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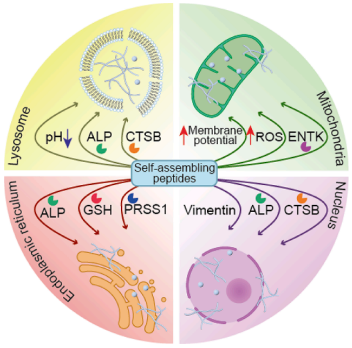
From cells to subcellular organelles: Next-generation cancer therapy based on peptide self-assembly

Huayang Liu, Huaimin Wang *

Department of Chemistry, School of Science, Westlake University, No. 600 Dunyu Road, Sandun Town, Hangzhou 310024, Zhejiang Province, China
Institute of Natural Sciences, Westlake Institute for Advanced Study, 18 Shilongshan Road, Hangzhou 310024, Zhejiang Province, China

GRAPHICAL ABSTRACT



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ABSTRACT

Due to the editability, functionality, and excellent biocompatibility of peptides, in situ self-assembly of peptides in cells is a powerful strategy for biomedical applications. Subcellular organelle targeting of peptides assemblies enables more precise drug delivery, enhances selectivity to disease cells, and mitigates drug resistance, providing an effective strategy for disease diagnosis and therapy. This reviewer first introduces the triggering conditions, morphological changes, and intracellular locations of self-assembling peptides. Then, the functions of peptide assemblies are summarized, followed by a comprehensive understanding of the interactions between peptide assemblies and subcellular organelles. Finally, we provide a brief outlook and the remaining challenges in this field.

1. Introduction

Cancer is a leading cause of death globally, responsible for nearly 10 million deaths in 2018, as reported by the World Health Organization (WHO) [1]. Traditional cancer treatments often come with numerous side effects. Postoperative pain is a common phenomenon after surgical treatment [2]. Typically, discomfort and fatigue persist throughout the entire recovery period. Chemotherapy and radiotherapy can also induce

* Corresponding author at: Department of Chemistry, School of Science, Westlake University, No. 600 Dunyu Road, Sandun Town, Hangzhou 310024 Zhejiang Province, China.
E-mail address: wanghuaimin@westlake.edu.cn (H. Wang).

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Table 1
Representative examples of functional peptides self-assembly in the lysosome.

No.	Stimulus	Morphology	Function	Ref.
L1	pH	Nanofiber	Enlarging the lysosome and increasing the permeability of the lysosome leading to cancer cell death	[74]
L2	pH	Nanofiber	Disrupting the lysosomal membrane and induce cellular apoptosis	[80]
L3	pH	Nanosheet	Disrupting the endosomal membrane and delivering antigenic peptide to cytoplasm	[82]
L4	pH	Nanofiber	Disruption of the lysosome, cisplatin sensitization, and cell death	[83]
L5	ACP	Nanofibrous network	Regulating the formation of sit-specific nanofibers through multistage self-assembly inside living cells	[84]
L6	ALP	Nanofiber	Selectively killing cancer cells by the formation of enzyme-triggered peptide assemblies due to the difference in endocytic uptake	[85]
L7	CTSB	Nanofiber	Inducing LMP and selective necroptosis	[86]
L8	CTSB	Nanoparticle	Contributing to the intracellular accumulation of the probe	[87]
L9	CTSB	Nanofiber	Disrupting lysosome, facilitating the release of siRNA for subsequent gene silencing	[88]
*L*10	CTSB	Nanofiber	LMP leaded to lysosomal damage, ROS generation and apoptosis	[89]

ACP, acid phosphatase; ALP, alkaline phosphatase; CTSB, cathepsin B.

diverse side effects, including hair loss [3], skin changes [4], nausea [5] and appetite loss [6]. New compounds or materials designed with specific functions to mitigate these drawbacks caused by surgeries and drugs in the cancer region have succeeded in some contexts [7–15]. Among them, peptides have been widely studied because of their programmable, low-cost, and biocompatible properties. Various functions, such as therapy, imaging, and drug delivery, can be achieved by designing in situ self-assembly peptides targeting cancer cells.

Molecular structure and the corresponding physicochemical properties can influence the formation of peptide assemblies. First of all, special secondary structures of peptides could contribute to the occurrence of self-assembly, including α-helix, β-sheet, and β-hairpin. α-helix is the main secondary structure in proteins [16], while most peptide fragments would lose the α-helix structure after separation from original proteins. The stable amphiphilic structure provided by α-helix could promote the self-assembly of peptides. β-sheet is a secondary structure of peptides arranged regularly, the binding between hydrophobic motif of peptide molecules is more likely to induce peptides self-assembly into nanofibrous morphologies [17,18]. Similarly, some peptides with an alternating sequence of hydrophobic and hydrophilic amino acids tend to form β-hairpin and subsequently assemble to nanofibrils [19,20]. Secondly, the widespread of non-covalent interactions play a role in self-assembling of peptides, including hydrogen bonding, π-π interactions, hydrophobic interactions and electrostatic interactions. These non-covalent interactions primarily depend on the residues of peptides. For example, electrostatic interactions are induced by the charged peptides containing aspartic acid, glutamic acid, arginine, histidine or lysine. However, π-π interactions only exist between aromatic amino acids, such as phenylalanine, tryptophan and tyrosine.

Self-assembling peptides could be designed using different modules, including a self-assembly module and a functional module. The self-assembly module could promote the self-assembly ability of peptides through the aforementioned interactions like diphenylalanine (FF) [21]. The functional module, typically drug molecules, could endow the self-assembling peptides with specific functions. Moreover, the targeting modules like triphenylphosphonium (TPP) [22] or RGD [23] enable peptides accumulate in target region and subsequently self-assembly at critical aggregation contentions. The responsive modules enable self-assembly of peptides being triggered by changes in environmental conditions such as temperature [24], light [25], pH [26], enzyme [27] and so on. The introduction of targeting modules or responsive modules enables peptides to exert their functions in targeting regions or under

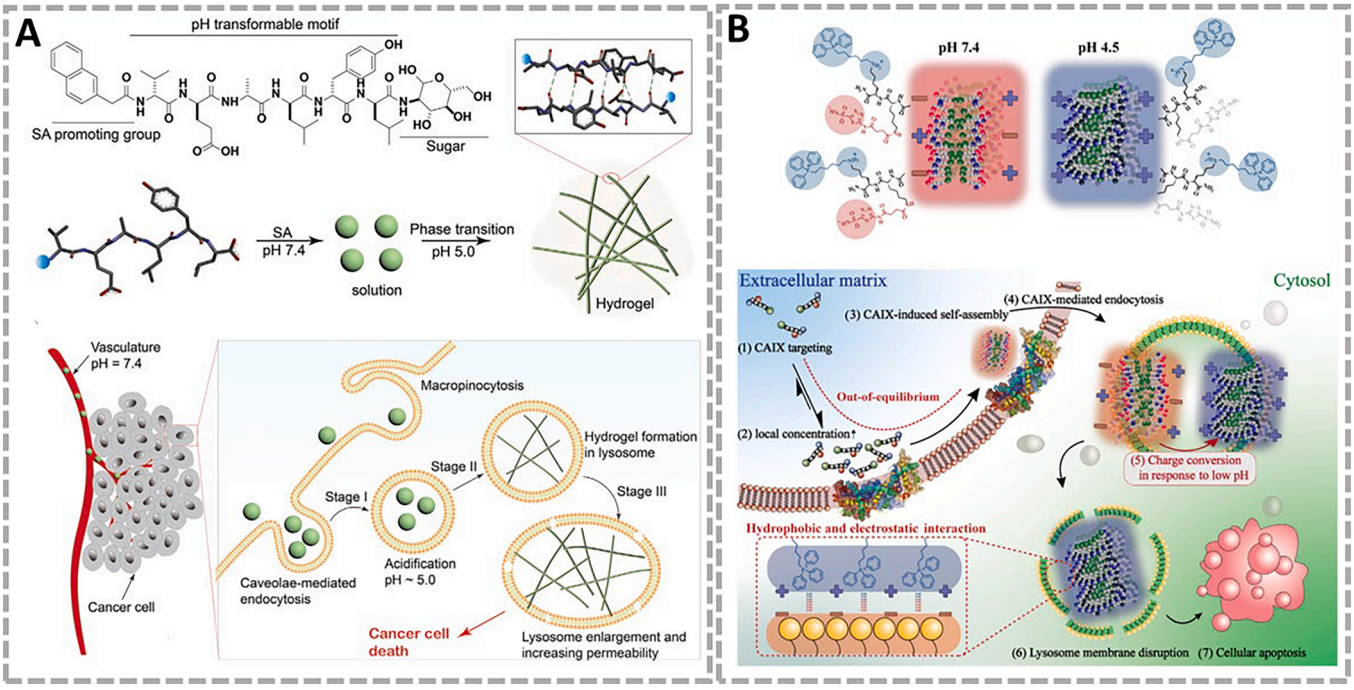

Fig. 1. pH-responsive peptide self-assembly in lysosome. (a) ph-triggered phase transformation of hexapeptide induces the enlargement of the lysosome and increases the permeability of the lysosome, leading to cancer cell death. Adapted with permission from Ref. [74]. (B) The protonation of acetazolamide at low pH triggers the formation of highly ordered chiral fibrous structure inside the lysosome, resulting in lysosomal membrane disruption and subsequent cell death. Adapted with permission from Ref. [80].

