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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.

Table 1
Comparison of the cell proliferation between the two groups (OD value).

Groups	*n*	12 h	24 h	48 h
Experiment group	5	0.22 ± 0.01*	0.15 ± 0.03*	0.09 ± 0.01*
Control group	5	1.14 ± 0.05	1.07 ± 0.02	1.09 ± 0.03

*$P < 0.05$, when compared with the control group.

3. Results

3.1. Effect of salinomycin on T24 metastasis and invasion

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 ($P < 0.05$). No obvious change of T24 cell proliferation at each timing point in the control group ($P > 0.05$). T24 cell proliferation at each timing point in the experiment group was significantly lower than that in the control group ($P < 0.05$) (Table 1).

3.2. Histological observation

The microscopic results showed that in the study group, at the time of 12 h, the number of T24 cell was decreased, the adherent ability was reduced, partial cells turned to an oval or circle shape, the transparency of the cytoplasm was reduced, and the number of cell nucleus was decreased; at the time of 24 h, a small part of T24 cells lost a normal shape, turning a circle shape, the number was significantly reduced, partial cell debris could be seen or there was an occurrence of irregular sprouting; at the time of 48 h, a large part of T24 cells lost a normal shape, a large amount of cells were floating, more cell debris, cell nucleus pycnosis, and cell membrane lysis could be seen. In the control group, at the time of 12 h, T24 cells entered a logarithmic phase, the number of cells was increased with a tight arrangement, some mitotic figure could be seen, the cells could grew well in the following timing point (Figure 1).

3.3. Effect of salinomycin on EMT process

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 ($P < 0.05$) (Figure 2).

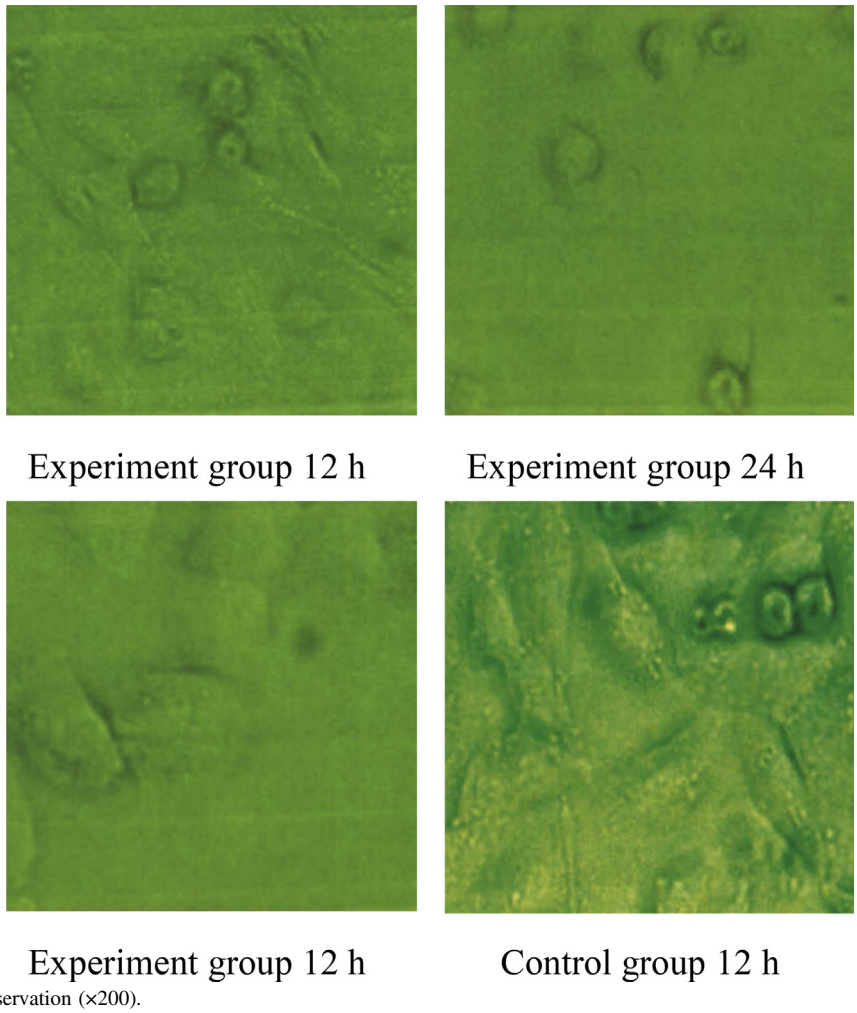

Figure 1. Histological observation (×200).

