ENGINEERING SMALL MOLECULE NANODRUGS TO OVERCOME BARRIERS FOR CANCER THERAPY

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Abstract
Small molecule nanodrugs consisting of pure drugs, drug-drug dimers, drug-drug conjugates, or drug derivatives could realize drug delivery by themselves without the aid of carriers, which have received abundant attention in recent decades. Avoiding the use of additional carriers, the yielded small molecule nanodrugs hold the following advantages: (a) high drug loading capacities (some of them could even reach up to 100%); (b) precise and tunable drug loading ration; and (c) no carrier-induced long-term toxicity. The past decade has witnessed rapid growth and advancements in this field. In this review, we will briefly introduce both in vitro "barriers" and in vivo barriers for drug delivery, and then summarize the most recent development of small molecule nanodrugs from the point of view how to engineer small molecule nanodrugs to overcome these barriers.

KEYWORDS
barriers, drug derivatives, drug-drug conjugates, drug-drug dimers, pure drugs, small molecule nanodrugs

INTRODUCTION
Chemotherapy is one of the widely used and most effective therapeutic methods for cancer treatment that could prolong life or even heal the disease. However, during chemotherapy a number of side effects such as hair loss, osteoporosis, headache, trouble breathing, neuropathy, and so forth may be caused because the healthy cells were harmed when the rapidly growing cancer cells were killed. In order to reduce collateral toxicity to healthy cells and further enhance their therapeutic efficiency, nanomedicines have been engineered, which ushered in a new era for drug delivery. Up to now, varieties of nanoparticles including micelles, liposomes, vesicles, inorganic nanoparticles, viral nanoparticles, and so forth loaded with drugs were reported. With these nanoformulation platforms, pharmacokinetics and biodistribution of anticancer drugs could be changed resulting in improved therapeutic efficiency and low systemic toxicity. Several of these delivery systems have been approved for use in clinic. However, carriers-based drug delivery systems always suffer from low drug loading ratio, batch-to-batch variation in drug loading, and long-term toxicity of carriers, which greatly hinder their...
rapid clinic translation. Generally, drug loading ration of most of the existing nanomedicines is less than 10% associated with many carrier materials. Drug encapsulation through noncovalent way will lead to batch-to-batch variation in drug loading. And long-term nanotoxicity will be caused by the accumulation of carriers.

In order to overcome these limitations, small molecule nanodrugs with high and precise drug loading ration and negligible nanotoxicity were developed. Different from traditional drug delivery systems, most small molecule nanodrugs consisting of pure drugs, drug-drug dimers, or drug derivatives could realize drug delivery by themselves without the aid of additional nanocarriers, which are just excipients without direct therapeutic function. Thus, the drug loading capacities of small molecule nanodrugs are excellent. Some of them even reach up to 100%. Besides, the drug loading ratio of small molecule nanodrugs is precise and tunable because of the well-defined structure of small molecules and cocktail self-assembly process. And there is no concern on carrier-induced long-term toxicity for avoiding the use of additional carriers.

Based on the distinctive merits of small molecule nanodrugs, the past decade has witnessed rapid growth and advancements in this field. In this review, we will summarize the most recent development of small molecule nanodrugs from the point of view how to engineer small molecule nanodrugs to overcome both in vitro “barriers” and in vivo barriers. First, we will briefly introduce both in vitro “barriers” and in vivo barriers during the development and delivery of small molecule drugs. Then, we will talk about how to precisely control the nanodrug formulation of small molecule nanodrugs through the molecular preparation and self-assembly process to overcome the in vitro “barriers.” To clarify, the in vitro “barriers” we mentioned here specifically refer to barriers to scaling up process, namely, precise control of nanodrug formulation during clinical translation of small molecule nanodrugs. Finally, we will summarize the strategies to engineer small molecule nanodrugs to overcome the in vivo barriers including blood barrier, tumor tissue barrier, and tumor cell barrier. Hopefully, with this review new thoughts and ideas could be inspired to pave the way for a next generation of therapeutics nanopolymers for the treatment of cancer.

2 | BARRIERS OF NANOSIZED DRUG DELIVERY SYSTEMS

As a promising drug delivery system, nanosized drug delivery system has been developed tens of years. Several nanodrugs for cancer therapy have been approved by Food and Drug Administration, in order to accelerate the clinic translation of nanodrugs from bench to bedside. Deeper thinking of both in vitro “barriers” and in vivo barriers that slow down this process will help in rational designing of nanosized drug delivery systems.

2.1 | In vitro “barriers”

The first barrier hindering the clinic application of nanodrugs is the formulation preparation. Up to now, many nanosized drug delivery systems contain nanocarriers and cargoes. Some of them suffer from batch-to-batch variation and quality control. For example, for some polymer-based carriers it is very hard to synthesize polymers with well-defined molecular weight, which might have some influence on the reproducibility of nanocarrier with respect to the component and sometimes the size. In addition, the drug loading ratios of cargoes loaded through noncovalent ways are hard to precisely control. These variations will greatly hinder the clinic translation of nanodrugs. Thus, nanodrugs with high drug loading capacity, precise molecular structure, and tunable self-assembly properties are highly demanded for translation from bench to bedside.

