

Tsa Inhibits the Migration and Invasion of Mda-Mb-231 Cells through Mapk / P38 Signaling Pathway

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Abstract: objective: to investigate the effect of trichostatin a (tsa) on the migration and invasion of breast cancer mda-mb-231 and its possible molecular mechanism. Methods: mda-mb-231 cells were cultured in vitro until the logarithmic growth period, and treated with tsa at different concentrations of 20, 40, 80 and 160 nmol / l for 72 hours. Cell viability was detected by cck-8, apoptosis was detected by flow cytometry, migration and invasion were detected by scratch test and transwell cell. Western blotting was used to detect the protein levels of mmp-2, mmp-9, p-erk, p-jnk and p38 mapk. Results: tsa could inhibit the activity of mda-mb-231 cells in a dose-dependent manner ($p < 0.05$); tsa could inhibit the migration and invasion of mda-mb-231 cells in a dose-dependent manner ($p < 0.05$). Western blotting results showed that tsa down-regulated mmp-2 and mmp-9 protein expression levels, and up-regulated p-p38 mapk and e-cadherin protein expression levels ($p < 0.05$). Tsa had no significant effect on p-erk and p-jnk protein expression levels, and the difference was not statistically significant ($p > 0.05$). After p38 mapk inhibitor sb203580 combined with tsa, the cell scratch healing rate increased and the proliferation activity increased with the increase of mmp-2 and mmp-9 protein expression levels, and the decrease of e-cadherin protein expression levels. (<0.05). Conclusion: tsa can migrate and invade breast cancer mda-mb-231 cells. It down-regulates mapk / p38 signaling pathway, mmp-2 and mmp-9 protein expression, and up-regulates e-cadherin protein expression.

1. Introduction

Breast cancer is the most common malignant tumor in women worldwide and the leading cause of death from women's cancer diseases. According to statistics, there were more than 2 million new cases in 2018, accounting for about a quarter of the total cancers in women [1]. The occurrence of breast cancer is closely related to sex hormone level, abnormal regulation of transcription factors, abnormal regulation of cell proliferation and apoptosis and many other factors [2].

Trichostatin a (tsa), which belongs to hydroxamic acids, is one of the most effective histone deacetylase inhibitors (hdacis). Previous studies have shown that tsa can inhibit the proliferation and induce apoptosis of a variety of cancer cells, such as lung cancer, colorectal cancer, gastric cancer and breast cancer, so it has become a potential anti-tumor drug. However, there are few reports on the effect of tsa on the migration and invasion of gastric cancer cells. In this study, mda-mb-231 cells of breast cancer were cultured in vitro. Cck8 detection, flow cytometry and western blot were used to explore the effect of tsa on the migration and invasion of mda-mb-231 cells and its molecular mechanism of action, so as to provide theoretical basis for the search and development of new treatment methods for breast cancer.

2. Materials and Methods

2.1 Material

MDA-MB-231 of breast cancer was purchased from Shanghai cell bank of Chinese Academy of Sciences. Inhibitor sp203580 was purchased from CSN. DMEM high glucose medium, fetal bovine serum and trypsin were purchased from GIBCO. Ripa lysate and BCA protein concentration

determination kit were purchased from biyuntian Biotechnology Co., Ltd. Trichostatin A, MTT, DMSO and DAPI were purchased from sigma company. Cell cycle staining solution and annexin V / PI cell apoptosis detection kit were purchased from BD company. E- E-cadherin, matrix metalloproteinase (MMP) -2, MMP-9. Phospho-MAPK family antibody kit, β -actin monoclonal antibody, and HRP-labeled goat anti-rabbit IgG were purchased from Abcam. Horseradish peroxidase labeled goat anti-rabbit secondary antibody, horseradish peroxidase labeled goat anti-mouse secondary antibody, and ECL developing solution were purchased from BIO-RAD Company. Cell culture dishes, 6-well plates, 96-well plates, etc. were purchased from Corning, USA. PBS and absolute ethanol were purchased from Beijing Tiangen Biochemical Technology Co., Ltd.

