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Original Article

Down-regulation of FoxM1 by thiostrepton or small interfering RNA inhibits proliferation, transformation ability and angiogenesis, and induces apoptosis of nasopharyngeal carcinoma cells

Lizhu Jiang[1,2], Peng Wang[3], Lili Chen[1], Hongyan Chen[1]

[1]*Department of Otolaryngology, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China; Departments of* [2]*Otolaryngology,* [3]*Osteology, Dazu District People's Hospital, Chongqing 402360, China*

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Abstract: Nasopharyngeal carcinoma (NPC) is a head and neck malignant tumor rare throughout most of the world but common in Southern China. Forkhead box M1 (FoxM1) transcription factor has been shown to play important role in the development and progression of human cancers. We have previously found that FoxM1 was overexpressed in NPC patients and was associated with development of NPC. However, the exact functional significance of FoxM1 and its inhibitor thiostrepton in NPC is little known. The purpose of this study was to investigate in vitro activity of down-regulation of FoxM1 by thiostrepton or siRNA against NPC cell line. FoxM1 inhibition by thiostrepton or siRNA inhibited proliferation of NPC cells by down-regulation of cyclin D1 and cyclin E1. Transformation ability of NPC cells was suppressed by thiostrepton. FoxM1 inhibition by thiostrepton induced apoptosis of NPC cells by down-regulation of bcl-2, up-regulation of bax and p53, and inducing release of cytochrome c accompanied by activation of caspase-9, cleaved caspase-3 and cleaved PARP. In addition, FoxM1 inhibition by siRNA transfection also down-regulated expression of bcl-2 and up-regulated expression of bax, p53, cleaved caspase-3 and cleaved PARP. Furthermore, FADD and cleaved caspase-8 expression were up-regulated by thiostrepton or FoxM1 siRNA, and expression of cIAP1 and XIAP was inhibited by thiostrepton. At last, FoxM1 inhibition by thiostrepton reduced the expression of HIF-1α and VEGF, and transfection of FoxM1 siRNA decreased VEGF expression but not HIF-1α. Collectively, our finding suggest that FoxM1 inhibition by thiostrepton or siRNA suppresses proliferation, transformation ability, angiogenesis, and induces apoptosis of NPC.

Keywords: FoxM1, thiostrepton, proliferation, apoptosis, oncogene, nasopharyngeal carcinoma

Introduction

Nasopharyngeal carcinoma (NPC), an Epstein-Barr virus associated malignancy, is the most common head and neck cancer in China with an incidence of 30-80 per 100,000 people per year in southern China [1]. Radiotherapy with or without chemotherapy is the mainstream treatment for NPC. However, there are limitations in these therapeutic applications for their undesirable side effects and local recurrence and distant metastasis [2]. Thus, challenges of NPC treatment exists and novel therapeutic targets and new approaches for NPC treatment are urgently needed [3].

The forkhead box M1 (FoxM1), a member of the Fox transcription factor family, plays important roles in maintaining homeostasis between cell proliferation and apoptosis [4]. Dysfunction of FoxM1 prevents differentiation, and this ultimately guides undifferentiated cells toward malignant transformation [5]. Overexpression of FoxM1 has been shown to be associated with carcinogenesis of tumor development in various cancers [6-9]. On the other hand, suppression of FoxM1 expression inhibited growth and induced apoptosis of cancer cells [10, 11]. Since FoxM1 suppression appears to inhibit tumorigenesis and progression, chemical compounds that target FoxM1 may act as anticancer drugs [12-15]. FoxM1 inhibitor thiostrepton, a natural product with antibiotic properties isolated from *Streptomyces azureus*, is known to directly interact with FoxM1 and inhibit the binding of FoxM1 to genomic target sites [16].

We have previously found that FoxM1 was overexpressed in NPC patients and was associated with development of NPC. However, there are still questions whether inhibition of FoxM1 by thiosrtrepton or small interfering RNA (siRNA) could suppress growth, induce apoptosis and inhibit transformation ability of NPC cells through modulating the expression of its target genes. We have therefore investigated the role and mechanism of FoxM1 and thiostrepton in NPC cells *in vitro*. In this study, we have identified FoxM1 may be a useful therapeutic target for NPC and negative regulation of FoxM1 by thiostrepton may contribute to NPC treatment in the future.

Materials and methods

Cell culture

The NPC cell line C666-1 was cultured in RPMI-1640 medium (Invitrogen, USA) with 10% fetal bovine serum (Gibco, USA), 100 μg/mL streptomycin and 100 U/mL penicillin (Hyclone, USA). Cells were cultured under a 5.0% CO_2 atmosphere. Thiostrepton (Sigma, USA) was dissolved in DMSO (dimethylsulfoxide).

Cell viability studies by CCK-8 assays

Cells ($5×10^3$) were seeded in 96-well plates containing complete medium and incubated for 24 h. Different concentrations of thiostrepton were added, and DMSO was used as control. After 24, 48 or 72 h, 10 μl of the CCK-8 (Beyotime Inst Biotech, China) solution was added to each well and went on with incubation for 2 h in an incubator. The absorbance was measured at 450 nm using a microplate reader (Tecan, Switzerland).

Cell growth studies by carboxyfluorescein succinimidyl ester (CFSE)

C666-1 cells were collected and washed twice by PBS, and stained with 5 μM CFSE (Dojindo, Japan) for 25 min at 37°C, according to the manufacturer's instructions. CFSE-labeled cells were then seeded in 6-well plates, treated with 4, 6, 8 μM thiostrepton, and harvested at different times (24, 48 and 72 h). The mean fluorescence intensity (MFI) was measured in each case, using flow cytometry (Beckman coulter, USA).

