

Extracellular Vesicles: The Next Generation Theranostic Nanomedicine for Inflammatory Bowel Disease

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Abstract: The recent rapid development in the field of extracellular vesicles (EVs) based nanotechnology has provided unprecedented opportunities for nanomedicine platforms. As natural nanocarriers, EVs such as exosomes, exosome-like nanoparticles and outer membrane vesicles (OMVs), have unique structure/composition/morphology characteristics, and show excellent physical and chemical/biochemical properties, making them a new generation of theranostic nanomedicine. Here, we reviewed the characteristics of EVs from the perspective of their formation and biological function in inflammatory bowel disease (IBD). Moreover, EVs can crucially participate in the interaction and communication of intestinal epithelial cells (IECs)-immune cells-gut microbiota to regulate immune response, intestinal inflammation and intestinal homeostasis. Interestingly, based on current representative examples in the field of exosomes and exosome-like nanoparticles for IBD treatment, it is shown that plant, milk, and cells-derived exosomes and exosome-like nanoparticles can exert a therapeutic effect through their components, such as proteins, nucleic acid, and lipids. Moreover, several drug loading methods and target modification of exosomes are used to improve their therapeutic capability. We also discussed the application of exosomes and exosome-like nanoparticles in the treatment of IBD. In this review, we aim to better and more clearly clarify the underlying mechanisms of the EVs in the pathogenesis of IBD, and provide directions of exosomes and exosome-like nanoparticles mediated for IBD treatment.

Keywords: extracellular vesicles, exosome, exosome-like nanoparticles, inflammatory bowel disease, theranostic

Introduction

Inflammatory bowel diseases (IBD) is a term of chronic autoimmune diseases of gastrointestinal tract, typically categorized into one of two subtypes: ulcerative colitis and Crohn's disease.¹ IBD is mainly characterized by intermittent recurrence and quiescence inflammatory remission, and it is apparently associated with increased colorectal cancer risk, high morbidity, and decreased life quality.²⁻⁴ Ulcerative colitis is confined to the colon, extend from the rectum to the cecum in a contiguous manner, shows superficial mucosal inflammation, and results in ulceration, severe bleeding, toxic megacolon, and fulminant colitis.^{5,6} In contrast, Crohn's disease affects any part of the gastrointestinal tract in a discontinuous manner, has transmural inflammation, and contributes to fibrotic stricture, fistulas, and abscesses.^{7,8}

The incidence of IBD is approximately 0.5–24.5 cases per 100,000 person-years for ulcerative colitis and 0.1–16 cases per 100,000 person-years for Crohn's disease.^{9,10} Indeed, more than 1.8 million patients in the United States and approximately 3.5 million patients worldwide are suffering from IBD.^{9,11} Unfortunately, the incidence of IBD is dramatically increased worldwide paralleled by the rise of industrial development, imposing heavy economic burdens both on their families and public healthcare.¹²

The current knowledge of IBD indicates that it is linked to the interaction of multiple factors, including environmental, genetic, intestinal microbiota, and immune response factors.^{13–16} It is known that the underlying mechanism of IBD is an inflammatory response caused by the abnormal response of the intestinal mucosal immune system, and the release of a large amount of pro-inflammatory factors.^{17,18} However, the intestinal immune system is an intricate signaling network, that can protect dietary antigens or beneficial substances, defend against harmful substances, and maintain immune homeostasis.^{19–21} The intestinal immune system consists of a single layer of epithelial cells linked by tight junctions and intercalated with immune cells. Intestinal epithelial cells (IECs) mainly act as a physical barrier and separate from gastrointestinal lumen contents. The immune cells are composed of the innate immune cells (granulocytes, macrophages, and dendritic cells (DCs)) and adaptive immune cell (B cells and T cells).¹ Cell trafficking can prevent the invasion of pathogens and maintain immune homeostasis. When the intestinal immune system is dysregulated, it elicits an abnormally intestinal inflammatory response and IBD. Although the etiology of IBD has been extensively studied, the pathogenesis of IBD is still ambiguous.

The IBD armamentarium mainly depends on non-targeted therapies, such as 5-aminosalicylates, glucocorticoid, and immunosuppressive agents, as well as targeted biologic therapies, such as anti-TNF antibodies (Infliximab (IFX), Adalimumab (ADL), Golimumab (GOLI), and Certolizumab pegol (CZP)), against the p40 subunit of IL-12 and IL-23 (Ustekinumab), and JAK signaling pathway inhibitors (Tofacitinib).^{22,23} Despite the beneficial effects of these medications, up to 30% of patients do not have a response to initial treatment, and in up to 50% of patients, the response is lost over time.²² Moreover, their side effects can not be ignored, such as allergic reactions and toxicities to healthy organs. Therefore, it is urgent to develop new medications with preferable therapeutic efficacy and limited side effects.

Recently, studies have reported that the extracellular vesicles (EVs), including exosomes, outer membrane vesicles (OMVs), and plant-derived exosome-like nanoparticles (PDENs), released by different types of cells in gastrointestinal tract, gastrointestinal microbiota, and edible plants, play an essential role in the pathogenesis and treatment of IBD.^{24–26} EVs have been shown to form a novel model of cell trafficking, implicating in cellular signaling to regulate biological processes, such as immunomodulation and regeneration.^{27–29} Moreover, it has been demonstrated that EVs are critical actors in intercellular communication either at a paracrine level or at a distance. In addition, their cargoes, including lipids, proteins, and RNA, play critical roles in immune system modulation, and possess the ideal delivery system in the treatment of IBD.^{30–33} Compared with synthetic drug carriers, exosomes and exosome-like nanoparticles originating from their own cells or diets, such as milk, edible vegetables, and fruits, have considerable advantages of biocompatibility, immune tolerance, and non-toxicity.^{34–36} Therefore, exosomes and exosome-like nanoparticle-mediated delivery systems have gained considerable attention in treating IBD.

In view of the vital role of EVs in IBD, herein we comprehensively reviewed recent advances in exosomes, PDENs, and OMVs in the pathophysiology of IBD, summarized exosomes or exosome-like nanoparticle-mediated delivery system in the treatment of IBD, and predicted future directions for research efforts of IBD.

Biogenesis, Composition, Uptake and General Functions of Exosomes

Exosomes are bilayer vesicular nanoparticles with heterogeneity and nano-size (30–150 nm), representing a component of a broader class of EVs released by cells due to environmental stimulation or self-activation (Figure 1).^{37–39} In the beginning, exosomes are simply regarded as inconsequential “garbage” via a process of the cells eliminating unwanted cellular components into the extracellular environment.⁴⁰ However, more recent studies have found that exosomes play important roles in the physiological and pathological processes, as well as essential mediators of cell-cell communication.^{40–42} In this section, we briefly discussed the characterization of exosomes.

