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Ferroptosis and Cancer Therapy Review

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Abstract

Ferroptosis is a type of intracellular iron-dependent cell death that is different from autophagy, necrosis, and apoptosis. Ferroptosis is necessary for tumour suppression, according to a number of studies, which opens up new therapy options for cancer. The establishment of resistance to cancer therapy is one of the most significant ongoing challenges. The subject of conquering drug resistance has been the focus of numerous preclinical and clinical studies. Interestingly, ferroptosis has been associated with treatment resistance for cancer, and ferroptosis stimulation has been demonstrated to reverse drug resistance. The current knowledge of ferroptosis-inducing and ferroptosis defence mechanisms analyses the functions and mechanisms of ferroptosis in tumour immunity and tumour suppression, conceptualises the various ways that cancer cells are vulnerable to ferroptosis, and investigates therapeutic approaches for ferroptosis targeting in cancer. Cancer is one of the most terrible illnesses that can kill a person worldwide. There are several ways to treat cancer, including surgery, chemotherapy, and radiation. Analysis of the

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sensitivity of cancer cells to ferroptosis, which is impacted by their elevated reactive oxygen species levels and particular mutation profiles, opens up new possibilities for improving the efficacy of already used cancer treatments. This review included the state of knowledge about the induction and defence mechanisms of ferroptosis, the function and mechanisms of ferroptosis in tumor suppression, and treatment approaches for tumor-induced ferroptosis.

Keywords: Ferroptosis, Cancer, Therapy, Tumor, Iron-dependent

Introduction

Ferroptosis is a type of intracellular iron-dependent cell death that is distinct from necrosis, apoptosis, and autophagy. The term "ferroptosis" has only recently been applied to this nonapoptotic kind of cell death, despite reports of ferroptosis-like cell death in recent decades (Yagoda *et al*., 2007; Yang and Stockwell, 2008; Cheah, 2006). Dixon *et al.* (2012) have reported that the lethal mechanism activated by the oncogenic RAS-selective deadly small chemical erastin differs from commonly regulated cell death. Ferroptosis, another name for erythrin-induced cell death, is marked by an increase in intracellular reactive oxygen species (ROS) and an imbalance in the redox state. The disruption of redox state balance in biological systems has been linked to a number of diseases (Droge, 2002). Ferroptosis has been linked to the pathological cell death linked to ischemia illnesses, cancer, and degenerative diseases. It is considered a nexus between redox biology and cell physiological activities (Stockwell, 2017). According to Yang *et al.* (2014), ferroptosis is particularly prone to renal cell carcinoma, and the major regulator of ferroptosis is glutathione peroxidase 4 (GPX4). According to Brigelius-flohe and Maiorino (2013), GPX4 has the ability to catalyze the reduction of lipid peroxides, an essential function that guards against excessive lipid peroxidation. In a xenograft mouse model, tumor development was decreased by GPX4-regulated ferroptosis, while renal cell carcinoma cell lines were sufficiently destroyed by GPX4 knockdown. In 2015, Jiang *et al.* found that the majority of human cancers originate as a result of the p53 tumour suppression pathway becoming inactive, which is connected to ferroptosis suppression (Jiang *et al*., 2015). By blocking cystine uptake via the cystine/glutamate antiporter, p53 activation reduced intracellular glutathione (GSH) synthesis and shielded tumor cells from ferroptosis. Alvarez *et al.* (2017)

also demonstrated that lung cancer cannot thrive in a high oxygen environment without ferroptosis resistance (Ubellacker, 2020). The suppression of nitrogen fixation 1 (NFS1) by the iron-sulfur cluster biosynthesis enzyme was found to be essential for preserving iron-sulfur cofactors in the presence of oxygen and averting ferroptosis in cells. In order to avoid ferroptosis, melanoma cells, according to Ubellacker *et al.* (2020), prefer to spread more through the lymphatic system than the blood. Because the lymphatic environment contains lower quantities of free iron and higher levels of GSH and oleic acid, melanoma cells that form metastases there are better able to withstand ferroptosis and survive subsequent bloodstream metastases. According to these data, ferroptosis is essential for suppressing tumors and may be used as a therapeutic tool for cancer.

Cancer Generation

Since cancer cells are more susceptible to oxidative stress than non-cancerous cells, they are better able to adapt to it (Fiaschi and Chiarugi, 2012). Since ROS-generating enzymes such lipoxygenases, cyclooxygenases, and NADPH oxidases are more active in tumours and because mitochondrial function is changed, it is thought that tumours experience higher amounts of oxidative stress. Many of these enzymes are impacted by tumor-intrinsic factors, including decreased function of tumour suppressors like p53 and increased growth factor and oncogenic signalling like Ras signalling. Furthermore, redox stress is also induced in cancer cells by extrinsic stimuli like radiation and chemotherapy. Unchecked ROS, however, have the potential to harm macromolecular structures, such as proteins and membrane lipids, which may cause senescence or even cell death. Consequently, endogenous antioxidant networks are essential to cancer cells in order to preserve redox homeostasis (Aboelella *et al.* 2021; Reuter *et al.* 2010; Trachootham *et al.* 2009). According to Perillo *et al.* (2020), lipid ROS are produced by metabolic interactions between membrane-lipid polyunsaturated fatty acids and oxidant radicals. This causes oxidative lipid damage, which may ultimately cause ferroptosis, the death of cells. Ferroptosis is the word used to characterize a type of nonapoptotic cell death linked to lipid peroxidation reactions and the disruption of antioxidant and redox pathways. This type of cell death differs from other cell death processes like apoptosis, necroptosis, pyroptosis, and necrosis in terms of morphology, phenotype, and biochemistry (Dixon *et al.*, 2012; Cao and Dixon, 2016; Conrad *et al.* 2018; Gaschler *et al.* 2018). Additionally, labile active iron is needed. Ferroptosis is primarily caused by dysregulated metabolism, which is manifested by three main symptoms:

(i) reduced antioxidant machinery;

(ii) availability of redox-active iron; and

(iii) spread of hazardous lipid hydroperoxides (Dixon and Stockwell, 2019).

Mechanisms of Ferroptosis

Two important cues that initiate membrane oxidative damage during apoptosis are excessive iron ion buildup and lipid peroxidation (Li and Li, 2020). According to Sun *et al.* 2022, the primary molecular mechanism of ferroptosis entails the equilibrium between antioxidant defenses and oxidative damage. Modifying the process of ferroptosis or its essential genes can impact programmed cell death in a direct or indirect manner. Ever since Yang *et al.* (2014) revealed the GPX4-centered mechanisms of ferroptosis, a growing number of investigations have been carried out to find new mechanisms controlling ferroptosis. A pathway that is not dependent on GPX4 has also been found. Ferroptosis can be triggered by a potent theoretical framework provided by these findings. The process can be broadly classified into the following pathways: the iron metabolism pathway, the canonical GPX4-regulated pathway Treatment failure and resistance to chemotherapy are frequently caused by dysregulation of ferroptosis. It has been shown that ferroptosis can be genetically or pharmacologically regulated to overcome chemotherapy resistance (Fig. 1). The key pathways leading to the reversal of chemotherapy resistance include the lipid metabolism pathway, the iron metabolism pathway, and the classical GPX4-regulated pathway. These pathways are presumably responsible for ferroptosis. More people are beginning to recognise iron metabolism and lipid peroxidation signalling as the two main mediators of ferroptosis. Furthermore, the mitogen-activated protein kinase (MAPK) pathway is stimulated, which promotes ferroptotic cancer cell death.

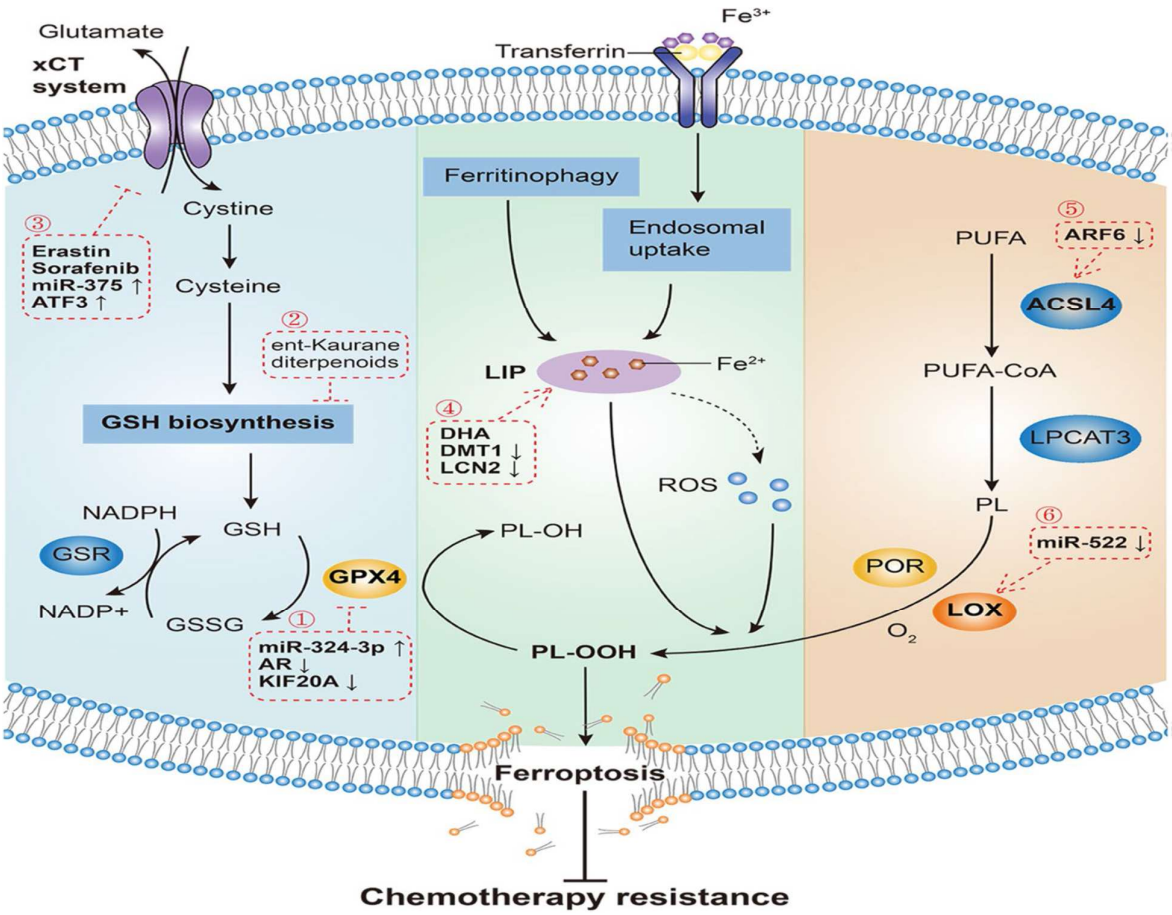

Figure 1: Overall mechanisms controlling the reversal of chemotherapeutic resistance and ferroptosis transfer of ferrin to the transferrin receptor and endocytosis of ferrin (Seiler, *et al.*, 2008)

