Tumor Microenvironment-Based Stimuli-Responsive Nanoparticles for Controlled Release of Drugs in Cancer Therapy

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Abstract: With the development of nanomedicine technology, stimuli-responsive nanocarriers play an increasingly important role in antitumor therapy. Compared with the normal physiological environment, the tumor microenvironment (TME) possesses several unique properties, including acidity, high glutathione (GSH) concentration, hypoxia, over-expressed enzymes and excessive reactive oxygen species (ROS), which are closely related to the occurrence and development of tumors. However, on the other hand, these properties could also be harnessed for smart drug delivery systems to release drugs specifically in tumor tissues. Stimuli-responsive nanoparticles (srNPs) can maintain stability at physiological conditions, while they could be triggered rapidly to release drugs by specific stimuli to prolong blood circulation and enhance cancer cellular uptake, thus achieving excellent therapeutic performance and improved biosafety. This review focuses on the design of srNPs based on several stimuli in the TME for the delivery of antitumor drugs. In addition, the challenges and prospects for the development of srNPs are discussed, which can possibly inspire researchers to develop srNPs for clinical applications in the future.

Keywords: cancer therapy; tumor microenvironment; stimuli-responsive; nanoparticles

1. Introduction

Cancer, also known as malignant tumor, is still one of the common causes of human death worldwide [1]. According to the GLOBOCAN database, the number of new cancer cases was estimated to be 19.29 million, and that of cancer deaths was approximately 9.96 million, in 2020 [2]. Numerous efforts from different fields have been made to explore effective and safe strategies to treat this disease. Among various treatments, chemotherapy is one of the most commonly used methods for cancer therapy at present [3]. For the past decades, researchers have been working to deliver anti-cancer drugs to tumor sites. However, the clinical application of conventional chemotherapeutic drugs is restricted, owing to the lack of selectivity, limited water solubility, poor targeting ability and serious systemic toxicity.

In recent years, nanomedicine has been extensively used in tumor-targeting drug delivery due to its unique molecular properties. Despite nano drug delivery systems’ (NDDS) enhancement of the efficiency of conventional chemotherapeutics, it is still an urgent problem to improve the bioavailability of drugs in tumor tissues, especially to enhance the cellular uptake and intracellular release of drugs. With a deeper understanding of the different properties between normal tissues and tumor tissues, srNPs were rationally
designed for drug delivery. Thus, to better understand the advances in the srNPs for anticancer therapy, this review briefly introduced the endogenous stimuli (i.e., low pH, high GSH concentration, overexpressed enzymes, excessive ROS and hypoxia) of the TME (Scheme 1), and summarized the application of srNPs in tumor therapy, aiming to provide inspiration for further research and facilitate the clinical translation of srNPs.

2. Stimuli in the TME

The TME is a cellular environment composed of tumor cells, fibroblasts, lymphocytes, immune cells, bone marrow-derived inflammatory cells, signal molecules, an extracellular matrix (ECM) and surrounding blood vessels [4,5]. All the cells embedded in the ECM consists of collagen and proteoglycan. In general, the tumor vessels are characterized by irregular shape as well as loose structure, and even lack the endothelial cells or basement membrane in the malignant lesions. Therefore, the TME provides suitable conditions for tumor cells to exchange materials and promotes their proliferation and metastasis. The TME is characterized by several features, such as acidity, high GSH concentration, hypoxia, overexpressed enzymes and excessive ROS, compared to the physiological environment [6].

2.1. Acidity

The lower pH in the extracellular matrix and interstitial space is a sign of malignant tumor, which is caused by excessive metabolites, such as carbon dioxide, lactic acid, as well as activated vacuolar-type (V-type) H(+)−ATPases (a proton pumps) [7,8]. In general, cancer cells produce large amounts of lactic acid due to their heavy reliance on glycolysis instead of oxidative phosphorylation, and this phenomenon is called the Warburg effect [9–11]. Typically, the pH value in the TME (pH 6.5) is lower than that in normal cells (pH 7.4), and the abnormal pH conditions further appeared in organelles, such as nuleosome (pH 5.5) and lysosome (pH 5.0) [12]. Acidic TME has been proved to facilitate the occurrence and metastasis of tumors. In addition, the abnormal pH is also one of the causes of tumor multidrug resistance (MDR), especially for weakly alkaline chemotherapeutic drugs [13]. However, the acidity of the TME and nuclear endosome/lysosome could also be utilized as endogenous triggers for srNPs.

2.2. High GSH Concentration

GSH is a thiol substance composed of glutamate, glycine and cysteine. It is the most abundant reductant in living cells, especially in some organelles such as cytosol, mitochondria and the nucleus. Normal concentration of GSH, with detoxification and
antioxidant effects, is crucial for the body to maintain immune system functions. The intracellular concentration of GSH is about 2–10 mM, which is significantly higher than its concentration in blood and the extracellular matrix (about 2–20 µM). In addition, tumor tissues showed 10 times higher GSH concentration than normal tissues. It has been reported that abnormal GSH levels are related to many human diseases, such as liver-related diseases, neurodegenerative diseases, epilepsy, diabetes and so on [14–16].

2.3. Hypoxia

As a hallmark of solid tumors, hypoxia is closely related to tumor invasion, metastasis and drug resistance. Due to the irregular shape of blood vessels in solid tumors, it is unable to deliver enough oxygen and nutrition to all regions, resulting in temporary or long-term hypoxia of tumor cells. The oxygen partial pressure in normal tissues is about 30 mmHg, while that in tumor tissues gradually decreases from the surface to the inside and reaches a low level (5 mmHg) in some regions, and the oxygen partial pressure in some solid tumors may be close to 0 mmHg. Moreover, Oxygen utilization also decreased with increased distance between tumor cells and blood vessels. Tumor cells in hypoxic areas divide more slowly than those in normoxic areas, making them less sensitive to chemotherapeutic drugs targeting cells that rapidly proliferate [17,18].

Moreover, hypoxia in the TME can also upregulate hypoxia-inducible factors (HIFs), protein dimerization consisting of HIF-α (oxygen-sensitive subunit) and HIF-β (constitutively expressed subunit), which can facilitate the growth and metastasis of tumors [19]. It has been reported that a HIF-α isoform stimulated tumor progression in some tumor models, such as kidney cancer and neuroblastoma. Under acute and severe hypoxia conditions (1–2% O₂), HIF-1α could be activated promptly to combine with HIF-β [20], so the stability of HIF-1α and transcriptional activity of HIF-1 are significantly enhanced in hypoxic TME, thus increasing the expression of vascular endothelial cell growth factor (VEGF) that can promote the growth of tumors with angiogenesis [21]. In addition, increased HIF-1α can also induce immune escape [22]. Although hypoxia provides favorable conditions for tumor progression, this characteristic also provides opportunities to develop srNPs.

