EFFECTS OF B-ELEMENE ON PROLIFERATION, MIGRATION AND INVASION OF COLORECTAL CANCER CELLS VIA WNT/B-CATENIN RELATED PATHWAY

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ABSTRACT

Objective: *To investigate the effect of β-elemene on the proliferation, migration, and invasion of colorectal cancer cells through Wnt/β -Catenin pathway.*

Methods: *Human colorectal cancer cell line HT-29 was randomly divided into a NC group, a 20 μg/ml β-elemene group, a 30 μg/ ml β-elemene group, and a 40 μg/ml β-elemene group. The optical density (OD) value of HT-29 cells at 0h, 24h, 48h, and 72h and the cell migration ability (cell migration distance) at 0h and 48h were analyzed. The invasive ability (number of penetrating cells) of HT-29 cells in each group at 48h were compared. The NC group and the 40 μg/ml group were further analyzed. The expression levels of Wnt/β-catenin signaling pathway-related proteins in HT-29 cells in β- elemene Group (β-Catenin, p53, MMP-2).*

Results: *At 0h, there was no significant difference in the OD value of HT-29 cells in each group (P>0.05). At 24h, 48h, and 72h, the OD values of HT-29 cells in the β-elemene concentration group were significantly lower than those in the NC group in a concentration-dependent manner (P<0.05). The migration distance of HT-29 cells in the β-elemene concentration group was significantly lower than that in the NC group (P<0.05). The number of HT-29 cells in the β-elemene concentration group was significantly lower than that in the NC group in a concentration-dependent manner (P<0.05). The expression levels of β-Catenin and MMP-2 in HT-29 cells in the 40 μg/ml β-elemene group were significantly lower than those in the NC group, and the expression level of p53 protein was significantly higher than that in the NC group (P<0.05).*

Conclusion: *β-elemene can inhibit the proliferation, migration, and invasion of colon cancer cells by blocking Wnt/β-catenin signaling pathway.*

Keywords: *β-elemene, Wnt, β-Catenin, colorectal cancer.*

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Introduction

Colorectal cancer usually occurs in people of middle age and older. The disease originates anywhere in the colon or rectum, particularly the rectum and sigmoid colon. Adenocarcinoma is the primary pathological type of colorectal cancer, which can spread to other organs and tissues mediated by blood circulation and lymphatic pathways[1]. The onset and evolution of colorectal cancer are characterized by changes in various types of gene levels, such as abnormal activation of proto-oncogenes and inactivation of oncogenes[2]. Although the current clinical treatment of colorectal cancer can alleviate the patient's condition to a certain extent, it can also lead to nausea, vomiting, reduced immune function, and other toxic side effects in patients; additionally, the risk of recurrence after treatment is very high[3]. Therefore, there is an urgent need to explore a safe and reliable anti-tumor drug to treat colorectal cancer. It is widely recognized that β-elemene has an anti-tumor effect. It has been reported that

β-elemene is a natural anti-angiogenic agent, which can block the expression of vascular endothelial growth factor, thereby inhibiting angiogenesis and achieving the purpose of inhibiting melanoma growth and metastasis[4]. Studies related to lung cancer have confirmed that β-elemene has the ability to block tumor cell proliferation, and its mechanism may be realized by increasing the expression level of p53 protein and then accelerating exosome release[5].

The wingless-type MMTV integration site family members (Wnt)/β-catenin signaling pathway plays a key role in embryogenesis, organ development, and other processes. Clinical studies have shown that abnormal activation of this pathway is closely related to the onset and evolution of tumors[6, 7]. However, there are few reports on the effect of β-elemene on the biological behavior of colorectal cancer cells by acting on Wnt/β-catenin. Therefore, this study will report on β-elemene.

Materials and methods

Experimental materials

Human colorectal cancer cell line HT-29 was purchased from Wuhan Purity Biotechnology Co., Ltd.

Main reagents and instruments

Reagents

High sugar DMEM was purchased from Nanjing Saihongrui Biological Technology Co., Ltd. MTT was purchased from Wuhan Baijiekang Biological Technology Co., Ltd. Transwell Branch was purchased from Shanghai Shengbo Biomedical Technology Co., Ltd. The BCA protein concentration kit was purchased from Nanjing Nuvezan Biotechnology Co., Ltd. Polyclonal antibodies against β-catenin, p53, and MMP-2 were purchased from Wuhan Bafel Biotechnology Service Co., Ltd. GAPDH antibody was purchased from Shenzhen Haodi Huatuo Biotechnology Co., Ltd. Fetal bovine serum was purchased from Shenzhen Rayward Life Technology Co., Ltd. Trypsin was purchased from Chuzhou Snorda Biological Technology Co., Ltd.

