptides were analyzed a LC-MS/MS system with a FullMS-ddMS^2 experiment (UltiMate 3000 coupled to an Orbitrap Q-Exactive).

Both Progenesis (Nonlinear Dynamics, Waters) and Mascot Matrix Suite were employed for raw data analysis (workflow described in *Figure 1a*), which evidenced 44 differentially expressed proteins (DEPs). Parallelly, a mRNA-seq experiment, run on the same samples, evidenced 18 differentially expressed transcripts (DETs) [3,4]. Two of them are mutually differentially expressed among DEPs and DETs, the Transferrin Receptor C (TFRC), and the Nucleoside Kinase 3 (NDK3) (*Figure 1b*), which are fundamental for the parasite to uptake mineral and metabolites from the host’s plasma. Their genes were validated with RT-qPCR, which confirmed their fold-change and significance, and their functional networks were characterized with bioinformatics tools like STRING and PANTHER (*Figure 1c*) [5]. The next step includes a biological validation with host’s directed compounds that inhibit these two proteins.

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.

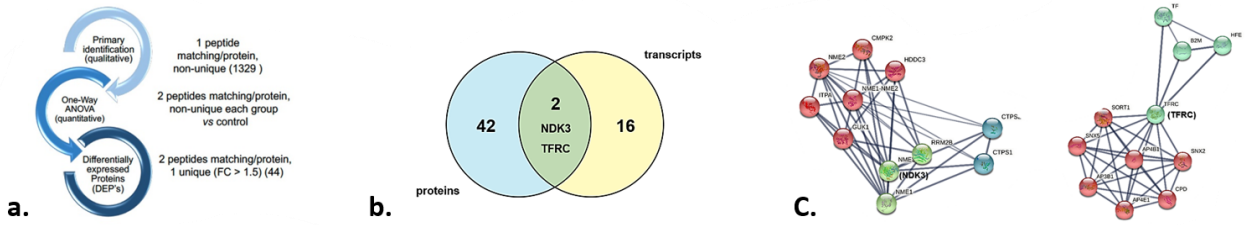

Figure 1. **a**. Workflow of protein identification. **b** identified DETs and DEPs from RNA-seq and proteomics experiments. **c** Network enrichment around NDK3 and TFRC

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