The Role of Macrophage Migration Inhibitory Factor (MIF) in Apoptotic Extracellular Vesicle Mediated Signaling in Breast Cancer

Kristy Hernandez[1,2], Safia Essien[1,3], and George T. Eisenhoffer[1,3]

1 - Department of Genetics, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030
2 – Cancer Prevention Research Institute of Texas Summer Undergraduate Program
3 – Genetics and Epigenetics Graduate Program, The University of Texas Graduate School of Biomedical Sciences at Houston, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030

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ABSTRACT

Background
The ability to prevent tumor repopulation after anticancer treatments has been a significant barrier to reducing morbidity and mortality of malignant tumors. Chemotherapeutic agents induce the death of rapidly dividing cells and dying cells can produce mitogenic cues that stimulate proliferation and promote repair. Whether these regenerative mechanisms are co-opted by tumor cells after cancer treatment is not well understood. Our data show that small membrane-bound vesicles generated during cell death (called apoptotic extracellular vesicles, or AEVs) serve as vehicles for the transfer of genetic information and the activation of signaling pathways that promote cell proliferation. We have identified the known mitogen and cytokine macrophage migration inhibitor factor (MIF) as enriched on apoptotic extracellular vesicles derived from epithelial stem cells in zebrafish. The goal of this project is to test if apoptotic extracellular vesicles from human breast cancer cells (bcAEVs) use MIF to evade the immune system and stimulate proliferation.

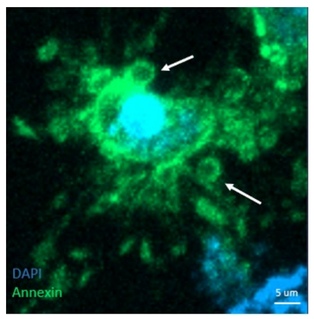
Methods
Apoptotic extracellular vesicles from MDA-MB231 breast cancer cells after exposure to chemotherapeutic agents were isolated by differential centrifugation for downstream studies. Immunogold labeling with transmission electron microscopy was used to determine the localization of the mitogenic and immunogenic protein Macrophage Migration Inhibitory Factor (MIF). Isolated bcAEVs were fluorescently labeled and injected into larval zebrafish where confocal microscopy was used to track interaction with macrophages expressing EGFP. Cell proliferation was also assessed using a BrdU incorporation assay. Chemical inhibitors against MIF were used to perturb the activity of specific breast cancer derived apoptotic bodies to assay for effects on proliferation.

Results
Foci of MIF were detected on the surface of the apoptotic extracellular vesicles (AEVs) purified from human breast cancer cells. Fluorescently labeled AEVs were tracked after injection into the zebrafish and were observed interacting with macrophages, providing a novel assay for probing interactions with the immune system *in vivo*. We observed an in increase in proliferation in larvae injected with bcAEVs, however before examination of MIF inhibition could be performed, further adjustments of drug controls timing and concentration are needed.

Conclusion
Here we show that the mitogenic and immunogenic protein MIF is present on the surface of bcAEVs and characterize the immunogenic and proliferative responses after introduction into zebrafish larvae. Future studies will inhibit MIF after delivery of the bcAEVs to the zebrafish larvae, with the goal of providing new insights into molecular pathways that can be leveraged against cancer by preventing the unwanted addition of new cells.

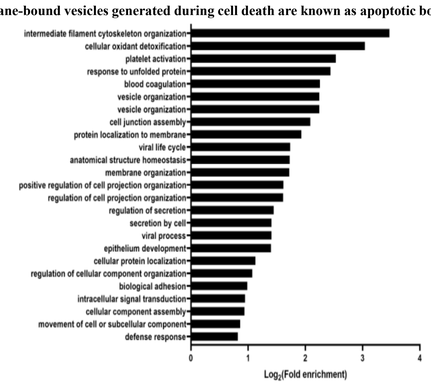
Keywords (less than 5)
Tumor repopulation, apoptosis, extracellular vesicles, MIF

