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Deep Dive Into MicroRNAs in Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD), which includes ulcerative colitis and Crohn's disease, is thought to develop in genetically predisposed individuals as a consequence of complex interactions between dysregulated inflammatory stimuli, immunological responses, and environmental factors. The pathogenesis of IBD has yet to be fully understood. The global increase in the incidence of IBD suggests a gap in the current understanding of the disease. The development of a new diagnostic tool for inflammatory bowel disease that is both less invasive and more cost-effective would allow for better management of this condition. MicroRNAs (miRNAs) are a class of noncoding RNAs with important roles as posttranscriptional regulators of gene expression, which has led to new insights into understanding IBD. Using techniques such as microarrays and real-time polymerase chain reactions, researchers have investigated the patterns in which patients with Crohn's disease and ulcerative colitis show alterations in the expression of miRNA in tissue, blood, and feces. These miRNAs are found to be differentially expressed in IBD and implicated in its pathogenesis through alterations in autophagy, intestinal barrier, and immune homeostasis. In this review, we discuss the miRNA expression profiles associated with IBD in tissue, peripheral blood, and feces and provide an overview of the miRNA mechanisms involved in IBD.

Lay Summary

We review the published studies on microRNA (miRNA) expression in inflammatory bowel disease, including miRNAs extracted from blood, tissue, and stool samples. We discuss the main mechanisms of miRNA involvement in inflammatory bowel disease and their potential use as biomarkers.

Key Words: microRNAs, IBD

Introduction

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is becoming a global disease due to the worldwide increase in its incidence and prevalence. Although advances have been made in understanding the pathogenesis of IBD, the leading causes still need to be fully understood. Studies in the last 2 decades have accumulated evidence that the syndromic nature of the disease results from interactions between host-derived and external components, including gut microbiota, the immune system, host genetic factors, and environmental factors.¹

Recently, significant attempts have been made to find novel and convenient alternative tools that are both noninvasive and cost-effective to facilitate the diagnosis and monitoring of IBD. These features are fulfilled by biomarkers, which may be surrogate indicators of intestinal inflammation and tissue damage. Many biomarkers have been proposed in the clinical approach to diagnosing IBD, but with some drawbacks.

C-reactive protein (CRP), for instance, is not specific and can be elevated in many other inflammatory and autoimmune diseases.² In addition to its low sensitivity of 0.49 (95% confidence interval, 0.72–0.98), it has been shown that around 15% of patients with IBD do not mount a CRP response during disease flares.³ It was recently coupled with 12 additional proteins to develop an endoscopic healing index to identify individuals with CD who were in endoscopic remission. This index had a high overall accuracy in identifying individuals in endoscopic remission. The area under the receiver-operating characteristic (ROC) curve values for endoscopic healing index were equivalent to those for fecal calprotectin (FC) but greater than those for serum CRP alone.⁴

FC has an overall sensitivity of 80% and a specificity of 76% for IBD diagnosis.⁵ The same study showed better accuracy with higher sensitivity and specificity of FC for CD than for UC. Other meta-analyses showed higher sensitivity levels (95%) and specificity (91%) for the diagnosis of IBD.⁶ FC is used as a complementary tool after establishing the diagnosis

Key Messages

- What is already known: miRNAs are involved in IBD pathogenesis, and many studies have been conducted to find miRNAs that can be used as biomarkers for IBD.
- What is new here: we review these studies to provide more understanding of the progress that has been achieved in this field.
- How this study can help in patient care: fecal miRNAs are promising biomarkers that could be used in the future.

to distinguish IBD types, monitor activity, and assess the response to the treatment for IBD. Lactoferrin also counts as a useful tool for assessing intestinal inflammation.⁷ However, neither FC nor lactoferrin allows for strict discrimination between CD and UC.

MicroRNAs (miRNAs) are noncoding RNAs that range in length from 18 to 23 nucleotides and influence posttranscriptional gene expression. They have a crucial role in the pathophysiology of various common disorders, including IBD.⁸ Because these miRNAs can be detected in different biofluids and are resistant to degradation by RNases, they are promising biomarker candidates for IBD.^{9,10} Despite encouraging results from several studies, further studies are needed to validate their adoption as biomarkers of IBD in clinical practice. The growing number of reported miRNAs detected in the intestinal biopsies, peripheral blood, saliva, and stool of IBD patients, and differentially expressed in CD and UC, suggest that they might be determining factors in the pathogenesis of IBD and could be a useful diagnostic and monitoring tool in IBD.

miRNAs in Tissues

Dysregulated miRNAs in UC Colon Tissue

Since the first study conducted by Wu et al,¹¹ which investigated miRNAs in the colon tissues of IBD patients in 2008 to establish a link between IBD and altered miRNA expression, many related studies have been carried out (Figures 1 and 2). The sigmoid colon biopsies were obtained during screening colonoscopies of patients with active colonic CD, chronic UC (active or inactive), microscopic colitis, infectious colitis, or irritable bowel syndrome and normal healthy patients to assess miRNAs in these samples.¹¹ Comparing active UC patients with healthy control subjects, Wu et al found that miR-16, -21, -23a, -24, -29a, -126, -195, and let-7f were upregulated, whereas miR-192, -375, and -422b were downregulated. When comparing CD tissues with healthy control tissues, there was no significant expression of active UC-associated miRNAs. This study showed that miR-192 is inversely correlated with the macrophage inflammatory peptide-2 alpha, an epithelial cell-derived chemokine, suggesting that miR-192 targets macrophage inflammatory peptide-2 and plays a negative regulatory role in inflammation.

Some of these miRNAs identified in the study of Wu et al¹¹ were reported in several other studies in the following years. For example, Feng et al¹² partially replicated the same results in 2012, identifying 2 miRNAs previously identified by Wu et al that were upregulated in UC and acted via different signaling pathways. When they compared the expression level of miR-21 and miR-126 in the sigmoid colon

biopsies of active UC patients and groups including inactive UC, healthy control subjects, and irritable bowel syndrome patients, they found a higher expression level in patients with active UC. The messenger RNA (mRNA) of the *NFKBIA* (coding for IκBα) and protein expression were lower in the active UC group compared with the other groups. The finding that miR-126 targets *NFKBIA* mRNA suggests that miR-126 could be involved in the pathophysiology of active UC. In a study conducted 5 years later, the authors focused on miR-21 and miR-126 found in a smaller cohort and took a further step to identify which colonic cells express these 2 most frequently reported altered miRNAs in IBD and their differential expression in UC and CD patients.¹³ The results were consistent with those reported by Feng et al, in which miR-21 and miR-126 levels were higher in UC colon biopsies compared with control subjects. On the other hand, only miR-21 significantly increased in UC biopsies compared with CD biopsies. The expression of the 2 miRNAs was not statistically significant in CD patients compared with control subjects. Takagi et al¹⁴ reported elevated levels of 7 miRNAs, miR-21, -155, -923, let-7a, let-7c, let-7d, and let-7g, in colon biopsy tissues of active UC patients compared with healthy control subjects. However, only miR-155 and miR-21 had significantly increased.

One of the early and leading studies in this field in 2010 investigated miRNAs that are differentially expressed between UC and CD patients' quiescent (noninflamed) colonic mucosa and healthy control subjects.¹⁵ The levels of miR-7, -26a, -29a, -29b, -31, -126-5p, -127-3p, -135b, and -324-3p were upregulated in UC patients' inflamed mucosa compared with healthy control subjects, with 4 of them being specific to inflamed mucosa. Only miRNAs with a >5-fold increased expression or a <0.2-fold decrease compared with control subjects were considered to minimize false classification. This may have resulted in the exclusion of miRNAs with a smaller but statistically significant altered expression.¹⁵ An attempt was made by Coskun et al¹⁶ to identify new miRNAs in IBDs that can be used as biomarkers. Endoscopic colonic mucosal punch biopsies were obtained from patients with quiescent UC or CD, active UC or CD, and healthy control subjects. There was an increase in miR-20b, -99a, -203, -26b, and -98 in colon biopsies from active UC patients compared with those in inactive UC patients, CD patients, and healthy control subjects. Two miRNAs (let-7e-3p and miR-125b-1-3p) were expressed more in quiescent UC than in active UC, CD, and control subjects. This was the first time that these miRNAs were identified in UC disease. Because miR-20b was expressed in both quiescent and active UC biopsies compared with control subjects, the researchers speculated that it might be used as a possible biomarker. This study identified IBD-associated target genes, including cytokine and kinase signaling pathways.

