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Carrier-free supramolecular nanomedicines assembled by small-molecule therapeutics for cancer treatment

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ABSTRACT

Nanomedicines have shown great promise in cancer therapy, but are challenged by limited drug loading, safety concerns of drug carriers, and complexity of function integration. Recently, carrier-free nanomedicines produced by supramolecular assembly of small-molecule therapeutic functionalities and their conjugates were proposed to address these issues. These nanomedicines achieve very high drug loading, enhanced tumor accumulation and improved therapeutic efficiency, and avoid carrier-related safety problems. In this review article, the applications of these nanomedicines in chemotherapy, photodynamic therapy, photothermal therapy as well as combination therapies will be reviewed. The concept of nanomedicine design and mechanism of supramolecular assembly will be discussed. Finally, future perspectives of carrier-free supramolecular nanomedicines for cancer therapy will be highlighted.

1. Introduction

Nanomedicines have shown promising advantages in cancer therapy, *i.e.* increased drug solubility, bioavailability and tumor accumulation, reversed drug resistance, reduced adverse effects, multi-function integration, and improved therapeutic responses [1–7]. Nanomaterials such as liposomes and lipid nanoparticles, proteins, polymer micelles and vesicles, dendrimers, and inorganic nanoparticles like graphenes and carbon nanotubes, gold nanoparticles were proposed as drug carriers to fabricate anticancer nanomedicines. However, only a few of nanoformulations succeed in approval by Food and Drug Administration (FDA). For example, PEGylated castor oil loaded with paclitaxel (PTX), liposomes loaded with doxorubicin (DOX), and human serum albumin nanoparticles loaded with PTX. The clinical translation of nanomedicines has been hindered by several challenges [8–11]. First, the safety concerns of nanocarriers should be addressed. The carriers such as polymers and inorganic nanoparticles are usually associated with difficulty in degradation, metabolization and excretion, and some of them were reported with immunogenicity, toxicity issues and other adverse effects. Second, the carriers usually occupy a high fraction of the prepared nanomedicine due to their large molecular weight or size, and thus relatively low drug loading is associated with most nanomedicines. Third, anticancer drugs are usually loaded by the nanocarriers *via* noncovalent interactions such as hydrophobic or ionic interactions, and might be released in a burst release manner during delivery. Therefore, well-controlled drug release by the nanomedicine is required. Fourth, nanomedicine usually requires functional integration, *i.e.,* tumor targeting and long blood circulation to improve its therapeutic response, but this will lead to increased complexities of the nanomedicines and difficulty in clinical translation [12–15].

In recent years, it is proposed that carrier-free supramolecular nanomedicines produced by small-molecule drugs and other therapeutic moieties may resolve these issues. These nanomedicines avoid carrier-related problems including degradation, metabolization, excretion and toxicity. They are usually fabricated by supramolecular assembly of drug conjugates without the incorporation of traditional drug excipient and vehicles [13,16–19]. As a result, very high drug loading (sometimes ~100%) can be achieved for these nanomedicines [20–22]. The drug molecules themselves participate in the formation of nanomedicines, avoiding burst release during delivery. In addition, stimuli-responsive spacers can be introduced into the conjugates to endow the supramolecular nanomedicine with controlled release characteristics. The carrier-free nanomedicines can be delivered to tumor tis-

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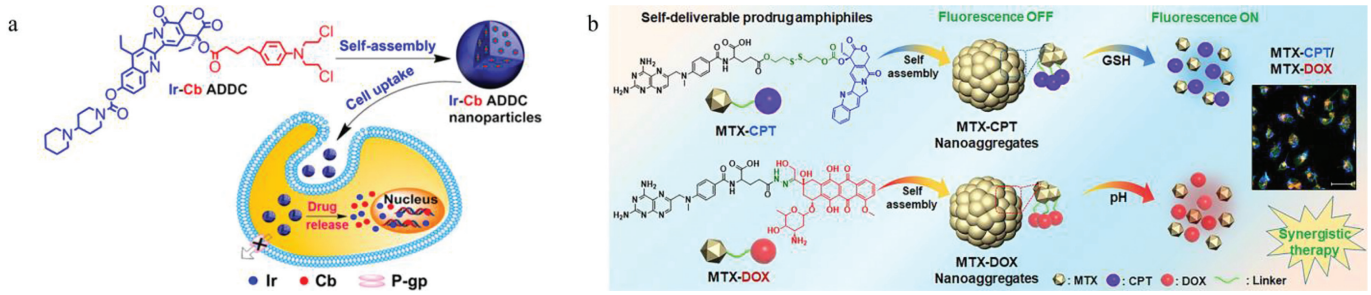

Fig. 1. The drug-free nanomedicines for chemotherapy assembled by drug-drug conjugates. (a) Ir-Cb amphiphilic drug-drug conjugate nanoparticles for cancer therapy. Reproduced with permission [29]. Copyright 2014, ACS. (b) MTX-CPT and MTX-DOX conjugate nanomedicines with stimuli-responsiveness for cancer therapy. Reproduced with permission [36]. Copyright 2018, Elsevier.

sues *via* the enhanced permeability and retention (EPR) effect or active targeting by the incorporated functional moieties [23–26]. Here, we reviewed recent progresses of carrier-free supramolecular nanomedicines designed for cancer therapy. We will focus on the preparation of carrier-free nanomedicines by supramolecular assembly of covalent or non-covalent conjugates of anticancer drugs, photosensitizers and photothermal agents. The applications of these nanomedicines in cancer chemotherapy, photodynamic therapy (PDT), and photothermal therapy (PTT) as well as combination therapies will be discussed.

2. Carrier-free nanomedicines for cancer chemotherapy

Chemotherapy represents the mostly adopted strategy to treat malignant tumors. Unfortunately, traditional chemotherapy suffers from several limitations including nonspecific selectivity, low accumulation in tumors, and adverse side effects for healthy tissues. With the help of nanotechnology, chemotherapeutic drugs could be precisely delivered to tumor tissues by nanodrug delivery systems [18,27,28]. Moreover, carrier-free nanomedicines are intended to integrate targeted drug delivery, stimuli-triggered drug release, and synergistic therapy into a single system *via* drug-drug conjugates, drug/drug non-covalent assemblies, pure drug nanocrystal-based assemblies, drug-ligand conjugates, and *etc.*

2.1. Drug-drug conjugates

Recently, carrier-free supramolecular nanomedicines which are formed by the covalent conjugates of drug molecules themselves, have been widely investigated with distinct advantages of ultrahigh drug loading, controlled release and negligible excipient-associated adverse effects. In a pioneer study, the hydrophilic drug irinotecan (Ir) was conjugated to the hydrophobic drug chlorambucil (Cb) *via* a cleavable ester bond to yield an amphiphilic prodrug (Fig. 1a) [29]. The Ir-Cb conjugate assembled into uniform nanoparticles (~88 nm) in water with very high drug loading. Benefiting from the nanoparticle characteristic, Ir-Cb nanoassemblies could overcome the multi-drug resistance (MDR) of cancer cells, and deliver both anticancer drugs into tumor tissues *via* the EPR effect, and kill the drug-resistant cancer cells *via* a synergistic anticancer effect. In a separate study, the anticancer drug cisplatin (CDDP) was bonded to vorinostat (SAHA), a histone deacetylase (HDAC) inhibitor *via* coordination interactions [30]. The conjugates further assembled into nanoparticles around 50 nm, and released platinum anticancer drug and SAHA triggered by endolysosomal acidity. The released SAHA from the supramolecular nanomedicine inhibits HDAC in tumor cells, and thus increase the anticancer activity of CDDP by blocking histone/DNA interactions. The cisplatin-SAHA nanomedicine also overcome the MDR of A549 cancer cells, and showed nearly 99% tumor inhibition *in vivo* with minimal system toxicity. SAHA was also conjugated to all-trans retinoic acid (ATRA) to obtain amphiphilic prodrug for supramolecular assembly [31]. The nanomedicine killed cancer cells *via* a synergistic effect of HDAC suppression and retinoic acid receptor activation. Similarly, two anticancer drug dasatinib were conjugated with a platinum(IV) prodrug *via* coordination chemistry [32]. The conjugate further assembled into nanoparticles around 120 nm, which showed both acid- and glutathione (GSH)-responsive release profiles. Anticancer drug methotrexate (MTX) was linked to gemcitabine (GEM) to prepare supramolecular nanomedicines [33]. Indomethacin (IND), a non-steroidal anti-inflammatory drug with multidrug resistance protein-1 transporter (MRP1) inhibition activity, was conjugated with anticancer drug PTX *via* a cleavable disulfide bond [34]. The amphiphilic conjugate assembled into nanomedicines around 160 nm, and the release of both IND and PTX can be triggered by intracellular GSH in tumor cells. The prepared nanomedicine reversed MDR on taxol-resistant A549 cancer cells by down-regulation of MRP1 proteins.

