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Original Research Article

Inhibitory effect and mechanism of action of tanshinone IIA on human bladder cancer cell J82

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Abstract

Purpose: *To investigate the therapeutic influence of tanshinone IIA on human bladder cancer cell J82, and the possible signal route involved.*
Methods: *Cell proliferative potential was measured using MTT assay, while the expressions of associated genes were determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and immunoblot assays.*
Results: *Tanshinone IIA decreased J82 cell survival rate by > 42 % and inactivated apoptosis by suppressing PI3K/AKT/mTOR signal route. Moreover, it decreased Bcl-2, but upregulated caspase and Bax ($p < 0.05$).*
Conclusion: *The inhibitory effect of TIIA on human bladder cancer suggests that TIIA can be developed into an anti-tumor agent.*

Keywords: *Tanshinone IIA, Human bladder cancer cell J82, Caspase-3*

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INTRODUCTION

Salvia miltiorrhiza is a rich source of the diterpenoid quinone tanshinone 11A (T11A) [1]. Studies have shown that T11A is effective in the management of various cerebrovascular and cardiovascular ailments [2–4]. Recent investigations have further revealed that tanshinones have antitumor [5], antioxidant [6], and anti-inflammatory properties [7]. Urothelial bladder carcinoma (UBC) is the most frequently occurring cancer of the urinogenital system [8]. It has been reported that the management of most UBCs involves surgery and intravesical chemotherapy [9]. Bladder carcinoma is the 6th most common malignancy in men, and the most frequent urologic cancer [10,11]. Recently, there has been a steady upsurge in cases of bladder carcinoma in China [11]. This study was focused on the anticarcinogenic influence of TIIA on human bladder cancer cell J82, and to unravel the possible underlying mechanism.

EXPERIMENTAL

Cell line, media and T11A

Tanshinone IIA (T11A) was purchased from AmyJet Scientific Company. Its purity was 98.6 %. Human bladder cancer cell J82 was kindly

obtained from Shanghai Huiying Biological Technology Co. Ltd. The J82 cells were maintained in RPMI-1640 containing 10 % FBS, streptomycin (1 %) and penicillin (1 %).

Cytotoxicity of tanshinone IIA

The test for cytotoxic effect of T11A on J82 cells was done using MTT method. The cells were seeded in a 96-well plate at a density of 4×10^3 cells/well, and were exposed for 48 h to graded doses of T11A ranging from 10 to 80 µg/mL). Thereafter, MTT (500 µg/mL) was added to each well, followed by incubation at 37 °C for 4 h. Then, the MTT was replaced with 120 µL DMSO. The absorbance of the formazan solution was measured spectrophotometrically at 570 nm, and the values obtained were used for computing the inhibition of cell growth.

Immunoblotting

Following lysis of the cells with protease suppressor-containing RIPA buffer, the protein content of the lysate was assayed with BCA method. Then, 30 µg protein samples were resolved on SDS-PAGE, followed by transfer to PVDF which was subsequently blocked by incubation for 1 h with 5 % de-fatted milk solution in TBST. This was followed by incubation of the membrane overnight with 1º antibodies for p-AKT, p-PI3K, and p-mTOR at 4 °C.

Then, the membrane was rinsed two times with TBST, followed by incubation for 1 h with HRP-conjugated 2º antibodies at laboratory temperature. Visualization of the protein bands was achieved with ECL kit, while the relative abundance of expressions were determined with TM Imager 600 System, with β-actin as internal control.

qRT-PCR

Total RNA was extracted from tumor tissues using Trizol reagent, and the RNA contents were determined spectrophotometrically at 260 nm. Following treatment of the extracted RNA with DNAase, it was reverse-transcribed to cDNA with PrimeScript™ Reverse Transcription kit bearing gDNA Eraser kit (Takara, China).

The cDNA product was used as template for quantitative PCR which was carried out using ABI 7500 RT-PCR system. The sequence of primers used are indicated in Table 1. The relative mRNA expressions were calculated with the $2^{-\Delta\Delta}$CT procedure, with β-actin as reference gene.

Table 1: Primer sequences used for real-time quantitative polymerase chain reaction

Gene	Forward (5 '–3 ')	Reverse (5–3')
Bcl-2	GGTGGGGTCATGT GTGTGG	CGGTTCAGGTACT CAGTCATCC
Bax	CCCGAGAGGTCTT TTTCCGAG	CCAGCCCATGATG GTTCTGAT
Casp ase-3	CATGGAAGCGAAT CAATGGACT	CTGTACCAGACCG AGATGTCA
β-actin	AGAAAATCTGGCA CCACACC	TAGCACAGCCTGG ATAGCAA

Statistical analysis

Results are presented as SD. Statistical analysis was done with ANOVA and thereafter with Dunnett's test using GraphPad Prism ver 6.0 software. Values of $p < 0.05$ were assumed to be statistically significant.

