Research Paper

The Mechanism of Resveratrol on Colorectal Cancer by Regulating AMPK/PGC-1-Alpha Signaling Pathway

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The objective of this study is to examine the impact of resveratrol on the activation of the AMP-activated protein kinase/peroxisome proliferator gamma receptor coactivator 1-alpha mechanism of signal pathway on colorectal cancer. Set up blank control group, colorectal cancer group and resveratrol group. The proliferation ability of three groups of cells was assessed using cell counting kit 8, protein expression was detected using Western blot, the expression level of related messenger ribonucleic acid was determined using quantitative polymerase chain reaction, and the migration and invasion ability of cells was assessed using the Transwell method. The resveratrol group exhibited a significantly elevated expression level of apoptotic proteins Fas, Bcl-2-associated X protein, and Fas ligand compared to the colorectal cancer group, and the expression level of B-cell lymphoma 2 was found to be significantly reduced compared to the colorectal cancer group. In the colorectal cancer group alpha, the relative expression of messenger ribonucleic acid and protein for mitogen-activated protein kinase and peroxisome proliferator-activated receptor-gamma coactivator was found to be significantly lower compared to the blank control group; the relative expression of messenger ribonucleic acid and protein in the resveratrol group cells, specifically mitogen-activated protein kinase and peroxisome proliferator gamma receptor coactivator 1-alpha, was found to be significantly higher compared to the colorectal cancer group. Resveratrol exhibits the potential to diminish the viability of colorectal cancer cells, impede the growth and metastasis of such cells, as well as prompt apoptosis in colorectal cancer cells. The mechanism potentially exhibits a correlation between the regulations of AMP-activated protein kinase/ peroxisome proliferator gamma receptor coactivator 1-alpha signaling pathway.

Key words: Resveratrol, protein kinase, signaling pathway, colorectal cancer, radiotherapy, chemotherapy, cytokines

Recently, the prevalence of colorectal cancer has been steadily rising due to the shifting patterns in individuals' lifestyles, dietary practices, occupational settings, and it has become a common malignant tumor of digestive tract in clinic[1]. At present, the clinical treatment of patients with colorectal cancer is still lack of radical treatment, mainly radiotherapy and chemotherapy, and surgical resection. In recent years, with the progress of targeted therapy, more and more malignant tumors begin to use targeted therapy in clinical treatment[2]. Therefore, in-depth study of the pathogenesis and etiology of colorectal cancer, looking for new treatment targets, to improve patient's clinical symptoms, slow down the progression of cancer, improve the quality of life of patients has important clinical significance. Resveratrol is a non-flavonoid polyphenol organic compound, which has good anti-tumor, anti-oxidative stress, anti-inflammatory, antibacterial and inhibitory effects on cytokines. The role of resveratrol in the prevention and treatment of gastric and lung cancer has been identified[3]. In addition, AMP Activated Protein Kinase/ Peroxisome Proliferator Activated Gamma Receptor Coactivator 1-Alpha (AMPK/PGC-1α)

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signal pathway is closely related to tumorigenesis and development, and is an important intracellular pathway for promoting proliferation and anti-apoptosis[4]. At present, resveratrol has been found to have a certain inhibitory effect on colorectal cancer, but the specific mechanism has not been fully explained. Based on this, this study explored the mechanism of resveratrol on colorectal cancer by regulating AMPK/PGC-1α signaling pathway, to facilitate the identification of a prospective therapeutic target for the clinical management of colorectal cancer.

MATERIALS AND METHODS

Materials and reagents:

Human normal intestinal mucosal cell line (FHC) and colorectal cancer HCT116 cells were from American Type Culture Collection (ATCC) cell bank. Resveratrol is purchased from Chengdu Munster Biotechnology Co., Ltd. AMPK, PGC-1α messenger Ribonucleic Acid (mRNA) primers and Beta (β)-actin primers (American Sigma company); immunohistochemically sheep anti-rabbit second antibody, Cell Counting Kit-8 (CCK-8) detection kit, quantitative Polymerase Chain Reaction (qPCR) detection kit, Annexin V- Fluorescein Isothiocyanate (FITC)/Propidium Iodide (PI) apoptosis kit apoptosis kit (Shanghai Biyuntian Co., Ltd.); Transwell chamber (Corning Co., Ltd.); artificial reconstructed basement membrane glue (Matrigel) purchased from American BD company; AMPK, PGC-1α, Fas, Bcl-2-Associated X Protein (BAX), Fas Ligand (FasL), B-Cell Lymphoma 2 (BCL-2) antibodies (Abcam Biotechnology Co., Ltd.).

