Advances in the study of regulators of ferroptosis in head and neck squamous cell carcinoma (Review)

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Received November 23, 2022; Accepted March 15, 2023

DOI: 10.3892/ijmm.2023.5248

Abstract. Head and neck squamous cell carcinoma (HNSCC), a common malignancy of the head and neck, is associated with a rapid progression, a high mortality rate and unsatisfactory curative effects. The treatment efficacy is unsatisfactory due to chemotherapeutic drug resistance, the lack of ideal therapeutic agents, as well as the absence of clinical prognostic models. Thus, the identification of novel potential therapeutic targets for its diagnosis and treatment is vital. Ferroptosis is an iron-dependent regulatory cell death mode different from traditional cell death modes, such as apoptosis and autophagy, and has notable therapeutic potential in cancer treatment. The study of ferroptosis in HNSCC is expected to solve this bottleneck problem. In the present review, the findings, characteristics and regulatory mechanisms of ferroptosis are summarized, with emphasis on the factors and drugs that regulate ferroptosis in HNSCC, in order to provide theoretical basis for the targeted therapy of ferroptosis in HNSCC.

Contents

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Key words: ferroptosis, head and neck squamous cell carcinoma, regulatory mechanisms, regulators

1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is a general term for squamous epithelial malignancies originating in the nasal cavity, oral cavity, pharynx and larynx, accounting for >90% of head and neck cancers, with rapid progression, extensive infiltration and a poor prognosis (1). The current treatment strategies for HNSCC are mainly based on radiotherapy, chemotherapy and surgery (2). However, patients with advanced or metastatic HNSCC are highly susceptible to recurrence and metastasis following surgery. These patients are usually treated with cisplatin-based concurrent radiotherapy and targeted drug-induced chemotherapy represented by cetuximab; the efficacy of this treatment however, is hampered by the widespread development of cisplatin resistance, thus rendering cisplatin-based chemotherapeutic regimens ineffective. Previous studies have shown that promoting ferroptosis in tumor cells is a more effective method of reducing cisplatin resistance in cancer cells (3), and it can enhance the sensitivity of tumor cells to radiotherapy and chemotherapeutic drugs (4).

Ferroptosis is a regulated form of cell death that differs from the traditional cell death modality, depending on lipid peroxidation, which is induced by iron ions and reactive oxygen species (5). Initially, researchers found that ferroptosis can cause multiple tissue damage via oxidative stress pathways, which are associated with the pathogenesis of several degenerative diseases, such as Alzheimer's disease (6), ischemia-reperfusion injury (7) and osteoarthritis (8). Based on the ability of ferroptosis to induce damage to multiple tissues, it has been suggested that ferroptosis has immense therapeutic potential in the treatment of cancer with active abnormal proliferation, and the induction of ferroptosis in tumor cells may lead to the development of novel treatment strategies (9).

Therefore, the present review summarizes the mechanisms of action of ferroptosis and its regulators in HNSCC, and also discusses the drugs regulated by ferroptosis in HNSCC, in order to provide a theoretical basis for the further use of ferroptosis in the treatment of HNSCC.

2. Data collection methods

Up to October 2022, the 'PubMed', 'Springer', 'Web of Science' and 'CNKI' databases were searched for original research

articles and reviews on the progress of ferroptosis in HNSCC. The terms for the search included the following: Ferroptosis, ferroptosis and HNSCC, regulatory mechanism of ferroptosis, iron metabolism, ferroptosis inducers, ferroptosis inhibitors, ferroptosis and glutathione peroxidase 4 (GPX4), ferroptosis and System Xc^-, and ferroptosis and iron (the detailed search strategies are presented in Table I).

3. The discovery and characteristics of ferroptosis

Ferroptosis is a regulated form of cell death which differs from apoptosis, necrosis and autophagy, which can be inhibited by iron chelators and antioxidants (10), characterized by the excessive accumulation of lipid peroxides and reactive oxygen species (ROS), discovered by the Stockwell laboratory at Columbia University in 2012 (11,12). The study of ferroptosis has been gradually applied to a variety of diseases, such as tissue ischemia-reperfusion, neurological diseases, acute renal failure and tumors (13).

Ferroptosis is caused by unrestricted lipid peroxidation and plasma membrane rupture, the two main features of which are iron ion accumulation and lipid peroxidation. Morphologically, ferroptosis is manifested by cell membrane fracture and effacement, mitochondrial atrophy, reduction or even the disappearance of mitochondrial ridges, and increased cell membrane density; biologically, it is manifested by the increased generation of ROSs, iron ion aggregation, the activation of mitogen-activated protein kinases (MAPKs), and the inhibition of cysteine-glutamate reverse transporter by decreasing cystine uptake and depleting glutathione (GSH); in immunologically, it is manifested by the release of damage-associated molecular patterns, which promote inflammatory responses (14).

4. Regulatory mechanisms of ferroptosis

It has been demonstrated that the sensitivity of cells to ferroptosis is associated with multiple regulatory mechanisms, mainly including three pathways: The regulation of iron metabolism, the regulation of the system Xc^-/GSH/GPX4 antioxidant system and the regulation of lipid peroxidation, which can directly affect the sensitivity of cells to ferroptosis (14) (Fig. 1).

