REVIEW

Exosomes in Food: Health Benefits and Clinical Relevance in Diseases

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ABSTRACT

Exosomes are membrane-bound organelles generally secreted by eukaryotic cells that contain mRNAs, microRNAs, and/or proteins. However, recent studies have reported the isolation of these particles from foods such as lemon, ginger, and milk. Owing to their absorption by intestinal cells and further travel via the bloodstream, exosomes can reach distant organs and affect overall health in both infants and adults. The potential role of food-derived exosomes (FDEs) in alleviating diseases, as well as in modulating the gut microbiota has been shown, but the underlying mechanism is still unknown. Moreover, exosomes may provide biocompatible vehicles for the delivery of anti-cancer drugs, such as doxorubicin. Thus, exosomes may allow medical nutritionists and clinicians to develop safe and targeted therapies for the treatment of various pathologies. The present review introduces FDEs and their contents, highlights their role in disease and infant/adult health, and explores their potential use as therapeutic agents. *Adv Nutr* 2020;11:687–696.

Keywords: exosome, food, cancer, inflammation, therapy

Introduction

The effect of food, particularly its bioactive components, on human health and disease has been extensively studied over the past few decades (1–3). Exosomes, also known as extracellular vesicles (EVs), are a type of bioactive component recently discovered in foods. Exosomes are small (50–300 nm), membrane-bound organelles secreted by the endosomal pathway of eukaryotic cells that facilitate intercellular communication (4). Their lumens contain mRNA, proteins, microRNAs (miRNAs), and long non-coding RNAs (5). Evidence suggests that mRNAs in target cells can translate into functional proteins (6). Additionally, miRNAs, proteins, and metabolites retain their biological activity in the recipient cells. Proteins and RNAs delivered by exosomes can affect the properties of recipient cells, thereby influencing diverse physiological and pathological functions. Exosomes from fruits, vegetables, and foods derived from animal sources have been successfully isolated, for example, exosomes derived from lemon have a diameter of $\leq$70 nm and are morphologically similar to mammalian exosomes (7). Food-derived exosomes (FDEs) can be absorbed in the intestine to act locally. Evidence has also indicated that FDEs may be delivered to other organs via blood flow and function distantly in the recipient cells. Although our understanding of the mechanistic details of selective packaging of FDEs and unloading of their cargo, along with the interaction with target cells is incomplete, these molecules have started to gain attention due to their potential roles in controlling physiological and pathological processes and as therapeutic vehicles. Here, we introduce FDEs and summarize the roles of FDEs in human health and disease, and envision their future applications. As FDEs have not been clearly categorized based on their intrinsic features, studies on FDEs often use synonyms such as "nanoparticles," "exosome-like nanoparticles," "nanovesicles," and "nanoshuttle." In this review, we use the term "food-derived exosomes (FDEs)" to accommodate all the terms mentioned in previous studies, emphasizing that intensive characterization of FDEs would allow their versatile applications.

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Abbreviations used: AMPK, adenosine monophosphate protein kinase, DSS, dextran sodium sulfate; EV, extracellular vesicle; FDE, food-derived exosome; ILV, intralumenal vesicle; miRNA, microRNA; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; siRNA, small interferring RNA.

FDEs

FDEs are a class of EVs found in food, which carry biomolecules for cell-to-cell communication. These small vesicles (50–300 nm) are surrounded by a phospholipid bilayer and are formed in multivesicular bodies in the cells, as intraluminal vesicles (ILVs). The fusion of a multivesicular body with the plasma membrane leads to the secretion of the ILVs to the extracellular environment, therefore becoming "exosomes" (8, 9).

FDEs are exposed to the environment of the digestive tract after food intake. However, the phospholipid bilayer surrounding the FDE protects it from the harsh conditions of the digestive tract, including the acidic environment of the stomach (10) (**Table 1**). Therefore, bioactive substances (miRNA, mRNA, metabolites etc.) in the lumen of the FDE are not accessible to the degradative enzymes of the gut, ensuring their stability. For instance, curcumin encapsulated in exosomes is 4 times more stable than free curcumin (11); therefore, stable FDEs are efficiently absorbed into the intestinal cells. The endocytosis of FDEs has been observed in both Caco-2 cells (human intestinal epithelial cell line) and CT26 cells (mouse intestinal epithelial cell line). Studies using fluorescence microscopy and flow cytometry have also demonstrated that FDEs can be taken up by intestinal macrophages in the mouse model (12) and by in vitro cultures of human macrophages (13). The FDEs absorbed in the intestine can affect the cellular properties locally, but FDEs also reach distal organs via blood circulation, where they function in host tissues and influence the systemic condition of the whole body. To enter the bloodstream, FDEs must cross the endothelial cell barrier of blood vessels. Studies in human umbilical cord vein endothelial cells (HUVEC), which is an established cell line model of blood vessels, showed that FDEs can be endocytosed by these cells. Moreover, a series of independent experiments described the accumulation of FDEs in several organs including the liver, spleen, brain, intestine, stomach, and lungs (**Figure 1**).

