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REVIEW

Emerging non-antibody−drug conjugates (non-ADCs) therapeutics of toxins for cancer treatment

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Cancer treatment

Abstract The non-selective cytotoxicity of toxins limits the clinical relevance of the toxins. In recent years, toxins have been widely used as warheads for antibody−drug conjugates (ADCs) due to their efficient killing activity against various cancer cells. Although ADCs confer certain targeting properties to the toxins, low drug loading capacity, possible immunogenicity, and other drawbacks also limit the potential application of ADCs. Recently, non-ADC delivery strategies for toxins have been extensively investigated. To further understand the application of toxins in anti-tumor, this paper provided an overview of prodrugs, nanodrug delivery systems, and biomimetic drug delivery systems. In addition, toxins and their combination strategies with other therapies were discussed. Finally, the prospect and challenge of toxins in cancer treatment were also summarized.

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1. Introduction

Cytotoxic drugs are widely used in cancer treatment[1]. Several highly toxic agents with half maximal inhibitory concentration (IC_{50}) values in the low pmol/L or low nmol/L range are widely used in cancer treatment due to their high efficacy against various cancer cells. However, the antitumor effects of clinically used toxins are limited by their non-selective toxicity to normal cells, leading to a low therapeutic index and a narrow therapeutic window[2–4].

Antibody–drug conjugates (ADCs) are an emerging strategy for targeted drug delivery, and take the lead in obtaining U.S. Food and Drug Administration (FDA) approval[5]. ADCs bring together the targeting advantages of monoclonal antibodies (mAbs) with the cytotoxic potential of small molecules to enhance specific drug delivery in tumor cells through the antibody-antigen interaction while sparing healthy tissues and/or cells from toxic damage[6–8]. Once mAbs selectively bind to the target antigen on the tumor cell, ADCs are internalized into cells and the payloads are released with the linker breaking or antibody hydrolysis, followed by free drug disrupting the function of the corresponding cellular structure[9]. The clinical results of ADCs are undeniably encouraging and preferable ADCs are designed continuously, but they still have shortcomings, including low drug loading capacity, poor delivery efficiency, possible immunogenicity, high cost of ADCs production, and low potential to penetrate solid tumors[10–13]. Consequently, non-antibody-mediated drug delivery may be an alternative strategy that can deliver toxins as safely and effectively as possible.

A variety of non-ADC drug delivery approaches have been widely reported and become research hotspots. Prodrugs are inactive or less active conjugates that are metabolized *in vivo* to release the parent drug[14]. As a widely used method of toxin delivery, a successful prodrug strategy could overcome multiple barriers of toxins, such as serious adverse effects, poor specificity, and insufficient cellular uptake efficiency[15–17]. In addition, with the development of nanotechnology and biomaterials, nanocarriers have shown remarkable advantages in toxin delivery[18], such as improved drug availability, promoted accumulation of toxic agents in tumor tissues *via* the enhanced permeability and retention (EPR) effect, and increased tumor targeting through appropriate modifications and controlled drug release[19]. In order to avoid the potential toxicity caused by exogenous materials, biomimetic drug delivery systems are emerging[20,21]. Highly efficient and low toxicity delivery of toxins has also been achieved by utilizing cell membranes[22–24], extracellular vesicles (EVs)[25,26], or living cells[27]. Furthermore, due to the complexity of tumor therapy, various studies have manifested that the combination of multiple antitumor agents or different therapeutic modalities could enhance antitumor efficacy, reduce toxicity, and overcome drug resistance to toxins[28].

In recent years, the development of novel approaches to the delivery of toxins for tumor therapy has been rapid and diverse. Here, various delivery approaches of toxins especially non-ADCs were summarized, mainly including prodrugs, nano-drug delivery systems (NDDS), biomimetic drug delivery systems, and combination strategies. These approaches have advantages and disadvantages (Table 1) that are important and valuable in the delivery of toxins. This review would be expected to provide new ideas for non-antibody mediated tumor treatment with toxins.

2. Prodrugs

Generally, free toxins cannot be applied directly to the treatment of tumors because of their non-selective toxicity to normal cells.

Table 1 The advantages and disadvantages of various toxin delivery strategies.

Strategy for toxin delivery	Advantage	Disadvantage
Small molecule–drug conjugates	• Modifying pharmacophore to reduce toxicity to normal tissue • Active targeting by connecting the small molecular ligands as targeting parts • Diffusing more easily in tumor tissues due to their low molecular weight	• Easy to be eliminate *in vivo* because of the low molecular weight • Active targeting is affected by the amount of antigen expressed • Complex synthesis process
Polymer–drug conjugates	• Passively targeting tumors through EPR effects • Prolonged circulation time	• Unclear mechanism of polymer clearance *in vivo* • Uncontrollable drug loading • Complex synthesis process
Noncovalently encapsulated NDDS	• Avoiding the selection of covalent binding sites and linkers • Masking the toxicity by encasing the toxin internally • Passively targeting tumors through EPR effects • Active targeting tumors through surface modification	• Potential toxicity caused by the encapsulated materials • Low drug loading • Drug leakage during drug storage and *in vivo* circulation
Self-assembling prodrug NDDS	• Stable and high drug-loading capacity • Requiring no or only a few excipients • Prolonged circulation time • Simple preparation process	• Sophisticated design is required to ensure the self-assembly capability of the prodrugs • Uncertain and unforeseeable fate *in vivo*
Biomimetic drug delivery systems	• Low immunogenicity and good biocompatibility • Natural targeting of tumors • Long circulation time	• Complex preparation process • High cost
Combination strategies	• Overcoming drug resistance • Enhanced anti-tumor effect	• Requiring complex and ingenious design

Table 2 SMDCs of toxins.

Target	Toxin	Ligand	Linker	Ref.
$\alpha_v\beta_3$ integrin	MMAE	Cyclo (DKP-RGD)	Glucuronide	31
$\alpha_v\beta_3$ integrin	MMAE	Cyclo [DKP-RGD] or Cyclo [DKP-isoDGR]	Val-Ala	32
$\alpha_v\beta_3$ integrin	MMAF	Cyclo [DKP-RGD] or Cyclo [DKP-isoDGR]	Val-Ala	32
$\alpha_v\beta_3$ integrin	DM1	iRGD	Disulfide	33
$\alpha_v\beta_3$ integrin	SN38	RGD	Neutrophil elastase-cleavable linker	34
Folic acid receptor (FR)	tubulysin B	Folic acid (FA)	Disulfide	35−38
FR	MMAE	FA	β-Galactosidase	39
FR	DM1	Vitamin folic acid	Disulfide	40
FR	PBD	FA	Disulfide	41
Carbonic anhydrase IX	MMAE	Acetazolamide derivatives	Val-Cit	42
Carbonic anhydrase IX	MMAE	Acetazolamide	Val-Cit	43
Carbonic anhydrase IX	DM1	Acetazolamide	Disulfide	44
Tyrosine receptor FGFR2	DM1	LLC2B peptide	Disulfide or Maleimide	45
Prostate-specific membrane antigen	MMAE	Glu-urea-Lys (DCL)	Val-Cit-PAB	46

By designing toxins as prodrugs with low toxicity, it is possible to apply them as drugs for therapy. Prodrugs refer to compounds obtained by modifying the chemical structure of a drug that is inactive or less active *in vitro* and releasing active parent drugs through enzyme or chemical conversion in the body to exert their effects[14]. Rational modification of the toxins to form prodrugs facilitates the therapeutic effect: (a) modifying pharmacophore to reduce toxicity to normal tissue[16]; (b) coupling target molecules and sensitive units to achieve targeted drug release[16]; (c) linking hydrophilic groups to improve the water solubility of toxins[17]; (d) prolonging the half-life ($t_{1/2}$)[29]. In this section, we will discuss the applications of small molecule drug conjugates (SMDCs) and polymer prodrugs of toxins in cancer treatment.

2.1. *SMDCs*

As special prodrugs, ADCs specifically guide drugs to tumor sites and weaken excessive toxicity of toxins in the systemic circulation. However, the insufficient cell penetration caused by high molecular weight limits the application of ADCs. It is a wise choice to use small molecular ligands [*e.g.*, peptides, aptamers (APt), and small molecules] as targeting parts that retain the advantages of ADC and correct their disadvantages. Compared with ADCs, SMDCs not only bind to the corresponding receptors on cancer cells efficiently but also diffuse more easily in tumor tissues due to their low molecular weight[30]. Next, we will discuss the application of different small molecular ligands in toxins delivery (Table 2)[31−46].

Transmembrane receptor $\alpha_v\beta_3$ integrin is commonly used as a receptor for the selective delivery of toxins. The $\alpha_v\beta_3$ integrin, associated with angiogenesis and tumor metastasis, overexpressing on various tumor surfaces and angiogenic endothelium (such as melanoma, glioblastoma, renal cell carcinoma, lung carcinoma, and breast cancer) but extremely limited in normal tissues, is an ideal target for anti-tumor[47]. RGD peptides containing arginine-glycine-aspartate sequence are renowned ligands of the $\alpha_v\beta_3$ integrin[48]. A variety of RGD peptides have been designed for the modification of toxins. Xie et al.[49]constructed a multifunctional SMDCs platform based on iRGD, a tumor-homing and -penetrating peptidic motif, and applied it to the delivery of potent toxin maytansinoid DM1 (Fig. 1). The results clearly showed that iRGD-tethered prodrug had good anti-tumor growth and metastatic effects in liver cancer xenograft mice. Under the influence of iRGD, the water solubility of the conjugates is greatly increased, which can be used for intravenous administration with the effect of anti-metastasis.

