Original Article

Down-regulation of JCAD Expression Attenuates Cardiomyocyte Injury by Regulating the Wnt/β-Catenin Pathway

(JCAD / coronary heart disease / Wnt/β-catenin signalling pathway / inflammation / vascular endothelial function)

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Abstract. Coronary heart disease (CHD) is one of the most commonly seen cardiovascular conditions across the globe. Junctional cadherin 5 associated (JCAD) protein is found in the intercellular junctions of endothelial cells and linked to cardiovascular diseases. Nonetheless, the influence of JCAD on cardiomyocyte injury caused by CHD is unclear. A model of H_2O_2-induced H9c2 cell injury was constructed, and JCAD mRNA and protein levels were assessed by qRT-PCR and Western blot. The impacts of JCAD on the proliferation or apoptosis of H9c2 cells were explored by CCK-8 assay, Western blot and TUNEL staining. The effect of JCAD on the inflammatory response and vascular endothelial function of H9c2 cells was detected using ELISA kits. The levels of Wnt/β-catenin pathway-related proteins were assessed by Western blot. H_2O_2 treatment led to a rise in the levels of JCAD in H9c2 cells. Over-expression of JCAD promoted H_2O_2-induced cellular injury, leading to notably elevated contents of inflammatory factors, along with vascular endothelial dysfunction. In contrast to over-expression of JCAD, silencing of JCAD attenuated H_2O_2-induced cellular injury and inhibited apoptosis, inflammatory response and vascular endothelial dysfunction. Notably, JCAD could regulate the Wnt/β-catenin pathway, while DKK-1, Wnt/β-catenin pathway antagonist, counteracted the enhancing impact of JCAD over-expression on H_2O_2-induced H9c2 cell injury, further confirming that JCAD acts by regulating the Wnt/β-catenin pathway. In summary, over-expression of JCAD promoted H_2O_2-induced H9c2 cell injury by activating the Wnt/β-catenin pathway, while silencing of JCAD attenuated the H_2O_2-induced cell injury.

Introduction

Coronary heart disease (CHD) is a heart condition caused by atherosclerosis of the coronary arteries and is a prevalent cardiovascular condition globally; the year-on-year increase in prevalence and death rates has become a serious threat to human health (Bae et al., 2021; Shaya et al., 2022). According to statistics, an estimated 8.88 million people lost their lives to CHD in 2019 globally (Xia et al., 2021). The progression of CHD is a long-term process, which is affected by a variety of risk factors, including obesity, hypertension, diabetes, physical inactivity, smoking and diet (Katta et al., 2021; Alpert, 2023). The main pathological feature of CHD is the spasm or atherosclerosis of coronary arteries, resulting in the narrowing or blockage of blood vessels and ultimately causing damage to cardiomyocytes due to ischaemia, hypoxia, or necrosis, and pathological changes in cardiac function or structure (Dong et al., 2019; Duggan et al., 2022). In recent years, studies have shown that in addition to direct cytotoxicity, oxidative stress can also promote the progression of CHD by regulating related signal transduction, leading to the formation of atherosclerotic plaques (Zhou et al., 2022; Yao et al., 2023). In addition, it is one of the main forms of early cardiomyocyte death in CHD (Dong et al., 2019). Thus, inhibition of oxidative stress and apoptosis may be a promising approach to the treatment of CHD. Currently, treatments for CHD include revascularization, medica-

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Abbreviations: ANOVA – analysis of variance, ATCC – American Type Culture Collection, CCK-8 – cell counting kit-8, CHD – coronary heart disease, DKK-1 – dickkopf 1, ET-1 – endothelin 1, GSK-3β – glycogen synthase kinase 3β, IL-1β – interleukin 1β, IL-6 – interleukin 6, JCAD – junctional cadherin 5 associated, NO – nitric oxide, sh-JCAD – JCAD short hairpin RNA, TNF-α – tumour necrosis factor α.

tion and exercise, but patient prognosis is still unsatisfactory (Tao et al., 2023; Yazdani et al., 2023). Therefore, we need new markers to provide more effective interventions to CHD patients.

Junctional cadherin 5 associated (JCAD) protein is an endothelial intercellular junction protein associated with coronary artery disease (Williams et al., 2019; Shigeoka et al., 2020). Recent global genomic studies have revealed a correlation between certain genetic variations near JCAD and cardiovascular disorders such as atherosclerosis and hypertension (Nikpay et al., 2015; Radhika et al., 2023). Xu et al. (2019) found that JCAD deficiency improved endothelium-dependent vasodilatory function and vascular inflammation and inhibited atherosclerosis in mice (Xu et al., 2019). Furthermore, it has been shown that JCAD promoted atherosclerotic thrombosis by modulating the PI3K/Akt pathway (Liberale et al., 2023). A review reported that JCAD may participate in atherosclerosis by regulating vascular inflammation and may affect thrombosis (Guzik et al., 2023). However, the influence of JCAD on CHD-induced cardiomyocyte injury is unclear.

