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Perspective

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Cancer Nanomedicines Based on Synthetic Polypeptides

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ABSTRACT: Nanomedicines are deemed as a most promising treatment modality for malignant cancers. Particularly, cancer nanomedicines based on synthetic polypeptides have gained interest because they possess excellent safety, unique hierarchical structure, and tailorable functionalities to suit for delivery of diverse drugs including synthetic drugs, peptides, proteins, and nucleic acids. A few polypeptide-based nanoformulations (e.g. NK105, NC6004, NK911, CT2103) are under phase I-III clinical investigation for treating patients with advanced solid tumors. In recent years, progress has been made in development of robust and high drug loading, tumor-targeting, membrane-disrupting, and/or stimuli-sensitive nanomedicines from de novo functional polypeptides, which afford not only better safety and reduced adverse effects but also further improved anti-cancer efficacy over clinical formulations. Moreover, virus-mimicking vehicles have been devised from polypeptides for efficient non-viral delivery of highly potent peptides, proteins, and nucleic acids, greatly advancing biotherapy for cancers. In this perspective, we highlight the state-of-the-art design and fabrication of cancer nanomedicines based on synthetic polypeptides, and at the end give our viewpoints on their future development for targeted cancer therapy and potential challenges for clinical translation.

KEYWORDS: Polypeptide; drug delivery; gene delivery; protein delivery; cancer therapy

1. INTRODUCTION

Nanomedicines are deemed as a most promising treatment modality for malignant cancers.[1-3] Particularly, cancer nanomedicines based on synthetic polypeptides have gained interest because they possess excellent safety, unique hierarchical structure, and tailorable functionalities to suit for delivery of diverse drugs including synthetic drugs, peptides, proteins, and nucleic acids. [4-6] Synthetic polypeptides with controlled molecular weights and high optical purity can be facilely obtained in large scale through ring-opening polymerization (ROP) of *α*-amino acid *N*-carboxyanhydrides (NCA).[7, 8] Especially, innovative initiators (transition metal complexes, silazane, lithium hexamethyldisilazide, etc.)[9-11] and optimized reaction conditions (nitrogen flow, cooperative polymerization, interface polymerization)[12-14] have been reported for precise synthesis of polypeptides under mild conditions. Remarkably, several micellar formulations based on poly(ethylene glycol)-*b*-polypeptide block copolymers (NK105, NC6004, NK911, *etc.*) developed by Kataoka *et al.* have been advanced into different phases of clinical trials to treat breast, pancreatic, and gastric cancers.[4] A prodrug of poly(L-glutamic acid)-paclitaxel (CT-2103) has entered phase III clinical trials and is expected to be the first synthetic polymer-drug conjugate to reach the market.[15] These first-generation nanomedicines are primarily based on simple synthetic polypeptides and have shown to improve drug solubility and pharmacokinetics and increase tumor drug concentration, greatly reducing side effects and widening therapeutic index compared with their clinical counterparts.[4, 16] In recent years, progress has been made in development of next-generation nanomedicines with robust and high drug loading, tumor-targeting, membrane-disrupting, and/or stimuli-sensitive functions from de novo functional polypeptides (**Figure 1**), which afford not only better safety and reduced adverse effects but

also further improved anti-cancer efficacy over clinical formulations. Moreover, virus-mimicking vehicles have been devised from synthetic polypeptides for efficient non-viral delivery of highly potent peptides, proteins, and nucleic acids, greatly advancing biotherapy for cancers. Notably, though there are several excellent reviews on peptide-based nanoassemblies,[17-19] as well as controlled synthesis and bioapplications of polypeptides including elastin-like polypeptides,[20-22] no perspective on recent strategies toward next generation nanomedicines based on synthetic polypeptides is available. In this perspective, we highlight the state-of-the-art design and fabrication of cancer nanomedicines based on polypeptides, and at the end give our viewpoints on their future development for targeted cancer therapy and potential challenges for clinical translation.

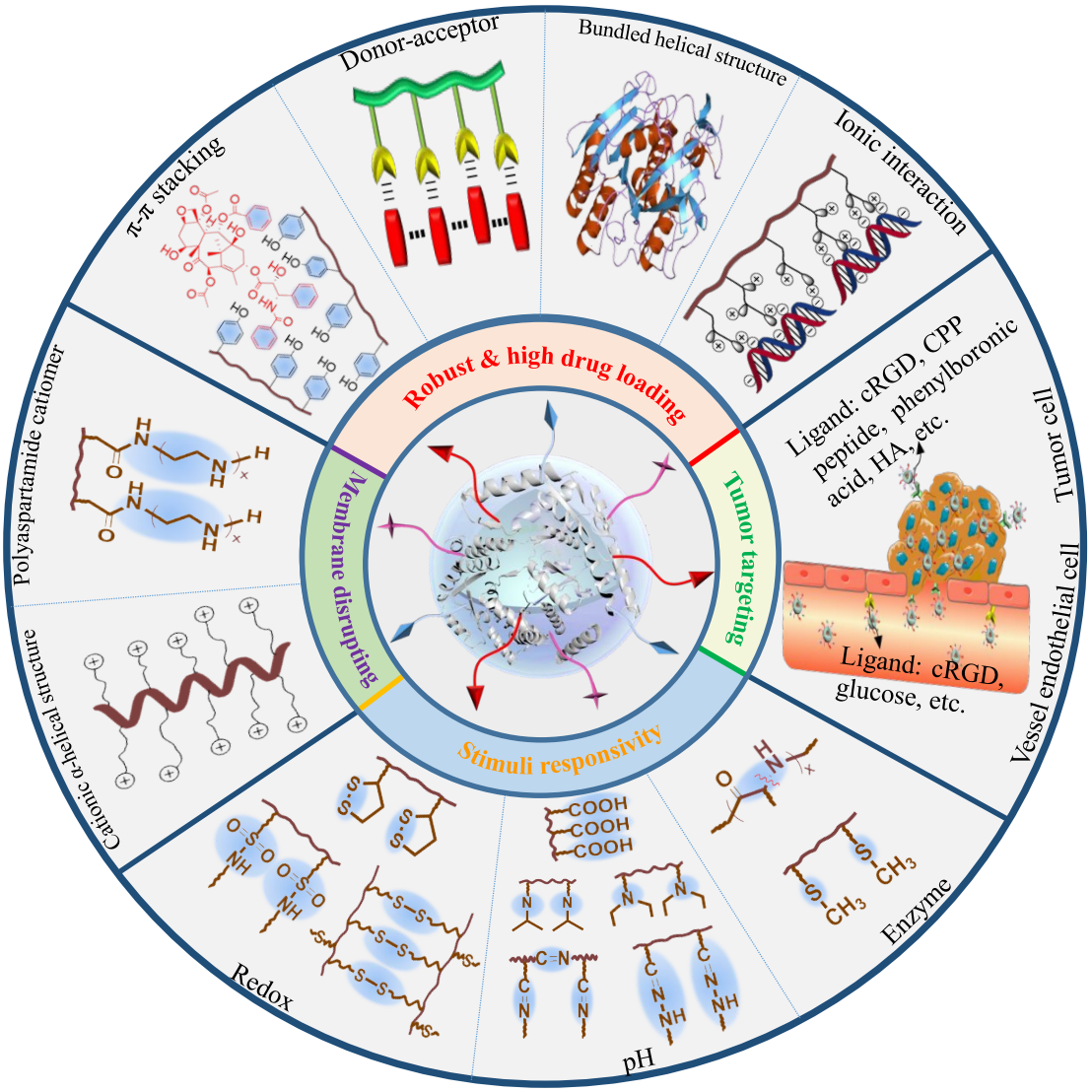

Figure 1. Next-generation nanomedicines with robust and high drug loading, tumor-targeting, membrane-disrupting, and/or stimuli-sensitive functions developed from de novo functional polypeptides.