Folic acid (FA) is another ligand that is commonly used in targeted tumor therapy. Compared with antibodies and peptides, FA shows the advantages of simple structure, easy preparation, and good stability. FA−drug conjugates bind to the folate receptor (FR) overexpressed in various tumors with high affinity and then internalize rapidly[30]. Christopher P and his co-workers[35,37,49] synthesized a series of FA−tubulysin B conjugates by coupling FA with tubulysin B, a highly toxic tubulin polymerization inhibitor (Fig. 2). These conjugates modified by FA were selectively taken up by cancer cells and exhibited promising antitumor activity. In particular, EC1456 enhanced the biostability of the peptide by using an optimized stable, water-soluble saccharopeptide spacer of all-D conformation. It was shown that EC1456 has significant anti-proliferative activity against FR-positive tumors, including models that were anticancer drug-resistant[38]. Recently, a Phase I clinical study of EC1456 was completed for the treatment of advanced solid cancer and non-small cell lung cancer (NCT01999738)[30].

Carbonic anhydrase IX (CAIX) is a sign of hypoxia, which is over-expressed in many solid tumors[50]. Small heteroaromatic sulfonamides such as acetazolamide have the capability of binding to CAIX[42]. DM1, monomethyl auristatin E (MMAE) and many other toxins have been successfully designed as SMDCs based on acetazolamide to target CAIX[43,51,52]. Krall et al.[51] coupled DM1 or duocarmycin derivatives, which were two toxins widely used in ADC development, with acetazolamide derivatives *via* disulfide bonds to target CAIX, a validated and accessible marker of renal cell carcinoma. The quantitative biodistribution studies revealed the contribution of the CAIX-binding moiety to the preferential accumulation of payloads at the tumor site. This prodrug demonstrated the feasibility of SMDCs targeting CAIX and provided a new idea for targeted delivery of potent cytotoxic drugs.

In addition, prostate specific membrane antigen (PSMA) is a type II transmembrane glycoprotein located on the surface of prostate epithelial cells[53]. Its expression is significantly up-regulated in prostate tumor cells compared to healthy cells,

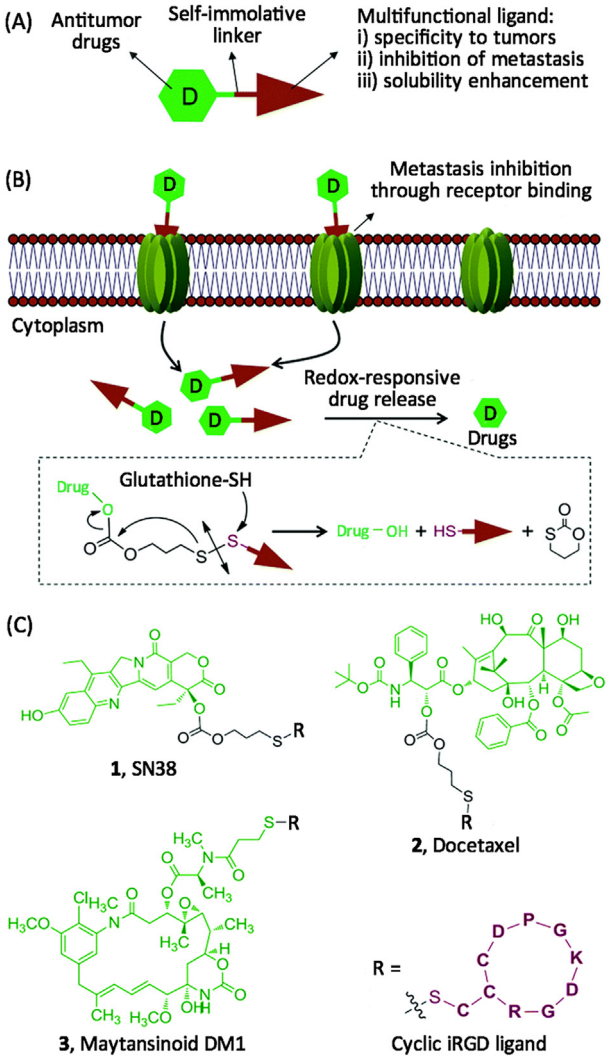

Figure 1 (A) Rational design of multifunctional prodrug conjugates composed of an anticancer chemotherapeutic (SN38, Docetaxel or DM1) and a multifunctional tumor-homing and penetrating peptidic motif *via* a self-immolating disulfide linker. (B) Tumor cell-specific recognition of prodrug conjugates by cell surface receptors followed by receptor-mediated endocytosis and cleavage of disulfide bonds responsive to intracellular thiols. (C) Molecular structures of synthesized prodrugs **1**–**3**. Reprinted with the permission from Ref. 33. Copyright© 2016 The Royal Society of Chemistry.

making it a promising target for drug delivery. A low-molecular-weight PSMA ligand, Glu-urea-Lys (DCL), has been applied to construct from rather simple to complex molecules by linking with monomethyl auristatin E through a dipeptide linker[46,54–56]. DCL is an inhibitor of folate hydrolase I activity, and can competitively inhibit the NAALADase activity of PSMA, so they can efficiently and specifically bind to PSMA on the surface of prostate cancer cells, and enter prostate cancer cells through internalization. PSMA-Val-Cit-PAB-MMAE showed a satisfying antitumor effect on 22Rv1 [PSMA (+)] xenografts at a single intravenous dose of 0.3 mg/kg, which was equivalent to that of docetaxel at a dosage of 10 mg/kg[46].

2.2. *Polymer–drug conjugates*

Active targeting *via* receptor or antigen may make it difficult to achieve satisfactory effects in cancer cells that express no or fewer related antigens. In addition, SMDCs are easy to eliminate *in vivo* because of their low molecular weight[57]. The conjugates formed by coupling toxins with macromolecular polymers can make up for such a problem by passively accumulating at the tumor site because of EPR effects and renal excretion can be reduced[58]. Polymer–drug conjugates with antitumor activity are not on the market yet, but several are in clinical trials. Most of them are *N*-(2-hydroxypropyl) methacrylamide– or polyethylene glycol (PEG)–drug conjugates[59]. EZN-2208 is a water-soluble, PEG-drug conjugate of the topoisomerase inhibitor SN38 (the active metabolite of irinotecan). EZN-2208 prolonged the exposure time of SN38 by linking SN38 to a multi-arm high molecular weight PEG, 40 k 4-arm-PEG, *via* a glycine linker. It has been shown that EZN-2208 has good clinical tolerability and anti-tumor activity in adult patients with advanced solid tumors[60–62]. Song et al.[63] also designed a trivalent PEGylated irinotecan prodrug, PEG-[Irinotecan]$_3$. The prodrug incorporated a 20 k PEG chain bound to a biodegradable oligo-peptidyl linker and three equivalents of irinotecan. The full-profile pharmacokinetics study of PEG-[Irinotecan]$_3$ showed that after intravenous administration to rats, PEG-[Irinotecan]$_3$ undergoes stepwise loss of irinotecan to form PEG-[Irinotecan]$_{3-x}$ ($x = 1, 2$) and PEG-[linker] during which time the released irinotecan undergoes conversion to SN-38. This behavior significantly prolonged the $t_{1/2}$ and tumor exposure time of both irinotecan and SN-38. In a colorectal cancer-bearing model in nude mice, the tumor concentrations of irinotecan and SN-38 produced by PEG-[Irinotecan]$_3$ were respectively 86.2 and 2293 times higher at 48 h than those produced by irinotecan. This supports the view that PEGylated prodrug PEG-[Irinotecan]$_3$ as well as the multivalent PEG modification strategy are promising.

In addition, dendrimers have received tremendous attention as one of the most widely used polymers. Dendrimers are a well-defined class of nanostructured macromolecules with narrow masses or sizes, polydispersity, and tree-like structures characterized by exponential numbers of discrete dendritic branches radiating out from a common core. These structural features make dendrimers amenable to extensive surface modifications, further facilitating their applications in the delivery of toxins. A wide variety of dendrimers have been developed for the design of polymer–drug conjugates[64]. For instance, the highly potent antimitotic agent MMAE significantly reduces cytotoxicity through cleavable disulfide linker spliced with dendritic polyglycerol and dendritic polyglycerol sulfate. These conjugates could be potential candidates with good tolerance *in vivo*[65].

Besides, the proportion of polymer is large in the conjugates, which contributes to effectively masking some disadvantages of small molecule parent toxins, such as instability, poor water solubility, high toxicity, etc.[66]. For instance, Olesen et al.[67] have prepared molecular, macromolecular, and supramolecular water-soluble prodrugs of MMAE which can be bioactivated by glucuronidase. Among them, a PEG glucuronide prodrug with a trityl group showed high QIC$_{50}$ values (*i.e.* QIC$_{50}$, calculated as a fold-ratio between the corresponding IC$_{50}$ values in the presence or absence of glucuronidase), which means that it can mask the toxicity of MMAE itself and be converted to the cytotoxic MMAE by enzymes. *In vivo*, antitumor effects mediated by the prodrug also displayed satisfactory statistical significance compared with the control.

