

RESEARCH COMMUNICATION

Effects of Emodin Extracted from Chinese Herbs on Proliferation of Non-small Cell Lung Cancer and Underlying Mechanisms

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Abstract

To aim of this was to observe emodin-mediated cytotoxicity and its influence on Rad51 and ERCC1 expression in non-small cell lung cancer (NSCLC). NSCLC cells were cultured *in vitro* with emodin at various concentrations (0, 25, 50, 75 and 100 $\mu\text{mol/L}$) for 48h and the proliferation inhibition rate was determined by the MTT method. Then, NSCLC were treated with emodin (SK-MES-1 40 $\mu\text{mol/L}$, A549 70 $\mu\text{mol/L}$) or 20 $\mu\text{mol/L}$ U0126 (an ERK inhibitor) for 48 h, or with various concentrations of emodin for 48 h and the protein and mRNA expressions of ERCC1 and Rad51 were determined by RT-PCR and Western blot assay, respectively. Emodin exerted a suppressive effect on the proliferation of NSCLC in a concentration dependent manner. Protein and mRNA expression of ERCC1 and Rad51 was also significantly decreased with the dose. Vacuolar degeneration was observed in A549 and SK-MES-1 cell lines after emodin treatment by transmission electron microscopy. Emodin may thus inhibited cell proliferation in NSCLC cells by downregulation ERCC1 and Rad51.

Keywords: Non-small lung cell cancer - emodin - ERCC1 - Rad51 - vacuolar degeneration

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Introduction

Lung cancer accounts for 13% (1.6 million) of the total cases and 18% (1.4 million) of the deaths in year 2008 (Jemal et al., 2011). Lung cancer is the leading cause of cancer death worldwide and can be classified into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC accounts for 85%-87% of all lung cancer worldwide (Fu et al., 2007). Platinum-based chemotherapy, the recommended standard first-line systemic treatment for advanced NSCLC, has limited efficacy and significant toxicity (Soria et al., 2011).

Emodin (1,3,8-trihydroxy-6-methyl-anthraquinone) is a natural anthraquinone derivative found in the roots and rhizomes of numerous plants (Ma et al., 2012), has been reported to exhibit anti-cancer effect on several human cancers such as lung cancer (Lai et al., 2009), chronic myelocytic leukemia (Chun-Guang et al., 2010), liver cancer (Hsu et al., 2010) and gallbladder cancer (Wang et al., 2010). However, the molecular mechanisms for the growth inhibition and cytotoxicity of emodin-treated NSCLC cells are poorly understood.

Excision repair cross-complementary 1 (ERCC1) has a leading role in the nucleotide excision repair (NER) process because of its involvement in the excision of DNA adducts (Vilmar and Sørensen, 2009). It can act as a heterodimer and function as an endonuclease to catalyze incision on the 5'-side of the damaged DNA (Ko et al.,

2011). Mammalian Rad51 is a central DNA recombinase in the homologous recombination repair (HRR) pathway (Ko et al., 2011). The recombinase protein Rad51 is overexpressed in many tumors (Raderschall et al., 2002), for example, in NSCLC, high expression of Rad51 in tumor tissue is associated with a poor prognosis (Takenaka et al., 2007). The inhibition of Rad51 expression has been shown to sensitize cancer cells to chemotherapeutic agents (Ko et al., 2008); however, whether Rad51 represents a new target for emodin-mediated decreasing lung cancer survival is still unclear.

In this study, we have investigated the role of emodin in enhancing the cell death in two lung carcinoma cell lines, SK-MES-1 and A549, and explored the molecular mechanisms involved in the cytotoxic effects. We also determined the roles of repair proteins ERCC1 and Rad51 in emodin-induced cytotoxicity. Emodin may be a novel and improved therapeutic modality for advanced lung cancer in the future, especially for patients in whom lung cancer cells are resistant to chemotherapeutic agents.

