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Review

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Metalloptosis: metal ions-induced programmed cell death based on nanomaterials for cancer therapy

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Abstract

Programmed cell death (PCD) is defined as regulated cell death controlled by an intracellular program. While apoptosis was once thought to be the only kind of PCD, current understanding has expanded to include other forms such as pyroptosis, autophagy, and necroptosis. These processes, especially apoptosis and necroptosis, serve as natural defenses that restrict cancer cells from surviving and disseminating. However, cancer cells have evolved various strategies to evade PCD, including genetic mutations and epigenetic modifications in key modulators of PCD pathways. With the continuous development of nanotechnology, emerging nanomaterials (NMs) are considered to break through this bottleneck due to their intrinsic physicochemical properties. Especially, new kinds of cell death induced by NMs, such as ferroptosis, cuproptosis, and calcium overload, show gratifying potential in cancer therapy, which is closely linked to the role of metal ions. Additionally, other metal ions-induced cell death such as sodium and zinc have also emerged in an endless stream. Hence, we propose the term "metalloptosis" to describe cell death induced by metal ions and summarize its application in cancer therapy through NMs. This review will delve into the critical design principles for engineering NMs involved in metalloptosis and provide a comprehensive summary of current metal ions-mediated cancer therapies, focusing on nanoplatforms and their mechanisms of action. We hope that this review will provide a new perspective on metal ions-mediated cancer therapy based on nanotechnology.

Keywords: Cancer therapy, Metalloptosis, Nanotechnology, Programmed cell death

1. Introduction

Programmed cell death (PCD) is a regulated, intracellular program essential for eliminating damaged, infected, or obsolete cells, thereby maintaining tissue homeostasis and development.[1] PCDs, categorized into apoptosis, pyroptosis, autophagy, and necroptosis based on distinct mechanisms, are intricately linked to human pathologies, including inflammatory disorders and malignant tumors. Among these, apoptosis is one of the earliest discovered forms of PCD, characterized by cell shrinkage and chromosomal DNA fragmentation.[2] The classical apoptotic pathways bifurcate into extrinsic and intrinsic categories.[3] The intrinsic apoptotic pathway is induced by intracellular stimuli such as oxidative stress and DNA damage, regulated by multiple kinds of proteins. Bcl-2 family proteins such as Bax and Bcl-2 modulate mitochondrial membrane permeabilization, facilitating the release of cytochrome *c*. This cytochrome *c*, in conjunction with procaspase-9 and apoptotic protease activating factor (Apaf-1), assembles into the apoptosome complex, a crucial regulator of the intrinsic pathway. On the other hand, the extrinsic apoptotic pathway is mediated by the interaction between death ligands and death receptors. Taking Fas as an example, Fas-associated death domain protein and procaspase-8/10 form the death-inducing signaling complex (DISC). DISC then activates the downstream effector caspases through self-cutting procaspase-8 and procaspase-3 into caspase-8 and caspase-3, respectively, ultimately leading to cell apoptosis.[4] Pyroptosis, distinguished by DNA fragmentation, cell blebbing, and leakage of cellular content, represents another kind of PCD that exhibits notable differences from apoptosis.[5] Gasdermin (GSDM) family proteins play a crucial regulatory role throughout the entire process of pyroptosis. Since the discovery and identification of the GSDM D protein in 2015, which remains in an inhibitory state under normal circumstances, the regulatory pathway of pyroptosis has progressively been elucidated. Upon activation, the GSDM D protein is cleaved by caspases, clipping to generate GSDM D-N (the N-terminal domain) and GSDM D-C (the C-terminal domain).[6,7] In addition to GSDM D, other GSDM family proteins such as GSDM A-C and GSDM E are also capable of inducing pyroptosis.[8–12] Autophagy is a relatively conservative way of regulating cell growth and death, which is crucial for maintaining intracellular homeostasis.[13] Serving as a physiologically cellular strategy, autophagy safeguards cell survival under stress conditions. Only under certain adverse circumstances, autophagy will over-activate and result in cell death.[14] It is mediated by some nutritional metabolic pathways through multistep lysosomal degradation, in which various

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kinds of proteins participate, such as autophagy-related (ATG) proteins and their core complexes. These proteins run through the entire process of autophagy from initiation, fusion, and degradation.[15] Necrosis initially seen as an accidental cell death has gradually been proven to have a programmatic regulation pathway, which is termed "necroptosis."[16] Different from necrosis caused by physical trauma, necroptosis can be affected by many receptors, such as Toll-like receptors,[17] interferon receptors,[18] tumor necrosis factor receptors,[19] and T cell receptors.[20] Besides, some chemical compounds such as necrostatin-1[21] can also induce necroptosis through metabolic toxic stress.

Intriguingly, PCD, especially apoptosis and necroptosis, is originally a natural self-protection mechanism formed by the body to prevent the survival or spread of malignant cells. However, cancer cells can successfully escape the recognition of PCD by regulating their own transcriptome and proteome. With the continuous development of nanotechnology, nanomaterials (NMs) are emerging to successfully treat cancer through inducing PCD in versatile ways thanks to their intrinsic physicochemical properties. The role of metal elements in NMs has aroused great interest among researchers. As research continually deepens, an expanding body of evidence underscores the significant role of metal elements in regulating biological processes, including cell growth and cell death. Various metal elements, including sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), and trace elements such as iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), cobalt (Co), and molybdenum (Mo), participate in many biological processes.[22] For instance, metal elements often act as cofactors to help some enzymes exercise their specific functions by stabilizing the structures of these enzymes and facilitating their catalytic activity. On the other hand, some metal elements such as Fe, Cu, and Zn are closely bound up with maintaining the structural integrity of proteins and other macromolecules within cells.[23] In terms of regulating cellular signaling pathways, Zn and Ca can participate in some signaling pathways of regulating some cell differentiation and gene expression[24]; Zn and Mg help the process advancement of DNA replication and repair[25]; Fe and Cu have been proven to be conducive to redox reactions through increasing the efficiency of transferring electrons between molecules.[26] However, disruption of the body's internal metal element homeostasis can cause a series of harmful consequences, such as cytotoxicity and various forms of cell death, which can significantly contribute to the onset of numerous diseases. Specifically, the deficiency of Fe ions can easily lead to anemia and cause low oxygen-loading in the body.[27] Similarly, the deficiency of Cu ions can also cause anemia, as well as bone abnormalities, and neurological dysfunction.[28] On the other hand, excessive metal ions can trigger different kinds of PCD through multiple pathways, including inducing oxidative stress response, disrupting cell membranes, causing protein inactivation, and splitting DNA fragments, ultimately making tissues and organs damaged.[29]

While the imbalance of metal ions can lead to various health issues, an interesting turn of events is observed in the field of nanotechnology. Many NMs, ingeniously designed, have been found to induce PCD. This property opens new avenues for research, especially in the potential therapeutic exploitation of NMs to target diseased or cancerous cells by manipulating PCD mechanisms. For example, recently proposed ferroptosis and cuproptosis based on NMs exert gratifying potential in cancer therapy. On the other hand, other metal ion-induced cell death including Ca, Na, and Zn (as well as termed ion interference therapy) has emerged in an endless stream. Therefore, we propose the term "metalloptosis" to describe the unique process of cell death induced by metal ions. This new definition aims to summarize existing metal ion-mediated cancer therapy, potentially paving the way for innovative research directions. It highlights the significant role that specialized metal ions in NMs could play in advancing cancer treatment strategies. The definition of metalloptosis will include the following aspects: (1) specific metal elements; (2) induced PCD; (3) specific signal pathways; and (4) specific detection methods or markers. Based on existing research, PCDs that meet the definition of metalloptosis include ferroptosis, cuproptosis, and calcium overload because all of them have specific signal pathways and specific detection methods or markers. Considering the current research status, some other metal ions (eg, Mg ions, Zn ions, Mn ions, and Na ions) can also induce PCD, while it is through some relatively universal signaling pathways, such as destruction of mitochondria, apoptosis, pyroptosis, necrosis, etc, we temporarily classify it as the definition of metalloptosis, hoping that as research deepens, we will discover that they have their own unique signaling pathways and detection methods or markers. In detail, this review will discuss the essential points of engineering NMs design pertinent to metalloptosis, and summarize existing metal ions-mediated cancer therapy consisting of nanoplatforms. It will also elucidate the underlying mechanisms of these therapies (including ferroptosis, cuproptosis, calcium overload, Zn ions-mediated cell death, Na ions-mediated cell death, etc), offering fresh insights and perspectives on metal ions-mediated cancer therapy through the lens of nanotechnology.

2. Design of engineering NMs in metalloptosis

As a kind of metal-dependent cell death, metalloptosis is closely linked to intracellular ion levels. Direct or indirect delivery of metal ions into cells is a common strategy to induce metalloptosis (Figure 1). Compared to small-molecule metalloptosis inducers, engineering NMs, with their unique physicochemical properties, offer significant advantages in enhancing drug stability, increasing tumor accumulation, reducing systemic toxicity, and achieving sustained release.[30] Therefore, this section will mainly focus on discussing the design strategies and the prominent role of engineering NMs in metalloptosis.

2.1 Engineering NMs for direct delivery of metal ions

Various NMs containing specific metallic elements have been employed to directly enhance intracellular metal ion levels, thereby inducing metalloptosis. The design of such NMs often requires attention to the following aspects: (1) The proportion of metallic elements in NMs should be appropriate, ensuring sufficient metal transport efficiency while maintaining viable biosafety and avoiding severe toxic side effects; (2) The modification of surface ligands should be strategically implemented to enhance the biocompatibility and stability of NMs. Additionally, ingenious surface modification can introduce new biofunctionalities such as targeted tissue specificity, while also enhancing interactions with biological membranes and biomolecules, thereby augmenting the therapeutic potential of the NMs. (3) The designed NMs should be stimulus-responsive, capable of releasing metal ions in a predictable manner upon exposure to specific external or internal environmental stimuli. These stimuli include physical factors (such as light, temperature, magnetic fields, and electric fields), mechanical factors (such as ultrasound), chemical factors (such as pH and certain molecules like

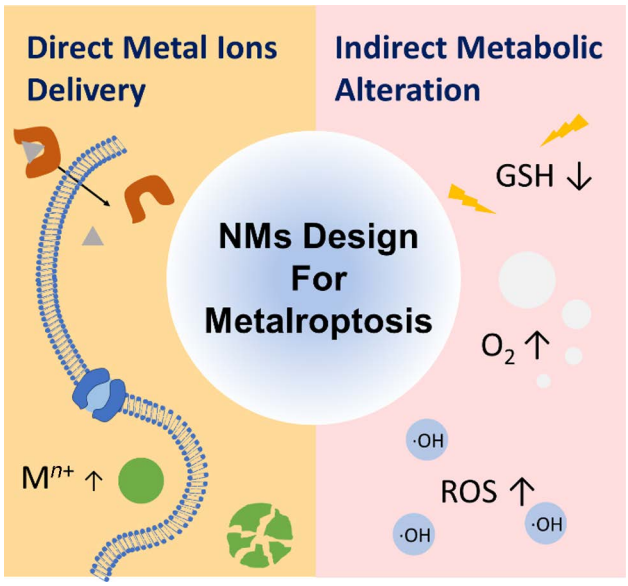

Figure 1. Scheme of engineering NMs in metalloptosis. Engineering NMs used for inducing metalloptosis can be categorized into 2 main types. The first type is utilized for direct metal ions delivery, such as NMs based on ionophores, inorganic NPs, and MOFs, which contain specific metal elements. The second type involves NMs used for metabolic alteration, which enhance the sensitivity of tumor cells to metal ions through pathways such as GSH depletion, ROS production, hypoxia modulation, oxygen generation, and affecting cell's ion channels, thereby causing or facilitating the occurrence of metalloptosis.

glucose), and biological factors (such as specific enzymes); (4) Through multifunctional design, additional capabilities such as imaging and synergistic therapeutic functions could be integrated into NMs.

Ionophores are well-known regulators of metal homeostasis, with many such molecules currently under clinical trials.[31] These molecules are capable of binding metal ions and facilitating their transport into cells, circumventing the standard metal ion importers.[32] The reversible nature of the binding between ionophores and metal ions lays the foundation for the targeted release of metal ions in response to the specific environment of tumor sites. However, due to their low molecular weight, ionophores are rapidly cleared and metabolized in circulation, which severely limits their uptake by tumor cells and, consequently, their therapeutic efficacy.[33] This challenge can be addressed by engineering ionophores into NMs, which has shown promising results in inducing metalloptosis. For instance, when elesclomol (ES), a well-known copper ionophore, was formulated into NMs in the form of the ES-Cu^{2+} complex, it overcame the limitations posed by the short half-life of small-molecule ES in the bloodstream, thereby enhancing copper accumulation in tumors and facilitating cuproptosis.[34]

Inorganic nanoparticles (NPs) containing specific metal elements are also extensively utilized for inducing metalloptosis. For example, iron oxide NPs,[35] amorphous iron NPs,[36] FePt NPs,[37] and iron carbide NPs[38] are employed in killing cancer cells through ferroptosis. Similarly, cuprous oxide NPs[39] and $Cu_2(PO_4)(OH)$ NPs[40] have been employed to initiate cuproptosis, while CaO_2 has been used to induce calcium overload.[41] These inorganic NPs often exhibit enhanced tumor tissue targeting, effectively reducing the adverse effects of metalloptosis on normal tissues. Additionally, they demonstrate prolonged circulation time in the blood as well, possessing a superior capability for metal ions delivery. This showcases the diverse potential of inorganic NPs in regulating metal homeostasis and advancing therapeutic applications.

Constructed from metal ions/clusters and organic linkers, metal-organic frameworks (MOFs) represent a category of highly ordered coordination polymers, distinguished by their porous crystalline structure.[42] MOFs, through ingenious design, customization, and modification of structural parameters, can achieve significant high surface areas, high-density active sites, and enhanced stability.[43] These features have led to their widespread applications in various fields including drug delivery, tissue engineering, and regenerative medicine, as well as antibacterial and anticancer treatments.[44–46] Furthermore, MOFs can be specifically tailored for efficient metal ions delivery to induce metalloptosis,[47,48] which will be discussed in detail in subsequent sections.

