Nicotine induces H9C2 cell apoptosis via Akt protein degradation

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Abstract. Smoking is highly associated with cardiovascular diseases. However, the effect of nicotine, a key ingredient in smoking products, on cardiomyocyte apoptosis remains controversial. The present study aims to clarify the role of nicotine on cardiomyocyte cell apoptosis and to investigate the underlying mechanism. In the present study, H9C2 cells were exposed to nicotine at various concentrations (0, 10 and 100 μM) for 48 h. Cell Counting Kit-8 and TUNEL assays were performed to assess cell viability and apoptosis, respectively, and reverse transcription-quantitative polymerase chain reaction and western blot analysis were used to investigate the mRNA and protein expression. PYR-41, a ubiquitin E1 inhibitor, was employed to investigate whether the ubiquitin-proteasome system was involved in the downregulation of Akt. An Akt1 overexpression plasmid was used to demonstrate the role of Akt in H9C2 cells apoptosis. Tetratricopeptide repeat domain 3 (TTC3) small interfering RNA (siRNA) was used to investigate the effect of TTC3 on Akt protein degradation. The results demonstrated that nicotine induced apoptosis in H9C2 cells compared with control cells ($P<0.05$). The protein level of Akt was downregulated by nicotine in a concentration-dependent manner ($P<0.05$). PYR-41 treatment restored the protein level of Akt. The cell viability was significantly improved by Akt overexpression when cells were exposed to nicotine at 10 μM, compared with control cells. Nicotine also upregulated the level of TTC3 mRNA ($P<0.05$) and the protein level of Akt, and cell viability was recovered by TTC3 siRNA. In conclusion, the current study demonstrated that nicotine induced H9C2 cell apoptosis via Akt protein degradation, which may be mediated by TTC3.

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Introduction

It is well established that smoking is a serious health concern and is associated with cancer and various other diseases (1-3). Cardiovascular disease is a frequently-occurring and common disease, with a higher incidence and mortality among smokers (4). Nicotine is the key ingredient in smoking products and there are two distinctive arguments regarding nicotine and apoptosis. Certain reports have indicated that nicotine promotes proliferation and inhibits apoptosis by acting on neuronal nicotinic acetylcholine receptors (nAChRs) (5,6). However, another report indicated that nicotine induces human cell apoptosis by influencing Hsp90 α expression (7). Therefore, the effect of nicotine on apoptosis requires further investigation. Previously, it was demonstrated that nicotine has a harmful effect on cardiomyocytes via the promotion of apoptosis (8), which is associated with various cardiology diseases and high cardiovascular mortality, however, the underlying mechanism is not well established.

Akt, a serine/threonine kinase, has an important role in cell survival, proliferation, migration and apoptosis. Akt is phosphorylated and activated by phosphoinositide 3-kinase (PI3K), which is mediated by the insulin pathway, and subsequently regulates fatty acid β-oxidation and promotes survival (9,10). An *et al* (11) reported that melatonin attenuates sepsis-induced cardiac dysfunction via activation of Akt. Due to the critical role of Akt, the regulatory mechanism of Akt is important. Various methods of Akt regulation exist, one of which is Akt protein degradation (12). A previous study demonstrated that ubiquitin-mediated Akt protein degradation leads to normal human lung fibroblast apoptosis (13). Tetratricopeptide repeat domain 3 (TTC3) and mitochondrial E3 ubiquitin protein ligase 1 (MUL1), which are important regulating factors of E3 ligases for Akt, have been reported to cause Akt ubiquitination and proteasomal degradation (14,15).

The present study, to the best of our knowledge, demonstrated for the first time that nicotine induced H9C2 cell apoptosis via Akt protein degradation. In addition, the results indicated that nicotine upregulated the level of TTC3 mRNA, which may be responsible for Akt protein degradation.

Materials and methods

Cell culture. H9C2 embryonic rat myocardium-derived cells were obtained from Shanghai Institutes for Biological

Table I. Primer sequences used for reverse transcription-quantitative polymerase chain reaction.

