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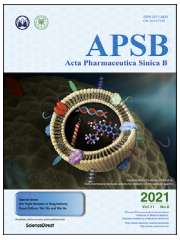

REVIEW

Protease-triggered bioresponsive drug delivery for the targeted theranostics of malignancy

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Malignancy

Abstract Proteases have a fundamental role in maintaining physiological homeostasis, but their dysregulation results in severe activity imbalance and pathological conditions, including cancer onset, progression, invasion, and metastasis. This striking importance plus superior biological recognition and catalytic performance of proteases, combining with the excellent physicochemical characteristics of nanomaterials, results in enzyme-activated nano-drug delivery systems (nanoDDS) that perform theranostic functions in highly specific response to the tumor phenotype stimulus. In the tutorial review, the key advances of protease-responsive nanoDDS in the specific diagnosis and targeted treatment for malignancies are emphatically classified according to the effector biomolecule types, on the premise of summarizing the structure and function of each protease. Subsequently, the incomplete matching and recognition between enzyme and substrate, structural design complexity, volume production, and toxicological issues related to the nanocomposites are highlighted to clarify the direction of efforts in nanotheranostics. This will facilitate the promotion of nanotechnology in the management of malignant tumors.

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1. Introduction

Despite remarkable progress in the prevention, diagnosis, treatment, and surveillance over the years, cancer remains a serious threat to human health and quality of life. Its morbidity and mortality are increasing year by year worldwide[1], especially in China[2]. Thus, more challenging theranostic methods are required to address this human public health impact. Currently, surgery remains the preferred option for the vast majority of solid tumors. Even so, limitations such as tumor accessibility, pathological complexity, preoperative spread, and metastasis make surgical resection inapplicable to all solid tumors[3]. Fittingly, chemotherapy interventions have emerged as standalone regimens or as an adjunct to other focal treatments, like surgery or radiotherapy[4]. Although a series of cytotoxic chemotherapeutics have become the first choice in clinical treatment against many kinds of cancer and have achieved significant progress, the toxicity to normal tissues, non-targeting, and the occurrence of drug resistance limit their clinical efficacy and application.

With research intensifying on the biological and pharmacological mechanisms of cancer at molecular level, a variety of molecular targeted drugs, gene remediation molecules, and immune microenvironment regulators have emerged to direct tumor signal transduction disturbances and metabolic abnormalities[5–8]. These novel drug models exhibit greater selectivity, targeting, histocompatibility, and therapeutic index than traditional toxic therapeutants, and they further improve the host's immune strength to remove tumor cells and resist foreign carcinogens. Despite some success, the identification complexity of carcinogenic and immunomodulatory targets, anchored by drugs, the instability, diversity, and mutation susceptibility of proto-oncogenes, greatly limit the progress and application of targeted treatment due to scanty drug targeting and concentration enrichment at the lesion[9–11].

To address this limitation, an alternative strategy is to integrate nanotechnology and stimulus responsiveness of tumor phenotype rather than intracellular signaling pathways, such as differential tumor-normal tissue physicochemical characteristics[12–15], distinct vascular structures[16], and altered enzyme expression which is highly active in tumors and less active in normal organs[17]. Among these, proteases, with intrinsic high specificity, strong catalytic activity, mild reaction conditions, significant expression and activity distribution in tumors, have been developed not merely as detective/prognostic biomarkers[18,19] or targets of molecular targeted therapy[20–22], but also be used in the intelligent presentation of bioactive substances by constructing nano-drug delivery systems (nanoDDS)[23–28].

The expanding understanding of cancer pathology and nanomedicine has outlined the prosperity of enzyme-stimulated drug delivery in cancer management. This review begins with a classified introduction to the structure and function of various tumor-related proteases based on the catalytic mechanism for substrate cleavage. Emphatically, the latest advances in protease-triggered nanoDDS in the targeted diagnosis and treatment of malignancies are discussed in terms of their classification criteria. In addition, some key obstacles such as the construction, scale production, and toxicity effect of nanocomposites, as well as the efficient matching of enzyme/substrate have been concerned to prospect the clinical potency of protease-responsive nanotheranostics.

2. Proteases in cancer

As proteolytic enzymes, proteases irreversibly catalyze the hydrolysis of peptide bonds, leading to protein disintegration in microseconds. About 588 proteases in humans are encoded by 7% of the protein-coding genome and are classified into five major categories according to their catalytic mechanism and decreasing order of abundance in the human body: the metallo-, serine, cysteine, threonine, and aspartic family of proteases[29,30]. These proteases cleave peptide bonds in different ways. Serine, cysteine, and threonine proteases covalently catalyze the lysing of peptide bonds through active side chains in the cleft. By comparison, metallo- and aspartic proteases hydrolyze peptide bonds by noncovalently forming highly active water molecules. Serving as natural "Swiss Army Knife", they can degrade proteins both spectrally and specifically to activate or passivate the substrates, thus playing an important role in multiple physiological events such as cell differentiation, cycle continuation, cell migration, hemostasis, tissue remodeling, immune response, wound healing, and programmed cell death[31]. However, their dysregulation can also lead to a wide range of diseases such as Alzheimer's disease[32], inflammation[33], and especially cancer[34]. Therefore, clarifying the structure and function of proteases and their hydrolysis mechanism is a prerequisite for analyzing them as therapeutic targets and theranostic responders, which is summarized in Table 1.

3. Protease-responsive nanoDDS for the targeted theranostics of malignancy

As the name implies, nanomedicine is the application of nanotechnology in the medical arena, that is, nanomaterials or devices are used in the diagnosis, treatment, monitoring, and prognosis assessment of diseases[35]. As the cornerstone of nanomedicine, varied nanomaterials have been developed as carriers to support diverse bioactive compounds such as hydrophilic or hydrophobic drugs, peptides, nucleic acids, proteins and so on[36–40]. Synergistic utilization of the passive targeting EPR effect (enhanced permeability and retention) determined by the irregular reticular structure of tumor vasculature and active targeting induced by highly expressed specific receptors/antigens on the tumor cell surface, physically encapsulated or chemically coupled drug-carrying nanosystems have shown significant advantages in cancer theranostics[41–44]. NanoDDS have multiple superiorities such as matrix diversity, flexibility of surface modification, improved stability and solubility, reduced drug leakage and immature biological effects, enhanced lesion/tissue absorption ratio, controlled pharmacodynamics and pharmacokinetics, minimized DDS-derived immunogenicity, and reasonable multicomponent co-loading capacity, which are closely related to the multicomponent dosing regimens, avoidance of drug resistance, enhanced therapeutic effect, improved patient compliance and life quality[45–48].

Considering the important roles of proteases in cancer pathology, a range of enzyme-responsive nanopreparations with multiple functional modules have been developed to provide advantages in optimizing the *in vivo* distribution of bioactive agents, improving diagnostic sensitivity, broadening the therapeutic window, and enhancing the synergy between diagnosis and treatment. In the following sections, progress in the construction of protease-induced nanovectors and their applications in targeted cancer theranostics in recent five years will be systematically discussed, along with the aforementioned classification criteria for proteases.

3.1. Matrix metalloproteinases-responsive nanoDDS for cancer theranostics

Matrix metalloproteinases (MMPs) represent a family of Zn^{2+}- and Ca^{2+}-dependent endopeptidases responsible for the

Table 1 Summary of tumor-related proteases and their characteristics.

Protease	Classification	Property	Function
MMPs	Gelatinases Matrilysins Archetypal MMPs Furin-activatable MMPs	The enzymatic potency of MMPs undergoes three processes: the synthesis of inactive pre-proenzymes, the removal of signal peptides to produce proenzymes, and the cleavage of the bridge structure leading to enzyme activation	Physiological activities: angiogenesis, wound healing, embryonic development, and tissue remodeling Pathological features: tumorigenesis, migration, invasion and metastasis
Serine proteases	uPA	uPA is secreted in a non-activated pro-uPA form and activated by binding to uPAR, and then it cleaves plasminogen into plasmin that exerts serine protease activity directly or reciprocally activates pro-uPA to uPA in a positive feedback loop	Induce ECM degradation and tissue remodeling Promote tumor growth, progression, and migration
	PSA	Active PSA is only localized near the prostate cells. It specifically recognizes and rapidly cleaves heptapeptide sequence based on semenogelin	Induce ECM degradation and tissue remodeling Promote tumor development
	Thrombin	It converts soluble fibrinogen into insoluble fibrin and increases malignant cell adhesion	Promote tumorigenesis, progression, and metastasis
Cysteine proteases	Cathepsins	They are lysosomal proteases sharing a common papain-like fold containing three α-helix domains and a β-barrel domain except for cathepsin C, as well as they show a tissue-specific distribution	Participate in antigen presentation, thyroid hormone liberation, epidermal homeostasis, precursor activation, keratinocyte differentiation, cell apoptosis, and bone remodelling Promote ECM degradation and tumor progression and metastasis
	Legumain	Legumain is synthesized as an inactive zymogen and then be catalytically activated in an acidic lysosome	Stimulate angiogenesis, enhance tumor progression and metastasis
Threonine proteases	Testes-specific protease 50	Its catalytic triplet is located near the opening of the pocket consisting of two sets of β sheets, which facilitates the substrate peptides to approach the threonine catalytic site	Participate in multiple cellular physiological processes Promote cell proliferation, stimulate tumor formation and progression
	Threonine aspartase 1	It is a "non-oncogene addiction" protease to crack the main regulator of mixed leukemic proteins and other human regulatory proteins	Be involved in head morphogenesis, segment recognition, spermatogenesis, and proliferation Affect cell proliferation, transformation, cycle progression, and tumor development
Aspartic proteases	Cathepsin D Cathepsin E Memapsin	They are synthesized as inactive zymogen progenitors and convert to mature active proteases under acidic pH without the assistance of other catalytic molecules.	Perform a series of physiological functions Promote cancer initiation and progression

MMPs, matrix metalloproteinases; uPA, urokinase plasminogen activator; uPAR, uPA receptor; ECM, extracellular matrix; PSA, prostate specific antigen.

degradation of extracellular matrix (ECM) components including collagen, laminin, fibronectin, elastin, and the basal lamina[49]. The enzyme potency of MMPs originates from a non-activated bridge structure between an unpaired cysteine in the highly conserved "Pro-Arg-Cys-Gly-X-Pro-Asp" sequence of the protease prodomain and the catalytic zinc. After breaking this bridge through proteolysis or chemical disruption, MMPs are activated for enzyme functions[50]. MMPs are associated with a variety of cellular physiological pathways and are defined as important regulators of tumorigenesis, progression, invasion, and metastasis. Among the 24 known MMPs, MMP-2, MMP-9, and MMP-14 are typically overexpressed in almost all types of cancer and are correlated to tumor invasion through degradation of collagen IV, laminin 5, and elastin[51–53]. Therefore, developing smart nanoDDS using MMP-2, MMP-9 or MMP-14 as stimulus has huge potential in cancer treatment and will be intensively evaluated below.

