The Journal of Nutrition
Recent Advances in Nutritional Sciences

Biological Activities of Extracellular Vesicles and Their Cargos from Bovine and Human Milk in Humans and Implications for Infants[1–3]

Janos Zempleni,* Ana Aguilar-Lozano, Mahrou Sadri, Sonal Sukreet, Sonia Manca, Di Wu, Fang Zhou, and Ezra Mutai

Department of Nutrition and Health Sciences, University of Nebraska-Lincoln, Lincoln, NE

Abstract

Extracellular vesicles (EVs) in milk harbor a variety of compounds, including lipids, proteins, noncoding RNAs, and mRNAs. Among the various classes of EVs, exosomes are of particular interest, because cargo sorting in exosomes is a regulated, nonrandom process and exosomes play essential roles in cell-to-cell communication. Encapsulation in exosomes confers protection against enzymatic and nonenzymatic degradation of cargos and provides a pathway for cellular uptake of cargos by endocytosis of exosomes. Compelling evidence suggests that exosomes in bovine milk are transported by intestinal cells, vascular endothelial cells, and macrophages in human and rodent cell cultures, and bovine-milk exosomes are delivered to peripheral tissues in mice. Evidence also suggests that cargos in bovine-milk exosomes, in particular RNAs, are delivered to circulating immune cells in humans. Some microRNAs and mRNAs in bovine-milk exosomes may regulate the expression of human genes and be translated into protein, respectively. Some exosome cargos are quantitatively minor in the diet compared with endogenous synthesis. However, noncanonical pathways have been identified through which low concentrations of dietary microRNAs may alter gene expression, such as the accumulation of exosomes in the immune cell microenvironment and the binding of microRNAs to Toll-like receptors. Phenotypes observed in infant-feeding studies include higher Mental Developmental Index, Psychomotor Development Index, and Preschool Language Scale-3 scores in breastfed infants than in those fed various formulas. In mice, supplementation with plant-derived MIR-2911 improved the antiviral response compared with controls. Porcine-milk exosomes promote the proliferation of intestinal cells in mice. This article discusses the above-mentioned advances in research concerning milk exosomes and their cargos in human nutrition. Implications for infant nutrition are emphasized, where permitted, but data in infants are limited. *J Nutr* 2017;147:3–10.

Keywords: biological activity, bovine milk, human milk, microRNA, noncoding RNA, exosomes, extracellular vesicles, infants

Introduction

The American Academy of Pediatrics recommends exclusive breastfeeding for the first 6 mo of life (1), but no more than 20% of parents in the United States follow this recommendation (2). Rates of exclusive breastfeeding are higher outside the United States where they may reach 39% (3). The majority of infants in the United States are fed by using formulas based on bovine milk or soy starting a few months into life (2, 4). Great efforts have been made by formula manufacturers to optimize the nutrient content in formulas through fortification. For example, infant formulas may be fortified with essential FAs and milk oligosaccharides, although the results of those efforts may be suboptimal (5, 6).

Many current publications suggest that extracellular vesicles (EVs)[4] in foods, specifically bovine and human milk, harbor a

---

[1] This material is based on work that is supported by the National Institute of Food and Agriculture (NIFA); the USDA, under award 2015-67017-23181; NIH grant 1P20GM104320 and NIFA 2016-67001-25301/NIH R01 DK107264; the Gerber Foundation; the Egg Nutrition Center; the University of Nebraska Agricultural Research Division (Hatch Act); and USDA multistate group W3002 (all to JZ).

[2] Author disclosures: J Zempleni, A Aguilar-Lozano, M Sadri, S Sukreet, S Manca, D Wu, F Zhou, and E Mutai, no conflicts of interest.

[3] The granting agencies had no influence on the study design; the collection, analysis, and interpretation of the data; the writing of the manuscript; or the decision to submit the manuscript for publication.

*To whom correspondence should be addressed. E-mail: jzempleni2@unl.edu.

[4] Abbreviations used: *bta, bos taurus*; DiR, 1,1′-Dioctadecyl-3,3,3′,3′-tetramethyl-indotricarbocyanine iodide; ESCRT, endosomal complex required for transport; EV, extracellular vesicle; MDI, Mental Developmental Index; miRNA, microRNA; MVB, multivesicular body; ncRNA, noncoding RNA; pri-miRNA, primary microRNA; siRNA, small interfering RNA; snRNA, small nuclear RNA; TLR, Toll-like receptor; V_{max}, maximal velocity.

Manuscript received August 15, 2016. Initial review completed September 19, 2016. Revision accepted October 20, 2016.

First published online November 16, 2016; doi:10.3945/jn.116.238949.

heterogeneous array of compounds with biological activities—for example, lipids, proteins, noncoding RNAs (ncRNAs), and mRNAs (7–12). Exosomes are a particularly important class of EVs because they protect labile cargos against degradation and provide a vehicle for cargo uptake through endocytosis of exosomes in virtually all tissues (7, 8, 13–15). This article reviews current advances in our understanding of the biological activities of milk EVs and their cargos, with an emphasis on infant nutrition whenever supporting data are available for human milk, formulas, and infants.

Extracellular Vesicles

The definition and nomenclature of the various classes of EVs are still somewhat in flux, but there is general consensus that exosomes, microvesicles, and apoptotic bodies are distinguished by size, biogenesis, and cargos (16). Among EVs, exosomes (size: 40–100 nm) (14) are of particular interest because their loading with RNA cargos is not a random process but involves sorting mechanisms that favor some cargos over others (17, 18). For example, the abundance of microRNAs (miRNAs) in exosomes secreted by immune cells have a pattern distinct from that in the secreting cells (19). Biogenesis of exosomes is initiated through the inward budding of vesicles (endocytosis) at the plasma membrane and continues during cargo recognition, sorting, and abscission events that involve the endosomal complex required for transport (ESCRT) (**Figure 1**) (18, 20–25). These events lead to the formation of multivesicular bodies (MVBs), which harbor exosomes. During MVB biogenesis, additional cargos, such as miRNAs, are sorted into the vesicles by mechanisms that are incompletely understood. The exosomes inside an MVB can either be secreted into the extracellular space or degraded in lysosomes (26). In addition to ESCRT-dependent processes, exosomes may also be generated in distinct, ESCRT-independent pathways; these alternative pathways depend on ceramide and neutral sphingomyelinase, the enzyme that converts sphingomyelin to ceramide (27). The extravesicular domains of exosome proteins are heavily glycosylated, and glycan features in exosome proteins and receptor cells appear to play essential roles in exosome endocytosis (13, 15, 28). Exosomes and their cargos are recognized for their roles in cell-to-cell communication (14, 29–31). For example, evidence suggests that miR-30d, secreted by the endometrium, is taken up by the preimplantation embryo and might modify its transcriptome in humans (32). However, the old paradigm that exosomes and their RNA cargos are obtained exclusively through endogenous synthesis has currently been challenged by the demonstration that dietary exosomes and RNAs may be bioavailable (15, 33–37).

