

Advances in ferroptosis in head and neck cancer (Review)

XINYI WANG, KUNPENG LI, TENG SONG, SULIANG XING, WEI WANG and YUHUI FANG

Department of Dermatology, Fuyang People's Hospital Affiliated to Bengbu Medical University, Fuyang, Anhui 236000, P.R. China

Received May 29, 2024; Accepted August 6, 2024

DOI: 10.3892/br.2024.1839

Abstract. Ferroptosis is an iron-dependent form of cell death that was discovered in 2012. It encompasses the coordinated orchestration of three fundamental biological pathways: Iron homeostasis, glutathione regulation and lipid metabolism. Head and neck cancer (HNC) is a heterogeneous group of cancers occurring on the mucosal surfaces of the upper respiratory and digestive tracts. Head and neck squamous cell carcinoma is the most common type of HNC, accounting for >90% of HNC cases, and has high morbidity and mortality rates. Despite improvements in diagnosis and treatment, the 5-year survival rate hovers at a dismal 50–60%, with recurrence afflicting nearly 30% of patients, highlighting the inadequacies of currently available treatments. Of note, research exploring the nexus between ferroptosis and HNC remains scarce; however, the present review endeavors to synthesize current knowledge surrounding ferroptosis. The present review elaborated on the normal physiological role of ferroptosis and discussed its potential involvement in HNC pathogenesis. Therapeutic strategies and prognostic paradigms for HNC that target ferroptosis were also reviewed. This review aims to provide direction to catalyze future investigations into ferroptosis in HNC.

Contents

1. Background
2. Normal mechanisms of ferroptosis
3. Ferroptosis and HNC
4. Conclusions and perspective

1. Background

Cell death has essential roles in normal physiological processes, as well as in disease pathology. Various physiological

phenomena, including embryonic development and the immune system's selection of B and T cells, necessitate cell death for their normal functioning. Normally, the clearance of dead cells operates seamlessly, but this system may become overwhelmed when a substantial number of cells die suddenly and accumulate, as observed during infections, chronic inflammation and tissue damage (1). Over recent decades, researchers worldwide have identified numerous forms of cell death, categorizing them into accidental cell death (ACD) and regulated cell death (RCD) based on their underlying mechanisms. ACD, caused by severe chemical, physical or mechanical stress, unfolds uncontrollably, whereas RCD can be modulated through pharmacological or genetic interventions (2). RCD, whether occurring under physiological or pathological conditions, has a crucial role in maintaining organismal homeostasis (3). Various forms of RCD occur spontaneously in the absence of external perturbations as a fundamental aspect of developmental processes or tissue turnover and are often referred to as programmed cell death (4). Recent investigations have characterized RCD into various modalities, encompassing programmed necrosis, intrinsic apoptosis, extrinsic apoptosis, ferroptosis, autophagy-dependent cell death, cellular pyroptosis, parthanatos, entotic cell death, netotic cell death, lysosome-dependent cell death, immunogenic cell death and necrosis driven by mitochondrial permeability transition (3). Among these, ferroptosis is an iron-dependent mode of cell death that was discovered in 2012 and is characterized by the activation of reactive oxygen species (ROS), iron aggregation, mitogen-activated protein kinase pathway activation, reduction of cystine uptake and glutathione (GSH) depletion (5). Iron, beyond its role as a regulator of enzyme activity, oxidizes a diverse array of substrates, inducing biological damage (6). Iron-mediated oxidative stress is a central mechanism in ferroptosis that causes an overwhelming accumulation of lethal lipid ROS (7,8). Ferroptosis is therefore an iron-dependent modality of regulated cell death characterized by an overwhelming iron-dependent accumulation of lethal lipid ROS (9). With the ongoing scrutiny of ferroptosis, evidence is accumulating that underscores its involvement in iron or ROS-related diseases, including cancer, neurodegenerative disorders, infections and inflammatory conditions (10,11).

Head and neck cancer (HNC) comprises a heterogeneous array of tumors that originate from the mucosal linings of the upper aerodigestive tract and constitutes a significant global health burden. With >880,000 new cases and >450,000 deaths annually, HNC ranks as the sixth most prevalent cancer worldwide. Among HNC, head and neck squamous cell carcinoma

Correspondence to: Dr Yuhui Fang, Department of Dermatology, Fuyang People's Hospital Affiliated to Bengbu Medical University, 501 Sanqing Road, Yingzhou, Fuyang, Anhui 236000, P.R. China
E-mail: yhfang34@163.com

Key words: ferroptosis, head and neck cancer, head and neck squamous cell carcinoma, tumor therapy

(HNSCC) is the predominant subtype, accounting for >90% of HNC cases. Characterized by rapid progression, extensive infiltration and poor prognosis, HNSCC poses considerable therapeutic challenges (12). Currently, the treatment strategies for HNC are mainly based on radiotherapy, chemotherapy and surgery (13). Long-standing evidence dating back to the late 1950s links tobacco use to HNSCC (14). Chronic heavy alcohol consumption is another independent risk factor for HNC, particularly HNSCC, and frequent alcohol consumption also potentiates the carcinogenic effect of tobacco (15). Furthermore, the escalating incidence of oropharyngeal cancers, attributed to human papillomavirus infection, adds complexity to HNSCC epidemiology (16). Despite advances in therapeutic strategies, the 5-year survival rate for patients with HNSCC remains dishearteningly low, hovering between 50 and 60%, with up to 30% of patients experiencing cancer relapse and treatment failure (17). Although the median age at diagnosis is typically ~60 years, a concerning trend has emerged with a rising incidence of HNSCC among adults younger than 45 years, primarily attributable to increased rates of oropharyngeal cancers associated with oncogenic human papillomavirus (18).

Of note, dual consequences of ferroptosis have emerged in tumorigenesis and tumor therapy, contingent upon tumor type and stage. Iron, a pivotal nutrient for cell proliferation and a cofactor for metabolic enzymes, can foster tumor initiation and growth. Furthermore, ferroptosis may trigger tumorigenesis by increasing the inflammatory response at an early stage (19). Conversely, recent experimental endeavors underscore the tumor-suppressive effects of ferroptosis inducers across diverse cancer models, highlighting the potential of ferroptosis as a promising target for anti-cancer strategies (20).

