

# Exosomes in Cancer Treatment

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# Introduction

- Extracellular vesicles (EVs) are important mediators in intercellular communication, both local and systemic, by transferring their components among different cells.
- EVs are heterogeneous membrane-bound vesicles that are classified by **size**, **density**, and **cellular origin**.
- Three major subclasses of EVs have been documented including
  - Microvesicles (MVs) or ectosomes
  - Apoptotic bodies
  - Exosomes

# Cont...

**Table 1** Types of extracellular vesicles

EVs	Size	Markers	Mechanism of biogenesis
Exosomes	30–150 nm	CD63, CD9, CD81, Tsg101	Generated by inward budding of the membrane of MVBs through ESCRT-dependent Or/and ESCRT-independent and released into the ECM upon fusion of MVBs with the plasma membrane
Exomeres	< 50 nm	Unknown	Unknown
Microvesicles or ectosomes	100–1000 nm	ARF6, Annexin A1	pinching off from membrane protrusions/the plasma membrane shedding
Apoptotic bodies	50–5000 nm	Phosphatidylserine	Generated from apoptotic cells following stimulation of apoptosis-related pathways

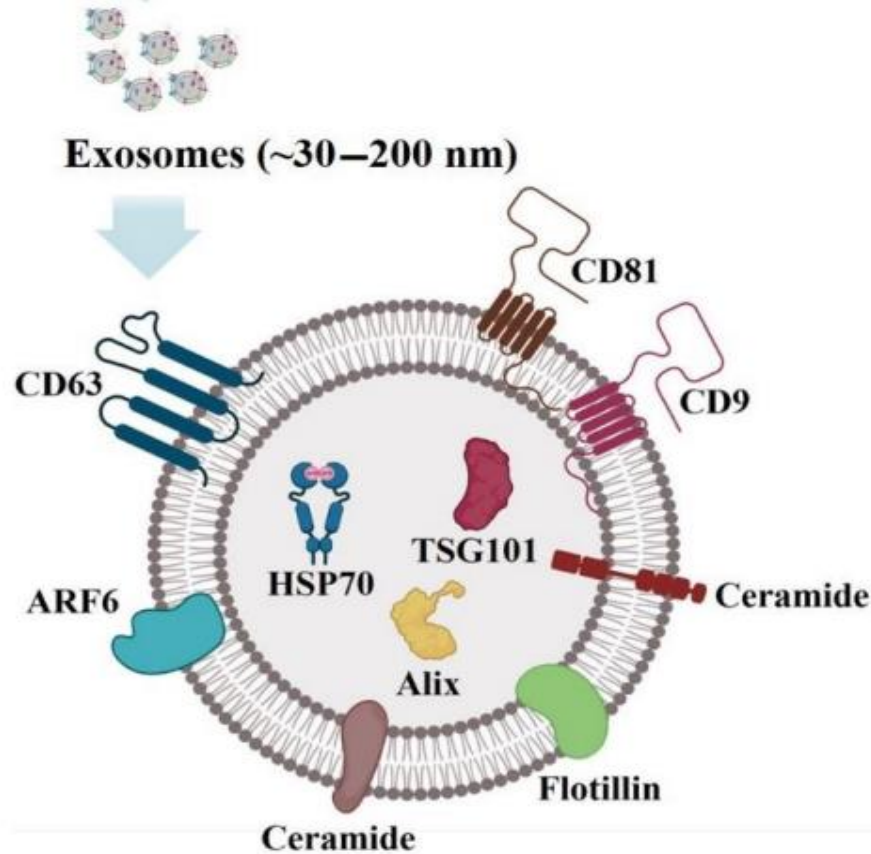
# Exosomes

- Exosomes have a lipid bilayer membrane that envelops many proteins, nucleic acids, lipids and cell debris.
- Exosomes were first discovered by Pan and Johnstone while investigating the maturation mechanisms of sheep reticulocytes into erythrocytes.
- Known as **junk** but more studies have developed into their true biological including:
  - ✓ Intercellular communication
  - ✓ Cell differentiation, growth and proliferation
  - ✓ Angiogenesis
  - ✓ Stress response
  - ✓ Immune regulation ,...

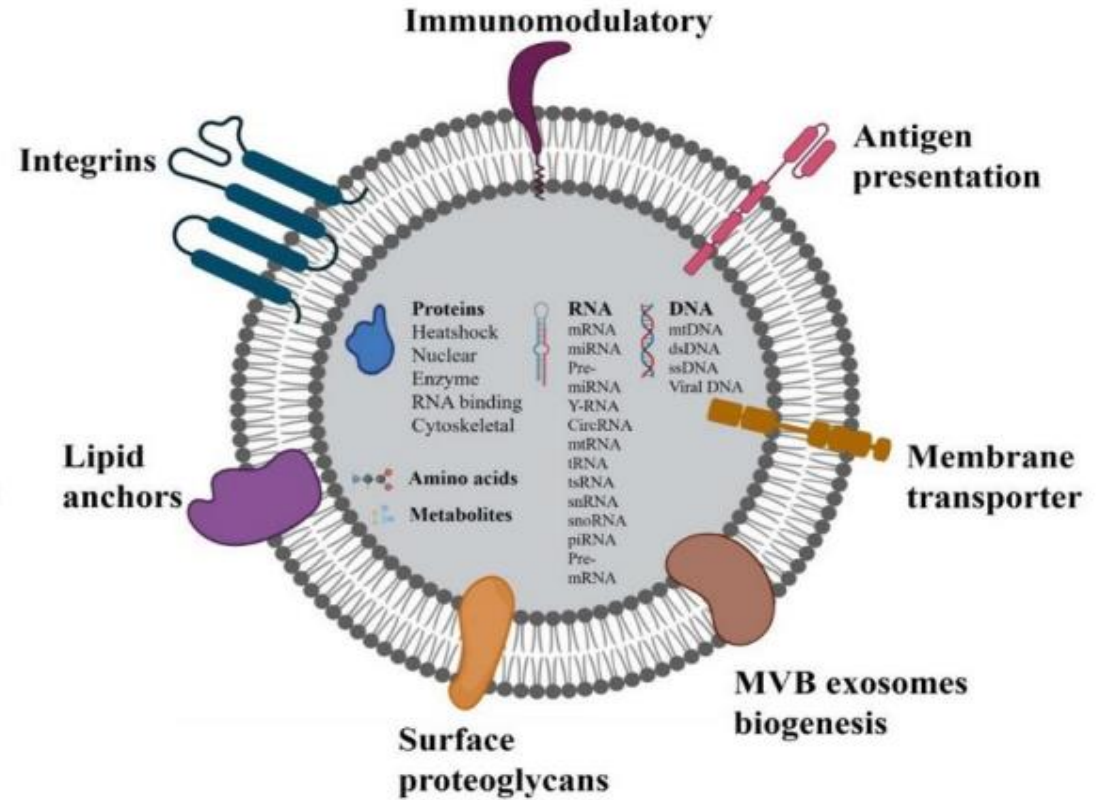
# Cont...

- Secreted by most cell types including
  - ✓ Immune cells
  - ✓ Neuronal cells
  - ✓ Epithelial cells
  - ✓ Endothelial cells
  - ✓ Cancer cells
  - ✓ Mesenchymal stem cells,...
- They are present in **various biological fluids** .
- Their content and physiological functions of exosomes vary depending on **the cells from which they are secreted**.

