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Research Article
Deoxyelephantopin Suppresses Invasion and Migration of Colorectal Cancer Cells Through Matrix Metalloproteinase-13

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Abstract

Background and Objective: Deoxyelephantopin (DET), the major constituent of *Elephantopus scaber*, has been shown to have anti-inflammatory effect *in vitro*. However, there is no direct evidence that DET can inhibit colorectal cancer cells migration and invasion. The anticancer activity of DET was evaluated by a MTT assay. **Materials and Methods:** Cell apoptosis were detected by Annexin V/PI staining. Cell invasion and transwell migration assays were used to observe the effect of DET on cells invasion and migration. **Results:** In the present study, data showed that DET treatment reduced colorectal cancer (CRC) cells migration and invasion and DET treatment decreased the protein levels of matrix metalloproteinase-13 (MMP-13). This is the first paper suggested DET-reduced MMP-13 expression can inhibit CRC invasion and migration. **Conclusion:** Therefore, the present study provides evidence that the DET reduced MMP-13 expression, leading to decrease the migration and invasion of CRC cells. This study provides a mechanistic insight into the inhibiting CRC functions of DET.

Key words: Deoxyelephantopin, colorectal cancer cells, proliferation, metastasis, matrix metalloproteinase-13

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Colorectal cancer (CRC) is the one of the most deadly malignancy in the world[1-3]. The current mode of treatment for colorectal cancer have declined in some Western countries, but the mortality rate in developing countries still have steady increased[4]. Mechanisms underlying the CRC are complicated and not fully understood. Therefore, the high efficiency new antitumor agents are required[5,6].

The past research has shown that *Elephantopus scaber* effectively induced human nasopharyngeal cancer cells apoptosis[7]. Deoxyelephantopin (DET), a major constituent of *Elephantopus scaber*, has recently been shown to induce many cancer cells apoptosis, including lung, breast, colorectal carcinoma and so on[8-10]. Considering the death from CRC is a result of metastatic disease[11-13], so evidence about the association between DET treatment and CRC metastasis is worthy of attention. However, little is known about whether DET could affect migration and invasion of CRC.

The tumor cells metastasis is a complex process and matrix metalloproteinases (MMPs) families are responsible for the metastasis process[14,15]. The MMP-13 overexpression has shown to associate with poor prognosis in many kind of cancer[16-20]. Many other types of invasive tumors, such as colorectal cancer[21] and chondrosarcoma cells[22]. It suggests MMP-13 might be associated with the regulation of tumor cell migration and invasion. However, little is known about whether DET could reduce MMP-13 expression in CRC.

In the present study, the effect of DET could inhibit the proliferation, cell apoptosis, invasion and migration in CRC cells, accompanied by the production of MMP-13 was investigated.

MATERIALS AND METHODS

Materials: Deoxyelephantopin (97% purity) was purchased from BioBioPha Co., Ltd. (Kunming, China). Calcein-acetoxymethyl ester (Calcein-AM), dimethyl sulfoxide (DMSO) were purchased from Beyotime Institute of Biotechnology (Nanjing, China). Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS) were purchased from GIBCO (Shanghai, China).

Cell culture: The human CRC cell lines HCT116 cells were obtained from American Type Culture Collection (ATCC) and were cultured in DMEM supplemented with 10% FBS, 100 units mL^{-1} penicillin and 100 μg mL^{-1} streptomycin and maintained at 37°C with 5% CO_2 in humidified atmosphere.

Determination of cell viability assay: The effect of DET on cell viability was determined by the cell counting kit -8 (CCK-8) assay was performed as previously described[23]. Briefly, cells were seeded in 96 well cell culture plates for 24 h, then cells were treated with different concentrations of DET for 24 h. Following treatment, 10 μL CCK-8 reagent was added to the medium and cells were further incubated at 37°C for 2 h. The absorbance was measured at 450 nm by a microplate reader.

Determination of cell apoptosis by flow cytometry: Cell apoptosis was determined by Annexin V-FITC/PIdouble staining apoptosis detection kit (Beyotime, Nanjing,China). Briefly, cells were treated with different concentrations of DET for 24 h. The cells were collected and resuspended in binding buffer (500 mL). Cells were incubated with Annexin V-FITC and PI according to manufacturer'sinstructions. Finally, the data were analyzed by flow cytometry (BD, San Jose, CA, USA).

