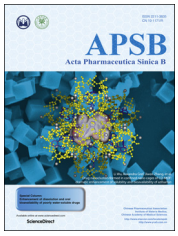
Acta Pharmaceutica Sinica B 2019;9(1):49–58

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



REVIEW

Emerging transporter-targeted nanoparticulate drug delivery systems

Hongyan Su, Yan Wang, Shuo Liu, Yue Wang, Qian Liu, Guangxuan Liu*, Qin Chen*

Department of Pharmacy, Cancer Hospital of China Medical University, Liaoning Cancer Hospital & Institute, Shenyang 110042, China

Received 28 July 2018; received in revised form 11 September 2018; accepted 4 October 2018

KEY WORDS

Transporter;
Nano-DDS;
Oral delivery;
Brain-targeting;
Tumor-targeting

Abstract Transporter-targeted nanoparticulate drug delivery systems (nano-DDS) have emerged as promising nanoplatforms for efficient drug delivery. Recently, great progress in transporter-targeted strategies has been made, especially with the rapid developments in nanotherapeutics. In this review, we outline the recent advances in transporter-targeted nano-DDS. First, the emerging transporter-targeted nano-DDS developed to facilitate oral drug delivery are reviewed. These include improvements in the oral absorption of protein and peptide drugs, facilitating the intravenous-to-oral switch in cancer chemotherapy. Secondly, the recent advances in transporter-assisted brain-targeting nano-DDS are discussed, focusing on the specific transporter-based targeting strategies. Recent developments in transporter-mediated tumor-targeting drug delivery are also discussed. Finally, the possible transport mechanisms involved in transporter-mediated endocytosis are highlighted, with special attention to the latest findings of the interactions between membrane transporters and nano-DDS.

*Corresponding authors.
E-mail addresses: guanxuan2004@126.com (Guangxuan Liu), chenqin0605@163.com (Qin Chen).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

https://doi.org/10.1016/j.apsb.2018.10.005

1. Introduction

The ultimate goal of designing drug delivery systems is to achieve better therapeutic outcomes with lower side effects. Current approaches include improving the physicochemical properties of formulations and/or addressing the complex fates of drugs following *in vivo* delivery[1–6]. With the rapid developments in nanotechnology and carrier materials, nanoparticulate drug delivery systems (nano-DDS) show great progress in drug delivery over the past few decades[3–9]. Drug molecules encapsulated in nanocarriers usually demonstrate totally different delivery characteristics instead of their intrinsic properties, due to the shielding effect of nano-DDS[8,10]. This permits development of more versatile drug delivery strategies as compared with only changing the physicochemical properties of compounds[10]. Therefore, rationally designing various nanocarriers and making specific modifications on the nano-DDS can achieve more effective drug delivery[7].

Among the various delivery strategies in the field of nano-DDS, developing smart targeted nanocarriers has long been a research focus for pharmaceutical scientists[11,12]. The ideal drug delivery outcome must be precisely delivering the therapeutic agents to their sites of action, especially for anticancer drug delivery[12–14]. Since most chemotherapeutic agents are cytotoxic compounds, which inevitably impose toxicity to the normal tissues[13], rational design of advanced nano-DDS with high efficiency and low toxicity is crucially important for anticancer drug delivery[15]. There is also encouraging results for the prospects of nano-DDS targeting to the intestinal absorption site or specific organs (*e.g.,* brain). These can significantly facilitate oral absorption or drug permeation of the blood–brain barriers (BBB)[16–19].

Traditional targeting strategies have mainly focused on modifying ligands on the surface of nano-DDS to recognize and interact with the specific receptors on cell membrane[20]. So far, the strategy seems to be working, but the targeting efficacy has been greatly limited by the variability and heterogeneity of membrane receptors[21–23]. Different patients with same disease may have different expression levels of receptors, and different receptors levels may also be found at different stages of the disease even for the same patient[22]. Therefore, receptor-based targeting strategies have not been brought to clinics, and it is necessary to seek for new target sites to develop targeted nanocarriers. Recently, membrane transporters have become emerging target sites for efficient drug delivery[24–27]. Transporters for glucose, amino acids, vitamins and ions are essential for cell nutrition[27]. In addition to these essential nutrients, transporters could also interact with a variety of drugs, thereby affecting the efficacy and safety of drugs[27]. Due to the important roles for cell nutrition, the expression levels of transporters are less variable than those of receptors[26,27]. In some specific cases, such as in tumors, the expression level transporters are usually upregulated to meet the enormous nutrition demand for uncontrolled growth of tumor cells[25]. Thus, transporters are an emerging target for designing tumor-targeting nano-DDS.

In the latest decades, the application of membrane transporters was not restricted to cancer therapy. Transporter-based drug delivery strategies have also been widely investigated in oral drug delivery and brain-targeting therapy[24–27]. Transporter-targeted prodrug strategies have been widely investigated, and several excellent relevant reviews have been published. Herein, we outline the recent advances in transporter-targeted nano-DDS (Fig. 1), including (i) emerging transporter-targeted nano-DDS developed to facilitate oral drug delivery; (ii) recent advances in transporter-assisted brain-targeting nano-DDS; (iii) recent developments in transporter-mediated tumor-targeting drug delivery; and (iv) possible transport mechanisms involved in transporter-mediated endocytosis.

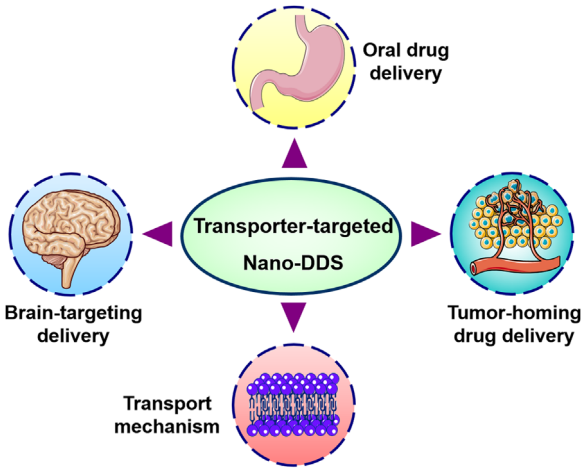


Figure 1 Emerging trends in the fields of transporter-targeted nano-DDS.

2. Transporter-targeted nano-DDS to improve oral absorption

Oral drug delivery has long been considered a natural and safe administration route, due to its good compliance[16,28–30]. However, a wide range of drugs cannot be administrated orally[16], since multiple barriers may be encountered during oral absorption process. These include water insolubility, inferior stability, poor drug permeability, and complex gastroenteric environments (*e.g.*, pH and metabolic enzymes)[16]. For example, the successful oral delivery of insulin would be profoundly important for diabetic patients, but orally-administered insulin has poor stability, resulting in inefficient oral absorption[31]. Furthermore, developing new strategies to facilitate the intravenous-to-oral switch in cancer chemotherapy has also attracted increasing attention[16]. In recent years, great progress has been made in designing transporter-targeted nano-DDS to improve oral absorption of peptide drugs and anticancer drugs[16,32].

2.1. *Improving oral absorption of protein and peptide drugs*

Oral delivery of large-molecule proteins and peptide drugs has long been a great challenge, due to their poor oral absorption caused by inferior stability and low permeability[33,34]. More recently, nano-DDS seems to be a viable approach by encapsulating proteins or peptides into nanocarriers[35–37]. Although nano-DDS could significantly improve the stability of proteins and peptides *via* a shielding effect, the oral absorption is still limited by the unsatisfactory permeability capability across the intestinal wall[37]. Various types of gastrointestinal transporters have been found to play important roles in essential nutrient uptake, and these transporters exist as natural targets for the efficient oral delivery of proteins and peptide drugs[36–41].

