# Tat-NTS Suppresses The Proliferation, Migration and Invasion of Glioma Cells by Inhibiting Annexin-A1 Nuclear Translocation

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**Research Article**

**Keywords:** Tat-NTS, glioma cells, MMP-2/9, NF-kB

**Posted Date:** March 11th, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-230574/v1



**Version of Record:** A version of this preprint was published at Cellular and Molecular Neurobiology on August 3rd, 2021. See the published version at https://doi.org/10.1007/s10571-021-01134-y.

# Abstract

Prevention of the nuclear translocation of Annexin-A1 with Tat-NTS (Trans-activator of transcription (Tat), nuclear translocation signal (NTS)) has recently been reported to alleviate neuronal injury and protect against cerebral stroke. However, the role that Tat-NTS plays in the occurrence and development of glioma still needs to be elucidated. Therefore, human glioma U87 cells were treated with various concentrations of Tat-NTS for 24 h, and cell proliferation, migration and invasion were determined with CCK-8 and Transwell assays, respectively. The results revealed that Tat-NTS significantly inhibited the nuclear translocation of Annexin-A1 in U87 cells, inhibited the proliferation, migration and invasion of U87 cells, and suppressed cell cycle regulatory protein expression and the activity and expression of MMP-2/MMP-9. Moreover, Tat-NTS reduced the level of p-p65 NF-κB in these cells. These results suggest that the Tat-NTS-induced inhibition of glioma cell proliferation, migration and invasion is closely associated with the induction of cell cycle arrest, the downregulation of MMP-2/-9 expression and activity and the suppression of the NF-κB signaling pathway. Thus, Tat-NTS may be a potential chemotherapeutic agent for the treatment of glioma.


# Introduction

Glioma is a common malignant brain tumor in the central nervous system. The 5-year survival rate of patients after diagnosis is less than 3%, and the prognosis is still very poor. Although the tumor can be treated by maximal safe surgical resection combined with radiotherapy, chemotherapy and biological therapy, the tumor can still relapse within a short period of time, which seriously threatens the life safety of patients. Glioma is still the most difficult problem in neurosurgery. In essence, glioma is a polygenic disorder, which affects its occurrence and development. Therefore, exploration of the underlying pathogenesis of glioma and discovery of effective biological products are urgently required.

Annexin-A1 (ANXA1)-mediated signaling has received increasing attention for its role in the development of human tumors (Foo et al. 2019). Recent investigations have proven that ANXA1 plays an essential role in membrane aggregation, proliferation, apoptosis, phagocytosis and carcinogenesis (Lim and Pervaiz 2007). ANXA1 is highly expressed in hepatocellular carcinoma (Masaki et al. 1996), adenocarcinomas of the esophagus (Kan and Meltzer 2009) and pancreas (Bai et al. 2004), lung adenocarcinoma (Liu et al. 2011) and glioma (Cheng et al. 2013) but is downregulated or lost in adenocarcinoma of the prostate (Patton et al. 2005), cervical cancer (Wang et al. 2008), thyroid cancer (Petrella et al. 2006), and head and neck cancer (Garcia Pedrero et al. 2004). Obvious overexpression of ANXA1 has been reported in glioma, and it functions as a tumor promoter in glioblastoma.

Tat-NTS is a newly discovered and synthesized small molecule peptide that inhibits ANXA1 nuclear translocation. Our previous studies have demonstrated that the amino acid residues from R228 to F237 in the repeat III domain of ANXA1 serve as a unique nuclear translocation signal (NTS) and are necessary for nuclear translocation of ANXA1 (Li et al. 2019). More importantly, we synthesized a cell-penetrating peptide derived by combining the trans-activator of transcription (Tat) domain with the NTS sequence.

This peptide has a good neuroprotective effect on nerve injury induced by oxygen and glucose deprivation in neurons in vitro and significantly improves prognosis in a rat model of cerebral ischemia. Moreover, Tat-mediated transduction has become a promising strategy for the treatment of numerous diseases, such as HIV-related diseases (Yuan et al. 2019b) and cerebral stroke (Jin et al. 2020). However, whether Tat-NTS can inhibit ANXA1 translocation into the nucleus in glioma cells and the regulatory role it plays in the occurrence and development of glioma remain to be further studied.

In this study, we explored the efficacy and potential functional mechanisms of Tat-NTS in glioma cells to elucidate the molecular mechanism by which Tat-NTS affects the migration and invasion of glioma and to provide an experimental basis for the study of Tat-NTS as a potential small molecule drug for the treatment of glioma. Our results indicated that Tat-NTS significantly inhibited the proliferation, migration and invasion of U87 human glioma cells, and this effect was partly due to the downregulation of MMP-2/MMP-9 expression and activity and the suppression of the NF-kB signaling pathway.

