Lipid nanoparticles for chemotherapeutic applications: strategies to improve anticancer efficacy

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Introduction: Chemotherapy remains the major form of treatment for cancer. However, chemotherapy often fails due to a variety of barriers, resulting in a limited intratumoral drug disposition. Recently, lipid nanoparticles (LNs, i.e., solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs)) have been shown to provide a favorable means for efficiently delivering drugs to tumor sites, while minimizing their side effects.

Areas covered: The delivery of drugs to tumors is restricted by a series of barriers, including the tumor abnormalities, strong adverse effects and poor specificity of cytotoxic drugs, and the induction of multidrug resistance (MDR). The present review summarizes the strategies using SLNs and/or NLCs to improve the anticancer efficacy of cytotoxic drugs, including passive targeting, active targeting, long circulating and MDR reversing. Specifically, the most significant *in vitro* and *in vivo* results on the use of SLNs and/or NLCs are highlighted.

Expert opinion: The future success of SLNs and NLCs for administration of cytotoxic drugs will depend on their ability to efficiently encapsulate and release drugs, the possibility for large-scale production, selective tumor cells targeting and increased antitumor efficacy with reduced tissue toxicity.

Keywords: anticancer drug delivery, enhanced anticancer efficacy, nanostructured lipid carriers, solid lipid nanoparticles

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1. Introduction

Today, cancer remains one of the leading causes of death and about 12.7 million cancer cases and 7.6 million cancer deaths occurred in 2008 [1]. The continuously increasing global burden of cancer presents an extraordinary challenge to the healthcare systems [2]. Over the past 20 years, great efforts have been made to improve cancer therapy. A current cancer therapy usually includes surgery, chemotherapy, radiotherapy, immunotherapy and biologic therapy. Chemotherapy plays a primary role in the treatment of malignancy that is no longer confined to one site or a limited region but has metastasized [3]. Traditionally, chemotherapy for cancer has focused on the identification of cytotoxic drugs, such as alkylating drugs, cytotoxic antibiotics, antimetabolites, plant alkaloids, topoisomerase inhibitors and other antineoplastic drugs [4]. Most of these drugs affect cell division by impairing mitosis and, thus, they can effectively target rapidly dividing cells. As tumor cells often undergo high growth fractions, they are more sensitive to chemotherapy [5]. Unfortunately, these drugs are also effective in killing healthy cells. Due to the low specificity, these cytotoxic drugs tend to have a narrow therapeutic window with high dose-limiting toxicities. In addition, they are conventionally administered close to their maximum tolerated dose [6]. These factors severely limit the clinical application of

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Article highlights.

- Chemotherapy often fails due to various barriers, including the strong adverse effects, poor specificity and induced multidrug resistance (MDR) of cytotoxic drugs and the tumor abnormalities.
- Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) provide promising prospects for improving the anticancer activity of cytotoxic drugs.
- The current trends in SLNs/NLCs mainly focus on active and passive targeting, long circulating, and MDR reversing.
- The all-in-one (targeting combined with long circulating function) LNs offer a new future direction for enhancing antitumor efficacy and reducing toxic effects of cytotoxic drugs.
- SLNs/NLCs exhibit great potential for delivering gene agents for cancer therapy, with a high transfection efficacy and low toxicity.
- Systematical investigations on physicochemical and physiological properties of SLNs/NLCs will lead to the introduction of SLNs/NLCs for clinically chemotherapeutic applications.

This box summarizes key points contained in the article.

cytotoxic drugs. Accordingly, a great deal of attention is being devoted to improving the antitumor efficiency and safety profile of cytotoxic agents, and investigating new ways to deliver both old and new therapeutic agents.

The use of lipid nanoparticles (LNs) has attracted the attention of many scientists to improve cancer chemotherapy. The relatively new systems include solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs), both of which are prepared based on lipid components other than phospholipids. Compared with other drug delivery systems, including liposomes and polymeric nanoparticles, SLNs and NLCs are potentially attractive due to their natural components and their ability to be produced on an industrial scale [7]. They can be prepared from a large variety of lipids including triglycerides, glyceride mixtures, lipid acids or hard fats and stabilized surfactants such as poloxamers, polysorbates, soybean phospholipid or lecithin. In addition, SLNs and NLCs both have important advantages, factors which make them excellent delivery systems for anticancer drugs [8].

The challenges of cancer chemotherapy will be addressed primarily in this review. In addition, the more recent trends and strategies involving the application of SLNs and NLCs as anticancer drug delivery systems to improve anticancer efficiency will be highlighted.

2. Challenges to the delivery of cytotoxic drugs to tumors

After systemic administration, cytotoxic drugs are distributed to different tissues via the blood circulation while being subjected to elimination. As demonstrated below, the delivery of drugs to tumors is influenced by the inherent and induced physiological factors, the physicochemical properties of the cytotoxic drugs and the drug carriers.

2.1 Inherent physiological barriers to the delivery of drugs to tumors

Many forms of malignant tumors are often characterized by uncontrolled cellular proliferation, which leads to abnormal tumor architecture and composition. These structural and functional abnormalities tend to be major obstacles limiting the uptake and permeation of anticancer drugs (**Figure 1**) [9]. First, the heterogeneous distribution of the tumor vasculature is a significant obstacle to the delivery of anticancer drugs [10]. As reported previously, the rapid proliferation of tumor cells usually causes blood vessels to separate, which results in collapsed vessels and reduced overall vascular density [11]. As a result, blood-borne oxygen and nutrients cannot reach the more distant cells. Hence, tumor cellular proliferation decreases as the distance from vessels increases. However, most cytotoxic drugs have toxic effects on rapidly proliferating cells. Therefore, slowly proliferating cells in the center of a tumor are less sensitive to cytotoxic drugs. In addition, the periphery of the tumor is usually highly vascularized, while the central region of the tumor is avascular [12]. The heterogeneities of the tumor vessels produce an uneven drug distribution and limit the delivery of antitumor drugs to the internal regions.

Second, tumor vessels are markedly different from normal tissues in their microscopic anatomical architecture. For instance, the blood vessels in the tumor are irregular, dilated, leaky or defective, and the endothelial cells are out of order with large fenestrations. Also, the perivascular cells and the basement membrane are frequently absent or abnormal in the vascular wall, and the lymphatic network is always impaired [13]. These unique tumor abnormities lead to increased accumulation of high molecular weight compounds or nanocarriers (such as liposomes, niosomes and SLNs/NLCs) in the tumor tissue, which is called the enhanced permeability and retention (EPR) effect. Generally, the unique EPR effect offers an opportunity for transporting more cytotoxic drugs into tumor tissue, by incorporating therapeutic drugs in nanoparticles. By contrast, fewer drugs are delivered into normal tissue lacking EPR effects. Thus, this EPR effect is considered to be a basic principle in passive tumor targeting drug delivery development. However, it also acts as a barrier since it offers an opportunity for the high extravasation and retention of macromolecules in the tumor interstitium. As a result, a tumor tissue exhibits a high interstitial fluid pressure (IFP) and a, subsequently, reduced transvascular pressure gradient. These changes have a significantly negative effect on the uptake and penetration of drug/drug carriers, while transvascular and interstitial transport is governed by convection [14,15].

