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REVIEW OPEN ACCESS

# How Do Host MicroRNAs Impact the Gut Epithelial Barrier During Inflammatory Bowel Disease?

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**Abstract**

**Introduction:** Inflammatory bowel disease (IBD) is a condition characterised by chronic inflammation of the gastrointestinal tract, a dysfunctional immune system and dysbiosis. Very little research explains the mechanisms underpinning the pathogenesis of IBD, but many studies have looked at the role of microRNAs and their impact on the epithelial barrier in which they increase intestinal permeability leading to an increase in inflammatory development in the barrier. The aim is to investigate the role of microRNAs on proteins that make up the tight junctions of the epithelial barrier.

**Methods:** This literature review will examine a range of review and primary studies and summarise how the microRNAs contribute to the degradation of the occludin protein, damaging of the epithelial gut barrier and the pathogenesis of inflammatory bowel disease.

**Results:** The author expects to have a thorough understanding of the effect of microRNA on occludin and how their relationship leads to the pathogenesis of IBD.

**Discussion:** Different primary studies examined different types of microRNAs and tested their effect on the occludin protein in the epithelial barrier, many of which caused its degradation and resulted in intestinal permeability.

**Conclusion:** Intestinal permeability can potentially lead to intestinal inflammation and the pathogenesis of inflammatory bowel disease. Future research can examine how microRNAs can affect other aspects of the intestine such as the microbiome and also look into potential therapeutic agents that can alleviate the symptoms or cure inflammatory bowel disease.

**Keywords:** microRNA; IBD; occludin; tight junctions; gastrointestinal tract

## Introduction

The gastrointestinal (GI) system plays a critical role in digestion, absorption, and regulating immunity for the human body. Its many components and features allow it to regulate movement of substances including ions, nutrients, water, and solute across the intestinal epithelium [1]. Equipped with a brush border, crypt, villi, and plasma membrane structure, each epithelial cell is joined together side-by-side by tight junctions and adherens junction proteins essential to the function of the epithelium [1]. Additionally, the GI tract creates an environment for over a 100 trillion commensal bacteria [2]. Many immune complexes have co-evolved with microbiota to protect the body against infection through producing pro- and anti-inflammatory cytokines and chemokines [2]. The GI tract and its many components contribute to its complexity and functioning to allow regulation of nutrients, waste, and immunity.

Dysregulated interactions between the microbiota and the immune system can bring about complex immune-mediated diseases, especially for individuals who are genetically susceptible [2]. These include inflammatory bowel disease (IBD), other autoimmune and metabolic diseases, and even cancer. IBD encompasses both Crohn's disease (CD) and ulcerative colitis (UC), disorders of the GI tract characterised by recurrent inflammation with disturbances in the gut microbiome and the breakdown of the tightly regulated intestinal barrier [2]. Although they're both characterised as IBD, UC affects only the colon and CD can affect anywhere between the mouth and the anus. Characteristic to IBD are microbiome disturbances that result in reduced bacterial diversity and an imbalance of certain types of commensal bacteria that contribute to a healthy metabolic profile [2].

### Tight Junctions

Tight junctions (TJs) are adhesion complexes that control the paracellular movement of ions, solutes, and water between the luminal (inside) and interstitial (outside) spaces of the small intestine [3]. The integrity of TJs assist with strengthening connections between adjacent cell membranes and other transmembrane and peripheral proteins [3]. Many intracellular signal transduction pathways such as protein kinase C, G, A, as well as MAPK

signalling contribute to the tight regulation of TJ barrier function [3]. Occludin, a type of TJ protein, is highly expressed at cell-to-cell junctions and contributes to the maintenance of TJs [3]. Claudin, composed of four transmembrane domains and two extracellular loops, is another TJ protein with 23 integral membrane proteins in its family [3]. Its extracellular loops contribute to interacting with adjacent cells to either form barriers against or pores for a selective group of molecules [3]. Lastly, zonula occludens (ZO), among the first TJ proteins to be discovered, can connect occludin and claudin to the actin cytoskeleton [3]. This review will focus on the effect of occludin regulation on TJ dysfunction and the pathogenesis of IBD.

In order to maintain its localization in the membrane, occludin needs to be phosphorylated at its serine and threonine residues otherwise, functioning in the cytoplasm of the intestinal epithelial cell ceases to occur [3]. As a result, phosphorylated occludin regulates TJ stability and permeability while also preventing damage to the TJ [3]. Contrastingly, inflammatory conditions can affect the expression of occludin and even downregulate it [4]. Using immunofluorescence labelling and confocal microscopy, a study was performed to see how TJ proteins, occludin and zona occludens-1 would be affected in the mucosal lining in control and UC patients [4]. Researchers found that mucosal tissue from patients with active UC had downregulated levels of occludin, according to the immunofluorescence labelling [4]. Furthermore, occludin expression in colon tissue of CD patients were similar to the expression of UC patients. Interestingly, occludin loss was specific to both types of IBD and not other intestinal disorders such as lymphocytic colitis [4]. Occludin proteins play a significant role in tight junction regulation and any damage that affects it could result in the pathogenesis of intestinal diseases.

