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Review article

# Stimulus-responsive nanoscale delivery systems triggered by the enzymes in the tumor microenvironment

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ABSTRACT

The tumor microenvironment is the cellular environment that is also described as the "soil" for supporting tumor growth, proliferation, invasion and metastasis, as well as protecting tumor cells from immunological recognition. Notably, tumor cells can grow much faster than other normal organs and invade surrounding tissues more easily, which results in abnormal expression of enzymes in the tumor microenvironment, including matrix metalloproteinases, cathepsins, phospholipases, oxidoreductases, *etc*. In opposite, due to the high selectivity and catalytic activity, these enzymes can promote nanoparticles to recognize tumor tissues more accurately, and the more accumulation of drugs at primal tumor sites will enhance therapeutic efficacy with lower systemic toxicity. Therefore, one promising antitumor strategy is to design stimulus-responsive nanoscale delivery systems triggered by the enzymes with the support of various nanocarriers, such as liposomes, micelles and inorganic nanoparticles, *etc*. In this review, numerous facts were cited to summarize and discuss the typical types of enzyme-stimulus responsive nanoscale delivery systems. More importantly, we also focused on their recent advancements in antitumor therapy, and offered the direction for further studies.

## 1. Introduction

Antitumor therapy is one of the biggest challenges in medicine, representing a heavy burden to human society. Currently, chemotherapy is widely considered to be an effective strategy for antitumor therapy. However, due to the systemic toxicity, lack of tumor targeting, and multidrug resistance, opportunities for broad application of chemotherapy in clinical practice are limited [1]. Studies in tumors and tumor microenvironments have revealed that the tumor microenvironment plays a pivotal role in tumor proliferation, invasion and metastasis by inducing abnormal characteristics, including hypoxia, tumorous acidity, abnormal expression of enzymes, high interstitial pressure, presence of inflammation and abnormal expression of tumor-related factors [2,3].

Based on these observations, a variety of microenvironment-responsive nanoparticles have been designed. Under the microenvironmental stimulation, loaded-drugs can be released responsively into desired tumor sites from various drug delivery vehicles, such as liposomes, polymer micelles, inorganic nanoparticles and other nanoparticles. Notably, their sizes, shapes and biocompatibilities can be controlled, and their surfaces can be modified with the support of nanotechnology [4]. Besides, due to the high selectivity and catalytic activity, stimulus-responsive nanoscale delivery systems triggered by the enzymes have exhibited more advantages than other drug delivery systems, receiving a tremendous amount of attention in the development of antitumor therapy.

Herein, we summarize and discuss the typical enzymes that have been employed in the enzyme-stimulus responsive nanoscale delivery systems. More importantly, we also focus on their recent advancements in antitumor therapy, and want to offer the direction for further studies.

## 2. The tumor microenvironment

The tumor microenvironment is the cellular environment, which is composed of tumor cells, immune cells, fibroblasts, bone marrow-derived inflammatory cells, lymphocytes, signaling molecules, the extracellular matrix and surrounding blood vessels [5,6]. Over 100 years ago, Stephen Paget introduced the "seed and soil" theory to explain the specific metastasis of breast cancer [7]. Further studies also revealed that the tumor microenvironment could provide the "soil" for supporting tumor growth, proliferation, invasion and metastasis, as well as avoiding immunological recognition.

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**Table 1**
A summary of enzymes employed in stimulus-responsive nanoscale delivery systems.

