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Is *Cryptosporidium* a hijacker able to drive cancer cell proliferation?

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ABSTRACT

The pathophysiological mechanisms of *Cryptosporidium* infection are multifactorial and not completely understood. Some advances achieved recently revealed that the infection by *Cryptosporidium parvum* induces cytoskeleton remodeling and actin reorganization through the implication of several intracellular signals involving, for example, PI3K, Src, Cdc42 and GTPases. It has also been reported that the infection by *C. parvum* leads to the activation of NF-κβ, known to induce anti-apoptotic mechanisms and to transmit oncogenic signals to epithelial cells. Despite the growing evidence about the hijacking of cellular pathways, potentially being involved in cancer onset, this information has rarely been linked to the tumorigenic potential of the parasite. However, several evidences support an association between *Cryptosporidium* infection and the development of digestive neoplasia. To explore the dynamics of *Cryptosporidium* infection, an animal model of cryptosporidiosis using corticoid dexamethasone-treated adult SCID (severe combined immunodeficiency) mice, orally infected with *C. parvum* or *Cryptosporidium muris* oocysts was implemented. *C. parvum*-infected animals developed digestive adenocarcinoma. When mechanisms involved in this neoplastic process were explored, the pivotal role of the Wnt pathway together with the alteration of the cytoskeleton was confirmed. Recently, a microarray assay allowed the detection of cancer-promoting genes and pathways highly up regulated in the group of *C. parvum* infected animals when compared to non-infected controls. Moreover, different human cases/control studies reported significant higher prevalence of *Cryptosporidium* infection among patients with recently diagnosed colon cancer before any treatment when compared to the control group (patients without colon neoplasia but with persistent digestive symptoms). These results suggest that *Cryptosporidium* is a potential oncogenic agent involved in cancer development beyond the usual suspects. If *Cryptosporidium* is able to hijack signal transduction, then is very likely that this contributes to transformation of its host cell. More research in the field is required in order to identify mechanisms and molecular factors involved in this process and to develop effective treatment interventions.

1. The ugly duckling: *Cryptosporidium* a peculiar parasite

Organisms in the genus *Cryptosporidium* are classified in the phylum Apicomplexa, class Conoidasida and order Eucoccidiorida. Their taxonomic position was challenged by molecular phylogenies, and a closer affinity to gregarines, representing an early branch at

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Table 1
Comparison of Apicomplexan parasites.

	Cryptosporidium	*Toxoplasma*	*Plasmodium*	*Theileria*
According to number of hosts in the life cycle	One host	Several hosts	Two	Two
Type of host cells	Epithelial cells	All type of nucleated cells	Hepatocytes and red blood cells	White blood cells
Localization within the cell	Intracellular but Extra-cytoplasmic	Intracellular	Intracellular	Intracellular
Auto-infection	Yes	No	No	No
Motility	Gliding motility	Gliding motility	Gliding motility	Gliding motility
Presence of apicoplast and mitochondion	No	Yes	Yes	Yes
Parasitophorus vacuole	Yes	Yes	Yes	No
Feeder organelle	Yes	No	No	No
Host actin remodeling	Yes	Yes	No	Yes

the base of the phylum, was shown (Certad et al., 2017).

As described in the Table 1, *Cryptosporidium* has characteristics, which differentiate it from other Apicomplexan such as *Toxoplasma, Plasmodium* and *Theileria.* The biological life cycle of *Cryptosporidium* alternates between asexual and sexual reproduction. However, in contrast to most other apicomplexan parasites, the entire life cycle of this parasite develops in one host for both asexual and sexual reproduction (Plattner and Soldati-Favre, 2008). Sexual reproduction ends with the production of oocysts, which are essential to transmission, and play a role in the continuous infection of the host. In fact, *Cryptosporidium* oocysts can mature within the host to be immediately infective. Therefore, the developmental cycle and the infection in the same host can be maintained (Plattner and Soldati-Favre, 2008).

Cryptosporidium parasites are a major cause of diarrhea worldwide. They affect humans and animals. Transmission is by the fecal–oral route *via* water or food contaminated with the parasite or by ingestion of oocysts after direct contact with humans and animals carrying the parasite (Ryan et al., 2021). Immunosuppressed individuals and children are particularly vulnerable to infection (Khalil et al., 2018). The main clinical manifestation of the infection is diarrhea and in fact, *Cryptosporidium* is a leading cause of severe pediatric diarrhea. A cohort study (GEMS) involving more than 20,000 children in Africa and Asia revealed that *Cryptosporidium* is one of the four main pathogens responsible for severe diarrhea and mortality in infants and toddlers (Khalil et al., 2018; Kotloff et al., 2013). Treatment options for patients with cryptosporidiosis are scarce. The only treatment for cryptosporidiosis approved by the Food and Drug Administration (FDA) is nitazoxanide. However, this drug has limited efficacy due to the fact that can allow the improvement of diarrheal symptoms without completely inhibiting the excretion of oocysts, and is non-effective in immunocompromised patients (Amadi et al., 2009). *Cryptosporidium* species have caused waterborne outbreaks of gastrointestinal disease around the world (Crawford and Kol, 2021).

