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Enhanced understanding of the involvement of ferroptosis in tumorigenesis: A review of recent research advancements

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ABSTRACT

Ferroptosis, a recently identified form of programmed cell death, is characterized by the accumulation of lipid peroxidation, reactive oxygen species, and elevated free iron levels, involving the regulation of glutathione metabolism, iron metabolism, lipid metabolism, and oxidative stress biology. Tumor metastasis, a critical hallmark of malignancy and a key contributor to cancer recurrence and mortality, has been extensively linked to iron dysregulation, highlighting the potential of agents inducing iron-mediated cell death as promising strategies for preventing and treating metastasis. This review offers a comprehensive understanding the regulatory mechanisms underlying ferroptosis and its crucial role in the three distinct stages of metastasis: invasion, circulation, and colonization.

KEYWORDS

Ferroptosis; Lipid peroxidation; Tumor metastasis; Iron; Glutathione metabolism; Reactive oxygen species

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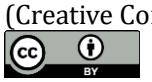

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1. Introduction

The prevalence of malignant tumors has emerged as a prominent peril to human well-being [1,2]. In 2022, Hanahan et al suggested that malignant tumors exhibit enduring proliferative signals, genomic instability, evasion of immune surveillance, and resistance to programmed cell death (PCD) [3]. Among them, the resistance of tumor cells to PCD is an important feature. The disruption of the regulatory mechanisms of PCD, including apoptosis, autophagy, necroptosis, and pyroptosis, has been demonstrated to facilitate the survival and uncontrolled proliferation of tumor cells [4-7] (Box 1). The increasing interest in PCD has led to more cases involving diverse pathways and molecules associated with cell death. The iron-dependent programmed cell death (PCD) modality, known as ferroptosis and introduced by Dixon et al [9] in 2012, is closely associated with aberrant iron metabolism and the accumulation of lipid peroxidation 8. Ferroptosis is lipid accumulation of reactive oxygen species (ROS) free radicals in tumor cells with oncogene mutations induced by small molecule compounds, including Erastin and RSL3 [9,10]. The morphological characteristics of ferroptosis include a reduction in mitochondrial volume, increased bilayer membrane density, disappearance of mitochondrial cristae, rupture of the outer mitochondrial membrane, or crumpling of the mitochondria [8]. These abnormalities are mainly due to the loss of selective permeability of the plasma membrane resulting from intense lipid peroxidation and oxidative stress. Ferroptosis morphological features, molecular mechanisms, and biochemical aspects distinguish it from other well-known PCD modalities such as apoptosis, autophagy, necrosis, and pyroptosis [11]. The current consensus is that lipid-based reactive oxygen species/phospholipid hydroperoxide (PLOOH) serves as the executing molecule in the initiation of iron death and that maintaining a delicate balance between oxidative damage and antioxidant protection during the synthesis and metabolism of this crucial molecule plays a central role in regulating iron death [12]. The occurrence of ferroptosis is characterized by the two key mechanisms of iron accumulation and lipid peroxidation, which induce oxidative damage to the cell membrane by promoting PLOOH production. However, antioxidant mechanisms can mitigate PLOOH levels and inhibit oxidative damage, thereby preventing cell iron death. Under normal circumstances, the two mechanisms maintain a dynamic equilibrium that ensures the body's metabolism (Fig. 1).

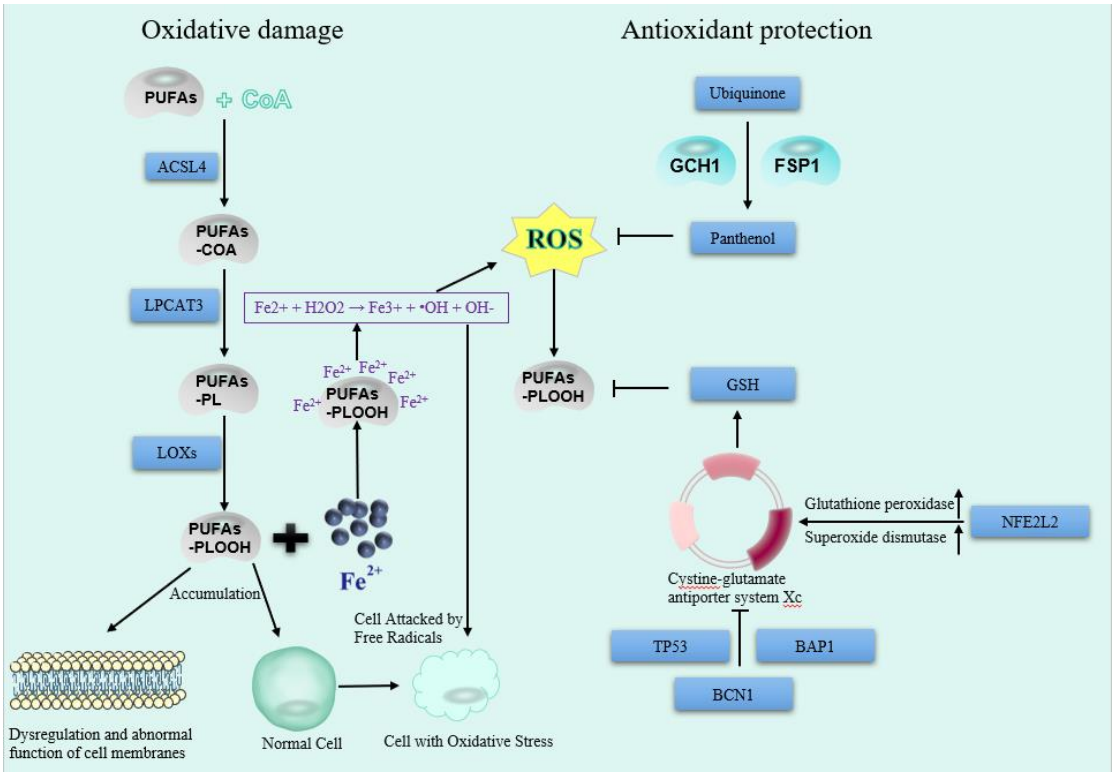

Figure 1. Balance of oxidative damage and antioxidant protection mechanisms in tumor cell ferroptosis.

However, when this equilibrium is disrupted, aberrant iron homeostasis occurs, leading to the onset of pathological conditions. The tumor cells have developed a robust antioxidant capacity in response to oxidative stress induced by rapid proliferation or various therapies, such as the hyperactivation of the Kelch-like epichlorohydrin 1/nuclear factor E2-related factor 2/antioxidant-responsive element signaling pathway and the enhanced activity of reactive oxygen species-scavenging enzymes like superoxide dismutase and catalase [13]. These adaptations contribute to tumor progression and confer resistance to therapeutic interventions. The latest research has demonstrated that ferroptosis plays a crucial regulatory role in the occurrence and progression of various cancers, making it a focal point and hot topic in the field of cancer treatment and prognosis improvement (Fig. 2). However, there is still a lack of systematic review of the regulatory mechanisms of ferroptosis and its relationship with malignant tumors. Therefore, this review will summarize the mechanisms of ferroptosis and its role in malignant tumors and provide new targets for clinical treatment of tumors.

Box 1. Ferroptosis

Biochemical properties: Iron-dependence; Lipid peroxidation; Non-apoptotic morphology; Lipid peroxidation-induced cell death; Inhibition by iron chelators

Morphological Characteristics: Ferroptosis is a type of programmed cell death that is characterized by morphological changes in the cell membrane. The morphological characteristics of ferroptosis include:

a. Membrane rupture: Ferroptotic cells exhibit membrane rupture, often observed as releasing intracellular contents.

b. Lipid peroxide accumulation: Ferroptotic cells accumulate lipid peroxides in the cell membrane, which can be visualized using fluorescent probes.

c. Mitochondrial shrinkage: Ferroptotic cells exhibit mitochondrial shrinkage, which is often observed as a reduction in mitochondrial size and number.

d. DNA damage: Ferroptotic cells exhibit DNA damage, which can be visualized using fluorescent probes for DNA breaks or comet assays.

e. Nuclear condensation: Unlike apoptosis, ferroptotic cells do not exhibit nuclear condensation or fragmentation. Instead, they exhibit a rounded and condensed nucleus.

Regulatory Pathways

Ferroptosis is a type of programmed cell death that is regulated by several different regulatory pathways. The main regulatory pathways involved in ferroptosis include:

a. Lipid peroxidation pathway:

The main event leading to ferroptosis is the accumulation of lipid peroxides in the cell membrane, which is catalyzed by the iron-dependent enzyme lipoxygenase. This pathway is regulated by the levels of iron and other nutrients and the activity of various enzymes involved in lipid metabolism.

b. GSH/GPX4 pathway:

The GSH/GPX4 pathway is a pivotal regulator of ferroptosis, as it is involved in detoxifying lipid peroxides. This pathway is regulated by the GSH and GPX4 levels and by the activity of various enzymes involved in GSH metabolism and GPX4 activation.

c. p53 pathway:

The p53 pathway is a significant regulator of cell cycle arrest and apoptosis, but it can also regulate ferroptosis through the transcriptional repression of GPX4. This pathway is regulated by the levels of p53 and its downstream targets, as well as by the activity of various enzymes involved in p53 signaling.

d. Nrf2/ARE pathway:

The Nrf2/ARE pathway is a key regulator of antioxidant response and cell survival, but it can also regulate

ferroptosis through the transcriptional activation of GPX4. This pathway is regulated by the levels of Nrf2 and its downstream targets and by the activity of various enzymes involved in Nrf2 signaling.

Overall, ferroptosis is regulated by several different regulatory pathways, including the Xc- /GPX4, MVA, sulfur transfer pathway, P62- Keap1-NRF2 pathway, P53/SLC7A11, ATG5- ATG7-NCOA4 pathway, P53-SAT1-ALOX15 pathway, HSPB1-TRF1, FSP1-COQ10-NAD(P)H pathway. These pathways are interconnected and coordinated to control the level of lipid peroxides in the cell membrane and to prevent ferroptosis from occurring.

Key genes

Ferroptosis is a type of programmed cell death regulated by several genes. The key genes involved in ferroptosis regulation include:

GPX4: GPX4 is a major regulator of ferroptosis, as it is involved in detoxifying lipid peroxides. The expression level of GPX4 is inversely correlated with the susceptibility to ferroptosis.

SLC7A11: SLC7A11 is a cystine/glutamate antiporter that imports cystine into the cell and exports glutamate out of the cell. The expression level of SLC7A11 is positively correlated with the susceptibility to ferroptosis.

ACSL4: ACSL4 is an acyl-CoA synthetase that catalyzes acyl-CoA formation from fatty acids. The expression level of ACSL4 is positively correlated with the susceptibility to ferroptosis.

