Review | Received 2 February 2026; Revised 7 March 2026; Accepted 10 March 2026; Published 30 March 2026
https://doi.org/10.55092/exrna20260003

From biomarkers to therapeutics: extracellular vesicle RNA as a pivotal player in inflammatory bowel disease management

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Highlights:

- EV-RNAs critically modulate IBD pathogenesis by regulating intestinal immunity, epithelial barrier integrity, and bidirectional host–microbiota crosstalk.
- EV-RNAs mediate systemic organ crosstalk, contributing to extraintestinal complications such as liver injury and cardiac dysfunction in IBD.
- EV-associated lncRNAs and miRNAs serve as promising non-invasive biomarkers for precise IBD diagnosis and disease activity monitoring.
- Engineered EVs, including MSC-derived and plant-based vesicles, offer targeted therapeutic platforms for delivering regulatory RNAs in IBD.

Abstract: Inflammatory Bowel Disease (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), is a group of chronic relapsing inflammatory disorders of the gastrointestinal tract with complex etiology and significant clinical challenges. Extracellular vesicles (EVs) act as key mediators of intercellular communication, carrying diverse RNA species—especially non-coding RNAs such as microRNAs and long non-coding RNAs—which have emerged as critical regulators in IBD pathogenesis and progression. This review synthesizes current understanding of how EV-associated RNAs modulate fundamental IBD-related processes, including inflammatory signaling, intestinal barrier function, immune regulation, and host–microbiota interactions. By integrating recent evidence from multi-omics studies and animal models, we highlight the promise of EV-derived RNAs as novel biomarkers and therapeutic targets. We further discuss advances in EV-RNA-based therapeutics and examine the challenges and future directions for translating these insights into clinical practice. By elucidating the multifaceted roles of EV-RNAs in IBD, this article aims to provide a theoretical foundation and inform future research toward precision diagnosis and personalized treatment strategies for IBD patients.

Keywords: EVs; EV-RNAs; IBD; molecular mechanisms; therapeutic potential

1. Introduction

Inflammatory Bowel Disease (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), comprises a group of chronic inflammatory disorders of the gastrointestinal tract. Its pathogenesis involves a complex interplay of genetic susceptibility, immune dysregulation, environmental factors, and alterations in the gut microbiota [1]. Globally, IBD epidemiology progresses through four defined stages: emergence, accelerating incidence, compounding prevalence, and prevalence equilibrium. Currently, early-industrialized regions are in the compounding prevalence stage and are projected to exceed 1% population prevalence by 2045, whereas newly industrialized nations are transitioning from accelerating incidence to compounding prevalence. Environmental exposures and demographic aging are primary drivers of these predictable transitions [2]. Recent advances in IBD research have highlighted the role of extracellular vesicles (EVs) as key mediators of intercellular communication within the intestinal microenvironment. These nanosized, membrane-bound vesicles are secreted by various cell types and transport bioactive molecules (including proteins, lipids, and RNA species such as non-coding RNAs) that influence recipient cell function [3]. Growing evidence suggests that EV-associated RNAs play pivotal roles in modulating immune responses, maintaining epithelial barrier integrity, and shaping microbial communities in the gut [4].

The recognition of EV-RNAs as critical regulators in IBD pathogenesis has opened new avenues for biomarker discovery and therapeutic innovation [5]. Enabled by high-throughput sequencing and multi-omics approaches, comprehensive profiling of EV-RNA cargo has revealed distinct expression signatures associated with disease activity and progression [6]. For example, dysregulated EV-derived long non-coding RNAs (lncRNAs) have been strongly correlated with IBD pathology, underscoring their potential as diagnostic and prognostic biomarkers [7]. Concurrently, targeting EV-associated RNAs offers a promising therapeutic strategy aimed at restoring mucosal homeostasis in IBD [8].

Mechanistic studies continue to delineate specific contributions of EV-RNAs to various aspects of IBD. EV-encapsulated microRNAs (miRNAs), for instance, have been shown to regulate epithelial repair processes and influence immune cell differentiation, thereby shaping the inflammatory milieu [9]. Similarly, EV-transported lncRNAs participate in maintaining intestinal barrier function and modulating host-microbiota interactions [10]. A deeper understanding of these molecular mechanisms is essential for translating EV-RNA biology into clinical applications.

From a translational perspective, EV-associated RNAs hold substantial promise as non-invasive biomarkers for early detection and disease monitoring in IBD [7]. Moreover, leveraging EVs as natural carriers for RNA-based therapeutics may offer novel treatment options, particularly for patients refractory to conventional therapies [11]. This review provides a comprehensive synthesis of current knowledge on EV-associated RNAs in IBD, focusing on their mechanistic roles in immune regulation, barrier restoration, and microbiota crosstalk. By integrating recent experimental and clinical insights, we aim to elucidate the therapeutic potential of EV-RNAs and outline future directions for their application in precision medicine for IBD.

2. Fundamental characteristics and categorization of EVs and their RNA

2.1. Definition and classification of EVs

EVs are heterogeneous, membrane-bound particles secreted by eukaryotic and prokaryotic cells and act as key mediators of intercellular communication. Based on biogenesis, size, and composition, EVs are classified into three main subtypes (Figure 1A); and apoptotic bodies (50 nm–5 μm), released during programmed cell death and containing cytoplasmic and nuclear fragments [12]. Each subtype carries a distinct molecular cargo—proteins, lipids, and nucleic acids including mRNAs and ncRNAs—that reflects the physiological or pathological state of the parent cell [13].

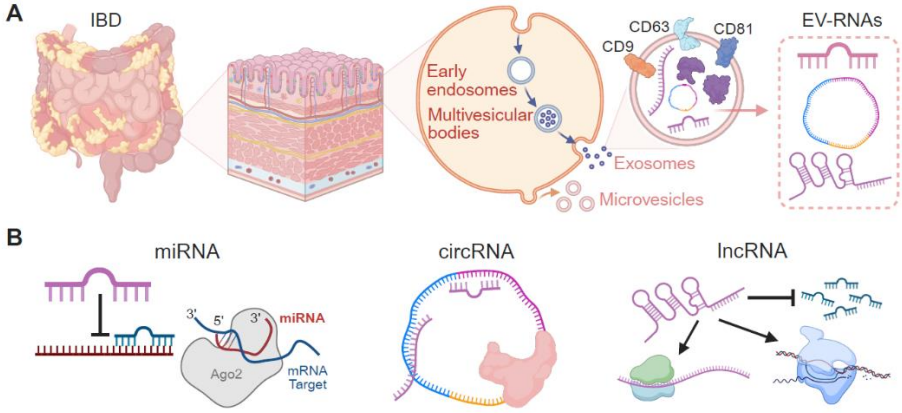

Figure 1. Biogenesis and major RNA cargo of EVs. **(A)** Schematic representation of the two primary pathways of EV biogenesis. Exosomes originate from the endosomal pathway through the inward budding of multivesicular bodies (MVBs) and subsequent fusion with the plasma membrane. They are characteristically enriched in tetraspanin markers such as CD9, CD63, and CD81. In contrast, microvesicles (or ectosomes) are generated by the direct outward budding and fission of the plasma membrane. **(B)** Overview of the diverse RNA species encapsulated within EVs. The predominant RNA cargo includes miRNAs, lncRNAs, and circular RNAs (circRNAs), each playing distinct roles in intercellular communication, post-transcriptional regulation, and epigenetic modulation, thereby critically influencing recipient cell function and phenotype. Created with BioRender.com.

EV function depends on their cellular origin and cargo. In the gastrointestinal tract, EVs facilitate crosstalk among epithelial cells, immune cells, and gut microbiota by transferring bioactive molecules that regulate immune responses, maintain mucosal homeostasis, and modulate inflammatory signals. Their cargo includes proteins involved in antigen presentation and signaling and regulatory nucleic acids such as miRNAs, which can reprogram gene expression in recipient cells [14].

