MEDICAL SCIENCE MONITOR

ANIMAL STUDY

e-ISSN 1643-3750
© Med Sci Monit, 2019; 25: 7538-7546
DOI: 10.12659/MSM.917115

Received: 2019.04.22
Accepted: 2019.06.05
Published: 2019.10.07

Xiaoai Jiedu Recipe Inhibits Proliferation and Metastasis of Non-Small Cell Lung Cancer Cells by Blocking the P38 Mitogen-Activated Protein Kinase (MAPK) Pathway

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

AE 1,2 Yuchao Wang*
ABC 2 Chunhua Xu*
CD 1,3 Bin Xu
CD 4 Li Li
DG 4 Wenting Li
FG 2 Wei Wang
FG 4 Mianhua Wu

1 Nanjing University of Chinese Medicine, Nanjing, Jiangsu, P.R. China
2 Endoscopic Center of Nanjing Chest Hospital, Nanjing, Jiangsu, P.R. China
3 Department of Respiratory Medicine, Nanjing Chest Hospital, Nanjing, Jiangsu, P.R. China
4 Institute of Oncology, The First Clinical Medical College, Nanjing University of Chinese Medicine, Nanjing, Jiangsu, P.R. China

* Yuchao Wang and Chunhua Xu contributed equally to this work

Corresponding Author: Mianhua Wu, e-mail: mianhuawu123@163.com

Source of support: This work was supported by the Nanjing Medical Science and Technology Development Foundation

Background: Lung cancer is the leading cause of cancer deaths in the world. Its major histopathological subtype is non-small cell lung cancer (NSCLC). Xiaoai Jiedu recipe (XJR) is a traditional Chinese medicine formula that can suppress growth and invasion of tumor cells. Here, we assessed the antitumor effect of XJR on NSCLC explored the underlying mechanisms.

Material/Methods: Three concentrations of XJR (low, middle, and high) were used to treat A549 cells. Cell Counting Kit-8 and colony formation assay were used to measure proliferation of A549 cells. Apoptosis was evaluated by Hoechst 33342 staining and flow cytometry. The expression of apoptosis-associated proteins was measured by Western blot analysis. Transwell and scratch wound healing assay were used to assess invasion and migration, respectively, of A549 cells. The expression of p38 MAPK pathway-associated proteins were measured using Western blot analysis.

Results: XJR suppressed proliferation and promoted apoptosis of A549 cells, especially in the high-dose group. The expression of Bcl-2 was reduced with increasing expression of Bax, cleaved caspase-3, and cleaved caspase-9. Invasion and migration abilities of A549 cells were inhibited after XJR treatment. XJR treatment decreased the expression levels of phosphorylated p38 (p-p38), p-ERK, and p-JNK in a dose-dependent manner.

Conclusions: The results demonstrated that XJR can inhibit proliferation, invasion, and migration, and induce apoptosis of NSCLC by blocking the p38 MAPK pathway, which shows the potential of XJR as a new treatment of NSCLC.

MeSH Keywords: Apoptosis • Carcinoma, Non-Small-Cell Lung • Cell Proliferation • MAP Kinase Signaling System

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/917115

2541 — 6 33

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Lung cancer is the most common type of respiratory system cancer and is notorious for its dismal prognosis, with a 5-year overall survival rate under 15% [1,2]. Non-small cell lung cancer (NSCLC) accounts for about 80% of lung cancer and its high morbidity and mortality pose a major public health challenge [3,4]. Similar to other types of cancer cells, NSCLC cells are characterized by sustained proliferation and resistance to cell apoptosis [5]. Despite comprehensive treatment strategies, including surgery, radiotherapy, and targeted therapy, the prognosis is still poor [6]. Therefore, there is an urgent need for identification of novel alternative therapeutic drugs to improve clinical survival rates.

Sustained proliferation of cells is one of the characteristic features in the development and progression of many kinds of cancers, including bladder cancer, breast cancer, gastric cancer, and NSCLC [7,8]. Apoptosis is a programmed cell death and is involved in numerous physiological and pathological processes, which can help to safeguard genomic integrity [9]. Therefore, the evasion of apoptosis is a prominent hallmark of cancer. Mounting evidence shows that dysregulation of apoptotic pathways can promote tumorigenesis [10,11]. The abnormal expression of apoptosis-related proteins, including Bcl-2, Bax, cleaved caspase-3, and cleaved caspase-9, were reported in a number of cancer studies [12]. It has been well documented that the p38 MAPK signaling pathway participates in the process of apoptosis in NSCLC cells, and the inactivation of p38 MAPK signaling can inhibit proliferation and induce apoptosis during NSCLC [13,14].

Traditional Chinese herbs have a long history of being used in the treatment of malignant tumors, and can be important sources of chemical anticancer medicine and many chemotherapeutic agents [15,16]. Xiaoai Jiedu recipe (XJR) is a classical formula consisting of 7 herbal Chinese medicines mixed in specific proportions: Hedyotis diffusa (20 g), Cremastra appendiculata (10 g), Bombyx batryticatus (10 g), Centipede (3 g), Akebia trifoliata Koidz (12 g), Radix pseudostellariae (15 g), and Radix ophiopogonis (12 g) [17]. A previous study reported that XJR could suppress the growth and invasion of tumor cells, possibly via affecting expressions of some genes in the chemokine signaling pathway [18]. However, the effect of XJR on NSCLC remains to be elucidated.

The aim of our study was to explore the effect of XJR on proliferation, apoptosis, and metastasis of human lung carcinoma cell line A549 cells and to clarify the possible underlying mechanisms by treatment with different concentrations of XJR.

