LNCRNA UCA1 REGULATING CELL AUTOPHAGY THROUGH AKT/MTOR SIGNAL PATHWAY FOR INHIBITING COLON CANCER CELL PROLIFERATION, PROMOTING CELL APOPTOSIS, AND INCREASING CELL CYCLE ARREST

Fangfang Lin[1,2], Zhangfa Song[3,*]
[1]Medical College of Zhejiang University, Hangzhou 310011, PR China - [2]Department of Pathology, Jiangshan People's Hospital, Jiangshan 324100, PR China - [3]Department of Anorectal Surgery, Shao Yifu Hospital Affiliated to Zhejiang University Medical College, Hangzhou 310016, PR China

ABSTRACT

Objective*: To analyze long-chain non-coding RNA (lncRNA) urothelial carcinoma-associated 1 (UCA1) regulating cell autophagy through the protein kinase B/rapamycin target protein (AKT/mTOR) signaling pathway, which inhibits colon cancer cell proliferation and promotes apoptosis and cell cycle arrest.*

Methods: *Caco-2 cells were cultured at the same concentration and transfected into si-NC, si-UCA1, and si-UCA1 + 100nmol LRAPA (autophagy inhibitor) groups. Each group was set up with four replicate wells. After 6 h, it was replaced with dual antibodies and the medium of serum to be cultured for 24 h. Cell proliferation was measured using the CCK-8 method in each group. The cell apoptosis and cell cycle changes in each group were detected using flow cytometry, away from light. The expression of microtubule-associated protein light chain 3 (LC3), p62, AKT, and mTOR of cells in each group were determined using Western blotting and PCR.*

Results: *The cell proliferation of each group was the same after 24 h. After longer periods of time, the differences in cell proliferation of each group were more obvious. At 72 h, compared with the Si-nc group, the proliferation ability of the si-uca1 and si-uca1 + Rapa group decreased significantly (P < 0.05), and the proliferation ability of the si-uca1 + Rapa group increased significantly (P<0.05). Compared with the Si-nc group, the apoptosis ability of the si-uca1 and si-uca1 + Rapa group was significantly higher (P<0.05), and the apoptosis ability of the si-uca1 + Rapa group was significantly lower than that of the si-uca1 group (P<0.05). In comparison to the Si-nc group, the G1 phase cells in the si-uca1 group were significantly reduced, and the S phase and G phase cells were significantly increased (P<0.05); compared with the si-uca1 group, the G1 phase cells of the si-uca1 + Rapa group were significantly increased, and the S phase and G phase cells were significantly reduced (P<0.05). In comparison to the Si-nc group, the expression level of LC3 and p62 in the Si UCA1 group was significantly lower, and the expression levels of Akt and mTOR were significantly higher (P<0.05)*

Conclusion: *lncRNA UCA1 may regulate cell autophagy through the AKT/mTOR signaling pathway, thereby inhibiting colon cancer cell proliferation, promoting apoptosis, and blocking the G2 phase of the cell cycle.*

Keywords: *lncRNA UCA1, AMPK/mTOR signaling pathway, cell autophagy, colon cancer, cell proliferation, apoptosis, cell cycle.*

DOI: 10.19193/0393-6384_2021_1_20

Received *November 30, 2019;* ***Accepted*** *January 20, 2020*

Introduction

Colon cancer involves malignant lesions in the colonic mucosa caused by genetic or environmental carcinogens and is a common form of malignant tumors worldwide[(1)]. According to statistics, in 2008, the number of colon cancer patients exceeded 1.2 million. Further, the number of deaths each year since has exceeded 100,000. The 5-year survival rate of patients with colon cancer is significantly reduced, and the 5-year survival rate of patients with advanced forms of the disease is only 8%, which has severely affected people's quality of life and health[(2)]. Therefore, the early diagnosis and treatment of colon cancer is a crucial clinical problem. Some studies have found that the occurrence and development of colon cancer are closely related to various factors such as the regulation of non-coding RNA, epigenetic modifications-such as gene methylation-and various tumor susceptibility genes, such

as p53[3]. Further, urothelial carcinoma associated 1 (UCA1) is a type of long non-coding RNA (lncRNA). Related studies have found that lncRNA UCA1 is abnormally expressed in bladder, breast, colorectal, gastric, hepatocellular carcinoma, and other tumor tissues, and plays an important role in tumor growth and metastasis[4]. According to certain studies, lncRNA UCA1 can promote the proliferation of breast cancer cells by inhibiting the expression of p27, but its role in colon cancer cells and its mechanism are still not clear[5]. To investigate both its role and mechanism in colon cancer cells, the following steps are conducted.