Instrument

The super clean workbench was purchased from Beijing Jiayuan Xingye Technology Co., Ltd. The inverted microscope was purchased from Beijing Jinda Sunshine Technology Co., Ltd. The CO2 incubator was purchased from Guangzhou Juenerg Nanobiotech Co., Ltd. The low-temperature centrifuge was purchased from Changsha Baxi Instrument Co., Ltd. The electric thermostatic water bath box was purchased from Hangzhou Nuoding Scientific Equipment Co., Ltd. The film transfer instrument was purchased from Guangzhou Huijun Biotechnology Co., Ltd.

Methods

• Human colorectal cancer cell line HT-29 was cultured with high glucose DMEM, and the culture dishes were placed at 37°C and 5% CO_2. The culture medium was replaced once every other day, and further studies were carried out when the cells were in the logarithmic growth phase.

• HT-29 cells in logarithmic growth phase were randomly divided into a NC group, a 20 μg/mL β-Elemene group, a 30 μg/mL β-Elemene group, and a 40 μg/mL β-Elemene group. Cells in each group were transplanted into 96 well plates at a density of 5×103 cells per well. HT-29 cells in NC group were not given any intervention, and HT-29 cells in the 20 μg/mL β-Elemene group, the 30 μg/mL β-Elemene group, and the 40 μg/mL β-Elemene group were given 20 μg/mL β-Elemene group, 30 μg/mL β-Elemene group, and 40 μg/mL β-Elemene group, respectively.

• The proliferation ability (Optical density [OD] value) of HT-29 cells in each group was detected by MTT method at 0h, 24h, 48h, and 72h, respectively. Each experiment was repeated 3 times.

• The migration ability of HT-29 cells (cell migration interval) was measured by scratch test at 0h and 48h, respectively. The invasion ability of HT-29 cells in each group was measured by Transwell test at 48h, and each experiment was repeated 3 times.

• After 48h, the expression levels of Wnt/β-catenin signaling pathway related proteins (β-catenin, p53, and Matrix metalloproteinase 2[MMP-2]) in HT-29 cells of the NC group and the 40 μg/mL β-elemene group were determined by Western blot and were compared.

Statistical methods

All relevant data were analyzed by SPSS23.0, and the measurement data such as the OD value of HT-29 cells in each group were expressed as ($x \pm s$). The value comparison between two groups was performed by t-test, and the comparison between multiple groups was performed by one-way analysis of variance. $P<0.05$ was considered to be significant.

Results

Comparison of HT-29 cell proliferation in each group

At 0h, there was no significant difference in OD value of HT-29 cells among all groups (P>0.05). At 24h, 48h and 72h, the OD value of HT-29 cells in the β-elemene concentration group was significantly lower than that in the NC group in a concentration-dependent manner (P<0.05). See Table 1.

Group	0h	24h	48h	72h
NC group	0.35±0.01	0.69±0.02	0.90±0.01	1.35±0.02
20 μg/mL β-elemene group	0.34±0.01	0.54±0.01[a]	0.69±0.02[a]	0.91±0.02[a]
30 μg/mL β-elemene group	0.36±0.01	0.47±0.01[ab]	0.55±0.01[ab]	0.75±0.01[ab]
40 μg/mL β-elemene group	0.35±0.02	0.39±0.02[abc]	0.45±0.02[abc]	0.59±0.01[abc]

Table 1: Comparison of OD values of HT-29 cells in each group (x±s).
Note: a means compared with NC group, P<0.05; b was compared with 20 μg/mL β-elemene group, P<0.05; c indicated that compared with 30 μg/mL β-elemene group, P<0.05.

Comparison of migration ability of HT-29 cells in each group

The migration spacing of HT-29 cells in the β-elemene concentration group was significantly lower than that in the NC group in a concentration-dependent manner (P<0.05). See Table 2.

Group	Migration spacing of HT-29 cells (cm)
NC group	0.51±0.11
20 μg/mL β-elemene group	0.40±0.08[a]
30 μg/mL β-elemene group	0.21±0.02[ab]
40 μg/mL β-elemene group	0.05±0.01[abc]

Table 2: Comparison of the migration ability of HT-29 cells in each group (x±s).
Note: a means compared with NC group, P<0.05; b was compared with 20 μg/mL β-elemene group, P<0.05; c indicated that compared with 30 μg/mL β-elemene group, P<0.05.

Comparison of the invasion ability of HT-29 cells in each group

The number of HT-29 cell penetration in the β-elemene concentration group was significantly lower than that in the NC group in a concentration-dependent manner (P<0.05). See Table 3.

Comparison of Wnt/β-catenin signaling pathway related protein expression levels in HT-29 cells in each group

The protein expression levels of β-catenin and MMP-2 in HT-29 cells in 40 μg/mL β-elemene group were significantly lower than those in NC group, and the protein expression level of p53 was significantly higher than that in NC group (P<0.05). As shown in Table 4.

