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Review Article

Visualization of ferroptosis in brain diseases and ferroptosis-inducing nanomedicine for glioma

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Abstract: A remarkable body of new data establishes that many degenerative brain diseases and some acute injury situations in the brain may be associated with ferroptosis. In recent years, ferroptosis has also attracted great interest in the cancer research community, partly because it is a unique mode of cell death distinct from other forms and thus has great therapeutic potential for brain cancer. Glioblastoma is a highly aggressive and fatal human cancer, accounting for 60% of all primary brain tumors. Despite the development of various pharmacological and surgical modalities, the survival rates of high-grade gliomas have remained poor over the past few decades. Recent evidence has revealed that ferroptosis is involved in tumor initiation, progression, and metastasis, and manipulating ferroptosis could offer a novel strategy for glioma management. Nanoparticles have been exploited as multifunctional platforms that can cross the blood-brain barrier and deliver therapeutic agents to the brain to address the pressing need for accurate visualization of ferroptosis and glioma treatment. To create efficient and durable ferroptosis inducers, many researchers have engineered nanocomposites to induce a more effective ferroptosis for therapy. In this review, we present the mechanism of ferroptosis and outline the current strategies of imaging and nanotherapy of ferroptosis in brain diseases, especially glioma. We aim to provide up-to-date information on ferroptosis and emphasize the potential clinical implications of ferroptosis for glioma diagnosis and treatment. However, regulation of ferroptosis in vivo remains challenging due to a lack of compounds.

Keywords: Ferroptosis, molecular imaging, nanomedicine, therapy, glioma, brain diseases

Introduction

During development and adulthood, damaged or unwanted cells are eliminated through the activation of regulated cell death pathways. In many developmental contexts, the caspase-dependent apoptosis pathway serves as the main executor of cell death [1, 2]. Apoptosis is also a desired outcome of traditional cancer therapies [3]. However, in specific circumstances like infection or trauma, cell death pathways other than apoptosis, such as ferroptosis, necroptosis, and autophagic cell death, appear to be accountable for cellular demise [4].

Some of these pathways have been linked to pathological cell death in the brain. For example, neuronal apoptosis plays a crucial role in the pathogenesis of Alzheimer's disease (AD). Furthermore, dysfunctional autophagy is closely related to AD. Several studies strongly suggest that autophagy is defective in AD and that impaired autophagy contributes to the development of the disease [5]. Parkinson's disease (PD) is characterized by the death of neurons in the substantia nigra pars compacta (SNpc), the region responsible for regulating motor function. Several studies have found similarities with ferroptosis in patients with PD, including decreased glutathione (GSH), lipid peroxidation, elevated iron levels, and increased levels of reactive oxygen species (ROS) (key features of ferroptosis) [6-8]. Furthermore, clinical trials have shown improvements in motor symptoms through the use of iron chelators [9, 10]. Abnormal activation of pathological cell death pathway is a common feature of neurodegenerative diseases. The molecular mechanisms

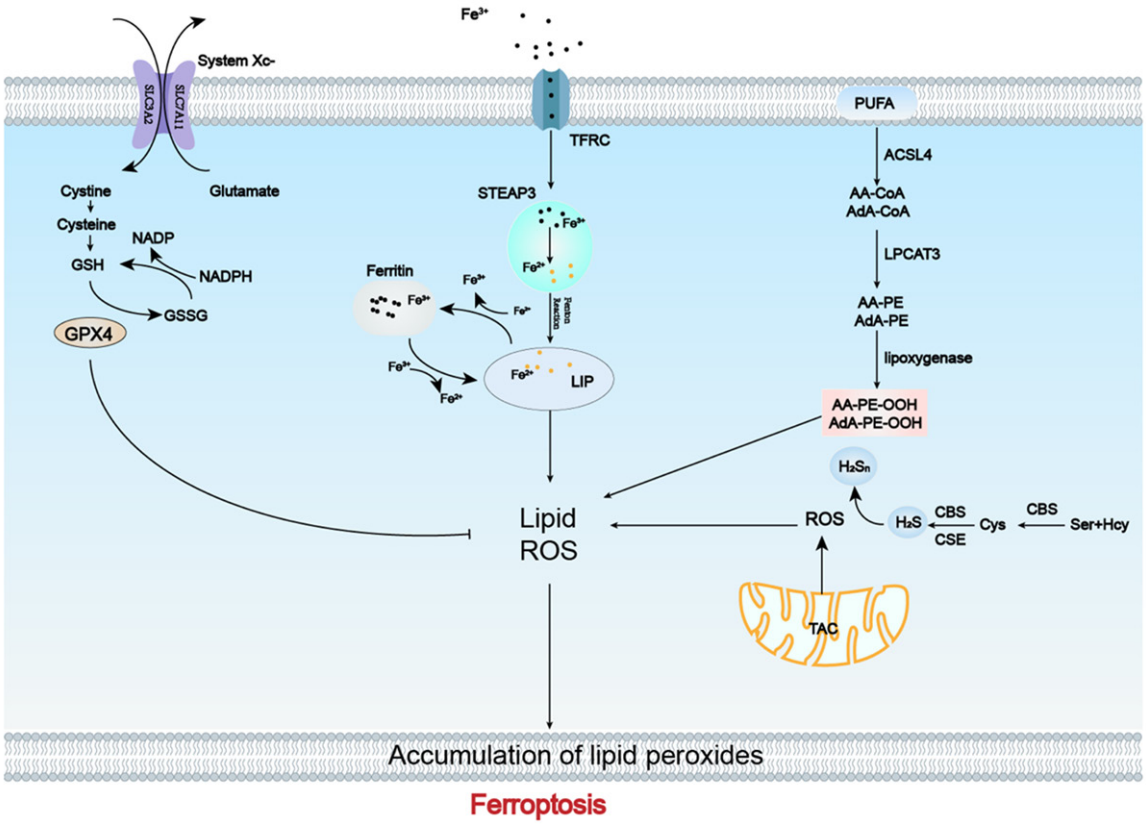

Figure 1. The main molecular mechanisms of ferroptosis. Abbreviations: ACSL4: Acyl-CoA Synthetase Long Chain Family Member 4; CBS: Cystathionine β-synthase; CSE: Cystathionine γ-lyase; Cys: Cysteine; GSH: Reduced Glutathione; GSSG: Oxidized Glutathione Disulfide; Hcy: Homocysteine; LIP: labile iron pool; LPCAT3: Lysophosphatidylcholine acyltransferase 3; NADP: nicotinamide adenine dinucleotide phosphate; PUFA: polyunsaturated fatty acids; System Xc: the glutamate/cystine antiporter; Ser: Serine; TFRC: transferrin receptor; TAC: tricarboxylic acid cycle.

underlying various forms of cell death are interconnected, as emerging evidence indicates the existence of intricate interactions between these mechanisms that contribute significantly to neuronal death [11, 12].