Materials and methods

Experimental cells

Caco-2 cells (Shanghai Boyan Biotechnology Co., Ltd.).

Experimental instruments and reagents

DMEM medium (Shanghai Limin Industrial Co., Ltd.); lipopolysaccharide (Shanghai Jingbang Industry Co., Ltd.; specification: 10 g); CCK-8 detection kit (Shanghai Huzhen Industrial Co., Ltd.); cell apoptosis detection kit (Beijing Biolab Technology Co., Ltd.); Flow cytometry (Miltenfa Biotech, Germany; type: MACSQuant 10); Beclin-1 antibody (Shanghai Caiyou Industrial Co., Ltd.); LC3 antibody (Shanghai Huabi Biotechnology Co., Ltd.); AKT antibody (Shanghai Yubo Biotechnology Co., Ltd.); mTOR antibody (Tuofei Biotechnology Co., Ltd.); Real-time quantitative PCR analyzer (Beijing Chenxi Yongchuang Technology Co., Ltd.; type: MA-1600Q); autoclave (Shanghai Boxun Medical Biological Instrument Co., Ltd.; type: BXM-150M); inverted biological microscope (Shanghai Cai Kang Optical Instrument Factory; type: XSP-7CC); and cryogenic high-speed centrifuge (Eppendorf China Co., Ltd.; type: 5427 R).

Experimental methods and observational indicators

Cell culture

Caco-2 cells were cultured in the DMEM cell culture medium. When the cells were cultured to about 75%, 2 mL of trypsin was added for digestion for about 15 s. When the cells became round and the intercellular space became large, fresh medium was added, a cell suspension was made, and the culture was put into the incubator for cultivation.

Cell transfection

10000 cells/well were inoculated into six-well plates in the good growth phase and in the logarithmic growth phase, and cultured at room temperature until the cell density reached about 75%. si-NC, si-UCA1, and si-UCA1 + 100nmol/LRAPA (autophagy inhibitor) groups were transfected at the same concentration, and four replicate wells were set in each group. After 6 h, the cultures were switched to the medium containing double antibodies and serum, and culturing was continued for 24 h in subsequent experiments.

Cell proliferation

The cell proliferation of each group was measured using the CCK-8 method. A cell suspension in the well-growth and logarithmic growth phase was seeded in a 96-well plate at 100 μL/well. After 24 h, 48 h, 72 h, 96 h, and 120 h, 10 μL of CCK-8 solution was added to each well, and the culture was continued for 2 h; further, the absorbance at 470 nm was measured.

Apoptosis

The cell suspension in the well-growth and logarithmic growth phase was inoculated into a six-well plate, resuspend adding a phosphate buffer, and cell apoptosis was detected in each group at room temperature, away from light, using flow cytometry.

Cell cycle

A cell suspension in a good growth state and in the logarithmic growth phase was uniformly seeded into a six-well plate, transfected when the cells grew to about 70%, and the cell cycle of each group was measured by flow cytometry

Variety

Cells were seeded into six-well plates, and cell transfection was performed when the cell density grew to about 75% to obtain si-NC and si-UCA1. The microtubule-associated protein light chain 3 (Light chain 3 (LC3)), p62, AKT, and mTOR expression in each group of cells was determined by Western blotting and PCR.

Statistical methods

The comparison of the enumeration data in this group of studies was performed using χ^2, expressed as [n (%)]. Measurement data were compared using independent sample t-tests between the two groups, and multiple sample averages were used between

groups, all of which were expressed as ($\bar{x}\pm s$). Cell proliferation was measured using the CCK-8 method. The cell apoptosis and cell cycle changes were measured using flow cytometry, away from light. The expressions of LC3, p62, AKT, and mTOR in each group of cells were determined using Western blotting and PCR. In this group of studies, SPSS 20.0 software was used for statistical data analysis, and the statistical result P<0.05 was considered as statistically significant.

Results

Comparison of cell proliferation in each group

The cell proliferation of the cells in each group was the same at 24 h. In longer periods of time, the cells in each group proliferated significantly.

At 72 h, in comparison to the si-NC group, the cell proliferation ability of the si-UCA1 and si-UCA1 + RAPA groups increased significantly (P<0.05); compared with the si-UCA1 group, the cell proliferation ability of the si-UCA1 + RAPA group was significantly higher (P<0.05). This information can be seen in Table 1.

