

ORIGINAL ARTICLE

Overexpression of FOXM1 is associated with metastases of nasopharyngeal carcinoma

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Abstract

Background. The forkhead box M1 (FOXM1) transcription factor plays an important role in the metastases of many cancers. Down-regulation of FOXM1 by its inhibitor, thiostrepton, can inhibit the metastatic potential of some cancers; however, there are few studies regarding the functional significance of FOXM1 and thiostrepton in the metastases of nasopharyngeal carcinoma (NPC) and the underlying mechanism.

Methods. Expression of FOXM1 in NPC, normal nasopharyngeal tissues, a NPC cell line (C666-1), and a nasopharyngeal epithelial cell line (NP69) was investigated by immunohistochemical staining, qRT-PCR, and Western blot. The correlation between FOXM1 expression and the clinical characteristics of patients was analyzed. Moreover, the effects of thiostrepton on expression of FOXM1 in C666-1 and NP69 cells, and the invasion and migration ability of C666-1 cells were examined. The expressions of MMP-2, MMP-9, fascin-1, ezrin, and paxillin were determined after treatment with thiostrepton.

Results. FOXM1 was overexpressed in NPC and C666-1 cells compared with normal nasopharyngeal tissues and NP69 cells. Overexpression of FOXM1 was associated with lymph node metastasis and advanced tumor stage. Moreover, thiostrepton inhibited expression of FOXM1 in C666-1 cells in a dose-dependent manner, but had a minimal effect on NP69 cells. Thiostrepton inhibited the migration and invasion ability of C666-1 cells by down-regulating the expression of MMP-2, MMP-9, fascin-1, and paxillin.

Conclusions. Overexpression of FOXM1 is associated with metastases of NPC patients. Thiostrepton inhibits the metastatic ability of NPC cells by down-regulating the expression of FOXM1, MMP-2, MMP-9, fascin-1, and paxillin.

Key words: Forkhead box M1 transcription factor (FOXM1), metastases, nasopharyngeal carcinoma, oncogene, thiostrepton

Introduction

Nasopharyngeal carcinoma (NPC), an Epstein–Barr virus-associated malignancy, is the most common head and neck cancer in China, with an incidence of 30–80/100,000 population/year in southern China (1). NPC has high invasive and metastatic features, and ~90% of patients show cervical lymph node metastasis at the time of initial diagnosis (2). Radiotherapy with or without chemotherapy is the mainstream treatment approach for NPC patients; however, there are limitations in these therapeutic applications due to

undesirable side effects, local recurrence, and metastasis (3). Thus, challenges in NPC treatment still exist, and novel therapeutic targets and new approaches for NPC treatment are urgently needed (4).

The forkhead box M1 (FOXM1), a member of the Fox transcription factor family, regulates a number of cell cycle key factors involved in the transition from the G1 to S phase, G2 to M phase progression, and transition to mitosis (5–7). It has been shown that FOXM1 represents a promising therapeutic target based on up-regulation in numerous human malignancies (8–13), while FOXM1 is suppressed in

non-dividing cells (14). It has been recently reported that FOXM1 is overexpressed in NPC and knockdown of FOXM1 expression diminishes NPC cell proliferation (15). Indeed, the functional significance of FOXM1 in NPC metastases has not been defined.

The FOXM1 inhibitor, thiostrepton, is a natural product with antibiotic properties isolated from *Streptomyces azureus* and is known to interact directly with FOXM1 and inhibit the binding of FOXM1 to genomic target sites (16). In addition, it has been shown that thiostrepton inhibits cell growth and induces apoptosis in a variety of human cancer cell lines by inhibiting FOXM1 expression (17-19). There are few studies that have focused on the effects of FOXM1 and thiostrepton on metastases of NPC, and the correlation between FOXM1 and thiostrepton, and MMP-2, MMP-9, fascin-1, ezrin, and paxillin.

In the current study we determined the expression of FOXM1 in NPC, normal nasopharyngeal tissues, a NPC cell line, and a nasopharyngeal epithelial cell line. The relationship between the expression of FOXM1 and clinicopathologic factors, such as TNM and clinical stage, was analyzed.

