



Extracellular vesicles-mediated interaction within intestinal microenvironment in inflammatory bowel disease

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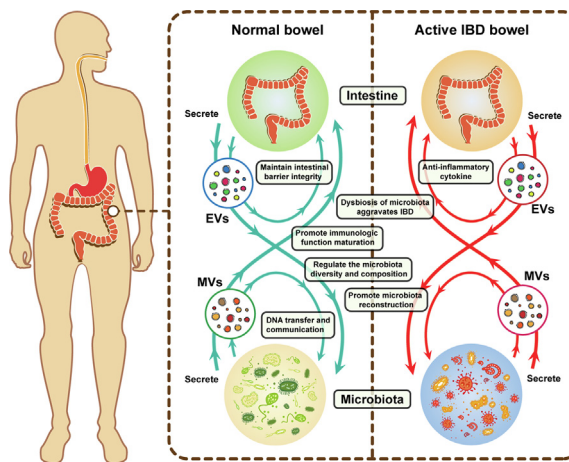
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HIGHLIGHTS

- EVs derived from different sources play modulatory functions in the intestine, especially interaction associated with microbiota.
- An EV-mediated interaction system was established to describe the possible mechanism of IBD pathogenesis and its cure.
- EVs-based treatments show great potential of clinical applications in IBD diagnosis and therapy.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: The intestinal tract is a complicated ecosystem with dynamic homeostasis via interaction of intestine and microbiota. Inflammatory bowel disease (IBD) is chronic intestinal inflammation involving dysbiosis of intestinal microenvironment. Extracellular vesicles (EVs), as vital characteristics of cell–cell and cell-organism communication, contribute to homeostasis in intestine. Recently, EVs showed excellent potential for clinical applications in disease diagnoses and therapies.

Aim of Review: Our current review discusses the modulatory functions of EVs derived from different sources in intestine, especially their effects and applications in IBD clinical therapy. EV-mediated interaction systems between host intestine and microbiota were established to describe possible mechanisms of IBD pathogenesis and its cure.

Key Scientific Concepts of Review: EVs are excellent vehicles for delivering molecules containing genetic information to recipient cells. Multiple pieces of evidence have illustrated that EVs participate the

Abbreviations: IBD, Inflammatory bowel disease; EVs, Extracellular vesicles; MVs, Membrane vesicles; IECs, Intestinal epithelial cells; GI, Gastrointestinal; UC, Ulcerative colitis; CD, Crohn's disease; SCFA, Short-chain fatty acid; FMT, Fecal microbiota transplantation; DSS, Dextran sulfate sodium; MSC, Marrow-derived mesenchymal stromal cells; NEAT1, Nuclear paraspeckle assembly transcript 1; Treg, Regulatory T cell; TNBS, 2,4,6-trinitrobenzenesulfonic acid; E-cadherin, Epithelial cadherin. EMT, Epithelial-mesenchymal transition; BMSC, bone marrow mesenchymal stem cell; TJs, tight junctions; ANXA1, Annexin A1; TLR, Toll-like receptor; *E. coli*, *Escherichia coli*; OMVs, Outer-membrane vesicles; CMVs, Cytoplasmic membrane vesicles; LPS, lipopolysaccharide; ECN, *E. coli* strain Nissle 1917; CRC, Colorectal cancer; PSMA7, proteasome subunit alpha type 7; hucMSC, Human umbilical cord MSC.

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interaction between host and microbiota in intestinal microenvironment. In inflammatory intestine with dysbiosis of microbiota, EVs as regulators target promoting immune response and microbial reconstruction. EVs-based immunotherapy could be a promising therapeutic approach for the treatment of IBD in the near future.

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Introduction

The gut hosts a complex microbiota that is currently considered to be a new organ that is strongly associated with host health and multiple diseases [1,2]. The microbiota in the intestine is initially comprised of microbes from the mother and then shaped by feeding and other environmental contacts [3]. Previous studies have shown that the contacts between the host and gut microbiota principally rely on metabolites [4,5], nucleic acids [6] and proteins [7,8].

Extracellular vesicles (EVs) and membrane vesicles (MVs) derived from host cells and microbiota, respectively, also participate in interactions in the intestinal microenvironment [9,10]. Accumulated evidence has proven that vesicles, as important means of cell–cell and cell–organism communication, are essential for intestinal homeostasis [11,12].

At present, inflammatory bowel disease (IBD) had affected more than 3.5 million people, with an increasing incidence worldwide [13]. The etiology and pathogenesis of IBD were very complex and remain unclear. In addition to genetic susceptibility and environmental pollution [14,15], several studies reported that dysbiosis of the gut microbiota of IBD patients with abnormal metabolism of intestinal epithelial cells (IECs) is associated with a changing abundance of cargo in EVs and MVs [16]. Moreover, EVs are involved in the formation of IBD and they have roles in the regulation of the intestinal mucosa and epithelial barrier function [17] and the export of cellular metabolites [10], as well as being important regulators of immune cell recruitment [18].

Here, we will review the possible role of EVs in the interaction between the gastrointestinal (GI) tract and gut microbiota in IBD patients, especially the mechanism of action and the effects of extracellular vesicles and bacterial membrane vesicles on IBD pathogenesis. In addition, we summarized the potential utility of EVs as diagnostic markers and as a novel therapeutic approach, as well as the application of modified EVs in IBD patients.

An overview of IBD and gut microbiome

IBD is a chronic inflammatory condition of the GI tract with two main subtypes, ulcerative colitis (UC) and Crohn's disease (CD), which are characterized by debilitating and chronic relapsing and remitting inflammation in the colon and GI tract, respectively. IBD patients have unique but inordinate microbial signatures [19], which commonly show reduced bacterial diversity [20] and a decreased abundance of Firmicutes [21–24]. Meanwhile, a significantly increased abundance of Bacteroidetes and Proteobacteria, mainly *Escherichia coli* [25], has been reported [22,23], as well as a decrease in obligate anaerobic producers, including *Faecalibacterium prausnitzii* and *Roseburia intestinalis*, which are specifically associated with short-chain fatty acid (SCFA) [26] production, in CD [27]. Decreased levels of SCFAs can inhibit histone deacetylase activity and modulate gene expression, cell proliferation and even the immune response [28]. In particular, reduced butyrate can aggravate colitis by increasing Treg cell production and decreasing the antibacterial activity of macrophages in GI [29].

In addition, some previous studies reported that the fungal microbiota was skewed [30] in IBD with the expansion of bacterio-

phages [31]. In pediatric IBD patients, the diversity of fungi also decreased, while Pacific *Candida* taxa were found to be increased in abundance [32]. The same results were also found in mature patients with an increased abundance of *Basidiomycota/Ascomycota* and decreased levels of *Saccharomyces cerevisiae* [30]. Another study indicated that the intestinal microenvironments of Crohn's disease-specific bacteria might favor the proliferation of fungi at the expense of bacteria. For bacteriophages, some previous reports indicated that bacteriophage levels were significantly different in IBD patients when compared to controls [33,34], such as *Caudovirales* [35], which was associated with a reduction in bacterial diversity by increasing the abundance of bacteriophage-exacerbated colitis via Toll-like receptor (TLR) 9 and IFN- γ [31].

