

Original Article

Tunicamycin induces apoptosis in non-small cell lung cancer cells through C/EBP homologous protein activation-mediated endoplasmic reticulum stress

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Abstract: Non-small-cell lung cancer (NSCLC) is one of the most common human cancers in the world that causes the most common cancer-related mortality in the world. Evidences have showed that Tunicamycin could inhibit growth of NSCLC through promoting apoptosis of NSCLC cells. However, underlying antitumor mechanism mediated by Tunicamycin is poorly understood so far in NSCLC cells. In the present study, tumor-inhibited efficacy and the anti-tumor molecular mechanism of Tunicamycin was investigated both in Non-small-cell lung cancer cells (NSCLCs) and xenografted mice. Tunicamycin exhibited cytotoxicity in A549 NSCLCs, which led to cellular atrophy and growth inhibition. Results showed that Tunicamycin inhibited migration and invasion through decreasing expression levels of vimentin, fibronectin and E-cadherin in A549 NSCLCs. Consistently, Tunicamycin significantly promoted apoptosis of A549 NSCLCs by up-regulation of cleaved PARP, activated caspase 9/3 and Bax and attenuating the expression of cyclin D1 and cyclin D2 in A549 NSCLCs. Notably, Tunicamycin increased expression levels of C/EBP homologous protein (CHOP) IRE1 α , ATF6 and p-eIF2 in progression of endoplasmic reticulum stress, which resulted in apoptosis in A549 NSCLCs. Mechanism analysis showed that knockdown of CHOP expression blocked Tunicamycin-mediated apoptotic activity in A549 NSCLCs. In vivo assay showed that Tunicamycin treatment significantly inhibited NSCLCs growth and prolonged survival of tumor-bearing mice. Overall, these findings indicate that Tunicamycin can induce NSCLC apoptosis through CHOP activation-mediated endoplasmic reticulum stress, which provide scientific evidences and suggest that Tunicamycin can serve as anti-cancer agent in the treatment of NSCLC.

Keywords: NSCLC, Tunicamycin, apoptosis, CHOP

Introduction

Non-small cell lung cancer (NSCLC) is one of the most common human cancers and famous for rapidly growth, easily migration, invasion and reoccurrence. Previous study has reported that tumor growth migration and invasion of NSCLC are the most important features in tumor metastasis, development and reoccurrence [1]. Currently, NSCLC occurrence has been closed to outbreak period duo to industrial pollution and destruction of the ecological environment in last century [2, 3]. NSCLC includes adenocarcinoma, large cell carcinoma and squamous cell carcinoma that is also the most frequent type of lung cancer and accounts for approximate 80% in the whole lung cancer cases [4-6]. Despite more and more therapeu-

tic improvements for NSCLC were put forward, the poor survival rate of patients with NSCLC was less than 15 percent in the overall 5 years, which makes greatest trouble for critical clinical problem [5, 7, 8]. In addition, migration and invasion of NSCLC is the chief culprits for poor survival rate in during treatment and recurrence for patient with NSCLC [9, 10]. Furthermore, NSCLC is one of the most common malignant lung tumors that has become a major public health problem and the leading cancer-cause of cancer-related mortality in the world [11]. Statistics has revealed that approximate 75% patients have been diagnosed in middle-late stage NSCLC, which presents lower 5-year survival rate [12]. In recent years, Tunicamycin has exhibited anti-cancer effects through regulation of apoptosis-related signal pathway in

many tumor cells [13]. However, the potential mechanisms of Tunicamycin-induced apoptosis have not been well understood in NSCLCs. Therefore, inhibition of migration and invasion of NSCLC is an efficient and hopeful therapeutic schedule for cancer patients [14, 15].

Tunicamycin is nucleotide antibiotic produced by *Streptomyces lysosuperficus* that has been widely used to inhibit breeding of the virus by inhibition of synthesis of glycoprotein sugar chain in the generation of lipid intermediate [16]. In recent year, Tunicamycin presents anti-tumor effects by inhibiting tumor cells growth and promoting apoptosis of tumor cells [17, 18]. Kim et al have showed that Tunicamycin could induce paraptosis potentiated by inhibition of BRAFV600E in FRO anaplastic thyroid carcinoma cells [19]. Balogh and his colleagues have suggested that Tunicamycin inducing apoptosis by overexpression of CREB protein contributes to tumor inhibition both in vitro and in vivo [20]. Jiang et al also have showed that Tunicamycin sensitizes human melanoma cells to tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis by up-regulation of TRAIL-R2 via the unfolded protein response and suggested that Tunicamycin can induce ER stress-mediated apoptosis by up-regulation of TRAIL-R2 expression in melanoma cells [21].

Currently, reports have showed that endoplasmic reticulum stress is associated with apoptosis of tumor cells through regulation of chaperones and oxidoreductases enter retrograde trafficking in various malignant cells [22]. Previous study has revealed that endoplasmic reticulum plays an important role in tumor immunity [23]. In addition, Arya *et al* have provided that novel evidence of endoplasmic reticulum specific localization of Hsp60 during apoptosis both in vitro and in vivo [24]. Furthermore, Giordano *et al* have indicated that Tunicamycin could induce endoplasmic reticulum stress in human hepatocarcinoma cells, which further inhibited growth of breast cancer cells [25]. Moreover, study has revealed that Tunicamycin enhances the apoptosis induced by tumor necrosis factor-related apoptosis-inducing ligand in endometriotic stromal cells in human prostate cancer cells [20]. These results suggest that Tunicamycin contributes to inhibition of tumor cells growth and promotion of apoptosis.