Materials and methods

Patients and tissue samples

A total of 65 histologically confirmed NPC patients were consecutively recruited between 2005 and 2008 from The First Affiliated Hospital of Chongqing Medical University in China. Twenty non-cancerous nasopharyngeal samples were collected from patients with clinical symptoms suggestive of NPC, but ruled out by biopsy. None of the patients received radiotherapy or chemotherapy prior to biopsy. Prior patient consent and approval from the Institute Research Ethics Committee were obtained. Among 65 patients with NPC, 45 were males and 20 were females (age range 33-75 years; median age 39 years). The clinical stage and histopathologic classification were based on the World Health Organization (WHO) classification, as follows: T1, 9 (13.8%); T2, 22 (33.8%); T3, 23 (35.4%); T4, 11 (16.9%); N-, 7 (10.8%); N+, 58 (89.2%); stage I, 7 (10.8%); stage II, 9 (13.8%); stage III, 30 (46.2%), and stage IV, 19 (29.2%). Furthermore, 10 NPC and 10 non-cancerous nasopharyngeal tissue samples were included in the cohort for immunohistochemistry to evaluate the expression of FOXM1 RNA and protein.

Immunohistochemical staining

Immunohistochemical staining was performed on 4- μ m paraffin tissue sections mounted on poly-

lysine-coated slides and dried at 37°C overnight. After the slides were deparaffinized in xylene and rehydrated conventionally, the endogenous peroxidase was blocked with 3% hydrogen peroxide in methanol for 20 min. Each slide was incubated with normal goat serum for 20 min at room temperature. The sections were incubated with rabbit anti-FOXM1 polyclonal antibody (1:100 dilution, sc-502; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) overnight at 4°C. After washing with PBS, the sections were incubated for 30 min with a horseradish peroxidase-labeled goat anti-rabbit secondary antibody (Boster Biological Technology, Ltd, Wuhan, China). Chromogen 3,3'-diaminobenzidine (Boster Biological Technology, Ltd) was used for 15 min to visualize immunolabeling, resulting in a brown precipitate. After washing, the sections were counterstained with hematoxylin. Positive and negative immunohistochemistry controls were routinely performed.

The immunostaining results were evaluated and scored independently by two pathologists without knowledge of the clinicopathologic outcomes of the patients. Immunostaining for FOXM1 was limited to the cytoplasm. Specimens with staining in >10% of the cells were considered positive. Immunohistochemical reactivity was graded on a scale of 0-3, according to the intensity of staining and percentage of immunopositive cells as follows: 0, no staining or <10% positive cells; 1, weak staining in >10% of tumor cells or moderate staining in 10%-40% of tumor cells; 2, moderate staining in >40% of tumor cells or strong staining in 10%-40% of tumor cells; and 3, strong staining in >40% of tumor cells (20).

Cell lines and chemical compounds

The NPC cell line, C666-1 (carries EBV), was cultured in RPMI-1640 medium (Invitrogen, Carlsbad, CA, USA) with 10% fetal bovine serum (Gibco, Grand Island, NY, USA), 100 μ g/mL of streptomycin, and 100 U/mL of penicillin (Hyclone, Logan, UT, USA). The immortalized normal human nasopharyngeal epithelial cell line, NP69, was grown in defined-KSFM medium supplemented with epidermal growth factor (EGF) (Invitrogen). Cells were cultured under a 5.0% CO₂ atmosphere. Thiostrepton (Sigma, St. Louis, MO, USA) was dissolved in dimethylsulfoxide (DMSO).

Quantitative real-time RT-PCR

Total RNA was extracted using Trizol reagent (Takara, Dalian, China) according to the manufacturer's instructions. Contaminating genomic DNA was