In recent years, numerous studies have revealed abnormalities in ferroptosis in various tissues of different cancer types and have demonstrated that certain drugs or natural compounds can induce ferroptosis in cancer (21). In addition, increased levels of transferrin (Tf) receptor (TfR)C1, which is responsible for cellular iron uptake, and decreased abundance of Tf, which is responsible for iron efflux, can lead to high intracellular levels of iron ions and the induction of ferroptosis in HNSCC cells. Furthermore, the TfR gene is located in a genomic region (3q29) that is frequently amplified in HNSCC (22). HNC is a complex cancer, for which new drugs, combination therapies and prognosis predictors need to be explored in depth. Targeting ferroptosis has potential in the anti-cancer field; therefore, exploring the role of ferroptosis in HNC is likely to provide a new direction for the diagnosis and treatment of HNC. However, despite the promising findings obtained to date, the relationship between ferroptosis and HNC remains relatively unexplored, with few studies addressing this nexus. The present review aimed to consolidate the existing understanding of ferroptosis, encompassing its physiological mechanisms and its involvement in HNC pathogenesis, treatment and prognosis. This review aims to provide a comprehensive resource to inform future studies investigating the intricate interplay between ferroptosis and HNC.

2. Normal mechanisms of ferroptosis

Ferroptosis, a finely orchestrated process, revolves around three pivotal pathways: Iron homeostasis, GSH regulation

(including its homeostasis and redox regulation), and lipid metabolism. GSH regulation and lipid metabolism both involve glutamate and interact with each other, and both can ultimately influence ROS accumulation to regulate ferroptosis. The iron homeostasis pathway regulates ferroptosis by controlling iron ion levels. However, certain pathways, such as the nuclear factor erythroid 2-related factor 2 (Nrf2)-related pathway, are associated with all three pathways.

Iron homeostasis. Recent findings have illuminated the central role of iron-dependent lipid peroxidation in ferroptosis, which hinges on the peroxidation of phospholipids containing polyunsaturated fatty acid (PUFA) moieties in conjunction with iron homeostasis. The induction of ferroptosis entails specific pathways involving redox-active iron and iron-dependent peroxidation enzymes (23). Iron metabolism is intricately governed by hepatic mechanisms, which control systemic iron homeostasis by producing and secreting factors pivotal for its regulation. Key proteins, such as Tf, ferritins, hepcidin and ferroportin (FPN), orchestrate this intricate regulation of iron homeostasis to provide redox control and maintenance of the systemic iron equilibrium (24). The hepcidin-FPN axis is a linchpin in governing extracellular iron homeostasis in both physiological and pathological states (25).

As an indispensable cofactor for various enzymes participating in redox reactions, the dual existence of iron as ferrous iron (Fe^{2+}) and ferric iron (Fe^{3+}) underscores its significance. Fe^{3+} binds to Tf in the serum and is then recognized by TfR in the cell membrane. Ferritin is a cytoplasmic iron storage protein composed of two subunits, ferritin heavy chain 1 (FTH1) and ferritin light chain, which regulate the storage of ferrous iron in cells. The process of ferritinophagy is the degradation of FTH1 mediated by nuclear receptor coactivator 4 (NCOA4) in autophagosomes, leading to the release of iron bound by ferritin into free iron, which increases the iron content and promotes ferroptosis. In addition, hepcidin, a central regulatory molecule involved in systemic iron homeostasis, is synthesized in hepatocytes and other cells and released into the circulation. It blocks iron release from duodenal cells and macrophages by binding to the iron export protein (FPN), a transmembrane protein whose function is to transport iron from the cell into the plasma. Under normal conditions, through the action of FPN, ferrous iron is transported to the extracellular environment and further oxidized to trivalent iron (26). In addition, Nrf2 is a regulatory factor for cell redox balance and a protective anti-oxidant response, as well as regulating glutamine metabolism related to ferroptosis (27). Wei *et al* (28) found that activation of the Nrf2/heme oxygenase (HO)-1 pathway can increase Fe^{2+} levels in cancer cells and induce ferroptosis. The regulation of iron homeostasis is therefore crucial in ferroptosis.

GSH regulation. In addition to processes related to iron homeostasis, ferroptosis is influenced by GSH homeostasis and redox regulation, a regulatory mechanism associated with important cellular pathways. GSH is the essential reducing substrate for glutathione peroxidase 4 (GPX4) and is crucial for inhibiting ferroptosis. GSH is essential for life as evidenced by the lethality caused by silencing γ -glutamate-cystine ligase, the rate-limiting enzyme in GSH synthesis (29).

The impact of GSH levels on ferroptosis emerged from the use of a ferroptosis inducer, erastin, which lowers the intracellular level of GSH. In sensitive cells, this activates a form of death morphologically identical to that induced by GPX4 knockout (9). The intracellular GSH concentration is meticulously regulated through a multifaceted homeostatic mechanism, wherein GSH steady-state levels are modulated by the kinetics of specific enzyme reactions (30). The GSH concentration is controlled by the rates of oxidation, conjugation, extrusion, uptake of thiol-containing precursors and re-synthesis. The cystine-GSH-GPX4 axis is a pivotal system in combatting ferroptosis in mammals. In the presence of catalytically active iron, the GSH/GPX4 axis assumes a critical role in neutralizing the generation of specific phospholipid hydroperoxides. The upstream component of this system is the cystine-glutamate countertransporter (System Xc⁻), which consists of the transporter protein solute carrier family 7 member 11 (SLC7A11), linked via a disulfide bond to the regulatory subunit, SLC3A2. Operating as an amino acid transporter on the cell membrane, System Xc⁻ imports cystine while exporting glutamate, thereby bolstering GSH synthesis (31). System Xc⁻ transports intracellular glutamate to the extracellular space and extracellular cystine into the cell, which is then transformed into cystine for GSH synthesis. GPX4 reduces the endogenous neutralization of lipid peroxide to lipid alcohol, ultimately reducing ROS accumulation (21). Inhibition of system Xc⁻ activates ferroptosis in cell lines because of a lack of intracellular cystine (32). Inhibition of SLC7A11, a subunit of system Xc⁻, by drugs such as erastin leads to GSH depletion and consequent inactivation of GPX4, which causes lipid peroxidation-mediated ferroptosis. In addition, Wu *et al* (33) found that Nrf2 enhances the resistance to ferroptosis in breast cancer cells by upregulating SLC7A11 levels. Hence, the intricate interplay of GSH homeostasis and redox regulation is a critical determinant in ferroptosis.

Lipid metabolism. Ferroptosis is intricately intertwined with glycolipid metabolism, encompassing glycolipid synthesis, storage, degradation, peroxidation, transport and β -oxidation. A pivotal step in ferroptosis promotion involves the peroxidation of PUFAs at the bis-allylic position (34). Under conditions of oxidative stress, increased synthesis of PUFA promotes lipid peroxidation. PUFAs can be classified according to the position of the first double bond on the methyl terminal end as ω -6 [e.g., linoleic acid (LA; 18:2), γ -LA (GLA; 18:3), dihydro-GLA (20:3), arachidonic acid (AA; 20:4) and adrenic acid (AdA; 22:4)] or ω -3 [e.g., α -LA (18:3), eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6)], and have amphiphilic properties to maintain the fluidity of the cell membrane. Among them, AA and AdA are the main substrates of lipid peroxidation in ferroptosis (35).