Third, the composition of the extracellular matrix (ECM) of a solid tumor may be a source of frictional resistance to drug transport [16]. The ECM of a solid tumor commonly consists of a denser network of fibers, such as collagen, elastin

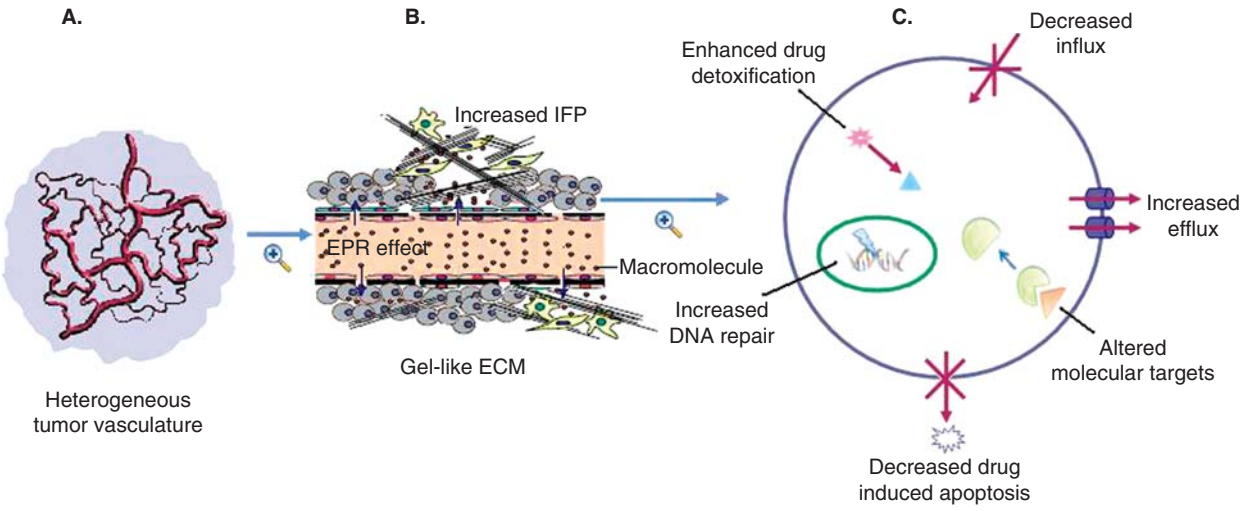

Figure 1. The obstacles limiting the delivery of anticancer drugs to tumors. A) The heterogeneous distribution of tumor vasculature leading to uneven drug distribution. **B)** Representation of tumor tissue: a) an increased IFP leads to a reduced transvascular pressure gradient and subsequently limits the transvascular and interstitial transport of drugs; b) a gel-like ECM, consisting of a denser network of fibers, and impaired interstitial drug transport. **C)** The mechanisms of MDR in tumor cells include increased drug efflux due to overexpression of drug efflux transporters, decreased drug influx, increased DNA damage repair, altered molecular targets, decreased drug-induced apoptosis or enhanced drug detoxification.
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and an increased number of fibroblasts and polysaccharides (such as hyaluronan, glycosaminoglycan). These components form a gel-like extracellular environment that impairs the interstitial transport of drugs. Moreover, a higher tumor cell density can reduce the interstitial transport of drugs. Many studies have reported that the penetration-resistance mainly occurs because the uptake by the first tumor cell layers encountered prevents drug diffusion to the subsequent layers [17]. Primeau *et al.* have shown that the concentration of doxorubicin decreases exponentially with the distance from the tumor blood vessels. Therefore, several courses of treatment need to be given to cancer patients to allow the cytotoxic drugs to diffuse to the more distant tumor cells.

Eventually, the low pH and hypoxia in the internal region of the tumor also contributes to the reduced effectiveness of cytotoxic drugs and some basic drugs are deactivated at low pH values and in a hypoxic environment.

2.2 Induced physiological barriers to the delivery of drugs to tumors

A high incidence of multidrug resistance (MDR) is a major reason for the failure of chemotherapy. MDR is particularly defined as tumor cells display resistance to a wide range of structurally and functionally unrelated drugs after treatment with one anticancer drug [18]. This can occur simultaneously or subsequently during the course of treatment. The acquired MDR is attributed to a variety of mechanisms, including increased drug efflux due to overexpression of drug efflux transporters, decreased drug influx, increased DNA damage repair, altered molecular targets, decreased drug-induced apoptosis, enhanced drug detoxification or elevated glycolipid levels (**Figure 1**) [19-21]. One of the most important mechanisms of MDR is the overexpression of transmembrane efflux transporters, such as P-glycoprotein (P-gp) and multidrug resistance-associated protein 1 (MRP1) [22]. These efflux transporters are members of the adenosine triphosphate (ATP)-binding cassette (ABC) transporter family and can produce the efflux of a broad range of cytotoxic drugs out of cancer cells, resulting in a lower intratumoral drug concentration. Consequently, tumor cells become cross-resistant and survive the cytotoxic insult even though a cytotoxic drug has marked *in vitro* efficacy [23]. High expression of P-gp and MRP1 exists inherently in tumors originating from various organs, including colon, liver, kidney, brain and pancreas. Therefore, MDR poses a significant challenge to effective chemotherapy.

2.3 Unique limitation of cytotoxic drugs

Cytotoxic drugs often display unique problems such as lack of stability, poor specificity, highly toxic effects on healthy tissue and the possibility of inducing MDR. Cytotoxic drugs work best in rapidly dividing and multiplying cancer cells. However, some normal cells divide and multiply quite rapidly, such as hair cells, bone marrow cells and cells lining the mouth and gastrointestinal tract. These may be affected by cytotoxic drugs and lead to side effects. Hence, side effects including tiredness, nausea and vomiting, anemia and hair loss are caused by almost all cytotoxic drugs. Moreover,

some agents exhibit severe selective toxicity, also known as dose-limiting normal tissue side effects, which make it impractical to achieve enhanced antitumor efficacy simply by increasing the drug dose. For example, cardiotoxicity is a well-known dose-limiting toxicity for anthracyclines and myelosuppression for taxoids [24,25]. These toxicities prevent further increase in the dosage or strength of the anticancer agent. Thus, there is an urgent need to develop suitable drug carriers, able to greatly reduce the toxicity and improve the therapeutic efficiency of cytotoxic drugs.

