Characterization of microRNAs in human breast milk and bovine milk from infected udders

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INTRODUCTION

Human milk provides complete nutritional components ensuring the development of newborns. Besides nutrients, milk offers specific immunoprotection to the growing infant via effectors including nucleic acids. For example, microRNAs (miRNAs) regulate gene expression at the post transcriptional level. Human milk miRNAs may play key roles in gut development, immunity and disease prevention in the neonate. Recent results obtained in our laboratory indicate that miRNAs are expressed during the intra-uterine and post-natal life.[1] Exosomal miRNAs in breastmilk remain active after ingestion by the newborn, hence offering direct cellular communication from mother to child.[2]

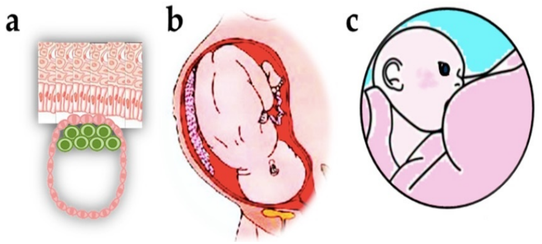
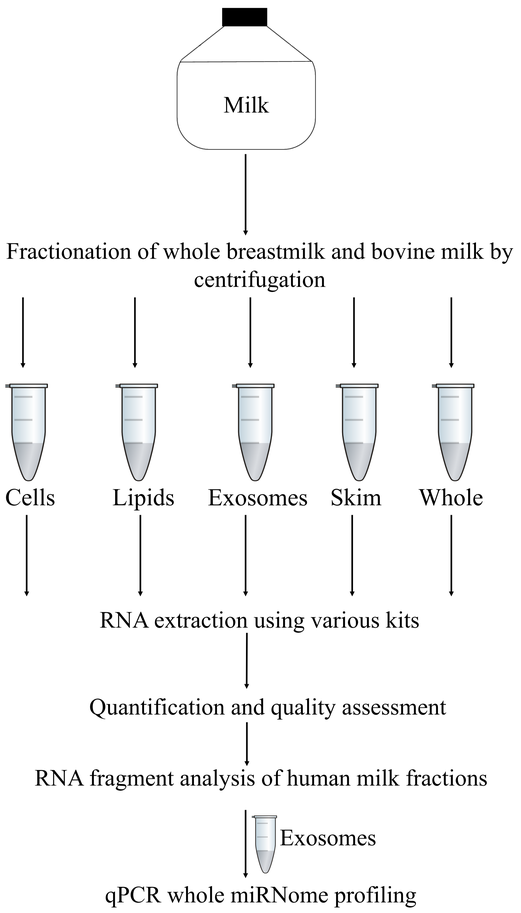


Figure 1. MiRNA expression during the intra-uterine (a,b) and post-natal life (c).

This research continues the ongoing characterization of miRNAs and depicts their abundance in different fractions of bovine and human milk. If human milk miRNA is implicated in gut health of premature infants, specific sequences will be tested as therapeutics for serious gastrointestinal inflammatory diseases involving neonates.

METHODS



RESULTS

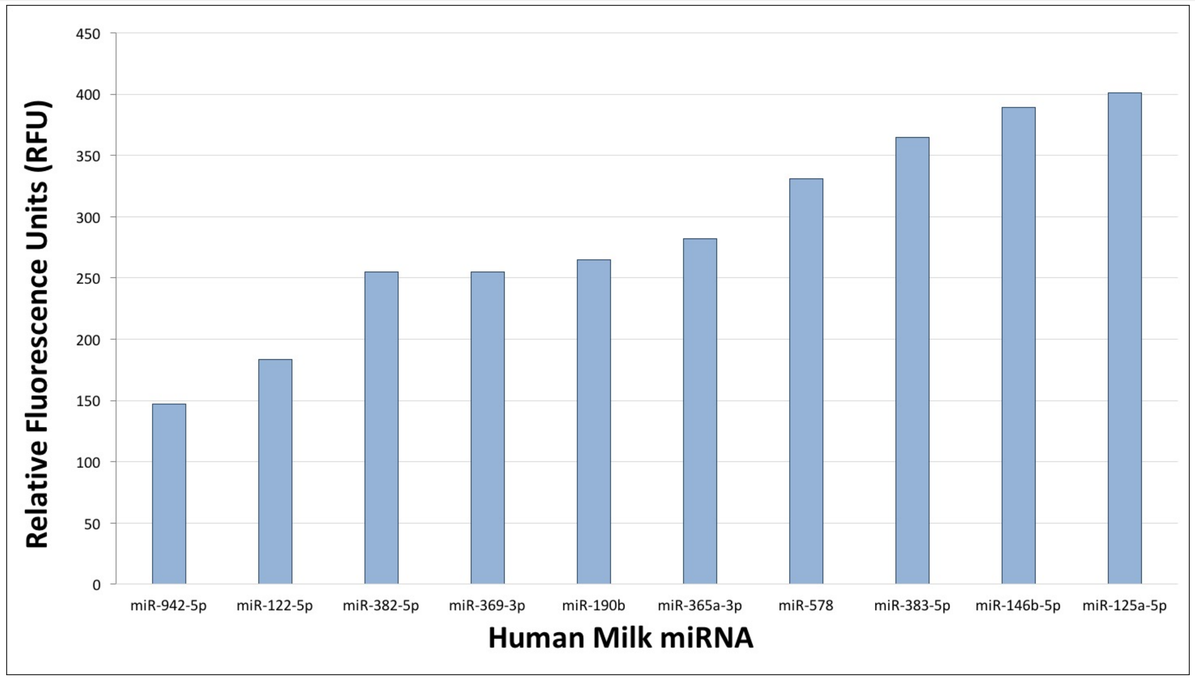


Figure 2. Relative fluorescence units of the 10 most fluorescent mature miRNAs found from a human milk exosome fraction. One 384 well plate was used from the miScript SYBR Green PCR kit (Qiagen). Prior to the real time PCR with the BioRad CFX384, a reverse transcription and pre-amplification were necessary to obtain 415.15ng of RNA. The RFU represents the fluorescence at 494 nm and 521 nm during the final 40th cycle.

