

Role of tRNA selenocysteine 1 associated protein 1 in the proliferation and apoptosis of cardiomyocyte-like H9c2 cells

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Abstract. Transfer RNA selenocysteine 1 associated protein 1 (Trnaup1) serves an essential role in the synthesis of selenoproteins, which have critical functions in numerous biological processes. Selenium deficiency results in a variety of diseases, including cardiac disease. However, the mechanisms underlying myocardial injury induced by selenium deficiency remain unclear. The present study examined the effects of Trnaup1 under- and overexpression in cardiomyocyte-like H9c2 cells, by transfection with small interfering RNA and an overexpression plasmid, respectively. Expression levels of glutathione peroxidase, thioredoxin reductase and selenoprotein K were decreased in Trnaup1-underexpressing cells, and increased in Trnaup1-overexpressing cells. Using MTT, proliferating cell nuclear antigen, annexin V and caspase-3 activity assays, it was demonstrated that reducing Trnaup1 expression levels inhibited the proliferation of H9c2 cells and induced apoptosis. Conversely, increasing Trnaup1 expression levels promoted cell growth. Western blot analysis revealed that the phosphoinositide 3-kinase/protein kinase B signaling pathway was activated in Trnaup1-underexpressing cells. Furthermore, the apoptotic pathway was activated in these cells, evidenced by relatively greater expression levels of B-cell lymphoma (Bcl-2)-associated X protein and reduced expression levels of Bcl-2. Taken together, these findings suggest that Trnaup1 serves a key role in the proliferation and apoptosis of H9c2 cells. The present study provides insight into the underlying mechanisms of myocardial injury induced by selenium deficiency.

Introduction

Selenium was discovered by the Swedish chemist Jöns Jacob Berzelius in 1817. It was considered a toxin until 1957, when the trace element was identified as an essential micronutrient for animals and humans (1,2). Selenium serves a role in numerous biological processes, including immune function, brain development and reproduction (3,4). *In vivo*, selenium is found in selenoproteins, which co-translationally incorporate selenium-containing selenocysteine (Sec), the 21st genetically encoded amino acid (5,6). Selenoproteins are critical for numerous physiological functions, including antioxidant defense, thyroid hormone production (4) and inflammation and immunity (7). Primary selenoproteins include glutathione peroxidases (GPxs), thioredoxin reductases (TrxRs) and iodothyronine deiodinases (8,9). Sec and selenoprotein biosynthesis requires a multitude of protein factors, including Sep (O-phosphoserine) transfer (t)RNA:Sec (selenocysteine) synthase (SepSecS), tRNA Sec 1 associated protein 1 (Trnaup1), and Sec insertion sequence binding protein 2 (SBP2) (10).

Trnaup1, originally named SECp43, is a highly conserved 43-kDa tRNA^{[Ser] Sec}-binding protein identified by affinity purification (11). It has been identified to interact with the selenocysteyl-tRNA^{[Ser] Sec}-Sec-specific elongation factor (EFsec) complex *in vitro*, and co-expression of Trnaup1 facilitates interaction between EFsec and SBP2 *in vivo*. In addition, Trnaup1 mediates Sec incorporation and upregulates selenoprotein mRNA expression levels (12). Furthermore, when SepSecS and Trnaup1 are co-expressed *in vitro*, Trnaup1 induces SepSecS to localize to the cytoplasm rather than the nucleus, where Trnaup1 typically resides (13). A previous study demonstrated that downregulation of Trnaup1 and SepSecS resulted in an extensive reduction of selenoprotein expression levels, whereas downregulation of SepSecS alone had no effect. Additionally, downregulation of Trnaup1 reduced GPx1 expression levels (14). These findings suggest that Trnaup1 serves a key role in the biosynthesis of selenoproteins.

Selenium deficiency results in a variety of cardiovascular diseases, including Keshan disease. Keshan disease, a cardiomyopathy endemic to China, is characterized by heart failure and severe cardiomyopathy, typically with arrhythmia and congestive heart failure (15,16). Our previous study

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demonstrated that reactive oxygen species accumulation and myocardial injury were features of a mouse model of selenium deficiency (17). However, the underlying mechanisms of myocardial injury induced by selenium deficiency remain unclear.