Under stimulation from dysbiosis and a disordered GI environment, the immune system and mucosal barrier integrity are impaired, leading to chronic inflammation and aberrant immune responses [36]. For instance, increasing numbers of pathogenic bacteria affect the permeability of the intestine and they are involved in the regulation of inflammatory gene expression [20,37], resulting in intestinal inflammation [38]. Accordingly, many therapies targeting microbiota have been developed to restore the health of a diseased microbiome through diet [39,40], prebiotics [41], antibiotics [42] and fecal microbiota transplantation (FMT) [28,43]. However, on account of the complexity of IBD, more detail should be elucidated in many more clinical experiments.

Origin, compositions and general functions of EVs

EVs were initially described nearly 35 years ago [44] and they play a crucial role in cell-to-cell communication. EVs are classified based on their cellular origin and/or biological function or biogenesis. According to their biogenesis, EVs are divided into three main classes: exosomes, microvesicles and apoptotic bodies, with characteristic markers respectively (Table 1) [45]. Exosomes are ~40 to ~200 nm (or ~30 to ~150 nm), single-membrane, endosome-derived sEVs secreted by most cells. Briefly, exosomes are a homogeneous population of vesicles formed with the intraluminal budding of the multivesicular body membrane [45]. MVs are ~200 to ~1000 nm EVs formed by budding of the cell membrane, and apoptotic bodies (500–2000 nm) are products of apoptotic cell disassembly [46,47]. Both microvesicles and exosomes are excellent vehicles for delivering molecules containing genetic information to recipient cells. Thus, in the following discussion, we mainly concentrate on the role of microvesicles and exosomes in IBD.

There is a characteristic subset of cell-derived components packaged in EVs, including proteins, nucleic acids, lipids, and glycoconjugates [48]. As one of the important components of EV components, proteins are abundant in variety and complex in structure and can be mainly divided into two types: structural proteins (such as Alix, TSG101, CD9, CD63, and Hsp90) and specific proteins (such as MCH-I, MCH-II, CD80, CD86, FasL, and TGF- β) [49]. In addition, nucleotides, approximately 200 bp in size, in EVs are also protagonists for EV functions, such as genomic DNA, mitochondrial DNA and RNA. These extracellular nucleic acids can be transferred to other tissues and cells by EVs. The number of species of RNA in

Table 1
Three major types of EVs.

Vesicle	Size(nm)	EV class	Origin	Protein markers
Exosomes	40–200 (30–150)	Small EV ^a	Endosomes	CD63 (CD81 CD9)
Microvesicles	200–1000	Large EV ^b	Plasma membrane	Annexin A1
Apoptotic bodies	500–2000	Large EV	Plasma membrane, endoplasmic reticulum	Annexin V

^a Small EVs are <200 nm in diameter.

^b Large EVs are >200 nm in diameter.

EVs are abundant, mainly including mRNAs and small regulatory RNAs [50], among which small noncoding RNAs are particularly enriched in exosomes [51,52]. Compared to RNA, EVs containing DNAs are more controversial. Accumulating evidence has indicated that EVs contain various DNAs, including single- and double-stranded DNA, genomic DNA and mitochondrial DNA, and even reverse-transcribed complementary DNAs [53–56], which may participate in the regulation of inflammation and are regarded as biomarkers of cancer, viral infection, and chemotherapeutic resistance [9].

However, the hypothesis that exosomes contain DNA was negated in a recent report [57]. In this research, investigators directly captured EVs by immunoaffinity to precisely characterize their constituents by virtue of high-resolution density gradient fractionation and found that small EVs are not vehicles of active DNA release. Under this circumstance, further exosomal DNA research needs more in-depth and comprehensive research with a high purity separation protocol.

In GI, EVs are mainly secreted by immune cells and IECs [58,59]. Abundant research has convincingly shown that EVs are important contributors to the communication between the microbiota [60], IECs [58], endothelial cells [61], and immune cells [59]. EVs are the main participators in vascular and epithelial barrier function in inflamed intestines and wound healing, as well as being important regulators of immune cell recruitment. Thus, in the following sections, we will discuss in detail the role of EVs in intestinal homeostasis, intestinal barrier integrity and immune cell function in the IBD-GI tract.

Extracellular vesicles from host cells in IBD-GI

Extracellular vesicles in GI contain different molecular particles according to their parental cells, which determine the function of exosomes. Exosomes from immune cells contribute to evasion of the immune system, and IEC-derived exosomes have been proven to be important regulators of IEC-induced immune tolerance (Fig. 1). These functions are critical in exosome-mediated immune responses, intestinal barrier modulation and gut microbiota shaping in IBD.

Extracellular vesicles-mediated immune responses

Extracellular vesicles are involved in immunomodulation via the functional transfer of nucleotides, proteins and other cargos between immune cells. In the active IBD intestinal microenvironment, increased recruitment of innate immune cells such as macrophages, monocytes, dendritic cells (DCs), neutrophils and T-cells, takes place. Under these circumstances, EVs are tightly related to macrophages in IBD (Table 2). For example, dextran sulfate sodium (DSS)-induced colitis mice treated with exosomes from human bone marrow-derived mesenchymal stromal cells (MSC-Exos) act mainly on colonic macrophages [62], which polarize M2b macrophages without favoring intestinal fibrosis [63]. In addition, nuclear paraspeckle assembly transcript 1 (NEAT1) was also proven to be involved in the inflammatory response by polar-

ization of macrophages [64]. Polarized M2b macrophage secretes exosomes to increase number of regulatory Treg cells and inhibit pro-inflammatory cytokine, resulting in attenuation of colitis [65] (Fig. 1). Exosomes from DSS-induced colitis mouse serum increase the phosphorylation of p38 and ERK and the production of tumor necrosis factor by macrophages [66]. Epithelial cells exposed to adherent-invasive *Escherichia coli* and extracellular vesicles from IBD patients' intestinal lumen induce increasing proinflammatory responses and the migration of a significantly greater number of macrophages [67,68]. In contrast, exosomes from visceral adipose tissue predispose the intestine to inflammation by promoting M1 macrophage polarization [69]. These studies support the hypothesis that exosomes are involved in the activation of macrophages. Furthermore, EVs have been found to have immunosuppressive effects, such as suppressing the activation of DCs to induce immune tolerance and participating in regulatory T cell (Treg) activation [18]. Activated Treg also secretes exosomes containing miR-195-3p, targeting pro-apoptotic Caspase 12, to alleviate IBD in mice [70]. IEC-derived exosomes are crucial regulators among sections, mediating the modulation of resident immune cells. In IBD development, IECs secrete epithelial cell adhesion molecule-dependent EVs with increased levels of TGF- β 1 in an ERK-dependent manner to maintain the intestinal tract immune balance and decrease IBD severity [11]. In addition, IEC-derived exosomes are capable of binding to HLA-DR4 molecules and inducing productive T-cell activation by preferentially interacting with DCs [71]. These EVs also contain abundant immunomodulatory molecules, such as higher levels of major histocompatibility complex class I and class II molecules, in IBD [58,72]. DCs are the only APCs capable of activating naive T cells, which contribute significantly to IBD pathogenesis [16]. Exosomes derived from DCs treated with IL-10 upregulate the levels of Tregs in the colonic lamina propria and relieve 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced colitis [73]. These immunomodulatory molecules are also essential in initiating adaptive immunity, including consolidating B-cell maturation [74].

