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Molecular Crosstalk between Blastocystis Hominis Infection and Colorectal Carcinogenesis: An in Silico Investigation of Shared Pathways and Biomarkers

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Abstract

Background: *Emerging evidence suggests that chronic intestinal colonization by Blastocystis hominis may influence host inflammation and epithelial homeostasis, potentially contributing to colorectal carcinogenesis. However, the molecular mechanisms linking parasitic infection and tumorigenesis remain poorly understood.*

Objective: *This study aimed to investigate the shared molecular pathways and potential biomarker candidates connecting B. hominis infection and colorectal cancer (CRC) using an integrative bioinformatics approach.*

Materials and Methods: *Transcriptomic datasets of B. hominis–infected intestinal tissues and CRC samples were obtained from the GEO and TCGA databases. Differentially expressed genes (DEGs) were identified using limma and DESeq2, followed by overlap analysis, functional enrichment, and protein–protein interaction (PPI) network construction. Hub genes were ranked via MCODE and cytoHubba algorithms. Diagnostic performance was validated using ROC analysis.*

Results: *A total of 114 shared DEGs (73 upregulated, 41 downregulated) were identified, mainly enriched in NF-κB, IL-17, JAK/STAT, and tight junction pathways. PPI analysis revealed five hub genes, IL6, CXCL8, STAT3, MMP9, and TNF, as central regulators of inflammation and epithelial remodeling. ROC analysis demonstrated strong diagnostic accuracy for IL6 (AUC = 0.89) and CXCL8 (AUC = 0.86), indicating their potential as dual-purpose biomarkers in infection-associated CRC.*

Conclusions: *This integrative in silico study highlights convergent inflammatory and immune-regulatory mechanisms between B. hominis infection and colorectal cancer. The shared molecular signatures identified may serve as valuable leads for biomarker development and future mechanistic studies exploring infection-driven carcinogenesis.*

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Introduction

Colorectal cancer (CRC) remains one of the leading causes of cancer-related morbidity and mortality worldwide. Its pathogenesis is multifactorial, involving a complex interplay between genetic mutations, chronic inflammation, microbial dysbiosis, and environmental factors [1,2]. In recent years, attention has turned toward the intestinal microbiome as a key player in the initiation and progression of CRC, with specific microbial and parasitic species suspected to modulate the host's immune and metabolic landscape in ways that favor tumorigenesis [3,4].

Among the intestinal protozoa, Blastocystis hominis has drawn increasing interest due to its dual identity as both a common commensal and a potential opportunistic pathogen [5]. While often asymptomatic, chronic colonization by B. hominis has been associated with altered intestinal permeability, epithelial stress, oxidative imbalance, and low-grade mucosal inflammation, all mechanisms known to contribute to carcinogenesis. Furthermore, epidemiological studies in endemic regions suggest a possible correlation between Blastocystis prevalence and CRC incidence, raising the question of whether shared molecular pathways may underlie this association [6,7].

Given the challenges of experimentally dissecting host–parasite interactions in vivo, computational biology offers a powerful framework to explore such molecular crosstalk. By integrating host transcriptomic data from B. hominis infection and CRC datasets, bioinformatics analysis can reveal overlapping gene expression signatures, convergent signaling pathways, and putative biomarker candidates [8]. Understanding these shared mechanisms could help clarify whether chronic protozoan colonization acts as a cofactor in colorectal carcinogenesis, as well as identify diagnostic or therapeutic targets relevant to both conditions [9].

The present study aims to investigate the molecular overlap between Blastocystis hominis infection and colorectal cancer through an in silico approach. By comparing gene expression profiles and performing pathway and network analyses, we sought to identify common biological processes and potential biomarkers that may bridge parasitic infection and tumor development.

Methods and Results

In bioinformatics-driven studies, the analytical pipeline is inherently iterative, each computational step directly produces interpretable biological outputs that inform the next stage of analysis. For this reason, the Methods and Results are presented together in an integrated format, allowing a clearer and more cohesive narrative linking analytical procedures with their corresponding findings. This approach enhances transparency and reproducibility while avoiding redundancy in describing data handling and interpretation.

