Aqueous extract of *Taxus Chinensis* (Pilger) Rehd inhibits lung carcinoma A549 cells through the epidermal growth factor receptor/mitogen-activated protein kinase pathway *in vitro* and *in vivo*

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Abstract

OBJECTIVE: To explore the anticancer mechanism of aqueous extract of *Taxus Chinensis* (Pilger) Rehd (AETC).

METHODS: The serum pharmacological method was used to avoid interference from administration of the crude medicinal herbs. Eight purebred New Zealand rabbits were used for preparation of serum containing various concentrations of AETC. Forty-eight Balb/c-nu mice were used for *in vivo* experiments. The effects of serum containing AETC on the proliferation of A549 cells and expression levels of the epidermal growth factor receptor/mitogen-activated protein kinase (EGFR/MAPK) pathway-related proteins *in vitro* were investigated. Additionally, the effects on the growth of A549 xenografts in nude mice, and expression levels of the EGFR/MAPK pathway-related proteins in the xenografts, were investigated.

RESULTS: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay revealed that the serum containing AETC significantly decreased the viability of A549 cells in a dose-dependent manner. Western blot showed that the serum containing various concentrations of AETC strongly reduced the levels of phospho-Jun N-terminal kinase (p-JNK) and phospho-extracellular signal-regulated kinase 1/2 (ERK1/2) while it increased the level of p-p38. However, no significant effects on the expression levels of JNK, ERK1/2, and p38 MAPK were found. In addition, an anticancer effect from AETC was observed *in vivo* in the Balb/c-nu mice bearing A549 xenografts.

CONCLUSION: AETC has significant effects on the growth of A549 xenografts and on the activity of the EGFR/MAPK pathway. Therefore, AETC may be beneficial in lung carcinoma treatment.
INTRODUCTION

Lung cancer is the leading cause of death in cancer patients worldwide,\(^1\) and non-small-cell lung cancer (NSCLC) accounts for more than 75%\(^2\) of lung cancers. With only a 20%-35% response rate and a median survival of 10-12 months, chemotherapy is still not effective enough for patients with advanced NSCLC.\(^3\) Therefore, targeting molecules are critical to cancer treatment and targeted therapy alone or in combination with other therapies is a promising strategy for treating cancers including NSCLC.\(^4\) Moreover, the use of complementary and alternative medicine (CAM) to relieve the side effects and improve the efficacy of chemotherapy is popular for treating cancer patients, particularly in Western countries.\(^5\) Traditional Chinese medicine (TCM) has a long history of treating diseases with advantages of low costs, multiple targets, and low toxicity. Therefore, TCM has been extensively used as an alternative medicine in many countries.

Taxus, a general term for Taxaceae Taxus species, is a natural anticancer plant. *Taxus Chinensis* (Pilger) Rehd is one of the subspecies of Taxus. A variety of extracts from *Taxus Chinensis* (Pilger) Rehd have been used as the anticancer compounds, such as paclitaxel, cephalomannine, baccatin III, 10-deacetyl baccatin III, and polyproprenols.\(^6-10\) Paclitaxel is the most effective anticancer ingredient extracted from Taxus species. According to our previous study, the aqueous extract of *Taxus Chinensis* (Pilger) Rehd (AETC) inhibits A549 cells by inducing apoptosis while paclitaxel induces cellular necrosis.\(^11\) Therefore, the active ingredient of AETC is not paclitaxel.

AETC has been used as a TCM formula for thousand years in China. It is bitter in flavor and nature with tropism to the heart meridian. AETC is usually used to treat cancer, kidney disease, and rheumatism alone or in combination with other herbs. Our previous studies have shown that AETC has inhibitory effects on A549 cells and induces cell cycle arrest and apoptosis.\(^12\) However, the underlying molecular mechanism has not been investigated. Therefore, the present study is designed to elucidate the possible mechanism of cell apoptosis induced by AETC.

We investigated whether serum containing AETC inhibited the viability of A549 cells or down-regulated epidermal growth factor receptor/mitogen-activated protein kinase (EGFR/MAPK) signaling in vitro. In the *in vivo* experiments, we explored whether AETC exhibited significant inhibitory effects on the growth of A549 xenografts and on the activity of the EGFR/MAPK pathway.

