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REVIEW

Research advances in peptide–drug conjugates

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Abstract Peptide–drug conjugates (PDCs) are drug delivery systems consisting of a drug covalently coupled to a multifunctional peptide *via* a cleavable linker. As an emerging prodrug strategy, PDCs not only preserve the function and bioactivity of the peptides but also release the drugs responsively with the cleavable property of the linkers. Given the ability to significantly improve the circulation stability and targeting of drugs *in vivo* and reduce the toxic side effects of drugs, PDCs have already been extensively applied in drug delivery. Herein, we review the types and mechanisms of peptides, linkers and drugs used to construct PDCs, and summarize the clinical applications and challenges of PDC drugs.

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1. Introduction

Drug delivery system is the system that delivers drugs to the target site for efficacy without causing toxic side effects to normal cells. The purpose of drug delivery system is to decrease the toxic side effects, enhance stability, improve bioavailability, reduce degradation, maintain stable and effective blood concentration, avoid fluctuations in blood concentration, and increase the concentration of drugs in target tissues[1−3]. Two strategies are applied to construct drug delivery systems: one is to use delivery carriers to improve the stability of the drug, and the other is a prodrug strategy in which the drug is covalently modified to temporarily limit the activity of the drug. Compared with the former, the prodrug strategy avoids the safety issues of immunogenicity and toxicity caused by delivery carriers and reduces the metabolic burden of patients on delivery carriers[4,5], and therefore has a wide range of applications.

Considered an emerging prodrug strategy in targeted drug delivery systems, peptide−drug conjugates (PDCs) are defined as drugs covalently linked to peptide sequences with certain functions through specialized linkers. The inherent chemical structure of PDCs is composed of three components: peptide, linker, and drug, whose mechanism of action varies depending on the kinds of peptides and linkers. Firstly, peptides specifically target cells by recognizing receptors on their surface, and PDCs enter the cell through receptor-mediated internalization. Then the linker breaks up in the cell under stimulation to release the drug and exert therapeutic effects[6]. Such a prodrug strategy can potentially improve drug targeting, reduce toxic side effects on other cells, increase drug stability in blood circulation, control drug release and improve drug bioavailability[7,8]. Compared to ADCs with similar construction strategies, the peptides contained in PDC have unique advantages[9−14]: (1) The small size of the peptide molecules results in higher drug loading, more easily penetrating tumor stroma and entering tumor cells. (2) Peptides are highly biodegradable without triggering any immunogenic reactions in the body. (3) Some of the targeting peptides can eliminate drug resistance in tumor cells by altering the cell entry mechanism to achieve effective killing of tumors resistant to drugs, and break the dilemma of ineffective treatment due to drug resistance that commonly exists in traditional chemotherapy. (4) The short peptide properties of PDCs make their structures more flexible and easier to be modified and conjugated, which can be coupled with various drugs, such as chemical, protein and peptide drugs, to prepare targeted agents, significantly reducing off-target toxicity and greatly improving the feasibility of PDC formulation platform technology. (5) The production process of peptide fragments is simple and easy to scale up. Therefore, PDC has great potential in developing targeted drug delivery systems.

The review will first highlight the categories and mechanisms of peptides, the classification of linkers and their cleavage mechanisms, as well as different drugs for the construction of PDCs. Then the summary of the status of PDC clinical development will be presented in the following section. Furthermore, we make predictions and perspectives on the possible barriers and challenges to the application of PDCs in the clinic. The purpose of the review is to provide guidance for the construction of novel peptide−drug conjugates.

2. Peptides in PDCs

In recent years, with the rapid advances in proteomics, peptide solid-phase synthesis and phage display technology, researchers have discovered or rationally designed an increasing number of novel peptides, significantly facilitating the progress of PDCs. Peptides, as an important part of PDCs, are the basis for achieving multiple functions of PDCs[15]. For instance, the solubility and drug-forming properties of hydrophobic drugs can be increased by linking water-soluble peptides; self-assembled peptides can spontaneously generate nanostructures of PDCs[16]; cell-penetrating peptides play a key role in enabling drugs to penetrate cell membranes and effectively reach their targets[17]. Peptides can be classified according to their functions[18]: cell-penetrating peptides (CPPs), cell-targeting peptides (CTPs), self-assembling peptides (SAPs), and responsive peptides.

2.1. *Cell-penetrating peptides*

Cell membrane is the natural barrier composed of lipid bilayer that restricts the entry of outside substances into the cell and guarantees the stability of the intracellular environment. Meanwhile, for the purpose of maintaining a stable internal environment, diverse physiological barriers exist in the human body, such as the blood−brain barrier (BBB)[19,20], the blood-eye barrier and the blood−fetal barrier. A major obstacle to the delivery of drugs is that they are incapable of penetrating cellular membranes and physiological barriers so that their effects are not realized inside cells. Cell-penetrating peptides refer to small, short molecular peptides that can enter cells without disrupting their membrane integrity, generally consisting of 5−30 amino acids. CPPs are rich in basic amino acids, including arginine and lysine. There are two common characteristics of CPPs which are positively charged and amphiphilic under physiological environments[21]. In recent years, CPP has been widely utilized in drug delivery systems (Table 1)[22−31]. The widely recognized mechanisms by which CPP penetrates cell membranes can be divided into two categories: endocytosis and direct translocation[32]. The process of endocytosis is energy-dependent with three main pathways: macropinocytosis, caveolae-mediated endocytosis (CvME) and clathrin-mediated endocytosis (CME)[33]. In contrast, direct translocation is a non-energy-consuming process, which is mediated by the interaction of positively charged CPP with negatively charged membrane components and phospholipid bilayers to promote direct permeation of the cell membrane[34]. Different types of models are available for direct penetration: barrel stave model, carpetlike model, toroidal pore model as well as inverted micelle model[35]. In this paper, cell-penetrating peptides are classified into linear and cyclic peptides based on their structures.

2.1.1. *Linear cell-penetrating peptides*

The linear peptide is the classical peptide structure in CPP, with simpler structure and easier to synthesize compared to the cyclic peptide, thus being the subject of experimental studies by many researchers. Human immunodeficiency virus-1 transcription activator (HIV-1 TAT) is the peptide derived from HIV-1 transcription activator protein. Since Frankel et al.[36] and Green et al.[37] first found in 1988 that TAT can rapidly enter cells across membranes and activate the replication of the virus genome, there has been extensive interest in TAT and its derived linear peptides. More recently, TAT linear peptides have also been widely applied in the construction of PDCs for the purpose of trans-membrane transport. Chen et al.[38] constructed three PDCs (NTD, d-NTD and q-NTD) in which TAT is covalently attached to one, two, or four doxorubicin, respectively, *via* a cathepsin B degradable

Table 1 Cell-penetrating peptide used in drug delivery.

Name	Sequence	Payload	Disease	Ref.
Polyarginine	RRRRRRRRR	Curcumin	Cancer	22
Transportan 10 (TP10)	AGYLLGKINLKALAALAKKIL	Vancomycin	Bacterial infection	23
Tumor-lineage homing CPP	RLYMRYYSPTTRRYG	Doxorubicin	Triple-negative breast Cancer	24
dNP2	CKIKKVKKKGRKKIKKVKKKGRK	Doxorubicin	Cancer	25
RVRR	RVRR	Olsalazine	Cancer	26
L-penetratin	RQIKIWFQNRRMKWKK	Insulin	Alzheimer's disease	27
Activable CPP (ACPP)	CRRRRRRRRRGGGPKKKKKK	Doxorubicin	Cancer	28
LH_2	Acetyl-LHHLCHLLHHLCHLAG-NH2	Paclitaxel	Triple-negative breast Cancer	29
Cancer cell line specific CPP	RLYMRYYSPTTRRYG	Gossypol	Cancer	30
T2	FKKFFRKLL	Paclitaxel	Cancer	31

tetrapeptide linker (-Gly-Phe-Leu-Gly-) (Fig. 1). The amount of doxorubicin in the conjugates was found to exert a remarkable influence on the release of doxorubicin, with the slowest rate of release from q-NTD while the fastest rate of release from NTD. Also, it was shown that q-NTD was the most effective in accumulating in cancer cells, while NTD showed the lowest concentration of intracellular accumulation. Curiously, when cells were assessed for viability with Sulforhodamine B (SRB) assay, d-NTD was found to be the most effective against HepG2 human hepatoma cells. Such results indicate that although the cell-penetrating peptide TAT can enhance the membrane penetration ability of the conjugates, the efficiency of intracellular accumulation and the rate of free drug release are two essential factors in predicting the efficacy of PDCs *in vitro*. Song et al.[39] prepared the PDCs TAT-CPT and TAT-2CPT with another antitumor drug camptothecin (CPT) through disulfide bonding, and found that the conjugates could exert antitumor effects through both CPT release and membrane disruption mechanisms. Lyu et al.[40] formed PDC by combining the sequence CYGRKKRRQRRR TAT with garcinia Cambogia acid (GA), which is extracted from the natural resin gamboges, to enhance the water solubility and targeted delivery of GA to bladder cancer tumor sites, which enhanced the anti-tumor activity of GA through reactive oxygen species-mediated apoptosis. PDCs based on TAT and its derived linear peptides have great potential applications in antitumor drug delivery.

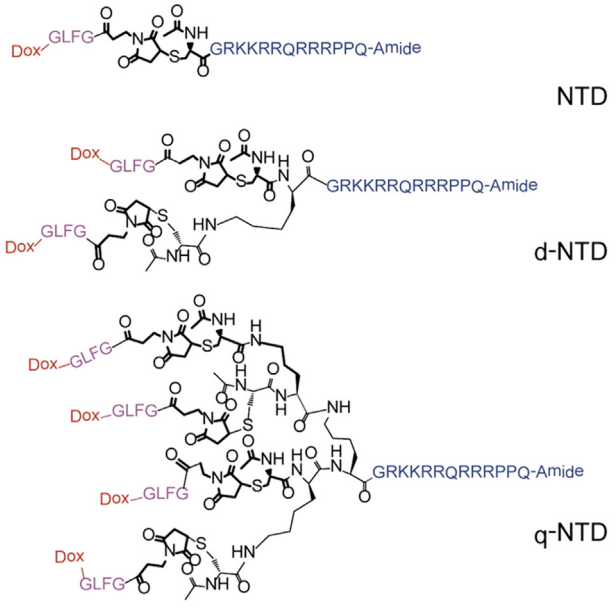


Figure 1 Chemical structures of the doxorubicin-Tat conjugates, including doxorubicin-Tat conjugates with 1 (NTD), 2 (d-NTD) and 4 (q-NTD) doxorubicin through a cathepsin B-sensitive GFLG linker. (Reprinted with the permission from Ref. 38. Copyright © 2014 Elsevier B.V.)

