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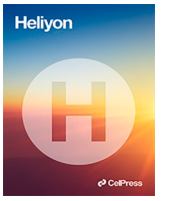

Research article

Impaired cardiomyocytes accelerate cardiac hypertrophy and fibrosis by delivering exosomes containing Shh/N-Shh/Gli1 in angiotensin II infused mice

Cong Wang [a], Zhiwei Lai [a], Huishi Tan [d], Hua Zhang [a], Lishan Tan [a], Qingyun Luo [a], Sanmu Li [a], Zibo Xiong [a,b], Guang Yang [a,b,c,*], Zuying Xiong [a,b,c,**]

[a] *Division of Renal Medicine, Peking University Shenzhen Hospital, Shenzhen Peking University-The Hong Kong University of Science and Technology Medical Center, Shenzhen, Guangdong, China*
[b] *Shenzhen Clinical Research Center for Urology and Nephrology, Shenzhen, Guangdong, China*
[c] *Institute of Nephrology, Shenzhen Peking University-Hong Kong University of Science and Technology Medical Center, Shenzhen, Guangdong, China*
[d] *Department of Gastroenterology and Hepatology, Guangzhou First People's Hospital, School of Medicine, South China University of Technology, Guangzhou, China*

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ABSTRACT

Backgrounds: Heart failure (HF) is characterized by progressive cardiac hypertrophy and fibrosis, yet the underlying pathological mechanisms remain unclear. Exosomes are pivotal in cellular communication and are key signaling carriers in HFs. This study investigated the roles of exosomes in HF.

Methods: Eight-week-old male mice were divided into three groups: a control group, an Ang II group receiving angiotensin II (Ang II) infusion for 4 weeks, and an Ang II + DMA group receiving Ang II and dimethyl amiloride (DMA) infusion. This study examined the associations between cardiac injury, exosomes, and their substrate Shh. Furthermore, we conducted cellular experiments to assess the effects of Ang II-induced injury in primary cardiomyocytes on other cardiomyocytes and fibroblasts, and to test the therapeutic effects of the exosome inhibitor DMA and the Shh signaling inhibitor cyclopamine (CPN).

Results: Ang II-induced cardiac hypertrophy and fibrosis, which were accompanied by exosome secretion and Shh upregulation *in vivo*. DMA relieved these cardiac lesions. Furthermore, cellular experiments revealed that Ang II-induced cardiomyocytes hypertrophy and activated cardiac fibroblasts by promoting the release of exosomes containing Shh/N-Shh/Gli1. Both DMA and CPN nullified fibroblast activation and proliferation.

Conclusions: Ang II-induced cardiomyocyte injury leads to cardiac hypertrophy and fibrosis through the release of exosomes carrying Shh signaling. The suppression of exosome secretion or the Shh pathway could offer new strategies for treating HF.

* Corresponding author. Division of Renal Medicine, Peking University Shenzhen Hospital, Shenzhen Peking University-The Hong Kong University of Science and Technology Medical Center, Shenzhen, Guangdong, China.

** Corresponding author. Division of Renal Medicine, Peking University Shenzhen Hospital, Shenzhen Peking University-The Hong Kong University of Science and Technology Medical Center, Shenzhen, Guangdong, China.

E-mail addresses: yakoaka@foxmail.com (G. Yang), xiongzy2005@163.com (Z. Xiong).

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1. Introduction

Heart failure (HF) is a syndrome characterized by cardiac dysfunction, with high morbidity and mortality rates affecting more than 37.7 million people worldwide [1,2]. Cardiac hypertrophy and myocardial fibrosis are significant pathophysiologic features of HF [3], and an imbalance in the expression of the renin-angiotensin system (RAS) is believed to be one of the primary causes [4,5]. The classical axis of the RAS, Angiotensin II (Ang II)/angiotensin type 1 receptor, is known to cause cardiac hypertrophy and myocardial fibrosis through its overactivation [6–8]. Despite the widespread use of RAS inhibitors in treating cardiovascular disease, they are not effective in reducing complications and mortality in patients with HF [9]. Therefore, developing effective therapeutic strategies for this disease remains a significant challenge.

Exosomes, extracellular vesicles with diameters ranging from 30 to 150 nm, facilitate intercellular substance exchange and information transfer [10,11]. Recent studies have demonstrated that Ang II can contribute to HF via exosomes. For instance, Ang II stimulates cardiac fibroblasts to release exosomes, leading to increased Ang II production and receptor expression in cardiomyocytes, thereby exacerbating cardiac hypertrophy [12]. Similarly, Ang II accelerates cardiac fibrosis by inducing epididymal white adipose tissue cells to secrete exosomes, facilitating the transport of miR-23a-3p to cardiac fibroblasts [13]. An *in vitro* study has shown that Ang II-treated human cardiomyocytes can promote extracellular matrix remodeling in atrial fibroblasts through exosome-induced polarization of M1 macrophages [14]. These studies indicate Ang II's role in HF progression, either by promoting local exosomal secretion within the heart or by triggering exosome release from distal organs. Yet, the mechanisms by which Ang II utilizes exosomal signaling to trigger cardiac injury in HF remain unknown.

The Sonic hedgehog (Shh) pathway is an evolutionarily conserved signaling pathway involved in organ development, maintenance of internal homeostasis, injury repair, and tissue fibrosis [15,16]. Multiple studies have revealed the profibrotic effect of Shh signaling in chronic fibrotic diseases of various organs such as lung, liver, kidney and skin [17,18]. However, Shh signaling plays a vital but controversial role in diverse cardiovascular diseases [19–21]. In previous studies, Shh has been shown to mediate cardiomyocyte proliferation and angiogenesis in ischemic cardiovascular disease [19,20,22]. Moreover, downregulating Shh signaling increased infarct size and cardiac function after myocardial infarction [23]. Whereas, another study indicated that inhibition of endogenous Shh signaling by cyclopamine in cardiac ischemia and reperfusion model mitigated ventricular dilation and improved cardiac output by reducing the fibrotic response [21]. It is well known that Ang II can induce fibroblast proliferation, myofibroblast differentiation and collagen deposition. In recent studies, the function of Ang II in fibrosis may be due to the activation of Shh pathway [24]. Typically, the dual lipid structure of Shh enables it to be encapsulated into exosomes, allowing it to exert various biological effects on local or distant cells [25,26]. However, the extent to which Shh signaling activation is involved in inducing cardiac injury in HF is yet to be fully elucidated.

