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The Effect of Fisetin on Human Triple Negative Breast Cancer Cell Line MDA-MB-231

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Abstract: This study selected Fisetin as an intervention drug, and took human triple negative breast cancer (TNBC) cell line MDA-MB-231 as the experimental object to explore the effects of Fisetin on the proliferation, migration and invasion of MDA-MB-231 cells. The effect of Fisetin on cell activity was determined by CCK8 assay and the drug concentration was determined. The effect of Fisetin on cell proliferation ability was determined by EDU staining and plate clone formation assay. The effect of Fisetin on cell migration and invasion was determined by Transwell assay. Through CCK8, EDU staining, and plate clone formation experiments, we found that the vitality and proliferation ability of MDA-MB-231 cells were significantly weakened. Through Transwell experiments, we found that the migration and invasion ability of MDA-MB-231 cells significantly decreased. Therefore, Fisetin can effectively inhibit the proliferation, migration, and invasion of MDA-MB-231 cells.

1. Introduction

Triple negative breast cancer (TNBC) is a subtype of breast cancer. It lacks the expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor 2 (HER2). It has a highly invasive, uncontrolled proliferation phenotype and poor clinical prognosis. Surgery combined with postoperative chemotherapy can effectively reduce the recurrence and mortality rates of TNBC. However, the treatment outcomes of TNBC are still unsatisfactory. To improve the survival rate and quality of life of TNBC patients, it is particularly important to find effective, safe, non-toxic or low toxicity new drugs.

In recent years, the role of traditional Chinese medicine in the prevention and treatment of TNBC has been increasingly valued due to its unique theoretical system and expertise in individualized treatment plans. Fisetin is a natural flavonol found in many fruits and vegetables, with multiple benefits such as antioxidant, anti-cancer, and neuroprotective effects. At present, domestic and foreign research has found that Fisetin exhibits anti-cancer effects in various types of cancer. Fisetin's study on colon cancer cells found that it can induce apoptosis and inhibit cancer cell growth by

inhibiting COX-2 and Wnt/EGFR/NF-κB signaling pathways[1]. Studies of Fisetin in prostate cancer revealed that Fisetin inhibited cell growth and suppressed DNA synthesis in a time-dose-dependent manner, while Fisetin induced apoptosis by modulating the mitochondrial apoptotic pathway[2]. Fisetin regulates the molecular mechanisms of Apaf-1, ERK, and COX-2 to induce apoptosis and inhibit cell growth in HeLa cells, as shown in a study of cervical cancer[3]. Fisetin can effectively reduce cell viability, inhibit clone formation, increase PTEN expression, and inhibit mTOR expression in human lung cancer cell line A549[4]. Fisetin inhibits DR3-mediated NF-κB activation, decreases MMP-9 expression, suppresses cell growth and induces apoptosis in pancreatic cancer [5].

The low toxicity and activity of Fisetin against cancer cells suggest that it has important application value, but large-scale trials are needed to evaluate its real chemotherapeutic effect. Whether Fisetin has an anti-TNBC effect is less reported in the domestic and international literature and lacks effective theoretical basis. In this study, we designed a series of in vitro experiments to observe the effects of Fisetin on TNBC cells by applying the existing mature bioassay technology, so as to provide an effective experimental basis for the search of effective and low-toxicity drugs to prevent and control TNBC as well as for clinical exploration.

2. Materials and methods

2.1 Cell culture and reagents

MDA-MB-231 cells were purchased from Procell Corporation (Wuhan, China). MDA-MB-231 cells were cultured in DMEM medium containing 10% fetal bovine serum and 1% penicillin/streptomycin mixture, and incubated in a 37 ℃, 5% CO_2 incubator. DMEM medium (Gibco), fetal bovine serum (FBS, Procell), penicillin/streptomycin mixture (dual antibody, Procell), 0.25% trypsin (Gibco), PBS buffer (Solarbio), Cell Counting Kit-8 (CCK-8, Dojindo), EdU-488 cell proliferation detection kit (Beyotime), 0.25% crystal violet staining solution (Solarbio).

