Review Article

Theme: Translational Application of Nano Delivery Systems: Emerging Cancer Therapy
Guest Editors: Mahavir B. Chougule and Chalet Tan

Polymeric Micelles: Nanocarriers for Cancer-Targeted Drug Delivery

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Abstract. Polymeric micelles represent an effective delivery system for poorly water-soluble anticancer drugs. With small size (10–100 nm) and hydrophilic shell of PEG, polymeric micelles exhibit prolonged circulation time in the blood and enhanced tumor accumulation. In this review, the importance of rational design was highlighted by summarizing the recent progress on the development of micellar formulations. Emphasis is placed on the new strategies to enhance the drug/carrier interaction for improved drug-loading capacity. In addition, the micelle-forming drug-polymer conjugates are also discussed which have both drug-loading function and antitumor activity.

KEY WORDS: cancer therapy; drug-interactive domain; dual-functional carrier; micelles; targeted delivery.

INTRODUCTION

Chemotherapeutic agents are typically water-insoluble, and their therapeutic outcome is compromised by the short circulation time and systemic toxicity. During the past century, tremendous efforts have been made to circumvent these limitations and improve the therapeutic benefit of anticancer therapeutics. This was originated from the concept of "magic bullet" that was proposed by Paul Ehrlich, recipient of the Nobel Prize for Physiology or Medicine in 1908, which suggests the benefit of targeted delivery of drug to the diseased cells. In the past decades, nanocarriers have emerged as an attractive research field in cancer therapy, including liposomes, micelles, and nanoparticles made of various materials. Polymeric micelles are extensively studied carriers for the delivery of poorly water-soluble drugs. By enhancing the aqueous solubility and prolonging the blood half-life of chemotherapeutic agents, the anticancer agents can passively accumulate in the tumor site through the leaky vasculature *via* the enhanced permeability and retention (EPR) effect (1,2). Compared with other drug carriers, micelles have the advantages of very small size (10–100 nm), which is critical for passive targeting to solid tumors, particularly the poorly vascularized tumors (3).

Based on the type of intermolecular forces driving the micelle formation, block copolymer micelles can be divided into several categories including hydrophobically assembled amphiphilic micelles, polyion-complex micelles, and micelles stemming from metal complexation (4). The hydrophobically assembled micelles usually consist of amphiphilic macromolecules that have distinct hydrophobic and hydrophilic domains, and the commonly used block segments of copolymers have been summarized (5). Upon exposure to aqueous medium, the amphiphilic molecules are spontaneously self-assembled into supramolecular core/shell structures, and water-insoluble drugs can be loaded into the hydrophobic cores.

Micelles have demonstrated a variety of shapes such as spheres, rods, vesicles, tubules, and lamellae depending on the relative length of hydrophobic/hydrophilic blocks as well as solvent environment (6–8). Morphology of micelles has significant impact on the pharmacokinetic properties of micelles. For example, worm-like filomicelles have shown ten times longer circulation time compared with the spherical counterpart made of similar material (9).

The most commonly used hydrophilic segment of micelles for drug delivery is poly (ethylene glycol) (PEG), with a molecular weight of 2–15 kDa. PEG is highly water-soluble, non-toxic and neutrally charged. PEG forms a hydrophilic corona on the surface of micelles which minimizes the non-specific interaction with blood components and prolongs the circulation time. Besides PEG, other polymers including poly(*N*-vinyl pyrrolidone) (PVP) (10) and poly(*N*-isopropyl acrylamide) (pNIPAM) (11) have also been used as hydrophilic portion of micelles.

Most polymeric systems involve the use of polymers as the hydrophobic domain including polyesters such as poly(lactic acid) (PLA) and polyamides such as poly (L-lysine) (PLL) and poly (beta-amino ester). Biocompatibility and biodegradability are two important prerequisites in designing these micellar carriers for clinical application. Polyesters and polyamides undergo enzyme-catalyzed hydrolysis *in vivo* and thus are considered biodegradable. There are several polymer

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micelle systems that have been studied in clinical phase (12). For example, Genexol-PM, a polymeric micelle formulation of paclitaxel, was evaluated in a phase I study in patients with advanced malignancies with emphasis on pharmacokinetics evaluation (13). Phase II clinical trial of Genexol-PM was performed in patients with metastatic breast cancer (14) and advanced non-small-cell lung cancer (15). Some other micellar formulations also have their phase I clinical trial completed, including paclitaxel-incorporated micellar formulation, NK105 (16), pluronic polymer-bound doxorubicin (SP1049C) (17) and NK911, a micelle-encapsulated doxorubicin (18).

