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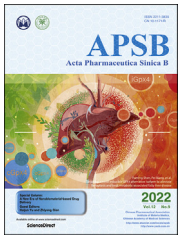

REVIEW

Non-apoptotic cell death-based cancer therapy: Molecular mechanism, pharmacological modulators, and nanomedicine

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Abstract As an emerging cancer therapeutic target, non-apoptotic cell death such as ferroptosis, necroptosis and pyroptosis, etc., has revealed significant potential in cancer treatment for bypassing apoptosis to enhance the undermined therapeutic efficacy triggered by apoptosis resistance. A variety of anticancer drugs, synthesized compounds and natural products have been proven recently to induce non-apoptotic cell death and exhibit excellent anti-tumor effects. Moreover, the convergence of nanotechnology with functional materials and biomedicine science has provided tremendous opportunities to construct non-apoptotic cell death-based nanomedicine for innovative cancer therapy. Nanocarriers are not only employed in targeted delivery of non-apoptotic inducers, but also used as therapeutic components to induce non-apoptotic cell death to achieve efficient tumor treatment. This review first introduces the main characteristics, the mechanism and various pharmacological modulators of different non-apoptotic cell death forms, including ferroptosis, necroptosis, pyroptosis, autophagy, paraptosis, lysosomal-dependent cell death, and oncosis. Second, we comprehensively review the latest progresses of nanomedicine that induces various forms of non-apoptotic cell death and focus on the nanomedicine targeting different pathways and components. Furthermore, the combination therapies of non-apoptotic cell death with photothermal therapy, photodynamic therapy, immunotherapy and other modalities are summarized. Finally, the challenges and future perspectives in this regard are also discussed.

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1. Introduction

Programmed cell death (PCD), which can be divided into apoptosis and non-apoptotic cell death, is an initiative cell death mode regulated by genes, and plays an important role in the development and homeostasis of multicellular organisms[1]. Among them, apoptosis is the first confirmed form of PCD, which is characterized by the activation of caspase, the resulting cell shrinkage, nuclear pyknosis, DNA fragmentation, as well as the formation of apoptotic bodies[2]. Inducing apoptosis is considered as one of the main strategies for cancer treatment since regulating apoptosis is closely related to the occurrence and suppression of tumor. For example, the overexpression of anti-apoptotic BCL-2 protein in tumor cells facilitates escaping from apoptosis and accelerates tumor occurrence, while the upregulation of the pro-apoptotic proteins BAX and BAK promotes tumor cell apoptosis[3]. In recent decades, many novel non-apoptotic cell death forms, including ferroptosis[4], necroptosis[5], pyroptosis[6], autophagic cell death[7], paraptosis, lysosomal-dependent cell death, and oncosis[8], have been discovered to reveal different signaling pathways and molecular mechanisms other than apoptosis, which provide potential targets and new strategies for cancer treatment[9–11].

Increasing evidence has proven the anti-tumor strategy based on non-apoptotic cell death is a direction to solve some existing problems in cancer treatment. On one hand, diverse forms of non-apoptotic cell death effectively bypass or overcome the resistance of tumor cells to apoptosis and provide alternative death pathways when the apoptotic pathway is defective, which significantly improve the anti-cancer efficacy[11]. On the other hand, triggering different PCD forms may lead to immune activation or suppression. For example, apoptosis induced by chemotherapy drugs, radiotherapy, and phototherapy can lead to tumor immune tolerance, which restricts the synergistic effect combined with immunotherapy[12]. On the contrary, the pro-inflammatory effects of necroptosis and pyroptosis can trigger a potent immune response[5,13]. Therefore, the immunogenicity of tumor cells can be maximized by inducing specific form of PCD to enhance the immunotherapy effects such as immune checkpoint blockade (ICB). However, many drug inducers that render non-apoptotic cell death have limitations such as low tumor specificity and poor biocompatibility, which may result in severe toxicity in normal cells and tissue. Furthermore, the low accumulation of the drug inducers at the tumor site may weaken their anti-cancer efficacy due to the short half-life in the blood circulation and rapid elimination by the kidneys.

Bio-nanotechnology shows unique benefits to enhance anti-tumor effect of non-apoptotic cell death inducers by various feasible strategies[14]. Nanomaterials possess the merits of increasing drug payloads and attaining targeted drug delivery, which enhance the therapeutic efficacy in selectively inducing non-apoptotic cell death of cancer cells. Hence, the development of nano drug delivery system loaded with non-apoptotic cell death inducers or other anti-tumor drugs is beneficial to improve therapeutic outcome. In addition, some nanomaterials can also exert anti-tumor effect by influencing the death forms of tumor cells. In general, the physicochemical properties of nanomaterials (such as chemical composition, size, shape, surface charge and the protein-binding capacity) may alternate cell proliferation or immune response[15]. It is worth noting that the shape of nanomaterials and the internalization degree of cell may mediate different cell death form, further affecting anti-tumor effects[16]. For example, the gold nanoprisms could trigger necrosis while the gold nanorods could trigger apoptosis and necroptosis with the light excitation due to the difference in the shape and the internalization rate[17]. Therefore, the physicochemical properties and shape of nanomaterials can be regulated through altering synthesis methods and surface modification to obtain nanomaterials with different non-apoptotic cell death-inducing functions for effective treatment of various cancers. Apart from the morphology, some compositions of nanocarriers facilitate the induction of non-apoptotic cell death by regulating the signaling pathway. For instance, iron-based nanomaterials are commonly utilized to trigger ferroptosis in tumor therapy by exogenously delivering iron into cancer cells, which disrupt the iron metabolism and promote Fenton reaction in tumor cells[18].

In this review, we briefly introduce the molecular mechanisms, signaling pathways, and various pharmacological modulators of non-apoptotic cell death including ferroptosis, necroptosis, pyroptosis, autophagy, paraptosis, lysosome-dependent cell death, and oncosis. Then we focus on the recent progresses of nanomedicines that induce non-apoptotic cell death in cancer cells (as shown in Fig. 1), and particularly the combination therapy strategies based on non-apoptotic cell death with other therapies such as photothermal therapy, photodynamic therapy and immunotherapy. Finally, insight and future prospective are also provided for the nanomedicine fabrication and innovating cancer treatment strategies based on non-apoptotic cell death.

2. Ferroptosis and its inducers for cancer therapy

2.1. *The molecular mechanisms of ferroptosis*

Ferroptosis is a new type of non-apoptotic cell death, which is closely related to the concentration of intracellular ferrous iron (Fe^{2+}) and can be inhibited by iron chelating agents. In 2012, ferroptosis was first termed by Scott J. Dixon et al.[19] and was defined as an iron-dependent cell death induced by erastin and RAS selective lethal factor 3 (RSL3). The underlying mechanism of ferroptosis is associated with the lipid peroxidation and accumulation of reactive oxygen species (ROS) in cells, which are mainly induced by excessive endogenous iron-mediated Fenton reaction and lipid oxygenase (LOXs)[20]. Among these processes, the systemic Xc^--GSH-GPX4 pathway plays a major role in ferroptosis. System Xc^- is a class of cysteine/glutamate antiporter that exists on cell membrane. Normally, cystine is transported into cells from system Xc^- and reduced to cysteine for glutathione (GSH) biosynthesis[21]. GSH is a cofactor and substrate of glutathione peroxidase 4 (GPX4), which oxidizes GSH to glutathione disulphide (GSSG) in a catalytic reaction. Meanwhile, the lipid hydroperoxide (LOOH) produced in the lipid oxidation process can be reduced to lipid alcohol (LOH), thus preventing the occurrence of lipid peroxidation[22]. When Xc^- is inhibited, GSH synthesis is blocked and GPX4 activity is inhibited, cytotoxic lipid peroxidation will accumulate and lead to ferroptosis. Among them, phospholipids containing polyunsaturated fatty acids (PUFA) are the main substrates in lipid peroxidation, which can be catalyzed by iron lipoxygenase (LOX) and promoted by iron-dependent Fenton reaction[23,24]. Recent studies have exhibited that excessive ROS production and accumulation of lipid peroxides may cause plasma membrane thinning, membrane pore formation and membrane rapture. It was also found that ferroptosis caused mitochondrial shrinkage, condensed mitochondrial membrane, and reduced or erased the mitochondrial cristae in cells[25]. In addition, activation of mitochondrial voltage-dependent anion channels 2/3 (VDAC2/3) and endoplasmic reticulum stress (*e.g.*, with erastin

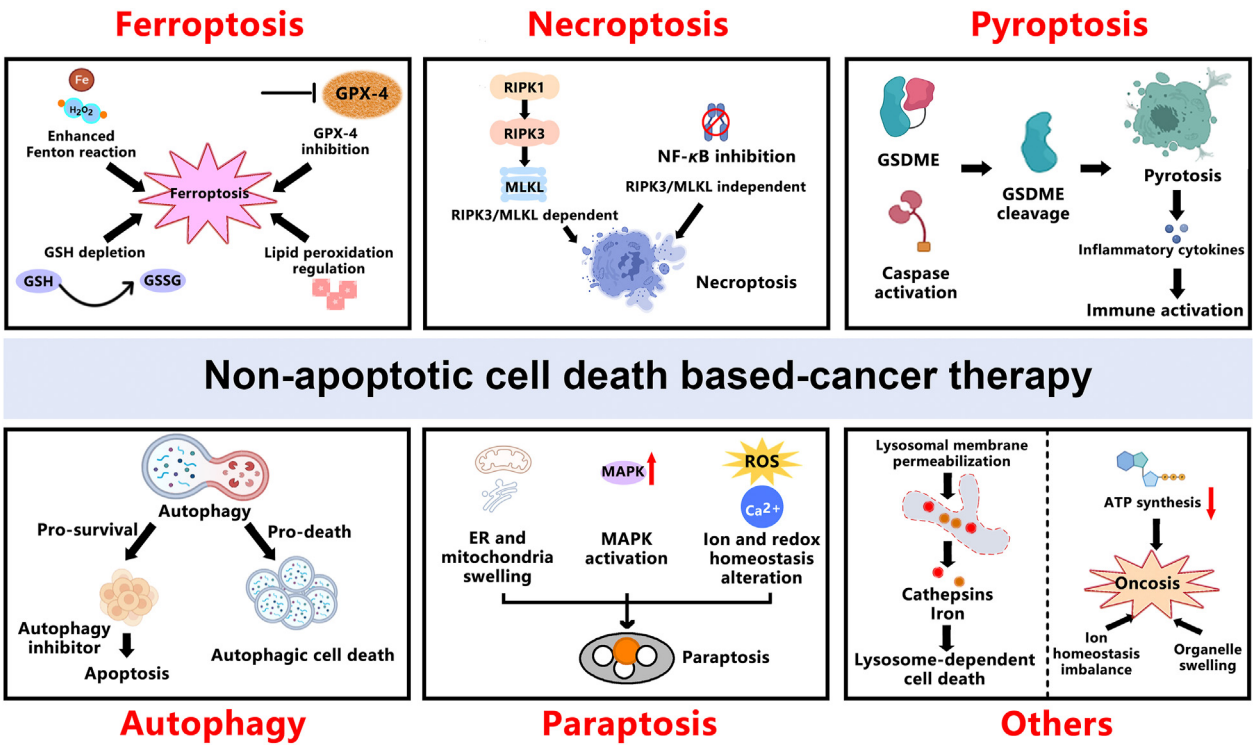

Figure 1 Schematic illustration of mechanisms and designing principles of cancer therapy based on non-apoptotic cell death comprising ferroptosis, necroptosis, pyroptosis, autophagy, paraptosis, lysosome-dependent cell death and oncosis.

treatment), can also cause ROS accumulation and mitochondrial damage, leading to ferroptosis[26,27].

2.2. *The pharmacological inducers of ferroptosis*

Ferroptosis, being considered as a hotspot for cancer treatment[4], plays an important role in suppressing various kinds of tumors, including colorectal cancer[28], head and neck cancer[29], pancreatic cancer[30], and liver cancer[31]. In recent years, many clinical drugs (sorafenib, sulfasalazine, etc.) and experimental compounds (such as erastin and RSL3) have been reported to induce ferroptosis in tumor cells. Apart from these conventionally used inducers, some natural compounds have also shown potent ferroptosis-inducing ability.

2.2.1. *Anticancer drugs and synthesized compounds*

According to different molecular mechanisms and signaling pathways, these widely used ferroptosis inducers can be mainly divided into three categories: blocking the Xc^- system, regulating the GPX4 or intervening in lipid peroxidation. The first type of ferroptosis inducers, such as sorafenib, sulfasalazine and erastin, are Xc^- system inhibitors with the ability of inhibiting glutamate absorption and rapidly consuming intracellular cysteine, which contribute to the hindrance of GSH synthesis and increased ROS levels for inducing ferroptosis[32–34]. The second type of inducers can directly or indirectly inhibit GPX4 for accumulation of lipid peroxides to induce ferroptosis. Among this type of inducers, RSL3, DPI compounds (DPI7, DPI10, DPI12, DPI13, DPI17, DPI18), and ML compounds (ML162, ML210) are direct inhibitors of GXP4. For example, RSL3 directly inhibits GPX4 by targeting the selenocysteine in the nucleophilic active site of GPX4 to trigger lipid peroxidation-induced ferroptosis[28]. In addition, the ferroptosis inducer FIN56 shows the ability to degrade GXP4. By depleting the GXP4 and the endogenous lipophilic antioxidant coenzyme Q10, FIN56 accelerates the production of lipid peroxides and thus causes ferroptosis[35]. Besides, 1,2-doxolane FINO2 can indirectly inhibit the enzymatic function of GPX4[36]. The third type of ferroptosis inducers promote the lipid peroxidation[29,37,38]. For example, FINO2 can directly oxidize ferrous iron with its peroxide moiety, and can induce lipid peroxidation independent of lipoxygenase activity, which ultimately leads to ferroptosis[36].

On the other hand, ferroptosis and autophagy normally function synergistically in inducing tumor cell death because autophagy can promote the degradation of ferritin and lead to the release of chelated iron in ferritin, which promotes the Fenton reaction mediated by iron ions and causes oxidative stress. Hou and co-workers have discovered that elastin activated the autophagy pathway in human fibrosarcoma cells HT-1080 and resulted in ferritin degradation, thus promoting cell ferroptosis[39]. Moreover, ferroptosis has also exhibited a synergistic effect when combined with cancer immunotherapy. For example, IFN γ released by $CD8^+$ T cells has been reported to inhibit the Xc^- system by down-regulating the expression of SLC3A2 and SLC7A11, which further promoted lipid peroxidation, sensitized tumors to ferroptosis, and improved the efficacy of immunotherapy[40].