Infection with *Cryptosporidium* begins after the ingestion of oocysts, carrying four invasive sporozoites. Sporozoites harbor the apical complex containing secretory organelles typical for Apicomplexa: micronemes, dense granules, and a single rhoptry (Guérin et al., 2021). It is unknown how the sporozoite enters the epithelial cell. The sporozoite lacks many components of the moving junction machinery that allows active invasion of other apicomplexan such as *Toxoplasma* and *Plasmodium*, and has also lost its apicoplast and mitochondrion, and is dependent on glycolysis for its energy requirements (Pinto and Vinayak, 2021). Once in the cell the parasite is intracellular but can be viewed as 'minimally invasive' (Valigurová et al., 2008) as its described below (Section 3).

2. Is *Cryptosporidium* responsible of oncogenesis?

Besides known clinical manifestations, in various animal groups and in humans, epidemiological and experimental studies *in vivo* and *in vitro* propose the hypothesis of a causal link between *Cryptosporidium* infection and digestive cancer (Kalantari et al., 2020; Sawant et al., 2020).

For instance, clinical studies in different countries showed a significant association between cryptosporidiosis and the presence of digestive neoplasia in humans (Sawant et al., 2020). Some of these studies are case reports (Sawant et al., 2020) but interestingly, case/control studies in different countries such as Lebanon (Osman et al., 2017), Poland (Sulżyc-Bielicka et al., 2018) and China (Zhang et al., 2020a) have reported a significant association between *Cryptosporidium* infection and colon cancer.

Additionally, a statistically significant increased risk of *Cryptosporidium* infection among cancer patients compared to controls was reported in a systematic review and meta-analysis compiling data on 3562 individuals from 19 cross-sectional and case-control studies [OR = 3.3; 95% CI: 2.18–4.98] (Kalantari et al., 2020). The same study showed that the occurrence of *Cryptosporidium* infection was related to colorectal malignancies (3.7 (2.10–6.50)) but unassociated with blood cancer (3.8 (0.49–29.60)) (Kalantari et al., 2020).

However, clinical evidences are based on few epidemiological data. Further case-control clinical trials focused on large patient cohorts investigating *Cryptosporidium* prevalence among patients with colon cancer *vs.* matched healthy controls, and thus evaluating infection by this parasite as a risk factor for oncogenesis should be conducted worldwide to clarify this question.

Moreover, an animal model of cryptosporidiosis was developed using SCID (severe combined immunodeficiency) mice. This model allowed to obtain a significant oocyst shedding during all the course of the experiment with both *Cryptosporidium muris* and *Cryptosporidium parvum* species. However, unexpectedly, the presence of adenocarcinoma in tumors located in the ileo-caecal region were

observed only in *C. parvum*-infected mice (Benamrouz et al., 2012, 2014; Certad et al., 2007, Certad et al., 2010b, Certad et al., 2012). In addition, it was shown that the carcinogenic potential of *C. parvum* was independent of the strain even if some isolates were more virulent than others inducing an earlier onset of neoplastic lesions, a higher mortality of infected animals and cholangiocarcinoma development (Certad et al., 2010a, Certad et al., 2012). Interestingly, *C. muris* was not able to induce this type of epithelial changes suggesting that the development of neoplasia is not necessarily due to the immunosuppression (Certad et al., 2007). Even more, SCID mice have been reported to develop spontaneous thymic lymphomas, but other tumor types are very rare (Huang et al., 2011). Moreover, the presence of neoplasia has been reported with other mice models of cryptosporidiosis. For instance, low-grade dysplasia was found in the biliary tree of IFN-γ knockout mice infected with *Cryptosporidium (*Stephens et al., 1999*)*. Similar histologic findings were linked with chronic *C. parvum* infection in NIH-III nu/nu mice (Mead et al., 1991). This carcinogenicity was also confirmed after infection of mouse enteric explants with this parasite (Baydoun et al., 2017).

At least 11 biological agents have presently been recognized by the International Agency for Research on Cancer (IARC) as major contributors to the number of cancers in humans worldwide. These agents include viruses and bacteria. Other pathogens, including parasites, are also considered oncogenic agents for humans. Among helminths, the widespread digenetic trematode *Schistosoma haematobium* has been associated to urinary bladder cancer, and the flukes *Opisthorchis viverrini* and *Clonorchis sinensis* are causally linked with cholangiocarcinoma (Cheeseman et al., 2016). Subsequently, the idea of parasites as a cause of cancer in vertebrates is slowly developing.

