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ORIGINAL RESEARCH

Circ_0001084/miR-181c-5p/PTPN4 Axis Mitigates Cardiomyocyte Injury by Modulating the TLR4/NF-κB Pathway: Insights into Therapeutic Potential for Myocardial Reperfusion Injury

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Background: Myocardial ischemia/reperfusion (I/R) injury significantly impacts the recovery of ischemic heart disease patients. Non-coding RNAs, including miRNAs, have been increasingly recognized for their roles in regulating cardiomyocyte responses to hypoxia/reoxygenation (H/R) injury. miR-181c-5p, in particular, has been implicated in inflammatory and apoptotic processes, suggesting its potential involvement in exacerbating cellular damage.

Methods: This study combined bioinformatic and experimental techniques to investigate myocardial injury. Gene expression data from the GEO database were analyzed, and HL-1 cardiomyocytes were used in a hypoxia/reoxygenation model to mimic reperfusion injury. Various molecular techniques have been applied to explore the underlying mechanisms, while statistical analyses have identified potential biomarkers and therapeutic targets.

Results: This study revealed significant upregulation of miR-181c-5p in cardiomyocyte H/R injury models, which inversely affected PTPN4 expression and activated the TLR4/NF-κB signaling pathway. Overexpression of PTPN4 inhibited this pathway. Notably, circ_0001084 was identified as absorbing miR-181c-5p, reducing its interaction with PTPN4 and subsequent pathway activation. This suggests a novel therapeutic pathway for myocardial I/R injury treatment, highlighting the interplay between non-coding RNAs and cellular stress responses.

Conclusion: circ_0001084 acts as a competing endogenous RNA for miR-181c-5p, enhancing PTPN4 expression and inhibiting the TLR4/NF-κB signaling pathway. These findings offer insights into the molecular mechanisms of myocardial I/R injury and potential therapeutic targets in ischemic heart disease.

Keywords: Myocardial, Circ_0001084/miR-181c-5p/PTPN4 axis, Cardiomyocyte hypoxia, Reoxygenation, TLR4/NF-κB pathway

Introduction

Myocardial ischemia/reperfusion (I/R) injury represents a pivotal challenge in the therapeutic landscape of ischemic heart disease, leading to an increase in cardiovascular complications post-infarct revascularization.[1–4] The pathophysiology of I/R injury is underscored by intricate molecular cascades, including the induction of apoptosis, programmed necrosis, exacerbated inflammatory response, generation of oxygen free radicals, and changes in mitochondrial membrane permeability.[5–7] To emulate I/R injury in a controlled setting, cardiomyocyte hypoxia/reoxygenation (H/R) models serve as key tool in vitro, reflecting the physiological and biochemical stresses encountered in vivo.

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The role of non-coding RNAs (ncRNAs) spanning microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) is increasingly being recognized for their regulatory capacity in cardiovascular pathology.[7,8] Specifically, miR-181c has been identified as significantly upregulated in the circulatory system of heart failure patients, with a concurrent upsurge in miR-181c-5p within myocardial tissues during the initial phases of myocardial infarction.[9,10] Experimental models have elucidated the cardioprotective potential of reducing miR-181c-5p levels, supporting the expression of anti-apoptotic proteins such as Bcl-2, thereby protecting mitochondrial integrity against apoptotic insults.[11–13] Moreover, miR-181c-5p has been implicated in indirectly mediating inflammatory responses through the TLR4/NF-κB signaling pathway,[14] further aggravating myocardial I/R injury.

PTPN4, a non-receptor protein tyrosine phosphatase defined by its PDZ and phosphatase domains, plays a pivotal role in regulating the inflammatory cascade by inhibiting TRAM tyrosine phosphorylation, thus curtailing NF-κB activation.[15,16] Within this regulatory framework, circRNAs, including circ_0001084, have been identified for their function as miRNA sponges, effectively sequestering miR-181c-5p and thereby alleviating the repression of downstream target genes.[16] Our investigations have highlighted the circ_0001084/miR-181c-5p/PTPN4 axis as a crucial modulator of the TLR4/NF-κB signaling pathway in the context of myocardial I/R injury, underscoring a novel insight into ncRNA-mediated cardiovascular disease modulation and offering a fresh perspective on therapeutic interventions to mitigate I/R injury.[17]

Extensive research depicts myocardial I/R injury as a multifaceted phenomenon, driven by ATP depletion, intracellular pH alterations, calcium dysregulation within mitochondria, and the activation of cell death pathways. These events are further intensified by NF-kappa-B activation and Cx43 degradation, cumulatively worsening myocardial infarction post-I/R injury.[18–20] Concurrently, the importance of developing novel therapeutic strategies to ameliorate I/R injury is emphasized, given the potential of various endogenous pathways identified in preclinical studies that, if activated at the right time, could substantially diminish cardiomyocyte damage and reduce infarct size.[21,22] Emerging insights into myocardial I/R injury underscore the critical involvement of ion channel dysregulation, reactive oxygen species, inflammation, and endothelial dysfunction in its pathogenesis,[23,24] adding complexity to the clinical management of ischemic heart conditions and emphasizing an integrative approach to uncover new therapeutic avenues.

This study aims to elucidate the molecular mechanisms underlying myocardial ischemia/reperfusion (I/R) injury, specifically investigating the role of miR-181c-5p and its interaction with PTPN4 and the TLR4/NF-κB signaling pathway. By examining the effects of circ_0001084 on miR-181c-5p and PTPN4 expression, this research seeks to identify potential therapeutic targets to mitigate cellular damage in ischemic heart disease.

Methods

Gene Expression Analysis

In our comprehensive study on myocardial injury, we integrated gene expression data analysis across mRNA, miRNA, and circRNA datasets sourced from the GEO database and processed through Illumina platforms. Each dataset underwent rigorous quality control, including intensity distribution, signal-to-noise ratio, and detection p-value checks. Samples with outlier characteristics were excluded to ensure high data integrity. Additional quality checks, such as boxplot and density plot visualizations, along with principal component analysis (PCA), helped verify data normalization and detect batch effects, thereby maintaining consistency across sample groupings.

Following preprocessing, which included background correction and normalization using the "limma" package and batch effect adjustments via the "SVA" package, we performed differential expression analysis. Genes were identified based on a statistical threshold of $p < 0.05$, and a fold change of 1.5, balancing sensitivity and specificity to capture genes of potential biological significance. This approach aimed to highlight genes relevant to myocardial injury while minimizing false positives, in line with standard practices in high-dimensional data analysis. To reduce non-biological variability, normalization across datasets and hierarchical clustering were conducted, ensuring that the observed variations likely represented biological rather than technical factors.

We then conducted enrichment analyses using the "clusterProfiler" package, focusing on GO and KEGG pathways, to explore the functions and pathways associated with differentially expressed genes in myocardial injury. Additionally, the Branch Least Absolute Shrinkage and Selection Operator (LASSO) algorithm helped us prioritize genes with high

prognostic value, identifying those with strong discriminatory power between injured and control samples. A subsequent literature review validated the clinical relevance of these genes in cardiovascular diseases, reinforcing their potential association with myocardial ischemia/reperfusion injury. In analyzing the GSE225245 and GSE242888 datasets, we observed significant transcriptional changes, with heatmaps and volcano plots illustrating clear differences in gene expression. The LASSO logistic regression algorithm identified key genes, such as *Ptpn4*, underlining their biological importance in myocardial injury. Our exploration of miRNAs and circRNAs revealed regulatory networks that significantly impact gene expression dynamics. Specifically, we identified miR-181c-5p, which interacts with *PTPN4* and regulates the TLR4/NF-κB pathway, a critical pathway in inflammatory responses during myocardial ischemia/reperfusion injury. Furthermore, circ_0001084 was identified as a competitive endogenous RNA (ceRNA) for miR-181c-5p, moderating its repressive effects on *PTPN4*.

The selection of circ_0001084 and miR-181c-5p was informed by both statistical and biological relevance, grounded in prior studies linking miR-181c-5p with the inflammatory and apoptotic pathways in cardiac injury. By binding to and modulating miR-181c-5p, circ_0001084 offers an additional layer of post-transcriptional regulation with therapeutic potential. This integrated, multifaceted analysis underscores the molecular mechanisms underlying myocardial injury and highlights potential biomarkers. Our study bridges data-driven findings with biological relevance, offering a foundation for further exploration of therapeutic strategies targeting gene regulation in cardiovascular injury.

Acquisition and Cultivation of Cardiomyocytes

The study was initiated with the procurement of HL-1 cardiomyocytes (strain CL-0605), sourced from mouse myocardium, provided by Procell Life Science & Technology Co., Ltd. While the HL-1 cell line offers a controllable in vitro model for examining hypoxia/reoxygenation injury mechanisms, validation of the circ _0001084/miR-181c-5p/PTPN4 axis in a single mouse cardiomyocyte line does not fully capture the complexities of ischemia-reperfusion injury in vivo. Future studies should expand this investigation to include additional cardiomyocyte cell lines, primary cardiomyocytes, and animal models to more robustly assess the therapeutic potential and physiological relevance of the circ _0001084/miR-181c-5p/PTPN4 axis in ischemia-reperfusion injury. These cells were cultured in a nourishing blend of DMEM/F12 supplemented with 10% fetal bovine serum and maintained in a stable environment at 37°C and an atmosphere of 5% CO2. The nutrient medium was refreshed every two–three days to sustain optimal cell growth and health.

The decision to utilize HL-1 cardiomyocytes in this study was based on their established relevance and suitability for in vitro cardiomyocyte research. HL-1 cells are a well-characterized, immortalized mouse cardiomyocyte line that retains the critical characteristics of cardiomyocytes, such as contractility, action potential generation, and expression of cardiac-specific genes. These attributes make HL-1 cells an advantageous model for simulating cardiac cellular responses under controlled conditions. In particular, the use of HL-1 cells allowed us to precisely control the hypoxia/reoxygenation (H/R) conditions, enabling detailed mechanistic studies at the molecular level that might be challenging to achieve with in vivo models due to their complexity and variability.

The choice to focus on in vitro studies rather than in vivo models is also influenced by ethical considerations and the intent to delineate fundamental molecular pathways, such as the TLR4/NF-κB pathway, in a simplified, controlled environment. In vitro systems provide the advantage of isolating cellular responses to specific manipulations (eg, miRNA and gene expression modulation), which is essential in the early stages of pathway characterization.

