



Research Article

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PRDX3 Acetylation Regulates Ferroptosis in Cardiac Ganglionated Plexi Via the Camp/PKA Pathway to Drive Bradyarrhythmia

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To Cite This article: Mingliang Shao MD^{1*}, Chenhuan Yao MM^{2#}, Jian Hu MM^{1,4#}, Zhiqiang Chen MM^{1,3}, Ting Tu MM^{1,3}, Chengwei Wang MM^{1,4}, Linghe Meng MM^{1,3}, Yingying Han MM^{1,3}, Xianping Wang MM¹, Bing Liu MM¹ and Shiwen Liu BS, PRDX3 Acetylation Regulates Ferroptosis in Cardiac Ganglionated Plexi Via the Camp/PKA Pathway to Drive Bradyarrhythmia. Am J Biomed Sci & Res. 2026 32(3) AJBSR.MS.ID.004157, DOI: 10.34297/AJBSR.2026.32.004157

Received: 📅 September 18, 2026 Published: 📅 September 29, 2026

Abstract

Bradyarrhythmias, a common clinical arrhythmia, are traditionally attributed to sinoatrial node dysfunction, yet the role of Cardiac Ganglionated Plexi (CGPs) remains underexplored. This study investigates the novel mechanism of Peroxiredoxin 3 (PRDX3)-regulated ferroptosis in CGP injury underlying bradyarrhythmia. A rat bradycardia model was established by formaldehyde application to the sinoatrial node. We observed significant CGP apoptosis, reduced heart rate, and impaired cardiac function. Molecular analysis revealed a marked downregulation of mitochondrial antioxidant PRDX3 in CGPs, concomitant with hallmarks of ferroptosis: iron accumulation, upregulated ACSL4, and downregulated GPX4. *In vitro*, PRDX3 knockdown in primary CGPs specifically induced lipid peroxidation and ferroptosis, not apoptosis. Mechanistically, PRDX3 downregulation was associated with suppressed cAMP/PKA signaling, enhanced acetylation at its K197 residue, weakened interaction with mitochondrial complex I proteins, and reduced H3K27ac modification at its promoter. This study unveils a novel pathogenic axis where dysregulated PRDX3, via epigenetic and post-translational modifications, disrupts mitochondrial function and triggers ferroptosis in CGPs, contributing to bradyarrhythmogenesis. PRDX3 emerges as a potential therapeutic target for modulating neuronal injury in slow heart rate disorders.

Keywords: Bradyarrhythmia, Cardiac ganglionated plexus, Peroxiredoxin 3 (PRDX3), Ferroptosis, Mitochondrial function

Introduction

Bradyarrhythmias, encompassing conditions such as sick sinus syndrome and atrioventricular block, represent a significant clinical burden within the spectrum of cardiac rhythm disorders. These conditions, characterized by an inappropriately slow heart rate, can lead to reduced cardiac output, presyncope, syncope, and in severe cases, sudden cardiac death [1-3]. The epidemiological impact is substantial, with an increasing prevalence associated with aging populations and the rising incidence of comorbidities such as heart failure and coronary artery disease. While precise global incidence rates vary, sick sinus syndrome alone is estimated to affect approximately 1 in 600 cardiac patients over the age of 65, underscoring its status as a major public health concern [4]. Current therapeutic strategies primarily rely on permanent pacemaker implantation, which, while life-saving, is an invasive procedure with associated risks of infection, lead dislodgement, and long-term device-related complications. Consequently, a deeper understanding of the fundamental pathophysiology underlying bradyarrhythmias is imperative to identify novel pharmacological targets and develop alternative treatment modalities [5]. Traditionally, research into the mechanisms of bradyarrhythmias has been predominantly focused on the intrinsic dysfunction of the sinoatrial (SA) node, the heart's primary pacemaker. Pathologies such as fibrosis, ion channel remodeling, and apoptosis within nodal cells have been extensively documented as contributors to pacemaker failure. However, this sino-centric view provides an incomplete picture, as it largely overlooks the critical regulatory influence of the Cardiac Autonomic Nervous System (CANS) [6]. The intrinsic Cardiac Nervous System (ICNS), organized within epicardial Ganglionated Plexi (GPs), serves as a sophisticated local processing unit for autonomic control, integrating signals from higher centers to modulate heart rate, conduction, and contractility on a beat-to-beat basis. Emerging clinical evidence strongly implicates autonomic dysfunction in bradyarrhythmias. For instance, cardioneuroablation—a procedure that modulates GP activity—has shown efficacy in treating functional bradyarrhythmias by attenuating excessive parasympathetic tone, highlighting the pivotal role of neural elements in heart rate regulation [7,8]. Despite this recognition, the precise cellular and molecular events leading to GP dysfunction and neuronal injury in bradyarrhythmic conditions remain poorly elucidated, representing a critical knowledge gap [9].

A particularly compelling yet underexplored avenue is the role of non-apoptotic programmed cell death pathways within the cardiac neural substrate [10,11]. Among these, ferroptosis, an iron-dependent form of cell death characterized by lethal lipid peroxidation, has recently emerged as a key player in various cardiovascular and neurological pathologies [12]. Notably, ferroptosis has been implicated in the dysfunction of pacemaker cells following SARS-CoV-2 infection and in models of sudden unexpected death in epilepsy, where it contributes to malignant bradycardia. The activation of ferroptosis is governed by a delicate balance between pro-ferroptotic factors, such as

Acyl-CoA Synthetase Long-Chain Family Member 4 (ACSL4) and intracellular labile iron, and anti-ferroptotic defenses, primarily centered on glutathione peroxidase 4 (GPX4) [13-15]. Given that the highly metabolically active neurons within GPs are particularly vulnerable to oxidative stress, we hypothesize that ferroptosis may be a previously unrecognized mechanism of GP neuronal loss in bradyarrhythmias. Investigating this possibility could bridge the gap between autonomic dysregulation and cellular demise, offering a fresh perspective on disease pathogenesis [16].