Ferroptosis is a novel mechanism of programmed cell death mediated by iron, which was first described in 2012 as distinct from other known forms of cell death, such as apoptosis [13]. Ferroptosis requires the production of lipid ROS and is involved in multiple processes, such as the Fenton reaction, iron-dependent ROS generation through the tricarboxylic acid cycle (TAC), and GSH depletion [14, 15].

Ferroptosis can occur through multiple pathways. First, as a prerequisite for ferroptosis, iron accumulation leads to sustained induction of lipid peroxidation through at least two mechanisms. For example, the iron-dependent Fenton reaction produces ROS, and excess iron activates iron-containing enzymes such as lipoxygenases, which peroxidize polyunsaturated fatty acids and drive ferroptosis. As such, many nanomaterials that regulate ferroptosis are based on iron overload. Second, antioxidant defense systems play a complementary role in inhibiting ferroptosis. As the predominant ferroptosis resistance factor, glutathione peroxidase 4 (GPX4) can convert toxic lipid peroxides into a non-toxic lipid form to protect against lipid peroxidation chain reaction and directly stop ferroptosis. In mammals, the cystine/glutamate transporter system significantly imports cystine into cells to produce GSH. The main metabolic pathways leading to ferroptosis are shown in **Figure 1**.

Ferroptosis is an essential cell death pathway that plays a crucial role in various nervous system diseases. The components of the ferroptosis pathway have emerged as promising targets

for drug development, with a number of drugs currently undergoing pre-clinical studies or clinical trials. For example, iron chelator by reducing oxidative damage associated with regional iron deposition is able to improve PD symptomology [9, 10]. Radical scavenger, the copper bis(thiosemicarbazone) complex Cu(II) (atsm), has the ability to inhibit peroxynitrite-driven toxicity, including the formation of nitrated α-synuclein oligomers. It has been shown to effectively reverse parkinsonian defects in animal models and holds promise as a potential treatment for PD [16]. Excess accumulation of iron in the brain may promote ferroptosis by accelerating lipid peroxidation, thereby exacerbating neuronal damage after ischemic stroke. Several candidate compounds, including iron chelators, natural and synthetic antioxidants, and others targeting iron-related signaling pathways, have been tested in animal or cellular models and have shown some protective effects against stroke [17].

Multiple modes of cell death may co-exist and interact with each other during the neurological disease process. It is thus necessary to develop imaging probe that can distinguish ferroptosis from other cell death modalities. Every kind of cell death has its own pathways which make rigorous challenges for the treatment of neurological diseases. A crucial step involves utilizing imaging probes to evaluate the severity of patients with iron deficiency anemia and prescribing suitable medication accordingly. Additionally, at the academic level, development of targeted imaging probe to identify new molecular targets is necessary to identify new ferroptosis targets [18].

In glioma, ferroptosis plays a pivotal role and has a significant impact on prognosis and therapy. Glioma is the most common primary tumor type in the central nervous system, comprising 40-60% of all primary intracranial neoplasms [19]. Gliomas are classified into four grades according to the World Health Organization criteria, with grade 1 and 2 gliomas being low-grade and grade 3 and 4 being high-grade gliomas. Despite the advances in technology and medicine, the prognosis of high-grade gliomas remains dismal, with a median overall survival of about three years for grade 3 glioma patients and only 15 months for grade 4 glioma patients [20, 21]. Glioblastoma (GBM) is the most common and aggressive subtype of grade 4 gliomas, with a five-year survival rate of about 5% [22, 23]. Hence, there is an urgent need to develop novel therapeutic strategies to improve the survival outcomes of glioma patients.

Recent advances in chemistry, molecular biology, and cancer immunology have revealed the role of ferroptosis in cancer development, drug resistance, and various modalities of cancer therapy [14]. For instance, researchers have discovered that ferroptosis can enhance the therapeutic effect of temozolomide in glioma-bearing mice [24], and that the ferroptosis inducer erastin can increase the sensitivity of GBM cells to temozolomide [25]. Furthermore, the administration of ferroptosis inducers was found to improve the efficacy of radiation in a xenograft murine glioma model [26]. These findings indicate that ferroptosis is significant for glioma chemotherapy and radiotherapy.

Ferroptosis, a regulated cell death mechanism, has diverse implications and requires detailed investigation into the biomarkers, microenvironments, and disease-related events. However, current imaging technology cannot obtain high-contrast images of glioma-driven ferroptosis. In the past decade, the rapid advancement of nanotechnology has provided a promising opportunity for diagnosing and treating gliomas. Several strategies have been developed to synthesize nanoparticles for glioblastoma therapy by enhancing ferroptosis, demonstrating the feasibility of this novel pharmacological target for cancer treatment.

This review provides a comprehensive overview of the recent progress on ferroptosis and its potential clinical applications in brain diseases. Firstly, we provided a brief introduction to the significance of cell death in brain diseases, as well as the molecular mechanism of ferroptosis and the factors that regulate its activation and suppression. Then, we showcase the current strategies for imaging brain disorders and nanotechnology-based therapy of ferroptosis in glioma and discuss their benefits and limitations. Finally, we offer some visions and suggestions for the future development of ferroptosis-based diagnosis and treatment of glioma. We hope that this review will inspire and inform readers about the critical role of ferroptosis in glioma research and clinical practice in the near future.

Biomarkers for ferroptosis imaging

Generally, morphological features caused by ferroptosis have been observed in vitro or in tissues, but it is difficult to distinguish ferroptosis from other types of regulatory necrosis based on these changes alone [27]. Therefore, assessing the level of ferroptosis involves detecting specific biomarkers [28, 29].