**Key words:** Taxus; Lung neoplasms; Receptor, epidermal growth factor; Mitogen-activated protein kinases; *In vitro*; *In vivo*

**MATERIALS AND METHODS**

**Materials**

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide (MTT), penicillin, and streptomycin were purchased from Sigma (St. Louis, MO, USA). F12-K medium, fetal bovine serum, and trypsin-EDTA (Ethylene Diamine Tetraacetic Acid) were purchased from Gibco BRL (Gaithersburg, MD, USA). Antibodies against epidermal growth factor receptor (EGFR), phospho-EGFR (p-EGFR), extracellular signal-regulated protein kinases (ERK1/2), phospho-ERK1/2 (p-ERK1/2), c-Jun N-terminal kinase (JNK), phospho-JNK (p-JNK), p38, and phospho-p38 (p-p38) were purchased from Cell Signaling Technologies (Beverly, MA, USA). Antibodies against β-tubulin were obtained from Sigma. Horseradish Peroxidase (HRP)-conjugated secondary antibodies against mouse IgG and rabbit IgG were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Enhanced Chemiluminescent Kits (ECK) were obtained from Millipore Corporation (Billerica, MA, USA).

*Taxus chinensis* (Pilger) Rehd (8 g/bag) was purchased from the Pharmacy of Zhejiang Provincial Hospital of Traditional Chinese Medicine, produced by Ningbo Taikang Bio-engineering Company (Ningbo, China). The main ingredient of this product is the powder of *Taxus Chinensis* (Pilger) Rehd tree bark. *Taxus Chinensis* (Pilger) Rehd is prepared into an aqueous extract and administered orally during TCM anticancer therapy. To evaluate the anti-tumor effects of *Taxus Chinensis* (Pilger) Rehd, the preparation of AETC used in this study was based on the TCM processing method.

Dry *Taxus Chinensis* (Pilger) Rehd was immersed three times in distilled water for 30 min. This raw solution was boiled for 30 min, then concentrated to 208 mg/mL. The undiluted solution was used as the high dosage for the animal experiment, and the 1:2 and 1:4 diluted solutions were used for the medium and low dosages, respectively. Samples were stored at -4°C for use.

**Animals**

Purebred New Zealand rabbits and Balb/c-nu mice were obtained from Hercynian poole-Rubicam Experimental Animals Co., Ltd. (production license number: SCXK, Shanghai, China, 2008-0016). All the animal experiments in this study were carried out in Zhejiang University of Traditional Chinese Medicine and approved by the Animal Ethics Committee. All animals were housed under controlled conditions (23°C, humidity 70%, 12 h light and 12 h dark) for 4 days before the experiment. Eight purebred New Zealand rabbits were used for preparation of the serum containing various concentrations of AETC. Forty-eight Balb/c-nu mice aged 5-6 weeks were used for the *in vivo* experiments. Nude mice were kept in a temperature-controlled and specific pathogen free (SPF) room of the experimental animal center.
Preparation of the serum containing AETC

Eight male purebred New Zealand rabbits, weighing about 2000 g, were randomly divided into four groups by random number method (2 rabbits per group). According to the formula of the equivalent dose of direct conversion: $d_{\text{atro}}(\text{mg/kg})=d_{\text{atro}}(W_{\text{human}})/R_{\text{human}} \times W_{\text{atro}}$, where $d_{\text{atro}}$ is the dose for New Zealand rabbit (mg/kg); $d_{\text{human}}$ is the dose for human (mg/kg); $W_{\text{human}}$ is the body weight for humans in general; and $W_{\text{atro}}=70$ kg, $W_{\text{atro}}=2$ kg, $R_{\text{human}}=0.1$, $R_{\text{atro}}=0.093$. The four groups were all treated by gavage. The low dosage was half the medium while the high dosage was two times the medium. The control group was treated with 10 mL distilled water by gavage. The low-dose group was treated with AETC at 186.65 mg·kg$^{-1}$·d$^{-1}$. The medium-dose group was treated with AETC at 373.3 mg·kg$^{-1}$·d$^{-1}$. The high-dose group was treated with AETC at 746.6 mg·kg$^{-1}$·d$^{-1}$. All rabbits were administered AETC intragastrically for 7 consecutive days. The rabbits’ blood serum was taken via cardiac puncture 1 h after the last administration. The sera of each group were mixed and placed at 56°C for 30 min to inactivate the complement, then filtered through a 0.22 μm pore filtrate for sterilization and kept at −20°C until use.