In addition to linear peptides derived from TAT, researchers have identified many other linear peptides used to penetrate physiological barriers. For the delivery of the antiviral drug porphyrin to the brain to treat viral brain infections, Mendonça et al.[41] in 2021 designed six peptide-porphyrin conjugates for penetrating the BBB, of which three PDCs were shown to be non-significantly cytotoxic or hemolytic and effective in crossing the BBB and inhibiting HIV *in vitro*. Employing the same strategy, Mendonça et al.[42] constructed eight peptide-porphyrin conjugates for the treatment of Zika virus (ZIKV) in 2022 and found that among them, PP-P1, with the ability to translocate across the blood–placental (BPB) and BBB barriers, showed particular effectiveness against ZIKV. Although linear membrane penetrating peptides have been widely used in the construction of many PDCs, however, their function and structure are too simple, resulting in disadvantages including poor stability, which makes them difficult to reach the target site in a complete and biologically active form, and low cell specificity, which may be taken up by non-specific cells, limiting their application as drug delivery tools[43,44].

2.1.2. *Cyclic cell-penetrating peptides*

Cyclic cell-penetrating peptide (cCPP), as a relatively new peptide, has shown great promise as a penetrating peptide for the construction of PDCs. Compared to linear peptides, cyclic peptides have significant advantages: (1) Cyclic peptides can significantly increase cell permeability. (2) Cyclic CPPs generally show higher resistance to protein hydrolysis. (3) Some cyclic CPPs exhibit effective endosome escaping. (4) Cyclic peptides have a higher affinity for target receptors. (5) Some cyclic CPPs have nuclear targeting properties[45]. Based on these advantages of cyclic CPP, El-Sayed et al.[46] coupled cyclic peptide $[WR]_5$ with the antitumor drugs paclitaxel (PTX) and camptothecin (CPT) to form PDCs, respectively. Compared with the parent drug, all of the PDCs are more soluble in water. In addition, in human breast cancer MCF-7 cell lines, the cytotoxicity of drugs and their corresponding PDCs was assessed. And the antiproliferative activity of PDCs was lower than that of the free hydrophobic drug in MCF-7 after an incubation period of 72 h, suggesting that CPT and PTX formed a prodrug. In later studies, they combined cabazitaxel (CBT) with cCPP with the help of an ester bond, and then

linked the integrin targeting (RGDC, TP1) peptides to the peptide−drug conjugate (cCPP-CBT) *via* disulfide bonds to form the conjugate TP1-cCPP-CBT (Fig. 2) to improve the targeting ability of the conjugate. In comparison with CBT, TP1-cCPP-CBT showed less toxicity (31−34-fold lower anti-proliferative activity) to natural human embryonic kidney (HEK-293) cells, demonstrating higher specificity of targeting. Also, cCCP enhanced the cell penetration of PDCs, making it easier to enter cells to release the parent drug CBT[47].

2.2. *Cell-targeting peptides*

Cell-targeting peptides are defined as peptides showing cell- or tissue-specific binding activity. Since Paul Ehrlich[48] first proposed the idea of targeted drug delivery in the early 20th century, it has become an increasingly popular study for researchers to improve the targeting of drugs. There are generally two categories of targeted drug delivery systems: passive targeting and active targeting. Passive targeting means that the drug accumulates passively in the focal tissue together with the blood circulation due to the nature of the delivery system itself (size, shape and charge, etc.) or the characteristics of the target tissue (poorly developed blood vessels and lack of lymphatic tissue return, etc.). On the other hand, active targeting is achieved by recognizing receptors or proteins specifically expressed in the target tissue and actively delivering the drug to the focal tissue through receptor−ligand interaction. Most of the PDCs based on cell-targeting peptides are delivered by an active targeting mechanism. A list of CTPs that have been applied to construct PDCs in recent years is given in Table 2[49−59].

2.2.1. *Arginine-Glycine-Aspartate (RGD) peptide*

As transmembrane glycoproteins are involved in cellular interactions with other cells or extracellular matrix, integrins have a significant impact on angiogenesis, leukocyte migration and tumor metastasis[60], hence being specifically expressed on tumor vascular endothelial cells. Arginine-Glycine-Aspartate (RGD) peptide, a tripeptide that specifically recognizes integrins $\alpha v\beta 3$ and $\alpha v\beta 5$, has been widely applied in the construction of cell-targeting peptides for PDCs. On the basis of RGD peptide, Kim et al.[61] described a switch therapy mediated by the tumor apoptotic protease caspase-3 (Fig. 3) which involves two different PDCs cleavable by caspase-3, RGDEVD-DOX (TPD1) and EMC-KGDEVD-DOX (MPD1), for the target of metastatic

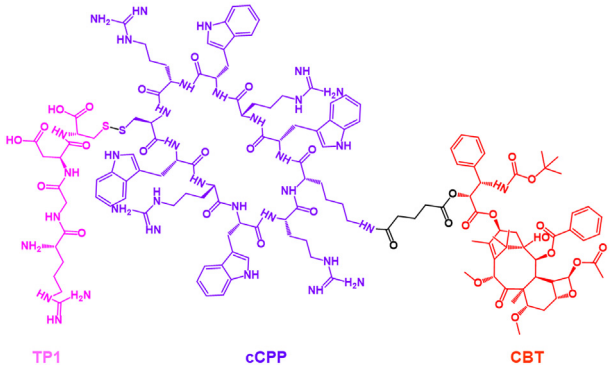


Figure 2 The structure of TP1-cCPP-CBT, in which integrin-targeting peptides TP1 improve targeting ability and cCPP enhance cell penetration ability as a cyclic cell-penetrating peptides, and CBT exert therapeutic effects.

triple-negative breast cancer (mTNBC). Firstly, TPD1 triggers the tumor cell-specific initial apoptosis and induces the expression of caspase-3 in the target tumor site by selectively targeting integrin $\alpha v\beta 3$ overexpressing cancer cells in metastatic tumor tissues *via* RGD peptide. Then activated by upregulated caspase-3 at the metastatic tumor site, MPD1 releases the cytotoxic drug DOX, which exhibits bystander-killing effects on neighboring cancer cells and further enlarges *in situ* apoptosis and increases tumor caspase-3 levels, resulting in recurrent activation of MPD1. Results showed an effective anti-tumor activity in the mTNBC mouse model. Based on the RGD sequence, the researchers further investigated to explore other derived linear or cyclic peptides with greater targeting capability. Feni et al.[62] combined CPP (sC18) with an integrin-targeting cyclic peptide (c[DKP-f3-RGD]) for delivery of the antitumor drug daunorubicin (DAU). The experiment results revealed that the toxic effects were more apparent in U87 cells expressing $\alpha v\beta 3$ in comparison to HT-29 and MCF-7 cells, thus demonstrating the targeting role of cyclic peptide (c[DKP-f3-RGD]) in PDC.

2.2.2. *Gonadotropin-releasing hormone (GnRH) peptides*

Gonadotropin-releasing hormone (GnRH), also called luteinizing hormone-releasing hormone (LHRH), has the primary role of stimulating the production and release of gonadotropins in adult men and women by specifically interacting with GnRH receptors on the surface of the anterior pituitary gonadotropic cells[63]. GnRH receptors have been found to be highly expressed in the pituitary gland, and also on the surface of tumor cells in some reproductive-related cancers (breast, prostate and ovarian cancers) and non-reproductive cancers (colon and lung cancers)[64]. Therefore, the construction of PDCs targeting GnRH peptides has attracted extensive attention. Schuster et al.[65] structured eight cleavable and four non-cleavable PDCs with the targeting peptide GnRH-III to deliver two chemotherapeutic agents with distinct action modes (paclitaxel and daunorubicin). Cellular anti-proliferative activity assays showed that A2780 ovarian cancer cells expressing high levels of GnRH receptors were growth inhibited by GnRH-III bioconjugates, while Panc-1 pancreatic cancer cells with low levels of GnRH receptors showed reduced activity. In addition, some analogues of GnRH can be employed as targeting peptides for the construction of PDC. Li et al.[66] combined three GnRH analogues [D-Cys^6, desGly^{10}, Pro^9-NH$_2$]:-GnRH [D-Cys^6, desGly^{10}, Pro^9-NHEt], -GnRH and [D-Cys^6, α-aza-Gly^{10}-NH$_2$]-GnRH with DOX to constitute three new GnRH-DOX conjugates I, II and III, respectively, through the combination of *N*-succinimidyl-3-maleimidopropionate. The results showed that all three conjugates displayed superior metabolic stability in human serum. Compared with free DOX, conjugate II exhibited reduced cytotoxicity to embryonic fibroblasts of GnRH receptor-negative 3T3 mice. In the tumor anti-proliferation assay, conjugate II showed a higher anti-proliferative effect on MCF-7 cells than conjugate I and conjugate III. Consequently, the targeting strategy of conjugate II is prospective for the chemotherapy of tumors over-expressing GnRH receptors.

2.2.3. *Somatostatin (SST) mimetic peptides*

Somatostatin receptor is a G protein-coupled cell surface receptor widely distributed in various tumor and angiogenic tumor vessels[67]. Somatostatin and its analogs quickly internalize into the cell and possibly translocate to the nucleus after binding to the receptor. Therefore, peptides based on somatostatin receptor

Table 2 Cell-targeting peptide used in PDCs.