The involvement of AA/AdA derivatives in ferroptosis requires the crucial roles of acyl-coenzyme A (CoA) synthetase long-chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3). ACSL4 catalyzes the conversion of free AA/AdA to CoA, facilitating their esterification into phospholipids, whereas LPCAT3 catalyzes the biosynthesis of AA/AdA-CoA and membrane phosphatidylethanolamine (PE), thereby forming AA/AdA-PE (36). Consequently, inhibition of ACSL4 or LPCAT3 attenuates

ferroptosis across various conditions. Of note, both ω -6 and ω -3 PUFAs influence ferroptosis, with ω -6 PUFAs restoring ferroptosis sensitivity in ACSL4-deficient cells (37), while dietary supplementation with a mix of ω -6 and ω -3 PUFAs promotes ferroptosis-related inflammatory bowel disease in mice (38). In addition, Delesderrier *et al* (39) reported that ω -6 PUFAs have relatively high susceptibility to lipid peroxidation and may therefore be involved in ferroptosis. By contrast, ω -3 PUFAs promote intracellular antioxidant synthesis and reduce the formation of hydroperoxides, which induce ferroptosis.

In addition, the activity of the arachidonate lipoxygenase (ALOX) family, consisting of six members (ALOXE3, ALOX5, ALOX12, ALOX12B, ALOX15 and ALOX15B), plays a tissue- or cell-dependent role in mediating the peroxidation of PUFAs to produce lipid peroxides (AA/AdA-PE-OOHs), which leads to ferroptosis. These lipid peroxides undergo secondary reactions, yielding electrophilic oxidative truncations prone to interacting with protein nucleophilic sites, thereby forming a network regulating ferroptosis sensitivity (36).

Lipid storage and degradation are also intimately linked to ferroptosis. Selective lipid droplet degradation via lipid autophagy, mediated by RAB7A, member RAS oncogene family, augments free fatty acid production, thereby promoting lipid peroxidation and subsequent ferroptosis (40). Tumor protein D52-dependent lipid storage inhibits RSL3 (a selective ferroptosis inducer)-induced ferroptosis in hepatocellular carcinoma (HCC) cells (40). Periplasmicin 2, a member of the lipodropin family, also inhibits ferroptosis in gastric cancer cells (41). Acetyl-CoA carboxylase α , a central enzyme involved in fatty acid β -oxidation and fatty acid biosynthesis, plays an environmentally-dependent role in promoting ferroptosis, suggesting that β -oxidation of lipids is also associated with ferroptosis (36). Magtanong *et al* (42) identified mono-unsaturated fatty acids (MUFAs) as suppressors of ferroptosis that act downstream of or in parallel with GPX4 and that also block the accumulation of plasma membrane lipid ROS. At the same time, exogenous MUFAs reduce PUFA incorporation into phospholipids, ultimately leading to a ferroptosis-resistant cellular state.

Besides, the redox regulation of ferroptosis includes not only the GSH/GPX4 axis, but also ferroptosis suppressor protein 1 (FSP1)-ubiquinol (CoQH2), cyclohydrolase-1 (GCH1)-tetrahydrobiopterin (BH4). The activity of FSP1 is not affected by intracellular GSH levels, GPX4 activity or p53 protein status. Instead, its activity is regulated by extra-mitochondrial ubiquinone (also known as coenzyme Q10 or CoQ10), a molecule that protects cells from iron-mediated oxidative damage. CoQ10 is effective in preventing lipid peroxidation in its reduced state, CoQH2, and FSP1 promotes the regeneration of CoQ10 by a mechanism that is dependent on NADPH (43). In addition, guanosine-5'-triphosphate (GTP) is involved in a key, GPX4-independent regulatory system for iron oxidation via the GCH1-BH4 pathway. BH4 is biosynthesized by GTP via three enzymatic steps catalyzed by GCH1, 6-pyrrolyltetrahydropterin synthetase and heptapterin reductase, respectively. GCH1 plays a rate-limiting role and its expression level has a major impact on the ability of cells to resist iron toxicity. If GCH1 expression is genetically or pharmacologically inhibited, it leads to BH4 deficiency, which in turn causes intracellular peroxide accumulation and iron

deposition. Conversely, overexpression of GCH1 increases BH4 biosynthesis, thereby decreasing ROS production (43). From these findings, lipid metabolism emerges as a pivotal player in the intricate orchestration of ferroptosis.

3. Ferroptosis and HNC

Ferroptosis in the prognosis of HNC. Examination of the GEPIA database revealed the survival rate of patients with tongue squamous cell carcinoma (TSCC) with high levels of brain abundant membrane attached signal protein 1 (BASP1) to be low, and that of patients with TSCC with low BASP1 levels to be high. Subsequent statistical analysis of paraffin-embedded TSCC tissues revealed that the level of BASP1 was positively associated with the TSCC clinical stage and T-grade, indicating its potential use as a prognostic indicator (44). BASP1 is an inhibitor of ferroptosis in HNSCC cells that influences the immuno-oncological microenvironment and is a potential predictive biomarker for anti-tumor immunotherapy. Combining induced ferroptosis with BASP1 inhibition is a promising therapeutic strategy for overcoming immunotherapy resistance (45). To date, these biomarkers regarding the relationship between HNC and ferroptosis have not been validated and used in a clinical setting, and their sensitivity and specificity in the prognosis of HNC cannot be determined. In addition, a 16-DNA methylation signature associated with ferroptosis has been developed as a biomarker to assess the prognosis of patients with HNSCC, including those with OSCC (46). Furthermore, Wu *et al* (47) have described a novel long non-coding RNA signature related to ferroptosis that is independent of expression levels and that offers potential for predicting patient prognosis and clinical applications in HNSCC.

Using a nasopharyngeal carcinoma (NPC)-affiliated HNSCC database, the role of ferroptosis-related genes in HNSCC and NPC was investigated and it was found that ferroptosis-related genes may affect the tumor immune microenvironment in NPC. Screening identified the ferroptosis-related gene autophagy protein 5 (ATG5), as a significant independent prognostic marker. High expression of ATG5 in patients with HNSCC is associated with worse overall survival and poorer response and survival rates following immune checkpoint inhibition therapy. Consequently, ATG5 is a key immune infiltration-associated ferroptosis-associated factor that has potential to be a prognostic biomarker and therapeutic target in NPC and HNSCC (48).