Nevertheless, the contribution of intracellular eukaryotic parasites to cancer development has been largely neglected until now. It was also suggested that *Plasmodium falciparum* could play a co-factor role in the development of Burkitt lymphoma (Johnston et al., 2014). However, only the Apicomplexan genera *Cryptosporidium* and *Theileria* have been shown to induce cell transformation in experimental models (Cheeseman et al., 2016).

Herein, we summarize and discuss potential mechanisms that could explain how *Cryptosporidium* would be able to hijack signaling pathways of the cell, eventually driving to oncogenesis.

3. *Cryptosporidium* is located at a unique host-parasite interface

The intestinal epithelium is formed by a single layer of epithelial cells that functions as a physical barrier. Within the barrier, epithelial cells are held together by the intercellular junctions. This barrier controls nutrient absorption and prevents the passage of microorganisms, luminal antigens, and toxins to the mucosal tissue (Certad et al., 2017).

Interaction between *Cryptosporidium* and the apical surface of the epithelial cell results in the localized activation of signaling cascades such as phosphoinositide 3-kinase (PI3K), Proto-oncogene tyrosine-protein kinase (Src), Cell division control protein 42 homolog (Cdc42) and GTPase (Chen et al., 2004a, 2004b), culminating in barrier disruption and polymerization of actin filaments in the region of host–parasite interaction with the consequent formation of a parasitophorous vacuole (PV) (Pinto and Vinayak, 2021).

The events after attachment and invasion differ between Apicomplexa parasites. Electron microscopy studies support that *Cryptosporidium* is “engulfed” by the epithelial cell in a PV to be located in an a peculiar position: intracellular stages neither penetrate under the host plasma membrane nor come into the close contact with the cytoplasm of epithelial cells (Kolářová and Valigurová, 2021). The PV compartment consists of a host cell membrane fold incompletely fused. An electron-dense band separates the cell cytoplasm from the modified part of the membrane fold. This band seems to function in parasite anchoring (Valigurová et al., 2008). In other apicomplexan parasites the PV consists of one or more membranes carrying parasite proteins that facilitate the exchange of nutrients and other molecules (Elliott and Clark, 2000).The presence of a feeder organelle at the interface between the parasite and the host is also a unique characteristic of *Cryptosporidium*. This area functions as an exchange site. This observation has been supported by studies reporting the localization of a predicted ABC-transporter at this site (Perkins et al., 1999).

4. *Cryptosporidium* and the hijacking of host actin polymerization

After the invasion *C. parvum* induces cytoskeleton remodeling and actin reorganization of the host cell (Kolářová and Valigurová, 2021). Upon attachment to the host cell, *C. parvum* induces the aggregation and activation of c-Src (a proto-oncogene) and Phosphatidylinositol-3-kinase (PI3K). c-Src dependent activation of cortactin can either directly induce actin polymerization or activate the Actin Related Protein 2/3 (Arp2/3)complex of proteins. Additionally, activated PI3K induces GTPases activation, which is thought to act through neural Wiskott-Aldrich Syndrome protein (N-Wasp) to activate the Arp2/3 complex of proteins (Chen et al., 2001; Chen et al., 2004a, 2004b). Particularly, Cdc42 and Ras homolog family member A (RhoA), members of the Rho family of GTPases, were shown to be recruited to the host-parasite interface in an *in vitro* model of human biliary cryptosporidiosis (Chen et al., 2004a, 2004b).

Rho GTPases are well known as regulators of cytoskeleton dynamics, cell morphology and polarity, cell motility, vesicle trafficking, cell cycle progression, cell survival, cell growth, and differentiation and gene expression. They are also considered as key players controlling the dynamics of actin-based processes in the formation of lamellipodia, membrane ruffling, and filopodia (Martin et al., 2002). Thus, the Rho GTPase signaling can contribute to the hallmarks of cancer (Crosas-Molist et al., 2022). Interestingly, the signaling cascades activated at the interface between *Cryptosporidium* and the host are likely similar to these pathways found in the formation of lamellipodia/filopodia (Lendner and Daugschies, 2014). Consistently, dilation of intercellular spaces with extensive development of lateral membrane extensions at the intercellular junctions of the ileo-caecal epithelia of mice infected with *C. parvum* have been previously described by us (Fig. 1).

