Novel Therapeutic Savior for Osteosarcoma: The Endorsement of Ferroptosis

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Ferroptosis has recently been discovered as an iron-dependent and non-apoptotic regulated mechanism of cell death. The induction of ferroptosis in tumor cells improves tumor treatment, making it a current research hotspot. Mechanistically, it starts by lipid peroxidation, iron accumulation, reactive oxygen species (ROS) production, and glutathione deprivation, highlighting novel treatment opportunities for many tumors and neurodegenerative disorders. Several tumor cell lines are resistant to ferroptosis inducers, even when the ferroptosis key enzyme glutathione peroxidase 4 (GPX4) is blocked, indicating that other important elements are also involved in this process. Ferroptosis-suppressor-protein 1 (FSP1) was discovered to be one of these elements in addition to a few others such as ferroptotic gatekeepers like GTP cyclohydrolase 1 (GCH1) and dihydroorotate dehydrogenase (DHODH). Osteosarcoma is the most common primary malignant bone tumor observed most frequently in children and adolescents. Several studies demonstrated that ferroptosis plays a critical role in the treatment of osteosarcoma, in particular drug-resistant osteosarcoma cells. We outlined four primary regulators involved in ferroptosis in this article, reviewed previously published studies of ferroptosis in osteosarcoma to provide covert insights about osteosarcoma treatment, and highlighted several critical issues to point out future research possibilities.

Keywords: ferroptosis, osteosarcoma, drug resistance, GPX4, FSP1, GCH1, BH4, DHODH

INTRODUCTION

Osteosarcoma is the most common primary malignant osseous tumor accounting for the largest proportion (60%) of orthopedic malignant tumors that commonly affect children and those younger than 20 years (1, 2). Distal femur and proximal tibia are the most common sites of osteosarcoma burst. However, the pathogenesis of osteosarcoma remains unclear, and it is considered to be related to the combination of genetic susceptibility, virus infection, ionizing radiation, and chemical toxins (3). Clinically, the main manifestations are swelling, pain, and dysfunction of adjacent joints, which can aggravate pain and affect patients’ sleep at night (4). Several studies demonstrate that 80% of
patients with osteosarcoma have a local invasion or distant metastasis when diagnosed (5). The lung is the most common organ for tumor metastasis, accounting for 85% of cases, and 90% of patients with tumors die because of metastasis (6). Current therapeutic strategies for osteosarcoma include surgical resection, radiotherapy, chemotherapy, and immunotherapy with a five-year survival rate of 70% (7). The prognosis of osteosarcoma is still unoptimistic. Tumor cells are resistant to chemotherapeutic drugs. Drug resistance is a critical factor contributing to therapeutic failure and tumor recurrence. Therefore, extensive research on elucidating the mechanisms involved in osteosarcoma and identifying relative molecular targets as well as treatment methods is warranted.

Cell death is a fundamental biological process that pervasively takes place in all living organisms (8). Cancer cells evade quintessential immune surveillance-mediated cell death and then, due to overwhelming proliferation, eventually cause dysregulation in the body (9). The five widely accepted forms of cell death are necrosis, apoptosis, necroptosis, pyroptosis, and ferroptosis (8). Pressing engagement of unknown stimulation or toxic factors concerning the unit of life could trigger uncontrolled necrosis. Additional aforementioned types are accordingly ascribed to regulated cell death (RCD) (10). As a newly identified type of cell death, ferroptosis is a research hotspot, and it mechanistically occurs via lipid peroxidation, iron accumulation, reactive oxygen species (ROS) production, and glutathione deprivation, highlighting the novel treatment opportunities for drug-resistant tumors and neurodegenerative disorders (11). The gatekeepers of ferroptosis include glutathione peroxidase 4 (GPX4) (12), ferroptosis suppressor protein 1 (FSP1) (13, 14), GTP cyclohydrolase 1 (GCH1) (15, 16), and dihydroorotate dehydrogenase (DHODH) (17). Except for these four, other pathways such as acyl-CoA synthetase long-chain family member 4 (ACSL4), adenosine monophosphate activated protein kinase (AMPK)-ACC2, and NF2-YAP are also important family member 4 (ACSL4), adenosine monophosphate activated four, other pathways such as acyl-CoA synthetase long-chain family member 4 (ACSL4), adenosine monophosphate activated protein kinase (AMPK)-ACC2, and NF2-YAP are also important.

