MINIREVIEW—MOLECULAR PHARMACOLOGY IN CHINA

Tumor Microenvironment Targeting and Responsive Peptide-Based Nanoformulations for Improved Tumor Therapy

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ABSTRACT

The tumor microenvironment participates in all stages of tumor progression and has emerged as a promising therapeutic target for cancer therapy. Rapid progress in the field of molecular self-assembly using various biologic molecules has resulted in the fabrication of nanoformulations that specifically target and regulate microenvironment components to inhibit tumor growth. This inhibition process is based on differentiating between biophysicochemical cues guiding tumor and normal tissue microenvironments. Peptides and peptide derivatives, owing to their biocompatibility, chemical versatility, bioactivity, environmental sensitivity, and biologic recognition abilities, have been widely used as building blocks to construct multifunctional nanostructures for targeted drug delivery and controlled release. Several groups of peptides have been identified as having the

ability to penetrate plasma membranes, regulate the essential signaling pathways of angiogenesis and immune reactions, and recognize key components in the tumor microenvironment (such as vascular systems, stromal cells, and abnormal tumor biophysicochemical features). Thus, using different modules, various functional peptides, and their derivatives can be integrated into nanoformulations specifically targeting the tumor microenvironment with increased selectivity, on-demand response, elevated cellular uptake, and improved tumor therapy. In this review, we introduce several groups of functional peptides and highlight peptide-based nanoformulations that specifically target the tumor microenvironment. We also provide our perspective on the development of smart drug-delivery systems with enhanced therapeutic efficacy.

Introduction

Tumors are composed of tumor cells and their microenvironment, including various stromal cells, extracellular matrix (ECM), soluble cytokines, and tumor vascular systems. The interaction between tumor cells and the surrounding microenvironment occurs at all stages of tumor progression. Hypoxia results in tumor vascular endothelial cells being activated, with aberrant expression of cell surface and secreted proteins, including integrins and matrix metalloproteinases (MMPs), that facilitate the construction of malformed and dysfunctional new

blood vessels (Carmeliet, 2000). Cancer-associated fibroblasts (CAFs), the most abundant cell type in the tumor microenvironment, actively participate in ECM remodeling and in promoting tumor growth (Neri et al., 2016). Tumor-associated macrophages (TAMs) are the predominant inflammatory cells in malignant solid tumors. Most of the TAMs polarize toward M2-like macrophages, which are characterized as immunosuppressive and play an important role in angiogenesis (Rogers and Holen, 2011). In addition, all stromal cells and ECM in the tumor microenvironment can form physiologic barriers, sheltering tumor cells and contributing to drug resistance (Khawar et al., 2015).

Improved therapeutic outcomes can potentially be achieved by synergistically targeting tumor cells and regulating the tumor microenvironment. Compared with the normal tissue environment, the tumor microenvironment exhibits stromal cell abnormalities, aberrant protein expression, acidosis, and hypoxia. These differences provide multiple targets for the selective delivery of therapeutic agents to tumors (Ji et al., 2013).

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ABBREVIATIONS: Ang, angiotensin; CAF, cancer-associated fibroblast; CPP, cell-penetrating peptide; CTL, cytotoxic T lymphocyte; DEAP, 3-diethylaminopropylisothiocyanate; Dox, doxorubicin; ECM, extracellular matrix; FAP- α , fibroblast activation protein- α ; FGF-12, fibroblast growth factor-12; GEM, gemcitabine; K-FGF, Kaposi fibroblast growth factor; LCST, low critical solution temperature; MMP, matrix metalloproteinase; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PEG, poly(ethylene glycol); TAM, tumor-associated macrophage; VEGF, vascular endothelial growth factor; VEGFR2, VEGF receptor 2.

The emergence and progression of nanotechnology provide a novel toolbox for designing the next generation of intelligent therapeutics with the design of well defined structures with flexible composition. By carefully selecting the building blocks and modulating the physicochemical properties of different nanoformulations, elevated tumor accumulation, on-demand drug release, and enhanced therapeutic outcomes can be achieved. With deeper understanding of the features of key components within the tumor microenvironment and the mechanisms by which these components promote tumor progression, therapeutic nanoformulations have been designed to specifically target and regulate the tumor microenvironment (Danhier et al., 2010). In addition, the physicochemical differences between normal tissues and the tumor microenvironment, such as acidosis and hypoxia, have also been taken advantage of to construct well controlled nanosystems with high therapeutic efficacy (Mura et al., 2013).

Endowed with low immunogenicity, facile modification, ample bioactivity, and the ability to penetrate tissues, peptides have served as promising building blocks for the construction of advanced nanoformulations. Many peptides used in tumor therapy are derived from functional domains of proteins and exhibit specific bioactivities, such as receptor binding, structural sensitivity to changing local physiologic or chemical conditions, penetration of the plasma membrane, and activation or inhibition of cellular pathways. Several research groups are screening more efficient functional peptides (Laakkonen et al., 2002; Porkka et al., 2002; Cieslewicz et al., 2013; Gautam et al., 2014). Owing to their salient bioactive functions, peptides hold great potential to become one of the most extensively used building blocks for the construction of nanoformulations for cancer treatment. Peptides can be incorporated into modules of nanoformulations that include targeting ligands, responsive cleavage sites, internalization mediators, and therapeutic molecules. In addition, taking advantage of the solid-phase peptide synthesis method, a sophisticated technique with high synthetic efficiency and moderate reaction conditions, one can easily obtain desired peptides with defined functions. In this review, we describe several groups of functional peptides with distinct bioactive properties and discuss progress focused on the design and fabrication of peptide-based nanoformulations specifically targeting the tumor microenvironment.

Functional Peptides

Tumor Microenvironment Targeting Peptides. Functionalization with tumor-targeting motifs is an excellent strategy for increasing the accumulation of therapeutic agents

within tumor tissues. With advancements in screening techniques, such as the phage-displayed peptide library and the one-bead one-compound combinatorial library, peptides have been identified that possess tumor-homing capacity. These peptides can specifically bind to various receptors that are specifically expressed or overexpressed in tumor tissues. Compared with antibodies, targeting peptides are much smaller, which makes them more efficient in tissue penetration and relatively easy to fabricate at much lower cost. In comparison with other small molecular targeting agents, peptides may be more straightforward for molecular design and more biocompatible. A brief summary of some targeting peptides is provided in Table 1.

