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Review article

Self-assembling prodrug nanotherapeutics for synergistic tumor targeted drug delivery

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1. Introduction

Clinical application of small molecule drugs such as chemotherapeutics are greatly impeded by their poor solubility in aqueous solutions, short blood circulation times, inadequate efficacy, lack of tissue-specificity and severe systemic adverse effects [1–4]. Tremendous efforts have been made to develop various drug delivery systems to address these limitations and strengthen tumor targeted delivery with an aim to enhance therapeutic efficacy while improving drug tolerance [5–7]. Over the past several decades, the self-assembling prodrugs (SAP) approach has emerged as a powerful therapeutic platform for enhancing tumor targeted therapy [8–44]. Compared to free drugs, well-designed SAP nanotherapeutics (SAPNs) are equipped with a number of advantages in addressing these unmet clinic needs: 1) strengthened physicochemical properties such as enhanced solubility and chemical stability, 2) improved pharmacodynamic profiles and attenuated side effects by favorably altering their pharmacokinetics (PK) and tumor uptake and 3) as effective carriers for delivering poorly water-soluble drugs in vivo in addition to ameliorating their systemic non-specific toxicities as well as boosting their tumor

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ABSTRACT

Self-assembling prodrugs represents a robust and effective nanotherapeutic approach for delivering poorly soluble anticancer drugs. With numerous intrinsic advantages, self-assembling prodrugs possess the maximum drug loading capacity, controlled drug release kinetics, prolonged blood circulation, and preferential tumor accumulation based on the enhanced permeability and retention (EPR) effect. These prodrug conjugates allow for efficient self-assembly into nanodrugs with the potential of encapsulating other therapeutic agents that have different molecular targets, enabling simultaneous temporal-spatial release of drugs for synergistic antitumor efficacy with reduced systemic side effects. The aim of this review is to summarize the recent progress of self-assembling prodrug cancer nanotherapeutics that are made through conjugating therapeutically active agents to Polyethylene glycol, Vitamin E, or drugs with different physicochemical properties via rational design, for synergistic tumor targeted drug delivery.

Statement of Significance

All current FDA-approved nanomedicines use inert biomaterials as drug delivery carriers. These biomaterials lack any therapeutic potential, contributing not only to the cost, but may also elicit severe unfavorable adverse effects. Despite the reduction in toxicity associated with the payload, these nanotherapeutics have not been met with limited clinical success, likely due to the monotherapy regimen. The self-assembling prodrug (SAP) has been emerging as a powerful platform for enhancing efficacy through co-delivering other therapeutic modalities with distinct molecular targets. Herein, we present a comprehensive review article summarizing three unique approaches of making SAP for synergistic drug delivery: pegylation, vitamin E-derivatization, and drug-drug conjugation. These SAPNs may inevitably pave the way for developing more efficacious, clinically translatable, combination cancer nanotherapies.

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targeted accumulation for more efficacious combination cancer therapy\[45,46\]. By improving the aqueous solubility, promoting the formation of pertinent nano-sized therapeutics and controlling the release at the same time, the conjugated prodrug and its encapsulated payloads can passively target and enhance the accumulation at tumor sites via the EPR effect [47–49].

Most of the existing carriers from conventional drug delivery systems utilize “inert” excipients which have no pharmacological activity, but impose additional safety concerns and add to the overall cost [50–52]. Conversely, synergistic or additive anticancer effects have been discovered between the therapeutic prodrug nanocarriers and delivered anticancer drugs following the intracellular uptake of the drug-laden SAP and the release of the biologically functional therapeutics from the conjugates [7,53–55].