FTH1 and FTL: FTH1 and FTL are ferritin proteins that store iron in the cell. The expression level of FTH1 and FTL is negatively correlated with the susceptibility to ferroptosis.

TFR1: TFR1 is a transferrin receptor that mediates iron uptake from the extracellular environment. The expression level of TFR1 is positively correlated with the susceptibility to ferroptosis.

Overall, GPX4, TFR1, SLC7A11, NRF2, NCOA4, P53, HSPB1, ACSL4, FSP1, and FTL are the key genes involved in ferroptosis regulation. These genes play essential roles in controlling the level of lipid peroxides in the cell membrane and preventing ferroptosis from occurring.

Apoptosis

Biochemical properties：

Apoptosis is a form of programmed cell death essential for developing and maintaining multicellular organisms. It is characterized by the activation of a cascade of biochemical reactions that lead to the death of the cell. The biochemical properties of apoptosis can be described as follows:

Caspases: Caspases are a family of proteases that play a central role in apoptosis. They are activated by upstream signals such as DNA damage or the activation of death receptors. Caspases cleave and activate other proteins, leading to the degradation of cellular components and, ultimately, the death of the cell.

Cytochrome c: Cytochrome c is a mitochondrial protein that is released into the cytosol during apoptosis. It binds to Apaf-1, a protein that is part of the apoptosome complex, which triggers the activation of caspases.

Bcl-2 family proteins: The Bcl-2 family of proteins plays a crucial role in regulating apoptosis. They can be divided into anti-apoptotic proteins (Bcl-2, Bcl-xL, and Mcl-1) and pro-apoptotic proteins (Bax, Bak, and Bad). The balance between these two groups determines whether a cell will undergo apoptosis or survive.

DNA fragmentation: During apoptosis, DNA fragmentation occurs by activating caspases. This fragmentation is visualized as DNA laddering on agarose gel electrophoresis.

Cytoplasmic shrinkage: Apoptotic cells undergo cytoplasmic shrinkage due to the loss of mitochondrial membrane potential and the activation of ion channels that lead to water efflux.

Plasma membrane blebbing: Apoptotic cells exhibit plasma membrane blebbing, which is characterized by the formation of membrane protrusions or blebs on the cell surface. This process is mediated by actin cytoskeletal rearrangements and involves the activation of membrane-associated proteins such as annexins.

Intracellular organelles degradation: Apoptotic cells undergo degradation of intracellular organelles, including

mitochondria, Golgi apparatus, and endoplasmic reticulum. This degradation is mediated by caspases and other proteases.

Overall, the biochemical properties of apoptosis involve the activation of caspases, the release of cytochrome c, the regulation of Bcl-2 family proteins, DNA fragmentation, cytoplasmic shrinkage, plasma membrane blebbing, and intracellular organelle degradation. These processes are critical for the execution of apoptosis and for maintaining homeostasis in multicellular organisms.

Morphological Characteristics:

Nuclear condensation: During apoptosis, the nucleus undergoes condensation, becoming smaller and denser. This is due to the chromatin condensation and the formation of nuclear membrane protrusions called nuclear envelope invaginations.

Cytoplasmic shrinkage: Apoptotic cells undergo cytoplasmic shrinkage due to the loss of mitochondrial membrane potential and the activation of ion channels that lead to water efflux. The blebbing of the plasma membrane also accompanies this shrinkage.

Plasma membrane blebbing: Apoptotic cells exhibit plasma membrane blebbing, which is characterized by the formation of membrane protrusions or blebs on the cell surface. This process is mediated by actin cytoskeletal rearrangements and involves the activation of membrane-associated proteins such as annexins.

Chromatin condensation: During apoptosis, the chromatin undergoes condensation, forming clumps or threads that are visible under a light microscope. This condensation is due to the interaction between histones and DNA, leading to the formation of DNA-histone complexes.

Cytoplasmic fragmentation: Apoptotic cells undergo cytoplasmic fragmentation, which is characterized by the cleavage of the cell into smaller fragments or bodies. This fragmentation is mediated by caspases and other proteases.

Nuclear envelope invaginations: During apoptosis, nuclear envelope invaginations form on the nuclear membrane, leading to the exposure of nuclear contents to the cytoplasm. This process is mediated by nuclear envelope proteins such as lamins and lamina-associated proteins.

Overall, the morphological characteristics of apoptosis involve nuclear condensation, cytoplasmic shrinkage, plasma membrane blebbing, chromatin condensation, cytoplasmic fragmentation, and nuclear envelope invaginations. These changes are critical for the execution of apoptosis and for maintaining homeostasis in multicellular organisms.

Regulatory Pathways:

Extrinsic pathway: The extrinsic pathway is triggered by the binding of death receptors on the cell surface to their ligands, such as tumor necrosis factor (TNF) or Fas ligand. This binding activates caspase-8, which initiates the cascade of events leading to apoptosis.

Intrinsic pathway: Intrinsic pathways are triggered by intracellular stressors, such as DNA damage or mitochondrial dysfunction. This pathway is regulated by Bcl-2 family proteins, which are divided into anti-apoptotic proteins (Bcl-2, Bcl-xL, and Mcl-1) and pro-apoptotic proteins (Bax, Bak, and Bad). When intracellular stressors are present, the balance between anti-apoptotic and pro-apoptotic proteins is disrupted, leading to the activation of caspase-9 and the initiation of apoptosis.

Apaf-1/caspase-9 pathway: The apoptosome complex is formed when cytochrome c is released from mitochondria into the cytosol. Cytochrome c binds to Apaf-1, which activates caspase-9, leading to the cascade of events leading to apoptosis.

IAP pathway: Inhibitors of apoptosis proteins (IAPs) are a family of proteins that inhibit apoptosis by blocking the activation of caspases. IAPs are negatively regulated by Smac/DIABLO, which is released from mitochondria during apoptosis. Smac/DIABLO binds to IAPs, leading to their degradation and the activation of caspases.

TP53 pathway: The tumor suppressor protein p53 plays a crucial role in regulating apoptosis by activating transcription of pro-apoptotic genes or inhibiting transcription of anti-apoptotic genes. When p53 is activated by DNA damage or other stressors, it triggers apoptosis by upregulating pro-apoptotic genes or downregulating anti-apoptotic genes.

Overall, the regulatory pathways of apoptosis involve the extrinsic pathway, intrinsic pathway, Apaf-1/caspase-9 pathway, IAP pathway, TP53 pathway, Death receptor pathway, mitochondrion pathway, endoplasmic reticulum pathway Caspase, P53, and Bcl-2 mediated signaling pathway. These pathways are interconnected and regulate the activation and execution of apoptosis in response to different cellular stressors.

Key genes

BCL2: BCL2 is an anti-apoptotic gene that encodes a protein that inhibits the activation of caspases, which are enzymes involved in the execution of apoptosis.

BAX: BAX is a pro-apoptotic gene that encodes a protein that promotes the activation of caspases and the initiation of apoptosis.

BAD: BAD is a pro-apoptotic gene that encodes a protein that interacts with Bcl-2 and inhibits its anti-apoptotic function.

CASPASE-3: CASPASE-3 is a caspase enzyme that is activated during apoptosis and plays a central role in the execution of apoptosis by cleaving and degrading cellular proteins.

CASPASE-8: CASPASE-8 is another caspase enzyme that is activated during the extrinsic pathway of apoptosis and initiates the cascade of events leading to apoptosis.

CASPASE-9: CASPASE-9 is another caspase enzyme that is activated during the intrinsic pathway of apoptosis and initiates the cascade of events leading to apoptosis.

P53: P53 is a tumor suppressor protein that plays a crucial role in regulating apoptosis by activating the transcription of pro-apoptotic genes or inhibiting the transcription of anti-apoptotic genes.

SMAC/DIABLO: SMAC/DIABLO is a mitochondrial protein that is released during apoptosis and binds to IAPs, leading to their degradation and the activation of caspases.

Overall, the key genes of apoptosis include BCL2, BAX, BAD, CASPASE-3, CASPASE-8, CASPASE-9, P53, and SMAC/DIABLO. These genes play a crucial role in regulating the activation and execution of apoptosis in response to different types of cellular stressors.

Necroptosis

Biochemical properties：

Necroptosis is a form of programmed cell death that is regulated by a series of biochemical properties. The biochemical properties of necroptosis can be described as follows:

Receptor-interacting protein kinase 1 (RIPK1): RIPK1 is a protein kinase that is essential for necroptosis. It interacts with the necroptosis-inducing receptor, TNFR1, and activates downstream signaling pathways that lead to necroptosis.

Receptor-interacting protein kinase 3 (RIPK3): RIPK3 is another protein kinase that is essential for necroptosis. It interacts with RIPK1 and phosphorylates it, activating downstream signaling pathways that lead to necroptosis.

Mixed lineage kinase domain-like (MLKL): MLKL is a protein that is involved in the execution of necroptosis. It is a downstream target of RIPK3 and is phosphorylated by RIPK3, leading to its activation. Once activated, MLKL forms pores in the plasma membrane, leading to cell lysis and necrosis.

Z-DNA binding protein 1 (ZBP1): ZBP1 is a protein involved in the necroptosis regulation. It binds to the DNA of necroptotic cells and triggers the degradation of RIPK1 and RIPK3, inhibiting necroptosis.

Receptor-interacting protein kinase 3 (RIPK3) and MLKL: RIPK3 and MLKL are the two main proteins involved in the execution of necroptosis. They interact with each other and form a complex that activates downstream

signaling pathways that lead to necroptosis.

Overall, the biochemical properties of necroptosis involve the interaction of RIPK1, RIPK3, and MLKL, as well as the regulation of ZBP1. These biochemical properties are essential for the activation and execution of necroptosis in response to different types of cellular stressors.

Morphological Characteristics:

Cell swelling: Necrotic cells undergo significant swelling due to the loss of membrane integrity and the influx of water and ions into the cell.

Plasma membrane rupture: Necrotic cells have a disrupted plasma membrane, which allows the release of cellular contents, including DNA fragments and inflammatory mediators, into the extracellular environment.

Cytoplasmic vacuolation: Necrotic cells exhibit vacuolation of the cytoplasm due to the loss of membrane integrity and fluid accumulation within the cell.

Nuclear condensation: Necrotic cells undergo nuclear condensation, which is a hallmark of necrosis. The nucleus becomes densely packed and forms a crescent-shaped structure.

Granulation of the cytoplasm: Necrotic cells exhibit granulation of the cytoplasm due to the accumulation of cellular debris and organelles within the cell.

Overall, the morphological characteristics of necroptosis include cell swelling, plasma membrane rupture, cytoplasmic vacuolation, nuclear condensation, and cytoplasm granulation. These morphological changes are essential for the activation and execution of necroptosis in response to different types of cellular stressors.