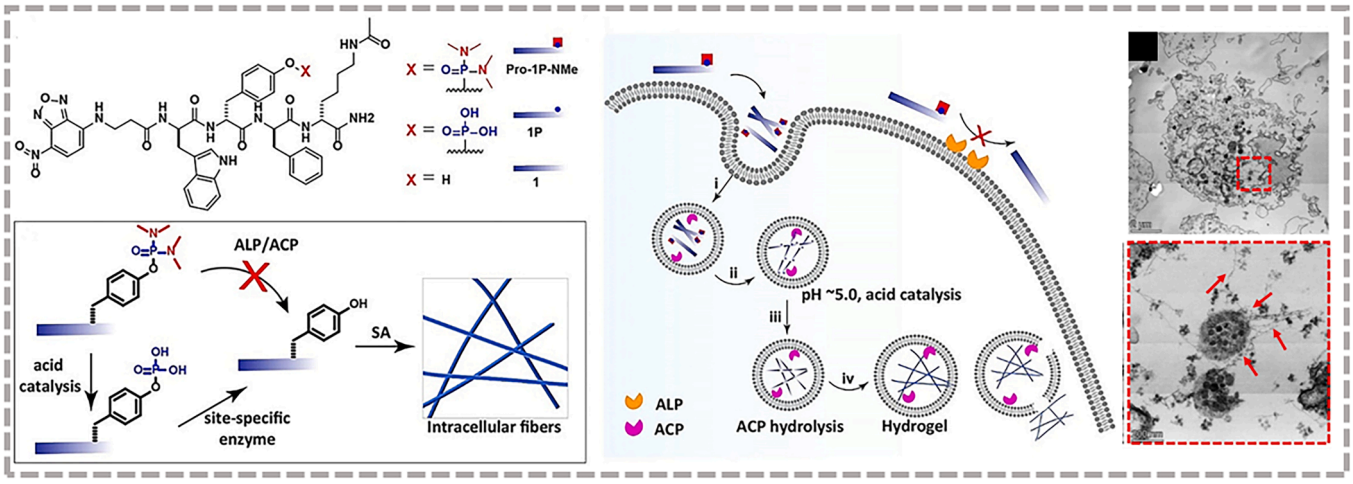

Fig. 2. ACP specifically removes the phosphate groups, leading to the subsequent formation of peptide-based nanofibrous networks inside the lysosome. Adapted with permission from Ref. [84].

specific stimuli. Thus, through this modular design approach, the structure and morphology of self-assembling peptides are programmable and controllable for various biological applications. Self-assembling peptides could form functional materials applied in various fields. For example, the hydrogel formed by self-assembling peptides could mimic the extracellular matrix for supporting cell migration, proliferation, tissue regeneration and angiogenesis [28,29]. Besides, drug molecules and anti-inflammatory factors could be loaded in this supramolecular structure, in order to deliver drug and modulate inflammation [30–32]. In recent years, in situ self-assembling peptide materials targeting cells are also being developed to address various challenges in the field of cancer therapy.

Peptides entering into cancer cells encounter the cancer cell membrane primarily. Peptides could be uptake by cells through various mechanisms, including direct translocation and endocytosis. These peptides could translocate across the cell membrane known as cell-penetrating peptides (CPPs) or protein transduction domains (PTDs). CPPs via direct translocation are often shorter than 30 amino acids and designed with positive charged amino acids, such as arginine and lysine [33]. Some of them would exhibit a secondary structure of α-helix and β-sheet which provide peptide molecules with a hydrophilic structure [34,35]. The positive charge and hydrophilic structure of CPPs enhance the interaction with lipid molecules and help them translocate membrane. Some peptides could be uptake via endocytosis, the efficiency of this mechanism could be influenced by various factors, including sequences, charge, hydrophobicity and size. Moreover, some certain peptide segments derived from proteins or antibodies could be internalized by cells through receptor-mediated endocytosis. Several advances have been achieved by designing peptides to target specific receptors on the cancer cell membrane [36–46], leading to the subsequent formation of self-assemblies on the cell membrane due to the accumulation of precursors with a high localized concentration. Alternatively, the overexpressing enzymes on the membrane, such as alkaline phosphatase (ALP) [47–56], matrix metalloproteinase-2 (MMP-2) [57], matrix metalloproteinase-9 (MMP-9) [58,59], and fibroblast activation protein-α (FAP-α) [60,61], could be utilized to initiate self-assembly of peptide through enzymatic reaction. These in situ extracellular peptide assemblies anchor to the cancer cell membrane and could prevent cancer cell metastasis or induce cell death.

In general, drug molecules are loaded by capsule or covalent conjugation and then delivered into cells. However, physical encapsulation could lead to drug leakage in blood circulation system. Synthetic polymer systems like liposomes [62] and poly-(lactic-co-glycolic) acid [63] are limited to proteins or other unstable substances. Synthetic inorganic systems including nanocarbon materials and gold nanoparticles are often toxic and difficult to degrade [64,65]. Peptide-drug covalent conjugates alter the original drug's chemical structure, resulting in significant changes in pharmacological activity [66]. The co-assemblies of peptides and drugs provide a novel method addressing these issues. The co-assembly process between drug and peptide molecules mainly driven by noncovalent forces, such as hydrophobic interactions, π-π interactions, electrostatic interactions, hydrogen bonds, and Van der Waals force [67,68]. The main dominant force is related to the residues of peptides and drug chemical structure. The microenvironmental stimulus, including pH and enzymes, could influence these forces, leading to structural changes of peptide-drug co-assemblies and subsequent controlled drug release [69] or retention [70]. These superiorities provide potential applications for peptide-drug co-assemblies in the field of tumor therapy. Some peptides could also enter cells independently and self-assemble under intracellular microenvironment. Moreover, subcellular organelles, such as lysosomes, mitochondria, endoplasmic reticulum, and nucleus, can also serve as specific targets to confine assemblies formation and exert functions.

The strategy of subcellular organelles self-assembly peptides exhibits superior therapeutic efficacy without common side effects associated with conventional treatment strategies. Conventional clinical anticancer drugs typically have several modes of action [71], including alkylation of DNA, inhibition of enzyme activity or genetic translation and so on. These modes affect both cancer cells and normal cells, leading to significant side effects. Thus, the selectivity of anticancer drugs is crucial for clinical therapy. While targeted molecular drugs could achieve selective killing, some shortages like drug efflux and acquired drug resistance, still limit their applications. The system of self-assembling peptides is designed according to the microenvironmental difference between cancer cells and normal cells, such as pH, enzymes, membrane potential and so on, leading to specifically target cancer cells and even subcellular organelles. Peptide self-assemblies targeting subcellular organelles could deliver anticancer drugs effectively to tumors or the intracellular region where drugs exert effects, which holds great potential for addressing the issues of conventional anticancer drugs. This mechanism could prolong the retention time and promote therapeutical efficacy of drugs [72,73]. Meanwhile, Intracellular self-assemblies of peptide could influence cellular functions, resulting in cell death without inducing drug resistance [74]. Similarly, the strategy of utilizing peptide self-assemblies could improve the stability and targeting ability of radiosensitizers [75,76] or gene therapeutic agents [77].

However, in vivo stability of peptide is a crucial factor for determining their therapeutic efficacy. Peptides are susceptible to numerous proteases present in the body, which could cause rapid degradation and clearance from the system resulting in the loss of therapeutic

