CHAPTER 11

Polymer-Based Prodrugs for Cancer Chemotherapy

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11.1 Introduction

Chemotherapy has achieved great success in cancer treatment during recent past decades, but it is still challenged by poor solubility, low tumor selectivity, and associated toxicity of most anticancer drugs.[1] The prodrug strategy is one of the most commonly used chemical/biochemical strategies towards improving the therapeutic index of anticancer drugs.[2]

A prodrug is defined as a chemically modified drug derivative that is inactive or less active but metabolized *in vivo* to release the parent active component in the pharmacological environment[3] to improve the drug's desirability, including water solubility, patient acceptability (*e.g.* decreasing pain on injection), and pharmacokinetics (absorption, biodistribution, metabolism, and elimination; ADME). A typical prodrug normally consists of three parts (Figure 11.1): (1) the parent drug exerting therapeutic effects; (2) the chemical linker bridging the parent drug and the modifier; and (3) the modifier endowing the prodrug with

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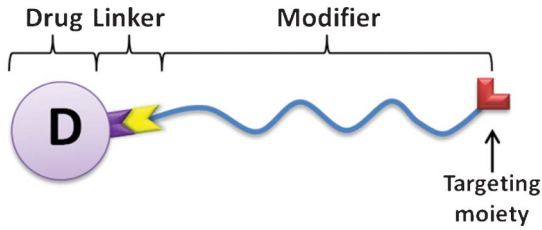

Figure 11.1 Sketch of a typical polymer prodrug.

various properties and functionalities. In this chapter, we focus on the low molecular weight drugs chemically modified with polymers for cancer drug delivery, some of which are generally called polymer–drug conjugates.[4]

The ultimate goal of cancer drug delivery is to increase the tumor selectivity or targeting ability to enhance the therapeutic efficacy and reduce side effects.[5] According to the three stages of the cancer drug-delivery process discussed in Chapter 3, such an ideal anticancer prodrug should be able to sustainably circulate in the blood compartments, efficiently extravasate into the tumor and penetrate through the tumor tissue, and finally get into tumor cells and release the parent drug.[6] Most polymer-based prodrugs endow the parent drugs with adequate aqueous solubility and improved tumor targeting. However, current polymer prodrugs have some inherent drawbacks, *e.g.* low drug content and low tumor specificity, that limit their further translation from the benchtop to the bedside. Thus, design of novel prodrugs with desirable properties is needed. In this chapter, we briefly review the methods for preparing conventional polymer prodrugs and their associated problems, and summarize new strategies showing great promise and the remaining challenges in translational prodrugs.

11.2 Design of Polymer-Based Prodrugs

As shown in Figure 11.1, a polymer-based prodrug consists of three parts: the drug, the linker, and the modifier. The drug determines the potency, while the linker and modifier determine where the drug goes to exert the potency (targeting). Anticancer drugs good for making prodrugs must have at least one reactive site to be anchored to the polymer modifier *via* a linker. The parent drugs must have high potency to avoid using too much excipient(s). The most investigated anticancer drugs are doxorubicin (DOX), paclitaxel (PTX), camptothecin (CPT), and its derivatives (*e.g.* SN38).

11.2.1 Linkers

Common chemical linkers for the synthesis of prodrugs for cancer therapy, such as ester, amide, hydrazone, and disulfide bonds, have already been summarized by Mahato *et al.*[7] An ideal linker rendering the prodrug maximal

therapeutic efficacy and minimal toxicity would be the one that is stable in the blood compartments but labile in cancer cells.

The linker should first be stable in blood circulation to ensure low toxicity in circulation.[8] For example, the PK1 conjugate in which DOX was covalently bound to poly[*N*-(2-hydroxypropyl)methacrylamide] (PHPMA) *via* a blood-stable but lysosome-labile peptidyl linker had very low dose-limiting toxicity.[9] In phase I clinical and pharmacokinetic studies, PK1 had a maximum tolerated dose (MTD) of 320 mg m^{-2}, and no congestive cardiac failure despite individual cumulative doses up to 1,680 mg m^{-2}.[9] However, both the PHPMA-PTX prodrug named PNU166945[10] and the poly(methacryloylglycinamide) (PMAG)-CPT prodrug[11] experienced dose-limiting toxicity in phase I clinical study because their easily hydrolysable ester linkers released the drugs while in circulation.[12]

