Role of the TRPM4 channel in mitochondrial function, calcium release, and ROS generation in oxidative stress

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ABSTRACT

Ischemic heart disease is one of the most common causes of death worldwide. Mitochondrial dysfunction, excessive reactive oxygen species (ROS) generation, and calcium (Ca^{2+}) overload are three key factors leading to myocardial death during ischemia-reperfusion (I/R) injury. Inhibition of TRPM4, a Ca^{2+}-activated nonselective cation channel, protects the rat heart from I/R injury, but the specific mechanism underlying this effect is unclear. In this study, we investigated the mechanism of cardioprotection against I/R injury via TRPM4 using hydrogen peroxide (H_2O_2), a major contributor to oxidative stress, as an I/R injury model. We knocked out the *TRPM4* gene in the rat cardiomyocyte cell line H9c2 using CRISPR/Cas9. Upon H_2O_2 treatment, intracellular Ca^{2+} level and ROS production increased in wild type (WT) cells but not in TRPM4 knockout (TRPM4^KO) cells. With this treatment, two indicators of mitochondrial function, mitochondrial membrane potential ($\Delta\Psi m$) and intracellular ATP levels, decreased in WT but not in TRPM4^KO cells. Taken together, these findings suggest that blockade of the TRPM4 channel might protect the myocardium from oxidative stress by maintaining the mitochondrial membrane potential and intracellular ATP levels, possibly through preventing aberrant increases in intracellular Ca^{2+} and ROS.

1. Introduction

Ischemic heart disease (IHD) is one of the most common causes of death worldwide. Currently, IHD causes more than 30% of the annual deaths of people over 35 years of age [1]. Thus, IHD is a major health problem in imminent need of solutions. The pathology underlying IHD involves atherosclerosis of the coronary artery caused by plaques. Plaque accumulation narrows the artery, resulting in reduced or disrupted blood flow that alters oxygen metabolism in the heart, leading to a heart attack [2]. At present, percutaneous coronary intervention and coronary artery bypass graft are commonly used to resolve coronary artery blockage. Over the past 3 decades, these procedures have decreased IHD mortality dramatically by decreasing the infarct area [3]. However, mounting clinical evidence demonstrates severe adverse events following reperfusion, including myocardial stunning, microvascular obstruction, fatal arrhythmias, acute cardiac failure, and sudden death. Collectively, these outcomes are known as 'reperfusion injury.' Thus, ischemia-reperfusion (I/R) is recognized as a double-edged sword that can rescue the ischemic myocardium but also carries a risk of clinical complications that decrease patient survival [4—6].

The mechanism underlying myocardial ischemia-reperfusion (I/R) injury is complex and not fully understood. A decrease in myocardial oxygen caused by an ischemic event leads to mitochondrial dysfunction that results in attenuation of the mitochondrial membrane potential ($\Delta\Psi m$). This mitochondrial membrane depolarization leads to adenosine triphosphate (ATP) depletion and calcium (Ca^{2+}) overload, resulting in cell death [7,8]. Meanwhile, oxidative stress response is an important feature of myocardial I/R injury, which is manifested to generate reactive oxygen species (ROS). Although ischemic injury is initially resolved with the return of oxygen (O_2), a burst of ROS occurs during reperfusion that causes oxidative damage to lipids, proteins, and DNA. Increased ROS also causes further loss of mitochondrial function [9—11]. Thus, mitochondrial dysfunction, Ca^{2+} overload, and ROS overproduction are three key factors involved in the development of myocardial I/R

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injury.

The transient receptor potential melastatin member 4 (TRPM4), a nonselective monovalent cation channel, is impermeable to Ca^{2+} but regulated by the intracellular Ca^{2+} concentration [12]. The TRPM4 channel opens in response to increased intracellular Ca^{2+}, causing sodium (Na^+) influx that leads to membrane depolarization. These changes cause gradual swelling of cells that eventually leads to cell death [13]. ATP also mediates the activity of TRPM4 channels, which open in response to low intracellular ATP concentrations [13,14]. A study by Gerzanich et al. reports that low intracellular ATP led to the death of COS-7 cells expressing TRPM4 [15]. Taken together, these observations indicate that ATP modulates TRPM4 activity.

