Hypoxia-activated prodrugs and redox-responsive nanocarriers

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Abstract: Hypoxia is one of the marked features of malignant tumors, which is associated with several adaptation changes in the microenvironment of tumor cells. Therefore, targeting tumor hypoxia is a research hotspot for cancer therapy. In this review, we summarize the developing chemotherapeutic drugs for targeting hypoxia, including quinones, nitroaromatic/nitroimidazole, N-oxides, and transition metal complexes. In addition, redox-responsive bonds, such as nitroimidazole groups, azo-groups, and disulfide bonds, are frequently used in drug delivery systems for targeting the redox environment of tumors. Both hypoxia-activated prodrugs and redox-responsive drug delivery nanocarriers have significant effects on targeting tumor hypoxia for cancer therapy. Hypoxia-activated prodrugs are commonly used in clinical trials with favorable prospects, while redox-responsive nanocarriers are currently at the experimental stage.

Keywords: antitumor drugs, hypoxia, nanoparticles, redox-sensitive, tumor therapy

Introduction
Hypoxia is one of the key features of malignant tumors.1 It can significantly affect tumor treatment and prognosis because it can influence the expression of many genes.2 In recent years, being inspired by some antioxidants from plants3 and their antioxidant effects,4 several hypoxia-sensitive prodrugs have been designed for oxygen-selective chemotherapy on the basis of their ability to target the tumor hypoxia reductive microenvironment,5 and ultraviolet-visible spectrophotometry or high-performance liquid chromatography was performed to determine the quantitative assessment of drugs6 or dominant compounds in extracts.7 These prodrugs are nontoxic under normoxia but can be reduced to toxic chemotherapeutic drugs by specific hypoxia-responsive enzymes in cells. Therefore, prodrugs function through direct targeting of specific sites or indirectly by producing free radicals to kill tumor cells.

To overcome the low solubility of hydrophobic crude drugs,8 micro/nano-capsules or nano-micelles were introduced to control release.9 Liposome was the first commercial pharmaceutical formulation to encapsulate therapeutic agents.10 Naturally, apart from hypoxia-activated prodrugs, redox-responsive drug delivery systems have also been developed to target tumor cells.11 The concentration of reducing agents,12 such as glutathione (GSH), is much higher in the hypoxic tumor microenvironment than in normoxic cells. Thus, to target the tissue hypoxic microenvironment, redox-responsive bonds, such as disulfide bonds, are usually introduced to drug delivery nanocarriers assembled by artificial polymers or natural polysaccharides such as chitosan whose infrared spectra were determined.13

Both hypoxia-activated prodrugs and redox-responsive drug delivery nanocarriers are effective for targeting the hypoxic microenvironment (Scheme 1).
Hypoxia-activated prodrugs are usually used in clinical trials with favorable prospect, and the redox-responsive nanocarriers are commonly investigated at the experimental research stage. Here, we summarize the hypoxia-activated prodrugs and redox-responsive nanocarriers and discuss the advantages and disadvantages of these hypoxia-targeting strategies.

**Hypoxia-activated prodrugs**

There are four main types of hypoxia-activated prodrugs based on the skeleton structure, which are quinones, nitro-group, N-oxide analogs, and transition metal complexes. Quinone-based prodrugs, such as EO9 and RH1, are the earliest developed hypoxia-activated prodrugs, but with limited effects in clinical trials. Nitro-group chemicals include nitroaromatics and nitroimidazole. Nitroaromatic-based prodrugs include PR-104A, CB1954, SN29730, and KS119W, and nitroimidazole-based prodrugs include TH-302 and NQCQ-1. The most common N-oxide analog prodrug is the tertiary amine-oxides banoxantrone (AQ4N), whose reductive compound AQ4 can compete with topoisomerase II for binding to double-stranded DNA and subsequently leads to apoptosis and tumor cell death. Moreover, these prodrugs include particular aromatic di-N-oxides, such as trapazamin (TPZ) and SN30000. Lastly, prodrugs that are based on transition metal complexes include Co(III), Cu(II), V(II), and Fe(III), as well as other complexes.

**Quinones**

Quinones and their derivatives are the earliest developed hypoxia-targeting prodrugs. Quinones can be reduced to hydroquinones by two enzymatic steps, including CYP450-mediated one-electron transfer pathway and the DT-diaphorase-mediated two-electron transfer pathway (Figure 1). The functions of these drugs are dependent on structural modification. Mitomycin C (MMC) is the original quinone hypoxia-activated alkylating prodrug, which can be bio-reduced by the aromatization process. EO9, a derivative of MMC, is an indolequinone bioreductive antitumor drug that can be activated by DT-diaphorase under hypoxic and normoxic environments to induce DNA damage. To improve EO9’s pharmacokinetics and tissue penetration ability, the 2,3,5-position of indolequinone has been modified, and the derivatives were reported to maintain reasonable hypoxia sensitivity and low cytotoxicity. A series of indolequinone prodrugs have been designed to target quinone oxidoreductases. One of these compounds showed antiproliferative activities in the HT-29 and A549 cell lines with IC50 values of 0.386 and 0.263 mM, respectively. AZQ analogs, which are based on benzoquinone/benzimidazolequinone and naphthoquinone, have also been studied as potential bioreductive antitumor drugs.

In addition, quinones are used as small molecular drug delivery vehicles. For instance, indolequinones, naphthoquinones, and benzoquinones could release antitumor
agents by bioreductive- or radiochemical-mediated cleavage at the 3-position of the quinone ring.\textsuperscript{17,18} EO9 itself can be considered as a promoiety and is used to conjugate other antitumor drugs at the 3-position, in order to form a new dual-function prodrug. Moreover, the 2-position of the quinone ring can function as a substrate, as it is more sensitive than the 3-position during DT-diaphorase activation. Antitumor drugs can also be released from benzoquinones. The trimethyl lock, a trigger for molecular release, is activated by benzoquinones promoiety drugs, as well as naphthoquinones and benzoimidazolequinones. A series of seco-CBI-indole\textsubscript{2} N-acetyl O-amino derivatives have been investigated as promoiety of duocarmycin and CC-1065, which could be specifically cleaved from promoiety in hypoxic tumor regions.\textsuperscript{19} INDQ/NO system can generate NO through DT-diaphorase activation and induce DNA damage to inhibit tumor proliferation.\textsuperscript{20} Indolequinone SN38, coupled with target tumor ligand c(RGDrK) agents, has been designed to release SN38 under bioreductive conditions.\textsuperscript{21} Later, a new compound was synthesized by coupling SN38 and nitroaromatic groups, which could be specifically activated under hypoxia conditions in the A549 and HeLa cell lines. The released active drug could accumulate in tumor regions and inhibit tumor growth in a mouse tumor-bearing model.\textsuperscript{22}

