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A Mathematical and Systems Biology Framework Reveals the Regulatory Role of the SIRT1/FOXO1 Axis in Ferroptosis under Ischemia-Reperfusion Injury

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Abstract

Ischemia-reperfusion injury is a critical pathological process in flap transplantation, organ transplantation, and tissue repair, with ferroptosis recognized as one of the key mechanisms underlying irreversible cellular damage. To elucidate the role of the SIRT1/FOXO1 axis in ferroptosis regulation, this study constructed a ferroptosis regulatory network under ischemia-reperfusion conditions based on transcriptomic data analysis, systems biology, and mathematical modeling approaches. Through differential expression analysis, screening of ferroptosis-related genes, GO/KEGG enrichment analysis, and protein interaction network analysis, core regulatory nodes were identified. A ordinary differential equation model was further established to describe SIRT1-mediated deacetylation of FOXO1 and its regulation of anti-ferroptosis factors such as GPX4, SLC7A11, and FTH1. The results demonstrate that SIRT1 inhibits the ferroptosis process by promoting intranuclear activation of FOXO1, enhancing glutathione metabolism, lipid peroxide clearance, and iron homeostasis maintenance. When SIRT1 activity declines, lipid peroxide accumulation accelerates, potentially causing the system to cross a kinetic threshold and enter an irreversible damage state. This study provides a theoretical explanation for the ferroptosis regulatory mechanism in ischemia-reperfusion injury and offers a basis for targeting the SIRT1/FOXO1 axis to enhance flap survival.

Keywords: SIRT1; FOXO1; ferroptosis; systems biology; graph theory; ordinary differential equations3.

Introduction

ischemia-reperfusion injury (IRI) is a fundamental pathological process widely encountered in clinical practice and biomedical research, particularly in flap transplantation, organ transplantation, myocardial infarction, stroke, and reconstructive surgery [1]. During the ischemic phase, insufficient oxygen and nutrient supply leads to metabolic disruption, mitochondrial dysfunction, and ATP depletion. Upon reperfusion, the sudden restoration of blood flow paradoxically exacerbates cellular injury through excessive production of reactive oxygen species (ROS), inflammatory cascades, calcium overload, and endothelial dysfunction. These events collectively contribute to tissue necrosis, functional impairment, and even failure of therapeutic interventions.

Accumulating evidence indicates that multiple forms of regulated cell death are involved in IRI, including apoptosis, necroptosis, autophagy, and pyroptosis. Among these, ferroptosis has recently emerged as a critical contributor to IRI-associated tissue damage [2]. Ferroptosis is a distinct form of regulated cell death characterized by iron-dependent lipid peroxidation and oxidative membrane damage. Unlike apoptosis or necrosis, ferroptosis is driven by the imbalance between lipid peroxide generation and detoxification. The expansion of the intracellular labile iron pool promotes the Fenton reaction, generating highly reactive hydroxyl radicals that initiate lipid peroxidation of polyunsaturated fatty acids. When lipid peroxides accumulate beyond the detoxification capacity of antioxidant systems, particularly glutathione peroxidase 4 (GPX4), irreversible membrane damage occurs, ultimately leading to cell death.

The regulation of ferroptosis involves a complex network of metabolic and signaling pathways, including iron metabolism, lipid metabolism, and redox homeostasis [3]. Key molecular components such as solute carrier family 7 member 11 (SLC7A11), GPX4, and ferritin heavy chain 1 (FTH1) play essential roles in maintaining cellular resistance to ferroptosis by controlling cystine uptake, glutathione synthesis, and iron storage, respectively. Dysregulation of these pathways under IRI conditions leads to excessive lipid peroxidation and ferroptotic cell death, highlighting the need to understand upstream regulatory mechanisms [4]. Sirtuin 1 (SIRT1), a nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylase, is a central regulator of cellular stress responses [5]. It has been extensively implicated in modulating oxidative stress, mitochondrial biogenesis, inflammation, and cell survival [6]. Forkhead box O1 (FOXO1), a key transcription factor of the FOXO family, regulates genes involved in antioxidant defense, metabolism, and cell cycle control [7]. Previous studies have suggested that SIRT1 can enhance FOXO1 transcriptional activity through deacetylation, thereby influencing downstream antioxidant and metabolic pathways [8]. Emerging evidence further indicates that FOXO1 may regulate the expression of ferroptosis-related genes such as GPX4, SLC7A11, and FTH1[9]. However, despite these findings, the system-level regulatory mechanism by which the SIRT1/FOXO1 axis modulates ferroptosis under IRI conditions remains poorly understood [10].

