

Active Targeting Design and Preclinical Progress of Liposome-Based Nanocarriers for Cancer Therapy

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Abstract. Cancer remains a critical threat to human health, yet its clinical treatments are severely hindered by multiple limitations, such as solid tumor physical barriers, poor drug solubility, short in vivo circulation half-life, acquired drug resistance, and non-targeted side effects. These challenges have underscored the urgency of developing advanced drug delivery systems to improve therapeutic efficacy and safety. This paper focuses on actively targeted nanoliposomes as a promising strategy to overcome the drawbacks of traditional cancer therapies and passive targeting liposomes. Unlike passive targeting relying on the EPR effect, actively targeted liposomes are modified with ligands (antibodies, aptamers, peptides, etc.) that specifically bind to overexpressed receptors on tumor cells, thereby enhancing tumor cell recognition, endocytosis, and drug accumulation at lesion sites while reducing normal tissue toxicity. The paper further outlines two major cellular entry pathways of these liposomes (membrane fusion and endocytosis) and highlights that carrier physical properties determine the dominant entry route. Additionally, it summarizes key bottlenecks in clinical translation, including long-term safety validation, blood-brain barrier penetration, antigen/receptor heterogeneity, and large-scale manufacturing, as well as corresponding optimization frameworks for scenario-based delivery, immunoliposome construction and industrialization. This paper provides a comprehensive reference for advancing the clinical application of actively targeted liposomes in cancer therapy.

Keywords: Cancer therapy; liposome; nanoparticles.

1. Introduction

Cancer has now become the second leading cause of death worldwide and has an impact on human health [1]. Currently, the mainstream treatment methods for tumors include surgical removal, radiotherapy, chemotherapy, and immunotherapy [2]. However, these methods still have many practical limitations in clinical application: Firstly, for solid tumors, the existing treatment methods are difficult to effectively penetrate the tumor tissue barrier and achieve the desired therapeutic effect; Secondly, some drugs have poor solubility, which will affect their bioavailability and therapeutic effect; Thirdly, the half-life of chemotherapy drugs in the body is short, making it difficult to maintain an effective therapeutic concentration; Fourthly, tumor cells are prone to developing resistance to treatment, resulting in a gradual decline in therapeutic effect; Fifthly, the targeting of treatment is insufficient, making it difficult to precisely act on the tumor lesion; In addition, there may also be non-targeted side effects throughout the body or locally, causing damage to normal tissues and thereby affecting the tolerance of the treatment and the quality of life of the patient [1]. Due to the numerous defects of traditional cancer treatment methods, researchers are working to develop various nanotechnologies to provide more effective and safer cancer treatment solutions [3].

Liposomal nanoparticles, with their unique structural and functional advantages, have become highly promising carrier systems in the integrated field of cancer therapy and diagnosis [2]. In recent years, liposomal diagnostic and therapeutic technologies have developed rapidly, achieving breakthrough progress in both preclinical studies and clinical cancer models. Notably, hybrid liposomal systems have not only successfully received approval from the U.S. Food and Drug Administration (FDA) but have also entered the stage of commercial application, fully demonstrating their clinical translational value. As a clinically validated mature nanoplatform, liposomes can accurately deliver various therapeutic and imaging agents to targets, including comprehensive

diagnosis and treatment applications for solid tumors, precise treatment under imaging guidance, and multimodal combination therapy strategies. Through flexible screening and combinatorial regulation of diagnostic and therapeutic agents, it is possible to achieve precise customization of the diagnostic and therapeutic characteristics of liposomal engineering systems, further expanding their application boundaries in cancer treatment [4]. Despite the aforementioned advantages, traditional passively targeted liposomes still have limitations due to their delivery mode relying on the pathophysiological characteristics of tumors. To achieve more precise tumor-targeted therapy, actively targeted liposomes have emerged, and their definition, design core, and mechanism of action are as follows.

