Phytoestrogens on the Proliferation of Breast Cancer Cell MCF-7

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A cell is an organic whole containing biological macromolecules such as nucleic acid and protein. Human cells contain about 15,000 proteins, which play an important role in the development of cell life. This article aims to study the effect of phytoestrogens on the proliferation of breast cancer cells MCF-7. The estrogen-dependent MCF-7 cells described in this article are grown on average DMEM (containing calf serum 10) and connected to single cell culture. Five days before adding the test product, the cells were washed with PBS and converted into red-free phenol. High-sugar DMEM containing 5 parts of bovine embryo serum was cultured and treated with activated charcoal glucan glycosides. In the experiment, four dose groups of solvent control, estrogen control, anti-estrogens and two test substances were used for control, and the proliferation of MCF-7 cells was analyzed by integration method and flow cytometry. The experimental results in this article show that compared with the solvent control group, GS can significantly inhibit MCF-7 cell proliferation and cell DNA synthesis, and G/M can block the cell cycle and produce a similar inhibitory effect. 96mol/L zein treatment for 24 hours can significantly promote MCF-7 cell proliferation and cell DNA composition, promote cell cycle, and increase cell separation and proliferation index.

Keywords: Phytoestrogen Mechanism, Breast Cancer Cell MCF-7 Proliferation, Cell Culture, Cell Detection Method
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Breast cancer is one of the malignant tumors that seriously threaten women's physical and mental health, and its incidence in my country is increasing year by year. Although the research on breast cancer has become more and more extensive in recent years, it has been found that a variety of factors and signaling pathways are involved. The development of breast cancer is very rapid, but its pathogenesis is still unclear at the molecular level. Therefore, studying breast cancer-related molecules is still one of the current researches focuses.

The incidence of breast cancer is related to hormone levels in the body. Phytoestrogens are natural compounds extracted from plants that are structurally similar to estrogen and have the properties of estrogen. It can be connected to the estrogen receptor (ER), and the combination of different types and concentrations of phytoestrogens and ER shows different results. Preliminary discussion of its proliferation and estrogen-like effects will provide reference value for guiding people's diet and the application of phytoestrogens to medical treatment.

Jiang P proposed a two-layer red blood cell detection framework, including a complete convolutional neural network to extract candidate cell regions with an accuracy of about 97%, and a new marker watershed method based on morphology, marking and conditional skeleton extraction. Improve the segmentation of overlapping cells. But in computational molecular biology, the perfection of reliable and fully

automatic image analysis technology for red blood cell imaging is still a difficult problem [1]. Zhang Y cultured breast cancer cells MCF-7 under different oxygen concentrations to detect cell proliferation and invasion, and to detect the expression of miR-210, aiming to analyze the effect of hypoxia on breast cancer cell behavior and miR-210. It is of great significance in the development of multiple malignant tumors. However, due to the unstable nature of miR-210, the accuracy of the analysis results is not very high [2]. Gao Q performed genetic analysis on different areas of liver cancer to assess the level of heterogeneity within the tumor and link changes to responses to different drugs. He obtained HCC samples from 10 patients in the hospital who had undergone radical resection before receiving adjuvant therapy, and then collected 4-9 spatially different samples from each tumor, performed histological analysis, and isolated the cancer cells, and carried out low-passage culture. However, due to the backwardness and insufficiency of technical equipment, targeted therapies were not found to be effective for hepatocellular carcinoma [3].

The innovation of this paper is (1) proposes SSTR radionuclide imaging, which is currently an important method for the diagnosis and treatment of breast cancer. The SSTR in the endothelial cells of cancer cells is labeled with radionuclide, and the SSTR information is captured by the scintillation phenomenon to understand the patient's cancer cell range. (2) Establish a dSPE purification method by preparing nanocomposite materials as extractants to complete the enrichment and purification of target phytoestrogens, so as to achieve the detection concentration, and combine HPLC and MS detection methods to determine phytoestrogens.

