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MicroRNA-microbiome cross-kingdom networks drive inflammatory bowel disease through dynamic regulatory ecosystems (Review)

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Abstract. Inflammatory bowel disease (IBD) pathogenesis reflects complex interactions between host immunity and gut microbiome dynamics, with microRNAs (miRNAs) functioning as key mediators of cross-kingdom communication. Host-derived miRNAs modulate bacterial gene expression and reshape microbial communities, while gut microbiota influences host miRNA expression through microbial metabolites and multiple immune signaling. In IBD, dysregulated miRNAs disrupt immune homeostasis by affecting inflammatory responses, lymphocyte differentiation and epithelial barrier integrity. Yet many miRNAs exhibit context-dependent dual functions, complicating therapeutic targeting. Despite their biomarker potential for distinguishing IBD subtypes and tracking disease activity, clinical validation faces substantial obstacles including methodological inconsistencies, patient heterogeneity and temporal expression variability. Single-target miRNA therapeutics have yielded modest clinical outcomes, exposing the resilience of regulatory networks and compensatory mechanisms that limit intervention efficacy. The bidirectional architecture of miRNA-microbiome communication argues against reductionist approaches. Effective IBD management requires integrated strategies that address multiple regulatory nodes rather than isolated pathways. Advancing this field demands deeper investigation of temporal dynamics, spatial organization and network-level interactions. Such understanding will inform precision medicine strategies that restore regulatory equilibrium without compromising the adaptive capacity of host-microbiome systems. Progress depends on recognizing the integrated nature of these regulatory networks rather than treating components in isolation.

Contents

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Abbreviations: A. muciniphila, Akkermansia muciniphila; *B. thetaiotaomicron, Bacteroides thetaiotaomicron*; CD, Crohn's disease; CRC, colorectal cancer; DAMP, damage-associated molecular pattern; EMT, epithelial-mesenchymal transition; EV, extracellular vesicle; *E. coli, Escherichia coli*; *F. nucleatum, Fusobacterium nucleatum*; FMT, fecal microbiota transplantation; GELN, ginger-derived exosome-like nanoparticles; GEN, garlic-derived exosome-like nanovesicles; I3A, indole-3-carboxaldehyde; IBD, inflammatory bowel disease; LPS, lipopolysaccharide; *L. reuteri, Lactobacillus reuteri*; *L. rhamnosus, Lactobacillus rhamnosus*; miRNA, microRNA; NET, neutrophil extracellular trap; PRR, pattern recognition receptor; pri-miRNA, primary microRNA; pre-miRNA, precursor microRNA; SCFA, short-chain fatty acid; UC, ulcerative colitis

Key words: microRNAs, gut microbiome, inflammatory bowel disease, regulatory networks, cross-kingdom communication, precision medicine

1. Introduction

Inflammatory bowel disease (IBD) represents a complex, chronic intestinal autoimmune disorder characterized by enhanced gut permeability and dysregulated immune responses. This multifactorial condition is driven by diverse influences, including dietary factors, genetic predisposition and gut microbiome composition IBD encompasses two primary subtypes: Ulcerative colitis (UC), which manifests as superficial mucosal inflammation confined to the colon and rectum, and Crohn's disease (CD), characterized by transmural inflammation that can affect any segment of the gastrointestinal tract (1). These debilitating conditions predominantly affect young adults and adolescents, presenting with severe clinical manifestations including abdominal pain, diarrhea, malabsorption and systemic fatigue. Furthermore, IBD substantially elevates the risk of colorectal cancer (CRC) development and the incidence rate of CRC in IBD is approximately 18% after 30 years (2). However, the conventional understanding of IBD pathogenesis as a simple triad of genetic susceptibility, immune dysfunction and environmental triggers fundamentally underestimates the dynamic complexity of molecular communication networks that govern intestinal homeostasis. In this review, a triadic regulatory network model was proposed that emphasizes the interaction of host microRNAs (miRNAs), the gut microbiome and immune pathways as an integrated system driving IBD pathogenesis. Although recent genomic advances have identified over 250 genetic loci associated with IBD susceptibility (3), this reductionist approach fails to address a critical paradox: Why do genetically identical individuals exposed to similar environmental factors exhibit vastly different disease trajectories and therapeutic responses?.

The emerging recognition of miRNAs as pivotal regulatory molecules in IBD pathogenesis represents a paradigmatic shift from static genetic determinism toward dynamic epigenetic regulation. miRNAs are small, evolutionarily conserved, non-coding RNA molecules of ~22 nucleotides in length that function as post-transcriptional regulators. They exert their regulatory effects by binding to complementary sequences within mRNA 3' untranslated regions, thereby modulating protein synthesis and influencing diverse biological processes (4) (Fig. 1). Consequently, miRNAs have emerged as critical molecular determinants in IBD pathogenesis, possessing the unique ability to simultaneously influence host immune function and the intestinal microbial ecosystem (5). Nevertheless, current miRNA research suffers from three fundamental limitations that have hindered clinical translation: i) The overwhelming focus on individual miRNA-target interactions while ignoring systemic network effects; ii) the persistent separation of host and microbial miRNA functions despite mounting evidence of cross-kingdom regulation; and iii) the failure to account for temporal dynamics and context-dependent miRNA functions.

In this review, we propose a triadic regulatory network hypothesis, which posits that IBD pathogenesis emerges from dysregulated interactions within three interconnected regulatory systems. The first involves host-intrinsic miRNA networks that govern immune and epithelial cell functions (6). The second encompasses microbiome-derived regulatory mechanisms, including bacterial miRNAs and metabolite-mediated modulation of host miRNA expression (7,8). The third comprises cross-kingdom communication networks that facilitate bidirectional miRNA-mediated dialogue between host and microbial communities (9). This framework challenges the prevailing assumption that host and microbial regulatory systems operate as independent entities. Instead, we propose that IBD represents a failure of integrated cross-kingdom communication rather than isolated host immune dysfunction. This perspective fundamentally reframes therapeutic targeting from single-pathway interventions toward network-based precision medicine. Such an approach advances beyond current IBD therapies, which primarily alleviate symptoms and suppress inflammation but fail to restore the underlying regulatory balance governing host-microbiome interactions (10).

Building upon this conceptual foundation, three hypotheses may be derived that challenge current paradigms and demand mechanistic innovation (Fig. 2). i) It may be hypothesized that temporal dysregulation of miRNA expression cycles, rather than absolute expression levels, represents the primary driver of IBD pathogenesis. For instance, the microRNA *miR-16* in the gut exhibits diurnal rhythmicity in intestinal crypts, peaking and troughing over the day. This rhythmic miR-16 expression helps coordinate epithelial proliferation with feeding times, thereby maintaining mucosal homeostasis (11). This hypothesis challenges the current focus on static miRNA quantification while emphasizing the potential importance of circadian and ultradian rhythmic patterns that may be critical for maintaining intestinal homeostasis. ii) It may be proposed that IBD emerges from an evolutionary mismatch between rapidly evolving dietary and lifestyle factors and the slower adaptation of ancient miRNA-microbiome communication systems. This suggests that modern environmental triggers disrupt co-evolved regulatory networks, thereby explaining the rising IBD incidence in industrialized populations (12). iii) It was contended that current therapeutic failures may result not from inadequate drug efficacy but from adaptive network reorganization, wherein miRNA regulatory circuits undergo compensatory rewiring in response to single-target interventions. This phenomenon is being referred to as 'regulatory network resilience' and demands fundamentally different therapeutic approaches. Furthermore, specific miRNA signatures can effectively discriminate between UC and CD phenotypes, monitor therapeutic responses and potentially predict disease severity and long-term complications, including colorectal carcinogenesis (13).

Despite extensive research, three critical controversies remain unresolved and continue to impede clinical progress.

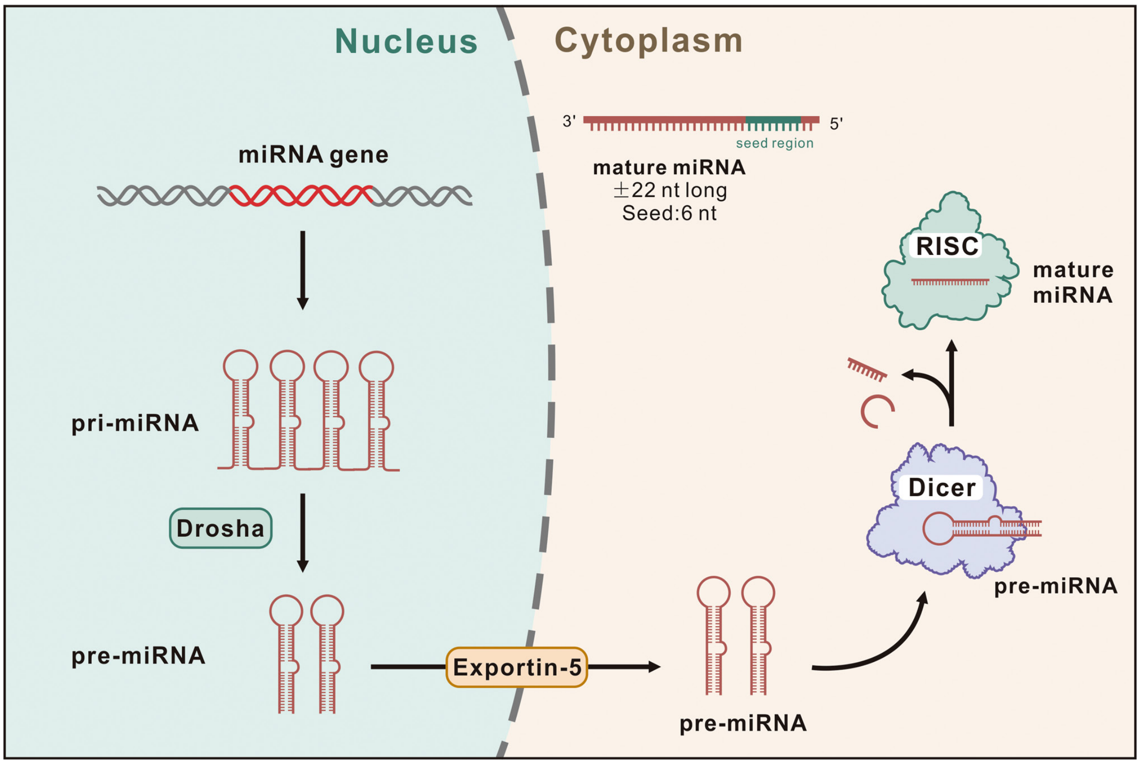

Figure 1. Eukaryotic miRNA biogenesis and processing pathway. The miRNA biogenesis pathway spans both nuclear and cytoplasmic compartments. In the nucleus (left panel), miRNA genes are transcribed to produce primary transcripts (pri-miRNA) containing characteristic stem-loop secondary structures. The nuclear ribonuclease Drosha processes pri-miRNA into shorter precursor molecules (pre-miRNA). In the cytoplasm (right panel), pre-miRNA is transported from the nucleus by Exportin-5. The cytoplasmic ribonuclease Dicer subsequently processes pre-miRNA into mature miRNA duplexes of approximately 22 nucleotides in length. The mature miRNA, containing a critical 6-nucleotide seed region for target recognition, is incorporated into the RISC to regulate target mRNA expression through complementary base-pairing interactions. This pathway represents the fundamental mechanism through which host cells generate regulatory miRNAs that participate in cross-kingdom communication with gut microbiota in IBD pathogenesis. miRNA, microRNA; pri-miRNA, primary microRNA transcript; pre-miRNA, precursor microRNA; RISC, RNA-induced silencing complex; nt, nucleotides.

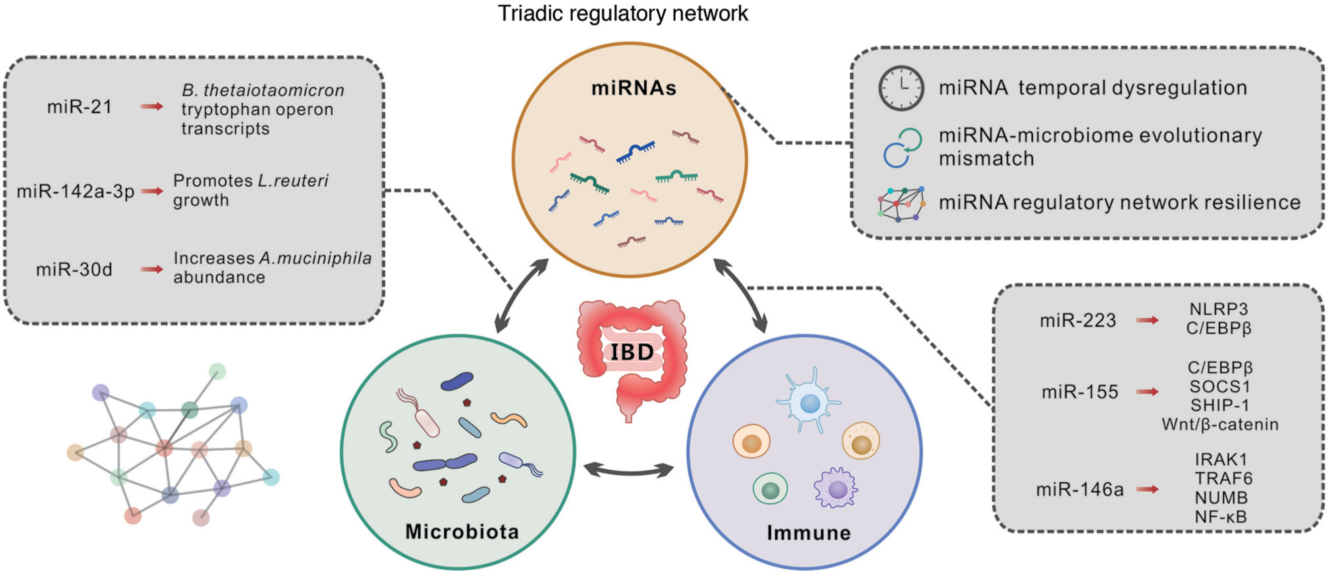

Figure 2. Triadic regulatory network model of miRNA-mediated IBD pathogenesis. Central miRNA pool (orange circle) mediates bidirectional communication between gut microbiota (green circle, bottom left) and immune system (blue circle, bottom right), with IBD pathogenesis resulting from network dysfunction. Cross-kingdom regulation examples: miR-21 targets *B. thetaiotaomicron* tryptophan operon, miR-142-3p promotes *L. reuteri* growth, miR-30d increases *A. muciniphila* abundance. Three novel hypotheses: Temporal miRNA dysregulation, miRNA-microbiome evolutionary mismatch and regulatory network resilience. Key miRNA targets in IBD: miR-223 targets NLRP3/C/EBPβ, miR-155 targets C/EBPβ/SOCS1/SHIP-1/Wnt/β-catenin, miR-146a targets IRAK1/TRAF6/NUMB/NF-κB. This framework emphasizes systems-level dysfunction requiring network-based therapeutic approaches. *A. muciniphila*, *Akkermansia muciniphila*; *B. thetaiotaomicron*, *Bacteroides thetaiotaomicron*; C/EBPβ, CCAAT/enhancer-binding protein β; IBD, inflammatory bowel disease; IRAK1, interleukin-1 receptor-associated kinase 1; *L. reuteri*, *Lactobacillus reuteri*; NF-κB, nuclear factor κB; NLRP3, NOD-like receptor protein 3; NUMB, NUMB endocytic adaptor protein; SHIP-1, SH2-containing inositol 5'-phosphatase 1; SOCS1, suppressor of cytokine signaling 1; TRAF6, TNF receptor-associated factor 6.

The cross-kingdom communication controversy persists despite compelling evidence demonstrating host miRNA regulation of bacterial gene expression. Intestinal epithelial cell miRNAs have been shown to shape gut microbiota composition in colitis models, and synthetic host miRNA mimics can alter specific bacterial transcripts and growth *in vitro* (14). Nevertheless, the physiological significance and therapeutic targetability of these interactions remain actively debated, with conflicting reports attributing observed effects to experimental artifacts rather than genuine biological phenomena (15,16). Equally problematic is the biomarker paradox. Although numerous studies have identified promising miRNA biomarkers for diseases diagnosis, systematic failures in clinical validation raise fundamental questions about the stability, specificity and biological relevance of circulating miRNAs as diagnostic tools (17,18). Perhaps most concerning is the therapeutic translation gap. Despite promising preclinical results, no miRNA-based therapeutics have yet reached clinical application for IBD. In animal models, delivery of certain miRNAs such as miR-146b or miR-26a mimics can attenuate intestinal inflammation (19), underscoring their therapeutic potential. However, translating these findings into clinical practice faces major hurdles, including efficient delivery of miRNA therapeutics to inflamed gut tissue, achieving precise targeting specificity, and ensuring long-term safety and stability in patients.

Rather than cataloguing individual miRNA-target associations, this review examines miRNA-microbiome interactions as a regulatory system and critically evaluates how its disruption contributes to IBD. Three questions organize this review: What is the mechanistic basis for bidirectional communication between host miRNAs and gut microbiota? Why have single-target therapies largely failed in clinical translation? And what obstacles must be overcome to develop effective miRNA-based interventions? We propose that IBD reflects a breakdown in host-microbiome communication, not merely host immune dysregulation. This view suggests that restoring regulatory balance across immune function, microbial ecology and their molecular dialogue may prove more effective than suppressing isolated pathways.