2.4 Widespread use of SLNs and NLCs as anticancer drug carriers

In the middle of the 1990s, SLNs began to be used for incorporating cytotoxic drugs [26]. Like other innovative carrier systems (e.g., lipid emulsion, liposome, polymeric nanoparticles), SLNs combine the advantages of physical stability, protection of labile drugs, controlled release and excellent tolerability. At the same time, SLNs avoid some associated problems, such as fast drug release of lipid emulsion, low drug capacity, high cost and drug leakage of liposomes, and the problem of large-scale production of polymeric nanoparticles [27]. Most importantly, SLNs exhibit greater versatility for loading various drugs with highly diverse physicochemical properties [26,28-31]. Besides the above physicochemical advantages, SLNs exhibit important physiological advantages, including: i) improved pharmacokinetics and drug biodistribution of cytotoxic drugs; ii) significantly improved anticancer activity of encapsulated cytotoxic drugs and iii) reduced side effects of cytotoxic drugs. Therefore, the SLNs have many benefits for the delivery of anticancer drugs to overcome some of the aforementioned obstacles.

The perfect lipid crystal matrix of SLN usually leads to low drug loading, limited adjustment of the drug release profile and undesirable drug expulsion during storage. NLC, a second generation of SLN, consisting of both solid and liquid lipids, has been developed to minimize or avoid some of the problems associated with conventional SLN [32]. NLC has an imperfect crystal or amorphous lipid matrix which allows drug loading in both the molecular form and in clustered aggregates at lattice imperfections. NLCs provide all the benefits of SLNs while, at the same time, offering the advantages of enhanced drug loading and less marked drug expulsion during preparation and storage [33]. Recent literature reports have demonstrated their potential as attractive delivery systems for anticancer drugs [34].

3. Strategies to enhance anticancer activity based on SLN/NLC and SLN/NLC-based drug delivery systems

So far, a number of cytotoxic drugs have been successfully incorporated into SLN/NLC and SLN/NLC-based systems. Preclinical studies involving cell culture or animal models have shown promising effects involving the improvement in the anticancer activity of cytotoxic drugs, along with minimizing their severe side effects. The current status and antitumor effects of anticancer drugs discussed in this review are listed in **Table 1**. The mechanisms for delivering drugs to tumors by LNs are similar to those in liposomes, including passive targeting, active targeting, long circulating and a combination of strategies (**Figure 2**) [35].

3.1 Tumor-specific targeting based on SLN/NLC drug delivery systems

As demonstrated earlier, most cytotoxic drugs exhibit narrow therapeutic windows due to a lack of tumor selectivity. Sometimes, the effective dose is close to the maximum tolerated dose for the cytotoxic agent. Therefore, the development of drug delivery systems able to target the tumor site is becoming a real challenge. Recently, SLNs/NLCs have been shown to exhibit great potential for tumor targeting. This is based on two different principles, namely, passive targeting and active targeting [36,37].

3.1.1 Passive tumor targeting

The unique feature of a tumor, the EPR effect, allows SLNs/NLCs to be able to distinguish between normal tissue and tumor tissue. The antitumor effect of SLN/NLC is usually better than that obtained with solution preparations. Tocotrienol (TRF)-loaded NLCs (TRF-NLCs) have been developed to increase their antiproliferative effects against neoplastic + SA mammary epithelial cells [38]. An improved cytotoxicity against A549 cells has also been obtained by encapsulating docetaxel in NLCs [39]. The inhibition rate of docetaxel NLCs was 90.36%, while that of commercial Duopafei® was only 42.74%, indicating that docetaxel NLCs are more effective inhibitors of tumor growth. However, the pore size of tumor vessels usually ranges from 100 to 780 nm [40], which sets the upper size limit for the extravasated LNs (< 200 nm). The effects of particle size on the EPR effect need further study.

After intravenous administration, SLNs/NLCs are quickly bound with opsonins in blood and subsequently cleared by the reticuloendothelial system (RES) within few minutes. Therefore, SLNs/NLCs are always concentrated in tissues with a rich RES, such as liver, spleen and lymph nodes. This passive targeting effect is favorable if the tumor is present in the RES. Moreover, the toxicity could be reduced because fewer drugs are distributed to other organs. For example, doxorubicin, a broad-spectrum antitumor drug with a therapeutic effect on lymph tumor, often produces serious cardiac toxicity. Three hours after i.v. administration of doxorubicin SLNs, the drug concentration was higher in the spleen and lower in the heart, compared with free drug solution. This indicated that doxorubicin SLNs exhibit lower drug accumulation in unexpected organs via the passive targeting effect [41].

Amazingly, several studies have found that cytotoxic drugs can be detected in the brain when loaded into SLNs, an indication that they have bypassed the blood–brain barrier (BBB) [41-43]. In the last decade, SLNs exhibit great potential for brain targeting, which were reviewed by Blasi *et al.* [44] and Kaur *et al.* [45]. Wang *et al.* have synthesized

Table 1. Overview, current status and antitumor effects of anticancer drugs discussed in this review.

Drug	Carriers	Focus of studies	Brief summary of the therapeutic effects	Ref.
Camptothecin	SLNs	Body distribution in mice	The AUC/dose was much higher in brain, heart and or gans containing reticuloendothelial cells	[42]
Docetaxel	NLCs	*In vitro* cytotoxicity against A549 cells *In vivo* antitumor efficacy in Kunming mice-bearing murine malignant melanoma (B16)	DTX-NLC was more cytotoxic against A549 cells The inhibition rate of DTX-NLCs increased to 90.36%	[39]
Docetaxel	SLNs	The pharmacokinetics and biodistribution in male albino rats	The drug concentration was also higher in the lung, spleen and brain, but lower in liver, heart and kidney	[41]
Docetaxel	Folate-PEG-NLCs	The pharmacokinetics in rats and tissue distribution in mice-bearing sarcoma-180 cells	Compared with the solution, the AUC_{0-t} of docetaxel was significantly increased and the MRT was prolonged. The docetaxel concentrations in tumor and kidney for folate-PEG-NLCs were significantly higher than for the solution	[67]
Doxorubicin	PEG-SLNs	The pharmacokinetics and tissue distribution in rabbits	Doxorubicin was still present in the blood 6 h after the injection of PEG-SLNs. PEG-SLNs significantly reduced the heart and liver concentrations of doxorubicin	[65]
Doxorubicin	PEG-SLNs	The pharmacokinetics and tissue distribution in rats	Doxorubicin was still present in the blood 24 h after injection of PEG-SLNs. PEG-SLN significantly reduced the heart concentration of doxorubicin	[47]
Doxorubicin	PLNs	*In vitro* cytotoxicity in MDA435/LCC6/MDR1 cells	PLNs produced a more than eightfold increase in MDR cell kill, compared with the solution	[73]
Doxorubicin	PLNs	Mechanistic study of enhanced uptake and retention in MDR cells	Some drugs physically associated with PLNs bypass the membrane-associated P-gp	[74]
Doxorubicin	SLNs	*In vitro* cytotoxicity in the P-gp-overexpressing cell line P388/ADR. *In vivo* efficacy in P388/ADR murine leukemia models in mice	SLNs resulted in a more than 10-fold greater cytotoxicity in P388/ADR cells, compared with the solution. SLNs had a longer median survival time (20 days), while that of the solution was 14.5 days	[75]
Doxorubicin	NLCs	*In vitro* cytotoxicity in MDR cells (MCF-7/ADR cells and SKOV3-TR30 cells)	The reversal potencies of MCF-7/ADR cells and SKOV3-TR30 cells were increased 6.4- and 2.2-fold, respectively	[34]

AUC: Area under the curve; Fr: Ferritin; FR: Folate receptor; 5-FU: 5-fluorouracil; HCPT: Hydroxycamptothecine; Hp: Hematoporphyrin; LNs: Lipid nanoparticles; LPN: Lipid-PEI (polyethyleneimine) hybrid nanocarrier; MDR: Multidrug resistance; MRT: Mean retention time; NLCs: Nanostructured lipid carriers; PEITC: Phenethyl isothiocyanate; PEG: Polyethylene glycol; P-gp: P-glycoprotein; siMCL1: MCL1-specific siRNA; siRNA: Small interfering RNA; SLNs: Solid lipid nanoparticles; TC: Tamoxifen citrate; TRF: Tocotrienol.