Target	Sequences/code name	Payload	Disease	Ref.
Synovial endothelium	CLRDHTSKC-NH	Sinomenine	Rheumatoid arthritis	49
Sortilin (SORT1)	TH19P01	Docetaxel	Epithelial ovarian cancer	50
Human epidermal growth factor receptor 2	Cyclo-GCG-RIKPRKGYTR	Camptothecin	Cancer	51
Fibroblast growth factor receptors	LLC2B	A maytansin derivative (DM1)	Cancer	52
Chemokine-like receptor 1 (CMKLR1)	YFPGQFAFS	Methotrexate (MTX)	Cancer	53
Vascular endothelial growth factor (VEGF)	CAAELAALEAELAALEGPWKGY PIPYGKLQFLIKKLKQLKVAC	Monomethyl auristatin E	Cancer	54
PC-3 tumors	LSNNNLR	Chlorambucil, Combretastatinor Camptothecin	Metastatic castration-resistant prostate cancer (mCRPC)	55
Somatostatin receptor type II (SSTR2)	RGD	Valproic acid and camptothecin	Pancreatic carcinoid tumor	56
Bone tissue	DDDDDD	Matrine derivative M19	Osteoporosis	57
Neuropeptide Y1 receptor (NPY1R)	[F^7, P^{34}]-NPY	Tesaglitazar	Type 2 diabetes and dyslipidemia	58
Transferrin receptors	CAHLHNRS	Doxorubicin	Cancer	59

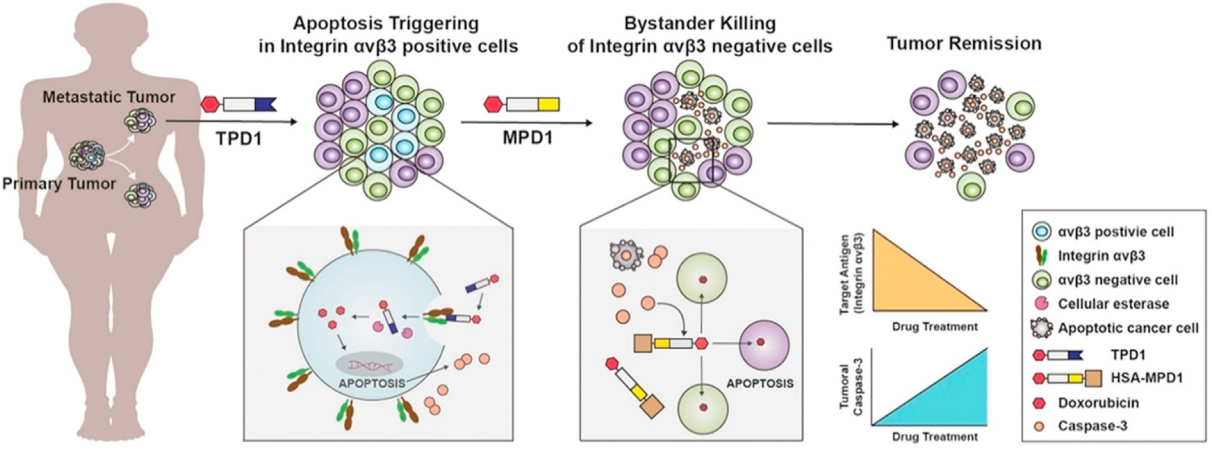


Figure 3 The schematic mode of a novel targeted chemotherapy strategy termed 'switch therapy' with two different caspase-3 activatable PDCs (TPD1 and MPD1) to treat mTNBC. (Reprinted with the permission from Ref. 61. Copyright © 2022 The Authors. Published by Elsevier B.V.)

(SSTR) and its analogs have great potential for the construction of PDCs. Redko et al.[68] reported five PDCs with SSTR2-specific backbone cyclic peptide 3207-86. Results showed that the free drug exhibited non-specific cytotoxicity to cancer cells and normal cells, while PDC exerted different cytotoxicity in different tumor cells, with no effects on negative control HEK cells. Therefore, the backbone cyclic SSTR2 peptide analogue 3207-86 is promising for constructing PDCs targeting solid malignant tumors. Similarly, Pryyma et al.[69] selected the octreotate (TATE-N_3), a circulation-stable octapeptide somatostatin analog agonizing somatostatin receptors, and constructed three peptide–drug conjugates by coupling them with amanitin. In the cell-based assay on target-positive Ar42J cells, the target activity of conjugates was reduced and the biological activity was increased by 1000-fold over the untargeted amatoxins[69].

2.3. *Self-assembling peptides (SAPs)*

Self-assembled peptides refer to peptides that selectively and spontaneously form one or more ordered structures from complex mixtures through non-covalent interactions (including van der Waals forces, electrostatic interactions, hydrogen bonding and stacking interactions)[70,71] without external stimulation. As basic component units of peptides, the types and arrangements of amino acids are the basis for the assembly capability of self-assembled peptides. In addition, the focus of designing self-assembled peptides is to exploit multiple intermolecular interactions, including hydrogen bonding, electrostatic interactions, π−π stacking, van der Waals forces, dispersion forces, metal−ligand complexes and entropic forces[72]. Peptides with self-assembly ability tend to be amphiphilic, aromatic, charge dispersed, and alternating arrangements of different and symmetrical charges. The main advantages of SAPs compared to common peptides are biocompatibility, biodegradability and multi-functionality. With the advantages of SAPs, Zhang et al.[73] designed a typical cationic anticancer peptide, R-lycosin-I, full of arginine positively charged and amphiphilic in nature, which can self-assemble into nanostructures in water. Based on this, an R-L-HCPT conjugate was formed by covalent coupling of R-lycosin-I with HCPT (DNA topoisomerase I inhibitor) *via* a glutamate anhydride junction, capable of

spontaneously forming nanoparticles with particle sizes of 40−60 nm in aqueous solution (Fig. 4). In relation to free HCPT or R-lycosin-I, R-L-HCPT nanoparticles exhibit superior anti-tumor growth and metastatic activity *in vitro* and *in vivo*. Apart from this, self-assembling peptides are also used in the preparation of hydrogels. Yang et al.[74] synthesized CRB-FFE-YSV based on two antitumor drugs: the small molecule drug chlorambucil (CRB) and tyroservatide (YSV), a peptide drug made up of L-tyrosine, L-serine, and L-valine. The monomer of CRB-FFE-YSV was conveniently converted into hydrogel structured with nanofiber during the heating-cooling process and has higher stability under enzymatic conditions. The experimental results showed that CRB-FFE-YSV could successfully enter tumor cells and the proliferation of tumor cells can be effectively inhibited. In addition, the CRB-FFE-YSV hydrogel expressed higher anti-tumor activity and excellent biocompatibility *in vivo* compared to free YSV, free CRB and mixed drugs.

2.4. *Responsive peptides*

Responsive peptides are peptides that undergo structural changes in response to external stimuli. Such changes occur at the structural level rather than simply linker breakage, resulting in triggered self-assembly or a switch of formulation type that facilitates drug delivery and release. Unlike self-assembled peptides whose self-assembly behavior is formed spontaneously, responsive peptides require external environmental stimuli to undergo structural changes. Usually, external environmental stimuli include temperature, pH, enzymes, etc. In general, when tissue or organ lesions occur, the physiological environment involved also changes. Take tumors as an example, on account of the excessive proliferation of tumor cells and incomplete development of vascular structure, tumor tissues are normally in a hypoxic environment, resulting in changes in their metabolic processes. In contrast to the aerobic metabolism of normal tissues, the metabolism of tumor tissues relies more on anaerobic glycolysis, and its metabolites, lactic acid and CO_2 from respiration, together cause the acidification of the tumor microenvironment. The weakly acidic nature of the tumor microenvironment has also become a new approach to targeting tumors. In addition, the unique microenvironmental characteristics of tumor sites accumulate some specific enzymes to provide thoughts for tumor targeting. In recent years, the construction of PDCs based on responsive peptides has attracted the interest of many researchers.

2.4.1. *Temperature responsive peptides*

Temperature responsive peptides are peptides that undergo changes in structure and properties at different temperatures. Some studies have reported a tumor treatment method called hyperthermia, in which heating the tumor tissue locally can

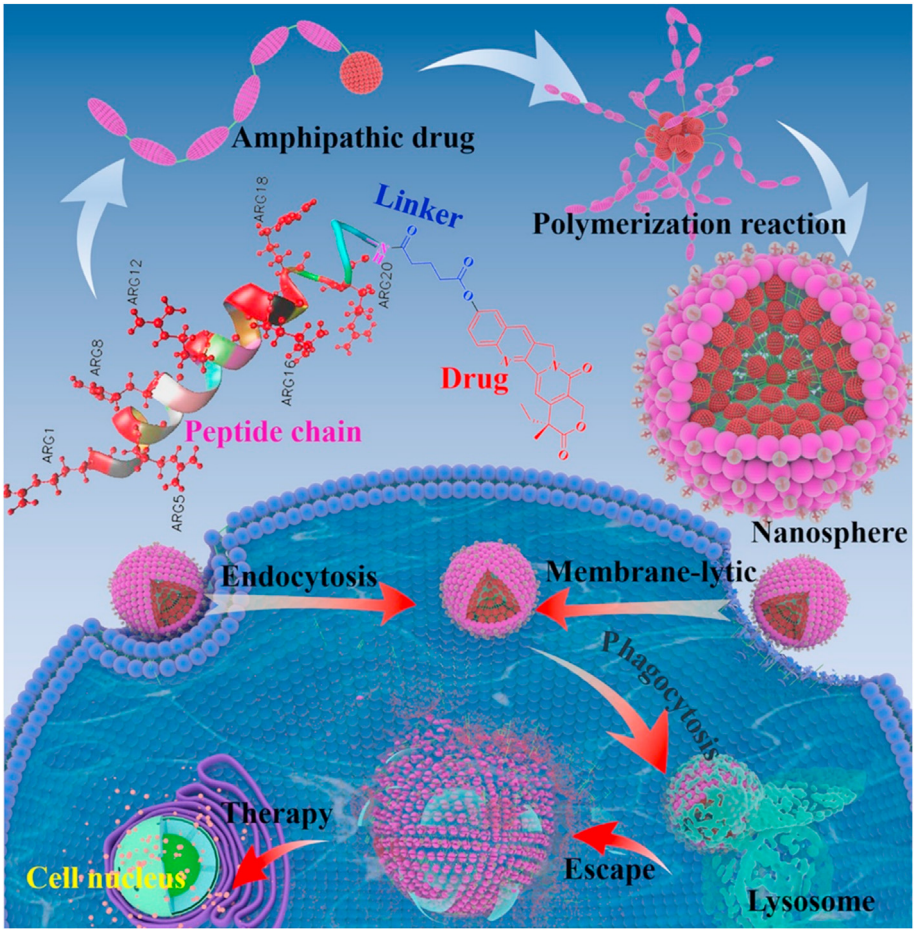


Figure 4 Chemical structure of the R-L-HCPT conjugates and a schematic illustration of the formation of R-L-HCPT nanospheres by self-assembly and the mechanism of transmembrane and exerting therapeutic effects. (Reprinted with the permission from Ref. 73. Copyright © 2020, American Chemical Society.)

