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Peptide-functionalized liposomes as therapeutic and diagnostic tools for cancer treatment

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Abstract

Clinically efficacious medication in anticancer therapy has been successfully designed with liposome-based nanomedicine. The liposomal formulation in cancer drug delivery can be facilitated with a functionalized peptide that mediates the specific drug delivery opportunities with increased drug penetrability, specific accumulation in the targeted site, and enhanced therapeutic efficacy. This review aims to focus on recent advances in peptide-functionalized liposomal formulation techniques in cancer diagnosis and treatment regarding recently published literature. It also will highlight different aspects of novel liposomal formulation techniques that incorporate surface functionalization with peptides for better anticancer effect and current challenges in peptide-functionalized liposomal drug formulation.

Keywords

Peptide; liposome; cancer; nanotechnology; bio-nanoparticle; active targeting; peptide-functionalized liposome; diagnostic liposomes

1. Introduction

Globally, cancer is a major public health concern; in the United States, it is the second-leading cause of death [1]. Cancer treatment with chemotherapeutic agents usually causes serious, undesired side-effects due to the harmful effects of chemotherapeutics on multiplying cells from normal, noncancerous tissues. Inefficient delivery is the leading cause of decreased drug effectiveness as well as serious adverse effects on non-targeted organs [2]. Therefore, researchers are now focusing on developing novel advanced drug delivery systems to improve the therapeutic index of chemotherapeutic agents by enhancing drug delivery and enabling the drug to reach a specific site of action with the total loaded amount [3, 4]. Progress in nanomedicine has enabled the design of nanoparticles consisting of different organic or synthetic materials for the diagnosis and treatment of cancer [5].

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Liposomes are nanoparticles that are capable of introducing both lipid-soluble and water-soluble drugs [6]. This encapsulation protects the drug from rapid degradation and reduces drug toxicity by making it unavailable to the systemic circulation. Liposomes can also improve the therapeutic index of a new or established drug by changing the drug's pharmacokinetic properties such as absorption and metabolism, increasing its biological half-life, or reducing its elimination. It has a hydrophilic exterior/interior and a hydrophobic layer that can enable it to incorporate various drugs and facilitate delivery to the desired site. To utilize this specialized vehicle, several anticancer drugs (including marketed) have been formulated to have special targeted delivery options.

Liposome surfaces can be functionalized using various methods to provide different beneficial properties for target-mediated cancer therapy, including increasing the stability of the entrapped compound while increasing the selectivity towards cancer cells. Liposomes can have specialized properties by virtue of their outer layer properties. These can achieve specific selectivity toward targeted cancer cell types, including specialized stimuli characteristics. Currently, various liposomal formulations are being developed, comprising various therapeutics compounds together with surface functionalization with active peptides to have specific delivery characteristics while maintaining their stealth property. Peptides as targeted agents enable the liposomal formulation technology to produce selective delivery characteristics in both treatment and diagnostic regimes while maintaining the biological properties of the peptide.

At present, liposomes have become one of the most popular delivery vehicles as an experimental model and commercially as a drug delivery system as they have low toxicity, biodegradability, biocompatibility, entrapment capacity of both hydrophilic and lipophilic drugs [7], and ease of target-specific drug delivery to malignant tissues [8]. Targeting particular cells and reduction of drug toxicity in the liposomal formulation has encouraged researchers to perform numerous studies focusing on liposome-based drug delivery [9–11]. The solubility of poorly water-soluble antitumor drugs can be increased through liposomes to improve existing cancer treatment regimens. Additionally, long-circulating liposomes can decrease the uptake by mononuclear phagocyte systems (MPS), which eventually increases a passive accumulation into the tumor area. Attaching targeted ligands (e.g., peptides) to the liposomal surface can also help to achieve active-tumoral targeting [12, 13]. The size of the nanoparticles has an effect on passive accumulation and effective retention on the targeted site of action (Fig. 1). These methods lessen drug deterioration and metabolic inactivation of a drug after treatment. Also, the amount of drug delivered and drug's bioavailability within the affected area increases, thus improving efficacy and/or reducing drug toxicity [14].

Peptide-functionalized liposomes that are tagged with imaging agents can be used to selectively and efficiently deliver diagnostic agents to the targeted site. For diagnostic purposes, peptide liposomes targeting particular receptors [16–18], irradiation-mediated diagnostic imaging with peptide-targeting liposomes [19], and peptide-conjugated theranostic liposomes possessing both therapeutic and diagnostic effects [20] can be effective methods for the detection of cancers at different stages. Some of these can be used for the development of personalized medicines [21, 22].

This review focuses on the advances in the last decade in liposomal formulation systems functionalized by active peptides for targeting effect containing therapeutic and diagnostic agents in the cancer treatment regime. The active peptides are mainly used in the reported liposomal formulations, either as a targeting agent (with or without biological actions) or cellular penetration facilitator, carrying chemotherapeutic and diagnostic agents into the targeted sites. In different segments we also cover some perspectives regarding peptide as a liposomal targeting agent, different properties of liposomal drug delivery system and specialized peptide-factionalized liposomal drug delivery systems for anticancer therapy. Surprisingly, very few attempts have been made to review peptide-functionalized liposomal systems covering different novel techniques and treatment approaches in anticancer therapy. Some of the previous reviews discussed particular targeting sites or particular treatment regimes with peptide-functionalized liposomal systems [23–25]. The current review comprehensively addresses different aspects of peptide-functionalized liposomal systems in anticancer therapy with recent updates, which we believe will present an overall scenario of novel anticancer approaches with targeted peptide-functionalized liposomal formulation systems and provide formulation scientists with an update on progress in this approach.

1.1 Bio-nanoparticles for targeted therapy

Microparticles and nanoparticles can also be used as an effective strategy for increasing oral bioavailability of peptides and proteins by protecting them from pH variations in different sites and enzymatic degradation from different proteolytic and other enzymes [26]. Macromolecules such as peptides or proteins can be encapsulated inside the polymeric carrier, which will protect them from pH and enzymatic degradation and also facilitate their absorption and control their release into the desired site [27, 28]. Nanoparticles can have sizes ranging from 1 to 1000 nm. However, a particle size around 200 nm is greatly desired for different drug delivery approaches [29, 30]. Nanoparticles can be generally classified as either nanospheres, a matrix system where a drug is evenly spread physically, or nanocapsules, in which the drug is encapsulated in vesicles of a polymeric membrane. The drug release and physicochemical properties of nanoparticles depend on the method of preparation [30, 31]. These can be pursued for targeted delivery of drugs and controlled drug release by changing the polymeric properties and surface chemistry [26]. Since peptides have the ability to bind to receptor proteins in the cells or on the surface of cells, peptides can be incorporated into liposomes and used for targeting or guiding the liposomes to particular target receptors or cells. A review of the literature suggests that peptides are used in liposomal delivery along with other drugs to enhance the effectiveness of the drug for treatment. Hence, a majority of the examples covered in this review are related to peptides that are used for enhancing the delivery of chemotherapeutic drugs and consequently increasing the therapeutic efficacy of drugs using peptide-conjugated liposomal formulations.

2. Formulation strategies for liposome-based drug delivery: peptide mediated receptor targeting for anticancer drug delivery

Liposomes are artificially prepared micro spherical vesicles composed of naturally derived phospholipids [10]. One of the major concerns regarding peptide drug delivery is *in vivo*

stability. Peptides are enzymatically degraded by various protease enzymes along with other enzymes, reducing their bioavailability [26]. Methods are being investigated to improve the plasma stability of peptides to increase their biological effect. Protection for peptides and proteins in the GI tract can be achieved to some extent through a lipid-based bilayer drug delivery system [40]. Encapsulation in the liposomal bilayer of proteins and peptides can prevent their degradation. In peptide-functionalized liposomes, PEGylation of the liposomal surface helps to protect the attached targeting peptide from degradation. Liposomes can also reduce the toxicity of the encapsulated drug by delivering the drug to the specific target site. This feature can be achieved through various processes, and peptide or peptidomimetic incorporation for the target-specific release is becoming a popular method for targeted drug delivery.

The phospholipid bilayer is the main structural feature of conventional liposomes. Currently, liposomal formulations are modified through a different mechanism, such as surface functionalization of the lipid layer. This bilayer has an amphiphilic nature with a hydrophilic polar head and lipophilic backbone. These liposomal nanoparticles have electrostatic potential, which also plays a role in stability as segregated particles can be negatively or positively charged. The overall charge of the liposome depends on the nature of the electrostatic potential of the polar head region. Two of the main lipid components used for liposomal formulation are natural or synthetic double-chain lipids (composed of a glycerol backbone and polar phosphate group); another component is sterols, e.g., cholesterol.

The lipids that are commonly used are either zwitterionic, such as phosphatidylcholine and phosphatidylethanolamine, or negatively charged, e.g., phosphatidylglycerol. Some positively charged lipids (e.g., 1,2-dioleoyl-3-trimethylammoniopropane (DOTAP) and *N*-[1-(2,3-dioleyloxy)propyl]-*N,N,N*-triethylammonium (DOTMA)) are commonly used for the delivery of genes due to their interactive property with the negatively charged deoxyribonucleic acid (DNA) [32] and negatively charged cell membrane phospholipid layer.

Degradation pathways for phospholipids in aqueous liposomal dispersions can be divided into two distinct categories–oxidative and hydrolytic degradation. Liposomal oxidative degradation can be prevented by the incorporation of completely synthetic and saturated phospholipids such as 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoylsn-glycero-3-phosphocholine (DPPC), and, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC). The level of oxidation of lipids can also be minimized by following some precautions during the preparation and storage of liposomes, which can affect the performance as well as create adverse effects on the phospholipid bilayer. Such initiatives include using freshly purified lipids and distilled solvents, taking care to avoid oxygen in the manufacturing processes, minimizing the production of high heat during various procedures, using nitrogen to deoxygenate the aqueous solutions, incorporation of antioxidant, e.g., α-tocopherol, as a component of the lipid layer, and storing the liposome suspensions in an inert environment [33].

One of the important components of liposomes is cholesterol, which has a modulatory effect on the characteristics of the lipid bilayer of the liposomes. It can maintain the stoutness of

the liposomal structure [34] and enhance the compactness of the bilayer by increasing the rigidity between the lipid bilayer structure containing a phospholipid molecule [35]. This structural integrity provides orderly arrangement of the bilayer in the hydrophobic region of the bilayer of the liposome while reducing micro polarity [36], providing lipid bilayer rigidity by reducing the flexibility of the encompassing molecules (particularly water-soluble molecules), and enhancing the micro viscosity of the lipid bilayers through compaction [37]. Cholesterol also plays a role in the morphological stability of liposomal formulation from gastrointestinal environmental stress through its rigidifying property [37]. Liposomal size can also be modulated through the incorporation of cholesterol into the liposome (liposomal size, including shape transition, increases with an increased amount of cholesterol); this incorporates fluidity and permeability, which eventually controls the release of water-soluble compounds from liposomes [38].

Another strategic factor in providing various advantageous properties and reducing some limitations of the liposome is enabling surface functionalization through various active components. To increase the stability of liposomes in the blood with increasing circulation time, polyethylene glycol (PEG) has been used to provide stealth properties in various formulations, including marketed drugs [39]. Other surface-active agents such as peptides, proteins, antibodies, aptamers, ligands, small molecules, or carbohydrates can be attached to the liposome outer layer for specificity and selectivity. These selective components can also be attached for targeted delivery of imaging agents (e.g,, Gd-DOTA-DSPE for MRI) for diagnostic purposes [40].

Peptide conjugation to the liposomal system has become one of the major parts of the nano-drug delivery technique in cancer therapy. This procedure helps to develop a targeting liposomal system with the feature that the peptide targeting functionality is conserved due to its attachment on the liposomal outer layer (Fig. 2). Most of the peptides have the structural and biological functionality to confer a targeting effect toward the tumor site. Peptide-functionalized liposomal formulations reported in the literature are targeted toward an extensive number of selective receptors that usually are overexpressed on the cancer cell, tissue, or cancer vasculature. These peptide targets can be differentiated into three distinct categories such as G-protein-coupled receptors (GPCRs), growth factor receptors (GFRs), and integrin receptors ($\alpha_v\beta_3$ and $\alpha_v\beta_5$) [23]. Neuroendocrine peptides are used as a target for GPCRs, which are membrane receptors overexpressed in tumor tissues such as gastrin-releasing peptide (GRP) receptors, gastrin/cholecystokinin (CCK_A and CCK_B), and somatostatin (SSTR2 or SSTR5), and neurotensin type 1 receptors (NTS1). A popular peptide target in liposomal formulation is the growth factor receptor family such as epidermal growth factor receptor (EGFR/ErbB/HER); among the four types of the tyrosine kinase receptors, some subtypes are overexpressed in different subclasses of cancer. One of the ideal cancer vasculature targets is integrin receptors that are overexpressed on tumor vasculature and angiogenic endothelium [24].

A somatostatin hormone analog octreotide (OCT), a cyclic octapeptide, was reported the first time in 2008 in the liposomal formulation to target somatostatin receptor type 2 (SSTR2), a receptor subtype overexpressed vastly in small-cell lung cancer and breast cancer. The study reported a liposome formulation coupled with OCT on the liposomal

surface and containing a natural anticancer agent cantharidin. *In vitro* and *in vivo* results indicated increased antitumor efficacy of the formulation with reduced drug toxicity [41]. A study reported an OCT-conjugated liposome containing two anticancer agents, doxorubicin (DOX) and platinum complexes. The results indicated that prepared liposomes represent a new target selective cargo system for delivery of platinum based drug (e.g. cisplatin) and cytotoxic drug DOX on cells overexpressing the SSTR2 and SSTR5 somatostatin receptors [42]. Another combinational stealth liposomal system comprising OCT loaded with DOX and combretastatin A-4 (CA-4) efficiently entrapped the drugs and provided a synergistic anticancer effect with targeted therapy [43]. Bombesin (BN) peptide, which targets GRP receptors overexpressed in various human cancers, e.g., pancreatic, ovarian, and breast cancers, had been used in the lipid layer of theranostic liposomes in two different formulations [44, 45]. Another peptide, CCK8, was utilized in two liposomal formulations containing DOX where the CCK8 peptide was targeted to selectively bind to cholecystokinin subtype receptors A (CCK1-R) and B (CCK-2R) [46] where CCK1-R has been observed to be highly overexpressed in certain neuroblastomas, gastroenteropancreatic neuroendocrine tumors, and meningiomas, while CCK-2R was found to be overexpressed in small-cell lung cancers, astrocytomas, thyroid cancers, and stromal ovarian cancers [47].

The EGFR receptor family (EGFR, HER-2, and HER-3) has been found to be overexpressed in various forms of cancers such as non-small cell lung cancer (NSCLC) [48] and breast cancer [49]. Two separate liposomal formulations were prepared to entrap doxorubicin and vincristine distinctively with surface modification by αHER-2 Fab' fragments with targeted Asn-Gly-Arg (NGR) peptides to deliver the drug selectively into endothelial cells in tumor vasculature and tumor cells against HER2-positive breast cancer [50]. EGFR-targeted-peptide ligand D4 was detected through virtual peptide library screening and used with a liposomal formulation in which the EGRF-targeted peptide was conjugated with polyethylene glycol (PEG) moiety to insert into the liposomal layer [51]. These liposomes can also be applicable for the treatment of other solid tumors. Recently, HER-2 targeted peptide P6.1 (KCCYSL) in three multimeric forms conjugated with liposomes was investigated for selective delivery into the HER-2-overexpressed cell lines BT474 and MDA-MB-231. The P6.1 peptide, derivatized with different metal chelators (DOTA, NOTA, CB-TE2A, DAP) and labeled with radionuclide metal ions (^{111}In, ^{64}Cu, ^{99m}Tc), has been successfully used for *in vivo* imaging of HER-2 overexpressing tumor models [52–54]. Magnetic MRI experiments also revealed the potential of the liposomal formulation to function as a diagnostic tool [16].

