

PRDX2-PARK7 Axis Alleviates Ang II-Induced Iron-Dependent Oxidative Injury and Ferroptotic Phenotypic Changes in Vascular Smooth Muscle Cells *In Vitro*

Tao Zheng^{1,*}, Qingyun Zhou¹, Zixuan Wang¹, Taotao Huang¹, Guofeng Shao^{2,*}

¹Department of Vascular Surgery, Ningbo Medical Center Lihuili Hospital, 315040 Ningbo, Zhejiang, China

²Department of Cardiothoracic Surgery, Ningbo Medical Center Lihuili Hospital, 315040 Ningbo, Zhejiang, China

*Correspondence: zantno1@yeah.net (Tao Zheng); sgf1958@sina.com (Guofeng Shao)

Submitted: 23 May 2026 Revised: 24 July 2026 Accepted: 12 August 2026 Published: 23 October 2026

Background: Oxidative stress and ferroptosis-associated pathways have recently emerged as important mechanisms contributing to vascular smooth muscle cell (VSMC) injury; however, their molecular regulation remains poorly understood. This study aimed to determine whether the peroxiredoxin 2 (PRDX2)-Parkinsonism-associated deglycase (PARK7) axis protects against angiotensin II (Ang II)-induced VSMC injury and to elucidate the underlying mechanism.

Methods: *In vitro* injury models were established using human VSMCs exposed to Ang II. PRDX2 was overexpressed using plasmid transfection, whereas PARK7 was silenced by short hairpin RNA. Cell viability and proliferation were assessed using Cell Counting Kit-8 (CCK-8) and 5-ethynyl-2'-deoxyuridine (EdU) assays, respectively, and apoptosis was evaluated by flow cytometry. Ferroptosis-related parameters, including intracellular iron accumulation, reactive oxygen species (ROS) levels in culture supernatants, and glutathione (GSH) depletion, were assessed. Western blot analysis was performed to quantify the expression levels of PARK7, nuclear factor erythroid 2-related factor 2 (Nrf2), solute carrier family 7 member 11 (SLC7A11), glutathione peroxidase 4 (GPX4), and VSMC phenotypic markers, including α -smooth muscle actin (α -SMA), smooth muscle protein 22- α (SM22- α), and osteopontin (OPN). Protein-protein interactions were predicted using bioinformatics databases and validated by co-immunoprecipitation (CO-IP).

Results: Ang II stimulation markedly impaired VSMC viability and proliferation, promoted apoptosis, induced iron-dependent oxidative stress with dysregulation of ferroptosis-associated markers, and triggered phenotypic switching from a contractile to a synthetic state. PRDX2 overexpression significantly improved cell viability ($p < 0.001$), restored proliferation ($p < 0.05$), reduced apoptosis ($p < 0.001$), decreased iron and ROS levels in culture supernatants ($p < 0.001$), and restored GSH content ($p < 0.001$). Moreover, PRDX2 overexpression increased the expression of PARK7, Nrf2, SLC7A11, GPX4, α -SMA, and SM22- α ($p < 0.05$), while reducing OPN expression ($p < 0.001$). CO-IP analysis confirmed a direct interaction between PRDX2 and PARK7. Importantly, PARK7 knockdown attenuated the protective effects of PRDX2 overexpression against ferroptosis-associated changes and phenotypic maintenance.

Conclusion: The PRDX2-PARK7 axis alleviates Ang II-induced VSMC injury by attenuating iron-dependent oxidative stress and modulating ferroptosis-associated phenotypic changes, accompanied by the restoration of Nrf2, SLC7A11, and GPX4 expression levels.

Keywords: VSMC; ferroptosis; phenotypic switching; oxidative stress; PRDX2; PARK7

Introduction

Vascular smooth muscle cell (VSMC) dysfunction is a central pathological process underlying multiple cardiovascular diseases, including aortic aneurysm, atherosclerosis, and hypertensive vascular remodeling [1,2]. In these conditions, vascular wall integrity is compromised by extracellular matrix degradation, VSMC phenotypic switching and apoptosis, inflammatory cell infiltration, and oxidative stress [3]. Aortic aneurysm represents a prototypical example, in which degenerative changes in the elas-

tic layer of the tunica media constitute a major pathological feature. Among these mechanisms, VSMC dysfunction is a key determinant of aortic wall structural deterioration and critically contributes to disease initiation and progression [4]. Current clinical management of advanced vascular diseases relies largely on surgical or interventional approaches, such as open surgical resection and endovascular stent graft implantation for aortic aneurysm, with treatment timing guided by aneurysm size, growth rate, and patient's overall condition [5]. Despite these interventions, patients remain at considerable risk of complications and long-term

recurrence, highlighting the urgent need for deeper mechanistic understanding of VSMC pathobiology to facilitate preventive and early-stage pharmacological therapies.

Ferroptosis is an iron-dependent form of regulated cell death caused by excessive accumulation of lipid hydroperoxide [6,7] and is closely associated with redox imbalance and cardiovascular disease pathogenesis [8]. A recent study demonstrated that overexpression of aminolevulinic acid synthase 2 (ALAS2) attenuates oxidative stress-induced ferroptosis in aortic aneurysms through activation of the GATA-binding protein 1 (GATA1) pathway [9]. Targeting glutathione peroxidase 4 (GPX4) significantly inhibits ferroptosis and delays abdominal aortic aneurysm formation [10]. Ferrostatin-1 suppresses ferroptosis in VSMCs by activating the solute carrier family 7 member 11 (SLC7A11)/GPX4 axis, thereby mitigating abdominal aortic aneurysm development [11]. The peroxiredoxin (PRDX) family comprises important antioxidant proteins that have been implicated in the inhibition of aortic aneurysm formation [12]. Previous studies have indicated that PRDX2 is associated with ferroptosis regulation and may inhibit its progression [13]. Protein-protein interaction (PPI) analysis using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database revealed an interaction between PRDX2 and Parkinsonism-associated deglycase (PARK7), a ferroptosis-related protein involved in the regulation of nuclear factor erythroid 2-related factor 2 (Nrf2) signaling [14,15]. Nrf2 is a key regulator of ferroptosis, and its activation effectively suppresses ferroptotic cell death [16]. Based on these findings, the present study investigated the role of the PRDX2-PARK7 axis and its mechanistic involvement in ferroptosis during Ang II-induced VSMC injury, providing insight into its potential contribution to vascular pathological processes.

Materials and Methods

Cell Culture

Human VSMCs (JY524, ssrcc, Shanghai, China) were cultured (37 °C, 5% CO₂, humidity) (D165H, Rwdls, Shenzhen, China). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; C0891, Beyotime, Shanghai, China) supplemented with 10% fetal bovine serum (FBS; C0235, Beyotime, Shanghai, China) and 1% penicillin/streptomycin (CA005-010, GenDEPOT, Katy, TX, USA). All VSMCs were authenticated by short tandem repeat analysis, and mycoplasma contamination testing was negative.