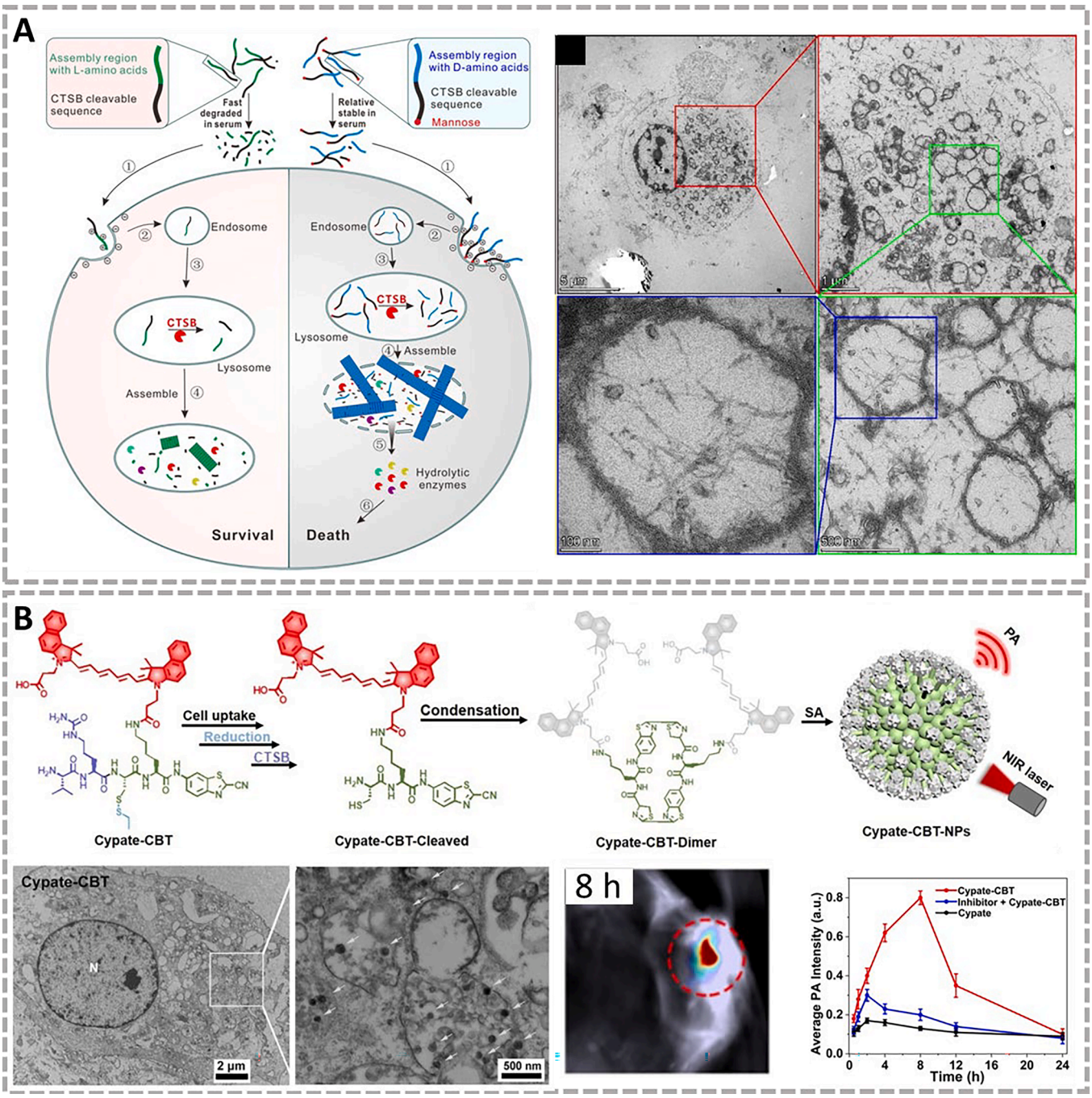

Fig. 3. Enzyme-triggered peptide self-assembly in the lysosome. (A) Left: D-amino acid substitution contributes to the stability of peptide in serum and C-terminal modification by mannose enhance the endocytosis to the peptide. Upon CTSB cleavage, peptides self-assemble in lysosomes that induce lysosomal membrane permeabilization (LMP), resulting in glioblastoma cancer cell death. Right: Peptides self-assemble into nanofibers in lysosomes of U87MG. Adapted with permission from Ref. [86]. (B) CTSB-triggered formation of Cypate-CBT nanoparticles in cells contributing to image CTSB-overexpressing tumors. Adapted with permission from Ref. [87].

effectiveness. Therefore, various strategies were carried out to address this issue. For example, L-amino acids could be substituted by D-enantiomers due to their less susceptibility to enzymatic degradation. Adding unnatural amino acids into sequences or protective groups to amino acids could increase the proteolytic resistance. These strategies could enhance the in vivo stability of peptides and subsequent therapeutic efficacy. Most peptide materials could be degraded by enzymes in vivo, and the cleaved short peptide fragments are generally biocompatible. However, some chemical modifications could enhance the proteolytic resistance of peptide, but might also exhibit a degree of cytotoxicity. Therefore, stimulus-triggered peptide functional materials with selective targets could effectively address these issues and enhance the therapeutic efficacy.

This review discusses the most recent strategies and highlights representative examples of functional peptide self-assembly within subcellular organelles over the past five years. We first introduce the cellular triggers that induce peptide self-assembly and related morphologies of peptide assemblies. Subsequently, we discuss the functions of these nanostructures formed in subcellular organelles. Finally, we provide a brief outlook on the challenges and potential applications of

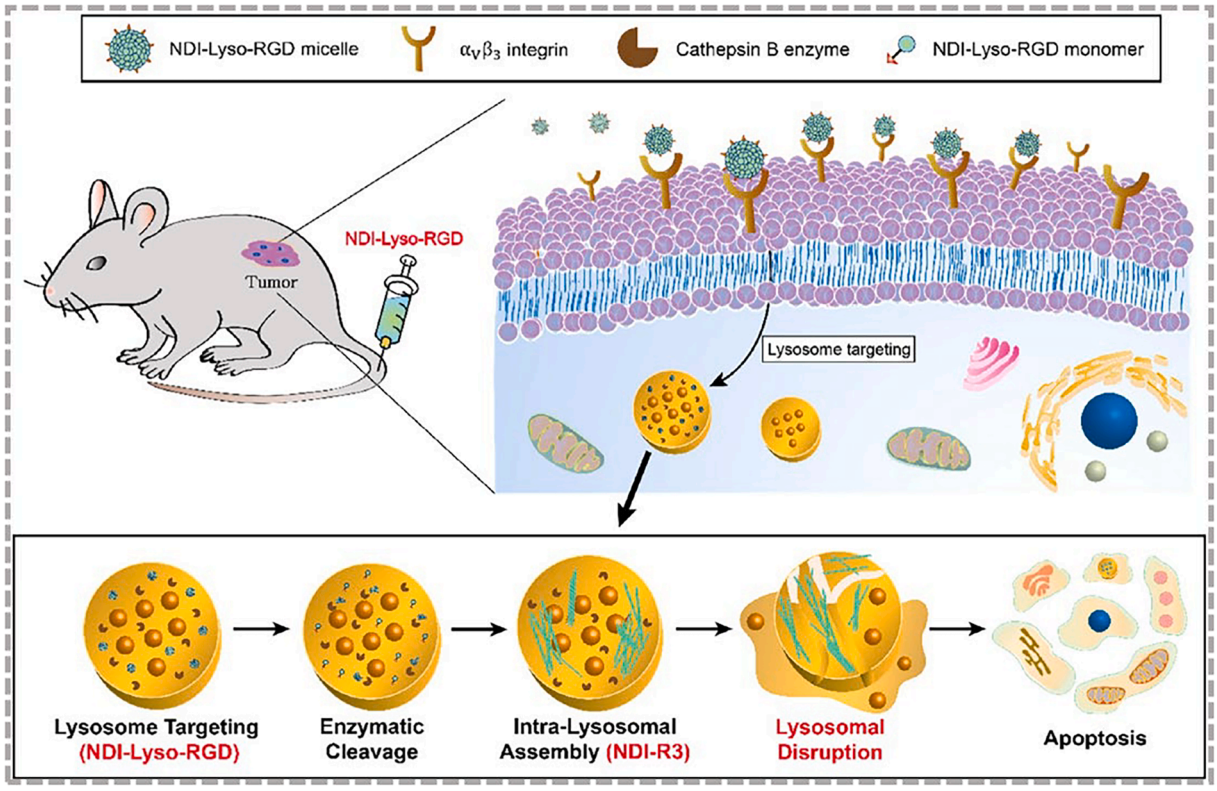

Fig. 4. CTSB-instructed peptides self-assembly in lysosomes destructs the integrity of lysosomal membrane, leading to lysosomal swelling and LMP. LMP further induces ROS generation and apoptosis. Adapted with permission from Ref. [89].

organelle peptide self-assembly.

2. Functional peptide self-assembly in subcellular organelles

Peptide self-assemblies in subcellular organelles have great potential to improve therapeutic efficacy and selective index because of their specific targeting and accumulation of increased retention. Generally, the design of these peptides is guided by the local environments. This section discussed the triggering stimuli, self-assembly morphologies, and their related functions from representative examples.

2.1. Lysosome

Lysosomes play an essential role as the waste disposal system in cells [78,79]. The complex microenvironment in lysosomes, such as pH and various enzymes, can be utilized to trigger peptide self-assembly (Table 1 and Figs. 1–4).

The pH inside the lysosome ranges from 4.5 to 5, lower than that in the cytoplasm [81]. The presence of protons in this acidic condition could induce the peptide self-assembly by protonation of some amino acids. For example, Wang et al. rationally designed aromatic capped glycopeptides that can be uptake by cells through endocytosis [74]. The acidic microenvironment in the lysosome protonated the glutamic acid on the peptide, weakening electrostatic interactions and strengthening other interactions between peptide molecules, for example, hydrogen bonding (Fig. 1A). Thus, the morphology of peptides changed from nanoparticles to nanofibers due to this mechanism. The formation of nanofibrous network enlarged the lysosomes and increased the permeability, leading to necroptosis and apoptosis of cancer cells (Table 1, L1). The changes of lysosome lead to cellular impairment, subsequently triggering necroptosis. Besides, the intracellular self-assemblies of peptides induce intrinsic cell stress, leading to the initiation of cell apoptosis. Liang et al. reported a polymer-peptide conjugate-based nanotransformer that could form nanosheets in acidic media [82]. The larger structures could induce membrane disruption and cytosolic delivery of the antigenic peptide (Table 1, L3), proving that the proton-driven transformable nanovaccines could be a powerful and safer cancer treatment. Ryu et al. reported peptide-based self-assembly fibers that respond to pH [80]. During endocytosis, the surface of nanofibers became positively charged and then the strong electrostatic and hydrophobic interactions between nanofibers and membranes leaded to the disruption of lysosomal membrane (Table 1, L2), inducing subsequent cellular apoptosis due to intrinsic cell stress (Fig. 1B). Lam's group designed peptide amphiphiles consisting of three parts: bis-pyrene as the hydrophobic motif, a peptide segment as the β-sheet forming motif and poly-D-Arg (8-mer) as the cell-penetrating motif [83]. The acidic microenvironment could change the conformation to nanofibrils within lysosomes, disrupting the lysosome, cisplatin sensitization, and cell death (Table 1, L4).