2.2 CCK8 experiment

MDA-MB-231 cells in logarithmic growth phase were taken, the cell concentration was adjusted, and 5000 cells per well in a volume of 100 μL were inoculated into 96-well plates, which were routinely cultured for 24 h and then dosed. Different doses of Fisetin (0, 5, 10, 20, 30, 40, 50 μmol/L) were added to the experimental group, equal dilutions of DMSO were added to the control group, and complete medium (without cells) was added to the blank group. CCK-8 reagent (10 μL/well) was added at 48h of incubation, and the incubation was continued for 2h. The OD value of cells at 450nm was detected by enzyme labeling instrument, and the cell viability was calculated: cell viability = (OD administration group - OD blank group)/(OD control group - OD blank group) 100%. The experiment was set up with 6 replicate wells in each group and repeated 3 times.

2.3 EDU staining experiment

MDA-MB-231 cells in logarithmic growth phase were seeded in 24 well plates. EDU medium (working concentration 1×) was added to label the cells, and the medium was discarded after incubation for 2h. The cells were incubated with 4% paraformaldehyde for 30 min at room temperature, and washed with washing solution (3% BSA in PBS) after discarding the fixative. Permeabilization solution (TritonX-100 in PBS) was incubated for 10 min on a shaker, and the washing solution was washed. The cells were incubated with click additive solution in the dark at room temperature for 30min, and washed with washing solution after discarding the staining reaction solution. DAPI reaction solution, incubate for 8 min at room temperature away from light, discard

the staining reaction solution, and add washing solution to wash. Image acquisition and analysis.

2.4 Plate cloning experiment

MDA-MB-231 cells (300-500 cells/per well) were inoculated into 6-well plates respectively, incubated in the incubator for 24 h and then administered according to the drug grouping, and cultured continuously for 14 d. The medium was discarded, and the cells were fixed with 4% paraformaldehyde for 10 min at room temperature, followed by staining with crystal violet staining solution for 45 min, and then photographed and observed for the number of colony formations after being rinsed with deionized water for drying.

2.5 Tanswell migration and invasion assay

The cell precipitate was collected, and resuspended by adding serum-free fresh culture medium. 600 μL of fresh culture medium containing 10% FBS was added to the culture wells of 24-well plate. 200 μL of cell suspension (containing 20,000 cells) was added into the transwell chamber. Then the cells were placed in the incubator at 37℃ with 5% CO_2. After 48h, the chambers were collected, and discarded the culture medium in the chambers. The cells were gently wiped off with a cotton swab and rinsed with PBS for 3 times. The chambers were fixed in 4% paraformaldehyde solution for 30 min, the fixative was discarded, rinsed twice with PBS, and stained with 0.25% crystal violet dye for 30 min. The chambers were rinsed in ultrapure water and dried at room temperature. Photographs were taken under a microscope with multiple fields of view and counted for graphing.

2.6 Statistical methods

Data were quantitatively analyzed using Graphpad, and Image J software was used for image analysis. Data were expressed as mean ±standard deviation. Comparisons between groups were made using the independent samples t-test. $p<0.05$ indicates a statistically significant difference. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$.

3. Results

3.1 Fisetin inhibits the activity of MDA-MB-231 cells

The effect of Fisetin (0, 5, 10, 20, 30, 40, 50 μM) on the activity of MDA-MB-231 after treatment for 48 h was examined by CCK-8, and the experimental results showed that the activity of MDA-MB-231 cells was significantly inhibited with the rise of the Fisetin dosage (****p<0.0001) (Figure 1). The IC50 value of Fisetin on MDA-MB-231 cells was 19.49 μM, so the concentration of Fisetin at 20 μM was chosen for the subsequent experiments.