Advances in isolation and characterization have refined EV classification. While differential ultracentrifugation remains common, it often yields heterogeneous preparations. Improved techniques such as size-exclusion chromatography, ultrafiltration, and immunoaffinity capture allow better separation based on surface markers and biophysical properties [15–17]. These approaches help define subtype-specific signatures: for example, exosomes are enriched in tetraspanins (CD9, CD63, CD81)

(Figure 1A) and endosomal sorting proteins, whereas microvesicles carry plasma membrane markers, and apoptotic bodies contain nuclear and organellar remnants [18].

In summary, EVs represent a diverse group of vesicles that differ in biogenesis, size, and cargo. Their role in transporting functional molecules between intestinal cells highlights their importance in gut homeostasis and disease, particularly in IBD.

2.2. Types and functions of EV-RNA

EVs carry a rich RNA cargo that includes miRNAs, lncRNAs, circRNAs, and mRNA fragments, each contributing to intercellular signaling and gene regulation. miRNAs (~22 nt) post-transcriptionally regulate gene expression and are selectively packaged into EVs for horizontal transfer, influencing recipient cell phenotypes [19,20]. LncRNAs (> 200 nt) participate in chromatin remodeling and transcriptional regulation; when loaded into EVs, they can act as competitive endogenous RNAs to sequester miRNAs [21,22]. CircRNAs, characterized by covalently closed loops, are highly stable in EVs and function as miRNA sponges or regulators of transcription and splicing [21,23] (Figure 1B). EV-associated mRNA fragments may also be translated in recipient cells, further altering cellular functions [24]. The selective incorporation of these RNA species into EVs is governed by specific molecular sorting mechanisms, which involve interactions with RNA-binding proteins, such as heterogeneous nuclear ribonucleoproteins (hnRNPs), that regulate the packaging and stability of EV-associated RNAs [25].

Functional studies have demonstrated that EV-delivered RNAs play important roles in diverse physiological and pathological settings, including immune regulation, cancer biology, angiogenesis, and tissue repair, underscoring their potential as biomarkers and therapeutic targets. For example, EV-transferred miRNAs can modulate inflammatory responses by targeting key signaling molecules in immune cells. Similarly, EV-derived lncRNAs have been shown to promote angiogenesis and tumor progression by regulating the expression of vascular endothelial growth factor A (VEGFA) [26,27]. Owing to their natural stability and cell-targeting properties, EVs represent promising vectors for RNA-based therapeutics. Ongoing research is focused on deciphering the mechanisms of RNA sorting, EV uptake, and functional RNA transfer to optimize their clinical application [20,28].

In summary, the diverse spectrum of EV-associated RNAs serves as critical mediators of intercellular communication, capable of modulating gene expression at multiple levels and thereby influencing a wide range of biological processes and disease states.

2.3. Progress in techniques for isolation and identification of EV-RNA

Advancements in EV-RNA isolation and characterization have been crucial for elucidating their molecular functions and therapeutic potential in complex disorders such as inflammatory bowel disease. While differential ultracentrifugation remains the gold standard for EV isolation due to its ability to separate vesicles by size and density while preserving RNA integrity, it is labor-intensive, equipment-dependent, and often yields heterogeneous populations [29,30].

Immunoaffinity-based methods have emerged as valuable alternatives, enabling selective isolation of EV subpopulations through surface markers. For example, magnetic beads functionalized with antibodies or aptamers targeting tetraspanins (e.g., CD9, CD63, CD81) enhance purity and reduce non-vesicular

contamination [31]. Resources such as the EV Antibody Database support reproducibility in these approaches through curated antibody validations [32].

Microfluidic platforms and size-based chromatography further improve isolation efficiency and scalability. Size exclusion chromatography (SEC), frequently combined with ultracentrifugation, provides gentle size-dependent separation that maintains vesicle structure and RNA content [33]. Advanced systems, including turbidimetry-coupled gradient SEC columns, enable high-resolution fractionation of EVs and membraneless condensates for detailed cargo profiling [34]. Additionally, microfluidic cationic lipoplex nanoparticle assays allow rapid and sensitive detection of exosomal RNAs with reduced sample volume and processing time compared to conventional qRT-PCR [35].

Following isolation, high-throughput sequencing and bioinformatics have transformed EV-RNA profiling. Small RNA sequencing comprehensively analyzes miRNAs, piwi-interacting RNAs (piRNAs), and other ncRNAs, revealing disease-related signatures and biomarker candidates [36,37]. Long-read nanopore sequencing captures full-length transcripts, offering enhanced insight into RNA isoform diversity and integrity [38]. Bioinformatics pipelines incorporating differential expression, gene set enrichment, and network modeling support functional annotation of EV-RNA cargo and identification of key disease pathways [39].

Innovative *in situ* labeling techniques, such as ascorbate peroxidase-mediated proximity biotinylation, enable selective tagging of RNAs and proteins within exosomes without prior isolation, opening new avenues for studying EV cargo dynamics in physiological and pathological contexts [40]. Similarly, methods like Controlled Level of Contamination sequencing (CoLoC-seq) improve specificity in subcellular transcriptomics by distinguishing membrane-enclosed RNAs from contaminants, refining localization studies relevant to EVs [41].

Together, these advances enhance our ability to characterize EV-RNA profiles, identify biomarkers, and develop EV-based diagnostics and therapeutics. Continued refinement and standardization will be essential for translating EV-RNA research into clinical applications in IBD and other diseases.

3. Overview of IBD

3.1. Clinical classification and epidemiology of IBD

IBD, primarily comprising CD and UC, is characterized by distinct pathological and clinical features. CD may affect any gastrointestinal segment and is characterized by transmural inflammation, often leading to complications such as strictures and fistulae. In contrast, UC is confined to the colorectal mucosa, with continuous superficial inflammation that elevates the risk of dysplasia and colorectal cancer [42]. Accurate classification is essential for diagnosis and management. The Montreal classification system, based on age at onset, disease location, and behavior, provides a standardized framework for assessing progression and personalizing therapy [1].

Globally, the incidence and prevalence of IBD continue to rise, notably in historically low-prevalence regions such as Latin America and Asia [43,44]. This trend reflects multifactorial interactions involving genetic susceptibility, environmental factors, immune dysregulation, and gut microbiome alterations. Urbanization and Westernized lifestyles are associated with increased risk, underscoring the role of environmental triggers [45]. A family history further elevates risk, highlighting genetic contributions [46].

IBD typically peaks in young adults (15–35 years), though incidence is rising in pediatric and older populations [47,48]. The disease follows a chronic, relapsing course marked by flares of abdominal pain, diarrhea, and weight loss, significantly impairing quality of life [49,50]. This unpredictable trajectory and potential for complications emphasize the need for early diagnosis and effective therapeutic strategies.

3.2. Pathological mechanisms of IBD

IBD pathogenesis involves aberrant immune activation, chronic intestinal inflammation, and epithelial barrier disruption. Central to this process is immune dysregulation, where inappropriate responses to gut microbiota drive infiltration of T cells and other immune cells into the mucosa. Pro-inflammatory cytokines such as IL-17 and TNF-α amplify inflammation and tissue injury [51,52]. Sustained inflammation contributes not only to clinical symptoms but also to complications like strictures, fistulas (particularly in CD), and architectural changes including fibrosis and dysplasia, increasing long-term colorectal cancer risk [53].

Genetic and environmental factors critically interact in IBD development. Genome-wide association studies have identified risk loci such as NOD2 and IL23R, which are involved in immune regulation and barrier function [54]. Environmental triggers such as diet, infections, and antibiotic use can disturb the gut microbiota, promoting dysbiosis that correlates with disease activity [55]. Dysbiosis may exacerbate inflammation and compromise barrier integrity (“leaky gut”), facilitating antigen translocation and perpetuating immune activation [56].

Cellular mechanisms including dysregulated apoptosis, impaired autophagy, and mitochondrial dysfunction also contribute. Epithelial apoptosis compromises barrier renewal, while autophagy maintains cellular homeostasis and modulates immune responses [57]. Mitochondrial dysfunction disrupts energy metabolism and promotes oxidative stress, further fueling inflammation [58]. These processes collectively highlight the importance of balanced epithelial integrity and immune regulation in IBD.