Bile acid transporters are widely expressed throughout the intestinal tract and have been investigated for oral delivery of proteins and peptide drugs, such as insulin[39–41]. For instance, Dr. Gan's research group developed deoxycholic acid-modified nanoparticles (DNPs) to overcome multiple intestinal barriers for oral

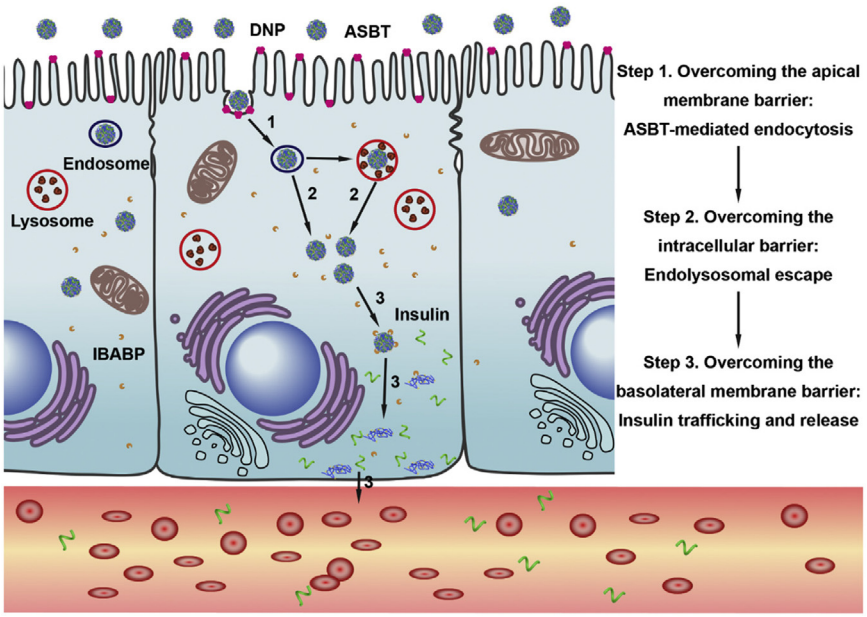


Figure 2 Schematic illustration of transepithelial transport of insulin from DNPs to overcome multiple barriers of the intestinal epithelium by exploiting the bile acid pathway. Reprinted with the permission from Ref. 39. Copyright © 2017 Elsevier Ltd. DNPs, deoxycholic acid-modified nanoparticles; ASBT, apical sodium-dependent bile acid transporter.

insulin delivery (Fig. 2)[39]. Deoxycholic acid-conjugated chitosan was designed and synthesized as the transporter-targeted carrier material into which insulin was encapsulated as DNPs[39]. Insulin-loaded DNPs were effectively internalized through apical sodium-dependent bile acid transporter-mediated endocytosis, thus surmounting multiple barriers of the intestinal epithelium[39]. More importantly, the stability of insulin in the epithelium was significantly improved due to the endosomal escape of DNPs[39]. Intracellular trafficking and basolateral release of insulin also occurred by interactions with a cytosolic ileal bile acid-binding protein[39]. As a result, the oral bioavailability of insulin was improved to 15.9% in type I diabetic rats after loading the lyophilized powder of DNPs into enteric capsules[39]. These results suggest that bile acid transporter-mediated endocytosis could play key roles in oral delivery of insulin by addressing the multiple barriers across the intestinal epithelium.

Amino acid transporters have also been used for targeting insulin delivery[41]. Specific transporters expressed in the small intestine are known to transport L-amino acids against a concentration gradient[41] Based on this rationale, L-valine-conjugated polylactic-*co*-glycolic acid (PLGA) nanoparticles were developed for oral delivery of insulin[41]. Cellular uptake experiments demonstrated that L-valine-conjugated PLGA nanoparticles showed distinct advantages over the non-modified nanoparticles[41]. Furthermore, the *in vivo* hypoglycemia test in streptozotocin-induced diabetic rabbits revealed that L-valine-conjugated PLGA nanoparticles could effectively reduce blood glucose levels in a sustained manner with clear therapeutic superiority *vs.* the non-modified nanoparticles[41]. The results suggest that L-valine-conjugated NPs are a promising nanoplatform for oral delivery of insulin across the intestinal wall.

2.2. *Facilitating the intravenous-to-oral switch in cancer therapy*

Most anticancer drugs are administrated intravenously, leading to poor compliance and high potential side effects[16,17]. Therefore, there is presently an intense research focus to discover methods for facilitating the intravenous-to-oral switch in cancer chemotherapy[16]. The common barriers hindering oral absorption of anticancer drugs include low water solubility, poor stability in the gastrointestinal tract, and limited permeability across the intestinal wall[16,17]. For example, the oral delivery efficiency of the taxane drugs (paclitaxel, docetaxel and cabazitaxel) is greatly limited by their low water-solubility, poor stability in gastrointestinal tract and good affinity with drug efflux pump P-glycoprotein (P-gp) transporter[16]. Although most transporters expressed in gastrointestinal tract helps to promote the oral absorption of drugs, the P-gp efflux pump acts in the opposite way[16]. Formulating chemotherapeutic agents into nano-DDS could significantly improve their water solubility and chemical stability in gastrointestinal tract[16], but the permeability of noncarriers remains unsatisfactory. Therefore, despite the promising application prospects of nano-DDS in oral chemotherapy, there is still a long way to further improve the permeability across the intestinal endothelial cells.

As mentioned above, a wide range of gastrointestinal transporters have been found to play important roles in essential nutrient uptake, and these transporters exist as natural targets for efficient oral delivery of both large-molecule proteins and small-molecule anticancer drugs. Among them, various types of transporters have been utilized for oral delivery of chemotherapeutic agents, including bile acid transporter, peptide transporter 1 (PepT1), organic cation transporter-2 (OCTN2), and sodium-dependent vitamin C transporter 1 (SVCT1)[42–52]. Dr. He's research group has made notable contributions in the field of transported-targeted anticancer drug delivery. Recently, they have published several research papers. These include: (i) PepT1-tageted nano-DDS. Dipeptide-modified PLGA nanoparticles were designed and developed to facilitate oral docetaxel delivery[42]; (ii) OCTN2-targeted nano-DDS. OCTN2 exists in small intestine as a Na^+-coupled absorption transporter where it mediates L-carnitine uptake. To exploit this, L-carnitine-modified PLGA nanoparticles containing encapsulated paclitaxel were shown to effectively target OCTN2 on

enterocytes to improve the oral absorption of paclitaxel[45]; and (iii) SVCT1-targeted nano-DDS. Ascorbate-modified PLGA nanoparticles were reported to target SVCT1 on epithelial cells for efficient oral delivery of therapeutic agents, and the targeting process and intracellular delivery fate of ascorbate-modified PLGA nanoparticles were documented and illustrated[46].

