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Original Research Article

Emodin inhibits proliferation and invasion, and induces apoptosis in human esophageal cancer cell line ECA109

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Abstract

Purpose: *To determine the anticancer effects of emodin in human esophageal carcinoma cell line ECA109.*
Methods: *Cell viability was determined by MTT assay, while cell invasion and apoptosis were measured by Transwell assay and flow cytometry, respectively. Expression levels of MMP-2, Bax, Bcl-2 and caspase-3 proteins were determined by Western blot.*
Results: *Flow cytometry data showed that the proportion of apoptotic cells was increased by emodin treatment. Apoptotic rates produced by 10, 20 and 50 μM emodin were 13.9 ± 3.8, 25.6 ± 6.2 and 39.8 ± 7.7 %, respectively. Transwell assay data revealed concentration-dependent suppression of the invasive rate of ECA109 cells by emodin (10, 20 and 50 μM) was 30.0 ± 4.5, 56.0 ± 6.8 and 69.0 ± 8.1 %, respectively. Furthermore, emodin treatment inhibited expressions of MMP-2 and Bcl-2 proteins, but induced the expression of Bax and caspase-3, when compared with control groups.*
Conclusion: *These results suggest that emodin inhibits cell proliferation and cell invasion, but induces cell apoptosis in human esophageal cancer cell line ECA109. Thus, emodin is a potential candidate for development of an effective chemotherapeutic agent against esophageal cancer.*

Keywords: *Emodin, Esophageal Cancer, Apoptosis, Cell invasion, Bax, Caspase-3*

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INTRODUCTION

Esophageal cancer is a common malignant cancer worldwide. In the past few decades, incidence of esophageal carcinoma has significantly increased, with the result that it currently affects more than 450,000 people [1,2]. Metastasis and recurrence are two key factors responsible for the high mortality of esophageal carcinoma. Even with radical resection treatment, the five-year recurrence rate of esophageal cancer is still above 40 % [3,4].

Emodin (1,3,8-trihydroxy-6-methylanthraquinone) is an active component of multiple traditional Chinese medicinal herbs, and it possesses antiviral, antimicrobial, immunosuppressive, hepato-protective, and anti-inflammatory properties [5,6]. Recent studies indicated that emodin suppressed the proliferation of various tumors such as hepatoma, gastric cancer, breast cancer and colorectal cancer, in which multiple genes and signaling pathways are involved [7-10]. However, not much is known about the effect of emodin on the growth of esophageal cancer cells.

In the present study, the effect of emodin on apoptosis and invasion of human esophageal carcinoma cell line ECA 109, and the underlying

mechanisms were investigated with a view to providing new insights into the treatment of esophageal cancer.

EXPERIMENTAL

Cell culture

Human esophageal cancer cell line ECA109 was obtained from GeneChem Company (Shanghai, China) and cultured at 37 °C in DMEM/F12 containing 10 % fetal bovine serum (FBS) in a humidified atmosphere with 5 % CO_2.

Cell proliferation assay

Standard tetrazolium bromide (MTT) assay was utilized to determine cell viability. Cells were seeded in 96-well plates (5×10^3 cells/well) with different concentrations of emodin (0, 10, 20 and 50 μM) for 24 h. Thereafter, 50 mL MTT (Sigma) solution (2 mg/mL in PBS) was added to each well, and the plates were incubated for 4 h at 37 °C. To dissolve the resultant violet formazan crystals, each medium was removed, and the cells were incubated with 200 μL dimethyl sulfoxide (DMSO) in the dark for 30 min. Absorbance was measured at 570 nm in an automated microplate reader with DMSO as blank. All assays were performed in quintuplicates and repeated at least three times.

Assay of apoptosis

Cell apoptosis was assayed by Annexin-V-FLUOS Staining Kit (Roche, USA) following the protocol provided by the kit manufacturer. The cells were then analyzed by flow cytometry using FACS Calibur (BD Biosciences, Bedford, MA, USA).

Cell invasion assay

Cell invasion was assayed in a 24-well Transwell chamber coated with Matrigel (BD Biosciences) on the upper surface of the membrane with a pore size of 8 μm (Sigma). Cells (1×10^4 cells/well) were suspended in culture media (100 μL, serum-free) and then seeded into the upper Transwell chamber. The lower chamber was covered with 10 % FBS. After 24 h incubation, the invaded cells were fixed, stained and counted under a microscope (Olympus).