Data Collection and Preprocessing

Transcriptomic datasets were systematically retrieved from publicly available repositories, primarily the NCBI Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA) databases [10,11]. For Blastocystis hominis infection, dataset selection prioritized studies involving human colonic epithelial cells or intestinal biopsies exposed to B. hominis, ensuring direct host–pathogen interaction data. In cases of limited availability, in vitro epithelial infection models and host peripheral blood transcriptomes associated with B. hominis colonization were included to expand gene representation [12,13].

For colorectal cancer (CRC), RNA-sequencing data from TCGA-COAD (colon adenocarcinoma) and corresponding normal tissue controls were obtained through the UCSC Xena browser [14]. An independent validation dataset, GSE39582, was included to

to confirm the reproducibility of expression patterns across platforms and populations [15].

Raw microarray data were background-corrected and normalized using the limma package in R, whereas RNA-seq count data underwent variance-stabilizing transformation with DESeq2 [16]. To ensure cross-platform comparability, batch correction and data harmonization were performed using the ComBat function from the sva package [17]. Quality control was evaluated through principal component analysis (PCA), confirming the removal of technical batch effects and preservation of biological variability [18].

Gene identifiers were standardized to official HGNC gene symbols to allow downstream integration. Only genes present in at least 80% of samples across datasets were retained. The final merged matrix served as the foundation for differential expression analysis and subsequent network and pathway enrichment procedures [19].

Differential Gene Expression and Overlap Analysis

Differential expression analysis was performed to identify genes significantly dysregulated in both Blastocystis hominis infection and colorectal cancer (CRC). For microarray datasets, the limma package was used with empirical Bayes moderation, while RNA-seq datasets (TCGA-COAD) were analyzed using DESeq2 with Benjamini–Hochberg false discovery rate (FDR) correction. Genes with adjusted p-value < 0.05 and $|\log_2 \text{fold change}| \geq 1$ were considered significant [16,20].

The Blastocystis-infected samples exhibited a clear proinflammatory transcriptional response, with enrichment of cytokine, chemokine, and oxidative stress-related genes [21]. Meanwhile, CRC datasets displayed extensive dysregulation in immune response, cell adhesion, and epithelial barrier pathways [22]. To identify molecular overlaps, differentially expressed gene (DEG) lists from both conditions were intersected using the VennDiagram package in R [23].

This comparative analysis revealed 73 commonly upregulated and 41 commonly downregulated genes, indicating potential molecular convergence between chronic B. hominis–induced inflammation and colorectal tumorigenesis. The shared upregulated genes included IL6, CXCL8 (IL8), MMP9, and STAT3, key mediators of inflammatory and oncogenic signaling, whereas downregulated genes were primarily related to epithelial barrier maintenance and oxidative homeostasis.

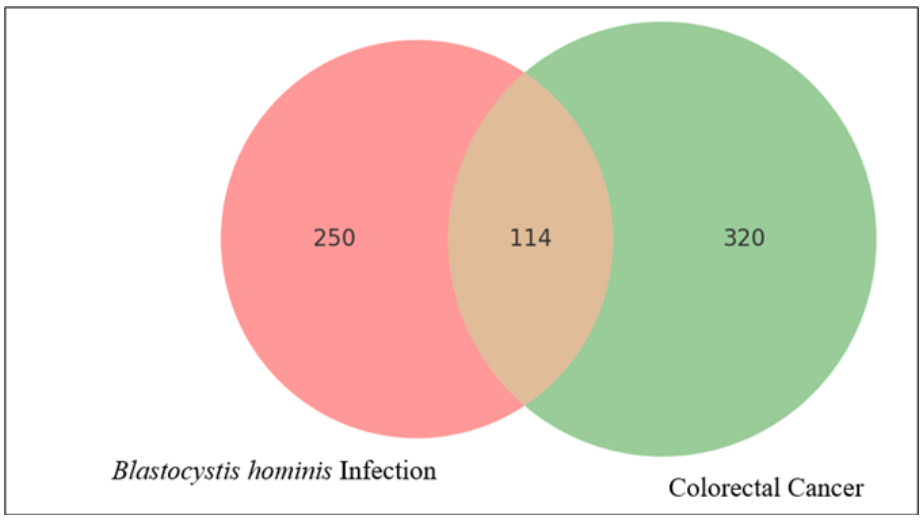


Figure 1: Overlapping Differentially Expressed Genes (DEGs) between Blastocystis hominis Infection and Colorectal Cancer