Overall, studying the correlation between the expression levels of ferroptosis-related genes and prognosis, alongside the construction of precise prognostic models, holds promise for providing diversified treatment options for patients with HNC.

Ferroptosis in targeted therapy for HNC. The main treatments currently available for HNC include radiotherapy (RT), chemotherapy and surgery. In RT, ROS are produced when water molecules absorb ionizing radiation. These ROS further interact with PUFA, triggering a process of lipid peroxidation and peroxidation of membrane phospholipids, which may ultimately lead to ferroptosis (34). It may thus be expected that RT can inhibit tumor progression by inducing iron-associated death. Furthermore, RT resistance

may decrease the effectiveness of RT, so it is necessary to increase the radiosensitivity. Feng *et al* (49) suggested that ferroptosis inducers enhance the radiosensitivity of cells by inducing ferroptosis, blocking the activation of the ferroptosis defense system, increasing the total intracellular iron content, promoting the production of ROS, decreasing the concentration of glutathione and increasing the radiation resistance to lipid peroxidation in cancer cells. Ma *et al* (50) showed that the use of iron-oxide nanocarriers could enhance the anti-tumor effect of cisplatin while reducing the side effects produced by ROS. Cisplatin can produce hydrogen peroxide (H_2O_2) in the cytoplasm through a series of reactions in the tumor microenvironment (TME). Subsequently, H_2O_2 can be converted to toxic hydroxyl radicals via the Fenton reaction catalyzed by iron ions, which triggers apoptosis and ferroptosis in tumor cells. It may be assumed that ferroptosis, as a newly discovered form of cell death, can be combined with other cancer therapeutic modalities to inhibit tumor progression, which will need to be explored by future experiments in HNC. However, the majority of HNC-based studies investigating the role of ferroptosis in HNC treatment involve targeted therapy.

In HNSCC, upregulated Nrf2 can directly bind to the promoter region of SLC7A11, thereby inducing System Xc-. This facilitates the translocation of extracellular cystine into the cell, subsequently elevating GSH levels and upregulating GPX4 expression. Elevated GPX4 then inhibits ROS and lipid peroxidation levels, ultimately diminishing the sensitivity of HNSCC cells to ferroptosis. This leads to increased resistance to RT (51). Modulation of the Nrf2/SLC7A11/ferroptosis pathway is therefore a potential therapeutic avenue to enhance HNSCC sensitivity to RT.

Analysis of The Cancer Genome Atlas database revealed elevated CDH4 expression in HNSCC and oral squamous cell carcinoma (OSCC) tissues compared with normal tissues. In the CDH4 overexpression group, GSH was elevated, oxidized glutathione (GSSG) was decreased and the GSH/GSSG ratio was elevated, which is consistent with the ferroptosis mechanism. These findings confirmed that CDH4 can decrease the sensitivity of cells to iron death and suggested that the effect of CDH4 on cell proliferation may be due to its inhibition of ferroptosis. CDH4 enhances the epithelial-mesenchymal transition (EMT) pathway, thereby diminishing OSCC cell sensitivity to ferroptosis and promoting tumor proliferation, invasion and metastasis (52). Conversely, ferroptosis significantly affects the overall survival of patients with HNSCC, primarily by regulating extracellular matrix structure, humoral immune response and vascular smooth muscle contraction, potentially via modulation of cancer stem cell proliferation.

Several other genes, including ACSL1, SLC39A14, TFRC and *homo sapiens* prion protein, exhibit close associations with ferroptosis, HNSCC development and long-term patient prognosis, underscoring their potential as therapeutic targets (53). Notably, GPX4 is a negative regulator of ferroptosis and its inhibitors induce ferroptosis in tumor cells. Compounds that directly inactivate GPX4 and promote ferroptosis are considered class II ferroptosis agonists. For instance, (1S,3R)-RSL3 is a ferroptosis inducer with selective cytotoxic effects on tumor cells with RAS mutations, which occur in several cancers, including colorectal cancer, embryonal carcinoma, alveolar rhabdomyosarcoma and melanoma (54,55). RSL3 and