Table 1. Overview, current status and antitumor effects of anticancer drugs discussed in this review (continued).

Drug	Carriers	Focus of studies	Brief summary of the therapeutic effects	Ref.
Doxorubicin	Brij 78-based LNs	*In vitro* cytotoxicity in MDR cells (NCI/ADR-RES and MDA-MB-435/LCC6MDR1)	The LNs had six- to eightfold lower IC_{50} values in MDR cells	[84]
Doxorubicin and GG-918	PLNs	*In vitro* cytotoxicity in MDA435/LCC6/MDR1 cells	The doxorubicin and GG-918 co-encapsulated PLNs demonstrated the greatest anticancer activity in the MDR cells	[85]
Doxorubicin	SLNs	*In vitro* reversal of MDR in NCI/ADR-RES cells	The cell viability was significantly reduced to approximately 12%, a threefold decrease in viability	[90]
5-FU	Fr-SLNs	*In vitro* cytotoxicity in MDA-MB-468 breast cancer cells *In vivo* antitumor efficacy in BALB/c mice-bearing MDA-MB-468 tumors	Ferritin-SLNs had an IC_{50} of 1.28 μM, while non-targeted SLNs had an IC_{50} of 3.56 μM. Ferritin-SLNs produced an effective reduction in tumor growth of MDA-MB-468 tumor-bearing BALB/c mice compared with free 5-FU	[61]
Hp-stearylamine	FR-targeted SLNs	*In vitro* cytotoxicity in FR (+) KB cells	FR-targeted SLNs exhibited an IC_{50} of 1.57 μM in KB cells, while non-targeted SLNs had an IC_{50} of 5.17 μM	[59]
HCPT	Octreotide-PEG-NLCs	The pharmacokinetics in rats and *in vitro* cellular uptake in SMMC-7721 cells	The octreotide-PEG-NLCs had a $t_{1/2}$ approximately 10-fold longer than the free solution and the highest uptake in tumor cell (SMMC-7721)	[68]
Paclitaxel Prodrug	FR-targeted SLNs	*In vitro* cytotoxicity in KB and M109 cells *In vivo* antitumor efficacy in BALB/c mice-bearing M109 tumors	In FR (+) KB cells, FR-targeted SLNs exhibited a lower IC_{50} of 0.61 μM, and produced greater tumor growth inhibition and animal survival in mice-bearing FR (+) M109 tumors	[58]
Paclitaxel	Brij 78-SLNs F68-SLNs	The pharmacokinetics in male KM mice	Compared with paclitaxel injection ($t_{1/2}$, 1.36 h), Brij 78-SLN and F68-SLN exhibited rather slow drug elimination with $t_{1/2}$ values of 4.88 and 10.06 h, respectively	[64]
Paclitaxel	NLCs	*In vitro* cytotoxicity in MDR cells (MCF-7/ADR cells and SKOV3-TR30 cells)	The reversal power for MCF-7/ADR cells and SKOV3-TR30 cells was 34.3- and 31.3-fold, respectively	[34]
Paclitaxel	Brij 78-based LNs	*In vitro* cytotoxicity in MDR cells (NCI/ADR-RES and MDA-MB-435/LCC6MDR1)	The IC_{50} value was over ninefold lower than that of taxol in MDR cells	[84]
Paclitaxel	PEGylated Brij 78-based LNs	*In vivo* efficacy in mice-bearing NCI/ADR-RES cells	The tumor volume in NCI/ADR-RES-bearing mice hardly changed during the course of the study after i.v. injection of paclitaxel-loaded PEGylated Brij 78-based LNs	[84]

AUC: Area under the curve; Fr: Ferritin; FR: Folate receptor; 5-FU: 5-fluorouracil; HCPT: Hydroxycamptothecine; Hp: Hematoporphyrin; LNs: Lipid nanoparticles; LPN: Lipid-PEI (polyethyleneimine) hybrid nanocarrier; MDR: Multidrug resistance; MRT: Mean retention time; NLCs: Nanostructured lipid carriers; PEITC: Phenethyl isothiocyanate; PEG: Polyethylene glycol; P-gp: P-glycoprotein; siMCL1: MCL1-specific siRNA; siRNA: Small interfering RNA; SLNs: Solid lipid nanoparticles; TC: Tamoxifen citrate; TRF: Tocotrienol.

Table 1. Overview, current status and antitumor effects of anticancer drugs discussed in this review (continued).

Drug	Carriers	Focus of studies	Brief summary of the therapeutic effects	Ref.
Paclitaxel	Cationic SLNs	*In vitro* anticancer effects in KB cells and *in vivo* anticancer effects in KB cell-xenografted mice	The human siMCL1 and paclitaxel co-loaded SLNs exhibited the greatest *in vitro* anticancer effects, and significantly inhibited the growth of tumors in KB cell-xenografted mice	[95]
PEITC	Chitosan-SLN microparticles	*In vitro* cytotoxicity in a MDR Calu-3 cell line	Enhanced cytotoxicity was obtained in the presence of the efflux inhibitors	[87]
siRNA	Folate-LPNs	Uptake efficiency in FR expressing PC3 cells	Folate-LPNs exhibited a high siRNA transfection rate and selectivity on PC3 cells	[72]
TC	Tristearin SLNs	The pharmacokinetics in rats	The $t_{1/2}$ of SLNs in plasma was about 3.5-fold higher than that of the free tamoxifen	[66]
TFR	NLCs	*In vitro* cell culture studies of the antiproliferative effect in neoplastic + SA mammary epithelial cells	The IC_{50} was twofold lower than that of the reference solution	[38]

AUC: Area under the curve; Fr: Ferritin; FR: Folate receptor; 5-FU: 5-fluorouracil; HCPT: Hydroxycamptothecine; Hp: Hematoporphyrin; LNs: Lipid nanoparticles; LPN: Lipid-PEI (polyethyleneimine) hybrid nanocarrier; MDR: Multidrug resistance; MRT: Mean retention time; NLCs: Nanostructured lipid carriers; PEITC: Phenethyl isothiocyanate; PEG: Polyethylene glycol; P-gp: P-glycoprotein; siMCL1: MCL1-specific siRNA; siRNA: Small interfering RNA; SLNs: Solid lipid nanoparticles; TC: Tamoxifen citrate; TRF: Tocotrienol.

