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## **MicroRNA in Inflammatory Bowel Disease**

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#### Abstract

Idiopathic inflammatory bowel disease (IBD) is a complex set of disorders that predominantly includes ulcerative colitis and Crohn's disease. The pathogenesis of IBD is multifactorial including genetic, infectious, and immunologic factors. MicroRNAs belong to a class of noncoding small RNAs that posttranscriptionally regulate gene expression, and they are an emerging class of genetic modifiers of IBD. Here, we focus on the use of unique microRNA expression patterns as biomarkers to classify and prognosticate disease severity in both mucosal tissue and serum from patients with either ulcerative colitis or Crohn's disease. Furthermore, we discuss specific micro-RNAs with respect to their roles in IBD pathogenesis and fibrosis. We also discuss the role of microRNAs in IBD-associated carcinogenesis, including their role as biomarkers, tumor suppressors, and oncogenes. Finally, we discuss the emerging therapeutic applications of microRNA manipulation to lessen the effect of IBD and its sequelae. Recent discoveries of the diverse roles of microRNAs in IBD pathogenesis have the potential to provide new targeted therapeutics for personalized medicine.

Keywords: inflammatory bowel disease, ulcerative colitis, Crohn's disease, micro-RNA, pathogenesis

### 1. Introduction

Idiopathic inflammatory bowel disease (IBD) is a group of chronic and recurrent inflammatory disorders that primarily involve the gastrointestinal tract. It predominantly includes ulcerative colitis (UC) and Crohn's disease (CD). The pathogenesis of IBD is multifactorial and not completely understood, but genetic, epigenetic, infectious, physiological, and immunological factors may all play important roles in the genesis and progression of the diseases [1–3]. A large number of genes have been linked to IBD susceptibility, pathogenesis, and carcinogenesis, and recent work has suggested that microRNAs can undertake the same roles.

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MicroRNAs are encoded within the genomes of a wide variety of eukaryotes, and more than 2500 human mature microRNAs have been curated in miRBase database since their discovery in 1993 [4, 5]. MicroRNAs are evolutionarily conserved, single-stranded noncoding RNA molecules of 19–24 nucleotides, which represent a class of regulatory RNAs suppressing gene expression at a posttranscriptional level. MicroRNAs concurrently modulate the expression levels of dozens or more messenger RNA (mRNA) targets and any given mRNA sequence may be targeted by several different microRNAs creating intricate regulatory networks to fine-tune a cell's function [6–8]. At the time of publication, microRNAs are predicted to directly regulate the expression of at least 30% of the entire mammalian genome [9]. MicroRNAs have been found to be involved in the normal functioning of multiple pathophysiological networks and in the pathogenesis of a broad spectrum of human diseases, ranging from neoplastic to inflammatory conditions [10–15].

In this chapter, we focus on the role of microRNAs in IBD as recent publications have indicated that microRNAs play critical roles in the pathogenesis of IBD and IBD-associated carcinogenesis and may serve as critical future targets for personalized medicine.

## 2. MicroRNAs show a diverse array of aberrant expression in IBD

Extensive literature has shown that microRNAs undergo dysregulation in both tissue and peripheral blood of patients with IBD with a goal of discovering biomarkers and crucial initiators of pathogenesis.

#### 2.1. MicroRNAs are differentially expressed in the mucosal tissue in UC

A search for biomarkers in UC in both non-affected and actively inflamed mucosa has identified a large number of microRNAs that show aberrant expression (**Table 1**) [16–28]. When compared to controls, a large number of microRNAs have been found to be upregulated including, but not limited to, miR-let-7e\*, miR-let-7f, miR-7, miR-7i, miR-16, miR-20b, miR-21, miR-23a, miR-24, miR-29a, miR-29b, miR-31, miR-98, miR-125b-1\*, miR-126, miR-126\*, miR-127-3p, miR-135b, miR-142-3p, miR-146a, miR-150, miR-155, miR-195, miR-196a, miR-206, miR-223, miR-324-3p, miR-375, miR-422b, miR-548a-3p, miR-650, miR-663, and miR-4284. The decreased microRNA profiles include miR-143, miR-145, miR-188-5p, miR-192, miR-194, miR-194b, miR-196b, miR-200b, miR-215, miR-216b, miR-320a, miR-346, miR-375, miR-489, miR-548e, miR-559, and miR-630. Seven microRNA candidates have been identified by at least two independent groups, miR-21 [16–18, 26, 27], miR-29a [16, 19], miR-31 [19, 24], miR-146a [23, 24, 27], miR-155 [17, 23], miR-192 [16, 27], and miR-375 [16, 27], making them the most promising candidates for biomarkers of UC.