The tumor vasculature is the first checkpoint that therapeutic systems encounter when circulating into the tumor. This makes vascular constituents, including endothelial cells, pericytes, and blood components, attractive targets. RGD and NGR, the first two targeting peptides screened via phage-displayed peptide libraries (Pasqualini et al., 1997; Arap et al., 1998), are widely used in targeted delivery of therapeutic agents to tumor tissues. These short tripeptides bind to integrin $\alpha_v\beta_3$ and $\alpha_v\beta_5$ and aminopeptidase N, respectively, which are overexpressed on vascular endothelial cells of tumor angiogenic blood vessels, resulting in remarkable accumulation of cargoes within the tumor. Other than RGD and NGR, functional peptides such as IF7, F3, cytotoxic T-lymphocyte (CTL), and CREAK also exhibit the ability to specifically target tumor blood vessels. The receptor for IF7 targeting is annexin1, a highly specific surface marker of tumor vasculature (Hatakeyama et al., 2011). F3, which is derived from the N-terminal fragment of human high-mobility group protein 2, possesses high affinity for nucleolin proteins expressed on the surface of tumor cells and endothelial cells (Porkka et al., 2002). The fibrin-fibronectin complex of plasma clots in tumor vessel walls and interstitial spaces, formed as a result of leaky blood vessels, provides another well recognized target for tumor targeting. The cyclic nonapeptides CLT1 and CLT2 strongly accumulate in tumor blood vessels and stroma via their interaction with fibrin-fibronectin (Pilch et al., 2006). Another peptide, CREAK, binds to clotted plasma proteins and induces additional local clotting, thereby producing new targets for additional peptide binding (Simberg et al., 2007). This self-amplifying process is an excellent feature for enhanced targeting efficiency.

Tumor stromal cells with unusual protein expression are also good candidates for targeting. Lyp-1, a cyclic nonapeptide,

TABLE 1
Brief summary of targeting peptides

Targeting Position	Names	Sequence	Receptors	References
Tumor vasculature	RGD	RGD	Integrin $\alpha_v\beta_3$ $\alpha_v\beta_5$	Pasqualini et al., 1997
	NGD	NGD	Aminopeptidase N	Arap et al., 1998
	IF7	IFLLWQR	Annexin 1	Hatakeyama et al., 2011
	F3	KDEPQRRSARLSAKPAPPKPEPKPKKAPAKK	Nucleolin	Porkka et al., 2002
	CTL1	CGLIIQKNEC	Clotted plasma protein	Pilch et al., 2006
	CTL2	CNAGESSKNC	Clotted plasma protein	Pilch et al., 2006
	CREAK	CREAK	Clotted plasma protein	Simberg et al., 2007
TAM	Lyp-1	CGNKRTRGC	P32/gC1q	Uchida et al., 2011
MSC	M2pep	YEQDPWGVKWWY	Unclear	Cieslewicz et al., 2013
	WAT	CSWKYWFGE	Unclear	Daquinag et al., 2011
ECM	a	CRRHWGFEC	MMP-2/9	Koivunen et al., 1999
	a	CTTHWGFTLC	MMP-2/9	Koivunen et al., 1999

MSC, mesenchymal stromal cell.

^aNo specific names.

has been verified to selectively bind to p32/gC1q, a receptor abundant on the surface of TAMs, as well as on tumor lymphatics and tumor cells (Laakkonen et al., 2002; Fogal et al., 2008; Uchida et al., 2011). When expressed in normal tissues, p32 exists intracellularly as a mitochondrial protein and escapes peptide recognition. One group identified a unique M2-selective peptide, called M2pep, that specifically recognizes murine M2 cells, including TAMs, and has low affinity for other leukocytes (Cieslewicz et al., 2013). Another targeting molecule, WAT, is a cyclic peptide that homes to adipose stromal cells (Daquinag et al., 2011), which belong to the mesenchymal stromal cell lineage.

In addition to cytokines and enzymes, ECM presents other appealing therapeutic targets. MMPs, overexpressed by endothelial cells and tumor cells, play an important role in tumor growth, angiogenesis, and metastasis. Two peptides, CTTHWGFTLC and CRRHWGFEFC, have been shown to selectively target MMP-2 and MMP-9 (Koivunen et al., 1999). The two peptides display high affinity and also inhibit enzymatic activities of MMP-2 and MMP-9 (Koivunen et al., 1999). Specific targeting with simultaneous inhibition suggests the potential for enhanced targeting and antitumor efficacy.

Microenvironment Responsive Peptides. Since proteinases and peptidases are abundant in the tumor interstitial space, therapeutic nanosystems fused with peptides that are specific substrates of these enzymes can be designed to control the release of therapeutic agents within the tumor microenvironment. As described already herein, MMPs, with an elevated expression in tumor ECM, are crucial in tumor progression. Therefore, making use of MMP-cleavable sequences can readily achieve drug release or active site exposure. The substrate peptide for MMP-2 is GPLGIAGQ; this sequence is cleaved into GPLG and IAGO by MMP-2. One group developed a liposome modified with cell-penetrating peptide (CPP) and a poly(ethylene glycol) (PEG)-conjugated antibody (Gao et al., 2013). PEG was linked to the liposome via a MMP-2 responsive sequence. In the presence of MMP-2, long PEG chains were removed from the liposomes. As a result, the exposed CPP mediated the internalization of the liposomes. In another example, the MMP-2 and MMP-9 sensitive sequence, PVGLIG, can be cleaved between glycine and leucine (Gao et al., 2013). This sequence can also be used for construction of tumor niche-responsive nanoformulations. fibroblast activation protein- α (FAP- α) is another accessible protease that is specifically expressed on the surface of CAFs, a major cellular component in the tumor microenvironment. FAP- α selectively cleaves the sequence GPAX (X designates any amino acid) between proline, and alanine (Ji et al., 2016b). Microenvironment responsive peptides are listed in Table 2.

The sustained Warburg effect and limited clearance of metabolic acids leads to a more acidic pH within the tumor microenvironment than in normal tissues. The relative acidity has an important implication on specific responses of the tumor microenvironment. pHLP is a peptide that has striking features of low pH sensitivity and tumor targeting (Andreev et al., 2007). This peptide, which is part of the bacteriorhodopsin C helix, inserts across the membrane as an α -helix at low pH. In a basic or neutral environment, the peptide is largely unstructured and has low affinity for cell membranes. Polyhistidines also possess the ability to respond to the acidic tumor environment; this is because an imidazole group on

