Advances in liposome research in the field of antitumor

Xiaochuang Xu*

China Pharmaceutical University, Nanjing 210000, China

Abstract. Liposomes, as biocompatible and safe nanocarriers with easily modified surfaces, can be well used in the field of antitumor. Their targeting properties have contributed to the reduction of drug dosage and non-target cell toxicity. To further exploit the targeting properties of liposomes, passive targeting liposomes, active targeting liposomes and physicochemical targeting liposomes have been constructed by surface modification. This paper summarizes the mechanisms of action of different types of targeted liposomes and describes the specific role of liposomes in overcoming tumor resistance, immunotherapy and helping drugs to cross the blood-brain barrier, and summarizes the current development issues and future directions.

Keywords: Liposomes; antitumor; targeted modifications.

1. Introduction

Since discovered by Bangham in 1965, liposomes have been used to encapsulate and transport molecules for the treatment of various diseases. In recent years, liposomes have received increasing attention as a new biomedical technology and are used in a wide range of applications including anti-tumor drug delivery, gene therapy, and immunotherapy. The use of liposomes for cancer therapy has great advantages, such as good biocompatibility, non-immunogenicity, controlled and slow release, reduced drug dosage and reduced toxicity to normal cells. Currently, four of the five liposomes approved for marketing in China are used in cancer treatment. In these decades, liposomes have undergone development from passively targeted liposomes, to actively targeted liposomes, to environmentally responsive liposomes and dual drug-loaded liposomes, with further refinement of their physicochemical properties, better targeting of tumors and gradual improvement in therapeutic efficiency1. This paper summarizes the characteristics and classification of liposomes with the targeting of liposomes as the core, and also synthesizes the research results in the past five years to introduce the application of liposomes in solving the important and difficult problems in the field of antitumor, hoping to provide theoretical support and reference for the research and development direction of liposomes in the field of antitumor.

2. Basic introduction to liposomes

2.1 Basic structure and characteristics of liposomes

Liposomes are closed vesicles with a phospholipid bilayer that act as a drug delivery system to encapsulate the drug. In aqueous solutions, the phospholipid molecules are inserted into the water with a hydrophilic head and a hydrophobic tail towards the air, which is disturbed to form a regular bilayer structure and connected to form a hollow vesicle structure1. Phospholipid bilayers can be one or more layers and are classified according to the number of layers as single-chambered liposomes, multi-chambered liposomes, and multi-vesicular liposomes. The vesicles of multicompartmental liposomes are concentric and the vesicles of multicapsular liposomes are not concentric3 . Due to the amphiphilic nature of the phospholipid bilayer, liposomes can encapsulate both water-soluble and lipid-soluble compounds. The former is encapsulated in a hydrophilic core enclosed by a hydrophilic head, while the latter is encapsulated in a sandwich formed by a lipophilic tail.

Liposomes have a similar composition to biological membranes, possessing good biocompatibility and biodegradability, which can be controlled by changing the lipid composition4. In the case of drugs, liposome-embedded drugs have increased solubility, stability, bioavailability, reduced toxicity, slow release and some targeting. Accordingly, liposomes are widely used in the treatment of tumors, where they can increase the proportion of drugs reaching the target site, reduce adverse drug reactions and, with modification, further enhance their targeting properties5, which can greatly

* Corresponding author: xuxiaochuang0827@163.com

increase the safety of clinical use. In addition to drugs, liposomes can also be loaded with large molecules such as DNA, RNA, enzymes, as well as antigens and vaccines, all of which are widely used in the anti-tumor field6. In addition, the ability of liposomes to load a variety of small molecules allows for the integration of contrast imaging with therapeutic agents7. This allows for real-time monitoring of therapeutic effects and provides an advantage for oncology treatment.

2.2 Preparation of liposomes

The preparation of liposomes is divided into passive and active drug loading methods depending on how the drug is loaded, as described below.

The passive drug loading technique is to dissolve water-soluble drugs in the aqueous phase and fat-soluble drugs in the organic phase, then use a certain way to mix, so that they form drug-loaded liposomes8. The common methods include the thin film method, reverse evaporation method, injection method, freeze-thaw method, freeze-drying method, compound emulsion method, etc.[1,9] Passive drug loading techniques are suitable for drugs with a good water or lipid solubility, i.e. most of the drugs. The advantages of this method are that it is simple to operate and there are no special additional steps, but the drug loading and encapsulation rate are low and the drug is prone to leakage. The addition of membrane permeation enhancers can promote the equilibrium of drug distribution10.

