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Gabriela Certad

► **To cite this version:**

Gabriela Certad. Is *Cryptosporidium* a hijacker able to drive cancer cell proliferation?. Food and Waterborne Parasitology, 2022, 27, pp.e00153. 10.1016/j.fawpar.2022.e00153 . hal-04474835

HAL Id: hal-04474835

<https://hal.science/hal-04474835v1>

Submitted on 22 Jul 2024

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

2 **Gabriela Certad**

3 U1019-UMR 9017-CIIL-Centre d'Infection et d'Immunité de Lille, Institut Pasteur de Lille, Université
4 de Lille, CNRS, Inserm, CHU Lille, F-59000 Lille, France;
5 Délégation à la Recherche Clinique et à l'Innovation, Groupement des Hôpitaux de l'Institut
6 Catholique de Lille, F-59462 Lomme, France

7
8 * Correspondence: gabriela.certad@pasteur-lille.fr
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10 **Abstract**

11 The pathophysiological mechanisms of *Cryptosporidium* infection are multifactorial and not
12 completely understood. Some advances achieved recently revealed that the infection by *C.*
13 *parvum* induces cytoskeleton remodeling and actin reorganization through the implication of
14 several intracellular signals involving, for example, PI3K, Src, Cdc42 and GTPases. It has also
15 been reported that the infection by *C. parvum* leads to the activation of NF- κ B, known to
16 induce anti-apoptotic mechanisms and to transmit oncogenic signals to epithelial cells.
17 Despite the growing evidence about the hijacking of cellular pathways, potentially being
18 involved in cancer onset, this information has rarely been linked to the tumorigenic potential
19 of the parasite. However, several evidences support an association between
20 *Cryptosporidium* infection and the development of digestive neoplasia. To explore the
21 dynamics of *Cryptosporidium* infection, an animal model of cryptosporidiosis using corticoid
22 dexamethasone-treated adult SCID (severe combined immunodeficiency) mice, orally
23 infected with *C. parvum* or *C. muris* oocysts was implemented. *C. parvum*-infected animals
24 developed digestive adenocarcinoma. When mechanisms involved in this neoplastic process
25 were explored, the pivotal role of the Wnt pathway together with the alteration of the

26 cytoskeleton was confirmed. Recently, a microarray assay allowed the detection of cancer-
27 promoting genes and pathways highly up regulated in the group of *C. parvum* infected
28 animals when compared to non-infected controls. Moreover, different human cases/control
29 studies reported significant higher prevalence of *Cryptosporidium* infection among patients
30 with recently diagnosed colon cancer before any treatment when compared to the control
31 group (patients without colon neoplasia but with persistent digestive symptoms). These
32 results suggest that *Cryptosporidium* is a potential oncogenic agent involved in cancer
33 development beyond the usual suspects. If *Cryptosporidium* is able to hijack signal
34 transduction, then is very likely that this contributes to transformation of its host cell. More
35 research in the field is required in order to identify mechanisms and molecular factors
36 involved in this process and to develop effective treatment interventions.

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38 **Keywords:** *Cryptosporidium*, pathogenicity, digestive cancer

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

48 Organisms in the genus *Cryptosporidium* are classified in the phylum Apicomplexa, class Conoidasida
49 and order Eucoccidiorida. Their taxonomic position was challenged by molecular phylogenies, and a
50 closer affinity to gregarines, representing an early branch at the base of the phylum, was shown
51 (Certad et al., 2017).

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

61 *Cryptosporidium* parasites are a major cause of diarrhea worldwide. They affect humans and animals.
62 Transmission is by the fecal–oral route via water or food contaminated with the parasite or by
63 ingestion of oocysts after direct contact with humans and animals carrying the parasite (Ryan et al.,
64 2021). Immunosuppressed individuals and children are particularly vulnerable to infection (Khalil et
65 al., 2018). The main clinical manifestation of the infection is diarrhea and in fact, *Cryptosporidium* is a
66 leading cause of severe pediatric diarrhea. A cohort study (GEMS) involving more than 20 000
67 children in Africa and Asia revealed that *Cryptosporidium* is one of the four main pathogens
68 responsible for severe diarrhea and mortality in infants and toddlers (Khalil et al., 2018; Kotloff et al.,
69 2013). Treatment options for patients with cryptosporidiosis are scarce. The only treatment for

70 cryptosporidiosis approved by the Food and Drug Administration (FDA) is nitazoxanide. However, this
71 drug has limited efficacy due to the fact that can allow the improvement of diarrheal symptoms
72 without completely inhibiting the excretion of oocysts, and is non-effective in immunocompromised
73 patients (Amadi et al., 2009). *Cryptosporidium* species have caused waterborne outbreaks of
74 gastrointestinal disease around the world (Crawford & Kol, 2021).

