Review Article

How exosomes in human breast milk may influence breast cancer risk

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Abstract: Breast milk has a primary function to deliver nutrition to a newborn, but may also be useful to monitor the health of the lactating breast. It is known that pregnancy increases a woman's breast cancer risk in the short term, and for women whose first pregnancy comes after age 30–35 years, for the long term. Cell-cell communication occurs in a variety of ways. A primary way for cells to communicate over distance is by the transfer of intracellular contents from one cell to another through the extracellular vesicles that cells secrete into body fluids. Exosomes are extracellular vesicles that are secreted by all cell types (with the possible exception of red blood cells) into a nearby body fluid, including milk. Exosomes provide cell-cell communication during physiologic processes such as lactation, but also in breast cancer. In this review we discuss what is known about milk exosomes, and how assessment of their contents may provide insight into the health of both the infant and the mother.

Keywords: Pregnancy-associated breast cancer; epithelial mesenchymal transition (EMT); transforming growth factor beta (TGFβ); breast milk; exosomes

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Introduction

The primary function of breast milk is to assist the newborn with its growth and immunity (1). On the other hand, information gained from the study of what is contained in milk may provide insight into the health of the human breast (2). Exosomes have been identified in the milk of many mammalian species (3). Most studies of milk exosomes involve how they assist the infant. There are relatively few studies addressing how exosomes might also influence the breast. As such, the primary focus of this review will be on studies that assess how exosomes may influence the mother's breast cancer risk, with a secondary focus on studies evaluating milk exosomes that influence the infant.

Exosomes are an important type of extracellular vesicle. They were first clearly described in 1983 (4), and the term "exosomes" was first used in 1987 (5). Nonetheless, analysis of exosome-like extracellular vesicles had been reported at least since the early 1970s. Exosomes contain a membrane and a variety of intracellular components, including proteins, mRNA, miRNA carbohydrates, and lipids, that can be transferred to other cells nearby or at distance (6). While some exosomes are quickly degraded, others are not and can travel in breast milk and other body fluids. Exosomes are known to influence immune function through their ability to present antigens to immune cells, thereby assisting in an infant's immunity (7,8). Exosomes are used by cancer cells to relay information which can foster tumor growth, migration, and cell breakdown (9). One possible mechanism by which exosomes may promote tumor growth is through their ability to synthesize ATP and transfer this ability to other cells, such as tumor cells (10).

Pregnancy influences breast cancer risk such that for at least five years after giving birth, a women's risk of developing breast cancer is higher than if she had never given birth (2). Long term breast cancer risk of a parous

woman whose first childbirth was in her 30's or later remains higher than a nulliparous woman for 30–50 years, but becomes lower than a nulliparous woman for a parous woman whose first childbirth was in her teens or early 20's (11). Thus, pregnancy increases short term breast cancer risk (compared to nulliparity) regardless of when a woman's first full term pregnancy (FFTP) occurs. Long term risk for parous women depends on the age when their first live birth occurred.

The reason(s) why parous women have an increased short term breast cancer risk remain speculative and are likely multi-factorial. First, there are higher levels during pregnancy of multiple breast growth promoting hormones, including estrogen, progesterone, and insulin like growth factor 1 (IGF1), which increase cell division of both normal and abnormal breast epithelial cells. Second, during breast involution at the end of lactation, the breast microenvironment mimics that of a healing wound, which is tumor promotional (11).

The reason why older first time mothers have a higher risk of developing breast cancer than younger first term mothers relates to the length of time the breast is exposed to mutagens and random mutational events before terminal differentiation, which occurs with a woman's FFTP (11). It is reported that an interval >16 years between menarche and FFTP increases a woman's risk of developing breast cancer (12).

Pregnancies that develop during or shortly after childbirth have been termed pregnancy-associated breast cancers (PABCs). PABCs occur in up to 40,000 women yearly. PABCs that develop after (but not during) childbirth tend to be more aggressive than breast cancers that occur at other times in a woman's life, on average being higher grade, with a higher proliferation rate, and more often hormone insensitive (11).

An important biologic process in both normal and malignant cells is epithelial mesenchymal transition (EMT). In health, EMT occurs in embryogenesis during mesoderm and neural tube formation and in wound healing. In neoplasia, EMT serves to facilitate the transition of non-tumorigenic cells to a malignant phenotype, and to transform well differentiated tumor cells into less well differentiated and more invasive and/or metastatic tumor cells. TGFβ is known to induce EMT in cancer, promoting tumor invasion and metastasis (13). The role of TGFβ in cancer development is more complex, as TGFβ can also induce apoptosis (14).