Methods:

Cell culture and treatment: Following resuscitation, the cells were incubated with Dulbecco's Modified Eagle Medium (DMEM) medium supplemented with 10 % fetal bovine serum, and subsequently incubated in a constant temperature incubator (37°, 5 % Carbon dioxide (CO_2)). Set blank control group, colorectal cancer group and resveratrol group. The cells in the blank control group were human normal intestinal mucosal cell line (FHC) without any treatment, the cells in the colorectal cancer group were HCT116 cells without any treatment, and the cells in the resveratrol group were cultured in the culture box for 48 h after adding 15 μg/ml of resveratrol. Repeat the experiment 6 times each time.

Detection of protein expression by Western blot: Colorectal cancer HCT116 cells were cultured in the incubator after 15 μg/ml resveratrol was added to the incubator for 48 h. After, each group of cells were homogenized at 4° to make 10 % homogenate, the protein concentration was determined by Bicinchoninic Acid (BCA) assay method, gel preparation, electrophoresis 90 min, gel cutting, transmembrane 90 min and milk sealing. Following the cleaning process, the samples were subjected to incubation with primary antibodies targeting AMPK, PGC-1α, BAX, FasL, Fas and Bcl-2, as well as secondary antibodies. Subsequently, the samples were developed and the obtained results were analyzed using Bio-Rad image laboratory software.

qPCR to detect the expression level of related mRNA: Colorectal cancer HCT116 cells were cultured in the incubator after adding resveratrol 15 μg/ml for 48 h. Each group of cells used RNA extraction kit to extract RNA, One Step Prime Script microRNA (miRNA) complimentary Deoxyribonucleic Acid (cDNA) synthesis kit was used to reverse transcription miRNA into cDNA, and miRNA fluorescence qPCR detection kit was used for quantitative real-time PCR. Complete the cycle according to the kit instructions. Following the completion of the reaction, the software was utilized to determine the relative expression of AMPK, PGC-1α and mRNA.

Detection of cell proliferation and apoptosis by CCK-8: A 100 l cell suspension is prepared in a 96-well plate and then pre-cultured. After 24, 48 or 72 h of incubation, 10 μl of CCK-8 is added to each sample and incubated for 4 h. Absorbance at 450 nm is measured by a spectrophotometer. The quantified result is equal to (Optical Density (OD) measurement of the experimental group-OD measurement of the control group))/(negative control-Mock).

Detection of cell migration and invasion by Transwell method: After the colorectal cancer HCT116 cells were added with resveratrol 15 μg/ml and cultured for 48 h, each group was adjusted to have a cell density of 5×10^5 cells/well inoculated in the upper chamber of the Transwell, in the lower chamber, medium containing 10 % fetal bovine

serum was added. The control group was given the same amount of DMEM culture medium, fixed, stained and then microscopically count the purple stained perforated cells and calculate the cell migration ability. For the detection of cell invasion ability, the upper chamber of the Transwell was first covered with Matrigel in an ultra-clean bench, and the subsequent steps were the same as that of cell migration.

Statistical methods:

Statistical analysis and processing of data were carried out using the Statistical Package for the Social Sciences (SPSS) 22.0 statistical software, and the measurement information was expressed by (x±s), and the comparison was made by t-test. Compared with blank control group, $^{a}p<0.05$ and compared with colorectal cancer group, $^{b}p<0.05$.

RESULTS AND DISCUSSION

The cell proliferation activity and cell migration observed in the colorectal cancer group were found to be significantly greater compared to those observed in the blank control group ($p<0.05$), while the cell growth activity and cell migration number of resveratrol in colorectal cancer group were significantly lower than those in colorectal cancer group ($p<0.05$) as shown in Table 1.

The incidence of cell invasion in the colon cancer group was significantly greater compared to the blank control group ($p<0.05$), the number of cell invasion in resveratrol group was lower than in colorectal cancer group, and the overall apoptosis rate in resveratrol group was higher compared to the colorectal cancer group ($p<0.05$) as shown in Table 2.

The resveratrol group exhibited significantly elevated expression levels of apoptotic proteins Fas, FasL and BAX compared to the colorectal cancer group. Conversely, the expression level of Bcl-2 in the resveratrol group was significantly diminished in comparison to the colorectal cancer group ($p<0.05$) as shown in Table 3.

The colorectal cancer group exhibited a significantly lower relative expression of MPK and PGC-1α mRNA compared to the blank control group. Conversely, the resveratrol group demonstrated a significantly higher relative expression of MPK and PGC-1α mRNA compared to the colorectal cancer group ($p<0.05$) as shown in Table 4.

The expression levels of MPK and PGC-1α protein were found to be significantly lower in the colorectal cancer group compared to the blank control group. Conversely, the resveratrol group exhibited significantly higher expression levels of MPK and PGC-1α protein compared to the colorectal cancer group ($p<0.05$) as shown in Table 5.