Use of miRNAs to Distinguish UC From CD

More comprehensive studies compared the expression of miRNAs between CD and UC vs control subjects, with interesting results. Most of these studies concentrated on finding a potential miRNA that can be used to diagnose IBD or to distinguish UC from CD. On the other hand, some of these studies were interested in certain miRNAs previously identified as IBD-associated miRNAs. Identifying miRNA target genes was also of interest for use as a therapeutic target in the future.

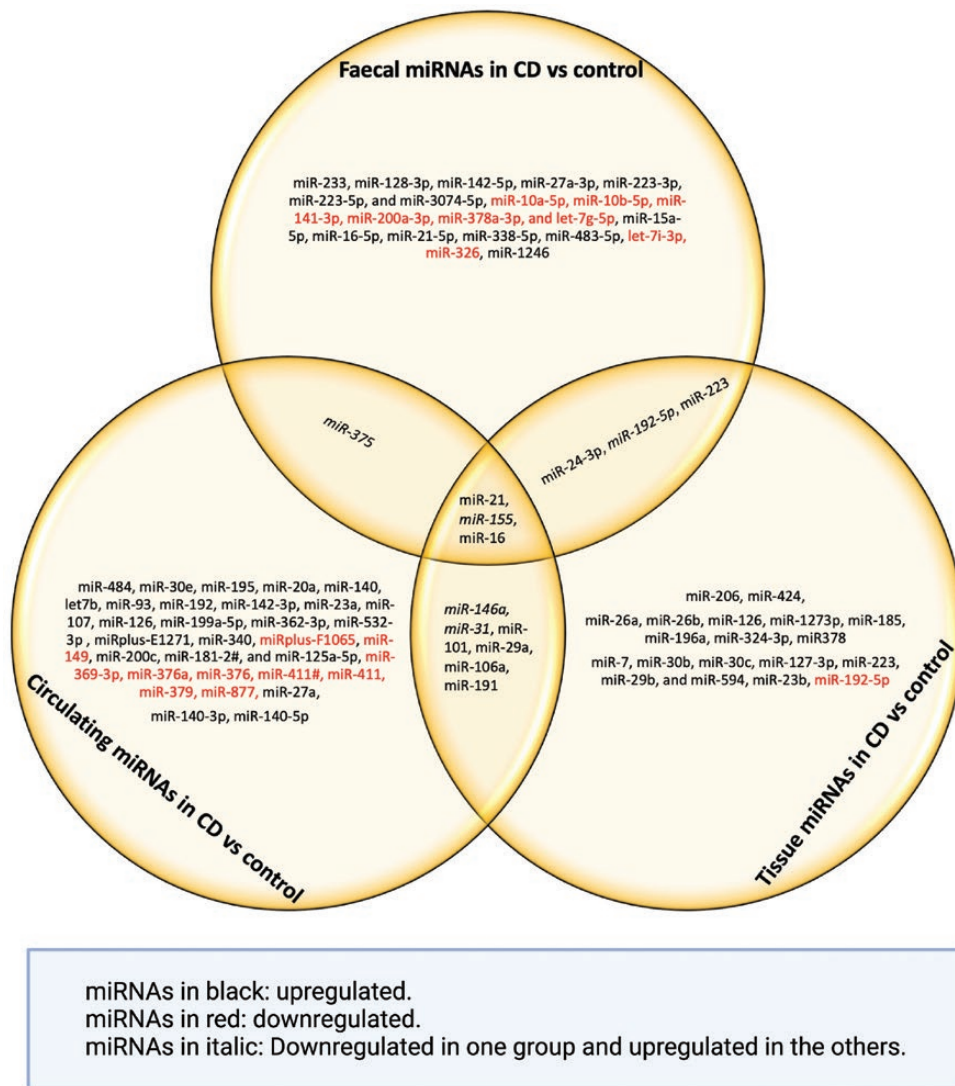


Figure 1. Summary of dysregulated microRNAs (miRNAs) in Crohn's disease (CD).

Lin et al¹⁷ tried to find new IBD-specific miRNA markers that distinguish IBD from other forms of enterocolitis. They studied miRNA expression in mucosal colonic samples obtained after colectomy from patients with UC, CD, and diverticular diseases (as control subjects) and other colitis patients. The study found that miR-31, -146a, -206, and -424 levels were higher in CD patients' fresh-frozen tissue samples than in control subjects, a finding seen in UC patients in the same study.¹⁷ This finding suggested a shared pathophysiological mechanism that mediates the development of these 2 diseases. However, in formalin-fixed, paraffin-embedded tissue, the differential expression of miR-206, -146a, and -424 was insignificant. This could be explained by the loss or alteration of signals during processing or by the possibility that these miRNAs are not actual IBD-specific markers. This was the first study to report miR-31 upregulation in formalin-fixed, paraffin-embedded tissues of colonic mucosa in both UC and CD with moderate sensitivity and specificity (71% and 67%, respectively), suggesting that it can be used as a biomarker for IBD regardless of disease activity and differentiating it from other forms of colitis. This finding of elevated miR-31 in UC and CD patients was supported later

by Schaefer et al,¹⁸ in which it was also suggested as a potential biomarker. This study analyzed miRNAs in biopsies, peripheral blood, and saliva. In matched colon biopsies from involved and noninvolved areas in UC and CD patients, miR-31 had the highest level of expression. However, only the CD pairs showed a statistically significant level of expression.¹⁸ In the CD-paired colon samples, 9 miRNAs (miR-21, -31, -101, -142-3p, -142-5p, -155, -223, -375, and -494) increased at a statistically significant level in endoscopically involved segments compared with those in endoscopically uninvolved segments. By doing a similar comparison in UC samples, 6 miRNAs (miR-21, -101, -142-5p, -146a, -155, and -223) were significantly elevated in samples of endoscopically involved segments. Schaefer et al also reported that the levels of miR-31, -101, and -146a were higher in colon biopsies from patients with CD compared with those from healthy control subjects. On the other hand, colonic biopsies from patients with UC had considerably higher miR-19a, -21, -31, and -101 than those from healthy control subjects. Certain miRNAs (miR-19a, -21, -31, -101, -146a, and -375) were suggested as biomarkers to identify IBD and distinguish UC from CD.