Group	Number of HT-29 cell penetration
NC group	99.15±10.45
20 μg/mL β-elemene group	61.45±5.15[a]
30 μg/mL β-elemene group	19.12±1.23[ab]
40 μg/mL β-elemene group	10.59±0.56[abc]

Table 3: Comparison of the invasion ability of HT-29 cells in each group (x±s).
Note: a means compared with NC group, P<0.05; b was compared with 20 μg/mL β-elemene group, P<0.05; c indicated that compared with 30 μg/mL β-elemene group, P<0.05.

Group	n	β-catenin	MMP-2	p53	GAPDH
NC	10	1.36±0.16	1.56±0.18	1.26±0.06	1.27±0.14
40μg/mL β-elemene	10	0.56±0.06	0.69±0.05	1.79±0.16	1.26±0.09
t		14.804	14.726	9.808	0.19
P		<0.001	<0.001	<0.001	0.849

Table 4: Comparison of β-catenin, p53 and MMP-2 protein expression levels in HT-29 cells of each group.

Discussion

In the early stage of colorectal cancer, there are no obvious symptoms; however, in the middle and late stages of development, cancer cells spread. This can lead to limitations in the daily life of patients with colorectal cancer and is manifested as emaciation, weakness, anemia, and other symptoms (8). Clinical reports suggest that colorectal cancer incidence and mortality after gastric cancer and esophageal cancer and its treatment is given priority to with surgery and supplemented by radiation and chemotherapy patients clinical curative effect and prognosis is not ideal, however, lead to colorectal cancer treatment stalled, so the search for a reliable and effective against colorectal cancer treatment is imminent.

Elemene, extracted from Curcuma zedoary, is one of the new second-class anti-tumor drugs in China. B-elemene is an active component of the drug, which plays a significant role in blocking tumor growth, promoting tumor cell apoptosis, and inhibiting tumor angiogenesis[9]. Due to its strong lipophilicity and low molecular weight, β-elemene can be widely distributed through many organs and tissues and can easily pass through the blood-brain barrier, which is conducive to blocking the

occurrence and development of many types of tumors, such as liver and gastric cancers[10]. Clinical studies have shown that β-elemene has the effect of single or combined therapy on some tumors, which can effectively reduce adverse reactions caused by radiotherapy and chemotherapy, reduce the recurrence rate of patients, and improve their survival time and quality of life[11]. It has been reported that β-elemene can significantly inhibit the proliferation of prostate cancer cells and glioma cells. Studies related to human renal cell carcinoma have confirmed that β-elemene can promote 786-0 cell apoptosis and block the growth of cancer cells through inhibition of Mitogen-activated protein kinase/Extracellular signal-regulated kinase (MAPK/ERK) signaling pathway, and phosphatidylinositol-3-kinase/serine-threonine kinase/Mammalian rapamycin targeting gene (PI3K/Akt/mTOR) signaling pathway[12]. Studies related to colon cancer have confirmed that β-elemene can block the proliferation of DLD-1 and HT-29 cells and induce apoptosis and autophagy of cancer cells. This may be related to the enhancement of reactive oxygen species, the induction of the activation of Adenosine 5' -monophosphate-activated protein kinase (AMPK), and the inhibition of mTOR phosphorylation[13]. The results of the MTT assay showed that β-elemene could significantly reduce the activity of HT-29 and inhibit the proliferation of human colorectal cancer cells in a concentration-dependent manner.

The results of the scratch assay and Transwell assay showed that β-elemene could block the migration and invasion of human colorectal cancer cells HT-29 in a concentration-dependent manner. The β-catenin gene is a proto-oncogene that is usually localized in the envelope and plays a key role in the Wnt/β-catenin signaling pathway, determining whether the pathway is turned on or off. Under normal physiological conditions, it can mediate the transfer of Wnt/β-catenin signaling pathway from the membrane to the cytoplasm or nucleus, and finally cause the activation of the signaling pathway. However, when the Wnt/β-catenin signaling pathway is in an abnormal state, the degradation of β-catenin is impaired. This leads to the aggregation of free β-catenin in the cytoplasm, which then metastases to the nucleus, and finally induces tumors[14]. P53 is a tumor suppressor gene, which can promote cell apoptosis by activating the transcription of many types of genes. For example, the increased expression level of p53 can lead to the decreased expression level of Bcl-2 and finally induce cell apoptosis[15]. MMP-2, a migration and invasion-related protein, is a member of the matrix metalloproteinase family, which can degrade collagen V, collagen VII, and gelatin in the basement membrane of extracellular matrix protein. This can lead to complete damage of the basement membrane, and ultimately induce invasion and migration of cancer cells. In this study, we found that β-elemene inhibited the expression of β-catenin and MMP-2 proteins related to the Wnt/β-catenin signaling pathway and increased the expression of p53 protein.

