An Efficient Magnetic Nanocatalyst Induced Chemo- and Ferroptosis Synergistic Cancer Therapy in Combination with T₁-T₂ Dual-Mode Magnetic Resonance Imaging through Doxorubicin Delivery

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Abstract

Excessive iron ions in cancer cells can catalyze H_2O_2 into highly toxic $\cdot\text{OH}$ and then promote the generation of reactive oxygen species (ROS), inducing cancer ferroptosis. However, the efficacy of ferroptosis catalyst is still insufficient because of low Fe(II) release, which severely limited its application in clinics. Herein, we developed a novel magnetic nanocatalyst for MRI-guided chemo- and ferroptosis synergistic cancer therapies through iRGD-PEG-ss-PEG modified gadolinium engineering magnetic iron oxide loaded Dox (ipGdIO-Dox). The introduction of gadolinium compound disturbed the structure of ipGdIO-Dox, making magnetic nanocatalyst be more sensitive to weak acid. When the ipGdIO-Dox entered into cancer cells, abundance of Fe(II) ions were released and then catalyzed H_2O_2 into highly toxic $\text{OH}\cdot$, which would elevate cellular oxidative-stress to damage mitochondria and cell membranes and induced cancer ferroptosis. In addition, the iRGD-PEG-ss-PEG chain coated onto nanoplatfrom were also broken by high expression of GSH, and then the Dox was released. This process not only effectively inhibited DNA replication, but further activated cellular ROS, making nanoplatfrom achieve stronger anticancer ability. Besides, the systemic delivery ipGdIO-Dox significantly enhanced T_1 - and T_2 -weighted MRI signal of tumor, endowing accurate diagnostic capability for tumor recognition. Therefore, the ipGdIO-Dox might be a promising candidate for developing MRI guided chemo- and chemodynamic synergistic theranostic system.

1. Introduction

Recently, the number of cancer patients has sharply increased, and cancer has been the second leading cause of death worldwide.¹ To date, the major means of treating cancer are surgery,² radiotherapy,^{3,4} chemical medication,⁵ and biological immunization therapy.⁶ Among these major treatments, chemotherapeutics still play an important role. Nevertheless, the clinical use of chemotherapeutic drugs has some obvious drawbacks, including their commonly induced toxicity,⁷ side effects,^{8,9} and acquired multidrug resistance (MDR),^{10–12} all of which dramatically limit the anticancer effect of chemotherapeutic drugs. In 2012, researchers found that excessive iron ions in cancer cells can catalyze H_2O_2 into highly toxic $\cdot\text{OH}$ and then promote the generation of reactive oxygen species (ROS). These ROS could cause the damage of protein, cytomembrane, and mitochondria, which accelerates the rate of the non-programmed death of cancer cells.^{13,14} This process, named ferroptosis, differs from the cell apoptosis induced by chemotherapeutic drugs because it does not lead to the acquisition of MDR. Therefore, the ferroptosis therapy might be a good approach for overcoming the current limitations of chemotherapy.

Currently, many studies on ferroptosis have been reported, and traditional ferroptotic agent mainly consist of Fe-based nanoparticles which readily release iron ions in cancer cells, such as iron oxide, ferric hydroxide and so on.^{15–17} Among abundance of Fe-based nanoparticles, magnetic iron oxide (MIO) cannot only provide iron ions to induce cancer ferroptosis, but possess excellent magnetic resonance imaging (MRI) ability because of its superparamagnetism, making MIO show huge potential application

for cancer theranostics.^{18–20} However, the unaltered MIO as ferroptotic agent and MRI contrast agent (MRICA) presents some challenges as follows: (1) the unaltered MIO has only T_2 MRI contrast ability, which is easily disturbed by calcification, bleeding, or metal deposits; (2) the catalytic activity of MIO is insufficient because of low response ability to tumor microenvironment. Therefore, it is necessary to adapt MIO through the design of structure and composition that makes them capable of high catalytic activity and multi-modal MRI.