Western blot

Cells were obtained and washed twice with PBS. Ice-cold radio-immunoprecipitation assay buffer (RIPA, Beyotime, Shanghai, China), containing freshly-prepared 0.01 % protease inhibitor cocktail (Sigma, Shanghai, China) was added so as to lyse the cells. Then the cells were incubated on ice for 30 min, and centrifuged at 13,000 rpm for 10 min at 4 °C. The supernatant (20 - 30 μg of protein) was run on 10 % SDS-PAGE gel and electrophoretically transferred to a polyvinylidene fluoride membrane (Millipore, Shanghai, China). The membrane was then blocked with blocking reagent and incubated with primary antibodies against MMP-2, Bax, Bcl-2, caspase 3 and GAPDH (1: 1,000 dilution), followed by incubation with the secondary antibody (1: 1,000 dilution, Beyotime, Shanghai, China). The blot was visualized under enhanced chemiluminescence (ECL, Thermo Scientific, Shanghai, China).

Statistical analysis

All the results are presented as mean ± standard deviation (SD, n = 3) and were analyzed statistically using SPSS18.0 software. Multiple comparisons were made by one-way ANOVA followed by Dunnett's test. $P < 0.05$ was considered statistically significant.

RESULTS

Emodin inhibits ECA 109 cell proliferation

The effect of emodin on viability of human esophageal cancer ECA109 cells was examined by MTT assay. Compared with the control group, the three concentrations of emodin used (10, 20 and 50 μM) significantly reduced ECA109 cell viability at 12, 24, 48 and 72 h ($p < 0.05$) in a concentration-dependent manner (Figure 1). Therefore, 10, 20 and 50 μM concentrations of emodin were used in subsequent experiments.

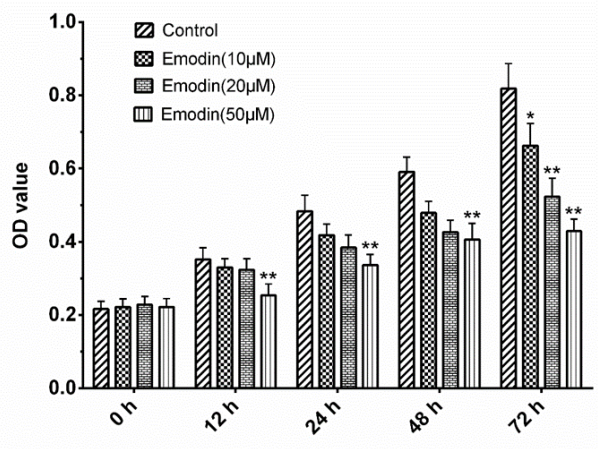

Figure 1: Effect of emodin on ECA 109 cell viability. Cells were treated with emodin (10, 20 and 50 μM) for 0, 12, 24, 48 and 72 h, and cell proliferation was measured by MTT assay. Data are presented as mean ± SD (n = 6); *$p < 0.05$; **$p < 0.01$ compared with the control group

Emodin induces ECA 109 cell apoptosis

The effect of emodin (10, 20 and 50 μM) on ECA109 cell apoptosis was determined using flow cytometry (FCM). As presented in Figure 2, while most ECA109 cells remained alive in the control group, those incubated with different concentrations of emodin for 24 h displayed significant concentration-dependent increases in the percentage of apoptotic cells (both AV and PI-positive).