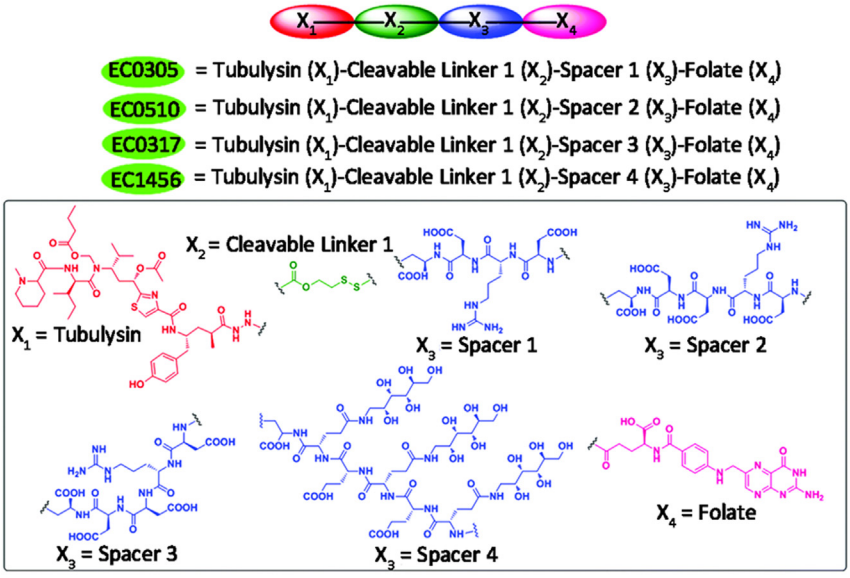

Figure 2 Tubulysin-based SMDCs (targeting ligand shown in pink; spacer shown in blue; cleavable linker shown in green; and therapeutic payload shown in red). Reprinted with the permission from Ref. 30. Copyright© 2021 The Royal Society of Chemistry and the Centre National de la Recherche Scientifique 2021.

3. NDDS

Since the last century, NDDS has gained wide attention due to its unique advantages. NDDS with various materials, functions, and morphologies have been extensively studied and applied in drug delivery, imaging, and diagnosis[19]. In recent years, NDDS have been increasingly developed for toxins delivery. A great many traits of NDDS are well suitable for non-ADC delivery of toxins: (a) based on the EPR effects or modified by targeting groups, NDDS penetrates blood vessels to accumulate in the tumor site and reduces drug exposure to normal tissues[68]; (b) the toxicity of the toxins can be effectively masked by the nano-carriers due to buried inside[69]; (c) NDDS is suitable for the delivery of various toxins such as hydrophobic, hydrophilic, and unstable toxins, possessing the ability to enhance their solubility and stability[70–72]; (d) NDDS with prolonged $t_{1/2}$ maintains effective blood concentration for a long time to reduce the frequency of administration[73]; (e) appropriate design of NDDS can be non-invasively across body barriers such as the blood–brain barrier[74]. In this section, NDDS-delivering cytotoxic drugs were divided into two types: noncovalently encapsulated NDDS and prodrug self-assembled NDDS.

3.1. *Noncovalently encapsulated NDDS*

The method of direct drug loading into nanoparticles has promising applications for a wide range of drugs[75]. Compared with the prodrug strategy, NDDS avoids the selection of covalent binding sites and linkers and only needs the selection of suitable materials as carriers according to the characteristics of the drug and the requirements for drug delivery. With the development of nanotechnology, more and more materials have been used as carriers for drugs, such as organic polymers, silica, proteins, etc.[76–78]. Some of the non-covalently wrapped toxin NDDS are summarized in Table 3[79–96].

3.1.1. *NDDS based on organic polymer*

The development of NDDS based on organic polymer was rapidly evolving. Various organic polymers have provided new options for the delivery of toxins. Poly (lactic-co-glycolic acid) (PLGA) is one of the most widely adopted NDDS carriers due to its ability to encapsulate and release drugs as well as passively target tumors[76]. PLGA has been applied to encapsulate SN38, pyrrolobenzodiazepines (PBDs), and other toxins[97–99]. The encapsulation of toxins in polymeric nanoparticles partly prevented the premature release of the drug before reaching the site of action, thus avoiding the non-selective cytotoxicity that occurred with the administration of free toxin, which in turn facilitated the enhancement of antitumor effect and safety[76].

To improve the targeting of NDDS, the surface of organic polymers was usually modified. Polymers modified with FA, biotin, hyaluronic acid (HA), and others would have the ability to actively target tumor cells. As previously mentioned, FA has a high affinity to FA receptors highly expressed in tumor cells. FA-modified D-α-tocopheryl polyethylene glycol succinate (TPGS) was used to modify the SN38-loaded PLGA nanoparticles to give them higher tumor accumulation and better antitumor effects compared to unmodified nanoparticles[82]. In addition, biotin and HA have high affinity to the highly expressed biotin receptor and CD44, respectively, in tumor cells. Biotin-targeted PLGA nanoparticles containing SN38 showed preferential anticancer properties against tumor cells with biotin receptor overexpression[100]. HA modification resulted in enhanced uptake of nanoparticles by CD44-expressing tumor cells, including cancer cells and tumor-associated macrophages[84].

Table 3 Noncovalently encapsulated NDDS of toxins.

Class	Toxin	Encapsulating material	Surface modification	Ref.
NDDS based on organic polymer	PBD	Poly (lactic-*co*-glycolic acid)	N/A	79
	DM1	Polylactide-D-α-tocopheryl polyethylene glycol 1000 succinate	Folate	80
	MMAF	Poly (1,4-phenyleneacetone dimethylene thioketal)	Bombesin, PEG	81
	SN38	Poly (lactic-*co*-glycolic acid)	D-α-Tocopheryl polyethylene glycol succinate (TPGS) and folate-TPGS	82
	SN38	Poly (lactic-*co*-glycolic acid)	Biotin	83
	SN38	Poly (lactic-*co*-glycolic acid)	Hyaluronic acid, methoxypoly (ethylene glycol)-*b*-poly (histamine methacrylamide)	84
	SN38	Methoxy-polyethylene glycol (mPEG)-chitosan	Hyaluronic acid	85
	SN38	Pluronic F127-polydopamine	N/A	86
	SN38	Polyamidoamine dendrimers	Cell-penetrating peptides, PEG	87
NDDS based on endogenous substance	DM1	Human serum albumin	N/A	88
	SN38	Bovine serum albumin	N/A	89
	camptothecin	Ferritin	Functional motif composed of hydrophobic peptides	90
NDDS based on inorganic materials	DM1	Mesoporous silica nanoparticles	Hydrochloride dopamine (PDA), PEG, epithelial cell adhesion molecule (EpCAM) aptamer (APt)	91
	DM1	Ultrasmall gold nanoparticles	Carboxylic acid terminated polyethylene glycol thiol ligands and 1-(2-mercaptoethoxy)-α-galactose C2 (αGalC2)	92
	MMAE	Silver nanoparticles	Prototypic CendR peptide (RPARPAR)	93
	SN38	Ultrafine iron oxide nanoparticles	Amphiphilic poly (ethylene glycol)-*b*-allyl glycidyl ether (PEG-*b*-AGE) polymer, RGD	94
	SN38	Single-walled carbon nanotubes	PEG	95
	SN38	Graphene oxide	N/A	96

N/A, not applicable.

Furthermore, the PEG is commonly used for surface modification of nanoparticles to overcome their rapid clearance in the reticuloendothelial system (RES). The PEG formed a hydrophilic environment around the nanoparticles, preventing them from being recognized by RES and thus increasing their $t_{1/2}$ and cycle time[101].

3.1.2. *NDDS based on inorganic materials*

In addition to organic materials, some inorganic nanoparticles with unique physicochemical properties also showed considerable attraction. Metal or non-metal inorganic materials NDDS have been widely applied for the delivery of toxins. The non-metallic material mesoporous silica occupied an important position in a variety of fields because of its good strength, thermal stability, and high specific surface area[77]. The modified mesoporous silica would be endowed with multiple functions, which would facilitate the targeted delivery of toxins. To mitigate the non-selective cytotoxicity of DM1, mesoporous silica nanoparticles (MSNs) loaded with DM1 and surface modified with hydrochloride dopamine (PDA), PEG, and epithelial cell adhesion molecule (EpCAM) APt were developed for targeted therapy of colorectal cancer (Fig. 3). In this system, the PDA coating was used as a pH-sensitive gatekeeper to control the release of DM1 from MSNs in response to pH stimuli. The PEG on the surface of nanoparticles could provide effective steric hindrance, increase circulating $t_{1/2}$, reduce the uptake of MSNs by RES, and enhance the EPR effect. EpCAM APt-directed active targeting could increase DM1 delivery to colorectal cancer as well as reduce toxicity and side effects by minimizing normal tissue exposure to DM1. The results confirmed that MSNs-DM1@PDA-PEG-APt may be a promising therapeutic platform for EpCAM-positive colorectal cancer[91]. In addition, non-metal inorganic materials based on carbon elements such as carbon nanotubes and graphene oxide have also started to be investigated recently in the field of toxin delivery[95,96]. These carbon nanomaterials, which have burst forth with the development of materials science research, have shown great potential in the delivery of toxins.

Metal-based inorganic materials were also employed for the delivery of toxins. For example, Li et al.[94] designed iron-based ultrafine iron oxide nanoparticles (uIONP) for SN38 delivery. SN38 encapsulated in the coating polymer exhibited pH-sensitive release. RGD-modified uIONP loaded with SN38 (RGD-uIONP/SN38) exhibited targeted cytotoxicity against $\alpha_v\beta_3$-integrin overexpressing U87MG glioblastoma cells with an IC_{50} of 30.9 ± 2.2 nmol/L. The efficacy study using an orthotopic mouse model of glioblastoma revealed that tumor-specific delivery of 11.5% injected RGD-uIONP/SN38 (10 mg Fe/kg), significantly prolonging the survival in mice by 41%, compared to those treated with SN38 alone ($P < 0.001$). Moreover, modified gold nanoparticles or silver nanoparticles have also been investigated for loading toxins to construct more targeted NDDS[93,102]. Although there were many studies on the NDDS based on inorganic materials, their potential toxicity of has not been fully studied. In particular, the possible biological incompatibility of intravenously injected inorganic materials limits the further application of NDDS based on inorganic materials[103].