Different integrin receptors such as $\alpha_4\beta_1$ [55], $\alpha_5\beta_3$ [56–59], and $\alpha_5\beta_1$ [60], have been targeted with peptide-functionalized liposomes loaded with different anticancer drugs such as doxorubicin (DOX) [61–63], paclitaxel (PTX) [59], cisplatin (CDDP) [64], docetaxel (DTX) [65], and 5-fluorouracil (5-FU) [66]. Peptide-functionalized liposomal formulations carrying anticancer agents have been used to target $\alpha_5\beta_3$ integrin which is overexpressed in different types of cancer, including lung cancer [57], glioma [58], and metastatic breast cancers [56].

3. Features of liposomes making them excellent targeting agents in tumors

Anticancer drugs can be delivered at the tumor site by passive targeting effect. Larger molecules and nanoparticles, such as liposomes, are accumulated in tumor tissues due to a characteristic feature of a tumor known as enhanced permeation and retention (EPR) effect [67]. The passive targeting of tumors by these nanoparticles is based upon the EPR effect, which is due to the nature of leaky vasculature resulting from rapid and defective angiogenesis in tumors shown in Fig. 3 [68, 69]. Other passive targeting is achieved using tumor microenvironment such as low pH at tumor site or using different stimuli that responds to the tumor microenvironment. Liposomes can become an effective drug delivery system by taking advantage of this effect.

3.1 EPR Effect:

Cancer cells interact with the immune system cells in such a way that it supports the growth of malignant cells instead of suppressing the cancer cell. Such an interaction between cancer cells and various immune cells forms a tumor microenvironment (TME) [70]. Most of the non-malignant cells in TME include the immune cells, tumor vasculature, lymphatics cells, fibroblast, and adipocytes, which secrete proteins and cytokines that play a vital function in advance and progress of cancer [71]. The vasculature system in tumor tissues has significant differences compared to the control. The blood vessels in the tumor tissues are heterogeneous and their functions are significantly impaired. The vasculature system is flawed with the deficiency of the basement membrane, and the blood vessels are varied in diameter, irregular forms, and bulges [72, 73]. The neovascularization in tumor tissues is the development of new blood vessels also called angiogenesis. This process ensures that the tumor receives adequate nutrition and oxygen from the blood supply [74]. The newly formed vasculature in tumor tissues, which is due to the defective vasculature supportive tissues, leads to the formation of leaky vessels and pores through endothelial gaps varying in diameter from 100 nm to 2 μM. Also, there is a compromised lymphatic system, which leads to poor waste removal from the tumor tissues, resulting in high retention and accumulation. This phenomenon of high permeability due to leaky vasculature and retention in tumor tissues is called an EPR effect. This EPR effect of the cancer cells can be used for passive targeting of therapeutic agents, nanocarriers for both anticancer effect and diagnostic purposes [75].

Knowledge of the EPR effect has enabled several efforts in the development of targeted delivery of the drug to the tumor site. Nanoparticles such as liposomes can be used as targeting agents as they are highly accumulated and retained in tumor tissues compared to normal tissues due to the EPR effect resulting from leaky and unusual vasculature. Moreover, the liposomal drug delivery system enables the escape of drug molecules from high renal clearance, increasing their half-life *in vivo* significantly and thereby increasing the chances of targeting effects on tumor tissues through the EPR effect. Mononuclear phagocyte system (MPS) in the liver and spleen can reduce the EPR effect of the nanoparticles, leading to poor delivery of drug molecules. The protein binding and nanoparticle aggregation may have a detrimental effect on their delivery by the EPR effect.

The effect of MPS on the liposomal drug delivery system can be resolved by employing the surface modification method on liposomes [77]. This might also help in solving the aggregation and protein binding problem and keeping the liposomes in blood circulation for a longer time. Coating the liposomes with polymers such as polyethylene glycol (PEG) has produced longevity in blood circulation. Rapid removal of liposomes from the circulation is prevented by the coating of PEG as it protects the liposomes from the MPS. As a result of its protection, liposomes remain in blood circulation for a longer period of time, which is required for efficient accumulation in the tumor [78]. A very detailed study on the EPR effect, its factors, and the mechanisms by which it is controlled is required for further knowledge on its use for the delivery approach of the liposomes and other nano-delivery approaches. Delivery of small molecules as a therapeutic and diagnostic approach for tumor-specific targeting is full of challenges owing to its rapid renal clearance, accumulation in normal tissues, and toxicity. Hence, nanoparticles systems such as liposomes can be designed as both passive and active targeting delivery systems to target cancer cells specifically through the EPR effect. The EPR effect will significantly limit the renal clearance and toxicity that makes liposomes superior delivery agents [79]. Targeting the tumor cells using the EPR effect must consider several factors, including particle size and shape, tumor perfusion, vascular permeability, vascular penetrations, retention and drainage from tumor tissues, and, cancer types. [80].

3.2 Liposomes making use of local stimuli for release of the drug:

Liposomes, because they are one of the most widely studied nanocarriers for targeted drug delivery, seem to have promising applications in stabilizing therapeutically active compounds, increasing the cellular and tissue uptake, and providing better biodistribution of therapeutic agents to target sites *in vivo* [81–83]. Tumor cells have a very peculiar nature in terms of their microenvironment and receptor expression. Such features of the tumor cell can be utilized for the formulation of better therapeutically efficacious liposomes. The tumor microenvironment has some unique features, including low pH, higher temperature, and unique enzymatic activity, that can serve as an endogenous stimulus. External stimuli like heat and light-triggered approaches have also been used for drug delivery approaches [84].

3.2.1 pH-triggered drug release by liposomes: The tumor microenvironment has lower pH (acidic conditions) compared to normal tissue environment; this is the result of excessive metabolites, mainly lactic acid and CO_2. This lower pH has been exploited for targeted drug delivery approaches using pH-responsive liposomes and nanoparticles. These pH changes can trigger the drug delivery from liposomes by protonation or deprotonation of lipid membranes, causing the destabilization of the lipid bilayer [85]. pH-sensitive lipids such as DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine) have polymorphic phase behavior that facilitates drug delivery by adopting the hexagonal state at lower pH, promoting the destabilizing of the lipid bilayer [86]. Dual pH-targeting liposomes have been used to significant effect to target the tumor cells specifically. Different types of lipid composition are formulated for pH sensitive liposomes [87, 88]. Apart from lipids, the use of pH-dependent peptides enables the release of liposomal contents at a lower pH [89].

Conventional liposomes exhibit slow release of the constituents after the internalization in the cell because of the PEG barriers. To prevent this problem, stimuli-responsive liposomal preparations can be introduced, which can degrade and release its constituents only upon exposure to certain conditions (Fig.4). pH-sensitive liposomes (pHSLip) have garnered success in enhancing liposome-mediated drug delivery, which is designed to release the active constituents based on the pH conditions of the microenvironment.

It is known that the average pH of the extracellular cancer microenvironment is between 6.0 and 7.0, while in normal tissues, it is 7.4 [91, 92]. This low extracellular tumor pH comes from the high glycolysis rate in cancer cells, which are more hypoxic than normal cells [93]. Hence, by utilizing pH-sensitive polymers and lipids, liposomes that can release the encapsulated active drugs in the presence of a low pH extracellular tumor microenvironment can be prepared. Hence, these pH-sensitive liposomal formulations overcome the limitations of the lack of selectivity of anticancer agents. Further, with the EPR effect of the liposome, they increase the efficacy of therapeutic entities such as siRNA, drugs, or radioisotopes.

Zhao et al. [87] reported that a tumor-specific, pH-responsive liposome could be prepared and targeted to the acidic environment of gliomas, where the pH-responsive liposome responds to the environment and triggers release of the drug (DOX). DOX-PSL-$H_7K(R_2)_2$ liposomal formulations were designed using $H_7K(R_2)_2$-modified pH-sensitive liposomes and evaluated for antitumor activity in mice with glioma tumor cells. It was observed that the liposomal formulation was able to deliver the active constituents of DOX in the acidic condition efficiently in *in vitro* and *in vivo* experiments.

Other important strategies reported that could be employed to target the acidic tumor extracellular environment include the use of cell-penetrating peptides (CPPs) and antimicrobial peptides (AMPs) based on liposomal formulations. Ding et al. formulated a liposomal formulation using PEG200-Hz-stearate on its surface along with CPPs. When it was compared with the PEGylated conventional liposomes, it was observed that the DOX accumulation in tumors increased up to 1.9-fold over the conventional liposomes and resulted in more cell apoptosis [94]. A similar strategy was also reported by Zhang et al., where a pH-responsive antimicrobial peptide was incorporated in the liposomal formulation; it was found that below pH 6.3, the liposome showed significantly improved efficiency in comparison to other liposomal formulations. Incorporation of paclitaxel (PTX) into the modified liposome further enhances the cellular toxicity [95]. Mozhi et al. described a liposomal formulation in which amphiphilic copolymer poly (β-amino esters)-poly (ethylene glycol) is conjugated with an antimicrobial peptide while DTX is encapsulated in the formulation. They found that once the liposome reaches the inside of the acidic endosomal compartment, the stimuli-responsive micellar carriers disassemble and release both peptides as well as DTX, which produces the antitumor effect [96].

3.2.2 Temperature-triggered release by liposomes: Hyperthermia is observed at the site of inflammation in tumor tissues compared to normal tissues [15] [15, 97]. Hence, this strategy can be utilized for the preparation of liposomal formulations in cancer therapy to increase drug tumor permeability and enhanced drug uptake. The hyperthermia has a profound effect on tumor permeability and drug uptake, and this property has been utilized

in the formulation of temperature-sensitive liposomes. Various studies have shown that local heating of tumor sites increases vascular permeability, blood flow, and pore size that results in enhanced extravasation of liposomes in tumor sites. Temperature-sensitive liposomes have been prepared from thermosensitive lipids and polymers that exhibit low critical solution temperature. The temperature at the tumor site is usually higher than the critical solution temperature of polymers, leading to precipitation of polymers and destabilization of lipid bilayers and releasing the liposomal contents [98].

In this technique, heat is applied to the tumor site, which causes an increase in microvascular pore size and blood flow and results in improved extravasation of drug-loaded nanocarriers. These types of temperature-sensitive liposomes can be prepared from thermoresponsive polymers or lipids with a low critical solution temperature (LCST) [99]. Some examples of temperature-sensitive polymer are poly (N-isopropylacrylamide (PNIPAM) and dipalmitoyl phosphatidylcholine. The polymer becomes water-soluble below its low critical solution temperature due to the formation of hydrogen bonds between the polymeric chain and the water molecules. Once the temperature is above the low critical solution temperature (generally at a tumor site), the polymer becomes insoluble and precipitates, disrupting the liposomes to release the drug [100]. Table 1 depicts some of the recent examples of thermosensitive liposomal formulation strategies used in cancer treatment.

3.2.3 Ultrasound-responsive peptide liposomes: In this approach, the tumor regions are exposed to an ultrasound wave, leading to localized and complete release of the active drug from the liposomal formulation. By utilizing low-frequency ultrasound (LFUS), the drug can be released from stabilized liposome formulations without disturbing its active physicochemical properties. In recent years, by using high intensity focused ultrasound (HIFU), local heating was induced, which causes a phase transition of the polymers and releases the drug from the liposomes [101]. The high-frequency sound waves can damage the liposomes and are able to produce chemical reactions that ultimately cleave chemical bonds between the peptides and the polymers [102]. Xie et al. [103] reported an ultrasound-responsive liposomal formulation using a combination of cell-permeable peptides and ultrasound strategy. Here, CPP-CPT conjugation was encapsulated in the liposome. Once the liposomes accumulate near the tumor region due to the EPR effects, ultrasound irradiation releases the CPP-CPT peptide-drug combination into the cells [103]. In another example in the literature, the cell-penetrating peptide-doxorubicin conjugate (CPP-DOX) was incorporated in peptide-modified nanobubbles (CPP-DOX/NGR-NB) and the penetration of CPP-DOX was temporally masked. It was found that the CPP-DOX/NGR-NB with ultrasound treatment? exhibited greater cytotoxic activity than that without ultrasound [104].

3.2.4 Magnetic field-responsive peptide liposomes: Magnetic liposomes use the magnetic force for the site-specific delivery of chemotherapeutic agents and maintain them at the site until the drug is completely released [105]. In this case, liposomes are magnetized by incorporating magnetites, such as Fe_3O_4 or γ-Fe_2O_3, that are less than 10 nm in size [97]. Applications of these liposomes include magnetic hyperthermia, transfection, and manipulation of cells and proteins. Because of their magnetic properties as well as their nanoscale size, they are also called superparamagnetic iron oxide nanoparticles (SPIONs)

[15]. Once an external magnetic field is exposed to the liposomes, the liposomes loaded with a drug with a ferromagnetic material can be guided to a particular target site where they release the drug [106].

3.2.5 Enzyme responsive liposomes: Elevated expressions and altered compositions of several enzymes such as matrix metalloproteinases (MMPs) are distinctive features of the tumor microenvironment that is hugely involved in the pathogenesis, invasion, metastasis, and progression of the tumor. In particular, MMP-2 and MMP-9 enzymes are found in the extracellular matrix sites of different types of cancers such as breast, colorectal, pancreatic, and lung [147]. These altered enzymes can be biomarkers for diagnosis of the types and stages of tumors and provide the platform for targeted specific delivery of nanoparticles such as liposomes by enzyme-triggered mechanism [107, 108]. Extensive design and use of MMPs responsive peptides, proteins, and polymers for delivery of drugs and imaging agents to the tumor have been studied [109]. In one of the studies of a multifunctional liposomal carrier composed of MMP-2-responsive components, it was shown that it significantly enhanced the tumor-targeting effect and internalization of the liposomes in the tumor [110]. Studies of cathepsin B- and D-responsive liposomes, glucose oxidase-triggered liposomes, and other enzyme-responsive liposomes are promising [111–113].

Site-specific enzymatical activation of prodrugs is another liposomal-targeted therapy to specifically target the tumor cells. In this case, an unfunctionalized prodrug, which can be converted to a cytotoxic drug by certain enzymes present on the extracellular site of tumor cells, is administered to the tumor site [114]. MMP-sensitive peptides are incorporated into liposome formulations, where the peptides act as linkers between the polymer and the lipid. The polymer hinders the uptake of liposomes due to a physicochemical barrier effect. Once the liposomes are in contact with MMPs at the tumor site, the peptide is cleaved, which leads to the cleavage of the polymer and is followed by uptake of the liposomes. Zhu et al. [110] reported a liposomal formulation containing two different types of lipopolymers: mAb 2C5-PEG(3400)-MMP2-cleavable peptide-1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and TATp-PEG(2000)-1,2-dioctadecanoyl-sn-glycero-3-phosphoethanolamine (DSPE). The DOPE forms a protective shield over the DSPE lipopolymer containing the TAT peptide. Once the peptide is cleaved at the tumor site, the long-chain PEG will emerge and remove its shielding effect. The hydrolysis of the liposome leads to the contact of the TAT peptide and increased uptake of liposomes [110].

Some other stimuli (external and internal)-responsive liposomal formulation strategies such as redox/thiol responsive liposomes [115, 116] and light-responsive liposomes [117–119] have been reported in the literature.

4. Peptide-functionalized liposome-mediated delivery approaches in cancer therapy

Functionalized peptides are being attached to the surface of liposomal formulation for both treatment and targeting purposes, which eventually increases the therapeutic efficacy of cancer treatment. These procedures also maintain the stability of the peptide drug, as well as in the case of cancer therapy, facilitate the targeted delivery of liposomal components into

the tumor site. Different drug molecules are encapsulated or attached along with the peptide-functionalized liposomes for the combinational effect or synergistic effect (in addition to targeting) in different treatment purposes (Fig. 5) e.g., multidrug-resistant (Table 2).

