The role of ferroptosis in radiotherapy and combination therapy for head and neck squamous cell carcinoma (Review)

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Abstract. Head and neck squamous cell carcinoma (HNSCC) is a highly aggressive, heterogeneous tumour usually caused by alcohol and tobacco consumption, making it one of the most common malignancies worldwide. Despite the fact that various therapeutic approaches such as surgery, radiation therapy (RT), chemotherapy (CT) and targeted therapy have been widely used for HNSCC in recent years, its recurrence rate and mortality rate remain high. RT is the standard treatment choice for HNSCC, which induces reactive oxygen species production and causes oxidative stress, ultimately leading to tumour cell death. CT is a widely recognized form of cancer treatment that treats a variety of cancers by eliminating cancer cells and preventing them from reproducing. Immune checkpoint inhibitor and epidermal growth factor receptor are important in the treatment of recurrent or metastatic HNSCC. Iron death, a type of cell death regulated by peroxidative damage to phospholipids containing polyunsaturated fatty acids in cell membranes, has been found to be a relevant death response triggered by tumour RT in recent years. In the present review, an overview of the current knowledge on RT and combination therapy and iron death in HNSCC was provided, the mechanisms by which RT induces iron death in tumour cells were summarized, and therapeutic strategies to target iron death in HNSCC were explored. The current review provided important information for future studies of iron death in the treatment of HNSCC.

Contents

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1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is a general term for squamous epithelial malignant tumors originating from the nasal cavity, oral cavity, pharynx, or larynx. HNSCC accounts for ~90% of all head and neck cancers and is characterized by high invasiveness and a poor prognosis (1). P53, as a transcription factor, can play its role in tumor suppression by activating the expression of numerous target genes (2). However, p53 is one of the most commonly mutated genes, which frequently harbors missense mutations. These missense mutations are nucleotide substitutions that result in the substitution of an amino acid in the DNA binding domain. Most p53 mutations in HNSCC are missense mutations and the mutation rate of p53 reaches 65-85% (3,4). Mutant p53 in HNSCC can interact with proteins and have effects on HNSCC proliferation, migration, invasion, immunosuppression and

metabolism. Studies have shown that mutant p53 can alter metabolic pathways, including reactive oxygen species (ROS), autophagy and lipid metabolism pathways (5,6). Current treatments for HNSCC include more precise surgical treatments, such as radiation therapy (RT) and chemotherapy (CT) (7). Early-stage HNSCC can be treated using radical RT or surgery, whereas advanced HNSCC should be treated with RT, surgery and CT, as well as multidisciplinary management of toxicity and side effects and follow-up (8). However, despite the extensive use of these treatments for the management of HNSCC, the five-year overall survival of patients with HNSCC has not changed significantly, and the recurrence rate for advanced HNSCC has remained high at ~50% (9). RT involves the use of α, β, γ, and X rays [ionizing radiation (IR)] to eliminate tumor cells and inhibit tumor cell proliferation, with the ultimate goal of achieving radical cure of tumors and controlling tumor progression (10). Direct transmission of radiation through the surface of superficial tumors may lead to irradiation of the normal tissues behind the tumor, leading to severe damage of the normal tissues, particularly those that are sensitive to radiation. Therefore, limiting the irradiation of normal tissues is an important challenge in the development of tumor RT. Currently, the developmental direction of clinical RT technology is to improve RT technology, change the local RT mode, maximize the accuracy of irradiation of tumor tissues, and avoid damage to normal tissues. Notably, patients with advanced HNSCC are radioresistant; thus, increasing their radiation dose to therapeutic levels increases the risk of damage to the surrounding vital organs and causes severe side effects. Therefore, protecting organ function is important in improving the curative effects of RT and reducing its side effects in patients with HNSCC.

Ferroptosis is a regulated form of cell death. Unlike the traditional mode of cell death, ferroptosis is caused by the accumulation of iron ions and ROS-induced lipid peroxidation (11). It is closely related to the occurrence and development of numerous human diseases, such as cancer, viral infections and degenerative diseases. Previous studies have shown that ferroptosis plays an important role in RT-induced cell death and tumor suppression, and that promoting ferroptosis in tumor cells enhances the sensitivity of the cells to radiation and CT drugs (12). Clinically, RT usually needs to be combined with CT, targeted therapy, or immunotherapy to eliminate tumor cells (13-15). In the present study, the mechanisms of ferroptosis and its regulatory factors in HNSCC were reviewed, and the mechanism underlying RT-induced tumor cell death in HNSCC was discussed to provide a theoretical basis for further improving the radiosensitivity of HNSCC.

