sciendo

Review • DOI: 10.2478/ahem-2022-0038 • AHEM • Vol. 76 Issue 1/2022 • 254-267

AHEM

Advances in Hygiene and Experimental Medicine
Postępy Higieny i Medycyny Doświadczalnej

The functional role of miRNAs in inflammatory pathways associated with intestinal epithelial tight junction barrier regulation in IBD

Review

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Received 18 August, 2021, Accepted 28 December, 2021

Abstract

Inflammatory bowel disease – Crohn's disease and ulcerative colitis – is an immune-mediated chronic disorder with still not fully elucidated complex mechanisms of pathogenesis and pathophysiology. Intestinal epithelial barrier (IEB) dysregulation is one of the major underlying mechanisms of inflammatory process induction in IBD. Proper IEB integrity is maintained to a large extent by intercellular tight junctions, the function of which can be modified by many molecules, including miRNAs. MiRNAs belong to noncoding and non-messenger RNAs, which can modulate gene expression by binding predicted mRNAs.
In this review, we summarize and discuss the potential role of miRNAs in the regulation of inflammatory signaling pathways affecting the function of the intestinal epithelial barrier in IBD, with particular emphasis on therapeutic potentials. The aim of the review is also to determine the further development directions of the studies on miRNA in the modulation of the intestinal epithelial barrier in IBD.

Keywords

inflammatory bowel disease • microRNA • tight junction protein • intestinal barrier • intestinal inflammation • tissue therapy

1. Introduction

Inflammatory bowel diseases (IBD) are immune-mediated chronic idiopathic inflammatory disorders of the gastrointestinal tract that encompasses Crohn's disease (CD), ulcerative colitis (UC), and indeterminate colitis [1]. In both CD and UC, incidence peaked in the second to fourth decade of life with higher prevalence in Western countries, but the gap between East and West became diminished. Recent data have shown no gender-specific prevalence in UC, while in CD differences in gender predisposition include increasing female incidences in the western population and males in the eastern population [2]. Prevalence of IBD in North America and Europe has reached 0.3% with further estimation above 0.6% in North America in the next 10 years [3, 4]. In the pathogenesis of IBD are involved both heritable genetic factors, responsible for the regulation of different pathways, and environmental factors associated with adaptive immune response, the composition of the microbiome, inflammation, or intestinal barrier function [5].

1.1. Intestinal epithelial barrier in IBD

The intestinal epithelial barrier (IEB) consists of a single layer of epithelial cells connected by multiple intercellular junctions, including tight junction (TJ), adherent junction (AJ), and desmosome, which are the major protein complexes of the apical junctional complex (AJC) [6]. IEB acts as a physical barrier between luminal components and underlying tissue, limiting access of microbes, antigens, and toxins to the internal environment, while allowing ions, water, and nutrients to be absorbed [7]. Moreover, the epithelial cells play a significant role in bacteria phagocytosis, toxin neutralization, wound repair initiation, and innate and adaptive immunity activation [8]. Intercellular TJs are essential complexes that form a continuous sealed space of the IEB, guarding its proper functioning [7]. TJs are the apical complexes composed of transmembrane proteins: claudins (CLDN), occludin (OCLN), junctional adhesion molecules (JAMs); cytoplasmic scaffolding proteins: cingulin, zonula occludens (ZO) family, and regulatory proteins [9]. These dynamic structures are responsible for regulating epithelial permeability and integrity

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[9]. Loss of the intestinal epithelial barrier function, manifested by increased permeability or impaired integrity, leads to the disruption of intestinal homeostasis caused by exposure of luminal contents to the host's tissues. Permanent lasting intestinal barrier dysfunction combined with an aberrant immune response are both significantly involved in the development of the inflammatory process in IBD [7, 10]. The defective intestinal barrier is commonly observed in IBD patients' colonic tissue, with the highest dysfunction for patients with active disease. It is directly correlated with persistence of intestinal inflammation [7, 11].

1.2. MicroRNA

MiRNAs belong to noncoding and non-messenger RNAs, 18–25 nucleotide segments of single-stranded RNA, which are endogenously expressed and which play an important role in enzymatic, structural, regulatory functions by silence target mRNAs [12, 13, 14, 15]. The miRNA binds to complementary sequences of mRNA 3-untranslated regions (3-UTR). However, 100% complementarity to the 3-UTR of the mRNA is not required for the regulation of gene expression. Therefore 1 miRNA can target a lot of mRNAs [16, 17]. The human genome contains 1917 annotated hairpin precursors and 2654 mature sequences of miRNA [18]. They are transcribed from intergenic or intronic regions of DNA by RNA polymerase 2 [19, 20]. MiRNAs can be co-expressed with a gene, which they regulate, because often miRNA are clustered on chromosomes proximal to gene clusters [21, 22]. Essential miRNA's role in relevant biological processes such as cell differentiation, apoptosis, cell proliferation, and carcinogenesis has been proved in many diseases [23, 24, 25, 26]. In IBD miRNAs are involved in the Th1/Th17 immune response, inflammation processes, apoptosis, interleukin expression, intestinal barrier function, and many other processes. Therefore miRNAs are important molecules in the pathogenesis of IBD [27, 28]. Knowledge about the role of miRNAs in IBD is growing steadily, helping to understand the pathogenesis of IBD and creating an opportunity for new diagnostic and therapeutic possibilities.

In this review, we summarize and discuss the potential role of miRNAs in the regulation of inflammatory signaling pathways affecting the function of the intestinal epithelial barrier in IBD, with particular emphasis on the therapeutic potential. The aim of the review is also to determine the further development directions of the studies on miRNA potential in the modulation of the intestinal epithelial barrier in IBD.