The active drug loading technique takes advantage of the fact that drug molecules can cross the lipid layer only when they are electrically neutral but not when they are in ionized form. It creates a pH gradient difference between the two sides of the lipid membrane, where the drug molecules are protonated after being transferred into the membrane and stay there[5,11]. The main methods are the pH gradient method, the ammonium sulfate gradient method, the calcium acetate gradient method and the ionic carrier gradient method. The active loading technique is suitable for amphiphilic substances and is characterized by good stability, a high encapsulation rate and a low leakage rate12 . Compared with passive drug delivery techniques, active drug delivery techniques have obvious advantages and have been more studied and applied in recent years.

In addition, new methods such as dual solvent replacement, CO_2 supercritical methods, staggered flow filtration and membrane contactor technology have also been introduced13. However, in the preparation of anti-tumor drugs, traditional methods such as thin film hydration, solvent injection and reverse evaporation are still used the most [14].

3. Classification of liposomes

In order to increase the targeting effect of liposomes and improve the targeting specificity, researchers have developed various surface modification methods to obtain liposomes with different targeting properties, which are classified into passive targeting liposomes, active targeting liposomes and physicochemical targeting liposomes according to their targeting mechanism, see Table 1.

Table 1. Classification of liposomes

Category	Surface Finishing	Targeted receptors	Loaded drugs	Treatment of medical conditions	Role	References
Passive targeting	PEG	-	Dulcimerin	Colon Cancer	Increased cancer cell suppression	15
	PEG	-	ANP0903	Lung Cancer	Effective delivery of drugs into cells, doubling the amount of drugs inside the cells	16
	Single chain PAS	-	-	-	Serum stability comparable to PEGylated liposomes, no "accelerated blood clearance" effect	17
Antibody modifications	Integrin $alpha_v$ $beta_6$ monoclonal antibody	Integrin $alpha_v$ $beta_6$	5-Fluorouracil	Colon Cancer	Promotes accumulation in cancerous tissues and apoptosis of tumour cells	18
	EGFR antibody	EGFR	miR-135a	Gallbladder cancer	Improved targeting and increased ability to target reproduced genes in vitro	19
	Cetuximab	HER1	Bee Toxin, Toadstool	Liver cancer	Killing effect on Sorafenib-resistant hepatocellular carcinoma cell lines with improved safety	20
	EGFR antibody	EGFR	Adriamycin	Glioblastoma	Helps cross the blood-brain barrier	21
	ENG-scFv	TnECs	α-1,3GT	Solid tumours	Increased targeting to overcome accelerated blood clearance	22
	Trastuzumab	HER2	Docetaxel	Breast cancer	Increases anti-value-added efficacy and improves drug delivery efficiency	23
	EGFR antibody, DSPE-PEG2000	EGFR	Irinotecan	Colorectal cancer	Increased targeting and enhanced anti-tumour action with slow release of drug	24

Ligand modifications	ApoB-100 protein	LDL receptors	Adriamycin	-	Increases the concentration of aggregates in tumour tissue and reduces cardiac side effects	25
	Folic acid	Folate receptor	Erythromycin, hypertrigonelline	Acute myeloid leukaemia	Increased targeting and good serum stability	26
	Transferrin	Transferrin receptor	Curcumin	Prostate cancer	Improves uptake of drugs by tumour cells	27
	LT7 peptide	Transferrin receptor	Docetaxel	Liver cancer	Improves drug accumulation and anti-tumour effects at the tumour site	28
	EGF	EGFR	Silver nanoparticles	Lung cancer, tongue cancer	Increasing the number of liposomes in targeted cells	29
	SP94	Liver surface-specific expressed protein	Radicicol	Liver cancer	Increasing the half-life of liposomes in vivo and reducing their non-selective toxicity	30
	AS1411, CTLA-4 aptamer	Nucleolin, CTLA-4 protein	-	Breast cancer, liver cancer, lung cancer, T-lymphocytoma	Enables T-cell redirection to tumour cells and promotes T-cell activation	31
	Galacto-biotin	Desialic acid glycoprotein receptor, biotin receptor	10-Hydroxycomptal	Hepatocellular carcinoma	Entry into hepatocellular carcinoma cells via desialic acid glycoprotein receptor-mediated endocytosis	32
	AS1411 adapters	pro-alpha collagen type I subunit	COL1A1 siRNA	Rectal cancer	Selective uptake in cancer cells via receptor-dependent endocytosis	33
Physical and chemical targeting	Polyethylene glycol (linked by hydrazone bond)	-	Gemcitabine	Pancreatic cancer	Hydrazone bonds break on pH change, enhancing tumour cell uptake and cytotoxicity	34
	Polyethylene glycol long chain	-	Tree Tongue Polysaccharide	-	Increases liposome membrane hydrophilicity, reduces plasma protein-membrane interactions and is rapidly released at 42°C	35
	HER2 antibody	HER2 receptor	Indocyanine Green, Doxorubicin	HER2-positive expressing tumours	Improves the ability of liposomes to internalise and accumulate in tumour cells and trigger drug release under infrared light irradiation	36

