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Nanomicellar carriers for targeted delivery of anticancer agents

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Abstract

Clinical application of anticancer drugs is limited by problems such as low water solubility, lack of tissue-specificity and toxicity. Formulation development represents an important approach to these problems. Among the many delivery systems studied, polymeric micelles have gained considerable attention owing to ease in preparation, small sizes (10–100 nm), and ability to solubilize water-insoluble anticancer drugs and accumulate specifically at the tumors. This article provides a brief review of several promising micellar systems and their applications in tumor therapy. The emphasis is placed on the discussion of the authors' recent work on several nanomicellar systems that have both a delivery function and antitumor activity, named dual-function drug carriers.

Chemotherapy is an important part of treatment for various types of cancers. Nevertheless, clinical application of anticancer drugs is beleaguered by problems such as poor water solubility, non-specificity and toxicity [1]. First, administration of poorly water-soluble drugs results in poor absorption and low bioavailability [2]. Second, the aggregation of water-insoluble drugs could cause local toxicity. Third, anticancer drugs are usually small-molecule drugs and are rapidly eliminated by the liver and kidneys. Furthermore, the broad tissue distribution of most anticancer drugs will lead to serious systemic toxicity. Currently, Cremophor EL (polyethoxylated castor oil)/ethanol (1:1, v/v) and certain surfactants are used to improve the solubility of anticancer drugs [3,4]. However, Cremophor EL can cause hyperactivity reactions, neuropathy and other serious side effects. In addition, the utility of conventional low-molecular-weight surfactants is limited by their high critical micelle concentrations (CMCs), which raises the concern of drug precipitation or burst release of drug upon dilution in the blood [5]. The advent of nanotechnology brings promises in drug delivery [6]. A number of macromolecular delivery systems such as **polymeric micelles**, liposomes, dendrimers and nanoparticles are under investigation to circumvent these limitations of chemotherapy and improve the potential of the anticancer drugs [7]. These vehicles can carry various types of drugs, protect them from degradation and minimize the undesirable side effect on normal tissues. Among the many studied delivery systems, polymeric micelles have gained considerable attention owing to ease in preparation, small sizes (10–100 nm), and the ability to solubilize water-insoluble anticancer drugs and effectively accumulate at the tumors [8,9]. Currently, several polymeric micelles incorporated with anticancer agents, NK012, NK105, NK911, NC-6004, SP1049C and

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Genexol-PM are under clinical evaluation [10–15], of which Genexol-PM has been approved by the US FDA for use in patients with breast cancer [16].

Many excellent reviews are available in the literature on the self-assembly of micelles, drug loading, and strategies of developing multi-functional systems for **targeted delivery** and sustained or stimuli-triggered drug release [17–21]. This review will give a brief review of several promising micellar systems and their applications in cancer therapy. The emphasis will be then placed on the discussion of several **dual-function micellar systems** that were recently developed in the authors laboratory [22–26]. In addition to a function of delivery, these micelles have antitumor activity by themselves and synergize with co-delivered anticancer agents.

Polymeric micellar systems

Polymeric micelles usually have the unique core-shell architecture composed of distinct hydrophilic and hydrophobic domains, with the structure of the copolymer usually being a di-block, tri-block or graft copolymer [5]. The hydrophobic core provides a loading space for poorly water-soluble drugs and the hydrophilic shell allows polymeric micelles gain stability in an aqueous environment [20]. The hydrophobic block should have good biodegradability and provide excellent compatibility with the loaded drugs. The most commonly used polymers for hydrophobic core formation are polyesters and polyamides. Polyesters used include polycaprolactone, poly(lactic acid) (PLA), poly(glycolic acid), and poly(lactide-*co*-glycolide), all of which have been approved by the FDA for use in various clinical applications [27–29]. Poly-amides used include poly(L-histidine), poly(L-aspartic acid) derivatives and poly(L-glutamic acid) derivatives. These biocompatible polyesters and polyamides display gradual degradation kinetics inside the body with the degradation time ranging from days to weeks. Polymeric micelles generally have very low CMCs. They range between 10^{-6} and 10^{-7} M, which are approximately 1000-times lower than low-molecular-weight surfactants [30]. The stability (CMC) of polymeric micelles varies depending on the type and molecular weight of the hydrophobic block. Generally, the higher the molecular weight of a hydrophobic block, the lower the CMC of the polymer.

One unique type of polymeric micelles composed of a cholic acid (CA) dendrimer as the hydrophobic block and PEG as the hydrophilic domain has been developed by Lam's group [31,32]. One such amphiphilic linear-dendritic copolymer, PEG_{5K}-CA₈, was composed of PEG, CA and lysine. They prepared paclitaxel (PTX)-loaded PEG_{5K}-CA₈ micelles with a tunable size of 20–60 nm. The most impressive properties of these micelles were excellent PTX loading capacity (~35%, w/w of drug polymer ratio) and superior stability (longer than 6 months). This is due to the planar structure and the facial amphiphilicity of CA and the dendritic configuration of the hydrophobic core. These PTX-loaded PEG-CA micelles demonstrated preferential tumor accumulation, excellent toxicity profiles and superior antitumor activity compared with Taxol (PTX dissolved in Cremophor EL/ethanol [1:1, v/v] mixture) and Abraxane (albumin-bound PTX), two FDA-approved PTX formulations [31]. This system has also been successfully applied to delivery of doxorubicin (DOX) to tumors [33].