2. Materials and Methods

This review is based on pertinent articles searched using PubMed, encompassing literature published over the last five years (2016–2021) in cases investigating the role of concerned miRNAs. However, in the other cases cited from the literature, older articles are also cited. The eligibility criteria were as follows: in vivo and in vitro studies; studies performed on humans, animals, and cell cultures; studies resulting in significantly altered tight junction protein expression and influences on intestinal barrier integrity caused by miRNAs associated with inflammatory pathways. Research that works with examined miRNA expression and its role only in cell cultures, without assays in animal models or human tissues, were excluded from this review. The key search words were as follows: inflammatory bowel disease, Crohn's disease, ulcerative colitis, microRNA, tight junctions, cell junctions, inflammation, intestinal epithelial barrier.

3. MiRNAs and their targets, signaling pathways in altered IEB function in IBD

3.1. TNFAIP3 and miRNA-23a

Tumor necrosis factor alpha inhibitor protein 3 (TNFAIP3)/A20 is a negative regulator of NfkB [29, 30, 31]. TNFAIP3/A20 plays an important role in the maintenance of epithelial barrier integrity by OCLN stabilization at the tight junction [32]. R. K. Felwick et al. examined miRNA-23a expression in CD and its role in the pathophysiology of CD. They demonstrated that miRNA-23a, by targeting TNFAIP3 mRNA 3'UTR, caused altered IEB function. miRNA-23a promotes an increase in the transcriptional activity of NFkB, but only in the presence of TNFa. Overexpression of miRNA-23a with TNFa leads to significant impairment of the intestinal epithelial barrier function. Moreover, upregulated expression of miRNA-23a throughout increased NFkB activity contributes to the significant pro-inflammatory effect. In active and inactive Crohn's disease, patient's biopsy miRNA-23a expression was significantly up-regulated. miRNA-23a expression positively correlated with loose stool frequency. There were no significant differences between TNFAIP3 mRNA expression in a Crohn's disease donors' group and a healthy cohort, but in encoded protein TNFAIP/A20 changes were observed. Using laser confocal microscopy (LCM) assayed for RNA strong immunostaining at the apical tight junction and subapical membrane regions of epithelial cells in healthy tissue was observed, while in active and inactive Crohn's disease tissue there was full or partial loss of staining in these cellular regions. In active and inactive Crohn's disease group, there was a significant decrease of TNFAIP3/A20 protein level at apical and subapical membrane regions [33].

3.2. ALK1 and miRNA-31-5p

Activin receptor-like type 1 (ALK1 or ACVRL1) is a cell-surface serine/threonine receptor for the TGF-beta superfamily of ligands [34]. Altered intestinal immunity associated with dysregulated TGF-beta signaling is known in the pathophysiology of IBD [35]. ALK1 is expressed in many tissues and cells, including human colonic intestinal epithelial cells (IECs) [36, 37, 38]. Toyonaga et al. examined the clinical impact of colonic ALK1 signaling dysregulation on intestinal barrier integrity and clinical outcomes in patients with CD. ALK1 signaling plays an important role in

regulating NOTCH target gene expression, the stemness, and the differentiation of human colonic IECs. Decreased ALK1 expression observed in CD patients colonic tissue contributes to lower NOTCH activity, higher colonic IECs stemness, and inhibited colonic IECs differentiation. Moreover, reduced ALK1 signaling impairs IEC barrier integrity by down-regulating tight junction protein expression – exactly tight junction protein 1 (TJP1), CLDN8, OCLN. ALK1 protein expression is directly regulated by miRNA-31-5p and significantly negatively correlated with miRNA-31-5p expression, increased in CD patient's colonic mucosa. Thus, downregulated ALK1 expression is caused by miRNA-31-5p overexpression. Toyonaga et al. also revealed that the subset of CD patients with markedly lower ALK1 expression (low-ALK1 CD subset) than the other CD patients (hi-ALK1 CD subset) have an increased risk of surgical resection and endoscopic relapse [39].

3.3. VDR and miRNA-675-5p

Data shows lncRNA gene H19 can play an important role in the development of inflammatory diseases and cancer [40, 41]. Vitamin D receptor (VDR) is a nuclear receptor whose signaling pathways play a significant role in the regulation of inflammation and carcinogenesis in different tissues through the mediation of the functions of 1,25(OH)2D3 [42, 43, 44, 45]. Investigations showed that 1,25(OH)2D3 protects the intestinal barrier, with injuries induced by destructive reagents and decreased VDR signaling perhaps playing an essential role in pathogenesis mechanisms of UC [46, 47, 48, 49, 50, 51]. Chen et al. investigated the expression and correlation of H19, VDR, and miRNA-675-5p in UC and miRNA-675-5p-induced effects on epithelial barrier [52]. In Caco-2 cells, miRNA-675-5p mimics transfection-caused significantly increased paracellular permeability, decreased TEER, and expression level of ZO-1, OCLN, and VDR. In contrast, miRNA-675-5p inhibitor co-transfection importantly impaired monolayer barrier disruption in Caco-2 cells with H19 upregulated expression, validating the important role of miRNA-675-5p in the effects of H19. Moreover, overexpression of H19 importantly correlated with decreased expression of VDR, which was also significantly impaired by miRNA-675-5p inhibitors. H19 overexpression caused decreased mRNA levels of tight junction proteins and miRNA-675-5p inhibitors impaired lower expression level of ZO-1. In UC tissues, related to healthy tissues, notably decreased VDR expression levels were observed, as well as significantly increased expression levels of H19 and miRNA-675-5p. Besides, miRNA-675-5p expression negatively correlated with the expression level of VDR. Chen et al. also proved that the direct target of miRNA-675-5p is VDR mRNA [52]. MiRNA-675-5p is transcribed from the first exon of H19 and the function of H19 can be modulated by that miRNA in many diseases [41, 53].