The SAP can be divided into several categories, including polymer-drug, lipid-drug, or drug-drug conjugates [56]. Owing to its high hydrophilicity, ease of formulation, low potential for toxicity, and biocompatibility, polyethylene glycol (PEG), has been extensively used as the hydrophilic segment for conjugation to lipophilic drugs (Embelin: EB; Vitamin E: VE; transfarnesylsiolesalic acid: FTS) to address solubility issues and bioavailability [57,58]. Moreover, these PEG-derived produgs are not only able to self-assemble to nanoparticles e.g. polymeric micromicelles, but can also co-deliver other water-insoluble chemotherapeutics in their hydrophobic core for synergistic anticancer activity [1,59,60]. Because of the steric hindrance provided by PEG hydrophilic corona, PEG-drug conjugates significantly improve a drug’s PK with prolonged half-life during blood circulation by avoiding the opsonization effect [61,62]. Furthermore, the PEG-drug nanotherapeutics revealed significantly improved tumor targeting efficiency through the leaky vasculature and impaired lymphatic drainage system via the EPR effect [5,61,63].

Recently, lipid-dativization appears to be another robust strategy for formulating hard-to-formulate drugs and addressing their intrinsic instability issues through facilitating their self-assembly into nanoparticles of various shapes [64–66]. It has been demonstrated that the doxorubicin (DOX)-derivatized α-α-tocopherol succinate prodrug (N-DOX-TOS) can readily form a 250 nm nanoassembly in aqueous solution upon stabilization by n-α-tocopherol poly(ethylene glycol) 2000 succinate and showed greater antitumor efficacy than free unmodified DOX [67]. Our own research has also shown that camptothecin-driven VE can form a nanofiber nanomedicine upon stabilization by a VE-based PEG carrier [9].

In addition, it has been reported that, through rational design based on the unique physicochemical properties of different drugs, amphiphilic hydrophobic drug-hydrophilic drug conjugates can self-assemble into nanotherapeutics with enhanced bioavailability, improved PK and enhanced antitumor efficacy [68]. The merit of this design is that instead of physically loading other drugs inside various nanocarriers for combination cancer therapy, the drug-drug conjugate already contains two distinctly pharmacologically active agents. Additionally, this amphiphilic drug-drug conjugation approach circumvents the nonuniform biodistribution of individual anticancer agents delivered through cocktail administration and ensures well-controlled temporal and spatial dual drug delivery vital for synergistic cancer elimination [45,46,69,70].

In this review, we will emphasize the progress regarding the PEG-drug, VE-drug, and drug-drug-based SAP conjugates for targeted drug delivery for combination cancer therapy.

2. PEG-derived SAP nanocarriers for synergistic drug delivery

Most of the nanocarrier components used in various drug delivery systems utilize “inert” excipients lacking any therapeutic activity [46,71]. The presence of large amounts of carrier materials not only increases the overall therapy cost, but also gives rise to unwanted side effects [72]. One of the most sophisticated designs of drug delivery systems is that the components forming the carriers can also have therapeutic potential. Following the intracellular delivery, the bioactive component of the delivery carriers can be liberated and cooperate with co-delivered drugs for improved synergistic cancer therapy [10]. Combination therapy with two or more drugs in a single nanocarrier working simultaneously at different molecular signaling pathways can not only maximize the anticancer efficacy, but can also be conducive to overcome drug resistance and counteract the side effects developed by monotherapy. Herein, some of the recently developed SAP amphiphilic nanocarriers with hydrophobic backbone composed of lipophilic anticancer therapeutics are discussed.

2.1. PEG-based embelin SAP as a dual functional nanocarrier for combination cancer therapy

Embelin (EB) (Fig. 1A) is a naturally occurring alkyl-substituted hydroxyl benzoinoquinone compound and a major constituent of Embelia ribes BURM. It has been shown to possess hepatoprotective, anti-inflammatory, and antidiabetic effects [73]. In addition, EB also exhibits notable antitumor activity in various types of cancers by inhibiting the activity of X-linked inhibitor of apoptosis protein (XIAP) [74,75]. EB has negligible toxicity in normal cells and thereby presents a good safety profile, as XIAP is particularly overexpressed in various drug-resistant cancer cells with a minimal role in healthy cells. Hence, XIAP has been shown to be an effective target for selectively inhibiting cancer cell growth [76,77]. Additionally through suppressing the NF-κB pathway, EB also has effects of downregulating the expression of several significant cancer promoting factors, such as XIAP, IAP/1/2, survivin, cFLIP, TRAF1, Bcl-2 and Bcl-L. [75]. However, owing to its long lipophilic acyl chain, EB is extremely hydrophobic, which renders poor aqueous solubility/bioavailability, unfavorable PK and unsatisfactory efficacy.