3.3. Current diagnosis and treatment status

Diagnosis of IBD remains reliant on invasive procedures, including endoscopy and imaging, along with nonspecific biomarkers such as C-reactive protein and fecal calprotectin [59–61]. While useful, these approaches lack disease specificity, creating a need for accurate, non-invasive biomarkers. EVs and their RNA cargo, particularly lncRNAs, have emerged as promising diagnostic tools capable of reflecting IBD-specific molecular pathways [7].

Therapeutically, IBD management includes anti-inflammatory agents, immunosuppressants, and biologics. Although corticosteroids, thiopurines, and anti-TNF therapies can induce remission, their efficacy varies and is often limited by safety concerns, immunogenicity, and loss of response over time [62,63]. These challenges highlight the demand for novel, targeted therapies with improved safety profiles. Recent developments focus on small molecules and pathway-specific agents that modulate precise inflammatory and immune mechanisms [64].

These limitations underscore the growing role of precision medicine in IBD. Personalized approaches integrating genetic, microbial, and environmental factors may optimize efficacy and reduce adverse events [65,66]. Additionally, EVs are being explored as natural delivery vehicles for therapeutic cargo, enabling localized treatment with reduced systemic exposure [67,68]. Continued progress

depends on developing more specific biomarkers (such as EV-RNAs) and patient-centered therapies to improve diagnostic accuracy, sustain remission, and enhance quality of life.

4. Regulatory mechanism of EV-RNA in IBD

4.1. The role of EV-RNA in regulating intestinal immune responses

EV-RNAs plays a pivotal role in orchestrating intestinal immune homeostasis by facilitating targeted intercellular communication and modulating key immune cell functions (Figure 2A).

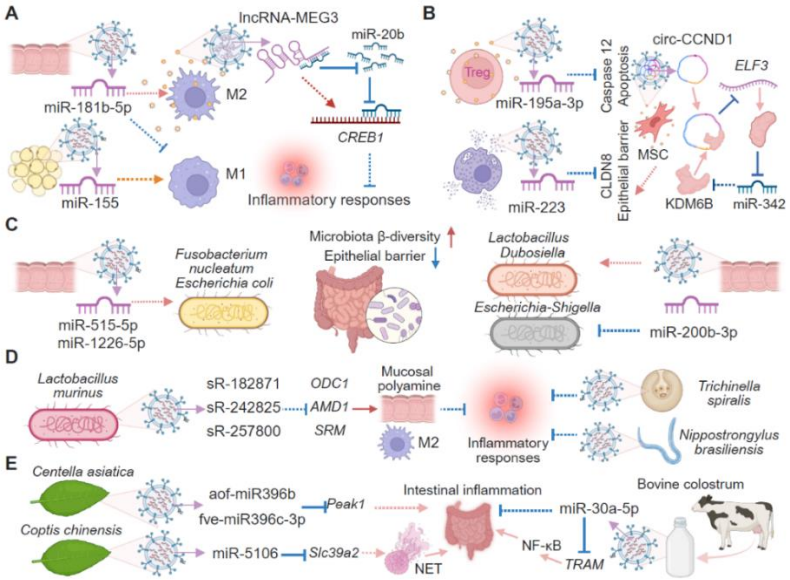

Figure 2. Mechanistic roles of EV-RNAs in IBD pathogenesis. **(A)** EV-RNAs modulate intestinal immunity. Colonic EV-derived miR-181b-5p promotes M2 macrophage polarization to resolve inflammation, whereas adipose tissue-derived exosomal miR-155 drives pro-inflammatory M1 polarization. M2 macrophage EVs deliver lncRNA MEG3 to epithelial cells, where it sequesters miR-20b-5p and enhances cAMP response element-binding protein 1 (CREB1) expression. **(B)** EV-RNAs regulate epithelial barrier integrity. Treg exosomal miR-195a-3p inhibits Caspase 12 to suppress apoptosis and promote repair, while mast cell exosomal miR-223 downregulates CLDN8 to impair tight junctions. EV-carried circ-CCND1 limits pyroptosis via the ELF3/miR-342-3p/KDM6B axis. **(C)** Host EVs shape the microbiota. Colonic EV-miRNAs (e.g., miR-515-5p, miR-1226-5p, miR-200b-3p) enter bacteria to modulate gene expression and growth. **(D)** Microbial EVs influence host physiology. *Lactobacillus murinus* bEVs deliver small RNAs (e.g., sR-182871, sR-242825, sR-257800) that downregulate polyamine biosynthesis enzymes (ornithine decarboxylase 1 (ODC1), adenosylmethionine decarboxylase 1 (AMD1), and selected reaction monitoring (SRM)) in epithelial cells, impairing mucosal repair. **(E)** Plant and food EV-RNAs exert cross-kingdom effects. *Centella asiatica*-derived EVs deliver aof-miR396b and fve-miR396c-3p to target host *Peak1*. *Coptis chinensis* EV-miR-5106 inhibits *Slc39a2* to restore zinc homeostasis, suppressing neutrophil extracellular trap formation (NETosis) and pyroptosis. Bovine colostrum EV-derived bta-miR-30a-5p targets TNF-receptor associated factor (TRAF)-related adapter molecule (TRAM) to attenuate NF-κB signaling. Created with BioRender.com.

For instance, the long non-coding RNA MEG3, encapsulated within extracellular vesicles derived from M2 macrophages (M2-EVs), demonstrates significant therapeutic potential in ulcerative colitis. M2-EVs deliver MEG3 into colonic epithelial cells, where it acts as a competitive endogenous RNA (ceRNA) to sequester miR-20b-5p, thereby relieving the miRNA-mediated suppression of the transcription factor CREB1. This MEG3/miR-20b-5p/CREB1 axis enhances epithelial viability, mitigates inflammatory responses, and reveals a novel EV-mediated regulatory pathway in IBD [69].

Similarly, miR-181b-5p, transported via colonic EVs, contributes to colitis resolution by regulating macrophage polarization. It suppresses pro-inflammatory M1 macrophage activation while promoting the anti-inflammatory M2 phenotype, thereby alleviating inflammation and restoring intestinal homeostasis in both acute and chronic colitis models [70]. In contrast, miR-155, delivered by adipose tissue-derived exosomes, exacerbates colitis by driving M1 macrophage polarization. This exosomal pathway illustrates a mechanistic link between high-fat diets, obesity, and worsened IBD outcomes. Notably, therapeutic delivery of a miR-155 inhibitor via exosomes can counteract this effect, promoting a shift toward the anti-inflammatory M2 phenotype and ameliorating colitis severity [71].

Collectively, these findings underscore the complex, context-dependent influence of EV-RNA on immune cell behavior and intestinal inflammation, positioning EV-carried RNAs as critical molecular mediators in both the pathogenesis and potential treatment of IBD.

4.2. EV-RNA in intestinal barrier function

EV-RNAs play a fundamental role in regulating intestinal epithelial integrity, acting as critical mediators that either support barrier maintenance or contribute to its disruption in the context of IBD (Figure 2B).

For example, miR-195a-3p, delivered by regulatory T cell-derived exosomes, mitigates IBD by directly targeting and downregulating the pro-apoptotic protein Caspase 12 in intestinal epithelial cells. This enhances cell proliferation, reduces apoptosis, and facilitates barrier repair, illustrating an exosome-mediated mechanism with therapeutic potential for restoring mucosal integrity [72]. Conversely, exosomal miR-223 derived from mast cells is transferred to intestinal epithelial cells, where it suppresses the tight junction protein CLDN8. This downregulation compromises epithelial barrier function, increases intestinal permeability, and contributes to IBD pathogenesis, highlighting how specific EV-RNAs can actively promote barrier dysfunction [73].

Beyond miRNAs, EV-encapsulated circRNAs also contribute to epithelial homeostasis. For instance, circ-CCND1 regulates pyroptosis, an inflammatory form of programmed cell death, through the ELF3/miR-342-3p/KDM6B signaling axis. By modulating this pathway, circ-CCND1 helps protect the intestinal epithelium from excessive inflammatory damage and maintains barrier function. Although detailed mechanisms underlying circ-CCND1-mediated epithelial regulation remain to be fully elucidated, the involvement of EV-circRNAs in pyroptosis represents a novel molecular avenue for preserving intestinal barrier integrity under inflammatory stress [74].