PHYTOESTROGENS ON BREAST CANCER CELLS

Phytoestrogens Extraction Method

Sample extraction method

The extraction of phytohormone samples is the basis for the establishment of analytical methods. Most phytohormones are enzyme compounds and have certain transformation and decomposition capabilities. This requires operations such as freeze-drying and liquid nitrogen grinding of fresh plant tissues before extraction The structural stability and biological activity of plant hormones [4]. Liquid plant samples usually choose the solvent extraction method. According to the principle of "similar compatibility", a solvent with similar polarity to that of the analyte is selected as the extractant. The extraction solvents frequently used at home and abroad are methanol, acetonitrile, acetone and Bieleski buffer Solution (60% methanol + 10% formic acid + 25% chloroform + 5% water) [5]. The study found that sample purification is difficult when acetone is used as the extractant, and acetonitrile interferes at the optimal detection wavelength of the analyte. When studying the extraction efficiency of Bieleski buffer on CTK, it was found that while extracting the analyte, in the buffer The chloroform also extracts the lipids in the matrix, which affects the extraction effect, so methanol is used as the best extractant for plant hormones [6].

Liquid extraction method

The principle of liquid extraction is to separate and purify the components through the different solubility of each component of the test substance in the solvent. The separation effect depends on the difference of the partition coefficient. Commonly used extraction solvents are diethyl ether, ethyl ether, n-butanol, ethyl acetate, but traditional liquid-liquid extraction has serious emulsification, and the consumption of organic solvents is large, time-consuming, complicated steps, and low sample recovery. Therefore, it is not suitable for trace plant hormone analysis [7].

With the advancement of science and technology, people have gradually improved the extraction method. The first proposed liquid-liquid micro-extraction (LLME) is to use a drop of 8μL of organic solvent as the extraction solvent and immerse it in the test sample to complete the enrichment of the target. The proposal of this method has received widespread attention, and finally the LLME extraction method has evolved into two application modes: hollow fiber membrane liquid-liquid microextraction

(HF-LLME) and dispersion liquid-liquid extraction (DLLME) [8]. Using the HF-LLME method, four plant hormones such as salicylic acid, IAA and ABA in the coconut milk were enriched. The enrichment multiple was more than 200 times, and the recovery rate was 88.3-119.1%. And the dispersive liquid-liquid microextraction (DLLME) technology was applied to the detection of plant hormones in green seaweed, the lowest detection limit was 0.2-1μg/mL, and the recovery rate was 80-120%. Finally, the DLLME technology was combined with a high-performance liquid-fluorescence detector (HPLC-FLD) to determine the IAA in the chlorella plants, and the detection limit was 0.02 ng/mL [9].

Method for Determination of Phytoestrogens

Gas chromatography is a detection method established based on the different polarities, boiling points, and the physical and chemical effects of the GC column of the analyte. Through the difference in the distribution coefficient of each component between the gaseous state and the solid state, each component can be separated and analyzed., Has the advantages of high separation efficiency, short analysis time and high sensitivity [10]. Under normal circumstances, the GC is combined with a mass spectrometer detector to improve the qualitative ability of the analyte through the rich structured information provided by the mass spectrometer.

Among the plant hormones discovered so far, only ethylene is volatile. All other plant hormones need to be derivatized before they can be detected by GC-MS. Derivative methods such as trimethylsilylation, acetylation and trifluoroacetylation are usually used. On this basis, the GC-ESI-MS method was established to detect the contents of IAA, IBA, JA and SA in tobacco plants, and then acetic anhydride was selected as the derivatization reagent to detect the cytokinin CTK. According to the experimental results, it is found that GC-MS has certain limitations. For example, the derivatization step is cumbersome, the derivatization reagents required for different plant hormones are different, the derivatized product is extremely unstable, and the fragment ion peak is weak, which increases the sample size. Difficulty in processi ng and detection. Compared with LC-MS, the sample needs high temperature treatment before detection, which causes some plant hormones to decompose into secondary products, and the method's reproducibility and stability are low, so it is difficult to separate and analyze multiple plant hormones at the same time [11].

