Review

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Targeting critical pathways in ferroptosis and enhancing antitumor therapy of Platinum drugs for colorectal cancer

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Abstract

Colorectal cancer (CRC) can be resistant to platinum drugs, possibly through ferroptosis suppression, albeit the need for further work to completely understand this mechanism. This work aimed to sum up current findings pertaining to oxaliplatin resistance (OR) or resistance to ascertain the potential of ferroptosis to regulate oxaliplatin effects. In this review, tumor development relating to iron homeostasis, which includes levels of iron that ascertain cells' sensitivity to ferroptosis, oxidative stress, or lipid peroxidation in colorectal tumor cells that are connected with ferroptosis initiation, especially the role of c-Myc/NRF2 signaling in regulating iron homeostasis, coupled with NRF2/GPX4-mediated ferroptosis are discussed. Importantly, ferroptosis plays a key role in OR and ferroptotic induction may substantially reverse OR in CRC cells, which in turn could inhibit the imbalance of intracellular redox induced by oxaliplatin and ferroptosis, as well as cause chemotherapeutic resistance in CRC. Furthermore, fundamental research of small molecules, ferroptosis inducers, GPX4 inhibitors, or natural products for OR coupled with their clinical applications in CRC have also been summarized. Also, potential molecular targets and mechanisms of small molecules or drugs are discussed as well. Suggestively, OR of CRC cells could significantly be reversed by ferroptosis induction, wherein this result is discussed in the current review. Prospectively, the existing literature discussed in this review will provide a solid foundation for scientists to research the potential use of combined anticancer drugs which can overcome OR via targeting various mechanisms of ferroptosis. Especially, promising therapeutic strategies, challenges ,and opportunities for CRC therapy will be discussed.

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Introduction

Colorectal cancer (CRC) is diagnosed routinely with distant metastases at a higher rate, thereby making it one of the most predominant tumors in the world.[1] A usual phenomenon associated with treatment failure of colorectal tumors following surgery is oxaliplatin resistance (OR).[2] Accordingly, the unmet need to reverse OR coupled with approaches to improve the effectiveness of anticancer drugs on CRC in a long-acting fashion, as well as prolonging patients' lives has attracted much attention from scientists and clinicians. As a new kind of cell death that depends on the accumulation of high concentrations of iron and lipid peroxidation (LPO), ferroptosis is characteristically associated with a buildup of reactive oxygen species (ROS) within cells, wherein it has become a hotspot in research involving reversal of drug resistance in tumor.[3]

The research on ferroptosis was first formally proposed in 2012. However, the research on ferroptosis is developing rapidly and attention has been increasing in the field of oncology, which is expected to become a hot issue in the future. The article published by Dixon et al. in 2012, which confirmed that ferroptosis is a significantly different form of regulatory cell death has been cited frequently.[4] This article is considered to be the most important and fundamental piece, which is pioneering research in this field. In 2014, Yang et al. found that glutathione peroxidase 4 (GPX4) can regulate the death of eosinophilic cancer cells through the ferroptosis pathway.[5] In 2016, studies found that ferroptosis inducers (FIN) could inhibit GPX4 by covalently targeting the active site selenocysteine, leading to the accumulation of polyunsaturated fatty acid (PUFA) hydroperoxides.[6] In 2017, Doll et al. found that ACSL4 inhibition could restrain the occurrence of ferroptosis.[7] In 2018, Ingold et al. found that GPX4 was selenium-dependent in preventing ferroptosis caused by hydrogen peroxide.[8] In 2019, studies showed that FSP1 was a key component of a nonmitochondrial co-antioxidant system that acted in parallel with the GPX4 pathway based on glutathione.[9] In 2020, Zou et al. revealed the role of the peroxisomal-ether-phospholipid axis in the development of susceptibility and avoidance of ferroptosis. They emphasized that PUFA-EPL was a unique functional lipid class, which was dynamically regulated in the process of tumor cell state transition and provided multiple regulatory nodes for therapeutic intervention of cancer.[10] Zhou and Jinku established a database of ferroptosis-related markers and regulators and ferroptosis-related cancer.[11] Moreover, a review published in Nature introduced the molecular mechanism of ferroptosis and tumor-related pathways and explored the application of ferroptosis in cancer treatment, such as systemic therapy and immunotherapy.[12] Therefore, more attention and research on ferroptosis will be carried out. Oxidative stress, inducers/inhibitors, synergistic antitumor effects, relationships with other cell death types, glutathione (GSH)/GPX4 and mechanisms of iron metabolism imbalance in ferroptosis, and ferroptosis in tumors are hot research directions. In conclusion, it is of positive significance to summarize the existing research results of ferroptosis and cancer and discuss the trends and hotspots of ferroptosis. The historical axis of landmark research achievements on ferroptosis and cancer is shown below (Figure 1).

Existing literature suggests that tumor resistance to several chemotherapeutics and targeted natural preparations could be reversed through ferroptosis induction in various

cancers.[13] In this regard, other works have hypothesized that resistance to ferroptosis in CRC cells may contribute to the OR. In this review, we will discuss tumor development that is related to iron homeostasis, namely levels of iron that ascertain cells' sensitivity to ferroptosis, oxidative stress, or LPO with key regulators of lipid metabolism in CRC cells that is connected with ferroptosis initiation. Moreover, the relationship between iron-induced LPO and CRC cells, especially the role of c-Myc/nuclear-factor erythroid-2-related factor-2 (NRF2) signaling pathways and NRF2-GPX4 mediated ferroptosis will be discussed. In addition, we will focus on genes and proteins associated with intracellular ferroptosis in the KIF20A-GPX4 pathway of CRC cells, wherein it is speculated to be the underlying mechanism of OR in CRC cells. What is more, possible drug-driven regulation of iron homeostasis and ferroptosis will be discussed. Besides, the possible use of small molecules, FIN, GPX4 inhibitors, or natural compounds coupled with anticancer drugs that can overcome OR via targeting various mechanisms of ferroptosis in CRC cells will be discussed as well.

Iron homeostasis and ferroptosis in CRC

In recent years, clinicians are facing challenges of resistance to platinum drugs by cancer cells during treatments. Thus, successful treatment of drug resistance will largely depend on a complete understanding of the mechanisms of resistance. Herein, our discussion will be centered on the relationship between LPO induced by iron and CRC cells that have been protected from ferroptosis, especially the role of c-Myc/NRF2 signaling in the regulation of iron homeostasis and NRF2-GPX4-mediated ferroptosis, which is related to several oxaliplatin drug resistances.

Disruption of iron homeostasis induces ROS production via c-Myc/NRF2 in CRC

Disrupting homeostasis of iron and generating abnormal levels of iron trigger oxidative stress in cells, which is usually induced when cellular antioxidants are overwhelmed by abundant ROS. Thus, cellular antioxidant systems could transfer ROS to non-reactive

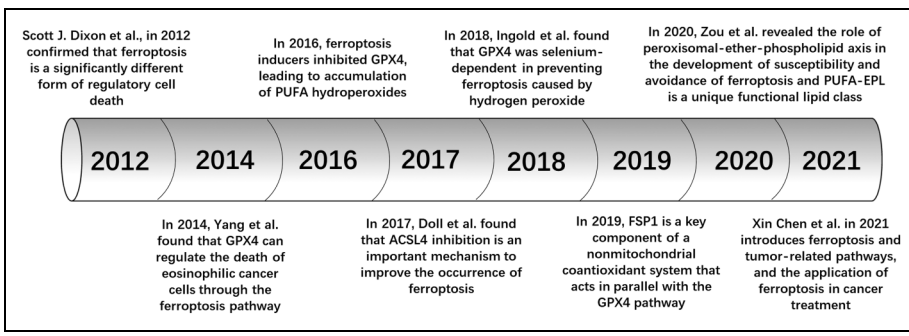

Figure 1. Combining the historical axis of landmark research achievements on ferroptosis and cancer.

water before they cause damage to cell membranes.[14] Intracellularly, NRF2 is a master regulatory transcription factor against oxidative stress.[15] Most importantly, the growth of CRC cells is promoted upon their exposure to iron mainly through NRF2 activation.[16] Physiologically, nuclear translocation of NRF2 occurs under oxidative stress conditions in cells, and it activates the expression of genes and leads to increased antioxidant proteins expression for the reduction of ROS, coupled with a decrease of the labile iron pool (LIP) to prevent the additional generation of ROS.[16] Also, control of iron import is accomplished by divalent metal transporter 1 (DMT1) and Transferrin receptor protein 1 (TfR1) degradation in lysosomes, proteasomes, vesicles of extracellular space, or endosomes from the cell membrane.[17,18] Thus, the homeostasis of iron can be ensured by controlling post-translational mechanisms. As a transcription factor, c-Myc represses FTH1/FTL expression while activating DMT1 and TfR1 expression to increase LIP.[19,20] Hence, disruption of iron homeostasis induces ROS production via c-Myc/NRF2 signaling mediated ferroptosis in CRC (Figure 2).

