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REVIEW

The Role of Erastin in Ferroptosis and Its Prospects in Cancer Therapy

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Abstract: Erastin was initially discovered as a small molecule compound that selectively kills tumor cells expressing ST and RAS^{V12} and was later widely investigated as an inducer of ferroptosis. Ferroptosis is a recently discovered form of cell death caused by peroxidation induced by the accumulation of intracellular lipid reactive oxygen species (L-ROS) in an iron-dependent manner. Erastin can mediate ferroptosis through a variety of molecules including the cystine-glutamate transport receptor (system X_C^-), the voltage-dependent anion channel (VDAC), and p53. Erastin is able to enhance the sensitivity of chemotherapy and radiotherapy, suggesting a promising future in cancer therapy. We hope that this review will help to better understand the role of erastin in ferroptosis and lay the foundation for further research and the development of erastin-based cancer therapies in the future.

Keywords: erastin, ferroptosis, system X_C^-, p53, VDAC, cancer

Introduction

Ferroptosis is an iron-dependent and non-apoptotic form of cell death defined in 2012. It is characterized by excessive accumulation of lipid peroxides and reactive oxygen species (ROS).[1] Ferroptosis differs from apoptosis, necrosis, and other types of cell death in terms of morphology, genetics, metabolism and molecular biology.[2] Ferroptosis can occur in many organ systems, such as testes, kidneys, heart, and brain.[3–5] Current knowledge indicates that ferroptosis occurs during various pathophysiological processes of the body, including degenerative diseases of the central nervous system, the antiviral immune response, arteriosclerosis, acute kidney injury, diabetes, and ischemia-reperfusion injury.[6] Although ferroptosis plays a vital role in maintaining the survival of normal cells and tissues, it is increasingly recognized that some oncogenic pathways are closely related to ferroptosis, making cancer cells extremely susceptible to ferroptosis.[7]

It has been found that ferroptosis can inhibit the proliferation of malignant cells in liver cancer, pancreatic cancer, prostate cancer, breast cancer, and other cancers.[8–11] In particular, some highly malignant cancer cells have been proved to be inherently vulnerable to ferroptosis, so inducing ferroptosis may become a new method of cancer treatment.[12] There are two main categories of ferroptosis inducers: the first type can play a role through the cystine-glutamate transporter (system X_C^-) and includes erastin, sulfasalazine, and glutamate while the second type can directly inhibit glutathione peroxidase (GPX) activity and includes RSL3 and DP17.[13,14] Among them, erastin differs from other ferroptosis inducers in that the latter usually trigger a single pathway, whereas erastin can trigger multiple molecules and the effect is efficient,

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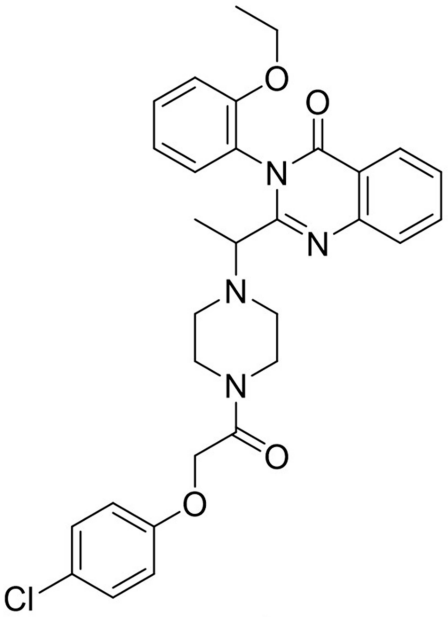
rapid, and lasting.[15] Since naturally non-apoptotic forms are induced, erastin-based cancer treatments promise to bypass the drawbacks of traditional therapies mediated by apoptosis. In this review, we first introduce the basic characteristics of ferroptosis, and then focus in detail on the mechanism and anti-cancer characteristics of erastin in inducing ferroptosis. It is expected to provide the basis for the potential of erastin as an anti-cancer drug in the future.

The Discovery of Erastin

In 2003, Dolma et al used large-scale screening experiments to explore the killing effects of various compounds on cancer cells. They found that camptothecin (CPT) and a novel small molecule compound from combinatorial libraries could selectively kill engineered cancer cells overexpressing Small T oncoprotein (ST) and oncogenic RAS. They named this new compound eradicator of RAS and ST (erastin).[16] (Figure 1) However, unlike the apoptosis induced by CPT, erastin-induced cancer cell death was found to be distinctly different. The classical apoptotic characteristics, including mitochondrial cytochrome c release, caspase 3 activation and DNA fragmentation, were not found in erastin-induced cell death, nor was erastin-induced cell death able to be inhibited by apoptotic inhibitors.[16–18] Erastin-induced cell death was, therefore, deemed to be a novel and non-apoptotic form of cell death.[17] Erastin was shown to kill human cancer cells exclusively, rapidly and irreversibly, without affecting normal cells of the same genotype.[15,16] Dixon et al named the cell death induced by erastin as ferroptosis.[1]



2-[1-[4-[2-(4-chlorophenoxy)acetyl]-1-piperazinyl]ethyl]-3-(2-ethoxyphenyl)-4(3H)-Quinazolinone

Figure 1 The chemical structure of erastin.

