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Review

Molecular mechanisms associated with ferroptosis during various pulmonary disorders and the possible therapeutic regimens for lung cancer

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ABSTRACT: Cell death is the natural process in which the old or unwanted cell is damaged and replaced by a newly functional one. Several factors trigger cell death and there are several mechanisms for this event such as apoptosis, autophagy, and ferroptosis. Ferroptosis as a novel type of programmed cell death is occurred due to cellular iron accumulation and the depletion in one of cellular antioxidant enzymes which is glutathione peroxidase 4 (GPX-4). Therefore, ferroptosis is characterized by lipid peroxidation and oxidative stress. Several metabolic pathways such as iron, amino acids, lipids metabolism, etc are considered as regulators for ferroptosis process. Several studies have proved the involvement of ferroptosis in lung diseases progression/depression that range from acute lung injury (ALI) to lung cancer. Based on large studies data the question that takes the scientists' attention is the use of anti-ferroptosis compounds because they could attenuate or worsen the disease symptoms?. In this review, the alterations in molecular mechanism regulated ferroptosis during the lung diseases development or prevention of and the role of its inhibitors or inducers during the treatment of lung diseases, especially lung cancer, was highlighted.

1. INTRODCTION

Unlike all known cellular death, apoptosis, autophagy, and other types of cell death, ferroptosis is a recently identified cell death type with distinct molecular mechanism and morphological changes. Lethal concentrations of lipid peroxides (LPOs) accumulate iron intracellularly during ferroptosis. Ferroptosis is directly connected to the metabolism of nutrients such as iron and amino acids beside lipid peroxides recycle [1]. Recent studies have shown that several lung diseases, including acute lung injury (ALI), chronic obstructive pulmonary disease (COPD), COVID-19, asthma, and pulmonary fibrosis (PF), are linked to elevated levels of iron and lipid peroxidation, as well as reduced glutathione and glutathione peroxidase4 (GPX4). Based on this research ferroptosis is strongly correlated with the development of these pathologies. In contrast, ferroptosis is a possible mechanism for damaging lung cancer cells [2]. Therefore, further research is essential to investigate the regulatory mechanisms of ferroptosis and its role in lung diseases.

Regulatory Mechanisms of Ferroptosis

There are several mechanisms that play a central role in ferroptosis regulation, and they are classified into stimulatory and inhibitory mechanisms that are summarized in **Figure (1)**.

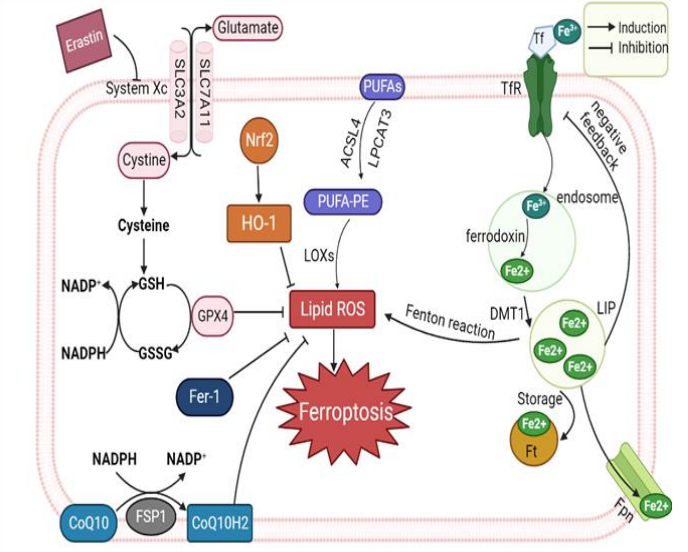

Figure 1. **The regulatory mechanisms of ferroptosis.** The figure shows the major regulatory mechanisms of ferroptosis either inducing or inhibitory pathways. CoQ10: coenzyme Q10; DMT1: divalent metal ion transporter-1; Fer-1: ferrostatin-1; Fpn: ferroportin; FSP1: ferroptosis suppressor protein 1; Ft: ferritin; GPX4: glutathione peroxidase 4; GSH: glutathione; GSSG: oxidized glutathione; LIP: labile iron pool; LOXs: lipoxygenases; NADPH: nicotinamide adenine dinucleotide phosphate; Nrf2: nuclear factor erythroid-derived 2; ROS: reactive oxygen species; TfR: transferrin receptor.

