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Review Article

Application of engineered exosomes in tumor therapy

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Abstract: Malignant tumors pose a significant threat to human health, and conventional cancer therapies are limited by inadequate targeting, leading to severe side effects. Exosomes, as extracellular vesicles mediating intercellular communication, exhibit advantages such as low immunogenicity, high biocompatibility, and low toxicity. After modification, engineered exosomes can be employed as targeted delivery vehicles in tumor therapy. This review summarizes the cellular origin, production methods, engineering strategies, and drug-loading routes of engineered exosomes, discusses their applications in cancer treatment, and delves into the challenges and issues in translating engineered exosomes to clinical practice, aiming to provide insights for exosome engineering research.

Keywords: Engineered exosomes, tumor therapy, clinical applications

Introduction

Cancer is a leading cause of mortality, posing a significant threat to human health [1]. Exosomes (EXOs) are nanoscale vesicles secreted by various cell types that encapsulate nucleic acids, proteins, and other cellular contents, playing a crucial role in intercellular communication. Due to their low immunogenicity, stability in blood circulation, high biocompatibility, low cytotoxicity, and strong ability to penetrate the blood-brain barrier, exosomes have been harnessed as carriers for therapeutic molecules in cancer treatment [2, 3]. However, native exosomes are prone to clearance by the body and non-specific uptake by off-target cells, as well as interactions with cellular membranes, thereby lowering their therapeutic efficacy [4, 5]. To address these limitations, bioengineering techniques have been employed to modify exosomes, aiming to enhance their specificity and clinical potential [6, 7]. This review summarizes the current technologies for engineering exosomes and their applications in tumor therapy, while also discussing challenges in translating engineered exosomes into clinical practice.

Production of engineered exosomes

Applicable source cells for engineered exosomes

Exosomes are extracellular vesicles, with cells serving as the “seed” source for their production. Although exosomes can originate from any cell, their quantity, cargo, membrane composition, and biological functions vary depending on the cell type [8]. Consequently, not all cells are suitable for generating engineered exosomes. Hence, cellular properties are carefully considered when selecting cell lines for exosome engineering. Common sources include mesenchymal stem cells (MSCs), tumor cells, human embryonic kidney cells 293 (HEK293), and dendritic cells (DCs).

MSCs are pluripotent stem cells found in various human tissues such as bone marrow, adipose tissue, placenta, and the umbilical cord, exhibiting self-renewal and multipotential differentiation capabilities. Exosomes derived from MSCs are produced in high quantities, immunologically less reactive, display minimal phenotypic changes, and exhibit lower host rejection; thus, MSC-derived exosomes often

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serve as carriers for delivering diverse small molecules, natural compounds, or chemotherapy drugs [9]. Furthermore, MSCs have inherent therapeutic potential, highlighting the potential of MSC exosomes for direct treatment [10]. Tumor cells are widely present in the parenchymal part of tumor tissues and are the main components of tumors. Tumor cell-derived exosomes can enhance tumor growth and metastasis and promote tumor cell drug resistance. In addition, tumor-derived exosomes can present tumor antigens to cytotoxic T cells and trigger the anti-tumor effects of T cells, thus inhibiting tumor growth [3]. The HEK293 cell line, derived from human embryonic kidney cells, is widely employed due to its high transfection efficiency, rapid growth, ease of culturing, and simple handling. Its high exosome production makes it an ideal model for generating drug-loaded exosomes [11]. Notably, some biopharmaceuticals produced using HEK293 cells have received regulatory approval from agencies such as the Food and Drug Administration (FDA) or European Medicines Agency (EMA), confirming the potential of HEK293-derived exosomes for standardized production and clinical application [12]. DCs are antigen-presenting cells that stimulate antigen-specific T-cell immune responses and are involved in both innate and adaptive immunity. Exosomes derived from DCs possess the ability to present antigens and immune-related proteins, thereby triggering anti-tumor immune responses and contributing to tumor regression [13]. Consequently, DC-derived exosomes have been employed in several preclinical and clinical studies for cancer vaccination and treatment [14]. In addition to the aforementioned cell-derived exosomes, a growing number of studies have investigated biofluid-derived vesicles such as milk, plasma, saliva, etc. [15-17], as well as plant-based exosomes (from various vegetables and fruits) [18], showing distinct physiological functions [19].