# Typical Exosomes



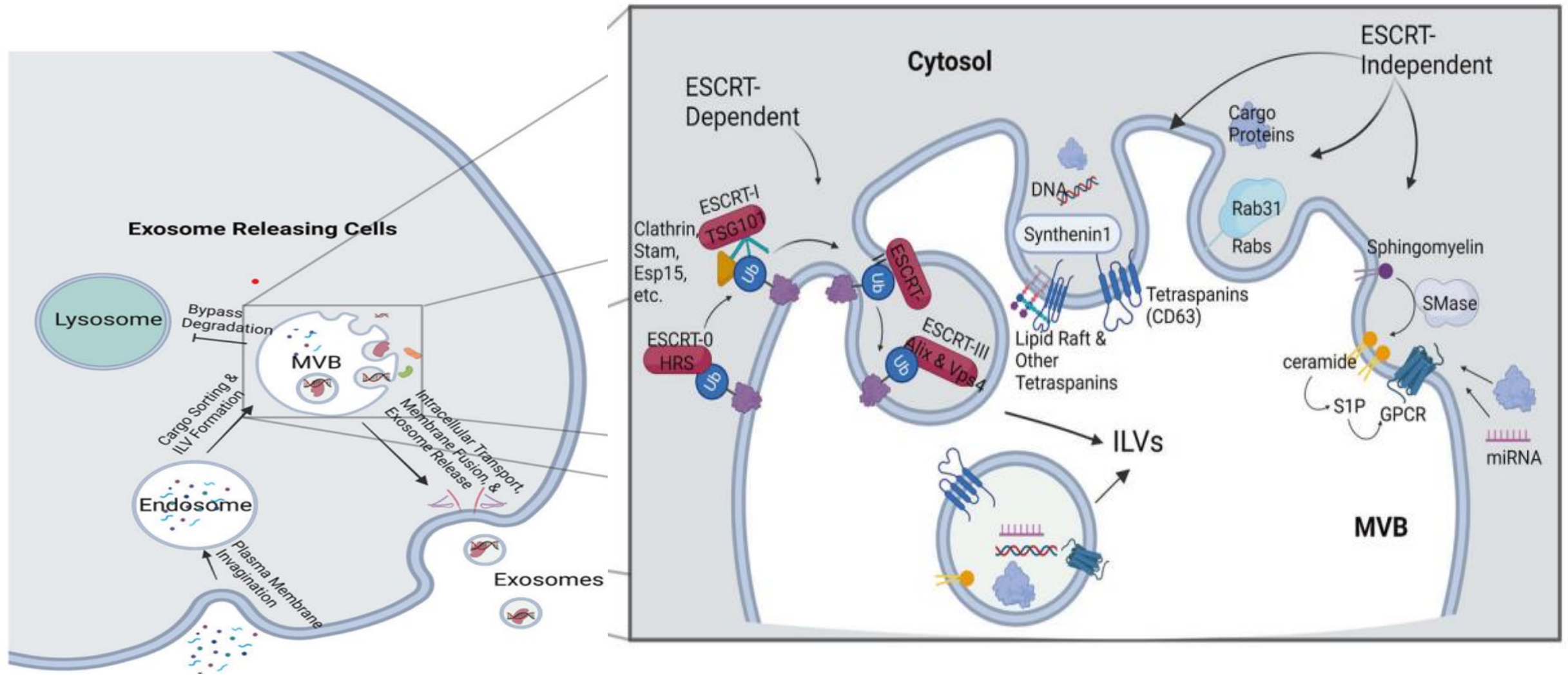
**Biomarkers of exosomes**

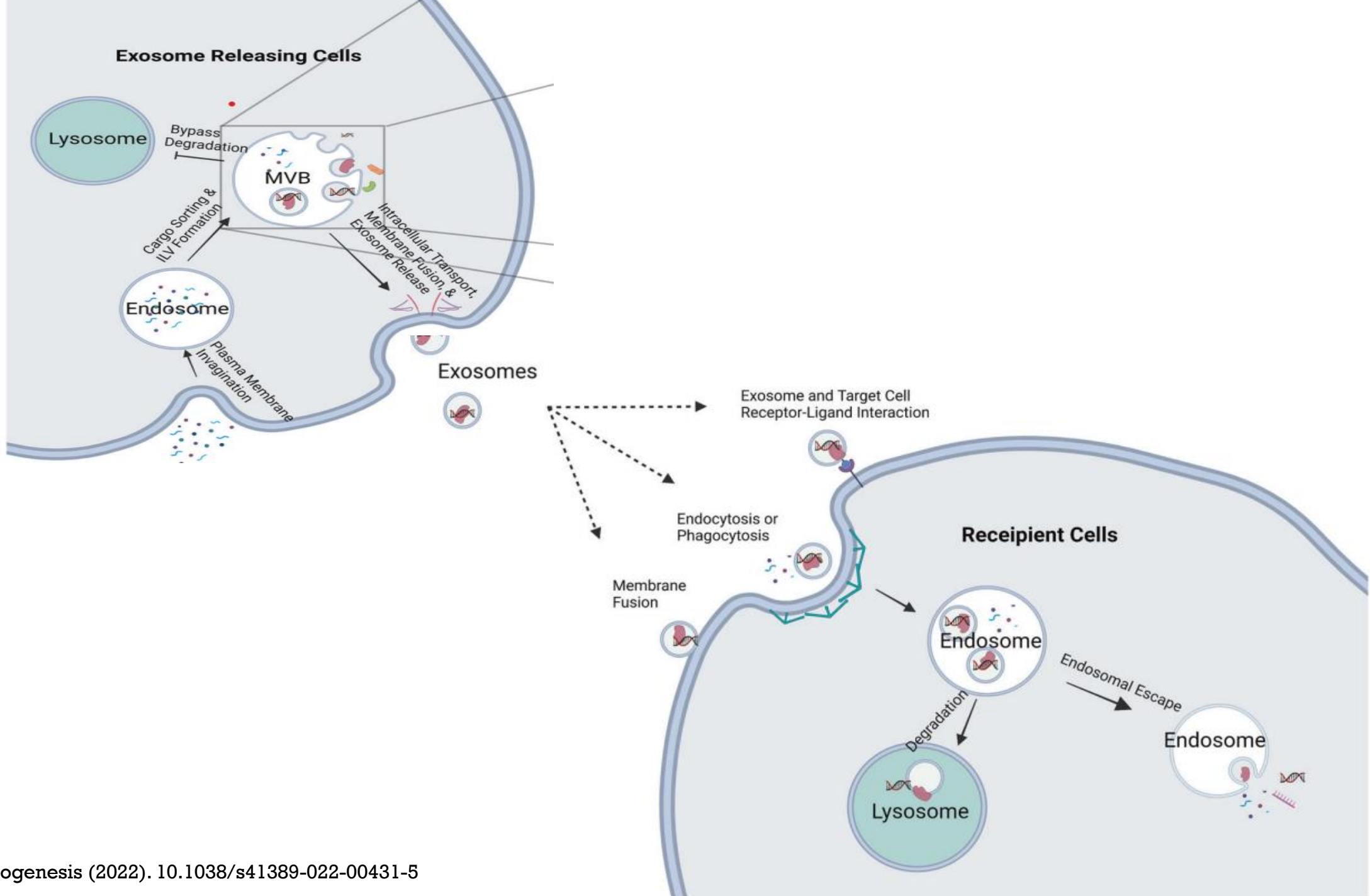


**Content of exosomes**



# Exosomes Biogenesis

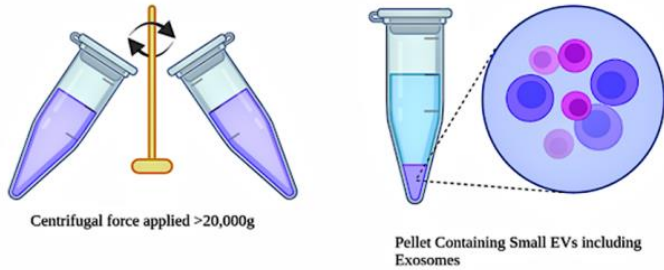




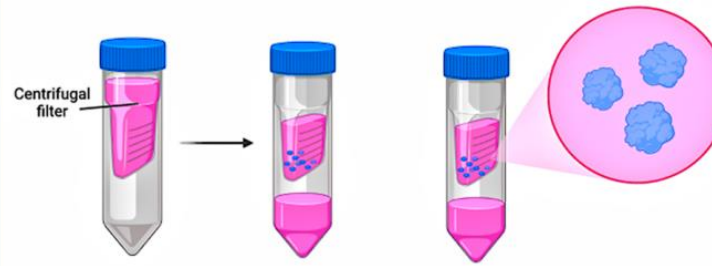


# Isolation Of Exosomes

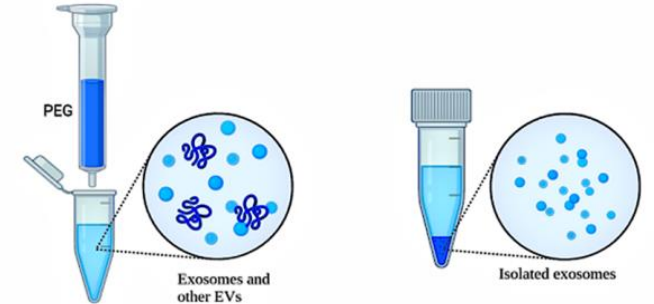
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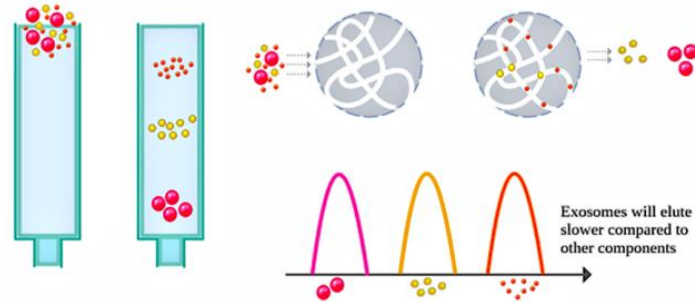
## Ultra-filtration



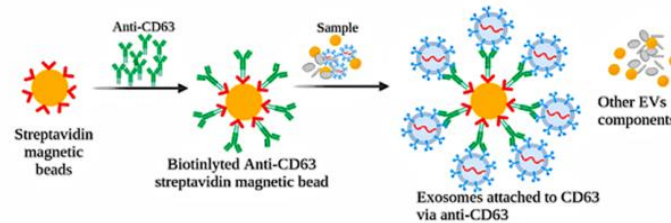
## Polymer precipitation method



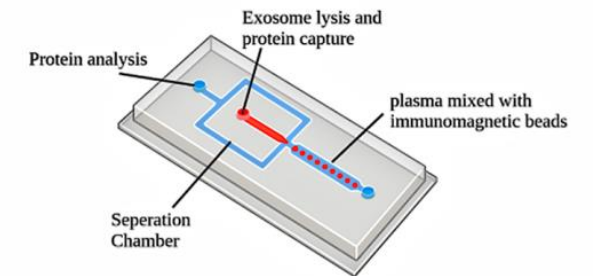
## Size-Exclusion technique



## Immunoaffinity capture methodology

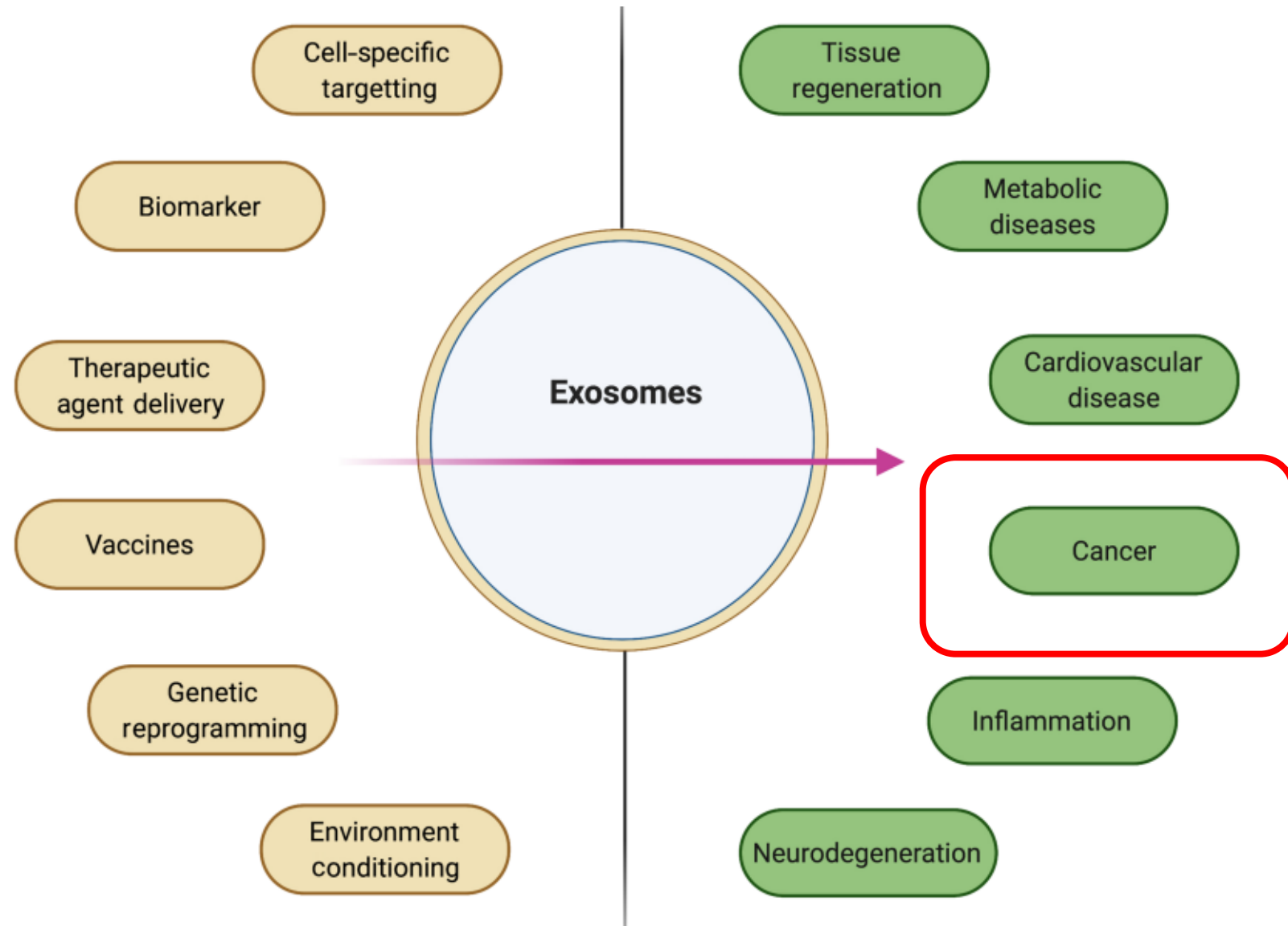


## Microfluidics- based isolation

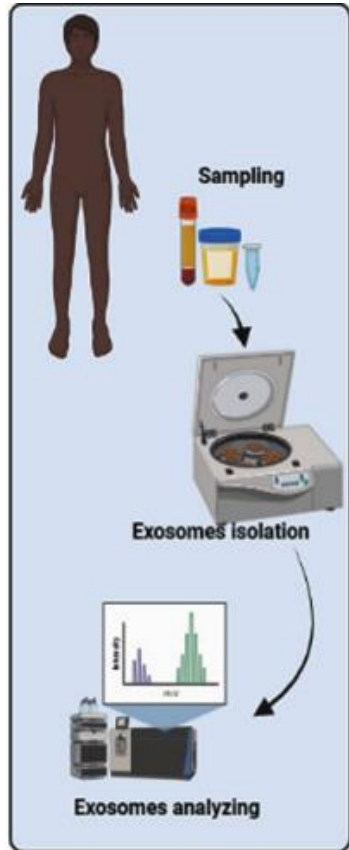


# Characterization Of Exosomes

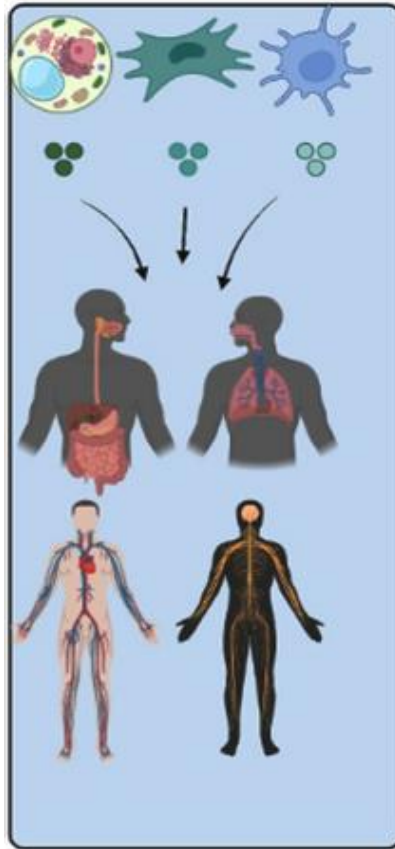
- Nanoparticle tracking analysis
- Dynamic light scattering
- Flow cytometry
- Western blot
- Electron microscopy



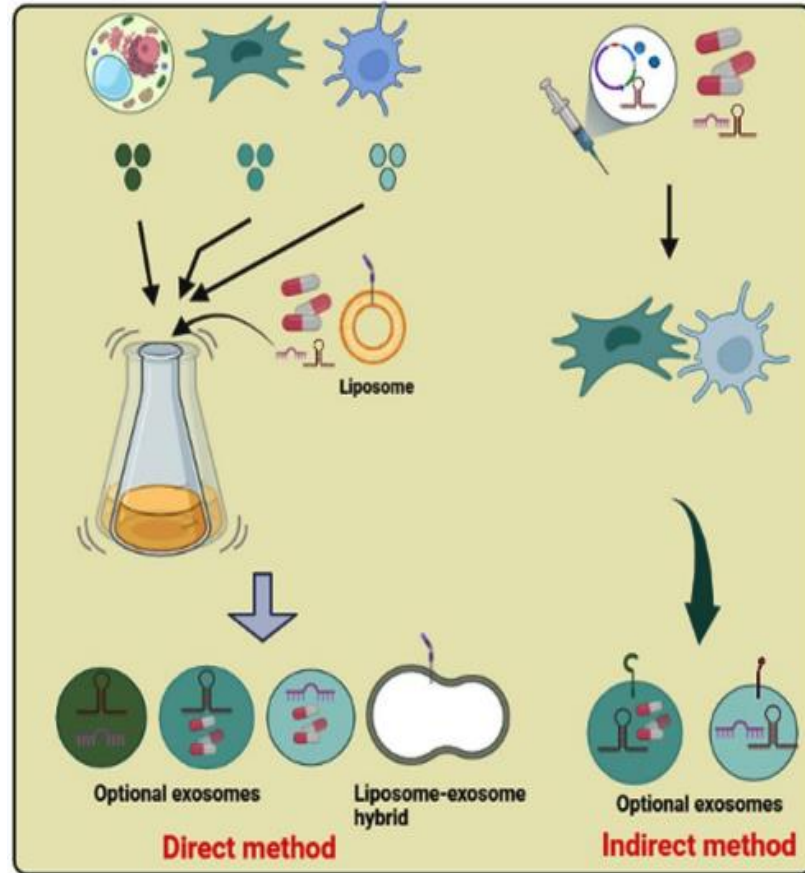
# Exosomes In Cancer Clinical Applications



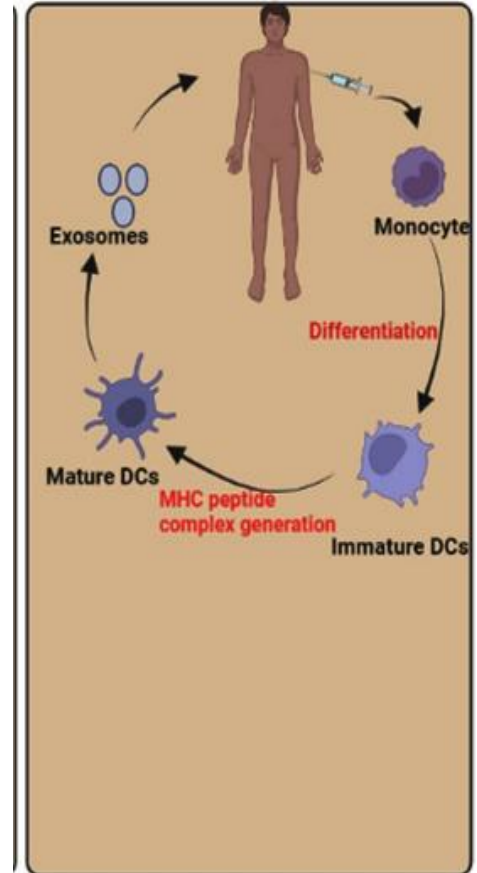
Biomarker



Exosome-therapy



Exosome drug delivery system



Exosome-vaccine

# 1. Exosomes In Cancer Diagnosis

- Early detection and effective treatment can reduce the mortality rate of cancer patients, as millions of people die from cancer each year.
- Traditionally, tissue specimens are routinely used to provide clinical diagnosis, prognosis and assessment for molecular changes.
- **Liquid biopsies** are non-invasive, can be obtained serially, and may facilitate early cancer detection.
- Exosomes secreted by tumor tissue in cancer patients circulate in the blood and play an important role in metastasis, early diagnosis and prognosis, and the corresponding response to treatment.

