Review Article
The emerging role of exosomes in the pathogenesis, prognosis and treatment of necrotizing enterocolitis

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Abstract: Exosomes are a subtype of extracellular vesicles. They contain bioactive molecules, including nucleic acids, proteins and lipids. Among the currently described exosomes, a majority are potential candidates for the diagnosis and treatment of necrotizing enterocolitis (NEC). In this work, we reviewed existing literature reports on exosomes and explored their roles in NEC. Exosomes derived from intestinal epithelial cells (IECs) participates in the development of intestinal diseases, thus can potentially be utilized as biomarkers for NEC. Besides, exosomes of human milk have been demonstrated to protect IECs from oxidative stress, stimulate intestinal stem cells activity, improve the proliferation and migration of IECs, and lower the incidence and severity of experimental NEC. Further, exosomes produced by stem cells can reduce the severity of experimental NEC and protect the intestinal barrier function during NEC. Conclusively, exosomes have been shown to influence the pathogenesis of NEC and exert a protective effect on NEC. However, additional investigations would be urgently necessary to comprehensively elucidate the underlying mechanisms of exosomes in NEC.

Keywords: Exosomes, necrotizing enterocolitis, intestinal epithelial cells, human milk

Introduction

Necrotizing enterocolitis (NEC), as acute inflammatory bowel necrosis, primarily attacks premature infants. The infant mortality as a result of NEC has been recorded as high as 30% [1]. Since the initial description of NEC, little changes have been observed in total mortality and treatment strategies [2]. Survivors may show serious sequelae, including gastrointestinal complications as well as severe neurodevelopmental retardation [3]. Among the possible risk factors involved in the development of NEC include: premature delivery, intestinal bacterial colonization and formula food feeding [2, 4]. Integrating infectious and proinflammatory components significantly contributes to the development of this disease [3]. However, the aetiology and pathogenesis of NEC remain obscure, implicating it as an elusive disease in terms of treatment and prevention. Therefore, new advanced strategies are urgently needed to prevent and treat this disease in preterm infants.

Moreover, exosomes are extracellular nanovesicles of endosomal origin acting as essential biological units both under normal and pathological circumstances. They encapsulate abundant components and play crucial functions in intercellular communication [5]. Notably, lipid bilayers of exosomes also protect their cargo from RNases and proteases, this implicates them as ideal delivery vectors for therapy [6]. Exosomes have been revealed to potentially exert regulatory functions on immune responses and alter the epithelial barrier function to protect the intestine against inflammation [7]. Proteomics of breast milk-derived exosomes revealed that these exosomes promote the development of the immune and gastrointestinal system [8]. Besides, breast milk exosomes have been reported to protect intestinal epithelial cells (IECs) against H_2O_2 induced cell toxicity [9]. Those indicate that exosomes have therapeutic potential with regard to NEC.

Therefore, this study provides a review of the currently available biological knowledge on

Table 1. Three types of extracellular vesicles

Vesicle type	Size (nm)	Morphology	Origin	Markers
Exosomes	30-150	Cup-shaped	Endolysosomal pathway; intraluminal budding of multivesicular body with cell membrane	CD9, CD63, CD81, CD82, HSP70, HSP90, Alix, TSG101, annexin, Rab GTPases
Microvesicles	50-1000	Heterogeneous	Cell surface; outward blebbing of apoptotic cell membrane	Integrin, selectins, CD40
Apoptotic bodies	500-4000	Heterogeneous	Cell surface; outward blebbing of apoptotic cell membrane	Phosphatidylserine, caspase 3

Data from Colombo et al. (2014) [11].

exosomes and their emerging role in NEC. Also, we share our opinion on the opportunities, future directions, and drawbacks with the use of exosomes in NEC diagnosis and therapy.

Exosomes: basic biological knowledge

Definition and classification

Although there are still controversies on nomenclature, the term "exosomes" generally refers to the lipid bilayer vesicles of endosomal origin formed through vesiculation of intracellular endosomes and release by exocytosis [5, 10, 11]. The first report about exosomes was availed in the early 1980s. when sheep reticulocytes matured into red blood cells, nanovesicles of endosomal origin loaded transferrin receptor [12]. In the past few years, some reports on the differentiation of red blood cells described exosomes as the bodies through which cells eliminate unwanted components [11]. However, interest in exosomes rose again in the late 1990s, as researchers speculated that exons might be an important medium for intercellular communication [13].

Exosomes are considered to be a subtype of extracellular vesicles, which differ from other types of extracellular vesicles, such as apoptotic bodies and microvesicles [14, 15]. The characteristics of exosomes and other extracellular vesicles are presented in **Table 1**. However, the nomenclature is still unclear and different terminologies are often used interchangeably across studies owing to the unclear biogenesis of extracellular vesicles. As previously described, inward budding of the endosome lumen results in the formation of intraluminal vesicles. Late endosomes harbouring intraluminal vesicles develop into multivesicular bodies. Subsequently, multivesicular bodies fuse with lysosomes and either is degraded

or fuse with the plasma membrane to release exosomes [11]. However, inconsistencies exist in extracellular vesicle purification protocols, also vesicle characterization has not been fully elucidated [11]. Therefore, to illuminate their physiological relevance and comprehensively understand the origin of the different populations of vesicles, their mechanisms of secretion and biogenesis need an in-depth assessment.

Exosome contents

Lipids are primary components of exosomes and have been revealed to be rich in diglycerides, cholesterol, phospholipids, glycerophospholipids, and sphingolipids or glycosylceramides [16]. A previous study revealed that the lipid composition of reticulocyte-derived exosomes was similar to that of the producing cells [17]. Some of the exosomal lipid compositions differ from the plasma membrane of the parent cell, partly because the exosome also contains lipids from the Golgi apparatus [18]. Their lipid composition depicts their unique rigidity. Exosomes as lipid carriers deliver to carry bioactive lipids to recipient cells. Besides, exosomal lysophosphatidylcholine as a chemoattractant for lymphocytes attract T lymphocytes and induce the maturation of dendritic cells to mediate immune response [19].

Moreover, exosomes contain genetic materials and proteins. The genetic material packaged within exosomes includes mRNAs, miRNAs, circRNAs, lncRNAs and DNAs [11]. The hTERT mRNA, a transcript of the enzyme telomerase, was reported to be shuttled from cancer cells via exosomes into telomerase negative fibroblasts. Consequently, it is translated into a fully active enzyme and transforms these cells into telomerase positive fibroblasts, thus creating non-malignant cells with telomerase activity [20]. As post-transcriptional inhibitors of

gene expression, miRNA plays a role in stimulating the degradation of functional inhibition of its mRNA targets. Exosomal miRNAs from the rat PC12 cells transported to human tumours could vary the expression level of the PTEN protein [21]. In addition, cardiovascular-expressed miRNAs have been found in human pericardial fluid exosomes whereby they promote therapeutic angiogenesis [22]. In addition, ZFAS1 lncRNA exists in exosomes and can be transported in exosomes to enhance the proliferation and migration of gastric cancer cells [23].

Previous reports have revealed that exosomes have cellular proteins, such as targeting and adhesion proteins (e.g. intergrins and tetraspanins), chaperones (e.g. Hsp60 and Hsp90), proteins involved in multivesicular body formation (e.g. TSG101 and ALIX), cytoskeletal proteins (e.g. actin and tubulin), cytoplasmic enzymes (e.g. pyruvate kinases and lactate dehydrogenase) [11, 24]. Exosomes also contain cell-specific proteins. For example, the surface of the exosomes secreted by T lymphocytes contain T cell receptors, granzyme and perforin, which activate of the immune response [25]. In recent years, studies on multifunctional properties of bioactive peptides derived from exosome have matured. Targeted MRM-MS has been utilized in detecting serum exosomes of cultured positive tuberculosis patients. Notably, 76 peptides representing 33 unique Mycobacterium tuberculosis proteins were found in these exosomes [26]. The highly purified CD81+ exosomes from human placenta were also revealed to contain more than 27 different peptides [27].

