JEIMP
E-ISSN: 2980-0617

The Journal of
European Internal Medicine Professionals

JEIMP
The Science

Review

Exosomes: Emerging Tools in Diagnostic and Therapeutic Applications—Challenges and Future Prospects

ORCID
Author(s) [1]Oktay Bağdatoğlu, [2]Sedat Gözel

Affiliation(s) [1]Bilkent City Hospital, Department of Nephrology, Ankara, Turkiye
[2]Hatay Education and Research Hospital, Department of Internal Medicine, Hatay, Turkiye

Corresponding Author Oktay Bağdatoğlu, M.D., Bilkent City Hospital, Department of Nephrology, Ankara, Turkiye
E-mail: obagdatoglu@yahoo.com

doi 10.5281/zenodo.13338077

J Eur Int Prof. Year; 2024, Volume: 2, Issue: 3 *Submitted at*: 25.07.2024, *Accepted at*: 18.05.2024, *Published at*: 19.05.2024

JEIMP belongs to "*The Foundation for the Management of Chronic Diseases***" and is supervised by the MKD Digital Publishing.** *www.jeimp.com and digitalmkd.com*

Abstract

Exosomes, nanoscale extracellular vesicles secreted by various cell types, have significant diagnostic and therapeutic potential, as accumulating evidence highlights their critical role in intercellular communication and therapeutic applications. This study provides a comprehensive overview of exosome biogenesis, their biological functions, and the mechanisms through which they influence pathological processes, particularly in cancer and cardiovascular diseases. The therapeutic potential of exosomes is explored in depth, highlighting their application in drug delivery, gene therapy, and regenerative medicine. Furthermore, the study discusses recent advances in engineering exosomes for enhanced therapeutic efficacy, including the development of strategies for targeted delivery and overcoming biological barriers. The dual role of exosomes in cancer biology—both as facilitators of tumor progression and as tools for therapy—is also elaborated upon, providing insights into their complex nature. Finally, the review addresses the challenges and future perspectives in exosome research, emphasizing the need for standardized protocols and further clinical trials to fully realize the potential of exosome-based therapies.

Keywords: Exosome, diagnostic biomarkers, therapeutic delivery, cancer, regenerative medicine

INTRODUCTION

Exosomes are nanoscale extracellular vesicles that have significant interest in recent years, due to their pivotal role in intercellular communication and their potential therapeutic applications (1,2). These vesicles are typically 30-100 nm in diameter and are secreted by almost all cell types into the extracellular space (3). Exosomes carry a variety of bioactive molecules, including proteins, lipids, RNA, and DNA, which they transfer to recipient cells, thereby influencing numerous physiological and pathological processes (1-3). While exosome promises new insights in contemporary medicine, it also carries clues about the personalized "future medicine". For instance, exosomes can be loaded with patient-specific therapeutic agents to enhance treatment efficacy and reduce side effects.

Exosomes are present in various body fluids, including blood, urine, and saliva, making them accessible for non-invasive diagnostic tests (4). The molecular content of exosomes reflects the physiological state of their originating cells, providing valuable information about the presence and progression of diseases. Exosomes originate from membrane invagination and are released by exocytosis, transmitting signals to target cells to facilitate cell-to-cell communication and maintain homeostasis. In an organ-specific view, the heart, a complex multicellular organ, contains resident cell types such as fibroblasts, endothelial cells, and smooth muscle cells. Effective communication between these different cell types and the immune system is essential for the dynamic equilibrium of the cardiac internal environment. Intercellular communication, mediated by exosomes and their contents, is a universal phenomenon that plays a crucial role in several pathological processes in cardiovascular diseases, including cardiomyocyte hypertrophy, apoptosis, and angiogenesis. Consequently, exosomes can serve as novel non-invasive diagnostic biomarkers in multiple diseases, such as atherosclerosis, myocardial ischemia, cardiac fibrosis, and ischemia-reperfusion injury.Exosomes derived from stem cells,

particularly mesenchymal stem cells (MSCs), have been shown to promote tissue repair and regeneration. They can enhance wound healing, reduce inflammation, and support the repair of damaged tissues in conditions such as myocardial infarction, stroke, and chronic wounds (5).

