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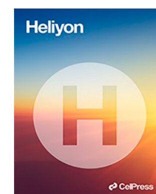

Research article

Sulfenylation of ERK1/2: A novel mechanism for SO_2-mediated inhibition of cardiac fibroblast proliferation

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ABSTRACT

Background: Endogenous sulfur dioxide (SO_2) plays a crucial role in protecting heart from myocardial fibrosis by inhibiting the excessive growth of cardiac fibroblasts. This study aimed to investigate potential mechanisms by which SO_2 suppressed myocardial fibrosis.
Methods and results: Mouse model of angiotensin II (Ang II)-induced cardiac fibrosis and cell model of Ang II-stimulated cardiac fibroblast proliferation were employed. Our findings discovered that SO_2 mitigated the aberrant phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) induced by Ang II, leading to a reduction of fibroblast proliferation. Mechanistically, for the first time, we found that SO_2 sulfenylated ERK1/2, and inhibited ERK1/2 phosphorylation and cardiac fibroblast proliferation, while a sulfhydryl reducing agent dithiothreitol (DTT) reversed the above effects of SO_2. Furthermore, mutant $ERK1^{C183S}$ (cysteine 183 to serine) abolished the sulfenylation of ERK by SO_2, thereby preventing the inhibitory effects of SO_2 on ERK1 phosphorylation and cardiac fibroblast proliferation.
Conclusion: Our study suggested that SO_2 inhibited cardiac fibroblast proliferation by sulfenylating ERK1/2 and subsequently suppressing ERK1/2 phosphorylation. These new findings might enhance the understanding of the mechanisms underlying myocardial fibrosis and emphasize the potential of SO_2 as a novel therapeutic target for myocardial fibrosis.

1. Introduction

Excessive proliferation of cardiac fibroblasts is a crucial pathological basis of myocardial fibrosis [1–4]. In response to injury or stress, cardiac fibroblasts exhibit adaptive proliferation, which is associated with increased collagen synthesis. Appropriate collagen production helps reinforce the heart scaffold during the initial period of cardiomyocyte damage [5,6]. However, excessive collagen deposition, generated by the over-proliferation of fibroblasts, leads to myocardial fibrosis and cardiac dysfunction, which participate in the pathogenesis of most myocardial diseases [7]. Therefore, clarifying the mechanisms underlying excessive proliferation of cardiac fibroblasts is important for the new therapeutic strategies of myocardial diseases. Recent research has shown that endogenous sulfur dioxide (SO_2) is a potential novel sulfur-containing gasotransmitter, alleviating myocardial fibrosis caused by myocardial infarction,

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diabetes, or excessive β-adrenergic stimulation [8]. Additionally, the knockdown of the endogenous SO_2-generating enzyme, aspartate aminotransferase (AAT), has been shown to significantly enhance the proliferation and migration of cardiac fibroblasts [9]. These studies suggested an important role of SO_2 in myocardial fibrosis. However, the mechanisms by which SO_2 inhibits cardiac fibroblast overproliferation remain unclear.

Mitogen-activated protein kinase (MAPK) pathways are widely investigated and reported to be involved in the pathogenesis of myocardial fibrosis [10–12]. Within the MAPK family, extracellular signal-regulated kinase 1/2 (ERK1/2) undergo the phosphorylation at threonine (Thr) 202 and tyrosine (Tyr) 204 residues, controlling cell proliferation, differentiation and apoptosis [13–15]. In particular, an increased phosphorylated-ERK1/2 (*p*-ERK1/2) is associated with cardiac fibroblast proliferation and myocardial fibrosis [16,17]. Previous studies have shown that SO_2 suppresses the proliferation of vascular smooth muscle cells (VSMCs) by inhibiting the ERK1/2 signaling pathway [18]. Moreover, AAT knockdown significantly activated ERK1/2 signaling in cardiac fibroblasts [9]. These above results suggest that the ERK1/2 signaling pathway may mediated the suppressive effect of SO_2 on cell proliferation. However, the mechanism by which SO_2 regulates the aberrant ERK1/2 activation remains unclear.

Sulfenylation is a chemical modification on the thiol group of cysteine residue in a protein [19], manifesting as the addition of a sulfenic acid (SOH) group to a cysteine (Cys) thiol, which is regarded as an important molecular mechanism for the biological effects of endogenous SO_2 [20–24]. For example, endogenous SO_2 suppressed the level of phosphorylated nuclear factor-κB p65 and subsequent p65 nuclear translocation and alveolar epithelial cell inflammation by sulfenylating Cys 38 at p65 [25]. In recent years, it has been found that oxidative modification of cysteine thiols can affect cellular physiological processes by influencing the onset of phosphorylation. For example, the sulfhydration of SIRT1 at Cys395/398 and Cys371/374 led to the dephosphorylation of NF-κB p65, and subsequently reduced inflammation response [26]. In a glioma cell study, nitric oxide suppressed the level of *p*-ERK1/2 via a nitrosylation on the thiol group of the cysteine residue in ERK1/2 [27]. Therefore, the above findings suggest a possible association between SO_2-induced sulfenylation and the ERK1/2 signaling pathway, which merits further investigation.

