



Seminar

Exosome – the novel clinical diagnostic and therapeutic delivery tool

Lixin Wang
(Year 2nd PHD)

Supervisor: Dr. Siaw Shi Boon
Department of Microbiology
The Chinese University of Hong Kong
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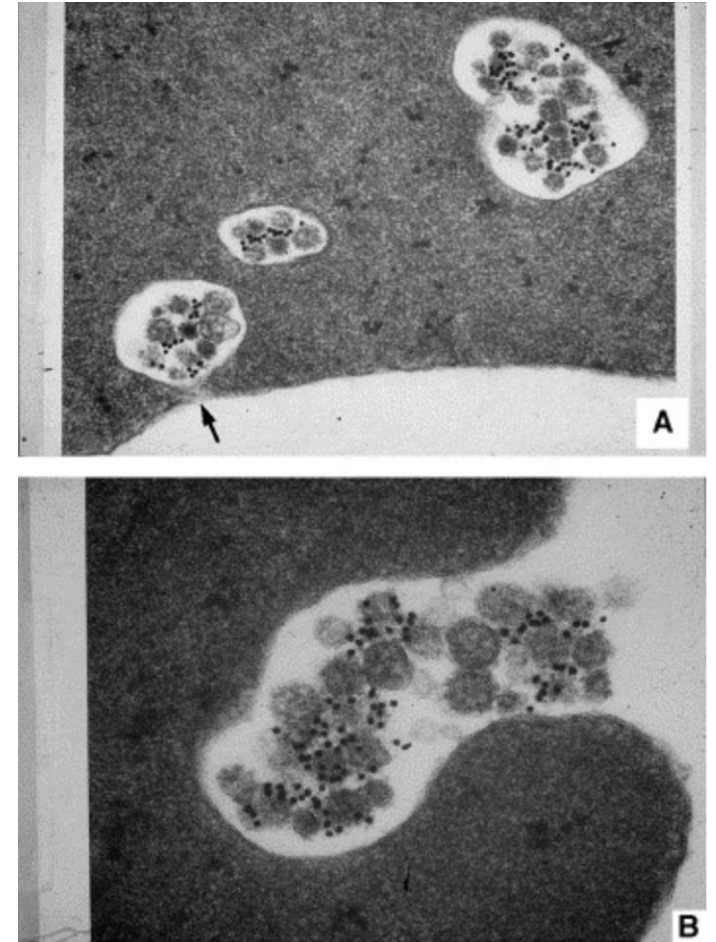


Discovery of exosomes



The road to the discovery of exosomes

- In 1981, Trams et al. discovered a group of vesicle-like structures with diameters 40-1000 nm smaller than those of multivesicular bodies by transmission electron microscopy ^[1]
- In 1987, Johnstone et al. used ultracentrifugation to isolate these membrane-bound vesicles from sheep reticulocytes. For the first time, these vesicle-like structures were named exosomes ^[2]



Exosomes in maturing sheep reticulocytes.

Johnstone RM , et al. Blood Cells Mol Dis. 2005 May-Jun;34(3):214-9.



The road to the discovery of exosomes

The Nobel Prize in Physiology or Medicine 2013



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James E. Rothman

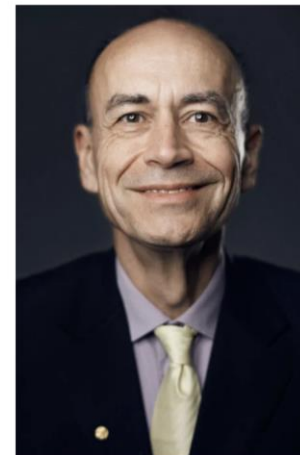
Prize share: 1/3



© Nobel Media AB. Photo: A. Mahmoud

Randy W. Schekman

Prize share: 1/3



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Thomas C. Südhof

Prize share: 1/3

The Nobel Prize in Physiology or Medicine 2013 was awarded jointly to James E. Rothman, Randy W. Schekman and Thomas C. Südhof "for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells"