The Wnt/β-catenin pathway is a well-known component of Wnt signalling that is essential for regulating cell proliferation, differentiation, and migration and is linked to a range of diseases, such as tumours, heart failure, atherosclerosis and CHD (Liu et al., 2020; Weerackoon et al., 2021). H_2O_2 can cause irreversible cell death through lipid peroxidation, DNA damage and changes in protein structure and function (Chen et al., 2023). It is often used to induce oxidative stress injury models in cardiomyocytes (Sun et al., 2021; Zhao et al., 2021; Tong et al., 2022). Therefore, in the first step in our study, we constructed a H_2O_2-induced rat cardiomyocyte (H9c2) injury model, detected the level of JCAD expression, and then explored the effects of over-expression or silencing of JCAD on the proliferation, apoptosis, inflammatory response and vascular endothelial function of H9c2 cells. Finally, we investigated the connection between JCAD and H9c2 cell injury caused by H_2O_2 through the Wnt/β-catenin pathway. The objective of this research was to elucidate the mechanism by which JCAD influences cardiomyocyte injury in CHD and to offer potential ideas for the clinical treatment of CHD.

Material and Methods

Cell culture and CHD model construction

H9c2 cells were obtained from the American Type Culture Collection (ATCC). DMEM (Gibco, Grand Island, NY) enriched with 10 % foetal bovine serum (Gibco) and 1 % penicillin/streptomycin double antibody (Gibco) served as the cell culture medium, and cells were cultured at 37 °C with 5 % CO_2. The cells were subcultured every three days and refreshed with new culture medium every other day. A cell injury model was constructed by exposing H9c2 cells to 200 μM H_2O_2 (Sigma-Aldrich, St. Louis, MO) for 12 h (Li et al., 2022).

Cell transfection and treatment

JCAD over-expression plasmid (JCAD), JCAD short hairpin RNA (sh-JCAD), or negative controls (Vector and sh-NC) were produced by Sangon Biotech (Shanghai, China), and the plasmids were transfected into H9c2 cells by utilizing Lipofectamine 3000 (Invitrogen, Carlsbad, CA). Forty-eight h after transfection, JCAD expression was detected by qRT-PCR. For the H_2O_2+JCAD+DKK-1 group, cells were treated with 200 μM H_2O_2 for 12 h and then treated with Dickkopf 1 (DKK-1, 100 ng/ml, HY-P72969, MedChemExpress, Monmouth Junction, NJ) for 2 h before transfection.

qRT-PCR

The Trizol reagent (Invitrogen) was utilized to conduct the RNA extraction. After that, cDNA was obtained by reverse transcription with the inclusion of AMV reverse transcriptase (Sigma-Aldrich). Then, the SYBR Green qPCR Mix kit (TAKARA, Tokyo, Japan) was applied for PCR amplification.

These were the primer sequences:
JCAD: F: 5'-GAGTAGTAGCAGGCGGGGT-3',
R: 5'-CTGTCCTACTGTGGGCATGG-3'.
GAPDH: F: 5'-GCGAGATCCCGCTAACATCA-3',
R: 5'-ATTCGAGAGAAGGGAGGGCT-3'.

Western blot

We used the RIPA lysate (Solarbio, Beijing, China) to lyse H9c2 cells and to obtain proteins, then the BCA kit (P0012, Beyotime, Shanghai, China) to determine the protein concentrations. After performing gel electrophoresis, the samples were moved to PVDF membranes (Invitrogen) and sealed for 1 h. After rinsing, the membrane was placed in a temperature of 4 °C for an overnight incubation with JCAD primary antibody (PA5-53677, 1 : 1000, Invitrogen), cleaved-caspase 3 primary antibody (PA5-114687, 1 : 500, Invitrogen), Bax primary antibody (MA5-14003, 1 : 100, Invitrogen), Bcl-2 primary antibody (MA5-11757, 1 : 50, Invitrogen), glycogen synthase kinase-3β (GSK-3β) monoclonal antibody (39-9500, 1 : 250, Invitrogen), p-GSK-3β monoclonal antibody (44-604G, 1 : 1000, Invitrogen) or β-catenin primary antibody (13-8400, 1 : 500, Invitrogen). On the following day, after being rinsed thrice, the membrane was cultured with goat anti-rabbit secondary antibody IgG (31460, 1 : 10,000, Invitrogen) for 1.5-h. Bands were developed by an ultra high sensitivity ECL kit (HY-K1005, MedChemExpress).

GAPDH (MA1-16757, 1 : 1000, Invitrogen) served as the internal reference, and the grayscale value of each protein band was obtained after image processing with Image J software.

CCK-8 assay

H9c2 cells were inoculated into a 96-well cell culture plate (1.5×10^4 cells/well), and after the cells adhered to the wall, the previous medium was taken out and replaced with 100 μl of complete medium mixed with

10 % CCK-8 reagent (Beyotime) in each well, then incubated for 2 h at 37 °C. The OD_{450} value of the cells was assessed utilizing a microplate reader (Thermo Fisher Scientific, Waltham, MA).

TUNEL staining

The apoptosis of H9c2 cells was identified by the TUNEL Apoptosis Detection Kit (C1091, Beyotime). H9c2 cells were exposed to 4 % paraformaldehyde (Solarbio) for 15 min and hydrated using a series of ethanol concentrations (100 %, 95 %, 75 % and 50 %) for 5 min each, and then rinsed twice with PBS. Then, 0.3 % Triton X-100 (Beyotime) was added dropwise, incubated for 5 min and rinsed two times with PBS. Afterward, the TUNEL working solution was incorporated and incubated at a temperature of 37 °C for 1 h.