While in vivo studies are undoubtedly necessary to understand the full translational potential and systemic effects of interventions, we believe our findings in HL-1 cells contribute foundational insights into the miR-181c-5p/PTPN4 axis and its effect on the TLR4/NF-κB signaling pathway. Future studies incorporating in vivo models will be critical to confirm these findings within the more complex physiological context of myocardial ischemia/reperfusion injury.

Experimental Treatment of Cells

HL-1 cardiomyocytes were prepared by seeding them into six-well plates at a concentration of 1.5 × 10^5 cells per well, allowing overnight adhesion. The experimental treatments involved transfecting cells with specific genetic constructs. Table 1 shows the primers and constructs used in the study. These transfections utilized Lipofectamine 3000 (Thermo

Table 1 Primers and Constructs Used in the Study

Primer	RNA/DNA Sequence
miR-181c-5p mimic	AACAUUCAACCUGUCGGUGAGUACUCACCGACAGGUUGAAUGUU
miR-181c-5p inhibitor	ACUCACCGACAGGUUGAAUGUU
Over-expression plasmids for circ_0001084 and PTPN4	

Fisher Scientific, USA) over a 24-hour period, including the corresponding negative controls (NC) for each construct. The effective expression of targeted genes was confirmed via RT-qPCR or Western blot assays.

In this study, the sample size for each experiment was set to a minimum of three biological replicates per experimental condition, consistent with standards for in vitro studies, to ensure sufficient representation of observed effects. This choice was based on preliminary assessments and feasibility constraints, including the resources and time required for each experimental setup. While a formal power analysis was not conducted due to the exploratory nature of the study, post-experimental analyses showed that the sample size used was adequate for detecting significant differences in gene expression and protein levels, particularly for high-magnitude effects related to key molecular targets, such as TLR4, NF-κB, and PTPN4. Future studies aimed at confirming these findings in more comprehensive models or with additional replicates could employ power analysis to optimize the sample size, particularly for experiments with more subtle expression changes.

Cardiomyocyte Hypoxia/Reoxygenation (H/R) Modeling

To simulate the effects of reperfusion injury, cells underwent an H/R model involving initial culture in serum-free, low-glucose DMEM under hypoxic conditions (94% N2, 5% CO2, 1% O2) for six hours, followed by reoxygenation under normoxic conditions (95% air, 5% CO2) in DMEM/F12 with 10% fetal bovine serum for 18 h.

Molecular Analysis Techniques

RNA Extraction and RT-qPCR

Total RNA was extracted using the Ultrapure RNA Kit (CWBIO, China) and converted to cDNA using the HiScript II Q RT SuperMix for qPCR kit (Vazyme, China); qPCR was performed using 2×SYBR Green PCR Master Mix on a CFX Connect Real-Time PCR Detection System (Bio-Rad) and also Ptpn4, U6, and β-actin: Specific primer sequences were used for each.

Expression levels were normalized to β-actin or U6, employing the 2^-ΔΔCT method, with analyses conducted in triplicates.[1] Table 2 shows the primers used for RT-qPCR analysis.

Western Blot Analysis

The Western blot assay protocol begins with the meticulous preparation of cell lysates from cultured cells. This process utilizes radio-immunoprecipitation assay (RIPA) lysis buffer, which is known for its efficacy in preserving protein integrity while efficiently lysing cells. The lysis is conducted at a controlled temperature of 4°C to prevent protein degradation.

Following lysis, the concentration of proteins in each sample was determined using the Bicinchoninic Acid (BCA) protein assay kit (E-BC-K318-M, Elabscience, China). This colorimetric assay is based on the reduction of Cu^2+ to

Table 2 Primers Used for RT-qPCR Analysis

Target Gene/Sequence	Forward Primer	Reverse Primer
miR-181c-5p (RT-qPCR)	GTCGTATCCAGTGCAGGGTCCGAGG	TATTCGCACTGGATACGACACTCAC
Circ0001084	ACTGGGAGAAGGGGAAGGA	AACTCTGGGCTCTGAAATGTG

Cu^1+ by protein in an alkaline medium, with the bicinchoninic acid forming a purple complex with the reduced copper, which is proportional to the protein present. After quantification, the protein samples were denatured to ensure that they unfolded into their primary structure, facilitating equal access for antibody binding.

The denatured protein samples were then subjected to Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), allowing for the separation of proteins based on their molecular weight. Following electrophoresis, the proteins were carefully transferred from the gel onto polyvinylidene fluoride (PVDF) membranes (IPVH00010, Millipore, USA), providing a stable medium for subsequent immunodetection.

To prevent non-specific binding of antibodies to the membrane, the PVDF sheets were blocked with a 5% solution of Bovine Serum Albumin (BSA) (A8020, Solarbio, China). This step is crucial for enhancing the specificity and clarity of the immunoblots.

After blocking, the membranes were incubated with primary antibodies specifically targeted against a series of proteins of interest. Table 3 shows the antibodies used for Western blot analysis. This incubation was carried out overnight at 4°C to allow for optimal binding of the antibodies to their target proteins. Following primary antibody incubation, the membranes were exposed to Horseradish Peroxidase (HRP)-conjugated secondary antibodies (ZB-2305, ZB-2301, ZSGB-BIO, China, dilution 1:2000). These antibodies are designed to recognize primary antibodies and are conjugated to an enzyme (HRP) that catalyzes the chemiluminescent reaction used for detection.

Visualization of the target proteins was achieved through the application of chemiluminescent substrates and subsequent exposure to a film or a digital imaging system (Chemi DocTM XRS+, Bio-Rad, USA). This process allows the capture of luminescent signals emitted upon the reaction of the substrate with HRP, directly correlating to the presence and abundance of the target proteins.

Finally, quantitative analysis of the protein bands was performed using ImageJ software, which involves measuring the grayscale values of the bands. This digital analysis allows for the precise quantification and comparison of protein levels between samples, facilitating a deeper understanding of protein expression and modification in response to experimental conditions.[2]

Fluorescence In Situ Hybridization (FISH)

In this study, a Fluorescence In Situ Hybridization (FISH) assay was conducted using a mmu_circ_0001084 FISH kit (MK10941, BOSTER, China) to visualize the localization of the mmu_circ_0001084 sequence within cardiomyocytes following various transfections and under conditions simulating hypoxia/reoxygenation (H/R). Initially, the cells underwent a series of preparatory steps, including washing with Phosphate-Buffered Saline (PBS), fixation in 4% paraformaldehyde to preserve cellular architecture, and membrane permeabilization with 0.5% Triton X-100 to allow probe entry. Subsequently, the cells were incubated overnight with a fluorescently labeled mmu_circ_0001084 probe, allowing for specific hybridization to its target sequence.

Table 3 Antibodies Used for Western Blot Analysis

Antibody	Catalog Number	Supplier	Dilution
NF-kB p65	AF5006	Affinify, China	1:1000
Phospho-NF-kB p65 (Ser536)	AF2006	Affinify, China	1:1000
IKKβ	AF6009	Affinify, China	1:1000
Phospho-IKK α/β (Ser180/Ser181)	AF3013	Affinify, China	1:1000
TLR4	19811-1-AP	Proteintech, China	1:1000
PTPN4	11131-1-AP	Proteintech, China	1:1000
TRAM	12705-1-AP	Proteintech, China	1:1000

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To enhance visualization, cell nuclei were stained with DAPI, a fluorescent stain that binds to DNA, providing a contrast that highlights nuclei in blue under a fluorescence microscope (CKX53, OLYMPUS, Japan). This meticulous process enabled the capture of detailed images, showcasing the precise localization of the mmu_circ_0001084 sequence within the cell context. Through fluorescence microscopy, researchers were able to observe the spatial distribution of this specific nucleic acid sequence, offering insights into its role and behavior in cardiomyocytes, especially under the stress of hypoxia/reoxygenation, a model relevant to understanding cardiac ischemia-reperfusion injuries.[3]

Apoptosis Analysis in Cardiomyocytes

Upon establishing a Cardiomyocyte Hypoxia/Reoxygenation (H/R) model, Apoptosis detection was meticulously carried out using 5 × 10^5 cells stained with an Annexin V-APC/7-AAD apoptosis kit (AP105-100kit, MULTI SCIENCES, China). This method hinges on the precise identification and quantification of apoptotic cells through flow cytometry, utilizing NovoCyte 2060R (Agilent, USA) to analyze the stained cells. While the quantification of apoptosis serves as an indicator of cell death, it does not fully capture the extent of cardiomyocyte injury. To provide a more comprehensive assessment, future studies should include a direct cell viability assay, such as MTT or trypan blue exclusion, to complement apoptosis and ROS measurements. The choice of using the Annexin V-APC/7-AAD kit for apoptosis detection was based on its established utility in distinguishing viable, early apoptotic, and late apoptotic cells, thereby providing initial insights into cell death mechanisms under H/R conditions. However, we acknowledge that this kit does not differentiate apoptosis from necrosis as conclusively as other apoptosis-specific markers might. In recognition of the reviewer's suggestion, future studies should incorporate additional apoptosis-specific markers, such as caspase-3 activation and the TUNEL assay, to enhance the specificity of apoptosis detection and allow for clearer differentiation between apoptosis and necrosis. These markers, including caspase-3 as an indicator of mitochondrial apoptosis and TUNEL for DNA fragmentation, would provide a more comprehensive understanding of the cell death pathways involved in the H/R model. Annexin V-APC/7-AAD staining differentiates between viable, early apoptotic, and late apoptotic or necrotic cells, enabling a comprehensive assessment of cell fate post-transfection and H/R model establishment, thus providing valuable insights into the cellular responses triggered by various experimental conditions.[1]

ROS Detection in Cardiomyocytes

Intracellular reactive oxygen species (ROS) levels were determined using an active oxygen detection kit (KGT010-1, KeyGEN Bio TECH, China), highlighting the oxidative stress status within the cells. Although ROS levels provide insights into oxidative damage, they do not comprehensively represent cardiomyocyte injury. Incorporating cell viability assays in future studies will offer additional clarity on the overall cell health under hypoxia/reoxygenation conditions. After treating the cells with 10μM DCFH-DA for 20 minutes at 37°C, a procedure designed to probe the presence of ROS, the cells were washed with PBS to remove excess dye. The fluorescence intensity of 2',7'-dichlorofluorescein (DCF), which reflects ROS levels, was quantified using flow cytometry. This step involved collecting data from over 10,000 events for each sample, offering a quantitative measure of ROS generation, an important indicator of cellular oxidative stress, which is particularly relevant in the context of the cardiomyocyte H/R model.[4]