Our previous study showed that inhibiting TRPM4 with 9-phenanthrol rescued rat myocardial I/R damage, as evidenced by decreases in LDH level, infarct size, and ventricular fibrillation [16,17]. The specific mechanism whereby TRPM4 inhibition protects against myocardial I/R injury is unknown. Here, we hypothesize that blockade of TRPM4 channels in cardiomyocytes during I/R affects 1) $\Delta\Psi m$, 2) mitochondrial ATP production, 3) intracellular Ca^{2+} level, and 4) intracellular ROS production. To test this hypothesis, we performed CRISPR knockout of the TRPM4 protein in rat H9c2 cardiomyocytes and treated the cells with exogenous oxidative stress stimulus, hydrogen peroxide (H_2O_2), to mimic I/R injury.

2. Materials and methods

2.1. Cell culture

The H9c2 rat cardiomyocyte line was purchased from American Type Cell Culture (Manassas, VA, USA). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA) in a humidified incubator (5% CO_2, 20% O_2) at 37 °C. Wild type (WT) and TRPM4 knockout (TRPM4[KO]) cells were seeded into 96-well plates (10^4 cells/well) for detecting level of cell viability, $\Delta\Psi m$, ATP, and Ca^{2+}. For the detection of ROS production, the cells were seeded into 48-well plates (2×10^4 cells/well), grown to 80% confluence.

2.2. Oxidative stress stimulation with H_2O_2

To mimic I/R injury, cells were treated with 500 μM H_2O_2 in DMEM supplemented with 10% FBS to induce cell damage (100 μl/well). Untreated control cells were incubated in fresh DMEM with 10% FBS at 37 °C for 4 h (100 μl/well). Cell viability, $\Delta\Psi m$, ATP concentration, intracellular Ca^{2+} concentration, and ROS production were compared between WT and TRPM4[KO] cells.

2.3. MTT assay of cell viability

The viability of WT and TRPM4[KO] cells was quantified using the MTT Cell Proliferation Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA). After 4 h of H_2O_2 treatment as described, the medium containing H_2O_2 was replaced with fresh DMEM (100 μl/well), and 10 μl MTT reagent (5 mg/ml) was added to each well. Samples were incubated in the dark at 37 °C for 3 h. The medium containing MTT reagent was removed, and the formazan crystals in each well were dissolved in 200 μl of dimethyl sulfoxide (Nacalai Tesque, Japan). The absorbance of the solution in each well was measured with a microplate reader at a wavelength of 570 nm.

2.4. Measurement of mitochondrial membrane potential ($\Delta\Psi m$)

The $\Delta\Psi m$ was determined using the JC-1 Mitochondrial Membrane Potential Assay Kit (Cayman Chemical Company, Ann Arbor, MI, USA). After 4 h of H_2O_2 exposure, the medium was replaced with JC-1 staining solution (100 μl/well). The cells were incubated in the dark at 37 °C for 20 min. The solution was replaced with 100 μl fresh DMEM, and JC-1 fluorescence was detected by fluorescence microscopy. Briefly, the JC-1 dye is a lipophilic, cationic dye (naturally exhibiting green fluorescence) that is extensively used for monitoring mitochondrial health in apoptosis studies. This dye enters the mitochondria, where it accumulates and forms reversible complexes called J aggregates. Healthy cells with a normal $\Delta\Psi m$ are detected as red fluorescent J-aggregates (excitation/emission = 540/570 nm). In contrast, J-monomers, which fluoresce green (excitation/emission = 485/535 nm), are formed in apoptotic or damaged cells due to insufficient intake of the JC-1 dye. The ratio of J-aggregates to J-monomers (red/green) represents the $\Delta\Psi m$, with mitochondrial depolarization indicated by a lower ratio [18]. The fluorescence intensity was measured using Image-J.

2.5. Measurement of ATP level

Intracellular ATP was measured using the Luminescent ATP Detection Assay Kit (Abcam, USA). After 4 h of H_2O_2 application, the medium in each well was substituted with 50 μl of detergent solution and shaken for 10 min in a bioshaker at 300 rpm. Reconstituted substrate solution (50 μl) was added to each well and shaken on a bioshaker at 300 rpm for 10 min. The samples were kept at room temperature in the dark for 10 min, and the luminescence was visualized and the intensity was measured using a Fujifilm LAS-3000 Imager (FUJIFILM, Japan).

2.6. Measurement of intracellular Ca^{2+}

Intracellular Ca^{2+} was measured using Fura-2-AM dye (Life technologies, USA). The cells were loaded with 5 μM Fura-2 reagent (100 μl/well) after H_2O_2 treatment, then incubated at 37 °C for 1.5 h, and washed twice with standard external solution (140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2 \cdot 2H_2O$, 10 mM D-glucose, and 10 mM HEPES, pH 7.4), to remove extracellular dye and maintained in standard external solution (100 μl/well). Fura-2 fluorescence was measured at wavelengths of 340 nm and 380 nm, and the intensity of fluorescence was calculated (AquaCosmos 2.6 analysis system).