2. Data collection methods

The PubMed (https://pubmed.ncbi.nlm.nih.gov/) and CNKI (https://oversea.cnki.net/index/) databases were searched for original research articles and reviews on the progression of ferroptosis after RT for HNSCC published until November 2023. The search terms included ferroptosis, ferroptosis and HNSCC, regulatory mechanisms of ferroptosis, ferroptosis and glutathione peroxidase 4 (GPX4), ferroptosis and solute carrier family 7 member 11 (SLC7A11). Information on ferroptosis was retrieved from the FerrDb database (http://www.zhounan.org/ferrdb/current/). FerrDb is the world's first database on ferroptosis regulatory factors and ferroptosis-associated diseases (16).

3. Cancer therapies

RT for head and neck cancer. RT is a common method of cancer therapy that involves the use of IR to eliminate tumor cells and inhibit tumor cell growth and metastasis (17). The clinical applications of radiation technology include palliative RT, conventional RT *in vitro*, stereotactic RT surgery, radionuclide therapy and intensity-modulated RT (IMRT) (18,19). IMRT is one of the most advanced and commonly used RT techniques. IMRT can minimize the amount of radiation normal tissues are exposed to and meet the treatment requirements for irregularly shaped tumor targets (20). Therefore, utilization of IMRT techniques for the treatment of patients with HNSCC can be adopted as an organ protection strategy, especially in patients with locally advanced disease (21).

RT-induced tumor cell death can be divided into accidental cell death (ACD) and regulated cell death (RCD) (22). ACD is an uncontrolled passive cell death process, whereas RCD is a controlled cell death process. RCD includes apoptosis, autophagic cell death, necrosis, cornification, atypical cell death (including mitotic catastrophe), anoikis, paraptosis, pyroptosis, entosis, excitotoxicity and ferroptosis (23,24). IR emitted by RT can cause a variety of DNA damage, including base damage, DNA single-strand breaks and DNA double-strand breaks (DSBs), which can affect the integrity of DNA or alter its chemical properties (25). Among them, DNA DSBs have been reported to be the most deleterious effect triggering genome stability, eliminating cancer cells, leading to genome instability, apoptosis, altered cell cycle checkpoints or post-mitotic death, and are the main cause of RCD in tumour cells (26).

Combination therapies. RT and surgery can achieve similar curative effects in patients with early-stage HNSCC. However, due to the lack of effective biomarkers for early diagnosis of HSNCC, most patients are diagnosed at the terminal stage of the disease. For patients with recurrent or metastatic HNSCC (R/M HNSCC) who have lost the opportunity for surgery and RT, long-term control of tumor growth, distant metastasis and clinical symptoms are more important than aiming for a cure. Systemic CT, immunotherapy and targeted therapy can be administered as the primary treatments.

Concurrent CT for head and neck cancer. With the development and advancement of CT, various platinum-based drugs have been revealed to exert certain therapeutic effects on HNSCC. Drugs used for the treatment of HNSCC include cisplatin, bleomycin, fluorouracil and methotrexate. These drugs act mainly through a cytotoxic mechanism to control the proliferation of tumor cells. The most desirable drugs from the drug screen are platinum-based drugs, which constitute the highest proportion of cancer drugs evaluated in clinical trials. In the TAX 323 (EORTC 24971) trials, patients with distant metastasis that occurred during TPF therapy (docetaxel + cisplatin + 5-fluorouracil) showed significantly prolonged progression-free survival and median overall survival (27).

Several clinical trials have shown that concurrent chemo-RT (CRT) for advanced HNSCC to increase local tumor control is relatively simple and can improve survival. Brizel *et al* (28) compared patients with advanced HNSCC who underwent surgery and received CRT, targeted therapy combined with CRT, and other treatments, and found that CRT is improved compared with RT alone for the treatment of locally advanced metastatic HNSCC.