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

83 **2. Is *Cryptosporidium* responsible of oncogenesis?**

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

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

93 Additionally, a statistically significant increased risk of *Cryptosporidium* infection among cancer
94 patients compared to controls was reported in a systematic review and meta-analysis compiling data
95 on 3562 individuals from 19 cross-sectional and case-control studies [OR = 3.3; 95% CI: 2.18-4.98]
96 (Kalantari et al., 2020). The same study showed that the occurrence of *Cryptosporidium* infection was
97 related to colorectal malignancies (3.7 (2.10–6.50)) but unassociated with blood cancer (3.8 (0.49–
98 29.60)) (Kalantari et al., 2020).

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

103 Moreover, an animal model of cryptosporidiosis was developed using SCID (severe combined
104 immunodeficiency) mice. This model allowed to obtain a significant oocyst shedding during all the
105 course of the experiment with both *C. muris* and *C. parvum* species. However, unexpectedly, the
106 presence of adenocarcinoma in tumors located in the ileo-caecal region were observed only in *C.*
107 *parvum*-infected mice (Benamrouz et al., 2012, 2014; Certad et al., 2007, 2010a, 2012). In addition, it
108 was shown that the carcinogenic potential of *C. parvum* was independent of the strain even if some
109 isolates were more virulent than others inducing an earlier onset of neoplastic lesions, a higher
110 mortality of infected animals and cholangiocarcinoma development (Certad et al., 2010b, 2012).
111 Interestingly, *C. muris* was not able to induce this type of epithelial changes suggesting that the
112 development of neoplasia is not necessarily due to the immunosuppression (Certad et al., 2007).
113 Even more, SCID mice have been reported to develop spontaneous thymic lymphomas, but other
114 tumor types are very rare (Huang et al., 2011). Moreover, the presence of neoplasia has been
115 reported with other mice models of cryptosporidiosis. For instance, low-grade dysplasia was found in
116 the biliary tree of IFN- γ knockout mice infected with *Cryptosporidium* (Stephens et al., 1999). Similar
117 histologic findings were linked with chronic *C. parvum* infection in NIH-III nu/nu mice (Mead et al.,

118 1991). This carcinogenicity was also confirmed after infection of mouse enteric explants with this
 119 parasite (Baydoun et al., 2017).

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

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

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

134 **Table 1. Comparison of Apicomplexan parasites**

	<i>Cryptosporidium</i>	<i>Toxoplasma</i>	<i>Plasmodium</i>	<i>Theileria</i>
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

135

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

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

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

147 The events after attachment and invasion differ between Apicomplexa parasites. Electron
 148 microscopy studies support that *Cryptosporidium* is “engulfed” by the epithelial cell in a PV to be
 149 located in an a peculiar position: intracellular stages neither penetrate under the host plasma
 150 membrane nor come into the close contact with the cytoplasm of epithelial cells (Kolářová &
 151 Valigurová, 2021). The PV compartment consists of a host cell membrane fold incompletely fused.
 152 An electron-dense band separates the cell cytoplasm from the modified part of the membrane fold.

153 This band seems to function in parasite anchoring (Valigurová et al., 2008). In other apicomplexan
154 parasites the PV consists of one or more membranes carrying parasite proteins that facilitate the
155 exchange of nutrients and other molecules (Elliott & Clark, 2000). The presence of a feeder organelle
156 at the interface between the parasite and the host is also a unique characteristic of *Cryptosporidium*.
157 This area functions as an exchange site. This observation has been supported by studies reporting the
158 localization of a predicted ABC-transporter at this site (Perkins et al., 1999).