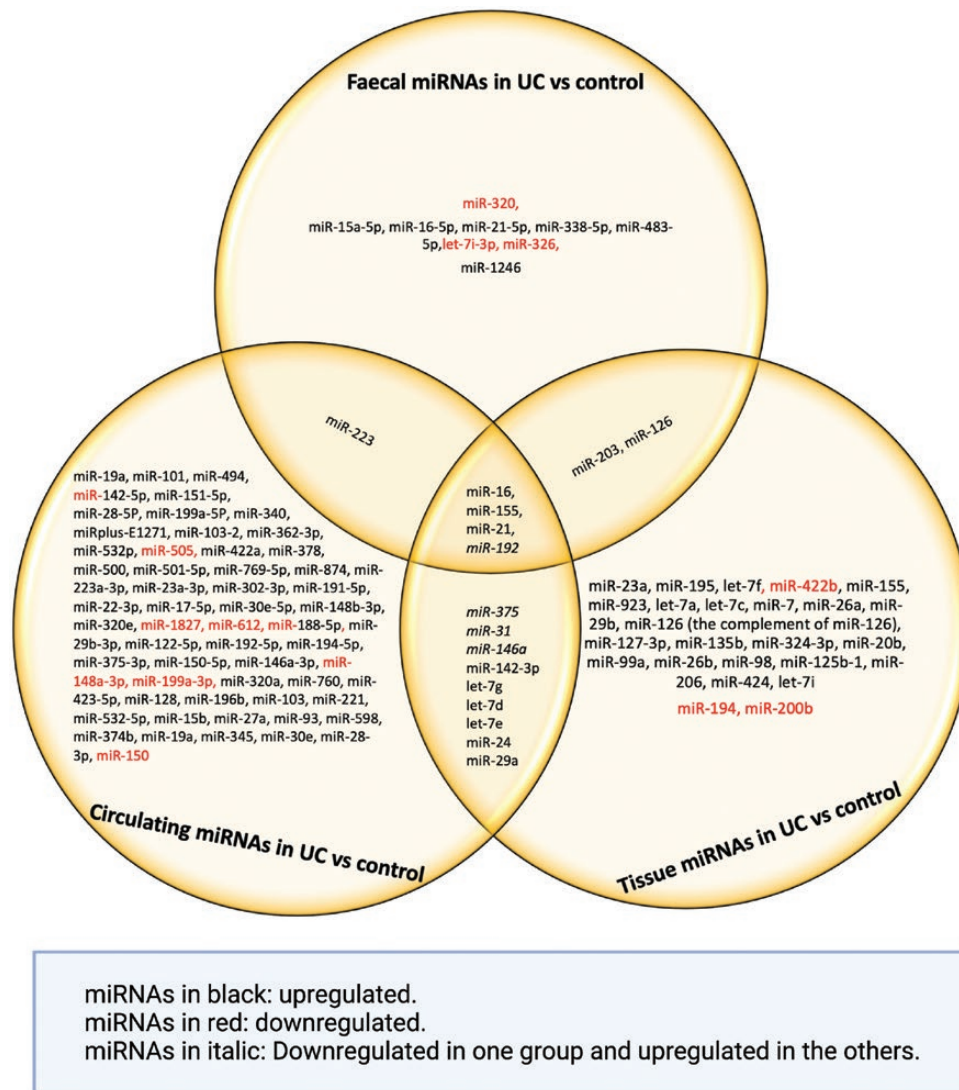


Figure 2. Summary of dysregulated microRNAs (miRNAs) in ulcerative colitis (UC).

Despite being one of the most commonly and repeatedly reported miRNAs in the analysis of tissues in IBD, miR-21's association with the disease has long been inconclusive. A systematic review and meta-analysis of 20 articles were thus conducted recently by Yan et al,¹⁹ including 459 patients with CD, 540 patients with UC, and 511 non-IBD control subjects, to determine the association between IBD risk and the expression of miR-21. Compared with non-IBD control subjects, the expression of miR-21 in colon tissue was significantly higher in patients with UC and CD. On the other hand, miR-21 expression was not significantly different between UC and CD patients. The increased level of miR-21 expression was linked with the disease activity in UC; however, this was not seen in CD.

According to Fasseu et al,¹⁵ several miRNAs were shown to be expressed more in the inflamed mucosa of CD patients compared with healthy control subjects, including miR-26a, -26b, -126-5p, -155, -1273p, -185, -196a, -324-3p, and -378. Among these miRNAs, 5 were expressed exclusively in inflamed tissues. The miRNAs that were reported in noninflamed mucosa were miR-7, -26a, -30b, -30c, -127-3p, -155, -223, -324-3p, -29a, -29b, -126-5p, and -196a. In this

study, 6 miRNAs (miR-150, -196b, -199a-3p, -199b-5p, -223, and -320a) with differential expression in UC and CD mucosal tissues were identified. These and another 9 miRNAs were examined for their ability to discriminate between UC and CD. This panel of 15 miRNAs successfully identified 15 (94%) of 16 patients as UC or CD.

In 2013, Iborra et al²⁰ investigated the miRNA profiles in the mucosa and serum of patients with CD (9 active CD and 9 inactive CD) and UC (9 active UC and 9 inactive UC). The patients included had different stages and localization of the disease. Seven miRNAs (4 upregulated and 3 downregulated) were expressed differentially in the mucosa of active CD compared with inactive CD. Five tissue miRNAs (2 upregulated and 3 downregulated) could differentiate active UC from inactive UC. miR-140-3p, expressed in the tissue of active CD, coincided with one of the miRNAs exclusively expressed in the blood of patients with CD (both active and inactive CD). Mucosal miRNA expression patterns in active UC did not correspond with those observed exclusively in active CD, suggesting that tissue miRNA expression patterns might be used to discriminate between the 2 diseases.

Regional Expression of miRNAs

Interestingly, the regional expression of miRNAs in CD has been reported. Ileal and sigmoid biopsies from patients with chronic active ileal and colonic CD were examined to assess miRNA expression.²¹ The expression of miR-16, -21, -223, and -594 was elevated in patients with chronically active terminal ileal CD compared with healthy control subjects. miR-23b, -106a, and -191 were overexpressed in Crohn's colitis compared with healthy control subjects. The miRNA expression in the terminal ileum in patients with active CD was further studied but focused on comparing inflamed and noninflamed terminal ileal mucosa with the healthy control subjects.²² They found that miR-192-5p was downregulated in inflamed mucosa compared with the mucosa of healthy groups. miR-495-5p was considerably elevated in noninflamed mucosa compared with healthy mucosa, but let-7b-5p was reduced. Inflamed mucosa had a significantly greater level of miR-361-3p and a significantly lower level of miR-124-3p than noninflamed mucosa. In 2014, Zahm et al²³ were the first to study miRNA expression in rectal biopsies from children with IBD, including 18 UC patients and 12 CD patients. Along with rectal mucosal biopsies, blood samples were collected to assess whether alterations in rectal miRNAs are reflected in patients' sera. Four miRNAs in rectal biopsies (miR-192, -194, -200b, and -375) were significantly decreased in pediatric UC patients compared with control subjects, whereas 4 miRNAs (miR-21, -142-3p, -146a, and let-7i) were significantly upregulated. Only miR-24 levels were differentially expressed between UC and CD patients. They were higher in UC samples than in CD samples in rectal mucosal biopsies, with a sensitivity of 83.3%, specificity of 85.7%, and ROC analysis of 84%.

In conclusion, several miRNAs, in addition to the most commonly reported miR-16, -21, -126, and -192, were dysregulated in isolated tissues from UC and CD patients. They are summarized in [Table 1](#).

miRNAs in Blood, Serum, and Saliva

Despite providing major advances in understanding the pathophysiology of IBD, obtaining miRNAs endoscopically for use as diagnostic markers remains an invasive diagnostic method. In contrast, circulating miRNAs appear to have greater potential for use as less invasive biomarkers. Therefore, multiple studies profiled miRNA expression in blood, serum, or plasma ([Table 2](#)). Among the first studies to investigate the potential for circulating miRNAs to be noninvasive biomarkers of pediatric CD was the work by Zahm et al,²⁴ which suggested that damage to the highly vascularized intestinal epithelia with lymphocyte-rich lamina propria may lead to an increase in epithelium-specific miRNAs in the bloodstream. Twenty-four miRNAs that were elevated in CD patients' serum were also examined and found to be unchanged in celiac disease patients' serum compared with serum samples from healthy control subjects, suggesting that the elevation of miRNAs is not due to inflammation-mediated damage to the small intestine mucosa. Zahm et al²⁴ reported 11 elevated miRNAs in pediatric CD patients' serum samples compared with normal control subjects. These are miR-16, -484, -30e, -106a, -195, -20a, -21, -140, -192, -93, and let-7b. These serum miRNAs offer promising diagnostic potential with sensitivities of 70% to 83%