2.2 | In vivo barriers

Well-defined nanodrugs still face many barriers including blood-level barriers, tumor tissue-level barriers, and tumor cell-level barriers. There are many excellent reviews that have already summarized all in vivo barriers in delivery systems functioning inside the body. Therefore, here we will only briefly go through these barriers and spend more effort on how to rationally design small molecule nanodrugs to overcome these barriers.

When the nanodrugs are injected intravenously, the blood circulation is the first in vivo barrier the delivery systems face. Proteins in plasma might attach to the surface of the nanodrugs through noncovalent interaction forming protein corona, which, to some extents, will change the in vivo circulation. The protein corona will also influence the drug release behavior. What is more, nanodrugs with protein corona will be uptaken by phagocytic cells as shown in Figure 1. After the uptake of phagocytic cells, either nanodrugs will be degraded by mononuclear phagocytes system or they will accumulate in liver and spleen, causing severe side effects.

After the blood circulation, those nanodrugs must penetrate the tumor tissue, then they can reach the region of interest. Different from normal tissues, tumor tissue has high interstitial pressure, deficient blood supply, acidic and hypoxic microenvironments, and so forth. All these characteristics of tumor tissue greatly hinder the drug delivery process, resulting in poor cancer treatment efficiency.
Even though those nanodrugs conquer all the tumor tissue barriers, some of them cannot accomplish their mission until they target certain subcellular organelle because many drugs can only function when they reach nuclei or the cytosol. Thus, successful delivery of anticancer drugs still faces several cellular-level barriers including the cell membrane, endosome or lysosome trapping, inaccurate subcellular localization, and drug resistance.

3 | OVERCOMING THE BARRIERS OF NANOSIZED DRUG DELIVERY SYSTEMS

With the development of nanotechnology especially the small molecule nanodrugs, people have developed a variety of strategies to overcome all these barriers. In the following part, we will first summarize the current ways to overcome the in vitro “barriers.” Then, introduce how rational design of small molecule nanodrugs could overcome the in vivo barriers. With that, hopefully, more motivating thoughts could be inspired to accelerate the clinic translation of small molecule nanodrugs.

3.1 | Small molecule nanodrugs overcoming in vitro “barriers”

Precise control of the nanodrug formulation is a great challenge for the clinic transition of nanosized drug delivery system. Different from traditional drug delivery systems, small molecule nanodrugs consisting of well-defined small molecule conjugates with high drug loading capacity, tunable drug loading ratio, and diverse morphologies exhibit great potential in cancer therapy.

The drug loading capacities of self-delivery small molecule nanodrugs are relatively high, in general around 50%, compared to those of carrier-based drug delivery systems because of avoiding the use of carriers. For some special small molecule conjugate designs, the drug loading capacity could even reach 100%. For example, Zhu et al.19 developed a drug-drug conjugate between methotrexate and gemcitabine with 100% drug loading ratio, which could self-assemble into stable nanoparticles for synergistic combination chemotherapy. Besides, small molecule assembly from supramolecular interaction could also result in 100% drug loading ratio.27 Gefitinib and tripeptide tyroservatide could co-assemble into supramolecular drug/drug delivery system via multiple intermolecular interactions, including hydrogen bonding and π-π stacking.20 Similarly, Zhu et al.28 reported clofarabine and raltitrexed could self-assemble into nanoparticles with 100% drug loading ratio through molecular recognition. Interestingly, single drug, retinoic hydroxamic acid, has been studied for nanodrug formation by itself with effective inhibition of tumor growth.29 Moreover, the component of small molecule nanodrugs could be facilely tuned through a cocktail assembly for synergistic combination therapy. For example, two amphophilic
drug-drug conjugates, gemcitabine-chlorambucil and irinotecan-chlorambucil, could co-assemble into ternary cocktail nanodrugs and the component could be adjusted by changing the feed ratio of two drug-drug conjugates in the assembly process.\(^{22}\)

Another attractive property of small molecule nanodrugs is their tunable size and morphology,\(^{30-32}\) which can meet the requirements of different tumors. Both the size and morphology of small molecule nanodrug can be changed using different preparation condition or by tuning their conjugation structures, which will greatly accelerate their clinic applications. For example, Lee et al.\(^{33}\) prepared the pure nanodrugs using curcumin as model drug with controllable size ranging from 20 to 200 nm through an ice-template-assisted approach as shown in Figure 2, which has the potential of extending to other hydrophobic therapeutic drugs. For drug derivatives, Kasai et al.\(^{34}\) reported that anticancer agent SN-38 modified by different fatty acids with different hydrophobicity could assemble into nanoparticles with different sizes. However,
no correlation of particle size and hydrophobicity has been observed by the reprecipitation method. Different from the reprecipitation method, amphiphilic oligopeptide-drug conjugates (hybrid prodrugs contained both camptothecin (CPT) and a capecitabine analogue) developed by Cui et al.\(^{35}\) could assemble into supramolecular nanostructures in water with different size and morphology, which strongly depended on the intermolecular interaction. Increasing the ratio of CPT in the hybrid drug could provide stronger π-π interactions that helped the one-dimensional growth of hybrid prodrugs in filamentous structures. It provided an alternative way to control the morphology of the nanoassemblies. Apart from these fiber- or micelle-like morphologies, varieties of liposome-like small molecule nanodrugs have been developed.\(^{36-43}\) Many of these nanodrugs have similar conjugate structure containing phospholipids.