Upon reaching the intracellular target, the prodrug must efficiently release the parent drug to exert its pharmaceutical action because only the liberated drug becomes active.[13] Stable prodrugs, *e.g.* drugs bonded to poly(lactic-*co*-glycolic acid) (PLGA)[14] or poly(L-aspartic acid) [P(Asp)],[15] showed low or even no anticancer activity. It is preferable that the linker be cleavable in the tumor microenvironment. This is achieved by using labile linkers responsive to the tumor's extracellular or intracellular stimuli (Figure 11.2).[16] Lysosomal pH-labile linkers (*e.g.*, amide, hydrazone, or *cis*-aconityl bonds) ensure the intracellular drug release in lysosomes and are widely used.[17] For instance, DOX was conjugated to the P(Asp) block of its block copolymer poly(ethylene glycol) (PEG)-*b*-P(Asp) *via* acid-labile amide[18] or hydrazone linkers.[19] DOX

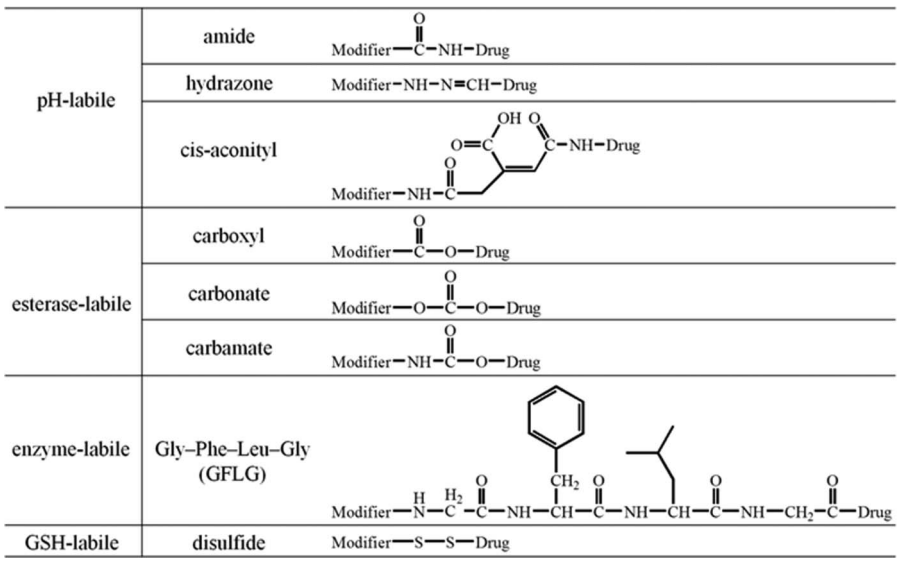

Figure 11.2 Commonly used extracellular and intracellular stimuli-labile linkers in prodrugs.

conjugated to PHPMA and biodegradable star polymers *via* such linkers demonstrated a fast DOX release at pH 4.[20] An interesting example is the dual pH-responsive prodrug PPC-Hyd-DOX-DA developed by Du and co-workers.[21] At tumor extracellular pH ($\sim$6.8), the prodrug reversed its surface charge from negative to positive *via* acid-labile amide bonds for fast internalization; at endosomal or lysosomal pH ($\sim$5.0), the drug was fast released due to the breakable hydrazone bonds. Esterases are ubiquitously distributed in the body and can readily hydrolyze ester bonds in prodrugs; thereby esterase-labile linkers, such as carboxyl, carbonate, and carbamate esters, are also employed.[22] For instance, in IT-101, CPT was conjugated to the linear β-cyclodextrin (β-CD) and PEG copolymer *via* the ester bond.[23] The conformation of released CPT was not changed compared to the parent CPT and the release rate of the conjugated CPT can be tuned.[24] Lysosomal degradable peptides [*e.g.*, glycylphenylalanylleucylglycine (GFLG)], which are cleavable by lysosomal enzymes, are also useful linkers in prodrugs.[25] Recently, the disulfide bond,[26] which can be cleaved by intracellular glutathione (GSH), has attracted increasing attention as linkers for intracellularly triggered drug release. The underlying rationale is the elevated intracellular GSH concentration, particularly in cancer cells, but low in the blood.[27]

11.2.2 Modifiers

The modifier may consist of a polymer chain and targeting moieties and others such as a tracer moiety. The main roles of the polymer chain are to endow the prodrug with water solubility and a long blood-circulation time for altered ADME and tumor targeting capability. The targeting moiety facilitates the prodrug's active targeting, as discussed in Chapter 2.