Regulation of iron metabolism. Iron is an essential trace metal element with redox activity in the human body; increased levels of iron and/or iron-binding proteins and dysregulated iron metabolism lead to a risk of carcinogenesis and promote tumor growth (15).

Iron in the body circulation is usually present and is transported as Fe^{3+}, stored in endosomes with transferrin (TF) in the presence of TF receptor 1, and is reduced to Fe^{2+} in the presence of iron oxidoreductase (STEAP3). When Fe^{2+} is in excess, it can be transferred to the cell plasma and eventually stored in ferritin to form labile iron pool. When the buffering capacity of ferritin is exceeded, a large amount of divalent iron ions (Fe^{2+}) catalyze hydrogen peroxide (H_2O_2), which generates toxic hydroxyl radicals (·OH) and thus triggers cellular lipid peroxidation (16). It has been found that the concentration of H_2O_2 and iron ions in a large number of tumor cells is generally low; thus, when the disorder of iron metabolism causes an increase of free iron in cells, iron catalyzes the production of ROS through the Fenton reaction, and ROS further promotes lipid peroxidation and causes lipid peroxide aggregation, which induces ferroptosis (17).

Cancer cells are more iron-dependent compared to normal cells (18), and some cancer cells exhibit iron ion aggregation. Thus, the regulation of ferroptosis via iron homeostasis can effectively kill cancer cells by increasing iron uptake, decreasing iron storage and limiting iron efflux to promote ferroptosis; and by preventing ferroptosis through iron chelators and antioxidants (19).

Cellular iron accumulation is one of the typical markers of ferroptosis, and the detection of changes in iron ions can indicate whether ferroptosis has occurred. The iron ion content is positively associated with ferroptosis, and by using the Fe^{2+} detection probe FerroOrange, flow cytometry or confocal microscopy to detect intracellular iron content, the orange fluorescence of FerroOrange is enhanced in cells in which ferroptosis occurs (20).

Regulation of the System Xc^-/GSH/GPX4 antioxidant system. Ferroptosis can be induced by exogenous or endogenous pathways. The exogenous pathway initiates ferroptosis mainly by inhibiting the functional activity of System Xc-. System Xc^- is a critical intracellular antioxidant system consisting of two subunits: Solute carrier family 7 member 11 (SLC7A11; xCT) and solute carrier family 3 member 2 (SLC3A2; CD98hc). SLC7A11 is responsible for the major transport activities, including cystine uptake and glutamate excretion; SLC3A2 functions as a chaperone protein. Cystine is exchanged into the cell at a 1:1 ratio with glutamate and is rapidly reduced to cysteine, which is involved in the synthesis of intracellular GSH and GPX4 (21).

The depletion of GSH, a key co-factor for the function of GPX4, disrupts cellular redox homeostasis, leading to the accumulation of ROS and ultimately inducing the onset of ferroptosis. Erastin can reduce GSH synthesis by targeting the inhibition of System Xc-, which reduces the cystine entering the cell and ultimately induces the onset of ferroptosis (22).

The endogenous pathway then activates ferroptosis by reducing the activity of GPX4. The basic function of GPX family members is H_2O_2 reduction at the expense of GSH, and GPX4 is the only enzyme that reduces cholesterol hydroperoxide and esterifies oxidized fatty acids (23). GPX4 is also a key regulatory component of the ferroptosis mechanism, and its reduced activity is the hallmark event for the onset of ferroptosis (24).

Regulation of lipid peroxidation. Normal cells tend to maintain a dynamic redox balance, whereas cancer cells are usually in an oxidative stress state with higher metabolic levels than normal cells, accompanied by ROS accumulation (25). The production of ROS is associated with damage to proteins, carbohydrates, lipids and nucleotides, which can eventually lead to the development of malignancies. The primary indicator of ferroptosis is the excessive accumulation of lipid peroxides and ROS, which is currently detected in laboratory mainly by the specific fluorescent probes, C11 BODIPY 581/591 and 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFH-DA) (26).

Table I. Summary of the search strategy used in the present study.

Items	Specification
Date of search	October 31, 2022
Databases and other sources searched	'PubMed' 'Springer' 'Web of Science' 'CNKI'
Search terms used	'ferroptosis' (title/abstract) 'ferroptosis' and 'HNSCC' (title/abstract) 'regulatory mechanism of ferroptosis' (title/abstract) 'iron metabolism' (title/abstract) 'ferroptosis inducers' (title/abstract) 'ferroptosis inhibitors' (title/abstract) 'ferroptosis' and 'GPX4' (title/abstract) 'ferroptosis' and 'System Xc^-' (title/abstract) 'ferroptosis' and 'Iron' (title/abstract)
Timeframe	2008-2022
Inclusion and exclusion criteria	Focus was placed on original articles and reviews in the English language about the regulatory factor of ferroptosis in head and neck squamous cell carcinoma; articles that had no information about HNSCC and ferroptosis were excluded

CNKI, China National Knowledge Infrastructure; HNSCC, head and neck squamous cell carcinoma; GPX4, glutathione peroxidase 4.