3.1.3. *NDDS based on endogenous substance*

In any case, what cannot be ignored is that the extensive use of carrier materials often leads to insufficient drug loading rate and

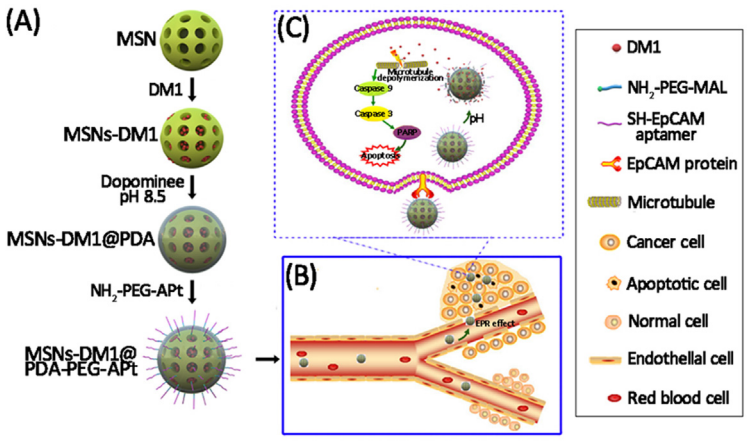

Figure 3 Schematic illustration of the synthesis of MSNs-DM1@PDA-PEG-APt bioconjugates (A) multifunctional MSNs-DM1@PDA-PEG-APt for targeted and controlled drug delivery (B, C). Reprinted with the permission from Ref. 91. Copyright© 2017 Ivyspring International Publisher.

potential toxicity associated with carriers. It is significant to select suitable materials as carriers to ensure safe and effective delivery of toxins. Endogenous substances like human serum albumin (HSA) and ferritin, are characterized by tumor tendency, biocompatibility, biodegradability and, non-immunogenicity, making them excellent candidates for drug carriers[78,104–106].

Influenced by the successful marketing of albumin-based formulations such as Abraxane®, the strategy of employing albumin as a carrier to deliver toxins has been widely developed. Wang et al.[88] effectively integrated the potent cytotoxic agent DM1 into biocompatible albumin nanoparticles. Taking advantage of the binding affinity to HSA, DM1 could be readily employed for albumin-based nanoassemblies. The results showed that HSA encapsulation significantly attenuated toxicity *in vivo* and enhanced the safety of DM1-based animal treatment. This work represented the first example of reformulation of a highly toxic DM1 with an HSA scaffold that had the potential to reduce the toxicity of the toxin and expand the range of cancer treatments available with albumin technology.

Ferritin, another endogenous protein, is a ubiquitously expressed iron storage protein. With a diameter inner cavity of 8 nm, ferritin has the potential space to encapsulate many drug molecules, thus providing protection to normal cells and reducing the side effects of toxins. Its outer diameter of 12 nm is suitable for the EPR effect. The disassembly and reassembly of the cage-like structure of ferritin are manipulated by pH changes to facilitate the encapsulation of therapeutic drugs within its structure. In addition, its self-assembly ability, symmetrical spherical structure, and high thermal stability as well as good biocompatibility and biodegradability propel a ferritin to be an excellent nano-drug carrier[105,106]. Some modifications to ferritin would also provide the ability to actively target as well as enhance the drug delivery capacity. For example, Wang et al.[90] redesigned the inner surface of a ferritin drug carrier (ins-FDC) by fusing the C-terminus of the human H ferritin (HFn) subunit with optimized hydrophobic peptides. The modified ferritin was effectively loaded with the hydrophobic toxin Camptothecin (CPT) and the hydrophilic Epirubicin. Dual-drug loaded ferritin nanocages showed high anti-tumor effects and a good safety profile, providing a new strategy for synergistic drug treatment of tumors.

3.2. *Self-assembling prodrug NDDS*

Noncovalently encapsulated NDDS typically use drug−carrier interactions to deliver toxins by physical encapsulation, which results in lower drug loading capacity and susceptibility to drug leakage during drug storage and *in vivo* circulation. With stable and high drug loading capacity, the self-assembled prodrug NDDS provides the possibility to reduce the systemic toxicity of highly toxic toxins and also provides good prospects for targeted delivery of toxins to specific tissues or cells[107]. Moreover, the self-assembled nanoparticles are designed due to the amphiphilicity of prodrugs and do not need the help of ingredients, which is beneficial to simplify the production process and improve drug loading *in vitro* and safety *in vivo*[107,108]. In short, vigorously researching self-assembling prodrug NDDS is expected to make a breakthrough in the anti-tumor treatment of toxins.

3.2.1. *Small molecule self-assembling prodrugs (SMSDs)*

The drug self-assembly process is driven by interactions between drug molecules (*e.g.*, hydrogen bonding, $\pi-\pi$ stacking, hydrophobic forces, and electrostatic interactions)[108]. The modification of toxins as prodrugs facilitates the balance of intermolecular forces and improves the ability of self-assembly. Combining the advantages of prodrug strategies and nanotechnology, SMSDs show significant advantages in delivering toxins: (a) nanosized formulations avoid rapid clearing or premature degradation of small molecule prodrugs *in vivo*; (b) the prodrug itself is both the drug being delivered and the drug carrier, resulting in a high drug loading capacity, while avoiding toxic side effects caused by the use of excessive carrier materials; (c) low molecular weight and small particle size allow toxins to easily penetrate tumor tissue and enter cells; (d) the preparation process of SMSDs is simple and easy to realize industrial production[107,109]. Given these advantages, SMSDs have become a research hotspot for toxin delivery in recent years.

SMSDs typically use functional side chains or chemical bonds to introduce "structural defects" into prodrug molecules to enhance their self-assembly capabilities. For instance, a novel hydrophobic SN38 prodrug was synthesized by attaching oleic acid to SN38 *via* disulfanyl-ethyl carbonate, which could self-

assemble into nanorods with high drug loading capacity (45%) and colloidal stability. These self-assembled nanorods showed remarkably high reduction sensitivity and potent *in vivo* antitumor activity[110] Additionally, another toxin, DM1, was modified by docosahexaenoic acid (DHA) to be able to self-assemble into nanoparticles, achieving the goal of effectively reducing the systemic toxicity and side effects of DM1 without sacrificing its antitumor effects[111].

In consideration of the high cytotoxicity of such toxins, it is necessary to achieve tumor homing as accurately as possible, therefore SMSDs with tumor-targeting ability were further discussed. Liang et al.[112] prepared a self-assembling prodrug of DM1 nanoparticles containing $\alpha_v\beta_3$-targeting peptide and reductively sensitive disulfide bond which have ligand-targeting and stimulus-responsive properties. The design incorporated the characteristics of the prodrug, nanoparticles, and active targeting, showing good therapeutic efficacy *in vitro* and *in vivo* without the unacceptable systemic toxicity caused by free DM1 (Fig. 4).

3.2.2. *Polymer self-assembling prodrugs*

Polymer–drug conjugates possess the advantages of enhanced drug solubility, increased efficacy, and improved pharmacokinetics, thus providing promising applications. Polymer self-assembling prodrugs mean that the polymer–drug conjugates could self-assemble into nanoparticles possessing the rational size of tumor accumulation and high drug loading. Taking β-cyclodextrin-based polymer prodrug of tubulysin A as an example, the self-assembled nanoparticles had a drug loading of up to 28%, a particle size of 100–130 nm, and increased the solubility of tubulysin A by 100 times[113].

Passive targeting of tumors relying on EPR effects alone may not be sufficient for toxin delivery. Therefore, functional macromolecules were gradually attracting the attention of researchers. Liu et al.[114] modified the polymeric prodrug $PEG_{2.4K}$-p$(HEMASN38)_{3K}$ of SN38 with a peptide cys-arg-gly-asp-lys (CRGDK) that specifically binds to neuropilin-1 overexpressed by tumor vessels and tumor cells to further improve the delivery and efficacy of SN38. The cellular entry of C-SN38 nanoparticles could be enhanced through binding interaction between CRGDK peptide and neuropilin-1 receptor expressing on the cell surface. Subsequently, active SN38 is released and exhibits cell-killing activity against tumor cells. As demonstrated by the results, compared to the non-functionalized control, the CRGDK-functionalized nanoparticles exhibited significantly enhanced tumor accumulation and penetration, as well as a significantly increased therapeutic activity. Combining active and passive targeting, novel functionalized polymer self-assembled prodrugs offer tremendous development potential in oncology therapy (Fig. 5).

4. Biomimetic drug delivery systems

Undoubtedly, NDDS has become one of the most widely researched toxin delivery strategies currently available. However, some carrier materials, especially polymers, are inevitably used in NDDS, which could have potentially toxic side effects. Because of the low immunogenicity and tumor targeting, biomimetic drug delivery system has been widely a concern by the scientific community. In addition to the endogenous substances as carriers mentioned in section 3.1.3., targeted delivery could also be easily achieved by using biocompatible cell membranes, and vesicles or directly using cells as drug carriers for delivery of toxins[115,116]. These biomimetic drug delivery systems compensate for the high immunogenicity and gradual complexity of conventional NDDS[117]. At the same time, the ingenious combination of traditional drug delivery systems with endogenous substances provides desirable biological functions, such as long circulation time and targeting tumors, further improves the efficiency of targeted tumor therapy and contributes to the implementation of precision medicine[118].

