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Research Article

# Exploration of the Mechanism of Arsenic Trioxide Inhibits the Proliferation of Bladder Cancer T24 Cells by Inhibiting Glutathione Peroxidase 4

**Kewen Zheng***

Department of Urology, The First Affiliated Hospital of Wenzhou Medical University, The First Clinical College of Wenzhou Medical University, Wenzhou City, R. P. China

## Abstract

*Background:* Bladder cancer is one of the most common malignancies globally, with high recurrence rates and limited therapeutic efficacy in advanced stages. Current treatments, including chemotherapy and immunotherapy, often face challenges such as drug resistance and severe side effects. Therefore, identifying novel therapeutic agents with improved efficacy and safety profiles is critical. Arsenic trioxide (ATO), a compound with established anti-cancer properties in other malignancies, has shown potential in modulating oxidative stress pathways, but its effects on bladder cancer remain underexplored. *Objective:* This study aims to investigate the inhibitory effects of ATO on the proliferation of bladder cancer T24 cells and elucidate the underlying molecular mechanisms, particularly focusing on the glutathione peroxidase 4 (GPX4)-mediated oxidative stress response. *Main Ideas:* The anti-proliferative effects of ATO were evaluated using the Cell Counting Kit-8 (CCK-8) assay, revealing a significant dose-dependent inhibition of T24 cell viability after 48 hours of treatment, with an IC50 value of 0.289 mg/L. Western Blot analysis demonstrated that ATO markedly reduced GPX4 expression, a key regulator of ferroptosis and oxidative stress. These findings suggest that ATO suppresses bladder cancer cell growth by disrupting redox homeostasis, potentially through GPX4 downregulation. The study provides mechanistic evidence linking ATO's anti-cancer effects to oxidative stress modulation in bladder cancer cells. *Conclusion:* Our results highlight ATO as a promising therapeutic candidate for bladder cancer, acting via GPX4-mediated oxidative stress pathways. These findings contribute to the growing body of research on redox-targeted therapies and warrant further investigation into ATO's clinical applicability, either as a monotherapy or in combination with existing treatments. Future studies should validate these results in vivo and explore synergistic strategies to enhance therapeutic outcomes. This work underscores the potential of ATO in developing novel, targeted approaches for bladder cancer management.

## Keywords

Bladder Cancer, Proliferation, Arsenic Trioxide, Glutathione Peroxidase 4

## 1. Introduction

Bladder cancer is one of the most prevalent malignancies worldwide, presenting significant challenges in both diagnosis and treatment. The quest for effective therapeutic approaches for this disease is paramount, particularly given the

*Corresponding author: zkw2121@163.com (Kewen Zheng)

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limitations of current treatment modalities. Recent studies have explored various compounds for their potential anti-cancer properties, among which arsenic trioxide (ATO) has garnered attention due to its demonstrated effectiveness against several cancer types [1]. However, the specific mechanisms through which ATO exerts its effects on bladder cancer cells, particularly the T24 cell line, remain inadequately characterized.

Existing research has established that ATO possesses anti-proliferative effects across a range of cancer cell lines, with emphasis on its ability to induce apoptosis and inhibit cell growth [2]. Yet, the majority of these studies have not systematically focused on bladder cancer, indicating a significant gap in the current understanding of ATO's role in this specific context. The limited exploration of ATO's mechanisms in bladder cancer cells, particularly regarding its interaction with crucial cellular pathways, highlights the necessity for further investigation to elucidate its potential therapeutic applications.

This study aims to fill this research void by providing a detailed analysis of ATO's effects on the proliferation of bladder cancer T24 cells, with a particular focus on its modulation of glutathione peroxidase 4 (GPX4) expression. GPX4 is an essential antioxidant enzyme that plays a critical role in maintaining cellular redox homeostasis [3]. Previous studies have suggested that alterations in GPX4 expression can influence cancer cell proliferation and survival, thereby implicating this enzyme in the potential mechanisms of action for ATO in bladder cancer [4].

To accomplish the research objectives, we will employ methodologies such as CCK8 assays to assess cell proliferation and Western Blot analysis to quantitatively evaluate GPX4 expression levels. These techniques are selected for their high sensitivity and reliability in providing meaningful data regarding cellular responses to pharmacological agents [5]. The primary goal of this investigation is to determine the IC50 value of ATO in T24 cells, elucidating its inhibitory effects on cell growth, while concurrently examining the regulatory role of GPX4 in this process.

In conclusion, this study endeavors to contribute significantly to the existing body of knowledge regarding ATO's effects on bladder cancer, particularly in T24 cells. By elucidating the underlying mechanisms and potential therapeutic implications, it seeks to provide a foundation for further clinical exploration of ATO as a viable treatment option for bladder cancer patients. Such insights are essential for advancing therapeutic strategies that can improve outcomes for individuals diagnosed with this challenging malignancy [6].

