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CD8+ T Cell-Induced Ferroptosis via a Positive Feedback Mechanism to Overcome Immune Resistance

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Abstract:

This research elucidates a novel strategy wherein CD8+ T cells overcome tumor immune evasion by initiating ferroptosis and establishing a self-sustaining positive feedback loop. Unlike traditional cytolytic pathways employed by CD8+ T cells (e.g., Fas-L/Fas and perforin/granzyme mechanisms), the interferon-gamma (IFN-γ)-facilitated ferroptosis pathway unveils a distinct immunoregulatory role. Ferroptosis is an iron-dependent, non-apoptotic form of cell death driven by excessive lipid peroxidation, loss of mitochondrial membrane integrity, and eventual plasma membrane disruption. Crucially, beyond direct tumor cell killing, the process leads to emission of damage-associated molecular patterns (DAMPs), which potently stimulate dendritic cell maturation and enhance antigen-specific T cell responses. This establishes a cyclic "immune activation--ferroptosis--immune reactivation" cascade, effectively alleviating immunosuppression within the tumor microenvironment (TME). Our comprehensive analysis demonstrates that CD8+ T cell-secreted IFN-γ downregulates SLC7A11, limiting cystine uptake and impairing glutathione (GSH) biosynthesis, thereby inactivating GPX4. Concurrently, IFN-γ upregulates ACSL4, promoting esterification of polyunsaturated fatty acids (PUFAs) into phospholipids and increasing susceptibility to lipid peroxidation. Together, these synergistic changes induce robust ferroptosis. This mechanism offers a transformative therapeutic perspective for tackling resistance to immune checkpoint inhibitors, with considerable theoretical and clinical implications for next-generation cancer immunotherapies.

Keywords: CD8+ T Cells; Interferon-gamma (IFN-γ); Ferroptosis; Glutathione (GSH); T Cell Dysfunction.

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1. Introduction

Cancer immunotherapy, particularly immune checkpoint blockade (ICB), has markedly improved outcomes for patients with various advanced cancers, representing a paradigm shift in oncology treatment. Nevertheless, primary and acquired resistance remain substantial challenges, constraining broader clinical application and leaving many patients without effective treatment options. Extensive research indicates that an immunosuppressive TME is a pivotal factor in ICB resistance, typified by exhausted effector T cells, infiltration of immunosuppressive cells (Tregs, MDSCs), and abundant inhibitory cytokines that collectively create a barrier to effective immune responses [1,2].

In recent years, ferroptosis has garnered considerable attention as a regulated cell death modality capable of circumventing apoptotic resistance mechanisms that often develop in treatment-resistant tumors [3]. It is molecularly characterized by collapse of the antioxidant system, especially dysregulation of glutathione (GSH) metabolism and inactivation of glutathione peroxidase 4 (GPX4), leading to iron-dependent lipid peroxide accumulation [4]. Of particular note, $CD8^+$ T cells are instrumental in inducing ferroptosis within the tumor microenvironment [5]. Upon antigen-specific activation, these cells secrete IFN-γ, which modulates the SLC7A11-GSH-GPX4 axis and ACSL4-mediated lipid metabolic reprogramming, culminating in ferroptosis. Simultaneously, ferroptotic cells release DAMPs that stimulate innate immunity, forming a positive feedback loop that enhances antitumor immunity through epitope spreading and enhanced antigen presentation.

This article systematically investigates the molecular mechanism whereby $CD8^+$ T cells, via IFN-γ signaling, trigger ferroptosis to reverse immune resistance, emphasize the crucial role of metabolic reprogramming and immunogenic cell death in reshaping the TME, offering a compelling rationale for combining ferroptosis inducers with existing immunotherapies to overcome treatment resistance. Additionally, it explores the translational potential of targeting this pathway and discusses challenges in application.

