Proceedings of the 3rd International Conference on Modern Medicine and Global Health
DOI: 10.54254/2753-8818/85/2025.20108

Exploring Iron Death in Tumor Microenvironment Around Glutathione Depletion and Its Application

Qingchi Zhang[1,a,*]

[1]*Harbin Institute of Technology University, Harbin, China*
a. zhangjun397@huawei.com
**corresponding author*

Abstract: Iron death represents a nuanced area of exploration within the realm of regulatory cell death (RCD), distinguished by its reliance on iron-mediated lipid peroxidation, setting it apart from conventional cell death pathways like apoptosis and necrosis. This process is hallmarked by an overload of intracellular iron and the accumulation of reactive oxygen species (ROS) due to unchecked lipid peroxidation, leading to cellular demise. The dual nature of iron death in cancer progression – acting both as a facilitator and inhibitor of tumor growth – underscores its complexity and the need for a deeper understanding of its molecular underpinnings. In the context of cancer biology, iron death's interaction with the tumor microenvironment (TME) and its impact on immune responses, particularly involving CD8+ T cells, have garnered significant attention. This form of cell death has been implicated in shaping the immune landscape within tumors, influencing the efficacy of cancer immunotherapies. Its potential to either promote or hinder carcinogenesis depending on the specific cancer type and stage highlights the intricate balance that exists. The review aims to consolidate current knowledge on the pivotal mechanisms governing iron death, emphasizing its regulation and the dichotomy of its roles in cancer development. By examining how iron death can either drive or suppress tumorigenesis across various human malignancies, the text seeks to elucidate the factors that determine this outcome. Furthermore, it catalogues a range of pharmacological agents capable of inducing iron death, shedding light on their therapeutic potential and the future prospects of leveraging targeted iron death induction as a novel strategy in cancer treatment. Through this synthesis, the review contributes to our comprehension of iron death's multifaceted involvement in cancer pathophysiology and its untapped potential for clinical intervention.

Keywords: Iron death, glutathione depletion, tumor microenvironment cancer treatment.

1. Introduction

The concept of iron death, introduced by Brent R. Stockwell of Columbia University in 2012, signifies a novel and tightly regulated pathway of cell demise driven by iron accumulation. This mechanism distinguishes itself from traditional forms of cell death like apoptosis, necrosis, and autophagy, primarily through its inhibition of glutathione peroxidase 4 (GPX4) [1]. By preventing cells from neutralizing lipid peroxides, iron death creates an environment conducive to iron accumulation, heightened lipid oxidation, and the generation of reactive oxygen species (ROS), ultimately leading to cellular demise. Cells undergoing iron death exhibit several distinctive

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characteristics: an excess of iron, pronounced lipid peroxidation, elevated levels of ROS, and genetic modifications associated with the regulation of iron homeostasis and lipid peroxidation. Microscopically, these cells show reduced mitochondrial size, significant changes in membrane and crista structure, while the nucleus remains largely unaffected.As a non-apoptotic form of cell death triggered by iron-dependent lipid peroxidation, iron death has gained recognition for its potential role in cancer immunotherapy. It not only directly contributes to the death of tumor cells but also enhances the efficacy of immunotherapy by modulating immune cell activity within the tumor microenvironment (TME). This dual function—directly inducing tumor cell death and amplifying the therapeutic impact of immunotherapy—positions iron death as a promising target for improving treatment outcomes in various cancers, including pancreatic and breast cancers. Thus, manipulating and controlling iron death holds significant promise for advancing personalized cancer treatment strategies [2].

After its discovery, the role of iron death in regulating various cellular processes and different diseases, particularly cancer, has been extensively studied. The complex nature of the tumor microenvironment (TME) means that iron death can play a dual role in human tumorigenesis. On one hand, it can act as a tumor suppressor by directly causing the death of cancer cells. On the other hand, its effects on the TME can either promote or inhibit tumor growth depending on the context.

Given the tumor-inhibitory effects of iron death, developing more specific inducers of this process could represent a promising strategy for cancer treatment. These inducers would need to be carefully designed to target cancer cells while minimizing off-target effects on normal tissues. By enhancing our understanding of the molecular mechanisms underlying iron death and identifying key regulators, researchers can develop targeted therapies that exploit this pathway to improve cancer treatment outcomes. This approach holds significant potential for advancing personalized medicine and addressing some of the challenges associated with current cancer therapies.