2. Mechanisms of miRNA in host-microbiome communication

miRNAs have emerged as pivotal molecular mediators orchestrating the complex bidirectional communication between host cells and the gut microbiome. Host-derived miRNAs are actively secreted into the intestinal lumen through multiple mechanisms, including packaging within extracellular vesicles. Once in the lumen, these miRNAs can traverse bacterial cell walls and directly influence microbial gene expression patterns, subsequently reshape the composition and functional activity of the microbiome. Studies have shown that specific miRNAs, such as let-7b and miR-21 in feces can directly regulate the composition and function of the intestinal microbiota, increase its pro-inflammatory potential, and further drive chronic inflammatory responses (20). A landmark study by Liu *et al* (14) demonstrated that fecal miRNA-mediated inter-species gene regulation facilitates host control of the gut microbiota. miRNAs are abundant in mouse and human fecal samples and are present within extracellular vesicles. Intestinal epithelial cells (IECs) and Hopx-positive cells were identified as the predominant fecal miRNA sources through cell-specific loss of the miRNA-processing enzyme Dicer. Critically, these miRNAs can enter bacteria such as *Fusobacterium nucleatum* and *Escherichia coli*, specifically regulate bacterial gene transcripts, and affect bacterial growth. Confocal microscopy studies using fluorescence-labeled (Cy3) miRNA mimics demonstrated that miRNAs entered bacteria and co-localized with bacterial nucleic acids, providing the temporal and spatial basis for bacterial gene expression regulation. Several specific miRNAs have been identified as regulators of bacterial growth: miR-515-5p elevated the proportion of *Fusobacterium nucleatum* 16S rRNA/23S rRNA transcripts, while miR-1226-5p upregulated the level of yegH mRNA in *Escherichia coli*. Both miRNAs promoted the growth of these bacteria, which have been implicated in colorectal cancer development. IEC-miRNA-deficient (Dicer1$^{\Delta IEC}$) mice exhibited uncontrolled gut microbiota and exacerbated colitis, while wild-type fecal miRNA transplantation restored fecal microbes and ameliorated colitis. Oral administration of synthetic miRNA mimics affected specific bacteria in the gut, suggesting that miRNAs may be used therapeutically to manipulate the microbiome for disease treatment (14). Conversely, this communication pathway operates bidirectionally, as gut microbiota profoundly influences host miRNA expression profiles. Studies utilizing germ-free mice colonized with microbiota from pathogen-free mice revealed differential expression of miRNAs in both the ileum and colon following colonization, with specific miRNAs involved in intestinal cell communication, signal transduction, and inflammatory responses (8). In the colon, the upregulated Abcc3 gene was identified as a potential target of miR-665, establishing a mechanistic link between microbiota colonization and miRNA-mediated gene regulation. Additionally, germ-free mice display abnormally expressed miRNAs in the amygdala and prefrontal cortex, with miR-182-5p, miR-183-5p, and miR-206-3p identified as targets of gut microbiota influence, suggesting the involvement of the microbiota-gut-brain axis in miRNA regulation (21). The gut microbiota regulates host miRNA expression primarily through microbial metabolites, including lipopolysaccharide (LPS), butyrate, bacterial amyloids, bile acids, and tryptophan-derived indole compounds. Bacterial components like LPS and flagellin trigger miR-146a expression in intestinal epithelial cells through TLR4/MyD88 signaling, establishing a direct molecular link between gut microbiota sensing and epithelial gene regulation. Once induced, miR-146a dampens cytokine production and promotes immune tolerance, essentially acting as a brake that prevents the epithelium from overreacting to constant microbial exposure (22). Short-chain fatty acids (SCFAs), particularly butyrate and propionate, can effectively regulate host miRNA expression through their activity as histone deacetylase (HDAC) inhibitors, thereby exerting a profound influence on epithelial barrier integrity and immune response modulation. Butyrate has been shown to alter the expression of 44 miRNAs in human colon cancer cells, including significant downregulation of the miR-106b family, which normally targets the tumor suppressor p21. By inhibiting HDAC-mediated histone deacetylation, butyrate increases chromatin accessibility at target gene promoters, thereby modulating miRNA expression profiles involved in intestinal homeostasis and carcinogenesis (23). Another paradigmatic example of this intricate regulatory mechanism is butyrate and propionate, which impair key B cell functions via an epigenetic mechanism. This mechanism involves HDAC inhibition at specific miRNA host genes, leading to upregulation of miRNAs targeting Aicda and Prdm1 and thereby suppressing the expression of Aicda and Prdm1 (24). This SCFAs-mediated regulation of B cell class-switch DNA recombination and plasma cell differentiation through miRNA modulation represents an important mechanism by which the microbiome influences systemic immunity. Bile acids, metabolized by both host and gut microbiota, have emerged as important regulators of miRNA expression. The transcriptional activity of farnesoid X receptor (FXR), a key bile acid sensor, is modulated by miRNAs including miR-34a and miR-22, which silence SIRT1 expression and thereby reduce FXR transcriptional activity. Notably, FXR exhibits negative autoregulation by inducing the transcription of miR-22, establishing a feedback loop between bile acid signaling and miRNA expression (25,26). Importantly, the gut microbiota has been shown to regulate white adipose tissue inflammation and obesity via a family of tryptophan-derived metabolite-associated miRNAs, and microbial regulation of hippocampal miRNA expression has implications for transcription of kynurenine pathway enzymes (27,28). These findings collectively establish that miRNA-mediated communication between the host and gut microbiota represents a fundamental regulatory mechanism with far-reaching implications for intestinal homeostasis, immune function, and disease pathogenesis. Understanding these intricate interactions provides new avenues for therapeutic intervention in microbiome-related disorders.

3. Extension of the triadic model

To extend the triadic regulatory network framework, additional emerging models that transcend traditional single-target approaches may be considered. These hypotheses remain preliminary but offer insights into dynamic regulatory ecosystems contributing to IBD pathogenesis.

Temporal regulatory oscillation in miRNA-microbiome interactions. IBD pathogenesis may involve not only static alterations in miRNA levels but also dysregulation of their temporal rhythms (29). Emerging evidence suggests that synchronized oscillations of miRNA expression, aligned with circadian or ultradian cycles and feeding patterns, are essential for maintaining intestinal homeostasis (30). Preliminary data suggest that disruptions in miRNA temporal dynamics, rather than changes in absolute abundance, may drive microbiome dysbiosis and inflammation (31-33). According to this perspective, the normally rhythmic dialogue between host and microbiota, mediated by periodic miRNA release and corresponding microbial responses, becomes desynchronized in IBD. Although direct *in vivo* evidence remains limited, early observations, including altered circadian miRNA expression patterns in experimental models, support this concept (34). Further investigation is needed to determine whether restoration of proper miRNA oscillations can reinstate the host-microbiome equilibrium.

Exogenous miRNA integration hypothesis. Emerging evidence indicates that miRNAs of dietary or exogenous origin may influence host-microbe interactions (35). The Exogenous miRNA Integration Hypothesis proposes that dietary miRNAs, particularly plant-derived miRNAs consumed in food, become functionally integrated into host regulatory networks, thereby creating hybrid regulatory circuits that evolved to sense and respond to nutritional inputs (36). The demonstration that orally administered miRNAs can alter gut microbiota composition and ameliorate experimental colitis in murine models provides initial evidence for this cross-kingdom regulatory effect (37). This mechanism may explain how modern processed diets, which are depleted of natural miRNAs, could contribute to microbiome dysbiosis and IBD development. However, this hypothesis remains controversial, as conflicting studies question whether the dietary miRNA effects observed in experimental systems reflect genuine physiological integration or methodological artifacts. The stability and bioavailability of exogenous miRNAs in the human gastrointestinal tract remain under investigation and preliminary findings require confirmation through independent studies employing standardized protocols (38). Resolution of this controversy will necessitate robust evidence, including the detection of bioactive dietary miRNAs in human intestinal samples and demonstration of their causal relationship with microbiome alterations. Currently, the exogenous miRNA hypothesis represents a compelling extension of the triadic network model, suggesting that environmental miRNAs may constitute an additional regulatory layer, although rigorous validation remains essential.

4. miRNA modulation of gut microbiota composition

The human gut microbiota consists of dense, diverse bacterial communities where dominant groups like *Bacteroidales* coexist through complex competitive and cooperative interactions, utilizing specialized systems to break down dietary polysaccharides and produce metabolites that influence host health. These microbial communities undergo dynamic shifts throughout life, with intense competition during early colonization shaping which strains establish long-term residency, while factors like diet, spatial niches, and interbacterial warfare through toxins and secretion systems continuously sculpt community composition and stability in the adult gut (39). The gut microbiome serves essential physiological functions, including maintenance of mucosal homeostasis, competitive exclusion of pathogenic microorganisms and preservation of epithelial barrier integrity through regulation of intercellular junction proteins. These functions are critical for preventing microbial dysbiosis associated with gastrointestinal disorders such as IBD (40). Despite the fundamental importance of gut microbiota regulation, the precise mechanisms through which host cells control microbial community composition remain incompletely understood. Evidence from Rothschild *et al* (41) indicates that only 1.9 to 8.1% of microbiota variation can be attributed to heritable genetic factors, suggesting that non-genetic influences, including dietary patterns and epigenetic modifications mediated through miRNA pathways, play predominant roles in determining gut microbiome diversity. Recent research has demonstrated that host cells can fundamentally reshape intestinal microbiota composition through miRNA-mediated regulatory mechanisms. Host-derived miRNAs can directly influence bacterial growth dynamics, thereby modifying the microbial landscape within the gut ecosystem. Host-derived miR-21 demonstrates the ability to bind to diverse gut microbes and enhance tryptophan synthesis transcripts, which subsequently regulate intestinal functions, including immune response modulation and epithelial permeability (42). Research by Liu *et al* (14) has provided evidence that fecal miRNAs can directly regulate bacterial gene expression and influence microbial growth patterns within the intestinal environment. Their investigations revealed that miR-515-5p and miR-1226-5p can enter bacterial cells, co-localize with bacterial nucleic acids and promote proliferation of CRC-associated bacterial species, including *Fusobacterium nucleatum* and *Escherichia coli*, through direct modulation of gene expression profiles. Supporting evidence for the role of miRNAs in host-microbiota interactions comes from experimental studies demonstrating that genetic ablation of Dicer, an essential enzyme for miRNA biogenesis, in IECs results in profound alterations in intestinal microbiota composition and increased susceptibility to dextran sulfate sodium (DSS)-induced experimental colitis in murine models. Specific host-produced miRNAs can exert suppressive effects on microbial growth. For example, the absence of CRC-associated miR-21 in knockout mice led to excessive proliferation of intestinal *Lactobacillus* species, while direct administration of human miR-21 demonstrated inhibitory effects on *Lactobacillus reuteri* growth *in vitro* (43). These findings collectively underscore the importance of miRNAs in mediating host-microbiota interactions and their potential for influencing gut microbiota composition and functional capacity.

In addition to the well-established role of dietary habits in shaping intestinal microbial communities, accumulating evidence demonstrates that food-derived miRNAs significantly contribute to gut microbiome formation and regulation. Ginger-derived exosome-like nanoparticles (GELN) containing bioactive miRNAs effectively mitigate DSS-induced experimental colitis in mice by modulating intestinal microbial composition. In this context, miR-7267-3p functions as a selective modulator that promotes beneficial bacterial taxa, including *Lactobacillaceae* and *Bacteroidales S24-7*, while suppressing potentially pathogenic *Clostridiaceae*. This miRNA exerts its effects by targeting monooxygenase-encoding genes in *Lactobacillus rhamnosus*, thereby promoting bacterial proliferation and increasing intracellular indole-3-carboxaldehyde (I3A) levels in GELN-treated mice. Elevated I3A levels stimulate IL-22 production, consequently strengthening the colonic mucus barrier and enhancing gut barrier function (44). Additional evidence demonstrates that miR-30d alleviates murine experimental autoimmune encephalomyelitis by modulating gut microbiome composition, specifically through promoting *Akkermansia muciniphila* growth and enhancing bacterial lactase expression (45) (Fig. 3). Furthermore, oral administration of miR-142a-3p mitigates DSS-induced colitis by enhancing *Lactobacillus reuteri* populations, a key genus for maintaining gut homeostasis. The beneficial effects of miR-142a-3p are abolished by concurrent antibiotic treatment, indicating that its therapeutic action is mediated through microbiome modulation rather than direct anti-inflammatory effects on host cells (46). The demonstrated effects of dietary miRNAs on gut microbiota challenge the prevailing assumption that miRNA-mediated regulation is exclusively endogenous. These observations suggest that dietary miRNAs may integrate into host regulatory networks to form hybrid regulatory systems capable of sensing and responding to environmental nutritional signals (47). This mechanism potentially explains the contribution of modern processed diets, which lack natural miRNAs, to microbiome dysbiosis and IBD pathogenesis. Nevertheless, controversies regarding cross-kingdom miRNA stability and biological activity persist, as conflicting evidence precludes definitive determination of whether observed effects constitute authentic regulatory mechanisms or experimental artifacts. For example, controlled feeding studies in mice and humans found no significant uptake of plant miRNAs like miR-168a from a rice-containing diet, despite earlier claims of its presence (48). An early study reported that rice-derived miR-168a survived digestion, entered the murine circulation, and downregulated hepatic LDLRAP1 (49), thereby increasing serum LDL, widely cited as emblematic evidence of cross-kingdom miRNA activity. However, subsequent independent studies in mice and humans largely failed to detect meaningful dietary uptake or gene-regulatory effects of miR-168a, indicating that such cross-kingdom transfer is not robust under routine conditions and remains contentious (50). Resolution of these uncertainties requires the development of standardized experimental protocols and implementation of independent validation studies.

5. Influence of miRNA on host immune responses

The innate immune system constitutes the primary defense against pathogenic invasion, providing rapid, broad-spectrum responses to diverse challenges while engaging in bidirectional crosstalk with adaptive immunity. Innate immune responses are regulated through intricate signaling networks, with specific miRNAs functioning as molecular switches that govern immune homeostasis. Mast cells and eosinophils represent critical effector populations in innate immunity whose functional responses are tightly regulated by specific miRNAs. miR-221/222 are markedly upregulated following mast cell activation (51). These miRNAs modulate cell cycle progression. Upon IgE-antigen stimulation, miR-221 enhances mast cell adhesion, migration, degranulation, and cytokine production, with these effects mediated through transcriptional remodeling of cytoskeletal components and increased cortical actin accumulation (52). In eosinophils, miR-223 plays a dual regulatory role governing both proliferation and differentiation. miR-223 expression increases progressively during eosinophilopoiesis, with targeted deletion resulting in hyperproliferative eosinophil progenitors and delayed CCR3 expression (53). miR-223 has emerged as a particularly significant biomarker for IBD and robust evidence supports its potent anti-inflammatory properties through multiple mechanisms (54). This miRNA alleviates intestinal inflammation in murine models by suppressing the NOD-like receptor (NLR) family pyrin domain containing 3 (NLRP3) inflammasome (55). miR-223-deficient mice develop more severe colitis with enhanced NLRP3 inflammasome activation,