The drug-free nanomedicines assembled by drug-drug conjugates also allow cancer targeting *via* the conjugated drug moieties in the nanomedicine. For example, erlotinib, an epidermal growth factor receptor (EGFR) inhibitor was conjugated with a natural compound curcumin *via* an oligo(ethylene glycol) (OEG) spacer [35]. The amphiphilic erlotinib-curcumin conjugate assembled into nanoparticles around 105 nm, which could be delivered into tumor tissues *via* a combination of EPR effect and EGFR targeting activity of erlotinib. Benefiting from the synergistic anticancer effect of both erlotinib and curcumin, the prepared nanomedicine showed much prolonged median survival time in a pancreatic cancer model. Similarly, the anticancer drug MTX with folate receptor targeting capability was conjugated with camptothecin (CPT) and DOX *via* stimuli-responsive linkages such as disulfide and hydrazone bonds (Fig. 1b) [36]. The MTX molecule is relatively hydrophilic relative to CPT and DOX, and thus assembled on the nanomedicine surface, which may facilitate cancer targeting *via* folate receptor recognition for cancer therapy. After internalization, the acid and GSH inside cells trigger the rapid release of both anticancer drugs for cancer therapy.

2.2. Drug/drug non-covalent assemblies

Besides covalent conjugates, different drugs can be also assembled into a single nanomedicine *via* non-covalent interactions. Inspired by the Watson-Crick base paring in nucleic acids, an anticancer drug clofarabine (CA) bearing purine moiety was bonded to a hydrophobic anticancer drug raltitrexed (RT) bearing quinazoline *via* multiple hydrogen bonding recognition (Fig. 2a) [37]. The non-covalent CA-PT pairs further assembled into uniform nanoparticles with a hydrodynamic size around 32 nm, and a low critical aggregation concentration (CAC) of 12 μg/mL. Though CA-PT nanoparticles were formed *via* non-covalent interactions, they were stable under physiological conditions during long-term incubation. The

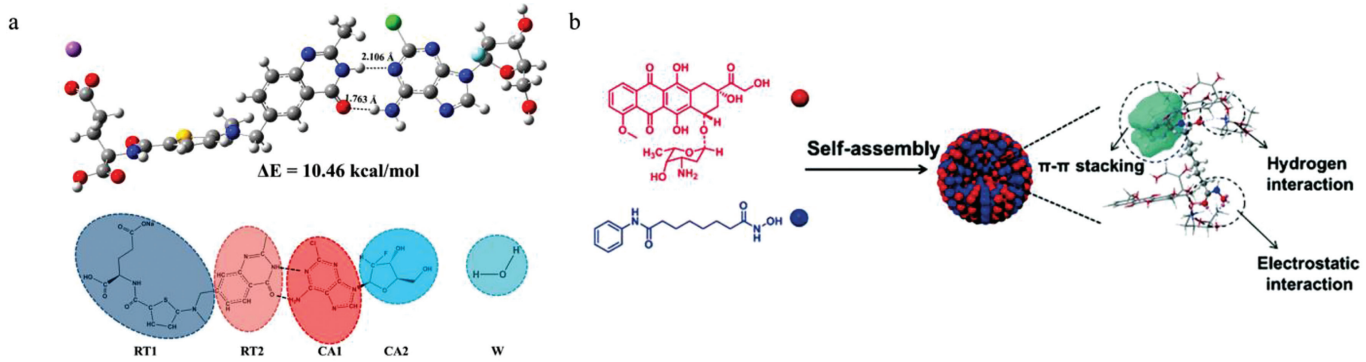

Fig. 2. The drug-free nanomedicines for chemotherapy assembled by drug/drug non-covalent interactions. (a) Non-covalent interactions between CA and RT. Reproduced with permission [37]. Copyright 2018, ACS. (b) DOX-SAHA nanomedicine assembled *via* multiple intermolecular interactions. Reproduced with permission [38]. Copyright 2018, RSC.

CA-PT nanomedicine showed much longer blood circulation time and increased tumor accumulation compared to both free CA and PT, and thus exhibited efficient tumor inhibition *in vivo*.

Similarly, SAHA was co-assembled with DOX *via* ionic, π-π stacking and hydrophobic interactions to form nanoparticles around 200 nm (Fig. 2b) [38]. Gefitinib, a targeted drug for the treatment of non-small-cell lung cancer (NSCLC) overexpressing EGFR, was co-assembled with a non-toxic tripeptide drug tyroservatide *via* hydrogen bonding and π-π stacking to form nanoparticles around 180 nm for NSCLC treatment [39]. Ursolic acid, a typical pentacyclic triterpenoid with anti-cancer activity, was co-assembled with PTX [40], MTX [41], and aspirin [42] *via* hydrophobic and hydrogen bonding interactions. Irinotecan and curcumin were assembled into fluorescent nanoparticles which allow self-monitoring and synergistic cancer therapy [43].

In a recent study, molecular dynamic simulation and quantum chemistry computation were used to investigate the co-assembly of five hydrophobic anticancer drugs including sorafenib, 10-hydroxycamptothecin (HCPT), docetaxel (DTX), ibrutinib, and lapatinib with a library of amphiphilic small molecules to form carrier-free nanomedicines [44]. The authors found that the formation of co-assemblies depends on the binding energy of small molecules with the drugs. When the binding energy was less than −20 kcal/mol, the amphiphilic small molecule can overcome the hydrophobic interactions between drug molecules and co-assemble with the drugs into nanomedicines. The lead carrier-free nanomedicines discovered in the library exhibited high drug loading and much improved bioavailability and therapeutic outcome compared to free anticancer drugs. The results provided an insight for the design of carrier-free nanomedicines by co-assembly of different small molecules.

2.3. Pure drug nanocrystal-based assemblies

It is reported that lots of anticancer drugs could form nanocrystals *via* supramolecular aggregation [13,16,17,45]. Curcumin, a natural phenolic compound with potent anticancer activity and potential photosensitizer were prepared into nanoparticles *via* reprecipitation [46]. The as-prepared nanoparticles were efficiently internalized by cancer cells, and the release curcumin generated efficient ROS upon laser irradiation and activated the JNK/caspase-3 signalling pathway for improved PDT. However, the pure drug nanocrystals are usually not stable in physiological conditions, and may further aggregate into precipitates. To improve the nanocrystal stability, several strategies were proposed. Anticancer drugs such as PTX (Fig. 3) [47] or carfilzomib [48] were assembled into nanoparticles *via* supramolecular assembly, and the assembled drug nanoparticles were further coated with metal-phenolic shells consisting of Fe^{3+} ions and tannic acid (TA) for sustained drug delivery [49]. Similarly, PTX nanocrystals were coated with amorphous IND *via* hydrophobic interactions to form supramolecular nanomedicines [50]. IND is a selective cyclooxygenase 2 (COX-2) inhibitor, which activates anticancer immune responses by COX-2 inhibition and M1/M2 macrophage polarization alternation. As a result, the nanodrug augmented the therapeutic outcome by PTX-mediated chemotherapy. DOX was assembled into nanoparticles and further homogeneously dispersed in lipiodol to prepare carrier-free nanomedicines with high stability [51].

