Review Article

Recent progress in cancer therapy based on the combination of ferroptosis with photodynamic therapy

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ABSTRACT

Current anticancer treatments have many limitations to achieving high efficacy. Hence, novel strategies that broaden therapeutic prospects must urgently be developed. Ferroptosis is an iron-dependent form of non-apoptotic programmed cell death that is induced by cellular antioxidative system inhibition. Photodynamic therapy (PDT) uses photosensitizers to generate reactive oxygen species and aggravate oxidative stress in tumor cells. Combining ferroptosis with PDT cooperatively regulates intracellular redox homeostasis, thus increasing cancer cell susceptibility to oxidative stress and yielding synergistic anticancer effects. In this review, various strategies for combining ferroptosis with PDT are comprehensively summarized and discussed, including mono-PDT and PDT-induced ferroptosis, combining PDT with small-molecule ferroptosis inducers, and combining PDT with metal-ion-induced ferroptosis. Additionally, the possibility of combining ferroptosis and PDT with other anti-tumor therapies is discussed. Finally, the prospects and challenges of combining ferroptosis with PDT in clinical cancer treatment are addressed. With increased understanding of the superiority of combination PDT with ferroptosis for cancer treatment, we hope that drug delivery systems based on this strategy will be further developed to increase anticancer efficiency and achieve successful clinical translation.

Keywords: antitumor, ferroptosis, photodynamic therapy, combination therapy, intracellular redox homeostasis

1. INTRODUCTION

According to the World Health Organization, cancer is the second leading cause of death globally and is responsible for the deaths of approximately 10 million people annually [1]. Moreover, cancer morbidity is likely to increase substantially in the coming decades [2]. Despite substantial efforts to improve anti-cancer treatments, the situation remains grim. Metastasis and tumor resistance to current clinical drugs are two of the greatest obstacles [3, 4]. Therefore, developing new therapeutic approaches is crucial.

Ferroptosis is a unique type of programmed cell death that is morphologically, biochemically, and genetically distinct from other well-known forms of cell death [5]. It can be triggered by cellular redox homeostasis, and the resulting accumulation of iron-dependent lipid peroxidation products is the primary cause of cell death. Lipid peroxidation causes the plasma membrane to rupture,

and cancer cells in epithelial-to-mesenchymal transition are particularly susceptible. This peroxidation promotes the destruction of metastatic tumors and persistent cancer cells, thus providing new therapeutic options for tumors that are resistant to existing therapies [6-8]. Moreover, owing to their vigorous metabolism and proliferation, cancer cells have a greater iron demand than normal cells and consequently are more susceptible to ferroptosis [9]. Therefore, ferroptosis selectively kills persistent cancer cells and has shown promise in decreasing the multiple drug resistance and metastasis of cancer cells.

Photodynamic therapy (PDT) is a promising anti-tumor therapy that uses reactive oxygen species (ROS) produced by photosensitizers (PSs) to kill cancer cells [10]. In comparison to conventional approaches, PDT has substantial advantages of on-demand administration, non-invasiveness, and avoidance of drug resistance. Importantly, it is a local treatment strategy
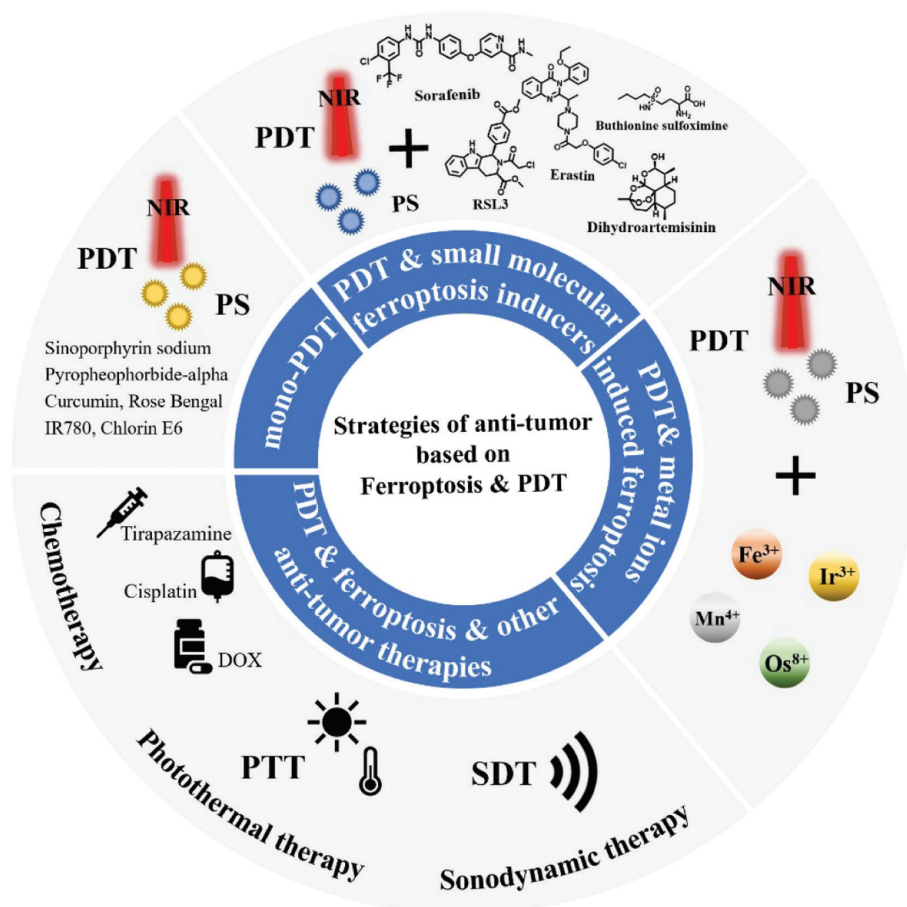
Scheme 1 | Strategies for anti-tumor therapy based on the combination of ferroptosis with photodynamic therapy (abbreviations: NIR: near-infrared, PS: photosensitizer, PDT: photodynamic therapy, PTT: photothermal therapy, SDT: sonodynamic therapy).

causing minimal damage to healthy tissue [11-13]. In addition, PDT boosts antitumor immunity by enhancing intra-tumoral T lymphocyte infiltration and secretion of numerous pro-inflammatory factors, such as IFN-γ, thus contributing to the long-term inhibition of cancer cell proliferation [14]. Therefore, PDT is substantially superior to traditional treatments, such as surgery and chemotherapy, which are not only immune unresponsive but even immunosuppressive [15, 16].

Nevertheless, ferroptosis and PDT each have limitations. For example, the extremely short half-life of $^1O_2$ and the hypoxic intratumoral microenvironment continually limit the efficacy of PDT [14, 17, 18]. Because high levels of inducers are required to trigger ferroptosis, the toxicity to normal tissues would be severe. However, these two strategies cooperatively elicit synergetic anti-tumor effects. Cellular ferroptosis enhances the efficacy of PDT by generating ROS through Fenton or Fenton-like reactions ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + (OH)^- + \bullet OH$) that continuously produce oxygen [19]. In addition, PDT also drives ferroptosis by triggering non-enzymatic lipid peroxidation with generated ROS [18, 20]. Ferroptosis and PDT

have synergistic rather than additive effects. Therefore, the combination of ferroptosis and PDT has high research value in anti-tumor therapy, particularly in resolving multidrug resistance and tumor metastasis [21].

In recent years, the combination of these two cancer treatment strategies has attracted extensive attention and shown broad application prospects for tumor treatment, particularly in solid tumors resistant to traditional therapies [22]. This review summarizes the research frontiers and development trends in this field, and systematically compares and discusses the strengths and limitations of these studies suggesting novel strategies for cancer therapy (**Scheme 1**) .

2. FERROPTOSIS COMBINED WITH PDT TO REGULATE INTRACELLULAR REDOX HOMEOSTASIS

ROS are by-products of aerobic respiration under physiological conditions. ROS cause DNA damage and genomic instability, thus driving the accumulation of tumor genetic alterations and promoting cancer progression

[23]. Cancer cells require considerable ROS to support their invasive development [24]. However, massive ROS cause cancer cell death through DNA damage and through lipid peroxidation, commonly known as oxidative stress [25]. To cope with these conditions, the antioxidative system works to maintain cellular redox homeostasis. Glutathione (GSH), an essential component of the intracellular antioxidant system, participates in numerous metabolic activities and physiological processes in the human body. As an antioxidant, GSH scavenges excess ROS by being oxidized to GSSG either directly or via an enzymatic reaction [26]. To adapt to cell growth and survive under oxidative stress, tumor tissue contains four times more GSH than normal tissue [27].

In addition to regulating oxidative stress, GSH plays an important role in drug detoxification, thus posing hurdles for chemotherapy or PDT in anticancer therapy. GSH mediates the detoxification of cisplatin, doxorubicin, and other agents, thus resulting in anticancer resistance [28]. Although photosensitizers are excited to increase intracellular ROS, in cancer, intracellular GSH has direct quenching effects, thus impeding effectiveness [29]. Consequently, cancer therapies targeting GSH metabolism have been extensively studied.

GSH is synthesized in the cytoplasm from cysteine, glutamate, and glycine through two ATP-dependent enzymatic reactions [30]. Multiple pathways are involved in GSH synthesis and metabolism, as described in detail previously [30-33]. Here, we focus on the system x_c^--GSH-GPX4 pathway, which is also relevant to ferroptosis (Figure 1). System x_c^- is a crucial glutamate/ cystine transporter for cystine absorption. Cystine is the oxidized form of cysteine and a rate-limiting substrate for GSH synthesis. GSH-dependent lipid hydroperoxide glutathione peroxidase 4 (GPX4) converts lipid hydroperoxides to nontoxic lipid alcohols, thus hindering PDT and ferroptosis [34]. The amount of intracellular GSH determines the normal function of GPX4, and GSH deficiency also leads to the inactivation of GPX4 [35].

Intracellular GSH depletion increases cancer cell susceptibility to oxidative stress and chemotherapeutic drugs, and has also been shown to increase the efficacy of ROS-based therapy [31]. Ferroptosis inducers disrupt the GSH-redox system by blocking system x_c^- or inhibiting GPX4 [36]. In addition, PDT generates substantial amounts of ROS, thereby disrupting the intracellular redox balance. Therefore, PDT and ferroptosis cooperatively regulate the intracellular redox balance by affecting the metabolism of ROS and GSH, thereby enhancing each other's anticancer efficacy. In the next section, we categorize these strategies according to the various ferroptosis triggering methods: mono-PDT and PDT-induced ferroptosis, combining PDT with small-molecule ferroptosis inducers, and combining PDT with metal-ion-induced ferroptosis (Table 1).