Another type of polymeric micelles that have been investigated involves the use of lipids as the hydrophobic core. For example, Torchilin's group has synthesized several PEG-diacyllipid conjugates, in which the hydrophobic segments are lipids of various acyl chains such as phosphatidylethanol-amine (PEG-PE) (19). Due to the strong hydrophobic interactions between the double acyl chains (20), the PEG-PE conjugates can form stable micelles with very low CMC value ($\sim 10^{-5}$ M) (21). These micelles can solubilize many types of poorly water-soluble drugs including paclitaxel (2), tamoxifen (2), porphyrin (2), camptothecin (22), and vitamin K3 (23). The PEG-PE micelles exhibit favorable stability, longevity in blood and tumor accumulation *via* the EPR effect (20,24). In contrast to liposomes (25), the small size of micelles enables them to effectively penetrate the vasculature of tumors, even for those with very low cutoff size (26).

Recently, Lam and colleagues have developed a series of micellar systems composed of PEG-cholic acid (CA) conjugates (27). A conjugate of eight CA molecules with one PEG5000 chain (PEG_{5K}-CA_8) was shown to load paclitaxel with high loading capacity (7.3 mg paclitaxel/mL) and a size of 20–60 nm (27). These paclitaxel-loaded PEG_{5K}-CA_8 micelles achieved improved antitumor effect and showed less toxicity in murine models of ovarian cancer compared to Taxol® and Abraxane® at equivalent paclitaxel doses (27). Phase I clinical trial of paclitaxel-loaded PEG_{5K}-CA_8 micelles demonstrated the superior anticancer efficacy and tolerance (28). In addition, compared to PEG_{5K}-CA_8 micelles, a similar micellar carrier PEG_{2K}-CA_4 was demonstrated to have higher doxorubicin loading capacity and more sustained drug release profile (28).

The last decade has seen significant progress in the development of various micellar systems for targeted delivery of anticancer agents (5,29,30). However, much improvement is still needed for the existing systems with respect to drug-loading capacity and formulation stability. Despite the structural differences among the reported micellar systems, most of them mainly rely on hydrophobic/hydrophobic interaction for drug incorporation into the hydrophobic cores. Such mechanism, while working well for some highly lipophilic drugs, may not provide sufficient drug/carrier interaction to effectively load other drugs. Various strategies have been proposed to introduce additional drug-interactive domains into the micellar system to improve the overall carrier/drug interaction. In addition, progress has been made in developing dual-functional carriers that demonstrate both delivery function and antitumor activity. The following two sections will summarize some of the works from our lab and other labs on developing improved micellar systems for anticancer agents.

MICELLES WITH BUILT-IN DRUG-INTERACTIVE DOMAIN AS IMPROVED DELIVERY SYSTEMS FOR ANTICANCER AGENTS

The drug-loading capacity of polymeric micelles is critically dependent on the compatibility between the drug and the micelle core (31). A series of studies have demonstrated that the drug-loading capacity of micelles can be greatly enhanced by optimizing chemical structures of the inner core segment for stronger drug/carrier interaction. A typical example of the strategy for enhanced compatibility between drug and block copolymer is the development of hydrotropic polymers. Hydrotropy refers to a phenomenon that the aqueous solubility of a poorly soluble compound is significantly enhanced by the presence of large amounts of a second solute, named hydrotrope (32). The hydrotropes aggregate only above a certain concentration, which is known as the minimal hydrotrope concentration (MHC) (33). Various studies have been carried out to elucidate the process of hydrotropic solubilization. Although the exact mechanism is not fully clarified, it may involve multiple non-covalent interactions including hydrophobic interaction, hydrogen bonding (34) as well as parallel stacking effect (35,36).

Most of the hydrotropes include an aromatic ring substituted by heteroatoms. The aromatic rings are basically hydrophobic, which can stack with each other or with the benzene rings in drug molecules (37). In addition, the polar groups may interact with drugs *via* hydrogen bonding (38). For example, nicotinamide, a typical hydrotrope, has been shown to form complexes with various hydrophobic drugs and enhance their water solubility (39). It has a pyridine ring that promotes the π-π stacking with drug molecules through its planarity (40). In addition, self-association of nicotinamide was shown to play a major role in the hydrotropic solubilization of riboflavin instead of complexation between the two species (32). Another example is *N*,*N*-diethylnicotinamide, which was shown to be the most effective hydrotrope for solubilizing paclitaxel among more than 60 structures tested (41). One drawback of hydrotropic solubilization is that high concentration of hydrotrope is usually required, which may cause various side effects. This is due to the high MHC of usual hydrotropes of about 1 M, compared to the critical micelle concentration (CMC) of typical micelles at 10^{-2}–10^{-3} M (42). To overcome this barrier, hydrotropic polymers have been developed which can dramatically enhance the local concentration of hydrotropes. Park's group has synthesized PEG block copolymers with hydrotropes linked with the hydrophobic domain of polymeric micelles, which appeared to be a versatile strategy to enhance the water solubility of various hydrophobic drugs of different structures (43,44). The key factors that affect the performance of hydrotropic polymers include the polymer backbone, type and orientation of hydrotropic moieties and the spacer groups connecting the backbone and hydrotropes. The review by Ooya *et al.* provides a comprehensive summary of the studies regarding the structure-activity relationship of hydrotropic polymers (36).