4.1 Doxorubicin-loaded peptide liposome:

Peptide-functionalized liposomal formulations have been prepared as a cargo to deliver various chemotherapeutic agents to cancer cells. Anthracyclines such as doxorubicin (DOX), daunorubicin (DNR), and their derivatives are widely used in various hematological and solid tumors; they are considered to be a first-line therapy for breast cancer, but can be associated with both cumulative and irreversible cardiotoxicity [64]. Also, cardiotoxicity can increase approximately fourfold if these are administered with other chemotherapeutic drugs [65]. For these reasons, the development of peptide-functionalized liposomal formulations is the focus in clinical and preclinical studies aimed at reducing the acute and cumulative cardiotoxicity, as well as mitigating other drug-related side effects (e.g., nausea, bone marrow depression, and alopecia) [66] while increasing drug efficacy. Doxorubicin (DOX) has been encapsulated in liposomal formulation alone or with other active components, facilitating targeting or synergistic effects. A targeted liposome was used to prepare arginine-rich cell-penetrating peptides (AR-CPP), which is a general approach for active tumor targeting. In a study by Deshpande et al., eight arginine units (R8) were used to attain optimal chain length for efficient translocation. In this study, dual factionalized liposome was prepared with surface modification through R8 and transferrin (Tf) to target A2780 ovarian cancer cells with R8-mediated intracellular delivery of DOX. In a different *in vitro* study, the researchers had found that dual DOX liposomes showed enhanced cytotoxicity. In comparison to other treatments, this dual DOX liposome was more effective in mitigating the tumor growth *in vivo* in an A2780 ovarian xenograft model [139].

Targeting liposomes to the vascular epithelial growth factor receptor 2 (VEGFR2) was done using a novel affinity peptide S1, which could specifically recognize and bind to the VEGFR2 receptor. The liposomal formulation was an effective nanoscale drug delivery system reported *in vitro* and *in vivo* by Han et al. Peptide S1 has 9 amino acids in the sequence, and the S1-functionalized liposomes (S1-LS) containing DOX showed promising VEGFR2-targeting drug delivery, which could be an effective method for cancer therapy and diagnosis [17]. In another study, peptide-conjugated DOX liposome has been prepared to recognize and bind effectively to the Claudin 7 (CLDN7) marker protein. In this study, the gastric tumor model was established with a tumor xenograft mouse model and an initial ionizing radiation dose was given, followed by a phage-displayed peptide library injection. Tightly bound peptides were recovered as well as a counterpart protein that was consequently recognized. The prepared peptide-conjugated liposome showed substantial improvement in therapeutic efficacy with the possibility of irradiation-mediated diagnostic imaging [19].

A promising approach for efficient drug delivery for cancer treatment was achieved through a spatiotemporally controlled heat-triggered ultrasound-mediated mild hyperthermia and thermosensitive liposome (Fig. 6).

Um et al. proposed an alternative mild hyperthermia approach for site-specific drug delivery because the conventional thermal approach can cause vascular damage, which decreases the extravasation of the drug into the tumor tissue and affects the therapeutic effectiveness in follow-up treatments. In this study, fatty acid-conjugated elastin-like polypeptide (FELP) had been used to prepare thermosensitive liposomes (FTSLs) that have a long blood circulation time with selective collapse at or above 40°C and have a synergistic effect together with mild hyperthermia for enhanced anticancer efficacy. In this system, unlike thermal-mediated drug delivery, which causes irreversible damage to the tumor vasculature; the use of acoustic treatment increased the permeability of tumor vessels for a short time through the acoustic cavitation effect along with the release of DOX in the tumor tissue. Thermal and non-thermal treatments on extravasation of the DOX-FTSLs and DOX were compared; *in vivo* near-infrared fluorescent (NIRF) dye live imaging of tumor tissue was conducted at 12 h post-injection of Cy5.5-DOX-FTSLs (Fig. 7) The researchers revealed that non-thermal acoustic treatment may be a safe alternative to thermosensitive liposome-involved hyperthermia for liposomal chemotherapy [140].

A pH-responsive liposomal formulation, which contained peptide $H_7K(R_2)_2$ as a targeting agent and also possessed a cell-penetrating peptide (CPP) characteristic in acidic conditions, was investigated. This peptide $H_7K(R_2)_2$-transformed pH-responsive liposome containing doxorubicin (DOX-PSL$H_7K(R_2)_2$) was investigated for targeted activity toward glioma tumor cells *in vitro* and antitumor properties *in vivo*. A selective targeting effect initiated by an acidic pH environment was found in these *in vitro* experiments in U87-MG and C6 glioma cells. pH-sensitive release of DOX from pH-responsive liposome was also confirmed by *in vitro* drug release in the study carried out in different pH conditions. Antitumor properties of DOX-PSL$H_7K(R_2)_2$ were found in the U87-MG orthotopic tumor-containing mice and C6 tumor-bearing mice examined in *in vivo* experiments [87]. Dai et al. reported liposome containing an integrin $\alpha_5\beta_1$ antagonist N-acetyl-proline-histidine-serine-cysteine-asparagine-amide (Ac-PHSCN-NH_2) as a novel targeting peptide, which is in clinical trials for cancer therapy. The liposomal formulation (PHSCNK-PL-DOX) also contains doxorubicin as a chemotherapeutic agent. PHSCNK-PL-DOX showed improved intracellular incorporation as well as enhanced cytotoxicity against melanoma B16F10 cells compared to control DOX-only liposome PL-DOX. This targeted formulation showed promising results in terms of better tumor inhibition and increased survival time compared to a control in an *in vivo* study, at the same time demonstrating lowered cardiovascular toxicity in tissue analysis. The pharmacokinetics and biodistribution results indicated that the integrin $\alpha_5\beta_1$ antagonist-incorporated liposomal formulation could be used as a potential therapeutic agent for targeted cancer therapy [141].

In an *in vivo* study by Ding et al., novel pH-sensitive liposomes CPPL(DOX) with doxorubicin-loaded cell-penetrating peptide (CPP) were studied in BALB/c nude mice containing tumors of human breast cancer. This study revealed some detailed information regarding the distribution of liposome CPPL(DOX) *in vivo* with histological examination on tumors as well as organ tissues including other bioaccumulation studies. The results showed that enhanced DOX buildup in tumors reaches up to 1.9-fold $(p<0.01)$, causing a relatively lower tumor growth ratio as well as more tumoral cell apoptosis through DNA disruption. Histological evidence showed promising signs indicating the absence of any inflammation or

necrosis on normal tissues. In contrast, large cellular damage with dissolving areas was found in tumor tissue on animals treated with CPPL(DOX) [94].

To overcome the problem of crossing the blood-brain barrier (BBB) when delivering the drug to the brain, glioma-targeted, dual-modified liposomes (DOX-TATAng-LIP) were formulated. Angiopep-2, a targeted ligand for low-density lipoprotein receptor-related protein-1 (LRP1) overexpressed in both glioma and BBB cell membranes was employed. TAT peptide was used to facilitate the penetrability of the liposomal formulation. *In vitro* results indicated that the drug DOX-TATAng-LIP not only facilitates permeability through transcytosis across BBB but increases glioma necrosis upon selective accumulation into the glioma cells [142].

The efficient delivery of the drug into the glioma is a challenging mission, and liposomal formulations have been successfully studied for this purpose. Glioma-targeted liposomal formulation have been prepared to facilitate the delivery of drug load into the tumor crossing the BBB and the blood-brain tumor barrier (BBTB). Proteolytically stable peptides ^{D}CDX and c(RGDyK) have been used; ^{D}CDX is a nicotine acetylcholine receptor (nAChRs) D-peptide ligand on the BBB and c(RGDyK) is an integrin ligand overexpressed on the BBTB and glioma cells. DOX-loaded double peptide-conjugated liposomes produced better survival rates with inhibition of glioma in glioma-bearing mice. The result suggested that the proposed liposomal formulation with multiple peptide ligands can achieve glioma-targeted delivery of DOX, crossing multiple barriers and accomplishing improved therapeutic efficacy of DOX treatment for glioma [143].

4.2 Daunorubicin-loaded peptide liposome:

Daunorubicin is an anthracycline antibiotic that is used as a chemotherapeutic agent for different types of leukemia. A daunorubicin (DNR)-loaded therapeutic liposome was also evaluated in various cancer treatment approaches. Low-density lipoprotein receptor (LDLR) overexpression in acute myeloid leukemia (AML) cells was targeted by a novel peptide AA_{13} (14 amino acids) that selectively binds to LDLR. As a general approach of linking the peptide to the liposome outer layer for targeted effect, AA_{13} was attached to the DSPE-PEG_{2000}-maleimide by the distal end connection. The AA_{13}-conjugated liposomes showed LDLR selectivity, including enhanced DNR cytotoxicity in AML cells. An *in vivo* study in BALB/c nude bearing the human acute promyelocytic leukemia cell line, NB4 xenografts revealed enhanced drug accumulation with DNR-loaded targeted liposomes in tumors compared to untargeted liposome, increased inhibition of tumor volume, and elongation in survival times [153].

DNR and emodin separately loaded dual-targeted liposomal formulations were evaluated in breast cancer therapy; they were modified with Arg_8-Gly-Asp (R_8GD) peptide for selective accumulation and destruction of vasculogenic mimicry (VM) channels expressing in most proliferating cancer cells. The combination therapy effectively inhibited MDA-MB-435S cancer cells as well as VM channel formation, decreasing cancer cell metastasis [154]. Dioscin is a natural steroid saponin and is known to induce apoptosis in cancer cells [169]. Combinational liposomal delivery of DNR and dioscin with surface treatment with a neutral cell penetrating peptide PFVYLI (PFV) was also applied for the improvement of targeted

tumor therapy with inhibition of tumor cell metastasis. The incorporation of dioscin mediates the augmentation of the repressing effect of DNR on lung cancer cell A549 and vasculogenic mimicry (VM) channels as well as tumor metastasis [155].

4.3 Docetaxel-loaded peptide liposome:

Docetaxel (DTX) is a cytotoxic chemotherapeutic agent used in the treatment of various cancers. Peptide molecules have been attached to liposomes for selectivity or other functional effects along with DTX as an anti-neoplastic agent. Yoon et al. reported the use of RIPL (IPLVVPLRRRRRRRRC) peptide for targeted delivery of RIPL peptide-conjugated liposomes (RIPL-L) into hepsin-expressing cancer cells. RIPL-L facilitated the accumulation of the liposome into the cancer tissue examined by fluorescent-conjugated probe. Compared to free DTX, liposome formulation DTX-RIPL-L showed significantly increased activity in tumor growth inhibition as well as lengthening the survival time of the experimental BALB/c nude mice bearing SK-OV-3 cell tumors [146].

Bi-functional liposomes prepared with two lipopeptides carrying docetaxel for targeting integrin ($\alpha_4\beta_1$) and ephrin (EphA2) receptors was also reported with improved activity of docetaxel in melanoma cells. Both the reported peptide-conjugated lipid molecules C_{16}-LDV and C_{16}-YSA (LDV and YSA are peptide molecules) were entrapped in three modified liposomal formulations LDV-DX (only LDV conjugated), YSA-DX (only YSA conjugated), and Lip. C16-LDV-YSA-DX or TL-DX (both LDV and YSA conjugated) containing docetaxel as an anticancer agent (Fig 8).

The therapeutic efficacy of docetaxel was significantly increased by the use of targeted liposomal formulations. Compared to DTX alone, TL-DX showed significant apoptotic death of melanoma cells, microtubule bundle formation, cell cycle arrest in G2/M + S, and inhibition of multicellular 3D melanoma spheroid growth. In fluorescence microscopic images, upon treatment of A375 cells with DX, LDV-DX, YSA-DX, and TL-DX liposome solutions and solution of DTX, the researchers found that TL-DX significantly increased intracellular microtubules bundle-like structure formation compared to only DX or with other liposomes, indicating that TL-DX significantly increased the efficiency of DTX into the melanoma cells (Fig. 9) [55].

Liposomal therapeutic efficacy was enhanced by incorporating active targeting agents as reported by Patel et al. An oral antifibrotic agent (telmisartan) was used to study its effect on tumor uptake and antitumor activity of a liposomes-targeting EphA2 receptor. Histidine-tagged EphA2 receptor-specific peptide (YSA) was used as a targeting agent to entrap docetaxel-loaded PEGylated liposomes (DPL) functionalized with a nickel-chelated phospholipid. The study revealed that pretreatment with telmisartan successfully increased tumoral uptake of liposomes. This approach had a promising effect that can be utilized with different types of solid tumors to give potent antitumor effects by compromising tumor barriers [147].

Combinations of drugs were also used in liposome formulations with dual-targeted peptides to deliver an effective anticancer effect. A study was reported by Yang et al. on dual peptide-modified liposomes in which docetaxel and siRNA were used in combination with two

receptor-specific peptides—specifically, neuropilin-1 receptor (tLyP-1) and low-density lipoprotein receptor-related protein receptor (Angiopep-2)—to target brain tumor and accumulation in the tumor (Fig. 10). This study revealed the co-delivery of two different drugs of different solubility characteristics, one a large hydrophilic molecule and the other a small lipophilic molecule. The first one involves endocytosis and subsequent release from endosome or lysosome, whereas the latter enters through passive diffusion of lipophilic small drugs upon release. This combinational peptide-modified liposome binds effectively to glioma cells, which facilitates internalization through specific receptor-mediated endocytosis and was found to be more effective than non-modified or single modified liposomes due to dual functionality of loading VEGF siRNA and DTX [148].

Liposomes also acted to facilitate drug retention properties of elastin-like polypeptide (ELP)-based self-assembling micelles and hybrid ELP/liposome nanoparticles, which have the characteristic of self-assembly in response to changes in temperature with a high loading of DTX and slow release have been reported by Zhang et al. They contained a gastrin-releasing peptide (GRP) on the outer surface working as a targeting ligand to target gastrin-releasing peptide receptor (GRPR), which is frequently overexpressed in prostate cancer cells. The researchers developed nanoparticles that showed rapid self-assembly in PBS at physiological temperature, and DTX was successfully entrapped into the nanoparticles at high concentrations. The reported nanoparticles had the potential for tumor retention by GRP activity, which was demonstrated *in vitro* using PC-3 cells in flow cytometry experiments. It also significantly reduced the cell viability of PC-3 cells [149].

4.4 5-Fluorouracil-loaded peptide liposome:

5-fluorouracil (5-FU) is an antimetabilite nucleoside analog used to treat various cancers. Different strategies have been used to facilitate the delivery of 5-FU to the target site as an effective therapeutic approach. In a recent study by S. Lakkadwala and J. Singh, a dual-functionalized liposomal delivery system was assessed to face the major hurdle of transporting the drug to the brain and crossing the BBB. For the enhancement of transport of the anticancer chemotherapeutic drug, 5-FU, across the BBB into the tumor cells, conjugation of the cell-penetrating peptide penetratin to transferrin-liposomes (Tf-Pen-conjugated liposomes) was evaluated. An *in vitro* cellular uptake study showed that the dual-functionalized liposomes are capable of higher cellular uptake in glioblastoma (U87) and brain endothelial (bEnd.3) cells monolayers. Similarly, higher apoptosis was found in U87 cells due to the action of dual-functionalized liposomes. This dual-functionalized liposome was also tested across a co-cultured endothelial barrier into a scaffold housing 3-d glioblastoma tumors and showed enhanced delivery of this therapeutic agent compared to the control [150].

A fibronectin-mimetic peptide was also used as a targeting agent in a PEGylated stealth liposome to target colon cancer cells. A novel peptide PR_b (peptide-amphiphile sequence) was designed to imitate the cell-attaching domain of fibronectin to target integrin $\alpha_5\beta_{1,}$ which is expressed in various cancer cells including colon cancer. 5-FU was used as a cytotoxic agent in the liposomal formulations as well as a positive control in various studies,

which revealed that the formulation was effective in targeted delivery and in delivering the therapeutic load directly to the colon cancer cells [66].