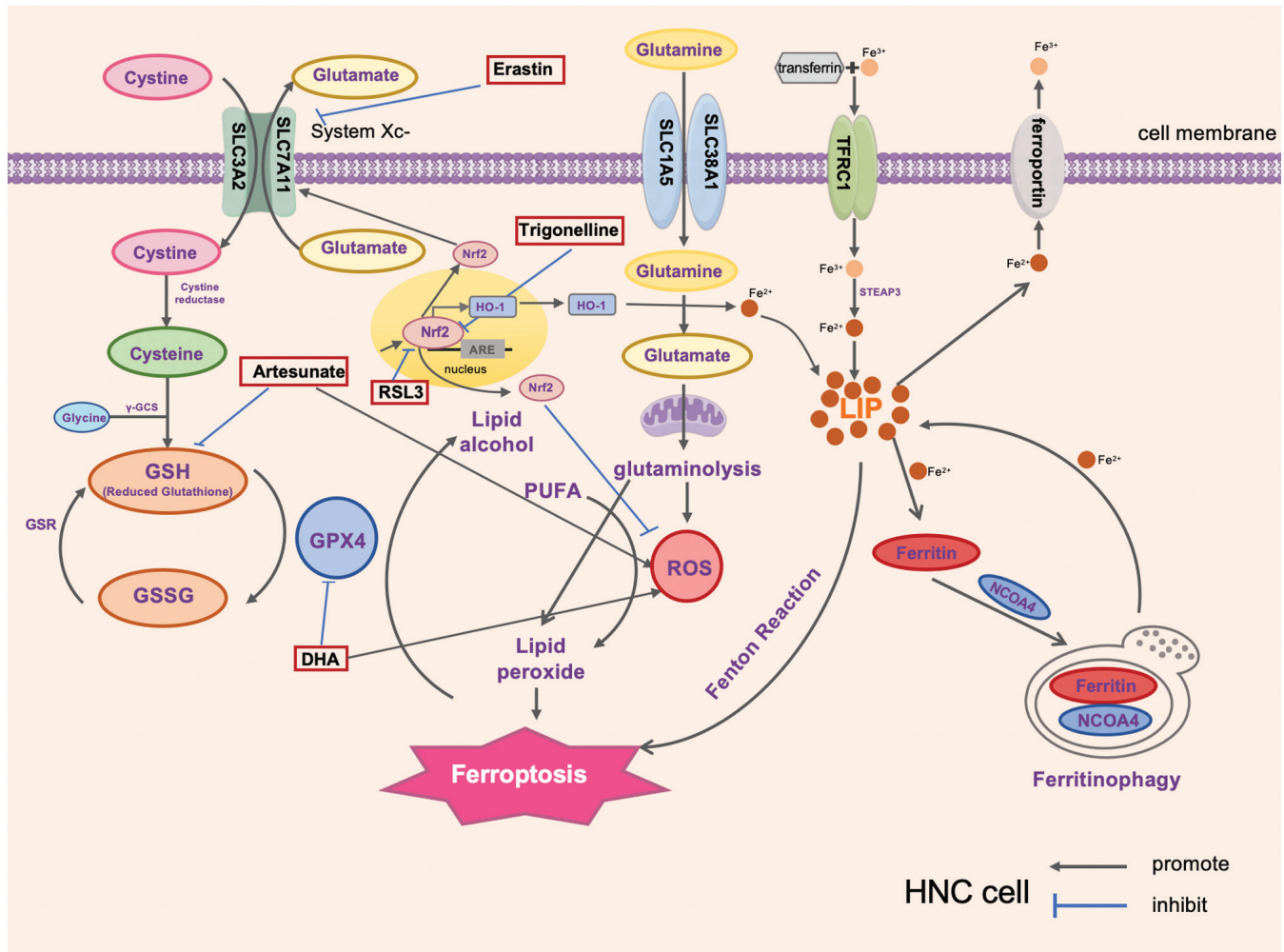


Figure 1. Critical ferroptosis factors in HNC and therapeutic drugs that target ferroptosis. HNC, head and neck cancer; SLC3A2, recombinant solute carrier family 3 member 2; γ -GCS, γ -glutamylcysteine synthetase; GSR, GSH reductase; GSH, glutathione; GSSG, oxidized GSH; GPX4, GSH peroxidase 4; Nrf2, nuclear factor erythroid 2-related factor 2; ARE, antioxidant response element; HO-1, heme oxygenase-1; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; TFR1, transferrin receptor 1; STEAP3, six-transmembrane epithelial antigen of prostate; LIP, labile iron pool; NCOA4, nuclear receptor coactivator 4; DHA, dihydroartemisinin; RSL3, a selective ferroptosis inducer.

trigonelline promote ferroptosis in HNC cells by inhibiting Nrf2 gene expression, which increases resistance to ferroptosis (56). In addition, artesunate and dihydroartemisinin (DHA), semi-synthetic derivatives of the traditional Chinese medicine component artemisinin, can also induce ferroptosis in HNSCC cells. Artesunate can overcome ferroptosis resistance in HNSCC by inhibiting the Nrf2-ARE pathway (57). By contrast, DHA has anti-tumor activity against HNSCC cells by inhibiting the cell cycle, inducing ferroptosis and apoptosis, and inhibiting angiogenesis (58). Furthermore, consistent with lipid-ROS accumulation and increased iron ions in ferroptosis, photodynamic therapy for oral tongue squamous cell carcinoma (OTSCC) with a supramolecular nanodrug consisting of the ferroptosis inducer erastin and photosensitizer chlorine 6 demonstrated enhanced anti-cancer effects by alleviating hypoxia and promoting ROS production. In addition, the ROS and O_2 concentration were increased in the tumor cells and SLC7A11 expression, which is upregulated in OTSCC, was inhibited. These findings indicate that the ferroptosis-promoting photodynamic therapy approach significantly enhances anti-cancer effects by alleviating hypoxia and

promoting ROS production (59). Fig. 1 summarizes some of the factors that target HNC to promote ferroptosis.

Drugs that target ferroptosis can be combined with radiotherapy, chemotherapy and immunotherapy for the treatment of cancers (34,49,50). It has been reported that mesenchymal cancer cells are metastasis-prone cells that have been found to be highly sensitive to ferroptosis (43). Therefore, it may be speculated that inducing ferroptosis can inhibit the metastatic spread of HNC. Raudenská *et al* (22) suggested that the mesenchymal subtypes subgroup of HNSCC characterized by elevated expression of EMT-related genes may be the most sensitive to ferroptosis. It may be hypothesized that patients with HNC with elevated expression of EMT-related genes may benefit more from ferroptosis-targeting therapies. Future in-depth studies in this area will help develop new drugs for HNC. In theory, targeting ferroptosis in HNC may be combined with other treatments to improve HNC outcomes. It may be speculated that criteria such as iron levels, ferroptosis-related gene expression and mutations may be available to determine the clinical applicability of ferroptosis-targeted therapy for cancer. To date, the ferroptosis inducers sulfasalazine, altretamine,

sorafenib and statins have been approved by the Food and Drug Administration as anticancer drugs for the treatment of cancers (60). However, the potential adverse effects of ferroptosis inducers during tumor therapy remain elusive and future in-depth studies in this area are still needed. In conclusion, further investigation into the expression of ferroptosis-related genes in HNC tumor cells holds promise for developing targeted therapeutic approaches aimed at inducing ferroptosis, thereby offering a novel direction for HNC treatment.

4. Conclusions and perspective

Ferroptosis, as a newly recognized form of cell death, has become an important research field in tumor development and treatment. In HNC, because cell death can act as a second messenger to guide the immune system and the tissue microenvironment to ensure tissue repair and homeostasis, the various cell death types can have multiple effects on treatment response (22). In HNC, however, the interplay between the various cell death pathways has not been identified. Both basic and clinical research has explored the role of ferroptosis in cancers, to advance cancer prevention, diagnosis, prognosis and treatment. Despite this growing interest, there remains a notable scarcity of studies focusing on ferroptosis in HNC. Current research predominantly centers on leveraging ferroptosis to target and sensitize HNC cells to chemotherapy and to unravel the underlying mechanisms.

It is evident that certain cells undergo adaptations to evade ferroptosis and understanding these adaptations is of paramount importance in identifying biomarkers sensitive to ferroptosis. The identification of such biomarkers promises to significantly augment our understanding of ferroptosis promotion in tumors, including HNC. However, there are potential mechanisms by which HNC cells may develop resistance to ferroptosis-inducing therapies. To the best of our knowledge, studies applying ferroptosis-inducing therapies to HNC are currently lacking, raising doubts about the resistance pathway.