and specificities of 75% to 100%. In the study conducted by Schaefer et al,¹⁸ the miRNA expression profile was compared between colon biopsies, blood, and saliva from UC and CD patients. Compared with normal samples, miR-21, -31, -146a, and -155 were considerably lower in CD blood samples, but miR-101 and miR-375 were significantly increased. In UC blood samples, miR-21, -31, and -146a levels were substantially lower than in normal samples, but miR-19a, -101, -142-5p, -223, miR-375, and -494 levels were greater. One miRNA (miR-101) in saliva was significantly higher in CD samples than in normal samples. In UC saliva samples, compared with those in normal samples, 3 miRNAs were significantly higher (miR-21, -31, and -142-3p), and 1 miRNA was significantly lower (miR-142-5p). Zahm et al²³ reported 3 miRNAs in the serum that were significantly elevated in both UC and CD patients compared with control subjects: miR-192, -21, and -142-3p. These serum miRNAs were found to have a sensitivity and specificity ranging between 75% and 80% and between 66% and 78%, respectively. However, these miRNAs could not differentiate UC from CD. According to Paraskevi et al,²⁵ some miRNAs might differentiate CD from UC. By using quantitative polymerase chain reaction (qPCR), this study identified that miR-16, -23a, -29a, -106a, -107, -126, -191, -199a-5p, -200c, -362-3p, and -532-3p were considerably overexpressed in the blood of CD patients compared with healthy control subjects. The expression level of miR-16, -21, -28-5p, -151-5p, -155, and -199a-5p was markedly elevated in UC patients compared with the healthy control subjects. Similarly, Wu et al²⁶ proposed that CD and UC patients may be distinguished from healthy persons by their miRNA expression patterns in a study that included 19 CD patients (14 active and 5 inactive) and 23 UC patients (13 active and 10 inactive). Patients with active CD had higher miR-199a-5p, -362-3p, -532-3p, and miRplus-E1271 in peripheral blood compared with healthy control subjects. Among these miRNAs, miR-362-3p showed the most significant difference in expression, with a 4.7-fold increase. The expression of miR-340-3p was increased in the peripheral blood of both active and inactive CD patients. At the same time, miRplus-F1065 was reduced only in the blood of inactive CD patients, and miR-149-3p decreased in both active and inactive CD patients. Only in patients with active UC was an increase in miR-28-5p, miR-151-5p, miR-199a-5p, miR-340-3p, and miRplus-E1271 in the peripheral blood. In contrast, inactive and active UC patients had elevated miR-103-2-5p, -362-3p, and -532p in peripheral blood samples. In both active and inactive UC patients, a single miRNA, miR-505-5p, was reduced in the blood. Eight miRNAs were confirmed by real-time qPCR to distinguish active UC from active CD: miR-28-5p, -103-2-5p, -149-3p, -151-5p, -340-3p, -505-5p, -532-3p, and miR-plus-E1153. When comparing platelet fraction–extracted miRNAs from 20 UC patients with 20 healthy control subjects, Duttagupta et al²⁷ identified 31 platelet-derived miRNAs with 92.8% accuracy, 96.2% specificity, and 89.5% sensitivity in differentiating UC patients from healthy people. Then, the expression of 7 platelet fraction–derived miRNAs was validated using qPCR (miR-188-5p, -422a, -378, -500, -501-5p, -769-5p, and -874). In 2015, Polyarchou et al²⁸ conducted a study in a larger cohort analyzing miRNAs in serum to assess their efficacy as diagnostic biomarkers in UC patients. miR-223a-3p, -23a-3p, -302-3p, -191-5p,

Table 1. Summary of studies of dysregulated miRNAs in tissues

Study	Disease	Upregulated miRNAs	Downregulated miRNAs
Wu et al ¹¹	Active UC patients vs HC subjects	<i>miR-16</i> , <i>miR-21</i> , <i>miR-23a</i> , <i>miR-24</i> , <i>miR-29a</i> , <i>miR-126</i> , <i>miR-195</i> , and <i>let-7f</i>	<i>miR-192</i> , <i>miR-375</i> , and <i>miR-422b</i>
Feng et al ¹²	Active UC patients vs inactive UC patients, irritable bowel syndrome, and HC subjects	<i>miR-126</i> and <i>miR-21</i>	
Thorlacius-Ussing et al ¹³	UC patients vs HC subjects UC patients vs CD	<i>miR-21</i> and <i>miR-126</i> <i>miRNA-21</i>	
Takagi et al ¹⁴	Active UC patients vs HC subjects	<i>miR-21</i> , <i>miR-155</i> , <i>miR-923</i> , <i>let-7a</i> , <i>let-7c</i> , <i>let-7d</i> , and <i>let-7g</i>	
Fasseu et al ¹⁵	Inflamed mucosa of UC patients vs HC subjects	<i>miR-7</i> , <i>miR-26a</i> , <i>miR-29a</i> , <i>miR-29b</i> , <i>miR-31</i> , <i>miR-126</i> (the complement of <i>miR-126</i>), <i>miR-127-3p</i> , <i>miR-135b</i> , and <i>miR-324-3p</i>	
Coskun et al ¹⁶	Active UC patients vs HC subjects, inactive UC patients, and CD Quiescent UC patients vs active UC patients, CD patients, and HC subjects Active and quiescent UC patients vs HC subjects	<i>miR-20b</i> , <i>miR-99a</i> , <i>miR-203</i> , <i>miR-26b</i> , and <i>miR-98</i> <i>miR-125b-1</i> and <i>let-7e</i> <i>miR-20b</i>	
Lin et al ¹⁷	CD patients and UC patients vs HC subjects	<i>miR-31</i> , <i>miR-146a</i> , <i>miR-206</i> , and <i>miR-424</i>	
Schaefer et al ¹⁸	CD paired colon samples (endoscopically involved colonic tissue compared with endoscopically uninvolved colonic tissue) UC paired colon samples (endoscopically involved colonic tissue compared with endoscopically uninvolved colonic tissue) CD patients vs HC subjects UC patients vs CD	<i>miR-21</i> , <i>miR-31</i> , <i>miR-101</i> , <i>miR-142-3p</i> , <i>miR-142-5p</i> , <i>miR-155</i> , <i>miR-223</i> , <i>miR-375</i> , and <i>miR-494</i> <i>miR-21</i> , <i>miR-101</i> , <i>miR-142-5p</i> , <i>miR-146a</i> , <i>miR-155</i> , and <i>miR-223</i> <i>miR-31</i> , <i>miR-101</i> , and <i>miR-146a</i> <i>miR-19a</i> , <i>miR-21</i> , <i>miR-31</i> , <i>miR-101</i> , <i>miR-146a</i> , and <i>miR-375</i>	
Fasseu et al ¹⁵	CD patients (inflamed mucosa) vs HC subjects CD patients (noninflamed mucosa) vs HC subjects	<i>miR-26a</i> , <i>miR-26b</i> , <i>miR-126</i> , <i>miR-155</i> , <i>miR-1273p</i> , <i>miR-185</i> , <i>miR-196a</i> , <i>miR-324-3p</i> , <i>miR-378</i> <i>miR-7</i> , <i>miR-26a</i> , <i>miR-30b</i> , <i>miR-30c</i> , <i>miR-127-3p</i> , <i>miR-155</i> , <i>miR-223</i> , <i>miR-324-3p</i> , <i>miR-29a</i> , <i>miR-29b</i> , <i>miR-126</i> , and <i>miR-196a</i>	
Wu et al ²¹	Chronically active terminal ileal CD patients vs HC subjects Crohn's colitis patients vs HC subjects	<i>miR-16</i> , <i>miR-21</i> , <i>miR-223</i> , and <i>miR-594</i> <i>miR-23b</i> , <i>106a</i> , and <i>191</i>	
Guo et al ²²	CD patients (ilium inflamed mucosa) vs HC subjects CD patients (noninflamed mucosa vs healthy mucosa) CD patients (inflamed mucosa vs noninflamed mucosa)	<i>miR-495-5p</i> <i>miR-361-3p</i>	<i>miR-192-5p</i> <i>let-7b-5p</i> <i>miR-124-3p</i>
Zahm et al ²³	UC patients (rectal biopsies) vs HC subjects	<i>miR-21</i> , <i>miR-142-3p</i> , <i>miR-146a</i> , and <i>let-7i</i>	<i>miR-192</i> , <i>miR-194</i> , <i>miR-200b</i> , and <i>miR-375</i>
Iborra et al ²⁰	Active CD patients (mucosa) vs inactive CD patients Active UC patients (mucosa) vs inactive UC patients	<i>miR-18a</i> , <i>miR-629</i> , <i>let-7b</i> , <i>miR-140-3p</i> <i>miR-650</i> , <i>miR-548a-3p</i>	<i>miR-422a</i> , <i>miR-885-5p</i> , <i>miR-328</i> <i>miR-630</i> , <i>miR-489</i> , <i>miR-196b</i>

Italicized miRNAs are also reported in stool in Table 3.