In summary, EV-RNAs operate as versatile molecular regulators of the intestinal barrier, capable of either enhancing repair or exacerbating damage. Their cell-specific origin and functional diversity highlight promising avenues for targeted interventions aimed at restoring epithelial homeostasis in IBD.

4.3. Potential roles of EV-RNA in intestinal fibrosis

EV-RNAs are increasingly recognized as key regulators in the pathogenesis of intestinal fibrosis, a process often resistant to conventional anti-inflammatory treatments in IBD. Intestinal fibrosis, a common complication of IBD, results in stricture formation and potential obstruction, representing a major clinical challenge. This pathological remodeling is primarily driven by persistent local inflammation, leading to excessive extracellular matrix deposition by activated mesenchymal cells [75]. Current anti-inflammatory therapies often fail to halt or reverse fibrosis, underscoring the urgent need for targeted anti-fibrotic interventions [76].

Emerging evidence points to the therapeutic potential of EV-RNAs in modulating fibrotic pathways. A recent study demonstrates that microvesicles derived from bone marrow mesenchymal stem cells (BMSCs) engineered to overexpress miR-200b can effectively mitigate intestinal fibrosis. These miR-200b-enriched MVs are internalized by intestinal epithelial cells and colon tissue, where they suppress epithelial-mesenchymal transition (EMT) by downregulating the key transcription factors zinc finger E-box-binding homeobox 1 (ZEB1) and ZEB2. Consequently, this leads to increased expression of the epithelial marker E-cadherin and decreased levels of mesenchymal markers such as vimentin and alpha-smooth muscle actin (α-SMA). The net effect is a significant reduction in collagen deposition and alleviation of fibrosis, as validated in both cellular and animal models [77].

Beyond the therapeutic potential of engineered EVs, accumulating evidence highlights that pathogenic EV-RNAs derived from disease-specific tissue microenvironments actively drive fibrogenesis in IBD. Notably, mesenteric adipose tissue hypertrophy, particularly the formation of creeping fat, is a hallmark of Crohn's disease [78]. Qian *et al.* demonstrates that exosomal miR-103a-3p derived from creeping fat adipose stromal cells (CF-Exos) promotes intestinal fibrosis by activating primary intestinal fibroblasts. Mechanistically, miR-103a-3p is enriched in CF-Exos and, upon delivery to recipient fibroblasts, directly targets the 3'-untranslated region of TGFBR3, a negative regulator of TGF-β signaling. This leads to downregulation of TGFBR3 expression, subsequent phosphorylation of Smad2/3, and enhanced fibroblast activation. Both *in vitro* and *in vivo* experiments confirm that knockdown of exosomal miR-103a-3p attenuates fibroblast activation and ameliorates intestinal fibrosis. Clinically, miR-103a-3p expression is upregulated in fibrotic intestinal tissues and creeping fat from Crohn's disease patients, with its levels positively correlating with fibrosis scores [79].

In summary, EV-RNAs are able to directly target the molecular drivers of fibrogenesis, offering a promising and precise therapeutic avenue for managing fibrosis in IBD beyond mere inflammation suppression.

4.4. EV-RNA-mediated bidirectional host-microbiota crosstalk in IBD pathogenesis

EV-RNAs have emerged as pivotal mediators of host-microbiota crosstalk in the gut, establishing a dynamic bidirectional communication network that profoundly influences intestinal homeostasis and disease processes such as IBD.

The host regulates the gut microbiota through fecal miRNAs, including miR-515-5p and miR-1226-5p, which are secreted within EVs by intestinal epithelial cells (Figure 2C). These host-derived miRNAs enter bacteria such as *Fusobacterium nucleatum* and *Escherichia coli (E. coli)*, where they modulate bacterial gene expression and growth dynamics. In experimental IBD models, impairment of epithelial miRNA secretion disrupts microbial balance, elevates microbiota β-diversity, and exacerbates dextran

sulfate sodium (DSS)-induced colitis. Notably, transplantation of fecal miRNAs from wild-type mice restores a healthier microbial composition and alleviates colitis, underscoring a novel host-directed regulatory axis in IBD pathogenesis [80].

Another example is miR-200b-3p, which is carried by colonic EVs and mitigates colitis through direct interaction with the gut microbiome (Figure 2C). This miRNA reprofiles the microbial community by enriching beneficial genera such as *Lactobacillus* and *Dubosiella* while suppressing opportunistic pathogens like *Escherichia-Shigella*. These changes contribute to the restoration of intestinal barrier function and the promotion of mucosal homeostasis in IBD [70].

This regulatory dialogue is bidirectional: while host EV-RNAs modulate microbial behavior, microbial EV-RNAs in turn influence host physiology. For instance, *Lactobacillus murinus* packages small RNAs, such as sR-182871, sR-242825, and sR-257800, into bacterial extracellular vesicles (bEVs). Upon delivery to host intestinal epithelial cells, these bacterial small RNAs downregulate key polyamine biosynthesis enzymes (ODC1, AMD1, SRM), leading to reduced mucosal polyamine levels (Figure 2D). This impairment hampers tissue repair and M2 macrophage polarization, thereby delaying recovery from DSS-induced colitis [81].

Beyond commensal bacteria, parasitic helminths also engage in EV-RNA-mediated cross-kingdom signaling. Extracellular vesicles from *Trichinella spiralis* muscle larvae (Ts-EVs) ameliorate trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice. Ts-EVs carry immunomodulatory miRNAs that dampen intestinal inflammation by suppressing Th1/Th17 responses and pro-inflammatory cytokines (e.g., TNF-α, IL-1β), while promoting Th2/Treg differentiation and anti-inflammatory cytokines (e.g., IL-10, TGF-β). They also strengthen intestinal barrier integrity by upregulating tight junction proteins such as occludin and ZO-1 [82].

Similarly, extracellular vesicles from *Nippostrongylus brasiliensis* (Nb-EVs) confer protection against TNBS-induced colitis in mice. Nb-EVs contain 52 immunomodulatory miRNAs predicted to target host genes involved in cytokine signaling pathways, including IL-6 and IL-17. These vesicles reduce pro-inflammatory cytokines (IL-1β, IL-6, IFNγ, IL-17a) and elevate the anti-inflammatory cytokine IL-10, thereby attenuating intestinal inflammation [83].

In contrast, EVs from the protozoan parasite *Giardia duodenalis* contain heat-stable small RNAs (sRNAs), including miRNAs and endo-siRNAs, which can exacerbate dysbiosis. These EV-sRNAs enhance pathobiont behaviors in gut bacteria such as *E. coli* by increasing swimming motility, adhesion to and invasion of intestinal epithelial cells, and disrupting biofilm formation. This RNA-mediated trans-kingdom communication contributes to microbiota imbalance, a hallmark of IBD pathogenesis [84].

Collectively, these studies illuminate a complex and bidirectional EV-RNA-mediated dialogue between the host and the intestinal ecosystem, encompassing commensal bacteria, probiotics, and parasites. This interkingdom communication network significantly shapes mucosal immunity, microbial community structure, and barrier function, offering novel mechanistic insights and potential therapeutic targets for modulating host-microbiota interactions in IBD.

4.5. Cross-kingdom regulatory mechanisms of plant and food EV-RNAs in IBD

Increasing evidence suggests that plant- and food-derived EVs are rich sources of regulatory RNAs that can cross species boundaries to modulate host physiology, offering promising therapeutic potential for IBD through direct molecular intervention in key inflammatory and repair pathways (Figure 2E).

Plant-derived exosomes represent a novel class of natural nanocarriers capable of delivering functional RNA across biological kingdoms. For instance, *Centella asiatica*-derived exosomes (CAEs) carry specific miRNAs, including aof-miR396b and fve-miR396c-3p, which ameliorate experimental colitis by targeting the host gene *Peak1*. Notably, Peak1 functions as a scaffold protein involved in cytoskeletal dynamics and cell migration—processes critical for intestinal epithelial restitution. By downregulating Peak1, these plant miRNAs attenuate DSS-induced intestinal inflammation, modulate immune-related gene expression, and contribute to the restoration of gut microbial balance. This multi-faceted effect—simultaneously targeting epithelial repair, immune modulation, and microbiome homeostasis—exemplifies how a single plant-derived EV-RNA cargo can coordinate complex therapeutic responses through a primary molecular target [85].