In addition to the aforementioned types, NMs based on single-atom nanozymes, hydrogels, and other coordination complexes can also be employed to directly deliver metal ions into cells, inducing metalloptosis.[49–52] This diversity in the design of NMs opens new avenues for exploring metalloptosis in cancer treatment.

2.2 Engineering NMs for indirect metabolic alteration

Metalloptosis has a strong connection with the metabolic processes within cells. By modifying the metabolic pathways specific to cancer cells, one can notably influence their responsiveness to metal ions. A combined strategy that integrates the induction of metalloptosis with metabolic reprogramming holds promising potential in the field of nanomedicine design. This method has been widely applied in various research contexts.[53,54] The design of these NMs for indirect metabolic alteration should thoroughly consider the interaction between cancer metabolism and the microenvironment, ingeniously utilizing the specific metabolic signaling pathways involved in various forms of metalloptosis for rational material design. Employing NMs to achieve glutathione (GSH) depletion,[54,55] ameliorate hypoxic conditions,[56] and provoke a burst of reactive oxygen species (ROS),[57] thereby initiating and enhancing metalloptosis, are all available strategies. For instance, by strategically employing NMs to deplete GSH, cancer cells are deprived of their primary antioxidant defense, which drastically increases their susceptibility to oxidative stress and metal ion toxicity. This depletion not only disrupts the redox homeostasis but also primes the cells for enhanced reactivity to subsequent treatments.[58] Moreover, addressing the challenge of tumor hypoxia can significantly improve the efficacy of metalloptosis. By utilizing NMs designed to improve oxygenation within the tumor microenvironment (TME), the oxygen levels are increased, which in turn facilitates the generation of ROS upon metal-ion interaction. This approach not only mitigates the limitations posed by hypoxic conditions but also synergizes with the oxidative stress induced by GSH depletion to further drive the metalloptosis process.

It is noteworthy that some engineered NMs can impact the cell's ion channels, thereby potentially inducing metalloptosis. Previous reports have demonstrated the possibility of stimulating the opening of membrane ion channels via optogenetic approaches, leading to an influx of extracellular ions and subsequent intracellular ion overload. For instance, Ai et al have strategically localized lanthanide-doped upconversion nanocrystals (UCNs) to cell membranes.[59] The emitted blue light from these UCNs could effectively activate light-gated ion channels,

inducing Ca^{2+} influx and triggering cell death in HeLa cells. Although research in this area is still limited, future studies are expected to clarify how NMs influence ion channel behavior. Understanding these interactions could potentially improve the use of these NMs in targeted cancer therapies and contribute to advancements in metalloptosis-based treatments.

The abovementioned strategies, when combined, offer a robust framework for designing next-generation nanomedicines that can efficiently target and eradicate tumor cells by exploiting their metabolic vulnerabilities. This comprehensive approach to cancer therapy highlights the innovative potential of integrating metalloptosis with metabolic alteration, establishing a new way for treatments in oncology.

Table 1

Ferroptosis-related genes and corresponding proteins.

Signaling pathways	Genes and proteins	Functions
Cystine uptake	SLC7A11 (solute carrier family 7 membrane 11)[60]	Promotes cystine uptake
	SLC3A2 (solute carrier family 3 membrane 2)[69]	Maintains SLC7A11 stability
Energy metabolism	SLC1A5 (solute carrier family 1 member 5)[70]	Regulates glutamine uptake
Fe metabolism	TF (transferrin)[71]	Delivers Fe ions
	TFR1 (transferrin receptor)[72]	Transfers Fe into cells
Lipid metabolism	GPX4 (glutathione peroxidase 4)[73]	Prevents lipids hydroperoxides

3. Strategic NMs for metalloptosis

3.1 Metal ions-specific-induced metalloptosis

3.1.1 Ferroptosis

Ferroptosis as a novel Fe-dependent PCD has been discovered in 2012,[60] which is morphologically and mechanistically distinct from common PCDs such as apoptosis, necrosis, and autophagy. Its prominent feature is an overload of lipid peroxides on cellular membranes, mitochondria shrinkage, and reduction.[60,61] In detail, hallmarks of ferroptosis are listed as follows. (1) Morphological features: ferroptosis is accompanied by cell membrane rupture, cytoplasmic swelling, chromosome condensation, autophagosomes increasing, and mitochondrial abnormalities.[60,62–64] (2) Biochemical features: Fe accumulation and lipid peroxidation are the 2 most important biochemical characteristics of ferroptosis. The accumulation of Fe ions in vivo can directly induce the Fenton reaction to generate excessive ROS, ultimately leading to oxidative damage-mediated ferroptosis. In addition to the classic Fe ions-induced ferroptosis, other metal ions such as Zn ions can also achieve this process,[65] which we will discuss in detail in the following sections. On the other hand, lipid peroxidation is mainly driven by free radical, which often occurs in the cell membrane. Its mechanism is that cell membrane lipids are oxidized and produce many metabolic products such as lipid hydroperoxides and reactive aldehydes.[66,67] These metabolic products significantly increase during the process of ferroptosis, which can be the most important biochemical detection marker of ferroptosis. (3) Genetic features: ferroptosis is regulated by various kinds of relatively specific genes and proteins, thus, their abnormal expression is often considered a detection marker for ferroptosis. These genes and proteins are often associated with substance uptake and metabolic pathways, including cystine uptake, oxidant metabolism, lipid metabolism, energy metabolism, and Fe metabolism[68] (Table 1). (4) Immune features: the immunological consequences of ferroptosis on the immune cells are mainly manifested as the loss of their functions. For instance, ferroptosis that occurred in T cells led to a decrease in their antiviral or antiparasitic function.[74] More importantly, the immune system's response is determined by ferroptosis-induced cell death. This is because various types of cell death can induce distinct immune reactions through the activation of different damage-associated molecular pattern (DAMP) signals. For instance, death cells induced by ferroptosis could activate the nuclear factor-κB pathway[75] and target lipid metabolism-related DAMP signaling,[76] eventually triggering the inflammatory response in peripheral macrophages.[77] In short, the discrimination of ferroptosis is clear and specific, which emphatically includes the following points: ROS generation, lipid peroxidation, Fe abundance, glutathione peroxidase 4 (GPX4) activity, GSH depletion, release of glutamate, and cystine uptake, SLC7A11 expression and so on. These indicators are essential for detection and study in the context of ferroptosis research.

In terms of mechanisms, Fe ions-induced ferroptosis was initially discovered, and as research progressed, researchers have gradually found that the ferroptosis pathway is not solely dependent on Fe ions, some Fe-independent pathways, such as ROS metabolism (particularly lipid peroxides) and GSH metabolism, can also induce ferroptosis. Hence, the metabolic pathways of ferroptosis are mainly summarized into the following 3 pathways: Fe metabolism, ROS metabolism, and GSH metabolism (Figure 2).[78] According to different triggering mechanisms of ferroptosis, different treatment strategies based on nanotechnology can be adopted.

3.1.1.1 Fe metabolism. Fe ions play an essential role in inducing ferroptosis whether through enzymatic or nonenzymatic reaction. Thanks to the Fe transport systems, intracellular Fe ions can always maintain a delicate balance under normal conditions. Extracellular Fe ions can be transferred into the cytoplasm by transferrin (TF) and its corresponding transferrin receptor (TFR) and stored in the ferritin.[61] On the other hand, intracellular Fe ions are exported outside the cell through ferroportin (FPN), which is the only known Fe ions exporter in mammal.[79] When Fe ions accumulate in the cytoplasm, disrupting the balance of Fe ions, it can trigger oxidative stress responses and the following ferroptosis, leading to cellular damage.[80] Interestingly, many studies have demonstrated that cancer cells have a higher demand for Fe than normal cells to maintain their rapid proliferation, specifically manifested as downregulation of FPN and upregulation of TFR1.[70,81] Based on the high dependence of cancer cells on Fe content, cancer cells are more susceptible to Fe overload or oxidative stress compared to normal cells, thus, inducing ferroptosis by altering the intracellular Fe ion content has become one of the emerging ways to treat cancers.[82]

At present, the majority of NMs triggering ferroptosis by changing the accumulation of Fe ions in the cytoplasm are Fe-based NMs. They can be internalized by cancer cells and degraded into ferrous (Fe^{2+}) or ferric (Fe^{3+}) ions in lysosomes, thereby directly boosting the intracellular Fe ions content. The excessive Fe ions can further trigger the Fenton reaction, inducing ferroptosis to kill cancer cells. For instance, the most common Fe-containing NPs are Fe_3O_4 NPs. Liang et al reported self-assembled ultrasmall Fe_3O_4 NPs with the surface modification

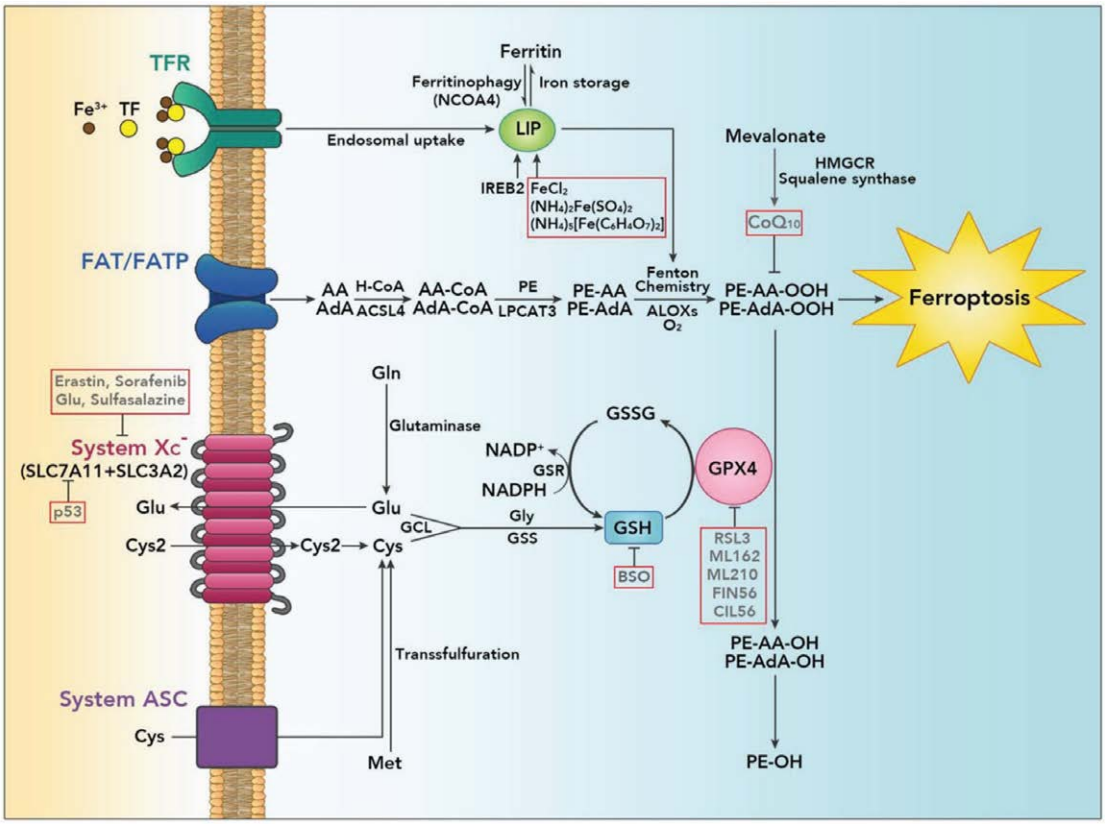

Figure 2. The regulatory mechanism of ferroptosis. (1) Fe metabolism: the transfer of Fe ions into the cell is dependent on transferrin receptor (TFR)-mediated endocytosis, and then activates the labile iron pool (LIP), which is also can be regulated by ferritin degradation (ferritinophagy). The increased LIP further promotes the Fenton reaction, enhancing cell sensitivity to ferroptosis. (2) ROS metabolism: in the process of lipid peroxidation, the fatty acid translocase (FAT) and fatty acid transport protein (FATP) participate in the generation of phospholipid hydroperoxides through the Fenton reaction, with the occurrence of ultimately induced ferroptosis. Meanwhile, GPX4 as a central regulator of ferroptosis, can combat lipid peroxidation by transforming toxic phospholipid acid into nontoxic phospholipid alcohols. (3) GSH metabolism: cystine (Cys2) and GSH metabolism constitute the principal line in the Fe-independent pathway of regulating ferroptosis. When oxidative stress occurs under extracellular conditions, cystine is taken up by system Xc^-, and the cystine is gradually reduced through GSH, which acts as an important intracellular antioxidant. This process can promote GPX4 to resist ferroptosis. Reproduced with permission from Liang et al.[78] Copyright 2019, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

of porphyrin-grafted lipid (PGL) to enhance ROS production and lipid peroxidation. These ultrasmall Fe_3O_4@PGL NPs with a diameter of ~10 nm exhibited good biosafety (Figure 3A). In terms of treatment mechanism, the Fe_3O_4@PGL NPs generated abundant ROS through porphyrin-mediated photodynamic therapy (PDT), which is beneficial for the occurrence of ferroptosis. Moreover, the results indicated that the content of ROS produced was the highest only when the coincubation of NPs endocytosed by HT-29 cells and RAW264.7 cells, which suggested that Fe_3O_4@PGL NPs could also enhance the anticancer ability of ferroptosis through modulating macrophages in the TME (Figure 3B, C).[83] To further enhance the catalytic activity of Fe-based NPs and improve their efficiency in inducing ferroptosis, Liang et al provided an effective therapy strategy to ameliorate the low ROS production efficacy of current Fe-based NPs through synthesizing body-centered cubic-ultrasmall single-crystal Fe NPs (bcc-USINPs) (Figure 3D). These bcc-USINPs remained stable in the normal physiological environment and reacted with weak acids in the TME due to their selective acidic etching of the Fe_3O_4 shell and the exposure of the Fe(0) core. The results of the catalytic activity analysis showed that bcc-USINPs exhibited superior ROS generation ability than other NPs (Figure 3E), which proved that bcc-USINPs have a stronger ability to induce ferroptosis than other NPs. More importantly, bcc-USINPs also triggered immunotherapy to enhance the synergistic effect of ferroptosis treatment. In detail, bcc-USINPs could efficiently induce immunogenetic cell death at a very low concentration (1 mg/kg) by promoting the maturation of dendritic cells (DCs) and enhancing the adaptive T cell response (Figure 3F–I). The bcc-USINPs-mediated ferroptosis treatment, when combined with programmed death-ligand 1 (PD-L1) immune checkpoint blockade immunotherapy, developed a strong immune memory, thereby potentiating the immune response. This Fe(0) delivery system based on bcc-USINPs provided an effective strategy for ferroptosis-based immunotherapy.[84] Considering that the efficiency of ferroptosis induced by Fe-based NPs is related to the penetration depth of NPs at the tumor site, Wang et al prepared a kind of stimuli-responsive size-switchable nanocapsules with Au-Fe_2C Janus NPs as ferroptosis agents and sorafenib (SRF) as the ferroptosis enhancer. These nanocapsules exhibited superior intratumoral accumulation compared to free Au-Fe_2C NPs, subsequently penetrating deeper into tumor regions. They disrupted the intracellular redox balance, eventually leading to efficient treatment through ferroptosis.[38]

It is worth noting that the induction of ferroptosis is not limited to Fe ions, and many other metal ions can also achieve ferroptosis through non-Fe ions-dependent pathways, such as Mn ions and Zn ions, which will be discussed in the following sections.

