Jotbody

Beyond affinity

Empowering Life Science with Precision

Jotbody EV Platform:

Targeted Nucleic Acid Delivery via Extracellular Vesicles in Metastatic Breast Cancer

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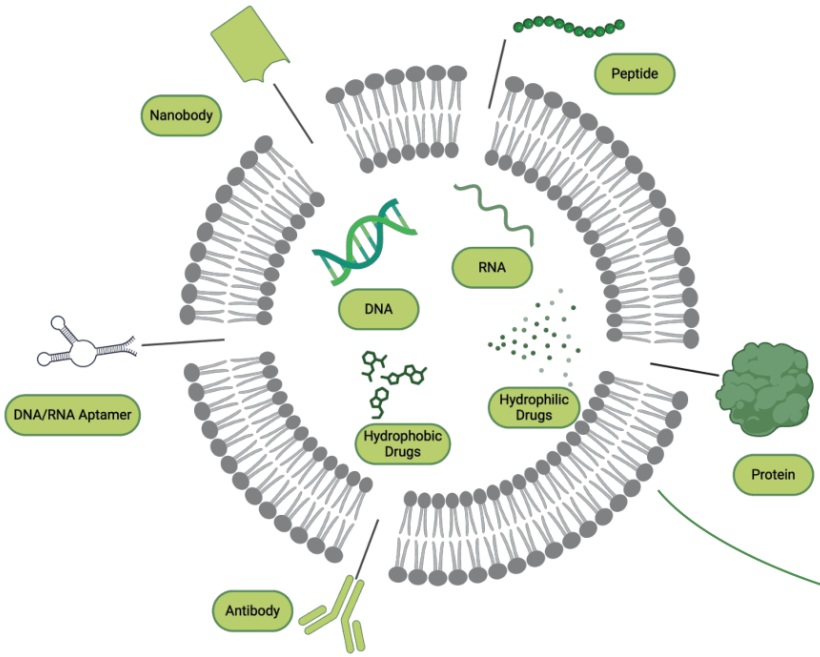
Introduction

Extracellular vesicles (EVs) are naturally released nanoparticles which act as messenger in cell to cell communcation. EVs deliver functional biomolecules from donor to recepient cells. With their natural biocompatibility, tissue-homing capabilities, and ability to transport functional molecules, EVs are an efficient and adaptable platform for drug delivery. However, naïve EVs suffer from non-specific uptake, which limits their therapeutic potential.

To transform EVs into a smart drug delivery system, We developed a platform for EVs' nucleic loading and surface modification, empowering EVs with the ability to deliver therapeutic nucleic acid and other molecules into targeted cells. As a proof of concept, antisense oligonucleotide (ASO) anti oncogene miR-125 was loaded into red blood cells derived EVs (RBCEVs) and a single domain antibdoy (sdAb) against EGFR was conjugated onto RBCEVs surface. The treatment successfully suppressed tumor growth in a mouse model of metastatic breast cancer, demonstrating the potential of Jotbody's platform for enhancing targeted nucleic acid-based therapies using EVs.

- **Targeted Delivery:** Jotbody's platform allows for conjugation of proteins, antibodies, antibody fragments, and single-domain antibodies.
- **Nucleic Acid Loading:** Jotbody's platform allows for effective loading of nucleic acids, including small RNAs and plasmids.
- **Enhanced Therapeutic Efficacy:** Enables precise and efficient delivery of genetic therapies directly to target cells, improving therapeutic outcomes while minimizing off target effects.
- **Safety and Biocompatibility:** Extensive testing confirms that our engineered EVs can be administered repeatedly without adverse effects.
- **Range of application:** The platform is dynamic and translatable to EVs of any source.

Jotbody's Smart Drug Delivery Platform



Methods

Every section of method follows the latest guidelines suggested by Minimal information for studies of extracellular vesicles (MISEV).

EV Isolation/Characterization

- Isolated RBCEVs from healthy human donors.
- Ultracentrifugation, Size exclusion chromatography (SEC), Particle Metrix ZetaView, Transmission electron microscopy (TEM), Western blotting for protein detection.