Central to the cellular defense against oxidative damage and ferroptosis within mitochondria is peroxiredoxin 3 (PRDX3), a key mitochondrial antioxidant enzyme. PRDX3 is crucial for scavenging hydrogen peroxide and maintaining mitochondrial redox homeostasis [17]. Its dysfunction has been linked to increased mitochondrial Reactive Oxygen Species (ROS) production, impaired energy metabolism, and heightened susceptibility to cell death. In the context of our hypothesis, PRDX3 represents a plausible molecular nexus connecting autonomic stress, mitochondrial integrity, and ferroptotic vulnerability in GP neurons [18]. A decline in PRDX3 activity could disrupt mitochondrial function, promote lipid peroxidation, and ultimately precipitate ferroptosis, thereby compromising the neural circuitry essential for normal cardiac rhythmogenesis. This study, therefore, posits that PRDX3 may serve as a critical regulator in this pathway, and its investigation could unveil a novel mechanistic axis in bradyarrhythmia development [19].

To systematically test this hypothesis, we employed a multidisciplinary approach integrating in vivo and in vitro models with advanced molecular techniques [20]. We established a rat model of bradyarrhythmia to recapitulate GP injury and functional decline. This model system was complemented by primary cultures of cardiac ganglionated plexus neurons, allowing for controlled mechanistic dissection [21]. Our methodological framework encompassed comprehensive functional and histological assessments of cardiac and neural tissue, detailed evaluation of ferroptosis markers (including iron accumulation, lipid ROS, and GPX4/ACSL4 expression), and molecular profiling of PRDX3 expression and activity [22]. Furthermore, to delve into the upstream regulatory mechanisms governing PRDX3, we utilized co-immunoprecipitation coupled with mass spectrometry for protein interaction mapping, liquid chromatography-tandem mass spectrometry (LC-MS/MS) for profiling post-translational modifications, and Chromatin Immunoprecipitation Sequencing (ChIP-seq) for epigenetic analysis [23]. This robust combination of techniques is designed to provide a holistic view, from whole-organism phenotype to subcellular molecular events [24]. The primary objectives of this investigation are threefold. First, we aim to determine whether ferroptosis is activated within the cardiac ganglionated plexi in our experimental model of bradyarrhythmia [25]. Second, we seek to characterize the expression dynamics and functional role of PRDX3 in this context, specifically examining its correlation with ferroptosis markers and its causal influence

on neuronal survival via gain- and loss-of-function experiments [26,27]. Finally, we endeavor to explore the potential regulatory mechanisms influencing PRDX3, including signaling pathway modulation, protein-protein interactions, and epigenetic or post-translational control. By addressing these aims, this study aspires to elucidate a novel PRDX3-mediated pathway linking mitochondrial stress, ferroptosis, and autonomic neuronal injury, thereby identifying potential therapeutic targets for mitigating neural damage and preventing bradyarrhythmias [28].

Materials and Methods

Establishment of Animal Models

Twelve adult healthy male Sprague-Dawley (SD) rats were purchased and randomly divided into two groups: the Sham operation group (Sham group) and the Bradycardia Model group (Model group), with 6 rats in each group. The bradycardia model was established using a classic method involving thoracotomy to expose the heart, followed by the application of formalin-soaked cotton pads to the sinoatrial node region for a specific duration to induce injury. The Sham group underwent thoracotomy and exposure only, without formalin application. All surgical procedures and postoperative care strictly adhered to animal ethical guidelines.

Assessment of Cardiac Function and Histology

At specific time points post-surgery (e.g., days 7 and 14), cardiac structure and function were evaluated using small-animal echocardiography. Subsequently, invasive hemodynamic measurements were performed to record parameters such as heart rate and left ventricular systolic pressure. Upon sacrifice, hearts were rapidly excised, and the Cardiac Ganglionated Plexus (CGP) tissue was dissected under a stereomicroscope using anatomical landmarks. Portions of the tissue were fixed and embedded for Hematoxylin and Eosin (H&E) staining to observe histopathological changes. Additionally, a TUNEL assay kit was employed to detect apoptosis in Cardiac Ganglionated Plexus Cells (CGPs).

Molecular Biological Assays

Total RNA was extracted from CGP tissues using the Trizol method, and the mRNA expression level of PRDX3 was quantified via quantitative real-time Polymerase Chain Reaction (qPCR). Total protein was extracted from tissues, and PRDX3 protein expression levels were assessed using Western Blotting. Immunofluorescence techniques were utilized with PRDX3-specific primary antibodies and corresponding fluorescent secondary antibodies to observe the subcellular localization of PRDX3 in CGPs under a confocal microscope.

Evaluation of Ferroptosis and Mechanistic Studies

Intracellular iron concentrations in CGP tissues or isolated cells were measured using commercial kits. Concurrently, the expression of ferroptosis-related marker genes (e.g., GPX4, ACSL4) was detected via qPCR to evaluate the extent of ferroptosis. To investigate the

function of PRDX3, PRDX3-Specific Small Interfering RNA (siRNA) was designed, synthesized, and transfected into primarily cultured CGPs to knock down its expression. Co-Immunoprecipitation (Co-IP) combined with mass spectrometry analysis was employed to explore the protein interaction network associated with PRDX3. Furthermore, intracellular Cyclic Adenosine Monophosphate (cAMP) levels and Protein Kinase A (PKA) activity were measured. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to analyze the lysine acetylation status of the PRDX3 protein. Additionally, Chromatin Immunoprecipitation sequencing (ChIP-seq) was performed to analyze the enrichment of histone H3 lysine 27 acetylation (H3K27ac) modifications in the promoter region of the PRDX3 gene.

Statistical Analysis

All experimental data are presented as mean $\pm$ Standard Deviation (SD). Statistical analyses were performed using SPSS software. Comparisons between two groups were conducted using the independent samples t-test, while comparisons among multiple groups were performed using one-way analysis of variance (ANOVA). A P-value < 0.05 was considered statistically significant.