It is routinely approach to achieve high catalytic activity through developing high-sensitive Fe-based nanomaterials to acidic tumor microenvironment (ATME). It was reported that amorphous iron nanoparticles (AFenPs) might be most efficient ferroptotic agent because iron ions were more easily released from AFenPs in the ATME compared to traditional Fe-based nanocrystal.^{21,22} In addition, Chen's group and our team found that gadolinium engineered iron oxide not only accelerated the release of iron ions, but generated abundance of oxygen vacancy defects, which significantly enhanced the catalytic efficacy of MIO, and then strengthened ferroptosis therapy.^{23,24} Although many of Fe-based ferroptotic agents have been developed, but the efficacy of ferroptosis therapy was still insufficient to induce cancer cell death because the dosage of iron ions required for ferroptosis therapy in tumor-bearing mice was very high (~ 75.0 mg iron/kg mice).²¹ Therefore, it was desired to achieve better anticancer ability through integrating chemotherapeutic drug into ferroptotic agent.

Herein, we synthesized magnetic nanocatalyst consisted of gadolinium species and MIO as ferroptotic agent and multi-modal MRICAs (GdIO) through one-pot solvent thermal method. Subsequently, the GdIO was used to load the doxorubicin (GdIO-Dox) and then the GdIO was further modified using iRGD-PEG-ss-PEG (ipGdIO-Dox). When the ipGdIO-Dox enter cancer cells, the iRGD-PEG-ss-PEG chain on the surface of ipGdIO-Dox were cleaved through depleting GSH, and then released Dox. The down-regulation of cellular GSH inactivated GPX4 expression, which further promoted ROS accumulation and then strengthened ferroptosis therapy. Meanwhile, the exposed GdIO quickly responded to ATME and then released abundance of iron ions, promoting cellular ROS generation and accelerating ferroptosis therapy. In addition, the released Dox could quickly enter nuclear, inhibit DNA replication, and then induce cancer apoptosis, which achieved the desired chemotherapy for cancer. Notably, the ipGdIO-Dox showed the excellent T_1 and T_2 relaxation rate, and its systemic delivery significantly enhanced the T_1 and T_2 signal intensity of tumor, achieved high quality of tumor MRI images, which would be beneficial to realize accurate diagnosis of cancer. Therefore, the ipGdIO-Dox might be a promising candidate for development of MRI guided chemo- and ferroptosis synergistic theranostic system.

2. Results And Discussion

2.1 Synthesis and Characterization of GdIO

The GdIO was synthesized through high temperature of solvent thermal decomposition with $\text{Fe}(\text{acac})_3$ and $\text{Gd}(\text{acac})_3$ as a precursor. As shown in Fig. 1a-d, the original MIO consisted of abundance of tiny

crystals and presented typical spherical shape with a size of about 150 nm. The high-resolution transmission electron microscope (HRTEM) image of MIO showed significant lattice fringe, and the interplanar spacing was 0.294 nm, which was assigned to (220) plane of spinel structure of magnetite. Nevertheless, after engineered by gadolinium species, the magnetic particles were etched, and showed the hollow structure, which provided enough capacity to load anticancer drug. In addition, it could be seen that the edge of GdIO transformed from spherical shape to cube, and its HRTEM images showed that there were two types of lattice fringes. The interplanar spacing in cube edge had no significant variation and was also 0.294 nm compared to MIO, indicating that the GdIO still contained the spinel structure of magnetite. It was surprising that the lattice fringe in the inner of GdIO was different with the cube edge, and its lattice fringe was 0.281 nm, which were assigned to (302) plane of $\text{Gd}(\text{OH})\text{CO}_3$ (PDF:24-0421), respectively. These results indicated that the GdIO was consisted of MIO and Gd species. Subsequently, the energy-dispersive X-ray (EDX) element mapping and line scanning analysis of GdIO confirmed the presence of Gd, Fe, O, N, and C elements, and showed that Gd and Fe species uniformly distributed into MIO. Besides, the signal of all elements in lumen region was weaker than that of edges.