A series of 4-nitrobenzyloxy carbonyl O\textsuperscript{6}-benzylguanine (O\textsuperscript{6}-BG) prodrugs were synthesized, which were considered as O\textsuperscript{6}-alkylguanine-DNA-alkyltransferase (AGT) inhibitors, which could be activated by reductase in the absence of O\textsubscript{2}.\textsuperscript{23} Three of these prodrugs could activate the reductase system under insufficient O\textsubscript{2} levels, and among these, the most water-soluble exhibited the highest nitro-group reduction efficiency. The same compound had little effect in a normoxic environment but showed enhanced cytotoxicity in a low O\textsubscript{2} environment, indicating that these AGT inhibitors can be more specifically released under hypoxic conditions. Comparable AGT inhibitors, 6-(benzyloxy)-2-(aryldiazenyl)-9H-purines,
Nitroaromatics/nitroimidazole

The earliest hypoxia-selective prodrugs were synthesized from nitroimidazole radioisotope. Figure 2 illustrates the hypoxia-selective mechanism of nitroaromatics/nitroimidazole.
and it could release bromo analogs of isophosphoramide mustard.\textsuperscript{35–37} SN30000 showed equivalent cytotoxicity to TPZ and TH-302 prodrugs but higher cytotoxicity than PR-104A and SN29428.\textsuperscript{38,39}

Pyrrolobenzodiazepine-containing TH-302 released similar hypoxia-activated compounds through the action of P450 reductase under both normoxic and hypoxic conditions. However, the cytotoxicity ratio of normoxic to hypoxic was still lower than TH-302.\textsuperscript{40} TH-302 could suppress canine lymphoma survival by downregulating HIF-1α and its downstream genes.\textsuperscript{41} Triggering Br-IPM release from TH-302 was accompanied by enhanced cytotoxicity when pO$_2$ was $<76$ mmHg, according to electron paramagnetic resonance monitoring.\textsuperscript{42} Nonpharmacological methods could exacerbate tumor hypoxia and therefore increase the in vitro and in vivo TH-302 cytotoxicity in pancreatic tumors.\textsuperscript{43} Tumor growth was efficiently inhibited by the combined treatment of ionizing radiation with TH-302 in pancreatic xenografts, but this inhibitory effect was not obvious in tumor stem cells.\textsuperscript{44} It has also been reported that TH-302 could overcome hypoxia-induced tumor radio-resistance, while combinatorial therapy with TH-302 and radiation could inhibit pancreatic tumor growth.\textsuperscript{45} Osteosarcoma tumor growth could be suppressed by the synergistic function of

**Figure 2** The hypoxia-activated mechanism of nitroaromatic/nitroimidazole prodrugs: PR-104, a phosphate ester pro-prodrug, could be triggered to release by phosphatases, and 5-hydroxylamino and 5-amino metabolites could be metabolized to release mustard moiety through NADPH:CP450 activation under bioreductive conditions. TH-302 can release bromo analog of isophosphoramide mustard.
TH-302 with doxorubicin (DOX), in which bone tumor burden, bone damage, and lung metastases could be simultaneously inhibited.\textsuperscript{46}

KS119 is one of the 1,2-bis(sulfonyl)hydrazine prodrugs, which can be reduced to the active intermediate 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)hydrazine, and then alkylated at the O\textsuperscript{6}-position of guanine. Compared with other AGT inhibitors, KS119 demonstrated the strongest cytotoxicity in cells expressing AGT and was able to overcome AGT-mediated drug resistance.\textsuperscript{47} BCCA621C and IC86621 can inhibit DNA double-strand repair, because their target is the DNA-dependent protein kinase (DNA-PK). BCCA621C was able to release DNA-PK inhibitor in hypoxia-selective cells, especially in mouse liver microsomes and NCI-H460 cells, and increased radiotherapy sensitivity, in contrast to normoxic cells.\textsuperscript{48} Both O\textsuperscript{2}-(2,4-dinitrophenyl)-diazeniumdiolate and oleanolic acid could be triggered by GST\textsubscript{π} enzymes to release NO in HCC tumor cells but not in normal cells. The released NO could induce apoptosis in HepG2 cells by arresting the cell cycle at the G2/M phase and activate mitochondria and MAPK-mediated pathways to enhance reactive oxygen species (ROS) generation.\textsuperscript{49} A series of 4-anilinoquinazoline derivatives, which were synthesized by conjugating 2-nitroimidazole to 4-anilinoquinazoline, can function as epidermal growth factor receptor (EGFR) inhibitors. These derivatives could be reduced under hypoxic conditions and induce apoptosis in tumor cells.\textsuperscript{50} Sulfonate prodrug (5-nitro-2,3-dihydro-1H-benzo[e]indol-1-yl)methyl contains releasable sulfonate groups which can act as alkylating agents for binding to the DNA minor groove. They exhibited 4,090-fold increased cytotoxicity during in vitro hypoxic conditions and showed strong antitumor effects in vivo.\textsuperscript{51}

N-oxides

N-oxides are considered as the most potential hypoxia-activated class of prodrugs because of their low cytotoxicity and can be selectively reduced, especially by reductases, to effective therapeutic compounds in a hypoxic environment (Figure 3). These features make them valuable for the synthesis of potent hypoxia-selective prodrugs. Two representative drugs, TPZ and AQ4N, in combination with

![Figure 3](image-url)  

\textbf{Figure 3} The hypoxia-activated mechanism of N-oxide prodrugs: two representative drugs, TPZ and AQ4N, are considered as the most potential hypoxia-activated prodrugs because of their low cytotoxicity and can be selectively reduced, especially by reductases.
radiotherapy and chemotherapy, have performed well in clinical trials.

TPZ is considered as an aromatic N-oxide and can be reduced by CP450 through the one-electron transport chain, which generates short-lived oxidation radicals that can cause DNA damage. Compared to quinones and nitroaromatics, the effective O₂ concentration range is broader in hypoxic cells under TPZ treatment. Mono-N-oxide metabolism is also involved in TPZ treatment, which might be induced by changes in electron transport chain. In addition, TPZ, which contains a nitrogen mustard at the 6-position of its structure, demonstrated that 4-oxide elimination could increase nitrogen mustard activation, which proved that TPZ could have a differential hypoxic cytotoxicity as a prodrug without masking the group’s activity. A series of TPZ analogs illustrated better water solubility and therapeutic effects.⁵² 3-Position modification could increase cytotoxicity and water solubility under hypoxic conditions,⁵³ while 7-position modification could increase the in vitro therapeutic effects. In addition, benzo-1,2,4-triazine 1,4-dioxide derivatives⁵⁴ and other heterocyclic aromatic N-oxides, such as quinoxaline 1,4-dioxide, imidazooxinozoline N-oxides, imidazopyridopyrazine N-oxides, oxadizole N-oxides, trazine N-oxides, and phenazine 5,10-dioxides, have been synthesized.