It has been shown that CD8⁺ T cells could promote ferroptosis in mouse melanoma tumor cells during cancer immunotherapy (61). Ferroptosis can reduce the number of immune cells to suppress the immune function of immune cells (62). Combined use of cinnamaldehyde dimer, which causes depletion of intracellular GSH in breast cancer cells, and sorafenib resulted in a significant enhancement of ferroptosis in 'cold' tumors and triggered a strong immune response *in vivo* (63). These studies have shown that ferroptosis can both promote and impair immune function. Fan *et al* (64) showed that inducing cancer cells to undergo ferroptosis may promote the expression of their immunogenicity and in turn the anticancer activity of immune cells. In addition, in small cell lung cancer, the release of interferon γ (IFN γ) from the CD8⁺ T cell populations reduces SLC3A2 and SLC7A11 expression, thereby promoting lipid peroxidation as well as ferroptosis of cancer cells (65). In HCC, inhibition of apolipoprotein C-1, a key protein in lipid metabolism, can also promote M1 polarization via the ferroptosis pathway and reshape the tumor immune microenvironment and improve anti-programmed cell death 1 immunotherapy for HCC (66). However, the existing studies did not point out the relationship between ferroptosis and immunotherapy for HNC and the role regarding the association

between HNC cells and surrounding stromal and immune cells affecting ferroptosis. Ferroptosis may play a role in immunotherapy for other cancers, and in the future, ferroptosis-related immunogenicity could broaden the scope of immunotherapy and provide personalized therapeutic options for patients with HNC. Certain non-coding RNAs may also be involved in ferroptosis: Huang *et al* (67) reported that long non-coding RNAs can affect ferroptosis by modulating GPX4 activity, Fe²⁺ levels, cysteine metabolism and ROS levels. The impact of hypoxia is a common feature of solid tumors and the hypoxic TME is of a certain relevance to ferroptosis. The related pathways between ferroptosis under hypoxia mainly include the Nrf2/HO-1 signalling pathway and the p62/kelch-like ech-associated protein-1/Nrf2 signalling pathway. Meanwhile, certain factors also participate in the occurrence of ferroptosis under hypoxia, such as hypoxia-inducible factor-1, NCOA4 and divalent metal transporter 1 (68). However, how non-coding RNAs regulate ferroptosis and how the hypoxic TME influences ferroptosis in HNC remains elusive.

As a more superficial tumor, HNC can be studied by targeting ferroptosis and by combining ferroptosis with light stimulation to further explore the clinical application of photodynamic therapy. The study of ferroptosis and its regulation with respect to cancer therapy has great potential to deliver therapeutic advances; however, few studies (including basic and clinical trials) related to ferroptosis in HNC have been reported recently. In-depth studies from multiple perspectives, such as epigenetics, gene mutation, TME and tumor immunity, are warranted to understand the regulatory mechanism of ferroptosis in HNC cells. Findings from such studies will provide new ideas and strategies for the treatment of HNC.

Acknowledgements

The authors would like to acknowledge Dr Shuangping Liu at the Chronic Disease Research Center (Medical College, Dalian University, Dalian, Liaoning, China) for her assistance with the editing and revising of the manuscript.

Funding

The present study was funded by the Bengbu Medical University Science and Technology Programme (2022byzd164) and the Scientific Research Fund Project of Anhui Medical University (2023xkj195).

Availability of data and materials

Not applicable.