ELISA

Logarithmic growth phase H9c2 cells were inoculated into a 6-well plate and cultured for 48 h. The supernatant was collected after centrifugation at 500 *g* for 5 min. The tumour necrosis factor α (TFN-α) ELISA kit (PT512, Beyotime), interleukin 1β (IL-1β) ELISA kit (PI301, Beyotime), interleukin 6 (IL-6) ELISA kit (PI330, Beyotime), endothelin 1 (ET-1) ELISA kit (EIAET1, Invitrogen) and nitric oxide (NO) ELISA kit (SP14504, Saipei Biotechnology Co. Ltd, Wuhan. Hubei, China) were utilized to identify the contents of TNF-α, IL-1β, IL-6, ET-1 and NO in the supernatants of H9c2 cells.

Statistical analysis

Each experiment was tested at least three times, and the results were recorded as the mean value with the corresponding standard deviation. Plotting was done with the aid of Prism software (Graphpad 9.0). SPSS 26.0 was employed to run the analysis of variance (ANOVA) and Student's *t*-test. A significant difference is denoted by $^{*}P < 0.05$.

Results

JCAD expression in H_2O_2-treated H9c2 cells

qRT-PCR was utilized to determine JCAD mRNA expression, and the findings revealed a notable up-regulation of JCAD mRNA in H9c2 cells following 12 h of H_2O_2 treatment (Fig. 1A). Moreover, the Western blot assay results further confirmed that H_2O_2 treatment led to a notable up-regulation of JCAD protein expression in H9c2 cells (Fig. 1B).

Effect of over-expression of JCAD on H9c2 cell injury caused by H_2O_2

We transfected JCAD in H_2O_2-treated H9c2 cells to explore the influence of over-expression of JCAD on H9c2 cells. Western blot and qRT-PCR results confirmed that the level of both JCAD mRNA and protein was significantly elevated in H9c2 cells after transfection with JCAD (Fig. 2A–B), which allowed for subsequent experiments. The CCK-8 assay was utilized to determine the impact of JCAD over-expression on H9c2 cell viability, and the findings demonstrated a considerable decline in H9c2 cell viability after H_2O_2 treatment, whereas over-expression of JCAD resulted in a further reduction in H9c2 cell viability (Fig. 2C). Apoptosis was detected in H9c2 cells by using TUNEL staining, and the findings revealed that treatment with H_2O_2 led to a notable rise in the amount of TUNEL-positive cells and a further rise in over-expression of JCAD (Fig. 2D). Western blot results revealed that H_2O_2 caused a rise in apoptosis marker proteins cleaved-caspase 3 and Bax, along with a decline in anti-apoptotic protein Bcl-2 in H9c2 cells, and the over-expression of JCAD strengthened the effect of H_2O_2, which suggested that over-expression of JCAD notably promoted the H_2O_2-induced H9c2 cell apoptosis (Fig. 2E). Moreover, H_2O_2 caused a significant rise in inflammatory factor levels in H9c2 cells, including TNF-α, IL-1β and IL-6. These inflammatory factor levels were further increased after transfection with JCAD (Fig. 2F–H). Notably, H_2O_2 caused a notable rise in the content of vascular endothelial active substance ET-1 and a significant decrease in NO content in H9c2 cells, and over-expression of JCAD intensified the effect of H_2O_2 (Fig. 2I–J). The above results indicated that over-expression of JCAD enhanced H_2O_2-induced cellular damage and promoted the inflammatory response and endothelial dysfunction.

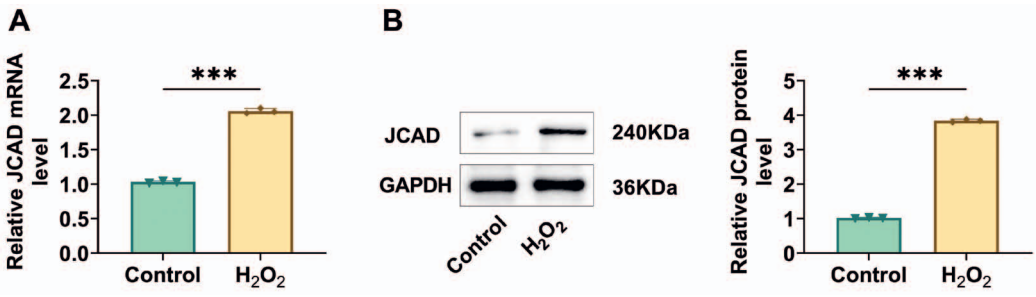

Fig. 1. JCAD expression in H_2O_2-treated H9c2 cells. (**A**) JCAD mRNA expression was assessed by qRT-PCR. (**B**) Examination of the JCAD protein level in H9c2 cells by Western blot.

