Impact of Tumor and Adipose-Derived EVs Isolated from Obese and Nonobese Mice on Metabolic Regulation and Metastatic Progression of Mammary Cancer Cells

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

Laith A. Rayyan[1], Emma J. Grindstaff[1], Dorothy Teegarden[2], Stephen D. Hursting[1,3], and Ximena Bustamante-Marin[1]

[1]Department of Nutrition University of North Carolina at Chapel Hill, [2]Department of Nutrition Perdue University, [3]Nutrition Research Institute

UNC NORC NUTRITION OBESITY RESEARCH CENTER

Introduction

Women with obesity have an increased risk of developing triple-negative breast cancer (TNBC) and its progression to metastasis[1]. Extracellular vesicles (EVs) are tissue-secreted nanoparticles that carry proteins, lipids, and nucleotides facilitating cell communication[2]. Proteomic analyses of EVs isolated from the mammary tumors and adipose tissue of obese and non-obese mice, identified the presence of pyruvate carboxylase (PC)[3]. This enzyme in known to promote angiogenesis, immune evasion, and metastasis[4]. We hypothesize that tumor and adipose-derived EVs increase the expression of PC and genes involved in the epithelial-to-mesenchymal transition (EMT) in non-metastatic murine mammary cancer cells.

Aims

1. To measure how obese EVs, isolated from mammary tumors and visceral adipose tissue (VAT) influence PC levels in mammary cancer cells.
2. To determine how obese EVs, isolated from mammary tumors and VAT influence the expression of EMT transcription factors in mammary cancer cells.

Methods

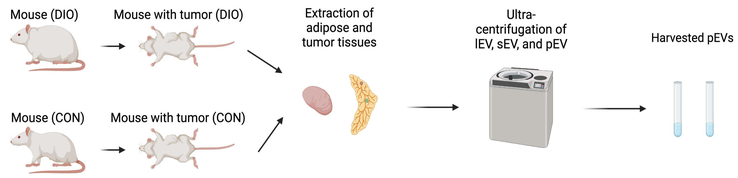

Figure 1: Schematic for mouse model, tissue collection, EV isolation, and purification. EVs were isolated from tumors and visceral adipose tissue by ultracentrifugation in combination with a density gradient.

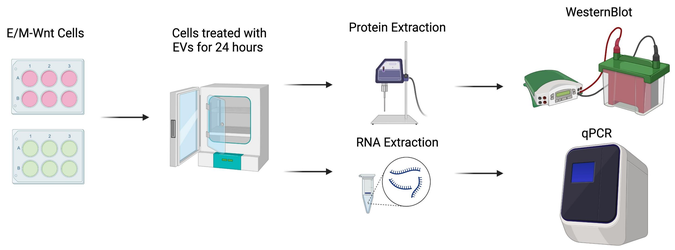

Figure 2: Schematic of cell treatment and subsequent experiments. E-Wnt and M-Wnt cells were treated with 25 µg or 50 µg of EVs. Following protein and RNA extraction, quantitative analyses were conducted through western blotting and qPCR.

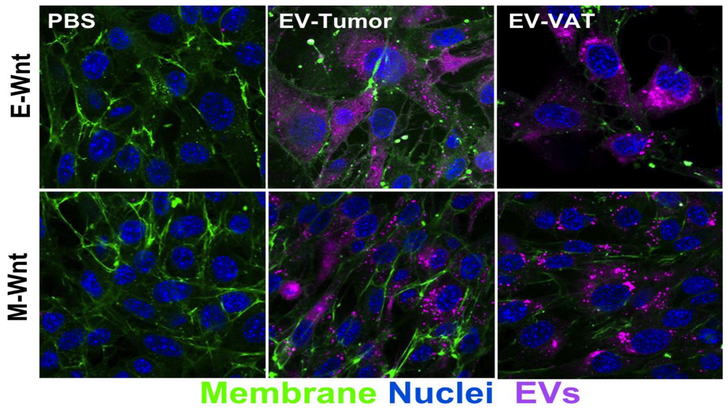

Figure 3: Confocal image of EVs' uptake in TNBC cell lines. EVs were stained with PKH26 and co-cultured with E-Wnt or M-Wnt cells. PBS was used as a negative control. After 24 hours of culture, the cell membranes were stained with CellBrite fix and the nuclei with Hoechst$_{33342}$. The cells were fixed using 2% PFA.