INTRODUCTION

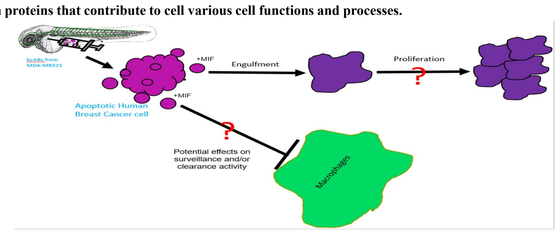


- AEVs are extracellular vesicles
- AEVs have been implicated in cell-to-cell communication
- AEVs are enriched in proteins that can modulate immunity and cell growth

Small membrane-bound vesicles generated during cell death are known as apoptotic bodies.



bcAEVs contain proteins that contribute to cell various cell functions and processes.



On the surface of purified epithelial stem cell derived apoptotic bodies, Macrophage Inhibitory Factory, was localized. MIF is a known mitogen and cytokine.. We will test if apoptotic extracellular vesicles from human breast cancer cells (bcAEVs) use MIF to evade the immune system and stimulate proliferation.

HYPOTHESIS

To test if apoptotic extracellular vesicles from human breast cancer cells (bcAEVs) use MIF to evade the immune system and stimulate proliferation.

METHODS AND RESULTS

bcAEV Isolation and injection into zebrafish

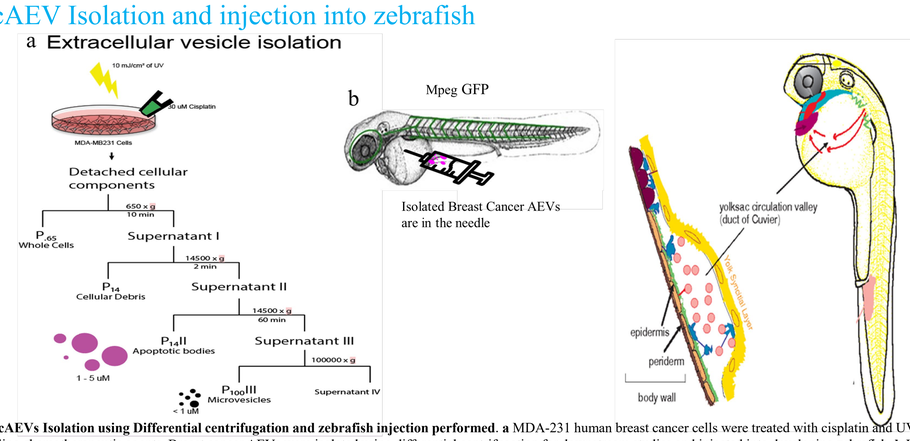


Fig.1 **bcAEVs Isolation using Differential centrifugation and zebrafish injection performed**. **a** MDA-231 human breast cancer cells were treated with cisplatin and UV treatment resembling chemotherapeutic agents. Breast cancer AEVs were isolated using differential centrifugation for downstream studies and injected into developing zebrafish. **b** Injection site is found at the Duct of Cuvier. Zebrafish lines used were Mpeg GFP.