The unique or overexpressed enzymes within lysosomes could trigger the self-assembly of functional peptides. Wang et al. employed a protection strategy to prevent peptide hydrolysis by phosphatase on the membrane or within the cytoplasm [84]. After endocytosis, the acidic microenvironment in lysosomes firstly hydrolyzed P-N bond to release the acid phosphatase (ACP)-cleavable phosphotyrosine (Fig. 2). Dephosphorylation enhance the hydrogen bonding and π-π stacking between residues, leading to the formation of nanofibrous networks within lysosomes. This multistage self-assembly of peptide achieved the precise control of the nanostructure formation in living cells (Table 1, L5). Xu et al. designed a phosphopeptide that could form nanofiber to achieve selective killing cancer cells due to the differences in endocytosis comparing to normal cells (Table 1, L6) [85]. After ALP catalysis, the micelles turned into nanofibers to deactivate the IAPs in cancer cells and protect normal cells. Due to the overexpression of cathepsin B (CTSB) in lysosomes of most solid tumors, Wang et al. investigated the self-assembly structures of a series of peptides from several views, including stereochemistry, C-terminal glycosylation, and regiochemistry [86]. After CTSB cleavage, the strong hydrophobic and π-π interaction

Table 2
Representative examples of functional peptides self-assembly targeting mitochondria.

No.	Stimulus	Morphology	Function	Ref.
M1	ALP	Nanoaggregate	TPP-peptide escape from the lysosome and induce mitochondrial dysfunction, resulting in cell death	[94]
M2	High localized concentration	Nanoparticle	Inducing dysfunction of mitochondria; physical damage to the mitochondria by biomineralization	[95]
M3	pH	Nanofiber	Releasing drug CPT and decreasing the mitochondrial membrane potential, resulting in apoptosis	[90]
M4	High localized concentration	Nanofiber	Disrupting mitochondrial membrane, resulting in apoptosis	[22]
M5	High localized concentration	Nanofiber	Co-assembling into fibers with chiral molecules, subsequently leading to drastic mitochondrial disruption	[96]
M6	ROS	Nanoparticle	Inducing apoptosis of senescent cells without harm to normal cells	[97]
M7	Casp3	Nanofiber	Inducing efficient cell apoptosis and pyroptosis	[100]
M8	ALP, reductase	Nanofiber	Disrupting mitochondrial membrane, increasing level of ROS and releasing Cyt C	[99]
M9	ENTK	Nanofiber	Releasing drug molecules into mitochondria by changing morphology of the self-assembly	[98]
M10	ENTK	Nanoparticle	Selectively delivering CLRP into the mitochondria of cancer cells, resulting in cell death	[101]
M11	SIRT5	Nanofiber	Activity-based imaging of SIRT5 in living cells; depolarizing mitochondria membrane potential and promoting ROS formation	[102]

ALP, alkaline phosphatase; ROS, reactive oxygen species; Casp3, caspase-3; ENTK, enterokinase; SIRT5, Sirtuin 5.

from self-assembly region of peptides lead to the formation of nanostructures. The most active peptide could form nanofibers with cytotoxicity against cancer cells upon CTSB catalysis, leading to lysosomal membrane permeabilization (LMP) and subsequent necroptosis (Table 1, L7) (Fig. 3A). In addition, Liang's group developed a photoacoustic (PA) probe, responsive to CTSB, which could self-assemble into nanoparticles and elevate PA signal in cancer cells by enhanced accumulation of the probe (Table 1, L8) [87]. This strategy developed by this group has the potential for cancer diagnosis at early stages (Fig. 3B). Lou et al. employed a peptide-conjugated-AIEgen (aggregation-induced emission luminogens) strategy that responds to extracellular MMP-2. After MMP-2 cleavage, the two parts, FC_{siRNA} and PyTPA, experienced separate processes. The former entered the cell and generated RNA to induce gene silencing after CTSB catalysis, while the rest of the part self-assembled into nanofibers and disrupted lysosome (Table 1, L9). The latter part produced reactive oxygen species (ROS) within the cancer cell. Together, these functions collaboratively inhibit tumor growth [88]. Ryu's group designed a lysosome-targeted peptide amphiphile that could change morphology from micelle to long fiber upon CTSB cleavage [89]. Hard fibers damaged the lysosomes including swelling and membrane permeabilization, leading to ROS generation, which causes severe damage to the cells. Subsequently, the damaged cancer cell undergoes apoptosis (Table 1, L10) (Fig. 4). Overall, the main challenge in development of self-assembling peptides targeting lysosomes is their stability under lysosomal microenvironment, including pH, enzymes, ionic strength and so on. Additionally, modulating lysosomal function and developing lysosomal imaging by self-assembling peptide system could potentially revolutionize our understanding and treatment of lysosomal-related diseases.

2.2. Mitochondria

Mitochondria, acting as a powerhouse of the cell, contribute to several cellular physiological activities. Hence, mitochondria have immense potential as targets to induce cancer cell death. Consequently, a series of peptides have been designed to interfere with mitochondrial function for cancer treatment (Table 2 and Figs. 5–7).

The elevated membrane potential in the mitochondria of cancer cells [91,92] provides a targeting condition for positively charged molecules because the changes of membrane potential changes the net charges of membrane to negative charge. This biochemical alteration could be applied in peptide materials design to selectively target cancer cells without affecting healthy cells [93]. Xu and coworkers engineered a peptide with TPP targeting mitochondria and enzyme-instructed self-assembly (EISA) targeting cells [94]. Upon ALP cleavage, TPP-peptides form nanostructures via non-covalent interactions and cancer cells uptake these assemblies via endocytosis (caveolae/raft-dependent pathway). Then, these nano-assemblies escaped from lysosome and target mitochondria by TPP, resulting in mitochondrial dysfunction and cell death (Table 2, M1). Ryu's group designed a dipeptide with TPP and trialkoxysilane [95]. Due to the targeting effect of TPP, the accumulation of the supermolecule in cancer cells is 10 times greater than in normal cells. Self-assembling nanoparticle causes the dysfunction of mitochondria and subsequently biomineralization via silicification damage mitochondria physically (Table 2, M2). Yu et al. integrated a segment of pro-apoptotic protein and drug camptothecin (CPT) into a cationic pentapeptide [90]. When passing through the acidic tumor microenvironment, the self-assembling nanofibers transforms into nanoparticles, which are then internalized by cells. Intracellular physiological pH changes the morphology back to nanofibers which subsequently targets mitochondria with release of drug CPT. The accumulation and retention of nanofibers in the mitochondria changes mitochondrial permeabilization, resulting in apoptosis of cells (Table 2, M3) (Fig. 5A). Ryu's group reported that the mitochondria-accumulating peptide (Mito-FF) could form nanofibers within mitochondria due to a high localized concentration, which destroyed the mitochondrial membrane and activated cell apoptosis [22] (Table 2, M4) (Fig. 5B). Moreover, the same group reported that Mito-FF could co-assemble with its mirror pair into superfibrils to disrupt the mitochondria dramatically (Table 2, M5) and induce cancer cell death selectively [96].