Dual Luciferase Reporter Gene Assay in Cardiomyocytes

To explore the molecular interactions, specifically the binding efficacy between circ_0001084 and miR-124-3p, a dual-luciferase reporter gene assay was employed. This assay used both wild type (Wt) and mutant (Mut) versions of circ_0001084, incorporated into the pmirGLO Report luciferase vector, which were then co-transfected into HL-1 cardiomyocytes along with a miR-181c-5p mimic or its control at a concentration of 25nM, using Lipofectamine 3000 (Thermo Fisher, USA) as the transfection agent. Subsequent analysis using the dual luciferase reporter assay kit (DL101-01, Vazyme, China) allowed for quantitative assessment of the interaction between circ_0001084 and miR-124-3p. This method not only delineates the direct regulatory effects of miR-124-3p on circ_0001084 but also enhances our understanding of the genetic mechanisms underlying the cardiomyocyte response to stress, such as that induced by the H/R model.[5]

Statistical Analysis

Data processing was conducted using SPSS version 19.0, with results expressed as the mean ± standard deviation for a minimum of three independent experiments. One-way ANOVA, supplemented by LSD post-hoc tests, facilitated the comparison of quantitative data across multiple groups, adopting an alpha level of 0.05 for statistical significance. GraphPad 8.0 and ImageJ were utilized for graphical and grayscale analyses, respectively.

Results

Analysis of Differential Gene Expression and miRNA Profiles in GSE225245 and GSE242888

Our differential gene expression analysis within the GSE225245 dataset revealed significant insights, illustrated by a heatmap (Figure 1A) and volcano plot (Figure 1B). The heatmap clearly distinguished upregulated and downregulated genes, indicating substantial transcriptional changes across groups. The volcano plot complemented this by mapping these genes, with upregulated genes to the right and downregulated to the left, emphasizing both statistical significance and fold changes. Together, these visuals highlight genes of potential biological significance, meriting further functional characterization to clarify their roles in biological processes and disease mechanisms.

To explore the functional implications of these differentially expressed genes (DEGs), we applied Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses (Figure 1C for GO and Figure 1D for KEGG). These analyses provided insights into biological processes, cellular components, molecular functions, and

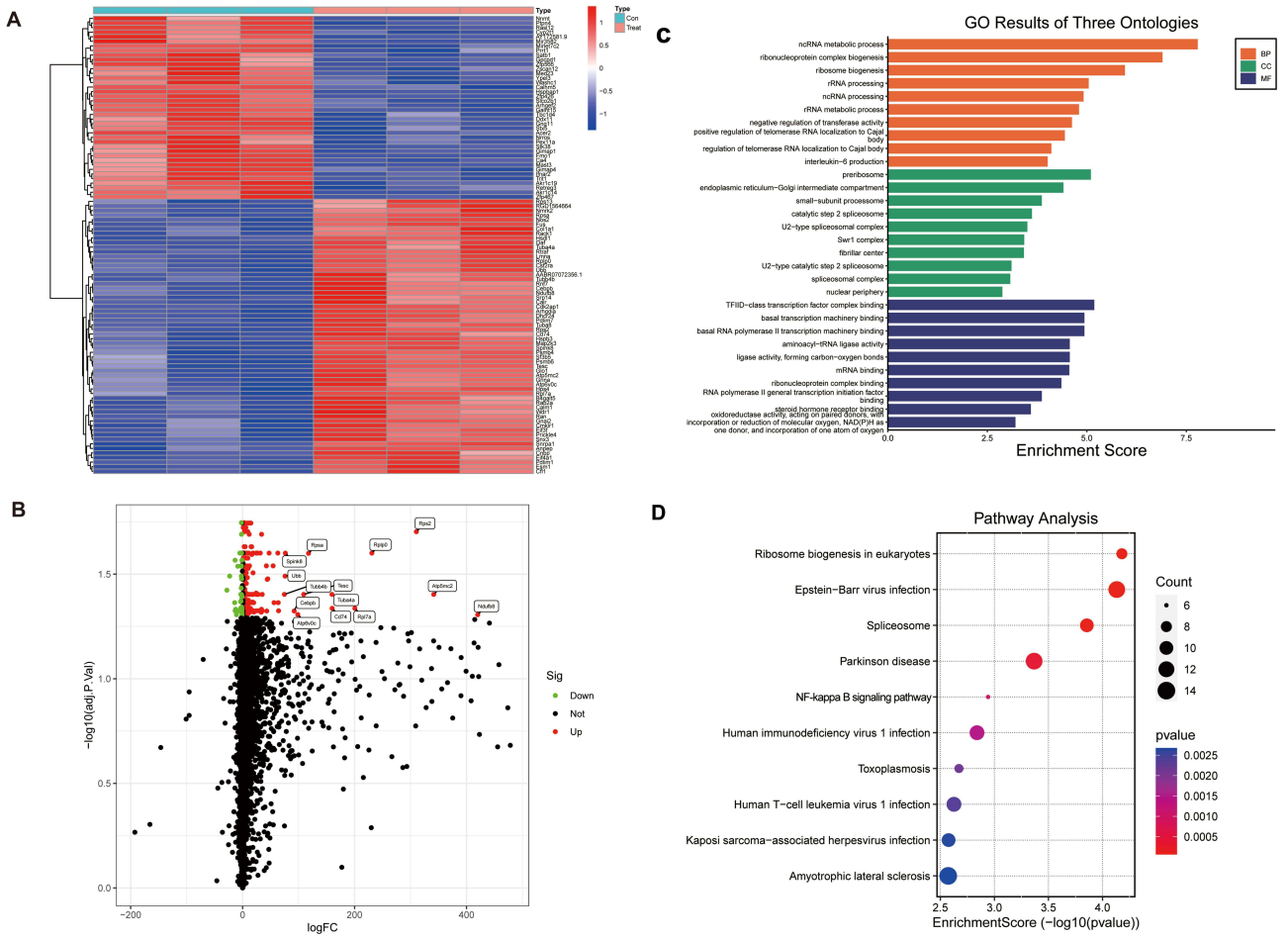

Figure 1 DEGs Volcano plot and heatmap of DEGs. (**A**) heatmap of DEGs in GSE225245 (**B**) Volcano plot of DEGs in GSE225245. GO and KEGG analysis of DEGs. (**C**) GO analysis. (**D**) KEGG analysis.

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pathways associated with the DEGs, forming a foundation for understanding their potential impact on biological and disease pathways. While this enrichment analysis offers valuable biological insights, it is constrained by the current scope of gene annotations and pathway knowledge.

Using the least absolute shrinkage and selection operator (LASSO) logistic regression, we identified key genes, with Ptpn4 emerging due to its differential expression (Figure 2A and B). This method proved effective in isolating crucial predictors from extensive data, although validating Ptpn4's biological role requires in-depth analysis to uncover its cellular function within this context.

Expression levels of miR-181c-5p and Circ_0001084 were analyzed via boxplots (Figure 2C for miR-181c-5p, Figure 2D for Circ_0001084), suggesting a complex regulatory interaction. The observed differences imply a regulatory network involving miR-181c-5p and Circ_0001084, with possible effects on cellular physiology. Further studies are needed to elucidate the functional implications of these expression patterns.

In the GSE242888 dataset, differential miRNA expression analysis confirmed miR-181c-5p upregulation, visualized through a heatmap (Figure 3A) and volcano plot (Figure 3B). This consistency reinforces miR-181c-5p's role in myocardial injury and TLR4/NF-κB pathway activation, highlighting it as a potential therapeutic target in cardiomyocyte hypoxia/reoxygenation injury. This comparison of datasets also revealed significantly altered miRNAs, indicating