2. ROBUST AND HIGH DRUG LOADING NANOMEDICINES BASED ON SYNTHETIC POLYPEPTIDES

Amphiphilic block copolymers could form core-shell type micelle structures in aqueous medium, facilitating the encapsulation of hydrophobic anticancer drugs in the micellar core.[23, 24] The weak hydrophobic interactions between drugs and hydrophobic segments in the micellar core, however, result in typically moderate drug loading and poor in vivo stability.[16, 25] Various physical interactions and covalent conjugation strategies have been exploited to enhance the loading and stability of drugs in polymeric micelles.[16] For example, Kataoka *et al.* reported that micelles prepared from PEG-*b*-poly(*β*-benzyl-L-aspartate) block copolymer (PEG-PBLA) had improved doxorubicin (DOX) loading and stability as a result of additional π-π stacking interactions, leading to considerably higher antitumor activity against mouse C26 tumor than free DOX.[26] We found that polytyrosine micelles formed from PEG-*b*-poly(L-tyrosine) (PEG-PTyr) enabled high loading of anthraquinone anticancer drugs including DOX and docetaxel (DTX).[27, 28] An exceptional DOX loading level of 63.1 wt.%, which represents the highest DOX loading ever reported for polymeric micelles, was obtained with polytyrosine micelles. Inspired by work on lipid nanoparticles,[29, 30] we further developed a novel polylipopeptide with pending long-chain alkyl groups, poly(*α*-aminopalmitic acid) (PAPA), from *α*-aminopalmitic acid.[31] Notably, polylipopeptide micelles formed from PEG-PAPA copolymer following loading 11.6 wt.% DTX exhibited a small size of 59 nm and a low critical micelle concentration of 2.38 mg/L. These polylipopeptide micelles displayed also decent loading of peptide drugs such as monomethyl auristatin E (MMAE) and

carfilzomib (CFZ) possibly through the "like dissolves like" principle.[32] For instance, a loading content of around 5.5 wt.% was achieved for MMAE, which was 55-fold higher than that of PEG-PLA micelles. Of note, MMAE-loaded polylipopeptide micelles exhibited over 10 times better toleration than free MMAE in mice and effective inhibition of HCT-116 colorectal tumor xenografts.[32] Interestingly, optically active PEG-poly(L- or D-glutamate) was reported to form bundled helical nanostructures with cisplatin (CDDP) in the core that exhibited superior stability and drug release in vivo to optically inactive PEG-P(D,L-Glu) counterparts, signifying the advantages of polypeptide secondary structure in overcoming the disintegration of micellar formulations in bloodstream.[33] In accordance, bundled helical nanomedicines showed better tumor accumulation and treatment efficacy against intractable pancreatic tumor. Yin *et al.* reported that amphiphilic polypeptide containing pending phenylboronic acid accomplished remarkable loading of amine-containing anticancer drugs like DOX, epirubicin (EPI) and irinotecan (up to ~50 wt.%) with nearly quantitative loading efficiency, due to strong electron donor-acceptor coordination.[34] Thus formed drug-loaded micelles exhibited uniform drug distribution and good lyophilization stability.

PEG-*b*-poly(L-lysine) grafted with cis-1,2-cyclohexanedicarboxylic acid (CCA) and lipoic acid (LA) (PEG-P(Lys-CCA/LA)) formed robust micelles with active loading of DOX via ionic interactions and crosslinking of the core.[35] Notably, these micelles exhibited pH and reduction dual-sensitive drug release behaviors, inducing enhanced inhibitory effect to both HeLa and HepG2 tumor cells. Micelles based on PEG-*b*-oligo(ethylenimine)-*b*-PGlu (mPEG-OEI-PGlu) copolymers were shown capable of co-loading DOX and CDDP via electrostatic and chelate interactions.[36] These dual drug-loaded micelles induced better treatment of metastatic lung cancer than single drug formulation.

The high specificity and potency of biopharmaceutics including protein drugs, small interfering RNA (siRNA), messenger RNA (mRNA), microRNA, and CASPAS9 render them

a potentially better treatment for many human diseases.[37, 38] The clinical translation of biopharmaceutics is, however, impeded by lack of efficient delivery vehicles.[39-42] Kataoka *et al.* reported that unit polyion complex (uPIC) composed of a single siRNA and dyad Y-shaped PEG-PLys block catiomer (YBC) that possesses matching number of positive charges with siRAN strand had small size of around 18 nm, superior tissue permeability in comparison with naked siRNA and lipid nanoparticles (LNP), and around 15-fold more siRNA/uPIC accumulation than naked siRNA in tumor sites in a subcutaneous stroma-rich pancreatic cancer (BxPC3) model (**Figure 2**).[43] The uPICs loaded with apoptosis-inducing siRNA against polo-like kinase 1 (PLK1) and an antisense oligonucleotide against turine upregulated gene1 (TUG1) exerted enhanced treatment of spontaneous pancreatic tumor and orthotopic brain tumor, respectively.

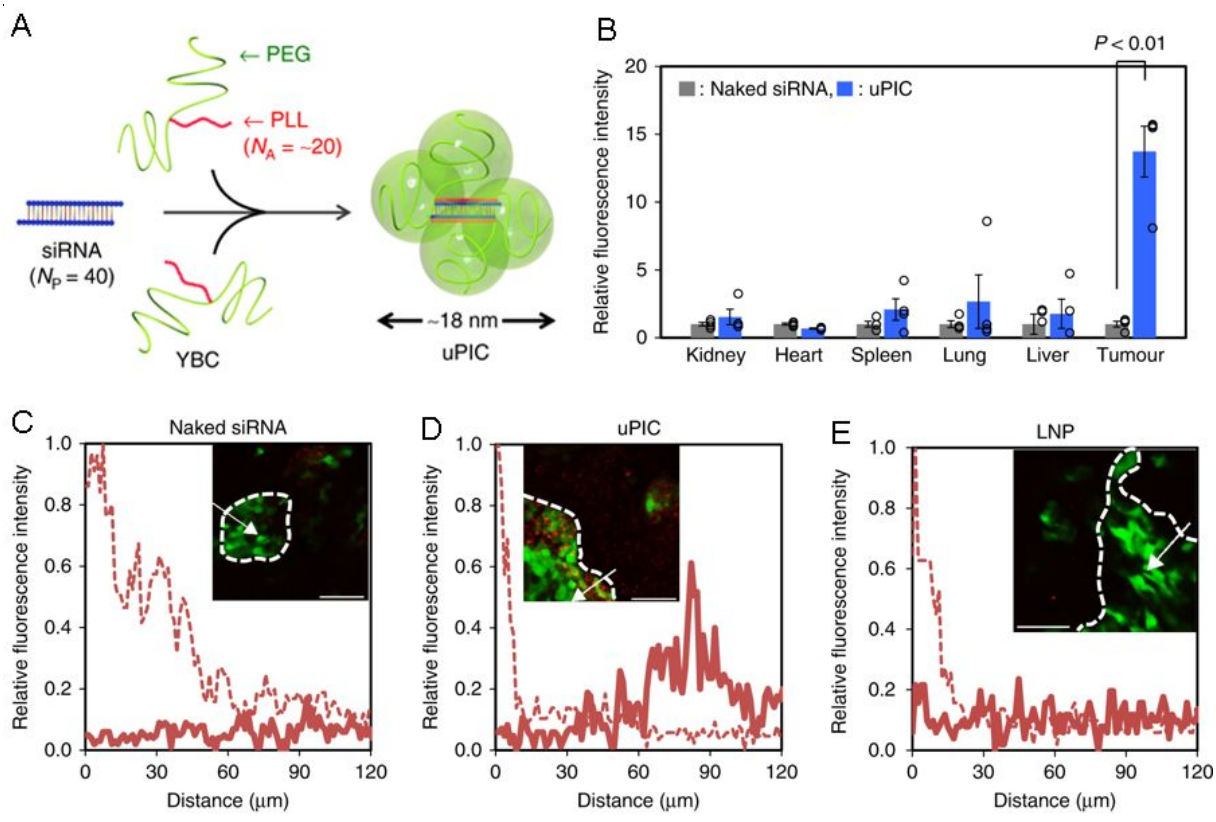

Figure 2. (A) Schematic illustration of uPIC formation from a single siRNA and dyad Y-shaped PEG-PLys block catiomer (YBC) through charge-matched ion-pairing. (B) Biodistribution at 48 h post-administration. (C-E) Spatial profiling of Cy5-siRNA in tumor

