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Protein Trafficking in *Plasmodium falciparum*-infected Erythrocytes

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Abstract

Malaria remains one of the world's most important infectious diseases. Malaria parasites make modifications to host erythrocytes that are essential to their survival and pathogenesis and are facilitated by parasite proteins exported to the host cytoplasm. These exported proteins form a functional trafficking complex in the host cytoplasm to transport virulence determinants to the erythrocyte surface; this complex, termed the Maurer's cleft, is thus essential for malaria virulence. Some of these exported proteins form a large protein complex that leads to profound structural and morphological changes in the host erythrocytes. In this report, I review the exported proteins in *Plasmodium falciparum*-infected erythrocytes. Because these proteins are closely linked to malaria pathogenesis, the information provided herein is important for our understanding of the molecular mechanisms involved in the pathogenesis of *P. falciparum* infection.

Introduction

Malaria remains one of the world's most important infectious diseases, affecting approximately 200 million people worldwide annually (WHO, 2018). When *Plasmodium falciparum*, one of the most virulent forms of the human malaria parasite, establishes infection in host erythrocytes, the parasites export numerous proteins to the host cell's cytoplasm and plasma membrane to remodel the host cell (Hiller *et al*., 2004; Maier *et al*., 2009). Some of these exported proteins form a large protein complex that leads to profound structural and morphological changes in the host erythrocytes (LaCount *et al*., 2005); for example, Maurer's clefts (Lanzer *et al*., 2006) and knobs (Wickham *et al*., 2001) are established in the cytoplasm and on the surface of the erythrocytes, respectively. Consequently, the infected erythrocytes become more rigid and adhere to the vascular endothelium, which prevents their clearance by the spleen and subsequently disrupts normal blood flow, resulting in severe malaria in humans (De Niz *et al*., 2016; Maier *et al*., 2008). This mini review discusses the exported proteins that are transported to the erythrocyte surface, including those associated with parasite adherence to the erythrocyte surface and severe malaria pathogenesis.

Exported proteins in *P. falciparum*-infected erythrocytes

The adherence of infected erythrocytes to the vascular endothelium is mediated by interactions between parasite adhesins on the erythrocyte surface and host endothelial receptors (Fairhurst *et al*., 2005; Janes *et al*. 2011; Waller *et al*., 2003). *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) is an antigenically variant adhesin that is transported to knobs on the erythrocyte surface (Waller *et al*., 1999). Knobs are macromolecular complexes of knob-associated histidine-rich protein (KAHRP) that anchor PfEMP1 to the membrane skeleton (Oh *et al*., 2000; Waller *et al*., 1999). Maurer's clefts are involved in the trafficking of PfEMP1 to the erythrocyte surface (Lanzer *et al*., 2006; Maier *et al*., 2008; Wickham *et al*., 2001). Many exported proteins, represented by skeleton binding protein 1 (SBP1) (Cooke *et al*., 2006; Maier *et al*., 2007), membrane-associated histidine-rich protein 1 (MAHRP1) (Spycher *et al*., 2008), ring-exported protein 1 (REX1) (Hanssen *et al*., 2008), subtelomeric variant open reading frame (STEVOR) (Przyborski *et al*., 2005), and PfEMP1 trafficking protein 1 and 5 (PTP1 and PTP5) (Maier *et al*., 2008; Rug *et al*., 2014) have been shown to reside in Maurer's clefts. Some of these exported proteins are essential for the intracellular transport of PfEMP1 to the erythrocyte surface, suggesting that they form a large protein complex in

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the Maurer's clefts that serves as protein trafficking machinery to transport exported proteins to their final destination (Rug *et al*., 2014). However, essential information regarding the interactions between these exported proteins is lacking, because of the technical difficulties of studying protein-protein interactions in the cytoplasm of *P. falciparum*- infected erythrocytes (Batinovic *et al*., 2017; Rug *et al*., 2014).

Motif sequences of exported proteins

The *P. falciparum* exportome was predicted to comprise approximately 400 proteins on the basis of the discovery of a motif sequence called PEXEL (*P. falciparum* exported elements) or HT (host-targeting sequence), which is conserved in the N-terminal region of many exported proteins (Hiller *et al*., 2004; Marti *et al*., 2004). However, many proteins that lack the canonical PEXEL/HT motif have been shown to be efficiently exported to the host cytoplasm (Heiber *et al*., 2013), thereby complicating the identification of the exported proteins that comprise the *P. falciparum* exportome. These PEXEL-negative exported proteins (PNEPs) include SBP1, MAHRP1, and REX1, all of which are indispensable for malaria virulence (Cooke *et al*., 2006; Hanssen *et al*., 2008; Maier *et al*., 2007; Spycher *et al*., 2008). Given the difficulty to predict and identify PNEPs on the basis of protein sequences, an alternative approach is needed to directly identify PNEPs based on their protein-protein interactions in the host cytoplasm.

