Exosome Nanovesicles for the Homing Delivery of Therapeutics into Autologous Cancer Cells

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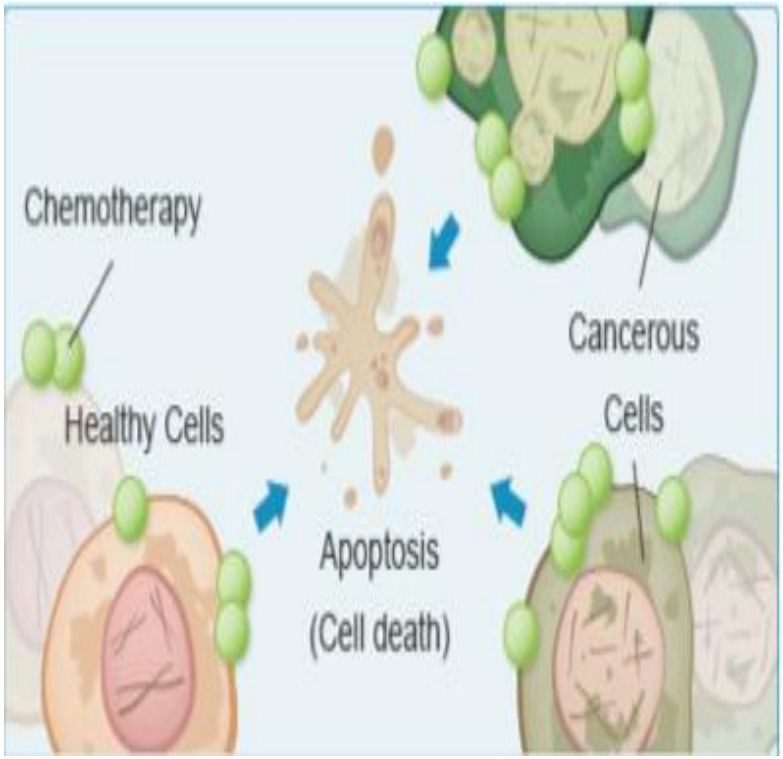
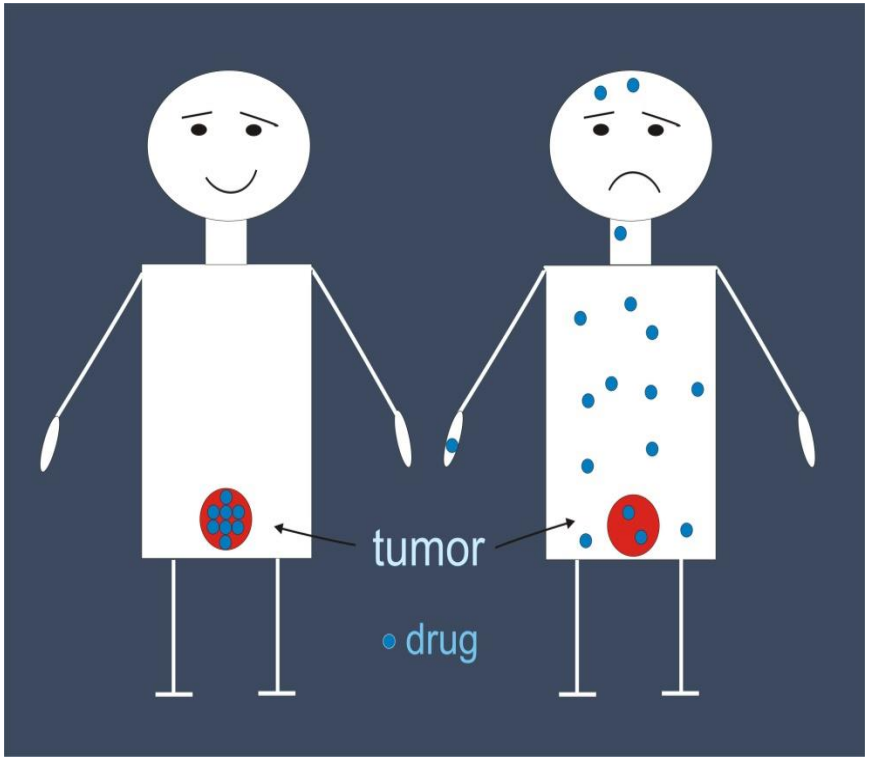
Outline

- Introduction
  - Nanoparticle-enhanced chemotherapy
  - Exosome for targeted drug delivery
- Experiment
  - Exosome-homing evaluation
  - Exosome-homing delivery
    - Fluorescent marker and anticancer drugs
    - Small interfering RNA
  - Exosome characterization
    - Surface proteins and miRNA
- Conclusions
- Future studies

Targeted-Delivery of Chemotherapy





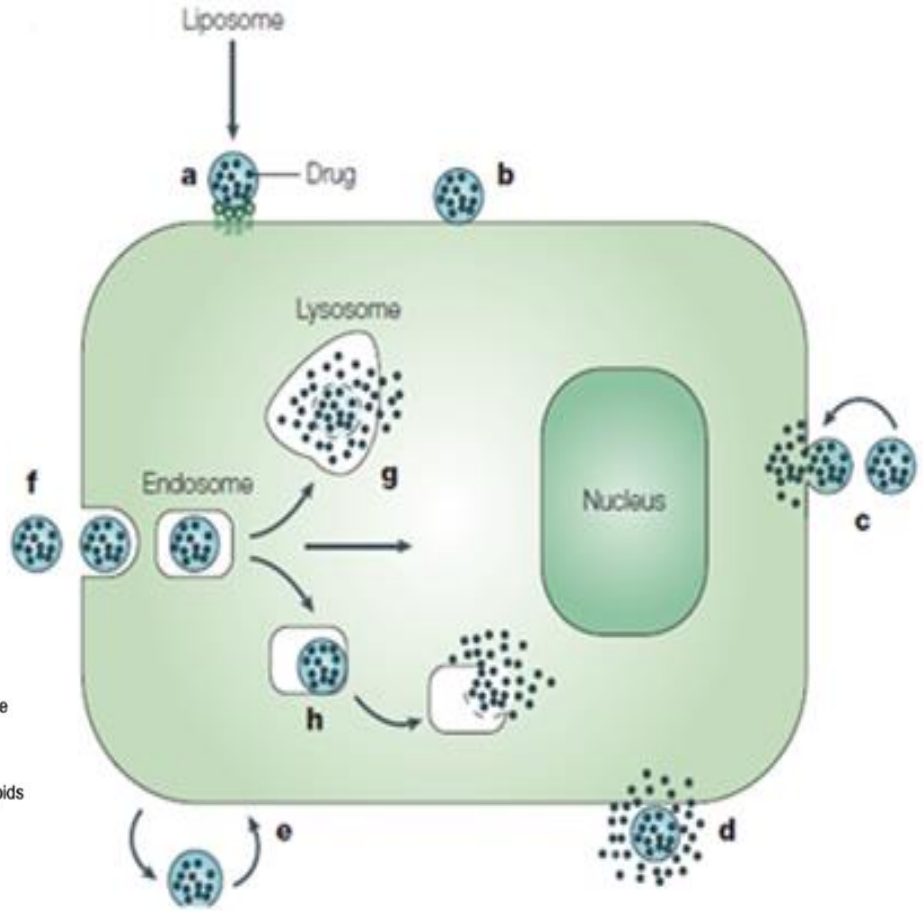
Traditional Chemotherapy

Targeted Chemotherapy

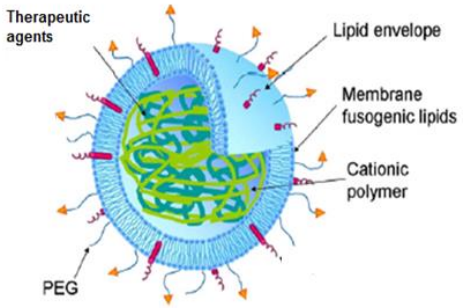
https://www.mesotheliomaguide.com/wp-content/uploads/2014/04/ChemoSideEffectCauses.png

Nanoparticles as Delivery Carriers

- Free anticancer drugs loaded into carrier
- Protection
- Pharmacokinetics
  - Fusion
  - Endocytosis uptake