EV Functionalization

- ASO or siRNA were transfected into RBCEV using Jotbody platform.
- Alpacas derived sdAbs (VHH) were covalently conjugated onto RBCEVs surface using Jotbody platform.

Results

Nanobody conjugated EVs show increased accumulation in target cells

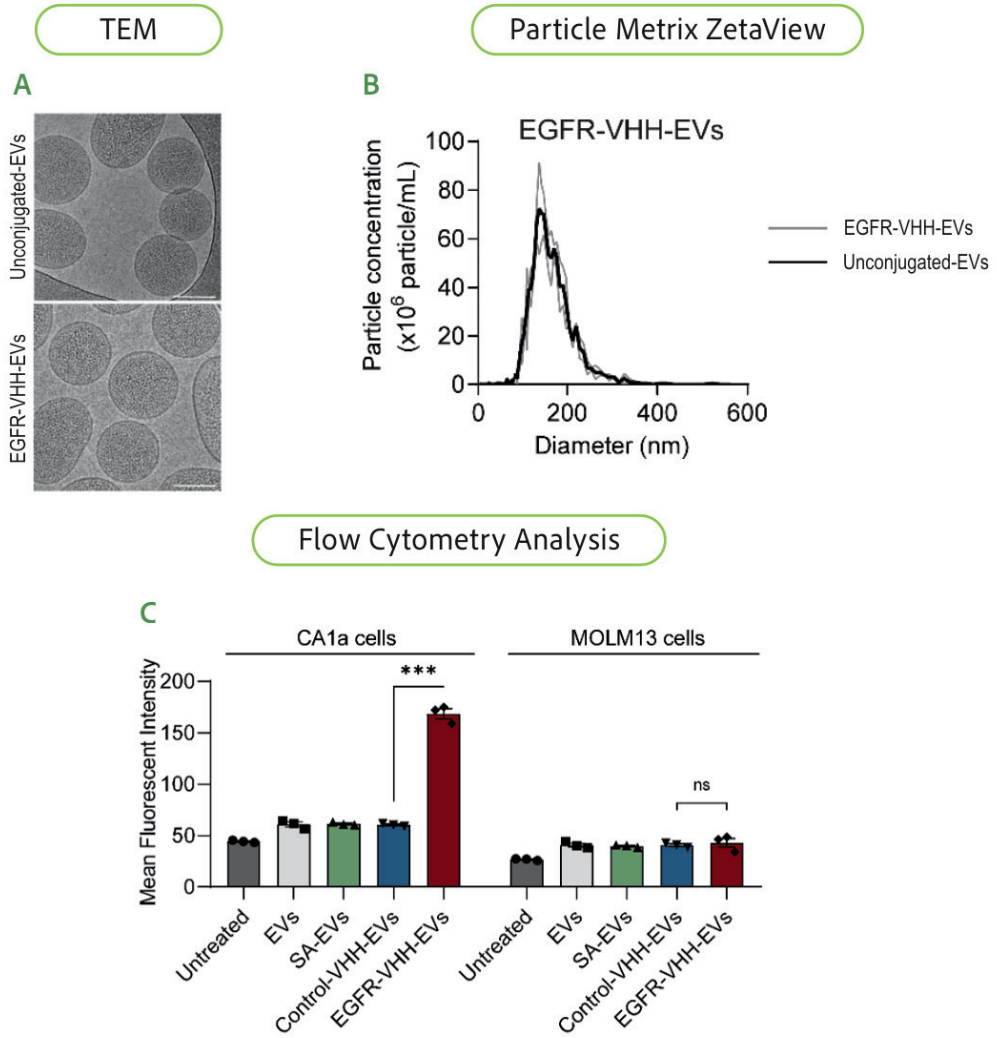


Figure A&B
VHH conjugation did not affect the naïve EVs structure, shape and size as confirmed by TEM and NTA.

Figure C
EGFR VHH was determinant to significantly increased the uptake of EVs into CA1a cells (EGFR+) but not into control cells MOLM13 (EGFR -) as evaluated by flow cytometry analysis.