Conventional bioinformatics approaches, including differential gene expression analysis, functional enrichment, and pathway annotation, have provided valuable insights into molecular alterations associated with IRI [11]. However, these methods are largely static and correlation-based, limiting their ability to capture the nonlinear dynamics and critical transitions inherent in ferroptosis. In particular, the shift from a compensatory state to an irreversible ferroptotic state involves complex feedback loops, threshold effects, and dynamic interactions that cannot be fully explained by traditional analyses [12,13]. To address these limitations, integrative approaches combining sys-

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tems biology and mathematical modeling have gained increasing attention. Mathematical models, particularly those based on ordinary differential equations (ODEs), enable the quantitative description of biochemical reactions and regulatory interactions over time. Such models are capable of revealing system stability, bifurcation behavior, and critical thresholds, thereby providing mechanistic insights into disease progression and therapeutic intervention.

In this study, we developed a comprehensive systems biology framework to investigate ferroptosis regulation in IRI. High-dimensional transcriptomic data were analyzed using linear modeling and empirical Bayes methods to identify differentially expressed genes. Ferroptosis-related genes were obtained through set intersection analysis, followed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment using hypergeometric distribution models. A protein–protein interaction (PPI) network was constructed to identify key regulatory nodes based on graph-theoretical metrics, including degree centrality, betweenness centrality, and closeness centrality. Furthermore, we established an ODE-based dynamic model describing the SIRT1-mediated deacetylation of FOXO1 and its downstream regulation of GPX4, SLC7A11, FTH1, and lipid peroxides.

The objectives of this study are fourfold: (1) to improve the robustness of differential expression analysis under small-sample conditions using empirical Bayes statistics; (2) to identify ferroptosis-related genes associated with IRI; (3) to determine key regulatory nodes within the ferroptosis network using graph theory; and (4) to construct a dynamic model of the SIRT1/FOXO1–GPX4/SLC7A11/FTH1–lipid peroxidation axis to provide a mathematical explanation for the transition from reversible cellular stress to irreversible ferroptosis.

Materials and Methods

Study Design: This study employed an integrative strategy combining theoretical modeling with systems biology to investigate the regulatory mechanism of ferroptosis under ischemia–reperfusion conditions. The overall workflow consisted of five interconnected components, including transcriptomic differential expression analysis, identification of ferroptosis-related genes, functional enrichment analysis, protein-protein interaction (PPI) network construction, and dynamic mathematical modeling. Briefly, microarray or RNA-seq datasets were first used to construct gene expression matrices, in which the expression level of each gene across samples was treated as a stochastic variable. Differential expression analysis between ischemia–reperfusion and control groups were then performed using an empirical Bayes framework to improve statistical robustness under small-sample conditions [14]. Subsequently, differentially expressed genes were intersected with known ferroptosis-related genes obtained from curated databases to generate a subset of ferroptosis-associated differentially expressed genes. Functional enrichment analysis, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG), was conducted to identify significantly enriched biological processes and pathways. Furthermore, a PPI network was constructed and analyzed using graph-theoretical approaches to identify key regulatory nodes. Finally, a system of nonlinear ordinary differential equations (ODEs) was established to describe the dynamic regulation of lipid peroxidation mediated by the SIRT1/FOXO1 signaling axis.

Differential Expression Analysis Based on Empirical Bayes Model: Let Y_{gj} denote the normalized log-expression value of gene *g* in sample *j*, where *g*=1,…,*G* and *j*=1,…,*n*. For each gene, a linear model was constructed as:

$$E(Y_{gj}) = X\alpha_g$$

where *X* represents the design matrix and denotes the gene-specific parameter vector. The contrast of interest, representing the expression difference between ischemia–reperfusion and control conditions, is defined as:

$$\beta_g = C^T \alpha_g$$

The estimated coefficient $\hat{\beta}_g$ and corresponding sample variance s_g^2 were obtained via ordinary least squares estimation. However, due to the limited sample size commonly encountered in transcriptomic studies, gene-wise variance estimates are often unstable and prone to inflation of false positives [15]. To address this issue, an empirical Bayes variance shrinkage approach was adopted, whereby individual gene variances were moderated toward a pooled prior estimate [16]:

$$\tilde{s}_g^2 = \frac{d_g s_g^2 + d_0 s_0^2}{d_g + d_0}$$

where d_g is the gene-specific degrees of freedom, s_0^2 is the prior variance, and d_0 represents the prior degrees of freedom. Based on the moderated variance, a moderated t-statistic was constructed:

$$\tilde{t}_g = \frac{\hat{\beta}_g}{\tilde{s}_g c_g}$$

where c_g is a constant determined by the design matrix. Differentially expressed genes were identified using the criteria $|\log_2 FC| \geq 1$ and false discovery rate FDR < 0.05. This approach effectively improves statistical power and reduces bias in small-sample transcriptomic analyses.

Identification of Ferroptosis-Related Differentially Expressed Genes: To identify candidate genes specifically associated with ferroptosis under ischemia-reperfusion conditions, a set-theoretic intersection approach was employed [17]. Let S_{DEG} denote the set of differentially expressed genes and S_{FRG} represent the set of ferroptosis-related genes obtained from public databases. The target gene set was defined as:

$$S_{target} = S_{DEG} \cap S_{FRG}$$

This step effectively reduces the high-dimensional gene space to a biologically relevant subset, thereby enhancing interpretability and reducing downstream computational complexity.