In conclusion, β-elemene can inhibit the proliferation, migration, and invasion of colon cancer cells, and its mechanism may be achieved by blocking the Wnt/β-catenin signaling pathway.

References

1) Shen MH, Chen LP, Ho TF, Shih YY, Huang CS, et al. Validation of the Taiwan Chinese version of the EORTC QLQ-CR29 to assess quality of life in colorectal cancer patients. BMC Cancer 2018; 18(1): 353.
2) Mur P, De Voer RM, Olivera-Salguero R, Rodríguez-Perales S, Pons T, Setién F, et al. Germline mutations in the spindle assembly checkpoint genes BUB1 and BUB3 are infrequent in familial colorectal cancer and polyposis. Mol Cancer 2018; 17(1): 23.
3) Grizzi F, Basso G, Borroni EM, Cavalleri T, Bianchi P, et al. Evolving notions on immune response in colorectal cancer and their implications for biomarker development. Inflamm Res 2018; 67(5): 375-389.
4) Sieniawska E, Michel P, Mroczek T, Granica S, Skalicka-Woźniak K. Nigella damascena L. essential oil and its main constituents, damascenine and β-elemene modulate inflammatory response of human neutrophils ex vivo. Food Chem Toxicol 2019; 125: 161-169.
5) Fan Q, Yang L, Zhang X, Ma Y, Li Y, et al. Autophagy promotes metastasis and glycolysis by upregulating MCT1 expression and Wnt/β-catenin signaling pathway activation in hepatocellular carcinoma cells. J Exp Clin Cancer Res 2018; 37(1): 9.
6) Uppada SB, Gowrikumar S, Ahmad R, Kumar B, Szeglin B, et al. MASTL induces Colon Cancer progression and Chemoresistance by promoting Wnt/β-catenin signaling. Mol Cancer 2018; 17(1): 111.
7) Zhang Z, Liu T, Yu M, Li K, Li W. The plant alkaloid tetrandrine inhibits metastasis via autophagy-dependent Wnt/β-catenin and metastatic tumor antigen 1 signaling in human liver cancer cells. J Exp Clin Cancer Res 2018; 37(1): 7.
8) Huang X, Xiang L, Li Y, Zhao Y, Zhu H, et al. Snail/FOXK1/Cyr61 Signaling Axis Regulates the Epithelial-Mesenchymal Transition and Metastasis in Colorectal Cancer. Cell Physiol Biochem 2018; 47(2): 590-603.

9) Cao M, Long M, Chen Q, Lu Y, Luo Q, et al. Development of β-elemene and Cisplatin Co-Loaded Liposomes for Effective Lung Cancer Therapy and Evaluation in Patient-Derived Tumor Xenografts. Pharm Res 2019; 36(8): 121.
10) Zhai B, Zeng Y, Zeng Z, Zhang N, Li C, et al. Drug delivery systems for elemene, its main active ingredient β-elemene, and its derivatives in cancer therapy. Int J Nanomedicine 2018; 13: 6279-6296.
11) Jiang Z, Liu J, Chen B, Mani R, Jacob JA. Cytotoxic effects of a sesquiterpene β-elemene on THP-1 leukemia cells is mediated via crosstalk between beclin-1 mediated autophagy and caspase-dependent apoptosis. Process Biochem 2019; 87: 174-178.
12) Zhan YH, Liu J, Qu XJ, Hou KZ, Wang KF, et al. β-Elemene induces apoptosis in human renal-cell carcinoma 786-0 cells through inhibition of MAPK/ERK and PI3K/Akt/ mTOR signalling pathways. Asian Pac J Cancer Prev 2012; 13(6) :2739-44.
13) Ahmad KA, Ze H, Chen J, Khan FU, Xuezhuo C, et al. The protective effects of a novel synthetic β-elemene derivative on human umbilical vein endothelial cells against oxidative stress-induced injury: Involvement of antioxidation and PI3k/Akt/eNOS/NO signaling pathways. Biomed Pharmacother 2018; 106: 1734-1741.
14) Li G, Su Q, Liu H, Wang D, Zhang W, et al. Frizzled7 Promotes Epithelial-to-mesenchymal Transition and Stemness Via Activating Canonical Wnt/β-catenin Pathway in Gastric Cancer. Int J Biol Sci 2018; 14(3): 280-293.
15) Li HL, Sun BZ, Ma FC. Expression of COX-2, iNOS, p53 and Ki-67 in gastric mucosa-associated lymphoid tissue lymphoma. World J Gastroenterol 2004; 10(13): 1862-6.

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