DLS results indicated that the hydrodynamic size of GdIO was approximate 357 nm, which was far larger than TEM observation. This result demonstrated that the pure GdIO might be aggregation in the distilled water. Remarkably, after iRGD-PEG-ss-PEG modification, the hydrodynamic size of ipGdIO significantly decreased and had only about 160 nm, indicated that the ipGdIO had excellent colloidal stability. The X-ray diffraction (XRD) pattern was used to analyze the structure and composition of the GdIO, and the result showed that the GdIO particles mainly consisted of Fe_3O_4 and $\text{Gd}(\text{OH})\text{CO}_3$, which was consistent with HRTEM observation. In addition, the full spectra of XPS further confirmed the presence of Gd, Fe, C, N, and O elements. $\text{Fe}2\text{p}$ spectra indicated that $\text{Fe}1/2\text{p}$ and $\text{Fe}3/2\text{p}$ peaks appeared at 710.5eV and 723.3eV, respectively, which were assigned to $\text{Fe}(\text{III})$ in the GdIO.²⁵ In addition, $\text{Gd}4\text{d}$ peaks appeared at 142.6 and 157.9 eV, further confirming the presence of $\text{Gd}(\text{III})$ in GdIO.²⁶

2.2 Magnetic Property and Catalytic Activity of ipGdIO

As we known, the magnetism of particle played a key role for achieving excellent MRI performance. Based on this, the magnetic property of the GdIO was investigated through superconducting quantum interference device (SQUID). The field-dependent magnetization curves indicated that GdIO showed high saturation magnetization with about 42.5 emu/g at 300K, implying that the GdIO had strong ability to shorten T_2 relaxation time of water molecules. Notably, when at 3K, the magnetization of GdIO gradually increased with magnetic field increase, and cannot reach to saturation, this might be closely associated with the presence of Gd species. In addition, there had no significant coercive force and remanence when at 300 K, indicating that the GdIO showed excellent superparamagnetism at room temperature. These results demonstrated that the GdIO had excellent magnetic property and could be a potential candidate as MRI contrast agent.

As previously described,²⁷⁻²⁹ Fenton-catalytic efficacy mainly depended on the concentration of iron ions. Therefore, the Fe release behaviors of the GdIO were investigated under different pH conditions. As

shown in Fig. 2f, the release amount of iron ions from GdIO gradually increased with pH decrease, and the maximal release amount reached to approximate 15.2%. It was noted that the Fe release rate of GdIO was significantly quicker than that of MIO, suggesting that the GdIO had stronger response ability to ATME. Based on this, it was worth expecting that the GdIO showed high performance of Fenton-catalytic activity. Next, the Fenton-catalytic activity of the GdIO was analyzed through electron spin resonance (ESR) spectra (Fig. 2h and 2i). After the GdIO treatment, there were significant peaks (1:2:2:1) assigned to hydroxyl radicals ($\cdot\text{OH}$). Moreover, the amount of $\cdot\text{OH}$ gradually increased with the GdIO concentration increase, implying that the Fenton-catalytic activity of the GdIO was dosage-dependent. In addition, the GdIO showed stronger ability to catalyze H_2O_2 into $\cdot\text{OH}$ when compared to traditional MIO, indicating that the GdIO might be a better ferroptosis agent than traditional MIO. Because of abundance of pore structures, the Dox could be effectively loaded into GdIO (GdIO-Dox). Subsequently, the GSH-responsive polymer (iRGD-PEG-ss-PEG) was coated onto the surface of the GdIO-Dox, and then fabricated ipGdIO-Dox. In Fig. 2e, the release amount of Dox in ipGdIO-Dox gradually increased with GSH concentration increase, indicating that the Dox release of ipGdIO-Dox was GSH dependent. This result demonstrated that the iRGD-PEG-ss-PEG chain on the surface of ipGdIO-Dox not only targeted tumor cells, but effectively depleted GSH, which would be beneficial to promote Dox release and up-regulate cellular ROS.