2.2 Cell Culture and Processing

MDA-MB-231 cells of breast cancer were cultured in DMEM medium containing 10% fetal bovine serum. The cell culture dish was placed in 37 °C and 5% CO₂ incubator, and the medium was changed every 2 days. The TSA powder was dissolved in dimethyl sulfoxide to prepare a stock solution with an initial concentration of 10 mmol / L, and the medium was diluted to a final concentration of 0.1, 1, and 10 μ mol / L according to the grouping for subsequent experiments.

2.3 Cck-8 Experiment

Cells in the culture dish were trypsinized and resuspended in complete medium. The cell suspension was seeded at a concentration of 5×10^3 cells / well in a 96-well plate with 3 replicates in each group, and 0.1, 1 and 10 μ mol / L TSA were added the next day. After 24 hours, CCK-8 reagent was added to the cell culture solution. , Incubate for 2h in the incubator, measure the absorbance at 450nm. The experiment was repeated three times.

2.4 Cell Scratch Test

The cells treated with 0, 10 μ mol / L TSA for 24 h were collected and seeded in 6-well plates at a concentration of 5×10^5 cells / well. After the cells are adhered to the wall, first use a marker to draw a horizontal line evenly on the back of the 6-well plate, and then use a disinfection pipette to scratch the culture line perpendicular to the horizontal line. PBS was rinsed to remove the scratched cells. Then add serum-free medium, culture for 24 hours, observe under inverted microscope ($\times 100$), take photos of scratch healing at 0 and 24 hours, and analyze the scratch healing rate with Image J software. The experiment was repeated three times.

2.5 Cell Invasion Experiment

The Matrigel was diluted with 4 °C precooled serum-free DMEM medium, and 100 μ l diluted Matrigel was added into Transwell chamber, and incubated in cell incubator for 4H. The cells treated with 0 and 10 μ mol / L TSA for 24 hours were resuspended in serum-free DMEM, and the cell concentration was adjusted to 2×10^5 cells / mL. 200 μ l of the cell suspension was added to the upper chamber of the cell, and 500 μ l of a medium containing 10% fetal calf serum was added to the lower chamber. After 24 hours of incubation, the Matrigel gel and non-membrane cells in the upper chamber were wiped off with a cotton swab, fixed with paraformaldehyde for 30 min, washed 3 times with PBS, and the nuclei were stained with DAPI staining solution. Observe the picture under an inverted fluorescence microscope at 100x and count the number of cells in each field. The experiment was repeated three times.

2.6 Western Blotting

The cells in the petri dish were digested with trypsin, the complete medium was resuspended, collected by centrifugation in an EP tube, and the total protein of the cells was extracted using a protein extraction kit. Adjust the protein concentration, fix the amount of protein per well and perform SDS-PAGE electrophoresis to transfer the target protein to the PVDF membrane. 1H was isolated from TBST solution containing 5% serum albumin and incubated overnight at 4 degrees (dilution was 1: 500). After two incubation, the ECL chemiluminescence droplet was loaded onto the PVDF membrane after 2h, and the image was exposed by gel imaging system. Image J software

is used to analyze the gray value of the target strip. The experiment was repeated three times.

2.7 Statistical Analysis

SPSS13.0 statistical software was used to analyze the data. The data of this study were expressed as mean \pm standard deviation, and single factor analysis of variance was used in multiple comparisons, and t-test was used in two comparisons. The difference was statistically significant ($P < 0.05$).

3. Result

3.1 Effect of Tsa on the Activity of Mda-Mb-231 Cells

The results of CCK-8 showed that in the experimental group, MDA-MB-231 cells were treated with TSA of 20, 40, 80 and 160 nmol / L respectively for 48 hours. Compared with the control group (0 nmol / L), the cell viability in the 80 nmol / L and 160 nmol / LTSA groups decreased significantly ($P < 0.05$). This indicated that TSA could inhibit the activity of MDA-MB-231 cells (Figure 1).