Groups	Cell proliferation				
	24h	48h	72h	96h	120h
si-NC group	0.23±0.01	0.64±0.06	1.49±0.05	2.74±0.09	3.15±0.11
si-UCA1 group	0.23±0.01	0.52±0.06	1.02±0.03[a]	1.61±0.12[a]	2.19±0.10[a]
si-UCA1 +RAPA group	0.23±0.01	0.59±0.07	1.19±0.02[b]	2.11±0.08[b]	2.84±0.13[b]
F	0.00	3.60	178.84	133.13	73.86
P	1.000	0.071	<0.001	<0.001	<0.001

Table 1: Comparison of cell proliferation in each group ($\bar{x}\pm s$).

Note: a denotes [a]P<0.05, compared with the si-NC group; b denotes [b]P<0.05, compared with the si-UCA1 group.

Comparison of cell apoptosis in each group

Compared with the si-NC group, the apoptosis ability of the si-UCA1 and si-UCA1+RAPA groups was significantly increased (P<0.05); the cell proliferation ability of the si-UCA1+RAPA group was significantly lower than that of the si-UCA1 group (P <0.05). This can be seen in Table 2.

Group	Cell apoptosis (%)
si-NC group	3.46±0.19
si-UCA1group	7.69±0.25[a]
si-UCA1+RAPA group	5.01±0.37[b]
F	233.36
P	<0.001

Table 2: Comparison of cell apoptosis in each group ($\bar{x}\pm s$).

Note: a denotes [a]P<0.0, compared with the si-NC group; b denotes [b]P<0.05, compared with the si-UCA1 group.

Comparison of cell cycle changes in each group

Compared with the si-NC group, the G1-phase cells in the si-UCA1 group were significantly reduced, and the S-phase and G-phase cells were significantly increased (P<0.05); compared with the si-UCA1 group, cells in the si-UCA1 + RAPA group in the G1 phase were increased significantly, and cells in S and G phases were significantly decreased (P<0.05). This is shown in Table 3.

Groups	Cell cycle (%)		
	G1phase	S phase	G phase
si-NC group	51.46±2.37	34.17±1.72	15.49±2.33
si-UCA1 group	33.15±2.94[a]	49.62±4.03[a]	24.41±2.16[a]
si-UCA1+RAPA group	45.55±3.28[b]	39.11±1.28[b]	19.29±1.44[b]
F	41.88	38.85	19.76
P	<0.001	<0.001	0.001

Table 3: Comparison of cell cycle changes in each group ($\bar{x}\pm s$).

Note: a denotes [a]P<0.0, compared with the si-NC group; b denotes [b]P<0.05, compared with si-UCA1 group.

Comparison of LC3, p62, AKT, and mTOR expression of cells in each group

Compared with the si-NC group, the expression levels of LC3 and p62 in the si-UCA1 group were significantly reduced, and the expression levels of AKT and mTOR were significantly increased (P<0.05). This is shown in Figure 1 and Table 4.

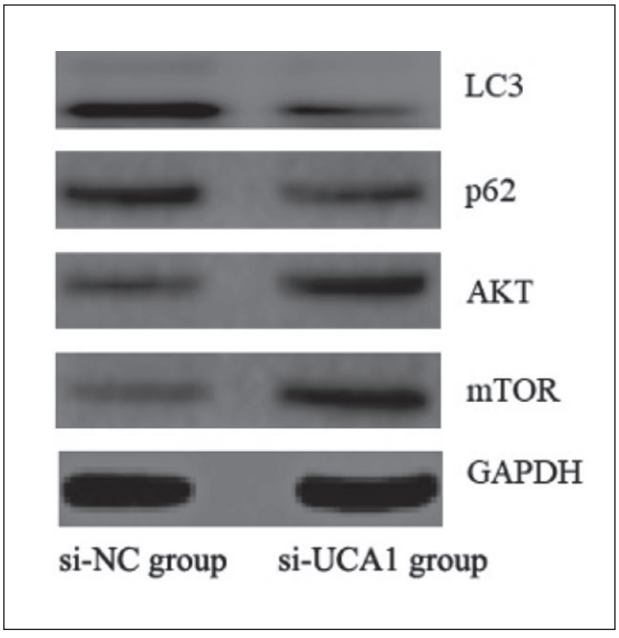

Figure 1: LC3, p62, AKT, and mTOR expression of cells in each group.