3.4. AhR and miRNA-124a

Aryl hydrocarbon receptor (AhR) belongs to the basic helix-loop-helix (bHLH) superfamily of ligand-inducible transcription factors, which are associated with cellular responses to environmental stimuli. AhR signaling modulates the development, function, and production of different cell types in the gastrointestinal tract and has an important role in the regulation of the immune response in IBD. AhR was implicated in several intestinal pathologies, such as intestinal inflammation, infection, and cancer [54]. The effect of AhR on the intestinal epithelial barrier may be directly or indirectly mediated through signaling with different cell types, including immune cells. AhR activation prevents intestinal barrier dysfunction by restoring the expression and distribution of the ZO-1, OCLN, and CLDN1 [55, 56]. AhR gene and protein expressions were downregulated in CD patients' inflamed colonic tissue, but not in UC patients, compared with tissue from uninflamed areas and healthy group [57]. Zhao et al. revealed AhR is a target gene of miRNA-124a. In murine colitis models and Caco-2 cells overexpression of miRNA-124a led to decreased AhR expression and impaired intestinal barrier function. Meanwhile, miRNA-124a inhibitors restored TJ protein expression, such as ZO-1, OCLN, JAM-A, Claudin-1 enhancing intestinal barrier integrity. Expression of miRNA-124a in colon biopsy tissues from CD patients and in colon epithelial cells was markedly upregulated [58].

3.5. MLCK and miRNAs

Myosin light chain kinase (MLCK) activated phosphorylation of the myosin II regulatory light chain (p-MLC) which is correlated with intestinal barrier tight junctions' permeability [59, 60]. Colon cancer-associated-transcript-1 (CCAT1), an lncRNA oncogene promoting cancer cell proliferation, migration, and its overexpression, was observed in inflammatory colonic tissues [61, 62]. Ma et al. examined the interaction between miRNA-185-3p, CCAT1, MLCK, and IEB integrity. Increased MLCK activity and p-MLC expression in Caco-2 cells may disrupt intestinal barrier function through changes to normally smooth arc-like shapes ZO1 and OCLN to irregular undulations of ZO1 and OCLN. Higher activity of MLCK and inflammatory pathways is associated with overexpression of CCAT1, which was significantly increased in IBD tissues compared with normal colorectal tissues. The study indicated that CCAT1 may play an important role in the modulation of miRNA185-3p stability by acting as a miRNA sponge. MLCK and CCAT1 have the same miRNA-185-3p response elements in their RNA regions. The analysis revealed that the expression of miR-185-3p was negatively correlated with the expression of CCAT1 in IBD patients. Up-regulated expression of CCAT1 is disturbing the interaction between miRNA-185-3p and MLCK leading to disruption of IEB function caused by increased activity of MLCK. The investigation demonstrated that irregular undulations of ZO1 and OCLN, which were induced by CCAT1

overexpression or miRNA-185-3p inhibitors could be gradually decreased by miRNA-185-3p. The expression of miRNA-185-3p was significantly decreased in IBD biopsy tissue compared with tissues obtained from healthy patients [63].

Shen et al. proved MLCK and c-JUN are direct targets of miRNA-200b. In Caco-2 cells stimulated by TNF-alfa miRNA-200b treatment led to a significant a decrease in IL-8 expression compared with untreated cells. Moreover, overexpression of miRNA-200b induced decrease of phospho-JNK (p-JNK), phospho-c-Jun (p-c-Jun), and total-c-Jun expression levels likewise AP-1 activity. MiRNA-200b could inhibit IL-8 synthesis caused by TNF-a. During TNFa-induced epithelial barrier disruption, negative changes in TJ proteins distributions CLDN1 and ZO-1 exhibiting irregularly undulating shapes were observed. Moreover, CLDN1 partial removal into cytoplasmic vesicles was observed. The investigation showed miR-200b overexpression maintains TJ morphology and attenuates rearrangements of CLDN1 and ZO-1 induced by TNF-a. MiRNA-200b overexpression treatment significantly increased TEER and dramatically decreased paracellular permeability after TNF-a stimulation. MiRNA-200b overexpression caused decreased expression levels of MLCK and p-MLC in trials with or without TNF-alfa stimulation, so miR-200b can successfully inhibit the MLCK/p-MLC pathway and preserve TJ epithelial barrier function. Thus, upregulated expression of miRNA-200b suppresses the intestinal barrier disruption induced by TNF-alfa upregulated JNK/c-Jun/AP-1 signaling pathway [64].

3.6. IL-9 and miRNA-21

IL-9 can modulate intestinal inflammation in animal models by regulating intestinal barrier function [65]. In DSS-induced colitis murine models, inhibitors of the IL9 impair inflammation [66]. Overexpression of IL9 was noted in UC tissue and a positive correlation was found of IL9 expression level with the activity of the disease [67]. Previous data demonstrated substantial down-regulation of CLDN8 in active IBD patients' tissues; additionally, CLDN8 seems to be one of the most important claudins in the pathology of IBD and its expression was the most reduced compared to other claudins genes in IBD [68, 69, 70]. Li et al. proved upregulation of IL-9 in colonic tissues of CD patients compared to the expression level in the no-inflammatory colonic tissue. In Caco-2 cells treatment with IL-9 reduced TEER, and co-treatment with a neutralizing antibody against IL-9 (anti-IL-9) significantly increased the tight junction protein expression. In treated cells a reduction of mRNA expression of CLDN8, CLDN1, ZO-1, and OCLN were detected. In murine models and cell cultures, IL-9 stimulation demonstrated significant overexpression of miRNA-21 and down-regulated expression of CLDN8. The investigation by using a miRNA-21 mimic and miRNA-21 inhibitor gave expected results in cell models that confirmed miRNA-21 directly targets CLDN8. In biopsy tissues of CD patients plentiful expression of miRNA-21, compared to the control group, was marked. Moreover, the expression of CLDN8 was significantly reduced in CD tissues, while up-regulated in the control group. Treatment with miRNA-21 inhibitor in TNBS-induced colitis in mice attenuated inflammation and revised permeability of the intestine barrier and other cardinal signs of colitis and restored CLDN8 level [71].