Gene	Accession no.	Primer sequence	
		Forward	Reverse
GAPDH	NM_017008.4	5'-GGCACAGTCAAGGCTGAGAATG-3'	5'-ATGGTGGTGAAGACGCCAGTA-3'
Total-Akt	NM_033230.2 NM_017093.1 NM_031575.1	5'-ATGGACTTCCGGTCAGGTTCA-3'	5'-GCCCTTGCCCAGTAGCTTCA-3'
MUL1	NM_001106695.1	5'-GCAAGTTACAGCCACCACCTGA-3'	5'-CCAGACTTTGTGTTGCTGCTGA-3'
TTC3	NM_001108315	5'-GTGGGCACAAGTTTCACAAAGG-3'	5'-AAGTGCATGGTGCATTAGTGAGG-3'

MUL1, mitochondrial E3 ubiquitin protein ligase 1; TTC3, tetratricopeptide repeat domain 3.

Sciences, Chinese Academy of Sciences (Shanghai, China). The cells were cultured in Dulbecco's modified Eagle's medium (Hyclone; GE Healthcare Life Sciences, Logan, UT, USA) containing 10% heat-inactivated fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and 100 U/ml penicillin and 100 μg/ml streptomycin at 37˚C in a humidified incubator with 5% CO_2 until 70-80% confluence, and sub-cultured (1:3 split ratio) using trypsin (0.25%) containing EDTA (0.02%).

Cell treatments. Nicotine (Sigma Aldrich, Merck KGaA, Darmstadt, Germany) was dissolved in DMEM containing 10% FBS to concentration of 10 and 100 μM, then 80% confluent cells were incubated with different concentrations of nicotine (10 or 100 μM) or standard medium for 48 h at 37˚C, or together with a PYR-41 pretreatment for 30 min. PYR-41 (Selleckchem, TX, USA) was dissolved in DMEM containing 10% FBS to concentration of 5 and 10 μM.

Cell viability assay. Cell Counting Kit-8 (CCK-8) assay was purchased from Dojindo Molecular Technologies, Inc. (Kumamoto, Japan) and was used to measure the cell viability in different nicotine treatment groups, according to the manufacturer's protocol. Briefly, cells were seeded in a 96-well plate at a density of $5x10^3$ cells/well with four replicates for each group and cultured overnight at 37˚C, cells were subsequently exposed to various concentrations of nicotine (10 or 100 μM) or standard medium as a control for 48 h at 37˚C. Following treatment, 100 μl fresh medium containing 10 μl CCK-8 solution was added to each well in an incubator for 30 min at 37˚C. The optical density of each well was determined at 450 nm by a microplate reader to calculate the cell viability.

Quantification of apoptotic cells. According to the manufacturer's protocol, the cell apoptosis at single-cell level was detected and quantified by TUNEL assay, using the *in situ* Cell Death Detection Fluorescein kit (Roche Molecular Systems, Inc., Pleasanton, CA, USA). Briefly, cells cultured on glass coverslips at 37˚C at 70-80% confluence, were treated with various concentrations of nicotine (0, 10 or 100 μM) for 48 h at 37˚C, washed with PBS, fixed with 4% paraformaldehyde in PBS for 1 h at 37˚C and permeabilized with 0.5% Triton X-100 in 0.1% sodium citrate for 2 min on ice. Following incubation with the enzyme solution mixture and label solution for 1 h at 37˚C in a dark, humid chamber, nuclei were counterstained with 100 ng/ml DAPI for 3-5 min at 37˚C. The percentage of cells undergoing apoptosis was determined with a fluorescence microscope and 25 random fields were quantified by an investigator who was blind to the treatment.

mRNA expression analysis. Total RNA was prepared from 95% confluent cultured cells using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. RNase-free DNase I (cat. no. RR047A; Takara Biotechnology, Co., Ltd., Dalian, China) was used to remove DNA contamination and cDNA was generated from 2 μg total RNA using the PrimeScript™ RT reagent kit with gDNA Eraser (cat. no. RR047A; Takara Biotechnology, Co., Ltd.), according to the manufacturer's protocol. The 20 μl reaction mixture was incubated as 50˚C of 15 min followed with enzyme inactivation by incubation at 85˚C for 5 sec. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was performed using a CFX96 Real-Time PCR System (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and the QuantiTect SYBR-Green PCR kit (Takara Biotechnology, Co., Ltd.), with the following An initial predenaturation step at 95˚C for 30 sec, followed by 40 cycles of denaturation at 95˚C for 5 sec and annealing at 60˚C for 30 sec. Data processing and normalization was undertaken as previously described (16). All RT-qPCR experiments were performed in triplicate. Primers used are listed in Table I.