3.1.1. *MMP-2-triggered drug delivery*

The strategy of functionalizing nanoDDS with specific substrates for the recognition of MMP-2 that are disorderly upregulated in tumor tissues has been recognized and approved to activate the bioactive components to improve theranostic performance. These intelligent systems are classified as follows in terms of their diagnostic and therapeutic modalities.

3.1.1.1. MMP-2-triggered cancer chemotherapy. Fay et al.[54] explored the cell-targeting specificity of MMP-2 sensitive surface-converting polyethylene glycol (PEG) coatings using a variety of detection methods such as optical, nuclear, and magnetic resonance imaging (MRI) techniques. Chakroun et al.[55] designed asymmetric reverse bolaamphiphile (RBA) supramolecular self-assemblies identified by tumor-specific MMP-2 for hydrogel degradation, to release anticarcinogens. Thereafter, considerable basic and clinical breakthroughs have been made in enzyme-responsive cancer chemotherapy, based on various favored chemotherapeutic agents.

Mesoporous silica nanoparticles (MSNs) are promising candidates for a new generation of nano delivery carriers. A PEG decorated folic acid (FA)-targeted MMP-2-triggered MSN nanocarrier, PGFMSN, was obtained for programmed doxorubicin (DOX) release by sequential encounters with MMP-2 recognition, FA targeting, and gelatin layer disengagement[56]. A gold (Au) nanoparticles (Au NPs)-biotin conjugate with an MMP-2-cleavable linker capped MSN was designed as a pH- and enzyme-responsive nanocarrier for controlled DOX delivery with excellent tumor destruction and minimal side effects[57]. Zhang et al.[58] proposed an envelope-type MSN to achieve hyaluronic acid (HA) receptor-mediated cell targeting and MMP-2/hyaluronidases bienzyme-responsive DOX release. Further, a pH/redox/MMP-2 triple sensitive nanohybrids based on DOX preloaded and responders assembled MSN exhibited enhanced intracellular DOX delivery and improved anticancer cytotoxicity[59].

NanoDDS loading DOX with other carriers have also emerged in recent years. Representatively, Chen et al.[60] prepared a biotin-PEG-*b*-poly(L-lysine, PLL)-peptide-DOX polymeric micelles to realize receptor-enhanced cell uptake and MMP-2-stimulated drug release for targeted tumor clearance. An MMP-2/ROS (reactive oxygen species) dual-sensitive DOX-encapsulated poly(L-methionine-*b*-L-lysine)-PLGLAG-PEG (MLMP) micelle was designed to exert MMP-2-urged bond breaks and ROS-driven DOX release, causing enhanced tumor inhibition with ideal targetability and *in vivo* circulation[61]. By coupling small dendrimer poly-L-lysine (DGL) with PEG-poly(caprolactone) micelles *via* an MMP-2-activated peptide, a size-adjustable nanosystem, DGL/DOX@PP was obtained to perform protease-induced thorough tumor permeability and tumor microenvironment (TME) regulation[62]. A well-defined block copolymer, PEG-GPLGVRGDG-P(BLA-*co*-Asp, PBLA: poly(β-benzyl L-aspartate)), was obtained *via* click reaction to encapsulate DOX for MMP-2-induced peptide cleavage, the dePEGylation of polymer carriers, and the exposure of RGD on the surface of nanoDDS, bringing about ligand-mediated endocytosis, increased drug release and deep penetration for effective therapeutic potency (Fig. 1)[63]. Another size-variable enzyme-responsive poly(amidoamine, PAMAM) dendritic molecules conjugated with HA were synthesized by click chemical reaction. When MMP-2 was encountered, a significant reduction in size from ~200 nm to ~10 nm promoted nanoconjugates extravasation, accumulation, diffusion, and penetration into solid tumors for excellent anticancer efficacy[64]. In another construction, DOX was covalently linked with a 12-amino-acid anti-HER2 peptide mimetic YCDGFYACYMDV-NH_2 (AHNP) to form an MMP-2 sensitive nanoconjugate for synergistic chemotherapy and biotherapy of HER2-positive breast cancer[65]. Apart from these, DOX-based nanoscale multidrug systems have also been developed to enable coordinated treatment of cancer, such as chemophototherapy[66,67].

Paclitaxel (PTX) is a recognized first-line anticancer star, but poor solubility, severe off-target toxicity, and annoying drug resistance still limit its clinical application. Nanotechnology plays an important role in drug delivery, especially introducing specific markers for tumor-activated therapeutic release. As a typical example, trilayer nanocapsules cross-linked by an oil-core based on a monodisperse oil-in-water nanoemulsion preloaded with PTX and an MMP-2-sensitive shell, were proposed for protease-triggered and tumor-selective drug delivery[68]. An enzyme-responsive triblock copolymer vesicle, PEG-GPLGVRG-*b*-poly(ε-caprolactone)-*b*-poly(3-guanidinopropyl methacrylamide, PEG-GPLGVRG-PCL-PGPMA), was demonstrated with asymmetrically distributed cell-penetrating PGPMA segments (91% inside and 9% outside). Upon entry into the MMP-2 environment, peptide linker breakage led to the dePEGylation of nanocarriers and the redistribution of PGPMA with 76% exposed to the carrier surface for enhanced cytotoxicity[69]. MMP-2-sensitive copolymers based on poly(lactic-*co*-glycolic acid, PLGA) and D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) were formed to present PTX for sensitive MMP-2 induction and enhanced antitumor potency[70]. Clinically approved PEG-*b*-poly(D,L-lactide, PEG-PDLLA), an amphiphilic block copolymer, was attached to MMP-2-cleavable peptide that facilitated the vector dePEGylation and cellular absorption, prolonged blood circulation, and improved antitumor activity[71]. Other multifunctional collaborative nanosystems, such as "all-in-one" polymer-lipid conjugate micelle[72], PTX@celecoxib core-shell nanoarchitecture (Fig. 2)[73], pH-sensitive PTX@sunitinib core-shell micelle[74], PTX/losartan in an amphiphilic gelatin matrix[75], PTX/2-acetylpyridine-4,4-dimethyl-3-thiosemicarbazone-copper(II) combination[76] and so on, have also been exploited for MMP-2-dependent on-demand programmed site-specific drug delivery and synergistic tumor-targeted treatment.

In addition, many other chemotherapeutics-derived nanoDDS, for example gemcitabine (GEM)[77,78], coumarin 6[27], trichosanthin[79], methotrexate (MTX)[80], pirfenidone[81], pirarubicin[82], cabazitaxel[83], and SN38[84], have also made extensive biomedical advances as stand-alone or multidrug combinations.

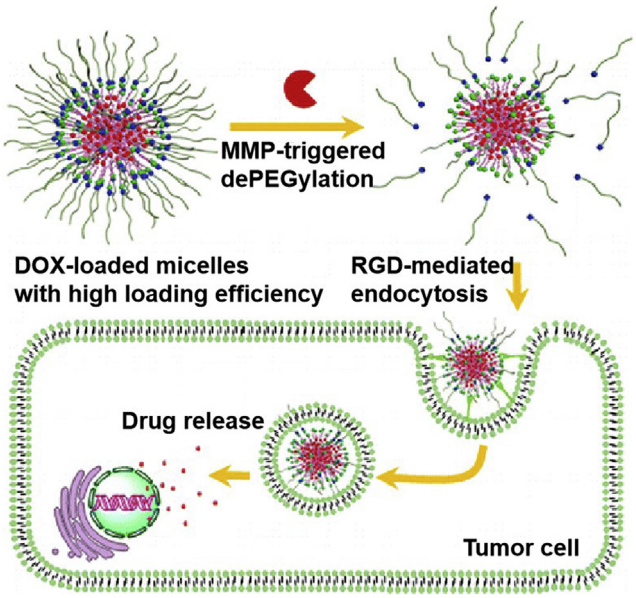

Figure 1 Schematic illustration of MMP-2-responsive dePEGylation and RGD-enhanced endocytosis of DOX-carried block copolymer micelles for targeted drug delivery and cell killing. Reprinted with the permission from Ref. 63. Copyright © 2016 American Chemical Society.

Multidrug resistance is a key stumbling block to the clinical progress of chemotherapeutic drugs. To solve this problem, a series of targeted measures have emerged, which mainly include synthesizing nanomaterials that can reverse drug resistance[85–88], encapsulating drugs into enzyme-responsive organelle-directed nanocarriers (Fig. 3)[89], or other size-controllable nanoconjugates[90]. Furthermore, some actively targeted and intelligently size-shrinking nanoplatforms have been constructed for accomplishing high accumulation, deep penetration, strong targeting, and improved efficiency in a variety of tumor types[91–95]. In order to overcome the premature release of physically encapsulated nanodrugs before they reach the tumor site, polymeric carrier-based prodrugs were created by conjugating therapeutics with the targeted group or protease-responsive peptide sequence to prolong blood circulation, improve tumor targetability, enhance cell internalization and intracellular distribution, and potentiate anticancer chemotherapy[23,96–98].