3,5-dioctanoyl-5-fluoro-2-deoxyuridine (DOFUdR) and incorporated it into SLNs (DO-FUdR-SLNs) [31]. After intravenous administration, the brain AUC (area under the curve) of DO-FUdR-SLN was 10.97- and 2.06-fold greater than that of FUdR solution and DO-FUdR solution, respectively. The overall brain targeting efficiency (TEC) of DO-FUdR-SLN was 29.84%, while the corresponding figure for FUdR solution was 11.77%. A more recent study found that significantly higher drug accumulations in brain were obtained by incorporating baicalein into tocol NLCs [46]. The brain targeting efficiency of SLNs can be influenced by the properties of SLNs, including the surface hydrophilicity, stabilized surfactant, surface charge, surface mobility and particle size. It is generally admitted that a more hydrophilic surface (especially for polyethylene glycol (PEG)-modified surface) can prevent quick clearance of particles by RES, which will also be discussed in Section 3.2. Approximately fivefold higher doxorubicin concentration in brain was found after 30 min of treatment with doxorubicin-loaded PEG-modified SLNs, compared with SLNs [47]. However, there was no detectable doxorubicin in the brain after the administration of doxorubicin solution. It is noteworthy that some commonly used stabilized surfactants for SLNs, such as poloxamer, polysorbate 80 and Brij 78, can enhance the brain drug uptake by inhibiting the efflux function of P-gp in BBB [48-51]. In addition, polysorbate 80-stabilized SLN was found to preferentially adsorb apoE and low apoCII. Thus, a high apoE/apoCII ratio was absorbed on the surface, contributing to high potential of polysorbate 80-stabilized SLNs to deliver drugs to the brain [50]. The surface charge of SLNs also has a significant effect on the brain targeting. For instance, the positively charged etoposide-loaded SLNs exhibited 14-fold higher drug deposition in brain than free etoposide and negatively charged SLNs after 4 h of injection [52]. A similarly increased brain drug uptake was also observed by incorporating clozapine or etoposide into positively charged SLNs [53,54]. Recently, an *in vivo* real-time fluorescence investigation indicated that SLNs exhibited higher brain accumulations than NLCs, which might be attributed to the higher molecular mobility of SLNs than NLCs. The higher mobility of SLNs might promote their penetration across the BBB [55]. In brief, SLNs/NLCs can improve the ability of a drug to cross the BBB and is a promising brain targeting system for treating central nervous system disorders.

3.1.2 *Active tumor targeting*

The distribution and permeability of vessels between different tumor sites may not be the same, and certain tumors do not exhibit an EPR effect [56]. An effective method (active targeting) is to attach the ligands, molecules that bind to specific receptors on the tumor cell surface, to the surface of SLC/NLCs to selectively deliver drugs to specific tumor cells. The ligands commonly used include folate, ferritin (Fr), monoclonal antibody, aptamers and peptides. Usually, folate receptors (FRs) are overexpressed in cancer cell membranes,

