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Luteolin inhibits the colon cancer HT−29 cell proliferation, migration and epithelial−mesenchymal transition: an experimental study

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ABSTRACT

Objective: To study the regulating effect of luteolin on colon cancer HT-29 cell proliferation, migration and epithelial-mesenchymal transition. **Methods:** Colon cancer HT-29 cells were cultured and randomly divided into two groups, control group were treated with serum-free medium without drugs and LUT group were treated with serum-free medium containing luteolin. After 24 h of treatment, cells were collected to extract RNA, and then fluorescent quantitative PCR method was used to determine the mRNA expression of proliferation genes, migration genes and epithelial-mesenchymal transition genes. **Results:** After 24 h of luteolin treatment, Lrig1, TSPYL5, Bim, SOX15 and DLC1 mRNA expression in LUT group were significantly higher than those in control group while RPS15a, Bad, TRPV5, TRPV6, PLD2, IBP, SphK1, FAK, Vimentin and N-cadherin mRNA expression were significantly lower than those in control group. **Conclusion:** Luteolin has inhibiting effect on colon cancer HT-29 cell proliferation, migration and epithelial-mesenchymal transition.

1. Introduction

Colon cancer is a common digestive tract malignancy in China and trends to occur in the younger. At present, surgical resection is the main method to treat colon cancer in clinic, but the long-term recurrence and metastasis of tumor may have adverse effect on postoperative survival rate[1]. The proliferation and migration of cancer cells are closely related to tumor recurrence and metastasis, and inhibiting the proliferation and migration of cancer cells is an important target for the treatment of colon cancer. Luteolin is a kind of flavonoid widely existing in many Chinese herbal medicines. Luteolin exists in chrysanthemum, scutellaria barbata, honeysuckle and other Chinese medicines. In recent years, the anti-inflammatory, anti-tumor and antioxidant value of flavonoids has received more and more attention, and many abroad studies have also confirmed that luteolin can inhibit the proliferation and invasion of gastric cancer, colon cancer, esophageal cancer and other gastrointestinal malignant tumor cells[2−4]. However, it is not clear about the effect of luteolin on colon cancer in China. In the following studies, we specifically analyzed the effect of luteolin on the proliferation, migration and epithelial-mesenchymal transition of HT-29 colon cancer cells.

2. Experimental materials and methods

2.1 Experimental materials

HT-29 colon cancer cell lines were bought in ATCC Cell Company, luteolin was bought in Sinopharm Chemical Reagent Co., Ltd., cell culture medium, fetal bovine serum and trypsin were purchased from Gibco Company, and Trizol lysate, reverse transcription kit and fluorescence quantitative PCR kit were purchased from Invitrogen Company.

2.2 Experimental methods

2.2.1 Cell culture and treatment

Colon cancer HT-29 cells were cultured in culture medium

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containing 10% fetal bovine serum, the cells were digested by trypsin after the density reached 80%, and the digested cells were inoculated in culture plate and treated in groups. Control group were treated with serum-free culture medium containing no drug, and LUT group were treated with serum-free culture medium containing 100 μmol/L luteolin.

2.2.2 Gene expression detecting

The cells were treated with different conditions for 24 h, then the culture medium was abandoned, the cells were kept and broken by Trizol lysate from Invitrogen Company, and the RNA in cells was separated and synthesized into cDNA with reverse transcription kit; the specific primers for Lrig1, TSPYL5, Bim, RPS15a, Bad, TRPV5, TRPV6, PLD2, SOX15, DLC1, IBP, SphK1, FAK, Vimentin and N-cadherin were designed, cDNA samples received fluorescence quantitative PCR amplification, the Ct value was read according to amplification curve and the mRNA expression was calculated according to the $2^{-\Delta\Delta Ct}$ formula.

2.3 Statistical methods

SPSS 19.0 software was used for t test of the differences in measurement data between two groups and $P<0.05$ indicated statistical significance in differences in test results.