Production and isolation of exosomes

The yield and cargo composition of exosomes can be controlled by modulating the culture conditions of their parental cells [20]. Therefore, identifying the optimal cultivation parameters for specific cell types and developing efficient isolation and purification techniques play a central role in enhancing exosome production and scalability. In terms of exosome production, exosomes isolated from serum-containing media comprise serum-derived impurities; however, serum-free media may impose significant stress on cells, altering exosome profiles [21]. Consequently, researchers employ serum-free media with alternative supplements or use exosome-depleted sera [22]. Furthermore, treatments such as hypoxia, starvation, oxidative stress, shear stress, heating, and electrical stimulation can impact exosome yields [23]. For instance, hypoxic conditions were found to stimulate the release of exosomes from pancreatic cancer cell lines MiaPaCa and AsPC-1, resulting in a noticeable shift in size distribution towards smaller particles [24]. Researchers have engineered 3D tissues containing stem cells or muscle cells, which were placed in bioreactors under flow stimulation or mechanical stretching. The findings revealed that mechanical forces significantly enhance cellular exosome production compared to static conditions [25]. For cell cultivation, common vessel types include multi-layer culture flasks, bioreactors, and hollow fiber bags [18]. For small-scale cultures, shake flasks, rotators, drums, wave bags, or bioreactors can be employed, whereas larger-scale cultures often utilize stainless steel bioreactors or platform shakers. Moreover, the choice of cell culture container also influences exosome output [26, 27].

Considering the diverse origins of exosomes, raw materials are often provided in bodily fluids or culture media, containing impurities such as nucleic acids and proteins that need to be purified. Common isolation techniques include ultracentrifugation, ultrafiltration, polymer precipitation, immunoprecipitation, size exclusion chromatography, and microfluidic separation [28]. Each method has advantages and drawbacks, leading to variations in exosome purity, yield, and physical-chemical properties depending on the chosen approach. A summary of this aspect is presented in **Table 1**. Following isolation, exosomes must be characterized to assess their purity, which is typically performed by methods like particle size and count determination (using transmission electron microscopy, nanoparticle tracking analysis, or imaging flow cytometry) or detection of specific surface markers and contents (immunoblotting, enzyme-linked immunosorbent assays, or polymerase chain reaction) [29]. Additionally, high-

Table 1. Exosome isolation techniques

Techniques	Principle	Advantages	Disadvantages	Ref.
Ultracentrifugation	Based on exosome density and size	High purity and homogeneity Suitable for large-scale exosome preparation Minimizing cross-contamination	More time-consuming Expensive instruments Not suitable for small volume	[19, 29]
Ultrafiltration	Based on exosome size	Fast and easy to operate	Low purity Reduced yield by filter clogging	[18, 19, 29]
Precipitation	Polymerenrichment	Easy to operate and high yield	Low purity and recovery rates	[18, 28, 29]
Immunoaffinity capture	Based on the specific binding of antibodies	Strong specificity High sensitivity, purity and yield little influence on exosome membrane structure	Harsh storage conditions More time-consuming	[6, 19, 28]
Size-exclusion chromatography	Based on exosome size	Complete exosome structure Uniform size Biological characteristics remain unaffected	Possibly mixed with particles of similar size Complex procedure	[18, 28]
Microfluidics	Based on the physicochemical and biological properties of exosomes, combined with acoustic, size, and immunoaffinity isolation	Fast and easy to operate High yield	Lack of large-scale testing No standardisation Low sample volume	[6, 19, 28]

throughput technologies such as next-generation sequencing and mass spectrometry are employed for comprehensive analysis of exosome composition and structure [30].