Cell cycle assay: HCT116 cells (1×10^6) were seeded in culture dishes and treated to DET for 24 h. The cells were then resuspended in 1 mL of buffer solution. Next, solution A (250 μL, trypsin buffer) was added for 10 min, then solution B (100 μL, trypsin inhibitor and RNase buffer) was added at room temperature for 10 min. Then PI solution (200 μL) was added for 10 min in the dark. The samples were analyzed by low cytometry (BD, San Jose, CA, USA).

Determination of the caspase-3 activity: Caspase-3 activity was measured by the CaspACE™ Assay System, Colorimetric (Promega, Madison, WI, USA) following the manufacturer's instructions. Briefly, cells were lysed with RIPA lysis solution (Beyotime, Haimen, China) and the lysates were centrifuged at 12,000 rpm for 10 min at 4°C and the supernatant fraction was harvested. For caspase-3 activity assay, an aliquot of culture supernatant was incubated with 200 μM of DEVD-pNA substrate at 37°C for 4 h. The absorbance was measured at 405 nm by a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Determination of cell migration and invasion assay: Cell migration assays were measured using transwell inserts (Costar, Cambridge, MA, USA). Cell invasion assays were investigated using transwell inserts precoated with 50 μL of 0.5% matrigel (BD Biosciences, San Jose, CA, USA) in CRC cells. Cells were treated with DET at different concentrations for 8 and 24 h (migration and invasion assay), cells remaining above the filters were scraped by a cotton swab. Cells that migrated to the underside of the filters were stained with calcein-AM

dye. The cell number of three fields per well was observed under light microscopy at a magnification of 100X.

Enzyme linked immunosorbent assay: The secreted MMP-13 levels in the culture supernatants were detected by MMP-13 Human enzyme linked immunosorbent assay (ELISA) Kit (Abcam, Cambridge, MA, USA) following the manufacturer's protocol.

Statistical analysis: All statistical analyses were carried by two tails student's test, or one-way ANOVA test. Data were presented as mean±standard deviation. A p-values less than 0.05 was considered statistically significant. Computer-based calculations were conducted using SPSS version 11.5 (SPSS, Inc., Chicago, IL).

RESULTS

DET inhibits proliferation of CRC cells: CCK-8 assay demonstrated that 1, 2 and 4 μM DET decreased HCT116 cells viability in a dose-dependent way; the IC_{50} value was 2 μM (Fig. 1a). Treatment of HCT116 cells with DET caused significant time-dependent reduction in cell viability with the IC_{50} values of 2 μM at 24, 48 and 72 h, respectively (Fig. 1b).

DET induces CRC cells apoptosis: The apoptotic effect of DET on CRC cells was detected by FITC-PI analysis. The data showed that DET induces apoptosis in CRC cells in dose-dependent manner as shown in Fig. 2a.

The activation of caspase-3 is involved in CRC cell apoptosis. Therefore, the effects of DET on caspase-3 activities

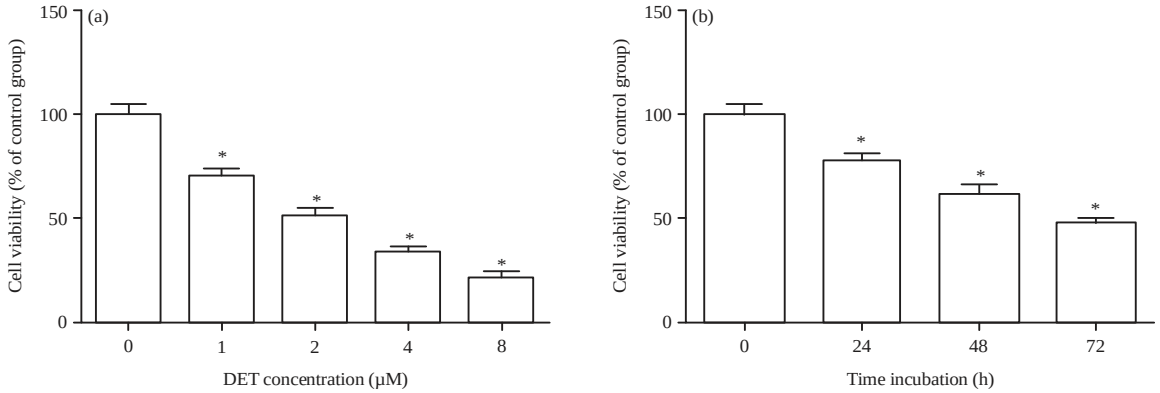

Fig. 1(a-b): Effect of DET on the cell viability of HCT116 cells, (a) Bar chart showed the percentage of cell viability after treatment with different concentrations of DET ranging from 0-4 μM on HCT116 cells for 72 h and (b) Bar chart showed the cytotoxicity of DET at different time incubation (24, 48 and 72 h) on HCT116 cells
The data expressed as Mean±SD of three independent experiments. *p<0.05, as compared with control