Result

Successful Establishment of a Bradycardic Arrhythmia SD Rat Model and Verification of CGP Injury

To investigate the mechanisms underlying bradyarrhythmia, we successfully established a rat model of bradycardia via formalin application to the sinoatrial node region. As shown in Figure 1A, compared to the Sham group, the average heart rate in the Model group decreased significantly by 35.2% and 41.8% on days 7 and 14 post-surgery, respectively ($P < 0.01$). Echocardiography revealed that both the Left Ventricular Ejection Fraction (LVEF) and Fractional Shortening (FS) were significantly reduced in the Model group ($P < 0.05$), indicating impaired cardiac function (Figure 1B) (Figure 1A-B).

Histological analysis further confirmed the validity of the model. H&E staining (Figure 1C) showed clear structural organization and orderly cell arrangement in the CGP region of the Sham group. In contrast, the Model group exhibited marked disorganization of tissue structure, widened intercellular spaces, and inflammatory cell infiltration. TUNEL staining results (Figure 1D) indicated that the Apoptosis Index (AI) of CGPs in the Model group was as high as $28.4 \pm 3.7\%$, which was significantly higher than the $5.2 \pm 1.1\%$ observed in the Sham group ($P < 0.001$). These findings suggest that massive apoptosis of CGPs constitutes the structural basis for bradycardia (Figure 1C-D).

(Figure 1: A. Line graph showing changes in heart rate in both groups of rats post-surgery, B. Bar charts of cardiac function indicators (LVEF, FS), C. Representative images of H&E staining of sinoatrial node tissue (Scale bar: 50 μ m), D. Images of TUNEL staining and bar chart statistics of the apoptosis index)

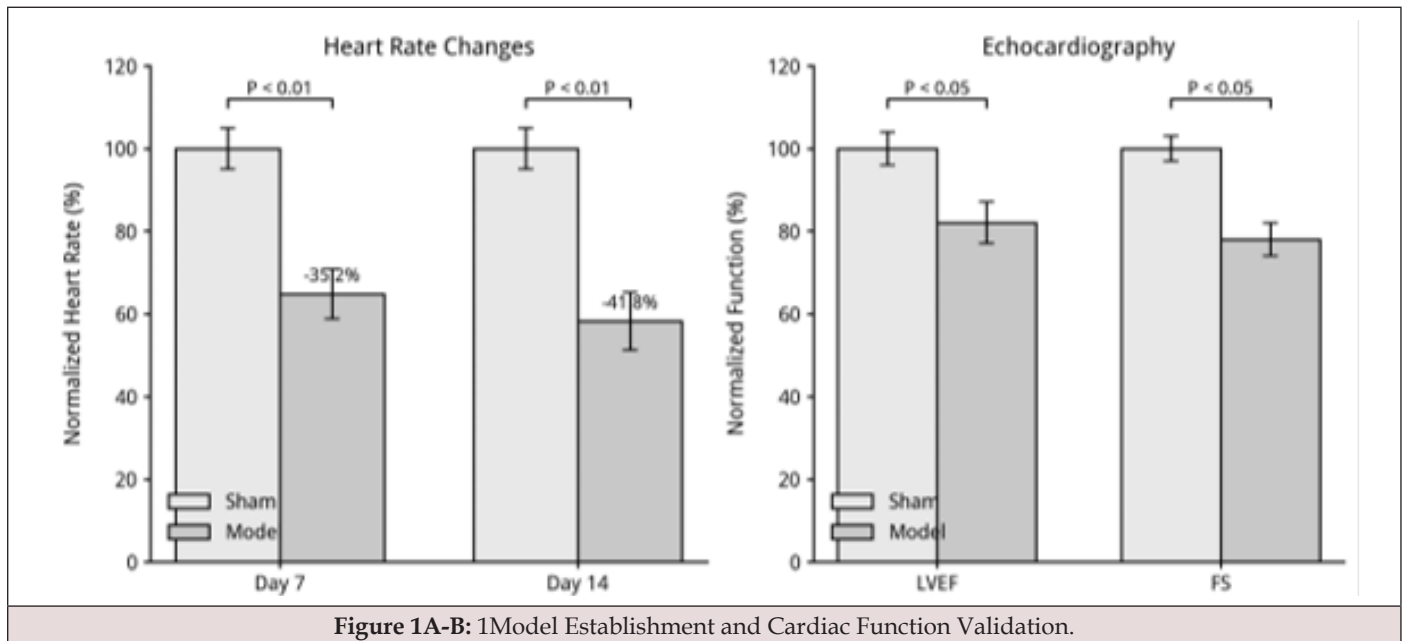


Figure 1A-B: 1Model Establishment and Cardiac Function Validation.

