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Mini Review

Function and Stage-Regulated Expression of the LmcDNA16 Gene Family Related to *Leishmania* Infectivity in Mammals

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Abstract

Leishmaniasis is a vector-borne neglected tropical disease caused by members of the genus *Leishmania*, heteroxenous parasites with a digenetic life cycle. They need two hosts to complete their life cycle: an invertebrate vector (sandfly) and a vertebrate as the definitive host. Metacyclogenesis is the biological process whereby *Leishmania* parasites transform from poorly infective forms into highly infective forms called metacyclic promastigotes. During this process of infectivity acquisition, several genes are specifically expressed, endowing the parasites with the capacity to infect the immune cells of the host. Some of these genes, like HASPs (HASPA1, HASPA2 and HASPB) and SHERPs (SHERP1 and SHERP2) belonging to LmcDNA16 gene family, are differentially expressed in the metacyclic forms. We aim to review the structure and function of these stage specific genes of LmcDNA16 locus, as well as their implication in the metacyclogenesis process.

Keywords: *Metacyclogenesis; Infection; Leishmania; LmcDNA16 Locus; HASPA1; HASPA2; HASPB; SHERP1; SHERP2*

Introduction

The World Health Organization (WHO) estimates that over one thousand million people are affected by at least one of the Neglected Tropical Diseases (NTD) such as schistosomiasis, river blindness (onchocerciasis), African trypanosomiasis, Chagas disease or leishmaniasis. Leishmaniasis is a group of clinical manifestations produced by *Leishmania* spp. infection. At least 20 species of *Leishmania* are responsible for the three clinical forms of disease: visceral, cutaneous and mucocutaneous. Recent data from the Global Health Observatory (GHO) place Leishmaniasis as an endemic disease in 98 countries on five continents [1]. It exhibits an incidence of 1.3 million new human cases annually, of which 300.000 are visceral and 1 million are cutaneous or mucocutaneous [2]. Although dogs and more recently cats, are well-known domestic reservoirs of the human disease caused by *Leishmania* spp., there are several orders of mammals (e.g. Edentata, Carnivora, Hyracoidea, Rodentia, Primates, Marsupialia, and Perissodactyla) that have been described as natural vertebrate hosts of *Leishmania* species [3]. *Leishmania* spp. need two hosts to complete their life cycle: an invertebrate vector, sandfly of the genus *Phlebotomus* (Old World strains) or *Lutzomyia* (American strains); and a vertebrate definitive host (dogs, wild mammals or humans). *Leishmania* parasites harbor two main different morphological forms during their life cycle: intracellular amastigotes in the mammalian hosts and motile promastigotes in the sandfly vector. In addition, among promastigote forms, metacyclic promastigotes, the mammal-infective stages, are likely the most studied ones [4]. Metacyclogenesis is a process whereby *Leishmania* transforms from poorly infective procyclic promastigotes into highly infective metacyclic promastigotes inside the insect vector [5]. The differentiation from non-infective procyclics into infective metacyclic parasites is a pre-requisite for resistance to complement-mediated lysis as well as intracellular survival. During this process of acquisition of infectivity, several genes are specifically expressed in the metacyclic promastigotes. Metacyclogenesis is also

related to drug resistance and infectivity. In 2011, Ouakad M., *et al.* tested the relationship between metacyclogenesis and pentavalent antimony (SbV) resistance in clinical lines of *Leishmania donovani* [6]. These authors demonstrated that metacyclogenesis was significantly higher in SbV-resistant clinical lines than in SbV-sensitive lines. The development of new methods of prophylaxis against leishmaniasis will be dependent on the biochemical and immunological characterisation of the parasite form that infects mammals, and a better understanding of the novel mechanisms involved in the control of gene expression in these organisms [7,8]. In this work, we aim to review the structure, function and expression of genes belonging to LmcDNA16 locus such as HASPA1, HASPA2, HASPB, SHERP1 and SHERP2 (Figure 1) involved in the metacyclogenesis process.