Similarly, extracellular vesicle-like nanoparticles (Cc-ELNs) isolated from *Coptis chinensis* deliver miR-5106, which exerts protective effects in IBD models. In neutrophils, miR-5106 targets and downregulates the zinc transporter gene *Slc39a2*, thereby reestablishing intracellular zinc homeostasis. Zinc homeostasis is increasingly recognized as a critical determinant of immune function; its disruption promotes neutrophil hyperactivation and tissue damage. By restoring zinc balance, miR-5106 inhibits neutrophil extracellular trap (NET) formation, reduces pyroptosis in intestinal epithelial cells, and promotes their proliferation. This mechanism is particularly noteworthy because it addresses a specific pathogenic process—neutrophil-driven tissue injury—rather than broadly suppressing inflammation, suggesting a targeted approach to IBD therapy [86].

Dairy products also serve as important sources of bioactive EVs. Bovine colostrum-derived exosomes are enriched with bta-miR-30a-5p, a miRNA that alleviates inflammation in intestinal epithelial cells by directly targeting and suppressing TRAM, an essential adapter protein in the TLR4 signaling pathway. The TLR4 pathway represents a central node in IBD pathogenesis, as it mediates responses to bacterial lipopolysaccharide and drives chronic intestinal inflammation. By targeting TRAM specifically, bta-miR-30a-5p attenuates downstream NF-κB activation and reduces expression of pro-inflammatory cytokines without completely abolishing TLR4 signaling—a distinction that may preserve host defense while limiting excessive inflammation. This fine-tuned regulation underscores the therapeutic potential of milk-derived EV-RNA cargo in intestinal inflammation [87].

Together, these findings highlight a growing recognition that EVs from dietary and herbal sources serve as natural delivery systems for regulatory RNAs that can target host inflammatory pathways, influence immune cell behavior, and promote tissue repair in IBD. What emerges from these studies is a consistent pattern: plant and food EV-RNAs appear to target key regulatory nodes (Peak1 for epithelial integrity, Slc39a2 for zinc homeostasis, TRAM for TLR4 signaling) that sit at the intersection of multiple pathogenic pathways. This strategic targeting may explain their potent effects despite relatively low abundance. Moreover, the evolutionary conservation of these target genes across species supports the feasibility of cross-kingdom RNA regulation as a therapeutic strategy. This cross-kingdom RNA communication not only expands our understanding of host-environment interactions but also opens innovative avenues for developing EV-based nutraceuticals and RNA therapeutics tailored for IBD management.

4.6. Gut EV-RNA-mediated systemic complications in IBD

Beyond its localized intestinal manifestations, IBD is increasingly recognized as a systemic condition in which gut-derived EVs and their RNA cargo play a crucial role in mediating pathological communication with distant organs, contributing to the development of extraintestinal complications.

IBD is frequently associated with liver injury, including conditions such as primary sclerosing cholangitis (PSC) [88]. The transcription factor cAMP responsive element-binding protein H (CrebH) has been shown to protect against IBD-induced liver damage by modulating the composition of plasma exosomes. In CrebH-deficient mice, levels of exosomal miR-29a-3p are significantly diminished. Under normal physiological conditions, exosomal miR-29a-3p suppresses the expression of tumor necrosis factor receptor-1 (TNF-R1) in hepatic endothelial cells, thereby inhibiting the upregulation of adhesion molecules such as mucosal addressin cell adhesion molecule-1 (MAdCAM-1) and subsequent lymphocyte infiltration into the liver. Thus, miR-29a-3p carried by circulating exosomes functions as a key molecular mediator within the gut-liver axis, attenuating hepatic inflammation associated with IBD [89].

Similarly, IBD has been linked to an elevated risk of cardiac disorders, including heart failure, myocardial infarction, and adverse cardiac remodeling, highlighting the existence of a gut-heart axis [90]. Research indicates that plasma exosomal miRNA profiles are markedly altered in patients with UC. Intestinal inflammation drives increased expression of miR-29b within colon epithelial cells and their secreted exosomes. These gut-derived exosomes, loaded with miR-29b, enter systemic circulation and are taken up by cardiomyocytes in the heart. Once internalized, exosomal miR-29b downregulates brain-derived neurotrophic factor (BDNF), a protein essential for normal cardiac contractility, and elevates apoptosis markers such as cleaved caspase-3. This molecular reprogramming contributes to structural and functional cardiac impairment, illustrating how gut-originating EV-RNAs can directly influence remote organ pathology [91].

Collectively, these findings underscore the role of gut-derived EV-RNAs as systemic signaling molecules that traverse biological barriers to modulate inflammation and cellular function in distant organs such as the liver and heart. This emerging understanding of organ-crosstalk mediated by EV-RNAs not only elucidates mechanisms underlying extraintestinal complications in IBD but also opens new avenues for biomarker discovery and therapeutic strategies aimed at intercepting pathological communication along the gut-liver and gut-heart axes. Relevant findings on the regulatory mechanisms of EV-RNA in IBD are summarized in Table 1.

Table 1. Diverse regulatory roles of EV-miRNAs (miRNAs, circRNAs, lncRNAs, microbiota-Derived sRNAs, and synthetic RNAs) in intestinal epithelial homeostasis and the disease processes of IBD.

Specific EV-RNA	Sample Source	Key Mechanisms/Targets	References
miRNAs			
miR-100	MSC EVs	Supports cell survival	[92]
miR-103a-3p	CF Exos	Targets TGFBR3, promoting Smad2/3 phosphorylation and fibroblast activation	[79]
miR-125b	MSC EVs	Suppress pro-inflammatory cytokines	[92]
miR-155	Visceral adipose tissue Exos	Promote M1 differentiation	[71]
miR-181b-5p	Colonic EVs	inhibited M1 macrophage polarization and promoted M2 polarization to reduce the levels of inflammation both in acute and remission of chronic colitis.	[70]

Table 1. *Cont.*

Specific EV-RNA	Sample Source	Key Mechanisms/Targets	References
miRNAs			
miR-195a-3p	Treg Exos	Targets Caspase 12; promote colonic epithelial cells proliferation and inhibited cell apoptosis.	[72]
miR-200b	BMSC Exos	Suppress the development of EMT by targeting ZEB1 and ZEB2.	[77]
miR-200b-3p	Colonic EVs	Interact with bacteria and regulate the composition of the microbiota, which contributed to intestinal barrier integrity and homeostasis.	[70]
miR-21	MSC EVs	Involved in the enhancement of anti inflammatory M2 macrophage polarisation by regulating the TGF-β inhibitor PTEN.	[92]
miR-223	HMCs Exos	Destroys intestinal barrier function by inhibition of CLDN8 expression.	[73]
miR-27a-3p	Engineered EVs	Suppresses PHB1 to reduce Th17 polarization and increases the number of $FOXP3^+$ regulatory T cells.	[3]
miR-29a-3p	Plasma Exos	Mediate MAdCAM-1 expression;led to prominent inflammatory signals in the liver.	[89]
miR-29b	Plasma Exos, epithelial cell Exos	Excessive plasma exosomal miR-29b suppresses critical proteins like BDNF in IBD, leading to cardiac impairment.	[91]
miR-30b-5p	intestinal EVs	Inhibits inflammation by suppressing key pro-inflammatory cytokines.	[64]
miR-378a-5p	hucMSC-Exos	Targets NLRP3 to attenuate colonic inflammation and promote tissue repair.	[93]
miR-433-3p	IECs EVs	Targets MAPK8 to mitigate intestinal inflammation.	[94]
miR-5106	Coptis Chinensis-derived EVs	Downregulated Slc39a2 expression, thereby restoring zinc homeostasis in neutrophils and reducing NET formation.	[86]
miR-515-5p /miR-1226-5p	Fecal EVs	Enter F. nucleatum and E. coli to regulate bacterial gene transcripts and affect bacterial growth.	[80]
miR-942-5p	Serum EVs	Regulating barrier function and cellular stress responses.	[95]
let-7a	MSC EVs	Associated with inhibition of apoptosis.	[92]
hsa-miR-16-5p, hsa-miR-4516	Salivary Exos	/	[96]
osa-miR166d-5p, gma-miR396a-3p	Tea-derived EVs	Target NF-κB pathway components (e.g., AKT1, IKBKB) in host immune cells.	[97]
aof-miR396b, fve-miR396c-3p	Centella Asiatica-derived EVs	Regulate immune cells and modulate gut microbiota.	[85]
bta-miR-30a-5p	Bovine Colostrum-derived EVs	Targets the NF-xB Signaling Pathwayto Alleviate Inflammation.	[87]
lncRNAs			
H19	Human plasma EVs	/	[10]
MEG3	M2 macrophage-Evs	Supports cell viability and decreases inflammatory cytokines through modulating the miR-20b-5p/CREB1 axis.	[69]
NEAT1	Human serum Evs	Induce macrophage activation by increasing p38 and ERK phosphorylation, resulting in enhanced TNF-α production in acute colitis.	[98]
circRNAs			
circ-CCND1	iPSC-MSC-Evs	Acts via ELF3/miR-342-3p/KDM6B axis to limit inflammatory cell death.	[74]
Microbiota-Derived sRNAs			
sR-182871, sR-242825, sR-257800	Lactobacillus murinus	sR-182871 targeting ODC1, sR-242825 targeting AMD1, and sR-257800 targeting SRM, resulting in lower polyamine levels.	[81]
Synthetic RNAs			
TNF-α siRNA	Engineered Ginger-Derived EVs	Reduces TNF-α level.	[99]