Gene silencing using small interfering RNA (siRNA)

For siRNA-knockdown experiment, double-stranded RNA duplexes that targeted the human FoxM1 gene (5'-GGUCCUGGACACAAUGAAUTT-3', 5'-AUUCAUUGUGUCCAGGACCTT-3') and negative control (NC) siRNA were purchased from Invitrogen. Cells were transfected by Lipofectamine 2000 reagent (Invitrogen) as described previously [17].

Anchorage-independent growth assay

First, 6-well plates were coated with a layer of 0.6% agar in medium supplemented with 20% foetal bovine serum. The cells, prepared in 0.3% agar and treated with 0, 4 μM thiostrepton, cultured for 3 weeks. Each experiment was repeated at least three times. The colonies were photographed (at a final magnification of 40×) and manually counted when colonies with over 50 cells.

Flow cytometric analysis of apoptosis

After treatment with 4, 6 and 8 μM thiostrepton, C666-1 cells were harvested and percentage apoptosis was measured by flow cytometry after staining with fluorescein-conjugated annexin V and propidiumiodide (PI) (Tianjin Sungene Biotech, China).

TUNEL assay

C666-1 cells were treated with 4, 6 and 8 μM thiostrepton for 48 h, and the apoptotic cells were measured using Tunel assay (Beyotime Inst Biotech, China) as described earlier [18].

Measurement of mitochondrial membrane potential

C666-1 cells were treated with 4, 6 and 8 μM thiostrepton for 24 and 48 h, washed twice with PBS, and incubated with 10 μg/mL JC-1 (Beyotime Inst Biotech, China) (37°C, 20 min), washed, and re-suspended in culture medium. Fluorescence analysis was conducted using flow cytometry.

Quantitative real-time RT-PCR

Total RNA were extracted using Trizol reagent (Takara, Dalian, China) according to manufacturer's instructions and contaminating genomic

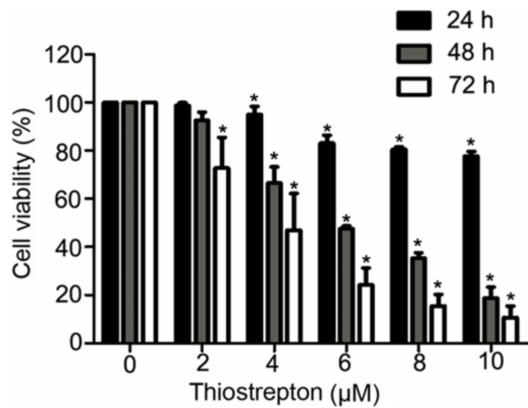

Figure 1. Thiostrepton inhibited viability of NPC cells. NPC cells were incubated with indicated doses of thiostrepton. Cell viability assay was performed using CCK-8 as described in Materials and Methods. $*P < 0.05$ *vs.* 0 μM group.

DNA was removed by incubation with DNase I (Takara, Dalian, China). RNA purity and concentration were determined by spectrophotometry. PCR was carried out according to the standard protocol on a Real-Time PCR-system (BioSystems) with SYBR Green detection. After an initial incubation of the 10 μl reaction mixture for1 min at 95°C, 39 cycles (95°C for 20 s, 58°C for 25 s) were performed for amplification. The specificity of amplification was confirmed by melting curve analysis. Each sample was tested in triplicate and the results were normalized to the level of gene GAPDH. The sequences of each set of primers were as follows: 5'-TGCAGCTAGGGATGTGAATCTTC-3' (sense) and 5'-GGAGCCCAGTCCATCAGAACT-3' (antisense) for FoxM1; 5'-TCTACACCGACAACTCCATCCG-3' (sense) and 5'-TCTGGCATTTTGG AGAGGAAGTG-3' (antisense) for cyclin D1; 5'-GAGCTGTTGGATCTCTGTGT-3' (sense) and 5'-CAAGGCCGAAGCAGCAA-3' (antisense) for cyclin E1; 5'-TACTCCCCTGCCCTCAACAAGA-3' (sense) and 5'-ACAACCTCCGTCATGTGCTGTG-3' (antisense) for p53; 5'-CCTTTTGCTTCAGGGTTTCAT-3' (sense) and 5'-GAGACACTCGCTCAGCT-3' (antisense) for bax; 5'-TGAACCGGCATCTGCACAC-3' (sense) and 5'-CGTCTTCAGAGACAGCCAGGAG-3' (antisense) for bcl-2; 5'-CAGTGGAGGCCGACTTCTTG-3' (sense) and 5'-ATGAACCAGGAGCCATCCTTT-3' (antisense) for caspase-3; 5'-CCCAGGGTCTTCGGATAG-3' (sense) and 5'-AGCGTGCTTCAGTTCATACA-3' (antisense) for PARP; 5'-CGTTCCTTCGATCAGTTGTC-3' (sense) and 5'-TCAGTGGTGGCAGTGGTAGT-3' (antisense) for HIF-1α; 5'-AAGATCCGCAGACGTGTAAATGTT-3' (sense) and 5'-CGGCTTGTCACATGCAAGTA-3' (antisense) for VEGF; 5'-CAGCGACACCCACTCCTC-3' (sense) and 5'-TGAGGTCCACCACCCTGT-3' (antisense) for GAPDH. $2^{-\Delta\Delta CT}$ method was employed to calculate the relative expressions.