Immunotherapy for head and neck cancer. Immune checkpoint inhibitors (ICIs) restore the antitumor immune response by blocking immune checkpoints. Inhibitory immune checkpoints, including programmed death receptor-1 (PD-1) and cytotoxic T lymphocyte-associated protein-4 (CTLA-4), are primarily expressed in T cells. Tumor-induced immune responses mediate tumor immune escape by upregulating the expression of immune checkpoints. ICIs can restore the antitumor immunity function of T cells through competitive inhibition of inhibitory receptors on T cells (29).

PD-1/PD-L1 and CTLA-4 inhibitors are the main immunotherapy agents for the treatment of R/M HNSCC. PD-1 inhibitors include pembrolizumab, nivolumab, camrelizumab, toripalimab, tislelizumab, avelumab and duvalumab, whereas CTLA-4 inhibitors include tremelimumab and ipilimumab. The PD-1 inhibitor pembrolizumab is currently used as a first-line treatment for HNSCC. The findings of the KEYNOTE-048 trial indicated that PD-L1 biomarkers are useful for selection of appropriate treatments for HNSCC (30). In 2016, the United States Food and Drug Administration approved the use of the ICIs (PD-1) pembrolizumab and nivolumab for the treatment of R/M HNSCC based on the results of the KEYNOTE-012 and CheckMate 141 trials (31-34), which indicated that anti-PD-1 monoclonal antibodies significantly prolong and improve overall survival in patients who show disease progression within 6 months of receiving platinum-based therapy (35-37). In a study on preoperative induction therapy plus postoperative adjuvant immunization, patients in the intermediate-risk group continued pembrolizumab monotherapy depending on whether positive surgical margins or lymphatic tissue metastasis was present. The patients who received pembrolizumab throughout the course of treatment demonstrated significantly improved one-year disease-free survival and overall.

Targeted therapy for head and neck cancer. Basic biochemical pathways and mutant proteins play important roles in targeted antitumor therapies by blocking tumor cell growth and survival (38). The epidermal growth factor receptor (EGFR) is an important member of the complex receptor tyrosine kinase family and plays a major role in cell signaling. EGFR is involved in cell and organism growth regulation and is highly expressed in most HNSCCs (39). Drugs currently used in the targeted therapy for HNSCC include the monoclonal antibodies cetuximab, panitumumab and zalutumumab; the small-molecule tyrosine kinase inhibitors gefitinib and erlotinib; and the dual-target tyrosine kinase inhibitor lapatinib. In 2016, the FDA approved the use of cetuximab in combination with RT for the treatment of patients with locally advanced HNSCC and R/M HNSCC that do not to respond to platinum-based CT (40). Cetuximab is a clinically effective monoclonal antibody against EGFR (41). These monoclonal antibodies enhance tumor antigen presentation by forming immune complexes that enhance the induction of tumor-specific T cells (42). In addition, cetuximab promotes natural killer cell antibody-dependent cytotoxicity and enhance the tumor cell-killing ability of complement-dependent cytotoxicity (43-45).

4. Ferroptosis

Ferroptosis is a form of iron-dependent cell death that was proposed in 2012 by Dixon *et al* (46). From a morphological and molecular biological perspective, ferroptosis is characterized by the presence of malformed small mitochondria, reduced mitochondrial crista, increased mitochondrial membrane density, rupture of the outer mitochondrial membrane, normal nuclear size, and a lack of condensed chromatin caused by overwhelming lipid peroxidation and oxidative disturbances in the intracellular microenvironment (13). Ferroptosis is mainly characterized by the presence of iron in the cell death execution and regulatory defense systems (47). Generally, the ferroptosis defense system can eliminate lipid peroxides and maintain a non-toxic state; however, if the amount of iron in the cell death execution system is higher than that in the ferroptosis defense system, the accumulation of lipid peroxides in the cell membrane increases to toxic levels, leading to ferroptosis (48). Ferroptosis gene regulators include drivers, repressors, markers and unclassified regulators (FerrDb; Fig. 1). Of these, only regulators play an important role in the ferroptosis regulatory network. The regulation network in the SLC7A11, GPX4 and acyl coenzyme A synthetase long-chain family member 4 (ACSL4) is highly expressed in the progression of the HNSCC cell line and reversible ferroptosis (Fig. 2A).