We hypothesized that Ang II could accelerate HF progression by stimulating exosome secretion from cardiomyocytes, thereby facilitating Shh transport. This study aimed to elucidate the role of exosomes in Ang II-induced HF and, secondarily, to assess the potential of the exosome inhibitor dimethyl amiloride (DMA) in mitigating Ang II-induced HF. To achieve this, both *in vivo* and *in vitro* studies were conducted utilizing an Ang II-induced HF mice model. Exosomes were isolated to assess Shh expression, and the capacity of DMA to reverse these effects was also investigated. This study is significant for revealing the pathogenesis of HF and for developing novel therapeutic strategies.

2. Methods

2.1. Ethics

Male C57BL/6 mice were purchased from Gempharmatech Company (Jiangsu, China) and were housed in a standard environment with ad libitum access to food and water. All studies were approved by the Animal Ethics Committee of Peking University Shenzhen Hospital (2022-577). Animals were anesthetized with isoflurane (R510, RWD, China) and sacrificed with an overdose of sodium pentobarbital.

2.2. Animals

Eight-week-old littermate mice were randomly assigned to one of three groups: (1) the control group infused with saline, (2) the model group infused with Ang II (690 ng/min/kg, Bachem, Torrance, CA) using osmotic pumps (Alzet 1004, DURECT, CA), and (3) mice infused with Ang II and DMA (20 mg/kg, A4562, Sigma, Germany). Osmotic minipump implantation was performed as previously described [27]. Mice were euthanized four weeks after Ang II infusion.

2.3. Blood pressure

Systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) were measured using tail-cuff volume pressure recording technology (CODA 8, Kent Scientific, CT) [28,29]. Mice were placed in a tubular holder and allowed to rest at 26 °C. Blood pressure readings were taken nine times on a warming panel once the tail base temperature reached 32 °C. The measurement process was monitored and analyzed using specialized software. The average blood pressure was recorded from the repeated measurements.

2.4. Western blot

Protein expression levels were analyzed using Western blotting (WB) [27]. The primary antibodies used were as follows: β-MHC (MAB90961, R&D Systems, MN), α-Actin (KM9006, Sungene, China), Fibronectin (Ab268021, Abcam, UK), Collagen I (772026, CST, MA), α-SMA (19245, CST, MA), Shh (S8231, Sigma, Germany), Gli1 (a23236, Abclonal, China), CD63 (Ab118307, Abcam, UK), TSG101 (Ab125011, Abcam, UK), c-Myc (ac1309, Abclonal, China), PCNA (a12427, Abclonal, China).

2.5. Histology

Paraffin-embedded heart sections were prepared, and subjected to Hematoxylin-Eosin and Masson trichrome staining, as well as immunohistochemical staining. The antibodies used were as follows: Collagen I (772026, CST, MA), α-Actin (KM9006, Sungene, China), and Shh (S8231, Sigma, Germany).

2.6. Cells

Primary neonatal rat ventricular cardiomyocytes (NRVCs) and cardiac fibroblasts were isolated and cultured [30]. The primary cells were cultured in DMEM/F12 (C11330500BT, Gibco, USA) medium supplemented with 10 % fetal bovine serum under standard conditions. Cardiomyocytes were treated with Ang II (1 μM) for 6 h, followed by incubation in serum-free medium. In some experiments, cardiomyocytes were pretreated with 50 μM DMA. Cardiac fibroblasts were incubated with cardiomyocyte-conditioned media or exosomes (Ctrl-Exo or Ang II-Exo, 20 μg protein/ml) in the presence or absence of cyclopamine (CPN) (10 μM, 4449-51-8, Chembest, China). The specific experimental steps are described in the Results section.

2.7. Cell proliferation

The assessment of cell proliferation ability was conducted using the 5-ethynyl-2′-deoxyuridine (EdU) assay. Briefly, cells were inoculated on coverslips of 12-well plates and then incubated with EdU working solution for 2 h. Subsequent fixation and staining were performed according to the instructions (C0078S, Beyotime, China), and the cells were photographed by fluorescent microscope.

2.8. Exosomes

Exosomes were isolated from the conditioned media collected from the above-mentioned groups using differential centrifugation. Specifically, the cellular supernatant was initially centrifuged at 300 g for 5 min, 2000 g for 20 min, and 10000 g for 30 min to remove cell debris. The supernatant was ultra-centrifuged at 110,000 g for 90 min. All the aforementioned procedures were conducted at 4 °C. The exosome pellets were then resuspended and quantified. For transmission electron microscopy (TEM), the exosomes extracted from conditioned media were examined according to established procedures [31]. Nanoparticle tracking analysis (NTA) was performed by Lemond Bio, China.

2.9. Fluorescent labeling

The cardiomyocytes were labeled with the cell tracker Dio (MB4239, Meilunbio, China), a fluorescent carbocyanine membrane dye, for 1 h and then washed thrice with phosphate buffered saline (PBS). Exosomes were obtained from the conditioned media of Dio-labeled cardiomyocytes and incubated with cardiac fibroblasts for 24 h, followed by detection via immunofluorescence.

2.10. Immunofluorescence

Primary cardiomyocytes and cardiac fibroblasts cultured on coverslips were fixed with 4 % paraformaldehyde for 15 min at room temperature. This was followed by permeabilization using 0.5 % Triton X-100 for 10 min, and then blocking with 10 % donkey serum in PBS for 30 min. Subsequently, the slides were incubated with different primary antibodies against the following antigens: β-MHC (MAB90961, R&D Systems, MN), Fibronectin (Ab268021, Abcam, UK), Shh (S8231, Sigma, Germany), and CD63 (Ab118307, Abcam, UK). After washing, the slides were incubated with cyanine dye-2 or cyanine dye-3–conjugated secondary antibodies (711165152, Jackson ImmunoResearch, USA). Nuclei were visualized by staining the cells with DAPI (S2110, Solarbio, China) according to the manufacturer's instructions. Imaging was performed using a Leica TCS SP2 AOBS confocal microscope (Leica Microsystems, Buffalo Grove, IL) or with an Olympus DP80 microscope equipped with an EMCCD camera (Olympus, Tokyo, Japan).