3.1.1.2. MMP-2-triggered cancer phototherapy. Photodynamic therapy (PDT) is a safe, effective and low damage clinical regimen, while the dark toxicity, poor water solubility, unsatisfactory half-life (<40 ns) and diffusion depth (<20 nm) of ROS in the living environment greatly limit its antitumor efficiency. To improve drug efficacy, multifarious intelligently responsive vehicles have been proposed for targeted deep delivery of photosensitizers, especially nanoDDS that can be sensitized by tumor-specific proteases. Representatively, a conjugate composed of MMP-2-activated peptide, cell penetrating peptide (CPP) and photosensitizer protoporphyrin (PpIX, Fig. 4)[99], and MMP-2/pH dual-responsive photosensitizer nano-prodrugs[100], were designed for purposeful drug release and tumor-targeted treatment.

3.1.1.3. MMP-2-triggered cancer gene therapy. Gene therapy has great anticancer potential by inserting therapeutic genes into plasmid vectors and delivering them to tumor lesions. Unsatisfactory *in vivo* efficacy limits the clinical transformation of widely used gene carriers such as lipoplex and polyplex. Against this disadvantage, nanotechnology has been skillfully used to construct various therapeutic gene delivery systems, typically such as PEG shielded MMP sensitive CPPs[101], envelope-type non-viral nanodevice for liver-targeting siRNA delivery[102], polyethylenimine (PEI)-based chlorotoxin-targeted melittin gene delivery to MMP-2 positive prostate cancer cells[103], MMP-2-cleavable PEG-β-cyclodextrin-PEI nanoconjugates for tumor suppressor microRNA miR-34a-induced breast cancer treatment[104], MMP-2-cleavable motif-included short amphiphilic peptide hydrogels for cell-demanded remedial peptide release into cervical cancer cells[105], MMP-2-sensitive cytotoxic peptide–dendrimer conjugates for enhanced intracellular enrichment, deep tumor penetration, promoted cell apoptosis and anti-glioblastoma (GBM) performance[106], multifunctional fusion proteins for targeting the release of interferon gamma[107], pro-apoptotic peptide[108], or cytotoxic enediyne[109] into MMP-2-dysregulated tumor tissues. Moreover, some promising nanocomplexes were also developed for the co-delivery of heat shock protein 70-specific siRNA (siHSP70) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)[110], sequentially responsive dual-targeted peptide presentation[111], MMP-2/glutathione (GSH) dual-stimulated DOX/miRNA-34a co-delivery to tumor mass[112], and MMP-2-triggered on-demand submission of neurotrophic growth factors (NTFs) to induce neural differentiation[113].

3.1.1.4. MMP-2-triggered cancer theranostics. Multipurpose all-in-one nanocomposites have attracted great enthusiasm for their opportunities to synergistically achieve sensitive diagnosis, targeted therapy, efficacy monitoring, and prognosis assessment of cancer. Many types of MMP-2-responsive nanotheranostics emerge at the right moment. For instance, DOX/AuNPs/quantum dots (QDs) nanoclusters for fluorescence imaging (FI)-mediated anticancer treatment[114], DOX-gelatin/epigallocatechin gallate/AuNPs nanoconjugates for FI-cooperated prostate cancer inhibition[115], pH-responsive Au nanoclusters for synergistic near-infrared (NIR) FI and chemo-PDT of MMP-2 positive lung cancer[116], MSN loaded with fluorescent and targeting peptides for FI-triggered tumor therapy[117], dual-modal photoacoustic (PA)/NIR FI-enhanced photothermal therapy (PTT)/PDT for lung cancer[118], and other theranostic nanosystems driven by additional optical principles including aggregation-induced emission (AIE)[119] and fluorescence resonance energy transfer (FRET)[120]. MSN- or 3D-printed biodegradable microswimmer-based magnetic nanoagents were designed for *in vivo* MMP-2-induced drug release and magnetic resonance imaging (MRI)[121,122]. DOX-encapsulated gold nanoclusters were presented for computed tomography (CT) imaging-visualized cancer chemotherapy[123]. The above MMP-2-encouraged representative achievements in cancer theranostics are summarized in Table 2[23,56–123]. In another work, chlorotoxin-conjugated radionuclide ^{131}I-labeled dendrimers were reported for CT-mediated cancer radiotherapy[124].

In addition, multiple MMP-2-activated nanocarriers have also been explored for other diagnostic and therapeutic applications, representative examples including the dual-targeting of the blood brain barrier (BBB) and glioma using MMP-2-responsive micelles that act as gene presentation vectors, to enhance the radiotherapy sensitivity of glioma[125], genetically-engineered attenuated *Salmonella* Typhimurium as anti-invasive vectors for the targeted

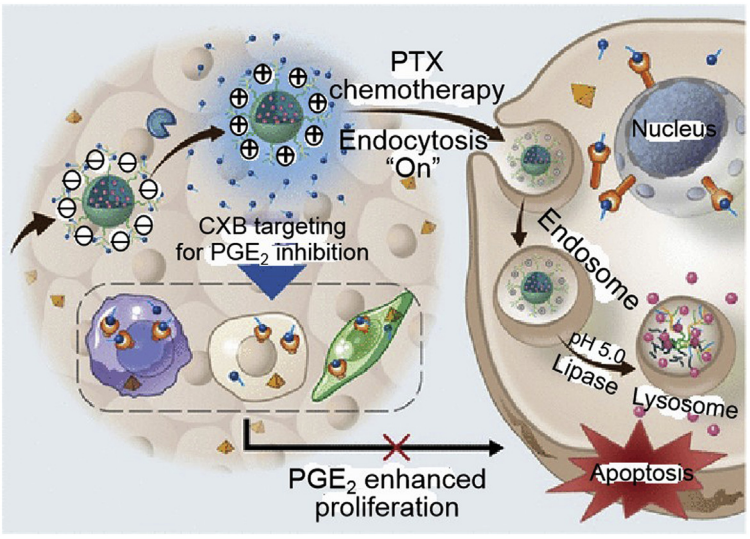

Figure 2 *In vivo* behavior of MMP-2-responsive nanoparticles loading PTX and anti-inflammatory celecoxib (CXB). Reprinted with the permission from Ref. 73. Copyright © 2019 American Chemical Society.

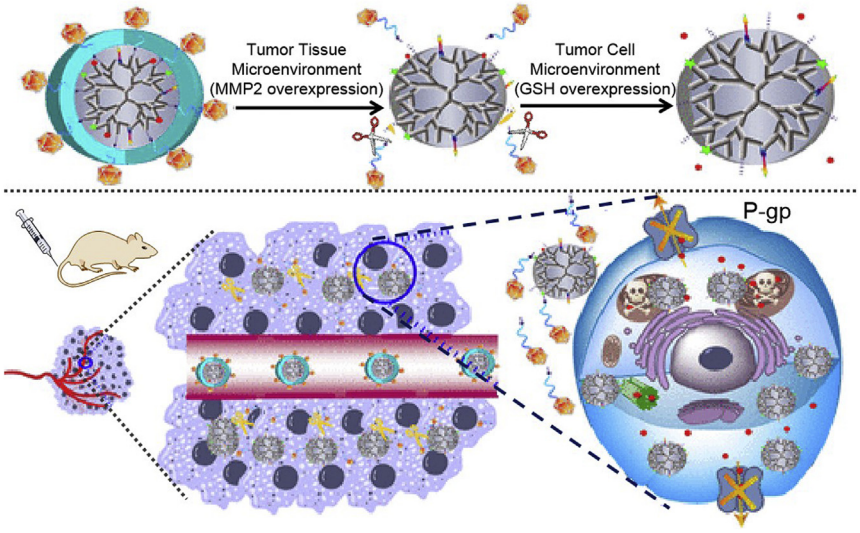

Figure 3 The mechanism of nanoconjugates targeting organelles with MMP-2/GSH dual responsiveness and overcoming drug resistance. Reprinted with the permission from Ref. 89. Copyright © 2018 American Chemical Society.

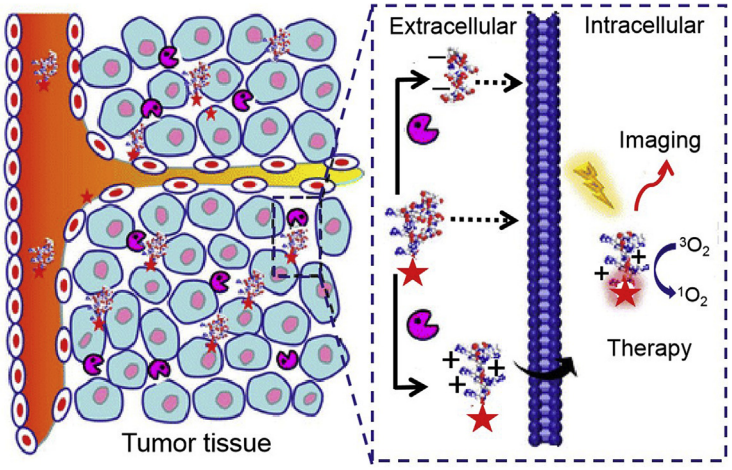

Figure 4 Action pathway of MMP-2-activated ACPP-PpIX nanocomposites for tumor imaging-synergized photodynamic therapy. Reprinted with the permission from Ref. 100. Copyright © 2015 American Chemical Society.

Table 2 Illustrative achievements of MMP-2-responsive nanoDDS and their applications in the targeted theranostics of malignancies.