In this study, we investigated the efficacy of Tunicamycin for NSCLCs growth and aggressiveness. To study the molecular mechanism involved in the proapoptosis effects of Tunicamycin, we analyzed Tunicamycin-mediated changes of endoplasmic reticulum stress in NSCLC cells and a mouse model. Here, we analyzed anti-tumor effects of Tunicamycin in xenografted mice, and show it leads to NSCLC apoptosis and inhibition of tumor growth.

Materials and methods

Ethic statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of Tianjin Hospital. All experimental protocols and animals were performed in accordance with National Institutes of Health and approved by Committee on the Ethics of Tianjin Hospital.

Cells and reagents

A549 cells were purchased from American Type Culture Collection (ATCC). Cells were cultured in DMEM (Sigma-Aldrich) medium (Gibco, CA, USA) supplemented with 10% fetal bovine serum (FBS) (Invitrogen, CA, USA). All cells were cultured in a 37°C humidified atmosphere of 5% CO₂.

MTT assay

A549 cells were incubated with Tunicamycin (0.50-2.50 mg/ml) in 96-well plates for 24, 48, 72 and 96 hours in triplicate for each condition with PBS as control. After the indicated time and dosage incubation, 20 µl of MTT (5 mg/ml) in PBS solution was added to each well, the plate was further incubated for 4 hours. Most of the medium was removed and 100 µl of DMSO was added into the wells to solubilize the crystals. The OD was measured by a BIO-RAD (ELISA) reader at wavelength of 450 nm.

Cells invasion and migration assays

A549 cells were incubated with Tunicamycin (0.20 mg/ml). For invasion assay, A549 cells were suspended as a density of 1×10^5 in 500 µl in serum-free DMEM. Cells were subjected to the tops of BD BioCoat Matrigel Invasion Chambers (BD Biosciences) according to the manufacturer's instructions. For migration

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assay, cells were subjected to a control insert (BD Biosciences) instead of a Matrigel Invasion Chamber. The tumor cells migration and invasion were counted in at least three randomly stain-field microscope every membrane.

Transfection of small interference RNA (Si-RNA)

All siRNAs were synthesized by Invitrogen (Shanghai, China) including Si-RCHOP (Kd-CHOP, sense, 5'-GAGCUCUGAUUGACCGAAU-3', antisense, 5'-CUCGAGACUAACUGGCUUA-3') or Si-RNA-vector (sense, 5'-GCAGUUGUACCAUGC-AUUA-3', antisense, 5'-CGUCAACAUGGUACGUA-AU-3'). A549 cells (1×10^6) were transfected with 100 pmol of Si-RCHOP targeting CHOP (Applied Biosystems) with Si-RNA-vector as control (Applied Biosystems) by using a Cell Line Nucleofector kit L (Lonza).

Apoptosis assay

A549 cells were grown at 37°C with 5% CO₂ until 90% confluence was formed. Apoptosis was assessed by incubation these cells with Tunicamycin (0.20 mg/ml) for 24 hours. After incubation, the tumor cells were trypsinized and collected. The cells were then washed in cold PBS, adjusted to 1×10^6 cells/mL with PBS, labeled with annexin V-FITC and PI (Annexin V-FITC Kit, BD), and analyzed with a FACScan flow cytometer (BD). The treatments were performed in triplicate, and the percentage of labeled cells undergoing apoptosis in each group was determined and calculated.

Animal study

Specific pathogen-free male Balb/c (eight weeks old) nude mice were purchased from Slack co., LTD (Shanghai, China). All rats were housed in a temperature-controlled environment (23±2°C) with 12 h light/12 h dark cycles. All mice were free access to food and water. Nude mice were implanted A549 cells into lamosina and were divided into two groups (n = 40 in each group). Treatments were initiated on day 6 after tumor implantation (diameter: 5-6 mm). Tumor-bearing mice were intravenously injected Tunicamycin (6 mg/kg) as PBS as control. The treatment was continued 14 times once time a day. The tumor volumes were calculated according to previous study [26].

Western blotting

A549 NSCLCs and tumors were homogenized in lysate buffer containing protease-inhibitor and were centrifuged at 2000x g at 4°C for 10 min. The supernatant of mixture were used for analyzing purpose protein. The purpose protein expression levels were incubated with rabbit anti-mouse primary antibodies: cyclin D1 (1:1000, ab134175, Abcam) and cyclin D2 (1:1000, ab81359, Abcam), vimentin (1:1000, ab28028, Abcam), fibronectin (1:1000, ab19-4395, Abcam), E-cadherin (1:1000, ab133597, Abcam), PAPR (1:1000, ab144322, Abcam), Bad (1:1000, ab90435, Abcam), Survivin (1:1000, ab203571, Abcam), P53 (1:1000, ab-1431, Abcam), Caspase-3 (1:1000, ab13847, Abcam), Caspase-8 (1:1000, ab108333, Abcam), Caspase-9 (1:1000, ab202068, Abcam), Bax (1:1000, ab32503, Abcam), Bim (1:1000, ab32158, Abcam), Bid (1:1000, ab62469, Abcam), Bax (1:1000, ab32503, Abcam), PERK (1:1000, ab217322, Abcam), CHOP (1:1000, ab11419, Abcam), eIF2α (1:1000, ab5369, Abcam), β-actin (1:1000, ab8226, Abcam) for western blotting. HRP-conjugated IgG (PV-6001, ZSGB-BIO, Beijing, China) were added after blocking (5% skimmed milk) for 1 hours at 37°C and then were visualized by using chemiluminescence detection system.

