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Perspective

Metal complexes induced ferroptosis for anticancer therapy

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ABSTRACT

Metal complexes for anti-tumor treatment have been developed rapidly in recent decades since the application of cisplatin in clinics. However, some tumor cells are resistant to apoptosis and not sensitive to metallodrugs that function through the apoptotic pathway. Recently, metal complexes have been reported to cause ferroptosis against tumor cells, which offers new opportunities for anticancer therapy. In this perspective, ferroptosis-inducing metal complexes and their working mechanisms are introduced, while the challenges and opportunities are also discussed.

Metals play essential roles in biological and physiological processes, while metal complexes have been used to diagnose and treat diseases [1]. Most of the anticancer metal complexes kill tumor cells through the apoptotic pathway [2]. However, some tumor cells can escape the apoptotic pathway, which is one of the reasons for multidrug resistance in cancer treatment. Developing metallodrugs that can cause other cell death pathways could serve as a promising strategy for combating apoptosis resistance. Ferroptosis, a new term coined by Dixon et al. in 2012, is an iron-dependent form of regulated cell death caused by excessive accumulation of lipid peroxidation [3]. Cells undergoing ferroptosis involve distinctive morphological features of shrunken mitochondria with increased membrane density. Biochemical and genetic characteristics of ferroptosis also differ from other regulated cell death pathways, which may lead to different effects on tumor progression and treatment response. Some drug-tolerant and apoptosis-resistant cancer cells are inherently sensitive to ferroptosis [4]. Therefore, the emergence of ferroptosis opens up a new research avenue for effective cancer treatment.

Iron and lipid metabolisms are important factors in the regulation of oxidative damage in ferroptosis. Both autophagic degradation of ferritin and activation of serotransferrin and lactoransferrin could promote ferroptosis by increasing intracellular free iron content. Excessive iron directly generates reactive oxygen species (ROS) through Fenton reaction or enzyme activation to cause lipid peroxidation. Different lipoxygenases (LOXs), cytochrome P450 oxidoreductase (POR) and NADPH oxidases (NOXs) could also enhance ferroptosis by mediating lipid peroxidation, whereas limiting the synthesis of polyunsaturated fatty acids (PUFAs) could inhibit ferroptosis [5]. Glutathione peroxidase 4 (GPX4) can directly convert phospholipid hydroperoxide to hydroxyphospholipid, thereby suppressing ferroptosis. The expression of GPX4 depends on glutathione (GSH), which is derived from cysteine. Normally, cysteine is obtained by reduction of cystine that enters the cell via system x_c^-, which contains two subunits of SLC7A11 and SLC3A2. Tumor suppressors, such as p53, BAP1, Kelch-like ECH associated protein 1 (KEAP1), function through induction of ferroptosis mainly via regulating SLC7A11 expression [4–6]. Furthermore, the GPX4-independent FSP1-$CoQH_2$ and GCH1-BH_4 systems are found to suppress ferroptosis as well [4,5].

Recent studies demonstrate the interaction between ferroptosis and the tumor microenvironment. Ferroptotic cancer cells could activate immune responses by releasing damage-associated molecular patterns (DAMPs), such as high mobility group box 1 (HMGB1), ATP, phosphatidylethanolamine, and KRAS-G12D [4,5]. Phosphatidylethanolamine navigates phagocytosis by targeting TLR2 on macrophages, whereas the uptake of KRAS-G12D drives macrophages pro-tumorigenic M2 polarization. On the other hand, immune cells could also regulate ferroptosis of tumor cells. $CD8^+$ cytotoxic T cells