Angiogenic endothelial cells were targeted through YIGSR peptide overexpressing laminin receptors attached in liposomes (YIGSR-SL). Liposome formulation YIGSR-SL showed significant effectiveness against lung angiogenesis and metastasis [151]. A similar study was done with cyclic RGD peptide targeting angiogenic endothelial cells which have a_vb_3 integrins overexpression and deliver the 5-FU to the targeted site [152].

4.5 Paclitaxel-loaded peptide liposome:

Paclitaxel (PTX) is another important drug in cancer chemotherapy. It is an alkaloid that prevents endothelial cell motility, proliferation, and mitotic spindle assembly, and also stabilizes microtubules [67]. The prototype of PTX was from a natural source [68] and has been used for decades in various types of cancer including ovarian, head and neck, breast, and non-small cell lung carcinoma [69]. As of now, numerous peptide-functionalized liposomal formulations containing PTX have been investigated as effective anticancer therapies. A recent study reported by Yin et al. showed that autophagy inhibition could help to reduce tumor growth and increase chemotherapeutic efficacy. The researchers prepared liposomes (PTX/HCQ-R8-dGR-Lip) modified with R8-dGR peptide co-loaded with PTX and hydroxychloroquine (HCQ) for targeted delivery by selective recognition of neuropilin-1 receptors and integrin αvβ3 receptors on B16F10 melanoma cells (Fig. 11). *In vitro* and *in vivo* analyses revealed that (PTX/HCQ-R8-dGR-Lip) showed more significant antitumor effect synergistically along with anti-metastatic effect. The results signified that a combination of chemotherapeutic drugs PTX and HCQ with R8-dGR peptide-modified liposomal formulation can have both autophagy-dependent and independent effects that may be useful as a promising treatment for primarily as well as metastatic melanoma [156].

Recently, a dual-active targeting liposomal formulation (Glu6-RGD-Lip) containing PTX focused on bone metastatic breast cancer was studied. For an efficient distribution of PTX into the desired site of action, a unique bone-directed glutamic oligopeptides-RGD peptide (Glu6-RGD) was designed and used as a liposomal ligand. It was found in *in vitro* and *in vivo* studies that Glu6-RGD-Lip formulation showed activity superior to that of PTX alone or only single-modified liposomes [56]. Another study with RGD peptide containing PTX co-loaded with curcumin was carried out for the treatment of lung cancer. It was found that a PTX and curcumin co-loaded RGD-modified liposomal formulation showed an improved antitumor effect *in vivo* compared to non-modified liposomes [57].

To overcome the multiple hurdles faced to deliver the drug into the glioma, one of the aggressive forms of malignancy, a PTX-loaded and TR peptide-modified liposome formulation (PTX-TR-Lip) was investigated. TR peptide is an integrin $\alpha_v\beta_3$-specific vector with a pH-responsive cell penetration attribute that facilitates permeation across the BBB. The *in vitro* results showed increased accumulation of TR peptide-conjugated liposome (TR-Lip) in the targeted site through transport across BBB, destruction of VN channels, and destruction of brain cancer stem cell (CSC) and glioma cells. An *in vivo* study revealed that TR-Lip could substantially increase glioma targeting and elimination of CSC as well as VM channels in tumor tissues. PTX-TR-Lip administration increased mean survival time

significantly compared to free PTX or other controls, which signifies its effectiveness as a therapeutic approach to brain glioma [170]. In another study, glioma targeting was also challenged through incorporating cyclic RGD peptide as targeting agent conjugated with a cell-penetrating peptide R8. Cyclic RGD peptide was conjugated to R8 through amide bond to obtain a tandem peptide R8-RGD [RRRRRRRR-c(RGDfK) The study showed that R8-RGD peptide-conjugated liposome (R8-RGD-lipo) had increased cellular uptake (~30-fold) compared to controls. *In vivo* study showed higher PTX delivery capacity of R8-RGD-lipo in C6 glioma-bearing mice and selective accumulation in glioma foci. The multifunctional peptide R8-RGD showed a unique approach to tackling difficult BBB drug transportation and targeting, while liposomal formulation provided the added advantage of selectivity demonstrated by *in vitro* and *in vivo* studies [59].

Pancreatic cancer treatment was evaluated with a dual functional lipid-albumin liposome formulation targeting pancreatic ductile adenocarcinoma (PDAC). PDAC has a glut of cancer-associated fibroblasts (CAFs) producing tumor stroma, which hinder drug delivery into the pancreatic tumor tissue. The small nanoparticle of PTX with albumin (HSA-PTX) that has substantial tumor penetration capacity was encapsulated in a FAP-α-responsive peptide CAP-conjugated thermosensitive liposome (CAP-TSL). Additionally, a photothermal compound IR-780 was incorporated into the liposomal formulation (CAP-ITSL) to give thermosensitive properties. The formulated HSA-PTX-loaded CAP-ITSL formulation produced efficient combinational photothermal chemotherapy, which could facilitate complicated PDAC treatment [157].

Enhanced chemo-immunotherapy was assessed against melanoma through the mechanism of cholesterol esterification inhibition in $CD8^+$ T cells. T-cell receptor development occurs with the help of cholesterol on CD8+ T lymphocytes (CTLs), which is cytotoxic to tumor cells initiating necrosis and apoptosis. pH-sensitive TH peptide-modified PTX and αGC immunoadjuvant co-entrapped liposomes (PTX/αGC-TH-Lip) were prepared, in which an acetyl-CoA acetyltransferase-1 (ACAT-1) inhibitor avasimibe was co-administered as chemo-immunotherapy [158]. The results indicated that the avasimibe and PTX/αGC-TH-Lip combinational therapy is a promising approach to upregulate the antitumor effects of chemo-immunotherapy by decreasing the suppression of $CD8^+$ T cells [158]. PTX and DOX combinations were studied for the targeted treatment of melanoma facilitated by transferrin and TAT-co-functionalized liposomes (Tf/TAT-PTX/DOXLP). *In vivo* study revealed the highest distribution into the targeted site of Tf/TAT-PTX/DOX-LP compared to other controls. Furthermore, dual PTX and DOX therapy showed a synergistic effect with enhanced therapeutic efficacy in melanoma treatment [159].

A lipophilic cell-penetrating peptide (CPP) with lower toxicity was incorporated-resulting in liposomal formulation PFV-Lip-PTX containing PTX was investigated in breast cancer treatment. *In vitro* and *in vivo* studies demonstrated that PFV-Lip-PTX produced reduced systemic toxicity and acted as a potential therapeutic candidate for tumor-targeted therapy in breast cancer treatment [160]. A combined liposomal strategy was reported by Xia et al., where PTX loaded, peptide-functionalized pH-sensitive liposome (PTX-TH-Lip) and losartan-loaded liposomal formulation (LST-Lip) were co-administered. Tumors consisted of a dense collagen circuit, which hinders the transport of drugs into tumors. Losartan can

inhibit this collagen network, but it could cause hypotension; consequently, it requires targeted administration that is mediated through liposomal formulation in this study. The results indicated that the combinational strategy could potentially exert enhanced therapeutic efficacy in breast cancer therapy [161].

4.6 Mitoxantrone loaded peptide liposome:

The peptide-functionalized liposomal formulation prepared by using mitoxantrone (MXT) [162], an anthracenedione antineoplastic drug, is commonly used in the treatment of different types of cancer, for example breast cancer, lymphomas, leukemias, and prostate cancer [171]. Studies indicated that MXT showed cardiotoxic potential, although less severe than free DOX due to its diverse mechanism of action [172]. In a recent study, luteinizing hormone-releasing hormone (LHRH) receptor-specific peptide conjugated liposomes containing MXT (LHRH-MTO-LIPs) revealed enhanced anticancer properties with inhibition of breast cancer cell growth *in vivo* compared with non-targeted MTO-loaded liposomes (MTO-LIPs) and free MXT [162].

5. Diagnostic agent-loaded peptide liposomes

Targeted delivery of diagnostic agents to tumors represents a significant advance in cancer diagnosis. Several studies have reported peptide-functionalized liposomes loaded with diagnostic agents–sometimes along with drug molecules–for cancer diagnosis and therapy. Song et al. reported dual-targeted paramagnetic liposomes functionalized with two angiogenesis-targeting peptide ligands, the αVβ3 integrin-specific RGD (Arg-Gly-Asp) and the neuropilin-1 (NRP-1) receptor-specific ATWLPPR (Ala-Thr-Trp-Leu-Pro-Pro-Arg) (A7R). Effective encapsulation of MRI contrast agent Gd-DTPA (gadolinium-diethylenetriamine pentaacetic acid) was achieved using prepared dual peptide-functionalized liposomes. The results showed that the highest SER (signal enhancement rate) was achieved through using dual targeting liposomes in MR imaging of mice bearing A549 cells *in vivo*. It was also found that dual-targeted liposomes were likely to exert a synergistic effect on the specificity of delivering Gd-DTPA to the tumor site and might be a potent system for molecular imaging of tumors [163].

RGD peptide-functionalized magnetoliposomes (cRGD-MLs) have shown great potential as magnetic resonance imaging contrast agents and as delivery vehicles for cancer therapy as evaluated by Ribeiro et al. It was found that cRGD-MLs can be visualized by both MRI and fluorescence imaging (FLI), although FLI was less sensitive due to lower depth penetration. Magnetoliposomes (MLs) has potential as a theranostic agent for the follow-up of antivascular therapies as they target tumor neovasculature [173]; however, T2-weighted fast spin echo MRI revealed some limitations due to potential misguiding changes in background signal (T2 values) caused by physiological changes (edema, hemorrhages, etc.) [164].

In a recent study, Panikar et al. reported novel peptide-conjugated ligand-targeted nanoliposomes (LTLs) for chemo-photodynamic therapy against HER2-positive breast cancer. The LTLs carried in their hydrophilic core methylene blue (MB)-attached NaYF4:Yb,Er upconversion nanoparticles (UCNPs) for NIR-activated bioimaging and leveraging visible emission for photoexciting MB for enhanced photodynamic therapy

(PDT). In addition, for chemotherapy, it contained DOX in the core as well. The *in vitro* and *in vivo* data suggested greater efficacy of LTLs as theranostic agents of breast cancer management [165].

Wang et al. developed a structure-based approach with various computational and analytical techniques to optimize cancer-targeting peptides for molecular imaging and therapy. A targeted L-peptide (12 amino acids) was identified to specifically target GRP78, a specific cancer cell-surface marker. Then the researchers conducted *in vitro* binding studies with cell lines and clinical cancer specimens, and *in vivo* tumor imaging and targeted chemotherapeutic studies. MicroSPECT/CT imaging was done to observe the uptake of peptide attached rhenium-188 (^{188}Re) radioisotope conjugated liposomes (^{188}Re-liposomes) and found greater uptake efficiency compared to control group. It was reported that the structure-based optimization strategy for peptides can be an effective tool for developing peptide drugs for cancer imaging and therapy [166]

In another study, a tetradecapeptide bombesin (BBN), which specifically binds to gastrin-releasing peptide receptors in humans, was used to prepare long-circulating and pH-sensitive liposomes (SpHL) containing technetium-99m (^{99m}Tc) isotope to produce ^{99m}Tc-HYNIC-βAla-Bombesin$_{(7–14)}$ as a radiotracer for breast tumor detection. *In vitro* and *in vivo* data suggested that pHLG-99mTc-BBN$_{(7–14)}$ presented promising characteristics suitable for a diagnostic component and could be a potential candidate for tumor identification [168].

A new approach of theranostic (possessing both therapeutic and diagnostic effects) liposomal formulation PTX/SPIO-SSL- H7K(R2)2 was reported containing PTX and iron oxide superparamagnetic nanoparticles (SPIO NPs) with pH-responsive peptide H7K(R2)2 modification where peptide H7K(R2)2 was used as a targeting ligand. SPIO NPs enabled the diagnostic imaging of the tumor site as a magnetic resonance imaging (MRI) agent and PTX worked to render an antitumor effect. PTX/SPIO-SSL-H7K(R2)2 liposomes showed effective antitumor activity with an MRI imaging effect producing dual functionality with a single therapeutic dosage form [20].

6. Limitations and challenges of peptide functionalized liposomes

6.1 Industry-scale production of functionalized liposomes:

Most of the liposomes that are clinically approved and have been manufactured on a large scale are very well characterized, which was possible due to their simple composition and structure. The scenario might not be the same in the large-scale production of these targeted liposomes. The functionalized liposomes have been studied mostly in a small laboratory setting where the product is a small amount. Shifting from the small scale to large scale comes with a massive challenge in the case of functionalized liposomes. The fact that it has been tremendously difficult to quantify the ligand conjugation with the liposomes accurately will undoubtedly lead to batch-to-batch variations in liposomes. This may result in variations in physicochemical properties of liposomes that affect their stability, biodistribution, and pharmacodynamics aspects of the functionalized liposomes [174]. Major challenges in the development of peptide-factionalized liposomes are shown in Fig. 12.

6.2 Characterization of functionalized liposomes:

Several methods have been outlined for the characterization of liposomes. The characteristics that have been measured include liposome size and polydispersity by dynamic and static light scattering, surface charge by measuring zeta potential, drug encapsulation efficiency by spectrophotometry techniques, and morphology as well as physical state by cryo-transmission electron microscopy and atomic force microscopy [175]. Proper methods to characterize functionalized ligand-directed liposomes are lacking, making it difficult to control batch-to-batch variability. Moreover, quantifying the exact amount of ligand incorporated is another challenge that may significantly affect the regulatory approval of such liposomes as it is challenging to control or correct the amount of ligand attached to the surface [176]. There is no proper development of biochemical and biophysical assays that can be employed to quantify the peptide or protein incorporated on to the surface [177]. Flow-cytometric approaches have been used to explore the ability to measure peptides and proteins functionalized on liposomes, but this technique has been semi-quantitative at best [178]. Hence, a uniform and well-documented method to characterize these liposomes is very much essential to developing a clinically approved candidate.

6.3 Strict storage conditions:

Liposomes as a formulation need stringent storage conditions. Liposome formulations must be stored in the refrigerator as their integrity is challenged at higher temperatures. Moreover, liposomes cannot be stored in the freezer as the ice crystals formed disintegrate their lipid bilayer [179].

6.4 Aggregation due to high peptide ligand density:

Functionalization strategy is very useful in producing the directly targeting liposomes, but it comes with great challenges. The surface ligand plays a vital role in increased uptake in the desired target site, and the ligand density on the surface of the liposomes must be optimum to get the desired effect. It has been observed that the high ligand density may be responsible for the aggregation of liposomes, which has a detrimental effect on drug delivery. Proper study and optimization of ligand density, type of cancer cells, and shape and size of liposomes are required to get the enhanced effect of functionalized liposomes [180].

6.5 Masking of peptide ligands by polymers:

Coating of the liposomal surface by PEG has dramatically solved the problem of aggregation and also aids in enhancing the blood circulation time of liposomes [78]. However, the use of PEG for surface modification also has several challenges and requires extensive optimization. Different liposomal formulations use a varied range of PEG length and density in the formulation; hence, a proper establishment for the use of optimum PEG in the various liposomal formulation is required. Longer PEGs, due to their tendency to attend globular structure, have a significant masking effect of the ligand interaction with the receptor due to steric hindrance. This problem must be addressed if one elects to use PEG, and it can be done by choosing the proper PEG. The consideration of binding affinity of the ligand to the receptor is also of prime importance [181].

6.6 Serum-protein binding:

The serum proteins are known to bind nonspecifically to the surface of the liposomes, altering their properties significantly. In the case of functionalized liposomes, such a shield of proteins around liposomes and plasma protein binding of ligand will block the binding of the ligand to the intended receptor, thereby reducing the targeting ability of these liposomes. This serum-protein binding will lead to altering pharmacokinetics, biodistribution, pharmacodynamics, and toxicity of liposomes *in vivo* [182, 183]. These factors must be taken into consideration during the formulation of the functionalized liposomes.