Iron metabolism. Ferroptosis is characterized by accumulation of iron ions and lipid peroxides. Iron is an essential trace element involved in redox activity in the human body. Increased levels of iron and/or iron-binding proteins and the dysregulation of iron metabolism contribute to the risk for cancer and promote tumor growth (49). Tumor cells are more dependent on iron than normal cells, and some tumor cells exhibit iron-ion aggregation. By iron, therefore, the steady state adjusts iron death by increasing iron intake; reduced iron can be stored and limit loss to promote iron death, as well as through the iron chelating agent and antioxidant to prevent death, effectively eliminating tumor cells (50). A recent study revealed that when iron metabolism disorder leads to increase in the amount of free iron in cells, the iron produced by Fenton's reaction catalyzes the production of ROS to further promote lipid peroxidation and induce ferroptosis (51).

Fatty acid oxidation. The underlying mechanism for ferroptosis is the iron-dependent accumulation of lipid peroxides. The ferroptosis defense system can inhibit lipid peroxidation under normal conditions. However, if the amount of iron in the cell death execution system is higher than that in the ferroptosis defense system, lipid peroxide rapidly accumulates in the cell membrane to toxic levels, triggering ferroptosis (48,52). Dysregulation of ferroptosis has been associated with numerous tumors, including HNSCC (53-56) (Fig. 2B). In the iron metabolism execution system, polyunsaturated fatty acids (PUFA) are produced in cells through the catalytic formation of PUFA-phospholipid-peroxide (PUFA-PL-OOH),

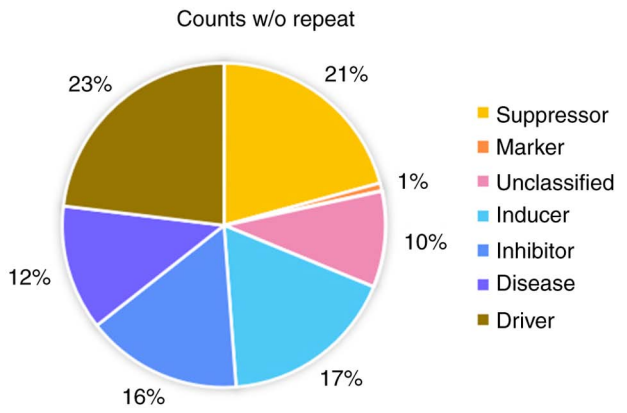

Figure 1. Proportions of various gene regulators of ferroptosis. Death regulation factor driving, suppressing, tags and unclassified regulators in a proportion of the total control factor.

causing the accumulation of the lipid peroxide in the cell membrane (47,57,58). This accumulation of lipid peroxides disrupts the integrity of the membrane, thereby inducing ferroptosis.

SLC7A11/GPX4 axon-dependent system. SLC7A11/reduced glutathione (GSH)/GPX4 signaling axis is considered to be the cell death defense system. The theory of ferroptosis was initially based on the findings of research on this pathway (59,60). SLC7A11, also known as the system Xc-, is a cystine/glutamate transporter that reverses transport of proteins by mediating the exchange of intracellular glutamate with extracellular cystine (61). Cystine is exchanged with glutamate in the cell in a 1:1 ratio and is rapidly reduced to cysteine, which is involved in the synthesis of GSH and GPX4 within the cell (62). GSH is a key cofactor in GPX4 function, and its depletion disrupts cellular redox homeostasis, leading to accumulation of ROS and ultimately inducing the onset of ferroptosis. Therefore, inhibiting the expression of SLC7A11 can induce ferroptosis. Moreover, GPX4 is a key regulator of ferroptosis. The basic function of the enzymes in the GPX family members is to reduce H2O2 at the expense of GSH. GPX4 is the only enzyme that reduces cholesterol, hydrogen peroxide and oxidized fatty acids. GPX4 can convert reduced GSH to oxidized glutathione and lipid hydrogen peroxide (L-H_2O_2, toxic) to lipid alcohols (L-OH, non-toxic), thereby promoting the decomposition of hydrogen peroxide and inhibiting ferroptosis (63,64). This indicated that reduced GPX4 activity favors ferroptosis (65,66). The SLC7A11/GPX4 axon-dependent system constitutes a ferroptosis defense mechanism that maintains non-toxic lipid peroxides levels, thus sustaining cellular viability.