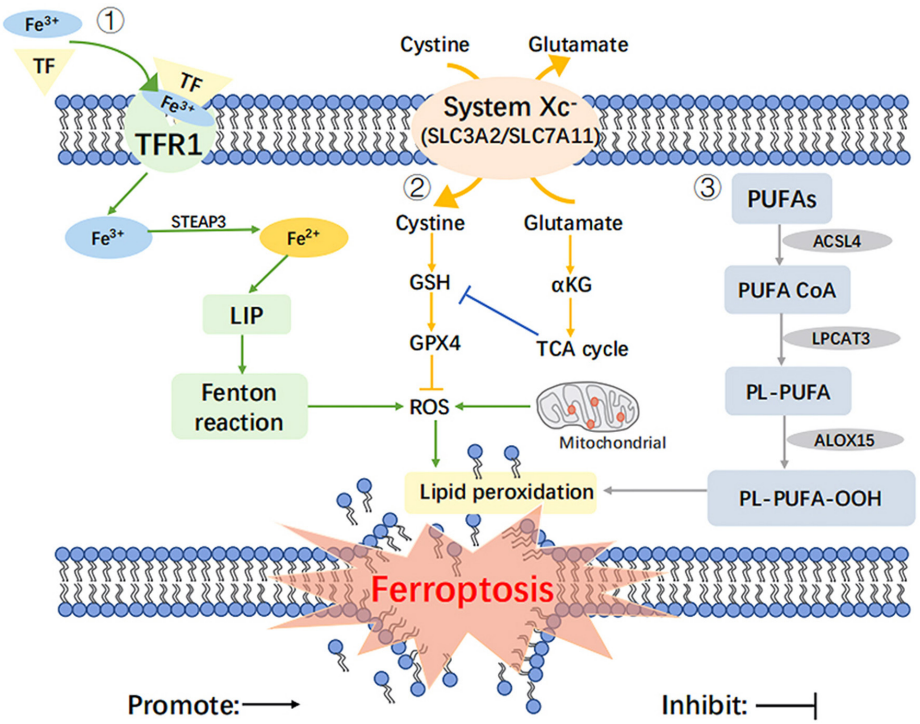

Figure 1. Regulatory mechanisms of ferroptosis. The regulatory mechanisms of ferroptosis include the following: i) The regulation of iron metabolism which involves TF, TFR1, LIP and the Fenton reaction; ii) the regulation of the System Xc-/GSH/GPX4 antioxidant system which involves GSH, GPX4, ROS, α-KG and the TCA cycle; iii) the regulation of lipid peroxidation which involves PUFAs, ACSL4, PUFA CoA, LPCAT3, PL-PUFA, ALOX15, PL-PUFA-OOH. TF, transferrin; TFR1, transferrin 1; LIP, labile iron pool; Fenton reaction, $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH$; GSH, glutathione; GPX4, glutathione peroxidase 4; ROS, reactive oxygen species; α-KG, α-ketoglutarate; TCA cycle, tricarboxylic acid cycle. PUFAs, polyunsaturated fatty acids; ACSL4, long-chain acyl-CoA synthetase 4; PUFA CoA, polyunsaturated fatty acids acetyl CoA; LPCAT3, lysophosphatidylcholine acyl-transferase 3; PL-PUFA, phospholipid polyunsaturated fatty acids; ALOX15, arachidonate 15-lipoxygenase; PL-PUFA-OOH, lipid hydrogen peroxide.

In the process of ferroptosis, polyunsaturated fatty acids (PUFAs) can be oxidized by intracellular ROS to produce unstable lipid hydroperoxides and lipid peroxides, reaching the lethal amounts and can lead to the destruction of bilayer lipid structures, thus inducing ferroptosis (27). The level of intracellular PUFAs determines the development of

ferroptosis (28,29). The enzymatic reaction of lipid peroxidation contains three key enzymes: Long-chain lipid acyl coenzyme A synthesis 4 (ACSL4) (30), lysophosphatidylcholine acyltransferase 3 (LPCAT3) (31) and arachidonic acid 15-lipoxygenase (ALOX15). Among these, ACSL4 catalyzes the conversion of free PUFAs into polyunsaturated fatty acids-acetyl coenzyme A (PUFA CoA), which is subsequently esterified by LPCAT3 into phospholipid-type polyunsaturated fatty acids (PL-PUFA), and finally ALOX15 participates in the peroxidation process of membrane phospholipids to lipid peroxides (32). In summary, disorders of lipid metabolism are closely related to cellular ferroptosis, and the accumulation of lipid oxides and ROS is necessary for ferroptosis to occur.

5. Ferroptosis regulators in head and neck squamous cell carcinoma

Cisplatin, one of the most widely used platinum compounds, is the clinical first-line chemotherapeutic agent used in the treatment of HNSCC (33). Although ~80% of patients with HNSCC are initially sensitive to this treatment, the resistance rate in subsequent treatment is as high as 70% (34), which severely diminishes the efficacy of cisplatin (35), and chemoresistance as a major cause of treatment failure in HNSCC can no longer be underestimated.