3. Results

3.1 Cell proliferation gene expression

Analysis of proliferation genes Lrig1, TSPYL5, Bim, RPS15a and Bad mRNA expression in two groups of cells was as follows: Lrig1, TSPYL5 and Bim mRNA expression in LUT group were significantly higher than those in control group while RPS15a and Bad mRNA expression were significantly lower than those in control group. Differences were statistically significant in Lrig1, TSPYL5, Bim, RPS15a and Bad mRNA expression in two groups of cells ($P<0.05$).

3.2 Cell migration gene expression

Analysis of migration genes TRPV5, TRPV6, PLD2, SOX15 and DLC1 mRNA expression in two groups of cells was as follows: TRPV5, TRPV6 and PLD2 mRNA expression in LUT group were significantly lower than those in control group while SOX15 and DLC1 mRNA expression were significantly higher than those in control group. Differences were statistically significant in TRPV5, TRPV6, PLD2, SOX15 and DLC1 mRNA expression in two groups of cells ($P<0.05$).

3.3 Cell epithelial–mesenchymal transition gene expression

Analysis of epithelial-mesenchymal transition genes IBP, SphK1, FAK, Vimentin and N-cadherin mRNA expression in two groups of cells was as follows: IBP, SphK1, FAK, Vimentin and N-cadherin mRNA expression in LUT group were significantly lower than those in control group. Differences were statistically significant in IBP, SphK1, FAK, Vimentin and N-cadherin mRNA expression in two groups of cells ($P<0.05$).

4. Discussion

Luteolin is a natural flavonoid with antitumor activity, and its effect on malignant biological behavior of colon cancer cells is not clear. Malignant proliferation is an important biological feature

Table 1.

Effect of luteolin treatment on proliferation gene expression in cells.

Groups	*n*	Lrig1	TSPYL5	Bim	RPS15a	Bad
LUT group	6	2.66±0.41	2.79±0.38	2.25±0.37	0.41±0.07	0.33±0.06
Control group	6	1.03±0.15	1.01±0.14	0.98±0.12	1.02±0.18	1.04±0.16
t		15.689	17.209	13.278	12.117	22.148
P		<0.05	<0.05	<0.05	<0.05	<0.05

Table 2.

Effect of luteolin treatment on migration gene expression in cells.

Groups	*n*	TRPV5	TRPV6	PLD2	SOX15	DLC1
LUT group	6	0.29±0.06	0.36±0.08	0.44±0.05	2.41±0.36	2.89±0.47
Control group	6	1.01±0.16	0.97±0.14	1.05±0.17	1.02±0.14	1.04±0.18
t		26.498	17.927	12.485	14.518	17.508
P		<0.05	<0.05	<0.05	<0.05	<0.05

Table 3.

Effect of luteolin treatment on epithelial-mesenchymal transition gene expression in cells.

Groups	*n*	IBP	SphK1	FAK	Vimentin	N-cadherin
LUT group	6	0.29±0.06	0.46±0.08	0.39±0.05	0.24±0.06	0.45±0.08
Control group	6	1.04±0.17	0.96±0.14	1.01±0.15	0.99±0.14	1.06±0.20
t		26.575	11.386	17.698	23.147	13.485
P		<0.05	<0.05	<0.05	<0.05	<0.05

of colon cancer cells, and multiple proliferation-related genes are associated with this biological feature. The products encoded by Lrig1 gene are transmembrane glycoproteins with repetitive leucine zipper structure, which can bind to EGFR and antagonize the cell growth effect mediated by it so as to inhibit cell proliferation[5]; TSPYL15 is a new tumor suppressor gene discovered in recent years. It plays an important role in maintaining cell morphology and viability, and TSPYL15 is lowly expressed in many malignant tumors; Bim is a Bcl-2 family member with pro-apoptosis and anti-proliferation activity, which can promote the release of cytochrome C from mitochondria into cytoplasm, and start the apoptosis cascade reaction[6]. RPS15a is a type of ribosomal protein involved in the composition of ribosomal small subunit, which can interact with translation initiation factor eIF-4F and promote the expression of various cyclin to accelerate cell cycle process and contribute to cell proliferation[7]; Bad is a Bcl-2 family member with anti-apoptosis and pro-proliferation activity, which can inhibit the activity of various pro-apoptotic molecules in Bcl-2 family and inhibit apoptosis so as to promote cell proliferation[8]. In order to define the luteolin impact on colon cancer cell proliferation, the above proliferation gene expression was analyzed in the study, and the results showed that Lrig1, TSPYL5 and Bim mRNA expression in LUT group were significantly higher than those in control group while RPS15a and Bad mRNA expression were significantly lower than those in control group. This indicates that the luteolin can increase the expression of anti-proliferation genes and decrease the expression of pro-proliferation genes so as to reduce the proliferation activity of colon cancer cells.

