

A Research on the Inhibition Mechanism of Y-27632 to Esophageal Cancer Cell EC9706 and its Significance to Epidemiology

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The purpose of this research is researching the the impact of ROCK caffeic acid phenethylester Y-27362 on cell growth and migration of esophageal cancer EC9706 and discussing the possible mechanisms. The main research method of this paper is culturing human esophageal cancer canopy EC9706 in vitro, adding 20umol/LY-27632 liquid (experiment group) and isometric PBS(control group) to culture for one hour, adopting cell counting, MTT experiment to test cell growth situation, using Transwell experiment to observe cell migration situation, employing Westem blot to detect the expression level of Cav-1, and making comparison of two groups. Finally it contrastively analyzes the results. The conclusion is that Rock caffeic acid phenethylester Y-27362 can restrict cell growth and migration of esophageal cancer EC9706, which provides a new idea and direction for curing esophageal cancer.

Key words: Esophageal cancer; preparation Y-27632, EC9706 cell.

Esophageal cancer is one of the highest morbidity and mortality of cancer. Chemotherapy is the main treatment means of terminal stage of esophageal squamous carcinoma. Cisplatin(DDP) is one of the most common chemotherapeutics. However, the side effect of DDP is serious and cancer cells often show the drug resistance, which restrict its clinical application and curative effect. Rock, Rho kinase, is Rho-associated coiled-forming protein kinase. An research found that ROCK showed an abnormal overexpression in cancer cells, such as breast cancer, prostatic cancer and lung cancer¹⁻³ and the overexpression showed orthogonality correlation with the grade of malignancy⁴⁻⁶. This experiment used Y-27632 that is the caffeic acid phenethylester of signal path to

deal with Esophageal cells EC9706 and measured the growth and migration of cells and the expression level of caveolin-1, stated whether Y-27632 can be used as the target therapeutic of Esophageal Cancer and made an initial discussion of possible mechanism from a new perspective.

MATERIALS AND METHODS

The human body esophageal cancer cell line EC9706, RPMI1640 medium, fetal bovine serum (Hangzhou Evergreen), dimethyl sulfoxide (DMSO), Cav-1 polyclonal antibody (CST), trypsin (Biyuntian biological technology co., LTD), thiazolyl blue(MTT)(CST) provided by this experiment.

Cell Culture

Firstly makes 100U/ml mediums with 90% RPI1640 nutrient solution, 10% fetal bovine serum and cyano-streptomycin respectively, culturing

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human esophageal cancer EC9706 on it and incubating on incubator under 37°C, 5% CO₂, medium is changed every 2~3 d, when cell wall adherent growth to the 80%~90% of bottom and passage, take out and conduct a test when cells are on logarithmic phase⁷.

Cell Counting

Cultivating EC9706 cell, inoculating in six pore plates to cultivate and incubate with the density of 1×10^5 /ml, then establishing the experimental group and control group. Add 20 μ mol/L Y-27632 solution into experimental group, and PBS with the same volume into control group, terminate the cultivation after 1h, digest the cells, extract a little of cell suspension, dropwise add into the edge of cover plate to make suspension full between counting plate and cover plate, stew for 3min, be care of the bubbles aroused inferior of cover plate, and also prevent suspension inflowing into the sideward grooves. The calculation with the total amount of the number of four big cell quantities, only record dexter and inferior line pressing cytoplasm and ignore the upward and right. Then calculate according to formula: cell population/ml=four big total cellular scores/ 4×10^4 .

MTT Experiment

Inoculate EC9706 cell cultivated with the density of 6×10^4 /ml into culture plate with 96 holes, 100 μ l/hole, set 5 ventral orifices of each concentration and establish control group; overnight culture under 37°C, 5% CO₂ incubator, after the growth condition of cell adhesion is favorable, add 20Y-27632 solution, add MTT solution (5 mg/ml) into each hole after culturing for 1h and continue to culture for 4h, then discard supernatant, each hole adds MDSO, shock 20~30min on MPP oscillator, measure each hole's optical density (OD) on the 492nm wave length of ELIASA. the inhibition ratio(%) of cell growth = average OD value of control group-average OD value of dosing group/average OD value of control group *100%.