System Xc-/GPX4 pathway

In mammalian cells, GPX4 is the principal enzyme that catalyses the reduction of phospholipid hydroperoxides (PLOOH). It is an essential regulator of cellular ferroptosis. GPX4 inhibits ferroptosis by maintaining membrane lipid bilayer equilibrium and reducing the toxicity of lipid peroxides. Additionally, according to Miao *et al.* (2022) it decreases harmful lipid peroxides (PL-OOH) to their equivalent alcohols (PL-OH) and transforms glutathione (GSH) to oxidised glutathione (GSSG). Iron mortality is caused by the buildup of intracellular peroxides when GPX4 is suppressed (Figure 1) (Wang *et al.*, 2022). By using siRNA knockdown of GPX4, Li *et al.* (2020) discovered that GPX4 is a central regulator of iron death. They also observed that a reduction in GPX4 expression significantly

accelerates the onset of cell death and the accumulation of ROS in the cells, which is consistent with the inhibition of the System Xc-system. GPX4 is a key hub gene on this pathway (Li *et al*., 2023). Pyrimidine ribonucleotide synthesis is catalysed by the mitochondrial endomembrane enzyme dihydroorotate dehydrogenase (DHODH) (Li *et al*., 2019). It has been found that DHODH, rather than cytoplasmic GPX4, controls mitochondrial ferroptosis in concert with GPX4. Additionally, DHODH can influence GPX4 by converting ubiquinone to ubiquinol, which can be utilized to inhibit tumors in tumors with low levels of GPX4 or in tumors with high levels of GPX4 effects in conjunction with iron oxidation inducers (Mao *et al*., 2021). Together, SLC7A11 and SLC3A2L, the two subunits of the cystine/glutamate reverse transporter (Chen *et al*., 2017), receive cystine, transform it into cysteine for the manufacture of glutathione, and release glutamate outside the cell. Through the activity of glutathione peroxidase, glutathione reduces reactive oxygen species, which in turn reduces glutathione synthesis and can cause oxidative damage to cells and even ferroptosis (Rochette *et al*., 2022). In glioblastoma, up-regulation of SL7A11 boosted anchorage-independent cell growth but had no effect on cell proliferation, according to Polewski's group (Polewski *et al*., 2016). SLC7A11 knockdown increased cell death in response to oxidative and genotoxic stress, and it also increased basal reactive oxygen species (ROS) and decreased glutathione synthesis (Yang *et al*., 2021). This discovery validates that disrupting the System Xc-system can result in a redox imbalance in glioblastoma and impede the viability of tumor cells. Furthermore, de Souza *et al*.'s experiments (De Souza, 2022) confirmed that inhibiting System Xc-in gliomas causes a rapid glutathione depletion, which impairs the tumor cells' ability to defend themselves against ROS, increases cell death, and triggers caspase-mediated apoptosis. These findings imply that inhibiting glioma cells' absorption of cystine uptake could be a practical means of eradicating tumor cells. In conclusion, the research that is currently accessible indicates that cancer cells can enhance the expression and functionality of the System Xc-/GPX4 system, which leads to the significant tumour progression, in order to better their own survival conditions.

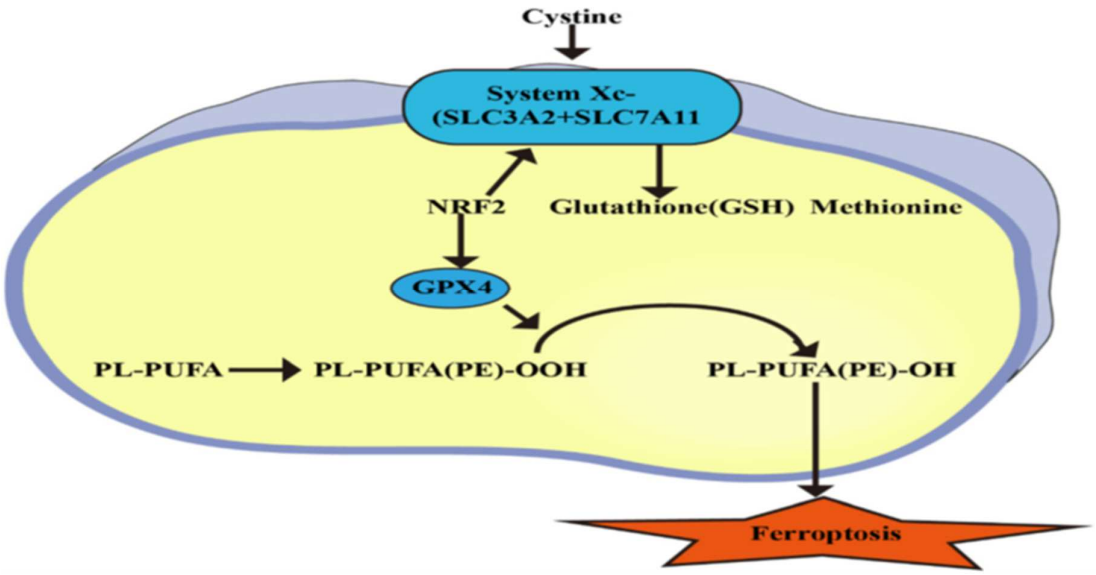

Figure 2: Pathway for System Xc- Signaling (Wang, *et al.*, 2022)

Iron metabolism pathway

A rise in the labile iron pool (LIP), a tiny Fe2+ pool, is one sign of ferroptosis, an iron-dependent form of cell death. It was discovered that serum transferrin's binding to the transferrin receptor and transferrin endocytosis caused the majority of cellular iron uptake (Richardson and Ponka, 1997). Ferroptosis results from the breakdown of ferritin by autophagy in fibroblasts and cancer cells, as demonstrated by Wen *et al.* (2016). By promoting ferritin degradation, or ferritinophagy, nuclear receptor coactivator 4 overexpression boosted the production of intracellular lipoproteins (Wen *et al.*, 2016). On the one hand, increased intracellular LIP may aid in the Fenton reaction, which releases free radicals (hydroxyl radicals) when phospholipids peroxidize to create PLOOH (Winterbourn, 1995). Nonetheless, the majority of ROS generation in cells is catalysed by iron. In the end, ROS generation causes ferroptosis and initiates lipid peroxidation (Dixon and Stockwell, 2014). It has been shown that, in comparison to normal cells, cancer cells require more iron to survive (Manz *et al.*, 2016). Ferroptosis induction may be a promising target for cancer therapy since it improves iron uptake and raises intracellular iron levels in rapidly growing cancer cells. According to a 2017 study by Alvarez et al., inhibiting NFS1 to boost intracellular LIP makes lung cancer cells more susceptible to ferroptosis and prevents lung tumour growth in vivo. Chang et al. (2015) also discovered that in cancer cells, the well-known IκBα inhibitor BAY 11-7085 increases LIP, activates heme

oxygenase-1, and improves ferroptosis. When coupled, the pathway of iron metabolism is altered to cause ferroptosis in cancer cells for therapeutic purposes.

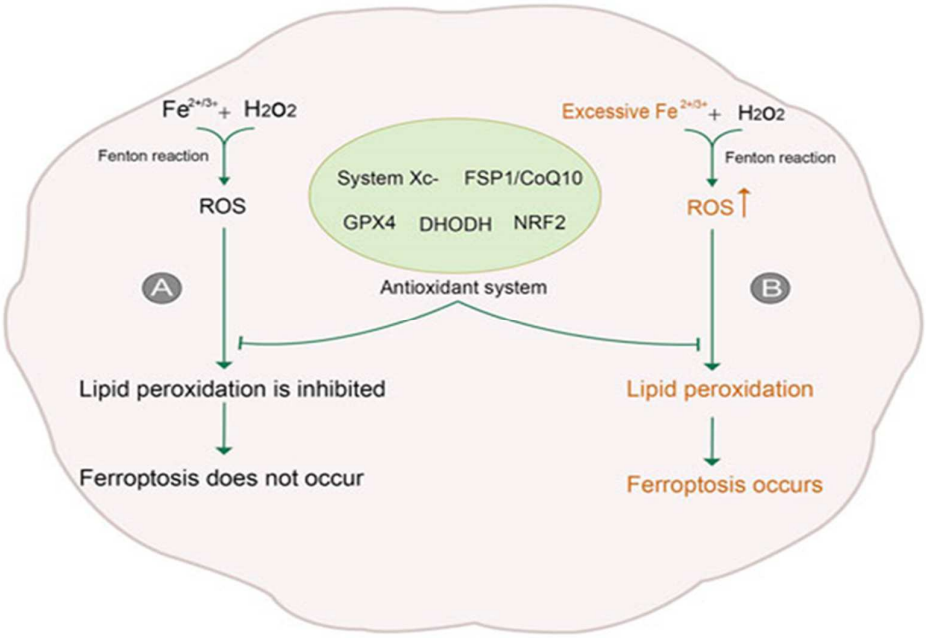

Figure 3: Fenton reaction of ferroptosis (Yan *et al.*, 2021)