Exosomal miRNAs are also important regulators of intestinal immune responses, modulating immune cell function and thereby coordinating the immune system in IBD (Table 2) [75]. For instance, exosomal miR155 and miR146a enter recipient DCs, mediating the repression of target genes [76]. In addition, T cells are activated by miR-1246 by activating proinflammatory nuclear factor in active IBD [77,78]. The NF- κ B signaling pathway is an important factor in the immune response activated in UC by targeting exosomal miRNAs, which promote the transcription of various proinflammatory cytokine genes, such as miR-16, an adenosine A2a receptor inhibitor mediating NF- κ B signaling pathway activation [79].

In summary, the functional molecules in exosomes that express anti- and proinflammatory factors mediate immune responses in IBD pathogenesis (Fig. 1).

Extracellular vesicles-mediated intestinal barrier modulation

Intestinal barrier dysfunction is another pivotal factor in IBD pathogenesis. The mechanisms of gut barrier maintenance are dis-

Table 2
A summary of EVs and their components in studies of IBD.

EVs component	Source and type of EVs	Target	Disease model	Effects and mechanisms	References
Immune response modulation					
–	BMSC-EVs	Macrophage	DSS-induced colitis	Promote M2-like macrophage polarization, regulate the JAK1/STAT1/STAT6 signaling pathway	[62]
–	BMSC-Exos	Macrophage	DSS-induced colitis	Exosomal-metallothionein-2 suppress NF-κB activation	[63 136]
NEAT1	Blood serum-Exos	Macrophage	DSS-induced colitis	NEAT1 inhibition promote macrophage polarization	[64]
–	M2b macrophage-Exos	Tregs	DSS-induced colitis	Suppress Inflammatory cytokine, regulate CCL1/CCR8 axis	[65]
–	Colitis blood serum-Exos	Macrophage	–	Upregulated phosphorylation of both p38 and ERK	[66]
–	IBD blood serum-Exos	Colonic epithelial cells	–	Increase translation of IL-8 protein in recipient cells, induced migration of macrophages	[67]
miR-155	Visceral adipose tissue-Exos	Macrophage	HFD/DSS-induced colitis	Promote M1 differentiation via transferring miR-155	[69]
miR-195a-3p	Treg-Exos	Caspase 12	DSS-induced colitis	Promote colonic epithelial cells proliferation and inhibited cell apoptosis	[70]
TGF-β1	IECs-EVs	Tregs	DSS-induced colitis	EpCAM-dependent IECs-EVs with increased levels of TGF-β1 alleviate IBD	[11]
–	IECs-EVs	DCs	–	Induce productive T-cell activation by interacting with DCs	[71]
IL-10	DCs-Exos	Tregs	TNBS-induced colitis	Increased levels of anti-inflammatory cytokine down-regulates mRNA expression of pro-inflammatory cytokines	[73]
miR-155	DCs-Exos	–	LPS-induced IBD	miR-155 enhances inflammatory gene expression	[76]
miR-146a	DCs-Exos	–	LPS-induced IBD	miR-146a reduces inflammatory gene expression	[76]
Intestinal barrier modulation					
miR-146b	–	NF-κB pathway	DSS-induced colitis	miR-146b-mediated inhibition of the ubiquitination of TRAF proteins upstream of NF-κB.	[81]
miR-200b	BMSC-Exos	EMT	TNBS-induced colitis	Suppress the development of EMT by targeting ZEB1 and ZEB2.	[82 83]
miR-223	HMCs-Exos	CLDN8	TNBS-induced colitis	miR-223 interacts with the IL23 pathway by inhibiting CLDN8	[85 86]
ANXA1	IECs-EVs	Epithelial FPRs	DSS-induced colitis	Target epithelial FPRs (FPR1 and FPR2/ALX) to promote wound repair	[91]
Gut microbial structure shaping					
–	TLR 2-deficient mouse serum EVs	TLR2/6 in probiotics	–	Inhibit the activity of Toll-like receptor (TLR)2/6 in probiotics leading microbial dysbiosis	[92]
miR-515-5p / miR-1226-5p	Fecal EVs	Bacterial gene transcripts	–	Enter <i>F. nucleatum</i> and <i>E. coli</i> to regulate bacterial gene transcripts and affect bacterial growth	[6]

IBD: Inflammatory bowel disease; EVs: Extracellular vesicles; Exos: Exosomes; BMSC: Bone marrow mesenchymal stem cell; DSS: Dextran sulfate sodium; HFD: High fat diet; DCs: Dendritic cells; TNBS: 2,4,6-trinitrobenzenesulfonic acid; EMT: Epithelial-mesenchymal transition; HMCs: Human mast cells; CLDN8: Claudin 8; ANXA1: Annexin A1; FPRs: Formyl peptide receptors; TLR: Toll-like receptor.

rupted in the inflammatory intestine, including downregulating epithelial cadherin (E-cadherin), reduced the thickness of the mucus layer, and dysfunction of goblet cells and Paneth cells [80]. IECs, a basic component of the intestinal barrier, are mainly affected by nucleotides in EVs from immune cells, the gut microenvironment and the ingesta. DC exosomes activate the NF-κB signaling pathway to improve intestinal barrier function in DSS-induced colitis via exosomal miR-146b [81]. lncRNA NEAT1 modulates the intestinal epithelial barrier to suppress the inflammatory response in IBD, including downregulating TNF-α and increasing the expression of CD206, IL-10, and arginase-1 [64]. In addition, epithelial-mesenchymal transition (EMT) occurs in the process of inflammation and cancer metastasis, while miR-200b plays an antifibrotic role in the inhibition of EMT by targeting Zeb1 and Zeb2 [82]. Jia and his group used bone marrow mesenchymal stem cell (BMSC)-derived microvesicles overexpressing miR-200b to prevent colon fibrosis induced by TNBS in colitis models [83]. On the contrary, another study showed a stimulating effect of exosomal miR-200b on colorectal cancer cell-derived xenografts to effective tumor growth, which indicated different roles of miRNA in the pathogenesis of IBD and colon cancer [84]. Exosomal miR-223 is a unique miRNA in IBD pathogenesis that breaches the integrity of intestinal tight junctions (TJs) by interacting with the IL-23 pathway by targeting Claudin-8 to promote IBD progression

[85,86]. miR-21, a characteristic marker in colonic cancer, is also regarded as one of the most dysregulated miRNAs with highly enriched expression in exosomes derived from various intestinal cells in IBD pathogenesis [87]. Several studies have proven that the expression of miR-21 is significantly increased and this has important implications in different phases of IBD pathogenesis [18,87]. Deletion of miR-21 resulted in the exacerbation of both the TNBS-induced and T-cell transfer models of colitis [88], whereas decreased levels of miR-21 in T cells obtained from colonic mucosa were reported during the recovery stage of UC [87]. These evidences proved that partial same pathogenesis was found in the process of IBD and gastrointestinal cancers, according with that the occurrence of IBD increases the risk of gastrointestinal tumorigenesis. Hence, some markers of colorectal cancer, such as the levels of exosomal miR-21, should also be considered in IBD pathogenesis.

