Milk-derived exosomes as regulators of various cell functions and drug delivery vehicles

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Abstract

Exosomes are members of a larger family of extracellular vesicles that play major roles in intercellular communication and cell functions. Milk exosomes are secreted from mammary gland epithelial cells of all mammals including dairy cows, undergo intestinal uptake in rodent and human and deliver their cargos to specific tissues. Recent studies reported that milk exosomes can successfully deliver various drugs both orally and intravenously. Here we examined the uptake and effects of milk exosomes in various cell types and analyzed the ability of these exosomes to deliver RNA-based therapies. Milk exosomes were isolated by differential centrifugation and analyzed by NTA and Western blot analysis for size, quantity and surface markers. Using confocal microscopy and ImageStream analysis, we demonstrated uptake of exosomes to various mouse and human cells, including primary muscle and tumor cells and different cell lines. Milk exosomes increased the proliferation of both human and mouse muscle satellite cells and the expression of MyoD. Treatment of various tumor cells with milk exosomes either decreased cell proliferation or had no significant effects. We next demonstrated that milk exosomes can deliver exon skipping antisense oligonucleotides into muscle cells and specific miRNA mimics into muscle and glioma cells. Exosomal delivery of miR-29c into muscle cells increased muscle differentiation and delivery of miR-145 into glioma cells decreased cell proliferation, indicating functional delivery of the miRNAs by these exosomes. In summary, milk exosomes exert various effects on mouse and human cells and represent a viable nano-carrier of RNA-based therapies for the treatment of various diseases.

Milk Exosomes

- **Milk is a rich and scalable source of extracellular vesicles including exosomes.**
- **Milk exosomes express a variety of proteins, lipids and RNA molecules, some are.**
- **Concerns were raised regarding the epigenetic impact of milk exosomes on human health.**
- **The stability of milk exosomes make them attractive candidate for the delivery of oral therapeutics.**
- **Intestinal uptake of microRNAs encapsulated in exosomes is an active, saturable process.**

Objectives

- **Characterize the internalization of milk-derived exosomes in various cell types**
- **Analyze the effects of milk exosomes on normal and tumor cells**
- **Study the use of milk exosomes in drug delivery**

Characterization of milk exosomes

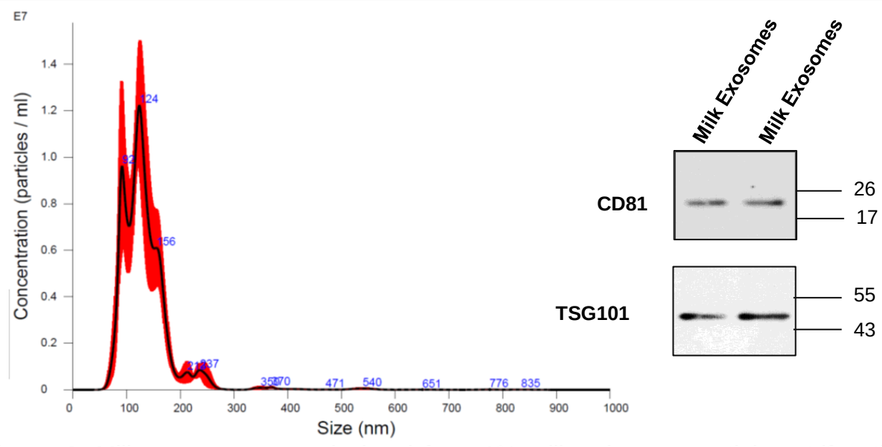

Figure 1. Milk exosomes were isolated from 1% milk using sequential centrifugation. Exosomes were analyzed by NTA and Western blot analysis.

