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Review Paper

The two faces of *Blastocystis* spp.: Is it the cause of colorectal cancer (CRC) or a consequence of it?

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Abstract

Introduction: Over the last few years, there has been an increase in the prevalence of *Blastocystis* spp. in colorectal cancer (CRC) patients. Moreover, various in vitro and in vivo studies have highlighted that intestinal colonisation of *Blastocystis* spp. has an influence on host immune responses leading to cellular apoptosis and membrane permeability. It has been suggested that *Blastocystis* spp. is an important risk factor for the worsening of CRC.

Aim: To present evidence concerning the association between CRC and *Blastocystis* spp.

Material and methods: A review of the literature was performed by searching Science Direct, PubMed, Scopus and Google Scholar databases up to December, 2023.

Results and discussion: Out of all in vitro and in vivo studies selected for this review, the majority of them have confirmed a significantly higher prevalence of *Blastocystis* spp. in colorectal cancer patients in comparison to the control groups. Several in vitro human colorectal carcinoma cell line studies have shown significant cytopathic and immunological effects of *Blastocystis* spp. Additionally, in vivo experimental animal model studies have shown that *Blastocystis* spp. infection significantly contributed to large intestinal polyp (colorectal adenoma) formation and the progression of colorectal carcinogenesis.

Conclusions: These studies strongly support suggestions that *Blastocystis* spp. could be an important factor to existing CRC development by influencing the host immune response and increasing oxidative damage.

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1. INTRODUCTION

Colorectal cancer (CRC) is currently the main subject of numerous research studies worldwide. In 2022, its high incidence and mortality rates placed it third among new cancer cases (9.6%) and second in terms of cancer-related mortality (9.3%).[1] It is estimated that the number of new cases will increase by 63.0%, while the mortality rate will increase by 73.4% by 2040.[2] Moreover, this increase in incidence rates has been found to be more noticeable among young adults as well as in nations at the stage of economic transition.[2] Cancer is defined as an uncontrolled, malignant cell proliferation caused by accumulated genetic mutations, associated with simultaneous protooncogene activation and due to deactivated tumor-suppressor genes.[3] The colorectal cancer usually develops over many years as a result of genetic changes of the sequence and epigenetic modifications in several genes. This initial multistage process is also known as the adenoma–carcinoma sequence.[4] The trigger leading to this disorder can be very complex and multifactorial in origin, and can consequently remain elusive in many cases. The initiation of cancer has been assigned to chemical and environmental carcinogens. However, several types of disease are linked to infectious biological agents, and many of these cancers develop in tissues with a high exposure to microbiota.[5] It is highly suggested that resident microorganisms specific for a tissue or body region can increase the risk of cancer, induce cancer development by influencing inflammation, cause DNA damage and apoptosis and modulate the immune response.[6,7]

Blastocystis spp. belongs to the stramenopile group and is the most common enteric protist in humans and animals worldwide.[8,9] *Blastocystis* spp. isolates are currently classified into 18S rRNA gene 28 subtypes (STs), among which, ST1-9 and 12 have been reported in humans, but ST1–4 are the most common *Blastocystis* spp. strains.[8,10,11] Although *Blastocystis* spp. infections are, in many cases, asymptomatic or patients may generally experience mild gastrointestinal symptoms and skin lesions, there are specific groups of individuals, such as children or elderly people, as well as patients with irritable bowel syndrome who could suffer serious blastocystosis symptoms.[12–14] The pathogenicity of *Blastocystis* spp. is determined by several factors including the infecting subtype, the interaction with the intestinal microbiota and the host's immune response.[15] Some studies have suggested that *Blastocystis* spp. is a member of the commensal gut microbiota of humans and other animals and may colonize the intestinal tract for a long time without causing symptoms by establishing itself.[9,16] There are many study results strongly suggesting that *Blastocystis* spp. colonization may be closely related to an anti-inflammatory response favoring changes in the bacterial composition of the gut microbiota.[16] Moreover, *Blastocystis* spp. pathogenicity is related to differences between the subtypes, including the release of cysteine proteases.[9,17] Some subtypes, such as ST1, ST4, and ST7, have been associated with health disorders in humans and, on the other hand, ST3 has been reported as a non-pathogenic subtype.[11,14,17] Additionally, *Blastocystis* spp. modulates the release of certain inflammatory cytokines, and consequently influences the host immune response.[18] Moreover, symptomatic *Blastocystis* spp. secretes high levels of cysteine proteases that modulate the downregulation of epithelial nitric oxide formation which has antiparasitic properties as well as cause cell apoptosis and host membrane permeability.[19]

2. AIM

The aim of this review is to summarize the association between *Blastocystis* spp. and CRC. Therefore, this paper includes chosen literature to (1) determine the prevalence of *Blastocystis* spp. in CRC patients; (2) review the effects of *Blastocystis* spp. isolates on in vitro colorectal carcinoma cell line study models; and (3) describe in vivo investigations of the *Blastocystis* spp. colonization effect on the intensification of colorectal carcinogenesis in experimentally infected animals.