2. Mechanism of iron death

Iron is a crucial micronutrient vital for the survival of microorganisms, plants, animals, and humans. Nevertheless, excessive iron can be detrimental, causing cellular damage through diverse mechanisms, one of which includes inducing cell death. Iron death refers to an iron-dependent, regulated form of cell demise characterized by uncontrolled lipid peroxidation leading to membrane damage. This process can be initiated either externally or internally. External triggers involve transporters that regulate the onset of iron death, such as inhibiting the amino acid antiporter system XC- or activating iron transport proteins like transferrin and lactotransferrin. Conversely, internal pathways typically involve suppressing the expression or function of intracellular antioxidant enzymes, notably GPX4. Besides small molecules and pharmaceuticals, certain stressors like extreme temperatures, low oxygen conditions, hypoxia, and radiation can also provoke iron-induced cell death. Aberrations in this regulatory mechanism are linked to protein disposal systems, including autophagy and the ubiquitin-proteasome pathway, and have been associated with numerous diseases, such as acute tissue injuries, infections, cancers, and neurodegenerative disorders [3].

2.1. Glutathione

Glutathione (GSH) is a ubiquitous tripeptide composed of glutamine, cysteine, and glycine, playing a pivotal role in maintaining cellular redox balance by serving as a major intracellular antioxidant. Its involvement extends beyond mere oxidation-reduction reactions; GSH is intricately linked with various physiological processes, including iron metabolism, where it acts as a sensor and regulator of iron levels, facilitates iron transport, and participates in the biosynthesis of iron-sulfur clusters and heme groups.

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The dynamic equilibrium between reduced glutathione (GSH) and its oxidized form, glutathione disulfide (GSSG), serves as a crucial indicator of the cell's redox status. This redox couple is fundamental in modulating numerous biological functions, given that a reducing environment is essential for proper cellular functioning.

Alterations in intracellular GSH concentrations have been implicated in diverse pathological conditions, such as viral infections, aging, and diseases associated with dysregulated Th1/Th2 immune responses. Notably, GSH depletion in antigen-presenting cells (APCs) disrupts antigen processing and diminishes the secretion of Th1 cytokines, highlighting GSH's pivotal role in shaping the immune response. Conversely, elevating cellular GSH levels can enhance the production of IL-12 and/or IL-27, promoting the differentiation of naive CD4+ T cells into Th1 cells, thereby skewing the immune response towards a pro-inflammatory profile.

Furthermore, GSH exhibits direct antiviral and antibacterial properties by inhibiting the replication and survival of numerous pathogens. These findings have spurred interest in developing molecules that boost GSH levels as novel immunomodulatory and antimicrobial therapeutics. By enhancing GSH availability, these agents could potentially restore immune balance, bolster host defense mechanisms against infections, and mitigate the impact of oxidative stress-related disorders.

In summary, the emerging understanding of GSH's multifaceted roles in regulating immune responses and combating pathogens underscores its potential as a target for immunotherapy. Future exploration into GSH-enhancing strategies may unveil new avenues for treating a wide array of diseases, leveraging the body's own defense systems to restore health and homeostasis [4].

2.2. GSH system

Glutathione (GSH), a tripeptide containing cysteine, is indeed considered one of the primary antioxidants in the human body. It plays a critical role in maintaining cellular redox balance and protecting against oxidative stress. The System XC^-, comprised of two protein subunits—SLC7A11 (also known as xCT) and SLC3A2 (also known as 4F2hc)—is an amino acid transporter responsible for importing cystine into cells while exporting glutamic acid. Once inside the cell, cystine is reduced to cysteine, which serves as the limiting precursor for GSH synthesis. This reduction step is crucial for GSH production, as cysteine availability directly impacts GSH levels.

Another source of cysteine comes from the trans-sulfuration pathway, which is the main metabolic route for sulfur-containing amino acids. This pathway interconverts methionine and homocysteine, with cystathionine β-synthase catalyzing the formation of cysteine from homocysteine. The trans-sulfuration pathway thus contributes to the intracellular cysteine pool necessary for GSH synthesis.

GSH is essential for the activity of Glutathione Peroxidase 4 (GPX4), a selenium-containing enzyme that acts as a central inhibitor of iron death. GPX4 reduces toxic phospholipid hydroperoxides (PLOOH) to non-toxic phospholipid alcohols (PLOH), thereby preventing the accumulation of peroxidized lipids that can trigger iron death. Iron death is a form of programmed cell death characterized by iron-dependent lipid peroxidation and can be induced by inhibiting either System XC^- or GPX4.

Research on the role and regulation of iron death has largely utilized inhibitors of System XC^- (such as erastin, sulfasalazine, and sorafenib) or GPX4 inhibitors (such as RSL3, ML162, and ML210). These inhibitors help elucidate the mechanisms underlying iron death and its regulation. Interestingly, genetic exhaustion or inhibition of GPX4 in various mouse tissues leads to different death-related pathological phenotypes, not limited to iron death alone. Depending on the tissue and context, GPX4 deficiency can result in apoptosis, focal necrosis, or necrotizing apoptosis, highlighting the diverse roles of GPX4 in cellular survival and death pathways.