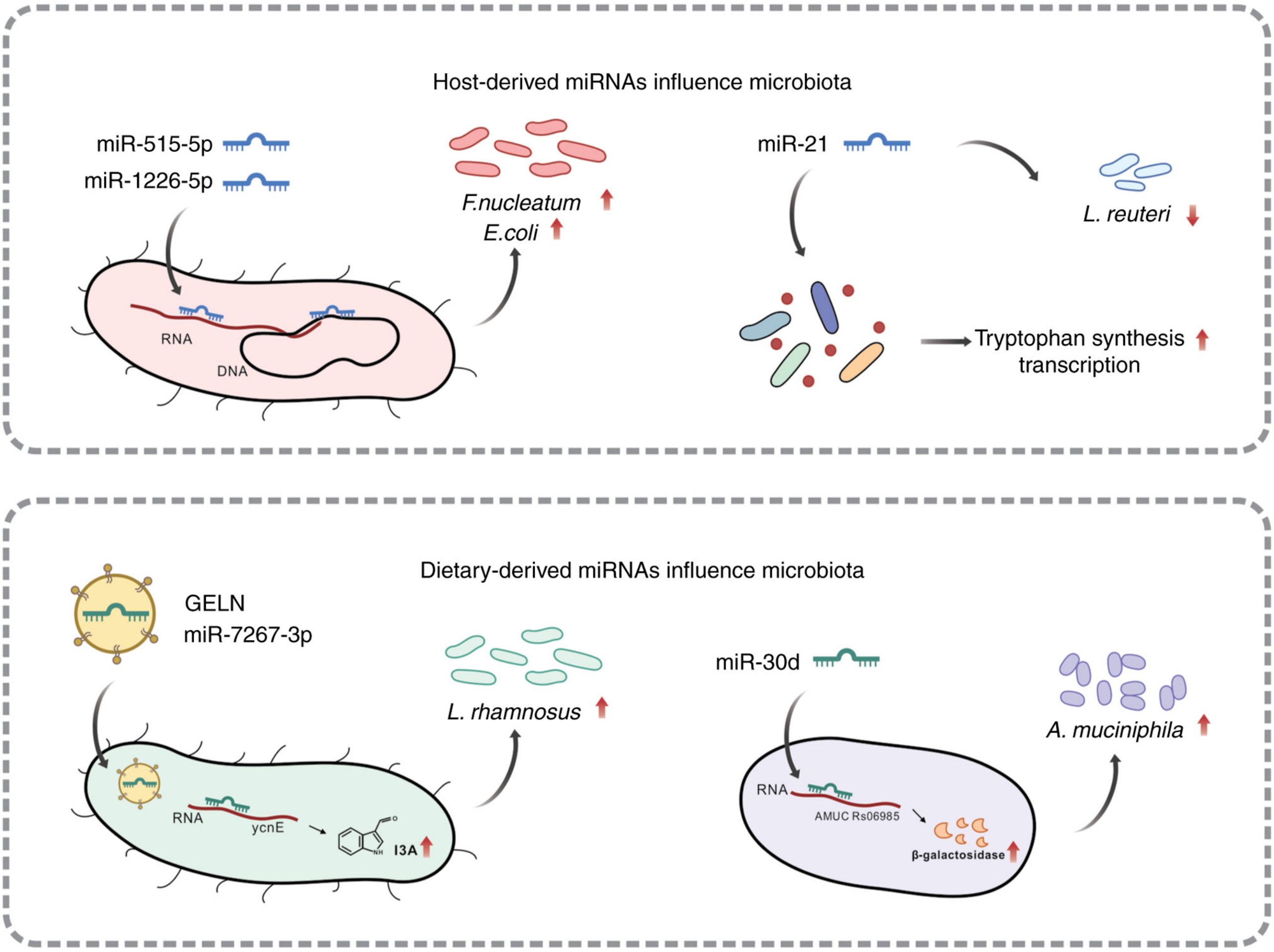

Figure 3. Host-derived and dietary-derived miRNAs modulate gut microbiota composition. Host-secreted miRNAs directly regulate bacterial gene expression and reshape microbial communities. miR-515-5p and miR-1226-5p promote pathogenic bacteria (*F. nucleatum*, *E. coli*) proliferation, while miR-21 enhances tryptophan synthesis in gut microbes but suppresses *L. reuteri* growth. Dietary miRNAs from GELN containing miR-7267-3p selectively promote beneficial *L. rhamnosus* growth by targeting monooxygenase and increasing I3A levels. miR-30d enhances *A. muciniphila* proliferation through upregulation of bacterial β-galactosidase expression, demonstrating cross-kingdom miRNA-mediated regulation of gut microbiome homeostasis. *A. muciniphila*, *Akkermansia muciniphila*; *E. coli*, *Escherichia coli*; *F. nucleatum*, *Fusobacterium nucleatum*; GELN, ginger-derived exosome-like nanoparticles; I3A, indole-3-carboxaldehyde; *L. reuteri*, *Lactobacillus reuteri*; *L. rhamnosus*, *Lactobacillus rhamnosus*; ycnE, monooxygenase gene; miRNA, microRNA.

whereas therapeutic administration of miR-223 mimics ameliorates disease severity (55,56). Furthermore, miR-223 regulates intestinal immune cell activity, as evidenced by the pro-inflammatory phenotype of immune cells from miR-223-deficient mice. Mechanistically, miR-223 directly targets CCAAT/enhancer binding protein (C/EBP)β mRNA to suppress pro-inflammatory gene expression in intestinal dendritic cell (DC)s and macrophages (57). Moreover, miR-223 exerts an anti-inflammatory effect by limiting calcium influx and mitochondrial ROS production in neutrophils to suppress IL-18-induced neutrophil extracellular trap (NET)s formation, while its exosomal transfer further reduces IL-18 production in macrophages, collectively restraining inflammatory amplification (58). These findings highlight the therapeutic potential of miR-223 in modulating intestinal inflammation. By contrast, miR-155 functions as a pro-inflammatory miRNA with a crucial role in mucosal immune homeostasis. miR-155 is upregulated in IBD and drives intestinal inflammation by suppressing SHIP-1, which unleashes AKT signaling and pro-inflammatory cytokine release. Blocking miR-155 restores SHIP-1 levels and effectively dampens the inflammatory cascade in experimental colitis (59). Further research has revealed miR-155 enhances the survival of activated innate lymphoid cell type 2 (ILC2) by preventing apoptosis, thereby sustaining type-2 immune responses during alarmin stimulation or helminth infection (60). miR-155 further promotes IL-33-induced ILC2 proliferation (61) and maintains IFN-γ expression in natural killer (NK) cells (62). In neutrophils, miR-155 drives NET formation through upregulation of peptidylarginine deiminase 4, whereas miR-223 exerts opposing effects (63,64). In IBD murine models, activated myeloid cells produce miR-155 through NF-κB-regulated pathways, triggering pro-inflammatory responses during early phases of the inflammatory cascade. Subsequently, miR-146a, an inducible anti-inflammatory miRNA, is transcriptionally activated by NF-κB following exposure to LPS, TNFα, or IL-1β. It then targets IL-1 receptor associated kinase 1 (IRAK1) and TNF receptor associated factor 6 (TRAF6) to dampen the very signaling cascade that triggered its expression, forming a negative feedback loop that fine-tunes innate immune responses (65). Delivering miR-146a via extracellular vesicles effectively reduces colonic damage, inflammatory cytokines, and disease severity in experimental colitis models (66). Indeed, miR-146a is widely recognized for its potent anti-inflammatory effects within the innate immune system through multiple mechanisms. miR-146a inhibits ILC2 proliferation and function, resulting in reduced IL-5 secretion in murine models (67). Additionally, miR-146a reduces NET formation processes associated with chronic inflammatory conditions in experimental murine models (68). miR-146a decreases IFN-γ expression in human NK cells (69) and reduces major histocompatibility complex class II and pro-inflammatory cytokine expression, including IL-6, in human and murine DCs and macrophages (70). miR-146b plays a particularly crucial role in regulating macrophage polarization within the intestinal microenvironment (71). miR-146b is downregulated in DSS-induced colitis and LPS-stimulated macrophages, exerting inhibitory effects by suppressing fibrinogen-like 2 (FGL2), an activator of p38-MAPK, NLRP3 and NF-κB-p65 signaling (72). Through FGL2 inhibition, miR-146b reduces M1 macrophage polarization and inflammatory responses *in vitro* and attenuates intestinal damage in IBD models *in vivo*. Furthermore, IL-10 stimulation enhances miR-146b expression in immune cells, whereas IL-10-deficient macrophages exhibit reduced miR-146b levels. Mechanistically, miR-146b targets interferon regulatory factor 5 (IRF5) mRNA to suppress bacterial toxin-induced IRF5 protein synthesis and pro-inflammatory macrophage polarization. Current evidence indicates that regulation of pro-inflammatory macrophage activity within intestinal tissues is primarily governed by the IL-10-miR-146b-IRF5 regulatory axis (71). miR-150 modulates NK cell maturation via c-Myb targeting and PI3K-AKT pathway suppression (69). miR-150-deficient mice exhibit decreased TGF-β levels, resulting in impaired intraepithelial lymphocyte production and differentiation (73). Distinct miRNA expression patterns characterize M1- and M2-polarized macrophages in human and murine systems. miR-155 drives macrophages toward a pro-inflammatory M1 phenotype during colitis by suppressing C/EBPβ and SOCS1, which fuels intestinal inflammation and worsens IBD pathology. Knocking out miR-155 shifts the balance toward anti-inflammatory M2 macrophages, dampening immune cell infiltration and protecting against colitis (74). miR-221-3p modulates inflammatory responses in TLR4-activated M2-macrophages by targeting Janus kinase (JAK)3. Aberrant miR-221-3p expression impairs anti-inflammatory responses and promotes M2-to-M1 phenotypic transition (75). Similarly, miR-125b (76) and miR-127-3p (77) facilitate polarization toward the M1 inflammatory phenotype. Conversely, let-7c has been shown to promote polarization toward the M2 anti-inflammatory phenotype in macrophages. let-7c overexpression decreases M1 markers (inducible nitric oxide synthase, IL-12) while increasing M2 markers folate receptor-β through C/EBPδ and p21-activated kinase 1 suppression (78). Beyond these extensively characterized miRNAs, numerous additional miRNAs have been identified as important modulators of innate immune cell maturation, specialization and proliferation. For instance, miR-183 have been demonstrated to suppress NK cell cytotoxicity by targeting cell surface proteins (79). Additionally, miR-34a (80) and let-7i (81) have been identified as promoters of DC activation and maturation in human and murine systems, respectively. These findings demonstrate the diverse regulatory roles of miRNAs in innate immunity under physiological and pathological conditions.

The development and function of the intestinal adaptive immune system are governed by intricate signaling networks that play fundamental roles in the differentiation and maturation of various adaptive immune cell populations, including T cells and B cells (5). These critical processes are extensively modulated by host miRNAs within the intestinal microenvironment. Dysregulation of these regulatory miRNAs can result in profound immune dysfunction and potentially trigger autoimmune pathological processes (82). Notably, miRNAs demonstrate remarkable versatility in their regulatory capacity, simultaneously managing multiple facets of adaptive immune system function. Previous research has established that CD is predominantly associated with T helper (Th)1-mediated immune responses, whereas UC is primarily driven by Th2-mediated inflammatory processes (1). Intestinal miRNAs exert a profound influence on CD4+ T-cell differentiation patterns in IBD pathogenesis. miR-17~92 enhances Th1 phenotypic commitment through IFN-γ upregulation (83). miR-17~92-deficient T cells failed to provoke severe colitis when transferred into Rag2$^{-/-}$ mice, showing markedly reduced weight loss and colon inflammation compared to wild-type controls. This impaired pathogenicity resulted from diminished Th17 differentiation and decreased IL-17A production by the knockout cells (84). miR-155 overexpression enhances Th1 differentiation, whereas its knockdown reduces CD4+ T-cell migration to colonic sites and promotes Th2 differentiation (85). And miR-31 induces Th1-type inflammation by suppressing IL-25 in the colon, thereby promoting IL-12/23-driven Th1/Th17 immune responses in colitis (86). Furthermore, it is worth noting that miR-146a can enhance Th1-mediated inflammation by promoting Th1 differentiation through post-transcriptional upregulation of the T-bet pathway and increasing key pro-inflammatory cytokines involved in atherosclerosis (87). miR-124 suppresses Th2 responses by shifting the Th1/Th2 balance toward Th1 through an IL-6R-dependent pathway (88). Conversely, miR-21 plays a crucial role in modulating Th2 responses by negatively regulating pro-inflammatory signals, thus contributing to the resolution of inflammation (89). miR-21 promotes Th2 differentiation by increasing Gata3 expression and decreasing Sprouty1 levels, acting as a key target of Bcl6 and enhancing Th2 responses in both conventional and Treg cells (90). miR-21-deficient mice exhibit elevated Th1 cytokines (IFN-γ, CXCL9) and reduced Th2 mediators (IL-4, CCL17) (91). Similarly, miR-29 suppresses Th1 differentiation by targeting the transcription factor T-bet (92,93). miRNAs sustain inflammatory responses by reinforcing T-cell activation and maintaining their pathogenic states. In psoriasis, miR-210 promotes Th17 and Th1 differentiation while blocking Th2 responses in CD4+ T cells through direct targeting of STAT6 and LYN, shifting the immune balance toward pathogenic inflammation (94). miR-24 displays distinct regulatory patterns compared to other miRNAs. It facilitates Th1, Th17, and iTreg differentiation while simultaneously suppressing Th2 responses by targeting an unconventional site in IL-4 mRNA: one nucleotide downstream of the stop codon, where the 3' end of miR-24 binds sequences that extend into the IL-4 coding region. This contrasts sharply with its cluster partners miR-23 and miR-27, which broadly inhibit multiple T cell lineages. miR-24 can counteract their suppressive effects, likely by targeting Smad7, a negative regulator of TGF-β signaling, which enhances the differentiation of TGF-β-dependent T cell populations (95). In chronic inflammation, miR-148a becomes upregulated in repeatedly activated Th1 cells and promotes their survival by suppressing Bim, a pro-apoptotic protein. Therapeutic blockade of miR-148a using antagomirs selectively eliminates these pathogenic Th1 cells from inflamed tissues while sparing protective memory T cells, offering a targeted approach for treating chronic inflammatory diseases (96).

The development and function of Th17 cells serve as crucial modulators in maintaining intestinal immune homeostasis and these processes can be significantly influenced by local microbiota and miRNAs (97). miR-155 promotes IL-17 production in Th17 cells (98) but does not enhance TGF-β and IL-10 release in regulatory T cells (Tregs), despite being regulated by the Treg-specific transcription factor Foxp3 (99). Targeted inhibition of miR-155 alleviates DSS-induced colitis by modulating Th17-mediated inflammation (100). Furthermore, intestinal miR-155 enhances DC cytokine production, driving Th17 cell differentiation and immune responses associated with autoimmune disorders (101). Conversely, miR-155 silencing reduces Th17 cells and inflammatory cytokines to suppress mucosal immunity and alleviate colitis (102). miR-34a suppresses Th17-mediated inflammation by directly targeting IL-6R and IL-23R to limit Th17 differentiation and expansion while also blocking CCL22-dependent Th17 recruitment to the colonic epithelium (103). miR-221 and miR-222 modulate Th17 activity downstream of IL-23 signaling by targeting MAF and IL-23R, thereby limiting Th17 expansion. Reduced expression of either miRNA increases intestinal susceptibility in murine models, consistent with IL-23 pathway suppression (104,105). miR-125a suppresses Th1/Th17 differentiation and cytokine production by targeting E26 transformation specific-1, transcription factor in CD4+ T cells. Its downregulation in IBD mucosa and exacerbation of trinitrobenzene sulfonic acid-induced colitis in miR-125a-deficient mice demonstrate its essential role in mucosal homeostasis (106). Additionally, miR-146a (107,108) and miR-106a (109) suppress Th17-cell differentiation and cytokine secretion, particularly IL-17, in the gastrointestinal tract. TNFα drives miR-106a expression in IBD, which suppresses IL-10 production and impairs regulatory T cell function. Genetic deletion of miR-106a attenuates intestinal inflammation by restoring Treg suppressive capacity and reducing pathogenic Th1 and Th17 responses (110).

Intestinal miRNAs also serve as critical regulators of Treg differentiation and maturation. miR-10a, an miRNA highly expressed in Tregs, is activated by TGF-β and vitamin A derivatives. miR-10a suppresses mRNA expression of Ncor2 and Bcl6 in intestinal Peyer's patches, preventing inducible Treg conversion to follicular helper T cells (Tfh). miR-10a also dampens DC activation by directly targeting IL-12/IL-23p40 and NOD2, thereby reducing the cytokine signals that drive inflammatory T cell responses. It also directly suppresses Th1 and Th17 differentiation in CD4+ T cells while leaving Th2 function intact, positioning it as a key brake on intestinal inflammation (83,111). Paradoxically, CD4+ T cells from miR-10a-deficient mice exhibit reduced susceptibility to DSS-induced colitis, as miR-10a limits IL-10 production by repressing Prdm1, the gene encoding Blimp1 (112). Beyond transcriptional control, miR-10a constrains Treg suppressive capacity by targeting Uqcrq, a component of mitochondrial complex III essential for oxidative phosphorylation, as well as amphiregulin, a molecule critical for epithelial repair. Treg-specific deletion of miR-10a enhances both suppressive function and barrier protection, resulting in attenuated colitis in experimental models (113). The miR-17~92 cluster of miRNAs, known for its oncogenic activity, enhances T cell-dependent humoral immunity by promoting Tfh development in germinal centers (114). Within this cluster, miR-17 and miR-19b augment pro-inflammatory Th1 responses while impairing Treg differentiation in murine models (115). Additionally, miR-92a promotes Th17 differentiation and suppresses Treg development by inhibiting Foxo1 (116). Beyond T cells, intestinal B cells contribute to miRNA-mediated regulation in adaptive immunity. B-cell miRNAs exhibit stage-specific expression, enabling subset classification based on distinct expression profiles (117). miR-150 controls pro-B to pre-B cell transition and its deficiency enhances B1 cell-mediated immunity through c-Myb upregulation (118). miR-155 promotes pathogenic IgG autoantibody production by lowering the activation threshold in B cells, thereby enhancing their ERK signaling, proliferation, and class-switch responses (119). And miR-146a restrains IgA class-switch recombination by suppressing Smad2, Smad3 and Smad4 in B cells, thereby limiting IgA production and preventing IgA-driven immunopathology (120) (Fig. 4). In brief, the emerging picture of miRNA regulation in immune responses reveals that traditional binary classifications of miRNAs as being pro- or anti-inflammatory inadequately represent the dynamic nature of immune regulation. Accumulating evidence indicates that miRNAs function as molecular switches redirecting regulatory networks between functional states rather than modulating individual pathways. This context-dependent behavior explains the apparent contradictions in miRNA function studies and suggests that therapeutic interventions should target network states rather than individual miRNAs.