3. MATERIAL AND METHODS

This article reviews the original papers that reported the prevalence and association of *Blastocystis* spp. with CRC patients. In vitro studies using *Blastocystis* spp. cultures and an in vivo research using an animal model published before December 2023 were included. Articles that were mentioned without specific findings of *Blastocystis* spp. association with CRC as well as conference papers were excluded. Science Direct, PubMed, Scopus and Google Scholar were searched using the following keywords: '*Blastocystis* infection' and 'CRC' and their MESH terms and synonyms. Additionally, manual searching through reference lists of included journal articles was done to find any missed studies during the online search.

4. RESULTS AND DISCUSSION

In vitro studies on *Blastocystis* spp. have shown that solubilized antigens of protist induce the proliferation of HCT116 human colorectal carcinoma cells.[20] Furthermore, Chen et al. reported that 17.3% of patients colonized with *Blastocystis* spp. had large intestinal polyps, called colorectal adenoma.[21] As a premalignant lesion, colorectal adenoma carries with it a high risk of developing CRC. Moreover, as a precancerous abnormality these polyps have also been found in animal models experimentally infected with *Blastocystis* spp.[22,23] All of these studies combined give a strong basis to conduct more research on *Blastocystis* spp. as a possible cause of CRC.

4.1. *Blastocystis* spp. prevalence in CRC patients

Based on the reviewed and selected articles from last decade, the prevalence of *Blastocystis* spp. in CRC patients was found to be between 12.2%–80.0%. The most frequently isolated subtypes were ST1 and ST3. In research from Malaysia

Table 1. Summary of the included studies reporting prevalence of *Blastocystis* spp. in CRC patients.

References	Country	Total case, *n*	Positive case, *n*(%)	Total control, *n*	Positive control, *n*(%)
Kumarasamy et al., 2014	Malaysia	204	43(21.1)	221	22(10)
Mohamed et al., 2017	Saudi Arabia	74	22(29.7)	80	12(15)
Toychiev et al., 2018	Uzbekistan	200	160(80)	200	36(18)
Sulżyc-Bielicka et al., 2021	Poland	107	13(12.2)	124	3(2.4)
Hawash et al., 2021	Saudi Arabia	75	20(26.7)	25	2(8)
Labania et al., 2023	United Arab Emirates	52 37 COGT+15 CRC	21(40.4) 12(32.4) COGT + 9(60.0) CRC	52	9(17.3)

Comments: *COGT – cancer of gastrointestinal tract; CRC – colorectal cancer.

