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ORIGINAL RESEARCH

TRPC5 Promotes Intermittent Hypoxia-Induced Cardiomyocyte Injury Through Oxidative Stress

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Purpose: Intermittent hypoxia (IH), a defining feature of obstructive sleep apnea (OSA), is associated with heart damage and linked to transient receptor potential canonical channel 5 (TRPC5). Nonetheless, the function of TRPC5 in OSA-induced cardiac injury remains uncertain. For this research, we aimed to explore the role and potential mechanism of TRPC5 in cardiomyocyte injury induced by intermittent hypoxia.

Methods: 30 patients with newly diagnosed OSA and 30 patients with primary snoring(PS) were included in this study. Participants were subjected to polysomnography (PSG) for OSA diagnosis. Echocardiography was used to evaluate the structure and function of the heart, while peripheral blood samples were obtained. Additionally, RT-qPCR was utilized to quantify the relative expression level of TRPC5 mRNA in peripheral blood. H9c2 cells experienced IH or normoxia. TRPC5 levels in H9c2 cells were determined via RT-qPCR and Western blotting (WB) methods. H9c2 cells overexpressing TRPC5 were subjected to either normoxic or intermittent hypoxia conditions. Cell viability was determined by CCK8, the apoptosis rate, reactive oxygen species(ROS) levels, and Ca^{2+} concentration were assessed by flow cytometry, and the protein levels of TRPC5, Bcl-2, Bax, and Caspase-3 were analyzed by WB. Mitochondrial membrane potential(MMP), mitochondrial membrane permeability transition pore(mPTP), and transmission electron microscopy(TEM) were employed to observe mitochondrial function and structure. After inhibiting ROS with N-acetylcysteine (NAC), apoptosis, mitochondrial function and structure, and the concentration of Ca^{2+} were further detected.

Results: TRPC5 and left atrial diameter (LAD) were higher in OSA individuals, while the E/A ratio was lower(all $P<0.05$). IH impaired cell viability, triggered cell apoptosis, and enhanced TRPC5 expression in H9c2 cells(all $P<0.05$). The effects of IH on apoptosis, cell viability, mitochondrial function and structure damage, and oxidative stress (OxS) in H9c2 cells were accelerated by the overexpression of TRPC5(all $P<0.05$). Furthermore, cell apoptosis and mitochondrial structural and functional damage caused by overexpression of TRPC5 were attenuated by ROS inhibition.

Conclusion: TRPC5 is associated with structural and functional cardiac damage in patients with OSA, and TRPC5 promotes IH-induced apoptosis and mitochondrial damage in cardiomyocytes through OxS. TRPC5 may be a novel target for the diagnosis and treatment of OSA-induced myocardial injury.

Keywords: TRPC5, oxidative stress, apoptosis, intermittent hypoxia, OSA

Introduction

Obstructive sleep apnea(OSA) involves repetitive upper airway obstruction, leading to interruptions in breathing, Intermittent hypoxia (IH), hypercapnia, or awakenings during sleep.[1] It is estimated that there are approximately 936 million individuals aged 30–69 years with OSA worldwide.[2] Studies have indicated that OSA is associated with heart-related complications, serving as a predictor for hypertension, coronary artery disease, heart failure, arrhythmia, and stroke.[3] Continuous positive airway pressure ventilation (CPAP) therapy is currently the recommended treatment for OSA and is particularly suitable for patients with moderate-to-severe OSA.[4] However, in some patients, the risk of cardiovascular events has not been reduced accordingly after CPAP therapy, despite improvement in intermittent

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hypoxia.[5] Therefore, it is important to elucidate the relationship between OSA and myocardial injury and to identify possible molecular mechanisms.

IH is the most fundamental pathogenic factor of OSA.[6] The major pathophysiological change in OSA is oxidative stress (OxS), induced by IH. It increases the reactive oxygen species (ROS) production and reduces antioxidant capacities,[7,8] and has been reported to promote myocardial injury.[9–11] Mitochondria are the main source of ROS[12] and intracellular Ca^{2+} stores, playing an important contribution in regulating intracellular Ca^{2+} concentration.[13] Ca^{2+} plays a crucial role as a key secondary messenger in cells, participating in apoptosis, autophagy, the cell cycle, and numerous other physiological processes.[14] Furthermore, Ca^{2+} is an important regulator of ROS, generating ROS through its involvement in the tricarboxylic acid cycle and the electron transport chain.[15] In turn, ROS affects intracellular Ca^{2+} signaling.[16] Excessive accumulation of ROS, which can stimulate the activation of mitochondrial calcium channels, promotes calcium homeostasis imbalance.[17]

Calcium channels are important for the maintenance of intracellular calcium homeostasis. TRPC5, part of the TRPC family, is a Transient Receptor Potential (TRP) Canonical Channel. As a nonselective calcium cation channel, it is expressed in the brain, heart, and kidney,[18] and is primarily involved in Ca^{2+} permeation, transporting extracellular free calcium into the cell. TRPC5 has been reported to be associated with myocardial injury and OxS. TRPC5 was elevated in myocardial tissue from patients with end-stage heart failure and positively correlated with myocardial injury.[19] Patients with non-valvular atrial fibrillation have significantly increased TRPC5 gene expression.[20] TRPC5 expression was upregulated in myocardial tissue from patients with end-stage idiopathic dilated cardiomyopathy.[21] Inhibiting TRPC5 may impair metastasis of colon cancer cells by reducing the production of the oxidative stress product −4--Hydroxynonenal(4HNE).[22] Inhibition of TRPC5 could attenuate oxidative stress and ameliorate cognitive dysfunction in a rat model of Parkinsonism.[23] Our previous study showed that TRPC5 is associated with myocardial injury in a rat model of intermittent hypoxia.[24] Still, the effect and the mechanism of TRPC5 in IH-induced myocardial injury remains uncertain.

Therefore, the aim of this study was to evaluate TRPC5 expression in peripheral blood mononuclear cells (PBMCs) from patients with OSA and its correlation with structural and functional cardiac damage. In addition, we investigated the potential mechanisms by which TRPC5 regulates myocardial injury in vitro, which provides new ideas for the diagnosis and treatment of myocardial injury caused by OSA.

Materials and Methods

Participants and Study Design

From February 2023 to April 2024, sixty individuals aged 18–75 years were recruited from the Health Examination Center of the First Affiliated Hospital of Xinjiang Medical University. Polysomnography (PSG) was employed for the diagnosis of OSA[25] in accordance with the clinical practice guidelines of the American Sleep Medicine Association. Based on the apnea-hypopnea index (AHI), individuals were categorized into the primary snoring group (PS, AHI<5 events per hour) or the OSA group (AHI≥5 events per hour). Exclusion criteria included: (1) central or mixed sleep apnea; (2) pulmonary fibrosis, chronic obstructive pulmonary disease, bronchial asthma, and other unstable respiratory system diseases; (3) conditions that could impact both the function and structure of the heart, like heart valve disease, cardiomyopathy, congenital heart disease, and severe arrhythmia; (4) diabetes, hyperthyroidism, hypothyroidism; (5) severe liver and kidney dysfunction, chronic inflammation, chronic wasting diseases, and malignant tumors; (6) other factors like cognitive impairment, sequelae of cerebrovascular diseases, and insomnia. Finally, 30 participants were included in each group.

Clinical data collection included age, gender, drinking and smoking history, and body mass index (BMI). Participants were required to fast for a minimum of 8 hours before blood sample collection. The Roche C8000 biochemical analyzer was utilized to analyze blood samples, measuring creatinine, uric acid, triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels. Simultaneously, cardiac troponin I (cTN-I), N-terminal pro-B-type (NT-proBNP), and creatine kinase isoenzymes (CK-MB) were assessed.

The research adhered to the Helsinki Declaration guidelines and received ethical approval from the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (ethical batch number: IACUC-20210326-08). All enrolled participants provided informed consent before participation.