Table 1. The common predicted targets for the top 5 most fluorescent miRNAs found from the real time PCR. The predicted targets were found on the miRBase database.

Human milk miRNA	Common predicted targets
miR-365a-3p	IQCK, POGZ, COG6, LOC144571, NDUFAF4
miR-578	DNM1L, ANO4, MINPP1, SLC17A6
miR-383-5p	TAOK3, NCKAP1, XRN2
miR-146b-5p	NOVA1, RHOXF2B, RHOXF2, TRAF6
miR-125a-5p	ORP9, CSNK2A1, ZNF543, FAM169B

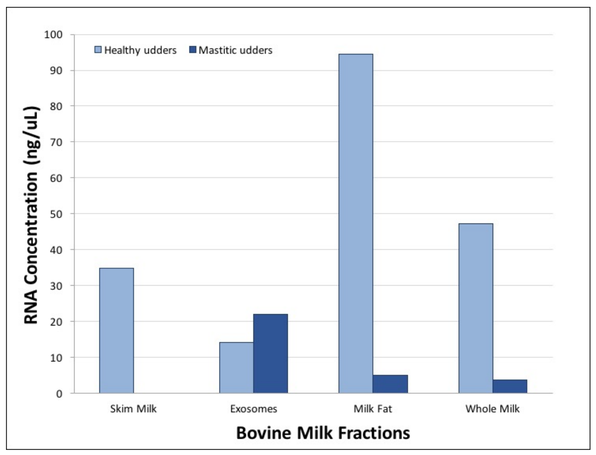


Figure 3. Total RNA concentrations (ng/uL) extracted from bovine milk. One or more quarters affected by a mastitic infection are compared to healthy bovine udders. The milk fractions coming from the healthy udders were extracted using the miRNeasy Serum/Plasma (Qiagen), miRCURY Cell&Plant (Exiqon) kits. RNA extraction from the mastitic udders was performed using the miRCURY Cell&Plant kit.

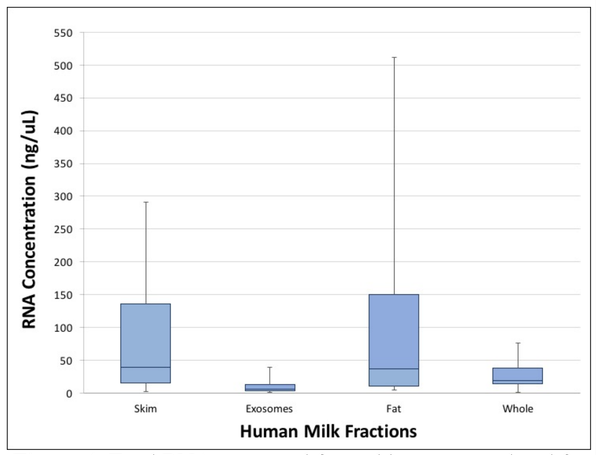


Figure 4. Total RNA recovered from skim, exosomal and fat fractions as well as whole milk from different human donors. The RNA was extracted using the miRNeasy Serum/Plasma (Qiagen), miRCURY Cell&Plant and miRCURY Biofluids (Exiqon) as well as TRIzol (Thermo Fisher Scientific).

DISCUSSION

Mostly uncommon miRNAs were identified in higher quantities with the real time PCR method. The stable core group of abundantly expressed miRNA in human breast milk samples include miR-148a-3p, miR-22-3p, miR-30d-5p, let-7b-5p, miR-200a-3p and miR-146b-5p.[3,4] From that group only the miR-146b-5p, an adipocyte-derived miRNA, was found as being one of the most abundant miRNA in the exosome sample. Its predicted targets may play a role in inflammation of the innate immune system.[5] Since it is transported to recipient cells via exosomes, a certain quantity could hypothetically be transferred to the blood circulation around the gastrointestinal tract, playing a key regulatory function in the newborn. Interestingly, this exosome sample showed many lipid regulatory miRNAs, including miR-122-5p, miR-125a-5p and miR-365. The presence of miR-122-5p in human milk exosomes might be associated with a role in the metabolism or synthesis of the lactating breast.[6] On the other hand, miR-125a-5p is involved with the regulation of oxysterol binding protein-related Protein 9 (ORP9), which induced a higher lipid intake from macrophages.[6]

Typically, a mastitic infection elevates the number of white blood cells and lymphocytes in the organism. A high RNA concentration could indicate an increased number of β-defensins, which are a part of the innate immunity system.[7] In this experiment, the observed decrease may be due to a RNase contamination, catalyzing the degradation of RNA into smaller components.

Conclusion: The results from the extraction of RNA into various fractions concur with other studies. As a result of the mastitic udder milk RNA extraction, further methods need to be optimized to properly quantify RNA from bovine milk. Although the actual roles of miRNA still remain unclear, their pathways may play a role in the innate immunity.

ACKNOWLEDGMENTS

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