2.3 The internalization, ROS Generation of ipGdIO in vitro

The internalization process of ipGdIO was investigated through CLSM observation. First, FITC molecules were rationally labeled into ipGdIO, and then was used to incubate cancer cells. As shown in Fig. 3a and 3c, MDA-MB-231 cells treated with pGdIO showed strong green fluorescence, indicating that pGdIO could be effectively internalized by cancer cells. Moreover, the ipGdIO-treated cells showed stronger green fluorescence than that of pGdIO, demonstrating that the targeting ligand promoted the uptake of ipGdIO. In addition, after the co-incubation with ipGdIO and amiloride, the green fluorescence of cell showed a slight decrease, indicating that the internalization of ipGdIO were partly inhibited. Remarkably, when the incubation with ipGdIO at 4 °C, the green fluorescence of cells significantly decreased. These results demonstrated that the internalization pathway of ipGdIO mainly energy- and clathrin-mediated endocytosis. Subsequently, the internalization process of ipGdIO were quantitatively analyzed through assessing Fe content in cells using ICP-MS. In Fig. 3b, it could be seen that the cell uptake of particle was dosage- and time-dependent. Notably, when treatment for 3h, the Fe content of MDA-MB-231 cells treated with ipGdIO (93.8 ng/10000cells) was higher 3-fold than that with pGdIO (23.2ng/10000cells), further confirming that ipGdIO had excellent targeting ability to MDA-MB-231 cells. In addition, after co-incubation with ipGdIO and amiloride, cellular Fe content decreased from 93.8 ng/10000cells to 85.6 ng/10000cells. Moreover, after incubation at 4 °C, the cellular Fe content further decreased to be only 60.3ng/10000cells. These results further demonstrated that the internalization of ipGdIO was realized through energy- and clathrin-mediated endocytosis, which was consistent with CLSM and flow cytometry result.

After entered cancer cells, it was very critical that whether ipGdIO activated cellular ROS. Based on this, we investigated cellular ROS level before and after ipGdIO treatment. As shown in Fig. 3d and 3e, the control group had no green fluorescence. The ultra-small iron oxide (USIO) was a classical ferroptotic agent, and was chosen as positive control. MDA-MB-231 cells treated with USIO only showed weak green fluorescence, but pGdIO had stronger green fluorescence, indicating that pGdIO had stronger ability to activate cellular ROS. In addition, when compared to pGdIO, the green fluorescence of MDA-MB-231 cells treated with ipGdIO was further strengthened, this was because the targeted ipGdIO could be more effective internalized by cancer cells, then result in more ROS generation. Remarkably, the use of either NAC (N-acetyl-L-cysteine, ROS scavenger) or DFO (Deferoxamine, ferroptosis inhibitor) alleviated cellular ROS production at different levels, suggesting that the Fe-activated ROS was closely associated with ferroptosis process.

2.4 Cytotoxicity, and Anticancer Mechanism of ipGdIO-Dox

The cellular ipGdIO-Dox not only activated excessive ROS, but released abundance of Dox. Then, they damaged mitochondria and inhibited DNA replication, resulting in cancer cell death. The cytotoxicity of ipGdIO-Dox was investigated through CCK-8 assay. As shown in Fig. 4a, all groups showed concentration-dependent effect on cell viability. In addition, the Dox seemed to have stronger inhibition ability on cell viability than pGdIO-Dox when concentration was less than 1 $\mu\text{g/mL}$. However, with concentration increase, the cytotoxicity of pGdIO-Dox was significantly stronger than Dox, this might be associated with the sustainable release of Dox from pGdIO-Dox. The ipGdIO-Dox showed the strongest cytotoxicity when compared to pGdIO-Dox and Dox, indicating that ipGdIO-Dox had the strongest anticancer ability. Notably, the use of NAC significantly decreased the cytotoxicity of ipGdIO-Dox, further suggesting that ROS-induced cancer cell death was an important pathway. In order to verify mechanism of cancer cell death, various inhibitors were used to treat cancer cells. As shown in Fig. 4b, both DFO and NAC partly recovered cell viability, confirming the presence of ROS-induced ferroptosis. In addition, the use of z-VAD also increased on cell viability, indicating that the ipGdIO-Dox induced apoptosis was also a vital approach for cancer cell death. In addition, the cycles of MDA-MB-231 cells treated with different samples were analyzed through flow cytometry. Compared to saline, all groups significantly inhibited S phase of MDA-MB-231, this was main because Dox effectively inhibited DNA replication in MDA-MB-231 cells.³⁰ Interestingly, the ipGdIO-Dox showed strongest inhibition ability on G2-M phase of MDA-MB-231, and the use of NAC could dramatically recover G2-M phase. This result indicated that high level of ROS might block G2-M phase³¹ and then induce cancer cell death.