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

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

170 Rho GTPases are well known as regulators of cytoskeleton dynamics, cell morphology and polarity,
171 cell motility, vesicle trafficking, cell cycle progression, cell survival, cell growth, and differentiation
172 and gene expression. They are also considered as key players controlling the dynamics of actin-based
173 processes in the formation of lamellipodia, membrane ruffling, and filopodia (Martin et al., 2002).
174 Thus, the Rho GTPase signaling can contribute to the hallmarks of cancer (Crosas-Molist et al., 2022).
175 Interestingly, the signaling cascades activated at the interface between *Cryptosporidium* and the host
176 are likely similar to these pathways found in the formation of lamellipodia/filopodia (Lendner &
177 Dauschies, 2014). Consistently, dilation of intercellular spaces with extensive development of lateral

178 membrane extensions at the intercellular junctions of the ileo-caecal epithelia of mice infected with
179 *C. parvum* have been previously described by us (Figure 1).

180

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

190

191 In addition, integrin $\alpha 2$ (ITGA2) and integrin $\beta 1$ (ITGB1) have been identified as potential receptors
192 that transduce *Cryptosporidium* signaling into the host cell (Zhang et al., 2012) (Zhang et al., 2012).
193 Integrins are primary sensors of the extracellular matrix (ECM) environment essential for cell
194 migration, growth, and survival (Hussein et al., 2015).

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

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

204 **Hallmarks of Cancer**

205 A comprehensible framework to organize the complexities of neoplastic disease, known as the
206 “hallmarks of cancer” was described (Hanahan & Weinberg, 2000). These hallmarks include
207 sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling
208 replicative immortality, inducing angiogenesis, and activating invasion and metastasis (Klöhn et al.,
209 2021).

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

213 **Figure 2. Relating the “hallmarks of cancer” (Hanahan & Weinberg, 2000) to the molecular mechanisms of**
214 ***Cryptosporidium* induced adenocarcinoma.**

215 1.(Chen et al., 2001), 2.(Chen et al., 2004), 3. (Liu et al., 2008), 4. (Castellanos-Gonzalez et al., 2008), 5. (Mele et al., 2004),6.
216 (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
217 et al., 2009), 12. (Deng et al., 2004), 13. (Benamrouz et al., 2014), 14. (Chen et al., 2007), 15. (Guesdon et al., 2015),
218 16.(Kumar et al., 2018), 17. (Zhang et al., 2020), 18. (Zhou et al., 2009), 19. (Gong et al., 2010). Illustration created with
219 BioRender.com.

220

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

225 Concerning the parasite, some virulence factors have been identified such as proteins involved in
226 oocyst disencystment, parasite motility, host cell adhesion (such as mucin-like glycoproteins and
227 thrombospondin-like adhesive proteins), sporozoite invasion of epithelial cells, PV formation,
228 intracellular multiplication, and host cell damage. Several molecules, such as phospholipases,
229 proteases and hemolysin H4 have been proposed as the cause of cellular damage (Bouزيد et al.,
230 2013).

231 Recently, miRNA candidates in *C. parvum* were identified targeting genes involved in several major
232 pathways involved in the biology, virulence, and pathogenesis of *C. parvum* (Ahsan et al., 2021).

233 Additionally, sequencing, annotation and comparative genomics of different isolates of *C. parvum*
234 allowed the identification of common single nucleotide variants (SNV) in the more virulent isolates.
235 These SNV were found in different families of genes already described to be implicated in parasite
236 virulence and parasite-host interaction, such as cysteine proteases, mucins, and transporters (ABC
237 and ATPase3). Many of these genes coded for membrane proteins, appeared to be destined towards
238 the secretory pathway or were implicated in the cytoskeleton remodeling (Audebert et al., 2020).

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

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

255 Concerning the host, potential signaling pathways may be hijacked by the parasite to induce
256 transformation have been explored. After infection of an animal model with *C. parvum*, alterations in
257 genes or proteins commonly involved in cell cycle, differentiation or cell migration, such as β -catenin,

258 Adenomatous polyposis coli (Apc), E-cadherin, (Kirsten rat sarcoma virus (Kras) and p53 were
259 investigated (Benamrouz et al., 2014). It was demonstrated immunohistochemical abnormal
260 localization of Wnt signaling pathway components and p53. Mutations in the selected loci of studied
261 genes were not found after high-throughput sequencing. An altered expression of proteins was
262 observed in tissues of infected mice with a decrease in an Apc and E-cadherin, and a cytoplasmic
263 accumulation of β -catenin in neoplastic cells but without its translocation to the nucleus. An
264 abnormal cytosolic p53 labeling in the adenomatous cells was also observed (Benamrouz et al.,
265 2014).