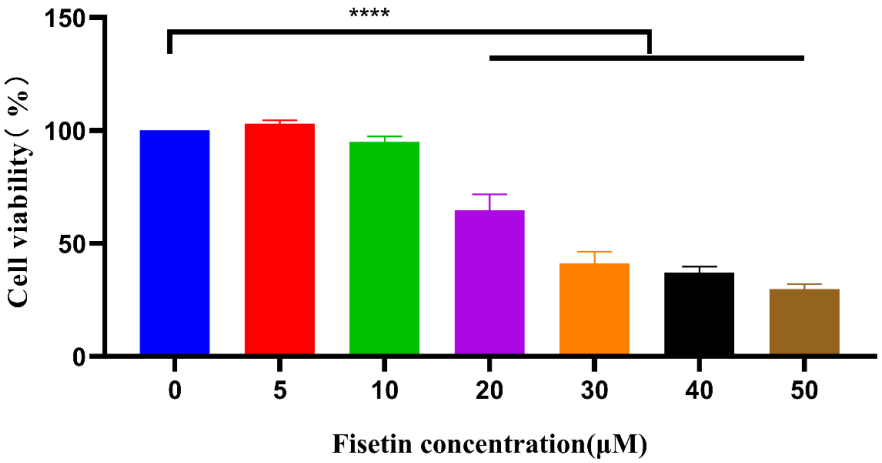

Figure 1: Effect of Fisetin on the activity of MDA-MB-231 cells

3.2 Fisetin inhibits MDA-MB-231 cell proliferation

EDU staining of MDA-MB-231 cells showed that the number of actively proliferating cells after 48 h of Fisetin-treated cells was significantly lower than that of the no-drug group, and the number of EDU-labeled green fluorescent cells was significantly reduced after Fisetin treatment (Figure 2A). Plate cloning experiments were performed on the cells, and the results showed that the number of cell colonies in the Fisetin-treated group was significantly lower than that in the non-drugged group (Figure 2B).

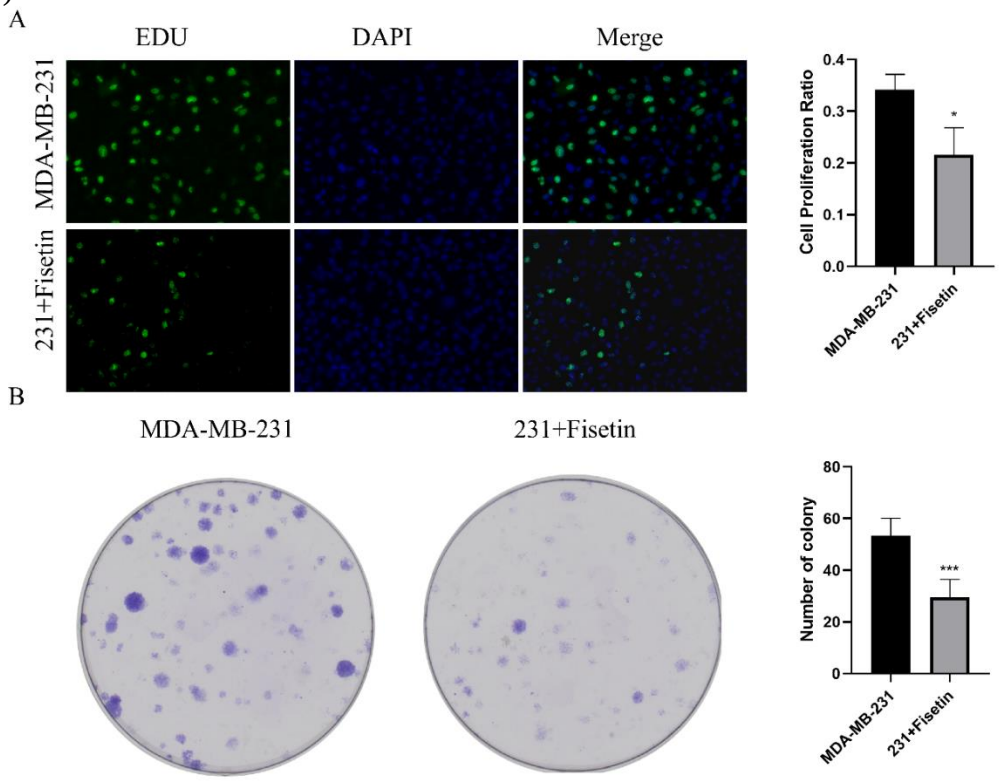

Figure 2: Effect of Fisetin on the proliferation of MDA-MB-231 cells (20×)