Besides hydrotropic effect, additional non-covalent interactions have been employed to improve drug-loading in polymeric micelles. For example, modification of poly(ethylene glycol)-poly(beta-benzyl L-aspartate) (PEG-PBLA) block

copolymer with benzyl ester on the PBLA chain enhanced the loading efficiency and stability of camptothecin-loaded micelles (45). Incorporation of hydrogen bonding urea-functional groups into block copolymers led to decreased CMC and improved stability of doxorubicin-loaded micelles (46). Similarly, the stability of micelles was enhanced with increased number of acid/urea groups in the micelles, which was due to improved hydrogen bonding between the carrier molecules and acid-amine ionic interaction between the drug and carrier (47). In addition, the loading of indomethacin and ibuprofen into polymeric micelles was dramatically improved by the acid-base interaction between hydrophobic segments of micelles and guest molecules containing carboxylic acid groups (48). Furthermore, Kataoka's group has shown that the encapsulation of cisplatin into polymeric micelles was facilitated by metal-ligand coordination (49,50). Inclusion of aromatic end groups (*e.g.* benzoyl and naphthoyl) has also been shown to improve the loading of paclitaxel into mPEG750-b-oligo(epsilon-caprolactone)$_5$-based oligomeric micelles (51).

We have recently demonstrated that the introduction of Fmoc as a "drug-interactive domain" can also significantly improve the drug-loading capacity of both emulsion and lipid-core micellar system (52). A series of PEGylated lipopeptide surfactants were designed and constructed to solubilize a synthetic antioxidant, JP4-039. Several ε-Boc lysine derivatives with various protective groups at α-NH$_2$ position were tested for their ability to solubilize JP4-039, among which the α-Fmoc-ε-Boc lysine was shown to be the most potent one (52). Incorporation of this drug-interactive motif Fmoc into drug-loaded emulsion led to significant increase in the formulation stability. We then designed another polymer-based micelle system, in which the Fmoc motifs were located at the interfacial region of lipopeptide surfactants with PEG$_{5K}$ as the headgroup and two oleoyl chains as the core-forming segment (53) (Fig. 1). The PEG$_{5K}$-(Fmoc-OA)$_2$ exhibited lower CMC value and significantly improved loading capacity for paclitaxel compared with an analogue without Fmoc motifs. The paclitaxel-loaded PEG$_{5K}$-(Fmoc-OA)$_2$ micelles showed increased anticancer effect over Taxol *in vitro* and *in vivo*. In addition, seven other drugs were effectively loaded into PEG$_{5K}$-(Fmoc-OA)$_2$ micelles, which suggests the utility and versatility of this platform for a broad range of drugs with different structures (53). Although the exact mechanism of carrier/drug interaction is not fully understood, the hydrophobic interaction and π-π stacking effect possibly contribute to the compatibility between the drug and carrier (Fig. 1).

PEG-DRUG CONJUGATES AS DUAL-FUNCTION CARRIERS FOR CANCER TARGETED DELIVERY

Rationale of Combination Therapy Using Polymer-Drug Conjugates as Carriers

As discussed above, improved drug-loading capacity, stability and tumor-specific distribution can be achieved by various strategies. However, most of the polymeric materials for drug delivery are "inert" and lack of therapeutic activity. In addition, the use of large amounts of carrier materials may impose safety concerns (54). Since Ringsdorf (55) proposed the concept of "polymeric prodrug" in 1975, the utility of polymer-drug conjugates in clinical therapy has been well

demonstrated. Interestingly, the conjugate of hydrophobic drug with hydrophilic polymers might be self-assembled into micelles, which can be potentially useful for the loading of another therapeutic molecule. Drug encapsulation with a biologically active carrier is an attractive strategy as it represents a unique form of combination. Combination therapy with multiple agents working at several signaling pathways at the same time could not only lead to maximized anticancer effect but also help to overcome the drug resistance (56). For example, the combination of PGA-paclitaxel conjugate with cisplatin (57) or carboplatin (58) has shown improved therapeutic benefits or reduced toxicity in Phase I clinical trial. In addition, the anticancer effect of *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-doxorubicin conjugate in combination with HPMA copolymer-mesochlorin e6 was shown to be more efficacious than either conjugate alone (59).

In contrast to the common combination regimen, drug-loading with bioactive carriers ensures the simultaneous arrival of multiple therapeutic agents at the same targeting site, and thus represents a promising strategy for better therapeutic outcome. These dual-function carriers not only deliver the drug to tumor site but also are biologically effective, which either enhances the therapeutic effect (60) or reduce the toxicity caused by the incorporated drug (61).

However, micellar systems based on drug-polymer conjugates are rarely used for the physical incorporation of another hydrophobic molecule. The following section summarizes some of the recent works from us and others, which demonstrate that such a strategy is not only feasible but also effective.