Lipid-core micellar systems are another special type of polymeric micelle composed of lipid moieties as a hydrophobic block capped with a hydrophilic segment such as PEG. One unique feature of **lipid-core micelles** is that they can be readily developed. In addition, the lipid-core micelles have a relatively low CMC and demonstrate better stability compared with the conventional low-molecular-weight surfactants. Torchilin's group first developed one such micellar system that is based on a PEG-phosphatidylethanolamine (PE) conjugate [34–37]. These PEG-PE micelles had a CMC value that was at least a 100-fold lower than

conventional surfactants. The presence of two fatty acyls contributes to the enhanced interaction among the lipid chains in the micelle core, which contributes to high stability [37]. This system has been used for the delivery of several hydrophobic drugs such as PTX, tamoxifen and camptothecin [34–37].

Most micellar systems utilize PEG as the hydrophilic segment of micelles. PEG is not biodegradable and does not form toxic metabolites. At the same time, PEG is commercially available in a variety of molecular weights from 1000 to 20,000 Da. A prominent advantage of PEG modification is to impart the *in vivo* longevity to drug carriers. First, PEG can reduce unwanted aggregation due to secondary interactions between polymeric particles. Second, the surface modification of polymeric carriers with PEG can reduce the binding of plasma proteins and minimize nonspecific uptake by the reticuloendothelial system, allowing the carriers to circulate in the blood for a long period of time. For example, amphiphilic diblock copolymers of PEG-b-poly(D,L-lactide-co-glycolide) (PEG-b-PLGA) demonstrated prolonged circulation times compared with the conventional PLGA nanoparticles [28]. In addition to PEG, several other biocompatible hydrophilic polymers have been developed as steric protectors, such as poly(acryloyl morpholine) (PAM), phosphatidyl polyglycerols and polyvinyl alcohol. PAM is a non-ionic hydrophilic polymer with similar properties to PEG, which has good polymerization efficiency, low toxicity and less immunological reactions *in vivo* [38,39]. PAM-modified liposomes have demonstrated prolonged circulation *in vivo* [40]. Similarly, phosphatidyl polyglycerol- or polyvinyl alcohol-coated liposomes have illustrated prolonged circulating times *in vivo* [41,42].

Passive & active targeting via micellar systems

Targeted delivery of anticancer agents to tumor tissues not only increases the therapeutic effect of the drugs, but also reduces the toxic effects on normal tissues. It is well known that tumor blood vessels have a high proportion of proliferating endothelial cells and that capillaries of tumor blood vessels become much more permeable than the capillaries of normal tissues. In addition, tumor tissues generally have a defective lymphatic drainage system, leading to enhanced accumulation and retention of macromolecules in tumors. Therefore, given long circulation times in blood, macromolecules and particles ranging from 10 to 400 nm are preferentially and passively accumulated at tumor and inflammatory tissues [43]. Since the passive targeting phenomenon was first discovered by Maeda and Matsumura [44], numerous studies have substantiated that a nanocarrier with the size of 20–200 nm preferentially extravasates into solid tumor tissues owing to the leaky tumor vasculature [45]. Besides, the effect of size on the biodistribution of polymeric micelles is organ- and tumor-type-dependent [46]. A recent study from Lam's group compared the passive targeting of nanoparticles of different sizes in a subcutaneous model of human ovarian cancer xenograft. It was reported that particles with a size of 154 nm were significantly taken up by the liver and lungs with limited accumulation at tumor sites. By contrast, particles with a respective size of 17 and 64 nm were much more effective in passive targeting to the solid tumor [32]. Cabral and colleagues compared the targeting efficiency of polymeric micelles of different sizes (30, 50, 70 and 100 nm) in both highly and poorly permeable tumors. While all of the tested polymeric micelles penetrated highly permeable tumors in mice, only the 30 nm micelles could penetrate poorly permeable pancreatic tumors to achieve a significant antitumor effect [47]. Perrault and colleagues conducted a similar study on biodistribution of gold nanoparticles (GNP) of different sizes in a mouse model of human breast cancer xenograft (MDA-MB-435) [48]. It was shown that GNP of approximately 60 and 100 nm were more effective in accumulating at tumor than the particles of approximately 20 nm. However, larger GNP mainly stayed near the vasculature while smaller GNP rapidly diffused throughout the tumor matrix. Thus, while there is a general consensus among many studies that sub-100 nm is critical for achieving

effective tumor targeting, the optimal size of a carrier is also affected by other factors including the type of tumor studied, the surface chemistry and shape of the nanoparticles examined, as well as the goal of the study. For example, the optimal size of the particles may be different depending on whether the therapeutic agent is targeted at the tumor or the tumor vasculature, or whether the delivered agent is aimed at tumor imaging or therapeutic application [49].

The further development of the targeted polymeric micelles involves the attempt to add an active targeting moiety capable of recognizing receptors on the tumors, which will facilitate the specific interaction with the tumor cells and subsequent intracellular delivery. Various types of ligands have been examined for active targeting including antibody, peptide, aptamer and small-molecule ligands [50,51]. For example, 2C5 antibody-targeted PEG-PE/PTX mixed micelles demonstrated enhanced tumor accumulation over free PTX or PTX in plain micelles, and displayed the ability to specifically recognize and bind to the tumor cells *in vivo* [52]. OA02-peptide-decorated micelles have also been prepared to facilitate targeted delivery of PTX to ovarian cancer cells that overexpress the α -3 integrin receptor [53]. The use of small-molecule ligands has the advantages of low cost and minimal immunogenicity. The folate receptor has been shown to be overexpressed in various types of cancers and folate-decorated delivery systems have been widely used for the targeted delivery of various types of therapeutics to cancers [54]. Small molecules specific for the prostate-specific membrane antigen are another type of promising ligand for active targeting of prostate cancer that overexpresses prostate-specific membrane antigen [55]. Recently, there also has been an interest in exploring sigma receptor ligands for tumor imaging and targeted therapy [56,57]. Sigma receptors are overexpressed in a variety of human tumors including non-small cell lung carcinoma, prostate cancer, melanoma and breast cancer [58]. Another interesting approach involves simultaneous incorporation of dual ligands into a nanomicellar system. It has been reported that incorporation of dual ligands can enhance targeting efficiency and cytotoxicity to A2780 ovarian cancer cells *in vitro* and *in vivo* compared with single ligand-targeted micelles [59].