Gene silencing with small interfering RNA (siRNA) and gene overexpression with plasmid vector. The siRNA against TTC3 mRNA (5'-UUGCAACUUGCUAGAAGAAUU-3') and scrambled siRNA (5'-UUAACGUUGAACGAUCUUCUU-3') were designed and purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). The Akt1 overexpression pCMV6-XL5 plasmid vector and control pCMV6-XL5 plasmid vector were designed by GeneCopoeia, Inc. (Rockville, MD, USA). H9C2 cells were plated onto 6-well plates at a density of $1.5x10^4$ cells/well and transfected with TTC3 siRNA (75 pmol) or pCMV6-XL5-Akt1 (2 μg) at 80% confluence at 37˚C using Lipofectamine 2000 according to the manufacturer's protocol (Invitrogen; Thermo Fisher Scientific, Inc.). At 48 h post-transfection, RT-qPCR was used to determine the expression levels

of TTC3 and western blotting was performed to determine the expression levels of Akt1. At 36 h post-transfection, the cells were subjected to treatment with nicotine (10 μM) or standard medium for 48 h at 37˚C. Subsequently, the cells were harvested for further analysis.

Western blot analysis. Cells were cultured in a 6-well plates until 95% confluence and were lysed in ice-cold radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology, Haimen, China) supplemented with 1% phenylmethylsulfonyl fluoride (Beyotime Institute of Biotechnology). Protein concentrations were detected with a bicinchoninic acid assay. Lysates (40 μg/lane) were separated on 12% SDS-PAGE gels, transferred to nitrocellulose filter membranes. Subsequently, the membrane was incubated with 10% skim milk for 1 h at 37˚C to block nonspecific binding, and incubated overnight at 4˚C with antibodies directed against Akt (cat. no. 9272; 1:1,000), phosphorylated (p)-Akt (ser473; cat. no. 4060; 1:1,000), caspase-3 (cat. no. 14220; 1:1,000), cleaved-caspase-3 (cat. no. 9664; 1:1,000; all from Cell Signaling Technology, Inc., Danvers, MA, USA), β-actin (cat. no. sc-130300; 1:500, Santa Cruz Biotechnology, Inc., Dallas, TX, USA). The blots were subsequently incubated with horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (cat. no. 7074; 1:2,000) or an anti-mouse IgG (cat. no. 7076; 1:2,000; Cell Signaling Technology, Inc.) for 1 h at 37˚C. Bands were visualized with an enhanced chemiluminescence kit (EMD Millipore, Billerica, MA, USA) in a ChemiDoc XRS system (Bio-Rad Laboratories, Inc.). The protein level was quantified with Quantity One software version 4.6.2 (Bio-Rad Laboratories, Inc.).

Statistical analysis. Data are presented as the mean ± standard error of the mean. SPSS 15.0 software (SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses. Statistical comparisons between two groups were performed with the unpaired Student's t-test. The differences between more than two groups were analyzed with one-way analysis of variance followed by Student-Newman-Keuls post hoc test. $P \leq 0.05$ was considered to indicate a statistically significant difference.