Material and Methods

Preparation of medicated serum

Decoction and concentration were used to concentrate the XJR consisting of 9 traditional Chinese herbs in a certain proportion into 2 g/ml filtrate, which was stored for later use. We obtained 24 adult Sprague-Dawley rats (200–250 g) from Shanghai SLAC Laboratory Animal Company (Shanghai, China). Rats were housed in a suitable environment with 24±3°C and 12-h light/dark cycle. All animals were given free access to food and water and were allowed to acclimate to the environment for at least 3 days before the experiment. All rats were randomly divided into 4 groups: Control (NC), low (L, 3.78 g/kg), middle (M, 7.56 g/kg), and high (H, 15.12 g/kg). Rats gavaged twice a day for 2 days with the corresponding dose of XJR. Within 1–2 h after the last gavage, all animals were sacrificed and blood samples were collected and centrifuged to separate serum, which was sterilized by passing it through a 0.22-micron bacteria-retentive filter and then inactivated at 56°C for 30 min to remove serum complement. Medicated serum was stored at –20°C. Our study was performed according to the guidelines for the Care and Use of Laboratory Animals and was approved by the Ministry of Science and Technology of China. All of the study protocols were approved by the Ethics Committee on Animal Experiments of Nanjing Chest Hospital.

Cell culture and treatment

Human lung carcinoma cell line A549 cells were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and incubated in RPMI 1640 medium (Gibco, China) containing 10% fetal bovine serum (Gibco, New York, USA) and 1% penicillin/streptomycin (Invitrogen, New York, USA) in a humidified incubator at 37°C and 5% CO_2. Cells were randomly divided into 4 groups – control, low, middle, and high – which were cultivated with medium containing different concentrations of drug serum.

Cell Counting Kit-8 (CCK-8) assay

A549 cells treated with medium containing different concentrations of drug serum were plated into 96-well plates (1×10^4) and then incubated at 37°C for 6 h. Cell viability was measured using CCK-8 assay (Shanghai Yi Sheng Biotechnology Co., China). At the indicated time points (24, 48, and 72 h), CCK-8 (10 µl) solution was added to wells for another 4 h at 37°C. Optical density (OD) value was detected at 450 nm on a microplate reader (Thermo Scientific, Waltham, MA, USA).

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

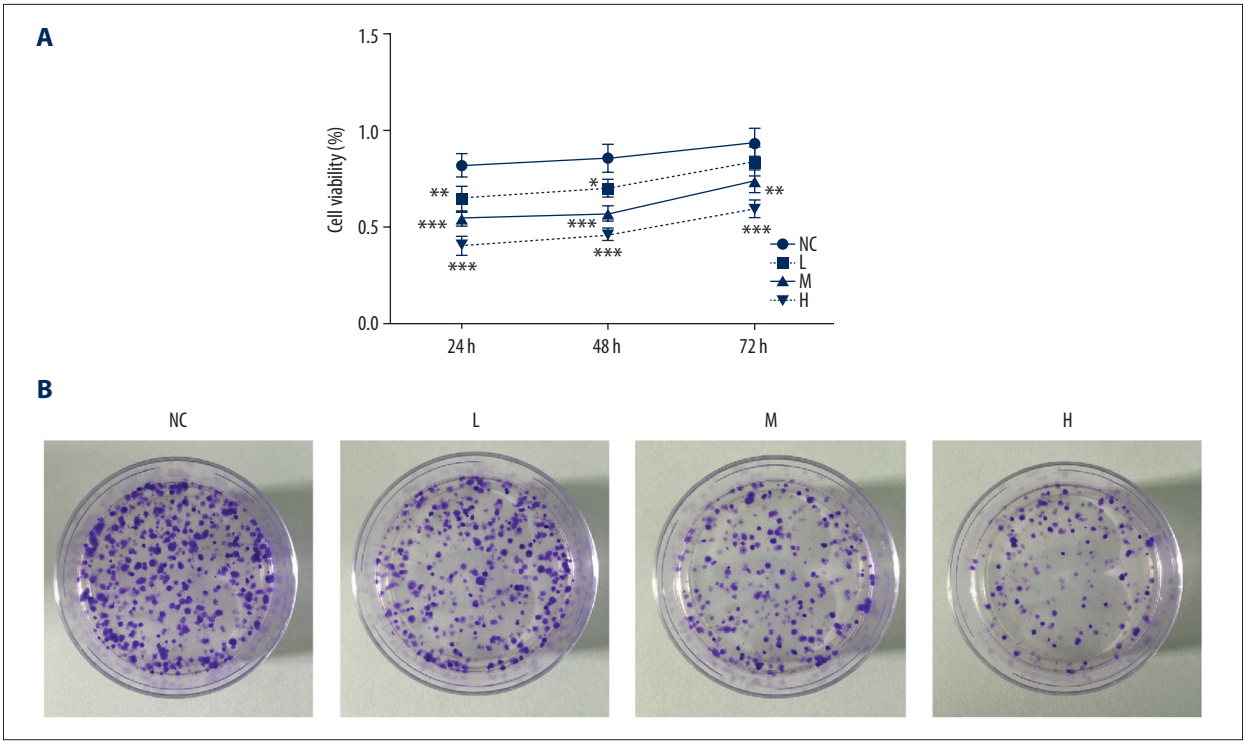

Figure 1. XJR inhibited cell proliferation in A549 cells. (**A**) Cell Counting Kit-8 assay was used to detect proliferation of A549 cells. (**B**) XJR inhibited the colony formation abilities of A549 cells. * P<0.05, ** P<0.01, *** P<0.001 *vs.* NC. NC – negative control; XJR – Xiaoai Jiedu recipe.

Colony formation assay

A549 cells were plated in 6-well plates at the density of 600 cells and cells were allowed to grow at 37°C for 7–12 days until the colonies were visible. Cells were then fixed and stained with 0.2% crystal violet to count the number of cells.

Cell apoptosis assay

The cell apoptotic rate was measured with flow cytometry analysis. A549 cells (5×10^5 cells/plate) were plated in 6-well plates. The Annexin V-PE/7AAD Staining Cell Apoptosis Detection kit (KeyGEN Biotech, China) was used to measure apoptosis of A549 cells, which was analyzed using FlowJo software (Becton-Dickinson, San Jose, CA, USA). Results are presented as the percentage of cells in each phase.

Hoechst 33342 staining

Cells were cultivated with 3 doses of XJR for 48 h, then they were fixed with 4% paraformaldehyde for 15 min. Subsequently, cells were stained with Hoechst 33324 reagent (Beyotime, Shanghai, China) for 5 min. Fluorescence microscopy (IX53, Olympus, Tokyo, Japan) was used to observe dells at 400× magnification.