2.4. Drug-ligand conjugates

Anticancer drugs can be conjugated with hydrophobic ligands to obtain amphiphilic bioconjugates [52,53], which could be further assembled into nanoparticles for cancer therapy. For example, squalene, a natural lipid precursor of cholesterol biosynthesis, was conjugated onto DOX *via* an ester bond [54]. The conjugate assembled into nanoassemblies around 130 nm, which showed esterase-responsive DOX release and much improved cellular uptake than free DOX. The squalenoylated DOX nanomedicine showed five-fold increase in maximum tolerance dose (MTD) of DOX, greatly decreased cardiac toxicity, and much increased anticancer activity on drug-resistant cancer cells. As a result, the prepared DOX nanomedicine exhibited high therapeutic efficacy on several tumor models.

Similar strategy could also be applied for carrier-free peptide delivery. Fluoro-lipids have shown unique features in cytosolic delivery including excellent self-assembly, efficient cell internalization and endosomal escape [55–62]. In this case, peptide drugs could be conjugated with fluorolipids to form amphiphilic bioconjugates and then assembled into nanoparticles for cancer therapy. For example, a proapoptotic peptide KLAKLAKKLAKLAK (KLA), which induces cell apoptosis by disrupting mitochondrial membrane, was modified with a fluoro-lipid *via* GSH-cleavable disulfide linkage, and the fluorous-tagged peptides were further assembled into nanostructures (Fig. 4) [63]. The fluorous-tagged KLA peptide nanoparticles were stable against enzymatic degradation and showed high anticancer efficiency. Moreover, the proapoptotic peptide-based nanomedicine efficiently inhibited tumor growth *in vivo*.

Alternatively, amphiphilic drug conjugates can be designed by conjugating hydrophilic small-molecule ligands such as OEG, peptides, lactose, and glucose onto hydrophobic anticancer drugs. For example, the hydrophobic anticancer drug 7-ethyl-10-hydroxyl-camptothecin (SN38) was conjugated with hydrophilic OEG *via* a thioether spacer. The conjugate assembled into nanoparticles around 100 nm, and the release of SN38 from the assemblies can

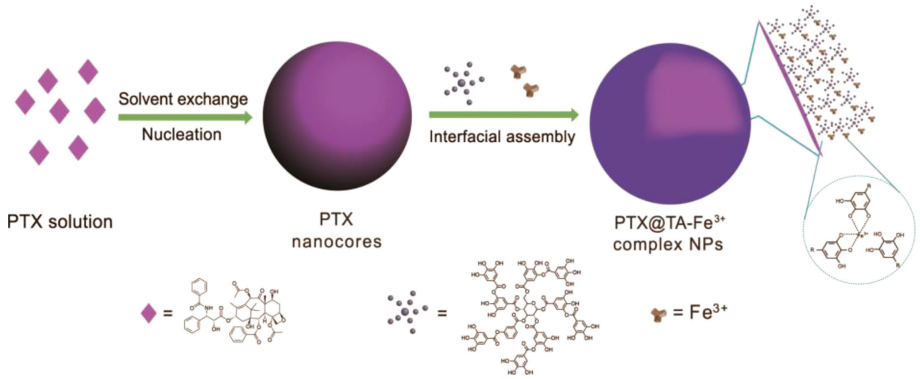

Fig. 3. The drug-free nanomedicines for chemotherapy assembled by pure drug nanocrystal. PTX nanocrystal stabilized by TA and Fe^{3+} coating. Reproduced with permission [47]. Copyright 2016, ACS.

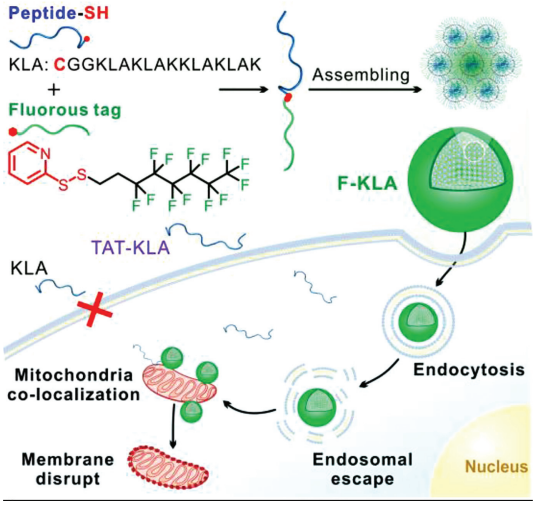

Fig. 4. Fluorous-tagged KLA peptide nanomedicine for cancer therapy. Reproduced with permission [63]. Copyright 2020, AAAS.

be triggered by both GSH and reactive oxygen species (ROS), and thus allow tumor redox heterogeneity responsiveness. In a separate study, the anticancer drug DOX was conjugated with a hydrophilic peptide (FRRG, cathepsin B-cleavable) to yield an amphiphilic prodrug, which assemble into nanoparticles around 200 nm (Fig. 5a) [64]. The assembled DOX-peptide nanoparticles showed selective toxicity on tumor cells overexpressing cathepsin B due to responsive DOX release, and thus showed promising therapeutic efficacy in the treatment of colon cancer with minimal adverse effects. Similarly, a CPT-based prodrug by conjugation of hydrophilic peptide RGD onto two CPT molecules *via* a matrix metalloproteinase 2 (MMP-2) cleavable spacer (PLGLAG) was synthesized, which was assembled into supramolecular nanostructures for cancer therapy [65].

To endow the conjugates with cancer targeting capability, a hydrophilic lactose moiety was conjugated to DOX *via* an acid-responsive hydrazone bond (Fig. 5b) [66], and the yielding DOX-lactose conjugate further assembled into nanoparticles around 200 nm with drug loading ratio as high as 61.7%. The lactose ligands on nanomedicine ensured enhanced cellular uptake by hepatocytes *via* asialoglycoprotein (ASGP) receptor mediated molecular recognition. Similarly, etoposide, a topoisomerase II inhibitor with anticancer activity, was conjugated to glucose *via* a ketal spacer (Fig. 5c) [67]. The etoposide-glucose conjugate assembled into uniform nanomedicines, and could release the bonded etoposide triggered by β-glucosidase or acid in a traceless manner.

Phytic acid (PA) is a natural occurring compound with six phosphate groups [68]. In an alternative strategy, anticancer drug CDDP was directly crosslinked with PA to form CPPA nanoparticles (Fig. 5d) [69]. PA on particle surface allow targeted delivery of CPPA nanoparticles to osteolytic lesions caused by bone tumors, and thus specifically deliver the platinum drug to bone surface adjacent to tumors. The released anticancer drugs triggered by tumor extracellular acidity efficiently killed cancer cells, while the released PA inhibited osteoclast activation to prevent osteolysis in the disease model.

Anticancer drugs can be also conjugated with β-cyclodextrin (β-CD) to fabricate supramolecular nanoassemblies. For example, CPT was conjugated to β-CD *via* a GSH-activatable disulfide linker (Fig. 6) [70]. The conjugates polymerize into supramolecular polymers *via* host-guest interactions between CPT and β-CD, and further assemble into nanomedicines. The nanodrug showed high therapeutic efficiency in the treatment of cancer and metastasis with decreased immunotoxicity. More importantly, the supramolecular nanomedicine could be facilely incorporated with other functionalities such as tumor targeting (cRGDfK peptide) and imaging modules (1,4,7-triazacyclononane-1,4,7-triacetic acid, NOTA) *via* similar host-guest chemistry. The NOTA ligand tagged on the nanomedicine could be coordinated with ^{64}Cu for positron emission tomography (PET) imaging.