6.7 Lack of accurate tumor models:

Functionalized liposome formulation depends on the type of tumor and its receptor expression, microenvironment, and vasculature system. Most of the targeted liposomes are studied *in vitro* on the 2D cancer cell model, which is not the perfect replica of the cancer disease model. The information obtained from using such a non-resembling model may ultimately lead to the failure of the liposomes *in vivo*. 3D tumor culture systems provide multicellular structure to study the liposomes. However, depending on the type of 3D tumor culture system the microenvironment may not be different compared to the 2D cell culture models. 3D models are known to bridge the gap between *in vitro* and *in vivo* models for drug screening [184]. The 3D model of cancer cell allows the cell to grow in clusters and layers and forms spheroids that resemble the cancer *in vivo* to a greater extent. Hence, the functionalized liposomes must be studied more on the 3D disease model to obtain reliable *in vitro* data. Moreover, the resistant tumor cells and models also need to be considered to obtain physiologically relevant information about the liposomes' effect on cancer. There are significant interactions between cancer cells and immune system cells to form a tumor microenvironment. Hence, one must select the animal model wisely and choose one that resembles the disease state very closely [185].

7. Conclusion and future directions

Peptide-functionalized liposomal formulations containing chemotherapeutic agents provide successful therapeutic approaches to overcoming difficult drug delivery problems, particularly in drug-resistant cancers. This is also very promising for producing lower toxicity in chemotherapy due to targeting effects and other attributes related to nanomedicines. Recent reported advancements in peptide-conjugated liposomal formulations showed clear evidence that combinational therapies with peptide-functionalized liposomes containing anticancer agents can indeed help to inhibit cancer progression through antitumoral effects compared to drugs, drug-containing liposomes, or peptides alone. Various formulations have been prepared with the peptide-functionalized liposomal system entrapping different anticancer drugs. However, these liposomal formulations are required to face further challenges for industrial production, considering the limitations of liposomal formulations, and must be proven for safety issues. An ideal liposomal formulation needs to protect the entrapped drugs from drug loss and prevent enzymatic degradation or serum binding for the effective treatment of cancer. The *in vivo* antitumor model needs to be carefully designed so it can depict the effectiveness of the

prepared liposomal formulation. Efficient formulation technologies need to be established with large-scale production capability for the peptide-functionalized liposomal system.

With increasing research on peptide-functionalized liposomal systems to treat and diagnose cancer, it can be anticipated that the knowledge of innovative peptide-functionalized liposomal delivery strategies will be enhanced in the future. It is undeniable that the development of peptide-functionalized liposomal systems represents a significant advance in cancer therapy and diagnosis. Future efforts are required to design precisely targeting peptide-functionalized liposomal systems to mitigate adverse outcomes of chemotherapy. Additionally, industrial development in peptide-functionalized liposomal formulations is needed to produce more efficient therapeutic or diagnostic agents to fight against cancer.

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Highlights

- Liposome surfaces can be functionalized for target-mediated cancer therapy.
- Surface-functionalized liposomes with peptides enhance therapeutic efficacy.
- Peptide-functionalized liposomes can be tagged with imaging agents for cancer diagnostic.
- Large scale production and storage of liposomes is still challenging

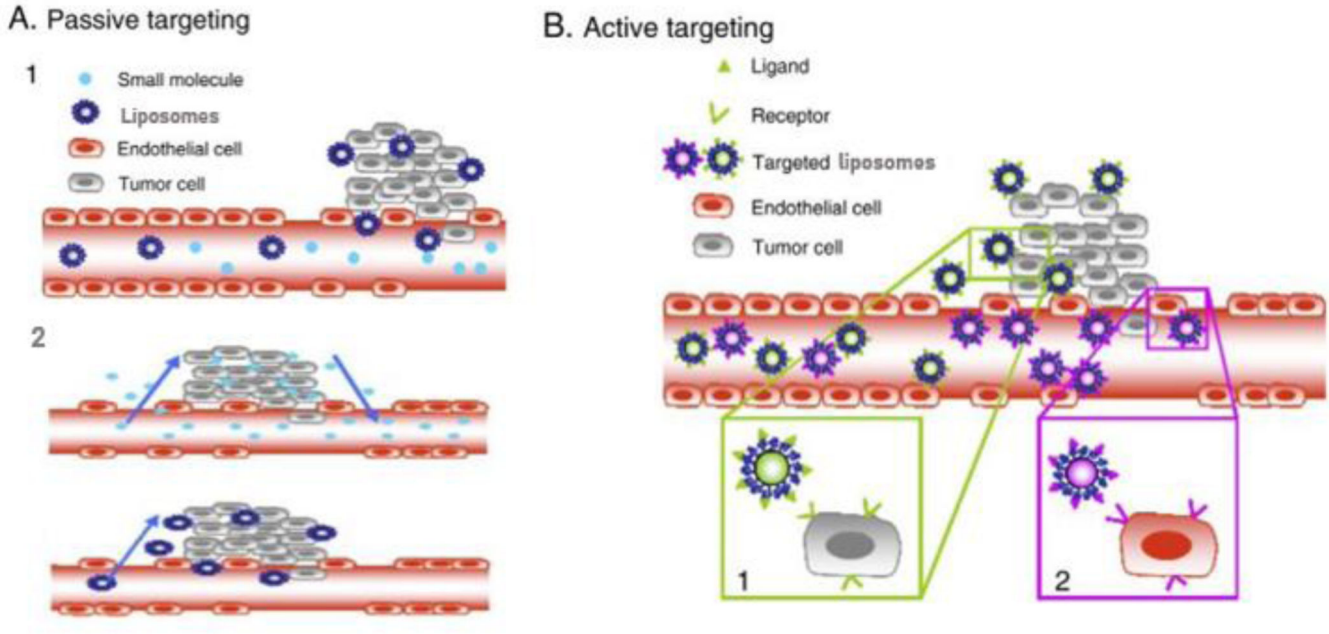

Fig 1.

A. Passive targeting of liposomes. (1) Selective accumulation of liposomes through leaky vasculature surrounding the tumors. (2) Size-dependent retention of liposomes in the tumor tissue. Drugs alone can diffuse back to the bloodstream, reducing drug concentration in the targeted site, while drug-loaded liposomes stay concentrated in the targeted site due to its particle size. B. Active targeting strategies. Targeting peptides grafted at the surface of liposomes bind to receptors (over)expressed by (1) cancer cells or (2) angiogenic endothelial cells (adapted with permission from [15]).

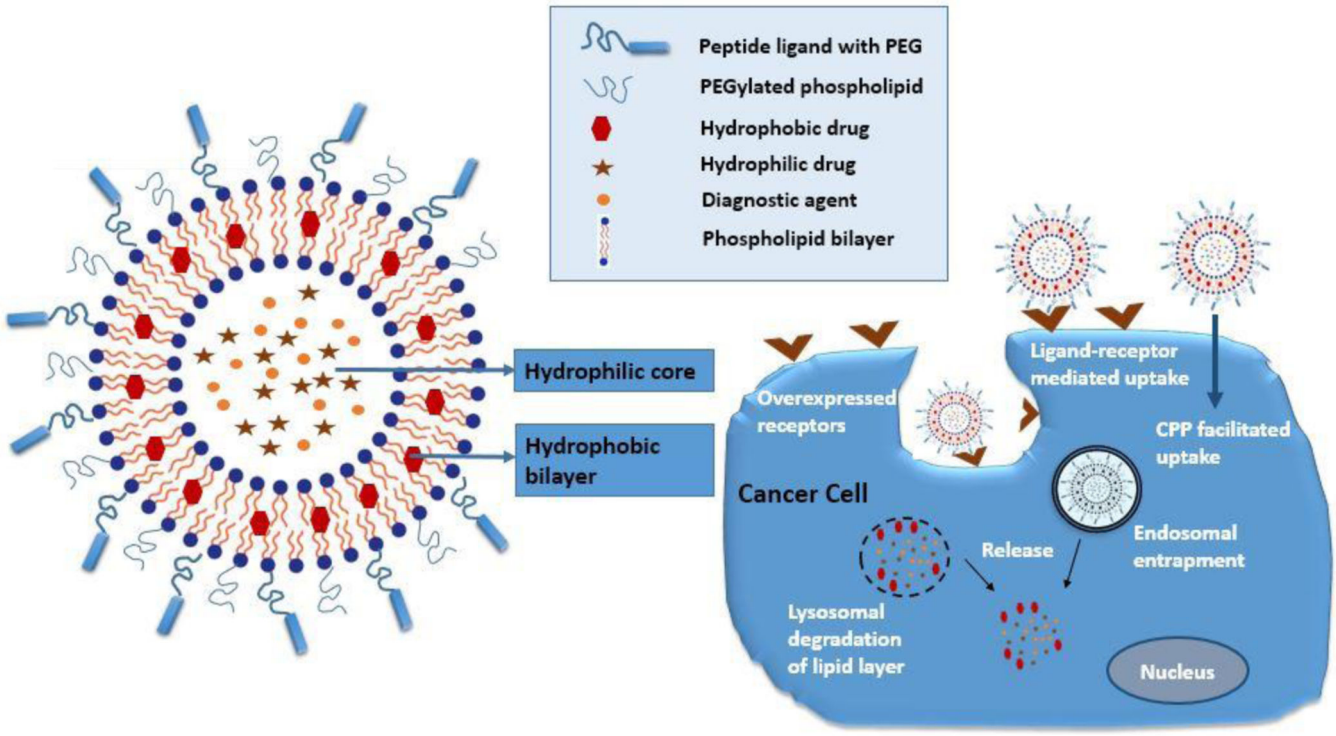

Fig. 2.

Schematic presentation of functionalized peptide-targeted liposomes drug delivery mechanism through an active targeting effect on targeted receptor overexpressed cancer cells. Active peptide-functionalized targeted liposomes either bind to the overexpressed receptors on the cancer cells selectively and go inside through receptor-mediated endocytosis or penetrate inside the cancer cells through cell-penetrating peptide (CPP)-mediated uptake. Drugs and diagnostic agents then can be released through endosomal or lysosomal degradation of the lipid layers.

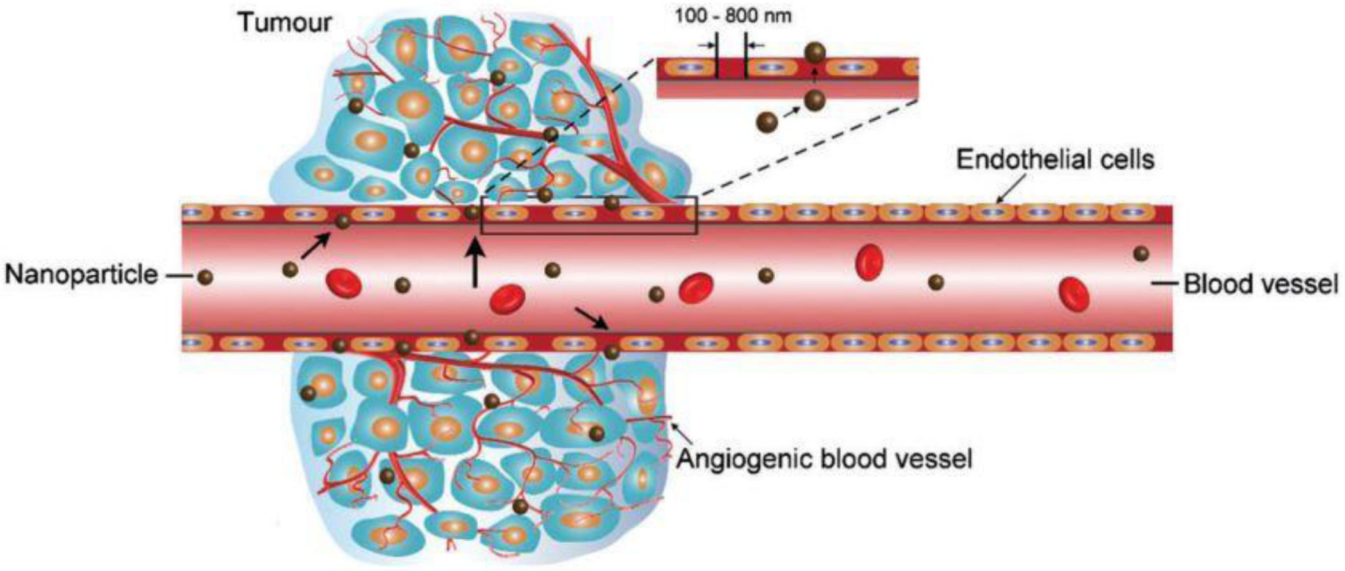

Fig. 3.

Schematic representation of the enhanced permeability and retention (EPR) effect. Large vascular fenestrae formation due to abnormality in the tumor site angiogenic vessels. (Reproduced with permission from [76]).

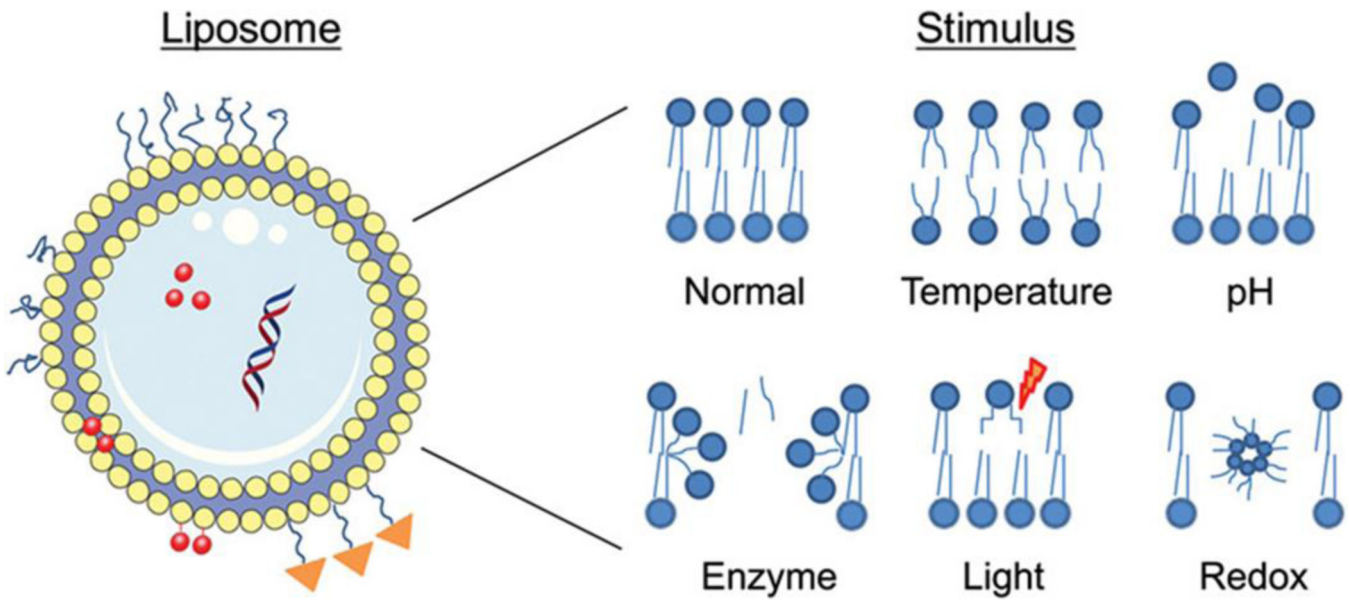

Fig. 4.
Controlled drug delivery system using stimuli-responsive liposomes. (Adapted with permission from [90])