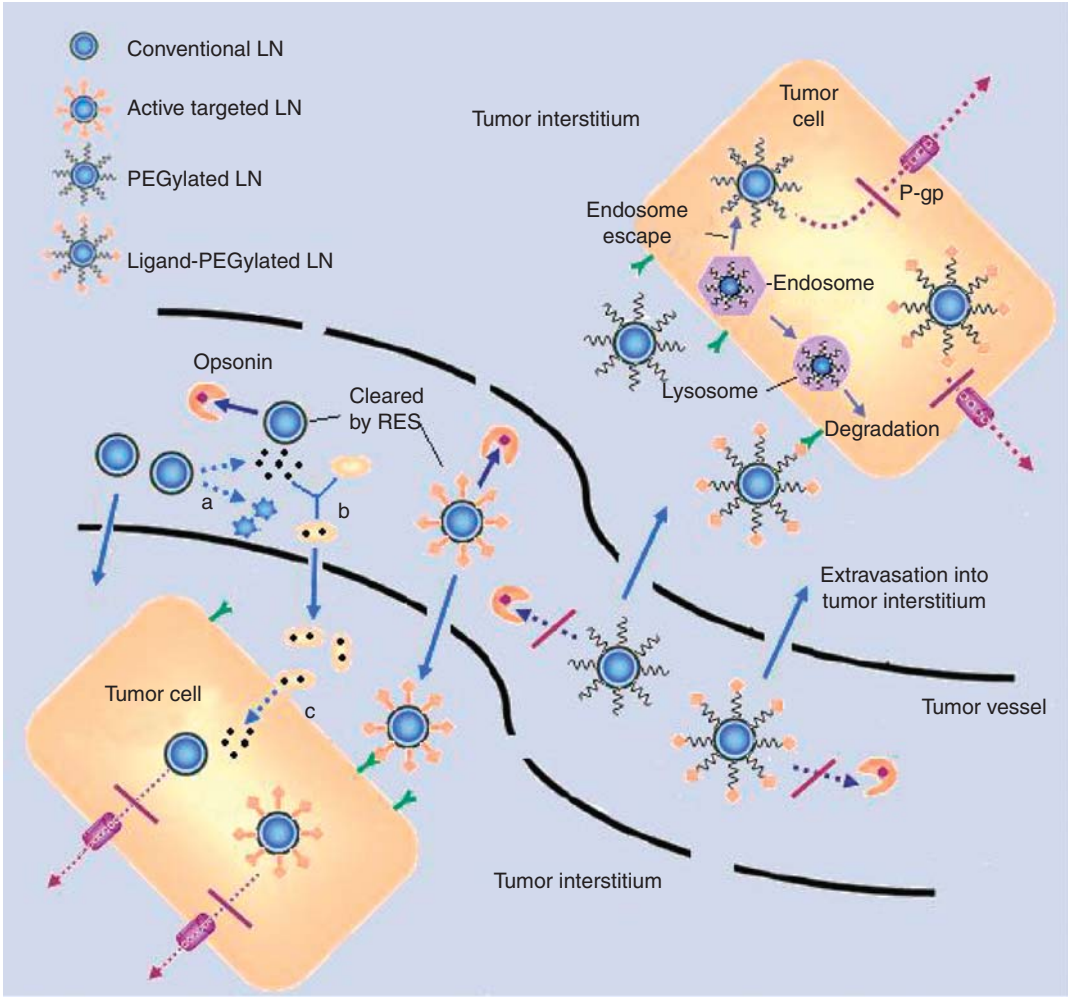

Figure 2. *In vivo* fate of lipid nanoparticles (LNs). 1) The conventional LNs and active targeting LNs, without PEG coating, exhibit a short $t_{1/2}$ due to their interaction with opsonins and are subsequently cleared by the RES, and only a small fraction of LNs could reach the tumor tissue via the EPR effect. Moreover, some LNs release drugs in plasma (a), and the released drugs get associated with plasma protein (b), entering the tumor cell by endocytosis (c). 2) The PEGylated LNs, with a surface modified by certain hydrophilic polymers, can hardly be recognized by RES. As a result, more PEGylated LNs could accumulate in tumor tissue and enter tumor cells. 3) The ligand-PEGylated LNs exhibit combined long circulating and tumor cancer-specific targeting functions. They can efficiently reach the tumor site and enter the tumor cells by receptor-mediated endocytosis. 4) Most LNs are internalized via phagocytosis. The internalized LNs allow the drugs to bypass the efflux action of membrane-associated P-gp and reverse MDR.
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which makes it possible to attach folate to the surface of LNs and, subsequently specifically target tumor cells [57]. Paclitaxel-7-carbonyl-cholesterol (Tax-Chol), a lipophilic prodrug of paclitaxel, has been incorporated into NLC, which also contained folate-polyethylene glycolcholesterol (f-PEG-Chol) as a ligand to target tumor marker FRs. In FR (+) KB (a human oral carcinoma cell line) cells, FR-targeted NLCs exhibited a greater than fourfold reduction in IC_{50} compared with non-targeted NLCs (0.61 vs 2.82 μM). Furthermore, FR-targeted NLCs showed greater tumor growth inhibition and animal survival was increased in mice FR (+) M109 tumors, compared with non-targeted NLCs [58]. A similar significantly reduced IC_{50} in FR overexpressing tumor cells was also obtained for FR-targeted SLN as a carrier of a lipophilic derivative of the photosensitizer hematoporphyrin (Hp) [59]. Transferrin receptor (TFR) is another kind of overexpressing receptor on the cancer cell membrane and, therefore, TFR may be a suitable target for cancer treatment [60]. Jain *et al.* prepared 5-fluorouracil (5-FU)-loaded ferritin-coupled SLNs (Fr-SLNs) for tumor targeting [61]. An *in vitro* cytotoxicity assay on Fr-SLN exhibited an IC_{50} of 1.28 μM, while a non-targeted SLN had an IC_{50} of

3.56 μM. Fr-SLN produced an effective reduction in tumor growth of MDA-MB-468 tumor-bearing BALB/c mice compared with free 5-FU. These findings confirm that ligand-linked LNs can efficiently target tumors. However, it should be noted that the targeted nanoparticles can be removed by the RES within minutes before they are able to bind to tumor cells. An integrated approach, which provides active targeting and long circulating LNs, will be described in the next part.

3.2 Development of long circulating SLNs/NLCs to avoid clearance by the RES

Despite the promising effect obtained with conventional LNs, their usefulness is limited by their rapid blood clearance and recognition by the RES [62]. For active targeting SLNs/NLCs, the brief circulation in the body reduces the opportunity to bind to specific receptors, and it is hard to achieve an active targeting effect. Therefore, it is necessary to prolong the circulating time of LNs, and this has been successful to some extent. The SLNs/NLCs with a surface modified by particular hydrophilic polymers is not easily recognized by the RES [63]. Some hydrophilic materials, like PEG, poloxamers or poloxamines and/or some amphipathic polymers, the hydrophobic part of which can lock into the inner of the SLNs/NLCs carrier and the hydrophilic part of which can cover the surface, are widely used. Some studies have indicated that there is a close relationship between the circulating time and the length of the polymer chain. Chen *et al*. prepared two kinds of long circulating SLNs, Brij 78-SLNs and F68-SLNs [64]. Compared with paclitaxel injection, Brij 78-SLNs and F68-SLNs exhibited rather slow drug elimination with a $t_{1/2}$ of 4.88 and 10.06 h, respectively. The PEG-DSPE (distearoylphosphatidylethanolamine) in the F68-SLNs has a longer hydrophilic chain, which can produce a stronger forbidden force on the plasma protein and prolong the circulating time. By encapsulating doxorubicin in PEG 2000-modified SLNs, doxorubicin could still be detected in rabbit blood more than 6 h after injection, while no doxorubicin was detectable when the drug solution was administered [47,65]. In the case of tamoxifen citrate (TC)-loaded SLNs, a long circulating effect was also obtained by an increased $t_{1/2}$ and prolonged mean residence time [66]. Unfortunately, due to the lack of studies on tumor-bearing mice, it is still unknown whether this kind of SLNs can lead to increased tumor drug concentrations.

Recently, more and more attention has been paid to active ligand conjugated stealth SLNs/NLCs. Docetaxel-loaded NLCs modified with an amphiphilic copolymer, folate-poly(PEG-cyanoacrylate-co-cholesteryl cyanoacrylate) (FA-PEG-PCHL), was prepared to produce a long blood circulating effect and improve the targeting ability of antitumor drugs [67]. The AUC was increased, clearance was decreased and the mean retention time (MRT) was prolonged for the FA-DTX-NLC group. The hydrophilic part PEG chains, attached to the surface of NLCs, can mask the surface charge, prevent the opsonin–nanoparticle interaction, and ensure efficient steric stabilization. The interference with the recognition by opsonin allows the drug carrier to achieve a prolonged blood circulation time. Su *et al.* developed a new conjugate, octreotide-polyethylene glycol (100) monostearate (OPMS), to enhance the targeted delivery of hydroxycamptothecine (HCPT)-loaded NLCs [68]. Octreotide was chosen to specifically bind to somatostatin receptors (SSTR) overexpressed in some tumors [69]. The OPMS-modified NLCs exhibited approximately a threefold longer $t_{1/2}$ than non-modified NLCs, and a 10-fold longer $t_{1/2}$ than free HCPT solution. Furthermore, florescence microscopy observations also showed the highest uptake for OPMS-modified NLCs in tumor cells (SMMC-7721). Interestingly, a greater degree of modification was found to lead to a longer circulating time and a higher drug uptake. This result can be explained as follows: first, with a high degree of modification, OPMS-modified NLCs exhibited a slower release rate of HCPT. Second, a higher degree of modification resulted in a greater fixed aqueous layer thickness (FALT), which could improve stability, prevent opsonization and macrophage uptake. Third, the highly modified NLCs exhibited an increased average surface density of PEG chains (SD_{PEG}), 0.592 PEG/nm^2, and a shorter distance (D), 1.30 nm, between two neighboring PEG grafting sites. The optimal SD_{PEG} for avoiding complement consumption should be around 0.5 – 0.67 PEG/nm^2 for one PEG, and a D value around 1 – 1.5 nm is suggested to avoid the adsorption of proteins [70,71]. It was appropriate for the highly OPMS-modified NLCs to maintain its resistance to RES, and have a prolonged circulation time. Further studies should be carried out on the antitumor effects *in vitro* and *in vivo*. Xue and Wong recently applied this integrated approach to deliver small interfering RNA (siRNA) for treating prostate cancer in an all-in-one manner [72]. A folate-coated lipid-PEI (polyethyleneimine) hybrid nanocarrier (LPN) achieved a longer circulation time, cancer-specific targeting, extended release and a less toxic form of siRNA therapy in an 'all-in-one' manner. The surface modification of SLN/NLC, that is, ligand-conjugated PEGylation, avoids clearance by the RES, prolongs the drug circulation time in blood, increases the exposure time of tumor cells to drug and enhances the specific targeting effect.