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

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

276 **6. Modulation of the immune response and apoptosis pathways**

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

280 However, *Cryptosporidium* can evade the host immune response by different strategies. For instance,
281 when residing inside the PV, the exposure of *Cryptosporidium* antigens is limited, thus the parasite

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

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

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

302 (Chen et al., 2001; Liu et al., 2008, 2009), as well as the modulated expression of pro-inflammatory
303 chemokines (Sawant et al., 2021), and Micro (mi)RNAs (Ming et al., 2017). In particular, these
304 miRNAs may modulate epithelial responses against the parasite at every step of the innate immune
305 network. However, *Cryptosporidium* may hijack host cell miRNAs to resist this immune response, as it
306 has been shown in viral and bacterial infections (Ming et al., 2017). For instance, the activation of

307 NF- κ B signaling, coupled with the induction of B7-H1 by downregulation of miR-513 in
308 *Cryptosporidium* infected cells may contribute to the apoptotic resistance of infected cells (Gong et
309 al., 2010). Consistently, a genome-wide miRNA expression analysis showed significant modulation of
310 miRNA expression profile of miR-942 in *C. parvum* gastro-intestinal epithelial cells through the
311 activation of the TLR4/NF- κ B pathway (Zhang et al., 2020). On the other hand, as explained above,
312 the expression of chemokines as CCL20 can be downregulated by the infection. Recently works have
313 confirmed that CCL20 is a target for miR-21 in infected epithelial cells as one of the immune evasion
314 strategies of the parasite (Guesdon et al., 2015) and it seems that the induction of miR-21 is also
315 dependent on the activation of NF- κ B signaling (Zhou et al., 2009).

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

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

329 Therefore, the ability of the parasite to evade the host innate immunological response by resisting
330 the upregulated expression of IFN γ -stimulated genes and downregulating the expression of key
331 players such as α -defensins favor a persistent *C. parvum* infection and alteration of the balance

332 towards cancer (Sawant et al., 2021). Innate immune signaling shares similarities with tumor
333 suppressor signaling, as both processes initiate cell cycle arrest and modulate apoptotic pathways.

334 **7. Similarities between *Cryptosporidium* and oncogenic pathogens**

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

345 For instance, oncogenic viruses such as Epstein Barr virus (EBV), hepatitis B virus (HBV), hepatitis C
346 virus (HCV), and herpesvirus, in a similar way as *Cryptosporidium*, can manipulate apoptosis
347 pathways, and inhibit the activity of proapoptotic proteins and signaling pathways, which facilitates
348 carcinogenesis (Zamaraev et al., 2020). The interaction of viruses with Receptor tyrosine kinases
349 (RTKs), directly or through adaptors such as Gas6, activates different mechanisms that facilitate
350 invasion, including receptor clustering in lipid rafts, lateral movement of viral particles to sites of
351 internalization near tight junctions, clathrin-mediated endocytosis, and actin cytoskeleton dynamics
352 (Haqshenas & Doerig, 2019) (Table 2).

353 As already discussed above, integrins play an important role in regulating cellular functions such as
354 cell adhesion, cell migration, and regulation of signaling pathways (Martin et al., 2002). Several
355 viruses harbor integrin-recognition motifs displayed on viral envelope/capsid-associated protein. As

356 adhesion molecules, integrins mediate cell-to-cell, cell-to-ECM, and cell-to-pathogen interactions.
 357 Adhesion of integrins to a solid surface is the first step in cell migration and motility. The key role of
 358 integrins in cell migration makes them essential for many important biological events, including
 359 embryonic development, inflammatory responses, wound healing, and cell transformation (Hussein
 360 et al., 2015).

361

362

363 **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 & Cohen, 2015; Scarth et al., 2021)
Activation of Mitogen-activated protein kinase (MAPK)/ Ras/Raf/c-Jun, NF- κ B, JAK-STAT, protein kinase C, Src, survivin and PI3K cascades	HBV, HPV, HCV	(Moore & Chang, 2010; Shlomai et al., 2014)
Modulation of apoptosis	EBV, HBV, HCV, Herpesvirus	(Zamaraev et al., 2020)
EMT activation	<i>Helicobacter pylori</i> , EBV	(Hofman & 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 & Chang, 2010)