The complex role of iron in CRC has been identified. The potential accumulation of a large amount of iron in the colon is due to the ability of small intestines to absorb more than 10% (as occurs under normal conditions) of the total iron.[21] Available data suggest that CRC risk is increased by oxidative stress induced through excess uptake of iron in cells.[22,23] Alternatively, advanced-stage CRC patients may suffer from intestinal chronic hemorrhage, which leads to chronic iron loss and even deficiency of iron stores.[24] Existing literature suggests different correlations between the clinic-pathological features of CRC patients and the expression of TfR1 from other kinds of cancers.[24,25] In addition, survival analysis showed that CRC patients that positively expressed TfR1 demonstrated a higher survival rate compared to patients who negatively expressed TfR1. Thus, these findings suggest that low expression of TFR1 may promote tumor development.[26,27] Hence, we need to explore the role of iron metabolism in the pathological process of CRC.

Iron homeostasis is maintained through the process of import, export, and storage, wherein the involvement of ferrous iron in ferroptosis occurrence is reported to be vital.[28] Based on this finding, other works have suggested that CRC cells are rich in and dependent on iron.[23] It is interesting to note that despite an increased iron level observed in CRC cells, the susceptibility to FIN is still less understood compared to other cancer types. Since iron is mostly bounded to ferritin, it prevents Fenton reaction that induces the generation of ROS.[29] Consequently, vast iron availability is not equivalent to an overload of iron. Ferroptosis seems to be controlled by the fragile balance between the level of ferritin and "free" iron. Significantly, it has been disclosed through previous work that restoration of CRC sensitivity to ferroptosis could be accomplished by breaking the balance by silencing GTP cyclohydrolase 1 (GCH1).[30,31] A drastic increase in the level of nuclear receptor coactivator 4 (NCOA4) has been confirmed after GCH1 suppression coupled with *in-vitro* and *in-vivo* treatments of erastin, along with degradation of FTH1 and production of LPO. Moreover, selective recognition and degradation of FTH1 by regulating NCOA4 via auto-lysosomes have been reported elsewhere.[32]

CRC sensitivity to ferroptosis is determined by iron levels

Ferroptosis is a new type of cell death that is different from autophagy, necrosis, and apoptosis.[33,34] It is controlled tightly by GPX4 and some regulatory proteins that are

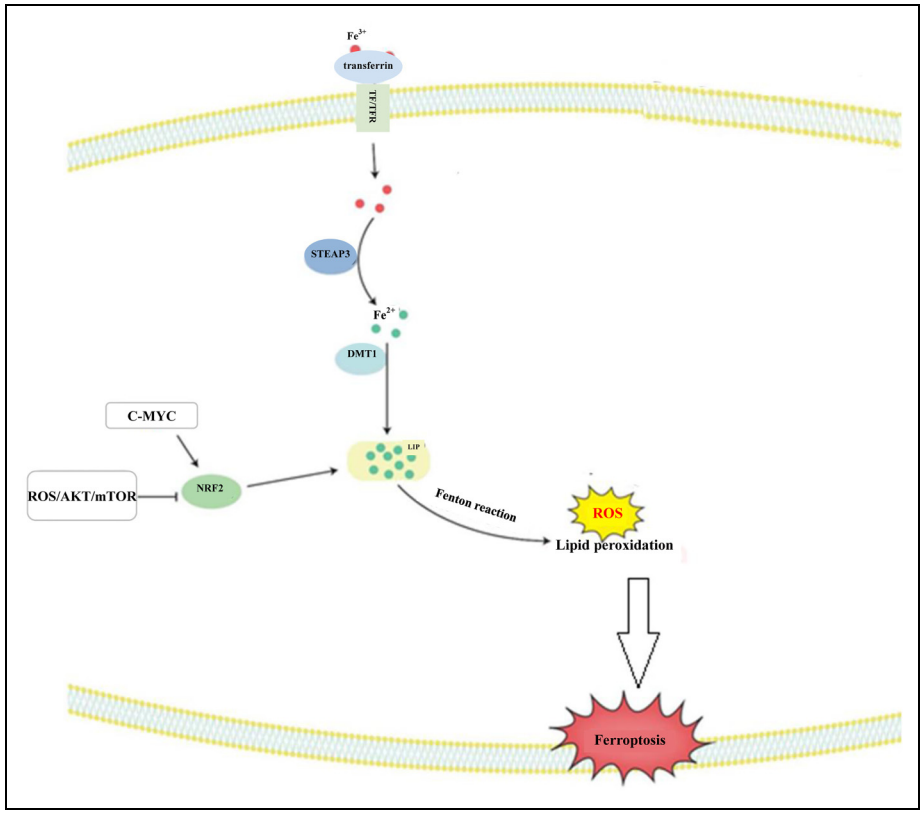

Figure 2. Roles and functions of iron homeostasis, LPO and ferroptosis in colorectal cancer (CRC). Regulation of the downstream transduction factor c-Myc/NRF2 of the ROS/AKT/mTOR pathway mediates the participation of TfR1 and DMT1 in metabolic homeostasis of iron in cancer cells.

responsible for transporting iron.[35] Recently, researchers have developed much interest in the novel approach of treating cancers like CRC through regulating ferroptosis. A cell's sensitivity to ferroptosis is determined by the expression of proteins (e.g. transferrin (Tf) TfR1, ferroportin, and ferritin) that regulate iron homeostasis and intracellular levels of iron.[36,37] Ferroptosis was triggered by increased levels of intracellular iron and ROS during breast cancer treatment with lysosomal disruptor siramesine, wherein the lysosomes are accordingly rich in iron.[37] In addition, cancer cells are sensitized to ferroptosis by extracellular iron (high amount of iron derived from diets or iron supplements).[38] Nanoparticles loaded with iron were developed for a different purpose but they showed antitumor potential in a serendipitous manner. For instance, doxorubicin-loaded nanoparticles with iron-saturated ferritin as an excipient caused ferroptotic-induced death in cell cultures of breast, cervical, CRC, leukemia, liver, and lung cancers.[39] Moreover, already approved iron-based nanoparticles for the treatment of iron deficiency could also

be used to treat tumors, and they showed promising therapeutic effects by acting as delivery carriers.

It is interesting to note that macrophages suppress tumor growth and spread by releasing hydrogen peroxides (which react with iron) into the microenvironment of the tumor.[40] The promotion of cellular ROS accumulation and increase of LIP level in cells is the underlying mechanism to trigger ferroptotic cell death (FCD) induced by RSL3. Mechanistically, elevated expression of transferrin has been observed in CRC cells after RSL3 treatment, amid reduced GPX4 expression, which suggested that the cell death was dependent on intracellular iron level.[32] Furthermore, GPX4 overexpression attenuated RSL3-induced FCD, thereby suggesting that suppression of GPX4 could determine ferroptosis induced by RSL3 in CRC cells.

LPO and lipid metabolism link to ferroptosis in CRC

As a unique class of programmed-cell death (PCD) that depends on iron, ferroptosis is defined by the accumulation of excessive oxidative stress and concomitant LPO. Historically, initial studies of LPO were related to injury to fats and alimentary oils in meat and its products,[41] but its role in various processes of pathology was soon discovered such as cancer. In general, the process of LPO is complex and non-radical species, free radicals, or oxidants attack double bond(s) between carbons of polyunsaturated lipids, which lead to the establishment/generation of lipidic peroxyl radicals and hydroperoxides (LOOH), and successively produce secondary products that have a longer half-life. Since ferroptosis has been demonstrated to occur unambiguously, it is imperative to simultaneously detect biochemical biomarkers of redox-active iron and LPO along with a deficiency in repairing peroxides of lipids.[42]