In addition, integrin α2 (ITGA2) and integrin ß1 (ITGB1) have been identified as potential receptors that transduce *Cryptosporidum*

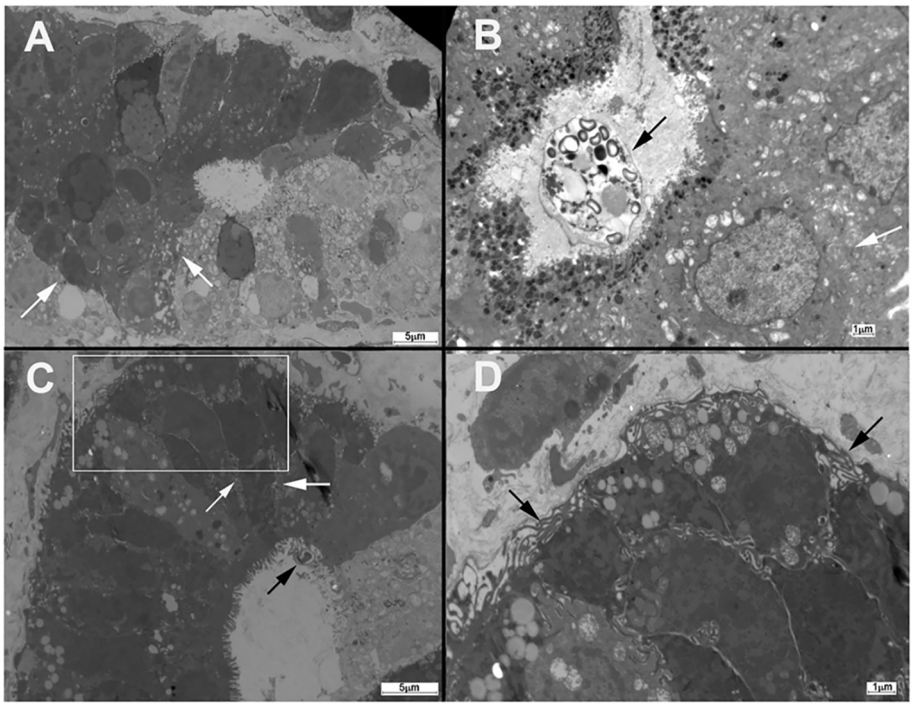

Fig. 1. Electron micrograph of ileo-caecal regions of dexamethasone-treated mice. (A) Electron micrograph of a section of normal non-neoplastic mucosa that shows normal intercellular junctions (white arrows). (B) In SCID mice that had been infected with *Cryptosporidium muris* (black arrow), alterations in the ultrastructure of intercellular junctions (white arrow) of gastric epithelial cells were not found. (C) Dilation of intercellular spaces with extensive development of lateral membrane extensions (white arrows) was observed at the intercellular junctions of the ileo-caecal epithelia of mice infected with *C. parvum* (black arrow). (D) Enlarged image of the area indicate by the white box in C, which shows lateral membrane extensions (black arrows). Scale bars: 5 µm (A,C); 1 µm (B,D). Source: image originally published in the journal *Diseases Models and Mechanisms* (Benamrouz et al., 2014) which allows to share, copy and redistribute the material in any medium or format, under the CC-BY license.

signaling into the host cell (Zhang et al., 2012) (Zhang et al., 2012). Integrins are primary sensors of the extracellular matrix (ECM) environment essential for cell migration, growth, and survival (Hussein et al., 2015).

The particular way the parasitic effector proteins involved in the process of this F-actin rearrangement, and their molecular targets it is not well understood. Recently, using live-cell imaging rhoptry protein 1 (ROP1), a parasite-secreted effector from roptries was found to bind the host protein LIM domain only 7 (LMO7), which is a cytoskeletal modulator that has been associated with the condensation of F-actin at cell junctions and in the terminal web of epithelia and sensory cells (Guérin et al., 2021). LMO7 directly binds to afadin and alpha-actinin to connect the nectin/afadin and E-cadherin/catenin complexes at the adherens junction suggesting a role in epithelial adhesion (Ooshio et al., 2004) and in invasiveness of tumors (Nakamura et al., 2005).

5. Molecular factors involved in *Cryptosporidium* induced colon cancer—hallmarks of cancer

A comprehensible framework to organize the complexities of neoplastic disease, known as the "hallmarks of cancer" was described (Hanahan and Weinberg, 2000). These hallmarks include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis (Klöhn et al., 2021).

Consistently, *Cryptosporidium* infection causes hyperproliferation, invasiveness and escape from apoptosis and immunological responses, presumably through the manipulation of host cellular pathways (Fig. 2).