Biological functions and therapeutic potential of exosomes

Mediating intercellular communication is considered to be a highly crucial biological function of exosomes. Exosomes carry and transfer biologically active material to recipient cells, exerting effects [28]. It is believed that exosomes mediate cell-cell information exchange through the following ways: (1) exosomes induces a signal complex that directly stimulates recipient cells by binding to cell surface ligand; (2) exosomes transfer receptors between cells; (3) exosomes deliver functional proteins, infectious particles or genetic information to recipient cells [6]. Once a recipient cell absorbs an exosome, the contents of the exosome impact the recipient cell by regulating key enzyme re-

actions, signaling pathways, cellular homeostasis, or other mechanisms. The mechanism by which this occurs and its effects are predominantly determined based on the pathophysiological status of the source cell and recipient cells [29].

At present, exosomes are considered to be important in the maintenance of normal physiology. For instance, exosomes derived from dendritic cells create a balance in pro- and anti-inflammatory T cells by inducing T helper cells to differentiate into regulatory T cells [30]. In blood circulation, exosomes participate in the coagulation cascade by providing a surface for the aggregation of coagulation factors [6]. However, similar properties of exosomes can associate them with the occurrence of pathological conditions. Tumor-derived exosomes, for example, can promote tumor growth, migration, invasion, microenvironment regulation, angiogenesis, immune manipulation and chemotherapeutic resistance [30]. Given the biological function and pathophysiologic effects of these nanovesicles, they are implicated as highly potential noninvasive biomarkers for diagnosis, therapeutic targets, biologic reagents, and drug delivery in treating disease. Exosome biogenesis, contents, and biological functions are highlighted in **Figure 1**.

Epithelial cell and neutrophil-derived exosomes in NEC progression

The intestinal epithelium is a huge interface between the luminal commensal microorganisms and the body. Epithelial injury and impaired barrier function resulting from inflammatory responses are common pathological features of gastrointestinal diseases, including NEC [31]. It has been reported IECs not only absorbed nutrition but also released exosomes containing immunomodulatory molecules. A previous study reported that IECs released immunomodulatory exosomes containing MHC class II and the Fas ligand into the mesenteric lymph following trauma/haemorrhagic shock and mediated post-traumatic immunosuppression causing dysfunction and depletion of dendritic cells [32]. Elsewhere, IEC-derived exosomes were reported to exert immunoregulatory effects on dendritic cells in an inflammatory bowel disease model [33]. IECs released exosomes from the apical and basolateral sides, which carried MHC class I and class II mol-
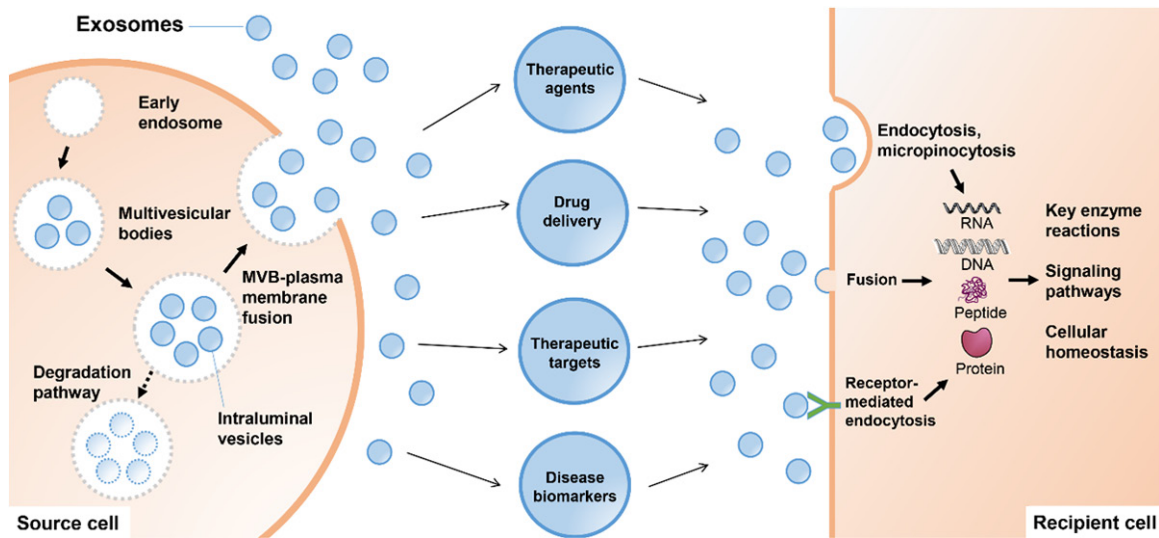
Figure 1. Exosome biogenesis, contents and biological functions. The plasma membrane is invaginated to form an early endosome, followed by budding of cargo (e.g., RNA, DNA, peptide and protein) into the endosomal membrane to form intraluminal vesicles within MVBs. MVBs then fuse with the plasma membrane to release exosomes. Exosomes are released by almost all cell types and are present in most if not all biological fluids. The biologically active cargo carried by exosomes can alter the phenotype of recipient cells. This process is mediated through endocytosis, membrane fusion and/or receptor-mediated endocytosis. The cargo of the exosome then affects the recipient cell by regulating key enzyme reactions, signaling pathways, cellular homeostasis, or other mechanisms. Exosomes may act as noninvasive diagnostic biomarkers, therapeutic targets, biological reagents and drug delivery systems. MVBs, multivesicular bodies.

ecules and were significantly increased in the presence of interferon gamma [34]. In particular, EVs derived from IECs have been demonstrated to activate wound repair circuits [35] and maintain the intestinal immune balance [32]. Also, epithelial cells release antimicrobial peptides in free form or in association with exosomes to kill parasites [36]. In addition, crypt-based immature IECs were previously revealed to transport the luminal peptidoglycan across the intestinal epithelia via a Toll-like receptor 2-mediated phagocytosis-multivesicular body-exosome pathway [37]. Notably, gasdermin D (GSDMD) has been shown to induces pyroptosis through the pore-forming activity of its N-terminal domain, cleaved by activated caspases associated with the release of IL-1β [38]. Furthermore, GSDMD mediated release of exosomes that contained polyubiquitinated IL-1β from IECs [39]. GSDMD-guided IL-1β secretion through exosomes has distinct physiological significance from pyroptotic IL-1β release because producing cells remain viable and continue to participate in the inflammatory response [39]. Additionally, Adherent-invasive *E. coli* (AIEC) are able to adhere to and invade IECs, survive and replicate within macrophages and induce a pro-inflammatory response [40].

AIEC infection of IECs induces secretion of exosomes that increase AIEC replication in exosome-receiving IECs and macrophages [40]. These findings suggest that exosomes of IECs may affect the expression antigen in the mucosal or systemic immune system via their inter-cellular communication functions and eventually impact NEC progression.