Environmentally responsive micellar systems

In addition to targeted delivery, another important consideration in the design of an optimal delivery system is controlled or triggered drug release upon reaching the tumor tissues or intra-cellular delivery to tumor cells. Ideally, polymeric micelles should be designed such that they are stable in the circulation or in normal tissues, but acquire the ability to disintegrate and release the entrapped drugs in selective areas with a unique environment, such as tumors and inflammation sites. Tumors and inflammation sites usually have mildly acidic environment. Acid-cleavable linkages such as acetal [60], orthoesters and hydrazones are stable at approximately pH 7.5 but hydrolyzed at an accelerated rate at pH values of 6 and below [61–63]. Thus, pH-sensitive polymeric micelles with acid-labile linkages are prone to disintegration and release of loaded drugs at the tumor sites [64]. For example, Kataoka's group reported coupling of an anticancer drug, adriamycin, to the core forming block through a pH-sensitive hydrazone bond. The micelles released adriamycin both time- and pH-dependently as the pH value decreased from pH 7.4 to 3.0 [62]. Another interesting example of pH-sensitive micelles was reported by Bae's group. They demonstrated that PTX-loaded cationic micelles could be shielded by electrostatically complexing with a negatively charged mPEG (2 kDa)-block-poly-sulfadimethoxine (4 kDa) at pH 7.4. This will render the particles stable in the blood circulation. Upon reaching the acidic tumor environment, the cationic micelles became de-shielded due to the protonation of polysulfadimethoxine and loss of negative charges. The regained positively charged surface of PTX-loaded micelles then facilitated interactions with the tumor cells, resulting in effective intracellular delivery of PTX [65]. Redox-potential is another significant difference

between the extra- and intra-cellular environment. It has been found that the disulfide bond linkage, though sufficiently stable in low glutathione (GSH) concentrations (2–20 μM) in the body, demonstrates a rapid cleavage (over a time scale of from minutes to hours) under the reducing conditions of high intracellular GSH concentrations of 0.5–10 mM [66]. Generally, the concentrations of GSH in tumor tissues are several times higher than those in normal tissues. As a result, polymers containing disulfide bonds illustrate the unique advantages of reduction-sensitive cleavage in the intracellular environment via thiol-disulfide exchange reactions. The disulfide linkages in these reducible polymers are located in the main or side chains, and act as cross-linkers [67,68]. Other environmentally responsive systems such as temperature-sensitive, magnetically sensitive and ultrasound-sensitive systems have also attracted increased attention recently [69].

Dual-function micellar system

PEG-derivatized vitamin E-based micellar systems

As discussed above, various types of polymeric micellar systems have been reported; however, most of them demonstrate no biological activity by themselves. One interesting approach in the design of a carrier is that components of the carrier system exhibit biological activity, either counteracting the side effects caused by the loaded anticancer drugs, or promoting synergistic effects with the incorporated drug. One example is the PEGylated vitamin E, D- α -tocopheryl PEG succinate (vitamin E TPGS or TPGS).

Vitamin E succinate (VE) D- α -tocopheryl PEG (α -TOS) is a derivative of vitamin E with unique biological properties. It demonstrates antitumor activity against various types of cancers, including oral, breast, lung and prostate [70,71]. A number of mechanisms are involved including inhibition of tumor cell proliferation and differentiation [72], inhibition of angiogenesis [73,74], suppression of NF- κ B activation [75,76] and generation of reactive oxygen species [77]. In addition, α -TOS selectively kills tumor cells without toxic effects on normal cells, such as intestinal epithelial cells, prostate cells and hepatocytes [69,78]. α -TOS can suppress tumor growth both alone and in combination with chemotherapy and/or radiation [79–81]. *In vitro*, α -TOS demonstrated enhanced cytotoxicity towards prostate cancer cells via additive or synergistic effects with DOX [80]. Treatment with low-dose calcitriol combined with α -TOS was significantly superior to the additive effect of each treatment in cultured prostate cancer cells and in animal models of prostate cancer [81]. α -TOS also synergized with PTX in inhibiting the growth of bladder cancer cells *in vitro* and *in vivo* through inhibiting the activation of NF- κ B [82]. In addition to directly potentiating the antitumor activity of anticancer drugs, α -TOS has also been reported to help overcome multidrug resistance via inhibiting the function of P-glycoprotein (P-gp) [83].

Poor water solubility of α -TOS limits its *in vivo* application. Conjugation of α -TOS with a PEG moiety can increase its solubility TPGS derivative of α -TOS. Interestingly, is a PEG_{1K} TPGS not only retains the biological activity of free α -TOS [84], but also demonstrates as an effective emulsifier, solubilizer, additive, permeability enhancer and absorption enhancer. In recent decades, TPGS has received increasing attention as an excipient in designing multifunctional drug-delivery systems. Examples of TPGS applications in nanomedicine platforms include prodrugs, emulsifiers, liposomes, nanoparticles of TPGS-based copolymers, and TPGS-based micelles [85–91].