Bile acid transporter-based nano-DDS have also been used for oral drug delivery[47–52]. For instance, taurocholic acid (TCA)-modified nanostructured lipid carriers (NLCs) were developed to improve oral bioavailability of curcumin by targeting bile-acid transporter[47]. *In situ* intestinal perfusion results showed that TCA-modified NLCs could significantly improve the absorption rate and permeability of curcumin[47]. *In vivo* pharmacokinetic studies revealed that TCA-modified NLCs showed a 15-fold higher area under the curve (AUC) in rats when compared with the unmodified NLCs after oral administration[47]. In addition to facilitating oral absorption of chemical compounds, TCA-modified nano-DDS was also successfully applied to improve oral delivery of therapeutic siRNA[48]. An AuNP-siRNA-glycol chitosan-TCA nanosystem was developed to selectively deliver Akt2 siRNA and for treatment of colorectal liver metastases[48]. The prepared TCA-modified nanosystem protected siRNA from degradation in the gastrointestinal environment, facilitated siRNA transport across enterocytes and enhanced accumulation of siRNA in liver[48]. *In vivo* pharmacological experiments showed potent therapeutic activity against colorectal liver metastases after oral administration of Akt2 siRNA-loaded TCA-modified nanoparticles[48]. These results suggest that transporter-targeted nano-DDS provides a versatile platform for both chemotherapeutic drugs and therapeutic genes.

3. Transporter-based brain-targeting nano-DDS

Drug delivery to the central nervous system (CNS) is still challenging due to the blood–brain barrier (BBB)[53,54]. The BBB is a natural defense barrier protecting the brain from harmful substances. Since only selected, neutral, lipophilic small molecules can diffuse into the CNS from blood[53], most drugs have traditionally been thought to be impermeable to the brain[53]. However, smart active targeting nano-DDS makes it possible to deliver many drugs to the brain. Approaches include receptor-and transporter-mediated targeting[55–59]. Herein, the emerging transporter-based brain-targeting nano-DDS is discussed, with special attention on the latest findings of specific transporter-based nanotechnology approaches.

3.1. Targeting to choline transporter

Choline, a polar and cationic molecule, plays key roles in biosynthesizing several important endogenous substances, such as lecithin.[60] Choline is also important for brain development[60]. However, this charged cation does not readily diffuse across the cell membrane. Therefore, a specific transport mechanism is required on plasma membranes to meet the cellular needs for choline[60]. Similarly, the choline transporter is also necessary to deliver choline across the BBB from plasma to brain tissues[60]. Based on this rationale, the choline transporter has been widely investigated and applied in brain-targeted drug delivery, resulting in development of several choline or choline derivative-modified nanocarriers[61–64].

For instance, a choline-modified doxorubicin (DOX)-PEG polymer conjugate was created in a micellar formulation for brain targeting and glioma therapy[61]. Micelles optimized to contain 20% choline demonstrated favorable cellular uptake, pharmacokinetics and biodistribution, resulting in potent *in vivo* antitumor activity[61]. A choline-modified nano-DDS also showed promise for brain-targeting gene delivery and glioma MRI diagnosis[62]. A choline transporter-targeting nano-DDS was also developed for efficient combination of gene therapy and chemotherapy[63]. A complex was prepared by intercalating DOX into TNF-related apoptosis-inducing ligand (TRAIL) DNA plasmid[63]. This DOX-TRAIL complex was then condensed with a choline derivative-modified nano-DDS for BBB penetration and glioma dual-targeting drug delivery[63]. The transporter-targeting co-delivery nano-DDS showed higher cellular uptake efficiency and cytotoxicity than unmodified nanoparticles, resulting in synergistic combination therapy[63].

3.2. Targeting to OCTN2 transporter

The OCTN2 transporter is overexpressed on both brain capillary endothelial cells and glioma cells[65,66]. It plays key roles in transporting L-carnitine from blood to brain across the BBB[65,66]. Long-term exposure of bovine brain capillary endothelial cells to carnitine resulted in a high accumulation of long-chain acyl carnitines, and acetyl-L-carnitine is of critical importance for brain function and energy supply[65,66]. Therefore, the OCTN2 transporter has attracted increased attention for rational design of brain-targeting prodrugs and nano-DDS.[67,68] For instance, Dr. Sun's research group developed L-carnitine-modified nano-DDS to target glioma cells for drug delivery across the BBB (Fig. 3)[68]. L-Carnitine was conjugated to poly (lactic-*co*-glycolic acid) (PLGA), and then L-carnitine-modified PLGA nanoparticles were prepared for glioma-cell targeting.[68] Modification of L-carnitine significantly improved the uptake of nano-DDS PLGA in the BBB endothelial cell line hCMEC/D3 and in the glioma cell line T98G[68]. Moreover, significant improvement of brain accumulation of L-carnitine-modified PLGA nanoparticles was observed[68]. Therefore, OCTN2 transporter-mediated brain-targeting strategy holds bright prospects for new drug delivery systems able to penetrate the BBB.

Recent study suggested that more than one transporter is involved in the brain accumulation of L-carnitine.[65] In addition to OCTN2 transporter, the amino acid transporter $ATB^{0,+}$ also functions in carnitine transport, and the expression of $ATB^{0,+}$ transporter in bovine brain capillary endothelial cells was confirmed by using RT-PCR technology[65]. These results suggest that $ATB^{0,+}$ could be used for brain-targeting drug delivery. Thus L-carnitine-modified nano-DDS could be also utilized as a dual-targeting nanoplatform by simultaneously targeting to OCTN2 transporter and $ATB^{0,+}$ transporter.

3.3. Targeting to glucose transporter

Glucose transport and utilization is critically important for brain activity[69]. Although glucose is an essential nutritional substance for brain, it cannot be synthesized by the brain[69]. As a result, the glucose transporter is overexpressed on the BBB to maintain the continuous high glucose and energy demands of the brain[69]. As such, the glucose transporter could also serve as a therapeutic target for drug delivery to the brain[69]. In recent years, a wide range of glucose transporter-based targeting strategies have been developed, including glucose transporter-targeted nano-DDS[69–71]. For instance, glucose-derived cholesterols were designed and

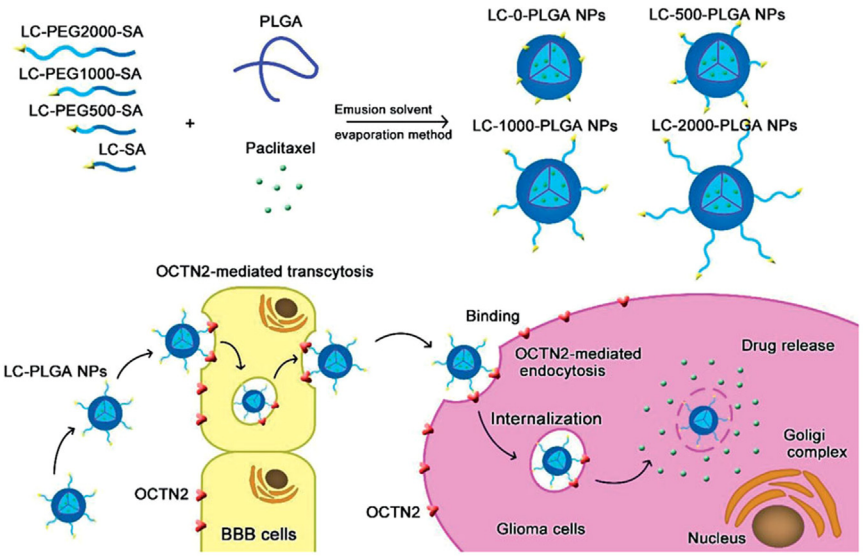


Figure 3 Graphic illustration of the composition of L-carnitine-conjugated nanoparticles with varied lengths of PEG spacers, and OCTN2-mediated BBB transcytosis and glioma targeting. Reprinted with the permission from Ref. 68. Copyright © 2017 Taylor & Francis Group.

synthesized, and glucose-modified liposomes were prepared with coumarin 6 loaded[70]. The *in vivo* biodistribution results suggested that glucose-modified liposomes demonstrated distinct advantages over the unmodified liposomes in terms of specific accumulation of coumarin 6 in brain[70]. Due to the simultaneous overexpression on both the BBB and glioma cells, the glucose transporter could be used for dual-targeting drug delivery[71]. For instance, a derivative of glucose (2-deoxy-D-glucose)-modified nano-DDS was developed for simultaneously targeting the BBB and glioma cells, resulting in efficient glioma treatment[71].