Lipid peroxidation

Lipid peroxidation, primarily affecting polyunsaturated fatty acids (PUFAs) in cell membranes, is catalysed by free radicals. Polyunsaturated fats (PUFAs) are particularly susceptible to peroxidation, which can harm lipid bilayers and impede membrane function (Doll *et al.*, 2017; Kagan *et al.*, 2017; Wenzel *et al.*, 2017). Lipid hydroperoxides (LOOHs) and reactive aldehydes (such as 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA) are byproducts of lipid peroxidation that develop during ferroptosis. Ferroptosis is facilitated by the up-and-down linked roles that various lipoxygenases, particularly ALOXs, play in mediating lipid peroxidation to generate the hydroperoxides (Wenzel *et al.*, 2017; Yuan *et al.*, 2016). Numerous lipids found in cell membranes, such as cardiolipin, phosphatidylcholine, and phosphatidylethanolamine (PE), are susceptible to oxidation (Yuan *et al.*, 2016). Many membrane electron transport proteins, most notably NADPH oxidase (NOX), aid in the production of reactive oxygen species (ROS) for lipid peroxidation during ferroptosis. In other circumstances, mitochondria are involved in the development of ferroptosis through the breakdown of glutamine, the tricarboxylic acid

cycle, the electron transport chain, and lipid production. While ferroptosis causes considerable changes to the mitochondria, cardiolipin peroxidation has not been detected during this process. Moreover, there is disagreement over the role mitochondria play in ferroptosis (Kapralov *et al.*, 2020; Gao *et al.*, 2015; Gao *et al.*, 2019). Lipid peroxidation may occur and evolve differently in different types of cancer cells. via lipid peroxidation and iron buildup. This network has particular importance as an oncology-related regulatory route for RCD.

Ferroptosis and Cancer Therapy

A novel method to treating cancer is ferroptosis and cancer therapy. Despite advancements in this area, cancer remains the second greatest cause of death worldwide. Currently, the primary method of treatment is using anti-cancer medications to cause cancer cells to undergo apoptosis. However, the therapeutic efficacy is limited since cancer cells have an innate and acquired resistance to apoptosis. The primary issue still impeding cancer patients' chances of recovery is drug resistance. One of the greatest strategies to prevent drug resistance in cancer cells is to induce ferroptosis (Hassania *et al.*, 2019; Elgendy *et al.*, 2020). It can be accomplished by employing exogenous molecules or medications, controlling extracellular physiological conditions (such as a high concentration of extracellular glutamate), blocking system xCT to widely induce ferroptosis, or focusing on cytogenesis in accordance with the variations between cancer cells and normal cells. Currently, a number of small compounds and FDA-approved therapeutic medications trigger ferroptosis in cancer cells, and numerous experimental models demonstrate how effective these inducers of ferroptosis are at preventing cancer. Simultaneously, ferroptosis can be efficiently triggered in tests by a range of treatments, highlighting the potential as a novel anti-cancer therapy. (Jiang *et al.*, 2020; Elgendy *et al.*, 2020; Hassania *et al.*, 2019).

Negative Regulators of Ferroptosis

GPX4: This enzyme breaks down lipid hydroperoxides into their respective alcohols and free hydrogen peroxide into water. It also breaks down reduced GSH into oxidised glutathione (GSSG). Erasin or BSO therapy can increase lysophosphatidylcholines and NADPH oxidation, which are indicators of ROS generation from lipids, while decreasing GSH and GSSG. Yang and colleagues (2014) However, GSH and N-acetylcysteine, a precursor to GSH manufacture, stop erastin lethality in U2OS cells (Figure 4a) (Yang *et al.*, 2014). But even in the absence of GSH depletion, RSL3 is capable of producing ROS.

RLS3 directly targets GPX4. The overexpression of GPX4 results in resistance to RSL3, while the iron, MEK, and ROS-dependent ferroptosis caused by GPX4 knockdown. (Yang *et al.*, 2014) Additionally, erastin has the ability to degrade GPX4 in a variety of cancer cell types, indicating a potential role for the protein degradation pathway in ferroptosis. Yu *et al.,* (2015) Ferroptosis's in vivo function is verified through the use of GPX4 conditional or inducible knockout mice. Acute renal failure is caused by GPX4 deletion in the kidney, although it can be prevented using ferrostatin-1 and necrostatin-1 (a necroptosis inhibitor). Friedmann *et al.*, (2014) Necrostatin-1 may suppress ferroptosis in the kidney in an off-target manner. Friedmann *et al.*, (2014) The induction of ferroptosis in the mice results in a partial loss of neurons in the brain. (Chen *et al.,* 2015) On the other hand, anemia is induced by RIP3-dependent necroptosis in animals whose hematopoietic cells lack GPX4, but not apoptosis or ferroptosis. (2015) Canli *et al.* These findings suggest that the GPX4 has context-dependent roles in cell death.

System Xc: Importing cystine, which is subsequently reduced to cysteine and utilized to create the primary antioxidant GSH, is System Xc's job in maintaining redox equilibrium. Sulfasalazine-induced inhibition of system Xc can result in ferroptosis, while β-mercaptoethanol-induced increases in cystine uptake into cells prevent erastin-induced ferroptosis in HT1080 cells (Figure 4a). (Dixon *et al.*, 2012) SLC7A11 and SLC3A2 make up the structural components of System Xc. Iron and ROS had no effect on erastin's overexpression of SLC7A11. (Dixon *et al.*, 2012) The anticancer efficacy of erastin is enhanced when SLC7A11 expression is suppressed by RNA interference (RNAi), but ferroptosis caused by erastin is lessened when SLC7A11 is overexpressed through gene transfection. (Dixon *et al.*, 2012) On the other hand, ferroptosis mediated by RSL3 does not require system Xc. After receiving treatment with erastin, sulfasalazine, and sorafenib, ferroptosis has been linked to multiple ER stress markers, including phosphorylation of eIF2α and overexpression of the ATF4 protein.(Dixon *et al.*, 2014) By suppressing SLC7A11 expression, p53, as will be explained later, acts as a positive regulator of ferroptosis in some cancer cells, hence reducing system Xc activity. (Dixon *et al.*, 2014) HSPB1: In a number of human cancer cells treated with erastin, the transcriptional factor heat shock factor-1 (HSF-1) substantially increases HSPB1 expression. (Sun *et al.*, 2015) Overexpression of HSPB1 prevents erastin-induced ferroptosis, while inhibition of HSF-1-dependent HSPB1 expression raises it. (Sun *et al*, 2015) HSPB1's role in controlling actin

dynamics and iron absorption depends on its phosphorylation. After HeLa cells are treated with erastin, protein kinase C (PKC) increases HSPB1 phosphorylation. This promotes ferroptotic resistance by obstructing cytoskeleton-mediated iron uptake and the generation of reactive oxygen species (Figure 4b). (Sun *et al.*, 2015) NRF2: In HCC cells, NRF2 has a role in preventing ferroptosis. (Sun *et al.*, 2015) NRF2-binding site interaction between Kelch-like ECHassociated protein 1 (Keap1) and p62 is responsible for the competitive inhibition of Keap1-NRF2 association, which maintains NRF2 protein stability after therapy with FINs (erastin, sorafenib, and BSO).(Sun *et al.*, 2015).

The transcription of genes encoding iron metabolism proteins (such as FTH1) and antioxidant proteins (such as quinone oxidoreductase 1 and heme oxygenase-1 (HO-1)) is stimulated in ferroptosis by increased NRF2 protein. Knockdown of NRF2 and these NRF2-targeted genes accelerates ferroptosis in HCC cells driven by sorafenib or erastin. (Sun and colleagues, 2015) On the other hand, erastin's stimulation of HO-1 expression may lead to a rise in HT1080 and fibroblast cell death, indicating that HO-1 plays two roles in ferroptosis. Regardless of whether lymphocytic choriomeningitis virus infection was present or not, mice with conditional ablation of GPX4 in T cells using Cd4-Cre had fewer CD8+ T cells in the spleen and lymph nodes (Know *et al.*, 2015). (Matsushita and associates, 2015) GPX4 T cells do not die from other forms of RCD, but they quickly accumulate lipid peroxides and undergo ferroptosis. (Matsushita and associates, 2015) Inducible deletion of GPX4 localized to neurons.