Targeted EVs specifically deliver the therapeutic cargo into target cells

Nucleic Acid Targeted Delivery

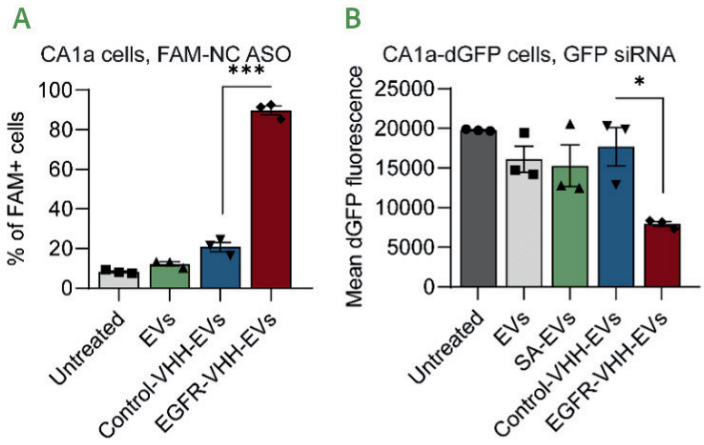


Figure A
Surface modification with EGFR nanobody specifically drives FAM-ASO loaded EVs into EGFR-positive CA1a cells.

Figure B
Surface modification with EGFR nanobody specifically drives siRNA loaded EVs into target cells resulting in reduction of GFP expression.

C

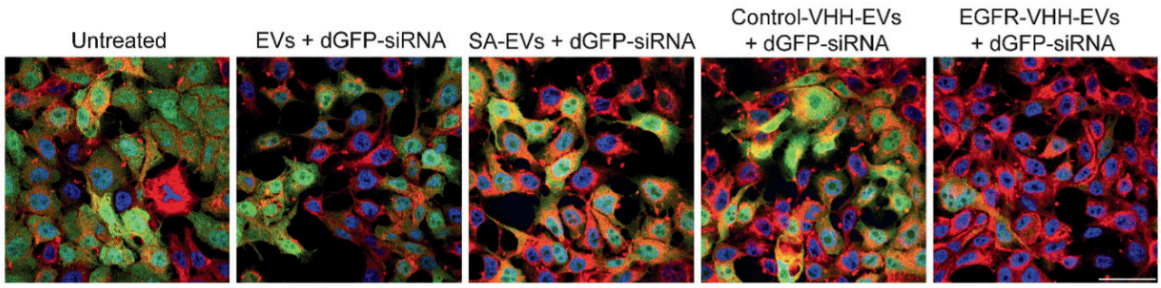


Figure C
EGFR-targeted EVs demonstrated the ability to efficiently deliver siRNAs, resulting in a 70% reduction in GFP signal.

D

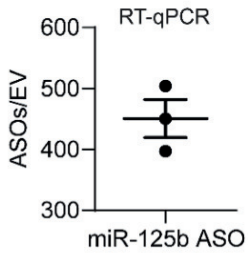
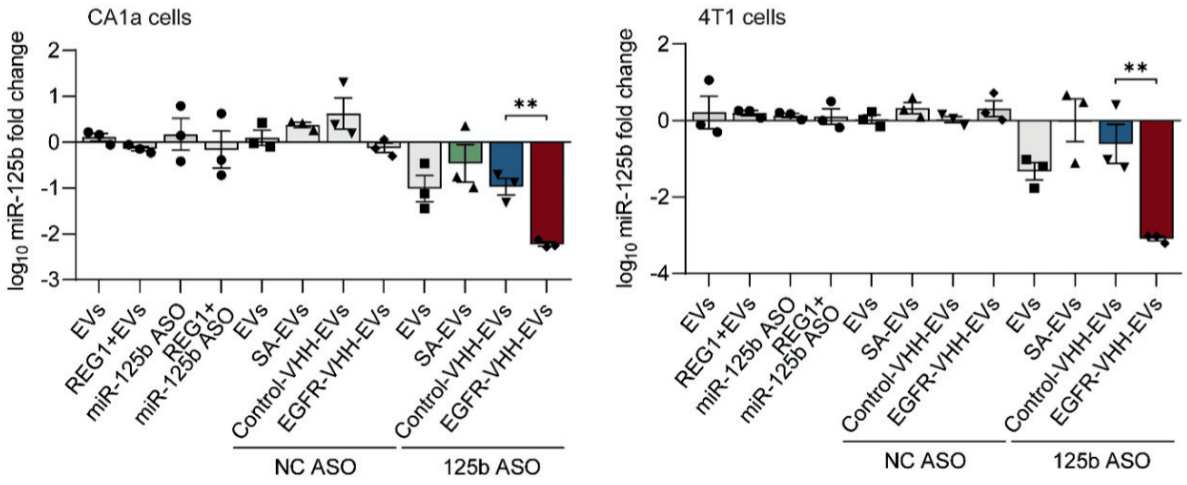


