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Review Article
Cryptosporidium-host Interaction Alters Regulation of OncomiRNAs and Tumor Suppressor miRNA Expression

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Abstract

The apicomplexan parasite *Cryptosporidium* is well-known for its capability to induce cryptosporidiosis, a severe diarrheal disease in human and animals. *Cryptosporidium* can also be a potential pathogen in human for cancer progression, particularly colorectal cancer. This review was designed to outline the information about the life cycle of the *Cryptosporidium*, the consequences of *Cryptosporidium* infection into the response mechanism in immune compromised host and finally the regulation of oncomiRNAs and tumor suppressor miRNAs upon *Cryptosporidium* infection. Host-*Cryptosporidium* interaction caused alteration of expression of a series of microRNAs or miRNAs as a result of controlling defense mechanism. Regulation of miRNAs in the infected cells may be identified as possible biomarkers in cancer progression. Upregulation of oncomicroRNAs or oncomiRNAs and the downregulation of tumor suppressor miRNAs in the host epithelial cells due to the *Cryptosporidium* infection may lead to cancer initiation on human.

Key words: *Cryptosporidium*, oncomiRNAs, tumor suppressor miRNAs, NF-κB, immune compromised patients, cancer

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cryptosporidium parvum (*C. parvum*) is a specie of genus *Cryptosporidium*, commonly known water borne and food borne parasite[1-4]. Even though the parasite is ubiquitously found in water, livestock and even in vegetables planted in wastewater, the harmful effect of *Cryptosporidium* is often being neglected[5]. It has been reported that *Cryptosporidium* can potentially cause cryptosporidiosis in human and animal[6-8]. Commonly, cryptosporidiosis is gastrointestinal abnormalities aroused after *Cryptosporidium* infection which is characterized by short-term or long-term diarrhea with abdominal pain[9,10].

Apart from gastrointestinal or colorectal epithelial cell lines, the parasite was also observed to infect the epithelial cells of biliary and pulmonary area[11,12]. *Cryptosporidium* infection possesses a nature of minimal invasion and shows a critical innate immune response against infection to the host defense mechanism. Hence, there is a high possibility of alteration of gene expression in infected host cells[13,14]. Therefore, *Cryptosporidium* species infectivity is one of major concerns in today's parasitological research.

Cryptosporidium belongs to the phylum apicomplexan which is particularly pathogenic in immune compromised patients. Several species of *Apicomplexa* are found to be responsible for introducing diseases in human[15] because of their ability to introduce a complex mechanism in the host cells in order to maintain their maximum growth and perseverance[16]. Infection of *Cryptosporidium* on epithelial cells leads to activation of innate and adaptive immune response mechanism within the host cells. *Cryptosporidium* was found to interfere with the signaling pathways of cytokine production and apoptosis pathway along with other and change the host cell defense mechanism, thus alter the immune system[15]. Nuclear factor kappa B (NF-κB) signaling pathway is the first line defense mechanism of epithelial cells in host to encounter *Cryptosporidium* infection[17,18]. List of numerous genes, cytokines or chemokines are involved in the process of NF-κB activation including regulation of microRNAs[19]. Interestingly, NF-κB pathway is considered as a mastermind player in the metastasis, initiation and development of human carcinogenesis[20].

The interrelation between cancer and *Cryptosporidium* was observed earlier, although, there was very little interest on that research initially. However, a couple of research work has been carried out later where evidence of the engagement of *Cryptosporidium* with colorectal patients has been established. The prevalence of *Cryptosporidium* was observed in immune compromised patients, where 12.6% of the 87 colorectal cancer patients' faecal samples were *Cryptosporidium* positive[21]. An *in vivo* study on mice upon *Cryptosporidium* infection observed that infection leads to polyps and adenocarcinoma in the gastrointestinal tract; hence *Cryptosporidium* has been identified as a possible agent to trigger the colorectal neoplasia[22,23].

