Deletion of the *P21* gene triggers changes in the invasion and replication of *Trypanosoma cruzi*

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P21 is a protein encoded by a single-copy gene and no orthologs are found in other trypanosomatids. Previous studies demonstrated the role of P21 during the infection of *Trypanosoma cruzi*. P21 operates as a signal transducer molecule, triggering a signaling cascade, which results in the alteration of the actin cytoskeleton of host cell, increasing the internalization of parasites. In addition, *T. cruzi* infected mice treated with recombinant P21 have shown increasing in leukocytes chemotaxis and fibrosis, but reduced angiogenesis and replication of intracellular amastigotes, indicating the role of P21 in pathogenesis of Chagas disease. However, the mechanisms underlying the role of P21 remain poorly understood. In this study, we generated P21 knockout parasites using CRISPR/Cas9 and analysed the phenotypic effects of the deletion of this gene. Our results showed that ablation of P21 reduced the growth rate of epimastigotes evaluated for 14 days. Furthermore, P21 knockout epimastigotes showed an increase in the length of the G1 phase and reduction in the S phase, resulting in a delay on cell cycle progression. Invasion assays performed with metacyclic trypomastigotes revealed that P21 knockout impairs parasites ability to invade HeLa cells when compared to the wild-type control. In contrast, intracellular replication rate of amastigotes is increased in P21 knockout parasites, observed after 72 hours of infection. Taken together, our data reveals the involvement of P21 during the different life stages of *T. cruzi*, demonstrating its importance throughout the parasite life cycle.

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