2.5. Metal ion-involved drug assemblies

Non-covalent drug-drug assemblies can be stabilized by metal ions *via* coordination interaction. For example, bortezomib (BTZ), a proteasome inhibitor for cancer therapy was bonded to natural polyphenols such as TA *via* dynamic boronate-catechol linkages, yielding amphiphilic BTZ-TA conjugates to further assemble into carrier-free nanodrugs (Fig. 7) [71]. However, the nanodrug formed by BTZ and TA is not stable in buffer solutions. To improve nanoparticle stability, Fe^{3+} ions are used to crosslink the residual catechol moieties in the nanoparticle *via* coordination interactions. The prepared supramolecular nanomedicine efficiently delivered BTZ into tumor tissues and inhibit tumor growth in several models. Besides, the incorporated Fe^{3+} ions allow the monitoring of nanoparticles *in vivo via* magnetic resonance imaging (MRI).

Similarly, Fe^{3+} stabilized nanomedicine assembled by pemetrexed (PEM) and pseudolaric acid B *via* coordination with carboxylate groups on these drugs were prepared for cancer therapy [72]. PEM in the nanodrug allows tumor targeting *via* receptor mediated endocytosis, while pseudolaric acid B exhibits anti-angiogenic effect to inhibit tumor growth. A nanomedicine consisting of MTX, artesunate (ASA) and gadolinium (Gd^{3+}) ions was prepared for combination chemotherapy [73]. The Gd^{3+} ions stabilized

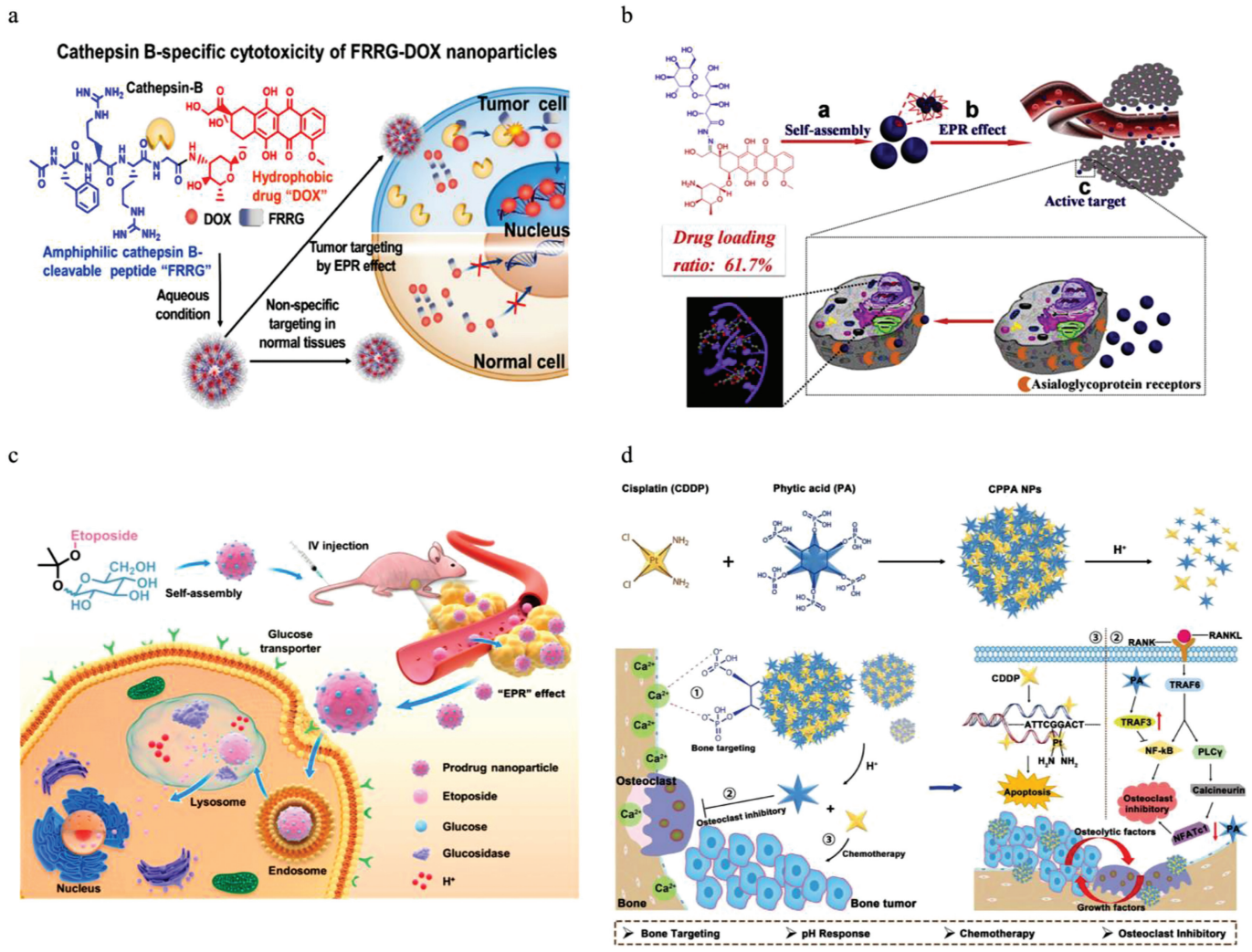

Fig. 5. The drug-free nanomedicines for chemotherapy assembled by drug-ligand conjugates. (a) DOX-FRRG conjugate nanoparticles for cancer therapy. Reproduced with permission [64]. Copyright 2019, Elsevier. (b) DOX-lactose conjugate nanoparticles for targeted cancer therapy. Reproduced with permission [66]. Copyright 2016, Elsevier. (c) Drug-free nanodrugs assembled by etoposide-glucose conjugates for targeted cancer therapy. Reproduced with permission [67]. Copyright 2020, ACS. (d) Carrier-free nanomedicine prepared by reactions between CDDP and PA for bone-targeted cancer therapy. Reproduced with permission [69]. Copyright 2020, Wiley.

the nanomedicine *via* coordination interactions with the carboxylate groups on both MTX and ASA, and the MTX allowed tumor targeting *via* folate receptor recognition as well as cancer chemotherapy, while the ASA could be activated by Fe^{2+} to induce excess ROS in tumor cells. As a result, the nanomedicine showed almost complete tumor elimination. In a separate study, a nanomedicine consisting of alendronate, calcium ions and cyclin-dependent kinase 7 (CDK7) inhibitor was prepared for the treatment of ovarian cancer [74]. Calcium ions stabilized the nanoparticles *via* coordination with the phosphate moieties on alendronates, and the nanomedicine efficiently induced cell apoptosis and inhibited tumor cell migration by affecting intracellular calcium homeostasis and CDK7 inhibition.

3. Carrier-free nanomedicines for cancer PDT

PDT, a noninvasive treatment of cancers, kills cancer cells by irradiating photosensitizer (PS) with laser of certain wavelengths, which converts oxygen in tumor tissues into highly reactive singlet oxygen ($^1O_2$). Although various nanodrug delivery platforms have been designed to improve the selective spatiotemporal distribution of PS and decrease the likelihood of off-target side effects, most of these widely used carriers have inherent drawbacks. Thus, it is of great importance to develop carrier-free strategies for cancer PDT [75–77].