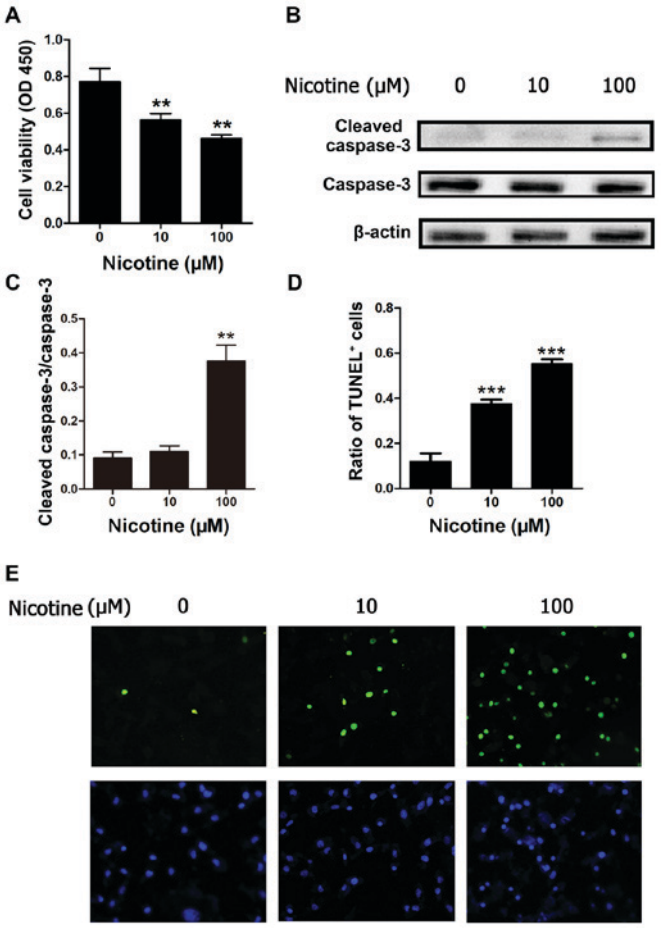

Figure 1. The effect of nicotine on H9C2 cell apoptosis *in vitro*. (A) Detection of cell viability following exposure to nicotine at concentrations of 0, 10 and 100 μM for 48 h (n=4 per group). (B) Representative western blots demonstrating the protein expression of caspase-3 and the active form cleaved caspase-3, which is a marker of apoptosis, when exposed to nicotine at concentrations of 0, 10 and 100 μM for 48 h. (C) Densitometric analysis of western blots was performed to quantify the protein expression of caspase-3 and the active form cleaved caspase-3 (D) Statistical analysis of the ratio of apoptotic cells detected by TUNEL staining (n=7 per group). (E) TUNEL staining for analysis of apoptosis following treatment of cells with 0, 10 and 100 μM nicotine. Upper, cell apoptosis was detected by TUNEL staining (green); lower, DAPI was used to stain nuclei (blue) (magnification, x400). Data are presented as the mean ± standard error of the mean. **P<0.01 and ***P<0.005 vs. 0 μM nicotine group. OD, optical density.

Results

Nicotine promotes H9C2 cell apoptosis in vitro. To investigate whether nicotine may promote cardiac myoblast cell apoptosis, the present study employed H9C2 cells to determine the role of nicotine on apoptosis in cultured cells. Cell viability was determined by CCK-8 assay. The viability of H9C2 cells exposed to nicotine at 10 and 100 μM was significantly reduced in a concentration-dependent manner after 48 h, compared with control cells (P<0.01; Fig. 1A). To further investigate the effect of the nicotine on H9C2 cells, the protein expression level of caspase-3, which is involved in apoptosis, was also investigated by western blotting. The results demonstrated that nicotine led to activation of caspase-3 (cleaved-caspase-3) in the 100 μM nicotine treatment, but not the 10 μM treatment group compared with the control (Fig. 1B and C). In addition, apoptosis was evaluated by TUNEL analysis. Compared with control cells, nicotine at 10 and 100 μM induced a significant increase in the number of TUNEL-positive cells after 48 h of treatment (Fig. 1D and E). These results indicate that nicotine may inhibit H9C2 cell viability and promote H9C2 cell apoptosis.

Nicotine induces Akt protein degradation through the ubiquitin-proteasome system in a concentration-dependent manner. To investigate the mechanism of nicotine-induced cells apoptosis, the present study investigated whether nicotine altered Akt and p-Akt protein levels. Similar to the reduction in cell viability, protein levels of p-Akt and total-Akt were reduced by a 48 h nicotine exposure in a concentration-dependent manner (Fig. 2A-C), compared with control cells. In order to further investigate the mechanism, the level of Akt mRNA expression was determined, and it was observed that Akt mRNA expression was unchanged following 48 h exposure to 10 and 100 μM nicotine (Fig. 2D). To investigate the involvement of the ubiquitin-proteasome system in the reduced