2.2.2. *Natural compounds*

A variety of natural compounds have also been reported to induce ferroptosis through multiple targets and mechanisms. For examples, some natural compounds can promote the lipid peroxidation by accelerating the iron-dependent Fenton reaction or LOX-catalyzed lipid oxidation. Artemisinin derivatives such as artesunate and dihydroartemisinin can trigger the ferritin degradation and cause elevated oxidative stress levels through the iron-dependent cleavage of the intramolecular bridges[32,33]. In addition, as exogenous lipid

supplementation contributes to promote the LOX-catalyzed lipid peroxidation, PUFA-conjugated linolenic acid has been verified to induce ferroptosis of triple-negative breast cancer (TNBC) cells, and markedly inhibit tumor growth and metastasis[20]. With different action mechanisms, honokiol (HNK) derived from *Magnolia grandiflora* has shown to decrease the GPX4 activity and increase ROS and Fe^{2+} levels in colon cancer cells, resulting in ferroptosis and tumor suppression[41]. A derivative of Jiyuan oridonin A (JDA) named a2 could induce ferroptosis in gastric cancer cells by reducing the GPX4 level, promoting the degradation of ferritin and ferrous iron accumulation[42]. Moreover, juglone found in *Carya cathayensis* could activate the HMOX1-mediated intracellular iron release and accumulation from heme degradation, consume GSH and promote the lipid peroxidation to induce ferroptosis[43].

2.3. *Ferroptosis-based nanomedicine*

Current strategies of designing nanomedicines that induce ferroptosis focus on increasing the accumulation of ROS and lipid peroxidation in tumor cells, which can be attained through these approaches: promoting the Fenton reaction, suppressing GPX4, depleting GSH, exogenously regulating the lipid peroxides, and further combination therapy. Based on the above approaches, numerous studies have been devoted to fabricating rational nanocarriers to deliver drugs, or to exploring special units and chemical bonds of the carriers for achieving therapeutic effect, thus promoting ferroptosis in tumor cells and realizing effective cancer treatment[44−68] (Supporting Information Table S1).

2.3.1. *Promoting Fenton reaction*

The Fenton reaction mediated by endogenous iron ions can result in highly toxic hydroxyl free radical production and lipid peroxidation, and then further trigger ferroptosis. Endogenous iron that mainly exists in ferritin is difficult to cause an effective Fenton reaction due to the low content in tumor cells. Therefore, the most common strategy of inducing ferroptosis is to design a nanocarrier that can supply iron from an external source. In recent years, many iron-based nanomaterials have been developed for cancer treatment, including iron oxide nanoparticles, iron-containing metal-organic framework (MOF), metal-organic networks (MONs), iron-drug complexes, and ferritin nanoparticles, etc. These iron-based nanomaterials can release chelated iron in the tumor site, and further promote the Fenton reaction to induce ferroptosis in combination with H_2O_2 or chemotherapeutic drugs (such as cisplatin, artesunate, and DOX). (1) A variety of iron oxide nanoparticles have been reported to accelerate the Fenton reaction and induce tumor cell ferroptosis. The supplemented Fe^{2+}/Fe^{3+} degraded by iron oxide nanoparticles and the externally delivery of H_2O_2 have been proven efficient to generate ROS. H_2O_2-loaded polymersome (H_2O_2/Fe_3O_4-PLGA) was developed by Li et al.[44] with Fe_3O_4 nanoparticles packed in the polymersome shell. After exposure to ultrasound, the encapsulated H_2O_2 in the core was released and reacted with Fe_3O_4 in the shell to generate hydroxyl radicals *via* the Fenton reaction, thus inducing ferroptosis and suppressing tumor growth. In addition, indirect production of H_2O_2 induced by active ingredient can further accelerate the Fenton reaction. Cisplatin was loaded in Fe_3O_4/Gd_2O_3 hybrid nanoparticles modified with lactoferrin and RGD dimer. After the nanoparticles entered tumor cells through the integrin $\alpha v\beta 3$-(RGD dimer receptor)-mediated internalization, Fe^{2+}, Fe^{3+} and cisplatin were released simultaneously to activate the NADPH oxidase (NOXs), increase the production of H_2O_2, and promote the Fe^{2+}/Fe^{3+}-mediated Fenton reaction, showing an excellent therapeutic effect on orthotopic brain tumor models[45]. (2) MOFs and MONs with lower cytotoxicity can also be designed as nanocarriers for ferroptosis-based cancer therapy. For example, pH-responsive core−shell MOFs (CS-MOFs) were prepared by coating highly biocompatible MIL-100 (Fe) on $Mn_3[Co(CN)_6]_2$ MOFs, in order to release artesunate (AS) and Fe^{3+} in the acidic tumor microenvironment. The released Fe^{3+} in tumor cells was further reduced to Fe^{2+}, thereby catalyzing AS to generate free radicals and ROS to kill the tumor cells[69]. Besides, metal−phenolic networks formed by the coordination between metal ions and phenolic molecules possess charming properties like pH-responsiveness and low cytotoxicity when used as ferroptosis-inducing agents. Den-DOX-tannic acid-Fe^{3+} nanocomplexes were fabricated by Guo's group[46] through mixing metal−phenolic network with a doxorubicin (DOX) loaded dendrimer (Den). The obtained nanocomplexes enhanced the Fenton reaction by supplementing ferrous iron and augmented the excessive ROS production *via* DOX to induce ferroptosis in drug-resistant cancer cells. (3) Iron−drug complexes have been widely explored as the nanomedicine for inducing ferroptosis, which can reduce the oxidative stress damage to the normal cells caused by Fe^{2+} ions before they are released at tumor site. DOX-Fe^{2+} was encapsulated in the core of amorphous calcium carbonate through a one-step approach, and the surface of the nanoparticles was conjugated with folic acid-modified and matrix metalloproteinase-2 (MMP-2)-sheddable polyethylene glycolylated polyamidoamine (PAMAM) dendrimer (Fig. 2A and C)[47]. Upon reaching the MMP-2 enzyme-abundant tumor microenvironment, the polyethylene glycol (PEG) moieties were removed to facilitate the nanoparticles to target tumor cells through folic acid ligands. Then the Fe^{2+} ions and DOX were released after the internalization and acidolysis of calcium carbonate internalization and acidolysis, promoting ferroptosis in tumor cells (Fig. 2B, D−F)[47]. (4) Different from the above-mentioned nanomedicine, ferritin nanoparticles can store and release iron in a controlled manner, which are considered as alternatives for inducing ferroptosis. Self-assembled ferritin nanoparticles with the ability of targeting tumor cells *via* the overexpressed transferrin receptors were prepared for DOX delivery by Yang's group[48]. The DOX-loaded ferritin nanoparticles increased the intracellular iron concentration and significantly augmented the ROS production, further promoting ferroptosis. Similarly, Cornel dots (αMSH-PEG-C′ dots), which were coated with polyethylene glycol and functionalized with α-melanocyte-stimulating hormone (αMSH), increased iron concentration by upregulating the expression of intracellular ferritin to result in the ROS generation, GSH depletion and ferroptosis[49].

2.3.2. *GPX4 suppression*

Glutathione peroxidase 4 (GPX4), a type of lipid repair enzyme which can reduce lipid peroxides to lipid alcohols, plays an important role in protecting cells from oxidative stress. Thus, suppressing or decreasing GXP4 can lead to the accumulation of lipid peroxides and eventually the occurrence of ferroptosis. At present, a variety of nanomedicines (such as exosomes and iron oxide nanoparticles) that can inhibit GPX4 have been fabricated for the ferroptosis-based cancer treatment. Among them, the most common design ideas are delivering GXP4 inhibitors such as elastin[50], sorafenib[51], and RSL[52] using nanocarriers to the tumor cells, and exploiting nanomaterials with GPX-4 inhibitory ability. Yu et al.[53] prepared folate-labeled exosomes (Erastin @ FA-exo) for targeted delivery of erastin, which inhibited the expression of GPX4 and upregulate the expression of cysteine dioxygenase (CDO1), triggering ferroptosis in

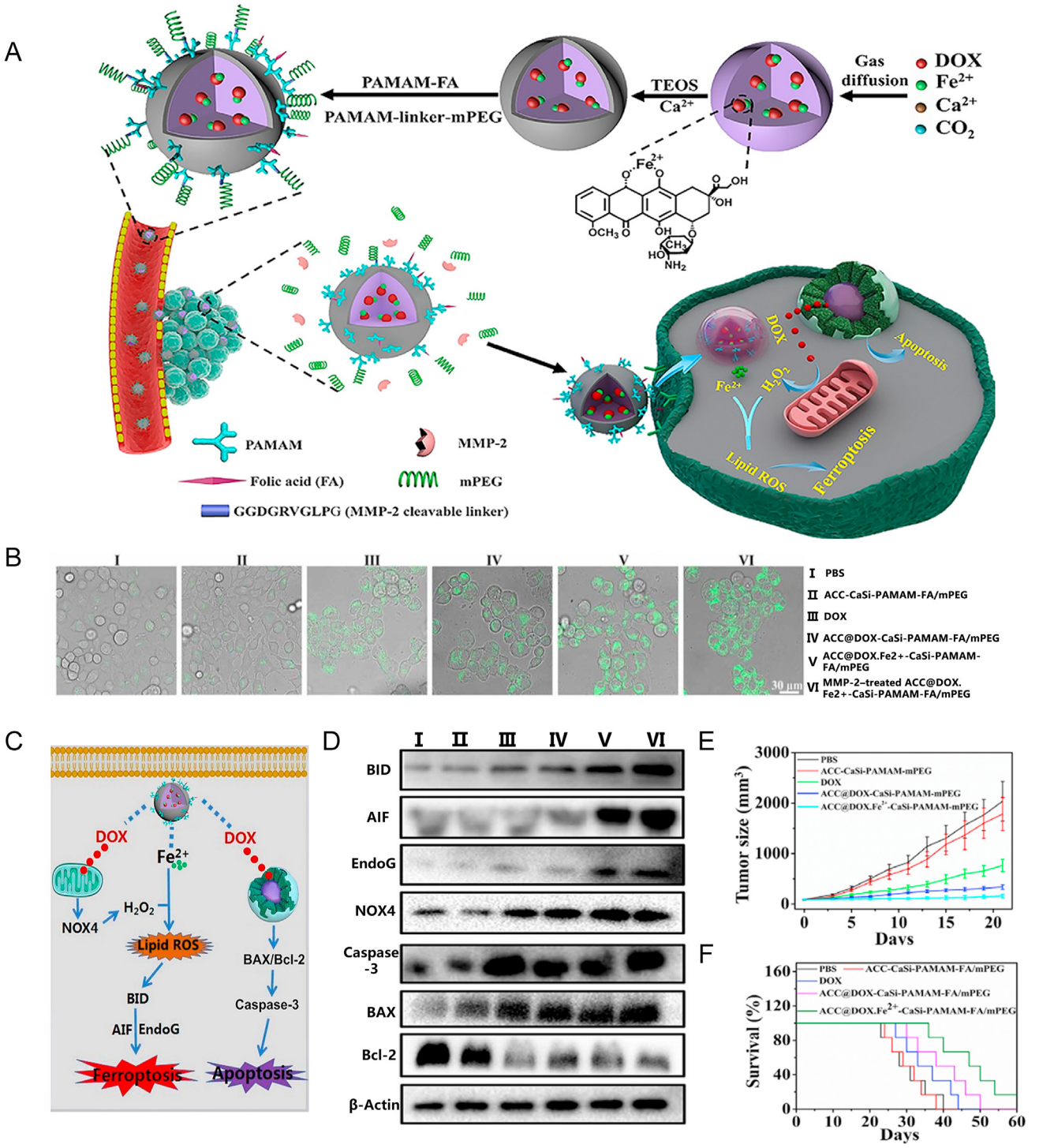

Figure 2 A strategy of chelate of chemotherapeutic drug and ferrous ions to promote ferroptosis by amplifying Fenton reaction. (A) Synthesis schematic depiction of amorphous calcium carbonate (ACC)-based nanoassembly and the therapeutic action of synergistically enhanced ferroptosis effect. (B) The level of lipid ROS (green fluorescence) observed by confocal laser scanning microscopy (CLSM) after incubation with various formulations with or without DOX/Fe^{2+}. (C) The anticipated molecular mechanism of the improved ferroptosis resulted from increasing generation of lipoperoxides. (D) Ferroptosis markers (BID, AIF, EndoG) and apoptosis makers (NOX-4, Caspase-3, BAX, BCL-2) expression after incubation with various formulations. (E) Tumor volume of ACC-based nanoassembly in mice with 4T1 tumors. (F) Survival curves of the mice. Reprinted with the permission from Ref. 47. Copyright © 2020 American Association for the Advancement of Science.

triple-negative breast cancer cells. Moreover, the further combination of GPX4 inhibitors and exogenous iron supplementation rendered the explosive accumulation of lipid peroxides, significantly improving the therapeutic effect with ferroptosis. For example, An and colleagues[52] physically encapsulated the GPX4 inhibitor RSL3 and ferric ammonium citrate (FAC) in a calcium phosphate (CaP) core-lipid shell hybrid nanocarriers. Such nanocarrier showed enhanced ferroptotic antitumor efficacy by markedly inhibiting

GPX4 activity and promoting hydroxyl radical generation. Additionally, GXP4 inhibitor sorafenib and magnetic iron oxide nanoparticles (MIONPs) were loaded in the photosensitizer IR780-Hex-modified chitosan oligosaccharide self-assembled nanoparticles (CSO-BHQ-IR780-Hex/MIONPs/Sor). The Fe^{2+} ions released by MIONPs facilitated the increase of hydroxyl free radicals and lipid peroxides, resulting in ferroptosis of tumor cells[51]. Similar effects have been achieved by this group's work in the same period, in which the sorafenib-loaded complex self-assemblies based on the photosensitizer Cy7-Hex and superparamagnetic iron oxide nanoparticles (SPION) were synthesized. The delivery of the GXP4 inhibitor sorafenib caused a burst of lipid peroxide. When combined with Fe^{2+} ions produced by SPION degradation under lysosomal acidity environment, the system showed a significant ferroptosis-inducing effect in drug-resistant epithelial cell carcinoma[54]. On the other hand, utilizing GPX4 inhibitor as a nanocarrier component is also an effective strategy to trigger ferroptosis in tumor cells. Liu and teammates[55] employed Fe^{3+} and tannic acid (TA) to form a network-like corona on the sorafenib (SRF) nanocores, which was dissociated in the acidic environment of the lysosomes, thereby allowing the release of SRF to inhibit GPX4 activity. At the same time, the released TA reduced Fe^{3+} to Fe^{2+}, realizing the iron redox cycle for keeping Fe^{2+} at a high level and thus effectively promoting ferroptosis.