However, there are many variables that differ between studies, including anatomical tissue location, degree of inflammation, prior medication, and platforms used to measure micro-RNAs that all may contribute to the high discordance between studies.

Status	Tissue type	Control	Aberrant microRNA expression	Reference
Active UC	Sigmoid, <i>n</i> = 15	Healthy	Decreased: miR-192 and 375 Increased: miR-let-7f, 16, 21, 23a, 24, 29a,	Wu [16]
			126, 195, and 422b	
	Sigmoid, <i>n</i> = 12	Healthy	Increased: miR-21 and 155	Takagi [17]
	Sigmoid, <i>n</i> = 12	Healthy	Increased: miR-21 and 126	Feng [18]
	Colon, nonspecific, <i>n</i> = 10	Healthy	Decreased: miR-188-5p, 215, 320a, and 346 Increased: miR-7, 31, 135b, and 223	Fasseu [19]
	Colon, nonspecific, <i>n</i> = 5	Healthy	Increased: miR-150	Bian [20]
	Colon, nonspecific, $n = 8$	Healthy	Decreased: miR-143 and 145	Pekow [21]
	Colon, nonspecific, $n = 20$	Healthy	Increased: miR-let-7e, 20b, and 98*	Coskun [22]
	Colon, <i>n</i> = 10	Healthy	Increased: miR-146a and 155	Beres [23]
Active or inactive UC	Colon, distal-most, $n = 10$	Healthy	Decreased: miR-194b, 216b, 548e, and 559 Increased: miR-31, 146a, 206, and 663	Lin [24]
	Sigmoid, <i>n</i> = 26	Healthy	Increased: miR-4284	Koukos [25]
Inactive UC	Sigmoid, <i>n</i> = 15	Healthy	Increased: miR-16, 23a, 24, 29a, 375, and 422b	Wu [16]
	Colon, nonspecific, <i>n</i> = 8	Healthy	Decreased: miR-188-5p, 215, 320a, and 346 Increased: miR-29a, 29b, 126*, 127-3p, 196a, and 324-3p	Fasseu [19]
	Colon, nonspecific, $n = 19$	Healthy	Increased: miR-20b and 125b-1*	Coskun [22]
Unknown	Colon, nonspecific, $n = 15$	Healthy	Increased: miR-21	Yang [26]
	Colon, nonspecific, $n = 12$	Healthy	Increased: miR-7i, 21, 142-3p, and 146a Decreased: miR-192, 194, 200b, and 375	Zahm [27]
Active UC	Colon, nonspecific, $n = 20$	Inactive UC	Increased: miR-98	Coskun [22]
	Colon, left or sigmoid, $n = 9$	Inactive UC	Decreased: miR-196b, 489, and 630 Increased: miR-548a-3p and 650	Iborra [28]

Table 1. Aberrant microRNA expression in human colonic tissue in ulcerative colitis (UC).

#### 2.2. MicroRNAs are differentially expressed in the mucosal tissue in CD

A search for biomarkers in CD has identified a large number of microRNAs that show aberrant expression (**Table 2**) [19, 23, 24, 28–32]. When compared to controls, miR-9, miR-9\*, miR-16, miR-21, miR-22, miR-23b, miR-26a, miR-29b, miR-29c, miR-30a, miR-30b, miR-30c, miR-31, miR-34c-5p, miR-106a, miR-122, miR-126, miR-126\*, miR-127-3p, miR-130a, miR-133b, miR-141, miR-146a, miR-146b-5p, miR-150, miR-155, miR-181c, miR-191, miR-196, miR-196a, miR-206, miR-223, miR-324-3p, miR-328, miR-375, miR-422a, miR-594, miR-663, and miR-885-5p have been found significantly upregulated [19, 24, 29, 31, 32]. The downregulated microRNA profiles include miR-let-7b, miR-7, miR-18a\*, miR-19b, miR140-3p, miR-194b, miR-216b, miR-548e, miR-559, miR-629, and miR-629\* [24, 30, 33].

