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Original Paper

Ciliary Neurotrophic Factor (CNTF) Protects Myocardial Cells from Oxygen Glucose Deprivation (OGD)/Re-Oxygenation via Activation of Akt-Nrf2 Signaling

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Key Words
Ischemic heart diseases • Oxygen glucose deprivation (OGD) • Myocardial cells • CNTF • Akt • Nrf2

Abstract.
Background/Aims: Oxygen glucose deprivation (OGD)/re-oxygenation (OGDR) exposure to myocardial cells mimics ischemia-reperfusion injuries. We studied the potential activity of ciliary neurotrophic factor (CNTF) on OGDR-treated myocardial cells. ***Methods:*** CNTF and CNTFR expression were tested by RT-PCR assay and Western blotting assay. Cell viability and death were tested by MTT assay and LDH release assay, respectively. Akt-Nrf2 signalings were tested by Western blotting assay and qPCR assay. ***Results:*** CNTF and its receptor CNTFR were functionally expressed in established H9c2 myocardial cells and primary murine myocardiocytes. Pretreatment of CNTF significantly attenuated OGDR-induced viability reduction and death in myocardial cells. Further studies show that in the myocardial cells CNTF activated NF-E2-related factor 2 (Nrf2) signaling to inhibit OGDR-induced reactive oxygen species (ROS) production and programmed necrosis, preventing adenine nucleotide translocator 1 (ANT-1)-p53-cyclophilin D (Cyp-D) mitochondrial association and mitochondrial depolarization. Nrf2 silencing or knockout almost abolished CNTF-induced H9c2 cytoprotection against OGDR. CNTF activated Akt in H9c2 cells and primary murine myocardiocytes. Conversely, Akt blockage by the pharmacological inhibitors not only blocked CNTF-induced Nrf2 Ser-40 phosphorylation and activation, but also nullified anti-OGDR actions by CNTF in myocardial cells. ***Conclusion:*** CNTF activates Akt-Nrf2 signaling to protect myocardial cells from OGDR.

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Introduction

Ischemic heart diseases are major threats to human health [1, 2]. Understanding the underlying pathological mechanisms and developing possible intervention strategies are urgent [1, 2]. Our group [3-5] and others [6-8] are using *in vitro* oxygen glucose deprivation (OGD) model to mimic ischemic myocardial cell injury. Sustained OGD (>1 h) and subsequent re-oxygenation (ODGR) exposure disrupts mitochondrial function, leading to profound reactive oxygen species (ROS) production, oxidative stress and myocardial cell necrosis (but not apoptosis) [6-8].

Ciliary neurotrophic factor (CNTF) is expressed in glial cells within the central and peripheral nervous systems [9-11]. CNTF promotes gene expression, cell survival or differentiation of sensory, sympathetic, ciliary and motor neurons [9-11]. CNTF binds to its receptor CNTFR, enabling the recruitment of glycoprotein 130 and LIFRβ (leukaemia inhibitory factor receptor), forming a tripartite receptor complex [9-11]. The complexation will lead to tyrosine phosphorylation, providing the docking sites for SH2-containing signaling molecules [9-11]. It will lead to activation of signaling cascades, including PI3K-Akt-mTOR, Erk-MAPK (mitogen-activated protein kinases) and Jak-STAT (signal transducers and activators of transcription) pathways [9-11]. CNTF and CNTFR expression and potential functions in myocardial cells have not been studied.

The nuclear transcription factor NF-E2-related factor 2 (Nrf2) is essential for the transcription and expression of key anti-oxidant enzymes [12, 13]. Nrf2-dependent enzymes, including *heme oxygenase-1 (HO1)*, *NADPH quinone oxidoreductase 1 (NQO1)* and *glutamate cysteine ligase catalytic subunit (GCLC)*, can inhibit ROS production and oxidative stress [14]. Our previous study has demonstrated that the Akt activator SC79 activated Nrf2 signaling to protect myocardial cells from OGDR [5]. Further, salidroside-induced myocardial cytoprotection against OGDR required Nrf2 signaling activation as well [3]. Here, we will show that CNTF prevents OGDR-induced myocardial cell death via activation of Nrf2 signaling.