# Materials And Methods

1. **Cells and Reagents**

The human glioma cell line U87 was obtained from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China) and cultured in DMEM (Gibco, Cat. #11965092, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, Cat. #16140089, USA) at 37°C with a 5% $CO_2$ humidified atmosphere. Cells in the logarithmic growth phase were used for experiments, and the maximum number of passages for cell lines was < 10. The Tat-NTS (NH2-YGRKKRRQRRR-RSFPHLRRVFCONH2) and the Tat-Scr (NH2-YGRKKRRQRRRFRLPSVHRFR-CONH2) peptides (99% purity) were synthesized according to our previous study (Li et al. 2019). The sources of antibodies were as follows: MMP-2 (#40994), MMP-9 (#3852), ANXA1 (#3299), p-p65NF-kB (#3031), p65NF-kB (#3034), b-actin (#3700), a-tubulin (#2144), Histone 3 (#4499), P21 (#2947), CDC2 (#28439), Cyclin A2 (#4656), Cyclin B1 (#4138) and horseradish peroxidase (HRP). The secondary antibodies (#7074 and #7076) were obtained from Cell Signaling Technology (Danvers, MA, USA). ANXA1 (sc-12740) was purchased from Santa Cruz Biotechnology Company, and Alexa Fluor 594-conjugated AffiniPure Goat Anti-Mouse IgG (H + L) was purchased from Jackson ImmunoResearch (West Grove, Pennsylvania, USA). Crystal violet dye (#DC079-1G) was obtained from Beijing Dingguo Biological Co., Ltd. (Beijing, China), and Transwell chambers (#3422) were purchased from Corning Lifesciences (Kennebunk, ME, USA). Matrigel (#356234) was obtained from BD Biosciences (Franklin Lakes, NJ, USA). The pore size of the Transwell chamber membrane was 8 mm.

2. **Cell Counting Kit-8 (CCK-8) Assay**

Cell viability was assessed by CCK-8 assay. Briefly, U87 cells were plated at a density of $4\times10^3$ cells in a 96-well plate. After culturing for 24 h, the cells were incubated with various concentrations of Tat-NTS for 24 h or a fixed concentration of Tat-NTS for 12, 24, and 48 h before the CCK-8 assay kit (#CK-04, Dojindo,

Kumamoto, Japan) was applied. Ten microliters of CCK-8 reaction solution was added to each well and subsequently incubated for an additional 4 h. The absorbance was then detected by an EnSpire Manager Spectrophotometer Plate Reader (Turku, Singapore) at 450 nm (excitation) and 600 nm (emission).

3. **Immunofluoresence Staining**

For immunofluorescence staining, stimulated U87 cells were gently washed with PBS solution, fixed in 4% paraformaldehyde solution for 30 min at room temperature, permeabilized with 0.1% Triton X-100 for 30 min, and then blocked for an additional 60 min in PBS containing 10% donkey serum. The cells were then incubated with primary antibodies [anti-ANXA1 (1:200, sc-12740)] overnight at 4°C. The cells were then rinsed with PBS containing 0.25% Tween-20 three times and subsequently incubated with secondary antibodies for 1 h at room temperature. DAPI was chosen for counterstaining of the nuclei. The cells were examined, and images were immediately collected under fluorescence microscopy (IX81, Olympus, Tokyo, Japan).

4. **Western Blot**

For extraction of whole-cell lysates, U87 cells were lysed in RIPA lysis buffer (Beyotime Institute of Biotechnology, Haimen, China) containing a protease inhibitor cocktail (Roche, Basel, Switzerland). The Nuclear and Cytoplasmic Protein Extraction Kit (Thermo Fisher Scientific) was used to prepare nuclear and cytoplasmic extracts following the protocols provided by the manufacturer. The Bicinchoninic Acid (BCA) Protein Assay Kit (Beyotime) was used to determine the protein concentrations of the extracts. Equal amounts of protein (30 mg) from each sample were loaded for immunoblot analysis. The proteins were separated by 10% SDS-PAGE and subsequently transferred to polyvinylidene fluoride (PVDF) membranes (#ISEQ00010, Millipore, Billerica, Massachusetts, USA), rinsed three times with Tris-buffered saline containing 0.1% Tween 20 (TBST), and then blocked with 5% nonfat milk in TBST at room temperature for 1 h. The membranes were subsequently incubated with the corresponding primary antibodies at 1:1000 in TBST at 4°C overnight. After washing with TBST three times, the membranes were subsequently incubated with horseradish peroxidase-conjugated secondary antibodies (1:10000) in TBST at room temperature for 1 h. After washing with TBST, the protein blots on the membranes were visualized by an Enhanced Chemiluminescence Kit (#RPN2232, Amersham, GE Health, UK) and photographed. ImageJ 1.4.3 (National Institutes of Health, Bethesda, MD, USA) was used to analyze the gray values of each band, and b-actin was used as a loading control.

5. **Migration and Invasion Assays**

First, the Transwell chamber was placed in liquid containing DMEM + 0.1% BSA (bovine serum albumin) for hydration in a 5% CO2 incubator at 37°C for 2 h. The glioma cells were harvested and counted and then inoculated in the chamber at a density of $5 \times 10^4$ cells/well. The medium in the upper chamber was DMEM + 0.1% BSA, and the medium in the lower chamber was DMEM + 15% FBS combined with 20 mM Tat-NTS or Tat-Scr. The effect of the treatments on the migration ability of glioma cells was observed, then the chamber was removed after 24 h incubation, and the cells were fixed on the chamber membrane

with 4% paraformaldehyde and then stained with 0.1% crystal violet; the cells that did not pass through the membrane were removed with a cotton swab. The cells were photographed under high magnification (200×), five random fields were observed from each well, and the results were analyzed. For invasion assays, the chambers were first coated with 90 ml of 0.8 mg/ml Matrigel, and the subsequent steps were similar to those of the migration assays.