Polysomnography (PSG)

All participants underwent at least a 4-hour PSG (Australia, Compumedics E-series). Monitoring indicators included blood oxygen saturation, chest and abdominal breathing movements, snoring, and oral and nasal airflow. After the monitoring, professional technicians conducted data analysis and recorded the AHI, oxygen desaturation index (ODI), average blood oxygen saturation ($MSaO_2$), and lowest arterial oxygen saturation ($LSaO_2$).

Collection of Peripheral Blood Specimens

To collect the blood, we used EDTA anticoagulant tubes. PBMCs were isolated from blood components using density gradient centrifugation. Through density gradient centrifugation, the PBMCs were separated from the blood components. Afterward, they were stored at −80 °C until being used.

Echocardiography

Expert ultrasound practitioners operated an American PHILIPS IE33 color Doppler ultrasonic diagnostic instrument while the individuals were positioned in the left supine posture. The echocardiogram was performed with the patient in a relaxed breathing state to observe the structure and morphology of each cardiac slice. Measurements followed the guidelines set by the American Society of Echocardiography,[26] including left atrial diameter (LAD), left ventricular end-diastolic diameter (LVEDD), left ventricular end-systolic diameter (LVESD), left ventricular posterior wall thickness (LVPWT), interventricular septal thickness (IVST), right ventricular diameter (RVD), right atrial diameter (RAD), e' peak, E peak, and A peak. Additionally, the E/A ratio and left ventricular ejection fraction (LVEF) were calculated.

Cell Culture and Treatment

Cells of the H9c2 line were acquired from Procell (CL-0089, Wuhan, China). In a 5% CO_2 humidified atmosphere incubator at 37°C, the cells underwent culture in DMEM medium (Procell, PM150210, China), supplemented with 10% fetal bovine serum (Gibco, 10099–141, USA), 100 mg/mL streptomycin, and 100 U/mL penicillin (Gibco, 15140122, USA). Two hours before IH treatment, the cells were exposed to N-acetylcysteine (NAC, MCE, HY-B0215, USA) for the experiments. Stock solutions of NAC at 1M were made in appropriate solvents and refrigerated at 4°C. Subsequent working solutions were prepared through the dissolution of the stock solution in the culture medium, with an equal amount of NAC added to every well. The NAC final concentration was 6 mM.[27,28]

IH Treatment

Upon reaching 70–80% confluence, H9c2 cells underwent IH stimulation, following a previously described method[29] with modifications. The cells were exposed to either IH (35 minutes at 1% O_2 followed by 25 minutes at 21% O_2, 1 cycle/hour) or Normoxia (21% O_2 balanced in 5% CO_2) for 24 hours.

RNA Isolation and Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)

RNA extraction utilized the Trizol reagent (Invitrogen, 10296028, USA), and subsequent cDNA synthesis employed the SuperScript III RNA PCR kit (ABI-Invitrogen, 11752050, USA). RT-qPCR was conducted with SYBR™ Select mix (ABI-Invitrogen, 4472920, USA) employing the StepOne™ Real-time quantitative PCR system (Applied Biosystems, StepOne Software, USA). Gene expression quantification utilized the $2^{-\Delta\Delta CT}$ method. For the endogenous control, we used β-actin.

Cell Transfection

H9c2 cells were growing in a 6-well culture plate at a density of 10^5 cells per well, and transfection took place upon reaching a confluency of 50–60%. H9c2 cells were then introduced into another 6-well plate, 48 hours after the addition

of TRPC5 lentivirus or a lentivirus vector-negative control. Afterward, fresh medium was added to replenish the cells. The efficiency of lentivirus transfection was confirmed by assessing green fluorescence, which was found to exceed 80%. qRT-PCR method was employed to validate the TRPC5 overexpression.

Cell Viability Assay

H9c2 cell viability was assessed using the CCK-8 kit (Beyotime, C0037, China). After seeding and culturing for 24 hours, the cells underwent incubation with CCK-8 solution at 37°C for 2 hours. Subsequently, we measured the optical density (OD) for each well at 450 nm.

Flow Cytometry Analysis

The H9c2 cell apoptotic rate was evaluated with the Annexin V-PE apoptosis Kit (Beyotime, C1062L, China). After IH or Normoxia treatment, 195 μL of binding buffer was added, followed by 5 μL of Annexin V tagged with FITC and 10 μL of PI. The mixture was then left to incubate in darkness at room temperature for 15 minutes. The early and late-stage percentages of apoptosis in the samples were determined using flow cytometry (BeamCyte-1026, BEAM DIAG). Within the flow cytometry scatter plot, Q3 indicated early apoptotic cells, whereas Q2 indicated late apoptotic cells.

Detection of Ca^{2+} and ROS

To measure intracellular Ca^{2+} concentration, the Fluo4-AM kit (Beyotime, S1061S, China) was employed. Following treatment, H9c2 cells were suspended in Fluo4-AM working solution at 37°C for 30 minutes in darkness. Using flow cytometry with excitation at 485 nm and emission at 530 nm (green), intracellular Ca^{2+} concentration was determined.

For the determination of ROS, the Reactive Oxygen Species Assay Kit (Beyotime, S003S, China) was utilized. Using flow cytometry, fluorescence intensity was monitored with excitation at 485 nm and emission at 530 nm.

Determination of Mitochondrial Membrane Potential (MMP)

In accordance with the manufacturer's guidelines, alterations in MMP were assessed using a JC-1 mitochondrial membrane potential assay kit (Beyotime, C2006, China). At 37°C, H9c2 cells (1×10^5) were treated with JC-1 staining buffer (10 mg/mL) for 20 minutes. Subsequently, the cells were rinsed with PBS and examined via flow cytometry. The proportion of aggregates, marked by red fluorescence representing the controlled MMP, compared to monomers, marked by green fluorescence indicating MMP loss, served as an indicator for unhealthy mitochondria.

Determination of Mitochondrial Membrane Permeability Transition Pore (mPTP)

Following the manufacturer's guidelines, alterations in mPTP were assessed utilizing an MPTP kit (Beyotime, C2009S, China). H9c2 cells (1×10^5) were subjected to Calcein AM (1μL/mL) staining solution at 37°C for 30 minutes. Subsequently, the cells underwent PBS rinsing and underwent flow cytometry analysis.

Transmission Electron Microscopy

H9c2 cells were harvested following treatment with various groups, then rinsed with PBS prior to fixation in 3% glutaraldehyde overnight at 4°C. Following this, cells underwent fixation in 1% osmium tetroxide for a duration of 1 hour. Once embedded in Epon resin, the sections were stained using uranyl acetate and lead citrate. Ultimately, slides were observed using a transmission electron microscope (Hitachi-7800, Japan).

Western Blot (WB)

Proteins underwent extraction and separation via 10% SDS-PAGE electrophoresis, followed by transfer onto PVDF membranes. Subsequently, the membranes were subjected to incubation with respective primary and secondary antibodies. The antibodies utilized were: anti-TRPC5 (1:1000, Abcam, ab240872), anti-Bcl2 (1:1000, Abcam, ab19695), anti-Bax (1:1000, Abcam, ab32503), and anti-Caspase3 (1:1000, Affinity, AF6311). Goat Anti-Rabbit IgG H&L (1:4000, Abcam, ab205718) and Goat Anti-Mouse IgG H&L (1:3000, Abcam, ab205719) were employed. The Enhanced

Chemiluminescence System (CLINX, ChemiScope6100) facilitated band visualization, while Image J software was employed to quantify band densities, normalized to β-actin.

Statistical Analysis

Data were analyzed using SPSS 25.0 software. Quantitative data that conformed to normal distribution was expressed as mean ± standard deviation, and an independent samples *t*-test was used for the comparison of the two groups. One-way ANOVA was used for the comparison of more than two groups, and the Bonferroni method was used for comparison between groups. Quantitative data not conforming to normal distribution was expressed as median (P_{25}, P_{75}), and a non-parametric test was used for comparison between groups. The chi-square test was used for qualitative data. Spearman's test was used for correlation analysis of non-normally distributed data. A significance threshold of $P < 0.05$ was applied for statistical interpretation.