done by Kumarasamy et al., the overall prevalence of *Blastocystis* spp. infection was 15.3% (65 out of 425). *Blastocystis* spp. was identified in 21.1% samples (43 out of 204) from CRC patients and the infection was significantly higher compared to control individuals (10.0%; 22 cases out of 221).[24,25] The number of positive samples containing ST3 was significantly higher in the CRC patient group when compared with control patients.[24] In another study conducted in Saudi Arabia, the prevalence of *Blastocystis* spp. among CRC patients was 29.7% (22 out of 74).[26] Mohamed et al. also isolated the protist DNA in 10.0% (16 out of 64) patients suffering from other cancers outside of the gastrointestinal tract (COGT), as well as in 15.0% (12 out of 80) non-cancer (NC) patients as a control group. *Blastocystis* spp. subtypes obtained from cancer patients were identified as having predominantly ST1 (54.5%) among CRC patients, ST2 (43.7%) among COGT patients, and less frequently ST5.[26] In a study reported by Toychiev et al., the frequency of *Blastocystis* spp. in CRC patients at various stages of cancer was almost four times higher than that found in the control group, in 80.0% (160 out of 200) and in 18.0% (36 out of 200), respectively.[27] Moreover, high-intensity infections were observed only in patients with CRC. The researchers found a significantly higher occurrence and intensity of the infection in patients with metastases in comparison with patients without metastases. Interestingly, the total number of CRC patients with *Blastocystis* spp. after surgery and chemotherapy was unchanged (75.0%) and differed significantly from this estimation in the control group (18.0%). The number of patients after chemotherapy with high-intensity infections (26.6%) was also stable. According to these findings, the authors strongly suggested that *Blastocystis* spp. might be associated with CRC pathogenesis.[27] Another study in Poland by Sulżyc-Bielicka et al. (2021) showed the lowest prevalence of *Blastocystis* spp. in patients with CRC when compared to other countries.[28] The prevalence of protist was five times higher in CRC patients than in the control group (12.4% and 2.5%, respectively). The predominant subtype in the CRC patient group was ST3, followed by ST1 and ST2.[28] The study from Saudi Arabia was designed to investigate the emergence of *Blastocystis* spp. infections in patients with various stages of CRC. This study found that 26.6% (20 out of 75) CRC patients were *Blastocystis* spp. positive and there was a significantly higher prevalence when compared to the healthy control group of non-CRC patients (8.0%; 2 out of 25).[29] Additionally, the study showed that *Blastocystis* spp. increases inflammatory cell infiltration and proinflammatory cytokines (tumor necrosis factor α) in CRC patients, especially in the higher stages of cancer, in comparison to the control group.[29] In more recent research carried out by Labania et al. in 2023 on cancer patients ($n = 52$) and NC participants ($n = 52$) also showed an association between *Blastocystis* spp. infection and CRC.[30] The cancer group was divided into a CRC ($n = 15$) group and a group with cancers outside the gastrointestinal tract (COGT; n=37). Also, this study revealed a significantly higher occurrence of *Blastocystis* spp. among CRC patients (60.0%) and an insignificant prevalence in COGT patients (32.4%) compared to NC individuals (17.3%) as a control group. The most common subtypes in the cancer groups, especially CRC individuals and in the NC group, were ST2 and ST3, respectively.[30] Summary of the included studies reporting prevalence of *Blastocystis* spp. in CRC patients is presented in Table 1. It is worth mentioning the unusual case of an invasive *Blastocystis* spp. infection presented by Janarthanan et al. in the United States.[31] During colonoscopy of the patient with severe symptoms, large ulcers in the colon with normal surrounding mucosa and multiple small shallow ulcers in the rectum were detected. Exudates with necrosis and pieces of colonic mucosa with severe acute and chronic inflammation were shown in the biopsy material. Additionally, focal acute cryptitis was revealed and, interestingly, several vacuolated and amoeboid cells were later identified as *Blastocystis hominis*.[31] *Blastocystis* spp. may cause, directly or indirectly, an inflammation in the intestine. Chronic inflammation may lead to the precancerous changes. As the researchers found the protist in the inflamed colonic crypts this may be a confirmation of *Blastocystis* spp. association with CRC.

4.2. Studies on experimentally infected animals

An animal model study conducted by Kumarasamy et al. showed evidence that *Blastocystis* spp. may significantly intensify Azoxymethane (AOM)-induced carcinogenesis by causing damage to the intestinal epithelium and encourage oxidative damage in rats experimentally infected with *Blastocystis* spp.[32] The authors found that the co-administration of *Blastocystis* spp. caused an increase in the number of hyperplastic aberrant colonic crypts compared to the control rats treated with the chemical carcinogen-AOM only. Histological changes of the intestinal mucosa, major dysplasia

as well as high levels of urinary oxidative indices were observed in AOM rats infected with *Blastocystis* spp. compared to the uninfected AOM-rats; and in two of them adenomas developed.[32] In another study, Ahmed et al. investigated the pathology induced in the gut of laboratory mice inoculated with *Blastocystis* spp. isolates obtained from symptomatic and asymptomatic patients CRC i non-CRC patients.[23] Histopathological examination of intestinal sections of all inoculated animals showed inflammatory cell infiltration and pathological changes to varying degrees. Vacuolar forms of *Blastocystis* spp. infiltrating the submucosa as well as polypoid formation were seen in sections from mice infected with *Blastocystis* spp. from CRC patients and from non-CRC symptomatic individuals.[23] Hussein et al. discovered a potential relationship between ST1 and ST3 intrasubtype infections causing chronic inflammation and precancerous polyps during immunosurveillance.[22] They evaluated the mucosal immune responses of the infected rat colons by symptomatic *Blastocystis* spp. isolates. Precancerous polyps were detected in 40.5% of the colons. The intrasubtypes of ST3 constituted 23.9% of these cases, followed by ST1, which showed polyps in 14.7% of cases, and ST4, which was less related to the colon abnormalities at – 1.9%.[22] Moreover, a mucin synthesized by goblet cells known as MUC2 was a polyp inducer in 38.9% of the infected colons. The low levels of MUC2 and goblet cell counts lead to a higher cell proliferation and a signifcant decrease in apoptosis then what is commonly associated with a precancerous colon.[33] High interepithelial lymphocyte (IEL) counts and high numbers of mast cells lead to a proinfammatory response which was signifcantly associated with polyp formation through excessive cytotoxicity that induces epithelial cell damage, particularly with ST1 and wild ST3.[34] Furthermore, the level of fecal secretory immune globulin A (sIgA) was low in polyp-inducing STs, which confirms the suggestions of a degradation effect of proteases secreted from invading *Blastocystis* spp. (Figure 1).[35]

