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REVIEW ARTICLE

Mitochondria-related signaling pathways involved in breast cancer regulate ferroptosis

Xinrui Dong [a,1], Ye Li [a,1], Xiaonan Sheng [a], Weihang Zhou [a], Aijun Sun [b,*], Huijuan Dai [a,*]

[a] *Department of Breast Surgery, Renji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai 200127, China*
[b] *Department of Thyroid and Breast Oncological Surgery, Xuzhou Medical College Affiliated Huaian Hospital, Huai'an, Jiangsu 223001, China*

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Abstract Ferroptosis is a novel form of regulated cell death characterized by iron-dependent excessive lipid peroxidation. The core organelle involved in ferroptosis is mitochondria. Mitochondria undergoing ferroptosis are distinct from normal mitochondria in terms of morphology, biochemistry, gene expression, and energy metabolism. An increasing number of studies have shown that mitochondria and their associated metabolic pathways mediate ferroptosis in the development and progression of breast cancer. In this review, we discuss the relevant research about ferroptosis in breast cancer and provide a comprehensive summary of mitochondrial regulation in ferroptosis from the perspective of lipid metabolism, oxidative phosphorylation, ion metabolism, glycometabolism, and nucleotide metabolism. We also summarize the application of mitochondrial metabolism-related pathways as ferroptosis treatment targets. Here we provide new insights into the relationship between mitochondria, ferroptosis, and breast cancer treatment.

* Corresponding author.
E-mail addresses: huaiansunaijun@163.com (A. Sun), daihuijuan1988@163.com (H. Dai).
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[1] These authors contributed equally to this work.

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Introduction

Breast cancer is the most common malignancy in women. According to GLOBOCAN2020, there are 2.26 million new cases of breast cancer and more than 680,000 deaths worldwide, which calls for more concern. Despite a large number of funds and staffing invested in breast cancer research, the etiology of breast cancer is not yet fully understood, and treatment resistance and early recurrence remain unresolved. Classical oncology theory considers that cancer cell growth relies not on mitochondrial aerobic respiration but on glycolysis to maintain energy metabolism while studies on the role of mitochondria in breast cancer are still under debate. The term ferroptosis was proposed in 2012 to refer to a novel form of regulated cell death,[1] raising the upsurge in the research of mitochondria associated with oxidative stress and iron ion regulation.[2] Herein, we focus on mitochondria to elaborate the regulation of ferroptosis by various metabolic pathways involved in mitochondria, conclude the role of mitochondria-related ferroptosis in the disease development and treatment of breast cancer, and shed new light on the relationship between mitochondria and ferroptosis in breast cancer.

Overview of ferroptosis

Iron is an essential trace element that plays a vital role in maintaining metabolisms, such as participation in oxygen transport, DNA biosynthesis, and ATP synthesis.[3] Iron is also an essential mediator of regulated cell death. Ferroptosis is characterized by the accumulation of lipid peroxidation resulting from iron ion pooling and is morphologically characterized by the smaller mitochondrial size, decrease or disappearance of mitochondrial cristae, and enhanced mitochondrial membrane density under electron microscopy.[4] Ferroptosis is exogenously regulated by the cystine/glutamate transport system (system xc^-) and the iron transport system, and endogenously regulated by intracellular antioxidant enzymes such as glutathione peroxidase 4 (GPX4).[5,6] Various cellular energy metabolic pathways through mitochondria are involved in the regulation of ferroptosis.

Mitochondria are the primary source of reactive oxygen species (ROS), and about 90% of ROS derived from mitochondria in eukaryotes.[7] System xc^- on the cell membrane exports intracellular glutamate in exchange for extracellular cystine and oxidizes it to cysteine,[8] which in turn forms glutathione (GSH) by combining with glutamate and glycine.[9] GSH acts as a reducing agent to form oxidized glutathione (GSSG) via GPX4 and, in turn, reduces lipid peroxides (L-OOH) to the corresponding lipid alcohols (L-OH).[10] On the other hand, extracellular ferric ions (Fe^{3+}) were transported into cells via transferrin (TFR) and then reduced to ferrous ions (Fe^{2+}),[11] which combine with L-OOH in a Fenton reaction to oxidize Fe^{2+} to Fe^{3+} again and generate many hydroxyl radicals (•OH).[12] Polyunsaturated fatty acids (PUFAs) on the mitochondrial membrane bind to •OH to generate PUFA radicals (PUFA•). These PUFA• are not stable and rapidly oxidized to PUFA peroxyl radicals (PUFA-OO•) and finally to PUFA hydroperoxides (PUFA-OOH).[13] These molecules with peroxidation capacity in the cascade reactions above all belong to ROS.[14] The accumulation of toxic lipid reactive oxygen species (L-ROS) destroys the phospholipid bilayers,[15] ultimately leading to cell death[16,17] (Fig. 1). Ferroptosis has been reported to be associated with neurodegenerative diseases (*e.g.*, Alzheimer's syndrome, Huntington's chorea, *etc.*), cardiomyopathies, and various cancers.[18–20]

