Probing the role of Arg97 in the parasite *Leishmania braziliensis* Hsp90 through site directed mutagenesis on the human counterpart

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Heat shock protein 90 (Hsp90) belongs to a family of ubiquitous proteins acting as molecular chaperones that stabilizes client proteins in a folded and functional state. Hsp90 plays a pivotal role in the life cycle control of the protozoan parasite *L. braziliensis* and is essential for survival and proliferation during the intracellular mammalian stage (the amastigote) (1). The N-terminal domain (NTD) contains the main structural determinants generating the ATP binding site in which ATP is hydrolyzed providing energy to perform the peculiar Hsp90 cellular activity. Molecules able to prevent ATP binding act as Hsp90 inhibitors and are able to block its chaperone function leading to client proteins degradation and subsequently to cell death. For this reason, Hsp90 is considered a promising target for the treatment of leishmaniasis (2). Nevertheless, the identification of new molecules selectively targeting *L. braziliensis* Hsp90 is very limited due to the high conservation of the ATP binding site between parasite and human proteins (3). In this investigation, we have established a reliable protocol for purification and crystallization of hHsp90-NTDα (hNTDα) and for expression and purification of *Lb*Hsp90-NTD (*Lb*NTD). Since no crystals have been obtained for *Lb*NTD, two variants of hNTDα, K112R and K112A, have been generated by site directed mutagenesis. In particular, the variant K112R represents the "*leishmanized*" protein in which the unconserved residue lysine 112 in the ATP binding site of hNTDα has been replaced by an arginine structurally matching this residue in *Lb*NTD. The K112A variant has been generated to deeply understand the role of this unconserved residue in human and parasite proteins. A vast variety of ADP and ATP analogues and cAMP have been used to probe the role of these residues. Our structural results strongly support that residue 112 in hNTDα and the corresponding Arg97 in the parasite counterpart are not crucial for substrate binding. At variance with previous investigations on *P. falciparum* NTD in which a prominent role in selectivity of the conserved parasite arginine has been reported, our analysis reveals that this residue is not exploitable for the development of inhibitors specifically targeting *Lb*Hsp90 over the human enzyme.

References: 1. Q. Li, Y. Zhou, C. Yao, X. Ma, L. Wang, W. Xu, Z. Wang, Z. Qiaoet *Parasitology research*. 2009, 105 (6), 1539-48. 2. A Hombach, J Clos *Parasitology* 2014, 141, 1156. 3. N Faya, D. L. Penkler, Ö.T. Bishop. *FEBS Open Bio* 2015, 5, 916–927.