Hormone Effects on Extracellular Vesicle Production and Loading from Breast Cancer Cells

Melinda Dae[1], Natalie Fan[1], Lan Pang[1], George A. Calin[1]

[1]Department of Translational Molecular Pathology, The University of Texas MD Anderson Cancer Center, Houston, TX

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Making Cancer History®

Introduction

Breast cancer is the second most prevalent cause of female cancer-related mortalities[1]. New therapies have increased 5-year patient survival rates to over 80% in certain countries, but more knowledge is needed to combat breast cancer. **MCF-7** is a patient-derived breast cancer cell line used for in vitro experiments that expresses estrogen and androgen receptors[2].

Extracellular vesicles (EVs) are crucial to future cancer research. These vesicles facilitate **cell-to-cell communication** and transfer proteins and RNA (such as small, noncoding microRNA) between cells[3].

This experiment focused on EV secretion levels and protein differentiations in these extracellular vesicles, before and after treatment with **17-β Estradiol** (17-βE)**.**

Hypothesis

Following treatment with 17-β Estradiol, EV secretion levels and miRNA content levels will increase significantly.

Aim

To gain more information about the effects of estrogen treatments on extracellular vesicle secretion and RNA contents.

Techniques

- Cell treatment for samples:
  - A. Control 48h (no hormones)
  - B. +17-βE 1 nM (concentration selected according to previous data from Calin laboratory)
- Western Blot for cell receptor detection in MCF-7 and MB231 (triple negative breast cancer cell line)
- Harvesting conditioned mediums and cell pellets after 48h incubation following treatment
- Extracellular vesicles quantification via NanoSight
- RNA isolation from cells pellet and EVs
- EV isolation via ultracentrifugation
- RT-qPCR of RNA from cells pellet and EVs for let-7a and let-7d miRNA expression levels (miRNA selected according to previous miRNA screenings from Calin laboratory)

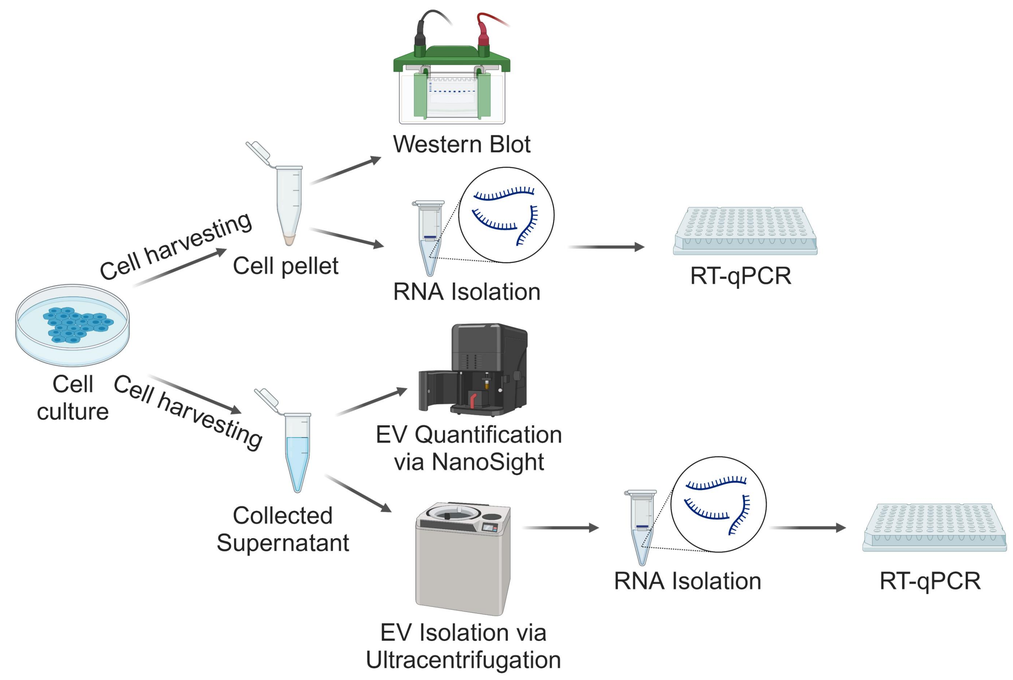

Fig. 1 Experimental workflow (created using Biorender)

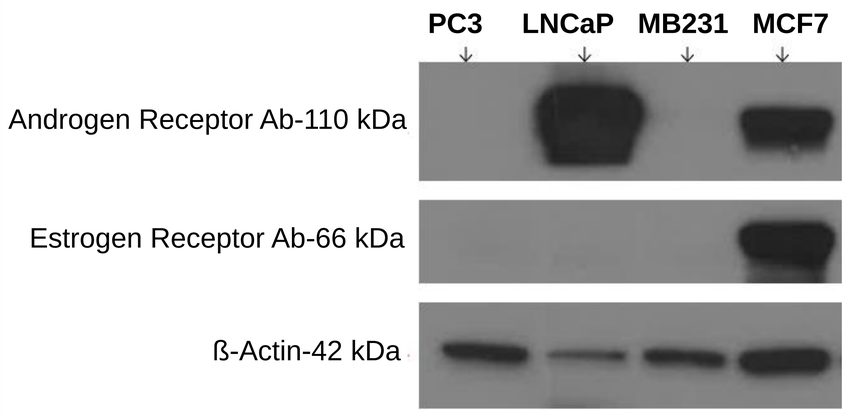

Fig. 2 Western Blot results show MCF-7 breast cancer cells are both estrogen receptor positive and androgen receptor positive, allowing for further research to treat these cells with 17-β Estradiol and, as part of the ongoing experimentation, DHT.