removed by incubation with DNase I (Takara). RNA purity and concentration were determined by spectrophotometry. PCR was carried out according to the standard protocol on a real-time PCR system with SYBR Green detection. After an initial incubation of the 10- μ L reaction mixture for 1 min at 95°C, 39 cycles (95°C for 20 s and 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 the *GAPDH* gene. The sequences for each set of primers were as follows: 5'-TGCAGCTAGGGATGTGAATCTTC-3' (sense) and 5'-GGAGCCCAG TCCATCAGAACT-3' (antisense) for *FOXM1*; 5'-CTTCCCTTTGGTCTTTCTGT-3' (sense) and 5'-GGTTGTCTGAAGTCAC TGCACAGT-3' (antisense) for *MMP-2*; 5'-CTCGGTAGGGA CATGCTAAGTAGAG-3' (sense) and 5'-CCTCT GGAGGTTTCGACGTGA-3' (antisense) for *MMP-9*; 5'-TCATGAGGAAGAGCTCTGAGT-3' (sense) and 5'-TCATGAGGAAGAGCTCTGAGT-3' (antisense) for *fascin-1*; 5'-TGCCGAAACCAATCA ATG TC-3' (sense) and 5'-GCTTCAGC-CAGGTAGGAA ATC-3' (antisense) for *ezrin*; 5'-CC AACAGCAG ACACGCATCT-3' (sense) and 5'-GGTGAGCTG CTCCCTGTCTT-3' (antisense) for *paxillin*; and 5'-CAGCGACACCCACTCCTC-3' (sense) and 5'-TGAGGTCCACCACCCTGT-3' (antisense) for *GAPDH*. The $2^{-\Delta\Delta CT}$ method was used to calculate the relative expressions.

Western blot

Protein samples were separated on precast 10% SDS polyacrylamide gels (SDS-PAGE). After electrophoresis, the proteins were transferred to PVDF membrane filters (Millipore Biotechnology, Billerica, MA, USA). The membranes were incubated overnight at 4°C with primary mouse polyclonal *FOXM1* antibody (Abgent, Inc., San Diego, CA, USA), primary rabbit polyclonal *fascin-1* (Boster Biological Technology, Ltd), or primary rabbit polyclonal *ezrin* (Cell Signaling Technology, Inc., Danvers, MA, USA), or primary rabbit polyclonal *paxillin* antibody (Immunoway Biotechnology Company, Newark, DE, USA). After washing three times in TBS-T, horseradish peroxidase (HRP)-conjugated secondary antibodies were used at a dilution of 1:5000 in TBS-T for 2 h at room temperature. After three additional washes with TBS-T, the immunoreactive bands were visualized with a chemiluminescence reagent (ECL; Millipore Biotechnology) and quantified using a Bio-Rad imaging system (Bio-Rad Laboratories, Inc., Hemel Hempstead, Hertfordshire, UK).

Cell invasion and migration assays

A Transwell chamber was used (8 mm, 24-well format; Corning, Lowell, MA, USA) to perform cell invasion and migration assays. For the migration assay, 5×10^4 cells in serum-free media were added to the upper chamber after treatment with 2, 4, or 6 μ M thioestrepton for 48 h. For the invasion assay, 1×10^5 cells in serum-free medium were added to the upper chamber coated with diluted Matrigel (Sigma-Aldrich, St. Louis, USA) after treatment with 2, 4, or 6 μ M thioestrepton for 48 h. Media containing 10% FBS were placed in the lower chambers. After 24 h of incubation at 37°C with 5% CO₂, the medium was removed from the upper chamber. The non-invaded cells on the upper side of the chamber were gently scraped off with a cotton swab, whereas the cells that had migrated or invaded through the membrane were stained with methanol and 0.1% crystal violet, imaged, and counted using an inverted microscope (Olympus, Tokyo, Japan) and quantified from visualizing five random fields at a magnification of $\times 200$. Experiments were independently repeated three times.

MMP-2 and MMP-9 assay

Cells were seeded to 50% confluence before treatment with thioestrepton or DMSO. The supernatants were then harvested after 48 h. MMP-2 and MMP-9 activity assays were performed using MMP-2 and MMP-9 ELISA kits (Colorfulgene Biological Technology, Ltd, Wuhan, China).

Statistical analysis

For statistical evaluation, SPSS software (version 14.0; SPSS, Inc., Chicago, IL, USA) was used. The Mann-Whitney *U* test, Kruskal-Wallis test, Student's *t* test, and two-way ANOVA were used for comparing groups. The Spearman correlation test was used to analyze the relationship between *FOXM1* expression and clinicopathologic characteristics. A $p < 0.05$ was considered statistically significant.