2.4. Overexpressed Specific Enzyme

Since physiological and metabolic processes in the human body depend on enzymes, the abnormal expression and activity of enzymes are the pathological basis of many diseases. Compared with that in normal tissues, some enzymes are overexpressed in tumor tissues, thus showing excessive secretion in the TME, such as matrix metalloproteinases (MMPs), hyaluronidases, β-Glucosidase, esterase [23,24]. By modification with specific enzyme substrates, srNPs can be cleaved by target enzymes and release drugs in the TME.

2.5. Excessive ROS

ROS include hydroxyl radicals (•OH), singlet oxygen (¹O₂), hydrogen peroxide (H₂O₂), peroxynitrite (ONOO⁻), superoxide anion (•O₂⁻) and so on [25]. There are several endogenous sources of ROS, while they are mainly produced by the incomplete reduction of oxygen and nicotinamide adenine dinucleotide phosphate oxidase in the mitochondria and plasma membrane [26]. As a signal molecule, ROS played an important role in protein translation, transcription and survival, as well as tumorigenesis and proliferation [27]. In an appropriate concentration, ROS are the significant signal molecules for multiple metabolic pathways, while excessive ROS might damage the tissues or organs and even induce serious diseases such as cancer. As a prominent feature of cancer, hypoxia significantly changes the ROS level in tumor tissues, so the ROS concentration in tumor cells (10⁻⁴ M) is much higher than that in normal tissues (2 × 10⁻⁸ M) [28,29].

3. Stimuli-Responsive Nanoparticles

As mentioned above, the TME possesses a variety of unique properties and plays a critical role in the occurrence, invasion and metastasis of tumors. However, the charac-
teristic difference between tumor tissues and normal tissues is also the theoretical basis for designing intelligent responsive NDDS [30,31]. srNPs can respond to various endogenous stimuli in the TME, and their properties, such as shape, size, surface charge and hydrophilicity, could be changed after reaching the tumor areas [32]. These changes can promote the accumulation, penetration, cellular uptake or drug release of nanoparticles, and ultimately enhance the anti-tumor therapeutic effect.

3.1. pH-Responsive Nanocarriers

Due to the Warburg effect, the acidic microenvironment of solid tumors can be used to achieve tumor-specific delivery of srNPs [1]. There are three common strategies for the construction of pH-responsive srNPs (Table 1). The first strategy is to use some specific molecular structures in the design of nanocarriers. The pKa values of these structures are close to the pH of the intercellular matrix, so their functional groups can be protonated in the TME with a lower pH, resulting in the destruction of the hydrophilic–hydrophobic balance of nanoparticles, thus causing structural changes, including rearrangement, expansion or disintegration [33]. Typical acid-sensitive groups include histidine, tertiary amine and sulfonamide structures. The second strategy is based on acid-labile chemical bonds, which can stably exist under neutral conditions and break under acidic conditions [34], enabling srNPs to disintegrate and release drugs in the TME. The third strategy is to use pH (low) insertion peptides (pHLIP), which could weakly interact with the cell membrane under neutral conditions and insert into the cell membrane and form stable transmembrane complexes in an acidic environment, thus promoting the cellular uptake of nanoparticles [35].

3.1.1. Hydrophobic-to-Hydrophilic Transition

There were numerous studies on the transition from hydrophobic to hydrophilic chemical groups in the TME [36–38]. For example, polymers with amino groups possess this property, as the amino groups in the structure can accept a proton and become hydrophilic when the pH value of the environment drops below its pKa [39]. The typical drug based on this concept is the polyhistidine nanomicelle developed by BAE et al. [40–42]. Poly-L-Histidine (polyHis) is a polypeptide containing imidazole groups, of which the pKa value is 6.5, thus, it shows a reversible hydrophilic–hydrophobic transition according to its protonated and deprotonated states. Bae et al. synthesized a pH-triggered micelle by using poly(histidine-co-phenylalanine)-b-poly(ethylene glycol) and poly(L-lactic acid)-b-poly(ethylene glycol)-folate [43]. The micelles loaded drugs through the hydrophobic interaction between the anticancer drug doxorubicin (DOX) and the deprotonated polyHis fragment, so it can exist stably in physiological environments with pH 7.4. However, when the nanomicelles reach the acidic TME, the polyHis block in the core gradually becomes unstable due to protonation, thus dissociating and selectively releasing the drug. Compared with pH-insensitive micelles, these nanomicelles were able to significantly enhance the antitumor efficacy of DOX [44]. PolyHis polymers are also used for achieving pH-responsive cellular uptake (Figure 1A) [45]. These nanomicelles are not affected by protein binding under blood circulation, and they are able to expose targeting ligands and bind to overexpressed cell surface receptors after being transported to the TME, thereby enhancing the uptake of nanodrugs by cancer cells.

More recently, PolyHis-based polymers were also used in gene anti-cancer therapy [46]. Zhao et al. developed a pH-triggered nanoplatform, PHD/PLL/siRNA nanoparticle (PHD/PLL/siRNA NP), via self-assembly of methoxy poly(ethylene glycol)-polyHistopoly(sulfadimethoxine) (PHD), poly-L-lysine (PLL) and PLK1 siRNA (siPLK1) [47]. Specifically, the spontaneous formation of PHD/PLL/siRNA NP is owing to electrostatic interaction between negatively charged PHD and siPLK1 and positively charged dendritic PLL. PHD/PLL/siRNA showed higher cellular uptake and efficient endo/lysosomal escape, which can be attributed to the pH-induced protonation and proton sponge effect of the imidazole ring of polyHis. In addition, the intracellular acidic microenvironment could
change the poly(sulfadimethoxine) (PSD) block from negatively charged to neutral, thus accelerating the disassembly of NPs and release of siPLK1. The results of the assay indicated that PHD/PLL/siRNA NP showed an excellent tumor elimination effect for non-small cell lung cancer therapy.

In addition to polyHis, protonation of tertiary amine groups can also alter srNPs from hydrophobicity to hydrophilicity in the acidic TME [48], thus changing the structure of nanoparticles and releasing the encapsulated drugs. For instance, Yang synthesized aliphatic polyester using enzyme polymerization [49], which could load DOX by self-assembly in aqueous solutions and release it in the TME. It was proven that the DOX release rate of micelles under an acid condition (pH 6.8) was significantly faster than that in a normal physiological environment (pH 7.4). The results of in vitro cellular uptake and cytotoxicity proved the feasibility of the drug delivery system. The pH-responsive release of drugs was owing to the transition of tertiary amine groups from hydrophobic to hydrophilic and the subsequent dissociation of structure. Zhang et al. designed a 4-diethylaminophenyl isothiocyanate (DAITC)-modified generation-five polyamidoamine (PAMAM) dendrimer (GDA) for delivering protein [50]. In this study, green fluorescent protein (EGFP) was encapsulated in GDA via complexation, and the hydrophobically modified GDA/EGFP can maintain stability at physiological pH (7.4) but disassemble at endolysosomal pH (6.0) due to the protonation of tertiary amines, thus showing high tolerability in serum and rapid release in the cytosol.