5. EV-RNA as a biomarker for IBD

5.1. The application potential of EV-RNA in IBD diagnosis

EV-RNAs, particularly lncRNAs and miRNAs, are emerging as promising non-invasive biomarkers for the diagnosis of IBD and for monitoring disease activity. The expression profiles of specific EV-RNAs in biofluids such as plasma and saliva correlate closely with IBD clinical activity, offering molecular signatures that reflect underlying inflammatory states. The expression profiles of specific EV-RNAs in plasma and saliva correlate strongly with IBD activity, offering molecular signatures that reflect underlying inflammation (Figure 3A).

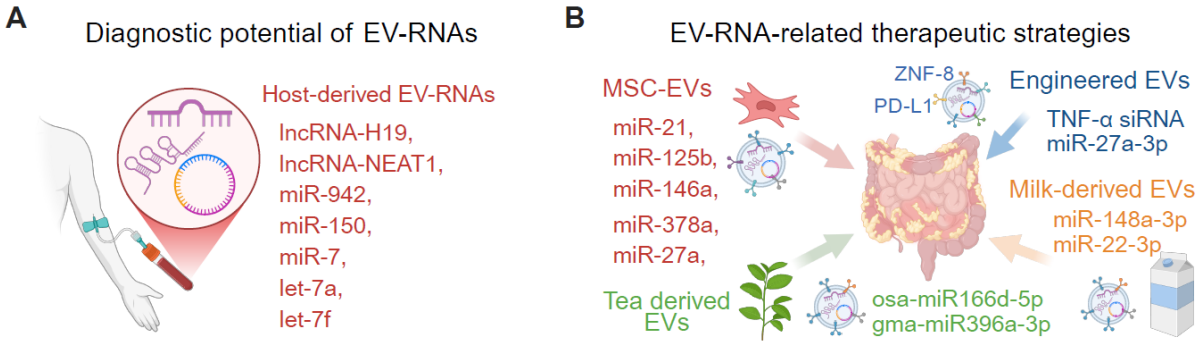

Figure 3. Diagnostic and therapeutic potential of EV-RNAs in IBD. **(A)** Circulating EV-RNAs serve as minimally invasive, disease-specific biomarkers for IBD. Their encapsulation within EVs confers stability in biofluids, enabling sensitive detection in plasma and saliva, and facilitating real-time monitoring of disease activity and therapeutic response. **(B)** EV-based therapeutic platforms offer novel treatment avenues. MSC-EVs deliver immunomodulatory miRNAs (e.g., miR-378a-5p, miR-21) that inhibit NLRP3 inflammasome activation and promote M2 macrophage polarization. Dietary EVs (e.g., from milk, tea) function as natural oral carriers for regulatory RNAs. Engineered EV platforms further enhance therapeutic precision through targeted cargo loading, surface modification, and tissue-specific delivery to modulate inflammatory and regenerative pathways in IBD. Created with BioRender.com.

One study identified the lncRNA H19 as a promising non-invasive diagnostic biomarker for active IBD. H19 expression was significantly upregulated in both inflamed intestinal tissues and plasma-derived EVs from patients with active IBD compared to those in remission, healthy controls, and individuals with other conditions such as rheumatoid arthritis or irritable bowel syndrome. Machine-learning analysis demonstrated that plasma EV-encapsulated H19 exhibits high sensitivity and specificity for distinguishing active IBD, with area under the curve (AUC) values of 0.95–0.97. These findings indicate that circulating EV-H19 is a stable and specific biomarker suitable for diagnosing and monitoring IBD disease activity [10].

Similarly, lncRNA NEAT1 is highly expressed in serum, intestinal tissues, and serum-derived exosomes from DSS-induced IBD mice. Elevated NEAT1 expression is also observed in TNF-α-stimulated inflammatory colon cell models. Mechanistic studies indicate that NEAT1 contributes to intestinal epithelial barrier dysfunction and promotes macrophage M1 polarization, likely mediated

through exosomal transfer. Consequently, NEAT1, particularly when detected in circulating exosomes, represents a potential novel molecular biomarker for IBD diagnosis [98].

Another investigation identified miR-942-5p as an EV-associated RNA that is rapidly upregulated in serum following transabdominal ultrasound in IBD patients. Its induction is more pronounced in patients with high-grade intestinal inflammation, especially in Crohn's disease, and correlates with clinical activity scores [95].

Furthermore, serum EVs carrying miRNAs such as miR-150-5p, miR-7-5p, let-7f-5p, and let-7a-5p show differential expression patterns in IBD patients. Their levels can be modulated by factors such as vitamin D supplementation, which influences inflammatory and oxidative stress pathways central to IBD pathogenesis. These miRNAs play important roles in immune regulation and redox balance, underscoring their utility as biomarkers for tracking disease activity and therapeutic response [100].

In addition to blood-based markers, salivary exosomal miRNAs have gained attention as convenient non-invasive biomarkers. A recent study highlighted hsa-miR-16-5p and hsa-miR-4516 as significantly elevated in the saliva of IBD patients compared with healthy controls. A logistic regression model combining these two miRNAs demonstrated robust diagnostic performance, achieving an AUC of 0.925 for discriminating IBD patients from healthy individuals and an AUC of 0.82 for distinguishing active disease from remission, offering a practical tool for IBD screening and activity monitoring [96]

In summary, EV-RNAs hold substantial promise as minimally invasive, dynamic biomarkers for IBD. Their inherent stability in biological fluids preserves RNA integrity and enables reliable detection in plasma and saliva. The disease-specific expression patterns of EV-RNAs, especially when analyzed using machine-learning-driven multi-marker panels, pave the way for the development of sensitive, specific, and non-invasive diagnostic platforms. To advance toward clinical implementation, future efforts should prioritize large-scale validation studies and the standardization of EV isolation and RNA detection methodologies, ultimately supporting earlier diagnosis, improved disease monitoring, and personalized treatment strategies in IBD.

5.2. Clinical translation challenges of EV-RNA detection technologies

The clinical translation of EV-RNA detection technologies faces several significant barriers. A primary challenge is the lack of standardized protocols for EV isolation and RNA analysis, which compromises data consistency and reproducibility across studies [101]. Current methods (including ultracentrifugation, precipitation, and microfluidic techniques) vary in yield, purity, and reproducibility, leading to discrepancies in EV-RNA characterization [102]. This methodological heterogeneity is further compounded by the biological diversity of EVs, which differ based on cellular origin and donor cell physiology [103]. Without uniform protocols, cross-study comparisons and biomarker validation, especially in complex conditions such as IBD, remain difficult.