Results

FOXM1 expression is up-regulated in NPC

First, 65 NPC specimens at different grades of malignancy and 20 non-cancerous nasopharyngeal tissue specimens were tested for *FOXM1* expression by immunohistochemistry. *FOXM1* was expressed predominantly in the cytoplasm of tumor cells. As is known, *FOXM1* utilization in adults is restricted to organs with proliferating cells involved in replenishing

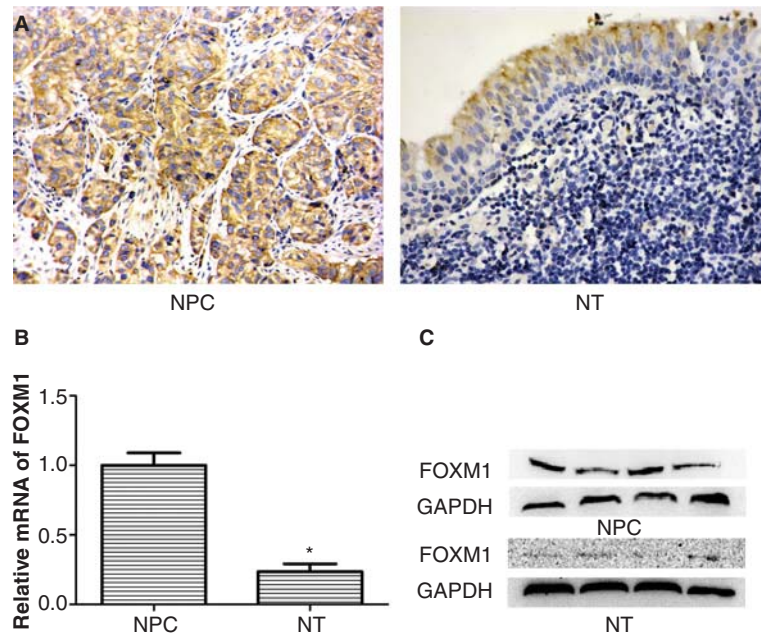


Figure 1. Expression of FOXM1 in NPC and normal nasopharyngeal tissues (NT). A: Overexpression of FOXM1 in NPC and low expression in NT as evidenced by immunohistochemical staining. B: Relative FOXM1 mRNA levels in NPC and NT were determined by qRT-PCR. C: Overexpression of FOXM1 in NPC and low expression in NT were determined by Western blot. Experiments were independently repeated three times. * $p < 0.05$.

differentiated cell populations (21). Therefore, low expression of FOXM1 was detected in normal nasopharyngeal epithelial cells, whereas there was nearly no expression of FOXM1 in submucous tissues (Figure 1A). Positive staining of FOXM1 was observed in 66.2% (43/65) of NPC samples and 40.0% (8/20) of normal tissues ($p < 0.01$) (Table I). To confirm the above results, the levels of FOXM1 mRNA and protein expression were determined by qRT-PCR and Western blot, respectively, in NPC and normal nasopharyngeal tissues. The expression of FOXM1 mRNA and protein was significantly up-regulated in NPC compared with normal nasopharyngeal tissues (both $p < 0.05$) (Figure 1B and C).

Higher level of FOXM1 expression in NPC patients is significantly correlated with metastases

Next, the relationship between FOXM1 expression and the pathologic features of NPC patients was determined. The positive expression of FOXM1 was not

related to age, gender, histologic classification, T stage, or M classification. Of note, an increased expression of FOXM1 was significantly correlated with lymph node metastasis ($p < 0.05$). Specifically, 41 of 58 tumors with lymph node metastasis (+) had high expression of FOXM1, whereas only two of seven tumors without lymph node metastasis – had low expression of FOXM1. Moreover, 73.5% of high-stage tumors (stages III and IV) expressed FOXM1, whereas this was the case in 43.8% of lower-stage tumors (stages I and II) (Table II). Spearman correlation analysis also showed that the expression ratio of FOXM1 was positively correlated with lymph node metastasis ($r_s = 0.273, p = 0.028$) and clinical stage ($r_s = 0.249, p = 0.046$).