Status	Tissue type	Control	Aberrant microRNA expression	Reference
Active CD	Sigmoid, $n = 5$	Healthy	Decreased: miR-19b and 629	Wu [29]
			Increased: miR-23b, 106a, and 191	
	Terminal ileum, $n = 6$	Healthy	Increased: miR-16, 21, 223, and 594	Wu [29]
	Colon, nonspecific, $n = 16$	Healthy	Increased: miR-9, 21, 22, 26a, 29b, 29c, 30b, 31,	Fasseu [19]
			34c-5p, 106a, 126, 126*, 127-3p, 130a, 133b,	
			146a, 146b-5p, 150, 155, 181c, 196a, 324-3p,	
			and 375	
	Colon, nonspecific, $n = 8$	Healthy	Decreased: miR-7	Nguyen [30]
	Colon, nonspecific, $n = 120$	Healthy	Increased: miR-196	Brest [31]
	Colon, nonspecific, $n = 15$	Healthy	Increased: miR-31 and 141	Huang [32]
	Colon, nonspecific, <i>n</i> = 10	Healthy	Increased: miR-146a and 155	Beres [23]
Active and	Colon, distal-most, $n = 9$	Healthy	Decreased: miR-194b, 216b, 548e, and 559	Lin [24]
inactive CD			Increased: miR-31, 146a, 206, and 663	
Inactive CD	Colon, nonspecific, $n = 8$	Healthy	Increased: miR-9*, 21, 22, 26a, 29b, 29c, 30a*,	Fasseu [19]
			30b, 30c, 31, 34c-5p, 106a, 126*, 127-3p, 133b,	
			146a, 146b-5p, 150, 155, 196a, 223, and 324-3p	
Unknown	Colon, nonspecific, <i>n</i> = 10	Healthy	Increased: miR-122	Beres [23]
	Colon, nonspecific, $n = 7$	Healthy	Increased: miR-21 and 375	Zahm [27]
Active CD	Colon, left or sigmoid, $n = 9$	Inactive	Decreased: miR-let-7b,18a*, 140-3p, and 629*	Iborra [28]
		CD	Increased: miR-328, 422a, and 885-5p	

Table 2. Aberrant microRNA expression in human colonic tissue in Crohn's disease (CD).

At least two independent groups have found similar dysregulation of miR-21 [19, 27, 29], miR-31 [19, 24, 32], miR-106a [19, 29], miR-146a [19, 23, 24], miR-155 [19, 23], miR-223 [19, 29], and miR-375 [19, 27].

#### 2.3. MicroRNAs are differentially expressed in blood samples in UC

Similar to the findings in tissue, microRNAs are also dysregulated in the peripheral blood of patients with UC (**Table 3**) [26, 28, 34–39]. When compared to controls, miR-let-7d, miR-let-7e, miR-let-7g, miR-let-7i\*, miR-plus-E1271, miR-15b, miR-16, miR-17-5p, miR-19a, miR-20b\*, miR-21, miR-22, miR-22-3p, miR-23a-3p, miR-24, miR-27a\*, miR-28-3p, miR-28-5p, miR-29a, miR-30e, miR-30e-5p, miR-31, miR-92a-1\*, miR-93, miR-103, miR-103-2, miR-103-2\*, miR-128, miR-138, miR-140-3p, miR-142-5p, miR-143\*, miR-146a-3p, miR-148b-3p, miR-150\*, miR-151-5p, miR-155, miR-181b, miR-188-5p, miR-191-5p, miR-196b, miR-199a-3p, miR-199a-5p, miR-221, miR-223, miR-223a-3p, miR-302-3p, miR-320e, miR-330-3p, miR-340\*, miR-345, miR-362-3p, miR-362-5p, miR-374b, miR-378, miR-378\*, miR-422a, miR-423-5p, miR-500, miR-501-5p, miR-532-3p, miR-532-5p, miR-550\*, miR-1946, miR-1271, miR-1274b, miR-760, miR-769-3p, miR-769-5p, miR-874, miR-941, miR-1246, miR-1271, miR-1274b,