Fatty acid (FA) is essential for cancer cells' proliferation at a high rate and acts as a precursor for the synthesis of plasma membranes and metabolites for the production of energy. Furthermore, some kinds of FA are essential for tumorigenesis and the progression of cancer. Monounsaturated FA (MUFA) and saturated FA (SFA) are normally biosynthesized by fatty acid synthase (FASN, a multienzyme and homodimeric protein) with palmitate (PA, C16:0) as the starting material.[43] In particular, long-chain FA (e.g. PA) is synthesized by FASN with the primer being acetyl CoA and a two-carbon donor being malonyl CoA, while the reducing power is NADPH. Further elongation of PA to stearic acid (SA, C18:0), while its desaturation to palmitoleic (C16:1n-9) and oleic (C18:1n-9) acids is catalyzed by desaturase enzyme, wherein the latter is elongated further to eicosatrienoic acid (C20:3n-9). Acetyl CoA is produced when FA (derived from the diet or de novo synthesis) is catabolized, after which the carrier of acyl groups enters the citric acid cycle to facilitate the production of ATP. On the other hand, the incorporation of FA into complex lipids such as phospholipids (PL), triacylglycerol (TAG), or esters of cholesterol (CE) takes place (Figure 3). Therefore, FA is initially activated by the enzyme acyl CoA synthetase (ACS), which is an important step for the abovementioned pathways.[44] Apart from producing NADPH as a by-product of oxidized FA, the metabolism of lipids has been directly linked to oxidative stress, wherein it is shown that expression of superoxide dismutase is reduced by knockdown of FASN, amid an increased generation of ROS and susceptibility to H_2O_2. Through that work,

it shows how FASN modulates the cytotoxicity induced by H_2O_2 in CRC SNU-C4 (KRASG12C) human cancer cells.[45]

Polyunsaturated fatty acids, such as linolenic acid (ALA), arachidonic acid (AA), docosahexenoic acid (DHA), and eicosapentenoic acid (EPA) are usually regarded as long-chain FA that contains two or more double bonds between their carbons. The preferred substrate form of PUFA for LPO is when it exits as a free FA or is esterified at the sn-2 position of PL, but acyl of the sn-1 position usually does not take part in oxidative reactions.[46] Indeed, the C-H bond of PUFA is weaker and susceptible to LPO wherein hydrogen of bis-allylic hydrogen with a penta-diene moiety can easily be abstracted.[47] It can be inferred from the results that the relevance of PUFA in CRC etiology derived from the potential generation of G > A transitions in K-ras oncogene.[48] Additionally, omega-3 PUFAs (n-3 PUFAs) and butyrate effects have been suggested to synergistically and potentially enhance the chemo-

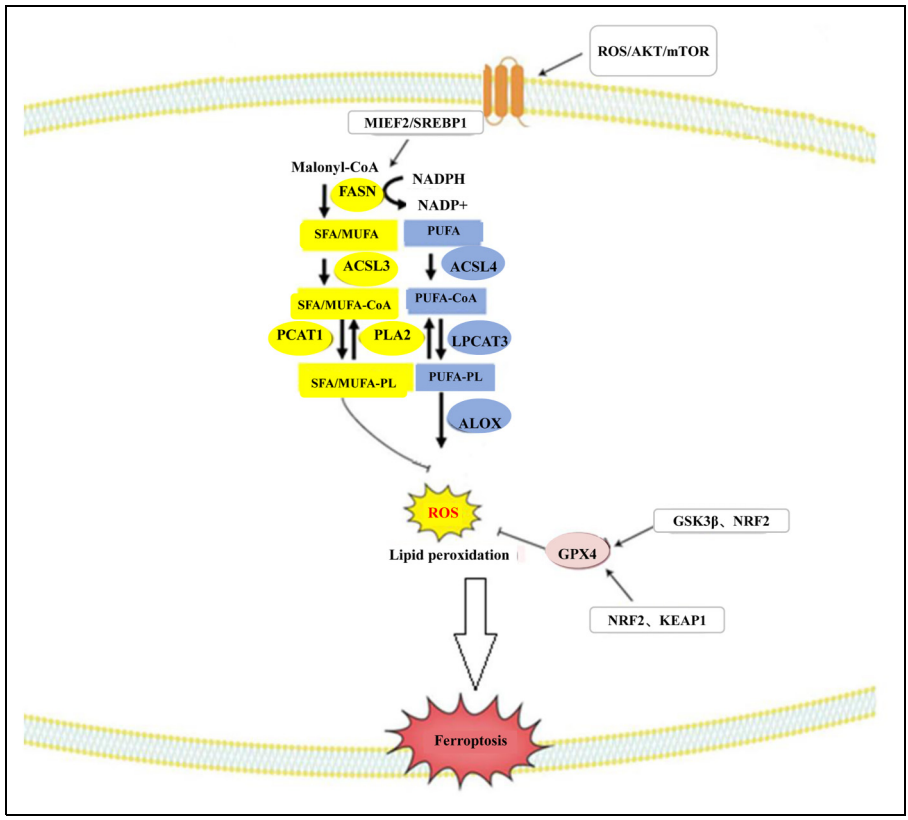

Figure 3. Roles and functions of lipid peroxidation, ferroptosis, and oxaliplatin-based chemotherapy associated CRC. Exposure of CRC cells to iron potentially induce activation of NRF2 and ROS, which results in increased accumulation of LPO and proteins associated with GSH as well as GPX4, amid prevention of iron-induced LPO and protection of CRC cells from ferroptosis. Ferroptosis in CRC cells interferes with lipid peroxidation and disrupt the metabolic balance of iron, thereby enhancing the chemosensitivity of drug-resistant cancer cells.

preventive effects of the aforesaid dietary ingredients. Herein, available evidence suggests that n-3 PUFAs and extremely fermentable fiber can alter vital pathways that are significant for preventing CRC, especially intrinsic or mitochondrial-mediated PCD that results from the accumulation of lipidic ROS (ferroptosis), while epigenetic programming is related to catabolism of lipids and β-oxidation-related genes.[49]

Evidently, PUFA oxidation during ferroptosis was established through the incubation of HT-1080 cells with alkyne labeled arachidonic acid (AA). Afterward, the obtained product was labeled with copper-catalyzed alkyne-azide click chemistry. In another work, prevention of oxidative products of AA breakdown (induced by Erastin treatment) was accomplished by treating with Ferrostatin-1 (Fer-1), a selective potent ferroptosis inhibitor.[50] Susceptibility to ferroptosis was increased when AA or other PUFAs were added to high-oleic oils, possibly because they were increasingly incorporated into PL.[51] Likewise, n-3 PUFA was found to purposefully inhibit oncogenic K-ras driven CRC.

Contrarily, MUFAs could not oxidize readily because they do not possess bis-allylic positions. Instead, their ability to potently suppress ferroptosis in cancer cells has been reported.[52] It is interesting to note that SFA and MUFA (dietary FA) but not PUFA potentially affected cancer, through their association with increased CRC risk coupled with particular K-ras codon 12 mutations.[53,54] On the contrary, upon their consumption, dietary n-3 PUFA (e.g. DHA and EPA) are incorporated into the PL of the plasma membrane, wherein their association with decreased risk of CRC has been established elsewhere.[55] Available genetic evidence supports the fundamental PUFA requirement for oxidation in ferroptosis, wherein it links particular genes for lipid metabolism to the accomplishment of ferroptosis.

The enzymatic defense against LPO of membrane PUFA is controlled by selenoprotein GPX4 (master regulator) since it is regarded as the only enzyme that can reduce esterified FA and cholesterol hydroperoxides that have been oxidized.[56,57] In general, inhibition of GPX4 results in a quick build-up of LOOH, but RSL3-induced cell death is blocked by its overexpression.[58] Nonetheless, the relationship between RAS status and GPX4 is still contentious. Exemplary, RSL3 and Erastin induced ferroptosis in human cancer cells that were engineered to lower express HRASG12 V compared to wild-type isogenic cells, but tumor cells without oncogenic RAS were susceptible to inhibition of GPX4 in HT29 CRC cells.[59] Furthermore, GPX4 overexpression meliorated RSL3 induced FCD, thus suggesting that suppression of GPX4 could be a fundamental determinant in ferroptosis induced by RSL3 in CRC cells.[59]

Interaction between chemo-resistance and ferroptosis in CRC

LPO and ferroptosis for chemo-resistance in cancer

Traditional cancer treatment options primarily fail because of chemoresistance in cancer cells. Since ferroptosis is associated with a high level of LPO, its crucial role in regulating tumor chemoresistance needs proper explorations. Studies found that the exposure of cells to high iron levels coupled with consumption of a high amount of GSH by excess iron led to ferroptosis.[60] The transporter x-c (SLC7A11) of cysteine/glutamate is associated with GSH levels in cells, while SLC7A11 suppression generally results in GSH depletion and

concomitantly induces ferroptosis.[61] Also, GSH serves as the GPX4 cofactor, which in turn plays a crucial role in deciding the level of cytoplasmic lipid oxidation.[62]

Existing literature has indicated that multidrug-resistant cancer can effectively be treated by targeting the LPO.[63] Besides, tumor with chemoresistance is regulated through the x-c/GSH/GPX4 axis. For example, cells that were more resistant to chemical drugs such as 5-fluouracil, cisplatin, and doxorubicin had increased GSH levels.[64,65] It is suggested that multidrug resistance protein-1, a transporter family member of ATP binding cassette (ABC) can be destroyed, amid suppression of GSH outflow and inhibition of ferroptosis.[66] Moreover, as a member of x-c system, SLC7A11 could mediate the uptake of cystine in cancer cells, while cystine is essential for the maintenance of GSH and geldanamycin resistance.[67] Thus, GSH is decreased during the induction of ferroptosis by inhibiting the system x-c.