Mitochondria-related signaling pathways involved in ferroptosis

Mitochondria are known as the "powerhouse" of cells. Mitochondria contain high iron levels.[21] Ferroptosis leads to significant morphological change and dysfunction in mitochondria.[4] As the energy metabolism center of fatty acid oxidation (FAO), tricarboxylic acid cycle (TCA cycle), and electron transfer chain (ETC), mitochondria and their related metabolic pathways play an essential role in ferroptosis (Fig. 2).

Lipid metabolism regulation in ferroptosis

The synthesis, storage, and degradation of fatty acids are dynamic processes. Fatty acid degradation is initially activated in the cytoplasm to form lipid coenzyme A and then transported into the mitochondria for β-oxidation. Lipid peroxidation in ferroptosis belongs to fatty acid degradation metabolism,[22] and mitochondria are the sites providing coenzyme A for lipid peroxidation.[23] Stearoyl coenzyme A desaturase (SCD1) is the rate-limiting enzyme that converts saturated fatty acids to monounsaturated fatty acids. Blocking SCD1 reduced the lipid antioxidant coenzyme Q10 in the mitochondrial electron transport chain and increased pro-apoptotic ceramide, thus inducing cellular ferroptosis and apoptosis.[24] 15-lipoxygenases (15-LOX) is an instrumental proinflammatory lipid peroxidative enzyme which oxidizes polyunsaturated fatty acids,[25] and it has been reported to damage the mitochondria directly.[26]

15-LOX accelerates the induction of ferroptosis by increasing the rate of lipid peroxidation through iron-catalyzed free radical reactions.[27] In addition to some enzymes involved in lipid metabolism, certain lipids can directly or indirectly alter the mitochondrial membrane lipid peroxidation level to facilitate or restrain ferroptosis. LOX promotes the synthesis of phosphatidylethanolamine (PE), which could induce ferroptosis, whereas tocopherols and tocotrienols can inhibit LOX and ferroptosis.[28] Exogenous monounsaturated fatty acids (MUFA) can inhibit iron death caused by Erastin and RSL3.[29] In summary, LOX and its corresponding lipids are crucial in initiating lipid peroxidation in ferroptosis. In contrast, inhibiting fatty acid β-oxidation with the ATP synthesis inhibitor etomoxir or oligomycin restores the sensitivity of renal cancer cells to ferroptosis.[30]

Oxidative phosphorylation regulation in ferroptosis

Oxidative phosphorylation proceeds in the inner mitochondrial membrane (IMM) of eukaryotic cells, where electrons from ETC complexes I and III on the IMM generate superoxide ($O_2^{\bullet -}$) and are converted to hydrogen peroxide (H_2O_2).[31] H_2O_2 is a potent oxidizing agent, which oxidizes