Cell invasion assay

The invasion ability of A549 cells was detected by Transwell assay performed in 24-well Transwell plates (Corning, NY, U.S.A.) with 8 μm-pore inserts coated with Matrigel (BD Biosciences, San Jose, CA). Cells were suspended in serum-free RPMI 1640 medium, and 200 μl suspension was added to the upper chamber. A total of 500 μl medium supplemented with 15% FBS was added to the lower chambers as a chemoattractant. Finally, the cells were stained with 1% crystal violet. Images were taken with a microscope (Zeiss, Oberkochen, Germany) and cells were counted in 5 randomly chosen fields.

Cell migration assay

The scratch wound healing assay was employed to evaluate the migration ability of A549 cells. Before the experiment started, cells (6×10^3 cells per well) were seeded into 6-well plates. By reaching a confluence of more than 75% for cultured cells, a 200-μl pipette tip was used to make a wound and then the floating cells were removed by washing with PBS. Then, the cells were incubated in serum-free RPMI 1640 medium and photographed with a camera. The images were captured using an inverted microscope at 0 h and 24 h. The migration rate was quantified by measuring the distance between the wound edges using ImageJ software.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

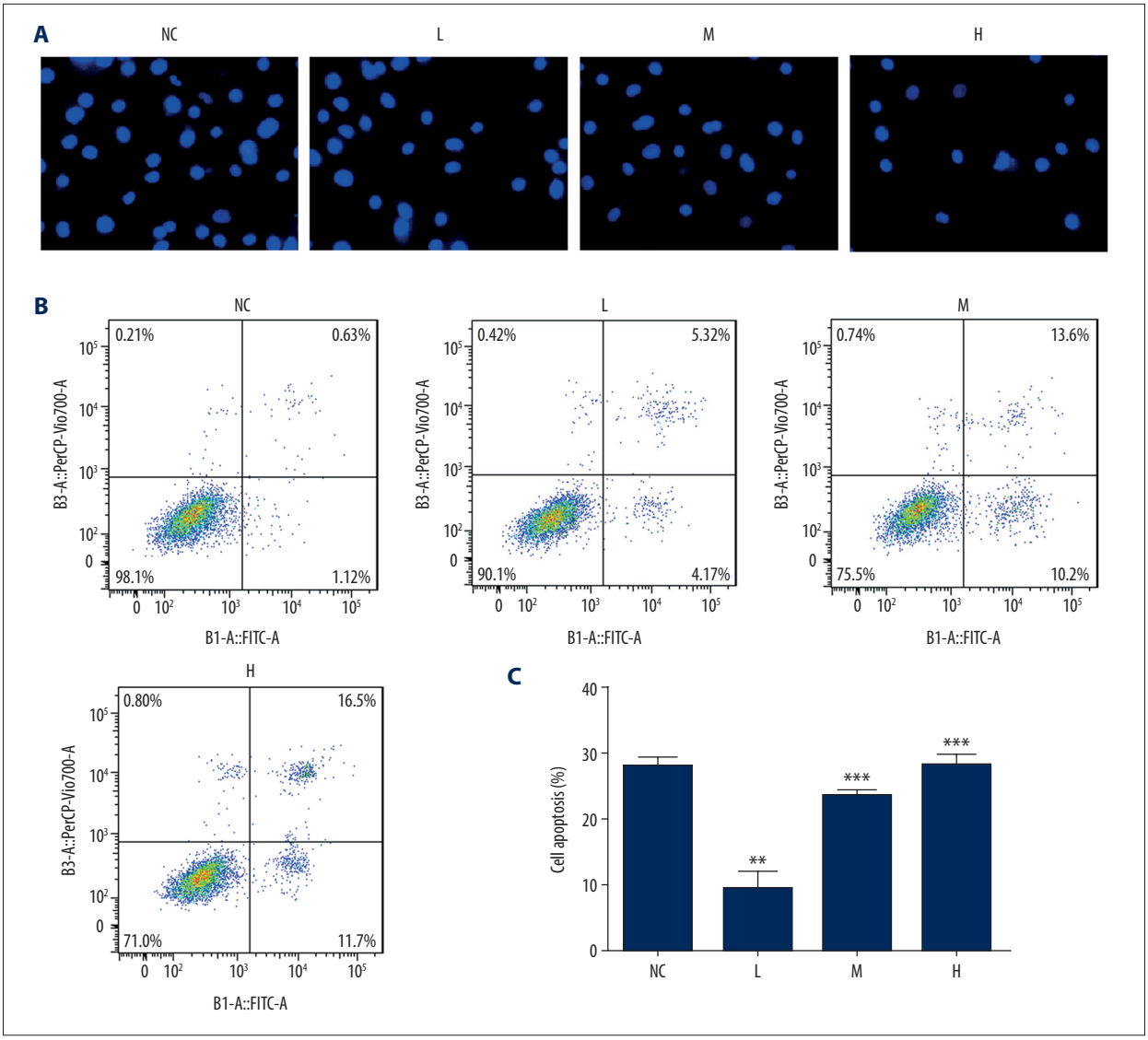

Figure 2. XJR induced apoptosis in A549 cells. (**A**) Changes in nuclear morphology during apoptosis were observed by Hoechst 33342 staining and visualized by fluorescence microscopy (magnification, ×20). (**B**) Cell apoptosis was assessed by flow cytometry analysis. (**C**) Cell apoptosis was quantified. ** P<0.01, *** P<0.001 *vs.* NC. NC – negative control; XJR – Xiaoai Jiedu recipe.