Cell Treatment

The PRDX2 overexpression plasmid (pRP[Exp]-EGFP-CMV>hPRDX2[NM_005809.6]) and its negative control (NC), as well as three short hairpin RNAs (shRNAs) targeting PARK7 (sh-PARK7#1, pRP[shRNA]-U6>hPARK7[shRNA#1],

5'-GTAGCCGTGATGTGGTCATTT-3'; sh-PARK7#2, pRP[shRNA]-U6>hPARK7[shRNA#2], 5'-ACTCTGAGAATCGTGTGGAAA-3'; sh-PARK7#3, pRP[shRNA]-U6>hPARK7[shRNA#3], 5'-GCAATTGTTGAAGCCCTGAAT-3') and the corresponding negative control shRNA (shNC, pRP[shRNA]-U6>Scramble, 5'-CCTAAGGTTAAGTCGCCCTCG-3') containing scrambled sequences, were obtained from VectorBuilder (Guangzhou, China).

For transfection of VSMCs with overexpression plasmids or shRNAs in 96-well plates, Lipofectamine 2000 (11668019, Invitrogen, Carlsbad, CA, USA) was used. VSMCs were seeded at a density of 5×10^3 cells per well and cultured until reaching 70–80% confluence. For each well, 0.4 µg of PRDX2 overexpression plasmid (or empty vector control) or 0.4 µg of shRNA plasmid (shPARK7#1, shPARK7#2, shPARK7#3, or shNC) was diluted in 50 µL of Opti-MEM reduced-serum medium (31985062, Gibco, Grand Island, NY, USA). Separately, 1.2 µL of Lipofectamine 2000 was diluted in 50 µL of Opti-MEM and incubated at room temperature for 5 minutes. The diluted DNA and lipid solutions were combined, mixed gently, and incubated for 20 minutes at room temperature. Subsequently, 100 µL of the DNA-lipid complex was added dropwise to each well containing cells in 200 µL of antibiotic-free complete DMEM. After 6 hours, the medium was replaced with complete DMEM containing 10% FBS and antibiotics. Transfection efficiency was subsequently evaluated by reverse transcription quantitative polymerase chain reaction (RT-qPCR).

Cells were divided into the following groups: Control, angiotensin II (Ang II), Ang II+NC, Ang II+PRDX2, Ang II+PRDX2+shNC, and Ang II+PRDX2+shPARK7. To establish an *in vitro* model of VSMC damage under aortic aneurysm circumstances, cells in the Ang II group were treated with 10 µM Ang II (4474-91-3, Proteintech, Wuhan, China) (48 h) [17]. Cells in the remaining groups were treated with 10 µM Ang II (48 h) following transfection with the corresponding overexpression plasmids or shRNAs.

RT-qPCR

RNA isolated from VSMCs using TRIzol reagent (A33250, Invitrogen, Carlsbad, CA, USA) was reverse-transcribed into complementary DNA (cDNA) using the Transcriptor First Strand cDNA Synthesis Kit (04379012001, Roche, Pleasanton, CA, USA). qPCR was performed on an ABI 7500 Real-Time PCR System (4351104, Applied Biosystems, Foster City, CA, USA) using SYBR Green PCR Master Mix (4344463, Applied Biosystems, Foster City, CA, USA). mRNA expression levels were normalized to β-actin and calculated using the $2^{-\Delta\Delta C_t}$ method [18]. The primer sequences are listed in Table 1.

Table 1. Primers used for RT-qPCR.

Genes	Forward primer (5'-3')	Reverse primer (5'-3')
PRDX2	GCTGGGCTGTGAAGTGCTGG	ACGCCGTAATCCTCAGACAAGC
PARK7	CGGGGTGCAGGCTTGTAAC	GACCACATCACGGCTACACT
β-actin	CCACCATGTACCCTGGCATT	GTCCTCGGCCACATTGTGAA

RT-qPCR, reverse transcription quantitative polymerase chain reaction; PRDX2, peroxiredoxin 2; PARK7, Parkinsonism-associated deglycase.

Cell Viability Assay

Cell viability was assessed using the Cell Counting Kit-8 (CCK-8; C0038, Beyotime, Shanghai, China) assay. Briefly cells (3×10^3 /well) were seeded into 96-well plates (FCP962, Beyotime, Shanghai, China) and incubated with 10 μ L CCK-8 reagent for 2 h. Absorbance (450 nm) was measured using a microplate reader (Bio-Rad, Hercules, CA, USA).

Cell Proliferation Assay

Cell proliferation was assessed using the BeyoClick 5-ethynyl-2'-deoxyuridine (EdU)-647Cell Proliferation Kit (C0071S, Beyotime, Shanghai, China). Briefly, VSMCs (5×10^3 cells/well) were seeded into 96-well plates and cultured overnight, followed by incubation with 10 μ M EdU for 2 h. Cells were then fixed with 4% paraformaldehyde (CF189021, Solarbio, Beijing, China) for 15 min and permeabilized with 0.3% Triton X-100 (P0096, Beyotime, Shanghai, China) for 15 min. After incubation with the click reaction cocktail provided in the kit at room temperature (RT) for 30 min in the dark, Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI; C1002, Beyotime, Shanghai, China). EdU⁺ cells (red fluorescence) were visualized using an inverted fluorescence microscope (200 $\times$, Leica, Wetzlar, Germany), and quantitative analysis was performed using ImageJ software (Version 1.53t; National Institutes of Health, Bethesda, MD, USA). The EdU-positive rate was calculated as follows: EdU⁺ rate (%) = (EdU⁺ cells / Total cells) $\times$ 100%.

Cell Apoptosis Assay

Apoptosis was assessed using the Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) Apoptosis Detection Kit (640914, BioLegend, San Diego, CA, USA). Following the indicated treatments, cells cultured in 6-well plates (FULA061, Beyotime, Shanghai, China) were collected for flow cytometric analysis. Briefly, VSMCs (1×10^5 cells/well) were washed with cold phosphate-buffered saline (PBS; C0221A, Bioinno, Taicang, China), resuspended in 195 μ L of binding buffer, and stained with 5 μ L Annexin V-FITC and 10 μ L PI (15 min, RT, darkness). Subsequently, 400 μ L of binding buffer was added, and apoptotic cells were analyzed using a NovoCyte Penton flow cytometer (Agilent, Santa Clara, CA, USA).

Iron Content and Glutathione (GSH) Assay

Cells (2×10^6 cells) were washed with cold PBS, lysed using radioimmunoprecipitation (RIPA) buffer (P0013, Beyotime, Shanghai, China), and centrifuged. GSH (A006-1-1) and iron content (A039-2-1) in the collected supernatants were quantified using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Absorbance values were measured at 520 nm (iron content) and 420 nm (GSH) using a spectrophotometer (Thermo Scientific, Waltham, MA, USA).

Reactive Oxygen Species (ROS)

ROS levels in culture supernatants were measured using a ROS enzyme-linked immunosorbent assay (ELISA) kit (J21799, Jilid, Wuhan, China). Absorbance was measured at 530 nm using a microplate reader (Bio-Rad, Hercules, CA, USA), and ROS concentrations were calculated based on a standard curve.

Western Blot

Proteins were extracted using RIPA lysis buffer, and protein concentrations were determined using a bicinchoninic acid (BCA) assay kit (P0009, Beyotime, Shanghai, China). Equal amounts of protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; P0690, Beyotime, Shanghai, China) and transferred onto polyvinylidene fluoride membranes (FFP24, Beyotime, Shanghai, China). The membranes were blocked (5% non-fat milk), incubated with primary antibodies (Table 2) overnight, and subsequently incubated with secondary antibodies (1 h, RT). Protein bands were visualized using an enhanced chemiluminescence (ECL) kit (KGC4603, KeyGene, Nanjing, China), and band intensities were quantified using ImageJ software (Version 1.53t; National Institutes of Health, Bethesda, MD, USA) with β -actin as the loading control.