MicroRNA and IBD

MicroRNAs are non-coding RNAs that control and regulate post-transcription and can influence the expression of TJ proteins such as occludin. Its biogenesis occurs in the nucleus through gene transcription by type II RNA polymerase [5]. The product is primary miRNA, which is later cleaved by endoribonucleases and becomes precursor miRNA [5]. The intermediate miRNA is transported to the cytoplasm where it is cleaved at its loop end by the Dicer complex, finally becoming mature miRNA and also forming a complex called miRNA-induced silencing complex [5]. A number of studies suggest that miRNAs play a critical role in regulating mRNAs post-transcriptionally. They bind to the 3' untranslated regions of mRNAs and can either inhibit or promote its degradation, silencing the gene [6].

MicroRNA in relation to IBD and tight junctions has been a topic of interest, with focus on microRNA-21 (miR-21). A number of studies have identified its upregulation in UC and CD patients, but its mechanism of action differs for both types of IBD. Yan et. al suggested that microRNA-21 levels are higher in UC and CD patients compared to non-IBD patients, but can only be used as a potential diagnostic marker in UC patients [7]. Targeting the microRNA-21 for therapeutic purposes resulted in protection against tissue injury and reduced inflammation, specifically when deleting it [7]. MicroRNA-21 is also said to be controversial because on one hand, it was reported to increase occludin expression resulting in intestinal TJ barrier enhancement [7]. Contrastingly, many studies have also shown the detrimental effect of the overexpression of microRNA-21on TJs such as occludin, claudin, and zona occludens, while also increasing intestinal permeability [7].

One such study investigated the effect of miRNA-122a on regulating occludin mRNA with TNF-α treatment. TNF-α is a proinflammatory cytokine that mediates inflammation in the gut and increases intestinal TJ permeability [8]. In regards to UC and CD, there are marked elevated levels of TNF-α leading to worsening inflammatory conditions and intestinal penetration of luminal antigens [8]. For the study, TNF-α dramatically increased the expression of miRNA-122a. Consequently, the over-expression of miRNA-122 caused a degradation of occludin mRNA and a subsequent decrease in the occludin protein [8]. MicroRNA-21 is also said to have decreased occludin levels resulting in TJ impairment [7]. Additionally, other pro-inflammatory cytokines such as interleukin -1β (IL-1β) can induce expression of miRNAs to trigger inflammation which can affect TJ protein expression.

## Methods

This review was carried out via a literature search of a variety of review and primary articles.

## Results

With specific focus on occludin, researchers examined the upregulation of different types of microRNA, and their effect on the degradation of occludin mRNA and subsequent TJ permeability [9]. miR-122, miR-200b-3p, and miR-200c-3p were the most successful in binding to the 3' untranslated region (UTR) and affecting the function of occludin [9]. In the context of interleukin-1β and specific focus on miR-200c-3p, a proinflammatory cytokine, there was a rapid increase of miR-200c-3p, and with the antisense inhibition of miR-200c-3p, IL-1β induced increase was inhibited, suggesting its requirement for increasing TJ permeability [9]. Antisense inhibition also inhibits the degradation of occludin mRNA. These results demonstrate that a proinflammatory cytokine induced increase of miR-200c-3p is responsible for occludin mRNA degradation and increasing permeability [9]. The *in vivo* approach compared human colonic tissue and its production of IL-1β and expression of miR-200c-3p levels in patients with active ulcerative colitis and patients with normal colonic tissue. There was a significant increase in IL-1β and

miR-200c-3p levels in inflamed tissues compared to normal colonic tissues [9].

In addition, miRNA-665 has been a topic of research due to its role in mucosal inflammation and cell apoptosis in CD and UC. Using a published expression profile, researchers found that miR-665 levels remained low in normal mucosal tissues, but increased four-fold in mucosal tissues affected by UC [10]. Furthermore. miR-665 expression substantially increased in colitis-induced, UC, and CD mucosal tissues compared to control groups with normal mucosal tissues. This increase was about 4-16-fold, suggesting miR-665's role in IBD progression. [10] Interestingly, researchers note that IBD is characterised by cell apoptosis in CD and UC patients [10]. They investigated miR-665's potential role in the process and found that apoptosis factors such as procaspase and TNF-α were induced and had significant increased activity in the presence of miR-665. Additionally, silencing miR-665 suppressed the apoptotic factors and resulted in reduced apoptosis [10]. Finally, they injected mice to see the overall effect of miR-665 on the colon which resulted in loss of crypt structure, shorter colon lengths, ulceration, a higher apoptosis index, and inflammatory cell infiltration, while inhibition had an opposite effect [10].