Capillary electrophoresis technology is a new type of separation and analysis method based on electrophoresis analysis mode established in the 1980s. According to its different separation media and separation characteristics, CE can be divided into capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), capillary electrochromatography (CEC) and other modes. It is widely used for the detection characteristics of plant hormones. The CEC detection method uses a quartz capillary tube as a separation pipe and uses a high-voltage electric field as a driving force to push the mobile phase into the separation column. The components to be tested are gradually separated due to the different migration rates and retention times of the components to achieve the detection purpose. This method combines the high efficiency of electroosmotic flow drive and the high sensitivity of HPLC, and has the characteristics of high selectivity, fast trace amount, and good repeatability.

Using the combination of high-pressure capillary electrochromatography and ultraviolet detector (pCEC-UV), an analytical method for the simultaneous rapid separation and detection of 5 endogenous and exogenous plant hormones was established. The detection limit was 0.2μg/mL, and the recovery rate was Up to 81.0-94.0%, showing good reproducibility. Selected OSD silicon wafer monolithic column as the separation column, combined with pCEC-UV detection method and online concentration technology to separate and analyze zeatin, ABA, IAA, etc. in corn. Compared with the traditional analysis method, it is enriched the efficiency is as high as 23 times, and the lowest detection limit is 0.09μg/mL, showing high sensitivity and selectivity [12].

Cell Proliferation Assay Method

MTT colorimetry

Take phenol red-free DMEM (containing 5CDT-FBS) to divide MCF-7 cells cultured for 4 days into 88 culture plates and culture for 24 hours. After the cells adhere to the wall, add several different concentrations (total volume of 200ml) of the test substance, and each dose group has four holes. At 18 hours, 36 hours, 54 hours, 72 hours, add MTT (5 g/L, 20 L/well), continue incubating for 4 hours, discard the medium, add 100 tons of DMSO to each well, use a wavelength of 490 The nm enzyme immunoassay measures the absorption (A) of each well, calculates the average A and the speed of multiplication, where the rate of increase PR can be expressed as:

$$PR = \frac{EX(A)}{CG(A)} \times 100 \qquad (1)$$

In the above formula, the average A value of the experimental group is represented by $EX(A)$, and$CG(A)$ represents the average A value of the solvent control group.

DNA synthesis by incorporation method

The treatment process is similar to the MTT colorimeter. After adding the test substance 12 hours and 56 hours, 37 kB $^3H - TdR$is added to each well, and the culture is continued for 16 hours (that is, 28 hours and 72 hours after adding the test substance). Collect the cells in the glass fiber filter paper with a cell collector, calculate the number of pulses per minute with the TOP count NXT instrument, and calculate the average and rate of DNA synthesis. The DNA synthesis rate$SR$is:

$$SR = \frac{EX(c)}{CG(c)} \times 100 \qquad (2)$$

In the above formula, $EX(c)$ represents the number of pulses per minute measured by the instrument in the experimental group, and the number of pulses per minute measured by the instrument in the solvent control group is represented by$CG(c)$.

Cell cycle method

MCF-7 cells were cultured in red phenol-free CME for 4 days, inoculated in a 22cm culture flask, and the cell concentration was adjusted to 8 cells/flask. After the cells were cultured for 36 hours, different concentrations of treatment factors were added after adherence. The cultivation ended after 72h. The collected cells were washed with PBS, centrifuged, and filtered with a 400-mesh screen. Adjust the cell concentration of each sample group to 10 cells/ml. Use flow cells to measure cellular DNA content and cell distribution. The cell is composed of several cycles of G_0/G_1, $G_2/M$and S. Then the cell proliferation index VAI can be obtained as:

$$VAI = \frac{S+G_2M}{\frac{G_0}{G_1}+S+G_2M} \qquad (3)$$

Immunochemical Method to Measure SSTR Expression

Cell pretreatment

Cell preparation

Cultivate the cells MCF-7 and MDA-MB-231 to 80~90% of the full bottle, then digest and collect the cells so that the cell density reaches 1×10 5 cells/mL and then inoculate them into a 6-well cell culture plate at 37°C, 5 After culturing for 24 hours under the condition of %CO 2 cell incubator, change the medium to start the dosing treatment when the confluence of the cells reaches 80%.