# Cont...

- Even though most cell types release exosomes, evidence supports
  - the **up-regulated exosome secretion** in several pathological conditions like **cancer**.
  - Under pathological conditions, cellular changes can be reflected in the **biological component of exosomes released by cells**.
- Exosomes carry different biomolecules, such as proteins, nucleic acids and lipids, which are encoded for different functions.

Exosomes play a **potential role as cancer biomarkers** because they are present in biological fluids, are similar to the parent cells and are circulating in a stable manner.



Sr. No.	Disease	Exosome source	Status	Remarks	Clinical Trial Identification
1	Pancreatic cancer	Ultrasound-guided portal venous blood exosomes	Recruiting	Safety of sampling portal venous blood, analyzing mRNA markers.	NCT03821909
2	Colon cancer	Curcumin conjugated with plant exosomes	Active, Phase I	Comparing exosome loaded curcumin on immune modulation, phospholipid profile of normal and malignant patients.	NCT01294072
3	Sarcoma	Blood samples	Recruiting	Evaluation of cancer pathogenesis, progression and treatment efficacy of exosomes	NCT03800121
4	Prostate cancer	Urine exosomes	Completed	Validation of non-digital rectal examination (DRE) exosome gene expression test of prostate cancer in biopsy.	NCT02702856
5	Pancreatic cancer	Blood samples from patients	Active, not recruiting	Exosome purification for RNA sequencing and proteomics	NCT02393703
6	Lung metastasis osteosarcoma	Blood samples	Recruiting	Identification of levels of circulating exosomal RNA with or without lung metastasis	NCT03108677
7	Gallbladder carcinoma	Exosomal blood samples	Recruiting	Establishing a correlation between exosome biomarkers and gallbladder carcinoma	NCT03581435
8	Stage IV pancreatic adenocarcinoma	Mesenchymal stromal cells-derived exosomes with KRAS G12D siRNA	Phase-I, not recruiting	Mesenchymal derived exosomes with KRASG12D in treating individuals with pancreatic cancer with KRAS G12D mutation.	NCT03608631
9	Pancreatic ductal adenocarcinoma	Portal vein blood	Completed	Test 3 CTC isolation methods and analyses for onco exosomes in pancreatic cell culture media by flow cytometry.	NCT03032913
10	Oral mucositis, head and neck cancer	Grape extract exosomes	Active, Phase-I, not recruiting.	Ability of plant exosomes to prevent oral mucositis in head and neck cancer.	NCT01668849
11	Non-small cell lung cancer (NSCLC)	Plasma exosomes	Not recruiting	New radiotherapy combined with immunotherapy	NCT02890849
12	Non-small cell lung cancer (NSCLC)	Dendritic cells derived exosomes	Completed Phase-2	No induction of T cells monitored in patients	NCT01159288
13	Thyroid cancer	Urine exosomal thyroglobulin and galectin 3	Active, not recruiting	Identifying urinary exosomal proteins (thyroglobulin and galectin 3)	NCT03488134
14	Colon cancer	Blood sampling	Recruiting	Novel ways of diagnosing and predicting the spread to other organs such as liver	NCT03432806
15	Prostate cancer	Urine samples	Active, not recruiting	Validated urine test to predict the incidence of high-grade prostate cancer in initial prostate biopsy	NCT03235687
16	Triple-negative breast cancer	Serum exosomes	Phase-I, recruiting	Assessing response to pembrolizumab in the primary tumor, circulating lymphocytes,	NCT02977468
17	Thyroid cancer	Urine exosomes	Active, not recruiting	Evaluation of new therapeutic mechanisms and medications for poorly differentiated or anaplastic thyroid cancer	NCT02862470

## 2. Exosomes In Cancer Therapy

- Despite the tremendous advances in cancer treatment over the past few decades, cancer remains one of the leading cause of death worldwide .
- Being double-layered, nano-sized, cell-free, and having their host derived nature exosome-associated treatments are potentially promising against cancer.
- In general, three exosome-related approaches were utilized:
  - Depletion of tumor-derived exosomes using exosome inhibitors
  - Administration of specific cell types-derived exosomes
  - Engineering exosomes as a vehicle to carry antineoplastic agents to selective target sites

# Cont...

## Depletion of tumor-derived exosomes using exosome inhibitors

- Tumor-derived exosomes have been reported to play a role in
  - tumor progression
  - angiogenesis
  - tumor invasion
  - mediating immune escape
  - inhibiting the anti-tumor response of receptor cells
  - multidrug resistance
- Therefore, depletion of cancer cell-derived exosomes would have therapeutic benefits in cancer patients.

# Cont...

- Current established exosome inhibitors are designed to target exosome biosynthesis or trafficking pathways.
- **Drugs inhibiting exosome biogenesis include**
  - Cholesterol synthesis inhibitor
  - Neutral sphingomyelinase inhibitor GW4869
  - Rab27A inhibitor tipifarnib
- **Inhibitors of exosome trafficking include**
  - Ras inhibitor manumycin A
  - Cytoskeleton reorganizing ROCK inhibitor Y27632
  - Cysteine proteinase inhibitor calpeptin

# Administration Of Specific Cell Types-derived Exosomes

- Exosomes can act as immunoregulating agents by modulating immune activation, antigen presentation, suppression, and surveillance.
- DCs, mesenchymal stem cells, NK cells, T cells and many other cells were used to produce the exosomes, each with unique anti-tumor functions.

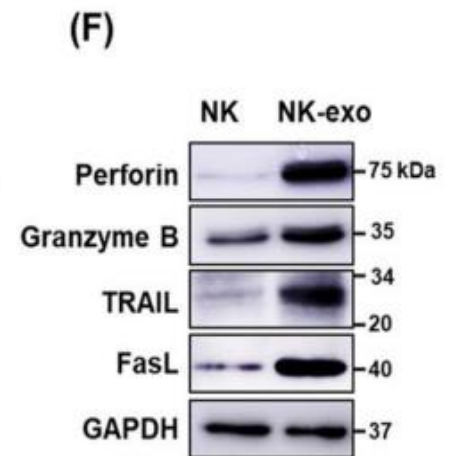
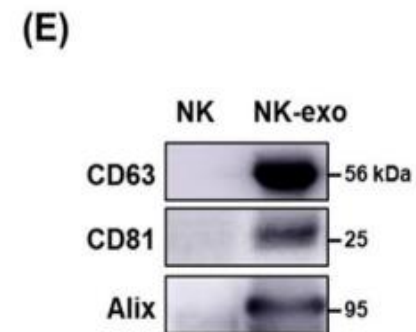
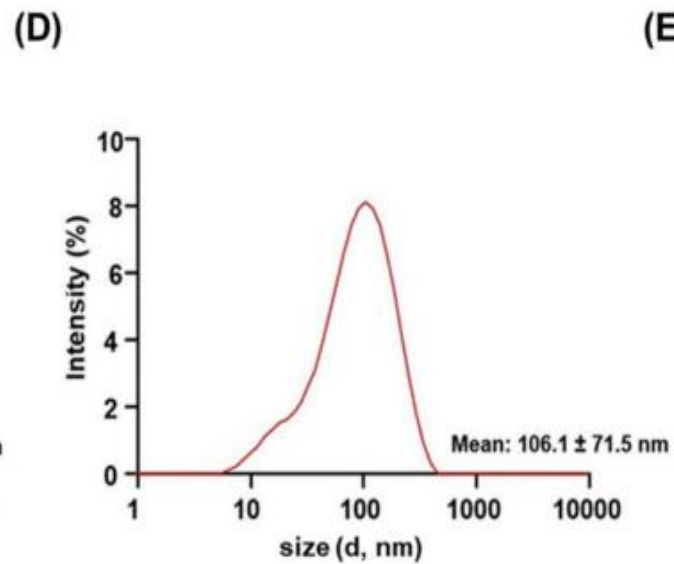
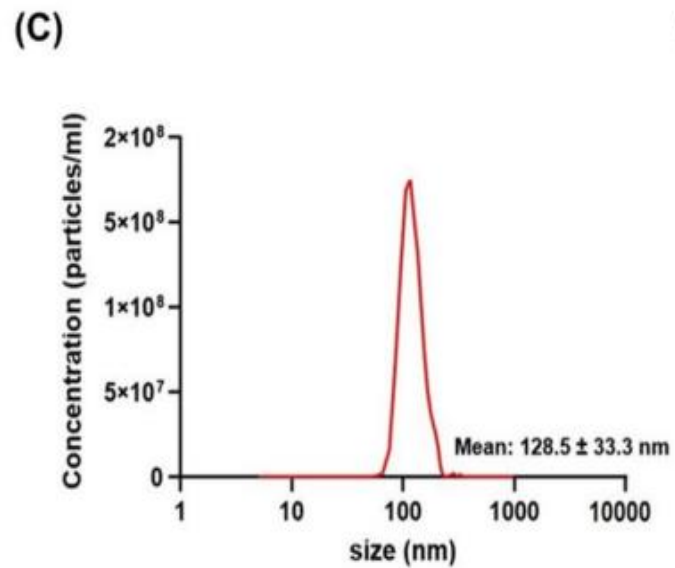
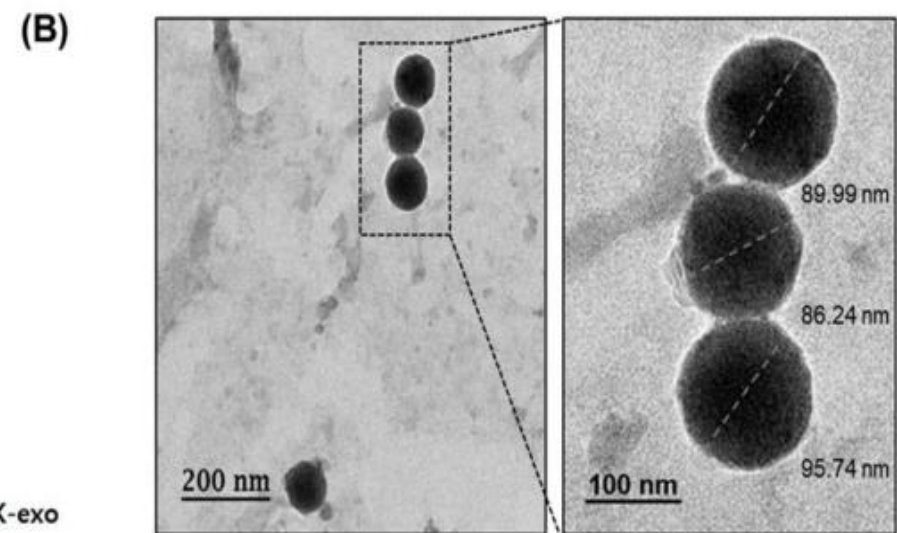
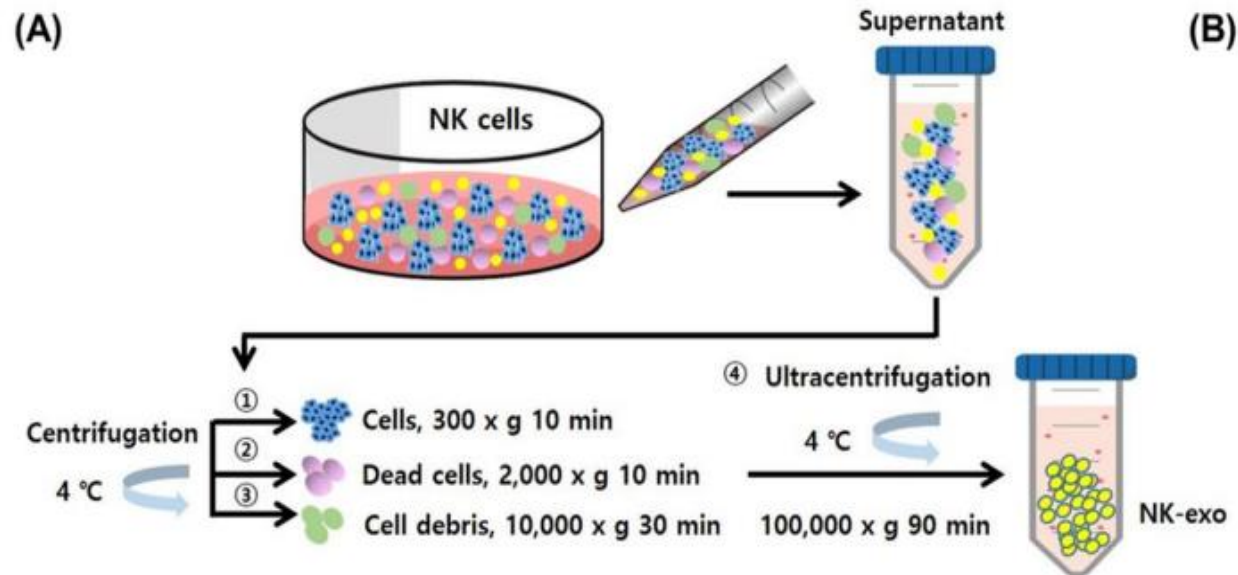
RESEARCH ARTICLE