Regulatory Pathways:

TNFR1-RIPK1-RIPK3 signaling pathway: TNFR1 is a receptor for tumor necrosis factor (TNF), which is a pro-inflammatory cytokine. Upon binding to TNFR1, TNF activates RIPK1 and RIPK3, phosphorylating each other and activating downstream signaling pathways that lead to necroptosis.

MLKL-dependent signaling pathway: MLKL is a protein that is involved in the execution of necroptosis. It is phosphorylated by RIPK3, leading to its activation. Once activated, MLKL forms pores in the plasma membrane, leading to cell lysis and necrosis.

ZBP1-dependent signaling pathway: ZBP1 is a protein involved in the necroptosis regulation. It binds to the DNA of necroptotic cells and triggers the degradation of RIPK1 and RIPK3, inhibiting necroptosis.

Receptor-interacting protein kinase 3 (RIPK3) and mixed lineage kinase domain-like (MLKL) signaling pathway: RIPK3 and MLKL are the two main proteins involved in the execution of necroptosis. They interact with each other and form a complex that activates downstream signaling pathways that lead to necroptosis.

The regulatory pathways of necroptosis involve TNFR1-RIPK1-RIPK3 signaling, MLKL-dependent signaling, ZBP1-dependent signaling, and RIPK3 and MLKL signaling. These signaling pathways are essential for the activation and execution of necroptosis in response to different types of cellular stressors.

Key genes

RIPK1: RIPK1 is a protein kinase that is essential for necroptosis. It interacts with the necroptosis-inducing receptor, TNFR1, and activates downstream signaling pathways that lead to necroptosis.

RIPK3: RIPK3 is another protein kinase that is essential for necroptosis. It interacts with RIPK1 and phosphorylates it, activating downstream signaling pathways that lead to necroptosis.

MLKL: MLKL is a protein that is involved in the execution of necroptosis. It is phosphorylated by RIPK3, leading to its activation. Once activated, MLKL forms pores in the plasma membrane, leading to cell lysis and necrosis.

ZBP1: ZBP1 is a protein that is involved in the regulation of necroptosis. It binds to the DNA of necroptotic cells and triggers the degradation of RIPK1 and RIPK3, inhibiting necroptosis.

FADD: FADD is a protein that is involved in the regulation of necroptosis. It interacts with RIPK1 and RIPK3, leading to their degradation and inhibiting necroptosis.

CASP8: CASP8 is a protein that is involved in the regulation of necroptosis. It interacts with RIPK1 and RIPK3, leading to their degradation and inhibiting necroptosis.

FLIP: FLIP is a protein that is involved in the regulation of necroptosis. It interacts with FADD and CASP8, leading to their degradation and inhibiting necroptosis.

Overall, the key genes of necroptosis include RIPK1, RIPK3, MLKL, ZBP1, FADD, CASP8, and FLIP. These genes play a crucial role in regulating the activation and execution of necroptosis in response to different types of cellular stressors.

Pyroptosis

Biochemical properties：

Pyroptosis is a form of programmed cell death characterized by the cell membrane's lysis and the release of pro-inflammatory molecules. The biochemical properties of pyroptosis can be described as follows:

Cytoplasmic swelling: Pyroptotic cells undergo significant cytoplasmic swelling due to the osmotic imbalance caused by the rupture of the cell membrane.

Plasma membrane rupture: Pyroptotic cells have a disrupted plasma membrane, which allows the release of cellular contents, including DNA fragments and inflammatory mediators, into the extracellular environment.

Cell lysis: Pyroptotic cells undergo cell lysis, which is characterized by the complete disruption of the cell membrane and the release of cellular contents.

Inflammasome activation: Pyroptosis is mediated by inflammasomes, which are multiprotein complexes that are formed in response to pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). The activation of inflammasomes leads to the formation of a pore in the cell membrane, allowing the release of pro-inflammatory molecules, such as IL-1β and IL-18.

Pro-inflammatory signaling: Pyroptosis triggers a pro-inflammatory signaling cascade that activates caspase-1 and caspase-11, which in turn cleaves and activates IL-1β and IL-18. This signaling cascade leads to the recruitment and activation of inflammatory cells, such as macrophages and neutrophils, which further amplify the inflammatory response.

The biochemical properties of pyroptosis involve cytoplasmic swelling, plasma membrane rupture, cell lysis, inflammasome activation, and pro-inflammatory signaling. These properties are essential for the activation and execution of pyroptosis in response to different types of cellular stressors.

Morphological Characteristics:

Cell lysis: Pyroptotic cells undergo cell lysis, which is characterized by the complete disruption of the cell membrane and the release of cellular contents.

Inflammasome activation: Pyroptosis is mediated by inflammasomes, which are multiprotein complexes that are formed in response to pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). The activation of inflammasomes leads to the formation of a pore in the cell membrane, allowing the release of pro-inflammatory molecules, such as IL-1β and IL-18.

Pro-inflammatory signaling: Pyroptosis triggers a pro-inflammatory signaling cascade that activates caspase-1 and caspase-11, which in turn cleaves and activates IL-1β and IL-18. This signaling cascade leads to the recruitment and activation of inflammatory cells, such as macrophages and neutrophils, which further amplify the inflammatory response.

The morphological characteristics of pyroptosis involve cell swelling, plasma membrane rupture, cell lysis, inflammasome activation, and pro-inflammatory signaling. These characteristics are essential for the activation and execution of pyroptosis in response to different types of cellular stressors.

Regulatory Pathways:

Inflammasome-dependent pathway: Pyroptosis is mediated by inflammasomes, which are multiprotein

complexes that are formed in response to pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). The activation of inflammasomes leads to the formation of a pore in the cell membrane, allowing the release of pro-inflammatory molecules, such as IL-1β and IL-18.

Cytokine-dependent pathway: Pyroptosis can also be triggered by releasing pro-inflammatory cytokines, such as TNF-α and IL-1β. These cytokines bind to their receptors on the cell surface, activating caspase-1 and caspase-11, which in turn cleave and activate IL-1β and IL-18.

Receptor-interacting protein kinase 3 (RIPK3)-dependent pathway: RIPK3 is an essential protein kinase for pyroptosis. It interacts with RIPK1 and MLKL, leading to their phosphorylation and activation. Once activated, MLKL forms pores in the plasma membrane, leading to cell lysis and necrosis.

Mixed lineage kinase domain-like (MLKL)-dependent pathway: MLKL is a protein that is involved in the execution of pyroptosis. It is phosphorylated by RIPK3, leading to its activation. Once activated, MLKL forms pores in the plasma membrane, leading to cell lysis and necrosis.

Receptor-interacting protein kinase 1 (RIPK1)-dependent pathway: RIPK1 is another protein kinase that is essential for pyroptosis. It interacts with RIPK3 and MLKL, leading to their phosphorylation and activation. Once activated, MLKL forms pores in the plasma membrane, leading to cell lysis and necrosis.

The regulatory pathways of pyroptosis encompass the inflammasome-dependent pathway, cytokine-dependent pathway, RIPK3-dependent pathway, MLKL-dependent pathway, and RIPK1-dependent pathway as a whole. These intricate pathways are indispensable for the initiation and execution of pyroptosis in response to diverse cellular stressors.

Key genes

Caspase-1: Caspase-1 is the main effector of caspase in pyroptosis. Inflammasomes and cleaves activate it and activates IL-1β and IL-18.

Caspase-11: Caspase-11 is another effector caspase in pyroptosis. Inflammasomes and cleaves activate it and activates IL-1β and IL-18.

NLRP3: NLRP3 is a protein that forms the inflammasome complex that mediates pyroptosis. It is involved in the activation of caspase-1 and caspase-11.

ASC: ASC is a protein that interacts with NLRP3 to form the inflammasome complex that mediates pyroptosis.

GSDMD: GSDMD is a protein that forms pores in the plasma membrane during pyroptosis. It is cleaved by caspase-1 and caspase-11 and is involved in the release of pro-inflammatory molecules.

MLKL: MLKL is a protein that forms pores in the plasma membrane during pyroptosis. It is phosphorylated by RIPK3 and is involved in releasing pro-inflammatory molecules.

RIPK1: RIPK1 is a protein that interacts with NLRP3 and MLKL to mediate pyroptosis. It is involved in the activation of caspase-1 and caspase-11.

The key genes involved in pyroptosis collectively encompass caspase-1, caspase-11, NLRP3, ASC, GSDMD, MLKL, and RIPK1. These genes play an indispensable role in the initiation and execution of pyroptosis triggered by various cellular stressors.

Autophagy

Biochemical properties：

Autophagy is a process by which cells recycle their own components through the lysosomes. There are several biochemical properties associated with autophagy, including the following:

Autophagosome formation: Autophagosome formation is initiated by forming a phagophore, a double-membrane structure that engulfs the cytoplasmic components to be recycled. The phagophore then expands and encloses the components, eventually fusing with a lysosome to form an autolysosome.

Lysosomal enzymes: Lysosomes contain hydrolytic enzymes that break down the engulfed components into

smaller molecules. These enzymes include proteases, lipases, and glycosidases, breaking down proteins, lipids, and carbohydrates.

Acidification of lysosomes: Lysosomes are acidic organelles due to the presence of hydrolytic enzymes. Acidification is essential for these enzymes' activity and the degradation of the engulfed components.

Protein degradation: Autophagy involves the degradation of proteins through the lysosomes. This degradation is initiated by the formation of autophagosomes, which engulf the proteins and fuse with lysosomes to form autolysosomes. The proteins are then broken down into smaller molecules by the hydrolytic enzymes in the lysosomes.

Lipid degradation: Autophagy also involves the degradation of lipids through the lysosomes. This degradation is initiated by autophagosome formation, which engulfs the lipids and fuse with lysosomes to form autolysosomes. The lipids are then broken down by the hydrolytic enzymes in the lysosomes into smaller molecules.

The biochemical properties associated with autophagy encompass the processes of autophagosome formation, activation of lysosomal enzymes, acidification of lysosomes, and degradation of proteins and lipids. These properties enable cells to recycle their own components efficiently through the lysosomal pathway and maintain cellular homeostasis.

Morphological Characteristics:

Autophagosome: Autophagosome is a double-membrane structure that forms around the cytoplasmic components to be recycled. It initially consists of a single membrane that expands and encloses the components and then fuses with a lysosome to form an autolysosome.

Lysosome: Lysosome is a membrane-bound organelle that contains hydrolytic enzymes that break down the engulfed components into smaller molecules.

Autolysosome: Autolysosome is a structure that forms when an autophagosome fuses with a lysosome. It contains both the autophagosome components and the lysosome enzymes, which break down the components into smaller molecules.

Acidic organelles: Autophagy occurs in acidic organelles, such as lysosomes, which are acidic due to the presence of hydrolytic enzymes.

Mitochondria: Autophagy can also occur in mitochondria, which are engulfed by autophagosomes and then broken down in the lysosomes.