2.7. Reactive oxygen species production assay

The level of intracellular ROS production in WT and TRPM4[KO] cells was determined using CM-H_2DCFDA (Invitrogen, Carlsbad, CA, USA), a chloromethyl derivative of H_2DCFDA. After 4 h of treatment with 500 μM H_2O_2, all cells were incubated with CM-H_2DCFDA (final concentration, 1 μM) in the dark at 37 °C for 20 min and rinsed twice with phosphate-buffered saline (PBS, Nissui Pharmaceutical, Japan). ROS production was assessed by fluorescence intensity using a fluorescence microscope (excitation/emission = 485/535 nm). The fluorescence intensity was measured using Image-J.

2.8. Statistical analysis

All data are presented as the mean ± standard error of the mean and were analyzed using Prism software (version 8.0, Graphpad Software, USA). Comparisons between two groups were conducted using Student's t-test. Results were considered statistically significant for p < 0.05.

CRISPR/Cas9 knockout of TRPM4 channels in H9c2 cardiomyocytes, DNA sequencing, and Western blot analysis for confirming knockout are described in [31].

3. Results

3.1. H_2O_2-induced cell death was ameliorated in TRPM4^{KO} H9c2 cells

The viability of H9c2 exposed to 4 h of 500 μM-H_2O_2 was monitored by MTT assay. As shown in Fig. 1A, the WT cells shrunk dramatically and were fewer in number after H_2O_2 treatment. In TRPM4^{KO} cells, no changes were observed in cell morphology or number after H_2O_2 treatment. The viability of TRPM4^{KO} cells was nearly triple than that of WT cells (0.84 ± 0.04 vs. 0.29 ± 0.06, normalized to untreated cells; $p < 0.0001$) after H_2O_2 treatment (Fig. 1B).

3.2. H_2O_2-induced mitochondrial dysfunction was attenuated in TRPM4^{KO} H9c2 cells

The ΔΨm in H9c2 treated with 500 μM H_2O_2 for 4 h was evaluated using JC-1 staining with fluorescence microscopy. JC-1 staining showed an aggregated pattern of energized mitochondria (red fluorescence) and a monomeric pattern for depolarized mitochondria (green fluorescence). As shown in Fig. 2A, compared to untreated cells, WT cells showed remarkably lower red fluorescence and higher green fluorescence after H_2O_2 treatment, indicating significant loss of ΔΨm. In sharp contrast, TRPM4^{KO} cells showed higher red fluorescence and lower green fluorescence, indicating that TRPM4^{KO} cells resisted the H_2O_2-induced ΔΨm depletion. The difference in the normalized fluorescence ratio, an indicator for the ΔΨm, between WT and TRPM4^{KO} was statistically significant (0.28 ± 0.06 vs. 0.72 ± 0.05, respectively; $p < 0.001$) (Fig. 2B).

Meanwhile, another indicator of mitochondrial function, intracellular ATP, was measured as well. After H_2O_2 treatment, the intracellular ATP concentration in TRPM4^{KO} cells was significantly higher than that in WT cells (0.79 ± 0.06 vs. 0.41 ± 0.02,

respectively; $p < 0.001$) (Fig. 2C). These results suggest that mitochondrial function can be protected from oxidative stress by inhibiting TRPM4 channel.

3.3. H_2O_2-induced intracellular Ca^{2+} overload was suppressed in TRPM4^{KO} cells

To investigate the involvement of TRPM4 in the regulation of intracellular Ca^{2+} in response to increased oxidative stress, we compared the intracellular Ca^{2+} levels in response to H_2O_2 exposure between WT and TRPM4^{KO} cells. Although a large increase in intracellular Ca^{2+} was observed in the WT cells in response to H_2O_2 treatment, this response was significantly inhibited in TRPM4^{KO} cells (Fig. 3A). In response to H_2O_2 treatment, the increase in Ca^{2+} was 0.19 ± 0.06 in WT but −0.03 ± 0.03 in TRPM4^{KO} cells, indicating no increase in intracellular Ca^{2+} in TRPM4^{KO} group after H_2O_2 exposure ($p < 0.01$) (Fig. 3B). These results suggest that the intracellular Ca^{2+} overload under oxidative stress involves the TRPM4 channel function.