Tandem reaction is a powerful strategy for introducing peptide assemblies to target mitochondria. Ryu and coworkers designed a peptide targeting integrin $\alpha_v\beta_3$-overexpressed senescent cells [97]. Elevated ROS level in mitochondria promoted disulfide bond formation, resulting in the formation of oligomers (dimers, trimers, and tetramers), which served as building blocks for self-assembly into nanosphere. The α-helix of peptide was stabilized by the self-assembling nanostructure (Fig. 6). The spherical nanostructures accumulate to mitochondria and interact with mitochondrial membrane. The damage of mitochondria subsequently induces apoptosis to achieve selective removal of senescent cells (Table 2, M6). Liang's group engineered a peptide-based molecule that responds to caspase-3 (Casp3). Upon cleavage, the porphyrin ring exhibits a strong propensity for face-to-face aggregation due to multiple noncovalent forces, including hydrophobic interaction, π-π stacking, and hydrogen bonding [100]. Subsequently, peptides forms nanofibers around mitochondria and after laser irradiation, the generated $^1O_2$ in cells could induce cell death with multiple modes, including apoptosis

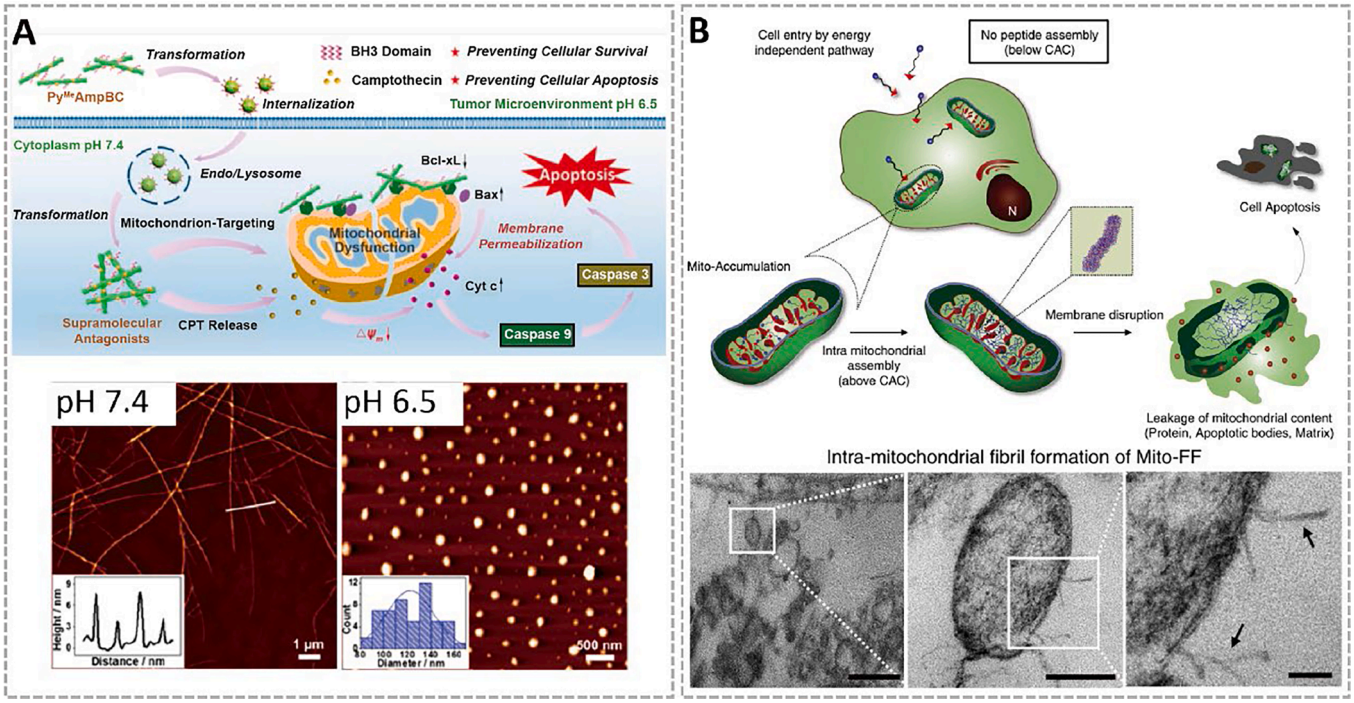

Fig. 5. Self-assembly of peptides targeting mitochondria. (A) Reversible changes in peptide morphologies that induced by pH trigger drug release, subsequently elevating mitochondrial permeability and inhibiting cellular viability. Adapted with permission from Ref. [90]. (B) The accumulation of peptides in the mitochondria induces the formation of self-assembled fibers, leading to subsequent apoptosis through the disruption of the mitochondrial membrane. Adapted with permission from Ref. [22].

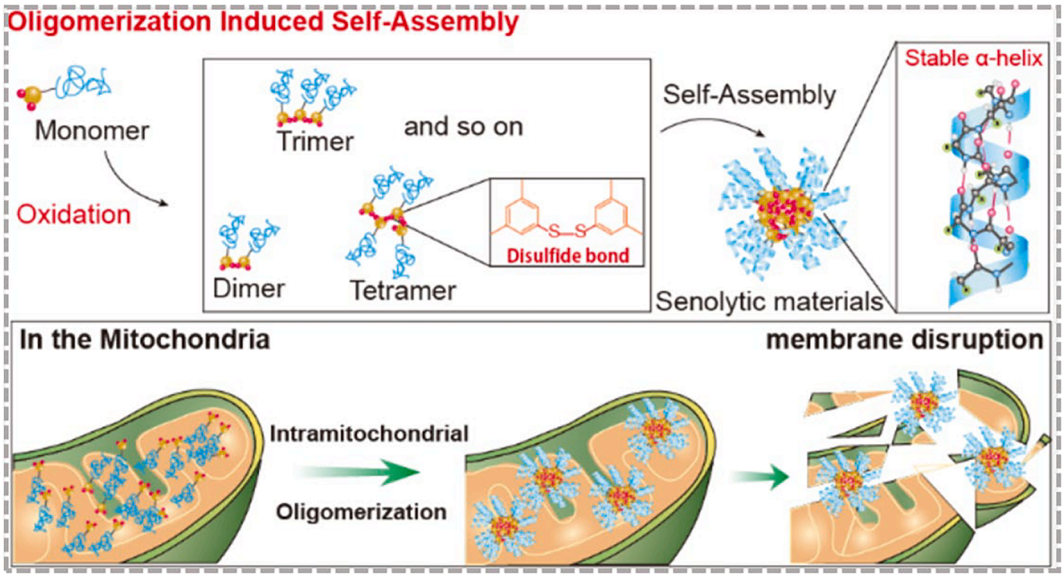

Fig. 6. Mitochondria-targeting peptides accumulated in mitochondria. Elevated ROS levels in senescent cells promote oligomerization to form spherical nanostructures, leading to mitochondrial destruction and apoptosis. Adapted with permission from Ref. [97].

and pyroptosis (Table 2, M7) (Fig. 7C). The porphyrin nanofibers greatly enhance cell apoptosis and elevated $^1O_2$ level activates pyroptosis via Casp1-dependent signaling pathway. With enhancement of these two types of cell death, this strategy exhibited great photodynamic therapy (PDT) effect. Yang and coworkers designed a peptide that can react stepwise with ALP and reductase to selectively self-assemble into nanofibers in the mitochondria of cancer cells [99]. The nanofibers can disrupt the mitochondrial membrane, leading to increased release of ROS and cytochrome *C* (Cyt C) (Table 2, M8) (Fig. 7B). Mitochondrial enzymes could also be employed to induce the self-assembly of peptides in the mitochondria. Diverging from cationic molecules that can accumulate in mitochondria, Xu's group designed an enzymatically cleavable peptide with a negatively charged motif for targeting mitochondria [98]. The micelles formed by the precursor, loaded with drug molecules, initially targeted to mitochondria. Upon the cleavage of enterokinase (ENTK), the micellar structure transformed into nanofibers, accompanied by the release of loaded drugs into mitochondria (Table 2, M9) (Fig. 7A). Based on this strategy, the same group invented a peptide-based nanoparticle for targeting mitochondria, loaded with chloramphenicol (CLRP), to escape the deactivation of glucuronidase in the