increase the vascular permeability at the tumor, thus enhancing drug delivery to solid tumors[75]. The use of hyperthermia in combination with PDC using temperature-responsive peptides enhances the killing effect on tumor cells. Elastin-like proteins (ELPs), a pentapeptide repeat unit (Val-Pro-Gly-Xaa-Gly, VPGXG) synthesized by Urry et al.[76], have specific temperature response. ELPs exhibit reversible phase transition behavior with temperature, exhibiting the soluble state at temperatures below their phase transition temperature T_t, and the insoluble aggregated state at temperatures above their T_t[77]. Based on this property of ELPs, Wang et al.[78] formed spherical micelles (IFNα-ELP$_{diblock}$) consisting of diblock copolymers by heat-induced self-assembly of interferon α and elastin (Fig. 5), which could significantly improve the protein hydrolytic stability of IFNα, and achieve a 124.3-fold longer circulating half-life of IFNα-ELP$_{diblock}$ micelles (54.7 h) than free IFNα (0.44 h). In addition to the heat-induced self-assembly of ELPs, the researchers also explored other methods. Abdelghani et al.[79] described an elastin-like polypeptide (ELP) diblock copolymer with multi-responsive assembly properties. Two methods were applied to make them self-assemble into micelles: one method was to trigger the self-assembly by adding divalent metal ions where it was found that Zn^{2+} was the most favorable ion. Also, the increasing concentration of Zn^{2+} could stabilize the nanoparticles within a wide temperature window (4−45 °C) and the copolymers exhibited pH responsiveness. Another method does not require the addition of metal ions but triggers co-assembly by rapidly heating the ELP above the transition temperature to produce stable nanoparticles. This new ELP system provides a multifunctional, modular nanocarrier platform that can adapt efficiently in response to different stimuli.

2.4.2. *The pH responsive peptides*

The pH-responsive peptides are peptides that differ in structure and properties as the pH of the environment varies. Some diseases can experience low pH at the site of the lesion, such as ischemia, arthritis, atherosclerosis, and tumors[80]. Since some peptides contain charged amino acids, they can respond to changes in pH, thus providing a new strategy for constructing PDCs. pHLIP is a peptide that undergoes conformational transformation under acidic conditions to enter cells through the cell membrane. pHLIP shows three different states under different pH conditions (Fig. 6): (1) at neutral and high pH conditions in the absence of lipid bilayers, pHLIP displays a water-soluble state (state 1); (2) at neutral and high pH conditions in the existence of lipid bilayers, most of pHLIP is bound to the membrane surface in a non-structured configuration (state 2); (3) in an acidic environment with a lipid bilayer, pHLIP is inserted across the cell membrane in the form of an α-helix (state 3). Song et al.[81] linked pHLIP with doxorubicin *via* disulfide bonds to form pHLIP-SS-DOX, a conjugate that is expected to reverse the phenomenon of tumor multidrug resistance (MDR) due to the unique trans-cellular pattern of pHLIP. In the cellular uptake study, pHLIP-SS-DOX

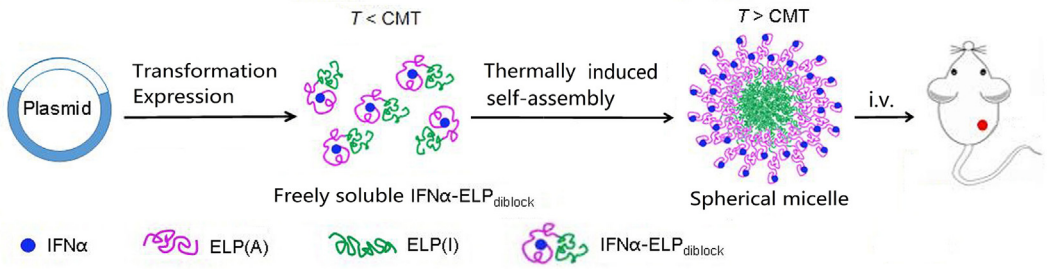


Figure 5 The schematic illustration of thermally induced self-assembly of IFNα-ELPdiblock into a spherical micelle. (Reprinted with the permission from Ref. 78. Copyright © 2020 Elsevier B.V.)

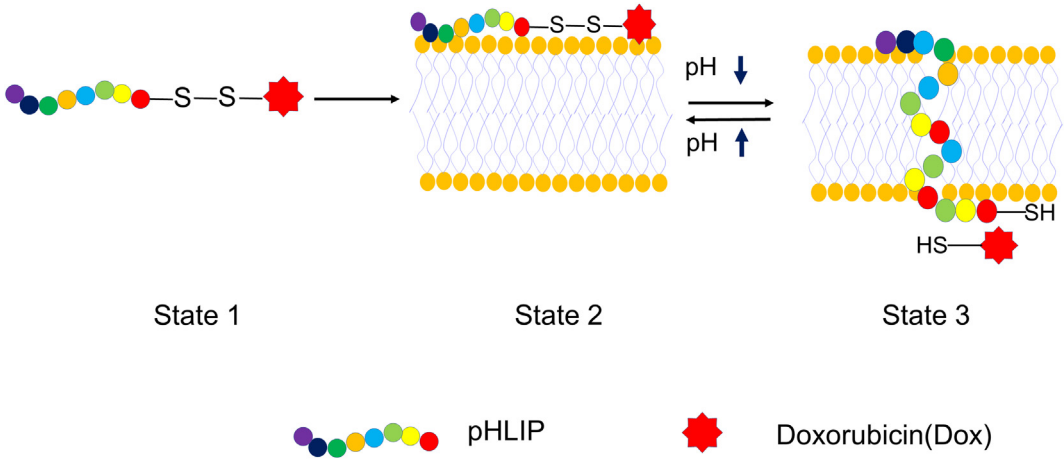


Figure 6 Three statements of pHLIP-SS-DOX under different pH conditions. State 1: In the absence of lipid bilayers, pHLIP displays a water-soluble state; State 2: At neutral and high pH conditions in the existence of lipid bilayers, pHLIP is bound to the membrane surface in a non-structured configuration; State 3: In an acidic environment with a lipid bilayer, pHLIP is inserted across the cell membrane in the form of an α-helix.

had similar levels of cellular uptake in drug-sensitive MCF-7 cells and drug-resistant MCF-7/Adr cells at pH 6.0, illustrating the ability of pHLIP-SS-DOX in targeting acidic tumor cells and reversing MDR. To further explore pHLIP, Burns et al.[82] used six pHLIP variants conjugated to a potent mitotic inhibitor Monomethyl auristatin F (MMAF). In spite of the fact that all PDCs exhibited significant pH-selective and concentration-dependent toxicity to tumor cells, their cytotoxicity profiles were remarkably different, with pHLIP(WT)-MMAF (AAEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG) exhibiting the strongest effect.

2.4.3. *Enzyme responsive peptides*

Enzyme responsive peptide is one of the more commonly used environmentally responsive peptides, whose application in drug delivery can be influenced by enzymes, thus it is often used to construct PDCs. Studies[83] have shown that a variety of specific enzymes appear and accumulate at the site of the lesion, among them are matrix metalloproteinases (MMPs), a group that has proven to play an important role in the development of malignant tumors. Based on this phenomenon, researchers have prepared a variety of peptide sequences that respond to MMPs. Ji et al.[84] conceived and prepared a self-assembled amphiphilic peptide drug conjugate (SAAPDC) 'two-in-one' nanofiber system (Fig. 7). They coupled doxorubicin with a β-amyloid-derived peptide oligopeptide (KGFRWR) to form an amphiphilic drug coupling, which behaves as an easily flowable liquid at room temperature and forms nanofibers in the lesion area upon administration. Then the prodrug inhibits the migration of MMPs and the proliferation of hepatocellular carcinoma with slow release from the nanofibers mediated by the diffusion and degradation of amphiphilic peptide drug conjugates. As a consequence, the nanofibers formed by self-assembly of DOX-KGFRWR exhibited specific response and inhibitory activity towards MMP-2 and MMP-9 enzymes. Similarly, a matrix metalloproteinase-2 (MMP-2) responsive chimeric peptide was designed by Zhang et al.[85] that could self-assemble into spherical nanoparticles when exposed to physiological environments, which can accumulate at tumor sites through the EPR effect. The chimeric peptides are specifically hydrolyzed by MMP-2 enzymes overexpressed in the tumor region, contributing to the spheroid-to-fiber conversion. And this conversion could enhance the accumulation and relaxation of contrast at the tumor site for dual-stage-amplified MRI and precise photodynamic therapy.

3. Linkers in PDCs

As the connecting bridge between drugs and peptides in PDCs, linkers determine the circulation time and stability of PDCs *in vivo*. The ideal linker should remain stable in circulation to avoid drug release prematurely while being able to release the drug rapidly and efficiently once it reaches the focal tissue. Meanwhile, the linker should have no influence on the affinity of the peptide towards its receptor and the activity of the drug[86]. Additionally, the synthesis process of linkers with peptides and drugs should be as simple as possible and remain stable throughout the synthesis process. The hydrophobicity of the linker should not be too strong to prevent PDCs from aggregating due to hydrophobicity, resulting in poor stability and reduced efficacy