1.(Chen et al., 2001), 2.(Chen et al., 2004a, 2004b), 3. (Liu et al., 2008), 4. (Castellanos-Gonzalez et al., 2008), 5. (Mele et al., 2004),6. (Sawant et al., 2021), 7. (Choudhry et al., 2009), 8. (He et al., 2021), 9. (Lantier et al., 2013), 10. (Lacroix et al., 2001), 11. (Liu et al., 2009), 12. (Deng et al., 2004), 13. (Benamrouz et al., 2014), 14. (Chen et al., 2007), 15. (Guesdon et al., 2015), 16.(Kumar et al., 2018), 17. (Zhang et al., 2020a, 2020b), 18. (Zhou et al., 2009), 19. (Gong et al., 2010). Illustration created with BioRender.com.

These abilities certainly makes *Cryptosporidium* a potential oncogenic agent involved in cancer development beyond the usual oncogenic suspects. The mechanisms that contribute to the development of *Cryptosporidium* induced digestive neoplasia are not totally understood, but are clearly multifactorial and involve both parasite and host factors.

Concerning the parasite, some virulence factors have been identified such as proteins involved in oocyst disencystment, parasite motility, host cell adhesion (such as mucin-like glycoproteins and thrombospondin-like adhesive proteins), sporozoite invasion of epithelial cells, PV formation, intracellular multiplication, and host cell damage. Several molecules, such as phospholipases, proteases and hemolysin H4 have been proposed as the cause of cellular damage (Bouzid et al., 2013).

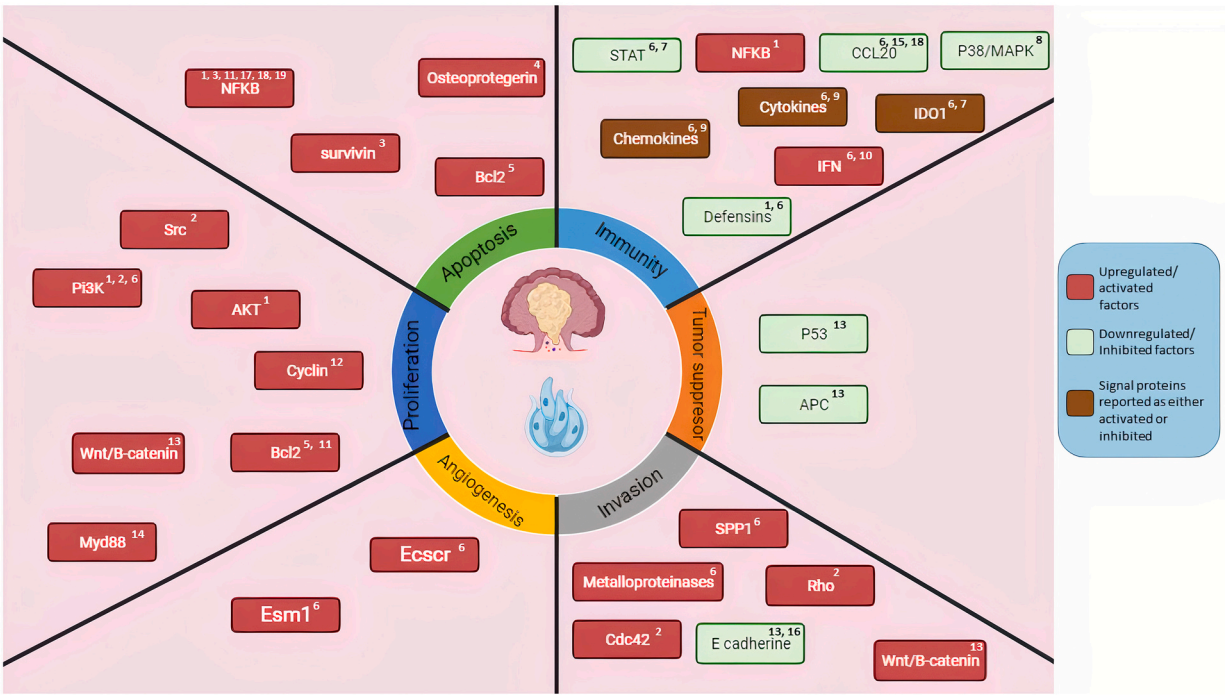

Fig. 2. Relating the "hallmarks of cancer" (Hanahan and Weinberg, 2000) to the molecular mechanisms of *Cryptosporidium* induced adenocarcinoma.

Recently, miRNA candidates in *C. parvum* were identified targeting genes taking part in several major pathways involved in the biology, virulence, and pathogenesis of *C. parvum* (Ahsan et al., 2021). Additionally, sequencing, annotation and comparative genomics of different isolates of *C. parvum* allowed the identification of common single nucleotide variants (SNV) in the more virulent isolates. Theses SNV were found in different families of genes already described to be implicated in parasite virulence and parasite-host interaction, such as cysteine proteases, mucins, and transporters (ABC and ATPase3). Many of these genes coded for membrane proteins, appeared to be destined towards the secretory pathway or were implicated in the cytoskeleton remodeling (Audebert et al., 2020).