Engineering modifications of exosomes

Most unmodified exosomes exhibit weak targeting capabilities and are easily engulfed by lysosomes upon entry into the body, restricting their application as gene-delivery vehicles in both clinical settings and research. To overcome these limitations, exosomes are often subjected to genetic engineering, chemical modification, or membrane fusion techniques for functional enhancement and improved stability [31].

The exosome membrane is composed of transmembrane proteins such as Lamp1 and GPI, and tetraspanins like CD63, CD9, and CD81 [32, 33]. Genetic engineering enables the fusion of targeted ligands with selected exosomal membrane proteins in plasmids, enhancing the targeting capacity of secreted exosomes when transfected into parent cells [34]. Alternatively, the protein composition and expression on the exosomal membrane can be modulated by inserting targeted epitopes into specific protein domains [35]. For instance, a study employed genetic engineering to fuse chondrocyte-affinity peptides (CAP) with the exosome surface protein Lamp2b, followed by transfection into dendritic cells. This resulted in exosomes effectively delivering microRNA-140 to chondrocytes and alleviating osteoarthritis progression in rat models [36]. However, this strategy involves intricate manipulations of the parent cells, which can be time-consuming and challenging. Moreover, introducing targeted groups onto the exosomal membrane may potentially disrupt the normal function of membrane proteins [35].

Chemical modification refers to the process of attaching target ligands to the exosome surface after the isolation step, which is performed either through bioconjugation reactions or lipid assembly [35]. Click chemistry is a biorthogonal conjugation technique enabling targeted ligands to covalently attach to exosome surfaces by converting the amine groups on exosomal membrane proteins to alkynes, which then react with azide-functionalized ligands via copper-catalyzed azide-alkyne cycloaddition (CuAAC) [37]. This method enhances exosome targeting without altering their size, internalization, or properties. However, the lack of site-specific control in alkynyl modifications can potentially impact exosomal protein function [38]. Alternatively, lipids or amphipathic molecules can be inserted into the exosomal lipid bilayer by self-assembly, followed by tethering of the target ligand to the surface. For instance, researchers have developed diacyl lipid-DNA aptamer (sgc 8) conjugates for cancer cell-specific therapy [39].

Furthermore, membrane fusion technology is a method that induces exosomes to fuse with other carrier membranes to generate hybrid exosomes through repeated freeze-thaw cycles, extrusion, ultrasound, polyethylene glycol (PEG) modification, etc. [40]. This approach is not limited to natural biological membranes but also facilitates the interaction between exosomes and liposomes. Payload capacity is enhanced by incorporating functional target ligands while maintaining efficient delivery of larger biomolecules, without compromising the exosomal membrane integrity [41]. For instance, hybrid exosomes formed by repeated freeze-thaw cycles between liposomes and exosomes can accomplish targeted delivery and expression of the desired product [42]. Moreover, fusing the membranes of two parent cells generates mixed cells that secrete exosomes with dual characteristics [43].

Exosome-mediated drug delivery

Exosomes can encapsulate various therapeutic molecules, including drugs, proteins, and nucleic acids [44]. Loading methods are typically classified into pre-loading and post-loading (**Figure 1**). Pre-loading refers to the manipulation of parent cells before exosome production, ensuring that drugs are encapsulated within the vesicles during their formation. This can be achieved by transfecting the cells with the drug or therapeutic molecules, which are then co-cultured directly with the medication, or genetically engineering the parent cells [45]. Consequently, the generated exosomes already contain the drug. Pre-loading methods help maintain exosome membrane integrity and stability; however, they are often complex, time-consuming, and challenging to control for precise drug-loading efficiency [35].