3.4. *Targeting to LAT1 transporter*

The LAT1 transporter is also overexpressed both on the BBB and glioma cells, which could be used for brain-targeting drug delivery.[72] For example, a glutamate-D-α-tocopherol polyethylene glycol 1000 succinate copolymer (Glu-TPGS) was synthesized to modify docetaxel (DTX)-loaded liposomes to enhance the BBB penetration and glioma therapy.[72] Glu-TPGS modified liposomes demonstrated effective higher cellular uptake, cell cytotoxicity and BBB penetration *vs.* unmodified formulations *in vivo*.[72] These results suggested that LAT1 transporter-mediated brain-targeting strategy provides another new option in designing brain glioma-targeting nano-DDS.

In summary, although the BBB is a major impediment to drug delivery in brain, various types of nutrient transporters open a window across this barrier to facilitate brain-targeting drug delivery for treating central nervous system diseases. Of greatest relevance may be that several important transporters (*e.g.*, glucose transporter and LAT1 transporter) are overexpressed on both the BBB and glioma cells, which providing the possibility of efficient treatment of brain glioma tumors[71–73].

4. Transporter-mediated tumor-homing drug delivery

Cancer remains an enormous challenge to human health[74,75], and many efforts have been made to address the consequences of malignant tumors[75–81]. One of the most characteristic features of tumor cells is uncontrolled and progressive proliferation, accompanied by the requirements for very large amounts of nutrients to maintain such abnormal growth[75]. As a result, various types of nutrient transporters overexpressed on tumor cells. These overexpressed membrane transporters as ideal natural targets for tumor-homing anticancer drug delivery[25,82]. Herein, the recent trends in transporter-based tumor-targeting nano-DDS are discussed, focusing on targeting to the overexpressed membrane transporters on tumor cells and the emerging transporter-based dual-targeting strategies.

4.1. *Targeting to the overexpressed membrane transporters on tumor cells*

Due to the wide overexpression of various transporters on tumor cell membranes, various transporter-mediated tumor-homing nano-DDS have been developed, including the glucose transporter[83–85], LAT1 transporter[72,86], OCTN2 transporter[87], amino acid transporter[88,89] SLC6A14 and $ATB^{0,+}$, and secreted protein acidic and rich in cysteine (SPARC)[90]. Among them, glucose transporter-targeted anticancer drug delivery strategies have been attracting increased attention, and several nano-DDS has been developed for efficient cancer therapy, including polymeric nanoparticles and nanomicelles[83–85]. For instance, redox-responsive tumor-targeting tri-layer nanomicelles were developed for hepatocellular carcinoma therapy (Fig. 4)[85]. The nanomicelles were modified with dehydroascorbic acid (DHAA) for specific recognition of the glucose transporter overexpressed on hepatocarcinoma cells[85]. As expected, these nanomicelles demonstrated significantly improved cellular uptake and accumulated distribution in hepatocarcinoma tumors, resulting in enhanced anticancer efficacy[85].

Amino acid transporters have also been widely explored as potential targets for cancer therapy, including SLC6A14[88,89] and $ATB^{0,+}$, and LAT1[72,86]. These specific transporters were found to be overexpressed in a wide range of tumors, including breast cancer, lung cancer, hepatocarcinoma and glioma[86–89]. A common strategy is to modify nano-DDS with specific amino acids recognizing and targeting the relevant transporter, thereby facilitating cellular uptake and tumor accumulation of nanoparticles. For example, glutamate-modified PLGA nanoparticles could

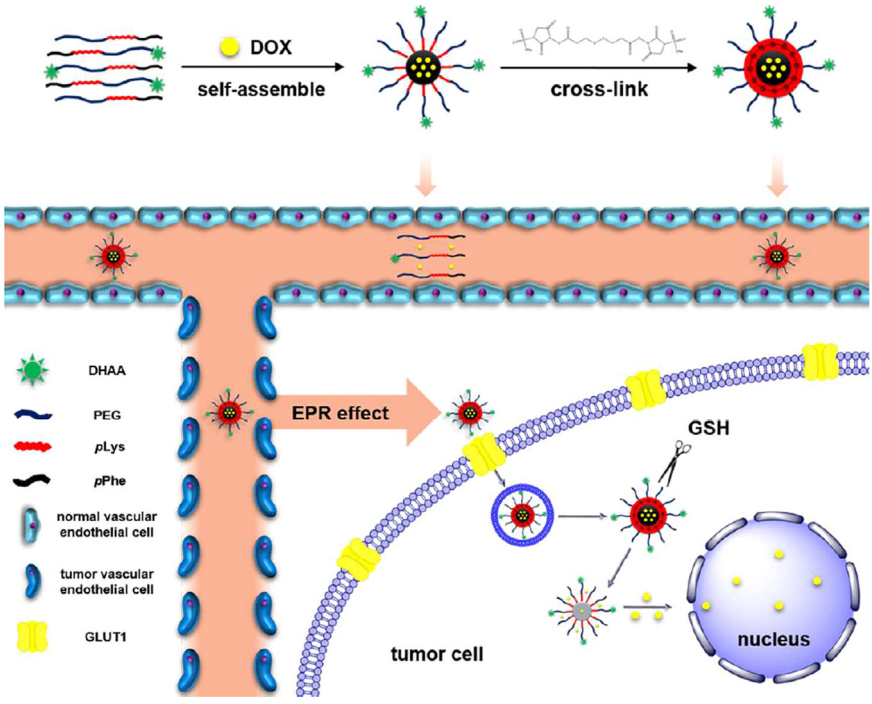


Figure 4 Illustration of stepwise synthesis, GLUT1-mediated endocytosis and GSH-triggered 3 intracellular drug release of DPL(s-s)P/DOX micelles. Reprinted with the permission from Ref. 85. Copyright © 2015 American Chemical Society.

readily recognize and bind with LAT1, promoting tumor accumulation and anticancer activity of chemotherapeutic agents[86]. In addition, lysine-modified liposomes demonstrated good targeting ability to $ATB^{0,+}$, facilitating tumor-homing delivery of DTX for hepatocarcinoma therapy[88].

4.2. *Transporter-based dual-targeting strategies*

Despite the tumor-targeting ability of transporter-based nano-DDS, tumor cells are heterogeneous in many aspects.[91–93]. The expression level of specific transporters may vary in different tumors or even in different regions in the same tumor[87,90]. Different expression levels have be found at different growth stages of one tumor[87,90]. Therefore, rational development of dual-targeting nano-DDS may be a solution to the challenges of tumor heterogeneity.

Recently, several transporter-based dual-targeting drug delivery strategies have been reported[87,90]. For example, the expression of both OCTN2 and $ATB^{0,+}$ on colon cancer cells was greater than on normal colon cells, leading to the development of dual-targeting L-carnitine-modified nano-DDS to target both transporters for colon cancer chemotherapy[87]. L-Carnitine-modified nano-DDS showed distinct improvement in cellular uptake and cytotoxicity of 5-fluorouracil in colon cancer cells (HCT116 and HT29 cells), resulting in enhanced antitumor efficacy in a 3D spheroid model[87]. In addition, the combination of transporter- and receptor-mediated tumor-homing drug delivery strategies could also effectively address the problems of tumor heterogeneity and enhance antitumor activity[90]. For instance, it was found that both the colon cancer cells and M2 macrophages overexpressed secreted protein acidic and rich in cysteine (SPARC) and mannose receptors (MR)[90]. Therefore, mannosylated albumin nanoparticles were designed to target both SPARC and MR, thereby acting on both cancer cells and M2 macrophages[90]. The dual-targeting nanosystem significantly improved the therapeutic outcomes[90]. Therefore, transporter-based dual-targeting nano-DDS holds promising prospects in response to tumor heterogeneity.