Basis of Ferroptosis

As early as the 1990s, Tan et al used glutamate to act on immortalized mouse nerve cells (HT-22) to study the effect of oxidative stress on neuronal cells. It was found that glutamic acid competed for the uptake of cystine, resulting in a decrease in glutathione and eventually oxidative cell death.[19] In 2008, Seiler et al identified lipid peroxidation as the key mediator of cell death in glutathione peroxidase 4 (GPX4) knockout cells. They speculated that GPX4 uses oxidative stress to activate a novel cell death pathway.[20] Additional research showed that this type of cell death could not be explained by either apoptosis and necrosis. In 2012, Dixon et al formally defined this mode of cell death as ferroptosis.

Iron plays an important role in many important metabolic processes in the body. Under physiological conditions, iron levels need to be properly balanced in the cell and are mainly regulated by transferrin and ferritin. Excessive ionic iron will cause "iron enrichment" and cause cell death, that is, ferroptosis.[21] Ferroptosis is an iron-dependent form of cell death characterized by the accumulation of intracellular lipid reactive oxygen species (L-ROS). Reactive oxygen species (ROS) is a collective name for a large class of molecules. They all contain oxygen atoms and are strongly oxidizing. ROS can react with the polyunsaturated fatty acids (PUFAs) of the lipid membrane and induce lipid peroxidation to form L-ROS. High concentrations of L-ROS can trigger oxidative stress in cells, causing oxidative damage.[22,23] Iron can contribute to the ROS pool in cells through the Fenton reaction, in which iron catalyzes the decomposition of H_2O_2 to generate hydroxyl radicals while enhancing the propagation of phospholipid oxidation and degradation of membrane lipids.[24] These all aggravate the formation of L-ROS and oxidative damage to cells.

Ferroptosis differs significantly from other forms of cell death (such as apoptosis, necrosis, and autophagy).[2,25] In terms of morphology, ferroptotic cells exhibit specific mitochondrial shrinkage and increased mitochondrial membrane density, while other typical characteristics of cell death are absent.[1,26] In terms of biochemical metabolism, the main manifestation is that ionic iron deposition causes membrane lipid peroxidation and excessive oxidative stress together

with the damaged intracellular redox homeostasis, with reduced antioxidant capacity and increased intracellular ROS, eventually lead to oxidative cell death.[27] Moreover, this death process can be inhibited by antioxidants and iron chelators.[1] Although many upstream pathways lead to ferroptosis, they lead directly or indirectly to an imbalance of production and degradation of intracellular L-ROS, and eventually, ferroptosis.[1,28]

The Relevant Pathways of Ferroptosis

Ferroptosis Is Induced by Inhibiting the Cystine-Glutamate Transporter System X_C^-

System X_C^- is a reverse transporter located in the plasma membrane. It is a heterodimer composed of a light chain subunit, xCT, encoded by *SLC7A11*, and functioning as the substrate-specific subunit, and a heavy chain subunit 4F2, encoded by *SLC3A2*, which is common to other amino acid transporters. System X_C^- transfers glutamate out of cells and cystine into cells at a ratio of 1:1.[29–31] Upon transfer into the cell, cystine is rapidly reduced to cysteine, which is then used in the synthesis of glutathione (GSH), a tripeptide composed of cysteine, glutamate, and glycine. The sulfhydryl structure contained in GSH can be oxidized and dehydrogenated, making GSH an important antioxidant and free radical scavenger in the body.[32] GPX is a peroxide-degrading enzyme, and GSH is an essential cofactor in its activation.[33] GPX plays a significant role in maintaining redox homeostasis and protecting cells from lipid oxidative stress leading to death. A variety of ferroptosis inducers can inhibit cystine absorption by inhibiting system X_C^-, resulting in reduced GPX activity. The consequence of this is a reduction in the cell's antioxidant capacity and hence increased L-ROS, ultimately leading to ferroptosis.[34] Therefore, inhibition of the cystine-glutamate transporter system X_C^- is an important pathway to induce ferroptosis.