Materials and Methods

Chemical and reagents

CNTF, LY294002, AZD5363 and MK2206 were purchased from Sigma-Aldrich (St. Louis, Mo). Antibodies of cyclophilin D (Cyp-D), adenine nucleotide translocator 1 (ANT-1) and p53, Nrf2, HO1, NQO1 and GCLC were provided by Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies for CNTF and CNTFR were obtained from Abcam (Shanghai, China). All other antibodies were purchased from Cell Signaling Tech (Beverly, MA). Cell culture reagents were obtained from Gibco Bio (Grand Island, NY). The 2′,7′-dichlorofluorescein diacetate (DCF-DA) fluorescent dye for ROS assay was provided by Roche Diagnostics (Mannheim, Germany).

H9c2 cell culture

As previously reported [3, 15, 16], the rat embryonic ventricular H9c2 myocardial cells were cultured in DMEM medium plus 10% fetal bovine serum (FBS) with necessary antibiotics. DNA fingerprinting and profiling were performed to confirm the cell line's origin every five months. Cells were subjected to mycoplasma and microbial contamination examination every month. Population doubling time, colony forming efficiency, and morphology were also examined to confirm the phenotype.

Primary culture of murine myocardiocytes

The detailed protocols of isolation and primary culturing of the murine myocardiocytes were described previously [3-5, 17]. In short, ventricles of C57BL6 mice (at P1) were minced and digested. The cell suspensions of primary myocardiocytes were filtered, cultured in FBS-containing M-199 medium, and plated for 30 min. The confluent monolayer with primary spontaneously beating cells was formed [17]. The protocols were approved by the Ethics Committee of Nantong University.

OGD/re-oxygenation

OGD/re-oxygenation (OGDR) protocol was described previously [3]. In short, cells were cultured in a pre-warmed glucose-free balanced salt solution [3]. The solution was then bubbled with an anaerobic gas mix (95% N_2, 5% CO_2). Cells were incubated in the solution for 4 h to produce OGD and then re-oxygenated.

MTT viability assay

Cells were seeded onto 96-well tissue-culture plate at a density of 3, 000 cells per well. Cell viability was examined by the 3-[4, 5-dimethylthylthiazol-2-yl]-2, 5 diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO) assay [3, 16]. The MTT optical density (OD) at 590 nm was recorded.

LDH assay of cell death

As previously discussed [5], cells were initially seeded onto 24-well tissue-culture plate at a density of 15, 000 cells per well. Following the applied treatment, cell death was determined by lactate dehydrogenase (LDH) assay via a commercial available LDH kit (Takara, Tokyo, Japan). LDH release (× 100 %) was calculated as follows: LDH in conditional medium/(LDH in conditional medium + LDH in cell lysates).

Western blotting assay

Western blotting assay was performed as described previously [3, 16]. Briefly, total cellular lysates (40 μg per sample of each lane) were separated by 10% SDS-PAGE gel, which were then transferred to the PVDF (polyvinylidene difluoride) blots (Merck Millipore, Darmstadt, Germany). After blocking in 10% non-fat milk, the blots were incubated with the applied primary and secondary antibodies. The ECL reagents (Pierce, Shanghai, China) were added to visualize the targeted protein bands based on the molecular weights, under x-ray films. The band intensity (total gray) was quantified using the the Image J software (from NIH), which was always normalized to that of loading control.

Mitochondrial immunoprecipitation (Mito-IP)

Following the treatment, the mitochondria of ten million H9c2 cells (per treatment) were extracted using the "Mitochondria Isolation Kit for Cultured Cells" from Thermo Scientific (Hudson, NH). The acquired mitochondria were then lysed [3]. Immunoprecipitation (IP) was performed via using the anti-Cyp-D antibody (see [18]), and immune complexes were captured with protein G-Sepharose. Afterward, the Cyp-D-bound IP lysates were subjected to Western blotting assay, p53-Cyp-D-ANT-1 association was detected [3, 18].

Real-time PCR

Total cellular RNA extraction by the TRIzol reagents (Biyuntian, Wuxi, China) and reverse transcription were performed as described previously [3] . Real-time PCR was performed via using a Bio-Rad IQ5 multicolor detection system. After amplification, melt curve analysis was performed to analyze product melting temperature. *GAPDH* was tested as internal control, using the $2^{-\Delta\Delta CT}$ method [19] for mRNA expression quantification. The primers for rat *HO-1, Nrf2, GAPDH, NQO-1* and *GCLC* were described previously [3]. The primers for murine *HO-1, Nrf2, GAPDH, NQO-1* and *GCLC* were from Dr. Jiang's group [3, 5, 20, 21]. mRNA primers for CNTF and CNTFR were designed and sequence verified by Genepharma (Shanghai, China).

