

## Research Progress of Nanoliposome Drug Delivery System in Breast Cancer Treatment

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### Abstract

In the research of breast cancer treatment, it is found that nanocarriers can carry the commonly used chemotherapeutic drugs for breast cancer and can release the drugs stably. Nanomaterials have a good targeting effect in breast cancer treatment. In terms of passive targeting, nanocarriers can take advantage of changes in the tumor microenvironment to enhance the penetration and retention effect, increase the local tumor drug concentration, and thereby improve the anti-breast cancer effect; in terms of active targeting, breast cancer molecular targeting and magnetic nanoparticle mediation Guided targeting can increase the intake of anticancer drugs by breast cancer cells. In addition, nanocarriers use the targeting effect to partially reverse the multidrug resistance of breast cancer, and use the silencing of related drug resistance genes to enhance the effect of reversal. For breast cancer metastasis, nano-drug carriers can reduce the risk of breast cancer metastasis, improve the effect of treating breast cancer bone metastasis, and have a certain therapeutic effect on breast cancer-related osteoporosis. To this end, the thesis briefly discusses the research progress of nanoliposome drug delivery system in breast cancer treatment. At the same time, the thesis uses  $\beta$ -element nanoliposomes to study breast cancer 4T1 cell targeted therapy.

### Keywords

Nanolipid Drugs; Breast Cancer; Targeted Drugs; Research Review.

## 1. INTRODUCTION

Breast cancer is a malignant tumor that occurs in breast epithelial tissues, which seriously threatens the physical and mental health of women around the world and even endangers their lives. Its incidence tends to be younger and has become a major public health problem in the current society. The US "2018 Cancer Data Report" suggests that breast cancer, lung cancer, and colorectal cancer are objects that women must pay attention to, especially breast cancer, which accounts for 30% of new cases and ranks first in the incidence of malignant tumors among American women. At present, the treatment methods for breast cancer mainly include surgery, chemotherapy, radiotherapy, endocrine therapy and other methods. Various treatment methods can achieve good therapeutic effects, but they also have various shortcomings or shortcomings. Chemotherapy is a treatment method that uses anti-cancer drugs to inhibit the division of cancer cells and destroy cancer cells. It plays an important role in the clinical treatment of breast cancer. However, the non-selectivity of chemotherapeutics to tumor tissues can easily cause systemic toxicity and accumulate in the tumor site. The chemotherapeutic drugs lower than the effective therapeutic dose will lead to the emergence of tumor drug resistance [1]. Therefore, it is very urgent and necessary to research and develop new tumor treatment methods. In recent

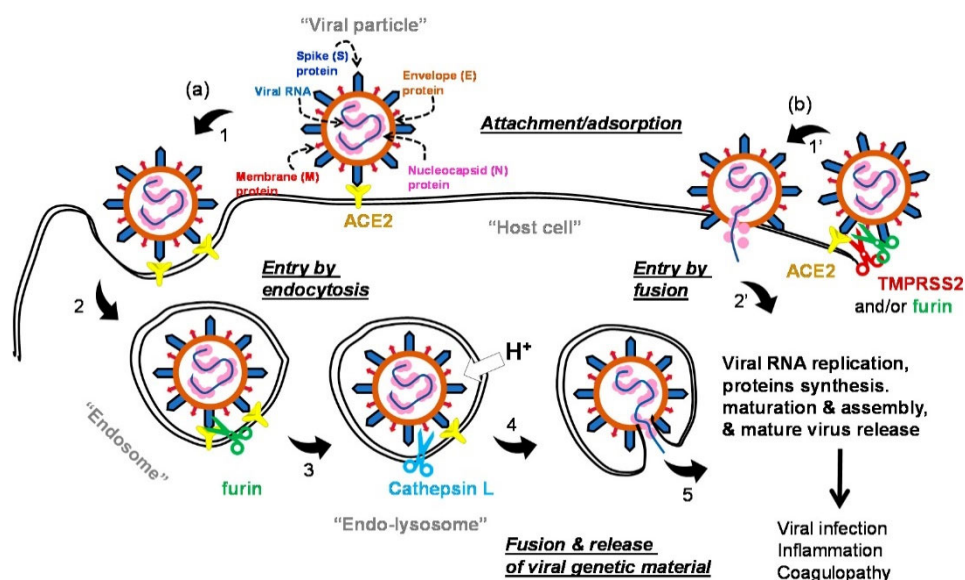
years, nanomaterials have shown great potential in tumor treatment. Due to their unique physical, chemical and biological properties, they can significantly reduce the adverse reactions of chemotherapy drugs and improve the effects of chemotherapy. They have gradually become a new type of biomedical field. Research hotspots. To this end, the thesis briefly discusses the research progress of nanoliposome drug delivery system in breast cancer treatment. At the same time, the thesis uses  $\beta$ -elemene nanoliposomes to study breast cancer 4T1 cell targeted therapy.