<https://www.nobelprize.org/prizes/medicine/2013/summary/>

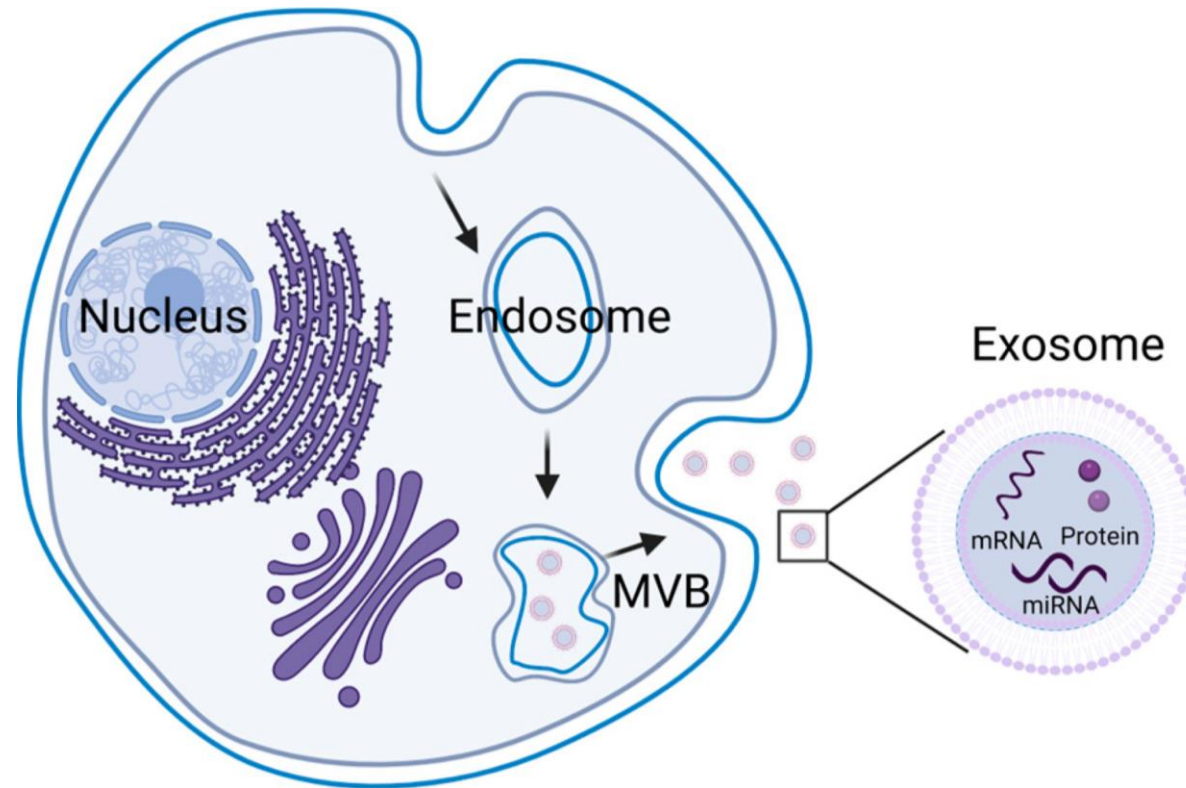


Exosome production and signaling delivery



Formation of exosomes

Endosomal pathway

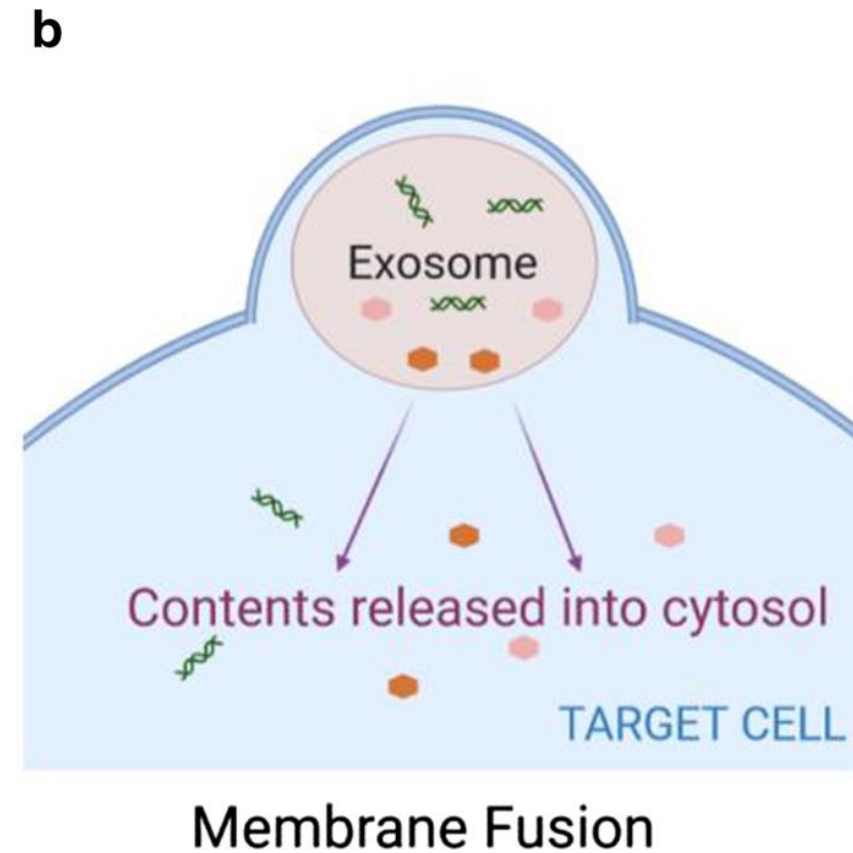
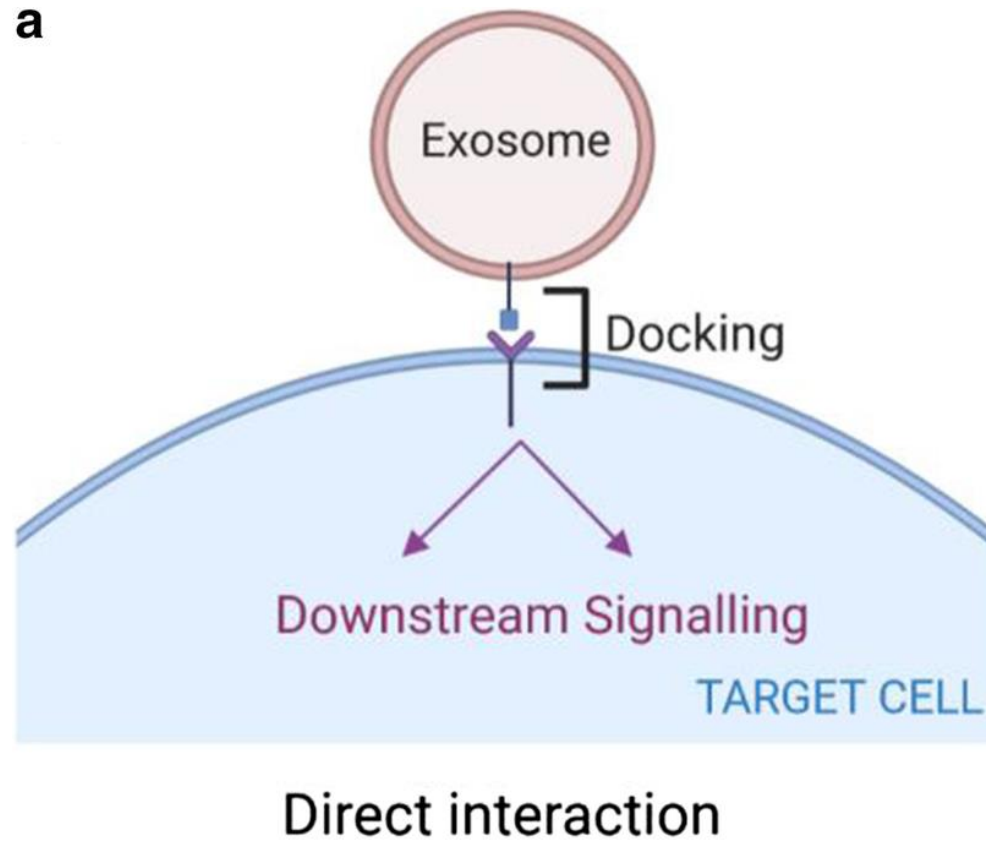


MVB: multivesicular bodies

Chen Q , et al. Molecules. 2022 Nov 12;27(22):7789.