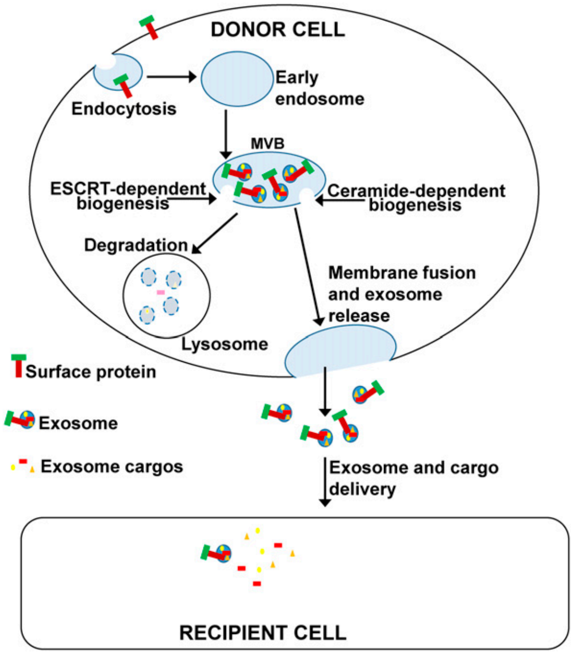

FIGURE 1 Schematic of exosome biogenesis in donor cells and delivery of exosomes and cargos to recipient cells. ESCRT, endosomal complex required for transport; MVB, multivesicular body.

Dietary Exosomes in Milk

Evidence suggests that exosomes in milk are bioavailable and deliver their cargos to tissues in humans, pigs, and rodents. The intestinal uptake of bovine-milk exosomes is facilitated by endocytosis in human colon carcinoma Caco-2 cell and rat primary small intestine IEC-6 cell cultures (15). Transport kinetics were modeled by using the Michaelis-Menten equation. In Caco-2 cells, Michaelis constant (K_m) and maximal velocity (V_{max}) were 55.5 ± 48.6 μg exosomal protein/200 μL medium and 0.08 ± 0.06 ng exosomal protein × 81,750 cells^{-1} × h^{-1}, respectively. In IEC-6 cells, K_m and Vmax were 152 ± 39.5 μg/200 μL and 0.14 ± 0.01 ng exosomal protein × 36,375 cells^{-1} × 30 min^{-1}, respectively. Approximately 140 mg exosome protein can be obtained from 1 L bovine milk; human-milk feeding studies suggest that the postprandial concentrations of miRNAs have a linear dose-response relation at volumes of bovine milk between 0.25 and 1.0 L (34). Glycosylation of exosome proteins and intestinal surface proteins is essential for intestinal uptake of milk exosomes, but the molecular identity of the glycan features is currently unknown (15, 28).

The percentage of milk exosomes that is disassembled in the intestinal mucosa for subsequent transfer of cargos to endogenous exosomes (as opposed to the passage of intact exosomes) is unknown, but it seems likely that both processes occur simultaneously, on the basis of the following observations. Studies in trans-well plates suggest that the ratios of miRNAs in bovine-milk exosomes change during passage in Caco-2 cells, consistent with repackaging of exosome cargos (15). On the other hand, there is also compelling evidence that suggests that a fraction of bovine-milk exosomes escapes dismantling in the intestinal mucosa. When bovine-milk exosomes were labeled with a cyanine-based fluorophore, 1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide (DiR), and administered orally to mice, absorption was apparent. DiR-labeled exosomes were detectable in all tissues examined, but the majority of exosomes accumulated in liver, spleen, and kidneys in athymic nude mice (35). Although that study constituted an important scientific advance, it failed to include important negative controls (i.e., free DiR and unlabeled exosomes). The accumulation of foreign (bovine) exosomes in resident macrophages in the liver and spleen contributes importantly to the distribution of milk exosomes among tissues in mice (38, 39). Importantly, human macrophages also take up bovine-milk exosomes and their RNA cargos (40). The delivery of milk exosomes to peripheral tissues is facilitated by their endocytosis in human vascular endothelial cells (41).

Cargos in Milk Exosomes

As noted above, EVs and, in particular, exosomes harbor a variety of cargos that play important roles in cell-to-cell communication, metabolism, and gene regulation and might also play a role in nutrition. Whereas there appears to be consensus about the bioavailability of milk exosomes, the biological activities and modes of action of the cargos encapsulated in milk exosomes are somewhat more controversial.

miRNAs. Genomic loci coding for miRNAs is usually transcribed by RNA polymerase II to yield primary miRNAs (pri-miRNAs) (**Figure 2**) (42). The RNase III enzymes, Drosha and Dicer, are essential for the synthesis of mature, bioactive miRNAs (43, 44). Drosha liberates hairpins from pri-miRNAs in the cell nucleus. The hairpins are exported into the cytoplasm for processing by Dicer, which removes the loop joining the 3′ and 5′ arms in pri-miRNAs to release an miRNA:miRNA duplex. The canonical model of gene regulation by miRNAs suggests that miRNA:miRNA (passenger:guide strand) duplexes bind to Argonaute proteins, where the passenger strand is then discarded and the guide strand is directed to complementary mRNA targets in the RNA-induced silencing complex (45). Mature miRNAs are ~22 nucleotides long, hybridize with complementary sequences in the 3′-untranslated regions in mRNA, and silence genes through destabilizing mRNA or preventing translation of mRNA (46–48). The sequence complementarity in the "seed region" (nucleotides 2–7) in miRNAs is of particular importance for binding to target transcripts, whereas perfect complementarity is not required in other regions. More than 60% of human protein-coding genes have been under selective pressure to maintain pairing to miRNAs, and 1881 high-confidence miRNAs are encoded in the human genome (49, 50). miRNAs have been implicated in virtually all physiologic (51) and pathologic (52) conditions. Noncanonical pathways of signaling by (milk) miRNAs have also been identified and are discussed below.