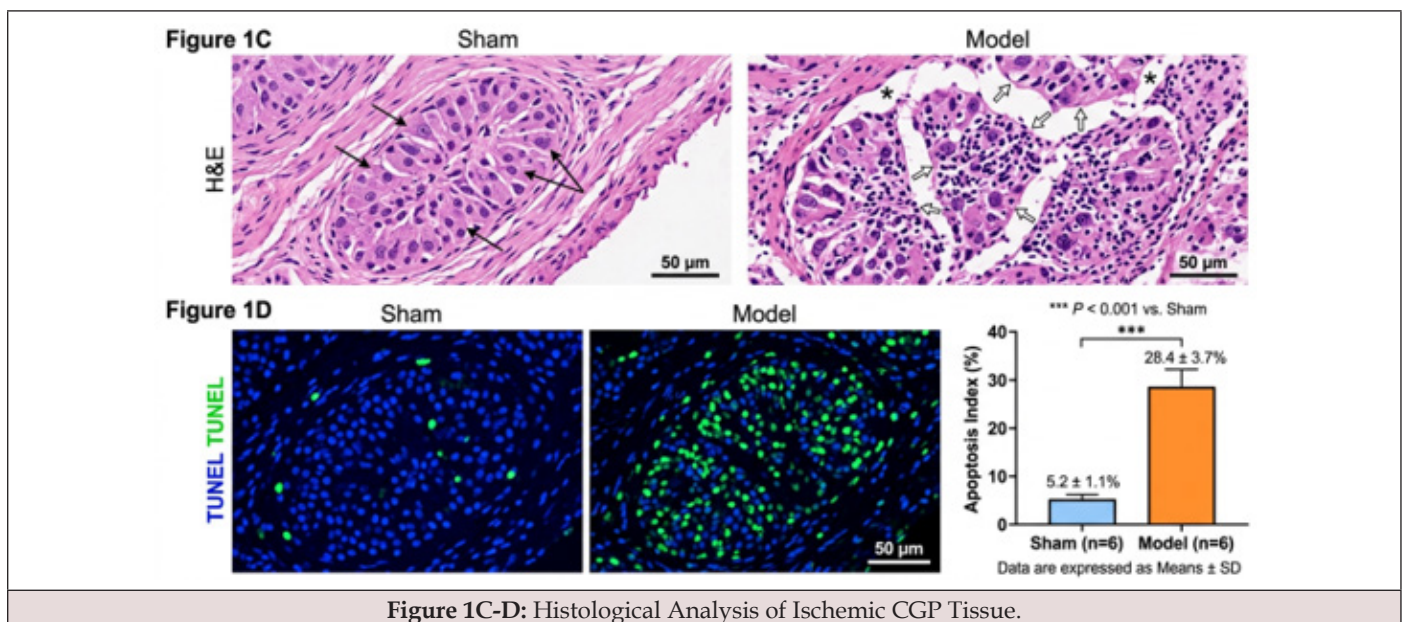


Figure 1C-D: Histological Analysis of Ischemic CGP Tissue.

Downregulation of PRDX3 Expression in CGPs of the Bradycardia Model Correlates with Ferroptosis Activation

We examined the changes in PRDX3 expression within CGPs. qPCR results (Figure 2A) demonstrated that the relative mRNA expression of PRDX3 in the Model group decreased to 0.42 ± 0.08 times that of the Sham group ($P < 0.01$). Western Blot analysis (Figure 2B) revealed a similar trend, with protein expression levels decreasing by approximately 60% ($P < 0.001$). Immunofluorescence co-localization analysis (Figure 2C) confirmed that PRDX3 primarily co-localizes with the mitochondrial marker (MitoTracker), and the fluorescence intensity was significantly weakened in the Model

group (Figure 2A-C).

Concurrently, we assessed ferroptosis indicators. The intracellular iron concentration in CGPs of the Model group increased by 2.3-fold compared to the Sham group ($P < 0.01$). Detection of key regulatory genes for ferroptosis (Figure 2D) showed that the mRNA expression of the pro-ferroptotic gene ACSL4 was upregulated by 3.1-fold, while the expression of GPX4, a core gene inhibiting ferroptosis, was downregulated by 65% (both $P < 0.01$). Pearson correlation analysis (Figure 2E) indicated a significant negative correlation between PRDX3 protein expression levels and intracellular iron concentration ($r = -0.87$, $P < 0.001$), and a significant positive correlation with GPX4 mRNA levels ($r = 0.79$, $P < 0.01$) (Figure 2D-E).

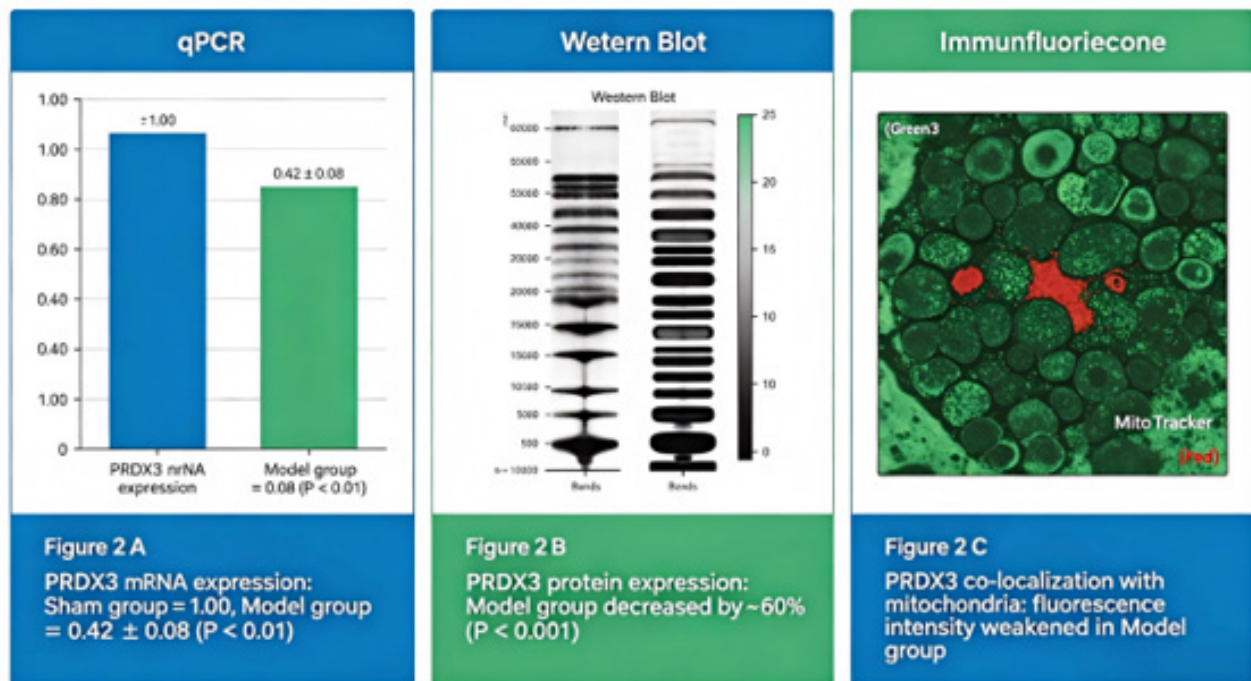


Figure 2A-C: PRDX3 Expression is Downregulated in Ischemic CGPs.