TABLE 2
Brief summary of the stimuli-responsive peptides and their applications

Stimuli	Peptide Sequence	Therapeutic Strategy	Result	References
Enzymes	GPLGIAGQ (MMP-2) PVGLIG (MMP-2/9) GPAX (FAP- α)	Linked protective PEG to CPP-modified liposome via MMP-2-responsive sequence	Enhanced target ability and internalization of nanocarriers in cancer cells	Zhu et al., 2012 Gao et al., 2013 Ji et al., 2016b
Acidosis	ACEQNPYWARADWLFTPTLLLDLALLVDADGEGTG	Combined pH LIP with a microRNA miR-155 via disulfide bond	Delivery of miR-155 specifically to tumor site and efficient uptake by tumor cells	Andreev et al., 2007
	H ₇	Conjugated cell-penetrating peptide (R ₂) ₂ and H7 modify to polymeric micelle	Activation of cell-penetrating peptide by response of H7 to the acidic tumor microenvironment	Zhao et al., 2016
	E ₄ K ₄	Mask to a cell-penetrating peptide via linking by an enzyme-responsive cleavable sequence	Cell-penetrating peptide exposed via E ₄ K ₄ charge transforming and linker cleaving	Huang et al., 2013a
Hyperthermia	VSSLESKVSSLESKVSKLESKSKLESKVSSLESK	Leucine zipper peptide-lipid hybrid nanovesicles loaded with Dox inside	Superior serum stability at physiologic temperature and increased drug accumulation in tumor	Al-Ahmady et al., 2012
	(VPGVG) ₄₀ (VPGXG) ₆₀ X=A;G; 1:1	Temperature triggered activation of the cell penetrating ability of ELP-penta-arginine copolymer	Thermal triggered copolymer assembly increased the local density of arginine and the cell penetrating activity at higher temperature	Macewan and Chilkoti, 2012

ELPs, Elastin-like polypeptides; X: any amino acid; |: cleavable site.

histidine, with a pKa of approximately 6.5, can be protonated under acidic conditions to make the peptide more hydrophilic (Zhao et al., 2016). The disturbed polarity usually leads to structural transformation of nanoformulations to exert favorable effects such as drug release and functional site exposure. Another group of pH-responsive peptides include sequences of carefully designed acidic and basic amino acids with isoelectric points between 6 and 7. One of the peptides includes an E₄K₄ sequence, which has a pI of approximately 6.4 and is negatively charged under physiologic conditions; in an acidic tumor microenvironment, this peptide is uncharged or slightly positively charged (Huang et al., 2013a). The changes in hydrophilicity or net charge can be used to alter conformations supported by electrostatic attractions to induce functional domain exposure or cargo release. These microenvironment responsive peptides are described in Table 2.

Mild hyperthermia is another feature of tumor tissue that occurs as a result of dysfunctional tumor vascular systems and poor heat exchange. Slightly higher temperatures, compared with the surrounding tissues, can potentially trigger particular peptides to respond to the tumor microenvironment. A leucine zipper peptide has been shown to form coiled-coil self-assembled aggregates composed of α -helix monomers; these dissociate into disordered, unstructured monomers at temperatures higher than 40°C. In this transformation, the peptide loses the ordered original structure, facilitating the release of drugs (Al-Ahmady et al., 2012). Elastin-like polypeptides are another group of temperature-responsive peptides that switch conformations in response to different temperature conditions (Macewan and Chilkoti, 2012). They possess low critical solution temperature (LCST), exhibit properties as soluble unimolecules below their LCST, and assemble into aggregates when the temperature is greater than the LCST. Amphiphilic Elastin-like polypeptides can also self-assemble into micelles with a hydrophobic core and hydrophilic corona (Macewan and Chilkoti, 2012).

Tissue-/Cell-Penetrating Peptides. Physiologic barriers such as vascular endothelial cells, tumor stromal cells, and ECM in tumor tissues are known to hinder the efficient penetration of nanosystems to reach targeted cells. The selective permeability of the plasma membrane leads to insufficient internalization of therapeutic systems. One type of tissue-penetrating motif, called the *CendR motif*, with a sequence R/KXXR/K and a second arginine or lysine residue on the C terminus of the peptide, has shown to bind to neuropilin-1, a membrane receptor expressed on endothelial cells, and to activate cell internalization and trans-tissue transportation (Wang et al., 2011). iRGD (CRGDKGPD) (Sugahara et al., 2010), one of the sequences within the CendR motif, exhibits tumor-specific penetrating ability as a result of the tumor-homing RGD motif. The RGD sequence mediates binding of iRGD to the tumor vascular targets, integrin $\alpha_v\beta_3$ and $\alpha_v\beta_5$. Subsequent proteolysis generates the C-terminal R/KXXR/K motif, which activates the neuropilin-1-dependent penetrating pathway. Another type of CPP, which is varied in size and sequence, interacts with the lipid bilayer and internalizes into cells via clathrin- or caveolin-mediated endocytosis, micropinocytosis, or an endocytosis-independent mechanism, such as the carpet model, inverted micelle model, barrel stave pore model, or toroidal model (Trabulo et al., 2010).

Most CPPs are derived from segments of natural translocating proteins with a large variety of sizes, sequences, and physicochemical properties. They can be classified into three main categories: cationic, hydrophobic, and amphipathic peptides. Cationic CPPs are positively charged and have a high affinity to negatively charged plasma membranes. The most commonly used cationic CPPs are poly-arginine and Tat-derived peptides (Takeshima et al., 2003; Walrant et al., 2011). Hydrophobic CPPs comprise nonpolar amino acids with low net charge and high lipid affinity. Signal sequence Kaposi fibroblast growth factor (K-FGF) and fibroblast growth factor-12 (FGF-12) belongs to the hydrophobic CPP category (Dokka et al., 1997; Nakayama et al., 2011). The remaining CPPs belong to amphipathic CPP class, which includes Antp, pVEC, penetratin, transportan 10, M918, VP22, and SAP (Derossi et al., 1994; Elliott and O'Hare, 1997; Fernandez-Carneado et al., 2004; Elmquist et al., 2006; El-Andaloussi et al., 2007; Amand et al., 2008; Islam et al., 2014). Amphipathic CPPs contain both polar and nonpolar amino acids and are thus both hydrophobic and hydrophilic in nature. There is a special group of amphipathic CPPs, including MPG, Pep-1, and S4₁₃-PV, that are synthesized by fusing segments of the HIV GP41 protein, reverse transcriptase of human immunodeficiency virus type 1, or DermaSeptin (Dermarite Industries, LLC, North Bergen, NJ) S4 peptide with the nuclear localization signal of simian virus 40 large T antigen (Hariton-Gazal et al., 2002; Morris et al., 2008). The resulting peptides form stable complexes with their cargo via noncovalent interactions and penetrate into the cell with high efficiency. A summary of the most widely used CPPs is presented in Table 3.

Therapeutic Peptides. Peptides have received considerable attention as therapeutic agents because of their high specificity, low toxicity, good tissue penetration, cost-effectiveness, and easy modification. Therapeutic peptides are commonly derived from chemokines, ECM proteins, growth factors, antigens, and other proteins, or they are identified via screening of a phage-displayed peptide library. They selectively target particular receptors to either activate essential signaling pathways or inhibit the receptors from interacting with their ligands. A great number of tumor therapeutic peptides have been identified, especially in the fields of antiangiogenesis and immunotherapy. Some examples are summarized in Table 4.