The present study hypothesized that Trnaulap may participate in selenium deficiency-induced myocardial injury. The role of Trnaulap in myocardial cell proliferation and apoptosis was investigated using H9c2 cells. Furthermore, the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway was examined to provide further insight into the molecular mechanisms underlying cardiomyocyte injury.

Materials and methods

Cell culture. The H9c2 rat cardiac myoblast cell line (purchased from the American Type Culture Collection, Manassas, VA, USA), was maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 5.5 mM glucose supplemented with 10% fetal bovine serum (Biological Industries, Kibbutz Beit Haemek, Israel) and 100 U/ml penicillin/streptomycin (Biological Industries) in an atmosphere of 5% CO₂ at 37°C.

Plasmids, small interfering (si)RNA and transfection. The open reading frame of Trnaulap was cloned into a pcDNA3.1(+) vector (pcDNA3.1(+)-Trnaulap; Thermo Fisher Scientific, Inc.), and transfection was performed using Lipofectamine[®] 2000 (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol, when cells were at 70–80% confluence. H9c2 cells transfected with the empty vector (H9c2-mock) or pcDNA3.1(+)-Trnaulap (H9c2-Trnaulap) were analyzed 24–72 h post-transfection. A total of three siRNAs targeting Trnaulap were obtained from Guangzhou RiboBio Co., Ltd. (Guangzhou, China). The sequences were as follows: 5'-GCC GAGAAGTGTTCATA-3' (Trnaulap siRNA-1), 5'-GGA CGAUGGCAUGCUGUAU-3' (Trnaulap siRNA-2) and 5'-GCAGACAUAUGAAGAGGUU-3' (Trnaulap siRNA-3). A negative control siRNA was additionally obtained from Guangzhou RiboBio Co., Ltd. Transfection was conducted using Lipofectamine 2000 according to the manufacturer's protocol, when cells were at 60–70% confluence. Analysis was conducted 24–72 h post-transfection.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA samples were isolated from cells 24 h post-transfection with TRIzol[®] (Thermo Fisher Scientific, Inc.) and reverse transcribed into cDNA using the High-Capacity cDNA RT kit (Applied Biosystems; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. qPCR was performed using SYBR[®] Green PCR Master mix and the 7500Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The primers for Trnaulap were as follows: Forward, 5'-AGGTTG GGGATGATGCACTG-3' and reverse, 5'-CGGGGATCTCTG ATGACACG-3'. Rat β -actin served as the endogenous control, with the following primer sequences: Forward, 5'-CCACCA TGTACCCAGGCATT-3' and reverse, 5'-CGGACTCATCGT ACTCCTGC-3'. The PCR was performed with an initial stage

of 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and elongation at 60°C for 30 sec. A dissociation curve was run for each plate to confirm the production of a single product. All assays were performed in triplicate. The relative RNA expression levels were determined using the 2^{- $\Delta\Delta C_q$} method (18).