tissues at the initial stage (dashed line) and 10 h (solid line) after systemic administration (red: Cy5-siRNA; green: GFP-BxPC3 cells).[43] Reproduced from ref 43 under Creative Commons Attribution 4.0 International License, https://creativecommons.org/licenses/by/4.0/ Copyright 2019 S. Watanabe et al.

Liposomes represent the most successful drug delivery system with more than 12 liposomal formulations in clinic, among which Doxil®, Marqibo®, and Onivyde® are routinely used to treat various tumors.[29] Act as superior alternatives to liposomes, polymersomes possess fascinating merits of improved stability, tailorable chemical permeability, and high structural versatility.[44-46] For example, PIC vesicles, termed as PICsomes, were developed from a block catiomer and a negatively charged counterpart.[47] PICsomes fabricated via assembly of rigid siRNA with flexible PEG-*b*-poly(*N*-(5-aminopentyl)-*α*,*β*-aspartamide) (PEG-P(Asp-AP)) at a molar ratio of 2:1 or 3:2 facilitated siRNA internalization into A549 lung cancer cells, inducing significant gene silencing with little cytotoxicity.[48] In a similar strategy, metallosomes were obtained from PEG-*b*-PGlu-cholesteroyl and (1,2-diaminocyclohexane) platinum(II) (DACHPt) through metal coordination.[49] PEG-*b*-P(Glu-*g*-mercaptosuccinic acid) (PEG-P(Glu-*g*-MSA)) with pending bidentate dicarboxylic groups was found to form small-sized polymersomes with almost quantitative loading of charged drugs like doxorubicin hydrochloride (DOX·HCl) likely via electrostatic interaction in the membrane.[50] We developed chimaeric polypeptide-based polymersomes (pepsomes) from asymmetric PEG-*b*-poly(L-leucine)-*b*-PGlu (PEG-PLeu-PGlu) copolymers for efficient encapsulation of DOX·HCl via ionic interactions with PGlu blocks in the vesicular core.[51]

In addition to physical interactions, covalent drug conjugation is another approach to improve drug loading capacity and stability. For example, podophyllotoxin (PPT) with

significant activity against P-glycoprotein was covalently linked to PGlu-g-PEG graft copolymer via ester bonds to increase its aqueous solubility and alleviate the off-target toxicity of PPT.[52] These PPT prodrug micelles exhibited over 13 times higher toleration and enhanced antitumor efficacy against MCF-7/ADR multidrug resistant breast tumor xenografts compared with free PPT. Lu *et al.* recently developed several site-specific topological protein-poly(γ-(2-(2-(2-methoxyethoxy) ethoxy)ethyl L-glutamate) (P(OEG_3-Glu)) conjugates by combining native chemical ligation and sortase A-mediated ligation under mild conditions.[53, 54] Interestingly, macrocyclization of interferon-P(OEG_3-Glu) conjugates was found to significantly improve tumor retention, penetration, and antitumor efficacy, possibly due to decreased steric hindrance.

3. MEMBRANE-DISRUPTING NANOMEDICINES BASED ON SYNTHETIC POLYPEPTIDES

The cell membrane and endosome membrane are the major barriers for application of intracellularly active drugs in particular nucleic acid drugs. Cheng *et al.* reported that water-soluble α-helical polypeptides bearing a cationic side-chain terminus have superior cell-membrane permeability over cell penetrating peptides including HIV-TAT and Arg9.[55] DNA complexes based on helical polypeptide poly(γ-4-((2-(piperidin-1-yl)ethyl)aminomethyl) benzyl-L-glutamate) (PPABLG) demonstrated around 12 and 9-fold higher transfection efficiency than commercial 25 kDa branched polyethyleneimine (PEI) in vitro and in vivo, respectively.[56, 57] Interestingly, the nano-complexes of PPABLG, Cas9 expression plasmids and sgRNA (P-HNPs) achieved 47.3% gene editing in cells, 35% gene deletion in vivo, and HeLa tumor growth suppression of >71%.[58] In addition, these α-helical polypeptides were shown to mediate efficient delivery of siRNA against epidermal growth factor receptor (EGFR) to U-87 MG glioblastoma tumor spheroids and xenografts, outperforming

commercial Lipofectamine 2000 (**Figure 3**).[59] Of note, helical polypeptides with varying cationic side chains including guanidines, quaternary ammoniums and phosphoniums also exhibited membrane-disrupting activities.[60-62] For example, helical polypeptide was also prepared by grafting p-toluylsulfonyl arginine to PLys that gave not only enhanced DNA condensation but also better interactions with cell membrane.[63] The nanocomplex with shVEGF exhibited significant antitumor effect and negligible pathological abnormalities in CT26 tumor-bearing mice.