6. miRNA in IBD pathogenesis

miRNA expression profiles in patients with IBD. miRNAs exhibit distinctive tissue-specific expression patterns and can be extracted from diverse biological tissues, including pulmonary tissues, through advanced RNA sequencing methodologies (121). These small regulatory molecules participate in post-transcriptional gene regulation by binding with sequence complementarity to mRNA molecules, thereby modulating protein translation (122). The primary regulatory mechanisms of miRNAs include suppression of target mRNA translation and reduction of mRNA stability, which collectively decrease the final protein output from specific mRNA transcripts. With the revolutionary development of miRNA microarray technologies and high-throughput RNA sequencing platforms, miRNAs have been identified in stable forms not only within human peripheral blood but also in various bodily fluids, including saliva, urine and fecal samples (123,124). This inherent stability renders miRNAs suitable as biomarkers for non-invasive liquid biopsy-based diagnostic strategies, particularly for cancer detection and monitoring (125).

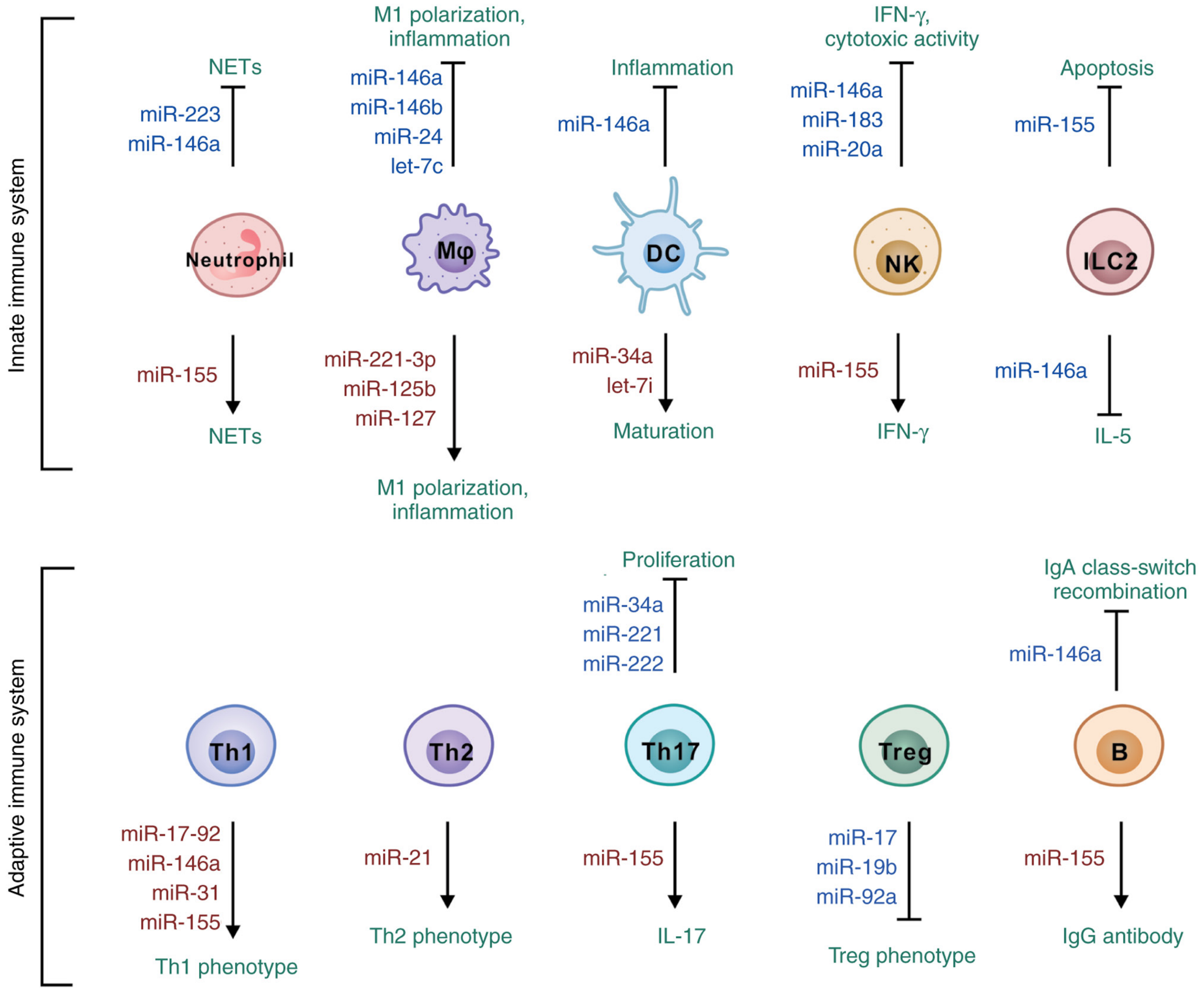

Figure 4. miRNA regulation of innate and adaptive immune responses in the intestinal microenvironment. miRNAs differentially regulate immune cell functions in inflammatory bowel disease pathogenesis. In innate immunity, miR-146a suppresses NETs and macrophage M1 polarization, while miR-155 promotes inflammatory responses. miR-34a and let-7i enhance DC maturation, whereas miR-146a, miR-183 and miR-20a inhibit NK cell cytotoxicity. In adaptive immunity, miR-17~92, miR-146a, miR-31 and miR-155 promote Th1 differentiation, miR-21 drives Th2 responses and miR-155 enhances Th17 IL-17 production. miR-17, miR-19b and miR-92a suppress Treg development, while miR-155 promotes B cell IgG production and miR-146a reduces IgA class-switching, demonstrating complex miRNA-mediated immune regulation in intestinal homeostasis. NETs, neutrophil extracellular traps; Mφ, macrophage; DC, dendritic cell; NK, natural killer cell; ILC2, innate lymphoid cell type 2; Th, T helper cell; Treg, regulatory T cell.

The majority of fecal miRNAs originate from IECs (14), providing a non-invasive approach to assess intestinal miRNA expression patterns. Accumulating evidence demonstrates that miRNAs regulate bacterial community composition within the gut ecosystem through modulation of bacterial gene expression (14,126). This regulatory relationship is bidirectional, as specific bacterial taxa and their metabolites, including butyrate, LPS and amyloid proteins, can modulate host gene expression (127). The regulatory networks involving miRNAs exhibit complexity, as individual miRNAs can target multiple mRNAs, while a single mRNA can be bound by numerous miRNAs. Target recognition regions for specific miRNAs often cluster spatially, resulting in cooperative repression that amplifies regulatory effects (128). In IBD pathogenesis, miRNAs participate in disease initiation and progression, serving as both diagnostic biomarkers and therapeutic targets. Current research quantifies miRNA levels in various body fluids and tissues using microarray profiling, reverse transcription quantitative polymerase chain reaction (RT-qPCR) and next-generation sequencing (NGS) (13). These regulatory molecules exert their pathological influence by modulating immune and inflammatory signaling cascades, thereby affecting IBD initiation and progression as well as colorectal carcinogenesis through regulation of immune signaling pathway components.

A pioneering investigation of miRNA alterations in patients with IBD identified 11 differentially expressed miRNAs in patients with UC compared to healthy controls. miR-16, miR-21, miR-29a and several other miRNAs exhibited elevated expression levels, whereas miR-192, miR-375 and other miRNAs demonstrated reduced expression in gut-related immune dysregulation (129,130). Subsequent studies have further characterized the complex changes in miRNA expression profiles in patients with IBD. miR-21 levels are elevated in UC compared to both controls and CD, with expression localized to lamina propria cells including macrophages and

T cell subsets. miR-126 is upregulated in UC and specifically expressed in endothelial cells, likely reflecting increased vascularization in inflamed tissue (131). It is worth noting that miR-21 promotes colitis progression and drives abnormal angiogenesis in CD by suppressing PTEN and activating the PI3K/AKT/VEGF pathway. Blocking miR-21 with antagonists reduces inflammation and restores normal vascular development in experimental colitis models (132). And miR-155 levels spike in CD patients who develop intestinal strictures, directly driving the fibrotic process. It works by suppressing HBP1, which unleashes Wnt/β-catenin signaling and triggers excessive collagen deposition in the gut wall (133). Several dysregulated miRNAs have emerged as potential biomarkers for distinguishing between CD and UC in both colonic tissue biopsies and non-invasive biological samples, including peripheral blood and fecal specimens (134). The persistent failure of miRNA biomarkers to achieve clinical validation despite extensive research reveals fundamental limitations in current conceptual frameworks. Accumulating evidence suggests that static miRNA expression levels are insufficient for disease diagnosis. Instead, IBD development appears to result from altered temporal patterns and impaired regulatory responses to environmental factors. This perspective explains why static measurements fail in clinical validation and suggests that diagnostic approaches must assess miRNA regulatory capacity rather than absolute expression levels. Furthermore, the assumption that specific miRNA profiles directly correlate with disease activity overlooks confounding effects of medication (135), diet (136) and microbiome composition (137), variables that may contribute more variance to miRNA expression than disease pathology itself (138).

Role of miRNA in IBD-associated inflammatory pathways. Microbiome-derived nucleic acids are present in detectable trace amounts in various human biological fluids, including saliva, stool, peripheral blood and plasma, suggesting their substantial potential as novel biomarkers for disease diagnosis and monitoring (139-141). The gut microbiome's profound influence on the host transcriptome represents an expanding area of diagnostic research, with investigations focusing on microbiota-derived miRNAs, although the molecular mechanisms underlying these interactions remain elusive (138,142). Experimental evidence demonstrates that the intestinal microbiota influences host miRNA expression through multiple regulatory pathways. For instance, specific pathogen-free-colonized mice exhibit altered miRNA expression levels in ileal and colonic tissues compared to germ-free controls (143). DNA microarray and miRNA expression analysis, combined with computational modeling, have identified that miRNA-665 suppresses ATP binding cassette subfamily C member 3, a gene involved in endogenous toxin metabolism and cellular detoxification (144). Additionally, miR-107 was downregulated by gut microbiota in immune cell populations, including DCs and macrophages, affecting MyD88 and NF-κB signaling pathways (145). Given that miR-107 specifically targets the IL-23p19 gene, it is hypothesized to modulate immune homeostasis and inflammatory balance. These findings suggest that the intestinal microbiota possesses the capacity to regulate host gene expression through sophisticated alterations of the host's miRNA expression profile (145).

The gut microbiome's surveillance against pathogens and tissue damage relies on pattern recognition receptors (PRRs) that detect pathogen-associated and damage-associated molecular patterns through two main families: membrane-bound TLRs and cytosolic NLRs (146). TLRs exhibit predominant or selective expression in specific cellular lineages, including immune cells (lymphocytes, DCs, macrophages and neutrophils) and non-immune cells (IECs and fibroblasts) (147). Upon ligand activation, TLRs and NLRs engage adapter proteins to trigger signaling cascades through NF-κB, IRFs, and MAPKs that regulate transcription of pro-inflammatory molecules, growth factors, and cytokines (148), pathways frequently dysregulated in IBD. The NLR family gene NOD2, mapped to chromosome 16q12.1, represents the first identified genetic susceptibility locus for CD and constitutes the first genetic determinant definitively associated with adult-onset IBD. NOD2 is expressed in various immune cell populations, including DCs, macrophages, monocytes and specialized Paneth cells, and its discovery has underscored the significance of PRR signaling pathways as potential therapeutic targets for IBD and related inflammatory conditions. miRNAs play crucial regulatory roles in modulating PRR signaling pathways and are increasingly considered as promising therapeutic targets for IBD intervention. miRNAs modulate NOD2 expression, thereby initiating complex cascades of inflammatory responses within intestinal tissues. Research has demonstrated that miRNAs regulate autophagy-related genes including NOD2, ATG16L1, and IRGM, which converge on autophagy pathways critical for IBD pathogenesis. Specifically, miR-30c and miR-130a are upregulated in enterocytes upon NF-κB activation during AIEC infection, resulting in decreased ATG5 and ATG16L1 levels, impaired autophagy, and increased intracellular AIEC burden with escalated inflammatory responses (5). miR-146a, a highly conserved anti-inflammatory miRNA, restrains colitis by directly targeting RIPK2 in myeloid cells, thereby weakening NOD2 inflammatory signaling and reducing production of IL-17 inducing cytokines in the colon. When miR-146a is absent, RIPK2 driven signaling becomes exaggerated, amplifies the IL-17 inflammatory axis, and leads to more severe colonic inflammation (149). miR-146a functions in γδ-T cells as a thymus imprinted brake that targets NOD1 and limits their shift toward IFN-γ production and IL-17 plus IFN-γ multifunctional states under inflammation (150). Furthermore, miR-146a regulates multiple genetic networks in IBD, and its deficiency worsens symptoms in a mouse model, while mimics alleviate the disease, highlighting its potential as a therapeutic target (151). Additional miRNAs, including miR-10a, miR-512, miR-320, miR-192, miR-495 and miR-671, directly target NOD2 expression and suppress the release of inflammatory cytokines. The expression of these regulatory miRNAs is dysregulated in patients with IBD, highlighting their roles in disease pathogenesis (126). miR-122 exerts a protective effect against intestinal epithelial injury by targeting and suppressing NOD2, thereby inhibiting NF-κB activation and reducing pro-inflammatory cytokine release. Downregulation of miR-122 in CD patients likely contributes to uncontrolled intestinal inflammation and disease progression (152). Conversely, NOD2 signaling also regulates miRNA expression. NOD2 activation enhances miR-29 expression in DCs, which subsequently regulates multiple immune mediator

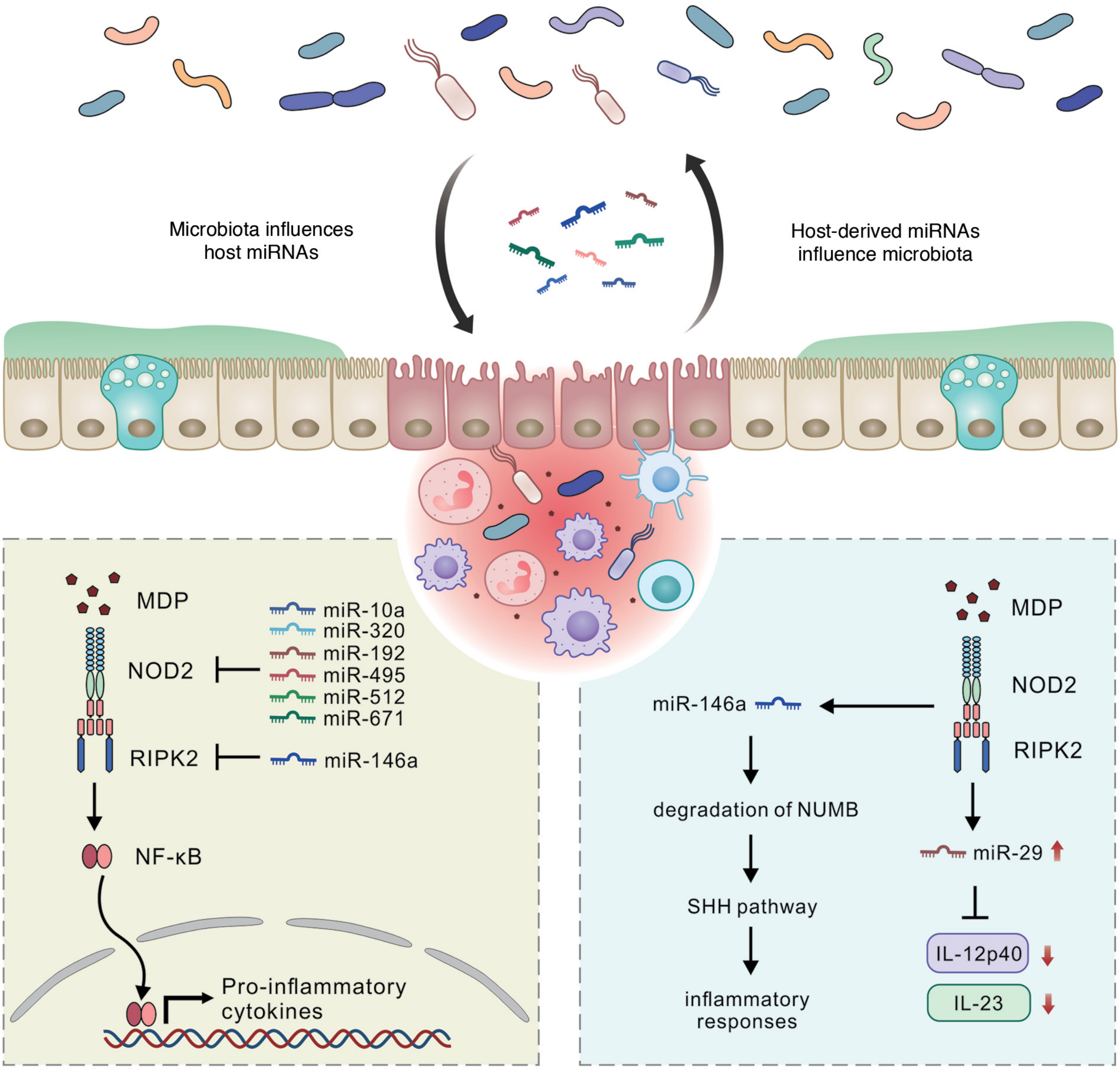

Figure 5. miRNA-mediated regulation of pattern recognition receptor signaling in IBD pathogenesis. Bidirectional miRNA-microbiome crosstalk regulates intestinal inflammation through modulation of PRR signaling pathways. Gut microbiota influences host miRNA expression, while host-derived miRNAs reshape microbial communities within the inflamed intestinal epithelium. Multiple miRNAs (miR-10a, miR-320, miR-192, miR-495, miR-512, miR-671) directly target NOD2 expression and suppress inflammatory cytokine release through inhibition of the MDP-NOD2-RIPK2-NF-κB signaling cascade. Conversely, NOD2 activation enhances miR-146a expression, leading to NUMB protein degradation and subsequent activation of the SHH pathway, promoting inflammatory responses. NOD2 signaling also upregulates miR-29 expression, which suppresses IL-12p40/IL-23 production and mitigates Th17 cell responses, demonstrating complex feedback mechanisms between PRR signaling and miRNA regulation in intestinal homeostasis and IBD pathogenesis. PRR, pattern recognition receptor; MDP, muramyl dipeptide; IBD, inflammatory bowel disease; NOD2, nucleotide-binding oligomerization domain 2; RIPK2, receptor-interacting protein kinase 2; NF-κB, nuclear factor κB; NUMB, NUMB endocytic adaptor protein; SHH, Sonic Hedgehog pathway.