Materials and Methods

Drugs and reagents

Emodin with purity >98% (Changzhou Longteng Biotech Co., Ltd), U0126 (ERK inhibitor) (Tianjin Bin Xin Bo O Bio-tech Co., Ltd), Fetal bovine serum (FBS) and DMEM high sugar (Hyclone), MTT (Amresco), DNA

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marker (Fermentas), RNA Isolation Kit and RT-PCR Kit (TaKaRa), PRO-PREPTM (Beijing SBS Genetech Co., Ltd), Bradford Protein assay kit (Shanghai Sangon Bio-tech, Co., Ltd), Rabbit polyclonal antibodies ERCC1 (FL-297) (SC-10785) and Rad51 (H-92) (sc-8349) (Santa Cruz, CA).

Cell lines and culture

Lung squamous cell carcinoma cells (SK-MES-1) were purchased from the Shanghai Institutes for Biological Sciences. Human bronchioloalveolar cell carcinoma cells (A549) were kindly provided by the Department of Pathology of Chongqing Daping Hospital. The two cell lines were cultured at 37 °C in a humidified atmosphere containing 5% CO₂ in DMEM complete medium supplemented with Penicillin (100 units/mL), Streptomycin (100 mg/mL), and fetal bovine serum (FBS) (10%).

Effect of emodin on the proliferation of SK-MES-1 and A549

Cell proliferation was determined by MTT assay. In brief, the SK-MES-1 and A549 cells in logarithmic phase were seeded into 96-well plate at a density of 1×10⁴/well and 1×10³/well followed by incubation at 37 °C for 24 h, respectively. Then, they were independently treated with emodin at different concentrations (25, 50, 75, 100 μmol/L). In the solvent control group, cells were treated with only equal volume of DMSO, and only medium was included in the blank control group. Five wells were included in each group and experiment was repeated at least three times. Incubation was performed for 48 h and then 20 μl of MTT (5 g/L) was added to each well followed by additional incubation for 4 h. The medium was removed and 150 μl of DMSO added followed by gently shaking for 10 min. The absorbance (A) was determined at 550 nm with a microplate reader. The proliferation inhibition rate and half maximal inhibitory concentration (IC₅₀) were calculated.

Proliferation inhibition rate (%) = $(1 - A_{UA} / A_{solvent}) \times 100\%$

Grouping and treatment

SK-MES-1 and A549 in logarithmic phase were collected. The following were carried out: (1) Cells were treated with emodin (SK-MES-1 40 μmol/L, A549 70 μmol/L) combined with U0126 (20 μmol/L) for 48 h, or alone. In the control group, cells were maintained in the medium without treatment or with only equal volume of DMSO; (2) Cells were treated with emodin at different concentrations (SK-MES-1 0, 20, 40, 80 μmol/L and A549 0, 35, 70, 140 μmol/L) for 48 h;

Observing the ultrastructure of NSCLC

To observe change of the ultrastructure of the two cells before and after emodin treatment by transmission electron microscope, the cells were treated with emodin (SK-MES-1 80 μmol/L, A549 140 μmol/L) for 48h and the control group cells were treated with complete medium with 10% FBS. Then, the cells were fixed with glutaraldehyde and osmium tetroxide dehydration in a

graded ethanol series, and flat embedding in Araldite. Ultrathin sections (40–60 nm) were placed on grids (200 mesh), and double-stained with uranyl acetate and lead citrate. The grids containing the sections were observed on a Hitachi H-7500 electron microscope.