Nanocarrier	Substrate	Drug	Diagnosis	Therapy	Tumor	Ref.
MSN-FA@Gelatin-PEG	Gelatin	DOX	–	Chemotherapy	HT29 human CRC cells	56
Au-biotin-peptide-MSN	KDPLGVC	DOX	FI	Chemotherapy	4T1 mouse breast cancer cells T47D human breast cancer cells	57
MSN@HA-gelatin-PEG	Gelatin, HA	DOX	–	Chemotherapy	MDA-MB-231 human breast cancer cells	58
MSN-gelatin-BAC	Gelatin	DOX	–	Chemotherapy	A549 human non-small cell lung cancer cells	59
Biotin-PEG-*b*-PLL-peptide-DOX polymeric micelles	CPLGLAGG	DOX	FI	Chemotherapy	SCC-7 mouse squamous cell cancer cells	60
poly(L-methionine-block-L-lysine)-PLGLAG-PEG	PLGLAG	DOX	–	Chemotherapy	NCI-H460 human lung cancer cells HT1080 human fibrosarcoma cells	61
PEG-GPLGVRGDG-P(BLA-*co*-Asp)	GPLGVRGDG	DOX	–	Chemotherapy	4T1 mouse breast cancer cells HT1080 human fibrosarcoma cells	63
Oil-in-water nanoemulsion	GPLGIAGQ	PTX	–	Chemotherapy	U87 human glioblastoma cells	68
PEG-GPLGVRG-PCL-PGPMA	GPLGVRG	PTX	–	Chemotherapy	HT1080 human fibrosarcoma cells	69
$TPGS_{3350}$-pp-PLGA	GPLGIAGQ	PTX	–	Chemotherapy	MCF-7 human breast cancer cells	70
PEG-GPLGVRGDG-PDLLA	GPLGVRGDG	PTX	–	Chemotherapy	4T1 mouse breast cancer cells H22 mouse hepatoma cells	71
R_9GPLGLAGE$_8$	GPLGLAG	PpIX	FI	PDT	SCC-7 mouse squamous cell carcinoma cells HT1080 human fibrosarcoma cells	99
Au-R_8-PLGLAG-EK_{10}	PLGLAG	ALA	FI	PDT	SCC-7 mouse squamous cell carcinoma cells	100
PEI-SPDP-chlorotoxin	Chlorotoxin	melittin gene	–	Gene therapy	PC-3 human prostate cancer cells NIH3T3 mouse embryonic fibroblasts	103
CD-PEI-GPLGIAGQ-PEG2000	GPLGIAGQ	miR-34a	–	Gene therapy	4T1 mouse breast cancer cells	104
Ac-I_3SLKG-NH_2 hydrogel	I_3SLKG	G(IIKK)$_3$I-NH_2	–	Gene therapy	HeLa human cervical cancer cells	105
PKT-*S*-PEG	GPLGIAGQ	KLAK	–	Gene therapy	U87 human glioblastoma cells	106
Nanoclusters containing DOX/azide@AuNP and Alkyne-MMP@QDs	CVGLPGD	DOX	FI	Chemotherapy	A549 human non-small cell lung cancer cells NIH3T3 mouse embryonic fibroblasts	114
Ce6-DOX-Au nanoclusters-MMP-2 polypeptide NPs	CPLGVRGRGDS	DOX, Ce6	NIR FI	Chemotherapy, PDT	A549 human non-small cell lung cancer cells	116
Au nanostars@BSA/I-MMP-2	GPLGIAGQ	IR-780 iodide	PA, NIR FI	PTT, PDT	A549 human non-small cell lung cancer cells	118
peptide-Fe_3O_4@MSN	PLGVR	Fe_3O_4, DOX	FI, MRI	Chemotherapy	HT1080 human fibrosarcoma cells NIH3T3 mouse embryonic fibroblasts	121
DOX@AuNCs	CRVGLPDC	DOX	μCT	Chemotherapy	A549 human non-small cell lung cancer cells	123

MSN, mesoporous silica nanoparticle; PEG, polyethylene glycol; PLL, poly(L-lysine); PBLA, poly(*β*-benzyl L-aspartate); PCL, poly(*ε*-caprolactone); PGPMA, poly(3-guanidinopropyl methacrylamide); TPGS, D-*α*-tocopheryl polyethylene glycol 1000 succinate; PLGA, poly (lactic-*co*-glycolic acid); PDLLA, poly(D,L-lactide); R_9, polycationic cell-penetrating peptide (CPP); E_8, polyanionic peptide; R_8, cationic CPP RRRRRRRR; EK_{10}, zwitterionic stealth peptide EKEKEKEKEKEKEKEKEKEK; PEI, polyethylenimine; SPDP, *N*-succinimidyl 3-(2-pyridyldithio) propionate; CD, *β*-cyclodextrin; PKT-S-PEG, KLAK, TAT (CPP) and MMP-2-sensitive peptide-PEG were conjugated onto dendrimers; BSA, bovine serum albumin; AuNCs, gold nanoparticle clusters; DOX, doxorubicin; PTX, paclitaxel; PpIX, protoporphyrin; ALA, 5-aminolevulinic acid; G(IIKK)$_3$I-NH_2, anticancer peptide; KLAK, cytotoxic peptide; Ce6, chlorin e6; Au, gold; NP, nanoparticle; QDs, quantum dots; FA, folic acid; HA, hyaluronic acid; BAC, *N*,*N*′-bis(acryloyl)cystamine; MMP-2, matrix metalloproteinase-2; PDT, photodynamic therapy; PTT, photothermal therapy; FI, fluorescence imaging; NIR, near-infrared; PA, photoacoustic imaging; MRI, magnetic resonance imaging; μCT, micro computerized tomography; CRC, colorectal cancer; –, not applicable.

therapy of orthotopic glioma[126], MMP-2/folate receptor (FR) dual-targeted polymeric micelles to improve tumor targeting, enhance anticancer efficacy, and overcome the resistance to molecular targeted therapeutics (Fig. 5)[127], and finally MMP-2-sensitive PEG hydrogel loaded with quinacrine sensitizing GBM cells to TRAIL for improved combination treatment efficiency[128].

3.1.2. *MMP-9-triggered drug delivery*

MMP-9, another gelatinase, is overexpressed in almost all known tumor entities. Its expression is directly related to the malignancy of tumors, thus opportunities for MMP-9 as a therapeutic target to inhibit its activity through specific siRNA or shRNA have been explored. Zhou et al.[129] designed an amphiphilic star copolymer based on dendritic poly(L-lysine) and hyperbranched polyglycerol *via* click reaction to co-load MMP-9 siRNA and docetaxel (DTX) for breast cancer therapy. A GSH-responsive chemotherapeutic self-sensitized polymeric prodrug consisting of hyperbranched poly(amido amine) (PAA) and MTX was synthesized to carry shRNA plasmid of MMP-9 for chemo/gene dual therapy of breast carcinoma[130]. Further, a similar transferrin (Tf)-targeted polymeric prodrug Tf-PAA-MTX was exploited to present MMP-9 shRNA plasmid and showed considerable expectations in chemotherapy gene therapy for nasopharyngeal carcinoma[131].

Except as a therapeutic target, the possibility of MMP-9 as an intelligent responder by using its proteolytic activity for drug release to tumor target has been confirmed and a variety of MMP-9-stimulated nanoDDS have been developed for cancer theranostics. The successful construction of various nanocarriers containing MMP-9-cleavable motifs, such as PLGA-*b*-PEG-based polymeric NPs[132] and PEG-derived polymeric nanogels[133], have played a key role in the enzyme-triggered chemotherapeutics delivery. Typically, MMP-9 catalyzed the hydrolysis of peptide micelles into nanofiber depots to facilitate DOX release[134]. MMP-9-susceptive tetrapeptide vesicles were established for targeting DOX to tumor lesion[135]. Combretastatin A4 nanodrug-upregulated MMP-9 expression promoted the selective release of DOX prodrug into human breast cancer cells[136]. A pH/MMP-9 sequentially responsive DOX-conjugated peptide was employed for structure transformation (from spherical to rod-like)-induced drug action process optimization and efficacy improvement[137]. Porta et al.[138] modified poly-(dimethyl siloxane)-poly-*b*-(methyl-oxazoline, PDMS-PMOXA) polymersome with an MMP-9-cleavable peptide and carried PTX for enzymatic digestion-enhanced pharmacological activity. Leukocyte-mimicking pluronic P123-lipid nanohybrids loading PTX were established to suppress the growth and metastasis of breast cancer, with 80.84% tumor inhibition and 10.62% metastatic foci in lung tissue[139]. Functionally similar, a reduction/MMP-9 dual-sensitive shrapnel methoxy PEG-peptide-vitamin E succinate NPs loading DTX was developed to inhibit breast cancer growth (81% of inhibitory rate) and metastasis (92% of inhibitory rate)[140]. MMP-9/cathepsin B dual-enzyme-cleavable GEM nanovectors and MMP-9-sensitive curcumin/p53 DNA co-delivery nanoDDS were designed for programmed pancreatic cancer suppression[141] and reversal of cisplatin resistance in ovarian cancer[142], respectively. Furthermore, MMP-9-activated chemotherapeutic-based combinations have also appeared, such as DTX/quercetin/imatinib synergy was proved to be promising for metastatic breast cancer therapy[143], PTX/thioridazine/PD-1 inhibitor were integrated into a cocktail strategy to improve breast cancer treatment[144].

An MMP-9-responsive cytotoxic T lymphocyte (CTL) vaccine delivering immune-tolerant elastin-like polypeptide (iTEP) was developed to enhance CTL vaccines and was considered an alternative to traditional dendritic cell-based vaccines (Fig. 6)[145,146]. Amphiphilic diblock copolymers containing Toll-like receptor 7 (TLR7) agonists and MMP-9 substrate peptide were prepared to enhance the immunotherapeutic efficacy and reduce immunotoxicity[147].

