Exosome Drug Delivery System in Cancer Treatment

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Abstract. Exosomes have garnered widespread attention in the field of cancer drug delivery due to their natural biocompatibility and targeting potential. Traditional chemotherapy methods face issues such as high toxicity and poor targeting, necessitating the development of more efficient delivery systems. In recent years, research has focused on drug loading strategies, surface modification techniques, and in vivo behavior evaluation of exosomes. However, technical challenges persist in terms of loading efficiency, targeting specificity, and safety. This article systematically analyzes the research progress of exosome drug delivery systems in three aspects: drug loading, tumor targeting efficiency, and pharmacokinetic and safety evaluation. It points out that current loading methods, such as electroporation and click chemistry, can enhance drug loading efficiency but suffer from issues such as membrane structure damage or unstable efficiency. Targeting strategies rely on parental cell sources and surface modification, making it difficult to achieve efficient and specific delivery. Additionally, animal models are difficult to fully simulate the human environment, affecting the reliability of preclinical data. To further optimize exosome drug delivery systems, theoretical basis should be provided by conducting in-depth research on humanized models, establishing standardized evaluation criteria, and carrying out a series of work centered around quality control and clinical translation, enabling them to play a greater role in precision cancer treatment.

Keywords: Exosomes, cancer treatment, drug delivery system, tumor targeting, pharmacokinetics.

1. Introduction

The current cancer treatment methods primarily encompass chemotherapy, radiotherapy, immunotherapy, and targeted therapy. However, these treatments all exhibit significant drawbacks, including substantial systemic toxicity, poor tumor targeting, and a high propensity for drug resistance. Traditional chemotherapy drugs exhibit low bioavailability and lack tissue selectivity, causing damage to normal tissues alongside tumor cells, leading to a range of adverse reactions. Furthermore, biological barriers such as the blood-brain barrier and the dense stromal structure within tumor tissues further hinder drug accumulation at the lesion site, thereby affecting treatment efficacy. Consequently, the development of efficient, low-toxic, and well-targeted drug delivery systems has become a focal point of current research.

Exosomes are extracellular vesicles with a membrane structure secreted by endogenous cells. They contain various biological macromolecules (including proteins, nucleic acids, phospholipids, etc.) and possess multiple biological functions. Exosomes can serve as one of the important modes of intercellular interaction by mediating the transfer of substances and signals between cells, and are considered to be one of the most promising drug carrier systems [1]. Current research primarily focuses on optimizing exosome drug loading technology, enhancing targeted delivery efficiency, and evaluating in vivo pharmacokinetics and safety. Despite the outstanding advantages of these systems in terms of biosafety and tissue penetration, they still encounter challenges such as fluctuating loading efficiency, complex production processes, and the absence of a standardized evaluation system.

In recent years, researchers have continuously improved the performance of exosome drug delivery systems through various strategies. In terms of drug loading, besides traditional pre-loading and post-loading methods, click chemistry and incubation methods can effectively enhance the loading efficiency [2]; In terms of targeted regulation, surface engineering modifications of specific ligands and environmental responsive elements have effectively enhanced the accumulation and penetration ability of exosomes at tumor sites [3]. In terms of safety assessment, computational models and multi-level experimental systems are combined to systematically evaluate their

immunogenicity and potential toxicity. These advancements provide important support for the clinical translation of exosome-based drug delivery systems.

This article aims to systematically review the latest research progress of exosomes as drug carriers, focusing on three aspects: optimization of drug loading strategies, improvement of tumor targeting efficiency, and pharmacokinetic and safety evaluation. It also points out the challenges faced by current technologies and future development directions.

2. Optimization of Extracellular Vesicle Drug Loading Technology

2.1. Introduction to Exosomes

Exosomes are a kind of extracellular vesicles with biofilm structure secreted by endogenous cells in the body. The particle size is generally between 30-150nm, which can be produced by different types of cells and has the biological characteristics related to the source cells.

Exosomes have a classic phospholipid bilayer structure, which can carry proteins, nucleic acids (miRNA and lncrna), lipids and other bioactive macromolecules to mediate the transmission of information between cells. Exosomes' surface adhesion proteins (tetrapeptide, integrin, CD11b/CD18 receptor, etc.) or specific ligands can promote exosomes to recognize target cells, and transport functional loads contained in exosomes to receptor cells through membrane fusion to function, and regulate various physiological or pathological processes [2].