3.1. PS-peptide assemblies

Peptides have advantages of structural diversity, facile synthesis, and inherent biocompatibility, and thus are attractive building blocks to co-assemble with PS for carrier-free PDT. Negatively charged PS chlorine e6 (Ce6) was co-assembled with diphenylalanine or an amphiphilic amino acid 9-fluorenylmethoxycarbonyl (Fmoc)-L-lysine *via* π-π stacking, ionic and hydrophobic interactions to form uniform nanoparticles of 100 nm or 200 nm, respectively (Fig. 8a) [78]. The assembled Ce6 nanoparticles showed enhanced cellular uptake by cancer cells and 4-fold increased efficiency on PDT, selective accumulation in tumor *via* EPR effect at 24 h post-injection, and thus allowed efficient tumor ablation by PDT. In a separate study, Ce6 was co-assembled with Fmoc-L-leucine (Fmoc-L-L) and manganese (Mn^{2+}) ions *via* coordination and hydrophobic interactions to form uniform nanoparticles around 100 nm (Fig. 8b) [79]. The prepared Ce6/Mn^{2+}/Fmoc-L-L nanoparticles showed GSH-responsive Ce6 release, which allowed enhanced photodynamic therapy under laser irradiation. In addition, the Mn^{2+} ions in the nanoparticles can be used to monitor

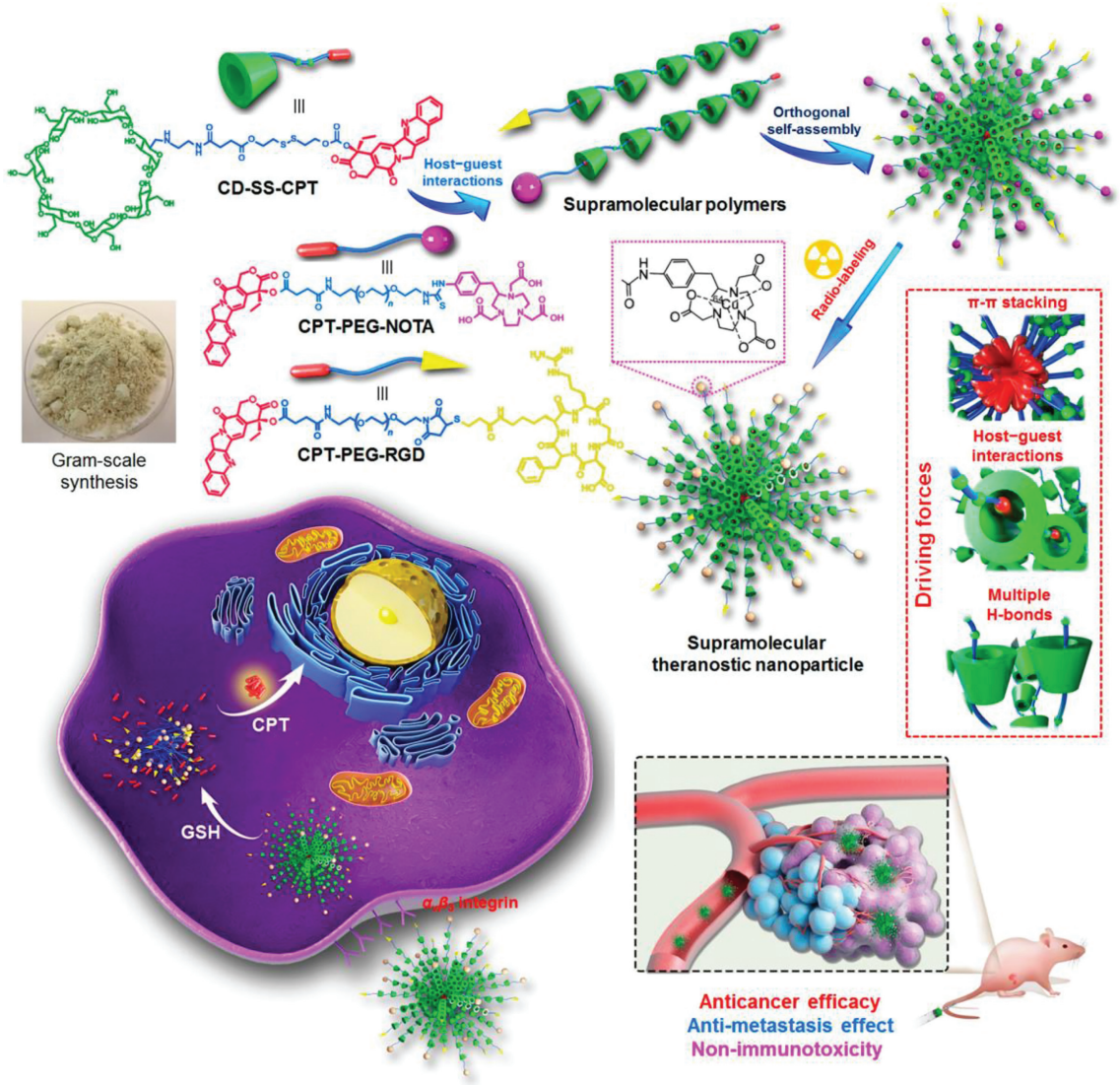

Fig. 6. The drug-free nanomedicine assembled by host-guest interactions between CPT and β-CD for cancer theranostics. Reproduced with permission [70]. Copyright 2018, ACS.

the therapeutic efficacy by MRI. Similarly, Ce6 was co-assembled with Fmoc-L-histidine and zinc ions (Zn^{2+}) *via* coordination to prepare metallo-nanodrugs (Fig. 8c) [80]. The metallo-nanodrugs showed GSH and pH-responsiveness, and much increased cellular uptake and tumor accumulation compared with free Ce6.

3.2. PS-enhancer assemblies

PDT kills cancer cells by converting oxygen into singlet oxygen. However, the hypoxia conditions in tumor tissues will hinder the PDT therapeutic efficacy. To overcome this limitation, various methods were proposed to improve the oxygen contents in tumor. In a recent study, atovaquone (ATO), an oxidative phosphorylation inhibitor was co-assembled with Ce6 *via* π-π stacking and hydrophobic interactions to prepare nanoparticles for oxygen-enhanced PDT (Fig. 8d) [81]. The released ATO from the nanomedicine can reduce oxygen consumption in tumor tissues by mitochondrial respiration inhibition, and therefore enhance Ce6-mediated PDT efficacy. Using the same strategy, lonidamine (Lon), a chemical that inhibits cell metabolism and mitochondrial respiration, was co-assembled with Ce6 to relieve the oxygen consumption in tumor and enhance PDT efficacy [76].

The oxygen-dependent PDT may accelerate tumor hypoxia by upregulation of hypoxia-inducible factor-1α (HIF-1α), which promotes tumor progression and metastasis. In this case, a HIF-1α inhibitor 3-(5′-hydroxy-methyl-2′-furyl)-1-benzylindazole (YC-1) was co-assembled with Ce6 *via* π-π and hydrophobic interactions to prepare nanodrugs with enhanced PDT efficacy [82]. It is known that the efficacy of PDT depends on ROS level in cancer cells, however, the induced ROS by PDT can be eliminated by intracellular GSH to protect the cells. To address this issue, the GSH inhibitor such as buthionine sulfoximine (BSO) was co-assembled with PS protoporphyrin zinc(II) (ZnPP) to prepare nanodrugs for improved PDT [83]. Besides BSO, the released ZnPP could inhibit heme oxygenase 1 to suppress the antioxidant defense system in cancer. As a result, BSO/ZnPP based PDT effectively inhibited tumor growth by producing more ROS. In a similar strategy, the PS pyropheophorbide-α was co-assembled with naphthazarin (Nap) for improved PDT [77]. The released Nap from assembled nanoparticles can down-regulate GSH in the cells to augment Pyro-mediated PDT. Simiarly, combretastatin A4 (CA4), an antiangiogenic agent with tubulin inhibition activity, was co-assembled with Ce6 for cancer therapy [84]. CA4 in the nanomedicine promoted the cellular uptake of Ce6 and promoted PDT *via* vascular disruption.