3. CURRENT STRATEGIES FOR COMBINING FERROPTOSIS WITH PDT

3.1 Mono-PDT and PDT-induced ferroptosis
PDT is a revolutionary anticancer strategy that eliminates tumor cells by light-exciting photosensitizers to
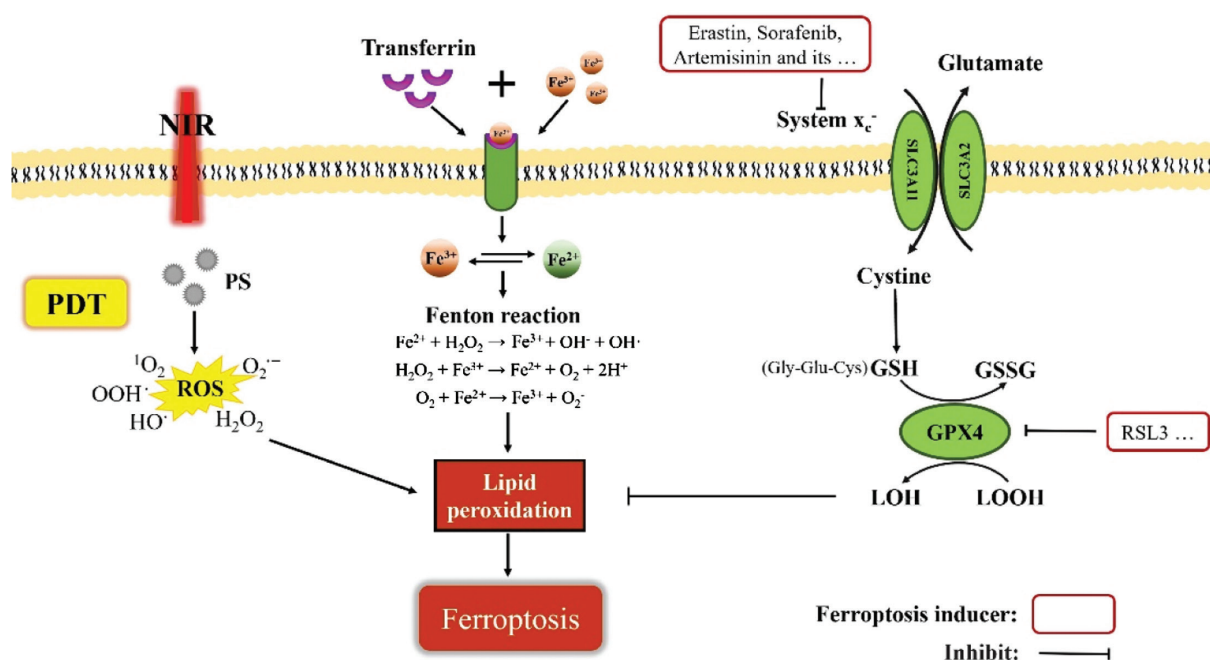

Figure 1 | Schematic overview of ferroptosis combined with PDT to regulate intracellular redox homeostasis.

Table 1 | Synergistic formulations based on ferroptosis and PDT

Preparation	Ferroptosis inducer	Photosensitizer	Cancer cell lines/animal models	References
Drug solution	/	Sinoporphyrin sodium	Cholangiocarcinoma	[41]
MH-PLGA-IR780 NPs	/	IR780	Osteosarcoma	[42]
PS-NO solution	/	Ce6	Cervical cancer	[43]
Au NRs/Cur/UCNPs@PBE NPs	/	Curcumin	Melanoma	[46]
SRF@Hb-Ce6 NPs	Sorafenib	Ce6	Mouse breast cancer, mouse liver cancer	[49]
Ce6@SRF@RDV NPs	Sorafenib	Ce6	Osteosarcoma	[50]
BCFe@SRF NPs	Sorafenib	Ce6	Mouse liver cancer	[51]
BNP@R NPs	RSL3	PPa	Mouse melanoma, mouse breast cancer	[16]
Ce6-erastin NPs	Erastin	Ce6	Oral tongue squamous cell carcinoma	[19]
Er/RB@Exos^{CD47} NPs	Erastin	Rose Bengal	Hepatic cancer	[59]
Drug solution	DHA	Ce6	Lung cancer	[47]
Ce6-PEG-PLA-NPs & BSO	BSO	Ce6	Mouse squamous cell carcinoma	[61]
p53/Ce6@ZF-T NPs	Fe^{2+}/p53	Ce6	Lung cancer	[67]
HCNPs	Hemin (Fe^{2+})	Ce6	Mouse breast cancer	[68]
PEG-Fns	Ferrihydrite	Ferrihydrite	Mouse breast cancer	[72]
Fe^{3+}@Au1Ag24@PbP nanoplatform	Fe^{3+}	Au1Ag24 nanoclusters	Mouse liver cancer	[73]
Fe_3O_4@PGL NPs	Fe_3O_4	porphyrins	Colon cancer	[75]
Drug solution	Os2	Os2	Cervical cancer	[82]
IrS NPs	Ir-SH	Ir-SH	Lung cancer	[83]
Drug solution	MitoIrL2	MitoIrL2	Pancreatic cancer, breast cancer	[84]

generate massive amounts of ROS. In contrast, the ROS produced by PDT initiate peroxidation of membrane lipids, thus leading to cellular ferroptosis, which is also characterized by uncontrolled iron-catalyzed lipid peroxidation [36, 37]. Hence, to some extent, mono-PDT can have synergistic effects in conjunction with the intrinsic iron pool [20]. Consequently, single-PDT-induced ferroptosis has been extensively studied, and ferroptosis inhibitors have been used to confirm the involvement of ferroptosis in tumor suppression beyond the effects of ROS generated by PDT [38-40].

Zhang et al. have used sinoporphyrin sodium to study cholangiocarcinoma and confirmed that PDT alone downregulates the expression of the cystine/glutamate transporter (system x_c^-), thus inducing ferroptosis [41]. In addition, to enhance the therapeutic effect, Wang et al. have used a polylactic acid-glycolic acid copolymer to load the photosensitizer IR780 (**Figure 2**) [42]. The micelle nanoparticles were then coated with human osteosarcoma cell membranes to obtain a biomimetic active targeting nanoplatform (MH-PLGA-IR780). The nanoplatform promotes ferroptosis by inactivating GPX4, and its effectiveness has been demonstrated in human osteosarcoma-bearing mice. Similarly, Guo et al. have

synthesized a Ce6 derivative (PS-NO) containing an NO donor [43]. High levels of NO produce reactive nitrogen species, which consume GSH and then disrupt intracellular redox homeostasis, thus causing oxidative stress and increasing cellular ROS levels [44]. Consequently, cancer progression is impeded through cell ferroptosis, and tumors are rendered vulnerable to chemotherapy, radiation, or immunotherapy [45]. The enhanced antitumor efficacy of PS-NO on the basis of the synergistic effects of ROS and reactive nitrogen species has been verified in Hela cells. Zhong et al. have loaded curcumin (as a photosensitizer) on gold nanorods (Au NRs) [46], then encapsulated Au NRs with upconversion nanoparticles (UCNPs) and modified them with phenylboronic double ester (PBE). Under near-infrared laser irradiation, the Au NRs/Cur/UCNPs@PBE elicit efficient photothermal therapy (PTT) and PDT. Further exploration of the mechanism mediating cell death has revealed that excess intracellular $^1O_2$ depletes GSH and causes ferroptosis beyond apoptosis. This study has indicated the possibility of integrating PDT, PTT, and ferroptosis in a single nanoplatform with high performance.

GSH deficiency may inhibit GPX4 activity and further improve the efficacy of ROS-based therapies [31, 45].
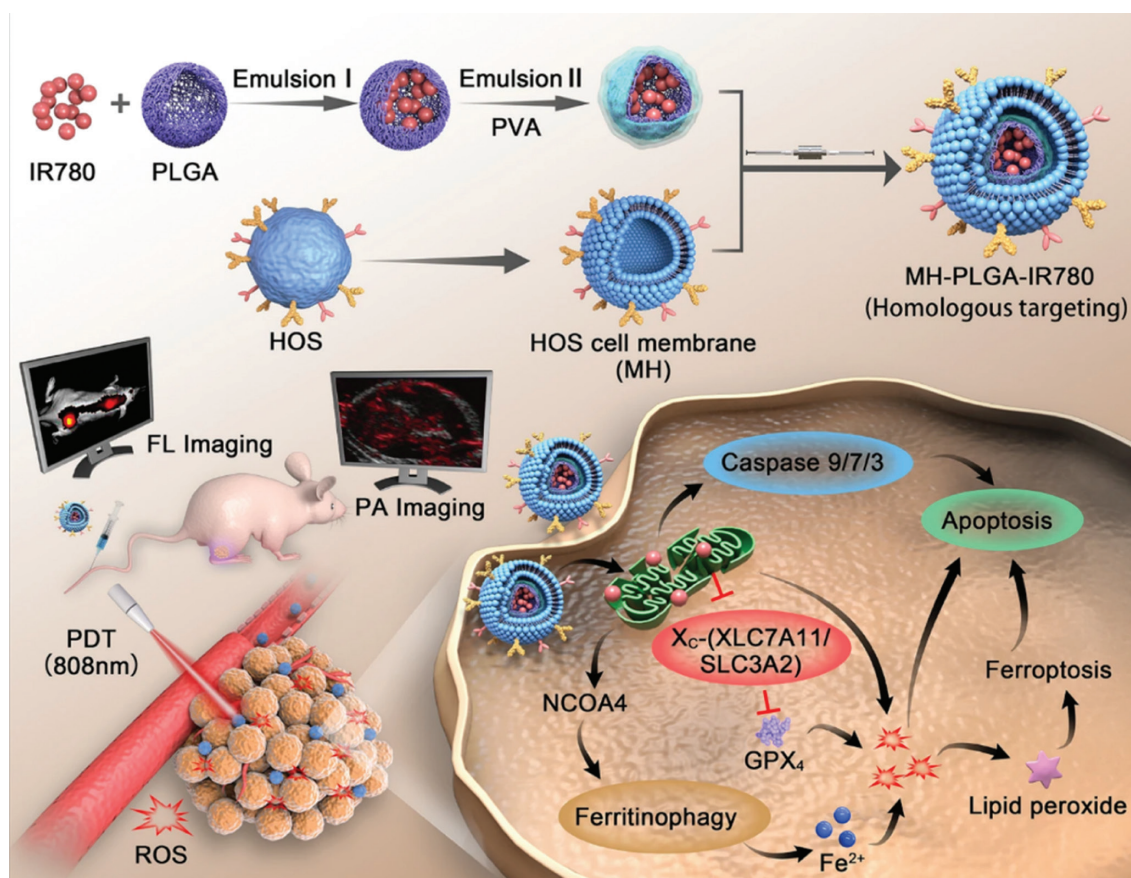
Figure 2 | Schematic diagram of the fabrication and anticancer mechanism of MH-PLGA-IR780 (abbreviations: HOS: human osteosarcoma cell, FL: fluorescence, PA: photoacoustic). Reproduced with permission, [42] copyright 2022 Springer Nature.

However, mono-PDT cannot always produce optimal therapeutic effects, possibly because the PDT-induced DNA damage response upregulates GPX4 and further degrades PDT-induced ROS [47]. Furthermore, PDT-induced ferroptosis may be weak. In addition, the tumor's hypoxic microenvironment would also hinder the efficacy of PDT. Combining photosensitizers with ferroptosis inducers selectively directs the development of cancer cells toward ferroptosis, thereby improving the anticancer effect of PDT [36].

3.2 Combining PDT with small-molecule ferroptosis inducers

As stated previously, the system x_c^--GSH-GPX4 pathway substantially affects the biosynthesis and metabolism of GSH as well as the cell anti-oxidation system (**Figure 1**). Small-molecule ferroptosis inducers often act on this pathway and disrupt the cellular antioxidant system, thus causing accumulation of lipid peroxides. These ferroptosis inducers exacerbate oxidative stress in PDT by increasing ROS and abrogating antioxidation. In addition, PDT-induced ROS directly trigger lipid oxidation [31, 36, 37, 48].