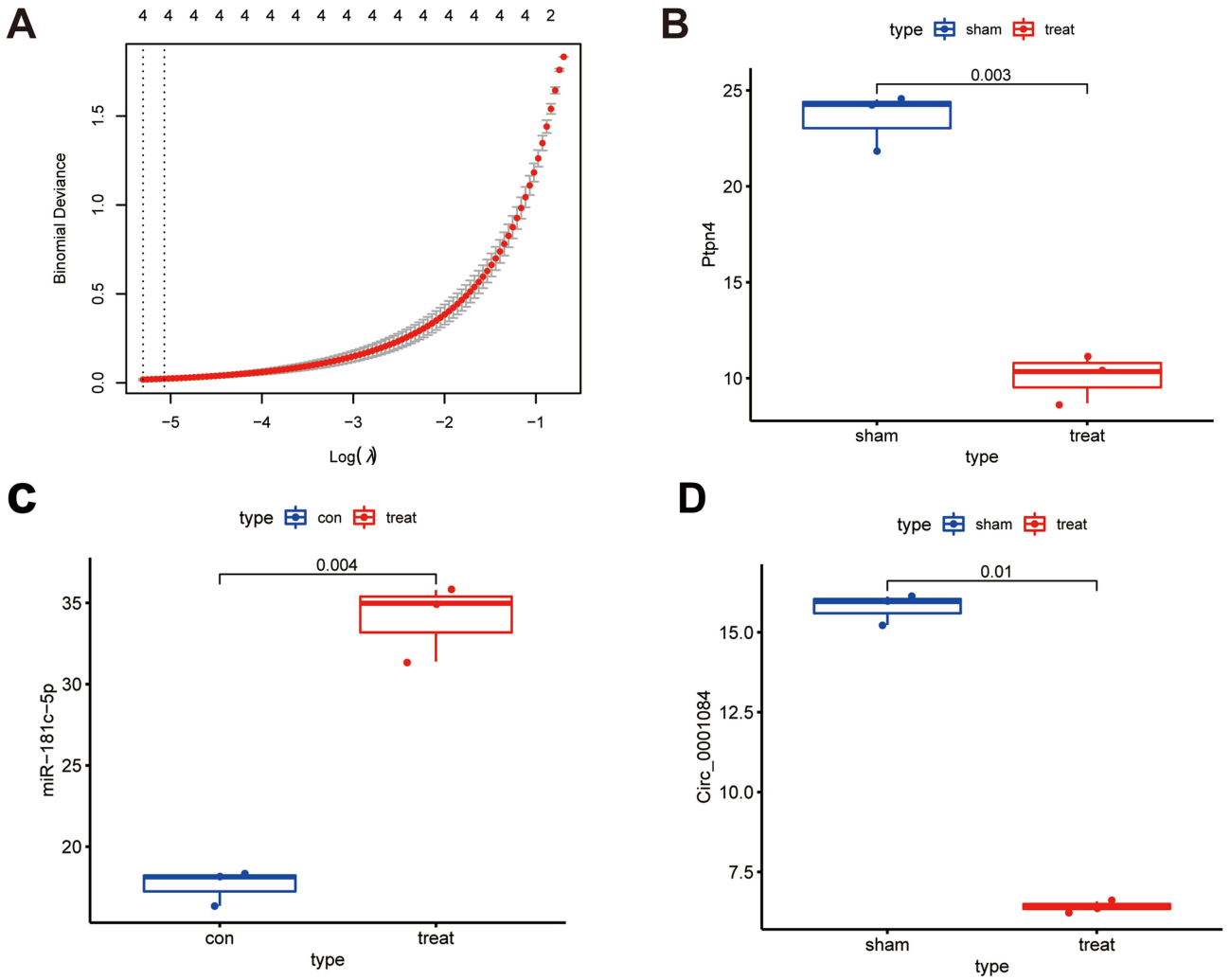

Figure 2 Screening of key genes. (**A**) key genes were screened by the least absolute shrinkage and selection operator (LASSO) logistic regression algorithm. (**B**) The Difference boxplot of Ptpn4. Box plot expression of genes. (**C**) The Difference boxplot of miR-181c-5p. (**D**) The Difference boxplot of Circ_0001084.

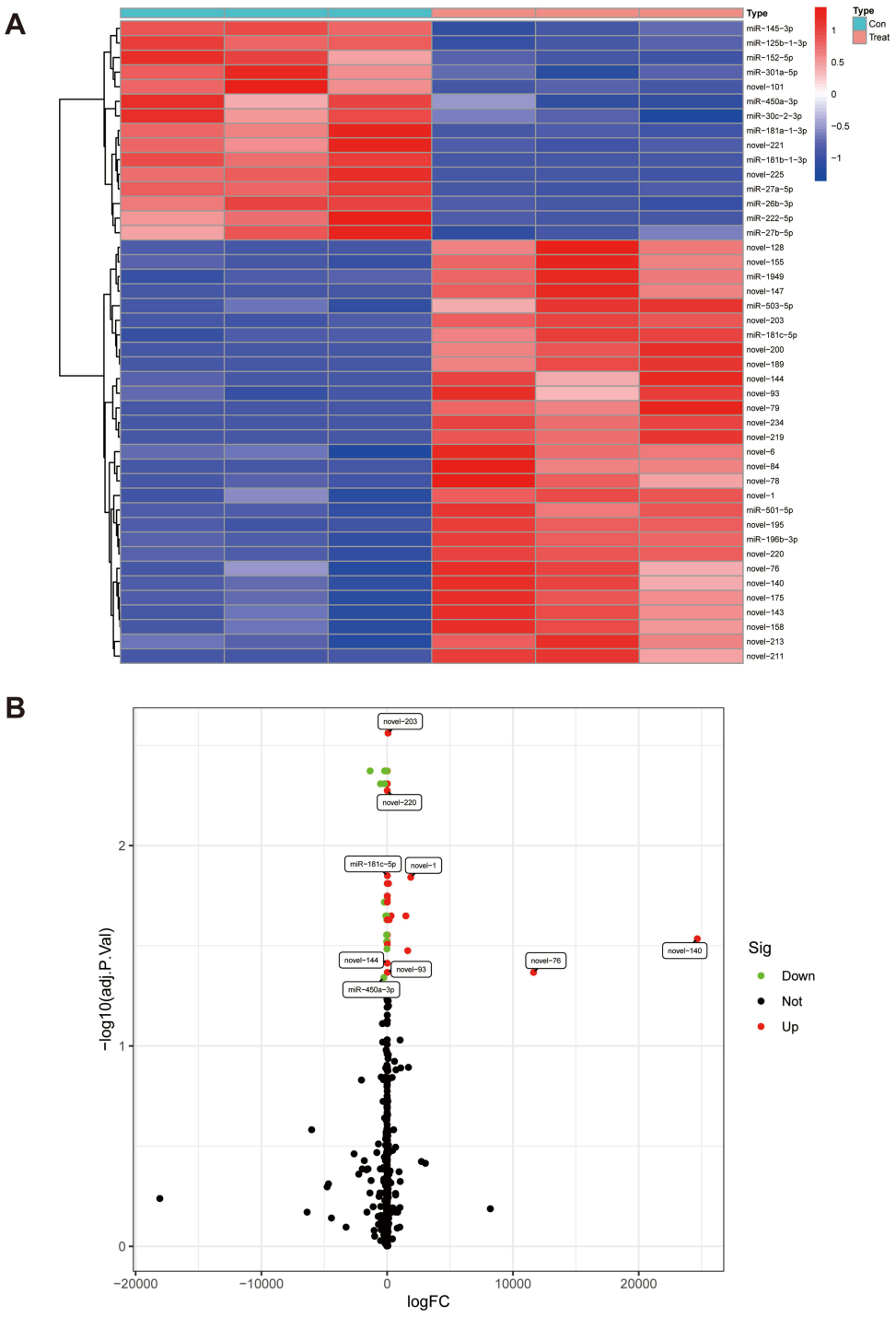

Figure 3 Volcano plot and heatmap of Differentially expressed miRNAs. (**A**) heatmap of Differentially expressed miRNAs in GSE242888 (**B**) Volcano plot of Differentially expressed miRNAs in GSE242888.

a regulatory network that influences gene expression and impacts biological processes. While further research is essential to define specific miRNA functions, this finding provides a basis for exploring their regulatory roles in gene expression.

In summary, our comprehensive analysis spans gene and miRNA expression, functional implications, and regulatory interactions, offering a holistic view of the underlying biological phenomena. However, translating these findings into biological relevance requires experimental validation and an intricate understanding of the complex biological systems involved.

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miR-181c-5p Significantly Elevated, PTPN4 Decreased, and the TLR4/NF-κB Signaling Pathway Activated in HL-1 Cardiomyocytes H/R Injury

Previous studies have shown increased miR-181c-5p expression in H9C2 cardiomyocyte models of hypoxia/reoxygenation (H/R) injury. Building on this, we explored miR-181c-5p's role in H/R injury by employing miR-181c-5p mimics and inhibitors in HL-1 cardiomyocytes (Figure 4A and B). Our findings confirm that miR-181c-5p is significantly upregulated in the H/R model (Figure 4C), correlating with a notable decrease in PTPN4 expression and activation of the TLR4/NF-κB signaling pathway evidenced by increased TLR4, TRAM, p-IKKβ, and p-p65 levels. Treatment with an miR-181c-5p inhibitor reversed these changes, suggesting therapeutic potential, whereas mimics showed no substantial effect (Figure 4D and E). Given apoptosis's primary role in cardiomyocyte death during H/R injury, we hypothesized that miR-181c-5p inhibition might reduce apoptosis. Flow cytometry revealed heightened apoptosis in the H/R model, which decreased with miR-181c-5p inhibitor treatment, while mimics led to further apoptosis increases (Figure 4F).[11,25] These results suggest that miR-181c-5p inhibition may protect against apoptosis, reinforcing its potential as a therapeutic target. Although apoptosis and ROS levels are limited markers of cardiomyocyte injury, they serve as accessible indicators of cellular stress response.

Our study further explored the circ_0001084/miR-181c-5p/PTPN4 regulatory axis in H/R-induced injury, revealing that circ_0001084, possibly acting as a miRNA sponge, reduces miR-181c-5p-mediated TLR4/NF-κB activation. This