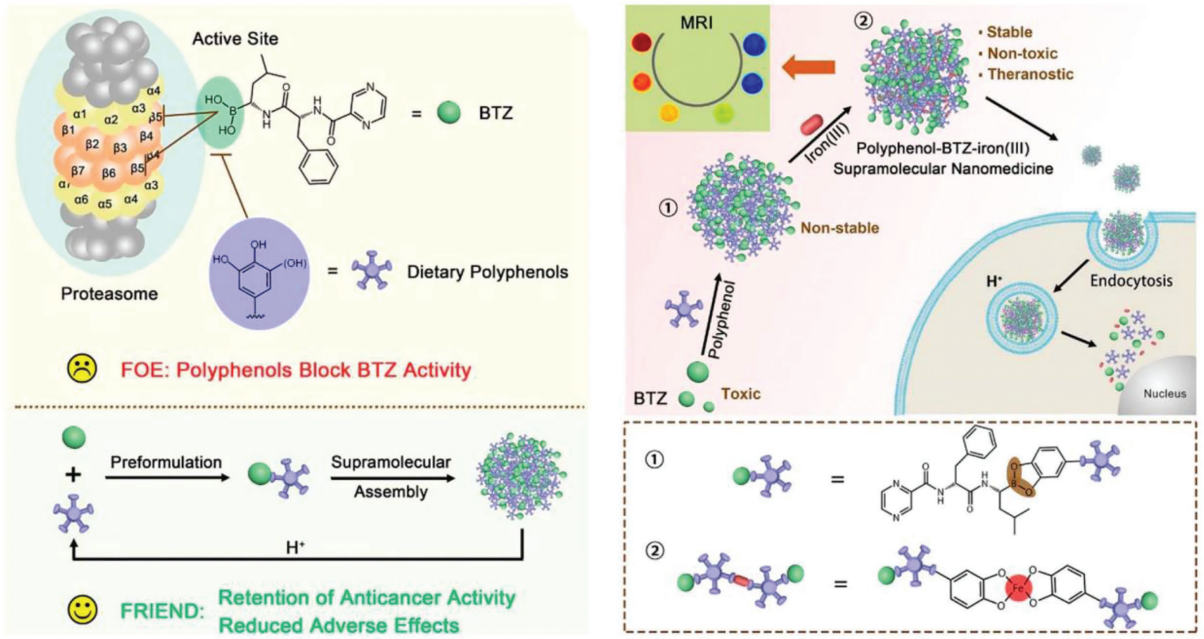

Fig. 7. Supramolecular BTZ-TA nanodrugs incorporated with Fe^{3+} ions for cancer therapy. Reproduced with permission [71]. Copyright 2018, ACS.

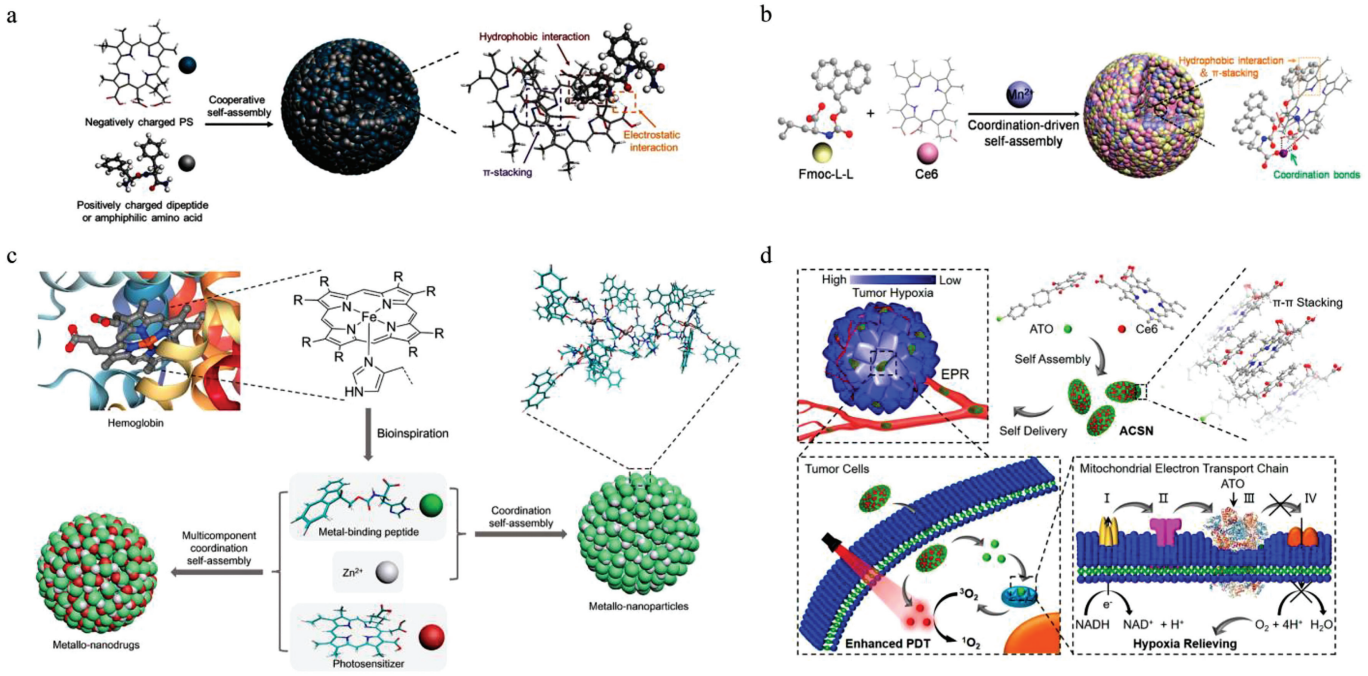

Fig. 8. Carrier-free nanomedicines for cancer PDT. (a) Photosensitive nanoparticles co-assembled by PS and peptides *via* non-covalent intermolecular interactions. Reproduced with permission [78]. Copyright 2016, Wiley. (b) Ce6 co-assembled with Fmoc-L-L and Mn^{2+} ions for MRI-guided PDT. Reproduced with permission [79]. Copyright 2018, ACS. (c) Supramolecular nanodrugs assembled by peptide, Zn^{2+} and PS. Reproduced with permission [80]. Copyright 2018, ACS. (d) Ce6-ATO co-assembled nanodrug for oxygen-enhanced PDT. Reproduced with permission [81]. Copyright 2020, ACS.

4. Carrier-free nanomedicines for cancer PTT

PTT is a promising therapeutic means against tumors with the advantages of time-spatial controllability, short-term therapeutic period, and limited drug resistance [85–91]. PTT depends on use of photothermal agents with high photothermal conversion efficiency, which absorb light energy and convert it into heat at local irradiated area. Traditional photothermal materials are suffering from limitations such as complicated fabrication processes, indistinct biodegradation, and potential biosafety, while carrier-free nanomedicine with high photothermal conversion efficiency provides an innovative strategy to overcome these shortcomings [92]. For example, purpurin-18 was covalently linked with a peptide consisting of an enzyme-cleavable sequence PLGVRG and an RGD targeting moiety [93]. The conjugate was not assembled under normal physiological conditions, but will be aggregate into nanostructures for enhanced tumor retention when the PLGVRG spacer was cut by gelatinase overexpressed in tumor. The assembled

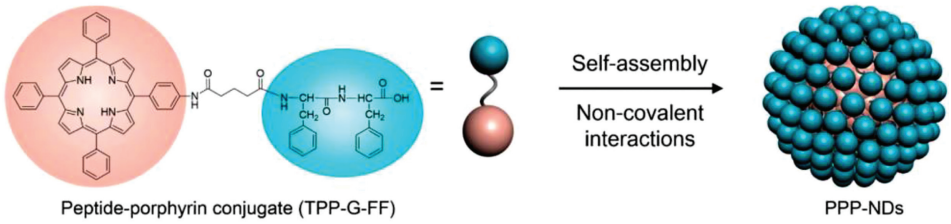

Fig. 9. Carrier-free nanomedicine assembled by amphiphilic peptide-porphyrin conjugates for cancer PTT. Reproduced with permission [94]. Copyright 2017, ACS.

