Introduction

- Osteosarcoma (OS) is the most common bone cancer in children. 1/3rd of all OS patients develop fatal metastatic disease, most often in the lungs.
- Metastasis is preceded by the formation of a pre-metastatic niche, whereby distant sites are "primed" for tumor cell seeding by tumor-secreted factors, one of which, are nano-sized extracellular vesicles called exosomes.
- Exosomes modulate stromal cell behavior at distant sites to promote metastasis.

We aim to determine the biodistribution of OS exosomes and their effects on lung stromal cells during OS pre-metastatic niche formation as a first step in identifying both earlier detection and novel therapeutic strategies for these high-risk patients.

Methods

Cell Culture: Human OS cell lines 143b, HOS, SAOS, U2OS, MG63.0, and MG63.2 were purchased from ATCC and maintained in their specified media with 10% FBS and penicillin/streptomycin.

Exosome isolation: Exosomes were isolated from supernatants harvested from OS cells cultured in exosome-depleted media by ultrafiltration followed by size exclusion chromatography, and quantified using a qNano particle counting and CD9 western blot. Exosomes were labeled with Cell Tracker DiL for imaging or PKH26 for flow cytometry.

Animal Studies: Animal studies were performed under CSU IACUC approval. Intravital imaging was done using the IVIS Spectrum following IV injection with labeled OS exosomes every 3 days for 2 weeks. In vitro Experiments: Donor human lung fibroblasts and alveolar macrophages were treated with OS exosomes for 72 hours and analyzed by ELISA.

Results

Human lung cells take up OS exosomes in vitro

- Alveolar Macrophages
- Lung Fibroblasts

Figure 3. Human lung fibroblasts and alveolar macrophages take up OS exosomes. (A) Human donor-derived alveolar macrophages and fibroblasts (B) were incubated with PKH26-labeled 143b exosomes and examined for uptake via flow cytometry.

Primary resident lung cells respond to OS exosomes

Figure 4. Lung cells respond to OS exosomes with cytokine production in vitro. (A) Donor human fibroblasts were treated for exosomes from 6 OS cell lines. (B) IL-6, IL-8, and MCP-1 were found to be highly upregulated after exosome treatment. (C) Human donor alveolar macrophages treated with 143b exosomes produce IL-8 assessed via ELISA.

Conclusions and Future Directions

- We can effectively detect OS exosome biodistribution in the mouse via non-invasive in vivo imaging.
- We observe OS exosome uptake and altered cytokine profiles in mice and primary resident lung cells.
- We intend to leverage our understanding of exosome biodistribution to determine if BAL fluid can serve as a predictor of metastasis.
- We aim to build on these studies to identify therapies that can impede pre-metastatic niche formation to slow lung metastasis.

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