PEG-Vitamin E Conjugates as Dual-Function Carriers for Cancer-Targeted Delivery

D-α-tocopheryl polyethylene glycol (PEG) 1,000 succinate (TPGS) is a PEG-derivatized natural vitamin E which has been approved by FDA as a safe pharmaceutical adjuvant for drug formulation. In recent years, the application of TPGS in drug formulations has been extensively studied, such as emulsifier in poly (lactic-*co*-glycolic acid) (PLGA) nanoparticles (62), solubilizer and permeation enhancer (63), TPGS-based liposomes (64), copolymers (65), and nanocrystal (66). By inhibiting the function of P-glycoprotein (P-gp), TPGS also helps to overcome the multidrug resistance (67) and enhance the oral bioavailability of anticancer drugs (68). In addition, TPGS-doxorubicin conjugate was developed as a prodrug for enhanced therapeutic effect (69).

TPGS forms micelles in aqueous solution, which were used for the dispersion of functional nanostructures such as carbon nanotubes (70,71), fullerenes (71), and iron oxide (72). However, with a relatively high critical micelle concentration (CMC) value of 0.2 mg/mL (73), TPGS micelles are not stable and easily dissociated upon dilution by plasma after intravenous injection. Therefore, TPGS is usually used together with other excipients to form mixed micelles. For example, PEG-phosphatidylethanolamine (PEG-PE) was mixed with TPGS at a molar ratio of 2:1 for the loading of camptothecin (CPT), which increased the CPT solubility by at least 50% compared to PEG-PE micelles without TPGS (74). Similarly, mixed micelles of 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-1,000] (DSPE-PEG) with
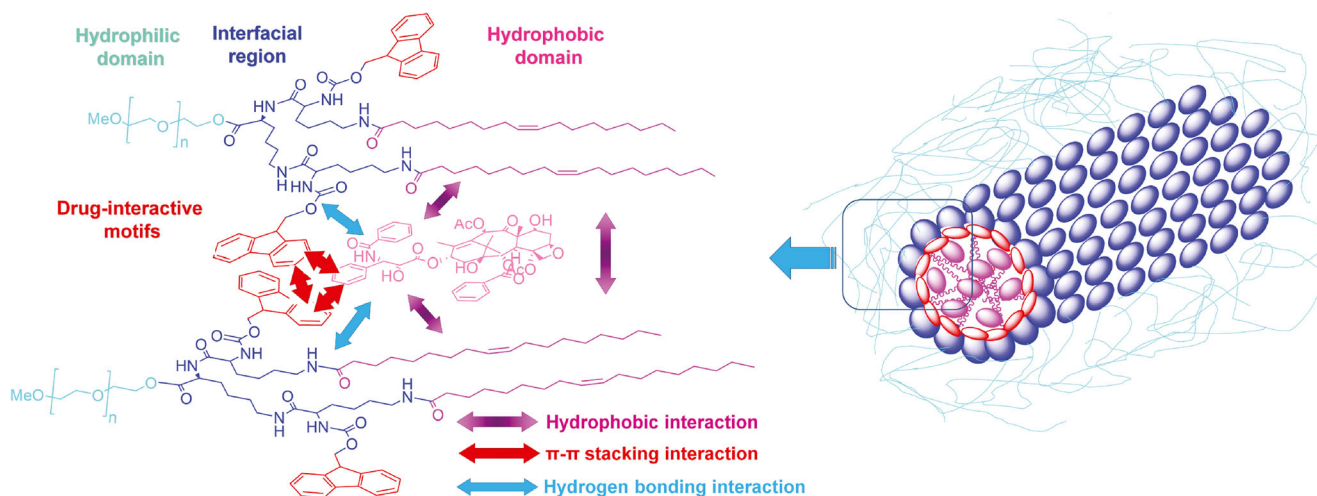
Fig. 1. Structure of PEG5000-Lys-(α-Fmoc-ε-oleoyl lysine)$_2$ and the postulated modes of carrier/drug and carrier/carrier interactions. Taken with permission from (53)

TPGS were prepared to encapsulate an anticancer agent, 17-allyamino-17-demethoxygeldanamycin (17-AAG) (75), resulting in controlled drug release and improved cytotoxicity compared with the free drug. In addition, TPGS also forms mixed micelles with Pluronic P105 (76), Pluronic P123 (77), and Pluronic F127/poly(butyl cyanoacrylate) (PBCA) (78). Compared with free drug or micelles without TPGS, those micelles showed improved solubility of hydrophobic anticancer drugs and increased cytotoxicity against MCF-7, MCF-7/ADR and HepG2 cell lines.