Interestingly, FDEs not only transfer their original cargo to the recipient cells but also incorporate into the host delivery system by interacting with endogenous exosomes. Manca et al. (21) labeled milk exosomes with DiR (a fluorescent lipophilic dye) and the miRNAs inside them with ExoGlow. Oral administration of these exosomes in mice showed the accumulation of DiR-labeled exosomes in the liver, whereas miRNAs were accumulated in the brain, kidney, and heart (21, 14). These authors have hypothesized that miRNAs in FDEs may be unloaded into endogenous exosomes, which then carry the cargo to other organs. Moreover, endogenous exosomes may redirect the accumulation of FDEs from one organ to another. For instance, intravenous-injected grapefruit-derived vesicles were accumulated in the liver, but if endogenous exosomes (from peripheral blood) were injected before the injection of grapefruit-derived vesicles, the grapefruit-derived vesicles were accumulated in the lungs, suggesting an interaction between FDEs and endogenous exosomes (22). Further investigation may lead to organ-targeted therapies, particularly therapies for lung disorders.

Another interesting subject of FDE research is their presence in cooked or processed foods. Deep sequencing analyses of pan-fried and pasteurized sirloin, bovine heart, and adrenals have confirmed the presence of miRNA that can potentially target mRNAs in human cells (23). However, whether the stability of miRNAs in cooked or processed food is due to encapsulation by FDEs needs further investigation.

Cancer

The role of FDEs in cancer remains an emerging area of research (**Table 2**). The uptake of FDEs by cancer

TABLE 1 Models of bioavailability and bioactivity of food-derived exosomes

Method/model	Source	Size (nm)
Human vascular endothelial cells (HUVEC) (14)	Cow milk	69
TNO GI[1] in vitro model (15)	Cow milk	200
RAW 264.7 cell line (12)	Ginger, carrot, grape, grapefruit	700
PKH26-labeled FDE administered in mice by gavage (16)	Ginger	102.3–998.3
Caco-2 (human intestinal epithelial cell model) (17)	Milk	30–120
HEK-293 cells (18)	Milk	–
Peripheral blood mononuclear cells (18)	Milk	–
CT26 mouse intestinal epithelial cell line (19)	Grapes	380.5
SW480 (colon cancer cell line) (7)	Citrus limon	50–70
A549 (lung cancer cell line) (7)	Citrus limon	50–70
LAMA84 (peripheral blood bone marrow cell line) (7)	Citrus limon	50–70
In vivo tumor xenograft (7)	Citrus limon	50–70
Mouse model (20)	Broccoli	18.3–118.2
Human feeding study (20)	Broccoli	18.3–118.2
Retro-orbital injection of DiR[2] -labeled exosomes in mice (14)	Cow milk	69

[1]TNO GI, TNO gastrointestinal model is a computer-simulated multi-compartment system to mimic various regions in the gut.
[2]DiR, dioctadecyl tetramethylindotricarbocyanine iodide is a fluorescent lipophilic dye.