2.3.3. *GSH depletion*

Glutathione (GSH) is an essential cofactor and substrate for GPX4. As the overexpressed GSH in tumor cells shows an antioxidant effect by protecting the tumor cells from oxidative damage, the consumption of GSH can indirectly suppress GPX4 and increase lipid peroxides, ultimately leading to ferroptosis. Currently, various strategies to construct nanomedicine based on GSH depletion have been developed to induce ferroptosis in tumor cells, including consuming GSH by elevating ROS, or reacting with GSH through chemical bonds of nanomaterials (such as disulfide bonds, manganese-oxygen bonds). Imidazoles containing disulfide bonds as organic ligands and zinc ions as coordination metals were used to prepare photosensitizer chlorin e6-loaded MOF nanocarrier, which caused intracellular GSH depletion through the thiol exchange reaction of disulfide bonds for indirect inactivation of GPX-4, enhancing antitumor PDT and ferroptosis[56]. In addition to the disulfide bonds, the Mn−O bonds in the nanomaterials can also be exploited to deplete GSH and induce ferroptosis as they can be decomposed by GSH in the tumor microenvironment. Tang et al.[57] loaded sorafenib with manganese-doped mesoporous silica nanoparticles (MMSN) loaded with sorafenib, in which the rupture of Mn−O bonds consumed GSH, while the release of sorafenib prevented GSH synthesis by suppressing the Xc^- transport system. This dual GSH depletion strategy effectively promoted ferroptosis in tumor cells. Similarly, arginine-rich manganese silicate nanobubbles (AMSNs) were prepared for taking advantage of the GSH depletion ability of Mn−O bonds. Compared with the conventional manganese silicate nanoparticles, the surface modification of arginine promoted the tumor homing capacity of nanoparticles, and the nanobubble structure increased the efficiency of GSH consumption, thereby effectively promoting tumor cell death *via* ferroptosis process[58].

2.3.4. *Exogenous regulation of lipid peroxides*

PUFAs in membrane phospholipids are the main source of lipid peroxides in ferroptosis process. Taking this into consideration, supplementing exogenous PUFAs or their lipid peroxides, such as octadecadienoic acid (linoleic acid), eicosatetraenoic acid (arachidonic acid) and docosahexaenoic acid (DHA) is becoming an effective strategy to construct nanomedicine for promoting lipid peroxidation and ferroptosis. For example, linoleic acid hydroperoxide (LAHP) was anchored on the surface of the iron oxide nanoparticles (IO-LAHP NPs) by Zhou's group[59]. The release of Fe^{2+} from the nanoparticles under acidic tumor microenvironment catalyzed LAHP to form excess singlet oxygen for ferroptosis promotion. Additionally, arachidonic acid was conjugated to the side chains of amphiphilic copolymer mPEG-PLys to form RSL3-loaded micelles. The micelles increased the concentration of ferroptosis-promoting precursor phosphoethanolamine-arachidonic acid (PE-AA) and the lipid peroxide PE-AA-OOH. At the same time, the redox-responsive release of RSL3 *via* the peroxidation of fatty acids in micelles suppressed GXP4, and synergistically promoted ferroptosis in drug-resistant cancer cells[60]. Moreover, Ou and co-workers[61] constructed low-density lipoprotein nanoparticles LDL-DHA for exogenous delivery of DHA, which effectively elevated the levels of intracellular ROS and lipid peroxides, and induced ferroptosis in HCC.

2.3.5. *Combination therapy*

The combination of ferroptosis with other cancer treatments such as chemotherapy, gas therapy, photothermal therapy (PTT), photodynamic therapy (PDT) and immunotherapy, holds great potential to reduce the side effects of nanomaterials or chemotherapeutic drugs and improve their antitumor efficacy, which offers a promising strategy for synergistic ferroptosis. (1) Some chemotherapeutic drugs have been proven to facilitate ferroptosis. For instance, doxorubicin mediated NOX4 signaling to supplement H_2O_2 for amplifying the ferroptosis effect of Fe^{2+}[47]. In addition, our group[70] developed an efficient gene carrier to co-deliver shMTHFD2 and shGPX4 plasmids for downregulating methylenetetrahydrofolate dehydrogenase 2 (MTHFD2) and GPX4, respectively. GPX4 downregulation mediated by shGPX4 and GSH depletion resulted by plasmid shMTHFD2 have been proven to enhance the therapeutic efficacy of GPX4 suppression. (2) For gas therapy, carbon monoxide (CO) is commonly employed to combine with ferroptosis inducers for promoting ferroptosis by acting on the mitochondria and accelerating the cell oxidation metabolism and ROS production. For instance, light-responsive CO prodrug diphenylcyclopropenone and the H_2O_2-responsive aminoferrocene-based prodrug were encapsulated in a photo-responsive polymer with mitochondria-targeting ability. The nanoparticles were degraded to release both CO and Fe prodrugs under ultraviolet irradiation, which augmented the production of ROS and triggered the Fenton reaction by generating CO in the mitochondria and Fe^{3+}/Fe^{2+} ions for synergistically enhancing ferroptosis[62]. (3) PTT and PDT have received extensive attention from researchers with the merits of low invasiveness and strong specificity to tumors. On one hand, PTT has been found to increase the permeability of tumor cell membranes to enhance the endocytosis of nanoparticles or drugs by tumor cells, contributing to reinforced tumor therapeutic efficacy[54]. A variety of nanomaterials such as hollow polydopamine nanoparticles, iron-based upconversion nanoparticles (UCNP) and metal−organic frameworks (MOFs), exert their strong NIR absorption and photothermal effect or rely on the loaded photothermal agents to elevate local temperature of the tumor site and trigger drug release, which may promote Fenton reaction and further ferroptosis[71,72]. On the other hand, PDT can serve as another source of ROS for the Fenton reaction to augment ferroptosis[73]. Qin and co-workers[74] fabricated a supramolecular nanoplatform through the interaction between chlorin e6-conjugated

β-cyclodextrin (Ce6-CD) and ferrocene (Fc)-modified FFVLG3C-PEG conjugates. Ce6 generated high level of ROS under laser irradiation to ensure constant occurrence of the Fenton reaction while Fc cascaded the Fenton reaction to produce hydroxyl free radical and oxygen for amplified PDT. (4) Ferroptosis have been found to promote antitumor immunity and immunogenic cell death (ICD) through the release of damage-related molecular patterns (DAMP), such as high mobility frame 1 (HMGB1). Based on this, ferroptosis can also be combined with immunotherapeutic agents such as immune checkpoint inhibitors, ICD inducer and adjuvant for combination chemo-immunotherapy, which can enhance the therapeutic effect[75]. Jiang et al.[76] developed the platelet membrane-camouflaged mesoporous magnetic nanoparticles Fe_3O_4-SAS@PLT for sulfasalazine (SAS) delivery. The nanoparticles showed immune evasion ability and targeted tumor metastasis with platelet (PLT) modification. The Fe_3O_4 nanoparticles and SAS synergistically induced ferroptosis, stimulated antitumor immune response and M1 polarization of macrophages, and showed effective promotion of PD-1 blockade therapy.

Tumor treatments based on induction of ferroptosis can be effectively applied to tumor cells that are resistant to apoptosis or chemotherapy. In addition, mesenchymal or metastatic cancer cells are highly sensitive to ferroptosis. For instance, the increased properties of epithelial mesenchymal transition (EMT) can help to induce ferroptosis of head and neck cancer cells[77,78]. Therefore, ferroptosis-based therapy is considered as a promising anti-tumor strategy. However, ferroptosis has an important impact on cell metabolism and tumor immune microenvironment. Ferroptosis may either promote or inhibit tumor progression and growth by changing the levels of related metabolic molecules and signaling molecules. The relevant mechanism needs to be further studied. For example, induction of ferroptosis can promote macrophage polarization towards M1 phenotype and then enhance anti-tumor immunotherapy, but it may also promote the progression of pancreatic tumors by stimulating KRASG12D-mediated M2 polarization of macrophage[79–81]. Based on these observations, when applying ferroptosis inducers and nanomedicines in antitumor therapy, it is necessary to consider the different phenotypes or types of tumor cells, and the specific effect and dosage of each non-apoptotic cell death inducer.

3. Necroptosis and its inducers for cancer therapy

3.1. The molecular mechanisms of necroptosis

Necroptosis is a caspase-independent form of PCD discovered by Teng et al.[82] in 2005. Necroptosis can be induced by various factors, including tumour necrosis factor alpha (TNF-α), Fas ligand (FASL), TNF-related apoptosis-inducing ligand (TRAIL) in the tumor necrosis factor superfamily, interferon-γ (IFN-γ), liposolysaccharide (LPS), double-stranded RNA (dsRNA), viral infection, etc. Binding of these ligands to their corresponding receptors would activate the receptor-interacting protein kinase and mixed lineage kinase-like pseudokinase related pathway, namely the RIPK1-RIPK3-MLKL pathway, which is a major mediator of necroptosis[83]. Among them, the binding of TNF-α and its receptor TNFR1 leads to the formation of complex I on the cell membrane with recruiting TNFR1-associated death domain protein (TRADD), TNF receptor-associated factor 2(TRAF2), RIPK1, E3 ligase apoptosis inhibitor CIAP1/2, etc[84]. Under the action of deubiquitination enzyme CYLD, RIPK1 is deubiquitinated, which leads to the release of complex I from cell membrane into the cytoplasm and the dissociation of RIPK1. Then, RIPK1 combines with Fas-associated death domain (FADD) and caspase-8 to form complex IIa, resulting in caspase 8-dependent apoptosis[85]. However, when the caspase 8 activity is inhibited by pan-caspase inhibitor (zVAD) or FLICE inhibitory protein (FLIP), RIPK1 can be recruited to complex II to form necrosome (consisting of RIPK1, RIPK3, and MLKL). Subsequently, the interaction of RIPK1 and RIPK3 through the RHIM domain leads to the phosphorylation of RIPK3 and induces the phosphorylation of MLKL and its transfer to the cell membrane, resulting in the formation of membrane pores and a large influx of calcium ions. Consequently, mitochondrial dysfunction, excessive ROS production, ATP depletion and a series of downstream events occur, causing the cell plasma membrane rupture, cell content spillage, swelling of cells and organelles in the process of necroptosis[86,87]. In addition, the binding of LPS and dsRNA to toll-like receptor (TLR), the activation of IFN-γ receptor, TRAIL receptor, Fas and other receptors can also involve in triggering necroptosis. For example, the activation of TLR3 or TLR4 bypassed the need of RIPK1 by binding with TIR-domain-containing adapter-inducing interferon-β (TRIF) and RIP3 comprising RHIM protein domains to form TRIF-RIPK3-MLKL necrosome, which induces RIPK3-dependent necroptosis[88]. Moreover, after viral infection of interferon, the DNA-dependent activator of interferon regulatory factors (DAI) was triggered to interact with RIP3 *via* RHIM domain, inducing RIPK1-independent necroptosis[89,90]. Studies have revealed that necroptosis induces effective anti-tumor immunity. For instance, necroptosis activated $CD8^+$ cells and promoted anti-tumor immune response by releasing DAMPs and activating the RIPK1-mediated NF-κB and the expression of its downstream target genes[91]. Moreover, Snyder and teammates[92] genetically engineered melanoma cells with high RIPK3 expression to induce necroptosis *in situ*, thereby activating the RIPK1/RIPK3/NF-κB pathway to enhance the $CD8^+$ T cell-mediated immune response and inhibit tumor growth.

3.2. The pharmacological inducers of necroptosis

Necroptosis has been reported to exert tumor therapeutic effect in various kinds of cancer cell lines including lung cancer, breast cancer, and liver cancer. In recent years, apart from the classic combinations of necroptosis inducers, such as TNF + cycloheximide + zVAD or TNF + inhibitor of apoptosis proteins (IAPs) +zVAD, many chemotherapeutic drugs (*e.g.*, sorafenib, cisplatin) and natural products (*e.g.*, shikonin, artesunate) have exhibited effectiveness in inducing tumor cell necroptosis[93].

3.2.1. Anticancer drugs and synthesized compounds

A variety of clinical drugs have been reported to induce necroptosis through RIPK, (mitogen-activated protein kinases) MAPKs and other different pathways. For instance, cisplatin induces TNF-α-mediated necroptosis by promoting the formation of RIP1/RIP3/MLKL necrosomes in esophageal squamous cell carcinoma cells KYSE140[94,95]. Another chemotherapeutic drug, sorafenib, induces necroptosis in multiple myeloma (MM) cells when caspase is inhibited by caspase inhibitor Z-Vad-FMK. Moreover, this necroptosis induction effect can also be inhibited by RIPK1 inhibitor necrostin-1[96]. In addition, dimethyl fumarate (DMF) can deplete intracellular GSH and elevate the ROS levels, and subsequently activate the MAPKs signaling pathway (including activating JNK, p38 and ERK), leading to necroptosis in CT26 colon cancer cells[97]. Furthermore, inducing necroptosis is beneficial to overcome apoptosis resistance of cancer cells for enhanced antitumor efficiency. For instance, by inducing necroptosis, the treatment of second mitochondria-derived activator of caspase (SMAC) mimetic

is shown to be effective in overcoming the apoptosis resistance of caspase-8-deficient colorectal cancer (CRC)[98].

3.2.2. *Natural compounds*

Studies have shown that some natural compounds, such as shikonin, artesunate and matrine, induce RIPK1-dependent necroptosis in tumor cells. For example, shikonin has been found to induce necroptosis in osteosarcoma, glioma, lung cancer cells by upregulating the expression levels of RIPK1 and RIPK3[99−101]. In addition, the necroptosis induced by artesunate in schwannoma cells RT4 could be inhibited by both caspase inhibitor and RIPK1 inhibitor. Also, artesunate induced phosphorylation of MLKL and increased RIP1 protein levels in cancer cells to induce RIPK1-dependent necroptosis[102]. Similarly, matrine derived from *Sophora flavescens* induced cell death in cholangiocarcinoma (CCA) cells QBC939 and Mz-ChA-1, which can be inhibited by necrostatin-1. Further study showed that matrine ultimately induced necroptosis *via* enhancing RIP3 expression, activating RIP1/RIP3/MLKL pathway and promoting the production of ROS[103]. In addition, Ophiopogon D' (OPD′) isolated from *Ophiopogon japonicus* can increase the levels of FADD, FasL, androgen receptor (AR) and prostate-specific antigen (PSA) in prostate cancer cells LNCaP, leading to the RIPK1-dependent necroptosis[104].

3.3. *Necroptosis-based nanomedicine*

In recent years, many nanomaterials, including metal sulfides[105,106], titanium dioxide[107], silica nanoparticles[108], and biological selenium nanoparticles[109,110] have been explored for necroptosis-based cancer treatment[17,105−121] (Supporting Information Table S2). For instance, it has been reported that silica nanoparticles regulated Z-DNA binding protein 1 (ZBP-1), causing both apoptosis and necroptosis in HCC cells[108]. And the biogenic selenium nanoparticles SeNPs activated TNF and interferon regulatory factor-1 (IRF1), to cause ROS production and ATP depletion, subsequently inducing necroptosis of prostate cancer cells[109,110]. In addition, when necroptosis combined with the induction/blocking of apoptosis and autophagy, PTT/PDT and other therapeutic approaches, antitumor therapeutic efficacy can be improved. Considering the fact that necroptosis is correlated to apoptosis and autophagy, there are effective methods to mediate tumor cell death by inducing both apoptosis and necroptosis at the same time, or promoting necroptosis by blocking autophagy and suppressing apoptosis with nanomaterials such as gold nanorods[111], gold nanoprisms[17], silver nanoparticles[112], and graphene oxide[113]. For example, graphene oxide-chloroquine nanoconjugates prepared by Arya et al.[113] induced autophagosome accumulation and activated necroptosis of A549 cells by blocking the autophagic flux. Furthermore, combining necroptosis inducers with phototherapeutic (PTT and PDT) agents can activate photothermal effect of nanomedicines and increase the ROS levels of tumor cell, which may effectively mediate tumor cell necroptosis with enhanced antitumor efficiency. Copper chalcogenide nanoparticles involving CuS-MnS_2 nanoflowers and CuS-NiS_2 nanoparticles have been reported to generate ROS and exert photothermal effects when being activated under light, triggering MLKL-mediated necroptosis of tumor cells[105]. Besides, other light-activated nanomedicine such as nitrogen-doped titanium dioxide (*N*-TiO_2) nanoparticles caused damage to the autophagosome−lysosome fusion, blocked the autophagic flux, and generated ROS in combination with PDT, effectively inducing RIPK1-mediated necroptosis[107].