To further facilitate the use of TPGS as a micellar formulation, several strategies have been developed to decrease its CMC. Feng's group has conjugated one tocopheryl succinate molecule with a PEG$_{2K}$ chain to generate TPGS$_{2K}$ for the delivery of docetaxel, which showed much lower CMC value compared with traditional TPGS (60). This improvement has enabled the formation of stable drug-loaded TPGS micelles without the help of other polymers or lipids. Another benefit of longer PEG chain is to further decrease the nonspecific uptake of TPGS$_{2K}$ micelles by RES. The study by Wang *et al.* showed that a conjugate of PEG$_{2K}$ with two vitamin E molecules exhibited further reduced CMC of 1.14 μg/mL (79), compared to that of TPGS$_{2K}$ (21.9 μg/mL) and TPGS (200 μg/mL) (60). Importantly, PEG$_{2K}$-Vitamin E$_2$ well retained the intrinsic activity of TPGS in inhibiting the activity of P-gp. Doxorubicin-loaded TPGS$_{2K}$ micelles showed greater cytotoxicity and tumor inhibitory effect than doxorubicin formulated in conventional TPGS (79). In light of this information, we have recently developed four PEG/vitamin E conjugates that differ in PEG molecular weight (PEG$_{2K}$ vs PEG$_{5K}$) and the molar ratio of PEG/vitamin E (1/1 vs 1/2), and their paclitaxel loading capacity was subsequently compared (80). Our data have shown that among all the four conjugates, PEG$_{5K}$ conjugate with two vitamin E molecules (PEG$_{5K}$-VE$_2$) showed the lowest CMC value, with highest loading capacity and stability. All the four conjugates retained the P-gp inhibition activity of TPGS. Delivery of paclitaxel *via* PEG$_{5K}$-VE$_2$ led to significantly improved antitumor activity compared with the commercial formulation Taxol® and other paclitaxel micellar formulations (Fig. 2).

PEG-Derivatized Embelin as a Nanocarrier for the Delivery of Paclitaxel

Embelin is an alkyl-substituted hydroxyl benzoquinone natural product discovered from the Japanese Ardisia Herb (Herba Ardisiae Japonicae) (81). Embelin was shown to possess a broad spectrum of biological activities including antidiabetic (82), anti-inflammatory (83), and hepato-protective effects (84). In addition, embelin exhibits antitumor activity in many types of cancers such as breast (85), colon (86), prostate (87), and pancreatic cancer (88). Through computational structure-based computer screening, Wang *et al.* (81) discovered that embelin is a potent inhibitor of X-linked inhibitor of apoptosis protein (XIAP), which partially explained its anticancer mechanism. XIAP is over-expressed in various types of cancer cells (89), particularly in drug-resistant cancer cells (90), while it plays a minimal role in normal cells.
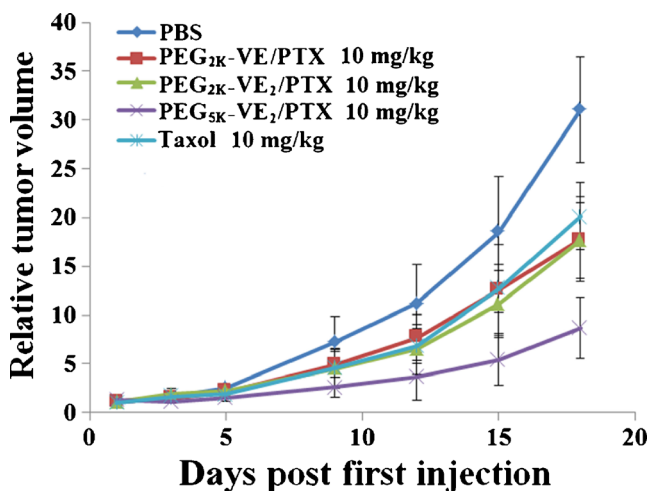

Fig. 2. Enhanced antitumor activity of PTX formulated in PEG$_{5K}$-VE$_2$ micelles. BALB/c mice were inoculated s.c. with 4T1.2 cells (2× 10^5 cells/mouse). Five days later, mice received various treatments on days 1, 3, 5, 9, 12, and tumor growth was monitored and plotted as relative tumor volume (mm^3). $P<0.02$ (PEG$_{5K}$-VE$_2$/PTX *vs.* Taxol, PEG$_{2K}$-VE/PTX or PEG$_{2K}$-VE$_2$/PTX). $N=5$. Taken with permission from (80)

Inhibition of XIAP has been demonstrated as an effective approach to selectively inhibit the growth of cancer cells (91). Embelin also inhibits NF-κB activation, which mediates the downregulation of several genes including surviving, XIAP, IAP1/2, TRAF1, cFLIP, Bcl-2 and Bcl-xL (92).