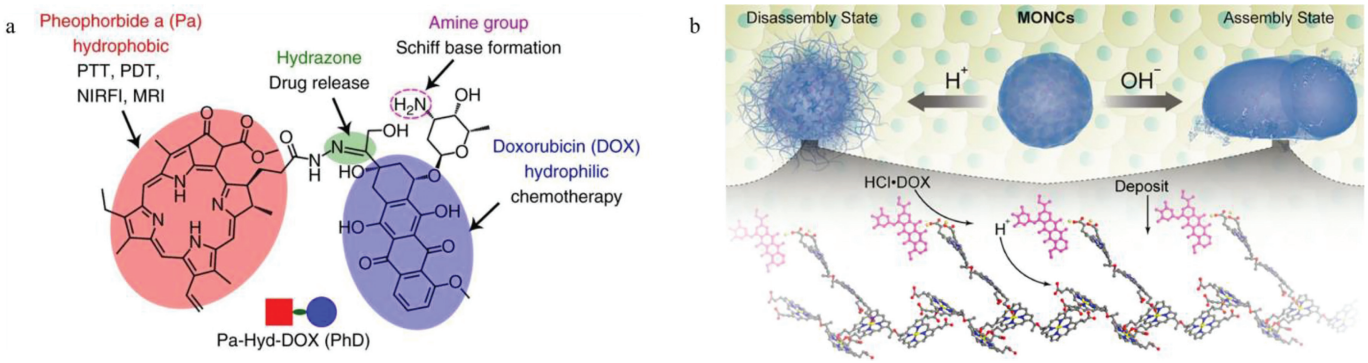

Fig. 10. Carrier-free nanomedicines for combined PDT and chemotherapy. (a) Carrier-free nanomedicine self-assembled by pheophorbide a-DOX conjugates for combination therapy. Reproduced with permission [99]. Copyright 2018, NPG. (b) Supramolecular nanodrug assembled by sinoporphyrin sodium, DOX and Fe^{3+} *via* non-covalent interactions for combination therapy. Reproduced with permission [103]. Copyright 2018, ACS.

purpurin-18 *in vivo* allowed efficient photothermal tumor ablation. In a separate study, porphyrin was conjugated with a dipeptide L-phenylalanine-L-phenylalanine (FF) *via* a glutaric acid spacer, and yielding amphiphilic conjugate was further assembled into uniform porphyrin nanodots around 28 nm *via* non-covalent hydrophobic interactions (Fig. 9) [94]. The assembled porphyrin nanoparticles showed fluorescence quenching and low singlet oxygen production under light exposure, but high photothermal conversion efficiency. As a result, the nanoparticles showed promising applications for cancer PTT. In an alternative strategy, photothermal dyes were co-assembled with other compounds *via* non-covalent interactions to prepare stable nanoparticles for PTT. For example, IR820 was complexed with ionic liquids *via* ionic interactions [95] or with the oxidative phosphorylation inhibitor ATO *via* hydrophobic interactions [96] to prepare stable nanoparticles for PTT. ATO in the later nanodrug regulated mitochondrial metabolism and inhibited the expression of heat shock proteins (HSPs), and thus augmented IR820-mediated cancer PPT.

5. Carrier-free nanomedicines for combination cancer therapy

Combination therapy is usually adopted to improve the therapeutic efficiency through synergistic effects, and it also allows minimalized administration of therapeutic agents or chemical drugs to reduce dose-related adverse effects [97]. The combination of PDT with chemotherapy, PTT with chemotherapy, PDT with PTT, and PDT with immunotherapy using carrier-free nanomedicines were discussed in this section.

5.1. PDT-chemo combination therapy

The synergistic therapy of PDT and chemotherapy showed exciting potentials for cancer treatment [98]. For example, a hydrophobic PS (pheophorbide a) was modified with a hydrazine group and further conjugated with DOX *via* an acid-cleavable hydrozone bond (Fig. 10a) [99]. The conjugates assemble into nanoparticles around 80 nm, and the assembled nanoparticles allow the ablation of tumor by *via* synergistic photodynamic and chemotherapy. Similarly, Ce6 was conjugated with a platinum(IV) anticancer drug [100], or a natural anti-cancer drug oleanolic acid (OA) [101], and further assembled into nanoparticles for combination therapy. A photosensitizer 2-(1-hexyloxyethyl)-2-devinyl pyropheophorbide-α was conjugated with anticancer drug CPT *via* a GSH-cleavable linkage. The formed nanomedicine possessed a high drug loading ratio of 59% and enabled cancer therapy *via* a combination of chemotherapy and PDT [102].

PS can also assemble with anticancer drugs *via* non-covalent interactions to form nanoparticles for combined PDT and chemotherapy. For example, the PS sinoporphyrin sodium was co-assembled with DOX and Fe^{3+} *via* hydrophobic and coordination interactions to form supramolecular nanoparticles around 140 nm (Fig. 10b) [103]. Compared with free sinoporphyrin sodium, the nanoparticles generated much more ROS (increased by ~3 fold) under laser exposure. After tumor accumulation, the mild acidity triggered the disassembly of nanoparticles into ultrasmall nanodrugs (5–10 nm) to enhance tumor penetration and therapeutic efficiency. Similarly, Ce6 was co-assembled with HCPT into nanorods (length: 360 nm; width: 135 nm) [104], with DOX into supramolecular nanomedicines below 100 nm [105], and with erastin, a ferroptosis inducer into nanomedicines around 150 nm for combined PDT and ferroptosis [106].

In a separate study, a nanomedicine consisting of curcumin, perylene and 5,10,15,20-tetra(4-pyridyl)porphyrin (H2TPyP) were prepared *via* non-covalent supramolecular assembly [107]. A fluorescent resonance energy transfer (FRET) effect from the perylene to H2TPyP is observed, while the green fluorescence from the anticancer drug curcumin is quenched in the nanomedicine. As a result, the nanoparticles emit near-infrared (NIR) fluorescence from H2TPyP upon laser irradiation. After nanoparticle disassembly, the green fluorescence from curcumin will be observed. This property allows the real-time monitoring of drug release and biodistribution, and the released curcumin and H2TPyP from the nanomedicine enable the ablation of tumor *via* combined chemotherapy and PDT.