Considering the synergistic effect of chemo- and ferroptosis therapy, some key proteins including Bcl-2, Bax, GPX4, and cleaved Caspase-3 were investigated through western blot analysis. The expression of Bcl-2 and Bax families were closely associated with the mitochondria-mediated apoptosis.^{32,33} As shown in Fig. 4c, the expression of Bcl-2 in cells treated with pGdIO-Dox significantly reduced compared to saline group, and corresponding Bax expression showed opposite result, demonstrating the presence of mitochondrial damage induced by ROS generation. After ipGdIO-Dox treatment, the expression of Bax was further up-regulated, and Bcl-2 was down-regulated, indicating that ipGdIO-Dox had the stronger

ability to damage mitochondria. In addition, the expression of cleaved caspase-3 in cells dramatically increased after ipGdIO-Dox treatment, further confirming that ipGdIO-Dox had the strongest ability to kill cancer cells. In addition, GPX4 protein could effectively eliminate cellular ROS through converting GSH into GSSG, and then avoid ferroptosis.^{34,35} Therefore, the expression of GPX4 was considered to be an important ferroptotic marker. In Fig. 4c, the expression of GPX4 significantly decreased after pGdIO-Dox treatment, confirming the presence of ferroptosis. Moreover, ipGdIO-Dox-treated cells showed the lowest GPX4 expression, indicating that the targeting ligand could further accelerate ferroptosis therapy. On the basis of above analysis, it could be concluded that ipGdIO-Dox effectively induced cancer cell death through the synergistic effect of chemo- and ferroptosis therapy.

2.5 Antitumor Activity, Biodistribution, and Pharmacokinetics of ipGdIO-Dox

The excellent performance of ipGdIO-Dox *in vitro* encouraged us to further explore its anticancer ability *in vivo*. Cancer-bearing mice were intravenously injected with ipGdIO-Dox at a dosage of 2 mg/kg, and then tumor volumes of mice were recorded. As shown in Fig. 5a and 5b, Dox showed significant inhibition on tumor volume when compared to saline group. In addition, the anticancer ability of pGdIO-Dox had a slight enhancement when compared to free Dox, which could be attributed to the synergistic effect of chemo- and ferroptosis therapy. It was noted that the ipGdIO-Dox showed the strongest tumor inhibition effect, this was because targeting ligand accelerated the ipGdIO-Dox accumulation in tumor site and then strengthened its anticancer activity. Remarkably, the use of NAC significantly weakened the anticancer effect of ipGdIO-Dox, confirming the involvement of ROS-mediated ferroptosis therapy. After treatment, the tumors of mice were excised, weighed, and then photographed. As shown in Fig. 5c, the tumor weight of mice injected with ipGdIO-Dox was the smallest, and had only 0.23 g when compared to other groups. In addition, the tumor weight of mice significant increased after NAC and ipGdIO-Dox co-injection. Meanwhile, the photos of tumor also showed the consistent result with tumor volume and tumor weight (Fig. 5e). These results demonstrated that ipGdIO-Dox could effectively inhibit tumor growth through synergistic action of chemo- and ferroptosis therapy, and be a promising candidate in clinical use.

At the end of treatment, the part of tumor tissues were cut into slices and then further analyzed through H&E staining and IHC staining. As shown in Fig. 5h, the necrotic areas of tumor tissues were as follows: Saline < ipGdIO-Dox + NAC < Dox < pGdIO-Dox < ipGdIO-Dox. In addition, IHC staining analysis showed the expression of apoptotic protein, and the order was as follows: Saline < ipGdIO-Dox + NAC < Dox < pGdIO-Dox < ipGdIO-Dox. These result further demonstrated that ipGdIO-Dox had the strongest anticancer ability. The body weight of mice were recorded, and the results indicated that all groups maintained a normal increase on body weight, and no significant difference was observed (Fig. 5d). The vital organs of mice in all groups including the heart, liver, spleen, lung, and kidney were also cut, and then analyzed through H&E staining. All groups had no significant pathological change (Figure S2). These results demonstrated that the ipGdIO-Dox had low side effects on the body. Besides, the biosafety of ipGdIO-Dox was also an important consideration for further clinical use. Therefore, the biodistribution and pharmacokinetics of ipGdIO were further investigated as shown in Fig. 5f and 5g. It could be seen that pGdIO-Dox and ipGdIO-

Dox mainly accumulated in liver and kidney tissue, this might be because the liver and kidney had abundance of reticuloendothelial system. Notably, the tumor accumulation of ipGdIO-Dox was significantly higher than that of pGdIO-Dox, confirming that ipGdIO-Dox had effective targeting ability to tumor. The pharmacokinetics curves indicated that the blood circulation time of Dox was very short and the half-time had only approximate 0.82 h. Nevertheless, the blood circulation time of pGdIO-Dox significantly increased when compared to free Dox. Notably, the ipGdIO-Dox showed the longest blood circulation time, which would be beneficial to promote the tumor accumulation of particles. Meanwhile, at post-injection 48 h, the ipGdIO-Dox could be completely excreted out from body, which decreased the potential risk for body because of long-term of accumulation. Based on above analysis, it was concluded that the ipGdIO-Dox had excellent biosafety and might be a promising candidate for further clinical use.