Most anticancer drugs are water insoluble, giving them poor bioavailability.[28] Anchoring drugs to water-soluble polymer chains makes them water soluble. For instance, PTX is an extremely water-insoluble anticancer drug ($<$0.01 mg mL^{-1}).[29] Conjugating PTX to a PEG[30] or poly(L-glutamic acid) (PGA)[31] resulted in highly water-soluble prodrugs with antitumor effects superior to PTX itself.[31,32]

It is generally assumed that by prolonging the blood circulation time, a polymer prodrug has more opportunity to pass through the hyperpermeable tumor blood vessels and extravasate into tumor tissue *via* the EPR effect.[1b] Prodrugs with a stealth property may evade the reticuloendothelial system (RES) screening[33] and thus circulate for a long time in the blood compartments, resulting in greatly increased tumor drug concentrations (10-fold or higher) and MTD relative to administration of the free drug.[34] The stealth character of a polymer prodrug is mainly determined by the polymer's properties. The polymer must be water soluble and, very importantly, not immunogenic. A very interesting example is the natural biopolymer dextran. It is usually assumed to be non-immunogenic, but drugs conjugated to dextran

and carboxymethyldextran can be captured by the RES, causing dose-limiting toxicity.[35] PHPMA[36] and PEG[37] are the most studied bio-inert polymers able to render prodrugs stealthy. For example, NKTR-102, a PEG prodrug of irinotecan (a chemotherapy drug that is metabolized to its active metabolite SN38 in the body), increased the half-life of SN38 90-fold (from 4 h to 15 days) and 10-fold in colorectal and lung cancer treatment, respectively.[38] Other PEG-based prodrugs of docetaxel (NKTR-105), SN38 (EZN-2208), and CPT (Pegamotecan) also showed longer half-life times and increased accumulation at tumor sites and thereby tumor growth suppression.[39]

Incorporating targeting ligands in the polymer modifier, such as antibodies, peptides, aptamers, and folic acid, may promote tumor targeting (building on the EPR effect) by receptor-mediated delivery,[40] as discussed in Chapter 2. For example, PK1 conjugated with galactosamine, named PK2,[41] delivered 3.3 $\pm$ 5.6% of the dose to the tumor and 16.9 $\pm$ 3.9% to the liver, whereas PK1 showed no obvious targeting.[42] Zhu *et al.*[43] and Borgman *et al.*[44] also proved that incorporating targeting ligands in polymer modifiers increased the prodrug's tumor accumulation. More examples have been reviewed elsewhere.[45]

11.2.3 Drawbacks of Current Polymer-Based Prodrugs

An inherent dilemma in polymer prodrugs is their drug-loading content *versus* the water solubility and thereby the stealth capability. A high drug-loading content in a nanocarrier reduces use of excipients and minimizes the related side effects, and should be considered as a demanding criterion to judge a prodrug's quality.[46] As most parent drugs are highly hydrophobic, the modifier or the water-soluble polymer chain must be sufficiently long to make the prodrug water soluble. Therefore, in most polymer prodrugs the drug content is less than 10 wt%.[47] Typical examples are PEG-DOX prodrugs using PEG with different molecular weights from 5,000 to 20,000 g mol^{-1}, which contain 2.7–8.0 wt% DOX.[48] A four-armed PEG-SN38 prodrug named EZN-2208, prepared by coupling SN38 to multiarm PEGs (Figure 11.3A), had an increased drug content, but only of 3.7 wt%, compared to the CPT-PEG-diol prodrug Pegamotecan.[39] As for PHPMA–drug conjugates, for instance PK1, the drug contents are also as low as 8 wt%.[49] Some prodrugs have high drug-loading contents. For instance, the prodrug CT-2103, PTX conjugated to PGA through its 2′-hydroxyl group (Figure 11.3B),[50] has a drug content of approximately 37 wt% and a PGA-20(S)-CPT prodrug has a 30–35 wt% drug content.[51] However, increasing the drug content not only lowers the prodrug water solubility but may also cause opsonization,[37] resulting in rapid blood clearance.

Another complication is also related to the polymer chain's molecular weight. To have a long blood-circulation time for passive tumor targeting, the molecular weight of a prodrug must be higher than the polymer's renal threshold, *e.g.* 40 kDa for PEG[52] and 45 kDa for PHPMA,[53] but the safety