Western blotting. Total protein samples were extracted from cells 72 h post-transfection using radioimmunoprecipitation assay lysis buffer (EMD Millipore, Billerica, MA, USA). Following incubation at 4°C for 15 min, the lysates were centrifuged at 6,000 \times g for 10 min at 4°C. Protein concentrations were determined using the Bicinchoninic Acid Assay kit (Pierce; Thermo Fisher Scientific, Inc.). Samples (50 μ g) were mixed with loading buffer, denatured for 5 min at 95°C and separated by 12% SDS-PAGE. The separated proteins were transferred onto polyvinylidene difluoride membranes (EMD Millipore), blocked with 5% non-fat milk in Tris-buffered saline/Tween-20 for 2 h at 25°C, and incubated with antibodies for 12 h at 4°C. The following primary antibodies were used: rabbit anti-Trnaulap (1:1,000; catalog no. 15053-1-AP), rabbit anti-PCNA (1:1,000; catalog no. 10205-2-AP) and rabbit anti-Bax (1:1,000; catalog no. 50599-2-Ig) from ProteinTech Group, Inc. (Chicago, IL, USA); rabbit anti-selenoprotein K (SelK; 1:1,000; catalog no. ab139949) and rabbit anti-thioredoxin reductase 1 (Txnrd1; 1:1,000; catalog no. ab16840) from Abcam (Cambridge, MA, USA); rabbit anti-GPx1 (1:1,000; catalog no. PA5-30593; Thermo Fisher Scientific, Inc.); rabbit anti-Bcl-2 (1:1,000; catalog no. B0774; Assay Biotechnology, Sunnyvale, CA, USA); rabbit anti-AKT (1:1,000; catalog no. 9272) and rabbit anti-phosphorylated (p)-AKT (1:1,000; catalog no. 4058) from Cell Signaling Technology, Inc. (Danvers, MA, USA); and mouse anti- β -actin (1:1,000; catalog no. BM0627, Boster Biological Technology, Wuhan, Hubei, China). The secondary antibodies used were goat anti-rabbit horseradish peroxidase (HRP)-conjugated IgG (1:2,000; catalog no. ZB-2301) and goat anti-mouse HRP IgG (1:2,000; catalog no. ZB-2305) purchased from Zhongshan Golden Bridge Biotechnology, Co., Ltd. (Beijing, China). The membranes were incubated with the secondary antibodies for 2 h at room temperature. The signals were detected by BioSpectrum 810 Imaging system (UVP, LLC, Upland, CA, USA) using enhanced chemiluminescence system (Pierce ECL Western Blotting substrate; Thermo Fisher Scientific, Inc.). The relative densities of bands were analyzed with Quantity One 4.62 software (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Cell proliferation assays. A total of 24 h post-transfection with siRNA or plasmid, H9c2 cells were seeded into 96-well plates at a density of 2,000 viable cells per well for cell proliferation assays, which were performed using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT; Sigma-Aldrich; Merck Millipore, Darmstadt, Germany), diluted to 5 mg/ml with serum-free DMEM. A total of 48 h after seeding, MTT was added to each well and incubated for 4 h at 37°C. Formazan crystals were dissolved in dimethyl sulfoxide, and absorbance at a wavelength of 490 nm was measured using a SpectraMax[®] M3 microplate spectrophotometer (Molecular Devices, LLC, Sunnyvale, CA, USA).

Flow cytometry. A total of 1×10^6 cells were seeded into a 60-mm dish 24 h prior to the experiment. A total of 48 h post-transfection, annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) staining were performed according to the manufacturer's protocol (Beyotime Institute of Biotechnology, Haimen, China). The stained cells were acquired using a FACSCalibur™ Flow Cytometer (BD Biosciences, Franklin Lakes, NJ, USA). All data were analyzed and visualized using FACSDiva™ 3.0 software (BD Biosciences).

Caspase-3 activity. Caspase-3 activity was determined using the Caspase-3 Activity assay kit (Beyotime Institute of Biotechnology), according to the manufacturer's protocol. Assays were conducted in 96-well microplates by incubating 10 μ l cell lysate with 80 μ l reaction buffer (1% NP-40, 20 mM Tris-HCl at pH 7.5, 137 mM NaCl and 10% glycerol) and 10 μ l 2 mM caspase-3 substrate (Ac-DEVD-pNA). The lysates were incubated at 37°C for 4 h. The absorbance of samples was measured at a wavelength of 405 nm using the SpectraMaxM3 microplate reader.

Mitochondrial membrane potential assay. Mitochondrial depolarization in the cells was measured using a Mitochondrial Membrane Potential assay kit with a JC-1 probe (Beyotime Institute of Biotechnology). Briefly, mitochondria were isolated from the cells following treatment, using the Cell Mitochondria Isolation kit (Beyotime Institute of Biotechnology), and incubated with 1 ml JC-1 staining solution (5 μ g/ml) for 20 min at 37°C, and subsequently with phosphate-buffered saline. The mitochondrial membrane potentials were measured using the relative quantities of dual emissions from mitochondrial JC-1 monomers or aggregates using the SpectraMaxM3 microplate spectrophotometer. The excitation wavelength was set at 485 nm. Fluorescence intensity was detected at wavelengths of 525 nm for monomers and 590 nm for aggregates. Mitochondrial depolarization was indicated by an increase in the 525/590 nm fluorescence intensity ratio.

Statistical analysis. All experiments were performed at least three times. Normal distribution data are presented as the mean \pm standard deviation, and differences between two groups were evaluated using the Student's *t*-test. One-way ANOVA followed by the Dunnett's multiple comparison test was used to examine differences of treated groups with the control group. $P < 0.05$ was considered to indicate a statistically significant difference. All statistical analyses were performed using SPSS software version 17.0 (SPSS, Inc., Chicago, IL, USA).