All these small molecule nanodrugs with high drug loading capacities, tunable drug loading ratio, and diverse size and morphology share precise chemical structure. Thus, their metabolism process can be easily monitored. Carefully engineering the precise conjugate structure of small molecule nanodrugs will greatly accelerate their clinic translation. In the following part, we will summarize the engineering of small molecule nanodrugs according to their precise conjugate structures including drug-drug conjugates, drug-drug dimers, and drug derivatives.

### 3.1.1 Drug-drug conjugates

Inspired by the amphiphilic assembly process in nature, amphiphilic drug-drug conjugates should also assemble into nanoparticles to work as self-delivery systems for cancer therapy. Yan et al.\(^{44-46}\) reported the pioneering work using hydrophilic anticancer drug irinotecan (Ir) and the hydrophobic anticancer drug chlorambucil (Cb) to synthesize the amphiphilic Ir-Cb conjugate via a hydrolyzable ester linkage (Figure 3A). Ir-Cb conjugates self-assembled into nanoparticles in water (Figure 3B). The Ir-Cb nanoparticles worked well in vivo showing longer circulation time in the bloodstream (Figure 3C) and better therapeutic efficiency compared to the drug mixtures (Figure 3D). Interestingly, these nanoscale particles can also overcome the multidrug resistance of tumor cells.

Since then, varieties of drug-drug conjugates have been reported. These amphiphilic drug-drug conjugates can be made through covalent linkers or supramolecular interactions between hydrophobic drugs and hydrophilic drugs.

For covalent conjugation method, a lot of anticancer drugs have been investigated to prepare the amphiphilic drug-drug conjugates, including irinotecan-doxorubicin conjugate,\(^{47,48}\) floxuridine-bendamustine conjugate,\(^{49}\) irinotecan-HIF-1α conjugate,\(^{50}\) methotrexate-CPT conjugate,\(^{51}\) irinotecan-bendamustine conjugate,\(^{52}\) irinotecan-chlorambucil conjugate,\(^{53}\) gemcitabine-chlorambucil conjugate,\(^{22,53}\) CPT-floxuridine conjugate,\(^{54}\) gemcitabine-CPT conjugate,\(^{55-57}\) methotrexate-CPT conjugate,\(^{58}\) methotrexate-doxorubicin conjugate,\(^{59}\) CPT-irinotecan conjugate,\(^{59}\) irinotecan-enediyne conjugate,\(^{60}\) doxorubicin-paclitaxel conjugate,\(^{61}\) and floxuridine-pseudolaric acid B conjugate.\(^{62}\) Most of these amphiphilic drug-drug conjugates can directly assemble into nanoparticles in water for cancer therapy. Interestingly, Zhang et al.\(^{61}\) reported the preparation of doxorubicin-paclitaxel prodrug nanoparticles with microfluidics. Many of these nanodrugs showed synergistic anticancer efficacy and some of them even could overcome the multidrug resistance of tumor cells.

For supramolecular interaction method, hydrophobic drugs and hydrophilic drugs form drug-drug conjugates via noncovalent interactions, such as hydrogen bonding, π-π stacking, hydrophobic interaction, van der Waals interaction, coordination, and so forth. For example, chemotherapeutic pharmaceuticals gefitinib (GEF) and tripeptide tyroservatide (YSV) have been used to construct supramolecular drug-drug delivery system through multiple intermolecular interactions including hydrogen bonding and π-π stacking. The assembled GEF-YSV nanoparticle showed excellent therapeutic efficiency for nonsmall-cell lung cancer. Besides, nucleosides analogue-based supramolecular nanodrugs prepared via molecular recognition can also greatly improve the therapeutic effect.\(^{20}\) As shown in Figure 4, Zhu et al.\(^{28}\) found that rational combination of the water-soluble anticancer drug clofarabine (purine nucleoside analogue) and the water-insoluble anticancer drug raltitrexed (quinazoline-based folate analogue) could self-assembled into supramolecular nanodrugs, which would induce the synergistic cytotoxicity based on their different mechanisms of action.

Apart from these traditional drug-drug conjugates, an amphiphilic Janus CPT-floxuridine conjugate (Figure 5) has been developed.\(^{63}\) Two hydrophobic CPT and two hydrophilic floxuridine were conjugated to multivalent pentaerythritol via hydrolysable ester linkage (Figure 5A). The Janus conjugates self-assembled into liposome-like nanocapsules for more efficacious combination chemotherapy (Figure 5B).