| Enzyme | Nanostructures | Sensitive site | Cancer types or models |
|---|---|---|---|
| MMP-2 [19] | Biotin-PEG-b-PLL(Mal)-peptide-DOX polymeric micelles | CPLGLAGG | SCC-7 |
| MMP-2 [20] | DOX–DGL–GNPs | Gelatin | 4T1 |
| MMP-2 [21] | PEG-pp-PEI-PE, PEG2000-peptide-PTX | PLGLAG | A549, HeLa |
| MMP-9 [22] | Magh@PNPs-PEG-RegaCP-PEG | MMP9 cleavable peptide regaCP | Pancreatic cancer |
| MMP-12 [23] | Paclitaxel-peptide nanoparticles | GPLGLAGGERDG | HT-1080 fibrosarcoma xenograft |
| MMP-14 [24] | Iron oxide nanocarrier | FITC-βAla-Cys-Arg-Ser-CitGly-HPhe-Tyr-Leu-Tyr | MMTV-PyMT tumor cells |
| Cathepsin B [25] | PDOX | Ac-FK-PABC-Dox | Hepatocellular carcinoma |
| Cathepsin B [26] | HPMA copolymer-DOX-OV-TL16 | GFLG | OVCAR-3 cells |
| Cathepsin B [27] | Lactose-containing HPMA copolymer-doxorubicin conjugate | GFLG | HepG2, Colo-205, SW-480, SW-620 |
| Cathepsin B [28] | Peptide-capped mesoporous silica nanoparticles | GIVRAKEAEGIVRAK | HeLa, MEFs WT |
| Cathepsin E [29] | Cathepsin E-activatable 5-ALA prodrug | RQAGFSL | PDAC cells |
| cathepsin L [30] | Lys(Ac)-Puro | K(Ac)-Puro | HCT116 xenograft |
| PSA [31] | L377,202/DOX | Nglutaryl-(4-hydroxyprolyl) AlaSer-cyclohexaglycyl-GlnSerLeu-CO2H | LNCaP, DuPRO-1, PC3, Hct116, MDA-MB435S, T24, MRC-5 |
| PSA [32] | Mu-HSSKLQ//L12ADT prodrug | MuHSSK(Fmoc)LQ peptide | LNCaP, HCT-116 |
| PSA [33] | 4-O-(Ac-HypSSChgQSSP)-dAc-Vin | 4-O-(Prolyl)-desacetylvinblastine | LNCaP, Colo320, T47D, |
| PSA [34] | A 5-fluorodeoxyuridine prodrug | His-Ser-Ser-Lys-Leu-Glu-OH (HSSKLQ) | LNCaP, TSU cells |
| PSA [35] | 2′-MuHSSKLQLAib-paclitaxel | MuHSSKLQ | CWR22Rv1, DU145, TSU |
| uPA [36] | 3′-N-(N*-Boc-Val-Leu-Lys) | D-Val-Leu-Ly | B16 Melanoma |
| uPA [37] | GaFK-Doxaz | N-acetyl-Gly-D-ala-L-Phe-L-Lys | MCF-7, MiaPaCa-2 |
| uPA [38] | Gold nanorods | LGGSGRSANAILEC | 4T1 cell tumors in mice |
| Trysin [39] | f-PRM/CHS nanocapsules | PRM | HeLa |
| Trysin [40] | Protamine/SCD supramolecular nanoparticles | Protamine | NA |
| PLA2 [41] | 1-O-DPPCV liposomes | Lipid | NA |
| α-amylase [42] | Dextrin-PLA2 conjugates | Dextrin | MCF-7, HT29, B16F10 |
| β-galactosidase [43] | Nanometric silica mesoporous supports capped with “Saccharides” | Starch derivatives Glucidex 47, Gludicex 39, and Glucidex 29 | HeLa, LLC-PK1 |
| β-galactosidase [44] | Doxorubicin-folate conjugate | Folate ligand | A549, HUVEC, KG-1, HL-60, A2780, HeLa, KB |
| Azoreductase [45] | Azobenzene-linked block polymer | Azobenzene | NA |
| Azoreductase [46] | G5-L(x)-DOX nanoconjugates | L1-L4 azo-linkers | HepG2, Hep3B |
| MPO [47] | GNP-corked NCNCs | NCNCs | B16 melanoma |

NA: not applicable.

It is especially difficult for tumor cells to maintain a suitable supply of oxygen and nutrients in the case of their rapid growth and excessive proliferation. Additionally, tumor cells rely heavily on glycolysis rather than oxidative phosphorylation to maintain biosynthetic functions, thus producing a large amount of lactic acid, which is known as the Warburg effect [8]. In this condition, the extracellular pH of the tumor site (pH 6.5–6.8) is usually lower than normal tissues (pH 7.4) [8,9]. Meanwhile, abnormal vascular networks provide insufficient blood for tumor sites, which results in hypoxia [10]. Various enzymes that participate in tumor progress are also upregulated, including matrix metalloproteinases (MMPs), cathepsins, phospholipases, oxidoreductases, *etc*. [11]. In opposite, these characteristics of the tumor microenvironment can provide novel pathways to achieve tumor-targeting delivery, enhancing therapeutic efficacy with lower systemic toxicity.

## 3. The nanoscale delivery systems in antitumor therapy

Recently, nanotechnology is an innovative technology and has been used successfully in medicine to improve the inapplicable properties of drugs or give new controllable characteristics for drugs, such as physicochemical properties or pharmacokinetic properties. In addition, nanocarriers are widely applied for tumor diagnosis and treatment, including liposomes, micelle dendrimers and other polymer carriers, as well as inorganic nanoparticles (such as gold nanoparticles, carbon nanotubes, or silicon quantum dots), macromolecules (such as antibody-drug conjugates, drug-polymer delivery systems) and so on. Antitumor drugs can be encapsulated inside of or at the core of nanocarriers via electrostatic adsorption, and they can also be connected to the surface or outer layer via covalent bonds [12,13]. In this manner, various nanoscale delivery systems have been designed to improve import characteristics of drugs, involving their physicochemical properties, half-life of blood circulation, biocompatibility, bioavailability and targeting, as well as overcoming other side effects of drugs. One example is that doxorubicin (DOX) loaded by liposomes can exhibit more advantages over free drugs in clinical practice.

In the past decades, various drug delivery strategies have been investigated in preclinical and clinical practices, containing hydrophilic polymer encapsulation for long-circulation, using the EPR effect for tumor penetration, surface modification with target ligands or membrane peptides for intracellular drug targeted delivery, and application of a sensitive stimulus for delivering or releasing drugs specifically. Furthermore, the targeted drug delivery systems can be also designed based on an antibody-antigen interaction or ligand-receptor binding on the surface of cells or vessels. Although monoclonal antibodies have achieved great success in antitumor therapy, it is difficult for them to infiltrate a solid tumor, which results in weakening their benefits in clinical practice [14,15]. Besides, small molecule ligands, such as folic acid and galactose, have been well-developed, but they still have poorer specificity and targeting abilities [16].