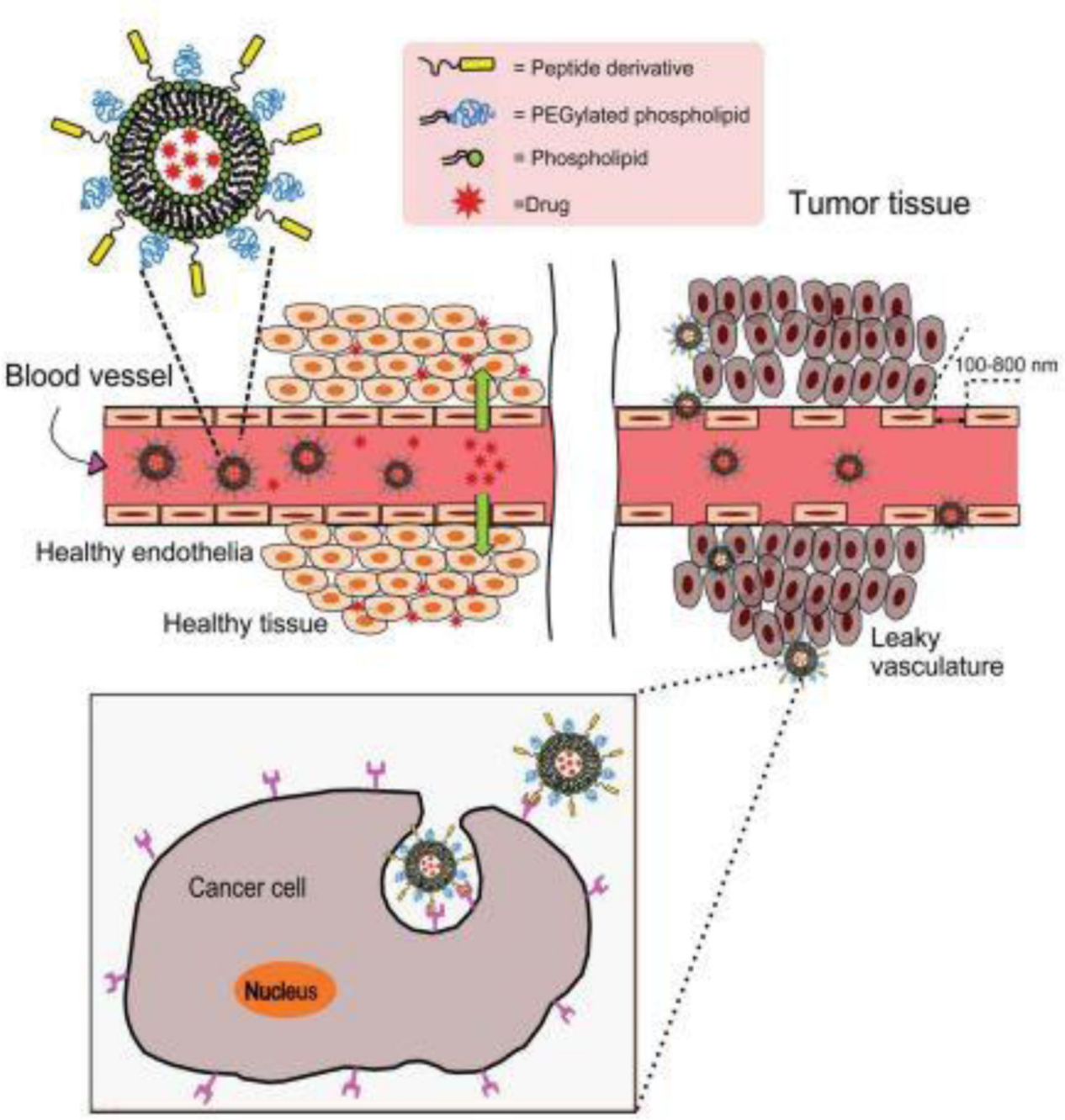

Fig. 5.

Schematic representation of peptide-functionalized PEGylated liposomes containing an active drug. Small drug molecules can efflux through the healthy blood vessels endothelial cells while the liposomal formulation remains contained in the vessel (left). In contrast, in the tumor tissue vessels (right) form large vascular fenestrae due to the rapid vascular growth which facilitate liposomal passage through the vessel and impaired lymphatic drainage helps extravasation also of large liposomal drugs. Upon accumulation, the bioactive peptide on the liposome outer layer binds to the overexpressed targeted receptor on the

cancer cells, which promotes intracellular uptake through receptor-mediated endocytosis and anticancer drug accumulation in vessels or in the nucleus (Reproduced with permission from [23]).

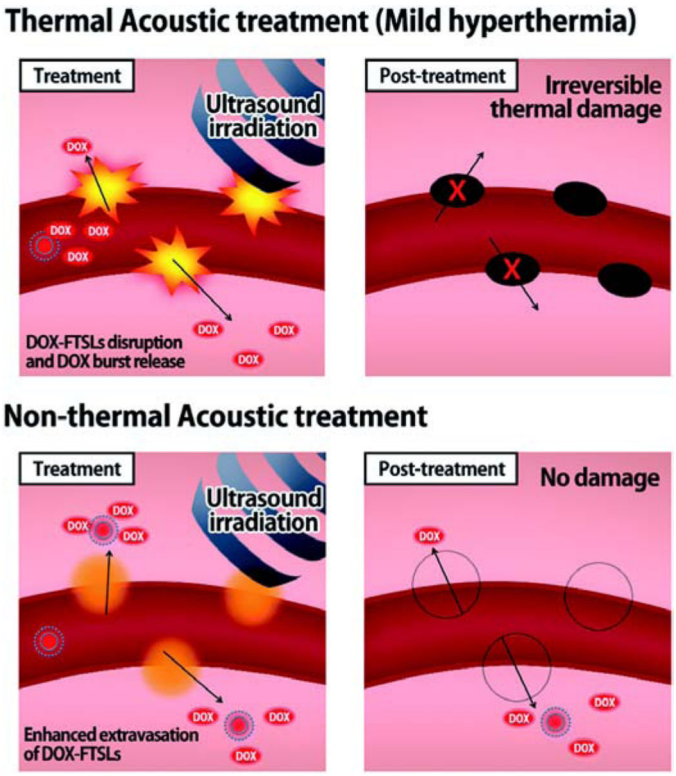

Fig. 6.

Schematic illustration representing the drug delivery mechanisms of thermal and non-thermal acoustic treatment using DOX-FTSLs. (Reproduced with permission from [140]).

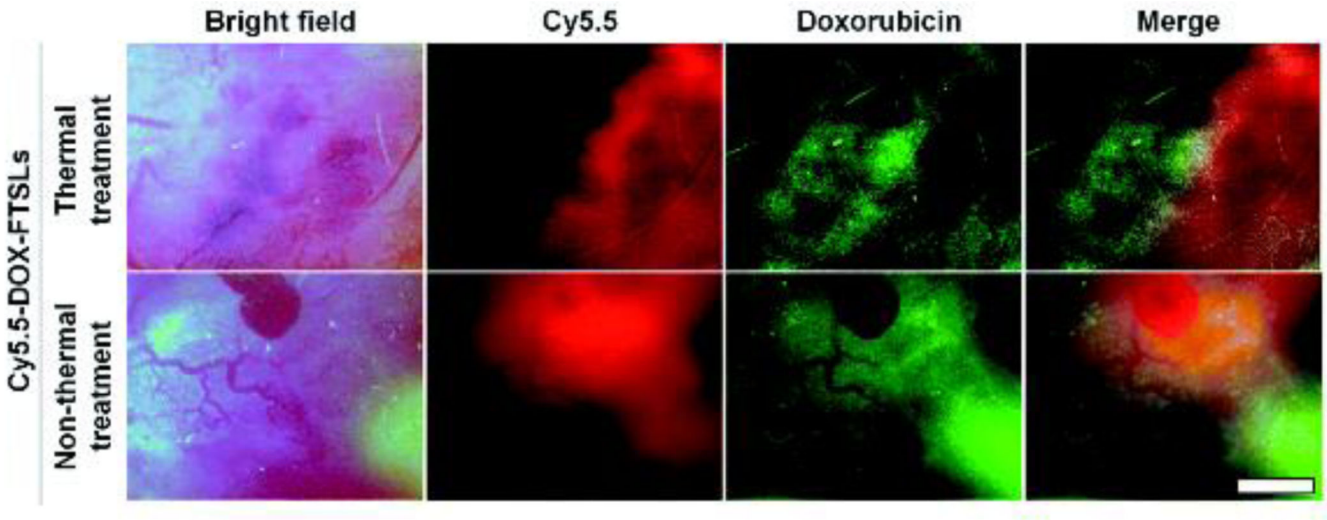

Fig. 7.

(a) In-vivo NIRF images of tumor vessels and tissues at 12 h after intravenous injection of Cy5.5-labeled DOX-FTSLs combined with thermal or non-thermal acoustic treatment (red: Cy5.5, green: DOX). Scale bar represents 150 μm. (Reproduced with permission from [140]).

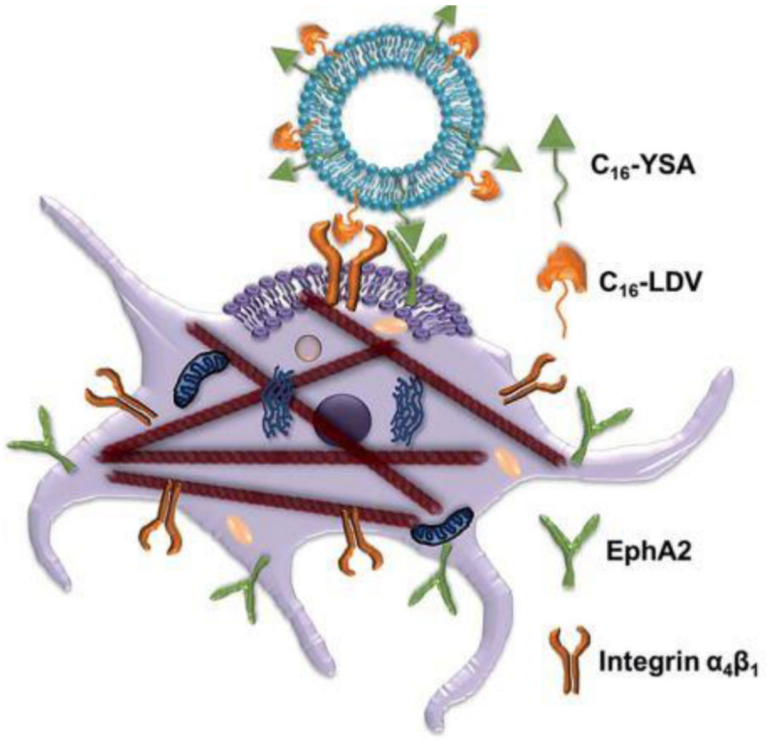

Fig. 8.

Schematic representation of dual functionalized liposome targets both integrin ($\alpha_4\beta_1$) and ephrin (EphA2) receptors of the melanoma cell. (Reproduced with permission from [55]).

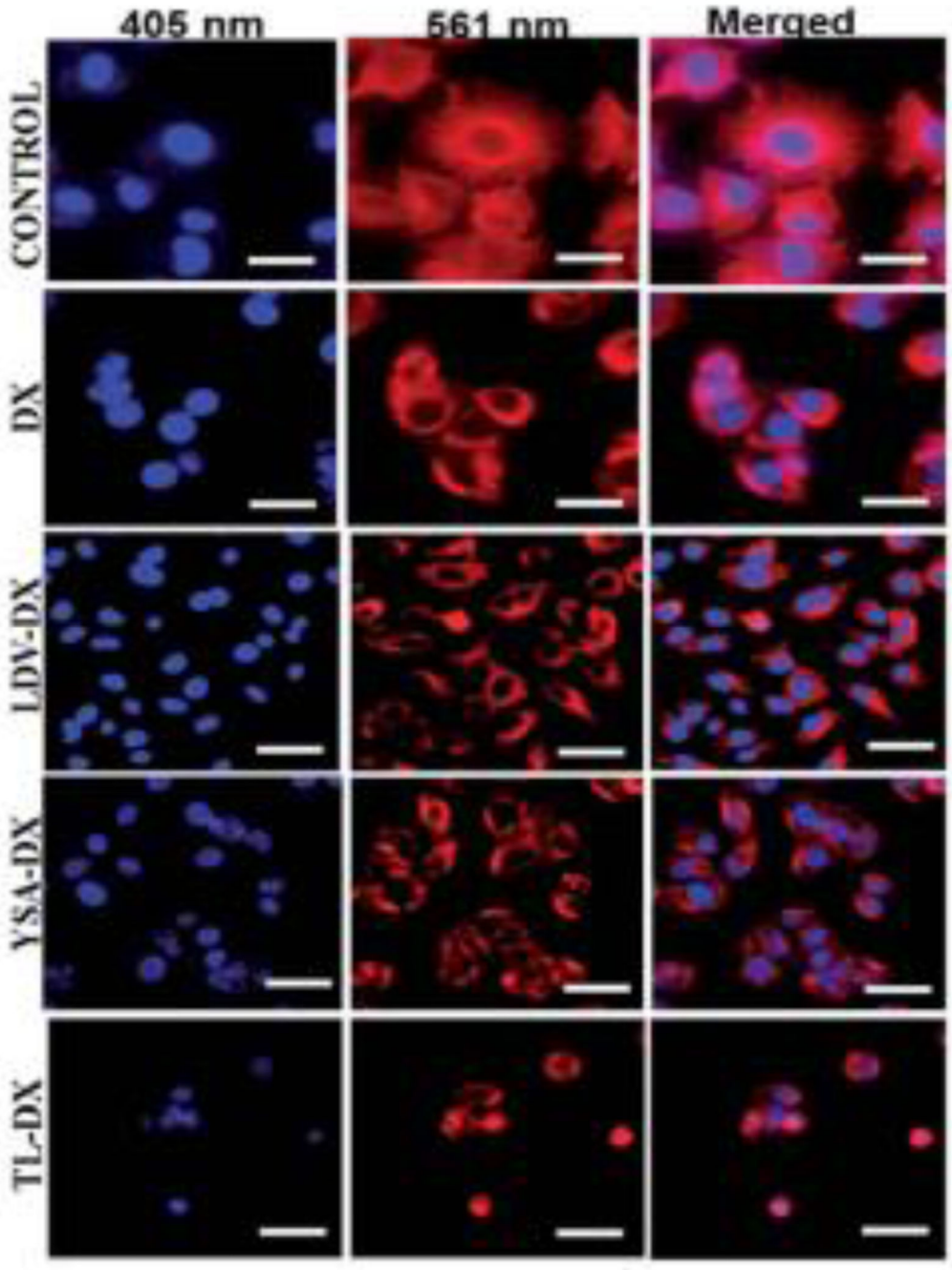

Fig. 9.

Fluorescent microscopic images of DX, LDV-DX, YSA-DX, and TL-DX on the microtubule network of A375 cell. TL-DX treatment caused higher microtubule formation and disruption. Scale bars correspond to 20 mm. (Reproduced with permission from [55]).