Western blot

Protein samples were separated on precast 10% SDS polyacrylamide gels. After electrophoresis, the proteins were transferred to PVDF membrane filters (Millipore Biotechnology, USA). The membranes were incubated overnight at 4°C with primary rabbit polyclonal cyclin D1, cyclin E1, bax, bcl-2, p53, cleaved caspase-3, cleaved caspase-8, caspase-9, cytochrome c, XIAP, cIAP1, FADD, HIF-1α, VEGF antibody (Immunoway Biotechnology Company, USA), cleaved PARP (Cell Signaling Technology, USA) or primary mouse polyclonal GAPDH antibody (Huaan Biotechnology Company, China). After washing three times in TBS-T, horseradish peroxidase (HRP)-conjugated secondary antibodies were used at a dilution of 1:4000 in TBS-T for 1 h at room temperature. After three additional washes with TBS-T, the immunoreactive bands were visualised with a chemiluminescence reagent (ECL, Millipore Biotechnology, USA) and quantified using Bio-Rad imaging system (Bio-Rad Laboratories, UK).

Statistical analysis

For statistical evaluation the SPSS software version 14.0 Sciences (SPSS Inc, USA) was used. Student's t-test and two-way ANOVA were used for comparing the groups. $P < 0.05$ in all cases was considered statistically significant.

Results

Thiostrepton causes inhibition of NPC cell viability

More than 90% NPC patients in China were undifferentiated, and EBV is consistently present in undifferentiated NPC [19]. Therefore, EBV positive cell line c666-1 is much better than other EBV negative NPC cell line being enrolled in current study. We firstly sought to determine whether treatment with thiostrepton leads to inhibition of viability of NPC cell line C666-1. C666-1 cells were treated with 0, 2, 4, 6, 8 and 10 μM thiostrepton for 24, 48 and 72 h, and cell viability was assayed using CCK-8 assay. It showed that thiostrepton inhibited

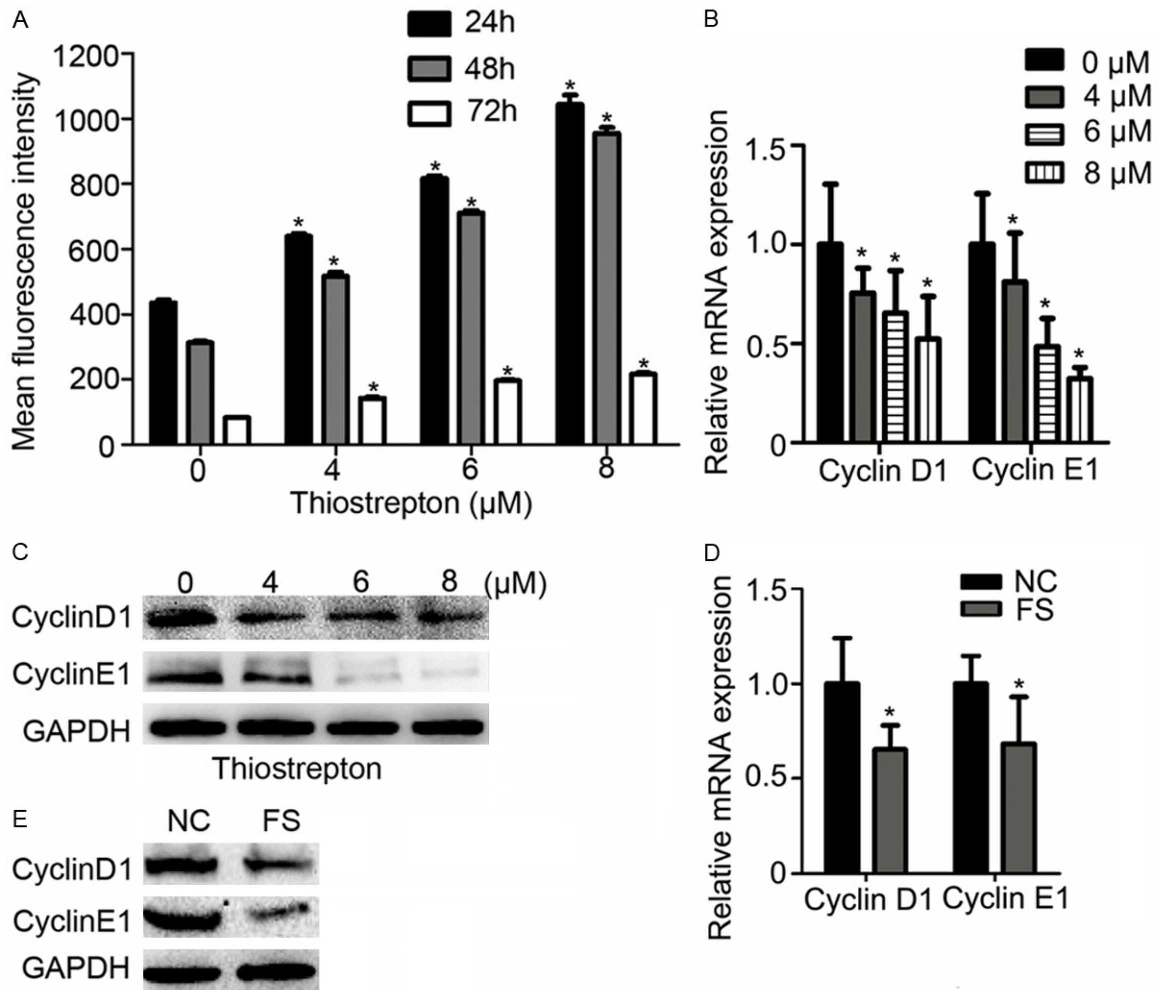

Figure 2. Thiostrepton inhibited the proliferation of NPC cells. A. NPC cells were treated with various concentration of thiostrepton for 24, 48 and 72 h, and stained with CFSE. Thiostrepton inhibited the division of NPC cells with higher fluorescence intensity. The graph displays the mean ± SD of three independent experiments. $^*P < 0.05$ *vs.* 0 μM group. B. Thiostrepton inhibited mRNA expression of cyclin D1 and cyclin E1 in NPC cells. C. Thiostrepton inhibited protein expression of cyclin D1 and cyclin E1 in NPC cells. D. Down-regulation of FoxM1 by siRNA inhibited mRNA expression of cyclin D1 and cyclin E1 in NPC cells. E. Down-regulation of FoxM1 by siRNA inhibited protein expression of cyclin D1 and cyclin E1 in NPC cells. NC: negative control, FS: FoxM1 siRNA.