Non-SLC7A11/GPX4 axon-dependent systems: The non-SLC7A11/GPX4 axon-dependent system consists of three regulatory pathways: (i) NAD(P)H-FSP1-CoQ is a newly discovered ferroptosis defense system (67). Ferroptosis suppressor protein 1 (FSP1), also known as apoptosis-inducing factor mitochondria-associated 2, is the oxidoreductase of ubiquinone (CoQ) and is mainly located in the plasma membrane (68-70). FSP1 acts mainly by catalyzing the reduction of CoQ into coenzyme Q (CoQH2) using NAD(P)H. Thereafter, CoQH2 exerts lipotropic and antioxidant effects, thereby inhibiting ferroptosis (71,72).

(ii) The GCH1-BH4-DHFR axis inhibits ferroptosis. Guanosine triphosphate cyclization hydrolase (GCH1) and guanosine-5'-triphosphate are the rate-limiting enzymes involved in the synthesis of tetrahydrobiopterin (BH4) (73). BH4, which is produced by dihydrofolate reductase through the reduction of dihydrogen biopterin (dihydrobiopterin, BH2), reduces the oxidation of endogenous free radicals and protects the lipid membrane from ferroptosis (74). The inhibition of GCH1 expression reduces BH4, thereby oxidizing iron and increasing GCH1 expression and BH4 synthesis, which inhibits ferroptosis (75).

(iii) The dihydrogen orotic acid dehydrogenase (DHODH)-CoQH 2 system inhibits ferroptosis by blocking mitochondrial lipid peroxidation (76). DHODH is mainly located in the mitochondrial membrane and inhibits ferroptosis through the reduction of CoQ into CoQH2, thus reducing the production of ROS.

5. Relationship between HNSCC treatment and ferroptosis

Relationship between ferroptosis and RT. DNA damage is one of the most important effects of IR in cells. IR-induced damage may directly affect cell proliferation and reduce the water content inside cells (~80% of the cell is water) to produce ROS and indirectly cause DNA damage (~60-70%) (77,78). This is because IR causes cytoplasm damage and generates highly active OH free radicals and other ROS, including O_2 and H_2O_2, which subsequently attack nucleic acids, lipids and proteins (79,80). Tumor cells are more susceptible to RT than normal cells owing to the high replication rate of tumor cells and defects in the DNA damage response (DDR) pathway (81,82). DNA DSB is the most serious type of DNA damage. Cell death may occur if a DSB is not repaired in a timely manner. In RT, the absorption of IR by water leads to the generation of ROS, which subsequently act on PUFA, leading to lipid peroxidation, peroxidation of membrane phospholipid lipids, and ultimately, ferroptosis (83). Therefore, RT can inhibit tumour progression by inducing iron death in tumour cells. It has been found that a variety of morphological features associated with iron death, such as shrunken mitochondria, increased mitochondrial membrane density and reduced mitochondrial cristae, were observed in tumour cells eliminated by radiation, including lung, breast, esophageal and ovarian cancers (84). Herrera *et al* (85) discussed the existing treatment of ovarian tumours with RT and the mechanisms by which RT mobilizes anticancer immunity. Lang *et al* (86) described iron death as a previously unappreciated mechanism of action of RT. Finally, the study named SLC7A11, a key regulator of iron mutations, as a mechanistic determinant of the synergistic effect of RT and immunotherapy (86). Furthermore, IR-induced ACSL4 expression increases PUFA-PL biosynthesis, which together with ROS, drives PUFA-PL peroxidation (PUFA-PL-OOH) and ferroptosis (87). SLC7A11 reduces ferroptosis by promoting the synthesis of GSH and reducing the production of L-OOH (88). RT increases the production of ROS, which can induce the activation the nuclear factor erythrocyte 2 related factor 2 (Nrf2)-heme oxygenase 1 (HO-1) pathway. The