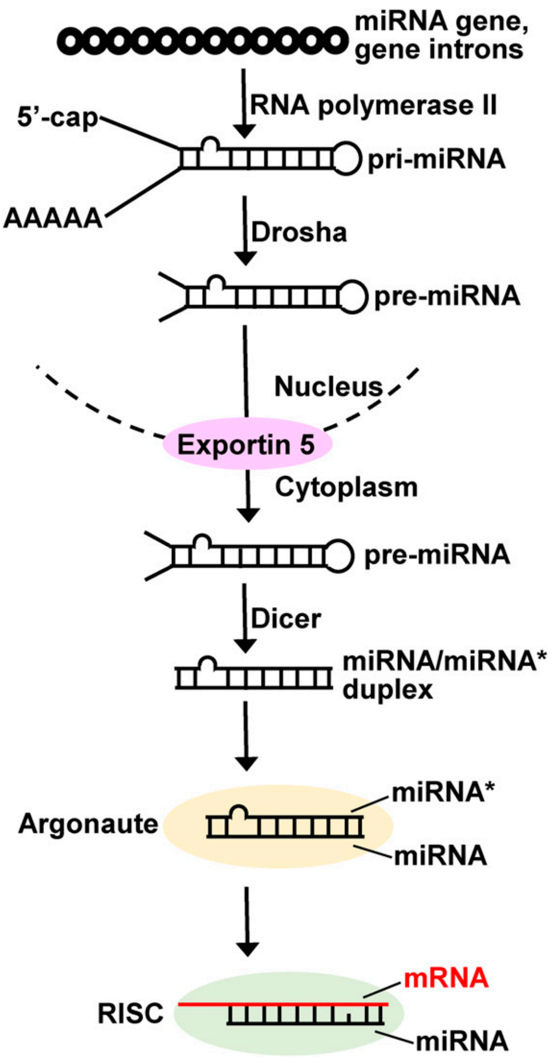

FIGURE 2 Schematic of miRNA maturation. miRNA* denotes the miRNA passenger strand that is typically degraded in miRNA maturation. AAAAA, poly adenine tail; miRNA, microRNA; pre-miRNA, premature microRNA; pri-miRNA, primary microRNA; RISC, RNA-induced silencing complex; 5′-cap, 5-prime cap.

The vast majority of the >400 miRNAs identified to date in bovine milk (7, 53, 54) have nucleotide sequences identical to those in humans (50) and therefore have a strong potential to regulate human genes. The expression of 14 miRNAs differed significantly between cows infected with *Staphylococcus aureus* and healthy controls (54). In addition, the expression of 8 miRNAs was distinct in grazing cattle compared with housed cattle, but these data were collected in plasma as opposed to milk, and the authors speculated that the effects might have been caused by the greater physical activity in grazing cattle (55). The binding of bovine miRNAs to human mRNAs might have implications for human nutrition and for infant formulas, particularly when considering that the content of select miRNAs in various infant formulas is <5% of that in mature human milk (J Zempleni, unpublished observations, 2014). Similar to bovine milk, 308 mature miRNAs and 639 precursor miRNAs have been identified in human milk by next-generation sequencing; in silico predictions suggest that these miRNAs target up to 9074 human genes (56, 57). Many of the miRNAs present in human milk are implicated in immune function (57, 58). Porcine milk also contains a large number of immune-related miRNAs, and the abundance of 10 immune-related miRNAs was greater in colostrum than in mature milk (8).

Traditionally, miRNAs have been considered endogenous regulators of genes—that is, miRNAs synthesized by a given host regulate the expression of genes in that host. An earlier report that MIR168a in rice is bioavailable in mice and humans (33) was initially dismissed because of concerns regarding "ineffective miRNA delivery" and sample contamination (59, 60). More recently, momentum is building in support of the theory that RNAs from plant sources are bioavailable (37, 61–70) and prevent inflammation in humans (71). Note that some genetically modified organisms utilize synthetic miRNA analogs, small interfering RNAs (siRNAs), to achieve gene knockdown in pests (72) [e.g., DvSnf7 siRNA in Monsanto's Smart Stax Pro corn (73)]. It is beyond reasonable doubt that the siRNAs in these organisms are biologically active (i.e., kill pests upon absorption). One could argue that insect digestive systems, unlike the human stomach, are not acidic (74). The counterargument would be that there is compelling evidence that miRNAs, encapsulated in EVs, survive harsh conditions such as low pH (7) and that bovine milk miRNAs resist digestion under simulated gastrointestinal tract conditions (75).

Importantly, our laboratory was the first, to our knowledge, to provide evidence that *1*) humans absorb biologically effective amounts of miRNAs from nutritionally relevant doses of bovine milk, *2*) physiologic concentrations of milk miRNAs affect human gene expression in vivo and in cell cultures, and *3*) endogenous synthesis of miRNAs does not compensate for dietary miRNA deficiency in mice (34). The bovine-specific *bos taurus* (*bta*)-miRs-143, -150, -378, 380-3p, and -1839 were detected in human plasma after a milk meal by using next-generation sequencing (76), which suggests that postprandial increases in plasma miRNA concentrations are caused by the absorption of miRNAs from bovine milk as opposed to milk-induced endogenous synthesis of miRNAs. This interpretation is consistent with the results from studies that assessed the transport mechanisms, bioavailability, and distribution of milk exosomes (see above), and with the detection of >50 plant-specific miRNAs in human plasma and serum (61).

Two recent studies in transgenic mouse models raised questions about the bioavailability of milk miRNAs and their signaling mechanisms, but the robustness of the mouse models that were used in these studies was questioned by an independent laboratory (77). One study used a transgenic mouse model that overexpresses miR-30b in mammary glands and secretes large amounts of miR-30b in milk to assess the bioavailability of miRNAs in mouse pups (78). The authors reported that the elevated concentrations of miR-30b in milk and stomach contents did not result in an increased concentration of miR-30b in select tissues and blood in transgenic mice compared with wild-type controls. However, the authors failed to assess whether miR-30b was encapsulated in exosomes, which is essential for their protection and transport (7, 15). The authors reported a 75% loss of miR-30b in the stomach of the mice with miR-30b overexpression (78), which is consistent with extra-exosomal localization and degradation of miR-30b by low pH in the stomach and enzymes in the intestine. In a second study, miR-375 knockout mouse pups were fostered to wild-type dams and only trace amounts of miR-375 were detected in the plasma of pups after milk feeding (79). The authors of that study acknowledged that "it remains possible that a small level of [miRNA and exosome] uptake does occur" but cautioned that "it is unlikely that milk [mirRNAs] function through canonical [miRNA] silencing." Note that miR-375 lacks a miRNA nucleotide sequence motif [(A/U)($C_{2–4}$)(A/U)] that is essential for miRNA packaging into exosomes (80). The authors of the miR-375 study proposed the enticing theory that noncanonical pathways contribute to miRNA signaling (79), which might explain how small changes in the concentrations of miRNAs elicit major changes in gene expression and phenotypes. Pathways have been identified that explain how femtomolar concentrations of miRNAs elicit biological effects through binding to Toll-like receptors (TLRs) or by surface antigen-mediated delivery of exosomes to immune cells (30, 81). Note that TLRs are widely appreciated for their ability to bind single-stranded (TLR3, TLR7, TLR8) and double-stranded RNA (TLR3) as part of antiviral response mechanisms (82–86), and that the majority of milk exosomes are delivered to macrophages (35). These observations are consistent with a recent report that suggests that MIR-2911 in honeysuckle has antiviral activity (70). The encapsulation of milk miRNAs in exosomes creates a scenario in which miRNAs are not diluted in the extracellular space but specifically delivered to receptor cells for local enrichment (30). Consistent with canonical and noncanonical signaling pathways, when human cell cultures were treated with synthetic miRNA:miRNA duplexes, human exosomes, and bovine-milk exosomes to produce femtomolar to nanomolar concentrations of miRNAs, reporter gene activities decreased compared with vehicle controls (17, 34). More than 400 species of miRNA in milk might have an additive effect when binding to TLRs.