Immunohistochemistry and immunofluorescence

NSCLC tumors from xenograft mice were fixed by using formaldehyde (10%) followed with embed in paraffin. Tumor tissues were fabricated to tumor sections. Antigen retrieval was performed in tumor sections and the sections were incubated with primary antibodies. Then, appropriate secondary antibodies were applied for specimens and specimens were visualized. A Ventana Benchmark automated staining system was used for observation of caspase-9, caspase-8, pro-caspase-3, TUNEL, ATF-6, CHOP and eIF2α in tumor tissues. For immunofluorescence, red fluorescent protein-labeled Apaf-1 or Cyto c (QIAGEN, Gaithersburg, MD) were used to incubate A549 cells and washed with PBS three times. Expression levels Apaf-1 or Cyto c were analyzed by fluorescence microscope (Canon, Japanese).

Statistical analysis

All data were expressed as mean and SD of triplicate dependent experiments and analyzed by

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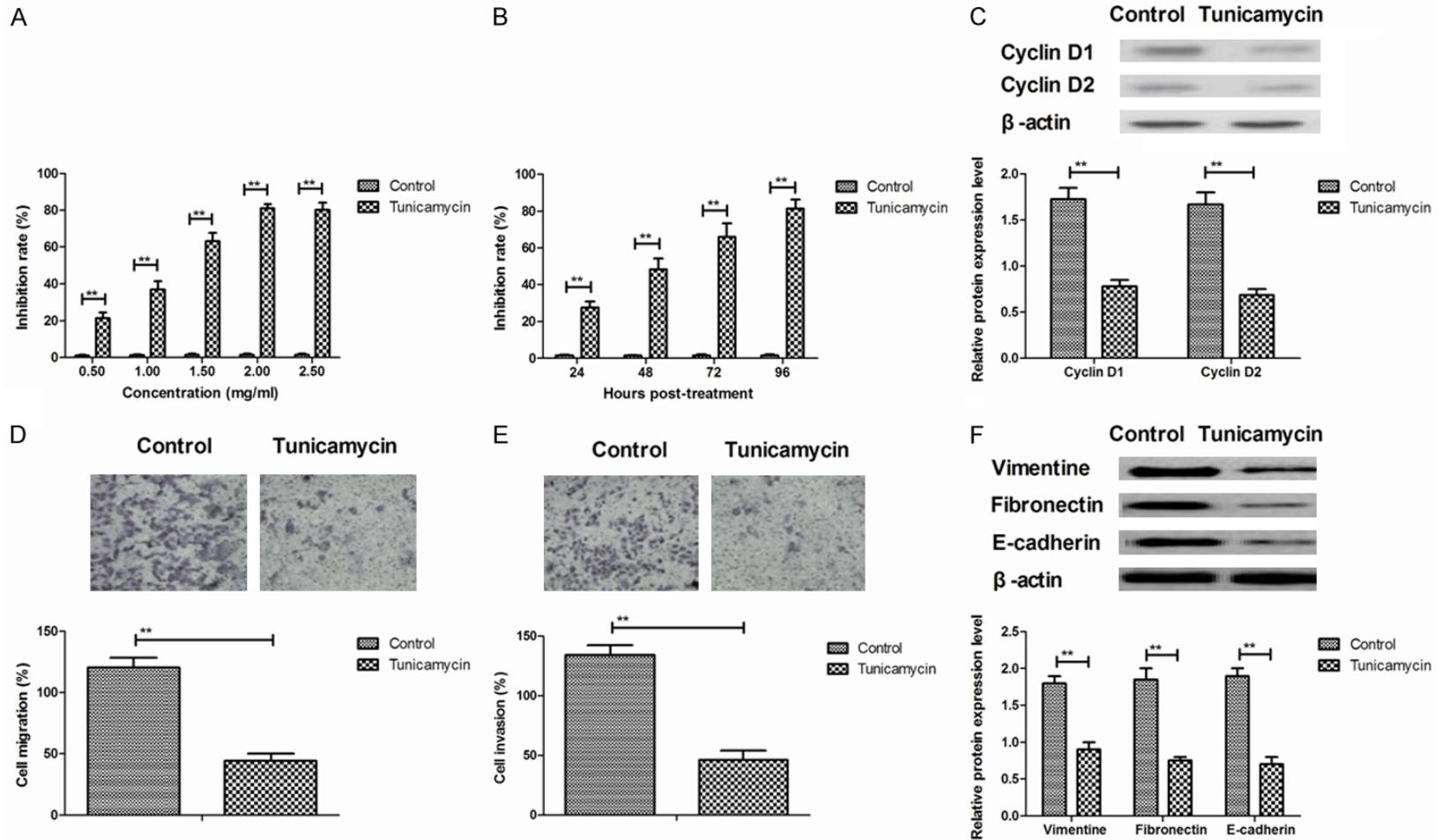


Figure 1. Effects of Tunicamycin on growth and aggressiveness of NSCLCs. (A) Tunicamycin inhibits growth in a dose-dependent manner. (B) Tunicamycin inhibits growth in a time-dependent manner. (C) Expression levels of cyclin D1 and cyclin D2 in A549 NSCLCs after Tunicamycin treatment. (D-E) Inhibitory effects of Tunicamycin on migration (D) and invasion (E) of NSCLCs. (F) Expression levels of metastasis-related vimentin, fibronectin and E-cadherin in A549 NSCLCs after Tunicamycin treatment.

