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# Nanomedicine as an emerging platform for metastatic lung cancer therapy

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**Abstract** Metastatic lung cancer is one of the most common cancers leading to mortality worldwide. Current treatment includes chemo- and pathway-dependent therapy aiming at blocking the spread and proliferation of these metastatic lesions. Nanomedicine is an emerging multidisciplinary field that offers unprecedented access to living cells and promises the state of the art in cancer detection and treatment. Development of nanomedicines as drug carriers (nanocarriers) that target cancer for therapy draws upon principles in the fields of chemistry, medicine, physics, biology, and engineering. Given the zealous activity in the field as demonstrated by more than 30 nanocarriers already approved for clinical use and given the promise of recent clinical results in various studies, nanocarrier-based strategies are anticipated to soon have a profound impact on cancer medicine and human health. Herein, we will detail the latest innovations in therapeutic nanomedicine with examples from lipid-based nanoparticles and polymer-based approaches, which are engineered to deliver anticancer drugs to metastatic lung cells. Emphasis will be placed on the latest and most attractive delivery platforms, which are developed specifically to target lung metastatic tumors. These novel nanomedicines may open new avenues for therapeutic intervention carrying new class of drugs such as RNAi and mRNA and the ability to edit the genome using the CRISPER/Cas9 system. Ultimately, these strategies might become a new therapeutic modality for advanced-stage lung cancer.

**Keywords** Lipid nanoparticles · Polymers · Drug delivery · siRNA and lung metastasis

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## 1 Introduction

According to WHO, lung cancer is the most common cancer leading to mortality worldwide [1], with an incidence of 1,378,400 deaths per year worldwide [2] and an overall 5-year survival rate of 15 % [3]. Lung cancer is manifested in one of two forms: small cell lung carcinoma (SCLC), which represents 13 % of the total lung cancer cases, and non-small cell lung carcinoma (NSCLC), which is considered significantly more aggressive, with low responsiveness to chemotherapy agents. Most NSCLC patients (around 80–85 %) present a metastatic disease form [4], characterized by both local and distant spread of cancer cells into the lymph nodes, lungs and other organs. The degree of lymphatic dissemination is an established prognostic predictor for these patients. Preferable first-line treatment for early-stage lung cancer patients combines operation for tumor resection and radiation treatment [5]. However, due to the deficiency in early-stage diagnostics, most lung cancers are only detected at advanced stages, with local tumor invasion or distant metastasis and are not suitable for surgery. Therefore, a systemic chemotherapy treatment modality that addresses the majority of lung cancers is currently the mainstay of advanced lung cancer treatment regimens, aimed at extending survival and improving quality of life [6].

### 1.1 Chemotherapy for patients with metastatic disease

The recommended treatment for patients with advanced NSCLCs involves systemic platinum-based chemotherapy (e.g., cisplatin, oxaloplatin) combined with taxens (such as Pacitaxel or Doxetaxel) or Gemcitabine [7].

Paclitaxel (Ptx) inhibits cell division by irreversibly assembling and stabilizing microtubules, thereby inhibiting microtubule disassembly and blocking cell proliferation [8]. Ptx is most commonly administered intravenously yet exhibits poor solubility in water (e.g., Taxol) and is therefore delivered with adjuvants, such as Cremophor EL, to increase its bioavailability. However, the castor oil-based Cremophor EL is associated with toxic events and can trigger severe side effects including hypersensitivity, neurotoxicity, and neuropathy [9, 10]. To reduce the toxicity of Cremophor EL and to improve the overall efficacy of paclitaxel, an albumin-bound formulation (Abraxane®) was developed, recently FDA approved for NSCLC in combination with carboplatin. Although Abraxane and Taxol are both effective drugs, however, each agent possesses unique drug delivery characteristics. Abraxane significantly improves the safety and overall response rate over Taxol but no significant improvement in overall survival.

The water-soluble drug doxorubicin (Dox) (e.g., Adriamycin® and Rubex®) is another type of chemotherapeutic agent commonly used to treat lung metastasis therapy, which also suffers from limited therapeutic potential in its free form. It is defined as an anthracycline antibiotic compound and also induces serious life-threatening side effects, such as cardiomyopathy and myelosuppression [11], which limit the permitted intravenous injection doses, resulting in lower effectiveness.

Gemcitabine (e.g., Gemzar®) is an antimetabolite agent, which interferes with DNA synthesis and prevents cell proliferation. While its therapeutic index is greater than other anticancer drugs, it also causes serious side effects such as myelosuppression, neutropenia, thrombocytopenia, and anemia [12].

Most of the conventional chemotherapeutic agents lack of specificity in that they fail to differentiate between healthy and cancer cells leading to severe adverse effects. In addition, cancer cells are notorious for their resistance to conventional chemotherapeutic drugs, such as Carboplatin and Cisplatin, which are widely use as first-line lung cancer metastasis treatment [13]. With time, the residual resistant cancer cells lead to tumor relapse and promote metastasis [14] while limiting the success of chemotherapy treatment and lower survival rates. Overall, cell resistance, lack of specificity and toxicity to conventional chemotherapeutic drugs present an urgent need for innovative, more efficient and effective treatment alternatives for lung cancer metastases.