The occurrence and development of tumors are usually associated with hypoxia, altered acid content, oxidation-reduction potential, abnormal expression of enzymes, and external stimuli (such as thermotherapy, magnetic field and ultrasound). Based on these special characteristics, microenvironment-stimulus responsive nanoscale delivery systems can be designed to deliver and release drugs specifically [17]. Among these systems, the stimulus-responsive nanoscale delivery systems triggered by enzymes are significantly more effective than the others. In recent studies, due to the high selectivity and catalytic activity, typical enzymes expressed abnormally in the tumor microenvironment have been used as biomarkers for tumor diagnosis, prognosis as well as the targets or stimuli for triggering drug release. Besides, a series of bioengineering technologies, such as genetic

engineering and cell engineering, are able to feutherly promote these systems to play a role in biomedical applications more reliably and efficiently.

## 4. The enzymes of the tumor microenvironment and the design of enzyme-stimulus responsive nanoscale delivery systems

### 4.1. The enzymes of the tumor microenvironment

Generally, most enzymes are proteins that are named after their substrates or types of catalytic reaction. Enzymes play a fundamental role in most physiological processes, such as angiogenesis, cell proliferation, migration, autophagy and apoptosis [18]. It has been found a group of enzymes express highly in tumor tissues, while they are not expressed or expressed at a relatively low level in normal tissues. Additionally, due to the mild reaction conditions, high specificity and lack of harm, stimulus-responsive nanoscale delivery systems triggered by the enzymes have become a focus of researchers. This review summarizes and lists the typical enzymes applied in enzyme-stimulus responsive nanoscale delivery systems, containing their nanostructures, sensitive sites and applications in Table 1.

### 4.2. Hydrolases and the design of its stimulus-responsive nanoscale delivery systems

Hydrolases are able to catalyze hydrolytic reactions, achieving a division of a larger molecule into smaller molecules. To date, hydrolases have been widely emerged as biological stimulators to trigger drugs release from nanocarriers specifically, since they have a high concentration in targeted tissues.

#### 4.2.1. Proteases

Recently, studies in cancer biology have established a strong association between proteases and tumor development. Proteases play an essential role in the catalytic reactions, irreversibly degrading proteins and maintaining the stability of internal environment, while abnormal hydrolysis of proteases will induce tumor hyperplasia, invasion and metastasis, tissue remodeling, inflammation and a series of tumor growth factors activation. In the tumor microenvironment, proteases mainly include matrix metalloproteinases, cathepsins, urokinase, prostate-specific antigen and trypsin.

*4.2.1.1. Matrix metalloproteinases*. Matrix metalloproteinases (MMPs) are considered to be members of protease family, and include 24 subtypes, such as collagenase, gelatinase, matrilysin and membrane-type [48]. Generally, MMPs are able to degrade the extracellular matrix [49], and take part in a series of physiological and pathological processes, including bone growth, nervous system development, inflammatory response [50] and tumor growth [51]. The most investigated MMPs contain MMP-2 (gelatinase A or collagenase IV), MMP-9 (gelatinase B), MMP-7 (matrilysin) and MMP-14 (membrane-type-1-MMP), whose expression in tumor tissues is 10 to 20 times higher than normal tissues [52]. In particular, MMP-2 and MMP-9 have the ability to degrade type IV and type V collagen, which can improve tumor diffusion, infiltration and migration. Previous studies revealed that MMP-2 could express highly in various types of cancers, such as oral, colon, breast and lung cancers [4]. Therefore, an enzyme-triggered delivery strategy based on modifying nanocarriers with the sensitive polypeptide sequences of MMPs will provide the potential to achieve specific delivery of drugs or genes to the desired sites. Lin Zhu et al. [53] conjugated paclitaxel (PTX) to PEG2000 with the MMP-2 sensitive peptide and constructed a novel type of targeted drug micelles. The PTX was loaded in the hydrophobic nucleus and coated with a hydrophilic polypeptide-PEG1000-PE shell. After administration, more nanoparticles would accumulate in the tumor sites through the EPR effect.

Notably, the typical MMPs-stimulus responsive nanocarriers also include liposomes, dendrimer carriers, nanogels, inorganic nanoparticles and nanoprobes. Kaiyong Cai et al. [54] reported on an MMP-13 responsive drug delivery system, MSNs-Peptide-BSA-LA@DOX. The MMP-13 substrate polypeptide sequence, PLGLAR, was employed as the sensitive intermedia linker, bull serum albumin (BSA) was employed as the end-capping agent of mesoporous silica nanoparticles (MSNs) and lactose acid (LA) was employed as the targeted part. After being triggered by MMP-13, DOX was released and delivered effectively, showing better efficacy and lower side effects than free DOX *in vivo* and *in vitro*. Sun Lu et al. [55] proposed a dual MMP-2 activatable and tumor cell-permeable magnetic nanoprobe to achieve concurrent drugs selectivity and intracellular tumor imaging. The nanoprobe was formed by self-assembly between a His-tagged fluorescent fusion protein chimera and nickel ferrite nanoparticles via a chelation mechanism.