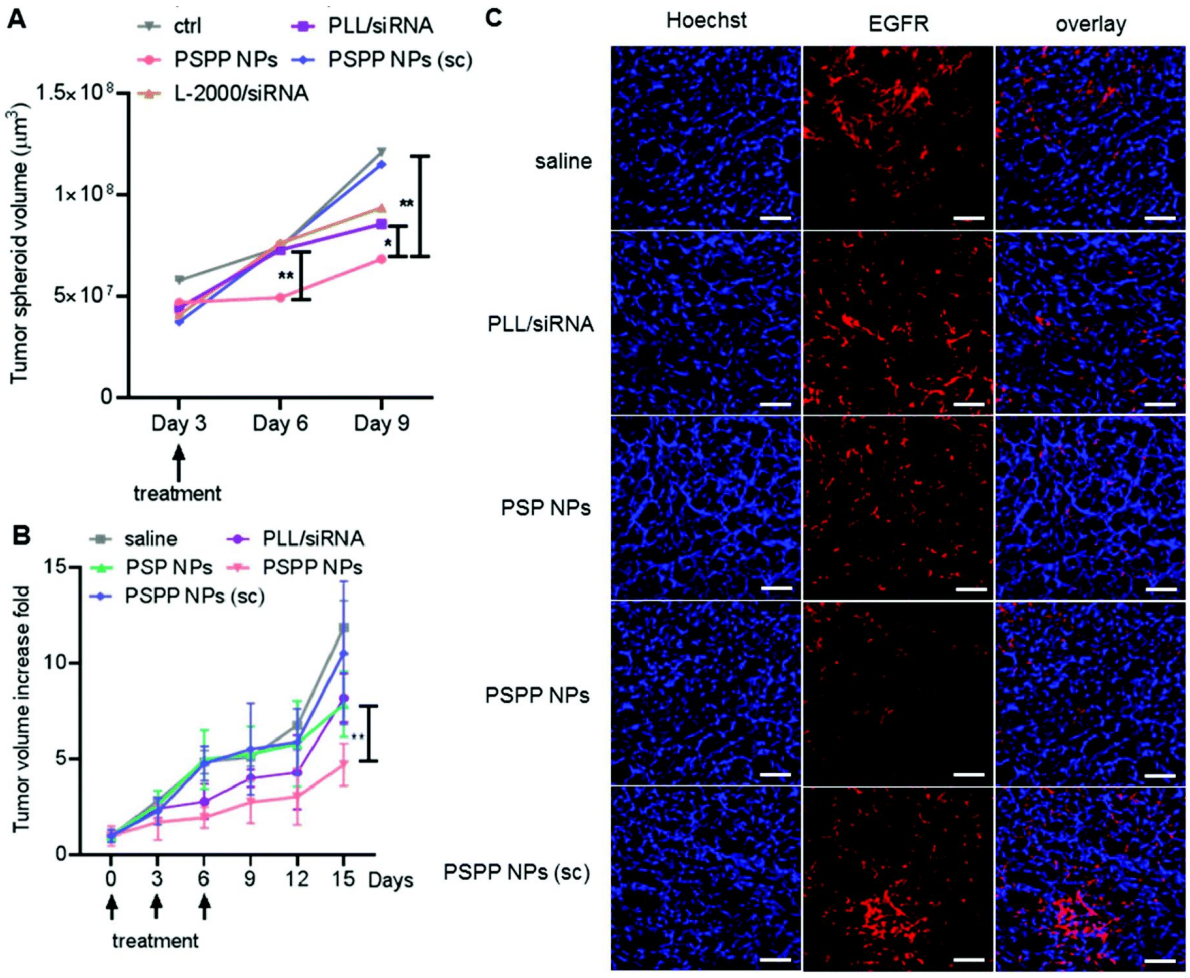

Figure 3. (A) Volume changes of tumor spheroids within 6 days following treatment. (B) Tumor volume changes in U-87 MG xenografts tumor-bearing mice treated with different nanoparticles. (C) EGFR immunostaining of tumor sections harvested on day 15.[59] Reproduced with permission of Royal Society of Chemistry, from ref 59, Copyright 2018; permission conveyed through Copyright Clearence Center, Inc.

Cationic and pH-sensitive polyaspartamide derivatives with varying numbers of aminoethylene units were obtained via aminolysis of poly(*β*-benzyl-L-aspartic acid) (PBLA). The pH-selective membrane destabilization conferred by the dual protonation state of aminoethylene units allowed endosomal escape of polyplexes with limited toxicity to the other cytoplasmic membranes lying at neutral pH. Notably, polyplex micelles formed from PEG-poly(*N*'-(*N*-(2-aminoethyl)-2-aminoethyl)aspartamide) (PEG-PAsp(DET)) successfully delivered plasmid DNA to several animal models including rabbit's clamped carotid artery,[64] rat lung,[65] and mouse skull.[66] More recent studies demonstrated that the conjugation of cholesteryl group at the *ω*-terminus of PEG-PAsp(DET) boosted the stability and endosomal escape of polyplex micelles, leading to significantly enhanced blood retention of mRNA or DNA and remarkable growth inhibition of subcutaneous human BxPC3 pancreatic adenocarcinoma.[67, 68]

4. ACTIVE TUMOR-TARGETING NANOMEDICINES BASED ON SYNTHETIC POLYPEPTIDES

The anti-cancer effect of nanomedicines can be further improved by installing targeting ligands such as peptides, antibodies, aptamers, and saccharides onto the surface of nanomedicines, facilitating efficient and specific cellular uptake via receptor-mediated endocytosis mechanism.[69-71] cRGD is a favorable ligand targeting toward $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, which are overexpressed in many tumors. We reported that cRGD-functionalized polylipopeptide-based micellar formulations developed from PEG-PAPA copolymers and chemical drugs (DTX, MMAE, etc.) showed efficient uptake and superb antiproliferative activity in B16F10 melanoma and HCT-116 tumor cells, respectively, leading to significantly more effective inhibition of both tumor models than non-targeted controls.[31, 32]

cRGD-decorated lipopepsomes (cRGD-LPP) revealed high loading and targeted delivery of DOX·HCl to orthotopic A549 human lung tumor in nude mice, resulting in significantly improved survival rate as compared to non-targeted counterpart and clinically used liposomal DOX (Lipo-DOX).[72] Robust and versatile nano-agents developed from PEG-*b*-poly(L-thyroxine) following cRGD functionalization have exhibited enhanced single photon emission computed tomography/computed tomography (SPECT/CT) dual-modality imaging and targeted radiotherapy in subcutaneous B16F10 melanoma and orthotopic A549 lung tumor.[73] Of note, selective cell penetrating CPP33 peptide-functionalized chimaeric lipopepsomes were developed from PEG-PAPA-PLys for selective delivery of siPLK1 to A549 human lung cancer cells in vivo, inducing effective tumor suppression and prolonged survival time.[74] In a recent study, antihuman tissue factor antibody fragment (Fab')-installed PIC micelles were constructed from PEG-PLys and siRNA, and the results showed that three molecules of Fab'-installed PIC micelles had the highest binding affinity and the most efficient gene silencing activity in pancreatic cancer BxPC3 cells compared with one or two molecule(s) of Fab'-installed PIC micelles, and exhibited high penetrability in BxPC3 spheroids.[75] Folate-targeted and DOX-loaded nanoparticles developed from diethylamine functionalized PEG-*b*-poly(*γ*-propargyl-L-glutamate) (PEG-PPLG) have been reported to possess enhanced tumor accumulation in folate-receptor positive KB xenografts compared to untargeted carriers, resulting in the suppression of tumor growth in vivo.[76] Kataoka *et al.* reported that phenylboronic acid (PBA)-installed micelles formed from PEG-PGlu and DACHPt exhibited sialylated epitope-targeted treatment of orthotopic and lung metastasis models of B16F10 melanoma.[77] Chen *et al.* developed sialyl epitope receptor and tumor microenvironment-recognizable polypeptide nanogels by functionalization with PBA and morpholine (MP) for targeted chemotherapy of highly metastatic malignancy.[78] DOX-loaded dual targeting nanogels (PMNG/DOX) exhibited a superior targeting effect and therapeutic

efficacy to both primary and metastatic B16F10 melanoma in comparison with nanogels modified with PBA only (PNG/DOX) (**Figure 4**).