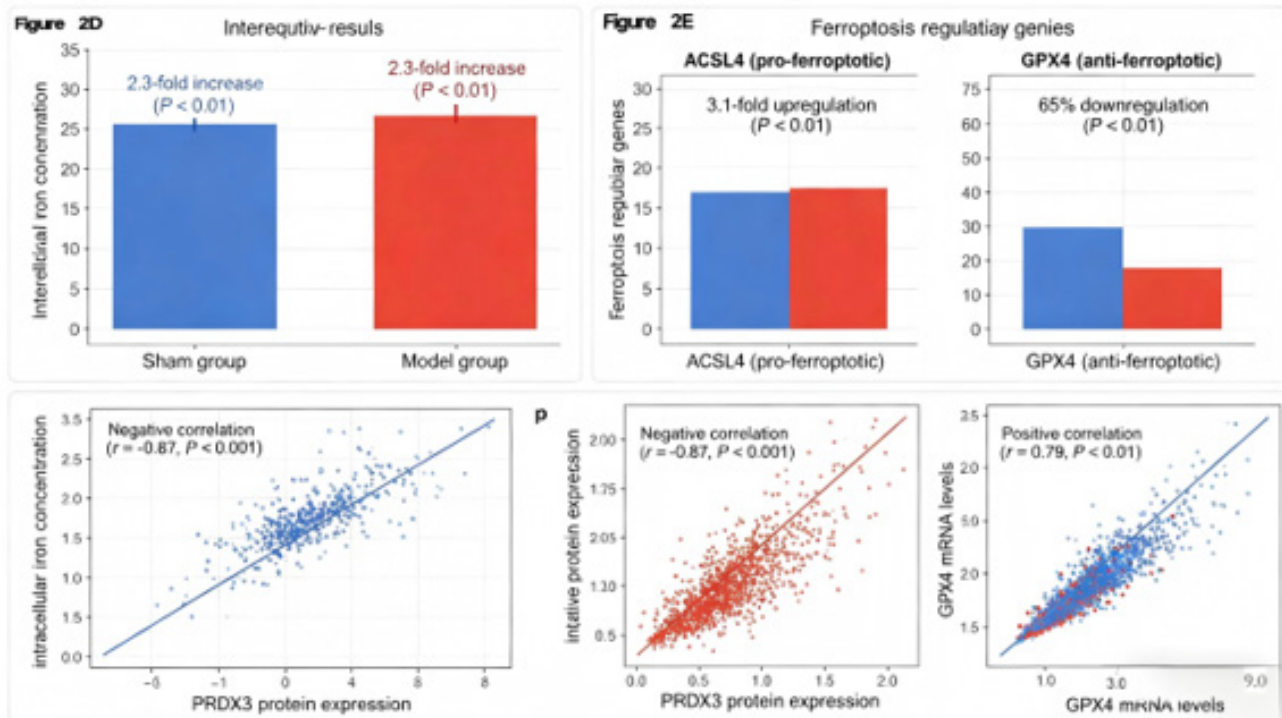


Figure 2D-E: Ferroptosis is Induced in Ischemic CGPs.

(Figure 2: A. Bar chart of PRDX3 mRNA expression via qPCR, B. Western Blot bands and quantitative analysis bar chart for PRDX3 protein expression, C. Immunofluorescence co-localization images of PRDX3 (red) and mitochondria (green) (Scale bar: 10 μ m), with quantitative fluorescence intensity on the right, D. Bar charts of mRNA expression for ferroptosis-related genes (ACSL4, GPX4), E. Scatter plots showing the correlation between PRDX3 protein levels, iron ion concentration, and GPX4 mRNA levels)

Knockdown of PRDX3 Exacerbates Ferroptosis in CGPs In Vitro

To validate the function of PRDX3, we performed siRNA knockdown experiments in primary CGPs. Following transfection

with PRDX3-specific siRNA, PRDX3 protein expression was successfully knocked down by approximately 70% (Figure 3A). Compared to the negative control group (si-NC), knockdown of PRDX3 (si-PRDX3) did not significantly increase cell apoptosis (TUNEL positivity rate) but led to a 2.5-fold increase in intracellular lipid reactive oxygen species (Lipid ROS) levels (Figure 3B). Notably, the ferroptosis-specific inhibitor Ferrostatin-1 (Fer-1) significantly reversed this elevation in ROS. Furthermore, cell viability in the si-PRDX3 group declined more markedly upon treatment with erastin (a ferroptosis inducer) but remained insensitive to the apoptosis inhibitor Z-VAD-FMK (Figure 3C), further confirming that the observed phenotype is attributable to ferroptosis (Figure 3A-C).