Polymer–drug conjugates have been intensively studied to improve pharmacokinetics and pharmacodynamics of a drug. PEG conjugates of PTX, DOX or camptothecin, PLA–PTX, and PLGA–PTX have been previously developed [92]. The TPGS–DOX conjugate, as a prodrug, was able to enhance the therapeutic effects and reduce the side effects through lowering drug accumulation in the heart compared with free drug [90,91]. Coupling of folic

acid to TPGS–DOX led to enhanced antitumor activity and further reduced gastrointestinal side effects owing to the improved targeted delivery [92]. TPGS was also examined as a component of copolymer PLA–TPGS for nanoparticle formulation of anticancer drugs [93,94], and as an excellent emulsifier in the preparation of nanoparticles of biodegradable polymers such as PLGA nanoparticles [95]. TPGS demonstrated an emulsification activity that is 67-times higher than that of polyvinyl alcohol. PTX formulated in TPGS-nanoparticles demonstrated a sustained therapeutic effect, which was more effective than Taxol formulation [96,97]. TPGS has also been used as a surfactant or component in liposomes. Replacement of lipid-PEG by TPGS for liposome surface coating led to a further improvement in circulation time [98,99], which was associated with an enhanced therapeutic effect in an animal model of brain tumor. Another study reported that the use of TPGS-containing liposomes improved the permeation of dextran through Caco-2 cells without any cytotoxicity effect [100].

TPGS has micellar properties and can incorporate water-insoluble drugs [9,88] and functional materials such as carbon nanotubes [101] and quantum dots (QT) [98]. TPGS can be applied as an efficient dispersing agent of carbon nanotubes in water [101]. TPGS mixed micelles showed a 2738-fold increase in the water solubility of QT than that of free QT in water [98]. TPGS can deliver water-insoluble drugs and/or hydrophilic drugs. Feng's group reported that the hydrophilic drug cisplatin could be effectively delivered to tumor cells as TPGS–cisplatin prodrug micelles [102]. More studies from this group have illustrated that D- α -tocopheryl polyethylene glycol 2000 succinate (vitamin E TPGS2K or TPGS2K) worked more effectively than TPGS as a micellar formulation for hydrophobic drugs. TPGS2K has been shown to be advantageous over conventional TPGS in terms of lower CMC (0.0219 mg/ml vs 0.2 mg/ml), higher drug loading capacity, enhanced *in vitro* cytotoxicity as well as a synergistic effect achieved between TPGS2K and docetaxel [9]. The study from Wang *et al.* suggested that a conjugate of PEG_{2K} with two vitamin E molecules performed better than that with one molecule of vitamin E for solubilization and delivery of DOX. DOX-loaded lysine-linked di-tocopherol PEG_{2K} succinate micelles facilitated the escape of intracellularly delivered DOX from P-gp-mediated efflux and were more effective in inhibiting tumor growth than similar TPGS–DOX mixed micelles [88]. The authors have synthesized four PEG–vitamin E conjugates that differ in the molecular weight of PEG (2K vs 5K) and the molar ratio of PEG/ vitamin E (1/1 vs 1/2), and compared their efficiency in the delivery of PTX *in vitro* and *in vivo* [22]. Our data demonstrated that the conjugates with two vitamin E molecules worked better than their counterparts with one molecule of vitamin E, either PEG_{2K}- or PEG_{5K}- system. In addition, PEG_{5K}-conjugates have lower CMC values and are more effective in solubilizing PTX compared with PEG_{2K}-counterparts (Table 1). PTX formulated in PEG_{5K}–VE₂ was more stable than the other three PTX formulations and demonstrated prolonged kinetics of PTX release as reported in a dialysis assay [22]. Finally, PTX-loaded PEG_{5K}–VE₂ demonstrated a significantly improved tumor growth inhibitory effect compared with PTX formulated in PEG_{2K}–VE or PEG_{2K}–VE₂, as well as Taxol in a syngeneic mouse model of breast cancer (4T1.2) (Figure 1). More studies are needed to better define the optimal structure of PEG–vitamin E as a micellar formulation for poorly water-soluble drugs.

PEG-derivatized embelin-based micellar systems

Embelin (EB) is one of the well-studied small-molecule natural products, which consists of the dihydroxyquinone core and a long hydrophobic tail (Figure 2). It has been demonstrated that EB possesses antioxidative [103], antidiabetic [103,104], antianalgesic [105,106], anti-inflammatory [105,106], anticonvulsive [107], wound-healing [108], colitis-protective [109] and hepatoprotective activities [110]. EB also demonstrates antitumor activity in various tumor cells [105,111–114], including those of breast [111], colon [112], prostate [113] and

pancreas [114]. One major mechanism involves the inhibition of the activity of X-linked inhibitor of apoptosis protein (XIAP) [115]. XIAP has been found to be overexpressed in various types of cancers [116], particularly drug-resistant cancers, and XIAP is a promising molecular target for the treatment of cancers [117]. In addition to blocking XIAP activity, EB could inhibit tumor cell growth and induces apoptosis through the suppression of Akt/mTOR/S6K1 [118] and STAT3 signaling cascades [119], downregulation of anti-apoptotic (Bcl-2, Bcl-xL, survivin, IAP-1/2, TNF- α) [111,120] and pro-proliferative (cyclin D1) proteins [112], activation of caspase-3/9 [111,115,121], cleavage of PARP [112], and inhibition of NF- κ B activation [122–124]. EB has been reported to have a minimal effect on normal prostate epithelial and fibroblast cells with low levels of XIAP [115].