2. Actively Targeted Liposomes and Their Core Design

2.1. Actively Targeted Liposomes

Actively targeted liposomes refer to carrier systems that are modified on the basis of traditional liposomes by attaching specific ligands to their surfaces, enabling them to recognize and specifically bind to specific receptors on the surface of tumor cells, thereby achieving targeted delivery of drugs to tumor cells. Their basic structure is derived from the natural properties of liposomes. Liposomes are artificial membranes prepared based on the phenomenon that phospholipid molecules can form stable lipid bilayer membranes in an aqueous phase. When a monolayer of lipid molecules spreads on the water surface, the polar ends insert into the water while the nonpolar tails face the air interface [5]. After stirring, an emulsion is formed, that is, clusters of lipid molecules with the polar ends outward and the nonpolar ends inward, also called micelles, or spherical liposomes composed of bilayer lipid molecules (Fig.1). Liposomes can be prepared using single or mixed phospholipids, and different membrane proteins or monoclonal antibodies can also be embedded, so liposomes can achieve targeted effects after processing and modification. In clinical treatments, liposomes show promising application prospects. Active-targeted liposomes, which encapsulate various drugs, enzymes, or other bioactive macromolecules within liposomes and precisely recognize receptors, hold promise for cancer treatment. As shown in Fig.2, targeted liposomes constructed through assembly and bioengineering techniques can integrate different therapeutic functions, providing an implementation path for the integration of cancer diagnosis and treatment (Fig.2).

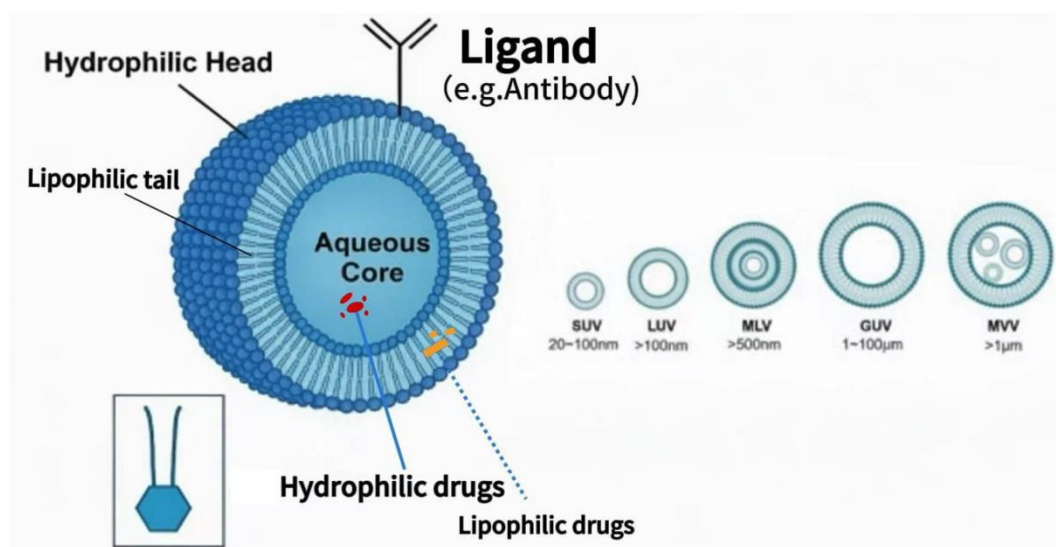


Fig. 1 Schematic diagram of the structure of liposomes [3]