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In summary, GSH and GPX4 are integral components of cellular defense mechanisms against oxidative stress and programmed cell death. Their regulation and dysfunction are associated with various pathological conditions, making them potential targets for therapeutic intervention [5].

3. Iron death as a tumor suppressor event

Iron-mediated cell death, or ferroptosis, is a complex process involving multiple key molecular players and mechanisms. System xc^- facilitates the exchange of glutamate for cystine, thereby sustaining intracellular glutathione (GSH) levels. GSH acts as a crucial antioxidant, offering protection against oxidative damage. Glutathione peroxidase 4 (GPX4) utilizes GSH to inhibit the peroxidation of lipids. The compound elastin disrupts system xc^- function, leading to reduced cystine uptake and subsequent depletion of GSH levels, ultimately enhancing lipid peroxidation. Iron is absorbed through the transferrin receptor (TfR), and once inside the cell, it can generate reactive oxygen species (ROS) via the Fenton reaction, further promoting lipid peroxidation. Polyunsaturated fatty acids (PUFA) and arachidonic acid (AA) contribute to iron-mediated cell death by exacerbating lipid peroxidation. Antioxidants such as N-acetylcysteine (NAC) and the GSH/thioredoxin reductase system (TXNRD) can mitigate oxidative stress and thereby inhibit iron-induced cell death. The delicate equilibrium between lipid peroxidation and antioxidative defenses ultimately determines whether a cell will undergo iron-mediated death.

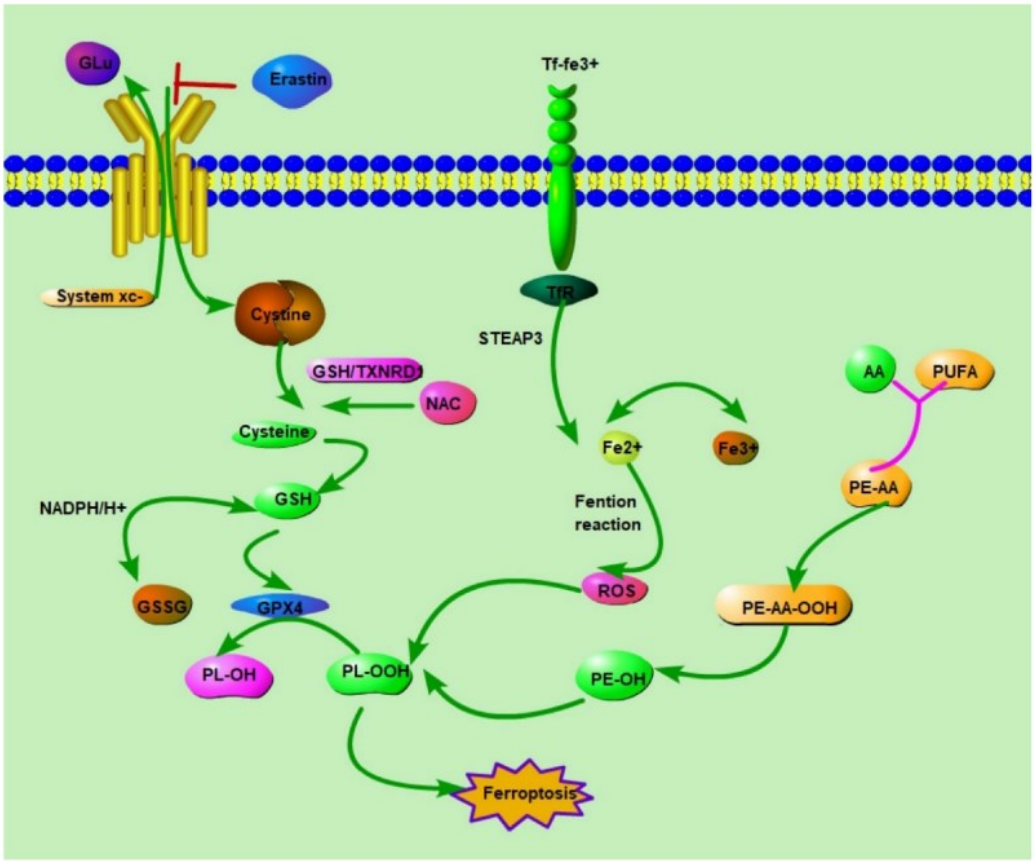

Figure 1: Key pathways and molecules involved in iron death in tumor cells [6].