PPI Analysis

The PPI relationship between PRDX2 and PARK7 was analyzed using the STRING database (<https://cn.string-db.org/>) [19]. All bioinformatics data used in this study were obtained from publicly available databases, and no restricted or confidential patient datasets were included. *Homo sapiens* was selected as the organism and the official gene symbols “PRDX2” and “PARK7” were entered

Table 2. Antibodies used in western blot analysis.

Antibody name	Source	Catalog number	Supplier	Type	Dilution
PRDX2	Rabbit	ab109367	Abcam	PA	1:1000
PARK7	Rabbit	ab76008	Abcam	PA	1:1000
Nrf2	Rabbit	ab62352	Abcam	PA	1:1000
SLC7A11	Rabbit	ab307601	Abcam	PA	1:1000
GPX4	Rabbit	ab125066	Abcam	PA	1:1000
β -actin	Mouse	ab8226	Abcam	PA	1:3000
α -SMA	Mouse	ab7817	Abcam	PA	1:1000
SM22- α	Rabbit	ab227566	Abcam	PA	1:1000
OPN	Rabbit	ab228748	Abcam	PA	1:1000
Goat Anti-Rabbit IgG H&L (HRP)		ab6721	Abcam	SA	1:5000
Goat Anti-Mouse IgG H&L (HRP)		ab205719	Abcam	SA	1:5000

Note: PA, primary antibody; SA, secondary antibody; Nrf2, nuclear factor erythroid 2-related factor 2; SLC7A11, solute carrier family 7 member 11; GPX4, glutathione peroxidase 4; α -SMA, alpha-smooth muscle actin; SM22- α , smooth muscle protein 22-alpha; OPN, osteopontin; IgG, immunoglobulin G; HRP, horseradish peroxidase.

into the multiple proteins search module. A combined score >0.4 was applied as the medium-confidence threshold for interaction inclusion, and isolated nodes were removed to declutter the network. The interaction between PRDX2 and PARK7 was evaluated based on the STRING combined score, which integrates evidence from multiple interaction prediction sources. No additional filtering or network expansion beyond the default STRING parameters was performed.

Co-Immunoprecipitation (Co-IP)

Co-IP was performed to evaluate the interaction between PRDX2 and PARK7. VSMCs (2×10^6 cells) were lysed in 500 μ L of ice-cold RIPA buffer (P0013, Beyotime, Shanghai, China) supplemented with 1 mM PMSF (ST506, Beyotime, Shanghai, China) and a protease inhibitor cocktail (P1005, Beyotime, Shanghai, China). Following centrifugation at 12,000 $\times$ g for 15 minutes at 4 $^{\circ}$ C, the supernatants were collected, and total protein concentrations were determined using a BCA assay kit (P0009, Beyotime, Shanghai, China). For each immunoprecipitation reaction, 500 μ g of total protein was diluted fivefold with IP buffer to a final volume of 750 μ L.

The diluted lysates were pre-cleared by incubation with 20 μ L of Protein A/G agarose beads (50% slurry; PR40025, Proteintech, Wuhan, China) for 1 hour at 4 $^{\circ}$ C with gentle rotation. After centrifugation, the pre-cleared lysates were transferred to fresh tubes and incubated overnight at 4 $^{\circ}$ C with 2 μ g of anti-PRDX2 antibody (ab109367, Abcam, Cambridge, UK), anti-PARK7 antibody (ab76008, Abcam, Cambridge, UK), or control IgG (ab216352, Abcam, Cambridge, UK) as a negative control.

The following day, 30 μ L of fresh Protein A/G agarose beads (PR40025, Proteintech, Wuhan, China) were added and incubated for 2 h at 4 $^{\circ}$ C with gentle rotation. The beads were washed thrice with 1 mL of IP buffer and once with 1 mL of IP buffer containing 0.1% Triton X-100. Bound proteins were eluted with 25 μ L of 2 $\times$ SDS loading buffer (LC2676, Thermo Fisher Scientific, Waltham, MA, USA) by boiling at 100 $^{\circ}$ C for 10 min and subsequently analyzed by western blotting.

Statistical Analysis

Intergroup differences were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Data are presented as mean $\pm$ standard deviation (SD). Statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). A p value < 0.05 was considered statistically significant.

Results

PRDX2 Overexpression Attenuated Ang II-Induced Iron-Dependent Oxidative Stress and Ferroptosis-Associated Changes in VSMCs

Transfection with the PRDX2 overexpression plasmid markedly elevated PRDX2 levels in VSMCs ($p < 0.001$, Fig. 1A–C). VSMCs treated with Ang II exhibited markedly reduced cell viability ($p < 0.001$, Fig. 1H) and proliferation (red fluorescence; $p < 0.001$, Fig. 1D,E), as well as increased apoptosis ($p < 0.001$, Fig. 1F,G). PRDX2 overexpression substantially improved viability ($p < 0.001$, Fig. 1H) and proliferation ($p < 0.05$, Fig. 1D,E) and diminished apoptosis ($p < 0.001$, Fig. 1F,G) in Ang II-treated cells. In VSMCs, Ang II exposure decreased GSH levels while increasing iron content and ROS levels in culture supernatants ($p < 0.001$, Fig. 1I–K). PRDX2 overexpression reversed these alterations ($p < 0.001$, Fig. 1I–K). Additionally, Ang II treatment increased the expression of osteopontin (OPN) while decreasing the expression of PARK7, Nrf2, SLC7A11, GPX4, α -smooth muscle actin (α -SMA), and smooth muscle protein 22-alpha (SM22- α) ($p < 0.05$, Fig. 2A–I). These effects were reversed by PRDX2 overexpression ($p < 0.05$) (Fig. 2A–I).

PRDX2 Alleviated Ang II-Induced Iron-Dependent Oxidative Stress and Ferroptosis-Associated Changes via PARK7

Analysis of the STRING database revealed a potential PPI between PARK7 and PRDX2 (Fig. 3A). Co-IP analysis validated the physical interaction between PRDX2 and PARK7n (Fig. 3B,C). PARK7 expression in VSMCs was greatly reduced by all three shRNAs targeting PARK7 (sh-PARK7#1, shPARK7#2, and shPARK7#3) ($p < 0.001$, Fig. 3D). The highest knockdown efficiency was observed with shPARK7#2, which was used in subsequent studies ($p < 0.001$, Fig. 3D). Ang II treatment induced higher apop-