miR-1296, and miR-4516 are upregulated in peripheral blood samples of patients with UC [26, 28, 34, 35, 38, 39]. When compared to controls, miR-150, miR-188-5p, miR-505\*, miR-612, and miR-1827 have been shown to be downregulated [28, 34, 38].

Status	Tissue type	Control	Aberrant microRNA expression	Reference
Active UC	Peripheral blood, $n = 13$	Healthy	Decreased: miR-505* Increased: miR-plus-E1271, 28-5p, 103-2*, 151-5p, 199a-5p, 340*, 362-3p, and 532-3p	Wu [34]
	Peripheral blood, $n = 88$	Healthy	Increased: miR-16, 21, 28-5p, 151-5p, 155, and 199a-5p	Paraskevi [35]
	Peripheral blood, $n = 62$	Healthy	Increased: miR-595 and 1246	Krissansen [36]
Active and inactive UC	Peripheral blood, <i>n</i> = 18	Healthy	Decreased: miR-150 Increased: miR-let-7d, let-7e, let-7g, 15b, 19a, 24, 27a, 28-3p, 29a, 30e, 93, 103, 128, 142-5p, 196b, 199a-3p, 221, 223, 345, 374b, 423-5P, 532-5p, 598, and 760	Iborra [28]
	Peripheral blood, <i>n</i> = 46	Healthy	Decreased: miR-188-5p, 612, and 1827 Increased: miR-17-5p, 22-3p, 23a-3p, 30e-5p, 148b-3p, 191-5p, 223a-3p, 302-3p, and 320e	Polytarchou [37]
Active UC	Peripheral blood, $n = 24$	Inactive UC	Increased: miR-23a-3p, 148b-3p, 320e, and 4516	Polytarchou [37]
Inactive UC	Peripheral blood, $n = 13$	Healthy	Decreased: miR-505* Increased: miR-103-2, 362-3p, and 532-3p	Zahm [38]
	Peripheral blood, $n = 10$	Healthy	Decreased: miR-505* Increased: miR-103-2*, 362-3p, and 532-3p	Wu [34]
Unknown	Peripheral blood, <i>n</i> = 20	Healthy	Increased: miR-let-7i*, 20b*, 22, 27a*, 31, 92a-1*, 138, 140-3p, 143*, 146a-3p, 150*,181b, 188-5p, 330-3p, 362-5p, 345, 378, 378*, 422a, 500, 501-5p, 532-5p, 550*, 720, 769-3p, 769-5p, 874, 941, 1271, 1274b, and 1296	Duttagupta [39]
	Peripheral blood, <i>n</i> = 15	Healthy	Increased: miR-21	Yang [26]

 Table 3. Aberrant microRNA expression in human peripheral blood in ulcerative colitis (UC).

Nine microRNAs have been found by at least two independent groups, miR-21 [26, 35], miR-28-5p [34, 35], miR-151-5p [34, 35], miR-199a-5p [34, 35], miR-345 [28, 39], miR-362-3p [34, 38], miR-505\* [34, 38], miR-505\* [34, 38], miR-532-3p [34, 38], and miR-532-5p [28, 39].

#### 2.4. MicroRNAs are differentially expressed in blood samples in CD

Similar to the findings in tissue, microRNAs are also dysregulated in the peripheral blood of patients with CD (**Table 4**) [28, 34–36, 38]. When compared to controls, a large number of microRNAs have been found to be upregulated in the peripheral blood including miR-plus-E1271, miR-let-7b, miR-16, miR-20a, miR-21, miR-23a, miR-27a\*, miR-29a, miR-30e, miR-93, miR-106a, miR-107, miR-126, miR-140, miR-140-3p, miR-140-5p, miR-188-5p, miR-191,

miR-192, miR-195, miR-199a-5p, miR-200c, miR-340\*, miR-362-3p, miR-484, miR-532-3p, miR-595, miR-877, and miR-1246. The significantly decreased microRNAs consist of miR-plus-F1065, miR-18a, miR-128, miR-140-5p, miR-145, miR-149\*, and miR-877.