These results confirm that beyond the genes involved in intracellular maintenance and damage to the host cell, genes involved in the initial interaction processes of *Cryptosporidium* oocysts and sporozoites with host epithelial cells can also be considered as parasite virulence factors (Audebert et al., 2020).

The role of long non-coding RNAs (lncRNA) delivered by *Cryptosporidium* into the host cell to manipulate host gene expression and pathogenesis has been also recently reported (Li et al., 2021). Recent studies describe that *Cryptosporidium* infection induces epigenetic histone methylations in infected epithelial cells through nuclear transfer of parasite RNA transcripts, resulting in modulation of transcription of genes important for cell proliferation, differentiation, and metabolism (Wang et al., 2017). For instance, nuclear delivery of Cdg7_FLc_0990 into the intestinal epithelial cells resulted in significant changes in expression levels of specific genes such as lipoprotein receptor-related protein (LRP5), solute carrier family 7 member 8 (SLC7A8), and Interleukin33 (IL33). These genes have been considered as keys to epithelial cell differentiation and metabolism through the Histone H3 lysine 9 (H3K9) methylation-mediated transcriptional suppression (Wang et al., 2017). The epigenetic modulation of a host's transcriptional program linked to host defense genes has been described for viral and bacterial infections facilitating the life cycle of pathogens (Wang et al., 2017).

Concerning the host, potential signaling pathways may be hijacked by the parasite to induce transformation have been explored. After infection of an animal model with *C. parvum,* alterations in genes or proteins commonly involved in cell cycle, differentiation or cell migration, such as β-catenin, Adenomatous polyposis coli (Apc), E-cadherin, (Kirsten rat sarcoma virus (Kras) and p53 were investigated (Benamrouz et al., 2014). It was demonstrated immunohistochemical abnormal localization of Wnt signaling pathway components and p53. Mutations in the selected loci of studied genes were not found after high-throughput sequencing. An altered expression of proteins was observed in tissues of infected mice with a decrease in an Apc and E-cadherin, and a cytoplasmic accumulation of β-catenin in neoplastic cells but without its translocation to the nucleus. An abnormal cytosolic p53 labeling in the adenomatous cells was also observed (Benamrouz et al., 2014).

Thus, for the first time it was reported that the Wnt signaling pathway, and particularly the cytoskeleton network, seems to be a key player for the development of the *C. parvum*-induced neoplastic process and cell migration of transformed cells (Benamrouz et al., 2014). In consistence, extensive downregulation of expression of key components of the adherent junctions of epithelial cells such as occluding, claudin 4, and E-cadherin was shown following *C. parvum* infection in different experimental models. ZO1, the adaptor protein that link epithelial tight junctions assembly to the actin cytoskeleton was also significantly decreased (Kumar et al., 2018).

Moreover, electron microscopic analysis performed on ileo-caecal region of infected SCID mice showed lateral and basal cytoplasmic extensions suggesting that a cytoskeleton network alteration is involved in the carcinogenic mechanisms induced by *C. parvum* (Benamrouz et al., 2014) (Fig. 1).

6. Modulation of the immune response and apoptosis pathways

Epithelial cells in response to *Cryptosporidium* infection produce cytokines and chemokines to attract immune cells to the infection site, and release antimicrobial peptides, which can kill the extra-cellular stages of the parasite (Laurent and Lacroix-Lamandé, 2017).

However, *Cryptosporidium* can evade the host immune response by different strategies. For instance, when residing inside the PV, the exposure of *Cryptosporidium* antigens is limited, thus the parasite becomes invisible to the immune system. This would be one of the first parasite immune evasion strategies (Valigurová et al., 2008). In addition, the key molecule of the host immune defense against *Cryptosporidium* is IFNγ. However, the parasite is able to circumvent the effect of Interferon gamma (IFNγ), after reducing Signal transducer and activator of transcription 1 (STAT1) production and Indoleamine-pyrrole 2,3-dioxygenase (IDO1) expression in order to restore the availability of tryptophan in the cells to allow the development of the parasite (Choudhry et al., 2009). Even if a recent microarray study reported high expression of IDO1 within a tumor microenvironment in a mouse model of cryptosporidiosis at 45 and 93 days PI. Authors proposed as explanation that upregulation of IDO1 would be due to inflammatory epithelial–mesenchymal transition (EMT) triggered by IFNγ and Tumor necrosis factor α (TNF-α) *via* STAT1 (Sawant et al., 2021).

