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Review article

Exosome Therapy for Cancer Treatment: Drug Delivery Tools

Running Title: Exosome Therapy of Cancer

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Abstract

Exosomes are vesicles that naturally transport molecules between cells and have emerged as promising systems for cancer therapy. Exosomes have a lipid bilayer membrane on their surface that contains glycoproteins, tetraspanins, and receptors. DNA and microRNA (miRNA) are enclosed inside the exosomes. Due to their ability to deliver drugs, proteins, and miRNAs, they offer a selective, stable alternative to traditional cancer treatments, which often have severe side effects. Exosomes can be engineered to target cancer cells more effectively, reducing tumor growth and enhancing immune responses. The method for isolating them is broken down into three main parts: (1) initial steps before isolation that involve obtaining fluids containing exosomes, focusing on protocols for organ explants and cell cultures; (2) The actual process of exosome isolation, which includes various gradient options; and (3) procedures conducted after isolation to assess the purity and quantity of the exosomal fraction. However, challenges like production efficiency and standardization still need to be overcome. Artificial exosomes, which combine the benefits of natural and synthetic systems, are being developed to meet these needs. Future research could focus on optimizing exosome engineering techniques and exploring their applications in various cancer therapies.

Keywords: Exosome, Drug delivery, Cancer Therapy

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Introduction

Exosomes are tiny extracellular vesicles that transport biological molecules such as proteins, microRNAs, and metabolites (1). Despite their small size and low biomolecule expression, their biological functions have recently been elucidated. Nevertheless, exosomes have quickly emerged as promising systems for drug delivery, particularly in cancer therapy. Current anti-tumor drugs often cause severe side effects, underscoring the need for more selective and stable delivery methods (2, 3). Exosomes, whether naturally sourced or synthetically engineered, offer a versatile platform for loading different types of molecules, including small compounds and therapeutic agents (4). Moreover, exosomes can be customized by selecting specific source cells or engineering them with affinity tags, enhancing their adaptability to the complex tumor microenvironment (5, 6).

Innovative cancer treatments are urgently needed to address metastatic cancer, which causes more than 8 million deaths annually worldwide. Exosomes naturally absorbed by cells can efficiently deliver drugs, therapeutic proteins, and microRNAs. As our knowledge of exosome formation, release, and uptake grows, interest in utilizing these vesicles as targeted delivery systems for cancer therapies has also risen (7). Exosome engineering allows for precise control over their contents and migration pathways, showing potential in cancer treatment. Studies using viral and non-viral methods have engineered parent cells to produce modified exosomes or alter exosome content after secretion (8). The results have been promising, demonstrating reduced tumor cell migration and proliferation, improved immune responses, increased cancer cell death, and heightened sensitivity to chemotherapy. However, to fully realize the potential of exosomes in clinical applications, standards for their production, isolation, and characterization must be established (9-11).

Basic Properties of Exosomes

Exosomes are a subset of extracellular vesicles (EVs), nanosized, membrane-bound structures secreted by cells. These EVs contain proteins, lipids, and nucleic acids specific to their cell of origin and can be categorized into exosomes, apoptotic bodies, and microvesicles (12, 13). Exosomes are produced through the endocytic pathway and are typically 40–100 nm in diameter, featuring a lipid bilayer containing various proteins, including heat shock proteins (HSPs), tumor-related genes, and fusion proteins (14, 15). They also carry nucleic acids, including messenger RNA (mRNA), microRNAs (miRNA), and noncoding RNAs, which help regulate gene expression and may play a role in cancer progression. Exosomes facilitate intercellular communication by transferring these molecules between cells, making them an attractive option for drug delivery **Figure 1 (**16-18).

Various cell types produce exosomes and are present in numerous bodily fluids. Mesenchymal stem cell-derived exosomes, which lack certain immune markers, are especially promising for therapeutic applications due to their ability to evade immune detection (20-23).