In this review, we will survey the latest developments and innovations in therapeutic nanocarriers, including liposomes and polymers, which are designed to efficiently deliver anticancer drugs and nucleic acids such as DNA & siRNA to metastatic lung cells and bear the potential to become candidates for the next-generation therapy for advanced-stage lung cancer. Emphasis will be placed on the latest and most attractive delivery platforms, which are developed specifically for lung metastasis therapy.

### 1.2 Therapeutic benefit of nanocarriers

One approach to target the delivery of drugs specifically to cancer cells is through directing cellular events at nanometer scale. Where current technologies require hundreds of thousands of cells to detect the presence of a tumor, nanotechnology approaches could radically lower this requirement, enabling much earlier diagnosis/treatment regimes. Through working on the nanoscale, it becomes possible to differentiate between healthy and cancerous cells thus significantly offering a wide therapeutic index with reduced adverse effects. One major clinical advantage of nanocarrier-based strategies over free drugs is specific delivery of large amounts of chemotherapeutic agents per recognition event.

Typically, nanocarrier-based approaches include a carrier, a targeting moiety that is bound to the carrier via specific conjugation chemistry, and a drug. Carriers may be composed of lipids, polymeric nanoparticles, inorganic nanoparticles, or dendrimers. Targeting moieties may include high affinity ligands, antibodies and nucleic acids, and they may be conjugated to the carriers utilizing a variety of chemistries. Given the wide array of potential nanocarrier-based strategies for targeting lung cancers, this review will focus only on lipid-based nanoparticles (LNPs) and on soluble polymer carriers conjugated to drugs via a degradable linker (Fig. 1).

A successful therapeutic drug delivery system should fulfill a number of requirements:

1. Constitute a depot for large amounts of sustained-release anticancer drugs
2. Increase circulation time in order to enable the drug sufficient time to reach its destination before clearance or degradation
3. Efficiently transfer its cargo to tumor/metastasis cells
4. Increased specificity and reduce toxicity.
5. Escape the immune system and increase treatment efficacy

These features will be discussed throughout this review.

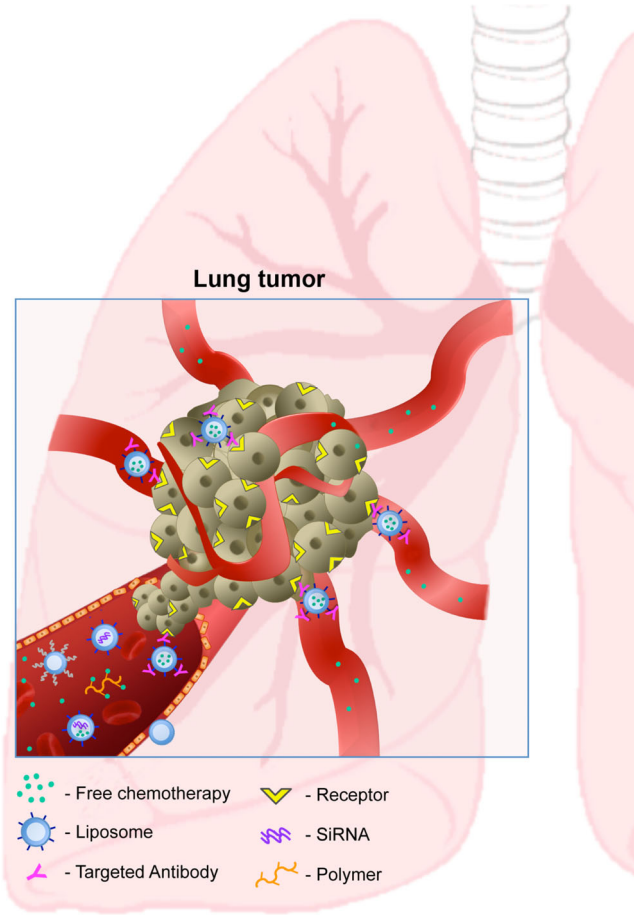


**Fig. 1** A schematic illustration of different nanocarrier-based treatment strategies in lung tumor metastasis: only small fraction (<1 %) of free chemotherapy (*green dots*) reaches the tumor metastasis microenvironment. Nanoscale carriers such as lipid-based nanoparticles (*blue*) or soluble polymers (*orange*) are loaded with chemotherapy (*blue particles with green dots inside*) or with siRNA (*blue particles with purple coils inside*) or combination of both can horn into the tumor blood vessels more efficiently due to EPR effect. In order to facilitate uptake of the nanocarriers into tumor/metastatic cells, a targeted nanocarrier can be designed using a targeting moiety such as a mAb or a natural ligand, which binds specific receptors that are overexpressed on the tumor cells and will enhance drug internalization into the tumor cell through receptor-mediated endocytosis

## 2 LNPs targeting lung metastatic disease

### 2.1 LNPs encapsulating chemotherapeutic agents

Lack of therapeutic efficiency along with adverse effects associated with conventional chemotherapeutic agents has given rise to the need for efficient and less toxic treatment alternatives for advanced NSCLC patients. The unique physicochemical properties of lipid-based nanoparticles (in the form of bilayer structures (liposomes)) or solid core lipid nanoparticles coupled with excellent biocompatibility makes them candidates as vehicles for drugs. These LNPs made of uniform lipid bilayers or solid cores can entrap various cytotoxic drugs: hydrophilic drug will be trapped in the aqueous region, while the lipophilic drug will be captured in the lipid leaflets. LNPs carry drugs safely to the destination-tumor site, release it in a gradual manner, and are then degraded [15, 16].