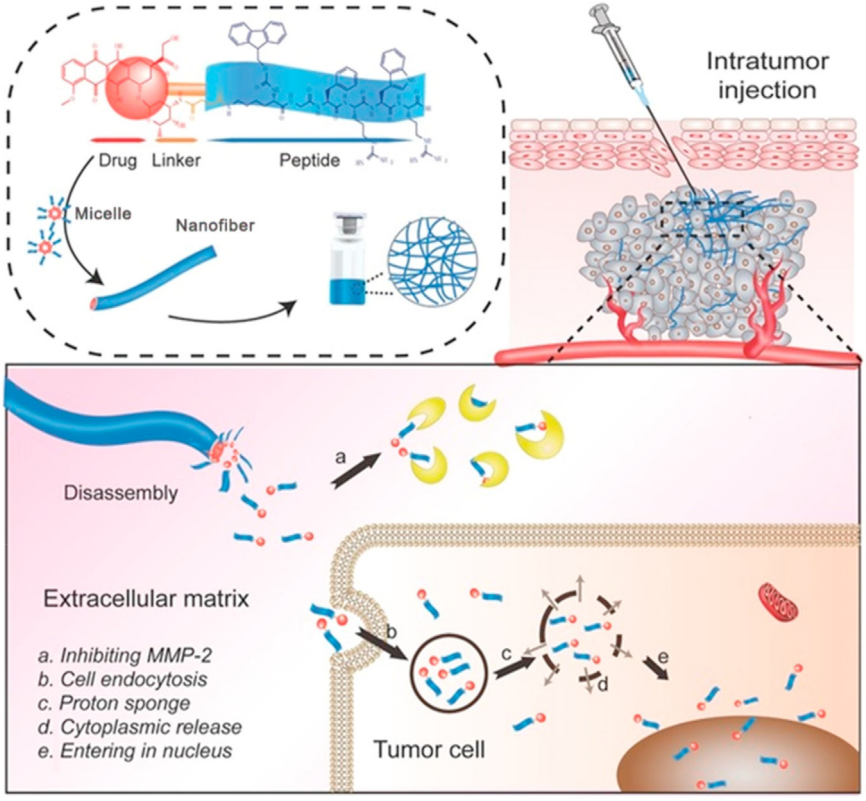


Figure 7 Schematic fabrication of multifunctional SAAPDC "two-in-one" nanofiber systems and disassembly in the presence of MMP-2 enzyme. (Reprinted with the permission from Ref. 84. Copyright © 2018 The Authors. Published by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.)

in vivo, as well as strong systemic toxicity and immune side effects[87]. According to the drug release mechanism and the cleavage behavior of linkers, linkers are mainly classified into non-cleavable linkers and cleavable linkers, and the latter includes pH-sensitive linkers, redox-sensitive linkers and enzyme-sensitive linkers according to the cleavage mechanism. Table 3[88–104] lists the specific classification of linkers and their structures.

3.1. *Non-cleavable linkers*

As the name suggests, the non-cleavable linkers refer to the linkers that are relatively stable and do not break during blood circulation. The greatest advantages of non-cleavable linkers over cleavable linkers are plasma stability, lower off-target toxicity, larger therapeutic window, and better drug resistance[105]. Non-breakable linkers are generally not responded to external stimuli, but release drugs after peptide metabolism. Hence, non-breakable linkers can remain stable in blood circulation before reaching the target site. Common non-cleavable linkers used to build PDCs include oxime bonds and thioethers. Randelovic et al.[88] formed GnRH-III-Dau conjugates by combining GnRH-III and its variants with the anticancer drug daunorubicin (Dau) using oxime bonds. In the three models of 4T1 mice, BALB/c mice and SCID mice with MDA-MB-231 human breast cancer and HT-29 human colorectal cancer, the GnRH-III-Dau conjugate displayed less toxicity than the free drug, indicating that the oxime bond is stable in the circulation. To better demonstrate the stability of thioether bonds in normal physiological environments, Liang et al.[90] designed three PDCs: RSSDOX, RSDOX and RVCDOX, with doxorubicin (DOX) as a model cytotoxic agent, cRGD as homing peptide and redox-sensitive cleavable disulfide (SS), non-cleavable thioether (S), and cathepsin B cleavable valine citrulline dipeptide (VC) as linkers respectively. From the drug release curves, it can be seen that the single thioether-linked RSDOX exhibited sustained and slow release behavior and there was no remarkable change in the drug release curve of RSDOX after the addition of a small molecule reducing agent (DTT), thus showing the relative stability of the thioether bond. Therefore, peptide–drug conjugates using such linkers usually do not cleave the linker before reaching the target tissue and do not affect the cytotoxic effect of the drug[106]. Certainly, the choice between non-cleavable and cleavable linkers depends on the design purpose of the delivery system and the properties of the drug.

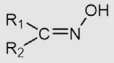
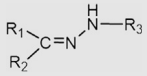
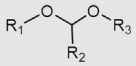
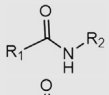
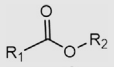
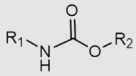
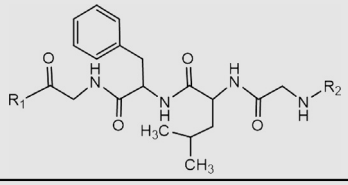
3.2. *Cleavable linkers*

Cleavable linkers are the most commonly used linkers in PDC construction, which can be cleaved in response to the physiological environment of the body or in the presence of enzymes to release drugs. Compared with non-cleavable linkers, the biggest advantage of these PDCs is that they can break specifically in the target tissue and release the drug rapidly to reach therapeutic concentration, reducing the toxic side effects to other tissues or organs which truly achieves the concept of intelligent drug delivery.

3.2.1. *The pH-sensitive linkers*

The pH-sensitive linkers are generally acid-sensitive linkers, which means that under acidic environments, the linker breaks to release the drug. As mentioned above, the tumor microenvironment is a weak acid environment (pH 6.5–6.9), while the pH of normal blood is in the range of 7.2–7.4. Taking advantage of this difference, researchers can design a variety of linkers that are stable in the blood circulation but break quickly under the acidic circumstances of the tumor microenvironment to achieve controlled release of drugs[107].

Table 3 The classification and structure of linkers.

Linker type	Name	Structure	Cleavage condition	Ref.
Non-cleavage	Oxime		Stable in the blood circulation and release drugs after peptide metabolism	88,89
	Thioether			90,91
Cleavage				
pH-sensitive	Hydrazone bond		Breaks under acidic environments	92,93
	Acetal bond			94
Redox-sensitive	Disulfide bond		Breaks in the presence of GSH	95,96
Enzyme-sensitive	Amide bond		Breaks in the presence of amidases	97,98
	Ester bond		Breaks in the presence of esterase	99,100
	Carbamate bond		Breaks in the presence of carbamate hydrolase	101,102
	GFLG		Breaks in the presence of proteases	103,104

Currently, the most widely studied pH-sensitive linker is the hydrazone bond[108]. Saghaeidehkordi et al.[92] designed **18-4**, a peptide that targets the keratin 1 (K1) receptor, a cell surface receptor on breast cancer cells, and linked it to doxorubicin through a pH-sensitive hydrazone bridge to constitute a PDC to investigate its efficacy against triple-negative breast cancer. The results showed that PDC-treated mice showed increased (1.4-fold) DOX in tumors and reduced (1.3−2.2-fold) DOX in other organs. As such, the PDC-treated mice showed significantly increased antitumor efficacy and reduced off-target toxicity in comparison to free DOX or saline-treated mice. Although hydrazone bonds are widely used in the construction of PDCs, their poor stability in blood circulation can have certain side effects. This linker may degrade slowly in the blood circulation, leading to premature release of the drug, thereby reducing its effectiveness and causing damage to other organ tissues. Therefore, there is a possibility to promote the stability of PDCs through the adoption of many chemical bonds that are similar to hydrazone bonds in the latter study.

Acetal is another very promising pH-sensitive linker, and studies have shown that it is expected that for every unit decrease in pH, the rate of acetal bond breakage is accelerated by 10 folds. Gillies et al.[109] designed four conjugates using different chemical structures of acetal to link model drug molecules to PEO. The kinetics of hydrolysis was studied by HPLC, and the half-life of the conjugates at pH 5.0 ranged from less than 1 min to several days, while in all cases the hydrolysis at pH 7.4 was slowest. Thus, it is expected that acetals will be used to construct pH-sensitive PDCs.

3.2.2. *Redox-sensitive linkers*

Glutathione (GSH) is a reducing agent found in the cytoplasm, and its intracellular concentration is roughly 1000 times higher than its extracellular concentration[110]. In tumors, the abnormal blood flow status causes a hypoxic environment, leading to increased activity of reductase, which increases the concentration of GSH. Due to its antioxidant properties, GSH can break a range of chemical bonds available for incorporation into junctions, such as disulfide bonds, thioesters, diselenides, and metal thiol junctions[16]. Among them, disulfide bonds are very stable in blood circulation and have been extensively applied in the construction of PDC drugs. Wu et al.[95] designed two PDCs, RGD-VC-CA and RGD-SS-CA, which consist of a tumor-homing peptide and a redox-sensitive cleavable disulfide linker or an enzyme-responsive dipeptide linker, respectively. Results demonstrated the superiority of RGD-SS-CA compared with RGD-VC-CA in drug release and cytotoxicity assays *in vitro*. In addition, it was found that RGD-SS-CA could significantly inhibit tumor growth after being injected intravenously into tumor-bearing mice *in vivo* anti-tumor assay. Liang et al.[96] synthesized amphiphilic peptide−drug conjugates (APDCs), utilizing the hydrophilic cRGD peptide as a homing peptide, and the hydrophobic drug medenosine (DM1) as a cytotoxic agent, with redox-responsive disulfide bonds (RSSD) and unbreakable thioether bonds (RCCD) as linkers, respectively. The APDC formed nanoparticles (APDC@NPs) by self-assembly, then were taken up by tumor cells *via* endocytosis mediated by $\alpha v\beta 3$ receptors through the accumulation of EPR effects at the tumor site, triggering intracellular drug release from APDC@NPs (Fig. 8A). There was a significant acceleration of DM1 release from RSSD@NPs after the addition of the reducing agent dithiothreitol (DTT), increasing from 27.0% to 78.6% within 48 h, as indicated by the drug release curves (Fig. 8B). Even without the reducing agent, approximately 20% of the drug was released within the same time. Conversely, the percentage of the cumulative release of RCCD@NPs kept below 4.0% with or without the presence of reducing agents. Thus, it can be expected that the mechanism of reduction-responsive release designed on the basis of disulfide bond breaking (Fig. 8C) can accelerate the release of the drug at the target site.