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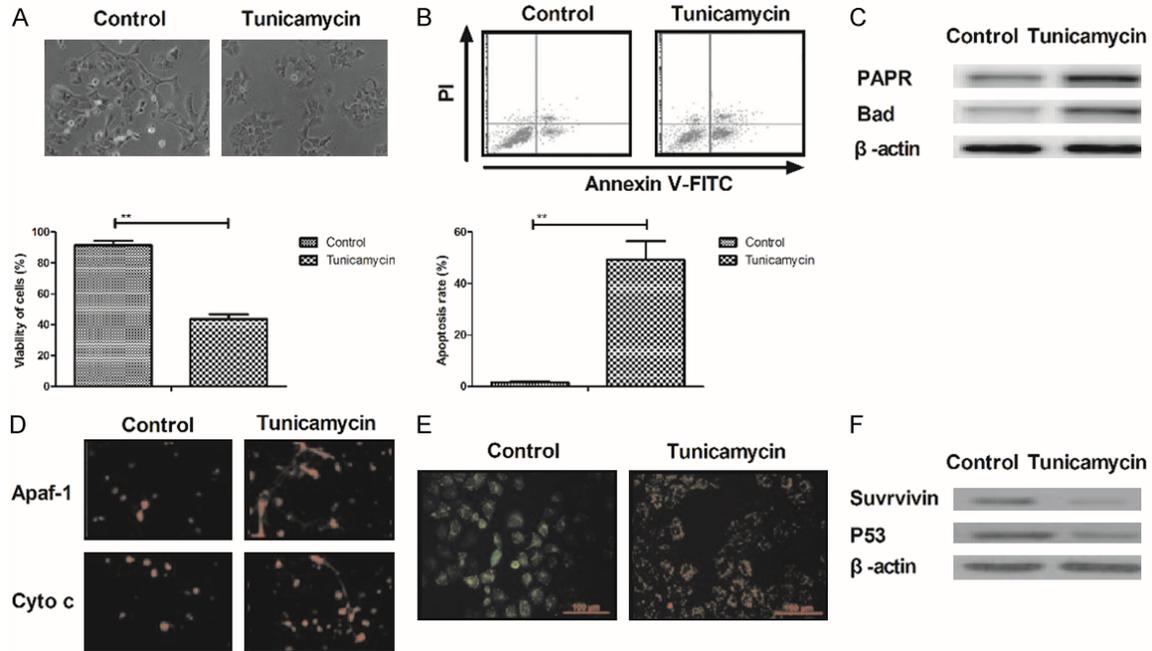


Figure 2. Tunicamycin induces apoptosis of NSCLCs through mitochondrial apoptosis signaling pathway. A: Affection of Tunicamycin on cellular atrophy of A549 NSCLCs determined by morphological analysis. B: Tunicamycin promotes apoptosis of NSCLCs. C: Expression levels of cleaved PARP and Bad in A549 NSCLCs after Tunicamycin treatment. D: Expression levels of Apaf-1 and Cyto c in A549 NSCLCs after Tunicamycin treatment determined by immunofluorescence. E: Analysis of mitochondria damage in A549 NSCLCs after Tunicamycin treatment. F: Expression levels of Survivin and P53 in A549 NSCLCs after Tunicamycin treatment determined by Western blot.

using student t tests or one-way ANOVA (Tukey HSD test). All data were analyzed using GraphPad software 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). * $P < 0.05$ and ** $P < 0.01$ were considered statistical differences.

Results

Tunicamycin inhibits growth and aggressiveness of NSCLCs

To clarify the inhibitory effects of Tunicamycin on NSCLCs, we detected growth and aggressiveness of A549 cells after incubation with Tunicamycin. Results demonstrated that Tunicamycin inhibited A549 cells in dose (0.50-2.50 mg/ml) and time-dependent (24-96 hours) manner in vitro (**Figure 1A, 1B**). Experiments revealed that 2.0 mg/ml of Tunicamycin could significantly suppressed A549 cells growth and reached maximum inhibition. Western blot showed that Tunicamycin markedly inhibited cyclin D1 and cyclin D2 expression in A549 NSCLCs (**Figure 1C**). Migration and invasion assays showed that Tunicamycin (2.00 mg/ml) significantly inhibited aggressiveness

of A549 cells (**Figure 1D, 1E**) compared to control group. We also indicated that Tunicamycin decreased expression levels of metastasis-related vimentin, fibronectin and E-cadherin in A549 NSCLCs (**Figure 1F**). These results indicate that Tunicamycin (2.00 mg/ml) could significantly inhibit NSCLCs growth and aggressiveness in vitro.

Tunicamycin promotes apoptosis of NSCLCs through mitochondrial apoptosis signaling pathway

To test if Tunicamycin can positively promoted tumor cells apoptosis, we analyzed apoptotic rate and apoptosis-related protein expression in A549 NSCLCs induced Tunicamycin (2.00 mg/ml) for 48 hours. We observed that Tunicamycin induced cellular atrophy of A549 NSCLCs determined by morphological analysis (**Figure 2A**). As shown in **Figure 2B**, apoptosis rate of A549 NSCLCs was promoted by Tunicamycin compared to control. Western blot showed that Tunicamycin administration up-regulated cleaved PARP and Bad in A549 NSCLCs (**Figure 2C**). Immunofluorescence assays demonstrat-