In addition, drug-polymer conjugates are widely applied in MMPs-stimulus responsive nanoscale delivery systems, since they can be degraded responsively and deliver drugs into desired sites. Polypeptides are commonly used to combine anticancer drugs with polymers or proteins to form drug-polymer conjugates. Seung-Ho Lim et al. [56] prepared the PEG-modified enzyme-stimulus responsive substrate-drug conjugates to target malignant glioma by using the MMP-12 substrate polypeptide to link PEG and DOX, suggesting the proliferation inhibition ability of PEG-peptide-ADR was superior to PEG-DOX through *in vitro* cytotoxicity experiments. Vivek Kumar Garripelli et al. [57] introduced MMP-2-sensitive polypeptides in thermal gel polymer carriers to increase the solubility and improve physicochemical properties of drugs.

Due to the biocompatibility and superior optical properties [58], a gold nanocarrier has been employed as an attractive vehicle for tumor targeting therapy. Mao Wei et al. [59] introduced gold nanoparticles (AuNPs) and MMP-2 cleavable peptides into gold nanoparticles clusters (AuNCs), which could enhance drug localization and improve micro computerized tomography in tumor theranostics. What's more, this strategy also exhibited high drug-loading rate and stability.

Previous studies have indicated that the smaller diameter of the nanoparticles is, the easier for them to penetrate tumor tissues. Recently, Han Min et al. [60] put forward a size-shrinkable drug delivery system based on MMP-2-sensitive HA end-conjugated poly (amidoamine) dendrimers. In the presence of MMP-2, macromolecules HA-pep-PAMAM (sensitive to MMP-2) showed a dramatic and fast size change from 200 nm to 10 nm (Fig. 1), furtherly confirming that the shrinking enzyme-responsive size of nanocarriers was a promising strategy, which could increase the effects on improving drug delivery and enhancing therapeutic efficacy *in vivo* and *in vitro*.

*4.2.1.2. Cathepsins*. Cathepsins are from a family of lysosomal cysteine proteases and have a strong association with tumor development [61]. The family has 11 members with molecular masses ranging from 20 to 35 kDa and can be divided into three different groups, including endopeptidases, aminodipeptidases and carboxymonopeptidases [62]. Especially, the cysteine and aspartyl cathepsin are often considered to be efficient targets usedful for designing prodrugs. In pathological conditions, active cathepsins are expressed on the cell surface and released to the extracellular space [63].

Cathepsin B (Cat-B) is mostly applied as a typical trigger for prodrugs, whose content and activity in various tumors is 3 to 9 times higher than normal tissues [64–68]. Generally, after synthesized and glycosylated in the endoplasmic reticulum and acidized in the Golgi apparatus, Cat-B is recognized by glycogen receptors on the lysosome surface and activated within the lysosome. However, due to the lack of recognition of glycogen, Cat-B secreted by tumor cells cannot be activated by the lysosome, which can only exist in the cytoplasm or outside cells in the form of an enzyme. Extracellular Cat-B involves in matrix degradation and tumor metastasis under the acidulous microenvironment of tumor tissues. To date, abnormal expression of Cat-B has been

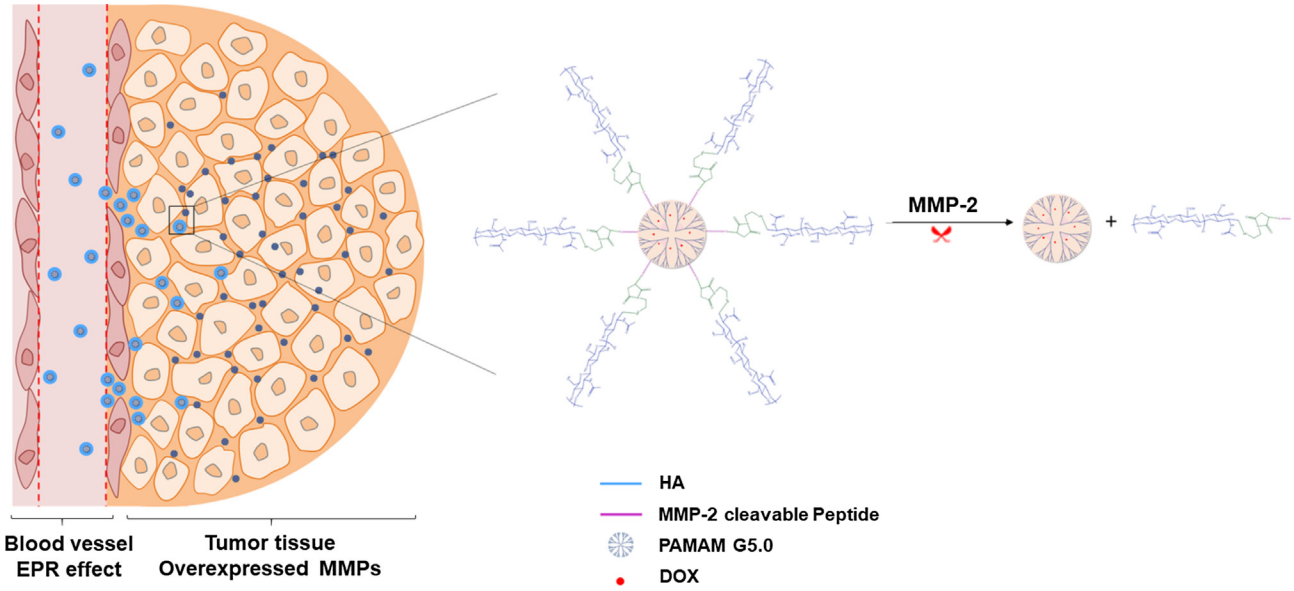


**Fig. 1.** Schematic illustration to show the size of size-shrinkable drug delivery system from 200 nm to 10 nm triggered by overexpressed MMP-2. Reprinted with permission from [60]. Copyright 2017 American Chemical Society.