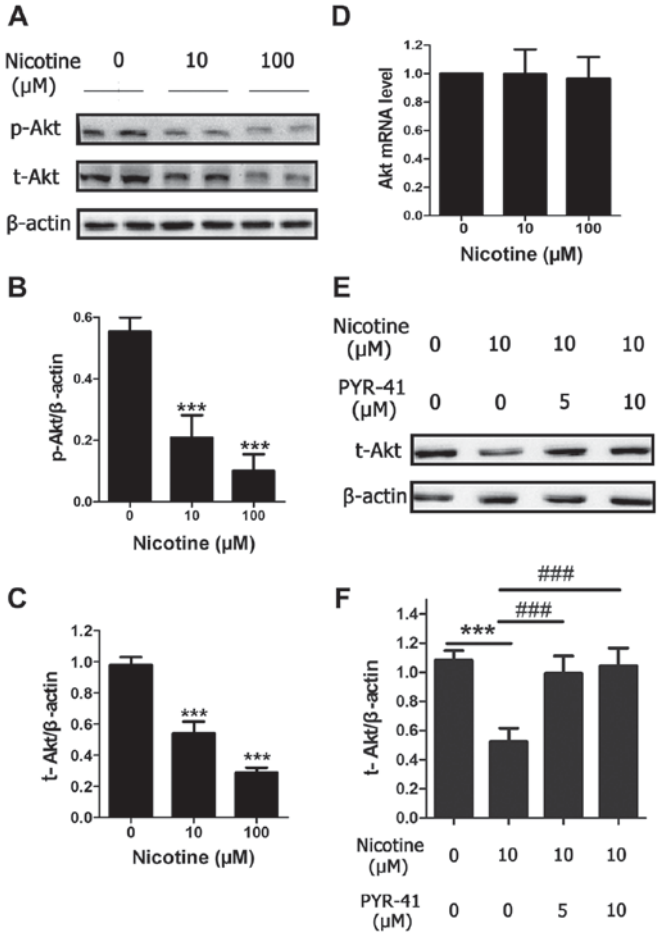

Figure 2. Nicotine induced Akt protein degradation via the ubiquitin-proteasome system. (A) Representative western blots revealing the protein expression of p-Akt and t-Akt when exposed to nicotine at concentrations of 0, 10 and 100 μM for 48 h. Densitometric analysis of western blots was performed to quantify the protein expression of (B) p-Akt and (C) t-Akt when exposed to nicotine at concentrations of 0, 10 and 100 μM for 48 h (n=8 per group). (D) Reverse transcription-quantitative polymerase chain reaction was performed to investigate the expression of Akt mRNA when cells were exposed to nicotine at concentrations of 0, 10 and 100 μM for 48 h (n=3 per group). (E) Representative western blots revealing the effect of PYR-41 (5 and 10 μM) on the protein expression of Akt when exposed to 10 μM nicotine for 48 h. (F) Densitometric analysis of western blots was performed to quantify the protein expression of Akt and to determine the effect of PYR-41 on Akt protein expression and therefore degradation (n=8 per group). Data are presented as the mean ± standard error of the mean. ***P<0.005 vs. 0 μM nicotine, $^{###}$P<0.005 vs. 10 μM nicotine and 0 μM PYR-41. p-Akt, phosphorylated-Akt; t-Akt, total-Akt.