Recent research has identified some drugs that can reverse chemoresistance *in vitro*; however, the majority of these drugs have cytotoxic defects that limit their clinical application, and thus, the search for a novel methods of chemotherapy sensitization has become a hot topic and difficult issue in HNSCC research (2). Previous studies have reported that the resistance of HNSCC to cisplatin can be reduced by inducing ferroptosis without affecting normal tissues (36-38). This may provide a new strategy with which to improve the post-operative survival of patients with HNSCC. Thus, exploring ferroptosis regulators in HNSCC is critical for improving the prognosis of patients with HNSCC.

Currently, in HNSCC, studies on ferroptosis have focused on the System Xc^--GSH-GPX4 pathway, which induces ferroptosis by directly or indirectly disrupting intracellular redox homeostasis (39,40) (Table II and Fig. 2).

Activation/inhibition of ferroptosis through the regulation of SLC7A11. SLC7A11 expression has been found to be higher than normal in a variety of cancer types (21,41,42) SLC7A11 can inhibit the occurrence of ferroptosis, and its expression level is positively associated with the clinical stage of patients with HNSCC (43); in human papillomavirus (HPV)-positive patients with HNSCC, the expression level of SLC7A11 has been found to be lower than that HPV-negative patients, and positive patients may be more responsive to radiotherapy and chemotherapy (44). It is hypothesized that the ferroptosis marker gene, SLC7A11, plays a crucial role in the development of HNSCC, and targeting SLC7A11 may selectively kill tumor cells while preserving normal cells (45).

In HNSCC, multiple factors regulate the expression of SLC7A11 and thus the occurrence of ferroptosis, and hotspot factors currently studied include non-coding RNAs (ncRNAs), nuclear factor erythroid 2-related factor 2 (NRF2), suppressor of cytokine signaling 1 (SOCS1) and interleukin (IL)-6, whose regulatory mechanisms are specified below.

ncRNAs. ncRNAs account for ~98% of the human transcribed genome and are involved in a number of physiological and pathological processes, including cancer, rendering them one of the hotspots of current research (46). It has been found that ncRNAs, particularly microRNAs (miRNAs/miRs) and circular RNAs (circRNAs), are closely related to the biological process of ferroptosis, and they can regulate ferroptosis in tumor cells through transcriptional or post-transcriptional levels by mechanisms involving glutamine catabolism, mitochondria-related proteins, iron metabolism, glutathione metabolism, amino acid metabolism, lipid peroxidation, cell cycle and the p53 signaling pathway (47). Therefore, ncRNAs and downstream target genes associated with cellular ferroptosis may become novel clinical markers and therapeutic targets for HNSCC.

a) miRNAs. It has been found that miRNAs can inhibit ferroptosis in tumor cells by regulating the glutamine catabolic pathway (48-50). miR-137 and miR-9 and other miRNAs achieve the inhibition of ferroptosis in tumor cells by inhibiting a link in the glutamine catabolic pathway, further revealing the molecular mechanisms of the miRNA regulation of ferroptosis in tumor cells, and promoting tumor cells by targeting related miRNAs glutamine metabolism can improve the sensitivity of tumor cells to ferroptosis (48,51). In addition, miRNAs can inhibit tumor cell ferroptosis by regulating mitochondria-related proteins. miRNA expression differences in tumor cells can affect mitochondrial function and energy metabolism, which in turn can achieve the regulation of tumor cell ferroptosis. Mitochondrial transferrin mainly mediates the entry of Fe^{2+} into mitochondria to maintain the coordinated intracellular distribution and utilization of iron (52,53).

Previous studies have demonstrated that miRNAs are key regulators of HNSCC carcinogenesis (54-56), and they have attracted widespread attention in HNSCC. Shen *et al* (57) revealed that miR-34c-3p is closely associated with the spread, migration, invasion and apoptosis of HNSCC. As illustrated in Fig. 2, miR-34c-3p inhibits the expression of SLC7A11 by binding to the 3' non-coding region of SLC7A11, which inhibits the transport process of System Xc^-. The reduction of cysteine affects the synthesis of cysteine and glutathione, which inhibits the expression of GPX4 and ultimately induces lipid peroxidation and ROS generation, leading to ferroptosis in HNSCC and inhibiting HNSCC cell growth (58).

b) circRNAs. circRNA, which account for >90% of the human transcriptome, but have minimal potential to encode proteins, are involved in the regulation of ferroptosis in tumor cells by adsorbing certain miRNAs, functioning as competing endogenous RNAs to regulate the expression of relevant miRNA target genes and thus, and their involvement in cancer ferroptosis (59). The potential regulatory mechanisms include mitochondria-related proteins, iron metabolism, glutathione metabolism and lipid peroxidation (60).

Some circRNAs have been reported to regulate head and neck tumorigenesis (61). As also illustrated in Fig. 2, Yang *et al* (62) in HNSCC found that circRNA FNDC3B further upregulated the expression level of SLC7A11 by adsorbing miR-520d-5p, inhibited the occurrence of ferroptosis, induced

Table II. Summary of ferroptosis regulators in HNSCC.