Apart from as necroptosis-inducers, nanomaterials such as solid lipid nanoparticles, cationic liposomes, polypeptide nanogels and other nanocarriers have also been extensively explored to deliver necroptosis inducers (*e.g.*, myricetin, shikonin, and mRIP3-pDNA) for necroptosis-based cancer treatment[114−116]. Ma's group[117] utilized the azoreductase specifically secreted by colonic bacteria to cleave the azo bond in the DMF-loaded sPCEG-azo polymer micelles, which triggered the release of DMF at the colon, and thereby caused the depletion of GSH, the elevation of ROS levels, the activation of the downstream signal MAPKs, and finally induced necroptosis of drug-resistant colon cancer cells. Additionally, myricetin (MYC) was loaded in solid lipid nanoparticles (MYC-SLNs) to trigger necroptosis by upregulating the expression of RIPK3 and MLKL in human lung cancer cells A549[114].

As the therapeutic effect of nanomedicine to induce RIPK3-dependent necroptosis is normally limited by the downregulation of RIPK3 expression in most tumor cells, another effective strategy is to design nanomedicine mediating necroptosis independent of the RIPK3/MLKL pathway for necroptosis-based cancer treatment. Nanobubbles have been reported to trigger RIPK3-independent necroptosis for cell-membrane rupture *via* the acoustic cavitation effect. PEG-CMD-Ce6 self-assembled nanobubbles were prepared by Um and colleagues[118] to encapsulate perfluoropentane (PFP) as a gas precursor (Fig. 3A)[118]. The ultrasound-mediated PFP cavitation effect promoted the tumor cell membrane rupture, leading to ROS production and damage-related molecular patterns (DAMP), including HMGB1 release, and the following necroptosis of the RIPK3-deficient CT26 cells in an RIPK3/MLKL-independent manner (Fig. 3B−D)[118]. In addition, the enzyme-catalyzed self-assembly (EISA), which combines enzymatic reaction and molecular self-assembly, can also induce RIPK3/MLKL-independent necroptosis for cancer treatment. *C*-Terminal methylated phosphotetrapeptide (pTP-Me), which assembled into nanofibers *via* the dephosphorylation of alkaline phosphatase at tumor sites was synthesized by Zhou and co-workers[119]. By inducing the expression of tumor necrosis factor receptor 2 (TNFR2) in Saos-2 cells and reducing the expression of three key proteins (PI3K, Akt and MEKK3) in the upstream signaling pathway of NF-κB, the nanofibers promoted the RIPK3/MLKL-independent necroptosis, and showed greater synergistic effect for tumor inhibition when combined with the NF-κB inhibitor BAY 11-7085. Besides, trypsin was utilized by Kim's group[120] to construct self-assembled nanofibers that selectively targeted the endoplasmic reticulum of tumor cells to induce ER stress, and combined proteasome and NF-κB inhibitor bortezomib (BTZ) to induce necroptosis in OVSAHO ovarian cancer cells.

In conclusion, inducing necroptosis can effectively overcome drug resistance and apoptosis resistance in some tumor cells. It also promotes the anti-tumor immunity by releasing DAMPs or activating T cells and dendritic cells (DCs), and enhances the anti-tumor immune microenvironment, showing great potential in cancer treatment. For example, in immune-tolerant pancreatic cancer, inhibition of RIPK1 kinase activity can promote tumor-associated macrophages to differentiate into immunogenic M1-like phenotypes, and show synergistic effect with PD-1 immunotherapy[122]. On the other hand, the release of cell contents and pro-inflammatory factors such as various interleukins (ILs) caused by necroptosis may also promote the occurrence of inflammatory reactions, causing damage and side effects to the surrounding healthy cells and tissues. Moreover, necroptosis may also promote tumorigenesis and tumor metastasis[123,124]. For example, necroptosis mediated by MLKL promotes tumor cell migration by activating a disintegrin

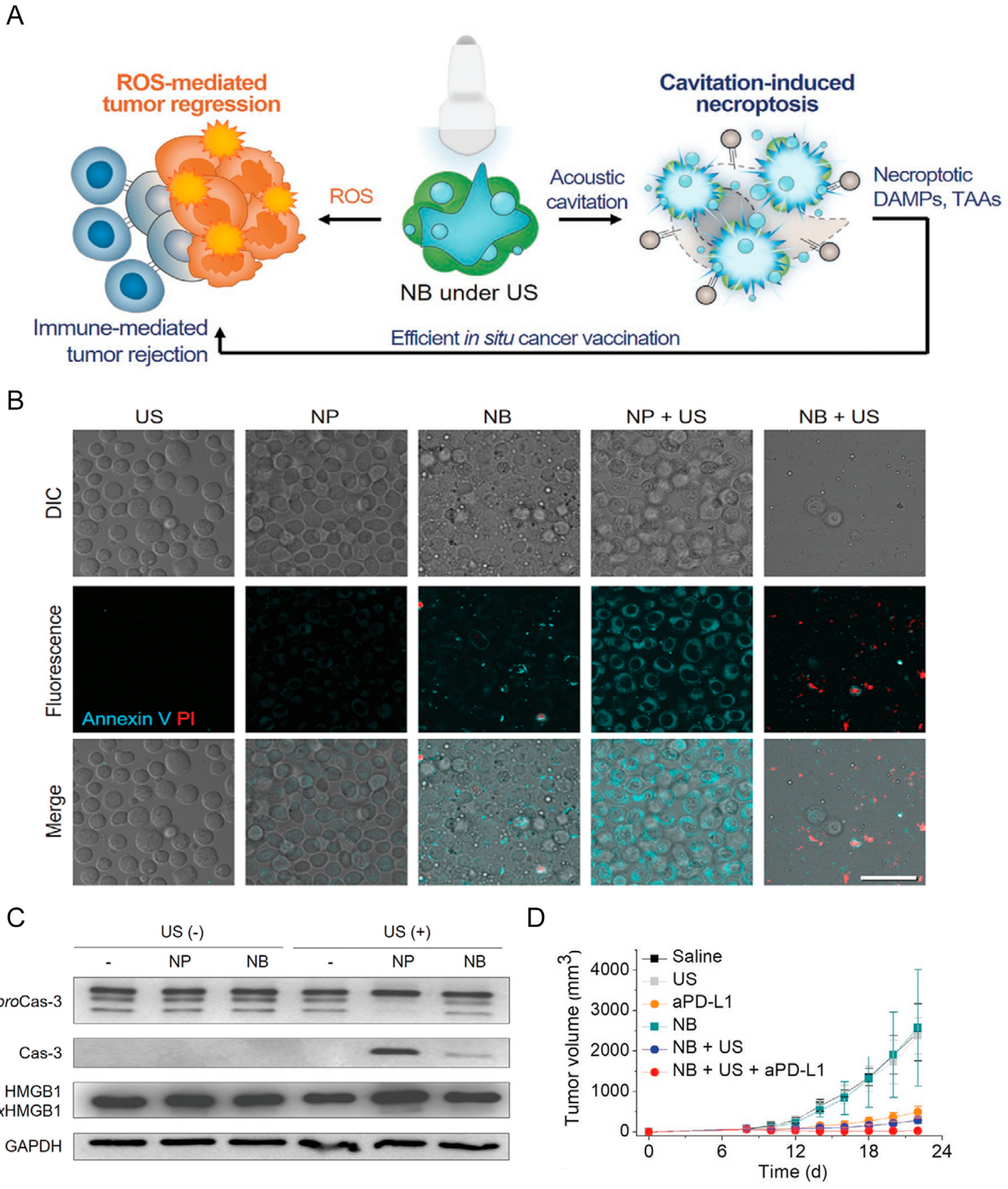

Figure 3 The self-assembled nanobubble encapsulating gas precursor perfluoropentane (PFP) and sonosensitizer Ce6 to induce RIPK3/MLKL-independent necroptosis based on sonoimmunotherapy. (A) Schematic illustration of the PVP@PEG-CMD-Ce6 NBs inducing necroptosis and immune response by causing ROS generation and cavitation effect under ultrasound irritation. (B) Confocal images of the cell membrane disruption of CT26 cells treated with NBs and US irritation. (C) The expression level of caspase-3 and HMGB1 in cells incubated with NP or NB, with/without US treatment. (D) The tumor volume of tumor-bearing mice under different treatments. Reprinted with the permission from Ref. 118. Copyright © 2020 Wiley-VCH.

and metalloproteinases (ADAMs)[125]. Therefore, more studies are needed to clarify the potential role and regulatory mechanism of necroptosis in cancer treatment, to avoid possible toxic side effects and immunosuppressive effects, and to promote the clinical application of necroptosis-based therapy. In addition, making use of the close relationship between necroptosis, autophagy, and apoptosis to determine drug combinations and develop new therapeutic schemes can help to overcome the resistance of tumor cells and achieve multi-channel tumor-killing effects.

4. Pyroptosis and its inducers for cancer therapy

4.1. The molecular mechanisms of pyroptosis

The concept of pyroptosis was put forward by Brad Cookson et al.[126] in 2001 to describe one form of caspase-1-dependent cell death discovered in *Salmonella*-infected macrophages[127]. Morphologically, pyroptosis is characterized by DNA fragmentation and chromatin condensation like apoptosis, as well as necrosis-like plasma membrane rupture. However, there still exist many distinctions between pyroptosis and other forms of cell death. For instance, compared with apoptosis, pyroptosis forms large vacuoles in the plasma membrane, which eventually leads to cell swelling and inflammation. Pyroptosis tends to undergo protrusions like apoptotic body in the first place and the following breakage is flatter than explosion-like necroptotic rupture[128,129]. In addition, pyroptosis is induced by a different molecular mechanism triggered by the lysis of Gasdermin family members (including GSDMD, GSDME, etc.) through caspase-1, caspase-4/5/11 and caspase-3 pathway. (1) The caspase-1-dependent pathway is mediated by the pattern recognition receptor on the surface of immune cells [PRR, mainly including NOD like receptor (NLR), AIM2 like receptor (ALRs) and Toll-like receptor (TLR), etc.][130]. PRR recognizes different pathogen-related molecular patterns (PAMP) from invading pathogens, DAMP released from damaged and dying cells, or other immunogenic substances, to recruit pro-caspase1 directly or to form specific inflammasomes and activate caspase-1 with the help of apoptosis-associated speck-like protein containing a CARD (ASC adaptor protein). The activated caspase1 can promote the maturation and release of inflammatory factors IL-1β and IL-18, and cleave GSDMD into carbon-terminal fragments and nitrogen-terminal fragments[131,132]. Subsequently, the nitrogen-terminal fragments of GSDMD are transferred to the lobules in the plasma membrane and combined with phospholipids, thereby inducing the formation of cell membrane pores, which eventually causes cell swelling and death[133,134]. (2) In the caspase-4/5/11-dependent pathway, the LPS on the surface of the gram-negative bacteria that infect the cells cause the multimerization of caspase-4/5/11 to form inflammasomes and cleave GSDMD, resulting in pyroptosis[135–137]. (3) In the caspase-3-dependent pathway, caspase-3 activated by chemotherapeutic drugs or DNA loss leads to the cleavage of GSDME, which in turn mediates pyroptosis in cells with high GSDME expression in a manner similar to GSDMD[138].

4.2. The pharmacological inducers of pyroptosis

Inducing pyroptosis is a very promising cancer treatment strategy, which has shown excellent effects in killing cancer cells and inhibiting tumor proliferation. Currently, the strategies to induce pyroptosis of cancer cells mainly include GSDMD cleavage, GSDME cleavage and GSDME supplement.

4.2.1. Anticancer drugs and synthesized compounds

In recent years, many drugs such as simvastatin and chalcone analogues have been found to mediate pyroptosis of cancer cells through the caspase1/GSDMD pathway or the caspase3/GSDME pathway[139,140]. For example, dipeptidyl peptidase DPP8 and DPP9 (DPP8/9) inhibitors can activate Nlrp1b in the inflammatory body sensor NLR family, and induce pyroptosis of acute myeloid leukemia (AML) cell line through caspase-1 pathway[141]. Simvastatin activates caspase-1-dependent GSDMD cleavage by activating NLRP3 to induce pyroptosis of H1299 and A549 cells. Similarly, cisplatin activates the caspase-3/GSDME pathway in A549 lung cancer cells with high GSDME expression to induce pyroptosis of lung cancer cells[142]. Chalcone analogs can induce caspase-3/GSDME-mediated lung cancer cell pyroptosis by promoting ROS production to inhibit proliferation[143]. In addition, some drugs induce pyroptosis through other pathways such as miR-497/PELP1/GSDMD, NF-κB/GSDME pathway, etc. For example, metformin can upregulate non-coding RNA miR-497 to inhibit oncogene protein PELP1, which successfully induces GSDMD-mediated pyroptosis of human esophageal cancer cells[144]. Thiopyran derivative induces GSDME-mediated pyroptosis of lung cancer cells by inhibiting the NF-κB pathway[145]. However, GSDME, the key protein in the process of pyroptosis, does not present in most tumors due to the methylation of the *DFNA5* (deafness autosomal dominant 5) gene, which can decrease or even eliminate the therapeutic efficacy of drugs inducing pyroptosis. Therefore, the problem of insufficient expression of tumor pyroptosis-related proteins can be solved by employing demethylation drugs such as decitabine, supplementing GSDM to reverse GSDME-related genes or delivering GSDM protein agonists and other methods to regulate the cellular GSDM levels. For example, employing epigenetic therapy to reverse the demethylation of related DNA such as *DFNA5* will restore the expression of GSDME and induce efficient pyroptosis of tumor cells[146].

4.2.2. Natural compounds

A variety of natural compounds have shown to mediate the cleavage of GSDMD and GSDME through different pathways and thus induce cell pyroptosis. For example, anthocyanins trigger pyroptosis in oral squamous cell carcinoma cells through the caspase1-GSDMD pathway to inhibit migration and proliferation[147]. In addition, cucurbitacin B (CuB) derived from the Cucurbitaceae plant can bind to TLR4 of non-small cell lung cancer (NSCLC) cells and activate caspase-1 and NLRP3 inflammasomes. It then causes the cleavage of GSDMD, and increases the mitochondrial ROS and Ca^{2+} level, thereby inducing cell pyroptosis and effectively inhibiting tumor growth[148]. On the other hand, paclitaxel can cause DNA damage in lung cancer cells A549, thereby activating caspase-8 and caspase-3, leading to the cleavage of GSDME by caspase-3 and the increase of its *N*-terminal fragment (GSDME-NT), ultimately inducing pyroptosis[142]. Similarly, kaempferol has been reported to cause intracellular ROS generation, activate caspase-3-mediated GSDME cleavage, promote the increase of IL-1β and ASC levels, and thereby trigger pyroptosis in glioblastoma (GBM) cells, showing good anti-tumor activity[149].