The morphological characteristics associated with autophagy encompass autophagosomes, lysosomes, autolysosomes, acidic organelles, and mitochondria, facilitating cellular recycling through the lysosomal pathway and maintaining cellular homeostasis.

Regulatory Pathways:

mTORC1-dependent pathway: mTORC1 is a protein complex that inhibits autophagy by phosphorylating and inactivating the autophagy-initiating kinase ULK1. mTORC1 activity is negatively regulated by the energy sensor AMPK, which is activated by low ATP levels.

Beclin-1-dependent pathway: Beclin-1 is a protein that promotes autophagy by interacting with the autophagy-initiating kinase ULK1. Beclin-1 activity is negatively regulated by Bcl-2, which is an anti-apoptotic protein.

Class III PI3K-dependent pathway: Class III PI3K is a lipid kinase that promotes autophagy by phosphorylating and activating the autophagy-initiating kinase ULK1. Class III PI3K activity is negatively regulated by the phosphatase PTEN, which is an inhibitor of PI3K signaling.

ATG proteins-dependent pathway: ATG proteins are a family of proteins that are essential for autophagy. ATG proteins are recruited to the autophagosome membrane and play a role in its formation and maturation.

UVRAG-dependent pathway: UVRAG is a protein that promotes autophagy by interacting with ATG proteins and recruiting them to the autophagosome membrane. UVRAG activity is negatively regulated by the protein p62,

which is an autophagy substrate.

Overall, the regulatory pathways involved in autophagy encompass the mTORC1-dependent pathway, Beclin-1-dependent pathway, Class III PI3K-dependent pathway, ATG proteins-dependent pathway, and UVRAG-dependent pathway. These pathways are indispensable for governing autophagy and its capacity to recycle cellular components.

Key genes

ATG1: ATG1 is a protein that initiates autophagy by forming a complex with ATG13 and ATG17. It is essential for the activation of the ULK1 kinase, which is the main autophagy-initiating kinase.

ATG5: ATG5 is a protein that is involved in the formation of the autophagosome membrane. It forms a complex with ATG12 and ATG16L1, which are essential for the recruitment of other ATG proteins to the autophagosome membrane.

ATG7: ATG7 is a protein involved in the conjugation of phosphatidylethanolamine (PE) to LC3, a protein essential for the formation of the autophagosome membrane.

BECN1: BECN1 is a protein that promotes autophagy by interacting with VPS34, a lipid kinase essential for the formation of the autophagosome membrane.

ULK1: ULK1 is a protein that initiates autophagy by forming a complex with ATG13 and ATG17. It is essential for the activation of the autophagy-initiating kinase.

PI3KC3: PI3KC3 is a lipid kinase that is involved in the formation of the autophagosome membrane. It phosphorylates PE, which is essential for recruiting other ATG proteins to the autophagosome membrane.

PTEN: PTEN is a protein that inhibits autophagy by dephosphorylating PI3KC3, which prevents the autophagosome membrane formation.

The key genes involved in autophagy, namely ATG1, ATG5, ATG7, BECN1, ULK1, PI3KC3, and PTEN, are essential for regulating autophagy and its capacity to recycle cellular components.

2. The occurrence and regulatory mechanism of ferroptosis

Dixon et al found that iron death-specific inducers such as Erastin and RAS-selective lethal small molecule 3 (RSL3) can cause programmed cell death 8. Furthermore 2008, Marcus Conrad and colleagues discovered that the genetic regulation of crucial genes governing redox status results in non-apoptotic cell death [14]. The electron microscopy revealed an observed thickening of the cell membrane, a wrinkling phenomenon in mitochondria, and a loss of mitochondrial cristae. There are also changes in biochemical characteristics, such as the accumulation of intracellular lipid peroxides and a decrease in reduced glutathione (GSH) [15]. This process can be reversed through iron death inhibitors, such as desferrioxamine (DFO), ferrostatin-1, and liproxstatin-1. In contrast, inhibitors targeting other forms of cellular programmed deaths, such as apoptosis and necrosis, are ineffective in inhibiting this novel type of programmed cell death [16]. This newly discovered iron-dependent mode of programmed cell death has been termed " ferroptosis." Either extrinsic or intrinsic pathways can trigger the process of ferroptosis. The extrinsic pathway is initiated by inhibiting cell membrane transporters, such as cystine/glutamate transporters (also known as the xc system), or by activating iron transporters like serotransferrin and lactotransferrin. The intrinsic pathway, conversely, is triggered by the inhibition of intracellular antioxidant enzymes such as glutathione peroxidase glutathione peroxidase 4 (GPX4) [17]. The delicate equilibrium between oxidative damage and antioxidant protection during the synthesis and metabolism of PLOOH lies at the heart of the iron death regulatory mechanism. The accumulation of iron and the peroxidation of lipids are two primary mechanisms that contribute to oxidative damage in cell membranes. Antioxidant mechanisms primarily involve both the classical GPX4-dependent pathway and the novel non-GPX4-dependent pathway [17]. The phenomenon of apoptosis has been extensively investigated in the past few decades; however, the development of therapeutic agents targeting key regulators of apoptosis, such as Caspase or BCL-2 family proteins, still poses significant challenges for clinical tumor treatment [18]. Anti-apoptosis

as a major feature of cancer, therefore, targeting the non-apoptotic regulated cell death (RCD) process may be an alternative strategy to inhibit tumor growth [19,20]. Three initial preclinical observations provide evidence for a correlation between specific oncogenic signals and the initiation of ferroptosis: (1) erastin, an ferroptosis activator, selectively triggers the death of cancer cells harboring mutant RAS but not wild-type RAS [21]; (2) The induction of cell death by erastin necessitates the activation of the RAS-RAF-MEK-ERK signaling pathway 9; (3) erastin-induced cell death also requires iron, which is well known to be very important for the proliferation of cancer cells 8. The subsequent studies have revealed an intricate signaling pathway that regulates iron metabolism by means of iron accumulation, lipid peroxidation, and membrane impairment (Fig. 2). This network has attracted significant attention as a potential new target for cancer therapy. The susceptibility of cancer cells resistant to conventional therapies or with a high propensity for metastasis to iron metamorphosis opens up new avenues for targeted therapeutic research [22,23]. We aim in this paper to provide insight into the mechanisms and functions of ferritin deposition in tumorigenesis and development as a potential therapeutic target. We describe tumor heterogeneity and signals associated with the iron apoptotic threshold and highlight potential therapeutic agents that could be used for clinical translation.

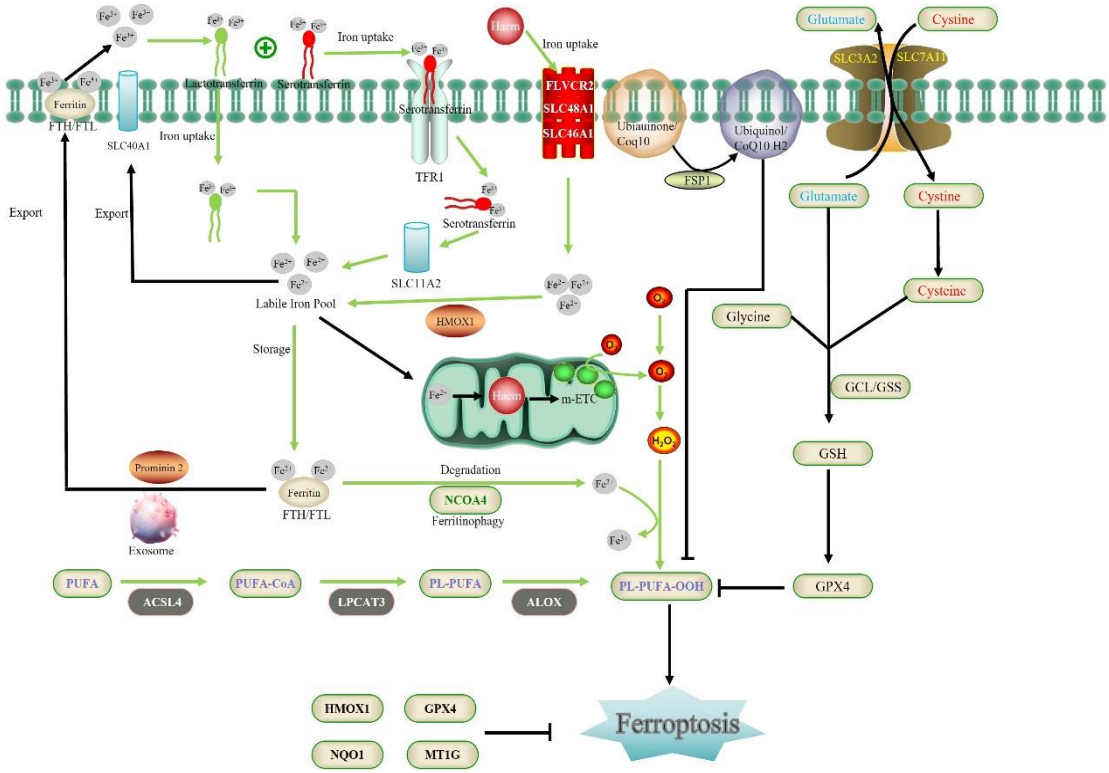

Figure 2. Molecular interactions implicated in the initiation and control of ferroptosis.

3. Iron accumulation and oxidative damage in ferroptosis

3.1 Iron accumulation

Iron accumulation and lipid peroxidation are critical signals that initiate oxidative membrane damage during iron oxidation ferroptosis 8. The central molecular mechanism underlying the role of ferroptosis involves the regulation of the delicate balance between oxidative damage and antioxidant defense [24]. The occurrence of ferroptosis is known to be crucial for the accumulation of iron proteins involved in maintaining iron homeostasis plays a vital role in regulating this process. These proteins' abnormal expression or dysfunction results in enhanced iron uptake, reduced iron storage, and limited iron efflux, leading to intracellular iron accumulation and subsequent

elevation of reactive oxygen species and lipid ultimately triggering the onset of cellular death caused by ferroptosis [10]. The growth of cancer cells, mainly cancer stem cells (CSCs), has been demonstrated to be predominantly reliant on the trace element iron compared to non-malignant cells. The epidemiological evidence suggests that a high dietary intake of iron is associated with an increased risk of developing various types of cancers, including hepatocellular carcinoma (HCC) and breast cancer [25-27]. These characteristics suggest that drugs that chelate iron (such as desferrioxamine) or enhance iron-mediated toxicity (such as drugs inducing iron metabolism, including sorafenib, sulfasalazine, statins, and artemisinins), may be utilized for the treatment of cancer patients.