5. Interactions between membrane transporters and nano-DDS

As discussed above, there is sufficient evidence for advantages of transporter-targeted nano-DDS for efficient drug delivery. These nano-DDS with specific modifications could effectively recognize and bind to the targeted transporters, and the nano-DDS could be internalized into cells *via* transporter-mediated endocytosis. Such a transporter-mediated cellular uptake mechanism of nano-DDS is definite. In addition, the mechanisms by which nano-DDS affects the expression levels of transporters have also attracted more attention in recent years[42,72,86–89]. Thus, illuminating the interactions between membrane transporters and nano-DDS is important for rational design of high-efficient transporter-based nano-DDS.

According to the recent studies[42,72,86–89], two possible fates of transporters in transporter-mediated cellular uptake were found: (i) recycling back to the cell membrane. Once the nano-DDS dissociates from the transporters, they can recycle back to cell membrane, which is important for maintaining the sufficient levels of transporters (Fig. 5)[42,72,86–89]; and (ii) degradation within endosome/lysosomes. If the transporter-mediated cellular uptake is an endosome-dependent pathway, the structure of transporter can be destroyed in endosome/lysosomes, which will decrease the transporter levels on cell membrane. So far, recent studies demonstrated that the two routes simultaneously exist[42,72,86–89]. For instance, the LAT1 protein of tumor cells incubated with LAT1-targeted nanoparticles were decreased at the beginning of cellular uptake, but the membrane LAT1 transporter increased once the nanoparticles were removed from the uptake medium,

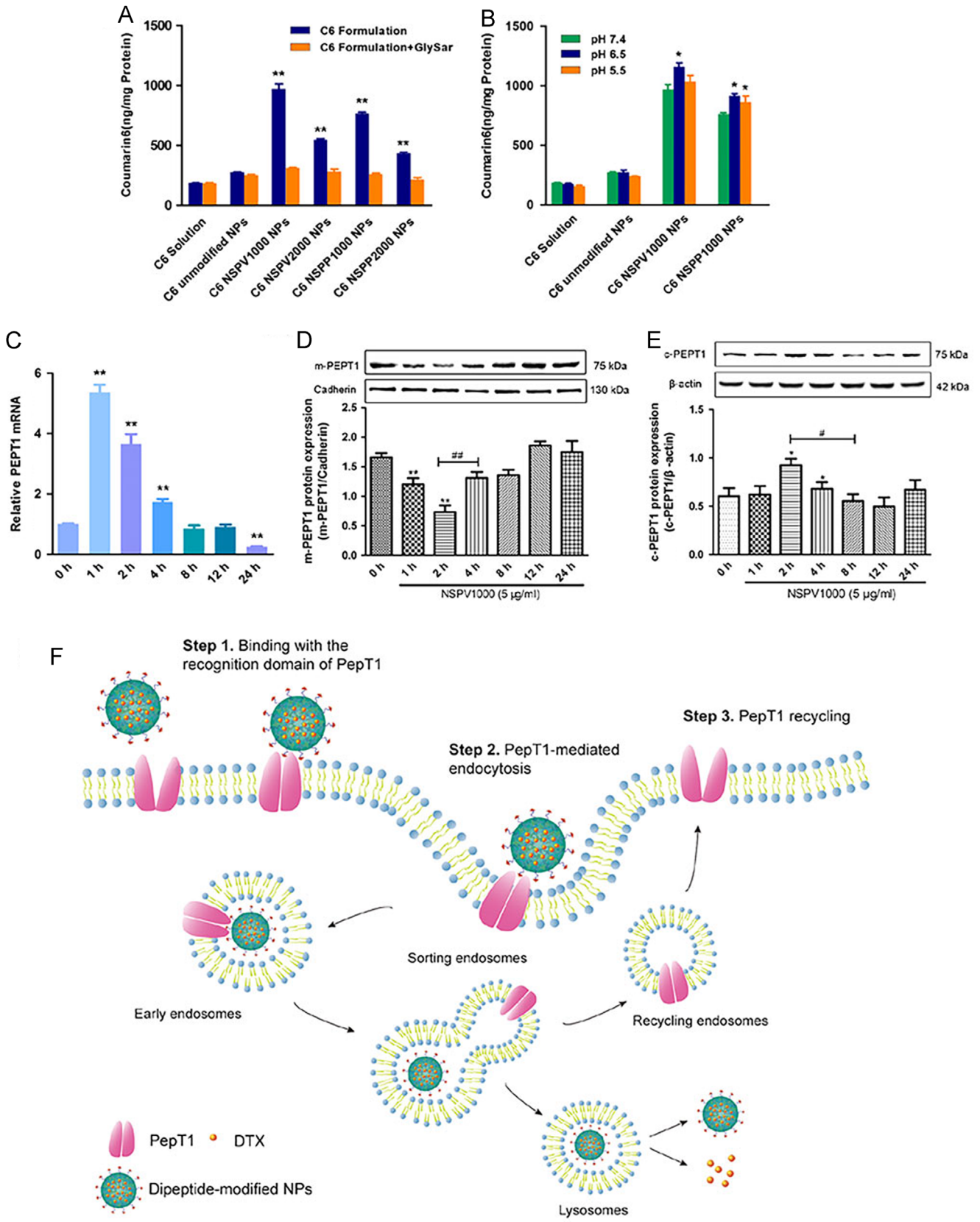


Figure 5 (A) Competitive study in hPepT1-Hela cells in the presence of typical substrate GlySar (GS); (B) Influence of proton in the culture medium on the cellular uptake of dipeptide modified NPs in hPepT1-Hela cells; The variation of relative PepT1 mRNA expression *versus* β-actin (C) and the variations of membrane and cytosol PepT1 protein expression (D,E) after treatments with NSPV1000 NPs for different time over 24 h. Data are shown as mean $\pm$ SD. $^{*}P<0.05$, $^{**}P<0.01$ *vs.* C6/DTX solution group or control group, $P<0.05$, $P<0.01$, $n=3$; (F) The schematic illustration of hypothesized mechanism of PepT1-mediated endocytosis. Reprinted with the permission from Ref. 42. Copyright © 2018 Taylor & Francis Group.

verifying evidence for the first situation[86]. Moreover, there was some evidence found for the degradation of transporters within cells[87–89].

6. Conclusions and perspectives

Transporter-targeted nano-DDS has emerged as a promising nanoplatform for efficient drug delivery. The basic strategies are modifying nano-DDS with specific substrates of transporters, including natural substrates (*e.g.*, choline, glucose, carnitine, vitamins and amino acids) and derivatives. In this paper, we reviewed the recent developments in transporter-targeted nano-DDS on the emerging transporter-targeted nano-DDS developed to facilitate oral drug delivery, transporter-assisted brain-targeting nano-DDS, transporter-mediated tumor-targeting drug delivery, and the specific transport mechanisms involved in the transporter-mediated endocytosis.