Cell-cell junctions maintain contact between adjacent cells (15). During EMT these contacts are broken down and/or reorganized, allowing the actin cytoskeleton to reshape. The epithelial (E)-cadherin protein is degraded (16), α-smooth muscle actin (SMA) is increased and reorganization of filamentous (F)-actin (17) within the cell is reorganized. Levels of vimentin, a protein which promotes cell migration and invasion, also increases (17).

Studies evaluating the presence and role of breast milk exosomes

Exosomes in human breast milk promote EMT

We determined if milk exosomes collected during breast involution, a time when the breast is remodeling and the microenvironment is tumor promotional, increased the aggressiveness of both benign and malignant breast epithelial cells (2). As only a minority of women develop PABC, we determined which protein(s) present in milk exosomes might drive this aggressiveness and selected milk samples for analysis with either a high or low concentration of the most interesting protein.

We measured the expression of six proteins in breast milk exosomes. We selected the proteins for analysis based on their presumed or known role in breast involution. Milk fat globule E8 (MFG-E8), or lactadherin, is secreted from exosomes and assists in the clearance of apoptotic mammary epithelial cells during involution (18). During involution, matrix metalloproteinase 2 (MMP-2), MMP-3 and MMP-9 assist in the reorganization of the breast back to its pre-pregnant state. MMPs have been reported to promote breast metastasis (19). Transforming growth factor beta (TGFβ) isoforms TGFβ1 and TGFβ2, which are upregulated during involution, can either suppress or promote tumorigenesis, depending on the situation (14). In preclinical studies of mammary involution, TFβs promoted apoptosis (20), whereas they promote the growth of tumor cells and stimulate cell proliferation in healing wounds (11). TGFβ isoforms are linked to breast cancer in preclinical models (21) and to prognosis in human breast cancer (22).

Of the six proteins analyzed, the greatest change in wean (from an involuting breast) compared to early milk protein (11) was in TGFβ2 which was increased (P=0.01) in wean milk. We next measured exosomal TGFβ2 in a number of wean milk samples, and added milk with high or low levels of TGFβ2 to immortalized benign (MCF10A) or malignant (MCF7) breast epithelial cells. There was evidence of a dose-response, with milk exosomes containing 1.5 ng/mL TGFβ2 having less effect than 10 ng/mL

pharmacologic TGFβ2 (2). Milk, with a high but not a low level of exosomal TGFβ2, modified cell morphology by disrupting cell-cell junctions and increasing filopodia formation. E-cadherin expression decreased while α-SMA and vimentin increased. Both the morphologic and protein expression changes are consistent with EMT. The changes in the benign MCF10A cells were consistent with transformation toward neoplasia, while the changes in MCF7 cancer cells were consistent with a more aggressive, invasive phenotype.

Evidence for plant miRNAs in human and porcine breast milk exosomes

Exosomes were isolated from six porcine milk samples and analyzed for the presence of plant miRNA species (3). Seventeen plant miRNA species were identified overall. Two of four human milk samples contained plant miRNAs, with 35 plant miRNA species identified between the two samples. Plant derived miRNAs in human milk were similar to those found in human serum (23). These miRNAs may influence human target mRNAs and thereby the person consuming these plants derived miRNAs. How the miRNAs in milk may specifically influence the breast compared to the remainder of the individual is uncertain.

Milk exosomes contain over 2,000 proteins

The first report of exosome-like proteins in bovine milk is from 1973 (24). A 2011 report which analyzed the proteome of exosomes isolated from the milk of mid-lactation cows yielded 2,107 proteins. The milk fat globule (MFG) membrane proteins butyrophilin, xanthine oxidase, adipophilin and lactadherin were the most abundant proteins found, though they represented <2% of the total milk protein content. MFG membrane proteins are thought to be derived from the plasma membrane. The remaining 98% of milk proteins are from the bovine ductal epithelial lining as well as from other cells in bovine milk (24). The presence of proteins from ductal epithelial cells suggests the exosomal miRNAs have potential physiological significance to mammary physiology.

Breast milk exosomes promote intestinal epithelial cell growth

Breast milk is known to prevent necrotizing enterocolitis (NEC), but the mechanism(s) underlying this are unknown. In an attempt to better understand how this occurs, exosomes were isolated from rat milk. The isolated exosomes, exosome free milk or control (phosphate buffered saline) was added to intestinal epithelial cells. Exosomal treatment increased epithelial cell proliferation somewhat more than milk lacking exosomes, and significantly more than PBS control (25).