Milk exosomes internalize in mouse muscle cells

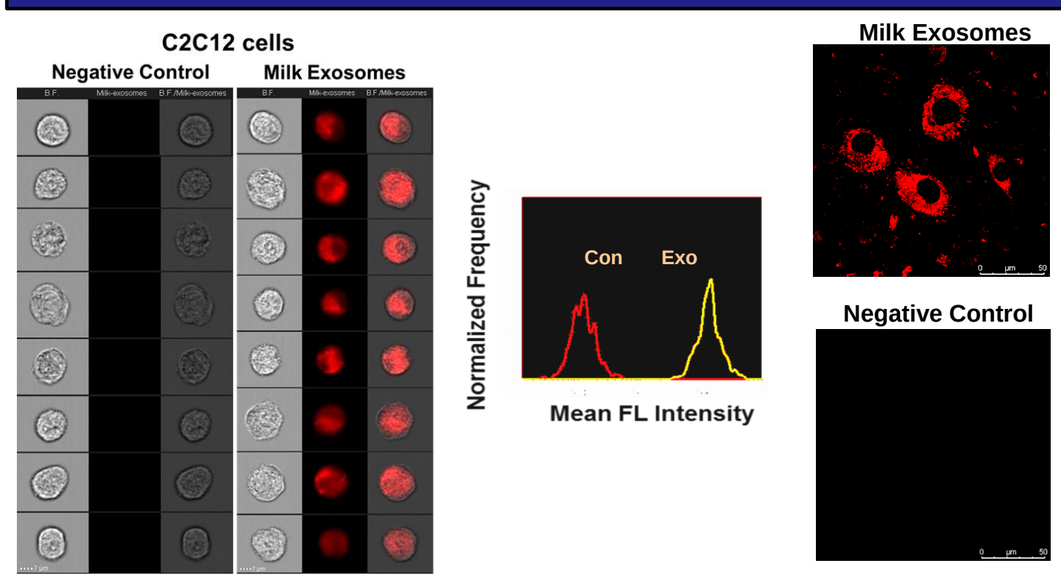

Figure 2. Milk exosomes were labeled with CellTracker Red and incubated with C2C12 for 24 hr. The internalization of the exosomes in the cells was analyzed using ImageStream analysis and confocal microscopy.

Milk exosomes induce proliferation of muscle and satellite cells

Figure 3. C2C12 and human satellite cells from healthy control and DMD patients were treated with milk exosomes. The proliferation of the cells was determined after 3 days of treatment. * P<0.001

Milk exosome effects on cancer cells

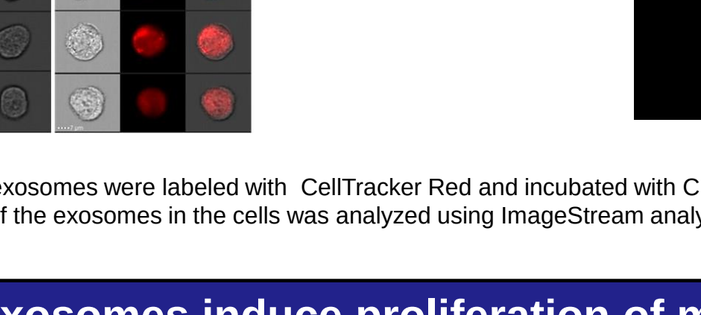

Figure 7. Exosomes were incubated with different tumor cell lines and cell proliferation was analyzed after 3 days. *P< 0.001

