Exploration of Mechanisms of Inhibition of Exosome Secretion by Novel Drugs in Ovarian Cancer Cells

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INTRODUCTION

Ovarian Cancer

- Most lethal gynecological cancer.
- High-grade serous ovarian carcinoma (HGSOC) most common.

Exosomes

- Extracellular vesicles that contain macromolecular cargo – such as nucleic acids, proteins, and lipids – that play a significant role in intracellular communication and modifying the extracellular microenvironment.
- Formed as intraluminal vesicles (ILVs) within multivesicular bodies (MVBs) within the plasma membrane to be packaged and sent for release in the extracellular space.
- Release mechanisms have a potential role in ovarian cancer cell progression.

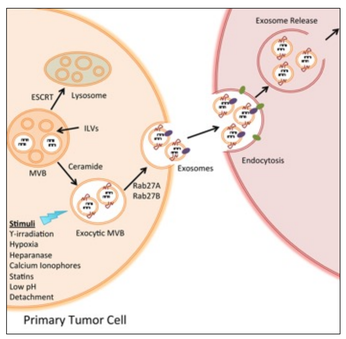

Figure 1. Exosome secretion pathway mechanisms. MVB biogenesis is governed by endosomal sorting complexes required for transport machinery (ESCRT)-dependent and ESCRT-independent pathways. ESCRT-independent pathways involve the conversion of sphingolipids to ceramide, that traffick the exosomes to be released. Rab27A and Rab27B traffick the exosomes to fuse with the membrane and to be released in the extracellular space. Exosomes may also fuse with the lysosomes in ESCRT-dependent mechanisms.

Tumor exosome inhibitors (TEXi1 and TEXi2)

- Have shown promise in inhibiting exosome release; however, the mechanisms at which they do so are unknown.

HYPOTHESIS & OBJECTIVE

Hypothesis

- TEXi1 and TEXi2 inhibit exosomes of ovarian cancer cells through similar mechanisms of known exosome inhibitors.

Objective

- To investigate the exosome secretion inhibition mechanisms of TEXi1 and TEXi2 compared to known exosome inhibitory drugs (MKT-077, Nexinhib20, GW4869).

SIGNIFICANCE / IMPACT

- Preventing ovarian cancer metastasis in the tumor microenvironment by using novel therapeutics to target and inhibit exosome secretion.

METHODS

- Model systems: Healthy fallopian tube secretory epithelial cells (FT33) as control, OVSAHO, MeSOV, OVCAR-3 and OVCAR-4 cancer cell lines (HGSOC) grown in exosome-free fetal bovine serum containing media.
- MTS cell viability assay for IC_{50} determination.
- Treatments include dimethyl sulfoxide (DMSO) as control, MKT-077, Nexinhib20, GW4869, TEXi1, or TEXi2 for 48 hours.
- Exosomes isolated using ultracentrifugation and filtration.
- Exosome protein markers confirmed through Western blotting.
- Nanoparticle tracking analysis using NanoSight NS300 utilized to quantitate isolated exosomes