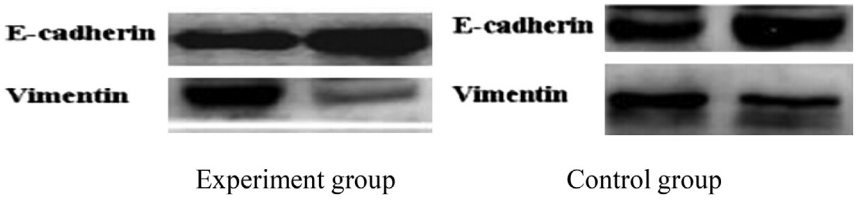

Figure 2. Determination of E-cadherin and vimentin expressions by Western blotting.

4. Discussion

Tumor is a neoplasm formed in an abnormal hyperplasia of monoclonicity arising from a lost regulation to the cell growth in a gene level under the effect of various factors [6]. The invasion and metastasis of tumor cells are a complicated process, i.e., cancer cells are separating from the primary tumor site, and establish a metastasis lesion in a remote place [7]. The typical metastasis process of cancer cells is that a remote metastasis is achieved by the lost cell growth inhibition ability and the degradation of extracelluar matrix components through altering the cell adhesive force and the activity of cell proliferation related enzymes [8].

Bladder cancer is characterized by a high recurrence rate, strong cell invasion ability, and a high mortality. Surgical operation is a main method to treat non invasive bladder cancer. A perfusion of chemotherapy or immunotherapy drugs to the bladder cavity is given according to the tumor staging and pathological grading after operation [9]. Chemotherapy can effectively reduce the recurrence rate of bladder cancer, but the chemotherapeutics are toxic and may pose some damage to the body immune functions. Patients will abandon the chemotherapy mostly due to intolerance, resulting in an unsatisfactory long-term efficacy [10]. Therefore, exploring a new method to treat bladder cancer is an urgent tissue required to be resolved in the clinic. With the development of molecular biology and genomics, new anticancer drugs are constantly emerging.

Salinomycin is a kind of monocarboxylic acid polyether type antibiotics, produced by the fermentation of Streptomyces albus, possesses a specific cyclic structure, and can form a complex compound with the pathogenic microorganisms and the extracellular cations of coccidian, especially K^+, Na^+, Rb^+, to alter the intracellular and extracellular ion concentrations. The complex compound is transported to the cells by the bacteria or transport protein to destroy the intracellular and extracellular ion balance, leading to an inactivation of cells [16–20]. Foreign researches demonstrated that salinomycin can inhibit the metastasis and invasion of breast cancer cells in rats, and induce the cell apoptosis [11]. Guo *et al.* reported that [12] salinomycin *in vivo* and *in vitro* can inhibit the expression of Wnt/β-catenin in the liver cancer cells. Mao *et al.* found that [13] salinomycin can inhibit the invasion and metastasis of gastric cancer cells. Antoszczak found that [14] salinomycin can inhibit the growth of osteogenic sarcoma cells. Calzolari *et al.* verified that [15] salinomycin can up regulate the E-cadherin expression in colon cancer cells. In the study, the metastasis and invasion abilities of serum bladder cancer cell line T24 in the experiment group was significantly reduced ($P < 0.05$), T24 cell proliferation was gradually decreasing, and T24 cell proliferation in each timing point was significantly lower than that in the control group ($P < 0.05$). The histological observation results showed that in the experiment group, salinomycin can significantly promote the apoptosis of bladder cancer cell line T24 and inhibit the proliferation, suggesting that salinomycin has a significant inhibition effect on T24 cell proliferation. Moreover, 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 ($P < 0.05$), showing that salinomycin can suppress EMT process through up regulating E-cadherin expression and down regulating vimentin expression in order to inhibit the metastasis and invasion of tumor cells.

The results in the study showed that the alterations of E-cadherin and vimentin expressions in the tumor cells are probably the main mechanism for changing the metastasis and invasion abilities of tumor cells. Salinomycin can suppress the expressions of E-cadherin and vimentin in the bladder cancer cells and has a significant inhibition effect on the metastasis and invasion of bladder cancer cells.

Conflict of interest statement

We declare that we have no conflict of interest.

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