RT-PCR

Cells in each group were collected and treated with BIOZOL. Total RNA was extracted by using chloroform - isopropyl alcohol. The absorbance of extracted RNA was measured at 260 nm and 280 nm followed by the calculation of ratio of A₂₆₀ to A₂₈₀. The extracted RNA was used as template and reverse transcribed into cDNA. The cDNA was then applied to amplification by PCR. The mixture (25 μl) for PCR included 2.5 μl of cDNA. β-actin served as an internal loading control. The conditions for gene amplification were pre-denaturation at 94 °C for 3 min, 30 cycles of denaturation at 94 °C for 30 s, X °C for 30 s and extension at 72 °C for 45 s, followed by a final extension at 72 °C for 10 min. X means annealing temperature. Annealing temperature of ERCC1, Rad51 and β-actin were 64 °C, 62 °C and 56 °C, respectively. The primers were as follows: ERCC1: 5'-GTC TGA CCA CCG TGA AGT CAG TC-3' (forward), 5'-AGT CAG GAA AGC CGG ATG CCA GA-3' (reverse), anticipated size of 490 bp; Rad51: 5'-GGC CAG GTT TCT GCG GAT GCT T-3' (forward), 5'-TGG CAT CTC CCA CTC CAT CTG CA-3' (reverse), anticipated size of 278 bp; β-actin: 5'-GTT AGT TGC GTT ACA CCC TTT CTT G-3' (forward), 5'-ACT GCT GTC ACC TTC ACC TGG CC-3' (reverse), anticipated size of 160 bp; The products were subjected to 1.5% agarose gel electrophoresis for identification and the optical density of each target gene was determined and normalized by that of β-actin serving as the relative expression of corresponding target gene.

Western blot analysis

Total protein was extracted and protein concentration was determined by Bradford method. Then, an appropriate amount of proteins was subjected to SDS-PAGE and transferred onto PVDF membrane followed by blocking in 5 g/ml non-fat milk at 37 °C for 1 h. The membranes were independently treated with rabbit antihuman polyclonal antibody (ERCC1 (1:200), Rad51 (1:200) and β-actin (1:500)) in 5% non-fat milk at 4°C overnight. After washing in TBST, these membranes were incubated with HRP conjugated goat anti-rabbit secondary antibody (1:1000) correspondingly at 37 °C for 2 h. Color development was done by enhanced chemiluminescence and representative photographs were captured using a chemiluminescence imaging system (ChemiDocXRS). The optical density and area of each band were determined with Quantity One 4.4.0 software. The optical density and area of each band were normalized by those of β-actin for semi-quantitation.

Statistical analysis

Data were expressed as mean ± standard deviation ($\bar{x} \pm s$). The normality test and homogeneity of variance test were performed by SPSS 17.0 software firstly. Comparisons among different groups were

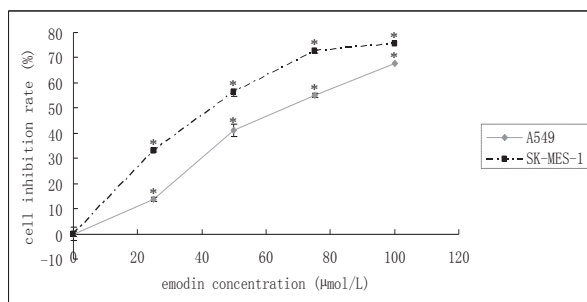


Figure 1. Cell Inhibition was Determined by MTT Assay. SK-MES-1 and A549 cells were treated with various concentrations of emodin (25, 50, 75 and 100 $\mu\text{mol/L}$) for 48 h. SK-MES-1 and A549 cells exposed to emodin showed an increase in inhibition of cell proliferation compared with control (* $P < 0.05$)

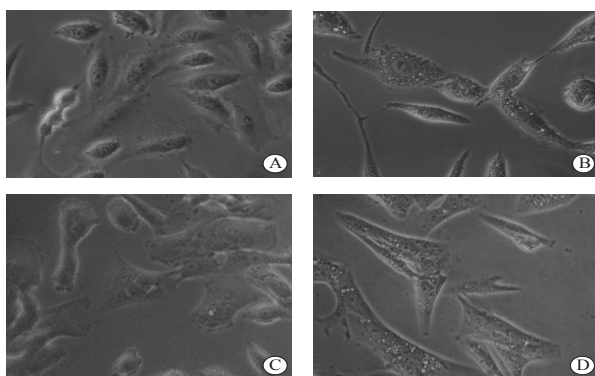


Figure 2. The Cells were Treated with Various Concentrations of Emodin for 48 h and Cell Morphology Changes were Observed by Light Microscope. 2A & C A549 and SK-MES-1 cells were cultured in DMEM-High Glucose medium supplemented with 10% Fetal Bovine Serum (FBS). 2B & D A549 and SK-MES-1 cells exposed to emodin at 140 $\mu\text{mol/L}$ and 80 $\mu\text{mol/L}$, respectively