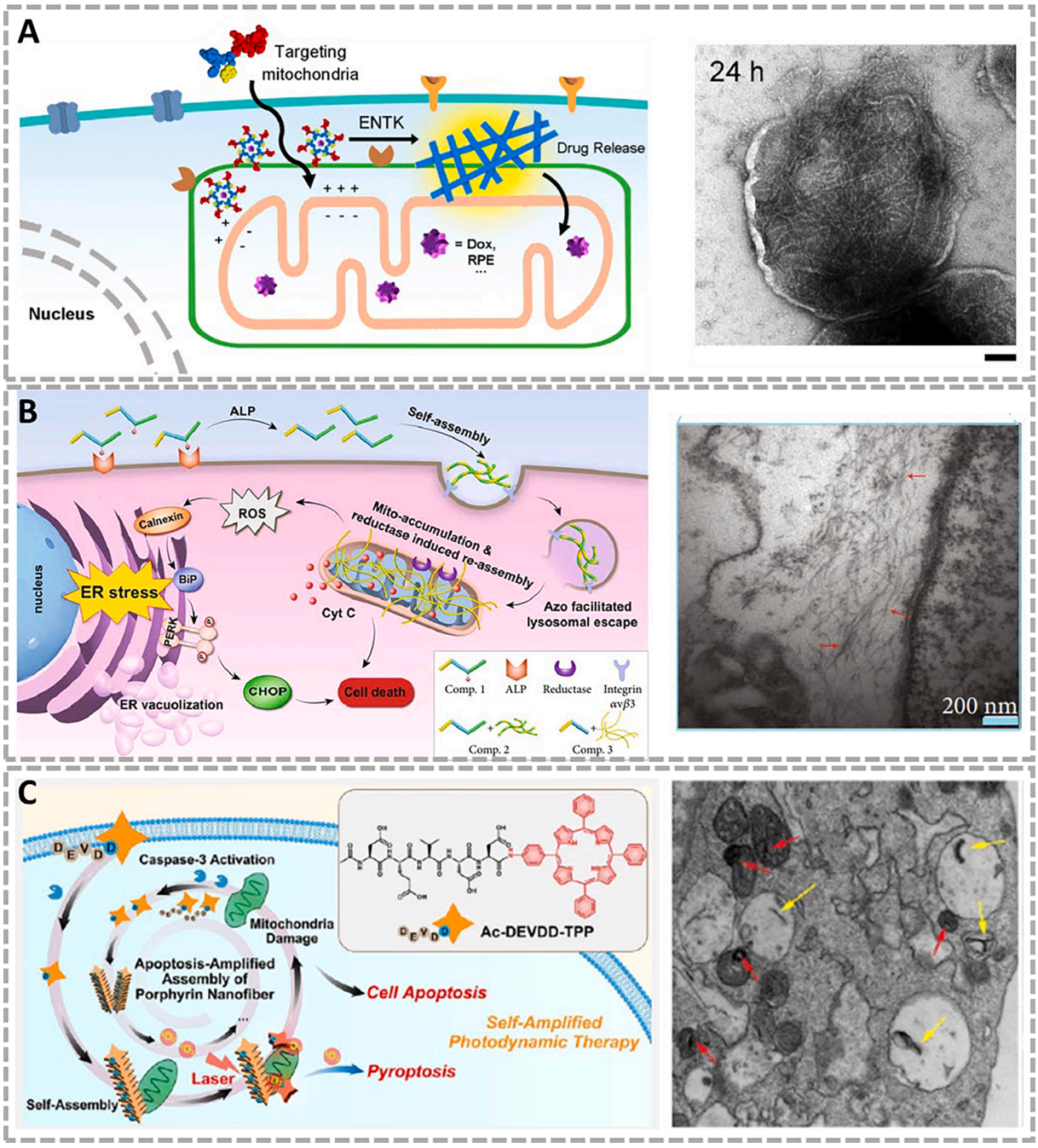

Fig. 7. Enzyme-triggered self-assembly of peptides targeting mitochondria. (A) Upon the cleavage of enterokinase (ENTK), peptides changed from micelles to nanofibers around mitochondria and released drug molecules. Adapted with permission from [98]. (B) The formation of nanofibers inside the mitochondria disrupts the mitochondrial membrane, leading to apoptosis. Adapted with permission from Ref. [99] (C) Upon caspase-3 (Casp3) cleavage and laser irradiation, the peptides self-assemble into nanofibers, subsequently resulting in apoptosis and pyroptosis. Adapted with permission from Ref. [100].

cytosol. The peptide is designed to respond to ENTK present in the mitochondria of cancer cells, achieving selectively targeting mitochondria of cancer cells (Table 2, M10). After ENTK cleavage, CLRP could be delivered into mitochondria to inhibit protein synthesis, resulting in an increased sensitivity of cancer cells to cisplatin [101]. Sirtuin 5 (SIRT5) is another specific mitochondria-localized enzyme. Sun and coworkers designed a SIRT5-recognized peptide that can form nanofibers in the mitochondria after SIRT5 catalysis. The hydrophobic part of molecules enhanced the fluorescent intensity of NBD, enabling activity-based imaging of SIRT5 in living cells. The nanofibers further depolarized the membrane potential of mitochondria and promoted ROS formation (Table 2, M11). Moreover, this strategy further enhanced anticancer activity when combined with three different chemotherapy drugs [102]. Currently, most self-assembling peptide systems lead to mitochondrial dysfunction, yet the mechanisms remain unclear. Investigating these mechanisms and exploring the potential to regulate mitochondrial function by self-assembling system is crucial for the future development of mitochondria-targeted therapies.

2.3. Endoplasmic reticulum (ER)

ER acts as a transportation system in cells and plays a vital role in protein synthesis, folding, modification, and transport. Peptide self-assembly targeting ER could influence various cellular physiological

Table 3
Representative examples of functional peptides self-assembly in endoplasmic reticulum (ER).

No.	Stimulus	Morphology	Function	Ref.
E1	Targeting ER and GRP78	Nanofiber	Inducing persistent ER stress to sensitize cancer chemotherapy; Inhibiting GRP78 refolding activity to promote endogenous protein aggregation	[104]
E2	Surface receptors	Nanosphere	Inducing M2-type macrophages repolarization by dual inhibition of ER and oxidative stresses	[106]
E3	ALP	Nanofiber	Utilizing intracellular peptide self-assemblies to modulate various protein–protein interactions	[103]
E4	ALP	Crescent-shaped assemblies	Disrupting the integrity of plasma membrane and inducing cancer cell death through ER stress.	[105]
E5	PRSS1	Nanofiber	Inducing ER stress and cell death	[109]
E6	GSH	Nanoribbon	Inducing combinatorial organelle dysfunction and cell death	[107]
E7	CES	Film-like nanostructure	Targeting ER and exhibiting synergistic anticancer effect with chlorambucil	[108]

GRP78, glucose-regulated protein 78; ALP, alkaline phosphatase; PRSS1, trypsin-1; GSH, glutathione; CES, carboxylesterase.

processes and then impact cancer cell's fates (Table 3 and Figs. 8 and 9).

Xu's group integrated short peptides with naproxen and cyclooxygenase-2 (COX-2) as a enzymatic substrate [103]. After dephosphorylation by ALP, these precursors form nanofibrous hydrogel on ER, resulting in the sequestration of COX-2 and protein-tyrosine phosphatase 1B (PTP1B), which provided a new strategy to modulate intracellular protein–protein interaction by functional self-assembly peptides (Table 3, E3) (Fig. 8A). Moreover, the same group designed a peptide selectively targeting cancer cells due to overexpression of ALP. The assemblies cause the disruption of cell membrane and accumulate on the ER, which disturb the protein-folding capacity of ER. Subsequently, continuous ER stress and activation of the caspase signaling cascade result in cancer cell death (Table 3, E4) [105]. Yu's group created a 19-mer peptide (RVMLIGKEIIYIIYEKDEL) comprising three motifs: a binding motif for inhibition of glucose-regulated protein 78 (GRP78), a self-assembly motif, and a targeting motif [104]. The accumulation of peptide nanofibers in ER could cause persistent ER stress and inhibit GRP78 refolding activity, sensitizing cancer cells to toyocamycin and enhancing the drug's anticancer efficacy (Table 3, E1) (Fig. 8B). Cai et al. developed a supramolecular peptide amphiphile drug-delivery system (SPADS) to reprogram macrophages and reshape the tumor immune microenvironment [106]. The peptide nanospheres released ER-targeting drugs within cells in response to intracellular ROS, and this dual inhibition of ER and oxidative stresses could repolarize M2-type macrophages, resulting in the inhibition of growth and metastasis of cancer cells (Table 3, E2). Using enzyme-instructed noncovalent synthesis of molecular condensates in the cellular environment for intracellular sequestration of enzymes is still a big challenge.

The overexpression of specific enzymes on the ER in cancer cells could serve as triggers for peptides to target ER and facilitate their therapeutic effects selectively. Xu's group reported a branched peptide as a substrate of trypsin-1 (PRSS1, and its cytotoxicity is correlated with the expression level of PRSS1 [109]. The overexpression of PRSS1 in OVSAHO (a high-grade serous ovarian cancer cell line) cleaved branched peptides and promoted their self-assembly into nanofibers on ER, resulting in selective ER stress and subsequent cell death (Table 3, E5). Yu et al. utilized the overexpression of GSH as a trigger to realize in situ self-sorting of peptide assemblies in living cells [107]. The selection of GSH could avoid the interruption between the two independent assembling process. Upon GSH cleavage, two peptides have a great tendency to self-assembly through β-sheet hydrogen bonding interactions. The assemblies of peptides could target ER and Golgi separately, leading to combinatorial organelle dysfunction and cell death (Table 3, E6) (Fig. 9A). In addition, Zhang et al. reported a L-/D-version peptide-based nanomedicine could target ER and be responsive to carboxylesterase (CES) commonly expressed in tumor [108]. Hydrolysis led to drug release, and the cleaved peptide segments (FFK, ffk) could self-assemble into film-like nanostructure on ER. After the treatment, several ER proteins, such as transmembrane sensor protein, chaperone proteins and oxidoreductase proteins exhibited upregulation, indicating the targeting effect of assemblies. Subsequently, enhanced ER stress triggered cancer cell death (Fig. 9B). This mechanism demonstrates synergistic anticancer effect with chlorambucil (Table 3, E7). ER is the largest organelle in most cells enclosed by a continuous membrane. However, there is a lack of studies investigating the interactions between self-assembling peptide systems and the major components of the ER membrane. Relevant understanding could provide crucial insights into mechanisms and fundamental researches for the future design.

2.4. Nucleus

The nucleus is a vital organelle in cells for the synthesis and assembly of ribosomes. Thus, functional peptide self-assembly targeting the nucleus has the potential to become a novel cancer therapeutic strategy (Table 4 and Fig. 8).