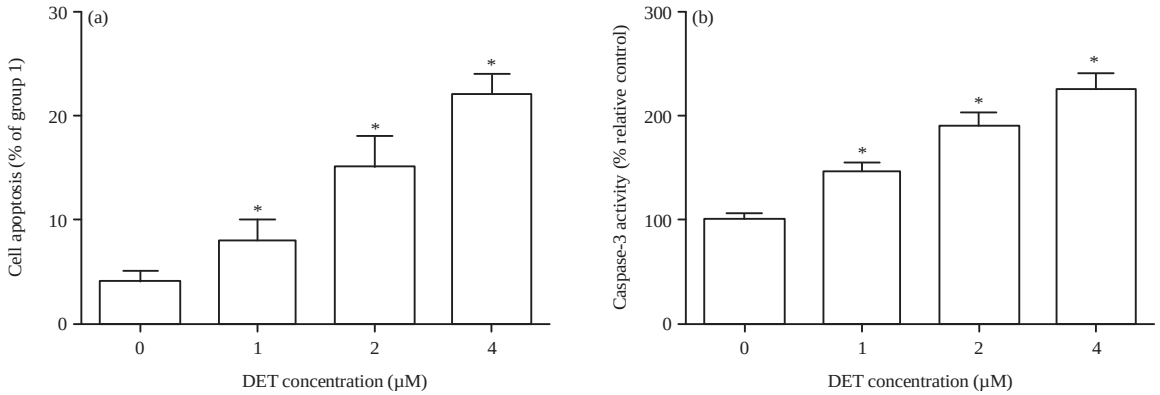

Fig. 2(a-b): Effect of DET on the cell apoptosis of HCT116 cells, (a) Bar chart showed the percentage of cell apoptosis after treatment with different concentrations of DET ranging from 0-4 μM on HCT116 cells and (b) The bar chart showed the activation of caspase-3 after treatment with different concentrations of DET ranging from 0-4 μM on HCT116 cells
The data expressed as Mean±SD. of three independent experiments. *p<0.05, as compared with control

in HCT116 cells were detected. Following DET treatment, the activation of caspase-3 was observed, with an increase of 239% compared with the control group (Fig. 2b).

Effect of DET on CRC cells cycle: The effect of DET on CRC cells cycle progression was detected by flow cytometry. The data revealed that DET increased HCT116 cells cycle arrest at S phase (Fig. 3). The number of cells in the S phase were significantly increased compared with the control group in a dose-dependent manner ($p<0.05$). So DET significantly down regulated the number of cells at G1 and G2/M phases (Fig. 3).

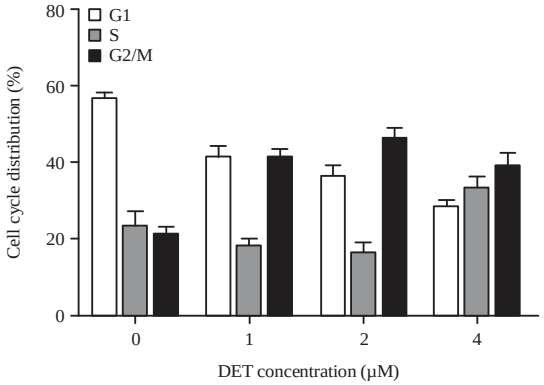

Fig. 3: Effect of DET on the cell cycle of HCT116 cells, The bar chart showed the cell cycle distribution after treatment with different concentrations of DET ranging from 0-4 μM on HCT116 cells

The data expressed as Mean±SD of three independent experiments. *p<0.05, as compared with control

DET decreases the migration of CRC cells: To evaluate the effect of DET on cellular motility, cell migration assays were used. The CRC cells were treated with different concentrations of DET (0-4 μM). After 24 h stimulation, 2-4 μM DET treatments significantly decreased the translocated cells (Fig. 4a). DET had its maximum impact at 4 μM ($p<0.05$) with a motility of 0.19-fold compared with untreated group (Fig. 4b).