ROS detection

H9c2 cells were seeded onto 6-well plates at 1×10^5 cells per well. Following the OGDR (or plus CNTF) treatment, ROS production was determined by the DCF-DA fluorescent dye assay (Invitrogen) [3]. Briefly, cells were stained DCF-DA dye at 5.0 μg/mL for 60 min under the dark. DCF-DA fluorescent intensity was recorded on a spectrofluorometer using excitation and emission filters of 488 and 530 nm, respectively [3].

Mitochondrial depolarization assay

H9c2 cells were seeded onto 6-well plates at 1×10^5 cells per well. Following the OGDR (or plus CNTF) treatment, mitochondrial depolarization ("ΔΨ") was tested by the mito-dye JC-1 (Sigma), which will aggregate to form the green monomers following mitochondrial depolarization [22]. Briefly, cells were stained with JC-1 (5.0 μg/mL), which were then washed, tested immediately on a spectrofluorometer. JC-1 fluorescence OD at 550 nm was recorded.

Nrf2 silencing by shRNA

H9c2 cells were seeded onto 6-well plates at 1×10^5 cells per well. The Nrf2 shRNA-containing lentivirus (with the sequence 5' CCACATTTTCTTAATGCTTTTGA), as described previously [3, 5], was added directly to cultured H9c2 cells at 20 μL/mL medium for 24 h. Cells were the culture in puromycin (0.25 μg/mL)-containing medium for another 96 h. Nrf2 knockdown in the selected stable cells was confirmed by Western blotting assay and qPCR assay. Control cells were transfected with same amount of scramble control shRNA (Santa Cruz).

CRISPR/Cas9-mediated Nrf2 knockout

H9c2 cells were seeded onto 6-well plates at 1×10^5 cells per well. The small guide RNA (sgRNA) targeting human Nrf2 (targeted DNA sequence, 5'-GGCATCTTGTTTGGGAATGT) was inserted into the lentiCRISPR-GFP plasmid. The lentiCRISPR-GFP-Nrf2 KO construct, provided by Dr. Li [23], was transfected to H9c2 cells by using the Lipofectamine 2000 reagent (Invitrogen, Shanghai, China). GFP-positive cells were thereafter FACS-sorted, and selected stable cells were subjected to Nrf2 knockout screening.

Statistical analysis

Data presented were mean ± standard deviation (SD). Statistical differences were analyzed by one-way ANOVA by Dunnett's Test (SPSS 21.0, Chicago, IL). A two-tailed unpaired T test was applied to test significance between two treatment groups. Values of p <0.05 were considered statistically significant.

Results

CNTF protects myocardial cells from OGD/re-oxygenation

First, by performing the RT-PCR assay, we showed that mRNA expression of CNTF and its receptor CNTFR were detected in both H9c2 myocardial cells and primary murine myocardiocytes (Fig. 1A). Further, CNTF and CNTFR proteins were also expressed in the myocardial cells (Fig. 1B). Oxygen glucose deprivation (OGD)/re-oxygenation (OGDR) is often applied to cultured myocardial cells. In line with our previous findings [3-5], OGDR exposure in H9c2 cells (for 24 h) induced potent viability (MTT OD) reduction (Fig. 1C) and cell death (LDH medium release, Fig. 1D). Significantly, pretreatment of CNTF (for 1 h) dose-dependently inhibited OGDR-induced cytotoxicity in H9c2 cells (Fig. 1C and D). CNTF at 5-100 ng/mL significantly inhibited viability reduction (Fig. 1C) and LDH release (Fig. 1D) by OGDR in