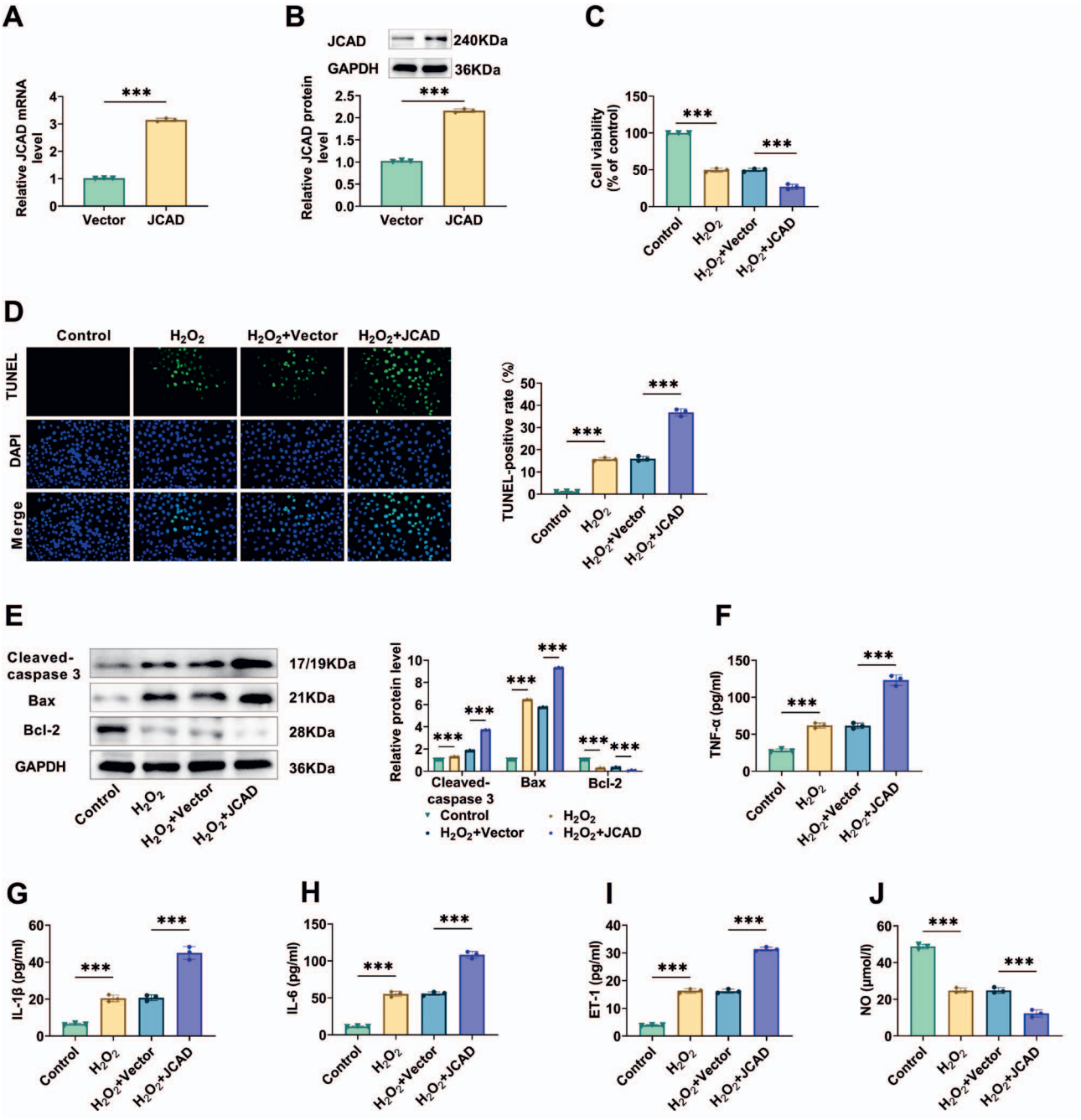

Fig. 2. Effect of over-expression of JCAD on H9c2 cell injury caused by H_2O_2. (**A–B**) Examination of JCAD over-expression efficiency by qRT-PCR and Western blot. (**C**) Cell viability was assessed by CCK-8 assay. (**D**) TUNEL staining was utilized to detect apoptosis. (**E**) Examination of cleaved-caspase 3, Bax and Bcl-2 expression by Western blot. (**F–H**) ELISA kits were utilized to detect TNF-α, IL-1β and IL-6 contents in H9c2 cell supernatant. (**I–J**) ELISA kits were utilized to detect ET-1 and NO levels in H9c2 cell supernatant.

Effect of JCAD silencing on H9c2 cell injury caused by H_2O_2

Next, sh-JCAD was transfected into H_2O_2-treated H9c2 cells to investigate the impact of JCAD silencing on H9c2 cells. After sh-JCAD transfection, both JCAD protein and mRNA expression were significantly decreased, as indicated by Western blot and qRT-PCR (Fig. 3A–B). Cell viability was determined by the CCK-8 assay after silencing of JCAD, and the findings indicated that the silencing of JCAD counteracted the inhibitory impact of H_2O_2 on H9c2 cell viability (Fig. 3C). Moreover, silencing of JCAD decreased the amount of TUNEL-positive cells (Fig. 3D), down-regulated cleaved-caspase 3 and Bax protein levels and up-regulated Bcl-2 expression (Fig. 3E), which suggested that silenc-