Yang and coworkers reported a simple strategy for constructing peptide-based supramolecular nanomedicines to deliver two drugs, 10-hydroxycamptothecine (HCPT) and cisplatin to the nucleus [112]. HCPT faces the challenges in entering the nucleus, whereas cisplatin could potentially address this due to its positive charge. This strategy exhibited synergistic effects of two drugs to inhibit cancer cells (Table 4, N1). Xu et al. reported a phosphopentapeptide showing no cytotoxicity to normal cells with micellar structure [110]. After dephosphorylation by ALP, the nanostructure turned into nanoribbon with a secondary structure of α-helix. Nanoribbon could only be formed under high concentration of ALP (over-expression) that assist in selectively killing cancer cells. The formation of nanoribbon is determined by dephosphorylation ratio, when the ratio reaches about 24 %, the significant self-assembling nanoribbon is observed. As the peptides contain transmembrane domains (LLLLY), the self-assemblies could interact with membrane which facilitates nuclear localization and leads to subsequent rapid killing of cancer cells (Table 4, N4), as shown in Fig. 10A. Due to the different expression levels of ALP in various cells, this strategy exhibits selectivity. Moreover, Xu et al. further applied this strategy to target osteosarcoma cells that could selectively kill osteosarcoma cells. From proteomics analysis, designed peptides could co-assemble with components of nucleosome, such as H4C1and H3C1, which assist peptides in targeting nucleus without inducing drug resistance (Table 4, N5) [113]. Liang et al. grafted several link peptides, including the CTSB-sensitive part (ALAL), nucleus-targeting part (YGRKKRRQRRR), and chrysin-modified polymers, onto hyaluronic acid to load rapamycin (RAPA) and 9-nitro-20(S)-camptothecin (9-NC) [111]. The amphiphilic structures of molecules induce the self-assembling of nanoparticles with a hydrophilic shell and a hydrophobic core. Due to the hydrophobicity of 9-NC, it is loaded in the core of nanoparticles. Upon enzymatic cleavage, secondary nanoparticles could target the nucleus and directly deliver 9-NC into the nucleus, resulting in enhanced anti-tumor efficacy combined with previously released RAPA (Table 4, N2) (Fig. 10B). Wang et al. designed a peptide targeting vimentin because Vimentin could form improper self-assembly in cancer cells. In the presence of vimentin, the nanostructures of molecule transformed from nanoparticles to nanofibers, forming β-sheet secondary structure inside which inhibits the

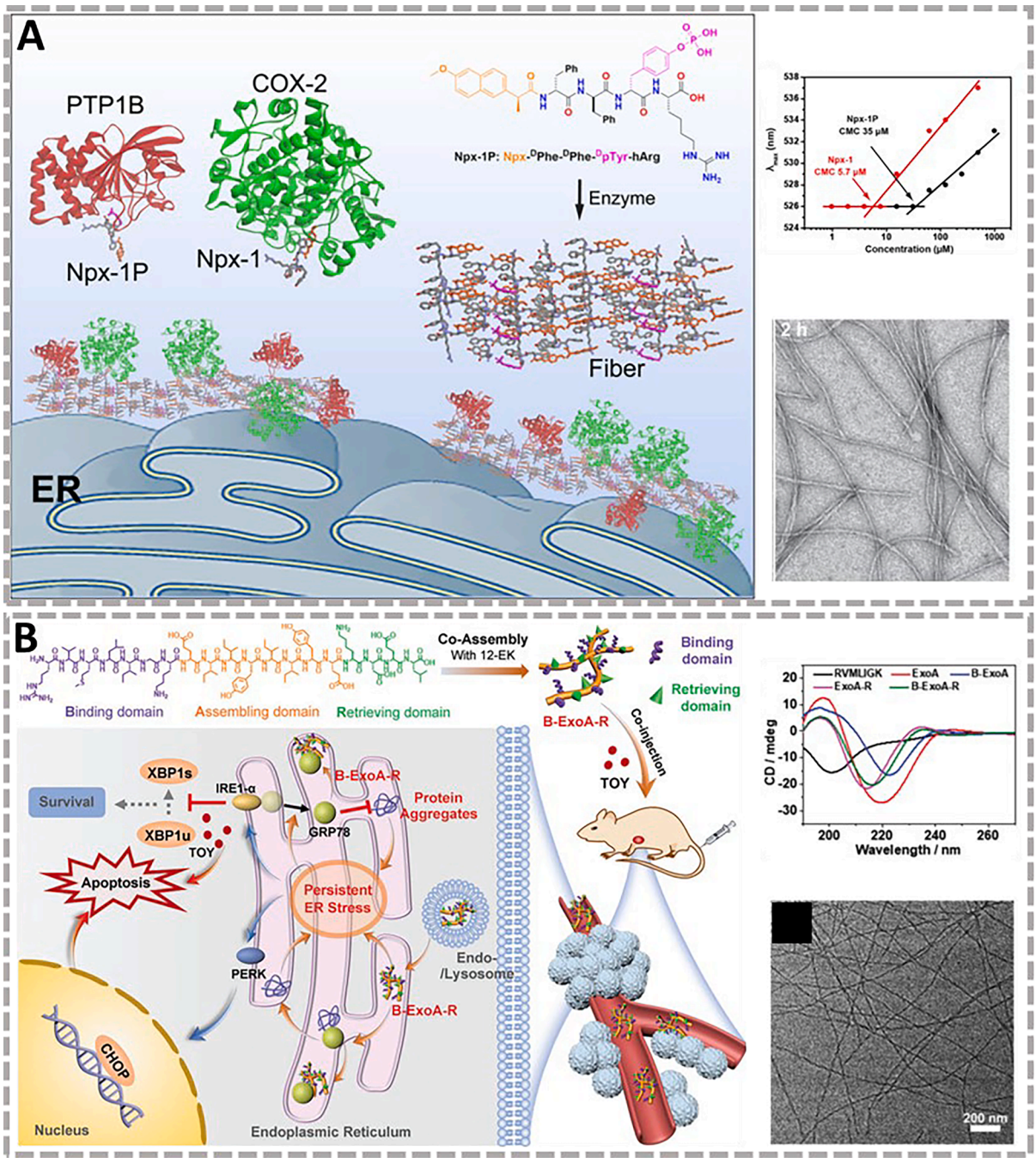

Fig. 8. Self-assembly of peptides on ER. (A) Dephosphorylated peptides can self-assemble into fibers and accumulate on the ER. Adapted with permission from Ref. [103]. (B) Peptide self-assemblies induce persistent ER stress within cells. Adapted with permission from Ref. [104].

migration and invasion of cancer cells (Table 4, N3) [114]. In addition, Yang et al. reported a drug-peptide amphiphile whose secondary conformation could be finely controlled by temperature and enzyme [115]. Enhancement of cellular uptake caused by α-helix secondary structure and nuclear accumulation contributed to the superior anti-cancer ability of this strategy (Table 4, N6). In summary, developing self-assembling peptide system specifically targeting nucleus could reduce the distribution of drugs in non-targeted tissues, lower side effects, and enhance therapeutic effects.

3. Conclusion and perspectives

In recent years, there has been significant and vigorous development in the in situ self-assembly of functional peptides in subcellular organelles. This work mainly discussed the morphology and functions of the peptide self-assembly under various stimuli (e.g., concentration, pH, enzymes, and receptors) in different organelles, including lysosomes, mitochondria, ER, and nucleus. Based on these findings, we envisioned that one could rationally design functional peptide self-assembly that is finely tuned at specific intracellular locations. These strategies mentioned above provide new potential ways for drug delivery, cancer therapy, imaging, and other related fields. The authors would also like to apologize for the omission of the excellent works because of the space limit.

Despite the promising results, there are still numerous challenges to address. First, the fundamental issues in this field still need to be explored. Most current studies only prove that self-assemblies appear in subcellular organelles. However, its assembly mechanism and