DET decreases the invasion of CRC cells: Matrigel transwell chamber assays were used to investigate the role of DET on CRC cell invasion. After DET treatment, CRC cells significantly reduced migration from the above chamber to the lower chamber compared with the control groups (Fig. 5a), which implied that DET could reduce the invasive ability in CRC cells. It was found that the invasive ability of cells translocated through Matrigel-coated filter invasion chambers was up to 0.23 fold compared with control group at 4 μM DET treatment (Fig. 5b). Therefore, DET notably reduced the ability migration and invasion in the CRC cells.

DET decreases MMP-13 expression: To examined whether DET stimulated MMP-13 expression, as shown in Fig. 6, the expression of MMP-13 was decreased with the DET treated. Meanwhile, the end of the peak of MMP-13 protein expression level was detected at the concentration of 2-4 μM DET treatment in cells.

DISCUSSION

Now a days the most of clinical drugs remain unsuccessful to treat human colorectal cancer because of drug resistance

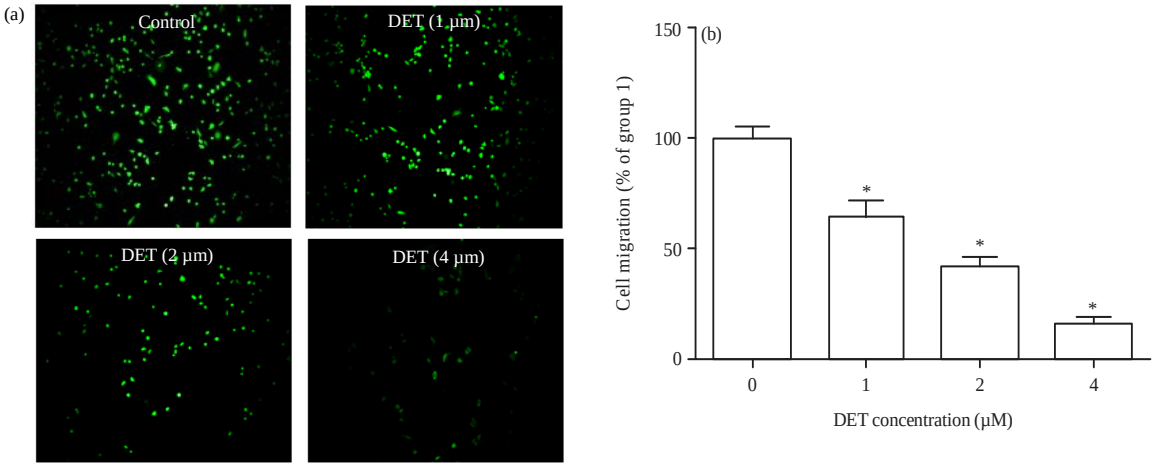

Fig. 4(a-b): Effect of DET on the cell migration of HCT116 cells, (a) Representative images of the transwell assay on HCT116 cells for 8 h and (b) Bar chart showed the percentage of cell migration after treatment with different concentrations of DET ranging from 0-4 μM on HCT116 cells for 24 h

The data expressed as Mean±SD of three independent experiments. *p<0.05, as compared with control