p53 Participates in Ferroptosis

p53 is a classic tumor suppressor that mediates tumor cell cycle arrest, aging, and apoptosis.[35,36] With the accumulation of research on the mechanisms of cell death, it has been found that p53 not only causes apoptosis, but that activation of p53 also plays an important role in regulating ferroptosis in certain cancer cells.[37,38] Activation of p53 was found to significantly reduce the expression of *SLC7A11* in cells, up-regulation of p53 reduced expression of *SLC7A11* at both the protein and mRNA levels and knockdown of the p53 gene eliminated the inhibition of *SLC7A11*.[39,40] Other studies have further demonstrated that the cell's antioxidant capacity is significantly reduced after p53 gene activation.[41] Zhang et al concluded that the inhibition of *SLC7A11* expression by activation of p53 led to a decrease in system X_C^- activity, which in turn regulated ferroptosis.[42] In addition to inhibiting the activity of system X_C^-, p53 can also mediate ferroptosis by directly targeting the diamine acetyltransferase *SAT1* and the mitochondrial glutaminase *GLS2* which is involved in the regulation of glutamine metabolism.[43,44]

However, in some cases, p53 can also reduce cell sensitivity to ferroptosis. Studies have found that p53 activates p21 in a transcription-dependent manner and delays the onset of ferroptosis.[45,46] In addition, Xie et al found that in colorectal cancer (CRC) cells, p53 can also inhibit ferroptosis by combining with dipeptidyl peptidase-4 (DPP4).[47] So far, it is believed that p53 is at the core of a powerful signaling network during ferroptosis. On the one hand, p53 can increase the sensitivity of cells to ferroptosis to eliminating abnormal cells and inhibiting tumorigenesis while on the other hand, p53 has another major function in protecting normal cells from various stress factors. When metabolic stress occurs, p53 can both reduce the cells' sensitivity to ferroptosis and protect them, allowing them to maintain normal physiological functions. At present, the mechanism of p53's regulation of ferroptosis under different influencing factors has not been fully studied. The role of p53 in the ferroptosis signaling regulatory network is complex. The specific mechanism of p53 in cancer treatment needs further study.

Other Pathways of Ferroptosis

GPX4 is a member of the GPX family and plays a critical role in maintaining intracellular redox homeostasis. Certain inducers of ferroptosis, such as RSL3 and DP17, have been found to act by direct inhibition of GPX4, leading to a decrease in the cellular antioxidant capacity, and eventually resulting in ferroptosis.[1] The voltage-dependent anion channel (VDAC) is an ion channel located in the outer mitochondrial membrane where it mediates and controls molecular and ion exchange between the mitochondria and the cytoplasm.[48,49] The permeability of VDAC can be altered by drugs, causing mitochondrial metabolic disorder, ROS production, and subsequent oxidative death.[50] Under oxidative stress conditions, the transsulfuration pathway transfers a sulfur

atom from methionine to serine, yielding cysteine. The cysteine then acts as a substrate for the synthesis of GSH which assists GPXs in maintaining redox homeostasis and preventing oxidative damage. Therefore, this pathway can inhibit the occurrence of ferroptosis.[51] The ferroptosis-suppressor-protein 1 (FSP1) is an oxidoreductase catalyzing the reduction of ubiquinone (also known as coenzyme Q10, CoQ10). Ubiquinone is a lipophilic free radical scavenger. FSP1 can use NAD(P)H to catalyze the regeneration of CoQ10. In this way, FSP1 can protect the ferroptosis caused by the loss of GPX4. The FSP1-CoQ10-NAD(P)H pathway is an independent parallel system, which cooperates with GPX4 to inhibit ferroptosis caused by the rise of L-ROS.[52] Nuclear factor erythroid 2-related factor 2 (Nrf2) is also an important regulator of antioxidant response in the body. Under normal conditions, Kelch-like ECH-associated protein 1 (Keap1) promotes the ubiquitination and proteasome degradation of Nrf2. However, under oxidative stress, Keap1 is activated abnormally, which leads to the destruction of the interaction between Nrf2 and antioxidant response elements, thus participating in the regulation of ferroptosis.[53–55] Heme oxygenase-1 and transferrin are also important sources of intracellular iron and participate in the regulation of ferroptosis.[43,56]

Erastin, Ferroptosis, and the Mitochondria

VDAC, AIF, and MitoQ

The VDAC proteins are porins with a beta-barrel structure spanning the outer mitochondrial membrane. There are three VDAC isoforms, VDAC1, VDAC2 and VDAC3 and together they make up the most abundant proteins of the outer mitochondrial membrane. The VDAC proteins control the flow of metabolites and respiratory substrates through the outer mitochondrial membrane. These metabolites enter the mitochondrial matrix where they are used for the production of ATP which is dependent upon the maintenance of the mitochondrial membrane potential ($\Delta\ \Psi$).[57,58] VDAC can alternate between the states of "open" and "closed". In the presence of sufficient oxygen, malignant cells will still use glycolysis as a primary source of energy. This is known as the Warburg effect. After VDAC is blocked by tubulin and closed, it restricts the flow of respiratory substrates into the mitochondria. This is conducive to the aerobic glycolysis of cancer cells, leading to the Warburg effect.[59]