3.1 Passive targeting

The Enhanced permeability and retention effect (EPR) of tumor cells allows passive targeting of liposomes, i.e. the special permeability of the lax capillaries formed during tumor growth is favorable for the enrichment of nanosystems such as liposomes37. Ordinary liposomes, consisting only of phospholipids and cholesterol, enter the human circulatory system and are easily recognized by the mononuclear phagocyte system (MPS) and cleared by the reticuloendothelial system, making it difficult to reach target sites outside of the macrophage system. A common approach to this problem is to modify the liposome surface with polyethylene glycol (PEG)38. PEGylation increases the spatial resistance of liposomes, improves their hydrophilicity, particularly by reducing MPS affinity, and prolongs the plasma half-life, hence the term long-circulating liposomes. This is why they are called long-circulating liposomes 39.

3.2 Active targeting

Active targeting is the most selective form of liposome delivery. By modifying ligands with high affinity for receptors, liposomes can bind to specific receptors, thereby increasing their targeting and reducing toxic effects on non-targeted organs or tissues.40 .

3.2.1 Antibody-modified liposomes

Antibodies are one of the first and most commonly used ligands for active targeting modifications of liposomes, also known as immunoliposomes. Compared to other delivery systems, immunoliposomes enable targeted drug delivery by specifically binding to antigens that are overexpressed or specifically expressed on the surface of tumor cells41. They have a higher drug loading capacity, greater affinity and specificity than other delivery systems.

The antibodies used for modification are intact antibodies, monoclonal antibodies, antibody fragments and single chain fragment variable (scFv), which were often used in the past, but were gradually phased out due to their large molecular weight affecting the liposome size and lack of specificity42. Monoclonal antibodies are obtained from hybridized tumor cells and are highly specific, reacting with only one antigenic determinant cluster, showing a strong specificity and a promising future41. Antibody Fab fragments are formed by protein hydrolases shearing IgG antibody molecules, which are small in size, highly tissue penetrating, have low immunogenicity and are not recognized by macrophages43. It has become the preferred clinical modification method42. ScFv are obtained by linking the heavy and light chain variable regions of an antibody with a short peptide gene. It is the smallest structurally functional fragment with antigen-binding activity and is the most advanced form of immunoliposome target antibody with high penetration power, structural stability and low preparation cost44.

3.2.2 Ligand-modified liposomes

The principle of ligand-modified liposomes is to modify certain non-immunogenic substances onto liposomes so that the liposomes can specifically bind to specific or overexpressed receptors on tumor cells and then enter the target cells by receptor-mediated endocytosis45. Ligands commonly used for modification include folic acid, human epidermal growth factor, proteins, peptides and glycans.

The folate receptor is a transmembrane glycoprotein that is overexpressed on the surface of a variety of cancer cells in the ovary, breast, kidney and lung, but is rarely expressed in normal tissues and organs and has a strong affinity for folate and folate derivatives. The folic acid-modified liposomes showed good tumor targeting and low toxicity to normal tissues and organs in vitro experiments.46

Transferrin receptors are transmembrane glycoproteins associated with iron transport and are highly expressed on the surface of tumor cells in pancreatic, rectal, lung and bladder cancers due to the high rate of tumor cell appreciation and increased demand for iron 47. In in vitro experiments, transferrin-modified liposomes have been shown to enhance drug uptake by target cells and to release more drugs into the nucleus27. LDL is a complex of proteins and lipids that plays an important role in cell division, with increased uptake in rapidly dividing cell lines such as adrenal, colon, lung, prostate and breast cancers. LDL-modified liposomes can increase targeting efficiency while having an excellent safety profile25.