Results

Modulation of Trnaulap expression levels in H9c2 cells. A total of three different siRNAs targeting Trnaulap were transfected into H9c2 cells. After a 24-h incubation, the most effective siRNA (siRNA-1) was determined by RT-qPCR (Fig. 1A; $P < 0.001$ for H9c2-siRNA1 compared with H9c2; $P < 0.001$ for H9c2-siRNA2 compared with H9c2; $P < 0.001$ for H9c2-siRNA3 compared with H9c2) and used for subsequent experiments. Western blotting analysis was performed

48 h post-transfection to confirm under- and overexpression of Trnaulap in H9c2 cells, compared with the control siRNA or H9c2-mock groups, respectively (Fig. 1B, $P < 0.01$ compared with Control siRNA; Fig. 1C, $P < 0.05$ compared with H9c2-mock). Modulating Trnaulap expression levels affected GPx1, Txnrd1 and SelK expression levels, demonstrating that Trnaulap affects the expression levels of a variety of selenoproteins (Fig. 1D, $P < 0.05$ and $P < 0.01$ compared with Control siRNA; Fig. 1E, $P < 0.05$ and $P < 0.01$ compared with H9c2-mock).

Downregulation of Trnaulap inhibits the proliferation of H9c2 cells. To examine whether Trnaulap affects the proliferation of H9c2 cells, an MTT assay was performed and proliferating cell nuclear antigen (PCNA) expression levels were measured. Compared with the control group, cells transfected with Trnaulap siRNA exhibited decreased proliferation (Fig. 2A, $P < 0.05$ compared with H9c2-siRNA). Conversely, Trnaulap-overexpressing cells exhibited increased proliferation, compared with mock-transfected cells (Fig. 2B, $P < 0.01$ compared with H9c2-mock). Expression levels of PCNA were reduced in Trnaulap-underexpressing cells, compared with control siRNA-transfected cells (Fig. 2C, $P < 0.05$ compared with H9c2-siRNA), and increased in Trnaulap-overexpressing cells compared with control mock-transfected cells (Fig. 2D, $P < 0.05$ compared with H9c2-mock).

Downregulation of Trnaulap induces apoptosis. To examine the role of Trnaulap in apoptosis in H9c2 cells, annexin V-FITC and PI double staining was performed, and flow cytometric analysis was performed to measure the apoptotic rate of cells (Fig. 3A and B, $P < 0.05$ compared with H9c2-siRNA). Compared with the control group, the apoptotic rate was increased in the Trnaulap siRNA-transfected group. No significant differences were observed in the apoptotic rates of cells between the Trnaulap overexpression and empty vector groups.

Additionally, a Caspase-3 Activity assay kit was used to evaluate the apoptotic status of the various groups. In the under-expression group, caspase-3 activity was markedly increased compared with the control group (Fig. 3C, $P < 0.01$ compared with H9c2-siRNA). In comparison, there were no significant differences between the overexpression and mock-transfected groups (Fig. 3C, $P = 0.08$ compared with H9c2-mock).

A Mitochondrial Membrane Potential assay was performed using a JC-1 probe. In cells with healthy mitochondria, JC-1 is aggregated and emits a red fluorescence. When the inner mitochondrial membrane potential is dissipated, JC-1 is disaggregated and emits a green fluorescence. Therefore, depolarization of the mitochondrial membrane potential is detected as an increase in the green/red fluorescence ratio. The Trnaulap underexpression group exhibited a depolarization of the mitochondrial membrane potential (Fig. 3D, $P < 0.05$ compared with H9c2-siRNA), whereas no significant differences were observed between the overexpression and mock-transfected groups (Fig. 3D, $P = 0.0569$ compared with H9c2-mock).

To further examine the role of Trnaulap in cell death, two apoptosis-associated proteins, Bcl-2 and Bax, were examined by western blotting. Bax expression levels were upregulated,