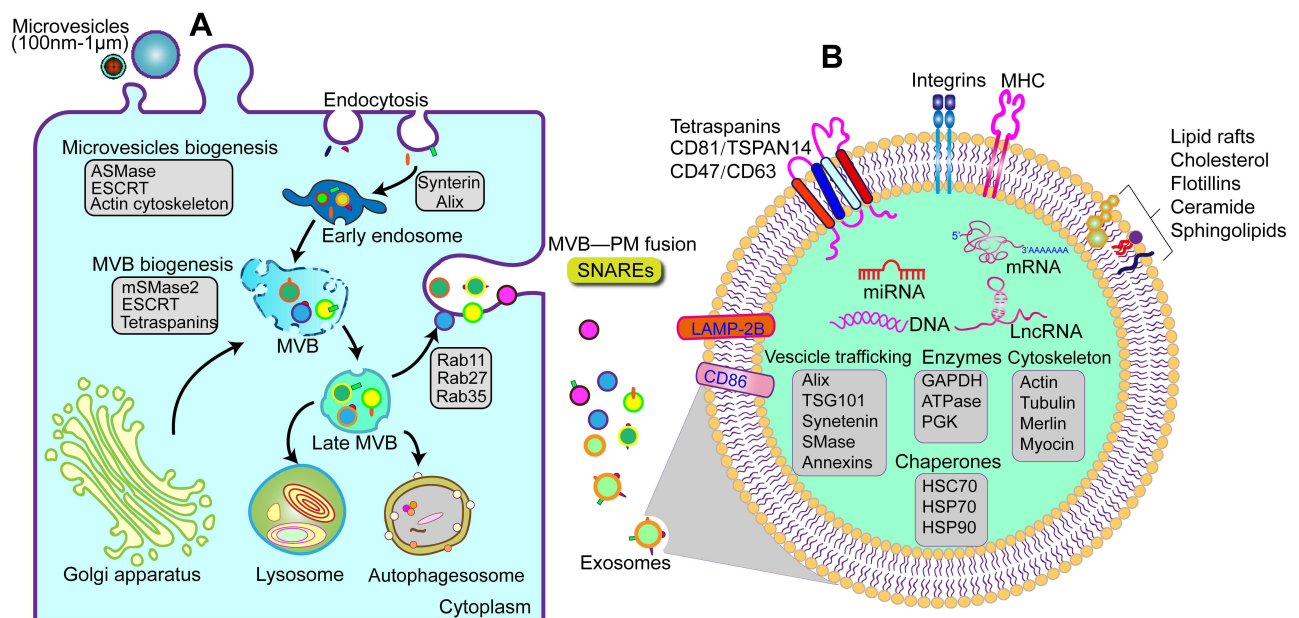


Figure 1 Biogenesis and composition of extracellular vesicles. **(A)** Exosomes are formed by the inward budding of endosomes into multivesicular bodies (MVBs) then formation of intraluminal vesicles (ILVs). MVB either fuses directly with lysosomes, or fuses with autophagosomes during autophagy, or which can secrete into the extracellular space as exosomes through fusion with cellular plasma membrane. RAB family members, such as Rab11, Rab27 and Rab35, have vital role in the exosome secretion through regulating MVBs trafficking and docking at the plasma membrane. In addition, soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein can induce the exosome secretion by recruiting MVBs docking at the plasma membrane and initiating membrane fusion. **(B)** The contents of released exosomes include proteins, DNA, mRNA, and miRNA.

The Biogenesis of Exosomes

Exosomes are derived from endosomal structures that take place via an endocytosis of invaginated endosomes from the plasma membrane⁴³ (Figure 1). Moreover, the formation and release of exosomes depend on an endosomal sorting complex required for transport (ESCRT)-dependent and ESCRT-independent machinery⁴⁴ (Figure 1). RAB family members, such as Rab11, Rab27 and Rab35, play a vital role in the exosome secretion through regulating multivesicular bodies (MVBs) trafficking and docking at the plasma membrane.⁴⁵ In addition, soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein can induce the exosome secretion by recruiting MVBs docking at the plasma membrane and initiating membrane fusion.⁴⁶ However, the processes underlying exosome biogenesis are intricate, and still not fully understood.^{43,47}

The Composition of Exosomes

Exosomes have been regarded as an evolutionarily conserved set of particles from parental cells, and are composed of various components, mainly including proteins, lipids, and nucleic acids^{43,48} (Figure 1). To date, a large variety of constitutive components have been identified in exosomes from different types of cells, and approximately 9769 proteins, 1116 lipids, and 6246 nucleic acids are obtained in the ExoCarta exosomes database (<http://www.exocarta.org>), indicating their complexity and potential functional diversities. However, the cargoes of exosomes are not completely unraveled, which needs to be understood in further studies.

The Uptake and Function of Exosomes

Exosome uptake may involve three mechanisms: interacting receptor-ligand, fusing with the plasma membrane of the target cell, and getting internalized by target cells.^{49–51} Scientists have recently discovered that exosomes play a crucial role in cell differentiation, programmed cell death, disease development and recovery, immune response, and inflammation by transporting cargoes of proteins, nucleic acids, and lipids (Figure 1).^{52–54} In brief, exosomes can communicate with target cells by the above-mentioned mechanisms, fuse with the plasma membrane, and release bioactive cargoes directly into the cytoplasm, leading to activation of the relevant signaling pathway and regulation of the specific gene

expression. Nevertheless, the underlying mechanisms of exosomes communicating with target cells remain largely undetermined.

Advantages of Exosomes as Drug Delivery Systems

Exosomes are continuously released into our bodily fluids as part of an intercellular communication network. They are, therefore, attracting increasing attention to improve the treatment of human diseases that are difficult to cure using conventional medicines or methods.^{55,56} The advantages of exosome-based drug delivery systems are largely realized: (1) The exosome membrane consists of two compartments, an aqueous core and a lipophilic shell formed by a lipid bilayer, making it possible to compartmentalize and solubilize both hydrophilic and hydrophobic materials.^{40,55} (2) Exosomes are coated by various cell membranes from our own body's cells, such as red blood cells (RBCs), platelets, white blood cells (WBCs) or cancer cells, and have displayed good biocompatibility, prolonged circulation, and tissue-targeting capacity.⁵⁷ (3) They are small enough and capable of crossing physiological barriers, such as the gastrointestinal barrier and blood-brain barrier, thus making it possible for targeted drug deliver.⁵⁶ Currently, a lot of exosome-based drug delivery systems have been completed in early clinical trials, most of which are concentrated in the field of cancer, inflammation, and autoimmunity disease.^{58–60} Nonetheless, the exosome-based drug delivery systems are challenged their widespread application, such as undetermined toxicity, undefined component, and commercial pharmaceutical productions.^{61–63}

Sources of Exosomes

The exosomes are ubiquitously present.⁶⁴ Therefore, this section will start with a brief description of the sources of exosomes involved in the pathogenesis and treatment of IBD.

Mesenchymal Stem Cell (MSCs)-Derived Exosomes

MSCs have unique biomedical properties due to their stemness, including differentiation, self-renewal, and colony formation. Moreover, MSCs are multipotent progenitor cells that can be isolated from bone marrow, umbilical cord tissue, amniotic fluid, adipose tissue, and dental pulp.^{65,66} Besides, MSCs express low levels of major histocompatibility complex (MHC) class I molecules and MHC class II molecules, which empower them to have low immunogenicity.⁶⁷ Importantly, MSCs have diverse functions, such as anti-inflammatory, anti-apoptotic, and neuroprotective actions, when producing and releasing a broad range of bioactive molecules (Figure 2).^{68,69,70,71} In addition, MSCs are amongst the largest cellular procedures of exosomes, which have amounts of cargoes, including 850 gene products and 150 miRNAs described, and play a vital role in the regulation of inflammation, metabolic disorders, and cell damages.^{72–74} Accumulating evidence has demonstrated that MSC-derived exosomes are considered to be a potential option for the treatment of human disease, such as Alzheimer's disease (AD), osteoarthritis, and autoimmune disease.^{75–77} Although MSC-derived exosomes show increasing potential for human disease, there is still a lack of evidence. Further research should focus on their functions and sorting mechanisms. Moreover, future clinical trials should be performed to verify the efficacy and safety of MSC-derived exosomes and establish a comprehensive theoretical basis for the clinical implementation.