pathways. NOD2 is essential for induction of the entire miR-29 family (including miR-29a, miR-29b and miR-29c), either independently or in conjunction with TLR2 or TLR5 signaling. miR-29 overexpression reduces IL-12p40/IL-23 expression and mitigates Th17 cells responses (153,154). NOD2 signaling enhances the expression of miR-146a, which targets the NUMB gene to relieve its suppression of Sonic hedgehog (SHH) signaling (Fig. 5). This cross-talk between NOD2, miR-146a, and SHH signaling contributes to the amplification of inflammatory responses in IBD (155). TLR4, an endotoxin recognition receptor expressed on both immune cells and IECs, is translationally suppressed by miRNA let-7i, whereas miR-21 indirectly modulates TLR4 signaling by targeting downstream components of the MyD88/NF-κB pathway (20). The expression of TLR2, which detects gram-positive bacterial components and is expressed predominantly in immune cells and colonic epithelial cells (40), is downregulated by miR-195 in THP-1 macrophages polarized to the pro-inflammatory M1 phenotype, impacting immune responses and inflammatory signaling pathways (156). miR-149, recognized for its anti-inflammatory properties, is associated with IBD pathogenesis. Its expression levels are reduced in the peripheral blood of patients with CD. miR-149 mitigates TLR-induced inflammation and cytokine production through targeted suppression of the MyD88 signaling adapter and NF-κB pathway (157). Collectively, these investigations highlight the roles of PRR signaling from IEC and myeloid cell populations in mediating responses to intestinal injuries or microbial infections. These findings also indicate the therapeutic potential of targeting specific miRNAs in clinical scenarios characterized by inadequate or dysregulated immune responses, such as those observed in IBD pathogenesis. Beyond their involvement in PRR signaling regulation, miRNAs are involved in transcriptional modulation of cytokine expression following PRR activation and in subsequent signal transduction cascades that activate both innate and adaptive immune response systems. The bidirectional regulatory relationships between miRNAs and PRR signaling pathways reveal that IBD pathogenesis cannot be explained by traditional linear inflammatory models. Accumulating evidence suggests that IBD results from the failure of adaptive regulatory networks to maintain homeostasis in the face of environmental challenges, rather than from aberrant activation of individual inflammatory pathways. This perspective explains why anti-inflammatory therapeutics provide temporary symptom relief but fail to achieve long-term remission: They suppress inflammatory outputs without restoring regulatory network function. Furthermore, the observation that identical genetic variants (e.g., NOD2 mutations) can result in different clinical phenotypes suggests that therapeutic efficacy depends on the restoration of network adaptability rather than the correction of specific molecular defects (158,159).

miRNA as biomarkers for IBD diagnosis. miRNAs are being investigated for their potential as non-invasive biomarkers for predicting disease progression and monitoring therapeutic responses in patients with IBD. Patients with active UC show distinct expression profiles of multiple miRNAs compared to healthy controls, with miR-21-5p, miR-16-5p and additional miRNAs showing elevated expression in the feces samples, while miR-141 and other selected miRNAs exhibited reduced expression in the biopsy samples, consistent with previous reports (134,160). Fecal miRNA profiling has demonstrated distinctly different composition in IBD patients, with miR-16-5p showing significantly increased expression both in UC and CD patients, while miR-21-5p elevation was specifically observed in UC patients (161). Additional studies have identified miR-223 and miR-1246 present at high levels in the stool of subjects with active IBD (162). Comprehensive fecal miRNA analysis in CD identified 17 significantly altered miRNAs including upregulation of miR-16-5p, miR-142-5p, miR-223-3p, miR-15a-5p, and miR-27a-3p, as well as downregulation of miR-10b-5p, miR-192-5p, miR-10a-5p, miR-375, and miR-200a-3p (163). Consequently, several miRNAs have emerged as diagnostic indicators for differentiating between UC and CD in colonic tissue biopsies and non-invasive samples, including peripheral blood, fecal specimens and saliva. A colon biopsy-based study in patients with IBD identified a diagnostic panel consisting of miR-19a, miR-21, miR-31, miR-146a and miR-375 as potential biomarkers for distinguishing between CD and UC (164). Peck *et al* (165), using NGS, demonstrated that a combination of miR-31-5p, miR-215, miR-223-3p, miR-196b-5p and miR-203 could categorize CD patients based on disease behavior, independent of inflammatory status. miRNA expression profiling in quiescent CD patients vs. healthy controls revealed site-specific differences across intestinal segments. In healthy tissue, the terminal ileum exhibited elevated expression of miR-31-5p compared to sigmoid colon (166). Additionally, nine miRNAs showed differential expression driven by both disease status and anatomical location, targeting immunoregulatory genes including NOD2 and IL6ST. These observations support the hypothesis that miRNAs may modulate inflammation-related gene expression through different mechanisms in IBD subtypes and anatomical locations (167,168). Recent pediatric IBD studies identified miR-29 as a distinguishing feature, with elevated ileal miR-29 levels strongly predictive of severe inflammation and stricturing behavior in treatment-naive pediatric CD patients (169). Pediatric patients with elevated miR-29 exhibited significantly lower Paneth cell counts, increased inflammation scores, and reduced levels of PMP22, indicating that miR-29 upregulation is associated with inflammation and Paneth cell loss. Another study identified miR-223 as a serum biomarker for IBD diagnosis, with elevated levels in patients with IBD compared to healthy controls (168). This miRNA correlated positively with disease activity measures in both patients with CD and UC, and exhibited stronger correlations with disease activity parameters in CD than conventional inflammatory markers such as the erythrocyte sedimentation rate and high-sensitivity C-reactive protein (170). A serum miRNA signature has been identified that not only indicates the development of colitis but also discriminates between inflammations of various origins, predicting UC with 83.3% accuracy (171). Recent innovations in biomarker discovery have revealed that extracellular vesicle (EV)-associated miRNAs can be dynamically modulated by diagnostic ultrasound, with miR-942-5p showing strong induction in higher grade intestinal inflammation and correlation with clinical activity in CD (172). Studies on extracellular vesicle-derived miRNAs have demonstrated that miR-181b-5p and miR-200b-3p play crucial roles in host-microbe interactions in colitis, with miR-181b-5p transplantation inhibiting M1 macrophage polarization and promoting M2 polarization to reduce inflammation (173).

Beyond their utility in monitoring disease activity in clinical practice, miRNAs also demonstrate potential as predictors of therapeutic response to various treatment modalities. In a study of patients with severe UC, Morilla *et al* (174) identified 15 miRNAs associated with responsiveness to corticosteroid therapy in patients with refractory disease, indicating the potential of miRNAs as predictive biomarkers for treatment response in IBD management. A validation study identified five candidate serum miRNAs (miR-126, let-7c, miR-146a, miR-146b, and miR-320a) associated with clinical response and mucosal inflammation in pediatric IBD patients, demonstrating their potential as pharmacodynamic and response monitoring biomarkers (175). In a study involving pediatric patients with IBD (including 17 cases of CD and 2 cases of UC) treated with either prednisone or infliximab therapy, the expression levels of miR-146a, miR-320a and miR-146b decreased with both therapeutic interventions, correlating with reduced inflammatory responses. By contrast, miR-486 responded to prednisone treatment but showed no response to infliximab therapy, illustrating the diversity in miRNA responses to different therapeutic modalities (160). Serum levels of miR-146a, miR-146b, miR-320a, miR-126, and let-7c change when pediatric IBD patients respond to anti-TNF-α or glucocorticoid therapy. These same miRNAs run high in inflamed gut tissue, making them promising candidates for tracking disease activity without repeated colonoscopies (175). It is noteworthy that miRNA biomarkers haven't reached clinical use so far because we've been approaching the problem wrong. Diseases like IBD emerge from dysfunctional regulatory networks, yet we keep measuring static molecular snapshots. This is why biomarker studies repeatedly fail validation. The solution is to test how these networks respond to standardized challenges like dietary shifts or controlled immune stimulation, similar to how glucose tolerance tests assess diabetes by measuring functional capacity rather than resting blood sugar. While miRNA expression profiles show real potential for distinguishing UC from CD and predicting treatment response, the field needs to identify IBD-specific signatures and evaluate their therapeutic value. But the critical shift must be from static measurements to dynamic assessments that capture the temporal complexity and network dysfunction underlying IBD pathogenesis.

7. Clinical applications of miRNAs in IBD management

Therapeutic potential of miRNA modulation in IBD. miRNAs are emerging as therapeutic agents due to their ability to regulate multiple gene targets within biological networks (19). Several miRNAs, including miR-29 and miR-126, show therapeutic potential in IBD treatment by modulating inflammatory pathways that share mechanistic similarities with approved biological drugs. miR-29 suppresses IL-23 production (153) while miR-126 inhibits leukocyte adhesion through vascular cell adhesion molecule 1 regulation (176). Additionally, miR-155 antagomirs suppress proteins within the JAK signaling cascade (177), resembling the therapeutic effects of JAK inhibitors currently employed in UC treatment. Two principal strategies have emerged for therapeutic miRNA intervention: i) Inhibiting upregulated pathogenic miRNAs using antagomirs; and ii) replacing downregulated beneficial miRNAs with synthetic mimics. Both strategies have demonstrated encouraging outcomes in preclinical IBD animal models and *in vitro* cellular systems, although clinical evidence from human studies remains limited.

miR-155 represents a particularly promising therapeutic target in IBD treatment, as its expression is significantly elevated in inflamed IBD mucosal tissues. Targeted inhibition of miR-155 shows substantial promise as an anti-inflammatory strategy by blocking the miR-155/NF-κB signaling axis, thereby suppressing pro-inflammatory cytokine secretion and modulating aberrant Th17-cell maturation (178). Similarly, TNF-α-driven miR-122a upregulation in intestinal epithelium promotes occludin mRNA degradation and barrier dysfunction in IBD. Anti-sense oligonucleotide-mediated miR-122a suppression prevents this pathogenic cascade, maintaining occludin expression and barrier integrity in both *in vitro* and *in vivo* models (179). Rawat *et al* (180,181) demonstrated that miR-200c-3p expression increases rapidly in intestinal epithelial cells during inflammation, where IL-1β triggers its upregulation and subsequent degradation of occludin mRNA through direct binding to the 3'UTR region. This process disrupts tight junction integrity and elevates intestinal permeability in both ulcerative colitis patients and experimental colitis models. Blocking miR-200c-3p with antagomirs restores occludin expression and prevents colitis progression, suggesting this microRNA represents a promising therapeutic target for preserving the intestinal barrier in IBD (180,181). Furthermore, targeted inhibition of specific miRNAs could serve as innovative therapeutic approaches for restoring compromised intestinal epithelial barrier function in IBD (182). The role of miR-146a in IBD presents a complex regulatory paradigm that requires careful consideration. This miRNA is upregulated in inflamed intestinal regions of patients with UC (183). However, conflicting data exist regarding the precise roles of miR-146a/b in intestinal inflammation. Certain studies suggest a pro-inflammatory role for miR-146a (184), while others demonstrate a regulatory role for miR-146b in facilitating macrophage transition from pro-inflammatory M1 to anti-inflammatory M2 polarization (185). This contradictory evidence highlights the need for comprehensive research to clarify miR-146 family member functions within the intestinal microenvironment and optimize the balance between their anti-inflammatory roles and mucosal barrier function regulation for potential IBD therapeutic applications (178).

Intestinal fibrosis in IBD results from fundamentally dysregulated tissue repair processes that lead to excessive extracellular matrix deposition and pathological tissue remodeling (186). This complication affects approximately half of patients with CD and frequently causes intestinal strictures requiring surgical intervention. It is now recognized as a self-perpetuating pathological process that persists independently of ongoing inflammation (186). From a clinical perspective, precise biomarkers for fibrosis detection and effective antifibrotic therapies remain critically absent. Emerging evidence has begun to elucidate the roles of miRNAs in fibrotic processes across various organ systems, including the gastrointestinal tract (187). Although the involvement of miRNAs in IBD-related inflammation has been increasingly well-characterized, their specific contributions to intestinal fibrosis remain incompletely understood. Nevertheless, miRNAs have demonstrated substantial potential as both profibrotic and antifibrotic regulatory molecules, representing promising targets for the diagnosis, prevention and treatment of pathological fibrogenesis (178). miR-155-5p is particularly implicated in IBD-associated fibrogenesis, especially in CD, where its expression correlates inversely with E-cadherin levels and significantly contributes to epithelial-mesenchymal transition (EMT). This miRNA is more abundant in CD tissues with fibrotic strictures. Through targeted inhibition of HMG-box transcription factor 1 (HBP1), it activates the Wnt/β-catenin signaling pathway, thereby increasing the expression of fibrosis-associated markers. In murine models, miR-155 mimics effectively induce fibrotic changes, whereas specific inhibitors significantly attenuate fibrosis development, establishing miR-155 as a promising therapeutic target for IBD-associated fibrosis (188). Conversely, *in vitro* studies demonstrate that miR-200b effectively ameliorate TGF-β1-induced intestinal fibrosis through targeted suppression of zinc finger E-box binding homeobox 1 (ZEB1) and ZEB2 transcription factors. Therapeutic delivery of miR-200b-containing microvesicles (miR-200b-MVs) significantly alleviates intestinal fibrosis by inhibiting pathological EMT (189). Similarly, miR-29b functions through direct downregulation of collagen synthesis pathways while simultaneously upregulating MCL-1 expression, which subsequently inhibits intestinal fibrosis development (190). Additionally, miR-130a-3p mimics effectively reverse the pathological effects of hsa_circRNA_102610, suggesting that exogenous miR-130a-3p may inhibit profibrogenic signaling through targeted suppression of hsa_circRNA_102610 (191). However, substantially more research is required to comprehensively elucidate profibrogenic miRNA networks and develop effective anti-miRNA therapeutic strategies for IBD-associated fibrosis.

The consistent inability to translate promising preclinical miRNA therapeutics into clinical applications indicates a fundamental misunderstanding of therapeutic targeting strategies in complex diseases. Accumulating evidence suggests that therapeutic interventions targeting individual miRNAs invariably induce compensatory network reorganization that not only negates therapeutic benefits but may also generate novel pathological states. This phenomenon provides an explanation for the systematic failure of single-target miRNA therapeutics in clinical translation, despite encouraging preclinical outcomes. Rather than targeting individual miRNAs, therapeutic strategies should prioritize the restoration of network adaptability through coordinated modulation of multiple regulatory nodes while maintaining the system's homeostatic capacity. Furthermore, the observation that identical miRNA perturbations can elicit opposing effects in different cellular contexts indicates that therapeutic efficacy depends on restoring context-specific responsiveness rather than achieving predetermined molecular endpoints.

miRNA-based diagnostic tools for IBD. The development of sophisticated miRNA-based diagnostic tools for IBD possesses substantial potential to improve the accuracy and efficiency of disease diagnosis and monitoring. miR-21 has emerged as a promising diagnostic biomarker for differentiating UC from CD. Both RT-qPCR and quantitative *in situ* hybridization (qISH) consistently demonstrate significantly elevated miR-21 expression in UC compared to CD tissue samples. These findings indicate that miR-21 functions not merely as a nonspecific inflammatory marker but is specifically associated with the distinct immunopathological processes underlying UC pathogenesis.

Detailed qISH analysis has revealed complex spatial distribution patterns of miR-21 expression, with predominant localization in inflamed lamina propria cell populations and specific epithelial cell subsets within architecturally compromised crypts, suggesting a multifaceted role for miR-21 in UC pathophysiology (192). This spatial heterogeneity reveals a fundamental limitation in current diagnostic approaches: The assumption that homogenized tissue measurements or systemic biomarker levels can accurately represent the spatially organized pathological processes characterizing IBD. Additionally, specific miRNAs, including miR-144, miR-519 and miR-211 have been recognized as significant modulators of gut microbiota composition and potential diagnostic indicators for CD, as demonstrated through comprehensive studies in adult patients (193). However, utilizing microbiome-modulating miRNAs as diagnostic tools presents a conceptual paradox: If these miRNAs actively shape the microbial environment influencing disease pathogenesis, their stability and reliability as diagnostic indicators remain questionable.