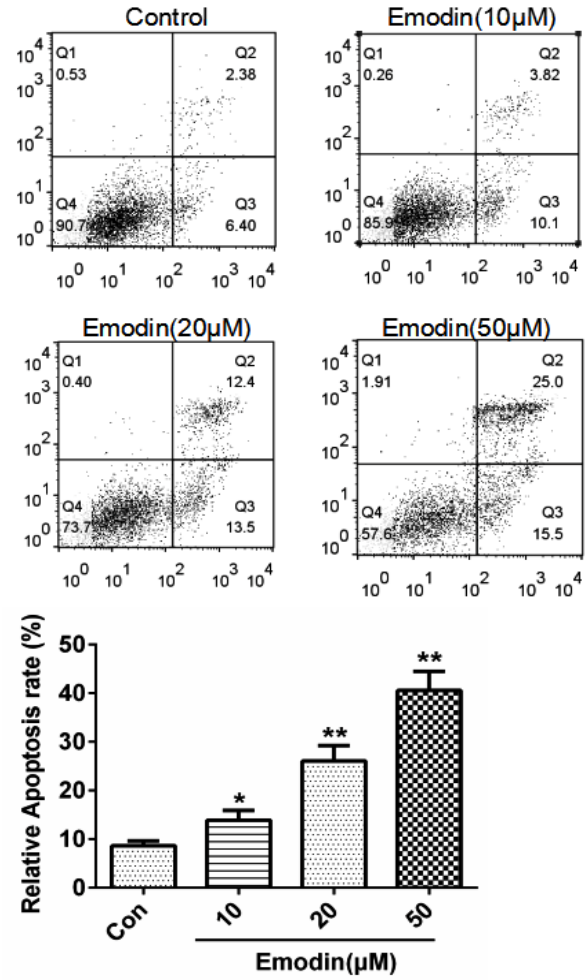

Figure 2: Effect of emodin on ECA 109 cell apoptosis. Cells were treated with emodin (10, 20 and 50 μM) for 24 h, and cell apoptosis was assessed by FCM. Data are presented as mean ± SD (n = 6); *$p < 0.05$; **$p <$ 0.01 compared with the control group

Emodin suppresses invasion of ECA 109 cells

Recent studies have demonstrated the central role of cell motility during tumor invasion and metastasis [11,12], hence, we investigated the effect of emodin on the invasive ability of ECA 109 cells using Transwell assay. As presented in Figure 3, Transwell migration assay demonstrated that emodin treatment significantly suppressed ECA 109 cell invasion in a concentration-dependent manner. The invasion rates of emodin – treated groups (10, 20 and 50 μM) were decreased by 30 ± 4.5, 56 ± 6.8 and 69 ± 8.1 %, respectively.

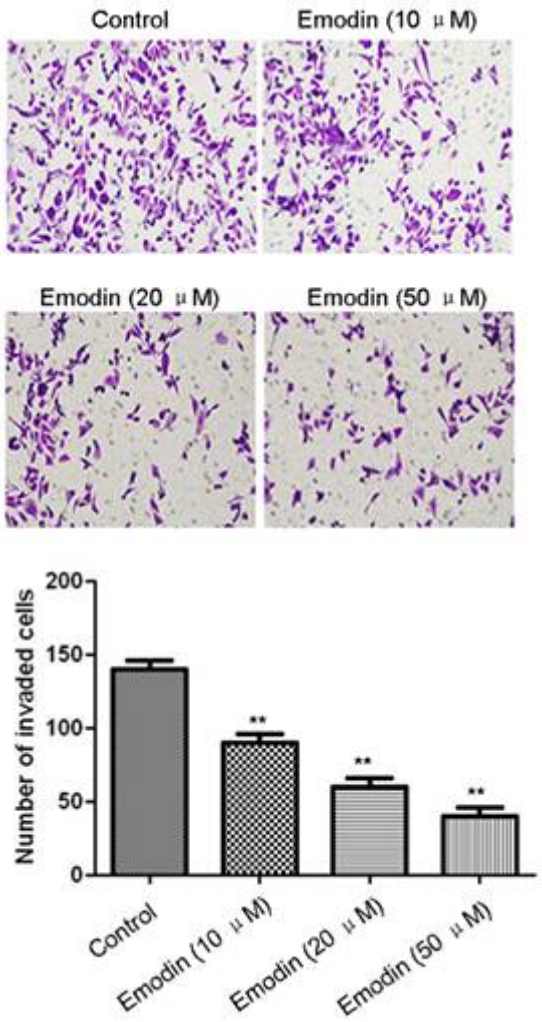

Figure 3: Effect of emodin on ECA 109 cell invasion. Cells were treated with emodin (10, 20 and 50 μM) for 24 h, and cell invasion was examined by Transwell assay. Data are presented as mean ± SD, n = 6; **$p <$ 0.01 compared with the control group

Emodin regulates expression of MMP-2, Bax, Bcl-2 and caspase 3

To elucidate the potential mechanism(s) involved in emodin-induced cell invasion, the expression levels of MMP-2, Bax, Bcl-2 and caspase 3 were measured by Western blot. It was found that MMP-2 and Bcl-2 protein levels were significantly reduced while those of Bax and caspase 3 were markedly elevated, indicating that emodin may inhibit ECA 109 cell invasion by down-regulating the expressions of MMP-2 and Bax and up-regulating Bcl-2 and caspase 3 expressions (Figure 4).