Exosomes from dendritic cells (DCs) are known to carry functional MHC-peptide complexes and various immune-stimulating molecules. They can present antigens to T cells, thus activating and modulating adaptive immune responses. This makes DC-derived exosomes (Dex) particularly interesting for therapeutic applications in cancer and other diseases due to their ability to stimulate immune responses against tumors (6). Exosomes exhibit dual functions in regulating inflammation. For instance, exosomes from M2 macrophages, which carry microRNA-148a, can downregulate inflammatory responses and alleviate conditions such as myocardial ischemia/reperfusion injury. On the other hand, exosomes from pyroptotic cells can promote inflammation by extending damage to adjacent cells through inflammasome activity (7).

Exosomes play a dual role in cancer biology, acting both as facilitators of cancer progression and as potential tools for therapeutic purposes. Tumor-derived exosomes (TDEs) are critical in promoting angiogenesis, which is the formation of new blood vessels that supply the tumor with nutrients and oxygen, thereby supporting its growth and metastasis. These exosomes carry pro-angiogenic factors such as VEGF (vascular endothelial growth factor) that stimulate blood vessel formation in the tumor microenvironment (8,9). Exosomes contribute to the preparation of the pre-metastatic niche, which is a conducive environment for metastatic tumor cells to colonize and grow. They transport molecules like miR-21 and TGF-β, which promote epithelial-to-mesenchymal transition (EMT), a process where cancer cells gain mobility and invasiveness. This facilitates their spread to distant organs (9-11). Exosomes can help tumors evade the immune system by carrying immune checkpoint molecules like PD-L1, which can bind to PD-1 on T cells, inhibiting their activity and allowing the tumor to escape immune surveillance (12). They can also carry FasL, which induces apoptosis in T cells, further suppressing the immune response against the tumor (13).

BIOGENESIS OF EXOSOMES

The biogenesis of exosomes begins with the formation of intraluminal vesicles (ILVs) within multivesicular bodies (MVBs), which are endosomal compartments. When MVBs fuse with the plasma membrane, ILVs are released as exosomes into the extracellular space. The structure of exosomes can be characterized by several features:

1.Lipid bilayer: Exosomes have a lipid bilayer membrane that provides structural stability and protects their cargo from degradation. This bilayer is enriched with cholesterol, sphingolipids, and ceramides, contributing to the unique biophysical properties of exosomes.

2. Surface markers: Exosomes express specific surface proteins such as CD9, CD63, and CD81, which are often used as markers for their identification and characterization. These proteins play roles in exosome formation, targeting, and uptake by recipient cells.

3. Internal cargo: The internal contents of exosomes include a complex mixture of proteins, RNAs (including mRNAs and miRNAs), and sometimes DNA. The cargo composition reflects the cell of origin and its physiological state, providing insights into cellular functions and pathological conditions.

Exosomes are formed through a complex process that involves the endosomal sorting complex required for transport (ESCRT) machinery and ESCRT-independent mechanisms (14,15).

•*ESCRT-Dependent Pathway*: The ESCRT machinery is composed of four main complexes: ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III. It starts with the ESCRT-0 complex, which recognizes and sequesters ubiquitinated cargo proteins in the endosomal membrane. ESCRT-I and ESCRT-II further sort these proteins into the budding vesicle within the endosome. ESCRT-II also helps in the assembly of the ESCRT-III complex. ESCRT-III is responsible for the scission of the vesicle from the endosomal membrane, leading to the formation of intraluminal vesicles (ILVs) within multivesicular bodies (MVBs). The MVBs either fuse with lysosomes for degradation or with the plasma membrane to release ILVs as exosomes into the extracellular space (14,15).

•*ESCRT-Independent Pathway*: This pathway relies on lipid components, especially ceramide, generated by the enzyme neutral sphingomyelinase, which induces the budding of ILVs into MVBs. Proteins like tetraspanins (CD63, CD9, CD81) are also involved in this process, facilitating the sorting and packaging of exosomal cargo.