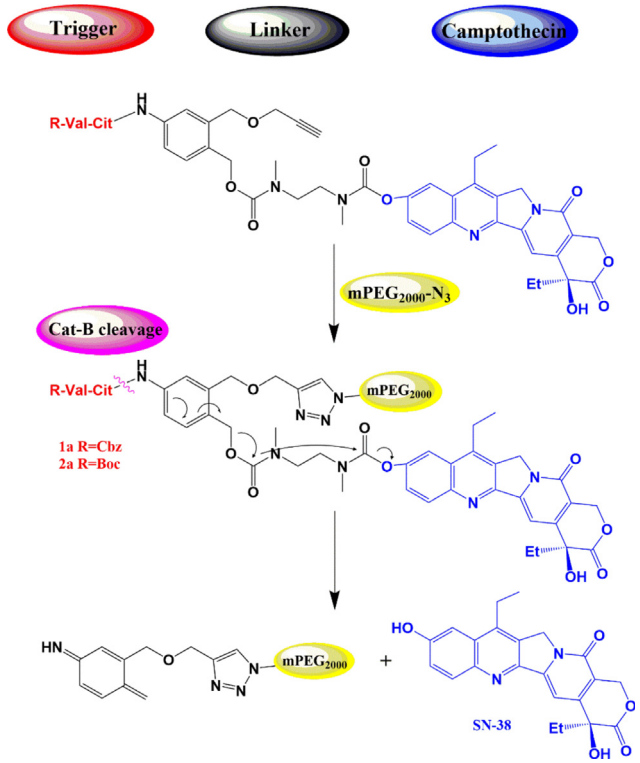


**Fig. 2.** Design of new Cat-B sensitive camptothecin loading nanoparticles equipped with a novel multifunctional linker. Reprinted with permission from [72]. Copyright 2016 American Chemical Society.

discovered in various cancer types. Besides, Cat-B has been reported to specifically hydrolyze Arg-Arg, Ala-Leu, Phe-Arg, Phe-Lys, Ala-Phe-Lys and Gly-Phe-Leu-Gly (GPLG) sequences [69,70]. Therefore, designing Cat-B-stimulus responsive nanoscale delivery systems also becomes a promising strategy in antitumor therapy. For example, the GPLG sequence was utilized in hydroxypropyl methacrylate (HPMA) copolymer conjugates to release drugs responsively in lysosomal compartments with the support of the cleavage between peptide spacer and Cat-B [71]. Furthermore, Zhang X et al. [72] introduced a Cat-B sensitive linker for synthesizing two Cat-B sensitive camptothecin conjugates (Fig. 2), indicating that the Cat-B sensitive linker could not only conjugate the camptothecin derivative, but also promote prodrugs to form biocompatible nanoparticles with enhanced water solubility.

*4.2.1.3. Urokinase.* Urokinase is a type of serine protease originally isolated from human urine, and it exists in the blood and in the extracellular matrix of many tissues. Urokinase is also known as urokinase plasminogen activator (uPA), which is encoded by the PLAU gene [73]. When recognized by its receptors on the cell surface, uPA will hydrolyze and activate serine protease plasminogen into plasmin. It is also found that uPA is able to participate in angiogenesis and tissue reconstruction during tumor invasion and metastasis [74], thus, uPA is often considered as an attractive drug target, and developing its inhibitors also brings a promising opportunity for antitumor therapy. P.K. Chakravarty et al. [36] combined the plasmin sensitive sequence, Val-Leu-Lys, with DOX to prepare the first plasminogen prodrug. B.L. Barthel et al. [37] combined the formaldehyde coupled DOX with the uPA sensitive sequence to prepare the prodrug, GaFK-DOX, which displayed excellent stability in plasma. Besides, GaFK-DOX could be hydrolyzed easily and released with a high dose of DOX, which significantly inhibited the tumor cells growth (average IC50 of 8 nM) (Fig. 3).

Recently, Gao N et al. [75] provided a novel strategy based on targeted theranostic nanoparticles to overcome the major obstacles in intraperitoneal chemotherapy. They found that intraperitoneal delivery of the uPA receptor targeted magnetic iron oxide nanoparticles (IONPs) could significantly enhance antitumor efficacy without obvious systemic toxicity.