TABLE 1: EFFECT OF RESVERATROL ON PROLIFERATION AND MIGRATION OF COLORECTAL CANCER CELLS

Group	n	Cell proliferative activity	Number of cell migration
Control	6	0.10±0.01	73.25±15.70
Colorectal cancer	6	0.22±0.04[a]	142.74±24.34[a]
Resveratrol	6	0.15±0.03[b]	106.48±17.44[b]
F		25.154	19.022
p		0.000	0.000

Note: Compared with blank control group, $^{a}p<0.05$ and compared with colorectal cancer group, $^{b}p<0.05$

TABLE 2: EFFECTS OF RESVERATROL ON INVASION AND APOPTOSIS OF COLORECTAL CANCER CELLSCELLS

Group	n	Overall apoptosis rate (%)	Number of cell invasion
Control	6	9.97±1.23	51.72±10.31
Colorectal cancer	6	9.51±1.13[a]	121.05±20.26[a]
Resveratrol	6	31.34±4.25[b]	93.12±16.35[b]
F		134.293	26.157
p		0.000	0.000

Note: Compared with blank control group, $^{a}p<0.05$ and compared with colorectal cancer group, $^{b}p<0.05$

TABLE 3: EFFECT OF RESVERATROL ON THE EXPRESSION OF APOPTOTIC PROTEIN IN COLORECTAL CANCER CELLS

Group	n	Fas	BAX	FasL	Bcl-2
Control	6	0.35±0.14	0.37±0.09	0.41±0.14	0.54±0.03
Colorectal cancer	6	0.33±0.17[a]	0.43±0.14[a]	0.44±0.14[a]	0.49±0.04[a]
Resveratrol	6	0.84±0.18[b]	0.86±0.16[b]	0.78±0.12[b]	0.31±0.03[b]
F		13.446	20.294	18.641	72.177
p		0.001	0.000	0.000	0.000

Note: Compared with blank control group, [a]p<0.05 and compared with colorectal cancer group, [b]p<0.05

TABLE 4: EFFECT OF RESVERATROL ON RELATIVE EXPRESSION OF AMPK AND PGC-1α mRNA IN COLORECTAL CANCER CELLS

Group	n	AMPK mRNA	PGC-1α mRNA
Control	6	1.65±0.40	1.76±0.43
Colorectal cancer	6	1.24±0.32[a]	0.83±0.35[a]
Resveratrol	6	2.47±0.54[b]	2.59±0.67[b]
F		12.744	18.451
p		0.000	0.000

Note: Compared with blank control group, [a]p<0.05 and compared with colorectal cancer group, [b]p<0.05

TABLE 5: EFFECT OF RESVERATROL ON MPK AND PGC-1α PROTEIN EXPRESSION IN COLORECTAL CANCER CELLS

Group	n	AMPK	PGC-1α
Control	6	0.57±0.08	0.52±0.09
Colorectal cancer	6	0.28±0.04[a]	0.30±0.03[a]
Resveratrol	6	0.86±0.28[b]	0.76±0.11[b]
F		15.521	45.156
p		0.000	0.000

Note: Compared with blank control group, [a]p<0.05 and compared with colorectal cancer group, [b]p<0.05

Colorectal cancer represents a prevalent form of malignant neoplasms within the digestive system, specifically affecting the colon or rectum, and is associated with significant morbidity and mortality rates[5]. With the aggravation of the aging problem and the change of people's living habits in our country, the incidence of colorectal cancer is gradually increasing, which is a serious threat to people's life and health[6]. At present, the pathogenesis and etiology of colorectal cancer have not been fully explained, but recent studies have found that age, genetic susceptibility, inflammatory reaction, immune disorder, physical and chemical radiation, and other factors are related to the occurrence and development of colorectal cancer[7]. At present, radiotherapy and chemotherapy and surgical resection are mainly used in clinic, although it can help some patients to cure cancer, but some patients are diagnosed later, lose the opportunity of surgical resection of tumor in the late stage, and are not sensitive to radiotherapy and chemotherapy, resulting in limited clinical treatment effect[8]. Colorectal cancer has the characteristics of high incidence, invasiveness, metastatic potential and recurrence rate, therefore, it holds immense clinical importance to specifically target neoplastic cells in individuals diagnosed with colorectal carcinoma[9]. Therefore, in-depth study of the pathogenesis and etiology of colorectal cancer, determine the molecular mechanism of growth and metastasis of colorectal cancer cells, and find new therapeutic targets, it has important clinical significance to improve the clinical symptoms, slow down the pathological progress of colorectal cancer and improve the quality of life. The objective of this study is to explore the mechanism of resveratrol on colorectal

cancer by regulating AMPK/PGC-1α signaling pathway, in order to provide a potential therapeutic target for clinical treatment of colorectal cancer.