Abbreviations: CD, Crohn's disease; HC, healthy control; miRNA, microRNA; UC, ulcerative colitis.

-22-3p, -17-5p, -30e-5p, -148b-3p, and -320e were elevated in UC patient serum samples compared with control serum samples, whereas *miR-1827*, -612, and -188-5p were downregulated. Interestingly, the platelet fraction-extracted miRNAs and serum-extracted miRNAs were different. There

was no overlap between the 2 reported signatures of circulating miRNAs in the 2 studies except for a single miRNA, *miR-188-5p*.^{27,28}

Jensen et al²⁹ measured miRNA levels in the plasma of CD patients by real-time qPCR, searching for circulating

Table 2. Summary of studies of dysregulated circulating miRNAs

Study	Disease	Upregulated miRNAs	Downregulated miRNAs
Zahm et al ²⁴	CD patient serum samples vs HC subjects	<i>miR-16</i> , miR-484, miR-30e, miR-106a, miR-195, miR-20a, <i>miR-21</i> , miR-140, let7b, <i>miR-192</i> , miR-93	
Schaefer et al ¹⁸	CD patient blood samples vs HC subjects	miR-101 and <i>miR-375</i>	<i>miR-21</i> , miR-31, miR-146a, and <i>miR-155</i>
	UC patient blood samples vs HC subjects	miR-19a, miR-101, miR-142-5p, <i>miR-223</i> , <i>miR-375</i> , and miR-494	<i>miR-21</i> , miR-31, and miR-146a
	CD patient saliva samples vs HC subjects	miR-101	
	UC patient saliva samples vs HC subjects	<i>miR-21</i> , miR-31, and miR-142-3p	<i>miR-142-5p</i>
Zahm et al ²³	UC and CD serum patients compared with HC subjects	<i>miR-192</i> , <i>miR-21</i> , and miR-142-3p	
Paraskevi et al ²⁵	CD patient blood samples vs HC subjects	<i>miR-16</i> , miR-23a, miR-29a, miR-106a, miR-107, <i>miR-126</i> , miR-191, miR-199a-5p, miR-200c, miR-362-3p, and miR-532-3p	
	UC patient blood samples vs HC subjects	<i>miR-16</i> , <i>miR-21</i> , miR-28-5p, miR-151-5p, <i>miR-155</i> , and miR-199a-5p	
Wu et al ²⁶	Active CD patient blood samples vs HC subjects	miR-199a-5p, miR-362-3p, and miR-532-3p, miRplus-E1271	
	CD patient (active and inactive) blood samples vs HC subjects	miR-340	
	Active CD patient blood samples vs HC subjects		miRplus-F1065
	Active and inactive CD patient blood samples vs HC subjects		miR-149
	Active UC patient blood samples vs HC subjects	miRs-28-5p, miRNAs-151-5p, miR-199a-5p, miR-340, and miRplus-E1271	
	Inactive and active UC patients vs HC subjects	miR-103-2, miR-362-3p, and miR-532p	miR-505
Duttgupta et al ²⁷	UC patients (platelet fraction) vs HC subjects	miR-188-5p, miR-422a, miR-378, miR-500, miR-501-5p, miR-769-5p, and miR-874	
Polytarchou et al ²⁸	UC patient serum samples vs HC subjects	<i>miR-223a-3p</i> , miR-23a-3p, miR-302-3p, miR-191-5p, miR-22-3p, miR-17-5p, miR-30e-5p, miR-148b-3p, and miR-320e	miR-1827, miR-612, and miR-188-5p
Jensen et al ²⁹	CD patient plasma samples vs HC subjects	miR-200c, miR-181-2#, and miR-125a-5p	miR-369-3p, miR-376a, miR-376, miR-411#, miR-411, and miR-379
Viennois et al ³⁰	UC patient serum samples vs control	miR-29b-3p, miR-122-5p, miR-192-5p, miR-194-5p, miR-375-3p, miR-150-5p, and miR-146a-3p	miR-148a-3p and miR-199a-3p
Yang et al ³¹	UC patient mucosal and serum samples vs HC subjects	<i>miR-21</i>	
Cordes et al ³²	Active CD and UC patients vs CD patients and UC patients in remission	miR-320a	
Iborra et al. ²⁰	CD patient serum (active and inactive CD) vs HC subjects	miR-27a, miR-140-3p, miR-140-5p, miR-16, miR-195 over	miR-877
	Active CD patients vs inactive CD	miR-188-5p, miR-877	miR-140-5p, miR-145, miR-18a, miR-128
	UC patient (both active and inactive UC) serum vs HC subjects	miR-760, miR-423-5p, miR-128, miR-196b, miR-103, miR-221, miR-532-5p, miR-15b, miR-27a, let-7g, miR-93, let-7d, miR-598, <i>miR-142-5p</i> , let-7e, miR-223, miR-19a, miR-345, miR-199a-3p, miR-24, miR-30e, miR-29a, miR-28-3p	miR-150

Italicized miRNAs are also reported in stool in [Table 3](#).

Abbreviations: CD, Crohn's disease; HC, healthy control; miRNA, microRNA; UC, ulcerative colitis.

miRNA in CD. When comparing CD patients with control subjects, miR-369-3p, -376a, -376, -411#, -411, and -379 were downregulated, while miR-200c, -181-2#, and -125a-5p were found to be elevated. miR-16 expression was substantially reduced in individuals with CD compared with control subjects, with ROC analysis showing an area under the curve (AUC) of 0.65 for discriminating CD from non-CD; they suggested that its serum reduction may be used to diagnose CD. Viennois et al³⁰ conducted a study assessing the alterations in serum miRNAs during the development of intestinal inflammation in *IL10^{-/-}* mice. From the 9 validated miRNAs, 7 were upregulated (miR-29b-3p, -122-5p, -192-5p, -194-5p, -375-3p, -150-5p, and -146a-3p) and 2 were downregulated (miR-148a-3p and miR-199a-3p). They showed that these serum miRNAs have an accuracy of 83% in distinguishing UC samples from control samples. Cordes et al³² reported that miR-320a in the blood could detect IBD activity with the ability to differentiate IBD from infectious colitis. Patients with active CD and UC had significantly higher miR-320a blood levels than patients in remission and healthy control subjects. The expression of miR-320a was strongly correlated with endoscopic disease activity.

In the study of Iborra et al,²⁰ 6 serum miRNAs (5 miRNAs were upregulated and only miR-877 was downregulated) were expressed specifically in CD patients (both active and inactive CD) when compared with healthy control subjects. Six serum miRNAs (2 miRNAs were upregulated and 4 were downregulated) were found to be differentially expressed when the active CD was compared with the inactive CD. Twenty-five miRNAs were found to be specifically expressed in the serum of UC patients compared with healthy control subjects, with 24 miRNAs showing an upregulation and 1 miRNA showing a downregulation (miR-150). No serum miRNAs were regulated specifically in active UC patients compared with inactive UC patients. There were 13 miRNAs expressed commonly in the peripheral blood of CD and UC patients.