Status	Tissue type	Control	Aberrant microRNA expression	Reference
Active CD	Peripheral blood, $n = 14$	Healthy	Decreased: miR-plus-F1065 and 149*	Wu [34]
			Increased: miR-plus-E1271, 199a-5p, 340*, 362-3p, and 532-3p	
	Peripheral blood, $n = 46$	Healthy	Increased: miR-let-7b,16, 20a, 21, 30e, 93, 106a, 140, 192, 195, and 484	Zahm [38]
	Peripheral blood, $n = 128$	Healthy	Increased: miR-16, 23a, 29a, 106a, 107, 126, 191, 199a-5p, 200c, 362-3p, and 532-3p	Paraskevi [35]
	Peripheral blood, $n = 57$	Healthy	Increased: miR-595 and 1246	Krissansen [36]
Active and inactive CD	Peripheral blood, <i>n</i> = 18	Healthy	Decreased: miR-877 Increased: miR-16, 27a*, 140-3p, 140-5p, and 195	Iborra [28]
Inactive CD	Peripheral blood, $n = 5$	Healthy	Decreased: miR-149* Increased: miR-340*	Wu [34]
Active CD	Peripheral blood, $n = 9$	Inactive CD	Decreased: miR-18a, 128, 140-5p, and 145 Increased: miR-188-5p and 877	Iborra [28]

Table 4. Aberrant microRNA expression in human peripheral blood in Crohn's disease (CD).

Six microRNAs have been found by at least two independent groups including miR-16 [28, 35, 38], miR-106a [35, 38], miR-195 [28, 38], miR-199a-5p [34, 35], miR-362-3p [34, 35], and miR-532-3p [34, 35].

# 2.5. Differential expression of microRNA that distinguishes UC from CD in indeterminate IBD

Although UC and CD have many overlapping features and symptomatology, they have distinct clinical, radiographic, endoscopic, surgical, and histologic findings. While most patients can be definitively classified as either UC or CD, 5–10% of IBD patients have equivocal features and are best classified as indeterminate colitis [40, 41]. The ability to determine which patients with indeterminate colitis act like CD would allow for better clinical and surgical management because patients with CD are much more likely to fail a pouch procedure.

Several microRNAs have been found to be differentially expressed between UC and CD, although the results have been inconsistent between most studies (**Table 5**) [19, 27, 34, 42].

In one study, a panel of five microRNAs (miR-19b, miR-23b, miR-106a, miR-191, and miR-629) was evaluated in 16 patients with a clinical diagnosis of indeterminate colitis. They found that 15 of 16 patients demonstrated UC-like expression patterns and concluded that microRNA

expression patterns in indeterminate colitis are far more similar to those of UC than CD [42]. These microRNA expression findings are similar to the data from long-term clinical followups where most indeterminate colitis patients act much more like UC patients, rather than CD patients. The possibility to test microRNA profiles prior to surgery, with the hope of improving pouch outcome, is promising.

Status	Tissue type	Control	Aberrant microRNA expression	Reference
Inactive UC	Colon, nonspecific, n = 8	Inactive CD	Decreased: miR-100a-3p, 100b-5p, 150, 196b, 223, and 320a	Fasseu [19]
Active and inactive UC	Colon, distal-most, n = 12	Active and inactive CD	Increased: miR-19b, 23b, 106a, 191, and 629	Lin [42]
Unknown UC	Colon, nonspecific, <i>n</i> = 12	Unknown CD	Increased: miR-24	Zahm [27]
Active UC	Peripheral blood, $n = 13$	Active CD	Increased: miR-plus-E1035, plus-F1159, and 3180-3p,	Wu [34]

Table 5. Differential microRNA expression between ulcerative colitis (UC) and Crohn's disease (CD).