To attenuate the nonspecific cytotoxicity and hemolytic activity of melittin, its MMP-9-sensitive prodrug was connected to perfluorocarbon NPs, which greatly enhanced the enrichment of nanoDDS in tumor sites and the prodrug decomposition restored pharmacological activity[148]. In addition to its important role in cancer therapy, MMP-9-specific fluorescent-labeled substrate peptides were introduced into nanodiamonds to enhance peptide stability and quantitatively respond to the efficacy of MMP-9 in

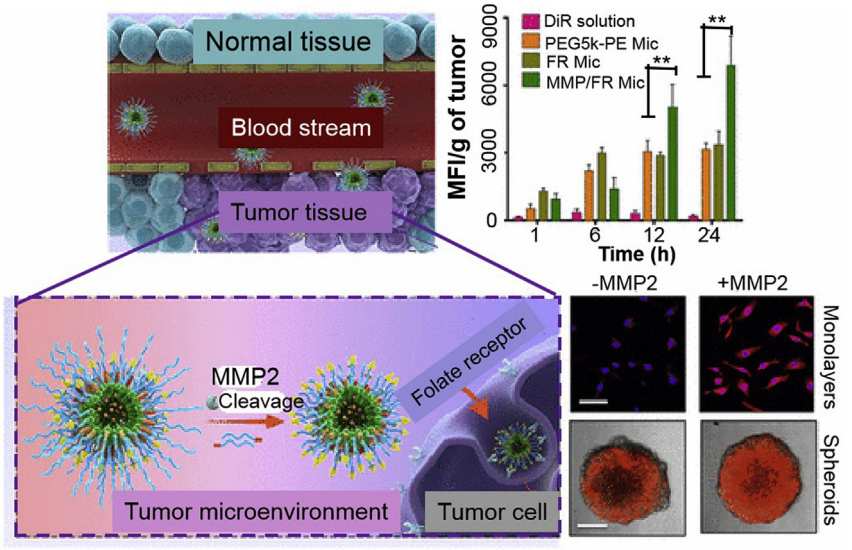

Figure 5 MMP-2-sensitive FR-targeted polymeric micelles directing dasatinib to tumor sites to increase anticancer potency and overcome multidrug resistance. Reprinted with the permission from Ref. 128. Copyright © 2017 American Chemical Society.

early detection of tumor metastasis[149]. The construction and anticancer applications of MMP-9-responsive nanocomplexes are illustrated in Table 3[135−149].

On the basis of exciting achievements in cancer theranostics using MMP-2 or MMP-9 as a single responder, a series of multipurpose polymeric nanoplatforms have also been exploited for the accurate theranostics of cancer with MMP-2/MMP-9 dual-sensitivity by intelligently loading and on-demand presenting various cytotoxic therapeutics such as DOX[150−154], PTX[155], DTX[156], camptothecin[157], bFGF[158], MMP inhibitors[159], immune modulators[160], and so on.

3.1.3. *MMP-14-triggered drug delivery*

MMP-14 is a primary endopeptidase highly expressed on the surface of many tumor cells and is closely related to cancer development. In recent years, it has been widely used as a responder to stimulate tumor theranostic events. Typically, Gao et al.[161] developed a graphene oxide (GO)/Au nanocomplex conjugated with NIR dye (Cy5.5) labeled MMP-14 substrate. After encountering MMP-14, this theranostic system exhibited sharp FI and PA signals, as well as excellent tumor inhibition against mouse squamous cell carcinoma. In another multifunctional nanosystem, ferumoxytol was conjugated with azademethylcolchicine (ICT) by an MMP-14-recognizable peptide (Ala-Cys-Arg-Ser-Cit-Gly-HPhe-Tyr-Leu-Tyr). The created nanotemplate selectively accumulated at breast cancer lesions and emerged acute MRI contrast and image-mediated efficacy monitoring[162]. Further, the therapeutic effect and cardiovascular toxicity of this protease-responsive ICT nanoprodrug were systematically evaluated in human breast, lung, prostate, and colorectal cancer. It concluded the remarkable clinical transformation potency of the MMP-14-sensitive nanotherapeutics[163].

3.2. *Serine proteases-responsive nanoDDS for cancer theranostics*

Serine proteases are the second most important family of proteases, accounting for approximately one-third of all proteases. Their catalysis is realized by a coordinated triad structure formed by the close arrangement of three amino acids of histidine (His57), serine (Ser195), and aspartic acid (Asp102)[164]. The serine proteases closely related to tumor progression and widely studied are urokinase plasminogen activator (uPA) and prostate specific antigen (PSA). Their utility in tumor-targeted drug presentation and disease treatment are discussed below, with a focus on the representative progress in the past five years.

3.2.1. *uPA-triggered drug delivery*

Overexpression of uPA is an important hallmark of many cancers. Its substrate specificity, that is, exclusively breaking the Arg-Ser bond in the Ser-Gly-Arg-Ser-Ala sequence of substrate peptides[165], contributes to the successful development of uPA-controlled tumor penetrating peptide[166] and fusion protein-based NPs[167] for effective drug loading and protease-driven payload delivery in tumor lesion. In order to achieve the super-early detection and good prognosis of pancreatic ductal adenocarcinoma (PDAC), a Gd^{3+}-based MRI contrast agent and cyanine dye Cy5.5 were combined with U11 (a pancreatic cancer-specific peptide) to form a dual-modal MRI/NIR FI nanoprobe for highly sensitive and spatially resolved detection of pancreatic precancerosis[168]. Matsumura et al.[169] prepared a clever PEGylated four-arm ^{64}Cu-bombesin analog tetramer connected by a substrate linker to achieve the desired positron emission tomography (PET) contrast and clearance enhancement in less time.

Some uPAR-driven smart nanosystems are also being explored for cancer treatment. Typically, a Wnt/uPAR co-targeting ultrasmall magnetic iron oxide nanoparticle (IONP) was designed to inhibit cancer stem cell phenotype and deliver DOX to chemoresistant breast cancer[170]. DOX nanocomplex coated with human serum albumin (HSA) and attached to the amino-terminal fragment (ATF) of urokinase was proposed to improve the anticancer efficiency and minimize the cardiotoxicity of DOX[171]. A pH-responsive uPAR-targeted DOX/curcumin nanosynergy was synthesized to combat the resistance and toxicity of DOX in the treatment of lung cancer[172]. A delicate fusion protein was developed to guide uPA-triggered scorpion toxin delivery for combating the growth and invasion of breast cancer[173].

Besides, uPA-stimulated theranostic nanocompositions have also played an important role in cancer management such as luteinizing hormone releasing hormone receptor/uPAR dual-targeting IONPs for the diagnosis and treatment of prostate cancer[174], uPAR-targeted PEGylated IONPs for MRI-guided drug delivery into peritoneal tumors[175], milk protein-protected uPA-targeted IONPs loading cisplatin for MRI-monitored effective therapy of pancreatic cancer with minimal side effects[176], pH responsive, uPAR-targeted MSNs for the identification of pancreatic cancer using multispectral optoacoustic tomography[177], iridium (Ir) complex-loaded Au nanostars functionalized with uPAR targeting module for photothermal/X-ray CT/PA three type diagnosis-synergized PTT/chemotherapy combination treatment for triple negative breast cancer (TNBC)[178]. Typical uPA-mediated cancer diagnosis and treatment events are briefly summarized in Table 4[168−178].

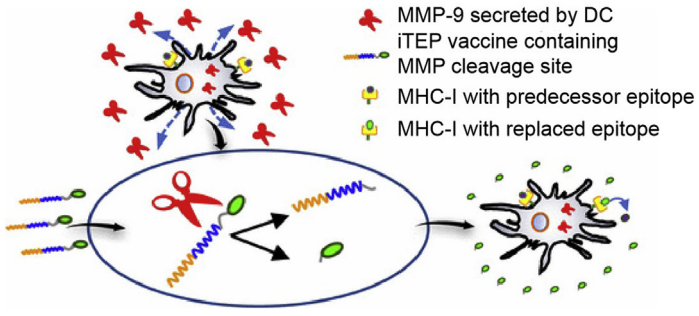

Figure 6 MMP-9 sensitized iTEP vaccine presentation for the direct exposure of CTL epitope onto MHC class I complexes located on the surface of dendritic cells. Reprinted with the permission from Ref. 146. Copyright © 2017 American Chemical Society.

Table 3 Representative development of MMP-9-sensitive nanoDDS and their applications in the targeted theranostics of malignancies.

Nanocarrier	Substrate	Drug	Diagnosis	Therapy	Tumor	Ref.
HWGF	HWGF	DOX	FI	Chemotherapy	MCF7 human breast cancer cells	135
CA4-NPs, MMP-9-DOX-NPs	GPLGL	DOX	–	Chemotherapy	4T1 mouse breast cancer cells	136
2-(Nap)-FFK$_{TPA-DOX}$ AGLDDRGD	AGLDD	DOX	FI	Chemotherapy	4T1 mouse breast cancer cells HepG2 human hepatoma cells MCF7 human breast cancer cells H22 mouse hepatoma cells	137
PDMS-PMOXA polymersomes	SRLSLPGC	PTX	FI	Chemotherapy	MCF7 human breast cancer cells	138
Leukocyte-mimicking pluronic-lipid nanovesicle hybrids	Pluronic P123	PTX	FI	Chemotherapy	4T1 mouse breast cancer cells	139
mPEG-peptide-VES copolymers	PLGLAG	DTX	–	Chemotherapy	4T1 mouse breast cancer cells	140
PEG-CycloRGD-CdSe/ZnS QDs	GGPLGVRGK, GFLG	GEM	FI	Chemotherapy	BxPC-3 human pancreatic cancer cells	141
Cationic nanostructured lipid carriers	Gelatin	DTX, Qu, IMA	FI	Chemotherapy, molecular targeted therapy	4T1 mouse breast cancer cells	143
PDB-PPV	PLGLAG	PTX, THZ, HY19991	FI	Chemotherapy, stem cell therapy, immunotherapy	MCF7 human breast cancer cells	144
Amphiphilic diblock copolymers	GPLGLAGGERDG	1V209	FI	Immunotherapy	4T1 mouse breast cancer cells	147
Perfluorocarbon NPs	GPQGIAGQ	Melittin	Ultrasonic imaging	Gene therapy	B16F10 mouse melanoma cells	148
Nanodiamonds	LGRMGLPGK	FITC, 5-TAMRA	FI	–	Huh7 human hepatoma cells SNU398 human hepatoma cells	149

Abbreviations: HWGF, His-Trp-Gly-Phe; NPs, nanoparticles; 2-(Nap)-FFK, 2-naphthylacetic acid-Phe-Phe-Lys; PDMS, poly(dimethyl siloxane); PMOXA, poly-*b*-(methyloxazoline); mPEG, methoxy polyethylene glycol; VES, vitamin E succinate; PDB, PEG-block-poly[(1,4-butanediol)-diacrylate-*β-N,N*-diisopropylethylenediamine]; PPV, mPEG-peptide-VES; DOX, doxorubicin; CA4, combretastatin A4; PTX, paclitaxel; DTX, docetaxel; GEM, gemcitabine; Qu, quercetin; IMA, imatinib; THZ, thioridazine; 1V209, 2-methoxyethoxy-8-oxo-9-(4-carboxy benzyl)adenine; QDs, quantum dots; FITC, fluorescein isothiocyanate; 5-TAMRA, 5-carboxytetramethylrhodamine; MMP-9, matrix metalloproteinase-9; RGD, Arg-Gly-Asp; TPA, 4-formylbenzoic acid; FI, fluorescence imaging; –, not applicable.