3.3 Strategies to overcome MDR based on SLN/NLC drug delivery systems

As mentioned in Section 2.2, MDR is one of the most serious challenges to cancer chemotherapy. The increased efflux rate of drug caused by overexpressing ABC transporters, typically P-gp and MRP1, is one of the major mechanisms of MDR in cancer cells. Therefore, current research into reversing MDR is mainly focused on blocking specific drug efflux.

Recently, SLNs and NLCs have shown potential promise to reverse MDR. Doxorubicin-loaded SLNs with a drug encapsulation efficiency of 60 – 80% and a particle size of 80 – 350 nm were prepared by Wong *et al.* for application to human MDR breast cancer cells (MDA435/LCC6/MDR1) and a mouse cell line (EMT6/AR1) [73,74]. Both cell lines were characterized by high expression of a classical membrane transporter, P-gp. Doxorubicin-loaded SLNs

showed more than an eightfold increase in killing MDR cancer cells but comparable efficacy on a wild-type cell line compared with doxorubicin solutions at the same drug concentration. A 10-fold greater cytotoxicity in a P-gp overexpressing cell line P388/ADR was found for doxorubicin SLNs with increased entrapment efficiency (> 80%) and a smaller particle size (< 100 nm) [75]. After 4 h of efflux, 15-fold more doxorubicin remained in the P-gp cells with doxorubicin SLNs compared with free doxorubicin. Furthermore, mice-bearing P388/ADR murine leukemia tumors, treated with the SLN formulation, had a longer median survival time (20 days) than animals given free drug solution (14.5 days) at a dose of 3.5 mg/kg. SLNs exhibited a significantly improved therapeutic effect in this MDR mouse model. The greater cytotoxicity compared with that in the previous study of the Wong group may be attributed to the smaller particle size. Since larger-sized particles will usually be taken up by the RES *in vivo*, particles with size less than 100 nm more easily accumulate in tumors [76].

Analogous results have also been reported for cytotoxic drug-loaded NLCs. In the study by Zhang *et al.*, NLC formulations of paclitaxel and doxorubicin were developed [34]. Compared with taxol and doxorubicin solution, the paclitaxel- and doxorubicin-loaded NLCs both exhibited high cytotoxicities in MDR cells (MCF-7/ADR cells and SKOV3-TR30 cells). The reversal power of the paclitaxel NLCs for these two kinds of cells was 34.3- and 31.3-fold, respectively, while that of doxorubicin NLC was 6.4- and 2.2-fold, respectively. Moreover, the percentage cellular uptake of paclitaxel NLC in MCF-7/ADR cells was two- to threefold higher than that of free drug solution over the same incubation time. These findings support the hypothesis that LNs (i.e., SLNs and NLCs) may be used to reverse MDR.

The mechanism of MDR reversal activity of drug-loaded LNs has been investigated and discussed in several papers. Drug in LNs probably enters tumor cells in two ways, that is, simple diffusion and phagocytosis [74]. The intracellular drug internalized via phagocytosis likely remains physically associated with LNs, which allows the drug to bypass the efflux action handled by the membrane-associated P-gp. As a result, more drug molecules are trapped within P-gp overexpressing cells after treatment with drug-loaded LNs compared with free drug solution. In addition, due to the membrane affinity of lipid materials and the nanoscale size of LNs, internalization of drug into tumor cells is enhanced, resulting in an increased intracellular drug concentration [77,78]. Moreover, it is noteworthy that some of the lipids and surfactants in LNs possess intrinsic P-gp inhibitory activities [79]. Poloxamers, usually used to stabilize LNs, were reported to enhance the cytotoxicity of doxorubicin in MDR cancer cells due to the inhibition of P-gp function [80-83]. Poloxamer is likely to inhibit P-gp through ATP depletion and membrane fluidization, which was reviewed in detail by Kabanov *et al.* [48]. Another P-gp reversing surfactant, Brij 78, was also found to enhance the cytotoxicity of doxorubicin and paclitaxel in P-gp overexpressing human cancer cells [84]. The tumor volume of NCI/ADR-RES bared mice remained almost unchanged following treatment with paclitaxel PEGylated Brij 78-based LNs. The enhanced anticancer efficiency can be partially attributed to the reduced drug efflux rate caused by the inhibition of P-gp function and ATP transient depletion of Brij 78. This positive finding (P-gp inhibitions of poloxamers and Brij 78) offers a novel therapeutic strategy to overcome MDR.

The simple encapsulation of anticancer drugs in LNs was useful but not enough to overcome the MDR problem. A few other strategies based on the SLN/NLC systems have been investigated to overcome MDR. Some researchers found that P-gp inhibitors, sometimes known as 'chemosensitizers', displayed a synergistic action with cytotoxic drugs. Wong *et al.* developed a polymer-lipid hybrid nanoparticle (PLN) system loaded with doxorubicin and P-gp-specific inhibitor GG-918 [85]. Interestingly, the greatest cytotoxicity against MDR cells was found after treating with PLNs loaded with two agents, while a lower cytotoxicity was obtained after co-administration of PLNs loaded with two single agents. The sequential or concurrent treatment of separate formulations of P-gp inhibitors and cytotoxic drugs or drug combinations cannot guarantee the co-action of drugs in the same tumor cells because of their different pharmacokinetics and tissue distribution. SLN provides a potential approach to loading cytotoxic drugs and chemosensitizer in the same carrier due to its ability to encapsulate drugs with a variety of properties [30,85]. To maximize the synergistic action, a chronological or sequential release of chemosensitizers and anticancer agents to the target site may be an alternative strategy to overcome efflux-mediated resistance. The modification of doxorubicin-loaded SLN with chitosan oligosaccharide gave the SLN some positive characteristics, such as improved drug loading, reduced burst drug release and a delayed release rate [86]. A novel chitosan SLN microparticle (CSM) system was also developed to co-encapsulate phenethyl isothiocyanate (PEITC), an antitumor agent, and efflux transporter inhibitors, such as tamoxifen, verapamil HCl or nifedipine [87]. CSM, a core shell-type delivery system, was incorporated with one fast releasing efflux inhibitor in the shell and a slower releasing anticancer agent, PEITC, in the core. The PEITC-loaded CSM produced enhanced cytotoxic expression of Calu-3 cells in the presence of the efflux inhibitors, due to the enhanced accumulation of PEITC through the modulation of the efflux transporters.