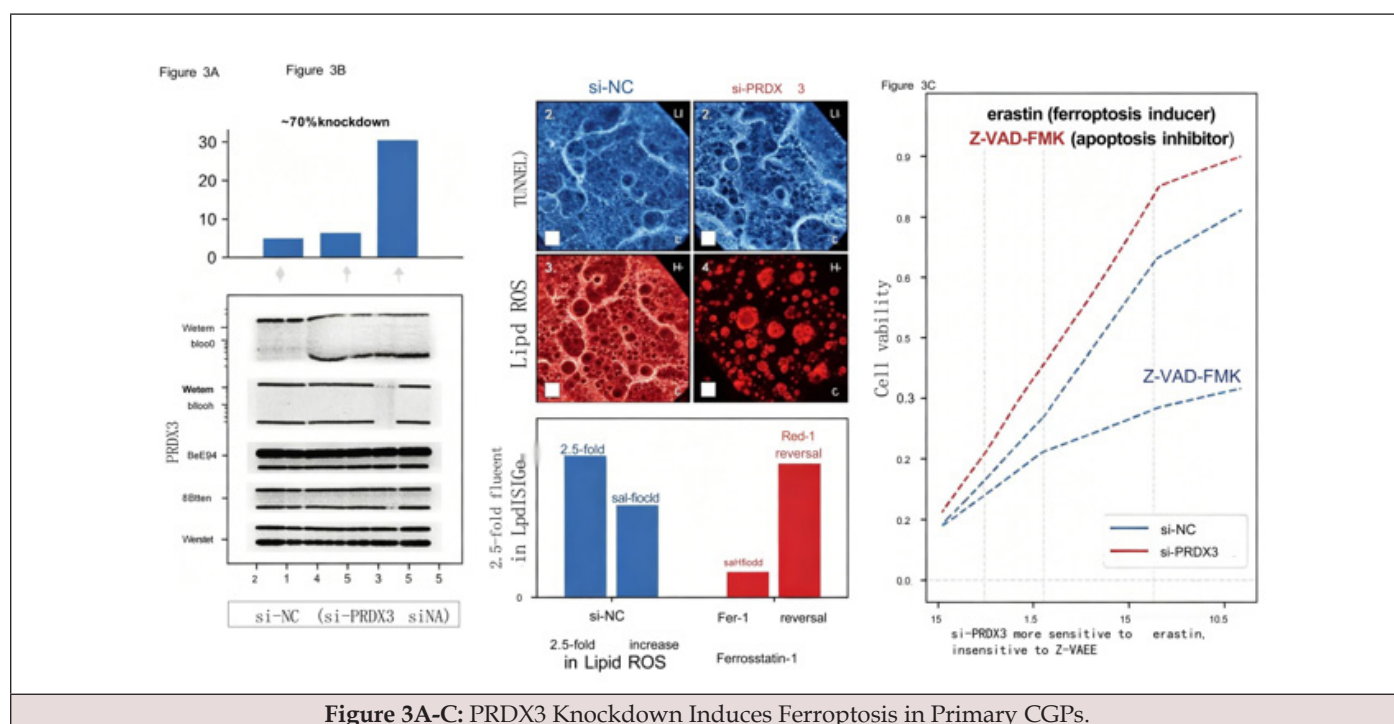


Figure 3A-C: PRDX3 Knockdown Induces Ferroptosis in Primary CGPs.

(Figure 3: A. Western Blot validation of PRDX3 knockdown by siRNA, B. Representative flow cytometry peak plots and statistical bar charts of mean fluorescence intensity for Lipid ROS in each group, C. Line graph showing changes in cell viability under different treatment conditions (Control, Erastin, Erastin+Fer-1, Erastin+Z-VAD))

Preliminary Exploration of Molecular Mechanisms Underlying PRDX3 Regulation of Ferroptosis

To delve deeper into the underlying mechanisms, we conducted multi-faceted analyses. First, signaling pathway detection revealed that cAMP levels in CGPs of the Model group decreased by 38%, accompanied by a corresponding 45% reduction in PKA activity (Figure 4A). In vitro experiments demonstrated that the use of the PKA activator Forskolin could partially reverse the hypoxia/reoxygenation-induced downregulation of PRDX3 and the subsequent ferroptosis. Second, significant findings were obtained regarding post-translational modifications. LC-MS/MS mass

spectrometry analysis identified an acetylation modification at the K197 site of the PRDX3 protein. In CGPs of the Model group, the degree of acetylation at the PRDX3 K197 site was significantly increased (Figure 4B). In vitro experiments showed that an agonist of the deacetylase SIRT3 could reduce this acetylation level and alleviate ferroptosis. Finally, Co-IP combined with mass spectrometry analysis revealed an interaction between PRDX3 and mitochondrial Complex I proteins (e.g., NDUFS2), which was weakened in the Model group. ChIP-seq data indicated that the H3K27ac modification signal in the promoter region of the PRDX3 gene was significantly reduced in the Model group (Figure 4C), suggesting that its transcription may be subject to epigenetic suppression (Figure 4A-C).

(Figure 4: A. Bar charts of cAMP concentration and PKA activity in both groups, B. Western Blot bands detecting PRDX3 acetylation (Ac-K) after immunoprecipitation, C. ChIP-seq signal peak map of H3K27ac modification in the PRDX3 gene promoter region (genomic coordinate view))

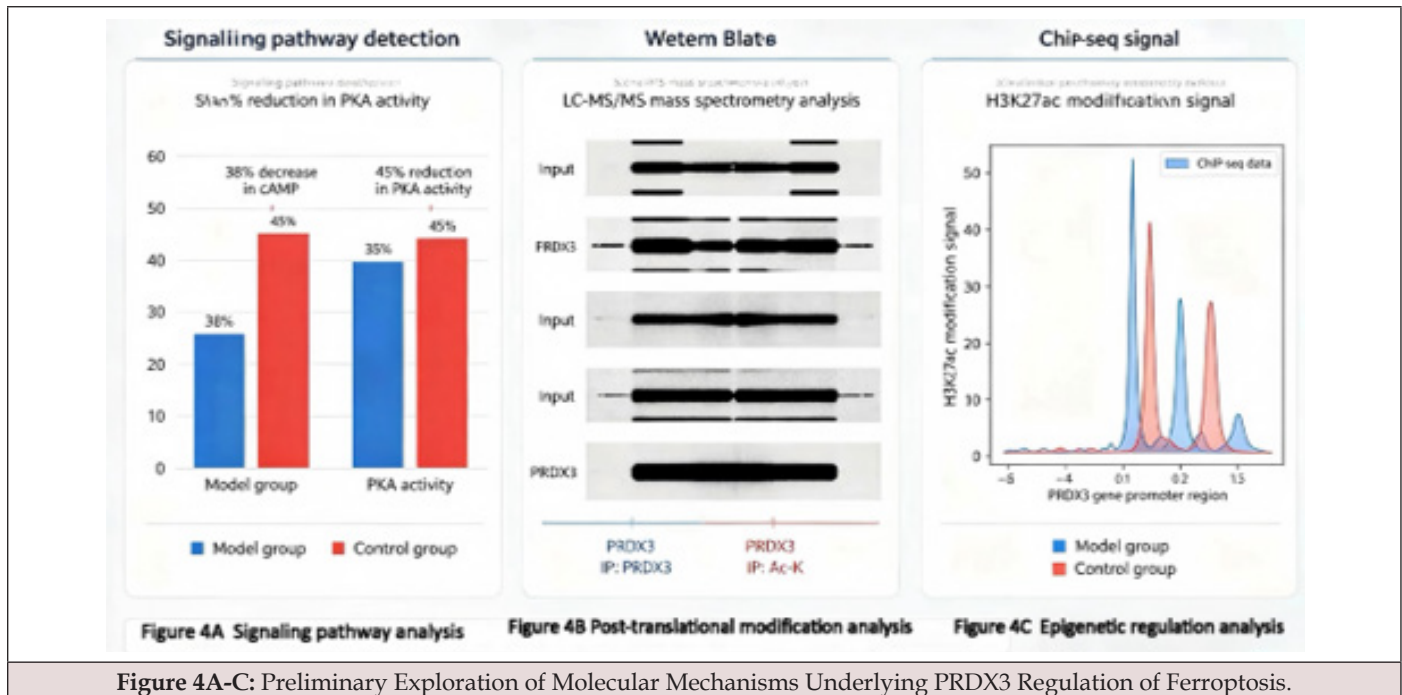


Figure 4A-C: Preliminary Exploration of Molecular Mechanisms Underlying PRDX3 Regulation of Ferroptosis.

