RNAi KNOCK DOWN OF AMASTIN GENES AFFECTS INTERACTION OF LEISHMANIA BRAZILIENSIS AMASTIGOTES WITH MACROPHAGE MEMBRANES


1Departamento de Bioquímica e Imunologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil; 2Departamento de Parasitologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil; 3Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil; 4Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Paraná, Curitiba, PR, Brazil; 5Departamento de Morfologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

Introduction and objectives: Amastins are surface glycoproteins encoded by large gene families present in the genomes of several trypanosomatids and highly expressed in the intracellular amastigote stages of Trypanosoma cruzi and various Leishmania species. These intracellular parasites are responsible for diseases that affect large numbers of people in the tropical world and for which new methods for disease control are urgently needed. Materials and methods: We perform sequence analyses of 52 amastin genes present in the genome of L. braziliensis, determine the mRNA levels of these genes as well as cellular protein localization using parasite expressing GFP fusions of different amastin genes. We have also knocked-down the expression of a group of delta-amastin genes and examined the effect of reduced amastin expression in macrophage infection. Results and Conclusion: Most members of all four amastin subfamilies present in the L. braziliensis are upregulated in amastigotes and encoded proteins that are localized in the parasite surface. Although primary sequence alignments showed no homology to any known protein sequence, homology searches based on secondary structure predictions indicate that amastins are related to a group of proteins that are components of eukaryotic tight junction complexes named claudins. By knocking-down the expression of amastins in L. braziliensis, their essential role during infection became evident. Amastin knockdown parasites showed impaired growth after in vitro infection of mouse macrophages and completely failed to produce infection when inoculated in BALB/c mice, an attenuated phenotype that was reverted by the re-expression of an RNAi-resistant amastin gene. Further highlighting their essential role in host-parasite interactions, which might involve the formation of “claudin-like” structures, electron microscopy analyses of macrophages infected with amastin knockdown parasites showed drastic alterations in the tight contact that is established between the surface of wild type amastigotes and the membrane of the parasitophorous vacuole.

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