The promotion of ferroptosis through integrated signaling pathways is facilitated by interventions targeting multiple levels, including enhanced iron uptake, diminished iron storage, and restricted iron efflux in experimental studies (Fig. 3). For example, the binding of transferrin to the transferrin receptor facilitates the transport of extracellular irons into the intracellular compartment, thereby exacerbating the onset of ferroptosis. Conversely, reducing the expression of transferrin receptor 1 and scavenging transferrin from the culture medium attenuate the sensitivity to Erastin, an inducer of ferroptosis [28]. The downregulation of iron-responsive element-binding protein 2 leads to an upregulation of downstream iron-storage-associated proteins (such as ferritin light chain, ferritin heavy chain polypeptide 1, and iron-sulfur cluster assembling enzyme), thereby attenuating intracellular ferroptosis through the reduction of intracellular irons. If the expression of the iron-responsive element-binding protein 2 negative regulator was interfered with cells showed the opposite effect 8. The NRC coactivator 4(NCOA4) selectively binds ferritin and facilitates its sequestration into autophagosomes for subsequent lysosomal digestion, thereby enabling the release of free iron ions through a process known as iron autophagy. Therefore, interfering with the expression of NCOA4 significantly inhibits the degradation of ferritin and the occurrence of ferroptosis [29-31]. Serum transferrin-mediated or milk transferrin-mediated iron uptake promotes ferroptosis through the transferrin receptor (TFRC) and/or an unidentified receptor, while iron efflux proteins solute carrier protein family 40 member 1 (SLC40A1), responsible for transporting iron ions out of the cell and reducing intracellular iron levels, inhibit ferroptosis [10,32]. Furthermore, the overexpression of SLC40A1 exerts an inhibitory effect on ferroptosis, while its knockdown enhances ferroptosis [33]. In the regulatory network of ferroptosis, ferritin acts as an iron storage protein. The autophagic degradation process enhances intercellular iron accumulation, thereby facilitating ferritin deposition (Table 1), while the exosome-mediated export of ferritin inhibits its deposition [29,30,34].Other proteins involved in iron metabolism, such as heat shock protein β1 (HSPβ1), NFS1 cysteine desulfurase (NFS1) [35], Iron-Sulfur Cluster Assembly Enzyme 26 (ISCU26) [36], CDGSH iron-sulfur domain 1 (CISD1), and CISD2 [37,38], along with several mitochondrial proteins participating in iron utilization during iron-sulfur cluster biogenesis, exert a negative regulatory effect on iron mutations by potentially reducing the availability of redox-active iron.

The elevation of intracellular irons concentration facilitates the accumulation of lipid peroxides through two distinct pathways. On the one hand, the generation of reactive oxygen species is catalyzed by the iron-dependent Fenton reaction, i.e., divalent iron ions (Fe2+) with redox activity can catalyze the generation of reactive oxygen molecules, such as hydroxyl radicals and hydroxyl radicals, from hydrogen peroxide, and the accumulation of these reactive oxygen species will directly contribute to the generation of PLOOH. On the other hand, by activating iron-dependent enzymes, including iron-containing lipoxygenase (LOX), cytochrome P450 oxidoreductase, etc, iron-dependent enzymes, such as LOX, catalyze the production of phospholipid hydroperoxides, which promotes ferroptosis 11. The findings of various studies have demonstrated that iron chelators effectively inhibit ferroptosis by reducing intracellular iron levels, thereby providing further evidence for the crucial role of ferroptosis in mediating cellular demise [39,40]. In conclusion, iron accumulation resulting from disorders in iron homeostasis is a significant mechanism promoting ferroptosis, and genes involved in regulating iron metabolism represent potential targets for modulating this process.

3.2 Lipid peroxidation

The unrestricted peroxidation of lipids is an additional crucial mechanism that facilitates ferroptosis. The process of lipid peroxidation initiates with phospholipids that contain polyunsaturated fatty acyl groups (PUFA-PL) within the lipid bilayer. The unsaturated acyl group of PUFA-PL abstracts a bisallylic hydrogen atom (located between the two carbon-carbon bonds) to generate a carbon-centered phospholipid radical, which subsequently reacts with a phospholipid hydroperoxide radical and abstracts a hydrogen from another polyunsaturated fatty acid (PUFA) to form PLOOH[41]. If GPX4 fails to reduce PLOOH into its corresponding alcohol, these substances will persistently oxidize PUFA-PL, generating more PLOOH. This chain reaction can potentially disrupt cellular membrane integrity, resulting in organelle and/or cell membrane rupture[42]. The results indicate that membranes containing PUFA-PL exhibit higher susceptibility to lipid peroxidation[43]. Dixon et al. (2015) identified two crucial enzymes involved in ferroptosis through a screen of haploid cell lines: acyl-CoA synthetase long-chain family member4 (ACSL4), responsible for PUFA synthesis and lysophosphatidylcholine acyltransferase 3 (LPCAT3), associated with lipid remodeling[44]. The primary role of ACSL4 is to facilitate the conjugation of long-chain polyunsaturated fatty acids (PU), such as arachidonic acid and adrenic acid, with coenzyme A. Subsequently, LPCAT3 catalyzes the esterification process for these substrates, leading to the formation of PUFA-phospholipids (PLs) within biofilms [45-47]. The knockdown of both enzyme genes in cells or the reduction of activity for both enzymes using inhibitors leads to a decrease in PUFA synthesis, which is a specific substrate for lipid peroxidation, thereby impeding the initiation of ferroptosis (Fig. 3). In contrast, when PUFA-PL levels are high in the cell membrane, cells are more susceptible to lipid peroxidation accumulation and thus more sensitive to ferroptosis inducers [46-48]. The iron-containing lipoxygenase (LOX) catalyzes the peroxidation of polyunsaturated fatty acid-phospholipids (PUFA-PL), generating PLOOH. In addition, inhibition of LOX expression effectively prevents Erastin-induced ferroptosis [43,47]. The findings of further studies have demonstrated the significant involvement of distinct LOX isoforms (ALOX5, ALOX12, ALOXE3, ALOX15, ALOX15B, etc.) in various physiological contexts. For instance, ALOX5, ALOXE3, ALOX15, and ALOX15B have been implicated in ferroptosis across various tumor cell lines of diverse origins (including BJeLR, HT-1080, and PANC1 cells), while ALOX12 plays a crucial role in p53-dependent ferroptotic cell death in the non-small cell lung cancer cell line H1299 [43,49,50].

Moreover, the susceptibility of PUFA, particularly arachidonic and adrenoic acids, to peroxidation during ferroptosis deposition leads to the destruction of the lipid bilayer and impacts membrane function. The biosynthesis and remodeling of PUFA in cell membranes necessitate the presence of two enzymes, namely ACSL. ACSL4 facilitates the CoA-dependent conjugation of unbound acid or adrenic acid, resulting in the formation of AA-CoA or AdA-CoA derivatives, respectively. LPCAT3 subsequently esterifies these derivatives into membrane phosphatidylethanolamine to generate AA-PE or AdA-PE[44,46,48]. The enzyme ACSL3 catalyzes the conversion of monounsaturated fatty acids (MUFA) into their acyl-CoA esters and facilitates their incorporation into membrane phospholipids, thereby conferring protection to cancer cells against iron metastasis[51]. The phosphorylation of beclin 1 by AMPK has been demonstrated to inhibit the production of reduced glutathione (GSH), thereby promoting iron metabolism[52], while the AMPK-mediated phosphorylation of ACAC restricts PUFA production and inhibits iron metabolism[53]. The findings of these studies expand our understanding of the functions of AMPK and unveil its involvement in ferroptosis in human cell lines derived from various tumor types. Additionally, research studies have indicated that specific membrane electron transport proteins, such as POR46 and NADPHases (NOXs), play a significant role in the generation of reactive oxygen species (ROS) during the process of ferroptosis and facilitate lipid peroxidation[54,55]. The involvement of the mammalian mitochondrial electron transport chain and tricarboxylic acid cycle, along with signals for glutaminolysis and lipid synthesis, has been observed in some instances of ferroptosis induction; however, the role of mitochondria in ferroptosis remains a subject of controversy[28]. Therefore,

a comprehensive investigation into the expression profiles of regulators involved in lipid peroxidation across various tumor types is imperative for informed patient selection when novel therapeutic approaches become available.

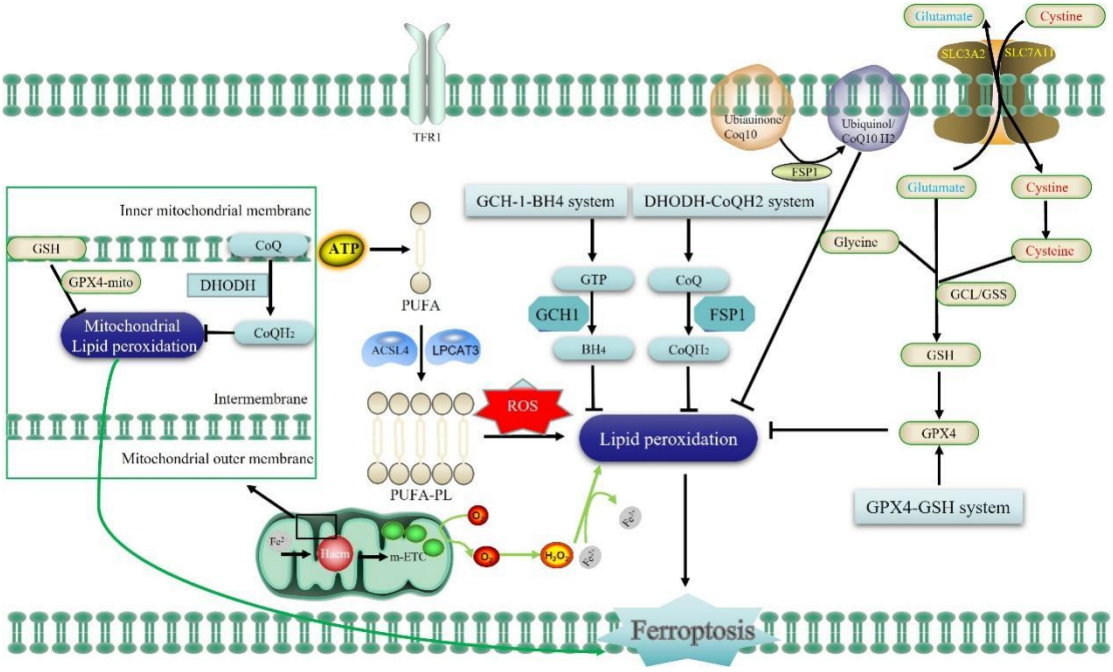

Figure 3. The synthesis of PUFA PLs, facilitated by ACSL4 and LPCAT3, is susceptible to peroxidation via both nonenzymatic and enzymatic mechanisms. The process of mitochondrial metabolism facilitates the production of reactive oxygen species (ROS), adenosine triphosphate (ATP), and/or polyunsaturated fatty acid-phospholipids (PUFA-PLs). The excessive accumulation of lipid peroxides on cellular membranes can trigger ferroptosis. Cells have developed at least four defense systems with distinct subcellular localizations to effectively detoxify lipid peroxides and thereby safeguard cells against ferroptosis.