### 3.1.2 Drug-drug dimers

Different from amphiphilic drug-drug conjugates, drug-drug dimers can also self-assemble into nanoparticles with high drug loading ration, controlled release behavior,
and enhanced therapeutic efficiency. A common way to construct drug-drug dimers is combining them through a cleavable disulfide linker. Up to now, several drug-drug dimers including paclitaxel dimers, \textsuperscript{64-66} doxorubicin dimers, \textsuperscript{67} and CPT dimers \textsuperscript{68} have been prepared by this way. The reason to use disulfide bond is that not only it is susceptible to the reductive environment, but also it can balance the competition between intermolecular forces involved in the self-assembly of nanomedicines.\textsuperscript{69} Other than disulfide linker, ester bond has also been used to develop drug-drug dimers. For example, glutamic acid-linked paclitaxel dimers have been synthesized to increase the solubility of paclitaxel in water for chemotherapy.\textsuperscript{70} Ortho ester can also be used as linker for indomethacin dimers, and they could accelerate drug release at mildly acidic environments.\textsuperscript{71} Wang et al\textsuperscript{72} had demonstrated that the linkers had great influence on the self-assembly of doxorubicin dimers. However, the length of linker did not have significant influence on the cytotoxicity as shown in Figure 6. Carefully choosing the linker and modified position of doxorubicin will greatly improve the therapeutic efficiency of doxorubicin dimers-based drug-drug conjugate nanoparticles.

3.1.3 Drug derivatives

Drug derivative-based nanodrugs are another important kind of small molecular nanodrugs. Similar to drug-drug conjugates and drug-drug dimers-based nanodrugs, drug derivative-based nanodrugs could also self-assemble from the amphiphilic drug derivatives directly. Up to now, a lot of molecules have been used to modify the anticancer drugs to tune their amphiphilicity for self-assembly delivery. Those molecules include the nonfunctional moieties and other kinds of functional molecules such as...
FIGURE 4  (A) The optimized structure of clofarabine (CA):raltitrexed (RT) complex and binding energy by DFT calculations and schematic of anticancer mechanism of CA:RT nanodrugs. (B) Effects of CA, RT, CA/RT mixture, and CA:RT nanoparticles on dATP, dTTP, dCTP, and dGTP pools in HeLa cells. Copyright 2018, American Chemical Society

FIGURE 5  (A) Schematic illustration of the chemical structure of Janus camptothecin-flouxuridine conjugate (JCFC) and its self-assembly into the liposome-like nanocapsule (NC) for cancer combination therapy. (B) In vivo antitumor effect of JCFC NCs on PC-3 tumor-bearing nude mice. Copyright 2017, Elsevier

oligopeptides, targeting ligands, imaging agents, lipids, and so forth. Small molecules without biological functions have been selected to conjugate with anticancer drugs to tune their lipophilicity and solubility for nanoparticle formation. Previously, the selection of small molecules for preparing small molecule modified anticancer drug conjugates has been seemingly random. Several molecules such as oligomer of ethylene oxide (OEG), retinoic acid, linoleic acid, lysophospholipid, dichloroacetate,
FIGURE 6  (A) The structures of doxorubicin drug-drug dimers with different linkers. (B) Cytotoxicity of different doxorubicin drug-drug dimer nanoparticles (DOX DDC NPs) against MCF-7 cells after 48 h incubation. Copyright 2018, Elsevier

FIGURE 7  (A) Amphiphilic camptothecin (CPT) prodrugs (OEG-CPT and OEG-DiCPT) and their self-assembly into nanocapsules to load other drugs. (B) In vitro cytotoxicity of CPT, OEG-DiCPT, DOX-HCl, and OEG-DiCPT/DOX-HCl to MCF-7 breast cancer cells determined by MTT assay. Copyright 2010, American Chemical Society

stearic acid, lauric acid, phospholipid, and so forth have been reported for self-assembled drug delivery. For example, Shen et al reported the concept of directly using ethylene oxide oligomer to construct nanocarriers in order to minimize use of inert materials, substantially increase the drug loading content, and suppress premature burst release. As shown in Figure 7A, one or two hydrophobic CPT molecule(s) were conjugated to an oligomer chain of OEG, forming amphiphilic CPT prodrugs (OEG-CPT and OEG-DiCPT). The prodrugs could form liposome-like nanocapsules with a CPT loading content as high as 40 or 58 wt%. Meanwhile, the resulting nanocapsules can load a water-soluble drug-doxorubicin salt (DOX-HCl), leading to a synergistic cytotoxicity to cancer cells (Figure 7B). However, the general rules on how to select these molecules have not been summarized. Actually, both Liang et al and Zheng et al have reported using different lipophilic tails to tune the polarity of the anticancer drugs for highly efficient drug delivery. The quantitative way to tune the polarity of drug conjugates has not been reported until Zhangetal used XlogP (the theoretical n-octanol-water partition coefficient calculated by the XLOGP3 method, representing the lipophilicity) and Hansen solubility parameters (representing the water-solubility) as significant factors to investigate the relationship between these parameters for self-assembly into nanoparticles. They selected paclitaxel as the model drug to prepare a series of small molecule-drug conjugates
and investigated the relationship between XlogP and Hansen solubility parameters for self-assembly processes as shown in Figure 8. Different fatty acids were conjugated with PTX (Figure 8A), and all of them could self-assemble into nanoparticles in the range 80-170 nm. The calculated XlogP values of these fatty acid-PTX conjugates increased with the chain length of fatty acids (Figure 8B). On the contrary, the Hansen solubility parameters decreased with the chain length of the fatty acids (Figure 8C). However, the IC50 value of PTX-based small molecule nanodrugs was increased along with the XlogP values (Figure 8D). Based on their results, they suggested that for designing self-assembling small molecule nanodrug, the XlogP value of the conjugated molecules should increase more than onefold compared with that of the parent drug, or their Hansen solubility of the polar solubility parameter and hydrogen bond solubility should decrease more than 10% compared with the parent drugs. However, the anticancer activity of the assembling nanodrugs will decrease along with the XlogP. Thus, a suitable XlogP value for designing small molecule nanodrugs is important. For these conjugates with higher XlogP value, a degradable linker should be imported to increase their anticancer efficiency. With that, more and more small molecule nanodrugs could berationally engineered in the near future for enhanced cancer therapy.