To address the limitations associated with EB, we developed a series of PEG-dativized EB conjugates, which differ in PEG length and amount of EB, bridging through the aspartic acid-mediated labile ester bond (Fig. 1A). Upon pegylation, the solubility of EB has been drastically increased from less than 1 mg/mL to more than 750 mg/mL. Equally important, PEG-EB conjugates retain similar cytotoxicity as the unmodified EB in three breast and prostate cancer cell lines. This effect is unlikely due to the polymer-drug conjugate surface activity as PEG-EB showed negligible hemolysis against red blood cells. More strikingly, PEG-EB produg not only self-assembles into spherical micromicelles (~20 nm) in aqueous medium, but can also efficiently encapsulate other hydrophobic drugs such as PTX (Fig. 1B) within its hydrophobic pocket to achieve synergistic cancer cell killing effect in four different breast and prostate cancer cell lines. This could be attributed to the efficient EB release from the conjugate following intracellular delivery, and consequently synergizing antitumor activity with PTX, which stabilizes the microtubes during mitosis. Moreover, we have demonstrated that the produg with two EB molecules and a longer PEG chain is markedly more effective for encapsulating PTX than the counterpart with a 1:1 PEG/EB molar ratio and a shorter PEG chain [6,58]. This is upheld by the fact that PEG5K-EB2 (0.35 μM) had a much lower critical micelle concentration (CMC) that of PEG35K-EB2 (4.9 μM) and at the same carrier/PTX molar ratios, PEG5K-EB2 showed smaller particle size with lower polydispersity, higher drug loading, and improved formulation stability than PEG35K-EB2. The strengthened formulation stability and drug loading in PEG5K-EB2 micelles is likely due to the improved PEG stealth effect, which exhibits greater steric hindrance. This prevents the ester linkage from being hydrolyzed under aqueous conditions, resulting in further stabilized micellar nanotherapeutics. Compared to the clinical PTX formulation, Taxol® (the PTX/PEG5K-
**EB₂ micellar formulation** displayed a much slower and controlled PTX release as evidenced by the $T_{1/2} = 34.1$ h vs $T_{1/2} = 6.57$ h in Taxol®. This delayed and more sustained PTX release from PEG₅₋ₓ_-EB₂ could be attributed to the strong interaction between the carriers and PTX. Additionally, EB has a benzoquinone ring and a long alkyl chain, which can form π-π stacking interactions and hydrogen bonding with PTX in addition to hydrophobic interactions, rendering enhanced overall carrier/PTX interaction. The close proximity of two EB lipidic chain in PEG₅₋ₓ_-EB₂ conjugate can facilitate the interaction of the carrier with PTX. Furthermore, PTX/PEG₅₋ₓ_-EB₂ presented a considerably higher maximum tolerated dose (MTD) as compared to Taxol [100 mg/kg vs 15 mg/kg], which is linked to slow release kinetics for PTX, lower nonspecific uptake by healthy organs, as well as the remarkable safety profile, in addition to the selectivity, anti-inflammatory and hepatoprotective activity of EB [6]. The increased MTD and enhanced safety allowed for maximal therapeutic efficacy to be achieved. In addition, PEG₅₋ₓ_-EB₂ was found to be capable of overcoming multi-drug resistance in NCI/ADR-RES cells through inhibiting the p-glycoprotein (p-gp) efflux pump [10]. Furthermore, near-infrared fluorescence (NIRF) imaging in prostate and lung cancer xenograft-bearing mice showed preferential tumor targeting for PEG₅₋ₓ_-EB₂ micelles (Fig. 1C) [6,10]. Most importantly, when compared to Taxol®, the co-delivery of PTX by SAP PEG₅₋ₓ_-EB₂ micelles significantly enhanced tumor reduction in murine models of breast and prostate cancers with no noticeable systemic toxicities (Fig. 1D). Consistent to literature [64,78,79], through anchoring the folic acid (FA), a tumor specific targeting ligand, onto the surface of PEG₅₋ₓ_-EB₂ nanomicelles, the intratumoral accumulation was greatly enhanced, which led to further boosted tumor growth suppression [10].