Sustained formation of new blood vessels is an important hallmark of tumor progression. Inhibiting angiogenesis and normalizing tumor vascular systems are the strategies most emphasized in therapy targeted to the tumor vasculature. Many peptides have been reported to target tumor blood vessels and block angiogenesis; these include T4, C16Y, and 6a-P (Rosca et al., 2011). T4 is a peptide identified by screening of a phage-displayed peptide library for high affinity to Tie2, an endothelial cell receptor kinase important in angiogenesis (Tournaire et al., 2004). Binding of T4 to Tie2 not only inhibits the interactions between Tie2 and angiopoietin 2 (Ang2) or angiopoietin 1 (Ang1) but also suppresses angiopoietin 1-dependent ERK activation and migration in human umbilical vascular endothelial cells. C16Y is an antiangiogenic peptide derived from one of the most potent angiogenic sites, C16 in laminin-1. This peptide eliminates the attachment of endothelial cells to laminin-1, disrupts tube formation, and inhibits angiogenesis in the tumor site (Ponce et al., 2003). 6a-P is a 20 amino acid peptide derived from vascular

TABLE 3
Sequences of several widely used CPPs

Categories	Names	Sequences	Origin	References
CenR motif Cationic	iRGD	CRGDKGPD	ECM proteins	Sugahara et al., 2010
	Nonarginine	R ₉	Synthetic	Walrant et al., 2011
Hydrophobic	TAT	YGRKKRRQRRR	Human immunodeficiency virus (HIV) type 1	Takeshima et al., 2003
	K-FGF	AAVALLPAVLLALLAP	K-FGF	Dokka et al., 1997
Amphipathic	FGF-12	PIEVCMYREP	FGF-12	Nakayama et al., 2011
	Antp	RQKIWFQNRMMKWK	Third helix of the antennapedia homeodomain	Derossi et al., 1994
	pVEC	LLILRRIRKQAHASK	Murine vascular endothelial-cadherin protein	Elmqvist et al., 2006
	Penetratin	RQKIWFQNRMMKWK	Antennapedia homeodomain in <i>Drosophila</i>	Amand et al., 2008
	TP10	AGYLLGKINLKALAALAKKIL	Synthetic peptide	Islam et al., 2014
	M918	MVTVLFRRLRIRACGPPVRV	Tumor suppressor protein p14RF	El-Andaloussi et al., 2007
	VP22	NAKTRRHERRRRLAIER	Herpesvirus structural protein	Elliott and O'Hare, 1997
	SAP	VRLPPVRLPPVRLPPP	Synthetic	Fernández-Carneado et al., 2004
	MPG	GALFLGLGAAGSTMGAWSPKKRKV	Combination of hydrophobic domain from HIV GP41 and NLS of SV40 large T antigen	Morris et al., 2008
	Pep-1	KETWWETWWTEWSQPKKKRKV	Combination of reverse transcriptase of HIV-1 and NLS of SV40 large T antigen	Morris et al., 2008
	S4 ₁₃ -PV	ALWKTLLKKVLKAPKKKKRKV	Combination of DenaSeptin S4 peptide with NLS of SV40 large T antigen	Hariton-Gazal et al., 2002

NLS, nuclear localization signal; SV40, simian virus 40; TP-10, transportan 10.

endothelial growth factor (VEGF). It binds to heparin sulfate proteoglycan, which is an important regulator in angiogenesis; the binding consequently inhibits the interaction between VEGF and heparin sulfate proteoglycan (Lee et al., 2010).

Because of its potential for remarkable specificity and efficacy, tumor immunotherapy has emerged as a promising tumor therapeutic strategy. Peptides play a significant role in tumor immunotherapy. Examples include peptide vaccines and immune checkpoint blocking peptides. Tumor antigens that can be recognized by cytotoxic T lymphocytes (CTLs) are usually expressed to a limited extent on tumor cells; however, peptides derived from tumor-associated antigens possess great potential to be recognized by CTL and can be delivered to tumor cells. For example, peptides derived from glypican-3 have efficiently stimulated CTL activity when loaded into tumors in vivo via intratumoral injection (Nobuoka et al., 2013). One of the mechanisms that allow tumor cells to escape from the immune system is overexpression of immune checkpoint proteins to inhibit T-cell attack. Using exogenous antagonists to block immune-suppressing pathways and activate the antitumor immune response of T cells. ^DPPA-1, a D-peptide antagonist, targets programmed death-ligand 1 (PD-L1) and efficiently disrupts the interaction between PD-L1 and its receptor, programmed death 1 (PD-1), unleashing the antitumor immune reaction (Chang et al., 2015). ^DPPA-1 is the first reported proteolysis-resistant peptide antagonist targeting the immune checkpoint protein human PD-1/PD-L1 (Chang et al., 2015).

Peptide toxins can also be used to regulate the tumor microenvironment by reducing the number of stromal cells to deteriorate the tumor-supporting stroma and break physiologic barriers for better drug perfusion. Numerous peptide toxins have been used to kill tumor cells, such as proapoptotic peptide KLA, phalloidin, and amanitin (An et al., 2010; Moshnikova et al., 2013; Jung et al., 2016), and they can also be used to induce stromal cell toxicity for enhanced drug perfusion and antitumor therapeutic efficacy.

Tumor Microenvironment Targeting and Responsive Peptide-Based Nanoformulations

As already described herein, peptides have exhibited various activities in the tumor microenvironment. For example, targeting peptides show high affinity to components of the tumor microenvironment, and responsive peptides are sensitive to tumor physicochemical conditions, CPPs are able to enhance cellular uptake, and therapeutic peptides can regulate pathways related to tumor progression. A number of these attractive building blocks have been used to construct nanoformulations specifically targeting tumor microenvironment and have achieved exciting treatment outcomes. In this section, we summarize recent work published by our group and others on the use of peptide-based nanoformulation strategies, especially for regulating tumor blood vessels and stromal cells.

Ligand Peptides Mediate Nanoformulations to Target Tumor Microenvironment

Herein, we review nanoformulations that are conjugated with targeting peptides against tumor vasculature,

TABLE 4
Examples of therapeutic peptides

Names	Sequences	Origin	Receptors	Mechanism	References
T4	NLLMAAS	Phage-displayed peptide library	Tie 2	Blocking the interaction between Ang1 and Tie2	Tournaire et al., 2004
C16Y	DFKLFAYYIKYR	Scrambled laminin-1 C16 sequence	Integrin $\alpha_v\beta_3$ and $\alpha_v\beta_1$	Antagonistically binding to integrin, blocking laminin-1 induced angiogenesis	Ponce et al., 2003
6a-p	KSVRGKGGQKRKRKKSRYSYK	Exon 6a-encoded domain of VEGF	HSPG	Inhibiting the binding of VEGF and HSPG, blocking the angiogenesis pathway	Lee et al., 2010
Glypican-3 peptide	FVGEEFTDV	Glypican-3	CTL	Loading to tumor cells and enhancing the recognition of tumor cells by CTL	Nobuoka et al., 2013
DPPA-1	NYSKPTDRQYHF	Phage-displayed peptide library	PD-1	Binding to immune checkpoint protein PD-1 and inhibiting the interaction between PD-1 and PD-L1	Chang et al., 2015
KLA	KLAKLAKKLAKLAK	Natural antibacterial peptide	Mitochondria membrane	Disrupting mitochondrial membrane and induce apoptosis (can be used in regulating stromal cell number in future)	An et al., 2010

HSPG, heparin sulfate proteoglycan.

lymphatics, and stromal cells and carry cargo, such as chemotherapy or gene therapy agents, for improved tumor therapy and decreased systemic toxicity.