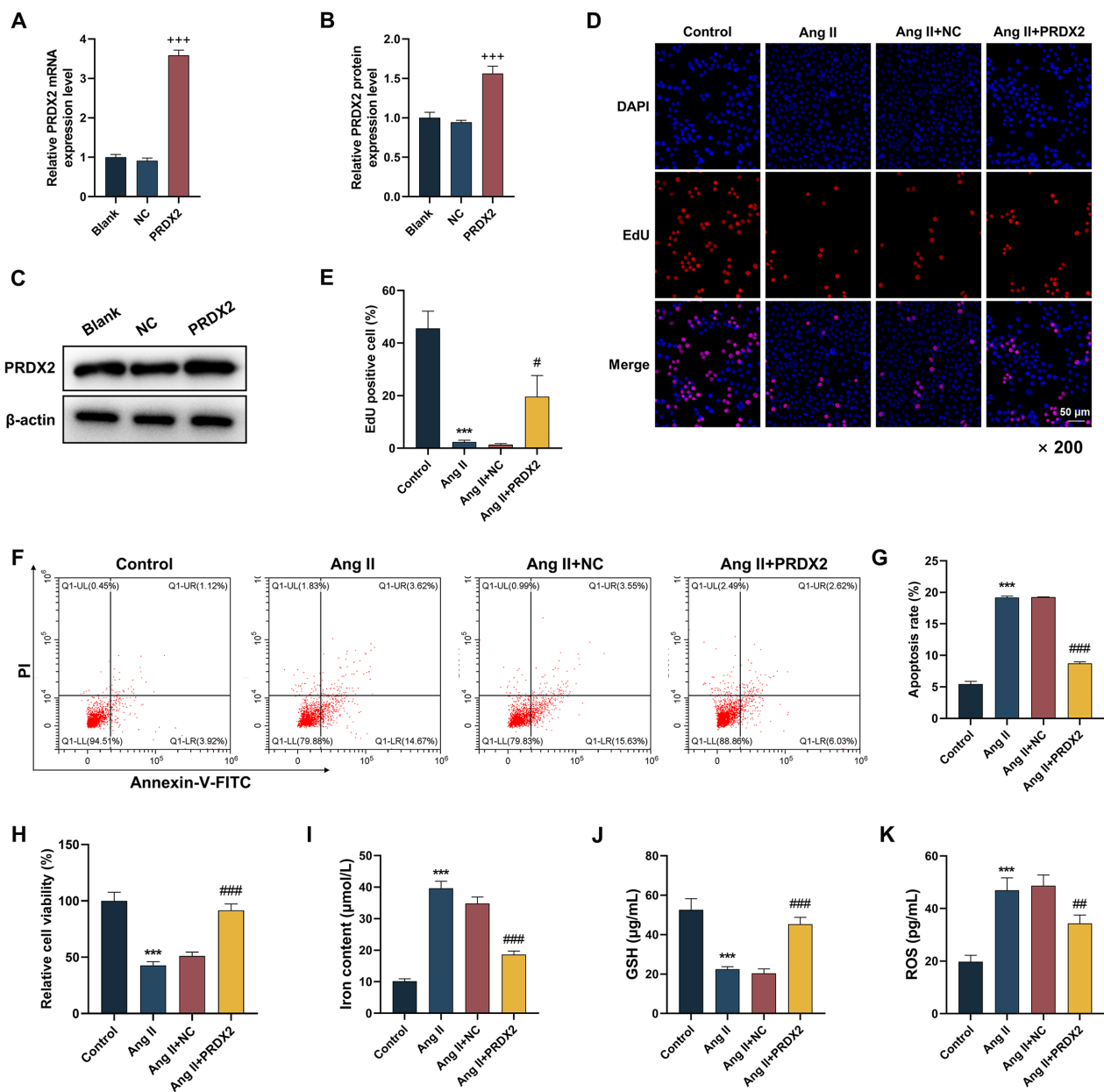


Fig. 1. PRDX2 regulates Ang II-induced VSMC viability and ferroptosis-related markers. (A) Transfection efficiency of the PRDX2 overexpression plasmid in VSMCs (RT-qPCR, normalized to β -actin). (B,C) Transfection efficiency of PRDX2 overexpression plasmid in VSMCs (Western blot, normalized to β -actin). (D,E) VSMC proliferation (EdU assay; EdU⁺ cells with red fluorescence). Magnification, 200 $\times$; scale bar, 50 μ m. (F,G) VSMC apoptosis (Flow cytometry). (H) VSMC viability (CCK-8 assay). (I–K) Intracellular iron content, GSH, and ROS levels in culture supernatants in VSMC. Data are mean $\pm$ standard deviation ($n = 3$ independent biological replicates). $^{+++}p < 0.001$ vs. NC; $^{***}p < 0.001$ vs. Control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. Ang II+NC. VSMC, vascular smooth muscle cell; EdU, 5-ethynyl-2'-deoxyuridine; CCK-8, Cell Counting Kit-8; GSH, glutathione; ROS, reactive oxygen species; NC, negative control.

tosis and reduced cell viability and proliferation (red fluorescence) ($p < 0.001$, Fig. 3E–I), whereas PRDX2 overexpression reduced apoptosis ($p < 0.001$), increased cell viability ($p < 0.001$), and significantly restored proliferation ($p < 0.05$) (Fig. 3E–I). However, compared with the Ang II+PRDX2+shNC group, the Ang II+PRDX2+shPARK7 group showed significantly reduced cell viability ($p <$

0.001), increased apoptosis ($p < 0.001$), and attenuated cell proliferation (red fluorescence) ($p < 0.05$) (Fig. 3E–I), indicating that PARK7 knockdown partially reversed the protective effects of PRDX2 overexpression on VSMC proliferation. In VSMCs, Ang II increased ROS levels in culture supernatants and iron content while decreasing GSH levels ($p < 0.001$, Fig. 4A–C). These alterations were reversed by