Ferroptosis is significantly distinct from other forms of regulated cell death, including apoptosis, necroptosis, and autophagic cell death, in terms of biochemistry [15, 28, 30, 31]. Ferroptosis is characterized by the accumulation of iron and ROS as well as the inactivation of the antioxidant defense system GSH-GPX4. Apoptosis is characterized by several biochemical features, including the activation of caspases, oligonucleosomal DNA fragmentation, and exposure of phosphatidylserine. The biochemical features of necroptosis consist of a drop in ATP levels, activation of receptor-interacting protein kinases 1 and 3 (RIP1 and RIP3), and the phosphorylation of mixed lineage kinase domain-like pseudokinase (MLKL). The biochemical features of autophagy involve the conversion of microtubule-associated protein 1 light chain 3 (LC3)-I to LC3-II and the degradation of substrates, for example, p62.

Because the original study defined ferroptosis as iron-dependent cell death, ferrous iron accumulation is one of the typical hallmarks of ferroptosis [32, 33]. The excessive accumulation of ferrous iron accelerates the disproportionation of hydrogen peroxide, leading to the formation of hydroxyl radicals. This subsequently triggers the excessive oxidation of lipids and other biomolecules, eventually inducing ferroptosis [34]. Additionally, the activation of iron-containing enzymes with lipid redox regulation also contributes to the induction of ferroptosis [35]. The iron chelator deferoxamine, inhibits ferroptosis by reducing cellular iron overload [13, 36]. Elevated intracellular or mitochondrial ferrous iron in ferroptotic cells or tissues, which can be monitored by iron assay kit [37].

ROS, including hydrogen peroxide (H_2O_2) and hypochlorous acid/hypochlorite ($HOCl/OCl^-$), are primarily generated as byproducts during mitochondrial oxidative phosphorylation. Elevated levels of ROS initiate lipid peroxidation, resulting in the degradation of lipid membranes and subsequent cell death [38]. As the biomarker of ferroptosis, ROS accumulation was assessed by fluorescent probe 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) [39].

The cellular antioxidant system is the main pathway limiting ferroptosis. The inactivation of the GSH-GPX4 antioxidant defense system leads to incomplete elimination of oxidative stress products and accumulation of lipid hydrogen peroxide [40, 41]. The import of cystine was abolished by erastin treatment, resulting in depletion of GSH and ultimately inducing ferroptosis [42]. As the hallmark of ferroptosis, GSH depletion was assessed by GSH assay kit [43].

Recent studies have found that the representative reducing substance hydrogen sulfide (H_2S) is involved in the process of iron death and is depleted during the process. Therefore, monitoring H_2S can achieve monitoring of iron death [44].

Ferroptosis imaging

Several ex vivo imaging techniques, such as transmission electron microscopy, immunohistochemistry, and western blotting, have been used to observe changes in tumor morphology or understand the pathophysiological changes in ferroptosis [45]. However, these diagnostic techniques have low sensitivity and poor selectivity, let alone their high invasiveness. Moreover, these conventional techniques cannot achieve real-time in vivo imaging of the critical molecules, pathophysiological microenvironments, or biological events to elucidate the pathogenesis of ferroptosis in animal models and humans. Furthermore, the crossing of the blood-brain barrier (BBB) and penetration into the brain pose significant challenges for delivering therapeutic agents and imaging probes [46]. Therefore, we will discuss in detail several imaging techniques that can monitor biological processes at the cellular and molecular levels for the qualitative and quantitative evaluation of ferroptosis in in brain diseases.

Fluorescence imaging

Fluorescence imaging is a non-invasive optical imaging method that can visualize intracellular metabolites, parameters, and biomolecules related to oxidative homeostasis and energy

metabolism in ferroptosis with real-time, noninvasiveness, and ultrahigh spatial resolution [45, 47]. However, its effectiveness can be influenced by tissue heterogeneities, depth location, and the animals' physiological state. Additionally, the optimal imaging duration is limited due to various factors. Iron plays a crucial role in ferroptosis, as it catalyzes the production of ROS and lipid peroxidation through the Fenton reaction and iron-containing enzymes (such as lipoxygenase). Although more than 95% of the cellular iron is bound to proteins, such as hemoglobin, myoglobin, cytochromes, or storage proteins, a high level of ferrous iron in the labile iron pool (LIP) can trigger ferroptosis. Therefore, detecting the change of iron ions in living organisms is essential for monitoring ferroptosis [48].

For in vivo imaging, the N-oxide reduction strategy has proven to be broadly useful in developing fluorescent probe for Fe^{2+} detection [49]. Another approach to developing Fe^{2+} probes based on reactivity is the incorporation of an endoperoxide motif featuring a bridged O-O bond sensitive to Fe^{2+}.

Recently, Qian et al. reported a two-photon fluorescent probe for Fe^{2+} detection in the brain of living epileptic mouse mode [50]. This probe used the dicyanoisophorone backbone as the basic fluorophore, adopting the classical N-oxide strategy to recognize Fe^{2+}. Due to excellent BBB-crossing and excitation and emission wavelengths, the probe FeP can efficiently reflect iron levels in the epileptic mouse brain. Their work indicated that Fe^{2+} levels are generally increased during epileptic seizure activity. They also revealed that dihydroartemisinin might exert its antiepileptic effects by modulating iron homeostasis in the brain and inhibiting ferroptosis (**Figure 2A**).

Recent studies demonstrate that the GPX4 antioxidation system protects cells from ferroptosis [51]. In 2022, Gu and colleagues proposed a series of 4,4-difluoro-boradiazaindacene (BODIPY)-based reducing substances-selective probes for monitoring GSH during ferroptosis [52]. Gu et al. designed a series of BODIPY-based probes for selective GSH detection, which was crucial for preventing ferroptosis. The probes WD-1-SH and WD-2-SH could image GSH changes in cells and tissues during erastin-induced and ischemic ferroptosis, respectively, as confirmed in live cells and animal models of middle cerebral artery occlusion (**Figure 2B**).