MIF is located on the surface of bcAEVs

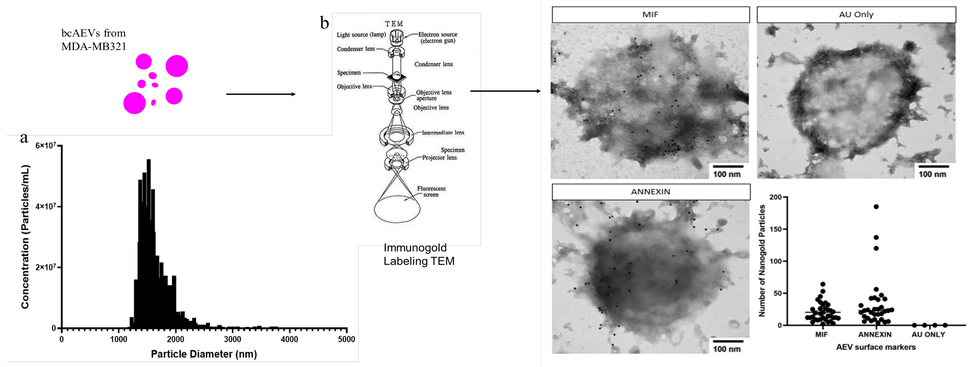
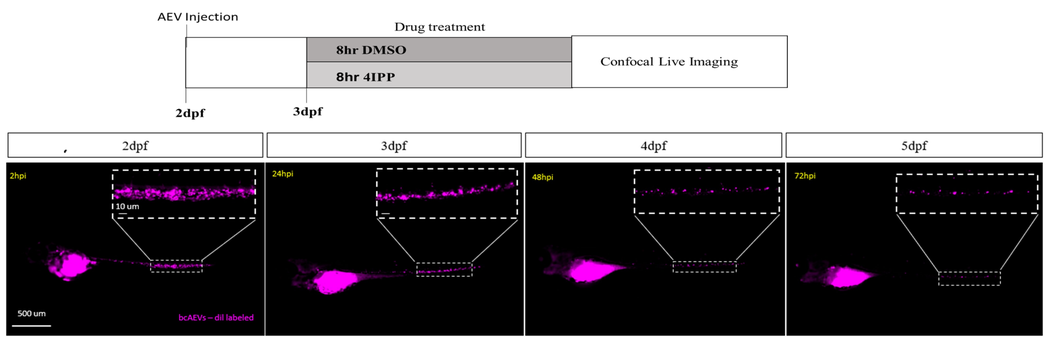
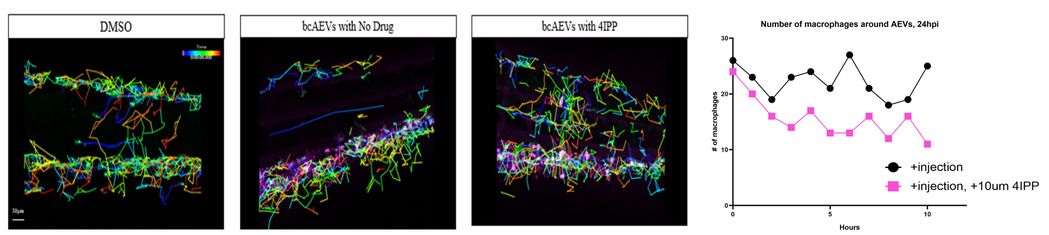


Fig. 2 **The Mitogenic and Immunogenic protein MIF is present on the surface of bcAEVs.** **a** Immunogold labeling with transmission electron microscopy was used to determine the localization of the mitogenic and immunogenic protein Macrophage Migration Inhibitory Factor (MIF) on purified apoptotic extracellular vesicles from human breast cancer cells. **b** The concentration and size of bcAEVs were quantified using tunable pulse resistance sensing with the qNano Instruments

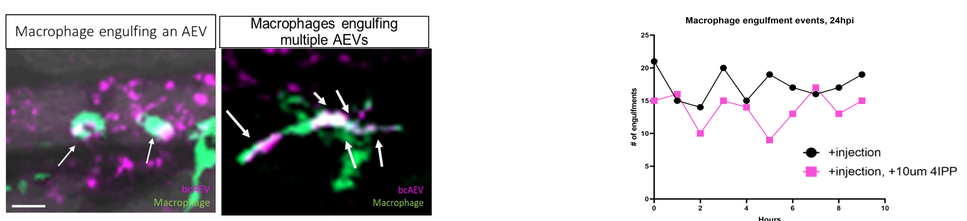
In vivo tracking of bcAEVs and interaction with macrophages