Plant-Derived Exosome-Like Nanoparticles (PDENs)

Despite a growing appreciation of the importance of exosomes in mammals, PDENs are detected earlier than mammal cell-derived exosomes.⁷⁸ Exosome-like nanoparticles of plant cells can be released through multiple pathways. For example, the fusion of MVBs with the plasma membrane (PM) can release intraluminal vesicles (ILVs) as exosomes; the vacuole fuses with the PM as a vacuolar pathway to release exosomes; and exocyst-positive organelle (EXPO) can also direct release exosomes (Figure 3). PDENs are mostly isolated from edible plants, such as ginger, broccoli, Citrus sinensis, lemon, and grapefruit (Figure 3).^{79–83} Recent evidence has demonstrated that PDENs can be delivered to other organs through blood flow and function distantly in the recipient cells. Moreover, PDENs have gained huge attention due to their potential to regulate physiological and pathological processes, and develop therapeutic vehicles.^{84,85} Although clinical trials are performed to investigate the efficacy and safety of PDENs for the treatment of human diseases, the number of studies is limited (such as NCT01294072, NCT01668849, and NCT03493984). Of note, there is no standard

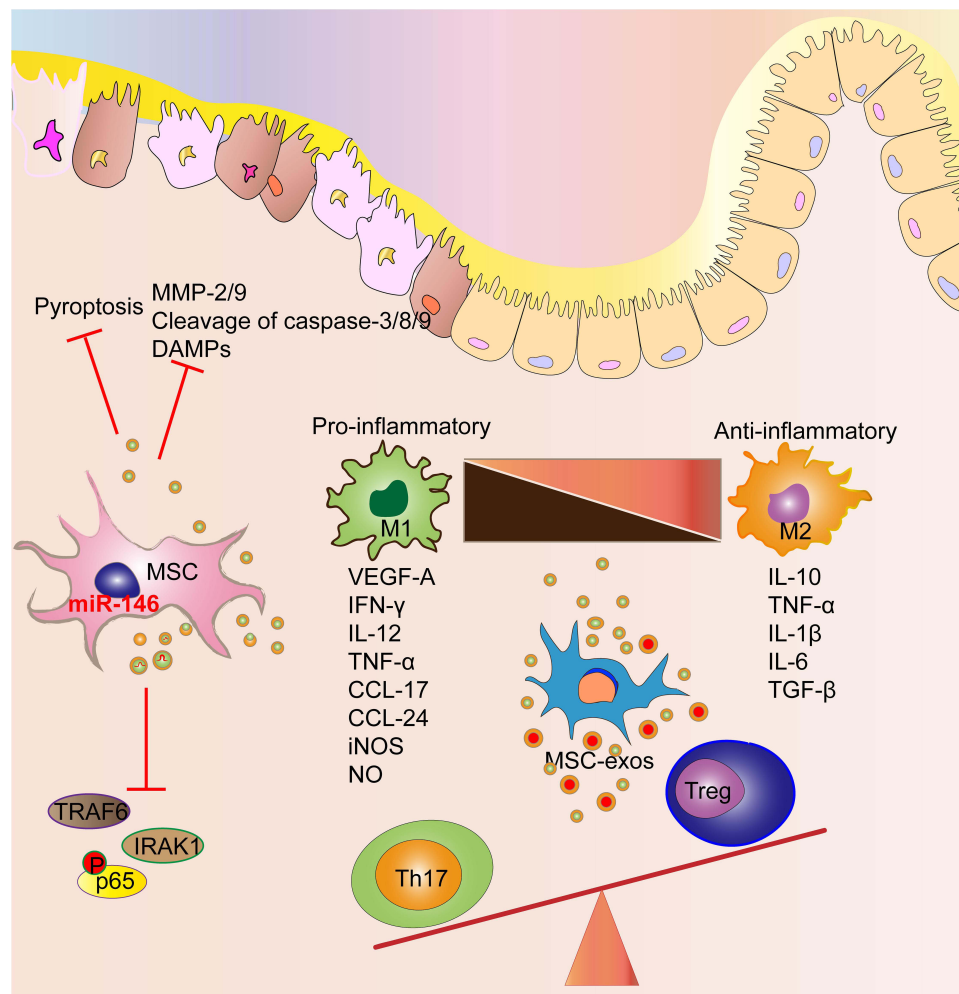


Figure 2 MSCs-Exo regulate the intestinal barrier, immune response and inflammatory responses, which provide an effective therapeutic strategy for IBD.

method for isolation, purification, and production of PDENs in clinical practice. Therefore, further research should formulate the protocols for isolating PDENs and perform significant clinical trials.

Milk-Derived Exosomes

Milk is a natural drink, has a large content of protein, fat, vitamins, and other nutrients, and can be used to supplement nutrition in daily life.^{86,87} Meanwhile, milk also plays an essential role in organism development and immune response.⁸⁸ Milk-derived exosomes are vital components of milk, released from mammary gland epithelial cells of all mammals including human and dairy cows.^{89,90} Accumulating evidence indicates that milk-derived exosomes play a crucial role in the appropriate maturation of the intestine and development of the gut microbiome, while deficiency of milk-derived exosomes contributes to metabolic and immunological disease in the newborn infants.^{91–93} Moreover, increasing studies show that milk-derived exosomes, as drug delivery systems, are conducted for the treatments of various diseases, including IBD.^{94,95} The details of milk-derived exosomes in the treatment of IBD will be discussed in the section about the therapeutic effects of exosomes on IBD.

Immune Cell-Derived Exosomes

The immune cells consist of innate cells (including granulocytes, macrophages, and DCs) and adaptive immune cells (including B cells and T cells).⁹⁶ Accumulating evidence has implicated that the immune cells secrete exosomes, and then engage in the transfer of information.⁹⁷ A great deal of attention has been paid to the role of immune cell-derived

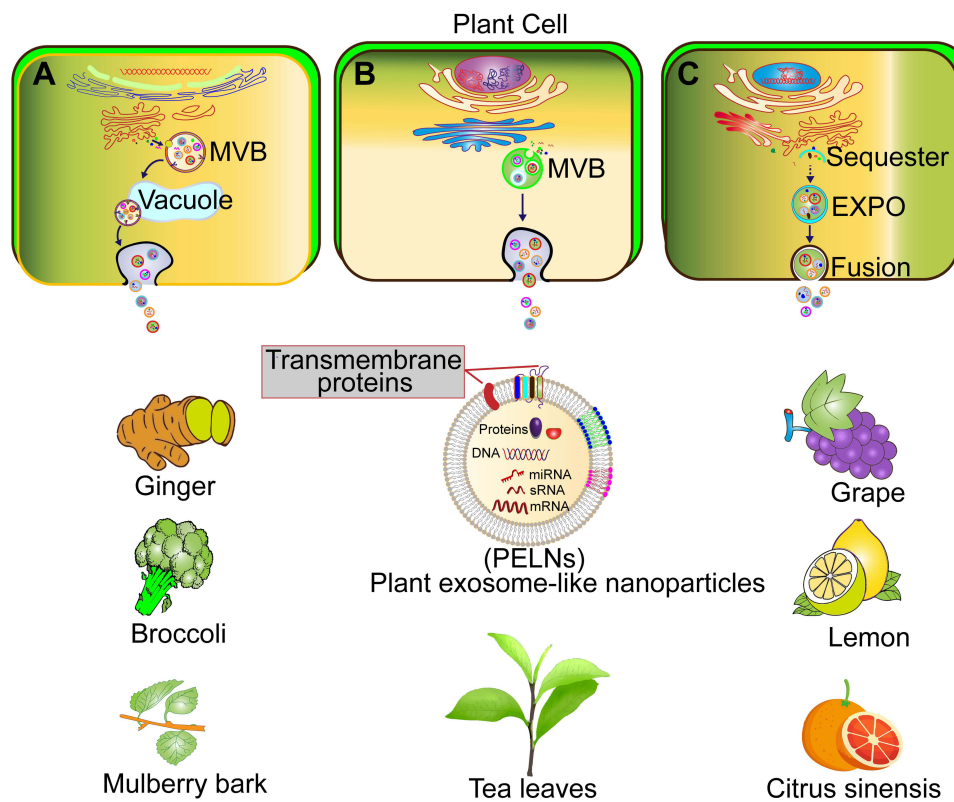


Figure 3 Biogenesis of plant-derived exosome-like nanoparticles and therapeutics application for IBD. (A) Vacuolar pathway; (B) MVBs pathway; (C) Exocytosis pathway by exocyst-positive organelle (EXPO).

exosomes both in healthy physiological and pathological processes.^{97–99} For example, B cell-derived exosomes bind with MHC class II, and induce antigen-specific T cell responses.¹⁰⁰ DC-derived exosomes can trigger regulatory T cells (Treg) activation while inhibiting T helper type 1 (Th1) cells, which modulate immune responses in IBD (Figure 4).³¹ The function of immune cell-derived exosomes in the IBD will be discussed in the section on exosomes and IBD.