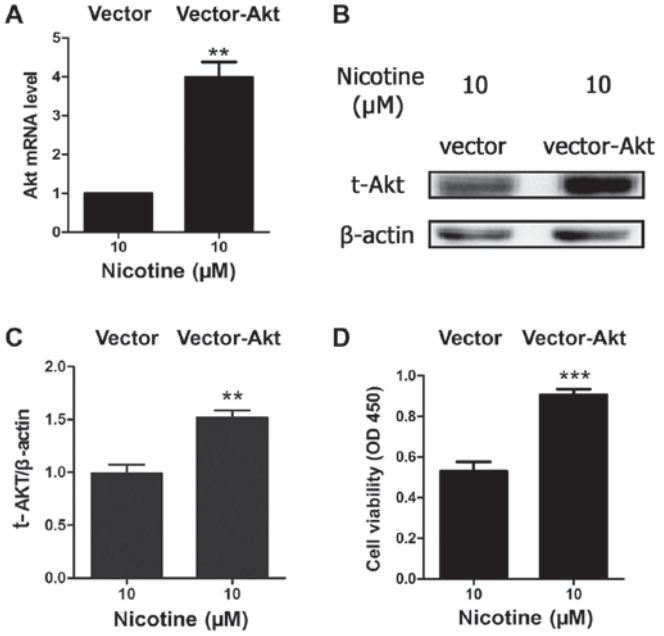

Figure 3. Cell viability was improved by Akt overexpression when exposed to nicotine. (A) Reverse transcription-quantitative polymerase chain reaction was performed to investigate the protein expression of Akt mRNA when H9C2 cells were transfected with vector or vector-Akt1 and exposed to nicotine at 10 μM (n=3 per group). (B) Representative western blots revealing the protein expression of Akt when H9C2 cells were transfected with vector or vector-Akt1 and exposed to nicotine at 10 μM. (C) Densitometric analysis of western blots was performed to quantify the protein expression of Akt when H9C2 cells were transfected with vector or vector-Akt1 and exposed to nicotine at 10 μM. (D) Detection of cell viability when H9C2 cells were transfected with vector or vector-Akt1 and exposed to nicotine at 10 μM (n=4 per group). Data are presented as the mean ± standard error of the mean. **P<0.01 and ***P<0.005 vs. vector. OD, optical density; t-Akt, total Akt.

protein expression of Akt following nicotine treatment, H9C2 cells were treated with different concentrations of PYR-41 (5 or 10 μM), which is a ubiquitin E1 inhibitor and also a valuable tool for investigating ubiquitination (17). After a 48 h period of nicotine exposure at 10 μM, which is the concentration that is closest to the levels observed in the blood of smokers, the reduction in the protein expression of Akt induced by 10 μM nicotine was significantly blocked by PYR-41 (Fig. 2E and F). Taken together, the results indicate that nicotine may cause Akt protein degradation via the ubiquitin-proteasome system in H9C2 cells.

Akt overexpression improves cell viability when exposed to nicotine. Due to the important role of Akt in cell survival and apoptosis, and as the results of the present study indicated that nicotine induced Akt protein degradation in a concentration-dependent manner, the present study further investigated the association between apoptosis and Akt. H9C2 cells were transfected with an Akt1 overexpression plasmid or vector-only. The results indicated that the level of Akt mRNA and protein were significantly upregulated in the vector-Akt group compared with the vector-only group when treated with 10 μM nicotine (Fig. 3A and B). Furthermore, the results demonstrated that Akt overexpression significantly inhibited nicotine-induced reductions in cell viability (Fig. 3C).

Nicotine induces Akt ubiquitination by TTC3. The results discussed in Fig. 2, where an inhibitor of ubiquitin E1 (PYR-41) was used, indicated that Akt protein degradation following exposure to nicotine occurred via ubiquitination and the ubiquitin-proteasome system. TTC3 and MUL1 are both E3 ligases that facilitate Akt ubiquitination. Therefore, to determine the type of E3 ligase against Akt that was involved in nicotine-induced Akt ubiquitination, the mRNA levels of TTC3 and MUL1 were determined using RT-qPCR in nicotine-exposed H9C2 cells. Nicotine augmented TTC3 mRNA expression in a concentration-dependent manner after 48 h of treatment (Fig. 4A). By contrast, MUL1 mRNA was unchanged under the same conditions (Fig. 4B). To further demonstrate the role of TTC3 in nicotine-induced Akt ubiquitination and degradation, TTC3 siRNA was used to inhibit