Functional Enrichment Analysis: Functional enrichment analysis was conducted to explore the biological significance of the identified ferroptosis-related genes [18]. The statistical significance of enrichment was evaluated using a hypergeometric distribution model [19]. Specifically, let *N* denote the total number of genes in the background genome, *M* the number of genes associated with a given pathway, *n* the size of the target gene set, and *k* the number of genes overlapping between the target set and the pathway. The probability of observing at least *k* overlapping genes is given by:

$$P(X \geq k) = 1 - \sum_{i=0}^{k-1} \frac{\binom{M}{i}\binom{N-M}{n-i}}{\binom{N}{n}}$$

Multiple testi false discovery rate, and pathways with FDR < 0.05 were considered significantly enriched. This analysis enabled the identification of key biological processes and pathways, including oxidative stress response, lipid metabolism, HIF-1 signaling, and ferroptosis-related mechanisms.

Protein-Protein Interaction Network and Graph-Theoretical Analysis: The ferroptosis-related gene set was mapped onto a protein–protein interaction network, which can be represented as a graph *G = (V, E)*, where *V* denotes the set of nodes (proteins/genes) and *E* represents the set of edges corresponding to functional or physical interactions [20]. To identify key regulatory nodes, three centrality measures were computed [21]. Degree centrality was defined as

$$C_D(v) = \deg(v) = \sum_{j=1}^{|V|} A_{vj}$$

reflecting the number of direct interactions. Betweenness centrality was calculated as $C_B(v) = \sum_{s \neq v \neq t} \frac{\sigma_{st}(v)}{\sigma_{st}}$, where σ_{st} denotes the total number of shortest paths between nodes *s* and *t*, and $\sigma_{st}(v)$ represents those passing through node *v*, indicating its role in information flow. Closeness centrality was defined as $C_C(u) = \frac{n-1}{\sum_{v=1}^{n-1} d(u,v)}$, measuring the efficiency of information dissemination across the network. These metrics collectively enabled the identification of hub genes and critical regulatory nodes.

Dynamic Modeling of SIRT1/FOXO1-Mediated Ferroptosis Regulation: To quantitatively describe the regulatory dynamics of ferroptosis, a nonlinear ODE-based model was constructed, integrating three core modules: SIRT1-mediated FOXO1 activation, FOXO1-dependent transcriptional regulation, and lipid peroxidation dynamics. Under ischemia–reperfusion stress, SIRT1 promotes the deacetylation and activation of FOXO1, which can be described as [22]:

$$\frac{d[FOXO_{act}]}{dt} = k_{deac}[FOXO_{ac}] - k_{deg}[FOXO_{act}]$$

Activated FOXO1 subsequently regulates the expression of anti-ferroptotic genes, including GPX4, which can be modeled using a Hill function:

$$\frac{d[GPX4]}{dt} = \frac{V_{max}[FOXO_{act}]^n}{K_d^n + [FOXO_{act}]^n} - \gamma[GPX4]$$

Finally, lipid peroxidation dynamics were modeled as the balance between production and clearance processes:

$$\frac{d[LPO]}{dt} = k_{Fenton}\frac{[Fe^{2+}]}{1+K_a[Fe^{2+}]}[PUFA][H_2O_2] + k_{LOX}[LOX][PUFA] - k_{cat}[GPX4][LPO]$$

This model captures the essential condition for ferroptosis initiation, namely that sustained lipid peroxide production exceeds GPX4-mediated detoxification, leading to irreversible accumulation of oxidative damage.

Results

Empirical Bayes Model Improves the Stability of Differential Gene Screening: The application of the empirical Bayes variance shrinkage approach resulted in a more stable and reliable identification of differentially expressed genes under small-sample conditions. Compared with conventional t-test-based methods, which rely solely on gene-wise variance estimates, the empirical Bayes framework effectively integrates global variance information across all genes, thereby reducing the influence of extreme or noisy variance estimates. Specifically, genes with artificially inflated variance under traditional methods exhibited moderated variance estimates after shrinkage, leading to a more balanced statistical distribution of test statistics. This moderation reduced the occurrence of spurious high fold-change genes driven by stochastic fluctuations. In contrast, genes with consistent expression differences across samples were preserved and more confidently identified as differentially expressed.

These observations suggest that the empirical Bayes model enhances both the sensitivity and specificity of differential gene screening. Importantly, the stabilized variance estimation provides a robust statistical foundation for downstream analyses, particularly in the context of ischemia–reperfusion transcriptomic data, where sample size is often limited and biological variability is high.