Contrastingly, there are some miRNAs that have inhibitory effects on IBD progression. When tested in a mouse model, miR-602 was downregulated in IBD intestinal tissues, suggesting a negative correlation. It was found that intestinal tissues from IBD mice decreased expression of miRNA-602 compared to control mice [11]. Additionally, overexpression of miRNA-602 resulted in the alleviation of IBD symptoms in mice [11]. Furthermore, IBD mice treated with miRNA-602 had similar colonic weight/length ratio to that of control mice and caused a significant inhibition of the mRNA expression levels of proinflammatory cytokines such as TNF-α, IL-1β and IL-6, but increased production of anti-inflammatory cytokines [11]. Finally, miR-602 promoted overexpression of occludin and other gastrointestinal epithelial proteins [11]. The results of this study suggest that contrary to other miRNAs, miRNA-602 has a protective role against IBD.

## Discussion

Inflammatory bowel disease has been a topic of interest for many researchers and its connection with microRNA and tight junction proteins has provided a wealth of information with disease pathogenesis and potential therapeutic treatments. MicroRNAs have also contributed to the phenomenon as it controls mRNA post-transcriptionally and can influence the function, integrity, and permeability of tight junction proteins. Studies have outlined that a malfunctioning intestinal barrier, characterised by increased permeability and weakening integrity of tight junctions, can result in intestinal inflammation through antigenic penetration. Many studies have investigated occludin depletion and the role it plays in IBD pathogenesis.

Several other studies have looked at the impact of other miRNA types on the expression of occludin and consequent inflammation in the intestinal barrier. Researchers examined the relationship between two types of noncoding RNA (ncRNA), long noncoding mRNA (PlncRNA1) and miRNA-34c, while studying their effect on TJ proteins such as occludin [12]. lncRNAs are longer than microRNAs with over 200 nucleotides, but have less sequence conservation [12]. Using a construction of the intestinal epithelial barrier, researchers found that overexpression of miR-34c decreased mRNA expression of occludin as well as other TJ proteins. When miR-34c was downregulated, the opposite effect occurred [12]. The results of this study suggest that miR-34c are associated and the knockdown of one facilitated the expression of the other.

One study looked at miRNA23a, and its effect on modifying the epithelial barrier while being induced by tumour necrosis factor (TNF), a proinflammatory cytokine [13]. They found that the expression levels of miRNA23a was higher in patients with Crohn's disease versus healthy patients with normal epithelium. Furthermore, this was also seen in patients who had inactive Crohn's disease in which there was no mucosal inflammation [13]. Examining the epithelium at a closer level, researchers looked at how the TNFAIP3/A20 protein, a negative regulator of nuclear factor kappa B (NFκB) signalling, was affected under the expression of miRNA23a. For the epithelium, its role is to maintain barrier integrity and cell survival and regulate immunological responses in the intestine [14]. Additionally, they found that increased miRNA23a levels in CD patients was correlated with decreased expression of TNFAIP3/A20 stabilised occludin, affecting the barrier integrity [13]. The results of this study suggest that a link exists between miRNA23a expression and transcription factors that regulate occludin at the epithelial barrier.

**Table 1.** Table outlining the type of microRNAs mentioned in the discussion, whether or not its expressed in UC or CD patients, and which cytokines it is activated by

| miRNA type | Expressed in which patients? | Activated by which cytokines? |
|---|---|---|
| miR-200c-3p | UC and CD | IL-1β |
| miR-34c | CD | IL-1β |
| miR-23a | CD | TNF |

## Conclusions

MicroRNA's impact on occludin and progression to pathogenesis of IBD has been a topic of many publications for over a decade and there is still much more to be done. Different miRNAs each contribute differently to occludin mRNA degradation and certain immune components such as cytokines, chemokines, and immune cells further influence IBD pathogenesis. Studying the various miRNA

types aids in discovering therapeutic approaches to mitigating the symptoms and effects of IBD and can lead to further development on potential cures against the disease. Further topics that researchers should explore is microRNA's affect on the intestinal microbiome and how any dysregulation with that could potentially affect occludin and thus IBD. There should also be emphasis on microRNA's significant effect on occludin in order to understand the specific tight junction and how its regulation occurs.

**List of Abbreviations Used**
GI: gastrointestinal tract
IBD: intestinal bowel disease
UC: ulcerative colitis
CD: Crohn's disease
TJ: tight junctions
TNF-α: tumor necrosis factor- alpha
NF: nuclear factor

**Conflicts of Interest**
The author consents that there are no conflicts of interest.

**Ethics Approval and/or Participant Consent**
No ethics approval required due to lack of participant consent and no other ethics to be taken into consideration.

**Authors' Contributions**
MO: performed the literature review, writing of the manuscript and gave final approval of the version to be published.

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