Regulation of microRNAs has extensively been reported to be the controller of the onset and progression of human cancer[24]. Infection of *Cryptosporidium* to the human epithelial cell lines exhibits alteration of wide range of microRNAs expression profile. Several *in vitro* studies showed that *Cryptosporidium* infection on cell lines caused upregulation of oncomiRNAs and simultaneously downregulates tumor suppressor miRNAs[3,7,25-42] (Table 1).

The rationale for this review is to understand subsequent mechanisms upon *Cryptosporidium* infection on epithelial cells. The elevated immune response causes the over expression of oncomiRNAs and simultaneously downregulation of tumor suppression miRNAs that enhanced the risk of initiation and progression of cancer. This review established the dysregulation miRNAs expression, one of the most important biomarkers of cancer regulation.

LIFE CYCLE OF *CRYPTOSPORIDIUM*

Cryptosporidium is referred to as a monoxenous organism that requires one host to complete life cycle. As a monoxenous organism the life cycle of the *Cryptosporidium* is completed within a host. Hosts apart, while remaining in the environment; the parasite exists as oocysts with thick outer wall which protect the organism in variable (high or low) temperature or such other environmental factors[43]. In desiccation the oocysts become inactivated, but the infective characteristic of the oocysts remain for several months even they pass through moist or cool climates[44].

Usually the sporozoites are attached to the host epithelial cells. Once *Cryptosporidium* reach to the intestine, excystation of oocysts is triggered by different gastrointestinal conditions such as temperature, pH etc.[45]. Excystation of oocyst causes the release of four sporozoites out of the shell into the gastrointestinal tract. Subsequently the attachment of the sporozoites occurs to the host epithelial cells. A parasitophorous vacuole is formed at the ileocecal junction, because of the invasion of sporozoites. The sporozoites invade onto the ileocecal junction, which results the engulfment of the sporozoites by the epithelial cell and thus a parasitophorous vacuole is formed. Parasitophorous vacuole is not only a host of the *Cryptosporidium*, but also, the existence of a number of different protozoan parasites may occur within it, but interestingly, there is a unique structural feature named 'feeder organelle' is contained in *Cryptosporidium*, due to which the cytoplasm of the parasite is separated from the cell[46].

Table 1: Regulation of miRNAs on *Cryptosporidium*-human epithelial cell line interaction and their associated pathway/targeted genes