C666-1 cell viability in a dose- and time-dependent manner (**Figure 1**) and the viability was significantly decreased when dose was 2 μM or above ($P < 0.05$) and the prolonged incubation enhanced the viability loss.

FoxM1 inhibition suppresses the proliferation of NPC cells by down-regulation of cyclin D1 and cyclin E1

We previously have demonstrated that FoxM1 was overexpressed in NPC tissues and NPC cell line compared with nasopharygeal normal tissues, and FoxM1 inhibitor thiostrepton significantly down-regulated FoxM1 expression in NPC cell line (data not show). Therefore, we here next investigated the role of FoxM1 inhibition in proliferation of NPC cell line C666-1. C666-1 cells was stained with CFSE and treated with 4, 6, 8 μM thiostrepton. The results showed that C666-1 cells division was delayed dramatically after treatment with thiostrepton (cells with higher CFSE fluorescence intensity). The inhibitory effect of thiostrepton on the cell division began at 24 h and became more obvious at 48 h (**Figure 2A**).

We detected expressions of oncogenes that were closely related to tumor proliferation. As shown in **Figure 2B** and **2C**, thiostrepton treatment significantly decreased cyclin D1 and cyclin E1 mRNA and protein expression. In addi-

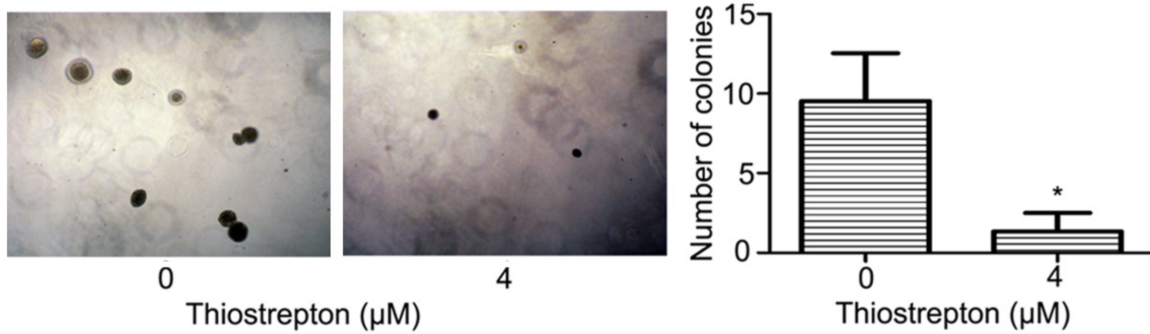

Figure 3. Thiostrepton suppressed clonogenicity of NPC cells. $*P < 0.05$ *vs.* 0 μM group.

tion, transfection of FoxM1-specific siRNA also down-regulated the mRNA and protein expression of cyclin E1 and cyclin D1 (**Figure 2D** and **2E**).

Thiostrepton inhibits transformation ability of NPC cells

We studied the effect of thiostrepton on cell transformation by soft agar colony formation assays using C666-1 cell line. As shown in **Figure 3**, C666-1 cells treated with 4 μM thiostrepton resulted in ~80% reduction in the number of colonies formed.

FoxM1 inhibition induces apoptosis of NPC cells

We used annexin V/PI dual staining for confirmation of thiostrepton-induced apoptosis in C666-1 cells. Cells were treated with 4, 6 and 8 μM thiostrepton for 24 and 48 h, and stained with annexin V/PI dual staining. It showed that thiostrepton induced C666-1 cell apoptosis in a dose- and time-dependent manner (**Figure 4A** and **4B**). As shown in **Figure 4A**, treatment of C666-1 cells with 4, 6 and 8 μM thiostrepton-induced 10.1%, 16.7% and 25.17% apoptosis, compared with only 5.76% in control group at 48 h. Furthermore, C666-1 cells were treated with various doses of thiostrepton for 48 h and apoptosis was measured by TUNEL analysis. As shown in **Figure 4C**, thiostrepton treatment resulted in apoptosis in a dose dependent manner.

Mitochondrial and caspase-mediated pathway are involved in apoptosis of NPC cells by inhibition of FoxM1

To activate the mitochondria apoptotic pathway, members of bcl-2 family are essential for regulating the mitochondrial integrity, and the increase in mitochondrial permeability transition is accompanied by a collapse in mitochondrial membrane potential [20]. In addition, p53 can directly activate pro-apoptotic bax to permeabilize mitochondria and engage the apoptotic program [21]. As shown in **Figure 5A** and **5B**, thiostrepton caused decreased bcl-2 expression and increased bax and p53 expression. We then tested the effect of thiostrepton on the mitochondrial membrane potential. C666-1 cells were treated with thiostrepton for 24 and 48 h and labeled with JC1 dye, and mitochondrial membrane potential was measured by flow cytometry. It showed that loss of mitochondrial membrane potential in C666-1 cells with a dose- and time-dependent manner as measured by JC1-stained green fluorescence depicting apoptotic cells (**Figure 5C**). It is known that once integrity of mitochondrial destroyed, cytochrome c could release from mitochondria into cytosol. Therefore, we detected the expression of cytochrome c in cytosol of C666-1 cells after treatment with thiostrepton. As shown in **Figure 5D**, higher level of cytochrome c was measured in cytosol.