Cell dosing treatment

Divide each type of cell into 5 groups. The blank control group will not do any treatment, the positive control group will be added with 10-3 M octreotide, and the experimental group will be added with 10-4 M, 10-3 M, and 10-2 M 1-methylacetyl. Endurea, test after 72 hours.

Immunohistochemistry

Climbing piece: Put the sterilized round cover glass into a 12-well cell culture plate, collect the log phase cells of each experimental group, then adjust the concentration of the cell suspension, and add 1 mL of cell suspension to each well Make the cells reach 1×10 5 cell/well; after incubating at 37 degrees Celsius with 5% carbon dioxide for 24 hours, take out the 12-well plate for ICC detection:

1) Aspirate the culture medium in the well, wash with PBS 3 times, 4% paraformaldehyde

500μL/well, fix for 30min at room temperature; wash 3 times with PBS, add 0.3% Triton 500μL/well, permeate the membrane at room temperature for 15min;

2) Wash with PBS 3 times and treat with 3% hydrogen peroxide for 10 minutes. 500μL/well goat serum to block 30mim; add primary antibody: SSTR (1:500), blank control with PBS instead of primary antibody, overnight at 4 degrees Celsius; add secondary antibody: wash 3 times with PBST, goat anti-rabbit biotin (1:1000) at room temperature 30min, SABC complex, 30min at room temperature;

3) Wash with PBST 3 times, DAB develops color (1:50) for 5 minutes, and rinse with tap water to stop DAB staining. After the glass slide is clamped out, observe and take pictures under the microscope.

BREAST CANCER CELL PROLIFERATION EFFECT EXPERIMENT

MCF-7 Cell Preparation Experiment

Cell culture

Slowly take out the culture flask from the 36 degrees Celsius, 5% carbon dioxide incubator, and observe the morphology of MCF-7 cells and the growth of MCF-7 cells under a microscope. Determine the freezing ratio according to the density of MCF-7 cell growth (Normally press 1:3 or 1:2).

Pour out the culture fluid in the culture flask and wash it repeatedly with 5-7ml PBS 3 times to completely wash off the FBS reagent in the remaining culture fluid (note the slow and gentle action), which will help the subsequent trypsin reaction; Add an appropriate amount of pancreatin to the culture flask (depending on cell density and pancreatin activity, usually 0.7-0.9ml is sufficient), gently shake the culture flask to make pancreatin fully contact with adherent cells, and leave it at room temperature for 1 min; under the microscope Observe carefully. When the cells are completely detached from the bottle wall, immediately add 4 ml of medium containing 10% FBS to the culture flask to stop further pulsation and prevent excessive pulsation (excessive pulsation can cause poorly-conditioned cells even When the cells die), use a sterile pipette to slowly (repeat 20 times) to form a cell suspension.

Remove the supernatant and add the prepared cell cryopreservation solution, gently pipetting until it is evenly mixed to form a cell suspension, and evenly distribute the cell suspension to the cryopreservation tube in 1.0-1.5ml, and freeze it. The outside of the storage tube is marked with the date, cell line type, etc.; after standing in a refrigerator at 5 degrees Celsius for 30 minutes, place in a refrigerator at -18 degrees Celsius for 2.5 hours, and then placed in a refrigerator at -77 degrees Celsius overnight, and then placed in a liquid nitrogen bottle for freezing spare.

Cell recovery

1) Prepare cell culture medium using DMEM medium containing 10% serum and 1% double antibody. After preparation, the medium is divided into 50 mL centrifuge tubes for use. Take the cells out of the liquid nitrogen tank and quickly put them in a 37°C water bath and let them dissolve. Prepare the necessary culture utensils and related equipment.

2) After the culture medium has melted, put it in a centrifuge at 1000 rpm and centrifuge for 10 minutes, remove and discard the culture medium, wash once with an appropriate amount of PBS, and centrifuge at 1000 rpm at room temperature for 5 minutes. Take out the centrifuge tube, pour out the PBS, add the culture medium and mix well and transfer to the culture vessel.