Milk exosomes internalize in human cancer cells

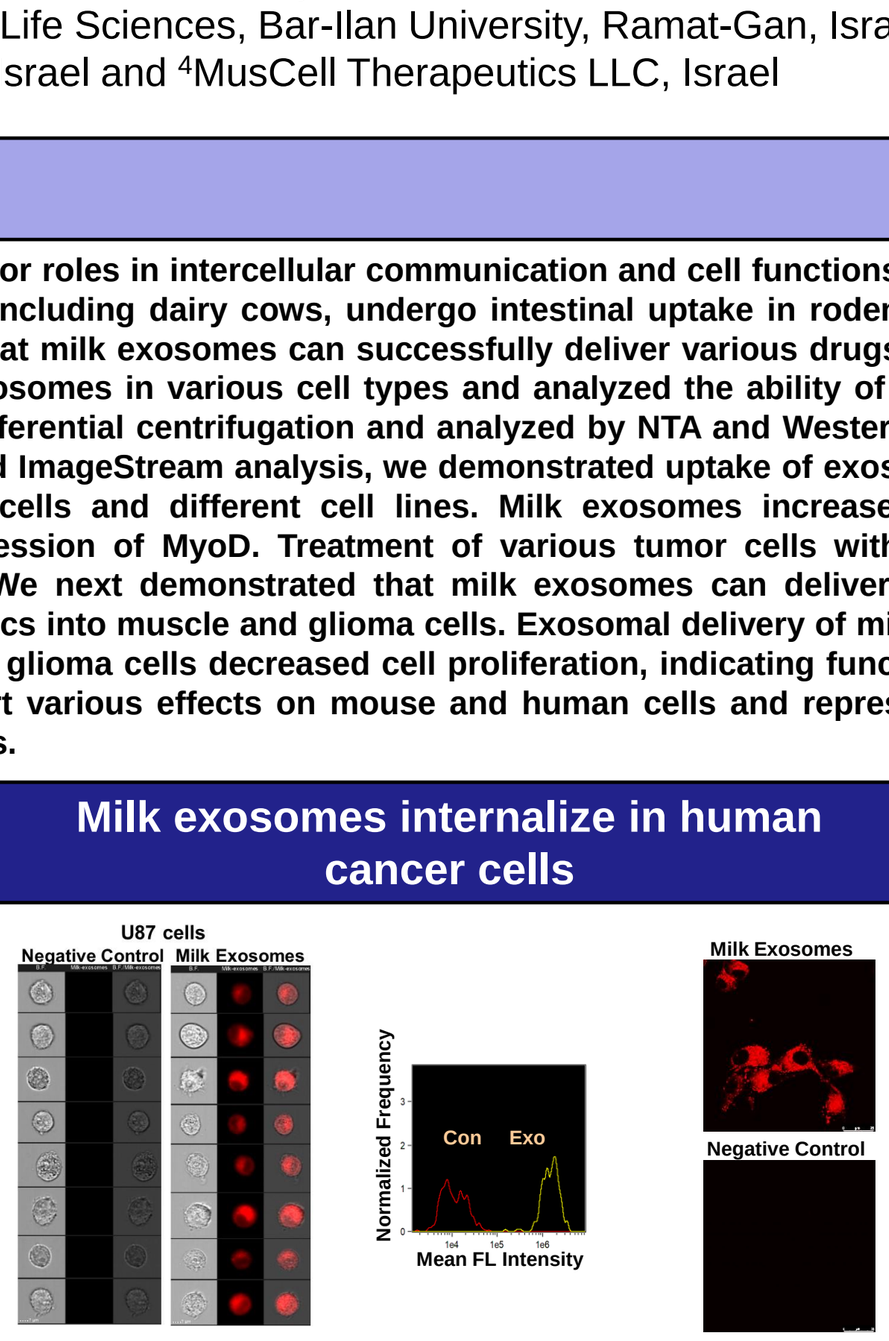

Figure 4. Milk exosomes were labeled with CellTracker Red and incubated with the human glioma U87 cells for 24 hr. The internalization of the exosomes in the cells was analyzed using ImageStream analysis and confocal microscopy.

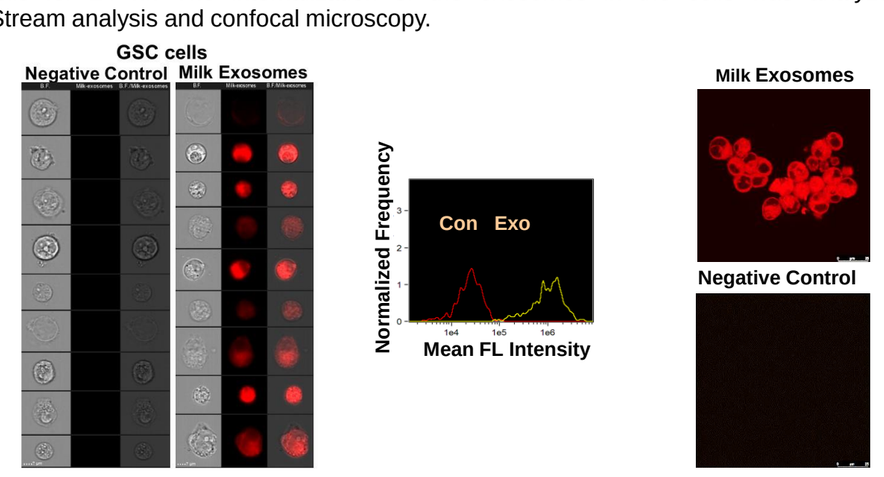

Figure 5. The internalization of milk exosomes was also analyzed in glioma stem cells that were isolated from fresh tumor specimens and were maintained in culture as tumor spheres.

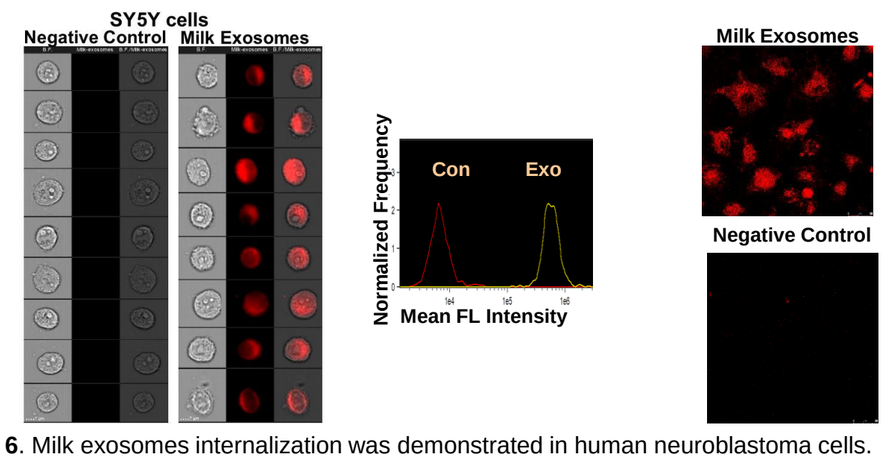

Figure 6. Milk exosomes internalization was demonstrated in human neuroblastoma cells.