3.2.2. *PSA-triggered drug delivery*

PSA, also known as glutamate carboxypeptidase II (GCPII), is the most prominent biomarker of prostate cancer for diagnostic and therapeutic targeting and is overexpressed in other solid tumors to promote their neovasculature and angiogenesis. Some first-line therapeutics have shown achievements in preclinical or clinical trials but have been associated with obvious side effects. Considering this, alternative targeted delivery schemes have been actively developed to enrich bioactive constituents in tumors for improved diagnostic and therapeutic outcomes by introducing PSA-recognizable ligands, aptamers, or antibodies into the nanoDDS.

To improve diagnostic performance, Machulkin et al.[179] synthesized a fluorescence-conjugated magnetite-Au NPs carrying PSA ligand for tissue-specific MRI. A distearoyl phosphatidyl ethanolamine (DSPE)-PEG-maleimide copolymer that bonded the anti-PSA single chain was labeled with ^{64}Cu for enhanced tumor targeting and PET imaging visibility[180].

Considerable achievements have also been made in PSA-mediated drug accumulation and efficacy improvement. Concretely, tumor lesions targeted delivery of PSA-specific motif-conjugated nanocomplexes carrying a variety of therapeutics either alone or in combination, mainly included DOX[181–185], PTX[186], DTX[187], SN38[188], camptothecin[189], thapsigargin[190], boron-containing reagents[191], zinc chelator[192], antibody-drug conjugates[193,194], kinase inhibitors[195], siRNA (Fig. 7)[196–200], miRNA[201], oligonucleotide[202], therapeutic proteins[203], and chemotherapy-based combination regimens with molecular targeted therapy[204], antibiotic therapy[205], gene therapy[206,207], and anti-inflammatory therapy[208].

PSA-targeted nanocomposites have also shown high levels of activity in imaging-synergized cancer treatment, typically such as FI-mediated chemotherapy[209] or PDT[210], PET-mediated chemotherapy[211], MRI/NIR FI-induced hyperthermia[212], ultrasound-guided gene therapy[213] or chemotherapy[214], and MRI-induced combination therapy[215]. Furthermore, some PSA-targeted

Table 4 The latest advances of uPA-cleavable nanoDDS and their applications in the targeted theranostics of malignancies.

Nanocarrier	Substrate	Drug	Diagnosis	Therapy	Tumor	Ref.
DGL-U11	U11	Gd^{3+}-DTPA, Cy5.5	MRI, NIR FI	–	PANC1 human pancreatic cancer cells	168
Four-arm PEGylated ^{64}Cu-bombesin analog tetramer	CGSGRSAG	^{64}Cu	PET	–	PC-3 human prostate cancer cells	169
Ultra-small magnetic IONPs	DKK1, ATF_{24}	DOX	NIR FI	Chemotherapy	MDA-MB-231 human breast cancer cells	170
ATF-HSA	ATF	DOX	FI	Chemotherapy	H1299 human non-small cell lung cancer cells H22 mouse hepatoma cells	171
U11-DOX/curcumin NPs	U11	DOX, curcumin	–	Chemotherapy	A549 human non-small cell lung cancer cells	172
ALA fusion protein	ATF	AGAP	–	Gene therapy	MDA-MB-231 human breast cancer cells	173
LHRH-AE105-IONPs	Modified LHRH peptide, AE105 peptide	PTX	MRI	Chemotherapy	PC-3 human prostate cancer cells	174
PEGylated IONPs	ATF	Cisplatin, DOX	NIR FI, MRI	Chemotherapy	PANC02 mouse pancreatic cancer cells	175
Milk protein-coated IONPs	ATF	Cisplatin	MRI	Chemotherapy	MIA PaCa-2 human pancreatic cancer cells	176
MSN-uPA NPs	Chitosan, uPA	GEM	MSOT	Chemotherapy	ASPC1, PANC1, and MIA PaCa-2 human pancreatic cancer cells	177
GNS@Ir@P-AE105	P-AE105	GNS, Ir complex	PT, PA, X-ray CT	PTT, chemotherapy	MDA-MB-231 human breast cancer cells	178

DGL, dendron-grafted poly-L-lysine; PEG, polyethylene glycol; IONPs, iron oxide nanoparticles; HSA, human serum albumin; ALA, ATF and AGAP linked with the uPA-cleavable linker gene; MSN, mesoporous silica nanoparticle; GNS, gold nanostars; DOX, doxorubicin; AGAP, one of the scorpion toxin polypeptides; PTX, paclitaxel; GEM, gemcitabine; Gd^{3+}-DTPA, Gd^{3+}-diethylene triamine pentaacetic acid; Cy5.5, cyanine dye; Ir, iridium; uPA, urokinase plasminogen activator; U11, tumor-targeting peptide; DKK1, Dickkopf-related protein-1; ATF, amino-terminal fragment of urokinase; LHRH, luteinizing hormone-releasing hormone; AE105, a potent nine-mer peptide antagonist of uPAR binding; P-AE105, a polyetherimide-AE105 peptide conjugate; PTT, photothermal therapy; MRI, magnetic resonance imaging; NIR, near-infrared; FI, fluorescence imaging; PET, positron emission tomograph; MSOT, multispectral optoacoustic tomography; PT, photothermal; PA, photoacoustic; CT, computed tomography; –, not applicable.

nanoDDS, particularly DTX-derived nanotheranostics, have already hinted valuable prospects in the clinical potential development[216], phase I[217], phase II and further clinical stages[218].

3.3. *Cysteine proteases-responsive nanoDDS for cancer theranostics*

Lysosomal proteases, mainly the cathepsin family and legumain, play a key role in the malignant evolution of diseases[219]. Cathepsins can be classified into three categories, namely cysteine (cathepsins B, C, F, H, K, L, O, S, V, W and X), serine (cathepsins A and G), and aspartic cathepsins (cathepsins D and E). Of these, cysteine cathepsins account for the majority. They share a common papain-like fold containing three α-helix domains and a β-barrel domain except for cathepsin C, in which the catalytic dyad Cys-His is localized and two separate domains define the substrate binding cleft[220]. The recognized function of cysteine cathepsins is their proteolysis of damaged or unwanted proteins in lysosomes for recycling[221]. Cathepsins B, L and S are key members of the cysteine protease group and play indispensable roles in many physiological events and pathological regulations of cancer. They have been widely used as responders in the intelligent delivery of imaging contrast agents and/or active therapeutics. Cathepsin B-induced drug delivery has been systematically described in our previous report[28]. Thus, the importance of cathepsin L, cathepsin S, and legumain, caspase 3 (the other two cysteine proteases) in motivating cancer management will be then highlighted.

3.3.1. *Cathepsin L-triggered drug delivery*

Cathepsin L is another well-established cysteine cathepsin upregulated in a multitude of human cancers[222]. Its expression level is closely related to tumor malignancy, invasiveness, grading and staging, and tumor occurrence. Upon glycosaminoglycan-mediated activation, the secreted cathepsin L degrades cell adhesion components directly or indirectly thus promotes tumor progression and metastasis[223,224]. Down-regulation of cathepsin L can induce apoptosis and inhibit metastasis in some tumor models including pancreatic cancer, lung cancer, liver cancer, etc. While in other tumors such as squamous cell carcinoma, cathepsin L exhibits significant expression positively dependent antitumor activity[225,226]. Hence, the importance of cathepsin L in stimulating drug migration and cancer management is outlined below.

For the past few years, considerable attention has been paid to the importance of cathepsin L in tumorigenesis and malignant progression. Meanwhile, its feasibility as a drug delivery target has also been developed. It is worth mentioning that Wang et al.[227] reported a cathepsin L-responsive acetylated azidomannosamine

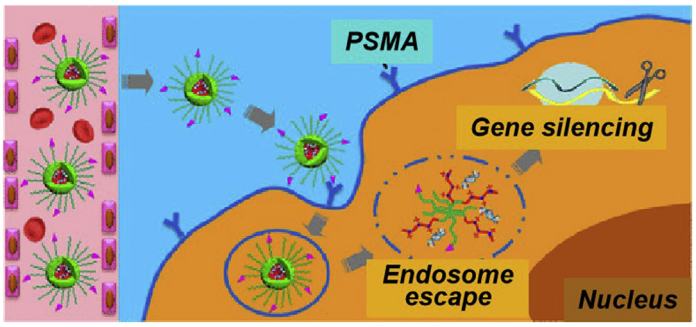

Figure 7 Schematic diagram displays the multifunctional envelope-type nanoplatform for PSA-targeted siRNA delivery and prostate cancer therapy. Reprinted with the permission from Ref. 200. Copyright © 2017 American Chemical Society.