It has been shown that release of cytochrome c activated the downstream caspases that are ultimately required to induce apoptosis [22]. We therefore determined whether thiostrepton-induced release of cytochrome c is capable of activation of caspase-9, cleaved caspase-3 and cleaved PARP. As shown in **Figure 5E** and **5F**, thiostrepton treatment resulted in the activation of caspase-9, cleaved caspase-3 and cleavage PARP in C666-1 cells.

Furthermore, we wonder whether transfection with FoxM1-specific siRNA could modulate the

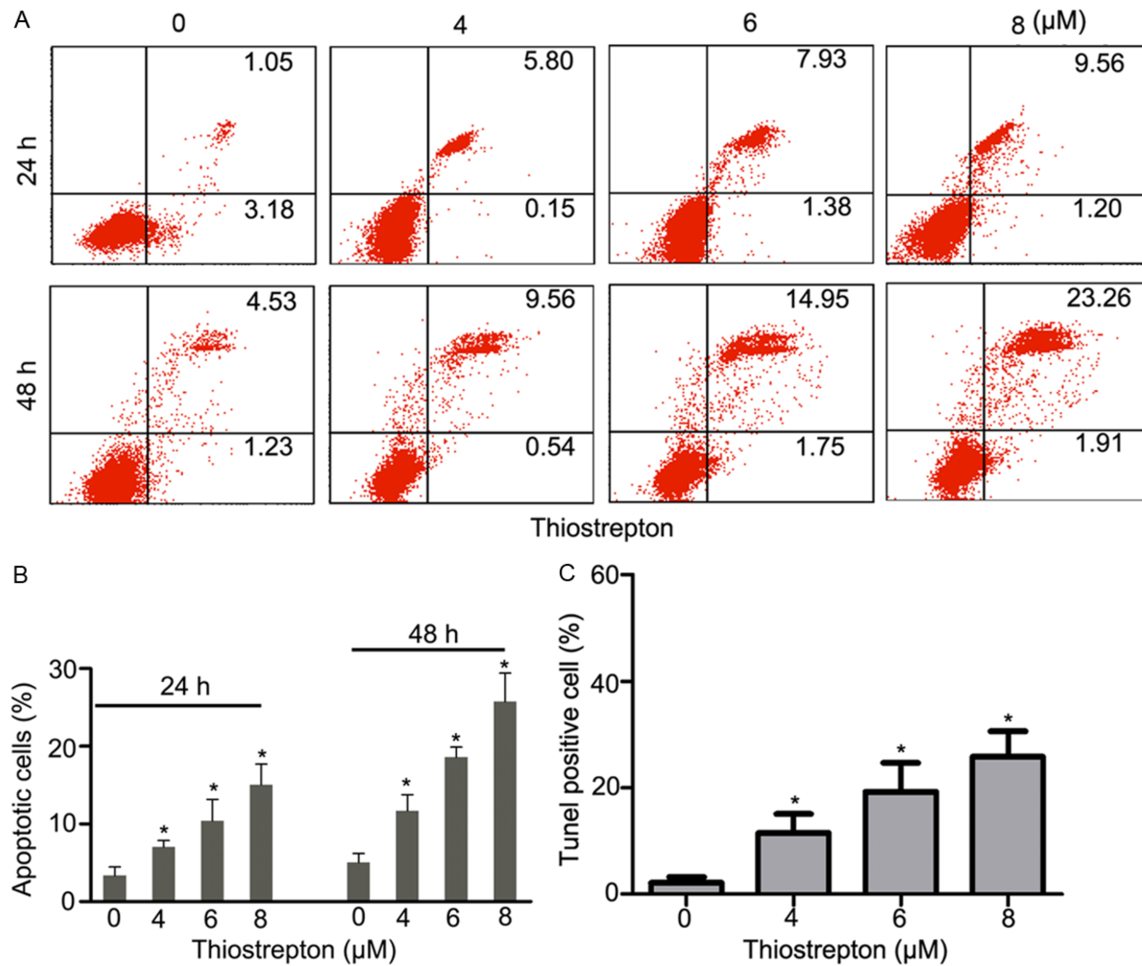

Figure 4. Thiostrepton induced apoptosis in NPC cells. A, B. NPC cells were treated with various doses of thiostrepton for 24 and 48 h and stained with flourescein-conjugated annexin-V and propidium iodide (PI) and analyzed by flow cytometry. C. NPC cells were treated with various concentrations of thiostrepton for 48 h and apoptosis was determined using tunel assay. *$P < 0.05$ *vs.* 0 μM group.

expression of the above genes. As shown in **Figure 5G** and **5H**, the mRNA and protein expression of FoxM1 and bcl-2 were down-regulated, and bax, p53, cleaved caspase-3 and cleaved PARP up-regulated.

Fas-dependent apoptotic pathway are involved in apoptosis of NPC cells by inhibition of FoxM1

The Fas-dependent apoptotic pathway is initiated by the Fas ligand or TNF-α, which initiate the extrinsic pathway. Fas-associated death domain (FADD) recruits and aggregates the pro- form of caspase-8, leading to the activation of caspase-8 [23]. We therefore sought to investigate whether thiostrepton treatment is capable of activation of FADD and cleaved caspase-8. As shown in **Figure 6A**, thiostrepton treatment resulted in the activation of FADD and cleaved caspase-8 in C666-1 cells. In addition, transfection of FoxM1-specific siRNA resulted in up-regulation of FADD and cleaved caspase-8 (**Figure 6B**).