Among these drug derivatives, drug oligopeptide conjugates are the most widely studied recently. Several review papers have already summarized the design principles of peptide-drug conjugate, including selecting the cytotoxic agents, linker technology, and oligopeptides. Moreover, both water-soluble drug-peptide conjugates and self-assembling drug-peptide conjugates for targeted drug delivery and cancer chemotherapy have been reviewed in 2016. Herein, we will summarize the recent advances of drug-peptide conjugate-based nanodrugs for cancer therapy. Doxorubicin and a hexapeptide as a matrix metalloproteinases (MMP) inhibitor (KGFRWR) have been reported to form a self-assembling amphiphilic peptide drug conjugate, which could assemble into nanofibers showing sustained release property for inhibiting the enzymatic activities of MMP-2 and MMP-9 and simultaneous inhibition of metastasis and tumor growth. Cui et al reported when CPT and capecitabine
conjugated to a short peptide (Sup35) to yield different prodrugs (CPT-Sup35-CPT, CPT-Sup35-capecitabine, and capecitabine-Sup35-capecitabine), the hybrid CPT-Sup35-capecitabine showed a synergistic effect and significantly enhanced potency against esophageal adenocarcinoma cells, which might help researchers to design combination chemotherapy based on two distinct drugs of different action mechanisms by the self-assembling hybrid prodrug strategy. Interestingly, Shen et al.66 found that self-assembly behavior and stability of 7-ethyl-10-hydroxycamptothecin and an arginine-glycine-aspartic acid-lysine peptide conjugate-based nanostructures were controllable by the relative hydrophobicity/hydrophilicity balance, surface charge density, and the conjugation position of 7-ethyl-10-hydroxycamptothecin. The optimized stable micelle formulation showed a prolonged blood circulation, significantly enhanced tumor accumulation, and improved cancer therapy. Moreover, other chemotherapeutic agents such as maytansinoid,87 CPT,88,89 and 5-fluorouracil90 have also been studied by similar assembling strategy for cancer therapy. Apart from those chemotherapeutic agents, photosensitizer has also been conjugated with chimeric peptide to develop photodynamic therapy-based self-delivery system. With the help of photosensitizer protoporphyrin IX, the self-assembled nanoparticle showed both mitochondria and plasma membrane dual-targeting and synergistic photodynamic therapy.91 Interestingly, amphiphilic functional peptides can assemble into nanostructure by themselves. Yan et al.92 took advantage of amphiphilic proapoptotic peptide KLAKLAKKLAKLAKGCK(Fmoc)2 combining with tumor-targeting hyaluronic acid and anticarcinogen doxorubicin to develop a self-delivery system, possessing precise tumor targeting, and exhibited fantastic antitumor efficacy as well as negligible side effects.

Very similar to functional oligopeptides, small molecular targeting ligands such as lactose93 and folate94,95 have also been used to prepare the amphiphilic drug derivatives for self-delivery cancer therapy. For example, Zhu et al.93 prepared the lactose-doxorubicin conjugate through a pH-responsive hydrazone group for targeted cancer therapy. Compared to the nontargeting glucose-doxorubicin nanoparticles, the targeting ligand-based lactose-doxorubicin nanoparticles displayed high cellular uptake and thus higher anticancer efficiency. Other than targeting ligands, several imaging agents including magnetic resonance imaging (MRI) agent96 and fluorescence imaging agent97 have been studied. The imaging agents could be used to figure out the nanodrug metabolism pathway96 or monitor the drug release process.97 As shown in Figure 9A, an esterase-responsive self-assembled Gd(DTPA-CPT) nanoparticles could accumulate in the tumor site through enhanced permeability and retention effect to enhance the therapeutic efficiency of CPT. With the help of MRI agent Ga-DTPA, the metabolism pathway of Gd(DTPA-CPT) nanoparticles was monitored by MRI (Figure 9B). Most interestingly, due to its inherent equilibrium, dissociation of the redundant Gd(DTPA-CPT) nanoparticles in blood circulation would result in effective clearance from the kidneys in the form of small molecules. Thus, Gd(DTPA-CPT) nanoparticles exhibited negligible chronic toxicity in the in vivo anticancer experiments.96 Moreover, chemotherapy drugs have also been conjugated to other therapeutic agents including photosensitizers98,99 to achieve chemo-photo therapy or multimodal imaging-guided trimodality cancer therapy.