To our knowledge, polymorphonuclear neutrophils (PMN) play a role in the development of tissue injury in NEC [41]. Interleukin-8 produced by epithelial cells attracts PMN to inflammatory sites and induces their activation, which is known to lead to intestinal necrosis [42]. Tissue-infiltrating PMNs have been reported to release exosome-like microparticles that trigger acute remodelling of epithelial junctions and reduce interepithelial adhesions, leading to enhanced recruitment of PMNs and further epithelial damage [31]. Besides, myeloperoxidase was also reported to be mobilized to the PMN surface and subsequently released in association with PMN-derived microparticles (PMN-MPs) upon PMN activation and binding to IECs. Myeloperoxidase is a potent inhibitor of wound closure following the binding of PMN-MPs to IECs. Microinjection of PMN-MPs into
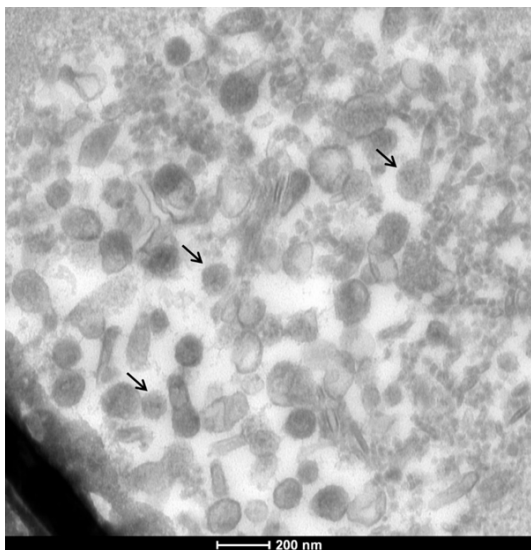
Figure 2. The representative microscopic image of human milk exosomes morphology. Samples were analyzed by electron microscopy.

the injured colonic mucosa can interfere with the healing of epithelial injury *in vivo* [43]. Notably, these findings suggest that PMN-MPs act as shuttles to protect and transmit active mediators to regulate cellular function during the progression of NEC. It is fair to assume that, by analysing changes in the exosome signature in NEC, markers and therapeutic targets for NEC can be identified.

Milk-derived exosomes in NEC prevention

Milk-derived exosomes

Breast milk or donor breast milk administration has been reported to prevent NEC [44-47]. A meta-analysis of 4 randomized clinical trials conducted between 1983 and 2005 showed that breast-feeding of premature infants was associated with a significant reduction (58%) in NEC incidence [48]. Moreover, a recent study revealed that the NEC incidence of premature infants fed exclusively human milk decreased by 77% compared with human milk supplemented with infant formula [49]. However, the mechanisms by which breast milk exerts these benefits remain unclear. It is important to identify the components of human milk that are involved in the prevention and control of NEC. Of note, exosomes as an inter-cell communication messenger have been found in human milk, and are rich in colostrum

and mature milk [50]. Similarly, in our group, we isolated exosomes from human milk, and the representative microscopy image is shown in **Figure 2**.

Previous studies found that milk exosomes can improve the development of intestines and exerts potential protective effects on NEC. Moreover, exosomes of porcine milk have been reported to significantly promote the expression of CDX2, IGF-1R, and PCNA, whereas it inhibited p53 gene expression, which is critical for intestinal proliferation [51]. Additionally, rat milk-derived exosomes were reported to significantly enhance IECs viability, promote proliferation, and stimulate activity of intestinal stem cells, which elevated Lgr5 gene expression [52]. Furthermore, yak milk-derived exosomes were revealed to promote the proliferation of IECs in a hypoxic environment, and showed higher activation of the HIF signaling pathway promoting IECs survival than cow-milk-derived exosomes [53]. Bovine milk exosomes were demonstrated to improve goblet cell expression and mucin production, reduced the expression of myeloperoxidase in experimental NEC [54] and exerted protective effects against oxidative stress in IEC-6 cells [55]. The uptake of bovine milk exosomes was shown to be mediated by endocytosis and dependent on cell and exosome surface glycoproteins in human and rat intestinal cells [56]. Notably, it was also shown that human milk exosomes preserved IECs from oxidative stress, which is one of the major factors causing cell apoptosis resulting from increased p53 expression [9]. Additionally, human milk exosomes protected intestinal stem cells from oxidative stress injury *in vitro*, which were potentially mediated via the Wnt/β-catenin signaling pathway [57]. Furthermore, we found that preterm breast exosomes significantly enhanced the proliferation and migration of IECs compared with full-term breast exosomes *in vitro* [58]. Our *in vivo* study has also confirmed the protective effects of preterm milk exosomes in a neonatal NEC animal model [58]. Breast milk-derived exosomes was then proved to significantly decrease the incidence and severity of experimental NEC [59]. Additionally, pasteurized breast milk-derived exosomes decreased the inflammation present in intestinal organoids exposed to injury in vivo [60]. Of note, colostrum-derived exosomes offer the best protective effect among different

periods breast milk on protecting intestine organoids against epithelial injury induced by LPS [61]. These findings suggest that exosomes in breast milk may exert potential protective effects against NEC. However, to comprehensively understand the protective role of human breast milk exosomes, it is important to identify the components of human milk that are involved in the prevention and control of NEC.

MiRNAs in milk exosomes

MiRNAs as critical post-transcriptional regulators of gene expression could be packaged in breast milk-derived exosomes [62]. The exosomal miRNAs could survive *in vitro* digestion and be absorbed by intestinal epithelia [63]. The miRNAs, including miR-4334, miR-219 and miR-219 in porcine milk exosomes have been reported to reduce LPS-induced proinflammatory responses and apoptosis in intestinal porcine enterocytes [64]. With the use of deep-sequencing technology to identify exosomal miRNAs in human milk, 4 of the top 10 miRNAs identified in human milk exosomes are designated as immune-related pre-miRNAs, and one of these miRNAs, miR-182-5p, was induced by interleukin-2 and promoted T cell-mediated immune responses [65]. One of the identified miRNAs is miR-22-3p, which has been confirmed to inhibit the activity of NF-κB, a key inflammatory signalling molecule that may cause NEC [66, 67]. Similarly, miR-148a-3p, which was revealed to be highly expressed in exosomes of preterm milk, can lower the response of inflammation as well as influence the embryonic cells differentiation [63]. Additionally, mothers with Type-1 diabetes exhibit an altered miRNA profile in human milk exosomes, of which some of the differentially expressed miRNAs including hsa-miR-4497 and hsa-miR-3178 could inducing pro-inflammatory cytokine expression [68]. Several miRNAs in NEC intestinal tissue have been reported to be under-expressed compared to control tissues, many of which play a role in intestinal goblet cell differentiation, prevention of excessive inflammation and maintenance of intestinal epithelial integrity [69]. These findings indicated that the exosomal miRNAs transfer from breast milk to infants through the digestive tract systemic circulation, and play an important role in the treatment of NEC. In addition, human milk-derived exosomes induced proliferation and epithelial mesenchymal transformation-re-lated changes, such as collagen type I and twist expression [70]. The positive effect of exosomes on normal cells may presents an aspect of their safety when utilized as a nutritional supplement to infant formula.

Proteins and peptides enriched in milk exosomes

A few researchers have conducted proteomic analyses of exosomes from breast milk. However, such studies are still in their initial stages. Proteomic approach (iTRAQ) was used to analyse milk-derived exosomes of human and bovine milk, and 575 differentially expressed exosome proteins were identified [71]. These proteins were highly enriched in the pathways, such as actin cytoskeleton, glycolysis/gluconeogenesis, leukocyte transendothelial migration, galactose metabolism, which are also important in NEC. Besides, high amounts of lactoferrin was found in human milk exosomes and could regulate cell survival and preserve infants from developing NEC [71]. In another study, proximity extension assay was also used to detect more than 100 proteins in breast milk-derived exosomes [72]. For instance, CX-CL5 as an attractor of neutrophils was present in milk exosomes and could regulate the immune system including influencing inflammation in the newborn gut [73]. In addition, KLK6, an important enzyme for immune cell differentiation and survival has previously been detected in milk exosomes [74].