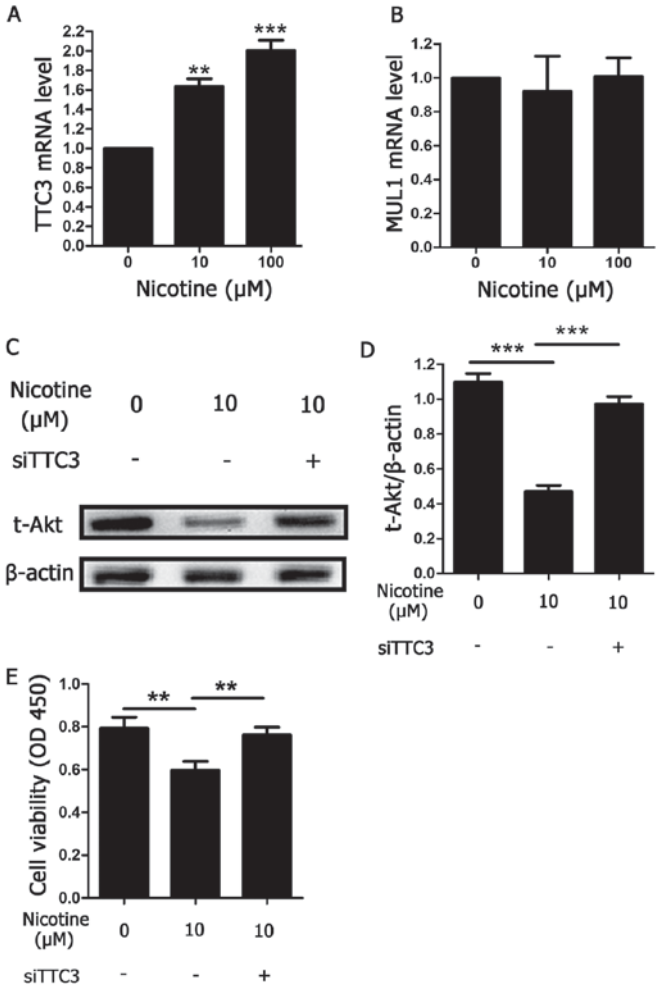

Figure 4. Nicotine induced Akt ubiquitination via TTC3. Reverse transcription-quantitative polymerase chain reaction was performed to investigate the expression of (A) TTC3 and (B) MUL1 mRNA when cells were exposed to nicotine at concentrations of 0, 10 and 100 μM for 48 h (n=3 per group). (C) Representative western blots revealing the effect of siTTC3 on Akt protein expression and therefore degradation when exposed to nicotine at 10 μM for 48 h. (D) Densitometric analysis of western blots was performed to quantify the protein expression of Akt and therefore determine the effect of siTTC3 on Akt protein degradation (n=8 per group). (E) Detection of cell viability when cells were exposed to nicotine at 10 μM for 48 h with or without siTTC3 (n=4 per group). Data are presented as the mean + standard error of the mean. **P<0.01 and ***P<0.005 vs. 0 μM nicotine TTC3, tetratricopeptide repeat domain 3; MUL1, mitochondrial E3 ubiquitin protein ligase 1; siTTC3, small interfering RNA targeting TTC3.

TTC3 mRNA expression. Knockdown of TTC3 expression by TTC3 siRNAs led to a significant reduction in Akt ubiquitination and degradation induced by nicotine, compared with nicotine-treated cells transfected with scrambled siRNA (Fig. 4C and D). Furthermore, the current study determined whether knockdown of TTC3 expression may affect nicotine-induced reductions in cell viability. Compared with scrambled siRNA, cell viability was significantly increased by TTC3 siRNA (Fig. 4E), indicating that apoptosis levels may have been reduced.

Discussion

The present study demonstrated that nicotine reduced the viability and induced H9C2 cells apoptosis, Akt protein expression was downregulated when exposed to nicotine at various concentrations for 48 h, PYR-41, a ubiquitin E1 inhibitor, restored the protein expression of Akt, Akt overexpression inhibited H9C2 cell apoptosis induced by nicotine, nicotine upregulated the expression of TTC3 mRNA, nicotine-induced reductions in the protein expression of Akt protein level were reversed when TTC3 was silenced by siRNA and nicotine-induced reductions in cell viability were inhibited when TTC3 was silenced by siRNA.

It is clear that smoking is associated with numerous diseases, particularly cardiovascular diseases. Recent study reported by Baber *et al* (18) reported that smoking was an independent predictor of major bleeding following percutaneous coronary intervention with drug-eluting stents. In addition, Stone *et al* (19) discovered that smoking was associated with hospitalization for heart failure. Furthermore, Das *et al* (20), using pigs as experimental animals, revealed that smoking induced myocardial injury by release of cardiac troponin-T and -I in the serum, oxidative stress, inflammation, apoptosis, thrombosis and collagen deposition in the myocardium. Sumanasekera *et al* (21) also reported that smoking induced reduced cardiac stem cell viability, migration reduction and led to exacerbation of the damage.