In the current study, we planed to determine the correlation between the sulfenylation and phosphorylation of ERK1/2 in the SO_2-inhibited myocardial fibrosis and cardiac fibroblast proliferation model. Moreover, we demonstrated the mechanism by which SO_2 potentially sulfenylated ERK1/2, then suppressed the phosphorylation ERK1/2 and inhibited cardiac fibroblast proliferation.

2. Materials and methods

2.1. Ethical approval statement

All animals were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., and bred in the Experimental Animal Center of Peking University First Hospital. The protocols were permitted by the Animal Ethics Committee of Peking University First Hospital (No. J2022020 for rats used in the extraction of primary cardiac fibroblasts, and NO. J201713 for mouse experiments) and were conducted in accordance with the Laboratory Animal Health Guide of US National Institutes.

2.2. Animals and treatments

C57BL/6N mice were implanted subcutaneously with osmotic minipumps (ALZET®) delivering 1.44 mg/kg/min angiotensin II (Ang II) for 4 weeks. Meanwhile, the mice were intraperitoneally administered of an SO_2 donor (a mixture of 0.54 mmol of Na_2SO_3 and 0.18 mmol of $NaHSO_3$ per kilogram of body weight was solubilized in saline) once a day for 4 weeks. At the indicated time points, the mice were euthanized by cervical dislocation after anesthesia.

2.3. Reagents

Ang II were purchased from Phoenix Pharmaceuticals (002-12, Burlingame, CA, USA). DAz-2 (13382, Cayman, Michigan, USA) was used to capture sulphenylated proteins. The sulfhydryl reducing agent dithiothreitol (DTT) was obtained from Beyotime Biotechnology (ST043, Shanghai, China). The 5-ethynyl-2′-deoxyuridine (EdU) incorporation assay kit (C0078S, Beyotime, Shanghai, China) was a commercial kit. The antibodies utilized in this study, including *p*-ERK1/2 (AF1891; 1:1000; Beyotime), ERK1/2 (AF1051; 1:1000; Beyotime), PCNA (10205-2-AP; 1:1000; Proteintech), His (TA150088Z; 1:1000; Origene), β-Tubulin (TA-10; 1:1000; Santa Cruze), and anti-rabbit/mouse antibodies (1:5000; Invitrogen; Thermo fisher), were commercially available.

2.4. Plasmids transfection

The pCDH-CMV-MCS-EF1-copGFP-T2A-Puro-Entry vector for $ERK1^{WT}$ (wild-type) and $ERK1^{C183S}$ (mutation of cysteine 183 to serine) were constructed by General Biol Technologies (Anhui, China) and subcloned into a 3 × His vector. Briefly, when cells reached 40–50 % confluency, plasmids (2 μg) were mixed with jetPEI™ reagent (Polyplus Transfection, France) for 12 min. The cells were incubated with complete medium containing the above transfection mixture for 6 h and then the medium was discarded. After the additional 48 h of incubation with fresh complete medium, the cells were checked and the successfully transfected cells were utilized for the experiments. Successful transfection of the plasmids was confirmed by detecting the expression of tag using anti-His antibodies.

2.5. Lentivirus packagement

The packaging plasmids (PAX2 and PMD2.G) and target plasmids were transfected into 293T cells using the transfection reagent. After incubation for 4–6 h, fresh medium was added to replace the medium. Viral particles were collected from culture supernatant after 48 h.

2.6. Extraction of cardiac fibroblast

Primary cardiac fibroblasts were separated from the ventricular tissue of Sprague–Dawley rats (150–180 g body weight). Rat was euthanized by cervical dislocation after anesthesia. The heart was rapidly excised from the body, washed three times, and minced into small pieces. The dissected heart pieces were attached to the bottom wall of the culture bottle. Subsequently, the cells were cultured in DMEM/F12 medium containing 20 % fetal bovine serum (FBS) (Clark, USA) and 1 % penicillin/streptomycin. After one week, the cells grew around the tissue and were passaged at approximately 80 % confluence.

2.7. Cell culture, transfection, and treatments

Primary cardiac fibroblasts at passages 2–4 were used for cell experiments. The cells were cultured in complete medium with 10 % FBS. When the cells grew to 70 % confluence, the medium was replaced by basal FBS-free medium and incubated for 24 h. Subsequently, the cells were stimulated with 1 μM Ang II in basal FBS-free medium, and supplied with 100 μM SO_2 donor for 24 h for further analysis. To investigate whether ERK1/2 sulfenylation was responsible for the suppression of ERK1/2 phosphorylation and cell growth by SO_2, cells were treated with 1 μM Ang II, 100 μM DTT, and/or 100 μM SO_2 donor. To further verify that sulfenylation of ERK1 Cys183 was responsible for the inhibitory impact of SO_2 on ERK1 phosphorylation and cardiac fibroblast proliferation, $ERK1^{WT}$ and $ERK1^{C183S}$ lentiviruses were respectively administered to fibroblasts, followed by the treatment with Ang II with or without SO_2 donor for 24 h.