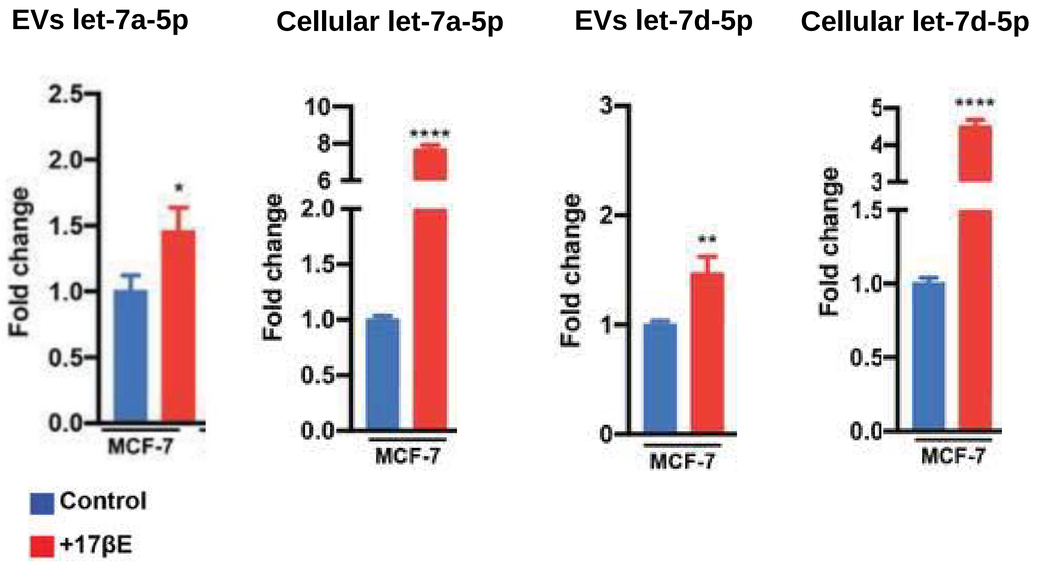

Fig. 3 RT-qPCR data shows a significant increase in let-7a-5p and let-7d-5p contents in EVs from MCF-7 cells after treatment with 17-β Estradiol 1nm.

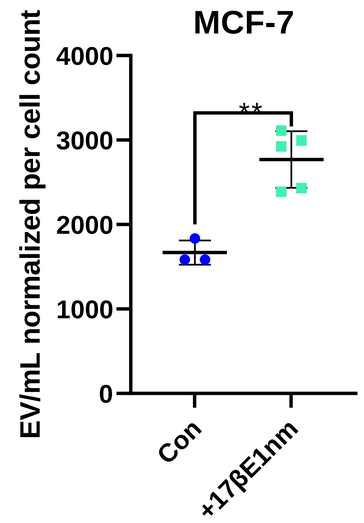

Fig. 4 EV quantification via NanoSight results show EV levels/mL normalized by cell counting data; after treatment, samples show significantly greater EV secretion levels.

Future Experiments

For ongoing experimentation, MCF-7 cells will be treated with **androgen** for 48h. We will collect the supernatant for androgen-treated MCF-7 cells and perform the following: EV Isolation via Ultracentrifugation, RNA isolation from EVs and cells, and RT-qPCR for let-7a-5p and let-7d-5p levels.

Conclusions

1. After treatment with 17- β Estradiol, MCF-7 cancer cells experienced significant **increases in EV secretion levels and let-7 miRNA content levels** within these EVs.

2. As depicted in Figure 3, within the secreted Evs and cells, treated samples had **increased let-7a-5p and let-7d-5p** content levels, changing by folds of up to 8.

3. As depicted in Figure 4, **EV secretion levels were significantly higher** for the sample treated with estrogen, increasing by approximately 1000 EVs/mL.

Acknowledgements

Special thanks to the members of Dr. George Calin's laboratory, Lan Pang and Dr. Calin for overseeing this project, as well as Natalie Fan. Many thanks as well to Ms. Nancy Strange, Ms. Tess Melancon, and Ms. Chandra Bartholomeusz, for overseeing the MD Anderson High School Summer Outreach Program.

References

1) Sun, Y., et. Al. (2017), *NCBI*. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5715522/
2) Drula, R., et al. (2023), *PNAS*. https://www.pnas.org/doi/10.1073/pnas.2122053120.
3) Zhao, Z., et al. (2021), *NIHS*. *https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8372011/*.