Moreover, the secondary amine group attached to the sulfone group in the sulfonamide possesses a near-neutral pKa, which can be utilized to design pH-responsive nanocarriers (Figure 1B) [51]. There exists electrostatic adsorption between negatively charged sulfonamides and positively charged polymers when pH is above 6.8, while in acidic microenvironments (pH below 6.8), the nanocarriers dissociate, as the sulfonamide is no longer charged, and the electrostatic interactions disappear. Kang and Bae designed a nanocarrier to transport and release nucleic acids using oligomeric sulfonamides (OS-ASs) [52], which could alter from hydrophilic to hydrophobic in a low pH environment. The nanocarriers were used to load the nucleic acid solution by formatting an OSA-polyplex. The results indicated that the OSA-polyplexes significantly enhanced DNA transfection by inducing endosomal release.

3.1.2. Acid-Labile Bond Cleavage

Several nanocarriers have been conjugated with acid-labile bonds, such as hydrazones [53,54], orthoesters [55,56], imines [57,58] and acetals/ketals [59,60], so as to develop a series of pH-responsive srNPs. The hydrazone bond is one of the most commonly used acid-sensitive bonds in nano delivery systems. At pH 7.4, the hydrazone is relatively stable, whereas it rapidly hydrolyzes in endosomes and lysosomes (pH 5–6) as its C=N double bond breaks under an acidic condition [61]. Etrych is the first researcher to study hydrazone linkage [62], and prepared pH-sensitive nanomedicine exhibiting advantages such as high drug loading capacity, simple preparation process, TME-responsive drug release and enhanced antitumor activity [63]. Zhou et al. connected amphiphilic conjugates DOX and β-sitosterol to the N-(2-hydroxypropyl) methacrylamide (HPMA) polymer using hydrazone as linkage [64]. The hydrazone linkage remains quite stable throughout the blood circulation at pH 7.4, while it breaks down quickly and releases 80% of its drug at pH 5.0. The pH-responsive cross-linked micelles showed significantly enhanced tumor permeability and anti-tumor efficacy in an H22 mouse xenograft model. The results proved that HPMA polymer micelles with hydrazone connections are feasible carriers for controlled-release nanodrugs.

Liao et al. developed the pH-sensitive hyaluronic acid-hydrazone linkage-DOX NPs (HA-hyd-DOX NPs) for targeted delivery of DOX. In specific, hydrophobic DOX was conjugated with the hydrophilic HA backbone by the pH-responsive hydrazone linkage, and the HA-hyd-DOX conjugates could self-assemble into HA-hyd-DOX NPs, which
can target tumors through a High Affinity of HA for overexpressed CD44 and achieve intracellular DOX release via cleavage of the hydrazone bond [65].

Not only a hydrazone bond, orthoester is also a kind of commonly used acid sensitive bond, due to its sensitivity to pH changes and its biocompatible degradation products. Huang et al. first reported polymers with orthoester as linkage [66,67]. Xu et al. synthesized a polymer poly((L-(2-(2-(dimethylamino)ethoxy))-L3-dioxolan-4-yl)methyl)methacrylamide) (PMAOE) linked by orthoester linkages to deliver DNA [68]. Drug-loaded nanoparticles were stabilized by electrostatic interactions between cationic amine groups and anionic DNA, and they were able to promote efficient release of DNA in the TME. Nuclear magnetic resonance (NMR) assay results showed that about 60% of the side chains underwent hydrolysis at pH 4.0 and slowly released adsorbed DNA.

The acid-labile imine linkage in an acidic environment has also been used as an acid-sensitive responsive bond for nano drug-loaded systems [69]. In 2017, Ding et al. fabricated dextran-doxorubicin (Dex-DOX) nanoparticles based on imine linkage (Figure 2A) [70]. Specifically, the hydroxyl groups on dextran were oxidized to aldehydes before being linked with DOX via imine linkage. The conjugate could self-assemble into uniform nanoparticles in an aqueous solution. The results proved that Dex-DOX could significantly improve cancer cellular uptake and enhance antitumor efficacy for B16-F10-bearing mice with excellent therapeutic safety. To further increase the stability of imine linkage under physiological conditions, benzoic-imide bonds were constructed by conjugation of the π-π bonds. Liao et al. developed the pH-responsive polymer nanogels with benzoic-imide cross-linkages synthesized by crosslinking of terephthalaldehyde (TFA) molecules with branched poly(ethyleneimine)-g-methoxy poly(ethylene glycol) copolymers for delivering indocyanine green (ICG) [71]. The nanogel encapsulating efficiently ICG retarded leakage of drugs at pH 7.8 by improving the stability of the drug in a neutral phosphate buffer, while it released ICG with the cleavage of benzoic-imide bonds in the nanogel when the pH was changed from 7.8 to 6.4, thus achieving controlled drug release.

In addition, Gillies and colleagues first reported the use of acetals as the connecting bonds of pH-responsive srNPs in 2004 [72]. Under acidic conditions, one of the oxygen atoms of the acetal bond is protonated and induces hydrolytic fracture, resulting in the transition of nanoparticles from hydrophobic to hydrophilic, thus accelerating drug release. Recently, Wagner et al. constructed pH-triggered mesoporous silica nanoparticles (MSNs) with acetal linker for loading and controlled release of resiquimod (R848), a Toll-like receptor 7 and 8 agonist, and OVA [73]. In this research, the carboxylated surface of MSNs enables them to connect with pH-induced capping composed of an acetal linker and biotin-avidin complex. The results of tests indicated that the R848-loaded MSNs showed rapid uptake by immune cells and efficient immune activation under acidic conditions. In addition, MSN-R848-OVAp activated an enhanced specific T-cell response by codelivery of the adjuvant and antigen. To prepare the polymeric material with acid-sensitivity and biodegradability, acetylated-dextran (Ac-Dex) is obtained by replacing 73% of hydroxyl groups in dextran with acetals [74]. Attractively, at 37 °C and pH 5.0, the half-life of FITC-dextran was approximately 10 h, whereas at pH 7.4 it was approximately 15 days. At present, a variety of drugs including DOX [75], plasmid DNA [76], siRNA [77,78] and antimicrobial agents [79] have been loaded into Ac-Dex-based nanoparticles.
Table 1. pH-responsive srNPs in cancer therapy.