2. Core Mechanism of $CD8^+$ T Cell-Induced Ferroptosis

The induction of ferroptosis by $CD8^+$ T cells is a multistep, finely regulated process involving sophisticated immune and metabolic crosstalk. As depicted in Figure 1, the core mechanism comprises three interlinked phases that create a self-reinforcing cycle of tumor cell elimination and immune activation:

First, antigen-stimulated $CD8^+$ T cells release IFN-γ, which binds to interferon receptors on tumor cells and suppresses expression of SLC7A11 through STAT1-mediated transcriptional regulation [5]. This cystine/glutamate antiporter subunit is critical for cystine uptake; its downregulation impairs GSH synthesis by limiting the availability of cysteine, a rate-limiting precursor for glutathione production. Given that GSH serves as an essential cofactor for GPX4 activity, its depletion leads to GPX4 functional inactivation [3,4], resulting in accumulation of lipid peroxides and subsequent induction of ferroptosis. This process is further amplified by the generation of reactive oxygen species through Fenton chemistry in the iron-rich tumor environment.

Second, IFN-γ signaling upregulates acyl-CoA synthetase long-chain family member 4 (ACSL4) through IRF1-mediated transcriptional activation. This enzyme catalyzes the incorporation of PUFAs into membrane phospholipids, particularly phosphatidylethanolamines [6]. The PUFA-phospholipids are highly susceptible to peroxidation by lipoxygenases, further driving the ferroptotic process through the generation of lipid hydroperoxides that disrupt cellular membrane integrity and function [3].

Third, cells undergoing ferroptosis release DAMPs—such as high-mobility group box 1 (HMGB1) [8], adenosine triphosphate (ATP), and heat-shock proteins—which activate dendritic cells via pattern recognition receptors (e.g., TLR4, P2X7) [7]. This enhances antigen cross-presentation through MHC class I molecules and promotes naïve T cell activation and differentiation into effector cells, effectively reversing local immunosuppression and establishing a reinforcing cycle of immunogenic cell death that expands the antitumor immune repertoire.

This multifaceted mechanism not only directly eliminates tumor cells but also remodels the TME to support sustained anti-tumor immunity through the creation of a more favorable immune contexture characterized by enhanced T cell infiltration and function.

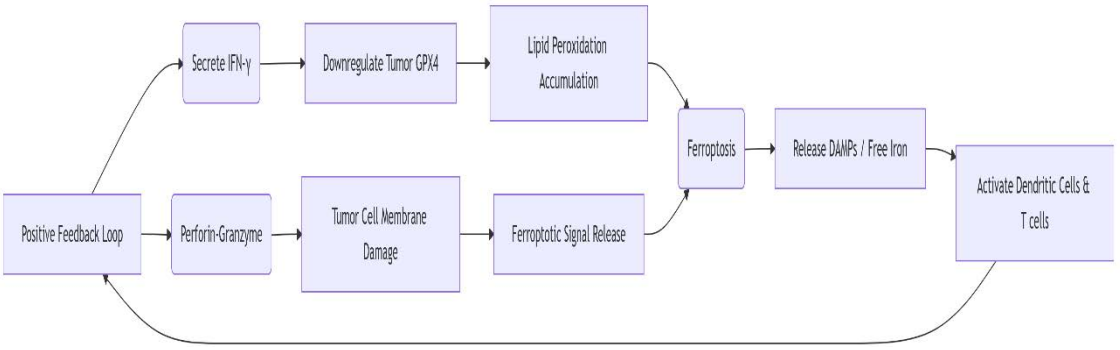

Fig. 1 Core Pathway of Ferroptosis Induction by CD8$^+$ T Cells

3. Implications of Ferroptosis in Overcoming Immune Resistance

Ferroptosis represents a promising means to counteract resistance to established cancer therapies, including immunotherapy. Mounting evidence indicates that the CD8$^+$ T cell/IFN-γ/ferroptosis axis can eradicate tumor cells that evade apoptotic stimuli, showing remarkable efficacy even in models resistant to immune checkpoint inhibition. Beyond direct cytotoxicity, ferroptosis fundamentally modifies the TME through DAMP release, which recruits and activates T cells and attenuates immune tolerance by reversing exhaustion phenotypes. The immunogenic nature of ferroptosis distinguishes it from other cell death modalities and provides a unique opportunity to overcome the immunosuppressive barriers that limit current immunotherapies.

Therefore, targeting ferroptosis—particularly in combination with ICB—holds significant potential for overcoming resistance. For instance, ferroptosis inducers such as RSL3 and erastin synergize with anti-PD-1/PD-L1 therapy, yielding tumor suppression rates exceeding 80% in otherwise refractory models, compared to less than 40% with monotherapies. This synergistic effect is particularly pronounced in tumors with high metastatic potential and those located in immune-excluded anatomical sites where traditional therapies show limited efficacy.