2.2. Overview of Existing loading Methods

In the development of drug delivery system based on exosomes, the effective encapsulation of therapeutic agents into exosomes is still one of the most significant technical obstacles. The current loading methods can be roughly divided into two categories: one is preloading (in the process of exosome biogenesis); The second is post loading (after exosome separation). The former method can encapsulate the target drug into exosomes at the same time in the process of exosome secretion through the modification of exosome derived cells. This method is simple to operate, but it may weaken the packaging ability of exosomes to a certain extent, and also cause the disorder of exosome biosynthesis pathway. After the exosomes are separated and purified, the drug is sent into the vesicles by physical or chemical means to complete the drug packaging. Electroporation, ultrasound and mechanical compression are all of this kind [2].

2.3. Challenges and Developments

Preloading strategy often faces the problems of low drug loading efficiency and easy to affect the natural process of exosomes; In the post loading method, electroporation is easy to cause the membrane structure damage and vesicle aggregation of exosomes. Although ultrasound and extrusion can improve the drug loading, it may damage the integrity and biological activity of exosomes. In addition, whether pre loading or post loading, the efficiency is significantly affected by the physical and chemical properties of the drug itself. Generally, hydrophobic drugs are easier to achieve efficient loading than hydrophilic drugs.

In addition to loading efficiency, the stability of drugs in exosome environment is also a major challenge. Some chemotherapeutic drugs are easy to degrade or chemically modify in the intravesicular environment, which affects their therapeutic effect. At the same time, the control of drug release behavior is also crucial: premature leakage during circulation will lead to off target toxicity, while insufficient target release will limit the efficacy.

In recent years, the progress of nanotechnology and bioengineering has promoted the development of hybrid loading strategies. For example, click chemistry can be used to fix the target molecule on the surface of exosomes through covalent bonds. The copper-catalyzed azide-alkyne cycloaddition reaction is one of the typical click chemistry methods. Compared with the traditional cross-linking methods (such as maleimide mercapto), this reaction has a stronger rate and yield, which can achieve precise location modification; And the reaction can be carried out in the aqueous phase, which is

suitable for the modification of biological macromolecules such as protein and nucleic acid. The whole process is gentle and will not affect the structural integrity and biological function of exosomes. Incubation method is relatively common and simple. In this method, drug molecules can be spontaneously embedded into exosomes depending on concentration gradient after CO incubation with exosomes. Incubation method can adjust and improve drug loading efficiency in vesicles according to the nature of drug molecules. Generally speaking, drug molecules with stronger hydrophobicity are more likely to be sucked into vesicles by exosomes through interaction with lipid bilayer on vesicle membrane. However, the loading efficiency of this method is generally not high, especially for large molecules such as miRNA, so it is often used in combination with other loading technologies in practical applications to improve the overall drug loading effect [2].

3. Evaluation of Tumor Targeting Efficiency

3.1. Characteristics and Influencing Factors

The natural "homing" ability of exosomes indicates that they may have potential application value in drug delivery in vitro and in vivo. Exosomes can accurately recognize and bind to specific cells, a process that depends on the cooperation of receptors on their surface and binding proteins and other signaling molecules in the surrounding environment. Therefore, exosomes from different cells can be targeted to move to specific tissues or organs and also be able to absorb co delivered drug carriers. At the same time, they can effectively protect the therapeutic molecules (such as miRNAs) carried by them and reduce their degradation in vivo [1].

Although the homing property of exosomes determines that they can shuttle and localize to tumor cells freely in vivo, the targeting efficiency and specificity of exosomes are far from reaching clinical standards, so they cannot achieve the expected therapeutic effect, and the targeting of exosomes needs to be strengthened from other aspects. Specifically, this "homing" effect of exosomes is significantly affected by their cellular origin to a large extent [2]. However, this advantage is also accompanied by certain risks, especially the potential hidden danger of transferring carcinogenic ingredients. This means that although tumor-derived exosomes have potential application value in targeted therapy, their safety still needs further research and evaluation to ensure its reliability and effectiveness in clinical application.