There are many molecules involved in oxidative regulation in mitochondrial metabolism. As an important oxidoreductase in the mitochondrial inner membrane, apoptosis-inducing factor (AIF) also participates in the removal of intracellular ROS. Knocking out the expression of AIF will cause a significant increase in intracellular ROS levels.[60] In addition, mice whose AIF expression level is knocked down by 80–90% are more sensitive to oxidative stress.[61] Therefore, AIF can effectively protect cells against oxidative stress. The mitochondria-targeted ROS scavenger mitoquinone (MitoQ) has powerful antioxidant properties, shown by its reduction of mitochondrial respiration and enhancement of glycolysis, thereby preventing lipid peroxidation, mitochondrial ROS production, and loss of organelle membrane potential. MitoQ is thus responsible for maintaining the integrity and function of the mitochondria. It is one of the most effective molecules preventing ferroptosis in different cell types.[62]

Erastin as an Antagonist of Tubulin Induces the Opening of VDAC

As early as 2007, Yagoda et al found that erastin can change the permeability of the mitochondrial outer membrane and that VDAC is the target of erastin.[17,63] Further research showed that erastin can reverse tubulin's inhibition of VDAC. Erastin can prevent and reverse the blockage of VDAC by cytoplasmic free tubulin in vivo and in vitro, allowing VDAC to open.[17] This opening of VDAC leads to three main biological effects: an increase of mitochondrial metabolism (the increase of $\Delta\psi$), a decrease in glycolysis and an increase of ROS production.[64] Since glycolysis and the inhibition of mitochondrial metabolism are metabolic characteristics of cancer cells, the promotion of VDAC opening by specific drugs and subsequent ROS production will affect most cancer cells.[63]

Inhibiting tubulin blockage of VDAC is expected to result in two independent but simultaneous effects: increased oxidative phosphorylation and ATP synthesis with reduced glycolysis leading to a reversal of the Warburg effect (the first hit) and increased ROS formation leading to oxidative stress (the second hit).[65,66] This anti-Warburg action can cause lethal or sub-lethal damage to cancer cells or can reduce cancer cell proliferation.[64] In addition, erastin can hyperpolarize mitochondria in cancer cells, which is followed by rapid depolarization, resulting in mitochondrial dysfunction.[64] One therapeutic advantage of erastin as a VDAC-tubulin antagonist is the specific killing of cancer cells; non-proliferating cells do

not have the high levels of free tubulin characteristic of cancer cells, so VDAC remains functional and is not regulated by free tubulin.[67]

In summary, the regulation of VDAC opening by erastin will have a significant effect on mitochondrial metabolism. This will first increase oxidative phosphorylation and ROS production followed by both indirect regulation of glycolysis and reversal of the Warburg phenotype-promoting aerobic glycolysis. This will increase ΔΨ, increase mitochondrial ROS and cause oxidative stress, eventually leading to ferroptosis.[68] Therefore, erastin represents a new pharmacological target which may become a new anti-cancer drug through regulating metabolism. (Figures 2 and 3)

Erastin Inhibits Ferroptosis Induced by System X_C^-

Reina et al found that erastin can cause compensatory transcriptional upregulation of *SLC7A11*.[67] Overexpression of *SLC7A11* through gene transfection reduced erastin-induced cell death, and inhibition of *SLC7A11* expression increased erastin's anti-cancer activity.[1] Thus, it appears that that erastin can indirectly reduce cellular uptake of cystine by direct inhibition of system X_C^-. Inhibition of system X_C^- by erastin indicates that besides altering the permeability of VDAC, erastin can also activate the classic ferroptosis pathway by acting on the system X_C^-.

When system X_C^- is inhibited, the consequent absence of cysteine, as a substrate for GSH synthesis, will result in diminished levels of GSH. Biochemical and metabolomic analyses showed that GSH was significantly depleted after erastin treatment.[13,69] GSH is a necessary cofactor for GPX4 to catalyze the degradation of hydrogen peroxide and hydroperoxide and inhibit the production of L-ROS. Therefore, the inhibition of system X_C^- by erastin indirectly leads to the decrease of GPX4 synthesis and the subsequent decrease of cell antioxidant capacity.[70] It was found that the activity of GPX4 was decreased in a variety of cancer cells treated with erastin.[71,72] The cell death caused by erastin inhibition of system X_C^- or GPX4 inactivation involves iron-dependent accumulation of L-ROS and consumption of PUFAs.[13,69] However, in cancer cells treated with erastin and GPX4-deficient mouse cells, the accumulation of L-ROS, consumption of PUFAs and subsequent cell death can be prevented by treatment with small molecular antioxidants, suggesting that L-ROS-mediated cell damage is essential for ferroptosis induced by erastin.[69,73]

In conclusion, erastin can prevent extracellular cystine from entering cells by inhibiting system X_C^-, which subsequently reduces the intracellular GSH level. GSH is an indispensable substrate for the antioxidant action of GPX4. Therefore, if the activity of GPX4 is reduced, redox homeostasis breaks down and L-ROS accumulates, leading to oxidative cell death, namely ferroptosis. (Figure 3)