Alkyl tertiary amine N-oxides AQ4N can be reduced to AQ4 by CP450 enzymes through two-electron reduction.⁵⁵ AQ4, an inhibitor of topoisomerase II, showed considerable therapeutic effects in preclinical trials. However, similar drugs such as nitroacrine with a tertiary amine side chain showed minor activities. Both amonafide and mitonafide contain a naphthalimide structure and could inhibit topoisomerase activity but without considerable effects in vitro. OCT1002, a deuterated analog of AQ4N, was not only able to inhibit LNCaP, 22Rv1, and PC3 tumor growth and revascularization but could also enhance the antitumor synergistic effects with other chemotherapeutic agents.⁵⁶ Synergistic liposomes containing both hypoxia-activated AQ4N and photosensitizer chlorin e6 have been introduced in hypoxic tumor therapy, in which hypoxia induced by photosensitizer chlorin e6 in PDT was used to subsequently trigger AQ4N activation.⁵⁷ Shen et al investigated AQ4N and gossypol combination chemotherapy in HA@AQ4N-Cu(II)-gossypol NPs and demonstrated that AQ4N could function as self-carrier material and auto-fluorescence indicator.⁵⁸

Phenazine 5,10-dioxides have also shown hypoxia-selective drug cytotoxicity. The mechanism is still unclear, and their cytotoxicity has just been demonstrated in vitro but with little effects in vivo. In a previous study, the function of 7-bromo-2-hydroxyphenazine-5,10-dioxide was studied in the cytosol of rat liver, using different enzyme inhibitors such as dicoumarol, ketoconazole, and menadione to determine which enzymatic pathways were involved. Activation of the natural product myxin, a bioreductive di-N-oxide, generates free radicals that cause DNA double-strand breaks.⁶¹ However, it does not show any obvious hypoxia-selective effects as the free radicals generated by phenazine 5,10-dioxides could not induce apoptosis in Caco-2 cells.⁶²

Moreover, several novel derivatives of phenazine 5,10-dioxides have been synthesized to investigate the inhibition rate between DNA and topoisomerase II, showing cytotoxicity in V79 cells in both hypoxic and normoxic environments.⁶³ Some of these derivatives illustrated cytotoxicity but did not exhibit hypoxia specificity. However, 7-fluoro-2-aminophenazine 5,10-dioxide showed hypoxia-selective cytotoxicity, and fluorescence tests indicated a stronger binding to DNA after hypoxia activation.⁶⁴

Many N-oxides based on ROS generation have been investigated. 3-Trifluoromethyl-quinoxaline 1,4-dioxides could release OH radicals through CP450 by one-electron reduction that could be suppressed by O₂.⁶⁵ Hence, its cytotoxicity enhancement depended on the concentration of O₂. 1,2,4-Benzotriazine 1,4-dioxides were significant hypoxia-selective antitumor prodrugs that function through a one-electron reduction to generate free radicals and induce DNA double-strand breaks.⁵⁴

**Transition metal complexes**

Transition metal complexes, especially Co(III) complexes,⁶⁶ are usually considered as potential targeting hypoxia prodrugs.⁶⁷ These complexes are coordinated with cytotoxic ligands of relatively low cytotoxicity for specific binding to their target sites. When the center metal ions are at a higher oxidation status, they are coordinated with ligands and cannot be readily metabolized in cells. However, when the center metal ions are reduced to a lower unstable reduced status, the corresponding ligands are triggered to be released (Figure 4). For instance, Co(III) can be reduced to Co(II) and release dissociated ligands as the active drugs. In fact, this procedure is reversible as Co(II) can be oxidized back to Co(III) in the present of O₂.

In order to study the drug release process, fluorescence coumarin Co(III) chaperones were used to target hypoxic regions. These anionic complexes could specifically release drugs in acidic and hypoxic regions. The releasing properties of C343haH, enable its application as a typical fluorophore tumor indicator instead of antitumor drug.⁶⁸ Two series of
Co(III) complexes with fluorescence auxiliary ligands have been shown to target hypoxia cells, according to assays on Eos photon-convertible green fluorescent protein (GFP) live hypoxic 3D cell models. After reduction of Co(III) to Co(II), the release of fluorescent ligands is induced, and fluorescence can be monitored to localize their targets.

Cobalt-mustard complexes, such as $\text{[Co(Racac)}_2\text{(DCE)}]^+$, are significant in vitro hypoxia-selective delivery systems. In order to design novel EGFR inhibitors, Co(III) complexes with hypoxia-responsive properties were synthesized to selectively release EGFR inhibitors in the hypoxic microenvironment of solid tumors. The related DNA minor groove alkylating agent, (1-(chloromethyl)-5-hydroxy-1H-pyrrolo[3,2-f]quinolin-3(2H)-yl)(5,6,7-trimethoxy-1H-indol-2-yl)methanone, could be synthesized with cyclam or cyclen auxiliary ligands, which is derived from cross-linking.

Figure 4 The hypoxia-activated mechanism of metal complex prodrugs: these complexes are coordinated with cytotoxic ligands of relatively low cytotoxicity for specific binding to their target sites. When the center metal ions are at a higher oxidation status, they are coordinated with ligands that cannot be readily metabolized in cells. When the center metal ions are reduced to a lower unstable reduced status, the corresponding ligands are triggered to be released.
to ethylene or N,N′-dimethyl analogs. These prodrugs demonstrated noticeable alkylating effects and marked hypoxia-selective cytotoxicity.

Co(III) complexes with H,bhnq ligands could be considered as bioreductive drugs. For instance, Co(III) complexes [Co(bhnq)(L1)]BF₄·H₂O and [Co(bhnq)(L2)]BF₄·H₂O [where L1: N,N′-bis(pyridine-2-ylmethyl) ethylenediamine and L2: N,N′-dimethyl-N,N′-bis(pyridine-2-ylmethyl) ethylenediamine] have been synthesized. Compared to nonmodified cyclen ligands, Co(III) complexes coordinated with N-substituted cyclen ligands and showed from 2- to 30-fold lower cytotoxicity and from 2- to 22-fold differences between hypoxic and normoxic cells.

Other Cu(II) or Fe(II) complexes are usually coordinated with tetradendate ligands, such as tris(2-methylpyridyl)-amine (tpa) or N,N-bis(salicylidene) ethane-1,2-diamine (salen). Novel Cu(II) complexes with 1,4,7-tetrazacyclododecanes as ligands showed 24-fold higher cytotoxicity in hypoxic rather than in normoxic in vitro conditions. Similar metal complex redox pairs such as reduction of Pt(IV) to Pt(II), Ru(III) to Ru(II), Cu(II) to Cu(I), and Fe(III) to Fe(II) were also commonly applied as hypoxia-triggered release strategies. The release mechanism was based on altered valence of center metal ions to release ligands. In addition, various metal complex prodrug nanocarriers have also been designed.

**Redox-responsive drug delivery nanocarriers**

**Nitroimidazole derivatives**

The first strategy to design a redox-responsive system was to use photo-induced electron transfer (PET) between fluorescent molecules and nitroimidazole (Figure 5A). 2-Nitroimidazole was commonly conjugated with (7-methoxycoumarin-4-yl) methyl carboxylates for assessing the photo-S₃₁-dependent cleavage process. In the presence of 2-nitroimidazole, there was no photo cleavage and consequently no fluorescence emission, due to the PET effects. Therefore, release of the antitumor drug etoposide (a topoisomerase inhibitor) could not be achieved in normal cells. However, in cancer cells, the hypoxia specificity of nitro to amino reduction attenuated PET and hence activated drug release, which was accompanied by blue fluorescence emission.