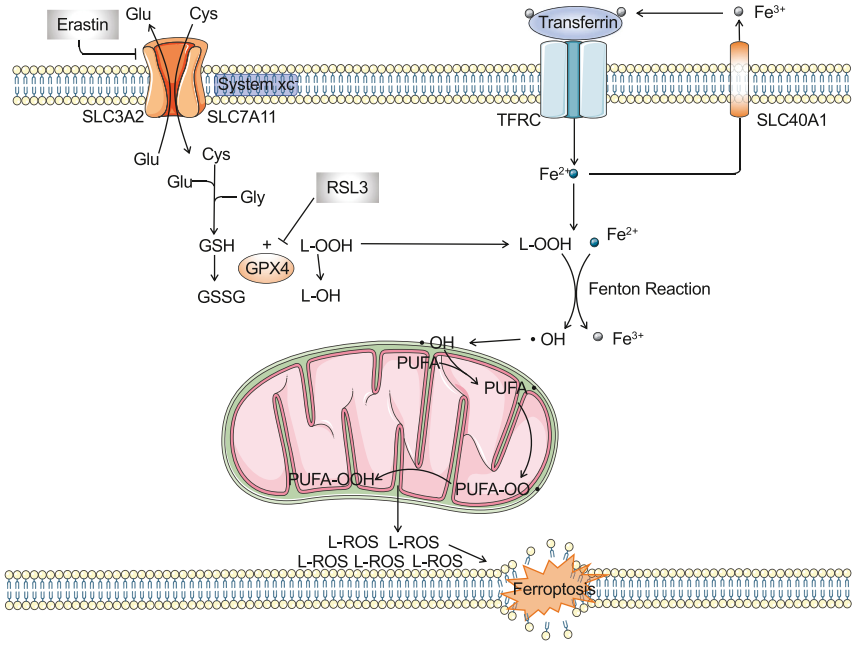

Figure 1 Overview of ferroptosis. The schematic figure shows that the canonical ferroptosis pathway is executed by phospholipid peroxidation associated with oxidative stress and iron ion regulation. Cys, cysteine; Glu, glutamate; Gly, glycine; GPX4, glutathione peroxidase 4; GSH, glutathione; GSSG, oxidized glutathione; L-OH, lipid alcohols; L-OOH, lipid peroxides; L-ROS, lipid reactive oxygen species; PUFA, polyunsaturated fatty acid; SLC3A2, solute carrier family 3 member 2; SLC7A11, solute carrier family 7 member 11; SLC40A1, solute carrier family 40 member 1; TFRC, transferrin receptor.

Fe^{2+} to Fe^{3+} and generates hydroxyl radicals to participate in ferroptosis. Inhibition of ETC complexes I—IV decreases the accumulation of lipid peroxides in Erastin-induced ferroptosis.[32] Reduced coenzyme II (nicotinamide adenine dinucleotide phosphate, NADPH) is also a critical electron donor in the ETC redox reaction and participates in the pentose phosphate pathway of glycometabolism. Pharmacogenomic studies of compounds with high cell-line-selective lethality reveal that intracellular NADPH abundance could predict cellular sensitivity to ferroptosis inducers. The underlying mechanism of resistance to ferroptosis inducers might be associated with ferroptosis-induced NADPH reduction.[33] Consistent with the high-throughput sequencing results, NADPH was also found to assist the ferroptosis regulator ferroptosis suppressor protein 1 (FSP1) to reduce coenzyme Q10 as a radical-trapping antioxidant that prevents lipid peroxidation propagation,[34—36] and NADPH produced from mitochondria may also halt lipid peroxidation and inhibit ferroptosis.[37]

Ion metabolism regulation in ferroptosis

In mitochondria, iron and calcium are predominant ions involved in ferroptosis. Mitochondria are the center of cellular iron metabolism, and iron ions are imported into mitochondria via the transferrin receptor on the mitochondrial membrane. The ferroptosis inducer Erastin binds to the voltage-dependent anion-selective channel (VDAC) on the mitochondrial membrane, increasing VDAC permeability and mitochondria generate NADPH oxidase 2 (NOX2)-related ROS, which leads to calcium overload,[38] and mitochondrial membrane potential increase along with depolarization, and eventually causes ferroptosis due to the loss of mitochondrial membrane stability.[39] Ferroptosis can also be mitigated by depletion of serum TRF or knockdown of the mitochondrial transferrin receptor.[40] Iron ion plays a pivotal role in heme production, and mitochondrial sideroflexin 2 (SFXN2) is also engaged in heme production. The knockdown of SFXN2 increases intracellular iron and accelerates the process of ferroptosis.[41] Mitochondrial heme oxygenase 1 (Hmox1) is significantly up-regulated in murine cardiac cells after Adriamycin treatment, resulting in heme degradation, Fe^{3+} accumulation, and finally induced ferroptosis. Reversely, mitochondria-targeted antioxidant MitoTEMPO significantly rescued Adriamycin-induced cardiomyopathy.[42] Mitochondrial ferritin (FtMt) could regulate iron redistribution between mitochondria and cytoplasm, maintaining mitochondrial iron homeostasis.[43] Once the ferrous iron levels were increased, the production of ROS generated, resulting in cellular macromolecule damage and finally triggering cell death, especially in some oxygen-dependent tissues such as the brain.[44] Iron and calcium (Ca^{2+}) in mitochondria also crosstalk with each other through ROS signaling.[45] VDAC permeability increases during ferroptosis, and the iron overload leads to excess Ca^{2+} entry into mitochondria, resulting in mitochondrial Ca^{2+} overload and disrupting mitochondrial function.[32] This is observed in neurodegenerative diseases such as Huntington's disease, Parkinson's disease, and Alzheimer's disease,[46] addressing the crucial role of ferroptosis. In contrast, the calcium overload also engaged in cellular antioxidant defense and ROS generation,[47] disturbing the mitochondrial iron and reactive oxygen homeostasis.[48] What's more, Ca^{2+} chelators reduce mitochondrial iron uptake and suppress either glutamine-