3) Place it in 37 degrees Celsius, 5% carbon dioxide cell incubator, and shake the cross back and forth to ensure that the cells are evenly distributed in the culture dish. Place gently. The next day, observe the cell status and proceed to the next experiment.

Cell passage

1) Observe the cell status in the vial culture flask under a microscope. It is observed that the cell growth is in good condition, and the culture medium is clear and normal for passage after it is full. Put the pancreatin and the culture medium in a 37°C water bath to preheat.

2) Pour out the medium in the vial in the ultra-clean workbench, add PBS to wash it once, then discard the PBS and add about 300uL trypsin, put it in the incubator for 1-2 minutes, and observe the cell status from time to time.

3) Shake the culture flask gently. When the cells in the flask are observed to flow back and forth like quicksand, add 1 mL of medium to stop the digestion, blow evenly with a large gun, and transfer to a 1.5 mL centrifuge tube. Centrifuge at 1000 rpm for 5 minutes, take out the centrifuge tube, discard the medium, add new medium, and put it into a new petri dish according to the proportion.

4) Put it back into a 37°C, 5% carbon dioxide cell culture incubator, and shake the cross back and forth to ensure that the cells are evenly distributed in the culture flask. Observe the cell status frequently, and be ready for the next experiment at any time.

Cell freezing

1) Observe the cell status in the vial culture flask under a microscope. It is observed that the cell growth status is good, the culture medium is clear and normal, and it is processed after it is full, and frozen when not needed. Prepare the cryopreservation liquid according to the number of cells you want to cryopreserve, and prepare according to the ratio of serum: medium: DMSO=7:2:1.

2) Remove the culture medium, pancreatin, and PBS from the constant temperature water bath, wipe them clean with 75% alcohol, and put them in the workbench. Take the cells out of the incubator, pour out the culture medium, wash once with sterile PBS, add trypsin, cover the bottom of the culture flask as the standard, and put it in the incubator for 2-3 minutes.

3) Observe the cell status, add culture medium to stop the digestion when the cells are completely detached, then gently blow evenly with a big gun, then transfer to a centrifuge tube.

4) Centrifuge at 1000 rpm for 5 minutes, remove the cells, pour out the medium, add 1 mL of the newly prepared cryopreservation solution, mix thoroughly by pipetting, transfer to the cryopreservation tube, and write the corresponding date, name of the cryopreserved cell and operation people. Transfer the cryopreservation tube to the freezer box and put it in the refrigerator at -80 degrees Celsius, and store it in liquid nitrogen after 2-3 days.

Cell Clone Formation Experiment

Clone formation refers to the formation rate of a cell clone, that is, the survival rate of seed cells. It indicates the number of adherent cells that survive and form clones after cell growth. Adherent cells may not be able to multiply and form clonal cells, and the cells that form clones must have adhesive and proliferative cells. The cloning rate reflects two important characteristics of cell number dependence and proliferation ability. In the case of specific gene knockdown, count the cells and control cells and transfer 2000 cells per well to a six-well plate for culture, change the medium at any time, and perform staining in 2-3 weeks to observe cell proliferation Status, you can record the difference between the knockdown group and the control group.

(1) Observe the cell status in the vial culture flask under a microscope. It is observed that the cell growth is in good condition, and the culture medium is clear and normal and processed after it grows up. Put the pancreatin and the culture medium in a 37°C water bath to preheat.

(2) Collect the cells according to the above-mentioned cell collection method, pour out the medium in the vial in the ultra-clean workbench, add PBS to wash once, then discard the PBS, add about 300μL of trypsin, put it in the incubator for 1-2 minutes, and observe from time-to-time Cell state.

(3) Gently shake the culture flask. When the cells in the flask are observed to flow back and forth like quicksand, add 1 mL of culture medium to stop the digestion, blow evenly with a large gun, transfer to a 1.5 mL centrifuge tube, centrifuge at 1000 rpm for 5 minutes, take out the centrifuge Tube, discard the medium.