Comparative strategies have been applied for the development of self-assembled nanocarriers to release hydrophobic drugs under hypoxic conditions. In these nanocarriers, the carboxymethyl dextran was treated as a backbone and conjugated to 2-nitroimidazole. In effect, the nanocarriers could release DOX more rapidly under a low oxygen environment rather than in normoxic conditions, due to nitro- to amino-group reduction. This induced phase changes occurred in nanocarriers and resulted in higher accumulation of DOX in hypoxic cells (Figure 5B). In addition, nanocarriers have been reported to selectively accumulate in hypoxic tumor regions as a result of the enhanced permeability and retention (EPR) effects. Therefore, these nanocarriers provide a potent drug delivery method for redox-responsive release of active drugs.

**Azo-group-based chemicals**

Azo-groups are commonly introduced in the process of hypoxic prodrugs fabrication. Nanocarriers have been specifically designed for hypoxia-induced siRNA uptake and silencing, and these include PEG2000, azobenzene, PEI (Mw: ~1.8 kDa), and 1,2-dioleyl-sn-glycero-3-phosphoethanolamine (DOPE) (Figure 5C). PEG2000 has been shown to extend the blood circulation time of NPs, which promotes their accumulation in tumors via EPR effects, but restricts their therapeutic effects. Therefore, to overcome this drawback effect, azo-benzenes were introduced to cleave PEG2000 from the PEI-DOPE NPs. The release of conjugated GFP was used as an indicator of siRNA targeting tumor hypoxia.

**Redox-responsive ferrocene**

Bioreponsive and self-healing supramolecular hydrogels have been synthesized based on host–guest inclusion for loading β-cyclodextrin as a host and propenoic acid conjugated to ferrocene as a guest (Figure 5D). The phase transformation of supramolecular hydrogels could be changed by oxidation-reduction due to interactions with ferrocene-β-cyclodextrin, which could promote the release of active drugs. The morphology of nanocarriers, which were composed of a poly(vinylferrocene)-bl-poly(methacrylate) shell and a hydrophobic core, could be altered by selective oxidation and further conversion of the polarity of their shells. Hence, the drug-controlled release can be triggered by the transition of hydrophilic to hydrophobic phase.

**Metal oxide NPs**

Metal oxide NPs were a series of stimuli-responsive-based delivery systems in biomedical applications, such as magnetic NPs based on ferric oxide, cobalt, or nickel, which were usually used in magnetic resonance imaging but also played significant roles in redox-sensitive drug delivery vehicles. For instance, superparamagnetic iron oxide NPs were introduced to deliver O₂-BG to brain tumors with redox-responsive coating layer through the peptide chlorotoxin receptor-mediated transcytosis.
Figure 5 Targeting hypoxia drug delivery and release strategies.


Abbreviations: CPT, camptothecin; DOPe, phosphoethanolamine; DOX, doxorubicin.
Folate-(PLGA-PEG-PLGA-urethane-ss) encapsulating DOX and $\gamma$Fe$_2$O$_3$ showed synergistic cytotoxic effects in tumor cells that were overexpressing folate receptors. These multiblock polymeric NPs could be triggered under redox environment and achieve excellent chemotherapeutic effects rather than serious side effects.\textsuperscript{32} Manganese dioxide could be used as the core coated with nanoscale coordination polymers that were constructed based on HF ions and a cisplatin prodrug, which could release cisplatin in reduction/acidic pH/H$_2$O$_2$-responsive multi-triggered conditions.\textsuperscript{83}

Selenium nanocarriers
Mesoporous selenium NPs have been synthesized to load DOX and to enhance their targeting effects by introducing redox-responsive properties for GSH-activated drug release. The synergistic effects induced by MSE and DOX enhanced the antitumor efficiency and decreased the drug side effects.\textsuperscript{84} Cross-linking of three diselenide-rich polymers (PEG-b-PES$_2$E, PEG-b-PUS$_2$e, and PEG-b-PBS$_2$e) to nanomicelles containing camptothecin (CPT) and DOX resulted in synergistic inhibition of tumor growth (Figure 5E). Visible light could induce diselenide metathesis, regeneration, and lipid bilayer formation, with increased sensitivity to GSH and ROS. In vitro and in vivo studies showed that tumor growth suppression could be achieved at low drug dosages due to their synergistic effects.\textsuperscript{85} A nanomicelle system delivering PTX was composed with diselenide-containing triblock copolymer, poly(ɛ-caprolactone)-bis(diselenide-methoxy poly(ethylene glycol)/poly(ethylene glycol)-folate) [PCL-(SeSe-mPEG/PEG-FA)]. Their cell internalization indicated that they showed an enhanced cell uptake through folate receptor-mediated endocytosis pathway in folate overexpressing 4T1 breast cancer cells rather than other cell lines that lacked FA.\textsuperscript{86}

Nanocarriers based on disulfide bonds
Reductive chemicals such as GSH have a significant role under redox conditions during tumor therapy, and dithiothreitol (DTT) is frequently used in vitro to replicate the redox environment.\textsuperscript{87} Disulfide bonds are often introduced to redox-responsive prodrugs and nanocarriers because they can be cleaved in a redox environment (Table 1).\textsuperscript{88,89} For instance, PEG, PEO, PCL, and PEI/HA are commonly used as nanocarrier fabrication materials (Figure 6).

### PEG
Disulfide bonds are frequently used in redox-responsive drug delivery micelles made from various PEG derivatives. Low-molecular-weight PEG, with amide group and disulfide