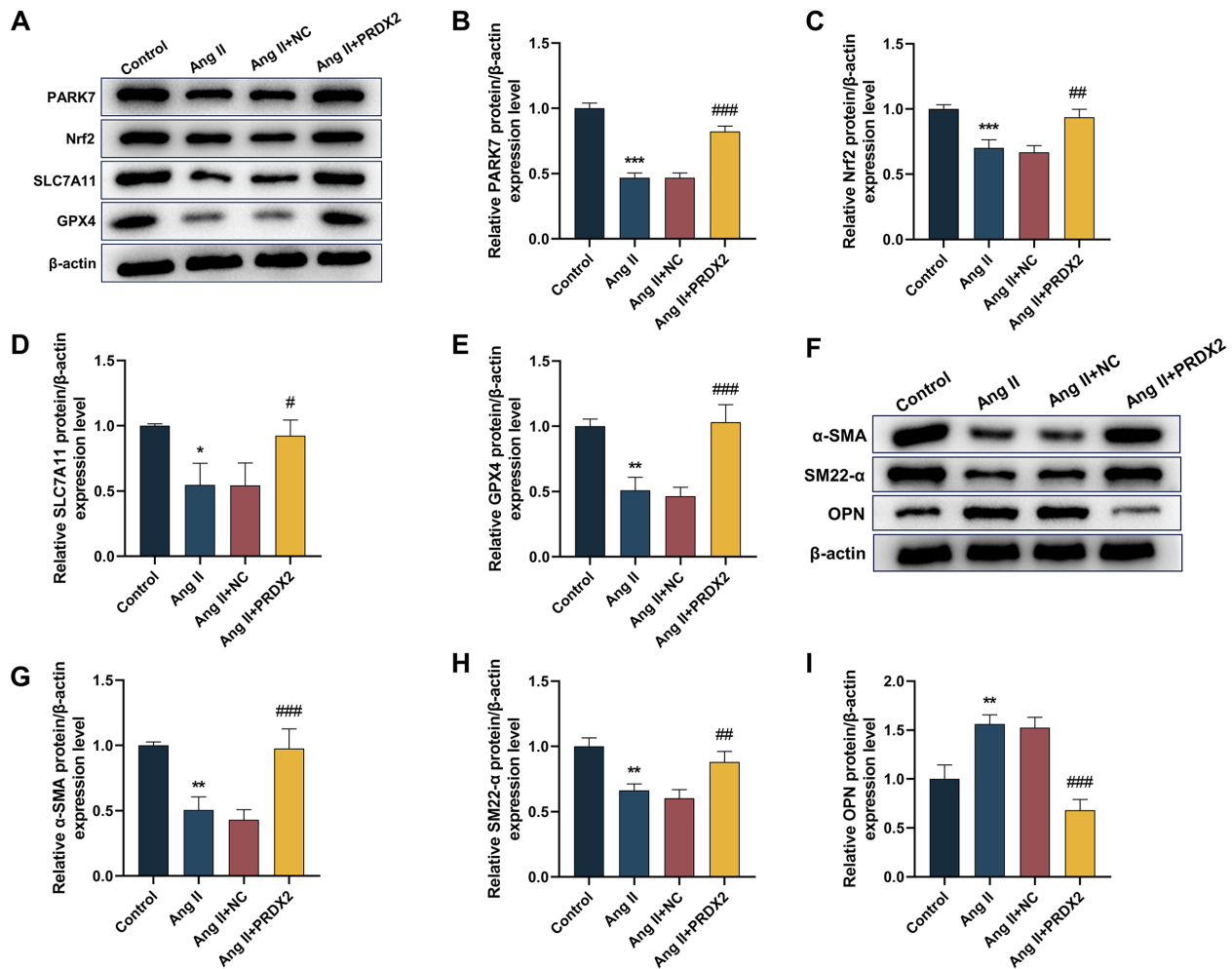


Fig. 2. PRDX2 regulates ferroptosis-related proteins and phenotypic switching-related proteins in Ang II-treated VSMC. (A–E) PARK7 (B), Nrf2 (C), SLC7A11 (D), and GPX4 (E) in VSMC (Western blot, β-actin as the internal control). (F–I) α-SMA (G), SM22-α (H), and OPN (I) in VSMC (Western blot, β-actin as the internal control). Data are mean ± standard deviation (n = 3 independent biological replicates). **p* < 0.05, ***p* < 0.01, ****p* < 0.001 vs. Control; #*p* < 0.05, ##*p* < 0.01, ###*p* < 0.001 vs. Ang II+NC.

PRDX2 overexpression, whereas PARK7 knockdown partially attenuated several protective effects of PRDX2 (*p* < 0.01, Fig. 4A–C). Ang II treatment increased OPN expression while decreasing Nrf2, SLC7A11, GPX4, α-SMA, and SM22-α levels (*p* < 0.05, Fig. 4D–K). These protein expression changes were restored by PRDX2 overexpression, and this effect was partially counteracted by PARK7 knockdown (*p* < 0.05, Fig. 4D–K).

Discussion

In this study, we found that PRDX2 interacts with PARK7 and that PRDX2 alleviates Ang II-induced VSMC injury through PARK7. PRDX2, a key member of the PRDX family, possesses strong antioxidant and free radical-scavenging capabilities and participates in cell proliferation, differentiation, apoptosis, and various signal-

ing pathways [20,21]. PARK7 is a multifunctional protein involved in transcriptional regulation, oxidative stress defense, mitochondrial function, and signal transduction [22]. It also functions as a protease, molecular chaperone, and deglycase, contributing to mitochondrial integrity and related antioxidant defenses [23]. Our further experiments demonstrated that PRDX2 alleviated Ang II-induced iron-dependent oxidative stress and ferroptosis-associated marker alterations in VSMCs through PARK7. A previous study reported that PARK7 inhibits ferroptosis via the Nrf2/GPX4 cascade [24]. Consistently, we observed that PRDX2 overexpression reversed the Ang II-induced downregulation of Nrf2 and GPX4, whereas PARK7 knockdown attenuated several protective effects observed after PRDX2 overexpression. These findings suggest that PRDX2-mediated protection is associated with PARK7-dependent restoration of Nrf2 and GPX4 expression, which