3.2. Research Status

The targeting ability of exosomes is related to the parental cell type. For example, tumor derived exosomes show better tumor targeting ability, but there are certain potential safety hazards; Exosomes derived from mesenchymal stem cells are relatively safer and reliable, but may not play a practical therapeutic role due to its low targeting efficiency [4].

The abnormal vascular system, high interstitial fluid pressure and dense extracellular matrix in the tumor microenvironment seriously limit the penetration and uniform distribution of exosomes in tumor tissues. In addition, the non-specific clearance of exosomes by the mononuclear phagocytic system (MPS), especially in the liver and spleen, further reduces the number of exosomes reaching the tumor area.

At present, the evaluation of targeting efficiency mostly relies on the in vivo imaging technology after labeling exosomes. This method cannot accurately distinguish the labeled exosomes from the actually delivered therapeutic cargo (refers to the active ingredients that are wrapped or loaded in a delivery system and are responsible for directly producing therapeutic effects), and there is a lack of standardized protocols between different studies, which makes it difficult to directly compare the results.

3.3. Optimization Strategy

The surface modification of exosome carriers can be divided into chemical bonding and genetic engineering. Compared with chemical bonding modification, which is complex and difficult to

control, exosomes can modify parental cells by genetic engineering, thereby improving the delivery ability and targeting specificity. Previous studies have achieved cancer targeting by engineering donor cells to express the transmembrane domain of platelet-derived growth factor receptor fused with ge11 peptide. In RAG2-/-mice, intravenously injected exosomes delivered let7a miRNA into transplanted breast cancer tissues expressing EGFR. The results showed that ge11-let7a-exos obtained by genetic engineering modification had good targeting to breast cancer tissues.

This study mainly analyzes two strategies: one is to construct a dual targeted system that simultaneously displays tumor specific ligands and microenvironment-responsive elements on the surface of extracellular vesicles. For example, PEGylated liposomes prepared based on supercritical assisted technology can prevent liposome particle aggregation due to the repulsive force generated on the PEG surface, and can remain stable in vitro for at least 70 days. Compared with unmodified liposomes, PEGylated liposomes have a drug controlled release time that is at least three times longer, and there is no significant initial burst release, indicating that PEGylated liposomes not only improve the circulation time of drugs in the body, but also enhance the stability of the structure, achieving the goal of controlling drug release [5]; The second is to establish a quantitative tracking system with cargo as the core, which can accurately monitor and evaluate the drug delivery and release process by labeling the extracellular vesicle membrane and encapsulated drug separately. For example, dual labeling with extracellular vesicle membrane dyes (such as PKH67) and drug fluorescent markers (such as DOX-Cy5) can be used to dynamically observe the distribution of extracellular vesicles in animals and the release of drugs at tumor sites using in vivo imaging systems; Further combining high-performance liquid chromatography-mass spectrometry (HPLC-MS) to quantitatively analyze the actual content of drug active ingredients in tumor tissues, in order to validate the targeted delivery efficiency and drug release kinetics at the molecular level [6]. This system can not only distinguish the distribution and metabolic pathways of extracellular vesicle carriers and the drugs they carry in the body, but also evaluate in real-time the true enrichment level and release dynamics of drugs at the lesion site during targeted therapy, providing quantitative theoretical basis for the optimization of delivery systems.

4. Pharmacokinetic and Safety Evaluation

4.1. Extracellular Vesicle Drug Delivery

After encapsulating drugs, the pharmacokinetic (PK) behavior of extracellular vesicles is significantly different from that of free drugs and artificially synthesized nanoparticles. Extracellular vesicles can significantly prolong the circulation time of drugs in the body, which is of great significance for improving drug efficacy and reducing side effects [5]. However, the pharmacokinetic characteristics of extracellular vesicles are influenced by various factors, including their source, isolation process, and administration route, all of which have a significant impact on the pharmacokinetic behavior of extracellular vesicles. The research conducted by Zhang Pingping and others from Nanjing Medical University shows that there are significant differences in protein content and function of human placental mesenchymal stem cell extracellular vesicles extracted under different cultivation methods, such as two-dimensional adherent culture, ultra-low adhesion plate static three-dimensional culture, and microgravity bioreactor dynamic three-dimensional culture. The results of protein mass spectrometry analysis showed that 145 differentially expressed proteins were screened from extracellular vesicles cultured from different dimensions, of which 15 proteins were significantly enriched. These differentially expressed proteins are involved in various biological processes such as cell aging, differentiation, and signal transduction, which in turn affect the biological functions of extracellular vesicles. Research has shown that differences in the origin of extracellular vesicles can lead to variations in the composition of their surface markers and contents, which may affect their distribution, metabolism, and biological effects in vivo [6,7]. In addition, the degree of optimization of the separation process and the choice of administration route will also affect its pharmacokinetic behavior.

