Synergy unleashed: *Leishmania donovani* GP63 paralogues cooperatively function to drive parasite infectivity at various pathogenic stages and regulate host survival mechanisms

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ABSTRACT

Leishmania donovani (Ld), the causative agent of visceral leishmaniasis, relies on finely tuned molecular strategies to thrive within both its insect vector and mammalian host. Central to its success is GP63, a zinc metalloprotease long recognized as a major virulence factor. While its role in promastigote attachment and invasion is well documented, particularly for cutaneous leishmaniasis, how GP63 functions in mammalian visceral infection has remained elusive. Importantly, in humans, after initial promastigote infection, subsequent parasite propagation depends exclusively on intracellular amastigotes arising from lysed macrophages. Classical GP63 expression and function in Ld amastigotes remain poorly understood, and GP63 null mutants reportedly retain infectivity in mice, raising fundamental questions about virulence factor complementation during mammalian infection. By combining comparative transcriptomics, CRISPR-mediated gene editing, and biochemical characterization, we uncover distinct and complementary roles of multiple GP63 paralogues in Ld. While both copies of GP63 encoded on

chromosome 10 (LdGP63_10.51 and 10.52), playing primary role in establishing cutaneous infection, were found to be functionally redundant for visceral Ld. In contrast, LdGP63_28 encoded on chromosome 28 proved essential for intracellular amastigote survival by suppressing host cell pyroptosis. Moreover, LdGP63_31 (chromosome 31) was found to primarily mediate promastigote attachment to the host macrophages with minimal contribution from LdGP63_28, facilitating initial infection establishment and amastigote genesis. Importantly, the absence of LdGP63_28 impacted amastigote infection more severely as compared to LdGP63_31. Structural and enzymatic analyses revealed divergent localization and substrate specificities to fulfil the functional requirements of these divergent proteases, which have evolved independently to carry out diverse functions in establishing infection. Collectively, this study indicates evolutionary divergence and functional specialization among GP63 isoforms in Ld by demonstrating that amastigote-specific and promastigote-specific GP63 isoforms synergistically mediate infection establishment and persistence.

GRAPHICAL ABSTRACT: Illustrating differential role of LdGP63_28 and LdGP63_31 in performing host entry and intracellular persistence.