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1.1. Iron Metabolism

There are several proteins; transferrin (Tf), transferrin receptor (TfR), ferritin (Ft), ferroportin1 (Fpn1) and divalent metal ion transporter-1 (DMT1), interact to each other to form the iron regulatory protein (IRP) system which is responsible for the maintenance of iron homeostasis. On the cell membrane and through endosomes formation, the binding Fe^{3+}- Tf complex to TfR enters the cells and then transferrin reduces Fe^{3+} to Fe^{2+}. DMT1 in endosomes disintegrated and released Fe^{2+} into the cytoplasm to form a labile iron pool (LIP). The formed LIP inhibits TfR via negative feedback inhibition. The cytoplasmic Fe^{2+} is then either stored as ferritin or released outside the cell via Fpn. In the case of excessive iron in LIP and the presence of H_2O_2, the Fenton reaction takes place producing hydroxyl radicals and ROS. Not only ROS-mediated oxidative stress can damage the cell, but also ROS can interact with membrane lipids producing peroxyl radicals, which then form more and more lipid peroxides (LPOs) promoting ferroptosis [3]. Ferroptosis can be considerably slowed down by antioxidants and ferrostatin-1 (Fer-1), showing that iron is crucial to the development and progression of ferroptosis [4].

1.2. Lipid Metabolism

LPOs are the key factor in inducing ferroptosis. Omega 3 and Omega 6 are two types of polyunsaturated fatty acids (PUFAs), which are mostly ingested through food, that are vital for human health. Acyl-CoA Synthetase Long-Chain Family Member 4 (ACSL4), in particular, is responsible for the esterification of PUFAs to acyl CoA. Phosphatidylethanolamine (PE)-containing arachidonic acid (AA)-CoA is created by ACSL4, which is then inserted in membrane phospholipids (PLs) by lysophosphatidylcholine acyltransferase 3 (LPCAT3). Lipoxygenase (LOX) oxidizes PUFA-PE resulting in lipid peroxidation. Hence excessive intake of Omega 6 and Omega 3 PUFAs leads to the accumulation of LPOs which are the main inducer of ferroptosis [5].

1.3. Nuclear transcription factor-2 (Nrf2)

Even at low expression level, Nrf2 is the principal regulator for cellular antioxidants status. To maintain its low expression under normal circumstances, Nrf2 is in a complex with Kelch-like ECH-associated protein 1 (Keap-1) that prevents its ubiquitylation and proteasomal degradation [5]. Once oxidative stress occurs, Nrf2 is upregulated and moves to the nucleus after dissociation from Keap-1. Inside the nucleus, Nrf2, as a transcription factor, upregulates antioxidant response elements-related genes: NADPH quinone oxidoreductase-1, and heme oxygenase 1 (HO-1) [3]. A cell with high levels of Nrf2 expression can resist ferroptosis and become more resistant to anti-ferroptosis chemotherapeutics [6].

1.4. Amino acid antiporter (System Xc-)

On the cell membrane, System Xc- which is a cystine/glutamate reverse transporter composes of two subunits: Solute carrier family 3 member 2 (SLC3A2) and solute carrier family 7 (cationic amino acid transporter, y+ system), member 11 (SLC7A11), that are joined by a disulfide bond [7]. At a 1:1 ratio, the System Xc receives extracellular cystine while concurrently exporting intracellular glutamate. Cysteine, the primary precursor in the production of glutathione (GSH), is converted from cystine inside the cell. Glutathione peroxidase 4 (GPX4) uses GSH as a substrate to degrade LPOs [3]. By reducing lipoxygenases (LOXs), preventing excessive activation and removing LPO created by iron accumulation through the Fenton reaction, GPX4 can considerably prevent LPO induced cell membrane damage [8]. Erastin induces ferroptosis via inhibition of SLC7A11 and cystine uptake to downregulate GSH synthesis. Downregulation of GSH inhibits GPX4 leading to accumulation of LPOs and damaging the cell membrane [3].

1.5. Ferroptosis suppressor protein 1 (FSP1)

FSP1 is an anti-ferroptosis protein located in the cell membrane. An oxidoreductase, FSP1 maintains the reduced form of coenzyme Q (CoQ) at the plasma membrane by reducing NADPH-dependent CoQ. By scavenging lipid peroxyl radicals and preventing their transport through the membrane, CoQ exerts an antioxidant effect [9]. FSP1 can drastically lower LPO synthesis in GPX4 mutant cells, proving that it is not GSH-dependent. The vulnerability of cancer cells to ferroptosis may rise when FSP-1 is inhibited. [10].