3.2.1 Sorafenib. Sorafenib (SRF) is an approved antitumor drug by the U.S. Food and Drug Administration. SRF directly inhibits system x_c^-, thus resulting in GSH depletion and GPX4 inactivation. When SRF coexists with iron-rich sources, the ferroptotic effect is amplified several times [49]. Several studies have examined the synergic cancer treatments using SRF and photosensitizers with different carriers. For example, Xu et al. have incorporated Ce6 with hemoglobin (Hb) to construct a 2-in-1 nanoplatform (SRF@Hb-Ce6) loaded with SRF (**Figure 3a**) [49]. As an endogenous protein, Hb carries oxygen and iron. The Hb-based nanoplatform provides iron for iron-dependent ferroptosis and oxygen for oxygen-dependent PDT. The encapsulation efficiency of SRF can reach 70%. To obtain tumor-specific drug release, an amphiphilic matrix metalloproteinase 2 (MMP-2)-responsive peptide has been inserted into a Hb-Ce6 micelle skeleton. HIF-1 expression is significantly diminished at tumor sites after Hb-Ce6 treatment (**Figure 3b-c**), thus indicating its hypoxia-allaying effect. The intracellular singlet oxygen level is enhanced with Hb-Ce6 group compared with Ce6 alone, owing to the oxygen-supplying effect of the Hb-based carrier. In addition, the synergistic
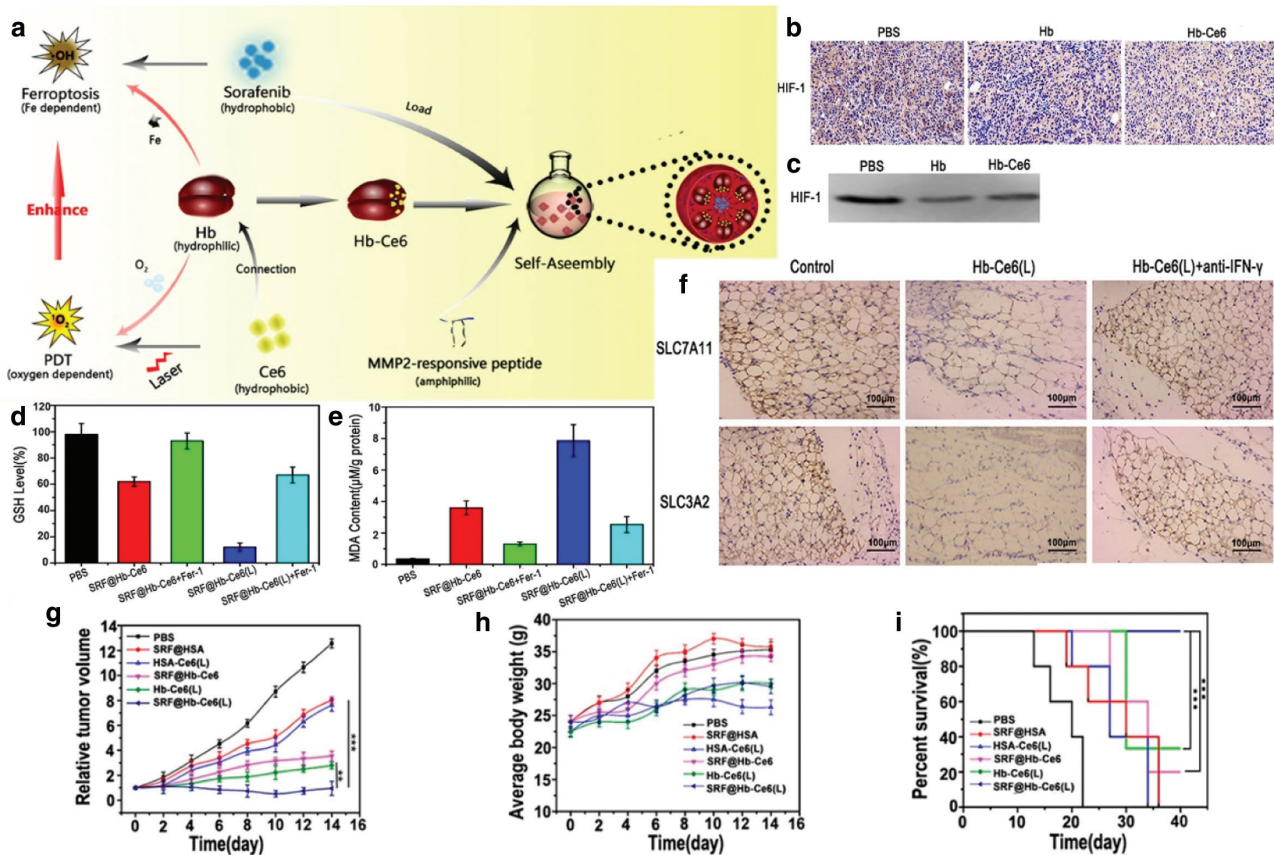
Figure 3 | (a) Schematic diagram of the fabrication and anticancer mechanism of SRF@Hb-Ce6. (b) Immunohistochemical assay of HIF-1 expression in tumor tissues in the PBS, Hb, and Hb-Ce6 treatment groups. (c) WB assay of HIF-1 expression at the cell level in the PBS, Hb, and Hb-Ce6 treatment groups. (d) GSH level in tumor tissue, determined with DTNB assays. (e) MDA content in tumor tissue. (f) Immunohistochemical assays indicating the expression of SLC7A11 and SLC3A2. (g) Tumor growth curves of mice during the 14 d treatments. (g) Animal weight changes in different groups during the treatment period. (i) Survival rates of mice in different treatment groups. Reproduced with permission, [49] copyright 2020 American Chemical Society.

mechanism of SRF-induced ferroptosis and Ce6-induced PDT has been extensively investigated, and significant interference with the intracellular redox balance has been observed. GSH levels in the laser group (SRF@Hb-Ce6 (L)) were approximately 50% lower than those in the PBS group but were only 30% lower without laser treatment (**Figure 3d**). Lipid ROS (malondialdehyde) with SRF@Hb-Ce6 (L) were approximately 2-fold higher than those with SRF@Hb-Ce6 (**Figure 3e**). These results have verified the interaction between PDT and ferroptosis produced by the nanoplatform. Beyond the influence on intracellular redox balance, a synergistic effect has also been observed in the tumor immune response. SRF@Hb-Ce6 infiltrates the tumor tissue by recruiting many T lymphocytes, thereby activating the immune system and leading to secretion of IFN-γ. Subsequently, IFN-γ decreases SLC7A11/SLC3A2 expression, significantly decreases tumor volume, and increases mouse survival rate (**Figure 3f**). In vivo results have indicated a significant decrease in tumor volume and a significant increase in overall survival (**Figure 3g-i**). Similarly, Liu et al. have designed a nanoplatform (Ce6@SRF@RDV) for the co-delivery of Ce6 and SRF to cancer sites by using a red-blood-cell-derived vehicle (RDV) [50]. RDV has multiple functions similar to those of Hb. Furthermore, the membrane structure of the RDV enables cellular uptake and biocompatibility. This novel nanosystem has higher cellular uptake rates, enhanced PDT and ferroptosis effect as well as improved safety compared with that without membrane coated. In another study, Wang et al. have constructed a novel hypoxia-responsive nanoreactor (BCFe@SRF) [51] by combining Ce6 with bovine serum albumin and ferritin via an azobenzene linker conjugate, then loading SRF. BCFe@SRF is degraded by PDT in a hypoxic environment, thus releasing ferritin for catalysis of the Fenton reaction, and destroying tumor antioxidant defenses by SRF, thereby enhancing antitumor effects.

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3.2.2 RSL3. RSL3 is a ferroptosis activator that induces ferroptosis in tumor cells by downregulating GPX4 [52]. RSL3 and PDT have been reported to induce immunogenic cell death (ICD) [16]. Song and colleagues have developed acidity-activatable dynamic nanoparticles that boost ferroptosis and PDT, and further promote cancer immunotherapy [16]. The nanoparticle (BNP@R) was fabricated by integration of a pyropheophorbide A (PPa) containing copolymer and an acid-labile copolymer for tumor-specific delivery of RSL3 (**Figure 4a**). BNP@R is activated by the tumor acidic microenvironment and triggers anti-tumor immune responses via PDT and GPX4-associated ferroptosis. The authors treated tumor cells with their developed BNP@R, then incubated them with IFN-γ, and observed greater ferroptotic cell death than that elicited by BNP@R alone, thus suggesting that IFN-γ-induced GSH bleaching promotes lipid peroxidation (**Figure 4b-c**). In vitro results have also confirmed that BNP@R induces PDT-based ICD,

and the lipid peroxidation generated by both the PDT and ferroptosis cumulatively promotes this ICD effect (**Figure 4d**). In vivo, the ability of BNP@R to promote intra-tumoral infiltration of CD8$^+$ T lymphocytes has also been validated (**Figure 4e**). In addition, the nanoparticles developed in combination with PD-L1 blockade further enhance tumor immune response and inhibit metastasis (**Figure 4f-h**).

Although the combination of ferroptosis and PDT-induced immunotherapy holds promise for long-term tumor control, several issues remain to be resolved. The inflammatory response of phagocytes may inhibit anti-tumor immunity and stimulate tumor growth [53]. Thus, further research is required to elucidate the distinct roles of both in tumor immunity. Few known PSs induce potent ICD, except for hypericin, 5-aminolevulinic acid, Rose Bengal (RB) acetate, and glycoconjugated chlorin; therefore, new PSs that induce ICD must be developed. To this end, Turubanova and colleagues have synthesized two
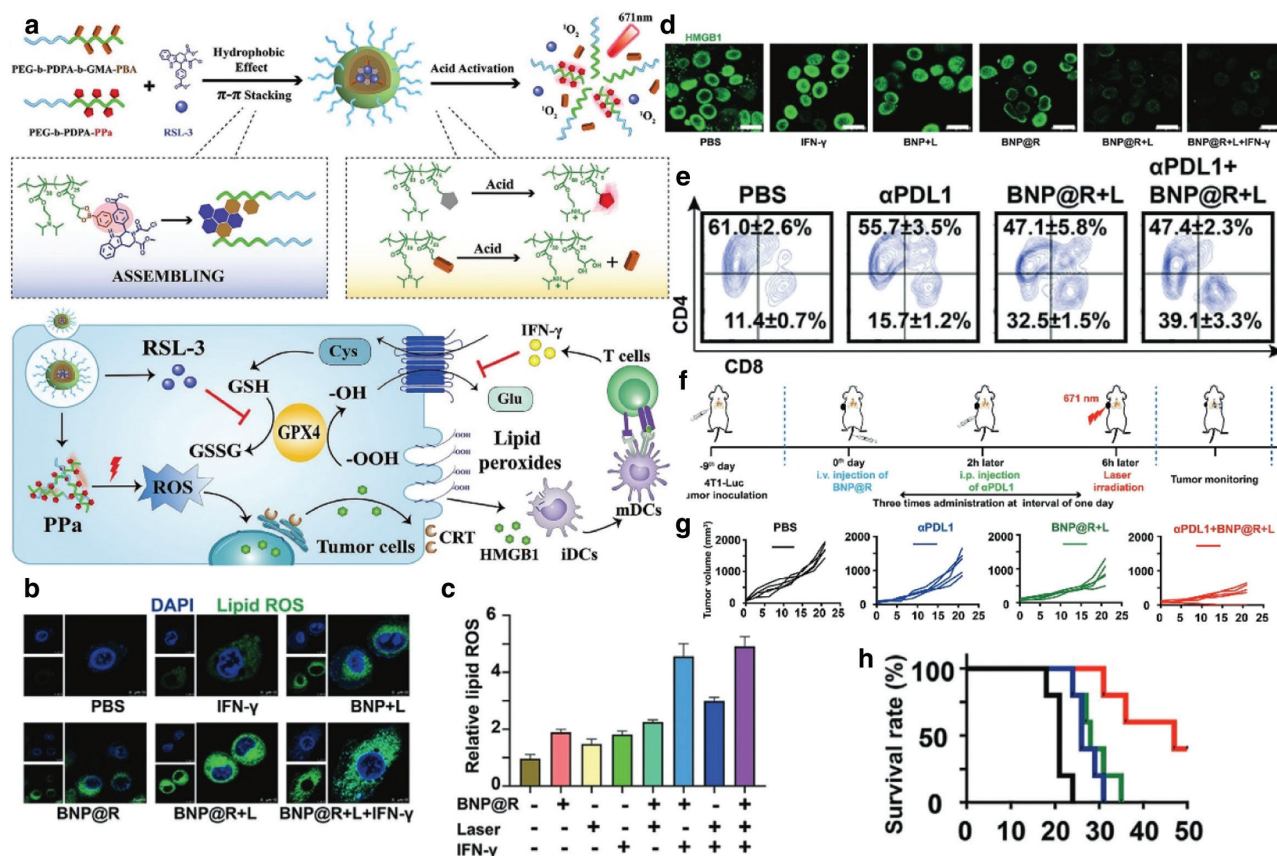