Exosomes in Body Fluids

Exosomes have been isolated from various physiological fluids, such as blood,¹⁰¹ urine,¹⁰² saliva,¹⁰³ amniotic fluid,¹⁰⁴ and breast milk.¹⁰⁵ Recently, exosomes can be used as biomarkers and indicators in diagnosing diseases, including IBD, due to their extensive existence in the above-mentioned biological fluids.^{30,106–108} Shao et al have reported that a serum exosome, pregnancy zone protein (PZP), is up-regulated in the IBD patients, and can be regarded as a promising biomarker of serological detection for IBD diagnosis.¹⁰⁸ Zhang et al have indicated that a salivary exosome, proteasome subunit alpha type 7 (PSMA7), is present at high levels in IBD patients and may be a promising indicator for IBD diagnosis.¹⁰⁹ Unfortunately, there are not many studies of body fluid-derived exosomes until now. Therefore, additional research on the body fluid-derived exosomes in IBD patients will be beneficial for a better understanding.

EVs in the Pathogenesis of IBD

EVs play crucial roles in the pathogenesis of IBD. Their cargoes not only facilitate or alleviate the pathogenesis of IBD, but also can be used as diagnostic and therapeutic markers.^{41,108–110} In this section, we will discuss the EVs derived from IECs, immune cells, and gut microbiota function in the pathogenesis of IBD.

Intestinal Epithelial Cells (IECs)-Derived Exosomes in IBD

IECs release exosomes to communicate with host immune cells and gut microbiota, and help maintain a homeostatic environment in the gut. The target and function of IECs-derived exosomes are to stimulate DCs, regulatory T cells (Treg),

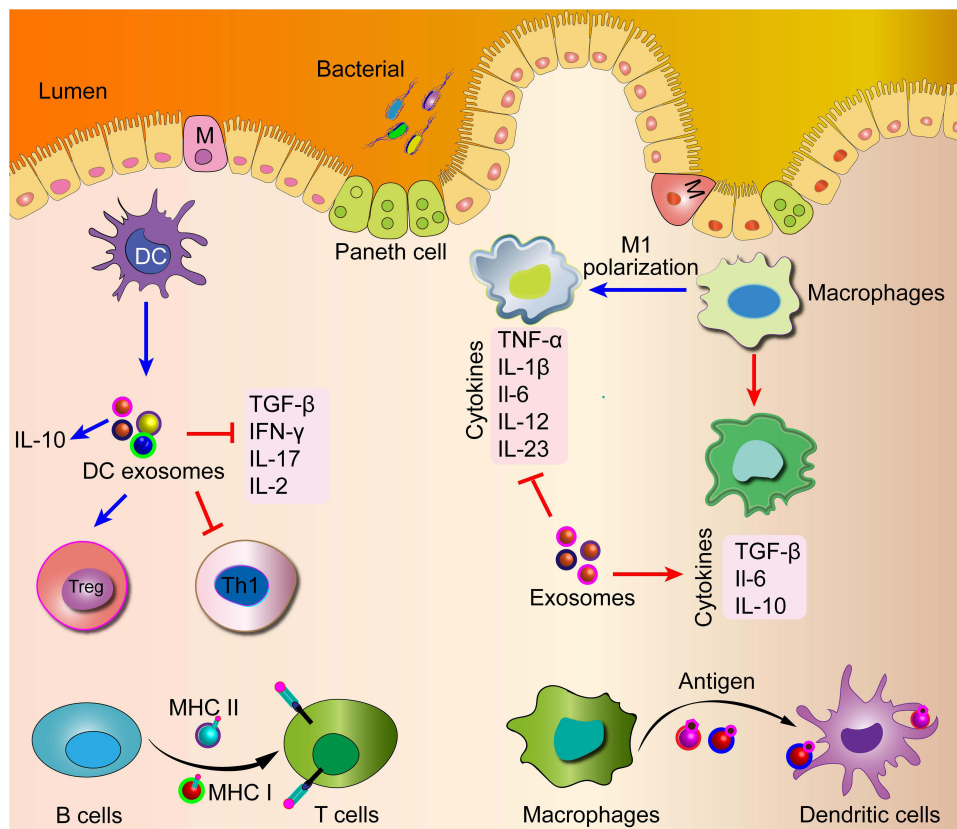


Figure 4 Exosomes derived from immune cells can modulate immune responses in the pathogenesis of IBD and also have been used in vaccine and drug delivery research.

and macrophage maturation with tolerogenic properties by immunoregulatory pathways, and maintain intestinal homeostasis (Figure 5).^{24,25,31} For instance, an IECs-derived exosome, TGF- β 1, can alleviate colonic inflammation in experimental mouse colitis through stimulating immunosuppressive DCs and Treg cells.¹¹¹ Interestingly, an IEC-derived exosome, $\alpha\beta$ 6, activates LTGF β in intestinal tolerogenic DCs, and Treg cells, and produces TGF- β 1.¹¹² In addition, an IEC-derived exosome, ANXA1, can activate the wound repair circuit, and promote the repair of intestinal mucosal wound in experimental mouse colitis.¹¹³ Although IECs are not primarily antigen-presenting cells (APCs), the IEC-derived exosomes can participate in antigen presentation within the intestinal mucosa and immunoregulatory process due to their expression of major histocompatibility complex I (MHC-1), MHC-II, and human leukocyte antigen-DM (HLA-DM).¹¹⁴ For example, MHC-II can activate B cell development, consolidate B cell maturation, and initiate adaptive immune.¹¹⁵

In the pathogenesis of IBD, an IECs-derived exosome, epithelial cell adhesion molecular (EpCAM), is greatly elevated, induces DC apoptosis, suppresses DC maturation, and inhibits the functions of DCs (Figure 5).¹¹⁶ IEC-derived exosomes, such as GSDMD, IL-1 β , and caspase-8, induce IECs pyroptosis, produce pro-inflammatory cytokines, and promote intestinal inflammation in experimental colitis model.¹¹⁷ However, myeloperoxidase (MPO), derived from IEC exosomes in IBD patients, as a defense protein, can induce the oxidation reaction by producing reactive oxidants.¹¹⁸ Exosomes isolated from IECs infected with adherent-invasive *Escherichia coli* can activate nuclear factor- κ B (NF- κ B) and MAPK pathways in naïve macrophages, resulting in an increased secretion of pro-inflammatory cytokines IL-6 and TNF- α .¹¹⁹ Of note, the important role of IEC-derived exosomes is to regulate the pro- and anti-inflammatory immune balance. Once the balance is disrupted in the intestinal immune system, it will lead to intestinal inflammation. Therefore, they may be promising targets for the treatment of IBD.