FERROPTOSIS GATEKEEPERS

GPX4

Glutathione peroxidase (GPX), a classical catalysis family bolstered by GSH oxidation to reduce hydrogen peroxide (H₂O₂) to water, is composed of eight paramount members containing GPX1 to GPX8 (36). Selenocysteine is considered a unique component serving as the central active site for the first four enzymes and is also indispensable for the function of GPX4 (37). In coordination with GSH, GPX4 is important for regulating ferroptosis, as it can neutralize ROS and counter oxidation (Figure 1) (12, 37). In the GSH/GPX4 antioxidant system, upstream system Xc⁻ comprises two subunits, solute carrier family 7 member 11 (SLC7A11) and SLC3A2, required for GSH/GPX4 biosynthesis, the antiporter transporting glutamate and cystine (38). The light chain SLC7A11 is demonstrated to be an effective therapeutic target for tumor treatment due to its unusual overexpression in many cancers. Generally, cellular GPX4 is prone to be inactivated by conventional class I ferroptosis inducers such as erastin, which specifically targets system Xc⁻ to cause GSH depletion, indirectly resulting in iron-dependent lipid ROS peroxidation and subsequent ferroptosis (39, 40). However, class II ferroptosis inducers such as RSL3 could directly react with GPX4 to cause anti-oxidation disorders and elicit ferroptosis (12). Mechanistically, GPX4 mainly plays a specific role in counteracting ROS generated from the Fenton reaction, a classical chemical reaction involving ferrous iron and H₂O₂, to prevent ferroptosis (41).

FSP1

Similar to GPX4, another promising protein named ferroptosis suppressor protein 1 (FSP1), earlier called apoptosis-inducing factor mitochondrial 2 (AIFM2), was discovered to mediate the ferroptosis protection pathway (13, 14). AIFM2 has been...
previously demonstrated to induce cell death in a caspase-independent manner, emerging as a potential therapeutic target for tumor treatment (42–44). Few reports have validated the key role of AIFM2 in inhibiting ferroptosis parallel to but independent of GPX4, whose name was then changed to FSP1. FSP1 is located on the human chromosome 10q22.1 and mediates p53-independent apoptosis (42). A majority of FSP1 protein adheres to the outer membrane of mitochondria, whereas the other part of FSP1 protein resides in the cytoplasm. Thus, the cytosolic FSP1 moves to the membrane when myristoylated to inhibit lipid peroxidation and arrest ferroptosis (13, 14). The N-myristoylation signal and flavoprotein oxidoreductase domain of FSP1 are essential to compete against ferroptosis. Moreover, FSP1 functions in collaboration with ubiquinone, also known as coenzyme Q10 (CoQ10). With a substantial utilization of NAD(P)H, FSP1 acting as an NADH-dependent CoQ10 oxidoreductase reduces ubiquinone to ubiquinol, which deals with oxidation through radical scavenging, thereby limiting lipid peroxidation and preventing the occurrence of ferroptosis (Figure 1). Overall, the deficiency of FSP1 and the restrained FSP1/CoQ10 signaling may play a critical role in causing cell death due to oxidative impingement.

GCH1
Recently, the GTP cyclohydrolase 1/tetrahydrobiopterin (GCH1/BH4) pathway was veriﬁed to inhibit ferroptosis in a GPX4-independent manner (15, 16). At the cellular level, BH4 plays a critical role in the activities of various enzymes (45). Mechanically, BH4 counteracts ferroptosis by scavenging lipid peroxidation, providing strong protection during the antioxidation process, whereas GCH1 is a rate-limiting enzyme for the de novo biosynthesis of BH4 from guanosine triphosphate (GTP) (46). Likewise, ferroptosis is sensitized by simultaneously inhibiting GPX4 function and a recycling system governed by dihydrofolate reductase (DHFR), whereby BH4 regenerates from BH2 (Figure 1) (15, 16). Theoretically, BH4 conﬁers robust defense against ferroptosis relying on the recycling shuttle loop of DHFR upon continuous replenishment by cellular hydrogen carriers. However, the underlying mechanisms of the GCH1/BH4 pathway are poorly understood in ferroptosis burst.