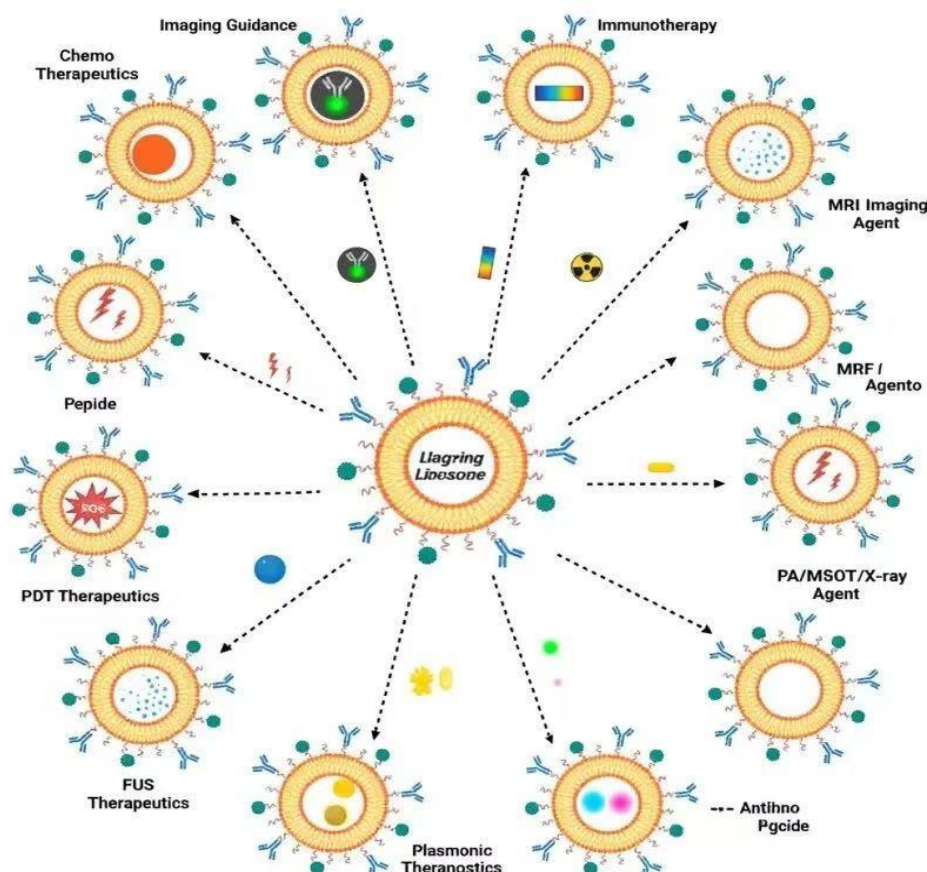


Fig. 2 Construction of Multi-functional Targeted Liposomes and Cancer Diagnosis and Treatment Mechanism Diagram [4]

2.2. Tumor-Specific Expression Characteristics

The passive targeting effect of liposomes arises from the disordered vascular architecture in solid tumors and their lack of functional lymphatic drainage. The combined effect of increased tumor vascular permeability and restricted lymphatic drainage, known as the enhanced permeability and retention (EPR) effect, enables liposomes to passively distribute and accumulate at tumor sites. However, passive targeting relies solely on the pathophysiological characteristics of tumor sites, which may lead to reduced therapeutic efficacy or off-target toxicity. One reason for this clinical inadequacy is that passive-targeting liposomes lack true specificity for tumor cells themselves. This limitation has prompted researchers to explore more precise targeting strategies, such as active targeting. Subsequent studies have demonstrated that active targeting interacts with tumor-specific biomarkers through molecular mechanisms, enabling direct targeting of tumor cells [6]. Active targeting leverages the interaction between liposome surface ligands and tumor cell surface-specific receptors. Tumor cells may overexpress certain receptors such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), folic acid (FA), integrins, CD44, CD13 and prostate-specific membrane antigens. Based on these receptors, specific ligands have been developed for liposome surface modification, including antibodies, nucleic acids (e.g., aptamers), proteins, peptides, small molecules, and carbohydrates [7].