Participation of IAP family in apoptosis of NPC cells by inhibition of FoxM1

We also examined whether thiostrepton treatment induces apoptosis by modulating the expression of inhibitor of apoptosis proteins (IAPs) that ultimately determine the cell's response to apoptotic stimuli. C666-1 cells were treated with 4, 6 and 8 μM thiostrepton for 48 h, and expression of cIAP1 and XIAP were detected by Western blot. As shown in **Figure 7**, thiostrepton treatment caused a dose-dependent down-regulation of cIAP1 and

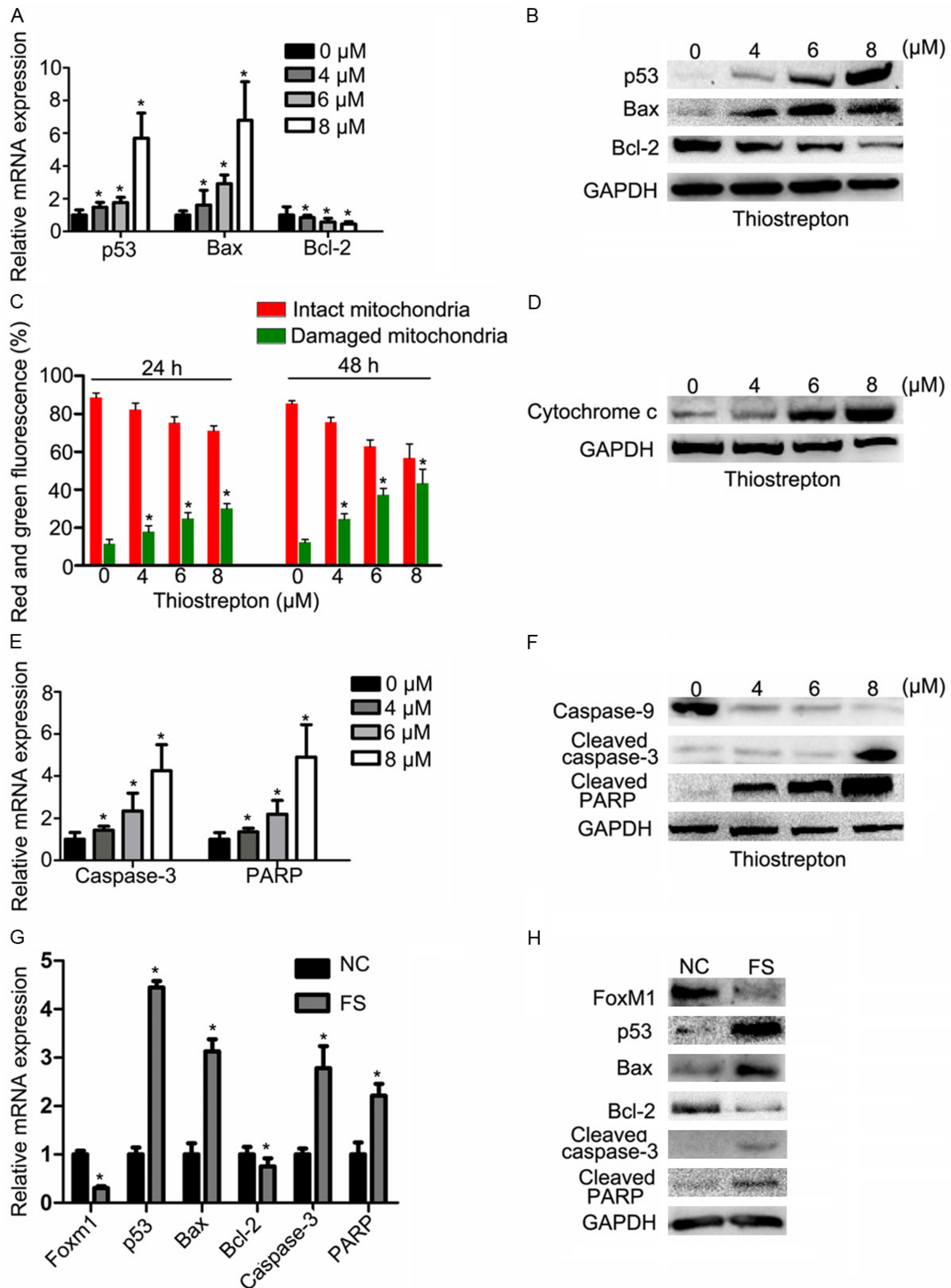

Figure 5. FoxM1 inhibition by thiostrepton or siRNA induced activation of the mitochondrial and caspase-mediated apoptotic pathway in NPC cells. A, B. Thiostrepton-induced activation of bcl-2, bax and p53. NPC cells were treated with 4, 6, and 8 μM thiostreopn for 48 h and the mRNA and protein expressions were detected by real-time RT-PCR and western blot. C. Loss of mitochondrial membrane potential in NPC cells with various concentrations of thiostrepton treatment for 24 and 48 h. Live cells with intact mitochondrial membrane potential and dead cells with lost

mitochondrial membrane potential. D. Thiostrepton-induced release of cytochrome c into cytosol after treatment for 48 h. E. Thiostrepton up-regulated the mRNA expression of caspase-3 and PARP. F. Thiostrepton-induced activation of caspase-9, cleaved caspase-3 and cleaved PARP. G. FoxM1 siRNA transfection down-regulated mRNA expression of Bcl-2 and up-regulated mRNA expression of Bax, p53, cleaved caspase-3 and cleaved PARP. H. FoxM1 siRNA transfection down-regulated protein expression of Bcl-2 and up-regulated protein expression of Bax, p53, cleaved caspase-3 and cleaved PARP. *$P < 0.05$ *vs.* 0 μM group or negative control. NC: negative control, FS: FoxM1 siRNA.