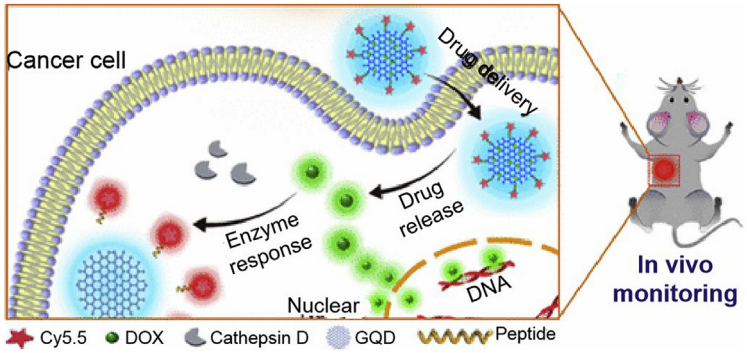

Figure 8 Graphene quantum dots (GQDs) loaded with DOX and conjugated with Cy5.5 for cathepsin D-responsive drug delivery and internal process visualization. Reprinted with the permission from Ref. 285. Copyright © 2017 American Chemical Society.

to mediate tumor-specific labeling *in vivo* that further enhanced DOX prodrug aggregation in tumor lesions and facilitated ablation of colorectal cancer (CRC), TNBC, and metastatic breast cancer in mice. In view of the significance of cathepsin L in promoting the proteolytic disassembly of reovirus which is critical for its antitumor activity, pretreatment with cathepsin L inhibitor greatly enhanced the sensitivity of reovirus-resistant tumor cells to reovirus-cationic liposome complex, thereby targeting reovirus to the cytosol and causing apoptosis[228].

3.3.2. *Cathepsin S-triggered drug delivery*

In the cysteine cathepsin family, cathepsin S has previously been detected in multiple tumor types, which often implies poor disease status and clinical outcomes. It is closely related to tumorigenesis and progression by stimulating angiogenic factors to promote angiogenesis[229]. In view of this, except as a therapeutic target, the properties of cathepsin S have been integrated into nanocomplexes for protease-susceptible oncologic imaging or targeted therapy.

In a typical manner, Fan et al.[230] synthesized a cathepsin S-degradable multi-block copolymers based on a water-soluble and biofriendly polymer, poly[*N*-(2-hydroxypropyl)methacrylamide] (HPMA), as the backbone. By coupling cathepsin S-sensitive peptide substrate (PMGLP) and radiolabeling with lutetium-177 (^{177}Lu), the nanocomposites showed highly sensitive single-photon emission computed tomography (SPECT)/CT imaging in PDAC. While capturing early lesions, the untargeted scavenging associated with mononuclear phagocyte system (MPS) tissues was enhanced without discounting the tumor localization and retention capability of the nanoagents. To further clarify the effect of block size on the *in vivo* bioefficacy of cathepsin S-fractured multi-block HPMA copolymer, dual-isotope labeling technique combined multiple cellular and *in vivo* strategies were implemented in PDAC cancer models. It is concluded that copolymers with smaller blocks exhibited faster enzyme cleavage kinetics than their larger counterparts. Moreover, the smaller ones enjoyed the superior MPS-related clearance and improvement in untargeted retention. However, this tendency did not affect the targeting and nanoenrichment at tumor sites. This discovery revealed the implication of nanoprofile for their internal functioning and biological performance, and provided a basis for designing nanotheranostics with a reasonable appearance[231]. Considering the overexpression and co-promotion of cathepsins S and L in tumor progression, they were focused as co-targets for enhanced diagnostic events and drug release. An endogenous small molecule protease inhibitor, stefin A, was embedded in the liposome matrix for bright tumor diagnosis in combination with effective treatment[232].

3.3.3. *Legumain-triggered drug delivery*

Legumain, with specific catalytic sequence His-Gly-Spacer-Ala-Cys, is another lysosomal cysteine protease from the legumain family (C13) and CD clan[233]. It is also a caspase and specifically cleaves the C-terminal substrate peptide of asparagines[234]. Legumain is mainly found in antigen presenting cells (APCs) and is upregulated in many human solid tumors, such as breast[235], ovarian[236], colorectal[237], prostate[238], and gastric[239] cancers. In addition, it can be expressed by tumor-associated macrophages (TAMs) and intratumoral blood vessels. Importantly, legumain

mediates tumor development and stimulates angiogenesis, tumor progression and metastasis in a number of ways. All of these provide references for the design of legumain-targeted prodrug and legumain-triggered nanoDDS.

As a representative, Ruan et al.[240] synthesized a bifunctional Au nanocomplex by synergistic amplification of receptor-mediated endocytosis and EPR effect. The NPs contained an legumain-activated AK peptide (Ala-Ala-Asn-Cys-Asp) and a integrin $\alpha v\beta 3$ receptor binding peptide (R8-RGD). After touching the glioblastoma site, the nanocomponents underwent a legumain-induced click cycloaddition to precisely locate and concentrate in lesion. This synergistic model greatly improved the anti-glioma efficacy of the loaded DOX. Similarly, modifying Au NPs with AK peptide and 2-cyano-6-aminobenzothiazole (CABT), the resulting nanocomposites were highly concentrated in the glioma site. After connecting DOX with pH-responsive groups, the obtained Au nanohybrids exhibited legumain-induced FI and acidity-enhanced antineoplastic effect[241]. To further suppress DOX-caused cytoprotective autophagy and enhance the immune response, hydroxychloroquine and anti-PD-L1 (programmed cell death ligand 1) antibody were simultaneously introduced into the above legumain-activatable Au NPs. This exquisite nano-combination effectively alleviated the immunosuppressive microenvironment and achieved exciting anti-glioma outcome without recurrence[242]. By covalently attaching the alanine-alanine-asparagine to the cyclic iRGD, the multitargeted tadpole-like peptide could transport DOX directionally. DOX-loaded multi-target liposomes specifically attacked tumor vascular endothelial cells to achieve unobstructed permeability. They substantially eliminated breast cancer without recurrence or metastasis due to exhausted tumor associated macrophage-related TME regulation[243]. This phenomenon was confirmed by another iRGD-mediated legumain-susceptible Au NPs for FI-guided ablation of breast cancer[244]. Beyond that, other strategies have also been used for legumain-enhanced cancer theranostics, such as an *N*-benzyloxycarbonyl-Ala-Asn-DOX prodrug for anti-cervical cancer treatment[245], a pH-triggered and legumain-digestible DOX-carbon dots prodrug for FI-monitored drug release[246], a substrate-bridged HA-DOX polygonal nanogel for CD44-targeted and legumain-responsive lung cancer killing[247], a legumain-cleavable tetrapeptide Ala-Ala-Asn-Leu conjugated 4-arm PEG-DOX polymeric prodrug for breast cancer-targeted drug delivery and enhanced antitumor index[248], a clever DOX-loaded liposome modified by fibronectin-binding peptide and legumain-cleavable peptide in tandem for tumor ecological microenvironment-disturbed breast cancer inhibition[249], and charge-driven self-assembled DOX-chitosan-PEG-PGA (poly(glutamic acid)) nanocomposites containing legumain substrate peptide for enhanced melanoma therapy[250].

In addition to DOX, other chemotherapeutics, targeted drugs or traditional Chinese medicine have also been successively exploited for legumain-induced cancer treatment. Jin et al.[251] co-coupled simvastatin and PTX using legumain-specific peptides to obtain TME-activatable liposomes. This multifunctional system effectively lowered cholesterol and reversed epithelial-mesenchymal transition (EMT)-related PTX resistance in non-small cell lung cancer (NSCLC). Cisplatin and indocyanine green (ICG) co-loaded nanopolymers, with legumain-responsive heptapeptide (Gly-Cys-Gly-Ala-Ala-Asn-Leu) upon their surface, showed excellent photothermal transformation-enhanced chemotherapeutic efficacy for gastric cancer[252]. On this basis, glutamic cisplatin-loaded Au@MSN core-shell NPs were functionalized with Gly-Cys-Gly-Ala-Ala-Asp-Leu to achieve protease-induced synergistic chemotherapy/PTT against malignant gastric cancer[253]. In order to overcome the drug delivery difficulty of metastatic breast cancer, inflammatory monocytes[254] or bioengineered macrophages[255] were exploited to anchor legumain-specific peptides and cytotoxic mertansine or soravtansine, respectively. The nanocomplexes could deliver therapeutics to preinvasive breast cancer and its lung metastase to achieve controlled anti-metastasis therapy. Further, legumain-sensitive melittin-mounted nanopolymers were designed to co-encapsulate IR-780 and sorafenib for oral available combinational chemotherapy and PTT of malignant gastric carcinoma[256]. Other perishable drugs, typically such as active protein trichosanthin, were cleverly genetically fused into an endogenous albumin scaffold *via* a legumain-cleavable substrate peptide. The dual-protein hitchhike prodrug was enriched at the tumor site by protease actuation and albumin mediation to remove orthotopic breast cancer[257].

3.3.4. *Caspase-3-triggered drug delivery*

Caspases are a 15-member family of cysteine proteases and are widely known for their important roles in programmed cell death and inflammation[258]. Among them, caspase 3 is the most typical executor of apoptosis. Under the initiation of caspase-8 or -9, the non-activated pro-caspase-3 is proteolytically cleaved to the active caspase-3, which migrates into the nucleus to cleave a variety of important intracellular functional proteins[259]. Commonly used therapies, such as chemotherapy, radiotherapy and immunotherapy, generally induce apoptosis by activating caspase 3. Therefore, up-regulated caspase-3 is considered by numerous researchers to be a positive indicator for evaluating the effectiveness of cancer treatments.

However, with further research, caspase-3 is depicted in a more refined functional spectrum in cancer development and treatment. It plays important roles in promoting the carcinogenesis after cytotoxic drug therapy[260], inducing tumor regeneration following radiotherapy through paracrine pathway[261], stimulating tumor growth by creating a pro-angiogenic microenvironment[262], and other such non-apoptotic effects. In a recent representative study, Zhou et al.[263] revealed the molecular mechanism by which caspase-3 stimulated the chemo-radiotherapy resistance and tumor metastasis in CRC by regulating EMT, and predicted the potential of caspase-3 inhibition synergized with cytotoxic regimen in enhancing the CRC treatment. Further, Bernard et al.[264] discovered and dissected the cleaved caspase-3's identity as a transcription factor. The transcriptional activity is determined mainly by a DNA-binding domain in the subunit of caspase-3 and an activated configuration. Caspase-3 acted directly on the promoter of multiple pro-angiogenic genes, especially vascular endothelial growth factor A (VEGFA), thereby inducing oncogenic angiogenesis and tumorigenesis by transcriptional regulation. Simultaneously, caspase-3 activation indirectly reduced a series of pro-apoptotic genes and caused resistance to cytotoxic therapeutics. Targeted inhibition of caspase-3 effectively sensitized chemotherapeutic agents and suppressed cancer progression and metastasis.