3.4. H_2O_2-induced increase in ROS production was lower in TRPM4^{KO} cells

To investigate the involvement of the TRPM4 channel in ROS production in response to exogenous ROS exposure, we compared intracellular ROS levels in response to H_2O_2 exposure between WT and TRPM4^{KO} cells. In WT cells, we observed more intense fluorescence in H_2O_2-treated than in untreated cells (4.30 ± 0.14 times higher) (Fig. 4A). In TRPM4^{KO} cells, no significant difference in fluorescence was observed between H_2O_2-treated and untreated cells (2.20 ± 0.63 times higher; $p < 0.05$ vs. WT) (Fig. 4B). These results suggest that the TRPM4 channel is involved in the oxidative stress induced ROS overproduction.

4. Discussion

Previous studies have shown that inhibition of TRPM4 by 9-phenanthrol ameliorates cardiac injury in the rat [16,17]. Although these studies clearly indicate involvement of the TRPM4
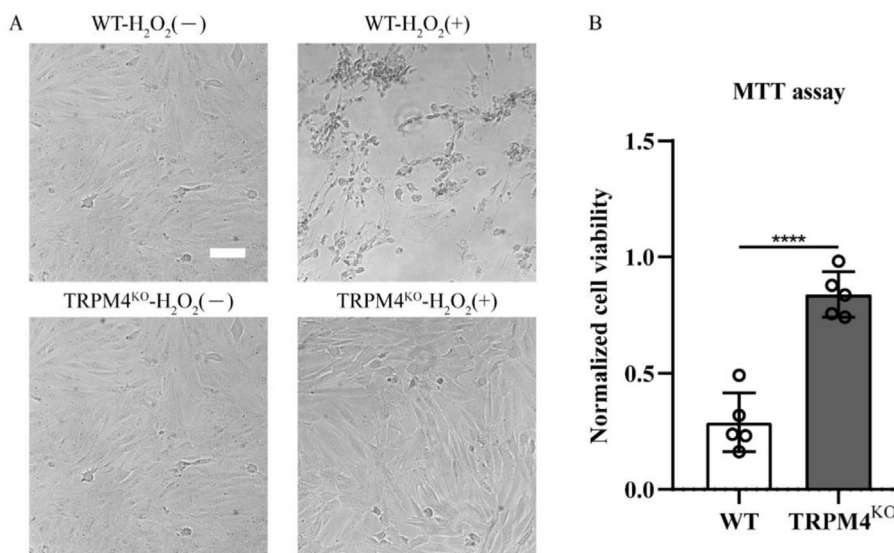