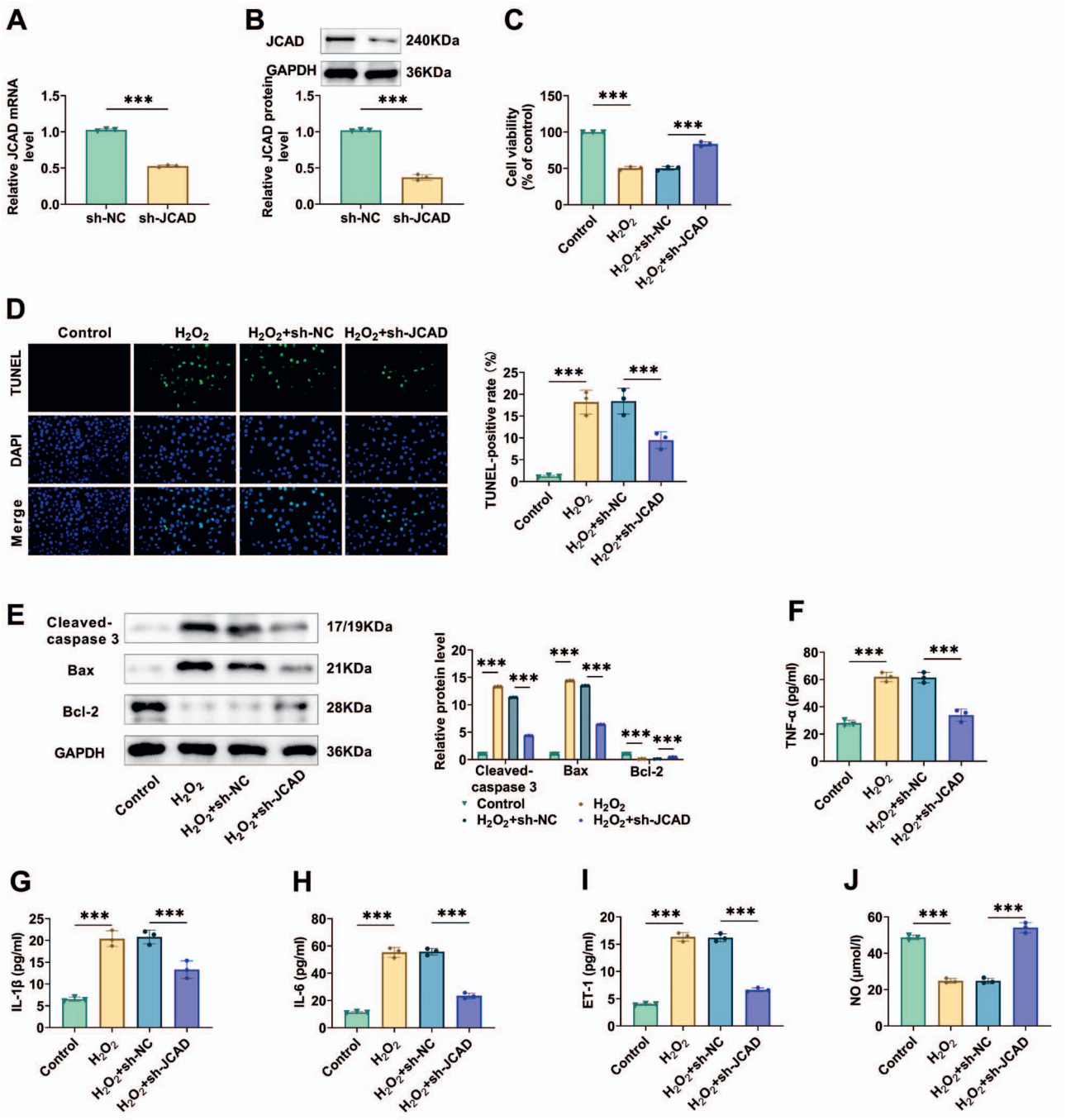

Fig. 3. Influence of JCAD silencing on H_2O_2-induced damage in H9c2 cells. (**A–B**) Examination of JCAD silencing efficiency by qRT-PCR and Western blot. (**C**) Cell viability was assessed by CCK-8 assay. (**D**) TUNEL staining was utilized to detected apoptosis. (**E**) Examination of cleaved-caspase 3, Bax and Bcl-2 expression by Western blot. (**F–H**) ELISA kits were utilized to detect TNF-α, IL-1β and IL-6 contents in H9c2 cell supernatant. (**I–J**) ELISA kits were utilized to detect ET-1 and NO levels in H9c2 cell supernatant.

ing of JCAD markedly inhibited apoptosis in H9c2 cells caused by H_2O_2. After transfection of sh-JCAD, the contents of TNF-α, IL-1β and IL-6 were notably reduced in H_2O_2-treated H9c2 cells (Fig. 3F–H) and the content of ET-1 was notably decreased, whereas the level of NO was notably elevated (Fig. 3I–J). In contrast to over-expression of JCAD, the above results suggested that silencing of JCAD attenuated H_2O_2-induced cellular damage, increased cell viability and inhibited apoptosis, inflammatory responses and endothelial dysfunction.