Bearing a long lipophilic chain, embelin is extremely hydrophobic and water-insoluble. In an attempt to explore the PEG modification as an approach to increase its water solubility, we have found that PEG-derivatized embelin forms micelles in aqueous solution (93). This is not a surprise considering the structural similarity between embelin and vitamin E (Fig. 3a–b). Interestingly, the antitumor activity of embelin was well retained after coupling with PEG chain (93). In addition, the PEG-embelin micelles are highly efficient in solubilizing various types of anticancer agent such as paclitaxel (93,94). Furthermore, PEG-embelin, at nanomolar range, showed synergistic effect with paclitaxel in several cancer cell lines tested (93). *In vitro* and *in vivo* studies have shown that the conjugate with two embelin molecules linked with one PEG chain is significantly more effective for loading paclitaxel than the conjugate with a 1:1 molar ratio of PEG and embelin. In addition, the PEG-embelin conjugates with longer PEG chain (PEG_{5K}) were shown to be more advantageous compared with the counterparts with shorter PEG chain ($PEG_{3.5K}$) (93,94). Near-infrared fluorescence (NIRF) imaging of PC-3 xenograft-bearing mice showed that PEG_{5K}-EB_2 micelles were selectively accumulated at tumor site with minimal distribution in major organs including liver and spleen (Fig. 4) (94). Delivery of paclitaxel *via* PEG_{5K}-embelin$_2$ micelles leads to superior antitumor activity compared to Taxol in murine models of breast and prostate cancers (94).

PEG-Farnesylthiosalicylate (PEG-FTS) Conjugate Micelles for the Delivery of Paclitaxel

S-trans,*trans*-farnesylthiosalicylic acid (Salirasib, or FTS) is a synthetic antagonist of Ras protein. By disrupting the anchorage of Ras on cell membrane (95), FTS is designed to
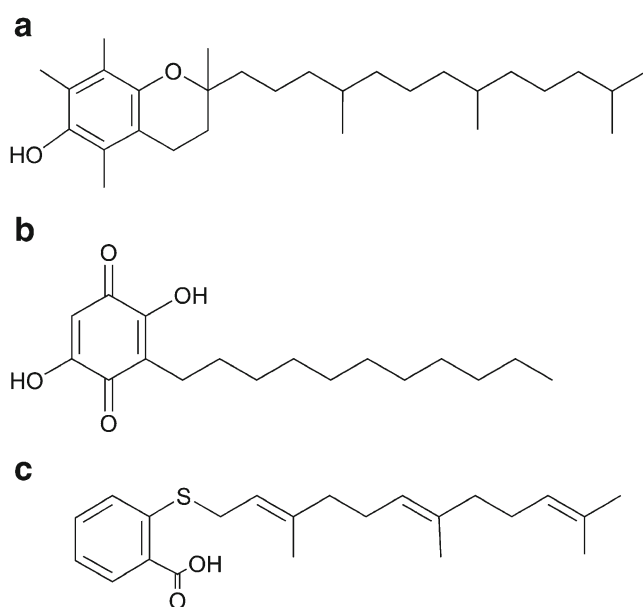
Fig. 3. Chemical structures of vitamin E (**a**), embelin (**b**) and FTS (**c**)

inhibit Ras-dependent growth of cancer cells. Approximately 20% to 30% of human tumors express permanently active oncogenic Ras (96), and the mutationally activated Ras was most commonly found in adenocarcinomas of the pancreas (90%), colon (50%), lung (30%), thyroid tumors (50%), and myeloid leukemia (30%) (97,98). Accordingly, FTS exhibited potent antitumor effect in various tumors such as pancreatic cancer (95), colon cancer (99), melanoma (100), and neurofibromatosis (101). In addition, Ras inhibitors demonstrated synergistic effect with other chemotherapeutics, which validated their application in combination therapy (102). For example, treatment of the resistant SW480 cells with FTS dramatically enhanced the sensitivity to gemcitabine and led to improved inhibition of tumor growth *in vivo* (103).

Similar to vitamin E and embelin, FTS is also a hydrophobic small molecule with a long hydrophobic chain and a functional group (–COOH) that can be readily used for further modification (Fig. 3c). The biological activity and chemical structure of FTS has prompted us to design the PEG-derivatized FTS conjugate as another dual-function micellar drug carrier (104). A labile ester linkage was used to facilitate the release of FTS and disassembly of the drug-loaded micelles following intracellular delivery to tumor cells. Our data have shown that the PEG-FTS_2 readily forms micelles in aqueous solution with a CMC of 0.68 μM. Paclitaxel can be efficiently loaded into those micelles, which are spherical in morphology with a uniform size of 20–30 nm. Ras protein downregulation (Fig. 5) and cytotoxicity of PEG-FTS_2 were comparable to free FTS as shown in 4T1.2 and HCT-116 cancer cell lines. The antitumor activity of paclitaxel-loaded PEG-FTS_2 micelles was shown to be significantly higher than that of Taxol in a syngeneic murine breast cancer model (104).