One of the major applications of EB in cancer treatment is in combination with other therapeutic agents to overcome drug resistance and/or achieve synergistic action. Mahato's group demonstrated synergistic action between bicalutamide and EB in androgen-independent human prostate cancer cells (C4-2), and the combination of bicalutamide and EB was more effective in treating prostate cancer compared with monotherapy *in vivo* [113]. However, bicalutamide exhibited agonist properties following prolonged treatment. Bicalutamide was then replaced with a new antiandrogen agent, ([S]-N-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-cyanophenyl)(methyl)amino]-2-hydroxy-2-methylpropanamide) (CBDIV17). The combination of CBDIV17 and EB resulted in supra-additive antiproliferative and apoptotic effects compared with control or mono-therapy *in vitro* and *in vivo* [125]. Devi's group reported that EB in combination with the classical apoptosis-inducing agent TRAIL caused a synergistic decrease in cell viability [126]. Synergistic effects of EB and ellagic acid was also demonstrated in pancreatic cancer [127]. In another study with HepG2/2.2.1 cells, Che *et al.* reported that combination of flavopiridol and EB could inhibit cell proliferation more effectively than each drug alone [128]. Recently, the authors have demonstrated that EB synergized with PTX at subeffective doses against DU145 prostate cancer cells [23].

EB is poorly water-soluble and the unfavorable pharmacokinetic/pharmacodynamic profile of EB limits its application. Several strategies have been developed to enhance its water solubility including an EB-phospholipid complex formulation [129] or the use of a micellar delivery system [23,24,113,130]. Mahato's group synthesized a polyethylene glycol-b-poly (carbonate-co-lactide) (PEG-b-p(CB-co-LA)) copolymer that is effective in codelivery of bicalutamide and EB [113]. They further extended this work to the codelivery of CBDIV17 and EB [125].

As a different approach to improving EB solubility, the authors developed a PEG (MW = 3500)-EB prodrug via a cleavable ester linkage [23]. The PEG_{3.5K}-EB conjugate was readily soluble in aqueous solution at >200 mg/ml. Interestingly, PEG_{3.5K}-EB self-assembles to form micelles that are effective in solubilizing several hydrophobic drugs including PTX, DOX and EB itself. A subsequent structure-activity relationship study demonstrated a similar conclusion as that with PEG-vitamin E conjugates:

- PEG conjugates with two molecules of EB were more effective than the conjugates with a 1:1 molar ratio of PEG/EB;
- Conjugates with PEG_{5K} worked better than their counterparts with PEG_{3.5K} [24]. The sizes of all PEG-EB conjugates were small (20–30 nm) and loading of PTX into the micelles demonstrated minimal effect on size [23,24].

In vitro studies reported that PEG-EB conjugates well-retained the EB activity, particularly for the conjugates with PEG_{3.5K} and/or one molecule of EB [23]. More importantly, PEG-EB synergized with PTX at subeffective concentrations [23]. It is likely that EB is released

from the conjugate following intracellular delivery and executes the antitumor effect by itself or synergizes with PTX in antitumor activity.

The authors evaluated the biodistribution and tumor-targeting efficiency of PEG_{5K}-EB₂ micelles in a mouse xenograft model of human prostate cancer (PC-3) using a hydrophobic near infrared fluorescence dye, 1,10-dioctadecyl-3,3,30,30-tetramethylindodicarbocyanine perchlorate, D-307. The dye was physically loaded into the PEG_{5K}-EB₂ micelles. Whole body near IR fluorescent imaging showed that a noticeable signal in tumor was observed as early as 2 h postinjection. The signal peaked at approximately 24 h and remained clearly visible 48 h after injection (Figure 3). By contrast, little fluorescence signal was observed in major organs including the liver and spleen (Figure 3). PTX-loaded PEG_{5K}-EB₂ micelles demonstrated an excellent safety profile with maximum tolerated dose of 100–120 mg PTX/kg, which was significantly higher than that (15–20 mg PTX/kg) for Taxol [24]. In addition, superior antitumor activity over Taxol formulation was demonstrated in both breast and prostate cancer models [24]. In a human prostate cancer xenograft model (PC-3), complete inhibition of tumor growth was achieved with minimal toxicity to the animals (Figure 4).

Studies conducted so far suggest that PEG-derivatized EB may serve as another effective and dual-function carrier to achieve synergistic activity with co-delivered anticancer agents [23,24]. One of the advantages of our synthesis scheme is its flexibility, which allows more systematic SAR studies to further optimize our delivery system. For example, one additional environmentally sensitive linkage may be introduced to PEG-EB conjugates to further facilitate the release of EB following intracellular delivery. The stability of the PEG-EB-based micellar system may also be improved via disulfide-mediated cross-linking. Finally, various EB derivatives with improved affinity towards XIAP have been reported [117]. Their utility as part of an improved dual-function carrier can also be tested. These studies are currently ongoing in our laboratory.