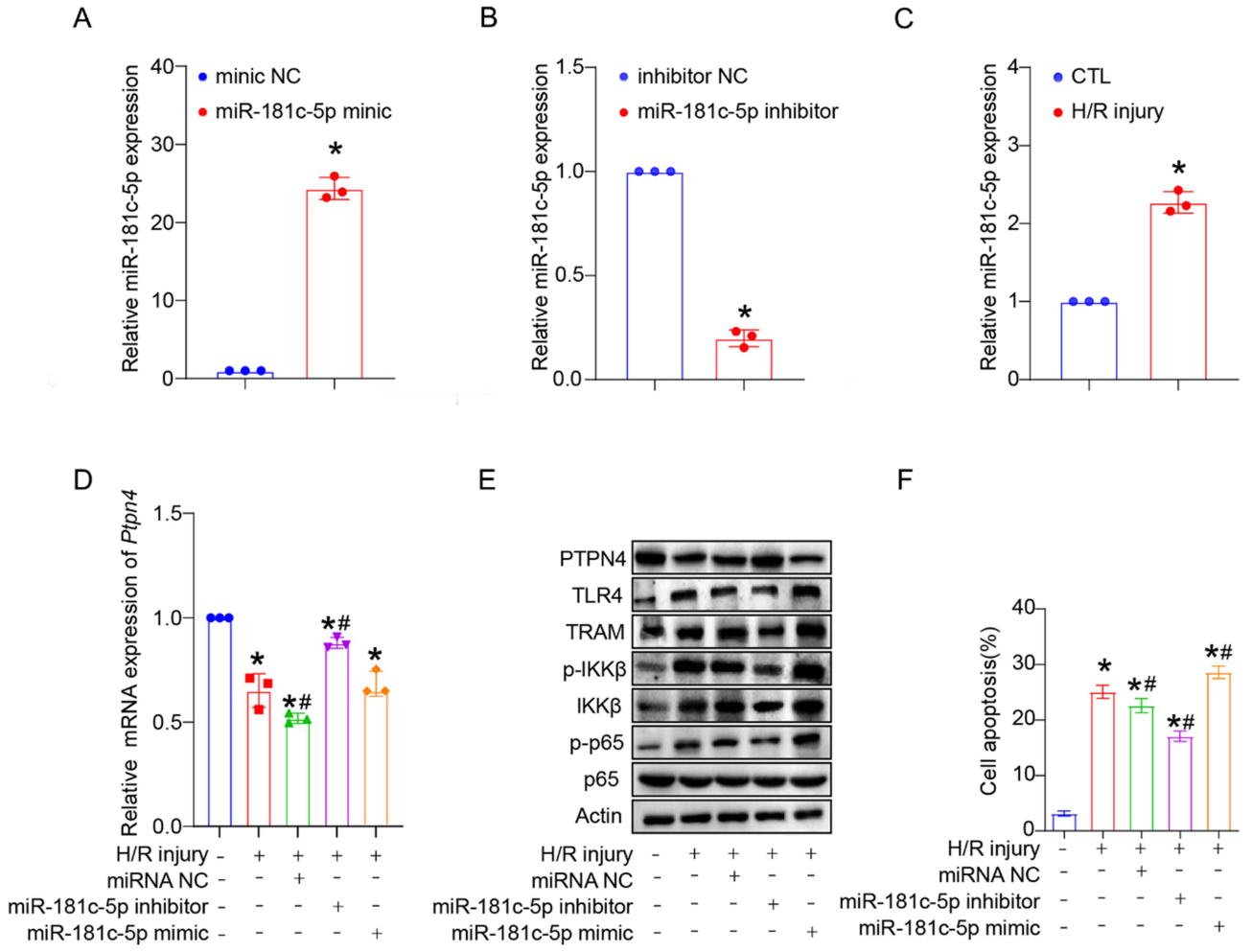

Figure 4 Expression of miR-181c-5p, PTPN4, and TLR4/NF-κB signaling pathways after cardiomyocyte H/R injury. (**A** and **B**) RT-qPCR detected miR-181c-5p expression after using of miR-181c-5p Mimic and inhibitor in HL-1 cardiomyocytes, (**C**) RT-qPCR detected miR-181c-5p expression in HL-1 cardiomyocytes H/R injury, (**D**) RT-qPCR detected PTPN4 expression in I/RI model by using miR-181c-5p mimic or inhibitor, (**E**) Western blot detected PTPN4 and TLR4/NF-κB pathway in HL-1 cardiomyocytes H/R injury by using miR-181c-5p mimic or inhibitor, (**F**) FC detected cell apoptosis in I/RI model by using miR-181c-5p mimic or inhibitor; The measurement data were expressed as mean±standard deviation, repetition=3, *P<0.05 compared with untreated group, # P<0.05 compared with only H/R injury group.

interaction diminished apoptosis and ROS in cardiomyocytes under H/R stress, positioning circ_0001084 as a promising target to modulate inflammation and apoptosis in ischemia-reperfusion injury. This work confirms and extends previous findings on individual axis components by elucidating their combined role in H/R injury pathophysiology.[6,7]

These results highlight miR-181c-5p's involvement in modulating H/R injury in cardiomyocytes by downregulating PTPN4 and activating TLR4/NF-κB signaling, suggesting that miR-181c-5p inhibition offers a therapeutic avenue. The absence of significant effects with miR-181c-5p mimics indicates potential additional regulatory mechanisms, warranting further investigation. This detailed exploration of the miR-181c-5p/PTPN4/TLR4/NF-κB axis provides nuanced insights into the molecular basis of H/R injury and highlights complex interactions between miRNAs and cellular signaling in cardiomyocyte stress responses.

PTPN4 Inhibits Activation of the TLR4/NF-κB Signaling Pathway in Cardiomyocyte H/R Injury

Earlier research highlighted miR-181c-5p's capacity to directly interact with the 3' UTR of PTPN4, thereby influencing PTPN4 expression and confirming its critical function as a downstream target of miR-181c-5p. Extending these findings, our study focused on the role of PTPN4 in the TLR4/NF-κB signaling axis in HL-1 mouse cardiomyocyte H/R injury models. By overexpressing PTPN4 (Figure 5A and B), we observed a notable decrease in the expression of key TLR4/NF-κB signaling proteins (TLR4, TRAM, IKKβ, p-IKKβ, and p-p65), which were otherwise elevated in the H/R injury context, as shown in Figure 5C. Additionally, apoptosis and reactive oxygen species (ROS) levels, which were significantly higher in the model group,

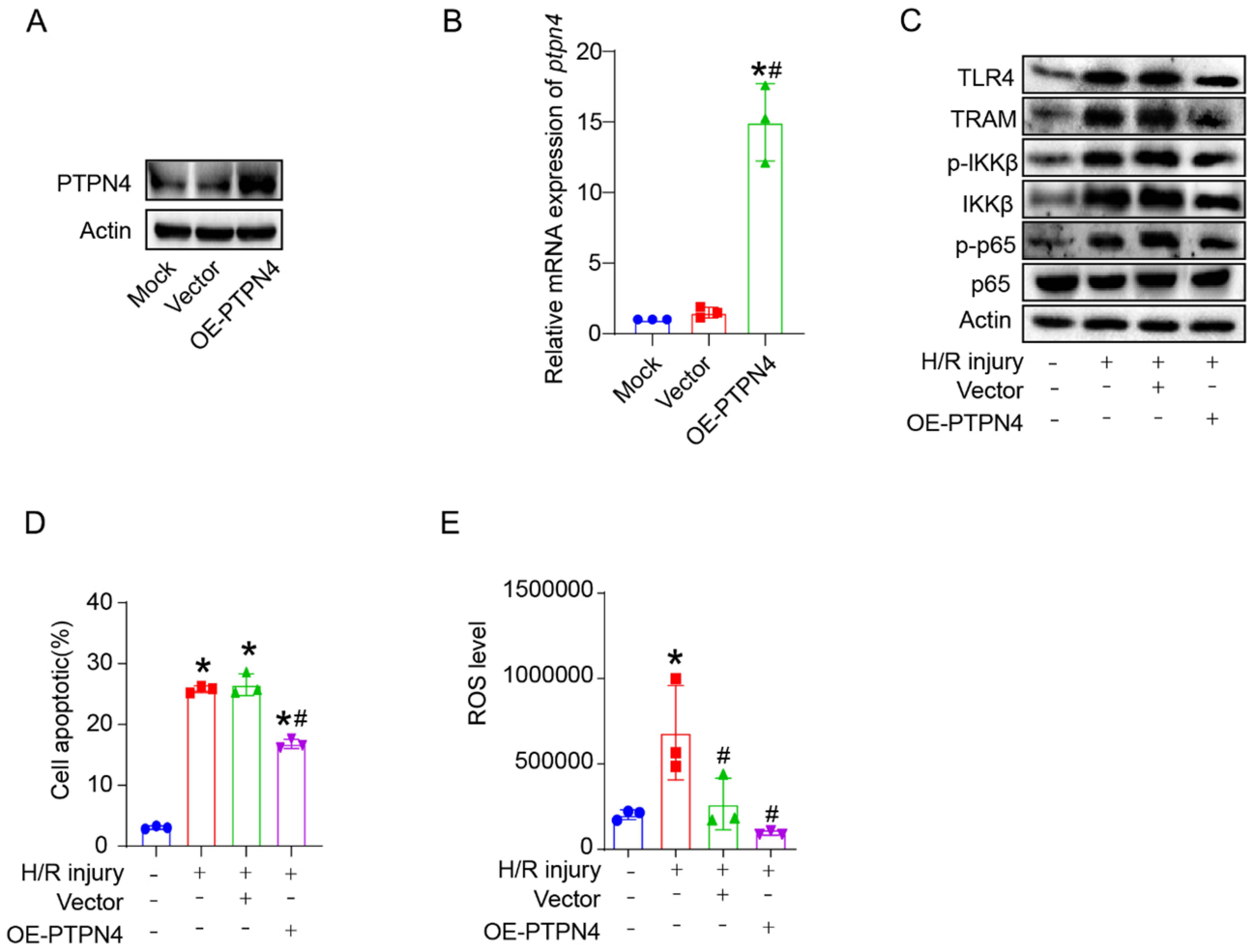

Figure 5 PTPN4 inhibits activation of the TLR4/NF-κB signaling pathway in cardiomyocyte H/R injury. (**A**) Western blot demonstrating expression of PTPN4 levels in HL-1 cardiomyocytes, (**B**) RT-qPCR quantification of miR-181c-5p expression in HL-1 cardiomyocytes, (**C**) Western blot detected expression of TLR4/NF-κB pathway levels after over-expression of PTPN4 in HL-1 cardiomyocytes, (**D**) (**E**) FC detected cell apoptosis and ROS levels after over-expression of PTPN4 in HL-1 cardiomyocytes; The measurement data were expressed as mean±standard deviation, repetition=3, *P<0.05 compared with untreated group, #P<0.05 compared with only H/R injury group.

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were markedly reduced following PTPN4 overexpression (Figure 5D and E). These outcomes suggest that PTPN4 serves as an inhibitory regulator within the TLR4/NF-κB pathway, potentially mitigating H/R injury by attenuating apoptosis and oxidative stress.[8,9]

Critically, this investigation underscores PTPN4's pivotal role in moderating the cardiomyocyte response to H/R injury through the modulation of the TLR4/NF-κB signaling pathway. The substantial reduction in both apoptosis and ROS levels following PTPN4 overexpression highlights its therapeutic promise. However, this study also signals the need for a deeper understanding of PTPN4's regulatory mechanisms and its interaction with miR-181c-5p. These insights not only reinforce the therapeutic potential of targeting PTPN4 in H/R injury management, but also require further research to unravel the complex regulatory networks governing cardiomyocyte survival and death pathways.