3.2 Small molecule nanodrugs overcoming in vivo barriers

After the formation of small molecule nanodrugs with precise chemical structures, nanodrugs only conquer the first barriers in their clinical translation. Upon systemic administration, small molecule nanodrugs will come across a series of biological barriers including barriers of blood, tumor tissue, and tumor cells during transport process in vivo. In the following part, we will summarize the published papers on overcoming in vivo barriers via engineering small molecule nanodrugs.

3.2.1 Small molecule nanodrugs overcoming blood barrier

Nanodrug formulations were engineered with hydrophobic drugs being transformed to possess amphiphilic features, which would prolong the blood circulation time of hydrophobic drugs. Ideal drug delivery systems should be those systems that have limited cytotoxicity and immune response and provide maximum delivery efficiency. Drug delivery systems with long blood circulation time could maximize the drug delivery to the region of interest. For traditional drug delivery systems, several strategies including surface modifications and tuning the physical properties of carriers have been used for prolonging blood circulation through immunosuppression. Surface modification such as PEGylation,100,101 negative surface charge, or zwitterionic modification102-104 have been widely used for prolonging the blood circulation (Figure 10). And many papers reported that the physical properties including the size and morphology105,106 of drug delivery system greatly influenced the retention time during systemic circulation. Similar to the traditional drug delivery systems, small molecule nanodrugs could be engineered through the same way to overcome blood barrier. For small
molecule nanodrugs, one important feature is their nanoformulation, which will greatly improve the blood circulation time of the delivered drugs in vivo. Almost all the reported small molecule nanodrugs with in vivo pharmacokinetic assay have shown that the blood retention time of small molecule nanodrugs is longer than that of free drugs. For example, a small molecule nanodrug consisting of amphiphilic lactose-modified doxorubicin exhibited improved pharmacokinetic compared to free doxorubicin. In detail, the concentration of lactose-doxorubicin conjugate was much higher than that of free doxorubicin at the same time up to 10 h.

Except for the nanoformulation strategy, surface modification could also be applied to extend the drug retention time in vivo. PEGylation is a regular way that several papers, such as PEGylation of amphiphilic triphenylphosphine-quercetin conjugates, paclitaxel, sorafenib, and chlorin e6 have already used for enhanced therapy. However, recent studies found that PEGylation of drug delivery vehicles might cause significant immune responses due to the complement cascade and accelerated blood clearance phenomenon, which needs to be confirmed in the small molecule nanodrugs. Thus, novel modification would be desirable for the development of small molecule nanodrugs. Zwitterionic modification has aroused extensive attention on account of their extended circulation time and negligible immunogenic response.
Figure 10  Schematic illustration of the design of drug nanocarriers that can achieve simultaneous stealth surfaces in circulation and enhanced cellular uptake in tumors. Copyright 2019, American Chemical Society

compared to PEGylation modification. Zwitterionic phospholipid has been widely used to prepare the amphiphilic drug derivatives. For example, artesunate was conjugated to phospholipid form dimeric artesunate phospholipid-based liposomes. Compared to the free drug artesunate, the blood clearance time of zwitterionic liposomes was significantly prolonged.

Physical properties such as size and morphology of drug delivery system are another important factors to consider when engineering small molecule nanodrugs. Although different sizes and morphologies (including liposome, micelle, nanofiber, etc. of small molecule nanodrugs have been developed, no systematic work has been done to investigate the relationship between pharmacokinetics of small molecule nanodrugs and their physical properties, which is important for the in vivo application of small molecule nanodrugs.

Rather than engineering small molecule nanodrugs themselves, recruiting biomacromolecules can also prolong the blood circulation time. As shown in Figure 11A, Chen et al reported a drug amphiphile consisting of CPT and an albumin-binding Evans blue (EB). Through systematic administration, the self-assembled nanodrugs with a diameter of 80 nm could bind to endogenous albumin resulting a 7-nm albumin/prodrug complex, which remarkably extend the circulation time (CPT-EB [6.5 h], CPT [0.05 h], 130-fold greater than CPT) (Figure 11B).

3.2.2 Small molecule nanodrugs overcoming tumor tissue barrier

During the circulation of small molecule nanodrugs, small molecule nanodrugs with a diameter ranging from 20 to 200 nm can extravasate and accumulate in the interstitial space of tumors by penetrating through the endothelial pores. Extravasation is a key step during the drug delivery process. Small nanodrugs with tunable size ensure the successful extravasation. Several methodologies have been developed to engineer the size of small molecule nanodrugs. An ice-template-assisted approach has been reported to prepare hydrophobic therapeutic drug with different diameter ranging from 20 to 200 nm, which could perfectly match the requirement of extravasation. For amphiphilic drug-drug conjugates and derivatives, the size and morphology of small molecule nanodrugs could be tuned via changing the polarity of drugs. For example, irinotecan derived with different fatty acid moieties could self-assemble into nanoparticles with different morphologies. However, no general rules have been reported to tune the size of the self-assembled small molecule nanodrugs. For drug dimers, Wang et al reported that the size of doxorubicin dimers could change when different linkers were used. And similar to drug-drug conjugates and drug derivatives, general rules should be further explored in the future to drive the clinic application of small molecule nanodrugs.

