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Current Status and Perspectives of Protease Inhibitors and Their Combination with Nanosized Drug Delivery Systems for Targeted Cancer Therapy

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Abstract: In cancer treatments, many natural and synthetic products have been examined; among them, protease inhibitors are promising candidates for anti-cancer agents. Since dysregulated proteolytic activities can contribute to tumor development and metastasis, antagonization of proteases with tailored inhibitors is an encouraging approach. Although adverse effects of early designs of these inhibitors disappeared after the introduction of next-generation agents, most of the proposed inhibitors did not pass the early stages of clinical trials due to their nonspecific toxicity and lack of pharmacological effects. Therefore, new applications that modulate proteases more specifically and serve their programmed way of administration are highly appreciated. In this context, nanosized drug delivery systems have attracted much attention because preliminary studies have demonstrated that the therapeutic capacity of inhibitors has been improved significantly with encapsulated formulation as compared to their free forms. Here, we address this issue and discuss the current application and future clinical prospects of this potential combination towards targeted protease-based cancer therapy.

Keywords: proteases, inhibitors, nanoscale drug-delivery system, combination therapy, cancer treatment

Plain Language Summary

Proteases include widely distributed enzymes that are crucial for protein homeostasis and regulate many cellular processes, such as gene expression, differentiation, immunological defence, migration and cell death. Recent studies have indicated that the balance between production, activation and inhibition of proteases is often disturbed in malignant tumors, leading to tumor progression and dissemination. Numerous proteases are therefore under investigation for therapeutic purposes and their inhibition properties may serve potential for anti-cancer strategies. However, since protease inhibitors have mostly been developed in the form of small molecules or peptides, until recently their insufficient metabolic stability has remained an obstacle. Consequently, formulating protease inhibitors with a nanoparticle-based delivery system offers a new perspective on protease-targeted therapies. Protease inhibitors loaded in nanocarriers may provide improved bioavailability, increased circulation time and accumulate specifically in the tumoral tissues. This novel concept for the administration of protease inhibitors expands their potential in biomedical applications and gives the oportunity to achieve more effective treatment.

Proteases and Their Inhibitors

Proteases are a complex of enzymes that hydrolyze peptide bonds and are responsible for the breakdown of proteins into their individual components.[1] Based on their amino acid

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core in the active site and the mechanism of peptide bond cleavage, proteases can be classified into six groups: cysteine, serine, threonine, glutamic acid, aspartate proteases and metalloproteases.[1,2] Their control over protein synthesis, turnover and function enables them to regulate many biological processes, such as protein digestion, fertilization, immunological response, wound healing, cellular growth, differentiation, migration and apoptosis.[3] Their enhanced activity and changes in expression have frequently been connected with disease propagation. Therefore, antagonization of these enzymes with designed inhibitors is a promising strategy against a wide range of protease-associated diseases such as parasitic,[4] fungal[5] and various infectious (eg, malaria,[6] human immunodeficiency virus [HIV],[7] hepatitis,[8] herpes[9]); inflammatory,[10] respiratory,[11] cardiovascular,[12] neurodegenerative disorders[13] as well as cancer disease.[14]

In the context of cancerogenesis, most extracellular proteases are involved in various processes associated with tumor development and progression by different mechanisms. Thus, they can act as the base for membrane and matrix degradation, inactivation of natural protease inhibitors and chemotherapeutics, cell viability regulation, immune response modulation and inflammatory cell recruitment.[15–18] Moreover, proteases localized within the intracellular compartments, including in the cytosol, nucleus, membrane and mitochondria are associated with many signaling pathways through which they can promote: adhesion, proliferation, migration, de-differentiation and epithelial to mesenchymal transition of cancer cells.[18–20] The release of proteases into various cellular compartments and their altered expression can result from different factors, including the activation of membrane receptors (such as the tumor necrosis factor receptor[21]) or the generation of reactive oxygen species.[22,23]

These diverse tumor-promoting roles of proteases has emphasized their potential and drawn much attention to them as molecular targets.[18,24] The inhibition of (1) matrix metalloproteases (MMPs), (2) the serine protease – urokinase-type plasminogen activator (uPA) system and (3) several cathepsins (Cts): CtsB, L, S, K, Z/X and D have all been recognized as promising approaches for anticancer therapy.[2,25]

Structural information on proteases and their active sites is vital to fit, design and introduce the inhibitor molecules to the biological systems. Proteolytic blocking of these enzymes by inhibitors can naturally occur through two different mechanisms that base on their interaction with the target protease: (i) irreversible trapping interaction, which initiates by cleaving an internal peptide bond in the inhibitor and causes its permanent conformational change (Figure 1A) and (ii) reversible tight-binding reactions, which directly bind to the active site of the protease[1,26,27] (Figure 1B).