Western blotting assay

After treatment with XJR for 24 h, A549 cells were harvested and lysed on ice in RIPA lysis buffer (Beyotime, Shanghai, China). Protein concentration was detected by using an Enhanced BCA Protein Assay Kit (Beyotime, Shanghai, China). We isolated 40 μg total protein samples by SDS-polyacrylamide gels (PAGE) and electroblotted them onto PVDF-membranes. All membranes were then blocked with 5% defatted milk and then incubated with primary antibodies. Subsequently, membranes were washed with TBST 3 times and incubated with HRP-labeled Goat Anti-Mouse IgG (H+L) antibody (A0216, Beyotime, Shanghai, China). All blots were then developed with an enhanced chemiluminescence (ECL) reagent and analyzed by ImageJ software, which was normalized by GAPDH. Anti-Bcl-2 (4223T), anti-Bax (5023T), anti-cleaved caspase-3 (9661T), anti-cleaved caspase-9 (9509T), anti-MMP2 (40994S), anti-MMP9 (13667T), anti-p38 (8690T), anti-JNK (9258T), anti-p-p38 (4511T), anti-p-ERK (4370T), anti-p-JNK (4668T), and anti-GAPDH (5174S) antibodies were from Cell Signaling Technology (Boston, MA, USA). Anti-ERK (sc-514302) was obtained from Santa Cruz Biotechnology (CA, USA).

Statistical analysis

Statistical data analysis was performed with SPSS 22.0 and GraphPad Prism 5.0. All data were analyzed from 3 or more independent experiments to achieve statistical analysis and all

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

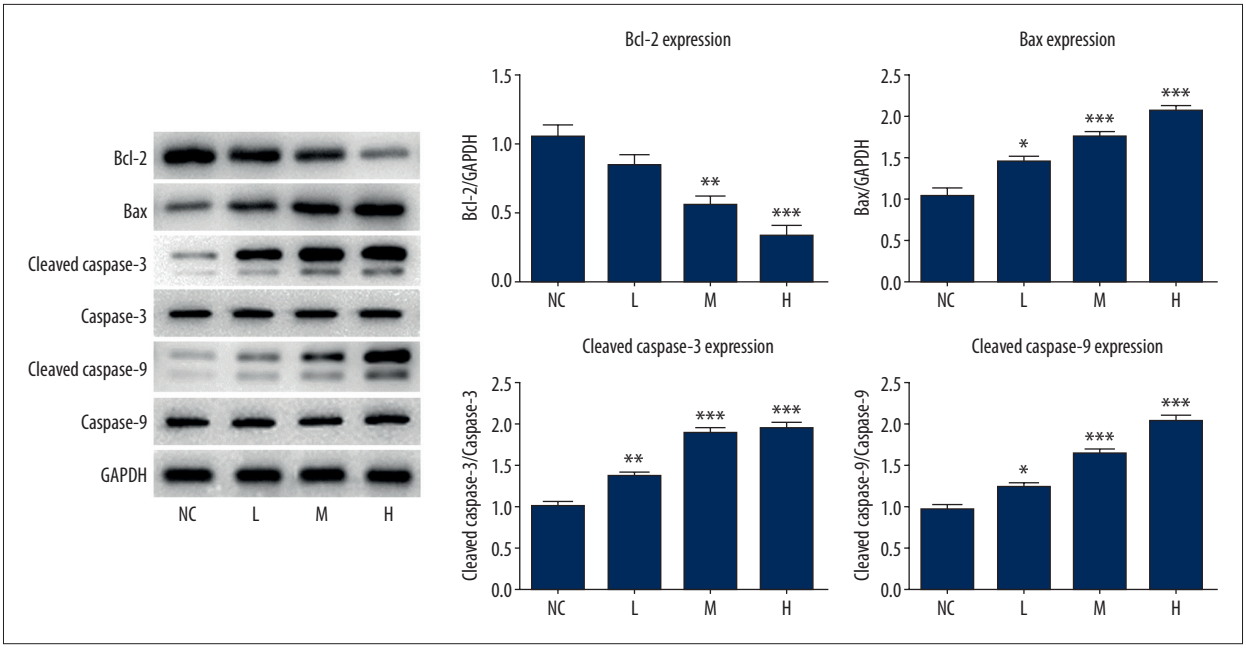

Figure 3. XJR affected the expression of apoptosis-related proteins. The expression levels of Bcl-2, Bax, cleaved caspase-3, and cleaved caspase-9 were measured by Western blotting. * P<0.05, ** P<0.01, *** P<0.001 *vs.* NC. NC – negative control; XJR – Xiaoai Jiedu recipe.

results are expressed as mean ± SD. Analysis of differences was performed with the two-tailed Student's *t* test and analysis of variance (ANOVA). P<0.05 was deemed statistically significant.

Results

XJR suppressed the proliferation of A549 cells

To determine the effect of XJR on A549 cell proliferation, a CCK-8 assay was used. As exhibited in Figure 1A, XJR suppressed the proliferation ability of A549 cells in a concentration-dependent manner compared to the NC group. To further confirm the inhibitory effect of XJR on the growth of A549 cells, a colony formation assay was used. From the results presented in Figure 1B, we found that XJR reduced the colony number of A549 cells, which was in accordance with the result of CCK-8 assay. Taken together, the above date indicated that XJR was able to suppress the proliferation of A549 cells.

XJR induced the apoptosis and regulated the expression of apoptosis – associated proteins in A549 cells

To assess whether the growth-repressive effect of XJR was related to apoptosis, Hoechst 33342 staining was used for observation. As shown in Figure 2A, increasing numbers of Hoechst 33342-positive cells were found following XJR treatment in a concentration-dependent manner. This indicated that XJR induces A549 cell apoptosis. To determine the apoptosis rate of cell, flow cytometry was employed. As presented in Figure 2B and 2C, the percentage of apoptotic cells were obviously increased following treatment with different doses of XJR versus that of the untreated group, and the high concentration of XJR exhibited the best inhibitory effect. Moreover, the expression of anti-apoptotic Bcl-2 protein was decreased in a dose-dependent manner with XJR treatment, accompanied by an increasing expression of pro-apoptotic Bax, cleaved caspase-3 and cleaved caspase-9 proteins (Figure 3). These findings indicated that XJR induced apoptosis of cells via modulating the expression of apoptosis factors.