Rescue Experiments to Verify the Effect of PTPN4 Regulation by miR-181c-5p on TLR 4/NF-κB Signaling in Cardiomyocyte H/R Injury

In our latest study, we aimed to elucidate the direct regulatory effect of miR-181c-5p on PTPN4 expression in HL-1 cardiomyocytes. To achieve this, we employed a dual approach by simultaneously transfecting HL-1 cardiomyocytes with a miR-181c-5p mimic and a PTPN4 coding sequence (CDS) overexpression plasmid. This strategy was designed to counteract the miR-181c-5p-induced reduction in endogenous PTPN4 levels, as shown in Figure 6A–C. Our findings

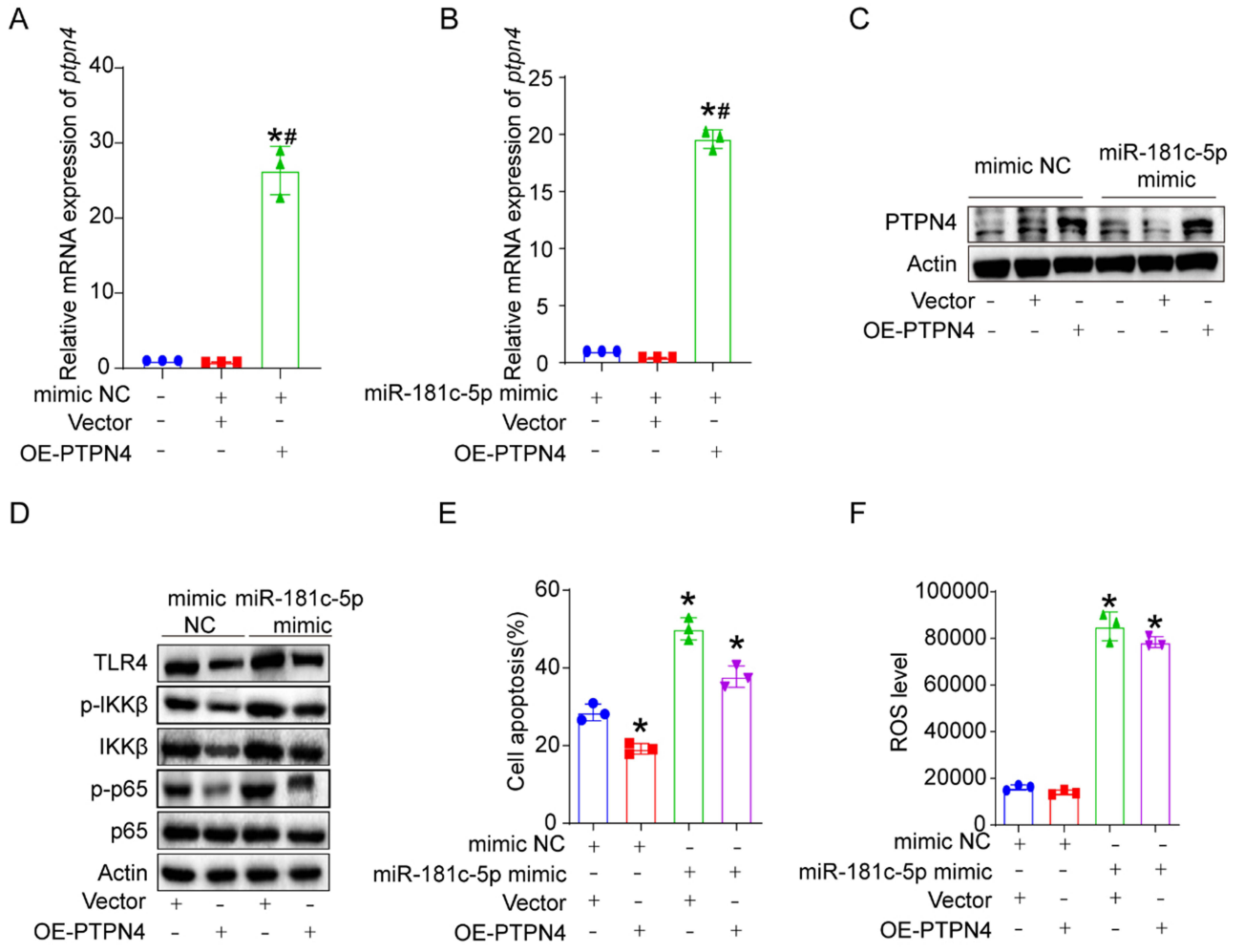

Figure 6 Effect of miR-181c-5p and PTPN4 overexpression on TLR4/NF-κB signaling, apoptosis, and ROS levels in HL-1 cardiomyocytes. (**A** and **B**) Relative mRNA expression of ptpn4 after transfection with miR-181c-5p mimic and PTPN4 overexpression vector (OE-PTPN4). (**C**) Western blot analysis of PTPN4 protein levels. (**D**) Western blot analysis of TLR4, p-IKKβ, IKKβ, p-p65, and p65 expression. (**E**) Apoptosis percentage as measured by flow cytometry. (**F**) ROS levels measured using a DCFH-DA probe. *Indicates a significant difference compared to the control group ($p < 0.05$), and # indicates a significant difference compared to the miR-181c-5p mimic group ($p < 0.05$).

revealed that compared to the control group (mimic NC+vector), the overexpression of PTPN4 (mimic NC+OE-PTPN4) led to a significant suppression in the expression levels of key TLR4/NF-κB signaling molecules, including TLR4, IKKβ, p-IKKβ, and p-p65. Conversely, these signaling molecules were elevated in the presence of the miR-181c-5p mimic alone. Notably, co-administration of the miR-181c-5p mimic with the PTPN4 overexpression construct (mimic+OE-PTPN4) effectively attenuated the upregulation of these signaling molecules, as shown in Figure 6D.

Further analysis using flow cytometry to assess apoptosis and reactive oxygen species (ROS) levels corroborated these findings, with similar trends observed across the different treatment groups (Figure 6E and F). These results lend additional support to the hypothesis that miR-181c-5p modulates the TLR4/NF-κB signaling pathway, primarily through the regulation of PTPN4 expression.[10]

Critically analyzing these findings, it becomes evident that miR-181c-5p plays a pivotal role in the activation of the TLR4/NF-κB pathway, which is a key mediator of inflammatory and stress responses in cardiomyocytes. The ability to mitigate miR-181c-5p-induced effects through PTPN4 overexpression not only confirms the direct regulatory relationship between miR-181c-5p and PTPN4 but also highlights the potential for targeted therapeutic interventions in conditions characterized by excessive activation of the TLR4/NF-κB pathway. This study further solidifies the understanding of the molecular dynamics at play in the cardiomyocyte response to stress and underscores the therapeutic potential of modulating miR-181c-5p and PTPN4 interactions.

Circle0001084 as a Potential Upstream Regulatory Progenitor of miR-181c-5p

Moreover, the exploration of non-coding RNAs, particularly circRNAs, has revealed their potential as upstream regulators of miRNA expression due to their unique structural features and functional versatility. In this context, circ_0001084 was identified as a potential upstream regulator of miR-181c-5p. The localization studies employing FISH indicated that circ_0001084 is predominantly present in the nucleus and is significantly downregulated in HL-1 cardiomyocytes under H/R stress (Figure 7A and B). This suggests that the decreased expression of circ_0001084 may lead to derepression of miR-181c-5p, subsequently affecting the TLR4/NF-κB signaling pathway and its downstream consequences. The potential regulatory role of circ_0001084 in the expression of miR-181c-5p underscores the complexity of the molecular networks involved in cardiomyocyte H/R injury and highlights the significance of targeting specific interactions within these networks to mitigate the adverse effects of such injuries on cardiac health.

In this study, circ_0001084 was identified as a potential upstream regulator of miR-181c-5p expression. Localization studies using FISH revealed that circ_0001084 predominantly resides in the nucleus and is significantly downregulated in HL-1 cardiomyocytes under H/R stress. This observation led to the hypothesis that the decreased expression of circ_0001084 could result in the derepression of miR-181c-5p, thereby influencing the TLR4/NF-κB signaling pathway and its downstream effects.

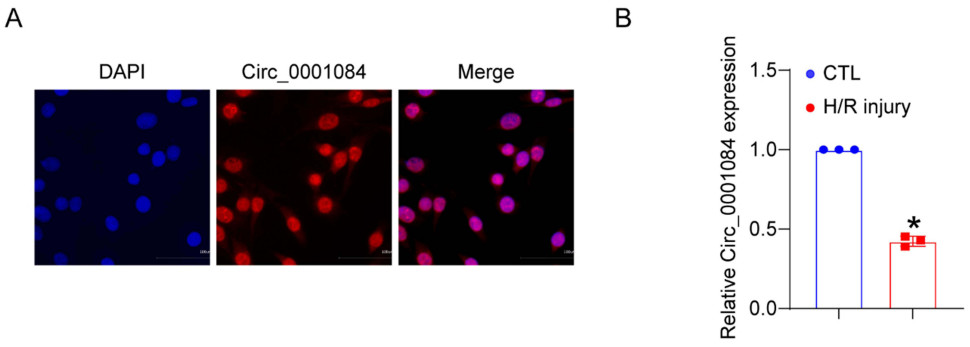

Figure 7 Circ_0001084 is a potential upstream regulatory progenitor of miR-181c-5p, (**A**) FISH detected the location of circ_0001084 in HL-1 cardiomyocytes, (**B**) RT-qPCR quantification of expression of circ_0001084 levels in H/R injury model; The measurement data were expressed as mean±standard deviation, repetition=3, *P<0.05 compared with CTL group.

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Circ_0001084 Reduces Mouse Cardiomyocyte H/R Injury by Adsorption of miR-181c-5p

To investigate the interaction between circ_0001084 and miR-181c-5p, we utilized wild-type (WT) and mutant (mut) circ_0001084 vectors and introduced them into HL-1 cardiomyocytes along with miR-181c-5p mimics. The assay revealed that miR-181c-5p significantly suppressed luciferase activity in cells transfected with the WT circ_0001084 vector, indicating a direct interaction, whereas the mutant vector showed no change in luciferase expression, illustrating the specificity of this interaction (Figure 8A). This indicated that the TGAATGT sequence is crucial for the binding between circ_0001084 and miR-181c-5p. Upon overexpressing circ_0001084 in HL-1 cardiomyocytes, we observed a notable increase in its expression (Figure 8B) and a consequent elevation in PTPN4 mRNA levels (Figure 8C), suggesting a regulatory role for circ_0001084 over PTPN4 and its potential protective mechanism against myocardial ischemia-reperfusion injury.