Results

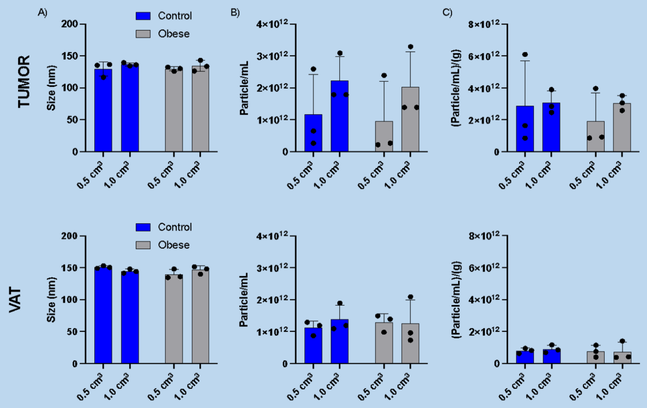

Figure 4: Characterization of EVs using nanoparticle tracking analysis. A) Median distribution of EVs isolated from visceral adipose tissue (VAT) and tumors in control and obese mice. B) Average particle concentration of EVs in particles per milliliter (particles/mL), C) EVs normalized by the mass of the tissue, expressed in (particles/mL/mg).

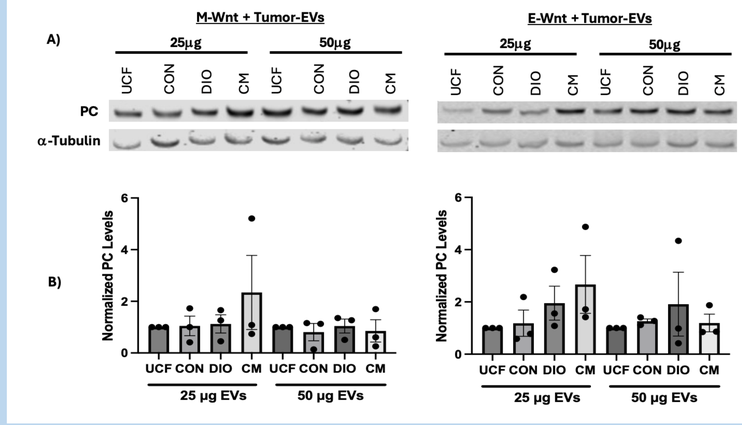

Figure 5: Analysis of PC levels in M-Wnt and E-Wnt cells following treatment with tumor-EVs. A) Western blot analysis of PC in M-Wnt and E-Wnt cells treated with 25 µg or 50 µg of EVs isolated from tumors of control mice (CON) or tumors from obese mice (DIO). α-Tubulin was used as a loading control. B) Densitometry Analysis of PC levels normalized to ultra-centrifugated media (UCF). CM, complete media.

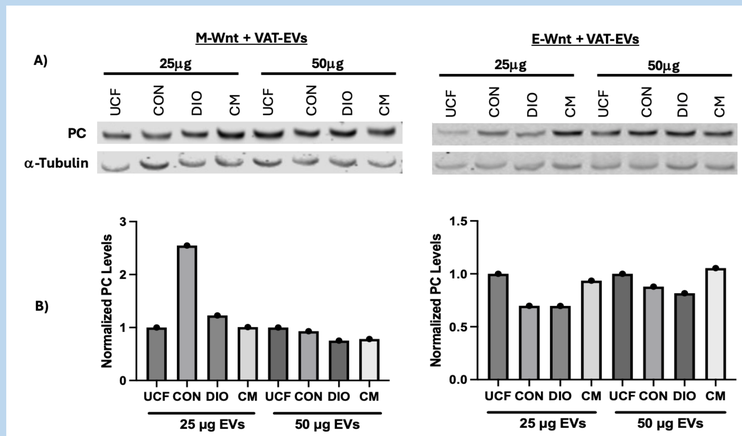

Figure 6: Analysis of PC levels in M-Wnt and E-Wnt cells following treatment with VAT-EV. A) Western blot analysis of M-Wnt and E-Wnt cells treated with 25 µg and 50 µg of total EV proteins. α-Tubulin loading control. B) Densitometry analysis of PC levels normalized to UCF media.