ROS are a group of molecules that include hydrogen peroxide (H_2O_2), hydroxyl radical (•OH), superoxide anion radical (O_2•), peroxynitrite ($ONOO^-$), and hypochloric acid/hypochlorite ($HClO/ClO^-$) [53]. A recent study proposed that HClO could induce ferroptosis by triggering aberrant lipid peroxidation and mitochondrial damage. After designing and synthesizing a highly efficient two-photon fluorescent probe (HCP) based on the quinoline scaffold with excellent BBB-crossing properties, the authors selectively detected endogenous HClO in the brains of kainic acid (KA)-induced epileptic mice (**Figure 2C**) [54]. As one of the most potent members of ROS, excess $ONOO^-$ irreversibly destroys multiple biological targets and mediates cell death, such as ferroptosis. Therefore, the detection of $ONOO^-$ may be able to reflect ferroptosis in brain [55].

H_2S is a potent reductant and nucleophile that can neutralize oxidants and electrophiles within cells [45]. H_2S plays a key role in maintaining intracellular redox balance and thus can serve as a marker of ferroptosis. James et al. designed a fluorescent probe named H_2S triggered and H_2S releasing near-infrared fluorescent probe (HL-H_2S) for the selective detection of H_2S during ferroptosis [56]. This probe comprised a benzyl thiocarbamate scaffold and an azide group as the H_2S-responsive unit. Interestingly, this study revealed that H_2S levels significantly declined in PC12 cells upon erastin-induced ferroptosis, while ferrostatin-1 could attenuate this effect. These findings suggested that H_2S regulated ferroptosis and that the probe could enable precise imaging and analysis of ferroptosis in situ (**Figure 2D**).

Magnetic resonance imaging

Magnetic resonance imaging (MRI) is a powerful technique for biomolecular imaging and clinical diagnosis, owing to its high resolution, deep tissue penetration, lack of ionizing radiation, and excellent soft tissue contrast [57]. However, conventional MRI relies on proton transverse relaxation rates to quantify brain iron, which shows overall low specificity for iron [58].

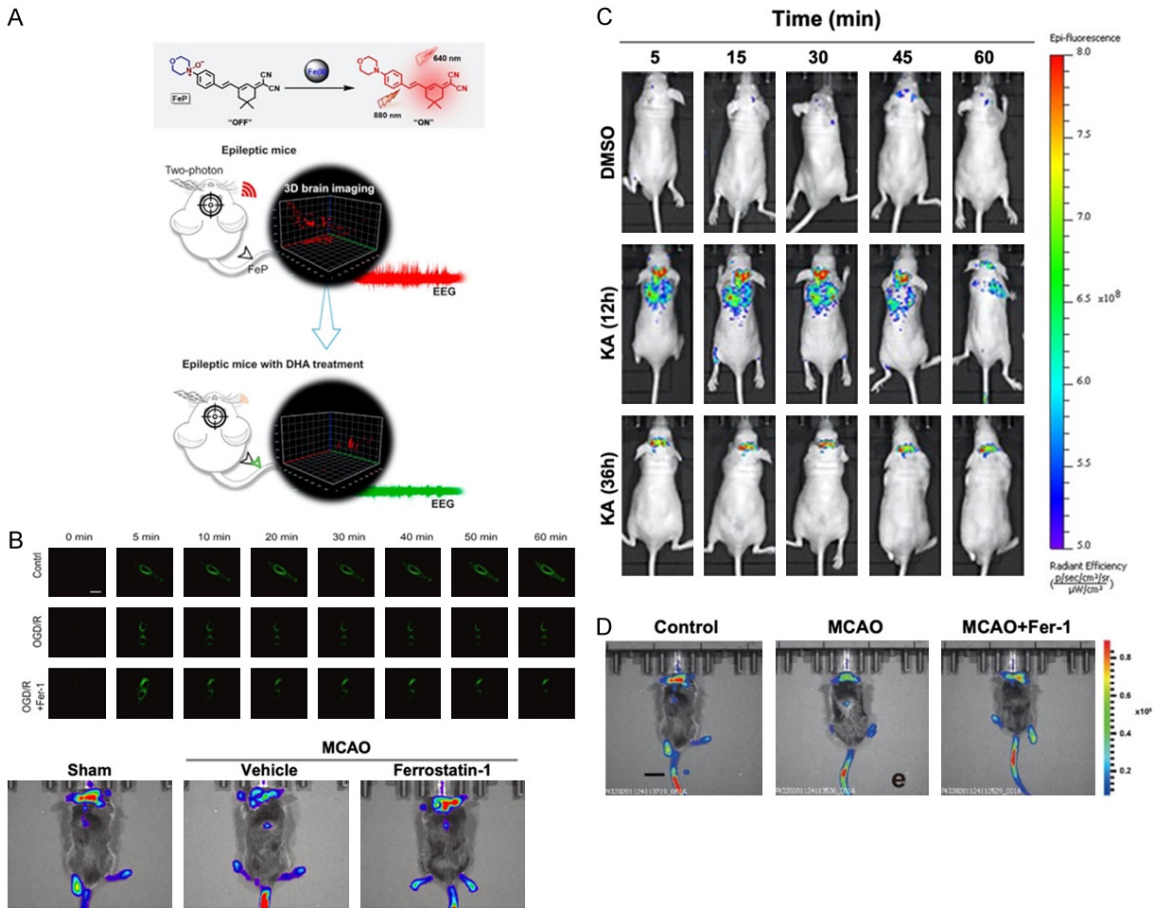

Figure 2. A. Proposed response mechanism of FeP (top). *In vivo* electroencephalogram (EEG) monitoring and animal behavioral studies, we demonstrated that dihydroartemisinin could exert potential therapeutic effects on the epileptic mice models (bottom). B. Visual imaging of MCAO in living brain of mice receiving different treatments: control group, MCAO group and Fer-1 group. Emission values were collected in the red channel (575~600 nm) with a 570 nm excitation wavelength. C. Fluorescence images of five groups of mice at 5, 15, 30, 45, and 60 min after i.v. injection with HCP, indicating that HCP was able to cross the BBB and image in live brains. Note that fluorescence signals in KA-induced epileptic brains were significantly higher than those in the control group. D. Visual imaging MCAO in living mice model. Mice using MCAO models with different treatments: control group (mice not undergoing MCAO); MCAO group (mice undergoing MCAO); Fer-1 group (injection of Fer-1 to mice tail veins). Abbreviation: MCAO: middle cerebral artery occlusion (Reproduced with permission from Shao C et al., Xiong X et al., Shao CW et al. and Liang T et al.) [50, 52, 54, 56].