Most importantly, another study suggested that enhanced ferroptosis could be an effective interventional approach to prevent multidrug resistance in cancer cells. It has been observed that ferroptosis induced by GPX4 inhibition could improve the effects of multiple kinds of anticancer drugs.[5] Also, the production of LPO by ACSL4 (fundamental lipid metabolism regulator) during the ferroptosis process has been described. Studies had suggested that the expression of ABC transporter regulated by ACSL4 contributed to the chemoresistance of breast cancer cells.[68] Moreover, overexpression of ACSL4 and ACSL1 could induce a shift of metabolic energy toward the utilization of glucose in CRC.[69] Also, cell proliferation in CRC was inhibited by suppressing ACSL1 or ACSL4, wherein a knockdown of ACSL4 elicited a more obvious effect.[69] Especially, Lysophosphatidyl-choline acyltransferase 3 (LPCAT3) and ACSL4 were promoters of ferroptosis induced by DPI7 and RSL3 based on a CRISPR genetic screening.[45]

Multi-drug resistant tumor cells (originated from a pool of persister cells) derive from the evolution of cancer cells during chemotherapy. Thus, relapse of the tumor can be prevented by targeting persister cells, which are in the mesenchymal state. Of note, mesenchymal state cells with high therapy resistance were found to be dependent on GPX4, which protects cells against ferroptosis.[70] Additionally, the association of a high mesenchymal state with zinc finger E-box-binding homeobox-1 (ZEB1) was revealed by a systematic investigation of cellular responses to FIN. ZEB1 is a transcriptional factor that has been described to fundamentally regulate adipogenic fate and lipid metabolism, which may increase the plasticity of metabolism, stemness, and colonization capacity of cancer cells.[71] Thus, lipid metabolism interactions with ferroptosis can modulate associations of drug resistance and mesenchymal-like phenotype. Additionally, exosomes secreted from stromal cells could promote chemotherapy in the tumor microenvironment (TME), like tumor-related fibroblasts, which resulted in reduced generation of LPO and ferroptosis in tumor cells.[63] Collectively, strategies to potentially sensitize tumor chemotherapy are highlighted by the aforementioned interaction between lipid metabolism and ferroptosis.

Role of NRF2/GPX4 signaling pathway for resistance to ferroptosis in CRC cells

Multiple pathways related to ferroptosis resistance in cancer cells have been reported. For example, ferroptosis resistance in cancer cells is induced under conditions of oxidative

stress and LPO.[45] Nevertheless, how LPO or oxidative stress causes ferroptosis resistance in cancer cells has not been clarified.

Possibly, NRF2 as a transcriptional factor can majorly regulate antioxidant response because redox homeostasis is maintained by downstream target genes. Noticeably, the cascade of ferroptosis is initiated by two FINs, with erastin suppressing system x-c and RSL3 inhibiting glutathione GPX4.[72] Moreover, LPO is prevented by many other enzymes and proteins. For instance, ferroportin, FTL, FTH1, GPX4 and NADPH could responsibly prevent LPO and hence induce ferroptosis.[73] Exemplary, FCD is triggered by RSL3 through the promotion of increased cellular level of ROS. Mechanistically, elevated expression of transferrin is found in RSL3-treated CRC cells along with reduced GPX4 expression, while GPX4 overexpression attenuates the RSL3-induced FCD. This finding implies that suppression of GPX4 by inhibiting NRF2 might act as a central factor in ferroptosis induced by RSL3 in CRC cells.[74] Therefore, the resistance of CRC cells to ferroptosis can be abolished by inhibiting the NRF2 pathway.

Based on available works, ferroptosis in cancer cells could be induced when cells were exposed to iron. Inferably, LPO induced by iron can be counteracted by increasing GSH levels.[75] In terms of mechanism, antioxidant genes are expressed upon activation of oncogenic RAS principally through a widely known master regulator of antioxidant response, nuclear factor-(erythroid derived-2)-like-2 (NFE2L2). Antioxidant response elements (ARE) are bonded to NRF2 within gene promoters that encode enzymatic antioxidants, namely, NADPH quinone oxidoreductase-1 (NQO1) and glutathione S-transferase A2 (GSTA2).[76] Furthermore, regulation of NRF2 activity is through a coordination of protein complex that comprises CLLIN3 (CUL3), ubiquitin ligase, Kelch-like epichlorohydrin (ECH) associated protein-1 (KEAP1), and other determinants.[45] Normally, NRF2 degradation is mediated by the aforementioned complex, which prevents its nuclear translocation. Nonetheless, induction of changes in the conformation of KEAP1 by oncogenic RAS results in transcriptional up-regulation of NRF2 and accompanying cytoprotection.[45]

Collectively, existing work has disclosed that NRF2 and ROS can be activated in CRC cells upon their exposure to iron, thus resulting in increased LPO status, and proteins associated with GSH and GPX4, thereby protecting CRC cells from ferroptosis (Figure 3).

Ferroptosis and OR in CRC cells are induced by the KIF20A-NUAK1-NRF2-GPX4 pathway

Through broad investigations, the mechanism underlying the pathological process of CRC resistance is discussed increasingly.[77,78] It has been confirmed that induction of ferroptosis in various tumors could reverse the resistance of several anticancer drugs and targeted formulations, thereby enhancing the therapeutic effects of such drugs.

As revealed by bioinformatical and cytobiological literature searches, high expression of KIF20A was seen in OR cell lines, and it showed a strong correlation with the survival rate of CRC patients. Therefore, KIF20A silencing could enhance the sensitivity of cells to oxaliplatin *in vitro* and *in vivo*, as well as inhibit the activation of NUAK1. Meanwhile, the potential reversal of oxaliplatin susceptibility of KIF20A silenced cells by an agonist

(ETC-1002) of NUAK1 had been shown. Furthermore, upregulation of PP1β expression was observed when NUAK1 was silenced, while downstream GSK3β Ser9 phosphorylation was downregulated, amid suppression of nuclear NRF2 import, inhibition of GPX4 expression (a central negatively regulatory protein for ferroptosis) and blockage of resistance in cancer cells. Also, the application of oltipraz (an agonist of NRF2) could reverse the sensitivity of NUAK1-silenced cells to oxaliplatin. Thus, ferroptosis in CRC cells could be suppressed through KIF20A-NUAK1-PP1β-GPX4 signaling pathway.[79]

Western blotting analysis affirmed that comparable to the unresistant cell line of CRC, expression of KIF20A was increased substantially in the resistant cell line. Furthermore, another work confirmed that high expression of KIF20A in resistant cell lines could promote upregulation of GPX4 expression intracellularly for the maintenance of redox balance in cells while inhibiting the ferroptotic process. Suppression of NUAK1 activation by silencing KIF20A had been suggested in another work, wherein stimulation of the pharmacological activation of the aforementioned kinase may reverse KIF20A-silencing induced susceptibility of cell lines to oxaliplatin. Later, the available findings affirmed that NUAK1 activation was abnormal in resistant CRC cells which resulted in suppression of protein phosphatase 1beta (PP1β) activity, while nuclear import NRF2 transcriptional activity was induced via regulation of GSK3β Ser9phosphorylation level, upregulation of intracellular levels of antioxidative compounds, maintenance of redox balance in tumor cells, and induction of resistance of cancer cell. A close correlation between activities of chemotherapies (including various targeted preparations and anticancer drugs) and disparity of redox status in cells was observed, while the increased transcriptional activity of NRF2 and its overexpression in cancer cells had been found in the resistance process, thus resulting in poor prognosis of patients.[80]

Collectively, ferroptosis induction may substantially reverse OR in CRC cells. Through existing investigations, the KIF20A-NUAK1 signaling pathway was highly expressed and activated in CRC cells, which in turn could inhibit the imbalance of intracellular redox induced by oxaliplatin and ferroptosis, as well as cause chemotherapeutic resistance in the cell via GSK3β-NRF2 signaling pathway. Prospectively, there is an unmet need for potential targets in the reversal of drug resistance in CRC cells. Clinically, treatment of CRC can be enhanced by making cancer cells sensitive to oxaliplatin through targeting KIF20A, NUAK1, or targets in the signaling pathway of NRF2-GPX4.

