Academic Journal of Science and Technology
ISSN: 2771-3032 | Vol. 12, No. 2, 2024

Research Progress of Cryptosporidium Adhesion and Gliding Motility Related Proteins

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Abstract: This review focuses on the complex molecular mechanisms involved in the adhesion and gliding motility of Cryptosporidium, emphasizing the importance of these processes in Cryptosporidium infection and summarizing the virulence factors involved. Subsequently, The manuscript meticulously examines the functional profiles and the chronicled research trajectories of adhesion-associated virulence factors, namely CSL, gp40/15, and gp900. In parallel, it explores the repertoire of factors implicated in gliding motility, including TRAP-C1, Cptsp4, and CpROM. Each factor is contextualized within the broader framework of the parasite's engagement with host defenses and its evasion strategies. Moreover, the review prognosticates the prospective research avenues that these factors are poised to influence, offering a measured appraisal of their viability as targets for vaccine and therapeutic development. The discourse encapsulates the current state of knowledge while venturing into the uncharted territories of potential, thereby establishing a robust foundation for future investigative endeavors aimed at demystifying the biological intricacies and pathogenic mechanisms of Cryptosporidium.

Keywords: Cryptosporidium; Virulence Factors; Adhesion; Gliding Motility.

1. Introduction

As an Apicomplexa phylum member, Cryptosporidium's broad host range and zoonotic nature pose a significant risk to public and veterinary health, affecting livestock, aquaculture, and related industries. Despite advancements in pathogenesis, epidemiology, clinical medicine, and immunology since the 21st century, understanding of Cryptosporidium's molecular interactions with hosts lags behind that of other apicomplexans, such as Eimeria, Plasmodium, and Toxoplasma(LENDNER and DAUGSCHIES, 2014). At the same time, research on the virulence and molecular mechanisms of Cryptosporidium is still in its infancy, with many issues remaining to be resolved. Therefore, the virulence factors of Cryptosporidium and its mechanism of interaction with the host have become a pivotal area of scholarly investigation in the contemporary era.

Cryptosporidium infects host cells through a sequence of events including excystation, gliding, adhesion, invasion, parasitophorous vacuole formation, intracellular survival, and induction of cellular damage. Parasitology has delineated key molecular factors in Cryptosporidium's pathogenicity, such as adhesins for host recognition, invasion factors for penetration, and cytotoxic elements leading to cellular dysfunction. These virulence factors are characterized by their surface localization or secretion, genetic diversity, telomeric or subtelomeric gene positioning often as multi-copy genes or gene family members, and susceptibility to post-translational modifications like glycosylation and lipidation(Bouzid, 2013). Among them, the proteins involved in adhesion and gliding motility are the most numerous and often play a unique role in the infection process, which is of great significance for the development of anti-Cryptosporidium drugs and vaccines.

2. Adhesion-related Virulence Factors

Adhesion is an essential stage where Cryptosporidium recognizes host epithelial cells and initiates the infection process, mediated by some special proteins located on the surface of the sporozoites. These proteins, known as adhesins, can bind to the receptors or membrane structures of host cells. According to current research, the variety of adhesins is the most abundant among all types of virulence factors of Cryptosporidium, and many also participate in gliding motility. The proteins that have been recognized to be involved in the adhesion of Cryptosporidium mainly include: gp40/15, (gp60), P30, GP900, TRAP, CSL, P23, CP47, CPS-00, CpClec, etc(Zhang et al., 2022).