The integration of advanced bioinformatics approaches with high-throughput sequencing technologies has facilitated the development of sophisticated miRNA signature panels, offering enhanced diagnostic accuracy compared to individual miRNA biomarkers. These multi-miRNA panels demonstrate improved sensitivity and specificity for IBD subtype differentiation while providing prognostic information regarding disease severity and therapeutic response potential. Machine learning approaches, particularly penalized support vector machines and random forest algorithms, have been successfully applied to analyze peripheral blood miRNA signatures, achieving classification accuracy of 97% for distinguishing CD from UC using a sparse model of 16 distinct miRNAs (194). Neural network analysis combining data from 9 miRNA candidates with five clinical factors achieved 93% accuracy in discriminating responders to steroids from non-responders in acute severe UC (174). The growing use of advanced computational methods to combine multi-omics information such as microbiome, genomics, and metabolomics is paving the way for precision medicine in IBD, enabling more accurate prediction of treatment responses and the discovery of meaningful biomarkers. However, the increasing complexity of these panels paradoxically reduces their clinical utility by creating systems that exceed the practical requirements for routine clinical implementation and complicate biological interpretation. The persistent discrepancy between diagnostic potential and clinical implementation reveals a fundamental misalignment between technological capabilities and clinical requirements. Effective IBD diagnostics should focus on assessing regulatory network function rather than static molecular signatures. Instead of measuring absolute miRNA levels, diagnostic tools should evaluate the dynamic responsiveness of miRNA regulatory networks to standardized physiological challenges, thereby providing functional assessments of regulatory capacity. This approach would help differentiate patients with intact regulatory networks, who may respond favorably to targeted interventions, from those with compromised network function and requiring more comprehensive therapeutic strategies. Furthermore, diagnostic tools must incorporate temporal measurements to capture disease progression patterns and therapeutic response dynamics, accounting for the inherently dynamic nature of IBD pathogenesis (Fig. 6).

Challenges in translating miRNA research to clinical practice. Targeting miRNAs for IBD therapeutic applications represents a promising yet challenging approach requiring consideration of multiple complex factors. Synthetic miRNA mimics designed to replicate beneficial endogenous miRNAs and specific antagomiRs engineered to suppress pro-inflammatory miRNAs may offer innovative therapeutic strategies without significantly increasing treatment-related toxicity. However, off-target gene repression mediated by miRNA-based molecules, resulting from partial sequence complementarity with non-target mRNAs, can potentially cause unintended clinical toxicity (195). The specific biological effects of individual miRNAs and particular microbiota species on inflammatory processes and carcinogenesis remain incompletely characterized and require further investigation before clinical translation (196). Additionally, the dynamic nature of the microbiota composition and the long-term consequences of

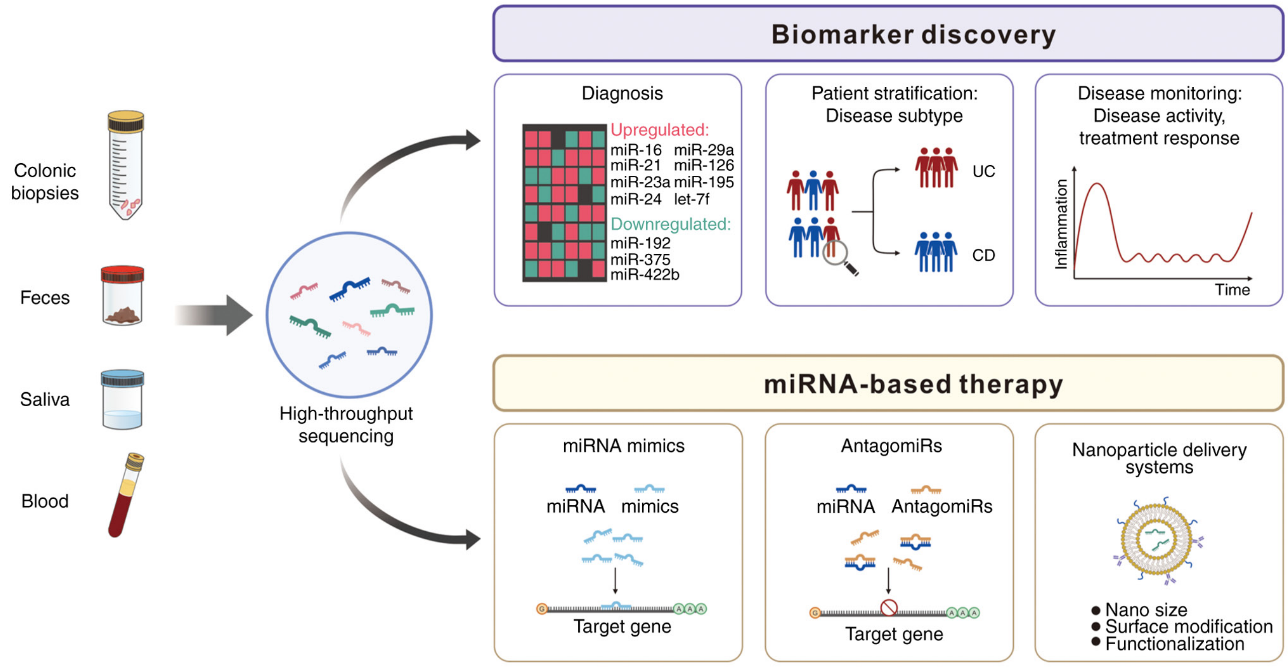

Figure 6. Clinical applications of miRNAs in IBD: From biomarker discovery to therapeutic intervention. High-throughput sequencing of miRNAs from multiple biological samples (colonic biopsies, feces, saliva, blood) enables comprehensive biomarker discovery and therapeutic development. For biomarker applications, distinctive miRNA expression profiles facilitate IBD diagnosis through identification of upregulated miRNAs. Patient stratification distinguishes UC from CD based on specific miRNA signatures, while disease monitoring tracks inflammation levels and treatment responses over time. For therapeutic applications, miRNA-based interventions include synthetic miRNA mimics to restore beneficial miRNA function and antagomiRs to inhibit pathogenic miRNA activity through complementary binding to target genes. Advanced nanoparticle delivery systems incorporating nano-size optimization, surface modification and functionalization enhance therapeutic miRNA stability, tissue specificity and clinical efficacy in IBD treatment. UC, ulcerative colitis; CD, Crohn's disease; IBD, inflammatory bowel disease; miRNA, microRNA.

microbiota modulation on miRNA expression profiles warrant careful consideration in therapeutic development (197). Critical translational challenges include developing efficient, targeted delivery systems capable of ensuring tissue-specific miRNA delivery while minimizing systemic exposure and adverse effects (198). The stability of therapeutic miRNAs in biological fluids and their bioavailability at target sites represent additional technical obstacles. Furthermore, establishing standardized protocols for miRNA quantification and quality control is essential for ensuring reproducible clinical outcomes.

Regulatory considerations present additional complexities. No miRNA therapy has yet reached Phase III or FDA approval and current guidelines for miRNA-based therapeutics remain limited, necessitating comprehensive safety assessment protocols and long-term monitoring strategies (199). The potential for immune activation in response to synthetic miRNA molecules and delivery vectors requires thorough evaluation in clinical trials. Given that subtle alterations in miRNA expression can profoundly affect intestinal epithelial function and tissue homeostasis (200), carefully designed strategies are essential for improving IBD treatment outcomes while minimizing adverse effects. Future research should prioritize personalized medicine approaches that integrate individual genetic backgrounds, microbiome compositions and disease-specific miRNA expression profiles to optimize therapeutic efficacy and safety in IBD management.

The persistent inability of miRNA therapeutics to achieve clinical success despite extensive research and substantial investment indicates fundamental limitations in current translation strategies that approach complex regulatory networks as collections of independent molecular targets. The field is increasingly recognizing that reductionist approaches have their limits. Instead, we need systems-level interventions that can fine-tune multiple network components without locking them into rigid regulatory patterns (201). This means rethinking how we develop drugs, shifting away from perfecting single molecules toward strategies that can nudge entire network states in the right direction. There's another layer to consider here: miRNA regulatory networks didn't evolve in isolation. They developed as adaptive systems to help organisms maintain balance when facing environmental pressures. This evolutionary perspective suggests our therapeutic approach should work with these systems, not try to override them (202). The development of network-compatible therapeutics that cooperate with endogenous regulatory systems represents a promising avenue for achieving clinical success in miRNA-based IBD therapeutics (203).

Comprehensive analysis of miRNA clinical implications reveals that current therapeutic and diagnostic approaches inadequately address the nature of regulatory network dysfunction in IBD. Rather than pursuing molecular specificity and pathway precision, clinical applications should acknowledge biological complexity and prioritize restoration of adaptive capacity at the systems level. This paradigm shift requires new metrics for therapeutic success (network adaptability rather than pathway inhibition), novel diagnostic frameworks emphasizing functional assessment over molecular quantification and innovative development strategies prioritizing systems compatibility over molecular optimization. Through this fundamental reconceptualization, the field can progress beyond the current pattern of promising preclinical results followed by disappointing clinical outcomes, toward transformative therapeutic approaches capable of addressing the multifaceted nature of IBD pathogenesis.

8. Technological advances in miRNA research

High-throughput sequencing for miRNA analysis. High-throughput sequencing technologies have revolutionized miRNA research by enabling comprehensive analysis of expression profiles across diverse biological systems and disease states. Yet this technological leap has created an unexpected challenge. The generated data complexity now exceeds our current interpretative capabilities (204). While these methodologies have provided unprecedented insights into regulatory networks governing miRNA function in health and disease, particularly in IBD and host-microbiome interactions, they have simultaneously exposed the limitations of existing analytical frameworks for capturing the dynamic, context-dependent nature of these networks.

Recent methodological innovations include the Parallel Single-Cell Small RNA and mRNA Co-profiling, version 2 (PSCSR-seq V2) technique. This method enables simultaneous sequencing of miRNAs and mRNAs at single-cell resolution, facilitating sensitive miRNA analysis alongside comprehensive mRNA expression data. The approach permits detailed coexpression analysis across thousands of individual cells (121). PSCSR-seq V2 has demonstrated considerable potential for revealing previously uncharacterized miRNA functions in cellular processes such as tumor suppression and metabolic regulation. This technology has also identified age-associated miRNAs like miR-29, which may serve as evolutionarily conserved markers for immunosenescence (121). However, a fundamental paradox emerges as technological resolution increases. Our enhanced capability to measure individual molecular events corresponds with a diminished capacity to interpret their collective biological significance. The miRTarBase database (205) represents another significant advancement, currently encompassing over 3,817,550 experimentally validated miRNA-target interactions (MTIs) from 13,690 peer-reviewed publications. This comprehensive resource documents miRNA interactions with therapeutic agents, their involvement in drug resistance mechanisms, and their potential as biomarkers for toxicity assessment and treatment guidance. The database incorporates oxidized miRNA sequences and integrates artificial intelligence models, including META's LLAMA3 framework, for efficient MTI identification, establishing it as an essential resource for molecular oncology research and drug development.

High-throughput sequencing has transformed how we study miRNAs in intestinal fibrosis. These methods systematically identify regulatory miRNAs, clarify their functional roles, and reveal underlying molecular mechanisms. By mapping miRNA-mRNA interaction networks, researchers pinpoint critical regulatory nodes in fibrotic processes, which guides the design of targeted therapies. Analysis of miRNA-regulated signaling pathways has yielded important mechanistic insights. For example, miR-155 targets HBP1, a negative regulator of Wnt/β-catenin signaling, which drives fibrotic tissue remodeling. These findings suggest new therapeutic strategies for managing intestinal fibrosis in patients with IBD (133,206). Combining single-cell RNA sequencing with miRNA profiling reveals cell type-specific expression patterns in inflamed intestinal tissues. Recent work identified the cellular sources of key miRNAs in IBD, showing that miR-145 localizes specifically to myofibroblasts in both CD and UC. Notably, myofibroblasts expressing miR-145 display enhanced tissue repair capacity and metabolic activity (167). miRNA sequencing also uncovers subtype-specific signatures in IBD. Elevated miR-31 characterizes ileal CD, while miR-215 levels predict penetrating complications. These stable markers classify patients into molecular subtypes with distinct clinical trajectories (207). Several limitations constrain current technologies. Data generation capacity has outpaced biological understanding (208). High-throughput approaches typically miss the temporal dynamics, spatial organization, and context dependency that define functional regulatory networks. Biological systems display distributed rather than hierarchical control, meaning single-node interventions often trigger compensatory mechanisms. Cell type-specific molecular signatures show dynamic plasticity in disease rather than stable, targetable features. Future methods must capture emergent network properties instead of simply cataloging individual molecular interactions (209). Overcoming these limitations requires analytical approaches that simultaneously measure molecular states, temporal trajectories, and functional responses across multiple organizational levels. Systems-level comprehension of miRNA regulatory networks depends on this shift.

Bioinformatics tools for miRNA-microbiome studies. Bioinformatics tools are now indispensable for analyzing the complex datasets generated in miRNA-microbiome research (210). However, the proliferation of computational methods has fragmented the field, with technical sophistication often concealing fundamental conceptual gaps and the absence of unified theoretical frameworks (211). Although these tools effectively identify patterns in high-throughput data and produce clinically relevant insights, they frequently reduce biological networks to collections of isolated statistical associations instead of capturing their inherently interconnected nature.

Advanced machine learning approaches have demonstrated capabilities in identifying disease-specific miRNA signatures with clinical applications. One study identified unique miRNA expression patterns and corresponding target genes in patients with sarcoma using The Cancer Genome Atlas data combined with Deep Neural Network analysis. This investigation revealed that specific miRNAs are involved in cancer development and can predict patient prognosis with high accuracy. The research highlights the potential utility of particular miRNAs, including miR-3688 and miR-3936, in tumor screening, prognosis prediction and therapeutic target identification, although large-scale clinical validation remains necessary (212). However, these machine learning successes raise a fundamental question: If complex diseases can be accurately predicted using molecular signatures, why have predictive models consistently failed to yield effective therapeutic interventions?

The development of specialized computational pipelines for integrating miRNA and microbiome datasets has enabled the identification of cross-kingdom regulatory interactions previously undetectable using conventional approaches. These bioinformatics frameworks incorporate statistical methods for handling microbiome data characteristics, including compositional effects, sparsity and high dimensionality. Network-based approaches have proven valuable for visualizing and analyzing interactions between host miRNAs and microbial communities, revealing regulatory relationships contributing to disease pathogenesis. However, these advances reveal a limitation in current computational paradigms: Treating cross-kingdom interactions as static network relationships overlooks the dynamic, context-dependent nature of host-microbiome communication determining functional outcomes. Predictive modeling utilizing machine learning algorithms has shown promise in identifying miRNA biomarkers for IBD diagnosis and prognosis. These methods integrate multiple data types, including miRNA expression profiles, clinical parameters and microbiome composition, to develop robust models for disease classification and therapeutic response prediction (213,214). Artificial intelligence approaches, including deep learning frameworks, have enhanced model accuracy and reliability while reducing computational time. However, increasing reliance on 'black box' algorithms creates tension between predictive accuracy and mechanistic understanding, potentially hindering the development of biologically-based therapeutic interventions (215). Cloud-based bioinformatics platforms have democratized access to analytical tools, enabling researchers worldwide to analyze complex miRNA datasets without extensive computational infrastructure. These platforms provide standardized pipelines ensuring reproducibility and enabling cross-study comparisons. User-friendly interfaces have made these tools accessible to researchers with diverse computational backgrounds, accelerating discovery in miRNA research. However, this democratization creates risks by enabling sophisticated analyses without requiring an understanding of underlying assumptions and limitations, potentially leading to data misinterpretation (216). The current bioinformatics landscape exhibits 'analytical sophistication without conceptual integration', where computational tools are applied to biological problems lacking adequate theoretical frameworks for interpretation (211). Advancing this field requires analytical approaches that capture temporal evolution, spatial organization and functional context of regulatory networks.

Innovations in miRNA delivery systems. Emerging therapeutic strategies for miRNA-based interventions involve sophisticated nanoparticle delivery systems specifically designed to improve molecular stability, enhance tissue specificity and minimize off-target effects that can compromise therapeutic efficacy and safety (217). However, the extensive emphasis on delivery system sophistication reveals a critical limitation: The underlying assumption that effective therapeutic delivery will overcome the intrinsic limitations of targeting complex regulatory networks through single-molecule interventions. These innovative delivery approaches represent critical technological advances that are essential for translating promising preclinical findings into effective clinical therapeutics, yet these approaches may not address the core challenges inherent in single-molecule interventions targeting complex regulatory networks. Indeed, recent reviews emphasize that even the most advanced nanoparticle systems still face issues of biodistribution, immunogenicity, and off-target repression, especially when applied to multi-target regulatory networks (187,218). The implementation of multi-panel biomarker analysis approaches, which have gained increasing popularity in CRC diagnostic applications, could be strategically expanded to include comprehensive miRNA profiling for predicting IBD disease progression and personalizing therapeutic treatments (180). This integrative approach offers direct anti-inflammatory therapeutic benefits while simultaneously paving the way for developing targeted, personalized IBD treatment protocols that account for individual patient molecular profiles and disease characteristics, although the correlation between molecular specificity and therapeutic efficacy remains to be fully established.