Immune Cells-Derived Exosomes in IBD

Accumulating studies have indicated that both the innate and adaptive immune responses participate greatly in the IBD pathogenesis.^{120,121} The innate immune response is quicker to trigger the phagocytic responses and antigen presentation,

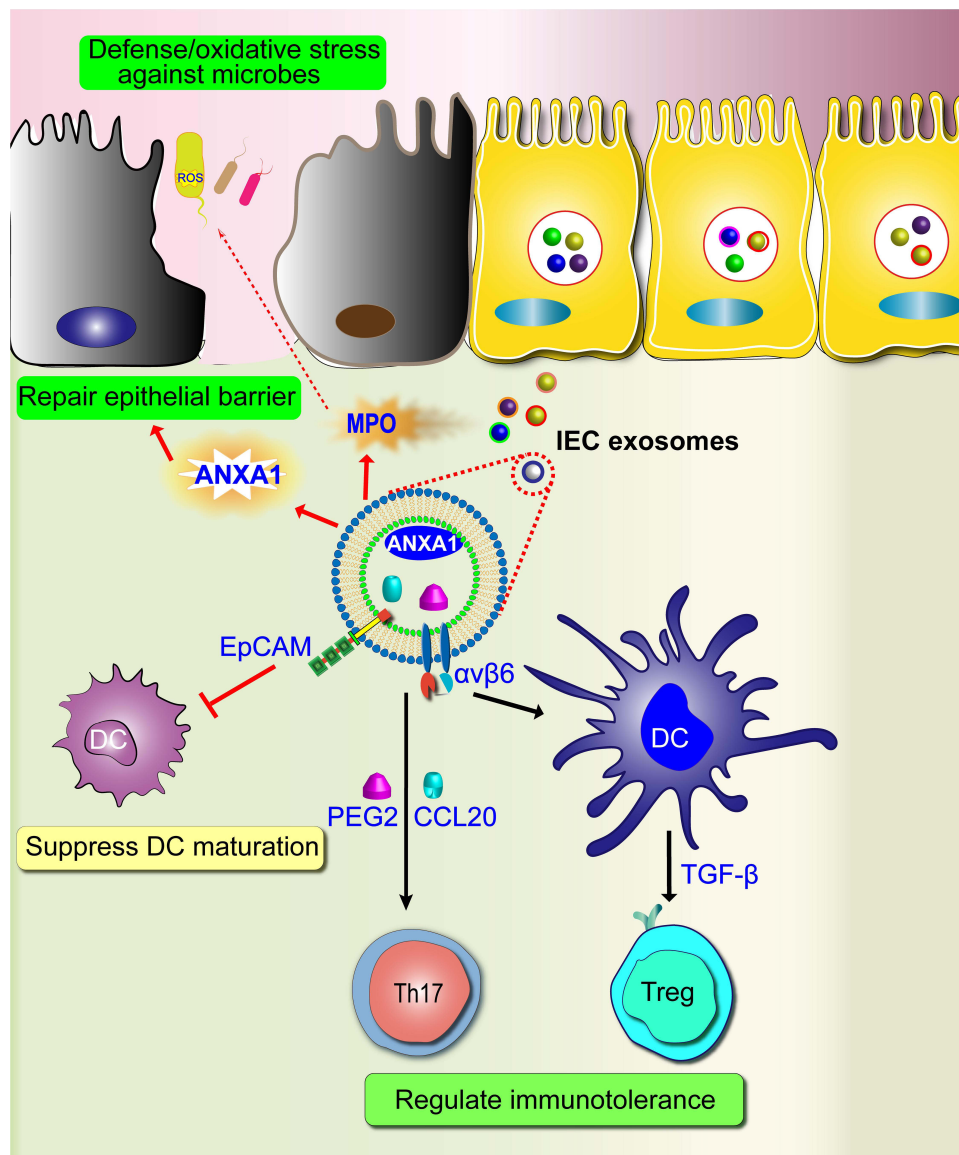


Figure 5 Function of IECs-derived exosomes in IBD. IECs-derived exosomes can promote antigen presentation, increases intestinal immunotolerance, repair epithelia barrier.

accompanied by the initiation of the adaptive immune system.¹²¹ This pertains to most immune cells, such as macrophages, DCs, neutrophils, and monocytes (Figure 4).¹²¹

Macrophages play a pivotal role in the maintenance of intestinal homeostasis and the pathogenesis of IBD (Figure 4).^{122–124} Macrophages are classified into two subsets with seeming different functions: M1 macrophages secrete higher levels of pro-inflammatory cytokines including TNF- α , IL-1 α , IL-12, and IL-6. On the contrary, M2 macrophages produce higher levels of anti-inflammatory cytokine of IL-10.^{122–124} Macrophage-derived exosomes are involved in the pathogenesis of IBD. An M1 macrophages-derived exosome, miR-21a-5p, can suppress the expression of E-cadherin, then promote ILC2 activation, and result in the destruction of the intestinal mucosal epithelium in experimental mouse colitis.¹²⁵ Yang et al have reported that M2 macrophage-derived exosomes can suppress the expressions of pro-inflammatory cytokines including IL-1 β , IL-6, and IL-17A, and exert protective effects on DSS-induced colitis.¹²⁶ Moreover, an M2 macrophage-derived exosome, miR-590-3p, can inhibit the secretion of pro-inflammatory cytokines, such as IL-1 β , IL-6, and TNF- α , promote epithelial wound repair, and attenuate DSS-induced colitis.¹²⁷ In addition, MSC-derived exosomes can attenuate colitis by suppressing the release of colon M1 macrophages and pro-inflammatory

cytokines, and promoting the polarization of M2 macrophages.¹²⁸ Therefore, the balance of M1/M2 macrophages may be an effective treatment strategy for IBD.

DCs are the most effective and professional APCs of the colonic immune system, which can capture antigens, and initiate an immune response, and DCs-derived exosomes may possess immune-stimulatory or suppressive effects (Figure 4).¹²⁴ DC-derived exosomes, CD80 and CD86, as costimulatory markers, can trigger naïve CD4⁺ T activation, thus amplifying the initiation of primary adaptive immune responses.¹²⁹ Moreover, a DC-derived exosome, TGF-β1, possesses strong immunosuppressive activity by inducing Treg cells in IBD mouse model.^{130,131} In addition, DC-derived exosomes treated with IL-10 can attenuate 2,4,6-trinitrobenesulfonic acid (TNBS)-induced mouse colitis by inhibiting the expressions of TNF-α, IL-2, and IFN-γ and promoting the expressions of IL-10 and Treg cells.¹³² It is conceivable that DC-derived may be a promising target for the therapeutic intervention of IBD.

Other immune cell-derived exosomes can also influence IBD progression in different ways (Figure 4). Neutrophil-derived exosomes can contribute to the decrease of epithelial cadherins and promote neutrophil recruitment in colitis.¹³³ Treg cell-derived exosomes can promote IEC proliferation, inhibit IEC apoptosis, and protect the intestinal barrier in IBD mouse model.^{134,135} Granulocytic myeloid-derived exosomes can alleviate IBD by diminishing the proportion of Th1 cells, and promoting the expansion of Treg cells.¹³⁶ These immune cell-derived exosomes may be the new therapeutic intervention for IBD in the future.