Rab GTPases are crucial for the trafficking of MVBs to the plasma membrane. Proteins like Rab27a and Rab27b mediate the final steps of exosome release. Actin and microtubule networks play a significant role in transporting MVBs within the cell. Moreover, tetraspanin, G1-coupled S1P1 receptors, membrane sphingolipids may have potential role in exosome biogenesis (14,16,17) (**Table 1**).

CLINICAL APPLICATIONS OF EXOSOMES

Exosomes are being explored for a wide range of clinical applications due to their natural role in intercellular

Table 1. Biogenesis pathways of exosomes

Biogenesis Pathway	Key Components	Mechanism
ESCRT-Dependent	ESCRT-0, ESCRT-I, ESCRT-II, ESCRT-III complexes	Involves sorting and scission of vesicles within endosomes leading to ILV formation
ESCRT-Independent	Lipid components (e.g., ceramide), tetraspanins (e.g., CD63, CD9, CD81)	Relies on lipid-induced membrane curvature for ILV formation

ESCRT; Endosomal sorting complex required for transport, ILV; intraluminal vesicles, CD63, CD9, CD81; tetraspanins, MVB; multivesicular body; CRISPR-Cas9; clustered regularly interspaced short palindromic repeats-associated protein 9

communication and ability to carry bioactive molecules (**Table 2**).

1. As a Diagnostic Biomarker

Cancer

Exosomes derived from tumors contain specific proteins and nucleic acids that reflect the tumor's molecular profile. For example, circulating exosomal miRNAs like miR-21 and miR-1246 are potential non-invasive biomarkers for various cancers (18). Epigenetic mechanisms play a crucial role in the functional regulation of gene expression. When these processes become dysregulated, it leads to altered gene function and potentially results in the malignant transformation of cells. Several key mechanisms are involved in epigenetic regulation, including DNA methylation, histone modifications, nucleosome positioning, non-coding RNA molecules, and the expression of specific microRNAs (19,20). Since dysregulated miRNA levels have been found in many malignancies and tumor-derived exosomes reflect the miRNA expression of the original tumor cells, tumor-associated exosomal miRNAs can serve as blood biomarkers, making them suitable for personalized cancer diagnosis and treatment (21).

miR-21: One of the most studied miRNAs, miR-21 is often found in elevated levels in tumor-derived exosomes. Its overexpression is associated with several cancers, including breast, colorectal, and non-small cell lung cancer (NSCLC). In breast cancer, exosomal miR-21 levels correlate with disease stage and tumor size, making it a potential marker for early detection and disease monitoring.

MiR-155: This miRNA is another critical biomarker found in various cancers, including breast and lung cancer. Exosomal miR-155 levels are linked to tumor aggressiveness and metastatic potential, offering insights into disease progression and patient prognosis (22).

MiR-1246: Associated with colorectal cancer, miR-1246 is found at elevated levels in exosomes from CRC patients, particularly in early-stage disease. Its presence in exosomes makes it a promising non-invasive biomarker for early detection (23).

Let-7 Family: The let-7 miRNA family, involved in regulating multiple oncogenes, is often downregulated in NSCLC. Exosomal let-7 levels are reduced in patients with advanced disease, highlighting its potential as a diagnostic marker for early-stage lung cancer (24,25).

Neurodegenerative Diseases

In neurodegenerative diseases like Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), exosomes derived from affected neurons contain specific proteins and RNA molecules that can be used as biomarkers for early diagnosis. Additionally, exosomes, particularly those derived from stem cells, show promise as therapeutic agents due to their ability to cross the blood-brain barrier, deliver therapeutic cargo, and modulate neuroinflammatory and neuroprotective pathways (26).

Increased levels of amyloid-beta (Aβ1–42) in exosomes isolated from Alzheimer's disease (AD) patients, reflecting the pathological process of amyloid aggregation in the brain (27).

Elevated levels of Phospho-T181-tau and Phosphor-S386-tau in exosomes are associated with AD progression and can predict the disease years before clinical onset (28).