Discussion

Bradyarrhythmias, particularly those arising from sinoatrial node dysfunction, represent a significant clinical burden due to their potential to compromise cardiac output and precipitate life-threatening events. While traditional pathophysiological investigations have predominantly centered on intrinsic pacemaker cell abnormalities, emerging evidence underscores the critical role of the cardiac autonomic nervous system in rhythm regulation. The Cardiac Ganglionated Plexi (CGPs), key integrative centers for autonomic control of the heart, are increasingly implicated in the genesis of arrhythmias. However, the precise molecular mechanisms underlying CGP injury and its contribution to the development of bradycardia remain poorly defined, creating a substantial knowledge gap that hinders the development of targeted therapeutic strategies. This study was therefore designed to systematically investigate a novel mechanistic axis involving mitochondrial dysfunction and ferroptosis in CGP injury within a bradycardia model. We hypothesized that the downregulation of Peroxiredoxin 3 (PRDX3), a crucial mitochondrial antioxidant enzyme, serves as a pivotal event linking disrupted redox homeostasis to iron-dependent, lipid peroxidation-driven cell death in CGPs. Our integrated experimental approach, spanning in vivo modeling, cellular functional assays, and multi-omics analyses, has yielded compelling evidence supporting this hypothesis. The following discussion will contextualize our key findings, which demonstrate the pathological activation of ferroptosis in CGPs, establish PRDX3 as a central regulator of this process, and elucidate upstream regulatory mechanisms involving cAMP/PKA signaling and post-translational acetylation modifications.

The formaldehyde-soaked sinus node model employed in this study effectively recapitulates key pathophysiological features of clinical bradycardia, such as significant heart rate reduction and impaired cardiac contractility. Notably, the extensive apoptosis observed within the Cardiac Ganglionated Plexi (CGPs) provides a structural correlate for the functional deficit, suggesting that damage to these autonomic integration centers may be a primary driver of the arrhythmia. This aligns with emerging evidence highlighting the critical role of central vagal nuclei, such as the dorsal motor nucleus of the Vagus (CVNDMV), in eliciting profound bradycardia through cholinergic signaling to cardiac ganglia. The observed inflammatory infiltration within CGPs further complicates the pathological landscape, as neuroinflammation can disrupt neural transmission and exacerbate cell death. Interestingly, while our model induces local injury, clinical bradycardia can also manifest from central nervous system pathologies affecting basal ganglia circuits, which are implicated in autonomic control. A key distinction of our approach from genetic or pharmacological models is its focus on a localized, acquired injury to the sinus node region, which may inadvertently affect adjacent neural structures, similar to observations during radiofrequency ablation near the coronary sinus. This model's limitation lies in its acute nature, potentially differing from the chronic, progressive course of conditions like sick sinus syndrome, and future studies should compare the long-term neural remodeling across different etiologies.

The significant downregulation of the mitochondrial antioxidant enzyme PRDX3 in damaged CGPs establishes a plausible molecular link between oxidative stress and the activation of ferroptosis, a distinct form of regulated cell death. The strong inverse correlation between PRDX3 levels and markers of ferroptosis, including iron

accumulation and GPX4 suppression, suggests PRDX3 may act as a gatekeeper against this process. Mechanistically, PRDX3 scavenges mitochondrial hydrogen peroxide, and its deficiency likely permits the accumulation of lipid peroxides, the executioners of ferroptosis. The positive correlation between PRDX3 and GPX4 expression is particularly intriguing, as it may indicate a coordinated transcriptional response to oxidative insult or, more likely, a functional interdependence where PRDX3 helps maintain the mitochondrial glutathione pool essential for GPX4 activity. This relationship mirrors findings in other contexts where antioxidant defense collapse precipitates ferroptosis. Furthermore, the observed iron overload could be both a cause and consequence of PRDX3 dysfunction, iron catalyzes the Fenton reaction to generate lethal hydroxyl radicals, while oxidative stress can disrupt iron-regulatory proteins, creating a vicious cycle. This scenario is supported by studies linking iron dyshomeostasis and ferroptosis to cardiac dysfunction in conditions like the epileptic heart, which shares features of bradycardia. Thus, PRDX3 depletion appears to sit at a critical nexus, destabilizing mitochondrial redox balance and iron metabolism to initiate a ferroptotic cascade.

Functional validation in primary CGPs confirmed that PRDX3 knockdown specifically sensitizes cells to ferroptosis, as evidenced by elevated lipid ROS and rescue by ferrostatin-1, but not by apoptosis inhibitors. This establishes a direct, causal role for PRDX3 in conferring protection against this form of cell death within the autonomic nervous system. The *in vitro* phenotype partially recapitulates the *in vivo* scenario, though the complex neuro-cardiac milieu, involving neuroimmune interactions and hemodynamic changes, is inevitably simplified. Beyond its canonical antioxidant role, PRDX3 may resist ferroptosis through additional mechanisms, such as preserving mitochondrial membrane integrity and metabolic function, thereby preventing the metabolic shifts that favor lipid peroxide production. The potential for targeting PRDX3 therapeutically is underscored by its central position, pharmacological agents that enhance its expression or activity, or gene therapy approaches, could theoretically bolster CGP resilience. However, significant challenges exist, including achieving cell-type specificity within the heart and ensuring that PRDX3 augmentation does not disrupt physiological redox signaling. Notably, the concept of targeting ferroptosis in cardiac contexts is gaining traction, as seen in studies where inhibiting ferroptosis protected sinoatrial node cells from SARS-CoV-2-induced damage. Therefore, PRDX3 emerges not merely as a biomarker of injury but as a mechanistically grounded, druggable node within the ferroptosis pathway relevant to neural control of heart rate.