2.1. Circumsporozoite-like Glycoprotein Antigen (CSL)

The circumsporozoite-like glycoprotein (CSL) antigen, a conserved protein of approximately 1300 kDa, was identified in 1997 by Langer and Riggs using monoclonal antibody 3E2. CSL's presence on the surface of Cryptosporidium parvum merozoites and sporozoites, as revealed by immunofluorescence, indicates its role in host cell adhesion during invasion(Zhang et al., 2022). In 1999, Riggs found that native CSL has a high affinity for human intestinal epithelial Caco-2 cells, can specifically bind to them in a dose-dependent, saturable, and self-exchange manner, and CSL specifically bound to the surface of living Caco-2 cells can inhibit the adhesion and invasion of sporozoites(Langer and Riggs, 1999). Catalina research team demonstrated that CSL-1, when combined with the immunomodulatory peptide FISEIAIIHVLHSR and adjuvant for mouse immunization, potently induced antibody production in 2018. This underscores CSL-1's potential in Cryptosporidium vaccine and drug development, with its adhesive mechanism warranting further investigation.

2.2. gp40/15（p60）

Gp40 and gp15, mucin-like glycoproteins originating from a shared precursor, reside on merozoites and sporozoites and form the adhesive gp60 complex, which dissociates during gliding motility. Strong and others identified the gp40/15

protein, uncovering its highly diverse gene locus with numerous single nucleotide and amino acid polymorphisms through nucleotide sequence analysis. This research laid the foundation for the current Cryptosporidium typing method based on gp60. In 2020, Zhaohui Cui et al. utilized indirect immunofluorescence microscopy to demonstrate gp40/15's association with the parasitophorous vacuole membrane(Cui et al., 2020). Yuexin Wang et al. employed bimolecular fluorescence complementation test and co-immunoprecipitation to identify ENO1 as an interacting host receptor for gp40, necessitating further mechanistic investigation. Recently, Muxiao Li et al. leveraged CRISPR/Cas9 for stable expression of gp40 and gp15 in Toxoplasma gondii, advancing Cryptosporidium mucin research(Li et al., 2024).

2.3. gp900

CAROLYN et al. first described gp900 in 1997, a glycoprotein exceeding 900 kDa with cysteine- and threonine-rich domains. This antigen is notable for its recognition by protective bovine colostral immunoglobulin and demonstrates high immunogenicity in mice and cattle(PETERSEN et al., 1992). The protein contains an N-terminal signal peptide(SP) that targets it to the endoplasmic reticulum and features a transmembrane domain near the C-terminus, which separates the long non-cytoplasmic domain from the short cytoplasmic domain(Li et al., 2022). In 1998, Barnes et al. identified gp900 in the anterior regions of merozoites and sporozoites using immunoelectron microscopy. In 2022, Xiaohui Li utilized immunofluorescence to reveal abundant gp900 in the microneme apices before and after sporozoite release, with minor presence at the cell membrane. Given the structural differences of this protein on the microtubules and the cell membrane, It can be proposed that the C-terminal domain of gp900 is cleaved in the secretory pathway before reaching the cell surface(Li et al., 2022). In 2023, Yang Ling et al. expressed and purified gp900 using BL21 cells and co-cultured it with RAW264.7 macrophages, observing upregulation of CD86, IL-6, and TNF-α. These findings imply that recombinant gp900 dose-dependently stimulates macrophages via the NF-κB and MAPK signaling pathways(Yang et al., 2024). Recent research indicates a pivotal lubricative function for gp900 in Cryptosporidium infection, highlighting its potential as a therapeutic and vaccine target.

3. Gliding Motility-Related Virulence Factors

Gliding motility in Cryptosporidium is facilitated by the inner membrane complex (IMC), actin, and adhesins, with myosin motors driving the rearward movement of adhesins to propel the parasite forward(Yahata et al., 2021). This process is complemented by proteases that cleave adhesins, enabling smooth progression, and involves key factors identified by the scientific community.