2.6 In Vivo Tumor MRI Diagnosis

As we mentioned earlier, the GdIO showed excellent magnetic property, which encouraged us to explore its MRI contrast performance. The T_1 and T_2 relaxation rate were very key indicator to assess the MRI contrast performance of particles, and it could be calculated via the slope of linear $1/T_i$ ($i = 1$ or 2) to metal ion concentration. As shown in Figure a, the T_1 relaxation rate of ipGdIO was $4.87 \text{ mM}^{-1}\text{s}^{-1}$ and $2.44 \text{ mM}^{-1}\text{s}^{-1}$ at 3.0 T and 7.0 T, respectively. Subsequently, the T_2 relaxation rate of ipGdIO was also measured and showed $279.2 \text{ mM}^{-1}\text{s}^{-1}$ and $384.5 \text{ mM}^{-1}\text{s}^{-1}$ at 3.0 T and 7.0 T, respectively. In addition, the T_1 images of ipGdIO significantly brightened with concentration increasing, and corresponding T_2 images also gradually darkened. These results indicated that ipGdIO had strong T_1 and T_2 contrast performance and had great potential as T_1 - T_2 dual-modal MRI contrast agent. Next, we further assessed tumor diagnostic ability of ipGdIO-Dox through 7.0 T MRI scanner. The T_1 - and T_2 -weighted images of mice at axial plane were acquired at different time after injection of pGdIO-Dox and ipGdIO-Dox with a dose of 10 mg/kg. The T_1 -weighted images (T_1 WI) of tumor tissue gradually brightened at post-injection (p.i.) of pGdIO-Dox and the brightest image appeared at p.i. 2 h. After that, the T_1 WI of tumor began to weaken, this might be because pGdIO-Dox began to be metabolized from tumor. Compared to pGdIO-Dox, the T_1 WI of tumor in mice injected with ipGdIO-Dox was significantly brighter, which could be attributed to excellent targeting ability. In addition, the MRI signal to-noise ratio change (ΔSNR) of tumor region was analyzed through MRlcro software. The greatest ΔSNR of ipGdIO-Dox appeared at p.i. 3h and was up to more than 65%, which was more 2-folds than that of pGdIO-Dox. In addition, the T_2 WI of tumor treated with pGdIO-Dox significantly darkened with extend time, and the darkest images also appeared at p.i. 2 h, which was consistent with its T_1 WI result. Remarkably, the tumor region seemed to be very bright at pre-injection of ipGdIO-Dox, this might be because the tumor of mice contained abundance of adipose tissues. However, after injection of ipGdIO-Dox, the T_2 WI of tumor gradually darkened, and the darkest image appeared at p.i. 3 h. In addition, the analysis of ΔSNR indicated that the maximal ΔSNR of ipGdIO-Dox was significantly higher than that of pGdIO-Dox, and it was about 1.5-folds of pGdIO-Dox. These results demonstrated that the systemic delivery of ipGdIO-Dox effectively enhanced the ΔSNR of T_1 WI and T_2 WI in tumor, which can accelerate the early diagnosis and accurate therapy of tumors. Therefore, it

could be concluded that ipGdIO-Dox might provide a promising candidate to achieve a low toxicity, effective, and accurate cancer theranostics.