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[Table 1: The properties of redox-responsive nanomicelles]
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<td>PAMAM-ss-PEG</td>
<td>10.12–16.90</td>
<td>–</td>
<td>DOX</td>
<td>B16</td>
<td>9.2</td>
<td>10.4</td>
<td>86.3–92.5</td>
<td>135</td>
</tr>
<tr>
<td>PEO-b-PMAC</td>
<td>PEO</td>
<td>84–126</td>
<td>4–21</td>
<td>DOX</td>
<td>T4</td>
<td>–</td>
<td>6.8</td>
<td>7.1</td>
<td>105</td>
</tr>
<tr>
<td>PEO-b-PMA</td>
<td>PEO</td>
<td>106.2–109.9</td>
<td>–</td>
<td>DOX</td>
<td>A2780</td>
<td>50.3</td>
<td>51.1</td>
<td>5 hours, 100%</td>
<td>106</td>
</tr>
<tr>
<td>PCL-SS-PEEP</td>
<td>PCL</td>
<td>70</td>
<td>3.1</td>
<td>DOX</td>
<td>A549</td>
<td>2.8</td>
<td>2.8</td>
<td>100 hours, 100%</td>
<td>108</td>
</tr>
<tr>
<td>HA-ss-DOCA</td>
<td>HA</td>
<td>119.3–253.8</td>
<td>34.7–161.3</td>
<td>PTX</td>
<td>MDA-MB-231</td>
<td>66.9</td>
<td>93.2</td>
<td>27.1–34.1</td>
<td>109</td>
</tr>
<tr>
<td>DOX-CC-HAMS</td>
<td>HA</td>
<td>148</td>
<td>–</td>
<td>DOX</td>
<td>SCC7</td>
<td>87</td>
<td>8.7</td>
<td>50 hours, 100%</td>
<td>111</td>
</tr>
<tr>
<td>HA-SS-COOh</td>
<td>HA</td>
<td>–110</td>
<td>–</td>
<td>DNA</td>
<td>HepG2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>112</td>
</tr>
<tr>
<td>LHRH-HA-cys-ADOX</td>
<td>HA</td>
<td>190.1–229.0</td>
<td>–</td>
<td>DOX</td>
<td>OVCAR-3</td>
<td>10.5</td>
<td>–</td>
<td>150 hours, 95%</td>
<td>132</td>
</tr>
<tr>
<td>MnO₂ nanosheets</td>
<td>HA/MnO₂</td>
<td>82.3–154.8</td>
<td>–</td>
<td>DNA</td>
<td>hMSCs</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>113</td>
</tr>
<tr>
<td>HMONs-ss-PAE</td>
<td>Silica</td>
<td>551.1</td>
<td>–</td>
<td>PTX</td>
<td>MCF-7</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>114</td>
</tr>
<tr>
<td>DS-Dox-PGEA</td>
<td>Silica</td>
<td>104</td>
<td>–</td>
<td>DOX</td>
<td>C6</td>
<td>2.3</td>
<td>–</td>
<td>7 days, 35%</td>
<td>120</td>
</tr>
<tr>
<td>RRMSN/DOX</td>
<td>MSNs</td>
<td>180</td>
<td>–</td>
<td>DOX</td>
<td>U-87MG</td>
<td>3.81</td>
<td>–</td>
<td>–</td>
<td>121</td>
</tr>
<tr>
<td>DOX@TTMSN</td>
<td>MSNs</td>
<td>103.2</td>
<td>–</td>
<td>DOX</td>
<td>U-87MG</td>
<td>4.36</td>
<td>–</td>
<td>–</td>
<td>122</td>
</tr>
<tr>
<td>MSNs-ss-SiRNA@DOX</td>
<td>MSNs</td>
<td>125</td>
<td>–</td>
<td>DOXsiRNA</td>
<td>MCF-7</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>123</td>
</tr>
<tr>
<td>pSiO₂-GSSG Ns</td>
<td>pSiO₂</td>
<td>–100</td>
<td>–</td>
<td>Amoxicillin</td>
<td>23.8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>124</td>
</tr>
<tr>
<td>MSe@DOX</td>
<td>MSe/HSA</td>
<td>82.1–120.4</td>
<td>–</td>
<td>DOX</td>
<td>MCF-7</td>
<td>–</td>
<td>18.7</td>
<td>2 hours, 78.5%; 10 hours, 81.3%</td>
<td>84</td>
</tr>
<tr>
<td>DOX@hpBPE</td>
<td>Hyperbranched polyphosphoester</td>
<td>107.7</td>
<td>–</td>
<td>DOX</td>
<td>MDA-MB-231</td>
<td>–</td>
<td>48.5</td>
<td>72 hours, 50%</td>
<td>99</td>
</tr>
<tr>
<td>HPAEG-AS1411</td>
<td>HPAEG/DNA</td>
<td>80.6</td>
<td>41–46</td>
<td>DOX</td>
<td>MDA-MB-231</td>
<td>–</td>
<td>49.30</td>
<td>60 hours, 90%</td>
<td>117</td>
</tr>
<tr>
<td>[p(His)ₙ-SS-PU-SS-p(His)ₙ]</td>
<td>Poly histidine/urethane</td>
<td>153–158</td>
<td>–</td>
<td>DOX</td>
<td>L929</td>
<td>13.6</td>
<td>19.3</td>
<td>42.6</td>
<td>130</td>
</tr>
<tr>
<td>Poly(styrene-alt-maleic anhydride)</td>
<td>82.2</td>
<td>7</td>
<td>DOXsiRNA</td>
<td>MCF-7 L929</td>
<td>8.6</td>
<td>–</td>
<td>24 hours, 51%</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>TPGL-SS-PLA</td>
<td>TPGL/PLA</td>
<td>128.5</td>
<td>–</td>
<td>PTX</td>
<td>A2780/T</td>
<td>3.11</td>
<td>–</td>
<td>168 hours, 90%</td>
<td>136</td>
</tr>
<tr>
<td>PTX-S-OA/TPGS₂ NPs</td>
<td>Oleic acid</td>
<td>100</td>
<td>–</td>
<td>SN38/PTX</td>
<td>KB-3–1</td>
<td>54.9</td>
<td>66.5</td>
<td>10 hours, 48%</td>
<td>139</td>
</tr>
<tr>
<td>HES-Ss-PTX</td>
<td>Hydroxethyl starch</td>
<td>151–205</td>
<td>–</td>
<td>PTX</td>
<td>4T1</td>
<td>6.3</td>
<td>12.0</td>
<td>12 hours, 80%</td>
<td>140</td>
</tr>
</tbody>
</table>

**Abbreviations:** CMC, critical micelle concentration; DLC, drug loading capacity; DLE, drug loading efficiency.
Hypoxia-activated prodrugs and redox-responsive nanocarriers

Figure 6 Redox-responsive nanomicelles containing disulfide bonds.

Disulfide bond cleavage could cause nanomicelle phase changes. Hence, cleavage of the new near-infrared (NIR) prodrug, DCM-S-CPT, can release fluorescence, which can be used in NIR optical imaging, and CPT which can be applied in chemotherapy. Adicyanomethylene-4H-pyran derivative has been used as an NIR fluorophore and conjugated by disulfide bonds to CPT as an antitumor drug. Hybrids of these molecules with PEG-PLA micelles have demonstrated good performance in tumor diagnosis and chemotherapy.93

Redox-sensitive self-assembly of the disulfide bond containing micelles mPEG-S-S-C-C could effectively deliver DOX, and the release was faster than that of the mPEG-C-C-C control, which did not include any redox-sensitive disulfide bonds.94 DOX release mainly occurred in the cytoplasm and then entered the cell nucleus, as observed by fluorescence confocal microscopy (FCFM). In contrast, DOX seldom entered the nucleus in the control. Tioguanine and tioguanine-generating prodrugs could be treated as antitumor and immunosuppressive agents. PEG-bl-poly (propylene sulfide) copolymers could form micelles by self-assembling with a free terminal thiol, causing propylene sulfide monomer ring-opening polymerization, and prodrug copolymers could be conjugated to tioguanine with disulfide bonds. Tioguanine prodrug micelles with the size of 18-40 nm were fabricated by self-assembly and responded to cysteine and serum simultaneously.95 Additionally, a series of shell-core structure disulfide-containing PEG micelles have been fabricated. For instance, micelles were synthesized with disulfides cross-linked P-(VCL-s-s-MAA)-PEG shells and carboxylic modified mesoporous silica (MSN) cores.96

