Original Research Article

Peiminine regulates the biological characteristics of colorectal cancer cells via P13K/Akt/mTOR and oxidative stress pathways

GuiLin Jin1, Zhen Luo2, Mingke Fen1, Lijun Xu3*
1Department of Experimental Specimen Center, 2Department of Tibetan Medicine, 3Department of Institute, University of Tibetan Medicine, Lhasa 850000, Tibet Autonomous Region, China

*For correspondence: Email: jinguilin9999@163.com

Sent for review: 11 December 2021 Revised accepted: 3 May 2022

Abstract

Purpose: To investigate the influence of peiminine on biological characteristics of colorectal cancer cells, and the underlying mechanism.

Methods: Two groups of cultured human colorectal cancer HCT-116 cells were used: peiminine and control groups. Peiminine group cells were exposed to the drug at a final concentration of 100 μmol/L. The effect of peiminine on cell proliferation was determined with CCK-8 method, while its effect on apoptosis was determined with flow cytometric method. Cell migration was determined with scratch test. The effect of peiminine on the expressions of proteins associated with the P13K/Akt/mTOR pathway and Wnt/β-catenin pathway in HCT-116 cells was determined with Western blotting assay.

Results: Cell proliferation was markedly reduced in the peiminine group, relative to control (p < 0.05). There was higher percentage cell apoptosis in peiminine-treated cells than in control. Moreover, cell migration potential was significantly lower in the peiminine-treated cells. There were significantly down-regulated levels of p-P13K, p-Akt and p-mTOR expressions in peiminine group, relative to the corresponding control expressions (p < 0.05). However, there were significantly higher relative expression of Wnt in peiminine group than in control cells, but β-catenin level was reduced, relative to the corresponding control level (p < 0.05).

Conclusion: These data indicate that peiminine suppresses the proliferative, apoptotic and migratory potential of colorectal carcinoma HCT-116 cells via regulation of P13K/Akt/mTOR and oxidative stress pathways.

Keywords: Peiminine, Colorectal cancer, Cell proliferation, Apoptosis, Migration

INTRODUCTION

Colorectal carcinoma is a common malignancy in the gastrointestinal tract [1]. In the early stage of colorectal cancer, there are no obvious symptoms. Consequently, most patients develop hematochezia, diarrhea and changes in defecation habits at the middle and late stages of colorectal cancer when their conditions have already deteriorated [2]. Studies have shown that the occurrence, deterioration and prognosis of colorectal cancer in patients are largely related to...
genetic factors, lifestyle and living environment [3].

The preferred treatment strategies for early and middle stage colorectal cancer involve combination of surgery and chemotherapy [4]. However, the prognosis and overall survival of patients with advanced colorectal cancer are poor. Therefore, various combination treatments are often used, including interventional therapy, molecular targeted therapy and Traditional Chinese Medicine (TCM) therapy, in order to take advantage of all therapeutic options [5].

At present, TCM therapy for advanced colorectal cancer has attracted a lot of interest, and it has high clinical value. Studies have demonstrated that TCM therapy not only mitigated the side effects of radiotherapy and chemotherapy, but also reduced postoperative tumor recurrence and metastasis, and effectively improved the prognosis and survival of patients [6]. Moreover, it has been reported that peiminine extracted from Chinese medicine *Fritillaria*, exerted analgesic and anti-inflammatory effects, and inhibited tumor growth and proliferation [7].

However, the anti-tumor mechanism of peiminine has not been elucidated. In particular, studies on changes in metabolites before and after PEMB treatment of HCT-116 colorectal carcinoma cells are still at the preliminary stages [8]. This study investigated the effect of peiminine on HCT-116 colorectal cancer cells, and the involvement of the P13K/Akt/mTOR pathway and oxidative stress metabolic pathway in the process.

**EXPERIMENTAL**

**Cells and culture conditions**

Human colorectal cancer HCT-116 cells were incubated in 96-well plates (2000 cells/mL) in DMEM containing 10% fetal bovine serum at 37 °C in a constant temperature incubator containing 5% CO₂. Then, the cells were assigned to peiminine and control groups. Cells in peiminine group were exposed to peiminine at a final concentration of 100 μmol/L. The control group was not treated.

**Drugs and reagents**

The drugs and reagents used in this study, and their sources (in brackets) were: peiminine (Shanghai Ulun Test Biotechnology Co. Ltd., mass fraction ≥ 98%, lot no. 20170227); neonatal calf serum (Zhengzhou Dening Biotechnology Co. Ltd); dimethyl sulfoxide (DMSO, Jinan Guangyu Chemical Co. Ltd.); Annexin V/FITC Reagent (Beijing Soleibao Technology Co. Ltd), and PI reagent (Wuhan Kanos Technology Co. Ltd).