Exosome signaling by direct interaction or membrane fusion



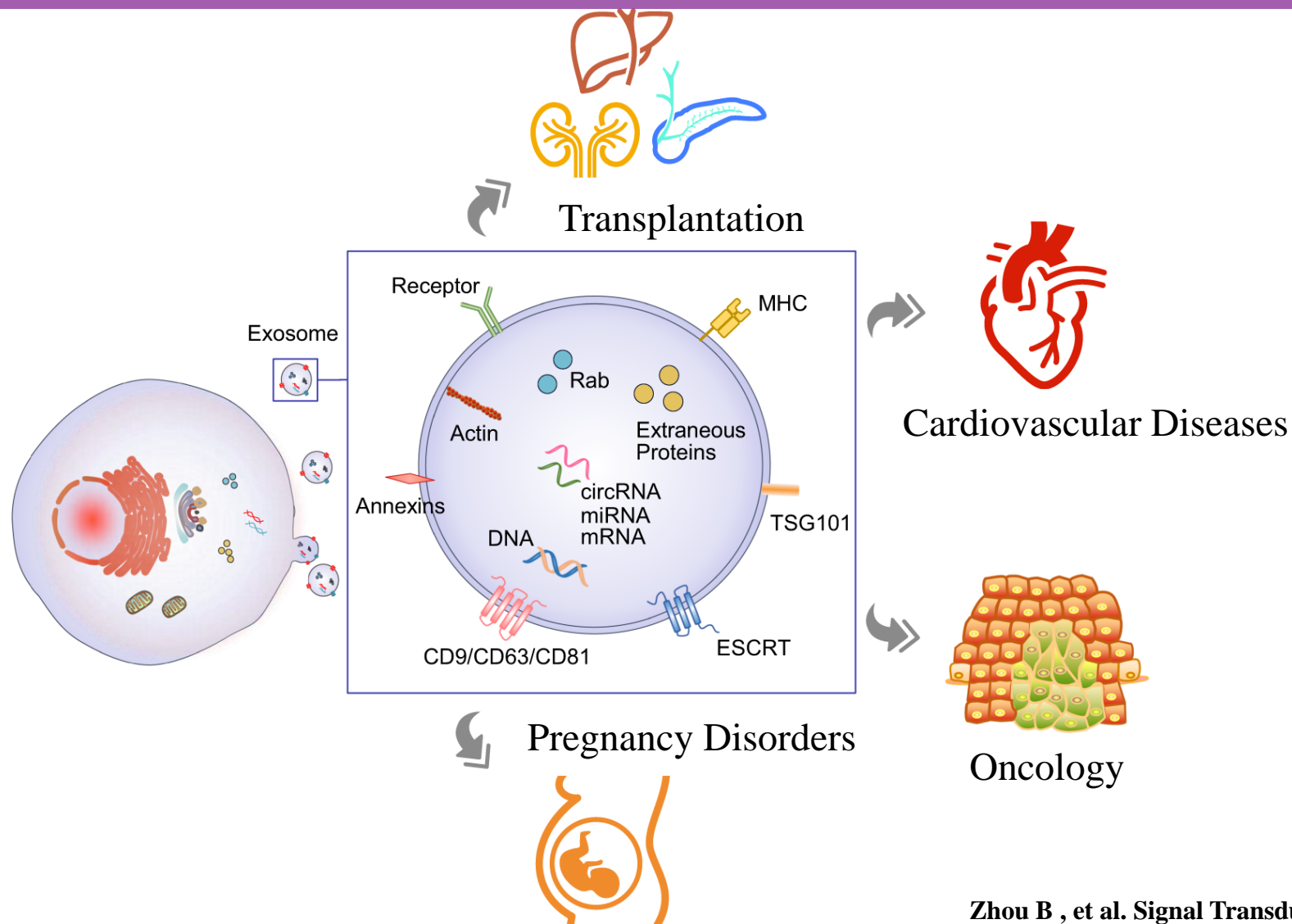
Gurung S , et al. Cell Commun Signal. 2021 Apr 23;19(1):47.



Application of Exosome in diagnosis



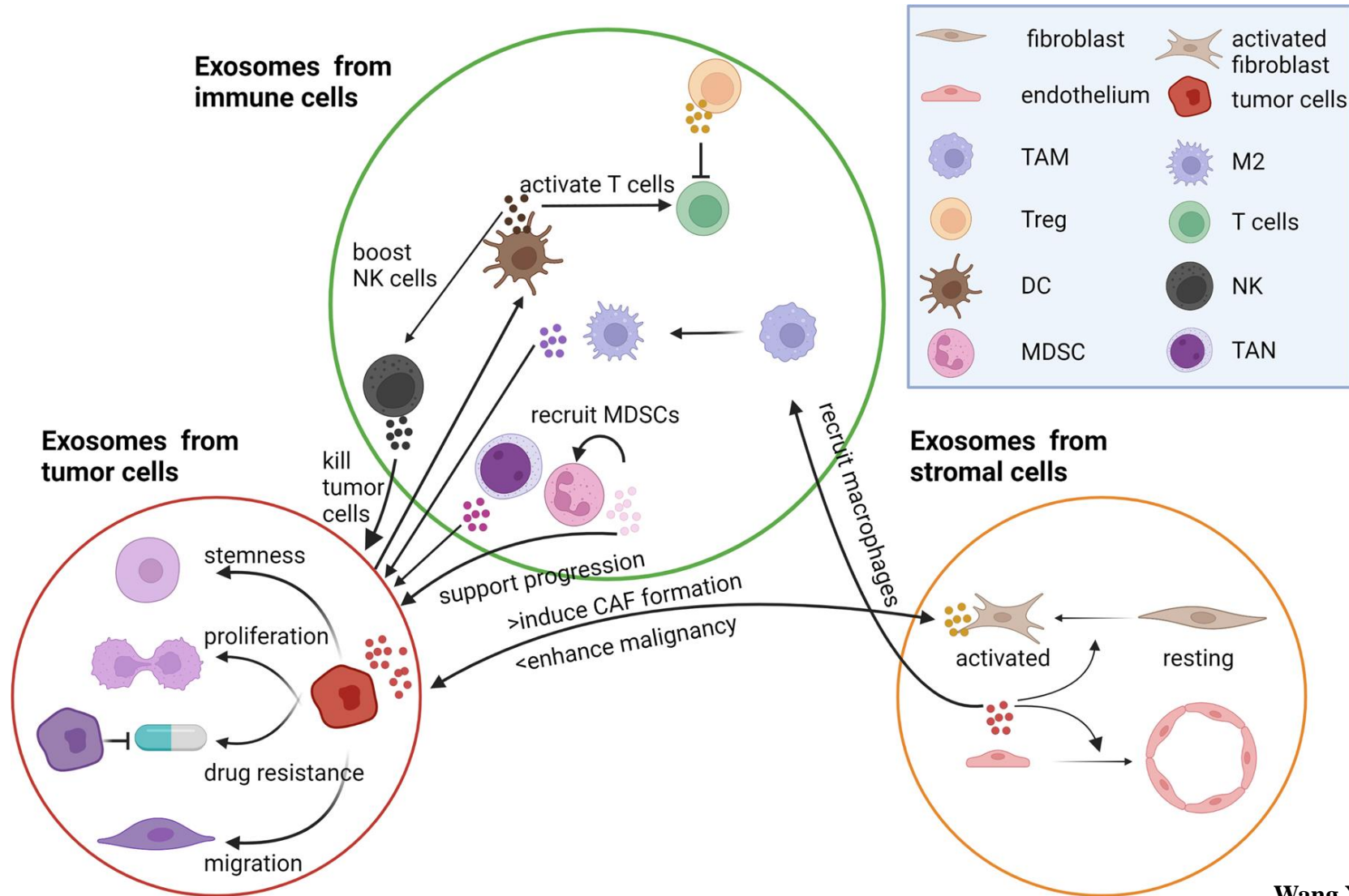
Application of Exosome in diagnosis



Zhou B , et al. Signal Transduct Target Ther. 2020 Aug 3;5(1):144.



Exosomes and cancer



TAM: tumor-associated macrophage
Treg: regulatory T cell
DC: dendritic cell
MDSC: myeloid-derived suppressor cell
M2: M2 macrophage
NK: natural killer cell
TAN: tumor-associated neutrophil
CAF: cancer-associated fibroblast.

Wang X , et al. Oncogenesis. 2022 Sep 15;11(1):54.