Another major hurdle is the intrinsic heterogeneity of clinical samples and patient populations. Differences in demographics, disease stage, and treatments can markedly influence EV profiles and RNA cargo in biofluids [104]. For example, the inflammatory milieu and intestinal state in IBD patients alter EV composition, complicating the identification of disease-specific RNA biomarkers [68,105]. Such variability requires nuanced interpretation and highlights the need for large-scale, multicenter validation studies that account for diverse clinical contexts [106].

Regulatory and logistical challenges also impede clinical adoption. Regulatory approval demands robust evidence of safety, efficacy, and reliability, yet the field lacks consensus on EV characterization and RNA analysis best practices [107]. Integrating EV-RNA assays into clinical workflows further necessitates specialized equipment and trained personnel. Overcoming these barriers will require collaborative efforts among researchers, clinicians, and regulators to establish clear guidelines for EV isolation, characterization, and biomarker validation. Addressing these issues is critical for harnessing the potential of EV-RNA technologies in personalized management of IBD and other diseases. Relevant findings on the application potential of EV-RNA in IBD diagnosis are summarized in Table 1.

6. The application prospects of EV-RNA in IBD treatment

6.1. Advantages of EVs as drug delivery vehicles

EVs possess unique biological properties that make them attractive candidates for drug delivery. Their high natural biocompatibility, derived from native biological membranes, minimizes immune rejection and toxicity compared to synthetic nanoparticles. This enhances circulation time and safety, supporting more effective therapeutic outcomes [108]. The lipid bilayer of EVs also protects encapsulated cargo (including RNA, proteins, and small molecules) from degradation, thereby improving stability and bioavailability [109].

A key advantage of EVs is their ability to cross biological barriers such as epithelial layers and the blood-brain barrier, enabling targeted delivery to specific tissues. This natural tropism, influenced by surface proteins and lipid composition, promotes accumulation at inflamed or diseased sites, increasing therapeutic precision [110]. Targeting can be further enhanced by engineering EV surfaces with ligands or antibodies specific to receptors on target cells [111].

EVs are also versatile carriers capable of delivering diverse therapeutic agents, from small-molecule drugs and nucleic acids (e.g., siRNA, mRNA) to proteins, allowing for personalized and combination therapies [112]. Their capacity to encapsulate both hydrophilic and hydrophobic compounds further broadens their utility across different disease contexts [113].

In summary, EVs offer a biocompatible, barrier-crossing, and multifunctional platform for drug delivery. These features support the development of safer, more effective therapeutics and hold significant promise for advancing precision medicine applications.

6.2. Therapeutic potential of MSC-derived EV-RNA in IBD management

EVs derived from mesenchymal stem cells (MSC-EVs) represent a promising cell-free therapeutic strategy for IBD, leveraging their ability to deliver functional RNA cargo that modulates immune responses and promotes tissue repair [114]. MSC-EVs, particularly exosomes, carry a diverse array of bioactive components, including ncRNAs, proteins, and lipids, that facilitate intercellular communication and immunoregulation. Compared with whole-cell MSC transplantation, MSC-EVs offer advantages such as greater stability, easier storage, reduced risk of tumorigenesis due to the absence of nuclei, and lower immunogenicity, thereby minimizing adverse effects like infusion-related toxicity [115].

Mechanistically, MSC-EVs exert their therapeutic effects through specific miRNAs that target key inflammatory and regenerative pathways in IBD. For example, adipose-derived MSC-EVs transport

miR-21, which promotes anti-inflammatory M2 macrophage polarization, while miR-125b and miR-146a suppress pro-inflammatory cytokines such as TNF-α and IL-6 via inhibition of NF-κB signaling. Together, these miRNAs enhance intestinal barrier integrity and mitigate inflammation in preclinical models, underscoring the immunomodulatory and regenerative potential of MSC-EV-delivered miRNA cargo [92].

Further illustrating this potential, exosomes derived from human umbilical cord mesenchymal stem cells (hucMSC-Ex) have been shown to alleviate DSS-induced colitis. A central mechanism involves the delivery of exosomal miR-378a-5p to macrophages, where it directly targets NLRP3 mRNA, thereby inhibiting NLRP3 inflammasome assembly and activation. This results in reduced caspase-1 activity, decreased maturation of IL-1β and IL-18, and suppression of gasdermin D-mediated pyroptosis, ultimately attenuating colonic inflammation and promoting tissue repair [93].

Innovative engineering approaches have further enhanced the therapeutic precision of MSC-EVs. In one study, EVs derived from Wharton's Jelly mesenchymal stem cells were dual-engineered to display PD-L1 on their surface and encapsulate miR-27a-3p. The surface PD-L1 engages PD-1 on T cells to suppress their activation, while miR-27a-3p targets and downregulates PHB1, which is a mitochondrial protein essential for Th17 cell metabolism and function. This combined strategy synergistically suppresses pro-inflammatory Th1/Th17 responses, promotes Treg expansion, and restores immune homeostasis in humanized mouse models of IBD, highlighting a sophisticated cell-free immunotherapeutic platform [3].

Collectively, these studies demonstrate that MSC-EVs, through their native or engineered RNA cargo, can precisely target multiple pathogenic pathways in IBD, offering a versatile, cell-free therapeutic avenue with significant potential for clinical translation in the management of refractory and immune-mediated intestinal inflammation (Figure 3B).

6.3. Dietary EV-RNA as a novel oral therapeutic strategy for IBD

Beyond MSC-EVs, orally administered EVs from dietary sources offer a novel route for targeted nucleic acid delivery to the gut. Oral administration is particularly suitable for IBD therapy due to direct access to inflamed intestinal sites; however, conventional nucleic acid drugs face challenges including enzymatic degradation, poor mucus penetration, and inefficient epithelial uptake [116].

Milk-derived extracellular vesicles (mEVs) exhibit intrinsic structural stability that allows them to withstand harsh gastrointestinal conditions and efficiently traverse the intestinal mucosa. The unique lipid profile of mEVs enhances their bioavailability and cellular internalization, establishing them as excellent natural nanocarriers for RNA-based therapeutics with improved efficacy and reduced systemic side effects [117]. Furthermore, mEVs are enriched in functional miRNAs that play key roles in intestinal health. For example, miRNAs such as miR-148a-3p and miR-22-3p inhibit NF-κB signaling, reduce inflammation, and protect against necrotizing enterocolitis (NEC). These EV-miRNAs resist digestive degradation, are internalized by intestinal epithelial cells, and enhance barrier function while alleviating oxidative stress. Studies in animal models confirm that milk EVs mitigate colitis, support goblet cell activity, and reduce intestinal damage, underscoring their therapeutic potential in maintaining gut homeostasis and treating IBD [118].

Tea-derived EVs (TEVs) also confer anti-inflammatory effects by reprogramming macrophage polarization. TEVs deliver plant miRNAs, notably osa-miR166d-5p and gma-miR396a-3p, into host macrophages. These miRNAs target the 3′-UTRs of AKT1 and IKBKB, leading to inhibition of the

NF-κB pathway. This results in reduced pro-inflammatory cytokine production and a shift from M1 to anti-inflammatory M2 macrophages, highlighting TEVs as natural therapeutic agents for intestinal inflammation [97].

Similarly, certain natural compounds can stimulate the release of host EVs with therapeutic properties. Cucurbitacin IIa alleviates colitis by promoting the release of host-derived EVs enriched with miR-30b-5p. Both these purified EVs and synthetic miR-30b-5p agomir significantly reduce inflammatory symptoms in DSS-induced colitis models, highlighting a miRNA-dependent therapeutic pathway [64]. In another approach, *Dendrobium officinale* polysaccharide (DOP) upregulates miR-433-3p in intestinal epithelial cell-derived small EVs (sEVs), reducing inflammatory cytokine production, thereby mitigating IBD progression [94].

Collectively, these findings underscore the significant potential of dietary and plant-derived EVs as versatile, biocompatible platforms for oral RNA delivery in IBD. By leveraging their natural stability, tissue-specific targeting, and inherent therapeutic cargo, such EV-based strategies offer a promising avenue for developing effective, patient-friendly therapies that address both inflammation and intestinal homeostasis in a targeted manner (Figure 3B).