**Determination of impact of peiminine on proliferative potential**

The influence of peiminine on proliferative potential of HCT-116 cells was determined with CCK-8 method. Cells in both groups were cultured at a density of 2000 cells/mL for 48 h with 10 μL of CCK-8 for 60 min. Thereafter, absorbance was measured at λ of 450 nm in an enzyme-plate detector.

**Measurement of apoptosis**

Apoptosis was measured flow cytometrically with Annexin V/PI. Peiminine solution was added to the HCT-116 cell plate, followed by incubation for 24 h. In place of peiminine, control cells had an equivalent volume of dimethyl sulfoxide. After culturing for 24 h, the culture medium was discarded, and the cells were washed thrice with phosphate buffer. Then, a single cell suspension was prepared and subjected to centrifugation, after which the cells were suspended again and mixed with 5 μL of Annexin V/FITC solution. The mixture was allowed to stand for 5 min, after which 10 μL of 20 µg/mL solution of PI was added. The mixture was well-shaken and allowed to stand for 10 min, after which phosphate buffer solution (400 μL) was pipetted into the reaction tube. The fluorescence of each of the two groups of cells was measured flow cytometrically.

**Cell scratch test**

A marker pen was used to evenly draw a horizontal line across the bottom of a six-well plate (at least 3 lines across each well). Thereafter, cells in the wells (1 – 4 × 10⁵ per well) were scratched with a pipette tip, washed thrice with phosphate buffer solution, and the liquid was placed in blood-free medium under a microscope and photographed. Then, the cells were placed in a 5% CO₂ constant temperature incubator at 37 °C for 24 h to observe the cell migration again, and photographed.

**Immunoblot assay**

After culturing with peiminine for 48 h, cells in control group were cultured with an equivalent volume of DMSO in place of peiminine for 48 h, after which total protein extraction was done with RIPA buffer solution. The protein content of the lysate was measured with BCA protein quantitative method. Then, the proteins were
resolved on SDS-PAGE, followed by electro-transfer to PVDF membranes. This was followed by incubation of the membranes overnight at 4 °C with polyclonal antibodies against MMP-9, MMP-2, P-P13K, P13K, P-Akt, Akt, P-MTOR and mTOR (all diluted 1:2000). Then, the membranes were incubated with 2° immunoglobulin linked to horse radish peroxidase at laboratory temperature for 30 min. The blots were subjected to enhanced electrochemiluminescence in the dark. The absorbance of each band was read, and the protein expressions were determined, relative to reference protein.

Statistical analysis

The data obtained in this study were analyzed using SPSS 21.0 software package. Measurement data are presented as mean ± standard deviation (SD). Inter-group comparison was done using independent sample t-test. Count data are presented as numbers and percentages (n (%)), and comparison between two groups was done using chi-square (χ²) test. Values of \( p < 0.05 \) were regarded as indicative of statistically significant differences.

RESULTS

Comparison of cell proliferation potential between the two groups

Cell proliferation was markedly lower in control cells than in peiminine group (\( p < 0.05 \); Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Parameter</th>
<th>( \chi^2 )</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peiminine</td>
<td>Proliferation (OD450nm)</td>
<td>0.73±0.14</td>
<td>1.00±0.12</td>
</tr>
<tr>
<td>Control</td>
<td>Apoptosis (%)</td>
<td>21.73±2.45</td>
<td>8.69±2.72</td>
</tr>
<tr>
<td>Peiminine</td>
<td>Healing (%)</td>
<td>63.52±7.96</td>
<td>83.47±5.82</td>
</tr>
</tbody>
</table>

Apoptosis

Apoptosis was significantly higher in peiminine-treated cells than in control cells (\( p < 0.05 \); Table 1).

Cell migration capacity

Table 1 shows that the degree of cell migration was markedly reduced in peiminine-treated cells than in control cells.

Effect of peiminine on the P13K/Akt/mTOR pathway in HCT-116 cells

The protein expressions of p-P13K, p-Akt and p-MTOR were markedly down-regulated in peiminine group, relative to control (\( p < 0.05 \); Table 2).

<table>
<thead>
<tr>
<th>Group</th>
<th>p-P13K</th>
<th>p-Akt</th>
<th>p-MTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peiminine</td>
<td>0.88±0.31</td>
<td>0.36±0.18</td>
<td>0.53±0.12</td>
</tr>
<tr>
<td>Control</td>
<td>0.52±0.26</td>
<td>0.24±0.11</td>
<td>0.28±0.10</td>
</tr>
<tr>
<td>( t )</td>
<td>3.446</td>
<td>2.203</td>
<td>6.198</td>
</tr>
<tr>
<td>( P )-value</td>
<td>0.001</td>
<td>0.036</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Relative expression levels of Wnt/β-catenin proteins ups

The relative expression of Wnt in peiminine group was markedly up-regulated in peiminine group, while relative β-catenin level in peiminine group was markedly low, relative to control values. These data are shown in Table 3.