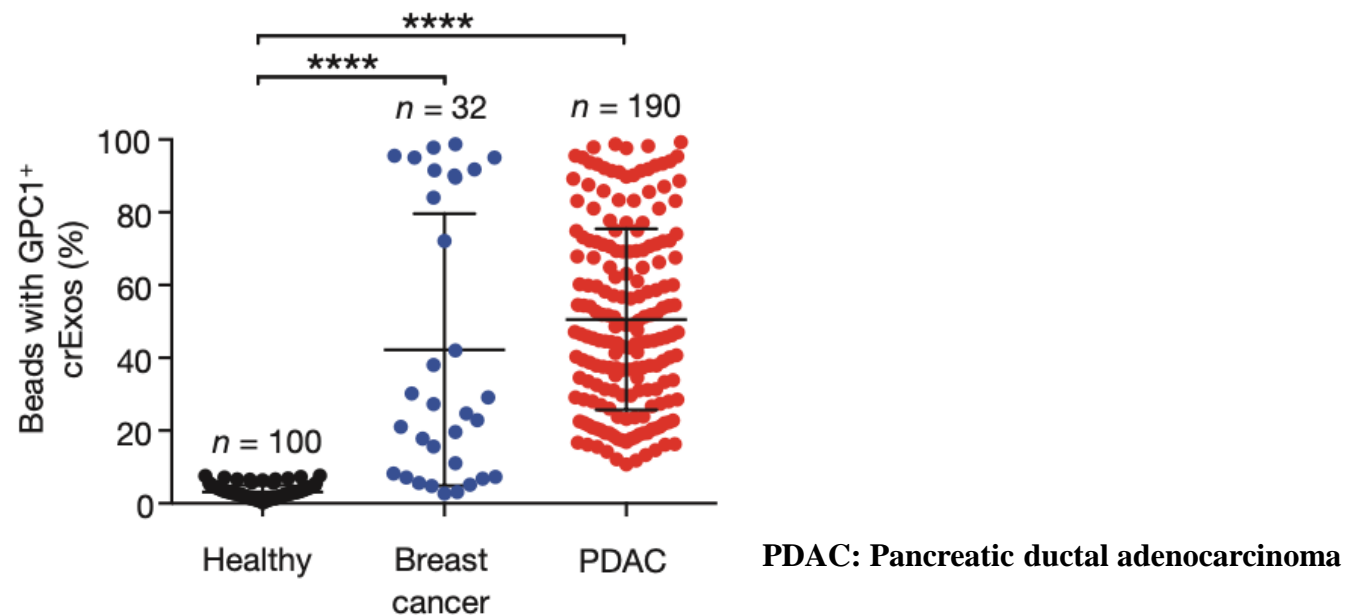


Exosomes and cancer diagnosis

nature

Article | Published: 24 June 2015

Glypican-1 identifies cancer exosomes and detects early pancreatic cancer

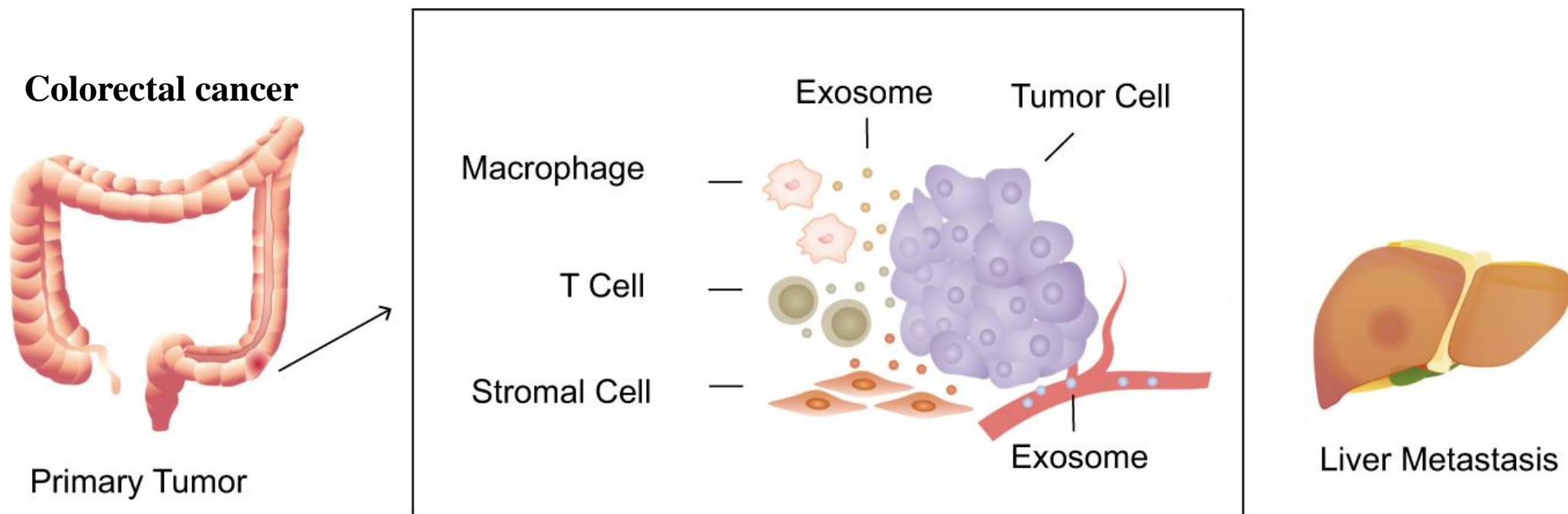


The combination of exosome glypican-1, CD82, and CA19-9 to establish an early screening test for pancreatic cancer.

Melo SA, et al. Nature. 2015 Jul 9;523(7559):17



Exosomes and cancer prognosis



Stromal cell-derived exosomes can carry bioactive molecules (e.g., miR-92a-3p) to colorectal cancer cells and enhance epithelial-mesenchymal transition and cell stemness to promote liver metastasis.

Zhou B , et al. *Signal Transduct Target Ther.* 2020 Aug 3;5(1):144.



Application of Exosome in Pregnancy Disorders

Fields	No. of patients	Source	Volume of body fluid	Targets	Exosome extraction	Extraction method	Detection method
Pregnancy disorders							
Pregnancy hypertension	98	Plasma	500 µl	miR-210	Exosome precipitation solution	NucleoSpin miRNA Plasma Kit	qPCR
Preeclampsia	47	Plasma	1 ml	miR-486-1-5p, miR-486-2-5p	Ultracentrifugation + ultrafiltration + OptiPrep gradient centrifugation	miRNeasy Mini Kit, TRIzol	NGS
	45	Plasma	1 ml	PLAP	Ultracentrifugation	ELISA	ELISA
Preterm birth	20	Plasma	1 ml	miRNAs	Ultracentrifugation	RNeasy Mini Kit	NGS
Congenital obstructive nephropathy	8	Amniotic fluid	10 ml	miR-300, miR-299-5p	Ultracentrifugation + sucrose gradient centrifugation	TRIzol	miRNA microarray

Zhou B , et al. Signal Transduct Target Ther. 2020 Aug 3;5(1):144.