2.11. Statistics

The data are represented as the mean ± SEM. Statistical analyses were performed with SPSS version 25.0, using one-way ANOVA for group comparisons, followed by either the Student-Newman-Kuels test or Dunnett's T3 procedure. A p-value ≤ 0.05 was considered to indicate statistical significance.

3. Results

3.1. DMA mitigates Ang II-induced cardiac injury and hypertension

The initial investigation focused on the effectiveness of DMA in ameliorating Ang II-induced cardiac injury. The present study found a notable increase in the heart weight to body weight ratio (HW/BW) in the Ang II group compared to that in the control group, which significantly decreased after DMA administration (Fig. 1A). Additionally, the impact of DMA on modulating Ang II-induced hypertension was assessed. The findings indicated that Ang II elevated SBP, DBP, and MAP, while DMA notably lowered these parameters (Fig. 1B). To elucidate the underlying mechanism, relevant protein expression was assessed through WB. Ang II notably upregulated the expression of the hypertrophic biomarkers β-Myosin Heavy Chain (β-MHC) and α-skeletal muscle actin (α-Actin) and the matrix proteins as Fibronectin, Collagen I, and α-SMA; however, DMA reversed these changes (Fig. 1C–F). Similar results were observed for tissue staining (Fig. 1G–J). These results suggested that DMA could mitigate Ang II-induced cardiac injury.

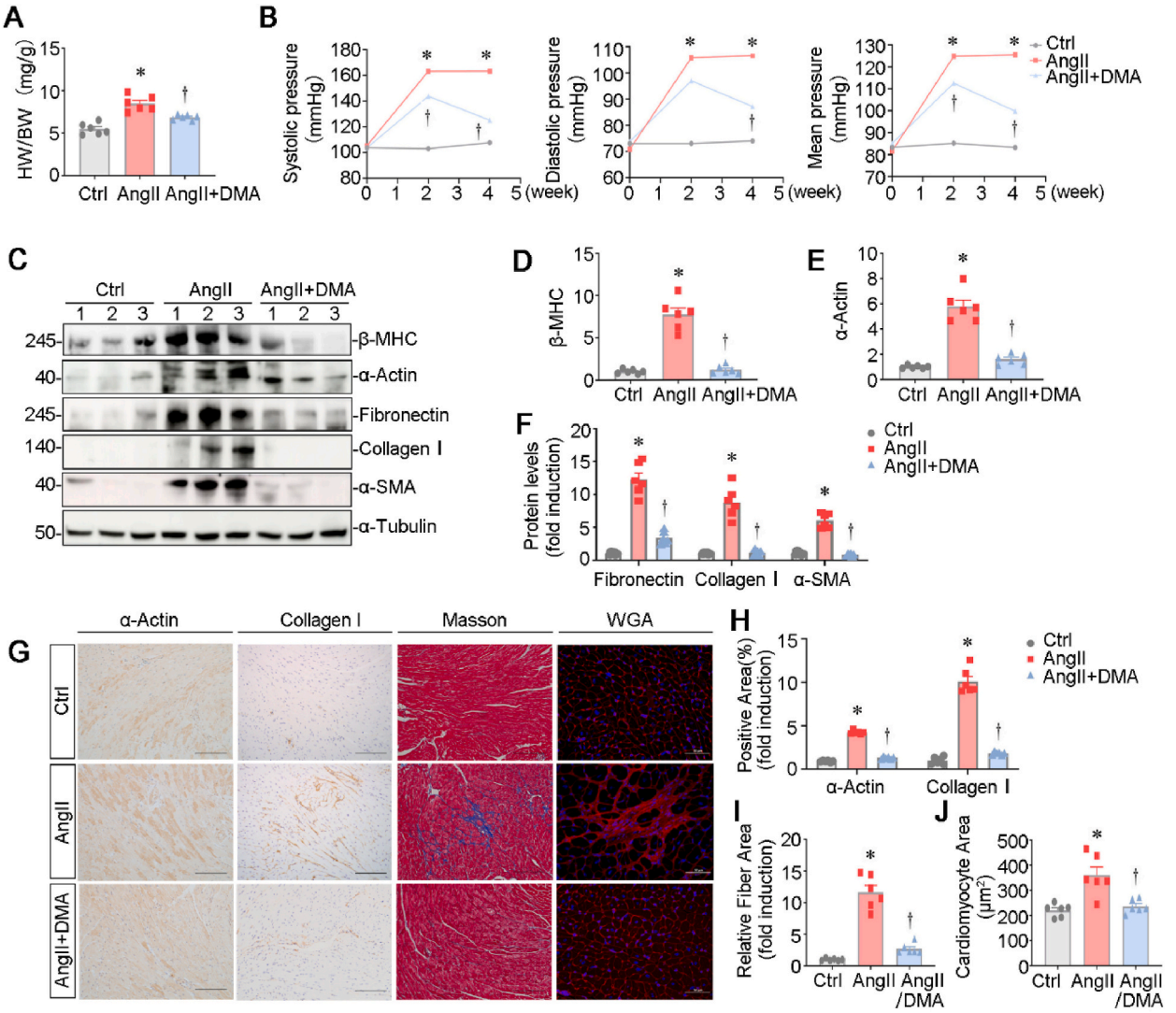

Fig. 1. Inhibition of exosome release attenuates cardiac hypertrophy and fibrosis in Ang II-treated mice. (A) Quantitative data on the heart weight-to-body weight ratio (HW/BW) in the three groups of mice. (B) Graphical representation of the SBP, the DBP and the MAP of mice in different groups over four weeks. Data are presented as mean ± SEM (n = 6). *$p \leq 0.05$ vs control; †$p \leq 0.05$ vs Ang II. (C) Western blot analyses showing that DMA alleviates cardiac hypertrophy and fibrosis in Ang II-infused mice. Representative Western blot (C) and quantitative data for β-MHC (D), α-Actin (E), Fibronectin, Collagen I, α-SMA (F) are presented. *$p \leq 0.05$ vs control; †$p \leq 0.05$ vs Ang II (n = 6). (G) Representative micrographs showing cardiac hypertrophy and matrix deposition in Ang II-infused mice after DMA treatment. Scale bar, 50 μm. (H) Quantitative data on α-Actin, Collagen I (H), Masson (I) and WGA staining (J) in the heart tissue of the three groups of mice are presented. *$p \leq 0.05$ vs control; †$p \leq 0.05$ vs Ang II (n = 6).