*4.2.1.4. Prostate-specific antigen.* Prostate-specific antigen (PSA) is a type of serine protease and belongs to the kallikrein-related peptidase family, which is encoded by the KLK3 gene in humans, thus, also known as kallikrein-3. PSA is commonly expressed in the epithelial cells of the prostate gland and can allow sperm to swim freely by degrading concentrated pancreatic hormone in semen [76]. When interacted with a plasma protease inhibitor, such as α-trypsin and α2-macroglobulin, PSA will be inactivated, so active PSA only exists around the prostate cells. Previous studies have found that the blood concentration of PSA will increase in the presence of prostate cancer or other prostate disorders [77]. Therefore, PSA is also used as a

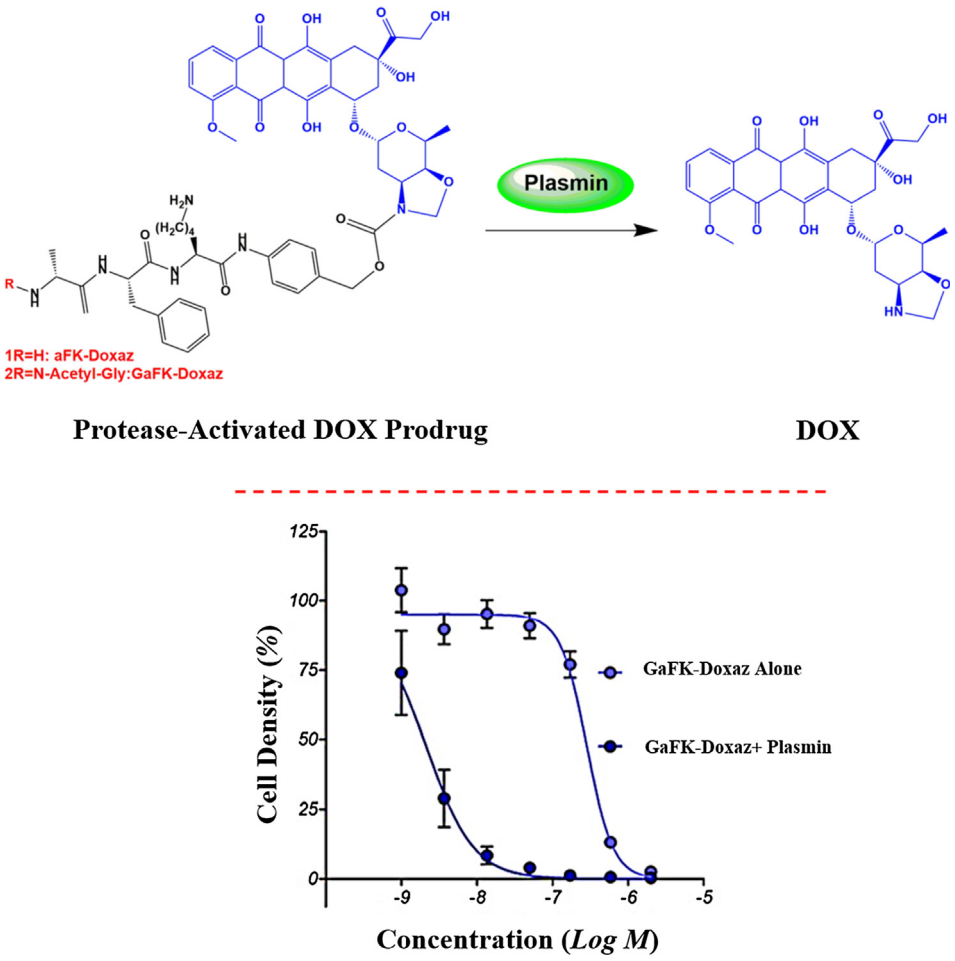


**Fig. 3.** Schematic illustration to demonstrate the progress of releasing DOX from the protease-activated prodrugs and powerfully inhibiting tumor growth (average IC50 of 8 nM). Reprinted with permission from [37]. Copyright 2012 American Chemical Society.

diagnostic strategy for serological diagnosis of prostate cancer [78]. So far, based on DOX [31], vinblastine [33], 5-floxuridine [34] and PTX [35], various prodrugs sensitive to PSA have been applied in targeted therapy for prostate cancer.

*4.2.1.5. Trypsin.* Trypsin is one of the most important digestive proteases from the PA clan superfamily, which can degrade dietary proteins into polypeptides and amino acids [79]. Radhakrishnan et al. prepared dual enzyme-stimulus responsive nanocapsules to load protamine and chondroitin, showing that the nanocapsules could be degradated responsively and deliver DOX into cervical tumor cells specifically [39].

*4.2.2. Phospholipases*

Phospholipases can hydrolyze phospholipids to fatty acids and other lipophilic substances, whose expression levels will increase in infectious disease and inflammation. Furthermore, as a part of the host defense mechanism, phospholipases can also overexpress in the invading peripheral region of tumors [80,81], which provide the potential for phospholipase to be designed as a stimulator in releasing drugs responsively. The phospholipase A2 (PLA2) family is the most studied, including secretory phospholipase A2 (sPLA2) and intracellular PLA2. Besides, the overexpressed PLA2 has been found in various disease conditions, such as cancer [82], immune disorders [83] and inflammatory tissue [84]. With the help of $Ca^{2+}$, PLA2 has the ability to hydrolyze cellular phospholipid membrane and other phospholipids, containing lipoprotein, pulmonary surfactant, microbial membrane and food composition. Andresen et al. [41] confirmed that long circulating liposomes could release drugs in large quantities under the functions of extracellular PLA2. Egeblad et al. [85] suggested that the effect of PLA2-stimulus responsive liposomes was 1.5 to 2 times higher than PEG-modified liposomes in inhibiting tumor growth. Kim et al. [86] put cyclodextrin on the surface of mesoporous silica nanoparticles with a polypeptide sequence (sensitive to phospholipase), which resulted in controlling the porous channels of nanoparticles. When the substrate degraded, cyclodextrin was removed from the surface to release drugs effectively. Recently, Christa L. Pawlowski et al. [87] utilized the vesicle destabilization triggered by clot-relevant PLA2 to develop platelet microparticle-inspired nanovesicles (PMINs), which were able to protect encapsulated thrombolytic drugs in circulation and avoid off-target uptake and function.