HEK293T cells were provided by the Cell Resource Center (Institute of Basic Medical Sciences, Beijing, China) and cultured in DMEM medium with 10 % FBS and 1 % penicillin/streptomycin. 293T cells were grown to 50 % confluence and transfected with wild-type or mutant plasmids using a transfection kit.

2.8. Western blotting

For protein extraction from cells and tissues, a lysis buffer was used. The protein concentration was assessed by the BCA method using a commercial kit (Beyotime, Shanghai, China). The proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred to nitrocellulose membranes. Subsequent to the transfer, non-fat milk was used to depopulate non-specific antigen binding sites. Thereafter, the membranes were exposed to the appropriate primary antibodies, followed by an incubation with secondary antibodies. Detection was performed using the FluorChem MultiFluor System (San Francisco, CA, USA). Band intensities were analyzed using ImageJ software. β-Tubulin preformed as a internal reference.

2.9. EdU incorporation assay

A commercial Click-iT® EdU Alexa Fluor® 594 Kit (Beyotime, Shanghai, China) was used to measure the proliferation of cardiac fibroblast. Cell suspensions were seeded (one day prior) into 8-wells cell chamber. Cells were labeled with 10 μM EdU for 2 h. Afterward, the cells were rinsed with 3 % BSA and then treated with 100 μl Click-iT® fixative for 30 min. Finally, the nuclei were labeled with Hoechst.

2.10. Biotin switch assay

Cells and tissues were lysed using a non-denaturing lysis buffer. A 5 mM DAz-2 was added to label sulfenylated proteins. The lysates were gently shaken at 37 °C for 2 h. The DAz-2 labeled protein was incubated with 250 μM p-biotin at 37 °C for 2 h. Subsequently, the sulfenylated proteins were enriched using UltraLinkTM Immobilized NeutrAvidinTM beads (Thermo Fisher, USA), followed by boiling the beads in loading buffer for western blot analysis.

2.11. Statistical analysis

Each cellular experiment was independently conducted at least three times. The results was expressed as the mean ± SEM. Statistical analysis utilized student's t-test to compare the two groups and ascertain statistical significance ($p < 0.05$). For data involving three or more groups, a one-way analysis of variance (ANOVA) followed by post hoc analysis was applied. Statistical analysis was performed using GraphPad Prism software (version 8.0, San Diego, CA, USA).

3. Results

3.1. SO_2 inhibited the level of p-ERK1/2 and cell proliferation but promoted ERK1/2 sulfenylation in cardiac tissues of Ang II-infused mice

To investigate the impacts of SO_2 on ERK1/2 phosphorylation, sulfenylation, and cell proliferation, Ang II-stimulated mice were treated with or without SO_2 donor. Western blot analysis showed that the ratio of *p*-ERK1/2 to ERK1/2 and the expression of PCNA protein in the Ang II-stimulated mouse heart were increased compared with those in control mice, but were decreased by SO_2 treatment (Fig. 1A–B and Supplementary Fig. 1A–B). In contrast, the biotin switch experiment showed that compared with those in control mice, there was a marked downregulation of sulfenylated ERK1/2 (SOH-ERK1/2) protein level in the hearts of Ang II-stimulated mice, whereas SO_2 treatment upregulated sulfenylated ERK1/2 (SOH-ERK1/2) protein levels (Fig. 1C and Supplementary Fig. 1C). These data provided evidence that SO_2 promoted ERK1/2 sulfenylation but inhibited ERK1/2 phosphorylation and cell proliferation in the cardiac tissues of Ang II-stimulated mice.

3.2. SO_2 inhibited ERK1/2 phosphorylation and suppressed cardiac fibroblast proliferation

We further explored the influence of SO_2 on ERK1/2 phosphorylation and cell proliferation in cardiac fibroblasts. Fig. 2A and Supplementary Fig. 2A showed that the level of *p*-ERK1/2 was increased in the cardiac fibroblasts treated with 1 μM Ang II for both 60 min and 120 min. Subsequently, treatment with 100 μM SO_2 donor attenuated the Ang II-activated *p*-ERK1/2 (Fig. 2B and Supplementary Fig. 2B). Moreover, we examined the effect of SO_2 on the proliferation of Ang II-treated cardiac fibroblasts using a EdU incorporation assay. The result showed that Ang II treatment for 24 h considerably increased the percentage of proliferating cardiac fibroblasts among the total living cells, which was suppressed by the SO_2 donor (Fig. 2C). Collectively, these results highlighted the considerable inhibitory effects of SO_2 on ERK1/2 phosphorylation and proliferation in cardiac fibroblasts.