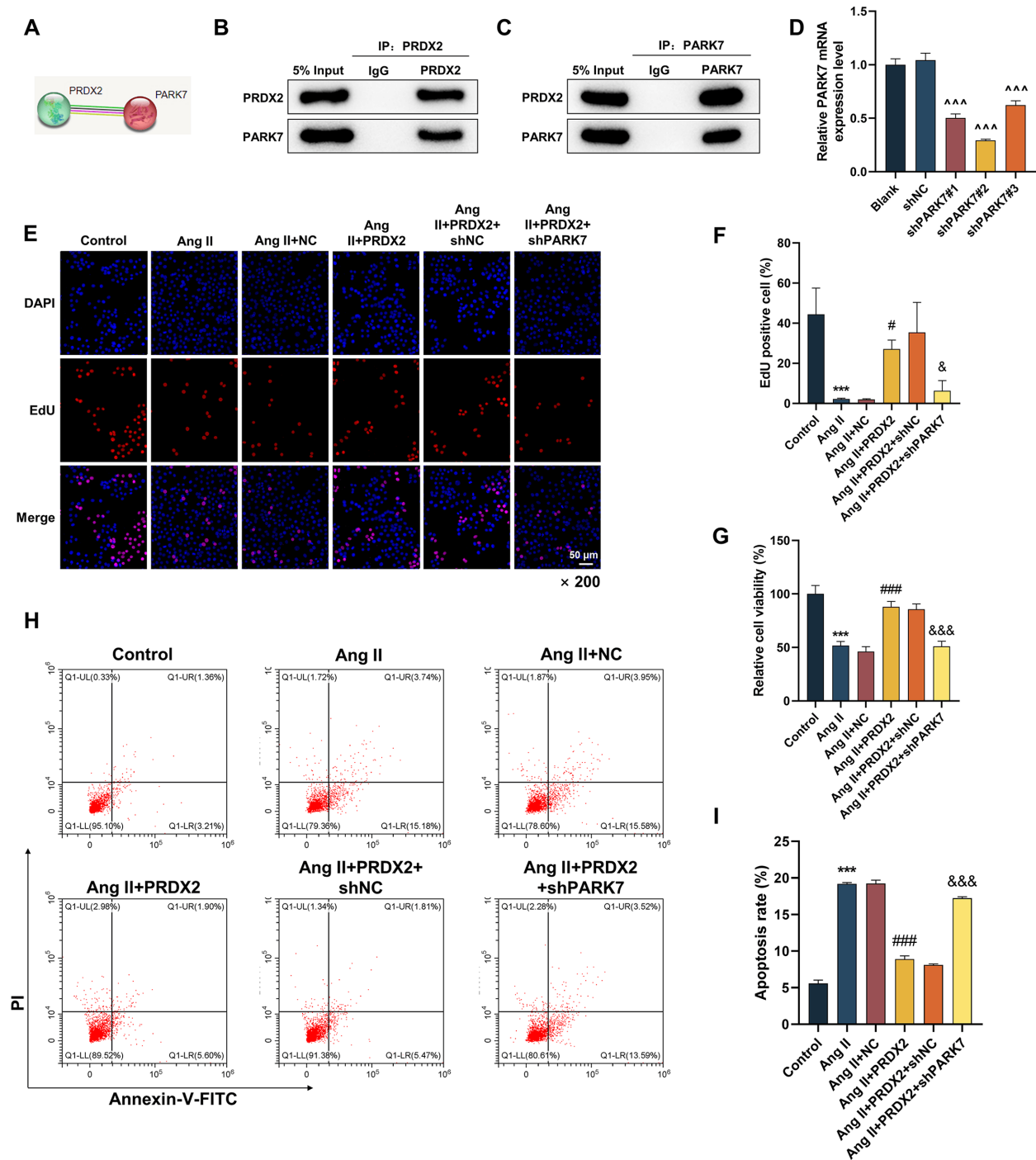


Fig. 3. PRDX2 and PARK7 regulate Ang II-induced VSMC viability and apoptosis. (A) STRING analysis of PRDX2-PARK7 interaction. (B,C) PRDX2 binding with PARK7 (Co-IP). (D) Transfection efficiency of short hairpin RNA (shRNA) targeting PARK7 (shPARK7#1, shPARK7#2, shPARK7#3) in VSMC (RT-qPCR, β -actin as the internal control). (E,F) VSMC proliferation (EdU assay). Magnification, 200×; scale bar, 50 μ m. EdU⁺ cells with red fluorescence. (G) VSMC viability (CCK-8 assay). (H,I) VSMC apoptosis (Flow cytometry). Data are mean $\pm$ standard deviation ($n = 3$ independent biological replicates). ^{^^} $p < 0.001$ vs. shNC; ^{***} $p < 0.001$ vs. Control; [#] $p < 0.05$, ^{###} $p < 0.001$ vs. Ang II+NC; [&] $p < 0.05$, ^{&&&} $p < 0.001$ vs. Ang II+PRDX2+shNC.

may reflect modulation of ferroptosis-related antioxidant defenses and reduction of VSMC injury in this *in vitro* model.

A hallmark of vascular pathology is VSMC phenotypic switching, whereby cells transition from a contractile to a synthetic state, accompanied by increased prolifer-

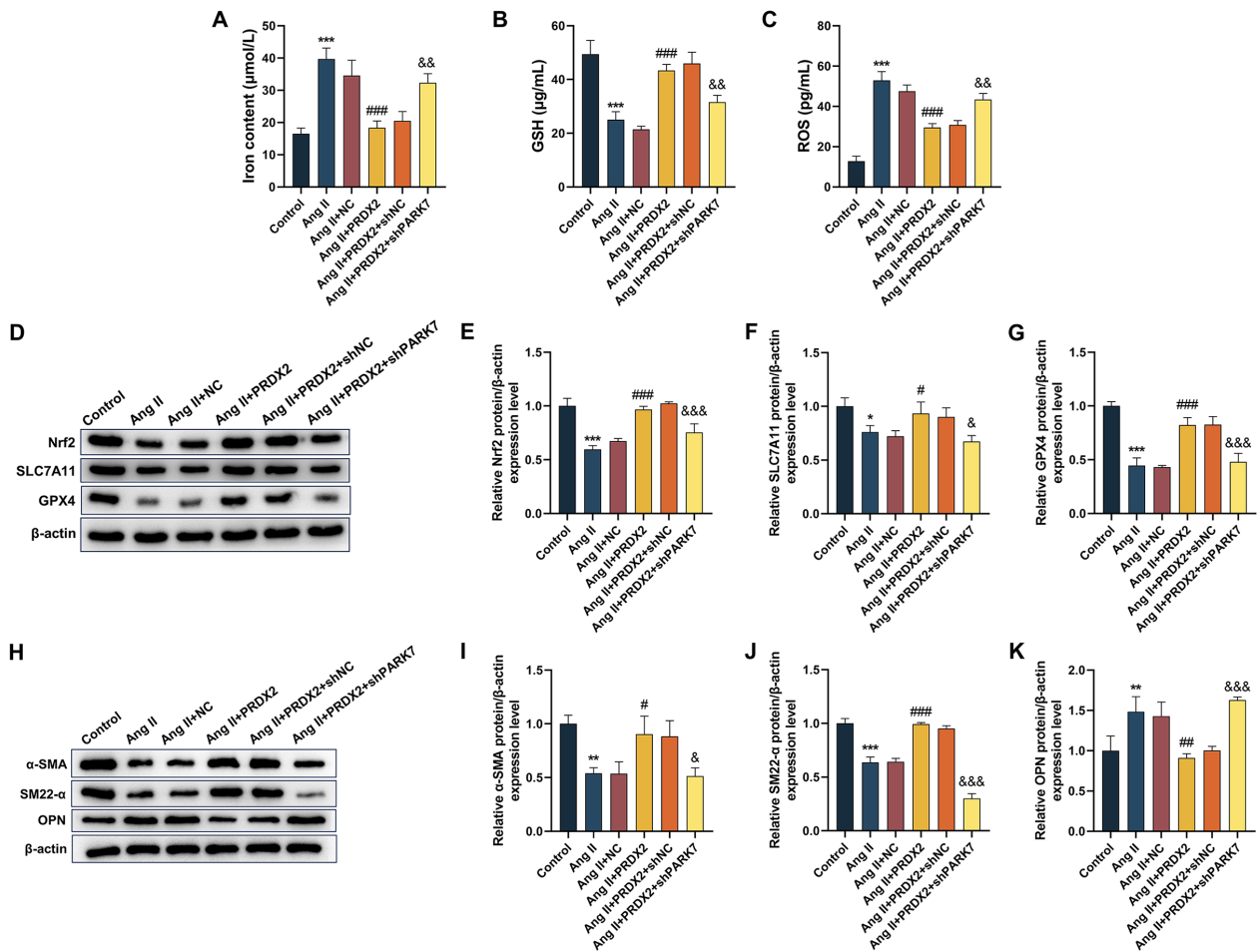


Fig. 4. PRDX2 and PARK7 regulate ferroptosis markers and phenotypic switching-related proteins in Ang II-treated VSMC. (A–C) Intracellular iron content, GSH, and ROS levels in culture supernatants in VSMC. (D–G) Nrf2 (E), SLC7A11 (F), and GPX4 (G) in VSMC (Western blot, normalized to β-actin). (H–K) α-SMA (I), SM22-α (J), and OPN (K) in VSMC (Western blot, normalized to β-actin). Data are mean ± standard deviation (n = 3 independent biological replicates). **p* < 0.05, ***p* < 0.01, ****p* < 0.001 vs. Control; #*p* < 0.05, ##*p* < 0.01, ###*p* < 0.001 vs. Ang II+NC; &*p* < 0.05, &&*p* < 0.01, &&&*p* < 0.001 vs. Ang II+PRDX2+shNC.

ation, migration, and extracellular matrix remodeling [25]. These changes compromise vascular wall integrity and contribute to the pathogenesis of various vascular remodeling diseases, including aortic aneurysm, atherosclerosis, and restenosis [26]. In this study, PRDX2 overexpression reversed Ang II-induced alterations in VSMC phenotypic markers, including downregulation of α-SMA/SM22-α and upregulation of OPN, whereas PARK7 knockdown attenuated this effect. α-SMA and SM22-α are characteristic markers of the contractile phenotype, which maintains vascular tone and exhibits low proliferative and migratory activity [27]. Downregulation of these proteins indicates loss of contractile characteristics and a shift toward the synthetic phenotype. In contrast, OPN is a hallmark marker of the synthetic phenotype, reflecting enhanced cell proliferation, migration, and extracellular matrix production [28]. Therefore, decreased α-SMA and SM22-α expression combined with increased OPN levels indicates a transition

from the contractile to the synthetic phenotype, representing a central pathological feature of vascular remodeling and aneurysm development.