The physical structure of LNPs is primarily defined by its phospholipids composition, which determines the chemo-physical features, such as size, shape, curvature, and charge. In the case of liposomes, the lamellae organization can take on a unilamellar vesicle (ULVs) or multilamellar vesicle (MLVs) form. The most common techniques for liposome preparation involve extrusion or microfluidic mixing steps [17]. Liposome size and lamella type can be manipulated using polycarbonate membranes with different pore sizes or by changing flow rate of the ethanol-dissolved lipids and aqueous drug solutions. Lipid-based nanocarriers have become a favorable platform for delivery of anticancer drugs mainly due to their non-toxic, biodegradable, and biocompatible nature [18, 19].

Liposomes, artificial and biodegradable phospholipid vesicles, represent a safe vehicle for loading various drugs and can be made from either non-charged or positively/negatively charged phospholipids that can be administrated via different types of routs such as intraperitoneally (i.p.), intravenously (i.v.), or intranasally. For example, neutrally charged liposomes, composed from dilauroylphosphatidylcholine (DLPC) lipids can deliver paclitaxel (Ptx) in an aerosol form, inhibited pulmonary metastases in murine renal carcinoma model and prolonged survival rates (25 %) in treated mice compared to untreated groups and free Ptx treated groups [20]. The aerosol liposomal delivery strategy significantly increased pulmonary concentration of Ptx in lungs compared to i.v. administration of free Ptx (33.4 and 1.3 mg h/g, respectively) and also the half-life of Ptx in circulation was significantly higher (35.5-fold) [20]. When treatment frequencies were increased from two to five times per week, aggressive behavior was observed, likely a sign of Ptx neurotoxicity.

However, metastatic cells are known to be resistant to most of the conventional chemotherapeutic agents with unsatisfactory effects of cytotoxic drugs on the metastatic foci [21]. This may be a direct consequence of their slower growth rate in comparison to primary tumor cells [22], hence making chemotherapy drugs better eliminators of highly proliferative cells. Additionally, subpopulations of cancer stem cells (CSC) possess highly potent self-renewal capacities together with a quiescence phenotype, rendering them drug-resistant phenotypes to most chemotherapy agents. Overall, conventional chemotherapy treatment may even encourage metastasis tumor formation, by way of resistant cancer cells that influence their micro-environment, which actually reduce therapeutic benefit of cytotoxic drugs and lead to lower survival rates [23]. Thus, a treatment based on a single chemotherapeutic agent may be useful only for primary tumor but not to metastasis lesions. There is an unmet need generating powerful treatments to simultaneously eliminate the primary tumor and its metastatic lesions.

A combined approach based on aerosolized liposome delivery strategy used sequential treatments with Ptx in combination with a vitamin E analog—α-TEA. Vitamin E has been shown to reduce lung metastasis incidence [24, 25]. Both compounds were encapsulated in DLPC liposomes. In a highly metastatic mammary tumor-bearing mice model (66cl-4-GFP), the combined treatment showed significantly synergistic antitumor and antimetastatic activities than the control or single-agent treatments. In the combined treatment group, a 95 % decrease in the average number of microscopic metastatic lung foci was reported compared to control groups (untreated/liposomes only). The aerosolized α-TEA liposomes demonstrated no clinical, biochemical, or hematological toxicity in the liver and kidneys. However, an increase in total number of lymphocytes was measured in comparison to animals treated with aerosolized empty liposomes [25].

The advantages of the aerosol-based liposome delivery method are rooted in the continuous and direct exposure of the lungs to active drug, along with an effective and highly tolerated treatment. Additionally, therapeutic doses are lower than other routes of administration (e.g., intravenous and intraperitoneal).

Most commonly used method for administration of drug-loaded nanocarriers is intravenous (i.v.) route. Cationic liposomes have been established as an efficient delivery platform for anticancer drugs via intravenous route. Their main advantages lie in their ability to serve as a driving force for cellular binding and endosomal escape. The endosomal escape is a critical barrier that must be overcome to effectively deliver drug to the cytosol. The most renown and senior clinically approved liposomal formulation is the Doxil® [26] This first nanodrug formulated with PEG 2000, cholesterol and DSPC form liposomes that encapsulate Dox, used to treat AIDS-related Kaposi's sarcoma, breast cancer, ovarian cancer and other solid tumors by i.v. administration. The PEG-2000MW chain coating prolongs circulation time and hence increases the probability of accumulation in the tumor tissues, mainly due to the enhanced permeability and retention (EPR) effect, an architectural defect in tumor vasculature causes leakiness of blood vessels and an inappropriate lymphatic drainage.