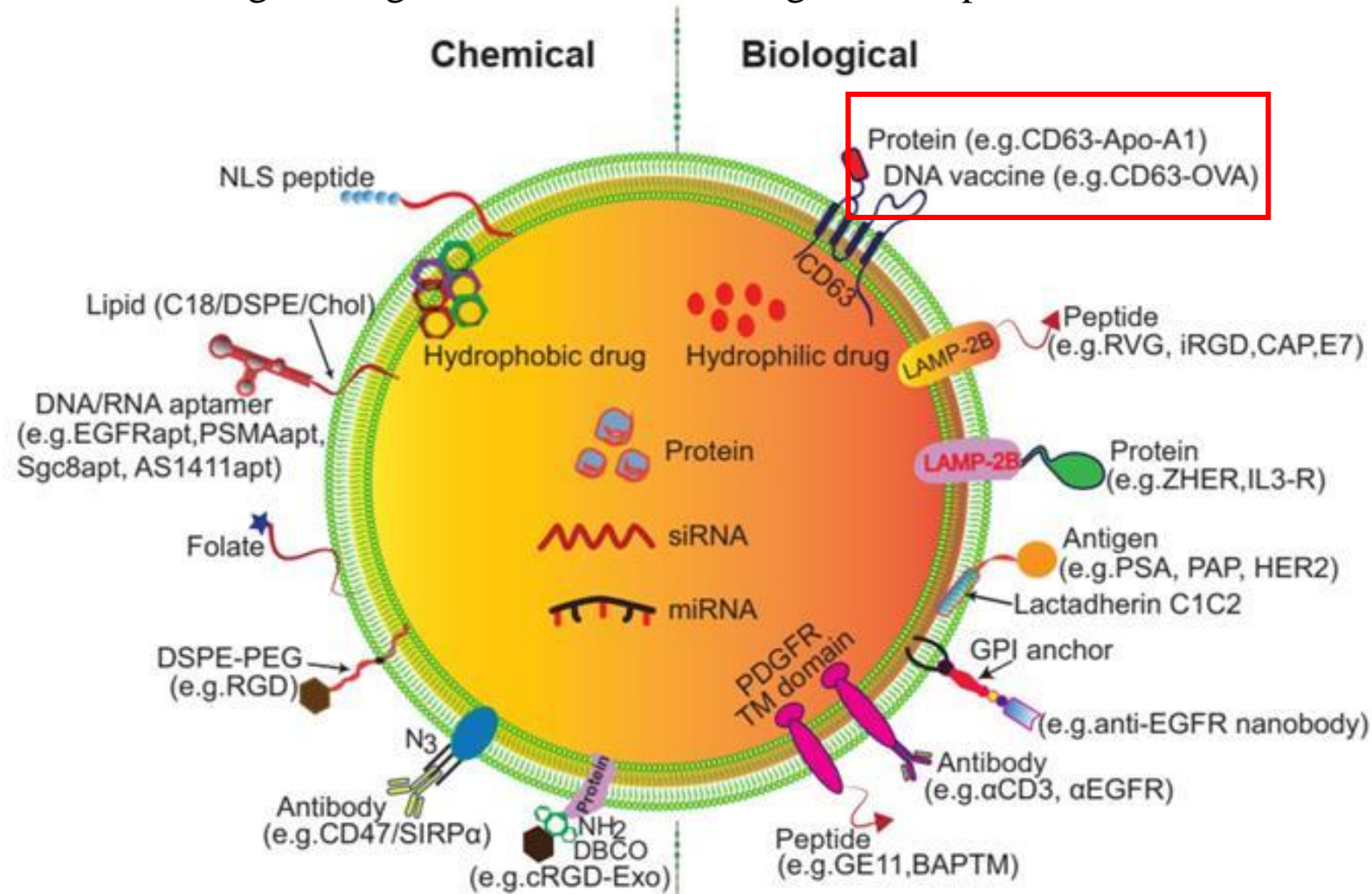


Application of Exosome in drug delivery



Application of Exosome in drug delivery

Surface engineering of exosomes via biological manipulation or chemical modification.

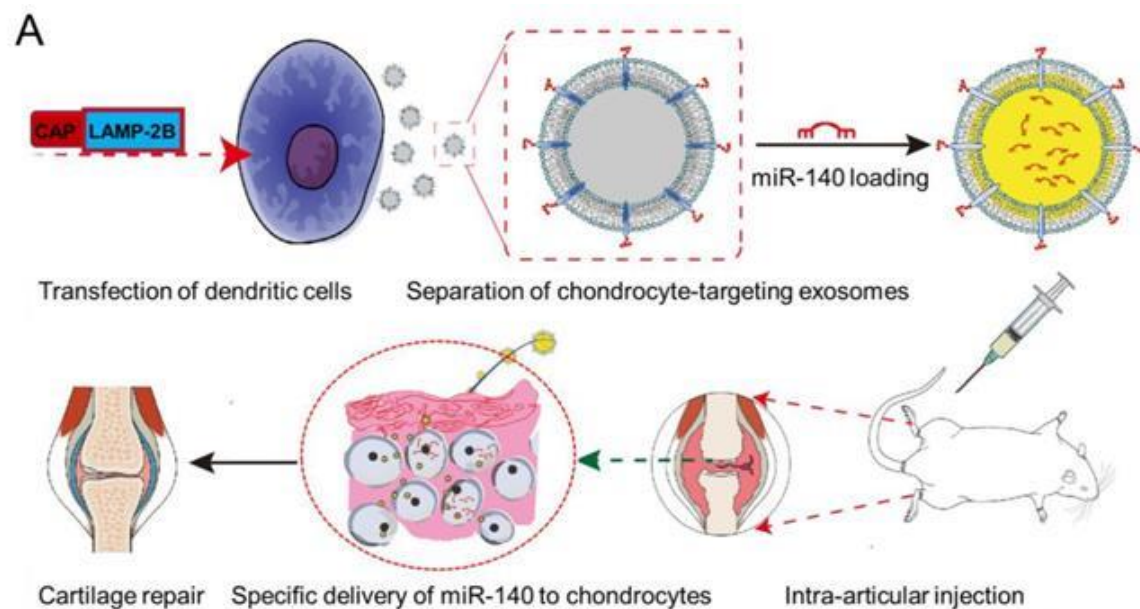


Liang Y , et al. Theranostics. 2021 Jan 1;11(7):3183-3195.