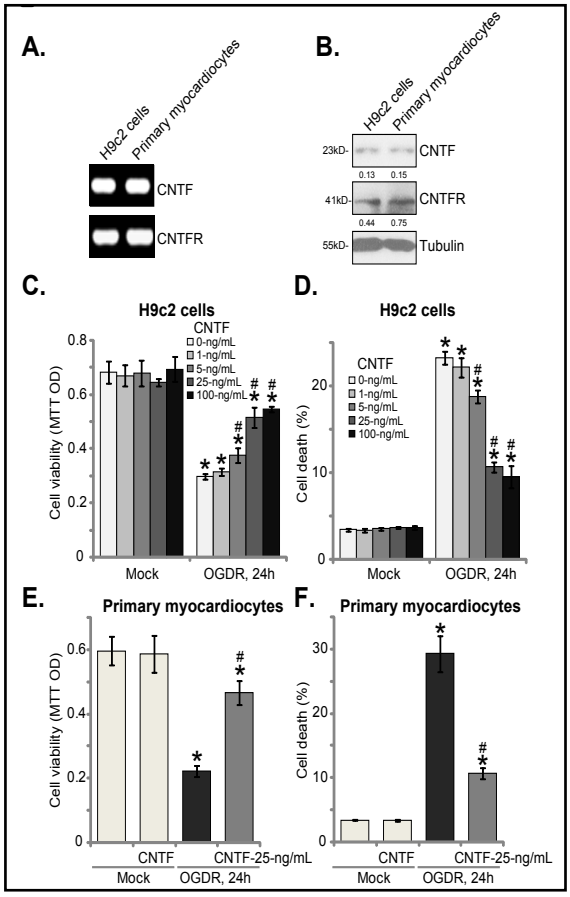

Fig. 1. CNTF protects myocardial cells from OGD/re-oxygenation. mRNA (A) and protein (B) expression of CNTF and CNTFR in H9c2 myocardial cells and the primary murine myocardiocytes were shown. H9c2 myocardial cells (C and D) or the primary murine myocardiocytes (E and F), pretreated with CNTF (1 h pretreatment), were maintained under oxygen and glucose deprivation (OGD) for 4 h, followed by 24 h of re-oxygenation. Afterwards, cell viability was tested by MTT assay (C and E); Cell death was examined by the LDH release assay (D and F). "Mock" stands for normal oxygen and glucose control (Same for all figures). "OGDR" stands for OGD/re-oxygenation (Same for all figures). Bars indicate mean ± standard deviation (SD, n=5). * p<0.05 vs. "Mock" cells. # p<0.05 vs. "OGDR" only treatment. Each experiment was repeated five times and similar results were obtained.

H9c2 cells. Notably, treatment with CNTF alone at tested concentrations (1-100 ng/mL) had no significant effect on cell viability and cell death (Fig. 1C and D). In the primary murine myocardiocytes, pretreatment with CNTF (25 ng/mL) attenuated OGDR-induced viability reduction (Fig. 1E) and cell death (Fig. 1F). CNTF single treatment was ineffective (Fig. 1E and F). These results show that CNTF protects myocardial cells from OGDR.