3.2. DMA mitigates Ang II-induced cardiac injury associated with the inhibition of exosome release and the upregulation of Shh

To investigate whether the therapeutic effect of DMA is related to exosomes and Shh, relevant indicators were tested. Compared to the control group, two widely accepted exosome markers, CD63 and TSG101, were significantly upregulated, including the expression levels of Shh and its downstream protein Gli1 (Fig. 2A–F). However, DMA reversed these results again. Furthermore, immunohistochemical staining demonstrated an increased Shh expression in hypertrophic cardiomyocytes induced by Ang II (Fig. 2G). These findings indicate a correlation between Ang II-induced cardiac injury and increased exosome release and Shh upregulation.

3.3. Medium from Ang II-treated cardiomyocytes induces hypertrophy of other cardiomyocytes and activation of cardiac fibroblasts in vitro

Injured cells can influence neighboring or distal cells through a paracrine mechanism. To explore the link between Ang II-induced cardiomyocyte injury and this mechanism, pertinent *in vitro* experiments were conducted (Fig. 3A). The NRVCs were exposed to Ang II for 6 h, after which the medium was replaced with serum-free medium and the culture was continued for 24 h. The resulting medium was then isolated and utilized to culture cardiomyocytes and cardiac fibroblasts separately. The results demonstrated that this medium upregulated the expression of the hypertrophic proteins β-MHC and α-Actin in normal cardiomyocytes (Fig. 3B–D). Similarly, this medium upregulated the expression of fibrotic proteins such as Fibronectin and α-SMA in cardiac fibroblasts (Fig. 3E–G). This finding suggested that injured cardiomyocytes can cause other cellular injuries via exocytosis factors.

3.4. Exosomes derived from injured cardiomyocytes induce hypertrophy of other cardiomyocytes and activation of cardiac fibroblasts in vitro

To elucidate the role of exosomes in this process, exosomes isolated from the aforementioned media were used to treat other cardiomyocytes or cardiac fibroblasts, respectively (Fig. 4A). The morphological characteristics, particle size, and concentration of these exosomes were determined using TEM and NTA assays (Fig. 4B–D). Subsequently, the effects of these exosomes on normal cells were tested. The results demonstrated that these exosomes upregulated the expression of the hypertrophic proteins β-MHC and α-Actin in normal cardiomyocytes (Fig. 4E–H). Similarly, these exosomes upregulated the expression of fibrotic proteins such as Fibronectin and α-SMA in cardiac fibroblasts (Fig. 4I–L). Furthermore, exosomes derived from cardiomyocytes were labeled with the fluorescent carbocyanine membrane dye Dio to track their uptake. The findings revealed that Dio-labeled exosomes were internalized by other cardiac fibroblasts (Fig. 4M). Consequently, exosomes derived from injured cardiomyocytes can be internalized by other cardiomyocytes and cardiac fibroblasts, activating injury-related signaling pathways.

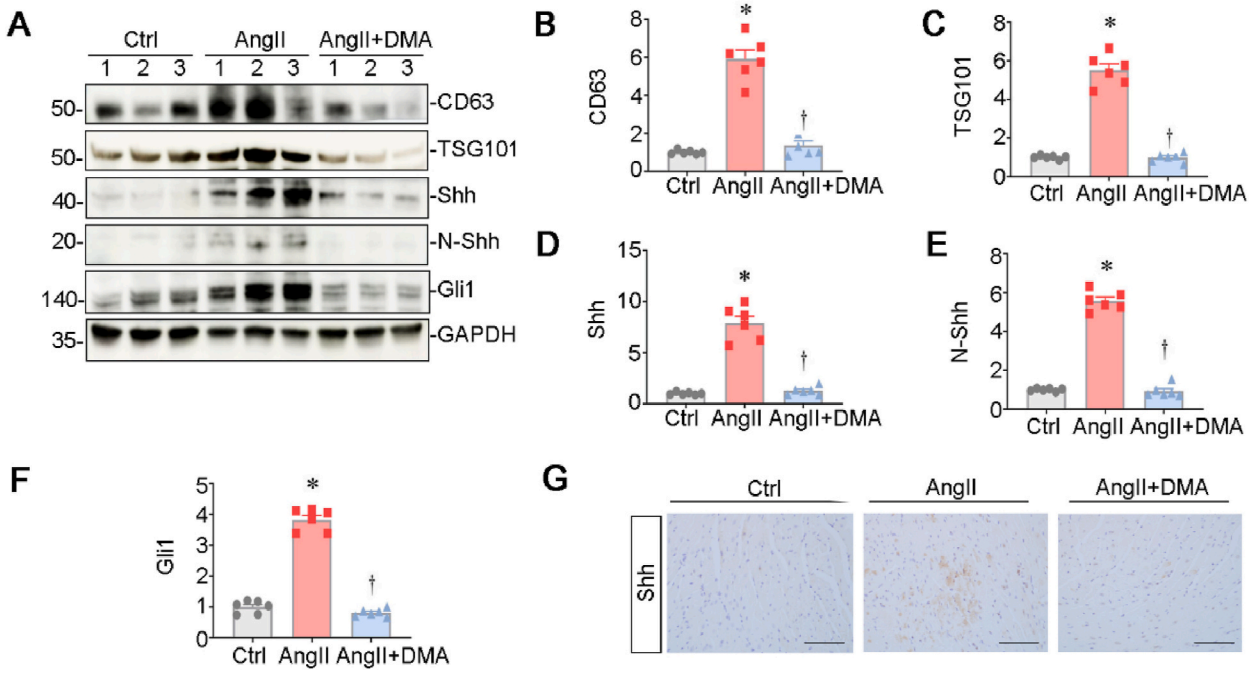

Fig. 2. DMA alleviates Ang II-induced cardiac lesions associated with inhibition of exosome secretion and Shh upregulation. (A) Western blot analyses demonstrating that DMA inhibits the exosome secretion and activation of Shh signaling after Ang II infusion. Representative Western blot (A) and quantitative data on CD63 (B), TSG101 (C), Shh (D), N-Shh (E), Gli1 (F) are presented. *$p \leq 0.05$ vs control; †$p \leq 0.05$ vs Ang II (n = 6). (G) Representative micrographs of immunostaining demonstrating the upregulation of Shh in the heart tissue of the Ang II infusion model. Scale bar, 50 μm.