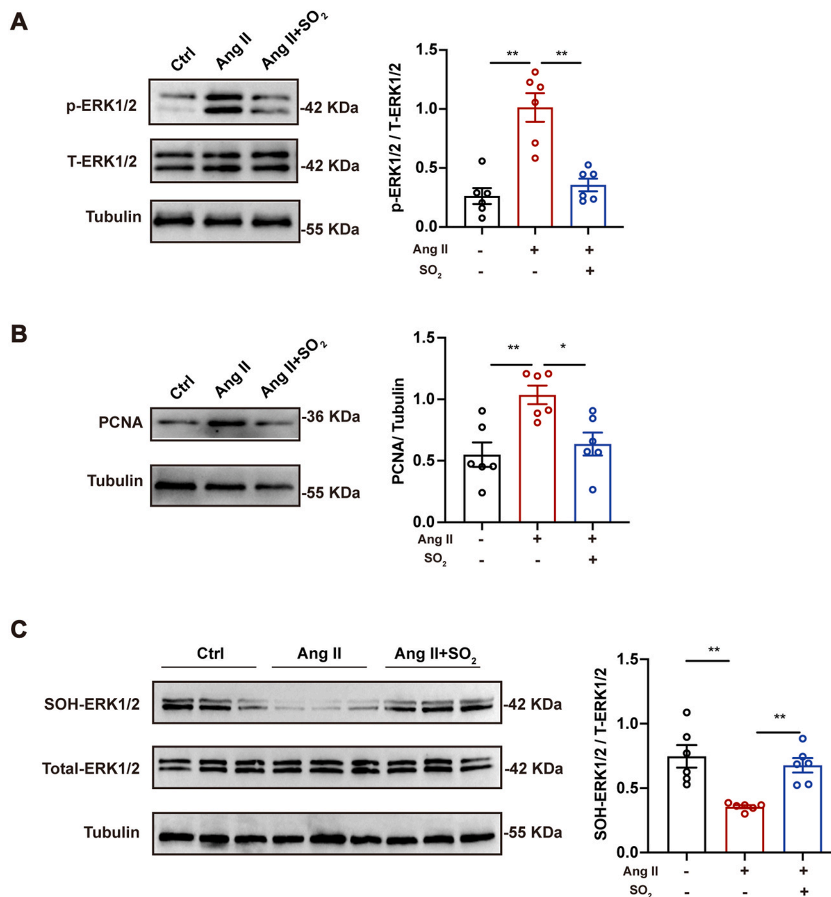

Fig. 1. SO_2 treatment inhibited ERK1/2 phosphorylation and cell proliferation while concurrently promoted ERK1/2 sulfenylation in cardiac tissues of Ang II-infused mice. (A) Western blot depicting *p*-ERK1/2 protein levels in hearts of mice with Ang II and SO_2 treatment (n = 6). (B) Western blot depicting PCNA protein levels in heart tissues of mice with Ang II and SO_2 treatment (n = 6). (C) Biotin switch assay showing the sulfenylation of ERK1/2 in heart tissues (n = 6). The results were expressed as the mean ± SEM by one-way analysis. * indicate $p < 0.05$ and ** indicate $p < 0.01$.