Thiostrepton inhibits the expression of FOXM1 mRNA and protein in C666-1, but has less of an effect on NP69

More than 90% of NPC patients in China have undifferentiated tumors; EBV is consistently present in undifferentiated NPC (22). Therefore, the EBV-positive cell line, C666-1, was more suitable for use in the current study than other EBV-negative NPC cell lines. Thiostrepton has been previously reported to inhibit the expression of FOXM1 in several cancer cell lines (18,23); however, the effects of thiostrepton on NPC and normal nasopharyngeal epithelial cell lines remain unknown. The NPC cell line, C666-1, and the transformed human nasopharyngeal epithelial

Table I. Expression of FOXM1 in NPC and normal tissue specimens.

Type	n	FOXM1				p value
		0	1	2	3	
NPC	65	22	13	20	10	< 0.01
Normal tissue	20	12	5	3	0	

Table II. Correlation between the clinicopathologic features and expression of FOXM1.

Variables	Total	FOXM1				p value
		0	1	2	3	
Age						
<50	39	13	8	12	6	0.961
≥50	26	9	5	8	4	
Sex						
Male	45	19	8	12	6	0.063
Female	20	3	5	8	4	
Histological classification						
I	1	0	1	0	0	0.598
II	60	20	11	19	10	
III	4	2	1	1	0	
T classifications						
T1	9	3	3	2	1	0.734
T2	22	6	4	8	4	
T3	23	10	3	7	3	
T4	11	3	3	3	2	
N classifications						
N0	7	5	1	1	0	0.029
N+	58	17	12	19	10	
M classifications						
M0	63	21	13	19	10	0.722
M1	2	1	0	1	0	
Clinical stage						
I + II	16	9	3	2	2	0.046
III + IV	49	13	10	18	8	

cell line, NP69, were treated with 2, 4, or 6 μ M thiostrepton for 48 h, and FOXM1 expression was detected by qRT-PCR and Western blot. The expression of FOXM1 mRNA in C666-1 cells was decreased in a dose-dependent manner after treatment with thiostrepton compared with the control group (Figure 2A). Similarly, the expression of FOXM1 protein in C666-1 cells was also decreased in a dose-dependent manner; notably, there was virtually no expression of FOXM1 protein after treatment with 6 μ M thiostrepton (Figure 2B). However, qRT-PCR analysis suggested that FOXM1 is expressed at a lower level in NP69 cells compared with C666-1 cells, and was minimally inhibited after thiostrepton treatment (Figure 2C).

Down-regulation of FOXM1 by thiostrepton inhibits invasion and migration of NPC cells

Because higher expression of FOXM1 in NPC patients was significantly related to metastases, we

hypothesized that down-regulation of FOXM1 could inhibit the migration and invasion capacity of NPC cells. C666-1 cells were treated with 2, 4, or 6 μ M thiostrepton in advance. An invasion chamber assay was used to verify the biological function of thiostrepton in NPC cell invasion. After 24 h, the invading cells were fixed, stained, and observed microscopically. As the representative micrographs clearly demonstrate, down-regulation of FOXM1 by thiostrepton can indeed inhibit NPC cell invasion *in vitro* in a dose-dependent manner (Figure 3A and B). Likewise, thiostrepton repressed the migration ability of NPC cells in a dose-dependent fashion compared with the control group (Figure 3A and B).

Thiostrepton decreased the expression of MMP-2, MMP-9, fascin-1, and paxillin in NPC cells

To determine the mechanism by which thiostrepton inhibited the invasion and migration of NPC cells other than direct repression of FOXM1, we first determined the expression of MMP-2 and MMP-9 mRNA and protein by qRT-PCR and ELISA in C666-1 cells. The expression of MMP-2 and MMP-9 mRNA and protein decreased with increasing concentrations of thiostrepton (Figure 4A and B). Furthermore, the expression of fascin-1, ezrin, and paxillin, essential components of cancer motility, was determined in C666-1 cells. The expression of fascin-1 and paxillin mRNA and protein decreased in a dose-dependent manner, and a significant reduction was observed at concentrations of 2 or 4 μ M (Figure 4A and C); however, the expression of ezrin mRNA and protein was not significantly down-regulated (Figure 4A and C).