Elevated levels of oligomeric and phosphorylated alpha-synuclein in exosomes from Parkinson's disease patients' plasma, serving as a potential biomarker for disease development and progression (29).

Increased levels of TDP-43 (both C-terminal fragments and full-length) in exosomes from Amyotrophic lateral sclerosis (ALS) patients, indicating its potential as a

Table 2. Therapeutic applications of exosomes

Therapeutic Application	Exosome Function
Drug Delivery	Engineered to deliver therapeutic agents (e.g., drugs, proteins, nucleic acids) to target cells
Gene Therapy	Used as carriers for delivering genetic material (e.g., CRISPR-Cas9, miRNAs)
Regenerative Medicine	Deliver proteins, lipids, and nucleic acids to promote tissue repair and healing

biomarker for ALS diagnosis and disease monitoring (30).

2. As a Therapeutic Vehicles

Drug Delivery

Exosomes can be engineered to deliver therapeutic agents, including small molecules, proteins, and nucleic acids, directly to target cells, enhancing treatment efficacy while minimizing side effects. Thus, exosomes offer a useful tool for a wide range of therapeutic applications, including chemotherapy, gene therapy, and photothermal therapy. Furthermore, their inherent ability for homotypic targeting and self-recognition may enhance their potential in personalized medicine (31,32).

Gene Therapy

Exosomes are emerging as powerful tools for gene therapy, providing a biocompatible and efficient means of delivering genetic material to target cells. Gene vectors, which are nucleic acids carrying therapeutic genes or gene-editing tools, face challenges such as degradation and immune response when administered directly. Exosomes, however, offer protection and targeted delivery, making them ideal carriers for gene therapy (33).

CRISPR-Cas9 system: Exosomes have been engineered to deliver the CRISPR-Cas9 system for precise gene editing. For example, exosomes from cancer cells can carry CRISPR-Cas9 components to target and modify genes in tumor cells, enhancing chemosensitivity and reducing tumor growth (34).

AAV Vectors (Adeno-Associated Virus): Exosome-associated AAV vectors (Exo-AAVs) have been developed to improve the delivery efficiency and reduce the immunogenicity of AAV-based gene therapies. These Exo-AAVs have shown promise in treating cardiovascular diseases, brain disorders, and certain cancers by efficiently delivering therapeutic genes to target tissues (33-35).

miR-140 for Cartilage Repair: Exosomes engineered to carry miR-140 have been used in gene therapy for cartilage repair. These exosomes selectively target cartilage tissue, promoting regeneration and repair, making them a promising tool for treating joint diseases (36).

PARP-1 Targeting in cancer therapy: The CRISPR-Cas9 system targeting the PARP-1 gene has been delivered via exosomes to selectively accumulate in ovarian cancer cells. This approach enhances the efficacy of cancer therapies by increasing the sensitivity of tumor cells to chemotherapeutic agents (37).

3. As a Regenerative Medicine

The therapeutic applications of exosomes have gained attention due to their ability to deliver proteins, lipids, and nucleic acids to target cells, thus influencing cellular behavior and promoting healing (38).

Heat Shock Protein 70 (HSP70): HSP70 is involved in stress responses and has been identified in exosomes, where it plays a role in protecting cells from damage and promoting cell survival.

ALG-2-interacting Protein X (ALIX): ALIX is crucial in the biogenesis of exosomes and helps mediate the sorting of specific cargo into these vesicles.

miR-21: This miRNA is often found in exosomes derived from stem cells and is involved in promoting tissue repair and reducing inflammation.

miR-126: Known for its role in angiogenesis, miR-126 in exosomes helps in promoting vascular repair and regeneration.

Glycosylphosphatidylinositol (GPI): GPI-anchored proteins are abundant in exosomes and contribute to the stability and targeting of these vesicles to specific tissues.

Exosomes carry various transcription factors that can influence the gene expression of recipient cells, thereby modulating cellular responses and promoting regeneration.