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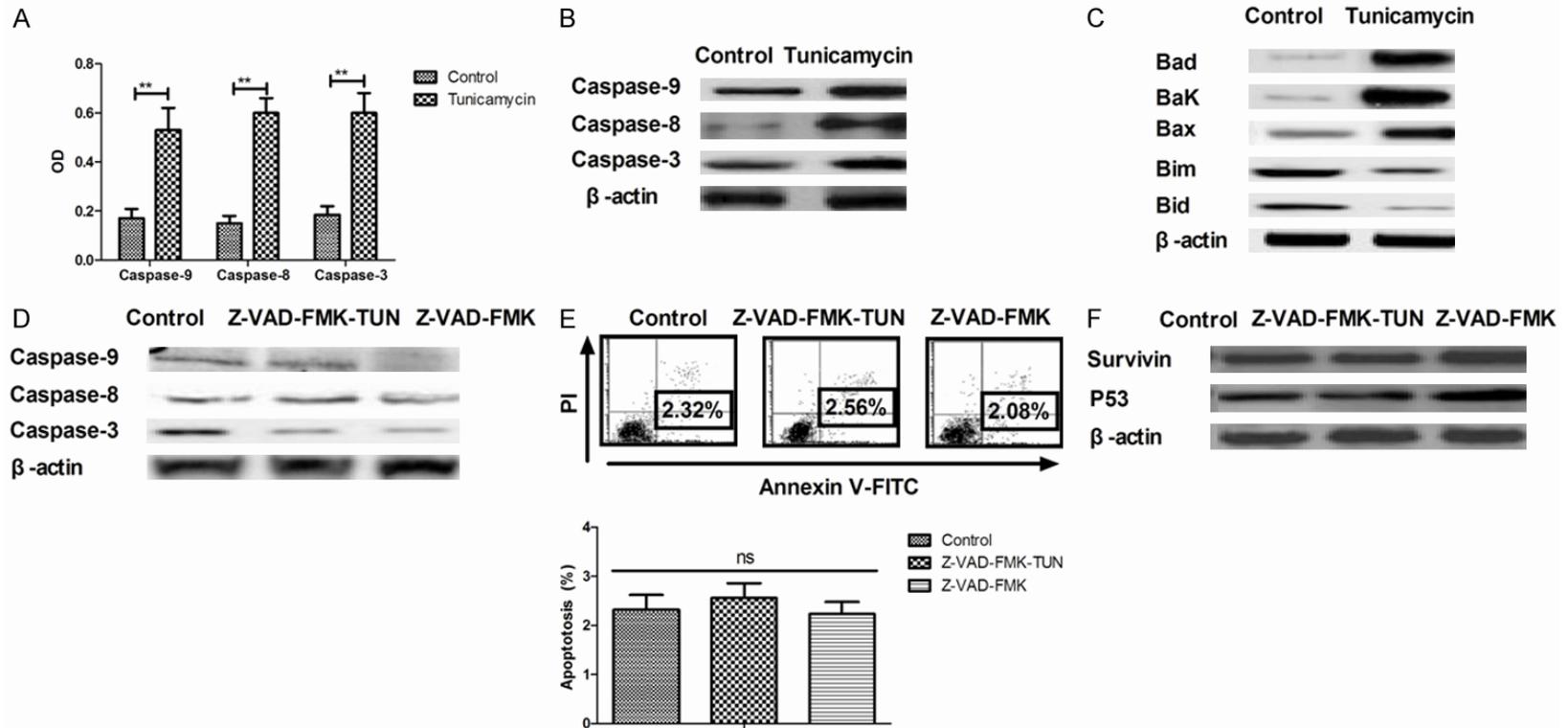


Figure 3. Tunicamycin activates apoptosis of NSCLC by caspase pathway under the regulation of Bcl-2 family proteins. A: Catalytic activity of caspase-9, caspase-8 and caspase-3 in A549 NSCLCs after Tunicamycin treatment determined by colorimetric assays. B: Expression levels of caspase-9, caspase-8 and caspase-3 in A549 NSCLCs after Tunicamycin treatment determined by Western blot. C: Expression levels of Bad, Bak, Bax, Bim and Bid in A549 NSCLCs treated by Tunicamycin. D: Effects of pan-caspase inhibitor (Z-VAD-FMK) on caspase-9, cleaved caspase-8 and cleaved caspase-3 proteins in A549 NSCLCs. E: Effects of pan-caspase inhibitor (Z-VAD-FMK) on Tunicamycin-induced apoptosis in A549 NSCLCs. F: Effects of pan-caspase inhibitor (Z-VAD-FMK) on expression levels of Survivin and P53 in A549 NSCLCs.