4. Mechanisms Underlying Immune Resistance and the Counteractive Role of Ferroptosis

Resistance to immunotherapy can be primary or acquired and involves diverse mechanisms that collectively create a formidable barrier to treatment success:

4.1 T Cell Exhaustion

Chronic antigen exposure in the TME leads to overexpression of multiple inhibitory receptors (e.g., PD-1, TIM-3, LAG-3, TIGIT), diminishing T cell effector function through epigenetic and metabolic reprogramming. Studies indicate that in non-small cell lung cancer (NSCLC) patients, the proportion of PD-1$^+$ exhausted T cells can be as high as 40-60%, and is significantly associated with poor response to anti-PD-1 therapy. Single-cell sequencing data further reveal that exhausted T cells co-express multiple inhibitory receptors and exhibit transcriptional programs distinct from functional effector cells, with their capacity to secrete effector molecules (e.g., IFNγ, TNFα, granzyme B) reduced by over 70% [1].

4.2 Immunosuppressive Microenvironment

Infiltration of Tregs and MDSCs, together with immunosuppressive cytokines (e.g., TGF-β, IL-10, IL-35), creates a formidable barrier to effector T cell activity. In some tumor types (e.g., pancreatic cancer), Tregs can constitute over 50% of CD4$^+$ T cells, and a Treg/CD8$^+$ T cell ratio higher than 0.5 is considered a key indicator of immunosuppression and poor prognosis. MDSCs consume arginine in the microenvironment via arginase-1 (Arg-1) and inducible nitric oxide synthase (iNOS), directly inhibiting T cell proliferation and function through nutrient deprivation and production of inhibitory mediators; experimental data show they can suppress approximately 60-80% of T cell responses in advanced tumors [2].

4.3 Defective Antigen Presentation

Tumor cells often downregulate MHC class I molecules and components of the antigen processing machinery to evade immune recognition. Approximately 30%-50% of solid tumors exhibit downregulation or loss of MHC class I expression, especially melanoma and colorectal cancer, through various mechanisms including β2-microglobulin mutations and epigenetic silencing [1]. CRISPR screening studies confirm that restoring MHC-I expression in tumor cells can increase T cell-mediated killing efficiency by 3-5

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times, highlighting the critical importance of this resistance mechanism.

4.4 Metabolic Interference

Tumor cells outcompete T cells for essential nutrients (e.g., tryptophan, arginine, glucose) and produce inhibitory metabolites like adenosine that create a metabolically hostile environment. The IDO1 enzyme, highly expressed by tumor cells and stromal elements, catalyzes the breakdown of tryptophan into kynurenine, which can induce T cell apoptosis and promote Treg differentiation. Analysis of clinical samples shows that a tryptophan/kynurenine ratio below 10 in tumor tissue often predicts poor response to immunotherapy. Furthermore, extracellular ATP is hydrolyzed to adenosine by the CD39/CD73 enzyme pair expressed on tumor cells and suppressive immune cells, inhibiting TCR signaling and promoting exhaustion; adenosine concentration in the tumor interstitium can reach 20-100 μM, sufficient to inhibit 90% of T cell activation through A2A receptor signaling [1].

4.5 Low Mutational Burden

Tumors with few mutations generate insufficient neoantigens to elicit a robust T cell response, limiting the available targets for immune recognition. Studies show that patients with a tumor mutational burden (TMB) higher than 10 mut/Mb can achieve an objective response rate (ORR) of 40-50% to ICB, while the ORR for patients with low TMB (<5 mut/Mb) is typically below 10%, highlighting the importance of antigen abundance in immunotherapy success [1].