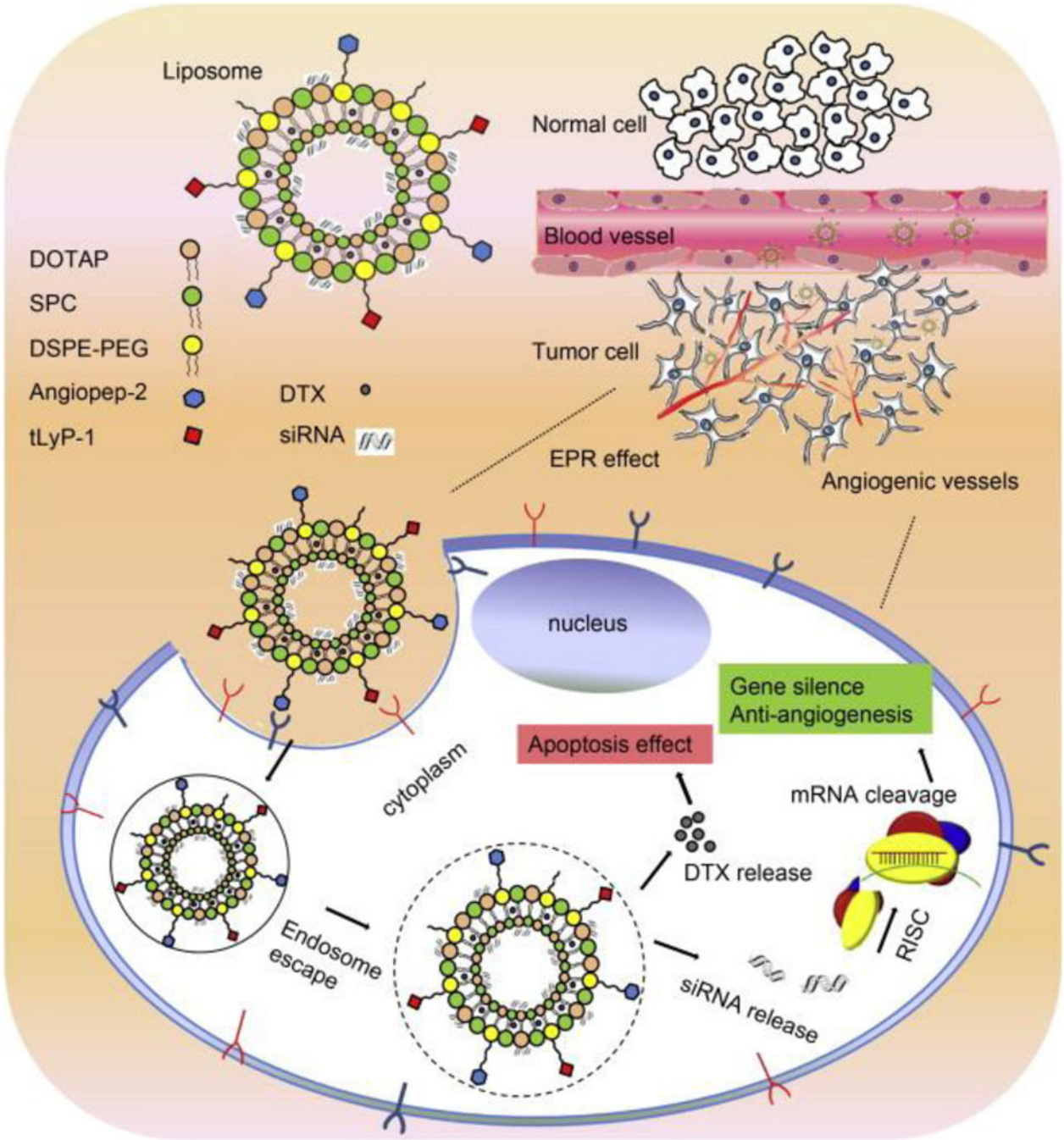

Fig. 10.

Schematic representation of two receptor-specific peptides, Angiopep-2 and tLyP-1, mediated liposomes and their tumor-targeting delivery mechanism. Tumor penetrations and delivery of the dual peptides-modified liposomes were achieved by targeting effect of peptides as well as via the EPR effect. Anti-angiogenesis gene (VEGF siRNA) and apoptosis-inducing chemotherapy (DTX) were used as a combinational therapeutic strategy. (Reproduced with permission from [148]).

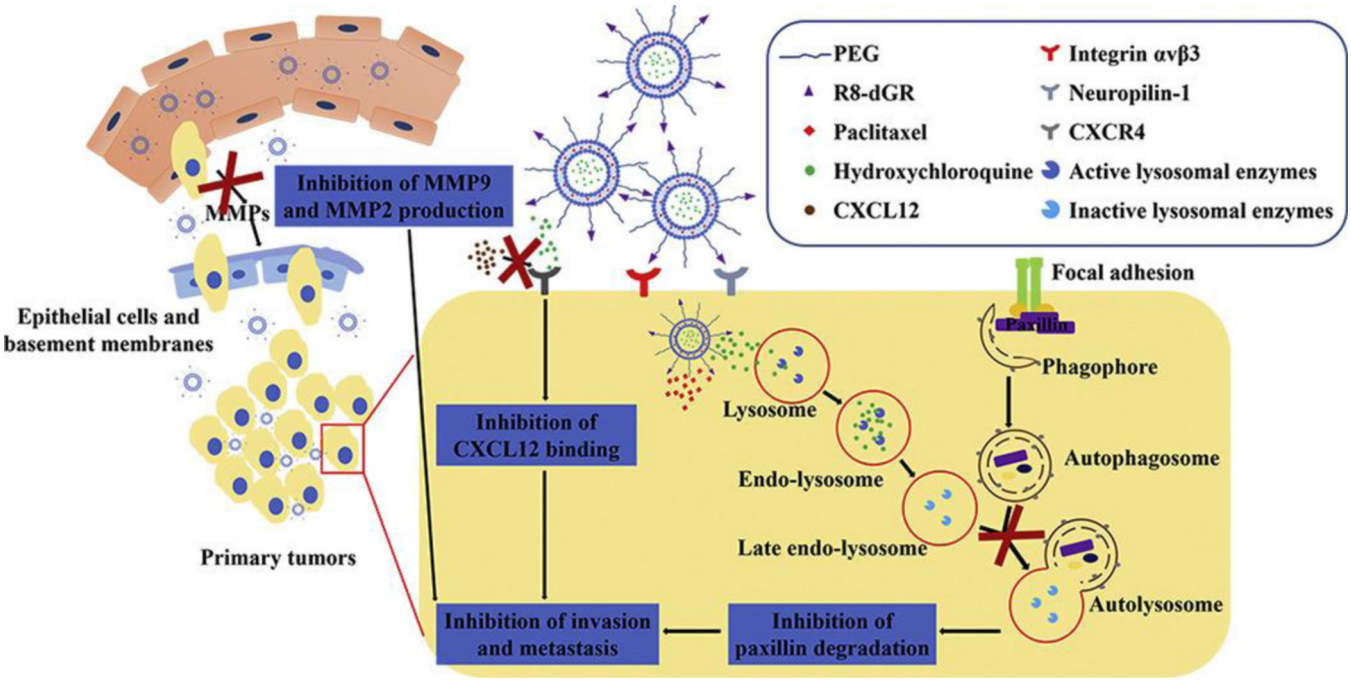

Fig. 11.

Schematic illustration of PTX/HCQ-R8-dGR-Lip delivered into tumor cells. (Reproduced with permission from [156]).

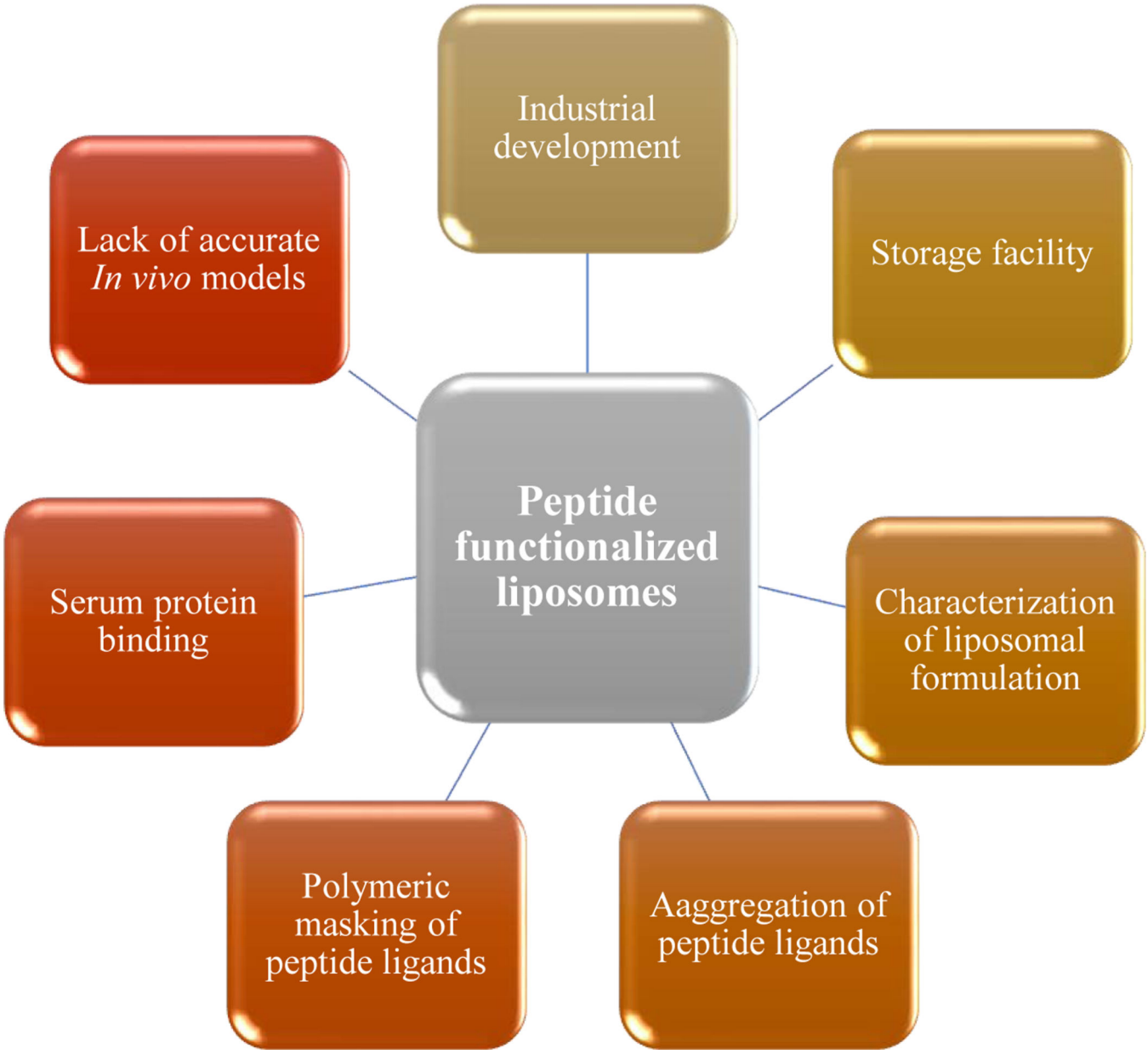

Fig. 12.
Challenges that need to be addressed and overcome in the development of peptide-functionalized targeting liposomal drugs.

Table 1

Stimuli-responsive peptide-functionalized liposomal formulations entrapping anticancer agents for targeted therapy of cancer

Surface attached peptide	Anticancer agent	Liposome formulation	Targeted cancer type	Peptide Target site/ Action	Size (nm)	Reference
CPP: $H_7K(R_2)_2$	DOX	DSPE-PEG-NHS	Brain (*in vitro* and *in vivo*)	Cell penetrating and pH responsive peptide targeting glioma tumor cells	92.19	[87]
CPP: GGRRRRRRRRR-amide	DOX	SPC-STR-EDCl-NHS-mPEG2000-hydrazone-stearate	Breast cancer (*in vitro* **and** *in vivo*)	CPP-facilitated delivery of anticancer agent in breast cancer.	121.25	[94]
Print3G	Calcein	DOPE:CHEMS:CHO L:PEG750-DSPE (43:21:30:6)	Breast cancer (*in vitro*)	Antagonist of an oncoprotein involved in breast cancer growth and invasion	162.6 163.8	[120]
CPP: [D]-H6L9	PTX	DSPE-PEG2000	Breast cancer	Integrin $\alpha_v\beta_3$ targeting by peptide and drug release by local hyperthermia	130–135	[95]
D[KLAKLAK]2 (KLA)	PTX	SPC:CHOL:PTX = 10:1:1 SPC:DKD:CHOL:PTX = 8:2:1:1	Lung cancer	Initiates apoptotic cell death	132	[121]
ELAAWCRWGFLL ALLPPGIAGGGC	Vaccine	DMPC:DMPG:Chol: DOPE	Breast cancer	Activation of cytotoxic T lymphocytes (CTLs)	126–142	[122]
CGKRKD(KLAKLAK)2	DTX	PBAE-PEG	Breast cancer (*in vitro*)	Angiogenic blood vessels in tumors	117	[96]
TDSILRSYDWTY TDSILRSYDGGG	DOX	NHS-PEG-DSPE	Lung metastasis	Non-small cell lung cancer (NSCLC) cells	65–75	[123]
H peptide RF peptide K peptide	miR-200 Irinotecan	DSPE:PEG	Colorectal cancer	Tumor neovasculature undergoing angiogenesis, and one mitochondria-targeting peptide	147–174	[124]
KSSPHSRN$(SG)_5$RG DSP	Calcein	DOPE:CHEMS:DSPE-PEG2000	Colon cancer	Cell-adhesion domain of fibronectin, specifically integrin $\alpha_5\beta_1$ receptor	98.36	[125]
NGR	DOX	DPPC:MSPC:DSPE-PEG_{2000}-NGR:DiO:: 85.2:9.7:5:0.1	Breast cancer and metastasis	CD13/ aminopeptidase N	107.8	[126]
CPP: CRGDRGPDC [iRGD]	DOX	DSPE-PEG2000:MPPC:DPPC	Liver cancer	$\alpha_v\beta_3$ integrins and neuropilin-1	84	[127]
CPP: CKRRMKWKK	siRNA	DPPC:MSPC:DSPE-PEG2000:: 87:3:10	Breast cancer (*in vitro* and *in vivo*)	CPP-facilitated delivery of anticancer agent	90	[128]
Cyclic RGD	DOX	DSPC/DPPC:DSPE-PEG (or DSPE-PEG-cRGD):cholesterol:E LP = 55:2:10:0.55	Breast cancer (*in vitro* and *in vivo* distribution)	$\alpha_v\beta_3$ integrin	181	[129]
CPP: CKRRMKWKK	DOX	DPPC: MSPC: (DSPE-PEG2000-NGR or DSPE-PEG2000) :: 87:3:10	Fibrosarcoma (*in vitro* and *in vivo* study)	CPP-facilitated delivery of anticancer agent	82–89	[130]
CREKA	DOX	DPPC, MSPC, DSPE-PEG, and DSPE-PEG-CREKA (86:10:2:2 molar ratio)	Breast cancer	Targeted clotted plasma proteins in tumor vessels and temperature triggered release of DOX	83.8	[131]

Surface attached peptide	Anticancer agent	Liposome formulation	Targeted cancer type	Peptide Target site/ Action	Size (nm)	Reference
CCRGDKGPDC	DOX	DSPE-PEG2000-maleimide	Breast tumor model	$\alpha_v\beta_3$ integrin	94.2	[132]
CPP: CGRRMKWKK	Campothecin	DSPE-PEG2000, DSPC and DPPC (molar ratio of 10:10:90)	Cervical cancer *(in vitro* and *in vivo*	CPP-facilitated and ultrasound triggered delivery of anticancer agent	189–190	[103]
CPP: CKRRMKWKK NGR: CYGGRGNG	DOX	DSPE-PEG2000-NGR	Breast cancer (*in vitro* and *in vivo*)	CPP-facilitated delivery of anticancer agent	195–202	[104]
AG73: CGGRKRLQVQLSIRT	DOX	DSPC and DSPE-PEG2000-OMe	Colon cancer cells in-vitro study	Syndecan-2 targeting combined with ultrasound trigger release	130–170	[133]
Elastin like polypeptide	DOX	DPPC:DSPE-PEG-2000:cholesterol:SA-ELP3-NH2 = 55:2:15:0.4125	Squamous cell carcinoma (*in vitro* and *in vivo)*	Facilitated stimuli-responsive release of anticancer agents	161.8	[134]
CPP: CKRRMKWKK	siRNA	DSPC and DPPC	*In vivo* distribution and cellular uptake in human breast adenocarcino ma cells	CPP-facilitated delivery of anticancer agent	201	[135]
CPP: CKRRMKWKK (derived from Penetratin)	DOX	DPPC:MSPC:DSPE-PEG2000 (87:3:10) Fe_3O_4	Breast cancer *(in vitro* and *in vivo* study)	CPP facilitated magnetic hyperthermia-triggered release of DOX	90–100	[136]
CPP: R8	PTX	Cholesterol:SPC: DSPE-PEG_{2K}-R8(35:65:0.8)	Breast cancer (*in vitro* and *in vivo* study	CPP facilitated and pH sensitive release of PTX	120	[137]
PEGylated cleavable lipopeptide (PCL) H-G-Trp(Boc)-I-P-V-Ser(tBu)-L-Arg-(Pbf)-Ser(tBu)-G-Glu(tBu)-Glu(tBu)-Glu(tBu)-Glu(tBu)PEG2000	DOX	POPC:Cholestrol:PCL (60:35:5)	Prostate adenocarcino ma (*in vitro* and *in vivo*)	MMP enzyme facilitated cleavage and ultrasound triggered release of DOX	127	[138]

Abbreviations: CPP, cell penetrating peptide; Chol, cholesterol; CHEMS, cholesteryl hemisuccinate; DSPE-PEG2000, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[poly(ethylene glycol) 2000]; DOX, doxorubicin; DTX, docetaxel; DPPG-Na, dipalmitoyl phosphatidylglycerol; DPPC, dipalmitoyl phosphatidylcholine; DOPE, Dioleoylphosphatidylethanolamine; DSPC, distearoylphosphatidylcholine; DSPE-PEG2000-OMe, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; mPEG2000-hydrazone-stearate (mPEG2000-Hz-stearate); HSPC, hydrogenated soy phosphatidylcholine; MSPC, 1-myristoyl-2-stearoyl-sn-glycero-3-phosphocholine; MPPC, 1-myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine; DSPE-PEG-NHS, 3-(N-succinimidyloxyglutaryl)aminopropyl; PBAE-PEG, poly(beta-amino ester) poly(ethylene glycol); PTX, paclitaxel; STR, stearate; SPC, soy phospholipids.