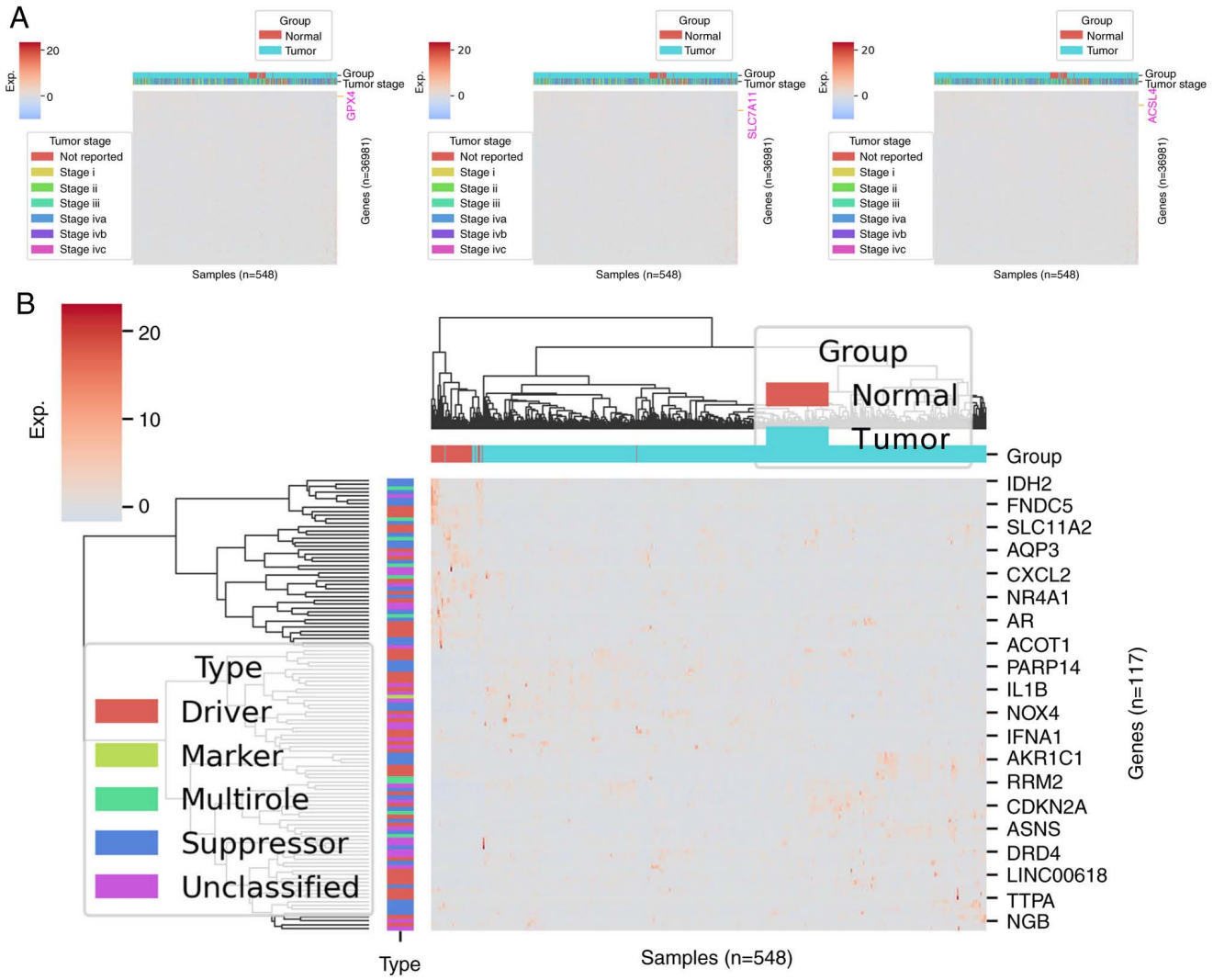

Figure 2. (A) Expression of GPX4, SLC7A11, ACSL4 in HNSCC. GPX4, SCL7A11 and ACSL4 were highly expressed in TCGA-HNSCC sample 548. (B) Ferroptosis-related regulators in HNSCC. A total of 548 samples were screened for the expression of regulatory factors related to ferroptosis in TCGA-HNSCC. GPX4, glutathione peroxidase 4; SLC7A11, solute carrier family 7 member 11; ACSL4, acyl coenzyme A synthetase long-chain family member 4; HNSCC, head and neck squamous cell carcinoma; TCGA, the Cancer Genome Atlas.

role of the Nrf2/HO-1 pathway in ferroptosis is bidirectional. A previous study showed that Nrf2 can activate SLC7A11, inhibit ferroptosis, and reduce the radiosensitivity of esophageal squamous cell carcinomas (89). Moreover, Wei *et al* (90) found that activation of the Nrf2/HO-1 pathway can increase Fe^{2+} levels in colorectal cancer cells and induce ferroptosis. Induction of ferroptosis in tumor cells is one way to enhance radiosensitivity. For example, pancreatic and renal cell carcinomas are sensitive to RT (91), which may be related to their dependence on cystine uptake (92). Inhibition of SLC7A11 and promotion of ferroptosis can increase the radiosensitivity of esophageal squamous cell carcinomas (93). A previous study found that IR may induce ferroptosis in tumor cells. This is because RT induces an increase in siderophiles, which can increase the sensitivity of tumor cells to RT.