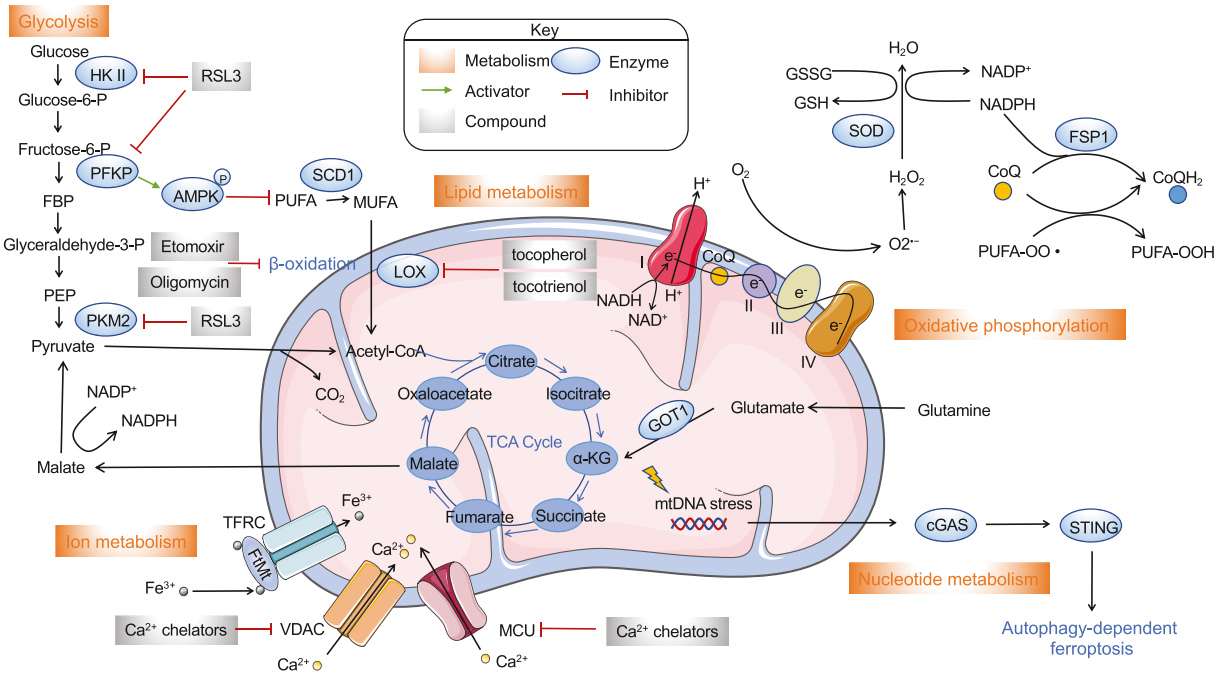

Figure 2 Mitochondria-related signaling pathways in regulating ferroptosis. Mitochondria-related metabolic pathway crosstalk plays an essential role in ferroptosis. Enzymes in lipid metabolism and lipids themselves are involved in ferroptosis regulation, which interacts with the glycolysis and TCA cycle. Intermediate products in the TCA cycle activate and further promote ferroptosis. ETCs in mitochondria drive proton motive force and provide $O2^{\bullet-}$ and thereby influence ferroptosis. In addition, the NADPH as an electron donor during the process of oxidative phosphorylation, glycolysis, and lipid metabolism also takes part in ferroptosis. Besides. Fe^{3+} and Ca^{2+} are critical in maintaining mitochondrial membrane stability. Genetic disorders and mtDNA stress could cause iron overload and subsequent ferroptosis. α-KG, α-ketopentane; AMPK, AMP-activated protein kinase; cGAS, cyclic GMP-AMP synthase; CoQ, coenzyme Q; $CoQH_2$, reduced ubiquinone; FBP, fructose 1,6-bisphosphate; FSP1, ferroptosis suppressor protein 1; FtMt, mitochondrial ferritin; GOT1, glutamic-oxaloacetic transaminase 1; GSH, glutathione; GSSG, oxidized glutathione; HK II, anti-hexokinase II; LOX, lipoxygenase; MCU, mitochondrial calcium uniporter; mtDNA, mitochondrial DNA; MUFA, monounsaturated fatty acid; $NADP^{+}$, nicotinamide adenine dinucleotide phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; $O2^{\bullet-}$, superoxide; PEP, phosphoenolpyruvate; PFKP, platelet-type phosphofructokinase; PKM2, pyruvate kinase; PUFA, polyunsaturated fatty acid; SCD1, stearoyl coenzyme A desaturase; SOD, superoxide dismutase; STING, stimulator of interferon response cGAMP interactor; TCA, tricarboxylic acid; TFR(C), transferrin; VDAC, voltage-dependent anion-selective channel.