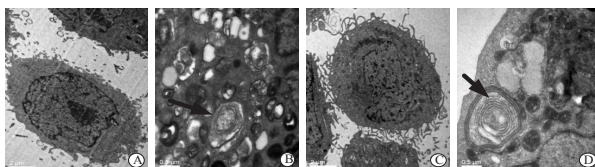


Figure 3. The Cells were Treated with Various Concentrations of Emodin for 48 h and Ultrastructural Changes were Observed by Transmission Electron Microscopy. 3A & C A549 and SK-MES-1 cells were cultured in DMEM-High Glucose medium supplemented with 10% Fetal Bovine Serum (FBS). 3B & D A549 and SK-MES-1 cells exposed to emodin at 140 $\mu\text{mol/L}$ and 80 $\mu\text{mol/L}$, respectively

performed one way or two way analysis of variance. Comparisons intra-group were done with SNK-q test. A value of $P < 0.05$ was considered statistically significant.

Results

Effect of emodin on the proliferation of NSCLC

Results showed emodin at different concentration had a suppressive effect on the proliferation of NSCLC, which was in a concentration dependent manner (Figure 1). Significant differences were noted in the proliferation of NSCLC among treatments with different concentrations of emodin ($p < 0.05$). The OD value of SK-MES-1 and A549 without treatment were 0.674 ± 0.021 and 0.474 ± 0.006 ,

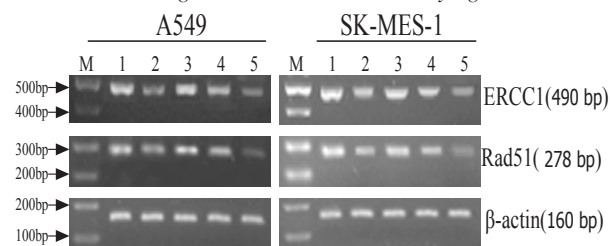


Figure 4. mRNA Expressions of ERCC1 and Rad51 in SK-MES-1 Cells and A549 Cells. Lane M: DNA marker; Lane 1: control group (cells without treatment); Lane 2: emodin group (A549 70 $\mu\text{mol/L}$, SK-MES-1 40 $\mu\text{mol/L}$); Lane 3: solvent group; Lane 4: U0126 group (20 $\mu\text{mol/L}$); Lane 5: emodin-U0126 cotreatment group (A549 70 $\mu\text{mol/L}$, SK-MES-1 40 $\mu\text{mol/L}$, U0126 20 $\mu\text{mol/L}$)

Table 1. mRNA Expressions of ERCC1 and Rad51 in NSCLC Treated with Emodin and U0126 (ERK inhibitor) (target gene/ β -actin, $\bar{x} \pm s$, n=3)

Group	A549		SK-MES-1	
	ERCC1	Rad51	ERCC1	Rad51
1	1.309 \pm 0.064	0.758 \pm 0.064	1.145 \pm 0.029	0.890 \pm 0.017
2	0.812 \pm 0.015 ^{abc}	0.518 \pm 0.062 ^{abc}	0.638 \pm 0.061 ^{abc}	0.530 \pm 0.085 ^{abc}
3	1.308 \pm 0.029	0.753 \pm 0.048	1.147 \pm 0.073	0.886 \pm 0.020
4	1.016 \pm 0.051 ^{abc}	0.673 \pm 0.056 ^{abc}	0.801 \pm 0.068 ^{abc}	0.671 \pm 0.074 ^{abc}
5	0.490 \pm 0.053 ^{ab}	0.225 \pm 0.016 ^{ab}	0.365 \pm 0.062 ^{ab}	0.220 \pm 0.085 ^{ab}