6.4. Engineered EV-RNA platforms for personalized IBD therapy

Emerging advances in bioengineering are enabling the design of tailored EV platforms that leverage RNA cargo and surface modifications for precise, personalized intervention in IBD, offering new strategies to restore immune and epithelial homeostasis.

Recent developments in intestinal modeling and EV engineering underscore the potential for targeted, RNA-based therapeutics in IBD. One study established an iPSC-derived air-liquid interface (ALI) culture system that recapitulates human intestinal epithelium polarity. This model revealed that polarized epithelial cells release EVs with distinct miRNA profiles from their apical and basolateral surfaces. Apical EVs were enriched in miRNAs associated with epithelial and immune cell communication, whereas basal EVs carried fibroblast-related miRNAs. These findings uncover a novel compartmentalized EV-mediated signaling network that regulates epithelial-mesenchymal and immune-stromal crosstalk, providing a valuable platform for investigating dysregulated intercellular communication in IBD and designing personalized therapeutic strategies [119].

Innovative engineering approaches further enhance the functionality of plant-derived EVs. For instance, a hybrid oral delivery system was developed using ginger-derived EVs coated on ZIF-8 nanoparticles to encapsulate TNF-α siRNA for ulcerative colitis therapy. The ginger EVs confer colon-targeting specificity, resist gastric degradation, and contain the intrinsic anti-inflammatory compound 6-shogaol. These engineered nanoparticles effectively deliver siRNA to intestinal macrophages, reduce TNF-α levels, promote epithelial barrier repair, and favorably modulate gut microbiota composition in mouse colitis models, demonstrating enhanced targeting, therapeutic efficacy, and biosafety [99].

Another sophisticated platform involves dual-engineered EVs designed for synergistic immunomodulation. These EVs co-present PD-L1 on their surface and encapsulate miR-27a-3p. The exosomal miR-27a-3p directly targets prohibitin 1 (PHB1), a mitochondrial protein essential for Th17 cell bioenergetics and function. By inhibiting PHB1, miR-27a-3p suppresses Th17 polarization and promotes the induction of $FOXP3^+$ Tregs. Combined with PD-L1-mediated checkpoint inhibition of

T-cell activation, these EVs reprogram inflammatory $CD4^+$ T-cell responses, attenuate intestinal inflammation, and preserve epithelial integrity in humanized colitis models, highlighting a potent cell-free immunotherapeutic strategy [3].

Together, these studies illustrate the rapidly evolving landscape of engineered EV-RNA platforms that combine precise cargo loading, surface functionalization, and tissue-specific targeting to address the complex pathophysiology of IBD. By integrating insights from advanced *in vitro* models and innovative nanoparticle design, such strategies hold significant promise for developing personalized, mechanism-driven therapies that can dynamically modulate immune and epithelial functions in patients with IBD (Figure 3B). Relevant findings on the therapeutic potential of EV-RNA in IBD management are summarized in Table 1.

7. Conclusion

The extensive body of research synthesized in this review unequivocally establishes EV-RNA as a pivotal and dynamic regulator in the pathophysiology of IBD. EV-RNAs, including miRNAs, lncRNAs, and circRNAs, serve as fundamental mediators of intercellular and cross-kingdom communication within the intricate intestinal microenvironment. Through these roles, they critically influence core disease mechanisms: they finely tune immune responses by regulating macrophage polarization and T-cell differentiation; they modulate intestinal epithelial barrier integrity, either promoting repair or exacerbating dysfunction; and they orchestrate complex bidirectional crosstalk with the gut microbiota. This multifaceted involvement positions EV-RNAs not merely as bystanders but as central actors driving inflammation, fibrosis, and even systemic complications, thereby offering a novel molecular lens through which to understand IBD.

In the realm of diagnosis, EV-RNAs present a transformative potential as non-invasive, specific, and dynamic biomarkers. Their encapsulation within EVs confers remarkable stability in biofluids like plasma and saliva, protecting them from degradation and enabling reliable detection. Specific signatures, such as elevated levels of EV-encapsulated lncRNA H19 or NEAT1, and distinct salivary exosomal miRNA panels, have demonstrated high sensitivity and specificity in distinguishing active IBD from remission or other conditions. These molecular profiles offer a real-time reflection of underlying mucosal inflammation and disease activity, potentially surpassing conventional biomarkers like CRP or calprotectin. The integration of machine learning with multi-EV-RNA marker panels paves the way for developing highly accurate, minimally invasive diagnostic platforms that could enable earlier detection, precise disease stratification, and proactive monitoring of therapeutic response.

Therapeutically, EV-RNAs offer a dual-pronged promise: as direct therapeutic targets and as natural vehicles for drug delivery. Targeting pathogenic EV-RNAs, such as inhibiting exosomal miR-155 from adipose tissue or supplementing beneficial species like miR-200b-3p from colonic EVs, represents a strategy to directly disrupt disease-driving pathways. Conversely, leveraging EVs as delivery systems capitalizes on their inherent biocompatibility, low immunogenicity, and ability to cross biological barriers. MSC-EVs deliver a cocktail of immunomodulatory miRNAs (e.g., miR-378a-5p, miR-21) that suppress NLRP3 inflammasome activation and promote M2 macrophage polarization, showing efficacy in preclinical colitis models. Remarkably, dietary and plant-derived EVs, such as those from milk, tea, or ginger, emerge as novel oral therapeutics. These natural nanocarriers can deliver functional RNAs

(e.g., bta-miR-30a-5p, osa-miR166d-5p) directly to the gut, exerting anti-inflammatory effects and modulating the microbiota, presenting a patient-friendly and targeted treatment avenue.

The future of EV-RNA-based applications is being further shaped by sophisticated bioengineering. Strategies such as engineering EVs to display targeting ligands (e.g., PD-L1) and encapsulate specific therapeutic RNAs (e.g., miR-27a-3p) enable precise, personalized immunomodulation. Hybrid systems, like ginger-derived EVs coated on nanoparticles carrying TNF-α siRNA, exemplify advanced designs that enhance targeting, stability, and synergistic efficacy. These engineered platforms move beyond natural cargo, allowing for the programmable loading of RNA therapeutics to correct specific molecular defects, heralding a new era of cell-free, precision medicine for IBD, particularly for patients refractory to conventional biologics.

However, the translation of this formidable potential from bench to bedside faces significant challenges that must be methodically addressed. A major hurdle is the lack of standardization in EV isolation, characterization, and RNA profiling techniques, leading to variability and hindering reproducibility across studies. The biological heterogeneity of EVs, influenced by cellular origin and patient-specific factors like disease activity and medication, adds complexity to biomarker identification. Therefore, large-scale, multicenter validation studies are imperative. Furthermore, the development of clear regulatory pathways for EV-based diagnostics and therapeutics, alongside ethical frameworks for data use, is essential for clinical integration. Collaborative efforts among researchers, clinicians, and regulators are needed to establish robust guidelines.

In conclusion, EV-RNA research is poised at the forefront of revolutionizing IBD management. By providing deep mechanistic insights into disease pathogenesis, offering superior biomarkers for precision diagnosis, and enabling the development of innovative targeted and engineered therapeutics, EV-RNAs hold immense promise. Realizing this promise will require sustained interdisciplinary collaboration to overcome technical and translational barriers. Through continued investment in foundational science, standardization efforts, and responsible clinical development, EV-RNA-based strategies can transition from compelling concepts to tangible tools, ultimately contributing to a new paradigm of personalized and effective care for patients with IBD globally.

Declaration of generative AI and AI-assisted technologies

During the preparation of this manuscript, the authors used generative AI tools only to improve language and readability. The authors take full responsibility for the content of the manuscript.

Acknowledgments

This work was funded by the National Natural Science Foundation of China (82400479) and the Research Project of Zhejiang Chinese Medical University (2025RCZXZK47).

Authors' contribution

Conceptualization, X.W.; investigation, R.R. and M.X.; writing—original draft preparation, R.R. and X.J.; writing—review and editing, R.R. and X.W; visualization (tables and figures), R.R., M.X. and X.W. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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