induced or cysteine-deprivation-induced ferroptosis.[1,49] Overall, the imbalance between iron and calcium was the key factor of disease,[50] indicating the potential role of their crosstalk in the development of ferroptosis.

Glycometabolism regulation in ferroptosis

Glycolysis is the first step of glycometabolism, and the TCA cycle is the third stage of aerobic respiration in eukaryotes. Warburg effect suggests that cancer cells metabolize most of their glucose via glycolysis by dysregulating mitochondrial function, while the ability of glycolysis is reduced when ferroptosis occurs.[1] RSL3 inhibited glycolysis by decreasing the production of three kinases in glycolysis: anti-hexokinase II (HKII), platelet-type phosphofructokinase (PFKP), and pyruvate kinase (PKM2).[51] This may be due to the increased permeability of VDAC, which also increases the entry of most metabolic raw materials, such as glutamate[52] and ATP[53] into the mitochondria. α-ketopentane (α-KG), an essential intermediate metabolite in the TCA cycle[54] links the TCA cycle to ferroptosis. α-KG can replace glutamine as a reducing agent in the oxidation reaction,[55] causing lipid ROS accumulation and ferroptosis. In addition, the downstream metabolites of α-KG in the TCA cycle, such as succinate, fumarate, and malate, can all be substitutes for glutamine in ferroptosis.[32] In contrast, the upstream metabolites of α-KG are impacted more by glucose than by glutamine.[32] In addition to α-KG, acetyl coenzyme A in the TCA cycle also affects ferroptosis. Lipid ROS levels can be inhibited by induction or simulation of energy stress through the cellular energy sensor AMP-activated protein kinase (AMPK) activation, which diminishes PUFA synthesis.[56]

Nucleotide metabolism regulation in ferroptosis

Mitochondria also contain a small amount of mitochondrial DNA (mtDNA). mtDNA depletion syndrome is a recessive genetic disorder caused by gene mutations, one subtype of which is the deoxyribonucleoside activating enzyme (DGUOK) mutation. Iron overload and the subsequent ferroptosis were found in patients with DGUOK mutation, which could lead to severe liver failure.[57] Apart from genetic disorders, zalcitabine, a nucleotide analog used as an antiviral drug, can activate the cGAS-STING1 pathway by inducing

mtDNA stress, and cause autophagosome formation, leading to autophagy-dependent ferroptosis via lipid peroxidation.[58]

Mitochondria-related pathways regulate ferroptosis in breast cancer

The effect of ferroptosis on the tumorigenesis of breast cancer

The growth of cancer cells depends on abnormal energy metabolism. Cancer cells are under persistent metabolic disturbance, such as oxidative stress caused by redox reactions of iron ions and thiol. Thus they are more likely to evade ferroptosis than normal cells.[59] Normal breast epithelial cells lack $\alpha 6\beta 4$ integrin, which inhibits the extracellular matrix (ECM), thereby maintaining cells susceptive to ferroptosis because of GPX4 suppression. However, breast cancer cells express $\alpha 6\beta 4$ integrin, protecting them from ferroptosis.[60]