To our knowledge, peptides and their precursors always have similar functions [75]. Modulation of immune responses by immunoregulatory agents, such as the natural or synthetic immunomodulatory peptides, has been suggested as a potential strategy to modulate immune system against infection and other immune-related diseases including NEC [76]. To comprehensively understand the protective effect of breast milk exosomes, we first performed a peptidomic analysis of full-term and premature breast milk exosomes. Many exosomal peptide precursors from the breast milk of full-term and premature infants are known to be associated with metabolic processes, developmental processes, immune system processes, biological adhesion and cell proliferation, which play important roles in the regulation of intestinal homeostasis [58]. It is speculated that these exosomal proteins and peptides trans-

ferred from breast milk to infants may have protective functions in the NEC.

Milk-derived exosomes as potential nano-drug carriers

There are various advantages of delivery of biologically active substance via human milk-derived exosomes: (1) exosomes protect molecules from low pH and high enzymatic activity environments, (2) capsulized pill delivery provides a higher local concentration, (3) efficient batch recognition and internalization by the intestinal epithelium is possible [46]. In particular, milk-derived exosomes are present in abundance and can be loaded with both hydrophilic and lipophilic agents [77]. It is reported when curcumin was mixed with milk exosomes in the presence of 10% ethanol: acetonitrile (1:1), 18-24% concentration of the drug can be provided. Notably, the formula stored at -80°C degree was stable for up to 6 months, which was determined by particle size analysis, anti-proliferative activity and drug loading [78]. Oral administration of milk exosomal curcumin in Sprague-Dawley rats proved that it was present in various organs at 3-5 times the levels obtained with its delivery as a free agent. Further, compared with the free curcumin administration mode, the system enhanced the anti-proliferative activity against a variety of cancer cell lines, including lung, breast and cervical cancer [78].

Furthermore, milk-derived exosomes have been assessed for oral chemotherapeutic drug paclitaxel (PAC), as an alternative to conventional intravenous therapy. Of note, the exosomal delivery method improved the efficacy and reduced toxicity of human lung tumour transplanted into nude mice. Importantly, oral PAC-loaded exosomes showed significant inhibitory effects on tumor growth. However, treatment with PAC at the same dose as PAC-loaded exosomes showed moderate but statistically insignificant inhibitory effects [79]. In another such study, Anthos loaded in milk exosomes was found to have significantly increased anti-proliferative activity against ovarian cancer cells, and was more effective than anthos alone and vector control in inhibiting tumor growth [80]. Thus, milk-derived exosomes can be harnessed as potential drug delivery systems for various molecules that are otherwise limited by their tissue bioavailability.

When these functional molecules (miRNAs, proteins, peptides and drugs) are added to milk exosomes or formula milk may be highly useful in NEC prevention. Ultimately, the future of exosomes as therapeutics relies on evaluating the optimal producer cells and the culture and stimulation conditions, as well as large-scale production of homogeneous exosomes. An in-depth understanding of exosome biology is essential to overcome these challenges and make advances in this field.

The therapeutic potential of stem cell derived exosomes in NEC

Stem cells have proved to be of great potential in the treatment of NEC. Recent studies reveal that intravenous or intraperitoneal administration of mesenchymal stem cells (MSCs) decreases the incidence of experimental NEC in rats [81, 82]. Previous studies indicate that despite improved function after MSC transplantation, both MSC engraftment and subsequent differentiation into appropriate cell types are rare. This signifies the presence of additional underling mechanisms [83]. A study revealed that exosomes secreted from MSCs carry critical signalling molecules and can thus potentially be utilized in disease intervention and treatment [84].

Further, a study using a rat model of NEC reported that intraperitoneal administration of exosomes isolated from bone marrow-derived MSCs reduced the severity and incidence of experimental NEC, and could protect the integrity of intestinal barrier [85]. Additionally, exosomes in other stem cells such as enteric neural stem cells, amniotic fluid-derived MSCs and amniotic fluid-derived neural stem cells have been reported to preserve the intestines from NEC [86-90]. These findings affirm that exosomes of different stem cells have potential function for NEC prevention and therapy. Notably, MSC derived exosomal miR-34a/c-5p and miR-29b-3p were found to improve intestinal epithelial barrier function by targeting the Snail/Claudins signaling pathway [91]. Another study reported that miR-200b in heme oxygenase-1-modified bone marrow MSC derived exosomes alleviates inflammatory injury of IECs by targeting high mobility group box 3 [92]. Although the other contents of stem cell-derived exosomes and mechanisms underlying the-

Table 2. Examples of the functional effects of exosomes/EVs in NEC

Source of exosomes/EVs	Target cells/tissues	Findings	Ref.
Neutrophils	IECs	Promoted epithelial injury and increased neutrophil recruitment.	[31]
Neutrophils	Intestinal tissues	Impaired epithelial wound healing.	[43]
IECs	Immune cells	Decreased colitis severity by inducing regulatory T cells and immunosuppressive dendritic cells.	[32]
IECs	Dendritic cells	Caused depletion and dysfunction of dendritic cells.	[33]
IECs	Epithelial wound	Accelerated the healing of murine colonic wounds after biopsy-induced injury.	[35]
IECs	Intestinal tissues	Participate in the inflammatory response.	[39]
IECs	IECs and macrophages	Increase AIEC replication in exosome-receiving IECs and macrophages.	[40]
Porcine milk	Intestinal tissues	Facilitated intestinal cell proliferation and intestinal tract development.	[51]
Rat milk	IECs	Promoted IEC viability, enhanced proliferation, and stimulated intestinal stem cell activity.	[52]
Yak milk	IECs	Promote proliferation of intestinal epithelial cells in a hypoxic environment.	[53]
Bovine Milk	Goblet cells	Enhance goblet cell activity and prevent the development of experimental necrotizing enterocolitis.	[54]
Bovine Milk	IECs	Protected against oxidative stress.	[55]
Bovine Milk	IECs	Mediated by endocytosis and was dependent on cell and exosome surface glycoproteins in human and rat intestinal cells.	[56]
Human milk	IECs	Potected IECs from oxidative stress.	[9]
Human milk	IECs	Enhanced the proliferation and migration of IECs and reduced the severity of experimental NEC.	[58]
Human milk	Intestinal tissues	Reduced the severity of experimental NEC.	[59]
Pasteurized human milk	Intestinal tissues	Decreased the inflammation present in intestine.	[60]
Human milk	Terminal ileum	Protected intestine organoids against epithelial injury induced by LPS.	[61]
BM-MSCs	Intestinal tissues	Reduced the incidence and severity of experimental NEC, and preserved the integrity of the gut barrier.	[85]
Human umbilical cord-MSCs	Intestinal tissues	Relieved colitis in mice.	[88]
Amniotic fluid-derived neural stem cells	Intestinal tissues	Reduced the incidence and severity of experimental NEC.	[89]
Neonatal enteric neural stem cells	Intestinal tissues	Reduced the incidence and severity of experimental NEC.	[89]
Gut microbiota	IECs/macrophages	Regulated intestinal UGT1A1 partially through secreting OMVs, which interacted with IECs directly or via activating macrophages.	[94]
Juice of grapes	Intestinal stem cells	Mediated intestinal tissue remodeling and protected against colitis.	[96]
Grapes, grapefruit, ginger and carrot	Intestinal macrophages and stem cells	Induced expression of genes for anti-inflammatory cytokines, antioxidants, and Wnt signaling activators.	[97]

