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Effect of salinomycin on metastasis and invasion of bladder cancer cell line T24

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ABSTRACT

Objective: To explore the effect of salinomycin on the metastasis and invasion of bladder cancer cell line T24 by regulating the related protein expression in the process of epithelial–mesenchymal transition (EMT), and to provide experimental basis for the treatment of urological tumors.

Methods: The bladder cancer cell line T24 was cultured *in vitro*. The rat bladder tumor model was established *in vivo*. The rats were randomized into two groups, among which the rats in the experiment group were given intraperitoneal injection of salinomycin, while the rats in the control group were given intraperitoneal injection of normal saline. The change of tumor cells in the two groups was observed. Transwell was used to detect the cell migration and invasion abilities, Real-time PCR was used to detect the expression of mRNA, while Western-blot was utilized for the determination of the expressions of E-cadherin and vimentin proteins.

Results: The metastasis and invasion abilities of serum bladder cancer cell line T24 after salinomycin treatment in the experiment group were significantly reduced when compared with those in the control group, and the tumor metastasis lesions were decreased from an average of 1.59 to 0.6 ($P < 0.05$). T24 cell proliferation in the experiment group was gradually decreasing. T24 cell proliferation at 48 h was significantly lower than that at 12 h and 24 h ($P < 0.05$). T24 cell proliferation at 24 h was significantly lower than that at 12 h ($P < 0.05$). T24 cell proliferation at each timing point in the experiment group was significantly lower than that in the control group ($P < 0.05$). The serum mRNA level and E-cadherin expression in the tumor tissues in the experiment group were significantly higher than those in the control group, while vimentin expression level was significantly lower than that in the control group ($P < 0.05$).

Conclusions: Salinomycin can suppress the metastasis and invasion of bladder cancer cells, of which the mechanism is probably associated with the inhibition of EMT of tumor cells.

1. Introduction

The invasion and metastasis of tumor cells are the basic characteristics of malignant tumors. Along with the progression of the disease, the cell adhesive capability is weakening, while the invasion ability of tumor cells in gradually increasing. The metastasis and invasion of tumor cells is a multi-factor, multi-stage, and multi-step process [1,2]. Experiments demonstrate that [3] the invasion and metastasis of tumor cells are closely associated with the process of epithelial–mesenchymal transition (EMT). EMT plays a vital role in embryonic development, tissue reconstruction, chronic inflammation, and various fibrosis diseases with a main characteristic of increasing vimentin expression and decreasing E-cadherin expression. E-cadherin is a member of calcium adhesin superfamily, and its function activity and expression intensity have a direct action on the separation and reattachment of cells. Vimentin is a kind of protein of intermediate filaments, can maintain the cell shape and the integrity of cytoplasm, and

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its abnormal expression will produce an important effect on the biological properties of cells [4].

Bladder cancer is a kind of urological tumor with a relatively high morbidity and recurrence metastasis rate. The early treatment for bladder cancer is mainly adopting local lesion resection, but postoperative metastasis occurs in 60% patients, 20% patients are progressed to the middle and advanced stage; moreover, the five-year survival rate is less than 50% [5]. Therefore, developing a new kind of anti-tumor drug is becoming a medical research emphasis currently. With the development of biopharmaceutical technology, a large amount of new antibiotics with unique molecular structures are constantly developing. In recent years, there are constant researches on salinomycin in inhibiting the metastasis and invasion of tumor cells, but there are fewer researches on salinomycin in inhibiting bladder tumor cells. In order to deeply comprehend that the salinomycin can inhibit the tumor cells by regulating the EMT process, a bladder cancer rat model is established in the experiment, and the rats were injected with salinomycin to observe the change of E-cadherin and vimentin expressions and the effect on the metastasis and invasion of bladder transitional cancer cell line T24.