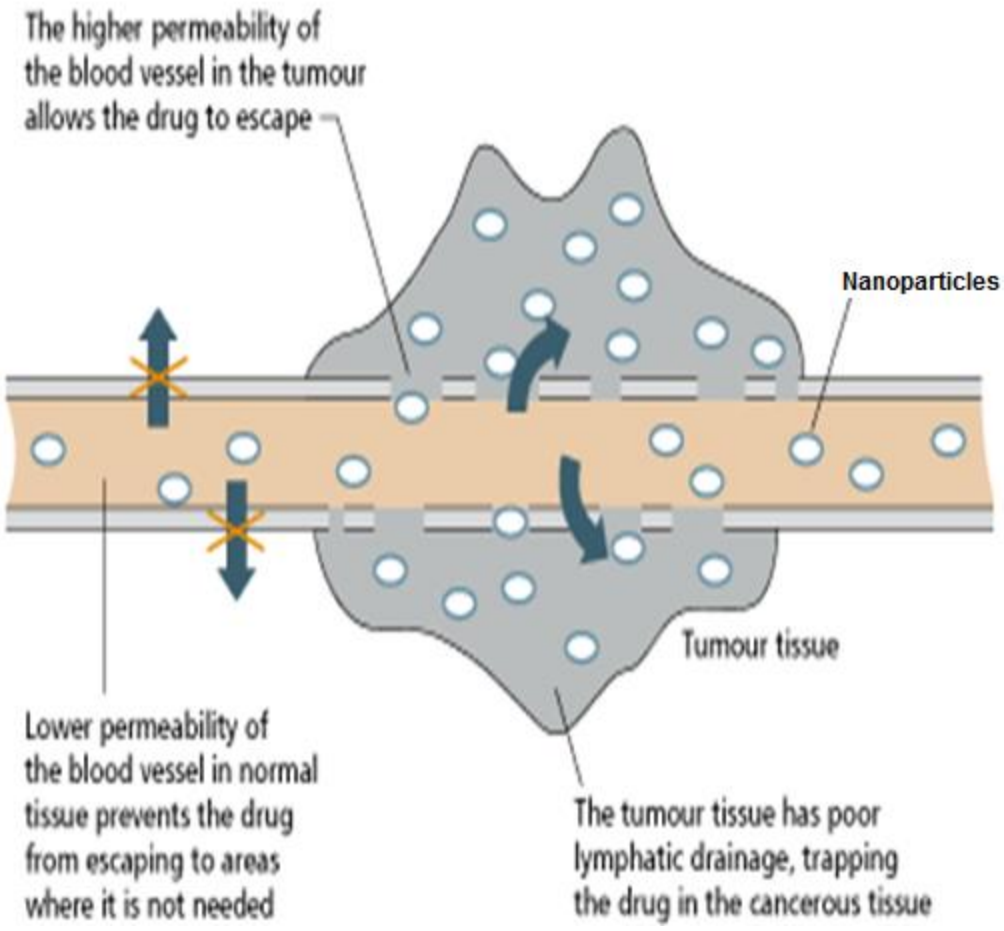
DOXIL®
(doxorubicin HCl liposome injection)



Torchilin (2005), Nature Reviews: Drug Discovery 4:145

Nanoparticles-Enhanced Delivery

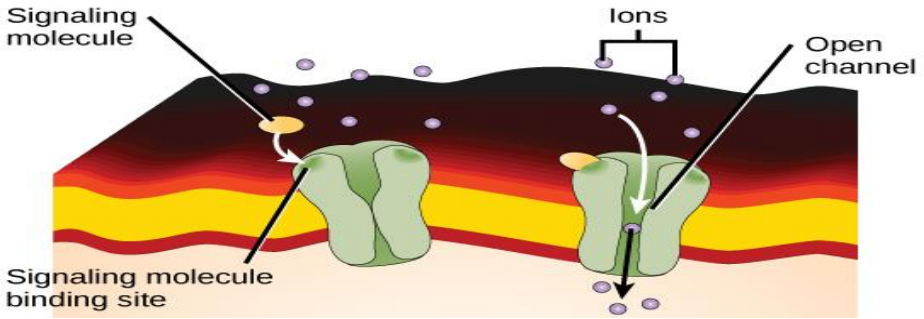
- Passive targeting
  - Enhanced permeability and retention (EPR)
- Limitation of nanoparticle-delivered anticancer drugs
  - Active targeting
  - Immunogenicity