A growing body of evidence indicates that iron death acts as a suppressor of tumors, influencing their cycle, proliferation, and progression. To date, aside from hematologic malignancies, hypoxia stands out as a distinctive characteristic of the tumor microenvironment (TME), prompting alterations

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in iron metabolism that subsequently impact the modulation of iron death. In hypoxic conditions, there's an elevation in the post-translational modification of iron regulatory protein 2, resulting in heightened expression of iron transporters and diminished expression of iron storage proteins. Carbonic anhydrase IX, pivotal in managing tumor hypoxia, sustains intracellular pH homeostasis by modulating the cystine/glutamate antiporter xCT, thereby shielding cancer cells from iron death. Research has revealed that the buildup of lactic acid within the TME, a byproduct of cancer cell glycolysis, can hinder iron death. Elevated lactic acid levels boost the expression of the hydroxycarboxylic acid receptor 1 and the monocarboxylic acid transporter 1, leading to decreased lipid peroxidation and suppression of iron death in hepatocellular carcinoma cells.

Moreover, inflammation associated with cancer in the TME can either facilitate or impede tumor iron death. Pro-inflammatory signals govern iron death across various cancer types through diverse signaling cascades. Recent investigations highlight a intricate interplay between iron death and immune effector cells within the TME, significantly influencing the efficacy of cancer immunotherapy.

To comprehensively unravel the intricate regulatory web of iron death in the TME, a multidisciplinary approach is imperative, incorporating computational biology, bioinformatics, and systems biology. These disciplines furnish robust tools and methodologies to decipher the complex interactions and pathways implicated in iron death, deepening our understanding of its role in oncology.

Computational biology employs mathematical modeling and computational algorithms to mimic biological phenomena. Within the context of iron death, such models can forecast how variations in lipid peroxidation, iron metabolism, and antioxidant defenses unfold under differing circumstances. By emulating these processes, scholars can pinpoint crucial regulatory hubs and prospective therapeutic interventions [7].

Bioinformatics harnesses computational strategies to interpret biological datasets. High-throughput techniques like genomics, transcriptomics, proteomics, and metabolomics generate vast amounts of data, which, when analyzed, can unveil the molecular underpinnings of iron death. Bioinformatics facilitates the identification of gene expression signatures, protein interactions, and metabolic shifts linked to iron death. For instance, integrating the transcriptomic profiles of colorectal cancer patients with the expression patterns of iron death-related genes can uncover biomarkers for patient subgrouping and predicting treatment outcomes.

The interplay between iron death and the TME is intricate and multidimensional, affecting not only the fate of tumor cells but also modulating immune cell functionality, thereby shaping tumor evolution and treatment responsiveness.

4. Application of iron death in CRC immunotherapy

In colorectal cancer (CRC), iron death exerts a dual impact, influencing both tumor cells and the tumor microenvironment (TME), thereby significantly affecting immunotherapy outcomes. Research has demonstrated that the expression of oncogenic fusion-associated arginine repeat proteins (OFARGs) is intimately linked to tumor responsiveness to immunotherapy. In immune desert type tumors, triggering iron death can augment the infiltration of immune cells, particularly CD8+ T lymphocytes, enhancing the tumor's immunogenicity. Conversely, in immune inflamed or immunologically rejected tumors, iron death might modulate the efficacy of immunotherapy by adjusting the quantity and functionality of immunosuppressive cells, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) [8].

Furthermore, the roles of co-activator-associated arginine methyltransferase 1 (CARM1) and heat shock protein family B (small) member 1 (HSPB1) in governing iron death underscore its significance within the tumor immune milieu. CARM1 fosters tumor advancement via methylating ACSL4;

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inhibiting CARM1 heightens tumor cells' susceptibility to iron death, promoting their immunogenic demise. Conversely, HSPB1 dampens iron death by curbing iron-induced reactive oxygen species (ROS) production. These discoveries highlight that targeting these molecules could fine-tune tumor cells' sensitivity to iron death, potentially boosting immunotherapy efficiency.

Integrating the notion of iron death into CRC immunotherapy strategies offers a novel avenue to augment treatment efficacy and surmount drug resistance. Studies exploring combinations of iron death inducers and immunotherapies reveal that paired drug regimens and nanotechnological approaches hold promise for amplifying antitumor immunity. A notable strategy involves concurrent delivery of dihydroartemisinin (DHA) and pyropentine (Pyro-Fe), which not only bolsters DHA's anticancer potency in vivo but also renders non-immunogenic CRC tumors susceptible to PD-L1 checkpoint blockade immunotherapy by boosting tumor immunogenicity. Additionally, nanotechnology applications introduce fresh prospects for precise targeting of iron death inducers, further enhancing therapeutic interventions [9].