3.7. IL-23 and miRNA-223

Previous studies showed the IL23/Th17 axis engagement in the regulation of IBD [72, 73]. Wang et al. proved that the IL23 pathway is associated with dysfunction of the intestinal epithelial barrier by interaction with miRNA-223. In the murine colitis model, IL23 treatment resulted in a decreased expression level of CLDN8 compared to untreated control. The same results were observed in cell models; IL-23 stimulation caused CLDN8 down-regulation and miRNA-223 upregulation. Inversely, co-treatment with anti-IL23P19 significantly increased CLDN8 expression level and attenuated up-regulated expression of miRNA-223. Epithelial barrier functions featured by TEER were attenuated after IL-23 stimulation and enhanced in trials using co-treatment with anti-IL23P19, which suggests an important role of the IL-23 pathway in cell barrier integrity. The study revealed that endogenous CLDN8 is inhibited by miRNA-223 in colonic epithelial cells; miRNA-223 mimics decreased the expression of CLDN8 and miRNA-223 inhibitor increased CLDN8 expression. In UC and CD biopsy samples, significantly higher expression of miRNA-223 compared to expression in healthy colonic mucosa and down-regulated expression of CLDN8 were noted. Treatment with miRNA-223 in a murine model of TNBS-induced colitis caused reactivation of CLDN8 in colitis tissues, reduction of important colitis signs, and enhancement of the intestinal epithelial barrier integrity. Thus, inhibitors of miRNA-223 could play a therapeutic role in IBD [68].

3.8. IL-21 and miRNA-423-5p

IL-21 plays an essential role in many processes involved in the progression of IBD, including interferon (IFN)-y production, Th1 cell differentiation [74, 75] promotion, and activation of transcription factors such as STAT1 and STAT3 regulation [76]. Wang et al. revealed IL-21 signaling pathway involvement in IEB disruption in IBD. They confirmed that IL-21 controls the level of CLDN5 by miRNA-423-5p, and 3'UTR of CLDN5 is a direct target of miRNA-423-5p. Up-regulated expression of miRNA-423-5p could attenuate CLDN5 level and miRNA-423-5p inhibitors can restore the level of CLDN5, both in Caco-2 cells and in a mouse model of acute colitis. CLDN5 transfection into Caco-2 cells restored the intestinal barrier function after forced miRNA-423-5p mimic stimulation by increasing epithelial barrier integrity and decreasing paracellular permeability. Therefore miRNA-423-5p could control the intestinal barrier function by targeting CLDN5. Moreover, miRNA-423-5p inhibitor treatment in a mouse model of acute colitis reduced inflammation by down-regulating the

expression of IL1B, IL6, TNF-a and inhibiting phosphorylation of the components of the NF-kappaB/MAPKs/JNK signaling pathway. MiRNA-423-5p is regulated by IL-21; IL-21 induces expression of miRNA-423-5p, and IL-21 neutralizing antibody blocked the expression of miR-423-5p. In colonic tissue from UC patients, miRNA-423-5p and IL-21 expression level were abnormally increased compared to the healthy group [77].

3.9. IL-1B and miRNA-200c-3p

Interleukin-1B is a proinflammatory cytokine increased in the course of IBD. IL-1B contributes to disruption of intestinal epithelial barrier function, playing an essential role in the development of IBD. The decrease of intestinal barrier tight junction integrity during IL-1B stimulation is partially caused by the up-regulated activity of MLCK induced by IL-1B [78, 79, 80]. Rawat et al. investigated the influence of IL-1B on IEB TJ permeability and TJ protein expression, and examined predicted miRNA to IL-1B-induced effects. Physiological concentration of IL-1B in Caco-2 cells leads to down-regulation of OCLN expression and increases TJ permeability. There was a strong correlation between the decreased OCLN expression level induced by IL-1B and the increased TJ permeability. IL-1B caused a significant increase of miRNA-200c-3p expression, while inhibitors of miRNA-200c-3p prevented OCLN down-regulated expression caused by IL-1B and increased TJ permeability. In the murine colitis model, IL-1B stimulation also caused a significant increase of miRNA-200c-3p expression, a decrease in OCLN mRNA level, and increased intestinal permeability. Inhibitors of miRNA-200c-3p transfected in vivo inhibited the up-regulated expression of miRNA-200c-3p induced by IL-1B, decreased OCLN expression, and inhibited impaired intestinal barrier integrity [81]. The pre-miRNA-200c-3p transfection caused miRNA-200c-3p overexpression [82], decreased OCLN mRNA level and degradation of intestinal tissue OCLN protein thereby enhanced intestinal barrier disruption in murine intestine. In a murine colitis model up-regulated expression of IL-1B, miRNA-200c-3p, and decreased OCLN expression was noted. Treatment with miRNA-200c-3p inhibitor resulted in inhibition of miRNA-200c-3p expression, a decrease of OCLN mRNA, and an increase of colonic permeability. In colonic tissue from active UC patients, a significant up-regulated miRNA-200c-3p and IL-1B expression was marked compared to the healthy colonic tissue from patients after colonic resection for noninflammatory conditions [81].