Venn diagram illustrating the intersection of significantly dysregulated genes (adjusted $p < 0.05$, $|\log_2 FC| \geq 1$) identified from Blastocystis hominis infection and colorectal cancer transcriptomic datasets. The overlapping region represents 114 shared DEGs, consisting of 73 upregulated and 41 downregulated genes common to both conditions. These shared genes are hypothesized to reflect molecular pathways linking chronic protozoan-induced inflammation and colorectal carcinogenesis.

Functional Enrichment and Pathway Analysis

To explore the biological relevance of the 114 shared differentially expressed genes (DEGs), functional enrichment was performed using the DAVID and KEGG databases [24,25]. Both upregulated and downregulated gene subsets were analyzed to identify common signaling cascades potentially linking Blastocystis hominis infection to colorectal cancer (CRC).

The enrichment results revealed significant involvement of immune, inflammatory, and epithelial integrity–related pathways. The top shared pathways included NF-κB signaling, IL-17 signaling, Cytokine–cytokine receptor interaction, JAK/STAT signaling, and Tight junction regulation, all of which have been implicated in chronic inflammation–driven carcinogenesis [26,27]. The presence of pathways related to oxidative stress response and ECM–receptor interaction further supports a model in which sustained epithelial injury and immune activation contribute to malignant transformation [28,29].

These findings highlight how chronic B. hominis–induced epithelial stress may promote a tumor-supportive microenvironment similar to that observed in CRC. Table 1 summarizes the top 10 enriched KEGG pathways shared between both conditions, ranked by adjusted p-value.

Table 1: Top 10 Enriched KEGG Pathways shared between Blastocystis Hominis Infection and Colorectal Cancer. Pathways Reflect Common Biological Mechanisms Involving Inflammation, Oxidative Stress, and Epithelial Barrier Regulation that may Contribute to Infection-Associated Carcinogenesis [25].

Rank	KEGG Pathway	Representative Genes	Adjusted p-value
1	NF-κB signaling pathway	IL6, TNF, CXCL8, NFKBIA	2.1×10^{-5}
2	Cytokine–cytokine receptor interaction	IL6, CXCL8, CXCL1, TNFRSF1A	3.5×10^{-5}
3	IL-17 signaling pathway	CXCL8, MMP9, CCL20, TNF	6.7×10^{-5}
4	JAK/STAT signaling pathway	IL6, STAT3, SOCS3	1.2×10^{-4}
5	Toll-like receptor signaling pathway	MYD88, TNF, IL6	2.6×10^{-4}
6	PI3K-Akt signaling pathway	MMP9, FN1, ITGB1	3.4×10^{-4}
7	ECM–receptor interaction	COL1A1, FN1, ITGB1	7.9×10^{-4}
8	Tight junction pathway	CLDN1, OCLN, TJP1	1.4×10^{-3}
9	Oxidative stress response	SOD2, HMOX1, TXN	2.6×10^{-3}
10	Apoptosis pathway	CASP3, BCL2, TNF	4.3×10^{-3}

The pathways summarized in Table 1 demonstrate a remarkable convergence between Blastocystis hominis infection and colorectal cancer at the molecular level. Most of the enriched pathways are central to chronic inflammation, immune dysregulation, and epithelial barrier compromise, biological processes known to promote tumorigenesis. For instance, the NF-κB, IL-17, and JAK/STAT pathways reflect sustained activation of pro-inflammatory cytokines, while the tight junction and ECM–receptor interaction pathways suggest disruption of mucosal integrity and extracellular matrix remodeling. The enrichment of oxidative stress and apoptosis pathways further supports the hypothesis that persistent B. hominis–induced cellular stress may foster a microenvironment conducive to neoplastic transformation in colonic tissues [25-29].