(4) Add an appropriate amount of medium to resuspend the cells and mix them thoroughly. Take

about 20 μL for counting. After counting, dilute the cells of each group to the same concentration and add them to a six-well plate to make 2000 cells per well.

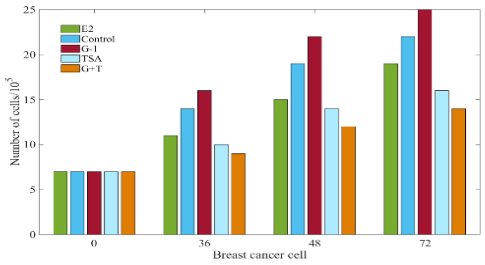
(5) Put it in the incubator for 2-3 weeks. When the cell clusters are visible to the naked eye, discard the culture medium, wash it with PBS, and then add 1 mL of anhydrous methanol to fix for 20 minutes.

(6) Discard anhydrous methanol, add 0.5% crystal violet for staining, and incubate at room temperature for 30 minutes. Discard the crystal violet solution, wash it with clean water, and then dry the culture plate. Record the number of clones of each group of cells for statistical analysis.

The Effect of Phytoestrogens on Breast Cancer Cells

In order to study the effect of phytoestrogens on the proliferation of breast cancer cells, we used MCF-7 (GPER positive and ERα/β positive) and MDA-MB-231 (GPER positive and ERα negative) cell lines as materials, and counted with a hemocytometer The growth curve was drawn by the method. According to the cell size, the inoculation amount of MCF-7 cells was 200 per dish, and the inoculation amount of MDA-MB-231 cells was 700 per dish. MCF-7 and MDA-MB-231 cells were cultured in phenol red and serum-free 1640 medium and DMEM medium, and cultured at 37 degrees Celsius and 5% carbon dioxide for three days to deplete

Figure 1.
Growth curve of breast cancer cells under TSA treatment



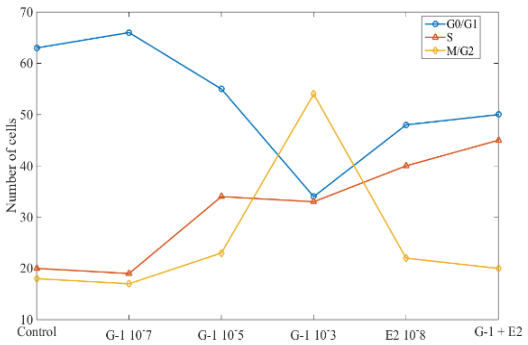
estrogen. Then the cells were cultured in 1640 medium and DMEM medium without phenol red and containing 10% fetal bovine serum, and were treated with E2 (10^-4 M), G-1 (10^-5 M) and TSA respectively. (10^-6M) processing, and the common processing of G-1 (10^-5M) and TSA (10^-6 M). Cells were collected at 36, 48, and 72 hours of treatment, and counted using a hemocytometer. The cells in each dish were counted three times, and the average value was taken. Each experiment was repeated 3 times, and the average value was used as the final result.

ANALYSIS OF RESULTS OF PHYTOESTROGENS ON BREAST CANCER CELLS

Analysis of the Effect of Phytoestrogens on the Proliferation of MCF-7

Through statistical analysis of the experimental part, the results are shown in Figure 1. From the data in the figure, it can be seen that E2 treatment

Figure 2.
The effect of phytoestrogens on the cell cycle



can significantly promote the proliferation of two breast cancer cell lines, and the number of cells is significantly higher when E2 treatment is 48h and 72h Compared with the control group (E2 vs control, P<0.01, n=3); TSA treatment can also significantly inhibit the proliferation of breast cancer cells. The number of cells in the treatment group at 36, 48, and 72 h was significantly lower than that in the control group (TSA vs control, P<0.01, n=3); G-1 as a specific agonist of GPER, the treatment result is similar to TSA, the number of cells after 36, 48 and 72h treatment was significantly lower than that of the control group

(G-1 vs control, P<0.01, n=3); G-1 and TSA have the most obvious inhibitory effect on cancer cell proliferation (G-1 +TSA vs control, P<0.01, n=3). The results show that G-1 can inhibit the proliferation of breast cancer cells after activating GPER, which is similar to TSA treatment and has a synergistic effect.