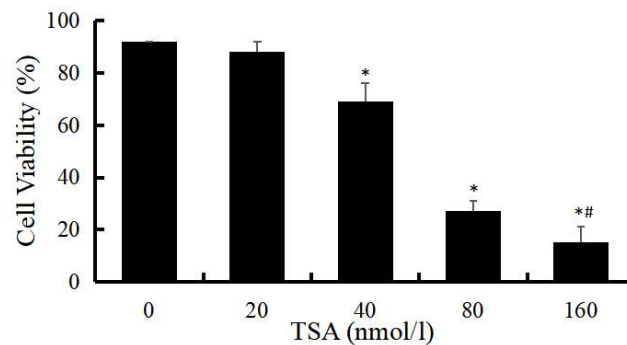


Fig.1 Effect of Tsa on the Cell Viability of Mda-Mb-231 Cells, Compared with Control Group,* $P < 0.05$, Compared with 80nmol / l Tsa Group,# $P < 0.05$

3.2 Effects of Tsa on the Migration and Invasion of Breast Cancer Mda-Mb-231 Cells

The results of Transwell experiment showed that in the experimental group, MDA-MB-231 cells were treated with TSA of 80 and 160 nmol / L for 48 hours, compared with the control group, the migration rate and invasion rate of the experimental group were significantly reduced ($P < 0.05$), and decreased with the increase of TSA concentration ($P < 0.05$). This indicates that TSA can inhibit the migration and invasion ability of breast cancer MDA-MB-231 cells in a dose-dependent manner (Figure 2).

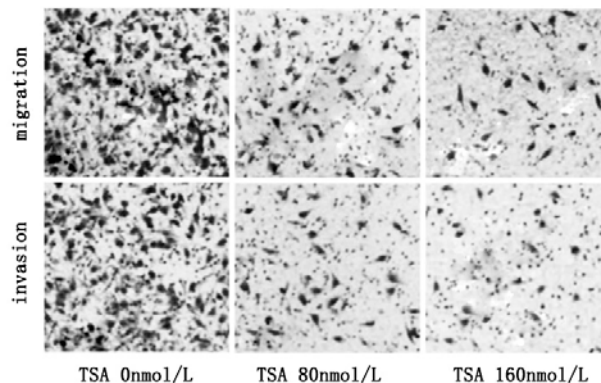


Fig.2 Effect of Tsa on Migration and Invasion of Mda-Mb-231 Cells

3.3 Effects of Tsa on e-Cadherin, Mmp-2, Mmp-9, P-P38 Mapk, P-Erk, P-Jnk Protein Expression in Breast Cancer Mda-Mb-231 Cells

The results of Western blot showed that, in the experimental group, the breast cancer MDA-MB-

231 cells were treated with 160 nmol / L of TSA for 48 hours, compared with the control group (0 nmol / L). The protein expression level was significantly reduced. However, the expression levels of p-p38 MAPK and E-cadherin were significantly higher ($P < 0.05$). TSA had no significant effect on the expression of p-ERK and p-JNK ($P > 0.05$). This suggests that TSA may down regulate MMP-2, MMP-9 and MMP-9, and up regulate the expression of E-cadherin protein through p38 / MAPK signaling pathway to inhibit cell migration and invasion (Figure 3).

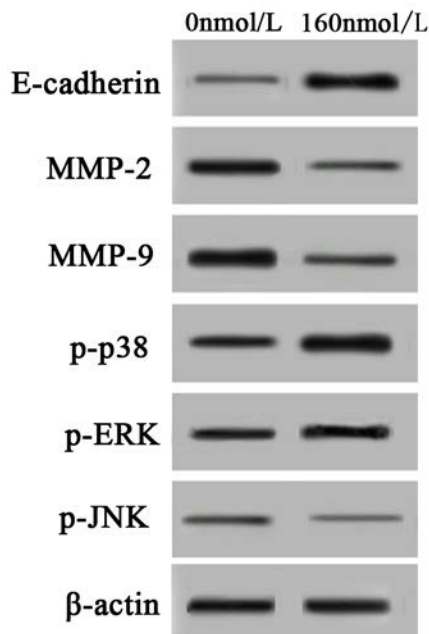


Fig.3 Effect of Tsa on Protein Levels of e-Cadherin, Mmp-2, Mmp-9, P-P38 Mapk, P-Erk and P-Jnk in Mda-Mb-231 Cells.