Results

Participant Characteristics

Table 1 provided a summary of the clinical and demographic features of the two patient groups. There were no significant differences between the two groups of age, gender, BMI, smoking history, drinking history, $MaSO_2$, uric acid, creatinine, TG, HDL-C, NT-proBNP, CK-MB, cTnI, LVEDD, LVESD, IVST, LVPWT, RVD, E peak, A peak, E/e' and LVEF. OSA

Table 1 Basic Information of Participants

	PS(n=30)	OSA(n=30)	*t* / $\chi 2$/*Z*	*P* value
Age(years)	56.53±10.83	58.37±8.79	−0.720	0.474
Male(n,%)	20(66.67)	23(76.67)	0.739	0.390
BMI(Kg/m^2)	25.21±4.11	26.36±2.66	−1.278	0.206
Smoking history(n,%)	19(63.33)	16(55.33)	0.617	0.432
Drinking history(n,%)	20(66.67)	19(63.33)	0.073	0.787
AHI(events/hour)	1.30(0.68,2.73)	19.99(14.40,36.02)	−6.645	<0.001
ODI(events/hour)	3.31(0.81,7.29)	15.88(8.50,24.37)	−4.828	<0.001
$MSaO_2$(%)	93.60(92.30,94.40)	93.40(91.90,94.00)	−1.213	0.225
$LSaO_2$(%)	84.50(81.80,90.00)	80.50(74.80,85.00)	−2.873	0.004
Uric acid (mmol/L)	307.93±87.46	327.66±81.43	−0.896	0.374
Creatinine (mmol/L)	63.68±14.69	69.07±12.68	−1.590	0.117
TC(mmol/L)	3.37(2.78,3.79)	3.86(3.18,4.52)	−2.403	0.016
TG(mmol/L)	1.27(0.92,1.53)	1.47(1.04,2.07)	−1.804	0.071
HDL-C(mmol/L)	0.95(0.81,1.16)	0.96(0.85,1.12)	−1.70	0.865
LDL-C(mmol/L)	2.45(1.66,2.68)	2.66(2.24,3.19)	−2.499	0.012
NT-proBNP(ng/L)	58.70(20.00,151.00)	81.95(25.70,284.25)	−1.247	0.212
CK-MB(ng/mL)	0.64(0.97,0.92)	0.62(0.44,0.87)	−0.177	0.859
cTnI(μg/L)	0.009(0.010,0.012)	0.010(0.009,0.012)	−0.683	0.494
LAD(mm)	33(32,37)	37(35,39)	−3.197	0.001
LVEDD(mm)	48(46,51)	49(48,50)	−0.693	0.488
LVESD(mm)	32(31,34)	32(32,34)	−0.525	0.600
IVST(mm)	9(9,10)	9(9,10)	−1.129	0.259
LVPWT(mm)	9(9,10)	9(9,10)	−0.916	0.360
RAD(mm)	33(32,34)	33(33,34)	−2.177	0.030
RVD(mm)	18(18,20)	19(18,20)	−0.764	0.445
E (m/s)	0.75(0.61,0.84)	0.68(0.56,0.83)	−0.771	0.441
A (m/s)	0.68(0.65,0.78)	0.77(0.68,0.84)	−1.873	0.061
E/A	1.03(0.78,1.31)	0.78(0.68,1.20)	−2.130	0.033
E/e'	7.16(6.63,7.99)	7.03(5.91,7.75)	−1.198	0.231
LVEF(%)	61.79(58.68,62.75)	61.90(58.71,62.95)	−0.163	0.871

patients had higher AHI, ODI, TC, LDL-C, LAD, and RAD, and lower $LSaO_2$ and E/A ratio. The observed differences were statistically significant (all $P<0.05$).

The Levels of TRPC5 mRNA Expression in the PBMCs of Participants

The relative levels of TRPC5 mRNA among participants are depicted in Figure 1. The findings indicated a notable rise in the relative TRPC5 mRNA expression among patients diagnosed with OSA ($P<0.05$).

Association of TRPC5 mRNA Expression with Sleep and Echocardiography Parameters

Table 2 presented the relationship between TRPC5 and sleep features, along with echocardiography parameters, in individuals diagnosed with OSA. The findings indicated a positive correlation between the expression levels of TRPC5 mRNA and AHI ($\rho = 0.476$, $P = 0.008$), ODI ($\rho = 0.415$, $P = 0.023$), as well as LAD ($\rho = 0.365$, $P = 0.045$), and a negative correlation with E/A ratio ($\rho = 0.0047$, $P = 0.042$).

IH Induced Myocardial Injury and Enhanced the Expression of TRPC5 in H9c2 Cells

After exposure to IH, flow cytometry was employed to assess the apoptotic rate of H9c2 cells. The findings revealed a notable rise in the apoptotic rate of H9c2 cells following exposure to IH (Figure 2A–B)($P<0.05$). Subsequent RT-qPCR and WB results (Figure 2C–E) showed an elevation in TRPC5 expression after IH treatment (all $P<0.05$), indicating a positive correlation between TRPC5 and IH-induced apoptosis of H9c2 cells.

Overexpression of TRPC5 Promoted IH-Induced Apoptosis in H9c2 Cells

Since TRPC5 is lowly expressed in the heart,[18] to examine its function in OSA, H9c2 cells were transfected with lentivirus to overexpress TRPC5. Post-transfection, RT-qPCR revealed a significant increase in TRPC5 mRNA expression in the oe-TRPC5 group ($P<0.05$), with no difference observed between the oe-NC and the Control groups (Figure 3A). Cell viability was assessed using CCK-8, which revealed that IH inhibited cell viability, and the inhibition was further enhanced by TRPC5 overexpression (Figure 3B) ($P<0.05$). Subsequent flow cytometry results showed that IH promoted apoptosis in H9c2 cells, which was exacerbated by TRPC5 overexpression (Figure 3C–D)($P<0.05$). WB results demonstrated IH-induced upregulation of Bax and Caspase-3, and downregulation of Bcl-2, with TRPC5 overexpression exacerbating these changes (Figure 3E–I) ($P<0.05$). Collectively, these findings suggested that TRPC5 upregulation exacerbates IH-induced apoptosis in H9c2 cells.

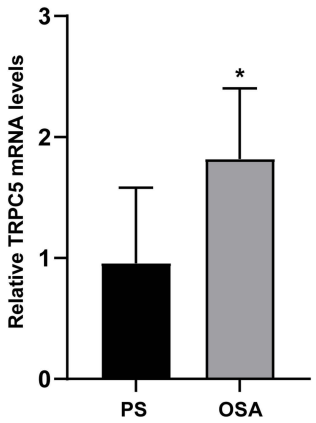

Figure 1 TRPC5 mRNA Levels in PBMCs of Participants. TRPC5 mRNA levels were identified through RT-qPCR analysis. Statistical significance (*$P < 0.05$) was observed compared to the PS group.