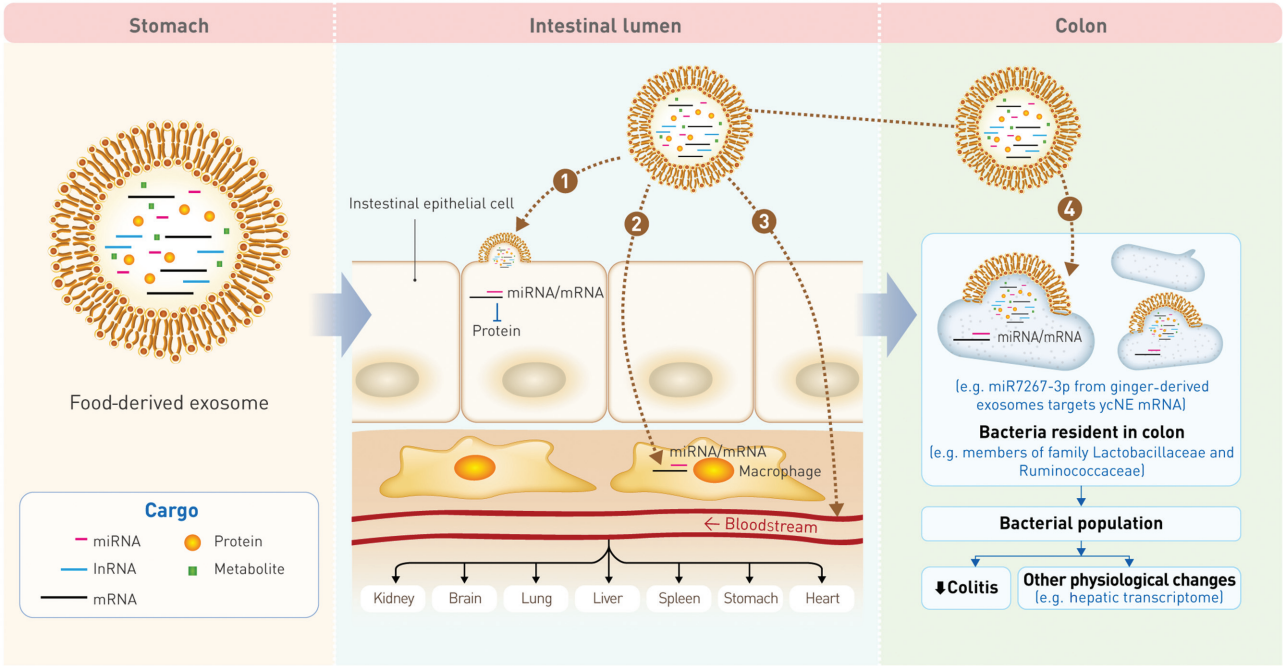

FIGURE 1 Possible fates of food-derived exosomes (FDEs) and their physiological effects on health and disease. Investigations involving FDEs demonstrate that they can empty their contents, particularly miRNA, in intestinal epithelial cells (1) and intestinal macrophages (2). Moreover, they can travel via the bloodstream to reach target organs e.g. lungs, liver (3). Bacteria in the large intestine can also take up FDEs, and directly or indirectly influence the host's health, such as reducing colitis (4). These findings suggest FDEs as potential candidates for designing nutritional therapies and carriers of synthetic/natural therapeutic agents against various pathological conditions.

cells can modify gene expression and reduce cancer-related phenotypes. For instance, lemon-derived exosomes increase TNF-related apoptosis inducing ligand (TRAIL)–mediated apoptosis, which is associated with reduced angiogenic cytokine secretion (e.g. vascular endothelial growth factor-α, IL-6, and IL-8) (5). Moreover, these FDEs target phosphatidic acid-preferring phospholipase A1 and acetyl-CoA carboxylase α (24, 25), revealing the targetable deregulation for cancer therapies. However, which molecules within FDEs have anti-cancer effects has not been investigated. In breast cancer rat models, for example, camel milk-derived exosomes increased the activity of antioxidant enzymes, including catalase, superoxide dismutase, and glutathione peroxidase, in tumors. This, along with a decrease in inducible nitric oxide synthase expression led to a reduction in tumor growth and metastasis (26). Although the transcript and lipid entities in camel milk-derived exosomes are known (27), the component that induces expression changes in xenografted tumors remains unspecified. It is noteworthy that there is compelling contradictory evidence claiming long-term exposure to bovine milk exosomes as a risk factor for cancer, and is supported by epidemiological studies (28). According to Banikezemi et al. [reviewed in (29)], milk exosomes have also been indirectly associated with the progression of hepatocellular carcinoma and melanoma by targeting miR-155, miR-21, and transforming growth factor-β (TGF-β). Given this discrepancy, in-depth investigations on which specific molecule within milk exosomes leads to cellular and molecular changes are yet to be performed. Further investigation is therefore needed to characterize the intrinsic features of bovine milk exosomes and their safety before using these FDEs in therapies or as drug carriers.