When small molecule nanodrugs arrive in the tumor region, they must diffuse into the deeper part to achieve effective chemotherapy. However, the composition of tumor tissues is significantly different from that of normal tissues. The tumor interstitial compartment is characterized by large interstitial space, high collagen concentration, low proteoglycan and hyaluronate concentrations, high interstitial fluid pressure and flow, absence of anatomically well-defined functioning lymphatic network, high effective interstitial diffusion coefficient of macromolecules, as well as large hydraulic conductivity and interstitial convection compared to most normal tissues, which will retard extravasation of small molecule nanodrugs, especially in large tumors, resulting in poor tumor treatment. To overcome this barrier, size-reducible small molecule nanodrugs can be developed to facilitate deep tumor penetration. Once small molecule nanodrugs extravasate from blood vessels, external stimuli will reduce their size, which will help the deep diffusion of nanodrugs. As shown in Figure 12, Yin et al reported a self-assembled phototheranostic nanodrug (PTN) based on multi-functional pentamethine indocyanine (ICy5) dye, cyclic Arg-Gly-Asp (RGD) peptide, and anti-cancer drug CPT (Figure 12A). Under light irradiation, the generated...
FIGURE 11  (A) Schematic illustration of transformative nanoparticles of amphiphilic CPT prodrug with prolonged blood circulation. (B) Blood half-lives of CPT-EB (6.5 h) and CPT (0.05 h). Copyright 2017, American Chemical Society

1O2 of PTN caused the degradation of ICy5 through the C-C cleavage of polyene chains resulting in size reduction of nanoparticles from 90 to 10 nm (Figure 12B), which lead to deep tumor penetration. Moreover, a “solution-particle” transition strategy developed by Feng et al123 could help the accumulation of nanodrugs in tumor tissue. The authors took advantage of the acidic tumor microenvironment through switching the morphology of anticancer drug from the unstructured solution to the spherical structure in response to tumor acidity. The zwitterionic drugs could prevent the nonspecific distribution during circulation, whereas they spontaneously formed into nanoparticles resulting in enhanced tumor accumulation. Actually, many self-assembled materials have been reported, which has already been summarized in this review.124

3.2.3 Small molecule nanodrugs overcoming tumor cell barrier

These small molecule nanodrugs trapped in tumor tissue cannot inhibit tumor cell growth until they are efficiently uptaken by tumor cell and then located in specific organelle or nucleus. When small molecule nanodrugs reach the exterior membrane of a cell, they will interact with components of the plasma membrane or extracellular matrix. Then they can enter the cell mainly through endocytosis. According to uptake process, endocytosis can be classified into two major types, namely, phagocytosis and pinocytosis. Phagocytosis mainly occurs in professional phagocytes responsible for host defense and the uptake of dead cells and cell debris.125 Thus, here we will only focus on pinocytosis of small molecule nanodrugs by tumor cells.

It is now well known that physicochemical properties of nanoparticles including size and shape, surface charge, surface functionalization, and surface hydrophobicity/hydrophilicity play an important role in directing the movement of nanoparticles across the cell membrane. All these physicochemical properties for sure will have great influence on the uptake of small molecule nanodrugs. For example, Han et al126 reported a tumor-triggered geometrical shape switch of chimeric peptide. As shown in Figure 13A, the chimeric peptide self-assembled into spherical nanoparticles at physiological condition. When accumulated at acidic tumor microenvironment, chimeric peptide underwent detachment of acid-sensitive 2,3-dimethylmaleic anhydride groups resulting in formation of rod-like nanoparticles, which accelerated internalization into tumor cells (Figure 13B). Moreover, surface charge switch127,128 of small molecule nanodrugs could also accelerate the cellular uptake process. It has been reported that an amphiphilic chimeric peptide with tumor acidity triggered the cellular/intracellular dual-target properties for in situ PDT in nuclei. With an acidic liable 2,3-dimethylmaleic anhydride-modified NLS peptide sequence, 2,3-dimethylmaleic anhydride could avoid the nonspecific adsorption, and tumor acidic microenvironment triggered the detachment of 2,3-dimethylmaleic anhydride, and then realized the enhanced tumor uptake. The surface of small molecule nanodrugs functionalized by targeting ligands could also accelerate the cellular uptake process through ligand-receptor interactions. As shown in Figure 14,
The fluorescence intensity of small molecule nanodrugs consisting of the targeting ligand (lactose, Figure 14B) and chemotherapy drug (doxorubicin)-treated cancer cells was higher than their analogue without targeting ligand (Figure 14C), indicating that the excellent targeting ability is the result of ligand modification. Up to now, few papers reported the surface hydrophobicity/hydrophilicity of small molecule drugs' influence on the delivery of drugs into cancer cells. Systemic research works could be done in the future to figure out this relationship. Rather than
these traditional strategies, enzyme-activated way, and self-assembled materials in cancer microenvironments could also promote the cellular uptake process.