Authors' contributions

XW designed and organized this manuscript. KL and TS wrote the basic sections of the manuscript. SX, WW and YF revised the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Bertheloot D, Latz E and Franklin BS: Necroptosis, pyroptosis and apoptosis: An intricate game of cell death. *Cell Mol Immunol* 18: 1106-1121, 2021.
- Tang D, Kang R, Berghe TV, Vandenabeele P and Kroemer G: The molecular machinery of regulated cell death. *Cell Res* 29: 347-364, 2019.
- Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P, Alnemri ES, Altucci L, Amelio I, Andrews DW, *et al*: Molecular mechanisms of cell death: Recommendations of the nomenclature committee on cell death 2018. *Cell Death Differ* 25: 486-541, 2018.
- Conradt B: Genetic control of programmed cell death during animal development. *Annu Rev Genet* 43: 493-523, 2009.
- Zhang X, Huang Z, Xie Z, Chen Y, Zheng Z, Wei X, Huang B, Shan Z, Liu J, Fan S, *et al*: Homocysteine induces oxidative stress and ferroptosis of nucleus pulposus via enhancing methylation of GPX4. *Free Radic Biol Med* 160: 552-565, 2020.
- Torti SV and Torti FM: Iron and cancer: More ore to be mined. *Nat Rev Cancer* 13: 342-355, 2013.
- Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, Fulda S, Gascón S, Hatzios SK, Kagan VE, *et al*: Ferroptosis: A regulated cell death nexus linking metabolism, redox biology, and disease. *Cell* 171: 273-285, 2017.
- Xie Y, Hou W, Song X, Yu Y, Huang J, Sun X, Kang R and Tang D: Ferroptosis: Process and function. *Cell Death Differ* 23: 369-379, 2016.
- Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, Gleason CE, Patel DN, Bauer AJ, Cantley AM, Yang WS, *et al*: Ferroptosis: An iron-dependent form of nonapoptotic cell death. *Cell* 149: 1060-1072, 2012.
- Chen X, Kang R, Kroemer G and Tang D: Broadening horizons: The role of ferroptosis in cancer. *Nat Rev Clin Oncol* 18: 280-296, 2021.
- Mahoney-Sánchez L, Bouchaoui H, Ayton S, Devos D, Duce JA and Devedjian JC: Ferroptosis and its potential role in the pathophysiology of Parkinson's disease. *Prog Neurobiol* 196: 101890, 2021.
- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 71: 209-249, 2021.
- Johnson DE, Burtress B, Leemans CR, Lui VVY, Bauman JE and Grandis JR: Head and neck squamous cell carcinoma. *Nat Rev Dis Primers* 6: 92, 2020.
- Hammond EC and Horn D: Smoking and death rates: Report on forty-four months of follow-up of 187,783 men. 2. Death rates by cause. *J Am Med Assoc* 166: 1294-1308, 1958.
- Hashibe M, Brennan P, Benhamou S, Castellsague X, Chen C, Curado MP, Dal Maso L, Daudt AW, Fabianova E, Fernandez L, *et al*: Alcohol drinking in never users of tobacco, cigarette smoking in never drinkers, and the risk of head and neck cancer: Pooled analysis in the international head and neck cancer epidemiology consortium. *J Natl Cancer Inst* 99: 777-789, 2007.
- Mehanna H, Beech T, Nicholson T, El-Hariry I, McConkey C, Paleri V and Roberts S: Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer-systematic review and meta-analysis of trends by time and region. *Head Neck* 35: 747-755, 2013.
- Canning M, Guo G, Yu M, Myint C, Groves MW, Byrd JK and Cui Y: Heterogeneity of the head and neck squamous cell carcinoma immune landscape and its impact on immunotherapy. *Front Cell Dev Biol* 7: 52, 2019.
- Sturgis EM and Cinciripini PM: Trends in head and neck cancer incidence in relation to smoking prevalence: An emerging epidemic of human papillomavirus-associated cancers? *Cancer* 110: 1429-1435, 2007.
- Dai E, Han L, Liu J, Xie Y, Kroemer G, Klionsky DJ, Zeh HJ, Kang R, Wang J and Tang D: Autophagy-dependent ferroptosis drives tumor-associated macrophage polarization via release and uptake of oncogenic KRAS protein. *Autophagy* 16: 2069-2083, 2020.
- Hassannia B, Vandenabeele P and Vanden Berghe T: Targeting ferroptosis to iron out cancer. *Cancer Cell* 35: 830-849, 2019.
- Mou Y, Wang J, Wu J, He D, Zhang C, Duan C and Li B: Ferroptosis, a new form of cell death: Opportunities and challenges in cancer. *J Hematol Oncol* 12: 34, 2019.
- Raudenská M, Balvan J and Masařík M: Cell death in head and neck cancer pathogenesis and treatment. *Cell Death Dis* 12: 192, 2021.
- Rochette L, Dogon G, Rigal E, Zeller M, Cottin Y and Vergely C: Lipid peroxidation and iron metabolism: Two corner stones in the homeostasis control of ferroptosis. *Int J Mol Sci* 24: 449, 2022.
- Gao G, Li J, Zhang Y and Chang YZ: Cellular iron metabolism and regulation. In: Chang YZ (ed). *Brain Iron Metabolism and CNS Diseases. Advances in Experimental Medicine and Biology*. Vol 1173. Springer Singapore, Singapore, pp21-32, 2019.
- Rochette L, Gudjoncik A, Guenancia C, Zeller M, Cottin Y and Vergely C: The iron-regulatory hormone hepcidin: A possible therapeutic target? *Pharmacol Ther* 146: 35-52, 2015.
- Fujimaki M, Furuya N, Saiki S, Amo T, Imamichi Y and Hattori N: Iron supply via NCOA4-mediated ferritin degradation maintains mitochondrial functions. *Mol Cell Biol* 39: e00010-19, 2019.
- Do MT, Kim HG, Choi JH and Jeong HG: Metformin induces microRNA-34a to downregulate the Sirt1/Pgc-1α/Nrf2 pathway, leading to increased susceptibility of wild-type p53 cancer cells to oxidative stress and therapeutic agents. *Free Radic Biol Med* 74: 21-34, 2014.
- Wei R, Zhao Y, Wang J, Yang X, Li S, Wang Y, Yang X, Fei J, Hao X, Zhao Y, *et al*: Tagitinin C induces ferroptosis through PERK-Nrf2-HO-1 signaling pathway in colorectal cancer cells. *Int J Biol Sci* 17: 2703-2717, 2021.
- Dalton TP, Chen Y, Schneider SN, Nebert DW and Shertzer HG: Genetically altered mice to evaluate glutathione homeostasis in health and disease. *Free Radic Biol Med* 37: 1511-1526, 2004.
- Oestreicher J and Morgan B: Glutathione: Subcellular distribution and membrane transport ¹. *Biochem Cell Biol* 97: 270-289, 2019.
- Rochette L and Vergely C: Coronary artery disease: Can amino-thiols be distinguished from reactive oxygen species? *Nat Rev Cardiol* 13: 128-130, 2016.
- Belalcázar AD, Ball JG, Frost LM, Valentovic MA and Wilkinson J IV: Transsulfuration is a significant source of sulfur for glutathione production in human mammary epithelial cells. *ISRN Biochem* 2013: 637897, 2014.
- Wu X, Liu C, Li Z, Gai C, Ding D, Chen W, Hao F and Li W: Regulation of GSK3β/Nrf2 signaling pathway modulated erastin-induced ferroptosis in breast cancer. *Mol Cell Biochem* 473: 217-228, 2020.
- Yang WS, Kim KJ, Gaschler MM, Patel M, Shchepinov MS and Stockwell BR: Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis. *Proc Natl Acad Sci USA* 113: E4966-E4975, 2016.
- Kagan VE, Mao G, Qu F, Angeli JP, Doll S, Croix CS, Dar HH, Liu B, Tyurin VA, Ritov VB, *et al*: Oxidized arachidonic and adrenic PEs navigate cells to ferroptosis. *Nat Chem Biol* 13: 81-90, 2017.
- Tang D, Chen X, Kang R and Kroemer G: Ferroptosis: Molecular mechanisms and health implications. *Cell Res* 31: 107-125, 2021.
- Doll S, Proneth B, Tyurina YY, Panzilius E, Kobayashi S, Ingold I, Irmeler M, Beckers J, Aichler M, Walch A, *et al*: ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nat Chem Biol* 13: 91-98, 2017.
- Mayr L, Grabherr F, Schwärzler J, Reitmeier I, Sommer F, Gehmacher T, Niederreiter L, He GW, Ruder B, Kunz KTR, *et al*: Dietary lipids fuel GPX4-restricted enteritis resembling Crohn's disease. *Nat Commun* 11: 1775, 2020.
- Delesderrier E, Monteiro JDC, Freitas S, Pinheiro IC, Batista MS and Citelli M: Can iron and polyunsaturated fatty acid supplementation induce ferroptosis? *Cell Physiol Biochem* 57: 24-41, 2023.
- Bai Y, Meng L, Han L, Jia Y, Zhao Y, Gao H, Kang R, Wang X, Tang D and Dai E: Lipid storage and lipophagy regulates ferroptosis. *Biochem Biophys Res Commun* 508: 997-1003, 2019.