Gut Microbiota-Derived OMVs in IBD

Gut microbiota-derived EVs also play a crucial role in the immunomodulation and intestinal barrier integrity of IBD.^{137,138} The gut microbiota commonly includes bacteria, fungi, and viruses.¹³⁹ In the present review, we will mainly focus on the bacterial extracellular vesicles (BEVs) in the IBD. Typical Gram-negative bacteria are released when the outer membrane lipid asymmetry leads to blebbing, resulting in the production of BEVs known as outer membrane vesicles (OMVs). BEVs are rich in various outer membrane proteins, such as DNA, RNA, virulence factors, membrane-bound proteins and polysaccharides, and peptidoglycans (Figure 6). Under physiological conditions, the gut microbiota interacts with the host, protects the intestinal barrier, and maintains intestinal immune homeostasis. For instance, *Lactobacillus rhamnosus* GG-derived EVs can protect the colonic tissue damage and alleviate intestinal inflammation via inhibiting the activation of TLR4-NF-κB-NLRP3 axis.¹⁴⁰ *Akkermansia muciniphila*-derived EVs can have a protective function on colitis, and ameliorate intestinal inflammation in the DSS-induced colitis model.¹⁴¹ However, the microbial diversity is prominently changed in the pathogenesis of IBD, indicating that the proportion of bacteria with anti-inflammatory properties is lower, whereas the proportion of bacteria with pro-inflammatory properties is relatively higher.¹⁴² Subsequently, alterations in the composition of intestinal bacteria can influence intestinal homeostasis and affect the intestinal barrier by these bacteria-derived exosomes mediating cross-talk between microbiota, intestinal epithelia, intestinal immune cells, and mucosal immunity. For example, EVs are derived from *Fusobacterium nucleatum* (Fn), a Gram-negative specific anaerobe, that can aggravate intestinal barrier disruption by promoting the differentiation of pro-inflammatory macrophages and accelerating IEC necroptosis.¹⁴³ *Escherichia coli* (E. coli)-derived OMVs, peptidoglycan, can increase the expressions of pro-inflammatory cytokines including NF-κB, IL-6, and IL-8, via activating nucleotide-binding oligomerization domain containing 1 (NOD1) signaling pathway in IECs.¹⁴⁴ Notably, exploring the function of probiotics and pathogenic bacteria, and balancing probiotics and pathogenic bacteria will be helpful in clarifying the relationship between gut microbiota and IBD, and identifying new therapeutic targets for IBD patients.

Therapeutic Effects of EVs on IBD

It is clear that EVs are involved in intestinal intercellular communication and the pathogenesis of IBD suggesting that EVs can be promising therapeutic options for IBD. The interest in EV research also propels the development of artificial nanoparticles, exosome-like nanoparticles, which are employed in the treatment of IBD. The application of IECs, intestinal immune cells, gut microbiota-derived exosomes, and OMVs in the treatment of IBD has been discussed in EVs in the Pathogenesis of IBD. Therefore, in this section, we will discuss MSC-, plant-, and milk-derived exosomes and exosome-like nanoparticles in the treatment of IBD.

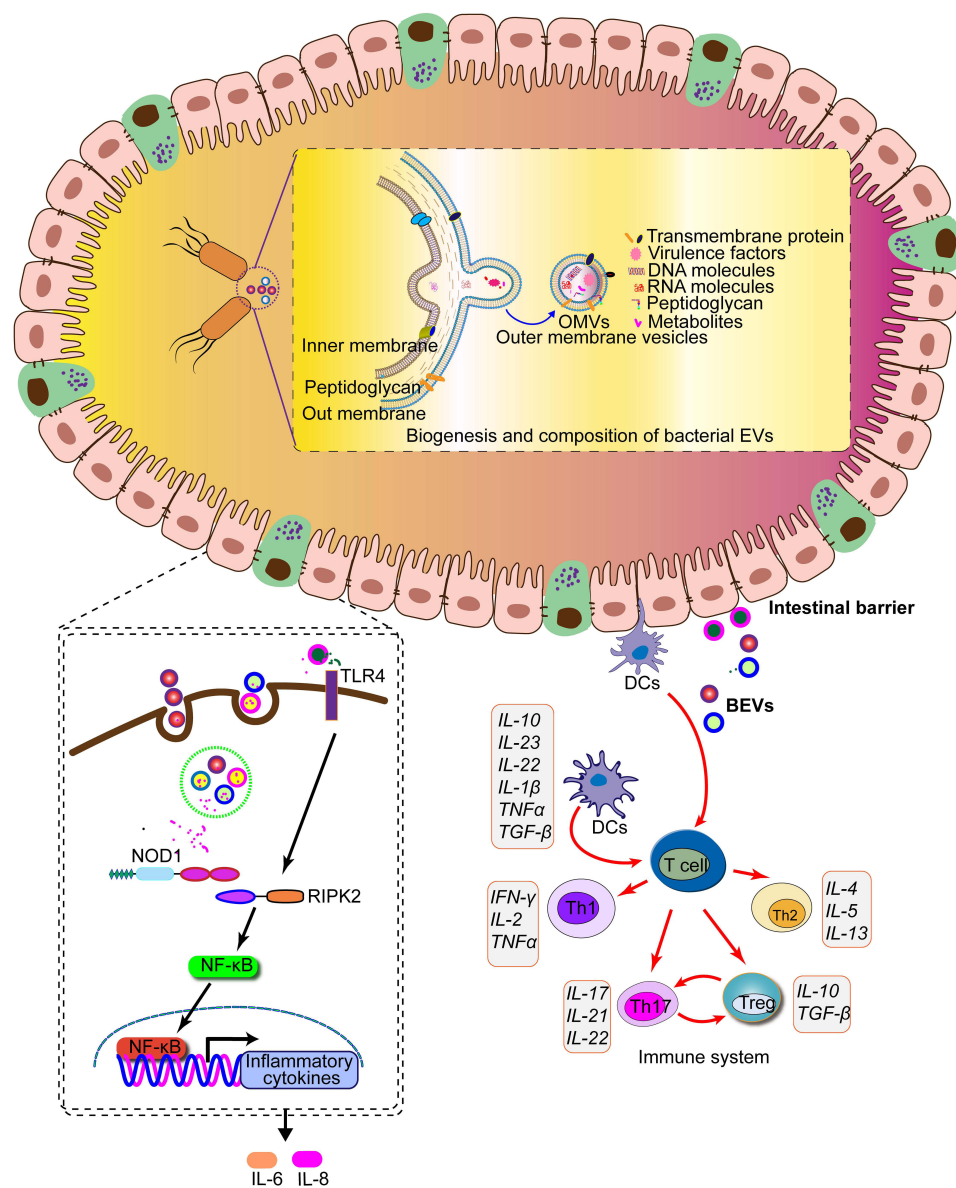


Figure 6 Schematic illustrate origin and composition of bacterial extracellular vesicles. The immunomodulatory effects and Inflammatory pathway network elicited by microbiota-derived BEVs in the gut were summarized.

Methods for Loading Drugs into EVs

Efficient loading of therapeutic cargo packaging into exosomes and exosome-like nanoparticles is a very critical step.^{145,146} Currently, different methods are commonly utilized for loading biological cargoes into exosomes and exosome-like nanoparticles including co-incubation, electroporation, and sonication (Table 1).¹⁴⁷ Co-incubation is a passive cargo loading technique, and the drugs move through a concentration gradient into the extracted exosomes and exosome-like nanoparticles.¹⁴⁸ In addition to co-incubation, the electroporation method uses an electric field to form temporary hydrophilic pores on the phospholipid membrane of the exosomes and exosome-like nanoparticles to load the biological cargoes.¹⁴⁹ Besides, sonication is an alternative method of loading drug into exosomes and exosome-like nanoparticles through drugs adhering to the surface of exosomes and exosome-like nanoparticles.^{94,150} However, there is a lack of horizontal comparison of the advantages and disadvantages of these loading methods in the current research.