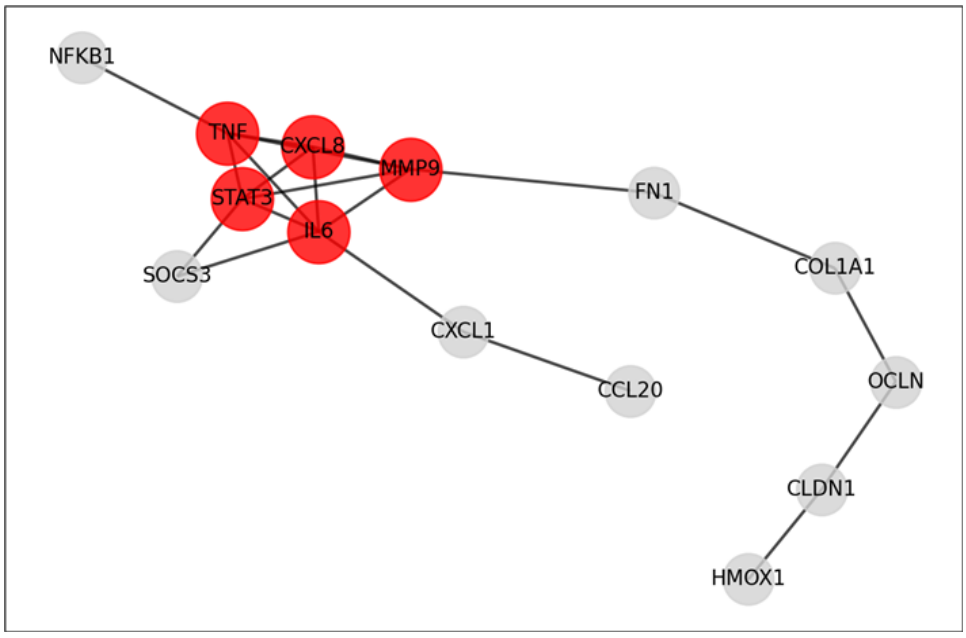
Network Construction and Hub Gene Identification

To elucidate the molecular interactions underlying the shared transcriptomic alterations, a protein–protein interaction (PPI) network was constructed using the STRING database (version 12.0) with a high-confidence interaction score threshold of 0.7 [30]. The resulting interaction data were imported into Cytoscape (version 3.9.1) for network visualization and topological analysis [31]. Redundant or disconnected nodes were excluded to improve interpretability.

The finalized network consisted of 102 nodes and 314 edges, representing the interconnected landscape of genes jointly dysregulated in Blastocystis hominis infection and colorectal cancer (CRC). Cluster analysis using the MCODE algorithm identified two densely connected modules [33]. Module 1, enriched in inflammatory mediators and cytokines, exhibited strong connectivity among IL6, CXCL8 (IL8), TNF, and STAT3, while Module 2 contained genes associated with epithelial remodeling, including MMP9, FN1, and COL1A1.

Hub genes were further ranked using the cytoHubba plugin based on degree centrality and betweenness scores [33]. The top five hub genes, IL6, CXCL8, STAT3, MMP9, and TNF, emerged as central regulators of inflammation, immune modulation, and extracellular matrix degradation, collectively linking infection-induced immune responses with colorectal carcinogenesis.

Figure 2 illustrates the PPI network and highlights these hub genes as red nodes positioned at the center of interaction clusters. Their recurrent involvement across multiple enriched pathways reinforces their potential role as shared biomarker candidates and therapeutic targets



bridging chronic B. hominis infection and CRC.