Mechanism	Compound	(Refs.)
Activation/inhibition of ferroptosis through regulation of SLC7A11	miR-34c-3p	(58)
	circRNA FNDC3B	(62)
	NRF2	(68)
	SOCS1	(75)
	IL-6	(78)
Activation/inhibition of ferroptosis through the regulation of GPX4	RSL3	(90)
	EMP1	(95)
	PER1	(105)
	Cav-1	(112)
Regulation of ferroptosis through iron metabolic pathways	FTH1	(75)

HNSCC, head and neck squamous cell carcinoma; SLC7A11, solute carrier family 7 member 11; GPX4, glutathione peroxidase 4; circRNA, circular RNA; NRF2, nuclear factor erythroid 2-related factor 2; SOCS1, suppressor of cytokine signaling 1; IL-6, interleukin 6; RSL3, RAS-selective lethal 3; EMP1, epithelial membrane protein 1; PER1, period circadian regulator 1 (period 1); Cav-1, caveolin-1; FTH1, ferritin heavy chain.

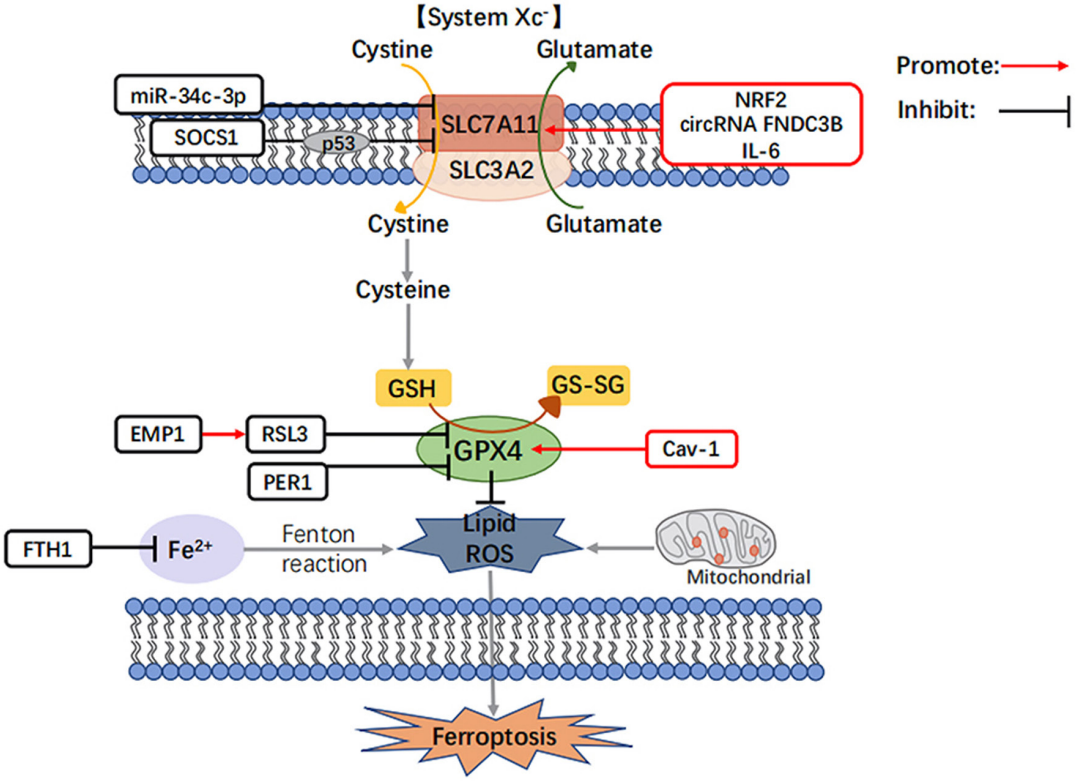

Figure 2. Roles and functions of the listed regulators in the regulation of ferroptosis in head and neck cancers. NRF2, nuclear factor erythroid 2-related factor 2; SOCS1, suppressors of cytokine signaling 1; IL-6, interleukin-6; GPX4, glutathione peroxidase 4; RSL3, an inhibitor of GPX4; EMP1, epithelial membrane protein genes 1; PER1, circadian rhythm protein 1, period-1; Cav-1, caveolin-1; FTH1, ferritin heavy chain.

epithelial-mesenchymal transition, and promoted HNSCC cell proliferation.

These studies have proven that ncRNA-based therapy may be used for cancer treatment by regulating ferroptosis; however, there is no mature technical means available for applying ncRNA-mediated tumor therapy to clinical practice. The in-depth study of the molecular mechanisms of the ferroptosis regulation of tumor cells by ncRNAs may provide potential targets and novel therapeutic strategies for cancer treatment. Thus far, circFNDC3B and miR-34c-3p have only been studied in HNSCC.

NRF2. The redox state of the cell is determined by the balance between the production of reactive substances and the subsequent reduction of a series of antioxidant defense systems. Excess reactive substances react to generate lipid peroxides, which are associated with the progression of a number of

diseases, including cancer, diabetes, cardiovascular disease and liver disease (63). The intracellular antioxidant defense system is used to control the level of reactive substances.