Ferroptosis-Related Gene Mapping Reduces the High-Dimensional Search Space: To improve biological interpretability, differentially expressed genes were intersected with ferroptosis-related gene sets, resulting in a refined subset of candidate genes [23]. This mapping process significantly reduced the dimensionality of the dataset while preserving genes most relevant to ferroptosis mechanisms [24]. From a systems perspective, this dimensionality reduction serves two key functions. First, it minimizes the influence of unrelated transcriptional noise, which is particularly important in high-throughput datasets where irrelevant signals may obscure meaningful biological patterns. Second, it enriches the dataset for genes directly involved in iron metabolism, oxidative stress regulation, and lipid peroxidation, thereby enhancing the mechanistic relevance of subsequent analyses. Notably, the resulting ferroptosis-related gene subset demonstrated more coherent functional patterns compared with the original differentially expressed gene set. This indicates that the intersection strategy effectively isolates a functionally connected gene module that is likely to play a central role in ischemia–reperfusion-induced ferroptosis.

Functional Enrichment Reveals Coordinated Activation of FOXO Signaling and Lipid Peroxidation Pathways: Functional enrichment analysis based on the hypergeometric distribution revealed that ferroptosis-related differentially expressed genes were significantly enriched in multiple biological pathways, including oxidative stress response, lipid metabolism, HIF-1 signaling, FOXO signaling, inflammatory response, and cell death pathways. The enrichment of oxidative stress and lipid metabolism pathways suggests that redox imbalance and membrane lipid remodeling are key events in ischemia-reperfusion injury. In particular, the significant enrichment of ferroptosis-related pathways supports the hypothesis that lipid peroxidation is a central downstream event driving cellular damage.

Importantly, the enrichment of the FOXO signaling pathway highlights a potential upstream regulatory mechanism. FOXO transcription factors are known to regulate antioxidant defense systems, and their activation may represent a compensatory response to oxidative stress. The concurrent enrichment of FOXO signaling and ferroptosis pathways indicates that these processes are not independent but rather interconnected components of a broader regulatory network. Furthermore, the involvement of HIF-1 signaling suggests a link between hypoxia adaptation and ferroptosis regulation, reflecting the complex interplay between oxygen availability, metabolic reprogramming, and oxidative damage. Collectively, these results indicate that ischemia-reperfusion injury is governed by a coordinated, multi-pathway regulatory system rather than a single linear process.

PPI Network Analysis Identifies SIRT1 and FOXO1 as Central Regulatory Hubs: The PPI network analysis revealed a non-random topological structure characterized by the presence of highly connected hub nodes. Among these, SIRT1 and FOXO1 exhibited elevated degree centrality, betweenness centrality, and closeness centrality, indicating their critical roles in maintaining network connectivity and regulating information flow. High betweenness centrality suggests that SIRT1 and FOXO1 act as bridging nodes connecting multiple functional modules, including oxidative stress regulation, metabolic pathways, inflammatory signaling, and ferroptosis defense mechanisms. This topological position implies that perturbations in SIRT1/FOXO1 activity could have widespread effects on multiple downstream processes.

In contrast, GPX4, SLC7A11, and FTH1 displayed network characteristics consistent with downstream effector genes. GPX4 directly mediates the reduction of lipid peroxides, SLC7A11 supports glutathione synthesis by facilitating cystine uptake, and FTH1 regulates intracellular iron storage to limit free iron availability. From a hierarchical

network perspective, these findings suggest that the SIRT1/FOXO1 axis functions as an upstream regulatory layer, while GPX4/SLC7A11/FTH1 constitute a downstream execution module responsible for suppressing ferroptosis. This layered architecture provides a mechanistic framework linking transcriptional regulation to biochemical processes involved in ferroptotic cell death.

ODE Model Reveals a Nonlinear Transition and Bifurcation-Like Behavior in Ferroptosis Dynamics: The ODE-based dynamic model revealed that ferroptosis progression is governed by nonlinear interactions between lipid peroxide generation and antioxidant defense mechanisms. Under conditions of high SIRT1 activity, FOXO1 deacetylation and activation are enhanced, leading to upregulation of GPX4, SLC7A11, and FTH1. In this state, the system maintains a dynamic equilibrium in which lipid peroxide generation is effectively counterbalanced by detoxification processes. Mechanistically, three coordinated protective pathways are activated: (1) GPX4-mediated reduction of lipid peroxides, (2) SLC7A11-dependent maintenance of glutathione synthesis, and (3) FTH1-mediated sequestration of intracellular iron, which reduces substrate availability for the Fenton reaction. Together, these mechanisms maintain lipid peroxide levels below a critical threshold.

However, when SIRT1 activity decreases under ischemia–reperfusion stress, the system undergoes a qualitative shift. Reduced FOXO1 activation leads to decreased expression of GPX4, SLC7A11, and FTH1, thereby weakening antioxidant capacity. As a result, lipid peroxide generation gradually exceeds the clearance capacity, leading to progressive accumulation. Notably, the model suggests the existence of a threshold-like transition resembling a bifurcation point. Once the system crosses this critical threshold, lipid peroxides accumulate rapidly, and the system transitions from a stable state to an irreversible ferroptotic state. Importantly, this transition is self-sustaining, meaning that even if external stress signals do not further increase, the system may remain locked in a high-LPO state.