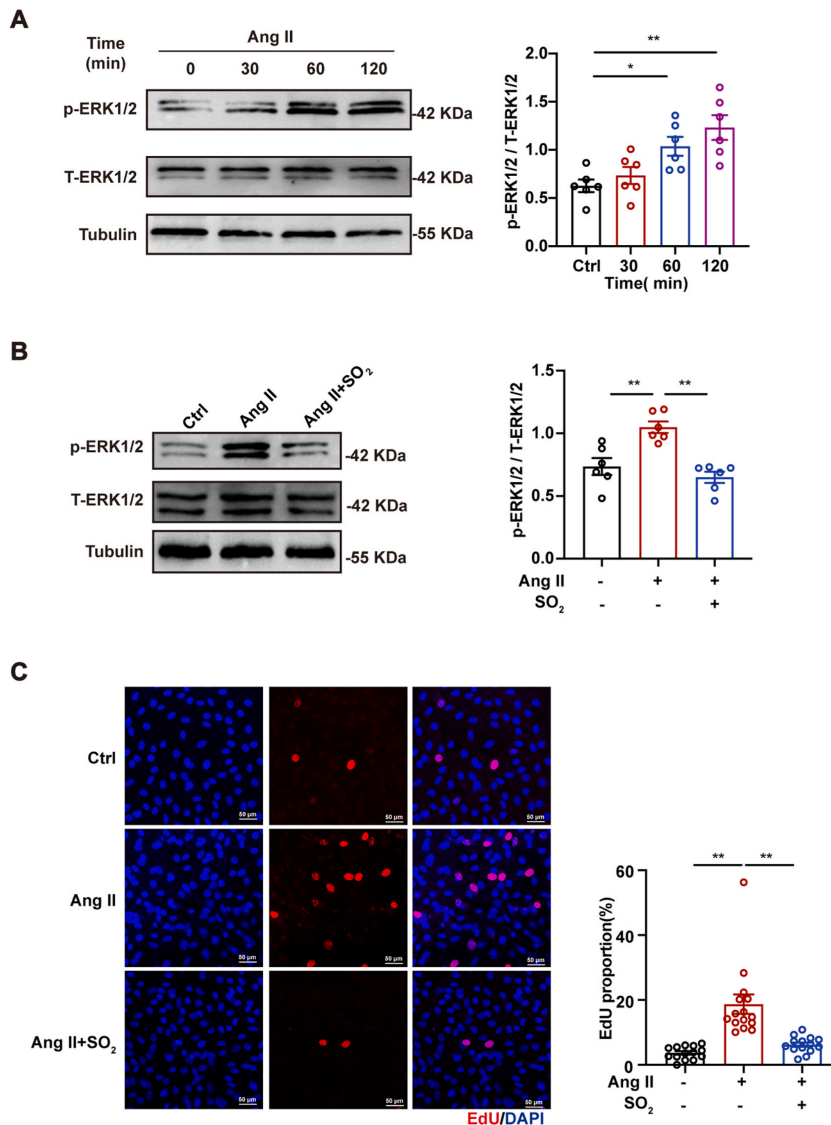

Fig. 2. SO_2 treatment suppressed ERK1/2 phosphorylation and proliferation of cardiac fibroblasts. (A) Western blot depicting ERK1/2 phosphorylation in Ang II-treated cardiac fibroblasts (n = 6). (B) Western blot was utilized to evaluate the phosphorylation of ERK1/2 in cardiac fibroblasts with Ang II and SO_2 for 60 min (n = 6). (C) EdU incorporation assay showing the proliferation of cardiac fibroblasts (scale bar = 50 μm). EdU (red fluorescence) and nuclear stained with DAPI (blue fluorescence) (at least 14 separated views). The results were expressed as the mean ± SEM by one-way analysis. * indicate $p < 0.05$ and ** indicate $p < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. SO_2 inhibited ERK1/2 phosphorylation and cell proliferation via sulfenylating ERK1/2 in cardiac fibroblasts

To elucidate the possibility that SO_2-induced ERK1/2 sulfenylation might be an upstream event of SO_2-suppressed ERK1/2 phosphorylation and cell proliferation in cardiac fibroblast, we employed 100 μM DTT, a thiol reducing agent, to cancel the ERK1/2 sulfenylation by SO_2 (Fig. 3A and Supplementary Fig. 3A). Along with DTT-diminished ERK1/2 sulfenylation, weak ERK1/2 phosphorylation and subsequent hypoproliferation in cardiac fibroblasts of the Ang II + SO_2 group were reversed by DTT (Fig. 3B, 3C and Supplementary Fig. 3B), which supported the hypothesis that ERK1/2 sulfenylation might mediate the SO_2-suppressed ERK1/2 phosphorylation and subsequent cardiac fibroblast proliferation.

3.4. Cysteine 183 in ERK1 is required for SO_2 -inhibited cardiac fibroblast proliferation

Both ERK1 and ERK2 serve as effector kinases in the MAPK signaling pathway in cardiac tissues. Previous research has indicated that overexpression of ERK1 rescues proliferation deficiencies in ERK1/2-knockouted embryonic fibroblasts, suggesting that ERK1