THERAPEUTIC EXOSOME PLATFORMS

Therapeutic exosome platforms can be broadly classified into naive exosomes and engineered exosomes:

1.Naive exosomes: These are naturally produced by cells without any genetic modification. They inherit the characteristics of their parent cells, which can be advantageous in maintaining low immunogenicity and high biocompatibility. MSC-derived naive exosomes are in clinical trials for treating inflammatory diseases, cardiac conditions, and wound healing.

2. Engineered exosomes: These exosomes are modified either during their biogenesis or after isolation to enhance their therapeutic properties. Engineered exosomes can be loaded with specific therapeutic molecules like siRNA, mRNA, proteins, or drugs using various techniques.

Engineering Exosomes for Therapy

To maximize the therapeutic potential of exosomes, various engineering techniques are employed:

1.Passive loading methods: Sonication and *Electroporation*: These methods temporarily disrupt the exosome membrane, allowing therapeutic molecules to be incorporated.

Freeze-Thaw cycles and extrusion: These physical methods facilitate the loading of bioactive agents by repeatedly freezing and thawing or forcing exosomes through membranes.

2. Active loading methods:

Genetic Engineering: Parental cells are genetically

modified to overexpress therapeutic molecules, which are naturally incorporated into exosomes during their formation.

EXPLOR Technology: This involves using light-sensitive protein interactions to load cargo proteins into exosomes. This method ensures high efficiency and control over the loading process.

CLINICAL TRIALS AND THERAPEUTIC DEVELOPMENT

Several biotech companies are advancing exosome-based therapies through clinical trials:

1. Codiak biosciences:
Oncology: Developing exosome-based siRNA therapies targeting KRAS mutations in pancreatic cancer. Clinical trials are underway to evaluate safety and efficacy.

2. Avalon Globocare:
Regenerative Medicine: Investigating MSC-derived exosomes loaded with miR-185 for treating conditions like Leucopla

SOURCES OF EXOSOMES

Exosomes are derived from various cell types, including but not limited to:

•Mesenchymal Stem Cells (MSCs): MSCs are a major source of exosomes, which are extensively studied for their regenerative and immunomodulatory properties. MSC-derived exosomes have shown promise in treating inflammatory diseases, promoting tissue repair, and delivering therapeutic agents (Table 3).

•Dendritic Cells (DCs): Exosomes from DCs are involved in immune responses and have potential applications in cancer immunotherapy.

•Tumor Cells: Tumor-derived exosomes play a crucial role in cancer progression and metastasis by transferring oncogenic molecules and modulating the tumor microenvironment.

•Other Cells: Exosomes can also be sourced from epithelial cells, neurons, and immune cells, each carrying specific cargo reflective of their origin and functional state.

Amplification of Exosome in Kidney Damage

EVs from damaged podocytes can induce apoptosis and profibrotic responses in tubular cells, promoting CKD progression.

EVs also play roles in tubulointerstitial inflammation and fibrosis by transferring pro-inflammatory miRNAs and proteins. Changes in EV cargo (e.g., miR-29c, miR-200b) can indicate kidney injury, fibrosis, and disease progression. Potential biomarkers identified in urinary EVs include aquaporin 1, fetuin A, ATF3, WT1, CD2AP mRNA, and various miRNAs. Circulating EVs from distant organs (e.g., heart, placenta) can affect kidney function and contribute to multi-organ dysfunction in conditions like pre-eclampsia and autoimmune diseases. MSC-derived EVs have shown potential in promoting kidney regeneration, reducing inflammation, and limiting fibrosis in models of AKI and CKD.

EVs from other sources (e.g., iPSCs, renal cells) also exhibit therapeutic effects by protecting mitochondrial function and reducing oxidative stress.

CONCLUSION

Exosomes represent a significant advancement in our understanding of intercellular communication and offer promising avenues for both diagnostic and therapeutic applications. Their ability to carry and deliver a diverse array of bioactive molecules makes them powerful tools in the fight against various diseases, particularly cancer and cardiovascular conditions. As the field of exosome research continues to expand, their role in drug delivery, gene therapy, and regenerative medicine is becoming increasingly clear. Despite the challenges associated with exosome-based therapies, including the need for standardized isolation and characterization methods, the potential for these nanoscale vesicles to revolutionize personalized medicine is immense. Ongoing research and clinical trials will be critical in translating these potential applications into practical, effective treatments, further solidifying exosomes as a cornerstone of modern medical science.