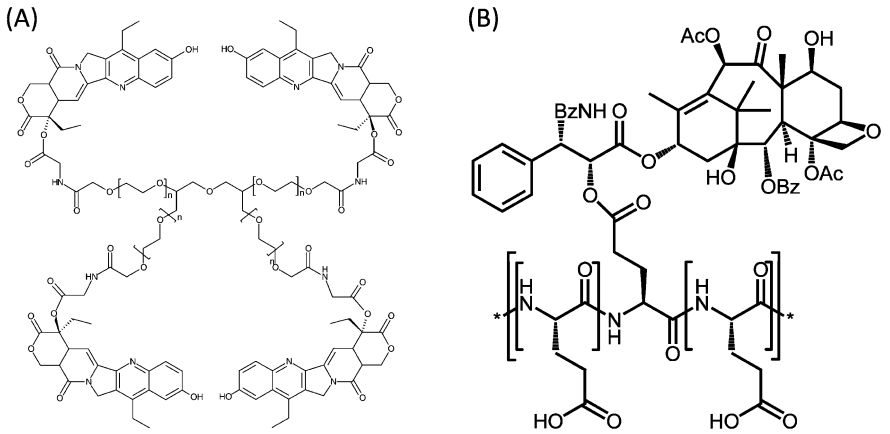

Figure 11.3 Chemical structures of EZN-2208 and CT-2103 polymer prodrugs. (Reproduced from Pasut and Veronese[39] and Singer[50b] with permission from Elsevier.)

prerequisite for *in vivo* applications is that the prodrug and the modifier must be smaller than the renal threshold if their excretion pathway is through the kidneys. The current strategy to reconcile these conflicting requirements is to choose a polymer molecular weight close to the threshold, for example 30 kDa for PHPMA, but this way inevitably shortens the blood circulation time of the prodrug. Therefore, new strategies resolving these dilemmas are required to design novel polymer prodrugs with higher drug content and better therapeutic indices.

11.3 New Strategies for Polymer Prodrugs

11.3.1 Self-Assembling Prodrugs

Given that prodrugs with a high hydrophobic drug content are not water soluble, we may take advantage of the hydrophobicity and directly use the drugs as the hydrophobic part and the modifier as the hydrophilic segment to make amphiphilic prodrugs that can self-assemble into vesicles or micelles as nanocarriers. An important advantage of this way is that even though a single prodrug molecule is small (several kDa) and far below the renal threshold, the formed vesicles or micelles are generally larger (tens of nanometers in diameter) than the kidney threshold ($\sim$5.5 nm) and thus can be retained for long blood circulation.[54] The second advantage of this strategy is that because the drug molecules are used as a part of the nanocarrier and replace some of the inert carrier materials, the nanocarrier's drug content is high. For instance, the anticancer drug CPT is very hydrophobic, with a water solubility of

2.5 μg mL^{-1}.[55] Conjugating one or two molecules to short oligomer chains of ethylene glycol (OEG) *via* β-thioester bonds produces an amphiphilic phospholipid-mimicking prodrug OEG-CPT or OEG-DiCPT (Figure 11.4). These prodrugs can self-assemble in aqueous solution into stable liposome-like nanocapsules of around 100 nm in diameter (Figure 11.4A). As shown in Figure 11.4, the hydrophobic wall of the vesicle contains no aliphatic chains like normal liposomes but is solely made of hydrophobic CPT drug molecules. Thus, the drug content is as high as 40 or 58 wt%. Another important characteristic is that since the nanocapsules or vesicles are made of the prodrug with well-defined structures, their drug content is fixed independently of the

