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# Regulatory effect of evodiamine on the malignant biological behaviors and Wnt/ β −catenin signaling pathway of colorectal cancer cell lines HT29

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ABSTRACT

**Objective:** To study the regulatory effect of evodiamine on the malignant biological behaviors and Wnt/ β -catenin signaling pathway of colorectal cancer cell lines HT29. **Methods:** Colorectal cancer cell lines HT29 were cultured and divided into blank control group and evodiamine group, and after different treatment, cell viability, proportion of different cell cycle as well as the contents of VEGFA, VEGFB, VEGFC, MMP3, MMP14, Wnt and β -catenin were detected. **Results:** (1) Cell viability: MTT value of evodiamine group was significantly lower than that of blank control group; (2) Cell cycle: proportion of both S phase and $G_2$/M phase of evodiamine group were lower than those of blank control group, and proportion of $G_0$/$G_1$ phase was higher than that of blank control group; (3) VEGF and MMP contents: VEGFA, VEGFB, VEGFC, MMP3 and MMP14 contents of evodiamine group were lower than those of blank control group; (4) Wnt/ β -catenin signaling pathway: Wnt and β -catenin contents of evodiamine group were lower than those of blank control group. **Conclusion:** Evodiamine can inhibit the proliferation of colorectal cancer cell lines HT29 and down-regulate the expression of VEGF and MMP, and the effect may be achieved by inhibiting the activation of Wnt/ β -catenin signaling pathway.

## 1. Introduction

Colorectal cancer is the common type of malignant tumor of digestive tract in our country, the incidence has been rising in recent years, and the main clinical treatment is surgical resection combined with postoperative chemotherapy. Oxaliplatin is the traditional chemotherapy drug that belongs to cell cycle nonspecific drug, mainly acts on intracellular DNA and influences its replication and transcription process. With the popularization of platinum-based chemotherapy regimen, the incidence of drug resistant cases also appears constantly, it causes serious influence to the survival of patients with colorectal cancer, and exploring more effective drugs with different mechanisms of action has urgent clinical needs. Evodiamine is the alkaloid extracted from fructus evodiae, and it has antitumor activity. In the following research, the regulatory effect of evodiamine on the malignant biological behaviors and Wnt/ β -catenin signaling pathway of colorectal cancer cell lines HT29 was analyzed.

## 2. Experimental materials and methods

### *2.1 Experimental materials*

Colorectal cancer cell lines HT29 were purchased from the cell bank of Chinese Academy of Sciences, culture medium, serum and other reagents for cell culture were purchased from Gibco Company,

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cell plates, culture flasks and other consumables were purchased from Thermo Company and MTS reagents were purchased from Promega Company.

### 2.2 Methods

#### 2.2.1 Cell culture and treatment methods

Cells were recovered, cultured with DMEM containing 10% fetal bovine serum until cell density grew to 70%-80%, then digested with trypsin and sub-cultured, and amplified cells were inoculated in cell plate and treated with different concentration of evodiamine.

#### 2.2.2 Cell viability detection

For cell viability detection, amplified cells were inoculated in 96-hole cell plate and treated with different conditions for 24 h, then MTS detection liquid was added in cell culture holes, cells were continuously cultured for 3-4 h, and then OD values at 490 nm were detected in microplate reader.

#### 2.2.3 Cell cycle detection

Digested cells were inoculated in 6-hole plate and treated with drugs, then culture medium was discarded, cells were washed with PBS twice, after that 0.25% trypsin was added for 2 min of digestion, digested cells were transferred to centrifuge tubes, re-suspended with PBS solution containing 10% fetal bovine serum, centrifuged and washed twice and finally fixed in -20 ℃ pre-cooled absolute ethyl alcohol for the night, ethanol was discarded after centrifuge the next day, cells were washed with PBS twice and then stained with propidium iodide for 30 min away from light, and finally flow cytometer was used to detect cell cycle.

#### 2.2.4 Protein content detection methods

Treated cells were taken, culture medium was discarded, protein lysis buffer was added, cells were fully disrupted to get protein suspension and appropriately dilute it, and then enzyme-linked immunosorbent assay kits were used to detect the contents of VEGFA, VEGFB, VEGFC, MMP3, MMP14, Wnt and β -catenin.

### 2.3 Statistical methods

SPSS 18.0 software was used to input data, measurement data was by t test and differences were considered to be statistically significant at a level of $P<0.05$.