Another enticing possibility for gene regulation by milk miRNAs is the formation of miRNA:mRNA duplexes (87). Note that it is unknown whether miRNAs are present in milk as miRNA:miRNA duplexes, as single-stranded miRNAs, or as miRNA:mRNA duplexes. The canonical pathway of gene regulation by miRNAs suggests that regulation is initiated through the binding of miRNA:miRNA duplexes to Argonaute proteins (see above). However, current evidence suggests that single-stranded miRNAs may also initiate mRNA degradation through the formation and subsequent binding of miRNA:mRNA duplexes to Argonaute 2, in addition to targeting mRNAs through the formation of miRNA:miRNA duplexes (87). The miRNA:mRNA duplex pathway is a transcript-specific alternative to the regulation of gene expression by the binding of single-stranded RNAs (miRNAs) to TLRs.

Our laboratory has produced initial evidence that apparently minor changes in the dietary intake of milk exosomes and their RNA cargos might cause major shifts in the gut microbiome, which might facilitate changes in gene expression and phenotypes, and constitutes another noncanonical miRNA signaling pathway (see below). The observations with regard to the effects of RNA cargos on the gut microbiome are preliminary and await peer review by independent scientists before publication. Taken together, observations with regard to TLRs and, perhaps, the gut microbiome suggest that theoretical calculations of copies of (dietary) miRNAs per cell (79) based on plasma concentrations of individual miRNAs may be an oversimplification of signaling by dietary miRNAs.

ncRNAs other than miRNAs. Bovine milk contains ncRNAs other than miRNAs. A recent analysis by next-generation sequencing revealed the presence of mostly transfer RNAs, but also some ribosomal RNAs, small nuclear RNAs (snRNAs), small nucleolar RNAs, repetitive sequences, and nonannotated sequences (54). Similar patterns were observed in porcine-milk exosomes (88). To the best of our knowledge, no such information is available for human milk. snRNAs play a role in the splicing of pre-mRNA in the spliceosome (89), whereas small nucleolar RNAs primarily guide modifications of ribosomal RNA, transfer RNA, and snRNA (90). The functions of these ncRNAs in bovine milk and human milk are unknown.

mRNAs. Approximately 19,000 and 2600 mRNAs were detected in bovine-milk whey exosomes and whey supernatant, respectively, by using DNA microarrays (40). In our own studies, we identified >3500 mRNAs in bovine-milk exosomes, ~100 of which contained an ATG start codon (D Wu and J Zempleni, unpublished observations, 2016). These observations are important, because mRNAs and expression plasmids in endogenous EVs can be translated to protein in receptor cells (91, 92). Evidence suggests that milk exosomes also deliver expression plasmids to receptor cells for subsequent translation (41). The translation of dietary mRNAs to proteins might have major implications for immune tolerance and food allergies in humans (93, 94). Note that the majority of mRNAs in endogenous exosomes are truncated and enriched in the 3′-untranslated region in human cell cultures and cannot be translated because they lack a start codon (95). However, close to 14% of transcripts in human cells are secreted in exosomes in nontruncated form. These observations in human cell cultures showed patterns similar to those observed in bovine-milk exosomes. Of the ~3500 mRNAs in bovine-milk exosomes noted above, ~4% contained a translation start codon and many were truncated (D Wu and J Zempleni, unpublished observations). Note that truncated mRNAs with a start codon may elicit an immune response, despite not yielding full-length proteins. Preliminary studies suggest that mRNAs in bovine-milk exosomes can be translated into protein in vitro. Future studies will determine whether bovine mRNAs can be translated to protein in human immune cells and in mouse models. To the best of our knowledge, no data are available for the content of mRNAs in human milk.

DNA. Exosomes also contain DNA (14). This observation is somewhat surprising when considering that exosomes originate in MVBs in the cytoplasm. The biological function of exosomal DNA is unknown.

Lipids and proteins. Two major public databases serve as catalogs for the composition and cargos of EVs in various species, ExoCarta and Vesiclepedia (9, 10). ExoCarta has a focus on proteins, lipids, mRNAs, and miRNAs in exosomes, whereas Vesiclepedia includes entries for EVs other than exosomes. The ExoCarta database has 81 entries for proteins in human milk (9), whereas it is unclear how many of the ~1000 entries for lipids in exosomes in the ExoCarta database trace back to the analysis of human milk. A similar number of entries for proteins and lipids can be found in Vesiclepedia (10). Note that glycoproteins on the surface of exosomes play important roles in the homing of exosomes and subsequent uptake by recipient cells (13, 15, 30). The biological and nutritional importance of lipids and proteins in human-milk exosomes remain to be discovered. Further exploration of the roles of exosome lipids and proteins in the regulation of genes and metabolism may prove to be an important line of research.

Stability of Milk Exosomes and Their Cargos

Chemical stability. Encapsulation in milk exosomes protects miRNAs against degradation by RNases and low pH (7, 8), but meaningful losses of miRNA cargos may occur when exosomes are sheared during homogenization of raw milk (96). Shearing of exosomes makes RNA cargos accessible for extraexosomal enzymes, causing their degradation. Additional losses of miRNA cargos have been reported during microwave heating of milk, and the extent of degradation appears to depend on the nucleotide sequence (96). No loss is observed during cold storage (96). In previous feeding studies of exosome-defined diets, miRNA cargos in milk exosomes were depleted through disruption of the exosome membrane by sonication (34). As described for microwaving, the extent of miRNA degradation depended on the nucleotide sequence in miRNAs. The observed degradation bias is consistent with previous studies, which reported that distinct miRNAs have distinct stabilities (97). Sonication-dependent degradation is specific for miRNAs in milk exosomes, because sonication of orange juice caused very little degradation of the rather labile ascorbic acid compared with the rather extensive degradation caused by pasteurization (98). It has been proposed to use the content of miRNAs in infant formula to assess the quality of the products (53).

Physiologic conditions. Various physiologic and pathologic conditions have been identified that cause a change in the concentrations of exosome cargos in milk. To date, the majority of studies used bovine milk as a model and focused on miRNA cargos. For example, 14 miRNAs are differentially expressed in milk from cows infected with *S. aureus* compared with uninfected controls (54). Immune-related miRNAs are present at higher concentrations in colostrum than in mature human milk, and the same pattern was observed in porcine and bovine colostrum and mature milk (8, 57, 58, 99).