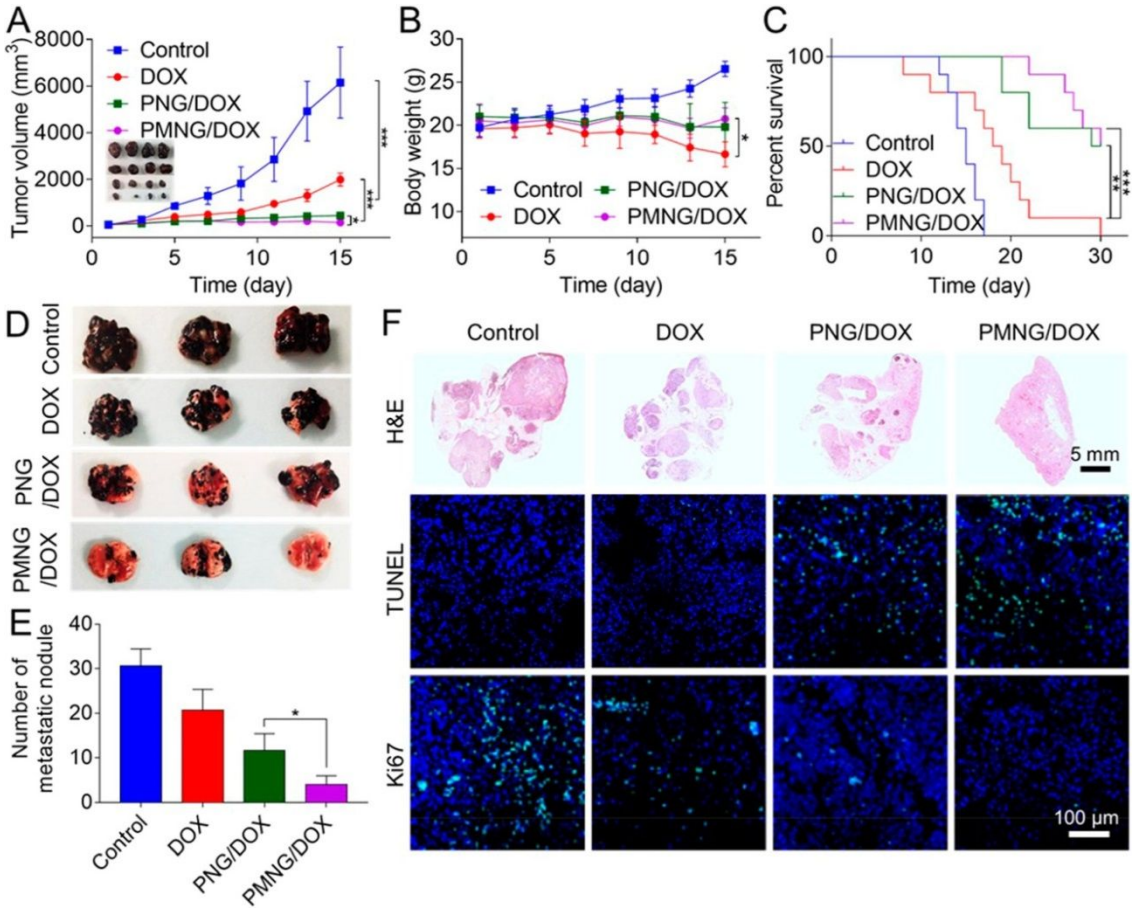

Figure 4. In vivo antitumor efficacy of PMNG/DOX on primary and metastatic melanomas. (A) Tumor volumes of primary B16F10 tumors after treatment with different formulations. (B) Body weight loss. (C) Survival rate. (D) Images of the lungs after treatments with different formulations. (E) Numbers of metastatic nodules with the diameters above 5 mm in D. (F) H&E, TUNEL, and Ki67 staining of lung sections at the end of experiments.[78] Reproduced with permission from ref 78. Copyright 2017 American Chemical Society.

The development of targeted nanomedicines based on poly(D,L-lactide-co-glycolide) (PLGA) has attracted particular interests because PLGA is one of the very few materials approved for medical use.[79] Interestingly, CD44-targeted nanomedicines could be facilely prepared from PLGA using functional polypeptide as a surfactant followed by covalent

coating with hyaluronic acid (HA).[80] Vitamin E-oligo(methyl diglycol L-glutamate) (VEOEG) is a novel surfactant that not only has excellent biocompatibility and biodegradability but also provides amino groups at the surface for immobilizing HA and shell-crosslinking. HA is known capable of targeting CD44 that is overexpressed in various malignancies.[81, 82] The results showed that HA coated PLGA nanoparticulate DTX was particularly robust and could efficiently target and suppress orthotopic human A549 lung cancer in mice (**Figure 5**).[83] In a following study, reductively cleavable surfactant VE-SS-OEG was developed to fabricate PLGA anticancer nanomedicines with sheddable HA coatings.[84] These PLGA formulations were stable against dilution and 10% FBS while quickly released DTX under reductive conditions. CD44-targeted nanoparticles were also obtained from HA-g-poly(γ-benzyl-L-glutamate) and HA-g-PTyr-LA conjugates that showed efficient and stable drug loading due to strong π-π stacking and superior selectivity and potent antitumor activity in CD44 receptor overexpressing MCF-7 and 4T1 breast tumors.[85, 86]

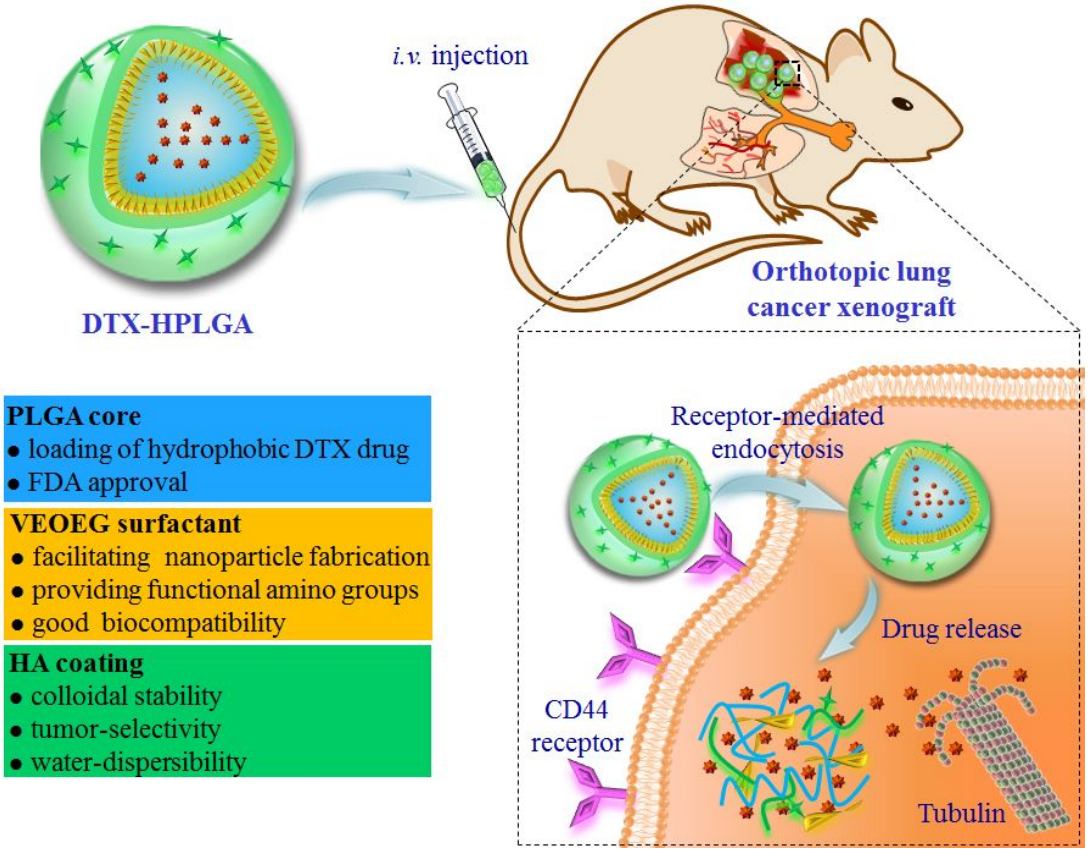

Figure 5. Illustration of hyaluronic acid (HA) coated PLGA nanoparticulate DTX that effectively targets and suppresses orthotopic human lung cancer.[83] Adapted from ref 83, Copyright 2016, with permission from Elsevier.