3.3 Fisetin inhibits MDA-MB-231 cell migration and invasion

In migration and invasion experiments, the number of MDA-MB-231 cells invading into the lower chamber of the cubicle was significantly reduced after Fisetin spiking treatment for 48 h (Figure 3A and 3B).The results of the Transwell assay indicated that Fisetin could effectively inhibit the migration and invasion ability of MDA-MB-231.

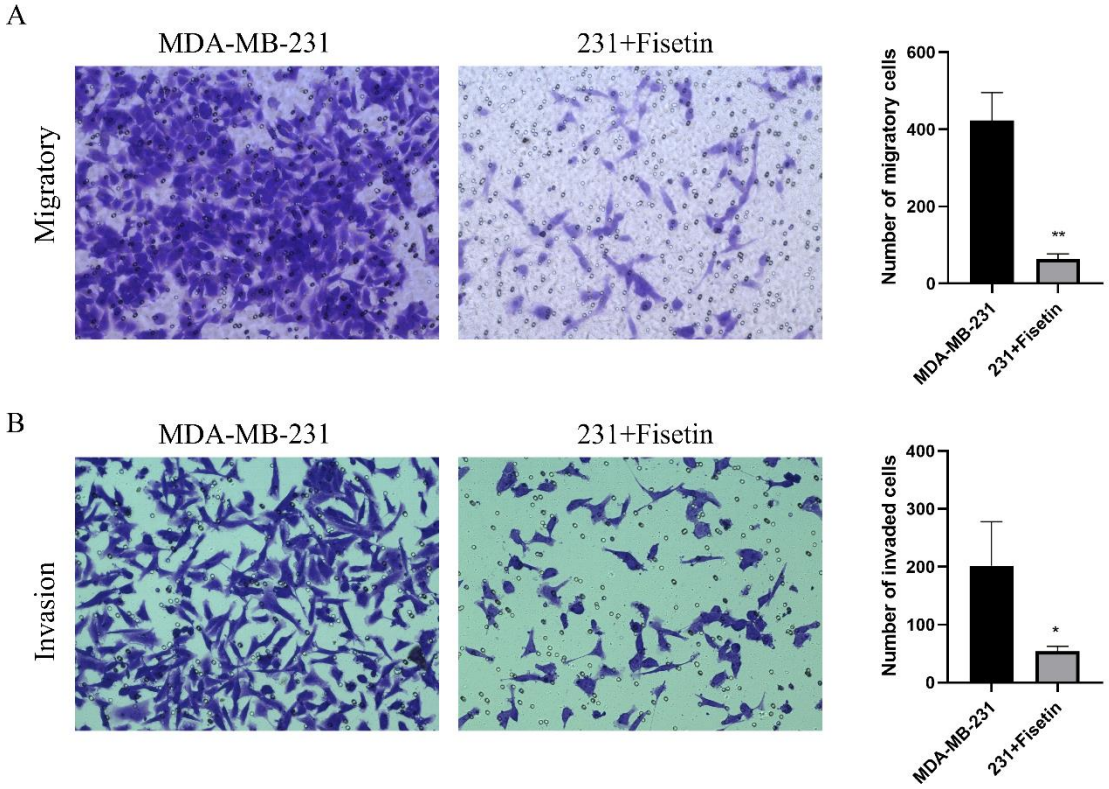

Figure 3: Effect of Fisetin on MDA-MB-231 cell migration and invasion (20×)

4. Discussion

TNBC itself is biologically more malignant than other pathologic subtypes of breast cancer, and is strongly invasive, with a very high probability of postoperative metastatic recurrence within 5 years. Currently, the clinical treatment of TNBC is mainly based on surgery and chemotherapy, but the results are still unsatisfactory. With the progress of breast cancer treatment, Chinese medicine has become one of the main modalities in the treatment of breast cancer by playing a unique advantage in the combination of Chinese and Western medicine treatment programs.