Table 2 Association of TRPC5 mRNA Expression with Sleep and Echocardiography Parameters in OSA Individuals

	ρ	*P* value
AHI(events/hour)	0.476	0.008
ODI(events/hour)	0.415	0.023
$LSaO_2$(%)	−0.065	0.733
LAD(mm)	0.365	0.047
RAD(mm)	0.145	0.446
E/A	−0.374	0.042

The Overexpression of TRPC5 Promoted IH-Induced Ca^{2+} Influx and the Oxidative Stress in H9c2 Cells

To explore the impact of TRPC5 on OxS, we assessed Ca^{2+} and ROS levels in H9C2 cells. Flow cytometry was used to measure intracellular Ca^{2+} and ROS levels in cells exposed to normoxia or IH conditions. The findings revealed that IH significantly elevated Ca^{2+} and ROS accumulation in H9c2 cells compared to the Normoxia group($P<0.05$). Furthermore, TRPC5 overexpression further amplified Ca^{2+} levels (Figure 4A–B) and ROS levels (Figure 4C–D) in H9c2 cells (all

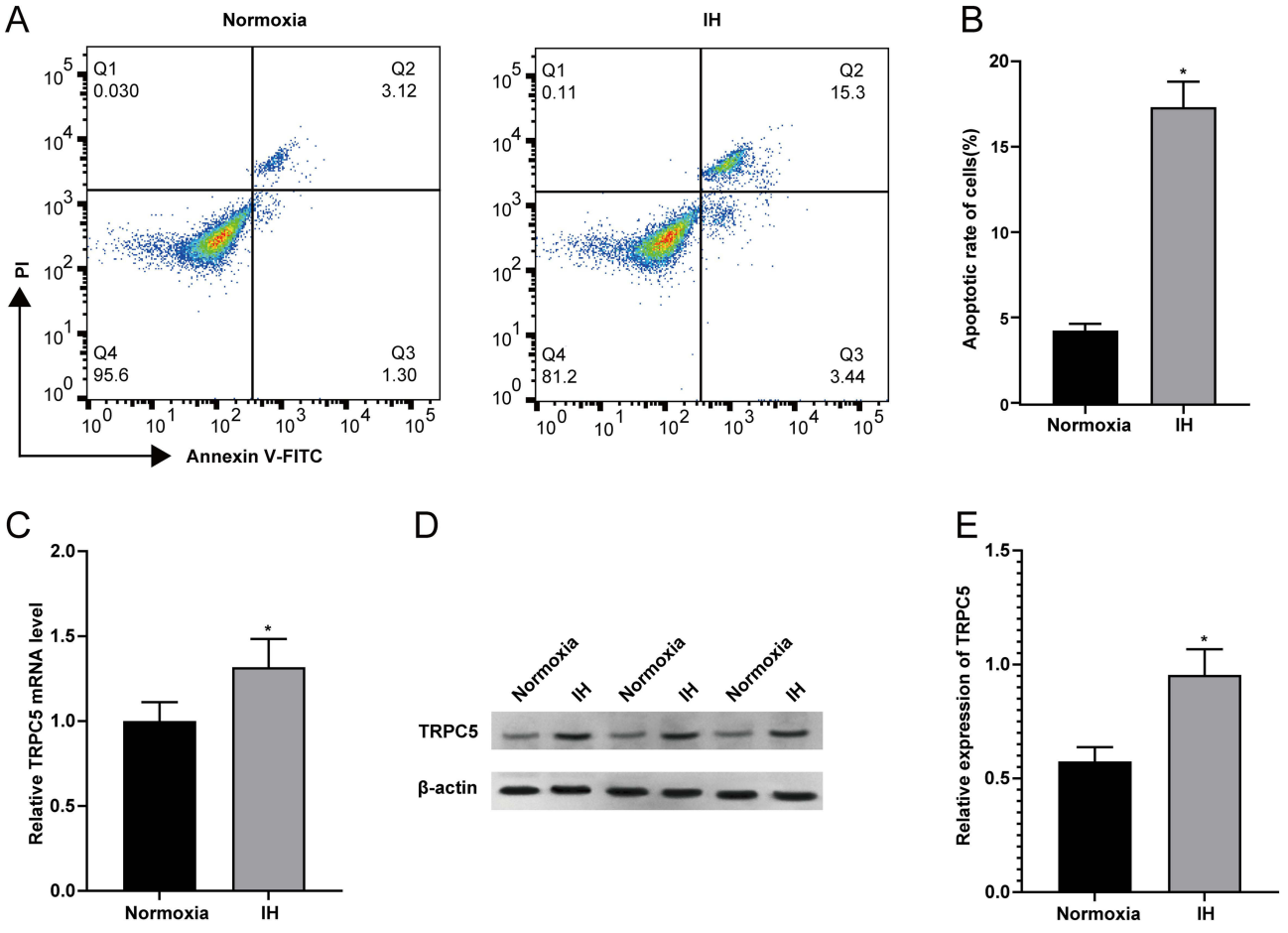

Figure 2 Impact of IH on Myocardial Injury and TRPC5 Levels in H9c2 Cells. (**A–B**) Flow cytometry was utilized to assess the apoptotic rate of H9c2 cells. In RT-qPCR (**C**) and WB (**D–E**) results, the difference in TRPC5 expression between IH and the Normoxia cohorts was observed. Statistical significance (*$P < 0.05$) compared to the Normoxia group.

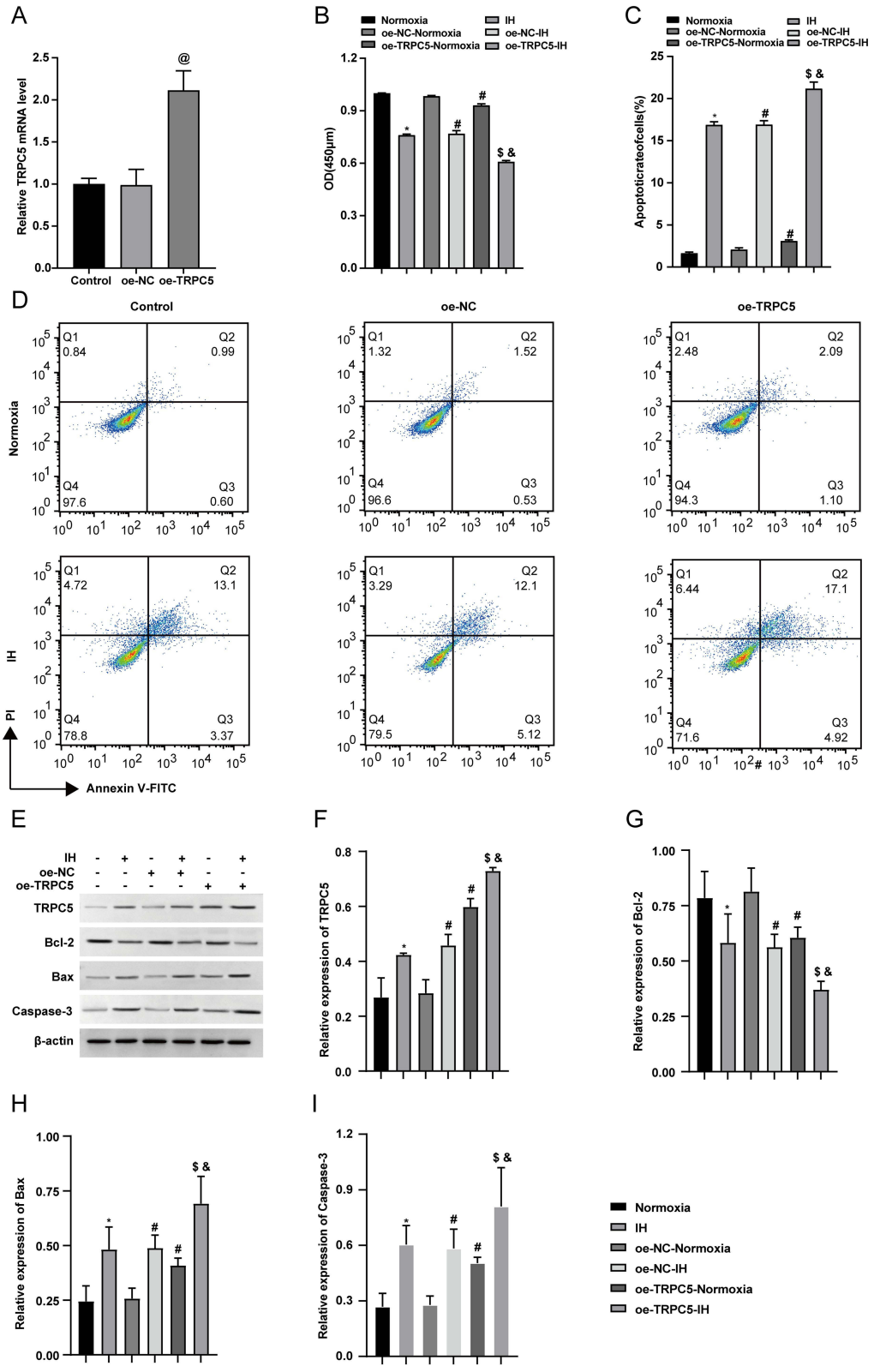

Figure 3 Overexpression of TRPC5 promoted IH-induced apoptosis in H9c2 cells. (**A**) The transduction efficiency was analyzed using RT-qPCR. (**B**) H9c2 cells viability were determined using the CCK-8 assay. (**C–D**) Through flow cytometry analysis, the apoptotic rate of H9c2 cells was measured. (**E–I**) WB results were employed to analyze the TRPC5, Bcl-2, Bax, and Caspase-3 levels. $^{*}P < 0.05$ compared to the Normoxia group, $^{\#}P < 0.05$ compared to the the oe-NC-Normoxia group, $^{\$}P < 0.05$ compared to the oe-TRPC5-Normoxia group, $^{\&}P < 0.05$ compared to the the oe-NC-IH group, and $^{@}P < 0.05$ compared to the control group.