IECs, intestinal epithelial cells; AIEC, adherent-invasive *E. coli*; NEC, necrtotizing enterocolitis; LPS, lipopolysaccharide; BM-MSCs, bone marrow-derived mesenchymal stem cells; UGT1A1, UDP-glucuronosyltransferase 1A1; OMV, outer membrane vesicle.
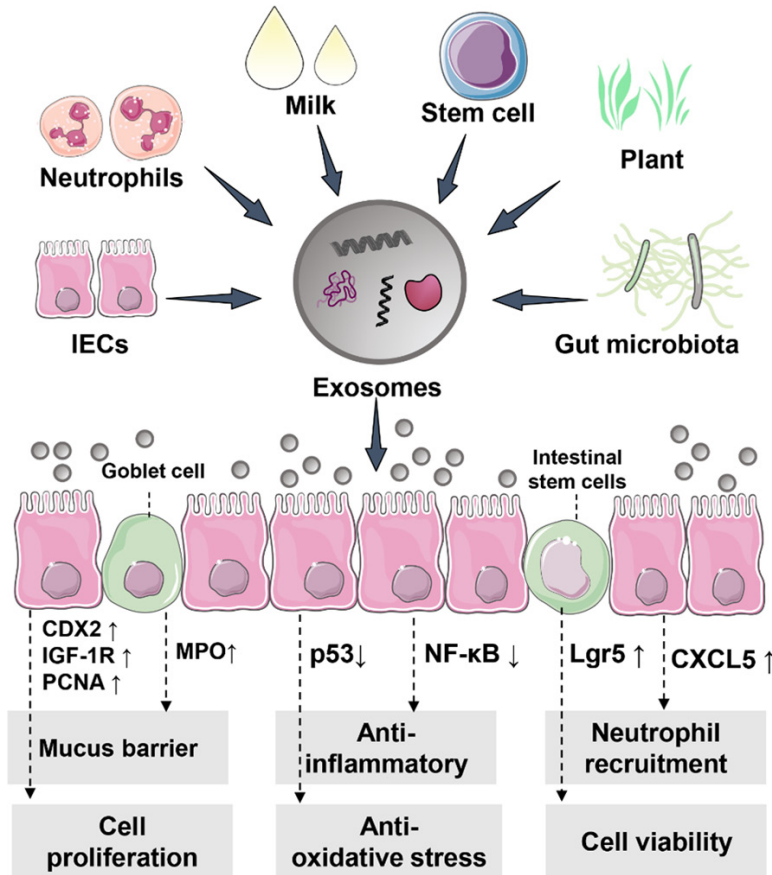
Figure 3. Different sources of exosomes in NEC. Exosomes from IECs and neutrophils transport bioactive substances to regulate cell function and intestinal homeostasis. Both human milk and stem cells exosomes have protective effects in animal models of NEC. Exosome-like nanoparticles of plant and gut microbiota indicate the potential of cell-free therapy in NEC. The positive effects of exosomes are mediated by multiple mechanisms. Exosomes protect mucus barrier, improve IECs proliferation and intestinal stem cells viability, reduce oxidative stress and inflammation of IECs, and increase neutrophil recruitment hence are ideal therapeutic agents in NEC. NEC, necrotizing enterocolitis; IECs, intestinal epithelial cells.

se therapeutic effects need further investigation.

Other exosome-like nanoparticles in NEC

After birth, the gradual colonization of gut microbes and the development of the body occur simultaneously. Environmental microbes constitute the most vital group of exogenous stimuli in the perinatal period. Recent studies have revealed that bacterial outer membrane exosome-like vesicles (OMEVs) can regulate inflammatory responses by interacting with immune cells; upregulate the expression of tight junction proteins in IECs to enhance barrier func-

tion; transfer virulence factors to host cells and lead to cell injury; stimulate the expression of endothelial cell adhesion molecules; serve as natural nanocarriers for immunogenic antigens, and enter systemic circulation to induce various immunological and metabolic responses [93]. Further, the gut microbiota has been proved to influence intestinal UDP-glucuronosyltransferase 1A1 partially by secreting OMEVs [94]. Based on these findings, it would be worthy to conduct a further assessment of whether and how bacterial OMEVs is associated with NEC.

Previously, studies demonstrated that plant cells can secret exosomes [95]. It has been demonstrated that grape derived exosome-like nanoparticles penetrated the intestinal mucus barrier and were absorbed by mouse intestinal stem cells thereby protecting them from colitis [96]. Besides, grapes, grapefruit, ginger and carrot edible plant-derived exosome-like nanoparticles were shown to induce the expression of genes that code for Wnt signalling activators, antioxidants and anti-inflammatory cytokines, all of which critically maintained intestinal homeostasis [97]. These findings may prompt the development of new and safe strategies for using edible plant-derived nanoparticles as an alternative drug delivery vehicle or as nanosize therapeutic agents.

Conclusions

By integrating the findings of previous studies, exosomes that play important roles in NEC are briefly summarized in **Table 2**. Multiple mechanisms of exosomes may have positive effects on NEC and they were show in **Figure 3**. Since

cargo related to the exosomes often reflects the various health and pathogenic states of released cells and tissues, efforts are being made to explore the possibility that the exosomes can be used as a biomarker and therapeutic target to diagnose and evaluate therapeutic success of NEC. However, further studies are underway to fully understand the underlying mechanisms. Since exosomes are specifically used to regulate the transfer of bioactive substances between cells, the possibility of using these vesicles for treatment is currently being studied. Notably, efforts are being made to develop technologies for encapsulating therapeutic peptides, nucleic acids and drugs into exosomes, as well as to protect them and improve their bioavailability and transportation into disease tissues. We believe that, eventually, exosomes containing beneficial molecules can be harnessed to benefit NEC patients.

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Disclosure of conflict of interest

None.

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References

[1] Fitzgibbons SC, Ching Y, Yu D, Carpenter J, Kenny M, Weldon C, Lillehei C, Valim C, Horbar JD and Jaksic T. Mortality of necrotizing enterocolitis expressed by birth weight categories. J Pediatr Surg 2009; 44: 1072-1075; discussion 1075-1076.

[2] Nino DF, Sodhi CP and Hackam DJ. Necrotizing enterocolitis: new insights into pathogenesis and mechanisms. Nat Rev Gastroenterol Hepatol 2016; 13: 590-600.

[3] Neu J. Necrotizing enterocolitis: the mystery goes on. Neonatology 2014; 106: 289-295.

[4] Chen Y, Koike Y, Chi L, Ahmed A, Miyake H, Li B, Lee C, Delgado-Olguín P and Pierro A. Formula feeding and immature gut microcirculation promote intestinal hypoxia, leading to necrotizing enterocolitis. Dis Model Mech 2019; 12: dmm040998.

[5] Kalluri R and LeBleu VS. The biology, function, and biomedical applications of exosomes. Science 2020; 367: eaau6977.

[6] EL Andaloussi S, Mäger I, Breakefield XO and Wood MJ. Extracellular vesicles: biology and emerging therapeutic opportunities. Nat Rev Drug Discov 2013; 12: 347-357.

[7] Bui TM, Mascarenhas LA and Sumagin R. Extracellular vesicles regulate immune responses and cellular function in intestinal inflammation and repair. Tissue Barriers 2018; 6: e1431038.

[8] van Herwijnen MJ, Zonneveld MI, Goerdayal S, Nolte-'t Hoen EN, Garssen J, Stahl B, Maarten Altelaar AF, Redegeld FA and Wauben MH. Comprehensive proteomic analysis of human milk-derived extracellular vesicles unveils a novel functional proteome distinct from other milk components. Mol Cell Proteomics 2016; 15: 3412-3423.

[9] Martin C, Patel M, Williams S, Arora H, Brawner K and Sims B. Human breast milk-derived exosomes attenuate cell death in intestinal epithelial cells. Innate Immun 2018; 24: 278-284.

[10] Gould SJ and Raposo G. As we wait: coping with an imperfect nomenclature for extracellular vesicles. J Extracell Vesicles 2013; 2.

[11] Colombo M, Raposo G and Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annu Rev Cell Dev Biol 2014; 30: 255-289.

[12] Harding C, Heuser J and Stahl P. Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. J Cell Biol 1983; 97: 329-339.

[13] Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ and Geuze HJ. B lymphocytes secrete antigen-presenting vesicles. J Exp Med 1996; 183: 1161-1172.