Apart from exogenous inhibiting molecules, the activity of proteases is also regulated by interactions with endogenous inhibitors. Among them, tissue inhibitors of metalloproteinases (TIMPs) are key regulators for targeting metalloproteases,[28] while serpins are directed against serine proteases[29] and cystatins are predominantly inhibiting cysteine proteases.[30] However, it is important to note that the majority of protease inhibitors may interact with more than one type of protease, eg, serpins act with serine protease but can also inhibit the cysteine proteases.[26]

Matrix Metalloproteinase Inhibitors

Two general classes of MMP inhibitors, which consist of endogenous (TIMPs) and synthetic agents, are mostly non-selective. A group of non-peptidic inhibitors, including MMI270, prinomastat, marimastat and batimastat was designed with a zinc-binding hydroxamate group.[31] Investigations to eliminate the significant limitations of early agents and decrease their side effects, low lability and specificity prompted the development of less toxic and more potent inhibitors.[31] The second generation of non-hydroxamate MMP inhibitors was designed with different peptidomimetic and non-peptidomimetic structures, such as carboxylic acids (tanomastat), thiols (rebimastat) and others.[31,32] Although the early inhibitors were improved, both the hydroxamate and non-hydroxamate groups failed to pass the early stages of human clinical trials.[33] Despite their enhanced inhibitory effects, the broad-spectrum synthetic MMP inhibitors did not meet the relevant criteria when tested in humans.[34] Moreover, there were several MMP inhibitor limitations leading to these failures including, metabolic instability, poor oral bioavailability and dose-limiting toxicities. Prolonged treatment with broad-spectrum MMP inhibitors was associated with unwanted side effects and this resulted in the use of lower, often inadequate, MMP inhibitor doses in trials.[35] After the development of more potent selective antibodies, high affinity to MMPs was achieved without side effects such as musculoskeletal syndrome.[36] One of the selective antibodies Andecaliximab (GS-5745; anti-MMP-9), has undergone clinical trials and been approved for its beneficial control over tumor growth and metastasis in a colorectal
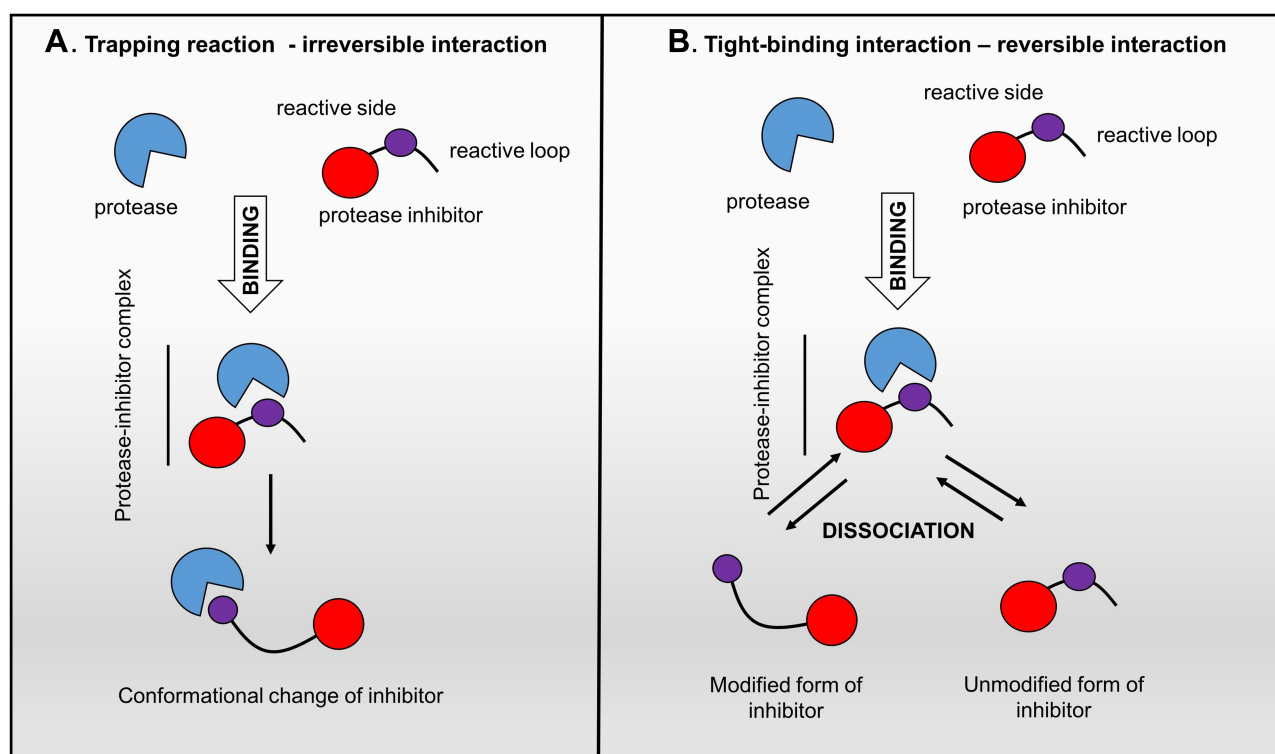
Figure 1 Protease inhibitor action mechanisms. Left panel: "trapping reaction" – the binding of inhibitor with protease activates the cleavage of an internal peptide bond, triggering an irreversible conformational change of the inhibitor; Right panel: "tight-binding interaction" – the reversible interplay of the inhibitor with the active site of protease is similar to the enzyme-substrate reaction.[27,134]