Figure 4 | (a) Schematic diagram of the fabrication and anticancer mechanism of BNP@R. **(b)** CLSM examination and **(c)** flow cytometric analysis of PDT and GPX4-inhibition-induced intracellular accumulation of lipid peroxides in B16-F10 tumor cells in vitro with a BODIPY-C11 fluorescent probe. **(d)** Flow cytometric examination of ferroptosis-induced CRT expression on the surface of the of B16-F10 cell membrane in vitro. **(e)** Tumor-infiltrating CD8$^+$ and CD4$^+$ T lymphocytes in total CD3$^+$ T cells. **(f)** Treatment schedule for PD-L1 blockade and nanoparticle-mediated ferroptosis induction in mice bearing 4T1 breast tumors. **(g)** Tumor growth and **(h)** survival curves of 4T1-tumor-bearing BALB/c mice subjected to different treatments. Reproduced with permission, [16] copyright 2021 John Wiley and Sons.

cyanol porphyrazines resembling porphyrins and phthalocyanines; these tetrapyrrole macrocycles have both high photodynamic activity and strong immunogenicity [54]. Future studies may use these new PSs combined with ferroptosis to elicit enhanced tumor immune responses.

3.2.3 Erastin. In 2012, erastin was found to induce cancer cells to undergo ferroptosis, a unique form of non-apoptotic cell death [55]. Numerous studies have since demonstrated that erastin induces GSH depletion by inhibiting system x_c^- function [48, 56-58]. Zhu and colleagues have developed a self-assembling nanoplatform composed of erastin and Ce6 [19]. In comparison to single Ce6-based PDT, Ce6-erastin nanoparticles increase tumor cell ROS. Ce6-erastin nanoparticles also significantly enhance antitumor effects. In another example, Du et al. have loaded erastin and a PS (RB) into exosomes (Er/RB@Exos[CD47]) to deliver both compounds to tumor sites with high specificity [59]. Exosomes have shown effective tumor distribution via CD47, thereby eliciting a "do not eat me" signal. In addition, the promising in vivo antitumor effect of Er/RB@Exos[CD47] have been confirmed, and combining ferroptosis inducers with PSs has been verified to enable use of a lower drug dose, thereby decreasing adverse effects.

3.2.4 Dihydroartemisinin. Beyond SRF, many drugs on the market have been demonstrated to induce ferroptosis. Dihydroartemisinin (DHA), a sesquiterpene lactone compound derived from the natural plant *Artemisia annua*, downregulates the expression of GPX4 and triggers ferroptosis in lung cancer cells [60]. Han et al. have found that the oxidative stress caused by Ce6 upregulates GPX4, thus degrading ROS and leading to drug resistance [47]. However, the downregulation of GPX4 by DHA-induced ferroptosis significantly enhances PDT efficacy. In vitro results have also shown that a combination of DHA solution and Ce6 solution has good anti-lung cancer efficacy, decreasing cell viability by 3 times and increasing the apoptosis rate by 3 times, thus providing a solid theoretical foundation for the improvement of PDT anti-lung cancer efficacy with the traditional Chinese medicine DHA.

3.2.5 Buthionine sulfoximine. Glutamate-cysteine ligase (GCL) is an enzyme that mediates GSH synthesis [31]. Buthionine sulfoximine (BSO) is a GCL inhibitor that decreases GSH levels by blocking GSH synthesis. Yoo et al. have combined Ce6-loaded nanoparticles (Ce6-PEG-PLA-NPs) with BSO [61]. Laser exposure to BSO more than doubles the accumulation of Ce6 in tumor tissue at each point in time, while decreasing GSH concentrations. This treatment significantly decreases the viability of cancer cells, as shown in SCC-7 cells. In vivo results have indicated that the combination of the preparation and BSO completely inhibits tumor growth within 14 days without causing substantial changes in mouse body weight.

In general, combining small-molecule ferroptosis inducers with PDT shows promise in improving redox interference-based cancer treatments. Among the various combination strategies, we expect that RDV or exosomes mainly based on biomimetics such as Hb may achieve better therapeutic effects because of their biocompatibility and internal functions. Furthermore, strong immune responses evoked by PDT aided by ferroptosis in these studies would shed new light on multimodal therapies (PDT-ferroptosis-immunotherapy) for achieving satisfactory therapeutic effects in cancer therapy.

3.3 Combining PDT and metal-ion-induced ferroptosis

Iron ions are indispensable in many physiological processes in the human body, and are relatively safe with low toxicity. Ferroptosis is an iron-dependent cell death [62] in which iron plays two main roles: (1) Sufficient iron constitutes an active pool, which produces large amounts of hydroxyl radicals through the Fenton reaction. The hydroxyl radicals promote the formation of lipid peroxides in a non-enzymatic manner [63]; (2) The lipoxygenase family members are the main enzymes that mediate lipase oxidation, and these lipoxygenases depend on iron supply to maintain their activity [64]. Therefore, interventions affecting iron homeostasis in tumor cells and the iron-overload state are also strategies to induce ferroptosis [9]. Hence, several studies have focused on the combination of iron-ion-based ferroptosis and PDT.

A tannic acid (TA) network with a polyphenol structure has been shown to reduce Fe^{3+} to Fe^{2+} [65, 66]. Exploiting this property, Yu et al. have designed a self-assembling nanoplatform (p53/Ce6@ZF-T, **Figure 5a**) [67]. The nanoparticles are composed of a p53 plasmid-complexed Ce6-poly(amidoamine), Fe^{2+}-containing mesoporous zeolitic imidazolate framework-8, and TA. After endocytosis by tumor cells, Fe^{2+} is released from the p53/Ce6@ZF-T and reacts with local H_2O_2, thereby generating toxic •OH via the Fenton reaction. TA, with its strong reductive ability, ensures the continuous supply of Fe^{2+} from Fe^{3+}. By-products from the Fenton reaction, such as O_2, alleviate hypoxia and therefore enhance Ce6-induced PDT (**Figure 5b**). Both the Fenton reaction and PDT amplify oxidative stress, thus leading to lipid peroxidation of the plasma membrane (**Figure 5c-d**). Moreover, the complexed p53 plasmid in p53/Ce6@ZF-T decreases SLC7A11 expression and suppresses GPX4 activity, thus promoting ferroptosis (**Figure 5e**). In vitro and in vivo studies have demonstrated remarkable antitumor efficacy.

The donors of iron are mostly inorganic particles, which may not be biocompatible and may lead to safety concerns. Chen et al. have directly combined hemin and Ce6 into nanoparticles (HCNPs) through self-assembly [68]. Hemin, the prosthetic group of the Hb, is composed of proto-porphyrin and ferrous atoms. Hemin has
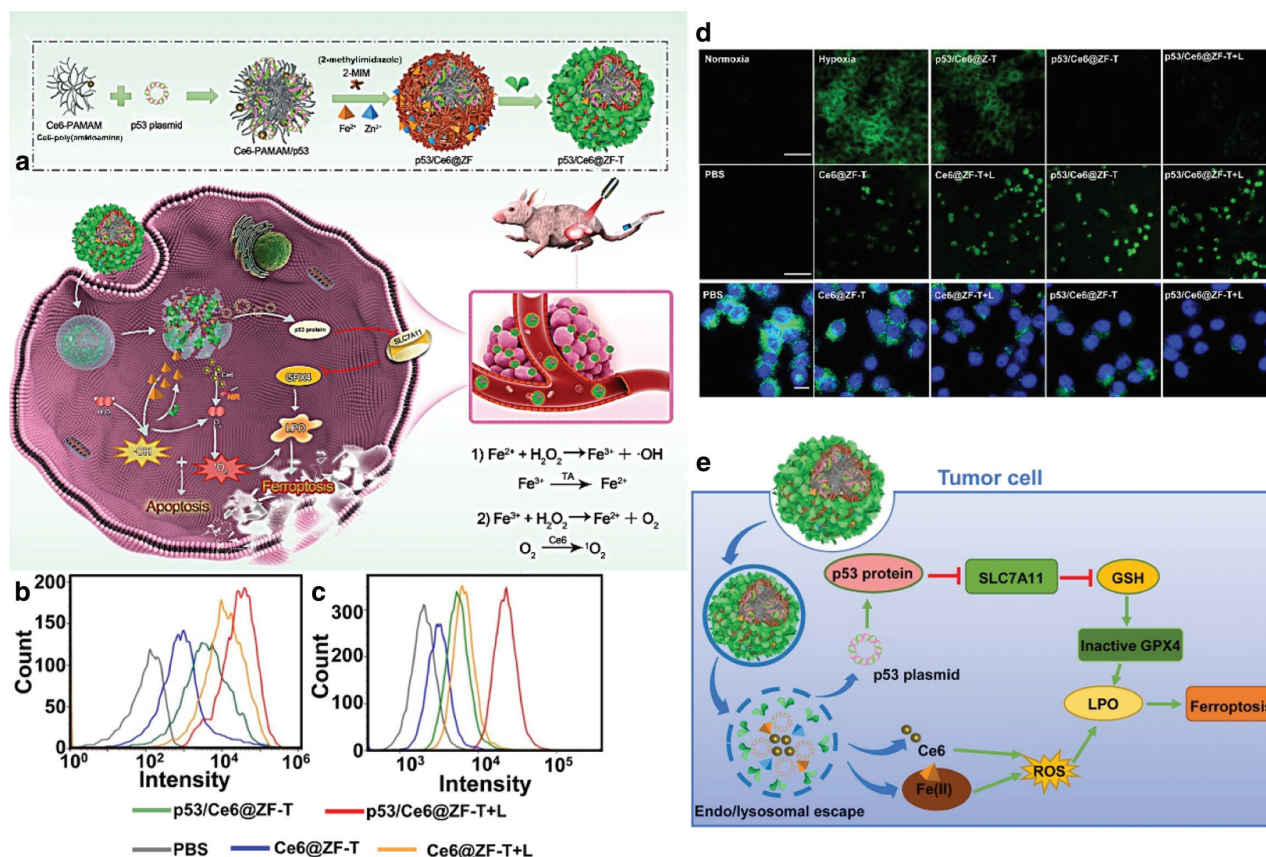
Figure 5 | (a) Schematic diagram of the fabrication and anticancer mechanism of p53/Ce6@ZF-T. **(b)** Intracellular ROS levels, determined by flow cytometry in H1299 cells with different treatments. **(c)** Intracellular LPO generation, determined by flow cytometry. **(d)** CLSM images of H1299 cells after incubation under normoxia with PBS or hypoxia with different treatments. **(e)** Illustration of the mechanism of p53/Ce6@ZF-T induced ferroptosis. Reproduced with permission, [67] copyright 2022 Elsevier.

multiple advantages, such as Fenton catalytic activity and GSH-depletion capability [69-71]. HCNPs have been validated to generate both O_2 and •OH, thus enhancing the PDT effect. In addition, HCNPs show strong GSH depletion ability, thus triggering Fe^{2+}-dependent ferroptosis. HCNPs have been found to have greater antitumor efficacy than Ce6 alone, thereby indicating their efficiency.