41. Sun X, Yang S, Feng X, Zheng Y, Zhou J, Wang H, Zhang Y, Sun H and He C: The modification of ferroptosis and abnormal lipometabolism through overexpression and knockdown of potential prognostic biomarker perilipin2 in gastric carcinoma. *Gastric Cancer* 23: 241-259, 2020.
42. Magtanong L, Ko PJ, To M, Cao JY, Forcina GC, Tarangelo A, Ward CC, Cho K, Patti GJ, Nomura DK, *et al*: Exogenous mono-unsaturated fatty acids promote a ferroptosis-resistant cell state. *Cell Chem Biol* 26: 420-432.e9, 2019.
43. Liu Y, Lu S, Wu LL, Yang L, Yang L and Wang J: The diversified role of mitochondria in ferroptosis in cancer. *Cell Death Dis* 14: 519, 2023.
44. Li Y, Wu T, Jiao Z and Yang A: BASP1 is up-regulated in tongue squamous cell carcinoma and associated with a poor prognosis. *Asian J Surg* 45: 1101-1106, 2022.
45. Pan X, Xu X, Wang L, Zhang S, Chen Y, Yang R, Chen X, Cheng B, Xia J and Ren X: BASP1 is a prognostic biomarker associated with immunotherapeutic response in head and neck squamous cell carcinoma. *Front Oncol* 13: 1021262, 2023.
46. Xu Y, Hong M, Kong D, Deng J, Zhong Z and Liang J: Ferroptosis-associated DNA methylation signature predicts overall survival in patients with head and neck squamous cell carcinoma. *BMC Genomics* 23: 63, 2022.
47. Wu C, Liu F, Chen H, Liu Q, Song C, Cheng K, Gao Z and Fan C: Identification of ferroptosis-related lncRNA pairs for predicting the prognosis of head and neck squamous cell carcinoma. *J Oncol* 2022: 7602482, 2022.
48. Shi M, Du J, Shi J, Huang Y, Zhao Y and Ma L: Ferroptosis-related gene ATG5 is a novel prognostic biomarker in nasopharyngeal carcinoma and head and neck squamous cell carcinoma. *Front Bioeng Biotechnol* 10: 1006535, 2022.
49. Feng Y, Li X, Yang B, Li M, Du Y, Wang J, Liu S, Gong L, Li L and Gao L: The role of ferroptosis in radiotherapy and combination therapy for head and neck squamous cell carcinoma (review). *Oncol Rep* 51: 79, 2024.
50. Ma P, Xiao H, Yu C, Liu J, Cheng Z, Song H, Zhang X, Li C, Wang J, Gu Z and Lin J: Enhanced cisplatin chemotherapy by iron oxide nanocarrier-mediated generation of highly toxic reactive oxygen species. *Nano Lett* 17: 928-937, 2017.
51. Feng L, Zhao K, Sun L, Yin X, Zhang J, Liu C and Li B: SLC7A11 regulated by NRF2 modulates esophageal squamous cell carcinoma radiosensitivity by inhibiting ferroptosis. *J Transl Med* 19: 367, 2021.
52. Xie J, Lan T, Zheng DL, Ding LC and Lu YG: CDH4 inhibits ferroptosis in oral squamous cell carcinoma cells. *BMC Oral Health* 23: 329, 2023.
53. Liu F, Tang L, Li Q, Chen L, Pan Y, Yin Z, He J and Tian J: Single-cell transcriptomics uncover the key ferroptosis regulators contribute to cancer progression in head and neck squamous cell carcinoma. *Front Mol Biosci* 9: 962742, 2022.
54. Codenotti S, Poli M, Asperti M, Zizioli D, Marampon F and Fanzani A: Cell growth potential drives ferroptosis susceptibility in rhabdomyosarcoma and myoblast cell lines. *J Cancer Res Clin Oncol* 144: 1717-1730, 2018.
55. Sui X, Zhang R, Liu S, Duan T, Zhai L, Zhang M, Han X, Xiang Y, Huang X, Lin H and Xie T: RSL3 drives ferroptosis through GPX4 inactivation and ROS production in colorectal cancer. *Front Pharmacol* 9: 1371, 2018.
56. Shin D, Kim EH, Lee J and Roh JL: Nrf2 inhibition reverses resistance to GPX4 inhibitor-induced ferroptosis in head and neck cancer. *Free Radic Biol Med* 129: 454-462, 2018.
57. Li S, Liu Y, Li J, Zhao X and Yu D: Mechanisms of ferroptosis and application to head and neck squamous cell carcinoma treatments. *DNA Cell Biol* 40: 720-732, 2021.
58. Lin R, Zhang Z, Chen L, Zhou Y, Zou P, Feng C, Wang L and Liang G: Dihydroartemisinin (DHA) induces ferroptosis and causes cell cycle arrest in head and neck carcinoma cells. *Cancer Lett* 381: 165-175, 2016.
59. Zhu T, Shi L, Yu C, Dong Y, Qiu F, Shen L, Qian Q, Zhou G and Zhu X: Ferroptosis promotes photodynamic therapy: Supramolecular photosensitizer-inducer nanodrug for enhanced cancer treatment. *Theranostics* 9: 3293-3307, 2019.
60. Wang H, Cheng Y, Mao C, Liu S, Xiao D, Huang J and Tao Y: Emerging mechanisms and targeted therapy of ferroptosis in cancer. *Mol Ther* 29: 2185-2208, 2021.
61. Wang W, Green M, Choi JE, Gijón M, Kennedy PD, Johnson JK, Liao P, Lang X, Kryczek I, Sell A, *et al*: CD8⁺ T cells regulate tumour ferroptosis during cancer immunotherapy. *Nature* 569: 270-274, 2019.
62. Chen X, Kang R, Kroemer G and Tang D: Ferroptosis in infection, inflammation, and immunity. *J Exp Med* 218: e20210518, 2021.
63. Zhou Z, Liang H, Yang R, Yang Y, Dong J, Di Y and Sun M: Glutathione depletion-induced activation of dimersomes for potentiating the ferroptosis and immunotherapy of 'cold' tumor. *Angew Chem Int Ed Engl* 61: e202202843, 2022.
64. Fan X, Fan YT, Zeng H, Dong XQ, Lu M and Zhang ZY: Role of ferroptosis in esophageal cancer and corresponding immunotherapy. *World J Gastrointest Oncol* 15: 1105-1118, 2023.
65. Niu X, Chen L, Li Y, Hu Z and He F: Ferroptosis, necroptosis, and pyroptosis in the tumor microenvironment: Perspectives for immunotherapy of SCLC. *Semin Cancer Biol* 86: 273-285, 2022.
66. Huang Y, Wang S, Ke A and Guo K: Ferroptosis and its interaction with tumor immune microenvironment in liver cancer. *Biochim Biophys Acta Rev Cancer* 1878: 188848, 2023.
67. Huang J, Wang J, He H, Huang Z, Wu S, Chen C, Liu W, Xie L, Tao Y, Cong L and Jiang Y: Close interactions between lncRNAs, lipid metabolism and ferroptosis in cancer. *Int J Biol Sci* 17: 4493-4513, 2021.
68. Gao X, Hu W, Qian D, Bai X, He H, Li L and Sun S: The mechanisms of ferroptosis under hypoxia. *Cell Mol Neurobiol* 43: 3329-3341, 2023.



Copyright © 2024 Wang et al. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.