### 2.2 Surface-modified LNPs

Non-specific toxicity to healthy cells is one of the major drawbacks in cancer treatment. Targeting metastasis tumor cells in treating NSCLC is achieved by conjugating small molecules, peptides, or mAbs to the surface of PEG chains by various conjugation chemistry techniques. Here, we have discussed some of surface-modified LNPs for targeted delivery of anticancer drugs to lung metastasis. A selective targeting approach based on tumor angiogenesis surface molecules is reported by Guan et al. Docetaxel loaded NPs modified with TH10 peptide , in efforts to target neural/glial antigen 2 (NG2), a proteoglycan, which is highly expressed in tumor-derived vascular pericytes lining the inside of blood vessels. The targeted NP exhibits a longer circulation time and was associated with significantly prolonged survival rates of B16F10-luc-G5 tumor-bearing mice, with no observed toxicity. A correlation between pericyte's density and decreased microvessel lung metastases density was demonstrated [27].

In order to facilitate drug penetration exclusively into tumor metastases and to increase cellular uptake, Qin et al. developed DSPE-PEG-liposomes with a unique surface modification based on chlorotoxin, a 36 aa peptide purified from the venom of the scorpion *Leiurus quinquestriatus*. Chlorotoxin binds the matrix metalloproteinase-2 (MMP-2) receptor, which is associated with cell surface expression of the voltage-gated chloride ClC-3 channels and considered a key factor in cell migration, critical to metastasis formation and development. This binding also decreased chloride ions currents, which inhibited tumor metastasis. A significant inhibition of A549 human lung adenocarcinoma cell migration was observed following exposure to chlorotoxin-modified liposomes [3].

Wang et al. decorated chitosan-NPs with the provascular agent bradykinin-potentiating peptide (BPP) and then loaded them with Cisplatin pro-drug. An increase in the penetration of BPP decorated chitosan-NPs and a tenfold increase in accumulation of Cisplatin exclusively in metastasized mouse lung in comparison to the free drug accumulation was observed. When compared with non-decorated particles, drug accumulation in the primary tumor was four times fold higher when delivered in BPP-coated NPs. In addition, H22 tumor-bearing mice treated with BPP-decorated chitosan-NPs exhibited prolonged survival, when compared to mice treated with free drug or non-decorated NPs [28]. The BPP-decorated chitosan-NPs demonstrated a cytotoxicity safety profile according to cell viability in two different tested cancer cell lines (SH-SY5Y and H22). However, the authors have not tested *in vivo* safety profile for these particles.

### 2.3 LNPs encapsulating nucleic acids

Liposomal delivery systems are promising carriers for delivery of negatively charged nucleic acids such as DNA, siRNA, and mRNA.

#### *2.3.1 LNPs delivering plasmid DNA*

As a well-known fact, cancer can occur due to mutations of some genes, also called tumor suppressor genes, such as p53, pigment epithelium-derived factor (PEDF), and phosphatase and tensin homolog (PTEN). Delivering of plasmid DNA (pDNA) encoding tumor suppressor genes can be utilized as a tool in cancer therapy. Cationic lipids can electrostatically bind to negatively charged nucleic acids making them an attractive tool for delivery of DNA in gene therapy applications

in early 1980s. Systemic administration of DNA-loaded liposomes has been demonstrated to effectively deliver gene cargo to the lungs [29]. Shi et al. adapted a combination of an anti-angiogenic and gene therapy, along with adenovirus-encoding pigment epithelium-derived factor (PEDF) encapsulated in 1, 2-dioleoyl-3-trimethylammonium propane (DOTAP) liposomes. PEDF endogenous protein is known for its ability to inhibit anti-angiogenesis activity and has been suggested to be deficient in malignancies [30]. Intravenous administration of DOTAP liposome-PEDF complex to B16F10 melanoma tumor-bearing mice led to a significant decrease in lung metastasis nodules and increased survival rate compared to control groups. No evidence of toxic effects, as determined by body weight and H&E staining of the liver, heart, spleen, and kidney were reported [31].

Additional DNA-loaded liposome strategy for metastasis treatment takes advantage of the pro-apoptotic effect of nitric oxide (NO). NO is typically expressed at low levels in all types of cancers, resulting in failure to induce apoptosis in these cells [32]. An alternative DNA treatment approach exploits the anticancer activity of inducible NO synthase (iNOS). Ye et al. took advantage of the prolong circulation time of small cationic DOTAP liposomes to mediate delivery of nitric oxide synthase gene together with low-dose Cisplatin to the lungs. Significant inhibition of lung metastases formation was measured in tumor-bearing mice i.v. administrated with the combined treatment (200.8±11.2 days compared to the group treated with low-dose Cisplatin alone, 133.6± 22.2 days). In addition, these mice had extended survival in comparison with mice injected with cisplatin only, and no visible signs of toxicity [29]. The combined treatment significantly improved the Cisplatin-mediated therapeutic index both in lung metastasis and tumor invasion. Although DNA delivery studies show promising tumor inhibition in rodent tumor models, severe immune activation due to CpG motifs present in pDNA hindered their usage in human clinical applications.