RT for DSB is the most effective method of damaging and eliminating cancer cells; however, the intrinsic efficiency of tumor cells in DNA damage repair may lead to cellular resistance and impair therapeutic outcomes. Genes and proteins involved in DSB repair are targets of cancer therapy because their alteration, interaction, translocation and regulation can affect the repair process and render tumor cells more sensitive to RT. Therefore, targeting DNA damage repair as a means of sensitizing cancer cells to RT is a promising strategy for precise and effective treatment of patients with cancer.

Ferroptosis and radiosensitization. RT can eliminate tumor cells to a certain extent and remains one of the most effective non-surgical treatments for numerous tumors. However, reduced effects of RT on tumor cells is usually unavoidable because of RT resistance (RR), which is the reduction in the effectiveness of antitumor treatment (94). Tumor cells may show increased expression of antioxidant defense system-related proteins and ferroptosis to control the RT-induced abnormal increase in lipid peroxides, which leads to ferroptosis and RR (95,96). RR can lead to tumor recurrence, poor treatment response, poor prognosis, decreased quality of life and an increased treatment burden. Therefore, increased radiation sensitivity helps to reduce the incidence of adverse reactions through RT-induced tumor cell death.

IR-induced radiobiological effects are closely associated with ferroptosis. IR can induce ferroptosis in tumor cells, and the ROS produced by ferroptosis are involved in the regulation of tumor cell radiosensitivity and the tumor microenvironment (TME) (97). Local hypoxia and inherent or adaptive RR of tumor cells may lead to decreased radiosensitivity. Some KEAP1 mutant tumors rely on ferroptosis defense mechanisms, such as adaptive upregulation of FSP1/CoQ and inhibition of PUFA-PL synthesis, to avoid IR-induced ferroptosis, and thus develop RR (98). Hypoxia has long been recognized as a key regulator of RR. Due to impaired ribonucleotide reductase activity at low oxygen concentrations, reduced nucleotide levels lead to accumulation of single-stranded DNA at stalled replication forks and under replication stress. At the same time, hypoxic environments can also lead to a DDR that activates ATR-mediated and ATM-mediated downstream targets such as p53, H2AX and CHK-1/2 (99,100). Subsequently, this downstream signalling can shift cells to a less radiosensitive phase by inducing cell cycle arrest (101,102). Besides, RT-induced expression of SLC7A11 and GPX4 contributes to RR as an adaptive response that protects cells from ferroptosis. Therefore, depletion of SCL7A11 and GPX4 can induce ferroptosis and achieve radiosensitization (103,104). Chen *et al* (105) reported that suppressor of cytokine signaling 2 (SOCS2), a potential prognostic predictor of RT, promotes ferroptosis and increases the radiosensitivity of tumor cells by increasing the ubiquitination and degradation of SLC7A11. Thus, SOCS2 can promote the radiosensitization of tumor cells *in vivo* and *in vitro* (105).

Inactivation of ACSL4 impairs the biosynthesis of PUFA-PL, which in turn causes RR. Ferroptosis inducers (FINs), such as erastin, FIN and sulfasalazine, block the activation of the ferroptosis defense system, increase total intracellular iron content, promote ROS production, reduce glutathione concentration, and increase lipid peroxidation in radioresistant tumor cells, thereby enhancing the radiosensitivity of the cells by inducing ferroptosis (106-108). A previous study demonstrated that FINs have a synergistic effect in tumor treatment (109). Class I FINs targeting SLC7A11, such as erastin and sulfasalazine (SAS); class II FINs targeting GPX 4, such as RSL3 and ML162; and class III FINs depleting CoQ and GPX 4, such as FIN 56, can induce tumor sensitivity to RT *in vitro* (110).

In radioresistant tumor cells, activation of the ferroptosis execution system or inhibition of the ferroptosis defense system can further enhance ferroptosis and inhibit the development of RR. Therefore, further research on the mechanisms related to ferroptosis are needed to clarify how to maximize the antitumor effects of targeted ferroptosis combined with radiation while minimizing damage to normal tissues.