### 2.2. PEG-based vitamin E SAP for synergistic drug delivery

The pegylated vitamin E (VE, Fig. 2A), p-a-tocopheryl polyethylene glycol 1000 (PEG₅₋ₓ) succinate (TPGS) system is another example of a dual functional nanocarrier [80–82]. VE, a lipophilic molecule with a long alkyl tail, is conjugated to PEG₅₋ₓ through a biodegradable ester bond, allowing it to self-assemble into micelles with a hydrophobic pocket where water-insoluble drugs can be incorporated. It has been demonstrated that VE has an antitumor effect against a wide spectrum of cancers via various mechanisms including induction of apoptosis, inhibition of tumor cell proliferation and differentiation, suppression of nuclear factor-kappa B (NF-κB) activation, and so forth [76,82–85]. Due to its tunable functionalities as an emulsifier, solubilizer as well as a permeability and absorption enhancer, TPGS has been approved by the FDA as a safe pharmaceutical adjuvant for drug formulation [81,84,86,87]. Additionally, based on its ability to inhibit P-glycoprotein (p-gp) drug efflux pumps, formulations with TPGS can reverse multidrug resistance (MDR) and improve the bioavailability of anticancer carriers [80,82,83,88]. Moreover, TPGS-based nanoformulations delivering PTX or other therapeutics exhibited potentiated antitumor activity in several animal cancer models [81,82]. Nevertheless, the high CMC value (0.2 mg/ml), renders it unstable in vivo, and unable to serve as an effective nanocarrier to encapsulate therapeutics [85]. In order to improve this drug delivery system, we have developed a number of improved TPGS conjugates by varying the PEG length and altering the ratio of PEG and VE (Fig. 2A). Systemic structure and activity relationship study (SAR) demonstrated that all of the optimized prodrugs (PEG₅₋ₓ-VE, PEG₅₋ₓ-V₄, PEG₅₋ₓ-VE₄, PEG₅₋ₓ-VE₃, and PEG₅₋ₓ-VE₂) retained the intrinsic activity of TPGS in inhibiting P-gp drug efflux pump [80]. Interestingly, PEG₅₋ₓ-VE₂ outperformed other conjugates in terms of PTX drug loading and formulation stability, leading to the highest level of delaying tumor growth even in comparison to Taxol® (FDA-approved PTX formulation). While PEG₅₋ₓ-VE₂ performed best among the four systems, the drug loading capacity (DLC) is still not adequate and within a single digit. To further optimize this SAP system, a fluorescently labeled doxorubicin (FMOC) drug-interaction motif was installed at the interfacial region of PEG₅₋ₓ-VE₂ [7]. FMOC has been shown to provide strong π-π stacking and hydrophobic interactions with other aromatic moieties, including itself (Fig. 2B) [89–92]. To test this hypothesis, Doxorubicin
(DOX) was chosen as the model drug since it has several aromatic rings. Upon Fmoc addition, PEG5k-Fmoc-VE2 can physically entrap up to 39.9% DOX, which was a drastic increase over the 2.9% DOX DLC for the PEG5k-VE2, and also higher than most of the existing DOX nanoformulations [7,10,60]. Because of reinforced carrier/carrier and drug/carrier interactions, the Fmoc-embedded formulation improved the PK and MTD when delivering DOX in vivo through controlled and sustained drug release, resulting in a prominently increased tumor growth reduction over DOX/PEG5k-VE2 and Doxil® (FDA-approved DOX nanoformulation) in breast, prostate, and drug-resistant murine cancer models (Fig. 2C).