Cell line and culture conditions

A549 human lung carcinoma cells obtained from Shanghai Institute of Cell Biology (Academia Sinica, Shanghai, China) were cultured in F12-K medium (Gibco, Gaithersburg, MD, USA) supplemented with 10% HyClone fetal bovine serum (Thermo company, Shanghai, China) were cultured in F12-K medium until fully confluent, then filtered through a 0.22 μm pore filtrate for sterilization and kept at −70°C. When the cells reached 70%-80% confluency, the control group was given serum, while other groups were given 10% serum containing AETC for 24 h in complete growth medium.

Cell viability assay

Cytotoxicity of the serum containing AETC in A549 cells was investigated using the standard MTT assay. A549 cells were seeded in a 96-well plate (Nest Biotech Co., Ltd., Shanghai, China)(200 μL/well) and incubated for 24 h at 37°C. The cells were treated with serum from the control group or treated with 10% serum containing various concentrations of AETC for 24 h at 37°C. Then, 20 μL of 5 mg/mL MTT in PBS (Keyi Biotech Co., Ltd., Hangzhou, China) were added to each well and incubated for 4 h at 37°C. The supernatant was removed after centrifugation. Finally, 150 μL of dimethyl sulfoxide (Sigma, St. Louis, MO, USA) was added with absorbance at 490 nm. Wavelength (A490) was measured by an ELX808 Micro Plate Reader (Bio-Tek Instruments Inc., Winooski, VT, USA). Relative cell proliferation inhibition rate (IR) = (1 – average A490 of the treatment group/average A490 of the control group) × 100%. The experiments were independently repeated three times.

Protein extraction and western blot analysis

10 exponentially growing A549 cells were cultured in a 6-well plate (Nest Biotech Co., Ltd., Shanghai, China). Cells were incubated in 10% serum from the control group or 10% serum containing various concentrations of AETC for 24 h at 37°C in an atmosphere containing 5% CO₂. After washing with ice-cold PBS, the cells were lysed in RIPA lysis buffer (Roche, Basel, Switzerland) containing a protease inhibitor cocktail. The extracts were centrifuged at 12 000 r/min for 5 min at 4°C to remove debris, and the protein concentration was determined using a Bradford assay (Bio-Rad, Hercules, CA, USA). The extracted protein samples (30 μg total protein/lane) were added in five volumes of sample buffer and subjected to denaturation at 100°C for 10 min. Then, the proteins were separated by 10% SDS-polyacrylamide gel electrophoresis at 250 mA for 2.5 h, and transferred onto a polyvinylidene fluoride (PVDF) membrane (Immunobilon-P, 0.45 mm; Millipore Co., Billerica, MA, USA). The membranes were blocked with 5% skim milk in Tris-buffered saline containing 1% Tween 20 (TBS-T, pH 7.4) at room temperature for 1 h, followed by incubation with primary antibodies (Cell Signaling Technologies, Beverly, MA, USA) (dilution 1: 3000) at 37°C for 1.5 h or at 4°C overnight. The membranes were washed three times with TBS-T for 10 min each at room temperature and incubated with a 1: 2000 dilution of HRP-conjugated secondary antibody for 2 h at room temperature. The membranes were then re-washed three times with TBS-T. The proteins were visualized using ECL and MP 4 + ChemiDocXRS protein mark detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). β-tubulin was used as the internal loading control. The densitometry readings of the bands were normalized to β-tubulin expression. The band intensity was analyzed using Quantity One software (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

In vivo experiment

0.2 mL A549 cells (10⁶ cells/mL) were subcutaneously injected into the nape of nude mice. After inoculation, 48 Balb/c-nu mice bearing A549 xenografts were randomly divided into four groups by random number method (24 rats per group). The treatments were: control group treated with 10 ml physiological saline; low-dose group treated with AETC (0.52 mg·g$^{-1}$·d$^{-1}$); medium-dose group treated with AETC (1.04 mg·g$^{-1}$·d$^{-1}$); and high-dose group treated with AETC (2.08 mg·g$^{-1}$·d$^{-1}$). The medium dosage was calculated according to the human and animal body surface area conversion method. The low dosage was half the medium while the high dosage was two times the medium. After 24 h, different treatments were given once a day via gastric gavage. The length and width of the xenografts were measured by vernier calipers every 3 days during the course of treatment. The volume of the xenografts was calculated by the standard formula,¹⁰ and the xenograft...
growth curves were plotted. The standard formula was 
\[ \text{volume} = a \times b^2 / 2 \] (a stands for length, and b indicates width). The above treatments were given for 7 weeks. All mice were sacrificed 24 h after the last administration. The weight of the primary subcutaneous xenograft and the inhibitory rate of the weight were then examined. The xenografts were stripped and stored in liquid nitrogen. 20 mg of the tumor tissues were cut down and washed three times with distilled water. The methods for protein extraction and western blot analysis were the same with the cell experiments. Western blot was used to detect the expression levels of EGFR, p-EGFR, JNK, p-JNK, p-ERK, p8MAPK, and p-p38MAPK in xenografts.

