ISSN 2706-6819 Vol.5, Issue 10: 53-56, DOI: 10.25236/IJFM.2023.051009

Effect and Significance of Quercetin on the Expression of CD44v6 and E-cadherin in Hela Cells

Manshu Lei, Quan Zhou, Aihua Chen, Wenfei Zheng, Wei Li

Department of Gynecology and Obstetrics, The People's Hospital of China Three Gorges University, Yichang, Hubei, 443000, China

Abstract: *Blocking tumor cell invasion and metastasis is one of the important ways to treat tumors. In this paper, HeLa cells were cultured with different concentrations of quercetin to evaluate the therapeutic effect of quercetin on human cervical HaLa cells cultured in vitro. RT-PCR was used to detect the expression of adhesion molecules CD44v6 and E-cadherin in human cervical cancer HeLa cells after quercetin action. The data showed that quercetin acted on HeLa cells , and with the increase of drug concentration, the proliferation inhibition rate of HeLa cells increased with dose-dependently (P<0.01).RT-PCR showed that quercetin significantly inhibited the expression of CD44v6, and enhanced the expression of E-cadherin with dose-dependence, which may ultimately have the effect of anti-metastasis of cervical cancer.*

Keywords: *quercetin; CD44v6; E-cadherin; Hela cells*

1. Introduction

Metastasis is a characteristic of malignant tumors, which seriously affects the efficacy and prognosis of tumor patients. Adhesion is a key link in the process of tumor metastasis. The first step in tumor invasion is a change in the adhesion molecules of tumor cells, which results in conferring the ability to metastasize the tumor. Subsequently, the adhesion of circulating tumor cells to the endothelial cells of the vasculature and the stroma occurs.[1] Cell adhesion molecules (CAM) play an important intermediary role in the mutual recognition and binding between cells and cells and extracellular matrix, and play a key role in growth, differentiation, inflammation, immune and other responses, thrombosis, and damage repair. In this paper, different concentrations of quercetin were used in HeLa cells to detect the expression of adhesion molecules CD44v6 and E-cadherin at the cellular level, and to explore the mechanism of quercetin inhibiting tumor invasion and metastasis.

2. Methodology

2.1 Experimental preparation

Quercetin was purchased from Sigma Company, USA. The main reagents included RPMI-1640 medium from Gibco, fetal bovine serum from Hangzhou SiJiQing Bioengineering Material Co., Ltd, trypsin from Sigma, thiazolyl blue (MTT) staining solution from Beijing Zhongshan, and Transwell insert from Corning-Costar. The main liquid in the experiment included:

(1) 10% of serum medium: 90% of 1640 medium, 10% of foetal bovine serum, penicillin (100u/ml), streptomycin (100ug/ml), value of pH is 7.2-7.4, stored at 4℃.

(2) 0.25% of trypsin: 0.5g of trypsin dry powder, dissolved in 200ml of calcium and magnesium-free PBS solution, adjusted the PH value of 7.2-7.4 with $NaHCO_3$, and then filtered with a filter to remove bacteria, and then stored at -20℃ for spare parts.

(3) PBS solution: NaCL is 4.00g, KCL is 0.10g, Na_2HPO_4 is 0.575g, KH_2HPO_4 is 0.10g, dissolved in 500ml of triple-distilled water, adjusted the PH value of 7.2-7.4 with $NaHCO_3$, autoclaved, and then stored at 4℃ for spare.

(4) Tetramethylthiazolium blue (MTT) stain: 25mg of MTT was added to 5ml of RPMI-1640 medium, mixed well and stored at 4℃, ready to use.

ISSN 2706-6819 Vol.5, Issue 10: 53-56, DOI: 10.25236/IJFM.2023.051009

2.2 Experimental Methods and Procedures

2.2.1 Cell Culture and passaging

HPV18-positive cervical cancer cells HeLa cells were grown in RPMI-1640 culture medium containing 10% calf serum and 100u/m penicillin, 100ug/ml streptomycin, and were routinely cultured in a closed incubator at a constant temperature of 37℃, 5% of CO_2 and 95% saturated humidity. When the color of culture medium changed from red to yellow or the cell density was large, the culture medium was changed. When the cell density reached 80%-90% of the culture bottle, the cells were passaged and cultured.

2.2.2 Cell processing and grouping

The culture solution was changed at 24h intervals. RNA was extracted from three bottles at 24, 48 and 72h after drug administration. Quercetin DMSO reservoir was diluted with RPMI-1640 culture medium on the day of experiment.

2.2.3 Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA extraction was sequentially divided into lysis, isolation, precipitation, elution, re-solubilisation and storage. The extracted RNA was placed in the refrigerator at -70°C for storage, or immediately used for reverse transcription. In turn, the quality of total cellular RNA was identified (formaldehyde denaturing gel electrophoresis clearly showed two bands of 18s and 28s, indicating that RNA was not degraded).