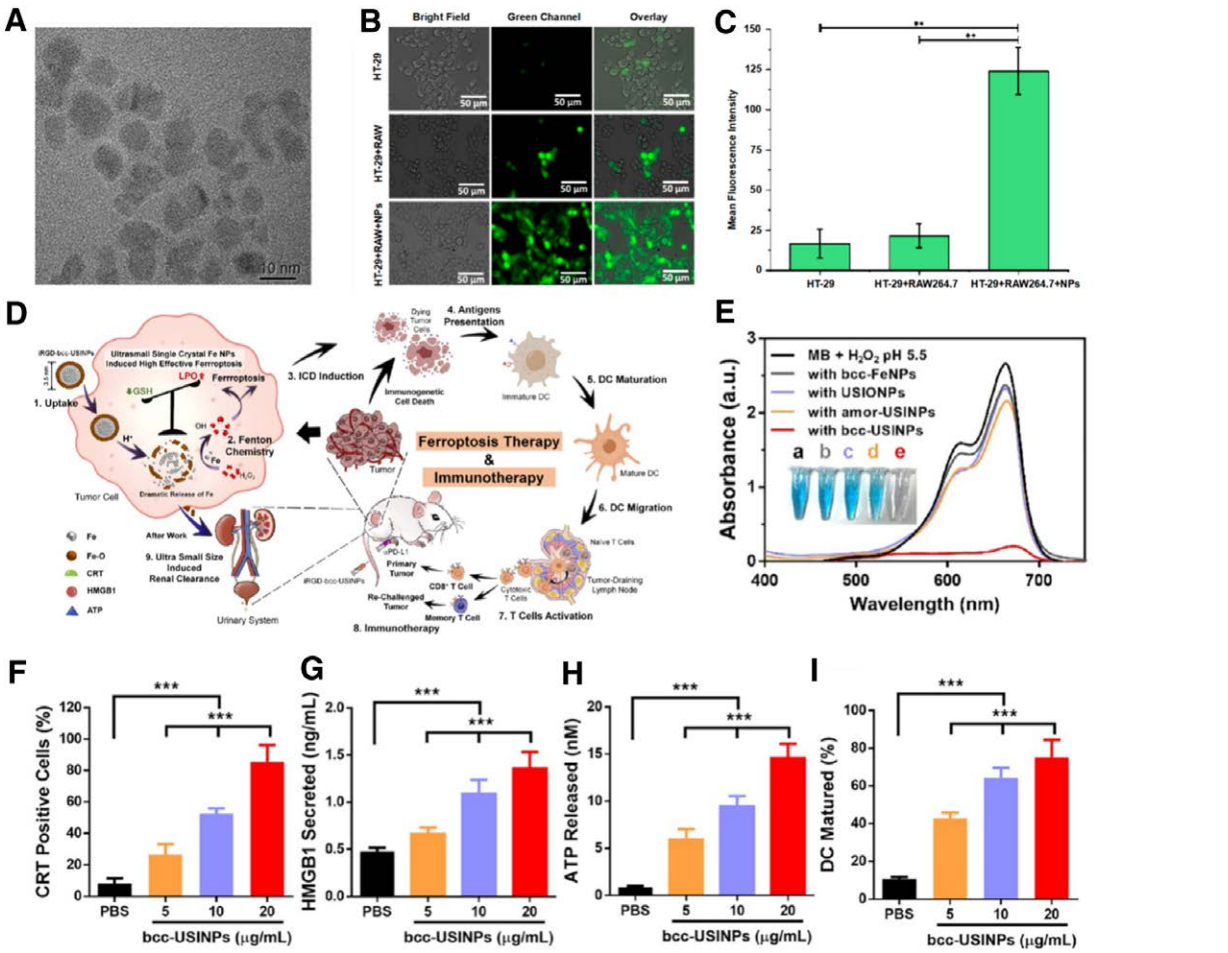

Figure 3. The NMs for ferroptosis via Fe metabolism. (A) Transmission electron microscopy (TEM) image of Fe_3O_4@PGL NPs. (B) Evaluation of intracellular ROS generation in different treatment groups for 3 days. (C) Quantification of intracellular ROS generation in (B). The values are presented as mean ± SD; **P < 0.01. (A–C) Reproduced with permission from Liang et al.[83] Copyright 2021, American Chemical Society. (D) Illustration of renal clearable bcc-USINPs for highly selective and effective ferroptosis therapy and immunotherapy. (E) UV/Vis absorption spectra of methylene blue solution in pH 5.5 buffer containing 1 mM H_2O_2 after adding bcc-Fe NPs, USIONPs, amor-USINPs, and bcc-USINPs at a concentration of 50 μg/mL [Fe]. (F) Flow cytometry quantification of calreticulin (CRT) on the surface of MC38 tumor cells. ***$P < 0.001$. (G) High mobility group box 1 (HMGB1) released from MC38 cells detected by an enzyme-linked immunosorbent assay (ELISA) kit ($n = 3$). ***$P < 0.001$. (H) Adenosine triphosphate (ATP) secretion that detected by an enhanced ATP assay kit ($n = 3$). ***$P < 0.001$. (I) Statistical analysis of DC maturation (gated on CD11c+ cells) after being incubated with PBS or bcc-USINP-treated MC38 tumor cells. ***$P < 0.001$. Data were expressed as means ± SD ($n = 3$). (D–I) Reproduced with permission from Liang et al.[84] Copyright 2021, American Chemical Society.

3.1.1.2 GSH metabolism. Reduced GSH is the major intracellular antioxidant in mammals, whose biosynthesis is subject to the influence of cystine and cysteine. In the triggering mechanism of ferroptosis, GSH actually is a negative regulatory factor. Blocking cystine acquisition from the extracellular environment or inhibiting the biosynthesis of GSH can trigger ferroptosis.[85] Many studies have demonstrated that GSH is strongly associated with cancer aggressiveness and chemoresistance, however, the existing therapeutic drugs targeting GSH cannot effectively scavenge GSH, so the relevant treatment can only serve as a supplementary strategy of cancer treatment.[86] The emergence of nanotechnology has brought a breakthrough opportunity to solve this problem. Wang et al prepared a kind of ferroptosis-inducing agent based on arginine (Arg)-capped manganese silicate nanobubbles (AMSNs) with the efficient consumption of GSH and inactivation of GPX4. In this nanosystem, taking advantage of the argininosuccinate synthetase deficiency present in cancers, Arg as the modified surfacing ligand allowed AMSNs to have good biocompatibility and tumor homing capacity, which further dramatically enhanced the efficiency of GSH depletion and GPX4 inactivation. AMSNs were degraded to release Mn ions of higher valence (Mn^{3+} and Mn^{4+}) in response to the weakly acidic conditions in TME. The released Mn^{3+} and Mn^{4+} were further reduced to Mn^{2+} in the presence of GSH, which precisely promoted the depletion of GSH. This depletion of GSH subsequently led to the upregulation of intracellular oxidative stress and the downregulation of GPX4 expression, collectively improving the treatment efficiency of ferroptosis. Meanwhile, the hollow structure also endowed AMSNs with the ability as drug delivery vehicles. Doxorubicin (Dox)-loaded AMSNs could suppress tumor growth more effectively compared with free Dox. In terms of bioimaging, Mn ions contributed to the T_1-weighted magnetic resonance imaging (MRI), ultimately achieving imaging-guided on-demand synergistic chemotherapy and ferroptosis treatment.[87]

In addition to ameliorating the inhibitory effect of GSH on ferroptosis, targeting other antiferroptotic signaling pathways also emerges as a potent strategic approach to enhance synergistic therapeutic effects. Ferroptosis suppressor protein 1 (FSP1) can mediate detoxification of lipid peroxides through decreasing nicotinamide adenine dinucleotide phosphate

(NADP+)/nicotinamide adenine dinucleotide phosphate (NADPH) ratio and catalyzing the recycling of coenzyme Q10 (CoQ10) to ubiquinol (CoQ10H2) for trapping lipid peroxyl radicals. The decreasing NADP+/NADPH ratio can further promote the conversion of cystine to cysteine and the biosynthesis of GSH and GPX4.[88,89] Consequently, devising a treatment strategy that is capable of simultaneously inhibiting both GSH- and FSP1-mediated ferroptosis-suppressing mechanisms holds considerable therapeutic potential. Li et al developed a Cu-tetra(4-carboxyphenyl)porphyrin chloride(Fe(III)) (Cu-TCPP(Fe))-based MOF nanosystem, incorporated with Au NPs via in situ nucleation and the ferroptosis inducer Ras-selective lethal small-molecule 3 (RSL3) via π–π stacking, meanwhile modified with polyethylene glycol (PEG) and internalizing RGD peptide (iRGD) for tumor-targeted drug delivery. Mechanistically, except for the classic Fe ions-induced ferroptosis, the introduction of Au NPs caused glucose depletion because of their glucose oxidase-like activities, eventually disrupting the pentose phosphate pathway and hindering reduced GSH biosynthesis. Meanwhile, Cu ions consumed GSH by oxidizing it to glutathione (GSSG), which could suppress the GSH-mediated antiferroptotic pathway in tumor cells for amplifying ferroptotic damage. Moreover, the loaded ferroptosis inducer RSL3 could deactivate the function of GPX4 to further result in pronounced ferroptotic damage. Nonetheless, Au NPs could also increase the NADP+/NADPH ratio and prevent the recycling of CoQ10 to CoQ10H2, impairing the FSP1-mediated antiferroptotic pathways (Figure 4A). Specifically, Au/Cu-TCPP(Fe) nanosheet was synthetized with a typical 2-dimensional morphology and its average size was between 100 and 200 nm (Figure 4B). The functional incorporation of the Au NPs and Cu species efficiently depleted the glucose and GSH in the tumor intracellular environment, which plays critical roles in the subsequent ferroptosis sensitization (Figure 4C). The results