Different susceptibilities to ferroptosis according to subtypes of breast cancer

Breast cancer can be divided into subtypes based on hormone receptors and human epidermal growth factor receptor 2 (HER2) status. Triple-negative breast cancer (TNBC) cell lines (MDA-MB-231, MDA-MB-468, MDA-MB-157) were more sensitive to RSL3 than other subtypes.[4] This may be interpreted that TNBC is more susceptible to cystine starvation, and system xc^- also depends on cystine/glutamate metabolism. Thus low cystine levels are more likely to induce ferroptosis in TNBC.[61] Further preclinical and clinical studies revealed that the positive regulator of ferroptosis, acyl-coenzyme A synthetase long-chain family member 4 (ASCL4), has higher expression in TNBC than other subtypes,[62] therefore, enhancing cellular sensitivity to ferroptosis.[4] Corresponding to this, ROS levels were significantly higher in TNBC than in hormone receptor-positive breast cancers, which originate mainly from mitochondria.[63] Iron deficiency activates the Notch pathway, triggering epithelial–mesenchymal transition (EMT) in mouse TNBC cells. CD44-mediated cellular iron endocytosis also increases iron-dependent demethylase activity, triggering EMT, and further facilitating cancer cell metastasis.[64] In addition, ferroptosis resistance is a feature of metastatic breast cancer cells, and GPX4 knockdown inhibited the tumorigenic and metastatic activity of TNBC cell line 4T1 in 27-hydroxycholesterol-resistant mice.[65] Tumor-associated macrophages modulate human hepatic leukemia factor and transactivated gamma glutamyltransferase 1 to promote ferroptosis resistance, resulting in TNBC proliferation, metastasis, and cisplatin resistance.[66] In general, inducing ferroptosis in breast cancer, especially in TNBC, offers a new idea for future treatment.

Targeting mitochondria-specific regulation to induce ferroptosis in breast cancer

There are various therapies according to breast cancer molecular subtypes, including endocrine therapy, chemotherapy, radiotherapy, targeted therapy, and immunotherapy. The current targets for ferroptosis in breast cancer treatment mainly focus on critical regulators of endogenous and exogenous regulatory pathways: inhibition of system xc^-, inhibition of GPX4, inhibition of Fe^{3+}, or inhibition of upstream regulatory factors of ferroptosis such as p53.[67] There are relatively few treatments targeting mitochondria, leaving a vast space for research. In particular, regulating various metabolic pathways involved in mitochondria may become a new direction for breast cancer treatment in the future (Fig. 3).

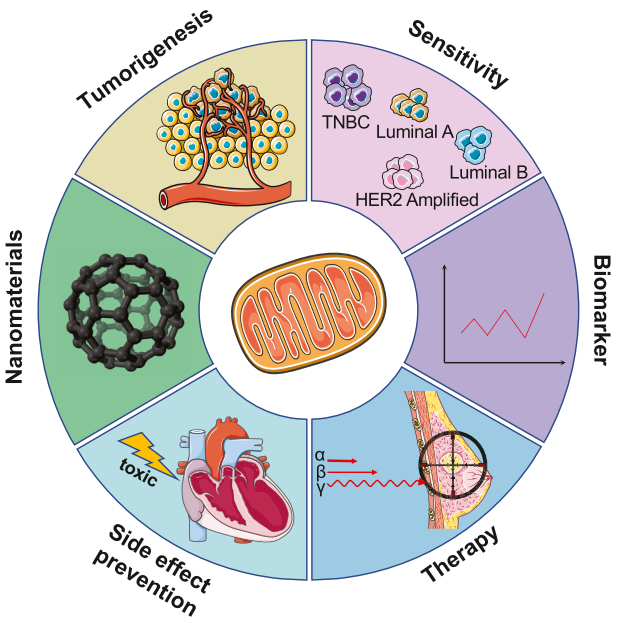

Figure 3 Applications of targeting mitochondrial metabolism in ferroptosis on the diagnosis and treatment of breast cancer. Mitochondria-specific regulations have a pivotal impact on virtually all aspects linked to the diagnosis and treatment of breast cancer: encompassing malignant transformation, breast cancer heterogeneity, biomarker monitoring, response to treatment, anticancer side effect surveillance, and nanomaterials research and development. HER2, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer.

Lipid metabolism as therapy targets

Lipid metabolism is the most critical mitochondrial metabolic pathway in ferroptosis. Therefore, there is a wide range of applications in breast cancer diagnosis and treatment targeting the regulation of mitochondrial lipid metabolism. Natural substance DMOCPTL, a derivative of parthenolide, was highly lethal to TNBC cells, exhibiting 15 times more than its parent compound PTL. DMOCPTL directly binds GPX4 and induces GPX4 ubiquitination, leading to mitochondria-mediated apoptosis by regulating early growth response 1.[68] It has also been reported that SCD1 expression can be used as a biomarker for breast cancer recurrence.[69] Herceptin, a HER2-targeted drug for breast cancer, increased mitochondrial ROS levels in rat cardiomyocytes and decreased GPX4 expression, inducing ferroptosis. The addition of the ferroptosis inhibitor

ferrostatin-1 reversed the elevation of ROS level.[70] This finding suggests that ferroptosis inhibitors could be used to prevent cardiotoxicity in $HER2^+$ breast cancer targeted therapy.