Moreover, the transfection of circ_0001084 led to a significant decrease in the expression of key proteins involved in the TLR4/TRAM/IKKβ/NF-κB signaling pathway (TLR4, TRAM, IKKβ, p-IKKβ, p65, and p-p65) compared to the model group, indicating a dampening effect on this pathway (Figure 8D). Additionally, both apoptosis and reactive oxygen species (ROS) levels, which were elevated in the model group, were significantly reduced following circ_0001084 overexpression (Figure 8E and F). These findings not only confirm the targeted interaction between

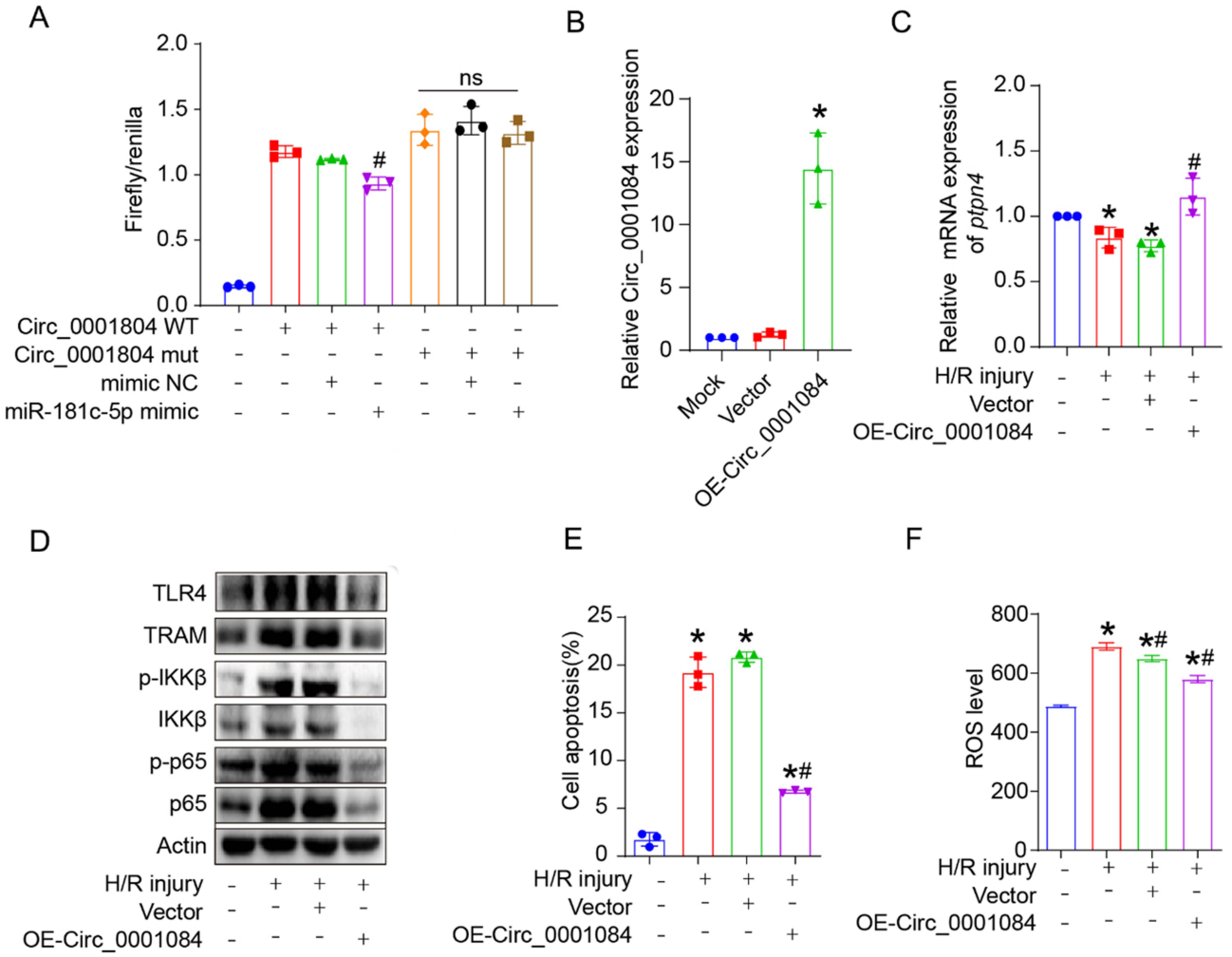

Figure 8 Role of circ_0001084 in regulating miR-181c-5p and TLR4/NF-κB pathway during cardiomyocyte H/R injury. (**A**) Dual luciferase reporter assay results for the interaction between circ_0001084 and miR-181c-5p. (**B**) Relative circ_0001084 expression levels following overexpression. (**C**) Relative mRNA expression of ptpn4 under different conditions. (**D**) Western blot analysis of TLR4, TRAM, p-IKKβ, IKKβ, p-p65, and p65. (**E**) Percentage of cell apoptosis as measured by flow cytometry. (**F**) ROS levels detected by DCFH-DA probe. *Indicates a significant difference compared to the control group ($p < 0.05$), # indicates a significant difference compared to the H/R injury group ($p < 0.05$), and "ns" denotes no significant difference.

circ_0001084 and miR-181c-5p, but also highlight the intricate regulatory network involving circ_0001084, miR-181c-5p, and PTPN4 and its influence on the TLR4/NF-κB signaling pathway and cardiomyocyte survival under stress conditions. The reduction in apoptosis and ROS levels upon circ_0001084 overexpression further supports its therapeutic potential in mitigating myocardial ischemia-reperfusion injury, suggesting a novel approach for cardiovascular disease treatment.

Rescue Experiments Verify the Effect of circ_0001084 on Regulating PTPN4 by Adsorption of miR-181c-5p on TLR4/NF-κB Signaling in Cardiomyocyte H/R Injury

To delve deeper into how circ0001084 modulates the TLR4/NF-κB signaling pathway following its interaction with miR-181c-5p, both circ0001084 and miR-181c-5p were co-transfected into HL-1 cells, with this process illustrated in Figure 9A, within the context of a cardiomyocyte hypoxia/reoxygenation (H/R) injury model. Our comparative analysis showed a significant increase in the expression levels of TLR4, TRAM, IKKβ, p-IKKβ, p65, and p-p65 in the group transfected with circRNA NC and miR-181c-5p mimic. Conversely, these levels were notably decreased in the circ0001084 plus mimic NC group, suggesting that circ0001084 overexpression can counteract miR-181c-5p mimic-induced activation of the TLR4/NF-κB signaling pathway, as detailed in Figure 9B.

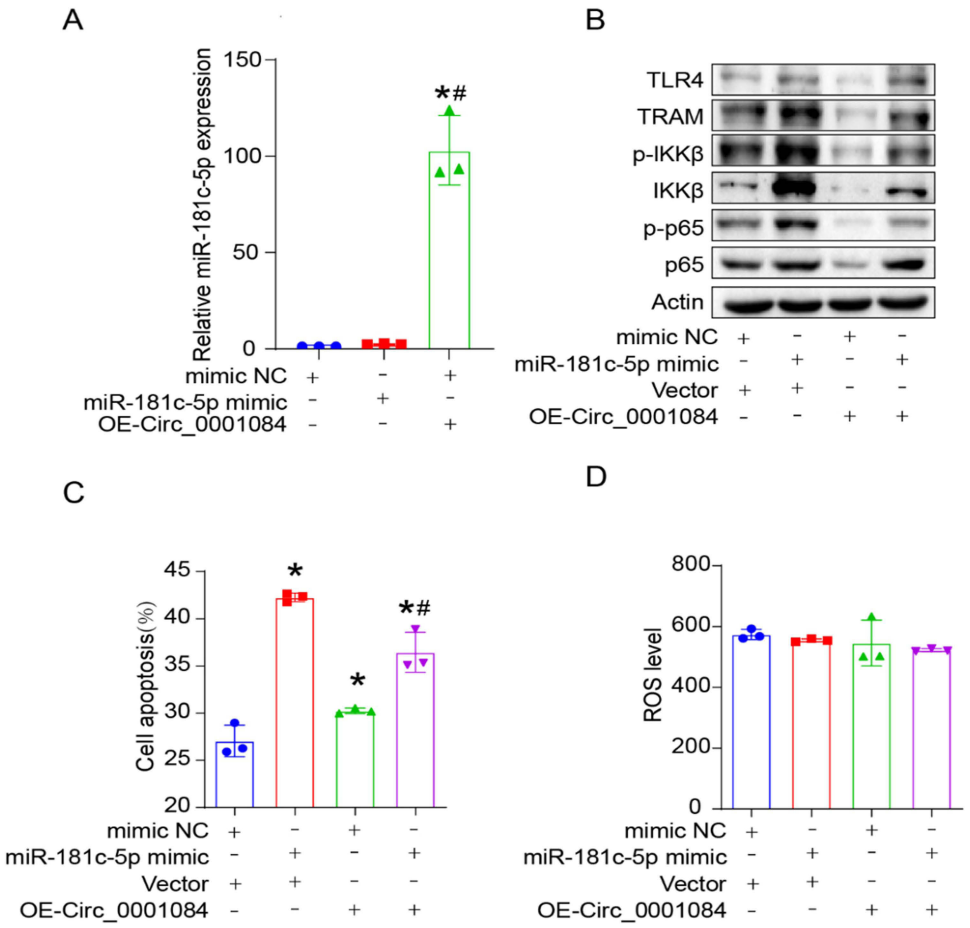

Figure 9 Rescue experiments verify the effect of circ_0001084 on regulating PTPN4 by adsorption of miR-181c-5p on TLR 4 / NF-κ B signaling in cardiomyocyte H/R injury; (**A**) RT-qPCR quantification of miR-181c-5p expression in HL-1 cardiomyocytes by transfection of circ_0001084, circ_0001084+Mimic NC, circ_0001084+ miR-181c-5p Mimic, (**B**) Western blot detected expression of TLR4/NF-κB pathway levels by transfection of circ_0001084, circ_0001084+Mimic NC, circ_0001084+ miR-181c-5p Mimic, (**C** and **D**) FC detected cell apoptosis and ROS levels in after over-expression of circ_0001084 by transfection of circ_0001084, circ_0001084+Mimic NC,circ_0001084+ miR-181c-5p Mimic; The measurement data were expressed as mean±standard deviation, repetition=3, *P<0.05 compared with mimic NC group, # P<0.05 compared with miR-181c-5p mimic group.

