Transfer of exosomes from cow's milk or pig plasma on Adipose Stem Cells

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Introduction

- Exosomes are small vesicles (ca. 100 nm in diameter) secreted by cells
- Exosomes → cargo for micro RNA (miRNA) → short RNA (ca. 20 nt) with important post-transcriptional regulation
- Exosomes → circulate in the body → taken-up by other cells → miRNA from one cell can affect the transcriptome of far-away cells
- Cow's milk has large amount of exosomes → miRNA in exosomes affect transcriptome of cells → animal-to-animal crosstalk
- Adipose stem cells (**ASC**) → adipocytes → effect on obesity
- ASC affected by circulating miRNA present in exosomes, maybe also dietary ones

The objective *were:*

1. *Assess if miRNA present in milk exosomes affect the transcriptome of ASC* → luciferase construct containing the target for miR29b and miR200c
2. *to determine if ASC take up circulating exosomes, including the ones present in milk* → fluorescent tracers

Our hypothesis *is that exosomes from milk are taken up from ASC and affect their transcriptome*

Original Method

Objective 1: Transfection of Adipose Stem Cells

We tested 8 commercial transfection reagents (Lipofectamine 3000, Lipfectamine Stem, EndoFectin, TransFectin, JetPRIME, Transfex, Effectene, PEI-NLS Peptide) with 4 different concentrations each and eGFP plasmid.

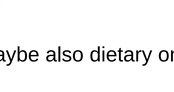
Ex: conditions for Lipofectamine 3000 were →

All reagents were used according to manufacturer's protocol.

µL Reagent/well	µg eGFP DNA/well
0.15	100
0.15	200
0.30	100
0.30	200

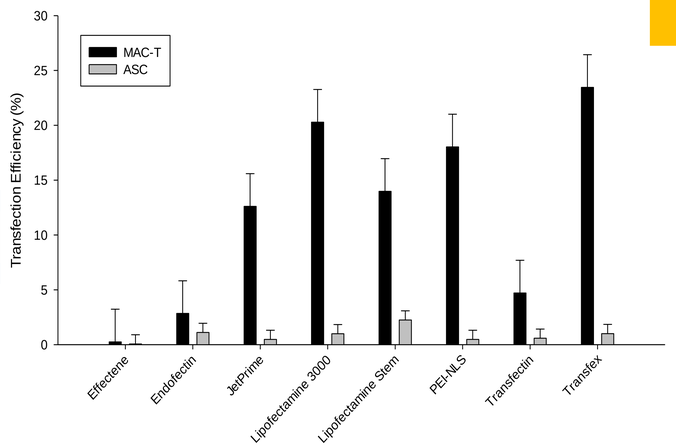
- ASC and bovine mammary epithelial cells (**MAC-T**) plated in 96 well plate 24 hours before transfection.
- Transfection reagents were added to each well and incubated for 24h.
- Cells were stained with Hoechst blue stain and images of cells were taken using fluorescence microscopy (blue=nuclei; green=transfected cells).
- CellProfiler was used to count the amount of stained cells and transfection efficiency was measured as the number of cells successfully transfected divided by total viable cells

Objective 2: Exosome Tracking



- ASC and MAC-T were plated in 96 well plate.
- Exosomes were labeled with SYTO RNASelect Green Fluorescent stain and BODIPY TR ceramide, and purified using spin columns (Thermofisher).
- Before adding exosomes, cells were stained with Hoechst
- Labelled exosomes were added to well and images were taken each hour for 24h using a robotic fluorescence microscope.

Results

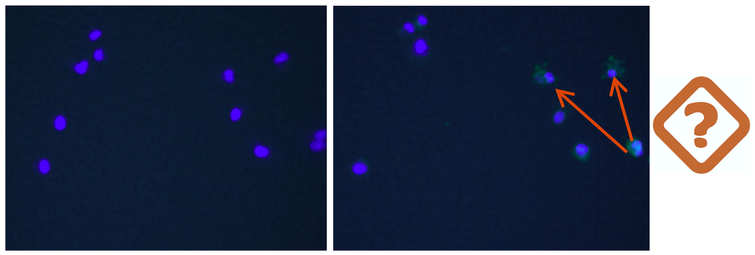


Objective 1

- MAC-T were used as a positive control and we achieved a maximum transfection efficiency of 23.4%, with TransfeX . ASC proved difficult to transfect with the highest efficiency at 2.2% with Lipofectamine Stem
- The second half of this experiment proved difficult due to the low transfectability of ASC

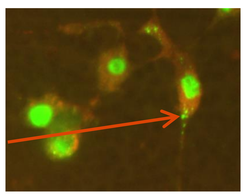
Objective 2

At first, it looked like exosomes were actually being taken up by ASC



Before and after exosomes added to cells: the green in the second picture looks like they could be exosomes…

…but when measured the staining in the membrane is drastically too large to actually be exosomes



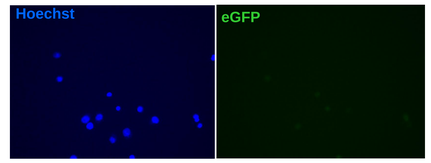
We thought that residual stain was remaining after the purification process, so we ran the exosomes through spin columns twice before adding to plate.
→ Residual staining still appeared, so we added <u>just stain</u> (without exosomes) to a well

The staining still appeared, proving that what we initially suspected were exosomes was simply excess stain in the membrane of ASC.

Next we questioned if uptake of exosomes was happening faster than our timecourse could capture → we added stained and purified exosomes to the plate and started capturing images within 30 seconds → Still, we were not able to clearly see exosomes being taken up

Discussion

Objective 1: ASC are naturally auto fluorescent (shown below on the right), so CellProfiler was unable to distinguish a legitimately transfected cell from a normal ASC in many of the images → for some of the reagents we had to hand count the transfected cells which may have impacted the accuracy of the data



Objective 2: Size of the exosomes and availability of correct equipment prevented us from addressing the hypothesis of this project → even at maximum magnification on our microscope (100x) exosomes were still too small to be visible

Conclusions

- **Objective 1**: our data were <u>inconclusive</u>; the transfection efficiencies for ASC is below the limit (2.5%) to use luciferase assay (*Osorio and Bionaz, 2017*)
- **Objective 2**: we were not able to physically see exosomes under the microscope so we are not able to state if they are or are not taken into the cell.

With the current methods and materials available we are unable to test our hypothesis → unable to continue the project any further.

Acknowledgements

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References

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