It is noteworthy that the PRDX2-PARK7 axis has been implicated in various vascular disease models. A previous study reported that PRDX2 knockdown significantly accelerates Ang II-induced abdominal aortic aneurysm progression, accompanied by increased aortic dilation, maximal diameter, and incidence [12]. Conversely, PRDX2 upregulation markedly reduces tumor necrosis factor-α (TNF-α)-induced ROS production, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) expression in carotid artery VSMCs [29]. Furthermore, PARK7 upregulation has been reported to inhibit pulmonary artery VSMC phenotypic switching, reducing excessive proliferation and migration and alleviating pulmonary hypertension [30]. Notably, ferroptosis activators exacerbate neointimal hyperplasia induced by

arterial ligation and promote VSMC transition from the contractile to the synthetic phenotype *in vivo* [31]. These findings indicate that the PRDX2-PARK7 axis may inhibit VSMC phenotypic switching by modulating ferroptosis, thereby preserving the contractile phenotype and reducing oxidative stress and extracellular matrix remodeling. However, whether these effects translate to complex vascular diseases, such as aortic aneurysms, requires further validation in *in vivo* and multi-cellular systems.

Notwithstanding these observations, several caveats should be considered. Primarily, all experiments were conducted using human VSMCs, and the *in vivo* relevance of these findings in aortic aneurysm models remains to be validated. Second, the long-term effects of PRDX2 or PARK7 modulation on VSMC function and aortic wall integrity were not assessed, which is critical for translating these findings into potential clinical applications. Finally, patient-derived samples were not included, limiting the direct clinical relevance of the results. Future studies should address these limitations by establishing animal models of vascular remodeling to validate the *in vivo* role of the PRDX2-PARK7 axis and by incorporating patient-derived VSMC samples or aortic tissue specimens to further evaluate its clinical potential.

Conclusion

Our *in vitro* study demonstrates that, under Ang II-induced stress, the PRDX2-PARK7 axis attenuates iron-dependent oxidative stress and modulates ferroptosis-related phenotypic changes in human VSMCs. We demonstrated that PRDX2 protects VSMCs from Ang II-induced injury in a PARK7-dependent manner, with concomitant restoration of Nrf2, SLC7A11, and GPX4 expression levels, as well as contractile protein expression. These findings are derived from an *in vitro* model and provide mechanistic insight into VSMC regulation under oxidative stress conditions, rather than direct evidence of a role in aortic aneurysm progression. The clinical and translational significance of the PRDX2-PARK7 axis in vascular remodeling and aortic aneurysm pathogenesis warrants further *in vivo* and patient-based studies.

Availability of Data and Materials

The datasets used or analyzed during the current study are available from the corresponding authors upon reasonable request.

Author Contributions

TZ, QYZ and GFS designed the research study; ZXW and TTH collected the data; TZ, QYZ, ZXW and TTH performed the experiments; TZ, QYZ and GFS analyzed the data; TZ and QYZ drafted the original manuscript; ZXW, TTH and GFS critically revised the manuscript for impor-

tant intellectual content. All authors gave final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This work was supported by Ningbo Top Medical and Health Research Program [grant number: 2022030107], Ningbo “Technology Innovation 2025” Major Special Project [grant number: 2022Z150], and Ningbo Natural Science Foundation [grant number: 2024J291].

Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Aherrahrou R, Baig F, Theofilatos K, Lue D, Beele A, Örd T, et al. Secreted Protein Profiling of Human Aortic Smooth Muscle Cells Identifies Vascular Disease Associations. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2024; 44: 898–914. <https://doi.org/10.1161/ATVBAHA.123.320274>
- [2] Qin HL, Bao JH, Tang JJ, Xu DY, Shen L. Arterial remodeling: the role of mitochondrial metabolism in vascular smooth muscle cells. *American Journal of Physiology. Cell Physiology*. 2023; 324: C183–C192. <https://doi.org/10.1152/ajpcell.00074.2022>
- [3] Elmarasi M, Elmakaty I, Elsayed B, Elsayed A, Zein JA, Boudaka A, et al. Phenotypic switching of vascular smooth muscle cells in atherosclerosis, hypertension, and aortic dissection. *Journal of Cellular Physiology*. 2024; 239: e31200. <https://doi.org/10.1002/jcp.31200>
- [4] Rombouts KB, van Merriënboer TAR, Ket JCF, Bogunovic N, van der Velden J, Yeung KK. The role of vascular smooth muscle cells in the development of aortic aneurysms and dissections. *European Journal of Clinical Investigation*. 2022; 52: e13697. <https://doi.org/10.1111/eci.13697>
- [5] Gilmore BF, Scali ST, D’Oria M, Neal D, Schermerhorn ML, Huber TS, et al. Temporal Trends and Outcomes of Abdominal Aortic Aneurysm Care in the United States. *Circulation. Cardiovascular Quality and Outcomes*. 2024; 17: e010374. <https://doi.org/10.1161/CIRCOUTCOMES.123.010374>
- [6] Zhou J, Zhang L, Yan J, Hou A, Sui W, Sun M. Curcumin Induces Ferroptosis in A549 CD133⁺ Cells through the GSH-GPX4 and FSP1-CoQ10-NAPH Pathways. *Discovery Medicine*. 2023; 35: 251–263. <https://doi.org/10.24976/Discover.Med.202335176.26>
- [7] Yan H, Wang S, Hu Y, Jing R, Liu L, Liu P, et al. AG8 induces apoptosis and ferroptosis through regulating caspase 3 and SLC7A11/GPX4 signaling pathway in triple-negative breast