4.3. *Pyroptosis-based nanomedicine*

The common strategies to fabricate pyroptosis-based nanomedicines as shown in Supporting Information Table S3 mainly[146,150−154] include the following three aspects. (1) Block copolymers, MOFs, liposomes, etc. are utilized to construct nano-systems for delivery of chemotherapeutic drugs or small molecules (cisplatin, doxorubicin, topotecan, iron ions, etc.) with pyroptosis-inducing ability to achieve effective cancer treatment based on pyroptosis. For example, arsenic trioxide was delivered by mPEG-PLGA-PLL triblock copolymer nanoparticles, which induced GSDME cleavage and increased GSDME-N expression in HepG2 and Huh7 cells expressing GSDME, thereby inducing pyroptosis[154]. In addition, iron-activated ROS have been proven to induce pyroptosis through the Tom20-Bax-caspase-GSDME pathway. Zhou et al.[155] combined iron with ROS inducer carbonyl cyanide metachlorophenylhydrazone (CCCP) to activate ROS production, as well as the oxidation and accumulation of Tom20 complex on the outer mitochondrial membrane, thus promoting pyroptosis in melanoma cells. In view of elevated concentrations of iron ions triggering ferroptosis, Ploetz and co-workers[153] prepared MIL-100(Fe) MOF with iron ions and trimellitic acid coated with lipids (Lip-MOF). The Lip-MOF was degraded in the weakly acidic tumor microenvironment and a large amount of iron ions were subsequently released to induce pyroptosis of Hela cells. Meanwhile, such nanomedicine activated the release of IL-1β, thus triggering a potent immune response. In addition to killing tumor cells and triggering anti-tumor immunity, nanomedicine based on pyroptosis can also effectively eliminate drug-resistant tumor cells. Serna and teammates[152] prepared a nanoparticle (T22-DITOX-H6) through self-assembly of the catalytic domain exotoxin (PE24) of the laryngeal toxin (DITOX) and the CXCR4 ligand T22, which targeted the overexpressed CXCR4 in colon cancer and induced cell pyroptosis. The obtained nanomedicine effectively eliminated anti-apoptotic cells in CRC tumors for improving therapeutic effect on drug-resistant tumors. (2) Some nanomedicines are aimed to upregulate the level of cellular GSDM and solve the problem of insufficient expression of tumor pyrolysis-related proteins for triggering pyroptosis in tumor treatment. DNA methyltransferase (DNMT) inhibitor decitabine was employed by Fan et al.[146] to upregulate the *DFNA5* gene associated with GSDME overexpression in tumor cells, and was combined with cisplatin-loaded nanoliposomes to induce pyroptosis, which significantly enhanced the anti-tumor immune effect to remove tumors and inhibited recurrence. (3) Combining pyroptosis inducers with light-responsive moieties can trigger pyroptosis in a controlled manner. By this approach, pro-inflammatory cell contents are secreted to activate systemic anti-tumor immunity. Indocyanine green (ICG) and decitabine (DCT) were encapsulated in the biomimetic nanoparticles (BNP) with tumor cell membrane coating[151] (Fig. 4A). ICG was activated by NIR light to generate heat and damage the cell membrane for releasing the payloads, which sharply increased the concentration of cytoplasmic Ca^{2+} and thus promoting cytochrome c release and activating caspase-3. On the other hand, DCT upregulated the expression of GSDME to enhance the cleavage of GSDME by caspase-3, triggering efficient pyroptosis of cancer cells and activating T cells, which showed the potential to be utilized for immunotherapy of primary and metastatic solid tumors (Fig. 4B−E)[151].

As a pro-inflammatory form of programmed cell death, pyroptosis also exhibits a dual regulatory effect on the tumor immune microenvironment, providing new potential targets for antitumor immunotherapy. For example, chronic inflammation caused by pyroptosis can promote tumor growth, while the induced acute inflammatory response can enhance the anti-tumor immune response[156]. Therefore, when combining pyroptosis-base cancer therapy with immunotherapy, it is important to ensure the immune-promoting effect induced by pyroptosis, which can reduce the side effects on normal cells and tissues, improve the anti-tumor efficiency, and enhance the safety of treatment. In addition, pyroptosis may have opposite effects on different types of tumor cells. For example, in breast cancer, the high expression levels of caspase-1, IL-1β, and GSDMD in the pyroptosis signaling pathway help to reduce tumor invasion and metastasis, and improve patient prognosis[157]. On the contrary, the high expression of GSDMD in adenoid cystic carcinoma may promote tumor metastasis and invasion[158]. Therefore, it is necessary to design different pyroptosis-inducing drugs and strategies for different types and stages of cancer. It requires studying the potential mechanisms and investigating the corresponding therapeutic effects of pyroptosis inducers to obtain the best therapeutic outcomes. On the other hand, there is a cross-regulatory effect between pyroptosis, necroptosis and apoptosis. For example, caspase-8 can activate caspase-3, 6, and 7 to induce apoptosis, and inhibit necroptosis by cleaving RIPK1 and RIPK3. The inactivation of caspase 8 then leads to the formation of inflammasomes and the cleavage of GSDMD to promote pyroptosis[159,160]. Therefore, combining pyroptosis with apoptosis or necroptosis and studying how to regulate the balanced relationship between them will help to overcome the tumor resistance of a single cell death pathway and obtain the synergistic effect when inducing tumor cell death.

5. Autophagy and its modulators for cancer therapy

5.1. *The molecular mechanisms of autophagy*

Autophagy is a type of programmed cell death based on the lysosome degradation of cytoplasmic components first proposed by Christian de Duve et al.[161] in 1967. Autophagy is characterized by the double-layer vesicles originated from the Golgi apparatus, endoplasmic reticulum, etc. in the cell encapsulating cytoplasmic components (such as damaged organelles and misfolded proteins) to form autophagosomes, which are combined with lysosomes to form autophagolysosome to degrade the contents. Under normal circumstances, a variety of cell pathology or stress factors such as nutritional deficiencies, oxidative stress, low cellular energy levels and hypoxia induce autophagy[162]. Autophagy is mainly regulated by mammalian target of rapamycin (mTOR) and autophagy-related genes (*Atg*). mTOR is a member of the phosphatidylinositol 3-kinase-related kinases (PIKKs) family and was discovered in 1991 as the target protein of the immunosuppressant rapamycin[163]. mTOR can participate in the formation of two different complexes mTORC1 and mTORC2. When the cell energy level is low, the main regulator of cell metabolism, AMP-activated protein kinase (AMPK), will be activated. The activated AMPK can suppress the mTORC1 activity by phosphorylation of TSC2 and Raptor, stimulate the dephosphorylation of unc-51 like autophagy activating kinase 1 (ULK1) and the formation of a ULK1 kinase complex to mediate the nucleation of intracellular double-layer vesicles[164,165]. Subsequently, ULK1 kinase causes Beclin1 phosphorylation, activating the class III phosphoinositide 3-kinase (such as VPS34, VPS15, etc.) to form PI3K complex (including Beclin1, VPS34, ATG14, VPS15). It also promotes the recruitment of ATG protein and the expansion of intracellular double-layer vesicles[166−168]. Next, ATG12 is activated by the E1 ubiquitin activating enzyme-

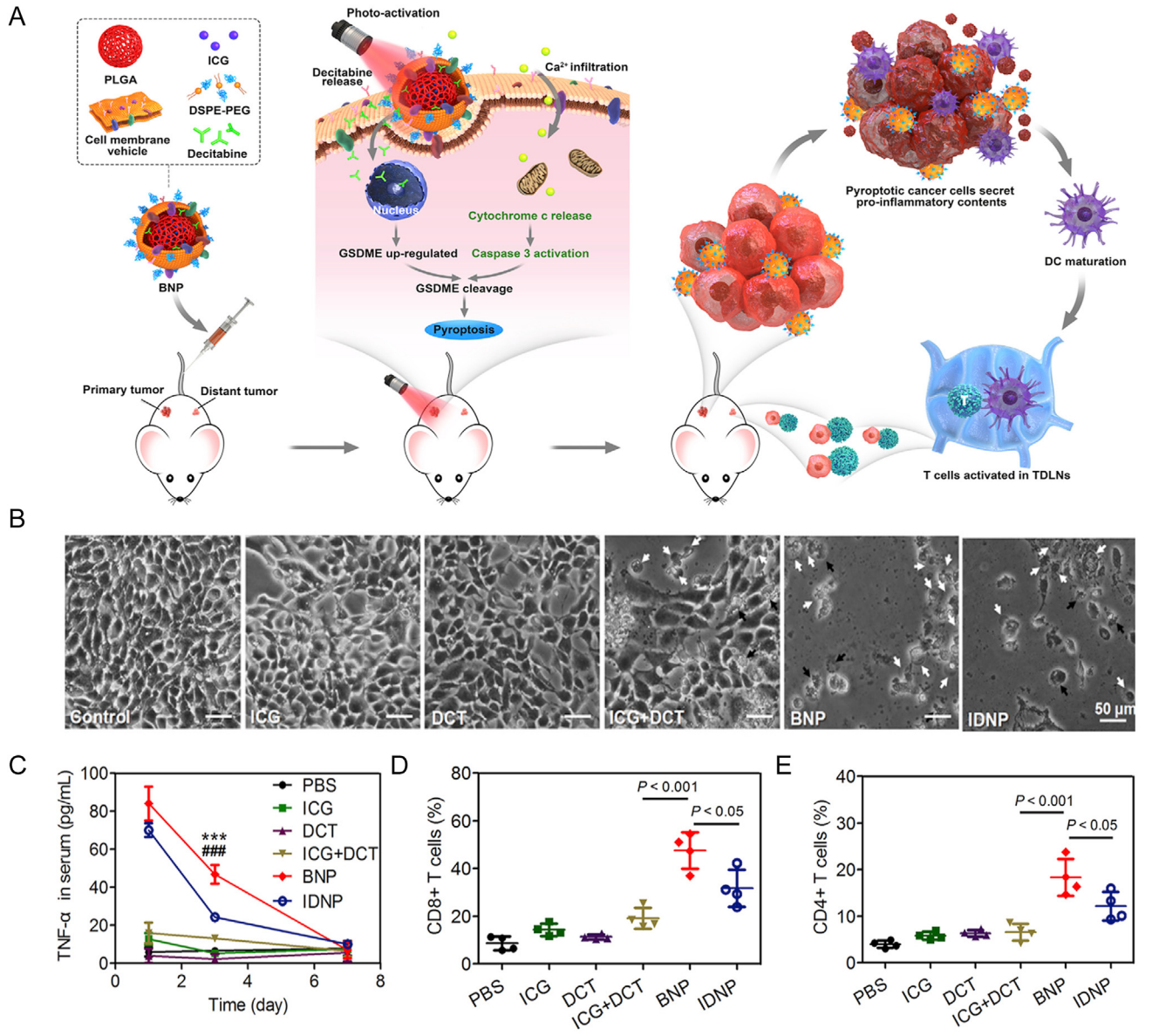

Figure 4 The biomimetic nanoparticles encapsulating ICG and decitabine to induce photo-activated pyroptosis in combination with immunetherapy. (A) The schematic illustration of the BNP to promote pyroptosis and enhance immune activation with ICG-mediated light triggering. (B) The morphology of 4T1 cancer cells incubated with different formulations. (C) The TNF-α level in 4T1 tumor-bearing mouse serum under different treatments. The percentages of (D) $CD8^+$ T cells in spleen and (E) $CD4^+$ T cells in distant tumors of mouse with indicated treatment. Reprinted with the permission from Ref. 151. Copyright © 2020 Elsevier.

like ATG7, and then is covalently linked to ATG5 after being transferred to the E2-like ubiquitin carrier protein ATG10. The ubiquitin-like system ATG5-ATG12 complex promotes the recruitment of microtubule-associated protein 1A/1B-light chain 3 (LC3) and the conversion to the lipidated form LC3-II. In this process, LC3 is cleaved by the cysteine protease Atg4 to form LC-I, which is then activated by ATG7 and transferred to E2-like ATG3. LC3-I is linked to phosphatidylethanolamine (PE) through the carboxyglycine group to form LC-II, which participates in the formation of autophagosome membranes, and works together with p62/SQSTM1 to mediate the formation of autophagosomes. After fusion with lysosomes, the contents of autophagosomes can be degraded[169]. On the other hand, in fasting condition, mTORC2 inhibition will induce autophagy in skeletal muscle cells through the Akt-FoxO3 pathway[170,171].

5.2. *The pharmacological modulators of autophagy*

Autophagy normally occurs when tumor cells are under pressure and stress, which plays an essential role in protecting tumor cells from death. It enables the degradation and reuse of damaged organelles and proteins in the lysosome to provide nutrients to cells, maintain cell homeostasis and metabolic balance. However, the excessive activation of tumor cell autophagy leads to excessive degradation of intracellular organelles and proteins, causing autophagic cell death[172]. Therefore, tumor treatment methods based on autophagy regulation are usually carried out from two aspects. (1) Specific ingredients are employed as protective autophagy inhibitors to facilitate tumor cell apoptosis by other chemotherapeutic agents. (2) Excessive autophagy activation with autophagy inducers leads to autophagic cell death of tumor cells.

5.2.1. Anticancer drugs and synthesized compounds

Currently, a variety of small molecule inhibitors of autophagy (including 3-methyladenine, chloroquine, or hydroxychloroquine, etc.)[173−175] have exhibited significant autophagy suppressive effect by reducing the level of cytoprotective autophagy. If the autophagy inhibitors are combined with chemotherapeutic drugs, cancer cell can be eliminated more effectively. For example, chloroquine or hydroxychloroquine can prevent the occurrence of autophagy by damaging autophagosome-lysosomal fusion in different kinds of tumor cells, such as HeLa cells and HCT-116 cells[173,174]. On the other hand, among a variety of autophagy inducers, JQ1, metformin, and rapamycin can induce autophagy in many cancers including bladder cancer, myeloma and pancreatic cancer by inhibiting the activity of mTOR to augment tumor cell death[176−178]. For example, JQ1 can down-regulate the mTOR levels through the LKB1/AMPK/mTOR pathway and induce autophagy in bladder cancer cells[177]. As one of the most commonly used autophagy inducers, rapamycin can induce autophagy in PC-2 cells and cause p53-dependent apoptosis by decreasing the level of mTOR and up-regulating the expression level of Beclin1[176].