JCAD regulates the Wnt/β-catenin pathway

To explore the molecular mechanisms by which JCAD affects H_2O_2-induced cellular damage, we evaluated the relevant signalling pathways. Western blot was utilized to measure the proteins involved in the Wnt/β-catenin

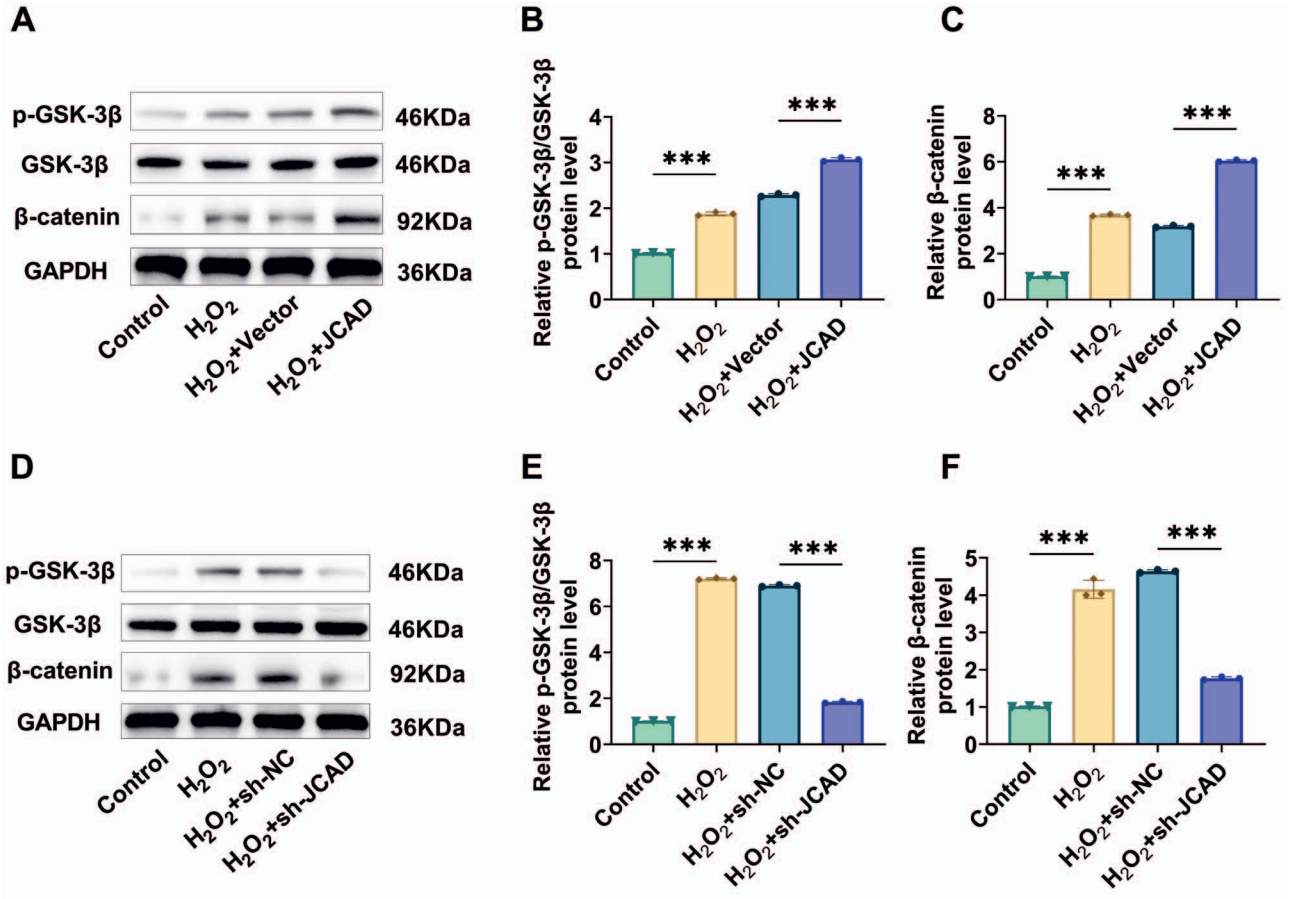

Fig. 4. JCAD regulates the Wnt/β-catenin pathway in H9c2 cells. (**A–C**) Examination of Wnt/β-catenin pathway-related protein levels after over-expression of JCAD by Western blot. (**D–F**) Examination of Wnt/β-catenin pathway-related protein levels after silencing of JCAD by Western blot.

pathway, revealing a significant increase in the phosphorylation level of GSK-3β and β-catenin protein expression in H9c2 cells after H_2O_2 treatment (Fig. 4A–C). This increase was further enhanced by over-expression of JCAD, indicating that the Wnt/β-catenin pathway was activated by the over-expression of JCAD. In contrast to over-expression of JCAD, silencing of JCAD led to a notable reduction in the phosphorylation level of GSK-3β and β-catenin protein expression in H_2O_2-treated H9c2 cells (Fig. 4D–F), suggesting that silencing of JCAD hindered the Wnt/β-catenin pathway.