Potentiality of NRF2-Pd-L1 axis for promotion of OR in CRC cells

In cancer stem cells, the role of NRF2 in protecting cells against inflammation and oxidative stress in many tissues has been reported.[81,82] Overexpression of NRF2 in various cancer types has been described, and it is linked with poor prognosis in patients.[83,84] As a classified family member of CD28-B7, programmed death ligand 1 (PD-L1) is normally expressed on cancer cells' surfaces.[85] Based on their potential clinical outcomes in different cancer types, several immunoglobulins against PD-L1 or PD-1 have attracted much attention in recent times.[86,87] Nonetheless, cancer cells have developed resistance to

these immunoglobulin-based treatment options. A combination of therapies for late-stage melanoma has been tested to evade the aforementioned challenge. To date, various PD-L1 upstream regulators have been reported. An experimental work by Yin et al. showed an upstream regulator of PD-L1 to be NRF2. Melanoma progression was suppressed by destroying or inhibiting NRF2 via a substantial upregulation of the activities of $CD4^+$ and $CD8^+$ cells. Also, the efficacy of anticancer drugs increased through the suppression of NRF2 jointly with an anti-PD-1 agent.[79] Available evidence collectively suggests that inhibition of NRF2 has the potential to successfully treat multiple cancers including CRC.

In another work, NRF2 was shown to be the activator of PD-L1 upstream transcription which modulated the expression of PD-L1.[88] Besides, co-regulations of PD-L1 and NRF2 pathways were described in melanoma.[79] Again, regulation of PD-L1 expression by NRF2 has been shown, wherein it can potentially serve as an alternative therapeutic option for PD-1-PD-L1 immunoglobulin-based therapy of melanoma. Experimental works on the relation of NRF2-PD-L1 axis and OR in CRC are limited. Thus, the significance of NRF2-PD-L1 axis to migration and resistance of CRC cells, coupled with the expression of CD80 and PD-L1, NRF2, and PD-L1 genes was ascertained using specimens of CRC biopsy. The authors observed significant overexpression of NRF2 in CRC samples compared to margin tissues.[89] Agreeably, various works on tissue detection during colonoscopy disclosed overexpression of NRF2 in the patients.[80] Also, overexpression of NRF2 is linked with the grade and stage of tumors.[90]

Notably, luteolin and brusatol chemically inhibited NRF2 which significantly reduced the expression of PD-L1 and NRF2 at the mRNA level, wherein this finding corroborates the idea that the expression of PD-L1 is positively controlled by NRF2. Systematically, expressions of PD-L1 and NRF2 at the mRNA level were substantially increased by tBHQ through activation of NRF2. In particular, expression of NRF2 at the mRNA level was increased by tBHQ (20 μM) via NRF2 activation.[91] Furthermore, based on related studies, it can be inferred that NRF2 silencing could substantially decrease the expression of PD-L1 and NRF2 at the mRNA level in resistant and sensitive cells, amid being dependent on time. Furthermore, an evaluation of the possible effect of NRF2 silencing on the efficacy of oxaliplatin to break down resistance to oxaliplatin in LS174 T/Res and SW480/Res cells was carried out. Through apoptosis and MTT assays, it was observed that the knockdown of NRF2 reversed the resistance of oxaliplatin in LS174 T/Res and SW480/Res cells. As observed earlier, transporters of ABC efflux (dependent on NRF2) were substantially downregulated by a recombinant NRF2-siRNA coupled with increased sensitivity to cisplatin, doxorubicin, and sorafenib in 143B and MG63 cells.[92]

Moreover, significant inhibition of cell migration was observed when oxaliplatin alone or in combination with NRF2-siRNA was to treat SW480/Res cells. Treatment of the cells with combined oxaliplatin and NRF2-siRNA achieved the best inhibition effects. Conclusively, the principal role of NRF2 in promoting chemoresistance in several tumors (including CRC) has been described, wherein one of the downstream targets of the NRF2 pathway is PD-L1. As a protein of immune checkpoint, PD-L1 exhibited a beneficial effect on the growth of tumors by inhibiting the immune system. Collectively, the available evidence suggests the potential of the NRF2-PD-L1 axis to

promote OR in CRC cells, and further research for a complete understanding of actual mechanisms in CRC resistance to oxaliplatin is needed.

Strategies to regulate chemo-resistance and ferroptosis in CRC therapy

FINs

In recent years, ferroptosis has been a hotspot in scientific research and its clinical relevance has been investigated. Several components approved by FDA have been demonstrated to induce ferroptosis in tumor cells.[93] The four classifications of FINs include: inhibition of x-c system-class I, direct inhibition of GPX4-class II, indirect inhibition of GPX4-class III, and increase in levels of iron-class IV. As an enzyme, GPX4 decreases peroxides of lipids, and deficiency of GPX4 can result in the conversion of lipid peroxides to toxic lipid form of ROS by free iron.[5,94] Being in class II, withaferin A (a natural agent for FIN) could increase iron pool intracellularly and suppress neuroblastoma xenografts growth.[95] Similarly, the interaction of the antimalarial drug artesunate with iron in lysosomes is identified to produce ROS, which results in ferroptosis. In this regard, enhanced survival of patients was observed in phase I trials of artesunate in several cancers.[96]

Moreover, the pattern of cell death induced by artesunate appeared to be a novel form of regulated cell death, that is, ferroptosis, based on its iron-dependence, ROS production, and caspase-independent cell death. Recent two reports also identified artesunate as a specific activator of ferroptosis and highlighted its potential to overcome the resistance of cancer.[97] Endoperoxide (FINO2) is the only known class IV FIN, which induces oxidation of ferrous iron. Nonetheless, the half maximal effective concentration (EC_{50}) was 20 μM for renal cancer cell lines and immortalized fibroblasts, and the lack of investigations *in vivo* limited its utility (Table 1).

Inhibitors of GPX4

Through high-throughput sequencing, the RSL3 compound was identified first to induce ferroptosis selectively in transformed cells that harbor activated HRAS.[121] In particular, RSL3 was identified to be a direct target of GPX4 through affinity purification experiments.[122] Compared to RSL3, ML162 (another inhibitor of GPX4) was found when various drugs were screened for compounds that can target HRAS.[98] Nonetheless, the use of ML162 and RSL3 in preclinical *in vivo* works and clinical trials is limited because of their poor pharmacokinetic parameters and nonselective binding to targets besides GPX4.[99] On the contrary, higher specificity and improved biological availability have been shown by ML210 (a prodrug inhibitor of GPX4) and its derivative (JKE-1674), amid potential exploitation of their health benefits for treating cancer.[100] Besides, withaferin A demonstrated its multifarious pro-ferroptotic effect through XMOX1 activation, GPX4 inhibition, ROS induction, and MAPK-RAS-RAF pathway inhibition.[95,101] On one hand, WA acts as a class II FIN,[15] which results in a rapid drop in expression and activity of GPX4. GPX4 protects membranes against LPO, and

Table 1. Therapeutic implications of regulating chemo-resistance and ferroptosis in cancer therapy.

Strategies	The various agents and their targets	Regulated Mechanisms in vivo, in vitro or clinical studies	Reference
Regulating chemo-resistance and ferroptosis in cancer	Ferroptosis inducers (FINs)	Withaferin A (a natural agent for FIN) could increase iron pool intracellularly and suppress neuroblastoma xenografts growth. Artesunate with iron in lysosomes is identified to produce ROS, which culminates in ferroptosis. In this regard, enhanced survival of recurrence-free was observed in phase I trials of artesunate in several cancerous cells amid efforts to continuously repurpose the drug in progressive clinical pipeline. Moreover, artesunate-induced ferroptosis, based on its iron-dependence, ROS production. Artesunate is a specific activator of ferroptosis, thus highlighting its potential to overcoming the resistance of cancer.	[95–97]
	Inhibitors of GPX4	RSL3 was identified to be directly targeted by GPX4, ML162 (inhibitor of GPX4) that can target HRAS. ML210 and its derivative (JKE-1674), amid potential exploitation of their health benefits for treatment of cancer. Withaferin A demonstrated its multifarious proferroptotic effect through XMOX1 activation, GPX4 inhibition, ROS induction and MAPK-RAS- RAF pathway inhibition. WA acts as a class II FIN, which resulted in a rapid drop in expression and activity of GPX4, and GPX4 protects membranes against lipid peroxidation, and its targeted degradation typically results in ferroptosis. A medium dose of WA induced a massive upregulation of HMOX1, which was followed by an increase in cellular labile Fe(II), and consequent ferroptosis.	[98–101]
	SREBP inhibitors	Statins may be a valuable candidate for inducing ferroptosis in tumor and the sensitivity of tumor cells to statin-induced cell death is influenced by SREBP2 inhibition. Significantly, preclinically and clinically that N-BPs, fatostatin and statins could respectively target FPPS, SREBP-2 and HMGCR either alone or combined with other drugs in various tumors. TVB-3664, while its particular human isomer (TVB-2640) is undergoing phase-2 clinical trial (NCT03808558) in patients with mutant K-ras LC. Therefore, it can be speculated that inhibition of both SREBP/FASN and statins may serve as an effective therapeutic approach to induce ferroptosis in this particular tumor type.	[45,102,103]
	Ferroptocide and auranofin	Ferroptotic induction by auranofin via suppression of thioredoxin reductase (TXNRD) activity, auranofin coupled with BSO-dependent inhibition of GPX4 has been used successfully to induce ferroptosis in small-cell lung cancer (SCLC). Alternatively, GPX4 degradation is induced by rapamycin (mostly utilized and characterized mTOR for autophagic inhibitor and inducer), which result in activation of autophagy-dependent ferroptosis in cell lines of pancreatic ductal adenocarcinoma (PDA). Another compound that is used to target the TXN-TXRD system is ferroptocide, which induces ferroptosis by	[104–106]