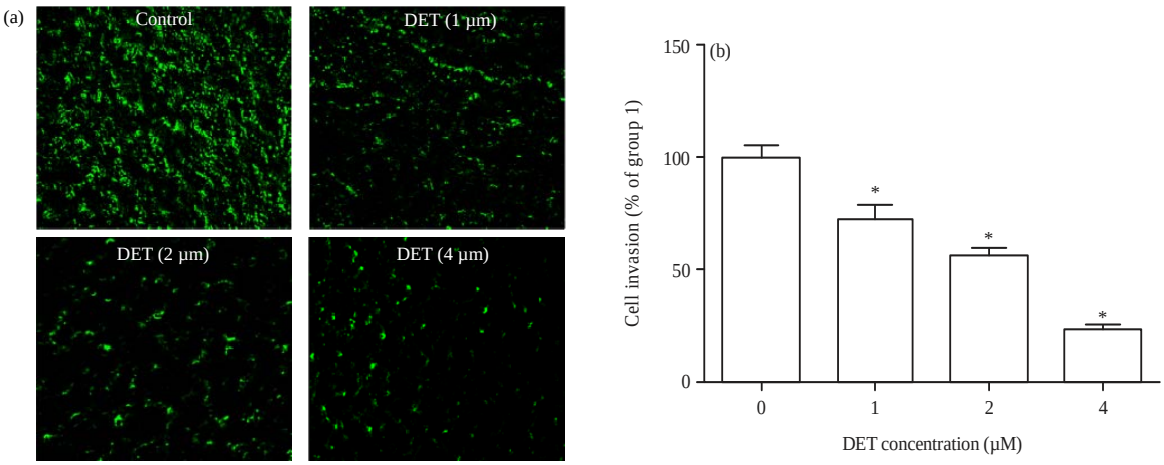

Fig. 5(a-b): Effect of DET on the cell invasion of HCT116 cells, (a) Representative images of the transwell invasion assay on HCT116 cells for 24 h and (b) Bar chart showed the percentage of cell migration after treatment with different concentrations of DET ranging from 0-40 µM on HCT116 cells for 24 h

The data expressed as Mean±SD of three independent experiments. *p<0.05, as compared with control

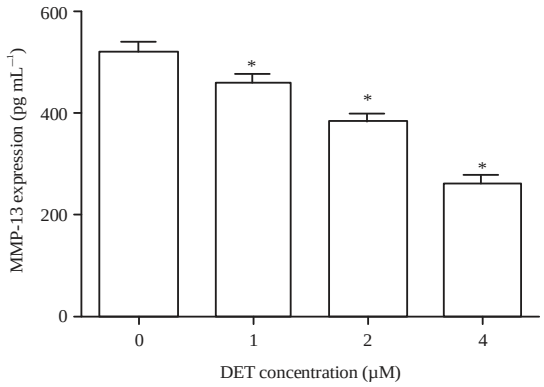

Fig. 6: DET reduces MMP-13 expression. The levels of secreted MMP-13 (active form) in the culture supernatants were measured by ELISA

The data expressed as Mean±SD of three independent experiments. *p<0.05, as compared with control

development and severe toxicity. The aims of this study were to investigate the effect of DET on the invasion and migration of CRC cells and the underlying mechanisms. These results showed that DET could reduce the migration and invasion of CRC cells associated with decreased MMP-13 expression. In addition, DET reduced CRC cells viability and induced CRC cells apoptosis. Taken together, these findings implicated DET may have beneficial effects by preventing CRC growth, inhibiting cell invasion and migration and inducing cell apoptosis.

DET has been shown to induce apoptosis and ROS generation in cancer cells[24]. DET mediated both ER stress induced paraptosis and apoptosis in triple negative breast cancer cells[25]. Moreover DET induces cell apoptosis via several signaling pathways which are derestrict in cervical cancer, lung cancer and nasopharyngeal cancer cells[26]. Further, DET induced apoptosis in osteosarcoma cells via ROS production, mitochondrial membrane changes and caspase activation; in addition, autophagy was participated in the roles of DET on osteosarcoma cells[27]. DET provoked cell apoptosis and cycle arrest in colorectal carcinoma, implying that DET is a potential anticancer candidate for colorectal carcinoma[9]. However, whether DET could inhibit proliferation of CRC remain unknown. The present study investigated whether DET could induce apoptosis and inhibit proliferation of CRC cells. The results showed that DET could effectively inhibit growth and induce apoptosis in CRC cells.

Considering cell invasion is the major characteristic of CRC[28,29], the relationship between DET exposure and CRC invasion and metastasis is worth of more attention. The local invasion is the first step for distant metastasis of tumor. Prevention of the local invasion may have a significant effect on the inhibition of distant tumor metastasis[30]. It is common knowledge that MMPs overexpression is closely connected with tumor invasion and MMP-13 has a key role in the MMP activation. Therefore, MMP-13 may be one of the DET responsive mediators and it inhibit the degradation of ECM which could lead to CRC migration and invasion.

CONCLUSION

It is concluded that the findings in this study revealed the exposure of DET may inhibit the migration and invasion of

colorectal cancer. This study highlights the effect of MMP-13 in regulation the DET reduced CRC migration and invasion. DET could be a therapeutic agent for the CRC treatment.

SIGNIFICANCE STATEMENT

This study discovers the DET (deoxyelephantopin) reduced MMP-13 expression, leading to decrease in the migration and invasion of CRC, (colorectal cancer cells) that can be beneficial for CRC treatment. This study will help the researcher to uncover the critical areas of CRC migration that many researchers were not able to explore. Thus a new theory on inhibiting CRC functions of DET may be arrived at.

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