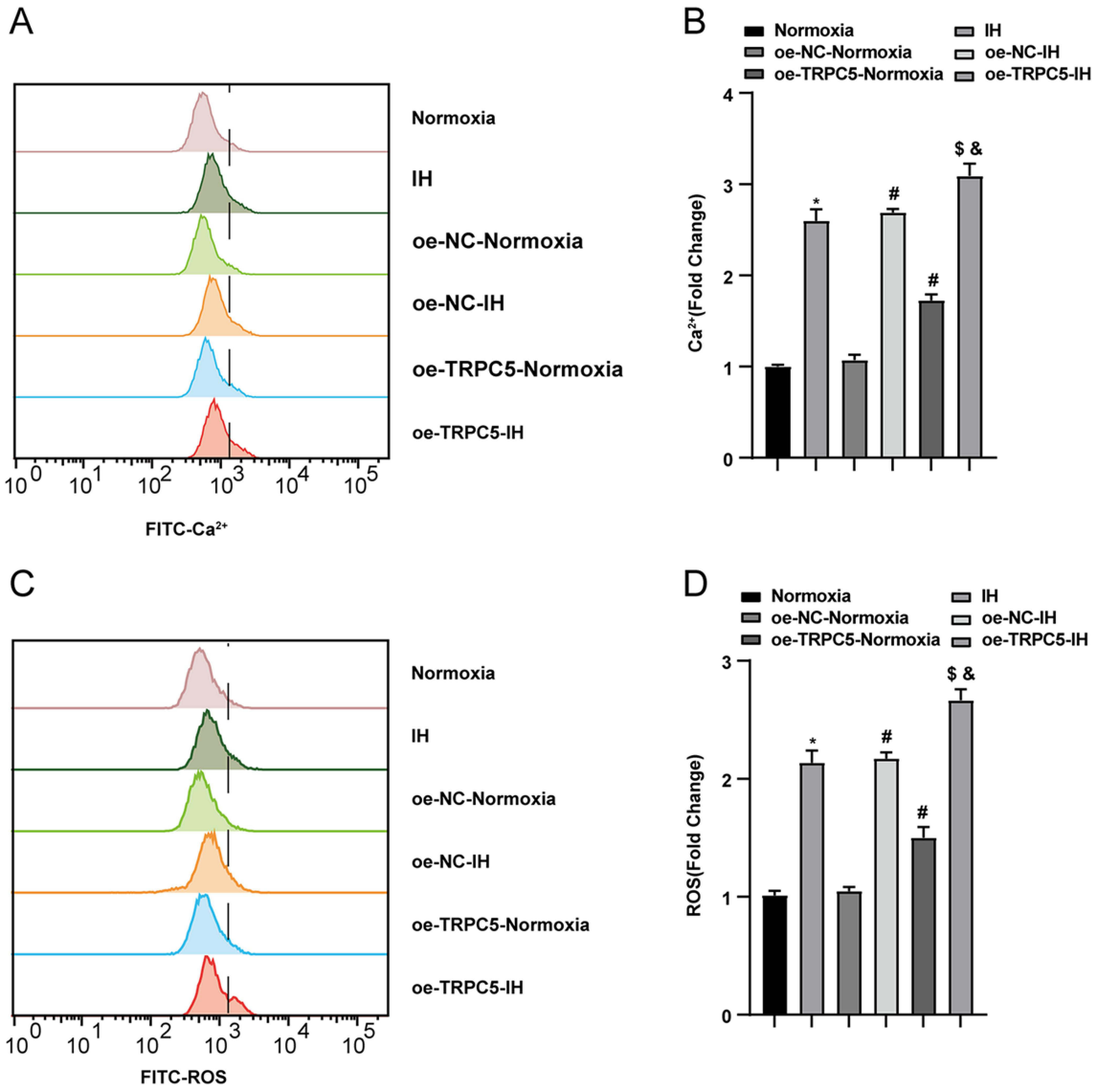

Figure 4 Overexpression of TRPC5 promoted IH-induced Ca^{2+} influx and the OxS in H9c2 cells. (**A–B**) Flow cytometry was employed to test the concentration of Ca^{2+} and the production of ROS (**C–D**). $^{*}P < 0.05$ compared to the Normoxia group, $^{\#}P < 0.05$ compared to the oe-NC-Normoxia group, $^{\$}P < 0.05$ compared to the oe-TRPC5-Normoxia group, $^{\&}P < 0.05$ compared to the the oe-NC-IH group.

$P<0.05$). These results collectively indicated that TRPC5 overexpression exacerbates cardiomyocyte injury by inducing intracellular Ca^{2+} overload and increasing ROS levels.

Overexpression of TRPC5 Promoted Mitochondrial Damage Induced by IH in H9c2 Cells

To investigate the impact of TRPC5 on mitochondrial structure and function, we examined the MMP, mPTP, and ultrastructural alterations of mitochondria. The findings indicated that IH induced a reduction in MMP and the opening of mPTP, with TRPC5 overexpression exacerbating these effects (Figure 5A–D) (all $P<0.05$). TEM results revealed that IH led to the loss of mitochondrial cristae, which was further intensified by TRPC5 overexpression (Figure 5E), suggesting that TRPC5 overexpression contributes to mitochondrial damage in H9c2 cells following IH.