Discussion

Regional and systemic metastases seriously affect the cure and survival rates of NPC patients. Recent studies have shown that FOXM1 and its inhibitor, thiostrepton, represented an attractive therapeutic target and novel anticancer drug in the fight against cancer (24–27). The current study showed, for the first time, that overexpression of FOXM1 is associated with NPC metastases. Thiostrepton inhibited the expression of FOXM1 in a NPC cell line, but had minimal effects on a normal nasopharyngeal epithelial cell line. Thiostrepton inhibited the migration and invasion of NPC cells through down-regulation of MMP-2, MMP-9, fascin-1, ezrin, and paxillin.

FOXM1 is frequently up-regulated in various human cancers and is able to enhance cell proliferation, migration, and invasion (28). Overexpression of FOXM1 has been reported in cervical intraepithelial

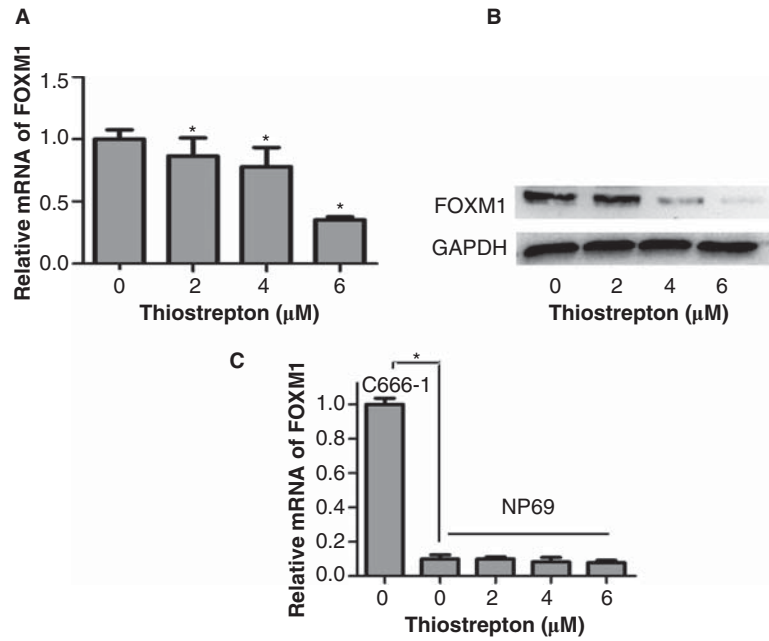


Figure 2. Thiostrepton significantly reduces the expression of FOXM1 mRNA and protein in a NPC cell line other than a nasopharyngeal epithelial cell line. A: The expression of FOXM1 mRNA in C666-1 cells was inhibited after thiostrepton treatment at 2, 4, or 6 μM for 48 h. B: Thiostrepton down-regulated the expression of FOXM1 protein in C666-1 cells. C: The expression of FOXM1 mRNA in NP69 cells was lower than in C666-1 cells and was less affected by thiostrepton. Experiments were independently repeated three times. * $p < 0.05$.