3. Conclusion

In summary, we developed a completely targeting Fe/Gd nanoplatfrom (ipGdIO-Dox) for chemo- and ferroptosis synergistic cancer therapy in combination with T_1 - T_2 dual-modal MRI. After entered cancer cells, the iRGD-PEG-ss-PEG chains on the surface of ipGdIO-Dox were cleaved through depleting cellular GSH, which quickly released Dox and up-regulated cellular ROS. The released Dox molecules quickly entered nuclear, and then inhibited DNA replication, inducing the apoptosis of cancer cells. In addition, the ipGdIO-Dox also effectively responded to weakly acidic microenvironment, and then released abundance of iron ions. These excessive iron ions catalyzed cellular H_2O_2 into highly toxic $OH\cdot$ through Fenton reaction, further elevating oxidative stress in cells. The excessive ROS not only damaged mitochondria, but inactivated GPX 4 expression and caused lipid peroxidase, resulting in cell ferroptosis. The introduction of gadolinium compound made ipGdIO-Dox possess excellent magnetic properties, and the ipGdIO-Dox showed strong T_1 - T_2 dual-modal MRI contrast performance. The systemic delivery of ipGdIO-Dox significantly enhanced the ΔSNR of T_1WI and T_2WI in tumor, and then improved the quality of MR images, allowing for achieving more detailed pathological information of tumor lesion. Therefore, this work provides a novel example of Fe/Gd nanoplatfrom for tumor-targeting chemo- and ferroptosis synergistic therapy in combination with T_1 - T_2 dual-modal MRI through delivering Dox.

Declarations

Availability of data and materials

The datasets and materials used in the study are available from the corresponding author.

Ethics approval and consent to participate

Mice used in this study were treated in accordance with the ethics committee guidelines of Shanghai Jiao Tong University.

Conflict of interest

The authors declare no competing financial interest.

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Figures

Figure 1

The TEM images of (a-c) MIO and (e-g) GdIO; High-resolution TEM images of (d) MIO and (h) GdIO; (i) Energy dispersive spectrometer (EDS) mapping images and corresponding line-scanning spectra of GdIO.

Figure 2

(a) The hydrodynamic size distribution of GdIO and ipGdIO; (b) XRD spectra of GdIO; (c) XPS full spectra of GdIO; (d) Field-dependent magnetization curve of GdIO at 3K and 300K; (e) The Dox release profile of ipGdIO-Dox with pH decreasing; (f) The Fe release behavior of ipGdIO under different pH conditions; (g) The Fe release curve of GdIO and MIO under pH 4.5 condition; (h) Hydroxyl radical detection in the mixture of DMPO and H₂O₂ treated with different concentrations of ipGdIO through ESR analysis; (i) ESR analysis for the catalytic activity of MIO and GdIO.

Figure 3

The internalization of MDA-MB-231 cells treated with different samples by (a) CLSM observation, (b) ICP-MS analysis, and (c) flow cytometry analysis; (d) ROS generation of MDA-MB-231 cells treated with different samples; (e) Green fluorescence signal of each group in (d). Scale bar: 100 µm.

Figure 4

(a) The viability of MDA-MB-231 cells treated with Dox, pGdIO-Dox, ipGdIO-Dox, and ipGdIO-Dox+NAC with different concentrations; (b) The viability of MDA-MB-231 cells treated with ipGdIO-Dox, ipGdIO-Dox+NAC, ipGdIO-Dox+DFO, and ipGdIO-Dox+z-VAD; (c) Western blot analysis of Cleaved caspase-3, GPX4, Bax, and Bcl-2 expression in MDA-MB-231 cells treated with saline, pGdIO-Dox, and ipGdIO-Dox at an equivalent Dox concentration of 1 μg/mL.

Figure 5

In vivo antitumor effect. (a,b) Tumor volume change, (c) corresponding tumor weight, and (d) body weight change of mice after treated with Saline, Dox, pGdIO-Dox, ipGdIO-Dox, and ipGdIO-Dox+NAC; (e) Tumor photo of mice after treatment; (f) The biodistribution of pGdIO-Dox and ipGdIO-Dox in vivo; (g) The pharmacokinetics of Dox, pGdIO-Dox, and ipGdIO-Dox in mice; (h) H&E staining and immunohistochemical staining of tumor after treatment with Saline, Dox, pGdIO-Dox, ipGdIO-Dox, and ipGdIO-Dox+NAC.

Figure 6

(a) Transverse relaxation rate and (b) longitudinal relaxation rate of ipGdIO-Dox measured by 3.0T and 7.0T MRI scanners; (c) T1 and T2 images of ipGdIO-Dox; (d) T1-weighted MR images of cancer-bearing mice at coronal planes after postinjection of pGdIO-Dox and ipGdIO-Dox.

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