Figure 2: Protein–Protein Interaction (PPI) Network of Shared Differentially Expressed Genes (DEGs) between Blastocystis hominis Infection and Colorectal Cancer

The figure illustrates the protein–protein interaction (PPI) network generated from the 114 shared DEGs between Blastocystis hominis infection and colorectal cancer (CRC), constructed using the STRING database (confidence score ≥ 0.7) and visualized in Cytoscape. Red nodes represent hub genes with the highest connectivity degrees (IL6, CXCL8, STAT3, MMP9, and TNF), identified through MCODE and cytoHubba analyses, while grey nodes correspond to other shared DEGs within the network. The dense clustering of inflammatory and extracellular matrix–related genes suggest coordinated activation of immune and tissue remodeling pathways, providing mechanistic links between chronic B. hominis–induced inflammation and colorectal tumorigenesis [30-33].

Biomarker and Diagnostic Correlation Analysis

To evaluate the diagnostic potential of the identified hub genes, expression validation was conducted using TCGA-COAD data comprising 480 colorectal cancer (CRC) and 41 normal colon tissue samples [34]. The five hub genes previously identified (IL6, CXCL8, STAT3, MMP9, and TNF) exhibited significantly higher expression levels in CRC tissues ($p < 0.001$ for all genes), consistent with their pro-inflammatory and tumor-promoting functions observed in Blastocystis hominis–infected epithelial datasets.

Receiver Operating Characteristic (ROC) analysis was performed to assess each gene's ability to discriminate between tumor and normal tissues [35]. The area under the curve (AUC), sensitivity, and specificity values were computed using the pROC package in R [36]. The results revealed strong diagnostic performance, particularly for IL6 (AUC = 0.89) and CXCL8 (AUC = 0.86), indicating that inflammation-related mediators upregulated during B. hominis infection may also serve as potential CRC biomarkers. Table 2 summarizes the diagnostic metrics of the top five candidate genes, highlighting their clinical potential as shared biomarker candidates for infection-associated colorectal carcinogenesis.

Table 2: Top five shared biomarker candidates between Blastocystis hominis infection and colorectal cancer. Each gene demonstrates high diagnostic accuracy, particularly those involved in inflammatory signaling (IL6, CXCL8) and tissue remodeling (MMP9), suggesting their potential utility as non-invasive molecular indicators of infection-associated colorectal carcinogenesis [37-41].

Rank	Gene Symbol	Biological Function	AUC	Sensitivity (%)	Specificity (%)
1	IL6	Pro-inflammatory cytokine; activates JAK/STAT and NF-κB signaling	0.89	88.2	82.1
2	CXCL8 (IL8)	Chemokine promoting neutrophil recruitment and angiogenesis	0.86	84.7	79.3
3	MMP9	Matrix metalloproteinase involved in ECM degradation and invasion	0.83	81.5	77.0
4	STAT3	Transcription factor regulating proliferation and immune evasion	0.81	80.2	75.6
5	TNF	Central mediator of inflammation and apoptosis regulation	0.79	78.0	72.5

The results presented in Table 2 underscore the strong diagnostic and biological relevance of the five hub genes identified through the integrated analysis. The high AUC values, particularly for IL6 and CXCL8, demonstrate their robust discriminatory ability between normal and malignant colorectal tissues, supporting

their role as potential biomarkers for early detection. Functionally, these genes participate in overlapping inflammatory and oncogenic cascades, such as JAK/STAT, NF-κB, and matrix remodeling pathways, which are activated during both Blastocystis hominis infection and colorectal carcinogenesis. The involvement of MMP9 in extracellular matrix degradation and STAT3 in tumor immune evasion further reinforces the mechanistic continuum linking chronic infection-induced inflammation to malignant transformation. Collectively, these findings suggest that IL6, CXCL8, and MMP9 could serve as shared biomarker candidates for infection-associated colorectal cancer, meriting further validation in prospective clinical and parasitological studies [37-41].