However, despite the rapid developments and promising application aspects of transporter-targeted nano-DDS, several concerns should be addressed in the future research in this field. These include: (i) for tumor-targeting drug delivery, although certain transporters have been found to be overexpressed on tumor cells, transporters are also of great importance for normal cells, and how to avoid the off-target distribution of nano-DDS in normal cells is still a big challenge; (ii) the underlying transport mechanisms of transporter-mediated endocytosis is still not entirely clear, especially for the endocytosis mechanism and definite intracellular fate of transporters; (iii) transporters have emerged as a new topic for active targeted drug delivery, but it is not known if transporter targeting has distinct advantages over receptor-mediated targeting for drug delivery; (iv) despite significant progress in animal models, how can we bridge the gap between preclinical animal models and clinical trials? Continuous study of the underlying mechanisms will contribute to the rational design of improved transporter-targeted nano-DDS in the future.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (No. 81703451) and the China Postdoctoral Science Foundation Grant (No. 2017M611269).

References

1. Ramasamy T, Ruttala HB, Gupta B, Poudel BK, Choi HG, Yong CS, et al. Smart chemistry-based nanosized drug delivery systems for systemic applications: a comprehensive review. *J Control Release* 2017;**258**:226–53.
2. Ruoslahti E. Tumor penetrating peptides for improved drug delivery. *Adv Drug Deliv Rev* 2017;**110**:3–12.
3. Luo C, Wang Y, Chen Q, Han X, Liu X, Sun J, et al. Advances of paclitaxel formulations based on nanosystem delivery technology. *Mini Rev Med Chem* 2012;**12**:434–44.
4. Jin K, Luo Z, Zhang B, Pang Z. Biomimetic nanoparticles for inflammation targeting. *Acta Pharm Sin B* 2018;**8**:23–33.
5. Zhou Y, Quan G, Wu Q, Zhang X, Niu B, Wu B, et al. Mesoporous silica nanoparticles for drug and gene delivery. *Acta Pharm Sin B* 2018;**8**:165–77.
6. Li R, He Y, Zhang S, Qin J, Wang J. Cell membrane-based nanoparticles: a new biomimetic platform for tumor diagnosis and treatment. *Acta Pharm Sin B* 2018;**8**:14–22.
7. Sun J, Luo C, Wang Y, He Z. The holistic 3M modality of drug delivery nanosystems for cancer therapy. *Nanoscale* 2013;**5**:845–59.
8. Jahangirian H, Lemraski EG, Webster TJ, Rafiee-Moghaddam R, Abdollahi Y. A review of drug delivery systems based on nanotechnology and green chemistry: green nanomedicine. *Int J Nanomed* 2017;**12**:2957–78.
9. Weng Y, Liu J, Jin S, Guo W, Liang X, Hu Z. Nanotechnology-based strategies for treatment of ocular disease. *Acta Pharm Sin B* 2017;**7**:281–91.
10. Mir M, Ishtiaq S, Rabia S, Khatoon M, Zeb A, Khan GM, et al. Nanotechnology: from *in vivo* imaging system to controlled drug delivery. *Nanoscale Res Lett* 2017;**12**:500.
11. Pezzini I, Mattoli V, Ciofani G. Mitochondria and neurodegenerative diseases: the promising role of nanotechnology in targeted drug delivery. *Expert Opin Drug Deliv* 2017;**14**:513–23.
12. Ahmadzada T, Reid G, McKenzie DR. Fundamentals of siRNA and miRNA therapeutics and a review of targeted nanoparticle delivery systems in breast cancer. *Biophys Rev* 2018;**10**:69–86.
13. Zhang S, Guan J, Sun M, Zhang D, Zhang H, Sun B, et al. Self-delivering prodrug-nanoassemblies fabricated by disulfide bond bridged oleate prodrug of docetaxel for breast cancer therapy. *Drug Deliv* 2017;**24**:1460–9.
14. Luo C, Miao L, Zhao Y, Musetti S, Wang Y, Shi K, et al. A novel cationic lipid with intrinsic antitumor activity to facilitate gene therapy of TRAIL DNA. *Biomaterials* 2016;**102**:239–48.
15. Shi J, Kantoff PW, Wooster R, Farokhzad OC. Cancer nanomedicine: progress, challenges and opportunities. *Nat Rev Cancer* 2017 20–37.
16. Luo C, Sun J, Du Y, He Z. Emerging integrated nanohybrid drug delivery systems to facilitate the intravenous-to-oral switch in cancer chemotherapy. *J Control Release* 2014;**176**:94–103.
17. Shekhawat PB, Pokharkar VB. Understanding peroral absorption: regulatory aspects and contemporary approaches to tackling solubility and permeability hurdles. *Acta Pharm Sin B* 2017;**7**:260–80.
18. Klyachko NL, Polak R, Haney MJ, Zhao Y, Gomes Neto RJ, Hill MC, et al. Macrophages with cellular backpacks for targeted drug delivery to the brain. *Biomaterials* 2017;**140**:79–87.
19. Ningaraj NS, Reddy PL, Khaitan D. Targeting brain tumors with nanomedicines: overcoming blood brain barrier challenges. *Curr Clin Pharmacol* 2018;**2**:110–9.
20. Meng J, Agrahari V, Youm I. Advances in targeted drug delivery approaches for the central nervous system tumors: the inspiration of nanobiotechnology. *J Neuroimmune Pharmacol* 2017;**12**:84–98.
21. Lajoie JM, Shusta EV. Targeting receptor-mediated transport for delivery of biologics across the blood–brain barrier. *Annu Rev Pharmacol Toxicol* 2015;**55**:613–31.
22. Bazak R, Houri M, El Achy S, Kamel S, Refaat T. Cancer active targeting by nanoparticles: a comprehensive review of literature. *J Cancer Res Clin Oncol* 2015;**141**:769–84.
23. Tambe V, Thakkar S, Raval N, Sharma D, Kalia K, Tekade RK. Surface engineered dendrimers in sirna delivery and gene silencing. *Curr Pharm Des* 2017;**23**:2952–75.
24. McKinlay CJ, Waymouth RM, Wender PA. Cell-penetrating, guanidinium-rich oligophosphoesters: effective and versatile molecular transporters for drug and probe delivery. *J Am Chem Soc* 2016;**138**:3510–7.
25. Kou L, Bhutia YD, Yao Q, He Z, Sun J, Ganapathy V. Transporter-guided delivery of nanoparticles to improve drug permeation across cellular barriers and drug exposure to selective cell types. *Front Pharmacol* 2018;**9**:27.
26. Santos RS, Figueiredo C, Azevedo NF, Braeckmans K, De Smedt SC. Nanomaterials and molecular transporters to overcome the bacterial envelope barrier: towards advanced delivery of antibiotics. *Adv Drug Deliv Rev* 2017;**136-137**:28–48.
27. Xu D, You G. Loops and layers of post-translational modifications of drug transporters. *Adv Drug Deliv Rev* 2017;**116**:37–44.
28. Adeoye O, Cabral-Marques H. Cyclodextrin nanosystems in oral drug delivery: a mini review. *Int J Pharm* 2017;**531**:521–31.