Proteins packaged in exosomes have also been demonstrated to mediate modulation of intestinal barrier function. Cellular prion protein, released from platelet-derived exosomes, maintains the function of the TJ barrier and protects lateral junctional complexes in IBD [89]. In addition, exosomal glucose-regulated protein 78 participates in cell-cell communication between colon cancer cells [90]. IECs secrete EVs containing Annexin A1 (ANXA1), a protein activating wound repair circuits, which was found to be signifi-

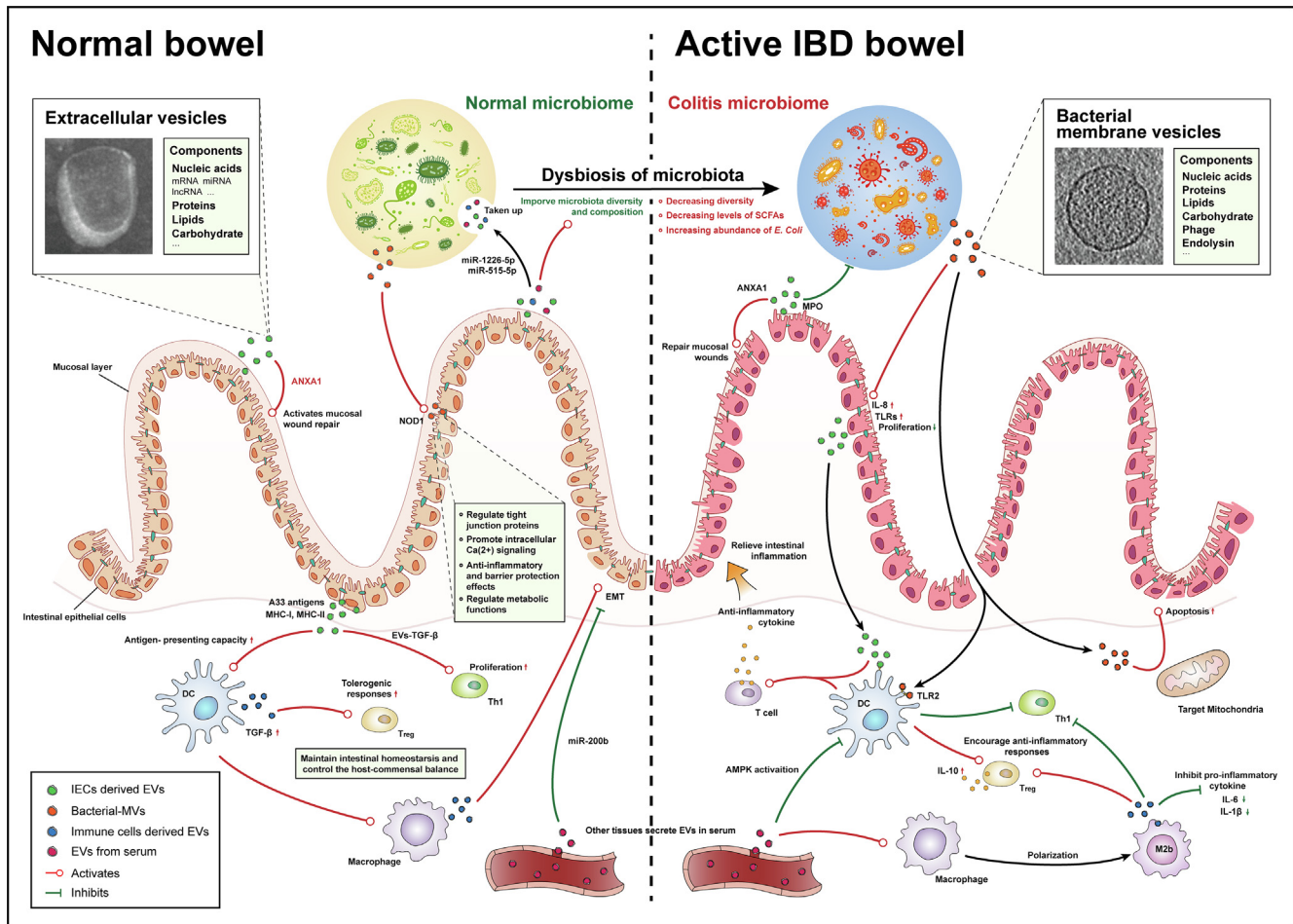


Fig. 1. The EVs-mediated interaction in intestinal microenvironment. In intestinal microenvironment, EVs from host and commensal contribute to intestinal homeostasis maintaining, maturation of immune cells and regulation of metabolic functions. Within inflammatory bowel disease, the balance of host-commensal is destroyed, accompany by dysbiosis of microbiota, dysfunctional immune response and intestinal barrier. Intestinal EVs directly or indirectly interact with immune cells, intestinal epithelial cells and gut microbiota, participating regulation of anti-inflammation response, restoring of mucosal barrier integrity and reconstitution of microbiota composition.

cantly increased in sera from active IBD patients, accelerating the recovery of intestinal barrier function [91].

The gut microenvironment and ingesta are also important factors in intestinal barrier modulation, and we will discuss them in a following section. In summary, exosomal nucleotides and proteins from host cells show crucial functions in maintaining intestinal barrier function and activating intestinal wound repair circuits in IBD pathogenesis (Fig. 1, Table 2).