Exosomal immunoregulatory content is influenced by when milk is collected as well as maternal lifestyle

Multiple reports suggest that milk exosomes provide factors that stimulate the infant's immune system. Exosomes in early milk (collected shortly after childbirth) are more numerous than in mature (2 months after childbirth) milk, they are enriched in HLA-DR molecules and have lower levels of HLA-ABC compared with those in mature milk (1). HLA-ABC molecules present antigens from inside a cell, whereas HLA-DR molecules present antigens from outside the cell. The primary function of HLA-DR is to present peptide antigens, potentially foreign in origin, to the immune system, and as such DR is a marker of immune stimulation.

Women with an alternative medicine lifestyle had lower mucin (MUC)-1 expression on their milk exosomes compared to milk from control mothers. MUC-1 is a cell surface protein that has been shown to protect the body from infections. Overexpression of MUC1 is associated with breast, colon, ovarian and pancreatic cancer (26). Thus, exosomal composition is influenced by when milk is collected (early in lactation or later), and by maternal lifestyle.

Immune related miRNAs are enriched in breast milk exosomes

Human breast milk exosomes were analyzed for the presence of miRNAs. Six hundred and two unique miRNAs were identified using deep sequencing technology. Of 87 immune-related pre-miRNAs, 59 (67.82%) were enriched in breast milk exosomes, and the endogenous immune-related miRNAs were more resistant to degradation than synthetic miRNAs. The authors speculate that the miRNAs are transferred from milk to the infant via the digestive tract, and thereby influence the development of the infant immune system (8).

Bovine milk exosomes can be transferred to human macrophages

The authors had previously demonstrated that miRNAs in rat milk were resistant to acidic conditions and RNase (27).

They determined if exosomes in bovine milk might influence human THP-1 monocyte/macrophage cells. Their studies demonstrate that bovine milk exosomes were incorporated into the monocyte/macrophage THP-1 cells, suggesting that exosomes in cow's milk may influence the human immune system.

Human milk exosomes are resistant to digestion and incorporated into human intestinal cells

To determine if human milk exosomes resist enzymatic digestion in the gastrointestinal tract, the authors added pepsin and pancreatin to the exosomes and found that a significant portion resisted digestion (28). They then incubated the exosomes with human intestinal epithelial cells and determined that the exosomes were incorporated into the enterocytes.

Bovine milk exosomes were also evaluated for their ability to be incorporated by intestinal cells (29). The authors found that the exosomes were incorporated both in Caco-2 human colon carcinoma and IEC 6 benign rat small intestine epithelial cells. Incorporation was more efficient in the malignant than in the benign epithelial cells. miR-29b and miR-200c accumulated in Caco-2 cells.

Summary

Exosomes are present in a variety of body fluids, including breast milk. The sources of these exosomes are diverse, some derived locally from the breast ductal lining, others from immune cells, while still others originate in plants that we consume. The composition of human milk exosomal content is influenced by when during lactation the milk is collected as well as maternal lifestyle. Exosomes are resistant to digestion. They are taken up by the intestine as well as immune cells. Exosomes in milk appear to serve a protective function by conferring immunity and stimulating the immune system of the newborn.

The contents of milk exosomes determine their effect. Exosomes are known to contain miRNAs, mRNAs, proteins and lipids. Milk exosomes with high but not low levels of TGFβ2 were found to transform benign breast epithelial cells toward neoplasia, and malignant, well differentiated breast epithelial cells toward a more invasive phenotype. As such, milk exosomes perform vital functions in health and disease. One of the great advantages of exosomes in milk is that the fluid is collected noninvasively, and sample volume generally does not inhibit screening the sample for disease identifiers. Understanding how exosomes do this can help us to better understand how to screen for disease, and act on the risk information accordingly.