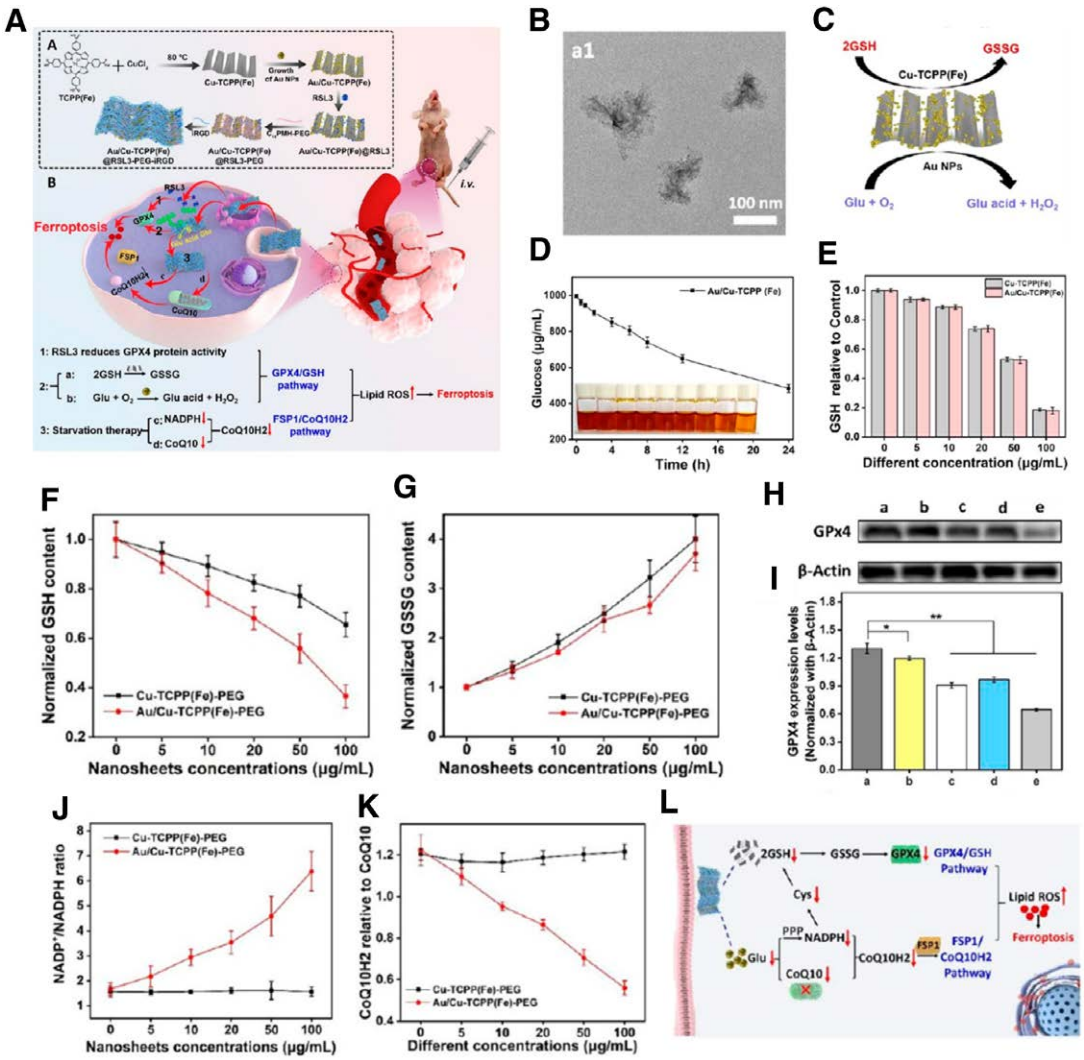

Figure 4. The NMs for ferroptosis via GSH metabolism and CoQ10H2 pathway. (A) Schematic illustration of the tumor-targeting composite nanosheet for sensitizing tumor ferroptosis via impairing GPX4/GSH and FSP1/CoQ10H2 pathways. (B) TEM image of Au/Cu-TCPP(Fe). (C) Schematic diagram of composite nanosheet catalysis. (D) Time-dependent GOx-like activity of Au/Cu-TCPP(Fe), $n = 3$. (E) Concentration-dependent GSH consumption activity of Cu-TCPP(Fe) and Au/Cu-TCPP(Fe), $n = 3$. (F) Relevant intracellular GSH content after Cu-TCPP(Fe)-PEG and Au/Cu-TCPP(Fe)-PEG treatment. (G) Relevant intracellular GSSG content after Cu-TCPP(Fe)-PEG and Au/Cu-TCPP(Fe)-PEG treatment. (H) Western blotting assay of the GPX4 protein expression after different treatments. (I) Relevant gray statistics of (H). (J) The ratio of intracellular NADP+/NADPH after different treatments. (K) The ratio of CoQ10H2 to CoQ10 in 4T1 cells after different treatments. (L) Mechanisms of composite nanosheets regulating 2 independent ferroptosis signaling pathways. (a) Control; (b) Cu-TCPP(Fe)-PEG (10 μg/mL); (c) Cu-TCPP(Fe)-PEG (50 μg/mL); (d) Au/Cu-TCPP(Fe)-PEG (10 μg/mL); (e) Au/Cu-TCPP(Fe)-PEG (50 μg/mL), $n = 3$. Reproduced with permission from Li et al.[90] Copyright 2021, American Chemical Society.

of evaluation experiments on the GOx-like catalytic activity demonstrated that the Au/Cu-TCPP(Fe) nanosheet consistently reduced the glucose concentration over a prolonged incubation time, with the final relative levels dropped by around 51.7% after 24 h (Figure 4D). On the other hand, as shown in Figure 4E, both Cu-TCPP(Fe) and Au/Cu-TCPP(Fe) could reduce the concentration of GSH in a dose-dependent manner. After incubation with a nanosheet (100 μg/mL) for 24 h, the concentration of GSH was decreased to around 81.4% and 82.1%, evidently suggesting the GSH-consuming ability of the Cu species in the nanosheet. Furthermore, the effect of the catalytic activity of composite nanosheets on 2 independent regulatory pathways of ferroptosis (GPX4/GSH pathway and FSP1/CoQ10H2 pathway) was systematically evaluated. In terms of GPX4/GSH pathway, the results indicated that the GSH-oxidizing capability of the Cu species in the Cu-TCPP(Fe)-PEG and Au/Cu-TCPP(Fe)-PEG nanosheets caused an evident decrease of the cellular GSH (Figure 4F) and increase of the cellular GSSG levels (Figure 4G), which was consistent with the results of reduced expression of GPX4 induced by both Cu-TCPP(Fe)-PEG and Au/Cu-TCPP(Fe)-PEG (Figure 4H, I). In the terms of FSP1/CoQ10H2 pathway, Au/Cu-TCPP(Fe)-PEG could enhance the ratio of $NADP^+$/NADPH (Figure 4J) and weaken the ratio of CoQ10H2 to CoQ10 (Figure 4K) in a dose-dependent manner. These changes suggested that Au NPs in the Au/Cu-TCPP(Fe)-PEG nanosheets lowered the abundance of CoQ10H2 by the consumption of glucose and the decrease of NADPH content, potentially compromising the antiferroptotic capability of FSP1 proteins. In brief, according to different signaling pathways induced by ferroptosis, this nanosystem promoted the occurrence of ferroptosis and optimized the cell lethal effect of ferroptosis, providing a strong rationale for the application of ferroptosis therapy in the clinic (Figure 4L).[90]

Moreover, as a central downstream regulator of ferroptosis in GSH metabolism, GPX4 combats with lipid peroxidation by transforming toxic phospholipid hydroperoxides into nontoxic phospholipid alcohols, with the generation of oxidized GSH (GSSG) as byproducts.[91] Many chemical or biological methods can intervene in the expression of GPX4 and have also shown initial effectiveness in inducing ferroptosis.[80,92,93] Therefore, targeting GPX4 is a powerful therapeutic strategy for ferroptosis-based cancer therapy[94] and various kinds of nanoplatforms have been developed for suppressing GPX4.[87,95,96] Among them, 2 common solutions are as follows: first, NMs can act as the delivery system to transport small-molecule inhibitors of GPX4 in vivo, which is capable of overcoming certain defects, such as rapid clearance from systemic circulation and poor tumor enrichment efficiency; second, NMs can reduce the expression of GPX4 by utilizing their own intrinsic physicochemical properties.[87,96] Inspired by industrial electro-Fenton technology featured with electrochemical iron cycling, Liu et al constructed a network-like corona spontaneously consisting of Fe^{3+} ion and naturally derived tannic acid (TA) to wrap the SRF nanocores, which is a typical small-molecule GPX4 inhibitor (Figure 5A). The results showed that the formed SRF@Fe^{III}TA (SFT) NPs could significantly decrease the level of GSH (Figure 5B) and GPX4 (Figure 5C) for ferroptosis initiation. Meanwhile, TA chemically reduced Fe^{3+} to Fe^{2+}, offering iron redox cycling to effectively sustain the Fenton reaction and produce the lipid peroxide that was required in ferroptosis (Figure 5D). In vivo results indicated that this rational material design could advance the antitumor effect of ferroptosis through the

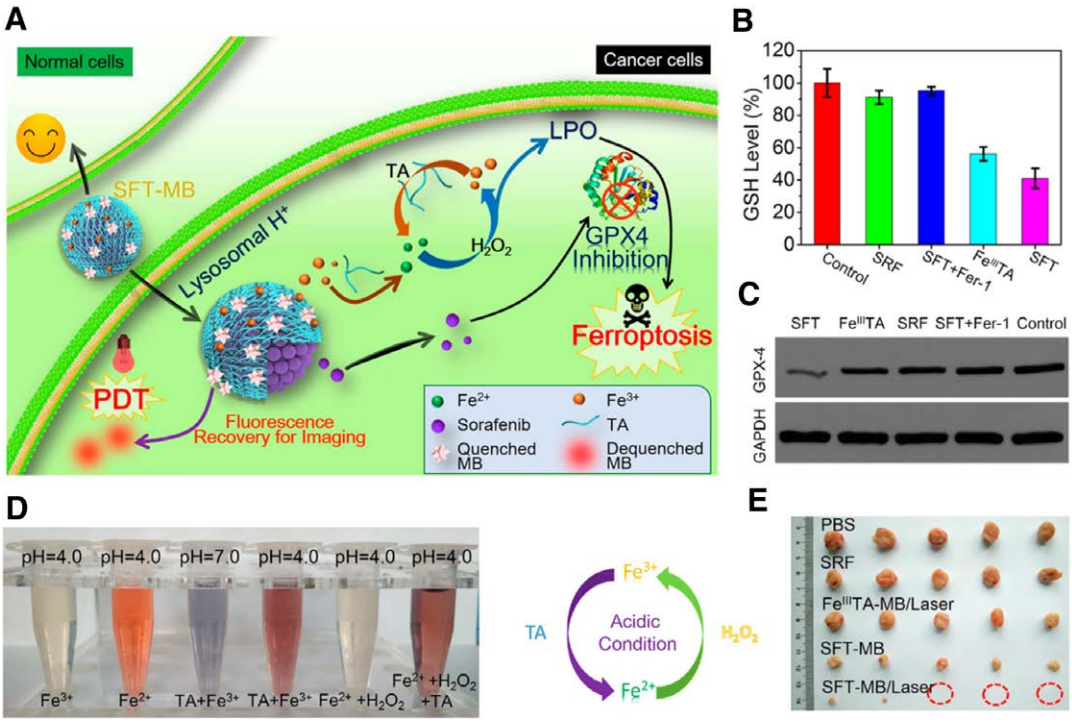

Figure 5. The NMs as drug delivery systems (DDSs) for ferroptosis via targeting GPX4. (A) Schematic illustration of SFT-mediated combination of ferroptosis and image-guided PDT. (B) Intracellular GSH level of 4T1 cells after treated with SRF, Fe^{III}TA, SFT, and SFT plus Ferrostatin-1 (Fer-1, a ferroptosis inhibitor) with an equivalent SRF dosage at 20 μM. (C) Western blot analysis of GPX4 expression in 4T1 cells after the treatment with SRF, Fe^{III}TA, SFT, and SFT plus Fer-1, respectively. (D) Observation on the iron redox cycling of SFT under the acidic condition. Fe^{2+} was detected using o-phenanthroline. An acidic environment triggered Fe^{2+} release from Fe^{III}TA, while TA under acidic conditions can reduce Fe^{3+} that originates from the oxidation of Fe^{2+} by H_2O_2 into Fe^{2+} again. (E) Photographs of tumors that obtained on the 14th day after treatments. Reproduced with permission from Liu et al.[95] Copyright 2018, American Chemical Society.

continuous internal supply of Fe ions combined with the specific GPX4 inhibitor (Figure 5E).[95] Moreover, the reconstituted NMs with docosahexaenoic acid were also proved to cause inactivation of lipid antioxidant GPX4 and accumulation of lipid hydroperoxides, with the consequence of significant cancer suppression.[96]

Interestingly, as the study continues to deepen, researchers have gradually discovered some new signaling pathways of ferroptosis. The latest research indicated that supplementation with dihydroorotate or orotate, the substrate and product of dihydroorotate dehydrogenase (DHODH), could attenuate or potentiate ferroptosis induced by inhibition of GPX4, respectively, and these effects were especially pronounced in cancer cells with low expression of GPX4 ($GPX4^{low}$). Inactivation of DHODH could lead to extensive mitochondrial lipid peroxidation and ferroptosis in $GPX4^{low}$ cancer cells and produce the same phenomenon in $GPX4^{high}$ cancer cells with the combination of ferroptosis inducers. All these results identified a new mechanism of ferroptosis mediated by DHODH and suggested a therapeutic strategy of targeting DHODH in cancer ferroptosis treatment.[97]

3.1.1.3 ROS metabolism. ROS, generated via various pathways, can promote the accumulation of oxidation products (particularly lipid peroxides), being acknowledged as the typical hallmark of ferroptosis.[98] Hence, it is an evident design strategy of NMs for ferroptosis to accelerate the generation of ROS. For instance, Guan et al reported a multifunctionalized nanoplatform with a ROS cascade for ferroptosis combined with chemotherapy/gas therapy in reversing multidrug resistance. Ferrocene used in this nanoplatform synergistically ameliorated the antitumor efficiency of ferroptosis through breaking the redox balance in the TME.[99] Moreover, Fe/Ni-layered double hydroxides (LDHs) were synthesized by a general and facile method to enhance apoptosis- and ferroptosis-induced cancer therapy. Compared with Fe-OH and Ni-OH, Fe/Ni-LDH exhibited exceptional catalytic activity with massive generation of ROS, •OH, and O_2•$^-$ radicals, which could prominently increase intracellular oxidative stress and accumulation of lipid peroxide (LPO), ultimately optimizing ferroptosis-mediated cancer therapy.[100] However, the current research on the regulation mechanism of ROS metabolism for ferroptosis has not been sufficiently validated; most studies are limited to detecting the content of ROS and markers of ferroptosis, without verifying the direct relationship between the 2 through rational experiments. For example, appropriate inhibitors can be used to block the other signaling pathways of ferroptosis such as Fe metabolism and GSH metabolism, to further clarify the regulatory role of ROS metabolism on ferroptosis. This is also important to enhance our understanding of the ferroptosis induced by ROS metabolism.