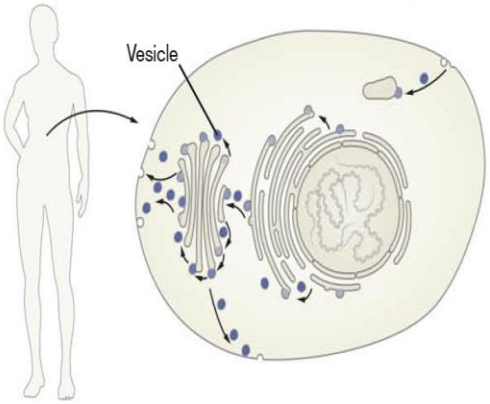


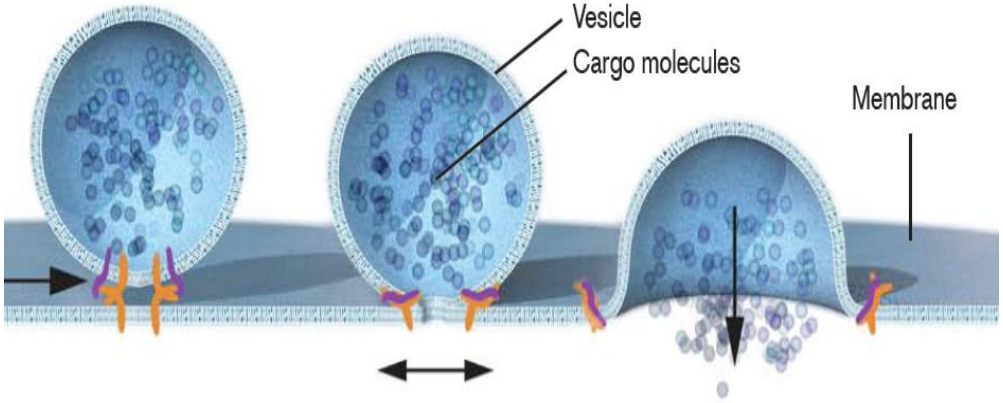
Dinsmore et al (2002), Science 298:1006

Extracellular Vesicles

- Garbage bins to novel carriers





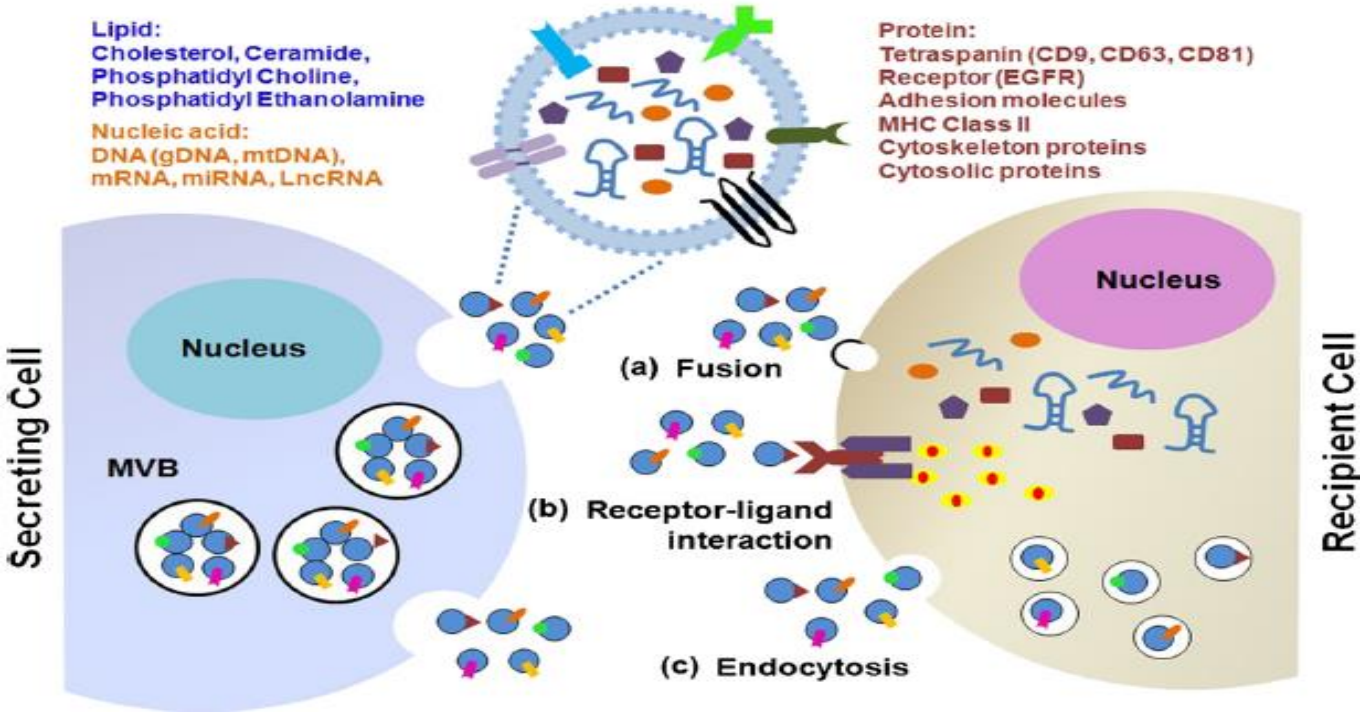


2013 Nobel Prize
Physiology or Medicine

Graça Raposo, and Willem Stoorvogel , J Cell Biology 2013, 373-383

Exosome Nanovesicles

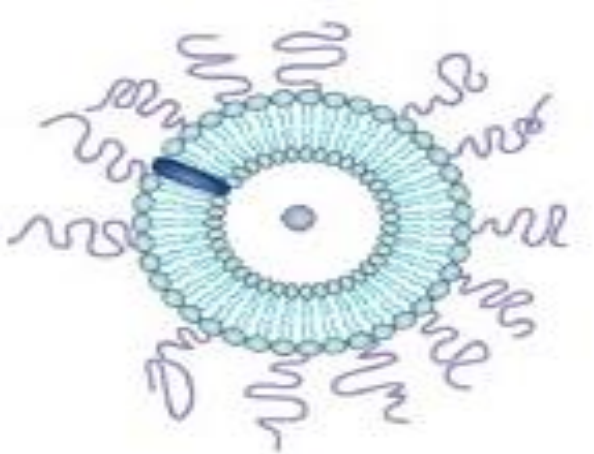
- Nanosize, abundant, and stable
- Crossing barrier
- Home selection

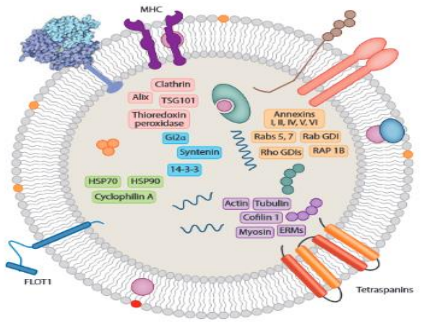


Zhang et al (2015), Journal of haematology & oncology 8:83

Exosomes as Drug Delivery Carriers

- **Alternatives to traditional nanoparticles**
  - Natural carriers
  - No immunogenicity
  - Long circulating time
  - Cross physiological barrier
  - Active home-targeting





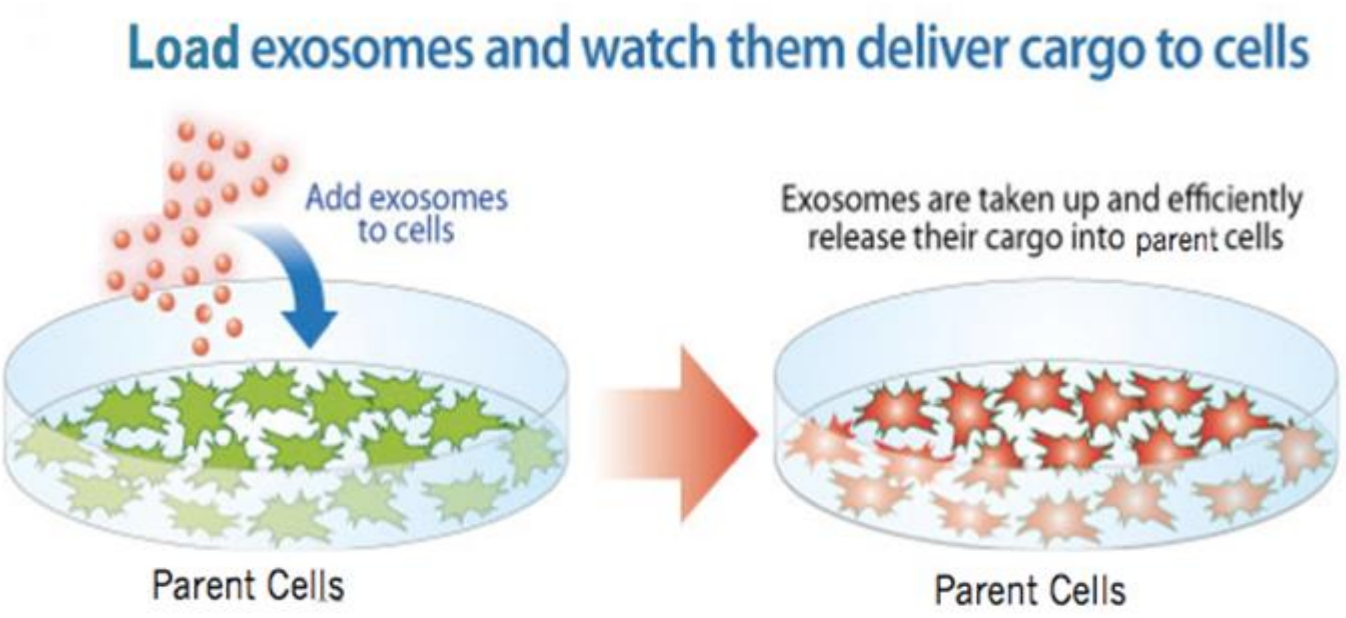
- **Borrowing self-secreted nanovesicles**
  - **Cancer cells**

Alvarez-Erviti et al (2011), Nature Biotechnology 29:341-5

Johnsen KB et al (2014), Biochimica et Biophysica Acta 1846:75-87

Hypothesis

- Cancer cell-derived exosomes could be used as a targeted carrier to deliver therapeutics into parental cancer cells



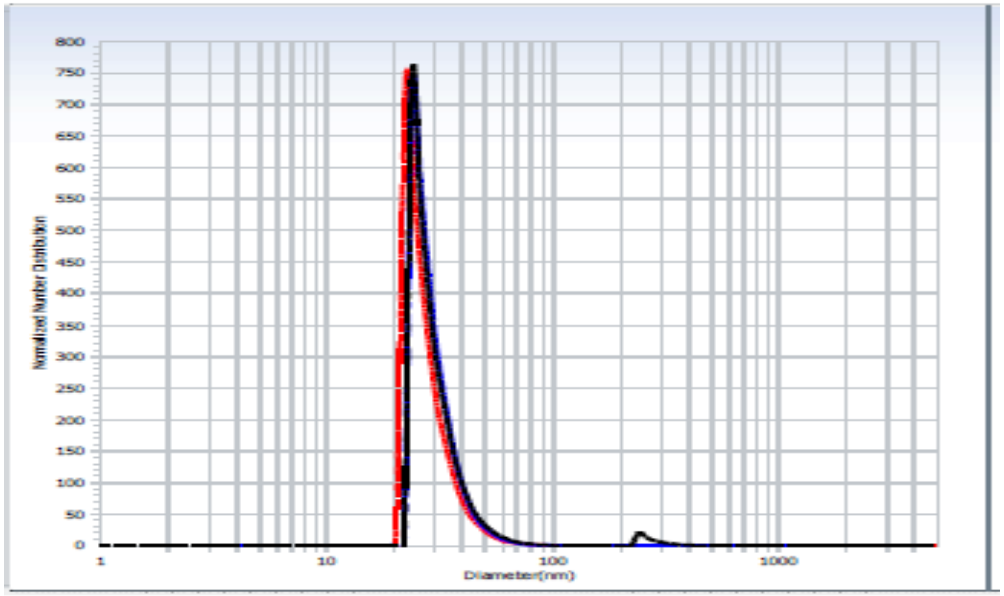
https://www.systembio.com/images/exosomes-deliver-to-cells.jpg

Experimental Design

I. Isolate exosomes from cell media
   - A549 exosome: adenocarcinoma A549
   - bEND.3 exosome: brain endothelial bEND.3
II. Assess home-selectivity of exosomes
III. Test cell uptake of exosome-delivered fluorescent maker
IV. Evaluate cytotoxicity of exosome-delivered anticancer drugs
V. Identify knockdown efficacy of exosome-delivered siRNA on ICAM-1