miRNAs	Regulation of miRNA	Methods	Type of cell	Pathway/target mRNA	Nature of miRNA	References
miR-15b	Upregulated	qRT-PCR, northern blot	H69	TLR4/NF-kB p65	Promote EMT and metastasis	Zhou *et al.*[25] and Sun *et al.*[26]
miR-16	Upregulated	qRT-PCR, northern blot	H69	TLR4/NF-kB p65	Tumor suppressor	Zhou *et al.*[25] and Chen *et al.*[27]
miR21	Upregulated	qRT-PCR, northern blot	H69	TLR4/NF-kB p65, CCL20	Oncogenic	Zhou *et al.*[25], Guesdon *et al.*[28] and Jainul *et al.*[3]
miR-23b	Upregulated	qRT-PCR, northern blot	H69	TLR4/NF-kB p65	Oncogenic	Zhou *et al.*[25] and Liu *et al.*[29]
miR-24	Upregulated	qRT-PCR, northern blot	H69	TLR4/NF-kB p65	Oncogenic	Zhou *et al.*[25], Sochor *et al.*[30] and Qin *et al.*[31]
miR-27b	Upregulated	qRT-PCR, western blot	H69	TLR4/NF-kB; KSRP	Oncogenic	Zhou *et al.*[32] and Wang *et al.*[33]
miR-30b	Upregulated	qRT-PCR, northern blot	H69	TLR4/NF-kB p65	Oncogenic	Zhou *et al.*[25] and Quintavalle *et al.*[34]
miR-30c	Upregulated	qRT-PCR, northern blot	H69	TLR4/NF-kB p65	Oncogenic	Zhou *et al.*[25], Quintavalle *et al.*[34] and Koutsaki *et al.*[35]
Mir-98	Downregulated	Northern blot, Bead-based miRNA luminex analysis, qRT-PCR	H69	SOCS4, CIS	Tumor suppressor	Zhou *et al.*[25] and Hu *et al.*[36]
MiR-125b	Upregulated	qRT-PCR, northern blot	H69	TLR4/NF-kB p65	Oncogenic	Zhou *et al.*[25] and Knackmuss *et al.*[37]
miR-221	Downregulated	qRT-PCR	H69	ICAM-1	Oncogenic	Gong *et al.*[38] and Zhang *et al.*[39]
miR-320	Downregulated	Bead-based miRNA Luminex analysis	H69	TLR4/NF-kB p65	Tumor suppressor	Zhou *et al.*[25] and Bronisz *et al.*[40]
miR-424	Downregulated	Bead-based miRNA luminex analysis, microarray, qRT-PCR, northern blot	H69		TLR4/NF-kB p65, HDAC	Zhou *et al*[25] and Zhou *et al.*[17]
Mir-503	Downregulated	Bead-based miRNA luminex analysis, microarray, qRT-PCR, northern blot, western blot	H69	NF-kB; HDAC	Tumor suppressor	Zhou *et al.*[17] Qiu *et al.*[41]
miR-513	Downregulated	qRT-PCR, Bead-based miRNA luminex analysis	H69	B7-H1		Gong *et al.*[38]
miR-Let-7	Downregulated	qRT-PCR	H69	TLR-4, MyD88/NF-kB SOCS4, CIS	Tumor suppressor	Hu *et al.*[36] Hu *et al.*[42]

The shape of the sporozoite itself starts to be more spherical gradually once the feeder organelle develops, later on, a trophozoite is formed. By asexual reproduction cycle, the trophozoite changes to Type I meront may either develops into a Type II meront or the Type I meront may produce 6-8 merozoites[47]. The redeveloped merozoites immediately infect the host again and join into a recycling asexual reproduction cycle. Some, as usual tend to develop to Type II meront[48].

On the other side, the Type II meronts start sexual production by releasing a set of four merozoites. Merozoites, which have released from Type II meronts are differentiated into macrogamonts. Also, after invasion the merozoites form microgamonts which further develop multi-nuclei to produce microgametes. Fertilization of the macrogamete is performed by the penetration of the free microgametes and thus a zygote is produced.

By the meiotic cell division process, four sporozoites are differentiated from one zygote. The sporozoites developed oocysts and the oocysts come out from the lumen. Two different types of oocysts namely, thin-walled oocysts and thick-wall oocysts of *Cryptosporidium* are produced inside lumen[48]. The oocysts with thin cell wall released sporozoites of *Cryptosporidium* directly into the lumen to infect onto the host cells. Thick-walled *Cryptosporidium* oocysts come out to be mixed into the faeces and have the capability to immediately infect the host cells or the faeces bring it into the environment[48].

IMMUNE RESPONSE UPON *CRYPTOSPORIDIUM* INFECTION

The epithelial cell lines are activated both adaptive and innate immunity reactions to encounter *Cryptosporidium* infection[18,49]. Upon infection, host epithelial cells expressed several types of pattern recognition receptors (PRRs) including and nucleotide binding oligomerization domain-like receptors (NLRs) and Toll-like receptors (TLRs)[19,50]. Associated adaptor proteins were brought into the site by the expression of these receptors and thus trigger the activation of nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways[51].

Innate immune reactions begin immediately after infection of *C. parvum* onto the epithelial cells. At the early stages, the inflammatory chemokines or cytokines are release due to inflammation caused by infection. Furthermore, the antimicrobial peptides are produced and the expression of adhesion molecules occurs[52]. Primarily the antimicrobial peptides (e.g., β-defensin 2 and nitric oxide) attempt to execute the *Cryptosporidium* or to inhibit its normal growth activity[32]. However, it is necessary to function PRRs to recognize pathogens and activate NF-κB signaling pathway in order to start up primary epithelial responses against infection[17,18].