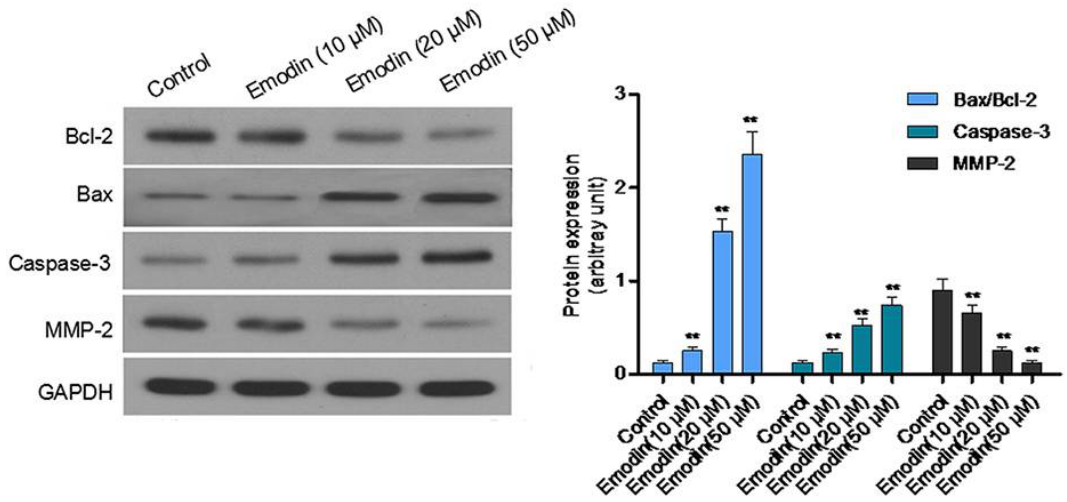

Figure 4: Effect of emodin on protein expressions of Bcl-2, Bax, Caspase-3 and MMP-2. Cells were treated with emodin (10, 20 and 50 μM) for 24 h, and protein expressions of Bcl-2, Bax, Caspase-3 and MMP-2 were measured by Western blot. Data are presented as mean ± SD (n = 6); $^{**}p < 0.01$ compared with the control group

DISCUSSION

Esophageal cancer is a prevalent malignancy worldwide. In particular, China is one of the top countries with a high incidence of esophageal cancer. Results of analysis indicated that 95 % of these esophageal cancers were in the form for squamous cell carcinoma (ESCC). Due to the poor prognosis of ESCC, it is important to elucidate the underlying mechanisms of OSCC, as well as its therapeutic targets. Traditional Chinese Medicinal (TCM) treatment, in conjunction with surgical operation, radiotherapy, or chemotherapy, can effectively alleviate cytotoxicity and strengthen immune functions. The combined use of Chinese and Western medicinal treatment is geared towards improving the quality of life and survival rate of OSCC patients by preventing recurrence and metastasis. Therefore, the search for effective anti-tumor products from Chinese medicinal herb is of vital importance to cancer therapy.

In the current study, it was demonstrated that different concentrations of emodin inhibited cell proliferation and invasion, and induced cell apoptosis in human esophageal cancer cell line ECA109. Emodin (10, 20 and 50 μM) significantly suppressed ECA109 cell proliferation in a concentration- and time-dependent manner. In addition, emodin (10, 20 and 50 μM) also induced concentration-dependent cell apoptosis after 24 h treatment. The release of several apoptotic factors, such as cytochrome c and AIF, can be elicited through activation of the intrinsic mitochondrial pathway, which results in mitochondrial apoptosis and activation of the mitochondrial death pathway. Cytochrome c release facilitates formation of the apoptosome containing the adaptor Apaf-1 and the initiator caspase-9 in the presence of dATP, which in turn activates the downstream effector caspase-3 to induce cell apoptosis [13,14]. AIF can elicit cell apoptosis independent of caspase-3 activation [15]. The results obtained in this study showed enhanced expression of activated caspase-3 proteins, indicating that emodin triggered caspase-dependent apoptosis which is activated by the mitochondrial death pathway and/or the death receptor pathway.

The first step in tumor cell invasion is the breakdown of the cytomembrane, which is known to be dependent on type IV collagen-degrading enzymes, mainly MMP-2 and MMP-9 [16]. Expressions of MMP-2 and MMP-9 have been reported to be associated with high potential of metastasis in several human carcinomas including esophageal cancer [17]. Results from Western blot demonstrated that emodin inhibited the expression of MMP-2, thus revealing the mechanism involved in the inhibition of cell invasion by emodin. This finding strongly suggests that emodin may be a promising drug target for different types of cancer.

CONCLUSION

The findings of this study show that emodin induces ECA109 cell apoptosis and inhibit cell invasion by regulating the activation and expressions of caspase-3, Bax/Bcl-2 and MMP-2, which are factors that play critical roles in esophageal cancer. Further studies are required to elucidate the possible mechanisms involved in these emodin-induced effects. Nevertheless, the findings of the present study show that emodin is

a good candidate for development of a novel and effective therapy for esophageal carcinoma.