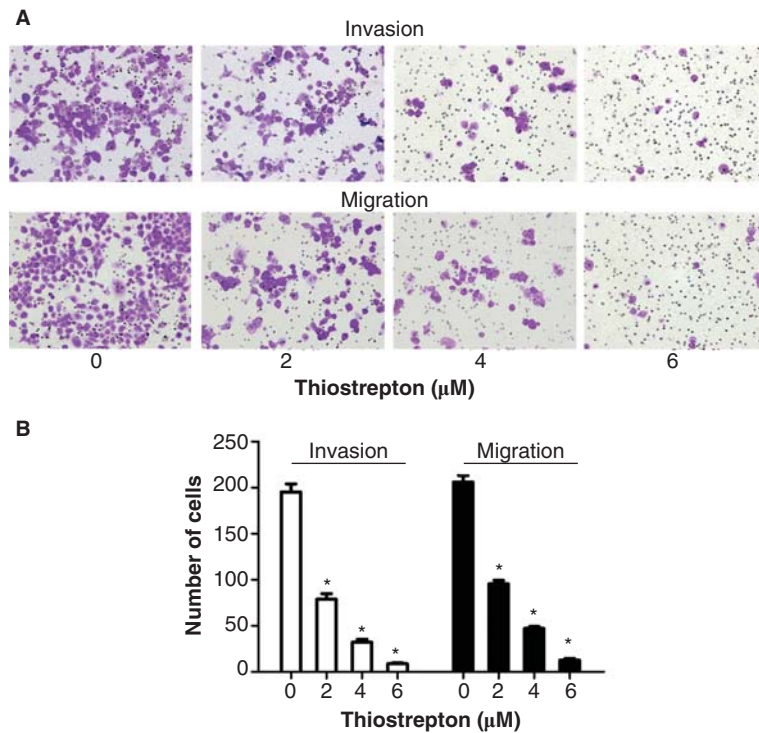


Figure 3. Thiostrepton reduces the invasion and migration activities of NPC cells. A: Invasion and migration assays were used to detect the invasion and migration ability of C666-1 cells following treatment with 2, 4, or 6 μM thiostrepton for 48 h. B: Manual counts of invasion and migration experiments. Experiments were independently repeated three times. * $p < 0.05$.

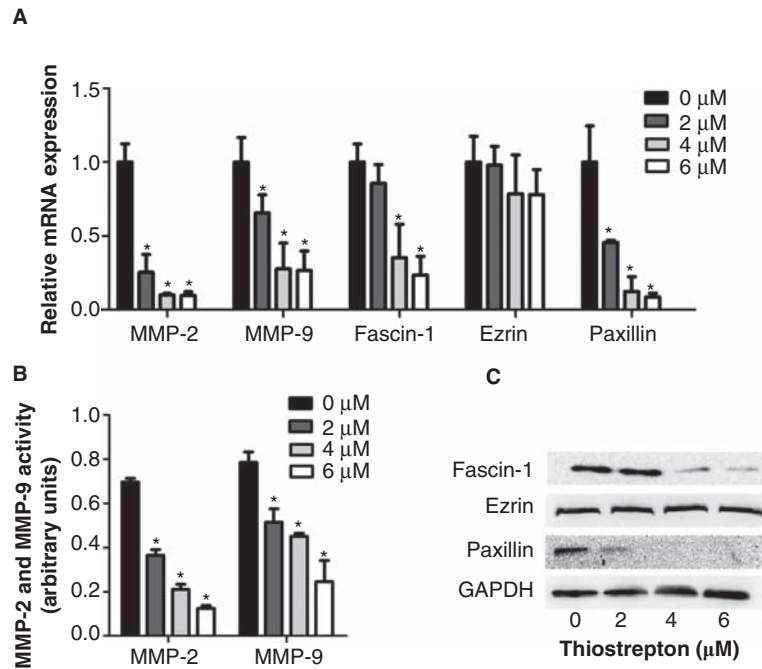


Figure 4. Thiostrepton reduces mRNA and protein expressions of metastatic associated genes in NPC cells. A: Relative MMP-2, MMP-9, fascin-1, ezrin, and paxillin mRNA levels were determined by qRT-PCR. B: An ELISA assay was used to detect protein levels of MMP-2 and MMP-9. C: Fascin-1, ezrin, and paxillin protein levels were determined by Western blot. Experiments were independently repeated three times. * $p < 0.05$.

neoplasia (CIN) and carcinoma compared with normal epithelium (12). Overexpression of FOXM1b promotes the growth and metastasis of gastric cancer cells and gastric tumorigenesis by transactivation of vascular endothelial growth factor expression and elevation of angiogenesis (13). In the current study we showed that FOXM1 is prominently expressed in 66.2% (43/65) of NPC tissue specimens, 69.8% (30/43) of which were > grade 2; however, only 40.0% (8/20) of normal nasopharyngeal mucous tissues expressed FOXM1. Taken together, these results suggest that FOXM1 is closely linked to nasopharyngeal carcinogenesis.