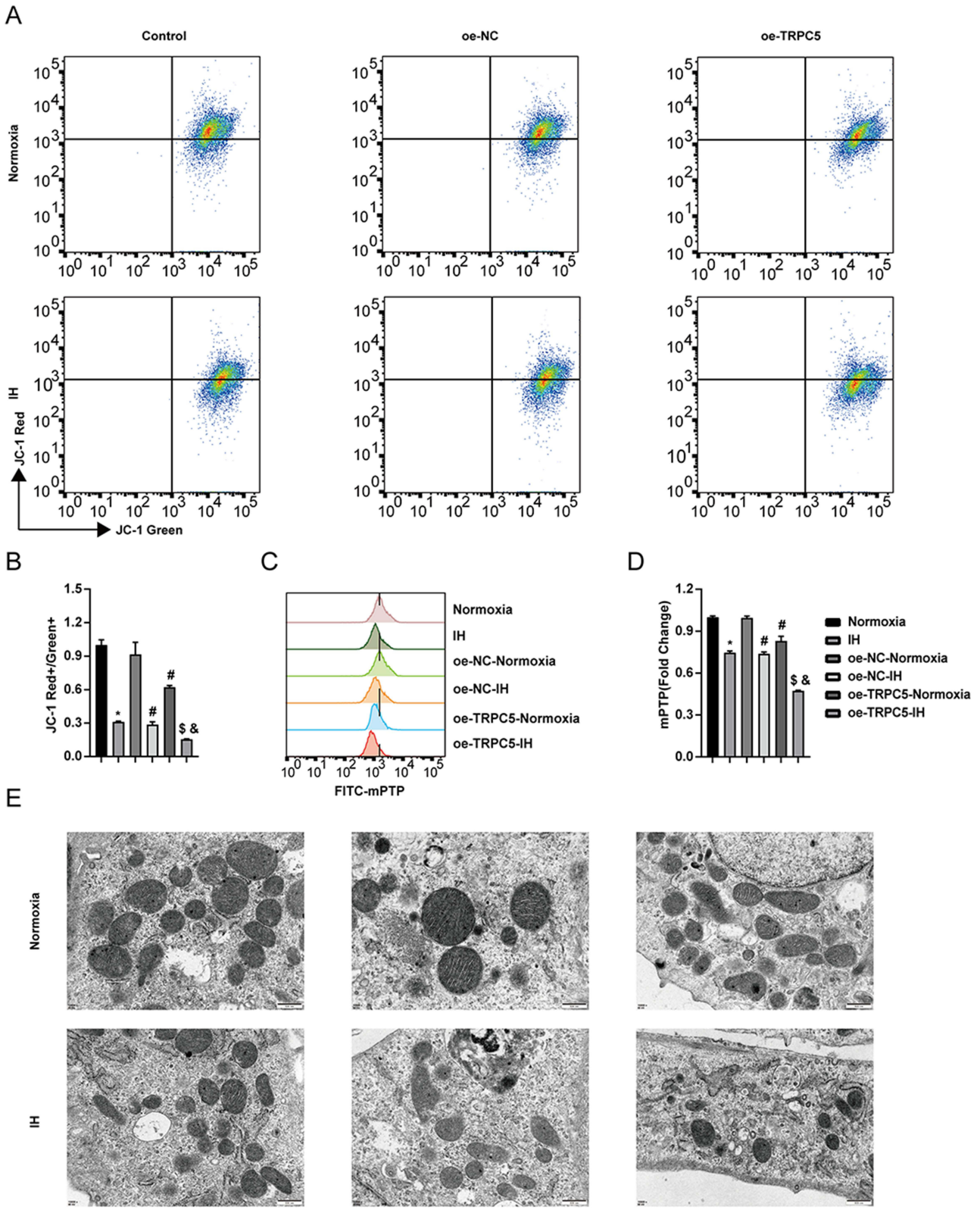

Figure 5 Overexpression of TRPC5 promoted mitochondrial damage induced by IH in H9c2 cells. Flow cytometry was used to measure the MMP (**A–B**) and mPTP (**C–D**) levels. (**E**) TEM images of mitochondria. *$P < 0.05$ compared to the Normoxia group, $^{\#}P < 0.05$ compared to the oe-NC-Normoxia group, $^{\$}P < 0.05$ compared to the oe-TRPC5-Normoxia group, $^{\&}P < 0.05$ compared to the the oe-NC-IH group.

NAC Alleviated the Damage to H9c2 Cells Caused by TRPC5 Overexpression Under IH

Since OxS is related to myocardial injury induced by IH, and TRPC5 is associated with OxS, we treated the H9c2 cells with NAC, a ROS inhibitor, to explore the mechanism of TRPC5 on cardiomyocyte injury during IH. The CCK8 assay revealed that NAC mitigated the decline in cell viability prompted by TRPC5 overexpression during IH (Figure 6A) ($P<0.05$). Flow cytometry assessed the apoptotic rate in each group, revealing NAC alleviating the apoptosis induced by TRPC5 overexpression under IH (Figure 6B–C)($P<0.05$). Additionally, WB results revealed the expression of apoptosis-related proteins. The findings indicated NAC's inhibition of the upregulation of Bax and Caspase-3, as well as the downregulation of Bcl-2 induced by TRPC5 overexpression following IH (Figure 6D–H)(all $P<0.05$). These results indicated the potential of NAC to alleviate H9c2 cell apoptosis resulting from TRPC5 overexpression during IH.

Flow cytometry was utilized to measure intracellular Ca^{2+} and ROS levels. The results revealed that NAC inhibited the increase in Ca^{2+} (Figure 7A–B) and ROS levels (Figure 7C–D) induced by TRPC5 overexpression after IH(all $P<0.05$).

We also assessed the MMP, mPTP, and ultrastructural changes of mitochondria. The findings revealed that NAC mitigated the decrease in MMP (Figure 8A–B) and the opening of mPTP (Figure 8C–D) in H9c2 cells induced by TRPC5 overexpression after IH(all $P<0.05$). The TEM results showed that NAC could alleviate the disappearance of mitochondrial cristae caused by TRPC5 overexpression under IH (Figure 8E).

All of the results indicated that inhibiting oxidative stress could alleviate the cardiomyocyte injury caused by TRPC5 overexpression under IH.

Discussion

Our study demonstrated that TRPC5 was upregulated and was related to structural and functional damage of the heart in OSA patients. It also showed that IH promoted cardiomyocyte apoptosis and upregulated TRPC5 expression. Overexpression of TRPC5 further promoted IH-induced cardiomyocyte apoptosis, disturbed Ca^{2+} metabolism, oxidative stress, and mitochondrial damage, whereas inhibition of oxidative stress partially reversed these changes.

Myocardial injury is a common complication of OSA, potentially leading to heart failure.[30] OSA has been linked to myocardial injury and diastolic dysfunction, with its severity positively correlated with diastolic dysfunction.[31] Additionally, left atrial volume is positively associated with OSA severity.[32] Our study revealed a larger LAD and lower E/A ratio in OSA patients, indicating myocardial injury, consistent with previous research. However, the mechanism of myocardial injury in OSA remains unclear.

TRPC5, as a non-selective cation channel, primarily promotes Ca^{2+} influx through protein tyrosine kinases or G protein-coupled receptors and is important in the calcium signaling pathway.[22] Studies have shown that TRPC5 is involved in myocardial injury. TRPC5 was found to be increased in the cardiac tissue of individuals suffering from end-stage heart failure.[19] TRPC5 knockout attenuated cholinergic-induced tachycardia, myocardial fibrosis, and cardiac hypertrophy.[33] Nevertheless, there has been no investigation into the correlation between TRPC5 and myocardial injury in OSA. Our research marks the inaugural effort to assess the association between OSA, myocardial injury, and TRPC5 levels in peripheral blood. The results showed that TRPC5 levels in PBMCs of OSA patients are associated with myocardial injury, suggesting that TRPC5 may be involved in myocardial injury in OSA.

Apoptosis is a natural biological process in which cells undergo programmed cell death following self-injury. Increased apoptosis is a manifestation of cellular injury, which is closely related to Ca^{2+} homeostasis.[34] As a second messenger, Ca^{2+} establishes a sophisticated and comprehensive network for information exchange between the mitochondria and endoplasmic reticulum, participating in various processes such as cell apoptosis, proliferation, and energy metabolism.[35] TRPC5 has been implicated in apoptosis. Knockout of TRPC5 alleviated neuronal apoptosis in mice induced by cerebral ischemia-reperfusion.[36] TRPC5 was involved in the injury and apoptosis of SH-SY5Y cells induced by $CaCl_2$.[37] HC070, an inhibitor of TRPC5, could suppress apoptosis in SH-SY5Y cells.[23] TRPC5 participated in the apoptosis of thyroid cancer via the Wnt/β-catenin pathway.[38] Our study demonstrated that IH promoted apoptosis and upregulated TRPC5 expression, indicating the potential role of TRPC5 in regulating apoptosis in H9c2 cells.