Innovative research has explored the therapeutic effects of naturally derived delivery systems, including garlic-derived exosome-like nanovesicles (GENs), which contain diverse bioactive components, including 127 known miRNAs with potential therapeutic applications. Comprehensive experimental investigations using DSS-induced colitis murine models demonstrated that Han-miR3630-5p contained within GENs could provide significant protection against colonic tissue damage through targeted inhibition of the TLR4/MyD88/NF-κB signaling pathway while simultaneously modulating gut microbiota composition. These findings indicate that GENs demonstrate considerable potential as preventive interventions for IBD due to their demonstrated anti-colitic properties and favorable safety profiles (219). The success of naturally derived systems raises an important consideration: Given that evolutionary processes have optimized miRNA delivery through biological vesicles, the rationale for pursuing fundamentally different synthetic approaches rather than biomimetic strategies warrants further investigation.

Advanced bioengineering approaches have led to the development of sophisticated encapsulation strategies for therapeutic miRNA delivery. A recent study demonstrated that encapsulating miR-31 mimetics within oxidized konjac glucomannan microspheres effectively alleviated DSS-induced experimental colitis in murine models (220). This innovative delivery system provides controlled release kinetics while protecting therapeutic miRNA from enzymatic degradation, thereby enhancing bioavailability and therapeutic efficacy. Targeted delivery strategies utilizing supercarbonate apatite (sCA) nanoparticles have shown remarkable promise for directing therapeutic miRNAs to specific cell populations within inflamed tissues. An experimental study demonstrated that miR-29 conjugated with sCA nanoparticles effectively targets DCs within inflamed colonic tissues, significantly ameliorating experimental colitis in murine models through intravenous administration of sCA-miR-29a-3p or sCA-miR-29b-3p formulations (221). This targeted approach facilitates preferential accumulation of therapeutic miRNAs in specific cell populations while minimizing systemic exposure and associated adverse effects. miR-223 overexpression achieved through specialized nanoparticle delivery systems significantly reduced experimental colitis severity (222), providing compelling evidence for the therapeutic potential of targeted miRNA delivery in inflammatory conditions. These delivery systems incorporate sophisticated targeting mechanisms that enable preferential accumulation in inflamed tissues while minimizing exposure to healthy tissues.

Recent innovations in delivery system design have yielded stimuli-responsive nanoparticles capable of releasing therapeutic payloads in response to specific inflammatory microenvironmental conditions, including pH alterations, oxidative stress and enzyme activities characteristic of inflamed intestinal tissues (223). These systems show improved specificity and reduced toxicity compared to conventional approaches. Targeting ligands, including antibodies, aptamers, and peptides, enable nanoparticles to preferentially target specific cell types or tissue regions, improving efficacy while reducing off-target effects. CD44-targeting peptide-modified LNPs demonstrated enhanced delivery to tumor cells (224). Lipid-based systems, including liposomes and lipid nanoparticles, offer biocompatibility, biodegradability, and protection of miRNAs from enzymatic degradation (225). These platforms can be engineered for controlled release and tissue-specific targeting, making them suitable for clinical translation in IBD treatment. The growing complexity of delivery systems raises questions about therapeutic design principles (226). Recent analyses suggest that increasingly intricate delivery mechanisms may indicate suboptimal target selection, this delivery system paradox highlights the need to reassess current approaches, and future development might benefit from identifying interventions inherently compatible with biological systems (227). Effective therapeutics should work with endogenous regulatory mechanisms rather than against them. Understanding and augmenting natural regulatory processes may prove more productive than relying solely on synthetic alternatives. Biomimetic approaches that leverage evolutionary optimization may offer advantages over purely engineered solutions (228,229). Table I summarizes key miRNAs involved in IBD pathogenesis, detailing their targets, experimental models employed and supporting evidence levels.

9. Cross-kingdom communication networks and systems approaches

Evolution of miRNA research in host-microbiome studies. The investigation of miRNA functions in host-microbiome interactions has evolved significantly over the past two decades, representing a paradigmatic shift in the understanding of cross-kingdom molecular communication. This evolutionary trajectory highlights the need to balance technological advancement with conceptual innovation to enhance the understanding of complex biological phenomena. Initially, research efforts were primarily concentrated on elucidating the fundamental functions of miRNAs in gene regulation within host cellular systems, with emerging recognition of their roles in mediating ecological interactions. This initial focus on reductionist approaches has evolved toward more integrative frameworks for understanding cross-kingdom regulatory networks.

The advancement of high-throughput sequencing technologies and computational analytical frameworks has enabled researchers to explore the complex interplay between miRNAs and diverse microbial communities inhabiting the human body. These technological capabilities have highlighted the challenge of translating molecular measurements into predictive models for biological outcomes, indicating opportunities for conceptual framework development alongside technical advances. Recent studies have identified viral miRNAs, particularly those derived from herpesviruses such as Epstein-Barr virus (EBV), as important regulatory molecules in host gene expression and disease pathogenesis. EBV-miR-BART18-3p represents a notable example of viral miRNA function, as it has been linked to promoting CRC development through enhancement of metabolic and lipogenic signaling pathways, increased CRC cell migration and invasion capabilities, and associations with advanced CRC disease stages. These findings suggest potential for this viral miRNA as both a diagnostic biomarker and therapeutic target (9), highlighting the complex role of viral-derived regulatory molecules in human disease processes. The discovery of viral miRNA regulation underscores the importance of considering viral and fungal components alongside bacterial interactions in host-microbiome research, as these may exert significant regulatory influences. Fungal microbiomes and their associated miRNAs remain understudied in terms of their interactions with host biological systems, representing an important knowledge gap in our understanding of host-microbiome communication networks. Although direct experimental evidence for gut fungal miRNAs affecting human gene expression remains limited, evidence exists for cross-kingdom small-RNA regulatory pathways that can significantly influence host gene expression patterns. Notable examples include bba-milR1 derived from the entomopathogenic fungus *Beauveria bassiana*, which has been demonstrated to suppress mosquito immune responses (230), and miRNAs from the plant pathogen *Fusarium oxysporum* that regulate gene expression in chickpea hosts (231). Additionally, research has revealed that miRNAs from the ectomycorrhizal fungus *Pisolithus microcarpus* can enhance its successful integration into *Eucalyptus grandis* root tissues during mutualistic symbiotic relationships (232). These studies collectively highlight the need for further research to elucidate the mechanisms through which diverse microbiomes interact with and influence host gene expression programs. The recognition of cross-kingdom miRNA communication expands the conceptual framework for understanding host-microbiome interactions beyond metabolite-mediated communication pathways.

Current trends in miRNA and microbiome research. Contemporary research in the miRNA-microbiome field has revealed bidirectional regulatory mechanisms wherein host-derived miRNAs, particularly those packaged within exosomal vesicles, can effectively modulate microbial gene expression patterns, while microbial metabolites such as SCFAs reciprocally influence host miRNA expression profiles (144). These interactions exhibit multi-layered, context-dependent characteristics that extend beyond simple bidirectional models. This intricate molecular crosstalk represents a fundamental mechanism of host-microbiome

Table I. Key IBD-related miRNAs: Targets, model systems and evidence strength.

miRNA	Primary target(s)	Target type	Experimental model	Evidence strength	Clinical relevance	(Refs.)
miR-21	*B. thetaiotaomicron* tryptophan operon; PTEN/PI3K/AKT	Cross-kingdom + host	Fecal microbiota co-culture; IL-10$^{-/-}$mice; colitis models; patient feces/biopsies	Moderate (Clinical validation + multiple independent studies)	Pro-inflammatory; diagnostic biomarker; elevated in active IBD	(20,42,131, 132,164,192)
miR-223	NLRP3 inflammasome; C/EBPβ; IL-1β/ IL-18	Host immune	Imune cell cultures; miR-223$^{-/-}$ mice; DSS colitis; patient serum/feces/biopsies	Strong (Genetic knockout + clinical validation + therapeutic delivery)	Biomarker for disease activity; therapeutic target	(54,55,57,168, 170,222)
miR-155	C/EBPβ; SOCS1; SHIP-1; Wnt/β-catenin	Host immune + fibrosis	CD4+T cells; immune cell cultures; CCD-18Co cells; miR-155$^{-/-}$ mice; DSS colitis; patient biopsies	Strong (Genetic knockout + clinical validation + dual functions)	Pro-inflammatory; pro-fibrotic; elevated in IBD mucosa	(59,74,85,100-102,133,178)
miR-146a	IRAK1; TRAF6; NUMB; NF-κB; TAB1	Host immune+ barrier	Epithelial cells and macrophages; miR-146a$^{-/-}$ mice; colitis models; patient biopsies	Strong (Genetic knockout + clinical correlation + inflammation regulation)	Upregulated in active UC mucosa; therapeutic target	(22,66,151, 155,184)
miR-146b	FGL2; IRF5; IL-10 pathway	Host immune	LPS-stimulated macrophages; DSS colitis; engineered *Lactococcus lactis*	Moderate (Clinical validation + innovative delivery system)	Macrophage polarization; upregulated in IBD tissue; probiotic-mediated delivery shows therapeutic potential	(71,72,175, 185)
miR-142a-3p	*L. reuteri* growth promotion	Microbial	DSS colitis + oral mimic ± antibiotics	Limited (Study validating microbiome-mediated mechanism)	Microbiome-mediated therapeutic effect	(46)
miR-29 family	IL-23p19; collagen synthesis; NOD2-regulated	Host immune + fibrosis	DCs and CD4+ T cells; fibroblasts; colitis models	Moderate (Therapeutic delivery system + multiple models + dual functions)	Anti-inflammatory; anti-fibrotic potential	(92,153,154, 190,221)
miR-10a	Prdm1 (Blimp1); IL-12/IL-23p40; NOD2	Host immune	CD4^{+} T cells; miR-10a$^{-/-}$ mice; DSS colitis; patient biopsies	Moderate-Strong (Genetic knockout + clinical samples + complex regulation)	DC and T cells function; IL-10 regulation	(83,111-113)

Table I. Continued.

miRNA	Primary target(s)	Target type	Experimental model	Evidence strength	Clinical relevance	(Refs.)
miR-122/ miR-122a	Occludin; NOD2	Host barrier + microbial	HT-29 cells; Caco-2 cells; *in-vivo*	Limited (*In vitro* and *in vivo)* validation) Strong (Clinical validation + therapeutic antagomir)	Epithelial homeostasis	(152,179)
miR-200c-3p	Occludin; epithelial barrier genes	Host barrier	Caco-2 cells; DSS colitis; patient biopsies	Moderate-Strong (Clinical validation + therapeutic antagomir)	Upregulated in active UC tissue; epithelial homeostasis; antagomiR therapeutic	(180,181)
miR-31	Inflammatory pathways; WNT and Hippo signaling	Host epithelial repair	miR-31$^{-/-}$ mice; OKGM delivery; patient biopsies	Moderate-Strong (Therapeutic delivery + clinical biomarker + multiple models)	Diagnostic panel for UC/CD differentiation; epithelial homeostasis; therapeutic delivery	(164-166, 220)
miR-215	Unknown in IBD context	Host	Patient tissue profiling (CD behavior)	Limited (Clinical correlation)	CD phenotype classification	(165)
miR-17~92 cluster	PTEN and IKZF4; Th1/Th17 promotion	Host immune	T cell cultures; T cell transfer colitis model	Moderate (Preclinical genetic models + limited IBD-specific data)	T cell differentiation; humoral immunity	(84,114, 115)
miR-125a	ETS-1; Th1/Th17 suppression	Host immune	CD4+ T cells; miR-125a$^{-/-}$ mice; patient biopsies	Strong (Genetic knockout + clinical validation + mechanistic clarity)	Downregulated in IBD patient mucosa; epithelial homeostasis	(106)
miR-106a	IL-10; NF-κB; Treg function	Host immune	miR-106a$^{-/-}$mice; miR106a$^{-/-}$ TNF$^{\Delta ARE/+}$; human PBMCs	Moderate (Genetic models + limited clinical data)	Treg dysfunction; IL-10 regulation	(109,110)
miR-200b	ZEB1; ZEB2; EMT inhibition	Host fibrosis	IEC-6 cells; TNBS-induced fibrosis; microvesicle delivery	Moderate (Antifibrotic model + delivery system)	Anti-fibrotic; EMT prevention	(189)
miR-149-3p	Inflammatory pathways; *A. muciniphila*	Host immune+ barrier + microbial	Caco-2 and SW480 cells; miR-149-3p$^{-/-}$ mice; patient blood	Moderate-Strong (Genetic knockout + clinical samples + dual host-microbe effects)	Reduced in CD patient blood; anti-inflammatory	(157)

A. muciniphila, *Akkermansia muciniphila*; *B. thetaiotaomicron*, *Bacteroides thetaiotaomicron*; C/EBPβ, CCAAT/enhancer-binding protein β; CD, Crohn's disease; DCs, dendritic cells; DSS, dextran sulfate sodium; EMT, epithelial-mesenchymal transition; ETS-1, E26 transformation specific-1; FGL2, fibrinogen-like protein 2; IBD, inflammatory bowel disease; IEC, intestinal epithelial cells; IKZF4, IKAROS family zinc finger 4; IRAK1, interleukin-1 receptor-associated kinase 1; IRF5, interferon regulatory factor 5; *L. reuteri*, *Lactobacillus reuteri*; LPS, lipopolysaccharide; miRNA, microRNA; NF-κB, nuclear factor κB; NLRP3, NOD-like receptor protein 3; NOD2, nucleotide-binding oligomerization domain-containing protein 2; NUMB, NUMB endocytic adaptor protein; OKGM, oral keratinocyte growth medium; PBMCs, peripheral blood mononuclear cells; PI3K, phosphoinositide 3-kinase; Prdm1, PR domain zinc finger protein 1 (also known as Blimp1); PTEN, phosphatase and tensin homolog; SHIP-1, SH2-containing inositol 5'-phosphatase 1; SOCS1, suppressor of cytokine signaling 1; Th, T helper cell; TNBS, trinitrobenzene sulfonic acid; TNF-α, tumor necrosis factor α; TRAF6, TNF receptor-associated factor 6; Treg, regulatory T cell; UC, ulcerative colitis; ZEB1, zinc finger E-box binding homeobox 1.

communication that has profound implications for health and disease states, and current research approaches are evolving from studying isolated molecular pathways toward more integrated analytical methodologies. Although a definitive causal mechanism has yet to be confirmed, multiple studies have documented a negative correlation between miR-122-5p levels and *Bacteroides uniformis* abundance in patients with type 2 diabetes, supporting the possibility that miR-122 may influence gut microbial community structure (including *Akkermansia muciniphila*) and thereby modulate metabolic health outcomes and inflammatory status (233). Current research emphasizes the crucial role of miRNA-microbiome interactions in the pathogenesis of diverse systemic diseases, including various malignancies, obesity, metabolic disorders and neurodegenerative conditions, with miR-21 and miR-155 identified as key regulatory molecules in these pathological processes. The gut microbiota's influence on host miRNA expression is mediated through sophisticated signaling pathways, including TLR and MyD88-dependent mechanisms, with beneficial bacterial species such as *Bifidobacterium* and *Lactobacillus* demonstrating the capacity to promote anti-inflammatory miRNA expression profiles (71). The study of specific miRNA-bacterial pairs provides a foundation for understanding broader network properties and ecological interactions.

This mechanistic understanding has opened new avenues for therapeutic intervention through targeted modulation of specific bacterial populations, although the complexity of network interactions requires careful consideration in therapeutic design. Advanced sequencing technologies, including long-read and single-cell sequencing methodologies, are employed to characterize microbial strain-specific responses to host miRNA molecules at high resolution (234). These technological advances enable researchers to identify previously unrecognized diversity in miRNA-microbiome interactions and to understand how different bacterial strains within the same species may respond differently to identical host-derived regulatory signals. These high-resolution measurements contribute to developing comprehensive theoretical frameworks for understanding multi-scale regulatory networks. Multi-omics integration approaches, encompassing metagenomics, metatranscriptomics and metabolomics analyses, represent an important contemporary development that enables comprehensive profiling of microbial functional capacities (235). These integrated analytical approaches provide holistic insights into the dynamic relationships between microbial community structure, functional activity and host regulatory responses, facilitating a more complete understanding of host-microbiome interactions in health and disease. Computational frameworks for analyzing multi-omics datasets enable researchers to construct interaction networks that reveal novel connections between specific microbial taxa, metabolic pathways and host miRNA expression patterns. These systems-level approaches provide valuable insights into the complexity of host-microbiome communication and are identifying novel therapeutic targets for intervention.

An emerging area involves the application of digital technology and data science to miRNA research and IBD management. Artificial intelligence and mobile health tools are being explored to integrate miRNA insights into patient care (236,237). Smartphone applications utilizing artificial intelligence algorithms are being designed to help patients with IBD track symptoms, inflammation markers and possibly biomarker data, including miRNA profiles (237-239). A recent study identified the essential information needs for an IBD self-management app, which cover domains such as disease knowledge, medication tracking, diet/nutrition and lifestyle habits, indicating opportunities for digital platforms to support patient care (240). Integrating miRNA information into these mobile tools may enable earlier therapeutic intervention and more personalized treatment strategies.