The postinternalization fate and intracellular localization of small molecule nanodrugs will greatly influence their final therapeutic efficiency, which is determined by the cellular internalization routes. Many internalized nanodrugs will be trapped in either in endosome or in lysosome. However, both endosome and lysosome are not the final therapeutic target. It is highly demanded to release these drugs in the case of degradation under hostile environment. Some drug delivery systems can efficiently impart neutralization effect after being protonated under the acidic conditions of endosome. This neutralization leads to an increase in endosomal pH, which will trigger the ionic transport into the lumen of endosome resulting in swelling and possible release of endocytosed nanodrugs, namely, proton sponge effect. Apart from the proton sponge effect, external stimuli, such as light, could also disrupt endosome to induce the enhanced cytotoxicity of anticancer drugs. For example, Xie et al. reported that small molecule nanodrugs self-assembled from porphyrin-paclitaxel conjugates could escape from endosome triggered by light irradiation. Under light irradiation, release of O2 damaged the endosome and then the released porphyrin-paclitaxel conjugates induced enhanced cancer therapy.

For those small molecule nanodrugs that can escape from or bypass endosome, the final subcellular localization will directly determine their therapeutic efficiency. Up to now, mitochondria and nuclear targeting small molecule nanodrugs have already been reported, for example, mitochondria-targeting small molecule nanodrug developed by Liu et al. through \( \pi-\pi \) stacking, O...p interaction, and electrostatic interaction between dehydroberberine and counter anions. When these nanodrugs reached cell membrane, anion exchange on the cell membrane facilitated cationic dehydroberberine entry into cells. Then, cationic dehydroberberine targeted mitochondria, led to mitochondrial dysfunction, and ultimately induced cell death. When subcellular or nuclear targeting ligands functionalize the small molecule nanodrugs, the functionalized nanodrugs have been demonstrated to direct drugs to the nuclear target. Han et al. prepared...
an amphiphilic chimeric peptide composed of an alkylated protoporphyrin IX, an acidic liable 2,3-dimethylmaleic anhydride-modified nuclear localization peptide sequence (PKKKRKV) using PEG segment as a linker. Tumor microenvironment triggered the detachment of the acidic liable group and realized the enhanced tumor uptake of detached nanoparticles due to the electrostatic interaction with negatively charged cell membrane. Then, nuclear localization peptide sequence was employed both as the cellular internalization accelerator and nuclear translocation resulting in efficient antitumor therapy in vivo. Apart from mitochondria and nuclei, more subcellular organelles could be explored for enhanced tumor therapy.

4 | CONCLUSIONS AND PERSPECTIVE

In this review, we briefly summarize both in vitro “barriers” and in vivo barriers of nanosized drug delivery system, followed by summaries of their physical properties including high drug loading, tunable size, and morphology. Then, we focused on how to engineer small molecule nanodrugs with these properties. Three major ways including drug-drug conjugates, drug-drug dimers, and drug derivatives were introduced with detailed examples. Through dedicated design, small molecule nanodrugs with nanoformulation, PEGylation, or zwitterionic modification could extend their pharmacokinetics. Then, engineering small molecule nanodrugs with transformable structures greatly helped the extravasation process. Once small molecule nanodrugs trapped in the tumor tissue, physicochemical properties of small molecule nanodrugs including size and shape, surface charge, and surface functionalization of small molecule nanodrugs directed their movement across the cell membrane. Finally, the cellular internalization routes and localization of small molecule nanodrugs determined their therapeutic efficiency. All these factors should be taken into consideration when small molecule nanodrugs are engineered.

Exceeding what has been done before, deeper works could be done to figure out the relationship between self-assembly behaviors and small molecule conjugate structure. More stimuli-responsive conjugate structures including light-responsive, self-immolative, and so forth are required for spatiotemporal control of anticancer drug release. Biocompatible materials such as oligonucleotides could conjugate with anticancer drugs to improve the programmable self-assembly of small molecule nanodrugs. Moreover, biomacromolecules such as phospholipids, antibodies, and proteins could involve in prolonging the circulation time of small molecule nanodrugs through rational design. Sometimes, cells in the bloodstream will also help to improve the circulation time. When evaluating the extravasation of small molecule nanodrugs, more clinically relevant tumor models should be used because in the clinic only a few tumor models such as Kaposi sarcoma and head and neck tumors present a strong enhanced permeability and retention effect. New strategies to modulate or to counterattack the abnormal tumor microenvironment could be explored to enhance the extravasation of small molecule nanodrugs. What is more, small molecule nanodrugs could also be used for treating diseases in the brain. When engineering this kind of delivery systems, rational design of molecular structures to overcome blood brain barriers should be carefully considered. For intracellular localization of small molecule nanodrugs, more subcellular targeting ligands could be developed to realize the precise delivery of drugs. Last but not least, our goal to create nanomedicines is to battle human disease. Thus, the ultimate clinical utility and translatability of nanomedicine should be carefully considered rather than designing a nanoparticle with multiple functions and struggling to adapt those functions to clinical use. 13

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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