### *2.3.2 LNP delivery of RNAi payloads*

Two major strategies to entrap small interfering RNAs (siRNAs) in LNPs have been proposed (Fig. 2). In the first and most common strategy, the LNPs possess a surface positive charge using a variety of synthetic cationic lipids such as DOTAP, *N*-[1-(2,3-dioleyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride (DOTMA), DC-cholesterol, and others [33, 34]. In these formulations, the siRNAs are not entrapped but electrostatically bound to the surface of these LNPs (Fig. 2a).

However, due to their high positively surface charge, cationic liposomes suffer from a fast clearance by macrophages in circulation and inefficient process of endosomal escape and intracellular cargo release in addition to immune response [35–37, 15].

A novel strategy to entrap siRNAs in LNPs was developed using ionizable lipids (such as Dlin-DMA/Dlin-KC2-DMA/Dlin-MC3-DMA), which have a $pK_a$ of 6.4–6.8 and thus could be charged at low pH to form a solid lipid core with siRNAs that are entrapped within the LNPs (Fig. 2b). Neutral or low surface charge at physiological pH avoids non-specific interaction with serum proteins and MPS clearance [38, 17, 39].

An attractive LNP-based therapeutic strategy is emerging in the last decade for tumors and lung metastasis that makes use of RNA interference (RNAi) molecules, which are naturally occurring molecules produce by most eukaryotic cells, which efficiently control gene expression in a complementarity-dependent manner. The common assumption is that this mechanism is designated to protect against pathogenic infections as well as regulate various biological pathways [40, 41]. This natural defense mechanism can be chemically mimicked to deliver synthetic RNAi molecules for therapeutic and diagnostic purposes. Since majority of the published work related to nanoscale drug delivery systems utilize siRNAs, we will detail this field exclusively in this review.

siRNA is chemically synthesized has a short RNA duplex of 19–23 nucleotides with various chemical modifications that increase its stability. siRNAs trigger sequence-specific mRNA cleavage of perfectly complementary targets [42, 16, 37]. The mechanism of siRNA-driven gene regulation, along with the high potency and safety profile of siRNAs, make them a safe drug candidate for personalized medicine [40]. Moreover, siRNAs are significantly more efficient than protein-based drugs, as they target single mRNA molecules, which underlie the translation of multiple copies of protein molecules, while protein-targeted therapies require direct inhibition of each active molecule. In addition, unlike protein-based drugs, siRNA synthesis is simple and does not necessitate cellular expression systems.

The small RNA molecule is very sensitive to degradation especially in the hostile body fluids [41, 36]. In addition, its negative charge impedes crossing biological barriers. Efficient delivery systems are therefore needed to deliver efficient amounts of small RNAs into specific tissues. Hattori et al. examined different cationic liposome formulations (DOTAP-cholesterol, DOTAP-DOPE, DDAB-cholesterol, and DDAB-DOPE) as siRNA delivery systems to lung metastasis. They systemically (i.v.) administrated DOTAP-based liposomes into mice bearing MCF-7-Luc lung metastases and found that the cationic liposomes accumulated in lung metastases and led to a gene knockdown effect while the naked siRNA accumulated in the kidneys only. All of the cationic lipoplexes exhibited primary accumulation of siRNA in the lung [43].

Kusumoto et al. exploited the influenza virus elements to construct an artificial influenza-like multifunctional envelope made of cationic lipids (DOTMA/EPC/Chol/STR-mPEG).