NRF2, the central transcriptional regulator in the oxidative stress response, plays a key role in cellular antioxidant responses, redox homeostasis and metabolic homeostasis, and is involved in lipid peroxidation and free iron accumulation (64); numerous factors of the ferroptosis cascade are target genes of NRF2 (65), indicating its critical role in mediating the ferroptosis response. When oxidative stress occurs, NRF2 activates and induces the expression of antioxidant genes to eliminate ROS, and if excessive ROS generation causes peroxidation, it initiates a cellular suicide program, in which ferroptosis is the most critical. Furthermore, a number of antioxidants such as GSH, heme oxygenase 1 and NAD(P)H quinone dehydrogenase 1 are downstream genes of NRF2 (66). Cancer and neurodegenerative diseases highly associated with oxidative stress render NRF2 a crucial therapeutic target for these diseases (64,67).

As illustrated in Fig. 2, in HNSCC, the high expression of NRF2 induces the high expression of SLC7A11 by directly binding to the promoter region of SLC7A11, which in turn induces System Xc^- to transport intracellular glutamate away and extracellular cystine in, and elevated cystine leads to an increase in glutathione, which in turn increases the expression of GPX4. In turn, the high expression of GPX4 suppresses ROS and lipid peroxidation levels, ultimately inhibiting the sensitivity of HNSCC cells to ferroptosis and leading to increased patient resistance to radiation therapy (68). Indeed, in other types of cancer, such as colorectal (69), lung (70), stomach (71) and ovarian cancers (72), NRF2 also functions as an oncogenic transcription factor, and cancer cells frequently exhibit the overexpression of NRF2, which plays a key role in counteracting environmental or intracellular stress (21,73).

Therefore, controlling the progression of HNSCC by modulating the NRF2/SLC7A11/ferroptosis pathway provides a potential therapeutic target for enhancing the sensitivity of HNSCC to radiotherapy, and exploring the combination of NRF2 inhibitors with radiotherapy remains a direction for future research.

SOCS1. SOCS1 is considered as a negative feedback regulator of cytokine signaling and is also involved in the formation of the E3 ubiquitin ligase complex (74), which plays critical regulatory roles in both cell growth and proliferation. As illustrated in Fig. 2, in HNSCC, SOCS1 functions as a 'ferroptosis activator' similar to miR-34c-3p by suppressing the transcription of SLC7A11 and thus the uptake of cystine (75).

IL-6. Smoking and betel nut chewing are two risk factors for HNSCC, both of which upregulate the expression of the pro-inflammatory cytokine, IL-6 (76), and oxidative stress caused by an imbalance of oxidants and antioxidants is considered to underlie the development of HNSCC after smoking (77). As also demonstrated in Fig. 2, Li *et al* found that IL-6 accumulation was associated with lipid peroxidation, which inhibited HNSCC ferroptosis *in vitro* and *in vivo* through the upregulation of SLC7A11 expression (78). In addition, IL-6 is secreted by immune cells or tumor cells and can promote tumor cell proliferation, epithelial-mesenchymal transition (EMT) through the activation of signal transducer and transcriptional activator 3 pathways, thus promoting cancer progression and inducing chemotherapy resistance, which is associated with a poor prognosis of patients with HNSCC (76,79). Thus, IL-6 can function as an inhibitor of ferroptosis that drives tumor progression, and it can potentially be used as a tumor prevention and therapeutic target.

Regulation of ferroptosis through the GPX4 pathway. Compared with normal tissues, GPX4 expression levels are significantly elevated in a variety of tumor tissues, such as triple-negative breast (80), gastric (81) and esophageal (82) cancer, leading to the hypothesis that GPX4 may be an oncogene. It has been shown to be a key regulator of ferroptosis; the overexpression or knockdown of GPX4 can affect the cell lethality of 12 ferroptosis inducers (83). GPX4 can affect tumor stem cell production and the EMT process by regulating ROS production (84,85). Viswanathan *et al* (86) demonstrated that GPX4 inhibitors in the majority of tumors can induce the ferroptosis of drug-resistant cells and prevent tumor recurrence; the combination of the targeted therapy of tumors with GPX4 inhibitors has provide new insight into resolving the issue of drug resistance in cancer cells (87).

In HNSCC, GPX4 is strongly associated with the poor prognosis of patients (88). A previous study demonstrated that the knockdown of GPX4 decreased cell viability, although the level of the caspase activity marker was not increased, indicating that non-apoptotic cell death occurred, which was reversed following pre-treatment with ferrostatin-1, suggesting that cells die via the ferroptosis pathway (89). Thus, in HNSCC, GPX4 can function as an inhibitor of ferroptosis and its downregulation induces tumor cell death; i.e., the modification of GPX4 expression may be a novel therapeutic approach for HNSCC.

In HNSCC, various genes such as RAS-selective lethal 3 (RSL3), epithelial membrane protein 1 (EMP1), period circadian regulator 1 (period 1; PER1) and caveolin-1 (Cav-1) regulate the occurrence of ferroptosis by regulating GPX4 expression and their specific regulatory mechanisms are described below.

RSL3. RSL3, an inhibitor of GPX4 that inhibits cysteine and glutamate transporter proteins, is a ferroptosis activator. As illustrated in Fig. 2, RSL3 functions as a 'ferroptosis activator' in HNSCC by downregulating GPX4 expression, inducing an increase in ROS generation, and catalyzing the lipid peroxidation of highly expressed unsaturated fatty acids in the cell membrane (90).