## 2. Methods and Material

### 2.1. Cell Culture and Experimental Design

The human bladder cancer cell line T24 was obtained commercially from Procell Co., Ltd. Frozen cryovials were rapidly thawed in a 37 ℃ water bath (thawing time <60 seconds). Cell suspensions were maintained in RPMI-1640 supplemented with 10% fetal bovine serum and antibiotics (penicillin 100 U/ml, streptomycin 0.1 mg/ml), with incubation under standard conditions (37 ℃, 5% $CO_2$ humidified atmosphere). Subculturing was performed upon reaching 70-80% monolayer confluence. For experimental purposes, cells were allocated into two treatment groups: (1) untreated control and (2) arsenic trioxide (ATO)-treated group.

### 2.2. Cell Culture and CCK-8 Assay

T24 cells were seeded into 96-well plates at a density of $5 \times 10^3$ cells per well and cultured for 48 hours. After treatment with the designated compounds or controls, 10 μL of CCK-8 reagent (Dojindo Laboratories, Japan) was added to each well, followed by incubation at 37 ℃ for 1–4 hours. The optical density (OD) was measured at 450 nm using a microplate reader. Cell viability was calculated by normalizing the OD values of treated groups to those of untreated controls. All experiments were performed in triplicate.

### 2.3. Western Blot Analysis of GPX4 Expression

To assess GPX4 expression levels in ATO-treated T24 cells, Western blotting was performed. Briefly, protein lysates (20 μg per sample) were separated by 10% SDS-PAGE and subsequently transferred onto PVDF membranes (Millipore, USA). The membranes were blocked with 5% non-fat milk in TBST at room temperature for 1 h, followed by incubation with primary antibodies: rabbit anti-GPX4 (1:1000 dilution, Abcam, ab125066) and mouse anti-β-actin (1:5000 dilution, Sigma-Aldrich, A5441) as loading control overnight at 4 ℃. After washing three times with TBST (10 min per wash), membranes were incubated with HRP-conjugated goat anti-rabbit IgG secondary antibody (1:5000 dilution, Abcam, ab6721) for GPX4 detection or HRP-conjugated goat anti-mouse IgG (1:5000 dilution, Abcam, ab6789) for β-actin detection at room temperature for 1 h. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection system (Pierce, USA), and band intensities were quantified using ImageJ software (NIH, USA) with normalization to β-actin expression levels.

### 2.4. Statistical Analysis

All statistical analyses were conducted using SPSS 25.0 (IBM Corp., USA). Quantitative data are presented as mean ± SD. Differences among groups were analyzed by one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons. A P-value < 0.05 was considered statistically significant.

## 3. Results

### 3.1. CCK Assay to Detect the Inhibitory Effect of Different Concentrations of ATO on the Proliferation of T24 Cell and IC50

The results of the CCK Assay of T24 treatment with ATO shows that the concentration of ATO increases from 0.025 mg/L to 0.5 mg/L, and the degree of inhibition of cell proliferation is positively correlated with ATO concentration as shown in Figure 2. Through the growth inhibition trend, we conclude that the IC50 of T24 cells treated with ATO is 0.289 mg/L.

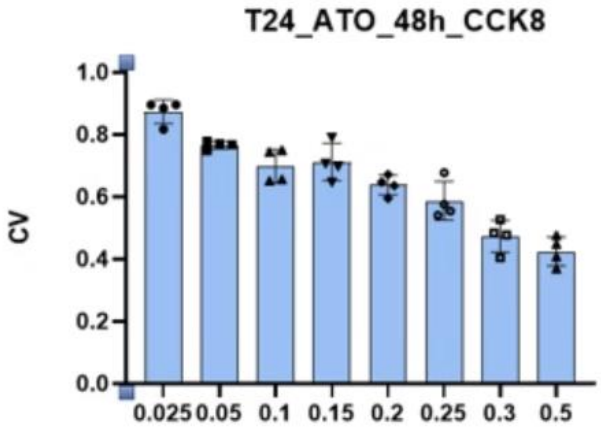


***Figure 1.*** *The inhibition of cell proliferation treated with ATO.*

### 3.2. Western Blot of GPX4 in Bladder Cancer T24 Cell Treated with ATO

Western blot results indicated that the expression of GPX4 of T24 cells decreased significantly 48 h after the treatment with ATO and nano-reaglar compared with negative control group ($P < 0.05$) as shown in Figure 2.

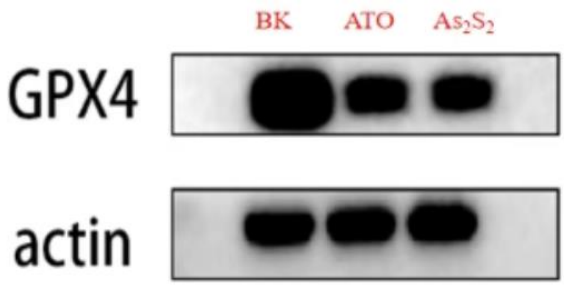


***Figure 2.*** *Effect of ATO and nano-reaglar on the expression of GPX4 in bladder cancer T24 cell.*

## 4. Discussion

Bladder cancer is a prominent urological malignancy characterized by high incidence and recurrence rates worldwide. It encompasses various histological subtypes, with transitional cell carcinoma being the most common, accounting for approximately 90% of cases. The disease poses significant challenges for effective treatment due to its multifactorial etiology and the development of resistance to conventional therapies. Current treatment modalities, including chemotherapy and immunotherapy, have shown limited efficacy, particularly in advanced stages of the disease, necessitating the exploration of novel therapeutic agents. Understanding the molecular mechanisms underlying bladder cancer proliferation and resistance is crucial for developing targeted therapies that can improve patient outcomes and reduce recurrence rates [7-9].