Circular redox-responsive micelles that incorporated PEG-A and PLA-B formed spherical micelles with a size ranging from 34 to 107 nm. These micelles could be triggered by DTT, and in 14 hours almost 60% of DOX was released, as compared to the control which released <30% of DOX. They were also triggered to release DOX in the HeLa cell line in the presence of GSH.97 Hypoxia-responsive 100 nm nanomicelles were fabricated by mPEG-PBLG-SS-DTX, which could be triggered to release 40% of docetaxel (DTX) when treated with 50 mM of DTT within 120 hours, while the control groups released only 10% of DTX. Cytotoxicity was observed in MCF-7/ADR and A549 cells with IC50 values 15-fold lower than the free DTX.98 Redox-responsive nanomicelles-loading DOX were constructed with PEGylated hyperbranched polyphosphoester (hbPPE), in which PEGylation could promote stability of nanomicelles. More than 50% of DOX was released from the DOX@ss-hbPPE within 72 hours when treated with 10 mM of DTT in vitro, in contrast to 23% release in the control group.99

A redox-responsive stomatocyte has been used for drug release triggered under reducing conditions. The disulfide bonds linked the hydrophilic PEG with the hydrophobic PS chains, which could be cleaved by GSH in order to release DOX.100 Star-shaped magnetic nanomicelles were self-assembled with four-arm PEG-PCL containing disulfide bonds. Both DOX and Fe₃O₄ were encapsulated, and PBA was treated as the active ligand to target salic acid (SA)-mediated endocytosis. Both PBA and external magnetic field could enhance cell uptake of star-shaped magnetic nanomicelles in vitro. These dual-targeting micelles could accumulate in tumor tissues by both magnetic guidance and SA-mediated endocytosis, and DOX release could be triggered by the redox environment to inhibit tumor growth.101

Internalization of redox-responsive PLGA-PEG NPs was studied based on FRET fluorophore pairs (DiO/DiL). The cell uptake efficiency of redox-responsive NPs on 3D cell culture models of lung cancer was higher than that of non-redox-responsive NPs, and the extracellular release of PEG fringe could promote the internalization of NPs.102 Redox-responsive PTX-loaded PEG micelles were modified with ABD035, a peptide with albumin-binding domain, targeting overexpressing albumin-binding proteins such as secreted protein acidic and rich in cysteine (SPARC) and gp60. The cellular internalization results indicated that the accelerated transport rate was due to SPARC- and caveolin-1-mediated pathways and therefore improved therapeutic effects on representative drug resistance model: triple-negative breast cancer by increasing cellular internalization as well as drug distribution.103 Polycarbonate-based NPs composed of PEG-b-poly(MPC)₅₉ azide-terminated α-lipoic acid, and 6-bromohexanoic acid were used in the redox-responsive drug delivery. The cellular internalization was investigated by CLSM and flow cytometry, indicating a high internalization efficiency of DOX-encapsulated NP systems.104

PEO
PEO is a similar polymer to PEG. Novel PEO-b-PMABC diblock copolymer has been synthesized by using dithioester-capped PEO in a reversible addition-fragmentation chain transfer polymerization process. The results demonstrated
that PEO-b-PMABC micelles were stable under physiological conditions but were cleaved into unbound copolymers with rapid DOX release under bioreductive conditions. The novel ionic polymer PEO-b-PMA and Ca\(^{2+}\) were treated as the core templates, while disulfide bonds were treated as ionic shells with cystamine being a degradable cross-linker. These gelatinous micelles with 50\% of DOX encapsulation rate have nanocarrier surface- and time-dependent degradation effects in cells, demonstrating increased release of DOX in human A2780 ovarian cells along with increased cytotoxicity.

**PCL**

Various redox-responsive PCL micelles with disulfide bonds have been investigated because of their favorable biocompatibility and biodegradability. Pyridyl disulfide-functionalized cyclic carbonate monomers could be polymerized by ring-opening copolymerization within four steps, during which the synthesized functional PCL chains with pendant pyridyl disulfide (PDS) chemical groups derive from 3-methyl-3-oxanemethanol. PCL-g-SS-PEG micelles were stable in a physiological environment but aggregated under redox-responsive conditions and released 82.1\% of DOX within 12 hours, while only 17.5\% of DOX was released without stimulation. FCFM showed that the DOX loaded in PCL-g-SS-PEG micelles could be delivered into the HeLa cell nucleus. A biodegradable ε- PCL-co-poly(ethyl ethylene phosphate) with disulfide bonds was synthesized to be triggered by GSH, resulting in enhanced DOX delivering efficiency and growth suppression in A549 cell lines.

**PEI/HA**

Both PEI and HA are suitable for nucleic acid delivery and gene therapy. Therefore, HA/PEI nanocarriers have been vastly used for delivering nucleic acids and antitumor agents. The intercellular PTX drug-controlled release from HA-ss-DOCA nanomicelles depended on HA-deoxycholic acid, whose drug encapsulation rate and drug loading rate were as high as 34.1\% and 93.2\%, respectively. HA-ss-DOCA micelles were stable under physiological conditions but dissociated in 20 mM of reducing agents. Furthermore, a conspicuous faster release rate of PTX was observed, resulting in increased in vitro cytotoxicity, while Nile Red release provided a specialized drug delivery monitoring system. HA-ss-DOCA micelles possessed higher uptake rates in vivo, especially in MDA-MB-231 cells overexpressing the HA surface receptor.

Bioreponsive NIR fluorescence and PDT agents were synthesized by conjugation of a photosensitizer and HA by disulfide bonds, which upon GSH triggering resulted in NIR fluorescence emission and cytotoxic compound release. Shell-core CC-HAM drug delivery micelles could also be triggered by GSH, and in vivo distribution analysis revealed tumor-targeting characteristic, improved DOX pharmacokinetics, and increased tumor accumulation. HA-SS-COOH could form DNA/PEI ternary polypeptides, and the fabricated procedure was accompanied by increases in size and zeta-potential. These HA-SS-COOH-coated ternary complexes showed 14-fold higher transfection efficiency and stability than naked nucleic acids. In addition, they showed higher accumulation in tumors of C57BL/6 tumor-bearing mice due to HA-targeting ligands, which also supported that HA derivatives can be applied in targeting gene therapy, as indicated by strong fluorescence intensity assessments. HA-PEI copolymers with disulfide bonds have been synthesized as gene nanocarriers, which were sensitive to acidic pH values and redox responsive conditions. These HA-ss-PEI/pEndo nanocarriers could transport nucleic acids to human mesenchymal stem cells and generate endostatin protein. Redox/ph-responsive MnO\(_2\) nanosheets have also been fabricated and synergistically functioned with HA to enhance biocompatibility. Cisplatin was absorbed by the nanosheets and delivered into tumor cells. The complexes were triggered in a low pH environment, and GSH could reduce MnO\(_2\) to Mn\(^{2+}\), whose biodistribution could be monitored by T1-MRI.

It is well known that HA can bind specifically to CD44. Responsive glycosaminoglycans (GAG) with alkane thiol were introduced to GAG:HA amphiphiles to form self-assembly micellar NPs, and CD44-mediated internalization was proven by loaded Nile Red among the three groups such as without blocking, specific CD44 blocking, and unspecific IgG1 blocking.