3.1.2 Cuproptosis

Copper is an essential trace element for the human body, which is primarily obtained from food. The current recommended copper intake for adults is 0.8 to 2.4 mg/d to maintain whole-body copper homeostasis.[101] Statistically, an adult human body contains about 100 mg of copper.[102] In one regard, copper is integral to various physiological processes in the body, acting as a catalytic and structural cofactor for a range of enzymes that facilitate critical biochemical reactions. Its role is particularly prominent in enzymes such as cytochrome *c* oxidase and superoxide dismutase 1, which are pivotal for cellular energy production and antioxidant defense, respectively.[103] Beyond these functions, copper is also vital for the synthesis of neurotransmitters,[104] iron metabolism,[105] peptide hormone maturation, and blood clotting.[102] While an optimal amount of copper is beneficial for the organism, excessive copper levels can precipitate a sequence of cellular metabolic disturbances that ultimately lead to cell death. Early research attributed this type of death to the accumulation of copper, which disrupts the delicate balance between the generation of ROS and the body's antioxidative defense mechanisms.[33] As of 2022, the notion of "cuproptosis" was formally published within the scientific community, representing a novel cellular demise pathway distinct from previously characterized mechanisms such as apoptosis, necrosis, and autophagy.[106]

Compared to ferroptosis, cuproptosis represents a relatively novel concept in the field of metal ions-mediated cell death research. It predominantly hinges on the intracellular accumulation of copper ions, which leads to the aggregation of lipoylated proteins and subsequent loss of iron-sulfur (Fe-S) cluster proteins. This sequence of events triggers proteotoxic stress, culminating in cell death. Specifically, researchers employed the ionophore elesclomol (ES) to facilitate the transport of Cu^{2+} into cells, where an excess of Cu^{2+} was subsequently transported to mitochondria and reduced to the more toxic Cu^{+} by the mammalian ferredoxin 1 (FDX1), a critical player in cuproptosis process. Besides reducing Cu^{2+}, FDX1 also lipoylates specific mitochondrial enzymes, such as dihydrolipoamide S-acetyltransferase (DLAT), crucial for maintaining normal cellular metabolism and mitochondrial functionality. When Cu^{+} binds to DLAT, it triggers the aggregation of this enzyme, subsequently impeding the tricarboxylic acid cycle. Concurrently, Cu^{+} diminishes the synthesis of Fe-S clusters. These dual actions collectively lead to cellular demise.[106] A detailed illustration of the cuproptosis mechanism is provided in Figure 6A, offering a visual representation of how these interactions converge to induce cell death.

The cuproptosis pathway is characterized by high levels of lipoylated proteins, including glycine cleavage system protein H, dihydrolipoamide branched chain transacylase E2, dihydrolipoamide S-succinyltransferase, and aforementioned DLAT.[107] These proteins' lipoylation is controlled by enzymes such as lipolytransferase 1, lipoyl synthase (LIAS), and dihydrolipoamide dehydrogenase, and FDX1, with FDX1 playing a key role in initiating the lipoylation process.[108,109]

The utilization of ionophores such as ES and disulfiram (DSF) to transport copper ions into tumor cells represents a direct and common method to induce cuproptosis.[110] Ionophores can traverse biological membranes by forming stable complexes with ions and, upon specific stimuli, release metal ions, thereby altering intracellular and extracellular ion concentrations.[111] However, the efficacy of existing small-molecule ionophores in inducing cuproptosis in tumor cells is somewhat limited due to their short blood half-life.[112] The advancement of nanotechnology offers a solution to this issue by constructing nanomedicines that incorporate ionophores alongside other components, enhancing the properties of the drug delivery system. Such nanomedicines not only address the aforementioned challenges but also equip therapeutic agents with multifunctional features. They enhance cuproptosis effectiveness by promoting synergistic treatments with immunotherapy and chemo-chemodynamic therapy, or by enabling image-guided approaches for tumor therapy.[34,113] For instance, Guo et al developed a nanomedicine, NP@ESCu, by encapsulating elesclomol-Cu with a ROS-responsive

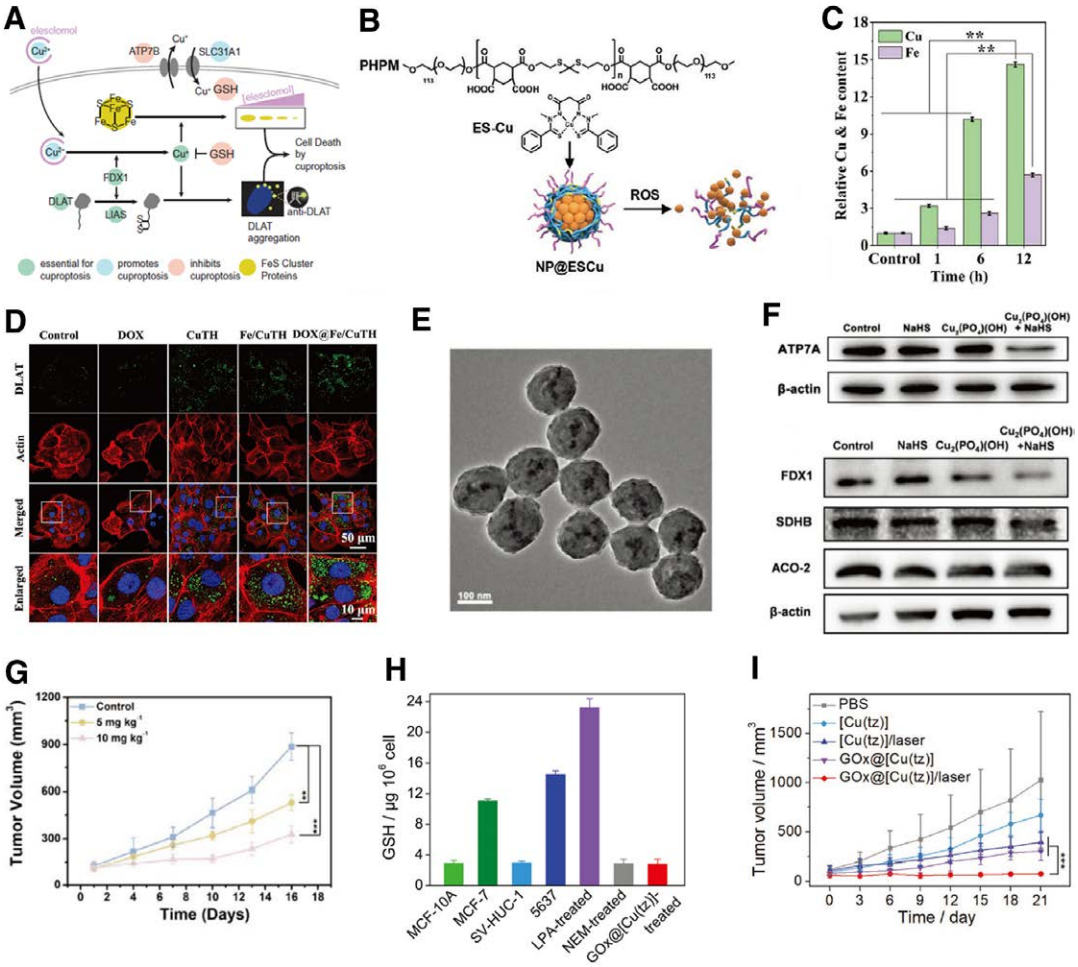

Figure 6. Mechanisms and NMs-induced activation of cuproptosis. (A) Schematic of mechanisms of cuproptosis. Reproduced with permission from Tsvetkov et al.[106] Copyright 2022, the Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. (B) Schematic diagram of ROS-induced release of ES-Cu from NP@ESCu. Reproduced with permission from Guo et al.[34] Copyright 2023, Wiley-VCH GmbH. (C) Changes of intracellular Cu and Fe levels in 4T1 cells after treatment at different times. $^{**}P < 0.01$. (D) Images of DLAT immunofluorescence in different treatment groups. (C, D) Reproduced with permission from Xu et al.[47] Copyright 2022, Wiley-VCH GmbH. (E) TEM image of $Cu_2(PO_4)(OH)$ NPs. (F) Western blot analysis on the expressions of ATP7A, FDX1, SDHB, and ACO-2. (G) Tumor volume curves of different treatment groups, $n = 5$. $^{**}P < 0.01$, $^{***}P < 0.001$. (E–G) Reproduced with permission from Zhao et al.[40] Copyright 2023, Wiley-VCH GmbH. (H) The intracellular GSH levels in the different cell lines or different treatments. (I) Tumor volume curves of different treatment groups, $n = 5$. $^{***}P < 0.001$. (H, I) Reproduced with permission from Xu et al.[54] Copyright 2022, Wiley-VCH GmbH.

polymer (PHPM), as shown in Figure 6B. Owing to the thioketal bonds in PHPM that were sensitive to ROS, NP@ESCu released its contents when exposed to elevated ROS levels within cancer cells, leading to cuproptosis. Furthermore, NP@ESCu enhanced PD-L1 expression on cancer cells, caused maturation of DCs, and enhanced $CD8^+$ T cell infiltration in tumor sites. It also triggered a shift from tumor-associated macrophages from the immunosuppressive M2 phenotype to the immunostimulatory M1 phenotype, thereby augmenting immunotherapy efficacy. The combined use of NP@ESCu and αPD-L1 in a bladder cancer mouse model demonstrated superior therapeutic outcomes, illustrating the potential of NP@ESCu as a multifaceted approach to cancer treatment.[34] In another study, researchers used DSF/Cu-based nanomedicine to induce cuproptosis. By encapsulating DSF/Cu complexes within a tumor TME-responsive core-shell dendrimer, the resulting CSTD-Cu(II)@DSF achieved MRI-guided synergistic therapy by combining cuproptosis and chemo-chemodynamic treatment.[113]

In addition to using ionophores for copper delivery, alternative NMs such as MOFs,[47,49,56] inorganic NPs,[39,40,50,114] and carbon nitride[115] can also facilitate copper ions delivery into cells, effectively triggering cuproptosis. For example, Xu et al explored amorphous metal-organic frameworks (aMOF) as cuproptosis carriers due to their unique hollow structure and amorphous properties. By incorporating Cu^{2+} and Fe^{3+} during the synthesis and modifying with Dox-loaded, functionalized hyaluronan, the resultant Dox@Fe/CuTH HaMOF enhanced the intracellular copper/iron concentration in tumor cells, inducing a synergistic antitumor effect through cuproptosis, ferroptosis, and apoptosis. The inductively coupled plasma–mass spectrometry (ICP-MS) data indicated that, with prolonged incubation of the Dox@Fe/CuTH with 4T1 cells, the intracellular copper content increased 14.6 times compared to the PBS-treated control group (Figure 6C). The rise in copper levels ultimately induced the occurrence of cuproptosis in tumor cells, as evidenced by the pronounced DLAT foci in cells treated with Dox@Fe/CuTH, attributed to abnormal oligomerization of DLAT (Figure 6D). Moreover, western blot results showed a significant reduction in the levels of FDX1 and LIAS in the Dox@Fe/CuTH-treated group compared to the control group. The destabilization of these proteins further confirmed the capability of the designed nanomedicine to induce cuproptosis.[47] Using a hydrothermal

method, copper hydroxyphosphate NPs ($Cu_2(PO_4)(OH)$ NPs) were synthesized (Figure 6E), which could respond to endogenously overexpressed H_2S to transform into ultrasmall copper sulfide NPs. This size transformation enhanced tumor cell uptake of the NPs, elevated intracellular ROS levels, and increased intracellular copper ion concentrations, ultimately leading to cuproptosis and pyroptosis. It is noteworthy that within an H_2S-enriched microenvironment, $Cu_2(PO_4)(OH)$ NPs were able to cause mitochondrial damage and consequently decreased the expression of copper-specific efflux proteins such as ATP7A (Figure 6F). This reduction led to decreased copper excretion, further facilitating the occurrence of cuproptosis. As shown in Figure 6F, the observed reduction in FDX1, succinate dehydrogenase B (SDHB), and aconitase-2 (ACO-2) levels following the treatment with $Cu_2(PO_4)(OH)$ NPs in an H_2S environment indicated the successful initiation of cuproptosis. After a 16-day treatment period, the tumors in H_2S-overexpressed colon cancer-bearing mice, treated with $Cu_2(PO_4)(OH)$ NPs, showed significant suppression, as depicted in Figure 6G.[40] By utilizing various NMs, these innovative approaches provide a range of mechanisms for "encapsulating" and "releasing" copper ions in cancer cells, thereby broadening the array of tools available for targeted cancer treatment via cuproptosis.

The above-discussed methods leverage nanoplatforms to elevate intracellular copper levels, inducing cuproptosis. As Figure 6A illustrates, there is a complex link between cuproptosis and cell metabolism. Modifying tumor metabolism to heighten copper sensitivity presents another viable strategy for inducing cuproptosis. Given that GSH, an endogenous copper chelator, can mitigate the therapeutic effect of cuproptosis by reducing free intracellular copper levels, incorporating components that deplete GSH or inhibitors blocking GSH synthesis into nanomedicines could potentially enhance the antitumor efficiency of cuproptosis. For example, researchers ingeniously utilized glucose oxidase (GOx) to respond to GSH in tumor cells, creating a nanomedicine (called GOx@[Cu(tz)]) based on nonporous copper(I) 1,2,4-triazolate ([Cu(tz)]) coordination polymer. This nanomedicine achieved dual depletion of glucose and glutathione, facilitating cuproptosis-based synergistic cancer therapy. From the intracellular GSH levels test in Figure 6H, it is evident that there was a significant decrease in GSH within tumor cells after treatment with GOx@[Cu(tz)]. Furthermore, in vivo animal experiments further confirmed that GOx@[Cu(tz)] exhibited excellent tumor suppression effects (Figure 6I).[54] Additionally, another study incorporated buthionine-sulfoximine (BSO) to inhibit GSH synthesis and catalase (CAT) to decompose endogenous hydrogen peroxide, constructing BSO-CAT@MOF-199@DDM nanomedicine that sensitized tumor cells to cuproptosis by alleviating hypoxia and reducing GSH synthesis.[56]

As a crucial subset of metalloptosis, research on cuproptosis is still in its infancy, with ongoing exploration into its mechanisms and the design of cuproptosis-inducing nanomedicines. Questions about the specific roles of key factors such as FDX1, specific compounds for cuproptosis, the resistance mechanisms of cuproptosis-inducing nanomedicines, the morphological features of cuproptosis, and the interaction with other cell death pathways remain open. Future designs should focus on optimizing nanomedicine transport efficiency, blood retention, tumor targeting, biocompatibility, and metabolic pathways. It is noteworthy that while the therapeutic efficacy of cuproptosis is limited when used in isolation, synergistic cancer treatments achieved through strategic NMs design can significantly enhance outcomes. Particularly, some copper-containing NMs exhibit unique properties such as exceptional photothermal conversion efficiency, catalytic activity, and MRI capabilities.[116,117] These features hold promise for utilizing imaging techniques to achieve precise spatiotemporal delivery of copper and multimodal synergistic cancer treatment.