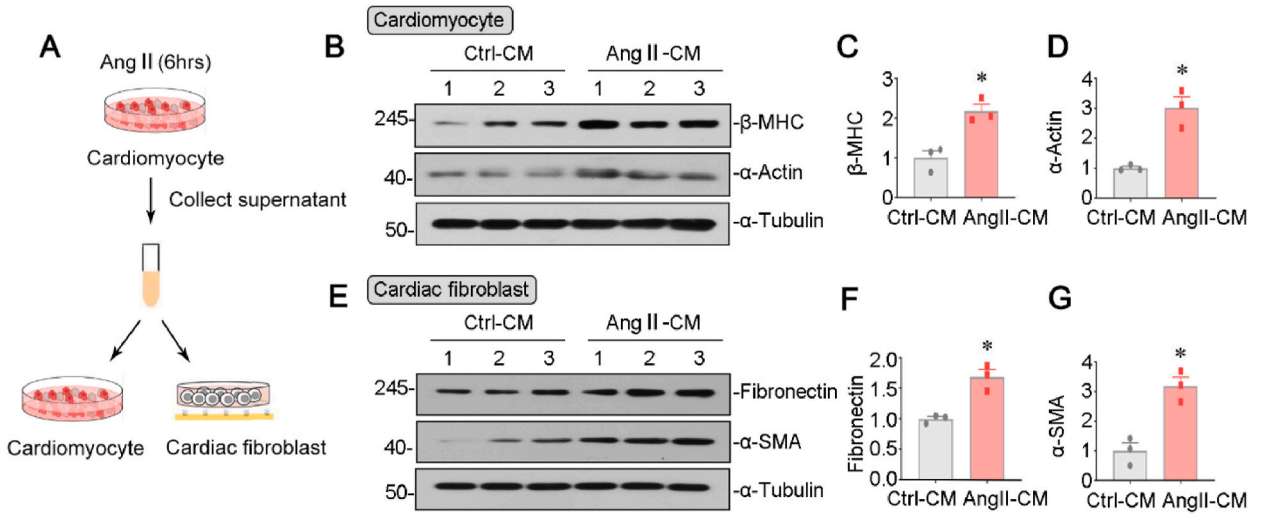

Fig. 3. Conditioned media from Ang II-treated cardiomyocytes promotes cardiomyocyte hypertrophy and cardiac fibroblast activation. (A) Experimental design. (B) Western blot analyses showing that protein levels of β-MHC and α-Actin in cardiomyocytes after incubation with conditioned media collected from Ang II-induced cardiomyocytes. (C–D) Quantitative data on β-MHC and α-Actin expression are shown for the indicated groups (n = 3). *$p \leq 0.05$. (E) Western blot analyses showing Fibronectin and α-SMA expression in cardiac fibroblasts incubated with conditioned media. (F–G) Quantitative data showing the protein levels of Fibronectin and α-SMA in the different groups (n = 3). *$p \leq 0.05$.

3.5. Inhibition of exosome signaling mitigates Ang II-induced cardiac fibroblast injury

To elucidate the relationship between exosome signaling upregulation and cardiac fibroblast injury, DMA was used to inhibit exosome secretion *in vitro*. Based on the results of the cell culture experiments described above, DMA was added or not added at the onset of cell culture (Fig. 5A). As expected, DMA significantly inhibited cardiac fibroblasts activation and proliferation, as evidenced by the expression of the fibrotic proteins Collagen I and Fibronectin, and the proliferative proteins c-Myc and PCNA (Fig. 5B–F). These data demonstrated that cardiomyocyte-derived exosomes are pivotal for mediating the activation and proliferation of cardiac fibroblasts.

3.6. Shh signaling packaged in cardiomyocyte-derived exosomes promotes cardiac fibroblast activation

Subsequently, the contribution of Shh signaling to this process was investigated. Western blot analysis of the exosomes showed that Ang II increased the secretion of exosomes (TSG101) and the expression of Shh signaling components (Shh, N-Shh, and Gli1), suggesting that Shh signaling was intact in these exosomes (Fig. 6A). Immunostaining corroborated similar results, showing induced expression of both Shh and CD63 in hypertrophied cardiomyocytes (Fig. 6B). To further investigate the role of Shh from exosomes in mediating fibroblast activation and proliferation, Shh signaling was blocked using CPN, a small-molecule inhibitor of the Shh pathway [32]. Exosomes were isolated from Ang II-induced cardiomyocytes and cultured into fibroblasts with or without CPN (Fig. 6C). As shown in Fig. 6D–H, inhibition of Shh signaling also blocked cardiac fibroblast activation and proliferation. Thus, Shh/N-Shh/Gli1 can be packaged in cardiomyocyte-derived exosomes and facilitate cardiac fibroblast activation.

4. Discussion

HF remains a significant public health concern, and its underlying injury mechanisms have not yet been fully elucidated. This study revealed that Ang II infusion induces myocardial hypertrophy and fibrosis, a process that is intricately linked to exosomes in impaired cardiomyocytes. Inhibition of exosomes appeared to alleviate Ang II-induced myocardial injury. Furthermore, Shh signaling was identified as a key factor in mediating cardiac fibroblast activation and matrix production. These findings contribute to our understanding of Ang II-induced cardiac injuries and could provide evidence for the development of novel therapeutic strategies for HF.