| Responsive Moiety | Nanoplatform                                                                 | Cargos | Application                                           | Tumor Model          | Refs. |
|-------------------|------------------------------------------------------------------------------|--------|-------------------------------------------------------|----------------------|-------|
| polyHis           | poly(L-lactic acid)-b-PEG-b-polyHis micelles                               | DOX    | pH-dependent drug release                              | MCF-7                | [40]  |
|                   | polymeric micelles constitute of two block copolymers of poly(L-lactic acid)-b-PEG-b-poly(L-histidine)-TAT and polyHis-b-PEG | DOX    | pH-dependent drug release and tumor targeted chemotherapy | A2780/AD, MCF-7, and A549 | [42]  |
|                   | A mixed micelle system composed of polyHis-co-phenylalanine-b-poly(PEG) and poly(L-lactic acid)-b-PEG-folate | DOX    | Reversal of multidrug resistance of cancer             | A2780/DOXR           | [43]  |
|                   | a mixture of polyHis/PEG-folate and poly(L-lactic acid)-b-PEG-folate       | DOX    | Reversal of resistant MCF-7 tumor                       | MCF-7/DOXR           | [44]  |
|                   | A micelle composed of polyHis-b-PEG and poly(L-lactic acid)-b-PEG-b-polyHis-biotin | DOX    | Increase of endocytosis.                               | MCF-7                | [45]  |
| tertiary amine    | mPEG/HCou-g-MPCL micelles                                                  | DOX    | pH-sensitive drug delivery                             | HeLa                 | [49]  |
|                   | GDA/EGFP                                                                     | EGFP   | pH-responsive cytosolic protein delivery                | 143B                 | [50]  |
| sulfonamide       | DNA/PEI/poly(methacryloyl sulfadimethoxine)-b-PEG                           | DNA    | Tumor specific gene delivery                           | A2780                | [51]  |
|                   | Oligomeric sulfonamides-incorporated poly(L-lysine)/DNA                     | DNA    | enhancement of nucleic acid delivery.                  | HEK293               | [52]  |
| hydrazone         | HPMA                                                                         | DOX    | pH-sensitive drug release                               | EL4                  | [62]  |
|                   | HPMA                                                                         | DOX    | pH-sensitive tumor chemotherapy                         | Hep G2, A549 and H22 | [64]  |
|                   | HA-hyd-DOX                                                                   | DOX    | pH-dependent drug release and tumor targeted chemotherapy | Hela                 | [65]  |
| orthoester        | PEG-b-PtNEA27/56/73                                                         | Nile Red. | Acid-sensitive and thermoresponsive drug release         | NA                   | [66]  |
|                   | PMAOE                                                                        | DNA    | pH-modulated release of gene                            | NA                   | [68]  |
| imine             | Dex-DOX                                                                      | DOX    | pH-sensitive tumor chemotherapy                         | B16F10               | [70]  |
| benzoic-imine     | benzoic-imine-containing PEI-g-mPEG                                         | ICG    | Acid-triggered photoinitiation release                  | NA                   | [71]  |
| acetal            | MSN – R848 – OVAp                                                           | R848 and OVA | pH-sensitive tumor immunotherapy                      | NA                   | [73]  |
|                   | Ac-DEX                                                                       | pyrene | pH-dependent drug release                               | NA                   | [74]  |
| pHLIP             | HauNS-pHLIP-Ce6                                                             | Ce6    | Tumor targeted PTT/PDT                                  | Hela                 | [80]  |
|                   | MONs                                                                         | DOX    | Tumor targeted chemotherapy                             | MDA-MB-231, MCF-7    | [81]  |
The insertion is mainly directional. Usually, the C-terminal enters the cytoplasm across the bilayer membrane, while the N-terminal remains outside the cell \[84,85\]. It was reported that the affinity of pHLIPs for the phospholipid bilayer at low pH is 30–50 times higher than that at high pH. In addition, the kinetic process of insertion of pHLIPs into the cell membrane is quite rapid, and the movement from the formation of the interface helix to the transmembrane can be completed in seconds to minutes \[86\].

3.1.3. pH(Low) Insertion Peptides

There is another typical reported approach to achieve the pH-triggered srNPs. The acidic TME can also activate pHLIPs, which are polypeptides with specific sequences that can be inserted into the cell membrane under acidic conditions \[35,82\]. These polypeptides are composed of two flanking sequences at the end and a transmembrane sequence in the middle (Figure 2A). The flanking sequence endows the protein with water solubility, while the transmembrane sequence is mainly composed of aspartic acid (ASP) and glutamic acid (Glu) residues, which can enable the pHLIPs to be more hydrophobic at acidic pH, thus enhancing the interaction with the cell membrane \[32\]. Through the mechanism of membrane-associated folding \[83\], pHLIPs could be triggered by the acidic TME and spontaneously form a helix to insert and span the cellular membrane (Figure 2B). Under neutral or alkaline pH conditions, pHLIPs generally exist in the form of unstructured monomers, so they can be soluble in aqueous solutions and reversibly associate with lipid bilayers or the outer surface of cell membranes. However, in the acidic microenvironment, the increased proton concentration could induce protonation of the carboxyl groups of ASP and Glu in the C-terminal and transmembrane sequences of pHLIPs, thereby enhancing their hydrophobicity. Thus, the protonation of residues triggers the formation of a transmembrane helix, which can insert itself into the hydrophobic layer of the cell membrane. The insertion is mainly directional. Usually, the C-terminal enters the cytoplasm across the bilayer membrane, while the N-terminal remains outside the cell \[84,85\]. It was reported that the affinity of pHLIPs for the phospholipid bilayer at low pH is 30–50 times higher than that at high pH. In addition, the kinetic process of insertion of pHLIPs into the cell membrane is quite rapid, and the movement from the formation of the interface helix to the transmembrane can be completed in seconds to minutes \[86\].
By utilizing this mechanism, Yu et al. developed pHLIP-coated hollow gold nanospheres (HauNS) containing chlorin e6(Ce6) by electrostatic approach, achieving desirable pH-driven controlled therapy [80]. As an efficient smart responsive delivery system, the HauNS-pHLIP-Ce6 could not only amplify the accumulation and retention effects in the tumor, but also induce enhanced photothermal therapy (PTT)/photodynamic therapy (PDT) simultaneously by a single NIR light. The pH-activated nanospheres were believed to be a promising theranostic platform against tumors. Additionally, Zhang et al. constructed mesoporous organosilica nanoparticles (MONs) modified with pHLIP (Figure 2C) [81], and these pHLIP-modified MONs loaded with DOX could achieve higher cellular uptake by MDA-MB-231 and MCF-7 cells and exert excellent cytotoxic effects against cancer cells in the low pH circumstances of the TME. The results indicated that the pHLIP-modified MONs could be employed as desirable nanocarriers for enhancing chemotherapy.