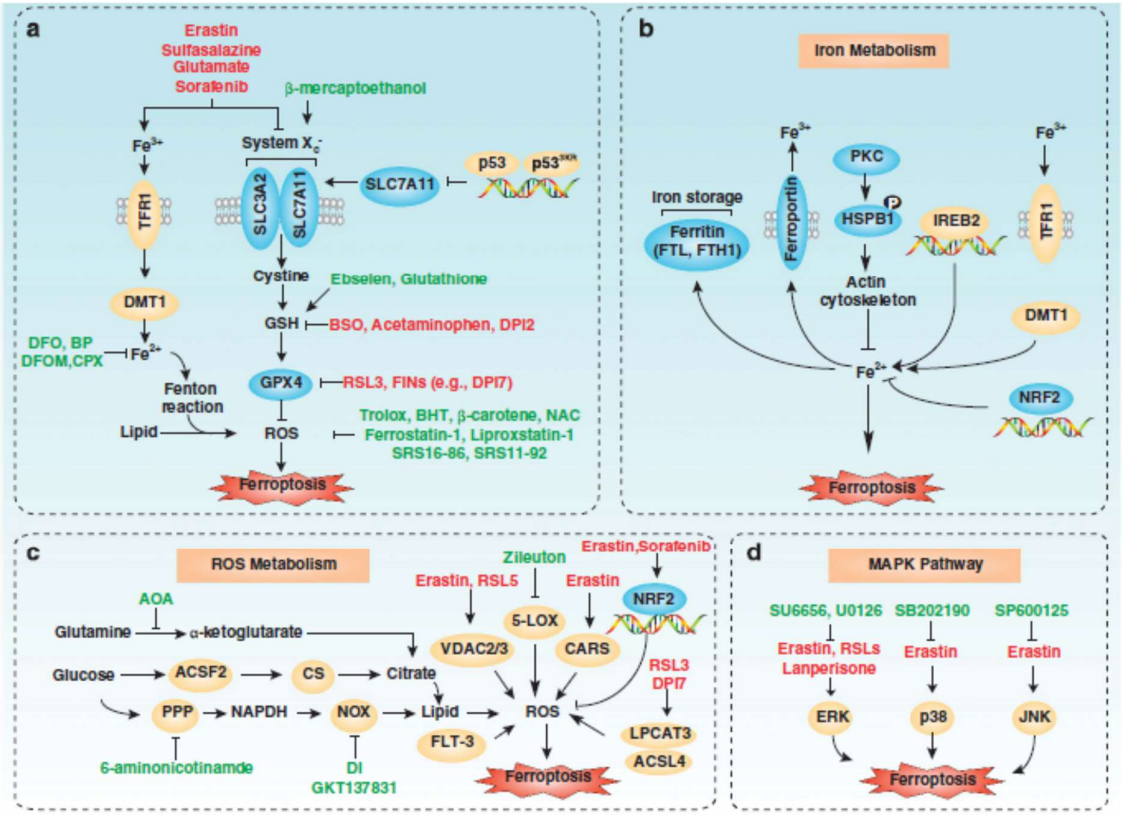

Figure. 4 Ferroptosis's molecular processes and signalling cascades. (a) Ferroptosis core regulators. (b–d) iron metabolism's roles (b), ROS metabolism (c), and the pathways of MAPK (d) in ferroptosis mechanism and functions of ferroptosis (Yang *et al.*, 2014).

Positive Regulators of Ferroptosis

VDAC2/3, but not VDAC1, reduces erastin-induced ferroptosis in Ras-mutant cells, implying that VDAC2/3 but not VDAC1 protects against ferroptosis (Figure 4c). Using affinity purification, erastin was found to directly target VDAC2/3. Yagoda and associates (2007) Higher levels of VDAC protein in cells translate into higher erastizin sensitivity. Yagoda and associates (2007) When isolated yeast mitochondria with a single mouse VDAC isoform 4 are exposed to erythrin, the rate of NADH oxidation decreases and NADH penetration into liposomes carrying human VDAC2 increases. Among others, Bauer (2011). Through its disruption of the VDAC-tubulin relationship, erythrin can increase aerobic glycolysis and decrease oxidative mitochondrial metabolism in human liver cancer cells (HepG2). This suggests ferroptosis regulation may include the cytoskeleton and energy metabolism (Maldonado *et al.*, 2013).

Ras: Ras-mutant cells, such as H-Ras-mutant designed cells, N-Ras-mutant HT1080 cells, and K-Ras-mutant Calu-1 cells, show gene-selective lethality in response to eryastasin. In 2007 Yamada *et al.* Ferroptosis induction, however, can occur in both Ras-dependent and -independent ways. Ras is required for the killing of pancreatic cancer cells, but not for the death of leukaemia cells when artesunate is used.Yu and colleagues (2015) Renal tubule cells, T cells, and fibroblasts are among the normal Ras wild-type cells that exhibit erasin sensitivity (Friedmann *et al.*, 2014; Skouta *et al.*, 2014; Masushita *et al.*, 2015; Jiang *et al.*, 2015; Linkermann *et al.*, 2014). Overexpressing mutant Ras in rhabdomyosarcoma cells (such as RMS13 cells) can even occasionally induce ferroptosis resistance to erastin and RLS3 (Schott *et al.*, 2015).

TFR1: The expression of TFR1 is elevated in ferroptosis-sensitive cells (like BJeLR cells) as compared to ferroptosis-resistant cells (like BJ cells).(Yang *et al.*, 2008) On the other hand, ferroptosis-sensitive cells (such as BJeLR cells) had downregulated expression of FTH1 and FTL, suggesting that iron storage influences ferroptosis as well.(Yang *et al.*, 2008) Ferroptosis caused by erastin in BJeLR cells is inhibited by TFR1 knockdown using shRNA, indicating that ferroptosis is prevented via suppression of iron uptake.(Yang *et al.*, 2008)Two crucial elements of complete foetal bovine serum are transferrin and glutamine, which cause fibroblasts to undergo ferroptosis in response to an amino acid shortage. (Gao *et al.,* 2015)

NOX: The NOX protein family converts oxygen into superoxide by moving electrons across cellular membranes. Calu-1 and HT1080 cells exhibit a partial reduction in erastin-induced ferroptosis in response to both the typical NOX inhibitor diphenyleneiodonium and the NOX1–/4-specific inhibitor GKT137831 (Figure 4c). The pentose phosphate pathway (PPP), a metabolic mechanism that proceeds in tandem with glycolysis, generates NADPH and pentoses. Pharmacological suppression of PPP by 6-aminonicotinamde (Figure 4c) or knockdown of two PPP enzymes (phosphoglycerate dehydrogenase and glucose-6-phosphate dehydrogenase) also partially prevents erastin-induced ferroptosis in Calu-1 cells.

p53: It has been discovered that ferroptosis in some cancer cells requires p53 activation. A crucial part of system Xc (Figure 4a), direct transcriptional repression of SLC7A11 is required for this process (explained later). Furthermore, SLC711A expression is inhibited

by p533KR (an acetylation-defective mutant) but not by other known p53 target genes (including p21 and BAX) that are implicated in pro-apoptotic and antiproliferative activities (Figure 4a). Even in the absence of cell-cycle arrest, apoptosis, or senescence, p533KR animals are nevertheless able to limit tumour growth.31. To carry out its function as a tumour suppressor, p533KR has to induce ferroptosis. Ferroptosis is another mechanism by which hyperactive p53 signalling is mediated, and it enhances embryonic lethality. (Jiang *et al.*, 2015) Murine double minute-2 (MDM2), an E3 ubiquitin ligase, controls the proteasomal breakdown of p53. According to Thomasova *et al.* (2015), ferrostatin-1 by itself is unable to stop MDM2 from being knocked down by RNAi-induced cell death, suggesting that mixed cell death types are in charge of p53-induced death.

CARS: In the event of cystine deficiency, ferroptosis is positively regulated by cysteinyl-tRNA synthetase (CARS). While CARS overexpression increases erastin sensitivity in a variety of cancer cell types, CARS knockdown prevents erastin-induced ferroptosis (Figure 4c). Yet, deletion of CARS is unable to stop the death of cells caused by FIN56, RSL3, or BSO, indicating that CARS controls ferroptosis at the cysteine biosynthesis level (Hayano *et al.* 2015)

AMPK signalling pathway

cellular It has been found that AMPK is triggered when cells are starved of glucose. This initiates a vigorous stress-repair process that neutralises reactive oxygen species generated by malnourished cells as a consequence of metabolic stress, shielding the cells from ferroptosis and averting renal ischemia reperfusion injury (Wang *et al.*, 2022). PUFAs required for lipid peroxidation are also suppressed during this time, along with lipid anabolism, and ferroptosis is related with both processes (Figure 5) (Ma *et al.*, 2022). during the same time, the mitochondria are primarily synthesising and catabolizing ATP. According to Lee *et al.* (2020), the AMPK pathway has been shown to play a partial tumour suppressor role in cell death experiments involving the induction of reactive oxygen species (ROS) and/or energy depletion through glucose starvation. However, the researchers also discovered that by lowering the ROS production, the AMPK pathway can be activated to shield cells from ferroptosis. Their research and several validations, however, have also demonstrated that AMPK inhibition suppresses ferroptosis and that, in some situations, AMPK's pro-tumorigenic effect is mediated by its capacity to inhibit ferroptosis. A highly

relevant putative regulator of colorectal cancer, TIGAR (Liu *et al.*, 2022) has been studied. It was discovered that TIGAR promotes tumour cells by inducing ferroptosis resistance in colorectal cancer cells via the AMPK signalling pathway, which in turn maintains the viability of tumour cells. These two results demonstrate that ferroptosis resistance in tumour cells is maintained by activation of the AMPK signalling system and that ferroptosis cannot be induced in cells by blocking this route.