In order to further understand the effect of phytoestrogens on the cell cycle progression of breast cancer, we used E2 treatment as a control to analyze the cycle progression of MCF-7 using flow cytometry. The specific results are shown in Figure 2, as expected. Compared with the control group, in the experimental group treated with E2 for 24 and 48 hours, the proportion of cells in S phase and G2/M phase was significantly increased (G-1 vs control, P<0.05, n=3); but G-1 treatment The proportion of cells in the S phase and G2/M phase of the group was also significantly increased, especially in the 36h and 48h groups treated with 10^-4 M G-1, the proportion of cells in the G2/M phase was as high as 39.1% and 64.4%, respectively , Indicating that phytoestrogens can block breast cancer cells in G2/M phase and inhibit their proliferation.

Analysis of the Influence of GS and ZEA on Cell Proliferation

In this paper, soybean isoflavones and zearalenone are used as representatives of phytoestrogens to act on breast cancer cells. The effect of phytoestrogens on breast cancer cells is judged by the number of cell divisions at 24h, 36h, 48h, and 60h. The results are shown in Table 1:

Table 1.
MTT method to measure cell proliferation

Group	24h		36h		48h		60h	
	A	PR(%)	A	PR(%)	A	PR(%)	A	PR(%)
GS	0.12	95.9	0.15	93.8	0.17	91.7	0.18	89.6
ZEA	0.13	104	0.17	106.7	0.19	107.8	0.22	110

The analysis of Table 1 shows that compared with the solvent control group, 96 mol/L GS can significantly inhibit the proliferation of T47D cells for 24 h. As the culture time is extended to 60 h, 70 mol/L GS can also significantly inhibit T47D cells. proliferation. ZEA is similar to E2 in that it can promote the proliferation of T47D cells, and as the time of action and dose increase, its proliferation effect shows a dose-effect and time-effect relationship.

Table 1 is more intuitive and concrete, and the graph is drawn as shown in Figure 3:

Figure 3.
MTT method to measure the effect of GS and ZEA on cell proliferation

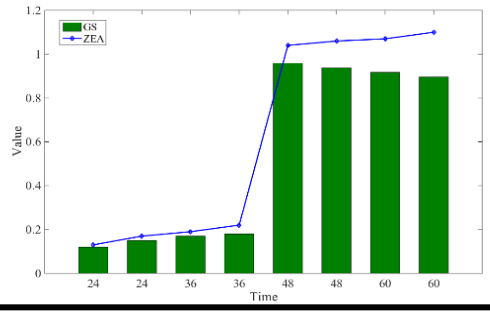
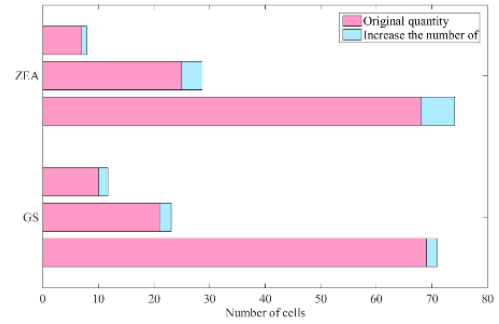


Figure 4.
The effect of GS and ZEA on the cell cycle



The test got similar results to 2.1. As shown in Table 2, compared with the solvent control group, the DNA synthesis of MCF-7 cells showed a significant downward trend with the extension of the G S action time and the increase of the action concentration. GS can significantly inhibit the DNA synthesis of MCF-7 cells under the condition of 96 mol/L for 24 h (PG 0.05). When the culture time is extended to 72 h, the 32-mol/L concentration group can also significantly inhibit MCF-7 Cellular DNA synthesis (P<0.05). The effect of ZEA on DNA synthesis of MCF-7 cells is similar to that of E, that is, the effect of promoting DNA synthesis of MCF-7 cells.