carcinoma model.[37] Nevertheless, the potential of anti-MMP antibodies has yet to be fully realized, because they undergo proteolytic degradation during circulation, limiting systemic therapeutic application and synthesizing the agent at a reasonable cost is difficult and relatively slow.[38,39]

Importantly, the failure of MMP inhibitor treatments was also partly due to the inhibition of MMP anti-targets that are critical in host protection.[40] It is tightly connected with the role of MMPs in the homeostatic regulation of the extracellular environment and controlling innate immunity.[41] Thus, the knock-down of MMPs, such as MMP-3, −8 and −9 enhanced tumorigenesis and metastasis in some animal models of cancer.[42,43] For a promising cancer therapy by MMP inhibition, the treatment must be selective against validated MMP targets.

Urokinase-Type Plasminogen Activator Inhibitors

Flavonoids are a group of secondary plant metabolites with a polyphenolic structure, which have been demonstrated to inhibit trypsin-like serine proteases such as uPA.[44] The natural flavonoid quercetin was found to inhibit serine proteases and show anti-metastatic effects against a broad range of cancer models such as prostate, cervical, lung, breast and colorectal cancers.[44,45] Research with uPA inhibitors developed based on aryl guanidine, aryl amidine or acyl guanidine backbones has shown their modest potency and poor selectivity.[46] One uPA inhibitor – mesupron has been studied in clinical trials and entered Phase II, presenting a suppressor effect on breast cancer metastasis and reduction of primary tumor growth.[47] However, its specificity is too low due to it targeting the S1 pockets, which are highly conserved among the serine protease family.[48] In contrast, peptide-based inhibitors (cyclic peptides) showed higher selectivity for contacting multiple other regions outside the S1 pockets.[49] Their unique structure provided by cyclization makes them more resistant to proteolytic degradation and better absorbed after oral administration.[48,50] Cyclic peptides are of considerable interest as uPA modulators because they exhibit much higher specificity than small-molecule inhibitors.[51] For example, a peptide-based uPA inhibitor CJ-463 (benzylsulfonyl-D-Ser-Ser-4- amidinobenzyla-mide) with Ki (inhibitory constant) value of 20 nM[51]

experimentally demonstrated good anti-tumor efficacy in a lung cancer animal mode.[51,52]