The principal finding of this study was that injured cardiomyocytes induce peripheral cell injury by delivering exosomes containing Shh/N-Shh/Gli1 which may not exclude other contents as miRNA, thereby accelerating cardiac hypertrophy and fibrosis. Generally, Shh is involved in regulating cell development and also promotes tissue regeneration and repair [33]. For instance, Shh mitigates intervertebral disc degeneration by suppressing oxidative stress and cellular senescence in the nucleus pulposus [34]. Additionally, Shh enhances neural repair after ischemic stroke [35], and it also facilitates the healing of pressure ulcers by activating the angiogenic properties of endothelial progenitor cells through the PI3K/AKT/eNOS pathway [36]. Similarly, studies focusing on the heart have indicated that upregulation of Shh promotes neovascularization, cell development, and proliferation [37–39]. Especially in myocardial infarction and ischemia and reperfusion injury, Shh exhibits the proangiogenic effect in both *vitro* and *vivo* model [40,41]. Collectively,

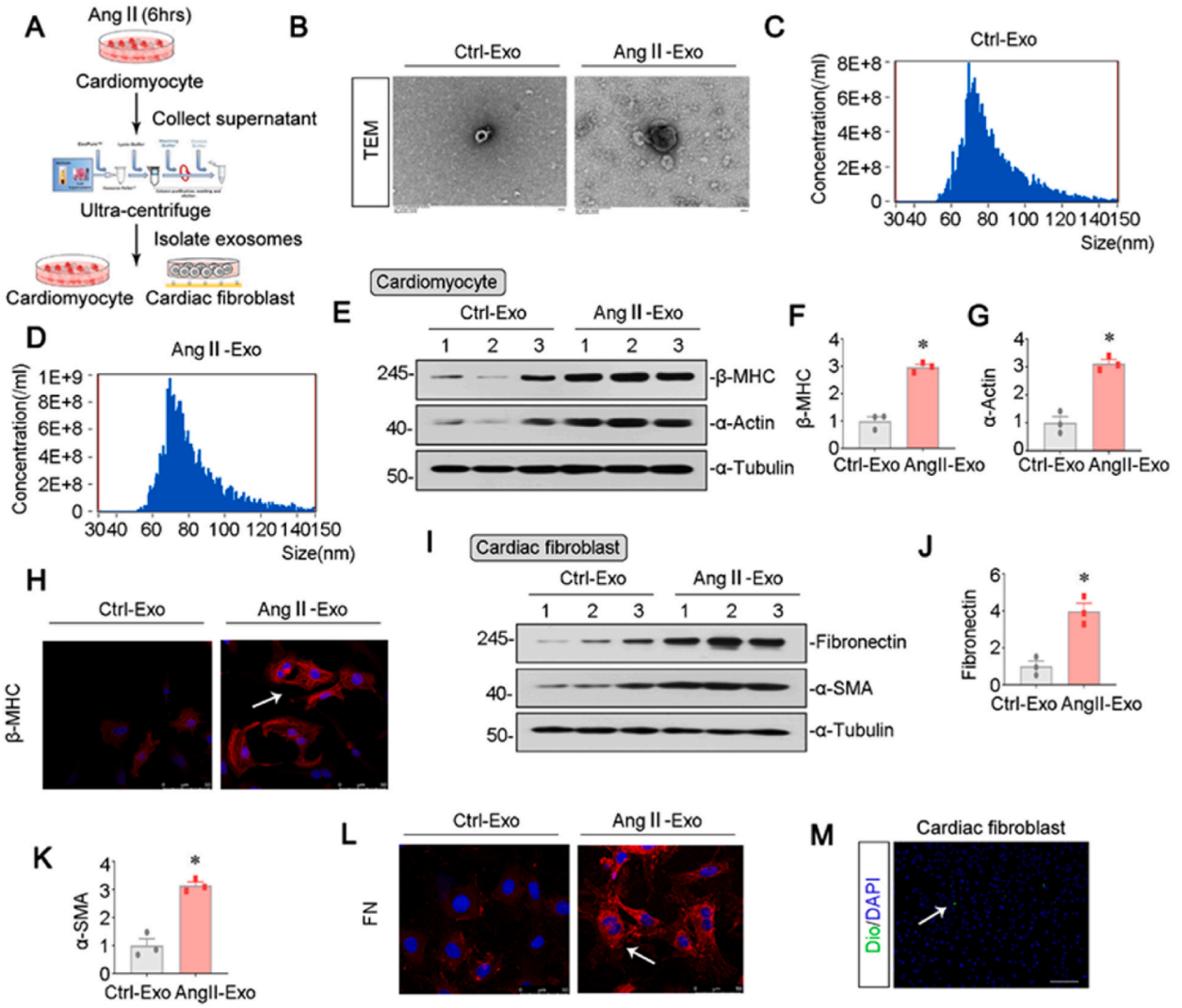

Fig. 4. Cardiomyocyte-derived exosomes induce the hypertrophy of cardiomyocytes and activation of cardiac fibroblasts. (A) We further treated cardiomyocytes and cardiac fibroblasts with exosomes isolated from the above-mentioned conditioned media for 24 h. (B) Transmission electron microscopy (TEM) image showing the exosomes isolated from the conditioned media of primary cardiomyocytes. Scale bar, 200 nm. (C–D) Graphical data showing the particle size and concentration of cardiomyocyte-derived exosomes in the different groups. Representative Western blot (E) and quantitative data on the protein levels of β-MHC (F) and α-Actin (G) are shown in different groups as indicated (n = 3). $^*p \leq 0.05$. (H) Micrographs confirming the immunofluorescence staining of β-MHC in cardiomyocytes treated with exosomes isolated from Ang II-induced cardiomyocytes. Arrows indicate positive staining. Scale bar, 50 μm. (I) Western blot analyses show Fibronectin and α-SMA expression in cardiac fibroblasts treated with exosomes separated from conditioned media. (J–K) Quantitative data on protein expression of Fibronectin and α-SMA are shown in different groups as indicated (n = 3). $^*p \leq 0.05$. (L) Representative images show the immunofluorescence staining of Fibronectin in cardiac fibroblasts incubated with exosomes isolated from Ang II-induced cardiomyocytes. Arrows indicate positive staining. Scale bar, 50 μm. (M) Fluorescent staining shows the intracellular transfer of cardiomyocyte-derived exosomes in cardiac fibroblasts. Cardiac fibroblasts were incubated with Dio (green), after which exosomes were isolated. Cardiomyocyte-derived exosomes were incubated with cardiac fibroblasts for 24 h. Arrows indicate cardiomyocyte-derived exosomes. Scale bar, 100 μm.