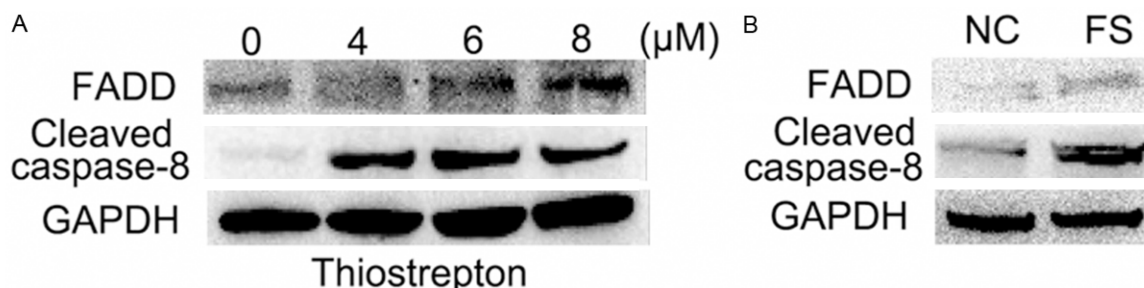

Figure 6. FoxM1 inhibition by thiostrepton or siRNA induced up-regulation of FADD and cleaved caspase-8 protein expression. A. Thiostrepton-induced up-regulation of FADD and cleaved caspase-8 protein expression. B. FoxM1 siRNA transfection up-regulated protein expression of FADD and cleaved caspase-8. NC: negative control, FS: FoxM1 siRNA.

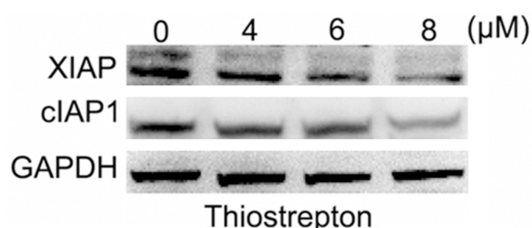

Figure 7. Thiostrepton-induced down-regulation of cIAP1 and XIAP protein expression.

XIAP. These results indicate that IAP may also be involved in down-regulation of FoxM1-induced apoptosis.

Effect of FoxM1 inhibiton on HIF-1α and VEGF

HIF-1α and VEGF are important factors that are involved in the angiogenesis of cancer. Therefore, we wonder whether inhibition of FoxM1 could suppress the expression of HIF-1α and VEGF. As shown in **Figure 8A** and **8B**, down-regulation of FoxM1 by thiostrepton inhibited the expression of HIF-1α and VEGF in a dose dependant manner. However, transfection of FoxM1-specific siRNA decreased VEGF expression but not HIF-1α (**Figure 8C** and **8D**).

Discussion

Convincing evidence has shown that elevated expression or activity of FoxM1 is associated with the development and progression of many cancers. Elevated expression or activity of FoxM1 is associated with the development and progression of many cancers. FoxM1 and its inhibitor thiostrepton represent an attractive therapeutic target and novel anticancer drug in the fight against cancer [12]. Previously, we have demonstrated that overexpression of FoxM1 was associated with development of NPC and thiostrepton significantly down-regulated FoxM1 expression in NPC cell line. However, little is known about the role of inhibition of FoxM1 by thiostrepton or siRNA in NPC.

Several studies have shown that thiostrepton inhibited the viability of cancer cells, and a reduction in FoxM1 expression resulted in inhibition of proliferation in cancer cells and dramatic decrease in tumor growth [10, 11]. In current study, thiostrepton significantly inhibited the viability of NPC cells in a dose- and time-dependent manner. CFSE staining showed a reduction in DNA synthesis in NPC cells following treatment with thiostrepton. In addition, inhibition of FoxM1 expression with thiostrepton treatment or transfection of siRNA also down-regulated tumor proliferate gene cyclin D1 and cyclin E1. These findings are consistent with the role of FoxM1 in cell proliferation. Moreover, we also found that thiostrepton significantly suppressed the transformation ability of NPC cells.

Down-regulated of FoxM1 by thiostrepton induce apoptosis of some cancer cells via intrinsic