4.1. *Cell membrane-camouflaged nanoparticles*

Nanoparticles camouflaged by cell membranes are the most common biomimetic drug delivery system. Wrapping cell membranes on the surface of conventional nanoparticles retains the corresponding biological functions of cells and has great application potential. Red cell membranes, tumor cell membranes, platelet membranes, and immune cell membranes were commonly used in cell membrane-camouflaged nanoparticles. Different cell membranes have different specific proteins and therefore have different biological functions[117]. For instance, red blood cells were the most numerous blood cells. Due to the advantages of long life and convenient extraction, the red cell membrane became the first cell membrane to be applied for membrane camouflage nanoparticles[119]. Hu et al.[120] showed that nanoparticles coated with red blood cell membrane exhibited a longer elimination half-life than that coated with the conventional PEG (39.6 h *vs*. 19.8 h). The excellent long circulation function was expected to help improve the passive targeting of nanoparticles to tumors.

In addition, based on the natural tendency of neutrophils, a common immune cell, to inflammatory tumor sites[121–123], neutrophil membrane-encapsulated nanoparticles could serve as a promising delivery system to improve the delivery of toxins to tumors. For another, neutrophil membrane-encapsulated nanoparticles could also counteract the interaction between neutrophils and circulating tumor cells (CTCs), thereby reducing the formation of neutrophil-CTC clusters and inhibiting tumor metastasis[124]. The hypoxia-responsive quinone-modified MMAE dimeric prodrug (hQ-$MMAE_2$) was encapsulated into PLGA nanoparticles, and then the neutrophil membrane was wrapped around the surface of PLGA nanoparticles (hQNM-PLGA) to make PLGA nanoparticles with the biological properties of neutrophils. After intravenous injection, hQNM-PLGA nanoparticles were recruited to inflammatory tumor sites following the intrinsic function of neutrophils, leading to improved stability of nanoparticles, increased tumor accumulation, and reduced leakage of MMAE in the systemic circulation. In the serious hypoxic environment of advanced breast cancer, hQ-$MMAE_2$ was degraded and MMAE was released, resulting in significant anti-tumor therapeutic effects. At the same time, hQNM-PLGA NPs could effectively block the formation of neutrophil-CTC clusters and thereby suppress tumor metastasis. These cell membrane-based bionic toxin delivery strategies incorporated the unique advantages of cells into NDDS, providing a promising approach to inhibit metastasis and treat tumors (Fig. 6)[24].

4.2. *Extracellular vesicles (EVs)*

Nowadays, cell-derived EV-based carrier systems have attracted considerable interest from researchers. EVs are heterogeneous groups of small, lipid-bound nanoparticles that are key mediators of many physiological processes. EVs are nanoparticles secreted by cells and have a phospholipid bilayer membrane structure[125]. EVs have been used in a variety of cancer therapeutic studies due

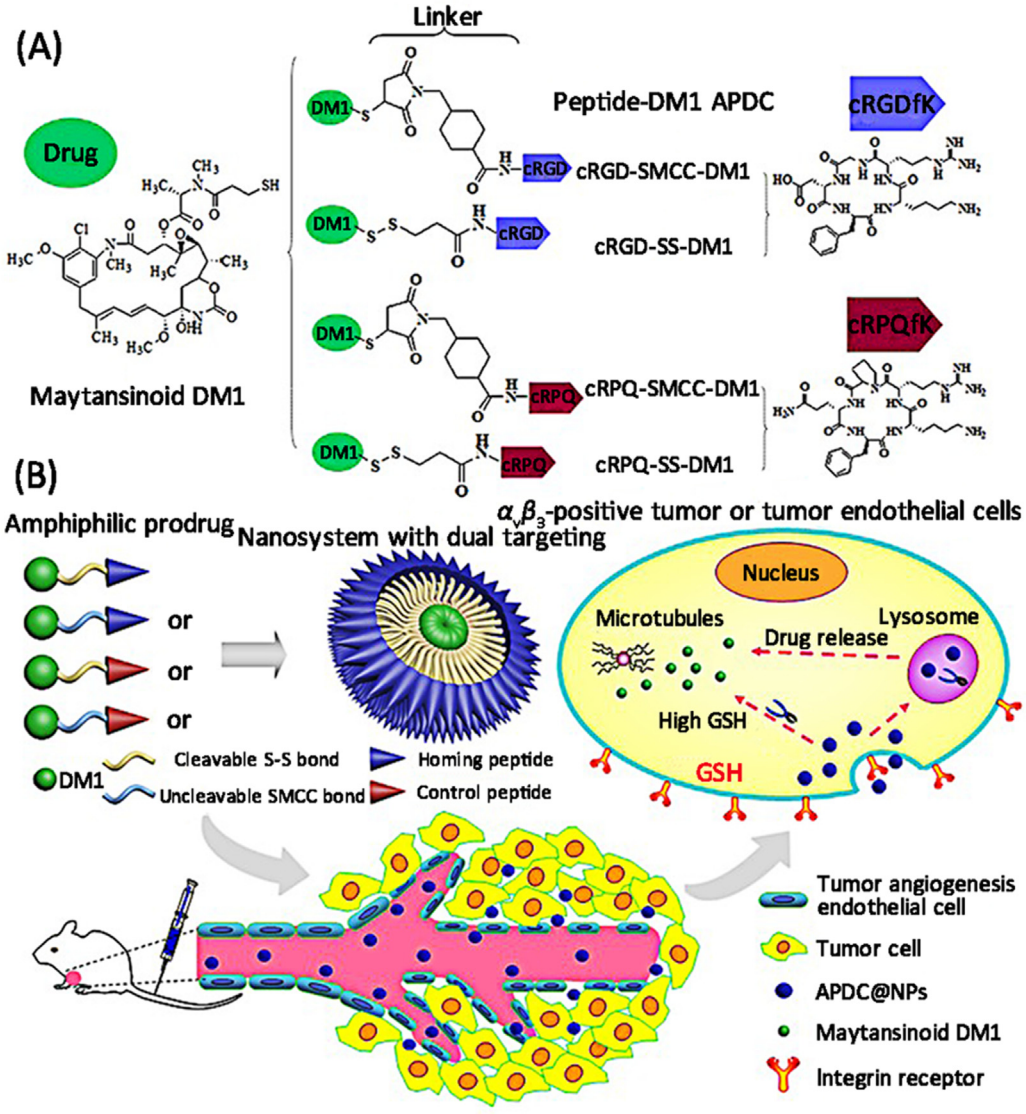

Figure 4 (A) Chemical structure of small molecule amphiphilic peptide-drug conjugates (APDCs): cRGD-SMCC-DM1 (RCCD), cRGD-SS-DM1 (RSSD), cRPQ-SMCC-DM1 (QCCD), and cRPQ-SS-DM1 (QSSD). (B) Schematic illustrations of the self-assembly of APDC nanoparticles (APDC@NPs), the accumulation of APDC@NPs at the tumor site by the EPR effect, their uptake by tumor cells or tumor angiogenesis endothelial cells by $\alpha_v\beta_3$ receptor-mediated endocytosis, and the triggered intracellular drug release from APDC@NPs. Reprinted with the permission from Ref. 112. Copyright© 2017 The authors.

to good structural stability, excellent biocompatibility, and natural transport capacity[126]. Si et al.[26] employed monoclonal antibodies modified EVs to deliver the natural cytotoxic marine compound verrucarin A with an IC_{50} of 2.2−2.8 nmol/L and the microtubule polymerization inhibitor DM1 with an IC_{50} of 3.1−4.2 nmol/L. This agent potently inhibited the growth of neuroendocrine tumors. The *in vivo* maximum tolerated dose study in non-tumor-bearing mice demonstrated a good safety profile for the delivery of potent chemotherapeutic agents by EVs.

4.3. *Living cells to deliver toxins*

Some cells (such as stem cells, and immune cells) have natural tumor-targeting functions without modification and are additionally good carriers. Unfortunately, the direct loading of small molecule chemotherapeutic drugs into cells causes the cellular carriers' death before reaching the target site due to their significant cytotoxicity. Consequently, using cellular delivery nanoparticles would retain the biological function of cellular carriers and avoid the leakage of drugs due to cell death during transport, which holds outstanding application prospects[117]. For example, nanoparticles with intelligent release based on inflammatory monocytes have been developed for the treatment of lung cancer metastasis. DM1 was conjugated with poly (styrene-co-maleic anhydride) by legumain-sensitive peptide and self-assembled into nanoparticles (SMNs), which were then phagocytized by $Ly6c^+$ inflammatory monocytes for drug delivery. The SMNs-laden monocytes (M-SMNs) could be preferentially delivered to the sites of metastases due to the autonomous metastasis-homing effects of monocytes and then activate the on-demand drug release as free drug molecules and drug-loaded microvesicles upon the differentiation into macrophages, thereby