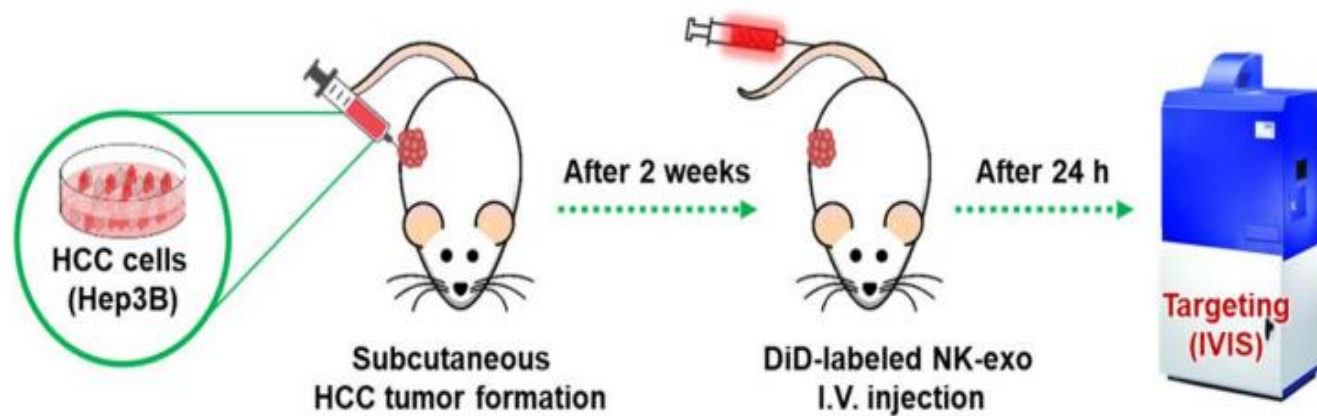
# Delivery of human natural killer cell-derived exosomes for liver cancer therapy: an in vivo study in subcutaneous and orthotopic animal models

Ho Yong Kim<sup>a</sup>, Hyun-Ki Min<sup>a</sup>, Hyeong-Woo Song<sup>a</sup>, Ami Yoo<sup>a</sup>, Seonmin Lee<sup>b</sup>, Kyu-Pyo Kim<sup>b</sup>, Jong-Oh Park<sup>a,c</sup>, You Hee Choi<sup>a</sup> and Eunpyo Choi<sup>a,c</sup>

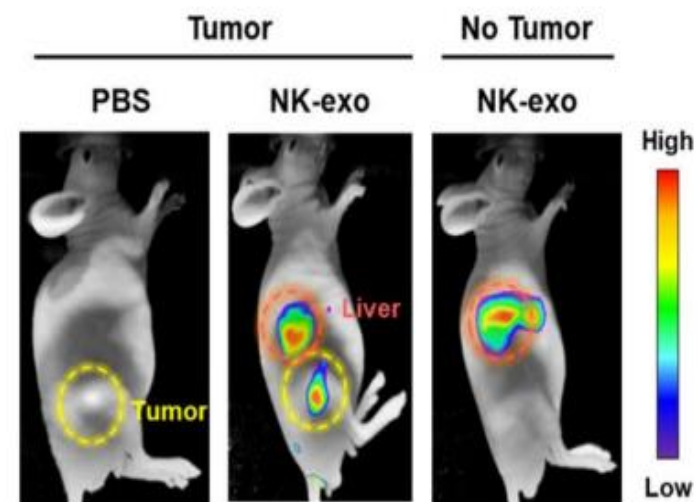




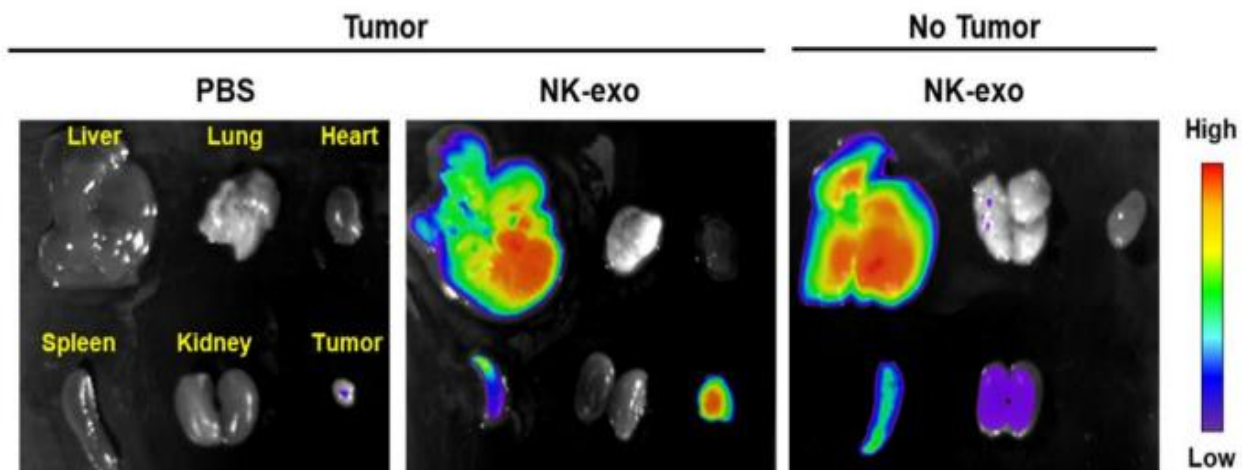
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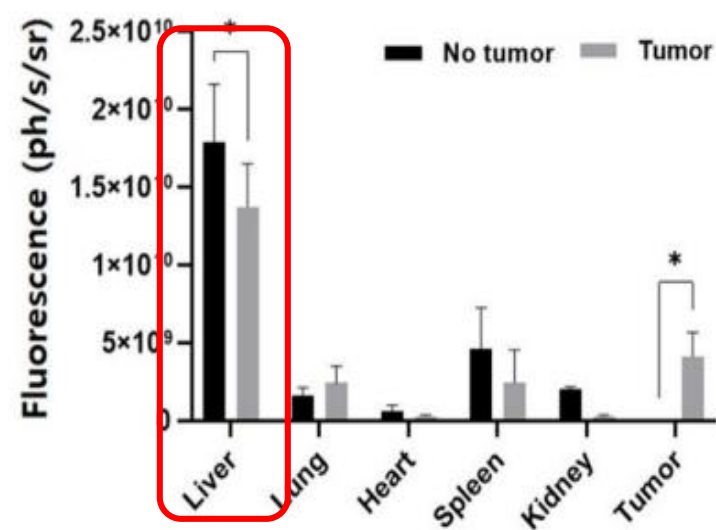
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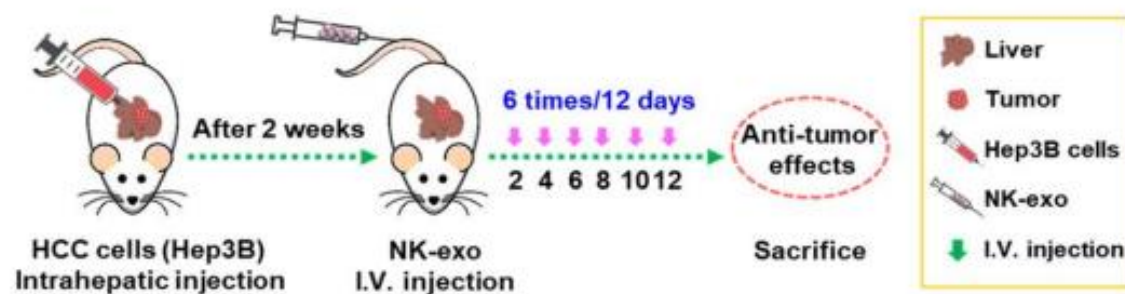
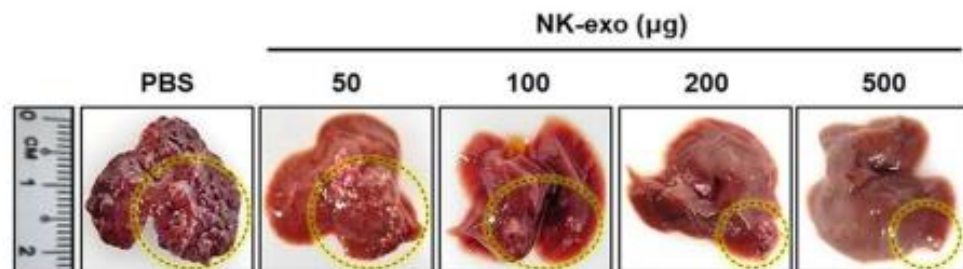
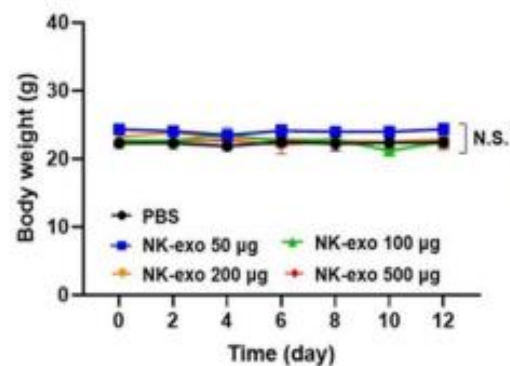
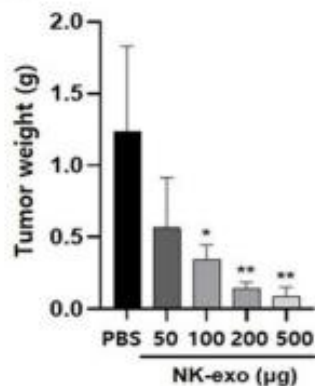
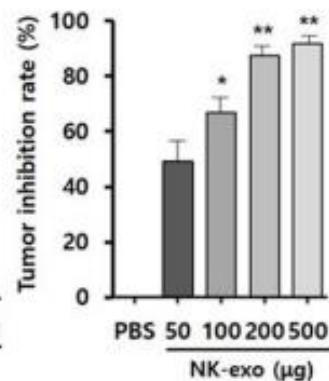
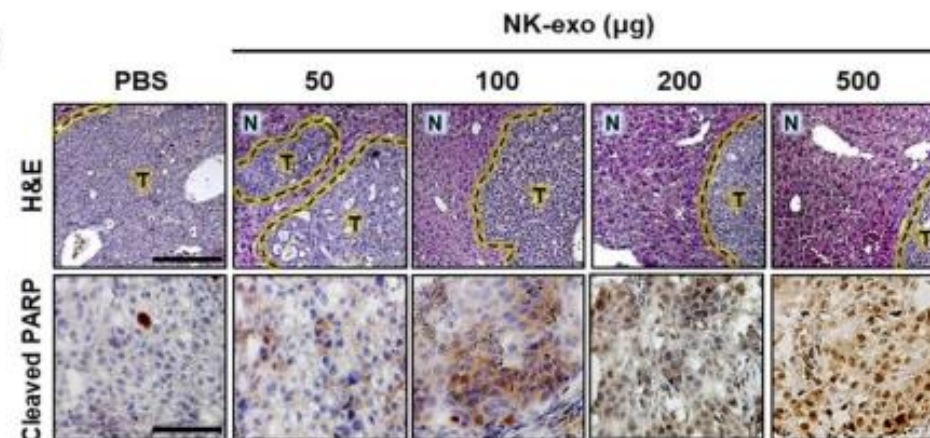
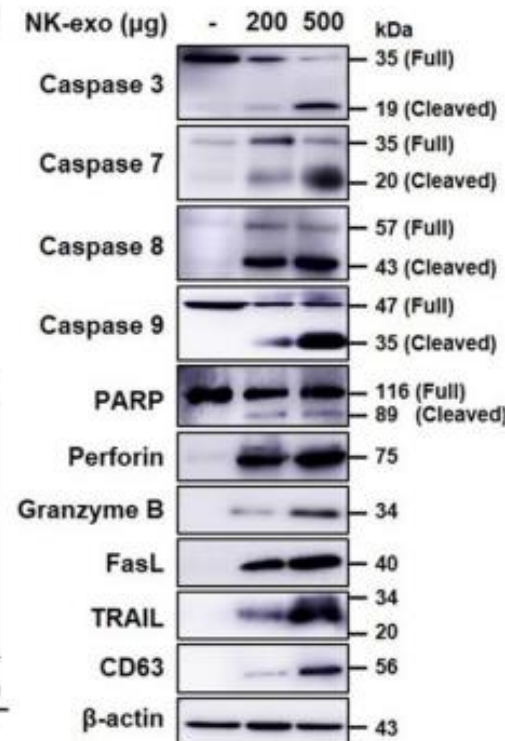
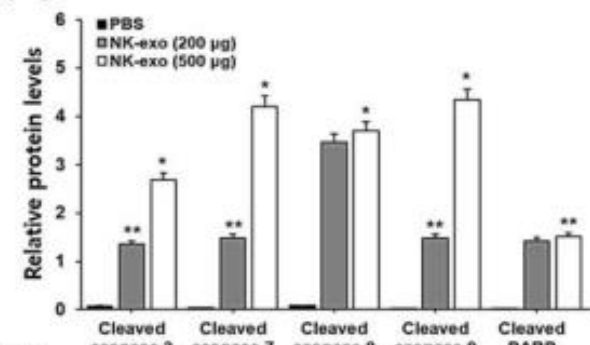
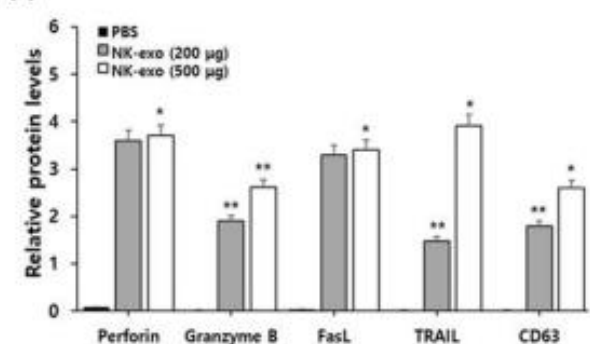


(C)



(D)



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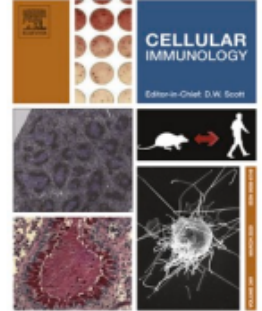