4.2. Pharmacokinetic Characteristics

Drugs that bind to extracellular vesicles typically exhibit longer blood circulation half-lives, but there are significant differences between different preparation batches and extracellular vesicle subtypes, making their in vivo behavior difficult to accurately predict, which poses challenges for clinical dose determination and treatment plan design [8].

Extracellular vesicles not only serve as delivery vehicles for drugs, but also possess various biological activities, such as participating in intercellular signaling. Although this activity contributes to functional realization, it may also trigger non targeted immune responses or biological molecule transfer. Although extracellular vesicles themselves have low immunogenicity, multiple injections may still activate the body's immune response, and the active ingredients carried by extracellular vesicles, such as proteins and RNA, pose a risk of metastasis in the body. Therefore, a systematic safety assessment is necessary before clinical application [2]. At present, commonly used animal models are difficult to fully simulate the real physiological environment in the human body, and there are species differences in the composition and interaction of extracellular vesicles with cells, which reduces the reliability of predicting preclinical experimental data. For example, in the experiment of Zhang Pingping et al., when explaining the results of animal experiments, it was also pointed out that the specific mechanism is "not yet clear" and further research is needed to verify the uncertainty of the translation of animal experimental results into clinical practice [7]. At present, a standardized toxicity assessment system applicable to extracellular vesicle therapy drugs has not been established.

4.3. Technical Strategy

To address the shortcomings of the PK variability and toxicity assessment system, this article proposes a multi-level safety assessment system based on existing research: firstly, in vitro experiments are conducted to evaluate the effects of extracellular vesicles on immune cell activation and non-target cell regulation; Secondly, using humanized mouse models (transplanted with human immune systems) for more clinical immunogenicity testing [9]. Finally, establish a high-sensitivity detection method to track non target molecule transfer mediated by extracellular vesicles.

In addition, 'Quality by Design' (QbD) is a concept that integrates modern management thinking into the drug development process, aiming to improve drug quality from the source. The key to implementing this concept lies in first constructing an accurate mathematical model to characterize the interaction between Critical Process Parameters (CPPs) and Critical Quality Attributes (CQAs), and then establishing the design space and supporting quality control system for the process flow. Therefore, the QbD concept can be introduced into the extracellular vesicle production process to identify CQAs closely related to efficacy and safety. By controlling these properties (such as specific membrane protein ratios or lipid compositions) during production, the consistency and controllability of extracellular vesicle drugs can be significantly improved, laying the foundation for future large-scale production and clinical applications [10].

5. Conclusion

This article provides a systematic review of the research progress of extracellular vesicle drug delivery systems in cancer treatment, focusing on three aspects: optimizing drug loading strategies, improving tumor targeting efficiency, and evaluating pharmacokinetics and safety. Research has shown that extracellular vesicles, as endogenous extracellular vesicles, have good biocompatibility, tissue penetration, and natural targeting potential, demonstrating significant advantages in the field of drug delivery. By improving the pre loading and post loading methods, combined with new loading technologies such as click chemistry and incubation, the drug encapsulation efficiency and stability have been effectively improved; The application of surface modification and genetic engineering strategies significantly enhances the enrichment ability of extracellular vesicles at the tumor site; The establishment of a dual labeling and quantitative tracking system enables precise monitoring of drug delivery and release processes. In terms of pharmacokinetics, extracellular vesicles can prolong drug

circulation time, but batch differences and preparation processes still affect the predictability of their in vivo behavior; The introduction of a multi-level safety evaluation system and quality-based design concepts provide quality assurance and risk control measures for clinical translation. Overall, the extracellular vesicle drug delivery system has important application prospects in precision cancer treatment. In the future, the combination of technological optimization and multimodal therapy is expected to further break through existing challenges and promote its clinical application.

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