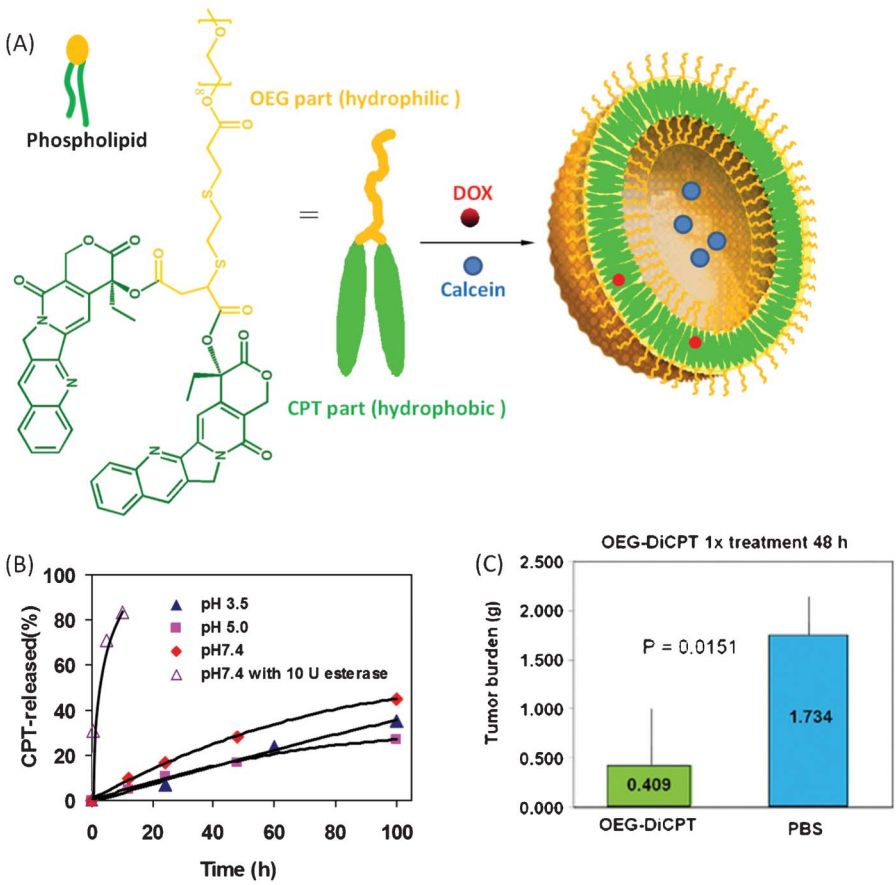

Figure 11.4 (A) Scheme of phospholipid-mimicking prodrugs OEG-DiCPT self-assembling into stable liposome-like nanocapsules to load other drugs. (B) CPT release kinetics from the OEG-DiCPT nanocapsules. (C) *In vivo* antitumor activity of OEG-DiCPT to xenografted intraperitoneal SKOV-3 ovarian tumors in BALB/c strain nu/nu mice. (Adapted from Shen *et al.*[56] with permission from the American Chemical Society.)

nanocarrier size and preparation methods. This character makes it easy to scale-up for translation, as discussed in Chapter 3. The nanocapsules have no burst release but can release CPT quickly once inside the cells (Figure 11.4B). *In vivo* tests showed that the nanocapsules had strong antitumor activity (Figure 11.4C).[56]

Another example is a self-assembling curcumin prodrug. Curcumin, a substance in turmeric, has been shown to have high cytotoxicity towards various cancer cell lines,[57] but its extremely low water insolubility and instability make its bioavailability exceedingly low and generally inactive in *in vivo* anticancer tests.[58] Using the same concept, we conjugated curcumin with two short OEG chains (Curc-OEG) *via* β-thioester bonds that are labile in the presence of intracellular glutathione and esterases and obtained an intracellular-labile amphiphilic surfactant-like curcumin prodrug: curcumin-OEG (Curc-OEG) (Figure 11.5).[59] Curc-OEG formed stable nanoparticles in aqueous conditions and served two roles: an anticancer prodrug and a drug

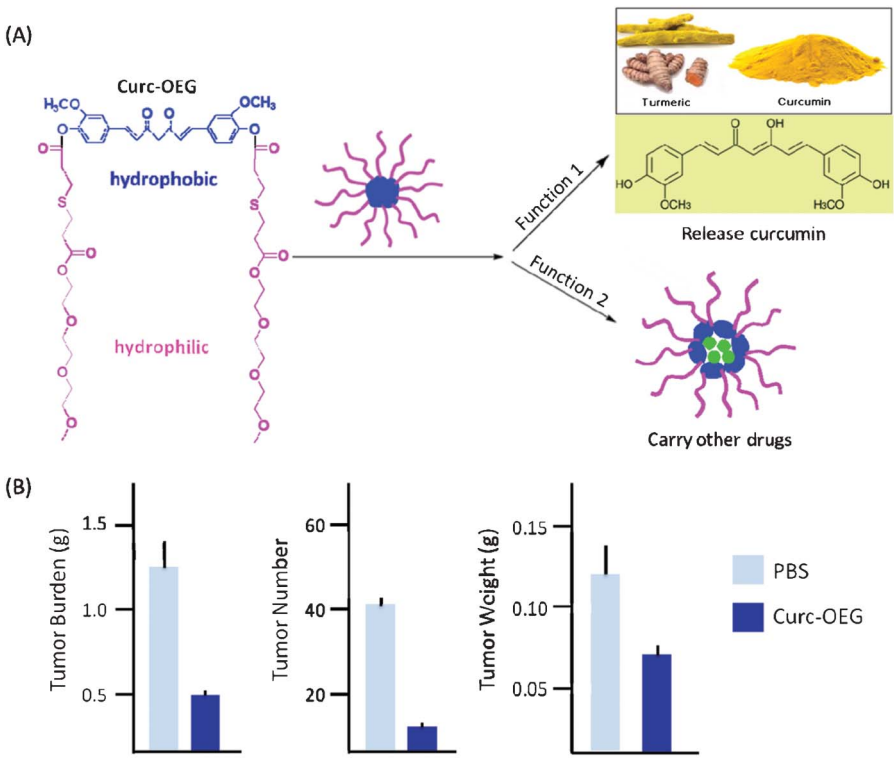

Figure 11.5 (A) Structure of Curc-PEG and its self-assembly into micelles to perform two functions: release curcumin and carry other drugs. (B) *In vivo* antitumor activity of Curc-OEG against SKOV-3 (*left* and *middle*) and MDA-MB-468 (*right*) carcinoma xenograft models. (Adapted from Tang *et al.*[59] with permission from Future Medicine.)