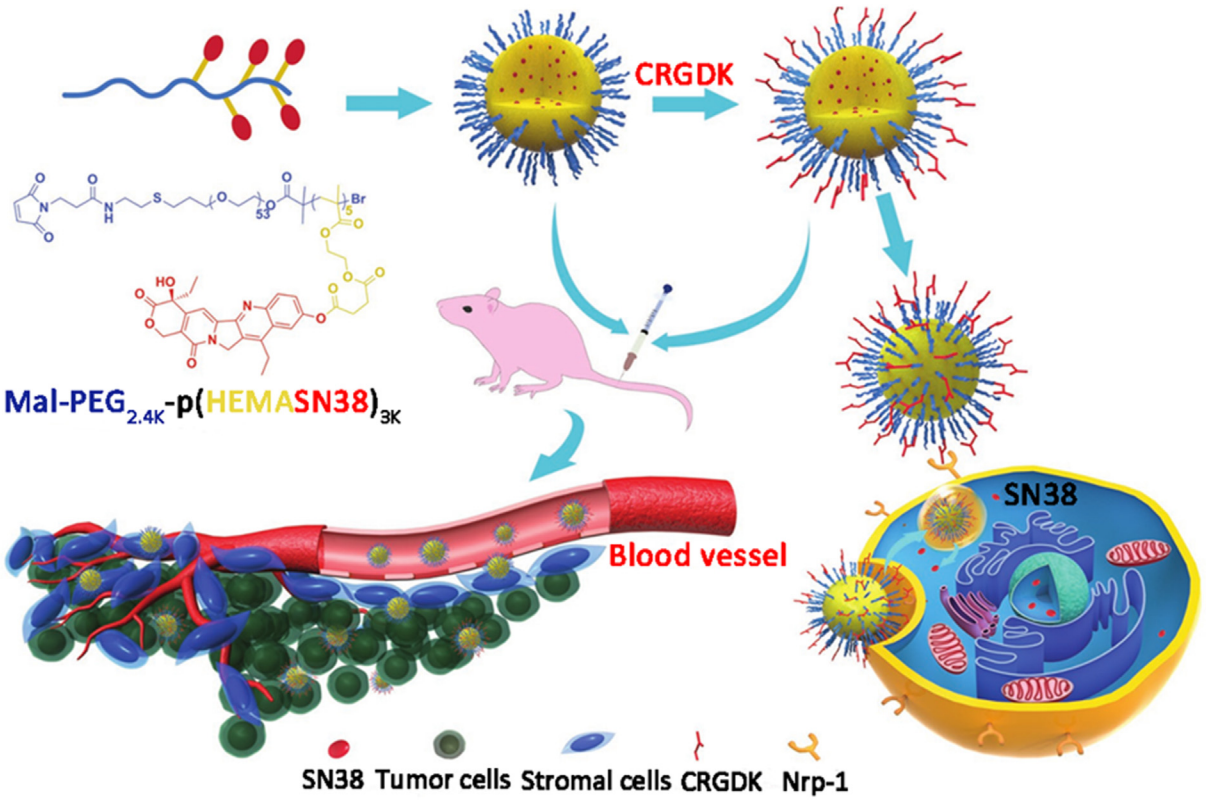

Figure 5 Illustration for the preparation of CRGDK-functionalized copolymer (C-SN38) *via* the reaction of the thiol group of CRGDK with the maleimide group of Mal-$PEG_{2.4K\text{-}p}$ $(HEMASN38)_{3K}$, and the self-assembled C-SN38 nanoparticles for targeted cancer therapy. Reprinted with the permission from Ref. 114. Copyright© 2018 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

enhancing the medical performance on breast cancer metastasis (Fig. 7). This drug delivery system was stable in blood circulation, but when monocytes reached the metastatic foci, they differentiated into legumain overexpressed macrophages to release DM1 derivative as free drug or drug-loaded microvesicles/vesicle that exhibited a high inhibition rate of lung metastasis of 77.8%[27].

The biomimetic drug delivery systems employed natural cells or cellular components as delivery carriers, significantly reducing immune clearance compared to conventional NDDS. Meanwhile, some unique properties, such as inherent targeting and good biocompatibility, gave biomimetic drug delivery systems a promising future. The safe biomimetic nanocarriers would be an outstanding choice for improving safety when delivering toxins. With the development of biotechnology, formulation technology, and high-sensitivity separation and analysis technology, the research about the drug loading capacity, stability, preparation procedure, and quality control of biomimetic drug delivery systems is getting more and more deeply, and the ideal biomimetic carrier system will be further developed[20,116–118].

5. Combination strategies

Toxins play an increasingly important role in cancer therapeutics. Multiple toxin delivery strategies have been developed and have shown successful outcomes. However, cancer is a complex and intelligent disease that has the characteristics of infinite proliferation, easy metastasis, and drug resistance, inducing the effect of mono-therapy is insufficient. Consequently, in scientific research and clinical application, multiple treatment options were usually used for combination treatment. Increasing research has manifested that a combination of dual or multiple agents could reduce toxicity, against drug resistance, and increase the antitumor effect[28,127].

5.1. The toxins are used in combination with chemotherapy drugs

Chemotherapy has always been regarded as one of the most important clinical approaches for fighting cancer. Toxins have been widely investigated and applied as chemotherapeutic agents because of their good anti-tumor activity. However, drug resistance due to multiple causes has emerged as one of the inescapable problems in chemotherapy. The combination of chemotherapeutic agents with different mechanisms would likely ameliorate the problem of drug resistance in chemotherapy[127]. For example, the combination of epidermal growth factor receptor (EGFR) inhibitors with SN38, the active metabolite of irinotecan, had a synergistic antitumor effect in SN38-refractory gastric carcinoma cells. The combination of an EGFR inhibitor and SN38 significantly increased the levels of apoptosis-associated molecules, caspase-6, p53, and DAPK-2, and led to apoptosis induction of irinotecan-resistant cells. The EGFR inhibitors increased the S-phase and decreased the UGT1A1 and ABCG expression in irinotecan-resistant cells. *In vivo*, antitumor assays showed that the SN38 and EGFR inhibitor lapatinib combination group suppressed the growth of OCUM-2M/SN38 xenograft tumors more effectively than either alone group[128].

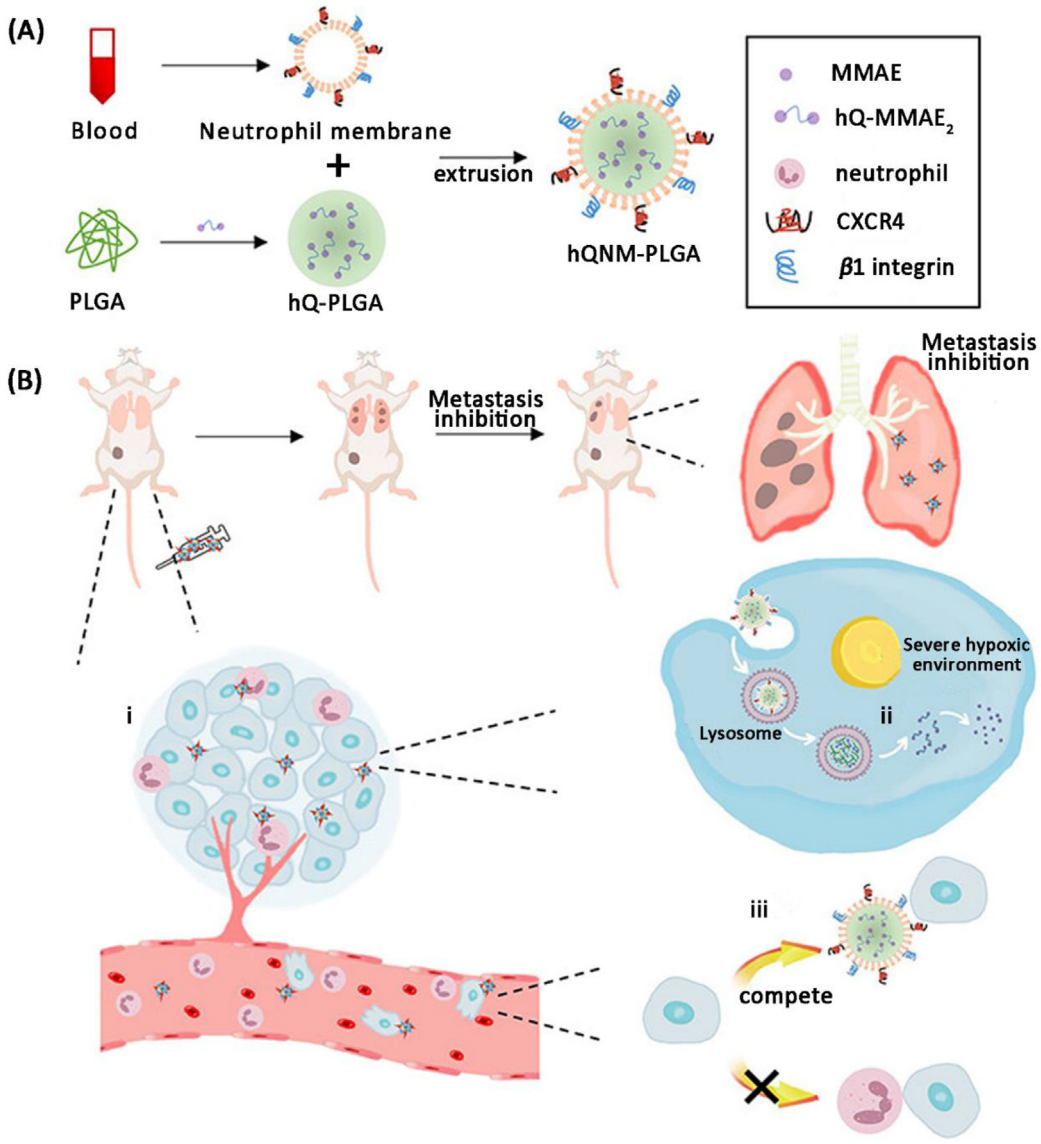

Figure 6 Schematic illustration of multi-site attack, neutrophil membrane-camouflaged PLGA NPs encapsulating hypoxia-responsive dimeric prodrug for enhanced cancer and anti-metastasis therapy. (A) The hQNM-PLGA NPs were prepared *via* the emulsion method, which contained neutrophil membranes and hypoxia-responsive dimeric prodrug hQ-MMAE2. (B) (i) hQNM-PLGA NPs could target hQ-MMAE2 delivery to tumor sites. (ii) hQ-MMAE2 degraded in the hypoxic environment to facilitate MMAE release, thereby eliminating the primary tumor cells to achieve good safety and anticancer therapy. (iii) hQNM-PLGA NPs disrupted the neutrophils-tumor cell clusters formation, thus inhibiting tumor lung metastasis. Reprinted with the permission from Ref. 24. Copyright© 2023 The authors.