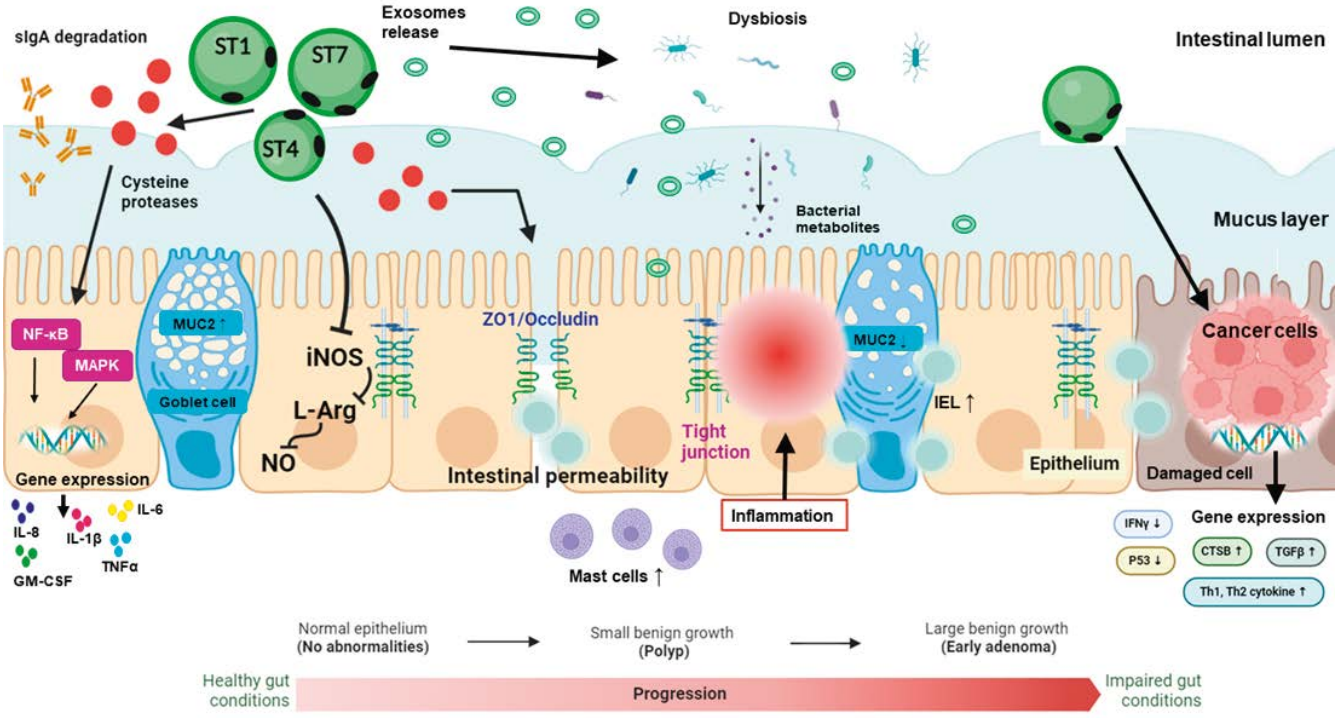

Figure 1. Direct and indirect simplified mechanisms of *Blastocystis* spp. association with colorectal cancer formation. Cysteine proteases produced by symptomatic *Blastocystis* spp. (STs1, 4, 7) are able to degrade sIgA, modulate the downregulation of epithelial nitric oxide formation, cause host membrane permeability. The exosomes released by protist are related to the gut microbiota dysbiosis and bacterial metabolites may increase the risk, or cause, an inflammation. *Blastocystis* spp. can influence the gene expression of proinflammatory cytokines by regulating the NF-κB and MAPK pathways. The low levels of MUC2 and goblet cell counts lead to a higher cell proliferation. High IEL counts and high numbers of mast cells lead to a proinfammatory response which was significantly associated with epithelial cell damage. As the cancer cells appear, *Blastocystis* spp. antigen causes the upregulation of T helper (Th)2 and Th1 cytokine gene expression as well as CTSB. *Blastocystis* spp. highly stimulates TGF-β gene expression and downregulates IFNγ and p53 gene expression in cancer cells. Comments: ST – *Blastocystis* subtype; sIgA – fecal secretory immunoglobulin A; NO – nitric oxide; NF-κB – nuclear factor κB; MAPK – mitogen-activated protein kinase; MUC2 – mucine; IEL – interepithelial lymphocyte; Th – T helper; TGF-β – transforming growth factor β; IFNγ – interferon γ; p53 – tumor protein P53; CTSB – cathepsin B; IL – interleukin; GM-CSF – granulocyte-macrophage colony-stimulating factor; TNFα – tumor necrosis factor alpha; iNOS – inducible nitric oxide synthase; L-Arg – L-Arginine; ZO1 – zonula occludens-1.

4.3. In vitro studies on colorectal carcinoma cell lines and *Blastocystis* spp.

The most reliable in vitro studies were aimed at observing the cytopathic and immunological effects induced by the *Blastocystis* spp. solubilised antigen on the human CRC cell line HCT116.[20,36,37] The authors speculated that *Blastocystis* spp. infection may have a supportive influence on the proliferation, invasiveness and metastatic properties of CRC cells. All of the studies findings showed increased cell proliferations in HCT116 cell-lines stimulated by *Blastocystis* spp. antigen.[20,36,37] One of the studies reported the cytopathic effect of *Blastocystis* spp. antigen on peripheral blood mononuclear cells.[20] Another in vitro investigation showed that the *Blastocystis* spp. antigen caused the upregulation of Th2 and Th1 cytokine gene expression.[36,37] In addition, *Blastocystis* spp. antigen highly stimulated transforming growth factor beta (TGF-β) gene expression and downregulated interferon γ and p53 gene expression in HCT116 cells.