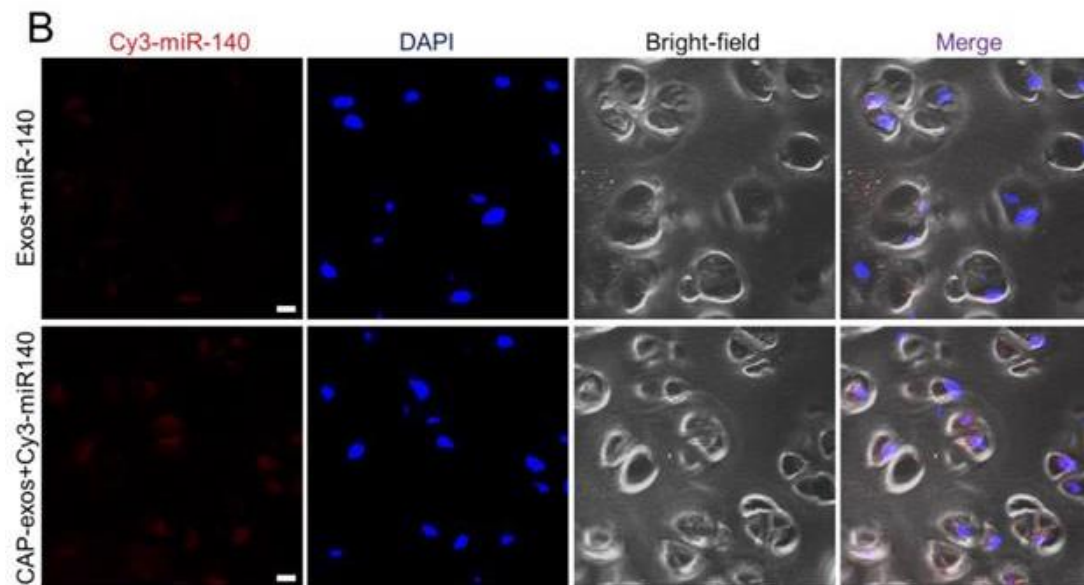
Application of Exosome in drug delivery

Chondrocyte-Targeted MicroRNA Delivery by Engineered Exosomes toward a Cell-Free Osteoarthritis Therapy



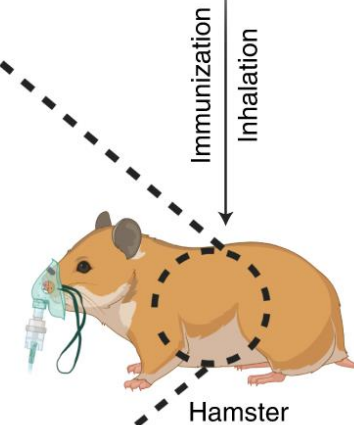
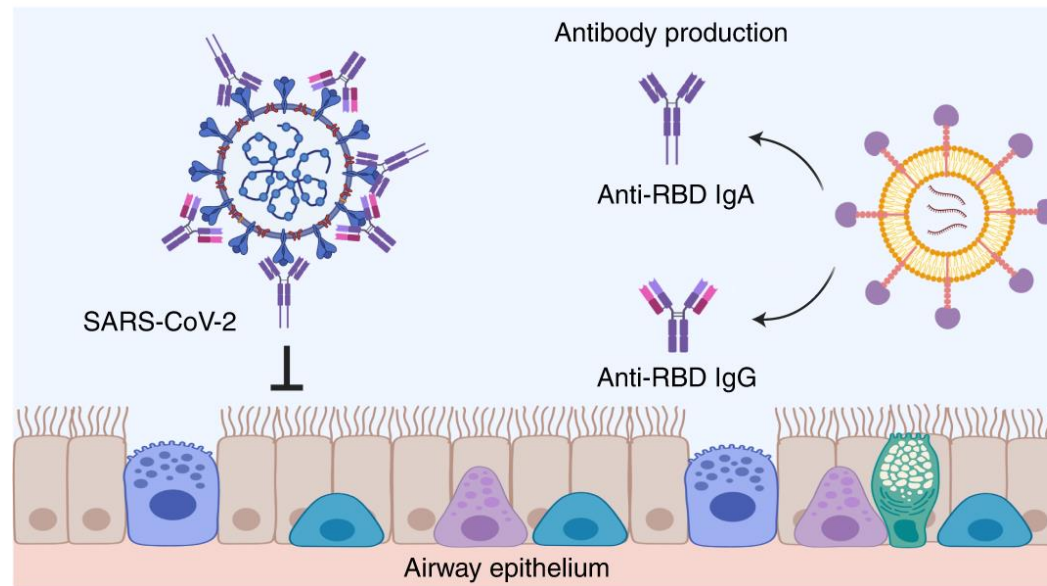
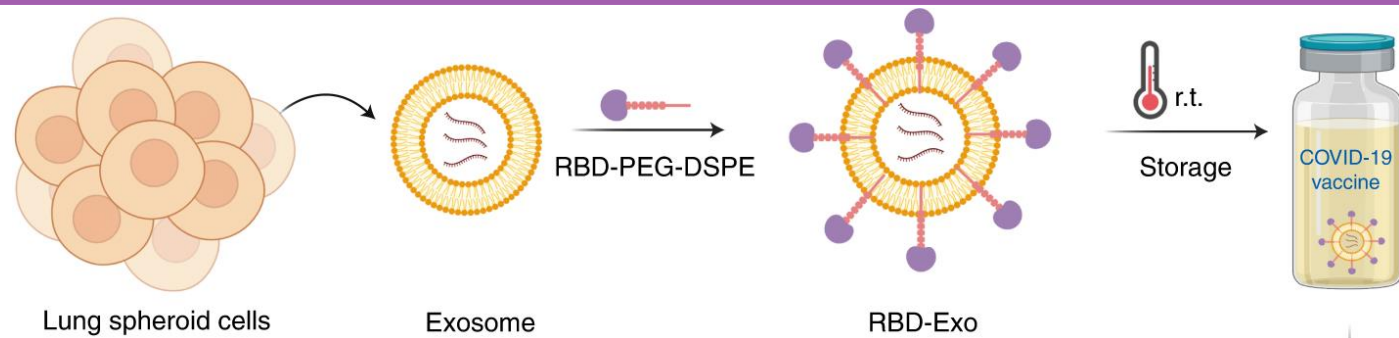
CAP: chondrocyte-affinity peptide

LAMP-2B: lysosome-associated membrane glycoprotein 2b protein





Application of Exosome in drug delivery



RBD: SARS-CoV-2 receptor-binding domain
DSPE-PEG-NHS: 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethylene-glycol)-N-hydroxysuccinimide

Unlike intramuscular vaccines, inhaled vaccines can induce the production of neutralizing antibodies against SARS-CoV-2 and triggered the mucosal immune system to generate antigen-specific secretory IgA and T-cell responses in the lung

Wang Z , et al. Nat Biomed Eng. 2022 Jul;6(7):791-805.



Summarize



Advantages

- 1.Biomarkers for Diagnosis:** Exosomes can carry specific proteins, lipids, and RNAs that reflect the health status of their parent cells, making them useful biomarkers for early disease detection, including cancer and other conditions.
- 2.Non-Invasive Collection:** They can be isolated from various body fluids (e.g., blood, urine, saliva), allowing for non-invasive sampling compared to traditional biopsy methods.
- 3.Targeted Drug Delivery:** Exosomes can be engineered to deliver therapeutic agents directly to target cells, enhancing the efficacy of treatments while minimizing side effects.



Limitation:

1.Isolation Challenges: The processes for isolating exosomes may affect their integrity and functionality.

2.Standardization Issues: There is currently a lack of standardized protocols for exosome analysis, making it difficult to compare results across studies.