10. Limitations and challenges

Although miRNA research in IBD demonstrates significant potential, several important limitations warrant consideration. Inter-patient variability in miRNA expression represents a significant challenge, as miRNA profiles can differ substantially among individuals, disease subtypes and even among intestinal regions in the same patient (167,241,242). This heterogeneity complicates the identification of universal biomarkers and indicates that diagnostic miRNA signatures may require personalization or contextual interpretation. Substantial technical challenges also exist in detecting and quantifying miRNAs, particularly from fecal samples. Fecal miRNAs are present at low concentrations and demonstrate variable isolation efficiency (243). Different studies report substantially different numbers of detectable fecal miRNAs due to variations in extraction methods, sequencing depth and analysis pipelines. This lack of standardization compromises reproducibility and highlights the need for consensus protocols and validation across larger cohorts (244,245). Furthermore, the complex interplay between host and microbiome introduces confounding factors that complicate the determination of causal relationships (246,247). Changes in the gut microbiota can alter host miRNA expression through mechanisms such as microbial metabolite-mediated regulation of host genes, while host miRNAs can concurrently shape microbial community composition (247,248). Disentangling these bidirectional effects remains inherently challenging. Consequently, observed miRNA alterations may represent consequences of microbiome shifts rather than primary drivers, or conversely. Rigorous experimental designs (e.g., gnotobiotic models, controlled dietary interventions) and advanced computational models will be required to dissect these interactions (249). The translation of miRNA findings into clinical practice faces practical challenges: Assays must be sufficiently sensitive, specific and cost-effective for routine use, and miRNA-based therapeutics must overcome delivery barriers and off-target risks (250,251). These limitations do not diminish the potential of miRNA research but rather identify areas where further work is needed, including large-scale validation studies, improved analytical techniques and integrated analyses accounting for host-microbe complexity. Addressing these challenges will be crucial for advancing miRNA-based IBD applications from bench to bedside.

11. Future directions in miRNA and IBD research

The field is actively exploring novel therapeutic and diagnostic strategies that leverage the miRNA-microbiome regulatory axis for clinical benefit (3). Current research efforts face the challenge of ensuring that investigations address key scientific questions while advancing toward clinical translation. A promising research direction involves the development of miRNA-based therapeutic interventions, including synthetic miRNA mimics and targeted anti-miRNAs, designed to modulate intestinal homeostasis and reduce inflammation in patients with IBD (5). Preclinical evidence demonstrates that miR-31 mimics have shown potential in reducing inflammatory responses in DSS-induced experimental colitis models through targeted modulation of pro-inflammatory signaling pathways. These findings provide a rationale for advancing miRNA-based therapeutics toward clinical development for IBD treatment applications, although the effectiveness of single-molecule interventions in complex regulatory networks requires further validation. Fecal microbiota transplantation (FMT) represents an emerging therapeutic approach with potential for IBD management, with evidence indicating that FMT can normalize dysregulated miRNA expression levels (including miR-23a and miR-150) while simultaneously reducing pro-inflammatory protein expression, thereby providing cytoprotective effects (252,253). These findings indicate the potential for developing personalized microbiome-based therapeutic interventions tailored to individual patient microbial compositions and miRNA expression profiles. The success of FMT interventions highlights the importance of considering both ecosystem-level and molecular targeting approaches in therapeutic development. An important research direction involves the investigation of dietary interventions supplemented with prebiotics or probiotics to regulate miRNA expression patterns and modulate microbial community composition (253,254). Technological advancements may provide higher-resolution insights into miRNA-microbiome interactions at the individual bacterial strain level, potentially identifying novel therapeutic targets previously undetected (246,255). The development of strain-specific therapeutic interventions could enable unprecedented precision in manipulating gut microbial communities to achieve desired therapeutic outcomes, although the complexity of ecological network responses requires careful consideration in therapeutic design.

Additionally, comprehensive *in vivo* experimental studies are needed to elucidate signaling pathways and identify bacterial taxa involved in miRNA regulatory mechanisms, to address knowledge gaps in understanding how miRNAs modulate microbial community dynamics in IBD pathogenesis (5,247). These mechanistic investigations are essential for translating scientific insights into clinical applications, while recognizing the importance of developing theoretical frameworks that capture emergent network properties alongside molecular characterization. Future research efforts may focus on developing predictive models that integrate patient-specific miRNA expression profiles, microbial compositions, genetic backgrounds and clinical parameters to optimize therapeutic selection and timing. Real-time monitoring systems for miRNA-microbiome dynamics could facilitate adaptive treatment protocols that respond to changing biological conditions. The development of predictive modeling and real-time monitoring should consider the inherent complexity of biological systems and the relationship between predictive accuracy and therapeutic efficacy. Emerging areas of investigation include tissue-specific delivery systems for therapeutic miRNAs, combination therapies targeting multiple miRNA-microbiome pathways and long-term safety assessments of miRNA-based interventions. These research efforts aim to translate mechanistic insights into clinical applications, thereby improving diagnostic accuracy, therapeutic efficacy and treatment outcomes for patients with IBD (178). Integration of artificial intelligence and machine learning approaches may accelerate the discovery of novel therapeutic targets and biomarkers, and facilitate the development of personalized treatment algorithms accounting for the interactions between host genetics, miRNA expression, microbial communities and environmental factors (213). These advances may contribute to transforming IBD management through precision medicine approaches tailored to individual patient characteristics and disease profiles.

Translating miRNA research into clinical practice requires navigating regulatory, commercial and economic barriers. Diagnostic tests must satisfy analytical and clinical validation standards before approval, while therapeutic development faces challenges in manufacturing scale-up, batch consistency and intellectual property protection (256,257). Clinical progress, though modest, is underway. ABX464 (obefazimod), an oral agent that upregulates miR-124, showed sustained efficacy in Phase 2 trials for moderate-to-severe UC, with reductions in IL-6 and improved endoscopic outcomes compared to placebo (258,259). Its advancement to Phase 3 trials indicates that miRNA-targeted strategies can navigate the translational pipeline (260). Current evidence points to three understudied areas: How regulatory networks adapt and reorganize under perturbation; how these networks evolve over time, beyond the static snapshots captured in most studies; and whether therapies can work with, rather than against, endogenous regulatory mechanisms. Progress in these areas will require closer integration of systems biology approaches with clinical investigation.

12. Controversies and unresolved challenges in miRNA research

Discrepancies in miRNA study results. Discrepancies in miRNA research findings, particularly within host-microbiome interactions, present significant challenges to establishing reliable and reproducible scientific conclusions for clinical translation. These inconsistencies extend beyond technical difficulties, revealing fundamental limitations in current research paradigms when investigating complex biological networks across multiple organizational scales and temporal dimensions. Such variations stem from methodological differences that significantly impact data interpretation and therapeutic translation, while simultaneously identifying critical scientific questions that remain inadequately addressed by current methodologies.

Methodological inconsistencies constitute a primary source of conflicting results in miRNA research. However, characterizing these as merely technical problems resolvable through standardization overlooks their deeper significance as indicators of conceptual limitations. The selection of analytical platforms, such as RT-qPCR vs. NGS technologies,

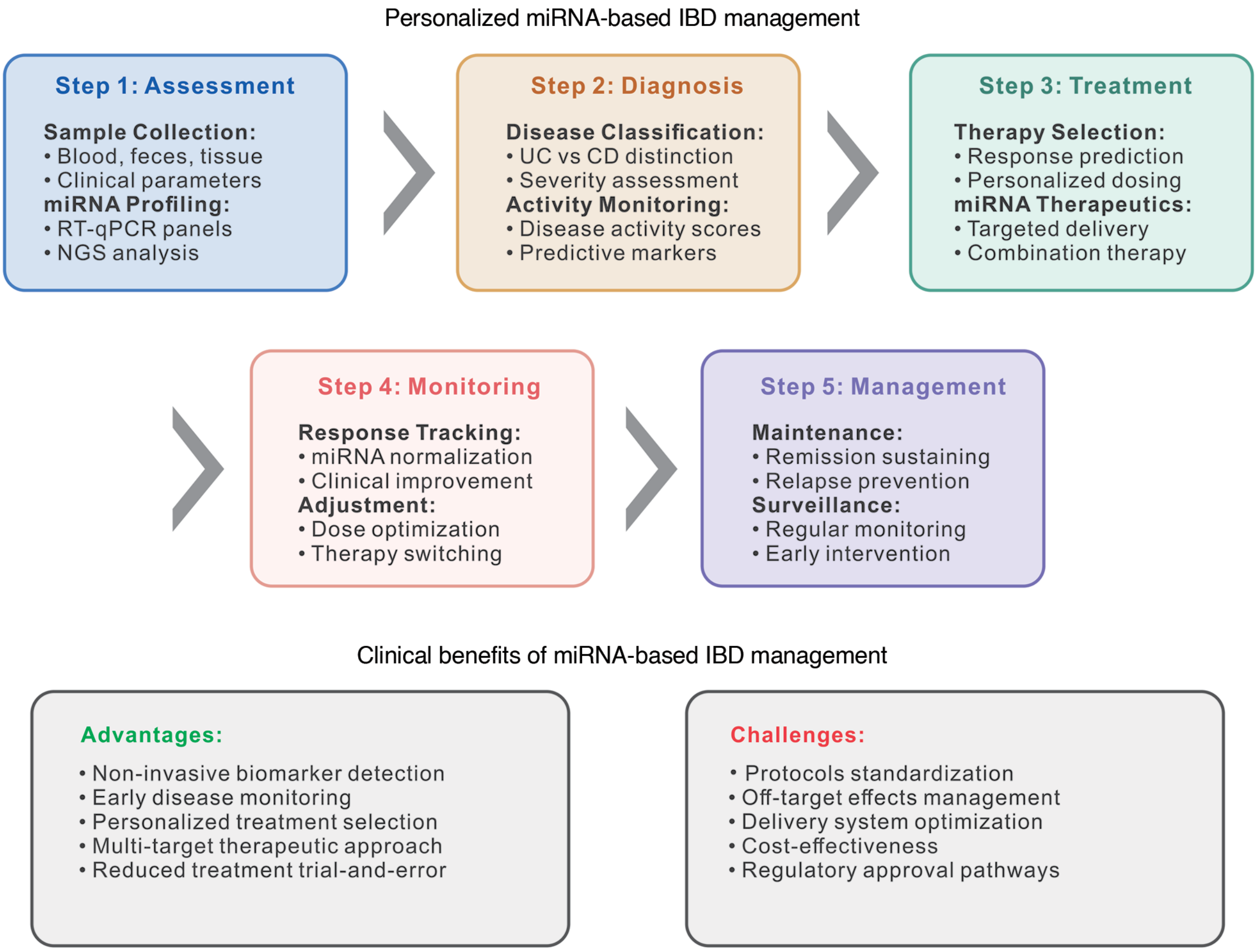

Figure 7. Personalized miRNA-based IBD management workflow. Five-step clinical workflow: i) Assessment: multi-sample collection (blood, feces, tissue) and miRNA profiling using RT-qPCR panels and NGS analysis; ii) Diagnosis: UC vs. CD classification, severity assessment and disease activity monitoring using predictive markers; iii) Treatment - response prediction, personalized dosing and targeted miRNA therapeutics with combination therapy; iv) Monitoring: miRNA normalization tracking, clinical improvement assessment, dose optimization and therapy switching; v) Management: remission maintenance, relapse prevention and regular surveillance with early intervention. Clinical benefits include non-invasive biomarker detection, early disease monitoring, personalized treatment selection, multi-target approaches and reduced trial-and-error treatment. Current challenges encompass protocol standardization, off-target effects management, delivery system optimization, cost-effectiveness and regulatory approval pathways. IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; RT-qPCR, reverse transcription quantitative PCR; NGS, next-generation sequencing.

frequently results in substantially divergent miRNA expression profiles (261). These technical discrepancies are amplified by variations in sample processing procedures, including RNA extraction methodologies, storage conditions and normalization approaches, which collectively contribute to irreproducibility in miRNA studies (262). Notably, methodological standardization alone may be insufficient if observed discrepancies reflect genuine biological complexity rather than technical inadequacies. Fundamental differences between analytical platforms create systematic biases affecting miRNA detection sensitivity, dynamic range and quantification accuracy. While RT-qPCR provides high specificity and quantitative precision for known miRNA targets, NGS enables comprehensive profiling but may introduce artifacts from library preparation and sequencing depth variations. These platform-specific characteristics require careful consideration when comparing inter-study results and underscore the need for standardized analytical protocols. Nevertheless, emphasizing platform standardization reveals an underlying assumption that biological phenomena can be adequately captured through single measurement approaches, regardless of technical sophistication. The debate surrounding cross-kingdom miRNA regulation remains contentious within the scientific community, with conflicting evidence regarding the physiological significance of these interactions. Several investigations support the hypothesis that plant-derived miRNAs, such as miR-168a, can influence gut microbiota composition and host health parameters through direct regulatory mechanisms (263,264). By contrast, other research groups attribute the detection of plant miRNAs in animal tissues to experimental contamination or suggest minimal physiological relevance (48). This disagreement reflects challenges in establishing appropriate experimental controls and developing sensitive detection methods capable of distinguishing genuine biological phenomena from technical artifacts. Furthermore, this controversy highlights limitations in current experimental frameworks for investigating phenomena that may operate through mechanisms distinct from conventional gene regulation. Additionally, computational models predicting miRNA-target interactions frequently yield inconsistent results and may overestimate the extent of microbial gene regulation by host-derived miRNAs. These predictive inconsistencies emphasize the need for standardized experimental protocols, refined computational algorithms and comprehensive validation frameworks to address these discrepancies and advance miRNA-microbiome research (265-267).

Debates on miRNA therapeutic efficacy. The therapeutic efficacy of miRNAs in modulating host-microbiome interactions remains contentious within scientific and clinical communities, primarily due to unresolved challenges concerning safety profiles, delivery mechanisms and treatment consistency. These debates, however, reveal a fundamental controversy that remains largely unaddressed: Current therapeutic failures may stem from inadequate conceptual frameworks rather than technical limitations alone. While these debates highlight uncertainties regarding the clinical viability of miRNA-based therapeutics for microbiome-related diseases, they often overlook a critical question: Can single-molecule interventions effectively modulate complex regulatory networks without inducing unpredictable compensatory responses?.

A primary concern involves the potential for substantial off-target effects in miRNA-based therapeutic interventions. Oliveira *et al* (268) highlighted that targeting microbial pathways with miRNAs risks disrupting beneficial microbiota due to the pleiotropic nature of miRNAs, the interconnectedness of microbial ecosystems, and our incomplete understanding of miRNA-microbiota interactions. The concept of 'off-target' effects may itself be misleading. In densely connected regulatory networks, any intervention propagates through multiple nodes, making distinctions between intended and unintended effects largely artificial. Delivery presents additional obstacles. miRNAs degrade rapidly in circulation, penetrate tissues poorly, and enter cells inefficiently. Current nanoparticle formulations address these problems only partially (199,254,269). Optimizing delivery systems may miss a deeper issue: therapeutic failure often reflects not inadequate delivery but the inherent difficulty of targeting single molecules within complex networks. Economic and practical barriers compound these biological challenges. Manufacturing costs for synthetic miRNAs and delivery vehicles remain high, standardized dosing protocols are lacking (270), and patient responses vary widely based on genetic background, baseline miRNA expression, microbiome composition, and disease severity. The absence of reliable predictive biomarkers complicates clinical trial design and result interpretation. The limited clinical success of miRNA therapeutics despite extensive research investment points to fundamental limitations in current therapeutic paradigms beyond technical obstacles (250). Reductionist strategies targeting single molecules may be poorly suited to diseases arising from network dysfunction. Therapies that restore regulatory flexibility rather than override it may prove more effective (Fig. 7).

As miRNA-microbiome research advances toward clinical translation, existing ethical frameworks prove insufficient. Two fundamental concerns emerge: Manipulating biological systems with unpredictable long-term consequences, and whether bypassing endogenous regulatory mechanisms creates new dependencies. Clinical safety poses the first challenge. Perturbing complex regulatory networks generates consequences beyond conventional off-target effects (195,271). Patients cannot provide truly informed consent when therapeutic outcomes exceed current predictive capacity. Microbiome interventions may produce delayed or cascading effects that extend beyond standard trial durations, creating temporal gaps that existing informed consent frameworks are ill-equipped to address. Dietary miRNAs in genetically modified foods extend these challenges to population-level exposures. Regulatory gaps leave consumers exposed to miRNA modifications without adequate oversight or labeling transparency (272-274). Food supply modifications affect entire populations without individual consent mechanisms, unlike clinical trials with defined participants and opt-out provisions. Diagnostic technologies introduce additional risks. Microbiome and miRNA profiling generates sensitive data revealing genetic predispositions and health conditions beyond intended disclosures (275). These datasets resist anonymization and enable new forms of discrimination, while data governance protocols remain inadequate. These challenges span clinical trials, food systems and data privacy, demanding integrated solutions. And progress requires transparent reporting standards, rigorous safety protocols and stakeholder engagement among researchers, clinicians, ethicists, patient advocates and community representatives. International coordination also becomes essential as research increasingly involves global collaborations.

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Availability of data and materials

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Authors' contributions

LeL, LiL, XL and HL conceived the study, conducted the literature review, visualization and drafted the original manuscript. HL, YJZ, SZ and LeL critically revised and proofread the manuscript. YZ, SZ and BL provided conceptual guidance and supervised the project. ZT, SM and CY contributed to literature screening and data extraction. LiL, HL and LeL secured the funding. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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