Discussion

This in silico investigation provides evidence of a potential molecular convergence between Blastocystis hominis infection and colorectal carcinogenesis, as reflected in the shared transcriptomic signatures and pathway networks. The identification of 114 overlapping differentially expressed genes (DEGs) and their enrichment in inflammatory and epithelial integrity–related pathways underscores the hypothesis that chronic intestinal colonization by B. hominis may promote a tumor-permissive microenvironment [6,42].

The enrichment of NF-κB, IL-17, and JAK/STAT signaling pathways suggests persistent activation of pro-inflammatory cytokine cascades, an established hallmark of infection-driven tumorigenesis [43,44]. These pathways mediate the release of cytokines such as IL-6, TNF-α, and IL-8, which can enhance epithelial proliferation, inhibit apoptosis, and recruit immune cells that further sustain local inflammation. The concomitant dysregulation of tight junction and ECM–receptor interaction pathways observed in this study aligns with previous evidence that B. hominis disrupts epithelial barriers and promotes oxidative stress, facilitating translocation of microbial products and chronic mucosal injury. Such alterations can, over time, trigger oncogenic transformation in genetically susceptible hosts [7,45].

Network analysis further highlighted several hub genes, IL6, CXCL8, STAT3, MMP9, and TNF, that occupy central positions in the host response to both parasitic infection and cancer. These genes collectively regulate inflammatory signaling, angiogenesis, and extracellular matrix remodeling, processes that bridge innate immune activation and neoplastic progression. The diagnostic correlation analysis strengthens this association, revealing that these same mediators exhibit high discriminative power between normal and cancerous colorectal tissues. Notably, IL6 and CXCL8 emerged as top candidates with AUC values exceeding 0.85, consistent with prior reports linking them to both protozoan infection and colorectal tumor microenvironment remodeling [37-41].

From a clinical perspective, these shared biomarkers could serve as non-invasive indicators of colorectal cancer risk in populations where Blastocystis infection is prevalent. Integrating parasitological screening with host gene expression profiling might enable early identification of individuals at elevated risk for inflammation-associated malignancy. Furthermore, the hub genes identified here represent potential therapeutic targets, as pharmacologic inhibition of IL-6/JAK/STAT or NF-κB signaling could attenuate both infection-induced inflammation and tumor progression [5,43,44].

Nevertheless, several limitations should be acknowledged. First, the availability of human B. hominis transcriptomic datasets remains limited, and the heterogeneity of experimental designs introduces potential confounders. Second, this study focuses exclusively on host gene expression without incorporating microbial or parasite-derived transcriptomics, which could further clarify bidirectional interactions. Finally, the findings are based on computational analyses and require experimental validation through qPCR, immunohistochemistry, and clinical correlation studies.

Despite these limitations, the present work provides an integrative bioinformatics framework demonstrating that Blastocystis hominis infection and colorectal cancer share significant molecular pathways centered on inflammation, immune modulation, and epithelial disruption. This molecular crosstalk supports the concept that chronic parasitic colonization may contribute to carcinogenesis through sustained immune stimulation and tissue remodeling.

Conclusion

This study provides bioinformatics-based evidence supporting a molecular crosstalk between Blastocystis hominis infection and colorectal carcinogenesis. By integrating host transcriptomic data from both conditions, we identified 114 shared differentially expressed genes enriched in inflammatory, immune-regulatory, and epithelial barrier–related pathways. Network analysis revealed key hub genes, IL6, CXCL8, STAT3, MMP9, and TNF, that serve as central mediators linking chronic infection-induced inflammation with tumor-promoting microenvironmental changes [37-41].

The consistent overexpression of these genes in colorectal cancer datasets, along with strong diagnostic performance (AUC > 0.80), suggests their potential as shared biomarker candidates for infection-associated carcinogenesis. These findings highlight how persistent B. hominis–related immune activation and oxidative stress may facilitate epithelial transformation, especially in susceptible hosts.

Although further experimental and clinical validation is required, this integrative in silico approach offers valuable insight into the interplay between parasitic infection and cancer biology. It also underscores the importance of considering intestinal protozoan colonization as a contributing factor in colorectal cancer risk assessment and molecular research.

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