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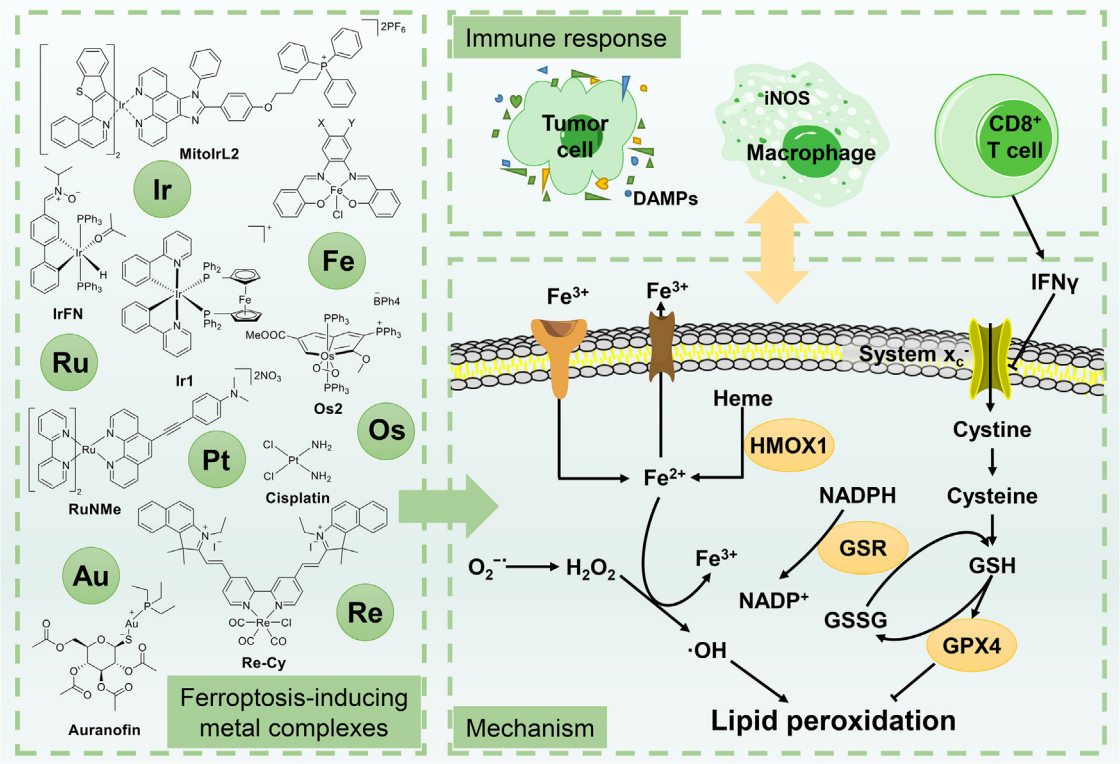

Fig. 1. Several metal complexes that induce ferroptosis and the related mechanisms.

were found to secrete IFN-γ which downregulates the expression of SLC7A11 and SLC3A2 to inhibit the cystine uptake in tumor cells, thereby sensitizing the tumor cells to ferroptosis [4,5]. Follicular $CD4^+$ helper T cells (T_{FH}) could promote immune B cell to produce long-lasting immune responses, while the amount of T_{FH} can be regulated by selenium-GPX4-ferroptosis axis [4,6]. Besides, pro-inflammatory M1 macrophages express more inducible nitric oxide synthase (iNOS) than anti-inflammatory M2 macrophages and are therefore more resistant to ferroptosis [4,5]. It can be seen that ferroptosis has great potential in tumor regulation and treatment.

Metallodrugs exhibit unique features due to their composition of metals and ligands. The different metal centers could show different coordination geometries and redox abilities, while the ligands could also show various biological activities [2]. Moreover, metal ions show the high affinity to biothiols, which might significantly interfere with the cellular redox equilibrium and cause dysfunction of proteins and enzymes. In addition, metal complexes could easily be sensitized by light to generate ROS, due to the spin-orbital coupling enhanced intersystem crossing. Therefore, metal complexes could easily induce redox imbalance and lipid peroxidation accumulation which might cause ferroptosis. In this perspective, we will introduce the recent advances in ferroptosis-inducing metal complexes and their applications in tumor treatment (Fig. 1). The main related mechanisms and therapeutic strategies are summarized in Table 1.