3.4 Inhibition of P38 / Mapk Signaling Pathway to Reverse the Inhibitory Effect of Tsa on the Migration and Invasion of Mda-Mb-231 Cells

To verify whether TSA mediates the migration and invasion of breast cancer cells through JNK / MAPK signaling pathway, small molecule inhibitor sb2035805 was used to inhibit JNK pathway. The experiment was divided into four groups: control group (TSA 0 μ mol /). TSA 160nmol / l group. Sp600125. TSA 160nmol / L + SB203580 group. The results showed that compared with TSA 160nmol / l group, cell activity, migration rate and invasion rate of TSA 160nmol / L + SB203580 group increased significantly ($P > 0.05$), MMP-2, MMP-9 and protein expression level increased significantly. The expression levels of p-p38 MAPK and E-cadherin proteins were significantly reduced, and the differences were statistically significant ($P < 0.05$). TSA had no significant effect on p-ERK and p-JNK protein expression levels, and the difference was not statistically significant ($P > 0.05$). This indicates that TSA mediates the migration and invasion of breast cancer MDA-MB-231 cells through the P38 / MAPK signaling pathway.

4. Discussion

HDACi is a new type of anti-tumor drug, which mainly exerts its activity by inhibiting histone deacetylase in the body. HDACi is a new type of anti-tumor drug that has been paid attention to in recent years, with strong targeting and low toxicity, which has obvious advantages over traditional cytotoxic drugs. TSA mainly inhibits HDAC enzyme activity and increases the expression of tumor suppressor genes through its hydroxamic acid chelating zinc ions at the HDAC activation site, and induces cell cycle arrest, cell differentiation and apoptosis. However, the effect of TSA on breast cancer cell migration and invasion and its mechanism are not clear. This study found that TSA inhibited human gastric cancer SGC-7901 in a dose-dependent manner at 20-160nmol / L to inhibit cell viability, migration, and invasion.

Epithelial-mesenchymal transition (EMT) has been widely studied in cancer and is considered to

play a key role in tumor invasion and metastasis. The down-regulation or deletion of E-cadherin expression is the main sign of the occurrence of EMT. E-cadherin is a calcium-dependent transmembrane glycoprotein that mediates cell-to-cell adhesion and can bind to Ca^{2+} to mediate cell-to-cell adhesion. It can also form a complex between catenin and actin and anchor it to the cytoskeleton to form a stable connection with adjacent cells. Therefore, E-cadherin plays an important role in maintaining intercellular connections and epithelial cell polarity. Extracellular matrix and basement membrane are important tissue barriers to limit tumor invasion and metastasis. Tumor cells can degrade extracellular matrix and basement membrane by secreting a variety of MMPs to invade peripheral tissues and blood vessels. A large number of studies have confirmed that down regulating the expression of MMP-2 or MMP-9 can inhibit the migration and invasion of cancer cells. In this study, TSA can inhibit the expression of MMP-2 and MMP-9 in MDA-MB-231 cells, and up regulate the expression of E-cadherin, which may be one of the important mechanisms of TSA inhibiting the migration and invasion of MDA-MB-231 cells.

MAPK signaling pathway can participate in the regulation of apoptosis, proliferation and migration, but whether TSA can inhibit the proliferation and migration of MDA-MB-231 cells through MAPK pathway has not been reported. In this study, 160 μ mol / L TSA significantly increased the phosphorylation level of p38 in breast cancer MDA-MB-231 cells, in order to further confirm whether the inhibition of TSA on the malignant biological behavior of breast cancer MDA-MB-231 cells depends on p38 MAPK signaling pathway. In this study, p38 MAPK inhibitor SB203580 was used to stem p38 phosphorylation. The results showed that down-regulation of p-p38 could reverse TSA's inhibition of breast cancer MDA-MB-231 cell activity and invasion and migration, suggesting that TSA may inhibit breast cancer MDA-MB-231 cell invasion by regulating p38 phosphorylation And migration.

5. Conclusion

In conclusion, this study preliminarily confirmed that TSA (160nmol/L) can inhibit the proliferation, migration and invasion of breast cancer 4T1 cells in vitro, and its mechanism may be related to TSA promoting the phosphorylation of p38 and down regulating the protein expression of MMP-2 and MMP-9, which provides the experimental basis and basis for the clinical anti-tumor application of TSA.

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