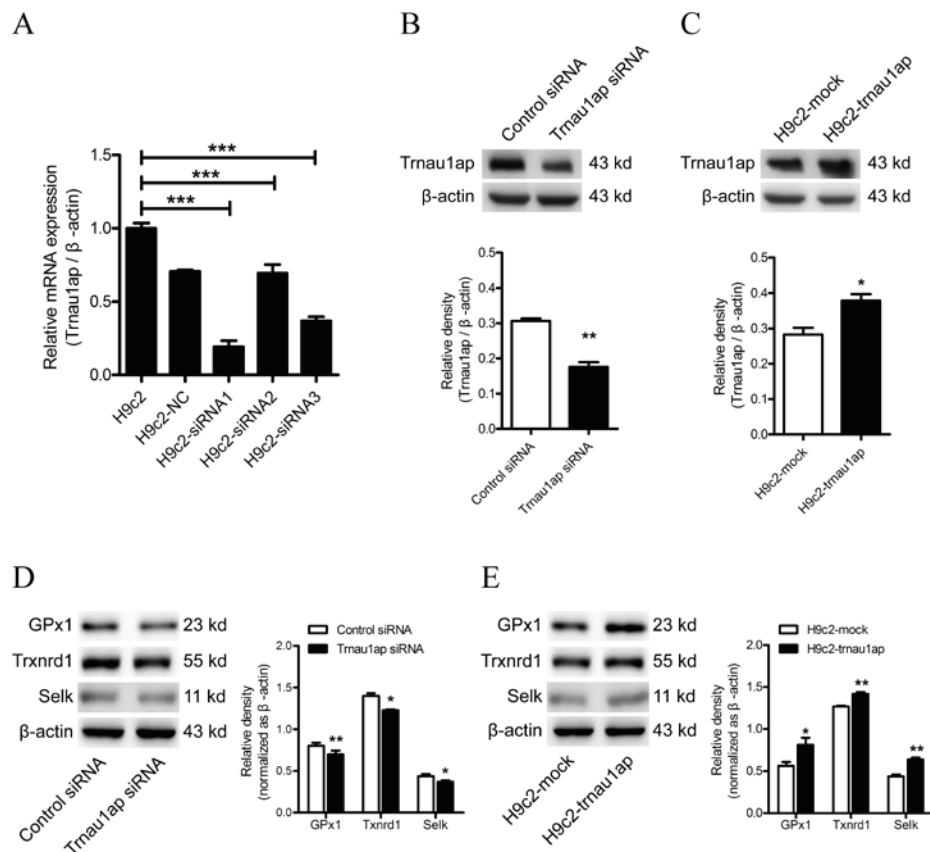


Figure 1. Expression levels of Trnaulap in H9c2 cells. (A) Expression levels of Trnaulap were determined by RT-qPCR 24 h post-transfection with Trnaulap siRNAs or control siRNA. Western blot analysis of Trnaulap protein expression levels in (B) H9c2 cells transfected with Trnaulap siRNA or control siRNA for 48 h and (C) H9c2 cells transfected with empty vector or pcDNA 3.1(+)-Trnaulap, 48 h post-transfection. Western blot analysis of GPx1, Txnrd1 and Selk levels in (D) Trnaulap-underexpressing, and (E) Trnaulap-overexpressing, cells. β -actin served as an endogenous control for western blotting and RT-qPCR. All data are presented as the mean \pm standard deviation (n=3). * P <0.05, ** P <0.01 vs. control siRNA or H9c2-mock, *** P <0.001. Trnaulap, transfer RNA selenocysteine 1 associated protein 1; siRNA, small interfering RNA; GPx1, glutathione peroxidase 1; Txnrd1, thiorexin reductase 1; Selk, selenoprotein K; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