Relationship between ferroptosis and other treatments. RT can eliminate tumor cells and activate antitumor immunity. The antitumor immune system activated by RT can further induce ferroptosis in tumor cells and inhibit tumor development. Jhunjhunwala *et al* found that dendritic cells are the most important antigen-presenting cells that can ingest, process and present antigens and activate $CD8^+$ T cells (111). Activation of $CD4^+$ T cells releases interferon gamma (IFN gamma) and activates the system Xc-, thereby promoting tumor lipid peroxidation and cell death (111,112). The combination of RT and some drugs can significantly induce ferroptosis in tumor cells compared with single drug therapy, leading to a considerable increase in the number of immune cells in the tumor tissue (113).

Cisplatin is a platinum-based drug commonly used as a first-line chemotherapeutic agent for the treatment of solid tumors. Owing to its wide antitumor spectrum and high curative effect, cisplatin is recommended by the World Health Organization for the treatment of cancer. Cisplatin can bind with guanine residues induced between multiple chain and chain stated, a crosslinking that leads to rapid cell death (114). In addition, Ma *et al* (115) found that iron-oxide nanocarriers enhance the anticancer efficacy of cisplatin and simultaneously reduce toxicity caused by generation of ROS. Cisplatin can produce H_2O_2 through a cascade in the cytoplasm of the cells in the TME. H_2O_2 can be further catalyzed by ferric iron ions into toxic hydroxyl free radicals generated by Fenton's reaction, leading to tumor cell apoptosis and ferroptosis (115).

Ferroptosis induced by the tumor suppressor p53 inhibits tumor development. The induction of p53 in the presence of lipid peroxidation may eliminate cells stressed by ferroptosis. Ferroptosis can induce cell death by releasing PUFA into the extracellular environment or by driving the expression of enzymes that stimulate PUFA-PL synthesis, such as LPCAT3 and ACSL4 (116). Sulfasalazine can inhibit the expression of glutathione by downregulating SLC7A11, thereby inactivating GPX4, causing ROS accumulation, and inducing ferroptosis to play a role as a tumor suppressor (117).

Ferroptosis is a newly discovered mode of programmed cell death that is closely related to RT and combined immunotherapy. Drugs can play a role in tumor suppression by inducing ferroptosis in tumor cells. The underlying mechanism involves inducing ferroptosis in tumor cells in a variety of ways and eventually inhibiting tumor progression.

6. Conclusion and perspective

HNSCC is the most common malignant head and neck tumor, with recurrence and metastasis rates of >65% and a five-year survival rate of <50% (118). The combination of surgery and RT has increased the survival rate for HNSCC over the past 20 years. However, the first-line therapeutic agents used for HNSCC, such as platinum-based agents, 5-fluorouracil, polyene paclitaxel, and cetuximab, have little effect on most patients. Therefore, effective treatment of patients with HNSCC to improve their quality of life and prognosis remains a challenge.

RT currently plays an important role in the treatment of HNSCC. Continuous improvement in RT technology will allow for more accurate dosing and mapping of the radiation area in patients with HNSCC. However, there are still certain limitations: How to make the radiation dose received by HNSCC more precise and reduce the damage to the surrounding normal tissues needs further exploration. In addition, RT resistance is a major barrier to improving the survival benefit of HNSCC treatment; therefore, methods to reduce RT resistance need to be explored. Ferroptosis plays a key role in radiation-induced cell death. Induction of ferroptosis

can enhance the radiosensitivity of tumor cells by inducing iron overload and lipid peroxidation, thereby maintaining the efficacy of RT.

Research on ferroptosis will help solve the major problems associated with HNSCC treatment and identify novel therapeutic targets and strategies for the diagnosis and clinical treatment of HNSCC. In the future, continuous research shall be conducted by the authors and improved experimental protocols will be utilized to further explore the effects of RT and other combined immunotherapies to improve the prognosis of patients with HNSCC and minimize treatment-related side effects.

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Authors' contributions

YF and XL wrote the manuscript. BY and ML were responsible for gathering the associated research and designing the review. YD, JW and SL collected and analyzed data. LGa, LGo and LL contributed to the study design, interpretation of the research articles, editing and critical revision of the manuscript. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare they have no competing interests.

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