These findings indicate that ferroptosis is not a simple linear process but rather a nonlinear dynamic transition driven by the imbalance between oxidative damage and antioxidant defense. This bifurcation-like behavior provides a theoretical explanation for the sudden and irreversible nature of cellular damage observed in ischemia-reperfusion injury.

Discussion

In the present study, we proposed an integrative theoretical framework to investigate the potential regulatory role of the SIRT1/FOXO1 axis in ischemia-reperfusion injury-associated ferroptosis. By combining empirical Bayes-based transcriptomic analysis, ferroptosis-related gene mapping, functional enrichment, PPI network topology, and nonlinear ordinary differential equation modeling, this study provides a systems-level interpretation of how ferroptosis may be regulated under ischemia–reperfusion conditions. Unlike conventional bioinformatics studies that primarily focus on differentially expressed genes and pathway annotation, our framework emphasizes the relationship between statistical gene screening, network organization, and dynamic system behavior. This approach allows ferroptosis to be understood not merely as the result of isolated molecular alterations, but as a nonlinear pathological transition driven by the imbalance between lipid peroxide generation and antioxidant defense.

A major methodological feature of this study is the use of the empirical Bayes model for differential expression analysis. Transcriptomic studies related to ischemia-reperfusion injury often suffer from limited sample size, biological heterogeneity, and high-dimensional gene expression features. Under such conditions, conventional gene-wise t-tests may generate unstable variance estimates, resulting in false-positive or false-negative findings. The empirical Bayes approach partially overcomes this limitation by borrowing information across the whole genome and shrinking gene-specific variance estimates toward a pooled prior distribution. This process improves the stability of statistical inference and enhances the reliability of downstream gene selection. Therefore, the use of empirical Bayes statistics is particularly suitable for ischemia–reperfusion-related omics studies, in which sample availability is frequently limited because of the complexity of clinical sampling, animal modeling, and tissue preservation.

The intersection between differentially expressed genes and ferroptosis-related genes further improves the biological specificity of the analysis. In high-throughput transcriptomic datasets, a large number of differentially expressed genes may reflect secondary, background, or nonspecific stress responses rather than mechanisms directly involved in ferroptosis. By mapping DEGs onto curated ferroptosis-related gene sets, the candidate gene space is reduced from a genome-wide scale to a more mechanistically focused subset. This strategy allows the analysis to concentrate on molecular processes closely associated with iron metabolism, lipid peroxidation, glutathione metabolism, oxidative stress, and antioxidant defense. From a systems biology perspective, such dimensionality reduction is not merely a filtering step; rather, it represents a biologically informed projection of high-dimensional gene expression data onto a disease-relevant regulatory module.

Functional enrichment analysis suggested that ferroptosis-related differentially expressed genes were mainly associated with oxidative stress, lipid metabolism, HIF-1 signaling, FOXO signaling, inflammatory regulation, and cell death pathways. This result is consistent with the pathophysiological characteristics of ischemia-reperfusion injury. During the ischemic phase, oxygen deprivation disrupts mitochondrial oxidative phosphorylation and promotes metabolic stress. After reperfusion, the sudden restoration of oxygen supply leads to excessive ROS generation, calcium overload, inflammatory activation, and endothelial dysfunction. These events collectively create a microenvironment that favors lipid peroxidation and iron-dependent oxidative damage. Therefore, the enrichment of oxidative stress and lipid metabolism pathways supports the hypothesis that ferroptosis represents an important downstream executor of ischemia–reperfusion-induced cell injury.

Among the enriched signaling pathways, the FOXO pathway deserves particular attention. FOXO transcription factors are key regulators of cellular stress resistance, antioxidant responses, metabolism, and cell survival. FOXO1, as a major member of this family, can regulate the expression of genes involved in redox balance and metabolic adaptation. In the context of ischemia-reperfusion injury, FOXO1 may function as a compensatory transcriptional regulator that attempts to restore intracellular homeostasis under oxidative stress. The enrichment of FOXO signaling together with ferroptosis-related pathways suggests that FOXO1 may serve as a bridge between upstream stress signaling and downstream anti-ferroptotic defense. This finding provides the biological basis for further focusing on the SIRT1/FOXO1 axis.

SIRT1 is a NAD^+-dependent deacetylase that plays a central role in metabolic regulation, mitochondrial homeostasis, inflammation, and oxidative stress responses. Under stress conditions, SIRT1 can modulate the activity of multiple transcription factors and co-regulators through deacetylation. FOXO1 is one of the important downstream targets of SIRT1. Deacetylation of FOXO1 may influence its nuclear

localization, DNA-binding activity, transcriptional selectivity, and protein stability. Therefore, SIRT1 does not simply act as an upstream enzyme but may function as a regulatory switch that determines whether FOXO1-mediated transcriptional programs are activated or suppressed. In this study, the SIRT1/FOXO1 axis was interpreted as an upstream regulatory module capable of influencing multiple anti-ferroptotic effector genes.