Some researchers have taken a different approach of using synthesis or preparation methods to directly endow iron with photodynamic properties. Yang et al. have developed a simple, rapid, and inexpensive synthetic method to make ferric ions into hydrated iron oxide (ferrihydrite) [72]. Ferrihydrite releases large amounts of Fe^{2+} under blue light irradiation, which promotes irreversible DNA fragmentation and ROS-associated ferroptosis. Similarly, Cheng et al. have used Au and Ag to fabricate Fe^{3+}-loaded nanoclusters (Fe^{3+}@Au1Ag24@PbP) [73]. Under near-infrared laser irradiation, Fe^{3+}@Au1Ag24@PbP not only achieves the combined therapeutic effects of PDT and ferroptosis, but also increases the temperature of the tumor

microenvironment and generates a photoacoustic signal.

As previously described, ferroptosis is a regulated cell death associated with lipid oxidation. Studies by Perez's group have shown that addition of polyunsaturated fatty acids induces ferroptosis in human cancer cells [74]. Given this, Liang et al. have engineered Fe_3O_4, porphyrins, and lipids into nanoparticles (Fe_3O_4@PGL NPs) [75]. They have found that extremely low doses of nanoparticles effectively destroy cancer cells, thus verifying the synergistic effects of the three substances.

The tumor microenvironment is hypoxic and mildly acidic with excess GSH and H_2O_2, thereby markedly affecting therapeutic effects [76-79]. Manganese oxide nanomaterials and their derivatives have unique properties, such as catalyzing H^+/H_2O_2 to oxygen (O_2), thereby relieving tumor hypoxia [80]. Chen et al. have used black phosphorus nanosheets (BNPs) to anchor MnO_2 nanoparticles and Fe_3O_4 nanoparticles, then coated them with M1 macrophage membranes to form an intelligent biomimetic nanoplatform [81]. Fe_3O_4 nanoparticles induce ferroptosis, and BNPs mediate PDT

under 670 nm laser irradiation, thus exhibiting synergistic antitumor effects. In addition, MnO_2 in the nanoplatform serves as an oxygen-generating reactor that enhances PDT efficacy. This combination of treatments has elicited strong anticancer effects and prolonged survival in tumor-bearing mice, through overcoming the tumor barrier (hypoxia) and enhancing M1-macrophage membrane-mediated tumor-targeting capability.

In addition to the commonly used iron, manganese, platinum, and copper, additional metal-based materials, including tellurium, molybdenum, cobalt, gallium, indium, and cerium, have been reported to save PDT-based ROS by depleting GSH and inducing ferroptosis [31]. Using metallic osmium, Lu et al. have developed an osmium-peroxygen complex (Os2) that is inactive in the dark but releases the peroxyl radical $O_2 \bullet -$ under light, even in the absence of oxygen, and converts into a cytotoxic osmium complex (Os1) [82]. In hypoxic tumors, Os1 is cytotoxic with or without radiation. Meanwhile, light-activated Os2 induces photocatalytic oxidation of endogenous 1,4-dihydro-nicotinamide adenine dinucleotide in living cancer cells, thus leading to GSH degradation, GPX4 downregulation, and lipid peroxide accumulation, and triggering ferroptosis. Moreover, in vivo studies have confirmed that Os2 effectively inhibits the growth of hypoxic solid tumors in mice. Similarly, Ke et al. have proposed an easy oxidative polymerization technique for biodegradable iridium (IrIII) coordination polymers (IrS NPs) [83]. Under light induction, IrS NPs generate singlet oxygen and superoxide anion radicals, which selectively accumulate in mitochondria, thus causing mitochondrial dysfunction and fragmentation, and triggering cell death through mixed apoptotic and ferroptotic pathways. Thus, the resistance to traditional therapeutic drugs can be overcome. This therapy has the potential to eradicate tumors. Yuan et al. have also synthesized a benzothiophene isoquinoline-derived cyclometalated iridium complex (MitoIrL2) by using iridium [84]. MitoIrL2 produce large amounts of $^1O_2$ after light irradiation, which in turn cooperate with the released iridium in inducing ferroptosis, thus providing a novel opportunity to overcome hypoxia-induced resistance in PDT.

Iron ions play key roles in inducing ferroptosis. Various types of strategies based on iron materials are widely used for ferroptosis, such as iron oxide, ferrihydrite iron-organic frameworks, and amorphous iron NPs. Unlike small-molecule ferroptosis inducers, these iron materials can be nano-sized or used as nano-carriers by themselves [85-87], thus providing advantages in combination with PDT. In addition, some iron or metal materials can serve as both ferroptosis promoters and PSs, and consequently have received increasing interest in combined anticancer therapy. However, much more research, including investigations of adverse effects and interindividual differences, will be needed before these iron/metal-based materials can be applied in clinical settings [88-91].

4. FERROPTOSIS AND PHOTODYNAMIC THERAPY COMBINED WITH OTHER ANTITUMOR THERAPY

4.1 Chemotherapy
On the basis of the tumor hypoxia microenvironment, delivery systems have been designed from different perspectives. One perspective is to provide oxygen to alleviate hypoxia. For instance, the SRF@Hb-Ce6 [49] and HCNPs [68] use hemoglobin and hemin, respectively, to provide oxygen for enhancing PDT, and iron for ferroptosis and improved biocompatibility. Another perspective exploits tumor hypoxia. Pan et al. have used PDT to aggravate the hypoxic tumor microenvironment, thus further activating the prodrug tirapazamine for chemotherapy [92]. They have constructed a nanoplatform incorporating the hypoxic-responsive prodrug tirapazamine (TPZ) into a polyvinyl pyrrolidone dispersed nanoscale metal-organic framework of Fe-TCPP (where TCPP is tetrakis (4-carboxyphenyl) porphyrin). The framework was then coated with cancer cell membranes to obtain PFTT@CM, a tumor-targeting agent (**Figure 6a**). PFTT@CM has synergetic PDT and ferroptotic effects, as detailed illustrated in the above examples, including enhanced PDT due to the oxygen-supplying and •OH-generating Fenton reaction, GSH depletion, and increased lipid peroxidation (**Figure 6b-d**). In addition, TPZ in the nanoplatform has chemotherapeutic effects. The drug loading efficiency of TPZ is 27.1 ± 7.4%. With the abundant ROS generated under laser excitation, tumor cells have been found to be more hypoxic with than without laser treatment, thus inducing TPZ activation. Additionally, TPZ causes nucleic acid damage that kills tumor cells. PFTT@CM with laser irradiation elicits greater nucleic acid damage, than TPZ alone and PFTT@CM without irradiation (**Figure 6e-i**). These results have indicated that PDT in combination with hypoxic-responsive TPZ yields superior anticancer effects. Tumor growth is significantly suppressed by PFTT@CM with laser with synergistic therapeutic strategies (ferroptosis, PDT, and chemotherapy). Beyond loading of chemotherapeutic agents into synergistic drug delivery systems, recent studies have demonstrated that certain classical chemotherapeutic drugs, such as cisplatin and doxorubicin, induce ferroptosis-like cell death [7]. For example, Fang et al. have found that DOX promotes free-iron release, thus leading to ferroptosis [93].

4.2 Photothermal therapy
PTT is a new anti-tumor therapy that uses photothermal agents to convert light energy to heat energy that kills tumor cells [94]. As a non-invasive treatment, PTT has the benefits of low toxicity, specificity, and repeatability [95]. PTT has substantial advantages over conventional tumor therapy [96]. Studies have shown that PTT enhances cellular uptake of Ce6, thus increasing the efficacy of PDT [97]. Therefore, PTT is frequently combined with PDT to enhance anti-tumor effects [98-100].
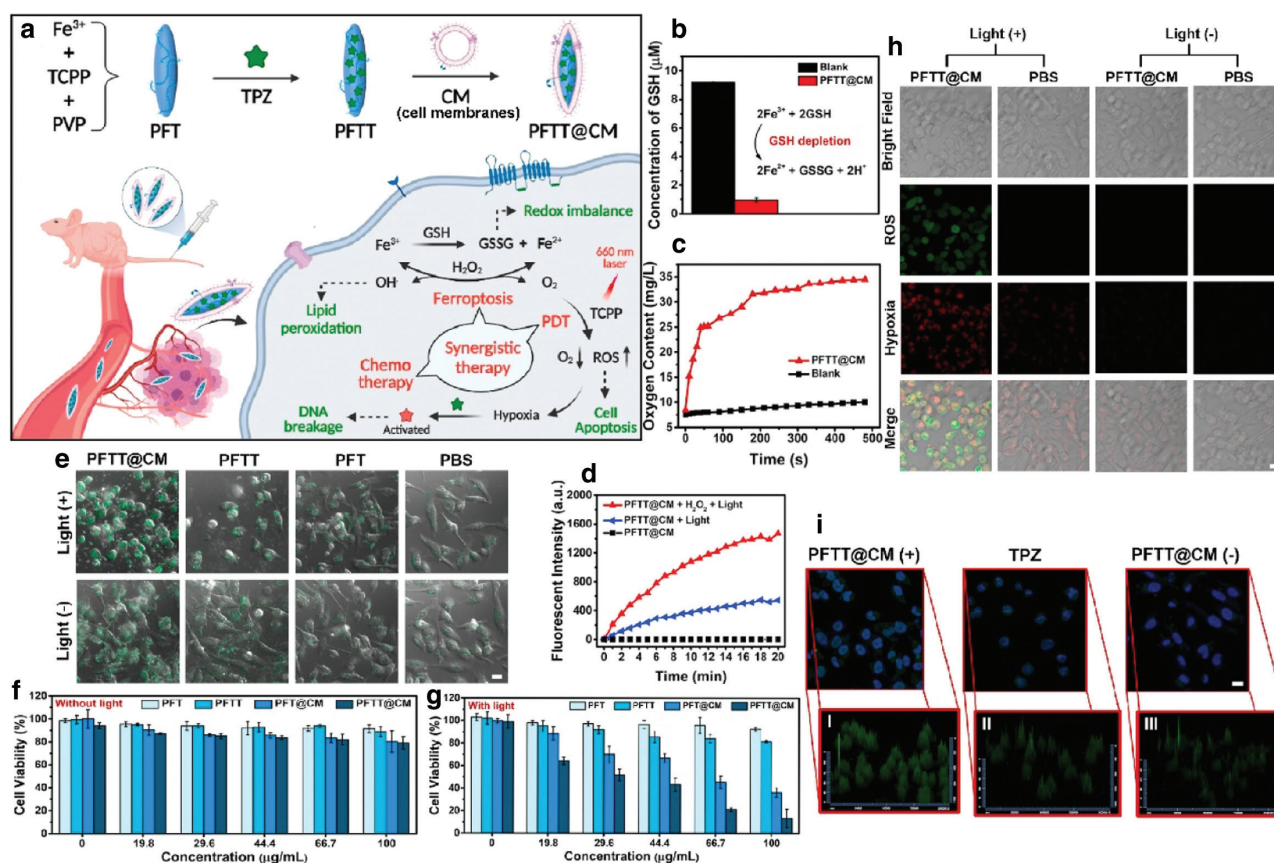
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Figure 6 | (a) Schematic diagram of the fabrication and anticancer mechanism of PFTT@CM. **(b)** GSH depletion ability of PFTT@CM, mediated by Fe^{3+}. **(c)** Time-dependent oxygen generation ability of PFTT@CM in H_2O_2 solution. **(d)** SOSG fluorescence changes in the presence of PFTT@CM + H_2O_2 + light, PFTT@CM + light, or PFTT@CM only. **(e)** Apoptosis detection of MDA-MB-231 cells after various treatments, with Annexin V-FITC as an indicator. Dark **(f)** and light **(g)** toxicities of PFT, PFTT, PFT@CM, and PFTT@CM against MDA-MB-231, measured with CCK8 assays. **(h)** ROS and hypoxia conditions of MDA-MB-231 cells treated with PFTT@CM, with PBS as a control, under light irradiation and dark. **(i)** CLSM images of MDA-MB-231 cells pretreated with PFTT@CM with (+) or without (–) light and TPZ, followed by labeling with TUNEL reagents and DAPI. Reproduced with permission, [92] copyright 2022 Elsevier.