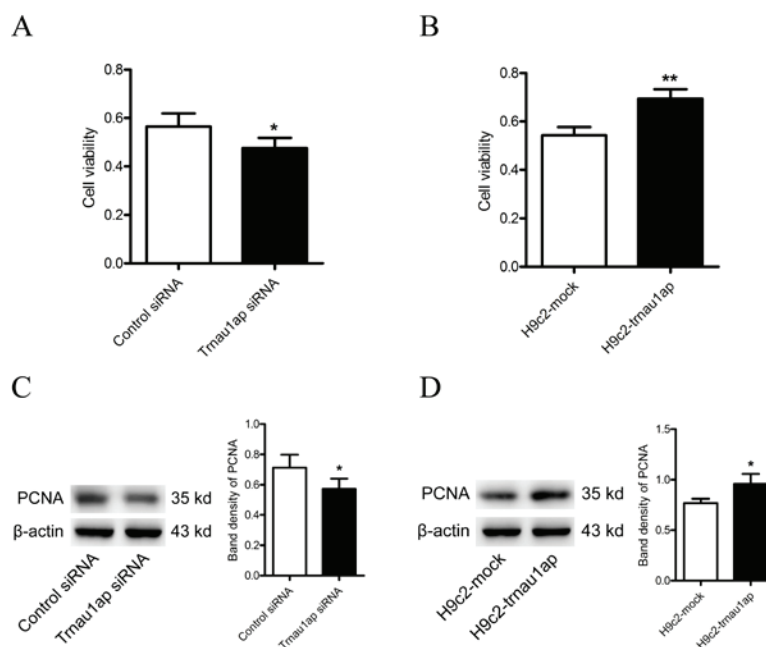


Figure 2. Effects of modulating Trnaulap expression levels on H9c2 cell proliferation. MTT assays were performed on cells with (A) Trnaulap knockdown by siRNA and (B) Trnaulap overexpression by Trnaulap transfection. Western blot analysis of protein expression levels of PCNA in (C) Trnaulap-underexpressing, and (D) Trnaulap-overexpressing, cells. β -actin served as an endogenous control. All data are presented as the mean \pm standard deviation (n=3). * P <0.05 and ** P <0.01 vs. control siRNA or H9c2-Mock. Trnaulap, transfer RNA selenocysteine 1 associated protein 1; siRNA, small interfering RNA; PCNA, proliferating cell nuclear antigen.

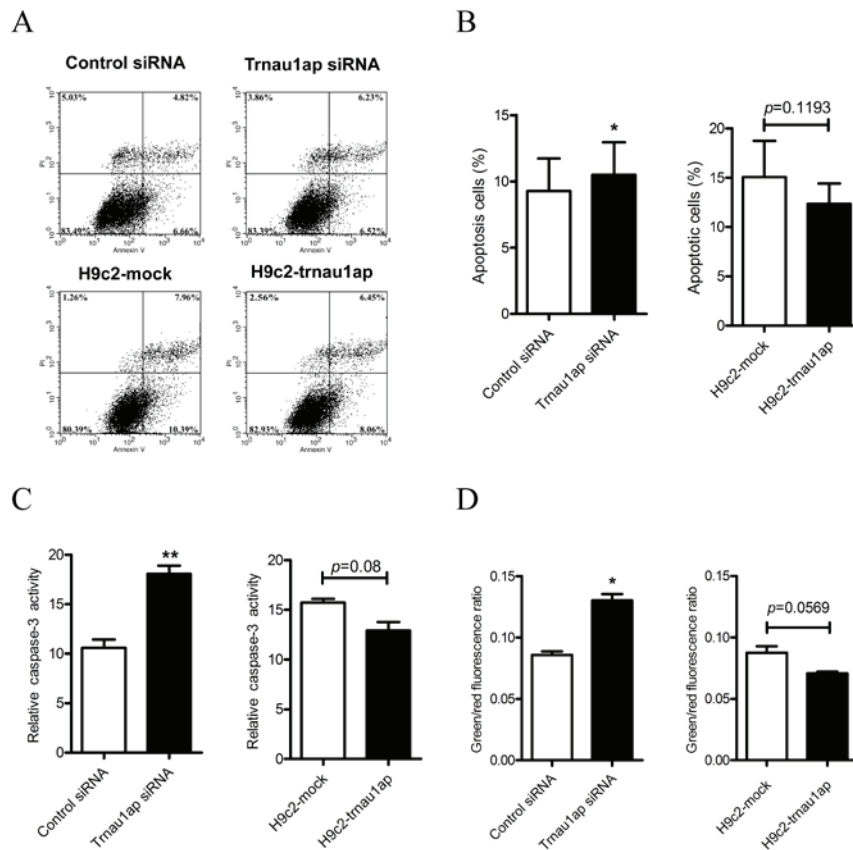


Figure 3. Effects of modulating Trnaulap expression levels on H9c2 cell apoptosis. (A) Apoptotic cells in the different groups were detected using annexin V-fluorescein isothiocyanate and PI staining 48 h post-transfection, and (B) the apoptotic rate was calculated. (C) Relative activity of caspase-3 in the different groups 48 h post-transfection. (D) Mitochondrial membrane potential in the four groups, measured using a JC-1 probe. Depolarization of the mitochondrial membrane potential is indicated as an increase in the green/red fluorescence ratio. All data are presented as the mean \pm standard deviation (n=3). *P<0.05 and **P<0.01 vs. control siRNA or H9c2-Mock. Trnaulap, transfer RNA selenocysteine 1 associated protein 1; siRNA, small interfering RNA; PI, propidium iodide; ns, non-significant.