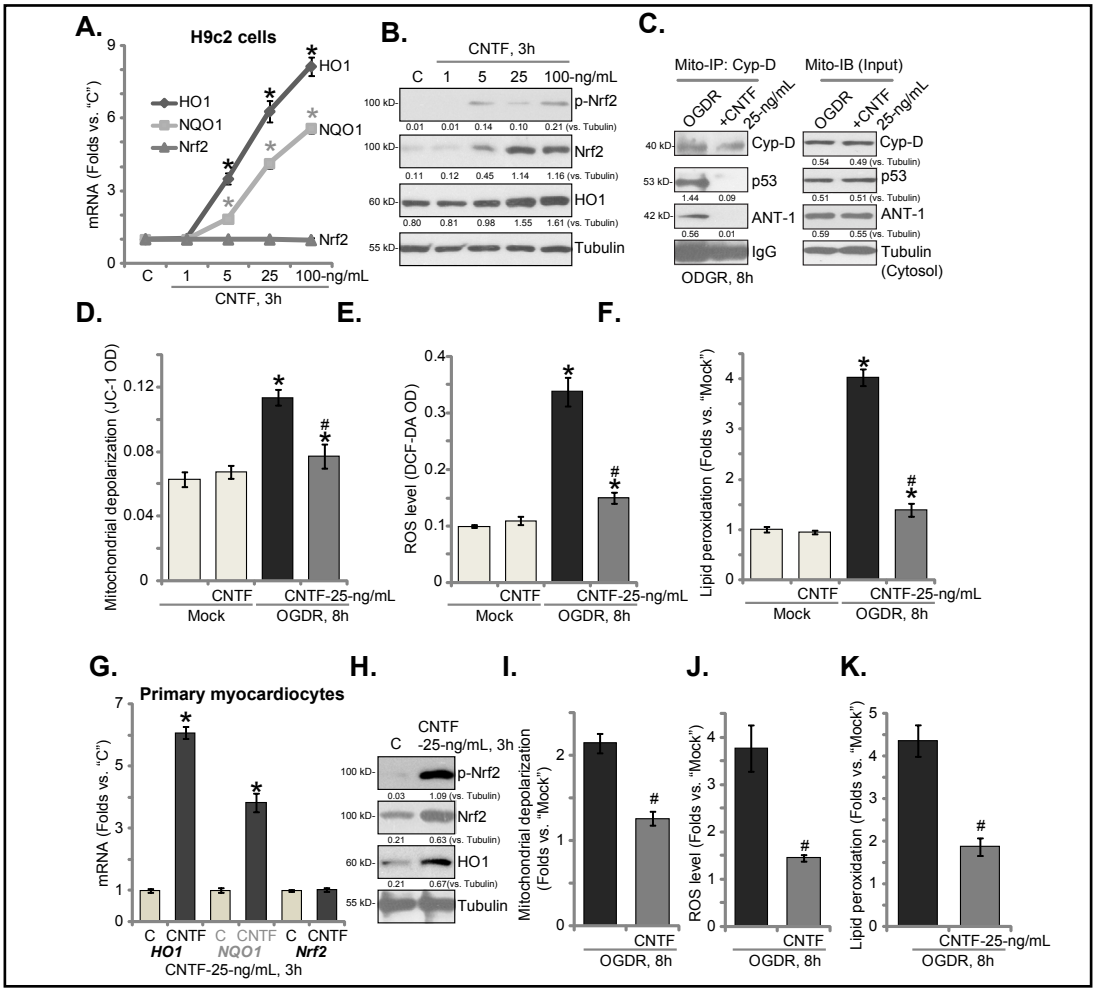

Fig. 2. CNTF activates Nrf2 signaling, inhibiting oxidative stress and programmed necrosis in myocardial cells. H9c2 myocardial cells (A-C) or primary murine myocardiocytes (G and H) with treated with CNTF at applied concentration for indicated time, mRNA and protein expression of listed genes were shown (A, B, G and H); The complexation and expression of listed proteins in the mitochondrial lysates were tested by mitochondrial immunoprecipitation assay ("Mito-IP" for Cyp-D-associated proteins) and mitochondrial immuno-blot assay ("Mito-IB", as "Input"), respectively (C); H9c2 myocardial cells (D-F) or primary murine myocardiocytes (I-K) were pre-treated with CNTF (25 ng/mL) for 1 h, maintained under oxygen and glucose deprivation (OGD) for 4 h, followed by 8 h of re-oxygenation; Mitochondrial depolarization, ROS production and lipid peroxidation were tested by JC-1 dye assay (D and I), DCF-DA dye assay (E and J) and TBAR activity assay (F and K), respectively. For Mito-IP assay, the amount of Cyp-D-bound p53 or ANT-1 was quantified (vs. Cyp-D, C). Expression of listed proteins were quantified and normalized to the loading control (B and H). Bars indicate standard deviation (SD, n=5). "C" stands for untreated control cells. * $p<0.05$ vs. group "C" (A and G). * $p<0.05$ vs. "Mock" cells (D-F, I-K). # $p<0.05$ vs. "OGDR" only treatment (D-F, I-K). Each experiment was repeated three times and similar results were obtained.

CNTF activates Nrf2 signaling, inhibiting oxidative stress and programmed necrosis in myocardial cells

Our previous studies have shown that activation of Nrf2 signaling potently inhibited OGDR-induced ROS production and subsequent programmed necrosis, thereby inhibiting cell death [3-5]. We tested whether CNTF could also activate Nrf2 signaling in myocardial cells. By performing the qPCR assay, we showed that treatment with CNTF dose-dependent increased mRNA expression of two primary Nrf2-dependent anti-oxidant genes, including HO1 and NQO1. Nrf2 mRNA expression level was unchanged (Fig. 2A), and CNTF dose-dependently induced Nrf2 phosphorylation (at Ser-40, a key site for activation [24-26]) and protein accumulation in H9c2 cells (Fig. 2B). HO1 protein expression was increased as well in CNTF (5-100 ng/mL)-treated cells (Fig. 2B). These results indicate that CNTF activates Nrf2 signaling in H9c2 cells.