3.2. GSH-Responsive Nanocarriers

Overexpressed GSH in the TME can also serve as a trigger switch for tumor responsive therapy [87]. Disulfide bonds (-SS-) and diselenide bonds (-SeSe-) and manganese dioxide (MnO₂) can break or disintegrate when incubated with GSH, so they could be used to design intelligent nanoplatforms to achieve tumor-specific drug release. Disulfide bonds have been widely used as a breakable bonding bond in nanocarriers to make nano preparations GSH responsive. For example, Chai et al. designed the GSH-responsive micelles, hyaluronic acid-ibuprofen (HA-ss-BF), prepared by conjugating ibuprofen (BF) to hyaluronic acid (HA) with a disulfide bond and self-assembling into micelles [88]. HA-ss-BF micelles could be employed as stimuli-responsive carriers for delivering DOX. Specifically, BF is used as an anticancer agent inhibiting the overexpressed cyclooxygenase-2 (COX-2) in cancer cells. Furthermore, HA-ss-BF micelles loaded with DOX could target cancer cells by recognition of CD44 receptors. Thus, HA-ss-BF micelles achieved GSH-responsive disassembly, targeted and on-demand release of drugs, as well as improved cellular uptake and excellent biodistribution (Figure 3A). Liu et al. developed GSH-responsive and DNA-based branched nanoplatforms for codelivery of gene editing components sgRNA/Cas9 targeting DNA and gene silencing component antisense targeting mRNA [89]. To be specific, a 3′ terminal extended single guide RNA (sgRNA) was prepared, which is capable
of complementary base pairing with complementary nucleic acid (antisense), and it was utilized to achieve coassembly of the sgRNA/Cas9/Antisense complex (RCA@NP) with antisense modified by two disulfide linkages as linker. The disulfide bonds enable RCA@NP to release antisense and sgRNA/Cas9 stepwise with the trigger of GSH and ribonuclease H enzymes (an intracellular RNA nuclease that can digest the RNA of RNA–DNA hybrids) in the intracellular environment, thus realizing synergistic antitumor efficacy. In addition, Ma et al. prepared ATRA-SS-HA nanomicelles by connecting hydrophobic all-trans retinoic acid (ATRA) and hydrophilic HA with disulfide bonds [90], which can self-assemble with curcumin (Cur) into Cur@ATRA-SS-HA. Such Cur-loaded nanomicelles can achieve GSH-triggered drug release due to the cleavage of disulfide bonds of nanomicelles in the TME, thus enhancing target esophageal cancer therapy.

Diselenide bonds also attract increasing attention as a GSH-responsive trigger. He et al. constructed diselenide-based GSH-responsive nanoparticles for triple-negative breast cancer-targeting (TNBC-targeting) treatment (Figure 3B) [91]. First, the paclitaxel (PTX) dimer prodrug PTXD-Se was synthesized with a diselenide bond serving as linkage, and it was subsequently encapsulated into an amphipilic copolymer. As a ligand of urokinase-type plasminogen activator receptor (uPAR) that expresses highly in TNBC ligand, uPA peptide was modified on the PTXD-Se NPs surface to obtain the uPA-PTXD NPs for further TNBC-targeting treatment. Since the diselenide bonds could be responsively cleaved by high GSH concentration and the uPA could bind with uPAR, the uPA-PTXD NPs showed GSH-controlled drug release and targeted tumor accumulation, exhibited significant antitumor efficacy and reduced systemic toxicity. Although both disulfide and diselenide bonds are highly sensitive to GSH, there is still debate as to which molecule is more sensitive. He et al. reported that the GSH-responsiveness of diselenide bonds is more sensitive than that of disulfide bonds [92], while Zhang et al. [93,94] believed that disulfide bonds possessed stronger reduction sensitivity than disulfide bonds. In any case, these srNPs triggered by GSH show enhanced antitumor activity and reduced toxicity, which have broad development prospects.

Besides disulfide bonds and diselenide bonds, MnO$_2$ is also could be utilized to design promising GSH-responsive nanocarriers. Mesoporous silica nanoparticles coated with MnO$_2$ achieved GSH-responsive release as the MnO$_2$ shell could be triggered to disassociate by abundant GSH in the TME, thus to deplete GSH and produce Fenton-like Mn$^{2+}$ for cancer imaging and self-reinforced chemodynamic therapy (CDT) [95]. Zhang et al. constructed HMnO$_2$@PEG/BLM nanoparticles, the polyethylene glycol (PEG)-modified hollow mesoporous MnO$_2$ nanoparticles loading bleomycin (BLM) that needs to be activated by metal ions to exert cytotoxicity [96]. When the nanoparticles reached the TME, they were degraded by the excessive GSH and produced Mn$^{2+}$, releasing BLM simultaneously, thereby forming Mn$^{2+}$-BLM in situ and activating the therapeutic activity of BLM. In addition, Mn$^{2+}$ could be utilized for in vivo magnetic resonance imaging (MRI). The nanoparticles effectively enhanced the antitumor therapeutic effects and avoided the adverse effects with GSH-responsive release and activation in situ of BLM (Figure 3C).

### 3.3. Hypoxia-Responsive Nanocarriers

Since hypoxia plays a key role in tumor angiogenesis, invasion, metastasis and immunosuppression [97], there has been increasing interest in developing nanocarriers for targeting the hypoxic TME recently. He et al. synthesized hypoxia-sensitive polyethylenimine-nitroimidazole (PEI-NI) micelles by self-assembly for codelivery of DOX and hyaluronic acid-Ce6 (HC) [98]. Under the hypoxic TME, the hydrophobic 2-nitroimidazole (NI) in micelles could be reduced to hydrophilic 2-aminoimidazole (AI) by a series of bio-reducing agents, enabling the micelles to release drugs by degradation to exert synergistic chemotherapy and PDT effects against cancer cells (Figure 4B). In addition, for accurate diagnosis and targeted treatment against tumors, Liu et al. constructed novel hypoxia-activatable polymeric micelles PEG-b-P(Asp-g-NIDH) consisting of 6-(2-nitroimidazole)hexylamine (NIDH) moieties grafted to PEG-b-poly (aspartic acid) (PEG-b-PAsp) for codelivery of...
ICG and DOX through self-assembly [99]. Owing to the presence of hypoxia-sensitive NIDH, PEG-b-P(Asp-g-NIDH) could achieve controlled drug release as well as synergistic chemotherapy and PTT/PDT effects, facilitating precision of photoacoustic (PA) imaging and eradication of malignancy (Figure 4A).

Another common hypoxia-sensitive structure is azobenzene (AZO). Perche et al. developed nanocarriers for siRNA delivery by introducing AZO structures between PEG and polyethyleneimine (PEI) (Figure 4C) [100]. After reaching the oxygen-deficient TME, the AZO bond broke and led to the disappearance of the shielding effect of the PEG-coating and the release of the encapsulated siRNA. Yang et al. synthesized a human serum albumin (HSA)-based hypoxia-responsive nanoparticle HCHOA by crosslinking AZO with oxaliplatin prodrug-conjugated HSA(HO) and Ce6-conjugated HSA(HC) [101]. The AZO group could be cleaved under hypoxic conditions, causing the rapid hypoxia-induced degradation of HCHOA in the TME. The hypoxia-triggered disassembly mode of HCHOA ensures its enhanced intratumoral penetration and PDT performance. Kulkarni et al. constructed the novel vesicle by self-assembly of di-block copolymer polylactic acid-AZO-polyethylene glycol for loading anticancer drugs gemcitabine (GEM) and erlotinib (ERL) [102]. The results indicated that drug-loaded vesicles could release encapsulated drugs in a hypoxic environment and significantly inhibit the proliferation of pancreatic cancer cells. To increase the penetration of drugs, nucleic acids, or probes into the core of tumors, Xie et al. designed pH-sensitive and size-shrinkable nanocarrier PAMAM-AZO-PEG (PAP) [103], which can encapsulate DOX in the core and absorb HIF-1α siRNA (si-HIF) on the surface with electrostatic bonding. In order to monitor the anti-cancer effect of DOX, the ROS probe also was combined with PAP+DOX. In the hypoxic TME, the PEG detached from