3.1.3 Calcium overload

Calcium ions (Ca^{2+}) signaling pathway is a fundamental molecular regulation pathway that controls many cellular biological processes such as cell proliferation and apoptosis. The endoplasmic reticulum (ER) that stores the primary intracellular Ca^{2+} coordinates with other organelles and the plasma membrane to maintain the normal level of Ca^{2+}. However, the imbalance of Ca^{2+} homeostasis has been considered as an important driving force in the initiation and progression of cancers.[118] The Ca^{2+} signaling pathway is finely tuned by complex Ca^{2+} channels, transporters, pumps, exchangers, and calcium-binding proteins.[119–122] Under normal circumstances, the concentration of Ca^{2+} in the cytoplasm is lower than the level of the extracellular environment.[123,124] The significant difference between intracellular and extracellular Ca^{2+} concentrations precisely creates an opportunity for Ca-mediated cancer treatment strategies.[119] In recent years, calcium overload as an emerging cancer therapy has been proved to disrupt the intracellular calcium homeostasis to cause irreversible cell damage.[125,126] Hence, calcium-based nanomaterials for inducing calcium overload have received increasing attention in the interdisciplinary field of medicine and materials.[41,127]

Due to the excellent biocompatibility, bioactivity, and biodegradability, calcium-based NPs[128–133] such as calcium carbonate ($CaCO_3$), calcium phosphate (CaP), calcium carbonate (CaC), calcium silicate (CaSi), and calcium fluoride (CaF_2) can directly and effectively increase the level of cellular basal calcium.[127] Their treatment mechanisms are also diverse, including calcium overload stress, ROS production, mitochondrial dysfunction, cell calcification, and abnormal immune response, which are all induced by abnormal changes in calcium concentration, especially increased intracellular Ca^{2+}. Among the various calcium-based materials employed for treating calcium overload in tumors, $CaCO_3$ stands out as the most prevalent choice. For instance, Li et al fabricated a kind of therapeutic nanoplatform (M@$CaCO_3$@KAE) consisting of $CaCO_3$ NPs loaded with Kaempferol-3-O-rutinoside (KAE) that could effectively disrupt calcium homeostasis regulation and facilitate calcium influx. These NPs were incorporated with the cancer cell membrane (M) to ensure the targeted delivery (Figure 7A–C). Because of the response to the weak acid of TME, M@$CaCO_3$@KAE NPs specifically released KAE and Ca^{2+}, which could break the balance of Ca^{2+} homeostasis through the role of KAE in promoting the introduction of Ca^{2+} and the continuous replenishment of Ca^{2+} by $CaCO_3$. The specific manifestation was the expression changes of calcium-regulated proteins (Figure 7D), indicating the serious consequences of calcium overload such as the magnified destruction of mitochondrial structure and functions, the increased cytoskeleton collapse and oxidative stress, and the ultimate cancerous cellular apoptosis (Figure 7E).[134] Although the currently reported Ca^{2+} nanogenerators,[137] including CaCO3[138] and calcium peroxide (CaO_2),[41] can impair the Ca^{2+} homeostasis of the mitochondrion to induce the calcium overload-mediated cancer therapy, there are still some limitations that need to be broken through. For example, the intramitochondrial concentration of Ca^{2+} can quickly return to a normal level owing

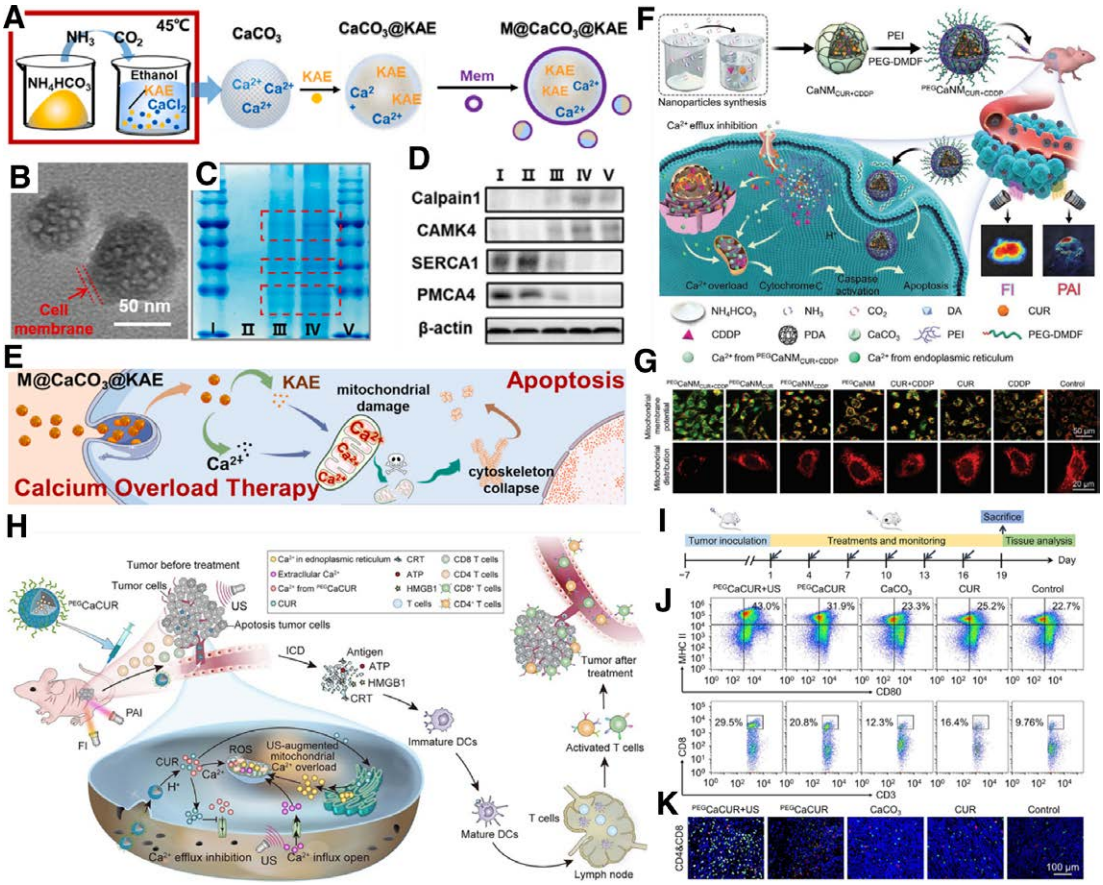

Figure 7. The $CaCO_3$-based NMs for calcium overload. (A) Schematic illustration of M@$CaCO_3$@KAE NPs synthesis. (B) TEM image of the M@$CaCO_3$@KAE NPs. (C) SDS-PAGE protein tracking of cancer cell membrane proteins. Samples from lane I to lane V are marker, $CaCO_3$@KAE NPs, M@$CaCO_3$@KAE NPs, cancer cell membrane, and marker, respectively. (D) Calcium-regulated proteins expression levels of A549 cells, I: control; II: $CaCO_3$ NPs; III: KAE; IV: $CaCO_3$@KAE NPs; V: M@$CaCO_3$@KAE NPs. (E) Schematic illustration of M@$CaCO_3$@KAE NP-mediated apoptosis. (A–E) Reproduced with permission from Li et al.[134] Copyright 2021, Elsevier Ltd. (F) Schematic representation of the functional pattern of multifunctional Ca^{2+} nanomodulator $^{PEG}CaNM_{CUR+CDDP}$. (G) Mitochondrial membrane potentials and distributions of MCF-7 cells treated with $^{PEG}CaNM_{CUR+CDDP}$ or other Ca^{2+} nanomodulators for 12 hours. (F, G) Reproduced with permission from Zheng et al.[135] Copyright 2021, Wiley-VCH GmbH. (H) Schematic illustration of photoacoustic/fluorescence dual-mode imaging-guided synergistic cancer therapy through US-augmented mitochondrial Ca^{2+} overload induced by calcium nanomodulator ^{PEG}CaCUR. (I) Treatment scheme of Ca^{2+} nanomodulators in vivo. (J) Populations of immune cells in draining lymph nodes after different treatments. (K) CD4 (red fluorescence) and CD8 (green fluorescence) T cells in tumor tissues after different treatments. (H–K) Reproduced with permission from Zheng et al.[136] Copyright 2021, American Chemical Society.

to natural Ca^{2+} excretion via the Ca^{2+} channel, resulting in an inferior anticancer effect. Hence, designing a kind of easily-prepared Ca^{2+} nanogenerator that could continuously release Ca^{2+} is an urgent problem to be solved. Based on the above issues, Zheng et al synthesized a kind of a multichannel Ca^{2+} nanomodulator ($CaNM_{CUR+CDDP}$) encapsulated with cisplatin (CDDP) and curcumin (CUR) in hybrid $CaCO_3$ NPs (Figure 7F). First, $CaCO_3$ NPs released a mass of Ca^{2+}, resulting in the excessive accumulation of Ca^{2+} in mitochondria; then, CUR further promoted the release of Ca^{2+} from the endoplasmic reticulum to the cytoplasm and inhibited Ca^{2+} efflux; finally, CDDP detrimentally affected the structural integrity and metabolic functionality of mitochondria by inducing DNA damage, leading to the mitochondrial dysfunction (Figure 7G).[135] In addition, calcium overload caused by $CaCO_3$ can be effectively managed in conjunction with other treatment methods, such as immunotherapy, improving the overall efficacy of synergistic therapy. Zheng et al further fabricated acid-sensitive PEG-decorated $CaCO_3$ NPs incorporating CUR (^{PEG}CaCUR) to systematically explore their immune function (Figure 7H). In accordance with the schematic diagram for in vivo treatment (Figure 7I), the ^{PEG}CaCUR group, when subjected to ultrasound (US), demonstrated a preferential enhancement in the maturation of DC and activation of CD8 T cells, in comparison to the other groups. This suggested the good effect of mitochondrial Ca^{2+} overload-induced immunogenic cell death (ICD) induced by ^{PEG}CaCUR NPs (Figure 7J, K).[136]

In addition to $CaCO_3$, calcium oxide (eg, CaO_2) is also an available Ca^{2+} nanogenerator. Zhang et al prepared a kind of pH-sensitive sodium-hyaluronate-modified calcium peroxide nanoparticles (SH-CaO_2 NPs) to induce an abnormal cytoplasm accumulation of free Ca^{2+}. Interestingly, these NPs created an artificial calcium-overloading stress in tumor cells, while normal cells were more tolerant of the adverse influence of NPs than tumor cells. Meanwhile, the enrichment of Ca^{2+} showed the potential to cause tumor calcification, which may further inhibit the growth of tumor tissue.[41]

3.2 Metal ions-nonspecific-induced metalloptosis

3.2.1 Zinc ions-induced cell death

Zinc (Zn) is a necessary trace element for the daily activities of the body, ranking second in abundance. It is closely associated with various biological functions, including cell cycle progression, signal transduction, gene expression, immune functions, meiosis, and numerous other physiological processes.[139]

Statistical data indicate that the activity of around 300 enzymes and the structural integrity of approximately 2000 transcription factors are zinc dependent.[140] Women typically have around 1.5 g of zinc in their bodies, while men have about 2.5 g, predominantly located in the bone and skeletal muscle.[141] Zinc homeostasis dysregulation is closely linked to cancer, with zinc ions not only induce ferroptosis or calcium overload but also directly cause apoptosis, necrosis, etc. Although zinc ions-mediated cell death has not yet been classified as a new type of PCD, it possesses distinct characteristics and will be the focus of discussion in this section.

Recent research increasingly highlights the significant role of zinc in ferroptosis. Zinc oxide NPs (ZnO NPs), approved as a safe substance by the United States Food and Drug Administration, exhibit good biocompatibility and cost-effectiveness, offering broad application prospects in the biomedical field.[142] A study by Zhang et al in 2020 demonstrated that ZnO NPs could trigger ferroptosis in human umbilical vein endothelial cells (HUVECs) by increasing ROS, lipid peroxidation, and depleting glutathione (Figure 8A–D).[143] Further investigations revealed that the zinc chelator N,N,N′,N′-Tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) could protect cells from erastin-induced cell death; supplementation with zinc alone ($ZnCl_2$) or together with the iron chelator deferoxamine significantly heightened the sensitivity of MDA-MB-231 (Figure 8E) and HT-1080 cells to ferroptosis.[144] Additional studies identified that ZIP7 plays a crucial role by regulating zinc transport from the ER to the cytosol, essential for ferroptosis. Blocking ZIP7's function genetically or chemically safeguarded cells against ferroptosis, a protection that was negated with the addition of zinc (Figure 8F).[144] These findings confirmed that Zn^{2+} and ZnO NPs are capable of inducing ferroptosis, suggesting that zinc administration could serve as a promising approach to promote the death of cancer cells.

Moreover, zinc overload can impair mitochondrial function and promote organelle death, coordinating with Ca^{2+}-induced damage to disrupt intracellular calcium homeostasis, ultimately leading to cell death.[146,147] While Zn^{2+} and Ca^{2+} are known to share many transport pathways, the precise nature of their interplay in the context of PCD remains to be clearly defined.