Phenotypes of Milk Exosome and Cargo Depletion

Little is known about the phenotypes caused by dietary exosome and cargo depletion. The plasma concentrations of miR-29b and miR-200c were 61% lower in C57BL/6 mice fed an AIN-93G diet containing sonicated, miRNA-deficient milk exosomes compared with mice fed a diet containing normal milk exosomes (34). This observation suggests that endogenous miRNA synthesis does not compensate for dietary miRNA depletion in wild-type mice. We currently study the phenotypes of feeding sonicated, miRNA-depleted diets in mice and have some promising preliminary leads (J Zempleni, unpublished observations). These studies include the role of the gut microbiome as a "transmitter" and "amplifier" of dietary exosomes and cargo signals. It is widely accepted that eukaryotic and prokaryotic micro-organisms communicate with their environment through EVs (100). An uncertainty of the ongoing studies is that phenotypes were caused by feeding sonicated exosomes, and it is currently not possible to attribute phenotypes to a particular class of exosome cargos or changes in exosome morphology. The changes in exosome cargos and morphology are an area of active investigation, but all that has been established as of today is that sonication causes a near-complete degradation of miR-29b and an ~80% degradation of miR-200c (34). The administration of porcine milk–derived exosomes by gavage in mice caused an increase in villus height and crypt depth and a change in gene expression patterns compared with controls. Consistent with these observations, porcine-milk exosomes increased proliferation rates in porcine jejunum IPEC-J2 cell cultures relative to controls (101).

Infant Nutrition

Little is known about the importance of exosomes and their cargos in infant nutrition. As discussed above, a few studies were conducted that cataloged exosome cargos in human milk and reported low concentrations of miRNAs in infant formulas relative to human milk. One longitudinal study compared 3 cohort of infants fed human milk, milk formulas, or soy formulas with regard to developmental endpoints (102), but it is not clear whether the effects of feeding were caused by exosomes and their cargos or other milk compounds. Breastfeeding was associated with a slightly higher score in the Mental Developmental Index (MDI) at ages 6 and 12 mo compared with infants fed milk or soy formulas. In addition, infants who were breastfed had higher Psychomotor Development Index scores, a lower probability to score within the lower MDI quartile, and a higher likelihood to score within the upper quartile for the MDI and Psychomotor Development Index, and a slightly higher Preschool Language Scale-3 score than infants fed formulas at ages 3 and 6 mo. However, that study was not controlled for the length of feeding and brand of infant formula and did not assess dietary exosomes and their cargos. In another study, a pathway-enrichment analysis was performed on the basis of the most abundant miRNAs in human-milk cells and fat portion (103). The analysis suggested that miRNAs in human milk likely affect immunity, growth and development, cell proliferation and apoptosis, lung epithelial progenitor cell differentiation, and epithelial-to-mesenchymal transition (103).

Future Outlook

Although evidence is mounting that exosomes and their cargos in milk and other dietary sources are bioavailable and elicit phenotypes in humans and animals, there are still voices of caution questioning the theory that dietary exosomes and their cargos are absorbed and alter gene expression through binding to mRNA in hosts (59, 60, 78, 79, 104). What are the concepts to be tested and the experiments to be conducted that would provide convincing evidence that dietary exosomes and their cargos have biological activity? We argue that the following studies need to be conducted and will yield compelling answers:

1. Exosome feeding studies should be conducted to determine whether dietary depletion elicits phenotypes.
2. Exosomes endogenously labeled with fluorescent proteins are powerful tools to track the bioavailability of dietary EVs in mouse feeding studies and should be further studied. Such studies should keep in mind that dietary exosomes may be absorbed, but a large fraction of their cargos may be transferred to endogenous exosomes in the intestinal mucosa. That said, dual labeling of exosomes and their cargos would be of particular interest. Note that motifs facilitating the repackaging of dietary exosomes cargos are incompletely understood.
3. The field of dietary exosomes and their cargos still has a strong focus on miRNA cargos, presumably because the first studies that were published in this area focused on miRNA cargos (33, 34). It may be prudent to assess the biological activities of cargos other than miRNAs.
4. Nucleic acid sequencing studies need to continue that unambiguously identify nonhuman sequences in human exosome feeding studies.
5. More research is needed to identify noncanonical pathways of exosome and cargo signaling; some promising leads have already emerged and are discussed above.

Acknowledgments

JZ, AA-L, MS, SS, SM, DW, FZ, and EM wrote the manuscript; and JZ had primary responsibility for the final content. All authors read and approved the final manuscript.