RESULTS

Exosome Inhibitors IC_{50} Determination using OVCAR-4-CD63-Nluc Model

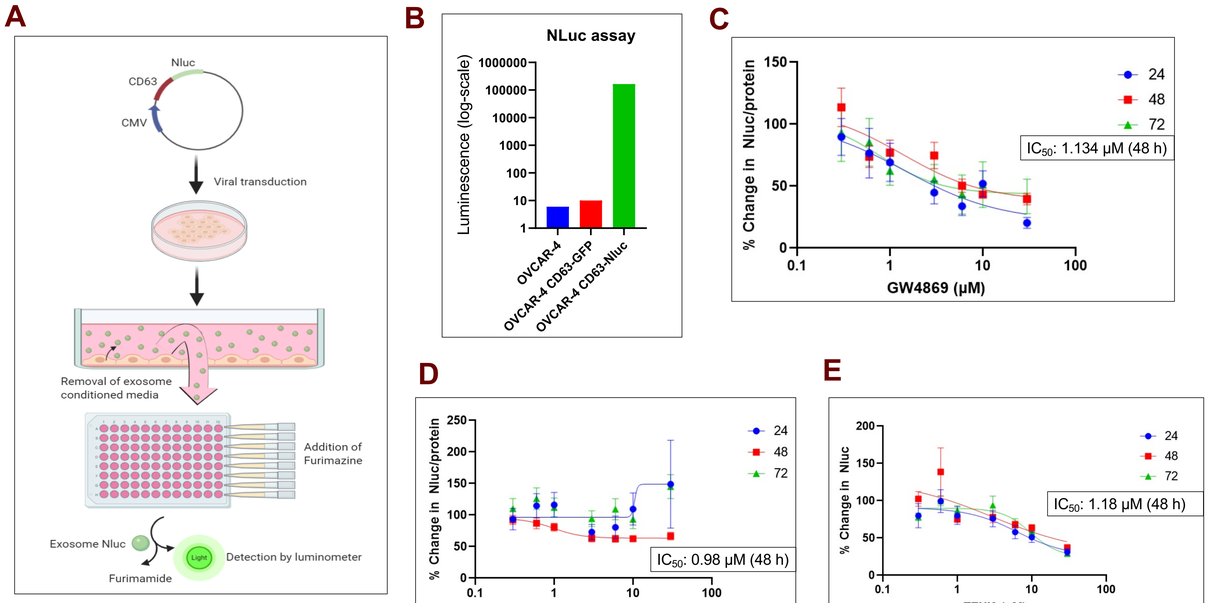

Figure 2. OVCAR-4-CD63-Nluc model and determining IC50 concentrations for drug treatments. A. OVCAR-4-CD63-Nluc model containing green luminescence protein on plasmid. **B.** MTS cell viability assay validating OVCAR-4-CD63-Nluc model compared with OVCAR-4 and OVCAR-4-GFP serving as controls to verify exosome concentration. **C, D, E.** Model used to treat cell lines with drugs GW4869, TEXi1, and TEXi2 at fixed time points (24, 48, and 72 hours) to determine IC_{50} values. MKT-077 and Nexinhib20 IC_{50} values calculated as 0.53 μM and 0.99 μM at 48 hours, respectively.

Nanoparticle Tracking Analysis

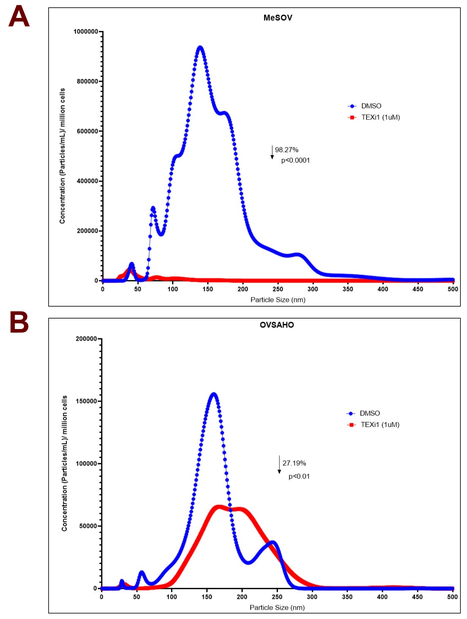

Figure 4. Nanoparticle tracking analysis for isolated exosome samples. A. Former NanoSight NS300 findings to quantitate the isolated exosomes in MeSOV and OVSAHO cell lines treated with TEXi1.

Exosome Isolation & Confirmation

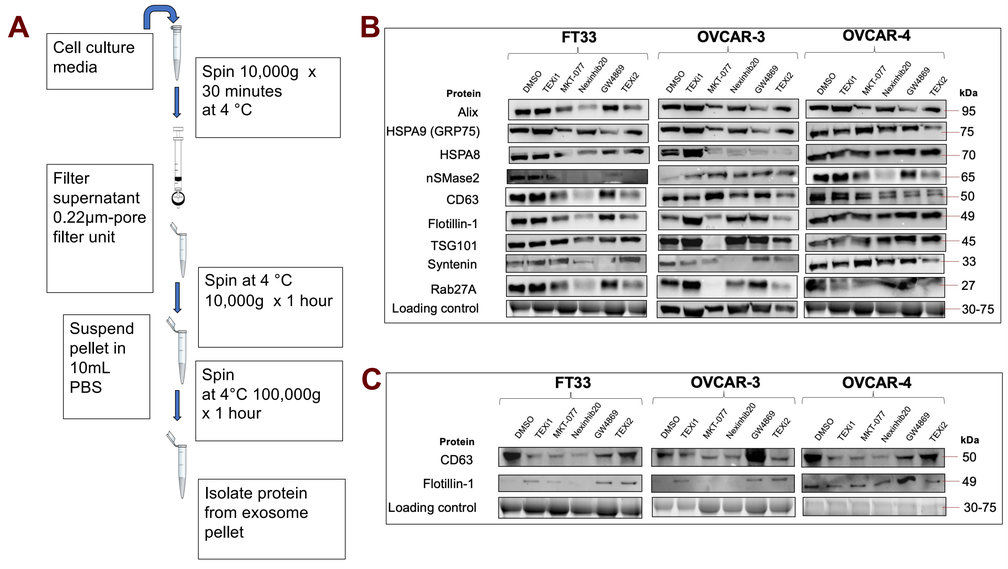

Figure 3. Exosome isolation methodology and Western blots of treated cells. A. Schematic illustration of the exosome isolation. **B.** Western blots confirming presence of ESCRT-dependent and ESCRT-independent proteins in whole cell lysates with added drug treatments: TEXi1 (0.98 μM), MKT-077 (0.58 μM), Nexinhib20 (0.99 μM), and GW4869 (1.134 μM) . **C.** Exosome Western blots confirmation.

DISCUSSION

Results Summary

- Exosome secretion proteins detected in immunoblots.
- Exosome CD63 levels decreased in TEXi1 and TEXi2 in OVCAR-3 and OVCAR-4 cell lines compared to solvent.

Limitations

- Low exosome concentrations loaded in Western blot.
- Time constraints.

Future Directions

- Treat OVCAR-4-CD63-GFP expressing cells with TEXi1 at IC_{50} and IC_{75} at fixed time points and image live cells using fluorescent microscopy.
- Isolate exosomes from plasma samples from MeSOV mouse model and perform Western blot to investigate TEXi1 treatment *in vivo.*
- Treat cells at IC75 concentration.

Societal Impact

- Known mechanisms of action for TEXi1 and TEXi2 can provide insight into developing new therapeutic agents for ovarian cancer metastasis.

ACKNOWLEDGEMENTS

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