PEG-derivatized *S-trans*, *trans*-farnesylthiosalicylic acid-based micellar system

S-trans, *trans*-farnesylthiosalicylic acid (FTS) (Figure 5) is a synthetic farnesylcysteine mimetic that acts as a potent and especially nontoxic Ras antagonist [131,132]. Constitutively active Ras caused by mutation in the Ras family of proto-oncogenes is present in one-third of human cancers [133], with the highest incidence of mutational activation of Ras being detected in pancreatic (90%) and colon (50%) cancers [133–135]. Ras is also activated in cancer cells by other mechanisms. In particular, hyperactivation of the EGF receptor tyrosine kinase activity causes persistent activation of Ras and Ras-mediated signaling [136]. The activated form of Ras constitutively activates its downstream effectors, contributing to cell transformation [137]. FTS can inhibit both oncogenically activated Ras and growth factor receptor-mediated Ras activation, resulting in the inhibition of Ras-dependent tumor growth [138]. FTS can inhibit Ras transforming activity and reverse the transformed phenotype of Ras-transformed fibroblasts [139]. FTS has demonstrated significant reduction of Ras levels in a wide array of established cancer models and inhibition of tumor growth in animals with no adverse toxicity [140]. One major mechanism involves affecting the membrane interaction of Ras by competing with Ras for binding to Ras-escort proteins, facilitating its degradation, and thus disrupting Ras protein to signal in the plasma membrane [139–141].

Similar to EB, the major application of FTS in cancer treatment is in combination with other drugs of different mechanisms of action [142–144]. The combined treatment of FTS and gemcitabine demonstrated a synergistic effect in inhibiting the growth of the Panc-1 tumor, resulting in an 81% reduction in tumor volume, which compared favorably to individual

drug treatments. In a clinical study, the combination of gemcitabine and FTS appeared to be well-tolerated and clinical response was demonstrated in patients with pancreatic adenocarcinoma [142]. The combination of FTS and a glycolysis inhibitor 2-deoxy-D-glucose demonstrated an additive effect on inhibition of cancer cell proliferation [143]. The combined application of FTS and histone deacetylase inhibitors such as valproic acid could synergistically inhibit the proliferation of cancer cells that express oncogenic K-Ras through downregulating Ras and blocking the expression of survivin and aurora-A [144]. Kloog's group demonstrated that FTS could chemosensitize Panc-1 pancreatic cancer cells and SW 480 colon cancer cells to cytotoxic drugs without developing drug resistance [145]. FTS also exhibits anti-inflammatory activity and can induce an increase in regulatory T cells in mouse splenocytes [146]. Combination of celecoxib and FTS enhanced the anti-inflammatory activity on T cells independent of prostaglandin E2 production [147]. The authors have recently demonstrated that FTS synergizes with curcumin in various types of cancers including breast and prostate cancer [Chen Y, Li S, unpublished data].

FTS has a hydrophobic nature and limited oral bioavailability [148]. Formulations for FTS have been rarely reported. One example is FTS-eluting fibers that exhibited controlled release characteristics and effectively induced brain tumor growth inhibition [148]. Our group has recently reported that coupling of the hydrophobic FTS to PEG leads to a significant increase in water solubility [25]. Similar to PEG-EB conjugates, PEG-FTS conjugates self-assemble to form micelles that are effective in solubilizing hydrophobic drugs. Again, the EB conjugates with PEG_{5K} work more effectively than their PEG_{3.5K} counterparts and the PEG-FTS₂ conjugates performed better than the PEG-FTS conjugates [23–25]. In addition, the authors have demonstrated that a conjugate with an ester labile linkage (PEG_{5K}-FTS₂ [L]) is more biologically active than a similar conjugate with a relatively stable amide linkage (PEG_{5K}-FTS₂ [S]) [25]. PEG_{5K}-FTS₂ (L) showed a level of cytotoxicity that was similar to that of free FTS in both 4T1.2 and HCT-116 cell lines, much more active than PEG_{5K}-FTS₂ (S) (Figure 6). It is unlikely that this is attributed to differences in the surface activity of the two conjugates, as both reported minimal hemolytic activity at the concentrations that were much higher than those used in the cytotoxicity study [25]. It is likely that FTS is much more readily released from PEG_{5K}-FTS₂ (L) by esterases following intracellular delivery. This was supported by the data that treatment of HCT-116 cells with PEG_{5K}-FTS₂ (L) also led to a significant decrease in the protein expression level of Ras (Figure 7). The authors have further demonstrated that PEG_{5K}-FTS₂ (L) was more active than PEG_{5K}-FTS₂ (S) in mediating PTX delivery and synergistic antitumor activity both *in vitro* and *in vivo* [25]. A similar result was achieved when PEG-FTS was used for the delivery of curcumin [Chen Y, Li S, Unpublished data]. These data strongly support our notion that PEG-FTS is a dual function carrier and that effective release of FTS is critical for synergistic action with co-delivered anticancer agents.

Dual-function micellar systems with drug-interactive domains

Despite the various differences among the reported micellar systems, essentially all of them are designed to load drugs through the principle of hydrophobic/hydrophobic interactions. However, most of the existing drugs or drug candidates are only moderately hydrophobic agents. The carrier/drug incompatibility may account for, among others, the limited drug loading capacity and formulation stability. Park's group has reported that inclusion of less hydrophobic and hydrogen bond-forming 'hydrotropic motifs' into the hydrophobic domain of polymeric micelles significantly improved the compatibility of the core-forming blocks with the drugs that were not entirely hydrophobic/lipophilic, leading to significant improvement in both drug loading capacity and the colloidal stability of drug-loaded micelles [149–151]. However, this concept has not been tested in lipidic systems. Interestingly, PEGylated surfactants with vitamin E, EB or FTS as a hydrophobic domain