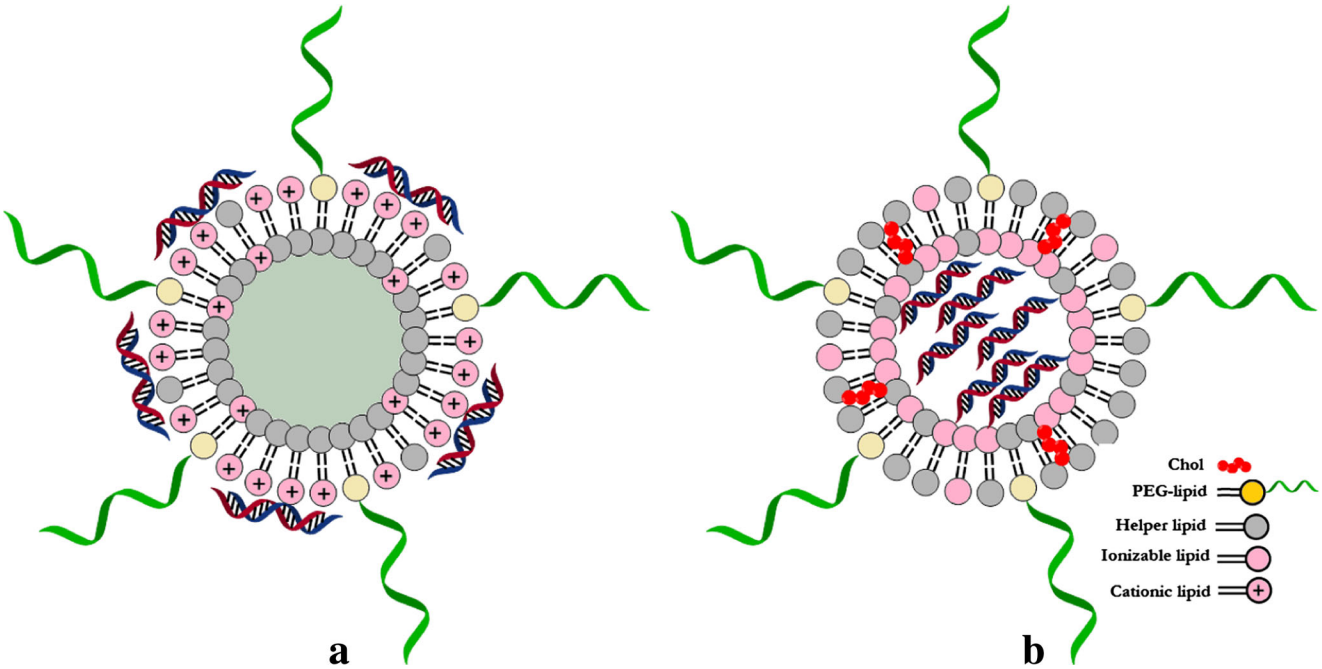


**Fig. 2** Two types of interactions between siRNA and liposomes: cationic liposomes with electrostatically complex siRNA on their surface (**a**) and siRNA entrapped in liposomes made of ionizable lipids (**b**)

For cargo, they used the CD31 siRNA as an endothelial specific marker. This lung endothelium-targeted multifunctional envelope-type nanodevice (MEND) contains surface-modified glutamic acid-alanine-leucine-alanine (GALA) peptide. The GALA peptide is similar to the hemagglutinin 2, an envelope component of influenza, which plays an important role in membrane fusion under acidic pH. The GALA peptide has a dual role as a targeting moiety to lung endothelium sialic acid-terminated sugar chains and as an efficient agent for delivery of siRNA cargo to the cytosol via endosomal membrane fusion. A significant 50 % reduction in the number of metastasis foci was demonstrated compared with the control groups. No toxic signs were measured 24 h post injection of 2 mg/kg siRNA [44].

Li et al. designed pegylated cationic liposomes made of DOTAP, cholesterol, and protamine for targeted delivery into metastatic tumors. Liposomes were loaded with luciferase siRNA through mixing the naked liposome solution with siRNA solution. Luciferase siRNA-loaded pegylated cationic NPs were decorated with a targeting moiety of anisamide, a small molecule ligand of sigma receptors, which are highly expressed on B16F10 tumors. Delivery efficiency of the targeted siRNA-loaded NPs was four fold higher than non-targeted NPs. Low doses of i.v. injected targeted NPs (150 μg/kg) caused up to 80 % knockdown of luciferase gene expression in whole lung metastasis. The formulation showed low immunotoxicity in serum cytokine induction assays [45]. In other report from same group, Wang and colleagues chose to silence CD47 for tumor treatment and lung metastasis inhibition. CD47 is overexpressed on cancer cells and used to evade recognition and phagocytosis by macrophages as cells expressing CD47 are recognized as 'self'. The proposed strategy was expected to block the anti-phagocytic signals by knockdown of CD47 expression. Indeed, intravenously delivered CD47 siRNA loaded in a anisamide coated LPH (lipid-protamine-hyaluronic acid) NPs encapsulated with CD47 siRNA to B16F10 lung tumor bearing mice, efficiently inhibited lung metastasis (27 %) compared to the untreated control mice [46].

Cationic/pegylated liposomal delivery systems suffer from toxic effects and higher doses raises safety concerns. Maksimenko et al. suggested binding Dox to squalene, a natural lipid, which increases drug stability in the circulation without the toxic side effects induced by cationic lipids. A 90 % inhibition in tumor growth was observed in squalene-Dox complex-treated pancreatic (MiaPaCa-2) and murine lung (M109) carcinomas, both highly aggressive cancer tumors and highly resistant to free Dox. In sharp contrast, only 3 % tumor inhibition was observed for the free Dox-treated cells [47]. The squalene-Dox complex did not cause cardiotoxicity, a known side effect of treatment with free Dox, in a hypertensive rat model. The squalene-Dox nanocomplex may provide a superior advantage in treating resistant cancer invasiveness and metastasis but serving both as a drug stabilizator in plasma and as a safety carrier that abolishes the cardiac toxicity of free Dox.