2. Ferroptosis and lung diseases

2.1. Ferroptosis and Acute lung injury (ALI) or acute respiratory distress syndrome (ARDS)

The pathophysiology of the frequent disease ALI/ARDS, which has a high mortality rate, is thought to involve oxidative stress, apoptosis, inflammation, and hypoxia [11]. High Fe^{2+}, reactive oxygen species (ROS), malonaldehyde (MDA), and low GSH were among the indicators of ferroptosis found in ALI animal models. Additionally, ferroptosis inhibitors may be able to lessen lung injury. These findings demonstrate that ferroptosis and ARDS are closely related [1]. Moreover, SLC7A11 and GPX4 were shown to be considerably downregulated with elevation of MDA and total iron levels in LPS-induced ALI. LPS treatment appears to promote ferroptosis because Fer-1 injection prevented LPS-induced ALI and raised the levels of SLC7A11 and GPX4 proteins in lung cells [12]. Furthermore, oleic acid-induced ALI model had low levels of total GSH, reduced GSH, GPX4 protein, and iron concentrations that are associated with low ferritin levels without affecting TfR1 which are resulting in lung tissue ferroptosis [13].

Several compounds that act as anti-ferroptosis can be used to prevent LPS-induced ALI. Where it is found that sevoflurane inhibits ferroptosis that took place in LPS-induced ALI in mice by decreasing MDA and Fe^{2+} and increasing GSH and GPX4 levels in the lung tissues [14]. Moreover, the treatment with panaxydol stimulates Keap1-Nrf2/ HO-1 pathway, which can also decrease ferroptosis in ALI caused by LPS [15].

The inhibitor of the apoptosis-stimulating protein of p53 (iASPP) that is used in intestinal ischemia reperfusion-induced-ALI mice through inhibiting lung tissue ferroptosis pathway. Where it is found that iASPP increased Nrf2, and GPX4 which consequently increased the expression of ferritin heavy chain (FTH1), NAD(P)H quinone dehydrogenase 1 (NQO-1), and HO-1 that was associated with the decrease of the expression of TF, Hypoxia-Inducible Factor (HIF)-1 and the other ferroptosis-related proteins [16].

2.2. Ferroptosis and Chronic Obstructive Pulmonary Disease (COPD)

Without a treatment, COPD has risen to become the fourth leading cause of illness and mortality worldwide [17]. COPD, often known as bronchitis or emphysema with obstruction of airflow, is a common chronic disorder characterized by pulmonary heart disease and respiratory failure. It is associated with abnormal inflammation that is brought on by the buildup of noxious gases and particles [2]. The toxic substances in cigarettes directly affect bronchoalveolar epithelial cells (BECs), which is a key risk factor for COPD [18]. Smoke from cigarettes (CS) can affect oxidative stress, inflammation, and iron homeostasis. After being exposed to CS, mouse and human bronchial epithelial cells produced higher levels of iron, Ft, serum Ft, and non-heme iron in lung cells. [3]. According to recent studies, a process known as ferritinophagy produces labile iron by degrading ferritin through autophagy. It has been demonstrated that CS stimulates the buildup of labile iron through ferritinophagy that is mediated by nuclear receptor coactivator4 (NCOA4), which causes phospholipid peroxidation and ferroptosis in human lung epithelial cells [17]. Additionally, human BEC (HBEC) peroxidation and ferroptosis are caused by low GPX4 activity levels and inadequate GSH, which are key factors in the pathophysiology of COPD [3]. By chelating intracellular iron and decreasing labile iron, deferroxamine (DFO) is reported to suppress ferroptosis in BECs. Additionally, Fer-1 decreased CS-induced cell death and lipid peroxidation, while liproxstatin-1 inhibited CSE-induced cell death. [17].

2.3. Ferroptosis and Asthma

Human airway epithelial cells (HAECs) and airway smooth muscle (ASM) cells are both dysfunctional in asthma, which is a common respiratory illness characterized by persistent inflammation and increased airway contractility and responsiveness. Recent research has shown that ferroptosis is the root cause of inflammation in the airways of asthmatic patients [19]. By increasing ROS levels and lipid peroxidation, house dust mites (HDM)-induced asthma has shown that ferroptosis may play a role [20]. Iron accumulates as a result of ferritinophagy, a process in which ferritin is broken down by autophagy. These actions define ferroptosis. according to Zeng et al., [21], airway epithelial cells have been found to exhibit ferritinophagy in asthma caused by HDM. In order to cure asthma, it may be possible to further investigate ferritinophagy regulatory targets.