[37] The exposure of investigated cell-lines to *Blastocystis* spp. antigen caused a significant upregulation of cathepsin B (CTSB).[36,37] The colon cancer cell proliferation under the influence of *Blastocystis* spp. antigen could be a result of higher levels of interleukin (IL)-6 and IL-8 expression (Figure 1).[36,37] In addition, *Blastocystis* spp. isolated from symptomatic individuals caused a more extensive inflammatory reaction as well as more enhanced proliferation of cancer cells, therefore, it is assumed to be more pathogenic as compared to asymptomatic isolates.[36] One article investigated some phenotypic characteristics – the surface ultrastructure, protein profiles and protease activity of *Blastocystis* spp. isolated from CRC patients, as well as symptomatic and asymptomatic non-CRC patients infected with the protist. This study demonstrated the presence of two protein bands of 230 KDa and 32 KDa in almost 42.9% of *Blastocystis* spp. CRC isolates, while in non-CRC isolates, there was a complete absence.[38] Several in vivo as well as in vitro studies have provided supportive data that *Blastocystis* spp. infections result in tissue damage that may lead to colorectal adenoma as a precursor lesion to colorectal adenocarcinoma.

5. CONCLUSIONS

(1) CRC ranks third in incidence and second in mortality among all cancers globally. The prevalence of *Blastocystis* spp. in CRC patients were found to be significantly higher compared to control individuals.
(2) Among *Blastocystis* spp. isolates there are some truly pathogenic subtypes, while others may be considered commensals. The pathogenicity of *Blastocystis* spp. is determined by the infecting subtype, the interactions with the intestinal microbiota and the host's immune response.
(3) Cysteine proteases secreted by the protist are involved in the disruption of the intestinal epithelial monolayer, which increase membrane permability.
(4) According to experimental evidence, *Blastocystis* spp. induces the proliferation of HCT116 human colorectal carcinoma cells, and is involved in large intestinal polyp (colorectal adenoma) formation causing a high risk of CRC development.
(5) Substantial precancerous abnormalities have been found in animal models experimentally infected with *Blastocystis* spp., which could induce carcinogenesis or exacerbate existing CRC via an alteration of the host immune response and increased oxidative damage.

Conflict of interest

Authors declare no conflict of interest.

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