The limited ability of passive nano-sized liposomes to deliver their cargo to tumor and metastasis lesions and the slow release and accumulation of the drug in tumor-metastasis regions are mainly attributed to the enhanced permeability and

retention (EPR) effect; an architectural defect in tumor vasculatures causes leakiness of blood vessels and an inappropriate lymphatic drainage. This phenomena result in extended circulation times of 10–200 nm in diameter particles [48, 49] next to the invasive tissues compared to non-delayed and less prolonged circulation time compared with normal tissues [48]. In order to provide increase specificity and enable better accumulation of the drug in the cancer lesions, active targeted liposomes are being developed. The ability to recognize unique proteins that is naturally and specifically coating the tumor and metastasis surface is a powerful tool as receptor-directed delivery systems increase the therapeutic window of the drug. The most challenging liposome design is required to effectively deliver drugs capable of destroying slow-growing metastatic foci and highly proliferative tumor cells. Conjugated monoclonal antibodies (mAbs) have been harnessed to increase drug accumulation in the metastatic/tumor microenvironments and to improve the therapeutic index of the drug. Conjugated mAbs-modified liposomes can upgrade the passive delivery system by transforming it into active homing vehicles, enabling tumor/metastatic cell recognition, enhanced cellular uptake through receptor-mediated interactions and prevention of tumor/metastatic progression.

Elbayoumi et al. conjugated a targeting moiety of anticancer mAb to the surface of pegylated liposomal Dox in order to increase the therapeutic index and to obtain larger drug depots in the tumor and metastasis microenvironment. They used the nucleosome-restricted active 2C5 mAb as their targeting strategy, enabling it to recognize specific receptor that is exclusively expressed on cancer cell surface. Dox accumulation was approximately twofold higher in targeted liposomal Dox-treated Lewis lung tumor-bearing mice, compared with those treated with non-targeted liposomes, and demonstrated significant inhibition of tumor growth and lung metastases. However, the targeted liposomes were more rapidly cleared from the circulation, possibly due to the Fc portion that shortens serum lifetime [50].

The stability of the liposomes as an efficient delivery platform for large quantities of anticancer drugs may also become an obstacle since it may cause unexpected toxicities. On the other hand, too little active drug concentration in the tumor/metastasis cells below the optimal threshold level, will decrease the desire anticancer effects and may lead to multiple drug resistance by exposing the tumor cells to low levels of drugs and in fact causing an active selection that enrich the resistant tumor cells while killing the sensitive cells [51, 52].

## 3 Polymer conjugates

A versatile group of conjugate molecules can carry and efficiently deliver drugs via covalent or non-covalent carrier-drug bonds. Conjugates improve stability of attached drug and address their low solubility within the circulation. When couple with conjugates resulting in size enlargement favors its penetration and accumulation in the tumor/metastatic microenvironment through EPR effect, when compared to the free drug, which is more rapidly cleared from the circulation [53] [54, 55]. Conjugate molecules increase the tissue and plasma's half-life of the drug cargo but can also provide sustained drug release, creating optimal cellular drug concentrations with a favorable therapeutic index [53].

### 3.1 Polymer conjugated chemotherapy agents

Conjugate polymers can be attached to chemotherapeutic or antiangiogenic agents [56]. Examples for chemotherapeutic drug conjugates, which are under clinical investigation particularly for NSCLC patients, include [*N*-(2-hydroxypropyl)ethacrylamide] (HPMA) copolymer-Dox [57] and poliglume (Xyotax), a large conjugate composed from Ptx and biodegradable poly-L-glutamic acid [58]. Short arginine-rich amino acid cationic sequence cell-penetrating peptides (CPPs) are a good example of cargo delivery conjugates for a variety of drugs in a non-receptor-mediated manner [59, 60]. But this peptide-based delivery platform suffers from low specificity and a limited therapeutic effect.

Designing peptide analogues may increase therapeutic effect in lung metastasis, Fujii et al. demonstrated anti-lung metastatic effect in B16-BL6 melanoma model in mice, using modified protease resistance (retro) Arginine-Glycine-Aspartic acid (RGD) peptide. The reversed arginine amino acid residue-modified peptide caused lung metastasis inhibition effect when compared to other modified peptides as well as the non-modified RGDS peptide. They found that one reason for the antimetastatic effect is due to the inhibition of tumor invasion [61].

Like passive targeted liposomes, polymers can be designed as passive delivery drug conjugates and penetration to the cancer cells is mainly attributed by the EPR mechanism or when including a targeting moiety to increase specificity to cancer cell microenvironment via an active cellular targeting strategy [18].

### 3.2 Targeted polymers

In order to increase tumor/metastatic microenvironment drug specificity, Shamay et al. adapted the CPP "switch on" approach. The CPP octaarginine (R8) was reversibly inhibited by masking positive charges with negatively charged polyanionic molecules. To this end, the R8 CPP was attached to the HPMA copolymer-D (KLAKLAK) 2-(PR-8). P-R8 conjugates served as an efficient Dox delivery strategy. The researchers demonstrated that polyanion complexation of P-R8 turned rendered it biologically inactive. The biological activity was restored following the addition of a stronger

polycation, which led to a significant increase in survival rate in B16F10 lung metastasis mouse model [62].