To overcome this limitation, Various MRI techniques, such as relaxation time mapping, quantitative susceptibility mapping (QSM), magnetic field correlation (MFC), and direct saturation imaging, can quantify iron content [59, 60]. Helpern et al. used magnetic field correlation (MFC) imaging, which measured the MFI produced by intravoxel tissue iron, mainly in the form of ferritin and hemosiderin iron [61]. It is worth noting that MFC imaging is more sensitive to ferric iron stored in ferritin, which constitutes most of the brain iron, than ferrous iron. Since ferritin is in equilibrium with free iron, MFC may also reflect this iron pool indirectly. Thus, MFC imaging may hopefully capture the changes of ferroptosis in the brain and enable early detection of related diseases. Additionally, QSM has recently emerged as a method to study the spatial distribution of magnetic susceptibility caused by dominant iron sources found within the human body, specifically ferritins. However, it remains challenging to differentiate between Fe^{2+} and Fe^{3+} using MRI. Therefore, Chen et al. developed an artemisinin-based MRI probe that can accurately map Fe^{2+} in acute cardiac/kidney injuries models

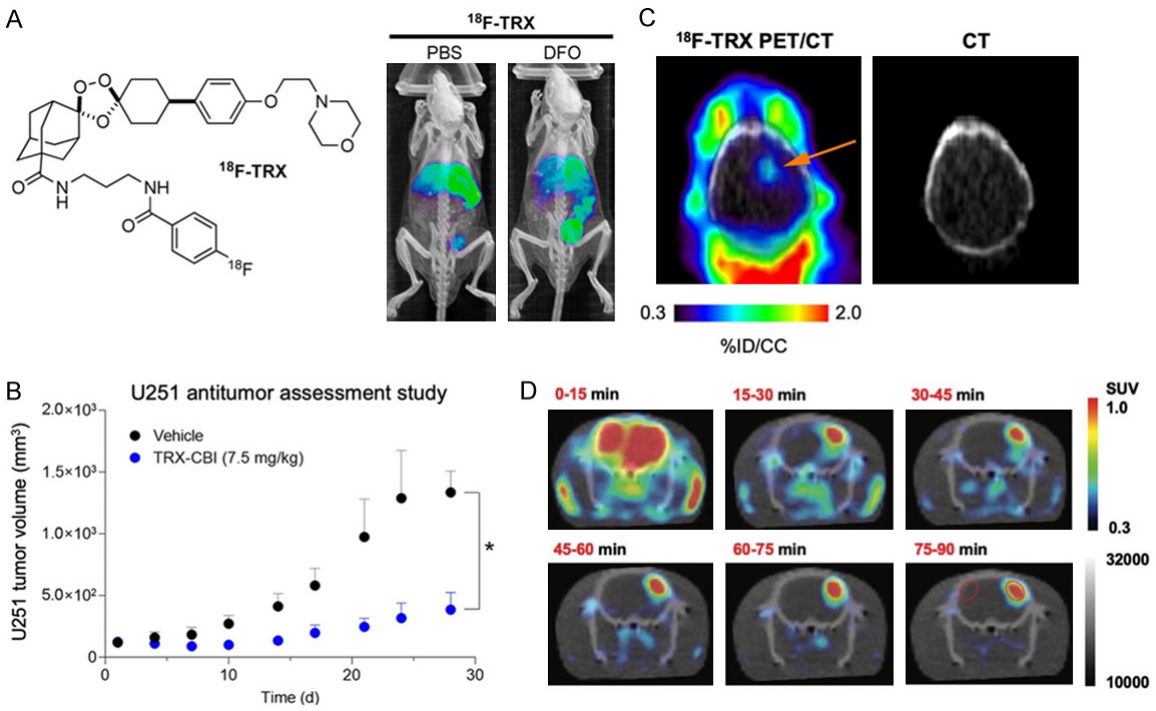

Figure 3. A. Structure of ^{18}F-TRX (left). Representative maximum intensity projections showing the biodistribution of ^{18}F-TRX in mice from PBS and DFO treatment arm from 50 to 60 min postinjection (right). B. Tumor volume data from antitumor assessment study show that U251, human glioma model with highest ^{18}F-TRX uptake among tumors surveyed, is responsive to treatment with TRX-CBI. C. ^{18}F-TRX PET/CT data showing radiotracer uptake in U87 MG tumor (arrow) implanted within right hemisphere of mouse brain. D. Typical PET imaging of ^{18}F-FDHM and/or its oxidized form in the rat brain. Data were collected for 90 min and integrated every 15 min. The red circles in the last image (75-90 min) indicate injection sites of SNP/saline. Abbreviation: TRX: trioxolane (Reproduced with permission from Muir RK et al. and Zhao N et al.) [65, 66].

[62]. MRI technology presented potential for distinguishing between Fe^{2+} and Fe^{3+} in cases of brain disorders.

Nanotherapy, a novel treatment approach for glioma, can induce iron accumulation in cells, which facilitates MRI [63]. Iron-based nanomaterials can act as effective MR contrast agents to help monitor therapy response in vivo in real-time. Therefore, combined with ever-involving nanotechnology, MRI is regarded as one of the most promising imaging modalities to visualize ferroptosis in glioma.

Positron emission tomography

Nuclear medicine imaging mainly consists of positron emission tomography (PET) and single photon emission computed tomography (SPECT). PET/SPECT imaging enables non-invasive and repeated quantitative imaging analysis of lesions or tumor areas in real-time from functional and metabolic perspectives [64]. The concentration of ferrous iron is associated with the progression of ferroptosis and the sensitivity to iron-targeted cancer therapies before ferroptosis occurs. Therefore, Evans et al. developed a novel radiotracer named ^{18}F-TRX (TRX = trioxolane) to quantify LIP in situ using PET imaging [65, 66]. 18F-TRX was derived from the structure of artefenomel, which reacted with the LIP in tissues and was sequestered within those cells. Moreover, the stable cellular accumulation of the ^{18}F signal was expected to be proportional to the LIP concentration. Many studies have shown that the LIP concentration strongly correlated with the iron-mediated cell death pathway ferroptosis sensitivity. Thus, ^{18}F-TRX has been used to monitor the LIP concentration by measuring ^{18}F-TRX uptake in a large panel of ten subcutaneous human tumor xenografts. Based on these data, ^{18}F-TRX has demonstrated high efficiency in predicting tumor drug sensitivity (**Figure 3A-C**).