Transell Experiment

As for single cell suspension, here with conventional method to use serum-free medium to prepare, cell/ml, each group of cells divided into two parts. Trypan blue exclusion assay, cell viability should larger than 95%. Add cell suspension (cells) and chemotactic factors into the upper ward

of Transwell culture plate and under, EMEM for 1h. Wipe away the cells on the surface of congealed fat, poly carbon gule and poly ester carbon membrane gently with wet cotton swabs. Take out of the upper wards carefully and mark it after bolting with wires, fix for about 30 min with ice-cold formalin. Use hematoxylin dye for 1min. Fetch the poly ester carbon membrane from upper wards carefully, put on neutral resin of glass slide and mount. Randomly sample five views of the cells attaches to poly ester carbon membrane under high power lens (*100) to count, wipe off average values. Repeat the experiment for three times.

Westem Blot Detection

Passage the logarithmic phase EC9706 cell and cultivate on the incubator under , make chemical reagent when cells grow to 80%~90% of the bottom of vessel, collect the cells after 1h, precool PBS washing cells for three times, add cell lysis buffer, ultrasonic cell disruption and bicinchoninic acid disodium (BCA) method to measure protein concentration. SDS-PAGE separates cell total protein, electrotransfer the proteins to PVDF membrane, seal the PVDF membrane with 5% skim milk powder/TBST solution, the protein loading quantity of sample is 30 μ g, Cav-1 working concentration is 1:500. working concentration is 1:1000, second antibody working concentration is 1:10000, ELC reagent shines, X-ray film exposures displays, marks, scan the stripes and makes gray analysis.

Experimental data process

Adapt SPSS 17.0 software to process data. Experimental data expresses with , make independent sample t detection; take cell growth curve as the impact and contrast of experiment to cell growth. Take as difference has statistical significance.

RESULTS

Cell Counting

After Y-27632 processing the cells 1h, count the growth of cells in different times, draw cell growth curve, the result shows with the increase of time, the amount of dosage group and control group both gradually increase, the cell proliferation of experimental group is slower than control group, the comparative differences of two groups have statistical significance.

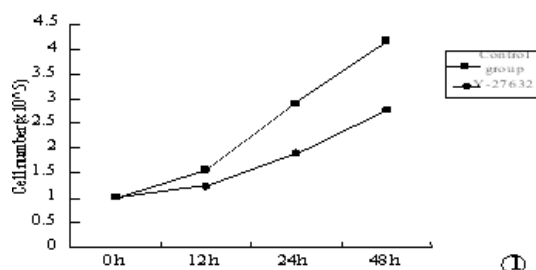


Fig. 1.

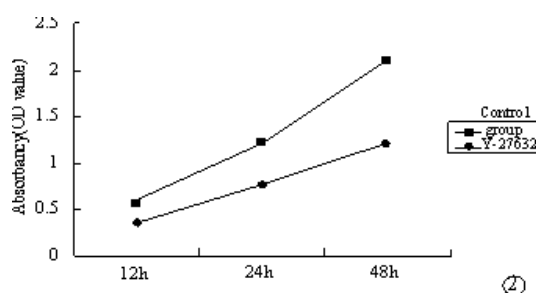


Fig. 2.

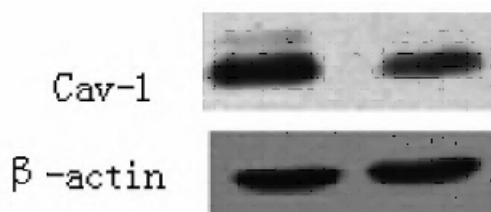


Fig. 3.