The LmcDNA16 locus

The LmcDNA16 locus of *Leishmania* major was first characterized as a gene family that contained 5 genes (HASPA1, HASPA2, HASPB, SHERP1 and SHERP2) linked within a 10 Kb region of the genome [9] (Figure 1A). Three of those genes are closely related in their DNA sequences: HASPA1, HASPA2 and HASPB encoding Hydrophilic Acylated Surface Proteins. SHERP genes (SHERP1 and SHERP2), encode small hydrophilic proteins that are localized in the endoplasmic reticulum and outer mitochondrial membrane (Small Hydrophilic Endoplasmic Reticulum-associated Protein) [10]. The LmcDNA16 locus is located on chromosome 23, from both Old and New World *Leishmania* species [11]. In addition, the LmcDNA 16 gene array is a region of genetic diversity between *L. major* strains [12]. The contribution of HASPs and SHERPs to parasite infectivity had been assessed. Two mutants (null and overexpressing parasites) were generated and both displayed different functions. In fact, in 2001 McKean., *et al.* removed the diploid LmcDNA16 gene locus by deletion of both alleles so that homozygous null mutants only exhibited HASP or SHERP gene expression depending on the deleted alleles. In the same way, they generated overexpressing parasites using plasmid constructions. Homozygous null mutants resulted as virulent as the wild type cells and similar results were obtained for intracellular survival. Nevertheless, although overexpressing mutants could change to infective stage, they couldn't survive within the intramacrophage environment. Furthermore, both null and overexpressing parasites showed increased sensitivity to complement-mediated lysis, suggesting therefore the alteration of their surface architecture [10]. Since null parasites were able to undergo metacyclogenesis *in vitro*, it is likely that HASP/SHERP do not play a role in parasite differentiation *in vitro* culture conditions. On the contrary, loss of both proteins in null parasites resulted in failure to produce metacyclic forms, and lower colonization of the stomodeal valve (SV) region in late-stage infections in the sandfly. Then, the genetic locus encoding HASP and SHERP proteins was shown to be essential for metacyclogenesis in *Phlebotomus papatasi*, that is, under *in vivo* conditions [11].

SHERP (Small Hydrophilic Endoplasmic Reticulum-associated Protein)

SHERP protein (57 amino acids) (Figure 1B) is localized in the endoplasmic reticulum (ER) and the outer mitochondrial membrane [13]. BLAST searches in the existing databases do not reveal any recognizable SHERP homologues. There is growing evidence suggesting that membrane lipid engagements and interactions with the vacuolar ATPase protein complex may drive the function of this unusual small protein. Moreover, SHERP may be involved in the vacuolar acidification associated with parasite autophagy in the vector due to its localization in mitochondrial and ER membranes (both are targets during autophagic digestion) and also to its potential interaction with a vacuolar ATPase *in vitro* [14]. Although SHERP mRNA is detectable in other parasite stages (procyclic and amastigotes), SHERP protein is exclusively and highly expressed in metacyclics [15]. In addition, it seems to be related to drug resistance. In fact, Ouakad., *et al.* (2011) observed SHERP overexpression in metacyclic forms from four antimony resistant clinical isolates [6].

HASP (Hydrophilic Acylated Surface Protein)

This family of hydrophilic acylated surface proteins includes four members: HASPA1, HASPA2 (codified by the smallest genes with 222 bp) (Figure 1C), HASPB1 and HASPB2 (codified by 534 bp-genes) (Figure 1D). This family of proteins shows only limited similarity to other homologue eukaryotic proteins.

HASPB was initially described in *Leishmania major*, as the second protein codified by the gene B of the LmcDNA16 gene family found on the surface of infective metacyclic and amastigotes forms [16]. Furthermore, HASPB was the first characterized surface peptide marker for infective stages during *Leishmania* cell cycle. In amastigote stage, it is expressed in the flagellar pocket likely indicating active protein secretion similar to the surface protein gp63 [17]. Due to the lack of a membrane-spanning domain for its direct attachment to the parasite membrane, its interaction with LPG for attachment to the parasite was postulated [9,16]. In *Leishmania major*, the deduced sequence

predicts a predominantly hydrophilic protein of 177 amino acids in length (18.7 kDa) characterized by repetitive elements, comprising 45% of the total molecule, that shares sequence identity with the peptidoglycan binding domain of protein A from Staphylococcus aureus [12].