3.2.3. *Enzyme-sensitive linkers*

Enzyme-sensitive linkers are defined as linkers that cleave and release drugs in the presence of specific enzymes, generally including chemical linkers and specific peptide sequence linkers. The chemical links represented by ester bonds, amide bonds and carbamates have been extensively adopted for the construction of PDCs. The nuclear endosomes and lysosomes of tumor cells are enriched with esterases and amidases, so the application of ester and amide bonds as linkers is very popular in the construction of targeted tumor PDCs. Gilad et al.[99] constructed a series of tumor-targeting peptide−drug conjugates by modifying the cyclic RGDfK pentapeptide to form amide and ester bonds by coupling chemical therapeutic drugs chlorambucil (CLB) or camptothecin (CPT) with primary amines on the peptide molecule, respectively. Böhme et al.[97] constructed PDCs by linking neuropeptide Y (NPY) analogs to methotrexate using amide bonds, and their antitumor activity depended on the amount of methotrexate linked by amide bonds. Besides, carbamate is another commonly used enzyme-sensitive chemical linker. The aliphatic amine-derived carbamate is sufficiently stable and can be hydrolyzed at a suitable rate to release the active drug[111,112]. Interestingly, carbamates are often known as trace-free linkers because upon their hydrolysis, CO_2 and the starting amine, as well as alcohol, are the only by-products generated[113,114]. To verify the effect of enzymes on drug release in PDCs containing enzyme-sensitive linkers, Sayyad et al.[101] compared indicators of stability and drug release of GnRH peptide-gemcitabine conjugates consisting of a carbamate as a linker or two amide bonds and an ester bond as a linker. They found that PDCs with two amide bonds and one ester bond as linkers released drugs at a greater rate than PDCs with carbamate as a linker as they are prone to the esterases and amidases present in high concentrations at the tumor site. The potential of these chemical linkers in the construction of PDCs is well illustrated by the use of ester bonds, amide bonds and carbamates for the selective release of drugs to tumors.

In comparison to chemical linkers, peptide sequences that can be specifically degraded by enzymes are more representative of enzyme-sensitive linkers. Unlike enzyme-responsive peptides in the peptides section that change the structure of PDCs in response to enzymes, peptides that act as enzyme-sensitive linkers can break and release the parent drug under the action of specific enzymes. Due to the presence of serum protease inhibitors and environmental pH, some proteases are usually inactive outside the cell and therefore the peptide sequence is more stable in the circulation[115]. In contrast, tumor cells contain abundant proteases in the nuclear endosomes and lysosomes that can hydrolyze peptide bonds, the most representative of which is cathepsin B that can hydrolyze Gly-Phe-Leu-Gly (GFLG). Petho et al.[103] linked the epidermal growth factor receptor (EGFR) targeting peptide GE11-D4 to daunomycin (DAU) *via* a GFLG peptide to ensure active drug release in human colon cancer HT-29 cells. Matrix metalloproteinases (MMP) are another enzyme with high concentrations in tumor cells, where overexpression of MMP2 and MMP9 is essential in metastatic tumor cells, facilitating tumor development

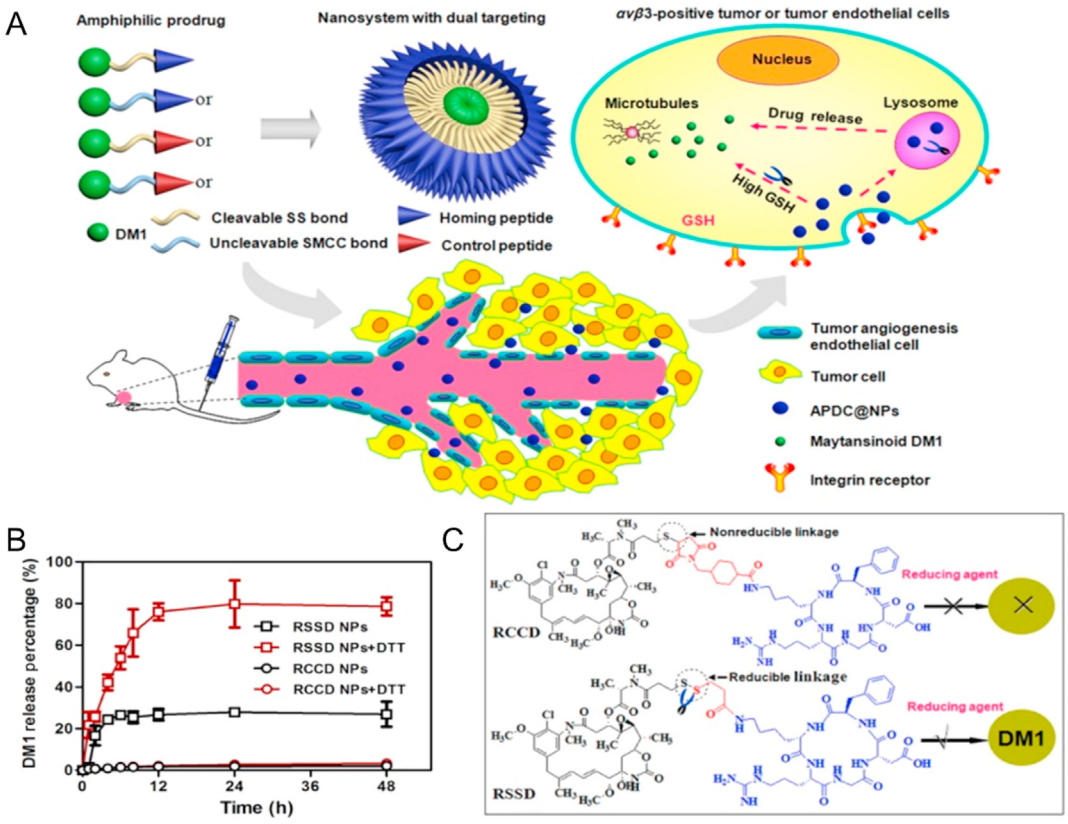


Figure 8 (A) Schematic illustrations of the self-assembly of APDC nanoparticles (APDC@NPs) and mechanism of cell entry. (B) Reduction-triggered drug release from the active targeting RCCD@NPs and RSSD@NPs with or without the presence of reducing agent DTT. (C) Reduction-based drug release mechanisms of thioether-linked and disulfide-linked APDC@NPs. (Reprinted with the permission from Ref. 96. Copyright © Ivyspring International Publisher.)

and angiogenesis, thus supplying nutrition to the tumor from newly born blood vessels[116]. With the high expression of MMP in tumors, Huang et al.[117] combined paclitaxel with a hexapeptide specifically acknowledged by MMP2 protein, and MMP2-specific protein hydrolysis of the conjugate occurred at the COOH terminus of the hexapeptide PVGLIG to release the drug. It was demonstrated that PVGLIG-paclitaxel conjugate dramatically strengthened the tumor specificity against HT-1080 and U87-MG tumor cells.

4. Drugs in PDCs

The payload of PDCs are drugs that exert cytotoxic or therapeutic effects, and most of these drugs have disadvantages including low water solubility, poor selectivity, short half-life and poor stability, which limit their clinical application[118]. Drugs that can be delivered using the PDCs strategy need to have *via*ble attachment sites. Also, the drugs ought to be free of pharmacological activity in the conjugated form, and when released in the focal tissue, they should go on to exert therapeutic effects with a clear mechanism of action and have strong pharmacological activity. After coupling with peptides, it can enhance the solubility of the drug, promote the drug selectivity, lengthen the circulation time in the body, optimize the bioavailability and prevent the side effects and toxicity of the drugs to other tissues. The drugs applied in PDC construction can be classified as chemical drugs, protein drugs and peptide drugs.

4.1. *Chemical drugs*

4.1.1. *Doxorubicin*

Doxorubicin (DOX) is a classical antitumor drug that enters cells by free diffusion and forms DOX-protease complexes with high affinity to proteases. Then DOX is carried into the nucleus through the protease and binds to topoisomerase to inhibit the synthesis of double-stranded DNA, leading to cell death. As a broad-spectrum antitumor drug, DOX has greater clinical application prospects. However, the side effects of nausea, vomiting, diarrhea and cardiotoxicity together with the multidrug resistance of tumors to DOX limit the clinical application of DOX[119]. Hence, Ziaei et al.[120] designed two PDCs formed by coupling a cell-targeting peptide of breast cancer (WxEAAYQrFL) to DOX with a succinimidyl thioether bond and a hydrazone linker, respectively. Both PDCs displayed similar toxicity to free drugs in cytotoxicity assays for triple-negative breast cancer cells but lower toxicity than free drugs for non-breast cancer cells. Consequently, the constitution of PDC enhances the selectivity of DOX for tumors and thus reduces cardiotoxicity. To overcome the multidrug resistance of tumors to DOX, the membrane-penetrating peptide TAT was linked to DOX *via* the enzyme cleavable junction GFLG to form PDC by Zhang et al.[121] The cell survival rate of this conjugate at a concentration of 50 μmol/L was 15% and 14% on drug-sensitive KB-3-1 cells and drug-resistant KB-V1 cells,

respectively, which suggested that this PDC could overcome the multidrug resistance of tumors to DOX to a certain extent.

4.1.2. Paclitaxel

Paclitaxel (PTX) is a cytotoxic drug extracted from *Taxus* and enables interaction with β-microtubule proteins, thereby causing the aggregation of microtubules causing cell cycle cessation in the G2/M phase and inducing apoptosis. PTX has shown superior therapeutic effects on cancers including breast, ovarian, esophageal and lung cancers. However, paclitaxel is poorly water-soluble, with difficulty in delivery to the body, and suffers from poor selectivity *in vivo* leading to off-target toxicity. Meanwhile, the P-glycoprotein-mediated efflux makes PTX more susceptible to multidrug resistance as compared to other chemotherapeutic agents, which can cause multiple adverse effects in the clinic, in terms of hematotoxicity, allergic reactions and neurotoxicity[122]. To improve the water solubility and targeting of PTX, Hua et al.[123] screened the CPP peptide sequence SynB3 and coupled it to PTX *via* an MMP-2 -sensitive linker (PVGLIG) to constitute a PDC capable of penetrating the BBB for targeted glioma release. As the result showed, SynB3-PVGLIG-PTX increased the PTX water solubility by forming a positively charged specific structure, and it could possess specific cytotoxicity with controlled release at glioma under specific hydrolysis by MMP-2 enzyme. Compared to free PTX, SynB3-PVGLIG-PTX efficiently inhibited the proliferation, migration and invasion of GBM cells both internally and externally.