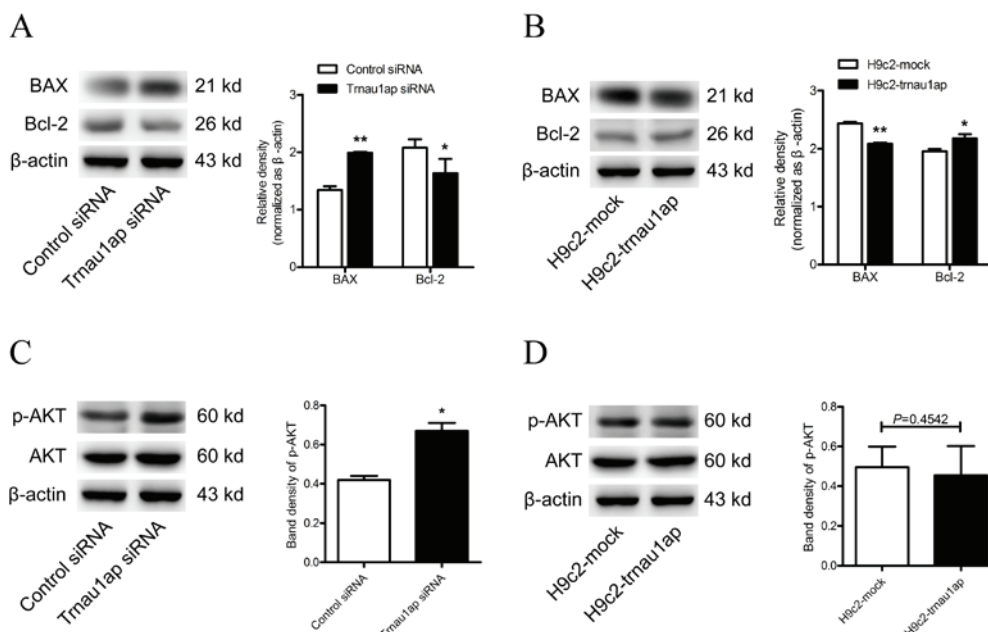


Figure 4. Effects of modulating Trnaulap expression levels on the expression of apoptosis-associated proteins. Protein expression levels of Bax and Bcl-2 in (A) Trnaulap-underexpressing, and (B) Trnaulap-overexpressing, cells were measured by western blotting. β -actin served as an internal control. The band densities of AKT and p-AKT in (C) Trnaulap-underexpressing and (D) Trnaulap-overexpressing cells were measured by western blotting. β -actin served as an internal control. The expression levels of p-AKT were normalized against total AKT. All data are presented as the mean \pm standard deviation (n=3). *P<0.05 and **P<0.01 vs. control siRNA or H9c2-Mock. Trnaulap, transfer RNA selenocysteine 1 associated protein 1; p, phosphorylated; AKT, protein kinase B; Bax, Bcl-2-X-associated protein; Bcl-2, B-cell lymphoma 2; siRNA, small interfering RNA; ns, non-significant.

whereas Bcl-2 expression levels were downregulated, in the underexpression group compared with the control group (Fig. 4A, $P < 0.05$ for Bcl-2, $P < 0.01$ for Bax compared with H9c2-siRNA). By contrast, Bax expression levels were downregulated, while Bcl-2 expression levels were upregulated, in the overexpression group compared with mock-transfected cells (Fig. 4B, $P < 0.05$ for Bcl-2, $P < 0.01$ for Bax compared with H9c2-mock). In addition, the PI3K/AKT signaling pathway was activated in Trnaulap-underexpressing cells compared with control cells after 48 h transfection (Fig. 4C, $P < 0.05$ compared with H9c2-siRNA), although no significant differences were observed in Trnaulap-overexpressing compared with mock-transfected cells (Fig. 4D, $P = 0.4524$ compared with H9c2-mock).

Discussion

The present study demonstrated for the first time, to the best of our knowledge, that modulating expression levels of Trnaulap affected the proliferation and apoptosis of rat cardiomyocyte-like H9c2 cells. Knockdown of Trnaulap by siRNA inhibited proliferation and increased apoptosis. Mitochondrial membrane potential depolarization was observed in the Trnaulap knockdown cells. In addition, the PI3K/AKT pathway was activated in cells with reduced Trnaulap expression levels. In comparison, Trnaulap-overexpressing cells exhibited an increased proliferative capacity.