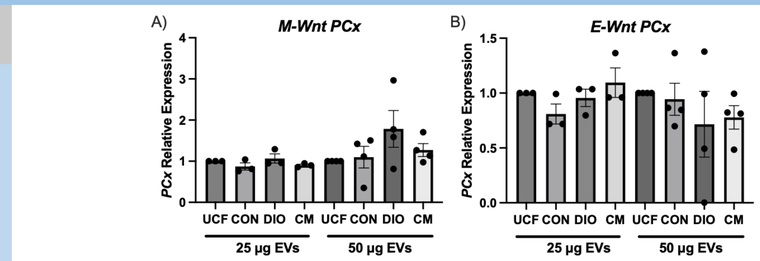

Figure 7: Expression of *PCx* in M-Wnt and E-Wnt Cells Subjected to EV Treatments. The cells were treated with 25 µg or 50 µg of EVs isolated from tumors of control mice (CON) or tumors from obese mice (DIO). Expression of *PCx* in A) M-Wnt cells and B) E-Wnt cells. One-way ANOVA analysis found no significant changes in *PCx* expression. *Rplp0* was used as a housekeeping gene.

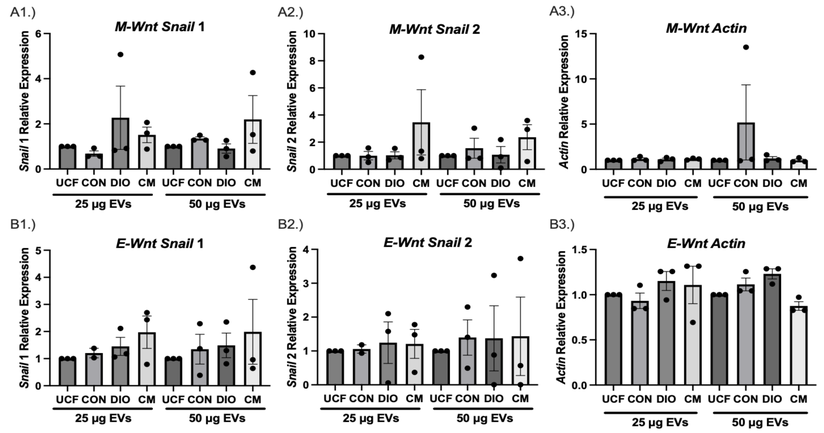

Figure 8: Differential Expression of EMT Markers and Actin in M-Wnt and E-Wnt Cells Subjected to EV Treatments. M-Wnt and E-Wnt cells were treated with 25 µg or 50 µg of EVs isolated from tumors of control mice (CON) or tumors from obese mice (DIO). The relative expression of A1., B1.) *Snail1*, A2., B2.) *Snail2*, and A3., B3.) *Actin*, were measured by qPCR. *Rplp0* was used as a housekeeping gene. The results are expressed as the mean ± SEM from three replicates.

Conclusion & Future Directions

- We confirmed the uptake of EVs by M-Wnt and E-Wnt cells after 24 hours. Upon treatment with EVs, we did not observe significant changes in PC levels, *PCx* expression, or expression of EMT transcription factors *Snail1* and *Snail2*.
- Future directions include increasing the cells' treatment with EVs beyond 24 hours to ensure EV content is delivered into the cells, promoting changes in the cellular microenvironment. Further experiments using the epithelial (E-cadherin) and mesenchymal (N-cadherin) markers and measuring cell proliferation and migration will provide evidence of the effects of EVs on EMT and tumor development.

References

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Acknowledgments

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