## 2. LITERATURE REVIEW

### 2.1. Passively Targeted Drug-loaded Nanoparticles

#### 2.1.1 Intake of RES system and controlled release of drugs

The so-called passive targeting means that the nanoparticles are taken up by RES (reticuloendothelial system, including liver, spleen, bone marrow, especially Kupffer cells in the liver) after entering the blood circulation. This capture and phagocytosis is affected by the size and surface properties of the nanoparticles [2]. In Kupffer cells, the carrier is degraded by the action of lysosomes, and the drug is re-released into the blood. Through continuous monitoring of the local blood concentration, the preparation process can be changed to form an ideal formulation with the best drug release curve. Taking doxorubicin, which is commonly used in the treatment of breast cancer, as an example (as shown in Figure 1 is the release principle of PBLG/PEO drug-loaded azithromycin), foreign scholars encapsulate it in polybenzyl glutamyl and polyethylene oxide copolymers. On PBLG/PEO, at 37°C, only 20% of the drug is released within 24 hours, and the average residence time in the blood is 3 times that of the free drug. It is possible to alleviate the cardiac toxic side effects caused by transient high blood concentration caused by a large-dose injection.



**Figure 1.** The release principle of PBLG/PEO-loaded azithromycin in the treatment of breast cancer

Some foreign scholars have proved through in vitro cell experiments that macrophages activated by IFN- $\gamma$  can enhance the cytotoxicity of anticancer drugs carried on nanoparticles, and analyzed that the reason may be that macrophages release cytotoxic factors after activation (Such as NO, etc.), which leads to an increase in tumor cell death. Magnetic nanoparticles and

molecular modification further strengthen the targeting effect on breast cancer [3]. In order to better play the role of tumor treatment, some targeting molecules are usually used to modify nanoparticles, such as anti-human breast cancer monoclonal antibodies, ligands, etc., to play a molecular targeting role and direct drug magnetic nanoparticles to tumor target areas. Studies have combined iron oxide nanoparticles with HER-2 antibody, and found that SK-BR-3 breast cancer cells uptake of antibody-bound iron oxide nanoparticles was significantly higher than that of normal cells, indicating that iron oxide nanoparticles bound to HER-2 antibody It has a highly effective targeting effect on SK-BR-3 breast cancer cells. Some scholars have used folic acid modified polyethylene glycol to wrap magnetic nanoparticles loaded with idarubicin polymer to improve the targeting of breast cancer cells.

### 2.1.2 Passive targeting of drug-loaded nanoparticles

Nanocarriers can take advantage of the enhanced penetration and retention effect of abnormal neovascularization of tumors, increase the accumulation of local nanoparticles in tumors, and improve the effect of anti-breast cancer. Due to the dense microvascular endothelial gaps in normal tissues, nanoparticles of appropriate particle size are difficult to penetrate the blood vessels of normal tissues, while the tumors have abnormal new blood vessels, and their blood vessel wall gaps are large. Nanoparticles can pass through the vascular endothelial pores and enter the tumor stroma, Play a certain passive targeting effect, this phenomenon is called enhanced osmotic retention (EPR) effect. Nanocarriers can use the local acidic environment of the tumor to increase the amount of drug released, thereby improving the effect of anti-breast cancer. Studies have found that the drug release rate of nanocarriers increases in an acidic environment, while the tumor is in an acidic environment that is relatively hypoxic [4]. Therefore, nanocarriers accelerate the release of local drugs around the tumor, thereby increasing the local blood drug concentration of the tumor, which plays a role in tissue targeting. Some foreign scholars have found that when pH=5, the drug release rate of polymethacrylic acid-polysorbate block copolymer loaded with doxorubicin is significantly faster than that under normal human pH (6-7.4). The release of the nano-drug carrier system at pH=4 is significantly faster than that at pH=7.4, achieving the effect of increasing the local drug concentration of the tumor.