In light of evidence showing the therapeutic efficacy of FDEs, clinical trials with FDEs have been performed. Exosomes derived from grape berries demonstrated the alleviation of oral mucositis during chemoradiation of head and neck cancer (NCT01668849) (24). FDEs also provide a solution for the transport of anti-cancer compounds as they overcome the limitations of bioavailability, stability, and safety posed by synthetic liposomes and exosomes from non-food sources. Exploiting this, milk exosomes have been utilized to encapsulate anti-cancer anthocyanins or paclitaxel (30, 31, 32). Lung cancer xenografts have shown a significant reduction in growth upon oral administration of anthocyanin- (30) or paclitaxel-loaded milk exosomes (32). Additionally, these exosomes changed ovarian cancer from cisplatin-resistant to cisplatin-sensitive by decreasing the expression of P-glycoprotein (31). The reassembly of grapefruit-derived exosomes carrying a STAT-3 inhibitor, JSI-124, reduced glioblastoma growth in the mouse brain (33). Doxorubicin or *Survivin* small interfering RNA (siRNA) has been incorporated into reassembled ginger-derived exosomes containing folic acid for targeting colon tumors and papilloma, reducing their volume via increased apoptosis and decreasing their proliferation in mice (34, 35).

TABLE 2 Therapeutic potential of food-derived exosomes in treating diseases[1]

Origin of FDEs	Drug	Route of administration	Therapeutic potential	Experimental validation
Ginger (34)	Doxorubicin	Intravenous	Colon cancer	Colon-26 xenograft
Camel milk (26)	N.A.	Media	Breast cancer	MCF-7
Bovine milk (30)	Berry anthocyanidins	Media	Ovarian cancer	OVCA432
		Oral gavage	Lung cancer	Mice xenograft
		Media	Breast cancer	MDA-MB-231, MCF-7
		Media	Pancreatic cancer	PANC1, Mia PaCa2
		Media	Prostate cancer	PC3, DU145
Milk (36)	Paclitaxel	Oral	Lung cancer	Lung xenografts
Grapefruit (37)	miR-17	Nasal	Brain tumor	Mice with GL-26 grafts
Ginger (34)	siRNA	Intravenous	Oral cancer	Mice xenograft (KB[2] cells)
Grape (38)	N.A.	Oral	Head and neck cancer oral mucositis	Humans (phase I)
Garlic (39)	N.A.	Media	Inflammatory disorders	Mouse macrophages
Ginger (40)	siRNA	Oral	Ulcerative colitis, inflammatory bowel disease	FVB[3] mice
Ginseng (41)	N.A.	Topical, oral	Hair growth	Hair follicle organ culture
Milk (42)	Curcumin	Media	Anti-cancer drug delivery	Caco-2 cells
Grapefruit (33)	Stat3 inhibitor JSI-124	Intranasal	Glioblastoma	GL-26 implanted C57BL/6J
	N.A.	Intravenous	Colon	CT26 implanted BALB[4]/c mice
				SW-620 implanted NOD/SCID[5] mice
Citrus limon (7)	N.A.	Intravenous	CML	NOD/SCID mouse xenograft
Grape (43)	N.A.	Oral	Colorectal cancer	Sprague-Dawley rats
Lavendar (39)	N.A.	Media	Inflammatory disorders	Mouse macrophages
Bovine milk (44)	N.A.	Transdermal	Multiple sclerosis	? (only hypothesis)
Fruit (not specified) (45)	Curcumin	Oral	Colon cancer	Humans (phase I)
Breast milk (46)	N.A.	Oral	Lysinuric protein intolerance	Suggestion (Letter to the Editor)
Vegetables (not specified) (47)	N.A.	Oral	Kashin-Beck disease	—
Apple (48)	N.A.	Media	—	Caco-2 and HEK293 cells
Bovine milk (49)	N.A.	Oral gavage	Arthritis	Mouse model
Ginger rhizome (39)	N.A.	Media	Inflammatory disorders	Macrophages
Bovine milk (50)	N.A.	Oral gavage	Necrotizing enterocolitis	Mouse necrotizing enterocolitis model

[1] Throughout this table, N.A. stands for not applicable.
[2] KB stands for Keratin forming tumor cell line.
[3] FVB stands for Friend leukemia virus-B susceptible albino mouse strain.
[4] BALB stands for Bagg Albino (inbred mouse strain).
[5] NOD/SCID is a mouse-strain homozygous for combined immune deficiency spontaneous mutation $Prkdc^{scid}$ and lack B- and T-lymphocytes.