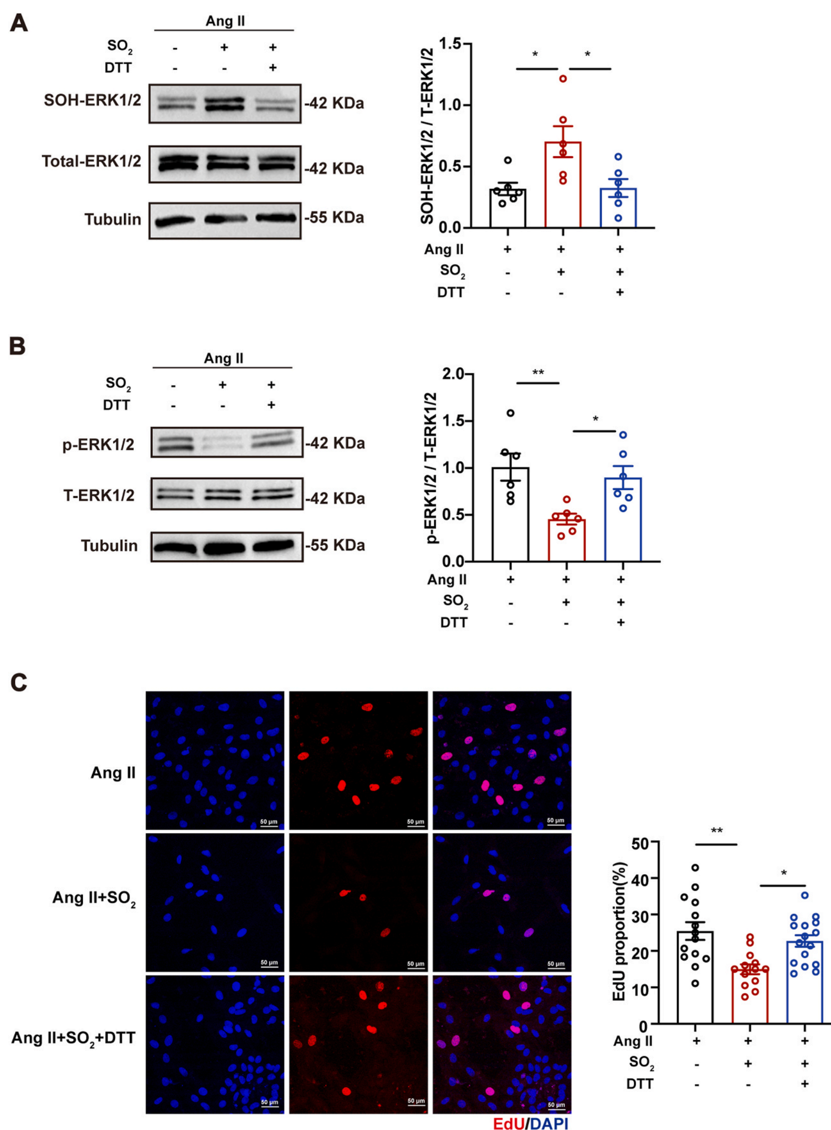

Fig. 3. SO_2 treatment inhibited ERK1/2 phosphorylation and cell proliferation in cardiac fibroblasts via sulfenylation. (A) Western blot showing the level of SOH-ERK1/2 in cardiac fibroblasts incubated with Ang II, SO_2 and DTT (n = 6). (B) Western blot analysis showing the ratio of *p*-ERK1/2 to ERK1/2 in cardiac fibroblasts incubated with Ang II, SO_2, and DTT (n = 6). (C) EdU incorporation assay showing the cardiac fibroblast proliferation (scale bar = 50 μm). EdU (red fluorescence) and nuclear stained with DAPI (blue fluorescence) (at least 13 separated views). The results were expressed as the mean ± SEM by one-way analysis. * indicate $p < 0.05$ and ** indicate $p < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

plays a more prominent role in cell proliferation [28,29]. Therefore, we focused on ERK1 to determine the exact sulfenylation site of $SO_{2.}$ Cys183 in ERK1 is reported to be highly sensitive to redox stimuli [27]. Therefore, an ERK1 mutant plasmid ($ERK1^{C183S}$) wherein Cys183 residue was replaced with serine was constructed in this study. Sulfenylated ERK1 levels significantly increased in 293T cells transfected with the $ERK1^{WT}$ plasmid after SO_2 treatment. However, SO_2 did not promote the sulfenylation of ERK1 in $ERK1^{C183S}$-transfected 293T cells (Fig. 4A and Supplementary Fig. 4A). These results demonstrated that Cys183 is the target of SO_2-induced ERK1 sulfenylation.

Furthermore, the significance of SO_2-induced sulfenylation Cys183 of ERK1 in the suppressive impacts of SO_2 on the ERK1/2 pathway and cell proliferation was explored (Fig. 4B, 4C and Supplementary Fig. 4B). In cardiac fibroblasts infected with $ERK1^{WT}$ lentivirus, SO_2 suppressed Ang II-stimulated the ERK1 phosphorylation and the percentage of EdU-positive cells. However, SO_2 did not alter these two indices in cardiac fibroblasts infected with the $ERK1^{C183S}$ lentivirus. Taken together, these results suggested that SO_2-sulfenylated ERK1 at Cys183 suppressed Ang II-stimulated ERK1 phosphorylation and inhibited cardiac fibroblast proliferation.