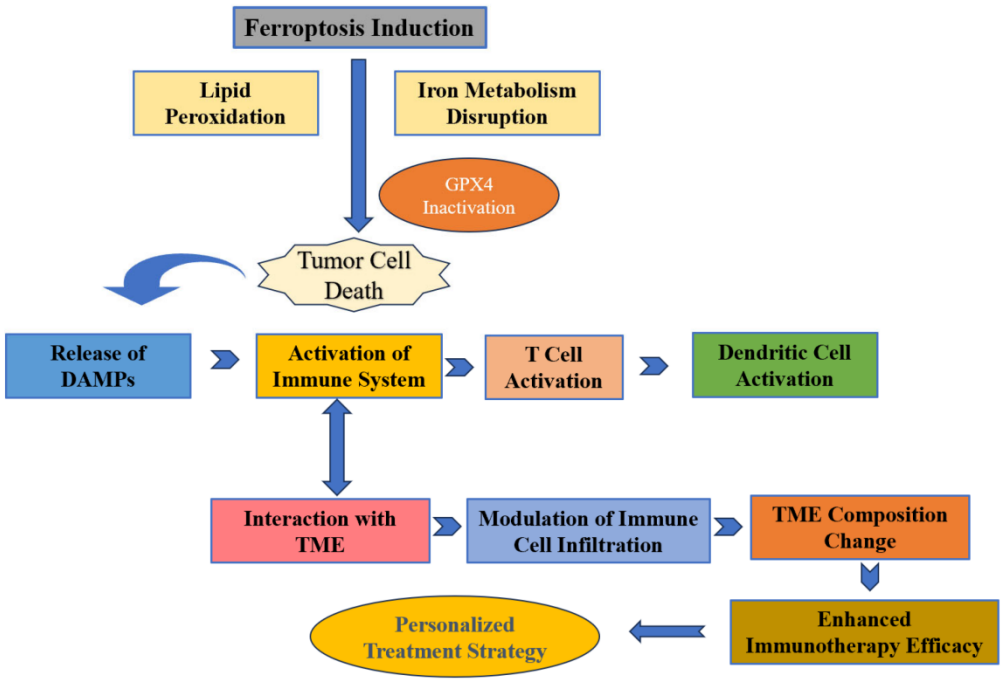

Figure 2: Ferroptosis and Immunotherapy [6].

Figure 2 outlines the series of events initiated by ferroptosis, illustrating its cascading effects on the tumor microenvironment (TME) and immunotherapy efficacy. Ferroptosis begins with the induction of lipid peroxidation and the inactivation of GPX4, culminating in programmed cell death specifically within tumor cells. This process results in the release of damage-associated molecular patterns (DAMPs), which serve as signals to activate the immune system. Consequently, there is an enhancement in T-cell and dendritic cell functionalities, fostering a more robust antitumor immune response. Additionally, these changes modulate the TME, creating a more favorable landscape for immunotherapy interventions. This sequence underscores the significance of ferroptosis as a novel mechanism of cell demise that holds promise for advancing personalized cancer treatment strategies, thereby garnering substantial interest in the field of oncology research [10].

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5. Conclusion

This review comprehensively analyzes the mechanisms, regulatory pathways, and therapeutic potential of ferroptosis. Ferroptosis plays a dual role in tumorigenesis, acting both as a tumor suppressor by inducing cancer cell death and as a tumor promoter under certain conditions in the TME. Key pathways such as glutathione depletion and GPX4 inhibition are highlighted as key mechanisms of ferroptosis regulation. In addition, the complex relationship between ferroptosis and TME components, including immune cells such as CD8+ T cells and immunosuppressive cells, highlights its key role in cancer progression and immunotherapy response. In tumors, ferroptosis has shown the potential to enhance tumor immunogenicity and immune cell infiltration, especially in immune-desert tumors, while also affecting immunosuppressive cell activity in immune-inflammatory and immune-rejecting tumors. Targeting key regulators such as ACSL4, CARM1, and HSPB1 to modulate ferroptosis sensitivity and improve the efficacy of immunotherapy. Furthermore, advances in nanotechnology and drug combinations, such as DHA and piroxicam (Pyro-Fe), highlight the potential of ferroptosis-based approaches to overcome drug resistance and enhance antitumor immune responses. These findings suggest that integrating ferroptosis into cancer treatment paradigms offers a promising avenue for improving treatment outcomes, especially in immunotherapy-resistant cancers.

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