5.2.2. Natural compounds

Studies show that some natural active ingredients (including oblongifolin C, olive leaf element, andrographolide, etc.) exert potent autophagy inhibition effect[179−181]. For instance, the natural compound oblongifolin C exerts anticancer activity by blocking the autophagosome-lysosome fusion, changing the lysosomal pH, downregulating the lysosomal cathepsin, and inhibiting lysosomal activity to prevent the autophagic flux of HeLa cells[181]. In addition, a variety of natural products such as curcumin, triptolide, etc. can induce excessive autophagy of tumor cells by inhibiting PI3K pathway, Akt pathway or other ways to enhance anti-tumor effect[182,183]. For example, curcumin downregulates the expression of PI3K, p-Akt and p-mTOR in gastric cancer cells and inhibits the PI3K pathway to induce autophagy[183]. Similarly, celastrol inhibits the activity of Akt and mTOR in glioma cells, leading to the induction of autophagy and ROS/JNK-dependent apoptosis[182].

5.2.3. Autophagy-based nanomedicine

Nanomedicine based on autophagy regulation for cancer therapy is mainly divided into two categories, including inducing autophagic cell death and inhibiting protective autophagy[184−201] (Supporting Information Table S4). On one hand, there are three common strategies for nanomedicine to induce autophagy. (1) Delivering autophagy inducers by nanocarriers is an effective method to mediate autophagy. The autophagy-inducing peptide Beclin1 and the antigen peptide OVA were employed for the copolymer−peptide conjugate complex NP-B-OVA preparation[184] (Fig. 5A) to induce dendritic cell autophagy. The complex also enhanced antigen cross-presentation and activated antigen-specific T cells for promoting anti-tumor immune response, thus effectively inhibiting tumor growth[184] (Fig. 5B−D). For timely overactivated autophagy to lead to more tumor cell death, Wang et al.[185] prepared autophagy-sensitive nanoparticles (ASN) by electrostatically combining the autophagy-sensitive C-TFG self-assembled micelles and the hyaluronic acid modified oxaliplatin prodrug (HA-OXA). Free OXA was released in the reduction microenvironment, which triggered ICD and activated autophagy. C-TFG micelles further responded to OXS-induced autophagy and released the autophagy inducer STF-62247, which promoted the excessive activation of autophagy to contribute to tumor cell death. (2) Some nanomaterials trigger autophagy by mediating the upregulation of ROS levels in tumor cells for oxidative stress production, or inducing mitochondria and other organ damage stress[186,189]. For example, Fe_3O_4 nanoparticles can inhibit the Akt-mTOR-p70S6K signaling pathway by increasing cellular ROS to reduce phosphorylated Akt, phosphorylated mTOR and phosphorylated p70S6K, thereby promoting autophagy and killing tumor cells[186]. The biomimetic bone-doped selenium-hydroxyapatite nanoparticles (B-SeHANs) designed by Li's group[189] can activate the ROS-mediated JNK pathway, inhibit the Akt-mTOR pathway, and induce autophagy and apoptosis to exert antitumor effect. In addition, a variety of metal-based nanoparticles, such as iron oxide nanoparticles lead to autophagic cell death by inducing mitochondrial damage, lysosomal damage, and endoplasmic reticulum stress-mediated tumor cell autophagy[187,190]. For example, the iron oxide nanoparticles synthesized by Khan and teammates[188] induced mitochondrial damage in A549 cells, mediated the increase of intracellular ROS levels, and activated the AMPK-mTOR-AKT signaling pathway to trigger autophagy and necrosis. (3) Combining autophagy inducers with cancer treatments such as PTT and PDT promotes autophagy activation and enhances tumor therapy efficiency. Zhou et al.[191] used polydopamine nanoparticles (PDA), autophagy-inducing peptide Beclin 1, PEG and cyclic Arg-Gly-Asp (RGD) peptides to synthesize PPBR NPs, where Beclin1 further enhanced PTT-induced autophagy, thereby activating autophagic cell death, and improving PTT efficacy. In the work of Wang's group[192], they prepared self-assembled dendrimers containing rapamycin and a photosensitizer (phthalocyanine, Pc). The ROS generated by Pc under irradiation destroyed the aldehyde group in the dendrimer to release rapamycin, which triggered autophagy and enhanced the efficacy of PDT to inhibit tumor growth.

On the other hand, some nanomedicines function by inhibiting the protective autophagy of tumor cells, which can be divided into two categories: (1) Using nanomaterials to deliver autophagy inhibitors (such as 3-methyladenine, hydroxychloroquine, etc.) is a practical strategy for tumor treatment based on autophagy inhibition. Chen et al.[193] used pH-sensitive zeolite imidazole ester framework (ZIF-8) nanoparticles to load the autophagy inhibitor 3-methyladenine (3-MA). The drug was released in the weakly acidic environment of the tumor, which downregulated the autophagy-related marker Beclin 1 and the ratio of LC3-II/LC3-I in Hela cells, thereby killing tumor cells by inhibiting autophagy flux and the formation of autophagosomes. (2) Nanomaterials with autophagy inhibition ability (such as gold nanoparticles coated with titanium dioxide, etc.) are fabricated for effective tumor treatment. For example, titanium dioxide-coated gold nanobipyramid (NBP/TiO_2) exerted autophagy inhibition effect by inhibiting the cathepsin B (CTSB) activity and cell proteolytic activity in the human glioblastoma cell U-87 MG, therefore inhibiting lysosomal degradation, blocking the autophagosome−lysosome fusion and suppressing autophagy flux. In addition, NBP/TiO_2 nanoparticles were combined with photothermal therapy and proteasome inhibitor Bortezomib (Bor) to play a synergistic anti-cancer effect[194].

Moreover, inhibiting cytoprotective autophagy caused by cancer therapy such as chemotherapy or radiotherapy, can overcome the resistance of tumors to chemotherapy drugs or radiation therapy as a strategy of therapeutic sensitization. Ruan et al.[195] constructed a functional drug delivery system D&H-AA&C based on agglomerable gold nanoparticles (AuNPs), which targeted the overexpressed legumain at the glioma site to release hydroxychloroquine (HCQ) and DOX. HCQ inhibited DOX-induced protective autophagy to sensitize glioma cells to DOX and synergistically repressed tumor cell proliferation (Fig. 6)[195]. Given that cancer cells acquire radiation resistance through cytoprotective autophagy, inhibiting

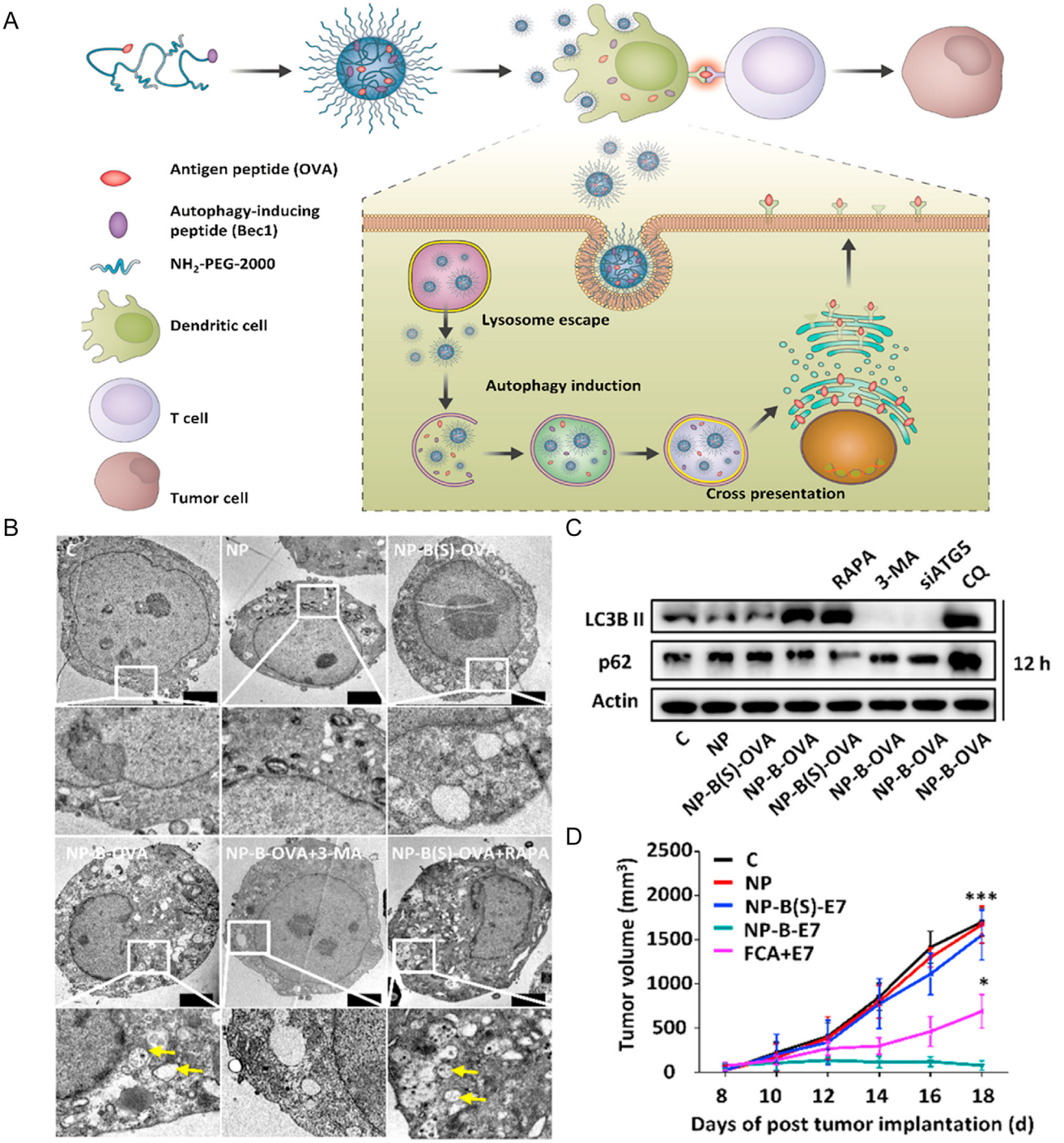

Figure 5 The self-assembled nanoactivators composed of Beclin1 peptide and the antigen peptide OVA to induce autophagy and enhance immune response. (A) Schematic depiction of the NP-B-OVA nanoactivators causing autophagy and promoting antigen cross-presentation as well as T cell priming. (B) The bio-TEM images of DC2.4 cells under indicated treatments. (C) The expression level of LC3B II and p62 of DC2.4 cells incubated with different formulations. (D) The tumor volume of TC-1-bearing mice under different treatments. Reprinted with the permission from Ref. 184. Copyright © 2019 American Chemical Society.

autophagy will improve the efficacy of radiotherapy. HCQ was loaded in hollow mesoporous silica nanoparticles HCQ-HMSNs for radiosensitivity, which caused lysosomal dysfunction in HCT116 colon cancer cells and prevented autophagosomes from fusing with lysosomes, thereby inhibiting radiation-induced cytoprotective autophagy and contributing to effective tumor inhibition[196]. Additionally, autophagy inhibition can enhance anti-tumor immune responses, and help to overcome the tumor immune resistance or drug resistance for better therapeutic effect. Some autophagy inhibitors such as HCQ promote the polarization of M1-like tumor-associated macrophages (TAM), which improves the sensitization of tumor cells towards chemotherapy. Thereby, the furin-responsive

aggregated AuNPs system (AuNPs-D&H-R&C) was developed for co-delivery of DOX and HCQ. This system responded to the furin overexpressed in breast cancer and caused *in situ* aggregations for limited cellular exocytosis and enhanced drug accumulation. The released HCQ and DOX under acidic environment induced tumor cell apoptosis and inhibited TAM autophagy synergistically. Moreover, HCQ mediated MI polarization of TAM, promoted the antitumor immunity, and helped to overcome DOX resistance, realizing combination chemotherapy and immunotherapy[202].

In conclusion, the current autophagy-based cancer treatments are mainly divided into two opposite strategies, inhibiting cytoprotective autophagy and inducing autophagy. Therefore, the use of small-molecule drugs and nanomedicines to rationally regulate autophagy of tumor cells for achieving ideal tumor suppression and therapeutic effects is the key to autophagy-based cancer therapy. Studies show that autophagy plays an important regulatory role in tumorigenesis, progression and metastasis, and has an important influence on the tumor immune microenvironment. For example, autophagy-related proteins such as Beclin1 and Atg7 participate in promoting autophagy, inhibiting tumor cell proliferation, and preventing tumorigenesis, tumor migration or tumor progression. However, autophagy defects of tumor cells can cause accumulation of damaged organelles under stress condition, leading to the accumulation of the autophagosome cargo protein p62/SQSTM1, and thus promoting oxidative stress, DNA damage and tumorigenesis[203]. In addition, the expression of Beclin1 in breast cancer cells is generally low, indicating that the lack of Beclin1 and the inhibition of autophagy may contribute to tumor development[204,205]. On the other hand, autophagy can promote the cell proliferation, migration and invasion of highly metastatic tumor cells by degrading paxillin and decomposing focal adhesions (FAs)[206]. According to various studies, autophagy often plays an important role in maintaining homeostasis of normal cells and inhibiting tumors in the early stages of tumors. And in the advanced stages of tumors, autophagy may show a tumor protection effect, helping tumor cells to overcome the stress environment and even chemotherapy resistance or radiotherapy resistance[207]. Moreover, autophagy can promote the secretion of various cytokines and immune cells such as B cells, T cells, DCs, which enhance anti-tumor immunity. The inhibition of autophagy may help advanced tumors to evade immune surveillance[208,209]. Therefore, it is the key and difficult point to choose the best autophagy regulation scheme for each stage and type of tumors, among which the related regulation mechanism, the development of new regulators and nanomedicines still need in-depth research. In addition, the use of nanotechnology to improve the specificity of autophagy modulators helps to reduce the impact of autophagy therapy on normal cells and achieve the desired antitumor effect.

6. Other forms of non-apoptotic cell death

Apart from the aforementioned ferroptosis, necroptosis, pyroptosis, and autophagy, other non-apoptotic cell death forms including paraptosis, lysosomal-dependent cell death, and oncosis also show great potential in tumor therapy. Recently, studies of nanomedicine based on these forms of non-apoptotic cell death have also provided novel practical strategies for designing antitumor agents, emerging as a hotspot in cancer treatment[210−220] (Supporting Information Table S5).

(1) Paraptosis: Paraptosis is a non-apoptotic programmed cell death form that is independent of caspase and was first named in 2000 with characteristics of cytoplasmic vacuolation, endoplasmic reticulum and mitochondrial swelling. It is induced by insulin-like growth factor I receptor (IGFIR) and is mainly mediated by MAPK kinase MEK-2 and MAP kinase JNK (Jun *N*-terminal kinase)-1[221,222]. Studies have found that a variety of small molecule inducers (such as I3M, δ-tocotrienol, loperamide)[221,223,224] and natural active ingredients (such as plumbagin, ginsenoside Rh2, curcumin)[225−227] can induce paraptosis and kill tumor cells by resulting in protein and Ca^{2+} homeostasis disequilibrium, and excessive ROS production[8,228,229]. For example, δ-TT induces mitochondrial swelling, ER expansion and cytoplasmic vacuolation, and increases the levels of p-JNK and p-p38 kinases in the MAPK cascade, thereby triggering paraptosis of prostate cancer cells[223]. In addition, curcumin induces cell paraptosis by decreasing mitochondrial membrane potential, increasing endoplasmic reticulum stress and cytoplasmic vacuoles in glioblastoma cell A172[225].