Extracellular vesicles-mediated gut microbial structure shaping

The importance of gut microbiota in the process of intestinal inflammation is undisputed. There are abundant conjectures about crosstalk between host and intestinal microbes. However, to date, a completely clear interaction network has not been described in IBD-GI. Several previous studies have proven that EVs from host cells participate in this network and play an important role in microbial reconstruction and dysbiosis (Fig. 1, Table 2). The activity of TLR 2/6 in probiotics is significantly reduced by EVs from TLR 2-deficient mouse sera, such as *Bifidobacterium* and *Lactobacillus*, leading to microbial dysbiosis [92]. Heat shock protein 70 (HSP70), a common component of exosomes, was able to stimulate proinflammatory responses by utilizing gram-positive (TLR2) and gram-negative bacteria (TLR4) receptors in a CD14-dependent fashion [93]. In addition, exosomal nucleic acids have also been proven to participate in microbial structure shaping. Liu investi-

gated fecal miRNAs between humans and mice and found that several miRNAs are enriched in fecal samples as well as in intestinal extracellular vesicles. These miRNAs, such as miR-515-5p and miR-1226-5p, can enter *Fusobacterium nucleatum* and *Escherichia coli* (*E. coli*) to regulate bacterial gene transcripts and affect bacterial growth [6]. Moreover, fecal miRNA from WT mouse transplantation reconstitutes the colonic microbes and ameliorates colitis in IEC-miRNA-deficient mice [6].

Compared to extracellular vesicle-mediated immune responses and intestinal barrier modulation, studies about exosomal functions in the gut microbiome are scarce. Nevertheless, exosome-mediated gut microbial reconstruction is a potential strategy for manipulating the microbiome, which may become an important application in IBD and other intestinal disease therapies.

Bacterial membrane vesicles in IBD-GI

In addition to EVs from host cells, numerous MVs from gram-negative and gram-positive bacteria, fungi, and protozoa play a significant role in the GI. In general, MVs are often referred to as outer-membrane vesicles (OMVs) because they were first found to originate from controlled blebbing of the outer membrane of gram-negative bacteria [94]. However, a recent review indicated that MVs can be categorized by their different parent cells, structure and composition, including OMVs, outer-inner membrane vesicles from gram-negative bacteria and cytoplasmic membrane

vesicles (CMVs) from gram-positive bacteria (Table 3) [10]. Regrettably, the vast majority of previous studies focused on OMV-related host pathology and immune tolerance.

Types and general functions of MVs

Different MVs have different structures and compositions depending on the diverse routes of the formation of MVs. OMVs are bilayered lipid membrane nanostructures 20–250 nm in diameter with an outer leaflet of lipopolysaccharide (LPS) and an inner leaflet of phospholipids, and they are purposefully secreted by gram-negative bacteria [94,95]. Their cargo can include biological components found within the parent bacterium, including LPS, periplasmic and membrane-bound proteins, DNA, RNA, enzymes, toxins and peptidoglycan [96]. Interactions between OMVs and epithelial cells can modulate the intestinal tract barrier [97,98] and cellular mechanisms that control proliferation [99] and apoptosis [100,101], and ultimately immune responses.

However, MVs were also observed in gram-positive bacteria without outer membranes and were thus named CMVs [10]. CMVs contain membrane and cytoplasmic components as well, also including DNA, RNA, enzymes, toxins and peptidoglycan, but no LPS.

Bacterial-MVs regulation of IBD-GI metabolism and immunity

Alterations in the intestinal microbiota have been involved in the pathogenesis and development of many diseases, as well as in IBD [102]. Homeostasis between the host and microbial communities in the intestine is implicated in the maturation and functions of IECs and various immune cells. Recent studies reported that an abundance of LPS-positive bacterial EVs was observed in patients with intestinal barrier dysfunction [103]. Consequently, bacterial EVs are also important regulators in IBD pathogenesis to modulate immunomodulation and the corresponding signaling pathways [98,104] (Fig. 1). *E. coli* is mainly a pathogenic bacterium that has been observed to be significantly enriched in IBD-GI. OMVs from *E. coli* stimulate transcriptional upregulation of TLRs in the IEC lines HT29-19A and Caco-2, leading to a mild proinflammatory response [105] and reducing the proliferation of HT-29 cells [106]. In addition, enterohemorrhagic *E. coli* O157-derived OMVs stimulate TLR5 and the TLR4/MD-2 complex signaling pathway to increase the production of IL-8 in IECs [107] while targeting mitochondria to induce endothelial and epithelial apoptosis [108]. On the other hand, probiotic *E. coli* strain Nissle 1917 (ECN) secretes OMVs that contribute to intestinal homeostasis by activating NOD1 signaling pathways and regulating the expression of TJ proteins to enhance barrier function in IECs [109,110]. Moreover, ECN-derived OMVs were proven to mediate anti-inflammatory and barrier protection effects and to relieve UC in experimental colitis [111].

Table 3
Three major types of bacterial MVs.

Vesicle	Origin	Cargo
OMV	Outer- membrane blebbing	Outer-membrane proteins, plasmids
OIMV	Explosive cell lysis and cell budding	Outer-membrane proteins, cytoplasmic (or inner) membrane proteins, RNA, DNA, plasmids
CMV	'Bubbling cell death' and bacterial autolysins	Cytoplasmic (or inner) membrane proteins, RNA, DNA, plasmids

OMV: outer-membrane vesicle; OIMV: outer-inner membrane vesicle; CMV: cytoplasmic membrane vesicles.

Probiotics are crucial components in the intestine that demonstrate significantly decreased abundance in IBD pathogenesis. In the case of *Bacteroides fragilis*, OMVs from *B. fragilis* can interact with DCs via TLR2 to induce the differentiation of IL-10-producing regulatory T cells and suppress TNBS-induced colitis [112]. OMVs also interact within DCs to induce IL-10 expression by regulatory T cells via the IBD-associated gene ATG16L1 and could relieve intestinal inflammation in a colitis model [12]. Note, some regulations of bacterial-MVs are found only in healthy individuals rather than IBD, such as *Bacteroides thetaiotaomicron* [113]. The bacterium *Akkermansia muciniphila* is a vital strain in the healthy human intestinal tract that has a crucial role in the regulation of gut barrier homeostasis and metabolic functions [114]. OMVs from *A. muciniphila* are involved in gut permeability via regulation of intestinal TJs to improve metabolic functions [97]. Stentz reported that *Bacteroides thetaiotaomicron*-derived mammalian InsP6 phosphatase was packaged inside OMVs, defending gastrointestinal proteases and interacting with intestinal epithelial cells to promote intracellular Ca²⁺ signaling [115].

Moreover, enteric pathogenic bacteria take advantage of OMVs to achieve bacterial invasion. For instance, OMVs from *Campylobacter jejuni*, a major cause of bacterial food-borne gastroenteritis, increase levels of bacterial adhesion and invasion via cleavage of IEC E-cadherin and occluding [116]. *Vibrio cholerae*, a major virulence factor of the diarrheal disease cholera, secretes OMVs with cholera toxin. These OMVs are internalized via the cholera toxin receptor on IECs [117] and activate the MAPK and NF-κB signaling pathways in a NOD1-dependent manner, resulting in increased expression of proinflammatory cytokines. In addition, IECs interacting with OMVs activate DCs to promote T cell polarization toward an inflammatory Th2/Th17 response [118].