DHODH
The FSP1-dependent CoQ10 reducing system, with antioxidant property, is termed as the endogenous antiferroptosis rudder (13, 14). The functions of FSP1 are strictly limited to cellular membranes according to current reports, and it was unclear whether mitochondrial membranes are subject to similar mechanisms. This question has now been satisfactorily answered by a recently published observation (17). Initially, in different types of cancer cells, lipid peroxidation drives the synthesis of pyrimidine bases in the presence of a speciﬁc enzyme dihydroorotate dehydrogenase (DHODH) located on mitochondria. DHODH catalyzes the conversion of dihydroorotate to orotate through an oxidative reaction by consuming ubiquinone and thereby generates ubiquinol to block ferroptosis, while this occurs independently in pyrimidine synthesis (Figure 1). Mitochondrial GPX4 possibly

FIGURE 1 | The four current known regulation systems in ferroptosis. Overall, lipid peroxidation in ferroptosis is under control of GPX4-, FSP1-, GCH1-, and DHODH-dependent systems. GPX4 is the most important gatekeeper for ferroptosis and bolstered through the sustainment of GSH and cystine transportation of system Xc− activation. System Xc− is composed of two essential subunits, SLC7A11 and SLC3A2. Generally, ferroptosis could be triggered by GPX4 inhibition directly or indirectly. Nonetheless, several cancer cell lines are resistant to GPX4 inhibition through activating additional regulation systems like FSP1/CoQ10 and GCH1/BH4 systems in the cytoplasm. These two independent manners play a critical role in mitigating cellular ferroptosis especially during loss of GPX4. However, the dysfunction of the three above-mentioned systems is observed in organelles such as mitochondria. Notably, then the fourth antioxidant system DHODH-mediated ferroptosis protection in mitochondria is revealed. In the inner membrane of mitochondria, DHODH suppresses ferroptosis via the conversion of ubiquinone to ubiquinol that fights against oxidative damage on the phospholipid membrane. A total of four gatekeepers presumably serve as potential targets for the treatment of osteosarcomas. Except the four pathways, other pathways are also important in regulating ferroptosis, such as ACSL4, AMPK-ACC2, and NF2-YAP pathways, which have been known to affect ferroptosis by regulating PUFA metabolism and cellular phospholipid composition. Lipoxygenases (ALOXs) and POR have been known to affect ferroptosis by driving lipid peroxidation, which play opposite roles to GSH–GPX4, FSP1–CoQ10, GCH1–BH4, and DHODH.
plays a redundant role in cell survival compared to DHODH; however, it repairs the lipid membrane damage (47). Potent DHODH inhibitors are sensitive to cancer cell lines with low GPX4 expression levels.

FERROPTOSIS IN OSTEOSARCOMA

Recent studies have shown that ferroptosis is involved in tumor growth and malignancy (Table 1). Isani et al. first described ferroptosis-like cell death with iron-dependent and non-apoptotic characteristics in osteosarcoma cell line D-27 (55). After treatment with *Artemisinin annua* extract, D-27 cells showed cell death presented with ballooning phenotype instead of fragmented nuclei distinct from previous apoptosis as well as necrosis patterns and possessed aberrant iron levels. The study identified that ferroptosis sensitization could be induced by dampening STAT3/Nrf2/GPx4 signaling to enhance the sensitivity of osteosarcoma cells to cisplatin (54). This was the first study focused on drug-resistant osteosarcoma and revealed a novel approach to make osteosarcomas more sensitive to the drug by utilizing ferroptosis inducers or STAT3 inhibitors. The vital gatekeeper of ferroptosis, GPX4, was first reported to be involved in osteosarcoma. Moreover, phenethyl isothiocyanate (PEITC), an isothiocyanate that is effective against various cancers, was reported to stimulate osteosarcoma ferroptosis by impairing iron metabolism, redox balance, and GSH-iron-ROS regulation (52, 53). This form of osteosarcoma ferroptosis was observed to be controlled by the MAPK signaling pathway. Ultrasound-activatable doxorubicin (DOX)-Fe(VI)@HMS-HEPEG (DFHHP) nanoparticles synergistically boosted the growth suppression of hypoxic osteosarcoma by inducing ferroptosis with notable GPX4 downregulation (51). Thereafter, three independent studies respectively demonstrated that the regulators EF24, KDM4A, and tirapazamine could mediate ferroptosis through GPX4 directly or indirectly (48–50). The overall picture of ferroptosis in osteosarcoma is incomplete, and further studies are warranted.