Currently, nanomedicine that induces paraptosis of tumor cells mainly exerts anti-tumor effects by causing increased intracellular ROS levels, cytoplasmic vacuolation, and organelle swelling. The complex of 8-hydroxyquinoline (HQ) and Cu^{2+} has been proven as an effective proteasome inhibitor to trigger paraptosis. HQ-conjugated block copolymer micelle PEG-PHQMA/DOX was designed to respond to the rich copper ions inside the tumor or the copper ions supplemented from the outside to generate $Cu(HQ)_2$ complex *in situ* (Fig. 7A and B)[210]. Such nanomedicine effectively inhibited proteasome activity, induced cell mitochondrial swelling and cytoplasmic vacuolation, contributed to increased ROS levels, activated MAPK to induce cell paraptosis, which overcame apoptosis resistance and effectively inhibited tumor growth (Fig. 7C and D)[210]. Similarly, the metal ion ligand complex technology was utilized to synthesize $Cu(DDC)_2$ NP, which induced paraptosis in drug-resistant prostate cancer cells (DU145-TXR) by causing endoplasmic reticulum swelling and cytoplasmic vacuolation[211]. In addition, further combining PDT and other methods can promote the increase of intracellular oxidative stress level and synergistically induce the non-apoptotic death of tumor cells. Han et al.[212] employed hypoxia-responsive hyaluronic acid-nitroimidazole (HA-NI), oxygen regulator MnO_2 NPs and reduction-responsive poly (L-glutamic acid) derivative γ-PFGA to synthesize core/shell nanoparticles (GC@MCS NPs) for simultaneous delivery of GA and Ce6. Such nanoparticles responded to tumor hypoxia and redox microenvironment relieved tumor hypoxia and consumed GSH to promote PDT treatment and the release of GA and Ce6. Ce6 mediated PDT to produce high concentrations of ROS under light, and synergistically promoted GA to induce paraptosis of tumor cells.

(2) Lysosome-dependent cell death: Lysosome-dependent cell death (LDCD), first proposed by Christian de Duve in 1983, is a form of programmed cell death mediated by autolysis of cells and tissues caused by released lysosomal hydrolase (mainly cathepsin) when the lysosome is broken, which is characterized by the triggering of lysosomal membrane permeability (LMP) and the resulting release of lysosomal contents[230]. LDCD is mainly induced by LMP, and cathepsin (such as cathepsin B＼D) is the main executor[231]. Therefore, inducing LMP plays an important regulatory role in LDCD. At present, many LDCD inducers including phenothiazine, flavonoid FV-429, ciprofloxacin or norfloxacin mainly induce LDCD by causing lysosomal damage and LMP[232−234].

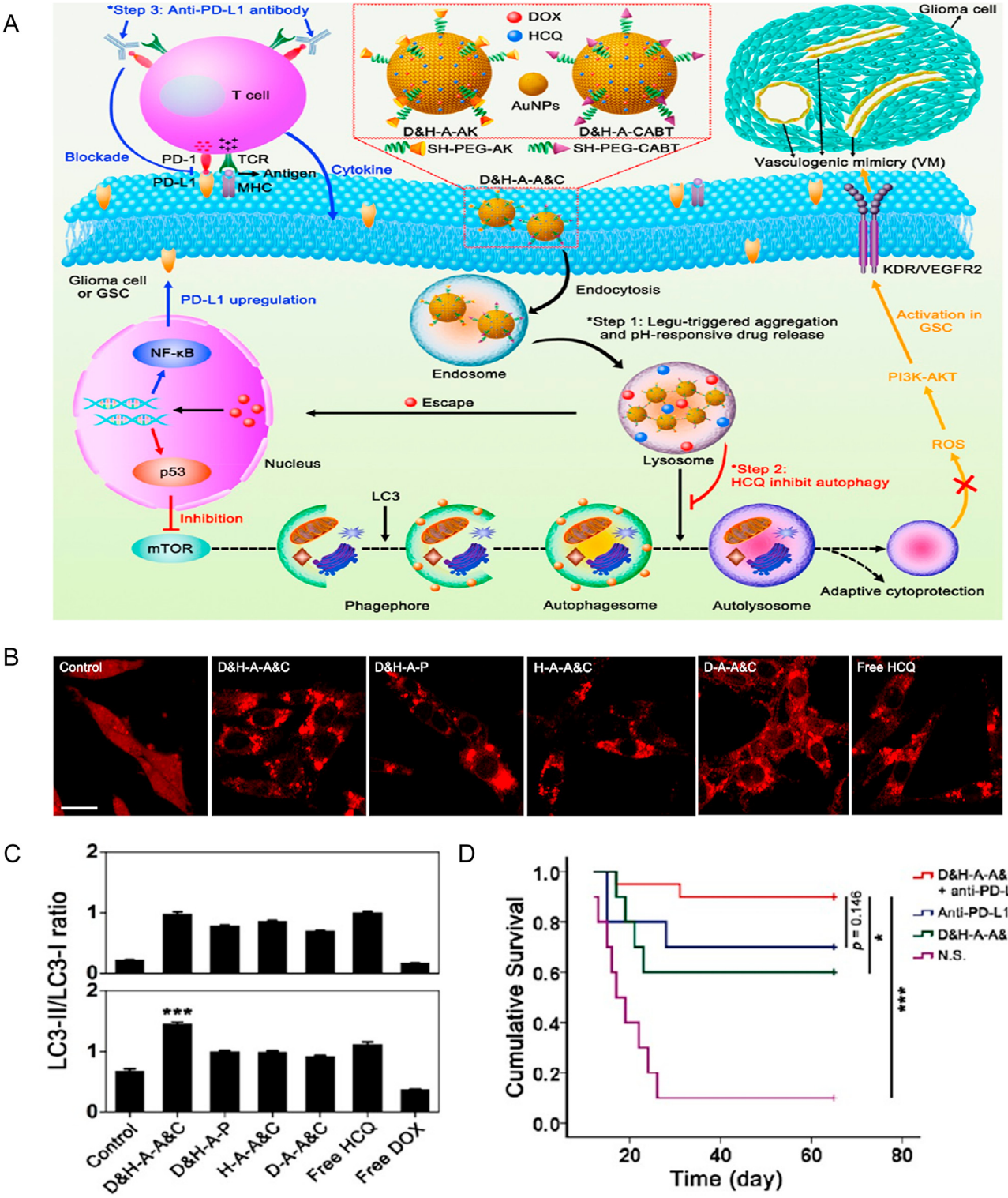

Figure 6 The gold nanoparticle-based drug delivery system loaded with DOX and HCQ to inhibit DOX-induced protective autophagy and suppress tumor cell proliferation. (A) Schematic illustration of the D&H-AA&C inhibiting cytoprotective autophagy and enhance immune response with anti-PD-L1 antibody treatment. (B) The confocal images of eRFP-LC3-expressing C6 cells treated under different treatments. (C) The semiquantitative ratio of LC3 expression in C6 cells with indicated incubations. (D) The Kaplan−Meier survival analysis of different formulations treating C6 glioma-bearing mice. Reprinted with the permission from Ref. 195. Copyright © 2019 American Chemical Society.

Among them, FV-429 can promote LDCD in T cell malignant tumor cells by inducing lysosomal damage and LMP, thereby inhibiting tumor progression[233]. Ciprofloxacin (CPX) or Norfloxacin (NFX) can cause LMP and lysosome damage and activate Bax and Bak to cause mitochondrial membrane permeability (MMP) and lysosomal-dependent cell death[232].

Nanomedicines targeting lysosomes, such as mixed-charge nanoparticles, iron oxide nanoparticles and metal composite nanomaterials, exert antitumor efficacy by causing lysosomal damage and LMP-induced LDCD[213–216]. For example, Borkowska et al.[216] used positively charged TMA and negatively charged MUA ligands to synthesize targeted mixed-charged nanoparticles [+/−]

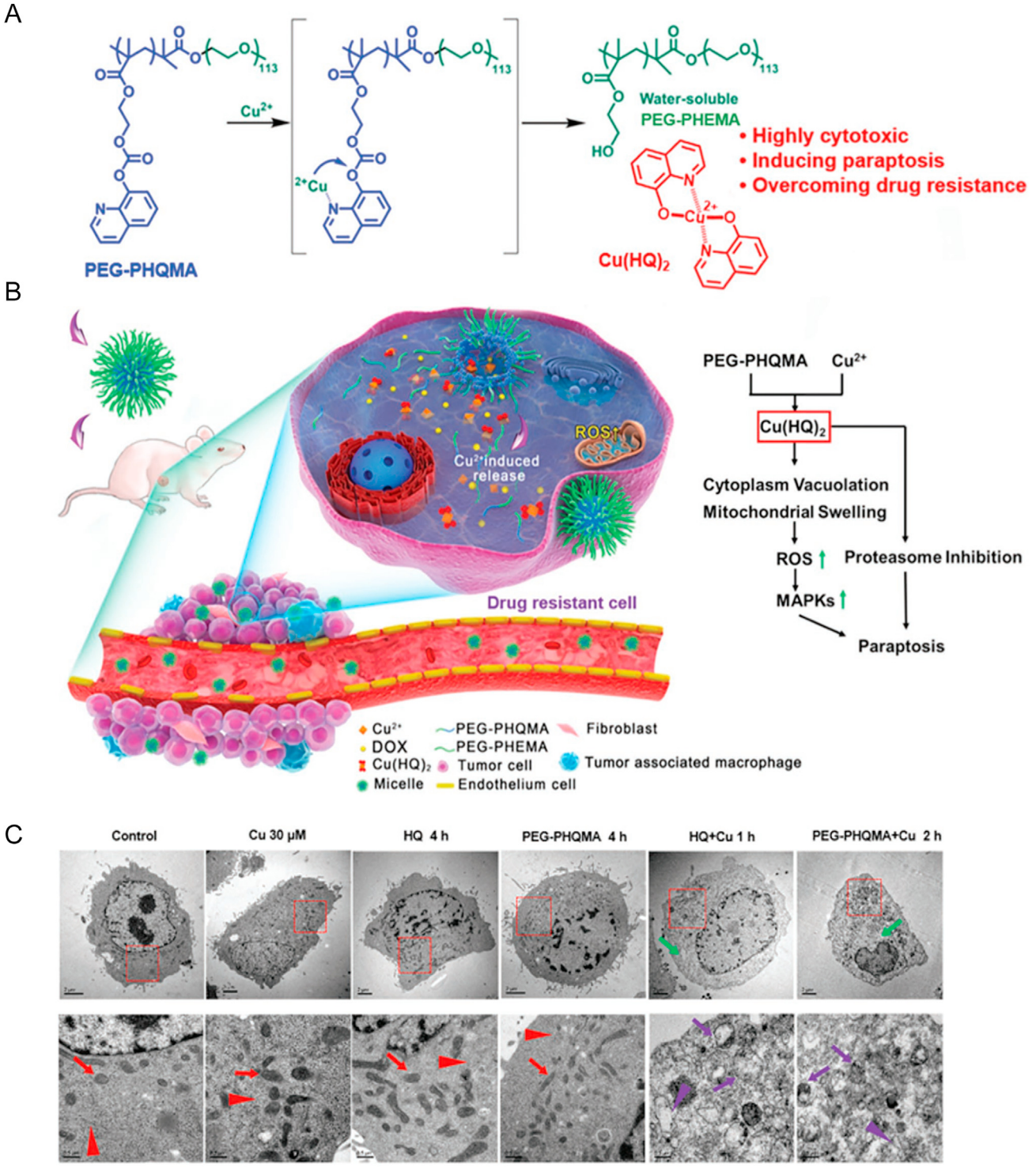

Figure 7 The 8-hydroxyquinoline conjugated block copolymer micelle to induce paraptosis by causing ROS generation and MAPKs activation. (A) Schematic description of the fabrication of PEG-PHQMA and (B) the mechanism of releasing HQ and generating cytotoxic $Cu(HQ)_2$ complex for paraptosis induction in response to the Cu^{2+} enriched environment at tumor site. (C) The TEM images of the paraptosis features of MCF-7/ADR cells with different treatments. Reprinted with the permission from Ref. 210. Copyright © 2018 Wiley-VCH.

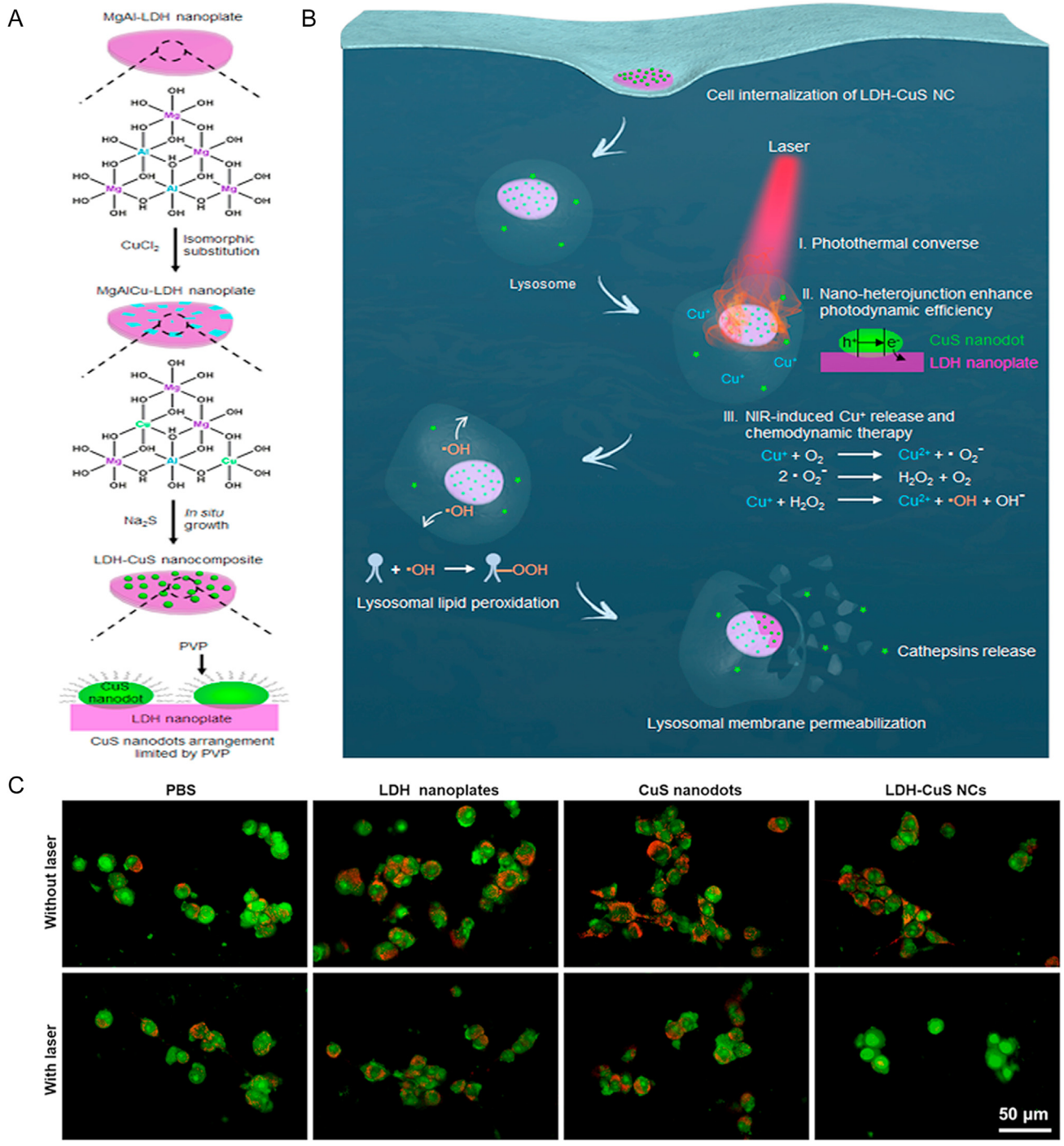

Figure 8 The double layered hydroxide-copper sulfide nanocomposites to induce lysosome-dependent cell death based on Cu^{2+} mediated Fenton reaction and photodynamic therapy. (A) Schematic diagram of the *in situ* synthesis method of LDH-CuS NCs. (B) Schematic illustration of the LDH-CuS NCs releasing Cu^{2+} under NIR irritation to induce lysosome-dependent cell death by causing photothermal effect and ROS generation. (C) The CLSM images of 4T1 cells under different treatments with lysosome stained in red fluorescence. Reprinted with the permission from Ref. 213. Copyright © 2020 American Chemical Society.