## 3. Results

### 3.1 Cell viability

24 h after treatment, MTS kits and microplate reader were used to detect cell OD values, and results of statistical analysis were as follows: OD values among five groups were statistically different; OD values of different dose of evodiamine group were all lower than those of blank control group; the higher the dose, the lower the OD value, and differences among groups were statistically significant ($P<0.05$).

**Table 1**

Comparison of cell viability among different treatment groups

| Group | OD values |
|---|---|
| Evodiamine (0.01 μmol/L) | 0.883±0.094 |
| Evodiamine (0.1 μmol/L) | 0.734±0.076 |
| Evodiamine (1.0 μmol/L) | 0.651±0.068 |
| Evodiamine (10 μmol/L) | 0.409±0.054 |
| Evodiamine (100 μmol/L) | 0.314±0.037 |
| Blank control | 1.183±0.142 |

### 3.2 Cell cycle

24 h after treatment, flow cytometer was used to detect cell cycle, and results of statistical analysis were as follows: proportion of both S phase and $G_2$/M phase of evodiamine group were lower than those of blank control group, and proportion of $G_0/G_1$ phase was higher than that of blank control group, and differences among groups were statistically significant ($P<0.05$).

### 3.3 Effect of evodiamine on VEGF and MMP expression in colorectal cancer cells

24 h after treatment, enzyme-linked immunosorbent assay kits

**Table 2**

Comparison of cell cycle among different treatment groups

| Group | $G_0/G_1$ phase | S phase | $G_2$/M phase |
|---|---|---|---|
| Evodiamine (0.01 μmol/L) | 54.48±5.38 | 33.12±3.85 | 12.40±1.32 |
| Evodiamine (0.1 μmol/L) | 59.34±5.86 | 30.23±3.42 | 10.43±1.18 |
| Evodiamine (1.0 μmol/L) | 65.53±7.78 | 27.79±3.75 | 6.68±0.84 |
| Evodiamine (10 μmol/L) | 72.84±8.14 | 23.45±3.25 | 3.71±0.49 |
| Evodiamine (100 μmol/L) | 80.14±9.52 | 18.34±2.10 | 1.52±0.14 |
| Blank control | 48.62±6.05 | 35.52±5.52 | 15.86±1.95 |

**Table 3**

Comparison of VEGF and MMP expression between two groups

| Group | VEGF molecules | | | MMP molecules | |
|---|---|---|---|---|---|
| | VEGFA (ng/mL) | VEGFB (ng/mL) | VEGFC (ng/mL) | MMP3 (ng/mL) | MMP14 (ng/mL) |
| Evodiamine | 35.48±4.28 | 19.33±2.18 | 53.38±6.95 | 46.59±5.91 | 39.40±4.83 |
| Blank control | 66.27±7.83 | 33.28±4.29 | 101.19±12.28 | 89.33±9.49 | 62.33±7.48 |
| *T* | 9.192 | 7.972 | 9.948 | 9.384 | 8.785 |
| *P* | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 |

were used to detect VEGFA, VEGFB, VEGFC, MMP3 and MMP14 contents of 100 μmol/L evodiamine group and blank control group, and results of statistical analysis were as follows: VEGFA, VEGFB, VEGFC, MMP3 and MMP14 contents of 100 μmol/L evodiamine group were lower than those of blank control group, and differences among groups were statistically significant ($P$<0.05).

### *3.4 Wnt/β-catenin signaling pathway*

24 h after treatment, enzyme-linked immunosorbent assay kits were used to detect Wnt and β-catenin contents of 100 μmol/L evodiamine group and blank control group, and results of statistical analysis were as follows: mRNA contents of Wnt and β-catenin of 100 μmol/L evodiamine group were lower than those of blank control group, and differences among groups were statistically significant ($P$<0.05).

**Table 4**
Comparison of Wnt and β-catenin expression between two groups

| Group | Wnt (ng/mL) | β-catenin (ng/mL) |
|---|---|---|
| Evodiamine | 29.45±4.10 | 34.41±5.04 |
| Blank control | 64.58±7.48 | 89.28±10.12 |
| T | 13.844 | 11.347 |
| P | < 0.05 | < 0.05 |