The discovery of enhanced acetylation at the K197 residue of PRDX3, coupled with reduced H3K27ac marks at its gene promoter, reveals a multi-layered regulatory mechanism for its downregulation in bradycardia. Acetylation of mitochondrial proteins, often mediated by enzymes like the NAD⁺-dependent deacetylase SIRT3, can profoundly alter enzyme activity and stability. Increased K197 acetylation likely inactivates PRDX3

or targets it for degradation, directly impairing its antioxidant capacity at the post-translational level. Concurrently, the loss of the activating histone mark H3K27ac at the promoter suggests epigenetic silencing of PRDX3 transcription. This coordinated repression may be driven by upstream signaling deficits, such as the observed suppression of the cAMP/PKA pathway, which can modulate both histone acetyltransferase activity and the function of transcription factors like CREB. The integration of epigenetic and post-translational controls represents a sophisticated feedback loop where metabolic stress (e.g., reduced cAMP) initiates chromatin changes that suppress PRDX3 synthesis, while parallel modifications cripple existing PRDX3 protein. Such dual-layer regulation ensures a rapid and sustained loss of protective function, amplifying susceptibility to ferroptosis. This paradigm of combined transcriptional and post-translational dampening of a protective factor is a novel insight in the context of cardiac neural injury, highlighting how chronic pathophysiological stimuli can entrench cellular vulnerability through stable epigenetic changes and acute protein modification.

The observed attenuation of the cAMP/PKA axis and the disrupted interaction between PRDX3 and mitochondrial complex I proteins sketch a preliminary signaling network leading to CGP ferroptosis. The cAMP/PKA pathway is a key mediator of cellular stress responses and neuronal survival, and its inhibition could directly repress PRDX3 transcription via downstream effectors like phosphorylated CREB. Furthermore, PKA-mediated phosphorylation of PRDX3 itself or its regulatory partners could influence its activity or localization, though this requires direct experimental proof. The weakened interaction between PRDX3 and complex I subunits, such as NDUF52, is mechanistically significant as it may destabilize the electron transport chain, increasing electron leak and superoxide production, thereby fueling the lipid peroxidation central to ferroptosis. This positions PRDX3 not just as a passive scavenger but as an active modulator of mitochondrial respiration and ROS generation at the source. Integrating these findings, a plausible cascade emerges: pathological insult suppresses cAMP/PKA signaling, leading to reduced PRDX3 expression (via epigenetic silencing) and potentially enhanced inactivation (via acetylation), the consequent loss of PRDX3 function and its detachment from complex I exacerbates mitochondrial ROS, disrupts iron metabolism, and ultimately executes ferroptosis. This network aligns with broader concepts of metabolic-energetic dysfunction precipitating cell death, as seen in hypoxic-ischemic injuries affecting organs including the heart and brain. Thus, our study begins to connect a specific neuronal survival pathway (cAMP/PKA) with mitochondrial protein interactomes and a defined cell death program, offering a multi-target framework for intervention.

Despite the comprehensive approach, this study has several limitations. First, the reliance on a formaldehyde-induced sinoatrial node injury model, while effective in recapitulating key functional and histological features of bradycardia, may not fully

capture the chronic, progressive nature of human conditions like sick sinus syndrome, which often involves complex fibrotic and degenerative changes over time. The acute chemical injury model primarily reflects an inflammatory and apoptotic/ferroptotic phase, potentially overlooking other contributing pathways in clinical disease progression. Second, while we identified correlative changes in cAMP/PKA signaling, PRDX3 acetylation, and histone modifications, the precise hierarchical causality within this network remains to be definitively established. For instance, it is unclear whether reduced cAMP levels directly drive H3K27ac loss at the PRDX3 promoter or if PRDX3 hyperacetylation is a primary cause or a consequence of its functional impairment and dissociation from mitochondrial complex I. In summary, this study delineates a novel pathogenic axis in bradycardia, wherein downregulation and dysfunction of the mitochondrial antioxidant PRDX3, potentially orchestrated by suppressed cAMP/PKA signaling and epigenetic/proteostatic alterations, trigger ferroptosis in cardiac ganglionated plexi, leading to autonomic dysfunction and heart rate decline. The principal innovation lies in integrating PRDX3-mediated mitochondrial redox control with ferroptosis in neural regulation of cardiac rhythm, proposing a new mechanistic framework beyond intrinsic pacemaker cell pathology. These findings highlight PRDX3 and the ferroptosis pathway as promising therapeutic targets. Future research should prioritize validating this axis in human sinoatrial node tissues and developing targeted interventions, such as PRDX3 activators or specific ferroptosis inhibitors, to assess their translational potential for treating neurogenic bradyarrhythmias.

Ethics Approval and Informed Consent

This survey research was reviewed and approved by the Institutional Review Board (IRB) of Wannan Medical University (Approval ID: IRB-2024-158). All methods were carried out in accordance with relevant guidelines and regulations.

Availability of Data

Not applicable.

Consent for Publication

The authors confirm that patient consent is not applicable to this article.

Sources of Funding. The study was supported by Scientific Research Project of Anhui Provincial Department of Education Key Project (No. 2024AH051932), Xuancheng City Health Commission scientific research project (No. XCWJ2022065), Key Natural Science Project of Bengbu Medical University (No.2024byzd88) and Bengbu Medical University Graduate Research Innovation Program Project (No. Byycx24077).

Disclosures

The authors declare no conflict of interest.

Authors' Contributions

Mingliang Shao, Chenhuan Yao and Ting Tu carried out the

studies, participated in collecting data, and drafted the manuscript. Chengwei Wang, Yingying Han, Zhiqiang Chen and Jian Hu performed the statistical analysis and participated in its design. Xianping Wang, Linghe Meng, Bing Liu and Shiwen Liu helped to draft the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

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