4.1.3. Other chemical drugs

Camptothecin (CPT), methotrexate (MTX), cisplatin (Pt) and cytarabine are other chemical drugs that are frequently used in the construction of PDCs. Camptothecin is derived from *Camptotheca acuminata*, a natural product that induces apoptosis through the inhibition of type I DNA topoisomerase. Chandna et al.[124] used a polyethylene glycol (PEG) polymeric carrier to link the anticancer drug camptothecin (CPT) with the tumor-targeting peptide LRHR and a cellular anti-apoptosis inhibitor (BH3 peptide) *via* a citric acid spacer to form a polymer−peptide−drug conjugate (PPDC) capable of exhibiting high activity against tumors in primary and metastatic cancers. The target of methotrexate is dihydrofolate reductase, which inhibits the metabolism of dihydrofolate by binding to it resulting in the interruption of pyrimidine and purine nucleotide synthesis and thus the inhibition of DNA biosynthesis[125]. Böhme et al.[126] utilized amides, esters, disulfide bonds, GFLG and novel disulfide-ester junctions to link neuropeptides targeting the human Y1 receptor to methotrexate to form PDCs as a means of overcoming the side effects of methotrexate. Cisplatin can bind to bases in the cell and change the chemical structure of DNA, thus affecting DNA replication. Conibear et al.[127] designed Oxali-Pt-Y-1, a specific PDC consisting of both cisplatin *via* thiol-maleimide and two β6 integrin-targeting peptides (P1) *via* CuAAC attached to a core peptide-PEG27 scaffold, with the promise of greatly enhancing the specificity of cancer treatment and imaging. In addition, some intestinal transporters can be fully utilized to enhance the absorption of oral PDC drugs, such as oligopeptide transporter 1, which plays an important role in the oral absorption of dipeptides and tripeptides from proteins[128]. Based on this, Sun et al.[129] connected an oligopeptide amino acid ester derivative with cytarabine to constitute a PDC, which increased the oral absolute bioavailability of cytarabine from 21.8% to 60.0% mediated by oligopeptide transporter 1, providing a new idea for the oral administration of PDC drugs.

4.2. Protein drugs

Apart from chemical drugs, protein-based drugs are gradually gaining popularity. Protein-based drugs represented by interferon and tumor necrosis factor can achieve effective anti-tumor therapeutic effects by inducing apoptosis and inhibiting intracellular protein synthesis. However, protein-based drugs also have disadvantages including poor *in vivo* stability, absence of targeting and low bioavailability. Therefore, peptide-protein drug conjugates consisting of peptides coupled with protein-based drugs have become a new idea to solve this problem.

Tumor necrosis factor (TNF) is a pro-inflammatory cytokine produced by macrophages or lymphocytes in the body and engaged in a wide range of chronic and acute inflammatory diseases, as well as killing tumor cells or necrosis of tumor tissue. To improve the targeting of TNF, Gregorc et al.[130] coupled TNF-α with NGR peptide to form NGR-hTNF, in which NGR can specifically recognize tumor neovascularization highly expressing CD13, allowing high concentrations of TNF-α to be pharmacologically effective in tumor tissues. From the phase II clinical trial, the combination of NGR-hTNF and DOX exhibited superior anti-tumor activity in recurrent small cell lung cancer patients, with a median progression-free survival of 3.2 months, in which anemia and neutropenia were the most common grade 3 to 4 adverse reactions. In addition, researchers have developed antibodies to TNF to inhibit the combination of TNF and its cognate receptors, thereby cutting off the subsequent physiological response[131]. Nevertheless, the half-time of TNF antibodies is too short in the blood, about 28 min[132]. To extend the circulation time of TNF antibodies *in vivo*, Conrad et al.[132] combined the heavy chain of TNF antibodies with elastin-like protein (ELP) to synthesize the conjugate ^{Nt}TNF-V_HH_{ELP}. The researchers found that the larger molecular weight of the coupling was not filtered out by the glomerulus, and therefore, the circulating half-life of ^{Nt}TNF-V_HH_{ELP} *in vivo* was considerably prolonged compared with that of the TNF antibody without ELP fusion.

Interferon (IFN) is a cytokine with broad-spectrum antiviral, antiproliferative and immunomodulatory functions, and is widely used in clinical practice to treat viral diseases and cancer. Nevertheless, the short half-life of IFN, its poor stability and frequent administration may lead to serious side effects and cause great suffering to patients. In addition, IFN is poorly targeted and cannot specifically kill tumor cells, which can bring serious toxic side effects such as neurotoxicity and immune disorders[133]. To improve the targeting of IFN, Li et al.[134] coupled the NGR targeting peptide with IFN-α2a to form IFN-α2a-NGR, with the finding of capability to target tumor vasculature. Compared with IFN-α2a, it significantly reduced the density of microvessels and induced apoptosis in vascular endothelial cells. Meanwhile, IFN-α2a-NGR downregulated the expression of VEGF and bFGF in tumor cells, thereby inhibiting tumor invasion, migration, angiogenesis and inducing endothelial cell apoptosis, thus being a promising anti-angiogenic agent.

4.3. Peptide drugs

Peptide drugs are the hot spot of new drug research and development in recent years. In comparison with traditional chemical and protein drugs, peptide drugs have obvious advantages: (1) peptide drugs generally have high activity, even at very low doses and concentrations; (2) in comparison with protein drugs, peptide drugs are smaller in molecular weight, easy to synthesize

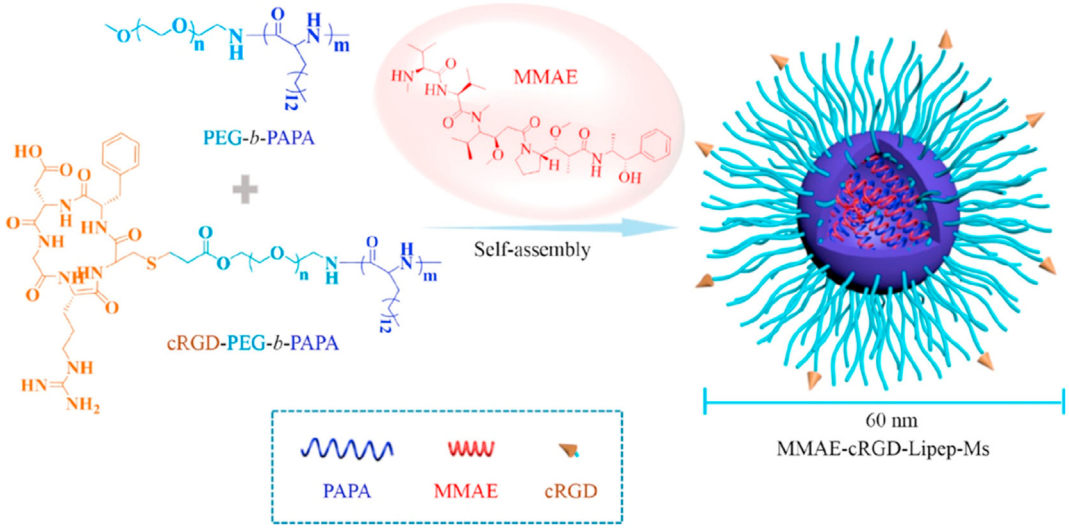


Figure 9 Illustration of cRGD-functionalized polylipopeptide micelles (cRGD-Lipep-Ms) that achieve enhanced and stable loading of mmae. (Reprinted with the permission from Ref. 135. Copyright © 2018, American Chemical Society.)

artificially and convenient for structural modification; (3) the synthesis of peptide drugs is highly efficient, and with the recent advances in technology, peptide solid-phase synthesis has become simple at a high degree of automation and easy to control; (4) fewer side effects. Most of the peptide drugs with the sequence homologous to human, plus the small molecular weight, no immunogenicity, will not cause immune reaction. However, the disadvantages of peptide drugs are also obvious. Unlike chemical and protein drugs, peptide drugs are physicochemically unstable, easily oxidized and hydrolyzed, as well as prone to agglomeration. Besides, the half-life of peptide drugs is shorter, and the clearance rate is faster, not easy to permeate the cell membrane. Aiming to address these drawbacks, many studies have applied PDCs for the delivery of peptide drugs. Qiu et al.[135] used cRGD peptides targeting integrins as model targeting ligands to construct cRGD-functionalized lipophilic peptide micelles (cRGD-Lipep-Ms), and then Monomethyl auristatin E (MMAE) peptide drug was loaded into the cRGD-Lipep-Ms core by the principle of similar phase solubility (Fig. 9). According to the results, compared to free MMAE, cRGD-Lipep-Ms exhibited more than 10-fold higher tolerability in mice and effectively inhibited the proliferation of $\alpha v\beta 5$ integrin overexpressing HCT-116 colorectal tumors with no significant systemic toxicity. Because of the large amounts of proteases in the gastrointestinal tract, most peptide drugs are hydrolyzed and therefore almost none of them should be used for oral administration. To realize the possibility of oral application of peptide drugs, Ahn et al[136] formed LMWC-exendin-4 couples by linking a glucagon-like peptide-1 (GLP-1) mimetic exendin-4 peptide to low molecular weight chitosan through a cleavable bond disulfide bond. From pharmacokinetic studies after oral administration, it was shown to have a C_{max} of 344 pg/mL, a T_{max} of 6 h and the bioavailability of 6.4% relative to subcutaneous. The conjugate produced high levels of hypoglycemic efficacy in a db/db type 2 diabetic mouse model. As such, LMWC-exendin-4 conjugate is expected to be used as an oral antidiabetic drug to treat type 2 diabetes and also provides a new idea to achieve great intestinal uptake efficiency and bioactivity in the case of other related peptide drugs.

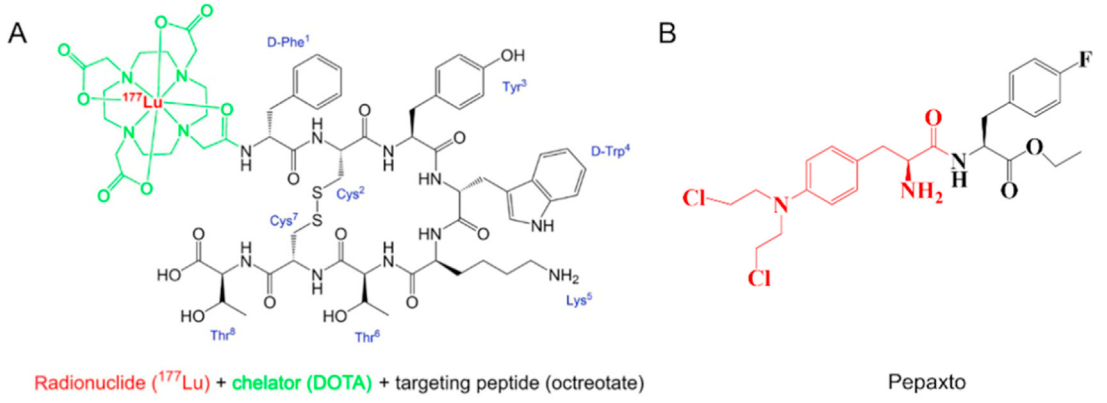


Figure 10 The structures of (A) [^{177}Lu] Lu-DOTA-TATE and (B) Pepaxto, respectively.