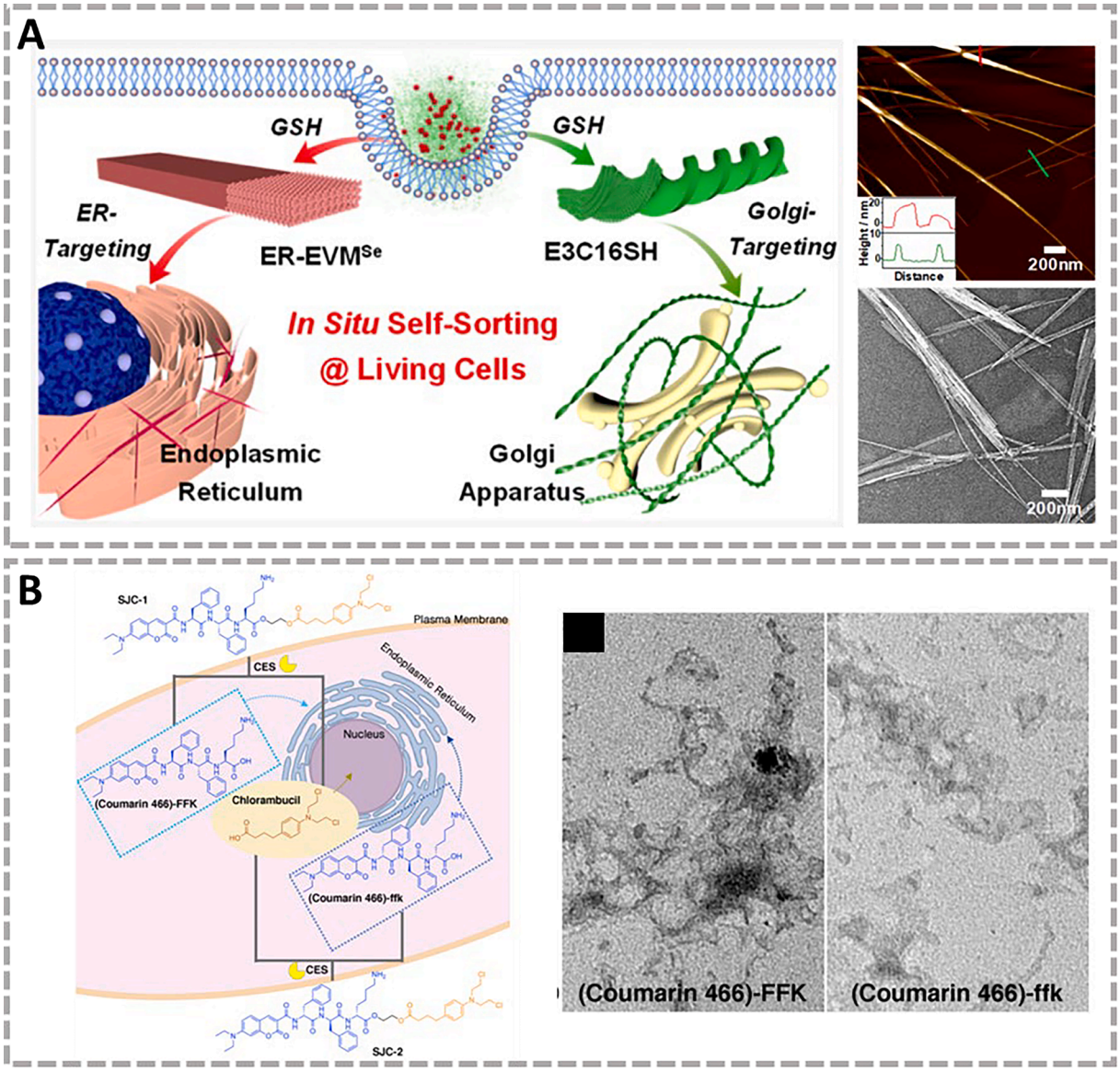

Fig. 9. Self-assembly of peptides on ER. (A) GSH-triggered peptide self-assemblies formation targeting to ER and Golgi, resulting in organelle dysfunction and cell death. Adapted with permission from Ref. [107]. (B) Simultaneous release of ER-targeting peptide self-assemblies and nucleus-targeting chlorambucil produces a synergistic anticancer effect. Adapted with permission from Ref. [108].

Table 4
Representative examples of functional peptides self-assembly targeting to nucleus.

No.	Stimulus	Morphology	Function	Ref.
N1	–	Nanofiber and nanoaggregate	Efficiently delivering two drugs to nucleus with dual synergistic effect	[112]
N2	CTSB	Nanoparticle	Utilizing secondary nucleus-targeting micelles deliver 9-NC to the nucleus directly, resulting in a significant enhancement of anti-tumor efficacy	[111]
N3	Vimentin	Nanofibrous network	Binding with vimentin and forming fibers to prevent skeletonization; inhibition of migration and invasion of cancer cells	[114]
N4	ALP	Nanoribbon	Significant intranuclear accumulation of peptide assemblies result in rapid killing	[110]
N5	ALP	Nanoribbon	Rapid killing osteosarcoma cells without toxicity to normal cells or inducing resistance	[113]
N6	ALP	Nanofiber	Enhancing cellular uptake and nuclear accumulation capability	[115]

CTSB, cathepsin B; ALP, alkaline phosphatase.

spatiotemporal assembly process are rarely studied, especially on time scales. Understanding these aspects could provide meaningful guidance in designing novel functional peptide self-assembly. Secondly, current triggering conditions for peptide self-assembly are simple, and the assembly process is generally achieved in one step. In future development, it is worth designing functional peptides that require multiple triggers to activate self-assembly behavior. This strategy could avoid cytotoxicity to normal cells and provide stronger selectivity to cancer cells. Thirdly, many questions that need to be resolved before developing clinical applications. The in vivo safety, stability and pharmacokinetics need further investigated, which are critical for determining the optimal dosage, administration route and treatment regimen. Besides, clinical tumors are generally at different stages and types which a complex situation. Currently, there is a significant lack of research on these issues. Finally, developing low-cost and scalable methods for manufacturing self-assembling peptides is crucial for promoting this novel cancer therapy.

Notwithstanding these challenges, the functional peptide self-assembly in subcellular organelles has been developed. In the future, it has the potential to offer new strategies for clinical cancer diagnosis and therapy.

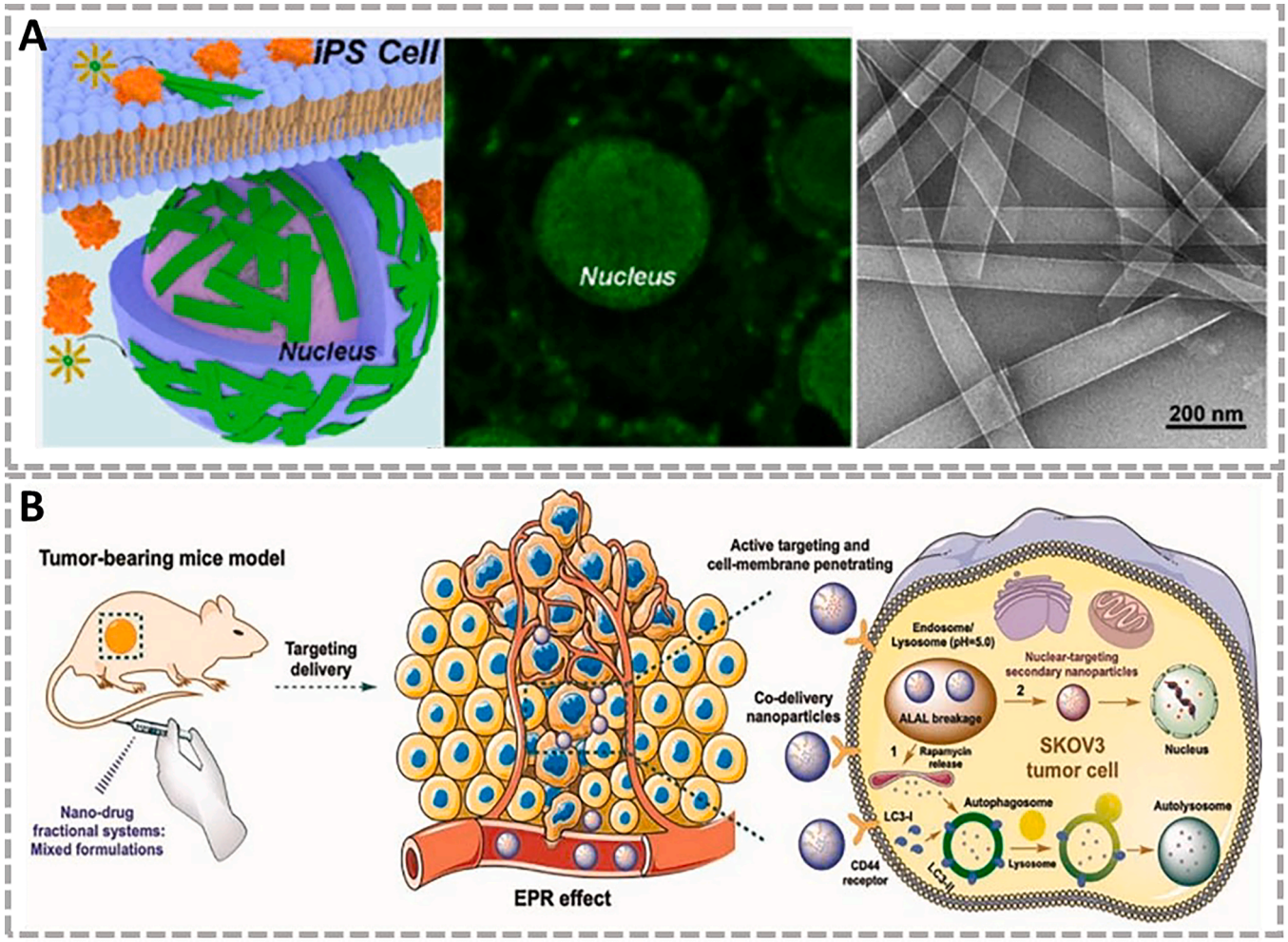

Fig. 10. Peptide self-assemblies targeting to nucleus. (A) ALP-cleaved peptides self-assemble into nanoribbons and accumulate in nucleus. Adapted with permission from Ref. [110]. (B) Upon enzymatic cleavage, intracellularly formed micelles can target the cell nucleus and directly deliver 9-NC into nucleus. Adapted with permission from Ref. [111].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.addr.2024.115327.

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