I: Particle Sizing of Exosome

Exosome origin	Particle size
Brain endothelial bEND.3 cell	48.4±3.3 nm
Lung adenocarcinoma A549 cell	25.5±1.0 nm

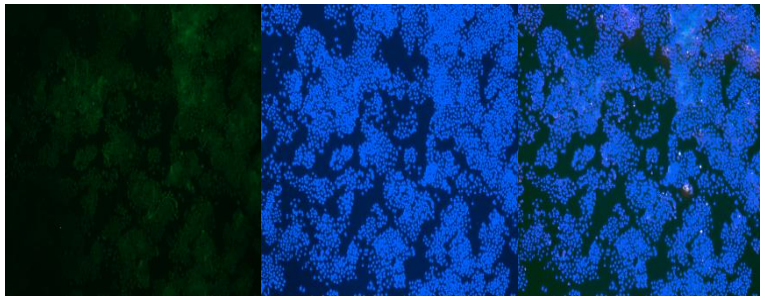


II: Home Selectivity of Exosomes

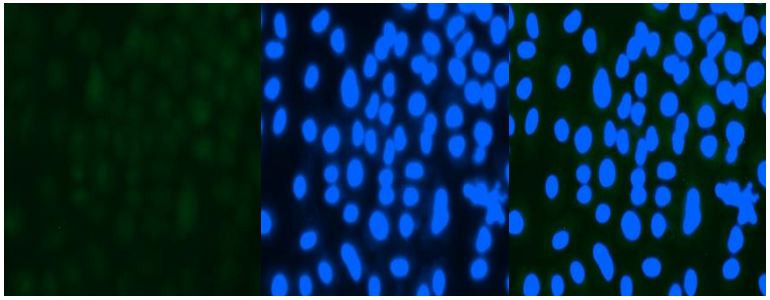
- Exosome labeled by Exo-Green® kit
- **A549 cell** staining and imaging

bEND.3 exosome

Exosome Nucleus Overlay



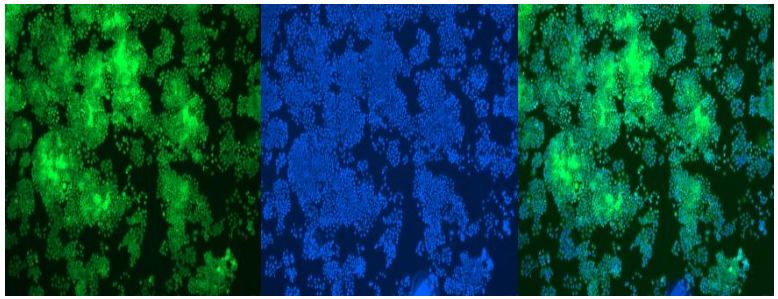
X 4

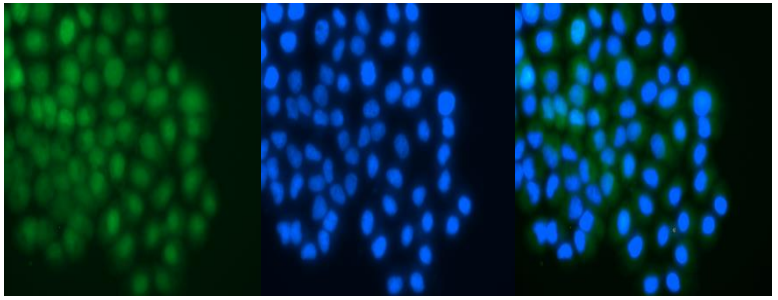


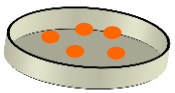
X 40

A549 exosome

Exosome Nucleus Overlay

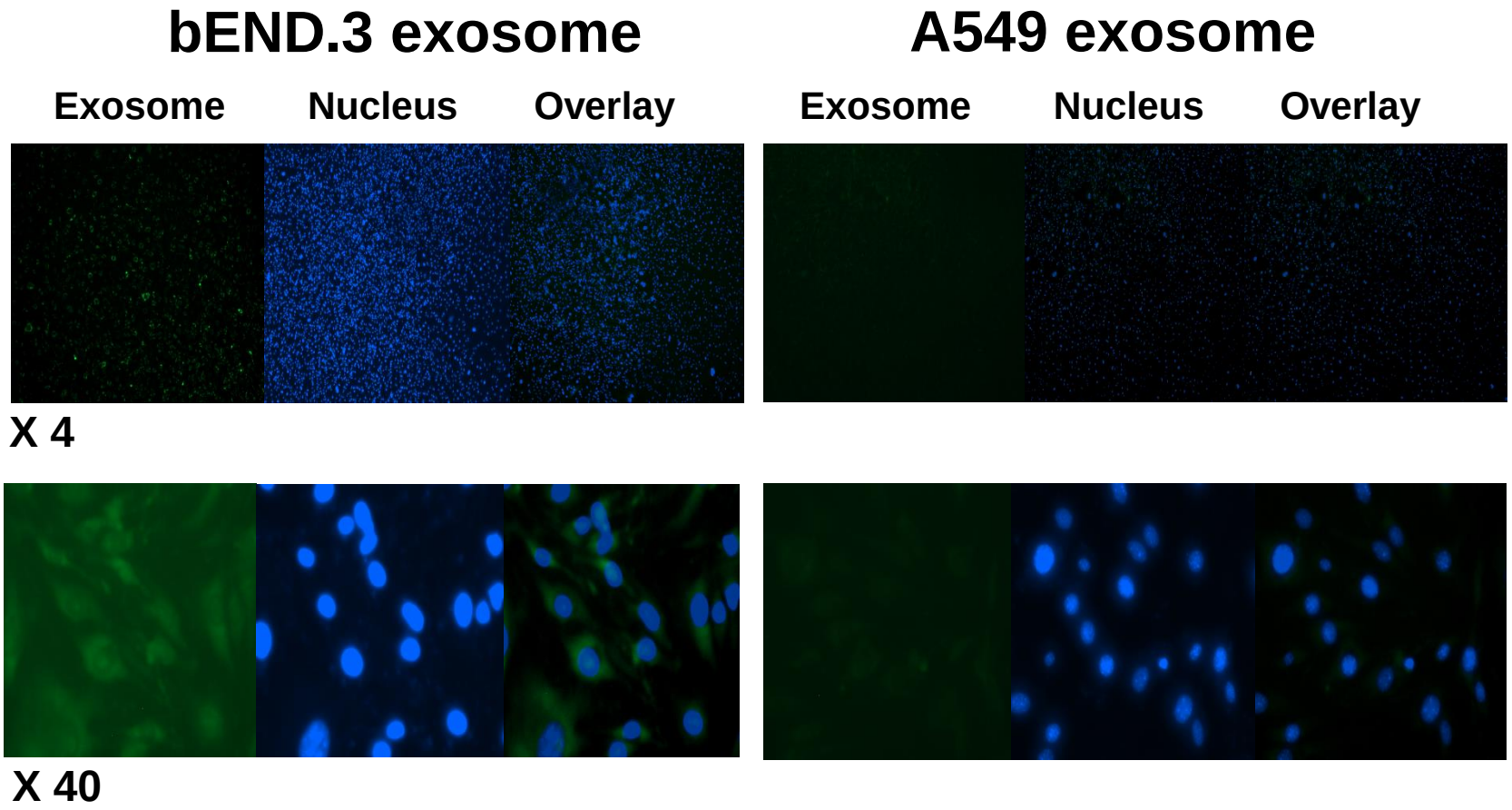






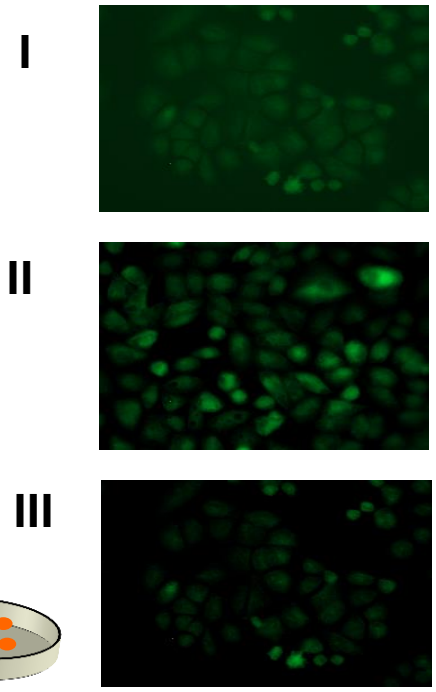
Home Selectivity of Exosomes

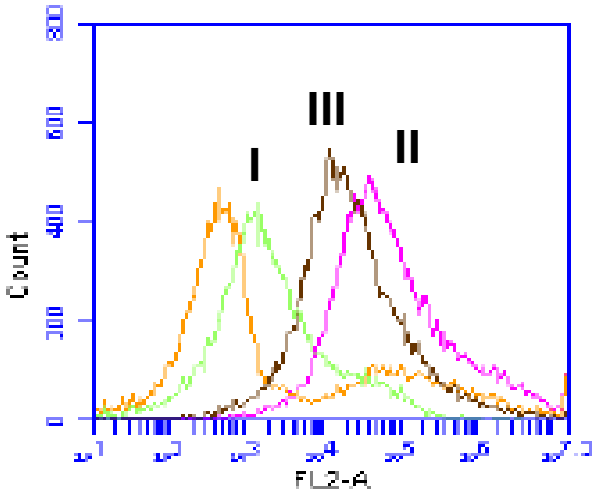
- Exosome labeled by Exo-Green® kit
- **bEND.3 cell** staining and imaging