Erastin Exacerbates Ferroptosis by Activating P53

Previous studies have confirmed that activation of the p53 gene can inhibit system X_C^- activity and cause ferroptosis.[74] Recent findings suggest that erastin is able to activate p53

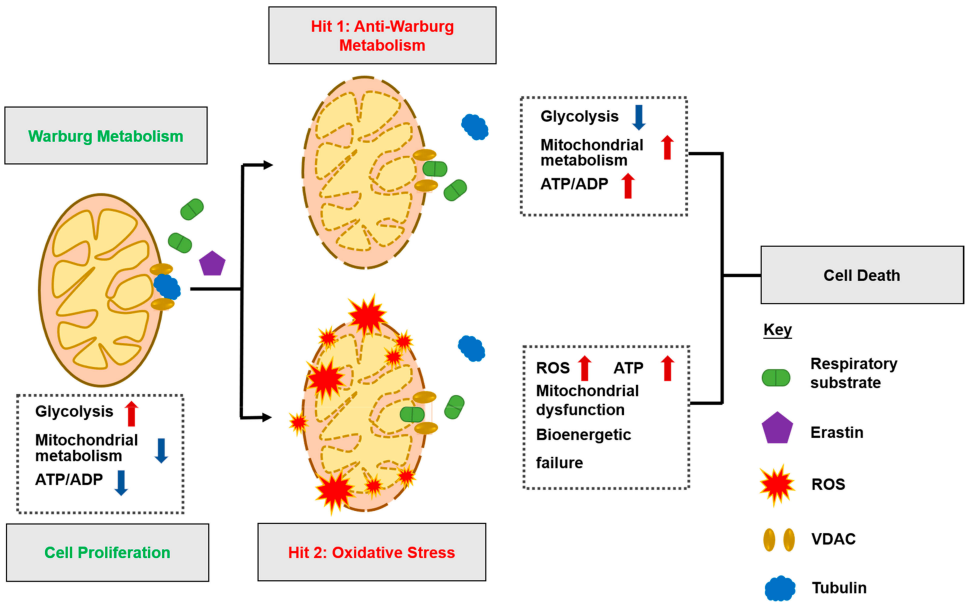


Figure 2 Erastin induces ferroptosis by altering the permeability of VDAC.