Other Drug-Polymer Conjugate Micelles for the Delivery of a Hydrophobic Chemotherapeutics

Several other micellar systems have been studied, which are based on polymer-drug conjugates such as polymer-curcumin and polymer-adriamycin conjugates. Curcumin is a natural polyphenol compound with promising anticancer application (105,106). It was reported that curcumin blocks NF-κB pathway (107) and in turn, induces apoptosis and inhibits the function of protein kinase C, epidermal growth factor receptor tyrosine kinase, and HER-2 (108,109). Curcumin has shown antitumor activity against various types of cancers including those of breast (110), colon (111), prostate (112,113), kidney (114), liver (115), lymphoid and myeloid tissues (116), and melanoma (117). In spite of all the anticancer activities, the potential application of curcumin is hindered by its poor water solubility and limited bioavailability. Furthermore, high drug dose is required for a desired therapeutic outcome due to its relatively low potency. Therefore, nano-sized delivery systems with high loading capacity and tumor-specific distribution represent an attractive strategy to address these issues.

In an attempt to increase the solubility of curcumin, various formulations have been developed such as cyclodextrin (118), nanoparticles (119), microparticles (120) and nano-sized complex (121). Polymer-curcumin conjugates were also synthesized as polymeric prodrugs. For example, Safavy *et al.* synthesized two conjugates including curcumin-PEG_{750} and curcumin-$PEG_{3.5K}$ (122). Both conjugates exhibited enhanced
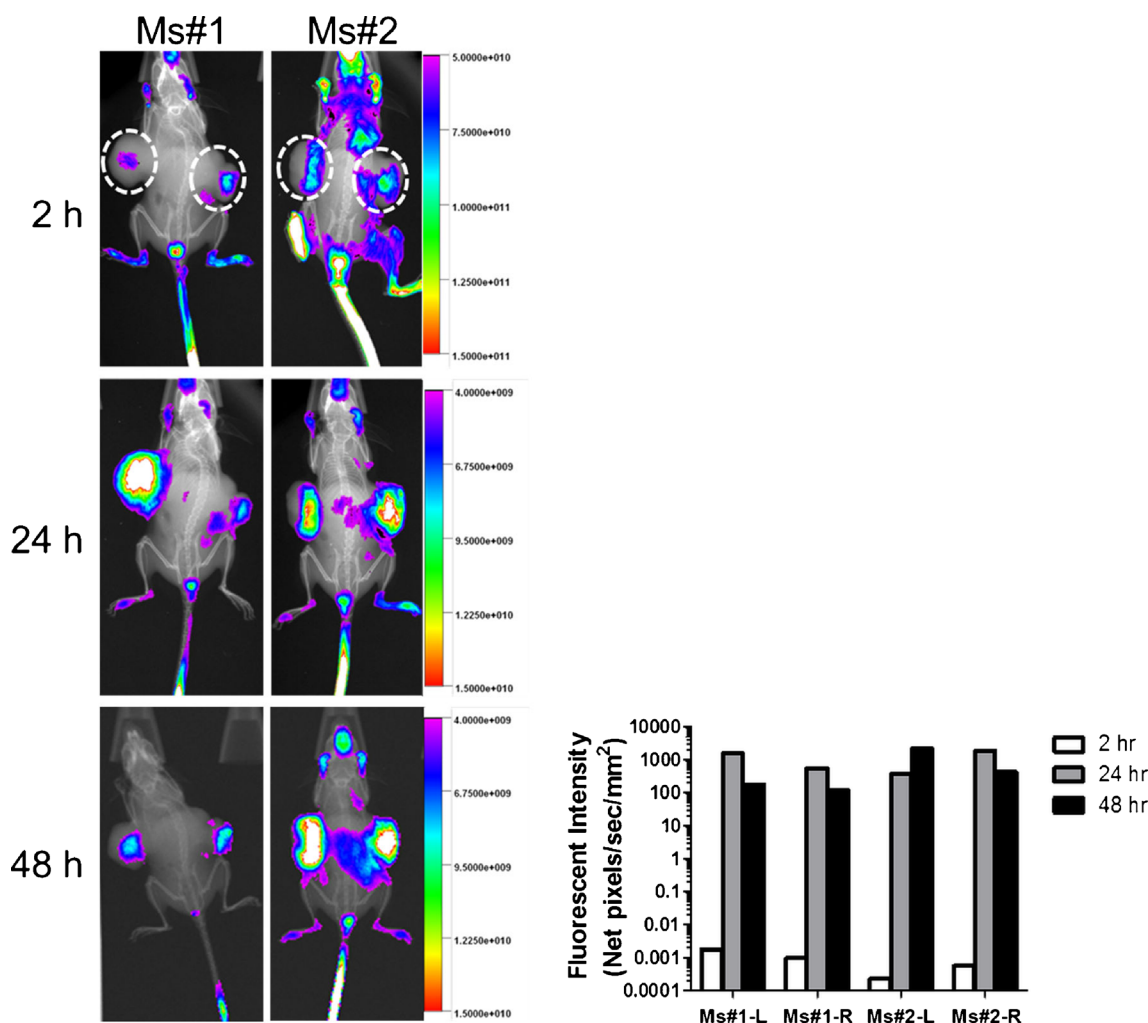
Fig. 4. *In vivo* NIRF imaging over time as indicated in prostate cancer. PC-3 xenograft-bearing mice at 2, 24, 48 h following i.v. injection of PEG$_{5K}$-EB$_2$ micelles co-loaded with PTX and DiD. Taken with permission from (94)