Fig. 1. H_2O_2-induced cell death was ameliorated in TRPM4^{KO} H9c2 cells. (A) The morphology of WT and TRPM4^{KO} cells treated with 500 μM-H_2O_2 for 4 h was observed via microscopy. Scale bar: 100 μm. (B) Cell viability as determined by MTT assay. After H_2O_2 exposure, cell viability was significantly higher in TRPM4^{KO} cells than in WT cells. Cell viability was normalized by dividing the absorbance of cells treated with H_2O_2 by that of untreated cells. Chart represents average of five independent experiments. Error bars represent SEM. ****$p < 0.0001$ vs WT.
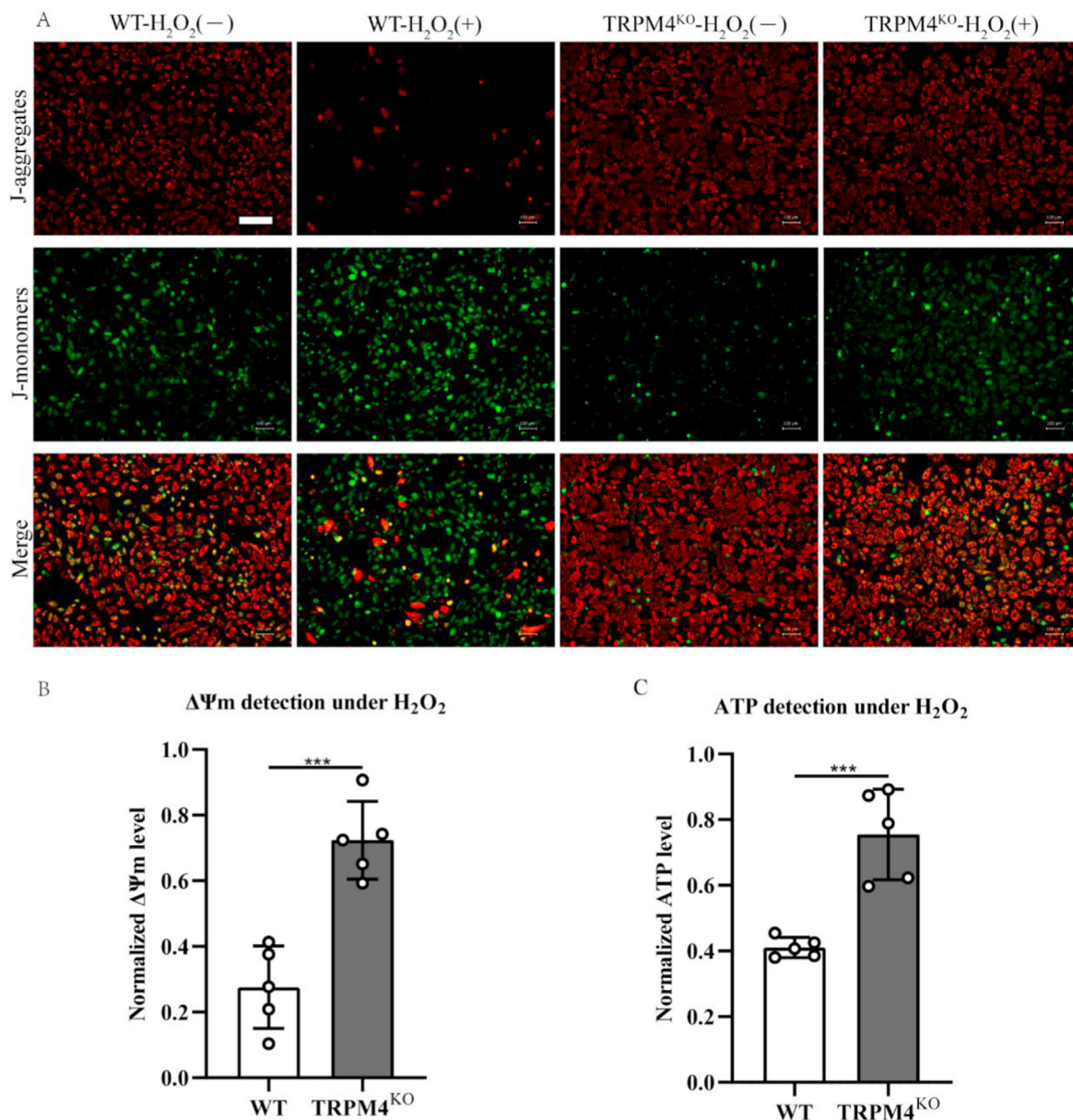
Fig. 2. H_2O_2-induced mitochondrial dysfunction was attenuated in TRPM4[KO] H9c2 cells. (A) Change in $\Delta\Psi m$ in WT and TRPM4[KO] cells after treatment with 500 μM H_2O_2 for 4 h was observed via fluorescence microscopy. JC-1 staining showed an aggregated pattern of energized mitochondria (red fluorescence) and a monomeric pattern for depolarized mitochondria (green fluorescence). Scale bar: 200 μm. (B) The $\Delta\Psi m$ was substantially preserved after H_2O_2 exposure in TRPM4[KO] cells. $\Delta\Psi m$ values were normalized by dividing the fluorescence intensity of H_2O_2-treated cells by that of untreated cells. (C) The level of ATP was effectively preserved in TRPM4[KO] cells after H_2O_2 exposure. The ATP level was normalized by dividing the luminescent intensity of H_2O_2-treated cells by that of untreated cells. Chart represents average of five independent experiments. Error bars represent SEM. ***$p < 0.001$ vs WT. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

channel in the pathogenesis of I/R injury, the underlying mechanism of such involvement is unclear. In this study, we investigated the mechanism of cardioprotection against I/R injury via TRPM4 using H_2O_2 as an I/R injury model in rat cardiomyocytes with and without TRPM4 knockout. Upon H_2O_2 treatment, intracellular calcium level and ROS production increased in WT but not TRPM4 knockout cells; in addition, the $\Delta\Psi m$ and intracellular ATP levels, indicators of mitochondrial function, both decreased in WT but not TRPM4 knockout cells. Taken together, these findings suggest that blockade of the TRPM4 channel protects the myocardium from oxidative stress damage by maintaining mitochondrial function, possibly by preventing aberrant increases in intracellular calcium and ROS.