Targeting Tumor Vasculatures. The most studied peptides targeting tumor blood vessels are the derivatives of RGD, an integrin $\alpha_v\beta_3$ and $\alpha_v\beta_5$ binding sequence present in various ECM proteins, including fibronectin and vitronectin. Some studies have suggested that modification of RGD ligands for use in nanoformulations carrying chemotherapeutic agents, siRNA, or small-molecule inhibitors leads to enhanced inhibition of angiogenesis and tumor growth (Danhier et al., 2012). Interestingly, an antimetastatic effect was observed when integrin $\alpha_v\beta_3$ and $\alpha_v\beta_5$ were targeted alone (Gvozdenovic et al., 2016); further studies are needed to confirm this promising strategy for inhibition of tumor metastasis.

VEGF, also known as vascular permeability factor, plays a vital role in the angiogenic process by binding to specific VEGF receptor 2 (VEGFR2, also known as KDR/Flk-1), a tyrosine kinase receptor. The binding of VEGF and its receptor then activates downstream signaling pathways, including FAK/paxillin and RAS/ERK, and results in the proliferation and migration of endothelial cells, consequently promoting angiogenesis and vascular growth (Liang et al., 2014). Therefore, the VEGF-VEGFR2 signaling pathway has been extensively explored as a target for tumor therapy. To enhance cellular uptake and antiangiogenic activities in vitro and in vivo, Fu and colleagues conjugated RGD peptide to selenium nanoparticles loaded with doxorubicin (Dox) for targeting tumor vasculature. This nanosystem disassembled under acidic condition in lysosomes after internalization, triggering drug release. In vivo experiments showed inhibition of MCF-7 tumor growth and angiogenesis. The antiangiogenesis effect resulted from apoptosis and cell-cycle arrest in endothelial cells through downregulation of the VEGF-VEGFR2 signaling pathway (Fu et al., 2016). Inhibition of VEGFR2 mRNA expression in new tumor blood vessels is also an attractive approach for antitumor therapy. For tumor-targeted VEGFR2-siRNA delivery, Liu and colleagues (2014) designed a self-assembling peptide nanoparticle system consisting of the targeting cyclopeptide c(RGDfk) with an 8-amino-3,6-dioxaoctanoic acid- β -maleimidopropionic acid modification (referred to as *RPM*) that is capable of encapsulating siRNA via intermolecular hydrogen bonding. The RPM/VEGFR2-siRNA showed high gene-silencing efficiency and minimal cytotoxicity in vitro and effectively suppressed neovascularization when delivered into zebra fish embryos. Administration of RPM/VEGFR2-siRNA to tumor-bearing nude mice significantly inhibited tumor growth and reduced the density of tumor vessels, accompanied by downregulated VEGFR2, at both the mRNA and protein levels in tumor tissues. No measurable immunogenicity of the nanoparticles was observed in mice (Liu et al., 2014).

In addition to RGD peptide derivatives, many other peptides can target tumor blood vessels. For example, Herringson and coworkers (2011) tested two targeting peptides, peptide WHSDMEWWYLLG, an antagonist for VEGFR-1, and peptide ATWLPPR, which specifically binds to neuropilin-1, a VEGFR-2 coreceptor. When engrafted into Dox-containing liposomes, the peptide WHSDMEWWYLLG promoted liposome aggregation and/or leakage of the encapsulated liposomal drug. ATWLPPR liposomes showed significantly enhanced tumor targeting efficiency, particularly when

TABLE 5
Nanoformulations targeting tumor microenvironment via conjugated ligand peptide

Targets	Therapeutic agents	Targeting ligands	Cell lines	Results	References
Integrins	RGD modified and Dox-loaded selenium nanoparticles	RGD	HUVECs and MCF-7 breast cancer cells	Selectively binding to tumor vessel and achieving antiangiogenesis.	Fu et al., 2016
Neuropilin-1	c(RGDfk) mediated nanoparticle encapsulated VEGFR2-siRNA	C(RGDfk)	HUVECs and A549 lung cancer cells	Inducing effective gene silence in vitro and inhibiting tumor growth in vivo	Liu et al., 2014
gC1qR	Peptide modified liposome loaded with Dox	ATWLPPR peptide	B16-F1 murine melanoma cells	Increased tumor targeting and suppressed tumor growth	Herringson and Altin, 2011
	LyP-1 conjugated to PEGylated liposomes loaded with or Dox	LyP-1	SPC-A1 lung adenocarcinoma cells	Enhanced inhibition effect on tumor cells in vitro and lymphatic metastatic tumors in vivo	Yan et al., 2012
M2-like TAM	Nanoformulations composed of (RNAi)-peptide biohybrid	M2pep	Mouse BALB/c macrophage J774.2 cells	Immunomodulating TAMs in tumor microenvironment and efficaciously eradicating tumor cells	Conde et al., 2015

HUVEC, human umbilical vein endothelial cell.

PE-PEG750 was used as the stabilizing lipid instead of PE-PEG2000 in the construction of the liposomes. Moreover, ATWLPPR liposomes inhibited the growth of B16-F1 tumors in mice (Herringson and Altin, 2011) (Table 5).

Targeting Tumor Lymphatics and TAMs. Lymphatic vessels in tumors are morphologically distinct from normal lymphatic vessels. As shown in Table 1, LyP-1 is a ligand peptide that targets tumor cells, tumor lymphatics, and TAMs in tumor hypoxic regions (Sánchez-Martin et al., 2011). To improve the efficiency of targeted therapy of lymphatic metastatic tumors, Yan and colleagues (2012) developed LyP-1-decorated and Dox-loaded PEGylated liposomes. Internalization of the LyP-1-decorated liposome was observed in tumor cells and metastatic lymph nodes, but not in normal lymph nodes. Peptide LyP-1 modification caused the liposomes to distribute adjacent to tumor lymphatics and TAMs in metastatic lymph nodes and also enhanced growth inhibition of tumor cells in vitro and lymphatic metastatic tumors in vivo (Yan et al., 2012).