1-control group (cells without treatment); 2-emodin group (A549 70 $\mu\text{mol/L}$, SK-MES-1 40 $\mu\text{mol/L}$); 3-solvent group; 4-U0126 group (20 $\mu\text{mol/L}$); 5-emodin-U0126 cotreatment group (A549 70 $\mu\text{mol/L}$, SK-MES-1 40 $\mu\text{mol/L}$, U0126 20 $\mu\text{mol/L}$); * $P < 0.05$ vs control group; ^b $P < 0.05$ vs solvent group; ^c $P < 0.05$ vs emodin-U0126 cotreatment group

and the OD value of SK-MES-1 and A549 treatment with only equal volume of DMSO were 0.680 ± 0.01 and 0.480 ± 0.007 respectively, so no particular difference was found between the two groups ($p > 0.05$). After emodin treatment for 48 h, MTT assay showed that the IC₅₀ of SK-MES-1 cells and A549 cells were (41.95 ± 1.27) $\mu\text{mol/L}$ and (70.18 ± 1.94) $\mu\text{mol/L}$, respectively. Thus, the emodin at 40 $\mu\text{mol/L}$ (SK-MES-1) or 70 $\mu\text{mol/L}$ (A549) was used in the following experiments.

Change in the cell morphology of NSCLC before and after treatment with emodin

Cell morphology changes in NSCLC was examined after emodin treatment for 48h by light microscope. Before SK-MES-1 cells and A549 cells treated with emodin, the cell volume was normal, strong light refraction of membrane, and there were little vacuole in cytoplasm (Figure 2A, 2C). After treatment with emodin for 48 h, the cell volume was smaller and longer, losing light refraction of membrane, and there were lots of vacuole in the cytoplasm (Figure 2B, 2D).

Change in the ultrastructure of NSCLC before and after treatment with emodin

Ultrastructural changes in NSCLC was examined after emodin treatment for 48 h by transmission electron microscopy. Normal appearance of mitochondria, nucleus and chromatin were seen in control NSCLC (Figure 3A, 3C). After emodin treatment, NSCLC exhibited typical autophagosome, lipid droplet, and ectatic endoplasmic reticulum et al. (Figure 3B, 3D). Arrowhead labeled autophagosome.

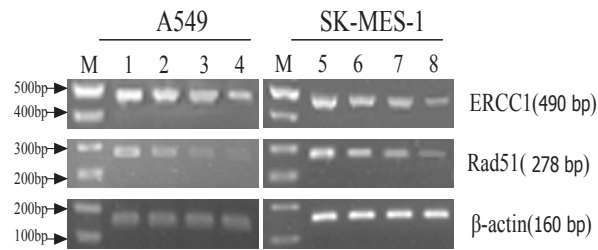


Figure 5. mRNA Expressions of ERCC1 and Rad51 in SK-MES-1 Cells and A549 Cells Treated with Emodin at Various Concentration. Lane M: DNA marker; Lane 1,5: control group (cells without emodin treatment); Lane 2: 35 $\mu\text{mol/L}$; Lane 3: 70 $\mu\text{mol/L}$; Lane 4: 140 $\mu\text{mol/L}$; Lane 6: 20 $\mu\text{mol/L}$; Lane 7: 40 $\mu\text{mol/L}$; Lane 8: 80 $\mu\text{mol/L}$

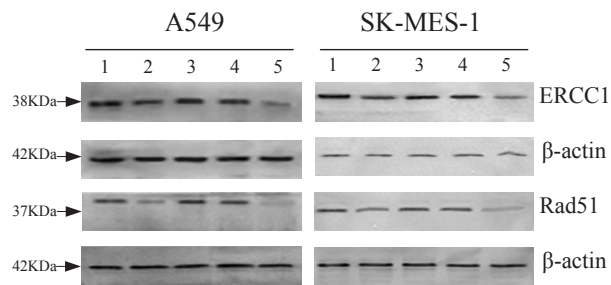


Figure 6. Protein Expressions of ERCC1 and Rad51 in SK-MES-1 Cells and A549 Cells. Lane 1: control group (cells without emodin treatment); Lane 2: emodin group (A549 70 $\mu\text{mol/L}$, SK-MES-1 40 $\mu\text{mol/L}$); Lane 3: solvent group; Lane 4: U0126 group (20 $\mu\text{mol/L}$); Lane 5: emodin-U0126 cotreatment group (A549 70 $\mu\text{mol/L}$, SK-MES-1 40 $\mu\text{mol/L}$, U0126 20 $\mu\text{mol/L}$)