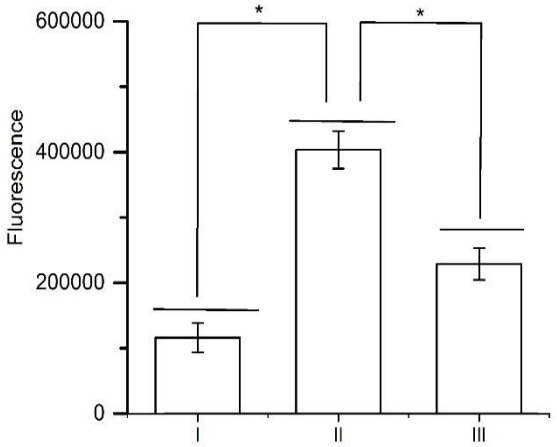


III: Cell Uptake of Rhodamine 123

- Loaded rhodamine 123 into exosomes
- A549 cancer cell
- Florescence microcopy
- C6 cell flow cytometry







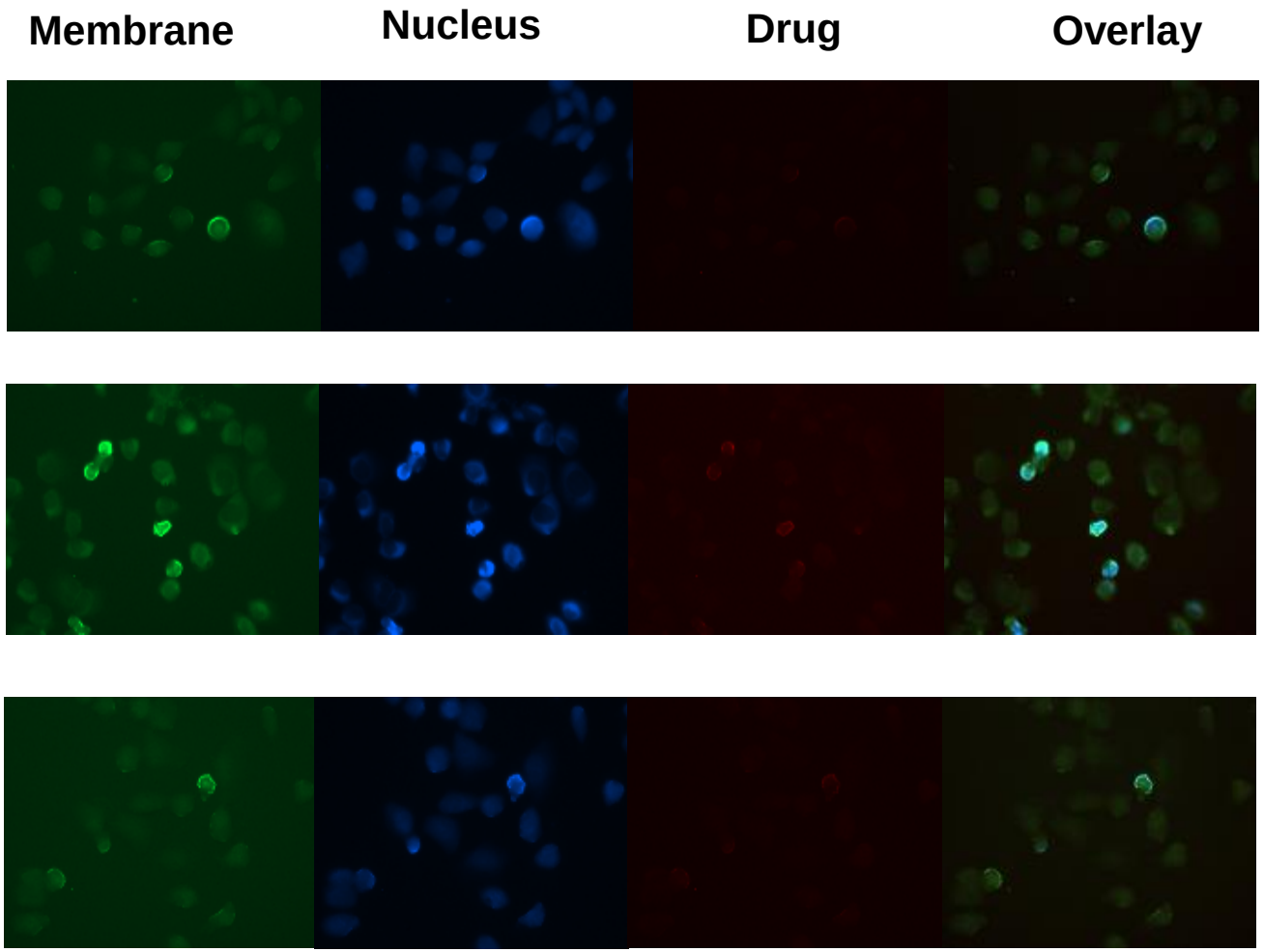
I: Rhodamine 123
II: Rhodamine 123 loaded into A549 exosome
III: Rhodamine 123 loaded into bEND.3 exosome

*** Results are significantly different, $p < 0.05$**

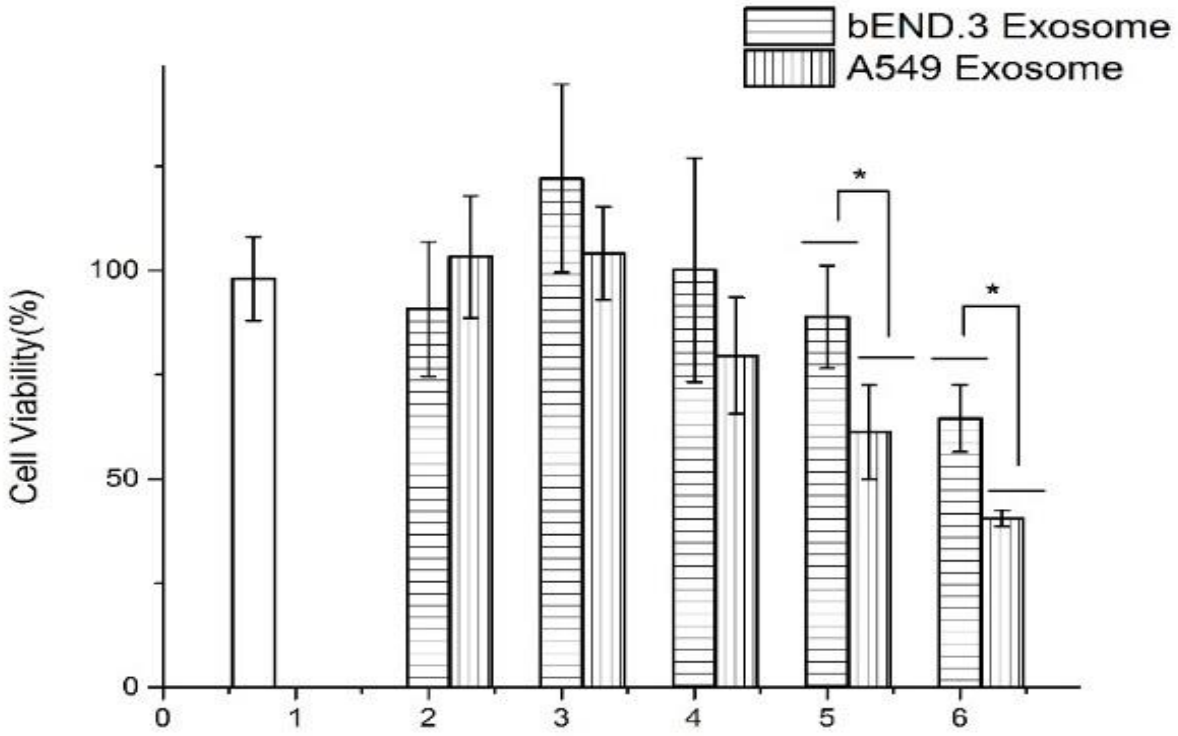
IV: Exosome-Delivered Anticancer Drugs

- Doxorubicin (auto-red fluorescence)
- Cell uptake assessed by staining and imaging
- Doxorubicin, methotrexate, and paclitaxel formulated into different amounts of exosome
  - Formulation having more loaded drugs in the higher amount of exosome
- A549 cells treatment
- Cytotoxicity evaluation of exosome-delivered anticancer drugs