In addition to direct empirical evidence, some studies proposed the potential involvement of FDEs in the anti-cancer activity of miRNAs. These studies reported some secreted miRNAs from plants with functions related to cancer. Given that secreted miRNAs are mostly preserved in exosomes, the miRNAs secreted from plant cells may also act through FDEs. The oral administration of plant miR-159 to mice reduced the proliferation of breast cancer xenografts by targeting human T cell factors (51). Despite the presence of exosome-encapsulated plant miR-159 in human sera and its negative correlation with the progression of breast cancer, the food source for miR-159 has not been found. Next-generation sequencing of small RNAs from coconut water also demonstrated the presence of miRNAs that target human genes involved in cancer-related pathways. The same study also successfully isolated and characterized the coconut-water-derived exosomes through dynamic light scattering and fluorescence staining with the phospholipid dye "Dil," indicating that these FDEs were 100–200 nm in diameter and surrounded by a phospholipid bilayer, similar to mammalian exosomes. However, how many miRNAs in coconut water are associated with exosomes remain unknown (52). Thus, the comprehensive and systemic analysis of biomolecules in FDEs from various sources needs to be performed in the near future.

Inflammation

In addition to cancer, FDEs also affect inflammatory responses, particularly in the intestines. For example, unidentified molecules in grape berry-derived exosomes promote

the proliferation of Lgr5+ stem cells in intestinal crypts via Wnt signaling activation, followed by a delay in inflammatory colitis in dextran sulfate sodium (DSS)-induced colitis murine models (43). Sulforaphane in broccoli-derived exosomes also reduces intestinal inflammation in colitis models, and broccoli-derived exosomes activate adenosine monophosphate protein kinase (AMPK) phosphorylation in CD11c+ dendritic cells (20).

From a clinical point of view, ginger-derived exosomes with bioactive gingerol and shogaol could be produced commercially on a large scale. These FDEs are well absorbed by the intestinal mucosa, causing a decrease in proinflammatory cytokines IL-1β and IL-6, and an increase in anti-inflammatory cytokine IL-10. Their efficacy has been experimentally demonstrated in mouse inflammatory bowel disease models and may provide a way forward for the treatment of Crohn's disease and ulcerative colitis in humans (53). Furthermore, the production and reassembly of lipids from ginger-derived exosomes are able to provide CD98-targeted siRNA-loaded ginger nanovesicles. The use of this siRNA carrier requires a dose 10,000 times lower than that of naked siRNA and is less toxic than the commercial "DC-Chol/DOPE" liposome formulation used for treating intestinal inflammation (43). Moreover, Chen et al. reported that the lipid components of ginger-derived exosomes alone inhibit inflammasome formation and assembly (39). Since inflammasome assembly is an essential characteristic of disorders such as diabetes mellitus 2 (54) and Alzheimer's disease (55), ginger-derived exosomes could be useful therapies against these disorders (Table 2).

Intestinal macrophages have been shown to take up FDEs from grapefruit, inhibiting the expression of proinflammatory cytokines such as TNF-α and IL-1β. These grapefruit-derived exosomes can be successfully loaded with methotrexate to delay inflammation in a DSS-induced colitis murine model (33). Human macrophages can also take up bovine milk exosomal miRNAs; however, the targets of these exosomal miRNAs warrant further investigation (56). In addition, shogaols in ginger-derived exosomes have been reported to prevent liver damage caused by alcohol. These FDEs activate Nrf2 via the Toll-like receptor/TIR-domain-containing adaptor-inducing interferon-β (TLR/TRIF) pathway to induce the upregulation of detoxifying enzymes in the liver for protection against reactive oxygen species. These results suggest a medical advantage, as no side effects have been reported (16).

Interestingly, exosomal miRNAs from ginger, grapefruit, and curcumin are preferentially taken up by specific bacterial species in the gut, depending upon their lipid composition. miR-7267 from ginger-derived exosomes was reported to regulate the mRNA and protein expression of ycNE in *Lactobacillus rhamnosus*. Since metabolites secreted by bacteria may influence other bacterial populations and ultimately the entire gut microbiome, the effects of FDEs on specific bacterial species could subsequently lead to the modulation of the inflammatory response in colitis. The microbiome of human subjects also changes after intake of ginger-derived exosomes (57). However, the correlations between bacterial population changes and physiological effects need to be further investigated before the clinical application of FDEs.

Nervous System and Musculoskeletal Disorders

Along with expanding research on the roles of FDEs in cancer and inflammation, researchers are now studying the effects of FDEs on disorders related to the nervous and musculoskeletal systems. Mutai et al. (58) found that the depletion of exosomes in a milk diet decreased spatial learning and memory by 130%. It also significantly decreased the acoustic startle response in female mice, establishing an association between milk exosomes and neurocognitive performance. These findings are consistent with that of a study reporting the accumulation of milk exosomal miRNAs, such as miR-34-a, in the mouse brain, suggesting that FDEs mediate the transfer of genetic regulators (21). Multiple sclerosis (MS) is a nervous system disorder in which the myelin-oligodendrocyte glycoprotein (MOG) in the neuronal myelin sheath deteriorates, causing nerve impulse abnormalities. Treatment of MS using bovine milk exosomes has been proposed from studies involving murine MS models. The surfaces of bovine milk exosomes contain the protein butyrophilin which shares 50% sequence homology with MOG. Due to this similarity, butyrophilin can be used to induce antigen-specific tolerance (44); however, the current lack of empirical evidence presents a new direction for further investigation.