Figure D
Comparison of Ct values revealed that each EV contained on average 450 miR-125b ASOs.

E



F

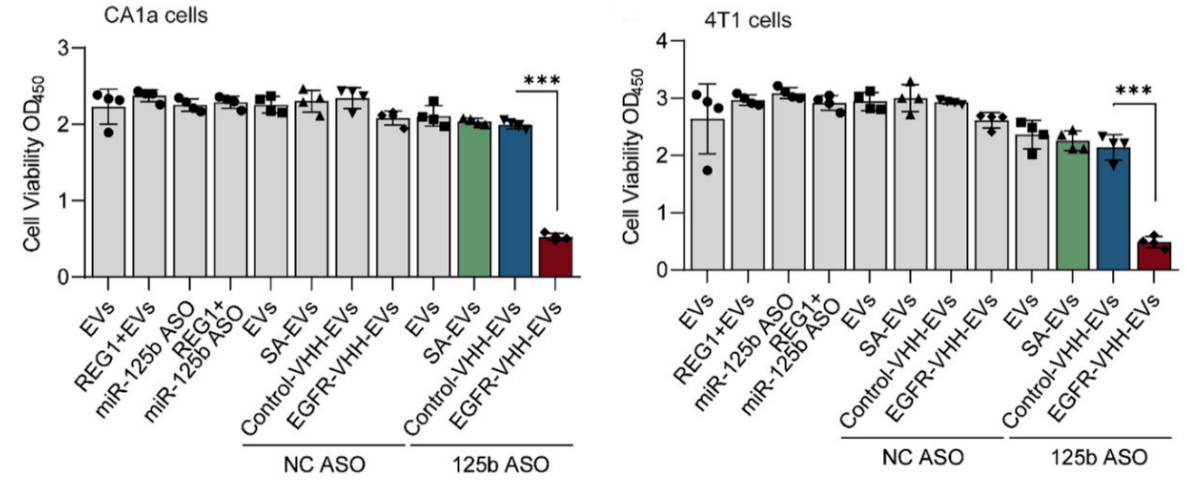


Figure E
EGFR-targeted EVs loaded with ASO (125b) significantly decrease the expression of the oncogenic miRNA 125 in both CA1a and 4T1 breast cancer cells.

Figure F
The decrease in miRNA 125 expression led to a reduction in cell viability in both CA1a and 4T1 cells.

Functionalized EVs suppressed th tumor growth in mouse model of breast metastatic cancer