Tao et al. have constructed a self-synergistic nanoplatform for tumor therapy [101]. They have used a silica nano-network to integrate the nuclear factor erythroid 2-associated factor (Nrf2) inhibitor brusatol, manganese dioxide, and the PS Ce6 in the nanoparticles, which were coated with folate (PEG-FA)-modified polydopamine (PDA) (brusatol/silica@MnO_2/Ce6@PDA-PEG-FA, BSMCPF). Ce6-mediated PDT induces high levels of ROS and stimulates Nrf2 expression, thus triggering antioxidant and antipyretic defense. Brusatol inhibits the activation of the Nrf2 pathway, thus inactivating GPX4 and ferritin heavy chain (FTH), enhancing PDT, and inducing ferroptosis. Compared with nanoparticles without brusatol, BSMCPF yields significantly higher levels of lipoxidation products and Fe^{2+}. In addition, the PDA in the nanoparticles shows market PTT effects. BSMCPF has been found to increase the temperature in tumor tissues from 31 to 52 °C within 10 min. This nanoplatform

exhibits promising antitumor effects with combinations of PDT, PTT and ferroptosis, and shows promise against hypoxia- and hyperthermia-associated resistance in anticancer treatments.

4.3 Sonodynamic therapy

The non-invasive treatment modality ultrasound (US)-triggered sonodynamic therapy (SDT) overcomes the barrier of the low tissue penetration depth of PDT [102]. Gan et al. have constructed a manganese porphyrin-based metal-organic framework (Mn-MOF), which triggers the release of oxygen (O_2), enhances PDT, and decreases intracellular GSH, thereby inducing ferroptosis through US [103]. A study has confirmed that porphyrin-based MOFs can be designed for efficient PSs in PDT, owing to their to high PS loading capacity and an absence of self-quenching. Mn-MOFs inhibit tumor growth and metastasis. In addition, Mn-MOFs effectively reshape

the tumor immune microenvironment by increasing the number of activated CD8+ T cells and mature DCs, and decreasing the numbers of myeloid-derived suppressor cells in tumor tissues.

Combining multiple therapeutic approaches into single agents to provide multi-modal delivery systems can decrease doses, avoid multidrug resistance, and achieve better efficacy [104]. However, optimizing the ratios of different drugs remains a challenge. The different metabolic rates of different types of drugs in different patients lead to complex pharmacodynamic/pharmacokinetic relationships, which require extensive clinical studies and leads to higher costs [105, 106].

5. CONCLUSION AND OUTLOOK

In conclusion, the combination of ferroptosis and PDT for cancer treatment has become a hotspots in the field of cancer therapy. Here, we summarized several strategies for ferroptosis combined with PDT, including mono-PDT and PDT-induced ferroptosis, combining PDT with small-molecule ferroptosis inducers, and combining PDT with metal-ion-induced ferroptosis. These examples demonstrate that PDT and ferroptosis have mutually reinforcing effects with high antitumor efficacy and safety, thereby providing a promising strategy for modern cancer treatment. Furthermore, we have presented other antitumor therapies in combination with these two treatments.

Studies have shown promising results in vitro and in vivo, but the design of drug delivery systems remains challenging [48]. First, the in-depth mechanisms of combined PDT and ferroptosis anticancer therapies must be further uncovered through various biochemical technologies, to provide a foundation for the future design of more effective and specific delivery systems. Second, although PDT is a controllable therapy with minimal invasiveness and adverse effects, ferroptosis may pose a risk of normal cell/tissue damage. Hence, developing combined delivery systems with tumor targeting ability for specific tumor microenvironments will be crucial. Third, significant differences exist between animal models and humans [107, 108], and further research on the correlations between them is required. Advances in technology such as organ-on-a-chip or organoids may be promising for evaluating these complicated synergistic strategies [109-111]. In addition, most studies have focused on the combined influence of PDT and ferroptosis on intracellular redox homeostasis. However, their ability to induce the tumor immune response makes these therapies attractive for treating cancer metastasis [112]; further studies are needed to provide detailed insights into their immunogenicity. Finally, because combination treatments contain several elements and must introduce different carriers, the safety problems of carriers cannot be overlooked. In addition to conventional methods, recent advances in microfluidic-assisted chemical synthesis and bioassays, as well as artificial-intelligence

systems that improve design hypotheses through feedback analysis, will accelerate carrier screening and the development of preparations [104, 105, 113]. Therefore, further research will be needed to investigate synergistic therapies to develop formulations with superior efficacy and fewer toxic adverse effects.

COMPETING FINANCIAL INTERESTS

The authors declare no competing interests.

REFERENCES

[1] Bermejo AV, Ragonnaud E, Daradoumis J, Holst P: Cancer Associated Endogenous Retroviruses: Ideal Immune Targets for Adenovirus-Based Immunotherapy. *International Journal of Molecular Sciences* 2020, 21:4843.

[2] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A: Global Cancer Statistics, 2012. *CA: A Cancer Journal For Clinicians* 2015, 65:87–108.

[3] Ganesh K, Massagué J: Targeting Metastatic Cancer. *Nature Medicine* 2021, 27:34–44.

[4] Ward RA, Fawell S, Floc'h N, Flemington V, McKerrecher D, Smith PD: Challenges and Opportunities in Cancer Drug Resistance. *Chemical Reviews* 2021, 121:3297–3351.

[5] Chen X, Kang R, Kroemer G, Tang D: Broadening Horizons: The Role of Ferroptosis in Cancer. *Nature Reviews Clinical Oncology* 2021, 18:280–296.

[6] Gao M, Deng J, Liu F, Fan A, Wang Y, Wu H, et al.: Triggered Ferroptotic Polymer Micelles for Reversing Multidrug Resistance to Chemotherapy. *Biomaterials* 2019, 223:119486.

[7] Angeli JPF, Krysko DV, Conrad M: Ferroptosis at the Crossroads of Cancer-Acquired Drug Resistance and Immune Evasion. *Nature Reviews Cancer* 2019, 19:405–414.

[8] Rodriguez R, Schreiber SL, Conrad M: Persister Cancer Cells: Iron Addiction and Vulnerability to Ferroptosis. *Molecular Cell* 2022, 82:728–740.

[9] Hassannia B, Vandenabeele P, Vanden Berghe T: Targeting Ferroptosis to Iron Out Cancer. *Cancer Cell* 2019, 35: 830–849.

[10] Meng Z, Zhou X, Xu J, Han X, Dong Z, Wang H, et al.: Light-Triggered In Situ Gelation to Enable Robust Photodynamic-Immunotherapy by Repeated Stimulations. *Advanced Materials (Deerfield Beach, Fla)* 2019, 31:e1900927.

[11] Huang JQ, Zhao LP, Zhou X, Liu LS, Zheng RR, Deng FA, et al.: Carrier Free O -Economizer for Photodynamic Therapy Against Hypoxic Tumor by Inhibiting Cell Respiration. *Small (Weinheim an Der Bergstrasse, Germany)* 2022, 18: e2107467.

[12] Wang S, Yu G, Yang W, Wang Z, Jacobson O, Tian R, et al.: Photodynamic-Chemodynamic Cascade Reactions for Efficient Drug Delivery and Enhanced Combination Therapy. *Advanced Science (Weinheim, Baden-Wurttemberg, Germany)* 2021, 8: 2002927.

[13] Xu X, Deng G, Sun Z, Luo Y, Liu J, Yu X, et al.: A Biomimetic Aggregation-Induced Emission Photosensitizer with

Antigen-Presenting and Hitchhiking Function for Lipid Droplet Targeted Photodynamic Immunotherapy. *Advanced Materials (Deerfield Beach, Fla)* 2021, 33:e2102322.

[14] Alzeibak R, Mishchenko TA, Shilyagina NY, Balalaeva IV, Vedunova MV, Krysko DV: Targeting Immunogenic Cancer Cell Death by Photodynamic Therapy: Past, Present and Future. *Journal For Immunotherapy of Cancer* 2021, 9: e001926.

[15] Donohoe C, Senge MO, Arnaut LG, Gomes-da-Silva LC: Cell Death in Photodynamic Therapy: From Oxidative Stress to Anti-Tumor Immunity. *Biochimica Et Biophysica Acta Reviews On Cancer* 2019, 1872:188308.

[16] Song R, Li T, Ye J, Sun F, Hou B, Saeed M, et al.: Acidity-Activatable Dynamic Nanoparticles Boosting Ferroptotic Cell Death for Immunotherapy of Cancer. *Advanced Materials (Deerfield Beach, Fla)* 2021, 33:e2101155.

[17] Hou Y, Fu Q, Kuang Y, Li D, Sun Y, Qian Z, et al.: Unsaturated Fatty Acid-Tuned Assembly of Photosensitizers for Enhanced Photodynamic Therapy via Lipid Peroxidation. *Journal of Controlled Release: Official Journal of the Controlled Release Society* 2021, 334:213–223.

[18] Wu H, Wang Y, Tong L, Yan H, Sun Z: Global Research Trends of Ferroptosis: A Rapidly Evolving Field With Enormous Potential. *Frontiers In Cell and Developmental Biology* 2021, 9:646311.

[19] Zhu T, Shi L, Yu C, Dong Y, Qiu F, Shen L, et al.: Ferroptosis Promotes Photodynamic Therapy: Supramolecular Photosensitizer-Inducer Nanodrug for Enhanced Cancer Treatment. *Theranostics* 2019, 9:3293–3307.

[20] Shui S, Zhao Z, Wang H, Conrad M, Liu G: Non-Enzymatic Lipid Peroxidation Initiated by Photodynamic Therapy Drives a Distinct Ferroptosis-Like Cell Death Pathway. *Redox Biology* 2021, 45:102056.

[21] Liu Z, Xie Z, Li W, Wu X, Jiang X, Li G, et al.: Photodynamic Immunotherapy of Cancers Based on Nanotechnology: Recent Advances and Future Challenges. *Journal of Nanobiotechnology* 2021, 19:160.

[22] Xie J, Wang Y, Choi W, Jangili P, Ge Y, Xu Y, et al.: Overcoming Barriers in Photodynamic Therapy Harnessing Nano-Formulation Strategies. *Chemical Society Reviews* 2021, 50:9152–9201.

[23] Cheung EC, Vousden KH: The Role of ROS in Tumour Development and Progression. *Nature Reviews Cancer* 2022, 22:280–297.

[24] Cheung EC, DeNicola GM, Nixon C, Blyth K, Labuschagne CF, Tuveson DA, et al.: Dynamic ROS Control by TIGAR Regulates the Initiation and Progression of Pancreatic Cancer. *Cancer Cell* 2020, 37:168–182.

[25] Poprac P, Jomova K, Simunkova M, Kollar V, Rhodes CJ, Valko M: Targeting Free Radicals in Oxidative Stress-Related Human Diseases. *Trends In Pharmacological Sciences* 2017, 38:592–607.

[26] Rossi R, Dalle-Donne I, Milzani A, Giustarini D: Oxidized forms of Glutathione in Peripheral Blood as Biomarkers of Oxidative Stress. *Clinical Chemistry* 2006, 52:1406–1414.

[27] Cheng X, Xu HD, Ran HH, Liang G, Wu FG: Glutathione-Depleting Nanomedicines for Synergistic Cancer Therapy. *ACS Nano* 2021, 15:8039–8068.