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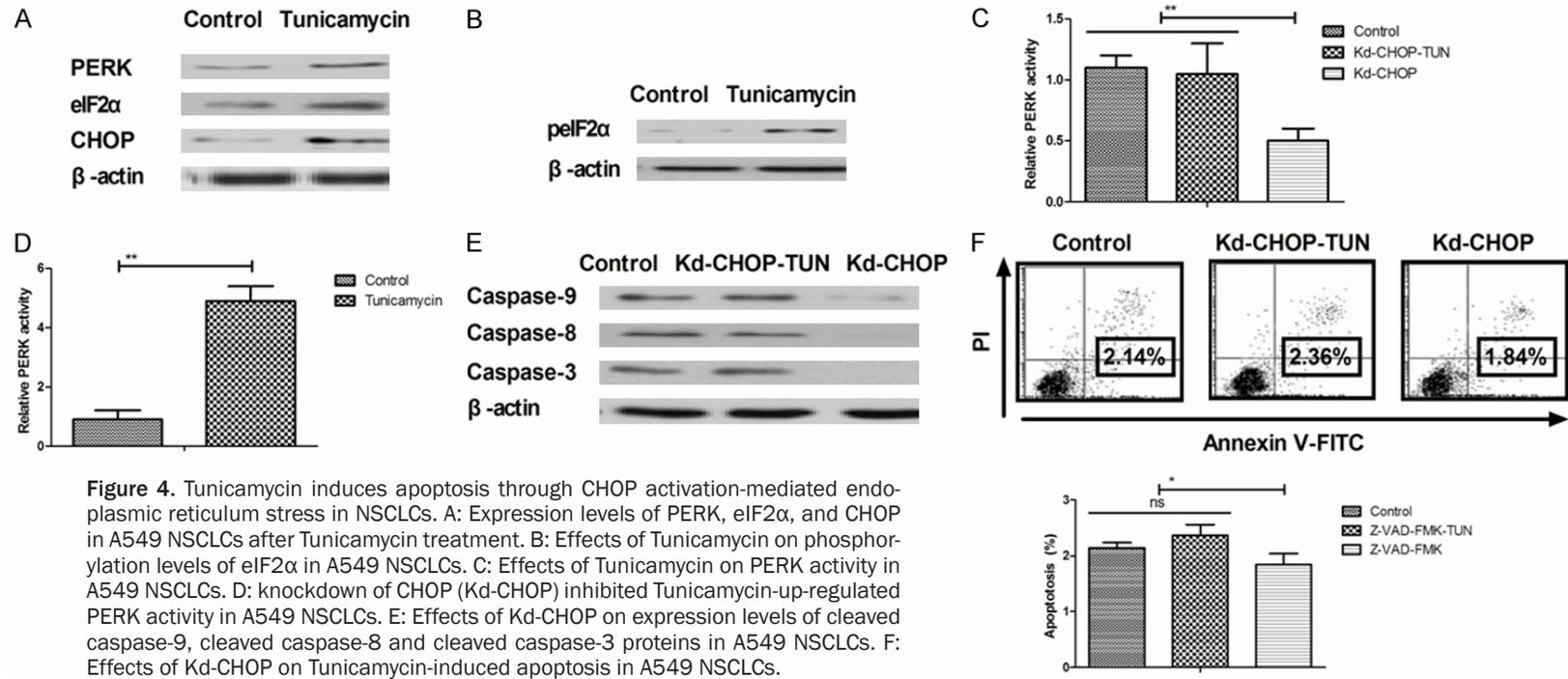


Figure 4. Tunicamycin induces apoptosis through CHOP activation-mediated endoplasmic reticulum stress in NSCLCs. A: Expression levels of PERK, eIF2 α , and CHOP in A549 NSCLCs after Tunicamycin treatment. B: Effects of Tunicamycin on phosphorylation levels of eIF2 α in A549 NSCLCs. C: Effects of Tunicamycin on PERK activity in A549 NSCLCs. D: knockdown of CHOP (Kd-CHOP) inhibited Tunicamycin-up-regulated PERK activity in A549 NSCLCs. E: Effects of Kd-CHOP on expression levels of cleaved caspase-9, cleaved caspase-8 and cleaved caspase-3 proteins in A549 NSCLCs. F: Effects of Kd-CHOP on Tunicamycin-induced apoptosis in A549 NSCLCs.

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ed that Apaf-1 and Cyto c expression levels were up-regulated by Tunicamycin administration in A549 NSCLCs (**Figure 2D**). As shown in **Figure 2E**, the ratio of green to red of JC-1 fluorescence was increased after being treated with Tunicamycin for 48 hours compared to control, which could further led to mitochondria damage. We also found that anti-apoptosis gene expression levels of Survivin and P53 were decreased in Tunicamycin-treated A549 NSCLCs (**Figure 2F**). These results suggest that Tunicamycin could induce apoptosis of NSCLCs through mitochondrial apoptosis signaling pathway.

Tunicamycin activates caspase pathway under the regulation of Bcl-2 family proteins

Previous reports have indicated that caspase family plays crucial roles in the induction of apoptosis [27]. To identify whether caspases were involved in Tunicamycin-mediated apoptotic machinery, we measured the catalytic activity and expression of caspase-9, caspase-8 and caspase-3 by colorimetric assays and Western blot, respectively. As shown in **Figure 3A, 3B**, the results demonstrated that Tunicamycin increased the catalytic activity and expression of cleaved PARP, cleaved caspase-9, cleaved caspase-8 and cleaved caspase-3 proteins in A549 NSCLCs. We observed that expression levels of Bad, Bak, Bax, Bim and Bid were up-regulated by Tunicamycin in A549 NSCLCs (**Figure 3C**). Mechanism analysis showed that pan-caspase inhibitor (Z-VAD-FMK) could prevent up-regulation of cleaved caspase-9, cleaved caspase-8 and cleaved caspase-3 proteins in A549 NSCLCs and blocked Tunicamycin-induced apoptosis (**Figure 3D, 3E**). Western blot showed that expression levels of decreasing of Survivin and P53 induced by unicomycin were impeded by Z-VAD-FMK in A549 NSCLCs (**Figure 3F**). These results suggest that Tunicamycin induces apoptosis by activation of caspase pathway under the regulation of Bcl-2 family proteins in A549 NSCLCs.