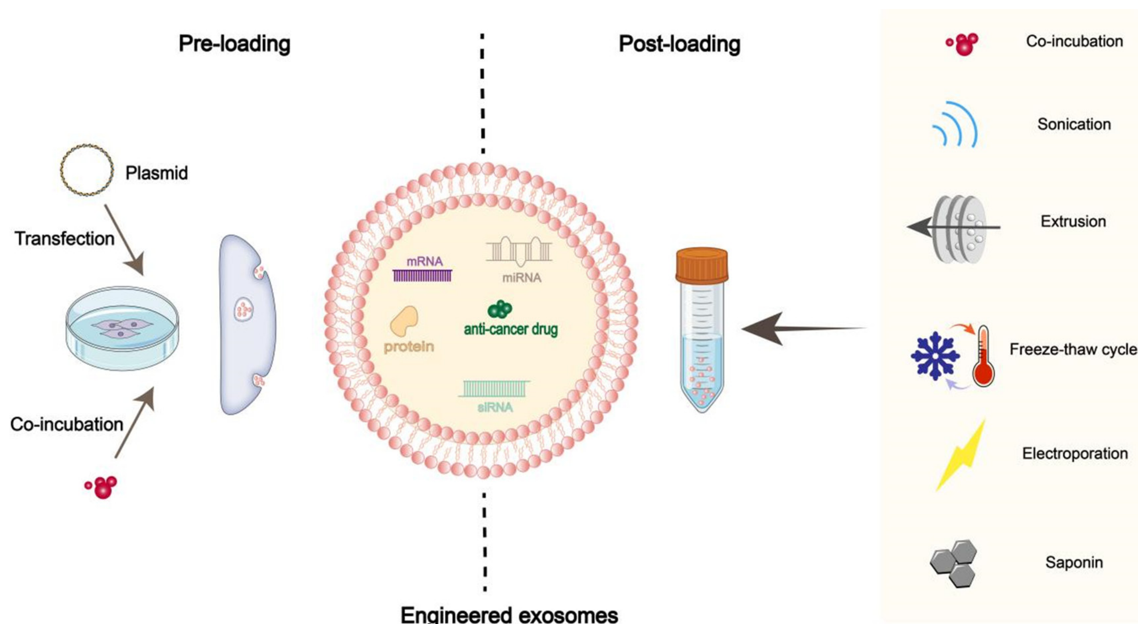

Figure 1. Loading methods of exosome: (1) Pre-loading: before exosome isolation. (2) Post-loading: after exosome isolation.

Post-loading refers to the passive or active loading of drugs onto exosomes produced by parent cells. Lipophilic drugs can be co-cultured directly with exosomes, allowing drug diffusion along concentration gradients, achieving drug encapsulation with minimal impact on exosome integrity; however, this method has limited efficiency due to the reliance on drug lipophilicity and concentration gradient [34, 44]. For hydrophilic drugs and large molecules, active encapsulation methods are often employed, such as physical or chemical modification of the lipid bilayer's hydrophobicity using techniques like ultrasound, extrusion, freeze-thaw cycles, or electroporation [46-48]. Alternatively, pores can be made in the lipid membrane using transfection reagents or surfactants like saponin, enabling the entry of non-permeable hydrophilic drugs or molecules into exosomes [49]. Additionally, exosomes can be modified by optogenetic reversible protein-protein interactions, controlling drug loading and release via light exposure [50].

Furthermore, liposomes can be conjugated with exosomes. The potential of liposomes as nanocarriers has been extensively tested in clinical trials, but their lack of bioactive signaling proteins in the lipid membrane limits their therapeutic potential and may cause toxicity [51]. Despite the lower yield and payload capacity of exosomes, they exhibit excellent cell targeting and low toxicity, underscoring their potential for drug delivery when combined with liposomes [52]. For instance, Lin et al. developed a hybrid exosome-liposome nanoparticle system that effectively encapsulates large plasmids, including CRISPR-Cas9 expression vectors, where they are taken up and expressed by MSCs [42].