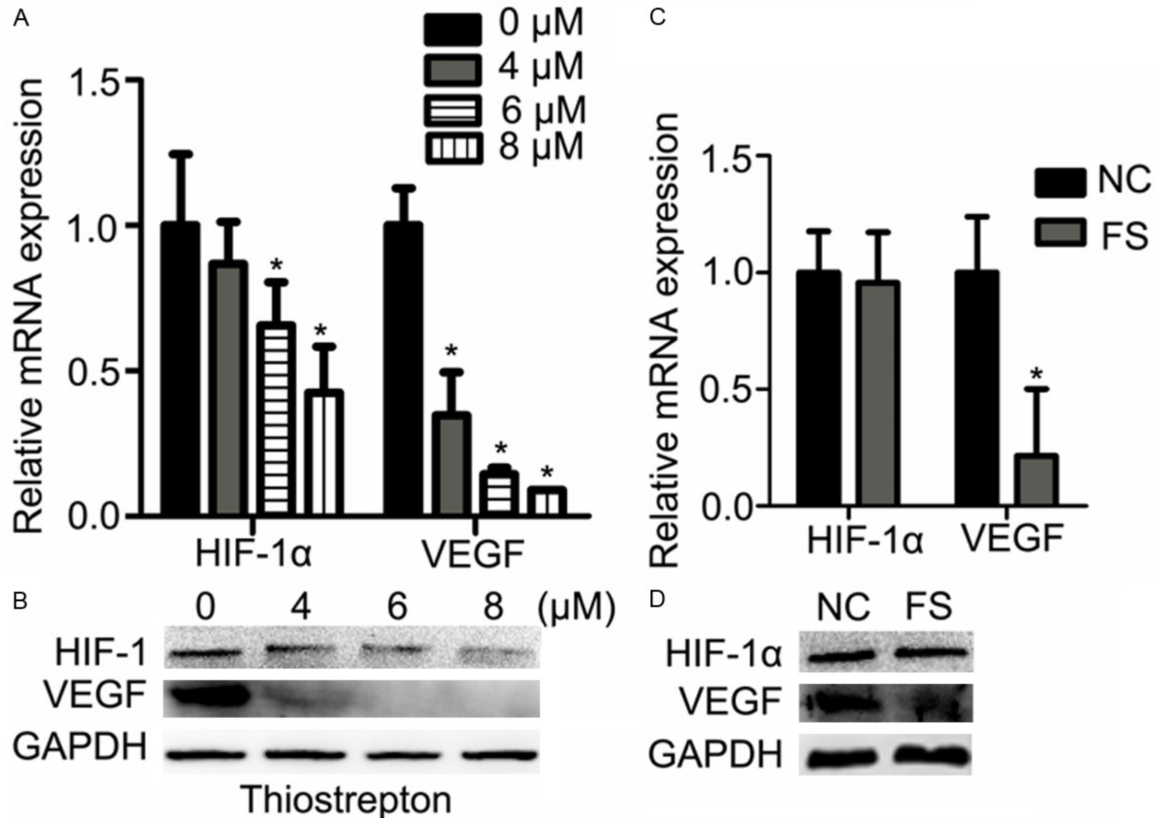

Figure 8. FoxM1 inhibition by thiostrepton or siRNA induced down-regulation of HIF-1α and VEGF expression. A. mRNA expressions of HIF-1α and VEGF were inhibited by thiostrepton. B. Protein expressions of HIF-1α and VEGF were inhibited by thiostrepton. C. FoxM1 siRNA transfection reduced the mRNA expression of VEGF but not HIF-1α. D. FoxM1 siRNA transfection reduced the protein expression of VEGF but not HIF-1α.

pathway or extrinisic pathway [10, 11]. In current study, we also found that thiostrepton induced apoptosis of NPC cells in a dose- and time dependant manner. Moreover, FoxM1 inhibition by thiostrepton or transfection of siRNA in NPC cells caused activation of p53 and bax, down-regulation of bcl-2, resulted in loss of mitochondrial membrane potential. Afterwards, our results showed that cytochrome c was released into cytosol, as cytochrome c expression increased in cytosol. Furthermore, we have also found that release of cytochrome c into cytosol resulted in activation of cleaved capsase-3, caspase-9 and cleaved PARP, eventually resulting in apoptosis. Therefore, these data suggest that FoxM1 inhibition in NPC cells causes apoptosis partly via the mitochondrial pathway and activation of caspase cascade. Moreover, our observation that inhibition of FoxM1 expression with thiostrepton treatment or transfection of siRNA activated FADD and cleaved caspase-8 suggests that extrinisic pathway also participate in apoptosis of NPC cells. Collectively, down-regulation of FoxM1 induces apoptosis of NPC cells via both of intrinsic pathway and extrinisic pathway.

IAPs have been shown to suppress caspase activity and protect cells from apoptosis [24]. Our results showed that NPC cells expressed IAPs including cIAP1 and XIAP, and thiostrepton treatment decreased the expression level of these molecules, suggesting that down-regulation of IAPs may be involved in thiostrepton-induced apoptosis.

HIF-1α and VEGF are important factors in angiogenesis of tumors. HIF-1α is the upper stream gene of FoxM1, and HIF-1α could band to the FoxM1 promoter and increased FoxM1 expression in cancer [25]. In addition, recent reports have documented positive correlations between expression of FoxM1 and VEGF [5]. In the present study, we found a significant reduction in VEGF expression by down-regulation of FoxM1 using thiostrepton and siRNA transfection.

Moreover, we also found a significant reduction in HIF-1α expression by thiostrepton but not FoxM1 siRNA. These data suggest that suppression of FoxM1 by thiostrepton or siRNA has potential for antiangiogenesis therapy.

In summary, the present study demonstrated that down-regulation of FoxM1 suppressed proliferation of NPC cells with reduced expression of cyclin D1 and cyclin E1. Down-regulation of FoxM1 inhibited transformation ability of NPC cells. FoxM1 inhibition induced NPC cell apoptosis via intrinsic pathway and extrinisic pathway as well as IAP family. FoxM1 inhibition suppressed the expression of VEGF. Based on these findings, we conclude that FoxM1 may serve as a new target for NPC therapy, and targeting FoxM1 by thiostrepton may synergize radiotherapy in NPC combinatorial treatment to improve the efficacy of currently available treatments.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Hongyan Chen, Department of Otolaryngology, The First Affiliated Hospital of Chongqing Medical University, 1 Youyi Rd, Chongqing 400016, People's Republic of China. Tel: 86-23-89012758; E-mail: chenhongyan1963@hotmail.com

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