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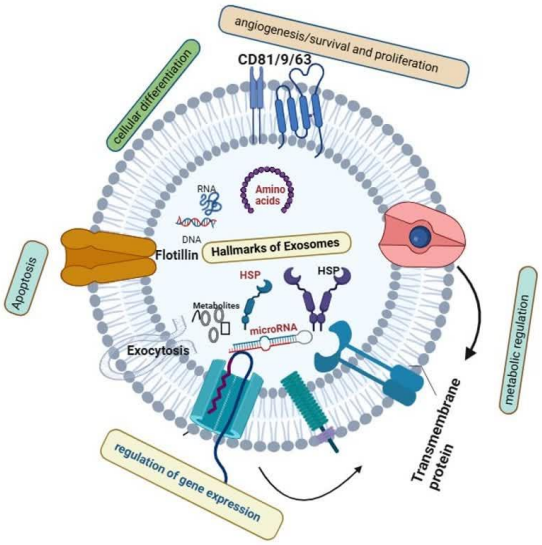
Figure1. Exosomes are extracellular tools that cause intercellular relations by transmission of biomolecules like nucleic acids, proteins, and fats. They are vital in biological pathways and contribute to health and disease conditions (19).



Exosome systems containing drugs have been created using physical techniques, cell engineering approaches, or chemical alterations. Physical encapsulation methods such as electroporation, extrusion, sonication, and freeze/thaw are straightforward techniques for loading high molecular weight cargo. However, these methods can compromise the structure of exosomes and result in lower loading efficiency. This means physical stimulation could potentially damage the exosome carrier, requiring a recovery period. Conversely, chemical modification processes can allow for tuning the exosome-based drug carrier without causing structural damage, enabling precise control.

Nevertheless, avoiding any byproducts and side reactions is crucial to ensure practical application in nanobiotechnology. Exosome-based drug delivery systems can transport various biomolecules through cell engineering techniques. However, this process is intricate, and the roles of different biomolecules must be understood before modifications can be made. Furthermore, encapsulation methods should be tailored to the specific properties of the drug. For instance, hydrophobic drugs can enter exosomes by mixing with them as they interact with the lipid bilayer of the exosomes. Conversely, hydrophilic drugs can not penetrate the lipid bilayer and cannot be encapsulated within exosomes (24).

Drug Delivery Vehicles for Cancer Therapy

Cancer is the second leading cause of death worldwide. Conventional treatments such as chemotherapy have significant side effects, mainly due to their non-selective nature, harming healthy tissues along with cancer cells (25, 26). Therefore, developing drug delivery systems (DDS) that more specifically target cancer cells is crucial for cancer treatment (27-29). Nanotechnology has advanced cancer treatment by creating drug carriers that accumulate in tumors while minimizing toxicity (30-33). However, challenges such as toxicity and poor biocompatibility remain. As natural DDS, exosomes provide an innovative alternative, offering advantages such as immune evasion and efficient cellular entry. Exosomes have also gained attention as drug carriers due to their role in intercellular communication (19, 34, 35). Donor cells can pass on external substances like microRNAs, mRNAs, proteins, and lipids to recipient cells through exosomes (36). Exosomes can enter tissues, spread through the bloodstream, and traverse the blood-brain barrier (BBB) (37). Exosomes help deliver substances in a way that avoids the P-glycoprotein

drug efflux mechanism, potentially lowering drug resistance (38).

Due to the non-specific nature of chemotherapy and the aggressive behavior of triple-negative breast cancer (TNBC), effective treatment strategies are lacking. Exosomes have emerged as promising drug carriers in this area. CD82, a molecule known for inhibiting tumor metastasis, is abundant in exosomes. The aptamer AS1411 selectively targets TNBC cells because of their high nucleolin expression. A "triple-action" exosome-mimetic nanovesicle system has been developed, combining CD82 overexpression, AS1411 conjugation, and doxorubicin (DOX) delivery. CD82 enrichment significantly reduces TNBC cell migration, AS1411 ensures specific targeting of TNBC cells, and DOX effectively suppresses cell proliferation and triggers apoptosis (39).