Innate immunity reactions triggered the adaptive immune responses by activating immune effector cells in the site of infection. Such cells include $CD4^+$ and $CD8^+$ lymphocytes, natural killer (NK) cells, macrophages, dendritic cells and innate lymphoid cells[53,54].

Although both immunity reactions (innate and adaptive immunity) are activated upon infection, but *Cryptosporidium* itself developed some counter immune defense mechanism on the host cells, continues replication within the host and survive in the host. To note that, either the production of interferon gamma (IFN-γ) or IFN-γ signaling function in the epithelial cells are considered as essential factors to activate innate and adaptive immunity[55]. But upon *Cryptosporidium* infection, researchers observed a reduction of signaling transductor and activator of an essential transcription factor of IFN-γ signaling pathway, namely signaling transductor and activator of transcription1α (STAT1α)[14]. In the infected host cell, the depletion of transcription 1α (STAT1α) caused inhibition of transactivation of IFN-γ-dependent gene[14]. Additionally, C-C motif chemokine ligand 20 (CCL20), a cytokine responsible for the clearance of parasite was observed downregulated in the epithelial cells upon *C. parvum* infection[28].

ELEVATED IMMUNE RESPONSE AND CANCER INITIATION

The first innate immune response against the *Cryptosporidium* infection is inflammation[20], but repeated infection can trigger the formation of tumor by disabling the immune system that acts against the tumor cells. Besides, the induction of cell proliferation and instability of genetic process can be the results of inflammation. These abnormalities to the epithelial cells further result to oncogenic mutations[20].

The defense mechanism starts with the activation of NF-κB pathway when *Cryptosporidium* infects on epithelial cells[56]. However, elevated NF-κB activation can be the potential regulator to produce colon cancer. Risk of colon cancer is high to inflammatory bowel disease (IBD) patients infected by *Cryptosporidium* due to the subsequent inflammation resulting from infection. Inflammation caused the secretion of tumor necrosis factor (TNF-α), interleukin (IL)-1, interleukin (IL)-17 and other pro-tumorigenic cytokines in order to induce NF-κB activity[57]. The downregulation of let-7 family and modulation of IL-6/STAT3 signaling in response to the inflammatory signals may lead to an uncontrolled proliferation of cells that results cancer initiation[58].

CRYPTOSPORIDIUM INFECTION IN IMMUNOCOMPROMISED PATIENTS

Cryptosporidium invasion onto the human gastrointestinal epithelial cell lines results toll-like receptor (TLR)/NF-κB signaling activation. This activation leads to possible secretion and production of chemokines and cytokines including prostaglandin E2, interleukin (IL)-8 and interleukin (IL)-13, antimicrobial peptides and nitric oxide as a defensive mechanism to kill *Cryptosporidium* or inhibit its intensification[59].

However, in immune compromised patients, there is insufficiency of T-cells (immune cells) containing a/b type T-cell receptor[60]. Thus, *Cryptosporidium* infection can alter the regulation of cellular mechanism and destroy complex regulatory network along with the regulation of miRNAs. Several studies confirmed that specific miRNA controlled different process of epithelial cells such as cellular differentiation regulation, cell death and proliferation, immunological response to microbial invasion, inflammatory responses, intracellular signaling pathways activation[61-63], where interference of any of these processes supported the onset and progression of cancer in epithelial cell including colorectal cell.

For colorectal cancer, numerous molecular signaling pathways involved to suppress the T-cells and its subsets. Deactivation of immunity of $CD4^+$ T cell is a result of canonical Wnt signaling in colorectal cell lines via suppression of IFN-γ and upregulation[64] of IL-17a. Decreased $CD4^+$ T cell count is also a detrimental factor in HIV patients since this results impairment in immune response mechanism[65].