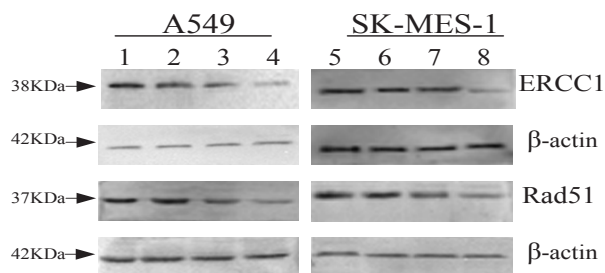


Figure 7. Protein Expressions of ERCC1 and Rad51 in SK-MES-1 Cells and A549 Cells Treated with Emodin at Various Concentration. Lane 1, 5: control group (cells without emodin treatment); Lane 2: 35 $\mu\text{mol/L}$; Lane 3: 70 $\mu\text{mol/L}$; Lane 4: 140 $\mu\text{mol/L}$; Lane 6: 20 $\mu\text{mol/L}$; Lane 7: 40 $\mu\text{mol/L}$; Lane 8: 80 $\mu\text{mol/L}$

Effect of emodin on the mRNA expressions of ERCC1 and Rad51 in NSCLC

In the experiment 1, the cells treated with emodin (SK-MES-1 40 $\mu\text{mol/L}$, A549 70 $\mu\text{mol/L}$) or 20 $\mu\text{mol/L}$ U0126 (ERK inhibitor) had significantly decreased mRNA expressions of ERCC1 and Rad51 when compared with those in control group ($p < 0.05$). There was no significant difference between cells treated with DMSO and without treatment ($p > 0.05$) (Figure 4 and Table 1). In the experiment 2, after treatment with emodin at different concentrations, the mRNA expressions of ERCC1 and Rad51 concentration-dependently decreased in the SK-MES-1 and A549 ($p < 0.05$). After the SK-MES-1 cells treated with emodin at 80 $\mu\text{mol/L}$ for 48 h, the mRNA expressions of ERCC1 and Rad51 significantly decreased

Table 2. mRNA Expressions of ERCC1 and Rad51 in NSCLC Treated with Emodin at Various Concentrations (target gene/ β -actin, $\bar{x} \pm s$, n=3)

Group ($\mu\text{mol/L}$)	A549		SK-MES-1	
	ERCC1	Rad51	ERCC1	Rad51
0	0.953 \pm 0.058	0.718 \pm 0.072	0.517 \pm 0.061	0.408 \pm 0.036
35	0.790 \pm 0.085 ^a	0.582 \pm 0.088 ^a	0.396 \pm 0.073 ^a	0.334 \pm 0.039 ^a
70	0.640 \pm 0.050 ^{ab}	0.471 \pm 0.072 ^{ab}	0.283 \pm 0.036 ^{ab}	0.227 \pm 0.053 ^{ab}
140	0.378 \pm 0.035 ^{abc}	0.323 \pm 0.019 ^{abc}	0.126 \pm 0.040 ^{abc}	0.110 \pm 0.027 ^{abc}

^a $P < 0.05$ vs control group; ^b $P < 0.05$ vs emodin group (A549 35 $\mu\text{mol/L}$, SK-MES-1 20 $\mu\text{mol/L}$); ^c $P < 0.05$ vs emodin group (A549 70 $\mu\text{mol/L}$, SK-MES-1 40 $\mu\text{mol/L}$)

Table 3. Protein expressions of ERCC1 and Rad51 in NSCLC Treated with Emodin and U0126 (target gene/ β -actin, $\bar{x} \pm s$, n=3)