work significantly better than those with simple lipids as micellar formulations [Lu J, Zhang X, Li S, Unpublished Data]. Vitamin E, EB and FTS all have an interfacial aromatic ring linked to an acyl chain, which may contribute to the improved formulation properties over the surfactants with simple lipid chains. These studies highlight the benefit of introducing additional structural variables to the traditional lipid-based excipients. The authors recently hypothesized that incorporation into a surfactant of a drug-interactive motif at the interfacial region will introduce an additional carrier/drug interaction mechanism, which shall lead to improvement in both drug-loading capacity and formulation stability [152]. This concept was tested with JP4-039, a mitochondria-targeted antioxidant and also a poorly water-soluble agent [153]. Our group has developed a simple screening process to identify agents that have potential drug-interactive activities based on the ability to prevent the formation of JP4-039 crystals in aqueous solution [152]. Among several motifs screened, the 9-fluorenylmethoxycarbonyl (Fmoc) moiety, a functional group that is routinely used for amino acid protection, was the most potent one [152]. The authors designed and synthesized a micelle-forming PEG-lipopeptide conjugate with a lysine branching segment and two Fmoc groups located at the interfacial region, and two oleoyl chains as the hydrophobic core. The authors found that incorporation of Fmoc motifs into a PEG-lipopeptide conjugate led to a significantly improved formulation with respect to both JP4-039 loading capacity and the stability of JP4-039-loaded micelles [152]. The authors then demonstrated that this micellar formulation can also be effectively used in formulating six other drugs of diverse structures including probucol, niclosamide, progesterone, cyclosporin A, nifedipine and griseofulvin [26]. These data strongly suggest that α -Fmoc behaves as a 'formulation chemophor', or a structural unit capable of interacting with many pharmaceutical agents. This has led us to test whether incorporation of a Fmoc motif can further improve the performance of the PEG_{5K}-FTS₂ micellar system. Indeed, this approach has led to 3.9- and threefold increase in drug loading capacity for PTX and DOX, respectively. In addition, the stability of drug-loaded formulations was significantly improved [Zhang X *et al.*, Unpublished Data]. More studies on the *in vitro* and *in vivo* characterization of the improved dual-function carrier are ongoing in our laboratory.

Conclusion

Several micellar systems have been developed in the authors laboratory that demonstrate potential as effective and dual-function carriers for poorly water-soluble anticancer agents. Despite the promising data, much remains to be done to further demonstrate their potential, in particular more toxicity and pharmacokinetic studies. It should be noted that although the three different systems discussed here share substantial similarity in design and performance both *in vitro* and *in vivo*, they shall find different application in the treatment of tumors with different molecular changes. For example, PEG-vitamin E-based systems should be more applicable for delivery of drugs to tumors in which overexpression of P-gp plays an important role in drug resistance. On the other hand, PEG-EB-based systems might be more suitable for tumors in which overexpression of XIAP is implicated in drug resistance. It remains to be tested whether mixed micelles composed of all of the different components can be used for the treatment of a tumor in which multiple mechanisms are involved in drug resistance. This will be addressed in the future.

Key Terms

Polymeric micelles

Nanosized colloidal particles formed by amphiphilic polymers dispersed in aqueous media

Targeted delivery	Selective delivery of drugs and other therapeutic agents to the designated sites. The goal is to increase the therapeutic efficacy of the drugs and reduce the side effects on normal tissues
Dual-function micellar systems	Drug carriers that have both a delivery function and pharmacological activity
Lipid-core micelles	Micelles that are formed using lipid moieties as hydrophobic blocks capping hydrophilic polymer chains

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Executive summary

Polymeric micelles as promising nanocarriers for the treatment of cancer

- Polymeric micelles usually have the unique core-shell architecture composed of distinct hydrophilic and hydrophobic domains.
- Long-circulating micelles can be prepared by grafting steric protectors such as PEG onto the micelles, which can reduce the nonspecific uptake by reticuloendothelial system.
- Nanoparticles with the size of 20–100 nm preferentially and passively extravasate into and accumulate at solid tumor tissues due to tumors leaky vasculature and poor lymphatic drainage.
- Incorporation of an active targeting moiety capable of recognizing receptors on the tumor cells will facilitate the specific interaction with the tumor cells and subsequent intracellular delivery.
- Stimuli-sensitive micelles are capable of disintegrating and facilitating drug release in response to changes in the local environment, such as decreased pH and increased temperature.

Development of dual function micelles to improve drug delivery

- An attractive approach in the design of a carrier is that components of the carrier system exhibit biological activity, either counteracting the side effects caused by the loaded anticancer drugs, or promoting synergistic effects with the incorporated drug.
- PEG-derivatized vitamin E, embelin and *S-trans, trans*-farnesylthiosalicylic acid represent three different dual-function nanomicellar carriers that facilitate the delivery of anticancer agents and synergize with co-delivered agents via distinct mechanism of action.
- Introduction of a drug-interactive motif at the interfacial region represents an effective strategy in improving the performance of lipid-core micelles such as PEG-*S-trans, trans*-farnesylthiosalicylic acid with respect to both drug loading capacity and the colloidal stability of the drug-loaded micelles.

Figure 1. Enhanced antitumor activity of paclitaxel formulated in PEG_{5K}-VE₂ micelles
 BABL/c mice were inoculated s.c. with 4T1.2 cells (2×10^5 cells/mouse). 5 days later, mice received various treatments on days 1, 3, 5, 9, 12 and tumor growth was monitored and plotted as relative tumor volume (mm³). $P < 0.02$ (PEG_{5K}-VE₂/PTX vs Taxol, PEG_{2K}-VE/PTX or PEG_{2K}-VE₂/PTX). N=5.
 PTX: Paclitaxel; VE: Vitamin E succinate.
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Figure 2.
Embelin.