2.3. PEG-derived S-trans Trans-farnesylthiosalicylic acid SAP micellar carrier

In addition to EB and VE-based PEG SAP nanotherapeutic carriers, PEG-derived trans-farnesylthiosalicylic acid (FTS, Fig. 3) has been developed. FTS is a synthetic first-in-class direct Ras antagonist designed to inhibit overactive cell growth in cancers by interfering the anchorage of Ras on cell membrane [93,94]. Nearly one-third of human cancers have constitutive Ras expression [95,96]. By targeting Ras, FTS has shown a noticeable antitumor effect with high selectivity in a wide range of cancers [97]. Similar to EB and VE, FTS contains a long lipophilic acyl chain that gives rise to poor water-solubility/bioavailability. Pegylation not only greatly ameliorates FTS’ aqueous solubility, but also enables the formation of nanocontainers that are capable of encapsulating other hydrophobic drugs, allowing for synergistic antitumor efficacy [98].

3. Vitamin E-based SAP cancer nanotherapeutics

Most of the current approaches are centered on improving the nanocarriers in order to augment their compatibility with the therapeutic payloads. These strategies cannot accommodate some agents with complex chemical structures, such as camptothecin (CPT), despite working well for a number of anticancer drugs that have relatively simple structures. CPT, a potent chemotherapeutic, showed remarkable antineoplastic activity against a wide array of cancers by suppressing the topoisomerase I in the S-phase of the cell cycle during DNA replication [99]. However, owing to its extremely poor solubility, intrinsic instability (under physiologic conditions due to the involuntary conversion of the active lactone form to the inactive carboxylate form) and systemic toxicities, its clinical application has not been granted. Moreover, few of the existing nanocarriers can be formulated with this drug. Polymer derivatization strategy has been attempted to address these shortcomings, but have been met with limited success. This is likely attributed to ineffective drug release because of the considerable steric hindrance posed by macromolecular polymer [100,101]. Instead of optimizing the nanocarrier component, we derivatized CPT with one molecule of VE, using either an ester bond or a disulfide linkage (Fig. 4A). Surprisingly, both CPT-
VE and CPT-S-S-VE prodrugs enabled efficient self-assembly into nanofibers with the stabilization of the PEG$_{5K}$-Fmoc-VE$_2$-based nanomicellar carrier (Figs. 4B and 4C). The formation of nanofibers of PEG$_{5K}$-Fmoc-VE$_2$/CPT-VE and PEG$_{5K}$-Fmoc-VE$_2$/CPT-S-S-VE, as compared to the spherical structures for PEG$_{5K}$-VE$_2$/CPT, PEG$_{5K}$-Fmoc-VE$_2$/CPT, PEG$_{5K}$-Fmoc-VE$_2$/CPT-VE, PEG$_{5K}$-VE$_2$/CPT-S-S-VE, and spherical shapes of the self-assembling prodrug described above, are likely due to strong π–π stacking interaction arisen from Fmoc moieties among carriers, Fmoc and aromatic rings in CPT and VE, and hydrophobic interactions between alkane chains among VE between/among carriers and/or prodrugs as evidenced by the fluorescence quenching, UV absorbance, FT-IR, and NMR analysis [9].