Cisplatin is used alone or in combination for different cancers treatments. $[Pt(NH_3)_2]^{2+}$ covalently binds to DNA to form cisplatin-DNA adducts, which result in replication and transcription inhibition, cell cycle arrest, and subsequent apoptosis. In 2018, Wu's group observed that cisplatin induced both ferroptosis and apoptosis in A549 and HCT116 [7]. Due to the high affinity of platinum complexes and thiol-containing biomolecules, cisplatin was found to reduce GSH levels and GPXs activity, which play an important role in ferroptosis. Additionally, combination of cisplatin and ferroptosis inducer erastin showed a better therapeutic effect.

Another FDA-approved gold drug, auranofin (AUR), is worth mentioning. Wang's group proposed that both AUR and TRi-1 induced ferroptosis as thioredoxin reductase (TXNRD) inhibitors [8]. Thioredoxin, a thiol-containing antioxidant, could compensate for the decrease of GSH and be reduced by TXNRD after reacting with ROS. High-dose of AUR triggered ferroptosis by weakening the total activity of TXNRDs and increasing lipid peroxidation and Ptgs2 mRNA levels, whereas GSH content and GPX4 expression were not affected. Meanwhile, AUR modulated hepcidin expression via the NF-κB/IL-6/STAT3 pathway, which could maintain iron homeostasis. Moreover, a mild pro-inflammatory effect caused by antiarthritic AUR was found in both wild-type mice and a mouse model of hemochromatosis. The mechanism of AUR indicating that thioredoxin system could be responsible for regulating ferroptosis.

Fe(III) and cisplatin-releasing nanoparticles have been designed for self-enhancing ferroptosis and apoptosis by combining chemotherapy and photothermal therapy (PTT) [9]. Cisplatin consumed GSH and activated NOXs, which could produce superoxide anion radicals (O_2^{-}) and H_2O_2. Fe(III)-polydopamine enabled nanocarriers to show photothermal effect, while the released Fe^{3+} induced Fenton reaction, depleted GSH and activated p53 to cause ferroptosis and apoptosis. Both iron ions and iron complexes can effectively induce ferroptosis. In 2019, Gust's group found that altering the 1,2-phenylenediamine substituents on a Fe(III) complex could modulate the extent of ferroptosis and necroptosis in HL-60 cells [10]. Of note, this is the first reported case of intact iron salophene complexes causing ferroptosis.

Wang et al. demonstrated for the first time that an anticancer iridium complex could induce ferroptosis [11]. Mitochondria-targeted iridium complex IrFN exhibited high cytotoxicity in A2780 cells through the HMOX1-mediated ferroptosis. Over-expression of HMOX1 promoted heme metabolism and increased intracellular iron content and ferritin production, thereby accumulating ROS and generating lipid peroxidation. Iridium complexes could also cause ferroptosis through photodynamic therapy (PDT). Yuan et al. constructed two iridium photosensitizers, IrL1 and MitoIrL2, as ferroptosis inducers [12]. Both of the two