Recent research has found that ferroptosis can reverse the above resistance mechanisms by inducing immunogenic cell death (ICD) and releasing DAMPs, enhancing antigen presentation and T cell priming through multiple parallel pathways. For example, in animal models, inducing ferroptosis can increase $CD8^+$ T cell infiltration within tumor tissue by 2-3 times and significantly reduce the Treg proportion (from ~25% to ~10%), effectively altering the immune contexture toward a more permissive state. Additionally, HMGB1 released by ferroptotic cells activates TLR4 signaling in dendritic cells, increasing IL-12 secretion by 4-5 times, strongly promoting T cell priming and Th1 polarization [8]. Therefore, combining ferroptosis inducers (e.g., RSL3, imidazole ketone erastin) with anti-PD-1 antibodies shows synergistic effects in various ICB-resistant models; the tumor inhibition rate in the combination treatment group exceeded 80%, while monotherapy groups were below 40%. These data highlight the great potential of targeting the ferroptosis pathway to overcome immune resistance through multimodal mechanisms [5,7].

5. Future Research Directions and Clinical Outlook

The $CD8^+$ T cell-ferroptosis axis provides a new framework for overcoming resistance to immunotherapy that merges metabolic targeting with immune activation. Central to this mechanism is the targeted exploitation of metabolic vulnerabilities in tumor cells (e.g., the SLC7A11-GSH-GPX4 axis) while simultaneously activating innate immunity to create a potent positive feedback loop that becomes self-sustaining.

Future investigations should prioritize several key areas:

Developing potent yet safe ferroptosis inducers with favorable pharmacokinetic properties and optimizing the combination and sequencing with ICB agents to maximize synergy while minimizing toxicity. The timing of administration may be critical, with sequential administration potentially superior to concurrent treatment in certain contexts.

Identifying predictive biomarkers—such as expression levels of ACSL4 and SLC7A11, IFN-γ signaling signatures, T cell infiltration status, and iron metabolism gene profiles—to guide patient selection and personalize treatment approaches. Multiplex immunohistochemistry and gene expression profiling may help identify patients most likely to benefit from combination strategies.

Elucidating mechanisms of tumor cell resistance to ferroptosis inducers, including compensatorypathways-liketheFSP1-CoQ10system, GTPcyclohydrolase-1-tetrahydrobiopterin pathway, and dihydroorotate dehydrogenase-mediated antioxidant defense, which may represent additional therapeutic targets [9,10].

Exploring the contributions of other immune cells (e.g., NK cells, macrophages, γδ T cells) to ferroptosis regulation to develop more comprehensive combination strategies that engage multiple arms of the immune system. Macrophages in particular may play dual roles in both inducing and resisting ferroptosis depending on their polarization state [11].

These endeavors will accelerate the clinical translation of ferroptosis-targeted therapies and potentially establish new standards of care for patients with immunotherapy-resistant cancers.

6. Conclusion

This study delineates a positive feedback mechanism whereby $CD8^+$ T cells alleviate immune resistance by inducing ferroptosis. Key observations include IFN-γ–mediated suppression of SLC7A11 and augmentation of

ACSL4, leading to GPX4 dysfunction and lethal lipid peroxidation. Moreover, DAMPs released during ferroptosis reignite antitumor immunity by activating dendritic cells and facilitating T cell priming.

More importantly, damage-associated molecular patterns (DAMPs) released by ferroptotic cells, such as HMGB1 and ATP, can effectively activate dendritic cells, promote antigen presentation and T cell priming, and reverse the immunosuppressive state of the tumor microenvironment. This process forms a positive feedback cycle of "immune activation-ferroptosi-immune re-activation," significantly enhancing the anti-tumor immune response.

From a theoretical perspective, this research reveals a new pathway by which $CD8^+$ T cells directly regulate tumor cell death modes, enriching the theoretical system of cross-regulation between immune cells and cell death in tumor immunology. In terms of clinical translation, targeting the ferroptosis pathway, particularly the combination of immune checkpoint inhibitors with ferroptosis inducers, represents a highly promising therapeutic strategy expected to overcome current challenges of resistance in immunotherapy. This strategy may demonstrate better therapeutic efficacy for patient populations with tumors exhibiting high ACSL4 expression and low SLC7A11 expression.

Areas for further development include developing highly efficient and low-toxicity ferroptosis inducers and optimizing the combination regimens with existing immunotherapies; establishing reliable biomarker systems for patient stratification; deeply exploring tumor cell resistance mechanisms to ferroptosis induction; and evaluating the safety and potential side effects of long-term ferroptosis induction. Through these efforts, the $CD8^+$ T Cell-Ferroptosis Axis is expected to become an important target for next-generation cancer immunotherapy, providing new solutions for overcoming immune resistance.

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