![Figure 3](https://www.biomedcentral.com/content/images/atoms/10.1186/s13287-019-1628-3_f3.png)

**Figure 3.** Design and responsive mechanism of GSH-triggered srNPs. (A) Preparation and in vivo release mechanism of HA-ss-BF Reproduced with permission [88]. Copyright 2020, Elsevier. (B) Schematic showing composition and GSH-triggered drug release of uPA-PTXD NPs. Reproduced with permission [91]. Copyright 2018 Ivyspring International Publisher. (C) Diagram illustrating formulation and application of HMnO@PEG/BLM. Reproduced with permission [96]. Copyright 2022, American Chemical Society.
positively charged PAMAM owing to the hypoxia-induced cleavage of AZO, enabling PAMAM to escape from endosomes through the proton pump effect, thus releasing loaded DOX and si-HIF. The results of the assay demonstrated that PAP+si-HIF+DOX can promote DOX penetration and silence HIF-1α expression and detect the elevated ROS level induced by DOX.

3.4. Enzyme-Responsive Nanocarriers

According to the literature, there were several enzymes overexpressed in tumor cells that could be utilized as endogenous stimuli for cancer imaging and treatment \[104,105\]. The advantage of enzymes as a reaction trigger are their high specificity for substrates, arousing increasing interest in developing enzyme-induced nanocarriers. The main endogenous enzymes studied include cathepsin, matrix metalloproteinase, phospholipase, glycosidase and so on (Table 2).

3.4.1. Cathepsin B

Cathepsin B (Cat-B) belongs to the family of lysosomal cysteine proteases, which are closely related to the development of cancer, and it is a typical stimulator, as its concentration in a variety of tumors is 3 to 9 times higher than that in normal tissues. Therefore, Cat-B-triggered srNPs have become new strategies for tumor treatment. Based on this, several Cat-B-cleavable peptides were designed, such as Glycine–Phenylalanine–Leucine.
-Glycine (GFLG) peptides [106,107] and Phenylalanine–Arginine–Arginine–Glycine (FRRG) peptides [108,109].

For instance, Cheng et al. created enzyme-responsive MSNs with a rotaxane structure serving as gatekeeper on the orifices (Figure 5B) [106]. The MSNs subsequently were modified by a multifunctional peptide containing Cat-B-responsive GFLG, exhibiting a high encapsulation rate for DOX. Thus, the DOX-loaded MSNs are capable of rapidly releasing the drug when the GFLG peptide was cleaved by excessive cathepsin B in the TME, resulting in superior antitumor activity. Song et al. developed Cat-B-degradable peptide nanoparticles (Arg–His–(Gly–Phe–Lue–Gly)₃ (RH–(GFLG)₃) for delivery of DOX [110]. Compared to the control group, the DOX-loaded RH–(GFLG)₃ exhibited enhanced stability, cell penetration and anti-cancer effects.

![Figure 5](image-url)  
**Figure 5.** Design and responsive mechanism of Cat-B-triggered srNPs. (A) Schematic representation of construct of FRRG-DOX nanoparticles and its tumor-targeting cytotoxicity due to the Cat-B-specific enzyme. Reproduced with permission [108]. Copyright 2019, Elsevier. (B) Cat-B-induced procedure and targeting intracellular application of the MSNs. Reproduced with permission [106]. Copyright 2015, American Chemical Society.

In addition, based on the Cat-B-cleavable peptide FRRG, Shim et al. designed a facile method for preparing the Cat-B-sensitive prodrug FRRG-DOX, which could release drugs with Cat-B-specific cleavage of prodrugs at the tumor site (Figure 5A) [108]. The results of human xenograft tumor models indicated that FRRG-DOX could improve targeting efficiency and antitumor efficacy against Cat-B-overexpressed cancer cells, and it did not cause severe toxicity in normal tissues owing to the low expression of Cat-B. Similarly, Cho et al. developed FRRG-monomethyl auristatin E (FRRG-MMAE) nanoparticles through self-aggregation [109], increasing the MMAE accumulation in tumors and enhancing the safety of therapy.

3.4.2. Matrix Metalloproteinases

In recent years, MMPs have become a hot research target in cancer therapy. MMPs are proteolytic enzymes whose basic role is to promote protein degradation and participate in regulating a variety of cell behaviors related to cancer biology. They belong to the zinc and
calcium-dependent endopeptidases family and are essential for tissue remodeling [111,112]. Under normal physiological conditions, the activity of MMPs is inhibited; however, in the TME, the abnormally high expression of some MMPs, such as MMP2, MMP7, MMP9 and so on, promotes the occurrence and metastasis of tumors [113]. Therefore, MMPs are widely used to achieve enzyme-triggered site-specific drug release. MMPs-responsive drug delivery can be achieved through constructing micelles, liposomes, dendrimers, nanogels and inorganic nanoparticles with MMPs-triggered structures [114]. Zhu et al. linked PTX with PEG\textsubscript{2000} through an MMP2-responsive peptide, constructing a novel targeted nanomicelle [115]. Specifically, PTX was loaded into the hydrophobic core of the micelles and was covered with hydrophilic PEG. After intravenous administration, nanomicelles could accumulate at the tumor through the retention effect (EPR effect) and enhance permeability of solid tumors, and then they could disintegrate to release PTX under the action of MMP2 in TME. Additionally, Zhou et al. designed a strategy to integrate the OXA-prodrug hexadecyl-oxaliplatin diethylene amine with 2,3-dimethylmaleic anhydride (HOAD) and PEGylated photosensitizer in MMP-2-activatable prodrug vesicles (MPV) into a nanoplatform (MPV-HOAD) [116]. This nanoplatform remained stable in the blood circulation, while after reaching tumors, the PEG corona was removed by MMP-2 and its surface charge was transformed from negative to positive under the acidic TME, subsequently improving penetration and accumulation of drugs in tumors, thus achieving an excellent antitumor effect.

Kalafatovic et al. developed an MMP-9-sensitive amphiphilic peptide that can form micelles through self-assembly for loading DOX [117]. Notably, in the TME, the cleavage of the MMP-9-triggered linkage in peptides could exert micelle reconfiguring to fibrillar nanostructures due to catalyzed hydrolysis of MMP-9, thus releasing DOX slowly and continuously. In addition, Liu et al. constructed an MMP-13-responsive MSNs-PLGLAR-BSA-LA@DOX [118]. Specifically, PLGLAR, the MMP-13 substrate polypeptide sequence, was used as a responsive linkage, bovine serum albumin (BSA) was used as end-capping to seal the MSNs and lactobionic acid (LA) was used as a targeting ligand. The results demonstrated that DOX in functionalized MSNs could be effectively released under the trigger of MMP-13 in the TME, thus enabling the nano-drug treatment group to exhibit stronger efficacy and lower toxicity compared with the free DOX group.