The PPI network analysis further supports this hierarchical regulatory model. SIRT1 and FOXO1 displayed characteristics of upstream hub nodes, whereas GPX4, SLC7A11, and FTH1 were positioned closer to downstream execution modules. This network organization is biologically meaningful. GPX4 is a core enzyme responsible for reducing lipid hydroperoxides and preventing membrane lipid peroxidation. SLC7A11, as a key component of system Xc^-, promotes cystine uptake and supports glutathione synthesis, thereby providing the reducing substrate required for GPX4 activity. FTH1 contributes to iron storage and limits the intracellular labile iron pool, reducing the availability of Fe^{2+} for the Fenton reaction. Together, these molecules form a downstream anti-ferroptotic defense system. In contrast, SIRT1 and FOXO1 may regulate the transcriptional and metabolic state of this defense system. This suggests a layered regulatory structure: SIRT1 acts as an upstream enzymatic regulator, FOXO1 functions as a transcriptional mediator, and GPX4/SLC7A11/FTH1 constitute the downstream ferroptosis-suppressive execution layer.

A key conceptual contribution of this study is the use of ODE-based modeling to explain the dynamic transition of ferroptosis. Conventional pathway analysis can identify which genes or pathways are involved, but it cannot adequately describe how a cell moves from a reversible stress state to an irreversible death state. Ferroptosis is not simply caused by a single molecular change; rather, it emerges from the dynamic imbalance between lipid peroxide generation and antioxidant clearance. The ODE model constructed in this study captures this essential feature by describing the relationships among SIRT1-mediated FOXO1 activation, FOXO1-induced anti-ferroptotic gene expression, and lipid peroxide accumulation.

Under conditions of sufficient SIRT1 activity, FOXO1 activation is enhanced, leading to increased expression of GPX4, SLC7A11, and FTH1. In this state, lipid peroxide production can be balanced by antioxidant clearance, and the cell remains in a relatively stable, low-LPO state. This state may correspond to a compensatory phase of ischemia-reperfusion injury, during which cells are stressed but still capable of maintaining redox homeostasis. However, when ischemia–reperfusion stress suppresses SIRT1 activity or disrupts NAD^+-dependent deacetylation, FOXO1 activation decreases. As a result, GPX4-mediated detoxification, SLC7A11-dependent glutathione synthesis, and FTH1-mediated iron sequestration may all be weakened. Once lipid peroxide production exceeds the clearance capacity of the system, LPO levels may rise rapidly and drive the cell toward ferroptosis.

This dynamic process may be understood as a bifurcation-like transition. In biological terms, this means that ferroptosis may not increase gradually in a linear manner. Instead, cells may tolerate oxidative stress within a certain range, but once a critical threshold is crossed, the system abruptly shifts from a survivable state to an irreversible death state. This concept is particularly important for ischemia-reperfusion injury because tissue damage often shows sudden deterioration after reperfusion. A cell or tissue may appear viable during the early stage of reperfusion, but rapid lipid peroxidation, mitochondrial dysfunction, and membrane damage may subsequently lead to irreversible injury. The proposed model provides a theoretical explanation for this phenomenon by suggesting that the balance between LPO generation and GPX4-dependent clearance determines the critical transition point of ferroptosis.

From a translational perspective, the SIRT1/FOXO1 axis may represent a promising therapeutic target for ischemia-reperfusion injury [25]. If SIRT1 activation enhances FOXO1-mediated transcriptional defense and subsequently upregulates GPX4, SLC7A11, and FTH1, then pharmacological or biological strategies aimed at activating SIRT1 may help suppress ferroptosis. Similarly, interventions that enhance FOXO1 antioxidant transcriptional activity or directly increase GPX4/SLC7A11/FTH1 expression may improve cellular resistance to lipid peroxidation. These strategies may be particularly relevant in flap transplantation and reconstructive surgery, where ischemia-reperfusion injury is a major cause of partial flap necrosis and poor postoperative recovery.

For flap survival research, this theoretical framework can be translated into a testable experimental design. In future animal experiments, random-pattern skin flap or axial-pattern flap models may be used to evaluate whether activation of the SIRT1/FOXO1 axis improves flap viability. The survival area of the flap, microvascular perfusion, histological injury, inflammatory infiltration, and oxidative stress levels could be assessed. At the molecular level, SIRT1, acetylated FOXO1, total FOXO1, nuclear FOXO1, GPX4, SLC7A11, and FTH1 could be measured by Western blotting, immunohistochemistry, immunofluorescence, or qRT-PCR. Ferroptosis-related biochemical indicators, including malondialdehyde, glutathione, Fe^{2+}, ROS, lipid ROS, and 4-HNE, could be used to evaluate the degree of lipid peroxidation and iron-dependent oxidative injury. If SIRT1 activation increases nuclear FOXO1 and restores GPX4/SLC7A11/FTH1 expression while reducing LPO accumulation, this would provide experimental support for the proposed model.