P. falciparum orthologues of exported proteins in *P. berghei*

P. falciparum orthologues of SBP1 and MAHRP1 were discovered in the rodent malaria *P. berghei* by De Niz *et al.* (De Niz *et al*., 2016) and disruption of these genes resulted in the decreased cytoadherence of *P. falciparum*-infected RBCs (iRBCs) to CD36 in a mouse model, suggesting that these genes are likely involved in the transport of an unidentified parasite ligand that allows binding of iRBCs to the vascular endothelium, similar to *P. falciparum* SBP1 and MAHRP1 (Cooke *et al*., 2006; Maier *et al*., 2007; Spycher *et al*., 2008).

Tryptophan-threonine-rich antigen

A tryptophan-threonine-rich antigen (termed TryThrA) was identified as a parasite protein that associates with SBP1 in the trafficking complex (Takano *et al*., 2019). TryThrA was previously characterized as a protein expressed on the merozoite surface that is involved in parasite invasion, a role that is supported by the inhibitory effect of synthetic peptides of TryThrA antigen on merozoite invasion of erythrocytes (Curtidor *et al*., 2006). My group found that TryThrA is expressed across the asexual cycle and localizes in Maurer's clefts. Moreover, my group demonstrated that its gene could be genetically disrupted without affecting parasite invasion, which is inconsistent with previous studies (Alam *et al*., 2015; Curtidor *et al*., 2006). This discrepancy could be explained by off-target effects of the synthetic peptides, or by alternative expression of molecules that could compensate for the loss of TryThrA in our knockout parasites. Further studies are warranted to elucidate the precise function of TryThrA in infected erythrocytes.

Membrane palmitoylated protein 1

Host-parasite protein interactions play an essential role in malaria progression and pathogenesis (Egan *et al*., 2015; Miller *et al*., 2002; Olszewski *et al*., 2009). My group has identified several host-parasite protein interactions in the host cytoplasm (Takano *et al*., 2019). Among three of the host factors my group identified (STOM, KPNB1, and MPP1), my group found that MPP1, a membrane palmitoylated protein 1 (also termed p55, 55 kDa erythrocyte membrane protein) was recruited into the Maurer's clefts (Takano *et al*., 2019). MPP1 is a member of the membrane-associated guanylate kinase (MAGUK) family and plays essential roles in the membrane organization of erythroid cells, composition of lipid rafts on erythrocyte membranes, and erythrocytopoiesis (Biernatowska *et al*., 2017; Egan *et al*., 2015; Lach *et al*., 2012; Quinn *et al*., 2009). Moreover, a previous proteomic study of microvesicles, which are secreted from the surface of *P. falciparum*-infected erythrocytes and likely bud from Maurer's clefts (Mantel *et al*., 2013), identified the presence of this protein. MPP1, therefore, likely contributes to the organization of the membranous structures of Maurer's clefts.

Plasmodium helical interspersed subtelomeric family

By using a series of knockout experiments and cytoadherence assays with potential SBP1-interacting proteins, my group identified *MAL8P1.4* as being involved in the cytoadherence of iRBCs to vascular endothelial receptors (Takano *et al*., 2019). MAL8P1.4 is a member of the *Plasmodium* helical interspersed subtelomeric (*PHIST*) family of exported proteins, which play diverse roles in parasite-infected erythrocytes (Kumar *et al*., 2018; Oberli *et al*., 2014; Oberli *et al*., 2016; Proellocks *et al*., 2014). Although the function of *PHIST* genes has not yet been fully elucidated, previous studies have revealed that specific PHIST proteins can bind to the acidic C-terminal (ATS) domain of PfEMP1, and that the depletion of genes that encode PHIST proteins results in decreased cytoadherence (Oberli *et al*., 2016; Proellocks *et al*., 2014). Moreover, the binding capacity of a PHIST protein differs for each PfEMP1 depending on the sequence of its ATS domain (Kumar *et al*., 2018; Oberli *et al*., 2016), suggesting that *PHIST* genes might have coevolved with specific ATS domains to create interaction pairs with maximum binding strength to transport a specific PfEMP1 to the erythrocyte membrane (Kumar *et al*., 2018; Oberli *et al*., 2014; Proellocks *et al*., 2014). In contrast, MAL8P1.4 does not localize to the surface of erythrocytes or a knob, and has a low binding affinity for the ATS domain of specific PfEMP1s (Oberli *et al*., 2014). Although there are many unknowns regarding the function of MAL8P1.4, given that multiple PHIST proteins are cooperatively and selectively involved in the transport of specific PfEMP1s to the erythrocyte surface (Oberli *et al*., 2016), the altered cytoadherence by *ΔMAL8P1.4* parasites may be due to the alternation of the PfEMP1 being transported and presented on the erythrocyte surface.

Conclusions

This mini review describes the functions of exported proteins in *P. falciparum*-infected erythrocytes, some of which can cause the onset of severe malaria. The information presented furthers our understanding of the molecular mechanisms for the pathogenesis of *P. falciparum* infections.

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