Application of engineered exosomes in tumor therapy

Transport of anticancer drugs

Exosomes, as native endogenous carriers, have low immunogenicity and low toxicity and can penetrate the blood-brain barrier. Engineered exosomes can be harnessed for delivering anti-cancer drugs, specifically targeting small molecules, nucleic acids, and proteins used in cancer therapy. Small molecules such as paclitaxel and doxorubicin can be loaded into exosomes for tumor therapy, and engineering techniques can enhance drug targeting, minimize metabolic degradation before reaching the target site, and improve delivery efficiency, thereby reducing toxicity and drug resistance [53]. For instance, Zheng et al. engineered exosomes derived from T cells express-

ing chimeric antigen receptors (CARs). The CAR-Exos conferred tumor-targeting ability and encapsulated the chemotherapy drug paclitaxel (PTX) to form PTX@CAR-Exos. Upon inhalation by lung cancer modeled mice, the modified drug-loaded exosomes successfully delivered PTX specifically to the tumor tissue, resulting in significant tumor volume reduction and extended survival, with lower toxicity to the mice; these findings demonstrate immense potential for cancer treatment [54].

Noncoding RNAs (ncRNAs), including long noncoding RNAs (lncRNAs), microRNAs (miRNAs), and circular RNA (circRNA), are involved in key cellular biological and pathological events, and changes in their expression are directly or indirectly associated with cancer progression. Many studies have mentioned the important role of ncRNA in cancer prevention and treatment, making it an interesting topic for cancer therapy [30]. However, due to some unstable physicochemical characteristics, they are easily cleared in circulating blood and have a difficult time entering target cells. In contrast, the delivery of ncRNAs by exosomes can prevent them from being broken down in vivo and achieve better therapeutic effects [3]. For instance, researchers employed miR-199a-laden lentiviral particles and puromycin selection to engineer miR-199a-modified adipose mesenchymal stem cells (AMSC-199a), from which miR-199a-encapsulated exosomes (AMSC-Exo-199a) were isolated. Experimentally, AMSC-Exo-199a facilitated the efficient transfer of miR-199a to hepatocellular carcinoma cells, significantly enhancing their sensitivity to doxorubicin in vitro and potentiating anti-tumor effects of the drug in vivo [55]. Similarly, Tao et al. employed ultrasound to load bcl-2 siRNA into exosomes (exosiBcl-2) for the treatment of gastrointestinal tumors. The results revealed that exosiBcl-2 effectively penetrated cell membranes, downregulated the expression of genes involved in tumor metastasis, induced apoptosis, and significantly inhibited tumor cell migration and invasion [56].

Exosomes, as therapeutic delivery vehicles for tumors, are not limited to carrying small molecules and RNA; they can also transport therapeutic macromolecules such as peptides and proteins [57]. Exosomes naturally participate in protein transport and shuttling. Therefore, utilizing exosomes for protein delivery can minimize their impact on biological activity while bypassing potential degradation by enzymes and the immune system [58]. In a previous study, exosomes were employed to facilitate targeted therapies with large biomolecules such as catalase (for hydrogen peroxide), superoxide dismutase, proteasomes, transferrin, and lactoferrin [59]. For instance, exosomes loaded with the antioxidant protein catalase were successfully delivered across the blood-brain barrier for Parkinson's disease treatment [60].

As biomarkers for tumor diagnosis

Exosomes are stably present in most biofluids, including plasma, urine, saliva, and milk [61], as they share similar membrane components and cargo to their secreting cells. The alterations in their contents can reflect the type and status of the source cell [62]. Consequently, exosomes can be isolated and purified from liquid biopsies, and analyzing their characteristic proteins or miRNAs may indicate the pathological or physiological state of the originating cells. Hence, exosomes may serve as biomarkers for early cancer diagnosis, therapeutic response assessment, prognosis, and disease progression [63-65].