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

366

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

382 8. Conclusions

383 It has been shown in **experimental models** that *Cryptosporidium* infection induces both *in vivo* and *in*
384 *vitro* development of digestive cancer even with very low inoculum. In addition, epidemiological
385 studies have shown a significant association between cryptosporidiosis and the presence of digestive
386 neoplasia in humans confirming the *in vivo* and *in vitro* observations. *Cryptosporidium* can sabotage
387 cellular pathways potentially being involved in cancer onset to favor a cellular environment
388 propitious for parasite replication. Some of these pathways deregulation can lead to a stimulation of
389 cellular proliferation, inhibition of apoptosis and immune evasion. If *Cryptosporidium* is able to hijack
390 signal transduction, then is very likely that this contributes to transformation of its host cell. Further

391 work is needed to confirm this hypothesis and elucidate the relationship between this apicomplexan
392 parasite and its host cell in order to understand the mechanisms used by the parasite to induce
393 transformation of its host cells and to develop effective treatment interventions.

394 **Funding**

395 This work was supported by the Centre National de la Recherche Scientifique (CNRS), the Institut
396 National de la Santé et de la Recherche Médicale, the Institute Pasteur de Lille, the Université de
397 Lille, the Groupement des Hôpitaux de l'Institut Catholique de Lille, the Institut Catholique de Lille,
398 the Centre hospitalier régional universitaire de Lille.