A549 Cell Uptake of Doxorubicin

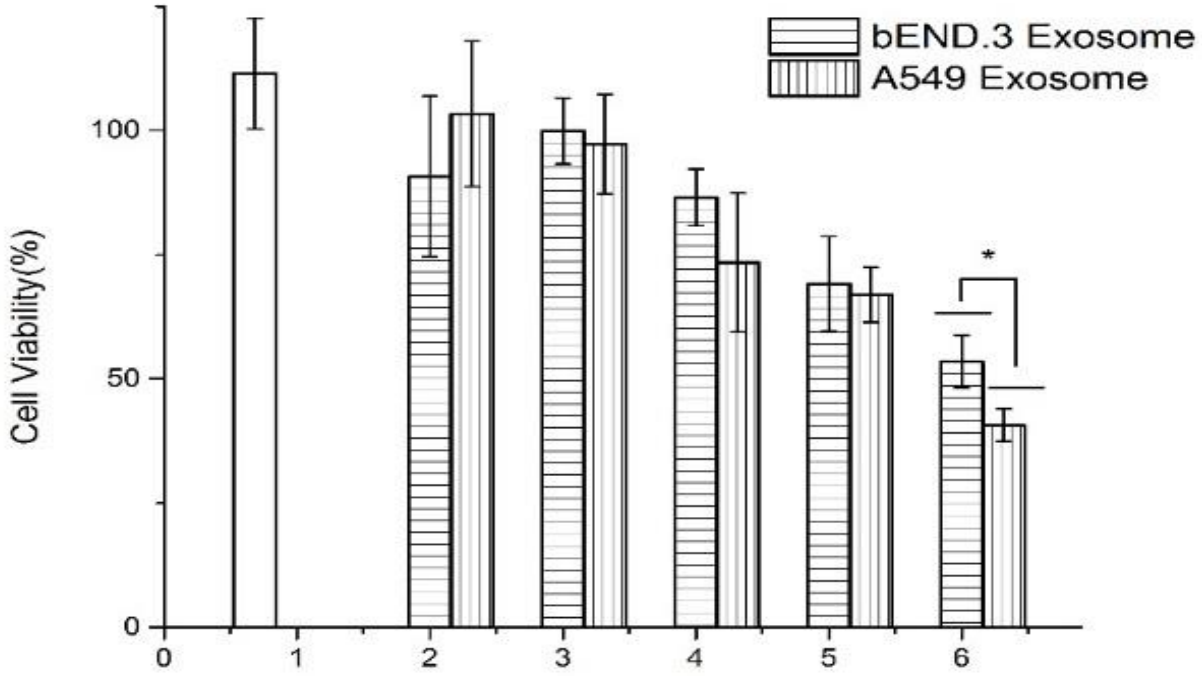


Increased Cytotoxicity of Exosome-Delivered Doxorubicin

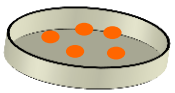


1: doxorubicin (1 μM), 2. 200 μg/ml exosome; 3. drug + 20 μg/ml exosome; 4. drug + 40 μg/ml exosome; 5. drug + 100 μg/ml exosome; and 6. drug + 200 μg/ml exosome, * Results are significantly different, $p < 0.05$).

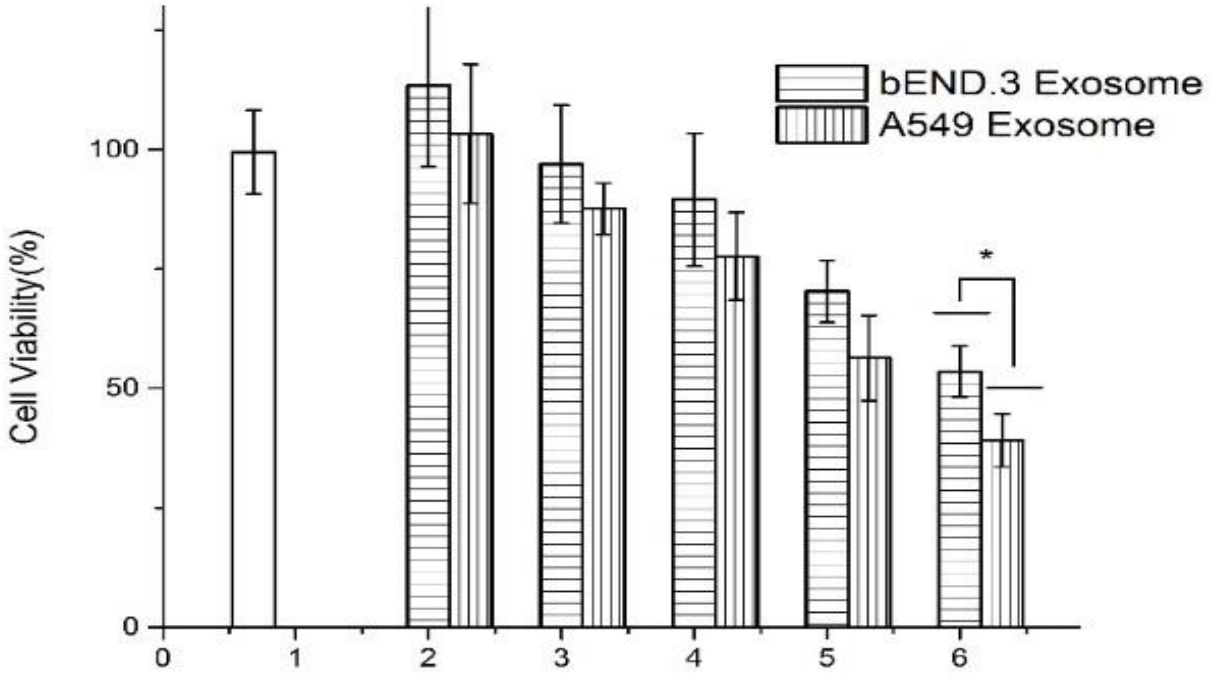
Increased Cytotoxicity of Exosome-Delivered Methotrexate



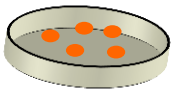
1: methotrexate (50 μM), 2. 200 μg/ml exosome; 3. drug + 20 μg/ml exosome; 4. drug + 40 μg/ml exosome; 5. drug + 100 μg/ml exosome; and 6. drug + 200 μg/ml exosome, * Results are significantly different, $p < 0.05$).