Milk exosomes deliver RNA-based therapies to muscle and cancer cells

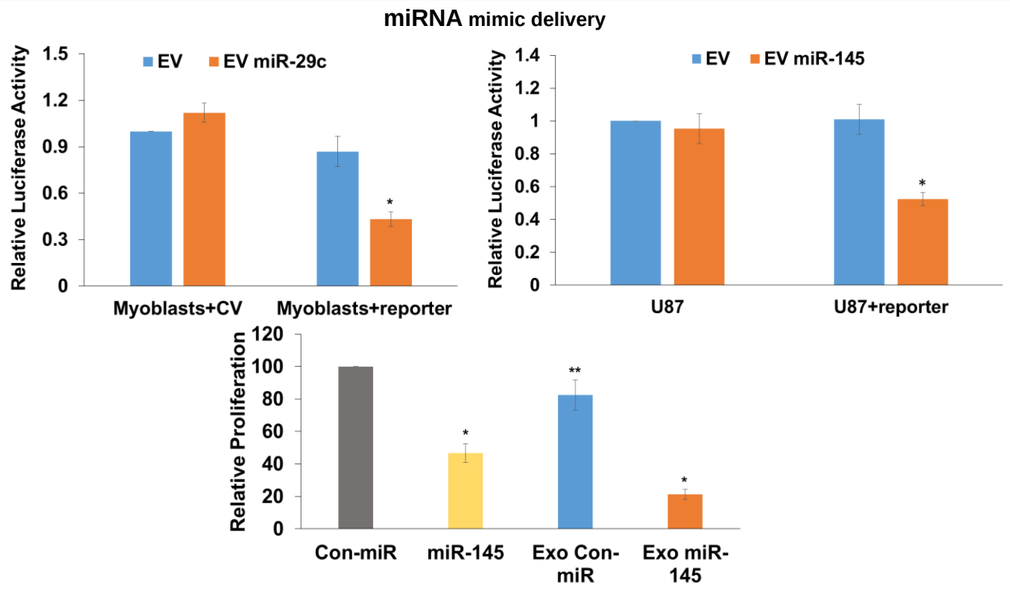

Figure 8. Exosomes were transfected with miR-29c or miR-145 and the delivery of these miRNAs to C2C12 and U87, respectively was analyzed using miRNA reporters. The effects of miR-145 transfection to U87 or exosomes transfected with miR-145 and incubated with U87 cells was analyzed on cell proliferation. *P<0.001 **P<0.01

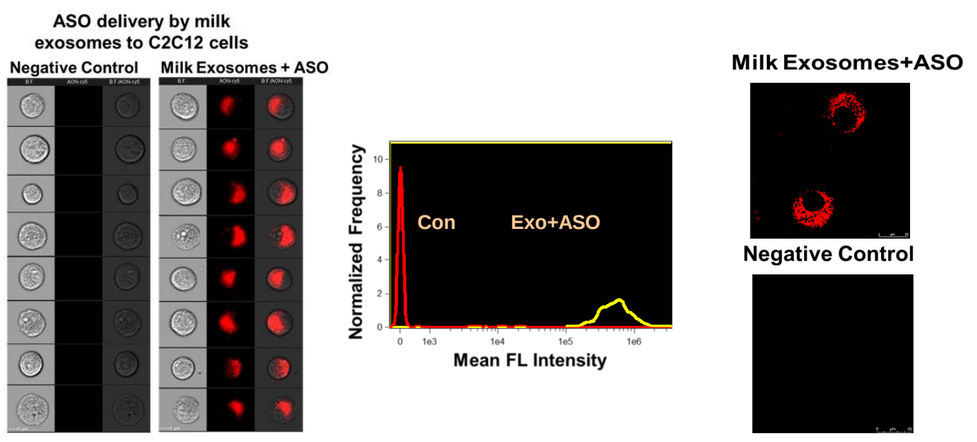

Figure 9. Exosomes were transfected with ASO-Cy5 (dystrophin exon skipping) using electroporation and incubated with C2C12 for 24 hr.

Conclusions

- **Milk exosomes have similar characteristics to human cell-derived exosomes with regards to size and marker expression.**
- **These exosome can internalize into a large variety of mouse and human cell types.**
- **The milk exosome induce proliferation of mouse muscle cells and human satellite cells derived from both healthy control and Duchenne muscular dystrophy patients.**
- **In contrast, these exosomes decreased the proliferation of most cancer cell examined.**
- **In addition, milk exosomes can efficiently and functionally deliver RNA-based therapeutics to both muscle and cancer cells.**
- **The effects of milk exosomes on various human cell functions and the mechanisms of their effects are currently being studied.**
- **The ability of milk exosomes to efficiently deliver RNA-based therapies are being analyzed in various pre-clinical disease models.**

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