DKK-1 partially reverts the influence of JCAD over-expression on H9c2 cell injury caused by H_2O_2

To further investigate whether JCAD affects H_2O_2-induced cell injury by regulating the Wnt/β-catenin pathway, H9c2 cells were exposed to Wnt/β-catenin pathway blocker, DKK-1 (100 ng/ml), and Western blot was utilized to determine Wnt/β-catenin pathway-related proteins. The findings indicated that JCAD over-expression resulted in heightened levels of GSK-3β phosphorylation and β-catenin expression in H9c2 cells, suggesting that over-expression of JCAD can activate the Wnt/β-catenin pathway, but these effects were partially attenuated by DKK-1 (Fig. 5A–C). The CCK-8 assay results suggested that DKK-1 attenuated the inhibitory impact of JCAD over-expression on the viability of H9c2 cells (Fig. 5D). The treatment of DKK-1 resulted in a noticeable reduction in the quantity of TUNEL-positive cells (Fig. 5E) and attenuated the suppressive impact of JCAD over-expression on cleaved-caspase 3 and Bax protein levels in H9c2 cells (Fig. 5F), indicating that DKK-1 significantly attenuated the JCAD over-expression-induced promotion of cell apoptosis. Moreover, DKK-1 effectively reduced the contents of TNF-α, IL-1β and IL-6 (Fig. 5G–I), reduced the level of ET-1 and increased the NO content (Fig. 5J–K). These results further confirmed that over-expression of JCAD promoted H_2O_2-induced H9c2 cell injury by activating the Wnt/β-catenin pathway.

Discussion

CHD is a prevalent cardiovascular condition that significantly impacts public health worldwide. Atherosclerosis causes development of plaques in the arteries, which can result in the formation of blood clots and nar-

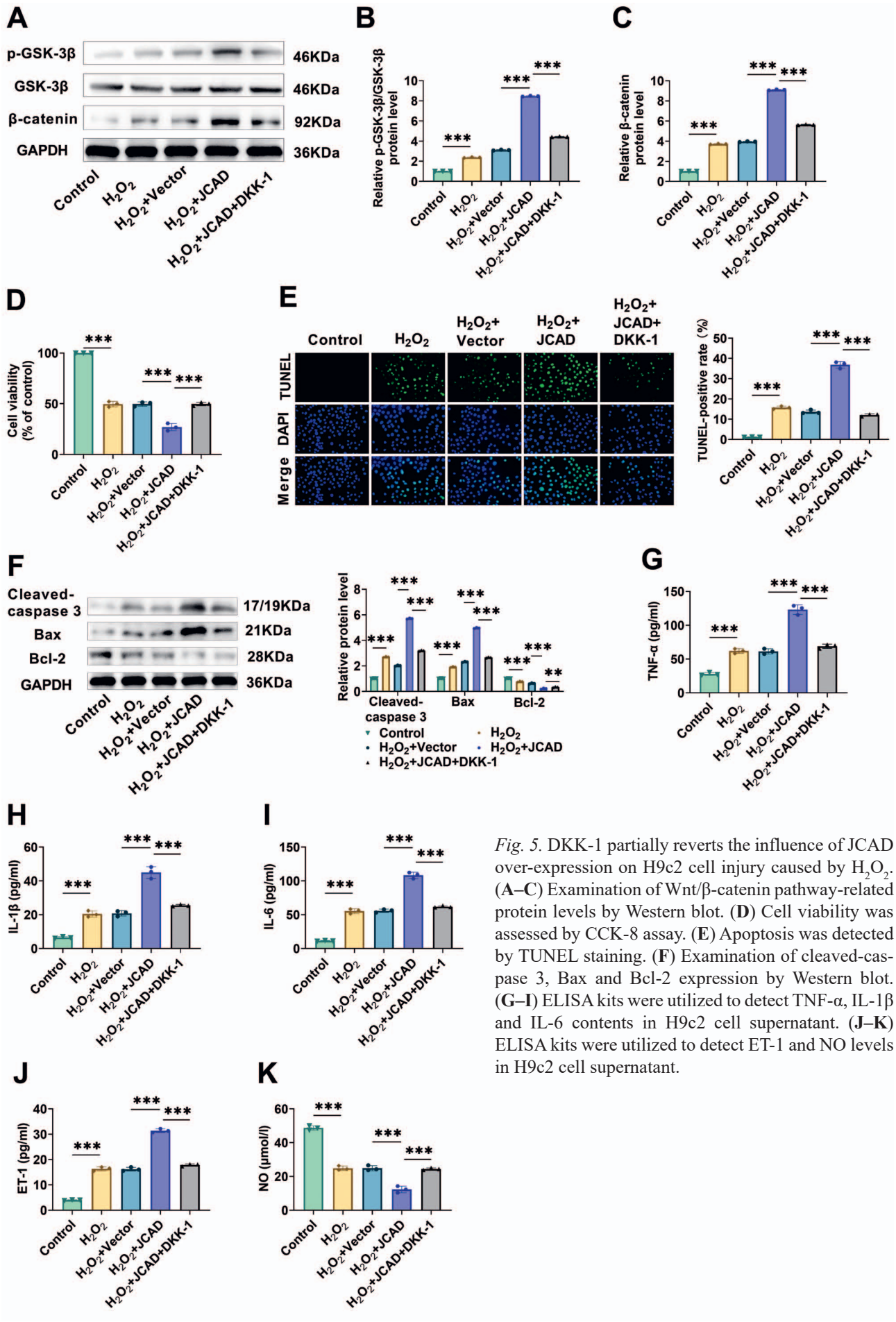

Fig. 5. DKK-1 partially reverts the influence of JCAD over-expression on H9c2 cell injury caused by H_2O_2. (**A–C**) Examination of Wnt/β-catenin pathway-related protein levels by Western blot. (**D**) Cell viability was assessed by CCK-8 assay. (**E**) Apoptosis was detected by TUNEL staining. (**F**) Examination of cleaved-caspase 3, Bax and Bcl-2 expression by Western blot. (**G–I**) ELISA kits were utilized to detect TNF-α, IL-1β and IL-6 contents in H9c2 cell supernatant. (**J–K**) ELISA kits were utilized to detect ET-1 and NO levels in H9c2 cell supernatant.