## 3. MicroRNA as a potential driver of pathogenesis and disease severity

Despite the vast numbers of microRNAs identified as deregulated in IBD, very few microRNAs are replicated in multiple studies. Here, we focus on the microRNAs with the best evidence as the driver of pathogenesis.

#### 3.1. MiR-21 has an essential role in IBD development and disease severity

MiR-21 has been consistently identified as being upregulated in active UC and CD, suggesting a central role in the pathogenesis of IBD [16–18, 26, 43]. One study showed that the deletion of critical DNA methyltransferases (DNMT1 and DNMT3b) caused the dysregulation of approximately 10% of all microRNAs, highlighting the epigenetic regulation of microRNAs by DNA methylation [44]. The genome-wide association studies (GWASs) using Illumina CpG methylation assays have shown that the miR-21 locus was hypomethylated, and subsequently overexpressed, in samples of peripheral blood in patients with active CD [43]. A miR-21 knockout mouse model has been developed to assess the importance of miR-21 in IBD pathogenesis. Similar to human IBD, mice treated with dextran sodium sulfate (DSS) developed a chronic colitis causing significant morbidity and mortality with elevated tumor necrosis factor alpha (TNF- $\alpha$ ) levels and miR-21 [45]. However, mice with genetic deletion of miR-21 in IBD pathogenesis, but further work is needed to clarify the mechanism of action.

The pathogenic effects of miR-21 in IBD are thought to be mediated through at least three separate mechanisms. First, miR-21 is thought to cause increased intestinal permeability in response to epithelial damage, a factor thought to initiate IBD. Despite no difference at baseline, treatment with DSS caused increased intestinal permeability in wild-type mice compared to miR-21 knockout mice [45]. Second, miR-21 is proapoptotic as the DSS-treated miR-21 knockout mice had less intestinal epithelial cell apoptosis than controls [45]. Several studies have indicated a role for miR-21 in the protection of free radical-induced apoptosis that linked the pro-survival phenotype to the inhibition of phosphatase and tensin homology (PTEN) with subsequent elevation of PI3K-Akt-mTOR activity [46-49]. Additionally, miR-21 has been shown to prevent renal tubular apoptosis by directly reducing levels of Rab11 protein [50]. The prevention of epithelial cell apoptosis may help maintain the epithelial cell barrier and limit intestinal permeability. Third, miR-21 has been associated with fibrosis in multiple disease models and has an emerging role in irreversible fibrosis of IBD. Multiple models of cellular injury have shown to be dependent on increased levels of TNF- $\alpha$  and subsequent induction of miR-21 [51, 52]. Increased serum levels of miR-21 were seen in human diseases with significant fibrosis, suggesting a role for miR-21 as a biomarker for disease activity [52, 53]. Elevated miR-21 expression is maintained throughout the development of dysplasia and carcinogenesis, but more controlled studies are needed to define its role in fibrosis [54].

#### 3.2. MicroRNAs are associated with fibrosis and strictures in CD

Transmural inflammation is a hallmark of CD leading to irreversible fibrosis and stricture formation that marks disease severity. Several studies have attempted to identify the role of microRNAs in CD-related fibrosis. MicroRNA profiling of the serum from patients with CD with and without strictures has implicated miR-19a-3p and miR-19b-3p as potential pathogenic markers [55]. The authors found that low levels of both miR-19a-3p and miR-19b-3p were strongly correlated with stricturing CD and were independent of other potentially confounding variables such as site, gender, age, disease duration, and activity [55]. The studies in liver fibrosis have implemented miR-19b as a negative regulator of the pro-fibrotic tumor growth factor- $\beta$  (TGF)- $\beta$ -signaling pathway, but these experiments have not been confirmed in the setting of CD-associated fibrosis [56].