Another probe named ^{18}F-labeled dihydromethidine (^{18}F-FDHM), which could cross the BBB and reacted with intracellular ROS, was reported in a recent study [67]. The authors used this probe for in vivo detection of ROS by microinfusing sodium nitroprusside, a ROS generator, into the right striatum of the brain. They observed a high level of radioactivity in the right brain one hour after injection, indicating the reaction of dihydroethidine with ROS (**Figure 3D**). This tracer might have potential applications in detecting ferroptosis, although the specificity of ROS increase in ferroptosis remained to be verified.

Ferroptosis-regulating nanomedicine for glioma

Ferroptosis is involved in many diseases and offers pharmacologically controllable therapeutic targets [68-70]. Many compelling findings indicate that drug-resistant cancer cells are susceptible to iron death. Mesenchymal and de-differentiated cancer cells often resist apoptosis induced by current therapies. Persistent cancer cells are a cause and source of drug-resistant tumors. They are susceptible to ferroptosis-based drugs, highlighting their importance as a novel therapeutic strategy in cancer treatment [71, 72]. In recent years, more studies have discovered that the inevitability of GBM drug resistance and recurrence may be due to the presence of cancer stem cells (CSCs) [73]. It was found that the iron uptake rate of GBM-CSCs was 2-3 times that of non-stem tumor cells [74], and it was believed that this change in iron metabolism might be associated with the drug resistance of GBM.

Nanotherapy for inducing ferroptosis in glioma cells

Nanoparticles can enhance tumor cell ferroptosis by various mechanisms, such as facilitating Fenton reactions, inhibiting GPX4, or delivering exogenous lipids. Li et al. developed angiopep-2 peptide-modified exosomes that could cross the BBB and targeted GBM [75]. These exosomes contained Fe_3O_4 nanoparticles that could release Fe^{2+} for sustained Fenton reactions. Moreover, they encapsulated siRNA of GPX4 (siGPX4) and DHODH inhibitor brequinar to impair the antioxidant capacity of tumor cells. This system represented a promising strategy for the treatment of glioblastoma (**Figure 4A**).

Combination of nanomedicine based on ferroptosis and conventional or emerging tumor therapy

Sonodynamic therapy (SDT) is an emerging anti-cancer approach that produces ROS that kills cancer cells, similar to photodynamic therapy. Focused ultrasound combines circulating microbubblesand enabled PIOC@CM to access the glioma and disintegrated the glioma Ce6 cell membrane through BBB-crossing [76]. Furthermore, hydrolysis of membrane phospholipids in the acidic tumor microenvironment could release Ce6 at the target site and generated large amounts of ROS, triggering SDT. Additionally, Fe_3O_4 provided exogenous Fe^{2+}, accelerating the Fenton reaction with many ROS, resulting in tumor cell ferroptosis. Ultimately, they realized a significant reduction in orthotopic gliomas after the second ultrasound pulse-triggered SDT, and ferroptosis was observed, even though the tumor could not achieve a complete cure (**Figure 4B**).

Cancer immunotherapy aims to stimulate the immune system to destroy tumors, which has minimal impact on the living body but potential adverse reactions and unequal efficacy of solid tumors cannot afford to ignore [77]. The combination of ferroptosis and immunotherapy is a promising approach to cancer therapy. For example, Liu et al. utilized Fe_3O_4 nanoparticles to connect with small interfering programmed cell death ligand-1 (siPD-L1) (Fe_3O_4-siPD-L1) and to further coat with the microglial membrane ($M_{\text{-BV2}}$) to form a biomimetic brain-targeted nanoparticle Fe_3O_4-siPD-L1@$M_{\text{-BV2}}$. Fe_3O_4-siPD-L1@$M_{\text{-BV2}}$ released siPD-L1 and inhibited the protein expression of programmed cell death ligand-1 (PD-L1) in orthotopic drug-resistant GBM cells [78]. Teff cell was activated by siPD-L1 and ferroptosis, which activated Fe_3O_4 to enhance the killing effect on drug-resistant GBM cells.

Traditional chemotherapy drugs induce drug resistance and fail to cross the blood-brain barrier, making them ineffective glioma treatments (Early termination of ISRCTN45828668, a phase 1/2 prospective, randomized study of sulfasalazine for the treatment of progressing malignant gliomas in adults). Accumulating evi-