The ability of T cell, $CD4^+$ is crucial in activation of T cell mediated NF-κB immune response mechanism[66] and NF-κB signaling pathway is assumed to be an active player in initiation, development and metastasis in human cancer[20]. Since, the defense mechanism is compromised in cancer and HIV patients, the *Cryptosporidium* prevalence is high in such types of patients[67].

ALTERATION OF MIRNAS UPON *CRYPTOSPORIDIUM* INFECTION

Cryptosporidium infection caused the activation TLR-4/NF-κB mediated signaling pathway and regulation of a number of miRNAs is associated to the pathway prior to infection. *Cryptosporidium* can modify the expression pattern of host epithelial cell line miRNAs by NF-κB p53 mediated signaling pathway activation[25]. The research identified miR-23b, miR-30b, miR-30c and miR-125b of cholangiocyte considerably upregulated along with a panel of miRNAs as a result of anti-microbial defense mechanism to counter the infection of *C. parvum*[25]. The study confirmed that, induced expression of selected miRNAs is due to transactivation of genes. Besides, a number of miRNAs of host biliary epithelial cells exhibit declined in expression[17] including miR-98.

Upregulation of TLR4 due to the *C. parvum* infection result downregulation[56] of let-7 miRNA. The subsequent inflammation produced by the *C. parvum* infection leads to an elevated expression of suppressors of inflammatory cytokine signaling (SOCS) and cytokine-inducible src homology 2-containing protein (CIS) and these alteration process is well maintained by downregulation of let-7 and miR-98[36,42]. The expression of NAD-dependent deacetylase sirtuin-1 (SIRT1) occurs due to the downregulation of let-7i, a miRNA precursor, in the H69 cell following infection[68].

The C-C motif chemokine ligand 20 (CCL20), a cytokine responsible for the clearance of parasite was observed downregulated in epithelial expression upon the *Cryptosporidium* infection resulting from the downregulation[28] of miR-21. When *C. parvum* was exposed to the cholangiocyte, reduction of miR-513 is observed that increase the expression of B7-H1[69]. *In vitro Cryptosporidium* infection on biliary epithelial cell line showed downregulation of miR-221 of host cells and this downregulation of miR-221 is associated with the upregulation of intercellular adhesion molecule-1 (ICAM-1)[38].

The miR-27b possesses a vital role in NF-κB signaling defense mechanism in infected host epithelial cell by targeting directly KH-type splicing regulatory protein (KSRP). The role of KSRP is to coordinate TLR4 mediated NF-κB signaling pathway. Hence, *Cryptosporidium* infection caused upregulation of miR-27b and this upregulation reduced the expression of KSRP in the epithelial cell line[32].

Activation of NF-κB and histone deacetylases (HDACs) dependent defense mechanism in infected epithelial cells downregulates miR-424 and miR-503. Increase in expression of CX3CL1 is due to the downregulation of miRNA-424 and miRNA-503[17].

CONCLUSION

The study established a relationship between the *Cryptosporidium* infection and regulation of miRNAs in the onset and progression of cancer. However, research on miRNAs regulation upon *Cryptosporidium* infection particularly in the *in vitro* gastrointestinal epithelial cells is inadequate. Therefore, further studies on miRNA and *Cryptosporidium* infection are vital to comprehend and reconfirm the mechanism of onset or progression of colorectal cancer by this parasite.

SIGNIFICANCE STATEMENT

This review discovered that there are significant dysregulation of oncomiRNAs and tumor suppressor miRNAs linked to the *Cryptosporidium* infection in the epithelial cells. Although the actual pathways for the dysregulation of tumor related with the miRNAs are needs to be understood, this review will be greatly beneficial for researchers to take as a reference for further studies. Current study proved that the development of cancer upon *Cryptosporidium* infection also causes the oncomiRNAs to be upregulated, while the tumor suppressor miRNAs are downregulated in the epithelial cell lines.

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