Group	A549		SK-MES-1	
	ERCC1	Rad51	ERCC1	Rad51
1	0.784 \pm 0.034	0.715 \pm 0.041	1.229 \pm 0.062	0.917 \pm 0.026
2	0.408 \pm 0.031 ^{abc}	0.340 \pm 0.068 ^{abc}	0.657 \pm 0.043 ^{abc}	0.539 \pm 0.087 ^{abc}
3	0.779 \pm 0.030	0.722 \pm 0.036	1.232 \pm 0.081	0.916 \pm 0.047
4	0.551 \pm 0.058 ^{abc}	0.565 \pm 0.024 ^{abc}	1.000 \pm 0.041 ^{abc}	0.760 \pm 0.065 ^{abc}
5	0.217 \pm 0.021 ^{ab}	0.162 \pm 0.049 ^{ab}	0.399 \pm 0.079 ^{ab}	0.316 \pm 0.013 ^{ab}

1-control group (cells without treatment); 2- emodin group (A549 70 $\mu\text{mol/L}$, SK-MES-1 40 $\mu\text{mol/L}$); 3-solvent group; 4-U0126 group (20 $\mu\text{mol/L}$); 5- emodin-U0126 cotreatment group (A549 70 $\mu\text{mol/L}$, SK-MES-1 40 $\mu\text{mol/L}$, U0126 20 $\mu\text{mol/L}$); ^a $P < 0.05$ vs control group; ^b $P < 0.05$ vs solvent group; ^c $P < 0.05$ vs emodin-U0126 cotreatment group

Table 4. Protein Expressions of ERCC1 and Rad51 in NSCLC Treated with Emodin at Various Concentrations (target gene/ β -actin, $\bar{x} \pm s$, n=3)

Group ($\mu\text{mol/L}$)	A549		SK-MES-1	
	ERCC1	Rad51	ERCC1	Rad51
0	1.880 \pm 0.071	0.893 \pm 0.036	1.169 \pm 0.045	1.753 \pm 0.077
35	1.448 \pm 0.086 ^a	0.741 \pm 0.038 ^a	0.838 \pm 0.049 ^a	1.350 \pm 0.056 ^a
70	0.862 \pm 0.038 ^{ab}	0.485 \pm 0.068 ^{ab}	0.581 \pm 0.063 ^{ab}	0.991 \pm 0.075 ^{ab}
140	0.286 \pm 0.019 ^{abc}	0.197 \pm 0.012 ^{abc}	0.159 \pm 0.013 ^{abc}	0.589 \pm 0.021 ^{abc}

^a $P < 0.05$ vs control group; ^b $P < 0.05$ vs emodin group (A549 35 $\mu\text{mol/L}$, SK-MES-1 20 $\mu\text{mol/L}$); ^c $P < 0.05$ vs emodin group (A549 70 $\mu\text{mol/L}$, SK-MES-1 40 $\mu\text{mol/L}$)

to 75.63% and 73.11%, compared to the control group (0 $\mu\text{mol/L}$), respectively. After the A549 cells treated with emodin at 140 $\mu\text{mol/L}$ for 48 h, the mRNA expressions of ERCC1 and Rad51 significantly decreased to 60.34% and 54.95%, compared with the control group (0 $\mu\text{mol/L}$), respectively (Figure 5 and Table2).

Effect of emodin on the protein expressions of ERCC1 and Rad51 in NSCLC

In the experiment 1, the cells treated with emodin (SK-MES-1 40 $\mu\text{mol/L}$, A549 70 $\mu\text{mol/L}$) or 20 $\mu\text{mol/L}$ U0126 had significantly decreased protein expressions of ERCC1 and Rad51 when compared with those in the control groups ($p < 0.05$). There was no significant difference between cells treated with DMSO and without treatment ($p > 0.05$) (Figure 6 and Table 3). In the experiment 2, after treatment with emodin at different concentrations, the protein expressions of ERCC1 and Rad51 were decreased in a concentration dependent manner in the SK-MES-1 and A549 ($p < 0.05$). After the SK-MES-1 cells treated with emodin at 80 $\mu\text{mol/L}$ for 48 h, the protein expressions of ERCC1 and Rad51 significantly decreased to 86.40% and 66.39%, compared to the control group (0 $\mu\text{mol/L}$), respectively. After the A549 cells treated with emodin at

140 $\mu\text{mol/L}$ for 48 h, the protein expressions of ERCC1 and Rad51 significantly decreased to 84.77% and 77.89%, compared with the control group (0 $\mu\text{mol/L}$), respectively (Figure 7 and Table 4).