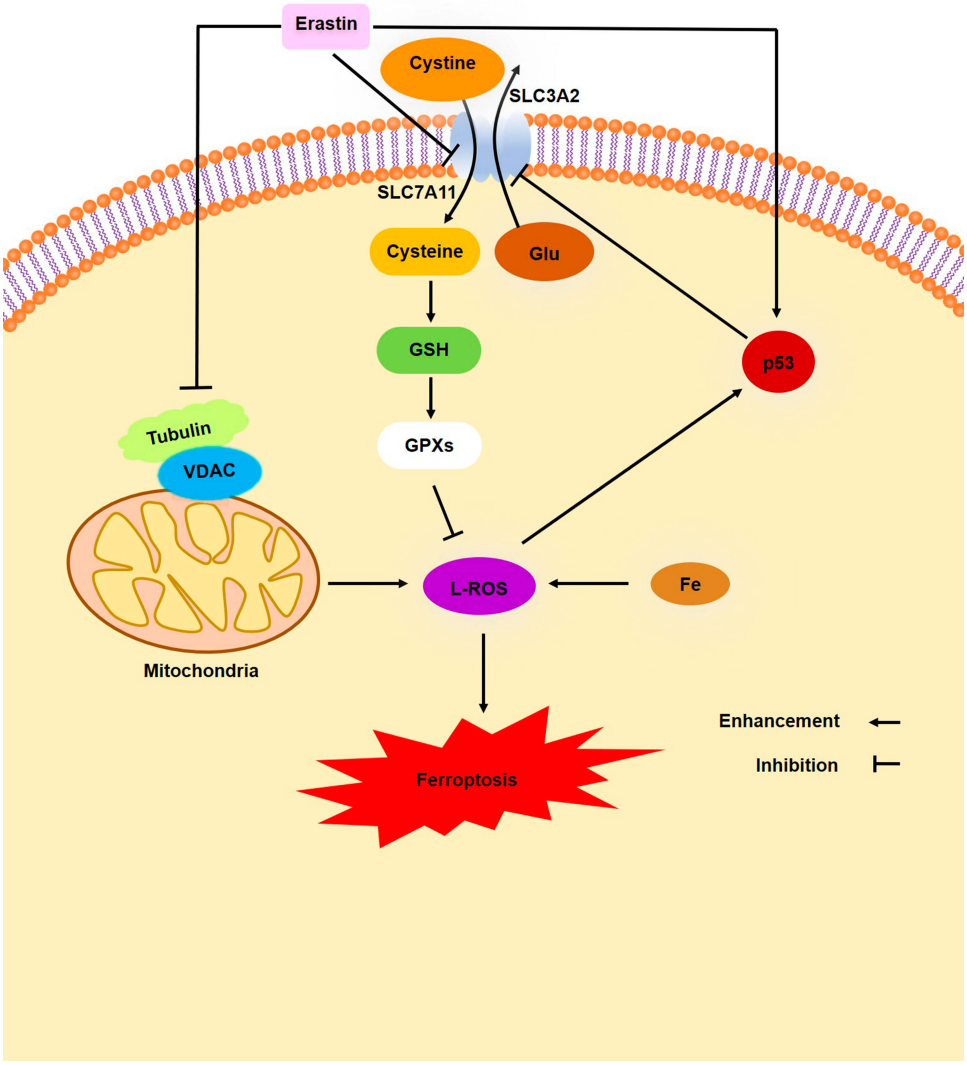


Figure 3 The relevant pathways of ferroptosis induced by erastin.

and thus can enhance ferroptosis. After erastin treatment of lung cancer A549 cells, p53 transcription products were significantly up-regulated and ROS levels were significantly increased. After pretreatment with the ROS scavenger N-acetyl-1-cysteine (NAC), erastin exposure did not significantly affect p53 activation, suggesting that p53 activation depends on the presence of ROS induced by erastin exposure. Therefore, it is not difficult to conclude that erastin treatment results in ROS production followed by p53 activation which subsequently activates the p53 downstream pathway. More importantly, this process forms a feedback loop: erastin causes an increase in ROS, which then leads to the activation of p53, which in turn, causes increased ROS. This exacerbates the key cytotoxic and cytostatic effects of erastin on A549 cells and eventually causes ferroptosis. However, this effect of erastin has not been found in normal lung cells, suggesting that it is specific for cancer cells[75] (Figure 3).

In 2015, Jiang et al constructed $p53^{3KR}$ mutant cells deficient in acetylation. These cells had lost the classic p53 function of inducing cell cycle arrest and apoptosis but had retained the ability to inhibit the transcription of *SLC7A11*. When erastin was used to treat the $p53^{3KR}$ mutant and p53-deleted cells separately, the mortality of $p53^{3KR}$ mutant cells was very high (> 90%) in contrast to that of the p53-deficient cells (≤ 10%). However, if the $p53^{3KR}$ mutant cells overexpressed *SLC7A11*, erastin treatment resulted in a significant reduction in the cell death rate (20%).[40] Wang et al constructed the $p53^{4KR98}$ model based on the $p53^{3KR}$ mutant cells. The $p53^{4KR98}$ model lost both the classical function of p53 and the ability to inhibit

SLC7A11 transcription. Erastin treatment significantly reduced both the cell death rate and tumor inhibition function of the $p53^{4KR98}$ model.[38] These results suggest that the activation of p53 by erastin may play an important role in tumor inhibition by inhibiting *SLC7A11* transcription and eventually ferroptosis.

Xie et al found that p53 wild-type CRC cells were not sensitive to erastin, but the sensitivity of CRC cells to erasin recovered after the inhibition of p53 activity by drugs or gene knockout. This is different from the previously documented effects of erastin on ferroptosis in other cancer cells.[47] As discussed above, the regulatory effect of p53 on ferroptosis is related to cancer cell types. The role of erastin in the activation of p53 and in increasing the sensitivity to ferroptosis is not applicable in all cells. However, this provides a broad scope for future research: to understand the regulatory effect of erastin on ferroptosis in the p53 pathway would be helpful, firstly, in identifying specific targets for the induction of cancer cells' death and, secondly, to inhibit ferroptosis of normal cells, to reduce the side-effects of chemotherapy. At present, it is unclear to what degree the p53 gene is involved in erastin-induced ferroptosis in cancer cells, requiring further study in the future.

Regulation of Lipid Metabolism by Erastin

Lipoxygenases (LOX) are non-heme iron dioxygenase, which can catalyze diallyl site oxygenation of polyunsaturated fatty acids in cell non-bilayer phospholipids. LOX-5 is a well-studied LOX isozyme and is a key enzyme for the synthesis of many highly active oxidized lipids. LOX-5-mediated polyunsaturated fatty acid oxidation plays an important role in ferroptosis.[76] Acyl-CoA long-chain synthetases are ligases responsible for the oxidation of long-chain fatty acids. One member of this family, ACSL 4, is expressed on the endoplasmic reticulum and mitochondrial outer membrane and is mainly responsible for the catalysis of lipids to form acetyl-CoA.[77] Research shows that ACSL4 is highly involved in ferroptosis. ACSL4 is involved in the synthesis of negatively charged membrane phospholipids such as phosphatidylethanolamine and phosphatidylinositol. They play an important role in lipid metabolism by incorporating polyunsaturated fatty acids into the cell membrane.[52,78] Therefore, ACSL4 plays an important role in the formation of ROS mediated by LOX, thus promoting ferroptosis.[76] Knockout of the GPX4 gene can lead to ferroptosis, while the double knockout of the GPX4 and ACSL4 genes can reverse GPX4 knockout-induced ferroptosis. This indicates that ACSL4 is necessary for the process of ferroptosis.[79] In addition, the expression of ACSL4 in ferroptosis-resistant cells was lower than that in ferroptosis-sensitive cells. Therefore, ACSL4 was also used as an indicator of ferroptosis sensitivity.[80]