## 2.2. Liposome Drug Delivery System

Liposomes are closed spherical vesicles surrounded by phospholipid bilayers, with diameters ranging from 25-1000nm. It was discovered and named by the British scholar Bangham in the 1960s. Gregoriadis and others first used liposomes as drug carriers in the 1970s. Liposomes have the functional characteristics of biological membranes, can fuse with cell membranes, can simultaneously encapsulate hydrophilic and hydrophobic drugs, and have the effects of slow and controlled release. They are the most mature nanocarriers currently studied.

Studies have shown that liposomes with a diameter of less than 100nm can avoid phagocytosis by the endothelial reticulum system and are easier to penetrate into tumor tissues, but the low drug loading is its main defect [5]. PEG-modified liposomes can increase drug loading and avoid RES phagocytosis, but the modified polymer compound increases the steric hindrance between the drug and cancer cells, and affects the absorption of liposomes by cancer cells. Doxi is now a PEG-modified liposome of adriamycin that has been marketed in the United States and Europe. Although Doxi reduces the cardiotoxicity of doxorubicin, the amount of drug released at the tumor site is very slow (less than 5% in 24h), resulting in the bioavailability of the drug being only 40%-50%. This shows that although PEG liposomes can concentrate on tumor sites, they will not automatically increase the anti-tumor pH value, sound waves, and enzyme-stimulated liposomes that release drugs. Thermosensitive liposomes were first proposed by Yatvin et al. The main component of the original heat-sensitive liposomes is dipalmitoylphosphatidylcholine (DPPC), which can promote liposome fusion, rupture and

release chemotherapeutic drugs under high temperature conditions (42°C). When 1-myristoyl-2-stearoyl lecithin (MSPC) and distearoyl phosphatidylethanolamine-polyethylene glycol (DSPE-PEG2000) are added to DPPC thermosensitive liposomes, they can increase significantly at 41°C. The permeability of the liposome membrane can improve the drug release rate and curative effect.

Recently, some scholars have further optimized LTSL liposomes, encapsulating doxorubicin and copper elements inside heat-sensitive liposomes (CuDox-LTSLs) to realize the multifunctional treatment of heat-sensitive liposomes. This composite system was injected intravenously into a mouse model of breast cancer twice a week. Under the guidance of ultrasound, the liposomes produced a thermal ablation effect on the tumor and stimulated the liposomes to release doxorubicin. The experimental results showed that the breast mass disappeared completely 53 days after treatment, and no cancer cells were found in pathological examination 8 months after treatment [6]. On the other hand, when the liposome surface is modified with breast cancer cell-specific expression molecules, the liposome can actively target breast cancer cells. Literature linked Her2 specific antibody-Trebizumab to temperature-sensitive liposomes to prepare heat-sensitive immunoliposomes. Experiments show that the absorption of heat-sensitive liposomes by Her2-positive breast cancer cells is 22 times higher than that of traditional liposomes. Like heat-sensitive liposomes, pH-sensitive immunoliposomes also significantly improve the drug release rate and targeting, but the leakage of drugs in the blood circulation is its main defect. Literature used functionalized single-walled carbon nanohorns as a hydrophobic drug carrier to wrap inside the phospholipid bilayer of pH-sensitive immunoliposomes, reducing the amount of drug leakage by half. Table 1 shows the current liposome nanoformulations for breast cancer treatment.

**Table 1.** Liposome nanoformulations for the treatment of breast cancer

| Formulation name    | Encapsulated drugs | Current stage              | Development country |
|---------------------|--------------------|----------------------------|---------------------|
| Doxil               | Adriamycin         | Listed                     | United States       |
| Myocet              | Adriamycin         | Listed                     | United States       |
| Caelyx              | Adriamycin         | Listed                     | Europe              |
| Lidod               | Adriamycin         | Listed                     | China               |
| Lipusu              | Paclitaxel         | Listed                     | China               |
| LEP-ETU             | Paclitaxel         | Phase I clinical trial     | United States       |
| MBT-0206            | Paclitaxel         | Phase I clinical trial     | Germany             |
| LEM-ETU             | Mitoxantrone       | Phase I clinical trial     | United States       |
| ATI-1123            | Docetaxel          | Phase I clinical trial     | United States       |
| INX-0125            | Vinorelbine        | Phase II clinical trial    | Canada              |
| Liposomal-Annamycin | Anthracycline      | Phase I/II clinical trials | United States       |
| Thermo Dox          | Adriamycin         | Phase II clinical trial    | United States       |

### 3. EXPERIMENTAL PROCESS

In this study,  $\beta$ -elemene nanoliposomes were prepared to study its inhibitory effect on breast cancer 4T1 cells.