carrier (Figure 11.5A). As an anticancer prodrug, the formed nanoparticles had a high and fixed curcumin-loading content of 25.3 wt%, and released active curcumin in the intracellular environment. Curc-OEG had high inhibition ability for several cancer cell lines due to apoptosis. Intravenously injected Curc-OEG had a bioavailability (the area under curve value) 250 times of that of the parent curcumin, and thus a high overall tumor concentration. As a result, it significantly reduced tumor weights and tumor numbers in the athymic mice xenografted with intraperitoneal SKOV-3 tumors and subcutaneous (mammary fat pad) MDA-MB-468 tumors (Figure 11.5B). Preliminary systemic toxicity studies found that Curc-OEG did not cause acute or subchronic toxicities in mouse visceral organs at high doses. As a drug carrier, Curc-OEG nanoparticles could carry other anticancer drugs, such as DOX, and ship them into drug-resistant cells, greatly enhancing the cytotoxicity of the loaded drug. Thus, Curc-OEG is a promising prototype that merits further study for cancer therapy.

11.3.2 Prodrug Micelles

Besides the phospholipid-mimicking amphiphilic prodrugs, another type of prodrug capable of self-assembly is the diblock copolymer in which one block is a water-soluble chain such as PEG and the other is conjugated with hydrophobic drug molecules as the water-insoluble block. Such block copolymers form micelles in aqueous solution with drug molecules anchored in the micelle core. For instance, the Matsumura group conjugated SN38 to the PGA block of a PEG-PGA copolymer.[60] The resulting conjugate PEG-PGA(SN38) containing approximately 20 wt% drug, named NK012, self-assembled into micelles with a diameter of 20 nm (Figure 11.6). NK012 was reported to eradicate liver metastases and achieve a significantly longer survival rate than CPT-11 ($P = 0.0006$). More recently, our group conjugated SN38 as the hydrophobic group to short poly(methacrylic acid) and obtained amphiphilic PEG-polySN38.[61] This prodrug formed nanoparticles with a high drug-loading content ($\sim$20 wt%) and tailorable sizes. Sub-100 nm nanoparticles were also self-assembled from a PEG-poly(L-lactide) (PEG-PLA)-based polymer–cisplatin prodrug in which cisplatin was anchored to the PLA terminal *via* a hydrazone bond.[62]

Another interesting self-assembly prodrug for continuous slow release of PTX was developed by Kwon and co-workers.[63] PTX was conjugated to PEG-p(Asp) through hydrazone linkers made from levulinic acid (LEV) or 4-acetylbenzoic acid (4AB) to give amphiphilic PEG-p(Asp-Hyd-LEV-PTX) and PEG-p(Asp-Hyd-4AB-PTX), which assembled into polymeric micelles with diameters of 42 and 137 nm, respectively. Mixing the two prodrugs produced micelles with diameters of 85 and 113 nm, respectively, having pH-dependent release. Detailed reviews can be found elsewhere.[64]

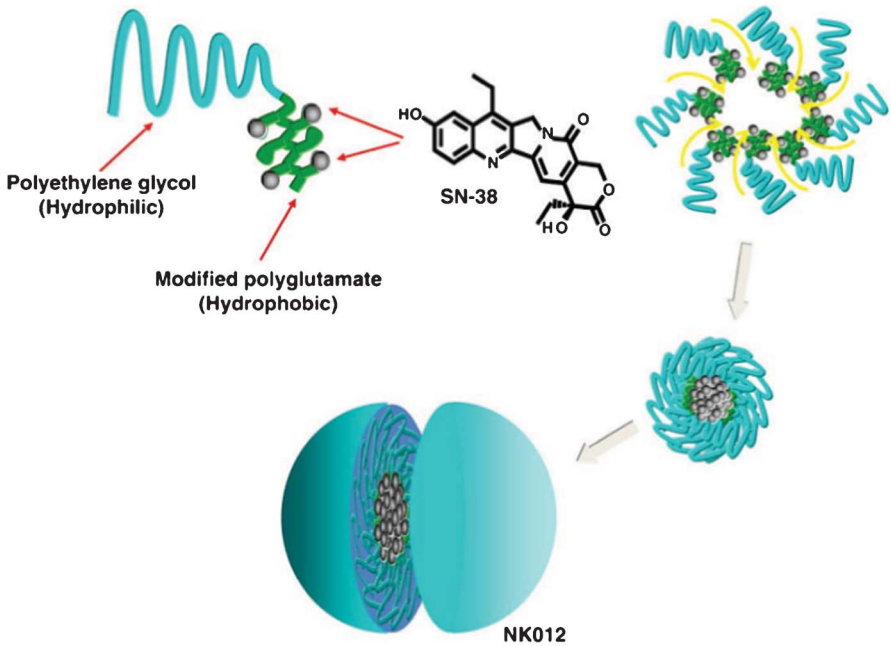

Figure 11.6 Synthesis and assembly process of NK012. (Reproduced from Matsumura[60a] with permission from Elsevier.)