Resveratrol is a non-flavonoid polyphenol organic compound, which has good anti-tumor, anti-oxidative stress, anti-inflammatory, antibacterial and inhibitory effects on cytokines[10]. Resveratrol can inhibit tumor angiogenesis, block cell cycle, induce autophagy and apoptosis, and enhance radio sensitivity of tumor cells through PI3K/AKT, AKT/GSK-3β/Snail and AMPK/PGC-1α signaling pathways[11]. It has been found that resveratrol has definite anti-tumor effects on many tumors[12]. In addition, resveratrol cannot only protect normal cells from adverse drug reactions, but also has no or low toxicity to normal cells, so it is expected to become a new generation of antineoplastic drugs[13]. The findings indicate a substantial increase in both cell proliferation activity and cell migration in the colorectal cancer group compared to the blank control group, while the cell proliferation activity and cell migration number of resveratrol in the colorectal cancer group were lower compared to the colorectal cancer group. The colon cancer group exhibited a significantly greater number of cell invasions compared to the blank control group. There is a suggestion that resveratrol possesses the potential to diminish the viability of colorectal cancer cells, as well as impede the proliferation, migration and invasion of such cells.

Fas and its ligand FasL are members of the cell surface receptor family known as tumor necrosis factor, and their interaction results in cell death mediated by the ligand. The reduction in Fas and FasL expression or the impairment of signal transduction pathways are associated with tumor progression[14]. The Bcl-2/BAX ratio is frequently employed as a means of assessing the extent of apoptosis in tumor cells[15]. The findings indicated a statistically significant increase in the overall apoptosis rate within the resveratrol group compared to the colorectal cancer group, the resveratrol group exhibited significantly elevated expression levels of apoptotic proteins Fas, BAX and FasL compared to the colorectal cancer group. Conversely, the expression level of Bcl-2 in the resveratrol group was significantly lower than that observed in the colorectal cancer group. It has been found that resveratrol can induce apoptosis in colorectal cancer cells, which may be through the mitochondrial pathway[16].

AMPK/PGC-1α signal pathway is a widely used signal transduction pathway in human body, this factor assumes a crucial function in the regulation of cellular processes such as growth, migration, differentiation and apoptosis[17]. AMPK belongs to serine/threonine protein kinase, which is an important factor in regulating cell energy homeostasis and inflammation. It is expressed in various metabolic organs and tissues. It can be activated by various stimuli by sensing changes in the state of cell energy metabolism, thus affecting multiple links of cell material metabolism to coordinate body metabolism and energy balance[18]. Research has revealed that the dysregulation of metabolism and energy balance leads to the activation of AMPK, which in turn governs the expression of downstream malonyl-Coenzyme A (CoA) and lipid synthesis genes through the process of phosphorylation, regulate the biosynthesis of fatty acids, thus inhibit inflammation and oxidative stress, and restore the energy balance of the body[19]. PGC-1α is a transcriptional coactivator of mitochondrial related genes. As a coactivator of nuclear transcription, it increases transcriptional efficiency by binding with other coactivators on different target genes, thus the regulation of crucial physiological processes, including fatty acid oxidation, oxidative phosphorylation, and mitochondrial biogenesis, is of significant importance[20]. PGC-1α can activate the transcription factor of mitochondrial DNA, Transcription Factor A Mitochondria (TFAM). The activated TFAM is capable of translocating across the mitochondrial membrane and entering the mitochondrial matrix, where it can interact with mitochondrial DNA. Subsequently, it forms a transcription initiation complex in conjunction Transcription-Factor Binding Motif (TFBM) and mitochondrial RNA polymerase, thus regulating mitochondrial biogenesis and function[21]. Research has substantiated that the excessive activation of the AMPK/PGC-1α signaling pathway significantly contributes to the regulation of proliferation, survival and invasiveness of many cancer cells[22]. The findings indicate a significant decrease in the relative expression of MPK and PGC-1α mRNA and protein in the colorectal cancer group compared to the blank control group. Conversely, the relative expression of MPK and PGC-1α mRNA and protein in the resveratrol group was significantly higher than that observed

in the colorectal cancer group. It is suggested that resveratrol can regulate AMPK/PGC-1α signal pathway and provide theoretical basis for clinical treatment of tumor metastasis and invasion.

To sum up, resveratrol exhibits the capacity to diminish the viability of colorectal cancer cells, impede the growth and metastasis of such cells, as well as prompt apoptosis within the colorectal cancer cell population. The mechanism potentially exhibits an association with the regulation of the AMPK/PGC-1α signal pathway.

Conflict of interests:

The authors declared no conflict of interests

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