In addition to pharmacological activation, genetic intervention strategies could further clarify the causal role of the SIRT1/FOXO1 axis. For example, SIRT1 overexpression or knockdown could be used to determine whether changes in SIRT1 activity directly affect FOXO1 acetylation and ferroptosis-related gene expression. FOXO1 silencing could be used to test whether the protective effect of SIRT1 depends on FOXO1. Similarly, GPX4 inhibition or SLC7A11 blockade could be used to determine whether downstream anti-ferroptotic effectors are required for the protective role of SIRT1/FOXO1. Such experiments would transform the current theoretical model into a mechanistically validated regulatory pathway.

Another important implication of this study is that ferroptosis intervention may require timing-specific strategies. If ferroptosis follows a threshold-dependent dynamic transition, early intervention before the system crosses the critical point may be more effective than late intervention after irreversible LPO accumulation has occurred. This means that SIRT1 activation or antioxidant defense enhancement may need to be applied during the ischemic phase, at the onset of reperfusion, or during the early reperfusion window. Once GPX4 activity is severely impaired and lipid peroxidation becomes self-amplifying, therapeutic rescue may become more difficult. Therefore, the dynamic model may help guide not only target selection but also the optimal timing of intervention.

This study also highlights the value of integrating bioinformatics and mathematical modeling in mechanism-oriented biomedical research. Bioinformatics analysis can identify candidate molecules and pathways, whereas mathematical modeling can describe the dynamic relationships among these components. In ferroptosis research, such integration is particularly useful because ferroptosis is governed by

multiple interacting modules, including iron metabolism, lipid metabolism, amino acid metabolism, mitochondrial stress, and antioxidant defense. A single pathway enrichment result cannot fully explain how these modules interact over time. By incorporating ODE modeling, this study provides a more mechanistic and dynamic interpretation of ferroptosis regulation.

Nevertheless, several limitations should be acknowledged. First, this study is based primarily on theoretical modeling and transcriptomic-level systems analysis. Although the proposed SIRT1/FOXO1-GPX4/SLC7A11/FTH1-LPO axis is biologically plausible, experimental validation is required to confirm the causal relationships among these molecules. Second, the ODE model simplifies the ferroptosis regulatory network by focusing on several core components. In reality, ferroptosis is also regulated by additional factors such as ACSL4, LPCAT3, FSP1, CoQ10, Nrf2, p53, mitochondrial metabolism, and inflammatory mediators [26]. These components were not fully incorporated into the current model. Third, model parameters such as reaction rate constants, degradation rates, Hill coefficients, and enzyme catalytic efficiencies were defined theoretically and require calibration using experimental or time-series omics data. Without parameter fitting and sensitivity analysis, the model should be interpreted as a conceptual dynamic framework rather than a fully predictive quantitative model.

Fourth, ischemia-reperfusion injury varies substantially across tissues and disease contexts. The molecular features of myocardial IRI, cerebral IRI, renal IRI, organ transplantation injury, and skin flap IRI may not be identical. Therefore, the generalizability of the SIRT1/FOXO1-centered ferroptosis model should be tested in specific tissue contexts. For flap transplantation in particular, vascular perfusion, endothelial injury, local inflammation, hypoxia gradients, and tissue edema may interact with ferroptosis-related pathways. Future studies should incorporate spatial factors and microenvironmental heterogeneity to better reflect the real biological condition of ischemic flaps.

Fifth, transcriptomic data alone cannot fully represent protein activity, post-translational modification, or metabolic flux [27]. This is especially relevant for SIRT1 and FOXO1 because their regulatory effects depend heavily on enzymatic activity, acetylation status, subcellular localization, and transcriptional activity rather than mRNA abundance alone [28]. Therefore, future studies should combine transcriptomics with proteomics, acetylomics, metabolomics, and lipidomics to obtain a more complete view of the SIRT1/FOXO1-ferroptosis regulatory axis. Lipidomics, in particular, would be valuable for directly measuring ferroptosis-related lipid peroxide species and validating the LPO dynamics proposed in the model.

Future research should proceed in several directions. First, time-series transcriptomic or proteomic datasets under ischemia–reperfusion conditions should be used to calibrate and refine the ODE model. By measuring SIRT1 activity, FOXO1 acetylation, GPX4 expression, GSH levels, Fe^{2+} concentration, and LPO accumulation at multiple time points, the model parameters could be estimated more accurately. Second, sensitivity analysis should be performed to identify which parameters most strongly influence ferroptosis transition. Such analysis may reveal whether SIRT1 activation, GPX4 upregulation, iron sequestration, or LPO clearance has the greatest impact on preventing ferroptosis. Third, bifurcation analysis could be applied to determine the critical threshold at which the system shifts from a stable survival state to a ferroptotic state. This would provide a more rigorous mathematical basis for the proposed nonlinear transition.