(Continued)

Table 1. (continued)

Strategies	The various agents and their targets	Regulated Mechanisms in vivo, in vitro or clinical studies	Reference
		binding covalently to TXN, dysregulation of TXN occurs in pancreatic cancer which regulates K-ras pathway, suggesting an effective approach to ferroptotic induction in RAS driven tumors.	
	Natural FIN compounds	Artemisinins that was discovered initially as natural antimalarial ingredient, have demonstrated antitumor potentials. Erianin is another natural compound that has demonstrated preclinical potential to induce ferroptosis in mutant K-ras LC models via induction of high intracellular calcium and iron levels. Besides, ferroptosis in mutant K-ras CRC has been discovered to be mediated by bromelain (through ACSL4 up-regulation), which is a proteolytic enzymatic mixture that is derived from stem of Ananas comosus L (commonly known as pineapple in the family Bromeliaceae). Tagitinin C (a sesquiterpene lactone that was extracted from Tithonia diversifolia) inhibited the growth of CRC cells (e.g. HCT116 cells) through induction of an oxidative microenvironment within the cells. Again, induction of ferroptosis by tagitinin C was followed by attenuated levels of glutathione (GSH) coupled with increased LPO accumulation. A substantially increased expression of heme oxygenase-1 (HO-1, a NRF2 downstream effector) was observed in tagitinin C treatment. Also, up-regulation of HO-1 culminated in increased LIP, thereby promoting LPO, amid induction of ferroptosis by tagitinin C via mediation of PERK-Nrf2- HO-1 pathway activation by ER stress. Combination treatment of erastin and tagitinin C enhanced the tumoricidal efficacy, which rendered ferroptotic insusceptible HCT116 cells sensitive to FCD. Additionally, tagitinin C synergistically exhibited cytotoxic activity with RSL3 (a ferroptosis inducer).	[107–110]
Combination Therapies	Vitamin C and cetuximab	Vitamin C could induce ferroptosis in cancer cells, wherein cetuximab and vitamin C co-treatment prevented the development of acquired cetuximab resistance in wild-type RAS-BRAF CRC. Combined treatment of cetuximab and β-elemene (a bioactive ingredient) was efficacious against mutant K-ras CRC cells by inducing ferroptosis. In addition, cetuximab could improve cytotoxic activity of RSL3 with the potential to promote ferroptosis induced 3 in mutant K-ras CRC cells. Besides, Nrf2/HO-1 played essential role in the promotion of RSL3-induced ferroptosis by cetuximab in mutant K-ras CRC cells. Collectively, these findings suggested that Nrf2/HO-1 activation in cells (that were treated with RSL3) was decreased by cetuximab, which could successively increase RSL3-induced levels of MDA and lipid ROS.	[74,111]
	Dp44 mT and DpC	Through transport of lysosomal Pgp, DpC and Dp44 mT overcame resistance to vinblastine and	[112–116]

(Continued)

Table 1. (continued)

Strategies	The various agents and their targets	Regulated Mechanisms in vivo, in vitro or clinical studies	Reference
		doxorubicin, wherein iron in lysosomes complexed with the compounds, to produce ROS which breaks-up the membrane of lysosome and induces apoptosis. In combination with 4-hydroperoxy-cyclo- phosphamide, doxorubicin, 5-fluorouracil, paclitaxel and tamoxifen, Dp44 mT improved synergistically the in-vitro cytotoxic effect against cancer cells of the breast	
	Chelators of Iron and chemotherapeutic drugs	The study has found that treating A549 and HCT116 cells with inhibitors of ferroptosis (deferoxamine-DFO, IRP2 knockdown and ferrostatin-1), could partly reverse toxicity induced by cisplatin, while alterations in mitochondrial structure were visually observed amid consistency with ferroptosis. Alternatively, another work has described the potential of cisplatin to deplete iron stores of tumor cells through direct IRP2 binding, thus inhibition of its binding to IREs coupled with increased levels of ferritin and decreased expression of TfR1could lower LIP. In an experiment conducted with mice, cytotoxicity of cancer cells was enhanced by combined DFO and cisplatin via augmentation of iron reduction in cultured cells and xenografts of CRC cells. As an agonist of HIF-1α, DFO could promote binding of HIF-1α to promoter of Gls1, wherein it increased expression of GLS1 but got rid of inhibitory activity of curcumol against the expression of GLS1. Also, EMT, invasion and migration of CRC cells were enhanced by DFO which also eliminated effects of curcumol. Moreover, the growth, metastasis and EMT of CRC cells in mice were successfully inhibited by curcumol, albeit abrogation of GLS1 or HIF-1α overexpression.	[117–120]

its targeted degradation typically results in ferroptosis.[40] On the other hand, a medium dose of WA induces a massive upregulation of HMOX1 followed by an increase in cellular labile Fe(II), and consequent ferroptosis.[101] Nevertheless, to fully establish the potentiality of withaferin A as an efficacious FIN, further research is needed to understand its pleiotropic property, multiple targeting dependences and susceptibilities of RAS-driven tumors, as well as the development of the drug into nanocarriers.[95]

Statins as SREBP inhibitors

As drugs to decrease cholesterol, statins could inhibit HMG-CoA reductase (HMGCR).[123] Besides, isopentenyl pyrophosphate (IPP, precursor of coenzyme Q10 and GPX4) formation is blocked by statins, which facilitates ferroptosis. The impact of the mevalonic acid pathway on various cancer signaling pathways is well known[124]; hence, several studies have explored the possible application of this pathway in tumor treatment,[125] like in RAS-driven tumors. Activation of RAS was initially observed to improve statins sensitivity, whereas susceptibility of tumor cells to statin therapy was closely associated with the expression of RAS and levels of prenylation, thus suggesting the potential of statins to treat cancers with high expression and variability of Ras.[126]

Possibly, these outcomes may be the result of statins inducing a feedback activation of sterol regulatory element binding transcriptional factor-1/2 (SREBP1/SREBP2) pathways, which subsequently activate genes that regulate the biosynthesis of mevalonic acid and lipids. Therefore, inhibitors of the Mevalonate pathway will block the maturation of selenocysteine tRNA and the synthesis of GPX4. In addition, another product of the Mevalonate pathway is Coenzyme Q10 (CoQ10), a powerful antioxidant in membranes, that can repress ferroptosis under oxidative stress.[102] Statins may be a valuable candidate for inducing ferroptosis in tumors, and the sensitivity of tumor cells to statin is influenced by SREBP2 inhibition.[127] Significantly, it has been shown preclinically and clinically that N-BPs, fatostatin, and statins could, respectively, target FPPS, SREBP-2, and HMGCR either alone or combined with other drugs, wherein various tumors have been treated with these treatment strategies.[103]

It is interesting to note that the SREBP1/FASN pathway was activated by mutant K-ras in lung cancer (LC), while mutant K-ras LC was selectively vulnerable to inhibition of FASN.[45] Actually, ferroptosis was purposely induced in mutant K-ras LC models by an inhibitor of FASN, TVB-3664, while its particular human isomer (TVB-2640) was undergoing phase-2 clinical trial (NCT03808558) in patients with mutant K-ras LC.[45] Therefore, it can be speculated that inhibition of both SREBP/FASN and statins may serve as an effective therapeutic approach to induce ferroptosis in this particular tumor type (Figure 4).

Ferroptocide and auranofin as FINs

Currently, a clinical trial (phase I and II, NCT01737502) study is being carried out to test the synergistic effect of rapamycin and auranofin (an antirheumatoid arthritis drug) on mutant RAS small and squamous LC. The potential of the two compounds to induce ferroptosis and synergism has been described. Ferroptotic induction by auranofin via suppression of thioredoxin reductase (TXNRD) activity has been reported,[104] wherein this

therapeutic strategy coupled with BSO-dependent inhibition of GPX4 has been used successfully to induce ferroptosis in small-cell lung cancer (SCLC).[105] Alternatively, GPX4 degradation reported in recent times was induced by rapamycin (mostly utilized and characterized mTOR for autophagic inhibitor and inducer), which resulted in the activation of autophagy-dependent ferroptosis in pancreatic ductal adenocarcinoma (PDA) cell lines.[106]

Another compound used to target the TXN-TXRD system is ferroptocide, which induces ferroptosis by binding covalently to TXN.[128] Notably, dysregulation of TXN occurs in pancreatic cancer that regulates K-ras pathway,[129] thus suggesting the possibility of this biological process serving as an effective approach to ferroptotic induction in RAS-driven tumors.