*4.2.3. Glycosidases*

Glycosidases, which are also known as glycoside hydrolases, can take part in the process of N-linked glycoproteins in the endoplasmic reticulum and Golgi apparatus, and also play a role in hydrolyzing carbohydrate structures in the lysosomes [88]. Recently, various glycosylated nanoparticles have been designed to release drugs selectively in desired tumor sites based on degrading in response to overexpressed glycosidases [89,90]. Bernardos A et al. [43] indicated that dye-loaded MSNPs could be encapsulated in pores more effectively with grafted molecules. In the presence of β-D-galactosidase, the sugar could be degraded to release the dye slowly. *In vivo* studies showed that starch-modified DOX nanoparticles could significantly reduce the cell survival rate. Jonathan Clarhaut J et al. [44] prepared the first β-galactosidase-responsive DOX-folate conjugates, which could selectively identify and

kill folate positive acute myelogenous leukemia blast cells without affecting normal endothelial cells, heart cells or blood cells from healthy donors. Recently, in order to meet the increasing demand for efficient drug delivery systems, Sergii Kolodych et al. [91] developed a novel type of β-galactosidase-cleavable linker for antibody-drug conjugates (ADCs) and synthesized a series of payloads, including galactoside linkers, monomethyl auristatin E (MMAE) and cysteine-reactive groups. When comnbined with galactoside linkers, ADCs also had advantages over trastuzumab that is a drug available on the market already for breast cancer treatment in mice model.

### 4.3. Oxidoreductases and the design of its stimulus-responsive nanoscale delivery systems

Oxidoreductases play an important role in oxidative stressby catalyzing the transfer of electrons from the reductant to the oxidant, which is also known as transferring from the electron donor to the electron acceptor [92]. Over the years, more attention has been given in employing oxidoreductases as therapeutic targets for disease treatment, such as Alzheimer's disease (AD) [93] and tumors [94].

#### 4.3.1. Azobenzene reductase

Azobenzene reductase, which is also known as azoreductase, can act on other nitrogenous compounds as donors by using $NAD^+$ or $NADP^+$ as acceptors. Due to the high expression in the human colon, azoreductase has been commonly applied in colon disease treatments. Rao et al. [45] prepared an azoreductase responsive assembled polymer by connecting azobenzene to an amphiphilic two-block copolymer via a covalent bond. In the presence of azoreductase and NADPH, the polymer could be degraded to release drugs specifically. Kopecek P et al. [95] obtained the azoreductase-responsive polymer with the support of copolymerization of HPMA and an azoreductase-sensitive 9-amino camptothecin prodrug monomer. It was hard to achieve the quick and efficient release of 9-amino camptothecin parent drug in the colon until the azo bonds were cracked via a series of intramolecular rearrangements. The *in vivo and in vitro* results furtherly confirmed the potential of this strategy in colon cancer therapy.

With the studies deepened, azoreductase-stimulus responsive nanoscale delivery systems can be also applied in other tumors. It has been found that azoreductase exists in the cytoplasm of liver cells. Medina et al. [46] prepared the azoreductase-stimulus responsive drug conjugate for hepatic cancer treatment based on linking DOX and PAMAM via an azo bond (Fig. 4). Due to the degradation of intracellular azoreductase, the conjugate could achieve targeted delivery of DOX and kill liver cells effectively. By comparison, the results of incubating nanocarriers and S9 cytoplasm isolated from rat cardiac myocytes suggested DOX was released in low amounts..

#### 4.3.2. Glutathione reductase

Glutathione reductase (GR), which acts as a key cellular antioxidant, can play an irreplaceable role in maintaining the normal oxidation level in humans [96]. Due to the excessive proliferation, tumor cells maintain long-term oxidative stress. In order to remove the large amount of oxidative substances produced in tumor cells, the content of GR increases much more than normal cells [97]. Recently, GR has been an attractive target for many pharmaceuticals. One typical strategy is that GR-sensitive chemical bonds are used to link the hydrophilic PEG with various hydrophobic chemical groups, and then prepare drug-loaded nanoparticles. After entering the tumor, GR-sensitive chemical bonds are broken responsively and achieve the rapid release of drugs [98].