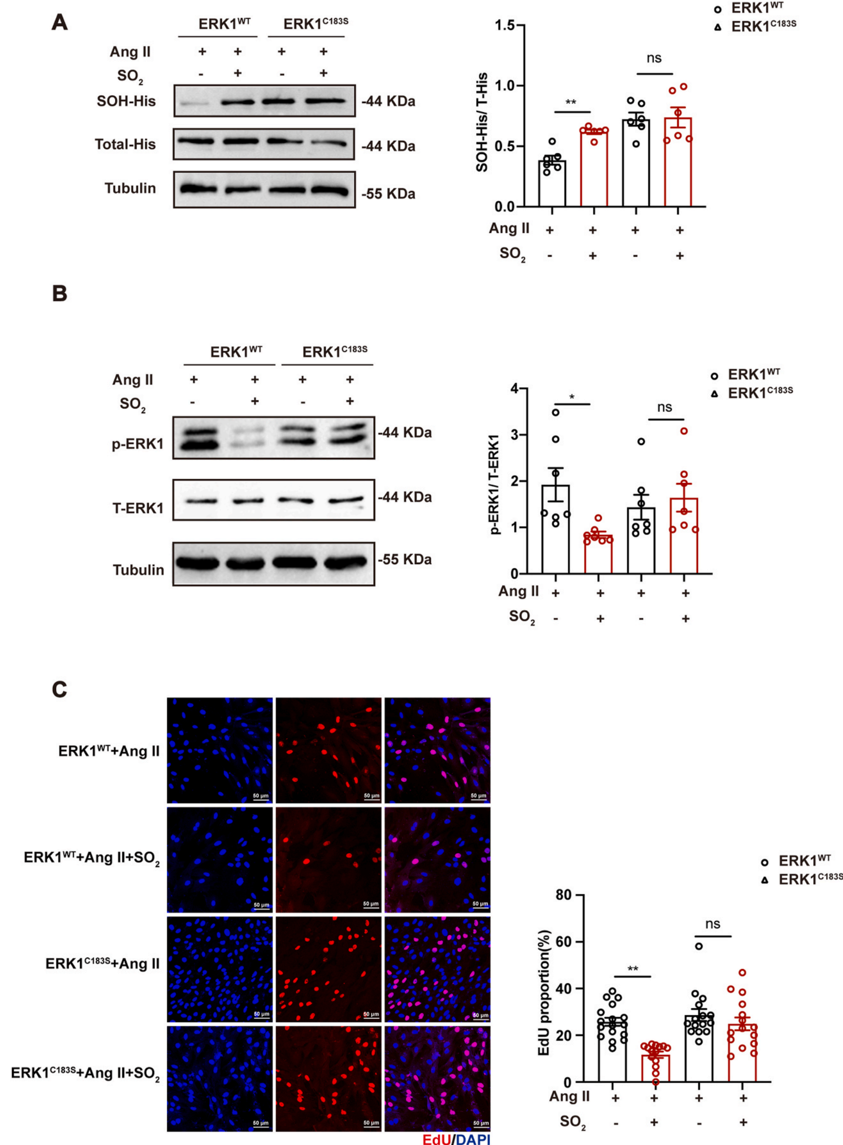

Fig. 4. SO_2 targeted the sulfhydryl groups on cysteine 183 in ERK1. (A) Sulfenylation of ERK1 in the 293T cells transfected with plasmids encoding the wild-type (ERK1^{WT}) or mutant (ERK1^{C183S}) ERK1 and treated with Ang II and SO_2 donor (n = 6). (B) Western blot showing *p*-ERK1 protein levels in the cardiac fibroblasts transfected with plasmids encoding the ERK1^{WT} or ERK1^{C183S} mutant and treated with Ang II and SO_2 for 1 h (n = 7). (C) EdU assay showing the growth ability of cardiac fibroblasts transfected with plasmids encoding the wild-type (ERK1^{WT}) or mutant (ERK1^{C183S}) ERK1 and treated with Ang II and SO_2 for 1 h. (scale bar = 50 μm). EdU (red fluorescence) and nuclear stained with DAPI (blue fluorescence) (at least 14 separated views). The results were expressed as the mean ± SEM by student's t-test and one-way analysis. * indicate $p < 0.05$ and ** indicate $p < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

In this study, we revealed that SO_2 sulfenylated ERK1/2, inhibiting ERK1/2 phosphorylation and cardiac fibroblast proliferation. Mechanistically, we identified the sulfhydryl group of Cys183 in ERK1 as a crucial target of SO_2 for the sulfenylation of ERK1 and the suppression of excessive cardiac fibroblast proliferation.

Many studies have confirmed that SO_2 can be endogenously produced by the metabolism of sulfur-containing amino acid. Moreover, endogenous SO_2 is found to be widely involved in controlling of physiological and pathophysiological processes as a new gaseous signaling molecule [8,30,31]. For example, endogenous SO_2 plays crucial roles in vasodilation, suppression of inflammation, and improvement of vascular collagen remodeling [32–35]. Furthermore, SO_2-mediated sulfenylation has been implicated in its biological effects, including control of inflammation and collagen remodeling [9]. Chen et al. found that SO_2 sulfenylated NF-κB p65 at Cys38, and thereby attenuated inflammatory responses in the lung tissues of rats with acute lung injury [25]. Huang et al. found that

the sulfenylation of SO_2 on Cys 64 in Smad3 was essential for SO_2-inhibited Smad3 phosphorylation and subsequent collagen synthesis in vascular smooth muscle cells [22]. Our study demonstrated that SO_2 significantly suppressed the proliferation of cardiac fibroblast. Considering that hyperproliferative cardiac fibroblasts were a major source of collagen accumulation, our findings demonstrated the vital role of SO_2 in the development of myocardial fibrosis.