2.2.4 2RT-PCR

After reaction mixture was water-bathed at 70°C for 5 min, adding 5×Reaction Buffer/(5ul), dNTP (10mM)/(2ul), Rnase Inhibitor/(25units (0.6ul)), M-MLV RT/(200U (1ul)), and Rnase-Free ddH2O/(up to 25ul). At the end of the reaction set for cryopreservation.

PCR: According to the formula: n moles = OD value × 100/number of bases, CD44v6 primers, E-cadherin, β-actin primers were synthesised and diluted to 20 pmol/μl, dispensed and stored in the refrigerator at -70℃. The base pair positions of the primers in the original sequence and the length of PCR products specifically amplified by the primers. (See Table 1 below)

Table 1: Primer sequences and amplified fragment lengths

Primer type	Nucleotide sequence	Amplified fragment length
CD44v6	Upstream 5'-GACACATATTGCTTCAATGCTTCAGC-3' Downstream 5'-TACTAGGAGTTGCCTGGGATGGTAG-3'	348bp
E-cadherin	upstream 5' -ATCCAAAGCCTCAGGTCATAAACA-3' Downstream 5' -AAGAAACAGCAAGAGCAGCAGAA-3	377bp
β-actin	upstream 5'-CGTCTGGACCTGGGCTGGCCGGGACC-3' Downstream 5'-CATGAAGCATTTGCGGTGGACGATG-3'	600bp

Add 10×PCR Buffer/(5ul), 2mmol/L dNTP/(5ul (0.2mmol/L each)), Taq DNA polymerase/(0.2ul (1U)), 25mmol/L MgCl2/(3ul (1.5mmol/L)), template DNA/(10pg~1ug), Sterilized ddH2O/(up to 50ul), etc., to the reaction tubes on an ice bath in turn. bout 20μl of Rnase-free liquid paraffin was added to the samples, and then the samples were put into the PCR instrument, and the PCR reaction was carried out according to the predetermined procedure. At the end of the reaction procedure, 9 μl of amplification product was taken for agarose gel electrophoresis analysis.

Electrophoretic identification of PCR amplification products: dissolve agarose in 1×TAE solution at a concentration of 1.5%, add EB at a final concentration of 0.5 μg/ml and mix well to prepare agarose horizontal electrophoresis plates. During the electrophoresis of PCR amplification products, DNA Marker was added and electrophoresed at the same time to determine the molecular weight of PCR amplification products. Detect under UV light after electrophoresis. The electrophoretic bands were analysed with a gel computer image analysis system, and the results were expressed as the ratio of the optical density of the sample to that of the internal reference.

3. Analysis

3.1 Detection of cell viability

After trypsin digestion, the concentration of HeLa cells was adjusted to 5×105 cells/ml, and

ISSN 2706-6819 Vol.5, Issue 10: 53-56, DOI: 10.25236/IJFM.2023.051009

inoculated in 96-well plates at 200ul per well, and then routinely incubated for 24h, the control was set up as before, and at the same time, quercetin was added into the cell culture medium at the three final concentrations of 20, 40, and 80umol/L, and then discarded the culture medium after 24h of incubation, and then 0ul of MTT (5 mg/ml) was added to each well, and then the incubation was continued for 4 hours. DMSO 150ul was added, and the absorbance values of each group were detected by enzyme immunoassay (A492) after shaking for 10 mins. The experiment was repeated three times, three duplicate wells was set up each time and concentration. The cell inhibition rate was calculated according to the formula cell inhibition rate (=1-mean A value of the experimental group/mean A value of the solvent control group)×100%.

3.2 Statistical methods

The data were processed statistically, and the results of the measurement data were expressed by $\bar{x}$ ± s and compared by ANOVA, with $P < 0.05$ being statistically significant.

4. Results

4.1 Changes in cell morphology

After 24 hours of quercetin action, inverted microscope observation of blank control group and solvent control group showed that there was no obvious change in cell morphology and no obvious reduction in cell number. The 20umol/L group showed a little cell shrinkage and rounding; 40umol/L group showed vacuolation phenomenon, a little cell floating on the cell surface. The 80umol/L group showed obvious reduction in cell number, and a large part of cells could be seen to be detached from the wall and suspended, or even disintegrated into fragmentation.

4.2 Changes in cell growth inhibition after quercetin action

Comparing the solvent control group with the blank control group, there was no significant difference in the A492 value ($P>0.05$), indicating that the solvent DMSO did not affect the proliferation of Hela cells. The proliferation of Hela cells was inhibited by different concentrations of quercetin (20umol/L, 40umol/L, 80umol/L) for 24h, and the inhibition rate increased with the increase of quercetin concentration. The difference between each group and the solvent control group was statistically significant ($p<0.05$) (See Table 2).