Moreover, the combination of toxins and chemotherapeutic agents with similar mechanisms is also beneficial to enhance the antitumor effect. Zhong et al.[129] reported a DM1-based prodrug self-assembled micelle loaded with docetaxel (DTX). Both DTX and DM1 were antimitotic agents, in which DTX works by inhibiting the formation of spindles, while DM1 acted as tubulins polymerization inhibitor. DM1 covalently combined with the cRGD functionalized poly (ethylene glycol)-*b*-poly (trimethylene carbonate) copolymer *via* a disulfide bond, which then self-assembled into micelle and physically encapsulated DTX by solvent exchange method, with the contents of DM1 and DTX are 24.9% (m%) and 12.5% (m%) respectively. Such micelle showed better tumor growth inhibition and longer survival time than a single drug in the B16F10 melanoma model. The combination index of DTX and DM1 was calculated to be 0.379, indicating a strong synergistic antitumor effect of the two drugs.

5.2. *The toxins are used in combination with radiotherapy*

With the advancement of medical technology, radiation therapy for tumors has also developed rapidly. It has been shown that radiotherapy sensitizers increase the sensitivity of tumor cells to radiotherapy in various ways, including inhibition of DNA damage repair, regulation of the cell cycle, and promotion of apoptosis. Coincidentally, toxins such as MMAE and DM1 could block tumor cells in the G2/M phase, which is the period when cells are most sensitive to radiotherapy[130–132]. Buckel's group designed an MMAE prodrug that contained a cRGD targeting peptide and

matrix metalloproteinases (MMP) targeted activatable cell-penetrating peptide (ACPP), named ACPP-cRGD-MMAE, as a radiosensitizer. Such ACPP-cRGD-MMAE with better affinity and selectivity for $\alpha_v\beta_3$ integrin overexpressed and MMP in tumors resulted in cancer cells being more sensitive to radiotherapy than surrounding normal cells. On the contrary, ionizing radiation also increased the permeability of tumor cells, the expression of integrin, and the activity of MMP. As a result, the combination strategy of the ACPP-cRGD-MMAE and IR exhibited an obvious reduction of cancer cell survival rate and significantly prolonged and strong tumor regression in PANC-1 or HCT-116 murine tumor models[133]. Additionally, Gao et al.[134] encapsulated nitrogenation prodrug of DM1 in poly (lactide-*co*-glycolic)-*block*-poly (ethylene glycol) (PLGA-*b*PEG) nanoparticles. The systemic toxicity of DM1 was suppressed by nanoparticle encapsulation and nitrosylation of the thiol group of DM1. After ionizing radiation, the level of intracellular oxidative stress increases, and the S−N bond breaks to release DM1 and NO that were oxidized to form highly toxic reactive nitrogen species such as peroxynitrites. Both DMI and reactive nitrogen species had strong cell-killing capacity; on the other hand, they made cancer cells more sensitive to radiation. Finally, the combination of nanoparticles and radiotherapy showed a tumor inhibition rate of 9.64-fold than that of radiotherapy alone, and significantly higher than that of free prodrug or DM1 nanoparticles (Fig. 8).

5.3. *The toxins are used in combination with phototherapy*

In addition to the combination of traditional chemotherapy and radiotherapy, the combination of phototherapy and toxin therapy has shown remarkable therapeutic effects. Phototherapy includes photodynamic therapy (PDT) and photothermal therapy (PTT), which can be realized by either photosensitizers or inorganic nano-materials with photosensitive properties[135−137]. They not only have therapeutic effects but also can be used for imaging to diagnose and analyze more accurately[138].

Chu et al.[139] prepared MPEG-(TK-CPT)-PPa self-assembled nanoparticles by attaching the toxin CPT and the photosensitizer pyropheophorbide-a (PPa) to poly (ethylene glycol) methyl ether (MPEG) concurrently with ROS-responsive thioketal (TK) and lipid linkage. This combination of toxin and photosensitizer prodrug nanoparticles prevented drug leakage during systemic circulation and allowed the simultaneous distribution of both drugs *in vivo*. The fluorescence signal generated by PPa contributed to the precise tracking and localization of nanoparticles. Under the guidance of imaging, a near-infrared laser locally irradiated tumor tissue upon reaching the strongest fluorescence. The ROS generated after irradiation not only cleaves TK linkage, thereby triggering controlled CPT release but also had cytotoxic effects on tumor cells. Ultimately, the combination of CPT-mediated chemotherapy and PPa-induced PDT enhanced the antitumor effect.

Except for photosensitizers, some inorganic materials also have photosensitive properties. Gold nanoparticles with localized surface plasmon resonance function could produce a PTT effect to destroy cells. Hosseinzadeh et al.[140] prepared MUC1-modified gold nanoparticles loaded with SN38-HA, which had dual antitumor effects. On the one hand, MUC1 modification enabled nanoparticles to deliver SN38 through active targeting and thus exerted cytotoxic effects. On the other hand, the photothermal properties of the gold core in the nanoparticles allowed the application of an external light source (*e.g.*, LED) to enhance their antitumor effects. The combination of toxin and photothermal gave the SN38-loaded gold nanoparticles the ability to overcome metastatic colon cancer. In addition, some studies have reported that gold nanoparticles had outstanding advantages in contrast[141], which would help to realize the visualization of toxin-photothermal synergistic therapy. Xu et al.[142] designed porous gold nanoparticles loaded with DM1 (DM1-mPEG/HER-PGNSs) possessing therapeutically relevant heating and significant thermo-chemotherapy capacities without inducing obvious organ damage. Simultaneously, mPEG/HER-PGNSs had a stronger computed tomography/photoacoustic contrast effect and enhanced photothermal imaging during photothermal treatment (Fig. 9).

Compared with the direct co-administration of photosensitizers and drugs, the tumor microenvironment can be improved by PTT, and then the toxins can effectively increase the tumor accessibility of drugs. Tan et al.[143] used bioinspired lipoprotein (bLP) with tumor homing ability to encapsulate photosensitizer DiOC18 (DiR)

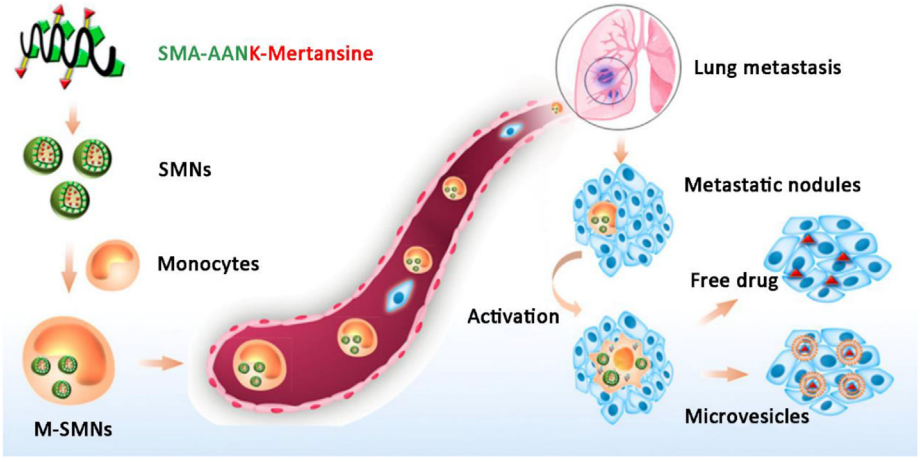

Figure 7 Schematic illustration of inflammatory monocytes loading legumain-sensitive nanoparticles (M-SMNs) to target lung metastasis of breast cancer and initiate metastatic-specific drug release to achieve anti-metastasis efficacy. Reprinted with the permission from Ref. 27. Copyright© 2017 American Chemical Society.