6. **Gelatin Zymography**

To measure MMP-2 and MMP-9 activity in U87 MG cells, gelatin zymography was performed as described previously (Morioka et al. 2019). Briefly, after incubation with various concentrations (2, 5, 10, 20 and 40 mM) of Tat-NTS or with Tat-Src (20 mM) for 24 h, the supernatants of glioma cells were collected, and the supernatants were then prepared with loading buffer (125 mM Tris-HCl, pH 6.8, 1% glycerol, 2% SDS, 0.01% bromophenol blue) without heating or reduction and separated by electrophoresis on an 8% SDS-polyacrylamide gel containing 0.1% w/v gelatin. After separation, the gels were gently washed thrice with distilled water containing 2.0% Triton X-100 for 1 h to remove SDS and further incubated with substrate buffer (50 mM Tris-HCl containing 10 mM CaCl2 and 0.02% NaN3, pH 7.6) at 37°C overnight, followed by staining with Coomassie Brilliant Blue R-250 for 1 h and decolorization in 20% methanol and 10% acetic acid until clear bands appeared. The density was measured with ImageJ software (Fuji Film, Tokyo, Japan).

7. **Statistical Analysis**

GraphPad Prism 5 software was used for statistical analysis. All data were expressed as the mean ± standard deviation. One-way analysis of variance was performed to analyze these data, and multiple comparisons between the groups were analyzed by Bonferroni's multiple comparison method. A value of $P < 0.05$ was considered to be statistically significant.

# Results

1. **Tat-NTS Inhibits The Proliferation of U87 Cells**

To investigate the influence of Tat-NTS on cell proliferation, cell viability was detected by CCK-8 assay after human glioma U87 cells were treated with vehicle (PBS) or Tat-NTS at different concentrations (2, 5, 10, 20 and 40 mM) for 24 h. The results showed that the proliferation of U87 cells was inhibited by Tat-NTS in a concentration-dependent manner; 10, 20 and 40 mM Tat-NTS significantly inhibited cell proliferation (Fig. 1A, $P < 0.01$), and the $IC_{50}$ of U87 cells treated for 24 h was 28.20 mM. Changes in cell viability from 20 mM Tat-NTS treatment for different durations were then observed. As shown in Fig. 1B, Tat-NTS significantly decreased cell viability after 12, 24 and 48 h. Therefore, 24 h was chosen as the treatment time for the following experiments.

2. **TAT-NTS Inhibits ANXA1 Nuclear Translocation**

After Tat-NTS treatment for 24 h, the total protein and cytoplasmic and nuclear protein fractions of glioma cells were extracted, and the expression of ANXA1 in each treatment group was detected by Western blot. The results showed that compared with the PBS group and Tat-Scr group, the amount of ANXA1 in the nucleus of the Tat-NTS group was significantly reduced, and that of ANXA1 in the cytoplasm was unchanged. Tat-NTS significantly inhibited the incorporation of ANXA1 into the nucleus, but it had no effect on the total expression level of ANXA1 (Fig. 2A and B). Furthermore, immunofluorescence staining analysis also showed that Tat-NTS significantly inhibited ANXA1 nuclear translocation compared with Tat-Scr or PBS (Fig. 2C).

3. **TAT-NTS Inhibits The Migration and Invasion of U87 Cells**

Next, we explored the effects of Tat-NTS on U87 cell migration and invasion. Transwell assays were used to detect the migration ability of cells in each group. The results showed that the numbers of penetrating cells in the PBS group and Scr group were similar, and there was no significant difference between these two groups ($P > 0.05$), while the migratory cell numbers of Tat-NTS-treated U87 cells were decreased by 64.86 ± 4.12% compared with those of Tat-Scr-treated cells (Fig. 3A and B, $P < 0.05$). Moreover, the number of invading U87 cells in the Tat-NTS group were decreased by 84.96 ± 3.18% compared with those in the Tat-Scr group, while those in the PBS group and Tat-Scr group were not significantly different (Fig. 3A and C). Tat-NTS significantly inhibited glioma cell invasion. Our results reflected that Tat-NTS inhibits the migratory and invasive abilities of U87 cells.

4. **The Effect of Tat-NTS on Cyclins and NF-kB Pathway**

To further explore the mechanisms of Tat-NTS inhibition on glioma cell proliferation, we detected the levels of cell cycle regulatory proteins by Western blotting. Previous studies have demonstrated that the NF-kB pathway plays an essential role in glioma cell migration and invasion (Wang et al. 2019; Kına et al. 2019). To investigate whether the inhibitory effect of Tat-NTS on migration and invasion was caused by NF-kB p65 downregulation, protein extracts were subjected to Western blot analysis. The results showed that Tat-NTS inhibited the expression of Cyclin A2 and CDC2 in glioma cells, while the expression of p21 protein was upregulated with increasing Tat-NTS concentration, but Tat-NTS had no impact on the expression of Cyclin B1 (Fig. 4A and B). Furthermore, the Western blot results showed that the phosphorylation level of NF-kB was significantly inhibited by treatment with Tat-NTS for 24 h in a dose-dependent manner, whereas there was no effect on the total NF-kB level, which suggested that Tat-NTS suppressed the activation of the NF-kB signaling pathway in human glioma cells (Fig. 4C and D).