Using circulating miRNAs is less invasive than studying miRNAs extracted from tissues. However, there is only a partial overlap when comparing miRNA profiles obtained from these different sources. In addition to the previously

mentioned miR-16, -192, and miR-21, only a few circulating miRNAs are associated with the IBD state in tissue (Figures 1 and 2).

miRNAs in Stool

Besides being a less invasive method and more easily collected, using fecal miRNAs as a biomarker also has other advantages. miRNA expression profiling in the blood largely reflects miRNA extrusion from various distant tissues and organs, in contrast to fecal miRNAs derived predominantly from intestinal epithelial cells.³³ These epithelial cells are shed continuously into the intestinal lumen as a normal physiological turnover process; consequently, miRNAs detected in the feces might reflect more precise results.^{34,35} Compared with miRNA expression in tissues and peripheral blood, the expression of fecal miRNAs was less explored by researchers. However, only some studies included stool as part of the analysis, and fewer were dedicated to studying only fecal miRNA expression (Table 3).

Schönauen et al³⁶ examined the expression of 4 circulating and fecal miRNAs (miR-16, -21, -155, and -223) in 43 patients with CD and 18 with UC. Among these patients, 38 had active disease, and 23 were in remission. Three of the 4 miRNAs (miR-16, -155, and -223) were expressed at a higher level in the feces of CD and UC patients compared with control subjects, with higher expression found in UC patients compared with CD patients. A subgroup analysis showed that all the miRNAs tested were expressed more in feces from IBD patients with active illnesses when compared with control subjects, except for miR-223 and miR-16, which were found to be higher when compared with IBD patients in remission. Interestingly, only fecal miRNAs were correlated with clinical parameters such as CRP or FC, but not serum miRNAs. The 2 most highly expressed miRNAs, miR-223 and miR-155, were further validated by PCR, and miR-223 was suggested as a potential fecal biomarker with 80% sensitivity and 93% specificity to distinguish patients with active disease from those in remission.

The expression of miRNAs was studied specifically in CD patients. Several fecal miRNAs were identified as potential

Table 3. Summary of studies of dysregulated fecal miRNAs

Study	Disease	Upregulated miRNAs	Downregulated miRNAs
Schönauen et al ³⁶	CD and UC patients vs control subjects	miR-16, miR-155, miR-233	
	Active CD and UC patients vs control subjects	miR-21, miR-155	
	CD and UC patients in remission vs control subjects	miR-16 and miR-223	
Wohnhaas et al ³⁴	CD patients vs control subjects	miR-15a-5p, miR-16-5p, miR-128-3p, miR-142-5p, miR-24-3p, miR-27a-3p, miR-223-3p, miR-223-5p, and miR-3074-5p	miR-10a-5p, miR-10b-5p, miR-141-3p, miR-192-5p, miR-200a-3p, miR-375, miR-378a-3p, and let-7g-5p
Ahmed et al ³⁷	UC patients vs control subjects	miR-21, miR-203, miR-126, and miR16	miR-320 and miR-192
Zhou et al ³⁸	CD and UC patients vs control subjects	miR-15a-5p, miR-16-5p, miR-21-5p, miR-338-5p, miR-483-5p	let-7i-3p and miR-326
Verdier et al ³⁹	Active CD patients vs control subjects and UC patients vs control subjects	miR-223 and miR-1246	

Abbreviations: CD, Crohn's disease; miRNA, microRNA; UC, ulcerative colitis.

biomarkers in a cohort of 52 patients with CD. Nine miRNAs (miR-15a-5p, -16-5p, -128-3p, -142-5p, -24-3p, -27a-3p, -223-3p, -223-5p, and -3074-5p) were upregulated, while 8 miRNAs (miR-10a-5p, -10b-5p, -141-3p, -192-5p, -200a-3p, -375, -378a-3p, and let-7g-5p) were downregulated.³⁴ Some of these miRNAs were negatively correlated with the Crohn's Disease Activity Index, while others were positively correlated.

In the study by Ahmed et al,³⁷ 4 miRNAs (miR-21, -203, -126, and -16) were upregulated in UC patients' feces, whereas 2 miRNAs (miR-320 and miR-192) were found to be downregulated. These were not seen in the stool of Crohn's patients.³⁷

Recently, a study by Zhou et al³⁸ was carried out to identify new possible miRNA biomarkers in the feces of IBD patients with better sensitivity. The study included 22 UC patients and 19 CD patients. Using microarray analysis, they found the overexpression of 5 miRNAs (miR-15a-5p, -16-5p, -21-5p, -338-5p, and -483-5p) and downregulation of 2 miRNAs (let-7i-3p and miR-326) in the feces of IBD patients when compared with the feces of healthy control subjects. However, when these miRNAs were verified by real-time qPCR, only 2 showed a significant increase compared with healthy control subjects. In both UC and CD patients' feces compared with healthy control subjects, miR-16-5p expression was significantly higher. It was overexpressed in the CD intestinal mucosa compared with its expression in healthy control subjects. However, only UC patients' feces showed an increase in miR-21-5p. An ROC curve analysis of miR-16-5p showed an AUC of 0.868 for CD and 0.853 for UC. The AUC of miR-21-5p for the diagnostic accuracy of UC was found to be 0.810 when the ROC curve was analyzed.

A large cohort screening study of fecal miRNAs in IBDs was conducted by Verdier et al³⁹ using NanoString technology. Although 150 fecal miRNAs were detected in the feces of both CD patients and control subjects, the levels were higher in CD samples than in control subjects. Only 2 miRNAs were found at a higher level in active CD patients' feces: miR-223 and miR-1246. Similarly, UC patients had higher levels of miR-223 and miR-1246 in their feces compared with healthy control subjects, with miR-223 showing higher levels in intestinal tissues and correlation with fecal calprotectin.

Mechanisms of miRNA Involvement in IBD

Several miRNAs are involved in the pathogenesis of IBD through interrelated mechanisms (Figure 3).^{40,41} These mechanisms include dysregulation of the intestinal epithelial barrier and autophagy inhibition, which lead to immune homeostasis disorder. These miRNAs regulate these molecular processes by targeting specific IBD-associated genes via complex pathways. With an increasing number of IBD-related miRNAs being identified, many studies have investigated the pathophysiological role of those miRNAs.

Dysregulation of the Intestinal Epithelial Barrier

Among the key mechanisms involving miRNA in IBD pathogenesis is the impairment of the intestinal barrier function.⁴² miR-21 is consistently detected in many studies and reported to be associated with IBD. Yang et al³¹ reported that the mucosal and serum levels of miR-21 were elevated in UC patients compared with healthy control subjects, and there was