these studies suggest that Shh plays a role in tissue repair by regulating cell development and proliferation. In the Ang II-induced hypertension model, the activation of Shh signaling accelerated proliferation and reduced apoptosis [42]. Besides, upregulated Gli, which is a downstream target gene of Shh signaling, may have exacerbated the pathological conditions of hypertension by promoting excessive proliferation of vascular smooth muscle cells [42]. Interestingly, the Shh pathway was also upregulated in silicotic fibrosis, which promoted Ang II-induced myofibroblasts differentiation [24]. It is understandable that Ang II leads to the autocrine of transforming growth factor-β1, which can activate the Shh signaling, and then promotes fibroblast proliferation and collagen deposition [43]. Therefore, Shh acts as a profibrotic factor in Ang II-induced chronic fibrotic diseases.

However, the present study revealed that Shh overexpression leads to cardiac hypertrophy and fibrosis, which are indicative of

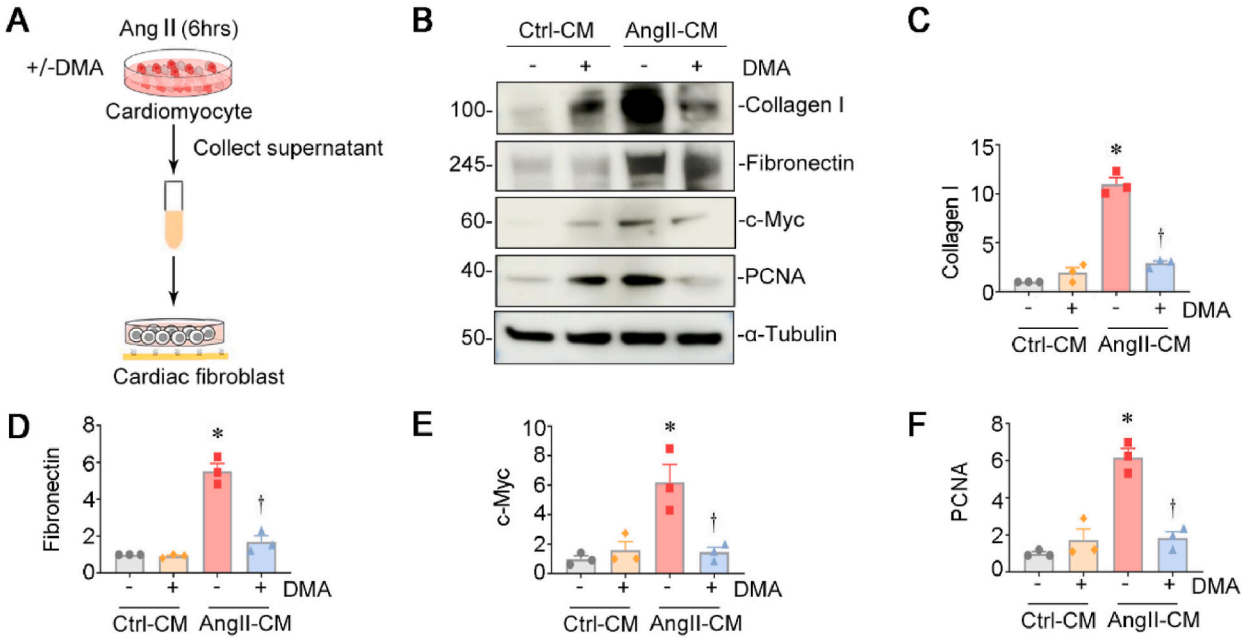

Fig. 5. Blocking exosome secretion mitigates cardiac fibroblast activation and proliferation *in vitro*. (A) A schematic diagram outlines the experimental design. We employed conditioned media obtained from Ang II-induced primary cardiomyocytes with or without DMA to treat cardiac fibroblasts for 24 h. (B) Western blot analysis shows the expression of fibrosis-related proteins and proliferation markers in cardiac fibroblasts after incubation with conditioned media collected from Ang II-induced cardiomyocytes with or without DMA treatment. (C–F) Quantitative data showing Collagen I (C), Fibronectin (D), c-Myc (E) and PCNA (F) expression in the indicated groups. *$p \leq 0.05$ vs sham control CM; †$p \leq 0.05$ vs Ang II CM (n = 3).

cardiac injury. This could represent a compensatory response to reduced cardiac function. It has been proposed that tissue regeneration ability diminishes with age, and therefore tissue repair typically replaces tissue regeneration after injury in adult individuals [44]. Consequently, the cardiac hypertrophy and fibrosis induced by Shh observed in this study might be reparative mechanisms for a heart with reduced function, aimed at preserving its functionality and structure. Alternatively, Shh signaling looks like to reveal dual function in heart disease. Short-term Shh upregulation post-injury may facilitate tissue repair, while long-term upregulation may increase the likelihood of tissue hypertrophy and fibrosis.

This study has several limitations. First, DMA only partially mitigated Ang II-induced cardiac injury and hypertension, indicating the importance of other unexplored mechanisms. Second, this study used a male model, which is associated with a greater risk of cardiovascular disease. Future studies should include female mice. Third, this study explored only the Shh pathway in exosomes and has not yet investigated other potential substrates like proteins, nucleic acids, and metabolites. Fourth, no animal studies of Shh inhibitors have been conducted. Fifth, the effects of exosomes from injured cardiomyocytes on distal organs remained unexplored. These areas warrant further investigations.

5. Conclusions

In conclusion, this study demonstrated that in Ang II-induced HF models, cardiomyocyte-derived exosomes promote cardiac hypertrophy and fibrosis through Shh signaling-mediated intercellular crosstalk. This study sheds light on a novel mechanism of intercellular communication in cardiovascular disease and provides substantial evidence for the development of new therapeutic approaches for HF.