[28] Cui Q, Wang JQ, Assaraf YG, Ren L, Gupta P, Wei L, et al.: Modulating ROS to Overcome Multidrug Resistance in Cancer. *Drug Resistance Updates: Reviews and Commentaries In Antimicrobial and Anticancer Chemotherapy* 2018, 41:1–25.

[29] Min H, Wang J, Qi Y, Zhang Y, Han X, Xu Y, et al.: Biomimetic Metal-Organic Framework Nanoparticles for Cooperative Combination of Antiangiogenesis and Photodynamic Therapy for Enhanced Efficacy. *Advanced Materials (Deerfield Beach, Fla)* 2019, 31:e1808200.

[30] Desideri E, Ciccarone F, Ciriolo MR: Targeting Glutathione Metabolism: Partner in Crime in Anticancer Therapy. *Nutrients* 2019, 11:1926.

[31] Niu B, Liao K, Zhou Y, Wen T, Quan G, Pan X, et al.: Application of Glutathione Depletion in Cancer Therapy: Enhanced ROS-Based Therapy, Ferroptosis, and Chemotherapy. *Biomaterials* 2021, 277:121110.

[32] Muri J, Kopf M: Redox Regulation of Immunometabolism. *Nature Reviews Immunology* 2021, 21:363–381.

[33] Rouhier N, Lemaire SD, Jacquot JP: The Role of Glutathione in Photosynthetic Organisms: Emerging Functions for Glutaredoxins and Glutathionylation. *Annual Review of Plant Biology* 2008, 59:143–166.

[34] Bersuker K, Hendricks JM, Li Z, Magtanong L, Ford B, Tang PH, et al.: The CoQ Oxidoreductase FSP1 Acts Parallel to GPX4 to Inhibit Ferroptosis. *Nature* 2019, 575:688–692.

[35] Chen X, Li J, Kang R, Klionsky DJ, Tang D: Ferroptosis: Machinery and Regulation. *Autophagy* 2021, 17:2054–2081.

[36] Mishchenko TA, Balalaeva IV, Vedunova MV, Krysko DV: Ferroptosis and Photodynamic Therapy Synergism: Enhancing Anticancer Treatment. *Trends In Cancer* 2021, 7:484–487.

[37] Homma T, Kobayashi S, Fujii J: Induction of Ferroptosis by Singlet Oxygen Generated from Naphthalene Endoperoxide. *Biochemical and Biophysical Research Communications* 2019, 518:519–525.

[38] Santos AFD, Inague A, Arini GS, Terra LF, Wailemann RAM, Pimentel AC, et al.: Distinct Photo-Oxidation-Induced Cell Death Pathways Lead to Selective Killing of Human Breast Cancer Cells. *Cell Death & Disease* 2020, 11:1070.

[39] Vadarevu H, Juneja R, Lyles Z, Vivero-Escoto JL: Light-Activated Protoporphyrin IX-Based Polysilsesquioxane Nanoparticles Induce Ferroptosis in Melanoma Cells. *Nanomaterials (Basel, Switzerland)* 2021, 11:2324.

[40] Li Y, Zhang R, Wan Q, Hu R, Ma Y, Wang Z, et al.: Trojan Horse-Like Nano-AIE Aggregates Based on Homologous Targeting Strategy and Their Photodynamic Therapy in Anticancer Application. *Advanced Science (Weinheim, Baden-Wurttemberg, Germany)* 2021, 8:e2102561.

[41] Zhang ZJ, Huang YP, Li XX, Liu ZT, Liu K, Deng XF, et al.: A Novel Ferroptosis-Related 4-Gene Prognostic Signature for Cholangiocarcinoma and Photodynamic Therapy. *Frontiers In Oncology* 2021, 11:747445.

[42] Wang Y, Zhang L, Zhao G, Zhang Y, Zhan F, Chen Z, et al.: Homologous Targeting Nanoparticles for Enhanced PDT Against Osteosarcoma HOS Cells and the Related Molecular Mechanisms. *Journal of Nanobiotechnology* 2022, 20:83.

[43] Guo X, Yu H, Shen W, Cai R, Li Y, Li G, et al.: Synthesis and Biological Evaluation of NO-donor Containing Photosensitizers to Induce Ferroptosis of Cancer Cells. *Bioorganic Chemistry* 2021, 116:105355.

[44] Deng Y, Jia F, Chen S, Shen Z, Jin Q, Fu G, et al.: Nitric Oxide as an All-Rounder for Enhanced Photodynamic Therapy: Hypoxia Relief, Glutathione Depletion and Reactive Nitrogen Species Generation. *Biomaterials* 2018, 187:55–65.

[45] Xiong Y, Xiao C, Li Z, Yang X: Engineering Nanomedicine for Glutathione Depletion-Augmented Cancer Therapy. *Chemical Society Reviews* 2021, 50:6013–6041.

[46] Zhong Y, Zhang X, Yang L, Liang F, Zhang J, Jiang Y, et al.: Hierarchical Dual-Responsive Cleavable Nanosystem for Synergetic Photodynamic/Photothermal Therapy Against Melanoma. *Materials Science & Engineering C, Materials For Biological Applications* 2021, 131:112524.

[47] Han N, Li LG, Peng XC, Ma QL, Yang ZY, Wang XY, et al.: Ferroptosis Triggered by Dihydroartemisinin Facilitates Chlorin e6 Induced Photodynamic Therapy Against Lung Cancerthrough Inhibiting GPX4 and Enhancing ROS. *European Journal of Pharmacology* 2022, 919:174797.

[48] Zaffaroni N, Beretta GL: Nanoparticles for Ferroptosis Therapy in Cancer. *Pharmaceutics* 2021, 13:1785.

[49] Xu T, Ma Y, Yuan Q, Hu H, Hu X, Qian Z, et al.: Enhanced Ferroptosis by Oxygen-Boosted Phototherapy Based on a 2-in-1 Nanoplatform of Ferrous Hemoglobin for Tumor Synergistic Therapy. *ACS Nano* 2020, 14:3414–3425.

[50] Liu W, Zhang J, Ding L, Ni W, Yuan J, Xiao H, et al.: RBC-Derived Nanosystem with Enhanced Ferroptosis Triggered by Oxygen-Boosted Phototherapy for Synergized Tumor Treatment. *Biomaterials Science* 2021, 9:7228–7236.

[51] Wang X, Wu M, Zhang X, Li F, Zeng Y, Lin X, et al.: Hypoxia-Responsive Nanoreactors Based on Self-Enhanced Photodynamic Sensitization and Triggered Ferroptosis for Cancer Synergistic Therapy. *Journal of Nanobiotechnology* 2021, 19:204.

[52] Li J, Liu J, Xu Y, Wu R, Chen X, Song X, et al.: Tumor Heterogeneity in Autophagy-Dependent Ferroptosis. *Autophagy* 2021, 17:3361–3374.

[53] Valashedi MR, Najafi-Ghalehlou N, Nikoo A, Bamshad C, Tomita K, Kuwahara Y, et al.: Cashing in on Ferroptosis Against Tumor Cells: Usher in the Next Chapter. *Life Sciences* 2021, 285:119958.

[54] Turubanova VD, Mishchenko TA, Balalaeva IV, Efimova I, Peskova NN, Klapshina LG, et al.: Novel Porphyrazine-Based Photodynamic Anti-Cancer Therapy Induces Immunogenic Cell Death. *Scientific Reports* 2021, 11:7205.

[55] Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, et al.: Ferroptosis: An Iron-Dependent form of Nonapoptotic Cell Death. *Cell* 2012, 149:1060–1072.

[56] Wang L, Liu Y, Du T, Yang H, Lei L, Guo M, et al.: ATF3 Promotes Erastin-Induced Ferroptosis by Suppressing System Xc. *Cell Death and Differentiation* 2020, 27:662–675.

[57] Koppula P, Zhuang L, Gan B: Cystine Transporter SLC7A11/xCT in Cancer: Ferroptosis, Nutrient Dependency, and Cancer Therapy. *Protein & Cell* 2021, 12:599–620.

[58] Ghoochani A, Hsu EC, Aslan M, Rice MA, Nguyen HM, Brooks JD, et al.: Ferroptosis Inducers Are a Novel Therapeutic Approach for Advanced Prostate Cancer. *Cancer Research* 2021, 81:1583–1594.

[59] Du J, Wan Z, Wang C, Lu F, Wei M, Wang D, et al.: Designer Exosomes for Targeted and Efficient Ferroptosis Induction in Cancer Via Chemo-Photodynamic Therapy. *Theranostics* 2021, 11:8185–8196.

[60] Chen GQ, Benthani FA, Wu J, Liang D, Bian ZX, Jiang X: Artemisinin Compounds Sensitize Cancer Cells to Ferroptosis by Regulating Iron Homeostasis. *Cell Death and Differentiation* 2020, 27:242–254.

[61] Yoo J, Jang SY, Park C, Lee D, Kwon S, Koo H: Lowering Glutathione Level by Buthionine Sulfoximine Enhances in Vivo Photodynamic Therapy Using Chlorin e6-Loaded Nanoparticles. *Dyes and Pigments* 2020, 176:9.

[62] Jiang X, Stockwell BR, Conrad M: Ferroptosis: Mechanisms, Biology and Role in Disease. *Nature Reviews Molecular Cell Biology* 2021, 22:266–282.

[63] Yan B, Ai Y, Sun Q, Ma Y, Cao Y, Wang J, et al.: Membrane Damage during Ferroptosis Is Caused by Oxidation of Phospholipids Catalyzed by the Oxidoreductases POR and CYB5R1. *Molecular Cell* 2021, 81:355–369.

[64] Yang WS, Kim KJ, Gaschler MM, Patel M, Shchepinov MS, Stockwell BR: Peroxidation of Polyunsaturated Fatty Acids by Lipoxygenases Drives Ferroptosis. *Proceedings of the National Academy of Sciences of the United States of America* 2016, 113:E4966–E4975.

[65] Liu T, Liu W, Zhang M, Yu W, Gao F, Li C, et al.: Ferrous-Supply-Regeneration Nanoengineering for Cancer-Cell-Specific Ferroptosis in Combination with Imaging-Guided Photodynamic Therapy. *ACS Nano* 2018, 12:12181–12192.

[66] Zhang L, Wan SS, Li CX, Xu L, Cheng H, Zhang XZ: An Adenosine Triphosphate-Responsive Autocatalytic Fenton Nanoparticle for Tumor Ablation with Self-Supplied HO and Acceleration of Fe(III)/Fe(II) Conversion. *Nano Letters* 2018, 18:7609–7618.

[67] Yu M, Yu J, Yi Y, Chen T, Yu L, Zeng W, et al.: Oxidative Stress-Amplified Nanomedicine for Intensified Ferroptosis-Apoptosis Combined Tumor Therapy. *Journal of Controlled Release: Official Journal of the Controlled Release Society* 2022, 347:104–114.

[68] Chen J, Chen F, Zhang L, Yang Z, Deng T, Zhao Y, et al.: Self-Assembling Porphyrins as a Single Therapeutic Agent for Synergistic Cancer Therapy: A One Stone Three Birds Strategy. *ACS Applied Materials & Interfaces* 2021, 13:27856–27867.

[69] Wang Y, Shi L, Ye Z, Guan K, Teng L, Wu J, et al.: Reactive Oxygen Correlated Chemiluminescent Imaging of a Semiconducting Polymer Nanoplatform for Monitoring Chemodynamic Therapy. *Nano Letters* 2020, 20:176–183.

[70] Robinson SR, Dang TN, Dringen R, Bishop GM: Hemin Toxicity: A Preventable Source of Brain Damage Following Hemorrhagic Stroke. *Redox Report: Communications In Free Radical Research* 2009, 14:228–235.