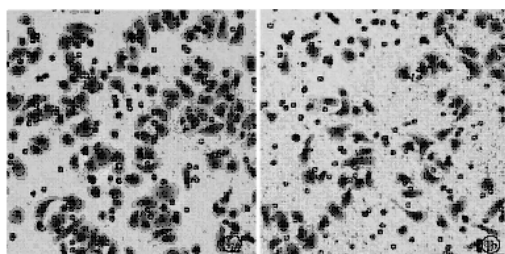


Fig. 4.

MTT Experimental Result

Detect the impact of Y-27632 on the cell growth of EC9706, the result shows the control group with PBS has no obvious growth inhibition effect to EC9706 cell, Y-27632 group has obvious inhibition effect to EC9706 cell, the comparative differences of two groups have statistical

significance ($p < 0.05$), with the increase of time, the absorbance of two groups gradually increase, the increase of dosage group absorbance is slower, the comparative differences of two groups have statistical significance ($p < 0.05$).

Western Blot Detection Result

After processing cells with 20 $\mu\text{mol/L}$ Y-27632 for 1h, the expression level of Cav-1 protein is dramatically declined, while the expression level of internal reference β -actin has no big change, through the analyzing of image processing system, gray value as reference, the Cav-1 expression level in Y-27632 cell is obviously lower than control group ($p < 0.05$).

Transwell Experiment

Observe the migration situation of dosage group and control group, the result shows after Y-27632 processing for 1h, the cell migration amount of Y-27632 group is (48 ± 9) , the control group's cell migration amount after processing with PBS is (108 ± 11) , the migration cell amount in Y-27632 group is obviously lower than control group ($p < 0.01$), hint that Y-27632 can obviously inhibit the migration ability of cells.

DISCUSSION

One Rho family history at least has fifty members and small G protein families, which is the clearest Ras relevant monomer GTP enzyme present. The researches of recent years have found that Rho family protein influences the transformation, survival, apoptosis, invasion and transferring of malignant cells⁸, its mechanism may through damaging cell polarity and cell junction, promoting the degradation of extracellular matrix, increasing the realization of the approaches such as angiogenic factors. Such as the activity of Rhoc or ROCK can obviously increase the invasion ability of various tumor cells, while when sealing ROCK function with ROCK inhibition can through many ways to inhibit the proliferation and transference of tumors⁹⁻¹¹. The researches of Hong zhenya *et al.*¹² found Rhoc or ROCK signal transduction pathway has close connection with in vitro invasion or migration of human ovarian carcinoma cells, interdict the expression of ROCK can inhibit the in vitro invasion ability of ovarian carcinoma cells effectively. This research shows ROCK can promote tumor growth, which is a tumor

promotor gap-associated protein.

Fossula is the flask-shaped sunken structure of plasma membrane found by Palade in 1953 under electron microscope, the diameter is about 50~100nm, which is determined as one kind of organelles, its function mainly is participate in the transmembrane transport and cell signaling transduction, Cav-1 is the structural protein of fossula, the research found in prostatic cancer, pancreatic cancer and esophagus cancer, Cav-1 expression increased significantly. and its expression level and neoplasm staging appears positive correlation, hint Cav-1 can promote the development of tumors. In preliminary researches we found that Cav-1 is high expression in human esophagus cancer TE13 cells, and Y-27632 can promote the growth and transference of TE13 cell, which is disaccord with present most of researches. To further research this result, we researched human esophagus cancer cell EC9706, then found that Cav-1 is expressive in EC9706, which hints that Cav-1 may promote the growth of EC9706 cell, at the same time use Y-27632 can inhibit the growth and migration of EC9706 cell obviously, and can reduce the expression level of Cav-1, which is disaccord with the research to TE13 cell previously, so is remaining to further study.

CONCLUSION

Esophageal cancer is one of the highest morbidity and mortality of cancer. And its prognosis is bad³⁻⁴ for the survival rate of five years for the patient of middle and late period is only 10%. The result of this research showed Y-27632 can used as the targeted cancer therapy of esophagus cancer and its mechanism may be connected with the expression level of Cav-1. The experiment sets forth the mechanism which inhibits the cell growth and migration of esophagus cancer from a new perspective to promote new idea for targeted cancer therapy of esophagus cancer.

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