Group	LC3	p62	AKT	mTOR
si-NC group	1.01±0.02	1.00±0.03	1.00±0.02	1.01±0.01
si-UCA1 group	0.21±0.03	0.17±0.01	4.15±1.13	8.23±1.79
t	44.376	52.494	5.574	8.067
P	<0.001	<0.001	0.001	<0.001

Table 4: Comparison of LC3, p62, AKT, and mTOR expression of cells in each group ($\bar{x}\pm s$).

Discussion

Colon cancer is a common malignant tumor in the digestive tract, and its diagnosis rate ranks third among common human cancers. Currently, surgical treatment is the most important treatment method for colon cancer. However, owing to the early hidden onset of colon cancer, most patients are in the middle and advanced stages at the time of diagnosis, losing out on significant treatment time. Therefore, the clinical treatment of colon cancer is often supplemented with chemotherapy after surgery. With the development of medical technology, the number of effective treatment methods for colon cancer has gradually increased, but the 5-year survival rate of patients is still low. Therefore, screening new molecular compounds to inhibit tumor growth, along with studying the changes in apoptosis and cell cycles-especially their specific mechanisms-through chemical genetics methods can promote drug development, laying a theoretical foundation for tumor treatment.

lncRNA is an RNA molecule that has wide applicability and can be used in several aspects of cell physiology, playing an important role in the formation, progression, and metastasis of cancer[6]. UCA1 is a type of lncRNA. Studies have found that the expression of lncRNA UCA1 in bladder cancer tissue is significantly higher than that in normal paracancerous tissue[7]. In addition, lncRNA UCA1 can also affect the activity of tumor cells through the phosphatidylinositol 3 kinase/protein kinase B (PI3K/AKT) signal pathway, heteronuclear riboprotein1, hnRNP1, microRNA -216b (Microrna-216b, miR-216b) among others[8]. lncRNA UCA1 can be used as a potential biomarker and therapeutic target for human cancer.

Proliferation and apoptosis are important activities in the process of cell growth, and cells always enter physiological states such as proliferation, differentiation, aging, and apoptosis during the change of cyclical matter. Some studies have found that certain lncRNAs play the important role of regulating pathological processes such as tumor cell proliferation and metastasis[9]. Apoptosis and autophagy have different morphological characteristics and physiological processes but have a very close relationship. Studies have found that, under certain conditions, apoptosis and autophagy can promote each other[10]. Cell autophagy is a catabolic balance mechanism caused by the phagocytosis and degradation of abnormal organelles, proteins, and pathogens by acid lysosomes in the cytoplasm[11]. It is widely found in eukaryotes and is a type of programmed cell death, participating in the regulation of various life activities such as cell survival and death, thereby maintaining the normal physiological state of cells. In recent years, an increasing number of studies have found that autophagy is an important factor in the growth of tumors through certain signaling pathways[12].

AKT is a threonine/serine protein kinase, located at the important intersection of multiple signal pathways, and usually exists in the regulatory network of eukaryotic cells. Studies have found that the expression level of AKT has an important relationship with the occurrence and development of tumors, and it can play the role of regulating the activation status of various downstream molecules[13]. mTOR is an important substrate of AKT and mainly exists in the form of mTORC1 and mTORC2 protein complexes in vivo. Many studies have found that mTOR is highly expressed in various tumor tissues such as lung cancer and liver cancer[14]. mTOR affects the process of initiating protein translation in cells, and it can provide cells with all the required substances to enter from the G0/G1 phase into the S phase. According to reports, when the AKT/mTOR signaling pathway is activated, cell proliferation is out of control, and cells cannot enter the normal apoptotic process, which significantly increases the chance of canceration of the cells[15]. Zhuang et al.[16] found that, after normal cells were exposed to carcinogens, the AKT/m TOR signaling pathway could be rapidly activated. This could lead to cells proliferating excessively, and the thresholds for apoptosis of damaged cells significantly increasing.

In this study, Caco-2 cells were treated, and the cell proliferation, apoptosis, and cell cycle changes were measured using CCK-8 and flow cytometry, respectively. The expressions of LC3, p62, ATG3, AKT, and mTOR were measured using PCR methods. It was found that the down-regulation of lncRNA UCA1 can significantly inhibit cell autophagy and cell proliferation, promoting apoptosis and the G2 phase arrest of the cell cycle. The AKT/mTOR

signaling pathway could be involved in the regulation of cell autophagy by UCA1.