NPs, which were endocytosed into the cell and then transported to the lysosome. Such nanomedicine exhibited pH-dependent self-assembly of nano-supercrystals, induced lysosomal permeability swelling and LMP, caused lysosomal membrane destruction and functional damage, and ultimately triggered lysosomal-dependent cell death. In addition, the further combination of magneto-thermal therapy, chemodynamic therapy and photothermal therapy enhances the effect of anti-tumor therapy based on LDCD. Domenech and colleagues[215] prepared epidermal growth factor (EGF)-bound iron oxide magnetic nanoparticles (IO-MNP), which entered cells and lysosomes through endocytosis by targeting the EGF receptor (EGFR) overexpressed on the surface of tumor cells, and locally generated heat under the alternating magnetic field to cause the rupture of LMP and lysosomes, increase the cytoplasmic

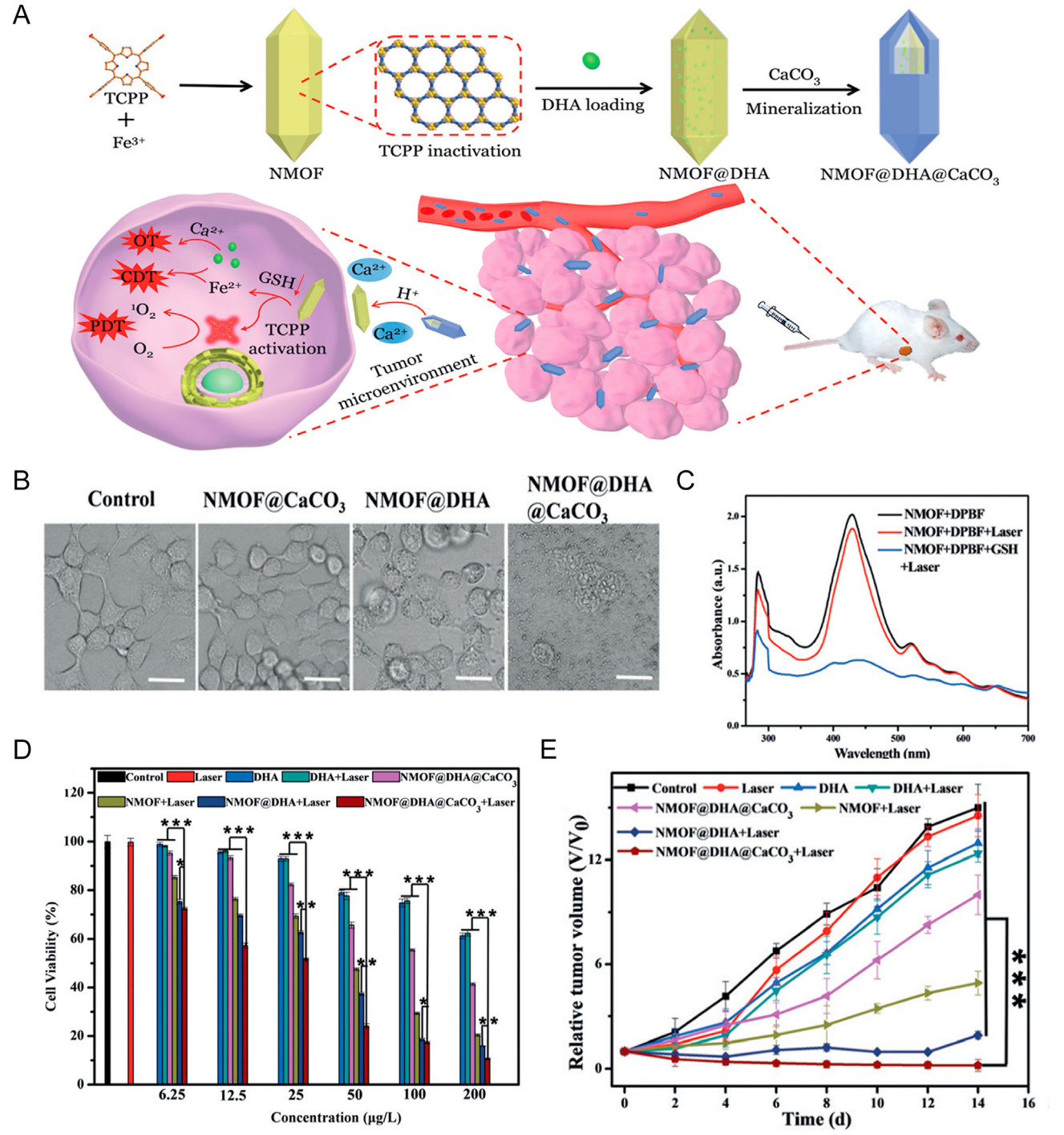

Figure 9 The $CaCO_3$ coated NMOF loaded with dihydroartemisinin to induce Fe^{2+}-DHA-mediated chemodynamic therapy, TCPP-mediated photodynamic therapy and Ca^{2+}-DHA-mediated oncosis. (A) Schematic description of the NMOF@DHA releasing Fe^{2+} and Ca^{2+} under acidic tumor and GSH enriched environment, and activating TCPP-mediated PDT for cancer treatment. (B) The morphology of 4T1 cells treated with different nanoparticles. (C) The UV−Vis absorption spectra of the $^1O_2$ probe DPBF under different treatments. (D) The cell viability of 4T1 cells treated with different formulations. (E) The relative tumor volume of 4T1 tumor-bearing mice under indicated treatments. Reprinted with the permission from Ref. 217. Copyright © 2019 Wiley-VCH.

activity of cathepsin B, and eventually lead to LDCD of MDA-MB-231 human breast cancer cell. Liu and co-workers[213] fabricated double layered hydroxide-copper sulfide nanocomposites (LDH-CuS NCs) with high photothermal conversion efficiency through a one-pot *in situ* growth strategy (Fig. 8A and B)[213]. LDH-CuS NCs induced LMP and LDCD *via* the lipid peroxidation process resulted by copper-mediated Fenton-like reaction and laser-induced photothermal effect (Fig. 8C)[213].

(3) Oncosis: Oncosis was first proposed by von Reckling-hausen in 1910 as a manner of cell death accompanied by cell swelling[235], which is mainly triggered by the depletion of

intracellular ATP to lead to the inactivation of Na^+/K^+-ATPase on the cell membrane and cause rapid increase of intracellular sodium and calcium ions. The imbalance of ion homeostasis eventually leads to organelle swelling and cytoplasmic swelling[236,237]. A variety of cell swelling inducers, including aspirin, Kahalalide F, Fluopsin C and other drugs, mainly induce oncosis by increasing cellular ROS levels and ATP depletion[238−240]. For example, aspirin can inhibit the level of Bcl-XL in tumor cells, and lead to ATP depletion, cytoplasmic vacuolation, thereby inducing oncosis and inhibiting breast cancer EMT6 tumor growth[240]. In addition, many natural compounds and their derivatives, such as dehydroabietic acid derivative QC4, berberine, and sodium artesunate, exhibit good abilities to induce oncosis[241−243]. For example, dehydroabietic acid derivative QC4 can induce ATP depletion, ROS generation, increase cytoplasmic free calcium ion concentration and lactate dehydrogenase (LDH) leakage in gastric cancer cells, and cause mitochondrial damage and cell membrane rupture, ultimately inducing oncosis and apoptosis[241].

Designing nanomedicine based on oncosis mainly focuses on employing nanomaterials to deliver oncosis inducers (such as artesunate, dihydroartemisinin), chemotherapeutic drugs (such as DOX) to tumor cells, or combining photosensitizers (such as TCPP, Ce6) with chemotherapy for effective therapy[217−220]. With the aid of photodynamic therapy, Li et al.[220] constructed branched polyethyleneimine (BPEI)-PEGylated ceria nanoparticles (PPCNPs-Ce6/FA) loaded with chlorin e6 (Ce6)/folic acid (FA). The nanoparticle entered cell by targeting the folate receptor on the surface of the tumor cell to elevate ROS production under near-infrared radiation, reduce the expression of P-glycoprotein (P-gp) to overcome cell resistance, as well as induce LMP, energy consumption and cell swelling for inducing oncosis of drug-resistant MCF-7/ADR cells. Further combined with chemodynamic therapy, Wan's group[217] constructed dihydroartemisinin (DHA)-containing $CaCO_3$-coated NMOF using $Fe_3O(OOC)_6$ metal clusters and TCPP as raw materials (Fig. 9A)[217]. Such nanomedicine released calcium ions and GSH-responsive NMOF@DHA in weakly acidic environment of the tumor, and then activated Fe^{2+}-DHA-mediated chemodynamic therapy, Ca^{2+}-DHA-mediated oncosis, and TCPP-mediated photodynamic therapy to synergistically induce 4T1 tumor cell death (Fig. 9B−E)[217].

7. Conclusion and future perspectives

Various forms of non-apoptotic cell death and their pharmacological modulators were briefly summarized. Then we further presented the latest progress of the nano drug delivery systems based on non-apoptotic cell death, including the fabrication and applications in cancer treatment. Nowadays, most chemotherapeutic drugs are confronted with the challenges to exert the anti-tumor activity sufficiently since tumor cells may escape from apoptosis to result in poor chemotherapy efficacy. Therefore, cancer therapy that induces non-apoptotic death has aroused more and more attention, which provides a novel strategy for effectively overcoming the apoptosis resistance in tumor treatment and the further chemotherapy resistance. However, it should be noted that there are still some existing problems and challenges confronted by non-apoptotic cell death. The low expression of positively ferroptosis-related factors (*e.g.*, LIFR[244], ACSL4[245], POR[246]) and the high expression of negative factors (MS4A15[247], RBMS1[248], GOT1[249], FSP1[250]) in specific cancer cells may result in resistance to drug-induced ferroptosis by various mechanisms, which diminish the therapeutic efficacy of ferroptosis-based therapy. For example, LIFR is normally downregulated in hepatocellular carcinoma, which activates NF-κB signaling pathway and upregulates iron-sequestering cytokine LCN2 to contribute to insensitivity to ferroptosis inducers. And RBMS1 is upregulated in lung cancer clinically to mediate ferroptosis evasion by interacting with the translation initiation factor. Therefore, more therapeutic strategies to regulate these related genes and proteins are required to be applied to overcome ferroptosis defense. Another concern for non-apoptotic cell death forms such as autophagy and necroptosis, is that they are regarded as a double-edged sword to dually inhibit or promote the development and metastasis of tumors. For instance, inducing autophagic cell death or inhibiting protective autophagy of tumor cell both can exhibit therapeutic effect in cancer treatment. Some studies also proved the role of necroptosis in promoting cancer progression and metastasis although necroptosis is effective for treatment of many cancers. Necroptosis-related RIP1 and RIP3 that are highly expressed in pancreatic ductal adenocarcinoma, suppress adaptive immune and promote progression through inducing expression of the chemokine attractant CXCL1 and upregulating cytoplasmic SAP130 and Mincle (the cognate receptor)[125]. In terms of pyroptosis, the effector GSDME is silenced in most cancer types due to the GSDME gene methylation, which makes it hard to trigger pyroptosis effectively. Moreover, most of the current pyroptosis inducers are poor in cell selectivity to render severe toxicity to normal cells considering potently releasing proinflammatory intracellular contents. For example, microcrystalline silica particles render significant inflammation and pyroptosis *via* NLRP3 inflammasome pathway and NF-κB signaling pathway in human airway epithelial cell, thus resulting in lung damage and even pulmonary fibrosis[251]. In view of it, the protein expression or functions of signaling pathways related to non-apoptotic cell death are diverse in different types of cancer cells[172]. The mechanism and signal pathways of non-apoptotic cell death in tumor cells still require to be thoroughly studied, to realize specific non-apoptotic death in tumor cells and achieve ideal therapeutic effect.

In cancer therapy based on non-apoptotic cell death, compared with small molecule inducers, nanomedicine can target tumor cells due to the tumor EPR effect and the ability to integrate targeting groups. Nanomaterials endow payloads with higher tumor specificity, targeting efficiency, blood circulation half-life and the accumulation rate at tumor site, which may reduce the toxic and side effects of the chemotherapeutic drug on normal cells and tissues, ensure the efficacy of the drug, and promote the clinical application of non-apoptotic cell death. In addition, the convergence of nanotechnology to combine non-apoptotic death with a variety of cancer therapies (such as chemotherapy, phototherapy, immunotherapy, etc.) further reduces the toxicity of nanomedicine, improves the therapeutic effect of cancer treatment based on non-apoptotic death compared with monotherapies, which provides more prospects for its application in cancer therapy. However, given that different types of tumors possess different sensitivities to the same nanomedicine that could induce non-apoptotic cell death, the therapeutic agent may also cause different degrees or even forms of non-apoptotic cell death. For example, iron-based nanomaterials can not only be employed to induce ferroptosis, but also be utilized to induce pyroptosis[69,153]. Therefore, it is in urgent need to rationally design nanomedicine based on non-apoptotic cell death and investigate the outcome of specific nanomaterial/drug combination for inducing a specific form of non-apoptotic cell death, or the same

nanomaterial/drug leading to different non-apoptotic cell death forms. Therefore, developing nanomedicine based on non-apoptotic cell death is a feasible strategy for effective cancer treatment, which provides in-depth understanding of anti-cancer mechanisms.

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Author contributions

Xuan Wang and Peng Hua did the literature search, analyzed related articles, and wrote the manuscript. Meiwan Chen, Xuan Wang and Peng Hua revised the manuscript. Peng Hua prepared the figures in the article. Chengwei He provided professional advice in the article. Meiwan Chen was the corresponding author and gave many explicit improving suggestions. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

Appendix A. Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2022.03.020.

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