Yuan et al found that HepG2 (human liver cancer cells) and HL60 (human promyelocytic leukemia cells) cells are highly sensitive to ferroptosis caused by erastin compared with LNCaP (human prostate cancer cells) and K562 (human chronic myeloid leukemia cells). The expression of ACSL4 mRNA and protein in HepG2 and HL60 cells were relatively high. After overexpression of ACSL4 in LNCaP and K562 cells, the cells' sensitivity to cell death induced by erastin was significantly increased.[80] This suggests that erastin regulates lipid peroxidation by regulating ACSL4, which leads to ferroptosis. In addition, an inhibitor of LOX-5, Zileuton, can inhibit erastin-induced ferroptosis by inhibiting the production of cytoplasmic ROS in HT22 cells.[81] So we speculate that erastin can regulate ferroptosis by regulating pathways other than GPX4 and affecting lipid metabolism and, more importantly, because ACSL4 is overexpressed in several different cancers, such as breast cancer, prostate cancer, colon cancer, and hepatocellular carcinoma.[82–85] This suggests that the induction of erastin is a specific anti-cancer pathway, only acting on cancer cells, and protecting normal cells from ferroptosis.

Pharmacodynamics and Safety Evaluation of Erastin

Due to its poor water solubility and unstable metabolism in the body, erastin is not suitable for direct use in vivo. Introducing other chemical groups into the aniline ring of erastin can result in compounds that are more soluble, stable, and better suited for in vivo administration. Examples of these include piperazine-erastin (PE) and imidazole ketone erastin (IKE).

Yang et al investigated the effects of PE on tumors in nude mice. They observed a significant reduction in tumor growth with no adverse effects or toxicity even at very high PE doses (60 mg/kg).[13] A study by Zhang et al using IKE treatment of a B cell lymphoma xenograft model reported stimulation of ferroptosis and inhibition of tumor growth with no adverse effects. The use of nanocarriers to enhance efficacy and selective delivery resulted

in stronger anti-tumor effects, also with no significant toxicity.[86] A further nanoparticle study by Li et al using ferritin-bound erastin and rapamycin also observed significantly controlled tumor growth with no obvious side effects.[87]

Other in vivo experiments have also shown that intraperitoneal injection of erastin analogs in tumor-bearing mice can significantly inhibit the growth of subcutaneous tumors in mice, and that the dose is well tolerated. Pharmacodynamic and toxicological studies have shown that according to the ratio of body surface area, erastin analogs are well tolerated at the indicated treatment dosages and thus have significant therapeutic potential.[88,89] Zille et al also believe that not only does erastin itself not have toxic effects, but it may prevent toxicity of the tumor to the central nervous system.[90]

In summary, the above studies confirm that erastin analogs can inhibit tumor growth in vivo and have minimal toxic and side effects. However, the use of erastin analogs alone is not enough to completely restrict the rapid growth of tumors in vivo. Based on current research results, combining erastin with other treatments such as radiotherapy and chemotherapy, or designing erastin analogues with higher bioavailability, greater metabolic stability, and more effective tumor invasion and accumulation rates will further optimize the therapeutic effect and reduce possible toxic and side effects.[86] It is worth noting that although current in vivo experiments with erastin provide very promising results, there is a need for further accurate pharmacokinetic and toxicological studies to provide a platform for further clinical trials in the future.

The Potential of Erastin in Clinical Applications

In Chemotherapy

Chemotherapy is one of the three main methods for the treatment of malignant tumors. However, due to the continuous and extensive use of chemotherapeutic drugs, tumors show different degrees of drug resistance.[91,92] This drug resistance of tumors to chemotherapy is a major factor leading to the failure of chemotherapy and poor prognosis.[93] Chemotherapeutic drugs eliminate cancer cells mainly by inducing apoptosis. Previous studies have confirmed that suppressed apoptosis or reduced susceptibility to apoptosis is an important mechanism of acquired drug resistance.[94] So, can we reverse drug resistance by other non-apoptotic cell death methods?

As described above, erastin can induce cancer cell death by a non-apoptotic and iron-dependent form of cell death. In addition to inducing cancer cell death itself, erastin can also be combined with chemotherapeutic drugs to enhance cancer cell sensitivity to chemotherapeutic drugs.[95] Erastin has been shown to enhance the sensitivity of lung cancer cells to cisplatin,[96] rhabdomyosarcoma cells to doxorubicin and actinomycin D,[97] glioblastoma cells to temozolomide,[98] for example. In addition, erastin can also eliminate the resistance of many types of chemotherapeutic resistant cells: it has been found to overcome the resistance of head and neck cancer cells and ovarian cancer cells to cisplatin,[15,99] and the resistance of non-RAS-expressing acute myeloid leukemia cells to cytarabine and doxorubicin hydrochloride (Adriamycin).[71] These results support the feasibility of using erastin as an anti-cancer drug in the clinic.