Table 1 Exosome Cargo Loading Method

Method	Cargo	Advantage	Disadvantage
Passive incubation	Small molecules drugs	Convenient	Low loading efficiency, limited to hydrophobic drug
Transfection	MiRNAs and siRNAs	Load large cargos	Need further purification, need transfection kit
Electroporation	MiRNAs, siRNAs, mRNA, DNAs, and proteins	Widely used and relatively easy to use	Aggregates, and low substantial retention rate
Sonication	MiRNAs, siRNAs and small molecules drugs	High loading capacity	Restricted to the loading of smaller non-biologic molecules
Freeze-thaw	MiRNAs, siRNAs and small molecules drugs	Convenient	May destroy the stability; low loading efficiency,
Dialysis	Small molecules drugs	High drug loading efficiency	May changes in size and charge
Saponin	MiRNAs, siRNAs, small molecules drugs, and proteins	Promote drug loading, and increase cellular uptake	Toxicity; destroy membrane integrity
Cholesterol-conjugated	ASO	Simply	Cargo may be degraded
Parental cells expression	TGF- β 1 and IL-10	Simply	Productivity of RNA is unstable
Parental cells incubation and extrusion	Small molecules drugs, miRNAs and siRNAs	Relatively simple	Low loading capacity

Abbreviations: ASO, antisense oligonucleotide; TGF- β 1, Transforming Growth Factor- β 1; IL-10, Interleukin 10.

Therapeutic Effects of MSC-Derived Exosomes Against IBD

Currently, MSC-derived exosomes have exhibited great potential in the treatment of IBD (Table 2). For example, human umbilical cord-derived MSC (HucMSC)-derived exosomes can promote the expressions of anti-inflammatory cytokine, such as IL-10, inhibit the levels of pro-inflammatory cytokines, including TNF- α , IL-1 β , and IL-6, and alleviate DSS-induced colitis.¹⁵¹ Moreover, human bone marrow MSC (hBM-MSC)-derived exosomes can upregulate the expression of anti-inflammatory factors, such as IL-10, IL-10, and TGF- β , decline the levels of pro-inflammatory factors, VEGF-A, IFN- γ , IL-12, TNF- α , CCL-24, and CCL-17, and ameliorate DSS-induced colitis by promoting the polarization of M2

Table 2 MSCs-Derived Exosomes for IBD Therapy

Classification of Exosomes	Non-Coding RNAs	Targets	Function	Ref.
HucMSCs-derived exosomes	miR-326	NF- κ B signaling pathway, neddylation-related enzymes	Inhibiting the neddylation and alleviating colitis	[29]
HucMSCs-derived exosomes	miR-378a-5p	NLRP3, IL-1 β , IL-18, and Caspase-1	Attenuating colitis by regulating macrophage pyroptosis	[151]
HucMSCs-derived exosomes	—	TSG-6	Restoring mucosal barrier repair and intestinal immune homeostasis	[156]
ADSC-derived exosomes	miR-132	Smad-7 and TGF- β /Smad signaling	Promoting VEGF-C-dependent lymphangiogenesis	[153]
HucMSC-derive exosomes	miR-378a-5p	NLRP3 axis	Regulating macrophage pyroptosis and protecting against DSS-induced colitis.	[157]
ADSC-derived exosomes	—	-	Improve inflammatory responses	[158]
hbMSCs-derived exosomes	—	Induction of IL-10	Improving mucosal inflammatory responses and maintaining intestinal barrier integrity	[152]
hucMSC-derived exosomes	miR-146a	SUMO1	Preventing colitis	[29]

Abbreviations: hP-MSCs, human placental mesenchymal stem cells; HMCs, human umbilical cord mesenchymal stem cells; hucMSCs, Human umbilical cord mesenchymal stem cells; ADSC, adipose-derived mesenchymal stem cells; hbMSCs, human bone marrow mesenchymal stem cells.

macrophages.¹⁵² In addition, human adipose MSC (hADSC)-derived exosomes can protect the intestinal barrier integrity, promote the IEC proliferation, and resist the intestinal inflammatory injury in DSS-induced colitis.¹⁵³ Moreover, MicroRNA-146, is a well-known anti-inflammatory miRNA, and exosomes obtained from MSCs overexpressing miR-146, can regulate NF- κ B p65 phosphorylation and inhibit the expressions of TNF receptor-associated factor 6 (TRAF6) and IL-1 receptor-associated kinase 1 (IRAK1), thereby inhibiting the release of inflammatory factors in macrophages, and reducing colonic inflammation²⁹ (Figure 2). Collectively, MSC-derived exosomes can protect the colitis mice model by restoring mucosal barrier repair and maintaining intestinal immune homeostasis.^{154–157} Excitedly, a randomized, double-blind controlled trial has demonstrated that MSCs-derived exosomes are an effective and safe option to treat complex perianal fistulas in patients with Crohn's disease who do not respond to conventional or biological treatments, or both.¹⁵⁸ Subsequently, MSC-derived exosomes are considered an effective therapeutic approach for IBD patients in clinical practice.^{159,160} Therefore, MSC-derived exosomes may provide an effective therapeutic strategy for IBD treatment.

Therapeutic Activity of PEDNs Against IBD

The edible PEDNs can also relieve intestinal inflammation and maintain intestinal immune homeostasis (Table 3). For instance, oral administration of tea leaf-derived exosome-like nanoparticles can decrease the levels of pro-inflammatory cytokines (TNF- α , IL-6, and IL-12), increase the amount of anti-inflammatory cytokine IL-10, restore disrupted intestinal barriers, and alleviate experimental mouse colitis.³⁴ Moreover, grape exosome-like nanoparticles can mediate impaired intestinal tissue remodeling, and protect against DSS-induced colitis by strongly promoting the proliferation of Lgr5^{hi} intestinal stem cells.¹⁶¹ In addition, broccoli-derived exosome-like nanoparticles can not only inhibit activation of intestinal DCs, but also induce tolerogenic DCs via activating the AMPK signaling pathway, contributing to prevent DSS-induced colitis in mice.⁸⁰ Furthermore, Zhang et al have discovered that oral administration of ginger-derived exosome-like nanoparticles can reduce the susceptibility of mice to DSS-induced colitis by increasing the survival and proliferation of IECs and promoting pro-healing factors including interleukin (IL)-10 and IL-22.¹⁶² Recently, Sriwastva et al have found that mulberry bark-derived exosome-nanoparticles can activate aryl hydrocarbon receptor (AhR) signaling and increase the amount of anti-microbial peptides (AMPs) in IECs, inhibiting intestinal inflammation, and altering gut microbiota composition in experimental mouse colitis.¹⁶³ Additionally, oral administration of turmeric-derived exosome-like nanovesicles can ameliorate mouse colitis and accelerate colitis resolution via decreasing the expressions of pro-inflammatory cytokines, including TNF- α , IL-6, and IL-1 β , and increasing the levels of the antioxidant gene, heme oxygenase-1 (HO-1) in mice colitis models.¹⁶⁴ Unfortunately, clinical trials are limited to the efficacy and safety of PEDNs for IBD patients.