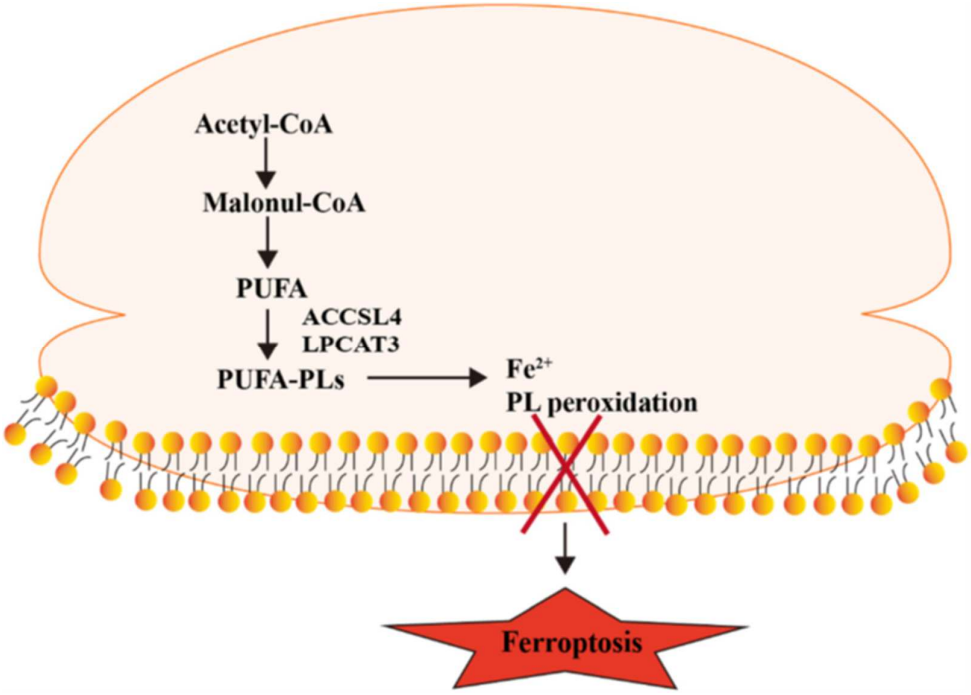

Figure 5: Signaling pathway of MAPK. (Lee *et al.*, 2020)

Ferroptosis And Cancer Genes

Since erastin (eradicator of RAS and ST) and RSL3 (RAS-selective Lethal 3) are the two most well-known inducers of ferroptosis and are, in theory, oncogenic RAS-selective lethal small molecules, ferroptosis and the RAS family of small GTPase, which includes HRAS, NRAS, and KRAS, are closely related (Yagoda et al., 2007; Yang and Stockwell, 2008). The connection between ferroptosis and RAS has been thoroughly examined in numerous investigations. According to Yamada et al. (2007), ferroptosis is highly sensitive in cancer cells expressing HRASV12, but erastin sensitivity is significantly decreased when KRAS is suppressed in KRAS mutant Calu-1 cells. A plausible reason could be that constitutive activation of the RAS pathway results in the downregulation of iron storage protein

expression and the upregulation of TFRC, a gene related to iron metabolism. However, in some cancer cell lines, more proof of the connection between erastin sensitivity and RAS mutation is not seen (Yang *et al*., 2014). However, overexpressing HRAS, KRAS, or NRAS in RMS13 rhabdomyosarcoma cells results in resistance to erastin and RAL3 (Schott *et al*., 2015). This suggests that RAS influences erastin sensitivity, but that ferroptosis sensitivity is not solely determined by the oncogenic RAS pathway (Dixon and Stockwell, 2019). Moreover, a number of investigations have demonstrated that HMGB1, or high mobility group box 1, is a crucial regulator of the ferroptosis that erastin produces (Ye et al., 2019). High susceptibility to RSL3-induced lipid peroxidation is mediated by ADP Ribosylation Factor 6 (ARF6), a member of the RAS superfamily (Ye et al., 2020). Oncogenic RAS's overexpression of NOX1 is thought to cause ROS to rise swiftly (Irani et al., 1997; Mitsushita et al., 2004). High iron diets and GPX4 deletion, which results in 8-OHG release, trigger macrophage infiltration and activation in mice with KRAS-driven pancreatic ductal adenocarcinoma (PDAC) (Dai et al., 2020).

Mitochondria

Evidence of altered mitochondrial shape in ferroptotic cancer cells—including decreased size, expanded cristae, and higher membrane density—provided the first clue that mitochondria were involved in ferroptosis (Dixon *et al*., 2020; Doll *et al*., 2017; Wang *et al*., 2020). However, ferroptosis was successfully triggered by erythrin therapy in cancer cells with reduced mitochondrial DNA that were unable to breathe (Dixon *et al*., 2012). Furthermore, ferroptotic inhibitory effect of ferrostatin-1 does not require mitochondria (Gascher *et al*., 2018). However, in conditions of either full amino acid deprivation or cysteine deprivation, glutaminolysis accelerated serum dependent oxidative cell damage and ferroptosis, which has been connected in studies to mitochondrial glutamine metabolism and ferroptosis (Gao *et al*., 2015). (Gao *et al*., 2019) looked into the function of mitochondria in a study that linked the mitochondria to cysteine deprivation-induced ferroptosis. Cysteine deficiency was alleviated by concentrating on the mitochondria, however GPX4 inhibition did not result in ferroptosis (Gao *et al*., 2019). It has been found that the electron transport chain (ETC), fumarate, succinate, malate, and other metabolite intermediates of the mitochondrial tricarboxylic acid cycle (TCA) pathway may change a cell's vulnerability to cysteine-induced ferroptosis. reduced susceptibility to Erastin treatment or cysteine deficiency because of TCA cycle or ETC dysfunction. It's important to note that in renal cancer, the inactivation of the tumour suppressor gene fumarate

hydratase (FH) increases the resistance to ferroptosis. This was suggested as the basic mechanism for its tumor-suppressive action, which states that a mutation in FH that causes lack of function is what causes the benefits of tumour growth. Another study that used a genome-wide CRISPR screen in combination with small-molecule mitochondrial inhibitors found a synthetic lethal link between GPX4 deletion and mitochondrial failure. The loss of GPX4 enhanced the toxic effects of mitochondrial inhibitors and made cancer cells more vulnerable to ferroptosis. In fact, the phenotype of ferroptosis and the synthetic lethal interaction were reversed in the GPX4 KO cells when mitochondrial-specific GPX4 was overexpressed. Furthermore, it has been demonstrated that both in vitro and in vivo mitochondrial dysfunction can promote the expression of GPX4. According to this study (To *et al*., 2019), mitochondria may play a significant role in the regulation of ferroptosis. Similarly, the susceptibility of cancer cells to ferroptosis has been shown to be regulated by the inner mitochondria membrane-localized enzyme dihydroorotate dehydrogenase (DHODH); deletion or inhibition of the enzyme enhanced or sensitised the cells to ferroptosis. It's interesting to note that strong mitochondrial lipid peroxidation was produced by both DHODH deletion and GPX4 silencing, but not by either one alone. This peroxidation may be prevented by antioxidants that specifically trap radicals in the mitochondria (Mao *et al*., 2021). It is important to keep in mind that the models used in the various research to eliminate mitochondria vary, which may help to explain some of the contradictory results about the function of mitochondria in ferroptosis. These included mitochondria DNA deletion (Dixon *et al*., 2012) versus eradication of the entire organelle (Gao *et al*., 2019) as opposed to ETC suppression by tiny molecules. All things considered, more research will be needed to fully understand the context-dependent functions of the mitochondria and their metabolism.

ACSL4 and LPCAT3 Axis

The genes for acyl-coenzyme lipid metabolism Two of the earliest genes discovered to be involved in the control of ferroptosis mediated by GPX4-inhibition are synthetase long-chain family member 4 (ACSL4) and lysophosphatidylcholine acyltransferase 3 (LPCAT3). The expression of the proferroptotic genes ACSL4 and LPCAT3, which encode proteins that alter the lipid architecture of the cellular membrane, was demonstrated to be necessary for ferroptosis. The acylation of long-chain fatty acids is an essential step in the synthesis of

long-chain polyunsaturated fatty acid-CoA (PUFA-CoA), which includes arachidonic acid-CoA (AA-CoA) and adrenic acid-CoA (AdA-CoA). The protein's function is what ACSL4 encodes. But these and other acylated AA are incorporated into membrane phospholipids by the LPCAT3-encoded protein (Kagan *et al.*, 2017; Doll *et al.*, 2017; Yuan *et al.*, 2016; Dixon *et al.*, 2017). There is a substantial correlation between the expression of ACSL4 and the sensitivity of cancer cells to ferroptosis; ferroptosis sensitivity is decreased by deletion of ACSL4, but it is sensitised by overexpression of ACSL4. As a result, downregulated ACSL4 transcript levels were found in ferroptosis-resistant cancer cell lines (Yuan *et al.*, 2016). The loss of LPCAT3 and ACSL4 decreased the amount of substrate available for oxidative lipid breakdown, which prevented PUFA from integrating into the membrane bilayer (Dixon *et al.*, 2017). Yuan *et al.* (2016) reported that the relationship between GPX4 and ACSL4 was demonstrated by the remarkable preservation of vitality in cells containing both enzymes. Thus, the membrane remodelling enzymes are significant regulatory nodes of ferroptosis susceptibility.

Ferroptosis Resistance

Olaparib, the well-known poly (ADP-ribose) polymerase (PARP) inhibitor olaparib has been licenced by the US Food and Drug Administration for use as maintenance and therapy in patients with advanced ovarian cancer who have genetic BRCA1/2 mutations. Olaparib has no advantage for people who do not have competent BRCA or inherited BRCA mutations. A catalytic subunit of the xCT system, solute carrier family 7 member 11 (SLC7A11) regulates the cellular defence mechanism against ferroptosis and stimulates the synthesis of GSH and cysteine supply. It has been documented that via inhibiting SLC7A11-mediated GSH production, PARP inhibition might encourage ferroptosis. Ovarian cancer cells and xenografts with BRCA-positive status are synergistically sensitized to the PARP inhibitor olaparib through enhanced ferroptosis caused by FINs (Hong *et al.*, 2020). According to this study, FINs plus PARP inhibitors may be a potential treatment option for ovarian cancer with BRCA-positive status. The therapeutic effectiveness of cetuximab and other anti-epidermal growth factor receptor (EGFR) antibodies is markedly reduced by RAS mutations that impact cetuximab. Approximately 50% of cases of metastatic colorectal cancer have these mutations. β-elemene, a naturally occurring chemical isolated from the Chinese plant Curcumae Rhizoma, is proposed as a novel inducer of ferroptosis. KRAS mutant metastatic colorectal cancer cells are susceptible to cetuximab and β-elemene combination treatment because it induces ferroptosis and

inhibits epithelial-mesenchymal transition. According to Chen *et al.* (2020), this may be a therapeutic option for patients with metastatic colorectal cancer who have RAS mutations. Gefitinib Clinical trials have demonstrated the beneficial effects of gefitinib on a range of tumour types; nevertheless, triple-negative breast cancer cells are resistant to the medication's clinical doses. Gefitinib sensitivity was increased by GPX4 inhibition through the promotion of cell ferroptosis. Following GPX4 silencing, malondialdehyde and ROS generation rose while GSH levels fell (Song *et al.*, 2020). Human triple-negative breast cancer cells are resistant to gefitinib, and GPX4 is an important regulator and possible target for treatment.