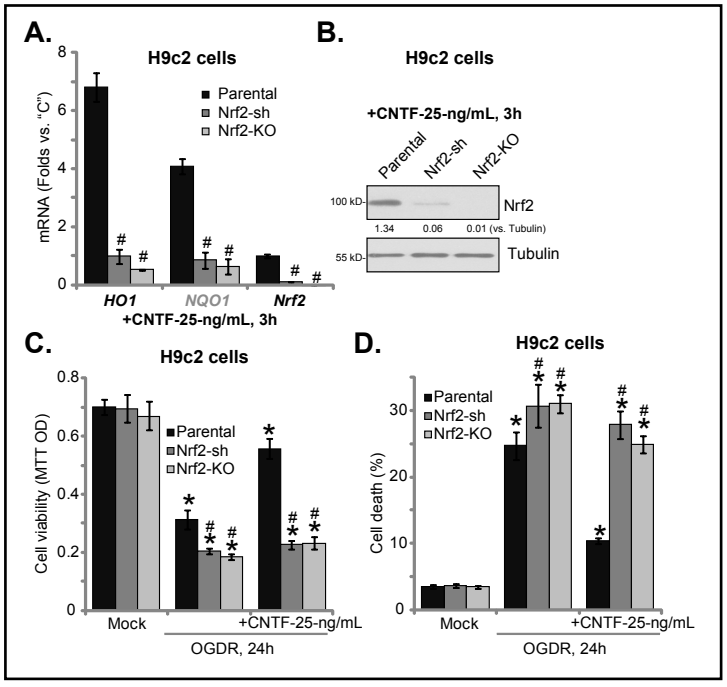

Fig. 3. Nrf2 silencing or knockout abolishes CNTF-induced myocardial cytoprotection against OGDR. Stable H9c2 cells with the lentiviral Nrf2-shRNA ("Nrf2-sh") or the Nrf2-knockout-CRISPR/Cas9 construct ("Nrf2-KO"), as well as the parental control cells ("Parental"), were treated with CNTF (25 ng/mL) for 3 h, mRNA (A) and protein (B) expression of listed genes were shown. Cells were pre-treated with CNTF (25 ng/mL) for 1 h, maintained under oxygen and glucose deprivation (OGD) for 4 h, followed by 24 h of re-oxygenation. Afterwards, cell viability was tested by MTT assay (C); Cell death was examined by the LDH release assay (D). Expression of Nrf2 was quantified and normalized to the loading control Tubulin (B). Bars indicate standard deviation (SD, n=5). * $p<0.05$ vs. "Mock" cells. # $p<0.05$ vs. "Parental" cells. Each experiment was repeated three times and similar results were obtained.

OGDR mainly induces programmed necrosis, but not apoptosis, in myocardial cells and other cells [5, 23, 27, 28]. Indeed, OGDR treatment in H9c2 cells induced programmed necrosis, which was evidenced by mitochondrial ANT-1-p53-Cyp-D association (Fig. 2C) and mitochondrial depolarization (JC-1 intensity increase, Fig. 2D), which was significantly inhibited by CNTF (25 ng/mL). The DCF-DA fluorescence dye assay results showed that OGDR-induced ROS production was significantly attenuated with pretreatment of CNTF (Fig. 2E). TBAR assay results further showed that lipid peroxidation following OGDR was alleviated by CNTF pretreatment (Fig. 2F). These results indicate that CNTF inhibits OGDR-induced programmed necrosis and oxidative stress in H9c2 cells.

In the primary murine myocardiocytes, CNTF (25 ng/mL) increased mRNA expression of HO1 and NQQ1 genes (Fig. 2G). Further, CNTF induced Nrf2 Ser-40 phosphorylation and protein stabilization as well as HO1 protein expression in the primary myocardiocytes (Fig. 2H). OGDR-induced mitochondrial depolarization (Fig. 2I), ROS production (Fig. 2J) and lipid peroxidation (Fig. 2K) were significantly attenuated by CNTF in the primary cells. Together, CNTF activates Nrf2 signaling, inhibiting oxidative stress and programmed necrosis in myocardial cells.

