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Molecular and functional characterization of the Tyrosine & Phosphoinositide phosphatase *Ld*TyrPIP_22 from the protist *Leishmania donovani*

Olympia Paraskevi TZIOUVARA

ABSTRACT

The single celled eukaryotic protozoan pathogen *Leishmania donovani/L. donovani* is one of the causative agents of the fatal disease visceral leishmaniasis (Kala azar). The pathogenicity of many microbial pathogens relies on subversion of host cell signaling pathways one of which includes phosphoinositide (PI) metabolism. While pathogenic bacteria PI phosphatases that modulate PI metabolism in the host already constitute attractive chemotherapeutic targets they are poorly studied in intracellular protozoan pathogens. The *Kinetoplastids*' phosphatome contains a subgroup of Atypical Dual Specificity/Lipid Phosphatases with no human homologues sharing a catalytic P-loop motif to the secreted virulence factors MptpB and LipA PI phosphatases from *Mycobacterium tuberculosis* and *Listeria monocytogenes* respectively. The *L. donovani* member of this family, encoded by the LDBPK_220120 gene locus and named in our work *Ld*TyrPIP_22, is highly conserved amongst *Leishmania* species and shares significant identity with protein tyrosine and PI phosphatases from pathogenic bacteria two of which (i.e LipA, MptpB) are considered druggable enzyme targets. Our previous work showed that a) recombinant bacterially expressed *Ld*TyrPIP_22-His has phosphatase activity and specifically dephosphorylates Ptyr, PI(3)P and PI(4)P and b) the endogenous *Ld*TyrPIP_22 partially colocalizes with *Ld*actin in *L. donovani* promastigotes. We present herein results regarding a) the localization of *Ld*TyrPIP_22 in promastigotes inside macrophages after *in vitro* infection and in axenic amastigotes by microscopy methods, b) a protocol for analysis of secreted proteins in the extracellular medium of cultured *Leishmania* promastigotes and amastigotes and data about the suspected secretion of *Ld*TyrPIP_22, c) exploration of the possibility transgenic *L. donovani* parasites that overexpress the recombinant *Ld*TyrPIP_22-RFP infect more efficiently macrophages *in vitro* and d) the construction of *Leishmania* specific expression plasmids pLexsy-sat2.1-*Ldtyrpip*$_{22}$*-6his* for expression of the r*Ld*TyrPIP_22-His protein as intracellular or secreted form and e) the construction of transgenic *Leishmania tarentolae* cells (non-human pathogen) overexpressing r*Ld*TyrPIP_22-His for isolation of the *Leishmania* produced enzyme forms for structural and further functional analysis