Accumulating evidence has demonstrated the importance of bacterial MVs in intestinal homeostasis and IBD pathogenesis (Fig. 1). However, the vast majority of studies have concentrated on the function of MVs from gram-negative rather than gram-positive bacteria. In reality, the abundance of obligate anaerobic *Clostridia* is significantly decreased in both CD and UC (introduced in chapter 2). Thus, the association between probiotic such as *Clostridia*-secreting CMVs and intestinal diseases needs more attention from researchers to demonstrate the mechanisms of probiotics and to develop new therapies related to CMVs and OMVs for IBD pathogenesis.

The interacted network of intestinal microenvironments

The interacted between host and gut commensal had been demonstrated in abundant previous studies. However, the roles of EVs-based interacted network in inflammatory intestine is not clear. We summarized a compendious interacted network to show the importance of EVs in intestinal microenvironments base on existing work and above description (Fig. 2). In normal bowel, EVs contribute maturation of immune cells and maintaining intestinal barrier integrity. Meanwhile, EVs regulate the microbiota diversity and composition to promote intestinal homeostasis indirectly. In this microenvironment, microbiota with stabilized community structure secrete beneficial MVs to promote immunologic function perfection. When the intestines are under inflammatory conditions induced by stress from intestinal microenvironments, cyclic EVs are transported to intestine targeting macrophages polarization and associated with microbial reconstruction. These EVs contain various biomarkers of IBD and promote expressing anti- or pro-inflammatory factors mediate immune responses. On the contrary, MVs from microbiota with dysbiosis aggravate dysfunctions of intestinal mucosal barrier and levels of inflammation.

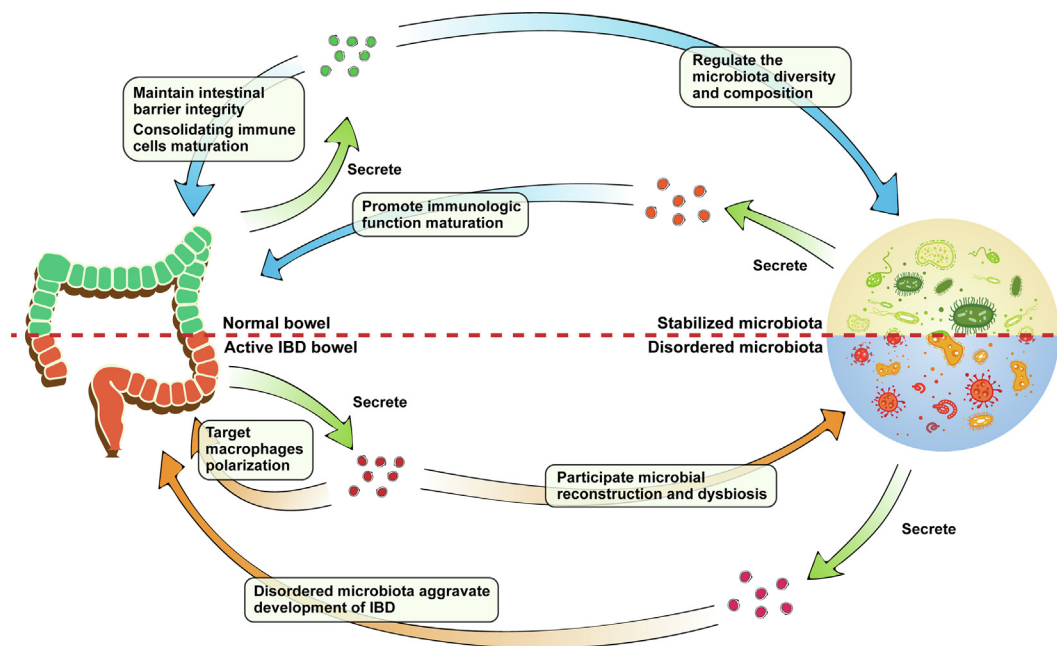


Fig. 2. A compendious host-EVs-microbiota loop in normal and inflammatory intestine. In normal bowel, EVs-based interaction between host and microbiota to maintain intestinal homeostasis. Under inflammatory conditions, intestinal EVs target macrophages to promote anti- or pro-inflammatory factors expression mediating immune responses. MVs from disordered microbiota aggravate development of IBD.

In summary, EVs-based interacted loop within intestine contributes homeostasis of normal intestine, whereas show double roles in IBD. However, the EVs-based interacted network in intestinal microenvironment, an extremely enormous ecosystem, is difficultly described clearly without abundant in-depth work. Consequently, more systematic work associated with functional EVs in intestinal microenvironment need to be reported to demonstrate the irreplaceable roles of host-EVs-microbiota loop.

The clinical potential of EVs and MVs in IBD

EVs play a basic role in cell-cell and cell-organism communication and have shown strong potential in clinical diagnosis and therapies. Therefore, a large number of studies have explored their therapeutic value with high expectations from academia and industries.

EVs as biomarkers in IBD clinical diagnosis

The advantages of EV-dependent diagnosis have been demonstrated in numerous studies. Compared to traditional histopathology diagnosis, regarding EVs as biomarkers in various diseases, they have higher targeting, provide more abundant biological information and have a reduced invasiveness of collection, which is more acceptable to patients, especially in cancer and inflammation diagnoses [119]. In human colorectal cancer (CRC), the proteomics and miRNA transcriptomics of serum have shown that multiple miRNAs and proteins are significantly altered in patients with gastrointestinal cancer [120–122]. Exosomal miR-19a with a significantly increased expression level was identified as a prognostic biomarker for the recurrence of CRC [121]. Likewise, SPARC and LRG1, extracellular matrix-related proteins, were found to promote metastasis in right-sided colon cancer serum [120]. The risk of intestinal cancer is markedly increased in IBD patients, as indicated in a number of studies [123–125]. Currently, many biomarkers are extensively used in the clinic, such as serum C-reactive protein levels, the expression of the NOD2 gene, and inflammatory cytokines, while none can be used separately for an accurate diagnosis of IBD due to nonspecificity [126].

Therefore, it is essential to establish an EV-dependent diagnostic system with high accuracy and convenience from the IBD patients' serum, luminal contents or feces. Calcium-dependent phospholipid-binding protein ANXA1 is an important proresolving mediator associated with epithelial wound repair via Rac2-dependent NADPH oxidase-1 and it is an inhibitor of inflammation [127–129]. Subsequently, ANXA1 was observed packaged in EVs derived from IECs and had elevated levels in sera from active IBD patients [91]. ANXA1 mimetic peptide encapsulated within targeted polymeric nanoparticles accelerated murine colonic wound healing and the recovery from experimental colitis [91]. Accordingly, increasing levels of ANXA1 in IEC-derived EVs may become a specific diagnostic approach for IBD clinical diagnosis. In addition, Zheng demonstrated that exosomes isolated from saliva contained proteasome subunit alpha type 7 (PSMA7), showed significant differences between IBD patients and healthy controls [130]. Compared to existing diagnostic methods, sampling saliva PSMA7 is more convenient and painless, avoiding the pain of colonoscopy in clinical diagnosis. However, the expression of PSMA7 was also found to be overexpressed in colorectal cancer [131], which demonstrated that higher purity is essential for a comprehensive analysis of EVs as potential biomarkers in IBD. Moreover, EVs containing nucleotides also have the potential to be biomarkers of IBD, such as exosomal miR214 and NEAT1 lncRNA, which are both found at high levels in active IBD [64,132].