3.10. BTG and miRNA-301A

The B-cell translocation gene (BTG)/TOB family of antiproliferation proteins are responsible for the regulation of genotoxic and cellular stress signaling pathways, cell differentiation, apoptosis, cell-cycle progression, gene expression, and also they are associated with protection mechanisms in oncogenic transformation [83]. He et al. demonstrated that miRNA-301A expression is significantly up-regulated in the peripheral blood mononuclear cells and colorectal tissues of IBD patients [84]. Early data showed that miRNA-301A could act as an NFkB activator in pancreatic cancer cells [85]. He et al. also examined the regulation of intestinal inflammation and epithelial barrier integrity by miRNA-301A [86]. BTG1 is a direct target of miRNA-301A; up-regulated miRNA-301A expression caused significantly down-regulated BTG1 mRNA expression in human cells. Overexpression of BTG1 caused inhibition of IL-1B-induced c-Jun phosphorylation and NFkB activation. Therefore miRNA-301A probably promotes NFkB activation by targeting BTG1. In IBD patients the levels of IEC-derived BTG1 are negatively correlated with miRNA-301A expression. In murine colitis model with MiRNA-301A gene knockout observed a smaller increase of epithelial permeability, IL-1B, IL-6, IL-8, TNF IEC production compared with the control colitis group and increased E-cadherin expression level in epithelia. In cell models, up-regulated miRNA-301A expression led to an increase of epithelial permeability, enhancement of paracellular permeability, transepithelial electrical resistance (TEER) reduction, and down-regulation of E-cadherin expression level. A positive correlation was found between increased miR-301A expression in IEC of IBD patients and mRNA level of NFkB p65. Moreover, miRNA-301A influences the activation of NFkB signaling in IEC by promoting that signaling and increasing the level of NFkB-associated proteins like IL-1B, IL-6, IL-8, and TNF. MiRNA-301A up-regulated expression is significantly induced by IL-1B and gently induced by IL-6. In intestinal epithelial cells (IEC) isolated from IBD patients compared with healthy control group cells, miRNA-301A expression level was importantly increased and BTG1 expression was decreased, both in CD and UC patients' cells. Also, there were significant differences between IEC isolated from the inflamed area and IEC from the healthy tissue area. In the IEC from uninflamed mucosa, miRNA-301A expression was similar to IEC from the healthy patients' control group and BTG1 expression was also down-regulated in inflamed mucosa compared to unaffected parts of the tissue. Apart from this, He et al. found a positive correlation between the IL-1B levels in IBD patient's serum and miRNA-301A expression in IEC from CD and UC patients as IL-1B mRNA and miRNA-301A expression in IEC. These results reveal the role of IL-1B in increasing miR-301A expression in IEC of IBD patients. MiRNA-301A up-regulated expression caused by IL-1B can occur through the JNK MAPK-dependent pathway. The promoter of miR-301A could be bound by c-Jun, and c-Jun phosphorylation is strongly induced by IL-1B. Summarizing, miRNA-301A could play a part in intestinal epithelial barrier disruption in IBD. In colonic tumor tissues from patients with CAC, miRNA-301A is increased compared with healthy controls. MiRNA-301A could putatively function as an oncogene [86].

3.11. STAT3 and miRNA-495

Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that belongs to a seven-member family of proteins responsible for peptide hormone signal transduction from the cell surface to the nucleus [87]. STAT3 expression in UC is localized in mucosal epithelial cells, lamina propria mononuclear cells, as well as in neutrophils granulocyte. STAT3 activity is regulated by phosphorylation through Janus kinase-signal transducer and activator of transcription (JAK) [88]. In UC STAT3 up-regulated expression could inhibit anti-inflammatory cytokine expression [89]. X.-Q. Chu et al. proved that STAT3 is a direct target of miRNA-495. Up-regulated miR-495 expression caused decreased STAT3 and JAK expression level, but increased expression of CLDN1. Therefore miR-495 inhibits the JAK/STAT3 signaling pathway by directed targeting STAT3 contributing to the maintenance of intestinal epithelial barrier functions. STAT3 positive cells in intestinal mucosa were statistically significantly more frequent in the UC group compared with the normal group. STAT3 and JAK expression levels in UC tissue of mice were increased, and miR-495 and CLDN1 expression were decreased [90].

3.12. EGFR and miRNA-122a

The epidermal growth factor receptor (EGFR) is a cell surface glycoprotein that binds the epidermal growth factor family of extracellular protein ligands. EGFR plays an important role in the activation of signaling pathways associated with cellular differentiation, proliferation, and survival [91]. Zhang et al. examined the role of miRNA-122a in regulating intestinal barrier function and revealed miRNA-122a overexpression could up-regulate the expression level of zonulin by directly targeting EGFR, leading to enhancement of intestinal permeability and inflammation in the murine model and Caco-2 cells [92]. Increased zonulin level associated with increased expression of miRNA-122a could be an indicator of intestinal permeability [93]. TNF-a and LPS treatment increase the miRNA-122a expression in cultured cells and enterocytes [94], whereas LPS treatment also elicits decreased expression of EGFR, CLDN1, OCLN, and increased zonulin expression [92]. Moreover, IL-6 and TNF-a expression in mice-miRNA-122a treatment group serum were both significantly increased compared to the control group. In colonic tissues of IBD patients, miRNA-122a and zonulin expression were significantly up-regulated relative to colonic tissues from the healthy group [92].

3.13. TLR4 and miRNA-375

Toll-like receptor 4 (TLR4) is a transmembrane receptor responsible for inducing the secretion of inflammatory cytokines by NFkB activation [95]. In healthy tissue, TLR4 is uncommonly expressed, but in UC and CD patients colonic tissue shows marked TRL4 overexpression [96, 97]. Wu et al. examined TLR4 and miRNA-375 expression in colonic tissue from IBD patients and from the healthy control group. In the IBD patients, colonic tissue miRNA-375 expression was decreased and TLR4 expression was increased compared with the healthy control group. A significant negative correlation between miRNA-375 and TLR4 mRNA levels was revealed. TLR4 mRNA is a direct target of miRNA-375. miRNA-375 mimics transfection led to a decrease in TLR4 mRNA level, and miRNA-375 inhibitors gave the opposite effect in TLR4 expression. Moreover, miRNA-375 inhibition caused increased paracellular permeability, decreased TEER, but also apoptotic rate elevation in Caco-2 cells. MiRNA-375 could play a protective role in the intestinal epithelial barrier during inflammation throughout the mediation of the TLR4/NFkB signaling pathway. LPS stimulation in Caco-2 cells induced TLR4 overexpression and upregulated NFkB protein level, but in the presence of high-level miRNA-375, TLR4 expression was downregulated. Additionally in LPS-induced Caco-2 cells, miRNA-375 inhibition led to overexpression of TNF-a, IL-1B, IL-6, IL-8 and decrease in the relative expression level of ZO-1, OCLN, and IL-10 [98].