Human epidermal growth factor receptor (EGFR) is a transmembrane protein with an extracellular receptor structural threshold that is overexpressed in solid tumors such as breast, gastric, colorectal, bladder, and prostate cancers 48. EGFR-targeted liposomes modified by human epidermal cytokines exhibit high toxicity to tongue and lung cancer cell lines and low toxicity to fibroblasts 49.

Peptides have good biocompatibility, high targeting, low immunogenicity and low toxic side effects30. They can specifically bind to integrin αv β3 receptor, CD13, HER-2 receptor, Tf receptor and so forth, such as RGD cyclic peptide, iNGR peptide, RVG peptide and KCCYSL peptide; cell-penetrating peptide is also a kind of peptide, which can directly penetrate the cell membrane barrier and deliver drugs into the cell without receptor, and has great potential in the application of anti-cancer drugs, such as BR2 peptide, R8 peptide, PFVYLI peptide and GALA peptide 50.

Sugar receptors have increased expression on the surface of deteriorating tumors, such as galactose receptors and mannose receptors. Galactose and mannose are widely available and have good biocompatibility, water solubility, target recognition and are inexpensive, and are mostly used for liposomal targeted modification of hepatocellular carcinoma therapeutics51 .

3.2.3 Nucleic acid aptamer-modified liposomes

Nucleic acid aptamers are short DNA or RNA fragments synthesized using in vitro screening methods with specific recognition functions. The ease and speed of production compared to antibodies and their smaller size also bring the advantages of low immunogenicity and high tissue penetration, making them an ideal alternative to antibodies52. Nucleic acid aptamers can bind specifically to tumor markers, which are substances specifically present and produced on malignant tumor cells, or produced by the host organism in response to tumor stimulation53. Also, modification of nucleic acid aptamers, including sugar modification, phosphodiester modification and 3' or 5' end-capping, can protect them from endogenous nuclease breakdown and improve their pharmacokinetics 54.

3.3 Physico-chemical targeting of liposomes

Physiochemically targeted liposomes take advantage of the characteristics of the tumor microenvironment or external forces to achieve targeted drug enrichment and selective release. The main liposome types are pH-sensitive, heat-sensitive, photosensitive, and magnetic liposomes55.

pH-sensitive liposomes are structurally destabilized at low pH (4.5-6.5) in tumor cells, causing drug release56. pH-sensitive lipids are used as the raw material for the preparation and polyethylene glycol phospholipids are modified on the liposomes to avoid degradation by lysosomes and to prolong the half-life57. The pH-sensitive liposomes release drugs accurately and rapidly and have good potential for application.

Photosensitive liposomes can be used to combine photothermal therapy58, and photochemotherapy59 together with chemotherapy. They are used as carriers to transport photosensitizers, phototherapeutic drugs and anti-tumor drugs, which, under specific light, have the effect of photothermal heating and the production of reactive oxygen species to kill tumor cells, while increasing the activity of anti-tumor drugs.

Thermosensitive liposomes can aggregate locally and release drugs rapidly at elevated local temperatures, but are less selective. Combining thermosensitive liposomes

with magnetic liposomes to make magnetically targeted thermosensitive liposomes that undergo a magneto-thermal transformation in a magnetic field to raise the temperature and cause drug release can greatly increase their targeting and controlled release ability, which is a hot topic of research in recent years [60].

4. Liposomes in antitumour applications

Cancer, or malignant tumors, is a serious threat to human life. Currently, the main methods used to control tumors are surgery, radiotherapy, chemotherapy, targeted therapy, and immunotherapy. These therapies have some drawbacks, for example, the toxicity of chemotherapy drugs to normal cells and the development of drug resistance in the long term; the low response rate and the short duration of immunotherapy[61,62]. Liposomes, as biocompatible targeting carriers, can compensate for some of these shortcomings and further contribute to oncology treatment. The following table provides a brief overview of the application of liposomes in antitumor therapy (Table 2).

Table 2. Liposomes in antitumor applications

Applications	Type	Modifications and drug delivery	Indications	References
Overcoming tumour drug resistance	Proactive targeting	KC26 polypeptide, simvastatin, paclitaxel	Non-small cell lung cancer	64
	Passive targeting	TKI and Adriamycin	Breast cancer	65
	Physical and chemical targeting	Paclitaxel, siRNA	Breast cancer	68
	Passive targeting	Cisplatin, siRNA	Ovarian cancer	68
Complementary Immunotherapy	Proactive targeting	AS1411, CTLA-4 aptamer	Multiple tumour cells	71
	Physical and chemical targeting	Ce6, SB-3CT	Melanoma	72
	Passive targeting	Metformin, oxaliplatin	Colorectal cancer	73
Auxiliary trans-blood-brain barrier	Proactive targeting	Ginsenoside Rg3, Paclitaxel	Glioma	76
	Proactive targeting	Transferrin, Penetrexed, Adriamycin, Erlotinib	Glioblastoma	77
	Physical and chemical targeting	PEG, fluorescent dye DiD	Brain tumours	78