Tumor neovasculature has emerged as a valuable target for precision cancer treatment with nanomedicines. Taking advantage of high expression of glucose transporter 1 (GLUT1) on tumor vascular endothelial cells and many cancer cells, glucose-decorated CDDP-loaded micelles (Glu-CDDP/m) were developed for targeted treatment of GLUT1-high human squamous cell carcinoma of the head and neck OSC-19.[87] Notably, glucose-decorated micelles could target the GLUT1 on the brain endothelial cells and trigger the passage of the nanocarriers through the blood-brain barrier (BBB) after recycling the GLUT1 to the abluminal side by intraperitoneal injection of glucose, achieving 6% of injected dose/g (% ID/g) of brain tissues with deep penetration in brains.[88] In another example, cRGD-installed micelles formed from PEG-PAsp(DET)-cholesteryl and a secretable anti-angiogenic gene sFlt-1 demonstrated significant tumor vasculature targetability via cRGD-integrin ($\alpha_v\beta_3$ and $\alpha_v\beta_5$) mediation mechanism, inducing effective suppression of tumor growth in BxPC3 pancreatic tumor models.[68]

5. STIMULI-RESPONSIVE NANOMEDICINES BASED ON SYNTHETIC POLYPEPTIDES

The ideal nanomedicines shall be stable during circulation while quickly release payloads once arriving at the target site. This on-off drug release might be achieved with stimuli-responsive polypeptides that undergo significant physical or chemical changes under tumor microenvironments or intracellular signals in tumor cells.[89-91] In particular, redox, acidic pH and enzyme-sensitive polypeptide nanomedicines have received most attention.

The intracellular concentration of glutathione (GSH) is about 100-1000 times higher than that in extracellular environment,[92] which makes GSH an interesting stimulus to trigger cytoplasmic drug release in the cancer cells. It was reported that reduction-responsive micelles developed from PEG-*b*-poly(*S*-tert-butylmercapto-L-cysteine) copolymers achieved fast release of DOX in breast cancer cells, resulting in improved antitumor efficacy toward MCF-7 breast tumor-bearing Balb/c nude mice compared with free drug.[93] Reduction-sensitive PIC micelles fabricated from polyaspartamide-SS-siRNA conjugate and PAsp(DET) demonstrated competent internalization by B16F10 cells and efficient siRNA release in tumor cells.[94] Chen *et al.* developed GSH-responsive DOX-loaded micellar formulations from PEG-PPLG grafted with sulfur dioxide (SO_2) and *N*-(3-azidopropyl)-2,4-dinitrobenzenesulfonamide to combat MCF-7/ADR human breast cancer.[95] In response to thiol compounds, these micelles could simultaneously release SO_2 and DOX, leading to increase of reactive oxygen species level in tumor cells that would induce oxidative damages of cancer cells and reversal of drug resistance. Recently, Ding *et al.* reported a conformation-directed micelle-to-vesicle transition from cholesterol-decorated PEG-*b*-PCys (PEG-PCys-Chol) as triggered by reactive oxygen species (ROS).[96] The oxidation of the thioether groups induced the packing transformation of PEG-PCys-Chol from *β*-sheet to *α*-helix. DOX-loaded PEG-PCys-Chol micelles displayed enhanced tumor inhibition due to the ROS-activatable cell internalization and drug release.

Stability is a practical issue for cancer nanomedicines.[24, 25] Interestingly, disulfide crosslinking has shown to be a unique strategy that not only enhances nanomedicine's extracellular stability but also triggers its intracellular drug release. For example, shell-disulfide-crosslinked micelles were fabricated by treating PEG-*b*-PLys-*b*-poly(L-phenylalanine) (PEG-PLys-PPhe) micelles with 3,3'-dithiobis(sulfosuccinimidylpropionate) (DTSSP).[97] DTX-loaded micelles exhibited

enhanced therapeutic efficacy in MDA-MB-231 tumor-bearing mice compared with free DTX and non-crosslinked micelles. Barz *et al.* reported that amphiphilic polysarcosine-*b*-poly(*S*-alkylsulfonyl-L-cysteine) (PSar-PCys(SO_2Et)) copolypeptide formed spherical or worm-like architectures depending on their secondary structure.[98] PEG-PLys-PCys afforded self-cross-linked nanoparticles that were able to co-deliver DOX and miR-129-5p to effectively overcome multidrug resistance (MDR) in cancer cells.[99] We reported that disulfide-crosslinked micelles formed from lipoic acid grafted PEG-PTyr and PEG-PLys conjugate exhibited high DOX loading, small size of 45 nm as well as reduction-triggered drug release.[100, 101] cRGD-decorated micellar formulations could effectively suppress growth of MDA-MB-231 human breast tumor without inducing obvious side effect.[100] Disulfide-crosslinked polypeptide nanogels were developed through copolymerization of Phe-NCA and Cystine-NCA using amino-terminated PEG as a macroinitiator.[102] DOX-loaded nanogels achieved swift drug release under high concentrations of GSH and efficient suppression of hepatoma growth in mice. Positively charged disulfide-crosslinked nanogels were developed from PLys-P(Phe-co-Cystine) for delivery of 10-hydroxycamptothecin (HCPT) to orthotopic bladder cancer in vivo.[103, 104] PLys with positive charge would endow nanogels with strong mucoadhesivity with negatively charged bladder mucosa as well as improve their cellular uptake.

The pH of physiological microenvironment is about 7.4, while decreases to 6.5-7.2 in the extracellular environment of tumors, and further decreases to 4.5-6.8 in the endo-/lysosomes of cancer cells.[105] In the past decades, various polypeptide-based nanovehicles functionalized with pH-responsive structural moieties have been developed. We reported that chimaeric polymersomes based on PEG-PLeu-PGlu while stable at physiological pH quickly felled apart at pH 5.0, likely due to alternation of ionization state of the carboxylic groups in PGlu that shifts PGlu blocks from random coil structure into α-helical structure.[51] MTT assays verified

that DOX·HCl-loaded polymersomes showed comparable antiproliferative activity to free DOX·HCl in RAW264.7 cells while higher anticancer activity toward drug-resistant MCF-7 cancer cells (MCF-7/ADR). Hammond *et al.* reported that pH-sensitive endosome-solubilizing polymersomes based on PEG-poly(L-glutamate) with pendant alkyl amine like diisopropylamine and diethylamine were able to encapsulate DOX·HCl at pH 7.4 while quickly released DOX·HCl at endosomal pH, resulting in efficient suppression of MDA-MB-468 breast tumor in Balb/c mice.[106] Kataoka *et al.* developed EPI prodrug micelles from PEG-poly(hydrazinyl-aspartamide) via a pH-sensitive hydrazone bond for effective treatment of GBM.[107] cRGD-decorated micelles achieved fast penetration into U87MG cell-derived 3D-spheroids and effectively suppressed the growth of an orthotopic GBM model. Moreover, pH-sensitive EPI-loaded micelles could effectively inhibit the spread of the primary breast tumor and the growth of axillary lymph node metastasis (ALNM), through selective accumulation and penetration in both primary and vascularized ALNM, as well as efficient drug activation triggered by the intratumoral acidic environment.[108] These EPI prodrug micelles could further encapsulate staurosporine (STS), an inhibitor for cancer stem-like cells (CSCs), for effective treatment of orthotopic 4T1-luc breast tumors and their recurrent EPI-resistant counterparts that correlated with CSC-associated sub-populations of breast cancer.[109]

pH-sensitive charge and size dual-rebound gene delivery system was constructed by covering polyplexes with aldehyde-modified polyethylene glycol (PEG) via Schiff base reaction that bestows long circulation and high tumor accumulation (**Figure 6**).[110] The rapid removal of PEG shell in an acidic tumor environment resulted in incompact structure and positively charged surface, facilitating tumor penetration and gene transfection. An antiangiogenesis therapeutic gene-loaded micelles demonstrated superior anticancer efficacy in mice bearing CT26 tumors. Shuai *et al.* developed pH-responsive polymer vector from

PEG-*b*-PLys-*b*-poly(aspartyl(*N*-(*N*′,*N*′-diisopropylaminoethyl))) (PEG-PLys-PAsp(DIP)) to co-deliver DOX and anti-BCL-2 siRNA.[111] The co-delivered siRNA served to suppress the expression of antiapoptotic BCL-2 and hence sensitizing the cancer cells to anticancer drugs and improving therapeutic effect in drug-resistant HepG2/ADM hepatic carcinoma. pH-activatable Mn^{2+}-doped CaP nanoparticles with signal-amplification capabilities were obtained using PEG-PGlu to interact with Mn^{2+}, Ca^{2+} and HPO_4^{2-} followed by hydrothermal treatment for non-invasive imaging of tumor malignancy.[112] At a low pH (e.g. in solid tumor), the CaP was observed to disintegrate and release Mn^{2+} ions that could bind to surrounding proteins, inducing increased relaxivity of Mn^{2+} and enhanced contrast. Interestingly, these nanoparticles could rapidly and selectively brighten solid tumors, identify hypoxic regions within the tumor mass and detect invisible millimeter-sized metastatic tumor in the liver.