Airway inflammation and asthma patients' hyperoxidative conditions also contributed to ferroptosis. When 15-LOX1 and Phosphatidylethanolamine binding protein 1 (PEBP1) work together to activate extracellular regulatory protein kinases, airway epithelial cells (AECs) undergo ferroptosis and autophagy [3]. Arachidonate 5-lipoxygenase (ALOX5) and arachidonate 15-lipoxygenase (ALOX15) expression is activated by IL-13 upregulation in HAECs, which promotes ferroptosis [19]. By altering the release of pro- and anti-inflammatory lipid mediators, IL-4 and IL-13 decrease the expression of GPX4 and increase the expression of ALOX15, which may help to boost the immune system's ferroptosis-sensitive condition. IL-6 promotes ferroptosis by disrupting iron homeostasis and inducing lipid peroxidation in HAECs. Inflammatory substances are released by activated macrophages in ferroptotic tissues. Eosinophils (Eos) are effector cells that also have immunomodulatory properties during asthma. Through an unusual mechanism, ferroptosis-inducing substances cause Eos ferroptosis and work in concert with glucocorticoids to cause Eos death. Glucocorticoid dosage and side effects may be decreased with treatment for Eos ferroptosis, which appears to be useful in reducing allergic airway inflammation like asthma [3].

2.4. Ferroptosis and Pulmonary Fibrosis (PF)

Excessive fibroblast proliferation and extracellular matrix (ECM) protein buildup are two features of the chronic, progressive illness known as pulmonary fibrosis. Bleomycin and LPS cause lung epithelial cells to undergo direct ferroptosis in the initial stages of inflammation. The phospholipid-lysophospholipid transacylase (tafazzin)-transcriptionally enhanced associate domain (TAZ-TEAD) signaling pathway is activated in the late fibrotic phase by transforming growth factor beta (TGF-β), increasing intracellular LIP and promoting the transition from fibroblast to myofibroblast. The development of scar tissue was linked to the conversion of fibroblasts into myofibroblasts, fibroproliferation, and an excessive buildup of ECM proteins in the lung parenchyma, which resulted in a pathological change of lung development and the degeneration of lung tissue. The iron chelator deferoxamine (DFO) and the ferroptosis inhibitor liproxstatin-1 (Lip-1) both reduced pulmonary fibrosis symptoms [22].

The expression of TGF- β, α-smooth muscle actin (α-SMA), and fibroblast activation protein-α (FAPα), two fibroblast markers, was increased in bleomycin-induced lung fibrosis. Ferrous iron (Fe^{2+}) accumulated in lung cells as well as 4-Hydroxynonenal (4-HNE), a biomarker for lipid peroxidation. These results imply that lipid peroxidation, iron overload, and fibroblast to myofibroblast transition are the mechanisms by which ferroptosis is triggered in bleomycin or LPS-induced PF [22].

2.5. Ferroptosis and Lung Cancer

More than 1 million subject dies from lung cancer, which ranks second among all cancers, in the year 2020. In accordance with the cell of origin, the WHO divided lung cancer into two primary groups. The first is small-cell lung cancer (SCLC), which is further divided into small-cell carcinoma and mixed small-cell carcinoma. In the United States, SCLC will account for 15% of all cases in 2020. In comparison to other types of lung cancer, non-small cell lung cancer (NSCLC) has a high death rate. Large cell carcinoma, lung squamous cell carcinoma (LUSC), and lung adenocarcinoma are the subsequent subtypes of NSCLC. [23]. In contrast to all lung diseases, ferroptosis is inhibited in lung cancer to promote cell proliferation and tumor growth. Ferroptosis inhibition is achieved by several mechanisms as shown in (Table 1). During cancer treatment, ferroptosis is induced to achieve cancer cell death. Different mechanisms are involved in inducing ferroptosis which is summarized in (Table 1). During small cell carcinoma, ferrous ions are decreased that associated with overexpression of SLC7A11 and GSH that confirmed the inhibition of ferroptosis in this case the ferroptosis inducer such as sulforaphane is used as potent inhibitor for SLC7A11 [24]. For Lung adenocarcinoma that are characterized by activation of PRIM2/SLC7A11 axis,