Similarly, in murine models of rheumatoid arthritis, it has been demonstrated that the oral intake of bovine milk exosomes containing miR-30-a, miR-223, miR-92-a, β-casein and β-lactoglobulin mRNAs may delay the onset of arthritis (49). This delay was supported by histological evidence of reduced joint inflammation and cartilage deterioration, as well as reduced serum molecular profiles of IL-6 and monocyte chemotactic protein-1 (49). In this regard, the oral intake of milk-derived exosomes by healthy mice led to reduced osteoclastic activity (bone resorption) and an increased number of osteocytes in the bone intertrabecular area (59, 60). These findings need to be further investigated in bone-related disease models such as Osteogenesis Imperfecta and Paget's disease.

In in vitro murine models of C2C12 myoblasts, bovine whey protein exosomes encapsulating miR-30b, miR-149, miR-2881, miR-214, let-7, miR-6520, and miR-16b led to enhanced muscle protein synthesis and increased the diameter of the muscle fiber by modulating the expression of eIF4A, p-Akt (Ser473), and p-AMPK (Thr172) (61). These results may enable the discovery of a clinical solution to alleviate the symptoms of muscular dystrophy and other muscle disorders. Mouse models lacking bovine milk exosomes in their diet showed a decrease in the expression of Rhobtb1 and Socs2 genes in skeletal muscles compared with that of mice fed an exosome-rich diet (62). Although Rhobtb1 is involved in Golgi body maintenance in breast cancer cells (63), Socs2 regulates muscle size via growth hormone signaling (64). Therefore, bovine milk exosomes might be

used as supplement additives for improving muscle health and growth. However, FDEs do not accumulate directly in the skeletal muscles, and therefore the FDE-induced gene-expression changes observed in the skeletal muscle may be attributed to cross-communication between this tissue and other organs. Accumulating evidence on the various effects of FDEs on the nervous system and musculoskeletal abnormalities would promote the improvement of therapeutic strategies and trigger clinical trials.

Infant Health and Disease

In addition to growing knowledge on the roles of FDEs in adult disorders, researchers are also exploring their roles in the health and pathological conditions of infants (**Table 3**). Infants mainly depend on breast milk, especially for the first 6 months (65). Infant formula and colostrum powder based on bovine milk or soy milk are also fed to a large population of infants due to insufficient milk secretion in mothers of preterm infants (66) or other medical or personal reasons (67). Exosomes are found in human breast milk as well as bovine milk and colostrum powder (68). These exosomes resist degradation in the stomach and intestine of preterm and full-term neonates, suggesting a bioactive role of exosomes in ensuring infant health (10). Several studies have revealed the effects of exosomes derived from breast milk on the immune function of infants. The analysis of breast milk-derived exosomes showed that the molecules they contain varies depending upon the maternal allergy status. For example, a lower concentration of mucin-1 was observed in the milk-derived exosomes of sensitized (allergy-induced) mothers compared with the non-sensitized mothers (69). The study also claimed that infants fed breast milk from allergic individuals with a higher concentration of human leukocyte antigen-ABC (HLA-ABC) were likely to develop allergies later, suggesting that allergy-related traits can be transferred from mother to infant via breast milk exosomes. These results further proposed a screening strategy for milk-derived exosomes to prevent allergy development in infants (63). Moreover, human colostrum-derived exosomes also caused an increase in the number of FOXP3 + CD4 + CD25 + T regulatory cells and greater IL-5 secretion in peripheral blood mononuclear cell culture (70). Bovine milk exosomes have an indirect effect on the gut microbiome and the changes observed in the gut microbiome have been correlated with transcriptomic changes of 69 genes in the liver (71). This study may have implications for treating hepatic disorders in infants. Moreover, independent studies have indicated that the same FDEs caused changes in amino acid metabolism and modulated the expression of hepatic amino acid transporters BCAAT1 and BCAAT2 (72). Further studies may provide a solution for low-birth-weight babies and protein accretion in muscles. In an infant animal model, rat milk exosomes could prevent the commonly occurring newborn disease "necrotizing enterocolitis" by increasing proliferating cell nuclear antigen expression and Lgr5+ activity to ensure the proliferation of intestinal epithelial cells (73). However, in these studies, the acting compound in the exosome was not identified.