TAMs are the predominant inflammatory cell components in the tumor microenvironment. Most polarize to become M2-like macrophages, which as immunosuppressive populations contribute to angiogenesis and tumor immune evasion and subsequently promote tumor proliferation and metastasis. TAMs have also been reported to be the major players in chemoresistance and radioprotection in tumors (Jinushi and Komohara, 2015). In clinical studies, high densities and activation levels of TAMs are associated with poor treatment outcomes. Therefore, TAMs have become appealing targets for the development of cancer therapy. The most common strategies in TAM-targeted therapy include those that aim to inhibit macrophage recruitment, reduce TAM survival, enhance the M1-like tumoricidal activity of TAMs, or block their M2-like pro-tumor activity. Conde and colleagues (2015) designed nanoformulations composed of an RNA interference (RNAi)-peptide hybrid that specifically targets murine lung TAMs using M2pep peptide and delivered anti-VEGF siRNA into TAMs as well as tumor cells simultaneously. This approach achieved high targeting efficiency via M2pep and promoted effective VEGF downregulation. Administration of the hybrid nanoparticles resulted in immunomodulation of the TAM population within the tumor microenvironment and efficient eradication of tumors with an increased overall survival (Conde et al., 2015) (Table 5).

Enhanced Tumor Penetration via Tumor Microenvironment Responsive Peptides

Based on the pathophysiological variations in tumor microenvironment, many on-demand responsive nanoformulations have been designed for the spatially and temporally controlled release of therapeutic agents in response to specific stimuli. Some excellent review articles have already extensively discussed the molecular design and development of nanoscale systems for tumor microenvironment response (Mura et al., 2013; Wang et al., 2014). In this section, we focus on the peptides that are responsive to the tumor microenvironment as tools to enhance the ability of nanoformulations to penetrate tumors.

CPPs have received tremendous attention for their abilities to improve drug penetration in vivo as a result of their capability to mediate the internalization of a variety of cargo

molecules into cells (Fonseca et al., 2009); however, the use of CPPs is limited in vivo owing to cell damage and low tissue specificity and to their positively charged nature (Zorko and Langel, 2005). Great efforts have been made to improve the selectivity of CPPs. Generally, CPPs are suppressed via electrostatically interacted shields or steric hindrance under physiologic conditions and are exposed to cells when they are circulating into tumor tissues. Based on this strategy, many nanosystems have been designed, including: 1) recovery of CPP functions via pH-activatable systems, 2) recovery of CPP functions via protease-activatable systems, and 3) a combination of these two strategies (Huang et al., 2013c).

pH-Activatable Tumor Penetration. Owing to the excess metabolic acid secreted from fast-growing tumors, the pH in tumor microenvironment is more acidic than that in normal tissue, which makes the design of systems responsive to low pH an effective tumor-targeting strategy. Several pH-responsive systems have been developed that use the amino acid histidine, which has a pKa value of 6.5 and can be protonated from a net negative charge to a net positive charge when translocated into the acidic tumor microenvironment. Zhang and coworkers (2011) designed a pH-responsive cell-penetrating carrier based on histidine-rich peptide via a pH-triggered charge conversion strategy. Yeh and colleagues reported another pH-responsive CPP, in which pH sensitivity was controlled by recombinant fusion to a histidine-glutamine oligopeptide (Yeh et al., 2016). Zhao and coworkers (2016) found that polyhistidine could respond to the acidic tumor microenvironment by exposing a CPP R4 sequence. In the literature mentioned herein, cell internalization of nanocarriers can be promoted by means of pH-triggered CPP surface-charge reversal from negative to neutral or positive.

CPPs that are pH-responsive can facilitate the active targeting and uptake of tethered nanoparticles. Shi and colleagues (2015) designed a novel peptide containing a targeting peptide c(RGDfK) and a pH-responsive CPP (AGYLLGHINLHHLAHL(Aib)HHIL-NH₂). Nanoparticles conjugated with this RGD-CPP peptide were not only able to actively target $\alpha_v\beta_3$ -overexpressing cells, compared with CPP-nanoparticles, but they also increased cellular uptake compared with RGD-nanoparticles. RGD-CPP nanoparticles loaded with paclitaxel showed significantly higher survival rates in B16F10 tumor-bearing mice compared with other groups (Shi et al., 2015).

Enzyme-Activatable Tumor Penetration. Expression of MMPs is often upregulated in tumor ECM, and MMPs can cleave specific sequences via enzymatic catalysis. Thus, MMPs may serve as a better target for broad-spectrum theranostic applications. Nanoparticles incorporated with CPPs can be modified with PEG via an MMP-2 cleavable spacer to mask positively charged CPPs. Linkage with PEG was able to enhance the stability of nanoparticles in serum and avoid nonspecific interactions (Harada et al., 2010). For example, one group of investigators developed a liposome modified with CPP and a PEG-conjugated antibody. PEG was linked to the liposome via a MMP-2 responsive sequence (Zhu et al., 2012). Through the enhanced permeability and retention effect, these liposomes could selectively accumulate at the xenograft tumor site, where PEG was subsequently detached as a result of cleavage of the linker by endogenous MMP-2. In this manner, CPP-mediated cell penetration of the liposomes was achieved.

Wang and coworkers developed a tumor specific drug-delivery system by encapsulating nanovesicles with an MMP-responsive polymeric network. This polymeric network could effectively prevent the cargo from nonspecifically leaking from the nanoparticles, with enhanced drug bioavailability. Tumor-overexpressed MMP-2 specifically triggered the disassembly of polymeric networks and released the functional nanoparticles in tumor tissues. By using a tumor-homing peptide iRGD conjugated to the nanoparticles as a target ligand, they were able to facilitate tumor targeting and penetration of the drug both in vitro and in vivo. These results demonstrate that synergistic therapeutic efficacy can be achieved based on improved tumor accumulation, penetration, and MMP-responsive drug release in the tumor microenvironment (Liu et al., 2015).

pH and Enzyme Double-Activatable Tumor Penetration. Another strategy reported for tumor microenvironment-specific recovery of the CPP effect is based on pH and protease double-activatable systems. Polyanionic peptides with pH-responsive properties are used to neutralize the CPP charge. In the tumor microenvironment the charges of these masking peptides is altered to result in CPP dissociation. For example, Jiang's group devised a pH-sensitive masking peptide with an isoelectric point of approximately 6.4 (Huang et al., 2013a,b). This peptide is negatively charged under physiologic pH, but it becomes predominantly uncharged or positively charged in the acidic tumor microenvironment. Since the conjugation of this masking peptide with CPP is weak, a peptide linker that is MMPs-cleavable was inserted between the masking peptide and CPP to form an intramolecular hairpin structure. When administrated in vivo, the hairpin structure remains intact in the circulation with the CPP blocked, maintaining the delivery system cell-impermeable and pharmacologically inactive in plasma. In the tumor microenvironment, the enzyme-responsive linker is cleaved by MMPs, and the hairpin structure disassociates. The exposed CPP can then penetrate the tumor. Based on this strategy, Jiang's group developed a series of tumor-targeting nanosystems with enhanced drug delivery and antitumor efficacy (Huang et al., 2013a,b).