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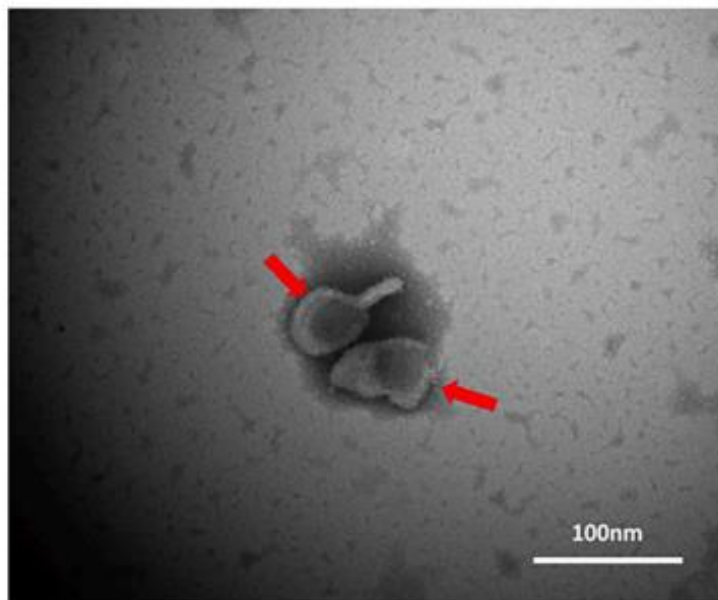
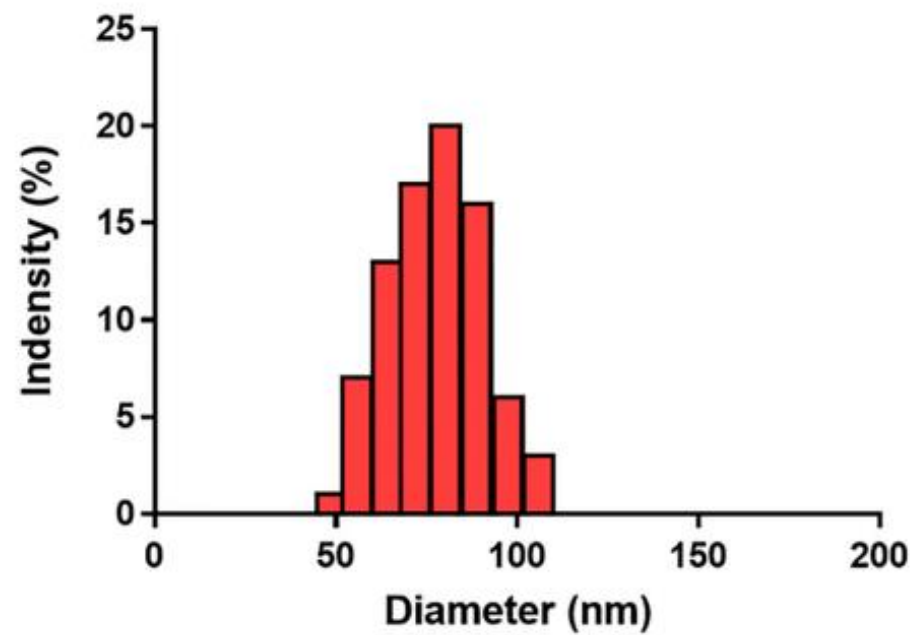
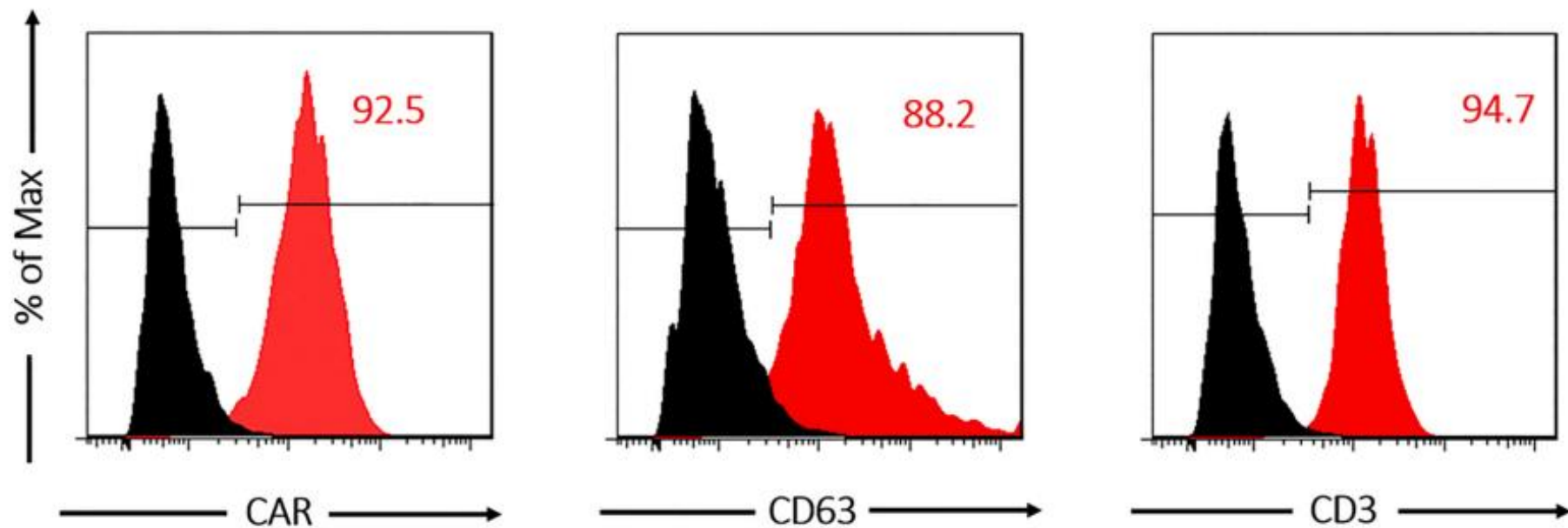


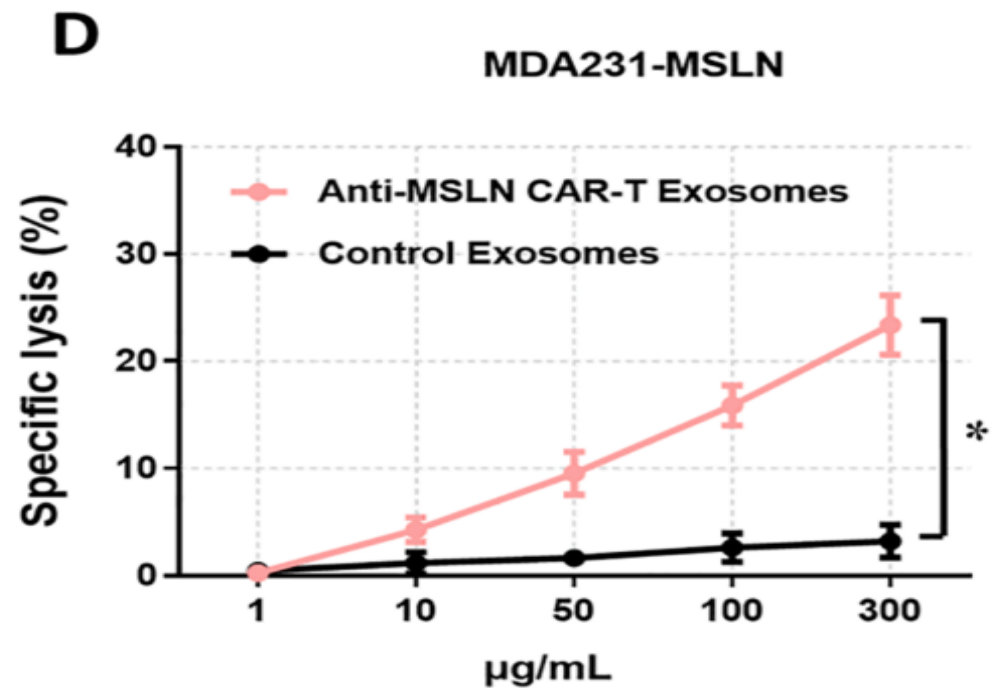
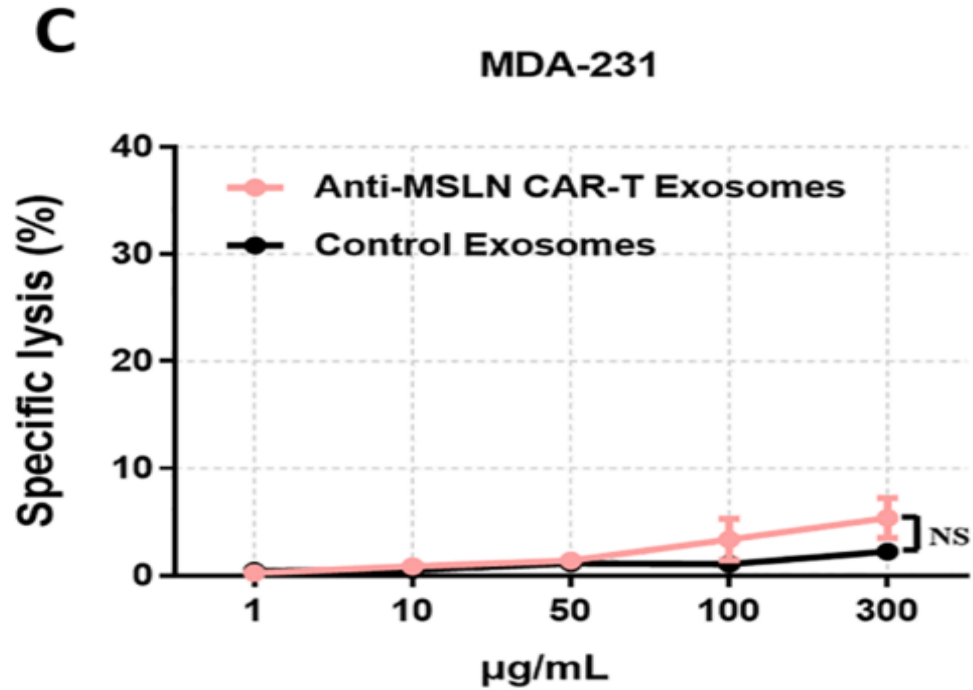
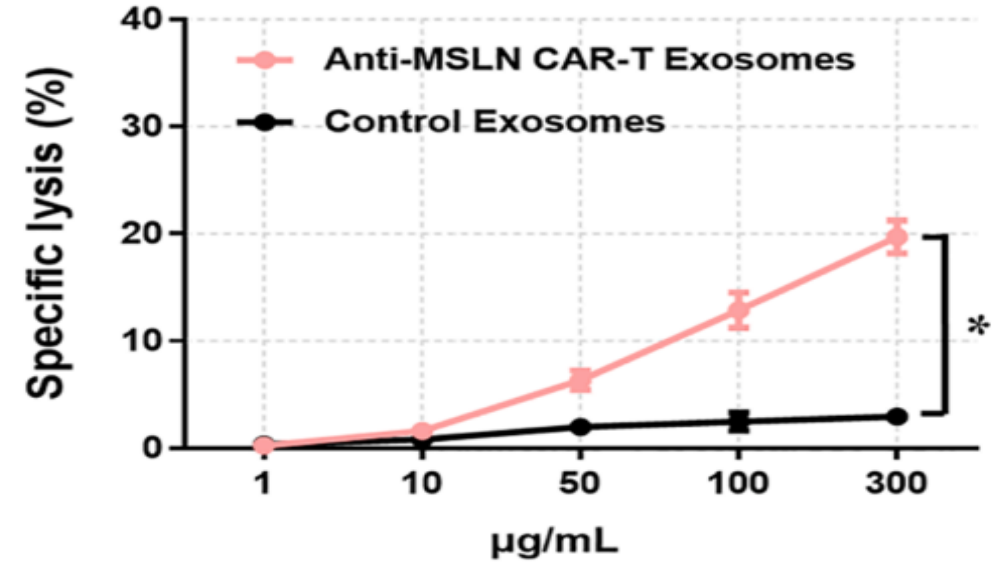
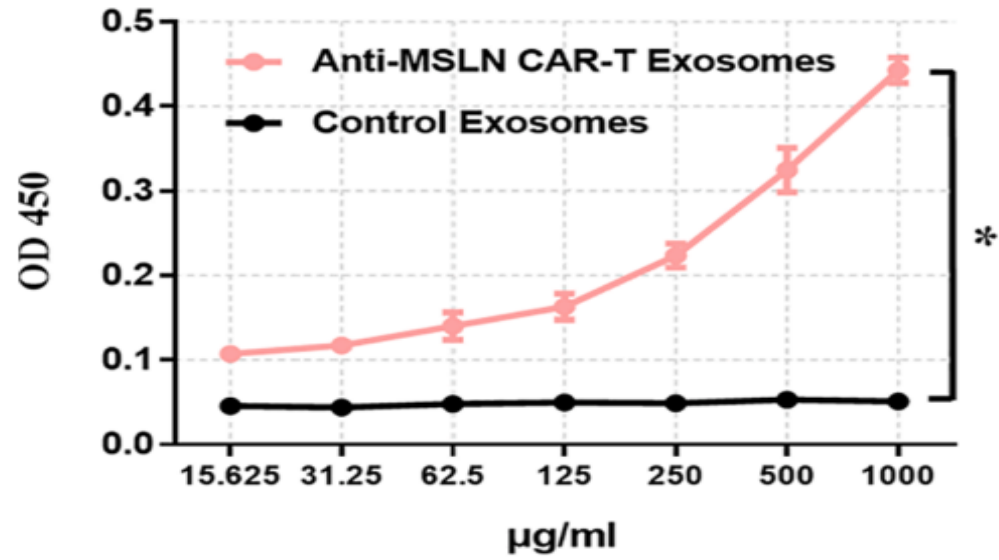
Research paper