Cathepsin Inhibitors

Cts are expressed as pro-enzymes with inactive form, which on cleavage of the pro-domain, are usually dissociated from mature enzymes, thereby activating their catalytic function, whereas endogenous and exogenous protease inhibitors regulate their activities.[53] The endogenic inhibitors including cystatins, thyropins, alpha2-macroglobulin, cytotoxic antigen 2β and members of the serpin family protect Cts from lysosomes that may be released, but they also possess other specialized roles.[53] Therefore, misbalanced ratio of inhibitor family members to Cts can promote tumor growth, invasion and metastasis.[18] Exogenous inhibitors can be divided into molecules isolated from animals, microorganisms, plants and fungi, monoclonal antibodies and small synthetic molecules. Among the natural inhibitors, the best-known is E-64 (1-[L-N-(*trans*-epoxysuccinyl)leucyl] amino-4-guanidinobutane), a wide-range peptidase inhibitor, isolated from *Aspergillus japonicus*, which serves as the base for more specific derivatives.[54] Owing to the low cell permeability, off-target binding and the irreversible nature of inhibition, development has been directed towards covalent and non-covalent reversible inhibitors.[55] The difference is based on the mechanistic approaches to enzyme inactivation. Generally, irreversible covalent inhibitors, permanently modify the protein of interest via the formation of a stable bond; its an off-rate that is slow relative to the rate of re-synthesis of the target protein.[52,56] Reversible inhibitors bind to the target protein using non-covalent or covalent interactions, depending on the binding strength; reversible covalent bonds increases the binding affinity to proteases by 10–1000 times.[57] While both reversible and irreversible inhibitors have their pros and cons, there has been a tendency to avoid irreversible, covalent inhibitors due to the risk of the immune response,[58,59] unpredictable side effects (such as the generation of allergenic modified proteins; haptens), non-specific, irreversible modification of off-target proteins; and the difficulty in tracking metabolites when covalently bound to proteins.[55]

Irreversible specific CtsB (CA-074 and CA-030[60]) and CtsL (CLIK-148 and CLIK-195[61]) inhibitors have been developed from the structure of epoxysuccinate derivatives using X-ray crystallography and in vivo test results have shown that they reduced breast cancer metastasis.[62]

Oral therapy using the reversible CtsK inhibitor (L-235) can protect against tumor-induced osteolytic lesions and cortical disruption. The administration of L-235 in an experimental model of breast cancer bone metastasis reduced skeletal tumor burden.[63]

Furthermore, several small molecule inhibitors of cathepsins have been tested in clinical trials with good outcomes observed. Following, a selected anti-CtsS antibody, Fsn0503, significantly blocked the invasion of different tumor cell lines with the most significant result for colorectal carcinoma cells (HCT116). Moreover, a better anti-tumoral effect of Fsn0503 was shown in vivo where it retarded the tumor vascularization.[64,65]

Future Aims for Protease Inhibitors Designing

Most protease inhibitors (eg marimastat, prinomastat, non-hydroxamates neovastat, rebimastat, and tanomasta), which have been tested in pre-clinical and clinical trials for cancer treatments, have failed due to poor outcomes and pharmacological effects.[33,34,66] Only a few, such as proteasome (multicatalytic protease complex) inhibitors: bortezomib, carfilzomib, ixazomib and MMPs inhibitor (doxycycline) have passed the Food and Drug Administration (FDA)-approval process. Using them with other therapies, including a combination of proteasome inhibitors with DNA-damaging drugs (eg doxorubicin and melphalan), has shown promise in the treatment of multiple myeloma and demonstrated the potential benefits for the development of antitumor therapy.[67,68]

Taken together, the in-depth exploration of natural inhibitors and synthesis of peptidomimetic antagonists, which control proteases, has provided many promising approaches for cancer studies.[46,69] However, therapy with protease inhibitor is complicated in practical applications since different types of cancers use various proteases at different stages of tumor.[3] As a result, no single inhibitor can be used on all classes of proteases. To develop suitable protease inhibitors successfully, the small molecule inhibitors need to be designed and tested against dedicated diseases and tissue.[70,71] In this context, transcriptomic and proteomic analyses are crucial to validate inhibitors as specific targets.[72,73] Additionally, comprehensive molecular experiments need to be performed to investigate the cross-talk between signal transduction pathways and protease activation cascades,[70,74] which will be a base for maximizing their specificity and approving their repressor potential in vivo.