3.Limited Understanding of Mechanisms: The biological mechanisms through which exosomes exert their effects are not fully understood, which can hinder their clinical application.



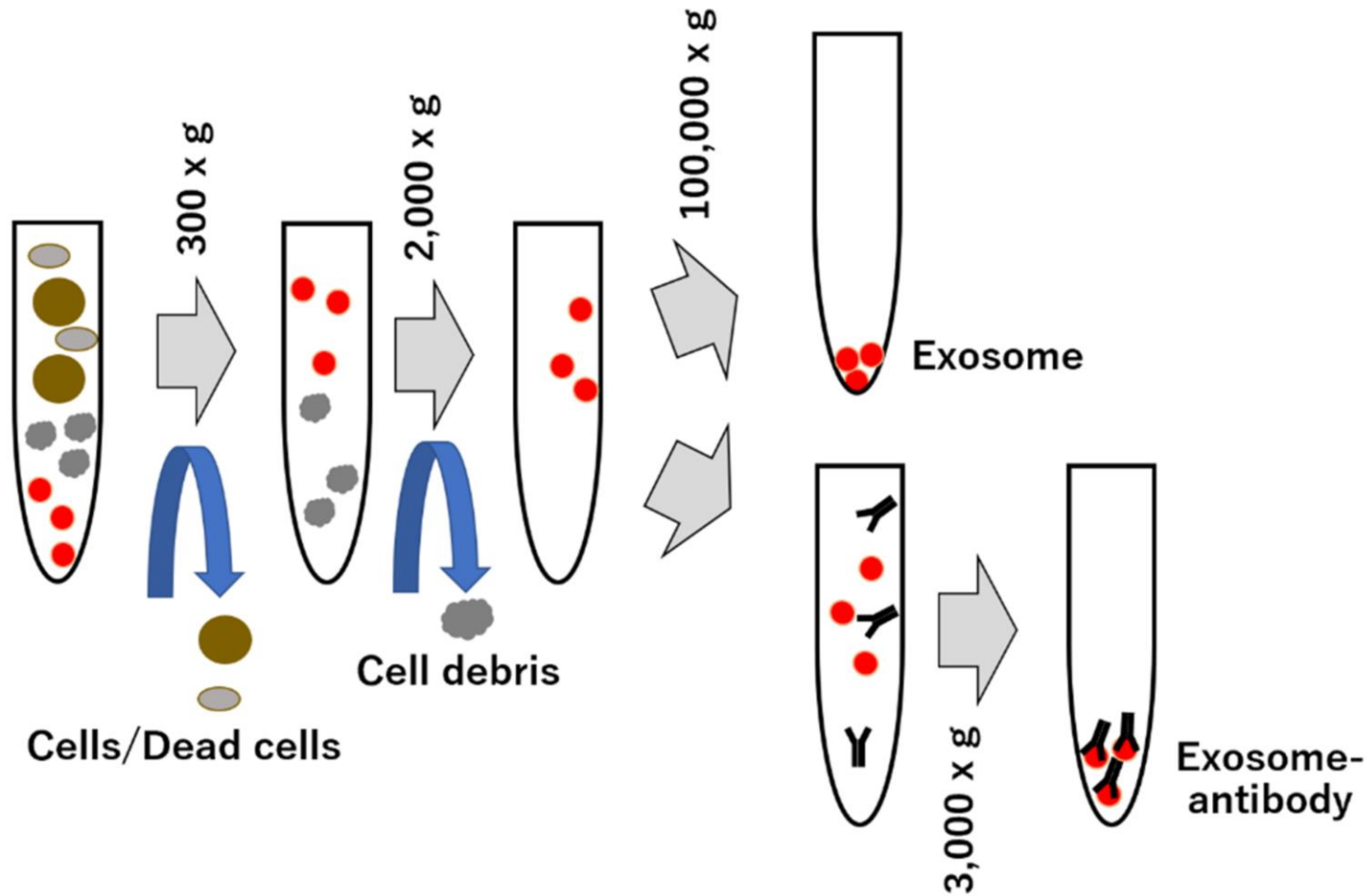
- [1] Johnstone RM. Revisiting the road to the discovery of exosomes. *Blood Cells Mol Dis*. 2005 May-Jun;34(3):214-9. doi: 10.1016/j.bcmd.2005.03.002. PMID: 15885604.
- [2] Chen Q, Wu D, Wang Y, Chen Z. Exosomes as Novel Delivery Systems for Application in Traditional Chinese Medicine. *Molecules*. 2022 Nov 12;27(22):7789. doi: 10.3390/molecules27227789. PMID: 36431890; PMCID: PMC9695524.
- [3] Gurung S, Perocheau D, Touramanidou L, Baruteau J. The exosome journey: from biogenesis to uptake and intracellular signalling. *Cell Commun Signal*. 2021 Apr 23;19(1):47. doi: 10.1186/s12964-021-00730-1. PMID: 33892745; PMCID: PMC8063428.
- [4] Matsuzaka Y, Yashiro R. Advances in Purification, Modification, and Application of Extracellular Vesicles for Novel Clinical Treatments. *Membranes (Basel)*. 2022 Dec 8;12(12):1244. doi: 10.3390/membranes12121244. PMID: 36557150; PMCID: PMC9787595.
- [5] Zhou B, Xu K, Zheng X, Chen T, Wang J, Song Y, Shao Y, Zheng S. Application of exosomes as liquid biopsy in clinical diagnosis. *Signal Transduct Target Ther*. 2020 Aug 3;5(1):144. doi: 10.1038/s41392-020-00258-9. PMID: 32747657; PMCID: PMC7400738.
- [6] Wang X, Huang J, Chen W, Li G, Li Z, Lei J. The updated role of exosomal proteins in the diagnosis, prognosis, and treatment of cancer. *Exp Mol Med*. 2022 Sep;54(9):1390-1400. doi: 10.1038/s12276-022-00855-4. Epub 2022 Sep 22. PMID: 36138197; PMCID: PMC9535014.
- [7] Melo SA, Luecke LB, Kahlert C , et al. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. *Nature*. 2015 Jul 9;523(7559):177-82. doi: 10.1038/nature14581. Epub 2015 Jun 24. Erratum in: *Nature*. 2022 Oct;610(7932):E15-E17. doi: 10.1038/s41586-022-05062-9
- [8] Zhou B, Xu K, Zheng X, Chen T, Wang J, Song Y, Shao Y, Zheng S. Application of exosomes as liquid biopsy in clinical diagnosis. *Signal Transduct Target Ther*. 2020 Aug 3;5(1):144. doi: 10.1038/s41392-020-00258-9. PMID: 32747657; PMCID: PMC7400738.
- [9] Zhou B, Xu K, Zheng X, Chen T, Wang J, Song Y, Shao Y, Zheng S. Application of exosomes as liquid biopsy in clinical diagnosis. *Signal Transduct Target Ther*. 2020 Aug 3;5(1):144. doi: 10.1038/s41392-020-00258-9. PMID: 32747657; PMCID: PMC7400738.
- [10] Liang Y, Duan L, Lu J, Xia J. Engineering exosomes for targeted drug delivery. *Theranostics*. 2021 Jan 1;11(7):3183-3195. doi: 10.7150/thno.52570. PMID: 33537081; PMCID: PMC7847680.
- [11] Liang Y, Xu X, Li X , et al. Chondrocyte-Targeted MicroRNA Delivery by Engineered Exosomes toward a Cell-Free Osteoarthritis Therapy. *ACS Appl Mater Interfaces*. 2020 Aug 19;12(33):36938-36947. doi: 10.1021/acsami.0c10458. Epub 2020 Aug 7
- [12] Wang Z, Popowski KD, , et al. Exosomes decorated with a recombinant SARS-CoV-2 receptor-binding domain as an inhalable COVID-19 vaccine. *Nat Biomed Eng*. 2022 Jul;6(7):791-805. doi: 10.1038/s41551-022-00902-5. Epub 2022 Jul 4. PMID: 35788687; PMCID: PMC10782831.





