PLASMODIUM KNOWLESI AS A MODEL FOR STUDY PLASMODIUM VIVAX METACASPASES

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Introduction: In the absence of a continuous *in vitro* culture system for *Plasmodium vivax, P. knowlesi* constitutes a primary model for studying *P. vivax* biology due to its close phylogenetic relationship. Metacaspases are cysteine proteases that seem to be essential for parasite survival and development, yet the dynamics of expression and functional activities of *Plasmodium* metacaspase remain poorly understood. **Objective:** This study aimed to characterize the expression profiles and catalytic activity of *P. knowlesi* metacaspases (*Pk*MCAs) and evaluate their suitability as models for *P. vivax* metacaspases (*Pv*MCAs) during intraerythrocytic development. **Methods:** Single-cell RNA sequencing data were obtained from asexual blood stages of *P. knowlesi* to assess the expression of *Pk*MCA1–3, as well as the unique substrate of metacaspases described, the *Tudor Staphylococcus Nuclease* (*Pk*TSN). Anti-*Pv*MCA1 antibody cross-reactivity was also assessed, and *Pk*MCAs activity was evaluated using a fluorogenic peptide substrate specific for metacaspases (Z-VRPR-AMC). **Results:** Distinct stage-specific expression patterns were identified: *Pk*MCA1 peaked in mature trophozoites; *Pk*MCA2 peaked in schizonts; and *Pk*MCA3 showed peaks in both stages. Expression of *Pk*TSN increased progressively during schizogony. Anti-*Pv*MCA1 antibodies successfully detected orthologous protein in the crude extracts of *P. knowlesi*. Functional assays showed that, similarly to *P. vivax*, metacaspase activity is detected in *P. knowlesi* extracts, which was calcium-independent and inhibited by metacaspase inhibitor treatment. **Conclusion:** The findings indicate that *P. knowlesi* has functional metacaspases that, besides constituting a model for studying *Pv*MCAs, could be explored to elucidate the role of metacaspases in *Plasmodium* biology.