In the mechanism underlying myocardial I/R injury, mitochondrial dysfunction and a significant accumulation of Ca^{2+} are triggers of cell apoptosis and damage. Impaired mitochondria usually exhibit membrane depolarization and ATP depletion [7]. According to Nilius et al. the TRPM4 channel is activated by an increase in intracellular Ca^{2+} and a decrease in ATP, leading to intracellular Na^+ overload, ending in cell death [19]. H_2O_2 induces sustained TRPM4 activity, resulting in deleterious processes that lead to cell death [20]. Thus, ischemia-reperfusion may activate TRPM4, further exacerbating I/R injury by promoting sodium influx. Thus, inhibiting the TRPM4 channel may prove useful for preventing a variety of I/R injuries. The following discussion addresses the protective effect of TRPM4 inhibition against myocardial I/R injury with respect to
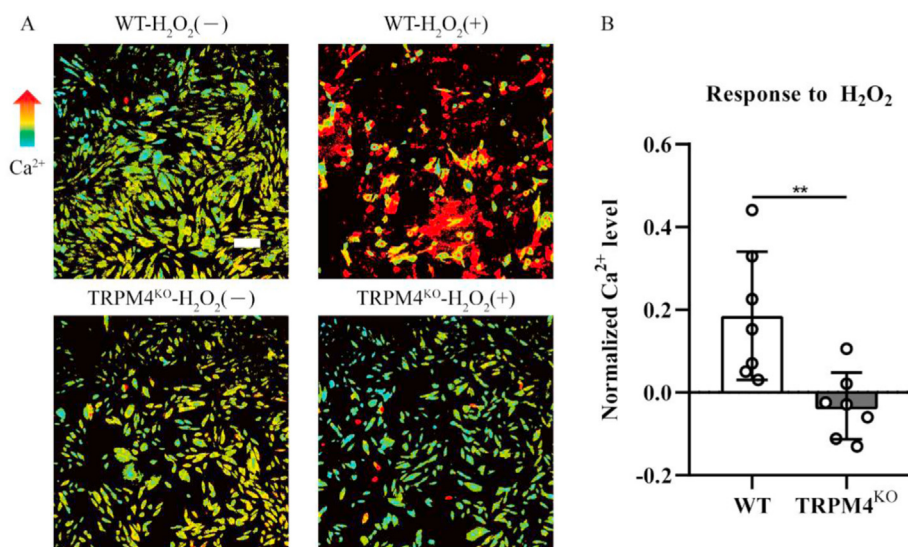
Fig. 3. H_2O_2-induced accumulation of intracellular Ca^{2+} was prevented in TRPM4[KO] H9c2 cells. (A) Changes in Ca^{2+} concentration in WT and TRPM4[KO] cells treatment with 500 μM H_2O_2 for 4 h was determined by fluorescence microscopy. Scale bar: 200 μm. (B) TRPM4 knockout in H9c2 cardiomyocytes prevented the intracellular Ca^{2+} overload under oxidative stress induced by H_2O_2. Ca^{2+} levels were normalized by dividing the difference in fluorescence intensity between H_2O_2-treated and untreated cells by the fluorescence intensity of untreated cells. Chart represents average of seven independent experiments. Error bars represent SEM. **p < 0.01 vs WT.
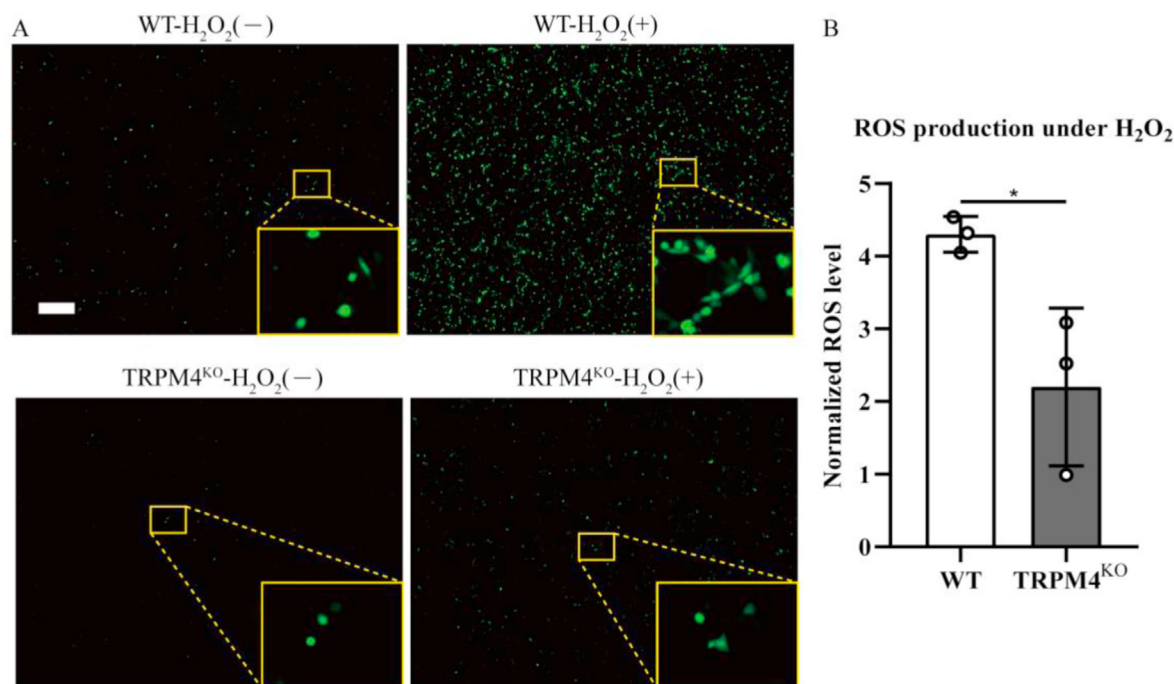
Fig. 4. H_2O_2-induced ROS production was lower in TRPM4[KO] cells. (A) Comparison of ROS production (green fluorescence) between WT and TRPM4[KO] cells treated with 500 μM H_2O_2 for 4 h using fluorescence microscopy. Scale bar: 100 μm. (B) The accumulation of ROS in response to H_2O_2 exposure was suppressed effectively in TRPM4[KO] cells. ROS levels were normalized by dividing the difference in fluorescence intensity of cells treated with and without H_2O_2 by that of untreated cells. *p < 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