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References

1. Torregrosa Paredes P, Gutzeit C, et al. Differences in exosome populations in human breast milk in relation to allergic sensitization and lifestyle. Allergy 2014;69:463-71.
2. Qin W, Tsukasaki Y, Dasgupta S, et al. Exosomes in human breast milk promote EMT. Clin Cancer Res 2016;22:4517-24.
3. Lukasik A, Zielenkiewicz P. In silico identification of plant miRNAs in mammalian breast milk exosomes--a small step

forward? PLoS One 2014;9:e99963.
4. Harding C, Heuser J, Stahl P. Receptor-mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. J Cell Biol 1983;97:329-39.
5. Johnstone RM, Adam M, Hammond JR, et al. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J Biol Chem 1987;262:9412-20.
6. Admyre C, Johansson SM, Qazi KR, et al. Exosomes with immune modulatory features are present in human breast milk. J Immunol 2007;179:1969-78.
7. Giri PK, Kruh NA, Dobos KM, et al. Proteomic analysis identifies highly antigenic proteins in exosomes from M. tuberculosis-infected and culture filtrate protein-treated macrophages. Proteomics 2010;10:3190-202.
8. Zhou Q, Li M, Wang X, et al. Immune-related microRNAs are abundant in breast milk exosomes. Int J Biol Sci 2012;8:118-23.
9. Hendrix A, Westbroek W, Bracke M, et al. An ex(o) citing machinery for invasive tumor growth. Cancer Res 2010;70:9533-7.
10. Bruschi M, Santucci L, Ravera S, et al. Human urinary exosome proteome unveils its aerobic respiratory ability. J Proteomics 2016;136:25-34.
11. Lyons TR, Schedin PJ, Borges VF. Pregnancy and breast cancer: when they collide. J Mammary Gland Biol Neoplasia 2009;14:87-98.
12. Li CI, Malone KE, Daling JR, et al. Timing of menarche and first full-term birth in relation to breast cancer risk. Am J Epidemiol 2008;167:230-9.
13. Kang Y, He W, Tulley S, et al. Breast cancer bone metastasis mediated by the Smad tumor suppressor pathway. Proc Natl Acad Sci U S A 2005;102:13909-14.
14. Wakefield LM, Roberts AB. TGF-beta signaling: positive and negative effects on tumorigenesis. Curr Opin Genet Dev 2002;12:22-9.
15. Huang RY, Guilford P, Thiery JP. Early events in cell adhesion and polarity during epithelial-mesenchymal transition. J Cell Sci 2012;125:4417-22.
16. Yilmaz M, Christofori G. EMT, the cytoskeleton, and cancer cell invasion. Cancer Metastasis Rev 2009;28:15-33.
17. Haynes J, Srivastava J, Madson N, et al. Dynamic actin remodeling during epithelial-mesenchymal transition depends on increased moesin expression. Mol Biol Cell 2011;22:4750-64.
18. Nakatani H, Aoki N, Nakagawa Y, et al. Weaning-induced expression of a milk-fat globule protein, MFG-E8, in mouse mammary glands, as demonstrated by the analyses of its mRNA, protein and phosphatidylserine-binding activity. Biochem J 2006;395:21-30.
19. Schedin P. Pregnancy-associated breast cancer and metastasis. Nat Rev Cancer 2006;6:281-91.
20. Flanders KC, Wakefield LM. Transforming growth factor-(beta)s and mammary gland involution; functional roles and implications for cancer progression. J Mammary Gland Biol Neoplasia 2009;14:131-44.
21. Bierie B, Gorska AE, Stover DG, et al. TGF-beta promotes cell death and suppresses lactation during the second stage of mammary involution. J Cell Physiol 2009;219:57-68.
22. Ghellal A, Li C, Hayes M, et al. Prognostic significance of TGF beta 1 and TGF beta 3 in human breast carcinoma. Anticancer Res 2000;20:4413-8.
23. Zhang L, Hou D, Chen X, et al. Exogenous plant MIR168a specifically targets mammalian LDLRAP1: evidence of cross-kingdom regulation by microRNA. Cell Res 2012;22:107-26.
24. Reinhardt TA, Lippolis JD, Nonnecke BJ, et al. Bovine milk exosome proteome. J Proteomics 2012;75:1486-92.
25. Hock A, Miyake H, Li B, et al. Breast milk-derived exosomes promote intestinal epithelial cell growth. J Pediatr Surg 2017;52:755-9.
26. Gendler SJ. MUC1, the renaissance molecule. J Mammary Gland Biol Neoplasia 2001;6:339-53.
27. Izumi H, Tsuda M, Sato Y, et al. Bovine milk exosomes contain microRNA and mRNA and are taken up by human macrophages. J Dairy Sci 2015;98:2920-33.
28. Lonnerdal B, Du X, Liao Y, et al. Human milk exosomes resist digestion in vitro and are internalized by human intestinal cells. FASEB J 2015;29:121.123.
29. 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.

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