Increased Cytotoxicity of Exosome-Delivered Paclitaxel

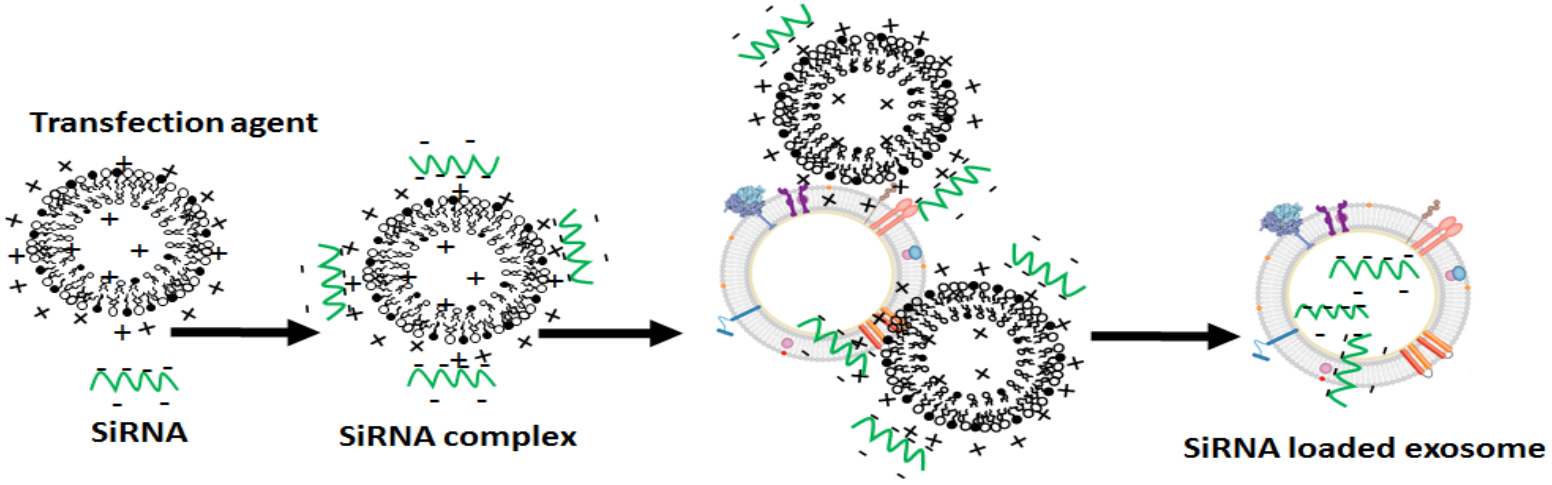


1. paclitaxel (50 μM); 2. 200 μg/ml exosome; 3. drug + 20 μg/ml exosome; 4. drug + 40 μg/ml exosome; 6. drug + 100 μg/ml exosome; and 7. drug + 200 μg/ml exosome, * Results are significantly different, $p < 0.05$).

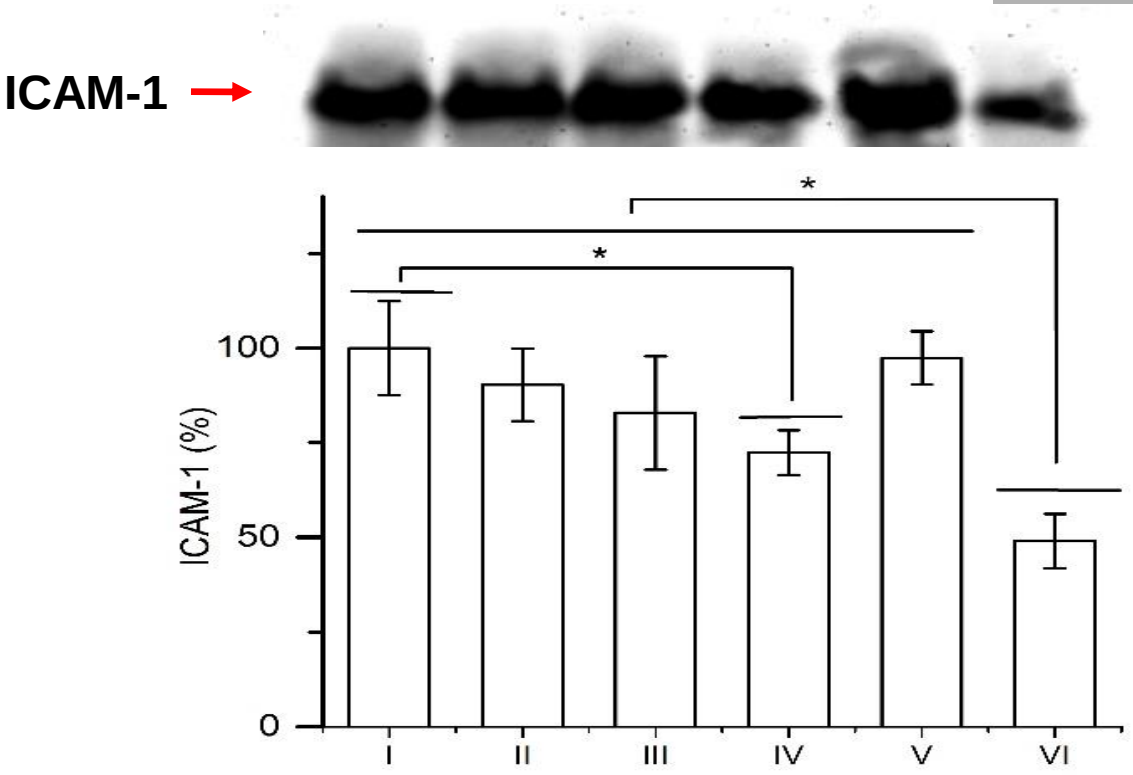


V: Exosome-Delivered Small Interfering RNA (siRNA)

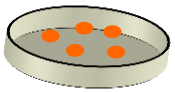
- Difficult to directly diffuse by incubation
- Form siRNA and transfection reagent (Lipofectamine®) complex
- Intercellular Adhesion Molecule 1 (ICAM-1)



ICAM-1 Knockdown by Selective SiRNA

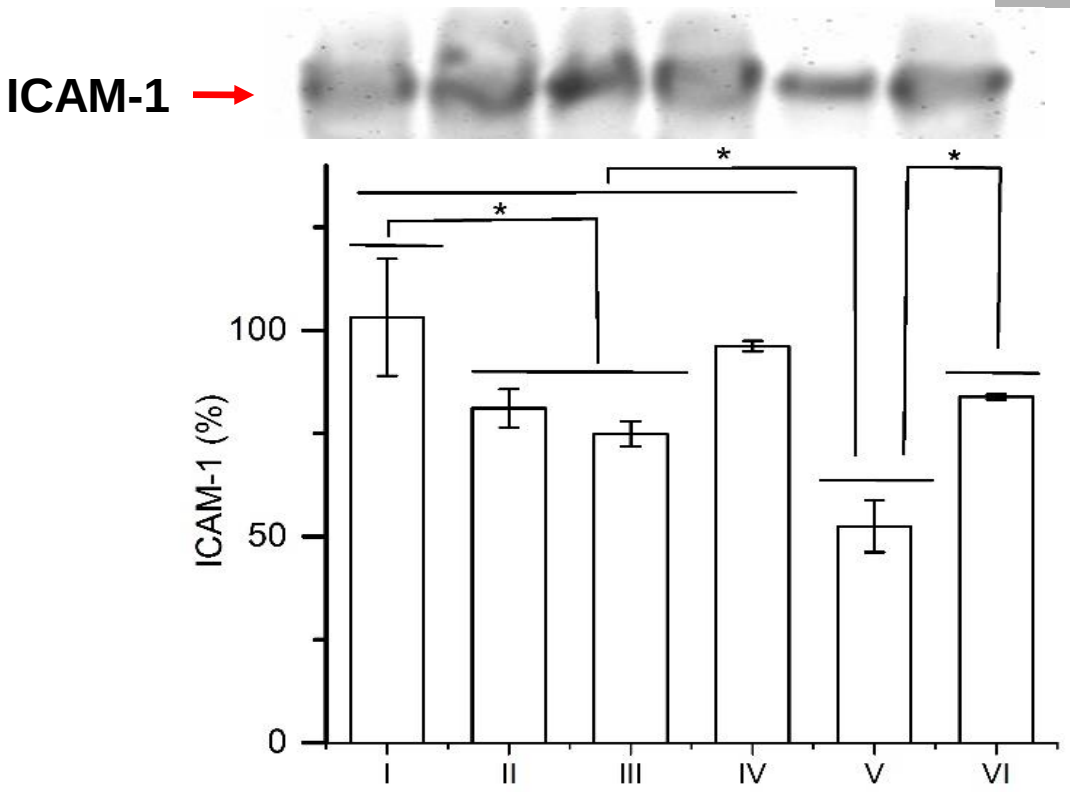


I. Buffer control
II. 60 µg A549 exosome
III. 60 nmole Scramble siRNA complex
IV. 60 nmole ICAM-1 siRNA complex
V. 60 nmole Scramble siRNA complex + 60 µg A549 exosome
VI. 60 nmole ICAM-1 siRNA complex + 60 µg A549 exosome