3.3 Antioxidation mechanism

The antioxidant enzyme GPX4 catalyzes the reduction of phospholipid hydroperoxides (PLOOH) reduction using glutathione (GSH) as a substrate, directly converting hydrogen peroxide to hydroxyphospholipids[42]. This mechanism positions GPX4 as a pivotal regulator in preventing ferroptosis in cancer cells. The reduced expression of GPX4 in tumor cells significantly induces cell death characterized by the accumulation of lipid reactive oxygen species, thereby highlighting the indispensable role of GPX4 as a crucial enzyme for promoting tumor cell survival[56]. The inhibitors RSL3 and ML162, which target GPX4, can accumulate lipid peroxides and induce ferroptosis[23]. Furthermore, in tumor-resistant cells, using CRISPR to knockout GPX4 or inhibit RSL3 resulted in cell death characterized by ferroptosis, effectively reversing resistance[57].

The expression and activity of GPX4 showed a positive correlation with the levels of GSH and selenium. The glutathione system plays a pivotal role in maintaining redox homeostasis and comprises glutathione (GSH), glutathione reductase, glutathione peroxidase, and glutathione transferase[58]. The synthesis of GSH involves two steps, utilizing cysteine glutamate and glycine[59]. The free cysteine in the bloodstream is oxidized as cystine, wherein two thiol groups are bonded through a disulfide linkage to create a distinctive dipeptide molecule that exhibits enhanced stability compared to an individual amino acid. Under conditions of redox stress, mammalian cells employ a specialized system known as the system xc- a dedicated cystine-glutamate transporter, to enhance cystine uptake

and GSH synthesis[60]. This system is primarily expressed in astrocytes and macrophages within the brain under normal physiological circumstances[61,62]. However, the expression of the system xc- is upregulated in a wide range of cancers, and its overexpression has been linked to chemoresistance and unfavorable prognosis in patients with glioblastoma[63,64]. The development of targeted systemic xc- inhibitors has emerged a promising strategy to effectively suppress tumor growth[65]. The system xc- is classified as a member of the SoLute Carrier 7 (SLC7) family, which consists of secondary active amino acid transporters[66]. It functions as a heterodimer composed of a light chain, also known as xCT or SLC7A11, and a heavy chain called 4F2hc. The transmembrane glycoprotein 4F2hc, also known as CD98hc or SLC3A2, plays a crucial role in facilitating the proper translocation of the transporter to the plasma membrane[67,68].

The multifunctionality of 4F2hc, encompassing integrin signaling and cell proliferation, leaves the role of heavy chains in transporter function relatively understudied[69]. The SLC7 family regulates the transport of amino acids in vivo[70] can be categorized into two subgroups: cationic amino acid transporters (CATs, including SLC7A1-4 and SLC7A14) and L-type amino acid transporters (LATs, including SLC7A5-13 and SLC7A15), to which the system xc- belongs[71,72]. As is shown in Fig. 4, The expression and activity of SLC7A11 in cells are positively regulated by the nuclear transcription factor E2-related factor 2 (NRF2) and negatively regulated by key regulators such as TP53, BAP1, and BECN1[73-76]. In most tumor tissues, the upregulation of SLC7A11 inhibits ferro, promoting unlimited cell proliferation. Moreover, erastin functions as a selective system xc- inhibitor that triggers ferroptosis by impeding cysteine uptake, diminishing GSH synthesis, and facilitating the accumulation of PLOOH 8. When the inhibition of system xc- occurs, certain cells compensate for the cysteine deficiency through the transsulfuration pathway, which is regulated by a family of aminoacyl tRNA synthetases, including cyst tRNA synthetase 1[77]. The polymorphic sites (rs384490, rs729662, rs2071101, and rs739) within the cysteine tRNA1 gene have been identified as being associated with an increased susceptibility to gastric cancer[78].

Non-GPX4-independent antioxidant pathways also play a crucial role in regulating iron-induced cell death, such as the pathway associated with ferroptosis suppressor protein 1 (FSP1)[79]. The studies have demonstrated that FSP1 exhibits nicotinamide adenine dinucleotide-ubiquinone redox activity, enabling it to catalyze the reduction of ubiquinone to ubiquinol for inhibiting lipid peroxidation and restoring the antioxidant function of vitamin E, thereby effectively preventing ferroptosis by GPX4 inhibition or knockdown[80,81]. The findings of another study demonstrated that GTP cyclohyase 1 (GCH1) exerts a protective effect against ferroptosis through the action of its metabolites, tetrahydrobiopterin and dihydrobiopterin. The antioxidant effects of tetrahydrobiopterin on PLOOH may be attributed to two pathways: direct scavenging of oxidized free radicals and reduction of lipid peroxidation through ubiquinol regeneration[82]. Although the precise role of GCH1 in safeguarding tissues and organs against ferroptosis remains elusive, knockout studies have demonstrated that mice lacking the GCH1 gene exhibit bradycardia and experience embryonic demise during mid-gestation[83]. In addition, Mao et al found for the first time that di hydroorotate dehydrogenase (DHODH) located in the inner mitochondrial membrane repaired lipid oxidative damage in mitochondria by regenerating ubiquinol through metabolomics analysis, thus inhibiting the ferroptosis pathway [84].

In conclusion, the system xc-/GSH/GPX4 plays a pivotal role in the antioxidant pathway that inhibits ferroptosis. Both the GPX4-dependent pathway centered on GSH metabolism and the non-GPX4-dependent pathway based on ubiquinol regeneration exhibit effects against intracellular lipid peroxidation, thereby exerting a protective effect against ferroptotic cell death. The mechanism of the classical GPX4 pathway in ferroptosis has been elucidated; however, further investigation is required to fully understand the role of non-GPX4-dependent pathways, particularly those associated with ubiquitin regeneration-related proteins (FSP1, GCH1, and DHODH), which primarily rely on ubiquitin regeneration.

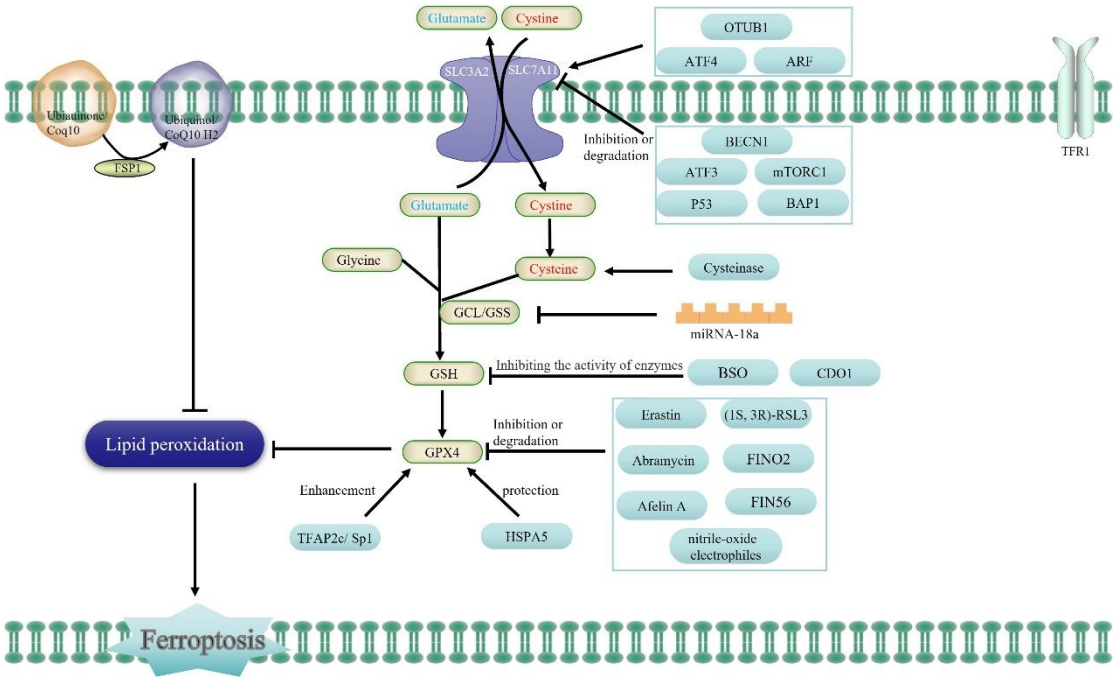

Figure 4. Antioxidant metabolism substances mediate the regulation of ferroptosis.

4. Ferroptosis and cancer

With the advancement of ferroptosis research, an increasing body of evidence suggests a close association between both malignant tumor development and drug resistance with iron death. Therefore, a comprehensive elucidation of the mechanism underlying iron death in malignant tumors may unveil novel potential targets for treating such malignancies.

4.1 The involvement of ferroptosis in carcinogenesis

The regulation of iron accumulation, lipid peroxidation, and antioxidant mechanisms prevents tumor cells from undergoing ferroptosis, leading to unrestricted proliferation of neoplastic cells and uncontrolled expansion of the tumor mass. The GPX4 enzyme is a crucial catalyst in lipid peroxides by GSH and plays a pivotal role in the survival of tumor cells. The knockdown of GPX4 in renal cancer cell lines impedes the elimination of lipid peroxides and triggers cell death characterized by an enrichment of lipid reactive oxygen species. This process can be reversed by DFO and the antioxidant vitamin E [34]. The GPX4-targeting compound RSL3 can induce cell death in colorectal cancer cells, exhibiting characteristics consistent with ferroptosis. Conversely, the overexpression of GPX4 has been shown to mitigate cell death [51,85]. Additionally, SLC7A11 functions as a subunit of the cystine-glutamate reverse transport system, known as System x-c, and exhibits significantly elevated expression levels in various tumor tissues, indicating its close association with malignant tumors [41, 52,86]. The downregulation of SLC7A11 expression in the HT-1080 tumor cell line enhanced Erastin-induced ferroptosis, whereas the upregulation gene conferred cellular protection[9]. In head and neck cancer, both pharmacologic inhibition and knockdown of SLC7A11 induced the development of ferroptosis[53]. Deubiquitinating enzyme BAP1 inhibits SLC7A11 gene expression by targeting the ubiquitination level of histone H2A in the SLC7A11 gene region, resulting in ferroptosis-like changes in tumor cells [41]. The oncogene p53 plays a crucial role in the survival of tumor cells by regulating multiple processes, including