CRediT authorship contribution statement

Cong Wang: Writing – original draft, Methodology, Funding acquisition, Formal analysis, Data curation. **Zhiwei Lai:** Methodology, Formal analysis, Data curation. **Huishi Tan:** Methodology, Formal analysis, Data curation. **Hua Zhang:** Methodology, Data curation. **Lishan Tan:** Funding acquisition, Formal analysis. **Qingyun Luo:** Methodology, Data curation. **Sanmu Li:** Software, Methodology. **Zibo Xiong:** Supervision, Project administration, Investigation. **Guang Yang:** Writing – review & editing, Project administration, Investigation, Funding acquisition. **Zuying Xiong:** Visualization, Supervision, Project administration, Investigation.

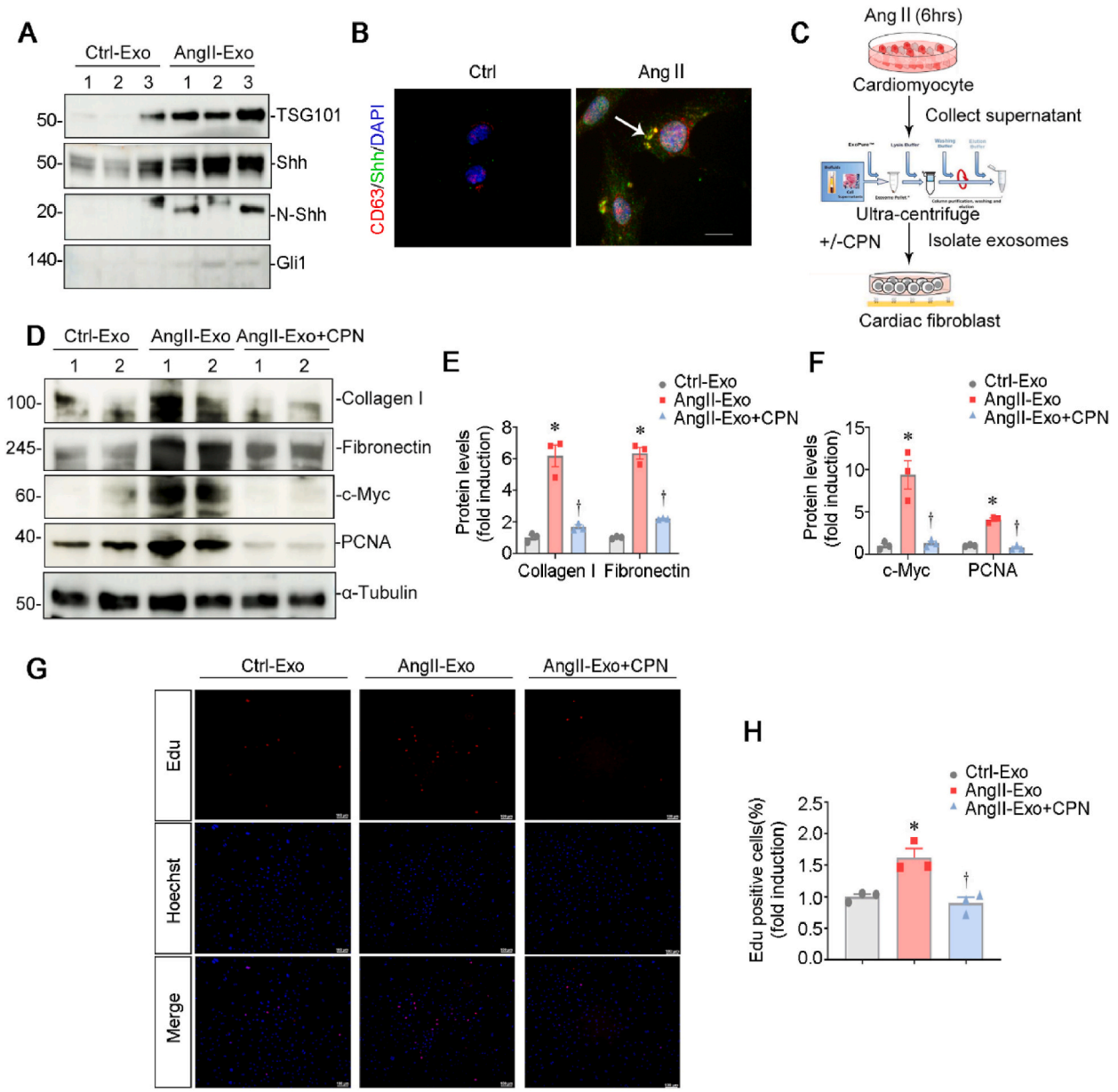

Fig. 6. Exosomal Shh promotes cardiac fibroblast activation and proliferation *in vitro*. (A) Western blot analysis confirmed the presence of TSG101 and Shh signaling proteins in exosomes isolated from Ang II-induced cardiomyocytes. (B) Double immunofluorescence staining showing the colocalization of Shh and CD63 in Ang II-induced cardiomyocytes. Arrows indicate positive staining. Scale bar, 25 μm. (C) A schematic diagram outlines the experimental design. We treated cardiac fibroblasts with exosomes isolated from conditioned media for 24 h with or without CPN. (D) Western blot analysis illustrates the expression of fibrotic and proliferative proteins in cardiac fibroblasts incubated with cardiomyocyte-derived exosomes. (E–F) Quantitative data showing the protein expression of Collagen I, Fibronectin, c-Myc, and PCNA in the different groups are shown. *$p \leq 0.05$ vs sham control Exo; †$p \leq 0.05$ vs Ang II Exo (n = 3). Representative images (G) and quantitative data (H) show the Edu staining in the groups mentioned above. Scale bar, 100 μm *$p \leq 0.05$ vs sham control Exo; †$p \leq 0.05$ vs Ang II Exo (n = 3).

Data availability

The data supporting this study are available upon reasonable request.

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Declaration of competing interest

The authors 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e39332.

Abbreviations

Ang II	angiotensin II
CPN	Cyclopamine
DBP	the diastolic blood pressure
DMA	dimethyl amiloride
MAP	mean arterial pressure
NRVCs	neonatal rat ventricular cardiomyocytes
NTA	nanoparticle tracking analysis
RAS	the renin-angiotensin system
Shh	Sonic hedgehog
SBP	the systolic blood pressure
TEM	transmission electron microscopy
α-Actin	α-skeletal muscle actin
β-MHC	β-Myosin Heavy Chain

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