In *vivo* Targeting Activity

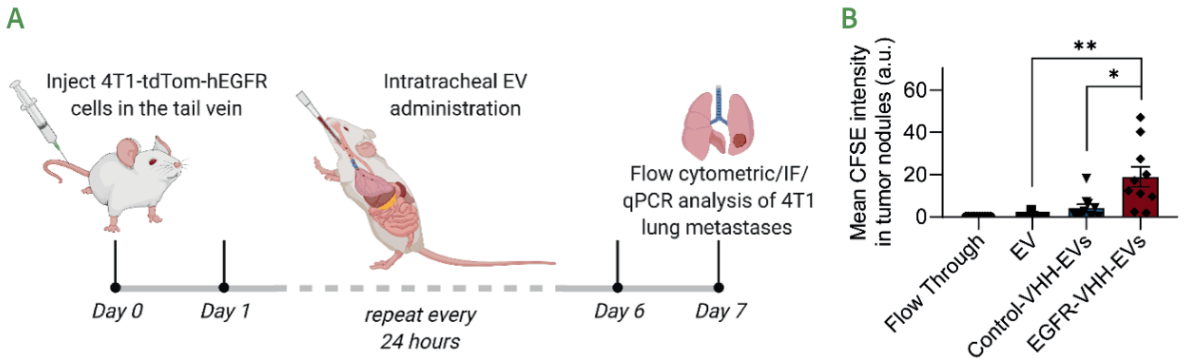


Figure A
We created an hEGFR-positive lung metastatic breast cancer model by systemically injecting 4T1-tdTomato-hEGFR cells into NSG-SGM3 mice for intratracheal EVs treatment.
Figure B
Upon intratracheal admistration, EGFR-targeted EVs showed significantly higher levels of uptake by tumor cells as compared to untargeted EVs.

In *vivo* Therapeutic Effect

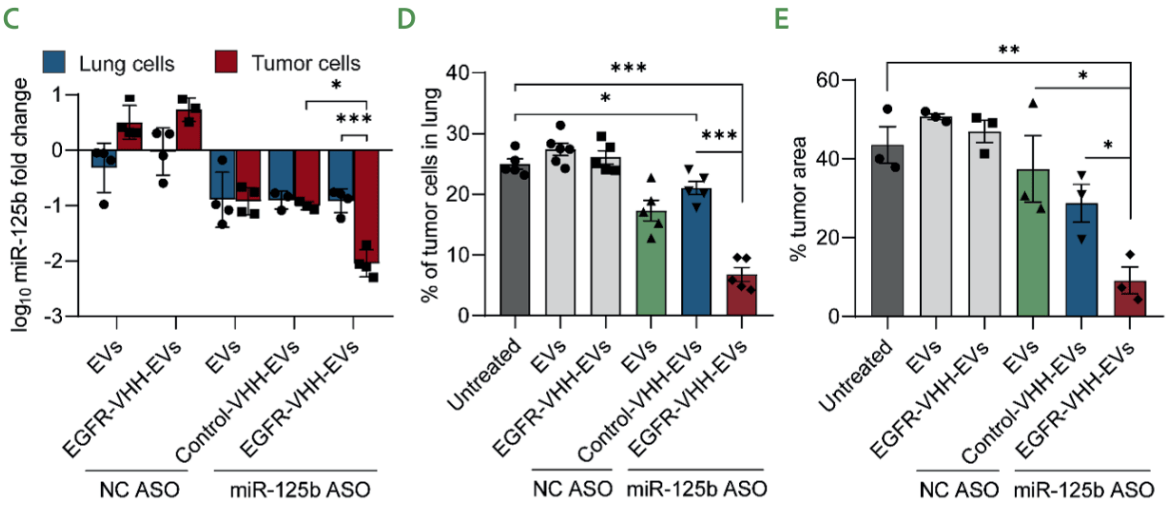


Figure C
EGFR-targeted miR-125b ASO-loaded EVs induce a significantly greater knockdown in tumor cells compared to healthy lung cells.
Figure D
EGFR-VHH-EVs loaded with miR-b ASO exhibited superior tumor-suppressive effects, leading to a significantly reduced percentage of tumor cells in the lungs compared to control groups.
Figure E
EGFR-VHH-EVs loaded with miR-125b ASO showed superior tumor suppression, significantly reducing lung tumor area compared to the control groups.