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Further investigation into the apoptotic response and intracellular reactive oxygen species (ROS) levels across different groups revealed that apoptosis was significantly higher in the circRNA NC+miR-181c-5p mimic group compared to the circRNA NC+mimic NC group. This increase in apoptosis was attenuated by circ0001084 overexpression, indicating the protective role of circ0001084 against miR-181c-5p-induced apoptosis. However, no significant differences were observed in the intracellular ROS levels among the groups (Figure 9C and D).

This series of experiments underscores the complex interplay between circ0001084, miR-181c-5p, and the TLR4/NF-κB signaling pathway in regulating the cellular response to H/R injury in cardiomyocytes. Specifically, the ability of circ0001084 to mitigate the pro-inflammatory and pro-apoptotic effects induced by miR-181c-5p highlights its potential as a modulating factor in cardiac injury scenarios. The lack of significant changes in ROS levels across treatments suggests that the protective effects of circ0001084 are primarily mediated through the modulation of apoptosis and specific signaling pathways rather than through a general reduction in oxidative stress. This finding could point towards more targeted therapeutic strategies for the treatment of myocardial ischemia-reperfusion injuries.

Discussion

The phenomenon of myocardial ischemia-reperfusion (I/R) injury has emerged as a paramount challenge in the post-therapeutic landscape, specifically after the re-establishment of blood flow to previously ischemic myocardial regions. This condition initiates a plethora of adverse molecular and cellular processes, including, but not limited to, apoptosis, inflammation, oxidative stress, and significant alterations in mitochondrial membrane dynamics. Such events are critical for understanding the pathophysiological aftermath of reperfusion therapy.[11–13] Our bioinformatics analysis provides a foundational understanding of the molecular landscape associated with myocardial ischemia/reperfusion (I/R) injury. By analyzing the GSE225245 and GSE242888 datasets, we identified differentially expressed genes and miRNAs that are potentially involved in I/R injury. Key findings included the upregulation of miR-181c-5p, which was consistently observed across datasets and was highlighted as a significant factor in the regulation of inflammatory pathways. Through pathway enrichment analysis, we found that these genes and miRNAs are intricately linked to the TLR4/NF-κB signaling pathway, suggesting a strong association with inflammatory and apoptotic responses in cardiomyocytes. Bioinformatics analysis laid the groundwork for identifying miR-181c-5p and PTPN4 as potential modulators of myocardial injury. Central to the molecular intricacies of these processes, miR-181c-5p emerges as a pivotal regulator, exerting substantial effects across diverse pathological landscapes, including neuroinflammation,[14,15] tumor progression,[16–18] and irritable bowel syndrome,[19] with its involvement predominantly mediated through pyroptosis.[20]

This study presents a novel and comprehensive regulatory framework for deciphering the pathogenesis of myocardial I/R injury, although the precise roles of circ_0001084 within cellular contexts require further elucidation. Nevertheless, RNA-based therapeutics targeting this axis in myocardial I/R injury are highly promising. Building upon the bioinformatics findings, our experimental investigations focused on understanding the molecular mechanisms through which miR-181c-5p and circ_0001084 influence the cardiomyocyte response to hypoxia/reoxygenation (H/R) injury. These results indicate that miR-181c-5p exacerbates H/R injury by downregulating PTPN4, thereby activating the TLR4/NF-κB pathway. Furthermore, we demonstrated that circ_0001084 functions as a competing endogenous RNA, sequestering miR-181c-5p and mitigating its repressive effect on PTPN4. This interaction suggests a novel regulatory axis, circ_0001084/miR-181c-5p/PTPN4, that could serve as a therapeutic target to modulate inflammation and apoptosis in myocardial I/R injury.

Identification of the circ_0001084/miR-181c-5p/PTPN4 axis opens new avenues for therapeutic interventions. Targeting this axis could enable the development of treatments aimed at reducing inflammatory responses and cellular damage in I/R injury. For instance, circRNA mimics or miRNA inhibitors can be designed to modulate miR-181c-5p activity, restore PTPN4 expression, and dampen the TLR4/NF-κB pathway. Future studies using in vivo models will be crucial to validate these findings and assess the translational potential of targeting non-coding RNAs in ischemic heart disease.

Detailed exploratory investigations using rat models of myocardial I/R injury and HL-1 rat cardiomyocytes subjected to hypoxia/reoxygenation (H/R) have underscored the role of miR-181c-5p in the promotion of inflammatory processes. This effect is mediated through the targeted degradation of PTPN4 mRNA, thereby disrupting its inhibitory action on the

TLR4/NF-κB signaling pathway. The resultant cascade facilitates the phosphorylation of key proteins, such as p65 and IκBα, culminating in the augmented expression of pro-inflammatory cytokines, notably IL-1β, IL-6, and TNF-α.

The involvement of the miR-181c-5p/PTPN4/NF-κB signaling pathway in modulating inflammatory responses was further validated in HL-1 mouse cardiomyocytes modeling H/R injury, highlighting the critical role of the pathway in the regulation of cardiomyocyte inflammation.[21] The exploration of long non-coding RNAs (lncRNAs) as potential upstream regulators has revealed their capacity to influence the miR-181c-5p interaction through mechanisms of competitive endogenous RNA (ceRNA), with lncRNA SNHG14 and MALAT1 identified in the context of nerve injury and myocardial infarction, respectively.[15,22] Furthermore, the research identified circ_0001084 was significantly downregulated in cardiomyocytes post-H/R injury, and its overexpression attenuated H/R injury by modulating the miR-181c-5p/PTPN4 axis.

This body of research presents inaugural evidence highlighting the circ_0001084/miR-181c-5p/PTPN4 axis as a pivotal regulator in ameliorating cell apoptosis and the production of reactive oxygen species (ROS) through modulation of the TLR4/NF-κB signaling pathway.[23,24] Identification of the circ_0001084/miR-181c-5p/PTPN4 axis as a modulator of the TLR4/NF-κB signaling pathway presents promising avenues for therapeutic intervention in myocardial ischemia/reperfusion (I/R) injury. Targeting this axis could offer a strategy for mitigating inflammatory and apoptotic responses in cardiomyocytes. For example, therapeutic approaches involving circRNA mimics or miRNA inhibitors can be explored to modulate miR-181c-5p activity, thereby restoring PTPN4 expression and inhibiting excessive TLR4/NF-κB pathway activation. This modulation may protect cardiomyocytes from hypoxia/reoxygenation-induced injury by reducing inflammation and apoptosis, ultimately improving recovery outcomes in ischemic heart disease.

Additionally, the upregulation of miR-181c-5p in response to H/R stress suggests its potential as a biomarker for the early detection of myocardial injury. Further research on miR-181c-5p and circ_0001084 could lead to diagnostic tools that monitor their expression levels as indicators of myocardial health, enabling more timely intervention strategies.

This study revealed the role of circ_0001084 in attenuating aspects of cardiomyocyte H/R injury by targeting miR-181c-5p and modulating the TLR4/NF-κB pathway, with reductions in apoptosis and ROS generation serving as initial indicators of the cellular stress response. Although flow cytometry for apoptosis and DCFH-DA probe-based ROS detection do not capture all facets of cardiomyocyte injury, they provide vital insights into cell death and oxidative stress pathways activated in H/R injury. Future studies should incorporate additional metrics, such as mitochondrial membrane potential or specific markers of necrosis, to offer a more comprehensive view of cardiomyocyte injury severity.

Future studies should focus on in vivo models and clinical samples to validate the protective effects of circ_0001084 and assess the therapeutic potential of modulating the circ_0001084/miR-181c-5p/PTPN4 axis. Investigating the pharmacokinetics and delivery methods of circRNA mimics and miRNA inhibitors in myocardial tissue is essential for translating these findings into viable therapeutic applications. Additionally, studies examining the broader regulatory networks involving circRNAs, miRNAs, and downstream signaling pathways in myocardial cells may further illuminate the complex molecular mechanisms at play, potentially uncovering additional targets for cardioprotective therapies.

Employing datasets GSE225245 and GSE242888, the study meticulously examined differential gene and miRNA expression patterns associated with myocardial injury, shedding light on the intricate molecular interplay involved. Through advanced biostatistical techniques, significantly differentially expressed genes (DEGs) and miRNAs were identified, offering insights into potential molecular mediators in the pathogenesis and progression of myocardial injury. The utilization of hierarchical clustering and volcano plot analyses effectively distinguished unique expression profiles and significantly dysregulated genes, affirming the efficacy of the analytical methods. Subsequent functional analyses, employing Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, delineated the biological processes and pathways implicated, furnishing a systemic overview of the molecular mechanisms of action.[26–28] The application of the LASSO logistic regression algorithm for key gene selection further highlighted genes such as Ptpn4 as potential markers or therapeutic targets, while the exploration of miRNA expression profiles emphasized the significance of post-transcriptional regulation.[29,30] Collectively, this study significantly enhances our understanding of the molecular underpinnings of myocardial injury and paves the way for the development of targeted therapeutic strategies, underscoring the paramount importance of integrated genomic and transcriptomic analyses in cardiovascular disease research.[25,31]

Conclusion

This study highlights a previously uncharacterized mechanism involving the circ_0001084/miR-181c-5p/PTPN4 axis and its regulatory effects on myocardial ischemia/reperfusion (I/R) injury. By combining bioinformatics with molecular experimentation, we demonstrated that circ_0001084 acts as a competing endogenous RNA, binding miR-181c-5p and thereby alleviating its inhibitory effect on PTPN4 expression. This interaction effectively reduces the activation of the TLR4/NF-κB signaling pathway, which exacerbates inflammatory and apoptotic responses in cardiomyocytes under hypoxia/reoxygenation (H/R) stress. Importantly, our findings suggest that modulating the circ_0001084/miR-181c-5p/PTPN4 axis may represent a novel therapeutic strategy for mitigating myocardial I/R injury, offering valuable insights into ischemic heart disease treatment. Future research should aim to further elucidate this regulatory network and evaluate its potential for clinical translation, with the ultimate goal of developing targeted therapies that address the inflammatory and apoptotic sequelae of I/R injury.

Data Sharing Statement

The raw data supporting the conclusions of this article will be made available by the authors without undue reservation.

Ethical Approval and Consent to Participate

Cell and animal experiments were approved by Jinan University Laboratory Animal Ethics Committee (NO: 20220825-01). Bioinformatics analysis on human data was approved by Medical Ethics Review Committee of the First People's Hospital of Zhaoqing City (B2023-05-13).

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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