Table 2.
DNA method to measure the effect of GS and ZEA on cells

Treatment	24h	60h

	Counts/min	SR(%)	Count/min	SR(%)
GS	141	97	182	90
ZEA	148	102	210	104

From the distribution of each phase of the cell cycle, as the concentration of GS increases, cells in G2/M phase gradually increase; GS 32~mol/L and 96~mol/L concentration groups for 72 h significantly increase cells in G2/M phase. The proportion of cells in S phase was significantly reduced (PG 0.05), and cells in G0/G1 phase also had a decreasing trend, indicating that GS can inhibit DNA synthesis and arrest the cell cycle in G2/M phase, which is different from the effect of TAM. The effect of ZEA on MC F-7 is similar to that of E. As the concentration increases, the proportion of cells in S phase increases significantly, suggesting that ZEA can promote DNA synthesis and promote cells in G0/G1 phase to S phase and increase cell proliferation index. The specific results are shown in Figure 4:

Comprehensive analysis can be found that 96 n mol/L ZE A can show a similar effect to E for 24 hours, that is, stimulate the proliferation of estrogen-dependent breast cancer cell line MCF-7. With the extension of the culture time to 96 h, 8 n mol/L ZE A can also significantly promote the proliferation of MCF-7 and increase the synthesis of DNA by MCF-7 cells, which promotes cells in G0/G1 phase to enter S phase and G/M during the period, the cell proliferation index increases. These effects are dose-dependent and time-dependent. G S can inhibit the proliferation of MCF-7 cells and decrease its DNA synthesis. With the increase of GS concentration, the distribution ratio of G/M phase cells gradually increased. After 32 F mol/L and 96 F mol/LGS acted on M CF-7 for 72 h, the ratio of S phase cells decreased significantly ($P<0.05$). And the proportion of cells in G0/G1 phase also tends to decrease, indicating that GS can inhibit DNA synthesis and block the cell cycle in G/M phase, but GS does not inhibit its division and proliferation index, which is different from the effect of TAM. In addition, in recent years, some foreign studies have shown that when mothers consume more soybeans, their infants have higher levels of soy isoflavones. Infants can also take in phytoestrogens through formulas that use soybeans as the main raw material. The academic effect remains to be clarified. Therefore, the in-depth study of plant hormones is of great significance for the comprehensive evaluation of the biological effects of environmental hormones.

CONCLUSIONS

The G-1 activated GPER studied in this article can inhibit the proliferation of breast cancer cells by inhibiting the phosphorylation of AKT, continuously activating ERK, changing the expression of apoptosis and cycle-related proteins in cells, and inducing apoptosis and cycle arrest of breast cancer cells, and Can significantly inhibit it.

Migrate Similar to the anti-tumor drug TSA, G-1 can inhibit the proliferation and migration of breast cancer cells and promote their apoptosis by activating GPER to up-regulate the histone acetylation level of breast cancer cells, affecting the cycle, and the expression of apoptosis-related proteins. G-1 is likely to become a new type of anti-tumor drug, and GPER may be another potential target for the treatment of cancer.

The research in this article shows that phytoestrogens have a significant effect on the proliferation of breast cancer cell MDAMB231, and can affect the cell cycle and induce apoptosis. The results show that USP18 plays an important role in the development of tumors. Its shortcoming is that it was found that phytoestrogens can cause cell proliferation inhibition. After further experiments, no corresponding downstream effector genes were found, which could not explain the cells further. Proliferation inhibition and the cause of cell cycle changes.

In this paper, in vitro cell experiments confirmed the proliferation of Cou, and preliminary explored its mechanism of this effect is related to ER, providing a theoretical basis for the risk assessment and practical application of food-borne Cou. However, the specific signal transduction pathways in cells are not yet clear, and further research is needed. In addition, the research in this article is conducted at the cell level in vitro. In future experiments, we will further conduct research in animal models and clinical trials, hoping to provide new ideas for breast cancer gene therapy and new

drug development.

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