Trnaulap, additionally known as SECp43, is a highly conserved protein with two ribonucleoprotein-binding domains and a polar/acidic carboxy terminus, and is critical for selenoprotein synthesis (11). A previous study reported that a decrease in the expression levels of Trnaulap induced a downregulation of GPx1 expression levels, and co-knockdown of Trnaulap and SepSecS induced a decrease in selenoprotein expression levels (14). A recent study identified no significant alteration in the levels of ^{75}Se -labeled hepatic proteins, or in levels of selenoproteins as determined by western blot analysis, in hepatocyte-specific Trnaulap knockout mice (19). However, in the present study, knockdown of Trnaulap in H9c2 cells reduced GPx1, Txnrd1 and SelK expression levels, and overexpression of Trnaulap increased selenoprotein expression levels, indicating that Trnaulap serves an important role in selenoprotein production. This discrepancy in findings suggests that the functional role of Trnaulap may vary according to tissue type and species.

Various selenoproteins are crucial antioxidant enzymes, including GPxs, TrxRs, SelK and selenoprotein W. Therefore, a decrease in selenoprotein expression levels, as in the Trnaulap knockdown group, may disturb cellular redox homeostasis, eventually leading to apoptosis.

PCNA, an essential cofactor of DNA polymerases, encircles synthesizing DNA and is used as a molecular marker of cell proliferation (20,21). Numerous studies have reported that the proliferation of cells and tissues is associated with PCNA levels (22-24). In the present study, PCNA levels were increased in Trnaulap-overexpressing cells, suggesting that a potential association exists between Trnaulap and cell proliferation.

Mitochondria serve critical roles in oxygen free radical detoxification (25). Furthermore, these organelles are important for apoptosis. When pro-apoptotic proteins, including

Bax and BH3 interacting-domain death agonist, translocate to the mitochondrial membrane, mitochondrial permeability transition pores promote cytochrome c release, triggering apoptosis (26). The present study revealed that mitochondrial membrane depolarization occurred in cells with decreased Trnaulap expression levels, suggesting that a reduction in Trnaulap levels promoted apoptosis via the mitochondrial pathway.

Apoptosis is an important cellular process during development, and is additionally involved in tissue homeostasis by eliminating damaged cells. Furthermore, apoptosis is involved in numerous diseases, including cardiovascular disease (27). Caspase (cysteine aspartase) family members serve primary roles in programmed cell death. In apoptosis, activation of caspase-3 is a critical event in the execution phase. Bcl-2 family proteins are key regulators of the apoptotic signaling pathway. The pro- and anti-apoptotic Bcl-2 family members present on the mitochondrial membrane determine apoptosis or survival (28). Bcl-2 is an anti-apoptotic protein, while Bax is pro-apoptotic. Numerous studies have revealed that the Bax/Bcl-2 ratio is an important determinant of whether cells undergo apoptosis (29-31).

Previous studies have demonstrated that selenium protects neuroblastoma cells from hypoxia-induced apoptosis (32), and additionally protects primary human keratinocytes from apoptosis induced by exposure to ultraviolet radiation (33). The present study revealed that reduced Trnaulap expression levels inhibited proliferation and induced apoptosis, suggesting that this protein regulates proliferation and apoptosis in H9c2 cells via its essential role in selenoprotein synthesis.

The PI3K/AKT signaling pathway serves a critical role in mediating survival and apoptosis (34,35). Oxidative stress has been demonstrated to activate the PI3K/AKT signaling pathway in a variety of cells (36). A previous study revealed that the PI3K/AKT signaling pathway was activated in the muscles of selenium-deficient chickens (37). The present study identified that the PI3K/AKT signaling pathway was activated in cells with reduced Trnaulap expression levels. This finding suggests that the downregulation of selenoprotein synthesis may induce apoptosis via increased levels of oxidative stress.

In conclusion, to the best of our knowledge, these results demonstrated for the first time that Trnaulap regulated proliferation and the mitochondrial apoptotic pathway in cardiomyocyte-like cells. However, additional studies using other cell types and species are required to clarify the functional roles of Trnaulap. Nonetheless, these findings provide insight into the mechanisms underlying myocardial injury induced by selenium deficiency.

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