mitochondrial function, Ca^{2+} overload, and ROS accumulation.

4.1. Mitochondrial function

Consistent with our hypothesis, under H_2O_2 exposure, a common chemical model of I/R injury, the mitochondria of WT H9c2 cardiomyocytes with intact TRPM4 activity showed a sharp decline in $\Delta\Psi m$. Conversely, the $\Delta\Psi m$ was preserved in TRPM4 knockout cardiomyocytes treated with H_2O_2 (Fig. 2A–B). As the $\Delta\Psi m$ is a known indicator of mitochondrial activity [21], this observation suggests that the decrease in mitochondrial membrane depolarization resulting from TRPM4 inhibition reflects the protection of mitochondrial function.

Impaired mitochondrial function decreases ATP production. After H_2O_2 treatment, the ATP level in TRPM4[KO] cells was almost twice than that of WT cells (Fig. 2C), indicating that TRPM4

inhibition preserves intracellular ATP production in the presence of H_2O_2. The health of cardiomyocytes depends on an adequate energy supply, which decreases during the process of I/R injury [11,22]. Thus, maintenance of energy is crucial to prevent myocardial I/R damage. Previous studies have shown that a sufficient ATP supply can suppress cell death related to TRPM4 activity [13,23]. Thus, the maintenance of normal intracellular ATP level by functional mitochondria decreases the risk of cell death from TRPM4 activation under oxidative stress. In summary, inhibiting the expression or activity of TRPM4 may be a useful option to rescue ROS-induced mitochondrial dysfunction and prevent H9c2 cardiomyocyte deaths.

4.2. Calcium overload

TRPM4 channel activity induced by elevated intracellular Ca^{2+} has been proposed to play a role in cell apoptosis and necrosis [13]. Intracellular Ca^{2+} overload is a major mediator of cell damage or death during myocardial I/R injury. The sodium-calcium exchanger (NCX) is a well-characterized ion transport protein expressed on the plasma membranes of all types of animal cells. Under normal physiological conditions, the NCX of cardiomyocytes maintains low intracellular Ca^{2+} concentrations by removing Ca^{2+} from the cell, thereby effectively regulating cardiac conduction and contractile function [24,25]. During ischemia-reperfusion, NCX can work in reverse, pumping Ca^{2+} into the cell, resulting in a rise in intracellular Ca^{2+}. This pathological process is attributed to an I/R-induced increase in H^+ concentration that activates the Na^+-H^+ exchanger to promote H^+ extrusion, leading to an increase in intracellular Na^+. The reverse-mode of NCX is activated by this elevated intracellular Na^+, causing intracellular Ca^{2+} overload [10,25,26]. Thus, the NCX is an important mediator of intracellular Ca^{2+} overload in myocardial I/R injury.