Natural FIN compounds

Various natural compounds have emerged as possible inducers of ferroptosis in tumors. Particularly, artemisinins, which were discovered initially as a natural antimalarial ingredient and isolated from *Artemisia annua*, have been demonstrated to own antitumor potentials.[107] Also, Erianin is another natural compound that is extracted from *Dendrobium chrysotoxum* Lindl and has demonstrated preclinical potential to induce ferroptosis in mutant K-ras LC models via induction of high intracellular calcium and iron levels.[108] Besides, ferroptosis in mutant K-ras CRC has been discovered to be mediated by bromelain (through ACSL4 up-regulation), which is a proteolytic enzymatic mixture derived from the stem of *Ananas comosus* L (commonly known as pineapple in the family Bromeliaceae).[109]

In another work, ferroptosis in HCT116 cells was induced when tagitinin C (a sesquiterpene lactone that was extracted from *Tithonia diversifolia*) inhibited the growth of CRC cells (e.g. HCT116 cells) through induction of an oxidative microenvironment within the cells.[110] Again, induction of ferroptosis by tagitinin C was followed by attenuated levels of GSH coupled with increased LPO accumulation. In terms of mechanism, activation of NRF2 by tagitinin C was accomplished through induction of stress in endoplasmic reticulum (ER) and oxidative stress in general. A substantially increased expression of heme oxygenase-1 (HO-1, a NRF2 downstream effector) was observed in tagitinin C treatment.[110] Also, upregulation of HO-1 led to increased LIP and LPO, amid induction of ferroptosis by tagitinin C via mediation of PERK-Nrf2-HO-1 pathway activation by ER stress.

Together with erastin (a well-established FIN), tagitinin C synergistically demonstrated effects on cytotoxicity and ferroptosis. Besides, ferroptotic-inducing effect of tagitinin C could be amplified by erastin because it could stimulate stress of the ER. Combination treatment of erastin and tagitinin C enhanced the tumoricidal efficacy, which rendered increase of sensitivity to FCD in ferroptotic insusceptible HCT116 cells. Additionally, tagitinin C synergistically exhibited cytotoxic activity with RSL3 (a FIN). Based on the ferroptotic-inducing potential of Tagitinin C, more efficient chemosensitizer can be developed with the hope that they may overcome resistant cancers, thus providing new treatment strategies for cancer therapy.

Combination therapies

Presently, there is an increasing trend to clarify the pathological process of CRC, wherein a series of OR-associated molecular mechanisms have been revealed in studies. Related

molecular mechanisms include excessive activation of the damage repair system of DNA, membrane transporter overexpression and resistance of CRC cells to autophagy, as well as the regulatory axis of c-Myc/miR-27b-3p-ATG10 that modulates chemoresistance and induction of protective autophagy by the axis of HSF1-miR-135b-5p to promote OR via the MUL1-ULK1 pathway.[130,131] In this regard, scientists have performed several experimental works involving basic and clinical models as well as oxaliplatin in combination with various anticancer drugs. However, unsatisfactorily results were observed since prolonged administration of combined drugs increased the toxic and side effects. Accordingly, novel means of reversing OR and the potential to exert their anti-CRC activity in a long-acting fashion, as well as the ability to prolong the survival rate of patients, are the hotspots.

Vitamin C or RSL3 combined treatment with cetuximab

Cancer progression and chemotherapy resistance are reported to be effectively prevented by activating ferroptosis. For example, vitamin C could induce ferroptosis in cancer cells, wherein cetuximab and vitamin C co-treatment prevented the development of acquired cetuximab resistance in wild-type RAS-BRAF CRC.[132] Combined treatment of cetuximab and β-elemene (a bioactive ingredient) was effective against mutant K-ras CRC cells by inducing ferroptosis.[111] In addition, cetuximab could improve the cytotoxic activity of RSL3 with the potential to promote RSL3-induced ferroptosis in mutant K-ras CRC cells. Besides, Nrf2/HO-1 played an essential role in the promotion of RSL3-induced ferroptosis by cetuximab in mutant K-ras CRC cells. Collectively, these findings suggested that Nrf2/HO-1 activation in cells (that were treated with RSL3) was decreased by cetuximab, which could successively increase RSL3-induced levels of MDA and lipid ROS. Agent of ferroptosis protected against cetuximab and RSL3-induced cell death, which suggested that ferroptosis led to cell death in the presence of cetuximab.[74] Hopefully, this result will facilitate the development of effective therapeutic options for patients with mutant K-ras CRC.

Dp44mT and Dpc overcome resistance

A complete understanding of the molecular mechanisms of resistance to chemotherapies brought opportunities for improving responses to tumor treatment. Exemplary, efflux of anticancer drugs from cancer cells is one of the principal reasons for multidrug resistance, amid upregulation of drug transporters expression, like resistance mediated by P-glycoprotein (Pgp), which quickly occurs through internalization, redistribution and its heightened expression via the activity of hypoxia-inducible factor 1-alpha (HIF-1a). The aforementioned processes could facilitate liposomal accumulation of the drug, thus creating a "*drug safe house*" beyond its treatment target, which eventually results in the efflux of the drug from tumor cells.[133] Transport of lysosomal Pgp, DpC, and Dp44 mT overcame resistance to vinblastine and doxorubicin,[112,113] wherein iron in lysosomes complexed with the compounds could produce ROS which broke the membrane of lysosome and induced apoptosis.[114,115] In combination with 4-hydroperoxy-cyclo-phosphamide, doxorubicin, 5-fluorouracil, paclitaxel, and tamoxifen, Dp44 mT improved synergistically the cytotoxic effect against cancer cells of the

breast *in vitro*.[116] Based on the promising anticancer effect of Dp44 mT, coupled with similar positive results from clinical trials of DpC, the next step may be an assessment of their combination with available anticancer agents.

Chelators of iron and chemotherapeutic drugs

Evaluation of chelators of iron in combination with common anticancer drugs like cisplatin has been carried out. Also, in combination with cisplatin, triapine has been evaluated in various clinical trials (www.clinicaltrials.gov). Evidently, existing findings support the safety of combinations of drugs with the additional advantage of improving the progress of free survival.[134] As reported, response to cisplatin was enhanced by triapine through disruption of homologous recombination repair accompanied by DNA

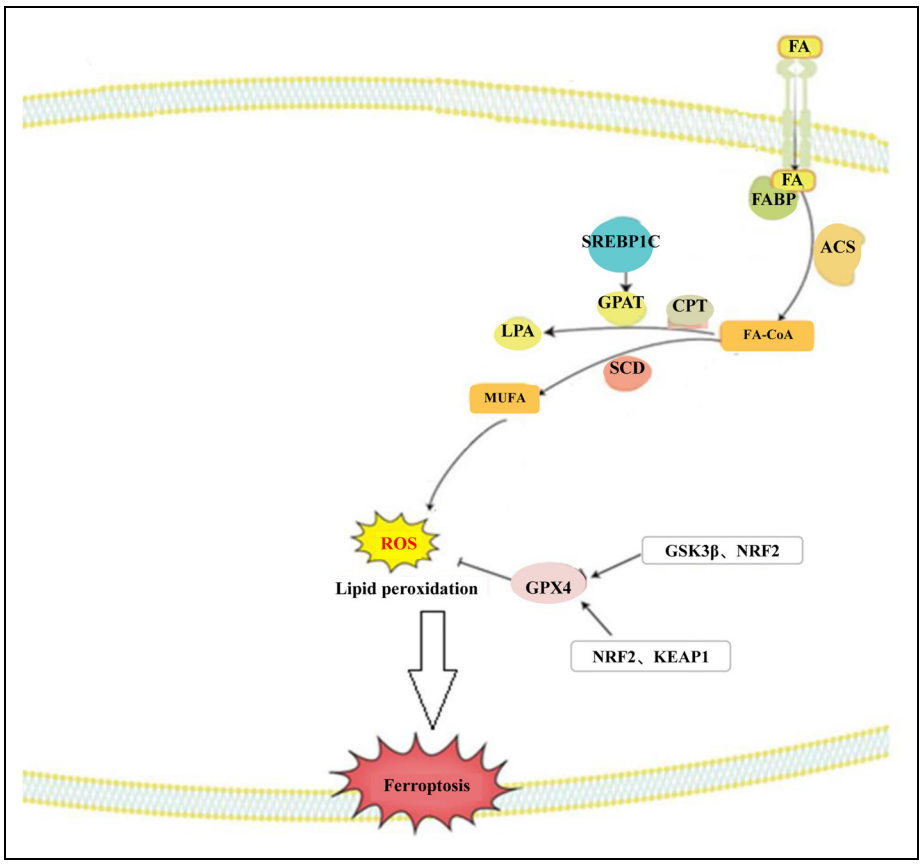

Figure 4. Roles and functions of lipid metabolism, enzymes and ferroptosis in colorectal cancer (CRC). The mechanism underlying the regulation of lipid metabolism and enzymes, such as transcriptional factor-1/2 (SREBP1/SREBP2) pathways in cancer cells, induces cell lipid peroxidation and ferroptosis.