[14] Todorova D, Simoncini S, Lacroix R, Sabatier F and Dignat-George F. Extracellular vesicles in angiogenesis. Circ Res 2017; 120: 1658-1673.

[15] Cho YE, Song BJ, Akbar M and Baek MC. Extracellular vesicles as potential biomarkers for alcohol- and drug-induced liver injury and their therapeutic applications. Pharmacol Ther 2018; 187: 180-194.

[16] Record M, Carayon K, Poirot M and Silvente-Poirot S. Exosomes as new vesicular lipid transporters involved in cell-cell communica-

tion and various pathophysiologies. Biochim Biophys Acta 2014; 1841: 108-120.

[17] Johnstone RM, Adam M, Hammond JR, Orr L and Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J Biol Chem 1987; 262: 9412-9420.

[18] Barile L and Vassalli G. Exosomes: therapy delivery tools and biomarkers of diseases. Pharmacol Ther 2017; 174: 63-78.

[19] Deng H, Sun C, Sun Y, Li H, Yang L, Wu D, Gao Q and Jiang X. Lipid, protein, and microRNA composition within mesenchymal stem cell-derived exosomes. Cell Reprogram 2018; 20: 178-186.

[20] Gutkin A, Uziel O, Beery E, Nordenberg J, Pinchasi M, Goldvaser H, Henick S, Goldberg M and Lahav M. Tumor cells derived exosomes contain hTERT mRNA and transform nonmalignant fibroblasts into telomerase positive cells. Oncotarget 2016; 7: 59173-59188.

[21] Zhou Y, Tian T, Zhu Y, Jaffar Ali D, Hu F, Qi Y, Sun B and Xiao Z. Exosomes transfer among different species cells and mediating miRNAs delivery. J Cell Biochem 2017; 118: 4267-4274.

[22] Beltrami C, Besnier M, Shantikumar S, Shearn AI, Rajakaruna C, Laftah A, Sessa F, Spinetti G, Petretto E, Angelini GD and Emanueli C. Human pericardial fluid contains exosomes enriched with cardiovascular-expressed microRNAs and promotes therapeutic angiogenesis. Mol Ther 2017; 25: 679-693.

[23] Pan L, Liang W, Fu M, Huang ZH, Li X, Zhang W, Zhang P, Qian H, Jiang PC, Xu WR and Zhang X. Exosomes-mediated transfer of long noncoding RNA ZFAS1 promotes gastric cancer progression. J Cancer Res Clin Oncol 2017; 143: 991-1004.

[24] Thery C, Ostrowski M and Segura E. Membrane vesicles as conveyors of immune responses. Nat Rev Immunol 2009; 9: 581-593.

[25] Okoye IS, Coomes SM, Pelly VS, Czieso S, Papayannopoulos V, Tolmachova T, Seabra MC and Wilson MS. MicroRNA-containing T-regulatory-cell-derived exosomes suppress pathogenic T helper 1 cells. Immunity 2014; 41: 89-103.

[26] Kruh-Garcia NA, Wolfe LM, Chaisson LH, Worodria WO, Nahid P, Schorey JS, Davis JL and Dobos KM. Detection of Mycobacterium tuberculosis peptides in the exosomes of patients with active and latent M. tuberculosis infection using MRM-MS. PLoS One 2014; 9: e103811.

[27] Burkova EE, Dmitrenok PS, Bulgakov DV, Vlassov VV, Ryabchikova EI and Nevinsky GA. Exosomes from human placenta purified by affinity chromatography on sepharose bearing immobilized antibodies against CD81 tetraspan-

in contain many peptides and small proteins. IUBMB Life 2018; 70: 1144-1155.

[28] Pegtel DM and Gould SJ. Exosomes. Annu Rev Biochem 2019; 88: 487-514.

[29] Camussi G, Deregibus MC, Bruno S, Cantaluppi V and Biancone L. Exosomes/microvesicles as a mechanism of cell-to-cell communication. Kidney Int 2010; 78: 838-848.

[30] Isola AL and Chen S. Exosomes: the messengers of health and disease. Curr Neuropharmacol 2017; 15: 157-165.

[31] Butin-Israeli V, Houser MC, Feng M, Thorp EB, Nusrat A, Parkos CA and Sumagin R. Deposition of microparticles by neutrophils onto inflamed epithelium: a new mechanism to disrupt epithelial intercellular adhesions and promote transepithelial migration. FASEB J 2016; 30: 4007-4020.

[32] Jiang L, Shen Y, Guo D, Yang D, Liu J, Fei X, Yang Y, Zhang B, Lin Z, Yang F, Wang X, Wang K, Wang J and Cai Z. EpCAM-dependent extracellular vesicles from intestinal epithelial cells maintain intestinal tract immune balance. Nat Commun 2016; 7: 13045.

[33] Kojima M, Costantini TW, Eliceiri BP, Chan TW, Baird A and Coimbra R. Gut epithelial cell-derived exosomes trigger posttrauma immune dysfunction. J Trauma Acute Care Surg 2018; 84: 257-264.

[34] van Niel G, Raposo G, Candalh C, Boussac M, Hershberg R, Cerf-Bensussan N and Heyman M. Intestinal epithelial cells secrete exosome-like vesicles. Gastroenterology 2001; 121: 337-349.

[35] Leoni G, Neumann PA, Kamaly N, Quiros M, Nishio H, Jones HR, Sumagin R, Hilgarth RS, Alam A, Fredman G, Argyris I, Rijcken E, Kusters D, Reutelingsperger C, Perretti M, Parkos CA, Farokhzad OC, Neish AS and Nusrat A. Annexin A1-containing extracellular vesicles and polymeric nanoparticles promote epithelial wound repair. J Clin Invest 2015; 125: 1215-1227.

[36] Laurent F and Lacroix-Lamande S. Innate immune responses play a key role in controlling infection of the intestinal epithelium by Cryptosporidium. Int J Parasitol 2017; 47: 711-721.

[37] Bu HF, Wang X, Tang Y, Koti V and Tan XD. Toll-like receptor 2-mediated peptidoglycan uptake by immature intestinal epithelial cells from apical side and exosome-associated transcellular transcytosis. J Cell Physiol 2010; 222: 658-668.

[38] Evavold CL, Ruan J, Tan Y, Xia S, Wu H and Kagan JC. The pore-forming protein gasdermin D regulates interleukin-1 secretion from living macrophages. Immunity 2018; 48: 35-44. e36.

[39] Bulek K, Zhao J, Liao Y, Rana N, Corridoni D, Antanaviciute A, Chen X, Wang H, Qian W, Mill-

er-Little WA, Swaidani S, Tang F, Willard BB, McCrae K, Kang Z, Dubyak GR, Cominelli F, Simmons A, Pizarro TT and Li X. Epithelial-derived gasdermin D mediates nonlytic IL-1β release during experimental colitis. J Clin Invest 2020; 15: 223-36.

[40] Larabi A, Dalmasso G, Delmas J, Barnich N and Nguyen HTT. Exosomes transfer miRNAs from cell-to-cell to inhibit autophagy during infection with Crohn's disease-associated adherent-invasive E. coli. Gut Microbes 2020; 11: 1677-1694.

[41] Emami CN, Mittal R, Wang L, Ford HR and Prasadarao NV. Role of neutrophils and macrophages in the pathogenesis of necrotizing enterocolitis caused by Cronobacter sakazakii. J Surg Res 2012; 172: 18-28.

[42] Neu J. Necrotizing enterocolitis. World Rev Nutr Diet 2014; 110: 253-263.

[43] Slater TW, Finkielsztein A, Mascarenhas LA, Mehl LC, Butin-Israeli V and Sumagin R. Neutrophil microparticles deliver active myeloperoxidase to injured mucosa to inhibit epithelial wound healing. J Immunol 2017; 198: 2886-2897.