Proteomic analysis of bovine milk exosomes revealed the presence of 2107 proteins, most of which were involved in immunological functions such as chemokine signaling,

TABLE 3 Significance of milk-derived exosomes in infant health and disease[1]

Source of milk/recipient	Cargo	Cited significance
Bovine/cell (74)	mRNA (αs1-casein, αs2-casein, β-casein, κ-casein, β-lactoglobulin) mi-RNA (miR-101, miR-125b, miR-150, miR-223, miR-24–1, miR-93)	Development of gastrointestinal health and immunity
Rat/rat intestinal cell (75)	—	Understanding of infant intestinal health
Bovine and human colostrum/N.A. (76)	920 kinds of protein (22% genes for stimuli response)	Useful for infant formulations and other dairy products
Bovine milk/N.A. (77)	Proteins for actin cytoskeleton, tight junctions, focal adhesions	Understanding of lactation and immune system
Human/child ≤2 y (69)	HLA-ABC[2], Mucin-1	Understanding the transfer of allergic tendencies from mother to child
Pig/piglet (78)	miR-200c, miR-21, miR-25–3p, miR-27b, miR-30a, miR-375	Pig as a model for diseases in breast and immune system development
Human/N.A. (79)	miR-17–92 cluster and its paralogs	Understanding of immune system development in newborns
Giant panda/N.A. (80)	Let-7, miR-30a, miR-148a dla-miR-1310, dla-miR-2916, dla-miR-319a (from bamboo)	Understanding of the mechanism of neonate development e.g. inner ear, neurodevelopment
Tamar wallaby/neonate (81)	Let-7 (f, a, i), miR-204, miR-30, miR-375	Understanding of developmental events in neonate with reference to lens morphogenesis, nervous system, hormone secretory organs
Human/N.A. (5)	Long noncoding RNA (SNHG8, GAS5, ZFAS1)	Understanding the mechanism of neonate allergies, asthma, autoimmune disorders, obesity development

[1]Throughout this table, N.A. stands for not applicable/not available.
[2]HLA-ABC represents Human Leukocyte Antigen comprising of molecules transcribed from locus A, locus B, and locus C.

cell receptor signaling, and B cell receptor signaling (77). However, improved methods of milk-exosome isolation have resulted in the observation of a smaller number of proteins (82). A total of 633 proteins with distinct functions have been identified from human milk exosomes (73). Studies have also shown that 575 proteins are differentially expressed between the colostrum and mature milk of bovines and humans, suggesting their role in fulfilling the unique requirements of infants during the different phases of development. Of these proteins, 22% are related to stimuli response, whereas others are involved in ribosome biosynthesis and regulation of the actin cytoskeleton (76). Moreover, protein-coding mRNAs with uncertain functions have been characterized in bovine milk exosomes (83), whereas no such findings have been documented in humans (84).

Recent RNA sequencing and quantitative real-time PCR analyses have demonstrated that exosomes in the breast milk of mothers of preterm infants carry 21 preterm-specific miRNAs, including miR-1307, which can resist the peculiar degradative environment of the preterm neonate gut (85). This miRNA is expressed in gastric tissues (86) and is involved in cell proliferation (87). Hence, miR-1307 in preterm milk exosomes might have specific roles in the early developmental stages of the gut of preterm infants. Studies on the function of exosomal miRNAs have been widely performed in mammalian animal models, due to the difficulty of conducting studies in human subjects. Studies on porcine milk exosomes demonstrated that miRNAs can be absorbed by the intestinal enterocytes of piglets. According to the Kyoto encyclopedia of genes and genomes pathway analysis, these miRNAs have roles in transcription, immunity, and metabolic processing (88). The uptake of bovine colostrum exosomes containing 7 immune-related miRNAs (miR-24a, miR-30d, miR-93, miR-106a, miR-181, miR-200a, and miR-451) by RAW264.7 cells, induced the overexpression of 3 of these miRNAs (miR106-a, miR181, and miR-451) and the production of cytokines IL-1β and IL-6 (68). Studies on other mammalian models, such as panda and wallaby, demonstrated changes in the miRNA composition of FDEs across different lactation phases according to the requirements of the developing infant. This suggests that the miRNA of these FDEs are signaling molecules transferrable to neonates (80, 81). Together, the results of the above-mentioned studies indicate that the bioactive compounds of FDEs affect infant health, paving the way for their application in therapy and milk-derived formulations.

