Identifying and exploiting deubiquitinating cysteine peptidase (DUBs) of *Leishmania*

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Deubiquitinating enzymes (DUBs) are a class of peptidases whose function is to cleave the post-translational modifier ubiquitin from proteins or ubiquitin-conjugates. DUBs play crucial roles in many biological processes such as protein degradation, gene regulation, epigenetics, trafficking, and DNA repair. Interfering with DUB function is considered a promising approach to selectively kill aberrant cells and DUBs are currently being pursued as anticancer drug targets. Leishmania also has a ubiquitin system and its genome suggest the presence of 20 DUB orthologues, however, the identity, function and essentiality of DUBs in L*eishmania* remains to be revealed. A chemical proteomics approach using a fluorescent ubiquitin-based probe was used for activity-based protein profiling, revealing the presence of many active DUBs in *Leishmania mexicana*. A number of stage-specific DUBs have been identified, including some that are active during differentation of procyclic promastigote to amastigote and some that have amastigote-specific activity. A previous RNAi screen in *T.brucei* identified a DUB (DUB1) that is essential for bloodstream form proliferation. The DiCRe inducible gene knockout system is being used to evaluate *L. mexicana* DUB1, with preliminary data suggesting that LmDUB1 is essential. Furthermore, active recombinant LmDUB1 protein has been expressed and purified using a baculovirus expression system and an HTS-compatible fluorescence polarisation assay developed based on the proteolysis of tetramethylrhodamine-labelled Lys(Ub)Gly. Our approach combines chemical and genetic screening to identify essential Leishmania DUBs as a starting point for drug discovery activities.