Traditional Chinese medicine has a significant therapeutic effect on TNBC, by regulating pathways such as Wnt/β - Catenin, NF - κB, PI3K-AKT, Hedgehog, Notch, and Hippo to inhibit the proliferation, invasion, and migration of TNBC cells, suppress the formation of EMT, reverse the resistance of chemotherapy drugs such as paclitaxel and platinum, enhance the sensitivity of tumor cells to chemotherapy drugs, and promote cancer cell apoptosis [6-12]. The promising therapeutic window and cost-effectiveness are two potential advantages of using naturally derived drugs. Fisetin is a natural flavonoid compound containing flavonol groups, commonly found in fruits and vegetables. And due to its extensive pharmacological properties, including cytotoxic activity against most cancers, Fisetin has received widespread attention in the scientific community. Fisetin can affect tumor metastasis or progression by stimulating or inhibiting specific molecular targets or biochemical

processes, indicating its potential for chemotherapy or chemoprevention. There are few domestic and foreign literature reports on whether Fisetin has anti TNBC effects. Therefore, Fisetin was chosen as the intervention drug for MDA-MB-231 cells in this experiment.

The aim of this study was to investigate the inhibitory effect of Fisetin on human MDA-MB-231 cells. Human MDA-MB-231 cells were treated with different concentration gradients of Fisetin, and it can be concluded by CCK-8 assay that the IC50 value for the inhibition of human MDA-MB-231 cells by Fisetin for 48 h was 19.49 μM. The higher the concentration of Fisetin, the lower the viability of the cells (Figure 1). To further verify the proliferative effect, EDU staining and colony formation assay were used in this study to verify the inhibitory effect of Fisetin on the proliferation of human MDA-MB-231. EDU labeled cells undergoing DNA replication, which appeared green under fluorescence microscope; DAPI labeled nuclei of cells, which appeared blue under microscope. The ratio of the number of green cells to the number of blue cells indicates the proportion of the number of cells undergoing replication to the total number of cells, and the higher value indicates the higher proliferation ability of the cells. The rate of cell clone formation indicates the number of wall-adherent cells that survive and form clones after cell inoculation. The cell clone formation was stained blue by crystal violet. At the same number of inoculated cells, the higher the number of cell clones, the stronger the cell proliferation ability. When MDA-MB-231 cells were treated with 20 μM Fisetin for 48h, the ratio of the number of cells undergoing replication to the total number of cells decreased, and the number of cell clones decreased, which indicated that Fisetin could effectively inhibit the proliferative ability of MDA-MB-231 cells, and the difference was statistically significant (Figure 2). Transwell chambers detect cell migration and invasion. The chambers are placed in a 24-well plate, and cells can deform through the holes in the chambers and run to the outside of the more nutrient-rich chambers and stick to the outside. By staining and counting the cells on the outside of the chambers, it is possible to determine the strength of cell migration and invasion. Treatment of MDA-MB-231 cells with 20 μM Fisetin resulted in a significant decrease in the number of cells crossing the chambers, indicating that Fisetin can effectively inhibit the migration and invasion ability of MDA-MB-231 cells (Figure 3).

Fisetin, as a natural medicinal ingredient, not only inhibits the proliferation ability of human MDA-MB-231 cells, but also effectively suppresses their migration and invasion. It shows potential anti-tumor activity in the treatment of TNBC and has low drug toxicity. The follow-up research will continue to carry out the in vivo and in vitro pharmacodynamics experiments of Fisetin, providing theoretical basis and experimental basis for the clinical use of Fisetin in the treatment of triple negative breast cancer.