The exosome purification

The exosome purification by ultracentrifugation and immunoprecipitation using antibody



Matsuzaka Y , et al. Membranes (Basel). 2022 Dec 8;12(12):1244.



Application of Exosome in Cardiovascular Diseases

Fields	No. of patients	Source	Volume of body fluid	Targets	Exosome extraction	Extraction method	Detection method	Findings
Cardiovascular diseases								
Myocardial infarction	20	Serum	1ml	miR-939	Ultracentrifugation	TRIzol	qPCR	Prognosis
	35	Plasma	5 ml	proteins	Ultracentrifugation	Trypsin	LC-MS/MS	Diagnosis
Coronary artery disease	180	Plasma	250 µl	miR-92a-3p	Ultracentrifugation	TRIzol	qPCR	Prognosis
Vascular disease	1060	Plasma	150 µl	Cystatin C, Serpin F2, CD14	ExoQuick™	Roche Complete Lysis-M	Immunoassay	Prognosis
Heart failure	20	Serum	–	miR-423-5p, miR-22, miR-320a, miR-92b	ExoQuick™ Exosome Precipitation Solution	Ethanol precipitation	qRT-PCR	Prognosis
	100	Serum	250 µl	miRNA	ExoQuick™ Exosome Precipitation Solution	ISOGEN II	qPCR	Prognosis
Stroke	131	Serum	–	miR-9, miR-124	ExoQuick™ Solution	Exosome RNA Purification Kit	qPCR	Diagnosis
Cardiac arrhythmia	37	Serum	30 µl	IL-1 β, P-selectin	Affinity capture	ELISA	ELISA	Prognosis

Zhou B , et al. Signal Transduct Target Ther. 2020 Aug 3;5(1):144.



Application of Exosome in Transplantation

Fields	No. of patients	Source	Volume of body fluid	Targets	Exosome extraction	Extraction method	Detection method	Findings
Organ transplantation								
Lung	30	BALF	–	SAGs, Collagen-V	Ultracentrifugation	–	Western blot	Diagnosis
		Serum	1ml		Total Exosome Isolation Reagent kit			
	12	BALF	20-60 ml	mRNAs	Ultracentrifugation	miRNeasy, miRCURY	RNA-Seq	Diagnosis
Heart	10	Serum	–	miR-142-3p	Ultracentrifugation	microRNeasy mini kit	qRT-PCR	Diagnosis
	48	Serum	200 µl	Proteins	Total Exosome Isolation	Trypsin	LC-MS/MS	Prognosis
Kidney	44	Urine	15 ml	CD3	Ultracentrifugation	iKEA	iKEA	Prognosis
	47	Urine	–	Tetraspanin-1, Hemopexin	Ultracentrifugation	RIPA buffer	LC-MS/MS	Diagnosis
	64	Plasma	–	mRNAs	exoRNeasy Serum/Plasma Midi Kit	exoRNeasy Serum/Plasma Midi Kit	qPCR	Diagnosis

Zhou B , et al. Signal Transduct Target Ther. 2020 Aug 3;5(1):144.



Application of Exosome in Transplantation

Classification:

Exosome:

A size range of 30–100 nm

Exosomes are of extracellular vesicles endosomal origin

Microvesicles:

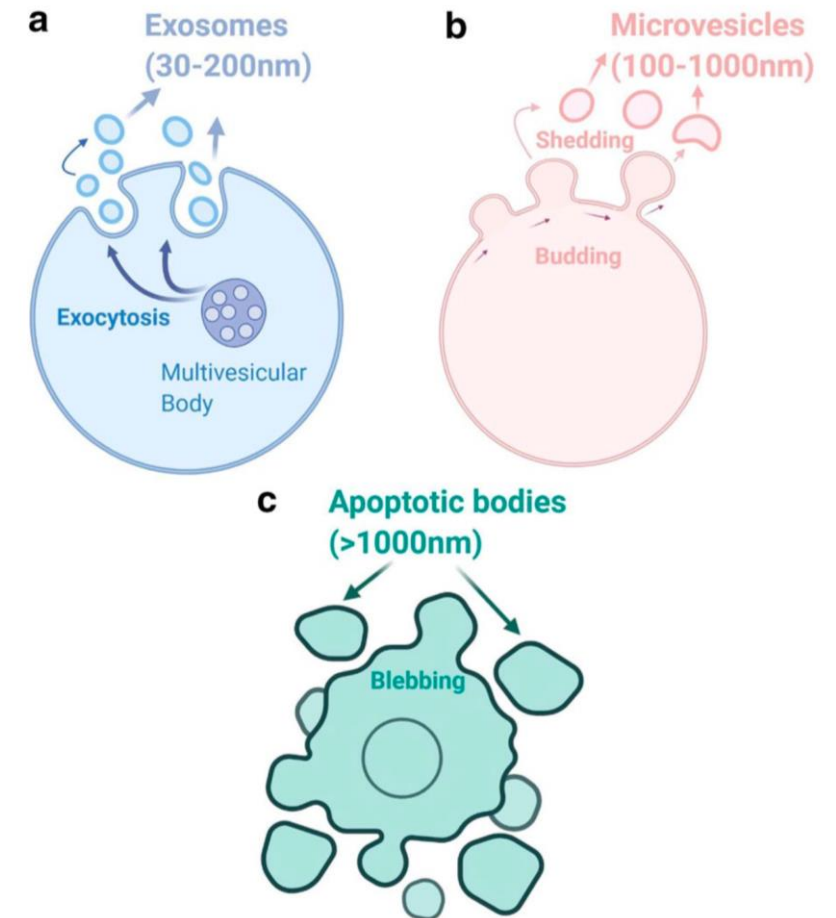
A size range of 50–1000 nm

Microvesicles typically form by membrane shedding, especially from injured or transformed cells

Apoptotic bodies:

A size range of 50–5000 nm

Apoptotic bodies result from the fractionation of the cellular content of cells that die by apoptosis



Gurung S , et al. Cell Commun Signal. 2021 Apr 23;19(1):47.