EMP1. EMP1 is a member of the epithelial membrane protein family, encoded by the peripheral myelin protein family, and is involved in the proliferation, migration and differentiation of tumor cells (91). It is mainly expressed in the squamous epithelium and exhibits differential expression levels in various normal tissues, such as esophagus, stomach and gallbladder (92). EMP1 plays a key role in cell adhesion, proliferation, apoptosis, tumor formation and ferroptosis (93), and its decreased expression is associated with the poor prognosis of patients with certain malignancies and is an independent predictor of ferroptosis (94).

As also illustrated in Fig. 2, Wang *et al* (95) reported that the overexpression of EMP1 in HNSCC cells intensified the downregulation of GPX4 by the ferroptosis activator, RSL3, increasing the levels of ROS and lipid peroxidation

in a time- and concentration-dependent manner, ultimately inducing the development of ferroptosis. In addition, EMP1 enhances the sensitivity of the tumor-targeting drug, gefitinib, and can be used as a biomarker of tumor chemotherapeutic resistance (96). Overall, EMP1 inhibits the malignant progression of HNSCC by activating ferroptosis in HNSCC and provides new markers which may be used to overcome the chemoresistance of tumors, contributing to the development of future therapeutic agents for HNSCC.

PER1. Period genes are present in almost all types of human tissues and organs and are involved in regulating a variety of important physiological and biochemical processes in organisms, and their abnormal expression is an important factor in the development of many diseases, including cancer (97). PER1 is one of the core genes of Period and is involved in regulating important physiological processes, such as apoptosis, autophagy, DNA damage repair and ferroptosis (98); its decreased expression in a variety of cancers, including gastric cancer (99), colon cancer (100), HNSCC (101) and non-small cell lung cancers (102) predicts that PER1 may be a key tumor suppressor gene (103).

In HNSCC, the decreased expression of PER1 is significantly associated with the TNM stage and poor prognosis of patients (104). PER1 negatively correlates with the expression of GPX4, a key regulator of ferroptosis, as shown in Fig. 2, which combine to form the PER1/GPX4 negative feedback pathway, thereby inducing the ferroptosis of cancer cells (105). In summary, the upregulation of PER1 gene expression can inhibit the proliferation, invasion and migration of HNSCC, which plays a similar role as RSL3 as a 'ferroptosis activator'; the in-depth study of this mechanism may provide a novel therapeutic approach for the treatment of HNSCC.

Cav-1. Caveolin is mainly composed of lipids and proteins, among which, Cav-1 plays a major biological role (106), functioning as an oncogene in a variety of tumors, including HNSCC, liver, colon, breast, kidney and lung cancer (107-109). It plays a critical regulatory role in substance transport, endothelial infiltration and tumorigenesis (110). Deng *et al* (111) demonstrated that Cav-1 was also involved in the regulation of ferroptosis. As demonstrated in Fig. 2, in HNSCC, Cav-1 is highly expressed and inhibits ferroptosis by upregulating GPX4 expression and suppressing ROS and lipid peroxidation. Lu *et al* (112) found that after knocking down Cav-1 in HNSCC, GPX4 expression was reduced, ferroptosis was activated, and cell proliferation, invasion and migration were all inhibited, suggesting that ferroptosis was negatively regulated by Cav-1. Overall, in HNSCC, Cav-1 functions as an inhibitor of ferroptosis and is likely to be a diagnostic marker of HNSCC (112).

Regulation of ferroptosis through iron metabolic pathways. Ferritin, the most critical form of iron storage in humans, consists of two polypeptide chains: The ferritin heavy chain (FTH1) and the light chain, which are essential for maintaining iron homeostasis and preventing iron overload, and also play a role in regulating the tumor microenvironment and immune metabolism (113). FTH1 is the major functional subunit of iron storage protein with iron oxidase activity that effectively reduces Fe^{2+} toxicity (114). Moreover, FTH1 is closely associated with the development of HNSCC, as illustrated in Fig. 2, where FTH1 expression levels are upregulated in HNSCC compared to normal tissues (75), which further inhibits the Fenton reaction by downregulating the expression of ferrous ions, thereby inhibiting cellular production of ROS and lipid peroxide aggregation, and ultimately negatively regulating ferroptosis (114). The further study of iron metabolic pathways may aid in the better understanding of the development of ferroptosis and may provide lead to the development of novel cancer therapeutics.

6. Therapeutic modalities regulating ferroptosis in head and neck squamous carcinoma

Drug treatment. Currently, drugs that have been experimentally validated to modulate ferroptosis in HNSCC include dyclonine and paclitaxel (PTX), and ferroptosis-based drug studies may alleviate current resistance to radiotherapy and chemotherapy in HNSCC and increase the sensitivity of the drugs.

Dyclonine, a covalent inhibitor of the cancer cell resistance gene, ALDH3A1 (115), plays a key role in inducing oxidative damage and cell death by promoting the accumulation of lipid peroxides in GSH-resistant cancer cells, and can reduce resistance to targeted therapy with the SLC7A11 inhibitor, sulfasalazine (116).