Table 2: Proliferation inhibitory effects of quercetin on HeLa cells as detected by MTT assay ($\bar{x}$ ± s)

Group Qu (μmol/L)	A492	Inhibition rate (%)
Blank control	0.990±0.009	-
Solvent control	0.981±0.010	0
20	0.643±0.074 **	34.76
40	0.537±0.082 **	46.13
80	0.372±0.068 **	62.47

Comparison with solvent control * $p<0.05$ ** $p<0.01$.

4.3 RT-PCR results

Take 5μl of RNA, add 95ul of DEPC water and mix well, and measure the OD value by UV photometer colorimetrically, and the OD260/OD280 between 1.7-2.0 can be used. RNA concentration = OD260×dilution/25(ug/ul). After measuring the OD260 and OD280 values of RNA samples, it was concluded that the ratios of all samples were greater than 1.8, which was in line with the requirements of RNA extraction, and electrophoresis appeared three complete bands (5S, 18S, 28S), suggesting that the RNA was complete. [2]

5. Discussions

CD44 family transcription products are mainly standard (CD44standard, CD44s) and variant (CD44variant, CD44v).CD44 mediates the adhesion of cells to the ECM through interaction with

ISSN 2706-6819 Vol.5, Issue 10: 53-56, DOI: 10.25236/IJFM.2023.051009

ligands, regulates cell accumulation and migration, and participates in physiological processes such as intracellular proliferative signalling, regulation of embryo formation and development, maintenance of organoids and tissue structure, and wound healing. CD44v is a facilitator of tumorigenesis and metastasis, of which the CD44v6 heterodimer containing exon v6 is the most closely related to tumor metastasis.[3] CD44v is a promoter of tumourigenesis and metastasis, in which CD44v6 isoforms containing exon v6 are most closely related to tumour metastasis. Tumour cells expressing CD44v6 are more likely to enter the lymphatic and circulatory systems, and to undergo vascular infiltration, lymph node metastasis, and distant metastasis.[4] High expression of CD44v6 in postoperative tissue specimens of non-small cell lung cancer and the expression level correlated with poor prognosis of patients. In this experiment, after quercetin treatment, the expression of CD44v6 in cervical HeLa cells decreased with the concentration of quercetin, indicating that quercetin can inhibit the expression of the adhesion molecule CD44v6 in a certain concentration range, which can inhibit the invasive ability of tumour cells.

E-cad plays an important role in the process of tumour infiltration and metastasis. When gene mutation or deletion, transcription or translation malfunction and other mechanisms can lead to E-cad abnormality, cell adhesion function is impaired, which can reduce cell differentiation, thus leading to invasive growth of tumour cells and detachment from the site of origin, and metastasize to local or distant places. At present, E-cad has been confirmed as a cancer cell metastasis inhibitory marker, and studies on breast cancer, gastric cancer, liver cancer, etc. have shown that the degree of tumour differentiation and staging are correlated with E-cad expression. The more poorly differentiated the tumour, the more poorly E-cad is expressed, while the expression of E-cad is more normal in well-differentiated cancer tissues, with better adhesion between cancer cells and less infiltrative growth[5].

6. Conclusions

In this experiment, after HaLe cells were treated with quercetin, the expression of E-cad was enhanced with the increase of quercetin concentration, indicating that quercetin could enhance the expression of E-cad. Based on the conclusion of the previous test of this topic, quercetin can inhibit the proliferation of tumour cells, and in this test, quercetin can affect the expression of adhesion molecules, and it is presumed that quercetin can play a role in inhibiting the invasion and metastasis of tumour cells. It was found by this assay that quercetin inhibited the proliferation in a dose-dependent manner and induced apoptosis in HeLa cells. Quercetin inhibits the expression of CD44v6 in HeLa cells in a dose-dependent manner. Quercetin enhanced the expression of E-cadhefin in HeLa cells in a dose-dependent manner.

References

[1] Li Lin, Zhu Quan, Lu Yin. Adhesion Molecules and Tumour Metastasis [J]. Chinese Pharmacology Bulletin. 2007, 05:568-571

[2] Yiqun Du.Research of preparation and biocompatibility evaluation of As2O3/Fe3O4 composite nanoparticles and its therapeutic effect of thermochemotherapy on cervical cancer [D]. Southeast University. 2007

[3] Lu Yihong. Cause of endometriosis to cell adhesion molecules [J]. Acta Medicinae Sinica. 2002. 03:432-433

[4] Zhou Laiyong. Preliminary study on the expression of SATB1 in non-small cell lung cancer [D]. Southern University of Science and Technology. 2009

[5] Zhang W, Li F, Zhang FY, Li L, Gao YH, Zhou BJ. Expression and significance of MMP-7 and E-cad in breast cancer [J]. Hebei Medicine, 2012, 34(09): 1288-1290