3.14. PP2A, Cdc42 and miRNA-320a

Protein phosphatase 2 (PP2A) is a major protein phosphatase holoenzyme that interacts with the TJ complex. The upregulated activity of PP2A causes dephosphorylation of the TJ proteins, ZO-1, OCLN, and claudin-1. In contrast, inhibition of PP2A induces the phosphorylation and recruitment of ZO-1, OCLN, and claudin-1. Therefore, the high activity of PP2A induces increased paracellular permeability and has an important role in the regulation of TJ function and assembly [99]. PP2A also can interact with JAM-A, and dephosphorylates JAM-A in the way of antagonization of aPKCζ-mediated phosphorylation of JAM-A at TJs [100]. Cell division cycle (Cdc)42 is a small GTPase of the Rho family that controls different cellular functions such as adhesion and transcription important to the formation of a functional intestinal epithelial barrier [101, 102, 103, 104]. Cordes et al. demonstrated probiotic E. Coli Nissle 1917 (EcN) treatment resulted in enhanced epithelial barrier integrity [105] and significantly upregulated expression of miRNA-320a, while enteropathogenic E. coli E2348/69 transfection caused the decrease of miRNA-320a level in T84 cells [5]. Moreover, both probiotic EcN and miRNA-320a significantly down-regulated PPA2 with its subunit R5B (PPP2R5B), Cdc42, and up-regulated the expression of JAM-A, whereas co-incubation with EPEC revealed inverse results. Trials with a high concentration of miRNA-320a co-incubated with EPEC revealed that miRNA-320a caused increased epithelial barrier integrity by attenuation of EPEC-induced PPP2R5B upregulation and JAM-A down-regulation. In murine colitis models, there was no correlation between disease activity and miRNA-320a tissue expression level; miRNA-320a was not significantly altered in DSS-induced colitis and not increased in T-cell transfer colitis, and colitic IL-10-/- mice. However, miRNA-320a is produced during proinflammatory stimulation, and as the elevated blood

level of miRNA-320a positively correlates with the histological damage in murine colitis models, miRNA-320a may be used for monitoring of disease activity. In active CD patients, miRNA-320a blood concentration was significantly higher in relation to patients with CD in remission and the healthy group [106].

4. Searching for new therapeutic opportunities

The standard strategy for IBD pharmacotherapy starts with stepwise, well-tolerated drugs and proceeds into aggressive immunosuppression. Surgical treatment may be necessary in case of failure of pharmacotherapeutic options [107]. Heterogeneity in the clinical course of both UC and CD causes difficulties in matching the best therapy tactics for all patients [108]. In CD, the risk of developing either stricturing or penetrating complications increases through the years of disease after CD diagnosis, and it may be associated with uncontrolled permanent inflammation; 70% of patients with CD undergo surgical treatment within 10 years of receiving the diagnosis [107, 109]. Therefore, the development of miRNA therapeutics based on targets, and regenerating the intestinal epithelial barrier, may be a promising venture. Recent data showed possibilities of intestinal barrier function restoration treatment in clinical trials [110, 111], but therapeutic strategies for IBD directly targeting the intestinal barrier currently do not exist [6]. A breakthrough in IBD therapy may be gained in the future by using the still-growing data about miRNA's role in the pathophysiology of IBD. MiRNA-based drugs are being developed in clinical trials, but none of them have yet reached the pharmaceutical breakthrough into clinical practice. What is needed is to develop efficient delivery systems of miRNA-based drugs, ensuring internalization into target cells or tissues to exert pharmacological effects. To gain access to intracellular targets and to guarantee protection against degradation by serum RNases in systemic administration of miRNA-based drugs, such medicines require formulation with specific materials or carriers using viral vectors, pDNAs, or intact cells. Proposed delivery systems of all RNA therapeutics may be implemented by dendrimers, lipid nanoparticles, lipopolyplexes, polymers, or antibodies. Accumulation and distribution into other target tissues, especially the liver and kidneys, have to be examined to determine safety profiles of microRNA drugs. Interaction of the proper microRNA with one distinct from the target in therapy mRNAs, conjugation of microRNA with specific ligands used in the formulation of miRNA drugs, metabolic stability, and internalization mechanisms to target cells of microRNA medicines, are other physicochemical and pharmacokinetic properties that require further examination in miRNA-based therapy development [112]. However, miRNA-based therapeutics, mainly miRNA mimics and inhibitors (antagomiRs), revealed positive feedback concerning their potentials in HCV infection [113, 114, 115], Alport syndrome [116, 117], and tumor suppression [118, 119]. On the other hand, it may be possible to modulate miRNA expression by interaction with components of RNA biogenesis; this could be used in potential miRNA-based therapies. Currently there are clinical trials using Abivax in IBD, a drug that produces ABX464 and contributes to up-regulation of miRNA-124, reducing the symptoms of IBD by modulation immunity and inflammation for patients refractory to corticosteroids and anti-TNF agents [120, 121, 122, 123]. After a Phase I dose–safety trial in the DSS-induced colitis animal model, ABX464 was taken to a Phase IIa clinical trial in patients with UC [122]. Phase IIa study with ABX464 with a total of 32 patients with moderate-to-severe ulcerative colitis was performed [123]. In this randomized (2:1) placebo-controlled double-blind induction study 32 patients received ABX464 50 mg once a day orally, or placebo, for 8 weeks in the induction phase. The primary endpoint was safety of ABX464 and key secondary endpoints included clinical remission (rectal bleeding sub-score = 0 and an endoscopic sub-score ≤1 and at least one-point decrease in stool frequency subscore from baseline to achieve a stool frequency subscore ≤1), endoscopic improvement (Mayo endoscopic score of 0 or 1), and clinical response and histological healing. Endpoints results at Day 56 were as follows: endoscopic improvement was -50% and 11% ($p = 0.03$), the reduction in the total Mayo Clinic Score (MCS) was –53% and –27% ($p = 0.03$) and reduction of partial MCS –62% and –32% ($p = 0.02$), the expression of miRNA124 was 7.69- and 1.46-fold ($p = 0.004$) for the ABX464 and placebo groups, respectively [123]. After the induction phase, 22 patients were included in an open-label long-term extension phase [124]. The primary endpoint was long-term safety of ABX464, and additional efficacy endpoints included clinical and endoscopic rates of response and remission. A total of 19 patients (86%) completed the 24 months of ABX464 therapy and showed the very good long-term safety and tolerability of 50mg given orally. At month 12 and 24 of ABX464 treatment 12/16 (75%), and 11/16 (68,8%) of the patients undergoing endoscopy achieved clinical remission, respectively. Clinical response was recorded in 15/16 (93.8%) at both month 12 and 24. In 13/15 (81.3%) and 7/16 (43.8%) endoscopic remission was shown at 12 and 24 months, respectively. Clinical remission was defined as MCS ≤ 2, with no individual subscore > 1; endoscopic remission was defined as Mayo endoscopic subscore = 0; endoscopic improvement defined as Mayo endoscopic subscore ≤ 1; and clinical response was defined as reduction ≥ 3 points in MCS and ≥30% change from baseline score. At month 12 the expression of miR-124 increased a mean of 215-fold in the rectal biopsy. ABX464 is currently in Phase IIb trials in UC and CD [124]. These impressive data of clinical efficacy and good safety profile make ABX464 an attractive candidate for further development. There are currently no other clinical trials on miRNA-based therapy in IBD in progress.