In this study, we investigate the antiproliferative effects of arsenic trioxide (ATO) on the bladder cancer cell line T24 and explore the underlying mechanisms, particularly focusing on the modulation of glutathione peroxidase 4 (GPX4) expression. Our research demonstrates that ATO significantly inhibits T24 cell proliferation, with an IC50 value identified at 0.289 mg/L. Additionally, ATO treatment results in a marked reduction in GPX4 expression, suggesting a potential link between oxidative stress regulation and cancer cell growth inhibition. These findings highlight ATO as a promising candidate for further exploration as a therapeutic agent in the management of bladder cancer, paving the way for future investigations into its clinical application and the elucidation of its precise molecular mechanisms.

The findings of this study indicate that arsenic trioxide (ATO) exerts a significant inhibitory effect on the proliferation of bladder cancer T24 cells, with an IC50 value determined at 0.289 mg/L. This result underscores the potential of ATO as a therapeutic agent for bladder cancer, given that it effectively suppresses cell growth at relatively low concentrations. The observed decrease in cell proliferation may be attributed to ATO's influence on critical cellular processes, particularly its modulation of the cell cycle and induction of apoptosis. It is plausible that ATO affects various signaling pathways, leading to G1/S phase arrest and enhancing apoptosis in T24 cells. Future investigations should focus on elucidating the specific molecular mechanisms underpinning these effects, including the role of oxidative stress and the potential involvement of apoptotic signaling pathways, as proposed in previous studies on other cancer cell lines [10, 5].

Additionally, this study reveals that ATO treatment results in a significant reduction in the expression of glutathione peroxidase 4 (GPX4) in T24 cells. GPX4 is a pivotal antioxidant enzyme that protects cells from oxidative damage, and its downregulation may compromise cellular redox homeostasis, thereby contributing to ATO-induced cytotoxicity. The interplay between GPX4 expression and cellular proliferation suggests that ATO may modulate the redox status of bladder

cancer cells, leading to increased susceptibility to oxidative stress and subsequent apoptosis. This finding aligns with literature indicating that GPX4 is a critical regulator of ferroptosis and other forms of cell death in cancer cells [3, 11, 12]. Thus, further research is warranted to explore the precise role of GPX4 in ATO's action and its potential as a therapeutic target in bladder cancer.

Moreover, the implications of ATO's action extend to its potential utility in overcoming chemoresistance in bladder cancer. As this malignancy is often characterized by its aggressive nature and propensity for recurrence, the identification of novel therapeutic strategies is crucial. The current study provides a foundation for future investigations into the combined use of ATO with existing chemotherapeutics, potentially enhancing the overall efficacy of treatment regimens. Understanding the synergistic effects of ATO with other agents could illuminate new avenues for bladder cancer therapy, particularly in cases where conventional treatments have failed [13, 14]. Therefore, this research not only contributes to the understanding of ATO's mechanisms of action but also highlights its potential as a promising candidate for clinical application in bladder cancer management.

This study has several limitations. First, the relatively small sample size and absence of clinical validation may restrict the broader applicability of our findings. Second, the lack of in vivo experiments limits the translational potential of our results from bench to bedside. Additionally, we did not assess potential off-target effects of ATO, which could influence its therapeutic efficacy and safety in bladder cancer treatment. Furthermore, our findings on GPX4 inhibition are based solely on T24 cells, and it remains unclear whether this mechanism extends to other bladder cancer cell lines or subtypes. Finally, inherent biological variability may introduce batch-to-batch discrepancies, affecting reproducibility. Future studies should expand to larger cohorts, vivo experiments, and diverse bladder cancer models to validate ATO’s specificity, efficacy, and safety.

In conclusion, our study demonstrates that ATO effectively inhibits the proliferation of bladder cancer T24 cells, likely through the modulation of GPX4 expression and subsequent alteration of the oxidative state within the cells. These findings not only provide insights into the molecular mechanisms underlying ATO's antitumor activity but also suggest its potential as a novel therapeutic agent for bladder cancer. Future research should focus on elucidating the clinical relevance of these findings and exploring the synergistic effects of ATO in combination with other therapeutic modalities.

## Abbreviations

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| ATO | Arsenic Trioxide |
| GPX4 | Glutathione Peroxidase 4 |
| CCK-8 | Cell Counting Kit-8 |

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## Author Contributions

Kewen Zheng is the sole author. The author read and approved the final manuscript.

## Conflicts of Interest

Non conflicts of interest.

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