RESULTS

Inhibitory influence of Tanshinone IIA on proliferative potential of J82 cells

Treatment with T11A markedly suppressed J82 cell growth in a dose-based fashion, with 80 µg/ml Tanshinone IIA producing about 56.79 % growth inhibition (Figure 1).

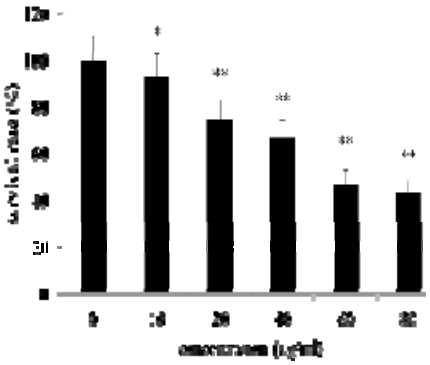

Figure 1: Effect of TIIA on J82 cell survival (%); *p < 0.05; **p < 0.01, vs control

Influence of tanshinone IIA on PI3K/AKT/mTOR signal route in J82 cells

The effect of tanshinone IIA on the PI3K/AKT/mTOR pathway in J82 cells was studied. As presented in Figure 2, the ratios PI3K/Actin, p-AKT/Actin, and p-mTOR/actin were reduced in J82 cells after treatment with TIIA at doses of 40, 80 µg/mL for 48 h, indicating that TIIA inactivated the PI3K/AKT/mTOR route in J82 cells.

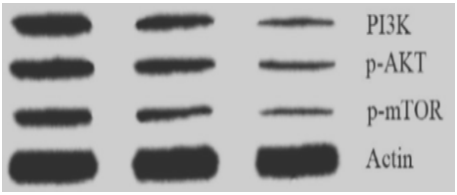

Figure 2: Effect of Tanshinone IIA on protein expressions of P-AKT, PI3K and p-mTOR

Effect of tanshinone IIA on apoptotic genes in J82 cells

Real-time qPCR was used to validate the outcome of MTT experiments. The results indicated that, relative to control, the T11A-treated cells had reduced Bcl-2 concentration and increased amounts of Bax and caspase-3. In addition, the effect of T11A was dose-dependent (Figure 3).

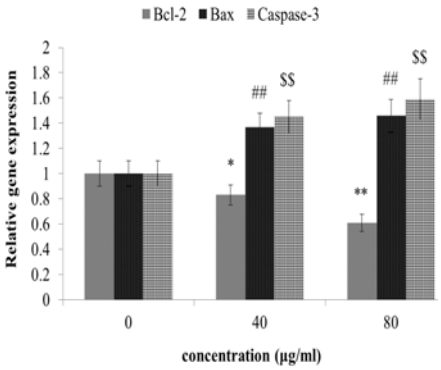

Figure 3: Effect of T11A on mRNA expressions of Bacl-2, Bax and caspase-3; $^{*}p < 0.05$; $^{**, \#\#, \$\$}p < 0.01$, vs control

DISCUSSION

Globally, bladder carcinoma accounts for the 7th most frequent malignancy in males, and the 17th most occurring carcinoma in females [12]. The disease affects various tissues of the urinary bladder. More than 70 % of fresh cases of bladder carcinoma are non-invasive, with high frequency of recurrence, notwithstanding localized treatment [12]. In addition, in spite of systemic treatment, the prognosis of about 25 % of new cases with metastasis to the muscle, is very poor [13]. However, not much is known about the etiology of bladder carcinoma.

Over the years, several studies on the phytochemical components of *S. miltiorrhiza* have led to the isolation and identification of more than 80 compounds, some of which comprise lipophilic diterpenoids [14]. The predominant lipophilic compounds are TIIA, TIIB, TI, and cryptotanshinone, with TIIA being the most prevalent [15].

Tanshinone 11A (T11A) exerts several pharmacological effects such as suppression of platelet aggregation, inhibition of inflammation, antioxidation and anti-carcinogenic properties [16,17]. In this study, T11A markedly reduced the viability of J82 cells.

The PI3K/AKT/mTOR signal route is involved in suppression of apoptosis [18]. In this pathway, P13K activates AKT through phosphorylation, thereby converting it to p-AKT [19]. In turn, p-AKT activates a series of downstream factors, including mTOR [20]. Through mTOR, p-AKT controls proliferative potential and growth of cells.
In this investigation, Tanshinone IIA markedly downregulated mTOR, AKT and P13K, as well as their activated forms, thereby suppressing the PI3K/AKT/mTOR signaling route. Thus, caspase-3 and Bax were upregulated, while Bcl-2 was suppressed, leading to cell death.

CONCLUSION

The present study has presented evidence that tanshinone IIA acts as an anti-tumor drug in bladder cancer *in vitro*. It IIA kills tumour cell J82 probably by suppressing the PI3K/AKT/mTOR signal route. This new finding on the inhibitory effect of tanshinone IIA suggests that it is a potential therapeutic strategy against bladder cancer.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Xianjun Zhang, Ziwen Lu designed all the experiments and revised the paper. Songzhe Piao and Tao Hong wrote the manuscript.

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