CPT-S-S-VE showed significantly higher intracellular (Fig. 4D) and intratumoral drug release (Fig. 4F) as compared to ester-bonded prodrug (CPT-VE) due to the high glutathione levels inside cancer cells and tumor tissue [9,46,71,102], which led to the higher anticancer activity both in vitro (Fig. 4E) and in vivo (Fig. 4G). Both CPT prodrug nanofibers demonstrated greater efficacy over free CPT in curbing breast tumor development in the syngeneic mouse cancer models (Fig. 4G). While the DLC for free CPT was only 0.65% when using the PEG$_{5K}$-Fmoc-VE$_2$ carrier, it was markedly increased to 9.2% for CPT-S-S-VE with improved formulation stability. This could be due to the fact that: 1) VE in prodrugs can function as a lipid anchor to facilitate its insertion/incorporation into the hydrophobic pocket formed by VE chains in nanocarrier; 2) the increased drug/carrier interactions such as π–π stacking between the Fmoc motif and the aromatic rings of CPT [9].

The VE-derivation has also been investigated for improving the compatibility of other common difficult-to-load drugs with existing carriers. It has been shown that DOX conjugated with η-a-tocopherol succinate (NDOX-TOS) formed a nanoassembly in aqueous solution after mixing with η-a-tocopherol poly(ethylene glycol) 2000 succinate with a much higher DOX DLC (34%) and led to superior tumor growth delay to free DOX [67]. Moreover, a recent discovery has demonstrated that PTX-conjugated VE, bridged via a disulfide linkage, led to spherical nanoparticles with improved PK and enhanced tumor killing effect in vivo as compared to Taxol® [103]. This approach was realized by the hypothesis that the insertion of a single disulfide bond into hydrophobic molecules can balance the competition between intermolecular forces, resulting in the consequent nanotherapeutic self-assembly.

### 4. Drug-Drug self-assembling nanomedicines for combination cancer therapy

In another approach to enhance the delivery of anticancer agents to tumor sites in the form of self-assembled nanotherapeutics, amphiphilic drug-drug conjugates (ADDCs) have been developed by conjugating a hydrophilic drug and a hydrophobic drug with labile linkage [27,68,104-107]. The advantages of this ADDC design is multi-faceted: 1) with coupling of a hydrophilic drug molecule, the hydrophobic drug solubility issue will be addressed; 2) since the conjugates are solely composed of drugs, ADDC alleviates the limited drug loading seen in traditional nanocarriers; 3) self-assembling ADDC can prolong the blood circulation of free drugs that are normally prone to rapid clearance mediated by opsonization [108] 4) improved tumor targeted accumulation with reduced non-specific toxicity to normal tissues based on the EPR effect; 5) decreased safety concern frequently encountered through the use of synthetic nanomaterials; [109] 6) can release drugs that have different molecular targets in a simultaneously temporal-spatial controlled fashion for synergistic anticancer activity; 7) can bypass drug efflux-pump-mediated drug resistance (eg., P-gp), as ADCC SAPs are taken up by cells via endocytosis, which is independent of the transporter-facilitated intracellular delivery.

Huang P. et al. constructed an ADDC using the water-soluble drug, Irinotecan (Ir) and the water insoluble drug, Chlorambucil (Cb) that self-assembled into spherical nanoparticle (Figs. 5A and 5B). The authors demonstrated that the release of free Ir and free Cb was accelerated at weakly acidic environment, indicating potential hydrolysis in the tumor microenvironment. It was shown that the in vitro cytotoxicity of Ir-Cb ADDC was dependent on the critical aggregation concentration (CAC). When the concentration of Ir-Cb ADDC was lower than CAC, it did not produce any significant cytotoxicity. However, at concentration higher than CAC,
Ir-Cb ADCC could produce significantly higher cytotoxicity compared to free Ir and free Cb. The authors attributed this observation to higher uptake of nanoparticles by the cells and subsequent intracellular release of Ir and Cb elicited synergistic effect. The Ir-Cb ADCC nanoparticles were also shown to accumulate to a greater extent in drug-resistant MCF-7/ADR cells by overcoming P-gp efflux pump. The blood retention time and biodistribution studies demonstrated that Ir-Cb ADCC nanoparticle could circulate for longer time in blood and achieved higher tumor accumulation via EPR effect compared to Ir and Cb (Figs. 5C and 5D).