Nexinhib20 and GW4869 have effectively inhibited RAB27A and neutral sphingomyelinase 2 (nSMase2). Notably, blocking nSMase2 and RAB27A reduced CD9, CD63, and Tsg101 RNA and protein expression levels. A combination therapy using cisplatin or etoposide with GW4869 or Nexinhib20 was tested on small cell lung cancer (SCLC) cell lines. This combined treatment significantly enhanced the effectiveness of first-line chemotherapy against SCLC cells. Additionally, suppressing exosome release with GW4869 and Nexinhib20 markedly decreased cell proliferation and strongly induced apoptosis in SCLC cells (40).

Artificial Exosomes as a Drug Delivery Vehicle

Artificial exosomes have been developed to overcome the challenges of natural exosome production and standardization (41, 42). These artificial vesicles, created using nanobiotechnology, combine natural and synthetic nanoparticles' benefits and show potential for drug delivery applications. Despite these advancements, hurdles such as large-scale production and drug loading remain (24, 43-46). Exosomes derived from lung cancer cells (LCCDEs) have garnered significant attention for their involvement in the development, diagnosis, treatment, and prognosis of lung cancer. LCCDEs can enhance cell growth and spread, influence blood vessel formation, alter immune responses against tumors during lung cancer development, manage drug resistance in treatment, and are now recognized as a key element in liquid biopsy evaluations for lung cancer detection (47). Following a stroke, brain cells can produce and release exosomes that traverse the blood-brain barrier (BBB) and can be found in peripheral blood or cerebrospinal fluid. Therefore, exosomes may be potential biomarkers indicating disease progression and aiding stroke recovery (48). Additionally, exosomes derived from mesenchymal stem cells (MSCs-Exo) retain the physiological functions of MSCs, such as tissue repair and regeneration, reduction of inflammatory responses, and immune system regulation. As a result, MSCs-Exo can serve as a natural drug delivery vehicle with therapeutic benefits and are being increasingly

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utilized in treating neurodegenerative and cardiovascular diseases (49).

Engineering Exosomes for Drug Delivery

Exosomes have advantages over synthetic systems, including their ability to fuse with cell membranes, improving drug delivery. Strategies to target exosomes to tumors include using peptides or antibodies to bind specific receptors on cancer cells (44, 50-52). However, avoiding rapid clearance by the immune system remains a challenge. Potential solutions include modifying exosomes to bypass immune detection or using metalloproteinases to alter exosome contents (53-55). The methods used for isolating exosomes should demonstrate high efficiency and be able to extract exosomes from different sample types. To assess the quality of the isolated exosomes, a range of non-optical and optical methods have been expanded to evaluate their size, size frequency, quantity, morphology, and biochemical composition (56). These include Ultracentrifugation, size, Immunoaffinity capture, precipitation, and microfluidics-based isolation techniques (57).

Enhancing the internalization efficiency of exosomes in tumor cells is crucial. A straightforward and practical approach involves modifying exosome membrane lipids to improve tumor cell uptake, leveraging the role of lipids in the interaction between tumor exosomes and cancer cells. This is achieved by incorporating amphiphilic phosphatidylcholine (PC) molecules into the lipid membrane of reticulocyte-derived exosomes (Exos) through simple incubation, resulting in PC-engineered exosomes (PC-Exos). Studies have shown that PC-Exos significantly increase tumor cell internalization and uptake—up to twice as much as unmodified Exos. When loaded with therapeutic agents, PC-Exos greatly enhance the accumulation of drugs or RNA within cancer cells, leading to improved in vitro anti-tumor effects (58).

Summary and Future Perspective

Exosomes offer significant advantages as drug delivery systems, with low immunogenicity, high safety, and minimal cytotoxicity (59, 60). However, challenges such as standardization, drug loading, and large-scale production must be addressed to realize their full potential. Artificial exosomes could offer scalable solutions, and future developments in this area could revolutionize cancer treatment (60-64). So, exosomes can penetrate different tissues and act as a tool for targeted therapy in different types of diseases.

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completed and revised it. Finally, all of authors read and approved final manuscript.

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