Migration and movement are other important malignant biological behaviors of colon cancer cells, and various migration-related molecules are related to this biological behavior. TRPV5 and TRPV6 are the TRP family members participating in the intracellular and extracellular calcium ion transport, the molecules themselves are highly selective calcium ion channels, and they can actively transport the extracellular calcium ion into the intracellular and regulate cell migration process through the biological effect of calcium ions[9]; the high expression of TRPV5 and TRPV6 can promote the migration of cancer cells during the malignant transformation of colonic mucosal epithelial cells[10]. PLD2 is a kind of phospholipase that can on the one hand, activate the downstream Wnt/ β -catenin pathway and promote cancer cell migration and movement, and on the other hand, increase the expression of a variety of MMPs, promote the hydrolysis of extracellular matrix and contribute to cell migration[11,12]. SOX15 is a member of the transcription factors SOX family, which contains HMG conservative motif and can identify ATATCCT sequences and play the role of transcriptional repressor to inhibit the expression of downstream pro-migration and invasion molecules and prevent cell migration. DLC1 is the molecule that regulates the activity of Rho GTP kinase, which can convert the active Rho-GTP kinase that binds to GTP into the inactive Rho-GTP kinase that binds to GDP, and thus inhibit cell migration[13]. In order to define the luteolin impact on colon cancer cell migration, the above migration gene expression was analyzed in the study, and the results showed that TRPV5, TRPV6 and PLD2 mRNA expression in LUT group were significantly lower than those in control group while SOX15 and DLC1 mRNA expression were significantly higher than those in control group. This indicates that luteolin can increase the expression of anti-migration genes and decrease the expression of pro-migration genes so as to reduce the migration activity of colon cancer cells.

Epithelial mesenchymal transition is an important biological pathway for colon cancer cells to move, migrate to and invade the surrounding tissue, the process is characterized by the transition from epithelial phenotype of cells to mesenchymal phenotype of cells, the intercellular polarity and adhesion mediated by epithelial phenotype will be weakened, and the cells obtain mesenchymal phenotype and easily leave the primary lesion and migrate to adjacent tissue[14]. IBP is a molecule with guanine nucleotide exchange activity, which can regulate cytoskeleton form and cadherin transcription through the downstream Rac1, RhoA, Cdc42 and other molecules, then promote the cell transition from epithelial phenotype to mesenchymal phenotype, and enhance epithelial mesenchymal transition process; FAK and SphK1 are the molecules that can interact with each other and affect cellular epithelial mesenchymal transition, SphK1 is able to adjust the content of sphingosine and promote the activation of FAK, and the activated FAK can catalyze tyrosine residue phosphorylation to start the activation of downstream signaling molecules, and thus accelerate the cellular epithelial mesenchymal transition process[15,16]. Vimentin and B-cadherin are markers of mesenchymal phenotype cells, and the above two markers are highly expressive during the enhancement of epithelial mesenchymal transition[17,18]. In order to define the luteolin effect on epithelial mesenchymal transition of colon cancer cells, the above epithelial mesenchymal transition gene expression was analyzed in the study, and the results showed that IBP, SphK1, FAK, Vimentin and N-cadherin mRNA expression in LUT group were significantly lower than those in control group. This indicates that the luteolin can regulate the expression of epithelial mesenchymal transition regulation genes and marker genes so as to inhibit the epithelial mesenchymal transition of colon cancer cells.

Above all, it can be concluded that luteolin can regulate the expression of proliferation genes, migration genes and epithelial mesenchymal transition genes in colon cancer HT-29 cells so as to inhibit the proliferation, migration and epithelial mesenchymal transition of cancer cells.

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