5. **Tat-NTS Inhibits The Expression and Activity of MMP-2 and MMP-9**

Matrix metalloproteinases (MMPs) are involved in the degradation of the extracellular matrix and components of the basement membrane (King 2016). Furthermore, previous studies have demonstrated that MMP-2 and MMP-9 play a vital role in the migration and invasion of glioma cells (Aroui et al. 2016; Pagliara et al. 2014); moreover, several reports have indicated that the NF-kB pathway affects the transcription of MMP-2/MMP-9 in cancer cells (Tang et al. 2016; Fong et al. 2010). Therefore, we

investigated whether Tat-NTS can regulate the expression and activity of MMP-2 and MMP-9. Western blot results showed that Tat-NTS downregulated the expression of MMP-2 and MMP-9 in a concentration-dependent manner (Fig. 5A and B). Gelatinase analysis showed that Tat-NTS inhibited the activity of MMP-2 and MMP-9 released into the cultured medium from glioma cells in a similar manner (Fig. 5C and D).

# Discussion

Glioma is a common malignant tumor in the central nervous system (Uhm and Porter 2017). It has the characteristics of invasive growth, a high recurrence rate, a high mortality rate and a low cure rate. Glioblastoma is the most common glioma. The median overall survival time of glioblastoma is approximately 14.6 months. Under standard treatment and diagnosis, the 5-year survival rate is less than 10% (Fatehi et al. 2018). At present, the main treatment methods include surgical resection of tumors combined with radiotherapy, chemical therapy and biotherapy. Although the treatment methods continue to improve, the prognosis of patients is still poor, and the median survival time of patients has not significantly improved. Therefore, it is urgent to find new agents to assist the surgical treatment of glioma. The NF-kB signaling pathway widely exists in cells and plays an important physiological role by regulating the cell cycle and the transcription of genes related to invasion and metastasis. Previous studies have indicated that the NF-kB signaling pathway is overactivated in gliomas, suggesting that this pathway is closely related to the occurrence and development of glioma (Ius et al. 2018; Yu et al. 2018). Therefore, the NF-kB pathway may become a promising target candidate for the treatment of glioma.

ANXA1 is a calcium-dependent phospholipid-binding protein that is widely involved in anti-inflammatory processes, signal transduction, cell differentiation and cell cycle regulation, tumor invasion and metastasis and other biological processes (Shao et al. 2019). ANXA1 is composed of a functional N-terminal region and a C-terminal core region. The C-terminal core region consists of four homologous repeats of 70 amino acid residues. In a previous study, we found that amino acid residues 228-237 in the repeat III domain are the region of the nuclear translocation signal (Li et al. 2019). Although ANXA1 plays an important role in promoting or inhibiting cancer in different tumors, its role in breast cancer is still controversial (Tu et al. 2017), and recent studies have shown that ANXA1 promotes the occurrence and progression of glioma (Tadei et al. 2018).

Transactivator of transcription (Tat) can translocate numerous proteins, peptides, plasmid DNA, siRNA, nanoparticles and other substances into cells in a short time with high efficiency (Guidotti et al. 2017; Wadia et al. 2004). HIV-I Tat (human immunodeficiency virus-1 transcription activator, Tat) has been widely studied (Yuan et al. 2019b). It can not only penetrate the cell membrane but also penetrate the blood-brain barrier, which is conducive to in vivo transport. Tat-NTS, as a newly discovered and synthesized inhibitor of ANXA1 nuclear translocation, is mainly composed of the trans-activator Tat and the amino acid residues of the nuclear translocation signal in the repeat III domain of ANXA1 (Li et al. 2019). Our results show that Tat-NTS can inhibit the proliferation, migration and invasion of glioma cells by inhibiting the nuclear translocation of ANXA1 and the phosphorylation of NF-kB protein. However, the

effect of Tat-NTS treatment on tumor growth in an animal model remains to be determined in future studies.

The aim of this study was to investigate the effects of Tat-NTS on the proliferation, invasion and migration of U87 glioma cells. We found that in glioma cells, Tat-NTS inhibited the nuclear translocation of ANXA1, inhibited cellular proliferation, caused G2/M phase arrest, and inhibited the expression and activity of matrix metalloproteinases (MMPs) by inhibiting the phosphorylation of NF-kB, thus inhibiting the migration and invasion of glioma cells. Recent studies have shown that the effect of ANXA1 on the invasion of glioma cells is related to the synthesis level of matrix metalloproteinases (Tadei et al. 2018). The results showed that the protein levels and activities of MMP-2 and MMP-9 in the cells treated with Tat-NTS were decreased, suggesting that Tat-NTS can inhibit the synthesis of matrix metalloproteinases by blocking the entry of ANXA1 into the nucleus, thus inhibiting the migration and invasion of glioma cells. These findings are similar to those of previous studies that showed that silencing ANXA1 inhibited the invasion of breast cancer cells via downregulation of MMP-9 (Kang et al. 2012). A change in cell cycle distribution is the basic biological characteristic of malignant tumors, and disruption of the cell cycle is the basic cause of uncontrolled proliferation (Yuan et al. 2019a). Therefore, regulation of cell cycle progression has become an important means of treating tumors as well as inducing apoptosis or necrosis. Cyclins are the main regulatory proteins of the eukaryotic cell cycle. They regulate the cell cycle by binding with cyclin-dependent kinases (CDKs) to form cyclin-CDK complexes (Malumbres and Barbacid 2009). When tumor cells are treated with chemotherapeutic drugs or radiation, they usually appear to undergo G1/S or G2/M arrest to repair damaged DNA. CDC2/cyclin B1 is one of the important factors regulating the cell transition from G2 phase to M phase. The dephosphorylation of CDC2 leads to the enhancement of cdc25 protein phosphatase activity and the initiation of mitosis (Butz et al. 2017). In this study, after treatment with Tat-NTS, the protein level of cyclin B1 was downregulated, and the activity of the CDC2/cyclin B1 complex was decreased, resulting in G2 phase arrest.