System X_C^- is strongly linked to drug resistance. The transport of system X_C^- causes an increase in intracellular GSH concentration, which has been confirmed to be one of the causes of chemotherapy resistance in tumor cells.[100] Therefore, system X_C^- can be a powerful and potential therapeutic target to overcome the drug resistance of cancer cells.[101,102] The inhibitory effect of sulfasalazine on system X_C^- has been demonstrated in small cell lung cancer,[101] liver cancer,[101] genitourinary tract cancer,[103] and rectal cancer.[104] Erastin has a much stronger inhibitory effect on system X_C^- than other system X_C^- inhibitors such as sulfasalazine, and is effective at low concentrations, so has the potential of reversing tumor resistance.[105]

In conclusion, there is convincing evidence for erastin's potential as an anti-cancer drug. It can be used as a new type of chemotherapeutic drug leading to cellular ferroptosis, as well as a chemotherapeutic sensitizer for various types of human cancer. It is thus an effective candidate drug.

In Radiotherapy

Radiation therapy is the second most crucial treatment for malignant tumors, second only to surgery. About 50–70% of patients with malignant tumors require radiotherapy during treatment.[106] However, potential organ damage is an insurmountable dose-limiting factor in radiotherapy. It is inevitable that some radiotoxic side effects may occur during or after radiotherapy.[107] In this context, improvement of radiotherapy efficacy as much as possible without increasing the dose has become an important way to break through these bottlenecks and is an urgent problem to be solved in the field of cancer radiotherapy.

Radiosensitizers can enhance damage to tumor tissues by promoting tumor cell apoptosis, regulating the cell cycle, accelerating DNA damage, and generating free radicals, thereby improving the efficacy of radiotherapy.[108] They can thus improve the therapeutic effects without increasing the dose of radiation.[109] Erastin increases the sensitivity of cancer cells to radiation besides its known induction of ferroptosis. Cobler et al found that erastin can increase the sensitivity of breast cancer cells to γ-rays in vivo and in vitro by inhibiting system X_C^-, and thought that erastin might prolong the duration of radiation-induced DNA damage.[110] Other studies also found that erastin enhanced X-ray-induced cell death of cervical cancer and lung cancer, and demonstrated the same effect in tumor-bearing mice.[111,112] More advantageously, most normal cells do not express *SLC7A11*,[110] so erastin may specifically increase the sensitivity of cancer cells to radiation, thereby increasing the death or proliferation of cancer cells and preventing radiation damage in normal cells. Cisplatin is a known radiosensitizer widely used in the clinic.[113] Erastin can increase the sensitivity of many cancer cells to cisplatin. Whether the combination of the two drugs will produce an additive effect and increase the sensitivity of cancer cells rapidly remains to be investigated. On the other hand, ionizing radiation has some effect on promoting the production of ROS mainly by destroying cellular DNA and causing cell damage.[114] As discussed above, the most significant feature of ferroptosis induced by erastin is the increase of ROS in cells. If erastin is used as a radiosensitizer, cancer cells can produce ROS through many other pathways besides ionizing radiation. Whether this effect will lead to the rapid increase of ROS in cells leading to the aggravation of cell peroxidation and death provides us with a reasonable hypothesis.

In conclusion, erastin can be used as a novel radiosensitizer to enhance the radiosensitivity of tumors, increase the radiosensitivity of radiation-resistant tumors, or reduce the radiation dose of normal tissues. It has excellent prospects for clinical application.

Conclusion

Erastin is a small molecule compound that can specifically kill human cancer cells without affecting normal cells of the same genotype, and this process is rapid and irreversible. Erastin, as a ferroptosis inducer, is different from other ferroptosis inducers which usually trigger a single pathway. Erastin can trigger multiple pathways: inhibits the action of the cystine-glutamate transport of system X_C^-, acts on VDAC to relieve the inhibitory effect of tubulin on VDAC, and may indirectly inhibit system X_C^- by activating p53, leading to ferroptosis. Erastin is more effective and fast-acting than other ferroptosis inducers, is effective at low concentrations and has long-lasting results. More importantly, erastin has great potential as a novel anti-cancer drug. Erastin can enhance the sensitivity of many cancer cells to various chemotherapeutic drugs and enhance the sensitivity of cancer cells to radiation. It can, therefore, be used as a new type of chemotherapy drug or chemotherapy sensitizer and radiotherapy sensitizer in cancer therapy. However, given the insufficient number of studies on erastin, further basic and clinical investigations should be conducted.

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Disclosure

The authors report no conflicts of interest in this work.

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