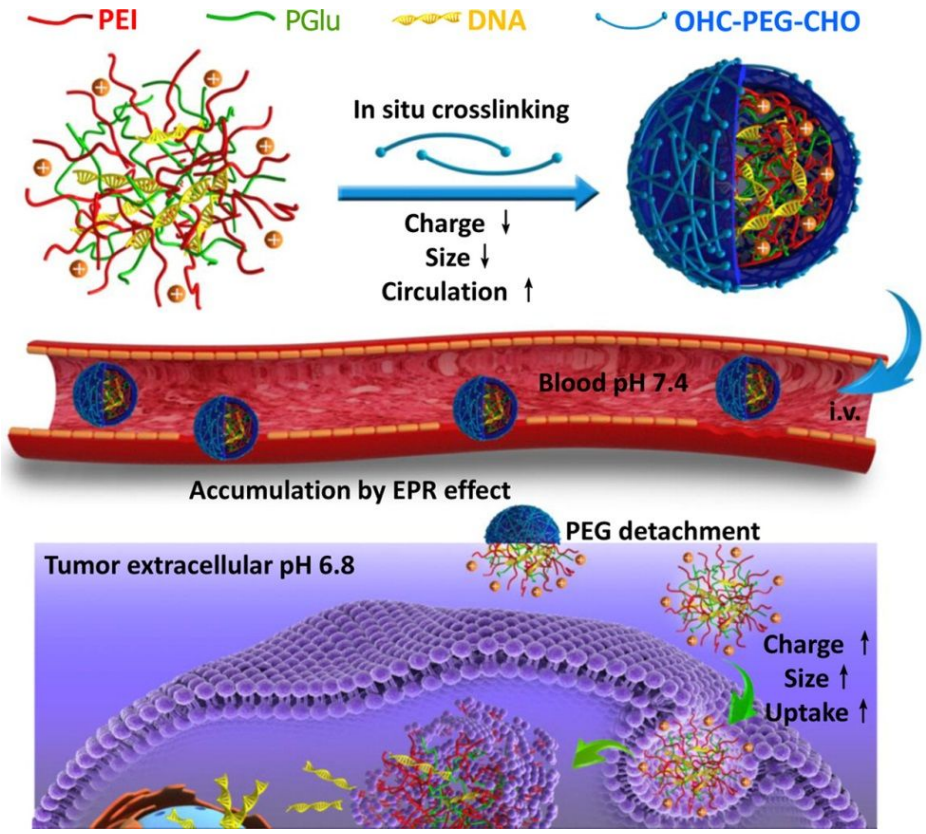

Figure 6. Schematic of the ultrasensitive pH triggered charge/size dual-rebound gene delivery system.[110] Reproduced with permission from ref 110. Copyright 2016 American Chemical Society.

Enzymes playing an important role in tumor invasion and remodeling are overexpressed in malignant tumors. Enzyme-sensitive nanomedicines have been energetically explored for enhanced tumor therapy.[113] In this sense, polypeptide-based nanomedicines are particularly interesting as polypeptides are usually degraded by enzymes. For example, polytyrosine micelles exhibited good colloidal stability against serum while released 90% of DOX under 6 U/mL proteinase K in 24 h or in HCT-116 colorectal cancer cells within 10 h.[27, 28] Interestingly, cRGD-functionalized DOX-loaded micelles demonstrated effective inhibition of tumor growth and improved survival rate in HCT-116 tumor-bearing mice. Deming *et al.* demonstrated that vesicles developed from amphiphilic poly(L-methionine sulfoxide)-*b*-P(Leu-co-Phe) (PLMetO-P(Leu-co-Phe)) exhibited obvious aggregation with irregular sheet-like structures and cargo (Texas Red labeled dextran) release in the presence of methionine sulfoxide reductases A and B that transformed hydrophilic and disordered PLMetO segments to α-helical poly(L-methionine) (PLMet) (**Figure 7**).[114]

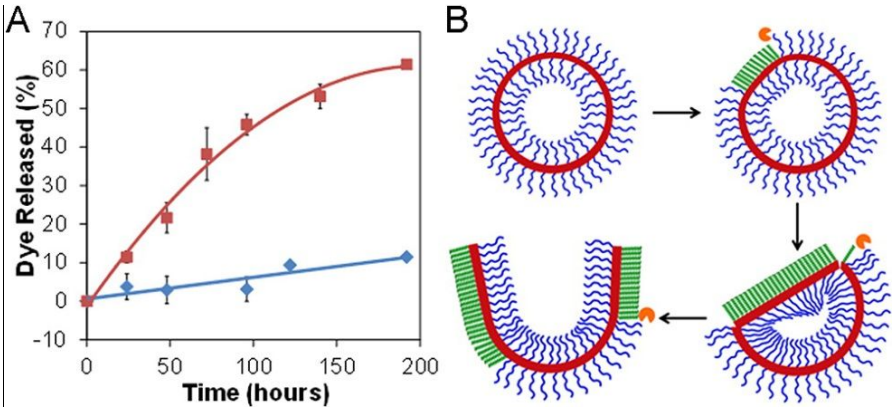

Figure 7. Enzyme-triggered release of probe molecules from methionine sulfoxide containing vesicles. (A) Plot showing cumulative release of Texas Red labeled dextran from vesicles over time. (B) Schematic showing a possible effect of enzymatic reduction of vesicle in the presence of methionine sulfoxide reductases A and B.[114] Reproduced with permission from ref 114. Copyright 2013 American Chemical Society.

In addition to single stimulus-sensitive systems, more sophisticated dual and multi-stimulus-sensitive polypeptide nanomedicines have also been constructed to further improve tumor therapy. For example, Lee *et al.* reported that pH and redox dual-responsive polypeptide micelles based on PEG-*b*-poly(2-(dibutylamino)ethylamine-L-glutamate) (PEG-SS-PNLG) copolymer significantly improved DOX release and treatment efficacy toward HepG2 tumors compared with free DOX.[115] Yan *et al.* reported that pH and reduction dual-responsive nanogels based on PEG-P(Glu-co-Cys) had high DOX loading efficiency of 96.7%, small size of 59 nm, and pH and reduction dual-triggered DOX release, inducing effective suppression of H22 hepatoma tumor in nude mice.[116] pH and reduction dual-sensitive unimolecular micelles were developed from a copolymer consisting of PEG, a hyperbranched polyester H40, and polyaspartamide containing pending mercaptoethylamine and imidazole groups (P(Asp-AED-ICA)), in which cationic P(Asp-AED-ICA) segments were conjugated onto H40 via a pH-sensitive aromatic imine bond.[117] GFPsiRNA-complexed micelles following the conjugation with GE11 peptide exhibited excellent GFP gene silencing efficiency in GFP-MDA-MB-468 TNBC cells without causing significant cytotoxicity.