11.3.3 Drug Polymers

An alternative to using the drug molecules themselves as a part of the nanocarrier is to directly use biofunctional drug molecules as monomers to prepare backbone-type polymer–drug conjugates, or *drug polymers*. One such example is polycurcumin (PCurc; Figure 11.7).[65] Inspired by the bihydroxyl functionality of the curcumin molecule, curcumin was used as a co-monomer to make the curcumin-containing PCurc by polycondensation polymerization. A series of water-soluble PCurcs were prepared with different covalent bonds (*e.g.*, ester, disulfide, acetal) with high and fixed curcumin-loading content and efficiency in comparison with previously developed curcumin-loading nanomedicines.[66] For instance, curcumin was condensed with divinyl ether to produce linear polycurcumin linked *via* acetal bonds. To make the polymer water soluble, PEG-diol was used as a co-monomer. Water-soluble polycurcumin, PCurc 8, could contain up to 25 wt% curcumin. The polymers were stable at neutral conditions, but hydrolyzed rapidly at lysosomal pH to release curcumin (Figure 11.7A), causing high toxicity to three cancer cell lines (even more cytotoxic than the parent curcumin itself) (Figure 11.7B) and remarkable antitumor activity in the SKOV-3 intraperitoneal xenograft tumor model (Figure 11.7C).

Another drug polymer we developed is the platinum(IV)-coordinated polymer using the platinum(IV) prodrugs DHP and DSP as co-monomers

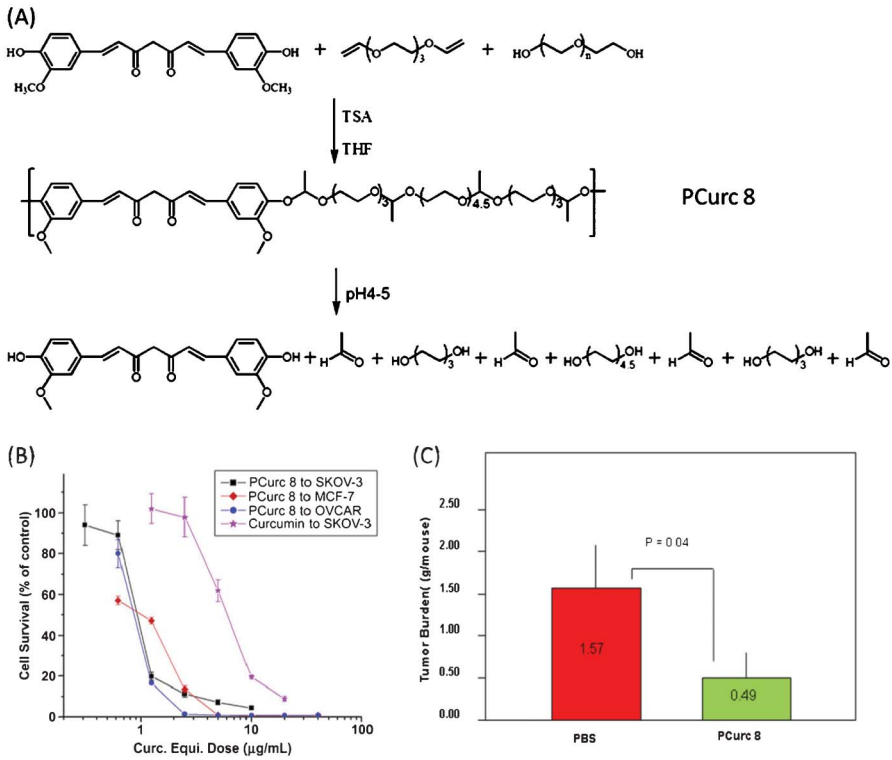

Figure 11.7 (A) Synthesis and acid-catalyzed hydrolysis of PCurc 8. (B) Cytotoxicity of PCurc 8 to SKOV-3, OVCAR-3, and MCF-7 cancer cell lines. (C) *In vivo* antitumor activity of Curc-OEG against SKOV-3 carcinoma xenografts. (Adapted from Tang *et al.*,[65] Cartiera *et al.*,[66a] and Shi *et al.*[66b] with permission from Elsevier and the American Chemical Society.)