The mechanism of action miRNA-based drugs in IBD has been presented in Figure 1.

5. Conclusions

In our review, we demonstrated the significant role of miRNAs in the regulation of inflammatory pathways associated with intestinal epithelial barrier function in IBD. The importance of many results was revealed, especially by validation through investigations, which examined the influence of proper miRNA mimics or inhibitors on cardinal signs of colitis and intestinal tissue damage using murine models of colitis.

In the vast majority of patients with Crohn's disease, it is necessary to undergo surgical treatment, with implications for the quality of life because of damaged intestinal structure caused by long-term uncontrolled intestinal inflammation. Searching for new methods of avoiding surgical resection in patients with active disease is one of the greatest therapeutic challenges in modern gastroenterology.

By examining miRNA's mechanisms, which regulate the expression of many cytokines or the negative effect for tight junctions' intestinal barrier integrity and gut homeostasis, scientists proved that therapies based on miRNA mechanisms have potential to reduce risk of surgical resections. Moreover, therapies based on regulation by proper miRNAs can maintain the remissions of both CD and UC.

Many positive effects have been observed in studies on the role of selected miRNAs in the regulation of the epithelial barrier function associated with inflammatory pathways. Inhibition or treatment with mimics of proper miRNA resulted not only in the significant enhancement of the intestinal epithelial barrier function but also in significant impairment of intestinal damage and colitis signs in murine models. In addition to inflammatory pathway regulation, miRNAs could also regulate tight junction protein expression, regardless of inflammation. These issues need further research, particularly on estimation of the importance of certain miRNAs in clinical practice.

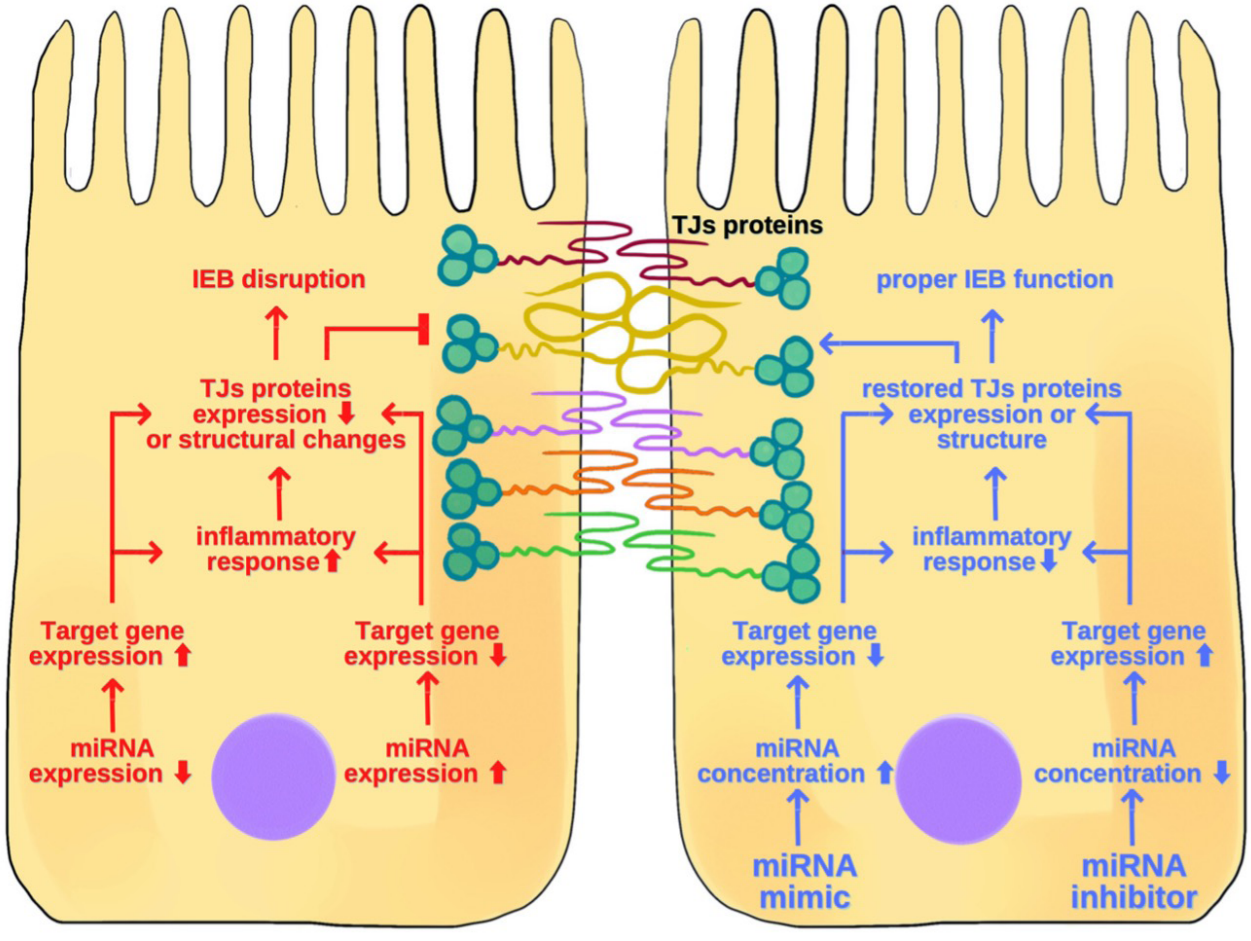


Fig. 1. Mechanism of action of miRNA-based drugs in IBD. The left side demonstrates the molecular effect of altered miRNAs' expression in IBD. The right side shows miRNA-based treatment response. miRNA–microRNA; TJs–tight junctions; IEB–intestinal epithelial barrier