Nrf2 silencing or knockout abolishes CNTF-induced myocardial cytoprotection against OGDR

In order to show that Nrf2 activation is required for CNTF-induced myocardial cytoprotection against OGDR, genetic strategies were applied. Nrf2-shRNA lentivirus was added to H9c2 cells. Puromycin was then added to select stable cells. Additionally, the Nrf2-knockout-CRISPR/Cas9 lentiviral construct (a gift from Dr [23].) was transfected to H9c2 cells to establish Nrf2-knockout cells. By performing the qPCR assay, we show that Nrf2 mRNA was significantly downregulated (over 90%) by Nrf2 shRNA (Fig. 3A). Nrf2 mRNA was completely depleted in Nrf2-knockout cells (Fig. 3A). Significantly, CNTF-induced mRNA expression of HO1 and NQO1 genes were blocked in Nrf2-silenced or -knockout cells (Fig. 3A). CNTF-induced Nrf2 protein stabilization was reversed by Nrf2 shRNA or knockout as well (Fig. 3B). Importantly, CNTF failed to inhibit OGDR-induced viability reduction (Fig. 3C) and cell death (Fig. 3D) in Nrf2-silenced or -knockout H9c2 cells. These results indicate that activation of Nrf2 is required for CNTF-induced cytoprotection against OGDR in H9c2 cells. Notably, in line with our previous findings [3, 5], OGDR-induced cytotoxicity was augmented in Nrf2-silenced or -knockout H9c2 cells (Fig. 3C and D), further supporting a cytoprotective function of Nrf2 in OGDR-treated cells.

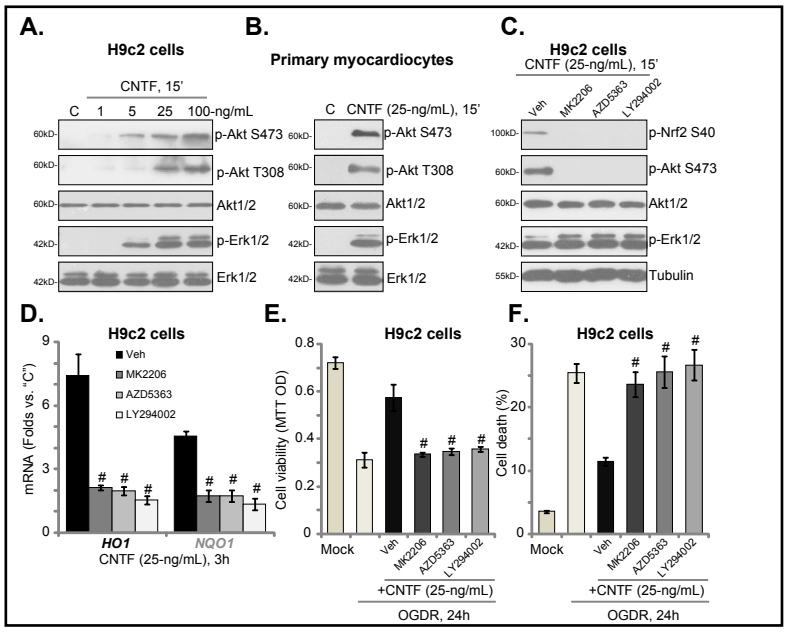

Fig. 4. Akt activation mediates CNTF-induced Nrf2 activation and myocardial cytoprotection against OGDR. H9c2 myocardial cells (A) or primary murine myocardiocytes (B) with treated with CNTF at applied concentration for indicated time, expression of listed proteins in total cell lysates were shown. H9c2 cells were pretreated with MK2206 (10 μM), AZD5363 (250 nM) or LY294002 (1 μM) for 30 min, followed by CNTF (25 ng/mL) treatment for indicate time, expression of listed proteins in total cell lysates were shown (C); HO1 and NQO1 mRNA levels were tested as well (D); Cells were also maintained under oxygen and glucose deprivation (OGD) for 4 h, followed by 24 h of re-oxygenation. Afterwards, cell viability was tested by MTT assay (E); Cell death was examined by the LDH release assay (F). "Veh" stands for the vehicle control (0.1% DMSO). Bars indicate standard deviation (SD, n=5). # $p<0.05$ vs. "Veh" cells (D-F). Each experiment was repeated five times and similar results were obtained.

Akt activation mediates CNTF-induced Nrf2 activation and myocardial cytoprotection against OGDR

We studied the potential upstream mechanism of Nrf2 activation by CNTF. Akt could be a upstream signaling of Nrf2 via phosphorylating Nrf2 at Ser-40 [21, 23, 26, 29]. Here, we found that CNTF dose-dependently induced Akt activation in H9c2 cells (Fig. 4A). Phosphorylated-Erk1/2, another major downstream of CNTFR [9, 10], was increased following CNTF treatment as well (Fig. 4A). In the primary murine myocardiocytes, CNTF (25 ng/mL, 15 min) induced phosphorylations of Akt (at both Ser-473 and Thr-308) and Erk1/2 (Fig. 4B). Therefore, CNTF activates Akt and Erk signalings in myocardial cells.

