



Preterm human milk exosomes have anti-inflammatory properties mediated

by miRNA-146a that is decreased in donor milk
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Background

- Breast milk (BM) contains protective components against inflammatory injury
- Exosome vesicles found in BM, are released in response to various stimuli
- Exosomes contain cytokines as well as microRNAs (miRNA), involved in various biological processes through regulation of posttranscriptional gene expression
- miR-146a was shown to be involved in immune tolerance by down-regulation of TNF- α

Objectives

- To examine whether preterm and term BM contains exosomes that contribute to anti-inflammatory regulation in preterm infants, as compared to donor milk (DM)

Methods

- Term, preterm (PT) BM samples (<32 weeks gestation) were collected in week 1 and 4 after birth, term donor (DM) samples and exosomes were isolated using differential ultracentrifugation method
- Level of exosomes in BM was determined by total protein recovery and exosome specific ELISA, and cytokine levels in exosomes were determined by ELISA
- Exosomes were co-cultured with THP-1 derived macrophages exposed to LPS. The supernatants were analyzed for TNF- α production
- We analyzed expression of selected miRNA in BM and DM exosomes using RT-PCR
- THP-1 derived macrophages were transfected with miR-146a mimic, the transfected cells were stimulated with LPS, and TNF- α expression and secretion were determined by qPCR and ELISA

Results

Figure 1. EV number in BM (term and preterm) and donor

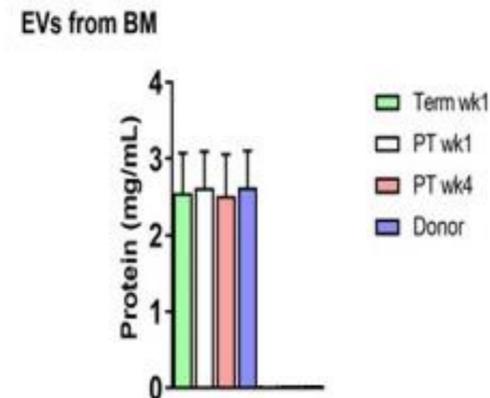


Figure 3. miRNAs content of EVs (miR-146a)

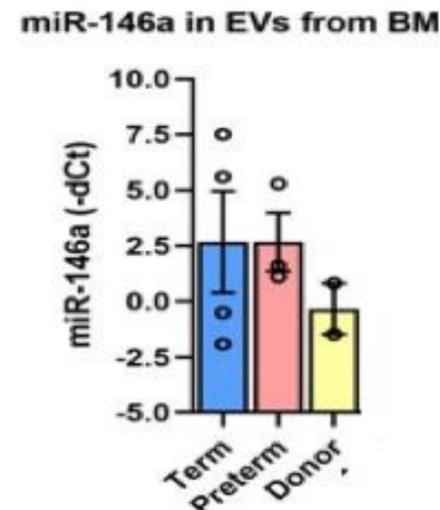


Figure 2. Exosomes from DM induced TNF- α in THP-1 cells more than preterm samples

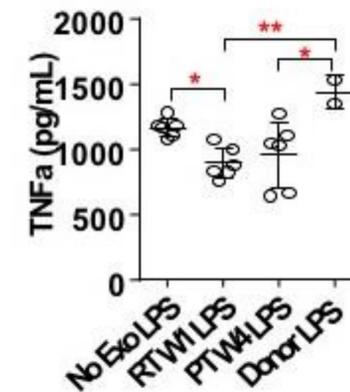
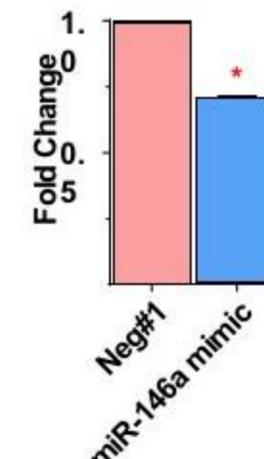


Figure 4. transfection of miR-146a mimic inhibits TNF- α expression



Results

- Exosomes are produced in Term, PT BM and DM, which was confirmed via electron microscopy, appearing as cup shaped with a diameter around 100nm.
- Exosomes isolated from PT BM inhibited LPS induced TNF- α production in macrophages. However exosomes isolated from DM exaggerated the TNF- α production
- DM exosomes had decreased miRNAs content, whereas PT and term exosomes contained several miRNAs including the anti-inflammatory miRNA-146a
- Exosomal miRNA-146a was significantly higher in term and PT milk compared to DM
- miRNA-146a transfection inhibited LPS induced TNF- α gene expression and protein secretion in macrophages

Conclusions

- Preterm human BM contains exosomes that contribute to anti-inflammatory immune regulation in the preterm infants mediated by miR-146a
- This novel finding illustrates the differential expression of immune regulatory molecules present in preterm BM compared to DM
- Clinicians should support using mother's breast milk and use donor milk only as an alternative

Future Studies

- Study whether BM derived exosomes can bind to gastrointestinal cells
- Study whether BM derived exosomes can modify gastrointestinal cell differentiation and their responses to infectious stimuli