Lysosomes are key organelles in cells, fundamental for metabolism and protein breakdown, and crucial for cell growth,

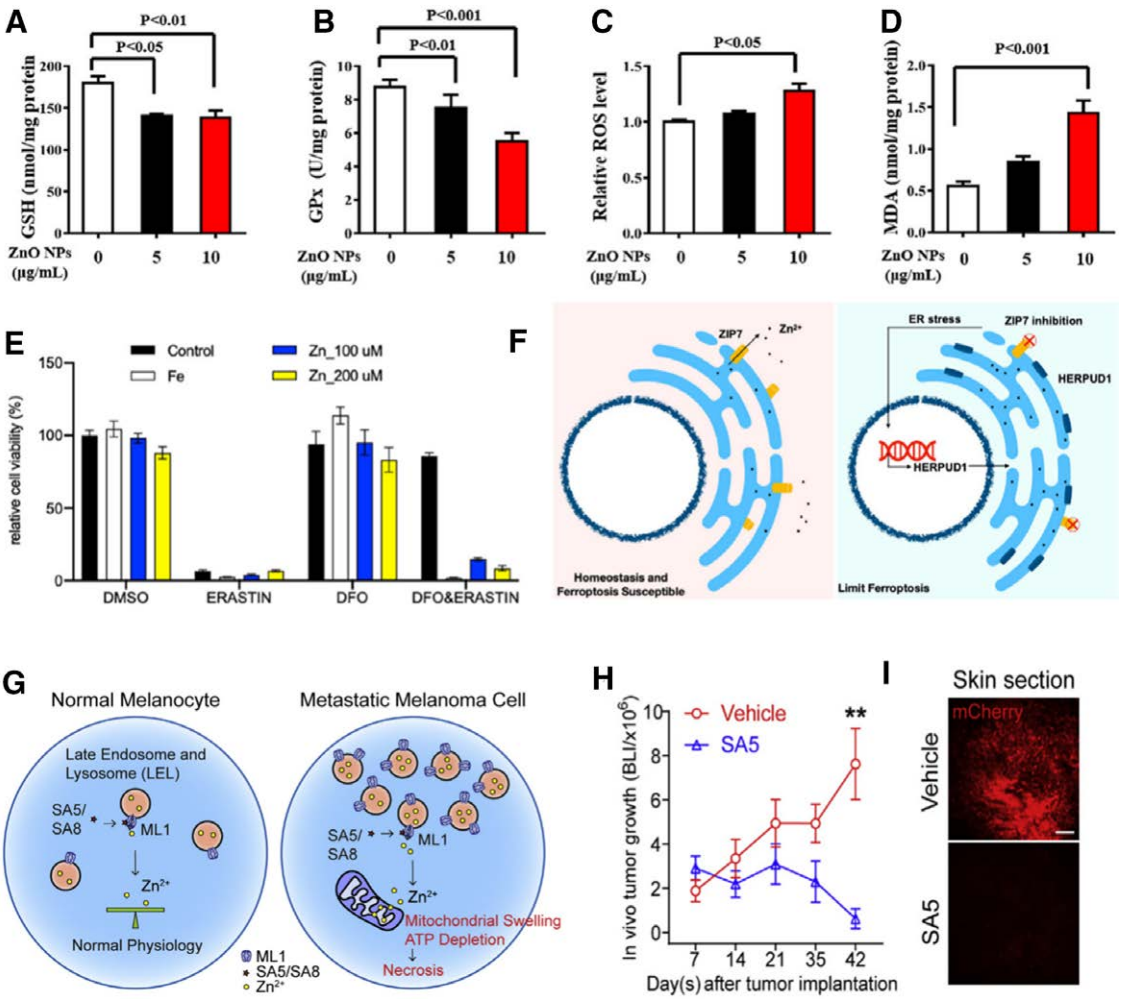

Figure 8. Illustration of zinc ion-mediated cell death and related nNMs. (A) GSH, (B) GPx, (C) ROS, and (D) MDA levels of HUVECs after the treatment with ZnO NPs at various concentrations. (A–D) Reproduced with permission from Zhang et al.[143] Copyright 2020, the author(s). (E) Relative cell viability of MDA-MB-231 cells after different treatments for 24 hours. (F) Illustration of ferroptosis resistance through ZIP7 knockdown. (E, F) Reproduced with permission from Chen et al.[144] Copyright 2021, the author(s). (G) Illustration of lysozincrosis in metastatic melanoma cells. (H) Tumor volume curves of different treatment groups, $n = 5$. $^{**}P < 0.01$. (I) Illustrative mCherry fluorescence images showcasing tumor cells (red) in skin sections from different treatment groups. (G–I) Reproduced with permission from Du et al.[145] Copyright 2021, the author(s).

division, and differentiation.[148] Unstable Zn^{2+} within cells is partly stored in lysosomes. Just as with iron, Zn^{2+} can also be released from its unstable reserve, influencing mitochondrial and lysosomal functions, thereby inducing zinc ions-mediated cancer cell death.[149] Mucolipin TRP channel 1 (TRPML1/MCOLN1; ML1) is a cation channel that allows the passage of Ca^{2+} and Zn^{2+}/Fe^{2+}, mainly found on the membranes of late endosomes and lysosomes in all mammalian cells.[150] It is essential for several lysosomal activities, such as transport, exocytosis, biogenesis, and heavy metal homeostasis. In metastatic melanoma cells with maladaptive upregulation of the ML1 lysosomal channel, Du et al discovered a kind of lysosomal Zn^{2+} release triggering nonapoptotic cell death, which they termed as lysozincrosis.[145] Upon treating these cells with TRPML-specific synthetic agonist (ML-SA), lysosomal Zn^{2+} release was triggered, leading to mitochondrial swelling/damage and rapid depletion of cellular ATP. This process induced necrosis, as illustrated in Figure 8G. As expected, administering ML-SA5, a specific type of ML-SA, significantly reduced tumor volume in MeWo-FmC-bearing mice over a 42-day treatment period without any relapse, demonstrating a more pronounced antitumor effect compared to the control group (Figure 8H, I).[145]

It is worth noting that the influence of zinc ions on cancer is notably varied, depending on the specific type of cancer involved.[140] Besides the aforementioned lysozincrosis in metastatic melanoma, studies indicate that ZnO NPs particularly induce cancer cell death through the apoptotic pathway in certain cancer cell lines, such as cervical carcinoma, lung carcinoma, head and neck squamous cell carcinoma, urinary bladder carcinoma, and head and neck squamous cell carcinoma.[151,152] Additionally, reports indicate that $ZnFe_2O_4$-based nanosystems could programmatically initiate and amplify the cyclic guanosine monophosphate-adenosine monophosphate synthase (cGAS)/stimulator of interferon genes (STING) signaling pathway in tumor cells, thereby inhibiting tumor progression and postoperative recurrence.[153]

In summary, current studies on zinc ions-mediated cell death mainly align with established PCD pathways, with no specific cell death mechanisms attributed to zinc ions induction yet identified. Research into the anticancer mechanisms of zinc ions-based nanomedicine, especially focusing on ZnO, remains relatively underexplored. Moreover, given the varying dependence of different cancer cells on zinc ions, establishing a universal principle for zinc ions-mediated cell death requires further investigation. However, it is undeniable that disrupting the zinc homeostasis in cancer cells represents a promising anticancer strategy.

3.2.2 Sodium ions-induced cell death

Sodium ions (Na^+), a kind of common physiological ion, are crucial for normal cell function, typically existing at concentrations an order of magnitude higher extracellularly (145 mM) than intracellularly (12 mM).[154] Conversely, intracellular potassium (K^+) levels are maintained at a higher concentration. This asymmetry in Na^+/K^+ concentration gradients is vital for amino acid transport, pH maintenance, and cell volume regulation.[155,156] Disrupting sodium homeostasis in tumor cells can induce significant osmotic changes, leading to apoptosis or necrosis. However, targeting tumor cells with excess Na^+ is challenging due to the strict cellular ion transport regulation of live cells and the short circulation lifetime of single-ion delivery. Ingeniously designed sodium-based NMs can bypass these hurdles, as they enter cells via endocytosis and release Na^+ internally, increasing osmotic pressure and causing selective tumor cell death while sparing normal cells due to their lower sodium levels.[157]

However, ionic compounds such as sodium chloride (NaCl) and sodium bicarbonate ($NaHCO_3$), selected for their availability and excellent biocompatibility, are challenging to encapsulate in NMs due to their high solubility in water.[158] This makes the delivery of sodium ions to tumor cells using NMs a complex task. It is able to overcome these challenges through kinds of preparation strategies, ensuring stable encapsulation and effective delivery within the TME. Jiang et al used sodium oleate and molybdenum chloride as precursors, with oleylamine as a surfactant, to synthesize pure-phase NaCl nanoparticles (SCNPs) via a microemulsion reaction in a hexane/ethanol mixed solvent.[157] Subsequently, the obtained SCNPs surface was modified with a layer of PEGylated phospholipid to enhance its water solubility, resulting in the phospholipid-coated PSCNPs (Figure 9A). PSCNPs could enter tumor cells via endocytosis, releasing a significant amount of Na^+/Cl^-, leading to increased osmolarity and rapid cell lysis. Interestingly, PSCNPs showed minimal toxicity toward normal cells like human prostate epithelial cell line (HPrECs) and mouse spermatogonial stem cells (C18-4). This selective cytotoxicity was attributed to the higher uptake by rapidly proliferating tumor cells and their inherently higher intracellular sodium concentrations ($[Na^+]_{int}$), making them more susceptible to PSCNPs-induced osmotic shock. Figure 9B illustrates the varying cytotoxic effects on different cells and their correlation with cellular $[Na^+]_{int}$, indicating that $[Na^+]_{int}$ plays a vital role in determining the susceptibility of cancer cells to PSCNPs. In vivo experiments demonstrated that PSCNPs suppressed tumor growth (Figure 9C) and induced apoptosis and necroptosis in cancer cells, with an increase in survival (Figure 9D). Moreover, the study also revealed that PSCNPs could induce ICD, simultaneously killing cancer cells and enhancing anticancer immunity. To further enhance the blood circulation time and sodium ions responsive release capability of NaCl-based nanomedicines, Li et al designed and synthesized a kind of GSH-responsive nanomedicines, termed NaCl@ssss-VHMS (Figure 9E), which comprised NaCl nanocrystals internally and a virus-mimicking hollow mesoporous tetrasulfide-organosilica (ssss-VHMS) externally.[158] The morphological advancements in NaCl@ssss-VHMS facilitated its rapid internalization into tumor cells via spike surface-assisted adhesion and invasion. Once inside, it degraded under the action of excessively produced GSH, releasing substantial amounts of Na^+/Cl^-, disrupting the ionic homeostasis of tumor cells, and inducing apoptosis, ferroptosis, as well as pyroptosis. The intracellular Na^+ and Cl^- ion levels were monitored using Na^+ (sodium-binding benzofuran isophthalate acetoxymethyl ester [SBFI-AM]) and Cl^- indicator probe (MQAE), respectively. The enhanced fluorescence signals of SBFI-AM and MQAE in NaCl@ssss-VHMS-treated HepG2 cells, compared to control groups, underscored its superior ion delivery performance (Figure 9F). In vivo imaging experiments also demonstrated the efficient accumulation of NaCl@ssss-VHMS within tumors (Figure 9G). In addition to NaCl, sodium citrate NPs have also been employed for sodium ion delivery, resulting in increased intracellular osmotic pressure and mediating tumor cell death.[160]

As another type of sodium-containing inorganic salt, sodium bicarbonate ($NaHCO_3$) NPs are also employed in sodium ions-mediated cell death. Distinct from the aforementioned NaCl NPs, $NaHCO_3$ NPs possess weak alkalinity, which allows

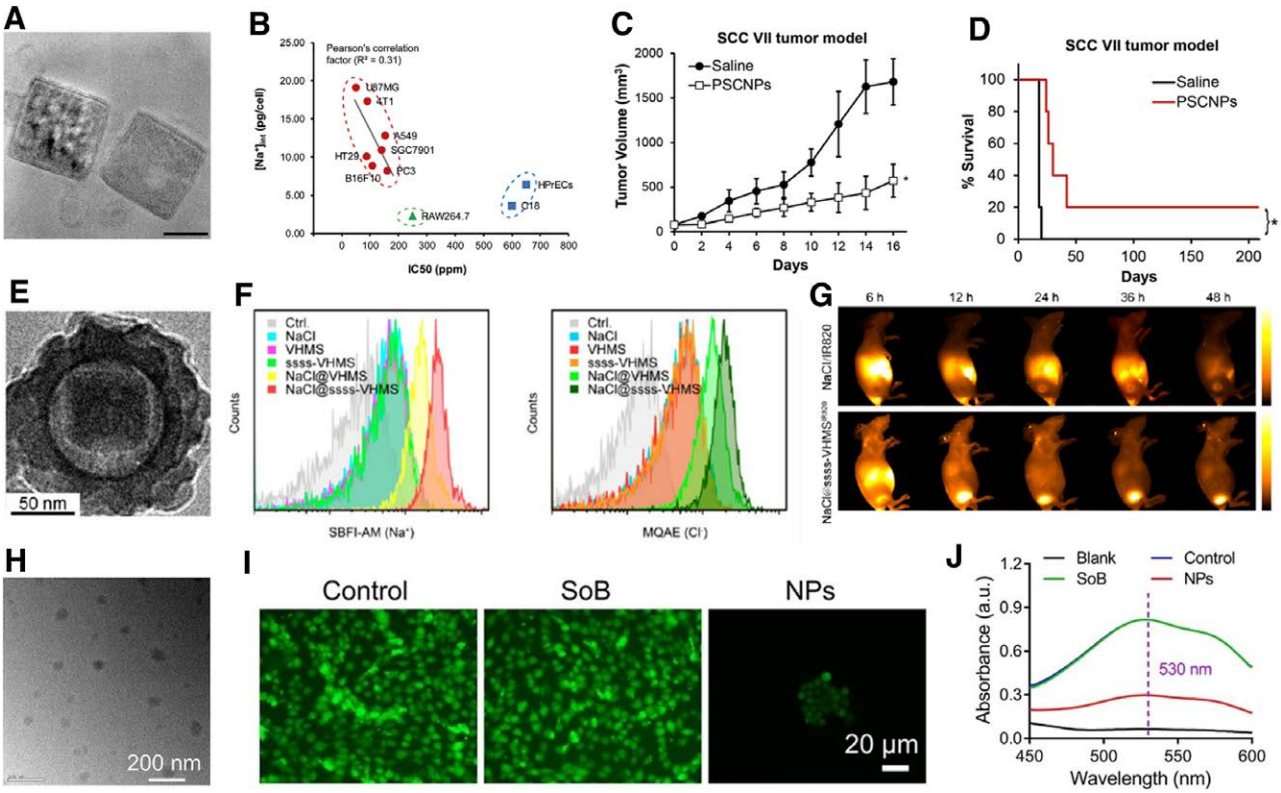

Figure 9. Illustration of sodium ions-mediated cell death and related NMs. (A) TEM image of PSCNPs. Scale bar, 40 nm. (B) The correlation between $[Na^+]_{int}$ and IC_{50}. (C) Tumor growth curves of different treatment groups. *P < 0.05. (D) Survival curves of different treatment groups. *P < 0.05. (A–D) Reproduced with permission from Jiang et al.[157] Copyright 2019, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (E) TEM image of NaCl@ssss-VHMS. (F) Flow cytometric analysis of intracellular Na^+ and Cl^- levels. (G) Time-lapsed near-infrared (NIR) fluorescence imaging of tumor-bearing mice after different treatments. (E–G) Reproduced with permission from Li et al.[158] Copyright 2022, American Chemical Society. (H) TEM image of $NaHCO_3$ NPs. (I) Intracellular pH detection after different treatments. (J) Extracellular lactic acid detection after different treatments. (H–J) Reproduced with permission from Ding et al.[159] Copyright 2023, Wiley-VCH GmbH.