apoptosis, autophagy, and the cell cycle. Additionally, there is evidence suggesting that p53 can also regulate ferroptosis in a bi-directional manner [54]. On the one hand, p53 induces iron death, mainly by inhibiting the expression of SLC7A11, which reduces cysteine uptake and thus GSH production, or by promoting the expression of spermidine/spermidine N1-acetyltransferase, which is ALOX15-dependent and contributes to the development of lipid peroxidation [55,56,87]. On the other hand, p53 exerts its influence by directly binding to dipeptidyl peptidase 4 (DPP4) and hindering its interaction with nicotinamide adenine dinucleotide phosphate oxidase 1 (NOX1). This inhibition effectively suppresses the production of reactive oxygen species (ROS) catalyzed by NOX1. Furthermore, p53 also induces the expression of cytokinin-dependent kinase inhibitory factor 1A (CKI1A), which enhances the de novo synthesis of intracellular glutathione (GSH). Moreover, p53 promotes the expression of glutaminase type 2 (GLS2), thereby elevating the intracellular levels of nicotinamide adenine dinucleotides (NAD+) and GSH. Consequently, these actions by p53 contribute to a decrease in the occurrence of ferroptosis [57-59]. The p53 KR mutation is characterized by its inability to undergo acetylation, resulting in impaired regulation of key cellular processes such as the cell cycle, senescence, and apoptosis. However, this mutation exhibits the capability to induce ferroptosis by binding to the promoter region of SLC7A11 and disrupting the normal transcription of the SLC7A11 gene [55]. In summary, inhibition of the ferroptosis pathway plays a vital role in promoting tumor cell survival and proliferation. GPX4 and SLC7A11 play critical roles in tumor growth, particularly in the context of tumor cells where their expression or activity is elevated. These proteins, when upregulated, can promote tumor proliferation by downregulating ferroptosis. In various biological settings, p53 exhibits bidirectional regulation of ferroptosis. However, the specific role of ferroptosis in different tumor types and stages of tumor development remains to be further elucidated.

4.2 Interactions between ferroptosis and tumor lipid metabolism

Ferroptosis is a recently discovered form of regulated cell death that is crucial in various biological processes. Emerging evidence suggests that ferroptosis is closely associated with tumor lipid metabolism, which is essential for cancer cell growth and survival [88]. To facilitate growth, cancer cells necessitate elevated levels of iron and lipid metabolism compared to normal cells, rendering them more susceptible to ferroptosis. In recent years, accumulating evidence has elucidated the intricate interplay between ferroptosis and lipid metabolism in the initiation, progression, invasion, metastasis, and development of therapy resistance in cancer [89,90] (Fig 5).

Our aim is to provide an overview of the current understanding of the interactions between ferroptosis and tumor lipid metabolism, highlighting their potential implications for cancer therapy. Tumor cells exhibit altered metabolic pathways compared to normal cells, contributing to their uncontrolled growth and proliferation. One of the key metabolic changes observed in tumor cells is the reprogramming of lipid metabolism. Recent studies have identified ferroptosis as a novel regulator of tumor lipid metabolism, suggesting that targeting these processes may offer new therapeutic opportunities for cancer treatment. The upregulation of genes involved in the synthesis of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), including ACLY, ACS, ACC, FASN, SCD1, and SREBP1, has been implicated in lipid metabolic alterations in cancer cells[91]. The statement can provide phospholipids for constructing cell membranes, thereby supplying lipid mediators essential for the proliferation and migration of cancer cells. The latest systematic review reveals that the genes and pathways implicated in dysregulated lipid metabolism in cancer encompass SCD1, FASN, and the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) signaling pathway[92]. The remodeling of the lipidome in cancer cells plays a crucial role in the initiation and execution of ferroptosis. Cancer cells exhibit a dysregulated mechanism that detects and regulates lipid composition, leading to an elevation in PUFA-PLs. This alteration facilitates cellular bending, endocytosis, and migration by inducing more flexible morphological changes[91].

Tumor cells exhibit distinct lipid metabolism patterns compared to normal cells. These alterations include

increased de novo lipogenesis, enhanced uptake and utilization of fatty acids, and altered membrane composition. For example, the expression of LPCAT3 and LPAAT3 promotes ferroptosis in cancer cells by increasing the proportion of PUFA-PLs in membrane lipids through preferential utilization of PUFA-CoA as a substrate[44]. Interestingly, the activation of SREBP1 in hepatocytes by LPCAT3-induced PL remodeling promotes the incorporation of PUFAs into the ER. Deficiency in LPCAT3 reduces the nuclear abundance of SREBP1, increases membrane saturation, and attenuates the lipogenic response to feeding, thereby contributing to resistance against ferroptosis[93]. Moreover, the oncogene stearoyl-CoA desaturase 1 (SCD1) encodes a fatty acid transporter protein primarily involved in regulating lipid metabolism and energy homeostasis[94]. The potential of SCD1 as a target for tumor therapy has garnered significant research attention, with evidence suggesting that inhibiting the expression or activity of SCD1 can effectively impede tumor cell proliferation, invasion, and metastasis. Elena Piccinin et al. demonstrated that targeting LXRs and SCD1 represents a promising novel approach for treating colorectal cancer[95]. Furthermore, SCD1 drives monounsaturation of fatty acids under normoxic conditions, but its activity is hindered by oxygen limitation[96]. Upregulation of SCD1 occurs in response to hypoxia during solid tumor growth, highlighting the importance of unsaturated lipids as essential nutrients for cancer survival under hypoxic conditions[97]. Oncogenic signaling pathways instigate the aforementioned alterations and contribute to neoplastic cell proliferation, viability, and resistance. Recent studies have revealed a complex interplay between ferroptosis and tumor lipid metabolism. On one hand, tumor lipid metabolism can influence ferroptosis by modulating the availability of lipid substrates for ROS production and iron sequestration[98].

For example, the mitochondrial elongation factor 2 (MIEF2) serves as a crucial regulator of mitochondrial division, and its elevated expression is indicative of an unfavorable prognosis in ovarian cancer patients[99]. The expression of sterol regulatory element-binding protein 1 (SREBP1) and its transcriptional target lipogenic genes, ACC1, FASN, and SCD1, is up-regulated by MIEF2 to promote fatty acid synthesis. Meanwhile, MIEF2 enhances cholesterol biosynthesis by upregulating the expression of sterol regulatory element binding protein 2 (SREBP2) and its transcriptional targets, HMGCS1 and HMGCR. The upregulation of SREBP1 and SREBP2 in OC cells involves an increase in mitochondrial reactive oxygen species (ROS) generation and subsequent activation of the AKT/mTOR signaling pathway mechanistically[100]. The afore-mentioned studies suggest that increased neolipogenesis contributes to an augmented supply of substrates for reactive oxygen species (ROS) generation, while alterations in membrane composition impact iron homeostasis and lipid peroxidation. On the other hand, ferroptosis can also impact tumor lipid metabolism by regulating the expression of key enzymes involved in lipid synthesis and degradation. Given the close relationship between ferroptosis and tumor lipid metabolism, targeting these processes may represent a promising strategy for cancer therapy.

Several compounds that can induce ferroptosis or modulate lipid metabolism have been identified, providing potential therapeutic options for cancer treatment. For example, inhibitors of de novo lipogenesis, such as etomoxir, have shown anti-cancer effects in preclinical studies[101]. Additionally, drugs that target iron homeostasis or lipid peroxidation, such as erastin and liproxstatin-1, have also demonstrated anti-tumor activity.[102] The interactions between ferroptosis and tumor lipid metabolism provide new insights into the complex biology of cancer cells and offer potential therapeutic opportunities. Further research is needed to fully understand the mechanisms underlying these interactions and to develop effective strategies for targeting ferroptosis and tumor lipid metabolism in cancer treatment.

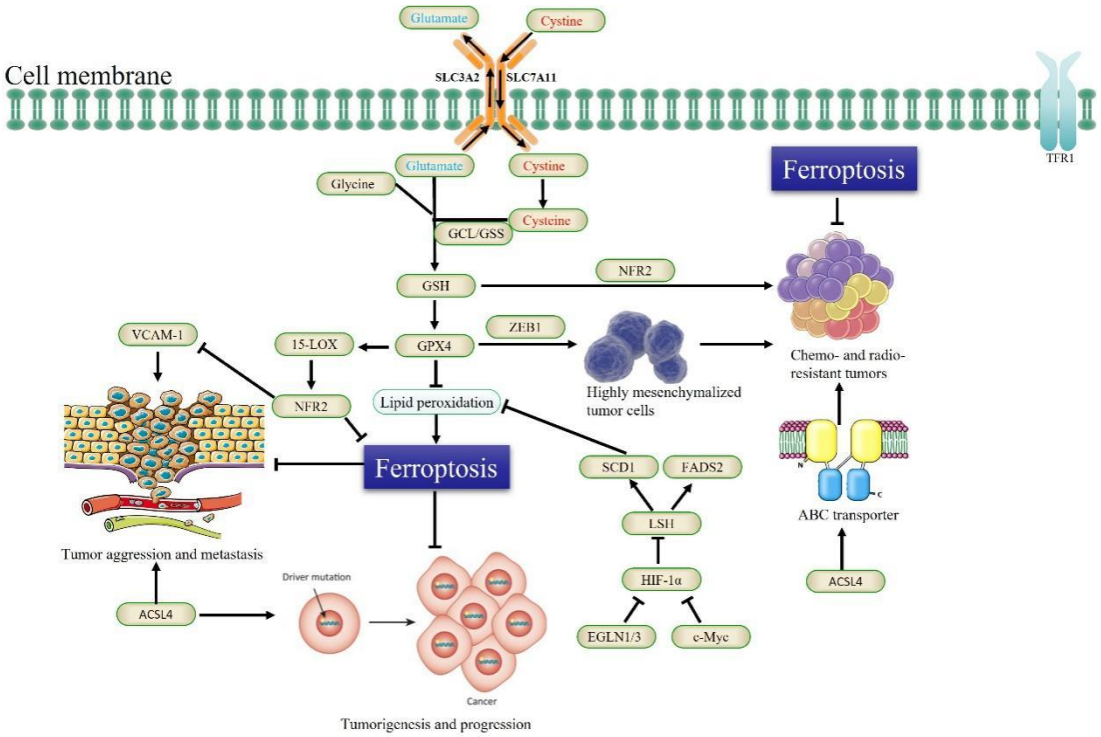

Figure 5. The interplay between ferroptosis and lipid metabolism in the context of tumor biology.