Several other microRNAs have been associated with TGF- $\beta$ -signaling pathway and fibrosis in CD. TGF- $\beta$  signaling has been shown to promote epithelial-mesenchymal transition, which leads to fibrosis in certain contexts. In patients with stricturing CD, the level of miR-29b was assessed in the mucosa overlying a stricture by comparing mucosa-overlying areas not affected by fibrosis [57]. Furthermore, the overexpression of miR-29b in fibroblast caused a decrease in TGF- $\beta$ -mediated collagen deposition [57].

The complex interactions in the microenvironment between the mucosa and underlying stroma in CD are highlighted by studies of miR-200b. In IBD, the ability of the mucosa to withstand damage and remain intact is a crucial mechanism to limit disease severity. Researchers found that the miR-200b level was decreased in the mucosa of UC and CD, which correlated with the extent of damage incurred by the epithelium [58, 59]. MiR-200b has been shown to inhibit TGF- $\beta$ -mediated epithelial-mesenchymal transition through the targeting of

ZEB1 and SMAD2 expression [59]. Additionally, the overexpression of miR-200b promoted the growth of epithelial cells by stimulating cyclin D1 production [59]. These studies support that miR-200b can protect against damage from CD in both epithelium and underlying stroma.

These recent observations are laying the foundation for how microRNAs are intricately involved in the formation of fibrosis and strictures in idiopathic IBD.

## 4. The role of microRNAs in IBD-associated carcinogenesis

Molecular mechanisms of IBD-associated carcinogenesis are poorly understood and are an exciting area of research within the field [60, 61]. Multiple epidemiologic and basic science studies have shown that the risk of IBD-associated colon cancer increases as the extent of the disease, severity of inflammation, and duration increase [62–64]. Colonoscopies with multiple spatially distinct biopsies are used to assess for IBD-associated dysplasia. Although histologic examination can reproducibly identify dysplasia, IBD-associated dysplasia is difficult to be distinguished from sporadic adenoma based on histologic appearance alone, but certain features and patient characteristics can be helpful in suggesting IBD-associated carcinoma [65].

Molecular alterations have been shown to lead to dysplasia and carcinogenesis and the abnormalities have been demonstrated in normal-appearing nondysplastic mucosa from UC patients who had a remote dysplastic lesion [66–71]. Using microRNA expression microarrays from tissue with IBD-associated dysplasia, the dysplastic epithelium revealed that 22 micro-RNAs (miR-31, miR-31\*, miR-96, miR-135b, miR-141, miR-183, miR-192, miR-192\*, miR-194, miR-194\*, miR-200a, miR-200a\*, miR-200b, miR-200b\*, miR-200c, miR-203, miR-215, miR-224, miR-375, miR-424\*, miR-429, and miR-552) were upregulated. Ten microRNAs (miR-122, miR-139-5p, miR-142-3p, miR-146b-5p, miR-155, miR-223, miR-490-2p, miR-501-5p, miR-892b, and miR-1288) have been found to be downregulated [72].

Studies of microRNAs may elucidate distinct pathways to identify IBD-associated dysplasia and subsequent carcinogenesis from sporadic mutations. As such, developing reliable molecular markers to distinguish sporadic adenoma from IBD-associated carcinogenesis will aid in the surgical management of IBD patients who are under consideration for a total colectomy.

#### 4.1. MiR-31 as an emerging biomarker of IBD-associated dysplasia

An assessment of the different anatomic locations in normal colon showed an equally low baseline expression of miR-31 [72]. MiR-31 is upregulated in both UC and CD, but not in other inflammatory conditions that have no association with dysplasia or malignancy [73]. Subsequently, the level of miR-31 was found to be 11-fold higher in IBD-associated dysplasia and carcinoma compared to that of IBD tissue without dysplasia, although no difference was seen in miR-31 expression level between IBD-associated dysplasia and IBD-associated carcinomas [72]. However, miR-31 was also found to be upregulated in sporadic colorectal adenocarcinomas [74–76].

The role of miR-31 in IBD-associated dysplasia or malignancy has only recently been examined and it is too early to determine if it is a useful marker of IBD-associated dysplasia or a generalized biomarker for carcinoma.