In summary, this study shows that Tat-NTS significantly inhibited the nuclear translocation of ANXA1, thus inducing cell cycle arrest and inhibiting cell invasion. Due to its small molecular weight and ability to readily cross the blood-brain barrier, Tat-NTS provides a new strategy for clinical application in glioma therapy.

# Declarations


### Acknowledgments

This work was supported by a research grant provided by the Excellent Youth Foundation of Health and Family Planning Commission of Wuhan Municipality (WX17Q10).


### Compliance with ethical standards

### Conflict of interest

The authors declare that they have no conflicts of interest to declare.

### Ethical approval

Not applicable. This article does not contain any studies with human participants or animals performed by any of the authors.

### Author contributions

ZZL, LL, and XL carried out the molecular biology analysis, participated in the design of the study, and drafted the manuscript. WQC and ZXL participated in the data analysis, manuscript editing, manuscript review and performed the statistical analysis. ZZL and ZXL conceived of and designed the study, and participated in the data analysis and coordination, and helped to draft the manuscript. All authors have read and approved the final manuscript.

### Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

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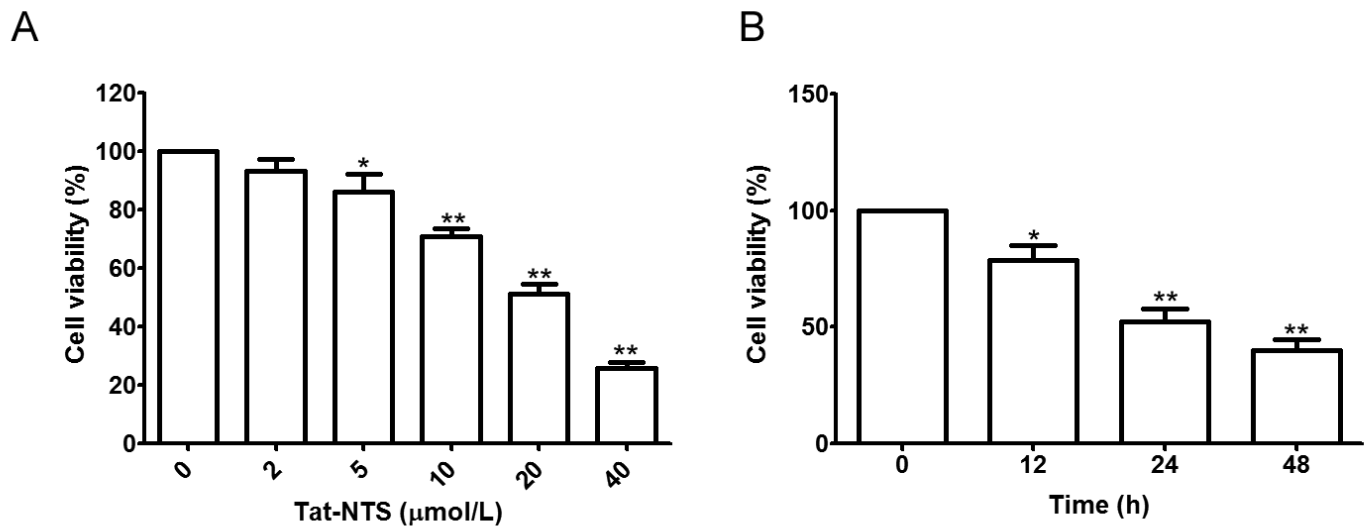
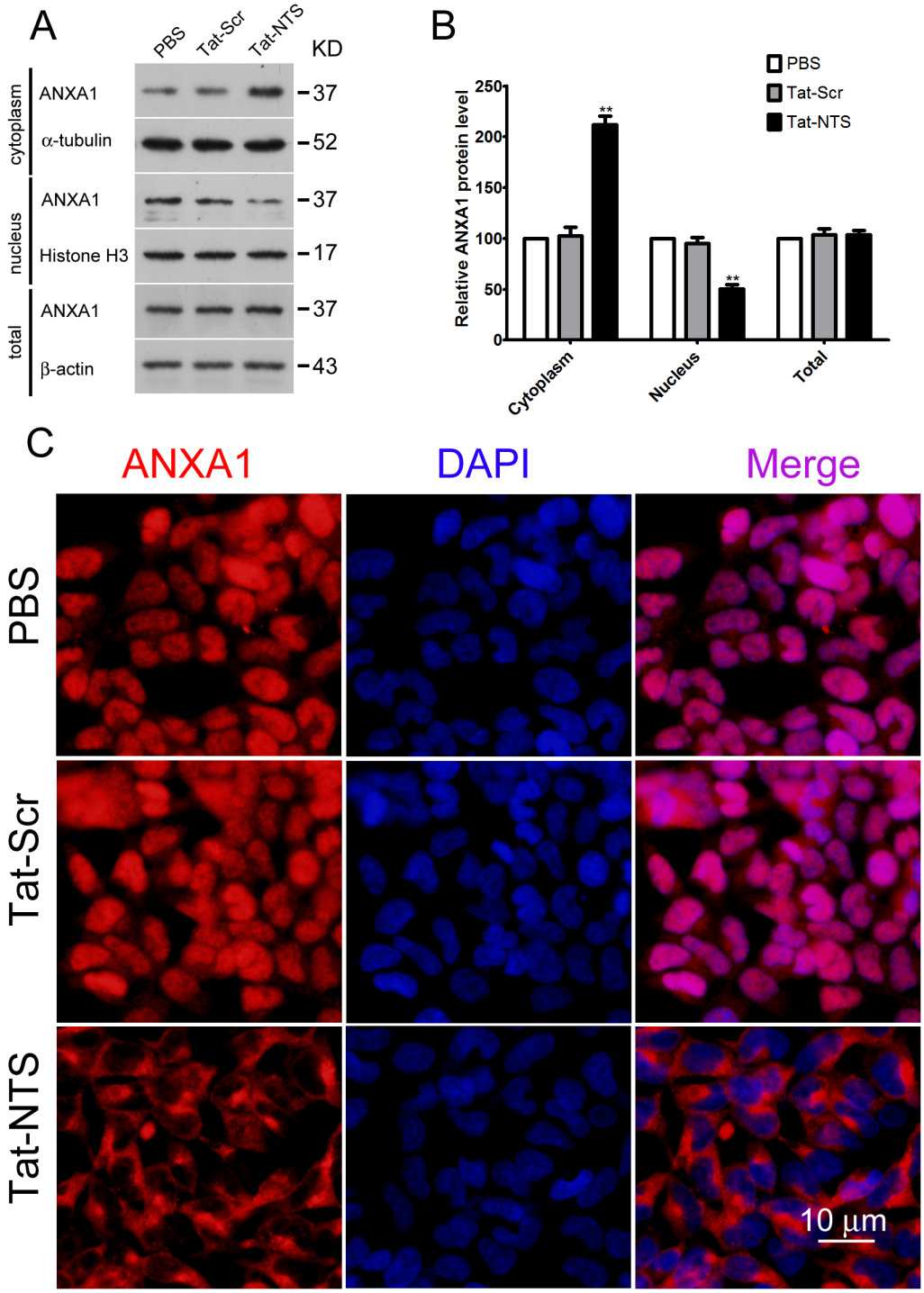
# Figures