3.4.3. Phospholipase

Phospholipases can hydrolyze phospholipids into fatty acids and other lipophilic substances. In addition, as part of the host defense mechanism, phospholipase is overexpressed in the surrounding invasive region of some tumors [119], which provides a specific stimulus for responsive drug release. At present, the research on the phospholipase A2 (PLA2) family is most extensive, including intracellular PLA2 and secretory phospholipase A2 (sPLA2).

Sun et al. designed sPLA2-degradable nanoparticles consisting of a liposome as the shell and two complementary cytokine-loaded DNA nanoclew (NCs) as cores [120]. Specifically, DNA NC cores were loaded with the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), a model cytokine, through Ni\textsuperscript{2+}-polyhistidine affinity between Ni\textsuperscript{2+}-modified DNA NCs and TRAIL, and the sPLA2-triggered liposome shell could protect the TRAIL-loaded DNA NC cores from degradation in the physiological environment and could be degraded by overexpressed sPLA2 in the TME. Thus, TRAIL-loaded DNA NCs could transform into nanofibers extracellularly and deliver TRAIL to death receptors on the plasma membrane of cancer cells, thereby activating the apoptotic signal (Figure 6B). The Andresen group developed an enzymatically-triggered prodrug liposome for delivering antitumor ether lipids (AELs) to tumor sites, and the liposomes also could be utilized to encapsulate various chemotherapeutics [121]. To be specific, sPLA2-sensitive masked AELs (proAELs) were synthesized, which could form liposomal membranes spontaneously in an aqueous solution. In the TME, owing to overexpressed sPLA2, the hydrolysis of ester bonds in proAELs led to the disintegration of liposomes, releasing activated cytotoxic
AELs and free fatty acids/encapsulated drugs. In another study, Lee et al. designed a strategy for the synthesis of sPLA2-sensitive phosphate micelles loaded with up-conversion nanoparticles (UCNP) [122], which could achieve targeted delivery of UCNP to prostate cancer cells for accurate bio-imaging. The biocompatible UCNP-loaded micelles exhibited precise drug release and reduced adverse effects. Ghavami et al. constructed sPLA2-responsive DSPC/DSPG/DSPE-PEG\textsubscript{2000} liposomes for targeted delivery of peptide nucleic acid (PNA), an antisense agent [123], to the tumor. Specifically, antisense octaarginine (a cell-penetrating peptide)-conjugated PNA (octaarginine-PNA) was prepared and encapsulated into the liposome. The PNA-loaded liposomes showed efficient sPLA2-induced release and excellent antisense effects against HeLa cells.

3.4.4. Glycosidases

Glycosidases participate in the occurrence of N-linked glycosylation in the Golgi apparatus and endoplasmic reticulum [124], and they also can hydrolyze carbohydrates in the lysosomes [125]. Thus, glycosidases could be utilized to design glycosidase-triggered nanocarriers for delivering drugs to the target tissues with high concentrations of glycosidases. In recent years, researchers have developed various glycosylated nanoparticles to selectively release drugs in the TME where cancer cells overexpress glycosidases. Bernardos et al. developed silica mesoporous supports (SMPS) modified with lactose or starch derivatives on the surface of nanoparticles to achieve enzyme-induced drug release [126]. The fluorescent dyes were effectively blocked in the pores of SMPS by the grafted saccharide molecules. With the triggering of $\beta$-D-galactosidase, the coated saccharides of MSNs were hydrolyzed and the entrapped dye was released. For efficient drug delivery, Clarhaut et al. synthesized a $\beta$-galactosidase-sensitive folate-DOX conjugate (FDC) consisting of a folate ligand, DOX and a galactoside trigger, which can selectively recognize folate receptor-positive acute myeloid leukemia (AML) cells and release the DOX due to the carbohydrate unit of FDC being degraded by the catalysis of intracellular $\beta$-galactosidase [127]. Rastegari et al. constructed Fe\textsubscript{3}O\textsubscript{4} magnetic nanoparticles ($\beta$-CD-MNPs) coated with $\beta$-cyclodextrin ($\beta$-CD) that were employed as enzyme-sensitive carriers for delivering prodigiosin (PG) to cancer cells (Figure 6A) [128]. The PG-loaded $\beta$-CD-MNPs were responsive to galactosidase, releasing drugs due to $\alpha$-glucosidase degradation, thereby realizing targeted drug release and improved drug retention to MCF-7/GFP and HepG2 cells.
### Table 2. Enzyme-responsive srNPs in cancer therapy.

| Stimulus | Responsive Moiety | Nanoplatform | Cargos | Application | Tumor Model | Refs. |
|----------|-------------------|--------------|--------|-------------|-------------|-------|
| Cathepsin B | GFLG | DOX@MSN- GFLG8/7RGDS / α-CD | DOX | Tumor targeted chemotherapy | HeLa | [106] |
| | | RH-GFLG<sup>3</sup> | DOX | Tumor targeted chemotherapy | HeLa | [110] |
| | FRRG | FRRG-DOX | DOX | Tumor targeted chemotherapy | HT-29 | [108] |
| | | FRRG-MMAE | MMAE | Tumor targeted chemotherapy | 4T1 | [109] |
| MMP-2 | GPLGAGQ | PEG<sub>2000</sub>-peptide-PTX | PTX | MMP-2-sensitive drug release | A549 | [115] |
| | GPLGLAG | MPV-HOAD | OXA pheophorbide a | MMP-2-sensitive PDT and cancer immunotherapy | CT26 | [116] |
| MMP-9 | GGFLG | MMP-9 responsive peptides in conjunction with DOX | DOX | MMP-9-triggered drug release and chemotherapy | MDA-MB-231-luc-D3H2LN | [117] |
| MMP-13 | PLGLAR | MSNs-PLGLAR-BSA-LA@DOX | DOX | MMP-13-triggered drug release and chemotherapy | HepG2 | [118] |
| sPLA2 | 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine liposome shells and TRAIL-loaded DNA NCs cores | TRAIL | Targeted Delivery of Cytokine | COLO 205 cells | [120] |
| | ester bonds in proAEL | proAEL | AEL | Tumor specific drug release for cancer therapy | KATO III | [121] |
| | phosphate | UCNP-loaded phosphate micelles | UCNP | Bioimaging of prostate cancer cells | 22Rv1 | [122] |
| | DSPC/DSPG/DSPE | DSPC/DSPG/DSPE liposomes | PNA | tumor targeted drug release for cancer therapy | HeLa | [123] |
| Galactosidase | Saccharides | SMPS modified with lactose or starch derivatives | [Ru(bipy)<sub>3</sub>]<sup>2+</sup> dye | Glycosidase-responsive intracellular controlled release of drug | HeLa | [126] |
| | Galactosidase | Carbohydrate unit | folate-DOX conjugate | DOX | Glycosidase-responsive chemotherapy | KG-1 and HL-60 | [127] |
| | α-glucosidase | β-CD | β-CD-MNPs | prodigiosin | Anticancer drug delivery | MCF-7/GFP/HepG2 | [128] |
After internalization by cancer cells, such a conjugate can disintegrate to release TAM and release due to thioether oxidation, thereby enhancing the potency of antitumor drugs. and in vivo assays proved that S-LPs exhibited efficient ROS-responsive targeted drug synthesized novel thioether phosphatidylcholines (S-PCs) and S-PC-based liposomes (S-LPs) for A549 cells. The results demonstrated that siRNA/ROS-PAMAM shows high gene transfection efficiency for loading siRNA(siRNA/ROS-PAMAM) [135], which can improve the release of siRNA under ROS-abundant conditions, reducing cytotoxicity for normal tissues. The experimental results demonstrated that siRNA/ROS-PAMAM shows high gene transfection efficiency for A549 cells.