2.3. Process of Liposome Recognizing Specific Receptors and Entering Cancer Cells

Liposomes deliver their drug payloads to cancer cells mainly via two pathways: direct membrane fusion and endocytosis-mediated interactions. In the fusion pathway, the liposome bilayer merges with the plasma membrane, allowing the encapsulated drug to be released relatively quickly into the cytoplasm and partially avoid lysosomal degradation. In contrast, endocytosis involves the cell engulfing the entire liposome into a vesicle, after which the release of the drug depends on

intracellular transport and, in many cases, requires endosomal escape to prevent lysosomal breakdown. Endocytosis itself can occur through multiple routes, with the dominant route influenced by the properties of the liposome, such as size, surface charge, lipid composition, PEGylation, ligand modification, as well as cell type and microenvironment [8]. Importantly, liposome stiffness affects the choice of the primary entry pathway. Softer, more deformable vesicles may achieve closer membrane contact and higher local curvature adaptation, making fusion—besides endocytosis—more favorable (dominant). As shown in Fig. 3, soft liposomes (45 kPa) can enter cancer cells through both fusion and endocytosis, with fusion as the main pathway, which can reduce the risk of drug degradation by lysosomes; while rigid liposomes (19 MPa) can only enter cells through endocytosis. This finding suggests that liposome stiffness can be used as an important regulatory parameter in active targeting design, and synergistically optimize drug delivery efficiency with ligand modification (Fig. 3) [8].

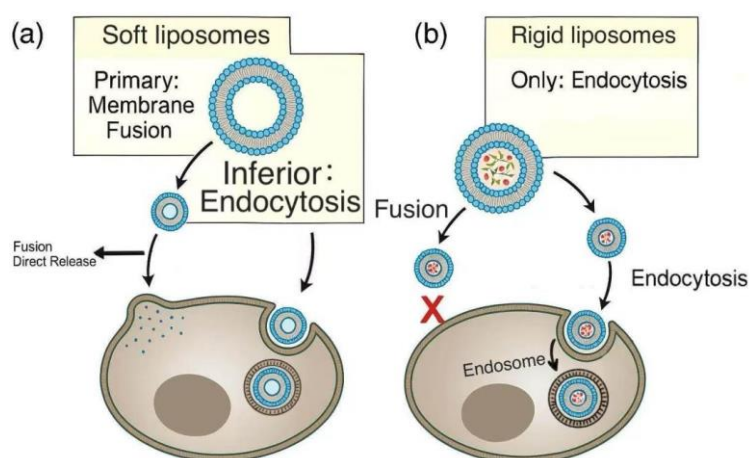


Fig. 3 (a) Soft NLP-45 kPa enters cancer cells primarily through fusion and secondarily via endocytosis. (b) Rigid NLG-19 MPa can only enter cancer cells through endocytosis [8].

3. Challenges and Future Optimization Directions

3.1. Core Challenges in Clinical Translation

Although Liposomal nanoparticles (NPs) have shown significant research value in the field of cancer treatment, their clinical application still faces considerable safety challenges. To ensure patient safety and efficacy, it is necessary to conduct in-depth studies on their pharmacokinetics, in vivo distribution, and long-term safety. In addition, extensive clinical trials need to be carried out in advanced animal models, followed by human trials, to verify their reliability and therapeutic effect in a clinical setting [9].

NPs are also regarded as an effective solution for drug delivery and targeted tumor cell therapy. However, due to the presence of the blood-brain barrier (BBB), delivering drugs to central nervous system cells still faces many challenges. Therefore, the mechanisms for drug delivery to the central nervous system cancer tissues need further investigation. This issue can be partially addressed by developing new approaches and using lipid-based compounds [10].

However, the clinical application of active targeted therapy is further constrained by antigen heterogeneity. Different cancer types, and even the same cancer at varying developmental stages, may exhibit distinct physiological and morphological characteristics, resulting in heterogeneous antigen expression. Receptor density represents another critical factor to consider in active targeted therapy. To achieve clear differentiation, the number of receptors in cancer cells must be significantly higher than those in normal healthy cells [11].

In the production process of active targeting technologies (especially immune liposomes), the main challenge lies in how to scale up the synthesis of nanomaterials. This issue involves two key aspects:

Firstly, how to achieve large-scale production of lipid-ligand complexes; Secondly, how to prepare liposomes with uniform particle size distribution and layered structure using lipid components. The binding of lipid-ligands usually requires multiple steps of synthesis, involving organic solvents. According to the current Good Manufacturing Practice (cGMP) standards, this will increase the complexity and cost of the production process - both in the binding process and the subsequent drug formulation process. It is particularly important to ensure the functional stability of the complexes under various processing conditions, because the introduction of nano-precipitated compounds will change the chemical composition of the nanoparticles, thereby causing uncertainties in their biological distribution, pharmacokinetics and pharmacodynamics [11].