Because TRPM4 is impermeable to Ca^{2+}, cell death resulting from TRPM4 activation is usually attributed to a rise in intracellular Na^+. An alternative hypothesis is that sustained intracellular Na^+ overload caused by TRPM4 activity causes NCX to work in reverse to cause Ca^{2+} accumulation in the cell that eventually leads to cell death [13]. Salcedo et al. reported that high level of intracellular Ca^{2+} in neurons during cerebral ischemia-reperfusion resulted in constitutive activity of TRPM4, resulting in neuron depolarization and eventual death [27]. Thus, accumulation of intracellular Ca^{2+} may be a common and important feature of I/R injury and TRPM4 activation. We observed that after H_2O_2 treatment, intracellular Ca^{2+} level increased approximately 20% in WT cells but decreased in TRPM4^{KO} cells (Fig. 3). This observation shows that the intracellular Ca^{2+} overload induced by oxidative stress was completely prevented by knockout of TRPM4 in H9c2 cardiomyocytes.

The initial ATP loss in myocardial I/R injury is caused by ATP consumption during anaerobic metabolism. This alteration hinders the activity of ATPases (Na^+/K^+ ATPase, sarcoplasmic reticulum/ endoplasmic reticulum Ca^{2+} ATPase) and also disrupts the transport of Ca^{2+} to the endoplasmic reticulum. As a consequence, Ca^{2+} accumulates in the cell, leading to Ca^{2+} overload. The mitochondrial permeability transition pore opens in response to the rise in intracellular Ca^{2+}, accelerating mitochondrial depolarization and causing a marked decrease in intracellular ATP [7,10]. Thus, a decrease in TRPM4 activity suppresses intracellular Ca^{2+} accumulation and may help to rescue dysfunctional mitochondria, preventing cell death under oxidative stress.

4.3. ROS overproduction

The results of this study and others suggest that the TRPM4 channel plays a role in the cellular injury mediated by ROS. Becerra

et al. observed that TRPM4 channel inhibition suppressed endothelial cell apoptosis caused by elevated intracellular ROS-induced by lipopolysaccharide treatment [28]. Salcedo et al. reported that elevated ROS production during I/R injury activated TRPM4, leading to neuronal death [27]. Furthermore, Ding et al. reported that exogenous ROS can significantly upregulate the expression of TRPM4, leading to extensive endothelial cell apoptosis; the TRPM4-inhibitor 9-phenanthrol suppressed this apoptosis [29]. In the present study, our results show that the H_2O_2-induced increase in ROS production was dramatically suppressed in TRPM4^{KO} cardiomyocytes (Fig. 4). Together, these findings suggest that suppression of TRPM4 expression may protect cardiomyocytes from the overproduction of ROS that causes cell death during I/R injury.

The link between the TRPM4 channel activity and mitochondrial dysfunction may be explained by ROS overproduction. We observed here that H_2O_2, a type of ROS, induced an increase in intracellular ROS. This result may result from 'ROS-induced ROS release (RIRR)' in mitochondria. In RIRR, overproduced ROS can damage mitochondria [30]. Interestingly, we observed that mitochondrial dysfunction, as evidenced by abnormal mitochondrial membrane depolarization and a marked decrease in ATP production, was resolved in TRPM4^{KO} cardiomyocytes. Therefore, the mechanism underlying cardioprotection by TRPM4 inhibition may involve the inhibition of RIRR that occurs in mitochondria.

Our findings indicate that inhibition of the TRPM4 channel rescues mitochondrial dysfunction during H_2O_2 exposure by preserving the mitochondrial membrane potential and ATP levels. In addition, decreased expression of TRPM4 protects cardiomyocytes against oxidative stress by limiting accumulation of intracellular Ca^{2+} and ROS. Inhibition of TRPM4 may be a valuable and novel strategy for suppressing myocardial ischemia-reperfusion injury.

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Author contributions

C.W. and K.T. wrote the main manuscript text. C.W. and J.C. performed the experiments. M.W. supervised the experimental framework. K.N. supervised the analyses. All the authors discussed the analyses and results.

Declaration of competing interest

No competing interests declared.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mex.2021.101404 .

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