### The exosomes derived from CAR-T cell efficiently target mesothelin and reduce triple-negative breast cancer growth

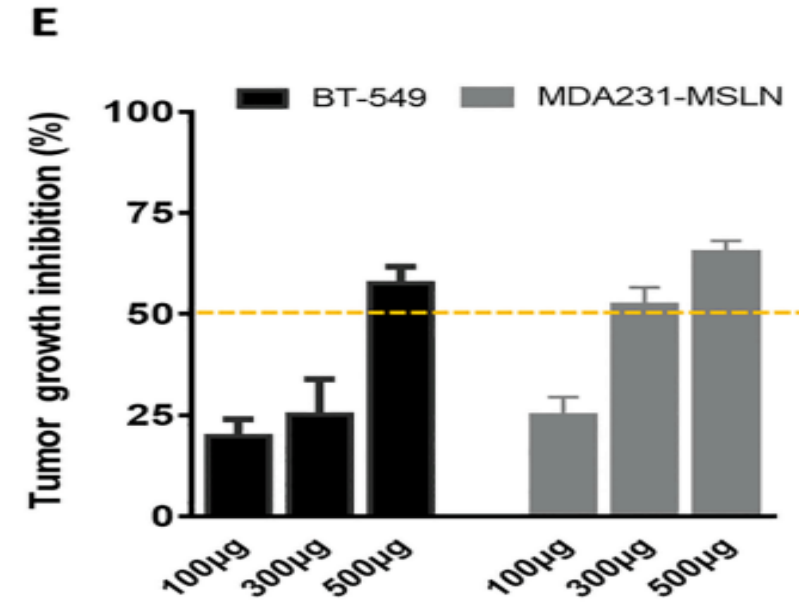
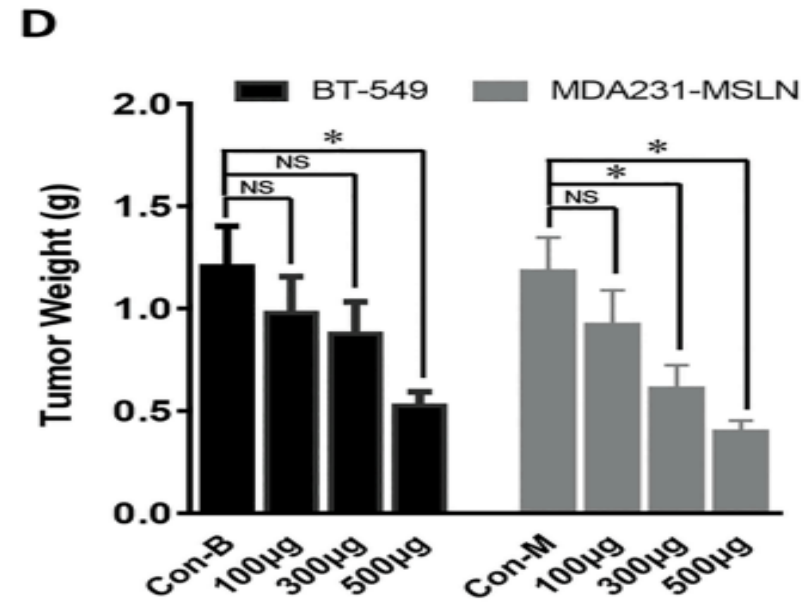
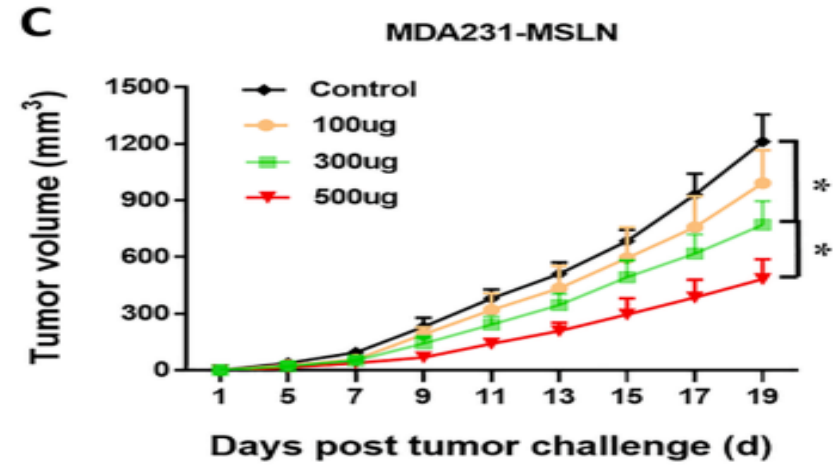
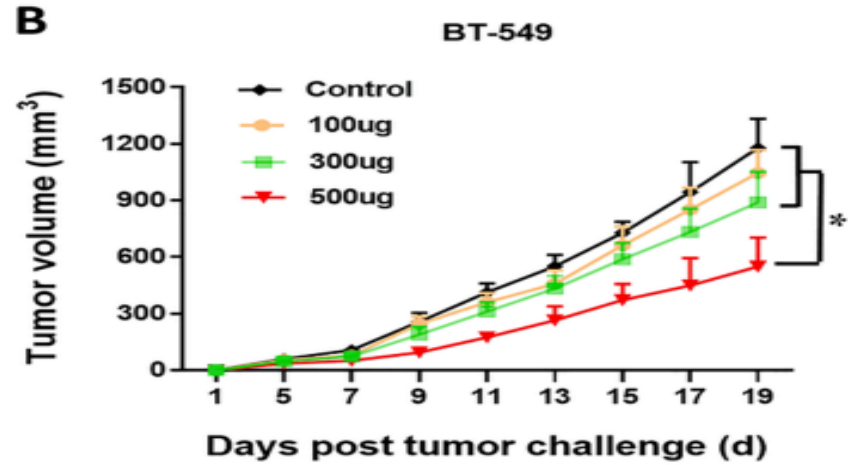
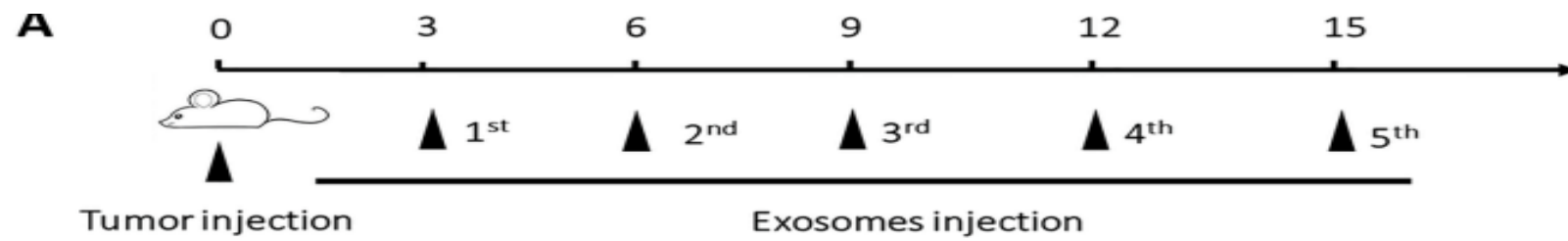
Pengxiang Yang<sup>a,b</sup>, Xingjian Cao<sup>c</sup>, Huilong Cai<sup>b</sup>, Panfeng Feng<sup>d</sup>, Xiang Chen<sup>c</sup>, Yihua Zhu<sup>c</sup>, Yue Yang<sup>b</sup>, Weiwei An<sup>b</sup>, Yumin Yang<sup>a,\*</sup>, Jing Jie<sup>c,\*</sup>



**B****C****D**







### 3. Exosome Vaccine

- Exosomes from immune cells and tumor cells have the potential to be used as a vaccine for cancer .
- Although cancer vaccines such as DC vaccines and peptide vaccines have become attractive anticancer immunotherapy options, some obstacles have hindered their successful application.
  - High cost of DC preparation
  - Storage of DC vaccines
  - Tumor-mediated immunosuppressive environment
  - Identification of specific tumor antigens
  - High degradation of antigen peptides
- Recently, **nanocarriers** have been considered as a new approach for vaccine delivery.

# Cont...

- Nanovaccines are made up of specific antigens (tumor-related proteins, peptides or nucleic acids) conjugated with possible nanocarriers.
- According to the manufacturing method, nanocarriers can be divided into three categories:
  - Synthetic nanocarriers
  - Semi-synthetic nanocarriers
  - Biogenic nanocarriers

# Cont...

- As biogenic nanocarriers exosomes are more advantageous nanocarriers because of their
  - Small size
  - Low toxicity
  - Biocompatibility
  - Phospholipid bilayer
  - Receptors similar to their origin
  - Delayed cycling
- Exosomes can serve as cell-free vaccination agents in advanced immunological approaches.

# Cont...

- **DC-derived exosomes (Dex)** have attracted attention as an alternative carrier for the delivery of tumor antigens .
  - Express tumor antigens, MHC, co-stimulatory molecules on their surface.
  - 10–100 times more enriched in peptide-MHC-II complexes than in DCs
  - Avoid high degradation
  - Easily dispersed in the lymph nodes
  - Cannot respond to immunosuppressive molecules
  - Favorable biocompatibility and biosafety in vivo
  - Stored in vitro for a longer period

**Table 2**

Functional difference among tumor vaccine therapies.

Functional difference	Peptide	DC	DNA/mRNA	Dex
Abundance of MHC-II complexes on surface	/	Fewer and low yields	/	10–100 times more enriched in peptide-MHC-II complexes than in DC
Storage of vaccine preparation	A low temperature maintains the activity of peptides.	A low temperature storage cannot efficiently maintain the activity of DCs.	The DNA/mRNA vaccines remain effective after several months when stored at low temperature.	Dex can be stored at – 80 °C for more than 6 months
Determination of molecular QC parameters	Clear quality parameters	Difficult in defining QC parameters	Clear quality parameters	Dex can be precisely designed for each donor patient.
Stability in circulation	Easily degraded in the body; short half -life	The membrane structure ensures the stability of vaccines in the circulation.	RNA is susceptible to ubiquitous RNase degradation.	The high levels of sphingomyelin and phosphatidylinositol in lipid composition helps increase the stability of Dex.
Delivery efficiency	Low	High	Low	High
Immunogenicity	Low	Increased Immunogenicity than peptide vaccine	low	High
Resistance to immunosuppression	Susceptible	Susceptible	Susceptible	No response





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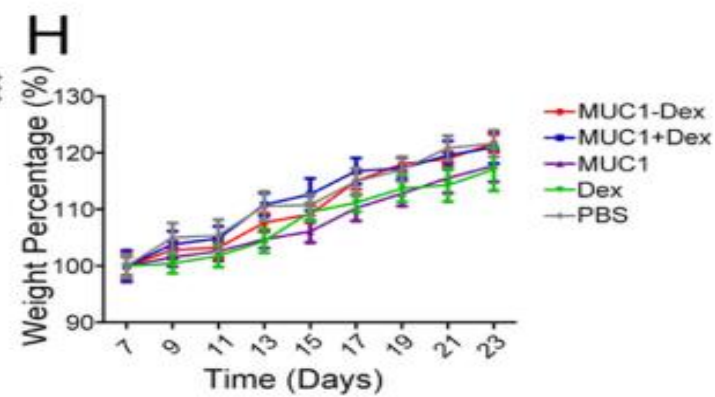
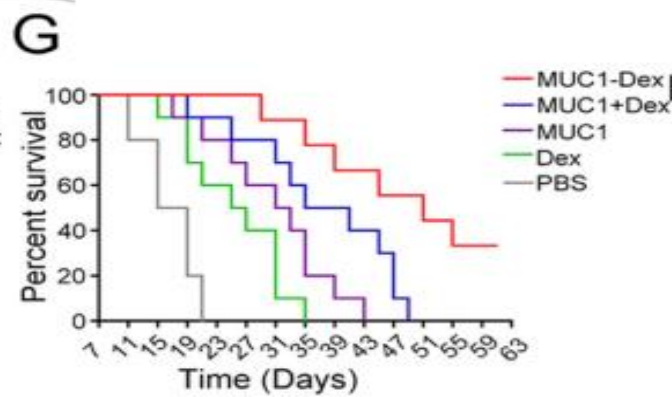
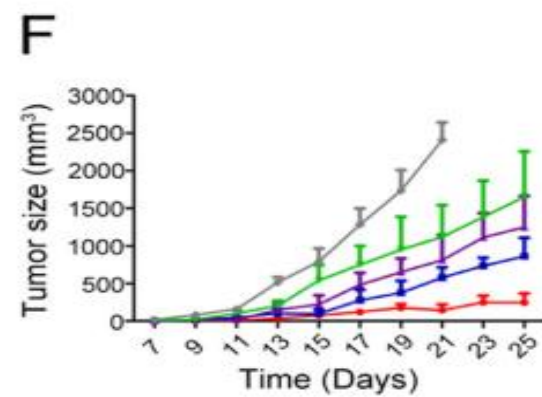
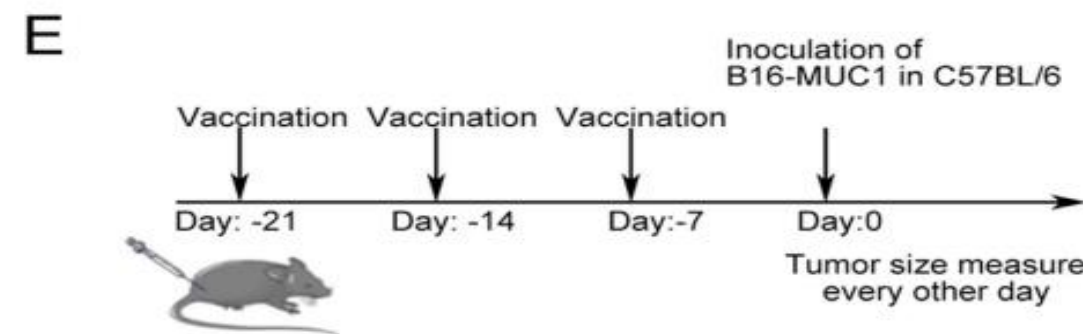
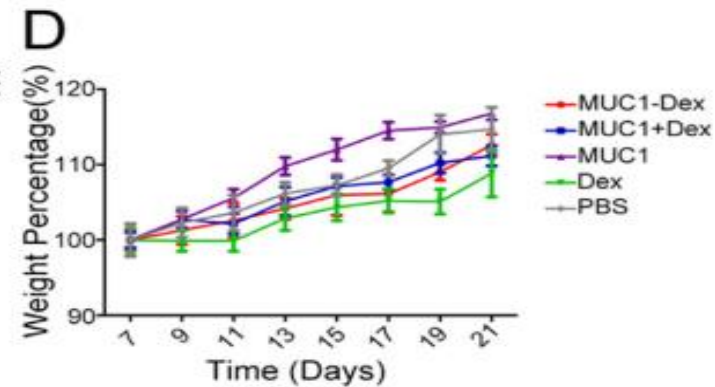
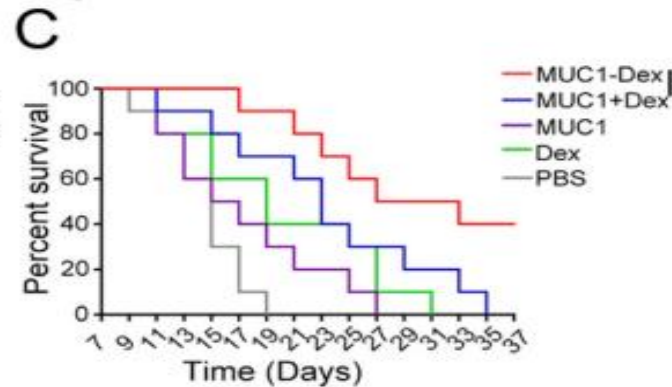
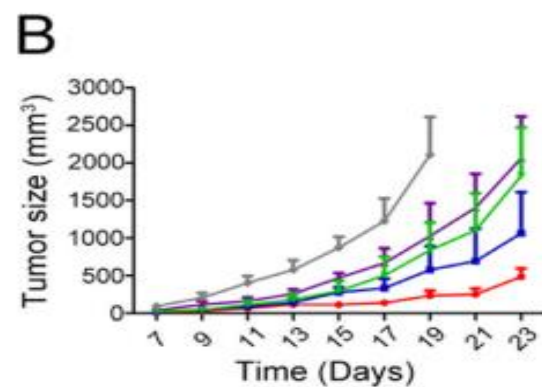
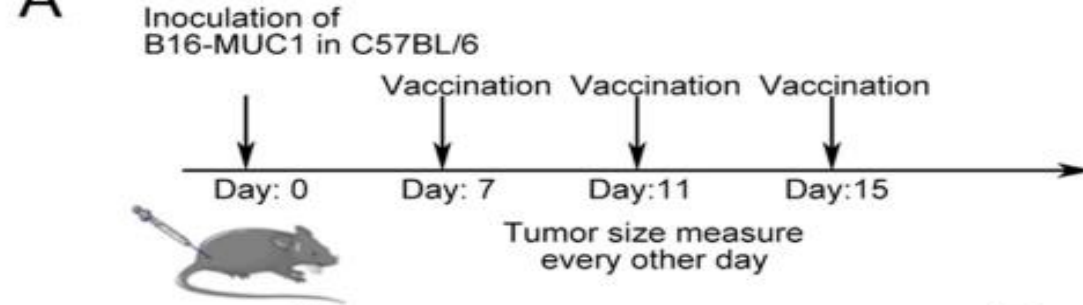