DECLARATIONS

Acknowledgement

None declared

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. *Hatogai K, Kitano S, Fujii S, Kojima T, Daiko H, Nomura S, Yoshino T, Ohtsu A, Takiguchi Y, Doi T, Ochiai A. Comprehensive immunohistochemical analysis of tumor microenvironment immune status in esophageal squamous cell carcinoma. Oncotarget 2016.*
2. *Guan C, Zhang J, Zhang J, Shi H, Ni R. Enhanced expression of early mitotic inhibitor-1 predicts a poor prognosis in esophageal squamous cell carcinoma patients. Oncol Lett 2016; 12: 114-120.*
3. *Kimura M, Kuwabara Y, Ishiguro H, Tanaka T, Takeyama H. Bypass Operation as Palliation for Unresectable Esophageal Cancer: Selection of Suitable Patients. Hepatogastroenterol 2015; 62: 794-796.*
4. *Chen L, Chen J, Xu B, Wang Q, Zhou W, Zhang G, Sun J, Shi L, Pei H, Wu C, Jiang J. B7-H3 expression associates with tumor invasion and patient's poor survival in human esophageal cancer. Am J Transl Res 2015; 7: 2646-2660.*
5. *Yin JT, Wan B, Liu DD, Wan SX, Fu HY, Wan Y, Zhang H, Chen Y. Emodin alleviates lung injury in rats with sepsis. J Surg Res 2016; 202: 308-314.*
6. *Lin W, Zhong M, Yin H, Chen Y, Cao Q, Wang C, Ling C. Emodin induces hepatocellular carcinoma cell apoptosis through MAPK and PI3K/AKT signaling pathways in vitro and in vivo. Oncol Rep 2016.*
7. *Zhang X, Chen Y, Zhang T, Y Zhang. Inhibitory effect of emodin on human hepatoma cell line SMMC-7721 and its mechanism. Afri Health Sci 2015; 15(1): 97-100.*
8. *Chihara T, Shimpo K, Beppu H, Yamamoto N, Kaneko T, Wakamatsu K, Sonoda S. Effects of Aloe-emodin and Emodin on Proliferation of the MKN45 Human Gastric Cancer Cell Line. Asian Pac J Cancer Prev 2015; 16(9): 3887-3891.*
9. *Huang PH, Huang CY, Chen MC, Lee YT. Yue CH, Wang HY, Lin H. Emodin and aloe-emodin suppress breast cancer cell proliferation through ERα inhibition. Evid-Based Complement Alternat Med; 2013.*
10. *Niu H, Hu Z, Liu H, Hu G, Yang B, Wu S, Li F. Long non-coding RNA AK027294 involves in the process of proliferation, migration, and apoptosis of colorectal cancer cells. Tumor Biology; 2016: 1-9.*
11. *Luo Q, Kiangs D, Zhang B, Song G. Cell stiffness determined by atomic force microscopy and its correlation with cell motility. Biochim Biophys Acta 2016; 1860: 1953-1960.*
12. *Monet M, Poet M, Tauzin S, Fouque A, Cophignon A, Lagadic-Gossmann D, Vacher P, Legembre P, Counillon L. The cleaved FAS ligand activates the Na (+)/ H (+) exchanger NHE1 through Akt/ROCK1 to stimulate cell motility. Sci Rep 2016; 6: 28008.*
13. *El ME, Ahmed MA, Ahmed AA. Attenuation of renal ischemia/reperfusion injury by acai extract preconditioning in a rat model. Life Sci 2015; 123: 35-42.*
14. *Borghetti G, Yamaguchi AA, Aikawa J, Yamazaki RK, de Brito GA, Fernandes LC. Fish oil administration mediates apoptosis of Walker 256 tumor cells by modulation of p53, Bcl-2, caspase-7 and caspase-3 protein expression. Lipids Health Dis 2015; 14: 94.*
15. *Xue Y, Yang G, Wang C, Li X, Du G. Effects of shRNA-Mediated SOX9 Inhibition on Cell Proliferation and Apoptosis in Human HCC Cell Line Hep3B Mediated by Ultrasound-Targeted Microbubble Destruction (UTMD). Cell Biochem Biophys 2015; 73: 553-558.*
16. *Radenkovic S, Konjevic G, Jurisic V, Karadzic K, Nikitovic M, Gopcevic K. Values of MMP-2 and MMP-9 in tumor tissue of basal-like breast cancer patients. Cell Biochem Biophys 2014; 68: 143-152.*
17. *Li Y, Ma J, Guo Q, Duan F, Tang F, Zheng P, Zhao Z, Lu G. Overexpression of MMP-2 and MMP-9 in esophageal squamous cell carcinoma. Dis Esophagus 2009; 22: 664-667.*