Figure 3. *In vivo* near-infrared fluorescence imaging over time as indicated in prostate cancer PC-3 xenograft-bearing mice at 2, 24 and 48 h following intravenous injection of PEG_{5K}-embelin₂ (PEG_{5K}-EB₂) micelles co-loaded with paclitaxel and 1,10-dioctadecyl-3,3,30,30-tetramethylindodicarbocyanine perchlorate, D-307. Reproduced with permission from [24].

Figure 4. Enhanced antitumor activity of paclitaxel formulated in PEG_{5K}-embelin₂ micelles
 Nude mice were inoculated subcutaneously with PC-3 cells (2×10^6 cells/mouse). A week later, mice received various treatments on days 1, 3, 7, 10, 13, 24 and 28, and tumor growth was monitored and plotted as relative tumor volume. $p < 0.005$ (20 mg/kg PTX/PEG_{5K}-EB₂ vs Taxol), $p < 0.01$ (10 mg/kg PTX/PEG_{5K}-EB₂ vs Taxol), $p < 0.05$ (20 mg/kg PTX/PEG_{5K}-EB₂ vs 10 mg/kg PTX/PEG_{5K}-EB₂), $N = 6$.
 EB₂: Embelin₂; PTX: Paclitaxel.
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Figure 5.
S-trans, trans -farnesylthiosalicylic acid.

Figure 6. Cytotoxicity of free *S-trans, trans* -farnesylthiosalicylic acid (FTS), PEG_{5K}-FTS₂ (L), and PEG_{5K}-FTS₂ (S)

(A) 4T1.2 mouse breast cancer cell line and (B) HCT-116 human colon carcinoma cell line. Reproduced with permission from [25].

Figure 7. Effects of *S-trans, trans*-farnesylthiosalicylic acid, PEG_{5K}-*S-trans, trans*-farnesylthiosalicylic acid₂ (L), and PEG_{5K}-*S-trans, trans*-farnesylthiosalicylic acid₂ (S) on total Ras expression by western blot analysis

Cells grown in medium containing 5% fetal bovine serum were treated with 0.1% dimethyl sulfoxide (control), PEG_{5K}-FTS₂ (L), PEG_{5K}-FTS₂ (S), and free FTS (at a FTS concentration of 10 μM), respectively, for 48 h. Total cell lysate was subjected to western blot analysis. Anti-Ras antibody was used to determine the total Ras levels in the cells.

FTS₂: *S-trans, trans*-farnesylthiosalicylic acid₂.

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Table 1
In vitro and *In vivo* characterization of free and drug-loaded dual function micelles.

Carriers	CMC (μM)	Size (nm)		<i>In vitro</i>	<i>In vivo</i>	Ref.
		Free micelle	Drug-loaded micelle			
TPGS	130.72	–	–	–	–	[9]
TPGS2K	8.65	33.0 ± 0.2	30.2 ± 0.4 (DXL)	Synergistic effect between TPGS2K and DXL	Not done	[9]
PLV2K	0.36	14.2 ± 4.3	16.4 ± 2.7 (DOX)	Similar to free DOX in MCF-7 cells but more potent than free DOX in resistant MCF-7/ADR cells	More potent than free DOX in murine breast cancer model 4T1	[88]
PEG ₂ K-VE	3.56	21.5 ± 0.7	34.4 ± 1.22 (PTX)	Similar to free DOX in MCF-7 cells but more potent than free DOX in resistant MCF-7/ADR cells	Comparable to Taxol in murine breast cancer model 4T1.2	[22]
PEG ₂ K-VE ₂	1.15	18.9 ± 0.1	26.1 ± 2.42 (PTX)	Similar to free PTX in MCF-7 cells but more potent than free PTX in NCI/ADR-RES cells	Comparable to Taxol in murine breast cancer model 4T1.2	[22]
PEG ₅ K-VE	0.58	19.7 ± 0.1	21.3 ± 0.60 (PTX)	Similar to free PTX in MCF-7 cells but more potent than free PTX in NCI/ADR-RES cells	Not done	[22]
PEG ₅ K-VE ₂	0.30	18.8 ± 0.1	20.1 ± 0.72 (PTX)	Similar to free PTX in MCF-7 cells but more potent than free PTX in NCI/ADR-RES cells	More potent than Taxol in murine breast cancer model 4T1.2	[22]
PEG _{3.5} K-EB ₂	4.86	22.8 ± 0.3	143 ± 17 (PTX)	More potent than free PTX in MDA-MB-231, 4T1, PC-3 and DU145 cells.	Not done	[23]
PEG ₅ K-EB ₂	0.35	20.6 ± 0.1	22.0 ± 0.28 (PTX)	More potent than Taxol in 4T1.2, PC-3 and DU145 cells	More potent than Taxol in murine breast cancer model 4T1.2 and human prostate cancer xenograft model PC-3	[24]
PEG ₅ K-FTS ₂	0.68	17.6 ± 0.9	24.93 ± 1.2 (PTX)	More potent than free PTX in 4T1.2, PC-3, DU145 and HCT-116 cells	More potent than Taxol in murine breast cancer model 4T1.2	[25]

CMC: Critical micelle concentration; DOX: Doxorubicin; DXL: Docetaxel; EB: Embelin; FTS: *S-trans, trans*-famesylthiosalicylic acid; PLV2K: Lysine-linked di-tocopherol polyethylene glycol 2000 succinate; PTX: Paclitaxel; TPGS: D- α -tocopheryl polyethylene glycol 2000 succinate; VE: Vitamin E succinate. Data taken from [9,22–25,88].