6. VIRUS-MIMICKING VESICLES BASED ON SYNTHETIC POLYPEPTIDES FOR CANCER BIOTHERAPY

Viruses are natural vehicles that are able to efficiently pack and transport certain payloads to target cells *in vivo*. The potential toxicity and safety issues, however, limit their wide applications. In recent years, virus-mimicking vehicles have been devised from polypeptides for efficient non-viral delivery of highly potent peptides, proteins, and nucleic acids, greatly advancing biotherapy for cancers. For example, Robust chimaeric pepsomes were constructed from asymmetric PEG-PAPA-PAsp triblock copolypeptide for high loading and targeted intracellular delivery of therapeutic proteins in vivo.[118] Chimaeric lipopepsomes

(CLP) exhibited high stability due to the existence of lipid-lipid packing of PAPA moieties in the membrane and nearly quantitative loading of cytochrome C and saporin mainly attributing to the electrostatic interactions with negative charged PAsp segments in the aqueous lumen. Saporin-loaded cRGD-modified CLP displayed a high potency in treating orthotopically xenografted A549 lung tumors at 16.7 nmol saporin equiv./kg. Similarly, chimaeric lipopepsomes containing positively charged moieties were developed from PEG-PAPA-PLys triblock copolypeptide for efficient encapsulation of negatively charged biopharmaceutics like polo-like kinase 1 specific siRNA (siPLK1) (**Figure 8**).[74] Interestingly, siPLK1-loaded selective cell penetrating peptide (CPP33)-functionalized CLP exhibited enhanced tumor accumulation, effective suppression of tumor growth, and considerably prolonged survival time of orthotopic A549 human lung tumor-bearing nude mice.

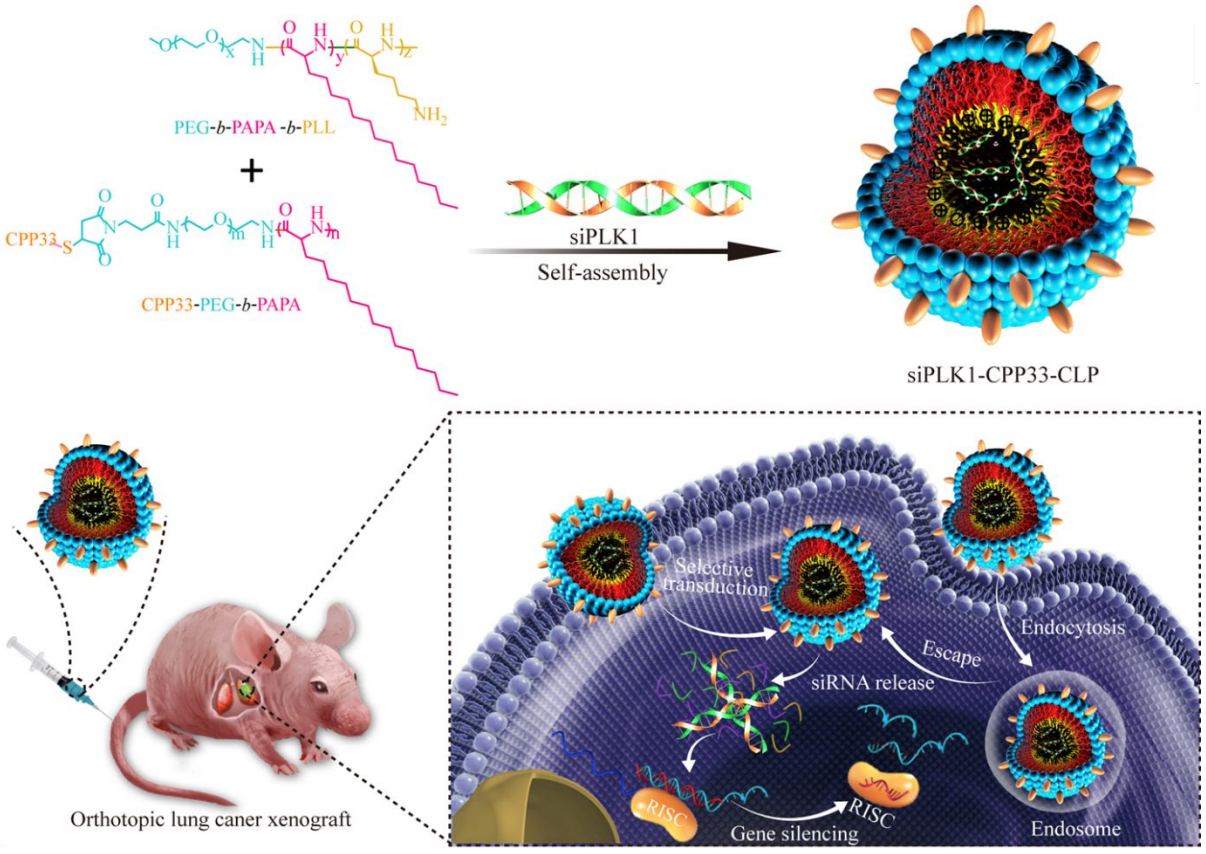

Figure 8. Illustration of CPP33-functionalized chimaeric lipopepsomes (CPP33-CLP) for efficient loading and selective delivery of siRNA to orthotopic A549 human lung tumor.[74]

Adapted from ref 74 with permission from John Wiley and Sons. Copyright 2019 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

7. Conclusions and Future Perspectives

The past decade has witnessed fast development of advanced cancer nanomedicines based on synthetic polypeptides. In addition to easy synthesis and good safety, polypeptides are characterized by their unique hierarchical structure and remarkable versatility which facilitates engineering with practically all kinds of functions needed for an ideal drug delivery system. Notably, functional polypeptides can be prepared not only from common natural amino acids and their derivatives but also from a selection of unnatural amino acids such as lipoamino acids. Depending on intended applications and drug entities, different forms of nano-vehicles like micelles, nanoplexes, polymersomes, nanoparticles and nanogels can be tailor-made from de novo polypeptides. While early development by our pioneers has led to translational studies of several polypeptide-based chemical nanomedicines, targeted delivery of biotherapeutics such as proteins, peptides, and nuclei acid drugs (e.g. siRNA) has gained more and more attention. In particular, vehicles like chimaeric pepsomes and membrane-disrupting polypeptides have appeared interesting as artificial viruses for cancer gene and protein therapy.

It should be noted, however, that despite recent achievements in the design of next-generation polypeptide-based cancer nanomedicines, none has moved toward clinical translation. Though many systems have been reported to bring about better treatment efficacy than clinically used formulations in the mouse tumor models, the benefits are limited by their sophisticated fabrication, potential toxicity and high cost. For successful clinical translation, new polypeptide-based cancer nanomedicines have to be simple, safe and functional, best leading to breakthrough in treatments for human malignancies that are intractable to date. The

development of multifunctional polypeptide vehicles as non-viral vectors for efficient parental delivery of biotherapeutics will continue to be an increasingly important subject of research that might give a high impact on cancer management. Moreover, polypeptide-based nanomedicines may also be developed for cancer combination therapy, e.g. combining chemotherapy with gene or immuno-therapy, to effectively treat inaccessible, resistant, metastatic as well as recurrent tumors. It is anticipated that with continuous effort, polypeptide-based nanomedicines may become an indispensable means for human cancer treatment in the future.

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Notes

The authors declare no competing financial interest.

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