Limitations

While exosomes hold great promise, there are several limitations and challenges that must be addressed to fully harness their therapeutic potential. One of the major hurdles in exosome research is the lack of standardized protocols for exosome isolation, characterization, and quantification. This lack of uniformity can lead to variations in experimental outcomes and complicates the comparison of results across different studies. The heterogeneity of exosomes, which can vary depending on their cell of origin and the physiological state of the donor cells, poses a challenge in understanding and controlling their biological effects. This complexity also complicates the development of exosome-

Table 3. Therapeutic applications of exosomes

Exosome Source	Key Characteristics
Tumor Cells	Promote angiogenesis, immune evasion, and metastasis
Mesenchymal Stem Cells	Promote tissue repair, reduce inflammation, and modulate immune response
Dendritic Cells	Activate and modulate adaptive immune responses
Other Cells	Reflect the physiological state of their origin and modulate various functions

based therapies. Although exosomes are generally considered to have low immunogenicity, the long-term safety of exosome-based therapies remains to be fully evaluated. There is also a need for more research on the potential unintended effects of exosome administration, particularly in the context of cancer, where exosomes can have both tumor-promoting and tumor-suppressing roles. Efficiently targeting exosomes to specific tissues or cells remains a significant challenge. Although progress has been made in engineering exosomes for targeted delivery, achieving precise and consistent targeting in a clinical setting is still difficult. The production of exosomes at a scale sufficient for therapeutic use presents another limitation. Developing scalable and cost-effective methods for exosome production without compromising their biological activity is crucial for the advancement of exosome-based therapies.

DECLARATIONS

Conflict of interest: None

Informed consent form: Not available

Funding source: No funding was received for the research.