References

1. Eidelman AI. Breastfeeding and the use of human milk: an analysis of the American Academy of Pediatrics 2012 Breastfeeding Policy Statement. Breastfeed Med 2012;7:323–4.
2. CDC. CDC national immunization surveys. Breastfeeding among U.S. children born 2002–2012. 2015 [database on the Internet; cited 2016 Jul 4]. Available from: http://www.cdc.gov/breastfeeding/data/nis_data/index.htm.
3. Cai X, Wardlaw T, Brown DW. Global trends in exclusive breastfeeding. Int Breastfeed J 2012;7:12.
4. US Department of Health and Human Services, National Institute of Environmental Health and Safety. Soy infant formula. Research Triangle Park (NC); 2013 [cited 2013 Nov 7]. Available from: http://www.niehs.nih.gov/health/topics/agents/sya-soy-formula/.
5. Kent G. Regulating fatty acids in infant formula: critical assessment of U.S. policies and practices. Int Breastfeed J 2014;9:2.
6. Vandenplas Y, Zakharova I, Dmitrieva Y. Oligosaccharides in infant formula: more evidence to validate the role of prebiotics. Br J Nutr 2015;113:1339–44.
7. Izumi H, Kosaka N, Shimizu T, Sekine K, Ochiya T, Takase M. Bovine milk contains microRNA and messenger RNA that are stable under degradative conditions. J Dairy Sci 2012;95:4831–41.
8. Gu Y, Li M, Wang T, Liang Y, Zhong Z, Wang X, Zhou Q, Chen L, Lang Q, He Z, et al. Lactation-related microRNA expression profiles of porcine breast milk exosomes. PLoS One 2012;7:e43691.
9. Mathivanan S, Fahner CJ, Reid GE, Simpson RJ. ExoCarta 2012: database of exosomal proteins, RNA and lipids. Nucleic Acids Res 2012;40:D1241–4.
10. Vesiclepedia version 2.1: CD36. 2013 [database on the Internet; cited 2014 Jul 15]. Available from: http://microvesicles.org/gene_summary?gene_id=948.
11. Record M, Carayon K, Poirot M, Silvente-Poirot S. Exosomes as new vesicular lipid transporters involved in cell-cell communication and various pathophysiologies. Biochim Biophys Acta 2014;1841:108–20.
12. Lydic TA, Townsend S, Adda CG, Collins C, Mathivanan S, Reid GE. Rapid and comprehensive 'shotgun' lipidome profiling of colorectal cancer cell derived exosomes. Methods 2015;87:83–95.
13. Escrevente C, Keller S, Altevogt P, Costa J. Interaction and uptake of exosomes by ovarian cancer cells. BMC Cancer 2011;11:108.
14. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol 2013;200:373–83.
15. Wolf T, Baier SR, 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;145:2201–6.
16. Lötvall J, Hill AF, Hochberg F, Buzas EI, Di Vizio D, Gardiner C, Gho YS, Kurochkin IV, Mathivanan S, Quesenberry P, et al. Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles. J Extracell Vesicles 2014;3:26913.
17. Stevanato L, Thanabalasundaram L, Vysokov N, Sinden JD. investigation of content, stoichiometry and transfer of miRNA from human neural stem cell line derived exosomes. PLoS One 2016;11:e0146353.
18. Abels ER, Breakefield XO. Introduction to extracellular vesicles: biogenesis, RNA cargo selection, content, release, and uptake. Cell Mol Neurobiol 2016;36:301.
19. Squadrito ML, Baer C, Burdet F, Maderna C, Gilfillan GD, Lyle R, Ibberson M, De Palma M. Endogenous RNAs modulate microRNA sorting to exosomes and transfer to acceptor cells. Cell Reports 2014;8:1432–46.
20. El Andaloussi S, Mager I, Breakefield XO, Wood MJ. Extracellular vesicles: biology and emerging therapeutic opportunities. Nat Rev Drug Discov 2013;12:347–57.
21. Schmidt O, Teis D. The ESCRT machinery. Curr Biol 2012;22:R116–20.
22. Babst M. MVB vesicle formation: ESCRT-dependent, ESCRT-independent and everything in between. Curr Opin Cell Biol 2011;23:452–7.
23. Hurley JH, Hanson PI. Membrane budding and scission by the ESCRT machinery: it's all in the neck. Nat Rev Mol Cell Biol 2010;11:556–66.
24. Adell MA, Teis D. Assembly and disassembly of the ESCRT-III membrane scission complex. FEBS Lett 2011;585:3191–6.
25. Morita E, Sandrin V, McCullough J, Katsuyama A, Baci Hamilton I, Sundquist WI. ESCRT-III protein requirements for HIV-1 budding. Cell Host Microbe 2011;9:235–42.
26. Hurley JH, Odorizzi G. Get on the exosome bus with ALIX. Nat Cell Biol 2012;14:654–5.
27. Bobrie A, Colombo M, Raposo G, Thery C. Exosome secretion: molecular mechanisms and roles in immune responses. Traffic 2011;12:1659–68.
28. Sukreet S, Zhang H, Adamec J, Cui J, Zempleni J. Identification of glycoproteins on the surface of cow's milk exosomes that mediate the uptake of exosomes into human colon carcinoma Caco-2 cells. ASN Scientific Sessions and Annual Meeting at Experimental Biology 2016; 2016 Apr 2–6; San Diego, CA. FASEB J 2016;30(Suppl 1):125.1.
29. Mittelbrunn M, Sanchez-Madrid F. Intercellular communication: diverse structures for exchange of genetic information. Nat Rev Mol Cell Biol 2012;13:328–35.
30. Bryniarski K, Ptak W, Jayakumar A, Pullmann K, Caplan MJ, Chairoungdua A, Lu J, Adams BD, Sikora E, Nazimek K, et al. Antigen-specific, antibody-coated, exosome-like nanovesicles deliver suppressor T-cell microRNA-150 to effector T cells to inhibit contact sensitivity. J Allergy Clin Immunol 2013;132:170–81.
31. Bryniarski K, Ptak W, Martin E, Nazimek K, Szczepanik M, Sanak M, Askenase PW. Free extracellular miRNA functionally targets cells by transfecting exosomes from their companion cells. PLoS One 2015;10:e0122991.
32. Vilella F, Moreno-Moya JM, Balaguer N, Grasso A, Herrero M, Martinez S, Marcilla A, Simon C. Hsa-miR-30d, secreted by the human endometrium, is taken up by the pre-implantation embryo and might modify its transcriptome. Development 2015;142:3210–21.
33. Zhang L, Hou D, Chen X, Li D, Zhu L, Zhang Y, Li J, Bian Z, Liang X, Cai X, et al. Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. Cell Res 2012;22:107–26.
34. Baier SR, Nguyen C, Xie F, Wood JR, Zempleni J. MicroRNAs are absorbed in biologically meaningful amounts from nutritionally relevant doses of cow's milk and affect gene expression in peripheral blood mononuclear cells, HEK-293 kidney cell cultures, and mouse livers. J Nutr 2014;144:1495–500.

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

36. Manca S, Giraud D, Zempleni J. Bioavailability and biodistribution of fluorophore-labeled exosomes from cow's milk after intravenous and oral administration in C57Bl/6J mice. ASN Scientific Sessions and Annual Meeting at Experimental Biology 2016; 2016 Apr 2–6; San Diego, CA. FASEB J 2016;30(Suppl 1):690.8.

37. Chen X, Dai GH, Ren ZM, Tong YL, Yang F, Zhu YQ. Identification of dietetically absorbed rapeseed (*Brassica campestris*, L.) bee pollen microRNAs in serum of mice. Biomed Res Int 2016; 2016:5413849.

38. Wiklander OP, Nordin JZ, O'Loughlin A, Gustafsson Y, Corso G, Mager I, Vader P, Lee Y, Sork H, Seow Y, et al. Extracellular vesicle in vivo biodistribution is determined by cell source, route of administration and targeting. J Extracell Vesicles 2015;4:26316.

39. Imai T, Takahashi Y, Nishikawa M, Kato K, Morishita M, Yamashita T, Matsumoto A, Charoenviriyakul C, Takakura Y. Macrophage-dependent clearance of systemically administered B16BL6-derived exosomes from the blood circulation in mice. J Extracell Vesicles 2015;4:26238.

40. Izumi H, Tsuda M, Sato Y, Kosaka N, Ochiya T, Iwamoto H, Namba K, Takeda Y. Bovine milk exosomes contain microRNA and mRNA and are taken up by human macrophages. J Dairy Sci 2015;98:2920–33.

41. Kusuma RJ, Manca S, Friemel T, Sukreet S, Nguyen C, Zempleni J. Human vascular endothelial cells transport foreign exosomes from cow's milk by endocytosis. Am J Physiol Cell Physiol 2016;310: C800–7.

42. Ameres SL, Zamore PD. Diversifying microRNA sequence and function. Nat Rev Mol Cell Biol 2013;14:475–88.

43. Lund E, Dahlberg JE. Substrate selectivity of exportin 5 and Dicer in the biogenesis of microRNAs. Cold Spring Harb Symp Quant Biol 2006;71:59–66.

44. Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Radmark O, Kim S, et al. The nuclear RNase III Drosha initiates microRNA processing. Nature 2003;425:415–9.

45. Schirle NT, Sheu-Gruttadauria J, MacRae IJ. Gene regulation: structural basis for microRNA targeting. Science 2014;346:608–13.

46. Chen K, Rajewsky N. The evolution of gene regulation by transcription factors and microRNAs. Nat Rev Genet 2007;8:93–103.

47. Jing Q, Huang S, Guth S, Zarubin T, Motoyama A, Chen J, Di Padova F, Lin SC, Gram H, Han J. Involvement of microRNA in AU-rich element-mediated mRNA instability. Cell 2005;120:623–34.

48. Djuranovic S, Nahvi A, Green R. miRNA-mediated gene silencing by translational repression followed by mRNA deadenylation and decay. Science 2012;336:237–40.

49. Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 2009; 19:92–105.

50. University of Manchester mirRBase 21. 2014 [database on the Internet; cited 2014 Aug 28]. Available from: http://www.mirbase.org/index.shtml.

51. Vojtech L, Woo S, Hughes S, Levy C, Ballweber L, Sauteraud RP, Strobl J, Westerberg K, Gottardo R, Tewari M, et al. Exosomes in human semen carry a distinctive repertoire of small non-coding RNAs with potential regulatory functions. Nucleic Acids Res 2014;42:7290–304.

52. Danielson KM, Das S. Extracellular vesicles in heart disease: excitement for the future? Exosomes Microvesicles 2014;2:1.

53. Chen X, Gao C, Li H, Huang L, Sun Q, Dong Y, Tian C, Gao S, Dong H, Guan D, et al. Identification and characterization of microRNAs in raw milk during different periods of lactation, commercial fluid, and powdered milk products. Cell Res 2010;20:1128–37.

54. Sun J, Aswath K, Schroeder SG, Lippolis JD, Reinhardt TA, Sonstegard TS. MicroRNA expression profiles of bovine milk exosomes in response to *Staphylococcus aureus* infection. BMC Genomics 2015;16:806.

55. Muroya S, Ogasawara H, Hojito M. Grazing affects exosomal circulating microRNAs in cattle. PLoS One 2015;10:e0136475.

56. Munch EM, Harris RA, Mohammad M, Benham AL, Pejerrey SM, Showalter L, Hu M, Shope CD, Maningat PD, Gunaratne PH, et al. Transcriptome profiling of microRNA by Next-Gen deep sequencing reveals known and novel miRNA species in the lipid fraction of human breast milk. PLoS One 2013;8:e50564.

57. Zhou Q, Li M, Wang X, Li Q, Wang T, Zhu Q, Zhou X, Wang X, Gao X, Li X. Immune-related microRNAs are abundant in breast milk exosomes. Int J Biol Sci 2012;8:118–23.

58. Kosaka N, Izumi H, Sekine K, Ochiya T. microRNA as a new immune-regulatory agent in breast milk. Silence 2010;1:7.

59. Snow JW, Hale AE, Isaacs SK, Baggish AL, Chan SY. Ineffective delivery of diet-derived microRNAs to recipient animal organisms. RNA Biol 2013;10:1107–16.

60. Dickinson B, Zhang Y, Petrick JS, Heck G, Ivashuta S, Marshall WS. Lack of detectable oral bioavailability of plant microRNAs after feeding in mice. Nat Biotechnol 2013;31:965–7.

61. Lukasik A, Zielenkiewicz P. In silico identification of plant miRNAs in mammalian breast milk exosomes—a small step forward? PLoS One 2014;9:e99963.

62. Beatty M, Guduric-Fuchs J, Brown E, Bridgett S, Chakravarthy U, Hogg RE, Simpson DA. Small RNAs from plants, bacteria and fungi within the order Hypocreales are ubiquitous in human plasma. BMC Genomics 2014;15:933.

63. Liang G, Zhu Y, Sun B, Shao Y, Jing A, Wang J, Xiao Z. Assessing the survival of exogenous plant microRNA in mice. Food Sci Nutr 2014;2:380–8.

64. Wang K, Li H, Yuan Y, Etheridge A, Zhou Y, Huang D, Wilmes P, Galas D. The complex exogenous RNA spectra in human plasma: an interface with human gut biota? PLoS One 2012;7:e51009.

65. Yang J, Farmer LM, Agyekum AA, Hirschi KD. Detection of dietary plant-based small RNAs in animals. Cell Res 2015;25:517–20.

66. Ju S, Mu J, Dokland T, Zhuang X, Wang Q, Jiang H, Xiang X, Deng ZB, Wang B, Zhang L, et al. Grape exosome-like nanoparticles induce intestinal stem cells and protect mice from DSS-induced colitis. Mol Ther 2013;21:1345–57.

67. Mu J, Zhuang X, Wang Q, Jiang H, Deng ZB, Wang B, Zhang L, Kakar S, Jun Y, Miller D, et al. Interspecies communication between plant and mouse gut host cells through edible plant derived exosome-like nanoparticles. Mol Nutr Food Res 2014;58:1561–73.

68. Mlotshwa S, Pruss GJ, MacArthur JL, Endres MW, Davis C, Hofseth LJ, Pena MM, Vance V. A novel chemopreventive strategy based on therapeutic microRNAs produced in plants. Cell Res 2015; 25:521–4.

69. Chin AR, Fong MY, Somlo G, Wu J, Swiderski P, Wu X, Wang SE. Cross-kingdom inhibition of breast cancer growth by plant miR159. Cell Res 2016;26:217–28.

70. Zhou Z, Li X, Liu J, Dong L, Chen Q, Liu J, Kong H, Zhang Q, Qi X, Hou D, et al. Honeysuckle-encoded atypical microRNA2911 directly targets influenza A viruses. Cell Res 2015;25:39–49.

71. Cavalieri D, Rizzetto L, Tocci N, Rivero D, Asquini E, Si-Ammour A, Bonechi E, Ballerini C, Viola R. Plant microRNAs as novel immunomodulatory agents. Sci Rep 2016;6:25761.

72. Heinemann JA, Agapito-Tenfen SZ, Carman JA. A comparative evaluation of the regulation of GM crops or products containing dsRNA and suggested improvements to risk assessments. Environ Int 2013;55:43–55.

73. Monsanto, Inc. Petition for determination of nonregulated status for corn rootworm protected and glyphosate tolerant MON 87411 maize. St. Louis: USDA Animal and Plant Health Inspection Service; 2013. [cited 2015 May 1]. Available from: https://www.aphis.usda.gov/brs/aphisdocs/13_29001p.pdf.