## Figure 1

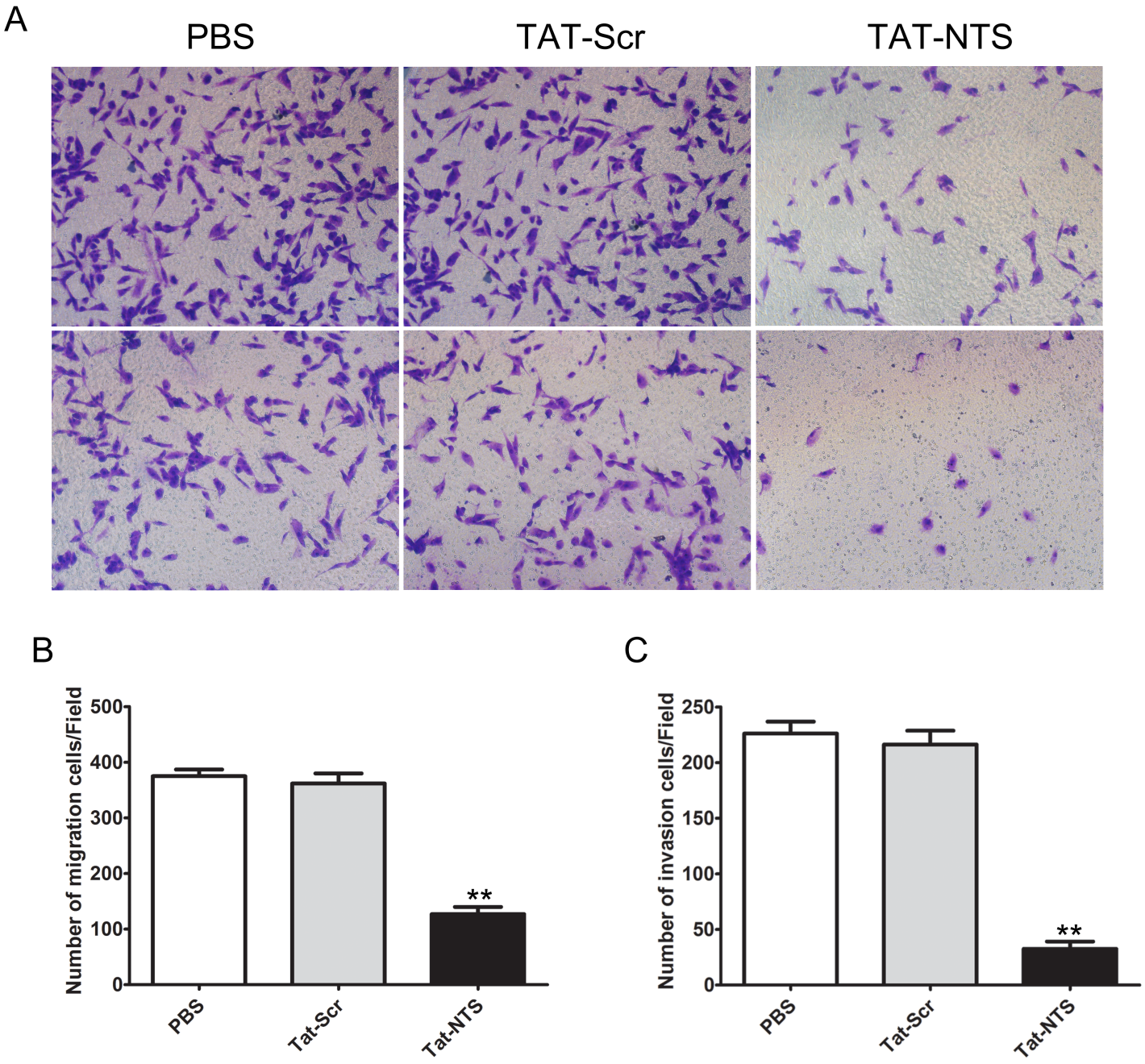
Effect of Tat-NTS on cell viability. Human glioma U87 cells were exposed to various concentrations of Tat-NTS (2, 5, 10, 20 and 40 M) or PBS (vehicle) for 24 h. Additionally, human glioma U87 cells were exposed to 20 M Tat-NTS for 12, 24 and 48 h. Cell viability was then analyzed by CCK-8 assay. Tat-NTS inhibited cell proliferation in a dose-dependent (A) and time-dependent manner (B). Mean ± SD. n = 5. * P < 0.05, ** P < 0.01 vs 0 mol/L or 0 h.