[44] Quigley M and McGuire W. Formula versus donor breast milk for feeding preterm or low birth weight infants. Cochrane Database Syst Rev 2014; Cd002971.

[45] Lucas A and Cole TJ. Breast milk and neonatal necrotising enterocolitis. Lancet 1990; 336: 1519-1523.

[46] Meinzen-Derr J, Poindexter B, Wrage L, Morrow AL, Stoll B and Donovan EF. Role of human milk in extremely low birth weight infants' risk of necrotizing enterocolitis or death. J Perinatol 2009; 29: 57-62.

[47] Hair AB, Peluso AM, Hawthorne KM, Perez J, Smith DP, Khan JY, O'Donnell A, Powers RJ, Lee ML and Abrams SA. Beyond necrotizing enterocolitis prevention: improving outcomes with an exclusive human milk-based diet. Breastfeed Med 2016; 11: 70-74.

[48] Ip S, Chung M, Raman G, Chew P, Magula N, DeVine D, Trikalinos T and Lau J. Breastfeeding and maternal and infant health outcomes in developed countries. Evid Rep Technol Assess (Full Rep) 2007; 1-186.

[49] Sullivan S, Schanler RJ, Kim JH, Patel AL, Trawoger R, Kiechl-Kohlendorfer U, Chan GM, Blanco CL, Abrams S, Cotten CM, Laroia N, Ehrenkranz RA, Dudell G, Cristofalo EA, Meier P, Lee ML, Rechtman DJ and Lucas A. An exclusively human milk-based diet is associated with a lower rate of necrotizing enterocolitis than a diet of human milk and bovine milk-based products. J Pediatr 2010; 156: 562-567, e561.

[50] Admyre C, Johansson SM, Qazi KR, Filen JJ, Lahesmaa R, Norman M, Neve EP, Scheynius A and Gabrielsson S. Exosomes with immune modulatory features are present in human breast milk. J Immunol 2007; 179: 1969-1978.

[51] Chen T, Xie MY, Sun JJ, Ye RS, Cheng X, Sun RP, Wei LM, Li M, Lin DL, Jiang QY, Xi QY and Zhang YL. Porcine milk-derived exosomes promote proliferation of intestinal epithelial cells. Sci Rep 2016; 6: 33862.

[52] Hock A, Miyake H, Li B, Lee C, Ermini L, Koike Y, Chen Y, Maattanen P, Zani A and Pierro A. Breast milk-derived exosomes promote intestinal epithelial cell growth. J Pediatr Surg 2017; 52: 755-759.

[53] Gao HN, Guo HY, Zhang H, Xie XL, Wen PC and Ren FZ. Yak-milk-derived exosomes promote proliferation of intestinal epithelial cells in an hypoxic environment. J Dairy Sci 2019; 102: 985-996.

[54] Li B, Hock A, Wu RY, Minich A, Botts SR, Lee C, Antounians L, Miyake H, Koike Y, Chen Y, Zani A, Sherman PM and Pierro A. Bovine milk-derived exosomes enhance goblet cell activity and prevent the development of experimental necrotizing enterocolitis. PLoS One 2019; 14: e0211431.

[55] Wang L, Shi Z, Wang X, Mu S, Xu X, Shen L and Li P. Protective effects of bovine milk exosomes against oxidative stress in IEC-6 cells. Eur J Nutr 2020; [Epub ahead of print].

[56] Wolf T, Baier SR and Zempleni J. The intestinal transport of bovine milk exosomes is mediated by endocytosis in human colon carcinoma caco-2 cells and rat small intestinal IEC-6 cells. J Nutr 2015; 145: 2201-2206.

[57] Dong P, Zhang Y, Yan DY, Wang Y, Xu X, Zhao YC and Xiao TT. Protective effects of human milk-derived exosomes on intestinal stem cells damaged by oxidative stress. Cell Transplant 2020; 29: 963689720912690.

[58] Wang X, Yan X, Zhang L, Cai J, Zhou Y, Liu H, Hu Y, Chen W, Xu S, Liu P, Chen T, Zhang J, Cao Y, Yu Z and Han S. Identification and peptidomic profiling of exosomes in preterm human milk: insights into necrotizing enterocolitis prevention. Mol Nutr Food Res 2019; e1801247.

[59] Pisano C, Galley J, Elbahrawy M, Wang Y, Farrell A, Brigstock D and Besner GE. Human breast milk-derived extracellular vesicles in the protection against experimental necrotizing enterocolitis. J Pediatr Surg 2020; 55: 54-58.

[60] Miyake H, Lee C, Chusilp S, Bhalla M, Li B, Pitino M, Seo S, O'Connor DL and Pierro A. Human breast milk exosomes attenuate intestinal damage. Pediatr Surg Int 2020; 36: 155-163.

[61] Gao R, Zhang R, Qian T, Peng X, He W, Zheng S, Cao Y, Pierro A and Shen C. A comparison of exosomes derived from different periods breast milk on protecting against intestinal organoid injury. Pediatr Surg Int 2019; 35: 1363-1368.

[62] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136: 215-233.

[63] Kahn S, Liao Y, Du X, Xu W, Li J and Lonnerdal B. Exosomal MicroRNAs in milk from mothers delivering preterm infants survive in vitro digestion and are taken up by human intestinal cells. Mol Nutr Food Res 2018; 62: e1701050.

[64] Xie MY, Hou LJ, Sun JJ, Zeng B, Xi QY, Luo JY, Chen T and Zhang YL. Porcine milk exosome MiRNAs attenuate LPS-induced apoptosis through inhibiting TLR4/NF-κB and p53 pathways in intestinal epithelial cells. J Agric Food Chem 2019; 67: 9477-9491.

[65] Zhou Q, Li M, Wang X, Li Q, Wang T, Zhu Q, Zhou X, Wang X, Gao X and Li X. Immune-related microRNAs are abundant in breast milk exosomes. Int J Biol Sci 2012; 8: 118-123.

[66] Takata A, Otsuka M, Kojima K, Yoshikawa T, Kishikawa T, Yoshida H and Koike K. MicroRNA-22 and microRNA-140 suppress NF-kappaB activity by regulating the expression of NF-kappaB coactivators. Biochem Biophys Res Commun 2011; 411: 826-831.

[67] Hunter CJ and De Plaen IG. Inflammatory signaling in NEC: role of NF-kappaB, cytokines and other inflammatory mediators. Pathophysiology 2014; 21: 55-65.

[68] Mirza AH, Kaur S, Nielsen LB, Størling J, Yarani R, Roursgaard M, Mathiesen ER, Damm P, Svare J, Mortensen HB and Pociot F. Breast milk-derived extracellular vesicles enriched in exosomes from mothers with type 1 diabetes contain aberrant levels of microRNAs. Front Immunol 2019; 10: 2543.

[69] Ng PC, Chan KY, Leung KT, Tam YH, Ma TP, Lam HS, Cheung HM, Lee KH, To KF and Li K. Comparative MiRNA expressional profiles and molecular networks in human small bowel tissues of necrotizing enterocolitis and spontaneous intestinal perforation. PLoS One 2015; 10: e0135737.

[70] Reif S, Elbaum Shiff Y and Golan-Gerstl R. Milk-derived exosomes (MDEs) have a different biological effect on normal fetal colon epithelial cells compared to colon tumor cells in a miRNA-dependent manner. J Transl Med 2019; 17: 325.

[71] Yang M, Song D, Cao X, Wu R, Liu B, Ye W, Wu J and Yue X. Comparative proteomic analysis of milk-derived exosomes in human and bovine colostrum and mature milk samples by iTRAQ-coupled LC-MS/MS. Food Res Int 2017; 92: 17-25.