Moreover, *C. parvum* infection can downregulate the expression of chemokines known to exert anti-microbial activity such as Chemokine (C—C motif) ligand 20 (CCL20) (Guesdon et al., 2015), defensin-α (DEFA4), and defensin-β (Sawant et al., 2021).

On the other hand, *Cryptosporidium* has developed strategies to modulate apoptosis and epithelial turnover depending on the developmental stages of the parasite in order to facilitate its growth and allow sufficient time for intracellular life cycle completion (Liu et al., 2009; Mele et al., 2004). Particularly, numerous studies have demonstrated an essential role for NF-κβ in the host cell response to *Cryptosporidium* infection (Laurent and Lacroix-Lamandé, 2017). This transcription factor, which can be activated by Toll-like receptors, has been implicated in anti- and pro-apoptotic signaling cascades.

(Chen et al., 2001; Liu et al., 2008, 2009), as well as the modulated expression of pro-inflammatory chemokines (Sawant et al., 2021), and Micro (mi)RNAs (Ming et al., 2017). In particular, these miRNAs may modulate epithelial responses against the parasite at every step of the innate immune network. However, *Cryptosporidium* may hijack host cell miRNAs to resist this immune response, as it has been shown in viral and bacterial infections (Ming et al., 2017). For instance, the activation of NF-κB signaling, coupled with the induction of B7-H1 by downregulation of miR-513 in *Cryptosporidium* infected cells may contribute to the apoptotic resistance of infected cells (Gong et al., 2010). Consistently, a genome-wide miRNA expression analysis showed significant modulation of miRNA expression profile of miR-942 in *C. parvum* gastro-intestinal epithelial cells through the activation of the TLR4/NF-κB pathway (Zhang et al., 2020a, 2020b). On the other hand, as explained above, the expression of chemokines as CCL20 can be downregulated by the infection. Recently works have confirmed that CCL20 is a target for miR-21 in infected epithelial cells as one of the immune evasion strategies of the parasite (Guesdon et al., 2015) and it seems that the induction of miR-21 is also dependent on the activation of NF-κB signaling (Zhou et al., 2009).

As a consequence of the infection other anti-apoptotic factors has also been described such as B-cell lymphoma 2 (BCL-2) (Liu et al., 2009; Mele et al., 2004), Osteoprotegerin (OPG) (Castellanos-Gonzalez et al., 2008) and survivin (Liu et al., 2009; Mele et al., 2004). As discussed before, previous findings have shown that apoptosis of host epithelial cells can be both induced and inhibited by *Cryptosporidium*. However, a recent RNA-seq analysis of monolayers of pig epithelial cells infected with *C. parvum* did not find upregulation of apoptosis related genes. These contradictory results leading to divergent conclusions could be due to the fact that different studies use different methodological conditions (Mirhashemi et al., 2018).

Moreover, a significant downregulation of p38/MAPK, MAP kinase-activated protein kinase 2 (Mk2), and Mk3 genes was found in an *in vitro* model of cryptosporidiosis (He et al., 2021). Suppression of MAPK signaling was linked with an impaired intestinal epithelial defense against *C. parvum* infection (He et al., 2021) (He et al., 2021). Interestingly, p38/MAPK has described as a tumor suppressor (Bulavin and Fornace, 2004).

Therefore, the ability of the parasite to evade the host innate immunological response by resisting the upregulated expression of IFNγ-stimulated genes and downregulating the expression of key players such as α-defensins favor a persistent *C. parvum* infection and alteration of the balance towards cancer (Sawant et al., 2021). Innate immune signaling shares similarities with tumor suppressor signaling, as both processes initiate cell cycle arrest and modulate apoptotic pathways.

7. Similarities between *Cryptosporidium* and oncogenic pathogens

Normal cells have protection programs, which safeguard them from getting out of control and developing cancer as follows: (i) cells divide only after receiving the appropriate signal; (ii) when cells accumulate many genetic anomalies, they commit suicide; (iii) cells divide a limited number of times, and, finally, (iv) they have adhesive properties that prevent the detachment and migration. However, oncogenic pathogens even if they are phylogenetically diversified, they can sabotage these programs in order to favor survival and transmission, and avoid the immune system (Ewald, 2009). When all these anti-cancer barriers are altered by a pathogen for its own multiplication and survival strategy, cells will then be transformed into neoplastic cells (Bañuls et al., 2013). In addition, there are several other mechanisms through which pathogens can transform cells into cancer cells, such as the induction of chromosome instability and of translocations, inflammation, *etc.* (Bañuls et al., 2013).

For instance, oncogenic viruses such as Epstein Barr virus (EBV), hepatitis B virus (HBV), hepatitis C virus (HCV), and herpesvirus,

Table 2
Common cellular targets between *Cryptosporidium* and some oncogenic pathogens.