LSH/GINS4 axis, or overexpression of NFS1, LSH or NAMPT [25], Dihydroartemesinin (DHA) or high-dose NMN could be used in order to control the expression or PRIM2 and modulate SIRT1/AMPK/ACC pathway [25,26]. Overexpression of GPX4, Nrf2 and HO-1 associated with loss of lncRNA are the common mechanisms linked with Lung squamous cell carcinoma (LUSC), therefore, drugs such as seramisine, lapatinib, Acetaminophine, RSL3 and Timosaponin-AIII could be used to induced ferroptosis and kill cancer cells [27,28,29,30]. During large cell carcinoma treatment ferroptosis inducer such as Erastin and Sinapine can be used to induced ferroptosis via upregulation of P53 or Tf and TfR [31,32].

2.6. Ferroptosis and Covid 19

The SARS-CoV-2 infection that gave rise to Covid-19, a pandemic illness, began toward the end of 2019. The lung and the throat are the major sites of infection for SARS-CoV-2 in human cells at various places [36]. Invasion of host cells and viral attachment to angiotensin-converting enzyme 2 (ACE2) can infect them directly, causing an increase in inflammatory reactions that can result in cell death and pulmonary problems. As a result of SARS-CoV2 binding to ACE2, increased pulmonary vascular permeability causes pulmonary edema and impaired lung function [25]. As a result, ARDS, hypercoagulation, and cytokine storm are pathological alterations in Covid-19 patients. [8].

Low amounts of serum transferrin and iron are present in Covid-19 patients [32]. Recent research suggests that SARS-CoV-2 generates iron excess via boosting the overexpression of hepcidin [23], since iron sufficiency hinders the virus' ability to finish its reproduction process while iron insufficiency facilitates it [19]. Lipid peroxidation and oxidative stress are brought on by the disruption of iron homeostasis in Covid-19 patients. As a result, the GSH/GPX4 antioxidant pathway is disturbed, which impairs the clearance of the resulting LPOs [3].

As a result, ferroptosis plays a role in the etiology of Covid-19, and iron chelators or anti-ferroptotic medications might potentially treat Covid-19 and other lung illnesses in patients.

3. Conclusion

Ferroptosis is a new type of programmed cell death with morphology, and biology that differ from other types. Ferroptosis is characterized by iron accumulation, excessive lipid peroxides, and ROS. Numerous studies have shown the implication of ferroptosis in the pathophysiology of lung diseases including ALI, COPD, PF, asthma, lung cancer, and Covid-19. In experimental models of ALI, Covid-19, COPD, PF, and asthma, MDA is elevated while antioxidant enzymes are downregulated, suggesting the stimulatory effect of ferroptosis on these diseases. Whereas ferroptosis inhibitors attenuate the oxidative stress and inhibit this process. Lung cancer is characterized by its resistance to ferroptosis. In contrast to other types of lung diseases, ferroptosis inducers inhibit the progression of lung cancer and have an ameliorating effect on cancer cells. To better understand the role of ferroptosis and to be used as a therapeutic target, animal model experiments should be done to understand the regulatory mechanisms involved in ferroptosis induction or inhibition during each disease and to deduce the main target for different treatments (synthesized compounds or natural products) in order to manage or treat lung diseases either by inducing or inhibiting ferroptosis.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Conflicts of interest

There are no conflicts to declare".