**Other materials**

Besides macromolecules such as PEG, PEO, PCL, and HA/PEI, comparable materials have also been investigated for drug loading in redox-responsive micelles. DOX-SS-COSO-SA nanomicelles with size of 62.8 nm could release DOX through disulfide bond cleavage and exhibited 34.8-fold higher cytotoxicity than the free DOX, in which stearic acid was grafted on the low-molecular-weight chitosan. Moreover, novel redox-responsive hyperbranched polymers have been prepared by reversible addition-fragmentation chain transfer and self-condensing vinyl polymerization, with the incorporation of DNA aptamer for targeting tumors.
10 mM of GSH could trigger almost 90% of DOX release, unlike the control which only released 5% of DOX within 60 hours. In HeLa cells, these polymer NPs were degraded in the cytoplasm and rapidly released DOX.\textsuperscript{117} Nanocicles loading dual antitumor drugs were constructed by co-loading of DOX and POEG-b-PSSDas on prodrug polymers attached to the oncogenic tyrosine kinase inhibitor dasatinib, which could be redox-responsive and showed enhanced antitumor effects both in vitro and in vivo.\textsuperscript{118}

Monodispersed mesoporous organosilica NPs cooperating with thioether bonds exhibited redox-responsive biodegradation phenomenon and improved the drug delivery efficiency as well as tumor suppressing ability.\textsuperscript{119} Mesoporous organosilica redox-responsive HMONs-ss-PAE has been synthesized to deliver P-gp modulator siRNA and chemotherapeutic DOX releasing drugs to overcome tumor drug resistance.\textsuperscript{120} Degradable silica NPs loading DOX (DS-DOX) were synthesized to co-deliver plasmid DNA (pDNA) and DOX. The surface of DS-DOX was modified with cyclodextrin-poly(glycidyl methacylate) (CD-PGEA), including one β-CD core and two ethanolamine-functionalized PGEA arms in order to absorb pDNA. After degradation of surface CD-PGEA, disulfide bonds in the center of DS-DOX were exposed and cleaved under hypoxia to release the pDNA and the active drug, which synergistically acted against tumor cells.\textsuperscript{121} Figure 7 illustrates the construction of hypoxia-responsive drug release of NPs by the introduction of the alkyl chain of stearic acid (C\textsubscript{18}) with thiol group in mesoporous silica NPs, in which DOX release could be prevented by the formed disulfide bonds under normoxia but could be triggered under hypoxia. In addition, the targeting RGD motifs have been modified to enhance tumor accumulation.\textsuperscript{122} Redox-responsive MSN capping with therapeutic and targeting peptides has been developed to enhance the RGD-induced cell endocytosis and release the loaded DOX by GSH activation, in order to induce tumor cells apoptosis.\textsuperscript{123} Transferrin can function as the gatekeeper of MSN for both active-targeting and controlled drug release. GSH was the key enzyme to trigger DOX release, and these nanocarriers demonstrated enhanced inhibition of tumor growth.\textsuperscript{124} In addition, MSNs were used to deliver siRNA and DOX. The surface of MSNs was modified by MPTMS/Py-SS-Py, and then DOX was loaded into the channels of MSNs. HS-siRNA was used to replace Py-SH in order to form a new disulfide linkage as a gatekeeper. In vitro, GSH could trigger siRNA and DOX release, and 49.4% and 78.8% of DOX was released when treated with 2 and 5 mM of GSH for 24 hours, respectively, while in the control group, only 14.8% of DOX was released. The siRNA also knocked down the expression of the target protein and demonstrated a synergistic effect with the chemotherapeutic agent.\textsuperscript{125}

**Multiple functional sensitive nanocicles**

Various dual- and even tri-functional reductively selective nanocicles have been obtained by different methods (Figure 8). Using the layer-by-layer method, self-fluorescence microcapsules were fabricated from the polysaccharide alginic acid and cystamine dihydrochloride, demonstrating pH- and redox-sensitivity.\textsuperscript{126} Experiments on both human and animal tumor models illustrated that the dual-sensitive HP-ICM could transport drugs in tumors. Comparable pH- and redox-responsive micelles were able to transport and release DOX under low pH and highly reductive conditions. Novel poly[2-(pyridine-2-ylsulfanyl)ethyl acrylate] nanocarriers possessed a free-radical polymerization side chains, which were coupled with PEG and cyclic RGD (cRGD) poly-peptides via disulfide bonds cross-linking to form RPDSG polymers. These 100 nm submicelles were stable in a physiological environment but released active drugs under reductive conditions.\textsuperscript{127} Reduction and pH dual-sensitive polypeptide micelles modified with copolymer PEG-b-poly(L-lysine) [PEG-P(LL-CCA/LA)] could be synthesized with lipoic acid (LA) and cis-1,2-cyclohexanedicarboxylic acid (CCA). Both PEG-P(LL\textsubscript{10}-CCA/LA\textsubscript{10}) and PEG-P(LL\textsubscript{14}-CCA/LA\textsubscript{10}) nanocicles loaded with DOX were triggered by 10 mM of DTT at pH 7.4. In vitro experiments demonstrated that DOX could be released in the endosome at pH 5.0 due to CCA cleavage, or released in the presence of 10 mM of GSH. PEG-P(LL\textsubscript{18}-CCA\textsubscript{4}/LA\textsubscript{14}) was able to release more DOX into the cell nucleus than PEG-P(LL\textsubscript{14}/LA\textsubscript{14}), as demonstrated by FCFM assays.\textsuperscript{128} PAA-SS-AD, PEG-AD, and PAA-CD, three constituents of self-assembled NPs loading DOX, containing disulfide bonds, released DOX through triggering by the reducing agent and the acidic medium. These DOX-loaded NPs were used to treat the animal model zebrafish and illustrated improved viability.\textsuperscript{129} A type of pH/redox dual responsive p(L-histidine)-SS-PU-SS-p(His) polymer was synthesized with pH-sensitive tiles p(His)\textsubscript{10} and redox-responsive disulfide bonds. The polymer chains could be self-assembled to form nanocicles for loading DOX with 19% loading efficiency. Further DOX release could be triggered by low pH or by treatment with 10 mM of GSH, and fluorescence indicated that the nanocicles could be biodistributed in the animal model within 72 hours and inhibit CT26 tumors.\textsuperscript{130} Reduction- and pH-responsive nanocicles, composed of
poly(styrene-alt-maleic anhydride) and short PEG chains, were used for delivering drugs and PLK-1 siRNA. These micelles were coated with BSA to enhance their stability. Micelle dissociation could be triggered by low pH and by 10 mM of GSH, resulting in tumor regression both in vitro and in vivo. DOX was grafted onto HA by using cis-aconityl and disulfide bond linkage and demonstrated both pH- and redox-responsiveness. These 229 nm micelles released 95% of the active drugs in 150 hours, in the presence of low pH and reductive agents, while the control release...