<table>
<thead>
<tr>
<th>Group</th>
<th>Wnt</th>
<th>β-catenin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peiminine</td>
<td>2.53±0.48</td>
<td>0.68±0.24</td>
</tr>
<tr>
<td>Control</td>
<td>1.01±0.27</td>
<td>1.03±0.36</td>
</tr>
<tr>
<td>( t )</td>
<td>8.727</td>
<td>2.558</td>
</tr>
<tr>
<td>( P )-value</td>
<td>&lt;0.001</td>
<td>0.019</td>
</tr>
</tbody>
</table>

DISCUSSION

Cell migration and proliferation are the main reasons for the poor outcomes in clinical treatment of colorectal cancer. Therefore, it is important to explore the potential of TCM in the prevention and treatment of tumor metastasis [9]. Tumor cells are heterogeneous, and each type of tumor, including colorectal cancer, has its own metabolic characteristics [10]. Indeed, different cancer cells within the same tumor have differences in metabolism. This provides an opportunity for research using metabolomics techniques [11]. It has been found that pebetin B plays an anti-lung cancer role by regulating the invasion and migration of human lung cancer A549 cells, thereby inhibiting the proliferation of the tumor cells [12].

Apoptosis, or type I programmed cell death, is crucial for the normal growth of organisms, as well as the stability of internal environment of tissues [13]. In terms of morphology, an apoptotic cell first loses contact with neighboring cells, contracts into a round shape, loses microvilli structure, and its endoplasmic reticulum expands...
into vacuole shape and merges with the cell membrane. When the nucleus contracts, nuclear chromatin is highly concentrated at the edge of the nuclear membrane, and nucleoli are cleaved, accompanied by the formation of ladder-like DNA subunit fragments [14]. Then, the cell membrane invades and divides the apoptotic cell into several cells; the subunits wrapped in the bilayer membrane structure are called apoptotic bodies [15].

In this study, the CCK-8 method was employed to assay the effect of peiminine on the proliferation of HCT-116 cells. It was found that peiminine treatment significantly decreased the proliferative potential of HCT-116 cells. This result indicates that peiminine inhibited the proliferative capacity of HCT-116 cells. Inhibition of proliferation of tumor cells is often accompanied by cell apoptosis. Based on the inhibition of proliferation of HCT-116 cells by peiminine, a further study on the effect of peiminine on cell apoptosis was carried out [16]. Flow cytometric analysis with Annexin V/PI showed that the percentage apoptosis of HCT-116 cells treated with peiminine was markedly increased, relative to control cells, indicating that peiminine enhanced apoptosis of HCT-116 cells.

Tumor cells fall off from the primary tumor, invade lymphatic vessels and blood vessels, and form new migration foci called tumor migration [17]. In this study, cell scratch test showed that the healing rate of peiminine group was significantly lower than that of the control group, suggesting that peiminine delayed the migration of tumor cells. The P13K/Akt/mTOR route is a major regulatory pathway in aerobic glycolysis and cellular biosynthesis. When tumor cells are activated by the P13K/Akt/mTOR pathway, cellular biosynthesis is correspondingly activated [18].

In this study, p-P13K, p-MTOR and p-Akt in group B were markedly down-regulated, relative to the corresponding expression levels in the control group. These results suggest that peiminine activated the P13K/Akt/mTOR pathway. Oxidative stress is vital tumor etiology and tumor treatment. Under normal physiological conditions, the regulation of oxidative stress response mechanism controls homeostasis in reactive oxygen species (ROS). When this balance is impaired, oxidative stress occurs and aggravates cell damage [19,20].

In the present study, the relative expression of Wnt in peiminine group was up-regulated, relative to control cell, while β-catenin in peiminine group was markedly down-regulated. These results suggest that peiminine regulated the biological characteristics of colorectal cancer cells via induction of β-catenin/Wnt oxidative stress signal route.

CONCLUSION

This study has demonstrated that peiminine suppresses proliferative and migratory potential of colorectal HTC-116 carcinoma cells and increases their apoptosis via activating P13K/Akt/mTOR and Wnt/β-catenin oxidative stress pathways. However, there is a need for further studies to ascertain the influence of peiminine on proliferation and invasion in an animal model.

DECLARATIONS

Acknowledgement

This study was financially supported by Establishment of multi-index quality evaluation system for Tibetan medicine “Fritillaria thunbergii” (no. BSDJS-20-06).

Conflict of interest

No conflict of interest is associated with this work.

Authors’ contributions

We declare that this work was performed by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. GuiLin Jin and Lijun Xu designed the study, supervised the data collection, and analyzed the data. GuiLin Jin interpreted the data and prepared the manuscript for publication. Zhen Luo and Mingke Fen supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0) and the Budapest Open Access Initiative (http://www.budapestopenaccessinitiative.org/rea d), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.
REFERENCES