4.1 Overcoming tumor resistance

Multidrug resistance in tumors is an important impediment to tumor therapy, with the main mechanisms being changes in drug uptake or efflux, enhanced DNA repair, enzyme system-mediated detoxification, mutations in drug targets and abnormal changes in signaling pathways63 . Liposomes can carry multiple drugs and overcome tumor multidrug resistance by inhibiting overexpressed transporter proteins and modulating signaling pathways in a variety of ways.

In non-small cell lung cancer, A549T cells exhibit strong paclitaxel resistance and invasive metastatic ability. Jin64 found that the combination of simvastatin and paclitaxel could exert synergistic effects on A549T, inhibit the integrin/FAK signaling pathway and reverse the resistance of A549T to paclitaxel. On this basis, simvastatin and paclitaxel co-loaded liposomes were prepared and modified by KC26 peptide which responded well to asparagine endopeptidase and successfully achieved tumor-associated macrophage targeting with significant tumor suppression effect.

The ABC (ATP-binding cassette) transporter consists of the nucleic acid structural domain and the transmembrane structural domain, which can help tumors pump out chemotherapeutic drugs, and its overexpression is a major cause of multidrug resistance in tumors. Small molecule tyrosine kinase inhibitors (TKIs) are a class of targeted antitumor drugs, and various TKI have been reported to effectively inhibit the efflux of ABC transporters to chemotherapeutic drugs, suggesting that TKI can be used not only as antitumor drugs but also as adjuvants for clinical treatment of drug-resistant tumors. Hao and colleagues65 developed the first membrane-fused liposomes co-loaded with TKI and adriamycin, which successfully achieved tumor cell membrane-fusion-mediated drug delivery and enhanced chemotherapeutic efficacy in drug-resistant tumors, with potential for clinical application.

Small nucleic acid-mediated gene silencing downregulates gene expression of overexpressed proteins that cause drug efflux, thereby overcoming multidrug resistance in tumors 66 . Naked micronucleic acids result in poor cellular uptake due to their negative charge and have poor stability in the blood and a short circulating half-life. Encapsulation with liposomes can significantly improve their in vivo transport properties, while also co-loading other chemotherapeutic agents to better inhibit tumor growth and metastasis 67. For example, cationic oligopeptide liposomes co-loaded with paclitaxel and siRNA targeting the Survivin gene mediated apoptosis and inhibited multidrug resistance in breast cancer cells; liposomes co-loaded with siRNA and cisplatin reversed multidrug resistance in ovarian cancer SKOV3 cells and inhibited tumor cell survival and metastasis68.

4.2 Adjunctive immunotherapy

Immunotherapy has a promising future in cancer treatment. Its mechanism of action is to selectively kill tumors by inducing or re-inducing cellular immune responses, particularly T cell-mediated tumor-specific

and tumor-associated antigen-directed cytotoxicity; or to combat cancer cells by increasing plasma concentrations of tumor-specific antibodies, natural killer cells, dendritic cells and macrophages 69 . However, some immunotherapeutic drugs have low water solubility, poor absorption and the ability to produce off-target toxicity, while liposomes are considered suitable carriers for immunotherapy due to their safety, modifiable surface and combination of therapeutic agents while preventing degradation of biological materials and improving biocompatibility and stability 70 .

Ren and colleagues71 constructed a liposome targeting both tumor cells and T cells for application in T-cell immunotherapy. The dual-affinity targeting liposomes loaded with AS1411 and CTLA-4 aptamers can target both nucleolin and CTLA-4 proteins, promote T cell redirection to tumor cells, have deep penetration and immunocidal effects on solid tumors without cytotoxicity, and are safe and suitable for a wide range of tumor cells.

Photodynamic therapy combined with immunotherapy can induce immunogenic death of tumor cells and improve the effect of immunotherapy. Liu and colleagues72 developed MMP-2 responsive peptide hybrid liposomes loaded with Ce6 and SB-3CT, which could activate the natural killer cell immune pathway and promote the body's immune clearance of tumor cells, while combined with photodynamic therapy, the liposomes were activated by laser after endocytosis by melanoma cells and targeted drug release to kill tumor cells, showing synergistic effects in melanoma cells and tumor-bearing mice in vivo.