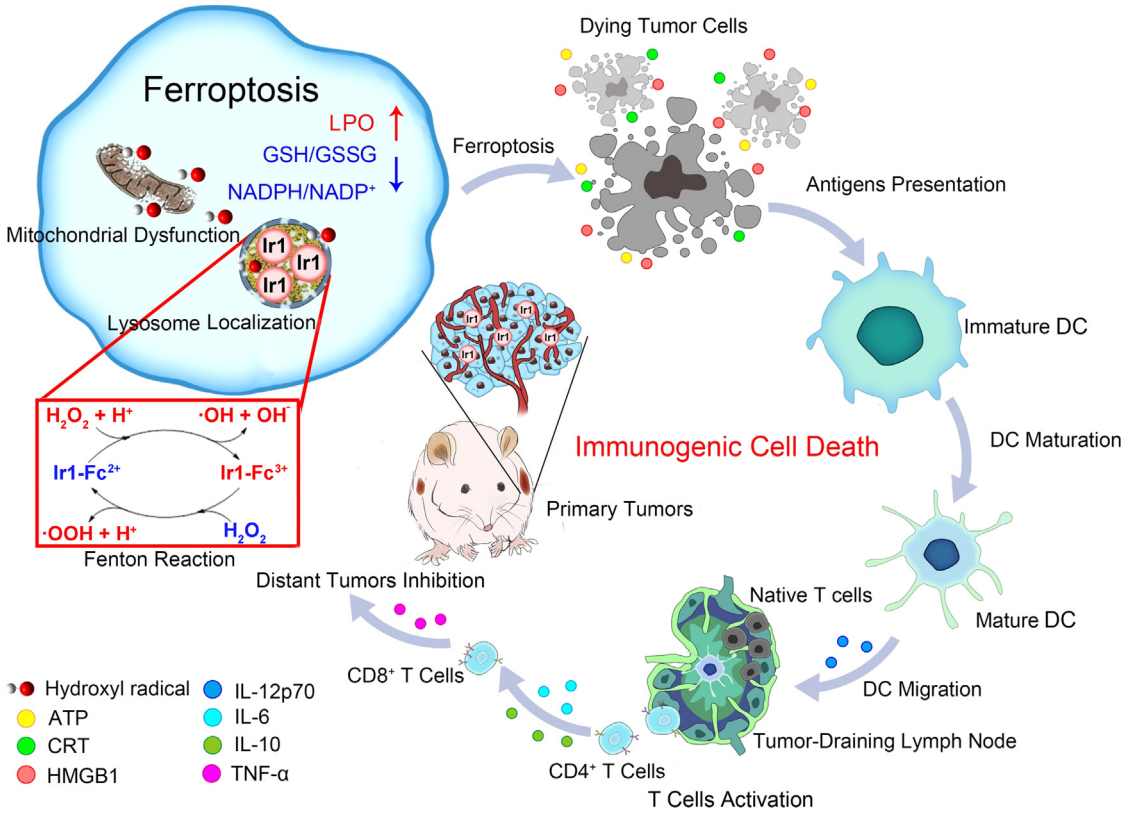

Fig. 2. Ir1 with Fenton-like catalytic ability produced ·OH leading to ferroptosis in cancer cells. Ferroptosis caused immunogenic cell death (ICD) and releases DAMPs to enhance cancer immune responses. Adapted with permission from Ref. [18]. Copyright 2021 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

Table 1
Proposed ferroptosis-inducing mechanisms and therapeutic strategies of different metal complexes.

Complex	Cell line	Mechanism	Therapeutic strategy
Cisplatin [7]	A549, HCT116	GSH-GPXs	Chemotherapy
AUR [8]	Huh7	TXNRDs	Chemotherapy
PtH@FeP [9]	4T1	GPX4, NOXs	Chemotherapy and Photothermal therapy
IrFN [11]	A2780	HMOX1	Chemotherapy
MitoIrL2 [12]	MCF-7	·OH, GPX4	Photodynamic therapy
IrS NPs [13]	A549	GPX4	Photodynamic therapy
Ir-g-C_3N_4 [14]	A375	GPX4	Photodynamic therapy
Os2 [15]	HeLa	GPX4	Chemotherapy and Photodynamic therapy
Re-Cy [16]	4T1	GPX4	Sonodynamic therapy and CO gas therapy
RuNMe [17]	MCF-7	GPX4	Photodynamic therapy
Ir1 [18]	MDA-MB-231	·OH, GPX4	Chemotherapy and Immunotherapy