Oxidative phosphorylation as therapy targets

Ferroptosis regulated by mitochondrial oxidative phosphorylation has less application in breast cancer treatment, which is just used as a biomarker for efficacy assessment. Manganese superoxide dismutase 2 (SOD2) is responsible for depleting oxygen radicals generated in ETC and converting them to H_2O_2.[71] SOD2 expression is significantly elevated in advanced and invasive breast cancers and can be used as a biomarker of breast cancer progression.[72] Breast cancer radiotherapy increases iron accumulation and ROS generation via increasing lysosomal membrane permeability. At the same time, overexpression of SOD2 can decrease cellular ROS levels, autophagy, and cell death, resulting in poor efficacy of radiotherapy.[73] MtDNA deletion and mutations have been demonstrated in various cancers, including breast cancer.[74] However, it remains unclear how to utilize the alteration in mtDNA in cancer therapy. MtDNA mutation frequency increases with aging like nuclear DNA, and mtDNA deletions and mutations are mainly related to respiratory chain dysfunction. Thus, mtDNA is also considered an indicator of oxidative damage. An analysis of lipid ROS and DNA in blood samples from breast cancer patients revealed higher levels of oxidative stress and damage compared to healthy controls and a higher level of SOD2, suggesting that SOD2 may protect mtDNA from ROS damage in breast cancer.[75] It illustrates that the bidirectional role of SOD2 in breast cancer is dual considering the application of SOD2 in oxidative phosphorylation-regulated ferroptosis in breast cancer: SOD2 expression may protect mtDNA in breast cancer patients, and it also needs to be noted that SOD2 overexpression has the potential to affect the efficacy of breast cancer radiotherapy. But in general, there is no doubt about the role of SOD2 as an inhibitor of ferroptosis in breast cancer treatment.[76] Overall, free radicals are directly generated in oxidative phosphorylation, which is an essential intermediate in ferroptosis induction. Therefore, increasing the specificity and sensitivity of mitochondrial oxidative phosphorylation-regulated ferroptosis to target tumor cells precisely is a challenging problem that remains to be solved.

Ion metabolism as therapy targets

The ions involved in regulating mitochondrial ferroptosis are mainly iron and calcium. Calcium is primarily used as a chelator to inhibit ferroptosis, thus, is mostly applied in neuropathy to protect cells from ferroptosis rather than to promote ferroptosis. The ion-related breast cancer treatment is mainly focused on iron-related targeted drugs. The newly discovered iron-sulfur proteins CISD1 and CISD2 have the function of promoting breast cancer proliferation.[77] Knocking down CISD1 and CISD2 or targeting breast cancer mitochondria with the mitogen derivative MAD-28 can disrupt iron-sulfur proteins, increase iron accumulation in mitochondria, and inhibit breast cancer cell growth.[78] MAD-28 is also highly selective, with iron accumulation in mitochondria occurring only in breast cancer cells. In contrast, normal breast cells are not affected by MAD-28, suggesting that MAD-28 may be a potential targeting agent for the induction of ferroptosis and anticancer therapy.

Nanomaterials as therapy targets

With the development of pharmacological technologies, there are many applications of new nanomaterials in breast cancer treatment due to their high efficiency and safety, which also includes the use of mitochondria-specific nanomaterials loaded with ferroptosis drugs. Sorafenib is an oral multi-kinase inhibitor with antitumor efficacy. Studies have shown that sorafenib induces ferroptosis and the depletion of intracellular iron stores is associated with sorafenib resistance.[79] Mitochondrial membrane-anchored oxidation/reduction response and Fenton-Reaction-Accelerable magnetic nanophotosensitizer complex self-assemblies loading sorafenib (CSO-SS-Cy7-Hex/SPION/Srfn) generates lipid peroxide burst, enhancing the ferroptosis-inducing therapeutic efficacy of sorafenib by 18-fold in breast cancer cells.[80] Another ferroptosis drug, simvastatin, achieved high efficacy through a feat of new nanomaterials as well. Simvastatin inhibits the 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, which regulates the *ab initio* metabolism of cholesterol by magnetic nanoparticles Fe_3O_4@PCBMA, thereby down-regulating the mevalonate metabolic pathway and the GPX4 pathway, inducing ferroptosis in TNBC cells without liver or kidney toxicity. These two pathways are also related to mitochondrial glycometabolism and lipid metabolism.[81] A Cu-tetra (4-carboxyphenyl) porphyrin chloride (Fe(III)) (Cu-TCPP(Fe)) metal—organic framework (MOF)-based nanosystem was reported to be integrated with Au NPs and loaded with RSL3, which impedes GSH biosynthesis by disrupting the pentose phosphate pathway of mitochondrial glycometabolism, affecting a variety of enzymes involved in ferroptosis, ultimately enhancing the function of RSL3.[82] Most of these ferroptosis nanomaterials targeting mitochondria are still under research, but the high-efficiency ferroptosis induced by the win—win cooperation of new nanomaterials and ferroptosis is the dawn for the future treatment of breast cancer, especially TNBC.