## Figure 2

Tat-NTS inhibited ANXA1 translocation to the nucleus in U87 cells. U87 cells were treated with PBS, Tat-Scr or Tat-NTS (20 □M) for 24 h. Nuclear and cytosolic protein extracts were then subjected to Western blot analysis with specific antibodies. (A): Representative images of Western blot analysis for ANXA1 levels in the cytoplasmic and nuclear extracts. □-Tubulin and Histone H3 were used as cytoplasmic and nuclear internal controls, respectively, while □-actin was used as a loading for total protein samples. (B):

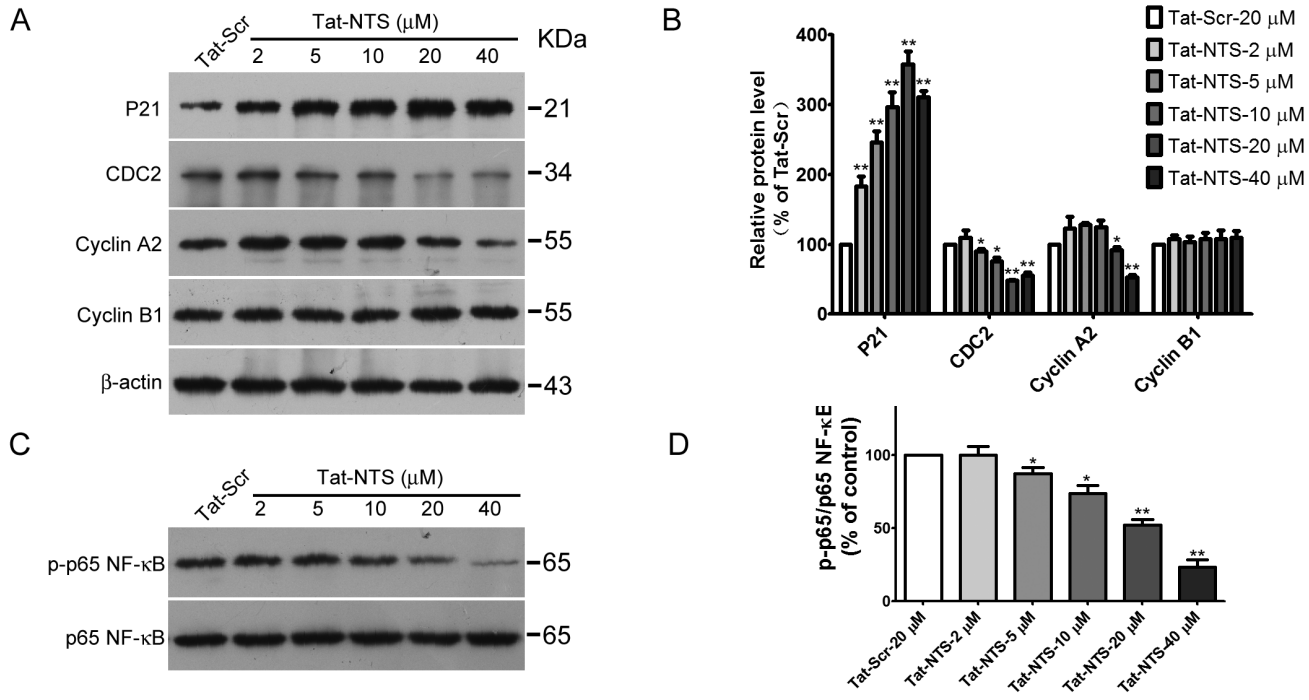
The relative level of ANXA1 protein was quantified by densitometric analysis. Data are represented as the mean ± SD from three independent experiments. **P < 0.01, versus the Tat-Scr control. (C): Representative immunofluorescence staining results of ANXA1 (red) and nuclei (blue) showing the subcellular distribution of endogenous ANXA1 in U87 cells. Scale bar = 10 □m.