Tumour-cell-extrinsic mechanisms

Tumor-associated macrophages (TAMs) that contains two immune suppressor cells that also contribute to immunotherapy resistance: regulatory T cells (Tregs) and tumor-associated macrophages (TAMs) (Sharma et al., 2017). A link was observed by Quezada et al. (2006) between the ratio of effector T cells to Tregs in the TME and the effectiveness of immunotherapy against anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4). T cells that are invading effectors are under the inhibitory control of tregs. According to this study, the combination with the tumour cell vaccine transduced with GM-CSF increases the ratio of effector T cells to Tregs, hence potentiating the effectiveness of CTLA-4 inhibition. Oweida *et al.* (2018), also showed in another study that Treg infiltration was connected to resistance to anti-PD-L1 immunotherapy. It has been demonstrated that antitumor immunity can be restored by addressing Treg depletion. Notably, GPX4 shields Tregs from ferroptosis, according to a recent study. Tregs lacking in GPX4 generate interleukin-1β and aid in the T helper 17 response, hence augmenting antitumor immunity (Xu *et al.*, 2021). When combined, these findings suggest that immunotherapy resistance may be reversed by causing Tregs to undergo ferroptosis through GPX4 suppression. TAMs are a subpopulation of cells that also appear to influence immune treatment responses. They can polarise into two main phenotypes: protumor M2 (TAM2) and antitumor M1 (TAM1). In TME, TAM2 is frequently the most prevalent subset of TAMs. Immune checkpoint inhibitors were shown to be more effective against pancreatic cancer when TAMs were reprogrammed through inhibiting CSF1/CSF1R (Zhu *et al.*, 2014). TAMs and Tregs are immune suppressor cells found in the TME that contribute to immunotherapy resistance

(Sharma et al., 2017). Quezada et al. (2006) found that the ratio of effector T cells to Tregs in the TME correlated with the efficacy of anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) immunotherapy. T cells that are invading effectors are under the inhibitory control of tregs. According to this study, the combination with the tumour cell vaccine transduced with GM-CSF increases the ratio of effector T cells to Tregs, hence potentiating the effectiveness of CTLA-4 inhibition. Oweida *et al.* also showed in another study that Treg infiltration was connected to resistance to anti-PD-L1 immunotherapy. It has been demonstrated that antitumor immunity can be restored by addressing Treg depletion (Oweida *et al.*, 2918). Notably, GPX4 shields Tregs from ferroptosis, according to a recent study. Tregs lacking in GPX4 generate interleukin-1β and aid in the T helper 17 response, hence augmenting antitumor immunity (Xu *et al.*, 2021). When combined, these findings suggest that immunotherapy resistance may be reversed by causing Tregs to undergo ferroptosis through GPX4 suppression. TAMs are a subpopulation of cells that also appear to influence immune treatment responses. They can polarise into two main phenotypes: protumor M2 (TAM2) and antitumor M1 (TAM1). In TME, TAM2 is frequently the most prevalent subset of TAMs. Immune checkpoint inhibitors were shown to be more effective against pancreatic cancer when TAMs were reprogrammed through inhibiting CSF1/CSF1R (Zhu *et al.*, 2014). A recent study found that TAM1 exhibited greater resistance to ferroptosis than TAM2, owing to its ability to increase levels of inducible nitric oxide synthase (iNOS)/NO•. Potentiating antitumor immunity in the TME, regulation of ferroptosis by iNOS/NO• decreased TAM2 survival without impacting TAM1 (Kapralov *et al.*, 2020). Furthermore, in a preclinical mouse model and in patients, Jiang *et al.* demonstrated a correlation between high TYRO3 expression and anti-PD-1/PD-L1 immunotherapy resistance. TYRO3 promoted the polarisation of TAM1 to TAM2 and prevented ferroptosis in tumour cells. Jiang et al. (2009) report that blocking TYRO3 also resulted in ferroptosis and altered TAMs, increasing the immunotherapy susceptibility of cancer cells. Inducing ferroptosis in the TME may be a cancer cell-extrinsic method to overcome immunotherapy resistance by decreasing immune suppressor cells.

Tumour-cell-intrinsic mechanisms

According to Sharma *et al.* (2017), there are a number of intrinsic mechanisms found in tumour cells that contribute to immunotherapy resistance. These processes include the following: loss of PTEN expression, expression of the WNT/β-catenin signalling pathway, loss of the interferon-gamma signalling pathway, and loss of tumour antigen expression.

Activation of the mitogen-activated protein kinase (MAPK) signalling pathway. These changes lead to the incapacity to mount potent antitumor immune responses. Recent findings suggest that the immunologically cold state could become responsive to checkpoint inhibition by causing immunogenic cell death, which would trigger the adaptive immune system (Green *et al.*, 2009; Demaria *et al.*, 2016). It's interesting to note that immunogenicity has been shown for ferroptosis. In preclinical models, Efimova *et al.* reported a unique method of inducing ferroptosis-dependent immunogenic cell death to activate the adaptive immune system. According to reports, early ferroptotic cells stimulate the phenotypic maturation of bone marrow-derived dendritic cells and emit damage-associated molecular patterns (adenosine triphosphate and high-mobility group box 1) (Efimoval *et al.*, 2020; Tang *et al.*, 2020). Additionally, an eat-me signal, 1-steaoryl-2-15-HpETE-sn-glycero-3-phosphatidylethanolamine (SAPE-OOH), was seen on the surface of ferroptotic cancer cells. Additionally, SAPE-OOH enrichment facilitated phagocytosis by targeting the toll-like receptor 2 on macrophages (Luo *et al.*, 2021). When coupled, inducing ferroptosis in cancerous cells can function as a vaccination to overcome immunotherapy resistance and activate antitumor immunity.

Protumour roles for ferroptosis

In contrast to earlier research, ferroptosis has also been demonstrated to promote tumor growth in specific circumstances (Fig. 6). For instance, it has been observed that in glioblastoma multiforme (GBM) in vivo, tumor-associated neutrophils can cause lipid peroxidation and iron-dependent tumor cell death. Although this resulted in more tumors being destroyed, the disease also became more aggressive. Myeloperoxidase granules were transferred intercellularly to the tumor cells by neutrophils, resulting in the ferroptotic death of the tumor cells. In turn, ferroptosis was decreased and the aggressiveness of the disease was reduced when GPX4 overexpression or ACSL4 silencing was applied to the tumors (Yee *et al.*, 2020). Other immune cells in the TEM have been identified to play similar roles. For instance, loss of Gpx4 in myeloid cells increased ROS generation and enhanced carcinogen-induced intestinal tumour invasion in a mouse investigation of the evolution of intestinal tumours caused by carcinogens. Increased macrophage recruitment to the tumor was seen by the authors (Canli *et al.*, 2017), which resulted in the production of higher amounts of peroxide in the TME. As a result, the intestinal cells experienced widespread DNA mutation events that aided in the growth of the tumor (Canli *et al.*, 2017). These results demonstrate how immune cell ferroptosis can act to encourage the

development of tumors and the advancement of illness. Examining the cancerous cells in a pancreatic tumorigenesis model, we discovered that ferroptosis stimulation stimulates the development and spread of tumors.

The KrasG12D oncogene is expressed in a pancreas-specific manner in an autochthonous model of pancreatic cancer initiation used by the authors (Dia *et al.*, 2020). The administration of a high-iron diet in conjunction with Gpx4 deletion accelerated the development of pancreatic tumors, as seen by enhanced tumor invasion and metastasis, a lower survival rate, and an amplified stromal response. Mechanistically, it was discovered that high-iron diets and Gpx4 deletion both encouraged the release of 8-hyroxyguanosine (8-OHG), an oxidized nucleoside base that later triggered the STING pathway. According to Corrales *et al.* (2016), the STING pathway is an innate immunological inflammatory response that detects cytosolic DNA and triggers a type I interferon-mediated inflammatory response. In the carcinogenesis model of pancreatic cancer driven by Kras, the STING response enhanced macrophage infiltration and polarization into a protumor phenotype. By limiting macrophages or blocking the STING pathway, liproxstatin-1, a ferroptosis inhibitor, was able to reverse the protumorigenic phenotypes and lower survival (Fig. 6). In a different investigation, ferroptosis was discovered to increase autophagy-dependent exosomal release of KRASG12D protein from pancreatic cancer cells utilizing a pancreatic cancer disease model (Dia *et al.*, 2020). According to Dai *et al.* (2020), extracellular $KRASG^{12D}$ was bound by and taken up by AGER/RAGE receptors on macrophages, which caused tumorigenic polarization of macrophages and encouraged the formation of pancreatic tumors. All things considered, these new findings about ferroptosis's function in the TME point to a dual effect that depends on the type of cancer, the cell type, the model, and the disease state, with ferroptosis either boosting or suppressing tumor growth. Therefore, more thorough and methodical research on ferroptosis that takes into account the whole range of the TME landscape is required in order to translate ferroptosis-regulating drugs as cancer therapeutics. This will help identify the circumstances in which ferroptosis encourages or hinders the growth of tumors in vivo. Moreover, the TME's proof of ferroptosis duality emphasises the need of limiting the induction of ferroptosis to particular cell types.