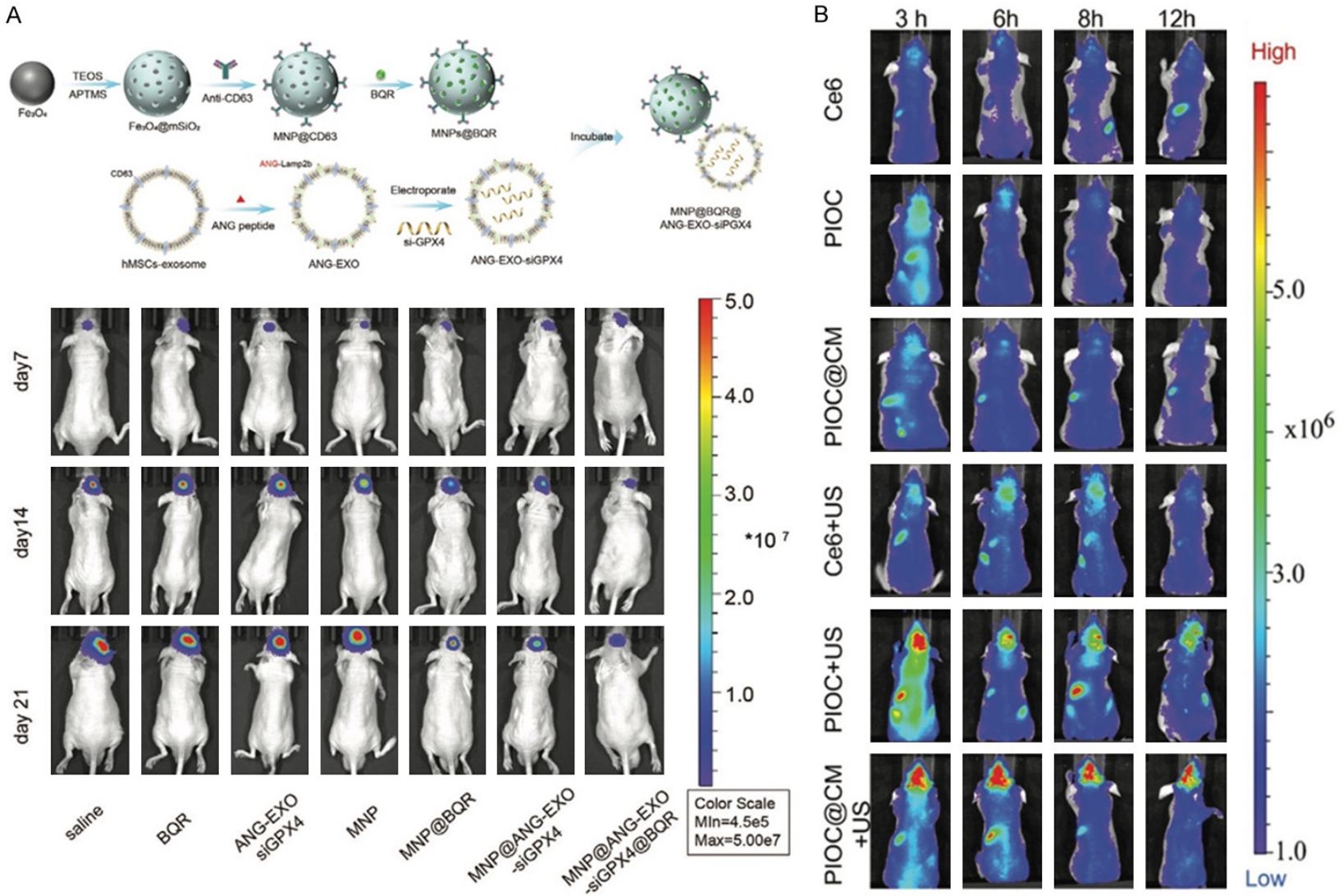

Figure 4. A. Schematic illustration of the design and synthesis of MNP@BQR@ANG-EXO-siGPX4 (top). Luminescence images of orthotopic LN229-Luc+ human GBM tumor-bearing nude mice following different treatments monitored on days 7, 14, and 21 (bottom). B. Real-time fluorescence imaging of mice bearing C6 tumors in different groups (Reproduced with permission from Li B et al. and Zhu M et al.) [75, 76].

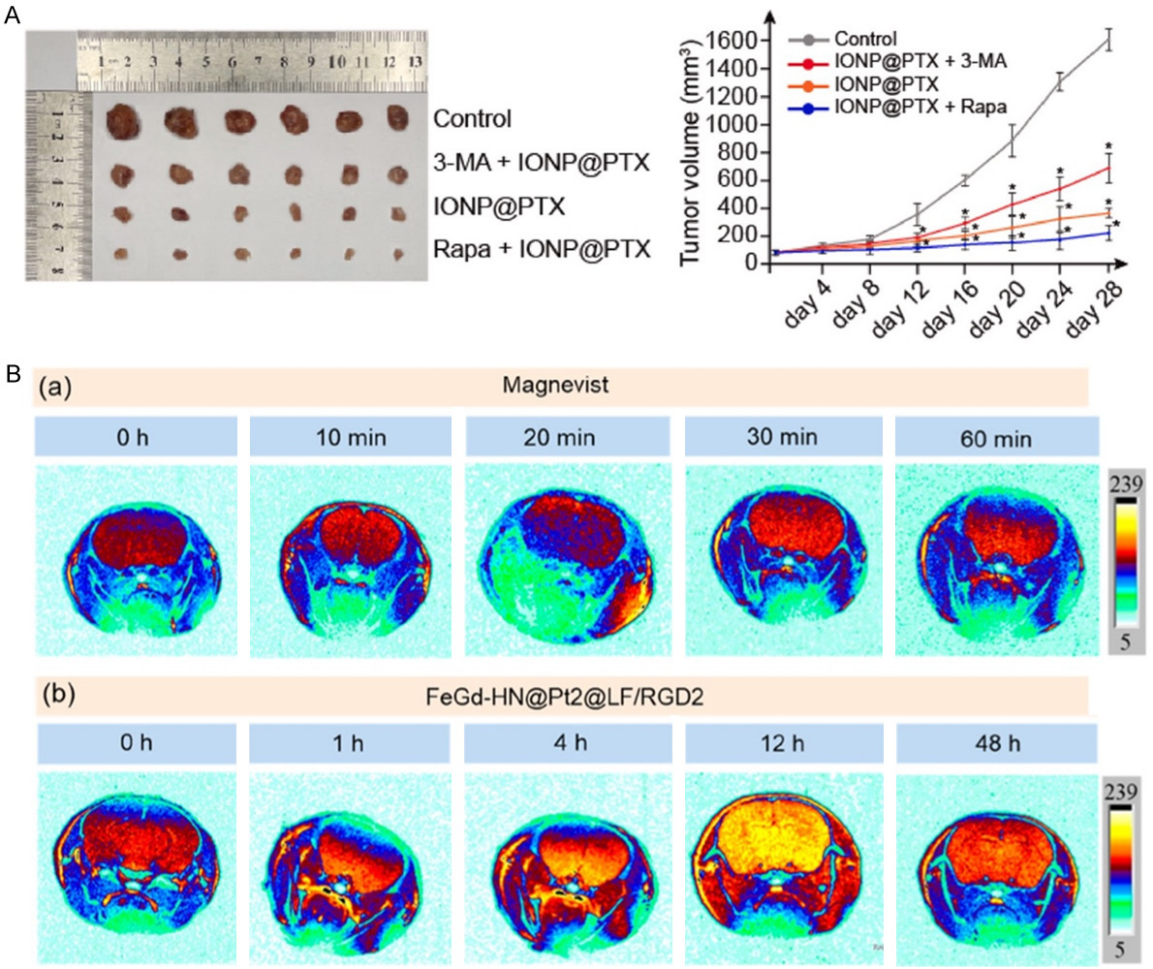

Figure 5. A. Inhibitory effect of IONP@PTX on GBM xenografts via autophagy-dependent ferroptosis pathway. Representative images of the resected tumors (left); Tumor growth curves of GBM xenografts (right). B. T1-weighted MRI images of mouse normal brains (without tumors) pre- or post-intravenous injection of Magnevist (a, upper) or FeGd-HN@Pt2@LF/RGD2 (b, lower). Abbreviations: 3-MA: 3-methyladenine; Rapa: rapamycin (Reproduced with permission from Chen H et al. and Shen Z et al.) [80, 83].