**Abbreviations:** GSH, glutathione; NIR, near-infrared; PTX, paclitaxel; ROS, reactive oxygen species.

Core-shell NPs loading DOX are composed of a hydrophobic core containing DOX and dendrimer PAMAM modified with SPDP, and a conjugated PEG-SH hydrophilic shell. These NPs have been modified with cRGD to enhance their targeting activity and could be triggered by redox and low pH values to enhance antitumor efficiency both in vivo and in vitro. A novel copolymer named TPGS-SS-PLA was developed to form 130 nm micelles to encapsulate PTX. cRGD was used to target tumor and enhance cell uptake. The PTX-loaded NPs showed dual pH-sensitivity and redox-responsiveness and could be triggered to release >80% of PTX in 7 days, in the presence of DTT, at pH 5.5. These NPs inhibited tumor growth and reduced cytotoxicity side effects in vivo.
pH- and hypoxia-responsive liposomes have been constructed by Chol-SS-COOH with modification of the CD-44 targeting HA. The liposome degradation and DOX release were triggered by both mild acidic conditions and reductive agents. Liposome administration with HA modification showed more intensive in vitro cytotoxicity than liposomes, and longer in vivo half-life time release of DOX than free DOX. Finally, in vivo tests revealed CD-44-mediated biodistribution, GSH triggered controlled release and more effective antitumor activity. A dual pH- and redox-responsive NP system was fabricated with charge-reversible pullulan derivative and poly(β-amino ester) for both gene and methotrexate delivery. Human hepatoma cells were treated with these NPs that entered cells through endocytosis but finally escaped from the endo/lysosomes and located the cytoplasm, and tetramethylrhodamine (TAMRA)-DNA was proven significantly internalized compared with naked DNA control, because the surface coating of pullulan showed specific affinity-binding ability to asialoglycoprotein receptors.

Novel redox-responsive prodrug nanoplatforms have been synthesized by conjugating PTX and oleic acid by thioether linking. PEGylated PTX-S-OA micelles showed 57.4% drug-loading efficiency and inhibited tumor growth in vivo. In contrast to the disulfide bond cleavage, PTX could be cleaved by both intracellular GSH and ROS, illustrating its dual GSH/ROS activity in the tumor microenvironment. α-Amylase and hydroxyethyl starch have been incorporated into redox-responsive NPs to deliver PTX via disulfide bond conjugation. HES-SS-PTX NPs could be degraded by α-amylase. Pharmacokinetic analysis demonstrated that these NPs had a longer half-life-enhanced accumulation in tumor regions and were triggered by reducing agents, thus illustrating higher antitumor efficiency than free PTX. A redox/ enzyme dual-responsive nanomedicine system was triggered to release NO to kill specifically tumor cells, whose procedure was monitored by flow cytometry, cooperating with a GSH S-transferases π responsive drug as NO donor, and its cellular internalization was confirmed by a time-dependent red fluorescent dye QM-2.

Amphiphilic PEG-P-SS-HP copolymers showed temperature and reduction sensitivity with 90% of PTX encapsulation rate and drug content >35%. Furthermore, DTT was required for rapid drug release, and the vehicles could decrease the PTX cytotoxicity side effects. Based on p(PEG-MEMA-co-Boc-Cyst-MMAm-co-VI)-b-PEG (PPBV), novel temperature, pH values, and reduction responsive polymer micelles were designed for drug delivery. These PTX-loaded nanomicelles demonstrated stability in PBS but the release of active drugs could only be triggered at certain temperatures, under sub-acidic or reductive conditions. Amphiphilic PEG-PCL chains with three disulfide bonds were incorporated in the triple-sensitive cleavable polymeric nanocarriers. Triple disulfide bonds improved the redox responsive sensitivity, and GSH could trigger the DOX release to inhibit tumor cell proliferation. Triple-responsive (protease/redox/pH) porous silica NPs have been developed as drug delivery vehicles. Oxidized GSH GSSG was conjugated on the surface of silica NPs to promote redox and protease sensitivity. Acid-triggered fluorescent molecules, such as ZnO quantum dots were attached to the channels of nanopores of porous silica NPs as imaging probes and drug release gatekeepers.

**Summary and perspective**

Since hypoxia is one of the marked features of malignant tumors, which is associated with several adaptation changes in the microenvironment of tumor cells, the exploitation of targeting tumor hypoxia is a research hotspot for cancer therapy. In this review, we described current hypoxia-activated therapeutic systems in two aspects: several hypoxia-activated chemotherapeutic prodrugs and redox-responsive nanocarriers. Hypoxia-activated prodrugs can be activated in tumor hypoxic tissue, which can avoid nonspecificity of current antineoplastic agents to minimize side effects and benefit to clinical propulsion. Currently available biomaterials or drugs have a significant role in targeting hypoxia tissue and curing human malignant tumors and other types of cancer. Because of the highly complicated microenvironment of tumors and drug cytotoxicity side effects, few drugs could easily pass the tests required for drug discovery and applied in clinical chemotherapy. In this stage, the hypoxia selectivity of many candidate drugs is not high enough to pass clinical trials. Therefore, few hypoxia-sensitive prodrugs have been circulated in the markets. It needs high throughput methodology to screen ideal candidates for clinical phases, and a deeper understanding of interactions between small molecules and targeted biomacromolecules is required, which can indicate the suitable conditions of the hypoxia-activated molecular structure transformations. But until now, limitations of medicinal development are the disadvantages of hypoxia-activated prodrugs. Hypoxia-active prodrugs with more sensitivity to tumor microenvironment should be designed to increase their specificity to tumor tissue. Hydrophilic hypoxia-activated prodrugs are more significant in clinical trails.

However, at this time, nanotechnology has provided a useful approach for delivering drugs that had been previously
eliminated, by increasing their tumor-specific cytotoxicity, hypoxia sensitivity, and preventing drug side effects. Accompanied with in-depth studies on the molecular regulation of the HIF-1 cell signaling pathway, hypoxia tumor regions can be targeted more accurately.146

It was widely known that the drug delivery systems could only deliver <5% of injected dose to tumor region.147 The redox-responsive drug delivery nanocarriers mainly use nanomicelles containing disulfide bonds to target hypoxia, in which the hypoxic cells are identified by various redox molecules such as GSH, cysteine, and homocysteine. Biocompatible macromolecules such as PEG, PEO, PCL, and HA/PEI are frequently used to fabricate these redox-responsive nanomicelles. Comparable active groups, such as nitroimidazole or azo-groups, can also be reduced by specific reducible enzymes in hypoxic cells. Besides, because all nanomicelles have a loose structure and show low encapsulation rates, research studies on redox-responsive nanomicelles are still at the experimental stage, and more clinical trials are required to assess their applicability in clinical practice. It is challenging to apply micro/nano system on patients rather than animal models. In addition, the release mechanism of nanomicelles was still not very clear and should be explored in vivo, in order to reveal the molecular process of inter- and/or intracellular responses to prodrugs. Nanoplatforms for multi-responsibility and theragnostic integration should be developed to provide tumor therapy with more specificity and precision. The reduction-sensitive polymers and prodrugs possessing indolequinone moiety for hypoxia-selective activation. In conclusion, these hypoxia-targeting strategies have potential therapeutic applications and should be further investigated in experimental and clinical research studies.

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**Disclosure**

The authors report no conflicts of interest in this work.

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