increased intestinal permeability in the UC mucosa and the Caco-2 cells model. miR-21 targets *RhoB* mRNA, resulting in *RhoB* depletion and tight junction dysfunction in intestinal epithelial cells. The intestinal permeability of Caco-2 cells grown in monolayers was significantly increased when miR-21 was overexpressed but dramatically lowered when miR-21 was knocked off.³¹ It was found that miR-21 control subjects intestinal epithelial tight junction permeability through the PTEN/PI3K/Akt signaling pathway.⁴³ Using the same cellular model, Ye et al⁴⁴ reported that tumor necrosis factor α (TNF- α) control subjects intestinal permeability by promoting miR-122a-mediated occludin mRNA degradation. Additionally, miR-122a regulates the mRNA of zonulin, a protein found in tight junctions in the intestine that may be used as an indicator of intestinal permeability.⁴⁵ Its overexpression promoted zonulin production and intestinal permeability by targeting the epidermal growth factor receptor, which was identified as a miR-122a target gene.⁴⁶ Aquaporin 3 mRNA, which codes for a water/glycerol transporter expressed at the basolateral membrane of colonic epithelial cells, is targeted by miR-874. Moreover, 2 tight junction proteins, occludin and claudin-1, were involved in miR-874-induced intestinal barrier dysfunction. When Caco-2 cells were treated with TNF- α , an essential mediator of inflammation in the gut, the overexpression of miR-874 further increased paracellular permeability.⁴⁶ Wang et al showed that miR-191a works through its direct target, zonula occludens-1 (ZO-1), one of the epithelial junctional proteins that maintain the permeability of the intestinal barrier.⁴⁷ This in vitro study showed that the ZO-1 protein inside intestinal epithelial cells was depleted following the overexpression of this miRNA and suggested that the TNF- α pathway induced this depletion. ZO-1 was also reported to be targeted by another miRNA.⁴⁸ Alcohol-induced overexpression of miR-212 in Caco-2 intestinal epithelial cells decreased ZO-1 protein expression, promoting intestinal hyperpermeability. This finding was replicated in an animal model in which alcohol-fed mice subjected to miR-212 knockdown techniques showed a substantial reduction in EtOH-induced intestinal hyperpermeability. On the other hand, some miRNAs strengthen the intestinal barrier and act as a protective mechanism against bowel injury. Haines et al⁴⁹ found that miR-93 may enhance the integrity of the intestinal epithelial barrier by targeting the protein tyrosine kinase 6. This protective action may be achieved by reducing TNF- α /interferon γ -induced epithelial permeability. Shen et al⁵⁰ reported that miR-200b could inhibit TNF- α -induced interleukin (IL)-8 synthesis and tight junction injury by targeting c-Jun and myosin light chain kinase. The effects of miRNA-31, which is highly expressed in IBD, on intestinal inflammation were investigated using experimental murine models with colitis induced by dextran sulfate sodium or TNBS.⁵¹ This experiment showed that miRNA-31 promotes epithelial regeneration through the WNT and Hippo signaling pathways. Conversely, miR-31 directly targets IL-25 mRNA. In IL-10 knockout mice as well as TNBS-induced colitis mice, IL-25 and miR-31 levels were inversely correlated.⁵² miR-31 was shown to directly target IL-25 and to regulate T helper 1/T helper 17 cellular-mediated mucosal inflammation in colitis.⁵²

Inhibition of Autophagy

Autophagy defects are among the mechanisms that play a crucial role in developing IBD. miRNAs regulate autophagy

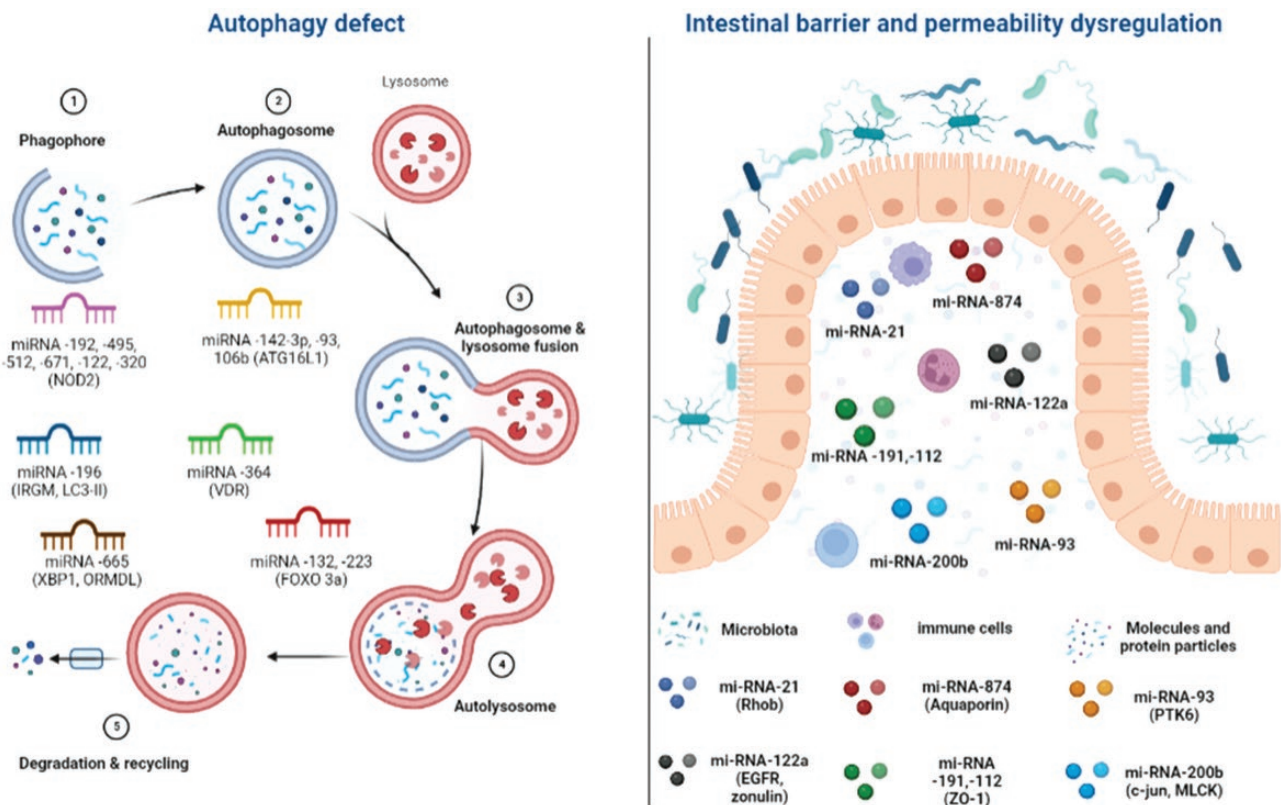


Figure 3. Summary of microRNA (miRNA) mechanisms and their target genes in inflammatory bowel disease (IBD). ATG16L1, autophagy-related 16-like 1; EGFR, epidermal growth factor receptor; IRGM, immunity-related GTPase family M protein; MLCK, myosin light chain kinase; NOD2, nucleotide-binding oligomerization domain containing 2; ORMDL3, ORMDL sphingolipid biosynthesis regulator 3; PTK6, protein tyrosine kinase 6; VDR, vitamin D receptor; XBP1, X-box binding protein 1; ZO-1, zonula occludens-1.

in the intestines by targeting autophagy genes via complex molecular and signaling pathways, which results in either the inhibition or stimulation of autophagy.⁵³

Some of these miRNAs are shared in regulating autophagy in both CD and UC, while others have been linked to one of these two. The literature review found some miRNAs that regulate autophagy in CD, including miR-192, -142-3p, -122, -93, -106B -20a, -30C, -130a, -20a, and -196. Chuang et al⁵⁴ were the first to report that miR-192 suppresses the nucleotide-binding oligomerization domain containing 2 (*NOD2*) expression. This gene has been strongly associated with susceptibility to CD and regulates autophagy and the nuclear factor kappa B (NF- κ B) pathway.^{55,56} The downstream signaling pathway of NF- κ B and messenger RNA expressions of interleukin-8 and CXCL3 were also inhibited by miRNA-192 mimics.⁴⁷ The study identified 3 miRNAs that suppress the *NOD2* gene: miR-495, miR-512, and miR-671. Chen et al⁵⁷ explored the mechanism through which miR-122 is implicated in the pathogenesis of CD. In this study, *NOD2* was identified as the target gene of miR-122. A series of interactions triggered by NOD resulted in the activation of the nuclear NF- κ B pathway, which subsequently activated many genes, including those involved in immune and inflammatory responses and apoptosis.⁵⁸ miR-142-3p regulates autophagy by targeting autophagy-related 16-like 1 (*ATG16L1*).⁵⁹ Lu et al⁶⁰ showed that the autophagy-mediated clearance of bacteria in epithelial cells was inhibited by miR-106b and miR-93 by targeting *ATG16L1*. miR-106b levels were elevated in intestinal epithelia from individuals