The in vivo antitumor efficacy study in MCF-7 tumor bearing nude mice showed that Ir-Cb ADCC nanoparticles produce highest reduction in tumor volume in comparison to Cb, Ir and Cb/Ir mixture (Figs. 5E and 5F) [27]. In addition, Liang et al. reported a Janus CPT-flouxuridine (FUDR, a hydrophilic chemotherapeutics) conjugate (JFCF) where two molecules of CPT were conjugated with two molecules of FUDR to produce a liposome-like nanocapsules (JFCF–NCs) around 115 nm in size with spherical morphology [68]. The CPT and FUDR were efficiently released at acidic pH and in the presence of esterase. JFCF–NCs can be internalized into a prostate carcinoma cell line rapidly with a significantly higher antiproliferative effect than free drugs. Moreover, when compared with free drugs and CPT and FUDR mixtures, JFCF–NCs elicited longer blood circulation time, increased tumor uptake, as well as enhanced tumor growth inhibition in prostate cancer [68]. In addition, Xu et al., has developed a supramolecular cisplatin-vorinostat nanodrug that can overcome drug resistance in synergistic cancer therapy [105]. Furthermore, Chlorambucil-Gemcitabine, Methotrexate-CPT, and CPT-Cytarabine ADCC self-assembling nanotherapeutics have been reported for efficacious combination cancer therapy [106-108].

5. Concluding remarks

A prodrug approach, particularly when they can self-assemble to form nano-sized drug delivery systems, is equipped with many advantages over parental drugs, such as 1) improved aqueous solubility that is more suitable for systemic administration; 2) enhanced intrinsic chemical stability through proper functionalization; 3) ameliorated bioavailability and pharmacokinetics; 4) boosted therapeutic efficacy (e.g., anticancer activity); 5) reduced non-specific systemic toxicities to healthy tissues. In addition, since a considerable portion of if not all the nanocarrier materials are comprised of therapeutically active prodrugs, SAP can constitutionally and drastically augment drug loading capacity and minimize the potential long-term adverse effects that could have been caused by using conventional nanomaterials. Furthermore, with the ability to concurrently encapsulate other therapeutic modalities, SAP can fulfill the precise and simultaneous temporal-spatial drug release, enabling the synergistic efficacy in combating cancers. Rather than improving the nanocarrier system for better drug packaging efficiency and formulation stability, SAP addresses formulation issues by having the drug anchored in carrier molecules during spontaneous self-assembly process. Moreover, by making drugs into SAP, they are able to circumvent the multi-drug resistance that often encountered by many free drugs, as which can be readily pumped out of cells by drug exporting transporters (e.g., P-gp), through nanoparticle-mediated endocytosis cellular uptake mechanism which can bypass the drug efflux transporters. Owing to these merits, a wide variety of SAPs have been developed, which has revolutionized the cancer treatment paradigm in the recent decades. Noteworthy, several aspects that are still needed to be taken into consideration when taking advantage of the SAP systems. First, prodrugs are not biologically active unless the active parent drugs are released. To boost the drug release, conjugation chemistry to anchor suitable linkers (e.g., ester bonds, disulfide linkages, and hydrazone bonds, etc.) should be carefully considered in order to utilize the distinct microenvironment in tumors (e.g., high hydrolysis and glutathione levels, and low pH) [67,110]. Additionally, the anticancer agents that either physically or covalently encapsulated to SAP systems ought to have different cancer molecular targets than those of the SAP’s in order to realize the synergistic anticancer activity. Last but not least, to allow the efficient self-assembly into nanodrugs, the constructed prodrug conjugates must be amphiphilic. Apart from chemotherapeutic agents, the SAP platform can also be applied to other therapeutic modalities that have similar functional groups, such as tyrosine kinase inhibitors, corticosteroids, anti-inflammatory agents, immunomodulators, and drugs that target infectious diseases for improved therapeutic efficacy and reduced side effects.
Declaration of Competing Interest

There are no conflicts of interest to declare.

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