74. Engel P, Moran NA. The gut microbiota of insects—diversity in structure and function. FEMS Microbiol Rev 2013;37:699–735.

75. Benmoussa A, Lee CHC, Laffont B, Savard P, Laugier J, Boilard E, Gilbert C, Fliss I, Provost P. Commercial dairy cow milk microRNAs resist digestion under simulated gastrointestinal tract conditions. J Nutr 2016;146:2206–15.

76. Shu J, Chiang K, Zempleni J, Cui J. Computational characterization of exogenous microRNAs that can be transferred into human circulation. PLoS One 2015;10:e0140587.

77. Melnik BC, Kakulas F, Geddes DT, Hartmann PE, John SM, Carrera-Bastos P, Cordain L, Schmitz G. Milk miRNAs: simple nutrients or systemic functional regulators? Nutr Metab (Lond) 2016;13:42.

78. Laubier J, Castille J, Le Guillou S, Le Provost F. No effect of an elevated miR-30b level in mouse milk on its level in pup tissues. RNA Biol 2015;12:26–9.

79. Title AC, Denzler R, Stoffel M. Uptake and function studies of maternal milk-derived microRNAs. J Biol Chem 2015;290:23680–91.

80. Shurtleff M. Cell-free packaging of microRNAs into exosomes reveals Y-box protein I as a critical sorting factor. Annual meeting of the American Society for Cell Biology; 2015 Dec 12; San Diego, CA [conference abstract]. American Society for Cell Biology 2015 ASCB Meeting abstracts. Mol Biol Cell 2015;26:B1110.

81. Fabbri M, Paone A, Calore F, Galli R, Gaudio E, Santhanam R, Lovat F, Fadda P, Mao C, Nuovo GJ, et al. MicroRNAs bind to Toll-like receptors to induce prometastatic inflammatory response. Proc Natl Acad Sci USA 2012;109:E2110–6.

82. Takeda K, Akira S. Toll-like receptors in innate immunity. Int Immunol 2005;17:1–14.

83. Potter JA, Garg M, Girard S, Abrahams VM. Viral single stranded RNA induces a trophoblast pro-inflammatory and antiviral response in a TLR8-dependent and -independent manner. Biol Reprod 2015; 92:17.

84. Tatematsu M, Nishikawa F, Seya T, Matsumoto M. Toll-like receptor 3 recognizes incomplete stem structures in single-stranded viral RNA. Nat Commun 2013;4:1833.

85. Zhang SY, Herman M, Ciancanelli MJ, Perez de Diego R, Sancho-Shimizu V, Abel L, Casanova JL. TLR3 immunity to infection in mice and humans. Curr Opin Immunol 2013;25:19–33.

86. Gibbert K, Francois S, Sigmund AM, Harper MS, Barrett BS, Kirchning CJ, Lu M, Santiago ML, Dittmer U. Friend retrovirus drives cytotoxic effectors through Toll-like receptor 3. Retrovirology 2014;11:126.

87. Janas MM, Wang B, Harris AS, Aguiar M, Shaffer JM, Subrahmanyam YV, Behlke MA, Wucherpfennig KW, Gygi SP, Gagnon E, et al. Alternative RISC assembly: binding and repression of microRNA-mRNA duplexes by human Ago proteins. RNA 2012;18:2041–55.

88. Chen T, Xi QY, Ye RS, Cheng X, Qi QE, Wang SB, Shu G, Wang LN, Zhu XT, Jiang QY, et al. Exploration of microRNAs in porcine milk exosomes. BMC Genomics 2014;15:100.

89. Guo Z, Karunatilaka KS, Rueda D. Single-molecule analysis of protein-free U2–U6 snRNAs. Nat Struct Mol Biol 2009;16:1154–9.

90. Maden BE, Hughes JM. Eukaryotic ribosomal RNA: the recent excitement in the nucleotide modification problem. Chromosoma 1997;105:391–400.

91. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9:654–9.

92. Kanada M, Bachmann MH, Hardy JW, Frimannson DO, Bronsart L, Wang A, Sylvester MD, Schmidt TL, Kaspar RL, Butte MJ, et al. Differential fates of biomolecules delivered to target cells via extracellular vesicles. Proc Natl Acad Sci USA 2015;112:E1433–42.

93. National Institute of Allergy and Infectious Diseases. Immune tolerance. 2014 [cited 2016 Jan 5]. Available from: https://www.niaid.nih.gov/topics/immunesystem/Pages/immuneTolerance.aspx.

94. National Institute of Allergy and Infectious Diseases. Food allergy. 2015 [cited 2016 Jan 5]. Available from: https://www.niaid.nih.gov/topics/foodallergy/Pages/default.aspx.

95. Batagov AO, Kurochkin IV. Exosomes secreted by human cells transport largely mRNA fragments that are enriched in the 3′-untranslated regions. Biol Direct 2013;8:12.

96. Howard KM, Jati Kusuma R, Baier SR, Friemel T, Markham L, Vanamala J, Zempleni J. Loss of miRNAs during processing and storage of cow's (*Bos taurus*) milk. J Agric Food Chem 2015;63: 588–92.

97. Mi QS, Weiland M, Qi RQ, Gao XH, Poisson LM, Zhou L. Identification of mouse serum miRNA endogenous references by global gene expression profiles. PLoS One 2012;7:e31278.

98. Tiwari BK, O'Donnell CP, Muthukumarappan K, Cullen PJ. Ascorbic acid degradation kinetics of sonicated juice during storage and comparison with thermally pasteurised juice. Food Sci Technol (Campinas) 2009;42:700–4.

99. Sun Q, Chen X, Yu J, Zen K, Zhang CY, Li L. Immune modulatory function of abundant immune-related microRNAs in microvesicles from bovine colostrum. Protein Cell 2013;4:197–210.

100. Wolf JM, Casadevall A. Challenges posed by extracellular vesicles from eukaryotic microbes. Curr Opin Microbiol 2014;22:73–8.

101. Chen T, Xie MY, Sun JJ, Ye RS, Cheng X, Sun RP, Wei LM, Li M, Lin DL, Jiang QY, et al. Porcine milk-derived exosomes promote proliferation of intestinal epithelial cells. Sci Rep 2016;6:33862.

102. Andres A, Casey PH, Cleves MA, Badger TM. Body fat and bone mineral content of infants fed breast milk, cow's milk formula, or soy formula during the first year of life. J Pediatr 2013;163:49–54.

103. Alsaweed M, Lai CT, Hartmann PE, Geddes DT, Kakulas F. Human milk miRNAs primarily originate from the mammary gland resulting in unique miRNA profiles of fractionated milk. Sci Rep 2016;6:20680.

104. Auerbach A, Vyas G, Li A, Halushka M, Witwer K. Uptake of dietary milk miRNAs by adult humans: a validation study. F1000Res 2016; 5:721.