Effects of Tunicamycin on CHOP activation-mediated endoplasmic reticulum stress in NSCLCs

To investigate Tunicamycin-induced apoptosis of NSCLCs, CHOP activation-mediated endoplasmic reticulum stress was analyzed in A549 NSCLCs. As shown in **Figure 4A**, Tunicamycin

increased PERK, eIF2 α , and CHOP protein expression levels in A549 NSCLCs. We demonstrated that phosphorylation levels of eIF2 α were up-regulated by Tunicamycin in A549 NSCLCs (**Figure 4B**). PERK activity was increased by Tunicamycin administration in A549 NSCLCs (**Figure 4C**). We observed that knockdown of CHOP (Kd-CHOP) abolished Tunicamycin-up-regulated PERK activity in A549 NSCLCs (**Figure 4D**). As illustrated in **Figure 3E**, Kd-CHOP decreased expression levels of cleaved caspase-9, cleaved caspase-8 and cleaved caspase-3 proteins in A549 NSCLCs. Additionally, apoptosis induced by Tunicamycin were canceled by Kd-CHOP in A549 NSCLCs (**Figure 4F**). These results indicate that Tunicamycin induces apoptosis through up-regulation of CHOP activation-mediated endoplasmic reticulum stress in NSCLCs.

In vivo anti-tumor efficacy of Tunicamycin in tumor-bearing mice

Anti-NSCLC tumor activity of Tunicamycin was further analyzed in tumor-bearing mice. Our data showed that Tunicamycin administration significantly inhibited tumor growth in 30-day short term observation compared to PBS-treated xenografted mice (**Figure 5A**). TUNEL assays revealed that Tunicamycin treatment increasing production of apoptotic bodies in tumors (**Figure 5B**). Immunohistochemistry showed that expression levels of caspase-3, caspase-8 and caspase-9 were up-regulated in tumors after treatment with Tunicamycin (**Figure 5C**). We also observed that expression and phosphorylation levels of eIF2 α were up-regulated by Tunicamycin treatment in tumors compared to PBS group (**Figure 5D**). Immunohistochemistry also identified that ATF-6 and CHOP expression levels were increased by Tunicamycin treatment in tumor in A549-bearing mice. Immunohistochemistry for caspase-3 and caspase-8 showed that Calotropin significantly promoted these tumor-apoptosis markers in experimental mice (**Figure 5E**). Long-term observation showed that Tunicamycin treatment significantly prolonged survival of tumor-bearing mice (**Figure 5F**). These results suggest that Tunicamycin treatment could inhibit tumor growth and prolong survival rate of A549-bearing mice.

Discussion

In the current study, the anti-NSCLC effects of Tunicamycin were investigated in NSCLCs and

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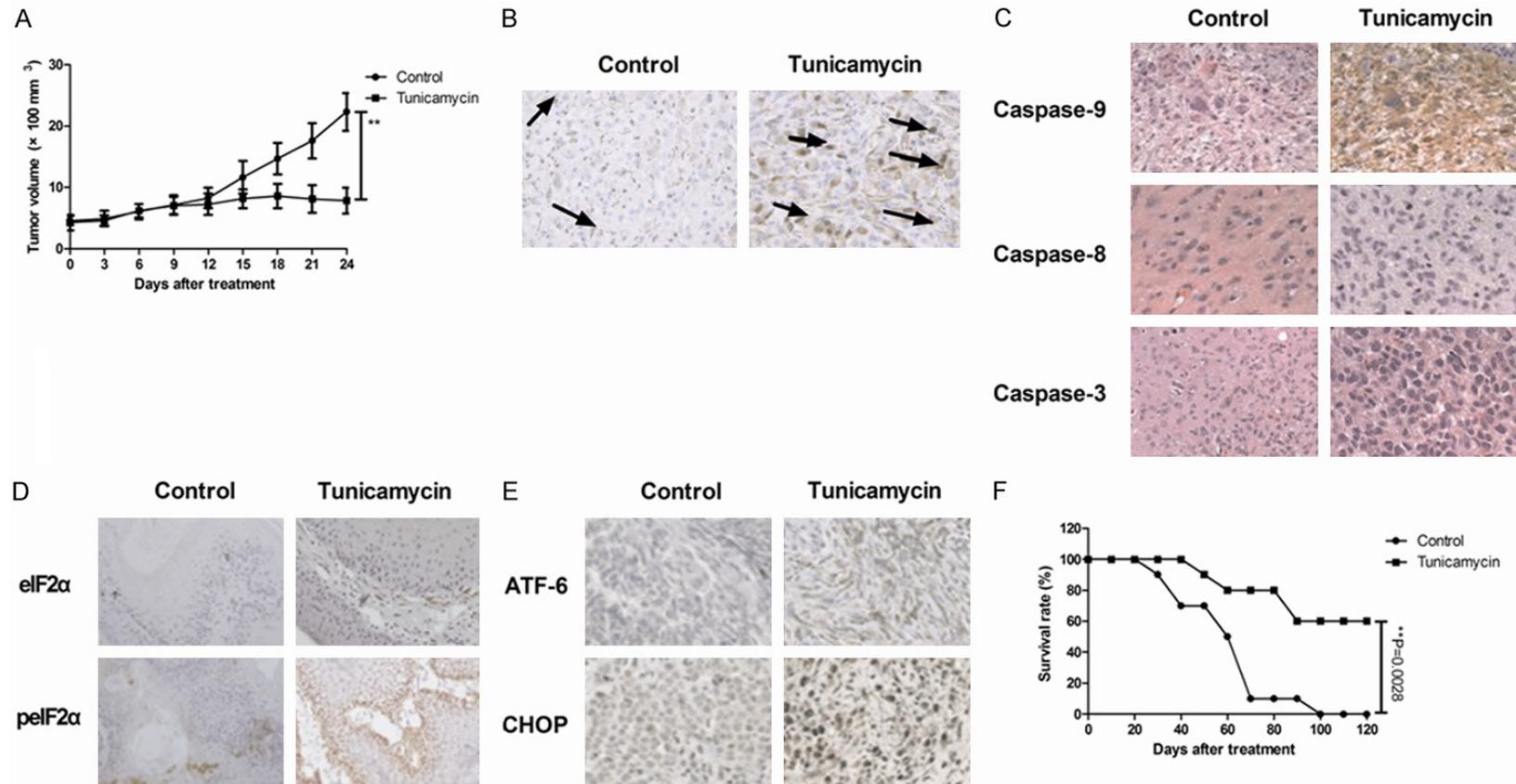