Table 1. MiRNAs collected in this review with their targets, marked expression in IBD, and altered TJ proteins expression. The table was divided into in vivo and in vitro examined effects on TJ proteins expression induced by altered miRNA expression; miRNA–microRNA; ZO-1–Zonula occludens-1; CLDN–Claudin; JAM-A– junctional adhesion molecules A; AJ–adherent junction; CD–-Crohn's disease; UC–-ulcerative colitis; IBD–inflammatory bowel disease; PBMCs–peripheral blood mononuclear cells

microRNA	Target mRNA	Expression in IBD	TJ proteins expression associated with miRNA-induced effect	Author/year
In vivo observed effects				
miRNA-124a	Aryl hydrocarbon receptor (AhR)	Overexpression in CD tissue	ZO-1 ↓, OCLN ↓, JAM-A ↓, CLDN1 ↓	Zhao et al., 2020 [58]
miRNA-31-5p	Activin receptor-like type 1 (ALK1)	Overexpression in CD tissue	CLDN8 ↓, TJP1 ↓, OCLN ↓	Toyonaga et al., 2020 [39]
miRNA-301A	B-cell translocation gene 1 (BTG1)	Overexpression in PBMCs and tissue in IBD	E-cadherin (AJ) ↓	He et al., 2017 [86]
miRNA-200b	Myosin light chain kinase (MLCK) and c-JUN	Decreased in IBD tissue	ZO-1 ↓, OCLN ↓	Shen et al., 2017 [64]
miRNA-21	Claudin 8 (CLDN8)	Overexpression in CD tissue	CLDN8 ↓	Li et al., 2018 [71]
miRNA-200c-3p	Occludin (OCLN)	Overexpression in UC tissue	OCLN ↓	Rawat et al., 2020 [81]
miRNA-495	Signal transducer and activator of transcription 3 (STAT3)	Decreased in UC tissue	CLDN1 ↓	Chu et al., 2018 [90]
miRNA-122a	Epidermal growth factor receptor (EGFR)	Overexpression in IBD tissue	CLDN1 ↓, OCLN ↓, Zonulin ↑	Zhang et al., 2017 [92]
miRNA-320a	Protein phosphatase 2 (PP2A) and Cell division cycle 42 (Cdc42)	Overexpression in serum of active CD	JAM-A ↑	Cordes et al., 2016 [106]
miRNA-423-5p	CLDN5	Overexpression in UC tissue	CLDN5 ↓	Wang et al., 2020 [77]
In vitro observed effects				
miRNA-185-3p	Myosin light chain kinase (MLCK)	Decreased in IBD tissue	ZO-1 ↓, OCLN ↓	Ma et al., 2019 [63]
miRNA-23a	Tumor necrosis factor alpha inhibitor protein 3 (TNFAIP3)	Overexpression in CD tissue	OCLN destabilization	Felwick et al., 2020 [33]
miRNA-675-5p	Vitamin D receptor (VDR)	Overexpression in UC tissue	ZO-1 ↓, OCLN ↓	Chen et al., 2016 [52]
miRNA-375	Toll-like receptor 4 (TLR4)	Decreased in IBD tissue	ZO-1 ↓, OCLN ↓	Wu et al., 2019 [98]
miRNA-223	Claudin 8 (CLDN8)	Overexpression in IBD tissue	CLDN8 ↓	Wang et al., 2016 [68]

Because of the ability of miRNAs to bind to more than one target, miRNAs must be thoroughly investigated. The targets of proper miRNA must be known to prevent adverse interaction potentials in therapeutics based on miRNA technology. The administration and pharmacokinetics of potential therapeutics are issues that require further investigation. MiRNA detection and measurement are currently one of the most discussed topics across the scientific community. There are many studies that focus on the sensitivity and specificity of miRNAs' quantification methods. The most common techniques for detecting small RNAs can be divided into many groups, and their combinations: polymerase chain reactions (PCR), microarrays, new generation sequencing (NGS), miREIA, and so on. Processes for miRNA quantification and validation of the results still need to be optimized.

To summarize, miRNAs interact with many signaling pathways associated with immune response and TJ epithelial barrier integrity. They can provoke inflammation, contributing to increased secretion of many cytokines, but also could be overexpressed during proinflammatory stimulation and thus be responsible for a cytokine-induced negative effect on TJ proteins expression and IEB function. Table 1 demonstrates miRNAs collected in this review with their targets, expression in IBD, and TJ proteins they affected. The table was divided into in vivo and in vitro examined effects on TJ proteins expression induced by altered miRNA expression.

Extensive research on the influence of miRNAs involvement in pathogenesis and pathophysiology of IBD results in better knowledge, but many mechanisms remain incomprehensible. Gathering certain information about miRNA regulatory pathways of intestinal epithelial barrier dysfunction in IBD may be useful in planning future clinical trials and studies focusing on miRNAs interactions. This growing knowledge creates new chances to find innovative therapies. Therefore, miRNAs are of great interest in the therapeutic field. Their potential in the diagnosis of IBD and as potential therapeutics should be examined in the future.

Authors' Contribution

B.O.: research concept and design, acquisition of data, data analysis and interpretation, writing – original draft preparation, writing – review and editing, visualization, literature review; **A.K.**: research concept and design, writing – review and editing, data analysis and interpretation, final proofreading and approval of the version for publication; **E.Ś.**: supervising the project, final proofreading and approval of the version for publication, funding acquisition.

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Conflict of Interest

The authors have no potential conflicts of interest to declare.

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