A study involving pulmonary squamous cell carcinoma (SCC) revealed that FOXM1 expression is correlated with poor histologic differentiation, lymph node metastasis, and advanced tumor stage (9). In gliomas, the level of FOXM1 expression is significantly higher in glioblastoma multiforme than anaplastic astrocytomas and low-grade astrocytomas (29); however, FOXM1 expression is not correlated with clinicopathologic parameters in non-small cell lung carcinomas (8). These findings suggest that the relationship between FOXM1 and clinicopathologic parameters might be tissue-specific. In the current study we showed that FOXM1 expression was more frequently detected in NPC with lymph node metastasis and advanced tumor stage. The results showed that 41 of 58 lymph node metastasis (+) NPC had

high expression of FOXM1, whereas only two of seven lymph node metastasis – NPC had low expression of FOXM1 and 73.5% of higher-stage (III and IV) NPC had high expression of FOXM1 compared to 43.8% of lower-stage (I and II) NPC. Moreover, during recent years it has been shown that overexpression of FOXM1 increases the invasion and migration potential of pancreatic and ovarian cancer cells *in vitro* (30,31). Therefore, we conclude that higher expression of FOXM1 is also associated with metastases of NPC patients.

In established animal models, FOXM1 is critical for the development and metastasis of chemically induced hepatocellular carcinoma and prostate, lung, and colorectal cancers (32-35). Down-regulation of FOXM1 by thiostrepton inhibits the metastatic potential of ovarian cancer cells (31). Moreover, thiostrepton specifically inhibits FOXM1 expression in breast cancer cells, but not in non-cancer cells (23). In the current study we also showed that thiostrepton inhibits the expression of FOXM1 in NPC cells in a dose-dependent manner, but has a minimal inhibitory effect on normal nasopharyngeal epithelial cells. In addition, we also showed that thiostrepton depresses the invasion and migration of NPC cells in a dose-dependent manner.

The expression of matrix metalloproteinases, particularly MMP-2 and MMP-9, has been strongly implicated in invasion (36,37). Recent studies have

reported that FOXM1 up-regulates the expression of MMP-2 and MMP-9 in osteosarcoma cells and transfection of FOXM1 into glioma cells directly activates the MMP-2 promoter, whereas inhibition of FOXM1 expression by FOXM1-siRNA suppresses activation (38,39). In addition, it is known that FOXM1 can be directly inhibited by thiostrepton, and thiostrepton can also inhibit the binding of FOXM1 to genomic target sites. In the current study it was shown that the expression of MMP-2 and MMP-9 was significantly down-regulated by thiostrepton. As mentioned above, FOXM1 directly or indirectly targets MMP-2 and MMP-9 in gliomas and osteosarcoma cells. Thus, we hypothesized that inhibition of FOXM1 by thiostrepton may also directly inhibit MMP-2 expression and indirectly inhibit MMP-9 expression in NPC cells.

The incremental motility of malignant cells is a critical step in metastasis, which is regulated by reorganization of actin cytoskeleton and regulation of focal adhesion (40-42). Fascin-1, ezrin, and paxillin are essential components of these cellular structures. Overexpression of fascin-1, ezrin, and paxillin has been reported in numerous human malignancies and is associated with metastasis in many cancers (43-46); however, there are few studies to date regarding the effects of thiostrepton on the expression of fascin-1, ezrin, and paxillin. In the current study we showed that thiostrepton significantly decreased the expression of fascin-1 and paxillin in NPC cells. In addition, we also observed that ezrin was not down-regulated after treatment with thiostrepton. Because fascin-1 and paxillin have not been reported as the direct or indirect target gene of FOXM1, the mechanisms responsible for these results were not established through the thiostrepton-FOXM1 pathway. Therefore, further studies are needed to determine whether or not FOXM1 directly targets fascin-1 and paxillin or indirectly through another endogenous gene.

In summary, we have characterized the expression of FOXM1 in NPC tissue specimens, its relationship with clinicopathologic factors, the effects of the FOXM1 inhibitor, thiostrepton, on invasion and migration ability of NPC cells *in vitro*, and the molecular mechanism involved. Our results suggest that overexpression of FOXM1 is involved in NPC development and metastases. Moreover, inhibition of FOXM1, MMP-2, MMP-9, fascin-1, and paxillin expression by thiostrepton suppresses the metastases of NPC cells. Based on these findings, additional studies are needed to elucidate the exact relationship between thiostrepton and FOXM1 and the above metastatic-related gene. In addition, it is possible that thiostrepton may synergize with chemotherapeutic drugs in NPC combination treatment to improve the efficacy of currently available treatments.

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