REFERENCES

1. Zhang Y, Bi J, Huang J, Tang Y, Du S, Li P. Exosome: A Review of Its Classification, Isolation Techniques, Storage, Diagnostic and Targeted Therapy Applications. *Int J Nanomedicine*. 2020;15:6917-6934. Published 2020 Sep 22. doi:10.2147/IJN.S264498
2. Li J, Zhang Y, Dong PY, Yang GM, Gurunathan S. A comprehensive review on the composition, biogenesis, purification, and multifunctional role of exosome as delivery vehicles for cancer therapy. *Biomed Pharmacother*. 2023;165:115087. doi:10.1016/j.biopha.2023.115087
3. Rajput A, Varshney A, Bajaj R, Pokharkar V. Exosomes as New Generation Vehicles for Drug Delivery: Biomedical Applications and Future Perspectives. *Molecules*. 2022;27(21):7289. Published 2022 Oct 27. doi:10.3390/molecules27217289
4. Bei Y, Yu P, Cretoiu D, Cretoiu SM, Xiao J. Exosomes-Based Biomarkers for the Prognosis of Cardiovascular Diseases. *Adv Exp Med Biol*. 2017;998:71-88. doi:10.1007/978-981-10-4397-0_5
5. Liu Q, Piao H, Wang Y, Zheng D, Wang W. Circulating exosomes in cardiovascular disease: Novel carriers of biological information. *Biomed Pharmacother*. 2021;135:111148. doi:10.1016/j.biopha.2020.111148
6. Xiong M, Chen Z, Tian J, et al. Exosomes derived from programmed cell death: mechanism and biological significance. *Cell Commun Signal*. 2024;22(1):156. Published 2024 Mar 1. doi:10.1186/s12964-024-01521-0
7. Dai Y, Wang S, Chang S, et al. M2 macrophage-derived exosomes carry microRNA-148a to alleviate myocardial ischemia/reperfusion injury via inhibiting TXNIP and the TLR4/NF-κB/NLRP3 inflammasome signaling pathway. *J Mol Cell Cardiol*. 2020;142:65-79. doi:10.1016/j.yjmcc.2020.02.007
8. Hicklin DJ, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J Clin Oncol*. 2005;23(5):1011-1027. doi:10.1200/JCO.2005.06.081
9. Katoh M. Therapeutics targeting angiogenesis: genetics and epigenetics, extracellular miRNAs and signaling networks (Review). *Int J Mol Med*. 2013;32(4):763-767. doi:10.3892/ijmm.2013.1444
10. Mashouri L, Yousefi H, Aref AR, Ahadi AM, Molaei F, Alahari SK. Exosomes: composition, biogenesis, and mechanisms in cancer metastasis and drug resistance. *Mol Cancer*. 2019;18(1):75. Published 2019 Apr 2. doi:10.1186/s12943-019-0991-5
11. Mastronikolis NS, Kyrodimos E, Spyropoulou D, et al. The Role of Exosomes in Epithelial-to-Mesenchymal Transition and Cell Functional Properties in Head and Neck Cancer. *Cancers (Basel)*. 2023;15(7):2156. Published 2023 Apr 5. doi:10.3390/cancers15072156
12. Wu Y, Fu H, Hao J, et al. Tumor-derived exosomal PD-L1: a new perspective in PD-1/PD-L1 therapy for lung cancer. *Front Immunol*. 2024;15:1342728. Published 2024 Mar 18. doi:10.3389/fimmu.2024.1342728
13. Whiteside TL. Immune modulation of T-cell and NK (natural killer) cell activities by TEXs (tumour-derived exosomes). *Biochem Soc Trans*. 2013;41(1):245-251. doi:10.1042/BST20120265
14. Krylova SV, Feng D. The Machinery of Exosomes: Biogenesis, Release, and Uptake. *Int J Mol Sci*. 2023;24(2):1337. Published 2023 Jan 10. doi:10.3390/ijms24021337
15. Winter V, Hauser MT. Exploring the ESCRTing machinery in eukaryotes. *Trends Plant Sci*. 2006;11(3):115-123. doi:10.1016/j.tplants.2006.01.008
16. Hurwitz SN, Conlon MM, Rider MA, Brownstein NC, Meckes DG Jr. Nanoparticle analysis sheds budding insights into genetic drivers of extracellular vesicle biogenesis. *J Extracell Vesicles*. 2016;5:31295. Published 2016 Jul 13. doi:10.3402/jev.v5.31295
17. Kajimoto T, Okada T, Miya S, Zhang L, Nakamura S. Ongoing activation of sphingosine 1-phosphate receptors mediates maturation of exosomal multivesicular endosomes. *Nat Commun*. 2013;4:2712. doi:10.1038/ncomms3712
18. Lohajová Behulová R, Bugalová A, Bugala J, Struháňanská E, Šafranek M, Juráš I. Circulating exosomal miRNAs as a promising diagnostic biomarker in cancer. *Physiol Res*. 2023;72(S3):S193-S207. doi:10.33549/physiolres.935153
19. Cao J, Yan Q. Cancer Epigenetics, Tumor Immunity, and Immunotherapy. *Trends Cancer*. 2020;6(7):580-592. doi:10.1016/j.trecan.2020.02.003