#### 3.1. Preparation of $\beta$ -element Nanoliposomes

We weighed 0.5 g of lecithin, 0.1 g of cholesterol, and 50 mg of  $\beta$ -elemene according to a mass ratio of 5:1, and dissolved them in 20 mL of chloroform. The lipid solution obtained was placed in a round-bottomed flask and used a rotary evaporator. At 45°C, the organic solvent chloroform

is removed by rotary evaporation, so that the lipid forms a thin film on the inner wall of the bottle. Then pour 1min of nitrogen into the round-bottomed flask, pour 10mL of phosphate buffer containing 0.1%FA-PEG2000 into the round-bottomed flask, and rotate it on a rotary evaporator at 45°C for 0.5-1h. , Wash off the lipid membrane. After being fully hydrated for 1 to 3 hours, it was filtered with 0.45 and 0.22 $\mu$ m membranes in sequence to obtain  $\beta$ -elemene nanoliposomes.

### 3.2. In Vitro Targeted Experiments

In the experiment, MCF-7 cells and 4T1 cells were incubated in a carbon dioxide incubator for 3 hours, the cells were taken out, and the cells were washed three times with PBS to remove the extracellular nanoparticles. Then place it under a confocal fluorescence microscope to observe the fluorescence signal of FITC in the cells [7]. The excitation wavelength of FITC is 488 nm. After confocal imaging, the cells were digested and dispersed evenly, and then the cells were collected. Use flow cytometry to detect the content of FITC in the cells to investigate the percentage of uptake by the cells. 1,000 cells were collected for each experiment.

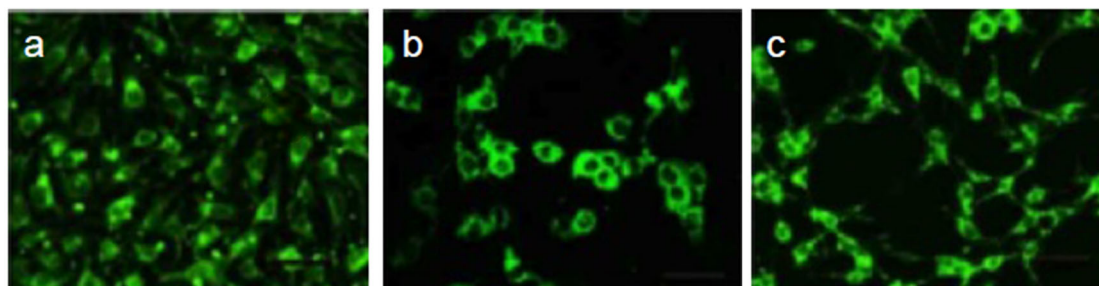
### 3.3. In Vivo Tumor Treatment Experiment

The 4T1 cells in the logarithmic growth phase were collected in the experiment, and 100  $\mu$ L of PBS cell suspension ( $10^7$ mL<sup>-1</sup>) was subcutaneously injected into the back of nude mice aged 4 to 5 weeks. Nude mice were randomly divided into three groups: control group (PBS),  $\beta$ -elemene group, and  $\beta$ -elemene nanoliposome group, with 10 mice in each group. Each group was injected into the tail vein, and then the tumor size was observed and measured every 3 days. Tumor volume=length $\times$ width $\times$ width $\div$ 2.

### 3.4. Experimental Results

#### 3.3.1 Targeted results

Figure 2A shows that a large amount of FITC red fluorescence is distributed in the cytoplasmic area, while only a small amount of FITC-labeled  $\beta$ -elemene nanoliposomes in the control group are distributed in the cytoplasmic area. In addition, the results of confocal microscopy showed that MCF-7 cells treated with  $\beta$ -elemene nanoliposomes had very little fluorescence. The results of flow cytometry detection and statistics are shown in Figure 2B and Figure 2C. The cell uptake rates of  $\beta$ -elemene group and  $\beta$ -elemene nanoliposomes are (21.6 $\pm$ 3.6)% and respectively (76.9 $\pm$ 3.1)%, the difference is statistically significant,  $t=26.023$ ,  $P<0.001$ .