ABBREVIATIONS

4-HNE: 4-Hydroxynonenal; ACC: Acetyl-CoA carboxylase; ACE-2: angiotensin converting enzyme2; ACSL4: Long Chain Family Member 4; ALI: acute lung injury; ALOX5: arachidonate 5-lipoxygenase; AMPK: 5' adenosine monophosphate-activated protein kinase; ARDS: Acute respiratory distress syndrome; ASM: Airway smooth muscle cells; BECs: bronchoalveolar epithelial cells; COPD: chronic obstructive pulmonary disease; CS: Cigarettes smoke; DFO: Deferoxamine; DMT1: divalent metal ion transporter-1; ECM: extracellular matrix; EGLN1: Egl-9 Family Hypoxia Inducible Factor 1; Eos: eosinophils; Fer-1: Ferrostatin-1; Fpn1: ferroportin1; FSP1: Ferroptosis suppressor protein 1; FSP1 KO: Ferroptosis suppressor protein1 knockout; Ft : ferritin; FTH1: ferritin heavy chain; GINS4: GINS complex subunit4; GPX-4: glutathione peroxidase 4; GSH: glutathione; HAEC: human airway epithelial cells; HDM: house dust mites; HIF-1: Hypoxia-Inducible Factor-1; HO-1: heme oxygenase 1; HSP90: Heat shock protein 90; iASPP: inhibitor of the apoptosis-stimulating protein of p53; IL: interleukin; IRP: iron regulatory protein; Keap-1: Kelch-like ECH-associated protein 1; LIP: labile iron pool; Lip-1: liproxstatin-1; LOX: lipoxygenase; LPCAT3: lysophosphatidylcholine acyltransferase 3; LPOs: Lipid peroxides; LPS: lipopolysaccharides; LSH: lymphoid-specific helicase; LUSC: lung squamous cell carcinoma; MDA: malonaldehyde; NAM: nicotinamide; NCOA4: Nuclear receptor coactivator4; NMN: Nicotinamide mononucleotide; NOK: novel oncogene with kinase-domain gene; NQO-1: NAD(P)H quinone dehydrogenase 1; Nrf-2: Nuclear transcription factor-2; NSCLC: Non-small-cell lung cancer; PEBP1: Phosphatidylethanolamine binding protein 1; PF: Pulmonary fibrosis; PRIM2: DNA Primase Subunit 2; PUFAs: Polyunsaturated fatty acids; ROS: Reactive oxygen species; SCLC: small-cell lung cancer; SIRT1: Sirtuin-1; SLC3A2: Solute carrier family 3 member 2; SLC7A11: solute carrier family 7 (cationic amino acid transporter, y+ system), member 11; STYK1: Serine/threonine/tyrosine kinase1 gene; Tf : transferrin; TfR: transferrin receptor; TFRC: transferrin receptor protein-1; TGF-β: Transforming Growth Factor beta.

Table 1. The mechanisms of ferroptosis resistance in types of lung cancer and possible treatments.

Types of Lung Cancer	Subtypes	Mechanism	Possible treatment	Mechanism of action	Reference
Small Cell Lung Cancer (SCLC)	Small cell carcinoma Combined small cell carcinoma	Low intracellular Fe2+ level Overexpression of SLC7A11 and GSH	Sulforaphane (SFN)	Inhibit SLC7A11 expression and increase iron levels	[24]
Non-Small Cell Lung Carcinoma (NSCLC)	Lung adenocarcinoma	PRIM2/SLC7A11 axis	Dihydroartemesinin (DHA)	Decrease PRIM2 expression	[26]
		EGLN1/c-Myc inhibits HIF1α and increases LSH LSH-GINS4 axis	—	—	[33]
		NFS1 overexpression	Suppression of NFS1	Inhibit cysteine transport and promote ferroptosis	[34,35]
		Overexpression of NAMPT that decrease intracellular NAM content	High-dose NMN	NAM overload that mediates SIRT1/AMPK/ACC pathway	[25]
	Lung squamous cell carcinoma (LUSC)	Loss of long noncoding RNA (lncRNA)	lncRNA P53RRA	Increase sensitivity of lung cancer cells to erastin-induced ferroptosis	[36]
		Nrf2/HO-1 pathway decreases ROS and LPOs	Lysosomal instability drug (seramesine) and dual tyrosine kinase inhibitor (lapatinib)	Trigger ferroptosis via reducing HO-1	[27]
			Acetaminophen	Sensitize NSCLC to erastin	[28]
		Overexpression of STYK1/NOK that upregulate GPX4	GPX4 knockdown	Induce ferroptosis	[37]
		Overexpression of GPX4	RSL3	-Suppress GPX4 -Induce Nrf2/HO-1 pathway -Disrupt autolysosome formation by lysosomal membrane destabilization	[29]
			Timosaponin-AIII (Tim-AIII)	Promote expression of and bind to HSP90 that induces GPX4 ubiquitination and degradation	[30]
	Large cell carcinoma	FSP1 mediates ferroptosis resistance in cells that lack GPX4	GPX4-KO FSP1-KO	Increase growth inhibition (ferroptosis)	[10]
		P53 knockdown	P53 expression	Inhibit SLC7A11 expression	[38]
			Erastin	Induce ferroptosis dependent on p53 pathway	[31]
		Low intracellular ferrous iron, ROS, and LPOs	Sinapine(SI)	Increase Fe2+ and LPOs via upregulating Tf and TfR	[32]

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