Furthermore, the molecular mechanisms by which endogenous SO_2 inhibited fibroblast proliferation were investigated. ERK1 and ERK2, the main members of the MAPK family, are essential for controlling cell proliferation [36]. Post-translational modifications, including phosphorylation and nitrosylation, are important control switches of regulating the ERK pathway [27,37–39]. Phosphorylation of Thr202 and Tyr204 residues of ERK1 activates the ERK1/2 signaling pathway. Subsequently, *p*-ERK1/2 interacts with downstream signaling pathways to promote cell proliferation [40]. ERK1/2 signaling is activated, as evidenced by the elevation of *p*-ERK1/2 in Ang II-induced cardiac tissues [41]. Moreover, ERK1 nitrosylation inhibits ERK1 phosphorylation, thereby regulating cell survival [27]. It was reported that Ang II inhibited endogenous SO_2 production in the mouse heart [42]. The current study showed the downregulation of sulfenylated ERK1/2 protein levels in the hearts of Ang II-induced mice, whereas SO_2 supplementation blocked the inhibitory impact of Ang II on ERK1/2 sulfenylation. These findings suggested that Ang II inhibited the sulfenylation ERK1/2 probably by downregulating endogenous SO_2 production. Moreover, our findings showed that SO_2-induced ERK1/2 sulfenylation parallelly occurred with the SO_2-suppressed ERK1/2 phosphorylation and cell proliferation in the myocardial tissue of Ang II-stimulated mouse and in the Ang II-stimulated cardiac fibroblasts, suggesting that there maybe existed a possible association between sulfenylation and phosphorylation of ERK1/2 controlled by SO_2 in cardiac fibroblasts. Subsequently, the intervention of a thiol-reducing agent, DTT, blocked the sulfenylation of SO_2 on ERK1/2 and then resurged SO_2-inhibited ERK1/2 phosphorylation and cardiac fibroblast proliferation, which further consolidated the upstream position of ERK1/2 sulfenylation by SO_2 and its suppressive effect on cardiac fibroblast proliferation.

Considering cys183 of ERK1 was susceptible to redox stimuli [38], we investigated if SO_2 sulfenylated ERK1 by targeting on cys183. The results showed that the mutation of cys183 to serine canceled the sulfenylation of ERK1 by SO_2. Simultaneously, in cardiac fibroblasts infected with lentivirus containing $ERK1^{C183S}$, the inhibitory effects of SO_2 on ERK1 phosphorylation and cell proliferation were blocked, suggesting that cys183 in ERK1 was required for SO_2-inhibited cardiac fibroblast proliferation. In summary, our findings demonstrate for the first time that SO_2-induced ERK1/2 sulfenylation is an upstream regulatory event inhibiting the ERK1/2 pathway and cardiac fibroblast proliferation. This is a novel post-translational mechanism by which SO_2 inhibits the proliferation of cardiac fibroblasts by targeting ERK1/2. These findings deepen the understanding of myocardial fibrosis pathogenesis. Our results might provide potential therapeutic insights into the treatment of fibrotic diseases.

However, our study has some limitations. First, our *in vivo* studies included only whole heart tissue which did not allows us to directly demonstrate effects on cardiac fibroblasts. Additional experiments would help to discriminate the effects in different cell types in the heart. Secondly, adult mice and rats were respectively used in our *in vivo* and *in vitro* experiments. In fact, the above animal and cell models were widely employed [43–48]. Although there was an inconsistency between the animal species, the important features of rat and mouse cardiac fibroblasts including the cellular biological characteristics and the reactiveness to various stimuli were very similar [49–54]. Thirdly, in our present study, we focused on adult cardiac fibroblasts, further studies on neonatal cardiac fibroblasts might provide more extensive significance in the future.

Data availability statement

Data will be made available on request.

Ethical statement

The protocols were approved by the Animal Ethics Committee of Peking University First Hospital (NO. J2022020 for rats used in extraction of primary cardiac fibroblasts, and NO. J201713 for mouse experiment) and were conducted in accordance with the Laboratory Animals Health Guide of US National Institutes.

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CRediT authorship contribution statement

Mei Ge: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lulu Zhang:** Validation, Software, Resources, Methodology, Investigation, Data curation, Conceptualization. **Junbao Du:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Hongfang Jin:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Boyang Lv:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Yaqian Huang:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Hongfang Jin reports a relationship with Editorial Board of Heliyon that includes: board membership. Co-corresponding author Hongfang Jin is a member of the Editorial Board for Heliyon. The paper was handled by the other editor and has undergone rigorous peer review process. Hongfang Jin was not involved in the journal's review of, or decisions related to this manuscript. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34260.

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