complexes could generate O_2^- and ·OH through type I photodynamic processes to trigger lipid peroxidation, which finally caused ferroptosis. The mitochondria targeting MitoIrL2 was able to induce synergism of ferroptosis and apoptosis, leading to a superior inhibition effect on apoptosis-resistant cancer cell lines. Recently, the first biodegradable coordination polymer formed by self-assembly of a disulfide bond containing iridium complex has been reported [13]. The high concentration of GSH in tumor cells could break the disulfide bond and release the complex monomer, while the decreased intracellular GSH exacerbated ferroptosis. Besides, mitochondria-targeting Ir(III) polypyridine complex functionalized graphitic carbon nitride nanosheets have also been demonstrated to affect GSH content, accumulation of lipid peroxides and GPX4 expression to mediating ferroptosis [14]. The above embodies the diversity and potential of ferroptosis through iron or redox homeostasis regulation by iridium complexes.

Other metal complexes have been found to cause ferroptosis as well. An osmium-peroxo complex Os2 was reported to activate the ferroptosis pathway [15]. Os2 releases Os1 with chemotherapeutic and phototherapy effects through radiation. In addition to generating ROS, Os2 and Os1 also photocatalyzed intracellular NADH, co-triggering ferroptosis by indirectly decreasing the reduction of oxidized glutathione (GSSG) to GSH. In addition, the newly developed sonodynamic therapy, which possessed better penetration depth than PDT, also showed its potential to cause ferroptosis. Zhang's group created a rhenium complex Re-Cy for sonodynamic and CO gas synergistic therapy of tumor cells [16]. Under ultrasound irradiation, Re-Cy significantly reduced GSH levels and down-regulated GPX4 expression, which lead to ferroptosis. Ruthenium complexes have also been found to induce ferroptosis via a GPX4-dependent pathway through photodynamic therapy [17].

Notably, an Ir(III) complex capable of inducing ferroptosis to enhance cancer immunity was recently reported by Mao's group (Fig. 2) [18]. The ferrocene group was introduced in an iridium complex to yield Ir1, which catalyzed the Fenton reaction to generate ·OH under acidic conditions. The increased ratios of $NADP^+$/NADPH and GSSG/GSH broke the cellular redox homeostasis, resulting in ferroptosis. RNA-seq

showed that Ir1 affected some pathways related to cancer, ferroptosis and immunity. The increase of DAMPs effectively caused immunogenic cell death, such as HMGB1, ATP and calreticulin. Furthermore, Ir1 could activate immune response to inhibit tumor *in vivo*, including increasing the percentages of $CD8^+$ and $CD4^+$ T cells, promoting the DCs maturation, and enhancing the secretion of pro-inflammatory cytokines (IL-12p70, IL-6 and TNF-α). This work opens up a new door for the development of metal complexes which show ferroptosis-mediated cancer immunotherapy.

In summary, metal complex-mediated ferroptosis has emerged as a promising strategy for anticancer therapy. The combination of chemotherapy, photodynamic therapy, photothermal therapy, sonodynamic and immunotherapy, as well as the combination of multiple cell death pathways, is promising to improve the anticancer efficiency of metallodrugs. Although several ferroptosis-inducing metal complexes that are effective in inhibiting tumor cells have been reported, most of them focused on the classic GPX4 pathway as the mechanisms responsible for ferroptosis. The in-depth investigation of ferroptosis-inducing mechanisms needs further exploration. New metal complexes that could trigger ferroptosis through GPX4-independent pathways are expected. Future efforts should also be devoted to investigating whether ferroptosis could be induced by other metallodrugs that have already entered clinic treatment. However, some cancer cells might possess certain pathways to escape from ferroptosis. In this regard, rational design on the ligand to block or interfere with the ferroptosis-resistant pathways is demanded. Moreover, the applications of ferroptosis-inducing metal complexes in other diseases are also worth investigation. In all, although the research field of ferroptosis-inducing metal complexes is still in its infancy, the current interesting studies have offered new strategies and opportunities for the development of effective metallodrugs against cancer.

Declaration of competing interest

The authors declare that they have no conflicts of interest in this work.

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