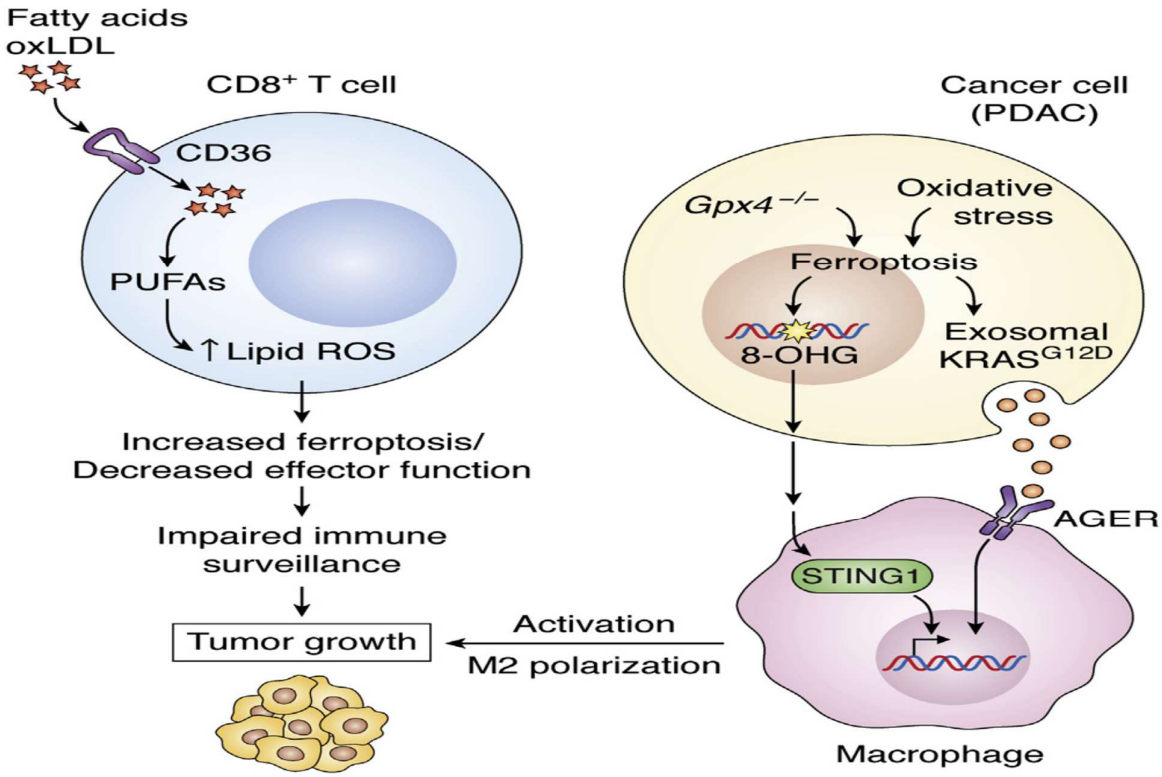

Figure 6: Protumour roles of ferroptosis in the tumour microenvironment. (Xu *et al.*, 2021)

Erastin Inhibits Ferroptosis Induced by System XC

According to research by Reina *et al.*, erastin can induce SLC7A11 transcriptionally upregulated in response. Pinto and Reina (2017) The anti-cancer effect of erastin was enhanced when SLC7A11 expression was inhibited, while erastin-induced cell death was decreased when SLC7A11 was overexpressed through gene transfection.1. Therefore, it seems that erastin can directly inhibit system XC, which in turn reduces cystine uptake by cells in an indirect manner. Erastin's inhibition of system XC suggests that, in addition to changing VDAC's permeability, erastin can act on system XC to activate the traditional ferroptosis pathway. Reduced amounts of GSH will occur when system XC is suppressed because cysteine, a substrate for GSH synthesis, will be absent. Following erastin treatment, GSH was found to be considerably reduced using biochemical and metabolomic tests. (Yang *et al.*, 2014; Skouta *et al.*, 2014) In order for GPX4 to catalyze the breakdown of hydroperoxide and hydrogen peroxide and prevent the development of L-ROS, GSH is a required cofactor. Thus, erastin's suppression of system XC indirectly causes a reduction in GPX4 production, which in turn causes a reduction in the antioxidant capability of cells.70

It was discovered that erastin treatment reduced GPX4 activity in a range of cancer cells.(Yu and others, 2015; Shiromizu *et al.*, 2019) Erastidin-induced suppression of system XC or GPX4 inactivation results in iron-dependent accumulation of L-ROS and PUFA consumption, which ultimately leads to cell death.(Yang *et al.*, 2014; Skouta *et al.*, 2014). However, the accumulation of L-ROS, consumption of PUFAs, and subsequent cell death in cancer cells treated with erastin and GPX4-deficient mouse cells can be prevented by treatment with small molecular antioxidants, indicating that L-ROS-mediated cell damage is necessary for ferroptosis induced by erastin.As per Skouta *et al.* (2014) and Seibt *et al.* (2018), In summary, erastin inhibits system XC, which lowers the intracellular GSH level and so blocks the entry of extracellular cystine into cells. GSH is a necessary substrate for GPX4's antioxidant activity. Thus, a decrease in GPX4 activity results in the breakdown of redox homeostasis and the accumulation of L-ROS, which in turn causes oxidative cell death, or ferroptosis. (Fig. 7) It has been established by earlier research that ferroptosis and system XC activity inhibition can result from p53 gene activation. In 2018, Ganapradeepan and colleagues According to recent research, erastin may be able to increase ferroptosis by activating p53.

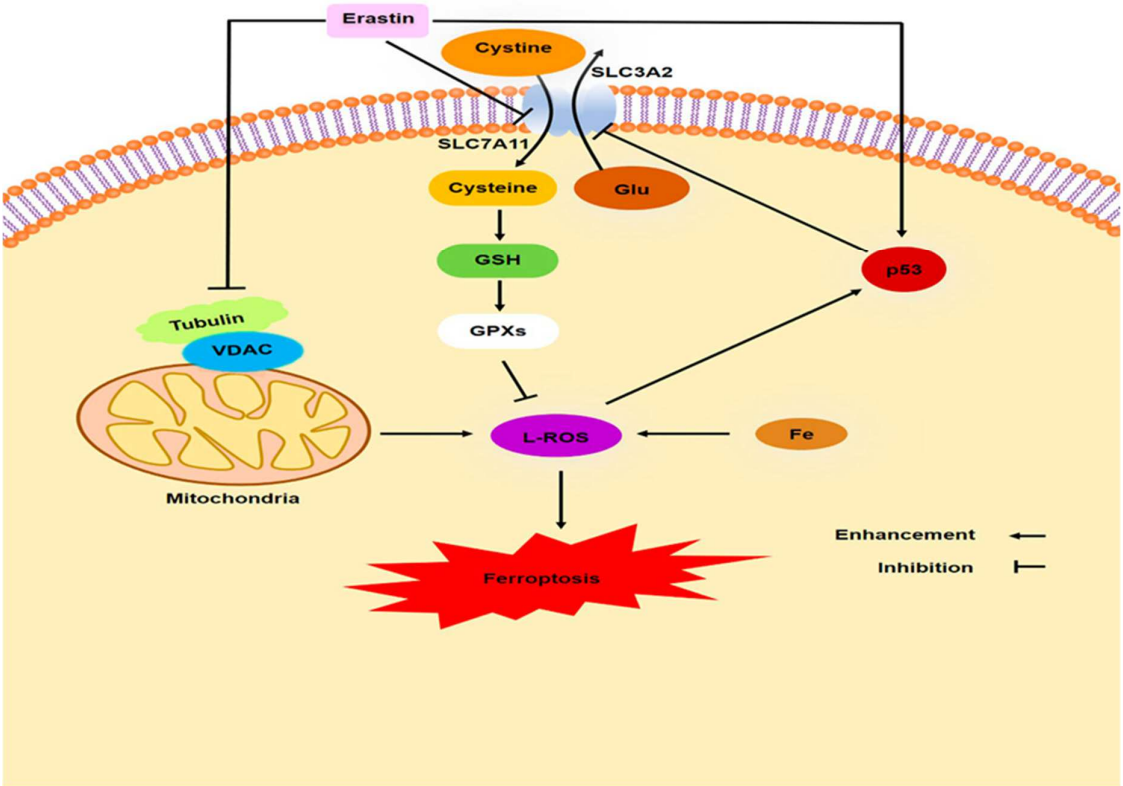

Figure 7: The relevant pathways of ferroptosis induced by erastin. (Huang *et al.*, 2018)

Current challenges to induce ferroptosis for cancer therapy

There has been a growing amount of study conducted to date on the mechanism of ferroptosis and its application in cancer therapy. To provide anti-tumor effects, a variety of ferroptosis-inducing drugs have been developed and manufactured (Lei *et al.*, 2022). Nonetheless, there are still many obstacles to overcome before ferroptosis inducers may be used in clinical settings, including limited therapeutic efficacy, significant medication side effects, and ambiguous detection techniques. Due to their short blood half-life, poor water solubility, and potential to produce drug resistance, chemical inducers utilising small compounds have far from satisfactory therapeutic efficiency (Shen et al., 2018). For example, erastin, a common system xc- inhibitor, has an unstable metabolism in the body and poor water solubility, which might result in unmanageable adverse effects (Gai *et al.*, 2020). An iron-chelating chemical licensed by the Food and Drug Administration (FDA) called deferoxamine is used to chelate iron ions and lessen the damage caused by many ferroptosis in normal cells and tissues. Nevertheless, the brief duration of its half-life (about 15 minutes) significantly restricts its build-up in cancer (Park *et al.*, 2022). Another significant component that has the most influence on treatment success is drug resistance, which has been shown in sorafenib-mediated hepatocellular carcinoma therapy and attenuated the drug's ability to induce ferroptosis (Tang *et al.*, 2022). Furthermore, a major obstacle to the use of ferroptosis in cancer treatment is the fact that the negative effects of the inducers now on the market are not insignificant. For instance, cisplatin suppresses GPX4 activity and downregulates intracellular GSH levels to cause ferroptosis (Guo *et al.*, 2018; Chen *et al.*, 2020). Nevertheless, its detrimental impact on the kidneys and eyes limits its extensive clinical use (Zhu *et al.*, 2020; Zhao *et al.*, 2020). Furthermore, one of the primary reasons for side effects is the poor targeting capacity of small-molecule inducers. Certain inducers of ferroptosis, such SAS and SRF, cause ferroptosis by preventing the downregulation of GSH and GPX4 that is induced by system xc. However, there are certain negative consequences associated with GPX4 downregulation in normal cells and tissues, including the formation of intestinal tumors and the demise of antigen-specific CD4+ and CD8+ T lymphocytes (Canli *et al.*, 2017; Matsushita *et al.*, 2015). Furthermore, there are significant problems that must be solved, including the identification and evaluation of ferroptosis-based treatment procedures. Nonetheless, there hasn't been much information released on the new diagnostic probes and imaging techniques that can be used to track this process, which is a promising avenue for uncovering ferroptosis's