Fourth, experimental intervention studies should be conducted to validate whether modulation of the SIRT1/FOXO1 axis affects ferroptosis and flap survival. Potential interventions may include SIRT1 activators, FOXO1 modulators, ferroptosis inhibitors, iron chelators, GPX4-enhancing strategies, or SLC7A11-related metabolic interventions. Combination therapy may also be considered because ferroptosis involves multiple coupled modules. For example, simultaneous enhancement of SIRT1 activity and inhibition of iron-dependent lipid peroxidation may produce stronger protective effects than targeting a single node. Finally, clinical translation should focus on identifying biomarkers that can indicate ferroptosis risk and therapeutic response. SIRT1 activity, FOXO1 acetylation, GPX4 expression, GSH depletion, MDA elevation, and Fe^{2+} accumulation may serve as candidate markers for evaluating ischemia-reperfusion injury severity and treatment efficacy.

In conclusion, this study provides a theoretical and systems-level explanation for the role of the SIRT1/FOXO1 axis in ischemia-reperfusion injury-associated ferroptosis. The findings suggest that SIRT1/FOXO1 may function as an upstream regulatory module that controls downstream anti-ferroptotic effectors, including GPX4, SLC7A11, and FTH1. The ODE model further indicates that ferroptosis may occur through a nonlinear, threshold-dependent transition driven by excessive lipid peroxide accumulation. These results not only deepen the mechanistic understanding of ferroptosis in ischemia-reperfusion injury but also provide a theoretical basis for developing SIRT1/FOXO1-targeted interventions to improve tissue protection and flap survival.

Conclusion

In conclusion, this study established an integrative analytical framework based on systems biology and mathematical modeling to investigate the potential regulatory role of the SIRT1/FOXO1 axis in ischemia–reperfusion injury-associated ferroptosis. By combining empirical Bayes-based differential expression analysis, ferroptosis-related gene set mapping, hypergeometric enrichment analysis, PPI network topology, and ordinary differential equation modeling, this study interpreted the SIRT1/FOXO1-mediated ferroptosis regulatory network from three complementary dimensions: statistical inference, network organization, and dynamic system behavior. This multi-level strategy provides a more comprehensive explanation than conventional bioinformatics analysis, which often remains limited to static gene screening and pathway annotation.

The proposed framework suggests that SIRT1/FOXO1 may function as an upstream regulatory module in the ferroptosis network. SIRT1 may promote FOXO1 deacetylation and transcriptional activation, thereby enhancing the expression of anti-ferroptotic effectors such as GPX4, SLC7A11, and FTH1. Through these downstream molecules, the system may maintain glutathione metabolism, promote lipid peroxide detoxification, and reduce free iron-mediated oxidative damage. Therefore, under relatively preserved SIRT1 activity, cells may remain in a compensatory and protective state in which lipid peroxide production is balanced by antioxidant clearance.

In contrast, when ischemia–reperfusion stress suppresses the SIRT1/FOXO1 axis, the expression and function of GPX4, SLC7A11, and FTH1 may be weakened. This disruption reduces the cellular capacity to clear lipid peroxides and maintain iron homeostasis. As a result, lipid peroxide generation may gradually exceed the antioxidant defense capacity, leading to rapid LPO accumulation. The ODE-based model further indicates that ferroptosis may not progress as a simple linear process, but rather as a nonlinear, threshold-dependent transition. Once the system crosses a critical point, cells may shift from a reversible stress state to an irreversible ferroptotic state.

Overall, this study provides a theoretical basis for understanding ferroptosis regulation in ischemia–reperfusion injury and highlights the SIRT1/FOXO1 axis as a potential target for therapeutic intervention. In the context of flap transplantation and tissue repair, activation of SIRT1, enhancement of FOXO1-mediated transcriptional defense, or restoration of GPX4/SLC7A11/FTH1 expression may represent promising strategies to reduce lipid peroxidation, inhibit ferroptosis, and improve tissue survival. Future studies should further validate this model using experimental data, including animal models of flap ischemia–reperfusion injury, molecular intervention experiments, time-series omics analysis, and quantitative parameter fitting. Such work will help transform the current theoretical framework into a more predictive and clinically applicable model for ferroptosis-targeted tissue protection.

Author Contributions

Zhengyang Qi contributed to conceptualization, methodology, formal analysis, mathematical modeling, visualization, and writing of the original draft. Ziheng Fang contributed to data curation, bioinformatics analysis, literature collection, and validation. Lin Zhang contributed to functional enrichment analysis, protein–protein interaction network analysis, formal analysis, and manuscript revision. Zijie Li contributed to investigation, data organization, result interpretation, and visualization support. Yong Lian contributed to supervision, project administration, resources, conceptual guidance, and writing—review and editing. All authors have read and approved the final manuscript.

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Data availability

All data are included within the article or Supplementary Information or available from the authors on request.

Conflict of Interest

The authors report no conflicts of interest in this work.

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