* Results are significantly different, $p < 0.05$

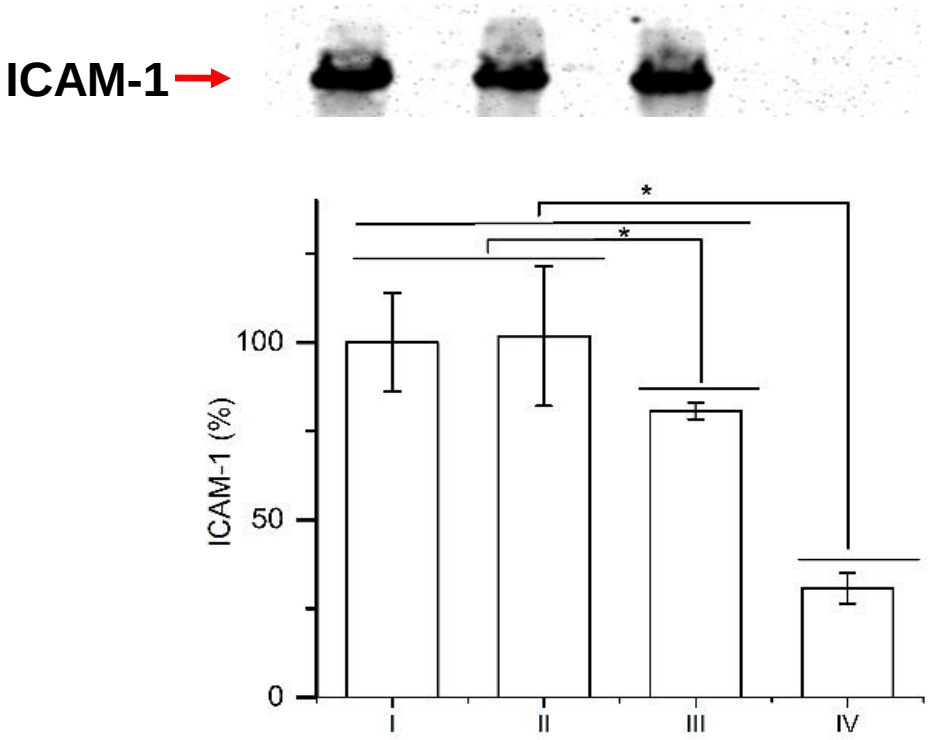
Inhibition From SiRNA Delivered by Autologous Cell-Derived Exosomes



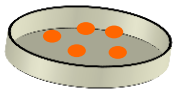
I. Buffer control
II. 60 nmole ICAM-1 siRNA
III. 60 nmole ICAM-1 siRNA complex
IV. 10 µl Transfection agent
V. 60 nmole ICAM-1 siRNA complex + 60 µg A549 exosome
VI. 60 nmole ICAM-1 siRNA complex + 60 µg bEND.3 exosome

* Results are significantly different, $p < 0.05$

Dose-dependent Effect of SiRNA Delivered by Exosomes



I. Buffer control
II. 240 µg A549 exosome
III. 240 nmole ICAM-1 SiRNA
IV. 240 nmole ICAM-1SiRNA complex + 240 µg A549 exosome



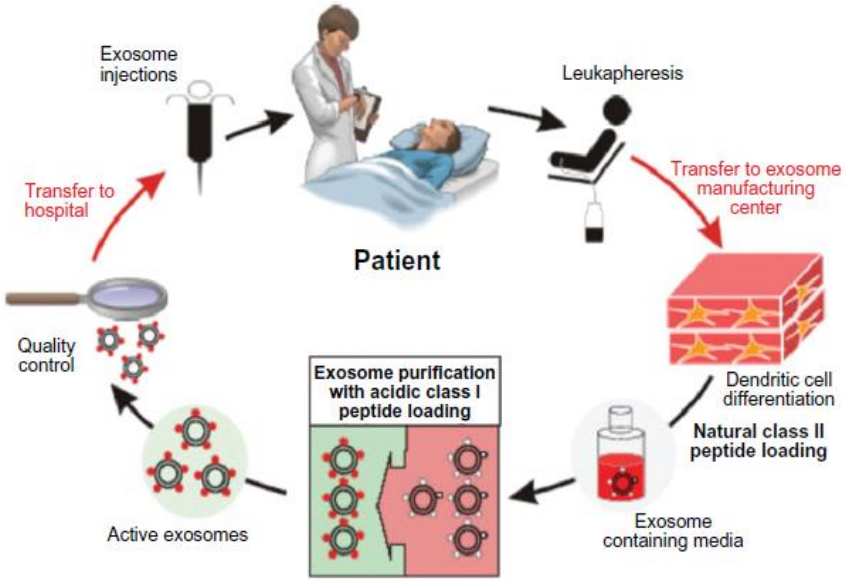
* Results are significantly different, $p < 0.05$

Conclusions

- Nanosized A549 exosomes showed parental cancer cell homing-selectivity.
- High fluorescence intensity was observed in the cells treated with exosome-delivered rhodamine 123.
- Exosome-delivered anticancer drugs significantly enhanced cytotoxicity on the autologous cancer cells.
- Exosome-delivered siRNA presented dose-dependent knockdown efficacy in cancer cells. ICAM-1 inhibitions were based on exosome original sources and formulation methods.

Future Direction

- New types of exosome
- Safety profiles



Personalized treatment

Tan et al (2010), International Journal of Nanomedicine 5:889-900

Acknowledgments

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  - Brittany Fogarty
  - Paige Martin
  - Ryan Hall
  - Joshua Allen
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  - Roger Phipps, Ph.D.
- **University of Cincinnati**
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MCF Maine Cancer FOUNDATION
Funding research. Finding hope.

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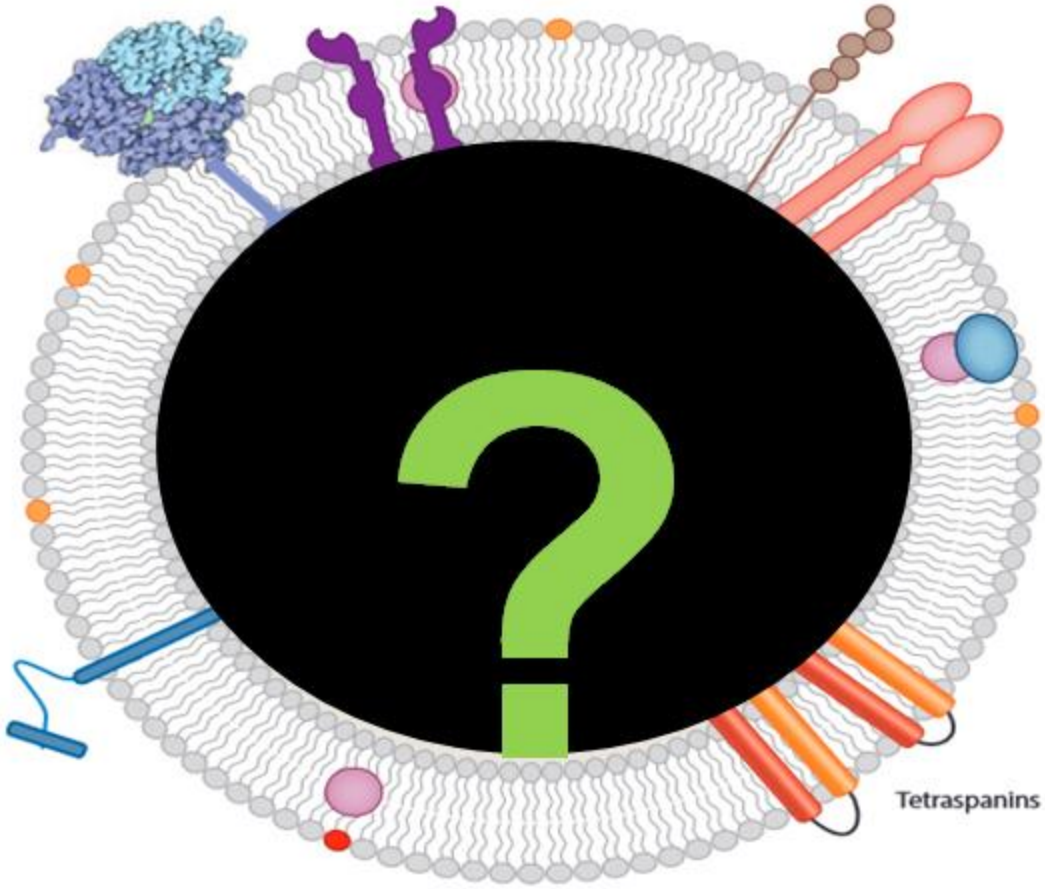
Questions