dence has suggested that combination therapy using standard chemotherapeutic agents and ferroptosis-inducing drugs could be a promising strategy to overcome drug resistance [79]. Paclitaxel (PTX) is a chemotherapeutic agent with promising antitumor activity, while clinical development is hindered by its limited water solubility and bioavailability. In order to improve the antitumor effect of PTX, nanoparticle delivery systems were used to solubilize medicines. In the nanoparticle IONP@PTX, PTX could activate the autophagy pathway and produced ROS, and iron oxide nanoparticles (IONP) could release excess Fe^{2+} [80] (**Figure 5A**). Ultimately, the nanoparticle produced excessive intracellular ROS production and ferroptosis to inhibit GBM cell migration and invasion. Furthermore, other chemotherapy drugs could also be used to design nanoplatforms, such as cisplatin (CDDP), which improved the therapeutic efficacy through a mechanism related to ferroptosis [81].

Radiotherapy produces excessive ROS resulting in oxidative stress, and diminishes the level of GSH, in addition to downregulating solute carrier family 7 member 11 (SLC7A11) and upregulating acyl-coa synthetase long chain family member 4 (ACSL4) [82]. Thereby, subsequent oxidative damage and reduced glutathione/oxidized glutathione disulfide (GSH/GSSG) ratio drop-induced ferroptosis. These results suggest that ferroptosis inducers may be effective radiosensitizers that can expand the efficacy

and range of radiation therapy indications, including glioma therapy. Currently, no application of nanoparticles as radio sensitizers in glioma is available.

Nanoplatform for the glioma theranostic

FeGd-HN@Pt@LF/RGD2 could be internalized into cancer cells by integrin αvβ3-mediated endocytosis and then released Fe^{2+}, Fe^{3+}, and CDDP upon endosomal uptake and degradation. Fe^{2+} and Fe^{3+} could directly participate in the Fenton reaction, while the CDDP could indirectly produce H_2O_2 to further accelerate the Fenton reaction (**Figure 5B**) [83]. The acceleration of the Fenton reaction generated reactive oxygen species to induce cancer cell death. The high R1 attributed to the dotted core-shell morphology of magnetic nanoparticles was used to assess and monitor the targeting effect of arg-gly-asp peptide dimer (RGD2) tumor response to ferroptosis therapy (self-MRI monitoring). Diffusion-weighted MR, which can quantify the water diffusion enhancement induced by cell death before tumor size or shape change is visible, was also used to monitor tumor response to ferroptosis therapy. The next decade will likely see a considerable rise in nanoparticles with combined diagnostic and therapeutic functions with apparent advantages in predicting and monitoring tumor response to ferroptosis therapy tumors [84].

Summary and outlook

In this review, we summarize emerging novel ferroptosis-based imaging probes. The imaging probes are based on monitoring several molecules, including iron, GSH, ROS, and H_2S, by the study of mouse models mimicking neurological diseases. Currently, different imaging methods possess both advantages and disadvantages, along with their own specific biomarkers that are suitable for imaging. Multimodal imaging combines the advantages of multiple imaging modalities for more accurate tumor detection. Besides, most existing probes focus on revealing the variations of a single parameter during ferroptosis rather than dissecting the relationship between ferroptosis and anti-tumor efficacy. Imaging probes targeting diverse markers can be comprehensively used to screen ferroptosis drugs and predict the therapeutic response to ferroptosis inducers.

With more and more people paying attention to ferroptosis over the past decade, visualization of ferroptosis based on specific molecular mechanisms of ferroptosis will provide an alternative strategy for diagnosing and treating diseases. As a ferroptosis-inducer, nanoparticles have many significant advantages. For nanoparticles, the main pathways to promote ferroptosis in tumor cells are through enhancement of Fenton response, inhibition of GPX4 or exogenous lipid. Firstly, nanoparticles can cross the brain-blood barrier. Secondly, Ferroptosis can strengthen the effectiveness of chemotherapy, radiation therapy, and immunotherapy. ROS generated by ferroptosis can modulate the tumor microenvironment, thereby inducing apoptosis, enhancing the ability of these treatments to kill cancer cells, and even effectively kill tumor cells. Nanoparticles can be combined with various therapies, such as immunotherapy, chemotherapy, and radiotherapy, and are a promising approach to induce ferroptosis in glioma. Finally, the nanoparticles loading probe can serve as a platform for integrating diagnosis and treatment.

Although there have been exciting advances in the development of nanomaterials that induce ferroptosis, problems still need to be urgently solved. Firstly, the biosafety of inorganic nanomaterials must be considered for their wide applications of iron. Second, due to the complexity of the interactions of ferroptosis and tumor microenvironment, the mechanism of iron-based nanomaterials entering tumor cells and their detailed regulatory mechanism involved in ferroptosis is not fully understood, according to the literature available. Therefore, this is very difficult for the treatment of glioma to choose the most suitable method and the most effective drug to achieve the best therapeutic effect of co-induction. Finally, the intrinsic susceptibility of untreated glioma to ferroptosis varies significantly among people and developmental stages. It is crucial to predict the sensitivity of glioma to drugs via various imaging methods. Therefore, it is believed that with the efforts of more researchers, ferroptosis will be well applied to clinical medicine to help solve some diagnosis and treatment problems.

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Disclosure of conflict of interest

None.

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