## 4. Discussion

Alkaloids are natural organic compounds, many of whom have antitumor effect. Fructus evodiae is a traditional Chinese medicine containing a variety of alkaloids, its taste is bitter and acrid, its nature is warm, and its main component is evodiamine. In recent years, studies have shown the antitumor effect of evodiamine on lung cancer[1], bladder cancer[2], tongue squamous cell carcinoma[3] and so on, and study of domestic DONG Li[4] and others believes that aqueous extract of fructus evodiae has the effect of inhibiting proliferation and promoting apoptosis for colon precancerous lesions in rats; study of foreign Chien CC and others[5] has confirmed the regulatory effect of evodiamine on colon cancer cell cycle. The main malignant biological behaviors of cancer cells lie in their strong proliferative capacity as well as the continuous invasive and infiltrative capacity to the surrounding tissue, which is also the biological basis of recurrence and metastasis in cancer patients. Recurrence rate in patients with colorectal cancer is high after surgery, and continuous proliferation of local residual malignant tumor cells is the key link of tumor recurrence and also the most important malignant biological behavior of colorectal cancer. In the research, MTS kits and flow cytometry were used to analyze the effect of evodiamine on colorectal cancer cell line proliferation at first. MTS detection kit is cell viability detection method developed by Promega Company based on MTT detection, the dye in the kit can specifically react with succinate dehydrogenase in mitochondria of viable cells and generate formazan, and then absorbance is read to accurately reflect the number of viable cells; flow cytometry detection methods can directly display cell cycle distribution. Experimental results showed that MTT value as well as the proportion of S phase and $G_2$/M phase of evodiamine group was lower than those of control group, and the proportion of $G_0/G_1$ phase was higher than that of control group. It indicated that evodiamine treatment could reduce cell viability of colorectal cancer cell lines and decrease the number of viable cells while make cell cycle largely arrest in $G_0/G_1$ phase and unable to perform the next replication.

Tumor cell proliferation is an active process of energy consumption that requires local capillary network to provide adequate nutrients and oxygen; the generation and regulation of capillary network has become the pathophysiological basis for colorectal cancer cells to complete malignant biological behaviors [6]. Tumor itself can secrete large amounts of pro-angiogenesis factors to directly mediate and participate in the occurrence and formation of local new vascular net. Among many pro-angiogenesis factors, vascular endothelial growth factor (VEGF) is the most powerful type that specifically includes VEGF-A, -B, -C and many other different subtypes and can directly recruit vascular endothelial cells and form new vascular net around tumor[7]. Apart from directly increasing the number of new blood vessels, tumor cells will continually invade surrounding vessel wall tissue, which leads to the occurrence of vessel wall remodeling, turns blood vessels with high resistance and low volume into blood vessels with low resistance and high volume, thus increases blood flow within tumor lesion and provides necessary nutrients for the completion of variety of biological behaviors of tumor cells[8]. Matrix metalloproteinase (MMP) is a zinc-dependent protease family, is the key molecule mediating tumor cell infiltration to the surrounding tissue, and includes MMP3, MMP14, MMP14 and many subtypes. Among them, MMP3 and MMP14 are considered to be the most closely related to colon cancer cell infiltration[9]. In the research, real-time PCR method was used to detect the effect of evodiamine on VEGF and MMP expression in colorectal cancer cell lines, and results showed that mRNA contents of VEGFA, VEGFB, VEGFC, MMP3 and MMP14 of evodiamine group were lower than those of control group. It indicated that evodiamine treatment could reduce VEGF and MMP expression and inhibit angiogenesis and local infiltration ability of tumor cells.

At present, numerous studies have confirmed that the activation of Wnt/β-catenin signaling pathway is closely related to the occurrence and development of colorectal cancer and β-catenin is the most important signal molecule in the signaling pathway[10–12]. When cells lack Wnt signal stimulation, intracellular β-catenin is affected by

GSK-3 β -APC-Axin complex, phosphorylated and degraded; when Wnt signal is activated, the phosphorylation-dependent degradation process of β -catenin is inhibited, intracellular β -catenin content increases, and it enters into the nucleus, forms transcription complex with TCF/LEF and implements the activation on the transcription of downstream target genes VEGF, MMP, survivin and so on, thus involved in the regulation of malignant biological behaviors of tumor[13,14]. In order to further clarify the molecular mechanisms for evodiamine to regulate malignant biological behaviors of colorectal cancer cell lines, the Wnt molecule and β -catenin molecule contents in Wnt/ β -catenin signaling pathway were detected, and results showed that mRNA contents of Wnt and β -catenin of evodiamine group were lower than those of blank control group. It indicated that evodiamine treatment could inhibit the activation of Wnt/ β -catenin signaling pathway in colorectal cancer cell lines, thus implementing the regulation on malignant biological behaviors of tumor by affecting the pathway.

Based on above discussion, it can be concluded that evodiamine can inhibit the proliferation of colorectal cancer cell lines HT29 and down-regulate the expression of VEGF and MMP, and the effect may be achieved by inhibiting the activation of Wnt/ β -catenin signaling pathway.

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