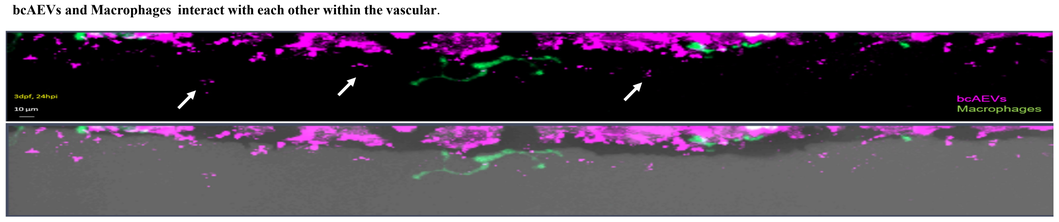
The Fluorescent intensity of AEVs decreases overtime possibly indicating clearance. Fluorescently labeled bcAEVs were injected into developing zebrafish 2pdf and imagined at 2hpi. Injected Zebrafish larvae continued to be imaged over time under homeostatic conditions until Larvae reached 5dpf. Fluorescent intensity of AEVs decreases in the circulation within the tail, while fluorescent intensity of AEVs injected in the yolk remains constant. This indicates possible AEV clearance by macrophages.



The Number of Macrophages around the Fluorescently labeled bcAEVs diminishes 24hpi with treatment of MIF inhibitor. Fluorescently labeled Macrophages were tracked frame by frame using Imaris. Each colored track represents macrophage movement The color of the tracks represents when the macrophages were present.



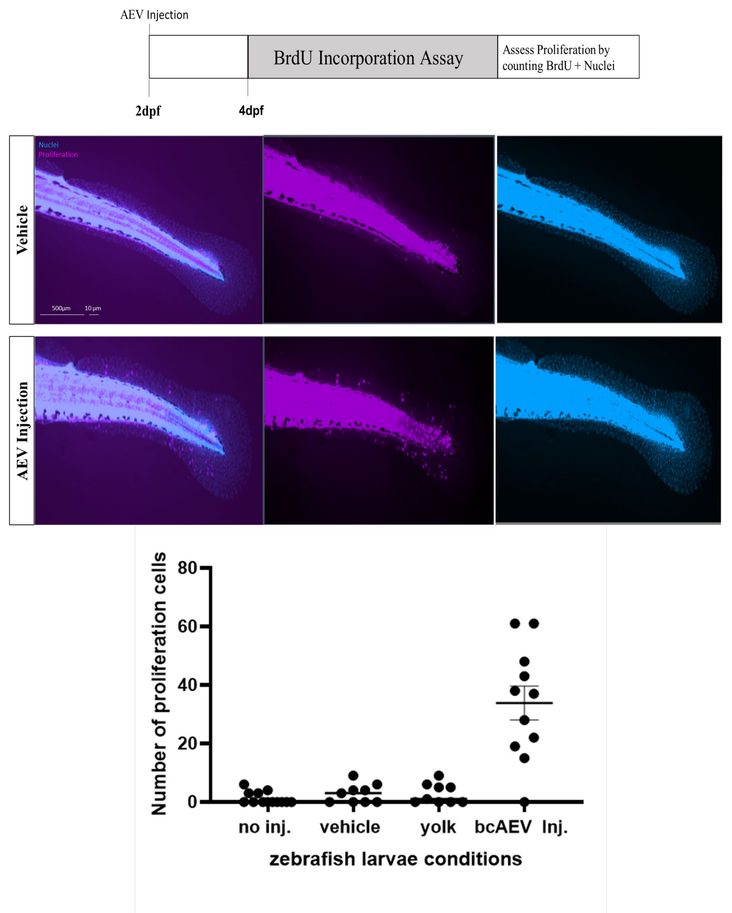
bcAEVs and Macrophages interact with each other within the vascular.



AEVs leave the vasculature to contact other cell types.

RESULTS CONTINUED

bcAEVs Stimulate Cell Proliferation



Prominent Stimulation of cell proliferation can be observed in bcAEVs injected zebrafish larvae condition. BrdU Incorporation Assay was performed to examine dividing cells. Four conditions were analyzed without 4IPP treatments.

CONCLUSION

- The Mitogenic and Immunogenic protein MIF is present on the surface of bcAEVs.
- We observed an in increase in proliferation in larvae injected with bcAEVs.
- bcAEVs and Macrophages interact with each other. Macrophages engulf bcAEVs.
- AEVs leave the Vasculature to possibly contact other cell types.

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