Nicotine, which is a key ingredient in smoking products, may be responsible for various risks associated with smoking (22). Marrs and Maynard (23) reported that nicotine is the classic nAChR agonist and it has also been used as an insecticide. Li *et al* (24) discovered that nicotine induced cardiomyocyte hypertrophy through the transient receptor potential cation channel subfamily C member 3-mediated Ca^{2+}/nuclear factor of activated T-cells signaling pathway. Furthermore, Zhou *et al* (8) reported that nicotine promoted cardiomyocyte apoptosis via oxidative stress and altered expression of apoptosis-associated genes. In the present study, the reduction of cell viability, the increased percentage of TUNEL-positive cells and the activation of caspase-3 indicated that nicotine also promoted H9C2 cell apoptosis.

Akt, a serine/threonine kinase, is a crucial molecule that is involved in various biological actions, including apoptosis, survival, proliferation and cell migration. Activated Akt, which is phosphorylated by PI3K, suppresses apoptosis by inactivating proapoptotic factors, maintaining mitochondrial integrity and stabilizing anti-apoptotic factors (25). In addition, Akt prevents apoptosis by phosphorylating apoptosis signal-regulating kinase 1 (26), which causes the activation of c-Jun N-terminal kinase and p38 mitogen-activated protein kinase (27). Furthermore, Akt leads to p53 ubiquitination and degradation by phosphorylating the ubiquitin ligase mouse double minute homolog 2, which leads to apoptosis inhibition (28). Stulpinas *et al* (29) previously demonstrated that inhibition of Akt kinase signaling pathways leads to muscle-derived stem cell death. Regarding the cardiovascular system, Kerr *et al* (30) discovered that endothelial Akt deletion induced retinal vascular smooth muscle cell loss and basement membrane deterioration, and resulted in vascular regression and retinal atrophy. In addition, Fujio *et al* (31) reported that Akt protected cardiomyocytes against ischemia-reperfusion injury. The results of the present study indicated that the protein level of Akt was downregulated when exposed to nicotine in a concentration-dependent manner, while the cell viability in

H9C2 cells was increased following nicotine treatment when Akt expression was upregulated.

There are various methods of Akt regulation, and one mechanism is Akt degradation. Kim *et al* (32) reported that non-thermal plasma induced Akt degradation via the ubiquitin-proteasome system, subsequently leading to head and neck cancer cell death. They also demonstrated that cigarette smoke induced normal human lung fibroblast cell apoptosis by Akt protein degradation via the ubiquitin-proteasome system (13). The present study demonstrated that PYR-41, a ubiquitin E1 inhibitor, restored the protein expression of Akt following nicotine treatment. Therefore, it was hypothesized that nicotine induced Akt protein degradation via the ubiquitin-proteasome system. The results of the current study also indicated that the cell viability was restored when Akt was overexpressed.

TTC3 is an Akt-specific E3 ligase that binds to Akt and facilitates its ubiquitination and degradation within the nucleus (14,33,34). The present study demonstrated that nicotine upregulated the level of TTC3 mRNA, whereas the level of Akt mRNA was not changed. Furthermore, when TTC3 was silenced by siRNA, the nicotine-induced reduction in the protein expression of Akt was significantly reversed, and the cell viability was also improved. Taken together, these results indicate that nicotine may induce Akt protein degradation by the ubiquitin-proteasome system via TTC3 upregulation.

In conclusion, the current study demonstrated that nicotine induced H9C2 cell apoptosis by facilitating Akt protein degradation, it was also demonstrated that inhibition of the degradation of Akt protein protected H9C2 cells from reduction of cell viability induced by nicotine. These results may contribute to the investigation of the mechanism of diseases caused by smoking and provide a novel therapeutic target for smoking-associated diseases. Due to the effect of nicotine on the apoptosis of H9C2 cells, and the role of nicotine on Akt protein degradation, we hypothesize that nicotine may aggravate apoptosis following ischemia/reperfusion, which requires further investigation.

Acknowledgements

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