For years now, scientists have focused on clarifying the routine pro-apoptotic effect of caspase-3 and exploring its non-classical cancer-promoting mechanism. This "double-edged sword" effect makes caspase-3 a crucial target for tumor-specific treatment and reversal of drug resistance, as well as a personalized strategy to conquer cancer invasion and metastasis. However, relatively few studies have used it only as a responsive switch for protease-induced anticancer therapeutics release.

3.4. *Threonine proteases-responsive nanoDDS for cancer theranostics*

Testes-specific protease 50 (TSP50) and threonine aspartase 1 are representatives of threonine proteases. Their importance is overwhelmingly reflected in their involvement in the whole process of tumor development and as effective anticancer targets. TSP50 was detected in human breast cancer cell-derived hypomethylated DNA fragments and abnormally activated in most breast and colorectal cancers[265,266]. Its transcripts are exclusively distributed in human testes. The catalytic triplet of TSP50 is composed of histidine at 153, aspartic acid at 206, and threonine at 310[267]. It is involved in a variety of cellular physiological behaviors including blood coagulation, body immunity, and protein processing. However, its dysregulation greatly promotes cell proliferation and tumor development[268]. TSP50 participated in multiple malignant processes of cancer, including stimulating cell proliferation *via* inhibiting activin signaling[269], promoting invasion and metastasis by inducing EMT[270]. It was also discovered as a potential predictor of early recurrence and poor prognosis in malignancies[271], and as a unique biomarker or treatment target for a variety of cancer therapies[272–275].

Threonine aspartase 1 is a strongly conserved developmental protease that is overexpressed in a variety of liquid and solid tumors. It is similar to type 2 asparaginases and N-terminal nucleophile (Ntn) proteases in structure, but shows unique biological functions including head morphogenesis, segment recognition, spermatogenesis, and proliferation[276]. Although the pathobiological mechanism has not yet been revealed, clarifying the structure-function relationship of threonine aspartase 1 is essential and has been widely revealed to explore its potential in diagnostic and therapeutic regimens[277,278].

All of the above threonine proteases, whether intrinsic in humans, derived from other organisms in nature, or converted by other proteases, are prominent candidates for the exploitation of their predictive, diagnostic, and therapeutic values, as well as their potential for protease-driven delivery of bioactive theranostic agents, which is currently less reported.

3.5. *Aspartic proteases-responsive nanoDDS for cancer theranostics*

Cathepsins D and E are widely popular aspartic proteases that are ubiquitous in the progression of many types of cancers. Even with inevitable extracellular secretion, they are largely confined to lysosomes for functions[279]. Cathepsin D is competent in a variety of physiological processes and is highly activated in a variety of tumors. Its upregulation, caused by altered trafficking-induced proenzyme increase, leads directly to poorer prognosis and shorter recurrence-free survival[280]. Cathepsin E belongs to the pepsin family and the endolysosomal pathway, which is significantly expressed in the immune cells, lymphatic system, gastrointestinal mucosa, epidermal keratinocytes, red blood cells, and tumor cells[281]. It exhibits regular cell-type specific localization and is also secreted into the ECM of activated phagocytes and cancer cells[282]. This differentiated cellular distribution of cathepsin E determines its cellular and tissue-specific biological efficacy, as well as overactivation-caused tumor development[283].

For the past few years, nanostrategies incorporating the responsiveness of these two proteases have been developed for the sensitive presentation of active therapeutics. Exemplarily, graphene QDs loading DOX was conjugated with Cy5.5 by a peptide that specifically responds to cathepsin D. The resulting nanoprobes programmatically released DOX and fluorescently tracked their internal processes (Fig. 8)[284]. Bhattacharya et al.[285] conjugated an N-terminal CPP, dexamethasone, and cathepsin D-activated peptide linker *via* hydrazone bond. This chemically stable conjugate could be efficiently ingested by retinal cells and exhibited cathepsin D-accelerated drug release and targeted therapy for ocular diseases. Another approach focused on the vectorization of cathepsin D inhibitors and the interpretation of their structure-function relevance[286,287]. For cathepsin E, recent studies emphasized exploring its feasibility as a molecular target for cancer theranostics and developing its clinical application potency[288].

3.6. *Other proteases-responsive nanoDDS for cancer theranostics*

Other proteases, such as furin, also play an indispensable role in the regulation of cancer processes and the promotion of theranostic programs. Furin is a proprotein convertase and mainly distributed in the cell surface or trans-Golgi body. It is dysregulated in microbial infections, fibrosis, Alzheimer's disease, and a variety of cancers such as breast cancer, head and neck cancer, NSCLC and rhabdomyosarcoma[289]. Furin performs a series of important physiological and pathological functions by cleaving the specific sequence (Arg-X-Arg/Lys−Arg↓) of paired basic amino acids in the substrates, or stimulating the activation of some protein precursors such as α- and β-secretases[290].

Based on the proteolytic properties of furin, a series of intelligent nanosystems have been developed for furin-responsive cancer detection and treatment. Li et al.[291] constructed an amphiphilic peptide, an Arg-Val-Arg-Arg-Phe-Phe-Phe-NBD (a nitrobenzoxadiazole) sequence. Among which, NBD was a hydrophobic environment-enhanced fluorescent chromophore. The Phe-Phe-Phe triplet provided hydrophobicity-promoted nano-assembly and cellular permeability. The hydrophilic Arg-Val-Arg-Arg sequence improved the membrane penetration and acted as a substrate for the specific detection of furin. The reasonably designed peptide self-assembled into a stable micelle and the fluorescence of NBD was lit to quickly detect furin *in vitro* and *in vivo*. This rapid response provided the possibility for furin-associated tumor recognition and progression evaluation. The substrates of furin are rich in basic amino acids, which give them the CPP potential. In view of this, furin substrates with different repeats[$(Arg\text{-}Val\text{-}Arg\text{-}Arg)_m$] were developed to deliver the cell-impermeable pro-apoptotic peptide KLAKLAKKLAKLAK and chemotherapeutic chlorambucil. After approaching the tumor lesion, the drug-carrying nanocomposites exhibited magnified cell permeability and enrichment, as well as furin-enhanced breast cancer elimination[292]. In addition, various other furin-responsive nanoplatforms were also rapidly evolving. As an example, furin substrate peptide-modified liposomes were designed to load vincristine for protease-specific rhabdomyosarcom treatment[293].

Summarizing the above analysis, the types of proteases and their structure-activity relationships have been gradually revealed. The clarity of protease function provides considerable application space for the development of enzyme-specific probes and inhibitors for cancer surveillance and treatment. Protease-mediated tumor-targeted enrichment of bioactive agents has shown promising progress. Moreover, this strategy will present an overwhelming prospect if the following considerations can be considered comprehensively. (1) Designing simple and clinically acceptable nanoplatform, and preparing them by easier, more environmentally friendly, and more

economical methods. (2) Exploring new proteases and synchronously analyzing the pathophysiological diversity of proteases reported. (3) Exploiting intelligent nanocomposites based on the heterogeneity of protease function. (4) Updating the tumor models according to the improvement of protease function.

4. Concluding remarks and future perspectives

Proteases underpin important physiological functions but dysregulate in a variety of disease-associated microenvironments and pathological cellular processes. It is extremely promising to exploit differentiated activity or expression of tumor-specific proteases as therapeutic targets or triggers. The thriving nanobiotechnology boosted enzyme-responsive DDS to be an overwhelming booster in targeted cancer management. In the present review, the origin, structure-function correlation, and pivotal physiological/pathological activities of cancer-related proteases are classified according to proteolytic mechanisms. Emphatically, recent advances in protease-stimulated cargo delivery and cancer theranostics are systematically explored.

Despite the encouraging achievements, several obstacles remain in the way towards the clinical transformation of enzyme-responsive nanoDDS: (1) the diversity, complexity, and patient variability of tumor-associated proteases; (2) substrate overlap and partial enzyme-substrate incompatibility; (3) cumbersome design and extreme construction of some nanocomplexes; (4) potential toxicity and biocompatibility consideration of nanoformulations. Therefore, further intensive and in-depth investigations such as refining the structure of enzymes and matching them to diseases, exacting the design and screening of substrate sequences, tailoring individual materials and integrating them into a delicate nanocomposite with negligible nanotoxicity and desired biocompatibility, are urgently needed to improve the theranostic efficiency of protease-responsive nanoDDS and facilitate their clinical transformation.

In conclusion, the significance of proteases in cancer ecology has made it necessary to elucidate the structure-activity relationship of proteases and their roles in the pathophysiological processes of cancer. A variety of specific substrates have been developed and engineered into multiple nanoprobes. These nanocomplexes have attracted considerable attention and achieved exciting efficiency in disease treatment. However, some limitations remain, such as the functional diversity of proteases, substrate sequence overlap, the construction complexity of nanocomposites, the difficulty of tumor modeling, and possible nanotoxicity. These obstacles can be addressed specifically through multidisciplinary approaches including oncology, biology, and materials chemistry. These advances will provide architectural and mechanistic insights for the new generation of nanotheranostics and ultimately promote their clinical applications in the targeted management of malignant tumors.

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Author contributions

Yanan Li, Cangang Zhang, Guo Li and Guowei Deng were responsible for reviewing relevant studies and writing the paper. Hui Zhang, Yongbing Sun and Feifei An provided instructional advice polished the contents of the article.

Conflicts of interest

The authors have no conflicts of interest to declare.

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