**Statistical analysis**

All the data are expressed as mean±standard deviation (SD). The statistical analyses were performed using SPSS 17.0 software (Chicago, IL, USA). Comparisons were made between each of the treatment groups and the control group by one-way ANOVA, and a difference of \( P<0.05 \) was considered to be statistically significant.

**RESULTS**

**Effects of serum containing AETC on the proliferation of A549 cells**

In the present study, after 24 h adherence, the A549 cells in the control group and the treatment groups were treated with 10% serum and 10% serum containing various concentrations of AETC, respectively. As shown in Table 1, the serum containing AETC markedly inhibited the proliferation of A549 cells in a dose-dependent manner, and the optical density in the treatment groups was significantly lower than that in the control group. Therefore, the proliferation of A549 cells is attenuated by serum containing AETC.

**Effects of serum containing AETC on expression levels of the EGFR/MAPK pathway-related proteins in vitro**

To determine the effects of serum containing AETC on the EGFR/MAPK pathway, the expression levels of ERK1/2, p-ERK1/2, JNK, p-JNK, p8MAPK, and p-p38MAPK were detected by western blot 24 h after A549 cells were treated with serum containing various concentrations of AETC. As shown in Figure 1, the level of p8MAPK expression in the treatment groups was significantly higher than that in the control group. Moreover, the expression levels of p-ERK1/2 and p-JNK in the treatment groups were lower than those in the control group. However, no significant changes in the expressions of ERK1/2, JNK, and p38MAPK were found. The levels of ERK, JNK, and p38 did not significantly change (\( P>0.05 \)). The levels of PERK and pJNK were significantly lower (\( P<0.05 \)), and the level of Pp38 was significantly higher than that in the control group (\( P<0.05 \)) (Figure 1).

**Effects of AETC on the growth of A549 xenografts in nude mice**

The inhibitory effect of AETC on A549 cells in vitro suggested that it might suppress the tumor growth in vivo. To confirm this hypothesis, animal experiments were performed. Twenty-four hours after the inoculation, different treatments were given once daily. The xenografts were palpable 5 days later. Inoculating A549 cells subcutaneously to establish the A549 xenograft model in nude mice was successful and the tumor formation rate was 100%. Two weeks later, the length and width of the xenografts were measured every 3 days during the course of treatment. The volume of the xenograft was calculated by the standard formula and the xenograft growth curves were plotted. As shown in Table 2 and Figure 2, the growth rate of the treatment groups was slower than that in the control group. Seven weeks after the treatment, the weight of the primary subcutaneous xenograft and the inhibitory rate of the weight were examined 24 h after the last administration, when all the mice were sacrificed. As shown in Table 3, different dosage of AETC exhibited a significant anti-tumor effect compared with the control group (\( P<0.01 \)). The inhibitory rates were 47.53%, 39.59%, and 46.47% in the low-dose, medium-dose, and high-dose AETC groups, respectively.

**Effects of AETC on expression levels of the EGFR/MAPK pathway-related proteins in xenografts**

To further confirm the effect of AETC on expression of the EGFR/MAPK pathway, we used western blot analysis to detect the level of EGFR, p-EGFR, ERK1/2, p-ERK1/2, JNK, p-JNK, p8MAPK, and p-p38MAPK in xenografts. Compared with the control group, densitometry showed that the levels of EGFR, ERK, JNK, and p38 were not significantly different (\( P>0.05 \)), the levels of p-EGFR, p-ERK, and p-JNK were significantly lower (\( P<0.05 \)), and the level of Pp38 was significantly higher than that in the control group (\( P<0.05 \)).