29. Liu L, Yao W, Rao Y, Lu X, Gao J. pH-Responsive carriers for oral drug delivery: challenges and opportunities of current platforms. *Drug Deliv* 2017;**24**:569–81.
30. Florek J, Caillard R, Kleitz F. Evaluation of mesoporous silica nanoparticles for oral drug delivery—current status and perspective of MSNs drug carriers. *Nanoscale* 2017;**9**:15252–77.
31. Luo YY, Xiong XY, Tian Y, Li ZL, Gong YC, Li YP. A review of biodegradable polymeric systems for oral insulin delivery. *Drug Deliv* 2016;**23**:1882–91.
32. Banerjee A, Qi J, Gogoi R, Wong J, Mitragotri S. Role of nanoparticle size, shape and surface chemistry in oral drug delivery. *J Control Release* 2016;**238**:176–85.
33. Choonara BF, Choonara YE, Kumar P, Bijukumar D, du Toit LC, Pillay V. A review of advanced oral drug delivery technologies facilitating the protection and absorption of protein and peptide molecules. *Biotechnol Adv* 2014;**32**:1269–82.
34. Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. *Nat Biotechnol* 2015;**33**:941–51.
35. Shan W, Zhu X, Liu M, Li L, Zhong J, Sun W, et al. Overcoming the diffusion barrier of mucus and absorption barrier of epithelium by self-assembled nanoparticles for oral delivery of insulin. *ACS Nano* 2015;**9**:2345–56.
36. Wong CY, Al-Salami H, Dass CR. Potential of insulin nanoparticle formulations for oral delivery and diabetes treatment. *J Control Release* 2017;**264**:247–75.
37. Ahmad J, Singhal M, Amin S, Rizwanullah M, Akhter S, Kamal MA, et al. Bile salt stabilized vesicles (bilosomes): a novel nano-pharmaceutical design for oral delivery of proteins and peptides. *Curr Pharm Des* 2017;**23**:1575–88.
38. Niu M, Tan Y, Guan P, Hovgaard L, Lu Y, Qi J, et al. Enhanced oral absorption of insulin-loaded liposomes containing bile salts: a mechanistic study. *Int J Pharm* 2014;**460**:119–30.
39. Fan W, Xia D, Zhu Q, Li X, He S, Zhu C, et al. Functional nanoparticles exploit the bile acid pathway to overcome multiple barriers of the intestinal epithelium for oral insulin delivery. *Biomaterials* 2018;**151**:13–23.
40. Zhang Z, Li H, Xu G, Yao P. Liver-targeted delivery of insulin-loaded nanoparticles *via* enterohepatic circulation of bile acids. *Drug Deliv* 2018;**25**:1224–33.
41. Jain A, Jain SK. L-Valine appended PLGA nanoparticles for oral insulin delivery. *Acta Diabetol* 2015;**52**:663–76.
42. Du Y, Tian C, Wang M, Huang D, Wei W, Liu Y, et al. Dipeptide-modified nanoparticles to facilitate oral docetaxel delivery: new insights into PepT1-mediated targeting strategy. *Drug Deliv* 2018;**25**:1403–13.
43. Gourdon B, Chemin C, Moreau A, Arnauld T, Baumy P, Cisternino S, et al. Functionalized PLA-PEG nanoparticles targeting intestinal transporter PepT1 for oral delivery of acyclovir. *Int J Pharm* 2017;**529**:357–70.
44. Gourdon B, Chemin C, Moreau A, Arnauld T, Delbos JM, Péan JM, et al. Influence of PLA-PEG nanoparticles manufacturing process on intestinal transporter pept1 targeting and oxytocin transport. *Eur J Pharm Biopharm* 2018;**129**:122–33.
45. Kou L, Yao Q, Sun M, Wu C, Wang J, Luo Q, et al. Cotransporting ion is a trigger for cellular endocytosis of transporter-targeting nanoparticles: a case study of high-efficiency SLC22A5 (OCTN2)-mediated carnitine-conjugated nanoparticles for oral delivery of therapeutic drugs. *Adv Healthc Mater* 2017;**6**:1700165.
46. Luo Q, Jiang M, Kou L, Zhang L, Li G, Yao Q, et al. Ascorbate-conjugated nanoparticles for promoted oral delivery of therapeutic drugs *via* sodium-dependent vitamin C transporter 1 (SVCT1). *Artif Cells Nanomed Biotechnol* 2017http://dx.doi.org/ 10.1080/21691401.2017.1417864.
47. Tian C, Asghar S, Wu Y, Chen Z, Jin X, Yin L, et al. Improving intestinal absorption and oral bioavailability of curcumin *via* taurocholic acid-modified nanostructured lipid carriers. *Int J Nanomed* 2017;**12**:7897–911.
48. Kang SH, Revuri V, Lee SJ, Cho S, Park IK, Cho KJ, et al. Oral siRNA delivery to treat colorectal liver metastases. *ACS Nano* 2017;**11**:10417–29.
49. Shan W, Zhu X, Tao W, Cui Y, Liu M, Wu L, et al. Enhanced oral delivery of protein drugs using zwitterion-functionalized nanoparticles to overcome both the diffusion and absorption barriers. *ACS Appl Mater Interfaces* 2016;**8**:25444–53.
50. Khatun Z, Nurunnabi M, Cho KJ, Byun Y, Bae YH, Lee YK. Oral absorption mechanism and anti-angiogenesis effect of taurocholic acid-linked heparin-docetaxel conjugates. *J Control Release* 2014 64–73.
51. Park J, Choi JU, Kim K, Byun Y. Bile acid transporter mediated endocytosis of oral bile acid conjugated nanocomplex. *Biomaterials* 2017;**147**:145–54.
52. Khatun Z, Nurunnabi M, Reeck GR, Cho KJ, Lee YK. Oral delivery of taurocholic acid linked heparin-docetaxel conjugates for cancer therapy. *J Control Release* 2013;**170**:74–82.
53. Banks WA. From blood–brain barrier to blood–brain interface: new opportunities for CNS drug delivery. *Nat Rev Drug Discov* 2016;**15**:275–92.
54. Zhao X, Chen R, Liu M, Feng J, Chen J, Hu K. Remodeling the blood–brain barrier microenvironment by natural products for brain tumor therapy. *Acta Pharm Sin B* 2017;**7**:541–53.
55. He Q, Liu J, Liang J, Liu X, Li W, Liu Z, et al. Towards improvements for penetrating the blood–brain barrier—recent progress from a material and pharmaceutical perspective. *Cells* 2018;**7**:24.
56. Patel MM, Patel BM. Crossing the blood–brain barrier: recent advances in drug delivery to the brain. *CNS Drugs* 2017;**31**:109–33.
57. Zhou Y, Peng Z, Seven ES, Leblanc RM. Crossing the blood–brain barrier with nanoparticles. *J Control Release* 2018;**270**:290–303.
58. Saucier-Sawyer JK, Deng Y, Seo YE, Cheng CJ, Zhang J, Quijano E, et al. Systemic delivery of blood–brain barrier-targeted polymeric nanoparticles enhances delivery to brain tissue. *J Drug Target* 2015;**23**:736–49.
59. Zhang TT, Li W, Meng G, Wang P, Liao W. Strategies for transporting nanoparticles across the blood–brain barrier. *Biomater Sci* 2016;**4**:219–29.
60. Allen DD, Lockman PR. The blood–brain barrier choline transporter as a brain drug delivery vector. *Life Sci* 2003;**73**:1609–15.
61. Li J, Yang H, Zhang Y, Jiang X, Guo Y, An S, et al. Choline derivate-modified doxorubicin loaded micelle for glioma therapy. *ACS Appl Mater Interfaces* 2015;**7**:21589–601.
62. Li J, Huang S, Shao K, Liu Y, An S, Kuang Y, et al. A choline derivate-modified nanoprobe for glioma diagnosis using MRI. *Sci Rep* 2013;**3**:1623.
63. Li J, Guo Y, Kuang Y, An S, Ma H, Jiang C. Choline transporter-targeting and co-delivery system for glioma therapy. *Biomaterials* 2013;**34**:9142–8.
64. Li J, Zhou L, Ye D, Huang S, Shao K, Huang R, et al. Choline-derivate-modified nanoparticles for brain-targeting gene delivery. *Adv Mater* 2011;**23**:4516–20.
65. Berezowski V, Miecz D, Marszalek M, Bröer A, Bröer S, Cecchelli R, et al. Involvement of OCTN2 and $B^{0,+}$ in the transport of carnitine through an *in vitro* model of the blood–brain barrier. *J Neurochem* 2004;**91**:860–72.
66. Miecz D, Januszewicz E, Czeredys M, Hinton BT, Berezowski V, Cecchelli R, et al. Localization of organic cation/carnitine transporter (OCTN2) in cells forming the blood–brain barrier. *J Neurochem* 2008;**104**:113–23.
67. Wang G, Chen H, Zhao D, Ding D, Sun M, Kou L, et al. Combination of L-carnitine with lipophilic linkage-donating gemcitabine derivatives as intestinal novel organic cation transporter 2-targeting oral prodrugs. *J Med Chem* 2017;**60**:2552–61.
68. Kou L, Hou Y, Yao Q, Guo W, Wang G, Wang M, et al. L-Carnitine-conjugated nanoparticles to promote permeation across blood–brain barrier and to target glioma cells for drug delivery *via* the novel organic cation/carnitine transporter OCTN2. *Artif Cells Nanomed Biotechnol* 2017;**3**:1–12.