With the development of methods for EV purification and omics analysis, a large number of exosomal biomarkers for IBD have been reported, including proteins and nucleotides [133]. Regrettably, there is no purification technology that is convenient, efficient, steady, and has a high rate of recovery and operability for EVs targeting clinical samples, which seriously restricts the development of clinical applications. On the other hand, there is still a lack of research on screening different cell- or tissue-specific EV markers, and the sorting mechanism of EVs in cells is not clear. Consequently, multiple problems of EVs associated with separation, preservation and application need further study.

The therapeutic effect of exogenous EVs on IBD

Recently, accumulated evidence about therapeutic methods associated with EVs from different cells and tissues has adequately demonstrated the unique advantage of EVs in the treatment of multiple diseases. In colorectal cancer therapy, tumor derived EVs were eliminated to inhibited tumor metastasis and immune escape in a large number of studies [134]. In inflammatory diseases therapy, EV-related treatment methods can be mainly divided into two categories, regarding EVs from stem cells, immune cells, ingesta and microenvironment as a new remedy, or considered nanocarriers to accomplish the purpose of targeted therapy.

Stem cells derived EVs

EVs from stem cells contribute to the self-renewal, immunomodulation, expansion and damage repair abilities of stem cells [135]. Compared to stem cell therapy, the application of SC-EVs avoids the potential risk of stem cell transplantation and is more convenient for transport and storage. Currently, MSCs-EVs have been proven in a number of experimental and clinical studies as potentially new remedies for the therapy of cancer and autoimmune and inflammatory diseases [136–139], including IBD. Several previous studies demonstrated that MSC therapy of colitis was mainly dependent on inhibition of colon macrophages, downregulation of proinflammatory cytokine levels and the suppression of NF- κ B signaling pathways in BMSC-EV treatment [62,140], which was especially amplified after inflammatory exposure [141]. Similar therapeutic effects were also observed for Adipose-, Olfactory ecto- and human umbilical cord MSC (hucMSC)-derived EVs injection of DSS-induced colitis [142–144]. In addition, Wu and colleagues showed that hucMSC-exosomes modulate inflammatory levels in DSS-induced colitis by regulating the ubiquitin modification level (Fig. 3) [145,146]. Moreover, MSCs-EVs are also excellent nanocarriers to transport small molecule drugs, which is elaborated on in the following section.

Consequently, therapy associated with MSCs-EVs is part and parcel of SCT, which represents a new, cell-free version of SCT, and has been investigated in various related diseases. Nevertheless, as a novel remedy for inflammatory diseases, a large number of experimental and clinical studies need to be done before MSC-EVs could be widely used in clinical therapy.

EVs from ingesta

In addition to EVs from host cells and the microenvironment, daily intake of EVs, such as from plants and milk, is also one of the primary sources of EVs, which are generally considered safe [18,147]. These ingesta-derived EVs possess anti-inflammatory potential and are more acceptable to the public owing to the popularity of dietetic therapy.

Breast milk is a sole nutritional supply to newborn infants and plays a crucial role in the development of the infant intestinal mucosal immune system and microenvironment [148]. Breast milk is also known to be a rich source of EVs, with early milk containing more EVs than mature milk [149], and these EVs participate in the maturation of the infant's immune system [150]. In GI, breast milk-derived EVs and their components (miRNAs) survive digestion in vitro and are taken up by intestinal cells [151–153]; numerous studies have also indicated that EVs, regardless of the origin (human or different animals' breast milk), promote the proliferation of intestinal epithelial cells, alter gut microbiota and enhance immunity of the intestine [154–158]. Hence, fresh milk may have the potential to prevent IBD [159] (Fig. 3).

Daily ingested food, such as fruits and vegetables, and derived exosome-like nanoparticles, also interact with the intestinal mucosa by osmosis (Fig. 3). Wang and colleagues demonstrated that exosome-like vesicles released from grapefruit were selec-

tively taken up by intestinal macrophages and modulated increasing levels of heme oxygenase-1 and inhibition of IL-1 and TNF- α [160]. Consequently, they further developed an EV-based oral delivery system to realize targeted drug delivery to macrophages [160].

Moreover, grape and broccoli secrete exosome-like nanoparticles to relieve DSS-induced colitis by inducing stem cell-mediated intestinal tissue remodeling and activating DC-activated protein kinase, respectively [161,162]. Ginger, the rhizome of *Zingiber officinale*, is one of the most widely used natural products and has been used as a spice and medicine for the treatment of some digestive tract problems [163]. Ginger-derived exosome-like nanoparticles were proven to be taken up by IECs and macrophages, contributing to protection of the intestinal barrier and reconstitution of the gut microbiota [163,164].

In summary, food-derived EVs are closely related to homeostasis of the intestine and microbiota (Fig. 3). Studies associated with these vesicles have achieved encouraging outcomes in experimental animal models and have been accepted by the public. However, no clinical trials have been published to date.

EVs as nanocarriers

With the development of nanotechnology, nanobased drug-delivery systems have attained considerable importance. Multitudinous nanobased drug formulations have been applied in experimental and clinical therapy to improve therapeutic efficacy. For instance, macrophage-targeted siRNA and 5-aminosalicylic acid are packaged in nanoparticles and delivered to inflamed areas [165,166]. However, the nanoparticles could not cross the border of the cytomembrane to realize efficient utilization of the drugs, as well as suffering from a deficiency of precise targeting.

Compared to other nanocarriers, EVs are highly regarded as vectors in the clinic, especially in cancer therapy, due to their nanoscale size, ideal native structure and characteristics [167–169]. The methods of modified EVs manufacture divided in two mainly types, parental cell modifying and direct EVs modifying (Fig. 3). In IBD, various effective proteins, RNA and small molecule drugs can be directly or indirectly packaged in transformed EVs from IECs, human embryonic kidney cells and immune cells, and then taken up by the intestine [64,76]. MSCs-EVs are also excellent carriers. miRNA-200b and miRNA-146a were packaged in MSC-EVs via recombinant lentivirus construction and showed attenuation of experimental colitis [83,170]. In addition, bone marrow-derived DCs were transfected with miRNA-155 and miRNA-146a mimics to secrete miRNA-overexpressing EVs, which were subsequently taken up by recipient DCs to regulate inflammation in LPS-injected mice [76]. Antibiotics are frequently used in inflammatory diseases to protect against bacterial infection. Researchers loaded antibiotics in exosomes from IECs to resist intracellular infections of methicillin-resistant *Staphylococcus aureus*, which is also an excellent application in IBD therapy [171].