Table 3 Features of Plant-Derived Exosome-Like Nanoparticles Regulate Intestinal Microenvironment

Source	Exosome Features	Mechanisms	Ref.
Ginger	~125 miRNAs, ginger bioactive constituents (6-gingerol and 6-shogaol).	Promoting proliferation of IECs, reducing the pro-inflammatory cytokines (TNF- α , IL-6 and IL-1 β), and increasing the anti-inflammatory cytokines (IL-10 and IL-22)	[85]
Mulberry bark	HSPA8 protein	Alleviate mouse colitis via the AhR/COPS8 pathway.	[163]
Lemon	Metabolites from lemon	Manipulate gut microenvironment	[83]
Citrus sinensis	–	Modulating inflammatory genes and maintaining a healthy intestinal epithelium	[82]
Broccoli	Lipid	Retardation DSS-induced colitis	[80]
Tea leaf	Lipid, protein, polyphenols and flavones	Induce reactive oxygen species, inhibit pro-inflammatory cytokines, and promote the production of anti-inflammatory IL-10	[34]
Turmeric	Lipid and protein	Decreasing the expression of the pro-inflammatory cytokines (TNF- α , IL-6, and IL-1 β), and increasing the levels of HO-1	[164]

Abbreviations: IECs, intestinal epithelial cells; DSS, Dextran Sulfate Sodium Salt; AhR, Aryl Hydrocarbon Receptor; HO-1, heme oxygenase-1; HSPA8, heat shock protein family A member 8; COPS8, constitutive photomorphogenic homolog subunit 8.

The Therapeutic Effect of Milk-Derived Exosomes on IBD

Milk-derived exosomes play an important role in the development of the digestive tract, which regulate intercellular signaling, inflammation and immune response, thus protecting against stress and various disease conditions including IBD.^{88,165,166} Milk-derived exosomes contain lipids, nucleic acid, and proteins, exerting biological function,¹⁶⁷ and they can be used as an oral natural drug delivery system in the treatment of various diseases including IBD.⁹⁴ MiR-148a, a milk-derived exosome cargo, plays a key role in promoting intestinal maturation, sustaining barrier function, and suppressing the activity of NF- κ B.⁸⁸ Moreover, insulin-like growth factor-1 (IGF-1), as an important milk-derived exosome cargo, can promote IEC proliferation, improve intestinal barrier function, and protect IEC from intestinal injury.^{168,169} In addition, milk-derived exosomes can restore the expression of the protein zonula occludens 1 (ZO-1), and decrease the intestinal permeability barrier in the DSS-induced mouse colitis.¹⁷⁰ Recently, Reif et al have reported that oral administration of cow and human milk-derived exosomes can attenuate the severity of DSS-induced mouse colitis by down-regulating pro-inflammatory cytokines TNF- α and IL-6.¹⁷¹ We have previously demonstrated that the NF- κ B signaling pathway is activated, and link with the pathogenesis of ulcerative colitis.¹⁷² In a recent study, oral administration of milk-derived exosomes can reduce intestinal epithelium disruption, inhibit infiltration of inflammatory cells and alleviate colonic inflammation by inactivating NF- κ B signaling pathway in the ulcerative colitis mice model.³⁵ On the other side, it has been demonstrated that milk-derived exosomes can deliver both hydrophilic and lipophilic small molecules including chemo drugs.¹⁷³ For instance, milk-derived exosomes, as a drug delivery system for loading curcumin can resist degradation by human digestive enzymes and possess enhanced intestinal permeability in vitro.¹⁷⁴ Although milk-derived exosomes have several advantages, such as no adverse immune, no inflammatory response, and considerable oral bioavailability, how to maintain their stability in the gastrointestinal fluid has not yet been fully understood. Indeed, the technique of scalability and stability of milk-derived exosomes has witnessed rapid progress over the past few years,¹⁷⁵ and they may provide a promising treatment strategy for IBD in the future.

Conclusions and Future Perspective

Accumulating evidence suggests that EVs play a pivotal role in the pathogenesis of IBD, and a better understanding of them can clarify the underlying mechanism in IBD, providing new insights into the therapeutic strategies of IBD. In the present review, we systematically discussed the current knowledge about EVs in the pathogenesis of IBD, and the feasibility of the application of exosomes and exosome-like nanoparticles for IBD treatment. Under normal condition, IEC-, intestinal immune cell- and gut microbiota-derived EVs primarily function to defend against pathogens, modulate the immune response, and maintain the intestinal hemostasis. Nevertheless, under pathological conditions, IECs, intestinal immune cells, and gut microbiota also secrete EVs and intervene in the pathological mechanisms of IBD, such as EpCAM, CD80, CD86, and so on. In addition, EVs crucially participate in the interaction and communication of IEC-immune cells-gut microbiota in the intestinal hemostasis. Therefore, once the imbalance is disrupted, it may contribute to immune response, intestinal inflammation and intestinal dysbacteriosis. However, further studies are required to identify the chemical contents of the EVs, and help clarify the pathogenesis of IBD and provide new therapeutic targets.

The use of exosomes and exosome-like nanoparticles as alternative modalities for IBD treatment is deemed to be safe, available, and cost-effective. The exosomes and exosome-like nanoparticles isolated from the edible plants, MSCs, milk and other cells can protect the intestinal barrier, maintain gut microbiota, and attenuate experimental mouse colitis. However, most current knowledge on exosomes and exosome-like nanoparticles for IBD treatment mainly comes from in vitro experiments and animal models of DSS-induced colitis. Although exosomes and exosome-like nanoparticles can be used as oral drug delivery vehicles due to their good biodistribution and inherent biocompatibility, how to target deliver the drug encapsulated by exosomes and exosome-like nanoparticles to the lesion site is still challenging. Moreover, clinical trials of exosomes and exosome-like nanoparticles for the treatment of IBD are still limited, and their administration route, dosage, and adverse events should be investigated before entering the clinical application.

Abbreviations

IBD, Inflammatory bowel diseases; EVs, extracellular vesicles; PDENs, plant-derived exosome-like nanoparticles; OMVs, outer membrane vesicle; PM, plasma membrane; TGF- β , transforming growth factor β ; IL-10, interleukin-10; IFX, Infliximab; ADL, Adalimumab; GOL1, Golimumab; CZP, Certolizumab pegol; EV, extracellular vesicle; ILVs, intraluminal

vesicles; ESCRT, Endosomal Sorting Complex Required for Transport; MVBs, multivesicular bodies; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; PS, phosphatidylserine; RBCs, red blood cells; WBCs, platelets, white blood cells; MSCs, mesenchymal stem cells; MHC, major histocompatibility complex; AD, Alzheimer's disease; IECs, intestinal epithelial cells; GDM, gestational diabetes mellitus; TDEs, tumor-derived exosomes; ANXA1, annexin A1; IGF-1, insulin-like growth factor-1; NF- κ B, nuclear factor- κ B; ZO-1, zonula occludens 1; PZP, pregnancy zone protein; PSMA7, proteasome subunit alpha type 7; TME, tumor microenvironment; DCs, dendritic cells; Treg, regulatory T cells; APCs, antigen-presenting cells; MHC-1, major histocompatibility complex I; HLA-DM, human leukocyte antigen-DM; EpCAM, epithelial cell adhesion molecular; MPO, myeloperoxidase; IL, interleukin; TNBS, trinitrobenesulfonic acid; NOD1, nucleotide-binding oligomerization domain containing 1; HucMSCs, human umbilical cord-derived mesenchymal stem cells; hBM-MSCs, human bone marrow-derived mesenchymal stem cells; hADSCs, human adipose tissue derived MSCs; GELNs, grape exosome-like nanoparticles; AhR, aryl hydrocarbon receptor; AMPs, anti-microbial peptides; MDEs, milk-derived exosomes.

Acknowledgments

This work was supported by Guangdong Basic and Applied Basic Research Foundation (No. 2018A0303100024), Three Engineering Training Funds in Shenzhen (No. SYLY201718, No. SYJY201714 and No. SYLY201801), Science and Technology Innovation Committee of Shenzhen (No. JCYJ20150403101028164, No. JCYC20170307100911479, No. JCYJ20190807145617113 and JCYJ20210324113802006), National Natural Science Foundation of China (No. 81800489).

Author Contributions

Rui-yue Shi, Li-sheng Wang and Jun Yao share co-corresponding authorship. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare no conflict of interest.

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