Table 4 Clinical research progress of PDCs.

Name	Application	Peptide	Linker	Drug	Molecular mechanism	Clinical status	Organization
ANG-1005	Various types of cancer	Angiopep-2	Succinic acid	Paclitaxel	Low-density lipoprotein receptor (LDLR) ligands	Phase III	AngioChem
zoptarelin doxorubicin	Various types of cancer	D-Lys6-LHRH	Amide	Doxorubicin	DNA topoisomerase II inhibitors, gonadotropin-releasing hormone receptor (GNRHR) ligands	Discontinued	AEterna Zentaris
NGR-hTNF	Malignant pleural	CNGRCG	Amide	hTNF	Tumor necrosis factor receptor modulators, angiogenesis inhibitors, aminopeptidase N modulators	Phase III	Molmed Spa
CBX-12	Various types of cancer	pH-Low insertion peptide	An intra-cellular cleavable linker	Exatecan	DNA topoisomerase I inhibitors	Phase I/II	Cybrexa
BCY-8245	Various types of cancer	Peptide targetingNectin-4	A cleavable linker	Monomethyl auristatin E	Drugs targeting nectin-4 and microtubule destabilizers (tubulin polymerization inhibitors)	Phase I/II	Bicycle Therapeutics
BT-1718	Breast cancer, non-small cell lung cancer and solid tumor	MT1-MMP binde	Disulfide	DM1	Drugs targeting matrix Metalloproteinase 14 (MMP-14; MT1-MMP) and microtubule destabilizers (Tubulin polymerization inhibitors)	Phase I/II	Bicycle Therapeutics
BTP-277	Endocrine cancer and small cell lung cancer	fCYwKTC C (2,7 SS)	Disulfide	DM-1	Microtubule destabilizers (Tubulin polymerization inhibitors) and somatostatin receptor type 2 (SSTR2) ligands	Phase I/II	Tarveda Therapeutics
G-202	Solid tumors	DγEγEγEγE	Amide	Thapsigargin	Sarcoplasmic/endoplasmic reticulum calcium ATPase 1 inhibitors	Phase II	Inspyr Therapeutics
CBP-1008	Advanced solid tumor	CB-20BK	Amide	MMAE	Tubulin inhibitors, transient receptor potential cation channel subfamily V member antagonists, folate receptor alpha antagonists	Phase I	Tong Yi Pharmaceutical (Suzhou) Co.
TH-1902	Anticancer drugs	TH19P01	Succinic acid	Docetaxel	Drugs targeting sortilin (neurotensin NTR3; NT3; Gp95) receptor and microtubule stabilizers (tubulin depolymerization inhibitors)	Phase I	Theratechnologies
SOR-C13	Advanced malignant solid	Folate	Amide	MMAE	Transient receptor potential cation channel subfamily V member antagonists	Phase I	Soricimed

5. PDCs for different diseases

In addition to the various types of cancer mentioned above, PDC drugs can also be applied to the treatment of other diseases. Inflammation, a common disease that afflicts millions of people worldwide, has a serious impact on people's quality of life. Naproxen, as a non-steroidal anti-inflammatory drug, can exert anti-inflammatory effects by inhibiting the synthesis of prostaglandins. However, due to the lack of selectivity, it brings some gastrointestinal adverse effects. To improve the selectivity of naproxen, Moreira et al.[137] constructed naproxen-dehydrodipeptide conjugates and found that they easily formed nanostructured fibrous supramolecular hydrogels. This hydrogel could be an optimal strategy for treating inflammation.

Moreover, PDC drugs are widely available for bacterial infectious diseases. The effective and safe treatment of bacterial infections is a major challenge for modern medicine. This is largely due to the limitations of current antibacterial drugs in terms of host toxicity, effective drug delivery, and increasing antibacterial resistance. To address these issues, Brankiewicz et al.[138] covalently linked fluconazole to a cell-penetrating peptide to form PDC, which showed a higher Candida-killing effect compared to free fluconazole. Similarly, in order to increase the lipophilicity of the hydrophilic antimicrobial peptide Polymyxin B (PMB), Dizdarević et al.[139] exploited the formation of imine bonds between the primary amino group of PMB and the carbonyl group of cinnamaldehyde, allowing it to be incorporated into self-emulsifying drug delivery systems for oral administration.

Furthermore, PDCs can be also applied to the delivery system of therapeutic drugs for diseases such as analgesia[140], malaria[141], and diabetes[142]. As long as the drugs satisfy the requirements of PDC construction, it is believed that more drugs for diseases can be delivered by this prodrug strategy in the future.

6. The progress of clinical application of PDCs

Although PDC has great application prospects, the PDC field is still in a depression of development. So far, only two PDC drugs have been approved for the market worldwide: One is Lutathera® ([^{177}Lu] Lu-DOTA-TATE), developed by Advanced Accelerator Applications S.A, a Novartis subsidiary, which was approved for market by the FDA in January 2018 with a view to treating somatostatin inhibitor receptor-positive gastroenteropancreatic neuroendocrine tumors. Lutathera® is formed by the complex coupling of the somatostatin hormone inhibitor targeting peptide (Tyr3)-octreotate with the radionuclide^{177} Lu and the bifunctional chelator DOTA (Fig. 10A). The therapeutic tracer [^{177}Lu]Lu-DOTA-TATE employs an emerging form of endoradiotherapy known as Peptide Receptor Radionuclide Therapy (PRRT): Lutathera® enters cells in combination with growth inhibitor receptors, and releases nucleotides to produce radiation to damage tumor cells, thus exerting therapeutic effects[143]. The other is Pepaxto (Melflufen), developed by Oncopeptipes, which was approved with accelerated marketing in February 2020 by FDA for use in multiple myeloma. Pepaxto is obtained by covalently linking DNA alkylating agents to peptides that target tumor cells highly expressing aminopeptidases (Fig. 10B). Due to its high lipophilicity, Pepaxto can be taken up by multiple myeloma cells, which are rapidly hydrolyzed intracellularly by aminopeptidases, releasing hydrophilic DNA alkylating agents. DNA alkylating agents can covalently bind to biomolecules (such as DNA, RNA, enzymes, etc.) in tumor cells, rendering them inactive and leading to tumor cell death[144]. However, on October 22, 2021, Oncopeptides announced the withdrawal of Pepaxto from the U.S. market, primarily because Pepaxto failed to reduce the risk of death in the ITT population in the confirmatory phase III OCEAN study (HR = 1.104).

Apart from the two drugs approved for the market, Table 4 lists the PDC drugs that are currently in clinical trials. ANG1005, a PDC drug developed by AngioChem that has entered Phase III clinical trials, is composed of three paclitaxel molecules with Angiopep-2 covalently attached. Angiopep-2 is a 19 amino acid peptide targeting low-density lipoprotein receptor-related protein 1 (LRP1). ANG1005 crosses the CNS barrier into the brain *via* LRP1-mediated endocytosis, and the linker is broken by lysosomal esterase, releasing paclitaxel to exert its drug effect[145]. According to the phase II clinical trial, ANG1005 has shown clear clinical efficacy in treating intracranial and extracranial breast cancer brain metastases patients, with clinical benefit rates of 77% and 86% respectively. Compared to the estimated survival of patients with breast cancer brain metastases without any treatment (2 months) and those with an aggressive treatment regimen (3–4 months), patients had significantly longer survival after ANG1005 treatment, with a median overall survival of 8 months[146].

7. Conclusions and perspectives

PDC drugs are first delivered to specific sites (active or passive targeting) through peptide targeting, then PDCs are effectively internalized into cells with membrane penetration of cell-penetrating peptides or receptor-mediated transmembrane action, and finally released payloads through specific cleavage of links in response to environmental stimulation or enzymatic action to exert drug effects. The review focuses on the classification of peptides, linkers and drugs in PDC drugs and their applications, and provides a summary and overview of drugs that have entered the market and clinical trials. From the number of PDC drugs on the market, it is clear that despite the many unique advantages of PDC, there are still many challenges and obstacles in the way of its clinical translation. First, considering safety concerns, it would be of great interest to illustrate the biocompatibility of PDC drugs. As is well known, peptides derived from natural amino acids are fully biodegradable in the form of water-solubility and are usually biocompatible with no adverse reactions. However, it is not known whether the biocompatibility and biodegradability of peptides can be retained after coupling with drugs. Therefore, it would be very encouraging to develop novel approaches for the biocompatibility and biodegradability of peptides after forming PDCs. Second, in terms of stability considerations, peptides have a short biological half-life, leading to restricted distribution and circulation time of PDCs *in vivo*, with limited time for drugs to enter focal tissue cells prior to peptide degradation. Although a number of methods are available to lengthen the biological half-life of peptides, involving head-to-tail cyclization, disulfide-linked cyclization, unnatural amino acid substitution, peptide mimetic, stapled peptides, and bicycle strategies, PDC drugs are small in size and easily cleared by the kidney. It is recommended to combine the prodrug strategy of PDC with the rational application of carrier materials to form a delivery system of suitable size and higher stability. Third, taking the effectiveness into consideration, linkers in PDCs require specific environments (temperature, pH, redox, enzymes, etc.) to break off and release the drug, with the drug sometimes not being released as a prototype drug or even not being released. In addition, there is no way to determine

the rate and efficiency of drug release in the target cells. Therefore, the efficacy of the PDC drug needs to be demonstrated with appropriate methods. Finally, from the perspective of the mode of administration, due to the presence of gastrointestinal proteases, similar to protein drugs, PDC drugs cannot be administered orally at present and can only be administered by invasive injection, which limits their clinical application. To address this limitation, it is highly recommended that the design of delivery systems for oral macromolecules should be considered, and appropriate carriers or structural modifications should be adopted to prevent the degradation of enzymes in the gastrointestinal system and to guarantee the absorption of PDC into the blood. The development of efficient PDCs requires the design and synthesis of multifunctional peptides, and we believe that with the progress and maturity of peptide screening technology and synthesis technology, more multifunctional peptides will be explored. The intelligent nanomedicine delivery strategy based on PDCs will also be widely applied in the clinic in the future.

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Author contributions

Liming Gong and Wei Huang designed the review. Liming Gong wrote the manuscript. Wei Huang and Zhonggao Gao revised the manuscript. Other authors participated in the search and collation of literature. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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