Discussion

Emodin is an active component found in the roots and rhizomes of numerous plants. This herb has been used in traditional Chinese medicine for the treatment of skin burns, infection, gallstones, hepatitis, inflammation, and osteomyelitis for years. The antiproliferative activity of emodin has been reported before, for example, emodin inhibited TNF- α -induced human aortic smooth muscle cell proliferation via the caspase- and mitochondrial-dependent apoptotic pathway (Heo et al., 2008) and emodin suppressed IL-1 β -induced mesangial cell proliferation via inactivating MKK3/6 and p38 MAPK (Zhou et al., 2006), moreover a report has shown that emodin could antagonize the signaling pathways ERK and AKT, which are essential for cell survival in A549 lung carcinoma cells (Su et al., 2005).

Some reports showed that emodin suppressed lung cancer cells proliferation via inactivating ERK1/2 signaling pathway (Ko et al., 2010; Su et al., 2010). Emodin at low doses of 2-10 $\mu\text{mol/L}$ and U0126 (an ERK1/2 inhibitor) cotreated additively, decreased cellular phosphor-ERK1/2 level (Chen et al., 2009). Ko et al. (2010) demonstrated that emodin induced cytotoxicity occurs via ERK1/2 inactivation and via ERCC1 and Rad51 could cause down regulation in human lung cancer cells. Our study showed the mRNA and protein levels of ERCC1 and Rad51 significantly decreased in the lung cancer cells cotreated with emodin and U0126 (ERK inhibitor), which was minimally decreased in those treated with emodin alone or U0126 alone. Therefore, the inactivation of ERK1/2 induced by emodin was partly responsible, for cell death in lung cancer cells.

In this study, we found that emodin significantly inhibited the proliferation of SK-MES-1 and A549 cells in a dose-dependent manner. After emodin treatment for 48 h, MTT assay showed that the IC₅₀ of SK-MES-1 cells and A549 cells were of (41.95 \pm 1.27) $\mu\text{mol/L}$ and (70.18 \pm 1.94) $\mu\text{mol/L}$, respectively.

High expression of Rad51 in tumor tissues is associated with an unfavorable prognosis in lung cancer (Qiao et al., 2005). A report showed that the down-regulation of Rad51 in lung cancer cells could increase the chemosensitivity of cells to gemcitabine (Tsai et al., 2010). Increased expression of ERCC1 in several cancers has been associated with more efficient removal of DNA adducts induced by platinum, leading to clinical resistance to platinum-based agents (Altaha et al., 2004). On the other hand, patients with NSCLC who had a lower level of ERCC1 were shown to have longer overall survival after treatment with platinum-based chemotherapy than patients who had a higher level of ERCC1 (Ceppi et al., 2006; Olaussen et al., 2006; Azuma et al., 2007). Emodin enhances cisplatin-induced cytotoxicity via down-regulation of ERCC1 and inactivation of ERK1/2 in the NSCLC (Ko et al., 2010). When the proliferation

of SK-MES-1 and A549 cells was inhibited, the mRNA and protein levels of ERCC1 and Rad51 corresponding decreased. Emodin suppressed the mRNA and protein levels of ERCC1 and Rad51 in a dose-dependent manner.

In this study, we observed that SK-MES-1 and A549 cells with emodin treatment both had vacuolar degeneration in cytoplasm. After emodin treatment, we found the typical autophagosome, lipid droplet, and ectopic endoplasmic reticulum et al. in SK-MES-1 and A549 cells by transmission electron microscopy. Consequently, there may be some other signaling pathway of that emodin could inhibit lung cancer cells proliferation, and hence further experiments are needed to prove it.

We therefore conclude that, one of the molecular mechanisms for the emodin inhibition cell proliferation in human lung carcinoma cells ERCC1 and Rad51 are downregulated and that ERK1/2 signaling pathway is inactivated in human lung carcinoma cells. Consequently, emodin can be viewed as a possible new agent for lung cancer treatment.

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