rowing of arteries. CHD is caused by the effects of atherosclerosis leading to reduced or blocked blood flow in the coronary arteries, and therefore, atherosclerosis is a common pathologic basis for CHD (Shao et al., 2020; Medina-Leyte et al., 2021). It has been shown that knockdown of JCAD in endothelial cells affects cell proliferation, apoptosis, migration and tube formation; additionally, high JCAD expression promotes endothelial dysfunction and atherosclerosis formation (Jones et al., 2018). Moreover, Xu et al. (2019) observed up-regulation of JCAD expression in endothelial cells of mouse and human atherosclerotic plaques. This suggested that *JCAD* may be a key gene associated with CHD. However, the effect of JCAD on CHD, especially CHD-induced cardiomyocyte injury, remains unclear. The H_2O_2-induced H9c2 cell injury model is a classical model for simulating CHD-induced cardiomyocyte injury (Zou et al., 2020). Our findings demonstrated a marked up-regulation of JCAD expression in H9c2 cells caused by H_2O_2. Increasing evidence suggests that activation of cleaved-caspase 3 and Bax and inhibition of Bcl-2 are the main mechanisms leading to cardiomyocyte apoptosis (Lu et al., 2021; Liu et al., 2022). The results of this research demonstrated a notable rise in the count of TUNEL-positive cells, an increase in cleaved-caspase 3 and Bax expression and a decrease in Bcl-2 expression in H9c2 cells. Over-expression of JCAD enhanced the effect of H_2O_2 and silencing of JCAD reversed the effect, suggesting that silencing of JCAD has a protective effect on cardiomyocytes.

Recent research has consistently demonstrated the significant impact of inflammation on the development of CHD (Yang et al., 2020; Dugani et al., 2021). Mutual adhesion between endothelial cells, leukocytes and platelets can exacerbate the inflammatory response, promote the formation of atherosclerotic plaques and affect the vascular microenvironment, which in turn causes endothelial dysfunction (Sagris et al., 2021; Montarello et al., 2022). Multiple chemokines and cytokines are involved in CHD progression, with TNF-α, IL-1β and IL-6 being the key mediators of inflammation (Tsioufis et al., 2022). We found that over-expression of JCAD led to significantly higher contents of TNF-α, IL-1β and IL-6 in H_2O_2-treated H9c2 cells and silencing of JCAD did the opposite, suggesting that silencing of JCAD ameliorated H_2O_2-induced inflammation in cardiomyocytes. ET-1 is a vasoconstrictor peptide associated with coronary endothelial dysfunction (Ford et al., 2020). NO and ET-1 have opposite effects on the vasculature, and NO/ET-1 dysregulation leads to constriction of coronary arteries, slowing of blood flow, aggregation of neutrophils on endothelial cells and release of a variety of mediators and oxygen free radicals, exacerbating myocardial ischaemia and hypoxia (Gupta et al., 2020, Wang et al., 2023). In the present study, H_2O_2 caused an increased ET-1 content and decreased NO content in H9c2 cells. Over-expression of JCAD enhanced the effect of H_2O_2 and silencing of JCAD attenuated it, further suggesting that silencing of JCAD ameliorates H_2O_2-induced endothelial dysfunction and has the potential to attenuate CHD.

Myocardial ischaemia and hypoxia are accompanied by pathological apoptosis and irreversible necrosis of cardiomyocytes, while the Wnt/β-catenin pathway is tightly linked to cardiomyocyte injury due to CHD (Desita et al., 2022; Law et al., 2022). For example, Liu et al. (2020) showed that in coronary artery disease, activation of Wnt signalling is associated with atherosclerotic plaque formation, endothelial dysfunction and the biological function of smooth muscle cells. According to Liu et al. (2023), paeoniflorin was found to improve atherosclerotic plaque lesions by blocking oxidized low-density lipoprotein-induced injury to endothelial cells via regulation of the Wnt/β-catenin pathway (Liu et al., 2023). Thus, we explored whether JCAD affects the biological functions of H9c2 cells by regulating the Wnt/β-catenin pathway. We found that JCAD was able to modulate the Wnt/β-catenin pathway, and the pathway blocker attenuated the promotional effect of over-expression of JCAD on H_2O_2-induced H9c2 cell injury, suggesting that JCAD acts by modulating the Wnt/β-catenin pathway.

Conclusion

Taken together, our research revealed that JCAD expression exhibited a notable increase in H_2O_2-treated H9c2 cells, and over-expression of JCAD reduced H9c2 cell viability and promoted apoptosis, whereas silencing of JCAD attenuated H_2O_2-induced cell injury, a mechanism that is mediated by regulating the Wnt/β-catenin pathway. Our study elucidated the potential mechanism of action of down-regulating JCAD to attenuate CHD cardiomyocyte injury using an *in vitro* cell model; however, there is a need for additional research on the influence of JCAD on cardiomyocyte injury *in vivo*. In conclusion, this study identified JCAD as a potential treatment target for CHD, offering a new reference for the clinical management of CHD.

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