underlying mechanism. As things stand, much work needs to be done before ferroptosis may be applied as a cancer treatment in a clinical setting. The field of ferroptosis inducer research has experienced a rapid growth in recent times. Numerous treatment approaches for cancer that involve inducing ferroptosis have been extensively documented. These include utilizing tiny molecules (erastin, sorafenib [SRF], sulfasalazine [SAS]), as well as related genes (e.g., plasmids containing shMTHFD2 and shGPX4; Liang *et al*., 2019; Yang *et al*., 2022). However, the current ferroptosis inducers' weak water solubility and instability severely restrict their potential for use in cancer therapy (Li *et al*., 2021). Fortunately, nanomaterials' designable, multifunctional, and adjustable qualities offer a variety of benefits to nano-systems that cause ferroptosis, including combination therapy, targeted distribution, and stimuli responsiveness (Wong *et al*., 2020; Wang *et al*., 2020; Zhamg *et al*., 2020). Specifically, responsive nanosystems based on different internal and external stimuli are more favorable for usage in cancer therapy because of their exact spatiotemporal control capability (Cook and Dcuzzi, 2021; Lu *et al*., 2017; Ovais *et al*., 2020). By inducing ferroptotic cell death, a number of responsive nanoplatforms have been developed and used to treat cancer (Ding *et al*., 2020; Li *et al*., 2020).

Tailoring nanomaterials to regulate ferroptosis in cancer therapy

Researchers are focusing on customizing nanomaterials to control ferroptosis in cancer therapy, given the limited practical uses of small-molecule inducers of ferroptosis. Many studies conducted in the last few years have created different functional nanomaterials to control ferroptosis (Li *et al*., 2021). Because of their ability to be controlled both spatially and temporally, stimuli-responsive nanomaterials have been utilized extensively to induce ferroptosis (Cai *et al*., 2019; Guan *et al*., 2022; Guo *et al*., 2020). Basically, they fall into two categories: (1) internal stimuli like ROS, GSH, pH, and glucose; and (2) external stimuli like radiation, light, ultrasound, and magnetic field (MF), as shown in Fig. 8. A number of responsive nanomaterials that cause ferroptosis are enumerated in this section, with a focus on stimulus-responsive nanosystems that cause ferroptosis in response to external or internal stimuli for cancer treatment.

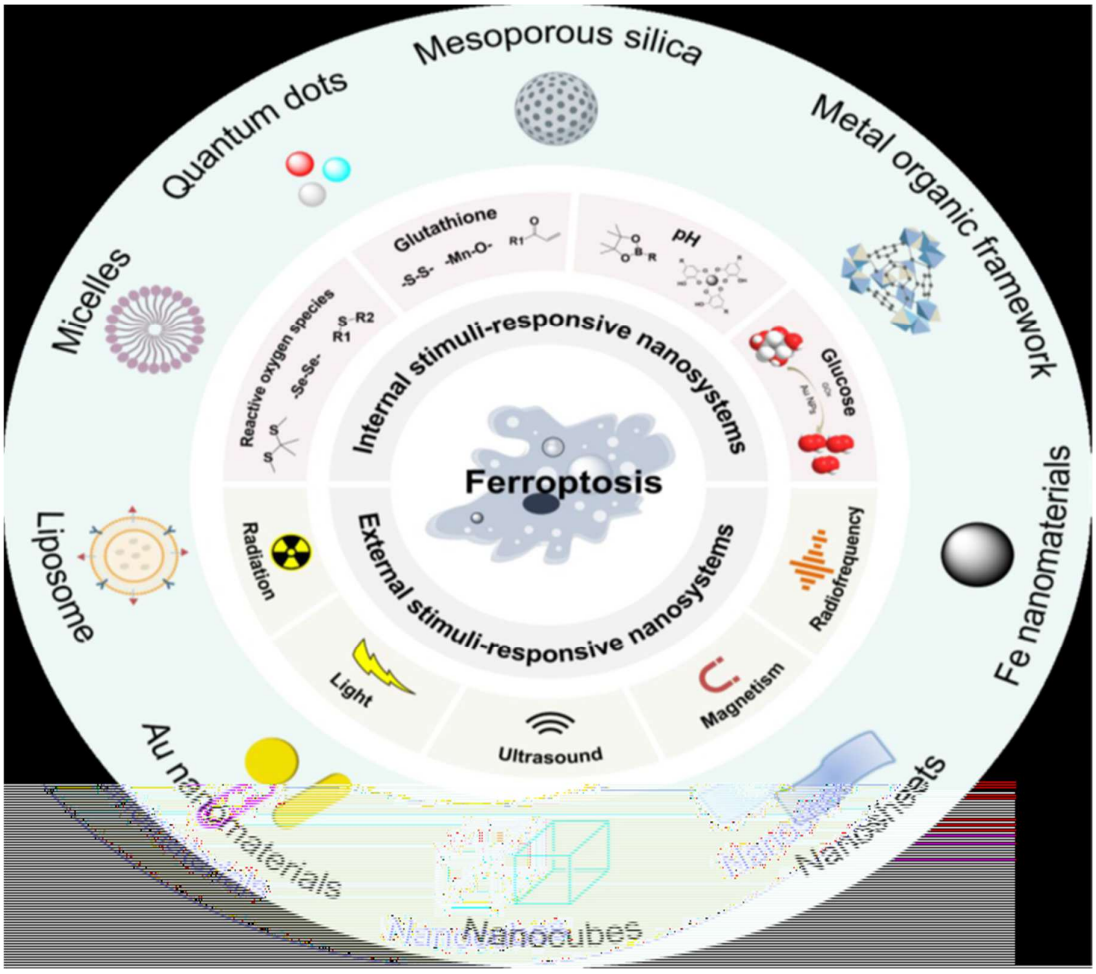

Figure 8: The class of nanomaterials that respond to stimuli and cause ferroptosis. (Guo *et al.*, 2020)

Redox Imbalance

The antagonistic relationship between antioxidant defense and oxidative damage is the primary mechanism of ferroptosis (Lie *et al.*, 2022). Ferroptosis results from an imbalance between the production of ROS and their degradation during lipid peroxidation (Tang *et al.*, 2021). The primary characteristic of ferroptosis and its necessary condition are the considerable intracellular ROS generation and the evident depletion of antioxidant agents. ROS come in a variety of forms, such as hydroxyl radicals, superoxide anions (O2), singlet oxygen (Lu *et al.*, 2022), hypochlorite anions (OCl−), hydrogen peroxide, and superoxide radical anions (O2•) (Cheung *et al.*, 2022; Tapeinos *et al.*, 2016). The major internal antioxidant system, known as the GPX4–GSH system, employs reduced GSH to detoxify lipid hydroperoxides through the phospholipid hydroperoxidase glutathione peroxidase 4

(GPX4), which is the main inhibitor of ferroptosis (Yang *et al.*, 2014). In conclusion, ferroptosis is a type of cell death brought on by intracellular redox imbalance-induced peroxidative toxicity (Stockwell and Jiang, 2020).

Conclusion

Since it was initially suggested in 2012, ferroptosis application in cancer therapy has drawn a lot of interest and been extensively covered in the media. Ferroptosis has always been regarded as an intriguing research topic, which has led numerous researchers to investigate it with the goal of creating successful anti-cancer treatments based on it. Research employing inducers of ferroptosis has exhibited promising anti-cancer properties. The range of agents that can elicit ferroptosis has been expanded, and the creation of these agents has been made easier by the swift progress made in nanotechnology in the medical field. Furthermore, responsive nanomaterials provide improved opportunities for ferroptosis induction for cancer therapy and enhance the therapeutic effect of small-molecule inducers due to their distinct physicochemical properties. They have been used to create a variety of stimuli-responsive nanomaterials that induce ferroptosis in cancer patients. Therefore, we have reviewed here stimuli-responsive nanomaterials that induce ferroptosis for the purpose of treating cancer, with a focus on external stimuli and internal stimulus responses in tumours. Despite the fact that ferroptosis inducers and their applications based on various stimuli-responsive nanomaterials have been extensively studied, there are still certain restrictions, challenges, open-ended issues, and areas that need further in-depth investigation and debate in order to enhance the current materials or create new ones. Here are a few viewpoints on the progress and difficulties of this study. First, there are a variety of obstacles and challenges associated with various external stimulation methods. For example, the primary issues in conventional radiotherapy that need to be resolved are the negative effects of ionizing radiation. High-Z metal nanomaterials have a high absorption of X-rays, which makes them a viable option for using as X-ray sensitizers to lower radiation dosesFerroptosis's limited ability to penetrate external stimuli effectively limits the application of this ROS source for deep tumours, which is the main barrier to therapeutic efficacy in light-triggered PDT. UV light has a penetration depth of around 10 mm, while NIR light has a penetration depth of about 1 cm. It is therefore possible to get around this limited penetration depth by creating

nanomaterials that are active in this wavelength range. By converting UV light sources into NIR light sources, two-photon technology may potentially improve the therapeutic effects of PDT. Organelle-targeted sonosensitizers may improve the ultrasound-mediated SDT, which has disadvantages similar to PDT. Researchers also need to take into account the impact that photosensitizers or sonosensitizers have on the surrounding normal tissue.

Recomendation

To precisely ascertain ferroptosis's possible use in the clinical setting, scientists should conduct additional research on the subject.

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