4.3 Ferroptosis and tumor drug resistance

Tumor drug resistance is a major challenge in cancer treatment, leading to poor patient outcomes and limited therapeutic options. The term "drug resistance" in the field of oncology refers to the inheritable capacity of cells to survive exposure to high concentrations of a pharmaceutical agent[103]. The concepts of "tolerance and persistence" describe cellular sensitivity and resistance towards drug interventions. The term "tolerance" refers to the capacity of cells to withstand temporary exposure to therapeutic drug concentrations, while "persistence" denotes the ability of a subset of cell clones to survive during treatment[104]. A wide range of intricate and multifaceted processes[105] characterizes the mechanism underlying tumor resistance. The genetic diversity and selective evolution of tumors typically classify them as genetic diseases, thereby attributing drug resistance primarily to genetic alterations[106]. The phenotype of a tumor can vary even within the same type, owing to the distinct genotypes present in its internal cells. This phenomenon is referred to as tumor heterogeneity, which represents the predominant factor contributing to tumor resistance[107]. The heterogeneity of tumors undergoes alterations during tumor treatment. This phenomenon indicates that the heterogeneity of the tumor has time and spatial specificity, and it also plays a key role in tumor drug resistance. The heterogeneity of tumors arises from genetic or epigenetic alterations in tumor cells or cells within the tumor microenvironment (TME), encompassing chromosomal instability, missense mutations in genes, and epigenetic modifications such as DNA methylation or histone changes that potentially contribute to the diversity observed among tumors (Fig. 6). For instance, in the context of chronic myeloid leukemia (CML), heterogeneity has been scientifically proven to be a contributing factor leading to the development of drug resistance[108]. The presence of BCR-ABL translocation is an established driving mutation in chronic myeloid leukemia. Subclones of leukemia cells harboring imatinib-resistant mutations within the BCRABL kinase domain can result in relapse among CML patients following treatment with imatinib[109]. The presence of tumor heterogeneity further contributes to developing resistance in lung cancer against epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs)[110]. The response of high-grade lung cancer to EGFR treatment is affected

by multiple subclone driven mutations in over 75% of non-small cell lung cancer (NSCLC) patients[111].

The overexpression of iron-generated proteins, such as divalent metal transporter-1 and transferrin receptor-1, is a common strategy employed by tumor cells to meet their increased iron requirements for rapid proliferation[112]. Furthermore, advanced cancer cells typically demonstrate heightened levels of oxidative stress and multiple genetic alterations, suggesting that these cancerous cells can be eliminated by either augmenting the production of ROS or inhibiting antioxidant defense mechanisms[113,114]. Recently, it has been postulated that modulation of crucial molecules involved in classical regulatory pathways of ferroptosis, including GSH, iron ions, ROS, GPx4, and LPO, may represent a potential strategy for overcoming tumor resistance[115-118].

Our objective is to investigate the correlation between ferroptosis and tumor drug resistance and explore the potential implications for cancer therapy. Emerging evidence indicates that ferroptosis may contribute to drug resistance in tumors. Several studies have demonstrated that cancer cells can develop resistance to various anticancer drugs by activating protective mechanisms against ferroptosis. The presence of iron is indispensable as a micronutrient, facilitating the optimal functioning of crucial enzymes[112]. The availability of free iron is crucial for electron transport, cellular respiration, cell proliferation and differentiation, and gene expression regulation in cancer cells. However, an excessive overload of labile iron pool poses a biochemical hazard due to its high capacity to induce ROS formation through the Fenton reaction, severely damaging key cellular biomolecules. The reported findings suggest that assessing free iron levels can serve as a criterion for determining the susceptibility of different cancer types to benefit from ferroptosis-promoting therapies[119]. The therapeutic potential of inducing ferroptosis may be more applicable to iron-rich tumors, such as PDAC , HCC[120,121], breast cancer [46], and NSCLC[122]. These tumors may exhibit increased sensitivity towards agents that promote ferroptosis. Additionally, subsequent studies have revealed that mutant RAS signaling enhances the cellular iron reservoir through transcriptional regulation of genes involved in iron metabolism[10]. Moreover, Duet et al. revealed that dihydroartemisinin, a ROS-donating drug, can potentially overcome cisplatin resistance in pancreatic ductal adenocarcinoma through the induction of ferroptosis[123]. The suppression of GPx4 occurs through androgen receptor ubiquitination induced by a curcumin analog, leading to ferroptosis and overcoming resistance to glioblastoma temozolomide[124]. The lipid metabolism of tumor cells is intricately linked to the susceptibility to ferroptosis. The ACSL4 enzyme, a crucial component in the process of ferroptosis, catalyzes the conversion of long-chain polyunsaturated fatty acids into acyl-CoA and subsequently participates in the enzymatic generation of LPO[46]. The inhibition of ADP ribosylation factor 6 (ARF6) activates ACSL4, thereby reversing gemcitabine resistance through the induction of ferroptosis[125]. The afore-mentioned findings present a promising opportunity for developing novel cancer treatments by overcoming drug resistance through the mechanism of ferroptosis. Ferroptosis represents a novel mechanism contributing to tumor drug resistance. Understanding the intricate relationship between ferroptosis and drug resistance could provide valuable insights into developing effective therapeutic strategies for cancer treatment. Further research is needed to elucidate the underlying mechanisms and identify potential therapeutic targets. Exploiting the ferroptosis pathway holds great promise for overcoming drug resistance and improving patient outcomes in cancer therapy.

4. Summary and outlook

The mechanism underlying iron-induced cell death in malignant tumors still poses numerous unanswered questions, such as the specific pathway by which peroxidation of polyunsaturated fatty acids triggers cellular demise. Which specific lipid types induce ferroptosis in diverse cancer subtypes, genetic backgrounds, and developmental stages. The immunogenicity, inflammatory response promotion, and mechanisms of ferroptosis: What is their correlation with the efficacy of immunotherapy in tumors?[126] The investigation of ferroptosis in the

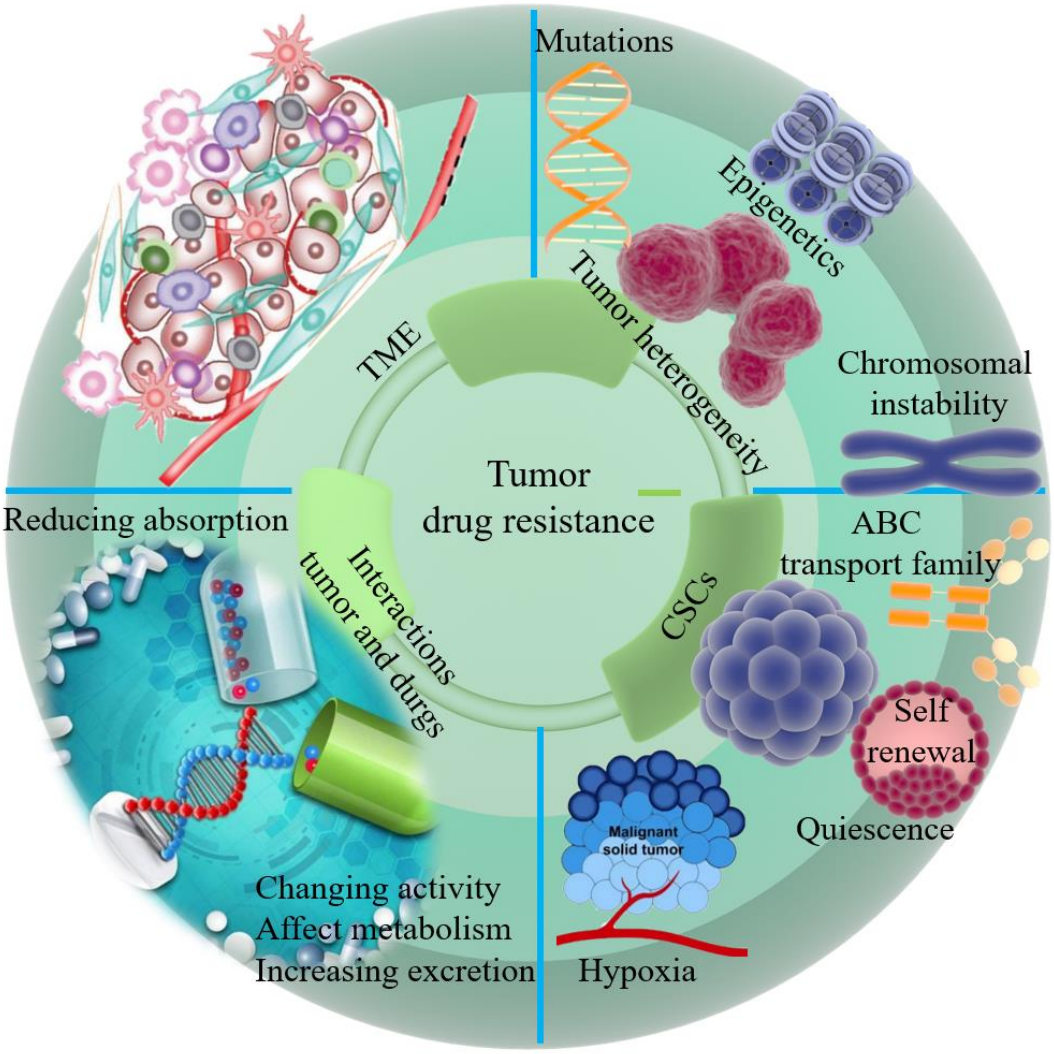

Figure 6. The Mechanisms underlying tumor drug resistance. Chromosomal instability, genetic mutations, and epigenetic alterations typically induce tumor heterogeneity. Cancer stem cells can evade drug treatments through upregulation of ABC expression, entering a dormant state via cell cycle arrest, enhancing DNA repair capacity, and establishing a hypoxic microenvironment Tumors can develop drug resistance through mechanisms such as decreased drug absorption, altered enzymatic activity, perturbed metabolism, and enhanced drug excretion. ABC, adenosine-triphosphate binding cassette; CSCs, cancer stem cells; TME, tumor microenvironment.

Context of tumors may, on the one hand, facilitate the discovery of additional mechanisms and molecules governing ferroptotic cell death, thereby contributing to a deeper understanding of this process; on the other hand, it has the potential to enhance our knowledge regarding tumor development and drug resistance mechanisms while offering a promising target for overcoming drug resistance or its reversal. Consequently, future research on ferroptosis in malignant tumors should focus on the following aspects. Investigate the regulatory mechanisms of ferroptosis in diverse malignant tumors with distinct genetic backgrounds and developmental stages to elucidate the pivotal molecules and pathways involved in ferroptotic cell death under various circumstances. The distinctive role of ferroptosis in tumor immunity, with a particular emphasis on the impact of ferroptosis on immunotherapy. The current research investigates the mechanisms underlying tolerance to chemotherapy, targeted therapy, and

radiation therapy, specifically focusing on ferroptosis. This includes the exploration of potential biomarkers and the development of targeted drugs. In the future, it is imperative to investigate the intricate mechanisms underlying ferroptosis further and employ targeted interventions in this pathway to overcome treatment resistance associated with ferroptosis, thereby advancing personalized tumor therapy and achieving precision treatment for cancer patients.

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Conflict of interest

The authors declare no competing interests.

Author contributions

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