To block Akt activation, Akt inhibitors were applied, including MK2206 [30, 31], AZD5363 [32, 33] and LY294002 [34]. As shown, the Akt inhibitors completely blocked CNTF (25 ng/mL, 15 min)-induced Akt activation (Fig. 4C). Consequently, Nrf2 Ser-40 phosphorylation by CNTF was blocked (Fig. 4C). Erk1/2 phosphorylation was not affected (Fig. 4C). Significantly, CNTF (25 ng/mL)-induced HO1 and NQO1 mRNA expression were largely inhibited by the Akt inhibitors (Fig. 4D). Functional assays showed that CNTF-induced anti-OGDR actions were almost nullified in by Akt inhibitors (Fig. 4E and F). Together, these results indicate that Akt activation mediates CNTF-induced Nrf2 Ser-40 activation and activation, protecting myocardial cells from OGDR.

Discussion

The cytoprotective function of CNTF in neuronal cells has been well-demonstrated [9-11]. Intracerebral administration of CNTF or the CNTF analogs protected neurons in rodent and primate models of Huntington's disease [35, 36]. CNTF activates downstream signaling cascades, including PI3K-Akt, Erk-MAPK and STAT3, which are essential for mediating its cytoprotective function [9-11]. The novel findings of this study are that CNTF and its receptor CNTFR are functionally expressed in established H9c2 myocardial cells and primary murine myocardiocytes. CNTF treatment in the myocardial cells induced activation of downstream signalings: PI3K-Akt and Erk-MAPK. Importantly, pretreatment of CNTF at only ng/mL concentrations significantly attenuated OGDR-induced viability reduction and death of myocardial cells. Thus, CNTF might be a novel and potent myocardial cytoprotective agent.

Inactivated Nrf2 associates with Keap1, leading to Cul3-dependent Nrf2 ubiquitination and proteasomal degradation [12, 13, 37]. Post-transcriptional modifications of Nrf2 are essential for its activation [14]. Nrf2 Ser-40 phosphorylation can induce Nrf2-Keap1 departure [38], enabling Nrf2 protein stabilization, accumulation, and nuclear translocation [21, 23, 26, 29]. Our results showed that CNTF activated Akt signaling, serving as a upstream signaling for Nrf2 Ser-40 phosphorylation and activation. Akt blockage not only blocked Nrf2 Ser-40 phosphorylation and activation, but also nullified CNTF-induced myocardial cytoprotection. Our results are consistent with recent findings. For example, Lee *et al.*, demonstrated that PI3K-Akt mediated sulforaphane-induced Nrf2 activation [39]. Further, the study by Xu *et al.*, showed that pyocyanin-induced Nrf2 activation is the downstream of PI3K-Akt [40]. Dr. Jiang's group has shown that Salvianolic acid A (SalA) and 3H-1, 2-dithiole-3-thione (D3T) induced Akt-dependent Nrf2 Ser-40 phosphorylation and activation, protecting human retinal cells from oxidative stress [20, 21]. We conclude that CNTF activates Akt downstream Nrf2 signaling to protect myocardial cells from OGDR.

OGDR mainly activates mitochondrial necrosis pathway ("programmed necrosis") in myocardial cells and other cells [3, 17, 18, 41-43]. OGDR will induce significant intracellular ROS production, which promotes p53 translocation to mitochondria, where it forms a complex with mitochondrial permeability transition pore components Cyp-D and ANT-1 [3, 18, 42, 43]. Cyp-D-p53-ANT-1 complexation promotes mitochondrial depolarization, mPTP opening, and eventually cell necrosis (but not apoptosis) [3, 17, 18, 41-43]. Here, CNTF potently inhibited OGDR-induced ROS production and programmed necrosis, preventing Cyp-D-p53-ANT-1 mitochondrial complexation and mitochondrial depolarization in myocardial cells. The blockage of mitochondrial programmed necrosis pathway by CNTF could explain its superior myocardial cytoprotective effect. The detailed mechanisms may warrant further studies.

Conclusion

In summary, CNTF activates Akt-Nrf2 activation to protect myocardial cells from OGDR.

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Disclosure Statements

The listed authors have no conflict of interests.

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