399 **Declaration of Competing Interest**

400 There are no conflicts of interest.

401

402 **Acknowledgements**

403 Thanks to Dr. Alejandro Arenas-Pinto for critical reading of the manuscript.

404

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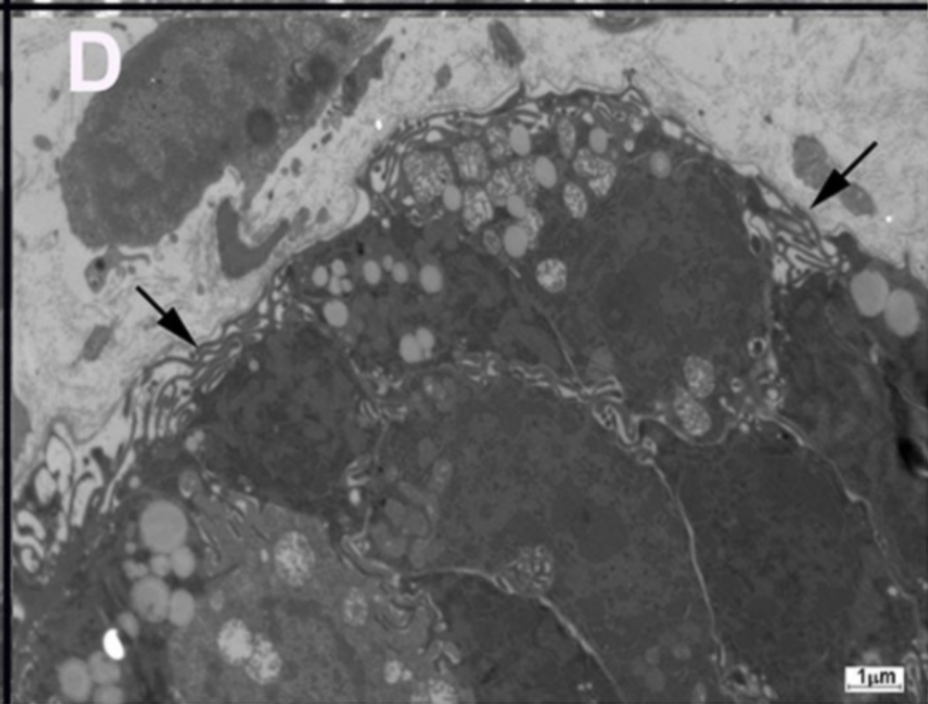
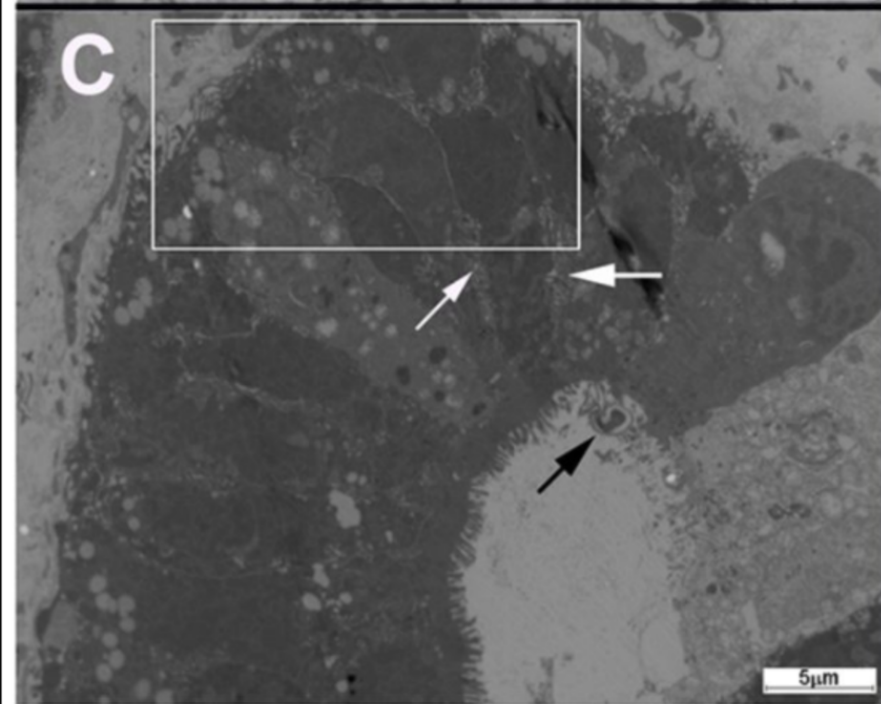
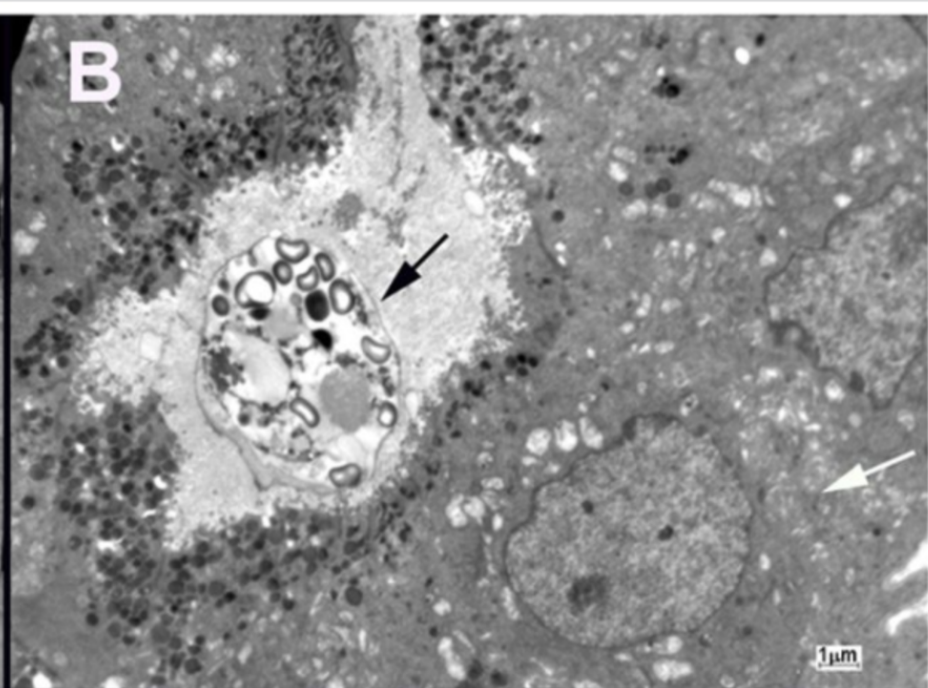
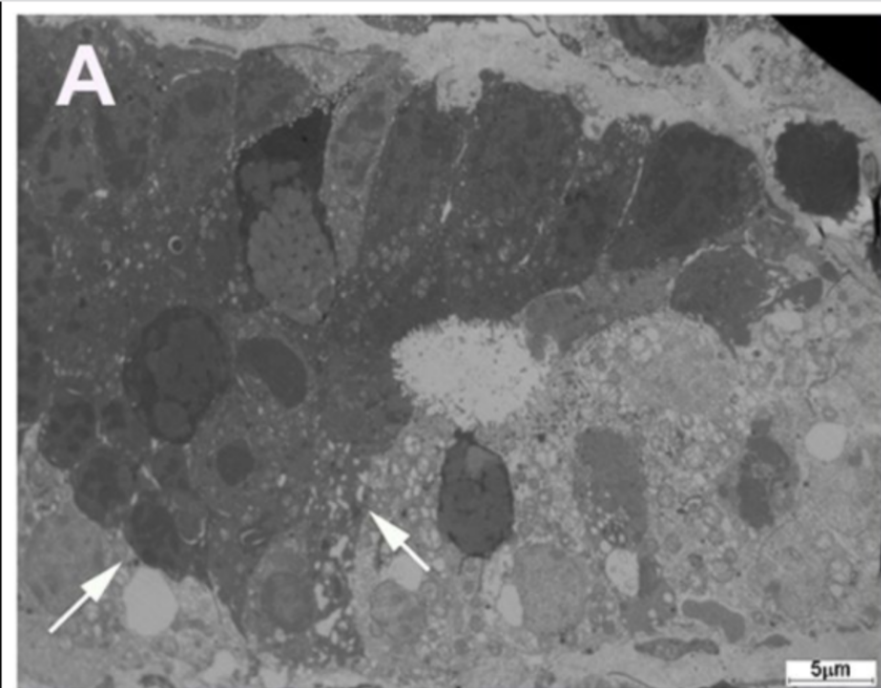
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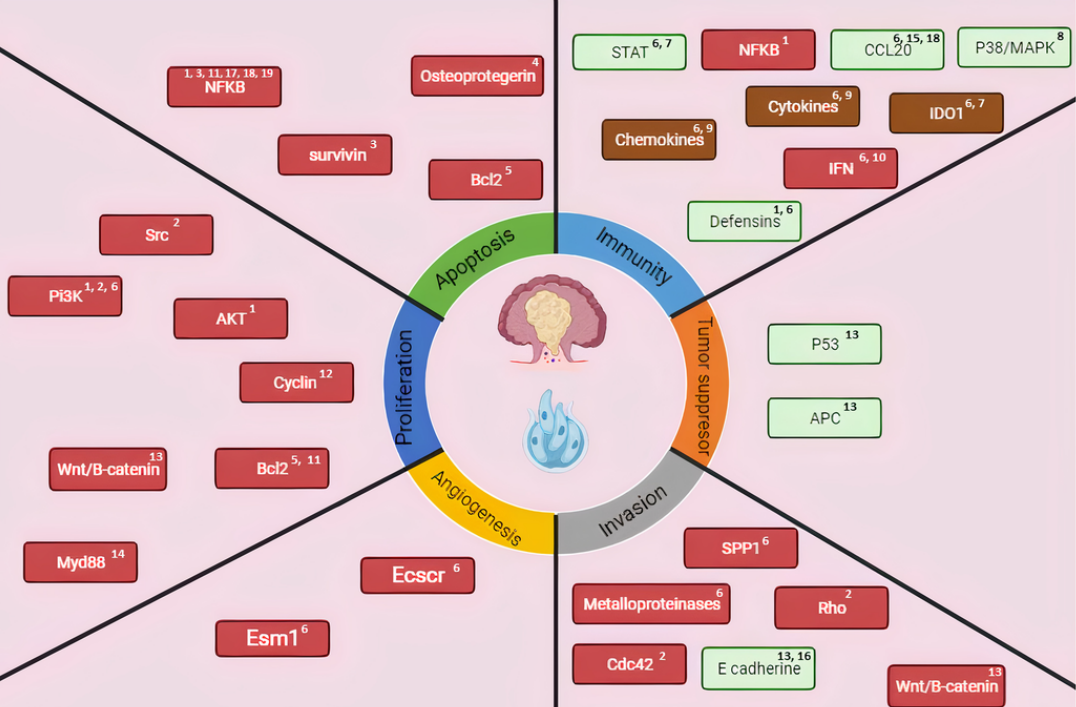
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1, 3, 11, 17, 18, 19
NFKB

Osteoprotegerin⁴

STAT^{6,7}

NFKB¹

CCL20^{6,15,18}

P38/MAPK⁸

survivin³

Bcl2⁵

Chemokines^{6,9}

Cytokines^{6,9}

IDO1^{6,7}

Src²

IFN^{6,10}

Pi3K^{1,2,6}

Defensins^{1,6}

AKT¹

P53¹³

Cyclin¹²

APC¹³

Wnt/B-catenin¹³

Bcl2^{5,11}

Myd88¹⁴

Ecsr⁶

SPP1⁶

Esm1⁶

Metalloproteinases⁶

Rho²

Cdc42²

E cadherine^{13,16}

Wnt/B-catenin¹³