2. Materials and methods

2.1. Materials

The human bladder transitional cancer cell line T24 was provided by the Experimental Center of Zhongshan University. Salinomycin was purchased from Sigma Aldrich. Transwell and matrigel were purchased from BD Biosciences. DMEM, DMSO, pancreatin, and fetal bovine serum were purchased from Gibco. Anti-mouse vimentin and E-cadherin were provided by Santa Cruz Biotechnology. TRIzol was purchased from *in vitro*-gen. PCR kits were purchased from TaKaRa. A total of 10 male rats weighing (6–10 g) were raised in SPF laminar flow cabinet of the Affiliated Hospital of Zhongshan University. All the manipulations abided by the related provisions of Experimental Animal Committee of Guangdong Province. The salinomycin (50 mM) used in the experiment was a mixed preparation of salinomycin and DMSO.

2.2. Methods

2.2.1. Cell culture and passage

T24 was taken from the liquid nitrogen container and quickly placed in the water bath at 37 °C for unfreezing. The nutrient solution (10 mL) was absorbed, added to the centrifuge tube, and centrifuged at 1000 r/min for 5 min. After T 24 was unfreezing, the thawing fluid was sucked, inoculated in DMEM nutrient solution containing 10% fetal bovine serum, placed in an incubator at 37 °C with a saturation humidity of 5% CO_2 for cultivation. The nutrient solution was changed every other day. The cell growth was observed under a microscope. The cells were sub-cultured using 0.25% pancreatin and 0.02% EDTA. Cells in a logarithmic phase were used for the experiment.

2.2.2. Animal model preparation and grouping

A total of 10 male rats were used in the experiment. After a routine anesthesia, the abdomen was opened. After a resuspension of high glucose medium not containing serum DMEM, and matrigel, the bladder transitional cancer cell line T24 was inoculated in the parenchyma of bladder in rats, and then the abdomen was sutured. After operation, the rats were randomized into the experiment group and the control group with five in each group. After operation, the rats in the experiment group were immediately given intraperitoneal injection of salinomycin with a dosage of 8 mg/kg, while the rats in the control group were given intraperitoneal injection of normal saline. A close observation was paid during the drug administration period. After 15 d, the rats were sacrificed by cervical dislocation, and the complete tumor tissues were stripped to observe the tumor growth and metastasis.

2.2.3. Detection of cell migration and invasion abilities by Transwell

Rat cells in each group were extracted by Trizol and inoculated in a six pore plate. The cell density was adjusted to 5×10^6/mL. A volume of 200 μL cells were taken and placed in Transwell chamber containing 500 μL 20% serum medium for cultivation. After 36 h cultivation, the medium was sucked, and the cells in the upper chamber were removed, fixed with 4% paraformaldehyde, stained with crystal violet for 15–30 min, and washed three times with PBS. The cells were observed under a microscope and recorded. During the invasion assay, 30 μL matrigel was added to the upper chamber, and the rest procedures were the same as the migration experiment.

2.2.4. Detection of mRNA expression by real-time PCR

RNA was extracted in strict accordance with the Omniscript RT kit instruction, and reversely transcribed to obtain cDNA. PCR was used to analyze the expressions of E-cadherin and vimentin. β-actin was used as an internal control. The primer sequences were as the following: vimentin upper stream primer: ATTTTCCCCTCGACAGCCGAT, vimentin down stream primer: TCCCAGGCGTAGACCAATA, E-cadherin upper stream primer: AGTCCACTGAGTAGCGCAGAC, E-cadherin down stream primer: CATTTCACGCATCTGGGGTTC. PCR reaction conditions were as the following: 95 °C 30 s, 95 °C 5 s, 60 °C 45 s, a total of 42 circulations. $2^{-\Delta\Delta Ct}$ was used to analyze the relative intensity of mRNA. The experiment was repeated three times and the average values were taken.

2.2.5. Determination of E-cadherin and vimentin expressions by western blot

RIPA lysate was used to provide proteins in the adherent cells. RIPA lysate and protease inhibitor were prepared according to a ratio of 100:1. After a lysis, the cells were placed on the ice for 1 h, vibratingly dissociated every 20 min, and centrifuged at 4 °C 12000 r/10 min. The supernatant was extracted, and western blot was used to determine the E-cadherin and vimentin expressions.

2.3. Statistical analysis

SPSS 17.0 software was used for statistical analysis. The measurement data were expressed as mean ± SD and t test was used. $P < 0.05$ was regarded as statistically significant difference.