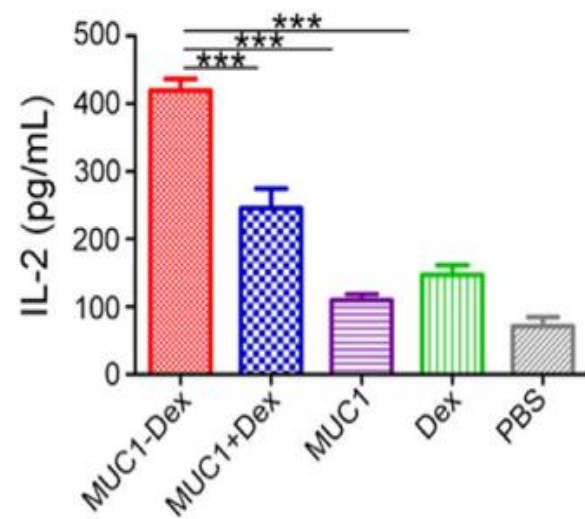
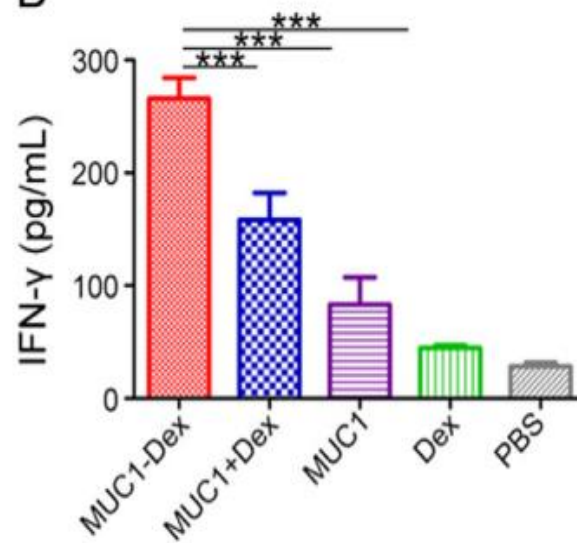
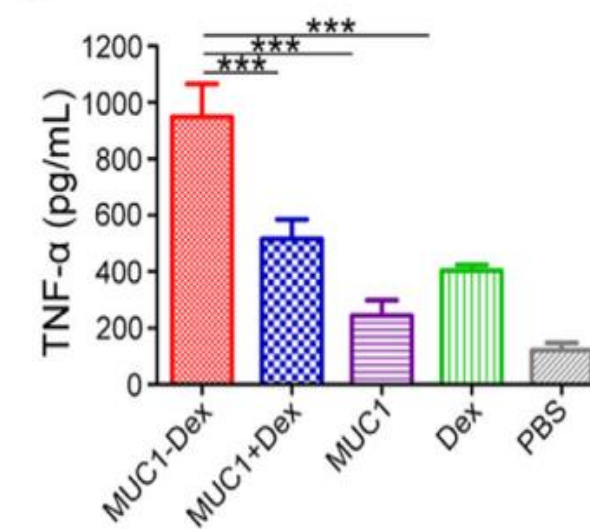
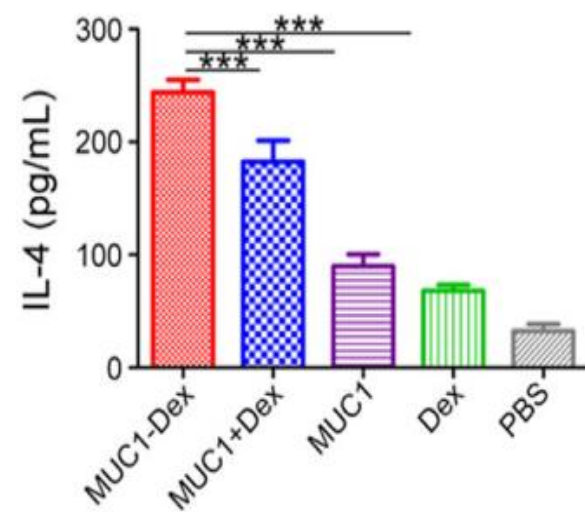
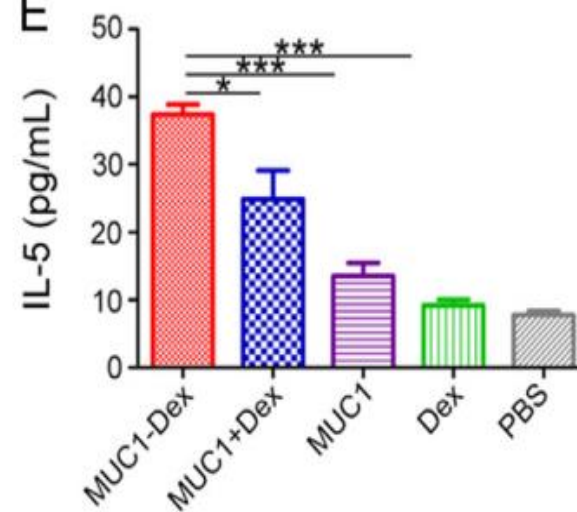
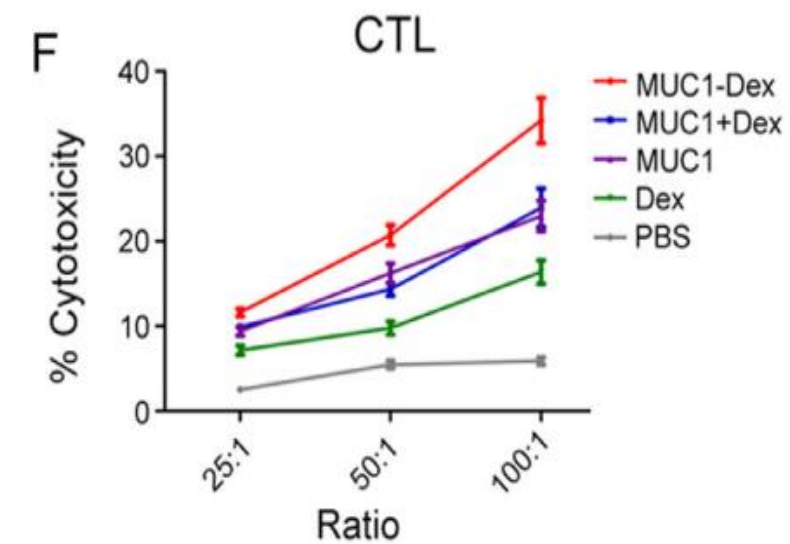
Full length article

### An efficient and safe MUC1-dendritic cell-derived exosome conjugate vaccine elicits potent cellular and humoral immunity and tumor inhibition *in vivo*

Haomiao Zhu, Kun Wang, Zhaoyu Wang, Danyang Wang, Xiaona Yin, Yonghui Liu\*, Fan Yu\*, Wei Zhao\*





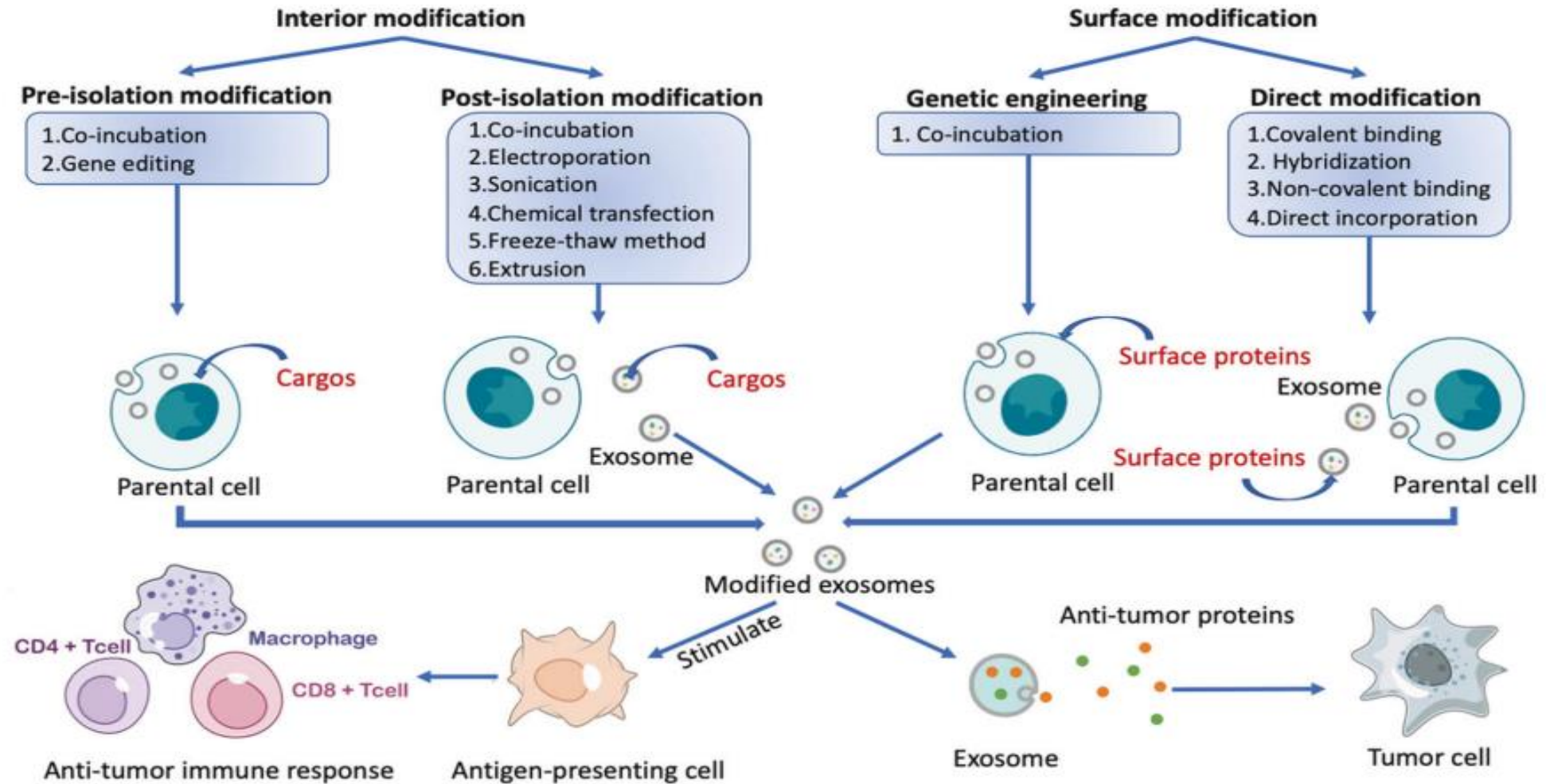
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## 4. Engineering Exosomes As Vehicle