As previously mentioned, exosomes contain a variety of proteins that bear the signature of their originating cells, which can be identified by immune assays and protein blot analysis [19]. For instance, Niu et al. isolated exosomes from sera of both healthy individuals and non-small cell lung cancer (NSCLC) patients; immunoblotting revealed significantly higher levels of alpha-2-HS glycoprotein (AHSG) and extracellular matrix protein 1 (ECM1) in NSCLC patients compared to healthy controls [66]. This study suggests that serum-derived exosomes harbor specific proteins with potential diagnostic and prognostic value for NSCLC, offering new strategies for tumor diagnosis and therapy. Moreover, differential expression of miRNAs in exosomes can serve as a basis for cancer diagnosis. Zhou et al. analyzed exosomal miRNAs from the plasma of both healthy individuals and endometrial cancer (EC) patients, indicating that EC patients had significantly higher miR-15a-5p, miR-106b-5p, and miR-107 levels compared to healthy subjects. Moreover, plasma-

derived exosomal miR-15a-5p was identified as a valuable biomarker for early detection and diagnosis of EC [67].

Exosome-delivered tumor antigens

Exosomes derived from antigen-presenting cells (APCs) and tumor cells can carry and present related complexes; this process represents a potential pathway to modulate immune responses for therapeutic purposes in cancer [68]. For instance, tumor-derived exosomes harbor a multitude of biomolecules, such as MHC class I and II molecules, co-stimulatory molecules, and various cancer-specific antigens, which are derived from the tumor cells [19]. Consequently, these exosomes can present tumor antigens to cytotoxic T cells, thereby activating their anti-tumor function and inhibiting tumor growth [3]. However, native tumor-derived exosomes may inadvertently induce immune cell suppression and tolerance, posing challenges in harnessing their full potential for cancer therapy. Evasion of the immune system hampers its application in cancer therapy [6]. To address this, researchers have engineered tumor-derived exosomes, modifying them into anti-tumor vaccines that can be presented to immune cells as antigens. The tumor-derived exosomes display enhanced anti-tumor immunogenicity and reduced tumorigenic effects, enabling long-term immunotherapy [69]. Additionally, exosomes can be combined with immune cells. Wang et al. introduced isolated nuclear components from tumor cells into activated M1-like macrophages to create chimeric exosomes (aMT-exos); these aMT-exos effectively infiltrated lymph nodes and stimulated T-cell activation through both classical antigen-presenting cell-induced immune stimulation and a unique "direct exosome interaction". This strategy holds promise for novel approaches in cancer immunotherapy [70].

Altering the tumor microenvironment

The tumor microenvironment (TME) serves as the "soil" for tumor cells and provides essential nutrients and material support for tumor growth and metastasis. The TME is composed of stromal components surrounding the tumor (such as cancer-associated fibroblasts, adipocytes, and extracellular matrix components like collagen, fibronectin, and laminin) and infiltrating immune cells (including lymphocytes, natural killer cells, and tumor-associated macrophages). The TME actively sustains and influences tumor progression and therapeutic outcomes [71]. Exosomes, as crucial mediators of cell-to-cell communication, play a pivotal role in modulating the TME [72]. Cancer-associated fibroblasts (CAF) are key components of the tumor stroma and can receive cargo from tumor-derived exosomes, which activate cellular signaling pathways, alter their biological functions, and reprogram energy metabolism [73]. These exosomes stimulate angiogenesis and lymphangiogenesis at the primary tumor site, facilitating metastasis [74, 75]. Moreover, tumor-derived exosomes can suppress the proliferation of CD8+ and CD4+ T cells, contributing to the establishment of an immunosuppressive microenvironment [76]. Considering the significant impact of exosomes on the TME, exosome engineering has emerged as a promising strategy for tumor therapy. For instance, researchers have successfully reprogrammed M2-polarized tumor-associated macrophages (TAMs) into an anti-tumor M1 phenotype, reversing the immunosuppressive TME [77]. Another target for exosome-based TME remodeling is fibroblast activation protein-α (FAP). Hu et al. developed tumor-derived exosome-like nanocapsules (EnVS-FAP) targeting the FAP gene. EnVS-FAP induces specific cytotoxic T lymphocyte (CTL) responses, thereby reshaping the immunosuppressive TME to inhibit tumor growth [78].

