**ABSTRACT**

Background
The ability to prevent tumor reappearance 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 observed interacting with macrophages, providing a novel assay for probing interactions with the immune system in vivo. We observed an 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 characterizes 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

**REFERENCES**

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**METHODS AND RESULTS**

**bcAEVs Isolation and injection into zebrafish**

**bcAEVs from MDA-MB231**

**MIF is located on the surface of bcAEVs**

**In vivo tracking of bcAEVs and interaction with macrophages**

**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 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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