XJR impeded the invasion and migration of A549 cells

Invasion and migration are 2 key phases involved in NSCLC metastasis. To study the effect of XJR on invasion and migration of A549 cells, a Transwell assay and a scratch wound healing assay were performed. The results demonstrated that the number of invasive and migratory OS cells in the XJR treatment group was decreased notably versus the negative control (Figure 4A–4D). In addition, MMP2 and MMP9 were found to be involved in cell invasion, migration, and cell-matrix adhesion, especially in the process of cancer metastasis. Thus, we measured the regulatory effects of XJR on the expression levels of MMP2 and MMP9. According to the results in Figure 5, the expression of the above proteins was downregulated in a dose-dependent manner. These data collectively indicate that XJR inhibits the invasion and migration of A549 cells.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

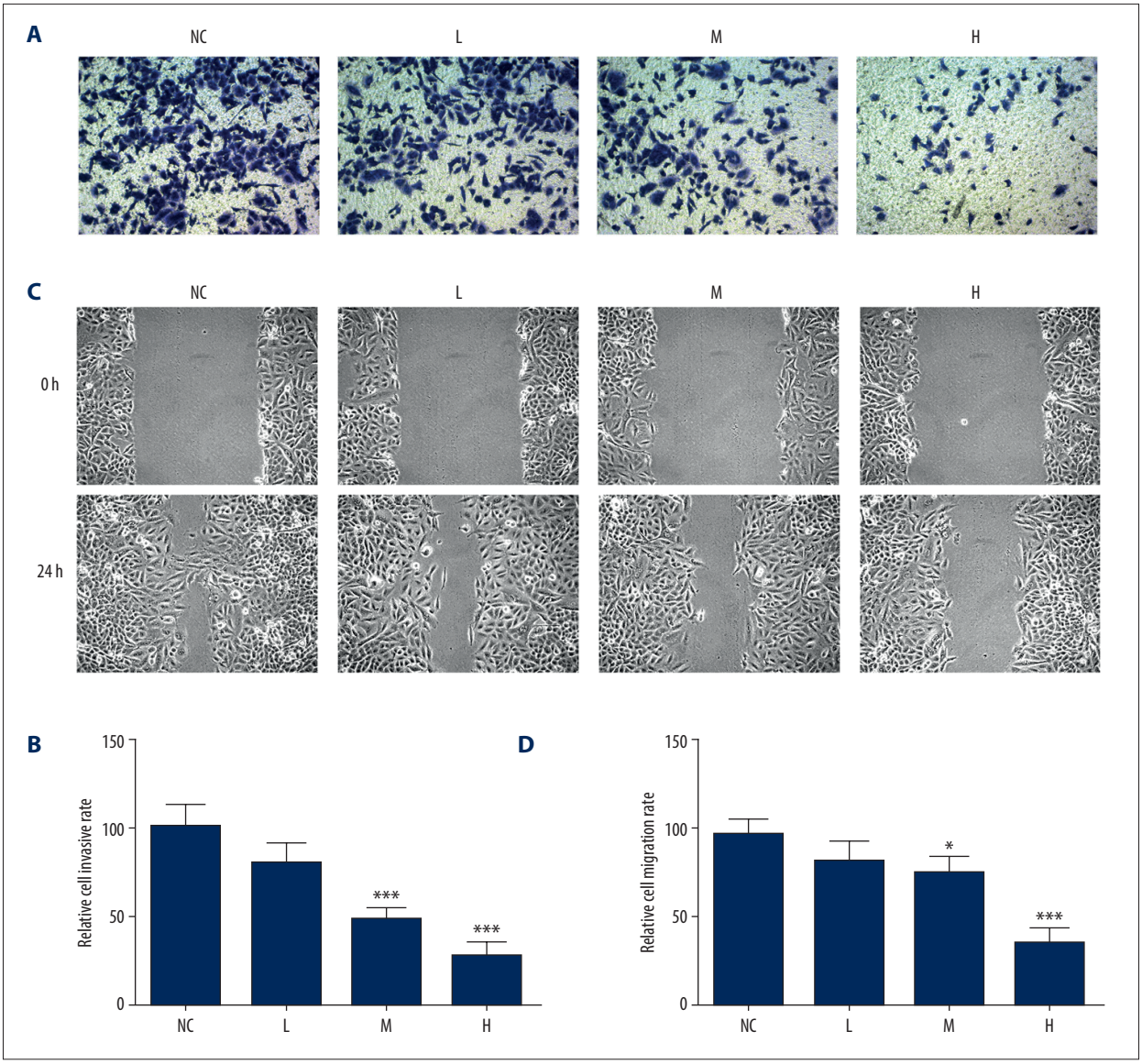

Figure 4. XJR inhibited invasion and migration in A549 cells. (**A**) Images of cell invasion of A549 cells (magnification, ×200). (**B**) Number of invading cells as determined with a counting chamber. (**C**) Cell migration was assessed by wound healing assay (magnification, ×100). (**D**) The number of migrated A549 cells after 24-h incubation. * P<0.05, *** P<0.001 *vs.* NC. NC – negative control; XJR – Xiaoai Jiedu recipe.

XJR suppressed the activation of p38 MAPK signaling in NSCLC cells

To further study the regulatory mechanisms of XJR in NSCLC, proteins in the p38 MAPK signaling pathway were measured using Western blotting. As exhibited in Figure 6, XJR inhibited the expression of p-P38, p-ERK, and p-JNK in a dose-dependent manner versus the NC group. The results indicated that XJR suppressed the p38 MAPK signaling pathway to exert its effects on A549 cells.