Cellular targets	Oncogenic pathogens	References
Cytoskeleton interaction	Herpesvirus	Wu et al. (2019)
Dysregulation of PI3K/AKT	HPV, Herpesvirus	Liu and Cohen, (2015); Scarth et al. (2021)
Activation of Mitogen-activated protein kinase (MAPK)/Ras/Raf/c-Jun, NF-κβ, JAK-STAT, protein kinase C, Src, survivin and PI3K cascades	HBV, HPV, HCV	Moore and Chang, (2010); Shlomai et al. (2014)
Modulation of apoptosis	EBV, HBV, HCV, Herpesvirus	Zamaraev et al. (2020)
EMT activation	*Helicobacter pylori*, EBV	Hofman and Vouret-Craviari (2012)
Wnt/β-catenin pathway activation	HBV	Cha et al. (2004)
Prevention of p53 nuclear localization	HBV, HPV	Cha et al. (2004); Moore and Chang, (2010)

Genes chosen for hallmarks activation represent available examples and are based on published data. HPV, human papillomavirus; EBV, Epstein–Barr virus; HBV, hepatitis B virus; HCV, hepatitis C virus.

in a similar way as *Cryptosporidium*, can manipulate apoptosis pathways, and inhibit the activity of proapoptotic proteins and signaling pathways, which facilitates carcinogenesis (Zamaraev et al., 2020). The interaction of viruses with Receptor tyrosine kinases (RTKs), directly or through adaptors such as Gas6, activates different mechanisms that facilitate invasion, including receptor clustering in lipid rafts, lateral movement of viral particles to sites of internalization near tight junctions, clathrin-mediated endocytosis, and actin cytoskeleton dynamics (Haqshenas and Doerig, 2019) (Table 2).

As already discussed above, integrins play an important role in regulating cellular functions such as cell adhesion, cell migration, and regulation of signaling pathways (Martin et al., 2002). Several viruses harbor integrin-recognition motifs displayed on viral envelope/capsid-associated protein. As adhesion molecules, integrins mediate cell-to-cell, cell-to-ECM, and cell-to-pathogen interactions. Adhesion of integrins to a solid surface is the first step in cell migration and motility. The key role of integrins in cell migration makes them essential for many important biological events, including embryonic development, inflammatory responses, wound healing, and cell transformation (Hussein et al., 2015).

The EMT is the most common type of cell reprogramming of oncogenic virus. EMT stimulates the dedifferentiation of epithelial cells into mesenchymal cells. This process can be due to the ability of a virus to neutralize tumor suppressor genes, or behave as oncogenes. Then, virus-induced mesenchymal cells can manipulate the microenvironment of the affected cells to promote proliferation and migration. These changes are related to modifications of transcription factors expressed by infected cells. In consequence, cytoskeleton, adhesive, and metabolic cellular components are modified, and the shape and behavior of the cell affected (Siddiqui et al., 2018) (Siddiqui et al., 2018). *Cryptosporidium* has a role in the morphogenesis, adhesion, migration and proliferation of epithelial cells sharing with oncogenic pathogens common signaling pathways, which lead to EMT. It has been suggested that these pathogens may be considered as EMT inducers that are able to cause a sustained activation of EMT regulating signaling pathways such as NF-κβ, MAPK and PI3K/AKT (Hofman and Vouret-Craviari, 2012). Consistently, AKT3, one of the isoforms of AKT that modulates various cellular responses *via* PI3K/AKT pathway was upregulated in a mouse model of cryptosporidiosis at day 93 post-infection. In addition, it was reported that *C. parvum* infection assists in development of an immunosuppressive tumor microenvironment (Sawant et al., 2021).

8. Conclusions

It has been shown in experimental models that *Cryptosporidium* infection induces both *in vivo* and *in vitro* development of digestive cancer even with very low inoculum. In addition, epidemiological studies have shown a significant association between cryptosporidiosis and the presence of digestive neoplasia in humans confirming the *in vivo* and *in vitro* observations. *Cryptosporidium* can sabotage cellular pathways potentially being involved in cancer onset to favor a cellular environment propitious for parasite replication. Some of these pathways deregulation can lead to a stimulation of cellular proliferation, inhibition of apoptosis and immune evasion. If *Cryptosporidium* is able to hijack signal transduction, then is very likely that this contributes to transformation of its host cell. Further work is needed to confirm this hypothesis and elucidate the relationship between this apicomplexan parasite and its host cell in order to understand the mechanisms used by the parasite to induce transformation of its host cells and to develop effective treatment interventions.

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Declaration of Competing Interest

There are no conflicts of interest.

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