with active CD. In contrast, *ATG16L1* levels were reduced compared with control subjects. Brest et al⁶¹ showed that miR-196 was overexpressed in intestinal epithelial cells within the inflamed ilea and colons in individuals with CD compared with healthy control subjects. miR-196 regulates the level of the immunity-related GTPase family M protein, which is critical for initiating and maturing xenophagy. When overexpressed, miR-196 inhibits autophagy at the initiation step and plays an important role in controlling intracellular pathogen degradation in human cells. This finding was further confirmed by monitoring the conversion of the autophagosomal marker LC3-II, which showed that miR-196 triggered a significant decrease in LC3-II conversion. Pierdomenico et al⁶² were the first to report the controlling role of miRNA-320 in *NOD2* expression. This cytoplasmic receptor recognizes bacterial wall components and triggers a cascade of signaling pathways that helps in bacterial clearance by using many mechanisms, including autophagy.^{62,63} They showed that miRNA expression was decreased by inflammation. *NOD2* expression was significantly increased with the inflammation-induced decrease of miRNA-320 in vitro and ex vivo. Upregulated *NOD2* expression stimulates the NF- κ B pathway, which subsequently increases the downstream transcription of proinflammatory cytokines (TNF- α and IL8). These findings were confirmed in inflamed mucosal colonic samples from CD and UC pediatric patients, in which CD and UC samples showed a significant decrease in miR-320 levels. At the same time, *NOD2* expression was significantly upregulated compared with noninflamed mucosal samples.

miR-29 expression was increased in human dendritic cells in response to *NOD2* signals. Because miR-29 downregulates IL-23, it was suggested that elevated IL-23 in CD might be due to the loss of miR-29-mediated immunoregulation in CD dendritic cells.⁶⁴ Chen et al⁶⁵ investigated the mechanism of miRNA-364 in IBD and found that this miRNA was induced by TNF- α , a proinflammatory cytokine that increases during mucosal inflammation. miR-364, in turn, inhibits its target vitamin D receptor in the intestinal epithelium and thereby inhibits autophagy.⁶⁵ Li et al⁶⁶ investigated the role of miR-665 in the progression of IBD. miR-665 was increased in inflamed mucosal biopsies from IBD patients and dextran sulfate sodium-induced colitis in mice and had a direct inhibitory effect on 2 genes involved in autophagy activation: X-box binding protein 1 and ORMDL sphingolipid biosynthesis regulator 3. Another interesting finding that might explain the chronic inflammation of IBD is the involvement of miR-665 in the stimulation of positive feedback regulation in the miR-665/ER/NF- κ B loop that leads to chronic gastrointestinal tract inflammation. Kim et al⁶⁷ reported that 2 miRNAs, miR-132 and miR-223, significantly increased in colonic mucosal biopsies from UC and CD. The mechanism involved in the pathogenesis of IBD was associated with initiating positive feedback cycles. These 2 miRNAs directly inhibit their target gene, *FOXO3a*, which activates the NF- κ B signaling pathway and produces proinflammatory cytokines. This study showed a similar positive feedback loop to the one shown by Li et al,⁶⁶ in which inflammatory signaling in response to lipopolysaccharide or TNF- α initiates a positive feedback loop by which activated NF- κ B induces inflammatory cytokines.

Fecal miRNAs and Intestinal Dysbiosis

The association between the alteration of intestinal microbiota homeostasis (dysbiosis) and IBD development has been established.⁶⁸ The fecal miRNA profiles are influenced by the gut microbiota composition.^{69,70} Interestingly, the gut microbiota is regulated by fecal miRNAs, which selectively target bacterial genes and inhibit bacterial growth.³² According to Viennois et al,⁷⁰ fecal miRNAs are likely to mediate the communication between the microbiota and the host in IBD. They identified 12 differentially expressed miRNAs in germ-free mice compared with conventional mice. The profile of these miRNAs was altered after germ-free mice were conventionalized via the transplantation of wild-type microbiotas. Significant associations between fecal miRNAs and IBD-associated bacterial species in the gut microbiota were also observed. Another example of miRNA-bacterial associations has recently been reported in relation to diet. In vegans and vegetarians, miR-425-3p, an miRNA involved in lipid metabolism, is highly expressed in the stool. Its expression level correlates with the relative abundance of specific microbial taxa, including *Akkermansia muciniphila*.⁷¹ This study, while not in the context of IBD, highlights that miRNA and bacterial species correlate and might be joint actors in the pathogenesis of dysbiosis-related disorders such as IBD, as recently reported.⁷² Further illustrating the connection between miRNA and microbiota, it was shown that the administration of 2 probiotics, *Lactobacillus fermentum* and *L. salivarius*, ameliorated colonic damage in a mouse model of colitis and concomitantly increased miR-143 expression in colonic tissue.⁷³ The diagnostic and therapeutic potential (for example, after probiotics intake or administration of miRNA

mimics) of miRNAs concerning gut microbiota has recently been reviewed.^{72,74}

Ji et al⁷⁵ reported 5 differentially expressed fecal miRNAs. miR-199a and miR-223-3p were upregulated in UC and CD (both active and inactive) compared with those in the healthy control subjects, while miR-1226, -548ab, and -515-5p were downregulated in both UC and CD patients. Subsequently, the effect of these fecal miRNAs on 3 well-known intestinal bacteria involved in IBD development (the pathogenic bacteria *Fusobacterium nucleatum* and *Escherichia coli* and the probiotic segmented filamentous bacteria) was studied. It was observed that some of these miRNAs accumulate in these bacteria and have the ability to inhibit or stimulate bacterial growth. As a result, dysregulation of these intestinal miRNAs might result in the imbalanced screening of intestinal microbiota, resulting in the onset of disease.

Overall, there is increasing evidence showing that the role of fecal miRNA in IBD is partially due to their interaction with the microbiota.

Conclusion

This review summarized multiple miRNA expression and pathogenesis aspects in CD and UC. Recently, there has been increasing interest in using miRNAs as a noninvasive diagnostic tool for IBD. None of the IBD-related miRNAs have been validated or used in clinical practice.

Here, we contribute to a better understanding by reviewing miRNAs expressed in tissue biopsies, circulating body fluids, and stool. Among these 3, fecal miRNAs are the least studied by researchers. However, they are being increasingly presented as determining actors in the pathogenesis of IBD by playing roles in inflammation, intestinal permeability, autophagy, and their interaction with the microbiota. By reviewing these studies, we observed that 4 dysregulated miRNAs are commonly expressed in tissues, blood, and stool in UC (miR-16, -155, -21, and -192) (Figure 2), while in CD, 3 dysregulated miRNAs are commonly expressed (miR-16, miR-155, and miR21) (Figure 1). Thus, they are interesting candidates as IBD biomarkers. miR-192 was specifically identified in UC patients and is a potential candidate for distinguishing between UC and CD. The fact that fecal miRNAs are the least invasive sampling method and might reflect more precise results provides bigger opportunities for future studies.

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Conflicts of Interest

L.P.-B. has received personal fees from AbbVie, Janssen, Genentech, Ferring, Tillotts, Pharmacosmos, Celltrion, Takeda, Boehringer Ingelheim, Pfizer, Index Pharmaceuticals, Sandoz, Celgene, Biogen, Samsung Bioepis, Alma, Sterna, Nestle, Enterome, Allergan, MSD, Roche, Arena, Gilead, Hikma, Amgen, BMS, Vifor, Norgine, Mylan, Lilly, Fresenius Kabi, Oppilan Pharma, Sublimity Therapeutics, Applied Molecular Transport, OSE Immunotherapeutics, Entera,

Theravance; has received grants from AbbVie, MSD, and Takeda; and owns stock options in CTMA. The remaining authors declare that the research was conducted without any commercial or financial relationship that could be construed as a potential conflict of interest.

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