The application of EVs as nanocarriers in IBD is not intensive and comprehensive. Some data have reported that the delivery ability of EV carriers can be increased via modification of their surface. For instance, exosomes fused with liposomes possess less immunogenicity and have superior colloidal stability [168]. In summary, as competitive nanocarriers for drug delivery, modified EVs have shown numerous possibilities in novel IBD therapy. However, it is not sufficient to accumulate evidence about experimental studies without clinical trials (Fig. 3). Ultimately, the wide application of EV-based drug delivery systems in clinical treatment depends on standardized production of either derived exosomes or modified exosomes as drug delivery systems.

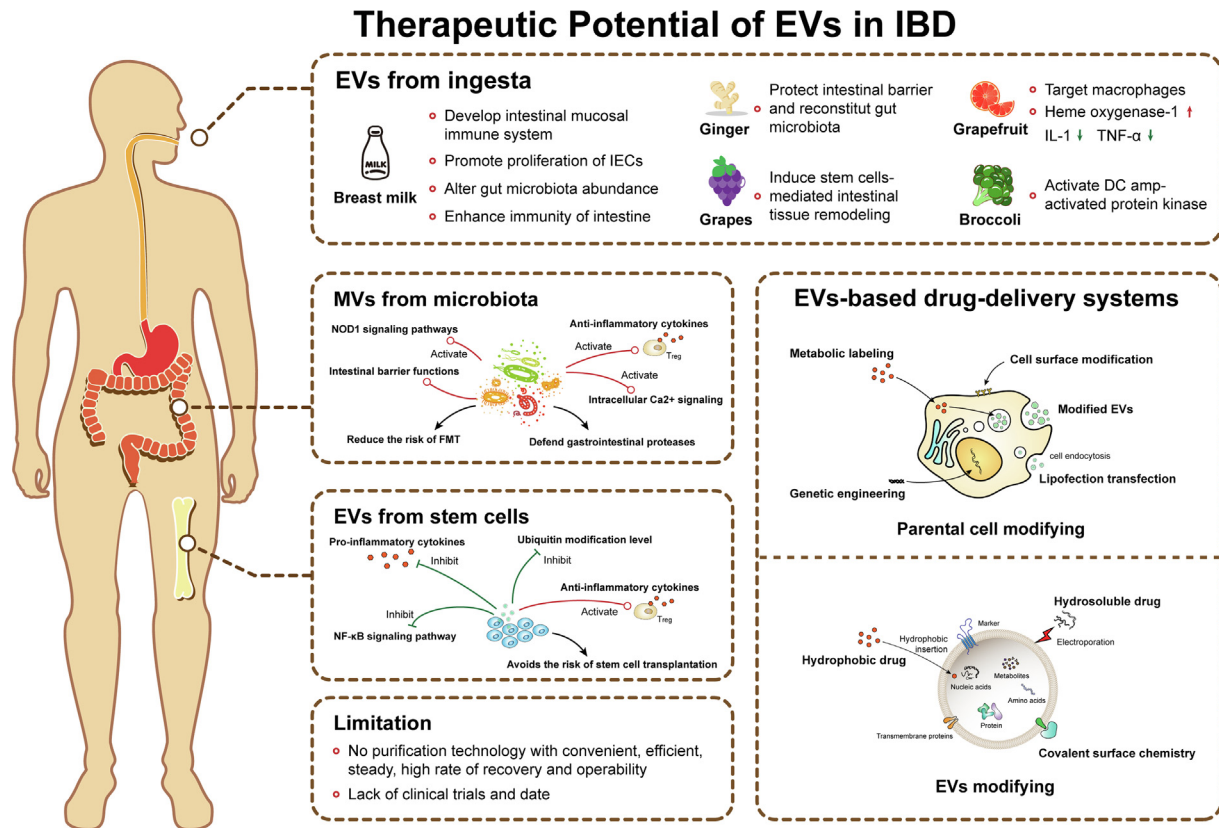


Fig. 3. The therapeutic potential of EVs in IBD. Both of modified and unmodified EVs have showed great clinical application potential in IBD therapy. Unmodified EVs are mainly secreted from stem cells, microbiota and daily ingested food. These EVs can be taken up by intestinal cells and bacteria to relieve IBD via anti-inflammation response regulation and balance of intestinal homeostasis. The technology of EVs modification can be divided in two mainly types, parental cell modifying and direct EVs modifying. Both of two modified methods can obtain EVs containing small molecule drug and functional nucleic acid. The EVs-based therapy can reduce the risk of existing clinical therapy for IBD, but a large number of experimental and clinical studies need to be done before these therapies could be widely used in clinical treatment.

MVs from microbiota

FMT is a popular novel therapy that uses intestinal flora reconstruction to repair disruption of the normal microbial communities, but it has a higher risk for IBD patients [172]. In contrast, transplantation of microbiota-derived MVs shows gentler growth and lower risk. The importance of bacterial MVs in intestinal homeostasis and IBD pathogenesis was minutely described in the previous section (Fig. 3). In addition, GI parasites, hookworms in particular, and their derived EVs, interact with host cells and bacteria. Hookworms have been used in clinical trials to treat IBD and celiac disease. *Trichinella spiralis* EVs was proved significantly ameliorating colitis via targeting inhibition of macrophage polarization [173,174]. Eichenberger and colleagues indicated that helminth EVs show great potential applications in the development of remedies for dysregulated immune system-induced chronic noninfectious disease treatment [175]. Evidence regarding EVs from microbiota as a remedial approach for IBD is comparatively scarce. More mechanisms of interaction associated with EV-based communication between the host and intestinal microenvironment need to be identified and applied in clinical therapy.

Future directions

Obviously, the highly potent modulation and therapeutic effects of EVs within the IBD intestinal microenvironment are clearly evident and well documented. Notwithstanding, there is still massive work to be done in future studies. EVs have been regarded as ideal vectors for drugs transfer and delivery, and has more prospects in

the applications of EVs. Accordingly, it is necessary to formulate standardized production, as MISEV 2018 [176], with regard to EVs modification, clinical preparation method, administration route and dosage, among other challenges. In addition, more details associated with mechanisms of EVs within the IBD need to be provided, so as to propose the possible pathogenesis related to EVs. As for the research of clinical application, future studies should try to combine with the clinical trials on the use of EVs-based diagnostic and therapeutic tool in IBD. Moreover, we believe that it is a prospective direction to modulate microbial structure or intestinal homeostasis targeting EVs-based interactive system in future studies.

Conclusion

In the current review, we mainly discussed the roles of EVs from different origins in the pathogenesis and progression of IBD, as well as their potential in clinical diagnosis and therapy. The development and study of EVs will build new understandings of cell–cell and cell–organism interactive communication and help develop novel therapeutic methods integrating safe, efficient and accurate targeting for the treatment and management of various conditions.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

CRediT authorship contribution statement

Qichen Shen: Conceptualization, Writing - original draft, Visualization. **Zhuizui Huang:** Data curation, Writing - review & editing. **Jiachen Yao:** Visualization. **Yuanxiang Jin:** Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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