Moreover, as an artificial class of drugs, protease inhibitors need to be designed with proper conformation to provide an extended interaction with specific proteases, while at the same time avoiding random degradation by the digestive tract, in plasma and inside cells.[46,75]

Furthermore, protease inhibitor delivery can incur problems due to (1) low membrane permeability,[59] (2) rapid elimination from plasma,[76] (3) high first-pass metabolism,[77] (4) very low oral bioavailability,[78] (5) fast renal clearance[79] and (5) short in vivo retention times.[80] To address these obstacles, finally, nanotechnology has been deployed to ensure the bioavailability of these inhibitors at the target site, thereby rendering improved pharmacokinetic and drug performance properties. In recent years, novel nanoparticle (NP) formulations such as non-traditional drug distribution platforms have been studied extensively for selective targeting and neutralizing proteases.[81–85]

Nanosized Drug Delivery Systems

Nanosized drug delivery systems are rapidly being developed in science and medicine where materials (liposomes, solid lipid NPs, dendrimers, polymers, silicon or carbon materials and magnetic NPs) in the nanoscale (10 nm to < 1000 nm) are used to serve as a scaffold for both diagnostic and therapeutic moieties.[86] NP-based drug delivery systems enable the encapsulation of a variety of therapeutic agents, such as protein-based drugs, nucleic acids, peptides and small-molecule inhibitors, thereby increasing their therapeutic index and clinical significance (Figure 2).[87] Considerable efforts have been devoted to improving novel nano-platforms toward the development of next-generation NP-based delivery systems.[88–91] Among nanomaterials, ultra-small fluorescent metal gold nanoclusters[92] (size < 2 nm) have gained increased attention for fluorescence imaging,[93–95] diagnosis[96] and cancer therapy.[97,98] Protein protected gold nanoclusters (GNC) as a dual-functional nanoplatform for drug delivery and fluorescence imaging of tumors have recently been developed. The GNC were conjugated with a reduction-sensitive cisplatin prodrug and then functionalized with folic acid target ligand, targeting breast tumors.[99] GNC could efficiently inhibit the growth of the primary tumor and suppress the metastasis of cancer cells. Insights for designing new GNC-based drug delivery systems are reported by Wu and coworkers who developed gold
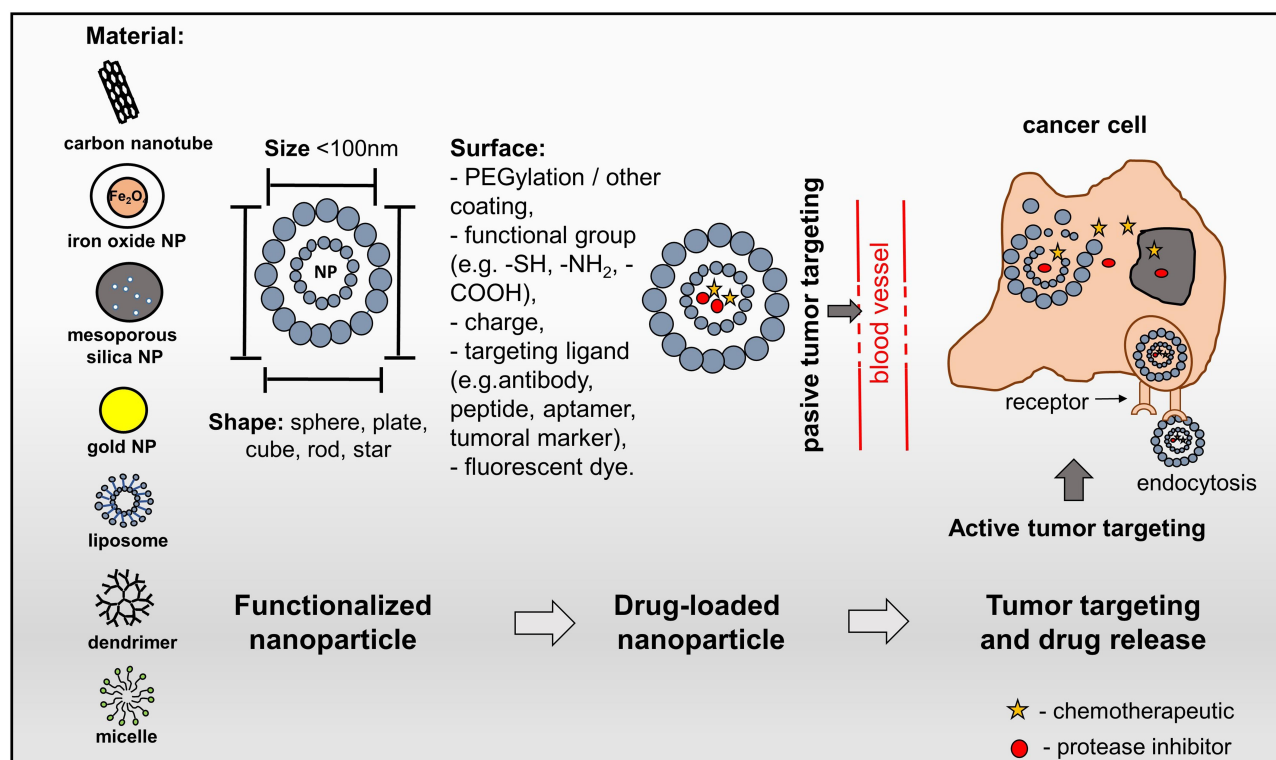