Discussion

NSCLC is one of the most common causes of cancer-associated death worldwide. Despite great progress achieved in NSCLC treatment, the clinical survival rates of patients remain poor. Therefore, to identify effective therapeutic targets for NSCLC is urgent. At present, traditional Chinese medicines have received wide attention for possible use in treatment of many diseases, including cancers. It is well known that Chinese herbs exhibit strongly anticancer ability due to the many bioactive components they contain. It has been well documented that the Chinese herbal medicine Wenxia Changfu formula

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

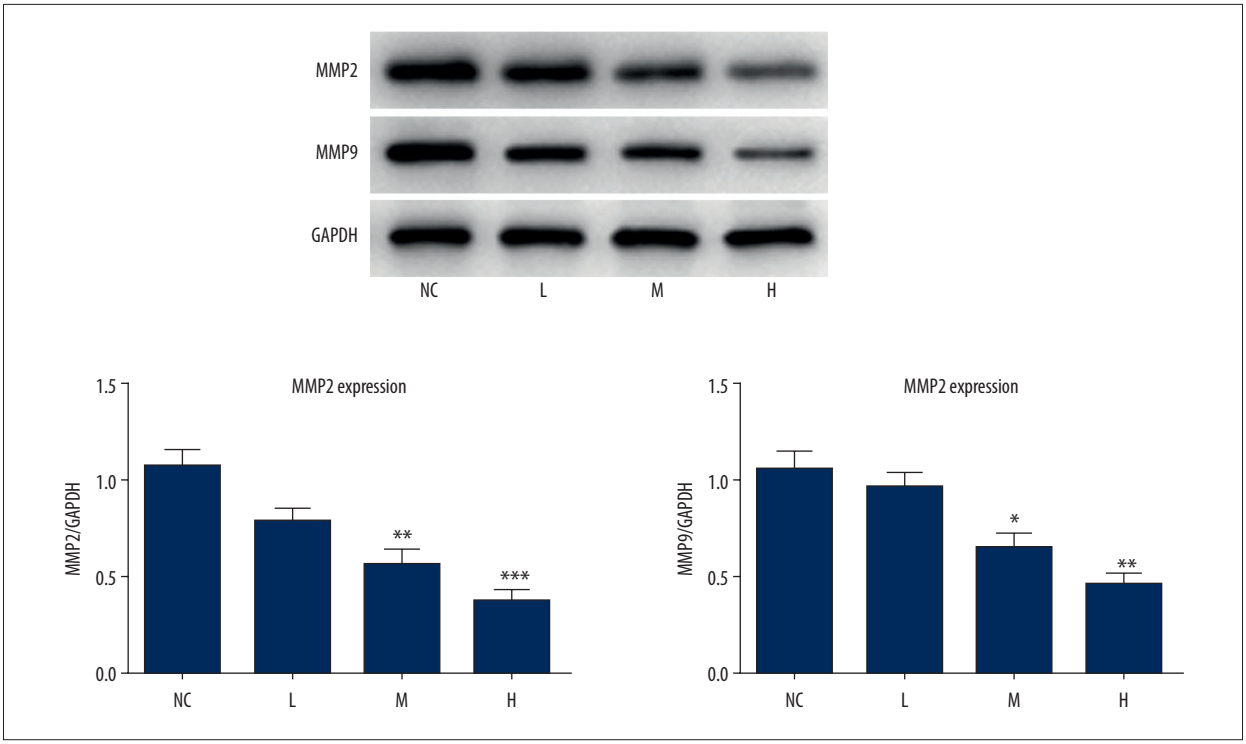

Figure 5. XJR downregulated the expression of MMP2 and MMP9 in A549 cells. The protein expression of MMP2 and MMP9 were measured by Western blotting. * P<0.05, ** P<0.01, *** P<0.001 *vs.* NC. NC – negative control; XJR – Xiaoai Jiedu recipe; MMP – matrix metalloprotein.

reverses cell adhesion-mediated drug resistance in lung cancer [19]. Fuzheng Kang-Ai decoction is a traditional Chinese medicine that can inhibit metastasis of lung cancer cells via the STAT3/MMP9 pathway [20]. In addition, accumulating evidence shows that Bu-Fei decoction can inhibit the progression of NSCLC [21]. Importantly, a previous study reported that XJR was able to inhibit the growth and invasion of tumor cells in H22 tumor-bearing mice [18]. However, the effect of XJR on NSCLC and its potential mechanisms remains to be elucidated. In the present study, we revealed the anti-NSCLC ability of XJR. We found that XJR could suppress the proliferation, invasion, and migration, as well as promoting apoptosis of A549 by blocking the p38 MAPK pathway, which demonstrates the potential of XJR as a new treatment of NSCLC.

Excessive proliferation of tumor cells contributes to the progression of cancers. Apoptosis is a programmed cell death that is involved in numerous physiological and pathological processes, which can help to safeguard genomic integrity. Our study results show the inhibitory effect of XJR in suppressing the growth of NSCLC cells through inducing apoptosis. Bcl-2 and Bax, 2 key proteins of the Bcl-2 family, play significant roles in the regulation of apoptosis as an anti-apoptosis genes and a pro-apoptosis gene, respectively [22,23]. In addition, it has been well documented that caspase family proteins, including caspase-3 and caspase-9, are predominant factors in the apoptotic pathway [24,25]. In our study, the level of Bcl-2 was downregulated accompanied with upregulation of Bax, cleaved caspase-3, and cleaved caspase-9 expression after treatment with XJR, which was consistent with many previous studies [22,26].

Metastasis is a hallmark feature of cancer and is becoming a serious obstacle in enhancing the survival rates of patients with NSCLC because of its high rate of distant metastasis [27,28]. Accumulating evidence supports that the processes of cell invasion and migration are essential for cancer metastasis [29]. Therefore, interfering in these processes is an efficient strategy to control NSCLC metastasis. Our present study revealed that XJR inhibited invasion and migration of A549 cells. Mounting evidence supports that MMP2 and MMP9 are implicated in cell invasion, migration, and cell-matrix adhesion in cancer [30]. Our results show that the expression levels of MMP-2 and MMP-9 were markedly decreased after treatment with different concentrations of XJR, as demonstrated above.

Previous research suggested that the p38 MAPK signaling pathway participates in the process of NSCLC [31]. Inactivation of the p38 MAPK signaling leads to anti-proliferation and anti-metastasis effects during NSCLC [13,32]. In addition, it has been reported that activation of p38 MAPK signaling promotes invasion and activates MMP-2 and MMP-9 expression [33].