[71] Xuan W, Xia Y, Li T, Wang L, Liu Y, Tan W: Molecular Self-Assembly of Bioorthogonal Aptamer-Prodrug Conjugate Micelles for Hydrogen Peroxide and pH-Independent Cancer Chemodynamic Therapy. *Journal of the American Chemical Society* 2020, 142:937–944.

[72] Yang Y, Tian Q, Wu S, Li Y, Yang K, Yan Y, et al.: Blue Light-Triggered Fe-Release from Monodispersed Ferrihydrite Nanoparticles for Cancer Iron Therapy. *Biomaterials* 2021, 271:120739.

[73] Cheng H, Wang X, Liu X, Wang X, Wen H, Cheng Y, et al.: An Effective NIR Laser/Tumor-Microenvironment Co-Responsive Cancer Theranostic Nanoplatform with Multi-Modal Imaging and Therapies. *Nanoscale* 2021, 13:10816–10828.

[74] Perez MA, Magtanong L, Dixon SJ, Watts JL: Dietary Lipids Induce Ferroptosis in Caenorhabditiselegans and Human Cancer Cells. *Developmental Cell* 2020, 54:447–454.

[75] Liang X, Chen M, Bhattarai P, Hameed S, Tang Y, Dai Z: Complementing Cancer Photodynamic Therapy with Ferroptosis through Iron Oxide Loaded Porphyrin-Grafted Lipid Nanoparticles. *ACS Nano* 2021, 15:20164–20180.

[76] Catalano V, Turdo A, Di Franco S, Dieli F, Todaro M, Stassi G: Tumor and its microenvironment: a synergistic interplay. *Seminars In Cancer Biology* 2013, 23:522–532.

[77] Li S, Zhang W, Xue H, Xing R, Yan X: Tumor microenvironment-oriented adaptive nanodrugs based on peptide self-assembly. *Chemical Science* 2020, 11:8644–8656.

[78] Yang G, Ji J, Liu Z: Multifunctional MnO Nanoparticles for Tumor Microenvironment Modulation and Cancer Therapy. *Wiley Interdisciplinary Reviews Nanomedicine and Nanobiotechnology* 2021, 13:e1720.

[79] Yang N, Xiao W, Song X, Wang W, Dong X: Recent Advances in Tumor Microenvironment Hydrogen Peroxide-Responsive Materials for Cancer Photodynamic Therapy. *Nano-micro Letters* 2020, 12:15.

[80] Ding B, Zheng P, Ma Pa, Lin J: Manganese Oxide Nanomaterials: Synthesis, Properties, and Theranostic Applications. *Advanced Materials (Deerfield Beach, Fla)* 2020, 32:e1905823.

[81] Chen K, Li H, Zhou A, Zhou X, Xu Y, Ge H, et al.: Cell Membrane Camouflaged Metal Oxide-Black Phosphorus Biomimetic Nanocomplex Enhances Photo-chemo-dynamic Ferroptosis. *ACS Applied Materials & Interfaces* 2022, 14:26557–26570.

[82] Lu N, Deng Z, Gao J, Liang C, Xia H, Zhang P: An Osmium-Peroxo Complex for Photoactive Therapy of Hypoxic Tumors. *Nature Communications* 2022, 13:2245.

[83] Ke L, Wei F, Xie L, Karges J, Chen Y, Ji L, et al.: A Biodegradable Iridium(III) Coordination Polymer for Enhanced Two-Photon Photodynamic Therapy Using an Apoptosis-Ferroptosis Hybrid Pathway. *Angewandte Chemie (International Ed In English)* 2022, 61:e202205429.

[84] Yuan H, Han Z, Chen Y, Qi F, Fang H, Guo Z, et al.: Ferroptosis Photoinduced by New Cyclometalated Iridium(III) Complexes and Its Synergism with Apoptosis in Tumor Cell Inhibition. *Angewandte Chemie (International Ed In English)* 2021, 60:8174–8181.

[85] Hao Y, Mao L, Zhang R, Liao X, Yuan M, Liao W: Multifunctional Biodegradable Prussian Blue Analogue for Synergetic Photothermal/Photodynamic/Chemodynamic Therapy and Intrinsic Tumor Metastasis Inhibition. *ACS Applied Bio Materials* 2021, 4:7081–7093.

[86] Yao X, Yang B, Wang S, Dai Z, Zhang D, Zheng X, et al.: A Novel Multifunctional FePt/BP Nanoplatform for Synergistic Photothermal/Photodynamic/Chemodynamic Cancer Therapies and Photothermally-Enhanced Immunotherapy. *Journal of Materials Chemistry B* 2020, 8:8010–8021.

[87] Chen Q, Ma X, Xie L, Chen W, Xu Z, Song E, et al.: Iron-Based Nanoparticles for MR Imaging-Guided Ferroptosis in Combination with Photodynamic Therapy to Enhance Cancer Treatment. *Nanoscale* 2021, 13:4855–4870.

[88] Singh A, Daemen A, Nickles D, Jeon SM, Foreman O, Sudini K, et al.: NRF2 Activation Promotes Aggressive Lung Cancer and Associates with Poor Clinical Outcomes. *Clinical Cancer Research: An Official Journal of the American Association For Cancer Research* 2021, 27:877–888.

[89] Qin JJ, Cheng XD, Zhang J, Zhang WD: Dual Roles and Therapeutic Potential of Keap1-Nrf2 Pathway in Pancreatic Cancer: A Systematic Review. *Cell Communication and Signaling: CCS* 2019, 17:121.

[90] Wang F, Lv H, Zhao B, Zhou L, Wang S, Luo J, et al.: New Insights for Future Treatments. *Journal of Experimental & Clinical Cancer Research: CR* 2019, 38:406.

[91] Valdiglesias V, Fernández-Bertólez N, Kiliç G, Costa C, Costa S, Fraga S, et al.: Are Iron Oxide Nanoparticles Safe? Current Knowledge and Future Perspectives. *Journal of Trace Elements In Medicine and Biology: Organ of the Society For Minerals and Trace Elements (GMS)* 2016, 38:53–63.

[92] Pan WL, Tan Y, Meng W, Huang NH, Zhao YB, Yu ZQ, et al.: Microenvironment-Driven Sequential Ferroptosis, Photodynamic Therapy, and Chemotherapy for Targeted Breast Cancer Therapy by a Cancer-Cell-Membrane-Coated Nanoscale Metal-Organic Framework. *Biomaterials* 2022, 283:121449.

[93] Fang X, Wang H, Han D, Xie E, Yang X, Wei J, et al.: Ferroptosis as a Target for Protection Against Cardiomyopathy. *Proceedings of the National Academy of Sciences of the United States of America* 2019, 116:2672–2680.

[94] Li X, Lovell JF, Yoon J, Chen X: Clinical Development and Potential of Photothermal and Photodynamic Therapies for Cancer. *Nature Reviews Clinical Oncology* 2020, 17:657–674.

[95] Ban Q, Bai T, Duan X, Kong J: Noninvasive Photothermal Cancer Therapy Nanoplatforms via Integrating Nanomaterials and Functional Polymers. *Biomaterials Science* 2017, 5:190–210.

[96] Liu Y, Zhu X, Wei Z, Feng W, Li L, Ma L, et al.: Customized Photothermal Therapy of Subcutaneous Orthotopic Cancer by Multichannel Luminescent Nanocomposites. *Advanced Materials (Deerfield Beach, Fla)* 2021, 33:e2008615.

[97] Tian B, Wang C, Zhang S, Feng L, Liu Z: Photothermally Enhanced Photodynamic Therapy Delivered by Nano-Graphene Oxide. *ACS Nano* 2011, 5:7000–7009.

[98] Shao W, Yang C, Li F, Wu J, Wang N, Ding Q, et al.: Molecular Design of Conjugated Small Molecule Nanoparticles for Synergistically Enhanced PTT/PDT. *Nano-micro Letters* 2020, 12:147.

[99] Lan G, Ni K, Lin W: Nanoscale Metal-Organic Frameworks for Phototherapy of Cancer. *Coordination Chemistry Reviews* 2019, 379:65-81.

[100] Sun Y, Zhang Y, Gao Y, Wang P, He G, Blum NT, et al.: Six Birds with One Stone: Versatile Nanoporphyrin for Single-Laser-Triggered Synergistic Phototheranostics and Robust Immune Activation. *Advanced Materials (Deerfield Beach, Fla)* 2020, 32: e2004481.

[101] Tao W, Wang N, Ruan J, Cheng X, Fan L, Zhang P, et al.: Enhanced ROS-Boosted Phototherapy against Pancreatic Cancer Nrf2-Mediated Stress-Defense Pathway Suppression and Ferroptosis Induction. *ACS Applied Materials & Interfaces* 2022, 14:6404–6416.

[102] Qian X, Zheng Y, Chen Y: Micro/Nanoparticle-Augmented Sonodynamic Therapy (SDT): Breaking the Depth Shallow of Photoactivation. *Advanced Materials (Deerfield Beach, Fla)* 2016, 28:8097–8129.

[103] Xu Q, Zhan G, Zhang Z, Yong T, Yang X, Gan L: Manganese Porphyrin-Based Metal-Organic Framework for Synergistic Sonodynamic Therapy and Ferroptosis in Hypoxic Tumors. *Theranostics* 2021, 11:1937–1952.

[104] Fan L, Zhao S, Yang Q, Tan J, Song C, Wu H: Ternary Cocktail Nanoparticles for Sequential Chemo-Photodynamic Therapy. *Journal of Experimental & Clinical Cancer Research: CR* 2017, 36:119.

[105] Raghavendra NM, Pingili D, Kadasi S, Mettu A, Prasad SVUM: Dual or Multi-Targeting Inhibitors: The Next Generation Anticancer Agents. *European Journal of Medicinal Chemistry* 2018, 143:1277–1300.

[106] Xiong Z, Jeon M, Allaway RJ, Kang J, Park D, Lee J, et al.: Crowdsourced Identification of Multi-Target Kinase

Inhibitors for RET- and TAU- Based Disease: The Multi-Targeting Drug DREAM Challenge. *PLoS Computational Biology* 2021, 17:e1009302.

[107] Robinson NB, Krieger K, Khan FM, Huffman W, Chang M, Naik A, et al.: The Current State of Animal Models in Research: A Review. *International Journal of Surgery (London, England)* 2019, 72:9–13.

[108] Doncheva NT, Palasca O, Yarani R, Litman T, Anthon C, Groenen MAM, et al.: Human Pathways in Animal Models: Possibilities and Limitations. *Nucleic Acids Research* 2021, 49:1859–1871.

[109] Tian C, Zheng S, Liu X, Kamei K-I: Tumor-on-a-Chip Model for Advancement of Anti-Cancer Nano Drug Delivery System. *Journal of Nanobiotechnology* 2022, 20:338.

[110] Picollet-D'hahan N, Zuchowska A, Lemeunier I, Le Gac S: Multiorgan-on-a-Chip: A Systemic Approach To Model and Decipher Inter-Organ Communication. *Trends In Biotechnology* 2021, 39:788–810.

[111] Loskill P, Wu JC: Stem Cell Based Human Organ-on-a-Chip Models for Drug Discovery and Development. *Advanced Drug Delivery Reviews* 2019, 140:1–2.

[112] Turubanova VD, Balalaeva IV, Mishchenko TA, Catanzaro E, Alzeibak R, Peskova NN, et al.: Immunogenic Cell Death Induced by a New Photodynamic Therapy Based on Photosens and Photodithazine. *Journal For Immunotherapy of Cancer* 2019, 7:350.

[113] Schneider G: Automating Drug Discovery. *Nature Reviews Drug Discovery* 2018, 17:97–113.