Figure 5. In vivo anti-tumor efficacy of Tunicamycin in tumor-bearing mice. A: Tunicamycin inhibits tumor growth in 30-day short term observation compared to PBS-treated xenografted mice. B: Tunicamycin induces production of apoptotic bodies in tumors. C: Immunohistochemistry analyzes expression levels of pro-caspase-3, caspase-8 and caspase-9 in tumors after treatment with Tunicamycin. D: Expression and phosphorylation levels of eIF2α in Tunicamycin-treated tumors. E: Immunohistochemistry analyzes ATF-6 and CHOP expression levels in Tunicamycin-treated tumors. F: Tunicamycin treatment prolongs survival of tumor-bearing mice. P** < 0.01.

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NSCLCs-bearing xenografted mice. Tunicamycin-mediated apoptotic signal pathways were also analyzed in A549 NSCLCs. The experimental results have demonstrated that Tunicamycin inhibits growth and aggressiveness of NSCLCs by suppressing fibronectin, vimentin and E-cadherin expression levels in A549 NSCLCs. The findings identified and defined Tunicamycin promotes apoptosis through caspase pathway-induced CHOP activation-mediated endoplasmic reticulum stress in NSCLCs.

Systematic review and meta-analysis have demonstrated that anti-cancer-induced apoptosis contribute to inhibition of NSCLC growth and aggressiveness [28, 29]. Report has showed that accelerates cyclin D1 degradation contributes to cell cycle arrest in colorectal cancer and mantle cell lymphoma models [30]. Russo et al have showed that ERK1/2 could activate Cyclin D2-derived peptide acts on specific cell cycle to lead to breast cancer cells death [31]. Our results have showed that Tunicamycin inhibits growth and aggressiveness of NSCLCs and decreased cyclin D1 and cyclin D2 expression in A549 NSCLCs, which may contribute to arrest A549 cells cycle [32, 33]. Results in this study have showed that Tunicamycin down-regulated expression levels of fibronectin, vimentin and E-cadherin in A549 NSCLCs that which have been reported to inhibit tumor migration and invasion [34, 35]. Tunicamycin down-regulating fibronectin, vimentin and E-cadherin expression in A549 NSCLCs may serve as an activator of gene transcription to inhibit tumor cell survival and invasion [36].

In this study, caspase signal pathway also was activated by Tunicamycin treatment in A549 NSCLCs. Activation of caspase signal pathway contributes to chemo-, radio- and bio-therapeutic regimens to kill tumor cells by inducing apoptosis, which is a cell-death procedure and involves in the activation of apoptotic signal molecules in tumor cells [37]. In this study, our findings suggest that Tunicamycin increases the catalytic activity and expression of cleaved PARP, cleaved caspase-9, cleaved caspase-8 and cleaved caspase-3 proteins in A549 NSCLCs. Up-regulation of caspase signal pathway promotes A549 NSCLCs apoptosis and further leads to growth inhibition both in vitro and in vivo. Data also demonstrate that expression levels of Survivin and P53 could be decreased

by Tunicamycin in A549 NSCLCs, and these benefits can be abolished by pan-caspase inhibitor (Z-VAD-FMK) [38, 39].

In this study, we analyzed the therapeutic implications of Tunicamycin and these findings indicate that Tunicamycin markedly induces apoptosis through up-regulation of CHOP activation-mediated endoplasmic reticulum stress in NSCLCs. A large numbers of studies have been reported the function of CHOP in endoplasmic reticulum stress-mediated apoptosis in tumor cells [40]. A study has demonstrated that PERK acting as a pro-adaptive protein kinase can be regulated indirectly by protein misfolding within the ER, who further contributes to tumor progression and represents a significant therapeutic target [41]. Another study has showed that increasing of PERK/eIF2 α /ATF4/CHOP signaling pathway enhances apoptotic cell death in tumor progression during endoplasmic reticulum stress, which provides promising anti-tumor therapeutic agents for human cancer [42]. Additionally, Petiwala et al have suggested that modulating CHOP expression has been identified to promote apoptosis and death and inhibit xenograft tumor growth [43]. Our results demonstrated that Kd-CHOP abolished Tunicamycin-up-regulated PERK activity, expression levels of cleaved caspase-9, cleaved caspase-8 and cleaved caspase-3, as well as apoptosis, which contributes to anti-tumor efficacy and long-term survival of tumor-bearing mice.

In conclusion, our findings indicate that Tunicamycin treatment activates pro-apoptosis gene expression and stimulates caspase pathway under the regulation of Bcl-2 family proteins (Bad, Bak, Bax, Bim and Bid) in NSCLC. We described Tunicamycin administration significantly inhibits the growth and aggressiveness and induces apoptosis of NSCLCs in vitro. The apoptotic mechanism was related to the Tunicamycin-mediated endoplasmic reticulum stress-induced intrinsic mitochondrial apoptotic pathways in NSCLCs. This study exhibits a clearly mechanism of the increasing CHOP activity stimulated by Tunicamycin, provides potential therapeutic strategy for the treatment of NSCLC.

Disclosure of conflict of interest

None.

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