F

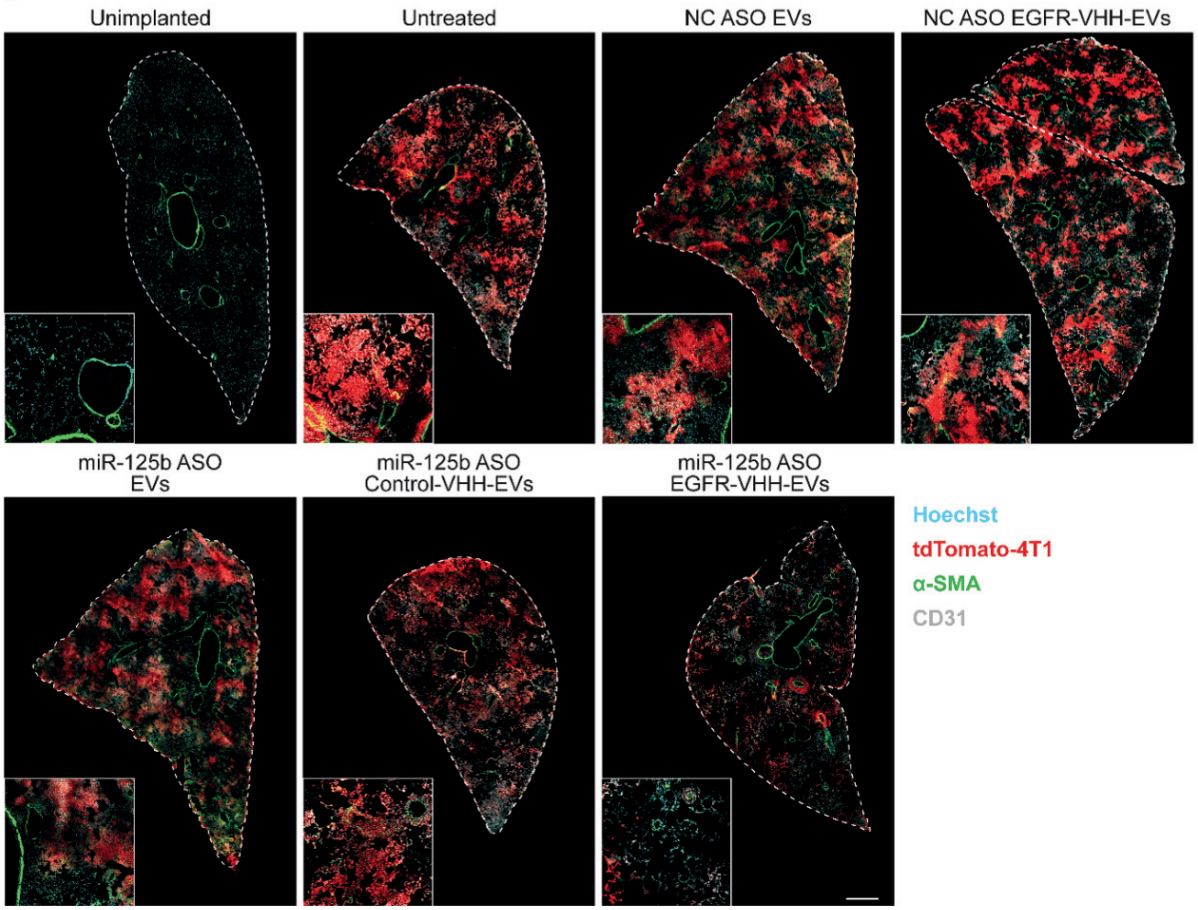


Figure F
Tumor nodules in the EGFR-targeted miR-125b-EVs treatment were significantly smaller and did not show high levels of colocalization with CD31, a wellestablished marker for blood vessel density in tumor tissue.

Conclusion

This modular and biocompatible approach to EV engineering presents an innovative drug delivery platform for the targeted delivery of diverse therapeutics. The method allows for stable conjugation of targeting moieties and therapeutic payloads, improving specificity, efficiency, and stability. The engineered EVs show significant potential in reducing side effects, overcoming cellular barriers, and delivering therapeutic molecules directly to diseased cells. This breakthrough paves the way for safe, scalable, and versatile EV-based drug delivery solutions, setting a new benchmark in nanomedicine.

Reference

Surface-engineered extracellular vesicles for targeted delivery of therapeutic RNAs and peptides for cancer therapy", Theranostics (2022); 12(7):3288-3315

Other related publication to learn further about Jotbody

Extracellular Vesicle Surface Display Enhances the Therapeutic Efficacy and Safety Profile of Cancer Immunotherapy, Molecular Therapy (2024) 32: 1-22
Red Blood Cell-Derived Extracellular Vesicles Display Endogenous Antiviral Effects and Enhance the Efficacy of Antiviral Oligonucleotide Therapy, ACS Nano 2023, 17, 21, 21639–21661
Covalent Conjugation of Extracellular Vesicles with Peptides and Nanobodies for Targeted Therapeutic Delivery", Journal of Extracellular Vesicles, 10.4 (2021): e12057

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