3.5. ROS-Responsive Nanocarriers

The higher level of ROS in cancer cells could also be used for the design and development of srNPs to enhance drug release at specific sites. In the design of ROS-responsive NDDS, the most commonly used groups are boric acid esters [129], thioketals [130], thioethers [131,132] and so on. A boric acid ester bond has been proven to be able to undergo ROS-induced degradation and its application in different fields has increased recently. For example, Lux et al. developed a novel ROS-responsive polyester containing a boronic ester that could degrade and release cargos through $\alpha$-tocopherol succinate-tamoxifen (TPGS-TAM) with an aryl boronic ester linker [134]. Sun et al. designed a self-amplified ROS-induced D-\(\alpha\)-tocopherol PEG$_{1000}$ succinate-tamoxifen (TPGS-TAM) with an aryl boronic ester linker [134]. After internalization by cancer cells, such a conjugate can disintegrate to release TAM and \(\alpha\)-tocopherol succinate (\(\alpha\)-TOS) that can subsequently increase ROS and further accelerate the release of TAM, thus achieving a robust anti-cancer effect.

Besides the boronic ester structure, thioketal was also used to design ROS-sensitive nanocarriers. Wang et al. constructed thioketal-core ROS-sensitive PAMAM dendrimers for loading siRNA(siRNA/ROS-PAMAM) [135], which can improve the release of siRNA in the TME. With thioketal employed as linkages, siRNA/ROS-PAMAM is cleavable in ROS-abundant conditions, reducing cytotoxicity for normal tissues. The experimental results demonstrated that siRNA/ROS-PAMAM shows high gene transfection efficiency for A549 cells.

Thioethers have also been utilized to design ROS-triggered srNPs. Du et al. synthesized novel thioether phosphatidylcholines (S-PCs) and S-PC-based liposomes (S-LPs) loaded with DOX for ROS-induced release of drugs (Figure 7B) [136]. The results of in vitro and in vivo assays proved that S-LPs exhibited efficient ROS-responsive targeted drug release due to thioether oxidation, thereby enhancing the potency of antitumor drugs.
Figure 7. Design and responsive mechanism of ROS-triggered srNPs. (A) Composite and \( \text{H}_2\text{O}_2 \)-degradable mechanism of polymeric nanoparticles. Reproduced with permission [133]. Copyright 2012, American Chemical Society. (B) Formulation and ROS-induced DOX release of S-LPs. Reproduced with permission [136]. Copyright 2019, American Chemical Society.

4. Conclusions and Perspectives

Tumor growth shows cellular and molecular heterogeneity [137], and it has been reported that a small quantity of progenitor cells and stem cells embedded in the perivascular region might be related to the growth and recurrence of tumors [138]. At the cellular level, malignant tumors are characterized by a complex mixture of benign cells, malignant cells, fibroblasts, stromal cells, vascular cells and infiltrating inflammatory cells. At the molecular level, the phenotype and gene expression profile of cancer cells are distinctly different from those of normal cells. This review comprehensively summarizes a series of intelligent nanoplatforms responsive to specific stimuli in the TME, including low pH, high GSH concentration, hypoxia, overexpressed enzymes and excessive ROS. Notably, the srNPs’ target tumor tissues and can be triggered by endogenous stimulation of the TME, thus showing enhanced anti-tumor efficacy and reduced toxicity. Additionally, the srNPs
play a crucial role in drug controlled-release against cancer cells as the active participant instead of the passive carrier, showing great development potential in improving disease treatment methods.

Compared with conventional drug delivery systems, TME-responsive nanoformulations control drug uptake and release in cancer cells and the TME through local stimulation, exerting excellent antitumor therapeutic efficacy. However, there are still some key problems to be solved before the srNPs are applied in clinical practice. Herein, we highlight some obstacles that must be eliminated as soon as possible and provide some suggestions for future applications.

Firstly, conditions of endogenous stimuli in some studies are imprecise, for example, the pH value used in some studies is lower than the actual pH of the tumor, and the concentration of the reducing agent used in vitro is higher than the actual concentration in vivo. Therefore, a better understanding of the differences between the normal physiological environment and the TME is essential to further develop stimuli-responsive nanocarriers. In addition, variability among patients and differences in the TME among diverse types of tumors hindered clinical translation of srNPs. To remove the obstacles caused by tumor heterogeneity, more triggers that are overexpressed in the TME should be found and studied, such as vascular endothelial growth factor (VEGF) [139], and multi-stimuli-responsive nanocarriers with tunable drug delivery should be developed to adapt to different tumors and different patients. In addition, exogenous stimuli [140], such as heat, light and ultrasound, also play an important role in targeted anti-tumor therapy, so research on these stimuli should also be promoted and explored in the future.

Secondly, numerous studies on srNPs in subcutaneous tumor-bearing mice models have been reported, but their therapeutic effect can rarely predict their safety and effectiveness in clinical trials [141]. Thus, more preclinical animal models that could highly simulate human TME need to be established, such as patient-derived tumor models [142], tumor metastasis models, in situ tumor models and tumor drug resistance models to further study the possible clinical applications of srNPs.

Thirdly, the potential systemic toxicity of srNPs is a severe challenge for their use in long-term treatments due to some non-biodegradable nanomaterials and relatively low accumulation efficiency at the target site. To overcome this obstacle, some strategies could be utilized to verify the biosafety of srNPs in the short term and in the long term. A promising approach is to develop metabolizable or biodegradable nanomaterials for the design of functional srNPs based on artificial intelligence and machine learning to avoid systemic toxicity [143]. In addition, a series of studies need to be comprehensively performed, such as the distribution of drugs intratumourally, scientific analysis of pharmacokinetics and pharmacodynamics, analysis of blood biochemistry and hematology, long-term toxicological evaluation and so forth [144].

Lastly, the sophisticated approaches and complex components for preparing srNPs severely hampered their clinical application due to the high cost, batch-to-batch variations and relatively poor stability of srNPs. The simple and feasible fabrication methods for srNPs should be innovated to ensure controllable cost, batch-to-batch reproducibility and maintained stability, thus facilitating the standardized large-scale production and quality control of srNPs for clinical translation.

To summarize, srNPs-responsive nanocarriers represent a promising future for the treatment of cancer. We firmly believe that in the near future, with the breakthrough of relevant research, multifunctional nano delivery systems for practical clinical applications will make great contributions to human health.

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