damage induced by cisplatin.[117] It is necessary for knowing the mechanism underlying the sequential combined treatment of cisplatin and triapine to be unique in order to attain synergism, wherein this suggests the importance of a drug administration schedule to maintain its efficacy. Nonetheless, the mechanism of cisplatin is still not understood clearly, especially its role in the metabolism of iron. Suggestively, cisplatin has been described to induce ferroptosis via GSH depletion and GSH peroxidase inactivation.[118] The authors reasoned that treating A549 and HCT116 cells with inhibitors of ferroptosis (deferoxamine-DFO, Iron responsive protein 2 [IRP2] knockdown and ferrostatin-1) could partly reverse toxicity induced by cisplatin, while alterations in the mitochondrial structure were visually observed amid consistency with ferroptosis. Notwithstanding, the findings of that study were not too convincing since minimal alterations were seen upon the reversal of toxicity induced by cisplatin. Alternatively, another work had described the potential of cisplatin to deplete iron stores in tumor cells through direct IRP2 binding, thus inhibition of its binding to IREs coupled with increased levels of ferritin and decreased expression of TfR1could decrease LIP.[119] In an experiment conducted with mice, the cytotoxicity of cancer cells was enhanced by combined DFO and cisplatin via augmentation of iron reduction in cultured cells and xenografts of CRC cells.

Based on available evidence, cisplatin seems to reduce iron intracellularly instead of triggering cell death induced by iron, amid the need for further investigation to provide some clarity. Despite evidence for various works on investigations of iron chelators in combination with anticancer drugs, their potential to increase the efficacy of targeted natural agents and immune-based treatment options has not been fully proven. The underlying reason for this trend has been postulated to be the stabilization of HIF-1α and increase in its expression which could result in increased cell proliferation, angiopoiesis and metastasis. Thus, combined treatment with a particular HIF-1α inhibitor is the ideal strategy to enhance the efficacy of iron chelators. As an agonist of HIF-1α, DFO could promote the binding of HIF-1α to the promoter of Gls1, wherein it increased the expression of GLS1 but got rid of the inhibitory activity of curcumol against the expression of GLS1. Also, epithelial–mesenchymal transformation (EMT), invasion, and migration of CRC cells were enhanced by DFO as well as eliminated effects of curcumol. Moreover, the growth, metastasis, and EMT of CRC cells in mice were successfully inhibited by curcumol, albeit abrogation of GLS1 or HIF-1α overexpression.[120]

Various FINs have been identified to increase chemosensitivity. For instance, the sensitivity of chemotherapeutics (e.g. doxorubicin/Adriamycin, temozolomide, cytarabine/ara-C, and cisplatin) was increased by erastin in certain tumor cells.[94] The combined anticancer effect of doxorubicin and dexamethasone was enhanced in multiple myeloma xenografts by PRIMA-1 (an agent with nongenotoxicity) by targeting mutant/deleted p53 and activating FCD.[113] Detoxification of chemotherapies importantly depends on GSH activity, and as such evaluation of specific inhibitors of GSH, buthionine sulfoximine (BSO) has been carried out in clinical trials, and it was used combined with anticancer drug melphalan in late-staged malignancies.[114] A well-tolerated BSO and combined treatment option was observed, which demonstrated some pharmacological effects, albeit lack of clarity of whatsoever clinical responses being due to induction of ferroptosis or inhibition of drug detoxification.[115] In conclusion, these agents have demonstrated the potential of determining the chemosensitivity of antitumor drugs, hence justifying the need to discover new cancer treatment options that can reverse drug resistance.

Conclusions

Ferroptosis in CRC cell interferes with lipid metabolism and disrupts the metabolic balance of iron in cancer cells, thereby enhancing the chemosensitivity of drug-resistant cancer cells. Exposure of CRC cells to iron potentially induces activation of NRF2 and ROS, which results in increased accumulation of byproducts of LPO and proteins associated with GSH as well as GPX4. Regulation of the downstream transduction factor c-Myc/NRF2 and the ROS/AKT/mTOR pathway mediates the participation of TfR1 and DMT1 in metabolic homeostasis of iron in cancer cells. Furthermore, the mechanism underlying the regulation of lipid and iron metabolisms in cancer cells is NRF2-GPX4 signaling cascade, which induces cell ferroptosis and promotes anti-CRC effects of oxaliplatin. In cancer therapy, one of the most popular approaches is targeting the cell death process. Ferroptosis is a newly coined term, leading to an apoptosis mechanism that exhibits distinct characteristics and has considerable promise in cancer treatment, as shown by the research. Ferroptosis plays a key role in OR and ferroptosis induction and may substantially reverse OR in CRC cells, which in turn could inhibit the imbalance of intracellular redox induced by oxaliplatin and ferroptosis, as well as cause chemotherapeutic resistance in CRC. Furthermore, fundamental research of small molecules, FIN, GPX4 inhibitors, or natural products for OR coupled with their clinical applications in CRC has also been summarized. Importantly, the potential molecular targets and mechanistic actions of small molecules or drugs are discussed as well. Suggestively, OR of CRC cells could significantly be reversed by ferroptosis induction, and the potential use of combined anticancer drugs may overcome OR via targeting various mechanisms of ferroptosis.

Future perspectives and clinical relevance

Ferroptosis is a biological process regulated by multiple genes, accompanied by a series of morphological and metabolic changes. Consequently, through in-depth research on the physiology, cytology, molecular biology, genomics, and bioinformatics of ferroptosis, more cancer therapeutic targets may be discovered. Although there have been some major advances in the development of iron-based therapeutics, their toxicity, short-half life, rapid metabolism, and emerging resistance are ongoing concerns. A lack of insight into the mechanisms of resistance to these therapies has somewhat hampered the generation and optimization of new analogs to overcome these issues. Metabolic studies will likely provide the information we need for determining the route and sites of drug "de-activation" and whether pro-drug strategies could circumvent it. It may also inform novel drug combinations with platinum-based drugs to improve tumor responses or help identify which patients are most likely to benefit from iron-based therapies. Besides, future works should be centered on the potential to target c-Myc/NRF2-mediated iron homeostasis or NRF2/GPX4 pathway signaling via the combined treatment of platinum-based drugs to modulate the fundamental regulatory protein in ferroptosis and blocked cellular resistance through activation of anticancer activity, amid the prospect of enhancing therapeutic strategies for CRC.

For prospective clinical applications, potently specific FINs, GPX4 inhibitors or natural products may be prepared as successful goals, while the potential of small

compounds to induce ferroptosis activation or development of a single subtype for clinical application of CRC might be envisaged. The potential of combined treatment of platinum-based drugs coupled with small compounds targeted delivery systems to enhance biological availability and effects has been highlighted based on available evidence. Additionally, secretion of exosome from stromal cells in TME (e.g. tumor-related fibroblasts) is promoted by chemotherapy, wherein it results in decreased accumulation of LPO and ferroptosis in tumor cells. Possible strategies for sensitization of platinum-based drugs may be through ferroptosis interactions with the metabolism of lipids, amid speculation that combining statins with inhibition of the SREBP-FASN pathway may efficiently induce ferroptosis in CRC.

Taken together, ferroptosis is involved in the resistance of multiple types of cancers to platinum-based drugs, and targeting ferroptosis is a promising strategy to overcome resistance to cisplatin or oxaliplatin. Experiments to be carried out in not too distant future can explore the development of payloads comprised of a couple of platinum-based drugs with the aim of successfully targeting homeostasis of iron or ferroptotic signaling via FA regulation and lipid metabolism. Through activation of the aforementioned platinum-based drugs via induction of ferroptosis and combined chemotherapies, drug resistance could be overcome through targeting multiple ferroptotic mechanisms, in particular, OR in CRC cells could be reversed substantially, amid the potential of such agents to potentially act as effective anti-CRC drugs.

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Author's contribution

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