- cancer. *Clinical Traditional Medicine and Pharmacology*. 2025; 6: 200222. <https://doi.org/10.1016/j.ctmp.2025.200222>
- [8] Zhao Z, Jia Y, Hou X. FRZB Attenuates Ferroptosis and Oxidative Stress in Diabetic Cardiomyopathy by Modulating the AMPK/PGC-1 α Signaling Pathway: A Novel Cardioprotective Mechanism. *Discovery Medicine*. 2025; 37: 2978–2989. <https://doi.org/10.24976/Descov.Med.202537203.249>
- [9] He Y, Wang X, Li D, Zhu Q, Xiang Y, He Y, et al. *ALAS2* overexpression alleviates oxidative stress-induced ferroptosis in aortic aneurysms via *GATA1* activation. *Journal of Thoracic Disease*. 2024; 16: 2510–2527. <https://doi.org/10.21037/jtd-24-370>
- [10] Shi Y, Zhao Y, Sun SJ, Lan XT, Wu WB, Zhang Z, et al. Targeting GPX4 alleviates ferroptosis and retards abdominal aortic aneurysm formation. *Biochemical Pharmacology*. 2025; 234: 116800. <https://doi.org/10.1016/j.bcp.2025.116800>
- [11] He X, Xiong Y, Liu Y, Li Y, Zhou H, Wu K. Ferrostatin-1 inhibits ferroptosis of vascular smooth muscle cells and alleviates abdominal aortic aneurysm formation through activating the SLC7A11/GPX4 axis. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*. 2024; 38: e23401. <https://doi.org/10.1096/fj.202300198RRR>
- [12] Jeong SJ, Cho MJ, Ko NY, Kim S, Jung IH, Min JK, et al. Deficiency of peroxiredoxin 2 exacerbates angiotensin II-induced abdominal aortic aneurysm. *Experimental & Molecular Medicine*. 2020; 52: 1587–1601. <https://doi.org/10.1038/s12276-020-00498-3>
- [13] Chen Y, Li S, Yin M, Li Y, Chen C, Zhang J, et al. Isorhapontigenin Attenuates Cardiac Microvascular Injury in Diabetes via the Inhibition of Mitochondria-Associated Ferroptosis Through PRDX2-MFN2-ACSL4 Pathways. *Diabetes*. 2023; 72: 389–404. <https://doi.org/10.2337/db22-0553>
- [14] Wang H, Liu H, Tang X, Lu G, Luo S, Du M, et al. Potentially functional variants of PARK7 and DDR2 in ferroptosis-related genes predict survival of non-small cell lung cancer patients. *International Journal of Cancer*. 2025; 156: 744–755. <https://doi.org/10.1002/ijc.35197>
- [15] Ouyang L, He T, Xing Y. Park7 protects retinal ganglion cells and promotes functional preservation after optic nerve crush via regulation of the Nrf2 signaling pathway. *Graefes' Archive for Clinical and Experimental Ophthalmology = Albrecht Von Graefes Archiv Fur Klinische Und Experimentelle Ophthalmologie*. 2023; 261: 3489–3502. <https://doi.org/10.1007/s00417-023-06099-w>
- [16] Yuan Y, Zhai Y, Chen J, Xu X, Wang H. Kaempferol Ameliorates Oxygen-Glucose Deprivation/Reoxygenation-Induced Neuronal Ferroptosis by Activating Nrf2/SLC7A11/GPX4 Axis. *Biomolecules*. 2021; 11: 923. <https://doi.org/10.3390/biom11070923>
- [17] Zhang L, Zhao Z, Quan X, Xie Z, Zhao J. Circ_0008285 silencing suppresses angiotensin II-induced vascular smooth muscle cell apoptosis in thoracic aortic aneurysm via miR-150-5p/BASPI axis. *Thoracic Cancer*. 2023; 14: 2158–2167. <https://doi.org/10.1111/1759-7714.15002>
- [18] Damgaard MV, Treebak JT. Protocol for qPCR analysis that corrects for cDNA amplification efficiency. *STAR Protocols*. 2022; 3: 101515. <https://doi.org/10.1016/j.xpro.2022.101515>
- [19] Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, et al. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Research*. 2023; 51: D638–D646. <https://doi.org/10.1093/nar/gkac1000>
- [20] Wang S, Chen Z, Zhu S, Lu H, Peng D, Soutto M, et al. PRDX2 protects against oxidative stress induced by *H. pylori* and promotes resistance to cisplatin in gastric cancer. *Redox Biology*. 2020; 28: 101319. <https://doi.org/10.1016/j.redox.2019.101319>
- [21] Liu J, Su G, Gao J, Tian Y, Liu X, Zhang Z. Effects of Peroxiredoxin 2 in Neurological Disorders: A Review of its Molecular Mechanisms. *Neurochemical Research*. 2020; 45: 720–730. <https://doi.org/10.1007/s11064-020-02971-x>
- [22] Kolisek M, Montezano AC, Sponder G, Anagnostopoulou A, Vormann J, Touyz RM, et al. PARK7/DJ-1 dysregulation by oxidative stress leads to magnesium deficiency: implications in degenerative and chronic diseases. *Clinical Science (London, England: 1979)*. 2015; 129: 1143–1150. <https://doi.org/10.1042/CS20150355>
- [23] Richarme G, Mihoub M, Dairou J, Bui LC, Leger T, Lamouri A. Parkinsonism-associated protein DJ-1/Park7 is a major protein deglycase that repairs methylglyoxal- and glyoxal-glycated cysteine, arginine, and lysine residues. *The Journal of Biological Chemistry*. 2015; 290: 1885–1897. <https://doi.org/10.1074/jbc.M114.597815>
- [24] Liao T, Xu X, Ye X, Yan J. DJ-1 upregulates the Nrf2/GPX4 signal pathway to inhibit trophoblast ferroptosis in the pathogenesis of preeclampsia. *Scientific Reports*. 2022; 12: 2934. <https://doi.org/10.1038/s41598-022-07065-y>
- [25] Cao G, Xuan X, Hu J, Zhang R, Jin H, Dong H. How vascular smooth muscle cell phenotype switching contributes to vascular disease. *Cell Communication and Signaling: CCS*. 2022; 20: 180. <https://doi.org/10.1186/s12964-022-00993-2>
- [26] Petsophonsakul P, Furmanik M, Forsythe R, Dweck M, Schurink GW, Natour E, et al. Role of Vascular Smooth Muscle Cell Phenotypic Switching and Calcification in Aortic Aneurysm Formation. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2019; 39: 1351–1368. <https://doi.org/10.1161/ATVBAHA.119.312787>
- [27] Lacolley P, Regnault V, Segers P, Laurent S. Vascular Smooth Muscle Cells and Arterial Stiffening: Relevance in Development, Aging, and Disease. *Physiological Reviews*. 2017; 97: 1555–1617. <https://doi.org/10.1152/physrev.00003.2017>
- [28] Castello LM, Raineri D, Salmi L, Clemente N, Vaschetto R, Quaglia M, et al. Osteopontin at the Crossroads of Inflammation and Tumor Progression. *Mediators of Inflammation*. 2017; 2017: 4049098. <https://doi.org/10.1155/2017/4049098>
- [29] Li J, Wang C, Wang W, Liu L, Zhang Q, Zhang J, et al. PRDX2 Protects Against Atherosclerosis by Regulating the Phenotype and Function of the Vascular Smooth Muscle Cell. *Frontiers in Cardiovascular Medicine*. 2021; 8: 624796. <https://doi.org/10.3389/fcvm.2021.624796>
- [30] Gao W, Shao R, Zhang X, Liu D, Liu Y, Fa X. Up-regulation of caveolin-1 by DJ-1 attenuates rat pulmonary arterial hypertension by inhibiting TGF β /Smad signaling pathway. *Experimental Cell Research*. 2017; 361: 192–198. <https://doi.org/10.1016/j.yexcr.2017.10.019>
- [31] Zhang S, Bei Y, Huang Y, Huang Y, Hou L, Zheng XL, et al. Induction of ferroptosis promotes vascular smooth muscle cell phenotypic switching and aggravates neointimal hyperplasia in mice. *Molecular Medicine (Cambridge, Mass.)*. 2022; 28: 121. <https://doi.org/10.1186/s10020-022-00549-7>