## Figure 3

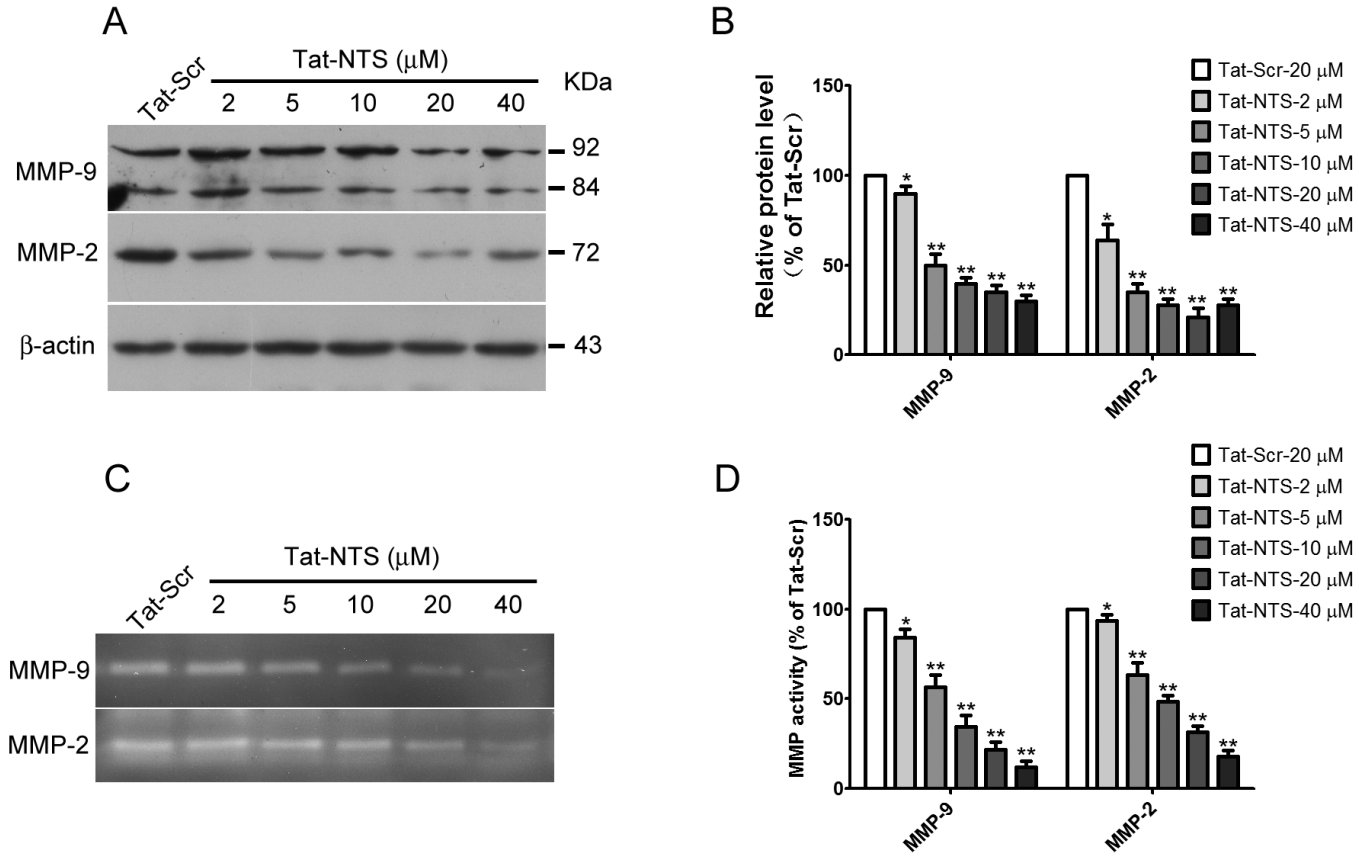
Effect of Tat-NTS on the migration and invasion capacity of glioma cells in vitro. (A): The cells were treated with PBS, Tat-Scr (20 □M) or Tat-NTS (20 □M) for 24 h, and cell migration was then determined

with a Transwell system. The migrating (upper panel) and invading (lower panel) cells on the bottom surface of the membrane were fixed and stained with crystal violet. Original magnification: 200x. (B and C): Quantification of migrating and invading cells. The migrating and invading cells were counted under a microscope in five random fields. Data are presented as the mean ± SD from three independent experiments. **P < 0.01, versus the Tat-Src group.




## Figure 4

Tat-NTS induced G2/M cell cycle arrest and inhibited the activation of the NF-κB signaling pathway in glioma cells. U87 cells were treated with the indicated concentrations of Tat-NTS (2, 5, 10, 20 and 40 μM) for 24 h, and the expression levels of P21, CDC2, Cyclin A2, and CyclinB1 and the phosphorylation level of p65NF-κB in total cell lysates were determined by Western blot. (A and C) Representative protein blots evaluated by Western blot analysis. (B and D) The densities of the protein bands were normalized to β-actin and p65 NF-κB, respectively. The data are shown as the mean ± SD from three independent experiments. * P < 0.05, ** P < 0.01 vs the Tat-Scr group.




## Figure 5

Tat-NTS inhibited the expression and activity of MMP-2 and MMP-9. U87 cells were treated with Scr control (20 □M) or different concentrations of Tat-NTS (2, 5, 10, 20□and 40 □M) for 24 h, followed by protein extraction and conditioned media collection. Cell lysates were analyzed by Western blotting, and conditioned media were measured by gelatin zymography assay. (A): Representative images of Western blot analysis of MMP-2 and MMP-9 expression in U87 cells. (B): Quantification of the relative protein levels of MMP-2 and MMP-9 in U87 cells. (C): Representative images of gelatin zymography of MMP-2 and MMP-9. (D): Relative quantification of the bands of MMP-2 and MMP-9 in U87 cells. n = 3. * $P < 0.05$, ** $P < 0.01$ vs the Tat-Scr group.