#### 4.2. MiR-214 as an emerging oncogenic driver of IBD-associated carcinogenesis

Recent evidence suggests that microRNAs are not just biomarkers, as they can be crucial for carcinogenesis. A high-throughput genomic screen of microRNAs has identified miR-214 as a positive regulator of NF-kB activity [77]. Elevated levels of miR-214 were found in human tissue from patients with active UC or CD [77]. Two groups have previously shown that miR-214 promotes the growth of malignant osteosarcomas by directly reducing levels of the tumor suppressor, phosphatase and tensin homology [78, 79]. Using in vitro and in vivo models, it was displayed that chronic disease activity initiated interleukin-6 causing STAT3-mediated transcription of miR-214 with subsequent reduction of PTEN, PDZ, and LIM domain 2 (PDLIM2) causing enhanced activity of oncogenic NF-kB [77]. Furthermore, an inhibitor of miR-214 was able to inhibit DSS-induced carcinogenesis [77]. These data support not only the role of miR-214 as an oncogene but also provide insight into the molecular mechanisms of its carcinogenesis.

# 4.3. MiR-124a as a tumor suppressor epigenetically inactivated in IBD-associated carcinogenesis

Chronic inflammation has been shown to cause hypermethylation of CpG islands within the promoter of genes causing decreased gene expression [80]. Epigenetic silencing of well-characterized tumor suppressor proteins, E-cadherin, p14, and hMLH1, has been documented in UC-associated carcinogenesis [81–84].

The role of miR-124a as a tumor suppressor has been shown by demonstrating the direct suppression of oncogenic cyclin-dependent kinase 6 (CDK6) in acute lymphoblastic leukemia, medulloblastoma, and hepatocellular carcinoma [85–87]. The evaluation of patients with UC showed a strong correlation between increased miR-124a-3 hypermethylation and increased CDK6 expression [88]. The levels of methylation were highest in patients who had pancolitis and long-standing disease, and the individual with the highest value was later found to have high-grade dysplasia [88]. These studies are supported by the usage of high-throughput genetic screens that identified miR-124a as a negative regulator of oncogenic STAT3 in human colonic epithelial cells [89]. The authors demonstrated that miR-124a was downregulated through hypermethylation in pediatric patients with UC and strongly correlated with elevated levels of STAT3 and increased disease severity [89].

In combination, these studies demonstrate that the long-standing inflammation of IBD has the power to epigenetically downregulate important microRNA tumor suppressors that alters the downstream targets of those events. These findings might open up novel pathways that can be assessed for prognosis and potential personalized medicine.

## 5. MicroRNA as potential therapeutic targets for IBD

The unique ability of microRNAs to posttranscriptionally regulate gene expression and affect multiple biological signaling pathways can lead to the development of antisense oligonucleotides as potential novel therapeutics in IBD. Multiple studies have shown that antisense oligonucleotides complementary to microRNAs can target specific microRNAs, abolishing their function in both in vitro cell models and in vivo animal models. Preclinical cell- and animal-based models have demonstrated that altering microRNA levels can modify the expression of either tumor suppressors or oncogenes as such to effect cancer growth [90–93]. Additionally, drug-induced microRNA changes have been shown to contribute to the therapeutic effect in in vitro models [94, 95].

With specific regards to the field of IBD, potential therapeutic targets consist of three general strategies: (1) to provide antisense oligonucleotides that inactivate microRNAs that are pro-inflammatory; (2) to replace the expression of tumor suppressor microRNAs; and (3) to provide antisense oligonucleotides that inactivate oncogenic microRNAs.

At the time of publication, no therapeutic manipulation of microRNAs in IBD has been published in either cell lines or animal models. However, a recent study has shown that the inhibition of miR-21, a central driver of IBD pathogenesis, slows the proliferation of a naso-pharyngeal carcinoma cell line [96].

Although the delivery of microRNA-targeted therapeutics is technically challenging, recent advances in delivery methods suggest that we may soon see an explosion of microRNA-targeted therapies being assessed in vivo for the first time [97–99].

## 6. Conclusions

In summary, the rapidly expanding knowledge of microRNAs in the pathogenesis and carcinogenesis associated with IBD has created an emerging interest in their potential role for personalized therapies.

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