Figure 2 Nanostructured drug delivery systems. Schematic representation of the nanocarriers used in smart drug delivery systems showing their structure: size, material, shape and surface. A smart drug delivery system uses passive targeting and active targeting. Passive targeting employs the enhanced permeability and retention to locate cancer sites. The accumulation rate of nanoparticles (NPs) in a tumor is higher than in healthy tissue due to the leaky endothelium of the tumor vasculature. Furthermore, a deficiency of the lymphatic system leads to the retention of NPs in the tumor. Active targeting uses the ligand-receptor interaction to locate cancer cells; drug-loaded NPs are conjugated with targeting ligands.[135,136]

nanocage/cluster hybrid structures displaying high antitumor efficacy owing to the combined actions of epidermal growth factor receptor signaling pathway blockage and photothermal therapy.[100] These have been developed as an all-in-one biomedical platform with proposed configurations with (i) significantly improved stability and solubility of treatments, (ii) prevention of degradation or premature interactions, (iii) improved drug absorption, (iv) targeted delivery of the drug to specific tissues, cells or subcellular compartments, (v) controlled drug distribution and pharmacokinetics.[101,102] Furthermore, by engineering the NP formulations, more properties could be incorporated into the NPs according to requirements of disease states, such as multi-drug co-administration, programmed drug-delivery or triggered drug release, thereby reducing adverse effects of inhibitor/drug combinations to the healthy tissues or organs and improving the quality of life for patients.[101,102]

Consequently, in this context, formulating protease inhibitors with nanostructured carriers presents new perspectives on protease-targeted cancer therapies. Currently, there are several promising studies in this field. The proteasome inhibitors, bortezomib- and carfilzomib-loaded different NP systems (such as PEGylated lipid micelle, liposomes, gold NPs, mesoporous silica NPs) have demonstrated superior therapeutic effects such as biocompatibility, higher efficacy, increased circulation time and decreased systemic toxicity as compared to free drug formulations in breast cancer models, non-small cell lung and colon carcinomas.[103,104]

Combination of Matrix Metalloprotease Inhibitors with Nanosized Drug Delivery Systems

Wide-range MMP inhibitor (marimastat), loaded into lyso-lipid-containing thermosensitive liposomes (LTSLs), has exhibited higher accumulation in tumor tissue accompanied by decreased cancer angiogenesis and metastatic potential. Compared to saline control, the treatment with marimastat-LTSLs of mice-bearing the human breast tumor presented a 20-fold decrease in tumor growth and lower metastatic potential. Moreover, the MMP-2 and MMP-9 expression and activity were remarkably reduced.[105] To conclude this effect, the encapsulation of active compounds into the bilayer of the liposomes facilitates the drug solubilization in aqueous media and also provides additional protection and control against drug degradation.[106]

Doxorubicin and Fab fragments (Fab'222–1D8) co-conjugated PEGylated liposomes directed against membrane type-1 matrix metalloproteinase led to reduced tumor growth and side effects, while prolonging the survival time of tested mice.[107] PEGylated liposomes contain a hydrophilic polymer polyethylene glycol (PEG) moiety onto the surface of the vesicle.[108] Herein the Fab′ fragments of antibody were conjugated at a distal end of PEG for sterically stabilization of doxorubicin-encapsulating liposomes. This modification significantly enhanced the cellular uptake of carriers into the HT1080 fibrosarcoma cells in comparison with the non-targeted liposomes. Systemic in vivo administration of Fab-doxorubicin structure into the tumor-bearing mice showed the notable suppression of tumor growth compared to control nanocarriers without Fab fragment.