- Parental exosomes or artificially modified exosomes used as a drug delivery vehicle have become appealing to researchers.
- Exosomes as delivery vesicles provide
  - Good tolerance
  - Proper internalization in distant cells
  - Reliable delivery of cargo such as proteins, mRNA, lipids, drugs and nucleic acids
  - An extended circulation half-life



# Modification Of Exosomes



RESEARCH

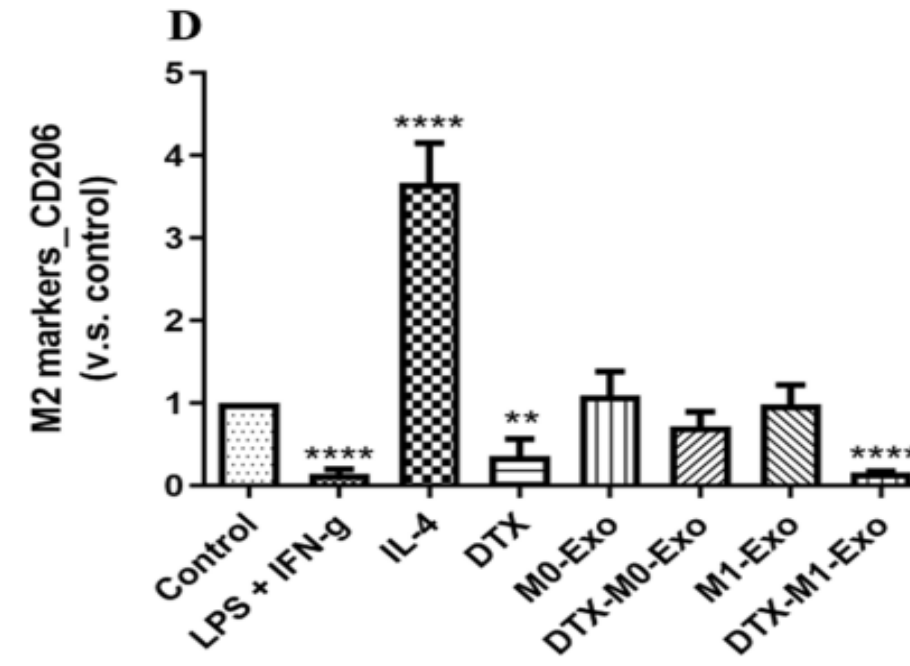
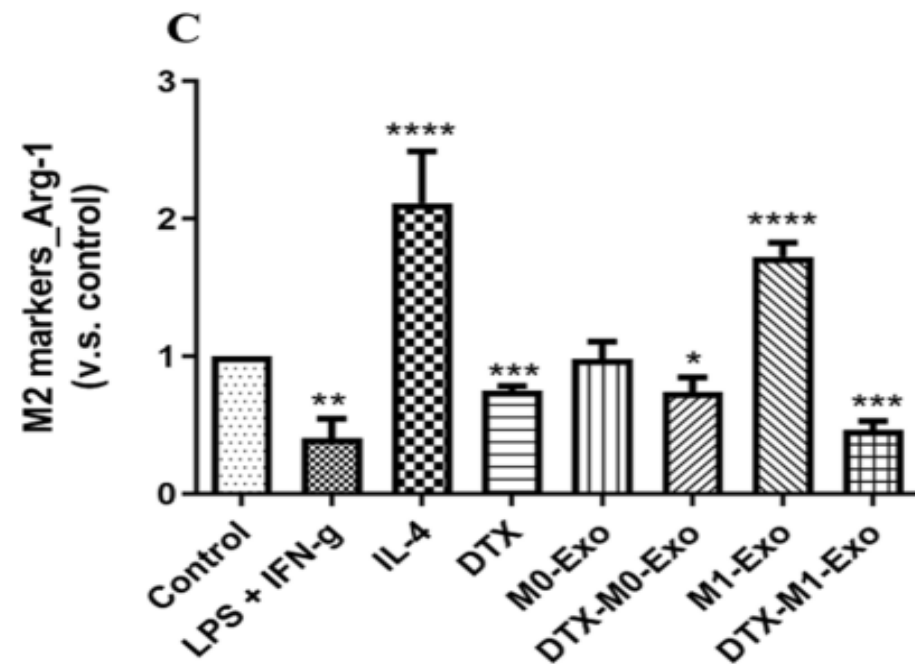
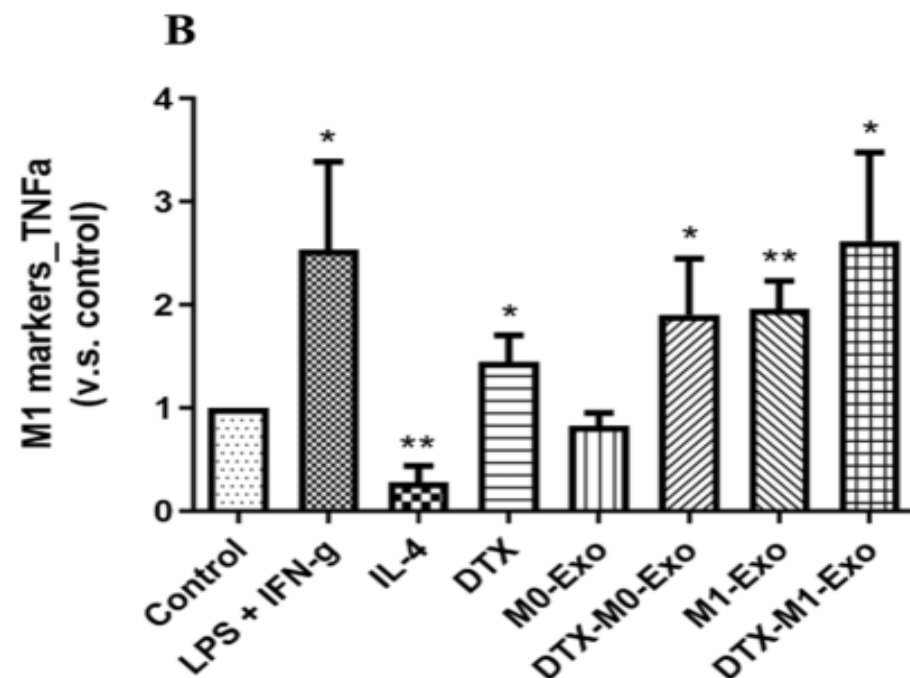
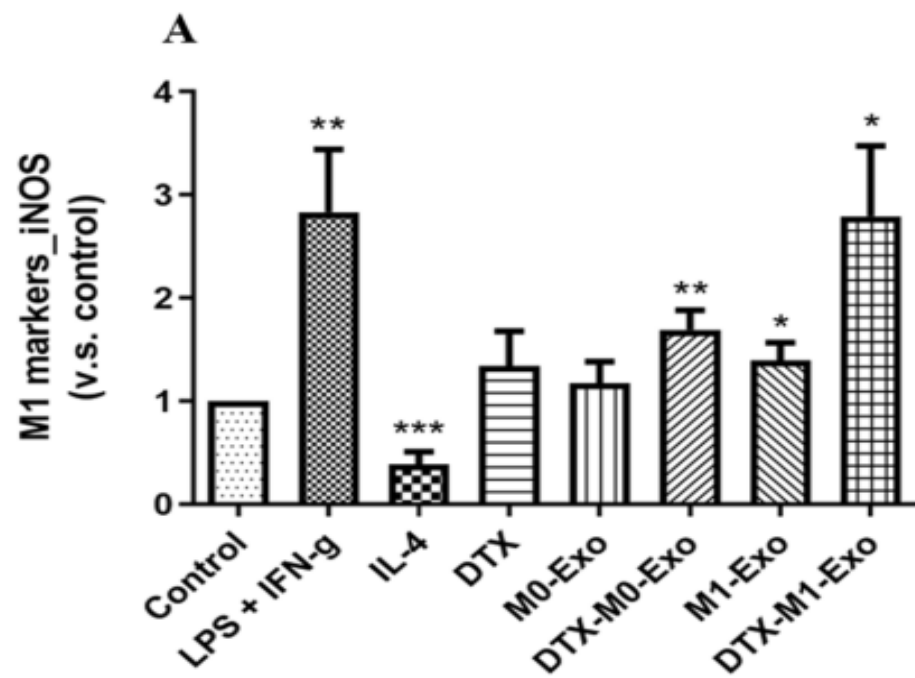
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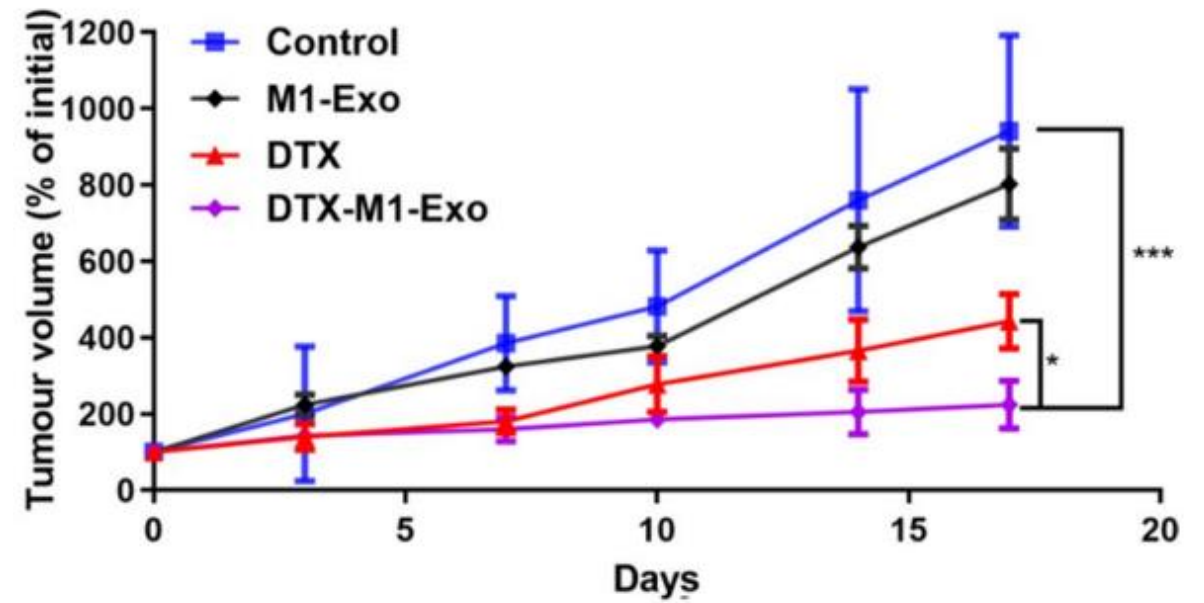
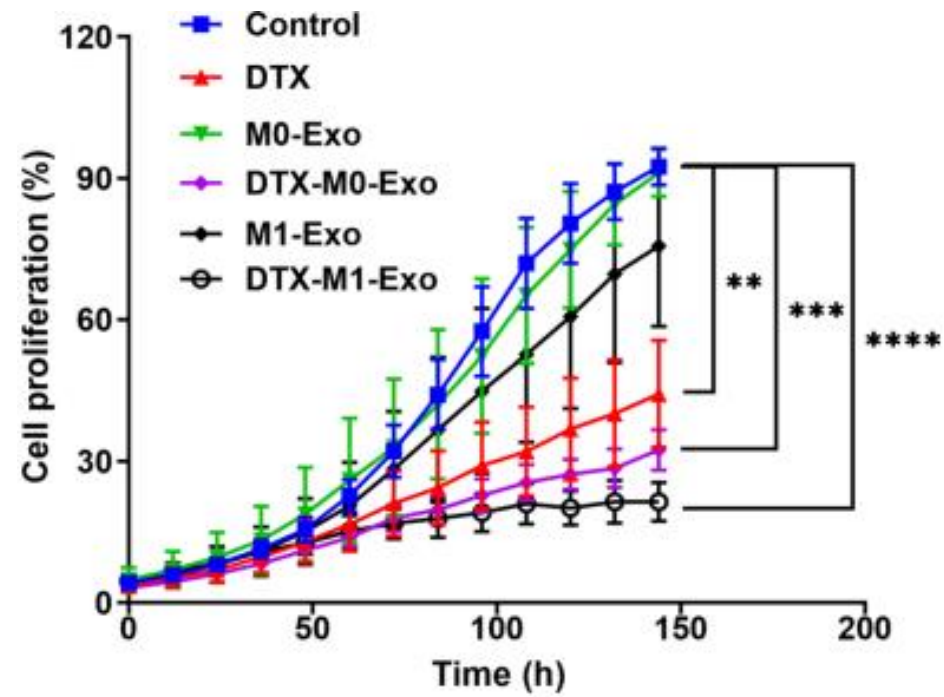
# Docetaxel-loaded M1 macrophage-derived exosomes for a safe and efficient chemoimmunotherapy of breast cancer



Yongmei Zhao<sup>1</sup>, Yuanlin Zheng<sup>1</sup>, Yan Zhu<sup>1</sup>, Hongyun Li<sup>1</sup>, Hongyan Zhu<sup>1\*</sup> and Tianqing Liu<sup>2\*</sup>







# Challenges And Future Trends

- Although therapies are under development, we still do not know the exact mechanism of exosome biogenesis.
- The source of EVs when developing engineered exosomes for therapeutic use, and MSCs, DCs, patient cancer cells and traditional cell lines are considered as basic candidates.
- Another challenge for clinical use is the fabrication of huge and stable engineered exosomes.
- Determining the potential of these natural vectors for use in cancer therapy is to improve the efficiency of the loading cargo when designing exosomes.

# Conclusion

- Exosomes derived from tumor, stromal and immune cells contribute to the multiple stages of cancer progression .
- Since specific contents within exosomes originate from their cells of origin, this property enables exosomes to function as valuable diagnostic and prognostic biomarkers.
- Furthermore, the applications of exosomes as direct therapeutic targets or engineered vehicles for drugs may open up new avenue for therapy.

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