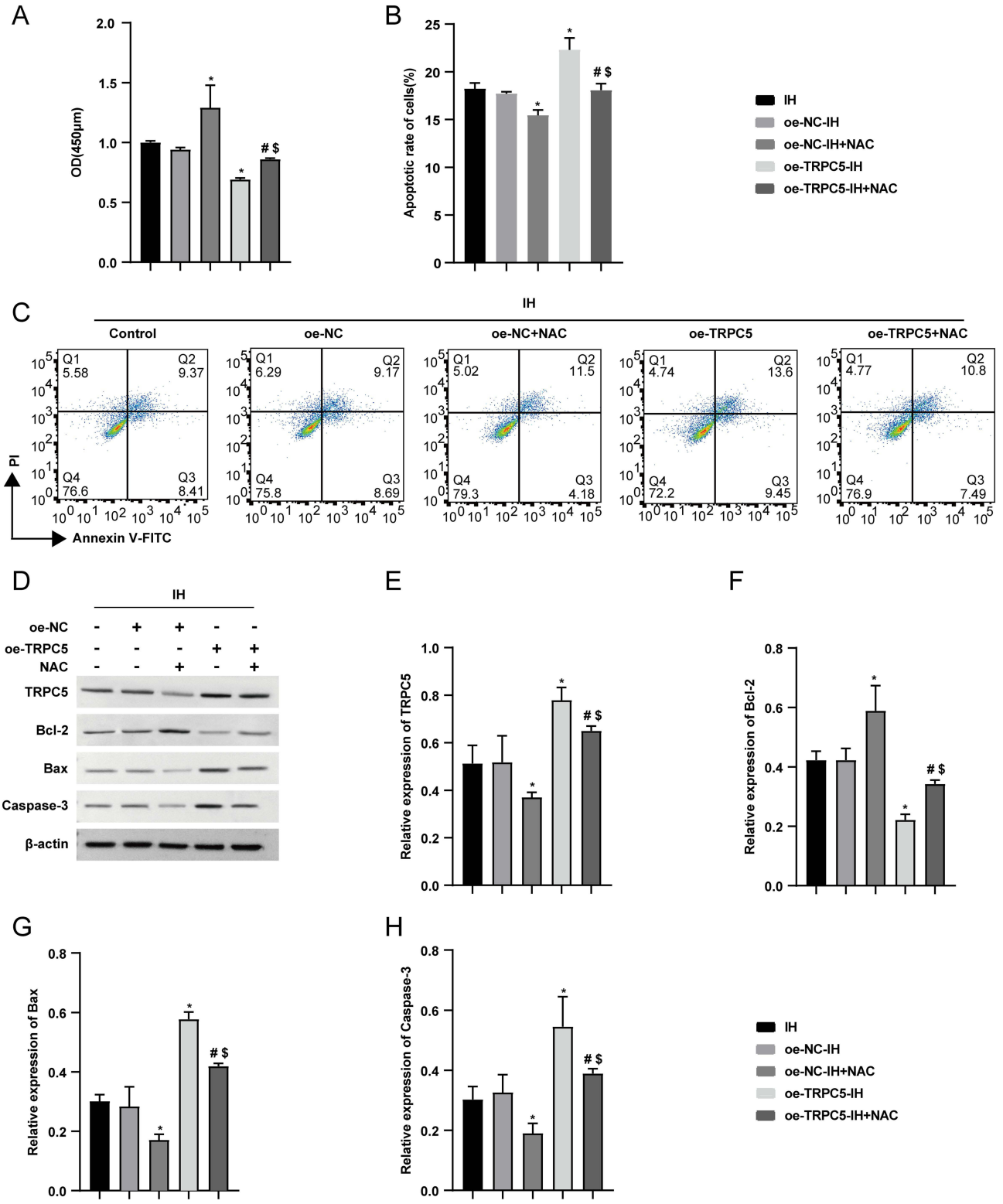

Figure 6 NAC mitigated H9c2 cell apoptosis induced by TRPC5 overexpression during IH. (**A**) H9c2 cells viability were determined using the CCK-8 assay. Flow cytometry was used to assess the apoptotic rates of H9c2 cells, including representative figures (**C**) and analysis (**B**). (**D–H**) WB results analyzed relative expression levels of TRPC5, Bcl-2, Bax, and Caspase-3. $^{*}P < 0.05$ compared to the oe-NC-IH group, $^{\#}P < 0.05$ compared to the oe-TRPC5-IH group, $^{\$}P < 0.05$ compared to the oe-NC-IH+NAC group.

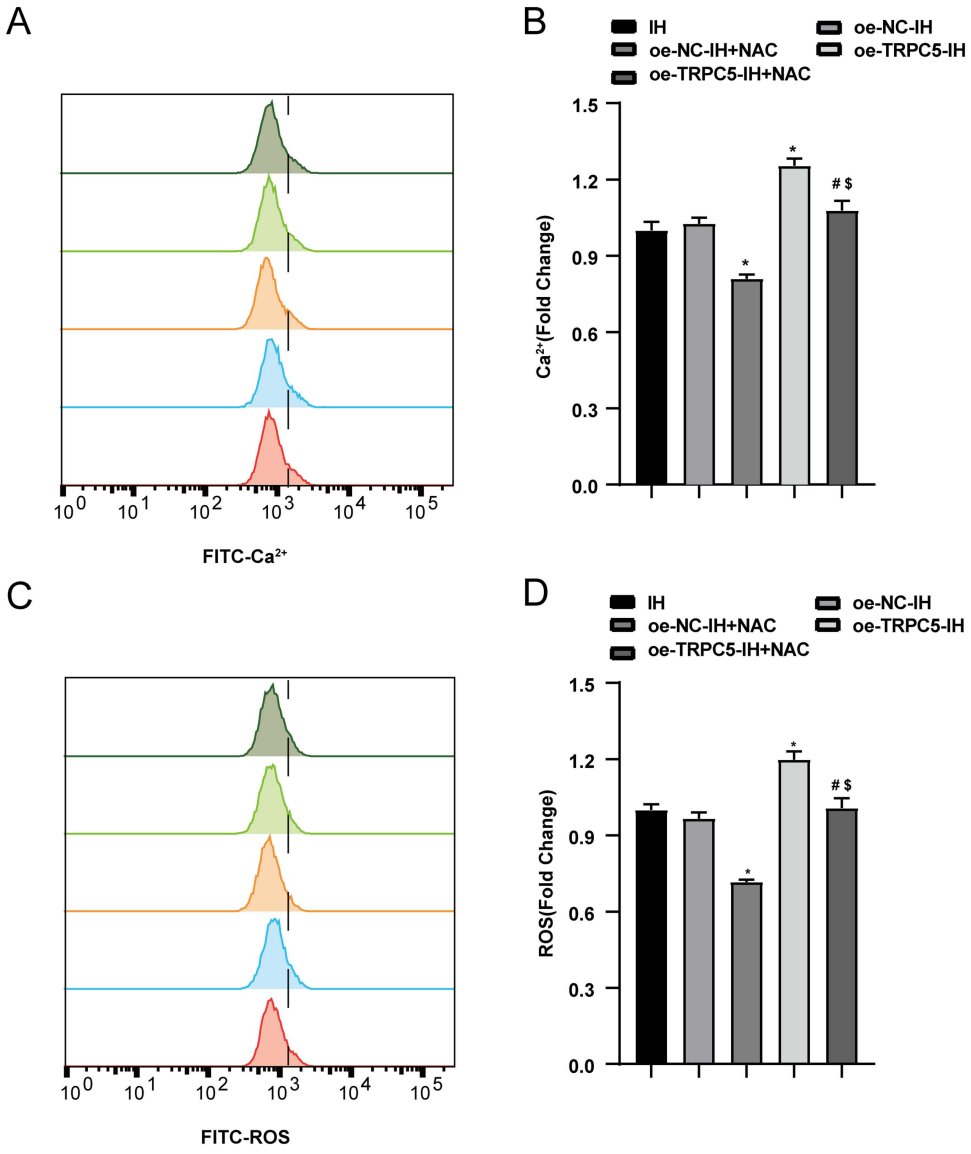

Figure 7 NAC alleviated the Ca^{2+} influx in H9c2 cells caused by TRPC5 overexpression under IH. Flow cytometry was used to measure Ca^{2+} concentration(**A–B**) and ROS production (**C–D**). $^{*}P < 0.05$ compared to the oe-NC-IH group, $^{\#}P < 0.05$ compared to versus the oe-TRPC5-IH group, $^{\$}P < 0.05$ compared to the oe-NC-IH+NAC group.

Overexpression of TRPC5 promoted apoptosis, mitochondrial damage, and elevated levels of Ca^{2+} and ROS. These findings align with previous studies.