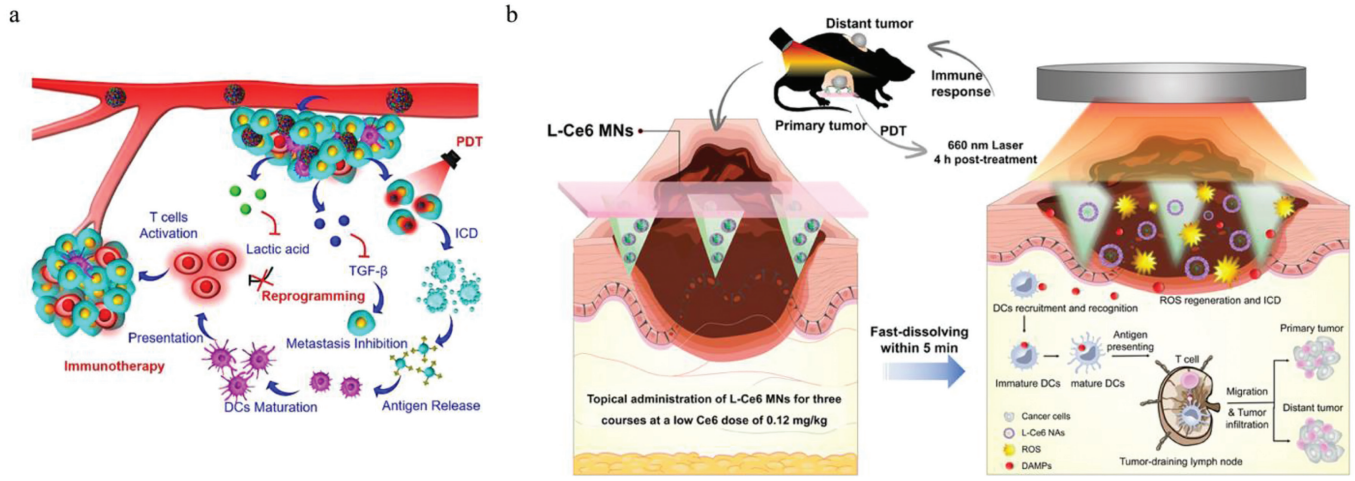

Fig. 11. Carrier-free nanomedicines for PDT-immuno combination therapy. (a) Nanodrugs assembled by Ce6, SB and Lon for PDT-amplified immunotherapy. Reproduced with permission [120]. Copyright 2022, ACS. (b) Nano-Ce6 integrated microneedle patches for enhanced photoimmunotherapy against melanoma. Reproduced with permission [121]. Copyright 2021, ACS.

5.2. PTT-chemo combination therapy

Besides PDT, chemotherapy was also combined with PTT in cancer therapy [108,109]. PTT reagent indocyanine green (ICG) was co-assembled with anticancer drug epirubicin (EPI) *via* ionic, π-π stacking and hydrophobic interactions to prepare nanoparticles with high drug loading and photothermal conversion efficiency. The nanoparticles showed improved cellular uptake and tumor accumulation compared to free ICT and EPI, and thus induced significantly increased therapeutic efficiency by combined PTT and chemotherapy [110]. Similarly, ICG was co-assembled with 10-HCPT to form nanoparticles for combined therapy [111]. Nanoparticles assembled by ICG and MTX were coated with amphiphilic anticancer drug CA *via* hydrogen bonding like Watson-Crick pairs [112]. The nanodrugs showed excellent serum stability and blood circulation time over seven days, as well as efficient tumor accumulation for combined therapy. Nanoparticles assembled by ICG, PTX, and ursolic acid *via* ionic, hydrophobic interactions were prepared for the same purpose [40].

In an alternative strategy, DOX was conjugated to the targeting ligand mannose *via* a ROS-sensitive thioketal linker to synthesize an amphiphilic prodrug [113]. The prodrug was further co-assembled with ICG to prepare carrier-free nanoparticles for combined therapy. The prepared nanodrug could be internalized by cancer cells *via* lectin receptor-mediated endocytosis. After cell internalization, NIR light irradiation on the nanoparticles generates ROS to trigger the cleavage of thioketal spacer between DOX and mannose, and followed by the release of DOX for chemotherapy.

5.3. PDT-PTT combination therapy

Combined PDT and PTT has also drawn extensive interests for cancer treatment [114]. A nanoparticle consisting of ICG, pheophorbide a and Cu^{2+} ions was designed for combined PDT and PTT [115]. Cu^{2+} ions in the nanoparticles coordinated with both ICG and pheophorbide a to form stable nanotheranosics, and also depleted intracellular GSH to augment ROS induced by both therapies. ICG in the nanoparticles showed increased photothermal conversion efficiency due to J-aggregation induced absorption redshift. Similarly, Zinc phthalocyanine (ZnPC) was co-assembled with ICG to prepare carrier-free nanodrugs for combined PDT and PTT [116]. Ce6 was co-assembled with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide (DiR) to prepare nanoparticles for the same purpose [117]. The PTT/PDT nanoparticles were also incorperated with anticancer drugs for combination therapy. For example, hydrophobic compounds Ce6, PTX were co-assembled with hydrophilic dye IR783 to prepare an "all-in-one" nanomedicine with a 70 nm size for combined cancer therapy [118].

5.4. PDT-immuno combination therapy

Carrier-free nanomedicine can be also incorporated with immune checkpoint blockade (ICB) for combined immunotherapies [12]. PS can be co-assembled with immune checkpoint inhibitors to prepare carrier-free nanomedicines for combined PDT and immunotherapy. For example, NLG919, a molecule inhibits the activation of indoleamine 2,3-dioxygenase 1 (IDO-1) to reverse immunosuppression in tumor tissues, was co-assembled with Ce6 *via* noncovalent interactions to prepare nanodrugs [119]. Ce6-mediated PDT induced immunogenetic cell death to activate cytotoxic T lymphocytes, and the co-delivered NLG919 promoted the immunoresponse *via* IDO-1 inhibition. Similarly, Ce6 was co-assembled with SB505124 (SB) and lonidamine (Lon) to prepare nanoparticles. SB505124 in the nanomedicine down-regulated transforming growth factor β (TGF-β), and lonidamine inhibited lactic acid efflux. The co-delivery of these small molecules in the nanodrug allowed efficient tumor ablation *via* PDT-amplified immunotherapy (Fig. 11a) [120]. In a seperate study, Ce6 nanoparticles were integrated into microneedle patches to treat melanoma (Fig. 11b) [121]. Ce6-mediated PDT efficiently ablated the primary tumor, which activated immunogenic cell death (ICD) and the release of danger-associated molecular patterns (DAMPs) to kill distant tumors *via* immunotherapy.

6. Conclusions and perspectives

In summary, carrier-free nanomedicines can be fabricated by supramolecular assembly of amphiphilic small-molecule functionalities such as anticancer drugs, photosensitizers and photothermal agents as well as their covalent or non-covalent conjugates. The conjugates could be fabricated with cleavable spacers and targeting moieties to endow the nanomedicines with stimuli-responsive and tumor targeting functionality. These prepared nanomedicines with a size of tens to hundreds of nanometres usually possess extremely high drug loading efficiency (approaches 100%) and improved blood circulation and tumor accumulation *via* EPR effect or

active tumor targeting. They can kill cancer cells *via* chemotherapy, PDT, PTT, immunotherapy or combination of these therapies.

However, although carrier-free nanomedicines have significant advantages such as ultrahigh drug loading, controlled release, negligible carrier-related toxicity and synergistic therapeutic effect, there are lots of disadvantages and challenges should be addressed. First, the large-scale industrial production of carrier-free nanomedicines with good quality control is challenging. The reproducible fabrication of carrier-free nanodrugs especially those formed by drug/drug non-covalent assemblies is very difficult. Second, the nanodrugs are usually prepared by supramolecular assembly of hydrophobic drug molecules, and the obtained assemblies are less stable in physiological conditions. These nanoparticles might be coated with phospholipids [122], red blood cell membranes [111], and PEG [123] to improve the stability of nanomedicines *in vivo*. Third, the assembly of small-molecule therapeutics are mainly governed by the equilibrium of the intermolecular forces. To precisely predict the self-assembly and co-assembly capacity and morphology of carrier-free nanomedicines remains a challenging task. The fourth challenge for carrier-free supramolecular nanomedicine is to precisely control drug ratios in multi-drug nanoparticles. It is worth noting that the finest formulation of nanoparticles could hardly correspond to the best synergistic ratio between the drugs. Therefore, great efforts are still needed to pave the way for clinical application of carrier-free supramolecular nanomedicines.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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