Targeting on ferroptosis to reverse drug resistance in breast cancer

Overcoming drug resistance is also a daunting challenge in breast cancer treatment. In addition to inducing ferroptosis in breast cancer treatment, ferroptosis has also been shown to correlate with drug resistance to breast cancer. Ferroptosis biomarkers could be applied to predict drug sensitivity in breast cancer. A cell death index model involving ferroptosis-related genes could predict resistance to the standard adjuvant chemotherapy regimen in breast cancer. Patients with a higher cell death index might be more sensitive to palbociclib.[83] GPX4 and ACSL4 expression has also been demonstrated to be a predictive factor for pathological complete response in breast cancer neoadjuvant chemotherapy. As ACSL4 increased and GPX 4

decreased, pathological complete response rates rose, indicating that ACSL4 inducer and/or GPX4 inhibitor might promote neoadjuvant chemotherapy efficacy.[84] Apart from its prognostic value, ferroptosis also has been proven to defeat drug resistance. For example, the inhibition of GPX4 has been proven to reverse gefitinib resistance in TNBC,[85] and divalent metal transporter 1 inhibition also induces ferroptosis and reverses multidrug resistance in breast tumors.[86] Fibroblast growth factor receptor 4 (FGFR4) is one of the FGFR family and was reported to co-expressed with HER-2 in breast cancer.[50] It was reported that m6A hypomethylation mediates FGFR4 up-regulation, and then accelerates cystine uptake and Fe^{2+} efflux, resulting in ferroptosis resistance in recalcitrant HER2-positive breast cancer. The application of FGFR4 inhibitor roblitinib could remarkably restore trastuzumab sensitivity in recalcitrant HER2-positive breast cancer.[87] These findings hold hope for developing novel therapeutics for drug resistance problems in traditional chemotherapy, targeted therapy, and maybe immunotherapy in the future.

Challenges in targeting ferroptosis in breast cancer

In addition to these applications, targeting ferroptosis is not without challenges to making it fully harnessed. First, up to now, there are few treatments targeting mitochondria and their related metabolic pathways due to their imperfect efficiency. The nanomaterials we discussed above could provide appreciable activity. Combining ferroptosis therapy with immunotherapy or other targeted therapy may be another resolution. Furthermore, will long-time use of ferroptosis therapy cause physiological change? If these ferroptosis inducers become available, the toxicity and side effect in humans remains a major hurdle. Based on existing studies, it seems that ferroptosis treatments might cause toxicities involved in the kidney, liver, and central nervous system.[88,89] Hence, the optimized dose and drug schedule need to be discovered cautiously. Finally, the development of predictive biomarkers still needs to be resolved. Ways such as testing biomarkers in patients' body fluids will be more convenient and cheaper. With the resolution of these issues, there will be a brighter future for ferroptosis-targeted therapies.

Conclusions

The process of ferroptosis is complex, involving the metabolism of almost every organelle and various molecules in the cell, and the regulatory network is even more intricate. Mitochondria, as the hub of signal transduction, play an essential role in glycometabolism, lipid metabolism, oxidative phosphorylation, and ion regulation for ferroptosis. However, the specific mechanisms of mitochondria in various tumor-related metabolisms in breast cancer are not yet clear. How the contact, crosstalk, and signaling regulatory networks between mitochondria and other organelles are carried out. A more in-depth understanding of mitochondria-specific governess in ferroptosis will advance our fundamental discovery of this cell death pathway as well as identify new therapeutic opportunities to target ferroptosis in breast cancer.

Author contributions

Conception and design: Huijuan Dai; Administrative support: Aijun Sun and Xiaonan Sheng; Manuscript writing: Xinrui Dong, Ye Li, and Weihang Zhou. Manuscript figures were drawn by Xinrui Dong and Ye Li. The final approval of the manuscript was done by all authors. Xinrui Dong and Ye Li contributed equally to this research.

Conflict of interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

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