Mitochondria are essential sources of cellular energy, and their abundance is high in myocardial tissue,[39] and closely linked to cardiovascular diseases.[40] Primarily, mitochondria generate energy through oxidative phosphorylation, playing a crucial role in maintaining cellular metabolic balance and vitality, with Ca^{2+} serving as a significant regulatory factor in this process.[39] When the concentration of Ca^{2+} remains within the normal range, mitochondria activate normally, sustaining stable respiratory chain function. However, excessive Ca^{2+} concentration can induce mitochondrial damage, resulting in decreased MMP and mPTP opening, ultimately leading to cell death.[41] TRPC5, a non-selective cation channel, is associated with mitochondrial damage. Blocking TRPC5 expression partially reversed the decrease in mPTP caused by MPP^{+} in SH-SY5Y cells.[42] Clemizole, a TRPC5 inhibitor, could ameliorate the decline in MMP and mitochondrial biosynthesis in Parkinson's rat models.[43] In our study, TRPC5 overexpression exacerbated MMP decline, mPTP opening, and mitochondrial cristae disappearance, suggesting that mitochondrial damage occurred, which is related to OxS.[44]

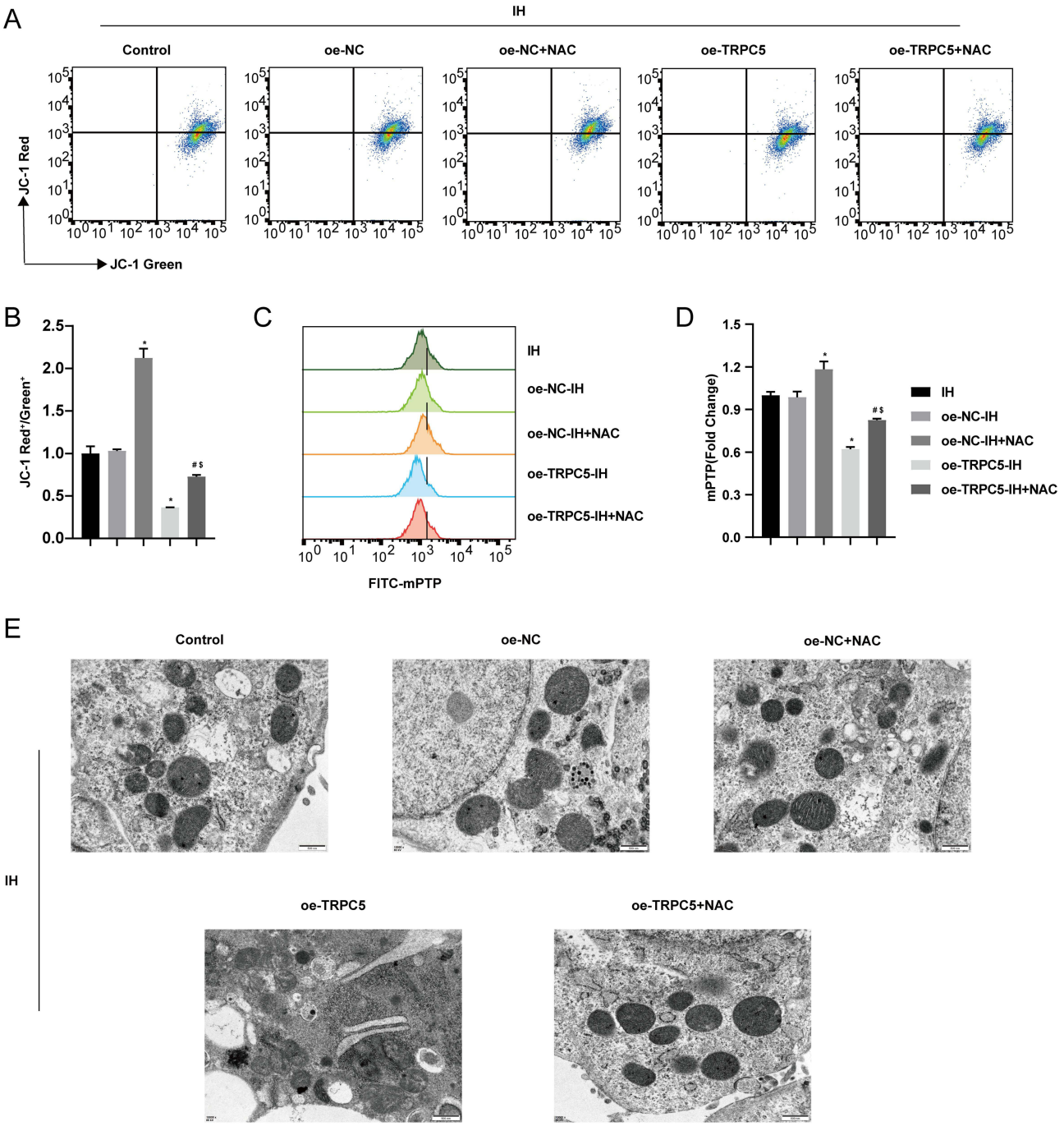

Figure 8 NAC alleviated the mitochondrial damage in H9c2 cells caused by TRPC5 overexpression under IH. Flow cytometry assessed the MMP (**A–B**) and mPTP opening (**C–D**). (**E**) TEM images of mitochondria. *$P < 0.05$ compared to the oe-NC-IH group, $^{\#}P < 0.05$ compared to the oe-TRPC5-IH group, $^{\$}P < 0.05$ compared to the oe-NC-IH +NAC group.

OxS arises from the harmful impacts of an imbalance between oxidative and antioxidant mechanisms[45] and is considered a key factor contributing to heart-related issues in individuals with OSA.[46] IH upregulated ROS levels in the heart of rats and promoted cardiomyocyte apoptosis, resulting in cardiac structural and functional damage.[24,47] This indicates that ROS levels may be the cause of IH-induced myocardial injury. It has been demonstrated that ROS levels are regulated by Ca^{2+}, and an imbalance in Ca^{2+} homeostasis results in OxS,[48] which eventually leads to injury. Disturbed Ca^{2+} metabolism promoted ROS production by affecting the electron transport chain,[49] which opened the mPTP, decreased the MMP, and ultimately led to apoptosis.[50] Our research illustrated that IH increased Ca^{2+}

concentrations and ROS, hindered cell viability, and induced apoptosis in H9c2 cells, suggesting a relationship between OxS and myocardial injury.

Studies have shown that TRPC5 is associated with OxS. TRPC5 knockout alleviated neuronal death due to the influx of Ca^{2+} and OxS.[51] Through depalmitoylation, TRPC5 shielded striatal neurons from OxS in Huntington's disease.[52] NU6027, an inhibitor of TRPC5, could alleviate the influx of Ca^{2+}, oxidative stress, and the loss of hippocampal dendrites induced by traumatic brain injury.[53] The present study revealed that TRPC5 promoted the influx of Ca^{2+} and the generation of ROS, suggesting that TRPC5 promotes OxS. To further elucidate the mechanism of TRPC5 and OxS, the ROS inhibitor NAC was used to inhibit OxS. After treatment with NAC, the apoptosis rate and cell viability significantly improved, the levels of Ca^{2+} decreased, and mitochondrial damage was alleviated.

However, our study has limitations: (1)The sample size of clinical data was small, which only demonstrated the correlation between TRPC5 and altered cardiac structural function in patients with OSA. (2)We did not conduct experiments with TRPC5 inhibitors to validate their impact on IH-induced myocardial injury. For future research, we need to expand the sample size and conduct a multicentre study to further validate our findings. Meanwhile, we need to use TRPC5 inhibitors for in vivo and in vitro experiments to validate our findings and enhance their credibility.

Conclusions

Our study is the first to investigate the relationship between TRPC5 and OSA-induced myocardial injury. The study revealed that TRPC5 is associated with myocardial injury in OSA patients. IH promoted apoptosis, and TRPC5 may exacerbate the injury by increasing ROS generation, worsening mitochondrial damage, and disrupting calcium homeostasis. This further elucidates the mechanism of TRPC5 in myocardial injury, suggesting that TRPC5 may be a promising target for the diagnosis and treatment of cardiovascular disease caused by OSA.

Data Sharing Statement

The data that support the findings of this study are available.

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Disclosure

The authors declare no conflicts of interest.

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