**Table 1** MTT Results among different groups

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<th>Group</th>
<th>n</th>
<th>Absorbance</th>
<th>Inhibitory rate (%)</th>
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<tr>
<td>Control</td>
<td>3</td>
<td>1.139333</td>
<td>-</td>
</tr>
<tr>
<td>Low-dose</td>
<td>3</td>
<td>0.914333</td>
<td>19.73×</td>
</tr>
<tr>
<td>Medium-dose</td>
<td>3</td>
<td>0.769333</td>
<td>32.46×</td>
</tr>
<tr>
<td>High-dose</td>
<td>3</td>
<td>0.658000</td>
<td>42.23×</td>
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Notes: the control group was treated with 10 mL distilled water by gavage. The low-, medium- and high-dose group were treated with AETC at 186.65, 373.3, and 746.6 mg·kg\(^{-1}\)·d\(^{-1}\) respectively. AETC were administered intragastrically for 7 consecutive days. MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; AETC: aqueous extract of *Taxus chinensis* (Pilger) Rehd. Compared with the control group, '\( P<0.05 \), ^{×}P<0.01.'
of p-p38 was significantly higher ($P < 0.05$) than that in the control group (Figure 3). Therefore, the activities of EGFR and ERK1/2 were possibly decreased while that of p38 increased in the xenografts. Additionally, AETC might inhibit A549 xenograft growth by suppressing the EGFR/MAPK pathway.

**DISCUSSION**

*Taxus chinensis* (Pilger) Rehd is an evergreen tree distributed in the southeastern region of China. Research into *Taxus* species has increased since the isolation of paclitaxel (Taxol) from the short yew (*Taxus brevifolia*) in 1971, which has a diterpenoid structure and strong anticancer effects. Our previous study demonstrated that AETC may inhibit the proliferation of A549 cells and induce G0/G1 phase cell cycle arrest and apoptosis. The growth curve assay showed that AETC inhibited the proliferation of A549 cells in dose- and time-dependent manners.

The inhibitory effect of serum containing AETC on the proliferation of A549 cells is of great value for exploring the related signaling pathways. Serum pharmacological research on compound herbal recipes has been performed for many years. This method involves removing the serum from animals administrated certain herbal medicine solutions intragastrically. The serum can better reflect real pharmacological effects in vivo, avoiding interference and simulating in vivo conditions from the crude medicinal herbs after administration. Within a certain range, the drug concentration in serum changes with the drug dosage administrated to the animal. There-
fore, we used the serum pharmacological method to explore the efficacy of AETC on A549 cells in vivo. The epidermal growth factor receptor (EGFR) is a transmembrane glycoprotein with a molecular weight of 170 kDa. It can interact with ligands, activate the downstream system, regulate cell proliferation, survival, adhesion, migration, differentiation, and promote wound healing. EGFR can also induce angiogenesis and stop apoptosis. The expression level of EGFR is higher in malignant tissues than that in normal tissues. It is reported that over-expression of EGFR is present in 40%-80% non-small-cell lung cancer (NSCLC) patients. Therefore, blocking or attenuating the EGFR signaling pathway is a promising strategy for controlling cancers including NSCLC.

Receptor down-regulation is the most prominent regulating way for EGFR signal attenuation, which involves the internalization and subsequent degradation of the activated receptor in the lysosomes. EGFR has six autophosphorylation sites (Y992, Y1045, Y1068, Y1086, Y1148, and Y1173) for regulating the activity of EGFR. It has been reported that Y1068 and Y1148 are the major autophosphorylation sites, while Y992, Y1045, and Y1086 are in the minority. The autophosphorylation site Y1068 is related to EGFR/MAPK. Interestingly, our in vivo study showed that AETC exhibited no significant influence on the degradation of EGFR/L-PKB.
AETC can decrease the p-EGFR to attenuate the EGFR signaling pathway, inhibit cell proliferation, adhe-