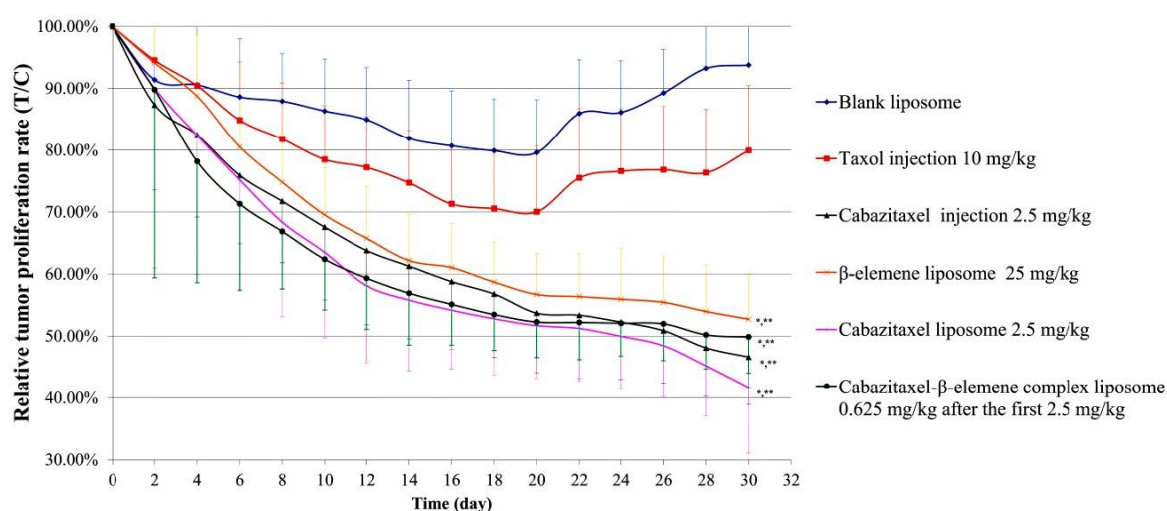
**Figure 2.** In vitro targeting of  $\beta$ -elemene nanoliposomes

#### 3.3.2 In Vivo Tumor Treatment Effect

As shown in Figure 3 and Table 2,  $\beta$ -elemene nanoliposomes significantly inhibited tumor growth during the one-month treatment period. Compared with the  $\beta$ -elemene nanoliposome group, the  $\beta$ -elemene group and the control group, the differences were statistically significant.

**Table 2.** In vivo tumor treatment effects of  $\beta$ -elemene nanoliposomes

| Group    | Control group | $\beta$ -elemene | $\beta$ -elemene nanoliposomes |           |
|----------|---------------|------------------|--------------------------------|-----------|
| Time (d) | 0             | 1.00±0.00        | 1.00±0.00                      | 1.00±0.00 |
|          | 4             | 1.62±0.05        | 1.12±0.02                      | 1.02±0.01 |
|          | 8             | 2.46±0.07        | 1.69±0.04                      | 1.04±0.02 |
|          | 12            | 3.11±0.08        | 2.11±0.03                      | 1.31±0.02 |
|          | 16            | 4.26±0.08        | 3.21±0.06                      | 1.89±0.05 |
|          | 20            | 6.53±0.11        | 3.71±0.04                      | 2.42±0.05 |
|          | 24            | 7.23±0.13        | 5.16±0.12                      | 3.22±0.07 |
|          | 28            | 9.46±0.09        | 7.66±0.12                      | 4.29±0.08 |

**Figure 3.** In vivo tumor treatment effect of  $\beta$ -elemene nanoliposomes

#### 4. CONCLUSION

Nanotechnology in breast cancer treatment research has the effects of slow-release drugs, precise targeting, reversing tumor multi-drug resistance, and enhancing drug efficacy. Nanoliposomes have been widely used to improve traditional cancer treatment strategies. The basic chemotherapeutic drugs using nanoliposomes as carriers can reduce the adverse reactions of chemotherapeutic drugs, and effectively prevent the rapid degradation of the drugs, which greatly changes the pharmacokinetics. After the liposome surface is modified with targeting molecules, it can be specifically enriched at the tumor site, thereby increasing the concentration of the drug at the tumor site.

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