Challenges

Promising evidence regarding our basic and applied knowledge of FDEs has led to efforts to devise protocols to isolate and engineer them for therapeutic and diet formulations. Although various methods have been used to isolate FDEs, they are generally isolated via ultracentrifugation followed by sucrose gradient centrifugation, which is a time-consuming laborious process, causes aggregation, and requires a large quantity of sample, making it inappropriate for large-scale application. Moreover, there is concern that this method disrupts FDEs, leading to the loss of their biological activity (89). The enrichment of exosomes based on surface biomarkers has also been widely used, particularly for surface CD63 and TSG101 (89), but to the best of our knowledge, the use of this method as an isolation strategy for FDEs is limited currently, because surface markers of FDEs have not been completely defined.

In addition to the identification of surface markers, the biochemical and physical characterization of FDEs including analysis of their morphology, size, charge, and lipids are still needed, particularly for plant-derived exosomes. The size, charge, and surface structure are further related to renal clearance and biodistribution (90). Although lipidomic studies in FDEs have shown cholesterol and sphingomyelin enrichment in exosomal lipid bilayers from mammals and phospholipid enrichment of FDEs from plant sources, an understanding of differences in uptake abilities based on the lipid layer composition of various exosomes is still lacking (91). The characterization of surface features of FDEs including lipid components and other factors may contribute to our understanding of how FDEs determine their target cells. Furthermore, it must be noted that data on edible FDEs from plants do not clearly indicate which particular cell type secretes exosomes and how processing (e.g. cooking) affects exosomal content. In addition, there is not a broadly accepted type of raw material (e.g. freshly obtained compared with market purchased) and therefore it is difficult to compare the results of the different studies. In the future, questions regarding the standardization of isolation and characterization techniques need to be addressed. It is important to gain a consensus among researchers for universally accepted nomenclature and methods.

Generally, studies presenting FDEs as promising therapies are based on mouse models. These models, with shorter generation times and $<$10% immune system similarity to that of humans, are not sufficient for demonstrating potential long-term effects, particularly in a physiologically relevant environment. Thus, it is necessary to develop improved animal models to understand the long-term effects and to validate the results of in vitro studies. Moreover, clinical trials must be performed to unveil the role of FDEs and estimate the efficacy of FDE-based drug carriers in humans.

Regarding the application of FDEs, bioavailability is also a matter of concern. It is not clear how FDEs are absorbed by cells and efficiently enter the blood circulation, although the passage of FDEs from the gut to the bloodstream has been proven. The targeting of FDEs to specific recipient tissues or cells is another challenge. Currently, determining the preference of an FDE for specific target tissue or cell type is still unresolved. Furthermore, the unpacking of cargo at the final destination of FDEs is not well understood. Therefore, close examination of the absorption, movement, and action of FDEs is required so that the detailed mechanism can be resolved. For clinical applications, it is also important to identify and validate the specific FDE-associated molecule responsible for the therapeutic effect. This will pave the way

for the reconstitution of these FDEs by precisely loading them with the relevant miRNA or protein at a higher concentration than those present in natural FDEs. It is also necessary to determine the yield required to impart a physiologically relevant effect, and the duration of the regulatory effect once the cargo is transferred to the target cell. Therefore, standardization procedures to determine dose-dependent efficacies and time gaps between doses, among other factors, need to be established. Stability/shelf life and storage conditions of FDEs from different sources are other issues that need attention. Stability is important if FDEs are used in nutrition supplements, for example, and it includes both physical and biological stability to retain particle size and activity, respectively. Lyophilization can enhance stability in general, but data on shelf life of FDEs after lyophilization is currently lacking. The advancement of our understanding of FDEs together with improvements in the loading techniques may enhance the use of FDE-based therapies, and allow comparative studies on the efficiency of FDE-based and already established therapies, opening a new paradigm in pharmaceuticals.

Conclusions

A growing body of evidence has revealed the effect of FDEs on both physiological and pathological events, and their potential use in treating human diseases and improving health are emerging, offering new therapeutic solutions. The importance of these novel applications is based on their advantages over existing therapeutic options, including market-available drugs, natural exosomes from non-food sources, stem cells, and synthetic liposomes. Promising results from the use of FDEs, directly or with manipulation, in disorders such as cancer, inflammation, gastrointestinal pathologies, and special conditions like pregnancy (92) pave the way for their application. Improvements in technical expertise and a deeper understanding of the mechanistic details will help the medical community provide a safe and efficient tool for resolving public health issues on a broader scale.

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