3.1. TRAP-C1

In 1998, Spano et al. identified Cptsp1, a thrombospondin-related adhesive protein homologous to Eimeria tenella's Etp100 and Toxoplasma gondii's MIC2, and designated it as TRAP-C1. The 76 kDa microneme protein, comprising 687 amino acids, is localized at the apical end of Cryptosporidium sporozoites and is crucial for their gliding motility and adhesion. Genetic studies by Stefan Kappe et al. have demonstrated TRAP's essential role in sporozoite invasion and gliding motility driving(Kappe et al., 1999). In 2002, Mingqi Deng et al. identified an apple domain in TRAP-C1, differentiating it from the Plasmodium falciparum TRAP and Eimeria tenella Etp100 proteins lacking this feature. By 2003, Pablo C. et al. had utilized recombinant TRAP-C1 in ELISA assays to demonstrate a robust and escalating humoral response in Cryptosporidium parvum TAMU and UCP oocyst-infected volunteers(Okhuysen et al., 2004), emphasizing the protein's immunogenic potential. As a potential virulence factor, TRAP-C1 holds significant promise for cryptosporidiosis prevention and treatment, particularly in the development of vaccines against Cryptosporidium.

In 2002, Mingqi Deng and colleagues identified twelve conserved Cryptosporidium parvum genes with TSP-1, apple, and EGF domains through TBLASTN analysis employing the TSP-1 domain as a query(DENG et al., 2002). These genes encode the CpTSP1-12 proteins, notable for their thrombospondin repeat sequences, highlighting a key advancement in the study of this pathogen. In 2023, Alan John et al. confirmed the cell surface and sporozoite secretion pathway localization of the CpTSP protein family using expansion microscopy and immunofluorescence(John et al., 2023). This family, represented by TRAP-C1, is now a focal point in Cryptosporidium research.

3.2. Cptsp4

The Cptsp4 protein, structurally akin to CpTSP5 but with heightened expression in early infection stages, consists of 488 amino acids with an N-terminal SP, PAN/APPLE domain, two TSP1 repeats, and lacks both introns and a C-terminal transmembrane domain(Wang et al., 2024). In 2018, Wang Kejie et al. demonstrated using Western blot and flow cytometry that TSP4-GST binds to HCT-8 cells in a dose-dependent and saturable manner, suggesting Cptsp4's role in sporozoite adhesion via heparan sulfate, a mechanism later confirmed in Cptsp7. Further, in 2024, Dongqiang Wang et al. revealed through immunostaining and enzyme-linked analysis that CpTSP4, stored in sporozoite micronemes, is secreted during sporozoite activation, gliding, and invasion, and is also associated with Cryptosporidium's unique central microtubules. The process is inhibited by kinesin-5 inhibitors SB-743921 and SB-715992, resulting in CpTSP4 accumulation(Wang et al., 2024). This study pioneers the discovery of the unique transport function of Cryptosporidium microtubules.

3.3. CpROM

Elisabetta et al, through the analysis of 100 recombinant peptides of Cryptosporidium parvum, identified a key rhomboid protein, CpROM, essential for sporozoite-mediated invasion. Extensive research on rhomboid proteases (ROMs) in other apicomplexan parasites, notably Toxoplasma gondii, underscores the critical role of TgROM 4 in facilitating gliding motility. Specifically, the targeted disruption of the TgROM 4 gene leads to increased adhesion to host cells and a characteristic rotational movement, thereby impeding the parasite's normal motility, which is a clear indication of the enzyme's non-redundant function in the gliding process. In 2016, Mingying Li et al. applied yeast two-hybrid and co-immunoprecipitation to demonstrate the interaction between

CpROM and CpMICs, suggesting CpROM's involvement in Cpgp900 cleavage(Li et al., 2016). Xin Gao et al. later characterized CpROM1 as a microneme protein localized to merozoites and the parasitophorous vacuole membrane. Quantitative RT-PCR analyses showed elevated CpROM1 transcription in oocysts and sporozoites versus intracellular stages, indicative of its role as a membrane-bound peptidase with hydrolytic capabilities. The function and mechanism of CpROM1 in gliding motility and invasion processes still need to be clarified. The latest research shows that CpROM1 is related to the release of extracellular vesicles(Bertuccini et al., 2024).

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