In conclusion, lncRNA UCA1 could regulate cell autophagy through the AKT/m TOR signaling pathway, thereby inhibiting colon cancer cell proliferation, promoting cell apoptosis, and blocking the cell cycle in the G2 phase.

References

1) Xu J, Liao K, Fu Z, Xiong Z. A new method for early detection of pancreatic cancer biomarkers: detection of microRNAs by nanochannels. Artif Cells Nanomed Biotechnol 2019; 47: 2634-2640.
2) Schmuck R, Gerken M, Teegen EM, Krebs I, Klinkhammer-Schalke M, et al. Gender comparison of clinical, histopathological, therapeutic and outcome factors in 185,967 colon cancer patients. Langenbecks Arch Surg 2020; 405: 71-80.
3) Nakayama H, Ohuchida K, Yonenaga A, Sagara A, Ando Y, et al. S100P regulates the collective invasion of pancreatic cancer cells into the lymphatic endothelial monolayer. Int J Oncol 2019; 55: 211-222.
4) Xiao C, Wu CH, Hu HZ. LncRNA UCA1 promotes epithelial-mesenchymal transition (EMT) of breast cancer cells via enhancing Wnt/beta-catenin signaling pathway. Eur Rev Med Pharmacol Sci 2016; 20: 2819.
5) Wang W, Yin Z. Diagnostic value of long non-coding RNA H19, UCA1, and HOTAIR as promising biomarkers in human bladder cancer. Int J Clin Exp Pathol 2017; 10: 11659-11665.
6) Cui M, Chen M, Shen Z, Wang R, Fang X, et al. LncRNA-UCA1 modulates progression of colon cancer through regulating the miR-28-5p/HOXB3 axis. J Cell Biochem 2019; 120: 376-388.
7) Wang Y, Zhang H, Li X, Chen W. Differential expression profile analysis of lncRNA UCA1α regulated mRNAs in bladder cancer. J Cell Biochem 2018; 119: 1841-1854.
8) Ananthanarayanan M. A novel long non-coding RNA (lncRNA) regulating Cholesterol and Bile acid homeostasis: A new kid on the block and a potential Therapeutic Target? Hepatol 2016; 64: 16-18.
9) Lee KH, Lee MS, Cha EY, Sul JY, Lee JS, et al. Inhibitory effect of emodin on fatty acid synthase, colon cancer proliferation and apoptosis. Mol Med Rep 2017; 15: 2163-2173.
10) Wang B, Qiao L, Shi Y, Feng X, Chen D, et al. ASPP2 inhibits oxaliplatin-induced autophagy and promotes apoptosis of colon cancer cells. Xi bao yu fen zi mian yi xue za zhi 2015; 31: 898-904.
11) He K, Zheng X, Li M, Zhang L, Yu J. mTOR inhibitors induce apoptosis in colon cancer cells via CHOP-dependent DR5 induction upon 4E-BP1 dephosphorylation. Oncogene 2016; 35: 148-157.
12) Hamraz M, Abolhassani R, Andriamihaja M, Ransy C, Lenoir V, et al. Hypertonic external medium represses cellular respiration and promotes Warburg/Crabtree effect. FASEB J 2020; 34: 222-236.
13) Song G, Xu S, Zhang H, Wang Y, Xiao C, et al. TIMP1 is a prognostic marker for the progression and metastasis of colon cancer through FAK-PI3K/AKT and MAPK pathway. J Exp Clin Cancer Res 2016; 35: 148.
14) Jahanafrooz Z, Mosafer J, Akbari M, Hashemzaei M, Mokhtarzadeh A, et al. Colon cancer therapy by focusing on colon cancer stem cells and their tumor microenvironment. J Cell Physiol 2020; 235: 4153-4166.
15) Dai Z, Wang L, Wang X, Zhao B, Zhao W, et al. Oxymatrine induces cell cycle arrest and apoptosis and suppresses the invasion of human glioblastoma cells through the EGFR/PI3K/Akt/mTOR signaling pathway and STAT3. Oncol Rep 2018; 40: 867-876.
16) Zhuang B, Min ZJ, Ni X, Wang TF, Wu DJ, et al. Inhibitory effects of lncRNA AK126393 on colon cancer cells and related mechanisms. J Mod Oncol 2019; 27: 924-928.

Corresponding Author:
Zhangfa Song
Email: zpty71@163.com
(China)