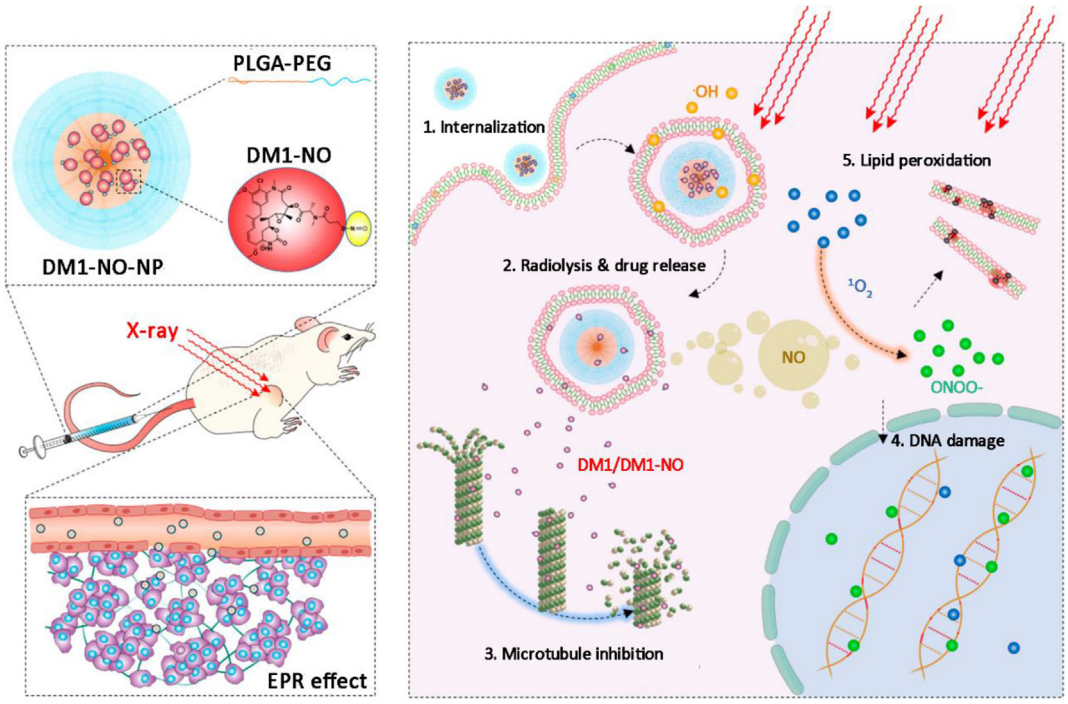

Figure 8 DM1-NO-encapsulated PLGA-*b*-PEG nanoparticles (DM1-NO-NPs) can accumulate in tumors through the EPR effect. In the presence of radiation and/or reduced pH in endosomes/lysosomes, the S−N bond was broken, releasing DM1 and NO. DM1 inhibits microtubule assembly, arresting cells at the more radiosensitive G2/M phase. Meanwhile, NO can react with ROS to form radicals such as peroxynitrites, causing DNA and lipid damage. The combined effects enhance the efficacy of RT. Reprinted with the permission from Ref. 134. Copyright© 2020 American Chemical Society.

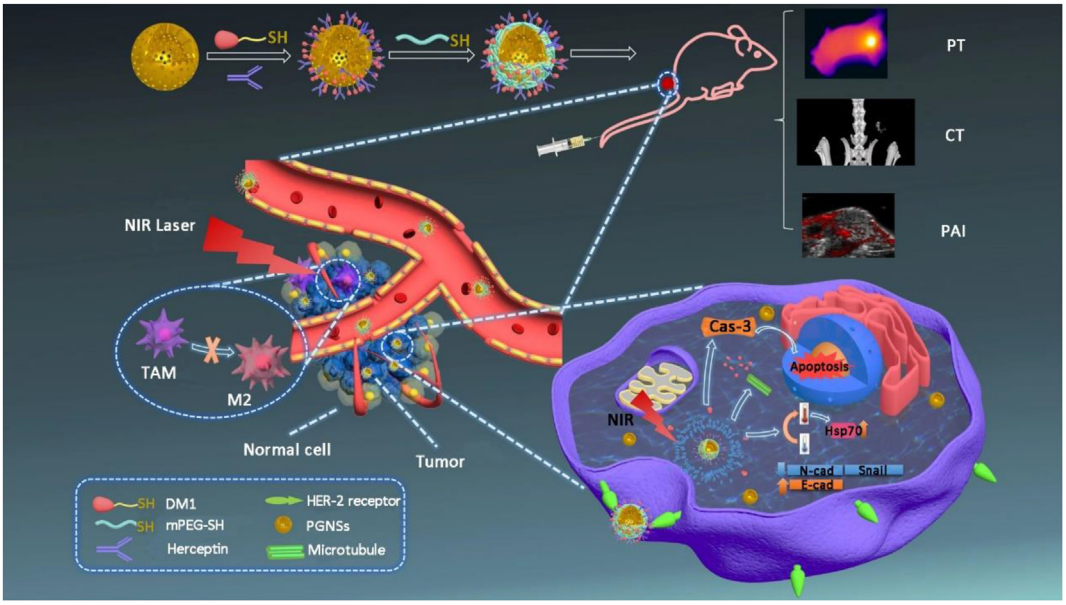

Figure 9 A DM1-doped porous gold nanoshell system for near-infrared accelerated redox-responsive release and triple modal imaging-guided photothermal synergistic chemotherapy. Reprinted with the permission from Ref. 142. Copyright© 2019 The authors.

and DM1 respectively. At first, DiR-bLP was injected, which was mainly retained in the tumor matrix and disrupted or destroyed the tumor stromal microenvironment barriers after irradiation, and then DM1-bLP could be delivered to the depth of tumor tissue (Fig. 10) with tumor accumulation increased by 4.27 times, and tumor accessibility increased by 27 times compared with the control

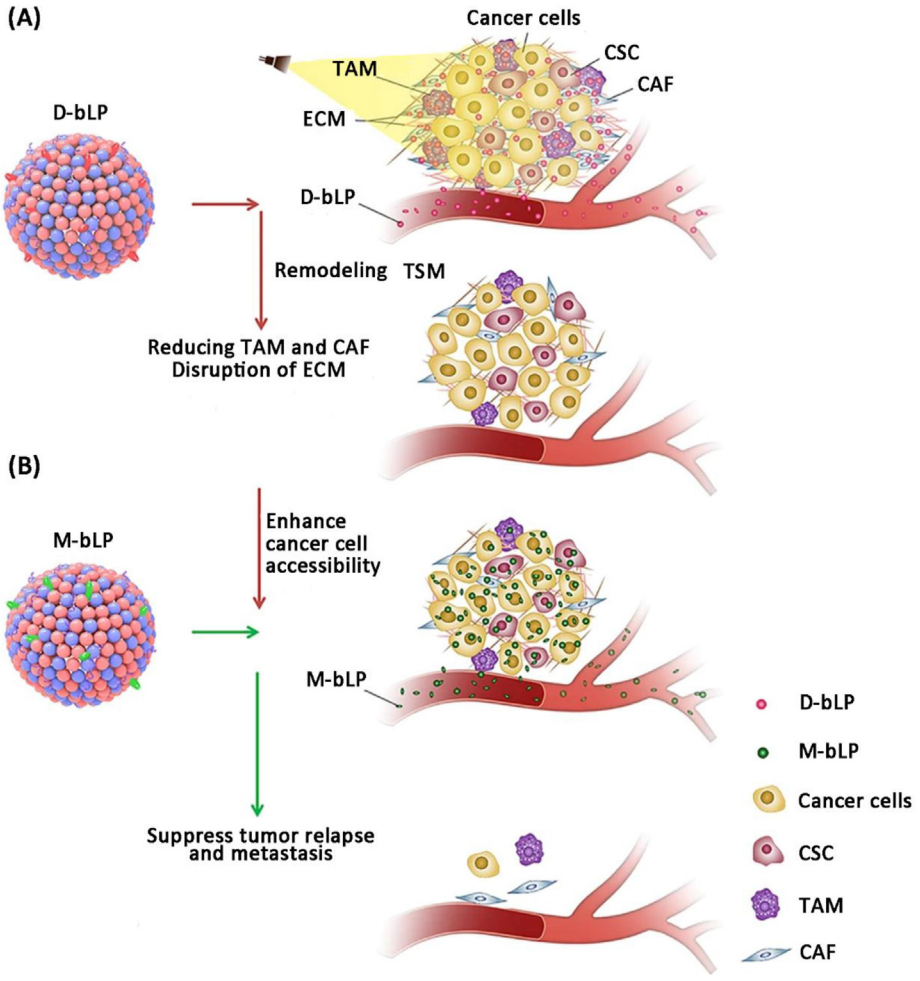

Figure 10 Schematic illustration of D-bLP-mediated photothermal remodeling of tumor stroma to enhance second M-bLP accessibility to cancer cells. (A) D-bLP-mediated photothermal effects cause drastic elimination of stromal cells and extracellular matrix components (*e.g.*, collagen I, fibronectin). (B) D-bLP-mediated tumor stromal microenvironments remodeling enhances the accumulation and penetration of second M-bLP in tumors and promotes their extravasation from tumor vasculature and accessibility to cancer cells, thus resulting in efficient suppression of tumor relapse and metastasis. CSC cancer stem cells. Reprinted with the permission from Ref. 143. Copyright© 2019 The authors.

group, inducing extremely outstanding tumor inhibition rate (95.9%) and lung metastasis inhibition rate (97.4%).

6. Conclusions

Toxins with high antitumor activity have been widely used for a long time. The extremely high cytotoxicity of toxins is a powerful guarantee for them as anticancer drugs, however, it also lays a safety risk for systemic toxicity. Consequently, the design of such delivery systems requires ensuring the maximum effectiveness of the toxin while minimizing side effects. Delivering toxins utilizing ADCs has now become the preeminent clinically recognized toxin delivery strategy. Although ADCs have their unique advantages, developing various delivery strategies is meaningful and conducive to broadening the clinical application of toxins. This paper focused on emerging non-ADC delivery strategies of toxins, mainly including prodrugs, NDDS, biomimetic drug delivery systems, and combination strategies. These delivery strategies, some of which are already in clinical trials, have great potential for clinical application and further development.

Unfortunately, the exploration of toxin delivery strategies also faces many challenges, such as the industrialization of chemical synthesis and formulations, the safety of nanocarrier materials and stability of formulations, and the correlation of *in vitro* studies with *in vivo* behavior. In recent years, rapid advancements in formulation technology and cross-disciplines have driven the development of non-ADC delivery systems for toxins[1]. More and more diverse designs of toxin delivery strategies (*e.g.*, stimuli-responsive prodrugs, novel actively targeted functionalized NDDS) will further benefit to improve the toxin efficacy and minimize side effects, and will also open up the possibility for clinical translation of toxin delivery systems. It is expected that highly effective and low-toxicity non-ADC toxin delivery systems with low production costs will be developed in the future with the continuous progress of research.

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Author contributions

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Conflicts of interest

The authors declare no conflicts of interest.

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