Another strategy, which combines a monoclonal nucleosome-specific antibody (mAb 2C5) expressed on carcinogenic cells with an MMP-2 cleavable peptide, provided additional selectivity for tumor cells and shielded a carried cell-penetrating peptide.[109] This system represents a liposomal nanocarrier in which two-sensitive bond between PEG and lipid undergoes cleavage in the tumor by the highly expressed extracellular MMP2. The conjugates combined with mAb 2C5 allow for the specific targeting of tumor cells. Additionally, cell-penetrating peptide triggering the enhanced intracellular delivery of the system after long-chain PEG removal and exposure. As a result, paclitaxel loaded PEG-poly-caprolactone NPs, which formulated with cell-penetrating peptides, attached through an MMP-2/9 cleavable sequence and showed longer survival in mice with glioblastoma than in controls.[110]

Combination of Urokinase-Type Plasminogen Activator Inhibitors with Nanosized Drug Delivery Systems

The novel formulation strategy, which encapsulated a high concentration of arsenic (As) in a stable precipitate with nickel acetate, was presented as a treatment that inhibited tumor cell growth in an orthotopictriple-negative human breast cancer xenograft model.[111] However, in further experiments, this composition showed a poor pharmacokinetic profile, including a short plasma half-life and dose-limiting toxicity, therefore As_2O_3 formulation served as a solution for these disadvantages.[112] To improve the antitumor properties of arsenic nanobins, then the anti-uPA antibody (ATN-291) was conjugated.[113] In experiments with ovarian cancer cells, uPA-targeted nanobins were internalized about four-fold higher when compared with

untargeted nanobins. The in vivo treatment with ATN-293-As$_2$O$_3$ nanobins decreased 47% of tumor burden in tested mice bearing orthotopic ovarian tumors.

Besides, several groups have demonstrated that NPs with different scaffolds (like liposomes, iron oxide, or gold NPs) conjugate with an inactive amino-terminal fragment (ATF) or 11-amino-acid sequence (U11) to target the expression of uPA in cancer cells.[114–116]

Iron oxide nanoparticles conjugated with ATF can work as the therapeutic system internalized via endocytosis pathway[117] and also provide probes for monitoring the drug delivery by using MRI.[118] Following, it was shown that ATF-conjugated iron oxide nanoparticles loaded with doxorubicin,[118] noscapine[119] or gemcitabine[120] efficiently inhibited the breast, prostate and pancreatic tumor growth, respectively. Furthermore, the retained MRI contrasting effect making them as useful for the theranostic approach.

In another technique, PEGylated polylactide-based NPs, activated by uPA-specific sequence, have been designed to hinder nonspecific internalization of cell-penetrating peptides (CPP) and improve the therapeutic potential of drug treatment with paclitaxel.[121] CPPs are a class of peptides with the ability to cross the cellular membrane without incurring significant cytotoxicity, therefore they became a good vectors for carrier systems.[122] Due to the ectopic expression of uPA in tumor tissues, the uPA specific sequence was added to the CPP-conjugated nanoparticles and protected them from nonspecific internalization. Next, the uPA cleavage allowed the CPP-conjugated nanoparticles to internalize. The uPA-activated CPP-conjugated nanoparticles loaded with paclitaxel showed greater therapeutic efficacy in an in vivo glioma mouse model.[121]

Combination of Cysteine Cathepsin Inhibitors with Nanosized Drug Delivery Systems