Peptide Self-Assembled Nanoformulations Targeting CAFs to Break Stromal Barriers

Despite the promising therapeutic potential exhibited by numerous antitumor nanoformulations, the heterogeneity among tumor cells and the presence of complex stromal cell barriers still present great challenges that limit tumor-targeting and cell-penetrating performance. Therefore, strategies to overcome tumor heterogeneity and to break stromal barriers are urgently needed. CAFs, the major stromal cell type in tumor microenvironment, play a key role in the formation of stromal barriers, leading to poor penetration for particulate therapeutics and also limiting access of pharmacologic drugs. Nanoformulations specifically targeting CAFs have shown promising results, as discussed in the following sections.

Our group developed peptide-based nanoformulations targeting and depleting CAFs to overcome the aforementioned obstacles. For example, a dual-mode nanomaterial that used CAF targeting combined with increased cellular uptake

Fig. 1. FAP- α responsive nanocarriers based on a cleavable amphiphilic peptide. (A) The structure of peptide cleavable amphiphilic peptide (CAP), which contains a TGPA sequence that can be cleaved by FAP- α . (B) Proposed mechanism of peptide self-assembly, drug-induced reassembly, and peptide and drug coassembly in the hydrophobic drug and amphiphilic peptide mixed solution. The components form the stable nanoparticles. The morphology of peptide assembly during Dox loading was observed by transmission electron microscopy. The assemblies transformed from mace-like (I) to spherical (II) with prolonged ultrasonication. (C) Drug-release profiles of CAP-Dox and uncleavable amphiphilic peptide (UAP)-Dox in the presence or absence of FAP- α . (D) Penetration of Dox into prostate tumor (PC-3 and CAF coimplanted) tissues after intravenous injection of different Dox formulations. Frozen tumor sections were stained with DAPI (blue) to label nuclei and CD31 (green) antibody to label tumor vasculature. Red: Dox. (E) Growth curves of PC-3 and CAF coimplanted prostate tumors in mice treated with different Dox formulations (used with permission from *Angew Chem Int Ed*, John Wiley & Sons; Ji et al., 2016b.). ** $P < 0.01$ vs. Control, Dox, and UAP-Dox groups.

coordinated by CPP and cholesterol improved the tumor penetration of chemotherapeutic drugs. This was achieved by depletion of CAFs and disruption of stromal barriers for the treatment of CAF-rich solid tumors (Ji et al., 2015). In another study, we reported a novel CAF-targeting drug delivery nanosystem based on a cleavable amphiphilic peptide designed to be specifically responsive to FAP- α , a membrane-bound serine protease specifically expressed on CAFs (Ji et al., 2016b). This cleavable amphiphilic peptide nanocarrier transformed from self-assembled nanofibers to spherical nanoparticles when loaded with hydrophobic drugs. The disassembly of these nanoparticles upon FAP- α cleavage resulted in efficient release of the encapsulated drugs specifically at tumor sites. This “transformer”-like drug nanocarrier could also disrupt the stromal barrier and enhance local drug accumulation (Fig. 1).

Since CAFs encompass a multifunctional stromal cell type, we found that selective inhibition, rather than complete depletion of CAFs, may be more appropriate for a safer long-term effective strategy. In one study, we developed a β -cyclodextrin (β -CD) modified MMP-2-responsive liposome loaded with the antifibrotic and anti-inflammatory agent pirfenidone and the chemotherapeutic drug gemcitabine (GEM) for CAF regulation; this formulation was used for targeted delivery of GEM in pancreatic cancer therapy (Ji et al., 2016a). When this nanoformulation reached the tumor site, the pirfenidone-loaded β -CD was able to discharge and accumulate in the

stroma after cleavage of the MMP-2 substrate peptide. The initial release of pirfenidone effectively downregulated fibrosis and decreased the stromal barrier. Subsequently, the RGD-containing liposomes loaded with GEM recognized tumor cells and penetrated into the tumor tissue, resulting in enhanced therapeutic efficacy. This combined antifibrosis and antitumor strategy may increase drug penetration and enhance chemotherapeutic efficacy, providing a potential strategy for the design of nanoformulations to improve the pancreatic tumor therapy (Fig. 2).

Therapeutic Peptide Self-Assembled Nanoformulations Targeting Tumor Vascatures

The short circulating half-life of small therapeutic peptides in vivo may limit their use in human clinical applications for tumor therapy (Talmadge, 1998). Strategies are needed for increasing the stability and activity of peptide drugs to improve their therapeutic outcomes. Apart from chemical modification, incorporating therapeutic peptides into nano-systems with adequate size, morphology, and surface properties can improve their stability and pharmacokinetics. Mediated by intermolecular forces, amphiphilic peptides composed of distinct hydrophobic and hydrophilic segments are able to self-assemble into particular nanostructures. A therapeutic peptide with the ability to inhibit CXCL chemokine

Fig. 2. A β -cyclodextrin (β -CD) modified MMP-2-responsive liposome loaded with the antifibrotic and anti-inflammatory agent pirfenidone (PFD) and the chemotherapeutic drug gemcitabine (GEM). (A) Illustration of the nanomedicine LRC-GEM-PFD (liposome with RGD and β -cyclodextrin containing GEM and PFD). (B) Immunohistochemical staining of collagen I and tumor growth factor- β (TGF) in pancreatic tumor tissue slices after LRC, PFD, and LRC-PFD treatment. (C) Penetration of rhodamine into pancreatic tumor (Panc-1 and PSCs coimplanted) tissues after intravenous injection of different PFD formulations. Red: rhodamine. (D) Tumor growth curves of PSCs/Panc-1 pancreatic tumors in mice treated with different GEM formulations (Used with permission from *ACS Appl Mater Interfaces*, American Chemical Society; Ji et al., 2016a.). * $P < 0.05$, ** $P < 0.01$ vs. Control.

receptor 4 function was demonstrated to form self-assembled nanoparticles that hindered CXCR4-mediated tumor metastasis (Tarasov et al., 2011).