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References

[1] Suh Y, Afaq F, Johnson JJ, Mukhtar H. A plant flavonoid fisetin induces apoptosis in colon cancer cells by inhibition of COX2 and Wnt/EGFR/NF-kappaB-signaling pathways. Carcinogenesis. 2009; 30(2):300-307.

[2] Haddad AQ, Venkateswaran V, Viswanathan L, Teahan SJ, Fleshner NE, Klotz LH. Novel antiproliferative flavonoids induce cell cycle arrest in human prostate cancer cell lines. Prostate cancer and prostatic diseases. 2006; 9(1):68-76.

[3] Ying TH, Yang SF, Tsai SJ, Hsieh SC, Huang YC, Bau DT, et al. Fisetin induces apoptosis in human cervical cancer HeLa cells through ERK1/2-mediated activation of caspase-8-/caspase-3-dependent pathway. Archives of toxicology. 2012; 86(2):263-273.

[4] Khan N, Afaq F, Khusro FH, Mustafa Adhami V, Suh Y, Mukhtar H. Dual inhibition of phosphatidylinositol 3-kinase/Akt and mammalian target of rapamycin signaling in human nonsmall cell lung cancer cells by a dietary flavonoid fisetin. International journal of cancer. 2012; 130(7):1695-1705.
[5] Murtaza I, Adhami VM, Hafeez BB, Saleem M, Mukhtar H. Fisetin, a natural flavonoid, targets chemoresistant human pancreatic cancer AsPC-1 cells through DR3-mediated inhibition of NF-kappaB. International journal of cancer. 2009; 125(10):2465-2473.
[6] Li L, Yang LL, Yang SL, Wang RQ, Gao H, Lin ZY, et al. Andrographolide suppresses breast cancer progression by modulating tumor-associated macrophage polarization through the Wnt/β-catenin pathway. Phytotherapy research : PTR. 2022; 36(12):4587-4603.
[7] Xie W, Zhang Y, Zhang S, Wang F, Zhang K, Huang Y, et al. Oxymatrine enhanced anti-tumor effects of Bevacizumab against triple-negative breast cancer via abating Wnt/β-Catenin signaling pathway. American journal of cancer research. 2019; 9(8):1796-1814.
[8] Kombiyil S, Sivasithamparam ND. In Vitro Anti-cancer Effect of Crataegus oxyacantha Berry Extract on Hormone Receptor Positive and Triple Negative Breast Cancers via Regulation of Canonical Wnt Signaling Pathway. Applied biochemistry and biotechnology. 2023; 195(4):2687-2708.
[9] Wu HT, Lin J, Liu YE, Chen HF, Hsu KW, Lin SH, et al. Luteolin suppresses androgen receptor-positive triple-negative breast cancer cell proliferation and metastasis by epigenetic regulation of MMP9 expression via the AKT/mTOR signaling pathway. Phytomedicine: international journal of phytotherapy and phytopharmacology. 2021;81:153437.
[10] Bao C, Liu T, Qian L, Xiao C, Zhou X, Ai H, et al. Shikonin inhibits migration and invasion of triple-negative breast cancer cells by suppressing epithelial-mesenchymal transition via miR-17-5p/PTEN/Akt pathway. Journal of Cancer. 2021; 12(1):76-88.
[11] Reimche I, Yu H, Ariantari NP, Liu Z, Merkens K, Rotfuß S, et al. Phenanthroindolizidine Alkaloids Isolated from Tylophora ovata as Potent Inhibitors of Inflammation, Spheroid Growth, and Invasion of Triple-Negative Breast Cancer. International journal of molecular sciences. 2022; 23(18).
[12] Yi YW, You KS, Han S, Ha IJ, Park JS, Lee SG, et al. Inhibition of IκB Kinase Is a Potential Therapeutic Strategy to Circumvent Resistance to Epidermal Growth Factor Receptor Inhibition in Triple-Negative Breast Cancer Cells. Cancers. 2022; 14(21).