PTX is a paclitaxel-like substance extracted from plants that exerts anti-mitotic and apoptosis-inducing effects (117); it also induces cellular autophagy (118), downregulates glutamine degradation-related genes (119), affects glutamine catabolism and shifts metabolic reprogramming to oxidative phosphorylation, which in turn regulates the onset of ferroptosis (90,120).

At this stage, the FDA has approved a number of drugs for clinical use that inhibit the development of epithelial-derived tumors, such as ovarian, endometrial and squamous lung cancers through ferroptosis, including GPX4 inhibitors (altretamine), iron ion activators (lapatinib) and SLC7A11 inhibitors (sorafenib) (121). However, although these drugs have not yet been used in the clinical treatment of patients with HNSCC, HNSCC also serves as a tumor of epithelial origin in which these drugs are likely to play a similar role; thus, further research is required into the use of these drugs in HNSCC.

Emerging therapies. Currently, the conventional treatment for HNSCC is surgical resection with drug therapy; however, the, treatment efficacy is poor. Photodynamic therapy (PDT) is one of the emerging therapies for HNSCC. With the participation of photosensitizers and the action of light, the body cells generate reactive oxygen radicals through electron transfer and energy transfer, and eventually interact with oxygen molecules. The generation of large amounts of intracellular ROS leads to lipid peroxidation, causing damage to the structure and function of cell and organelle membranes (122,123).

The non-invasive nature of PDT allows organ function to be preserved in patients with HNSCC. However, PDT is hampered by hypoxia in the tumor microenvironment (TME) due to high intracellular oxygen consumption and tumor vascular distortion. Therefore, increasing oxygen production in the TME may be a new approach to enhancing the efficacy of PDT. The study by Zhu *et al* (124) in HNSCC found that ferroptosis significantly enhanced the efficacy of PDT and enhanced the anticancer effect through the generation of ROS

and a sustained supply of O_2 through the Fenton reaction (124). In other words, ferroptosis offers new hope for overcoming PDT resistance associated with hypoxia in the treatment of HNSCC.

7. Conclusions and future perspectives

HNSCC is the most common malignant tumors of the head and neck, with a recurrence and metastasis rate >65% and a 5-year survival rate <50% (125). Although combined surgical resection and radiotherapy have improved the survival rate of patients with HNSCC to a certain extent over the past 20 years, in the majority of patients, first-line drugs used for HNSCC, such as platinum-based drugs, 5-fluorouracil, polyene paclitaxel and cetuximab have minimal effects (2). Therefore, the effective treatment of patients with HNSCC, and the improvement of the quality of life and prognosis of patients are issues that still need to be addressed. Several new therapeutic approaches have been developed for HNSCC, among which PDT is one of the emerging therapies widely used in the clinical treatment of HNSCC, and its treatment mechanism is consistent with ferroptosis; the combination of ferroptosis and PDT may lead to the development of a novel strategy for more effective HNSCC treatment in the future (124).

Since its discovery in 2012, numerous studies have demonstrated that ferroptosis plays a critical role as a novel cell death modality in various diseases (126). Although a number of pathways have been demonstrated to be involved in ferroptosis, to date, the mechanisms of ferroptosis *in vivo* are mainly focused on three pathways: Iron metabolism, the System Xc^-/GSH/GPX4 antioxidant system and lipid metabolism. Among these, the pathway involved in regulating HNSCC is mainly the System Xc^-/GSH/GPX4 pathway, which has been widely studied in various types of cancer, such as liver cancer and non-small cell lung cancer, and drugs targeting ferroptosis have been used in clinical trials (127).

There are numerous factors in the System Xc-/GSH/GPX4 pathway that can affect the occurrence of HNSCC, and in the present review, the factors that regulate ferroptosis in HNSCC have been described, based on existing studies. Among these, NRF2 has been most comprehensively studied in ferroptosis and HNSCC, and a large number of studies have shown that NRF2 exerts a more pronounced inhibitory effect in HNSCC compared with other influencing factors, and further clinical trials are required to verify its role in regulating ferroptosis in HNSCC (128). Likewise, a number of researchers have explored ncRNAs for factors that can affect HNSCC by regulating ferroptosis; however, the research is not yet mature and further investigations are warranted (129).

At this stage, the FDA has approved a number of drugs for clinical use that inhibit the development of epithelial-derived tumors, such as ovarian, endometrial and squamous lung cancers by targeting ferroptosis (130,131). While these factors that target ferroptosis have also been shown to be effective in *in vitro* studies on HNSCC, all of these studies need to be explored in more depth with regard to their effects on the development and progression of HNSCC to demonstrate whether they can also be applied to the clinical treatment of HNSCC. However, the emergence of these targeted agents is certainly a glimmer of hope for the current dilemma facing HNSCC.

Overall, ferroptosis studies help to address the major issues of HNSCC and provide novel therapeutic targets and strategies for the diagnosis and clinical treatment of HNSCC.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Natural Science Foundation of China (grant no. 31772551).

Availability of data and materials

Not applicable.

Authors' contributions

MY drafted the manuscript, and prepared the figures and tables. RG and XC participated in the literature search and in the analysis of the data to be included in the review. GS and FZ edited and revised the manuscript. All authors have read and approved the final version of the 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.

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