Encapsulated cystatin into PLGA (Poly(lactic-co-glycolic acid)) has been shown to be more stable compared to its free form, while strongly absorbed by human breast epithelial cell line, MCF-10A neoT and presenting significantly decreased CtsB activity. The created complex was able to pass from cellular membrane to the lysosomal compartments that are rich in proteases enzymes after five minutes through endocytosis.[123] At the other work, the same carriers were loaded with cystatin and labeled with the monoclonal antibody anty- cytokeratins, which are overexpressed in breast tumor cells. This selective

uptake of tested treatment was presented in co-cultures of invasive breast cells, enterocytes (Caco-2) and monocytes/macrophages. Additionally, the provided drug delivery system significantly inhibited the proteolytic activity and reduced the invasive and metastatic potential of tumor cells.[124] On the other hand, the broad-spectrum inhibitor JPM-565 has been successfully loaded in ~95 nm lipidated magnetic nanocarrier (ferri-liposomes) and tested in in vivo orthotopic murine models of primary mammary cancer (MMTV-PyMT). The results showed that the liposome-based application markedly decreased the cysteine Cts activity and, consequently, the tumor growth. Importantly, these nanocarriers possess contrast properties and were able to target the tumoral part and its microenvironment – stroma cells.[125] Next, the lipidated CtsB inhibitor (NS-629) was designed to target extracellular CtsB exclusively. NS-629 was incorporated through a lipid linker into the preformed sterically stabilized PEG-coated nanosized stealth liposomes, thereby forming a lipidated nanocarrier capable of specific CtsB targeting (LNC-NS -629). Encapsulated doxorubicin in LNC-NS-629 has demonstrated a 22-fold improvement in inhibitory activity as compared to its free form against mammary tumor cells.[126]

Future Aims for Nanosized Drug Delivery Development

Although remarkable efforts have been made in recent years toward the development of next-generation NPs to improve the pharmacological and therapeutic properties of traditional medicine, there are still rate-limiting steps that need to be addressed. NP-based targeting systems can be subjected to aggregation with their small size and large surface area, or be phagocytosed by macrophages, which may cause cytotoxic effects through their intracellular degradation.[127] Other important issues, including (1) low drug loading capacity,[128] (2) lack of control during the distribution of carriers,[129] (3) lack of biocompatibility,[130] (4) controlling the interaction with biological barriers,[131] (5) detecting and monitoring the exposure level[132] and (6) assessing the impact on the environment,[133] are also seen as significant drawbacks. In this context, novel techniques for developing and implementing nano-devices with approved quality and severe toxicity are needed. A real therapeutic breakthrough can be achieved solely by carrying out rigorous studies in the field of nano-therapy. As a result of these,

nanosized systems combining multifunctional properties with pharmacological targets may improve the curative effect of antagonist agents and thus contribute to making an effective cancer treatment tool.

Conclusions

Conventional chemotherapy uses individual anti-cancer agents or combinations for the treatment of tumor cells, which intervenes primarily with the macromolecular synthesis processes, interfering with DNA synthesis and mitosis and leading to the death of proliferating cancer cells. However, tumor cells often present challenges to chemotherapeutic agents and endow high levels of chemoresistance. Therefore, new therapeutic approaches are urgently needed. The protease inhibitors are promising candidates for anti-cancer agents. By combining them with NP-based delivery systems, they can be exploited as a powerful medical tool, which may help to overcome the chemoresistance. This novel therapy form may substantially improve drug bioavailability, increase circulation time and achieve specific accumulation of treatment in the tumor tissues. Eventually, the decreased doses will protect healthy tissues, reduce side effects and ultimately lead to better outcomes and a higher quality of life for patients.

Abbreviations

HIV, human immunodeficiency virus; MMP, matrix metalloprotease; uPA, urokinase-type plasminogen activator; Cts, cathepsin; Ki, inhibitory constant; TIMP, tissue inhibitors of metalloproteinase; FDA, Food and Drug Administration; NP, nanoparticle; GNC, gold nanocluster; LTSL, lysolipid-containing thermosensitive liposome; PEG, polyethylene glycol; As, arsenic; U11, 11-amino-acid sequence; ATF, amino-terminal fragment; CPP, cell-penetrating peptide; PLGA, poly(lactic-co-glycolic acid).

Acknowledgments

We thank Alexandr V. Bazhin for fruitful discussions in relation to this paper.

Funding

Disclosure

The authors report no conflicts of interest in this work.

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