Table 2

Peptide-functionalized liposomal formulations entrapping anticancer drug and diagnostic agents for targeted therapy of cancer

Surface attached peptide	Anticancer agent	Liposome formulation	Targeted cancer type	Peptide target site or activity	Size (nm)	Reference
Arginine unit (R8)	DOX	HSPC:Chol:mPEG-DSPE:RhPE (molar ratio 59:38.21:2:1)	Ovarian carcinoma (*in vitro* and *in vivo*)	CPP-facilitated delivery of anticancer agent	214.5	[139]
Peptide S1 (LIDHEWKENYFPLSF)	DOX	SPC:Chol: S1-PEG2000-DSPE and DOX (weight ratio 8:1:1:1 w/w/w/w)	Tumor-associated angiogenesis (*in vitro* and *in vivo*)	VEGFR2 receptor	143	[17]
P1 peptide (TVRTSAD)	DOX	DPPC: DPPG-Na: DPPE-PDP:Chol: Chol-PEG (15:15:30; 4:36 molar ratio)	Gastric cancer (*in vivo*)	CLDN7 marker protein	160	[19]
Fatty acid-conjugated, elastin-like peptide (FELP)	DOX	DPPC: DSPE-PEG: Cholesterol: FELP at a 55:2:15; 0.4125 molar ratio	Non-thermal acoustic cancer treatment (*in vitro* and *in vivo*)	Tumor site-specific drug delivery	134.97	[140]
pH responsive CPP $H_7K(R_2)_2$	DOX	DOPE: CHEMS: DSPE-PEG and DSPE-$PEGH_7K(R_2)_2$	Glioma tumor (*in vitro* and *in vivo*)	CPP-facilitated delivery of anticancer agents in gliomas	92.19	[87]
Integrin $\alpha_5\beta_1$ antagonist (Ac-PHSCN-NH2)	DOX	HSPC/DPPE /Chol/OHCPEG-CHO (15:5:10:10 mole ratio)	Melanoma B16F10 cells (*In vitro* and *in vivo*)	Targeted-drug therapy toward integrin $\alpha_5\beta_1$ receptor	96.0	[141]
Cell penetrating peptide (CPP)	DOX	SPC/Chol/DSPE-PEG2000 (100:50:8 mol/mol/ mol)	Human breast cancer tumor (*in vitro* and *in vivo*)	CPP-facilitated delivery of anticancer agents in breast cancer.	121.25	[94]
TAT cell-penetrating peptide	DOX	1.5% dsPe-PeG2000, 1% dsPe-PeG2000-tat, 2.5% dsPe-PeG2000-ang, 59% SPC, 36% CHO	Glioma cells (*in vitro*)	Low-density lipoprotein receptor related protein-1 (LRP1)	93.7	[142]
^{D}CDX and c(RGDyK) peptides	DOX	HSPC/cholesterol/mPEG2000-DSPE/DCDX-PEG3400-DSPE/ c(RGDyK)-PEG3400-DSPE (52/43/2/2/1, by mole)	Glioma (*in vitro* and *in vivo*)	Nicotine acetylcholine receptors (nAChRs) on the BBB and integrin highly expressed on the BBTB and glioma cells	93.9	[143]
APRPG	miRNA and DOX	DOPE, cholesterol, DPPC, and DCP-TEPA (4:4:3:1 as a molar ratio)	Colon (*in vitro* and *in vivo*)	VEGFR-1	163	[144]
RIV (DFDSDMDEDGDIDHDQ DGDQDHDPDKDIDRDM DIDQDMDTDI)	DOX and CA-4	Hydrogenated soybean phosphatidylcholine/ cholest erol, 55/45, mol/mol	Skin (*in vitro* and *in vivo*)	VEGFR-2	70–110	[145]
RIPL peptide (IPLVVPLRRRRRRRRC)	DTX	PC, TW80, and $DP_{2K}M$ (8.8:1:0.2 molar ratio)	Prostate and ovarian tumor (*in vitro* and *in vivo*)	Targeted delivery to hepsin-expressing cancer cells	162.4	[146]
LDV and YSA peptides	DTX	DOPC, Cholesterol, C16-LDV, C16-YSA, DX (16:4:8:8:1 ratio)	Melanoma cell (*in vitro*)	Integrin ($\alpha_4\beta_1$) and ephrin (EphA2) receptors	-	[55]

Surface attached peptide	Anticancer agent	Liposome formulation	Targeted cancer type	Peptide target site or activity	Size (nm)	Reference
Histidine tagged EphA2 receptor specific peptide (YSA)	DTX	DTX:DOGS-NTA-Ni:DOPC:Cholesterol:DSPE-PEG 2000 (0.5:5:20:5:2.5 weight ratio)	Lung cancer (*in vitro* and *in vivo*)	EphA2 receptor	189.3	[147]
Angiopep-2 and tLyP-1 peptides	DTX & VEGF siRNA	DOTAP:SPC:Chol:DSPE-PEG2000 (25:40:30:4, mol/mol)	Brain tumor glioma cell (*in vitro* and *in vivo*)	Protein receptor (angiopep-2) and neuropilin-1 receptor (tLyP-1)	110 – 150	[148]
ELP and tethered GRP	DTX	1.5× ELP-GRP/C/ACD-1/DTX	Prostate cancer cells (*in vitro*)	Gastrin-releasing peptide receptor (GRPR)	23.5 – 154.8	[149]
CPP Penetratin	5-FU and Tf	DOTAP/DOPE/ CHEMS /Pen-PEG(2000)-DSPE (43.5:43.5:5:4 mole %)	Brain tumor glioblastoma cell (*in vitro*)	Transferrin (Tf) receptors in brain tumors	178.12	[150]
PR_b	5-FU	(65-xy):35:x:y mol% of DPPC:CHOL:PEG:peptide-amphiphile, where x is the indicated mol% of PEG and y is the mol% of peptide-amphiphile	Colon cancer cells (*in vitro*)	Integrin $\alpha_5\beta_1$	80 – 150	[60, 66]
YIGSR peptide	5-FU	DSPC/CH/DSPE-PEG(2000)-MPB (6:4:0.5)	Angiogenic endothelial cells (*in vitro* and *in vivo*)	Laminin receptors	103	[151]
Cyclic RGD peptide	5-FU	DSPC/cholesterol/DSPE – PEG – RGD (56:39:5)	Angiogenic endothelial cells (*in vitro* and *in vivo*)	a_vb_3 integrins	105	[152]
Peptide AA_{13}	DNR	S100PC/CHOL/mPEG2000-DSPE (4:1:0.2, molar ratio)	Acute myeloid leukemia (AML) cells (*in vitro* and *in vivo*)	Low density lipoprotein receptor (LDLR)	95	[153]
$Arginine_8$-Glycine-Aspartic acid (R_8GD) peptide	DNR and emodin	EPC, Chol, DSPE-PEG2000, emodin and DSPE-PEG2000-R8GD at a mass ratio of 100:25:8:6:40	Cancer therapy (*in vitro* and *in vivo*)	Vasculogenic mimicry (VM) channels	100	[154]
PFV peptide	DNR and dioscin	EPC, Chol, DSPE-PEG2000, DSPE-PEG2000-PFV and dioscin (100:30:3:2:7, molar ratio)	Non-small-cell lung cancer (NSCLC) (*in vitro* and *in vivo*)	Vasculogenic mimicry (VM) channels and tumor metastasis	121.13	[155]
R8-dGR peptide	PTX	SPC, cholesterol and DSPE-PEG2000-OMe (molar ratio = 62:33:5)	Malignant melanoma (*in vitro* and *in vivo*)	Neuropilin-1 receptors and integrin $\alpha_v\beta_3$ receptors	100	[156]
Glu6-RGD peptide	PTX	SPC/cholesterol/Ligand Glu6-RGD-Chol (molar ratio = 62:33:3)	Bone metastatic breast cancer (*in vitro* and *in vivo*)	$\alpha_v\beta_3$ integrin	121.9	[56]
RGD peptide	PTX and CUR	PTX (5 mg), CUR (3 mg), CHOL (15 mg), DSPE-PEG 2000 (12.5 mg) and SPC (120 mg)	Lung cancer (*in vitro* and *in vivo*)	$\alpha_5\beta_3$ integrin	120.6	[57]
TR peptide	PTX	SPC, Cho, DSPE-PEG2000OMe, DSPE-PEG2000-peptide (59:33:2:6, molar ratio)	Glioma (*in vitro* and *in vivo*)	$\alpha_5\beta_3$ integrin	131.8	[58]
Peptide R8-RGD	PTX	SPC/cholesterol/DSPE-PEG2000/DSPE-PEG2000- R8-RGD (molar ratio ¼ 62: 33:4.2:0.8)	Glioma (*in vitro* and *in vivo*)	$\alpha_5\beta_3$ integrin	105.9	[59]

Surface attached peptide	Anticancer agent	Liposome formulation	Targeted cancer type	Peptide target site or activity	Size (nm)	Reference
CAP peptide	PTX and albumin nanoparticles	2.5 mg of DPPC, 0.1 mg of CAP, 0.51 mg of DSPE-PEG2KOMe, and 0.2 mg of IR-780	Pancreatic ductal adenocarcinoma (PDAC) (*in vitro* and *in vivo*)	Membrane biomarker FAP-α	123.9	[157]
TH peptide	PTX and αGC immunoadjuvant	Cholesterol/SPC/DSPE-PEG_{2000}/DSPE-PEG_{2000}-TH (molar ratio = 33:59:2:6)	Melanoma (*in vitro* and *in vivo*)	pH-responsive delivery of anticancer agent in melanoma	118.3	[158]
Cell-penetrating peptide TAT	PTX and DOX	SPC, CHO,DSPE-PEG_{2000}, DSPE-PEG_{1000}-TAT, DSPE-PEG_{3500}-Tf in 60, 33, 3, 2, 2 % respectively.	Melanoma (*in vitro* and *in vivo*)	CPP-facilitated delivery of anticancer agent	124.5	[159]
CPP PFVYLI	PTX	15.9 mg of EPC, 4.1 mg of Chol, 4.7 mg of PEG_{2000}DSPE and 1.5 mg of PFVPEG2000-DSPE	Breast cancer (*in vitro* and *in vivo*)	CPP-facilitated delivery of anticancer agent	120	[160]
TH peptide	PTX and LST	SPC, Cholesterol, DSPE-PEG_{2000}-OMe and DSPE-PEG_{2000}-TH (molar ratio = 59:33:2:6)	Breast cancer (*in vitro* and *in vivo*)	pH-responsive delivery of anticancer agent in breast cancer	109.3	[161]
Peptide $H_7K(R_2)_2$	PTX and SPIO NPs	PTX, EPC, cholesterol, DSPE-PEG, and DSPE-PEG-$H_7K(R_2)_2$	Breast cancer (*in vitro* and *in vivo*)	pH-responsive delivery of anticancer agent in breast cancer	168.30	[20]
Gonadorelin peptide	MXT	HSPC, cholesterol, and mPEG 2000-DSPE in a mole ratio of 90:10:0.4	Breast cancer (*in vitro* and *in vivo*)	Luteinizing hormonere-leasing hormone targeted delivery of anticancer agent	118.7	[162]
RGD and ATWLPPR peptide	Gd-DTPA	Egg PC/ cholesterol/ mPEG2000-DSPE at a molar ratio of 1.85/1/ 0.15	Tumor tissue - (*in vitro* and *in vivo*)	Angiogenesis targeting molecular imaging of tumor	103.50	[163]
cRGD peptide	Iron oxide (Fe_3O_4) nanoparticles	DMPC: DMPG (9:1) in TES buffer were mixed at a lipid/ Fe_3O_4 weight ratio of 1:5	Glioma and ovarian cancer (*in vitro* and *in vivo*)	$\alpha_5\beta_3$ integrin	57.8	[164]
Anti-HER2 peptide	Methylene blue (MB) attached NaYF4:Yb,Er upconversion nanoparticles (UCNPs)	500 μL soy lecithin (10 mM) and 47% cholesterol	Breast cancer (*in vitro* and *in vivo*)	HER2-positive breast cancer	90.0	[165]
L-peptide (RLLDTNRPLLPY)	Rhenium-188 (^{188}Re) radioisotope	Peptide-PEGylated-liposomes (1 ml) were added to a solution of ^{188}Re-BMEDA (50–250 MBq), and incubated at 60 °C for 30 min	Nasopharyngeal carcinoma (*in vitro, in vivo* and, *in silico*)	GRP78, a specific cancer cell-surface marker	-	[166]
Bombesin peptide	Technetium-99m (^{99m}Tc) isotope	DOPE, CHEMS, and DSPE-PEG2000 (lipid concentration 40 mM; molar ratio5.7:3.8:0.5, respectively	Breast cancer (*in vitro* and *in vivo*)	Different tumors including lung, prostate, breast, pancreas, and colon tumors, express receptors for these peptides [167]	124.1	[168]

Abbreviations: CPP, cell-penetrating peptide; Chol, cholesterol; CUR, Curcumin; CHEMS, cholesteryl hemisuccinate; DSPE-PEG2000, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[poly(ethylene glycol) 2000]; DOX, doxorubicin; DTX, docetaxel; DNR, daunorubicin; DPPC, DPPG-Na, dipalmitoyl phosphatidylglycerol dipalmitoyl phosphatidylcholine; DPPE-PDP, (N-[3-(2-pyridinyldithio)-1-oxopropyl]-L-a-dipalmitoyl phosphatidylcholine; DOPE, dioleoylphosphatidylethanolamine; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane chloride; DSPC, distearoylphosphatidylcholine; DMPG, 1,2-Dimyristoyl-sn-glycero-3-phosphorylglycerol sodium salt; DMPC, 1,2-Dimyristoyl-sn-glycero-3-phosphocholine; ELP, elastin-like polypeptide; EPC, egg yolk phosphatidylcholine; GRP, gastrin-releasing peptide; Gd-DTPA, gadolinium-diethylenetriamine pentaacetic acid; HSPC, hydrogenated soy phosphatidylcholine; LST, losartan; PTX, paclitaxel; RhPE, rhodamine-PE; SPC, soy phospholipids; SPIO NPs, superparamagnetic iron oxide nanoparticle; Tf, transferrin; TES, 2-[(2-hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulfonic acid; VEGF, vascular endothelial growth factor; 5-FU, 5-fluorouracil, MXT, mitoxantrone.