20. Iorio MV, Croce CM. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review [published correction appears in EMBO Mol Med. 2017 Jun;9(6):852. doi:10.15252/emmm.201707779]. *EMBO Mol Med*. 2012;4(3):143-159. doi:10.1002/emmm.201100209
21. Wang X, Tian L, Lu J, Ng IO. Exosomes and cancer - Diagnostic and prognostic biomarkers and therapeutic vehicle. *Oncogenesis*. 2022;11(1):54. Published 2022 Sep 15. doi:10.1038/s41389-022-00431-5
22. Xue X, Liu Y, Wang Y, et al. MiR-21 and MiR-155 promote non-small cell lung cancer progression by downregulating SOCS1, SOCS6, and PTEN. *Oncotarget*. 2016;7(51):84508-84519. doi:10.18632/oncotarget.13022
23. Wang S, Zeng Y, Zhou JM, et al. MicroRNA-1246 promotes growth and metastasis of colorectal cancer cells involving CCNG2 reduction. *Mol Med Rep*. 2016;13(1):273-280. doi:10.3892/mmr.2015.4557
24. Roush S, Slack FJ. The let-7 family of microRNAs. *Trends Cell Biol*. 2008;18(10):505-516. doi:10.1016/j.tcb.2008.07.007
25. Zhao B, Han H, Chen J, et al. MicroRNA let-7c inhibits migration and invasion of human non-small cell lung cancer by targeting ITGB3 and MAP4K3. *Cancer Lett*. 2014;342(1):43-51. doi:10.1016/j.canlet.2013.08.030
26. Huber CC, Wang H. Pathogenic and therapeutic role of exosomes in neurodegenerative disorders. *Neural Regen Res*. 2024;19(1):75-79. doi:10.4103/1673-5374.375320
27. Perez-Gonzalez R, Gauthier SA, Kumar A, Levy E. The exosome secretory pathway transports amyloid precursor protein carboxyl-terminal fragments from the cell into the brain extracellular space. *J Biol Chem*. 2012;287(51):43108-43115. doi:10.1074/jbc.M112.404467
28. Fiandaca MS, Kapogiannis D, Mapstone M, et al. Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: A case-control study. *Alzheimers Dement*. 2015;11(6):600-7.e1. doi:10.1016/j.jalz.2014.06.008
29. Zheng H, Xie Z, Zhang X, et al. Investigation of α-Synuclein Species in Plasma Exosomes and the Oligomeric and Phosphorylated α-Synuclein as Potential Peripheral Biomarker of Parkinson's Disease. *Neuroscience*. 2021;469:79-90. doi:10.1016/j.neuroscience.2021.06.033
30. Suk TR, Rousseaux MWC. The role of TDP-43 mislocalization in amyotrophic lateral sclerosis. *Mol Neurodegener*. 2020;15(1):45. Published 2020 Aug 15. doi:10.1186/s13024-020-00397-1
31. Baruah H, Sarma A, Basak D, Das M. Exosome: From biology to drug delivery. *Drug Deliv Transl Res*. 2024;14(6):1480-1516. doi:10.1007/s13346-024-01515-y
32. Koh HB, Kim HJ, Kang SW, Yoo TH. Exosome-Based Drug Delivery: Translation from Bench to Clinic. *Pharmaceutics*. 2023;15(8):2042. Published 2023 Jul 29. doi:10.3390/pharmaceutics15082042
33. Duan L, Xu L, Xu X, et al. Exosome-mediated delivery of gene vectors for gene therapy. *Nanoscale*. 2021;13(3):1387-1397. doi:10.1039/d0nr07622h
34. Grant EV. FDA Regulation of Clinical Applications of CRISPR-CAS Gene-Editing Technology. *Food Drug Law J*. 2016;71(4):608-633.
35. Hudry E, Martin C, Gandhi S, et al. Exosome-associated AAV vector as a robust and convenient neuroscience tool [published correction appears in Gene Ther. 2016 Nov;23(11):819. doi: 10.1038/gt.2016.65]. *Gene Ther*. 2016;23(4):380-392. doi:10.1038/gt.2016.11
36. Liang Y, Xu X, Li X, et al. Chondrocyte-Targeted MicroRNA Delivery by Engineered Exosomes toward a Cell-Free Osteoarthritis Therapy [published correction appears in ACS Appl Mater Interfaces. 2021 Dec 15;13(49):59591. doi: 10.1021/acsami.1c21472]. *ACS Appl Mater Interfaces*. 2020;12(33):36938-36947. doi:10.1021/acsami.0c10458
37. Kim SM, Yang Y, Oh SJ, Hong Y, Seo M, Jang M. Cancer-derived exosomes as a delivery platform of CRISPR/Cas9 confer cancer cell tropism-dependent targeting. *J Control Release*. 2017;266:8-16. doi:10.1016/j.jconrel.2017.09.013
38. Muthu S, Bapat A, Jain R, Jeyaraman N, Jeyaraman M. Exosomal therapy-a new frontier in regenerative medicine. *Stem Cell Investig*. 2021;8:7. Published 2021 Apr 2. doi:10.21037/sci-2020-037