Inspired by the peptide self-assembly concept, we designed a tumor microenvironment-responsive nanoformulation based on a tailor-designed amphiphilic peptide through conjugation of functional 3-diethylaminopropylisothiocyanate (DEAP) molecules to a short peptide (Zhao et al., 2014). The apparent pK_a of peptide conjugated with DEAP was approximately 6.9, which is close to the pH range within the tumor microenvironment. Protonation of the amphiphilic peptide at a weakly acidic pH led to a reduction in hydrophobicity, which triggered a pH-induced “intelligent switch” for the peptide self-assembled nanostructures, subsequently leading to their disassembly in the tumor microenvironment (Zhao et al., 2014). Furthermore, we recently developed a smart self-assembled nanoformulation using a therapeutic peptide known as C16Y (DFKLFVYIKYR). This peptide, with a hydrophilic head and DEAP as a hydrophobic tail, inhibits angiogenesis and tumor growth by targeting integrin $\alpha_v\beta_3$ and $\alpha_v\beta_1$ (Ding et al., 2015). DEAP-C16Y peptides self-assembled into spherical nanostructures under physiologic conditions and subsequently dissociated to release individual peptide molecules in weakly acidic tumors. Mechanistic investigation revealed that dissociated DEAP-C16Y peptides inhibited vascular endothelial cell migration and tubule formation through suppression of the focal adhesion kinase and PI3K-Akt signaling axis and also restrained tumor cell invasion via downregulation of invadopodia formation. The systemically administered DEAP-C16Y nanoformulations significantly decreased microvessel density, tumor growth, and distant metastasis formation in nude

mice, with effectively prolonged blood circulation of the therapeutic peptide in vivo. Additionally, the DEAP-C16Y nanostructures can be a smart and effective drug-delivery system of antineoplastic agents for targeted combination therapy. In this study, for the first time, we developed a self-assembled nanoformulation by using a therapeutic peptide as a building block. This peptide showed intrinsic antitumor activity and also provided a platform for combination therapy by encapsulating chemotherapeutic drugs in the nanoformulation, demonstrating a potential strategy for the future design of antitumor nanotherapeutics (Fig. 3) (Ding et al., 2015).

Numerous tumor microenvironment targeted nanosystems have been constructed and optimized using functional peptides to mediate cell penetration, target the tumor microenvironment, respond to tumor-specific enzymes for controlled drug release, or regulate cellular signaling transduction pathways. According to their functionalities, building blocks in nanoformulations can be classified into different categories and regarded as modules for nanosystem construction. For more efficient design and implementation of peptide-based drug delivery, controlled release, and targeting, as well as the regulation of the tumor microenvironment, we propose a modularized concept for peptide nanosystem construction (Fig. 4A). The self-assembled micelles, vesicles, and nanofibers are assembled from amphipathic monomers in a process that is generally mediated by various intermolecular forces. Typically, functional peptides are used as the hydrophilic heads, and lipophilic molecules such as cholesterol, alkyl chains, polymers, and lipids serve as the hydrophobic tails.

Fig. 3. A smart therapeutic peptide for inhibiting angiogenesis and tumor growth. (A) Schematic structure of the DEAP-C16Y peptide. (B) Proposed antitumor mechanism of DEAP-C16Y nanostructures. (C) Migration of human umbilical vein endothelial cell (HUVECs) treated by peptides in a transwell migration assay; cells were chemoattracted by 5% fetal bovine serum/RPMI-1640 medium. The number of migrated cells in each group was quantified. Tubule formation was assessed in HUVECs treated by C16Y or DEAP-C16Y. The total network length in each treatment group was quantified. * $P < 0.05$, ** $P < 0.01$ compared with the PBS group. (D) Growth curves and metastatic foci number of 4T1 tumors. Mice bearing 4T1 tumors were treated with DEAP-HSAF: a fragment of human serum albumin, DELRDEGKASSAKQ, nanostructures (negative control) or with C16Y or DEAP-C16Y nanostructures for indicated periods. Tumor volume was calculated every other day. Lung sections from 4T1-bearing mice were stained with H&E or proliferating cell nuclear antigens. The number of metastatic foci was quantified. * $P < 0.05$, ** $P < 0.01$ compared with DEAP-HSAF group. (E) Antitumor efficacy of DEAP-C16Y-Dox nanostructures. Mice bearing 4T1 tumors were treated with the indicated formulations every third day. Tumor volume was calculated every other day. The number of metastatic foci per lung was quantified (Used with permission from *Mol Cancer Ther*, American Association for Cancer Research; Ding et al., 2015). * $P < 0.05$, ** $P < 0.01$ compared with DEAP-HSAF group.

Such monomers, constructed with multiple modules, then self-assemble into nanostructures with diverse functions and physicochemical features. Apart from this self-assembled system, there is another common type of peptide-based nanostructure that uses preformed nanostructures, such as liposomes, polymeric particles, or inorganic nanostructures (e.g., gold, silicon, and ferric oxide) onto surfaces to achieve improved biocompatibility, active targeting, controlled release, or enhanced tissue penetration (Fig. 4B).

By proposing the concept of modularized construction, our intention is to highlight a strategy that categorizes the building blocks into different modules, depending on their functions. The desirable modules suitable for targeting environment and encapsulated therapeutic molecules can be selected and then combined to construct intelligent and well controlled nanoformulations. Modularized construction makes it easier to construct nanoformulations with promising specificity and therapeutic efficacy.

Conclusions

Compared with tumor cells, the surrounding tumor microenvironment is a more accessible target for antitumor therapy. The specifically expressed receptors and unique

physicochemical conditions within the tumor microenvironment provide many opportunities for nanoformulations to target tumor tissues. Many nanosystems have been developed to target the tumor microenvironment with the goal of achieving enhanced targeting efficacy and therapeutic outcomes. As discussed in this review, tumor progression is promoted by the

Fig. 4. Modularized construction of nanoformulations. (A) Amphipathic peptide consisting of a hydrophilic head and hydrophobic tail, based on various modules that self-assembles into nanostructures such as micelles, vesicles, and fibers in response to intermolecular forces. (B) Preformed nanostructures can be modified with functional modules for enhanced therapeutic efficacy.

sustained influence of the tumor microenvironment. In addition, stromal cells and ECM in the tumor microenvironment generate a natural sanctuary for tumor cells. Therapies that regulate the components in the tumor microenvironment, such as tumor blood vessels and CAFs, have been reported to efficiently suppress tumor growth with reduction of several underlying threats, such as invasion and metastasis. Moreover, the novel therapeutic strategy based on the dual targeting of tumor microenvironment and tumor cells has become increasingly attractive and has exhibited increased drug accessibility and elevated treatment efficacy. In particular, incorporated functional peptides are able to endow antitumor nanoformulations with high specificity, cell-penetrating ability, sensitivity to slight change in conditions, and potential for cellular pathway regulation and have achieved excellent therapeutic efficacy. In this review, we have proposed a modularized construction strategy for the assembly of peptide-based nanoformulations and have discussed its potential advantages and utility in anticancer nanosystem development. It should be noted that, despite the great advances in tumor microenvironment targeting and therapy, additional functional peptides need to be designed. A deeper understanding of the tumor microenvironment can also help us to make full use of its properties to develop antitumor nanoformulations with better specificity and stronger therapeutic efficacy.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Qin, Ding, Mujeeb, Zhao, Nie.

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