3.2. Key Optimization Strategies

In response to the aforementioned clinical translation challenges, targeted optimization approaches have been developed in current research, which are elaborated below from three core dimensions: blood-brain barrier penetration, antigen recognition accuracy, and large-scale production. For the issue of drug delivery to central nervous system tumors, a reusable optimization framework can be established: in scenarios where the blood-brain barrier can be bypassed, local administration routes with sustained-release effects are preferred; when systemic administration is necessary, a multi-stage delivery system is constructed through targeted enhancement of endocytosis, exogenous on-demand release or endogenous enzyme-triggered release, and the higher specificity, stronger internalization ability, and lower normal tissue toxicity brought by multi-stimulus responses are utilized to improve delivery efficiency[12]. To advance liposome ligand modification technology for specific antigen recognition and construct immunotherapy-effective liposome systems, modifications to the liposomes themselves can serve as a starting point. For instance, Rahman et al. embedded self-assembling chimeric nanobodies (cNPs) into the phospholipid bilayer of liposomes while loading 5-fluorouracil (5FU), creating immunoliposomes for cancer immunotherapy (5FU@cNBLP). The chimeric nanobody consists of a human single transmembrane domain, a flexible peptide linker, and a nanobody targeting human epidermal growth factor receptor 2. The blank immunoliposomes contained 1,2-dimonoacylglycerol-sn-3-phosphocholine (DMPC), DOPE, and cholesterol. To validate the antitumor activity of these immunoliposomes, researchers used SK-BR-3 tumor-bearing mice as models and compared the efficacy of different treatment regimens. Results showed that the 5FU@cNBLP group demonstrated significantly better antitumor effects compared to the 5FU@LP group. This study proposes a biophysical preparation strategy that enables efficient immunotherapy through a one-step liposome preparation method without chemical modifications, offering a valuable reference for designing anti-cancer immunoliposomes [13].

During the transition from laboratory-scale production to industrial-scale production of liposomes, scaling up requires ensuring repeatability, controllability, and compliance with regulatory requirements. The preparation process itself is rather complex and prone to defects, so the process needs to be continuously optimized to maintain the stability of product quality and performance. Regulatory agencies usually require strict and repeatable data to prove the safety, efficacy, and quality of the product, which further increases the difficulty of scaling up. In practical operations, methods that facilitate expansion and homogenization (such as ethanol injection, high-pressure homogenization, combined with ultrasound or extrusion to achieve smaller and more uniform particle size distribution) should be adopted first. At the same time, the control ranges of key process parameters and quality attributes should be clearly defined. Additionally, to improve drug loading efficiency and reduce leakage and batch-to-batch variations, active/passive drug loading methods (such as transmembrane ion gradients or pH gradients) can be used instead or in combination with passive drug loading methods. For example, Doxil uses the transmembrane gradient of ammonium sulfate for active loading, achieving a drug encapsulation efficiency of over 90%, thereby improving the consistency and industrial feasibility of the formulation [14].

4. Conclusion

Active targeting liposomes, which rely on ligand-receptor recognition, outperform passive targeting in achieving cell-specific recognition and endocytosis. This mechanism enhances tumor cell uptake, improves drug exposure at the lesion site, and reduces toxicity to healthy tissues. In clinical translation, active targeting is better suited for integration with image-guided therapies, stimulus-responsive systems and combination treatments. However, to improve the precision and controllability of treatment, challenges such as antigen heterogeneity, patient stratification, long-term safety verification, and large-scale production need to be addressed simultaneously. Future research can focus on the synergistic design of "ligand modification-physical property regulation-multi-stimulus response" and optimize schemes in combination with the individualized characteristics of clinical patients to promote the efficient translation of actively targeted liposomes from basic research to clinical application.

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