Hypoxia is a feature of the tumor microenvironment (TME) and has been shown to be associated with tumor immunosuppression. Metformin can regulate the metabolism of tumor cells, reduce their oxygen consumption and alleviate tumor hypoxia. Therefore, Song and colleagues 73 combined metformin with oxaliplatin, which can induce immunogenic cell death, to make Met-oxa liposomes, which could co-inhibit the growth of colorectal tumors in mice by activating anti-tumor immunity and reversing immunosuppression of TME.

4.3 Auxiliary trans-blood-brain barrier

The blood-brain barrier is an important structure for maintaining a stable environment in the brain, which prevents harmful substances from invading the brain, and on the other hand makes it difficult for drugs to be delivered to the brain74. The good biocompatibility of liposomes allows them to target drug delivery to brain lesions and does not damage the blood-brain barrier structure, which largely improves the bioavailability of drugs.75 This makes them the most studied and promising material for brain delivery.

Ginsenoside Rg3 has both therapeutic and targeting effects, and its use in the construction of brain-targeting liposomes can be a double benefit 76. Rg3 contains glucosyl residues and is a substrate for the glucose transporter (GLUT), which is overexpressed in glioma cells, thus significantly enhancing the tumor-targeting ability of liposomes and their anti-tumor activity.

Modification of cell-penetrating peptides reduces the difficulty of liposome entry into the brain. Transferrin receptors are overexpressed on the surface of brain endothelial cells and on glioblasts, and penetreotide is a cell-penetrating peptide that facilitates the transport of carriers into cells. Modification with transferrin and penetreotide containing liposomes of adriamycin and erlotinib significantly increased drug accumulation in the brain through a dual mechanism of receptor-mediated cell targeting and enhanced cell penetration, while the combination also significantly improved tumor inhibition 77.

The use of focused ultrasound to alter blood-brain barrier permeability is another non-invasive technique for drug delivery to the brain. Sophie and clooeagues78 applied the first rapid short pulse sequence to the delivery of liposomes, which were delivered uniformly throughout the brain at an acoustic pressure of 0.53 MPa without damage to brain tissue, for less aggressive or early stage brain tumors.

5. Summary and outlook

Among nanoparticle-mediated drug delivery, liposomes are the most successful drug carriers and the most approved type of nanomedicine by the US Food and Drug Administration. They are used in the treatment of breast cancer, lung cancer, liver cancer, gastric cancer, glioma and other tumors. It is also used in a variety of fields such as antiviral, anti-inflammatory, anti-malarial, neurodegenerative diseases, vaccines, nucleic acid drugs, diagnostics and other areas61.

Compared to the development of new anti-cancer drugs, the use of liposomes to combine existing drugs to improve their metabolic processes and effects is undoubtedly more efficient, convenient and less risky. The good biocompatibility of liposomes, the loading and protection of fat-soluble water-soluble drugs, the modifiable surfaces and their targeting functions make them very promising in the field of antitumor. In recent years, personalized cancer vaccines consisting of patient tumor-derived epitopes have been found to produce more specific cytotoxic T-cell responses, and there is much scope for liposomes in this regard80. However, even if some targeting is achieved through modification, there is still much room for improving the enrichment of liposomes within tumors 81. The potential for liposomes to enrich within the tumor is considerable.

At the same time, despite the intense research on liposomes, only a few have been approved for clinical use in cancer therapy and none have been approved for actively targeting liposomes82. The lack of active targeting of liposomes has not been approved. It can be seen that there are a number of issues between laboratory and clinical translation[83,84,85. For example, the degradation mechanism, pharmacokinetic and pharmacodynamic characteristics of liposomes need to be further investigated, and the pharmacokinetics and biodistribution are different from those of common drugs and are influenced by many factors, which are inaccurate when studied by traditional methods; the quality,

encapsulation rate and uniformity of surface ligand binding of liposomes are not easily guaranteed in scale-up industrial production; the lack of appropriate characterization methods for functionalized ligand-directed liposomes; the stringent storage requirements for liposomes, which must be stored in a refrigerator and not frozen; and the potential for complement activation-related allergic-like reactions associated with intravenous administration of liposomes. These pending issues are the direction of future research and require the concerted efforts of all researchers. Although there are still some problems in the clinical translation of liposomes, their future is immeasurable and it is foreseeable that they will be more widely used in the future.

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