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

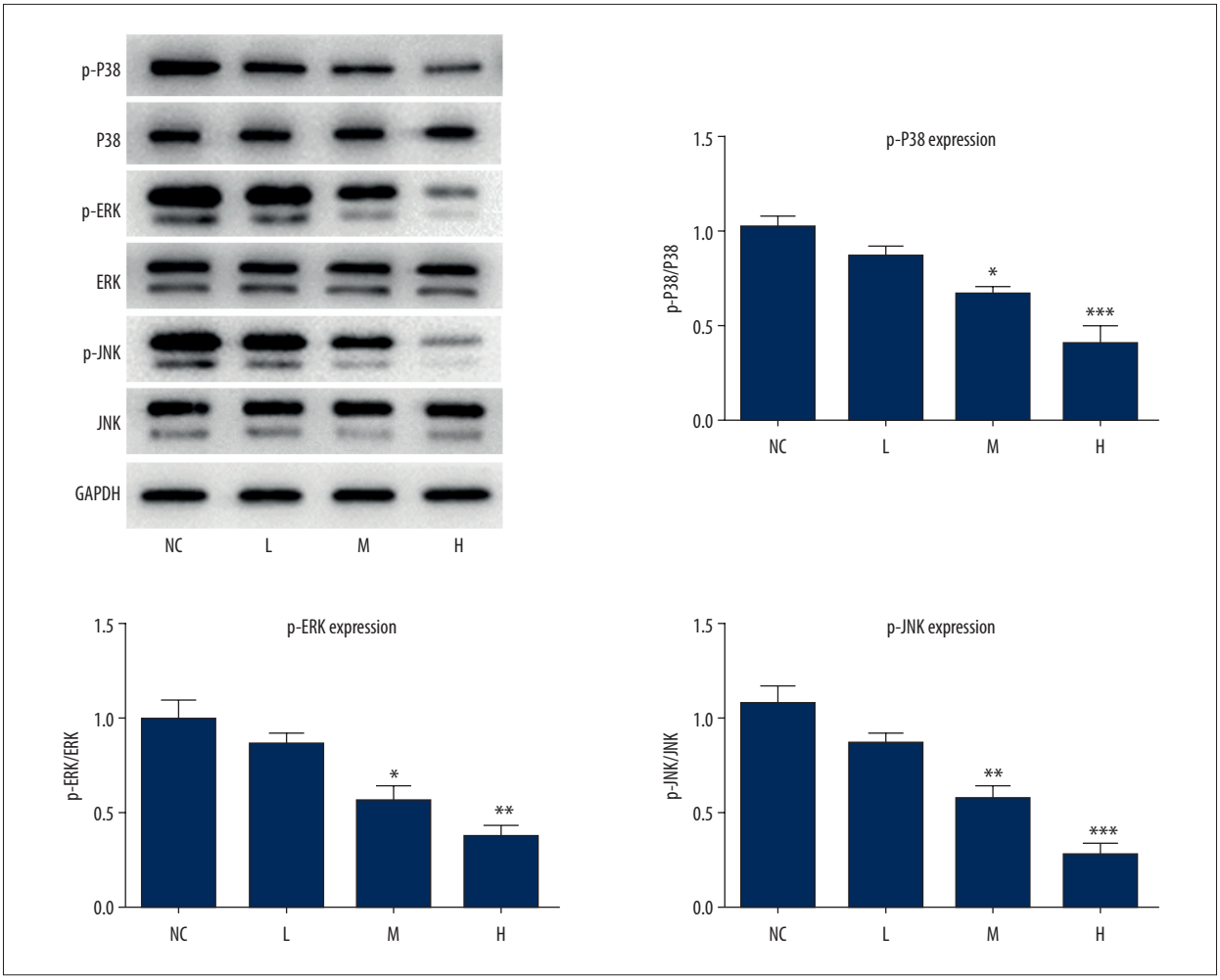

Figure 6. XJR suppressed activation of the p38 MAPK signaling pathway in A549 cells. The protein expression of p-P38, p-ERK, and p-JNK were measured by Western blotting. * P<0.05, ** P<0.01, *** P<0.001 *vs.* NC. NC – negative control; XJR – Xiaoai Jiedu recipe.

Therefore, we were interested in exploring whether XJR protects against NSCLC. The results indicated that the phosphorylation of p38, ERK, and JNK were attenuated in XJR treatment groups versus control. Our study suggests that XJR is a potent inhibitor of p38 MAPK in NSCLC cells.

Conclusions

The present study demonstrated that XJR plays a crucial role in progression of NSCLC, which is closely related to tumor proliferation, invasion, migration, and apoptosis, and the antitumor mechanism of XJR may be by inactivating the p38 MAPK signaling pathway. Our results demonstrated that XJR, a natural compound derived from herbs, could be an effective complementary and alternative agent for the therapeutic management of lung cancer.

Ethics approval and consent to participate

The study protocols were approved by the Ethics Committee on Animal Experiments of Nanjing Chest Hospital. All of the procedures were performed in accordance with the relevant policies in China.

Conflict of interest

None.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

References:

1. Hao H, Zhou Z, Li S et al: Shell feature: A new radiomics descriptor for predicting distant failure after radiotherapy in non-small cell lung cancer and cervix cancer. Phys Med Biol, 2018; 63: 095007
2. Shaw AT, Engelman JA: Ceritinib in ALK-rearranged non-small-cell lung cancer. N Engl J Med, 2014; 370: 2537–39
3. Gedvilaite V, Schveigert D, Cicenas S: Cell-free DNA in non-small cell lung cancer. Acta Med Litu, 2017; 24: 138–44
4. Ricciuti B, Brambilla M, Metro G et al: Targeting NTRK fusion in non-small cell lung cancer: rationale and clinical evidence. Med Oncol, 2017; 34: 105
5. Xu X, Chen D, Ye B et al: Curcumin induces the apoptosis of non-small cell lung cancer cells through a calcium signaling pathway. Int J Mol Med, 2015; 35: 1610–16
6. Saisho S, Yasuda K, Maeda A et al: Post-recurrence survival of patients with non-small-cell lung cancer after curative resection with or without induction/adjuvant chemotherapy. Interact Cardiovasc Thorac Surg, 2013; 16: 166–72
7. Zhang Z, Liu Y, Wang K et al: Activation of type 4 metabotropic glutamate receptor promotes cell apoptosis and inhibits proliferation in bladder cancer. J Cell Physiol, 2019; 234: 2741–55
8. Huang H, Han Y, Yang X et al: HNRNPK inhibits gastric cancer cell proliferation through p53/p21/CCND1 pathway. Oncotarget, 2017; 8: 103364–74
9. Fu Z, Yu W, Wang H, Chen X: Overexpression of RNF187 induces cell EMT and apoptosis resistance in NSCLC. J Cell Physiol, 2019; 234: 14161–69
10. Pu J, Tang X, Zhuang X et al: Matrine induces apoptosis via targeting CCR7 and enhances the effect of anticancer drugs in non-small cell lung cancer *in vitro*. Innate Immun, 2018; 24: 394–99
11. Yamaki A, Akiyama R, Murakami C et al: Diacylglycerol kinase alpha-selective inhibitors induce apoptosis and reduce viability of melanoma and several other cancer cell lines. J Cell Biochem, 2019; 120(6): 10043–56
12. Wang S, Long S, Xiao S et al: Decoction of Chinese herbal medicine fuzheng kang-ai induces lung cancer cell apoptosis via STAT3/Bcl-2/Caspase-3 pathway. Evid Based Complement Alternat Med, 2018; 2018: 8567905
13. Zhang Z, Rui W, Wang ZC et al: Anti-proliferation and anti-metastasis effect of barbaloin in non-small cell lung cancer via inactivating p38MAPK/Cdc25B/Hsp27 pathway. Oncol Rep, 2017; 38: 1172–80
14. Kang KA, Piao MJ, Madduma Hewage SR et al: Fisetin induces apoptosis and endoplasmic reticulum stress in human non-small cell lung cancer through inhibition of the MAPK signaling pathway. Tumour Biol, 2016; 37: 9615–24
15. Huang CH, Chang HP, Su SY et al: Traditional Chinese medicine is associated with a decreased risk of heart failure in breast cancer patients receiving doxorubicin treatment. J Ethnopharmacol, 2019; 229: 15–21
16. Lian F, Jin, Bao Q et al: Effectiveness of traditional Chinese medicine Jinlida granules as an add-on therapy for type 2 diabetes: A system review and meta-analysis of randomized controlled trials. J Diabetes, 2019; 11(7): 540–51
17. Ji Y LL, Li W, Zhu H et al: [Xiaoai Jiedu Formula inhibit the human liver cancer SMMC7721 transplanted tumor in nude mice by autophagy.] China Journal of Traditional Chinese Medicine and Pharmacy, 2019; 34: 1927–31 [in Chinese]
18. Wu MH, Li L: [Effect of xiaoai jiedu recipe on gene expression profiles in H22 tumor-bearing mice]. Zhongguo Zhong Xi Yi Jie He Za Zhi, 2013; 33: 1232–35 [in Chinese]
19. Fan RR, Wen ZH, Wang DW et al: Chinese herbal medicine for the treatment of cough variant asthma: a study protocol for a double-blind randomized controlled trial. Trials, 2019; 20: 3
20. Li L, Wang S, Yang X et al: Traditional Chinese medicine, Fuzheng KangAi decoction, inhibits metastasis of lung cancer cells through the STAT3/MMP9 pathway. Mol Med Rep, 2017; 16: 2461–68
21. Jiang ST, Han SY, Pang LN et al: Bu-Fei decoction and modified Bu-Fei decoction inhibit the growth of non-small cell lung cancer, possibly via inhibition of apurinic/apyrimidinic endonuclease 1. Int J Mol Med, 2018; 41: 2128–38
22. Ma L, Zhu Y, Fang S et al: Arenobufagin induces apoptotic cell death in human non-small-cell lung cancer cells via the noxa-related pathway. Molecules, 2017; 22(9): pii: E1525
23. Consensus development conference: Diagnosis, prophylaxis, and treatment of osteoporosis. Am J Med, 1993; 94: 646–50
24. Lim SL, Mustapha NM, Goh YM et al: Metastasized lung cancer suppression by Morinda citrifolia (Noni) leaf compared to Erlotinib via anti-inflammatory, endogenous antioxidant responses and apoptotic gene activation. Mol Cell Biochem, 2016; 416: 85–97
25. Tang Y, Yan G, Song X et al: STIP overexpression confers oncogenic potential to human non-small cell lung cancer cells by regulating cell cycle and apoptosis. J Cell Mol Med, 2015; 19: 2806–17
26. Xu Z, Zhang F, Zhu Y et al: Traditional Chinese medicine Ze-Qi-Tang formula inhibit growth of non-small-cell lung cancer cells through the p53 pathway. J Ethnopharmacol, 2019; 234: 180–88
27. Yuan S, Xiang Y, Wang G et al: Hypoxia-sensitive LINC01436 is regulated by E2F6 and acts as an oncogene by targeting miR-30a-3p in non-small cell lung cancer. Mol Oncol, 2019; 13: 840–56
28. Wei C, Dong X, Lu H et al: LPCAT1 promotes brain metastasis of lung adenocarcinoma by up-regulating PI3K/AKT/MYC pathway. J Exp Clin Cancer Res, 2019; 38: 95
29. Huang WT, Chong IW, Chen HL et al: Pigment epithelium-derived factor inhibits lung cancer migration and invasion by upregulating exosomal thrombospondin 1. Cancer Lett, 2019; 442: 287–98
30. Shi S, Luo W, Zhang R et al: CRTC2 promotes non-small cell lung cancer A549 migration and invasion *in vitro*. Thorac Cancer, 2018; 9: 136–41
31. Sahu V, Mohan A, Dey S: p38 MAP kinases: Plausible diagnostic and prognostic serum protein marker of non small cell lung cancer. Exp Mol Pathol, 2019; 107: 118–23
32. Zhang C, Zhu H, Yang X et al: P53 and p38 MAPK pathways are involved in MONCPT-induced cell cycle G2/M arrest in human non-small cell lung cancer A549. J Cancer Res Clin Oncol, 2010; 136: 437–45
33. Wu Z, He D, Zhao S, Wang H: IL-17A/IL-17RA promotes invasion and activates MMP-2 and MMP-9 expression via p38 MAPK signaling pathway in non-small cell lung cancer. Mol Cell Biochem, 2019; 455: 195–206

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]