them to release a substantial amount of Na^+ inside tumor cells while concurrently rectifying the acidosis of the TME.[161] In a recent study, Ding et al synthesized $NaHCO_3$ NPs using sodium oleate and ammonium hydrogen carbonate as precursors through a rapid microemulsion method (Figure 9H).[159] $NaHCO_3$ NPs were subsequently modified with distearoylphosphatidylethanolamine-poly(ethylene glycol) (DSPE-PEG) for enhanced biocompatibility. The resultant weakly alkaline $NaHCO_3$ NPs could modulate lactic acid metabolism through acid-base neutralization. Treatment with $NaHCO_3$ NPs significantly increased intracellular pH levels (Figure 9I) and markedly reduced the lactic acid concentration in the culture medium (Figure 9J), demonstrating the substantial potential of $NaHCO_3$ NPs in regulating lactic acid metabolism and maintaining cellular pH stability. Moreover, like the aforementioned NaCl NPs, $NaHCO_3$ NPs could also release significant quantities of Na^+ within tumor cells, leading to an increase in intracellular osmolarity. This process could trigger the pyroptosis pathway and ICD, thereby enhancing immune responses.

Currently, NMs employed in sodium ions-mediated cell death are known for their good biocompatibility and simple raw material components. They induce tumor cell death by disrupting the ionic homeostasis of tumor cells, leading to a sharp increase in osmotic pressure. Additionally, some of them can also regulate lactic acid metabolism through acid-base neutralization, thereby reversing the weakly acidic, immunosuppressive TME, offering broad application prospects. In this therapeutic strategy, the controllable preparation of NMs is crucial, ensuring prolonged blood circulation while preventing the off-target leakage and degradation of water-soluble sodium-containing compounds. Future developments could involve designing more comprehensive theranostic nanoplatforms, using imaging techniques to investigate the specific mechanisms and metabolic pathways in sodium ions-mediated cell death, such as fluorescent Na^+ reporter SBFI-AM or ^{23}Na MRI. Finally, sodium ions-mediated cell death holds significant research value in improving immune responses and achieving tumor immunotherapy, with current studies making beneficial explorations in this area.[157,159,160]

3.2.4 Other ions-induced cell death

Other essential metal ions, including magnesium (Mg), cobalt (Co), molybdenum (Mo), manganese (Mn), and potassium (K) ions, are also crucial for the growth and development of humans,[22] showing potential in inducing metalloptosis.

Magnesium ion (Mg^{2+}) as an essential trace element for the human body, participates in various physiological activities in cardiovascular, neurological systems, and muscles.[162] More importantly, Mg^{2+} can act as an activator involved in the synthesis of at least 200 enzymes[163] and regulate other momentous ion channels such as K^+ and Ca^{2+}.[164] Hence, abnormal levels of Mg^{2+} in the body can trigger various diseases, and some nanotheranostics based on Mg^{2+} have shown initial effectiveness in treating cancer. For instance, MgO NPs exerted a strong

Table 2

NMs inducing metalloptosis mentioned in this review.

Types of metalloptosis	Engineering NMs	Reference
Ferroptosis	Fe_3O_4@PGL	[83]
	bcc-USINPs	[84]
	Au-Fe_2C	[38]
	AMSNs	[87]
	Au/Cu-TCPP(Fe)-PEG	[90]
	SRF@Fe^{III}TA	[95]
	Fe/Ni-LDH	[100]
Cuproptosis	NP@ESCu	[34]
	CSTD-Cu(II)@DSF	[113]
	Dox@Fe/CuTH HaMOF	[47]
	$Cu_2(PO_4)(OH)$ NPs	[40]
	GOx@[Cu(tz)]	[54]
	BSO-CAT@MOF-199@DDM	[56]
Calcium overload	M@$CaCO_3$@KAE	[134]
	$CaNM_{CUR+CDDP}$	[135]
	^{PEG}CaCUR	[136]
Zinc ions-induced cell death	ZnO	[143,151,152]
Sodium ions-induced cell death	PSCNPs	[157]
	NaCl@ssss-VHMS	[158]
	$NaHCO_3$ NPs	[159]
Magnesium ions-induced cell death	MgO NPs	[165]
Cobalt ions-induced cell death	Co NPs	[176]
	iCoDMSNs	[177]
Manganese ions-induced cell death	Silica-coated MnO NPs	[184]
Potassium ions-induced cell death	KCl NPs	[186]

inhibitory effect on cancer cells[165] through the production of ROS because of the release of Mg^{2+} or that of ligands as well as byproducts.[166–168] The generated ROS further caused oxidative damage to DNA, protein denaturation, and lipid peroxidation, leading to the eventual cell death.[169] However, the detailed molecular mechanism of Mg^{2+}-mediated cell death still needs to be clarified.[170,171] Meanwhile, the biocompatibility and cytotoxicity of MgO NPs is also a key issue that potentially hinders the translation of MgO NPs in the field of cancer therapy,[172] which may be solved through the green synthesis routes of MgO NPs.[171,173] For instance, compared with Co oxide NPs, MgO NPs synthesized by the green method showed less toxicity and great bioavailability.[174] Moreover, the researchers have also found that introducing Mg^{2+} into NMs can promote the rolling circle amplification of DNA, ultimately achieving the antitumor effect of the functionalized DNA.[175]

Co and Mo have been reported to induce ferroptosis. Studies indicate that Co NPs significantly downregulated the expression of GSH, GPX4, and SLC7A11 proteins in Balb/3T3 mouse fibroblast cells, while leading to a notable increase in intracellular levels of ROS, MDA, cobalt, and iron.[176] However, ferrostatin-1 showed rescue effects on cells after treatment of Co NPs, suggesting a close association between Co NPs-induced cytotoxicity and ferroptosis.[176] In another study, cobaltous oxide nanodots were encapsulated within dendritic mesoporous silica NPs, followed by further surface modifications to yield iCoDMSNs. iCoDMSNs treatment increased transferrin receptor levels and reduced solute carrier family 40 member 1 (SLC40A1), leading to Fe^{2+} accumulation and subsequent ferroptosis.[177] Similarly, molybdenum sulfide NPs were able to generate ROS, thereby triggering lipid peroxidation and ferroptosis in cancer cells.[178] Additionally, the simultaneous integration of Co and Mo into NMs could enhance the induction of ferroptosis more effectively. Wu et al introduced a strategy of nonferrous ferroptosis, utilizing a $CoMoO_4$-phosphomolybdic acid nanosheet hybrid. This approach leveraged accelerated Mo(V)-Mo(VI) conversion to increase lipid peroxide levels, enhanced GSH consumption for GPX4 enzyme deactivation, and initiated a ROS surge, effectively inducing ferroptosis.[57]

Manganese-based NPs can induce ferroptosis in tumors through mechanisms such as GSH depletion, increased oxygen and ROS production, and enhanced lipid peroxidation. For example, when oral squamous cell carcinoma cells (Cal-27) were treated with MnO_2 nanoflowers under near-infrared radiation, there was an increase in cellular Fe^{2+}, an accumulation of LPO, and a downregulation of GPX4, effectively inducing ferroptosis.[179] Similarly, manganese silicate nanobubbles demonstrated a high capacity for depleting GSH, inducing ferroptosis through the inactivation of GPX4.[180] Furthermore, Mn can influence iron homeostasis by altering iron uptake and the expression of circulating transport and regulatory proteins; alterations in iron levels can, in turn, impact Mn balance and its biological roles.[181] Mn^{2+} may also contend with essential ions such as Ca^{2+} and Mg^{2+} for cellular entry via different transport mechanisms, suggesting its potential to trigger PCD by unbalancing ion equilibrium.[182] Moreover, Mn-containing NPs can stimulate innate and adaptive immunity through the cGAS-STING pathway, playing an important role in the immunotherapy of tumors.[183,184] As an illustration, hollow mesoporous silica-coated MnO NPs have been utilized in MRI-guided immune-chemodynamic therapy, where MnO NPs served in activating the cGAS-STING pathway for immunotherapy, upregulating ROS through Fenton-like reactions, and enhancing T1-weighted MRI.[184] This research effectively leveraged the unique properties of the Mn element for antitumor therapy.

As a vital physiological ion, potassium ions are integral to numerous crucial cellular functions, including metabolism, growth, and apoptosis.[185] Analogous to NaCl, KCl NPs are also applicable in cancer therapy. The intracellular release of a substantial amount of K^+ and Cl^- could alter the osmotic pressure within cancer cells, subjecting them to a hypertonic state. This change induced the rupture and subsequent death of the cancer cells.[186] Furthermore, potassium ionophores have demonstrated anticancer capability. For example, valinomycin, a natural ionophore, is able to pump intracellular potassium ions out of the cytosol, leading to mitochondrial damage and inducing apoptosis.[187] Additionally, a helical polypeptide-based potassium ionophore was shown to induce ER stress-mediated apoptosis in vivo.[188] However, it is important to note that the research on the antitumor applications of NPs constructed based on potassium ionophores remains comparatively limited.

Although the discussed metal elements in this section can mediate cell death, the exact pathways of their cancer-killing effects need more research. There is a current gap in definitive studies confirming these pathways. Future work must elucidate how these metals exert specific molecular interactions within cancer cells to maximize their therapeutic value. Unraveling these mechanisms will illuminate the details of metalloptosis and guide the development of targeted cancer treatments leveraging these metals' unique attributes.

4. Conclusion and perspective

Metalloptosis as a new form of cell death, is worthy of defining cautiously, because the present reported several types of metalloptosis, in addition to ferroptosis, cuproptosis, and calcium overload, the downstream signaling pathway of other types of metalloptosis (such as Mg ions-, Zn ions-, Mn ions-, and Na ions-mediated cell death) is still attributed to conventional PCD.

Table 2 summarizes the representative NMs mentioned in this review that induce metalloptosis. Hence, it is urgent to excavate more in-depth and specific cell death signaling pathways on metalloptosis. While the concept of "metalloimmunotherapy" has previously been introduced to the academic community, highlighting the inherent immunomodulatory properties of metal ions to enhance anticancer immune responses,[189] it is crucial to distinguish this from "metalloptosis." Metalloptosis specifically refers to a type of cell death mediated by metal ions, characterized by cellular damage and subsequent death triggered by specific metal ions. In contrast, metalloimmunotherapy leverages these immunomodulatory properties to bolster immune responses against cancers, focusing on enhancing immunological function rather than directly inducing cell death as observed in metalloptosis. Although both concepts underscore the critical role of metal ions in cancer research, they diverge fundamentally in their primary focus and application, highlighting the diverse therapeutic potentials of metals in oncology. Meanwhile, this is also inseparable from the advancements in imaging, artificial intelligence, analytical chemistry, and other fields, an increasing array of novel characterization and detection methods are available to directly or indirectly detect metalloptosis in cancers. Such interdisciplinary collaborations enhance our understanding of metal's anticancer mechanisms, offering new breakthroughs and progress in cancer treatment.

Furthermore, how to innovate the regulation signaling pathways of metalloptosis from a mechanistic perspective is a very challenging issue. Hence, in-depth studies on the interplay among different NMs components and their interactions with cancer cells are essential for a comprehensive understanding of metalloptosis. For instance, NMs with different sizes and morphologies often have varying cell phagocytic efficiency, which can directly influence their interaction with cancer cells. Composite NMs have synergistic effects between multiple elements, thus the affected signaling pathways always exhibit a networked pattern rather than a single pathway, which is beneficial for enhancing the anticancer effect of NMs, but poses certain challenges in accurately understanding the role of each element. Therefore, to delve deeper into the mechanism, the NMs designed often tend to be simple and controllable. For the composite NMs, the interactions among various metal ions in metalloptosis are intricate; for instance, zinc ions can induce lysozincrosis, and there are also reports suggesting their role in ferroptosis and calcium overload, as well as traditional PCD. Utilizing cell death inhibitors and ion chelators can further clarify different types of metalloptosis. Moreover, when exploring the anticancer mechanisms of NMs-induced metalloptosis, special attention should be paid to the metal degradation and release properties of the NMs. It is crucial to determine the specific metal concentrations, incubation time, and their impacts on cells, including morphology, metabolism, intracellular and extracellular ion content, and cell death markers.

Beyond oncology, metal homeostasis also plays a vital role in other diseases, such as neurodegenerative, cardiovascular, and metabolic disorders. Research into the mechanisms of metalloptosis can also provide new therapeutic targets for these diseases.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

Shuren Wang, Ran Ma, Zi Mei, and Yanglong Hou proposed the overall concept. Shuren Wang and Ran Ma wrote the paper. All authors contributed to the general discussion.

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