**TABLE 1 | Previous published studies regarding ferroptosis in osteosarcoma.**

| Year | Authors | Research object | Osteosarcoma cell lines | Target gate molecules | Major changes | Signaling pathways | Brief description |
|------|---------|----------------|-------------------------|----------------------|---------------|-------------------|------------------|
| 2021 | Yihua Shi et al. (48, | Tirapazamine | HOS, 143B, U2os | SLC7A11 | Iron accumulation, ROS production | / | Tirapazamine inhibits proliferation and migration of osteosarcoma cells under hypoxia by downregulation of SLC7A11 and GPX4 |
| 2021 | Meng Chen et al. (49) | KDM4A | HOS, 143B | SLC7A11 | GSH depletion, lipid peroxidation | / | KDM4A directly induces H3K9me3 demethylation to promote SLC7A11 transcription and dampen ferroptosis |
| 2021 | Hailijing Lin et al. (50) | EF24 | U2os, Saos-2 | GPX4 | Lipid peroxidation, iron accumulation, ROS production | HO-1/ GPX4 | EF24 upregulates HMOX1 to suppress GPX4 expression to induce ferroptosis |
| 2021 | Jingke Fu et al. (51) | DFHHP nanomedicine | Saos-2 | GPX4 | GSH depletion, iron accumulation, ROS production, reoxygenation | / | Ultrasound-activatable DFHHP nanomedicine plays a synergistic role in boosting the growth suppression of hypoxic osteosarcoma by the induction of ferroptosis |
| 2020 | Huanhuan Lv et al. (52) | β-Phenethyl isothiocyanate | HOS, 143B, U2os, MG-63 | GPX4 | GSH depletion, lipid peroxidation, iron accumulation, ROS production | MAPK | β-Phenethyl isothiocyanate alters iron metabolism and disturbs the redox balance in osteosarcoma to induce cell death by activating the MAPK signaling pathway |
| 2020 | Huanhuan Lv et al. (53) | PEITC | K7M2 | GPX4 | GSH depletion, iron accumulation, ROS production | MAPK | PEITC induces ferroptosis in K7M2 osteosarcoma cells by activating the ROS-related MAPK signaling pathway |
| 2019 | Qiang Liu et al. (54) | STAT3 | MG-63, Saos-2 | GPX4 | Lipid peroxidation, iron accumulation, ROS production | STAT3/ NH2/GPX4 | STAT3 inhibitors activate ferroptosis in osteosarcoma cells and increase sensitivity to cisplatin by impairing the STAT3/Nrf2/GPX4 signaling pathway |
| 2019 | Gloria Isani et al. (55) | Artemisinin and A. annua extract | D-17 | / | / | / | Artemisinin and A. annua extract show a more potent cytotoxic activity on osteosarcoma cells possibly through the induction of ferroptosis |