Besides the inhibition of efflux transporters, a few other strategies have also been exploited to overcome MDR. As reported by Liu *et al.* [88,89], high glucosylceramide synthase (GCS) activity also contributes to MDR, since GCS can glycosylate the pro-apoptotic ceramide to glucosylceramide. The accumulation of glucosylceramide will result in cell proliferation and MDR. Therefore, downregulation and/or blockade of GCS have been considered as a promising approach to overcome MDR and subsequently improve the cytotoxicity of conventional cytotoxic drugs [89]. Mixed backbone antisense glucosylceramide synthase oligonucleotide (MBO-asGCS), which downregulates the production of GCS, was loaded in SLNs to concurrently treat NCI/ADR-RES human ovary

cancer cells with free doxorubicin [90]. The cell viability significantly decreased to approximately 12%. This finding also suggested that SLN would be a potential carrier for genetic material. Furthermore, the aforementioned active targeting SLNs/NLCs help overcome MDR by specifically targeting cancer cells and enhancing endocytosis and, subsequently, bypassing or avoiding the pump efflux of P-gp.

3.4 Gene delivery for cancer therapy based on SLNs/NLCs

The advances in bioengineering in recent years have led to the discovery of many therapeutic genes. Increasing attention has been paid to the research and development of these genes for clinical therapy. The critical technique of gene therapy involves the development of efficient vehicles that can deliver foreign genes to target cells. Recently, the potential of SLN used as a vehicle for gene therapy has also attracted great interests highlighted in some current reviews [91,92]. del Pozo-Rodríguez *et al.* complexed the plasmid pCMS-EGFP with SLN vector and evaluated its transfection capacity *in vivo* after intravenous administration to mice [93]. This intravenous administration led to transfection in hepatic tissue and spleen. This finding indicated that the expression of foreign protein can be induced after intravenous administration of SLN–DNA vectors, supporting the potential of SLNs for gene therapy.

A novel cationic SLN has been produced to enhance p53 gene transfer to lung cancer cells. Plasmid DNA (pp53-EGFP)/SLNs complexes produce very high levels of wild-type p53 mRNA and protein expression levels in H1299 cells and enhance the efficacy of tumor growth inhibition. Hence, it is thought that a functional exogenous p53 gene could be efficiently delivered to human lung cancer cells, to subsequently induce apoptosis and inhibit tumor growth using SLNs as non-viral vectors [94]. Based on the study of Yu *et al.*, human MCL1-specific siRNA (siMCL1) and paclitaxel were co-loaded into cationic SLN (PcSLN) [95]. This kind of siMCL1–PcSLN exerted very high anticancer effects in human epithelial carcinoma KB cells *in vitro*, and significantly inhibited the growth of tumors in KB cell xenografted mice. These results demonstrated the potential of PcSLN for the development of co-delivery systems for anticancer drugs and therapeutic siRNAs.

Besides their high transfection efficiency, SLNs have other advantages as a promising gene delivery system, including low cytotoxicity [96], good storage stability, the possibility of steam sterilization and lyophilization [97]. Based on these findings, SLN-based vectors will be potential carriers of gene agents for cancer therapy, with a high transfection efficacy and low toxicity.

4. Conclusion

Cancer chemotherapy generally fails due to tumor abnormalities, severe side effects and unspecific distribution of cytotoxic drugs caused by the poor specificity, and high incidence of MDR. It is evident from the preceding discussion that SLNs and NLCs are an attractive choice as drug delivery systems for a variety of anticancer drugs. Their enhanced delivery characteristics for improved antitumor efficacy along with reduced side effects have been well documented for cancer chemotherapeutic agents. Amazingly, the smart NLCs, as the new generation of SLNs, offer much more flexibility in drug incorporation and modulation of drug release. These will be used to further improve the efficacy and side effect profiles of chemotherapeutic drugs for anticancer treatment. Furthermore, the successful surface modifications of SLNs/NLCs have opened up a new area for long circulating or active targeting.

5. Expert opinion

Various nanotechnology platforms are currently being developed with the aim of improving drug delivery for cancer chemotherapy. Among these systems, SLNs and NLCs, as relatively innovative lipid-based nanoparticles, are considered to be very versatile. The current trends in LNs mainly focus on prolonging the circulation time, developing active and passive targeting, and improving drug release profiles.

Although SLNs and NLCs are very attractive drug delivery candidates, detailed studies need to be carried out before the introduction of SLN/NLC-based formulations for clinical applications and their commercial marketing. The following issues need to be considered during the development of SLN and NLC systems for delivering cytotoxic drugs. Along with avoiding clearance by the RES, PEGylated SLNs/NLCs also reduce endocytosis by tumor cells. Meanwhile, there is often an increase in particle size that would reduce interstitial transport. It is still unknown whether, after injection into the systemic circulation, PEG polymers remain associated with the LNs. The role of the stealth technique in the pharmacokinetics of LNs needs to be better defined through more studies. Several *in vivo* antitumor efficacy studies using tumor-bearing animals indicate that SLNs and NLCs can increase the tumor drug concentration via the EPR effect. However, a Phase III trial of PEGylated liposomal doxorubicin HCl indicates that the liposomes provides a comparable therapeutic efficacy to doxorubicin, and only significantly reduced side effects due to reduced accumulation in healthy tissues lacking EPR effect [98]. The above differences between the antitumor results in animal and human trails may be attributed to differences in vasculature between spontaneous and implanted tumors. Therefore, whether SLNs/NLCs can lead to increased tumor drug concentrations via the EPR effect still needs to be investigated. A suitable predictive model to forecast the real fate of LNs *in vivo* needs to be designed for the development of LNs. Surface modification with ligands for targeting the cell surface is frequently applied for active targeting. Unfortunately, this specific binding limits further penetration of LNs into more remote tumor cells. It may be helpful to identify certain carrier-cell binding characteristics that would produce an optimal balance between selectivity and penetration. Other targeting strategies, such as targeting tumor vasculature or the

ECM surrounding the tumor microenvironment applied in liposomes, may provide new effective approaches for the development of SLNs/NLCs.

Despite the above challenges, several novel preliminary designs have shown positive results. For example, the co-encapsulation of cytotoxic drugs and a P-gp inhibitor into SLNs/NLCs provides a new approach to overcome MDR caused by P-gp-mediated cellular efflux. In recent years, an all-in-one (active targeting combined with long circulating function) NLC-based system has been designed to overcome many key limitations, such as fast clearance by the RES, and lower tumor specificity. These multifunctional PLNs represent one future direction for LNs. In addition, with the predominance of SLNs/NLCs for gene delivery, gene therapy alone or in combination with chemotherapy is becoming an important strategy for cancer treatment.

Since the application of SLNs and NLCs for cancer treatment is in the early exploratory stages, their physicochemical and physiological properties need to be demonstrated systematically. In our opinion, efforts should be focused mainly on two areas: i) industrial aspects such as formulations with controllable and stable drug release behavior, the reproduction, large-scale production and quality control of the natural components of SLN and NLC, regulatory excipient status (e.g., GRAS), and tolerability and ii) therapeutic aspects such as *in vivo* efficacy studies using tumor-bearing animals, the *in vivo* fate of LNs, clinical studies and novel approaches to enhance antitumor efficacy and reduce toxic effects on healthy tissue. It is anticipated that research and development involving SLN- and NLC-based systems will expand in the near future, with the ultimate goal being the development of SLN- and NLC-based nanoparticles that are safe, stable, efficient and cost-effective as future drug delivery systems for cancer treatment.

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Declaration of interest

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