#### 4.3.3. Peroxidase

Compared with other enzymes, there are much less studies releated to the use of peroxidase in stimulus-responsive drug delivery systems. Combining peroxidase with $H_2O_2$ to form strong oxidants can degrade

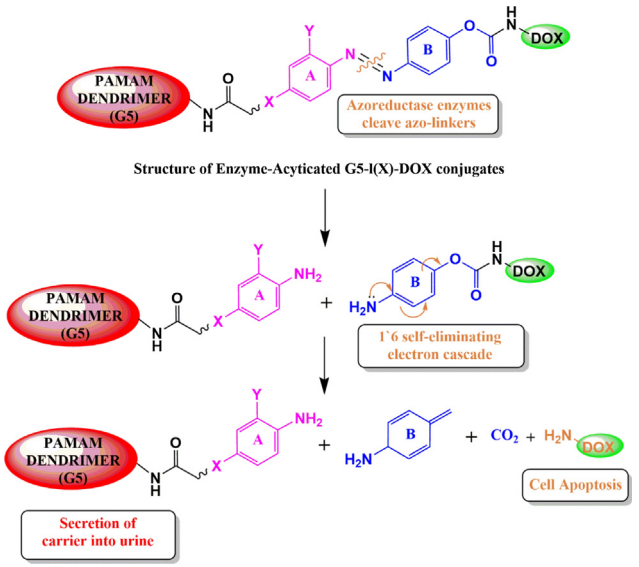


**Fig. 4.** Schematic drawing to show the degraded process of G5eL(x)e DOX conjugates. Reprinted with permission from [46]. Copyright 2012 Elsevier.

carbon nanomaterials [99,100]. Alexander Star et al. [47] adopted carbon nanotubes that were loaded with nitrogen as containers to package gold nanoparticles. After degrading the graphite cup shell by human myeloperoxidase (hMPO), the container would open effectively to release gold nanoparticles. Accordingly, the researcher delivered PTX to myeloid-derived suppressor cells (MDSC), which could inhibit the immunosuppressive potential of MDSC significantly.

## 5. Clinical progress in enzyme-stimulus responsive nanoscale delivery systems as anticancer drugs.

Metastasis will accelerate the dying process of tumor patients, which has become a worldwide challenge in the clinical antitumor treatment [51]. Therefore, it is extremely urgent to develop more effective drugs and novel therapeutic strategies in inhibiting tumor growth and metastasis. In the process of metastasis, enzymes of the tumor microenvironment can play an important role as regulatory molecules, such as MMPs. Herein, combined with related reports in recent years, we listed the typical antitumor drugs that have entered clinical trials in Table 2, in order to provide valuable references for the further studies. In this table, the drugs, responsive enzymes, cancer types, clinical trial stages and drug combinations are summarized.

## 6. Conclusion

Due to the higher selectivity and stronger therapeutic efficacy, but lower systemic toxicity, microenvironment-stimulus responsive nanoscale delivery systems triggered by the enzymes have attracted wide interest in antitumor therapy. Recent studies have furtherly confirmed that this strategy can exhibit more advantages in releasing drugs specifically and enhancing antitumor efficacy, compared to the conventional drug delivery systems. Therefore, we summarized and discussed the typical enzyme-stimulus responsive nanoscale delivery systems and their advancements in antitumor therapy, in order to offer the direction for further studies. Critically, because of the low, though not inconsiderable, expression levels in blood and normal tissues, these enzymes, such as MMPs, may also cause nanoparticles to degrade in advance and decrease the intracorporal circulation time, as well as affect normal tissues adversely. Multiple inhibitors of enzymes, such as MMPI, have been applied in antitumor therapy, while some inhibitors have poor clinical efficacy due to lack of selectivity. Thus, the degradation kinetics of enzyme-stimulus responsive nanoscale delivery systems need to

**Table 2**
Clinical progress in the enzymes of tumor microenvironment inhibitors as anticancer drugs [101].

| Drug names | Responsive enzymes | Cancer types | Clinical trial stages | Drug combinations |
|---|---|---|---|---|
| Ninlaro (ixazomib) [102] | Chymotrypsin | Multiple myeloma | Phase III | Lenalidomide + dexamethasone |
| Tanomastat [103] | MMP-9 | Ovarian carcinoma | Phase III | Paclitaxel, Platinum |
| Rebimastat (BMS-275291) [104] | MMP-1, -2, -3, -7, -9 and -14 | Non-small cell lung cancer | Phase III | Paclitaxel, Carboplatin |
| Disulfiram (DSF) [105] | MMP-2, -9 | Malignant melanoma, breast cancer, prostate cancer | Phase II | NA |
| Bisphosphonates (BPs) [106] | MMP-1, -2, -3, -7, -8, -9, -12, -13 and -14 | Breast cancer | Phase II | Zoledronic acid |
| Doxycycline hyclate [107] | MMP-2, -9 | Non-small cell lung cancer, Renal cell carcinoma | Phase II<br>Phase II | Interferon-α<br>Erlotinib |
| Neovastat (AE-941) [108] | MMP-2, -9, -12 | Non-small cell lung cancer | Phase III | NA |
| Genistein [109] | MMP-2, -9 | Breast cancer | Phase I | NA |

NA: not application.

respond in tumor sites specifically. Additionally, another problem is the expression levels of enzymes are different dependent on tumor sites, tumor types, tumor progress and even different patients. In conclusion, in order to find more accurate treatments and applications in clinical practice, designing enzyme-stimulus responsive nanoscale delivery systems requires researchers to not only sufficiently understand the *in vivo* distribution, expression levels and activities of targeted enzymes, but also consider the specificity of the pathological conditions.

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## Disclosure

The authors declare they have no conflicts of interest.

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