Ferroptosis has been extensively studied in various disorders and exhibits great potential in antitumor therapy (12, 56). Tumor diseases marked by angiogenesis, which accounts for metastasis and poor prognosis, are in most cases treated with surgical resection, radiotherapy, or combined chemotherapy (57). Herein, the evocation of ferroptosis could be regarded as a new approach to halt osteosarcoma growth and could provide support for the treatment of difficult tumors (20, 58). Extensive research has identified several ferroptosis inducers for treating tumors, including erastin modulating system Xc-, sulfasalazine, sorafinib manipulating system Xc-, and RSL3 acting on GPX4 (40, 59). Previous studies on the role of ferroptosis in osteosarcoma mainly targeted GPX4 and reported encouraging
results. Some tumor cell lines (possibly containing drug-resistant osteosarcomas) are also found to be resistant to ferroptosis burst when GPX4 is inhibited along with the acyl-CoA synthetase long-chain family member 4 (ACSL4) expression (18, 60). Therefore, induction of ferroptosis by blocking the FSP1/CoQ10 axis with concomitant GPX4 blockade may have positive effects on osteosarcoma treatment (13, 14). In addition, the level of FSP1 was found to be associated with tumor ferroptosis resistance, revealing that inhibitors of FSP1, earlier described as iFSP1, could be utilized to kill osteosarcoma cells, and the treatment could be further potentiated by the loss of GPX4 activity (14). Similarly, DHODH inhibitors are suggested to trigger ferroptosis through mitochondrial lipid peroxidation on those osteosarcoma cells with low GPX4 expression levels (17). However, abolishing the GCH1 network alone is not adequate to promote ferroptosis owing to CoQ10 interaction and the de novo biosynthesis of BH4 from GTP by GCH1 (46). In conclusion, targeting GPX4, FSP1, GCH1, and DHODH has a great potential for combating osteosarcoma.

DISCUSSION

Ferroptosis is a newly discovered form of regulated cell death with already identified four key regulators (61, 62). As a type of programmed cell death, ferroptosis induction offers an emerging strategy to treat tumors and other types of disorders. Although ferroptosis and its modulators, especially GPX4, have been extensively studied, much remains to be understood (12, 37). The mechanism of FSP1- and DHODH-mediated ferroptosis needs to be fully elucidated, and its regulators, inducers, or inhibitors are yet to be investigated. Apart from the aforementioned considerations, different cells may show diverse sensitivity toward ferroptosis or the possible FSP1 and DHODH inhibitors. Consequently, the exact dosage and method of delivering the possible FSP1 and DHODH inhibitors precisely are two major concerns that could limit the clinical application.

FSP1-mediated ferroptosis is also involved in inflammatory processes, hinting that ferroptosis is capable of recruiting immune cells in the tumor microenvironment (22, 63). The interaction between osteosarcoma cells and antitumor drugs is complicated. These drugs could induce cell death in various ways. The inflammatory process is an important component involved in osteosarcoma growth and development as tumor immunity is accompanied by inflammatory reactions (64).

Although cell death types are multifarious, several forms could be induced under the same situation. During the process of tumor cell death, inflammation could be induced and related inflammatory cytokines such as TNF-α, IL-1β, and IL-6 further cause immune cell infiltration, which further results in the elimination of dead cells. FSP1 is a bidirectional protein associated with apoptosis or ferroptosis (13, 14, 42, 43, 65). It was previously identified to induce apoptosis in a caspase-independent and p53-independent manner, while it also protects cells from ferroptosis. Therefore, FSP1 may be considered as a decision-maker for the mechanism of cell death. Overall, a high FSP1 expression level confers protection against ferroptosis while inducing cell death through apoptosis. This could explain why almost all normal cells have low FSP1 expression levels, and low FSP1 expression can avoid cell apoptosis. Ferroptosis is an inflammation-regulated necrosis contrary to apoptosis. It can trigger inflammation, which helps purge tumor cells. Targeting FSP1 to promote ferroptosis is an interesting and theoretical strategy in osteosarcoma therapy. A future direction can be set to study the synergistic effect of FSP1 inhibitors and immunotherapy in the treatment of tumors.

In conclusion, the concept of FSP1 and its fast growth have shed new light on antitumor therapies, drastically changing the landscape of tumor treatment. However, further research on the correlation of FSP1 with osteosarcoma growth and progression and the underlying mechanism of FSP1 in osteosarcoma pathogenesis is required. Further research will be crucial for developing ferroptosis-based therapeutic regimens (64).

AUTHOR CONTRIBUTIONS

SW and LC raised the idea for the article and critically revised the manuscript. CQ, TL, and DYL performed the literature search and data analysis. CQ, TL, LC, and SW were the major contributors in the drafting of the work. All authors contributed to the article and approved the submitted version.

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