Figure 3 Effects of AETC on expression of the EGFR/MAPK pathway-related proteins in A549 xenografts
1: the control group was treated with 10 mL distilled water by gavage; 2: the low-dose group; 3: the medium-dose group; 4: the high-dose group. A: EGFR; B: p-EGFR; C: JNK; D: p-JNK; E: p38; F: p-p38; G: ERK1/2; H: p-ERK1/2. The control group was treated with 10 mL distilled water by gavage; the low-, medium- and high-dose group was treated with AETC at 186.65, 373.3, and 746.6 mg·kg⁻¹·d⁻¹ respectively. AETC: aqueous extract of Taxus Chinensis (Pilger) Rehd; EGFR/MAPK: epidermal growth factor receptor/mitogen-activated protein kinase; EGFR: epidermal growth factor receptor; p-EGFR: phospho-EGFR; JNK: c-Jun N-terminal kinase; p-JNK: phospho-JNK; p-p38: phospho-p38; ERK1/2: extracellular signal-regulated protein kinases; p-ERK1/2: phospho-ERK1/2. Compared with the control group, a P<0.05; b P<0.01.
sion, migration, angiogenesis, and induce apoptosis. To explore the detailed mechanism, further in vitro studies have been performed to investigate the effect of AETC on the EGFR downstream pathway in vitro and in vivo. The mitogen-activated protein kinase (MAPK) pathway is one of the main downstream effectors of EGFR, including the ERK 1/2, the JNK/stress-activated protein kinase, and p38 MAPK. A number of studies have demonstrated that the EGFR/MAPK pathway is responsible for proliferation, survival, and metastasis of cancer cells, suggesting that identifying the approaches or drugs for inhibiting this pathway might have potential therapeutic value. To identify the signaling pathway(s) involved in the underlying mechanism, the phosphorylation/activation of cancer-related MAPKs including p38, JNK, and ERK1/2 in A549 lung cancer cells should be detected. JNK regulates many cellular processes, including cell proliferation, differentiation, survival, and migration. To date, thousands of papers have been published to directly or indirectly address the function of JNK in tissue homeostasis, cellular metabolism, inflammation, and carcinogenesis. JNK is activated in a subset of NSCLC biopsy samples and it promotes oncogenesis in bronchial epithelium. Moreover, the JNK pathway can stimulate NSCLC cell proliferation to cooperate with the PI3K pathway for maintaining cell survival.

The p38 MAPK signaling pathway has a negative regulatory function for its inhibitory effect on cell proliferation and tumorigenesis. ERK1/2 is also a crucial molecule in cell proliferation and carcinogenesis. It is activated by dual phosphorylation on both Thr202 and Tyr204 residues. The activated ERK1/2 has been reported in a variety of human tumor cell lines and epithelial cancer tissues including small- and non-small-cell lung cancers. According to our study, neither in vitro nor in vivo expression levels of cancer-related MAPKs (JNK, p38, and ERK1/2) changed significantly after AETC administration. At the same time, we found that p-p38 increased significantly while p-JNK and p-ERK1/2 significantly decreased. According to the previous report, p38 antagonizing the JNK-c-Jun pathway is a possible mechanism for suppressing tumorigenesis. However, how AETC suppresses the expression of p-JNK and p-ERK1/2 remains unclear. In the present study, we assume that the enhanced p-p38 is a down-regulation factor for p-JNK and p-ERK1/2.

The in vivo anticancer assay revealed that AETC effectively inhibited the growth of A549 xenografts, and the experimental results showed that the anticancer effects of AETC on A549 xenografts might be associated with the down-regulation of the EGFR/MAPK pathway. So far, we have only examined the Ras-raf-MAPK arm of EGFR signaling. However, there are several other signaling pathways known to be activated by EGFR, including the phosphatidylinositol-3-kinase and Janus kinase-signal transducers and activators of transcription pathways, which need to be further detected. Many of the chemotherapeutic agents for cancer treatment have side effects. Traditional Chinese medicines can be effective while avoiding adverse side effects. However, in most circumstances, neither the chemical entity nor the molecular mechanisms of action are well defined. Taxus chinensis (Pilger) Rehd is such a medicinal plant used traditionally as an anticancer material. The present study has demonstrated that serum containing AETC inhibits the proliferation of A549 cells in vitro and inhibits the growth of A549 xenografts in vivo by suppressing the EGFR/MAPK signaling pathway. This is the first study elucidating the anticancer mechanism of AETC. Based on these results, we believe that AETC may be a potential anticancer agent to be used worldwide.

REFERENCES

11 Yingfei X, Jing Z, Qiu Q. Study on mechanisms of inducing apoptosis with aqueous extract of Taxus chinensis and the inhibitory effect in human lung carcinoma A549...
<table>
<thead>
<tr>
<th>Year</th>
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<th>Title</th>
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</tr>
</thead>
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<tr>
<td>2012</td>
<td>Shu QJ, Li P, Wang BB</td>
<td>Experimental study on apoptosis induced by aqueous extract of Taxus chinensis in human pulmonary carcinoma cell A549 and its molecular mechanisms</td>
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