69. Patching SG. Glucose transporters at the blood–brain barrier: function, regulation and gateways for drug delivery. *Mol Neurobiol* 2017;**54**:1046–77.
70. Xie F, Yao N, Qin Y, Zhang Q, Chen H, Yuan M, et al. Investigation of glucose-modified liposomes using polyethylene glycols with different chain lengths as the linkers for brain targeting. *Int J Nanomed* 2012;**7**:163–75.
71. Jiang X, Xin H, Ren Q, Gu J, Zhu L, Du F, et al. Nanoparticles of 2-deoxy-D-glucose functionalized poly(ethylene glycol)-*co*-poly(trimethylene carbonate) for dual-targeted drug delivery in glioma treatment. *Biomaterials* 2014;**35**:518–29.
72. Li L, Di X, Zhang S, Kan Q, Liu H, Lu T, et al. Large amino acid transporter 1 mediated glutamate modified docetaxel-loaded liposomes for glioma targeting. *Colloids Surf B Biointerfaces* 2016;**141**:260–7.
73. An S, He D, Wagner E, Jiang C. Peptide-like polymers exerting effective glioma-targeted siRNA delivery and release for therapeutic application. *Small* 2015;**11**:5142–50.
74. Luo C, Sun J, Sun B, He Z. Prodrug-based nanoparticulate drug delivery strategies for cancer therapy. *Trends Pharmacol Sci* 2014;**35**:556–66.
75. Chen Q, Liu G, Liu S, Su H, Wang Y, Li J, et al. Remodeling the tumor microenvironment with emerging nanotherapeutics. *Trends Pharmacol Sci* 2018;**39**:59–74.
76. Luo C, Sun J, Liu D, Sun B, Miao L, Musetti S, et al. Self-assembled redox dual-responsive prodrug-nanosystem formed by single thioether-bridged paclitaxel-fatty acid conjugate for cancer chemotherapy. *Nano Lett* 2016;**16**:5401–8.
77. Luo C, Sun J, Sun B, Liu D, Miao L, Goodwin TJ, et al. Facile fabrication of tumor redox-sensitive nanoassemblies of small-molecule oleate prodrug as potent chemotherapeutic nanomedicine. *Small* 2016;**12**:6353–62.
78. Sun B, Luo C, Yu H, Zhang X, Chen Q, Yang W, et al. Disulfide bond-driven oxidation-and reduction-responsive prodrug nanoassemblies for cancer therapy. *Nano Lett* 2018;**18**:3643–50.
79. Chen H, Wang G, Sun L, Zhang H, Sun M, Sun J, et al. Regulating the alky chain length of fatty acid-didanosine prodrugs and evaluating its role in albumin binding. *Drug Deliv Transl Res* 2018;**8**:21–31.
80. Dai JT, Zhang Y, Li HC, Deng YH, Elzatahry AA, Alghamdi A, et al. Enhancement of gemcitabine against pancreatic cancer by loading in mesoporous silica vesicles. *Chin Chem Lett* 2017;**28**:531–6.
81. Tao W, Zhu X, Yu X, Zeng X, Xiao Q, Zhang X, et al. Black phosphorus nanosheets as a robust delivery platform for cancer theranostics. *Adv Mater* 2017;**29**:1603276.
82. Bhutia YD, Babu E, Prasad PD, Ganapathy V. The amino acid transporter SLC6A14 in cancer and its potential use in chemotherapy. *Asian J Pharm Sci* 2014;**9**:293–303.
83. Jiang X, Xin H, Gu J, Du F, Feng C, Xie Y, et al. Enhanced antitumor efficacy by D-glucosamine-functionalized and paclitaxel-loaded poly (ethylene glycol)-*co*-poly(trimethylene carbonate) polymer nanoparticles. *J Pharm Sci* 2014;**103**:1487–96.
84. Park JH, Cho HJ, Kim DD. Poly((D,L)lactic-glycolic)acid-star glucose nanoparticles for glucose transporter and hypoglycemia-mediated tumor targeting. *Int J Nanomed* 2017;**12**:7453–67.
85. Guo Y, Zhang Y, Li J, Zhang Y, Lu Y, Jiang X, et al. Cell microenvironment-controlled antitumor drug releasing-nanomicelles for GLUT1-targeting hepatocellular carcinoma therapy. *ACS Appl Mater Interfaces* 2015;**7**:5444–53.
86. Li L, Di X, Wu M, Sun Z, Zhong L, Wang Y, et al. Targeting tumor highly-expressed LAT1 transporter with amino acid-modified nanoparticles: toward a novel active targeting strategy in breast cancer therapy. *Nanomedicine* 2017;**13**:987–98.
87. Kou L, Yao Q, Sivaprakasam S, Luo Q, Sun Y, Fu Q, et al. Dual targeting of L-carnitine-conjugated nanoparticles to OCTN2 and $ATB^{0,+}$ to deliver chemotherapeutic agents for colon cancer therapy. *Drug Deliv* 2017;**24**:1338–49.
88. Luo Q, Gong P, Sun M, Kou L, Ganapathy V, Jing Y, et al. Transporter occluded-state conformation-induced endocytosis: amino acid transporter $ATB^{0,+}$-mediated tumor targeting of liposomes for docetaxel delivery for hepatocarcinoma therapy. *J Control Release* 2016;**243**:370–80.
89. Luo Q, Yang B, Tao W, Li J, Kou L, Lian H, et al. $ATB^{0,+}$ transporter-mediated targeting delivery to human lung cancer cells *via* aspartate-modified docetaxel-loading stealth liposomes. *Biomater Sci* 2017;**5**:295–304.
90. Zhao P, Yin W, Wu A, Tang Y, Wang J, Pan Z, et al. Dual-targeting to cancer cells and M2 macrophages *via* biomimetic delivery of mannosylated albumin nanoparticles for drug-resistant cancer therapy. *Adv Funct Mater* 2017;**27**:1700403.
91. Alizadeh AA, Aranda V, Bardelli A, Blanpain C, Bock C, Borowski C, et al. Toward understanding and exploiting tumor heterogeneity. *Nat Med* 2015;**21**:846–53.
92. Wu D, Wang DC, Cheng Y, Qian M, Zhang M, Shen Q, et al. Roles of tumor heterogeneity in the development of drug resistance: a call for precision therapy. *Semin Cancer Biol* 2017;**42**:13–9.
93. Marusyk A, Almendro V, Polyak K. Intra-tumour heterogeneity: a looking glass for cancer?. *Nat Rev Cancer* 2012;**12**:323–34.