Challenges and issues in the clinical translation of engineered exosomes

In contrast to synthetic vectors that can be mass-produced, exosome release is a natural process. Despite the significant advancements in exosome isolation and purification, large-scale production remains a significant challenge, hindering the clinical application of engineered exosomes. The clinical translation of exosomes necessitates the development of high-yielding, user-friendly, time-efficient, and standardized techniques for large-scale production and purification, as well as robust quality control standards and stable storage methods. However, existing methods fall short of meeting industrial production requirements.

The industrial production of exosomes begins with the cultivation medium containing exo-

somes. Parent cells need to be carefully selected based on their exosome yield, activity, targeting potential, and potential immunogenicity or oncogenicity. However, the optimal cell types yielding high quantities of exosomes with optimal safety remain unelucidated. The type of culture medium significantly impacts exosome production; serum-containing complete media, though rich in exosomes, are unsuitable for large-scale production due to contamination by heterologous exosomes. Serum-free media may provide a hostile environment for cells, whereas serum-depleted alternatives, although nutritionally adequate, struggle to completely eliminate exosomes, and even trace amounts result in product contamination [79]. Considering the clinical applications, animal-derived components like fetal bovine serum should be avoided. Some researchers have explored using human platelet lysate (hPL) as a serum substitute [80], but this carries the risk of pathogen contamination, and inter-donor variability inherent in alternative supplements introduces batch-to-batch inconsistencies that hinder standardization. In addition to the medium composition, the cellular microenvironment also plays a significant role; for instance, controlling factors such as temperature, oxygen concentration, and shear stress in bioreactors can regulate exosome yield and quality [22, 79]. However, the ideal environment for producing exosomes tailored to specific therapeutic targets has not yet been established.

The cell culture supernatant containing exosomes is harvested and is then subjected to isolation, purification, and characterization of exosomes. Numerous exosome separation techniques have been developed, but none can individually isolate large quantities of highly purified exosomes due to their heterogeneity and complexity [56]. Furthermore, these methods fail to differentiate exosome subpopulations, which may significantly impact clinical treatment outcomes [81]. Currently, relatively pure exosomes can only be achieved through a combination of multiple techniques, which involve laborious procedures and high costs, hindering large-scale production. Therefore, identifying and developing more straightforward isolation, purification, and quantification techniques to reduce costs and increase yields is essential in the clinical translation process.

Due to their natural origin in cells, the culturing conditions and manufacturing processes can significantly impact the functionality and biological properties of exosomes. Consequently, quality control measures are essential during exosome production, involving size assessments, contamination removal, detection of functional markers, and drug-loading capacity [82]. Exosomes are recommended to be stored at -80°C, but studies have shown that this may alter their biological activity [83]. Moreover, the associated high transportation costs make it unsuitable for large-scale industrial production. Some research suggests that freeze-dried exosomes can be stored at room temperature without compromising their functional characteristics [84]; however, further validation is needed to determine their clinical applicability in the future.

Conclusion

Engineered exosomes have been harnessed in research across various domains, including cancer therapy, showcasing promising therapeutic potential. As carriers, exosomes can be designed for targeted delivery. Acting as biomarkers, they provide abundant, stable, sensitive, and specific biological information. Moreover, they contribute to reprogramming tumor behavior and remodeling the tumor microenvironment, presenting novel strategies for cancer treatment. However, the application of engineered exosomes in clinical practice still faces numerous challenges. These include identifying effective sources and modification strategies to enhance their anti-tumor capabilities, streamlining and improving exosome production techniques to reduce costs and increase yields, and establishing standardized systems and guidelines for industrial-scale application. Achieving these goals requires substantial technological infrastructure, skilled personnel, and a robust quality management system, all of which necessitate collaborative efforts from diverse fields such as basic science, clinical medicine, and technology development.

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Disclosure of conflict of interest

None.

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