An interesting strategy for targeting metastasis exploits gelatinase A/B enzymes, which are known to play a major role in the development of cancer metastasis. Rutian et al. developed a smart copolymer based on a cleavable gelatinase peptide that was conjugated to NPs loaded with Docetaxel. The high concentrations of gelatinases in the tumor microenvironment result in cleavage at the peptide site and to NP collapse, enhancing cellular uptake of the anticancer drug by the tumor cells. The gelatinase-responsive drug delivery platform demonstrated an *in vitro* antitumor effect superior to that of the free drug or of the delivery vehicle without the peptide [63]. The contradicting role of metalloproteases in metastasis formation and in inhibition of tumor progression may lead to dangerous and unpredictable results when blocking their activity.

An additional intriguing study of inhibiting metastasis formation using a targeted peptide against galectin-3 is reported. Galectin-3 overexpression on cancer cells is known to contribute metastatic tumor formation by mediating tumor cell adhesion [64]. Intravenous injection of galectin-3-target G3-C12 peptide inhibited lung colonization in human tumor-bearing nude mice by 72 %, compared to untreated mice [65]. However, this method is limited, as it can only address the early stages of metastasis, but is inadequate in preventing prothrombotic states common to metastatic cancer cells, which are a significant cause of mortality in cancer patients [66]. HA uses solely as a chemotherapy drug's conjugate target agent to cancer cells to deliver chemotherapy drug, as demonstrated by Zhao Y. et al. [67]; *i.p*. injection of HA-rapamycin conjugates into a highly metastatic CD44-positive 4T1.2neu tumor model, increased survival rate, inhibited tumor growth, and reduced lung metastasis. The HA carrier/targeting is a potential therapeutic agent for highly CD44-positive metastatic cancer cells. HA is a glycosaminoglycan molecule that when covalently attached to NPs increases their circulation time by masking it from the immune system and by providing a very hydrophilic coating [68–70]. In addition, HA is a key CD44 and CD168 ligand, both of which are highly expressed on the surface of various tumors [69–71]. Hence, HA constitutes a highly attractive targeting agent.

### 3.3 siRNA conjugated polymers

In order to increase siRNA stability, a highly stable complex composed of linear polyethyleneimine (PEI) and a sticky siRNA (ssiRNA) was designed. Bonnet et al. intravenously injected TSA-Luc mammary tumor-bearing mice with a stable complex of ssiRNA against survivin and cyclin B1, key cell cycle regulators, both involved in cell proliferation and survival processes. A strong inhibition of lung tumor metastases was demonstrated. An insignificant inhibition (<20 %) was observed with the classic siRNA-PEI complex [72]. No additive inhibitory effect was demonstrated when two genes were simultaneously targeted. The therapeutic effect of Cisplatin was increased (up to 90 % tumor inhibition) when injected after cell cycle blockage with the ssiRNA-PEI complex treatment, compared with the 40 % inhibition when Cisplatin was injected alone. Mouse survival rate following co-treatment demonstrated a similar trend [72].

Shen et al. presented simultaneous inhibition of both metastasis and tumor cells by co-delivery of shRNA together with Ptx. The master regulator 'Twist' mediated tumor metastasis by promoting epithelial to mesenchymal transition (EMT), which occurs in metastatic sites. In order to block EMT, Twist shRNA (TshRNA) and Ptx were both conjugated with pluronic P85 and PEI polymer to form the D-α-tocopherylpolyethyleneglycol 1000 succinate complex. In the pulmonary metastasis mouse model, a significant synergistic inhibitory effect of both tumor growth and pulmonary metastasis formation was demonstrated. The $IC_{50}$ of free Ptx was 63-fold higher in comparison with the TshRNA-Ptx complex. In addition, the complex had a prolonged circulation time and promoted increased accumulation of Ptx and TshRNA in lung and tumor tissues [73]. Finally, an elegant approach to conjugate siRNA to triantennary *N*-acetylgalactosamine (GalNAc) induces robust RNAi-mediated gene silencing in the liver, most probably due to an uptake mechanism mediated by the asialoglycoprotein receptor (ASGPR) on hepatocytes. This promising strategy is currently under clinical investigation [74]. It will be interesting to see if this strategy could also be applied to lung tumors and their metastases.

## 4 Future prospective

Going forward, the development of different strategies to selectively deliver drugs to lung tumors and lung metastases is dependent on understanding the tumor biology, tumor microenvironment, and the interaction between the tumor cells and subsets of leukocytes. Particulate nanocarriers and polymer conjugates already increase the arsenal of drugs available to oncologists. These are currently based on passive tissue targeting, mainly EPR, and not active cellular targeting but new strategies utilizing a specific cell surface receptor as a way to target these nanocarriers into lung tumors or lung metastases showing great promise and need to be scaled up to be able translation into the clinic. In addition, new class of drugs, from the RNA family, including small interfering RNAs, microRNAs mimic, or anti-miRs, could effectively be used to modulate the function of specific gene or family of genes and are expected to be the next generation of pathway-specific medicine. In a broad sense, using mRNA to upregulate a

specific protein such as a tumor suppressor protein or the introduction of the CRISPER/Cas9 system to edit and delete a specific gene could represent future personalized medicine in combination of pathway-dependent molecules together with classical chemotherapeutic agents. These could provide new hope for patients suffering from aggressive lung tumor or lung metastases and may personalized the treatment that currently is not offered to these patients.

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