[72] Larssen P, Wik L, Czarnewski P, Eldh M, Lof L, Ronquist KG, Dubois L, Freyhult E, Gallant CJ, Oelrich J, Larsson A, Ronquist G, Villablanca EJ, Landegren U, Gabrielsson S and Kamali-Moghaddam M. Tracing cellular origin of human exosomes using multiplex proximity extension assays. Mol Cell Proteomics 2017; 16: 502-511.

[73] Rousselle A, Qadri F, Leukel L, Yilmaz R, Fontaine JF, Sihn G, Bader M, Ahluwalia A and Duchene J. CXCL5 limits macrophage foam cell formation in atherosclerosis. J Clin Invest 2013; 123: 1343-1347.

[74] Scarisbrick IA, Epstein B, Cloud BA, Yoon H, Wu J, Renner DN, Blaber SI, Blaber M, Vandell AG and Bryson AL. Functional role of kallikrein 6 in regulating immune cell survival. PLoS One 2011; 6: e18376.

[75] Shoji-Kawata S, Sumpter R, Leveno M, Campbell GR, Zou Z, Kinch L, Wilkins AD, Sun Q, Pallauf K, MacDuff D, Huerta C, Virgin HW, Helms JB, Eerland R, Tooze SA, Xavier R, Lenschow DJ, Yamamoto A, King D, Lichtarge O, Grishin NV, Spector SA, Kaloyanova DV and Levine B. Identification of a candidate therapeutic autophagy-inducing peptide. Nature 2013; 494: 201-206.

[76] Li M, Zhao J, Cao M, Liu R, Chen G, Li S, Xie Y, Xie J, Cheng Y, Huang L, Su M, Xu Y, Zheng M, Zou K, Geng L, Xu W and Gong S. Mast cells-derived MiR-223 destroys intestinal barrier function by inhibition of CLDN8 expression in intestinal epithelial cells. Biol Res 2020; 53: 12.

[77] Munagala R, Aqil F, Jeyabalan J and Gupta RC. Bovine milk-derived exosomes for drug delivery. Cancer Lett 2016; 371: 48-61.

[78] Aqil F, Munagala R, Jeyabalan J, Agrawal AK and Gupta R. Exosomes for the enhanced tissue bioavailability and efficacy of curcumin. Aaps J 2017; 19: 1691-1702.

[79] Agrawal AK, Aqil F, Jeyabalan J, Spencer WA, Beck J, Gachuki BW, Alhakeem SS, Oben K, Munagala R, Bondada S and Gupta RC. Milk-derived exosomes for oral delivery of paclitaxel. Nanomedicine 2017; 13: 1627-1636.

[80] Aqil F, Jeyabalan J, Agrawal AK, Kyakulaga AH, Munagala R, Parker L and Gupta RC. Exosomal delivery of berry anthocyanidins for the management of ovarian cancer. Food Funct 2017; 8: 4100-4107.

[81] Markel TA, Crisostomo PR, Lahm T, Novotny NM, Rescorla FJ, Tector J and Meldrum DR. Stem cells as a potential future treatment of pediatric intestinal disorders. J Pediatr Surg 2008; 43: 1953-1963.

[82] Tayman C, Uckan D, Kilic E, Ulus AT, Tonbul A, Murat Hirfanoglu I, Helvacioglu F, Haltas H, Koseoglu B and Tatli MM. Mesenchymal stem

cell therapy in necrotizing enterocolitis: a rat study. Pediatr Res 2011; 70: 489-494.

[83] Lai RC, Yeo RW and Lim SK. Mesenchymal stem cell exosomes. Semin Cell Dev Biol 2015; 40: 82-88.

[84] Baglio SR, Rooijers K, Koppers-Lalic D, Verweij FJ, Perez Lanzon M, Zini N, Naaijkens B, Perut F, Niessen HW, Baldini N and Pegtel DM. Human bone marrow- and adipose-mesenchymal stem cells secrete exosomes enriched in distinctive miRNA and tRNA species. Stem Cell Res Ther 2015; 6: 127.

[85] Rager TM, Olson JK, Zhou Y, Wang Y and Besner GE. Exosomes secreted from bone marrow-derived mesenchymal stem cells protect the intestines from experimental necrotizing enterocolitis. J Pediatr Surg 2016; 51: 942-947.

[86] Wei J, Zhou Y and Besner GE. Heparin-binding EGF-like growth factor and enteric neural stem cell transplantation in the prevention of experimental necrotizing enterocolitis in mice. Pediatr Res 2015; 78: 29-37.

[87] Watkins DJ, Zhou Y, Matthews MA, Chen L and Besner GE. HB-EGF augments the ability of mesenchymal stem cells to attenuate intestinal injury. J Pediatr Surg 2014; 49: 938-944; discussion 944.

[88] Mao F, Wu Y, Tang X, Kang J, Zhang B, Yan Y, Qian H, Zhang X and Xu W. Exosomes derived from human umbilical cord mesenchymal stem cells relieve inflammatory bowel disease in mice. Biomed Res Int 2017; 2017: 5356760.

[89] McCulloh CJ, Olson JK, Wang Y, Zhou Y, Tengberg NH, Deshpande S and Besner GE. Treatment of experimental necrotizing enterocolitis with stem cell-derived exosomes. J Pediatr Surg 2018; 53: 1215-1220.

[90] Pisano C and Besner GE. Potential role of stem cells in disease prevention based on a murine model of experimental necrotizing enterocolitis. J Pediatr Surg 2019; 54: 413-416.

[91] Li YY, Xu QW, Xu PY and Li WM. MSC-derived exosomal miR-34a/c-5p and miR-29b-3p improve intestinal barrier function by targeting the Snail/Claudins signaling pathway. Life Sci 2020; 118017.

[92] Sun D, Cao H, Yang L, Lin L, Hou B, Zheng W, Shen Z and Song H. MiR-200b in heme oxygenase-1-modified bone marrow mesenchymal stem cell-derived exosomes alleviates inflammatory injury of intestinal epithelial cells by targeting high mobility group box 3. Cell Death Dis 2020; 11: 480.

[93] Yu YJ, Wang XH and Fan GC. Versatile effects of bacterium-released membrane vesicles on mammalian cells and infectious/inflammatory diseases. Acta Pharmacol Sin 2018; 39: 514-533.

[94] Gao XJ, Li T, Wei B, Yan ZX, Hu N, Huang YJ, Han BL, Wai TS, Yang W and Yan R. Bacterial Outer membrane vesicles from dextran sulfate sodium-induced colitis differentially regulate intestinal UDP-glucuronosyltransferase 1A1 partially through toll-like receptor 4/mitogen-activated protein kinase/phosphatidylinositol 3-kinase pathway. Drug Metab Dispos 2018; 46: 292-302.

[95] Nielsen ME, Feechan A, Bohlenius H, Ueda T and Thordal-Christensen H. Arabidopsis ARF-GTP exchange factor, GNOM, mediates transport required for innate immunity and focal accumulation of syntaxin PEN1. Proc Natl Acad Sci U S A 2012; 109: 11443-11448.

[96] Ju S, Mu J, Dokland T, Zhuang X, Wang Q, Jiang H, Xiang X, Deng ZB, Wang B, Zhang L, Roth M, Welti R, Mobley J, Jun Y, Miller D and Zhang HG. Grape exosome-like nanoparticles induce intestinal stem cells and protect mice from DSS-induced colitis. Mol Ther 2013; 21: 1345-1357.

[97] Mu J, Zhuang X, Wang Q, Jiang H, Deng ZB, Wang B, Zhang L, Kakar S, Jun Y, Miller D and Zhang HG. Interspecies communication between plant and mouse gut host cells through edible plant derived exosome-like nanoparticles. Mol Nutr Food Res 2014; 58: 1561-1573.