HASPB requires N-terminal acylation for trafficking to and exposure on the plasma membrane and it is not regulated by phosphorylation [18]. Recombinant HASPB is a protective antigen against infection by *Leishmania donovani* and it may induce the production of IL-12 by dendritic cells in the absence of exogenous adjuvant. However, this response is not sufficient to induce a polarized Th1 response [19]. The same protein is also immunogenic in dogs and induces significant protection against canine leishmaniasis [20]. Sera from patients with visceral and cutaneous leishmaniasis recognize recombinant HASPB protein with high specificity and sensitivity [21,22]. In 2008, Marin., *et al.* demonstrated the lack of a functional HASP gene in L. (V.) panamensis, suggesting the probable loss of the complete gene family in species of the Viannia subgenus [23]. In contrast, an additional region called Orthologous HASP Locus (OHL) was identified. It encodes proteins that also contain amino acid repeats and localize predominantly to the amastigote (but not metacyclic) cytosolic membrane in *L. viannia* species. This orthologous protein, HASP-like protein, from L. (Viannia) braziliensis showed considerable genetic polymorphism in the repeated region among clones isolated from individual patients, and it was postulated that genetic variation may play a role in immune recognition [24].

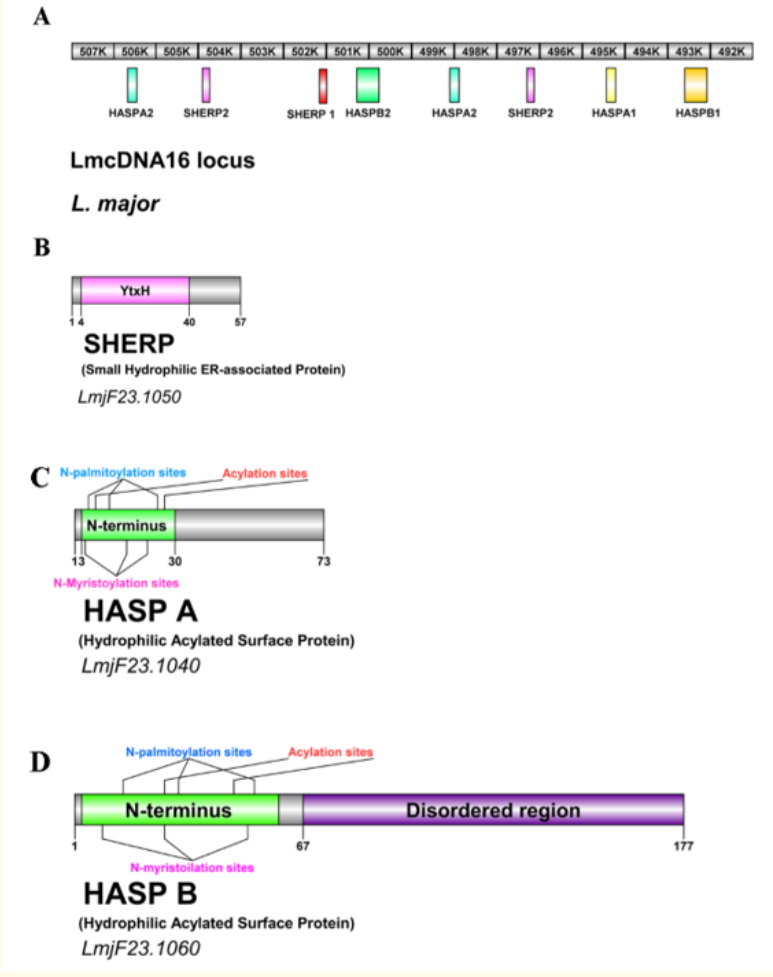

Figure 1: *The LmcDNA16 locus. (A) This locus contains five genes: HASPA1 (one copy), HASPA2 (two copies), HASPBs (HASPB1 and HASPB2, one copy of each gene), SHERP1 (one copy) and SHERP2 (two copies). (B) Structure of SHERP protein in Leishmania major. Only one conserved domain has been detected belonging to YtxH protein family, found in bacteria but functionally uncharacterized. (C and D) Structure of HASPA and HASPB in L. major. The schemes were illustrated using DOG 1.0 [25].*

Conclusions

Recently, several potential drugs and their mechanisms of action have been investigated [26-28]. Currently, key pathways need to be explored too. It is well known that metacyclogenesis remains a critical step for the completion of the parasite life cycle. During this process, *Leishmania* parasites differentiate from poorly infective procyclic promastigotes into highly infective metacyclic promastigotes within the insect vector. This acquisition of infectivity is related to the expression level of several specific genes in metacyclic promastigotes. Besides infectivity, metacyclogenesis is also associated to drug resistance. Therefore, these data reinforce the idea that the aforementioned members of LmcDNA16 gene family, implicated in metacyclogenesis, may be promising novel therapeutics targets against animal and human leishmaniasis.

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Competing Interests

The authors declare that have no competing interests.

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