water solubility and cytotoxicity against several human cancer cell lines in comparison with free curcumin (122). To further increase the drug content in the nanoparticles, Tang *et al.* synthesized a curcumin prodrug by attaching it with two short oligo (ethylene glycol) (Curc-OEG) chains. Beta-thioester bond was applied which can be selectively cleaved intracellularly by glutathione and esterase to release the drug (123). With a curcumin loading content of 25.3 wt.%, the Curc-OEG conjugate formed stable nanoparticles in aqueous solution and exhibited dramatic anticancer effect *in vitro* and *in vivo* without causing significant toxicity (123). Curcumin-polymer conjugates were also synthesized with hyaluronic acid (124) and polyvinylpyrrolidone (125). Most recently, Yang *et al.* (126) demonstrated that the covalent curcumin-polymer conjugates can be further used to physically encapsulate additional curcumin. Curcumin was loaded into the polymeric micelles,
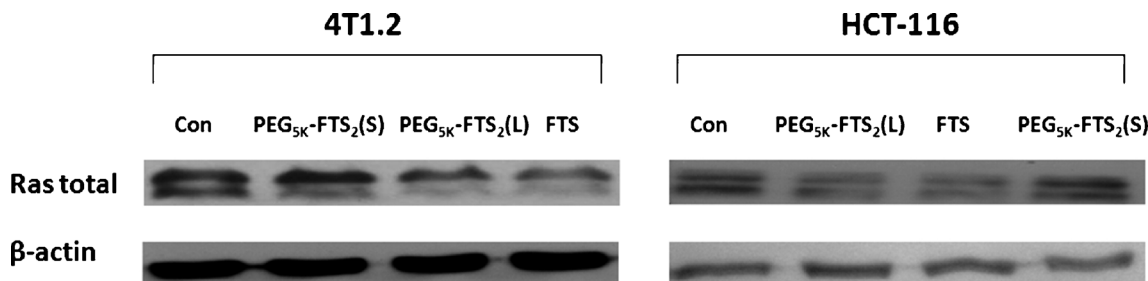

Fig. 5. Effects of FTS, PEG$_{5K}$-FTS$_2$ (L), and PEG$_{5K}$-FTS$_2$ (S) on total Ras expression by western blot analysis. Cells grown in medium containing 5% FBS were treated with 0.1% DMSO (control), PEG$_{5K}$-FTS$_2$ with liable linkage (*L*), PEG$_{5K}$-FTS$_2$ with stable linkage (*S*), and free 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. Taken with permission from (104)

which were synthesized by attaching multiple curcumin molecules to the hydrophobic poly (lactic acid) (PLA) backbone of PEG-PLA copolymer. Such micelles exhibited ten times lower CMC value and dramatically enhanced curcumin loading capacity (approximately fivefold) compared to traditional mPEG-PLA micelles (126).

Yokoyama *et al.* has reported that adriamycin (ADR)-conjugated polyethylene glycol–poly(aspartic acid) block copolymers (PEG-P[Asp(ADR)]) form micelles in aqueous solution and exhibited dramatic antitumor activity *in vivo* (127–129). However, the ratio of chemically/physically entrapped adriamycin was not analyzed, and certain amounts of adriamycin derivatives were incorporated in the micelles as impurities, which may cause side effects (130). The micelle preparation method was then improved which enabled the determination of this ratio and reduced the amounts of impurities (130,131). In addition, Yang *et al.* (132) developed a dual-drug system in which doxorubicin was chemically linked to the PLA end of polyethylene glycol-*b*-poly lactic acid (PEG-b-PLA). This conjugate was mixed with RGD-PEG-b-PLA, PEG-b-PLA and an antivascular agent combretastatin A4 to prepare the micelles. This dual-drug system significantly enhanced cellular uptake of the drug by B16-F10 cells and human umbilical vein endothelial cells, and achieved significant antitumor effect with increased lifespan of tumor bearing mice.

CONCLUSION AND FUTURE DIRECTIONS

Polymeric micelles have been extensively studied over the last decade as versatile and efficient drug delivery systems for cancer therapy. The design of polymer structure is increasingly sophisticated to improve the drug-loading capacity, tumor-specific uptake as well as anticancer effect. Various strategies have been developed to increase the drug/carrier interaction of polymeric micelles to maximize the drug-loading capacity, such as hydrotropic polymers and Fmoc-conjugated surfactants. In addition, several biologically active carriers have been developed as dual-functional carriers to improve the anticancer effect. More systematic studies on the structure-activity relationship of polymeric micellar systems are needed to better understand the mechanism of drug/carrier interaction and the effect of polymer structure on the drug-loading capacity. In addition, computational modeling may offer help in the tailored design of a polymeric carrier for each drug. These studies shall lead to the development of further improved micellar systems to advance the treatment of cancers.

ACKNOWLEDGMENTS

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