(Figure 11.8A).[67] The polymers had good water solubility and high and fixed platinum-loading contents, 27.7% for P(DSP-EDA) and 29.6% for P(DSP-PA), respectively, which were not achievable by any conventional conjugation methods. Additionally, the biodistribution results suggested that the conjugated platinum polymer might greatly minimize the liver and renal toxicity (Figure 11.8B).[67]

11.4 Future Challenges

Although polymer–drug conjugates or prodrugs have been investigated for several decades, the number of approved polymer-based prodrugs is still small.[68] Thus, the design of polymer-based prodrugs with more efficient drug delivery to achieve higher therapeutic efficacy is still a pressing need. Three aspects should be taken into consideration when designing a new prodrug:

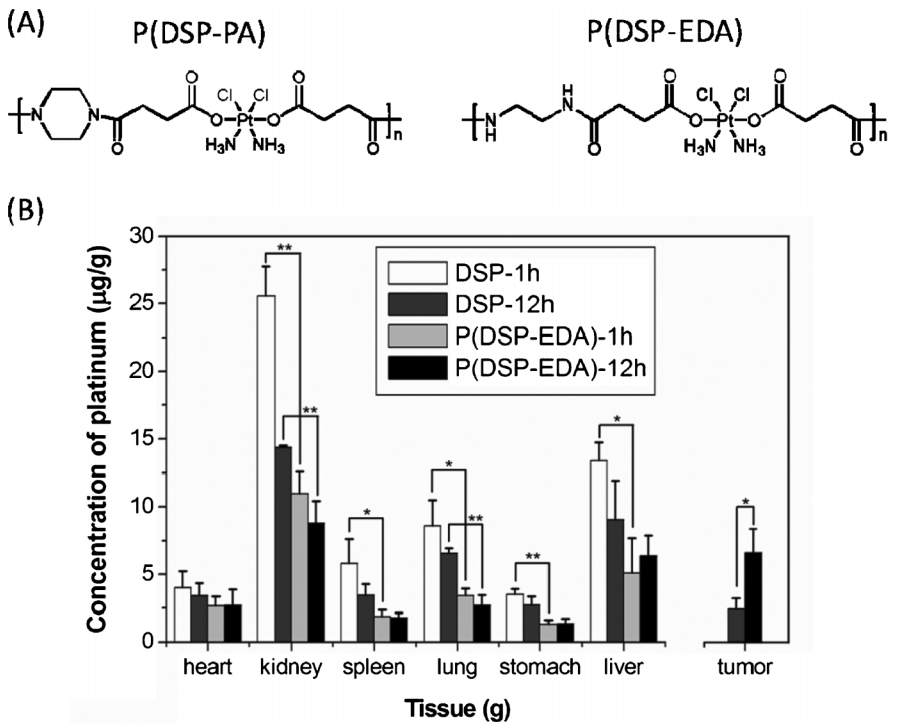

Figure 11.8 (A) Chemical structures of backbone-type platinum(IV) prodrugs. (B) The biodistribution of platinum in various organs and SKOV-3 xenograft tumors after a single i.v. administration of free DSP or P(DSP-EDA) at a DSP-equivalent dose of 20 mg kg^{-1}. (Adapted from Yang *et al.*[67] with permission from Elsevier.)

(1) the drug's configuration or structure should not be changed and its therapeutic efficacy should not be impaired; (2) the drug should remain tightly conjugated in circulation in order to reduce the drug-associated toxicity; and (3) the drug should be intracellularly released from conjugates efficiently once inside the tumor cells. The first aspect is the prerequisite of a conjugate, while the latter two aspects are the elementary 2R (*R*etention in circulation *versus* *R*elease in cells) rules[6] discussed in Chapter 3. Certainly, elementary 2S (*S*tealthy in circulation *versus* *S*ticky in tumor) rules[6] also should be addressed as further requirements of a superior prodrug.

Challenges remain even for those proved polymer–drug conjugates, including safety issues, good manufacturing process, and the cost-effective scale-up. Most of the proven conjugates use PHPMA or PEG as the modifier.[69] Although PHPMA and PEG are well tolerated clinically, their non-biodegradability is a significant disadvantage, especially in cases of chronic parenteral administration and high required doses.[69] Compared to other polymeric nanocarriers, the self-assembling prodrugs can be easily

formulated with a fixed composition and a relatively good manufacturing process, providing a novel design concept for translation of polymer-based prodrugs from benchtop to the bedside.

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