

# Emodin alleviates severe acute pancreatitis-associated acute lung injury by decreasing pre-B-cell colony-enhancing factor expression and promoting polymorphonuclear neutrophil apoptosis

HONGZHANG CUI<sup>1,2</sup>, SHU LI<sup>3</sup>, CAIMING XU<sup>4</sup>, JINGWEN ZHANG<sup>1</sup>, ZHONGWEI SUN<sup>1</sup> and HAILONG CHEN<sup>1</sup>

<sup>1</sup>Department of General Surgery, The First Affiliated Hospital of Dalian Medical University, Dalian, Liaoning 116011;

<sup>2</sup>Department of General Surgery, Affiliated Zhongshan Hospital of Dalian University, Dalian, Liaoning 116001;

<sup>3</sup>Department of Chinese Medicine, Dalian Municipal Central Hospital, Dalian, Liaoning 116033; <sup>4</sup>Department of Chinese Medicine, Dalian Obstetrics and Gynecology Hospital, Dalian, Liaoning 116083, P.R. China

Received June 24, 2016; Accepted April 20, 2017

DOI: 10.3892/mmr.2017.7259

**Abstract.** The present study aimed to evaluate the protective effects of emodin on severe acute pancreatitis (SAP)-associated acute lung injury (ALI), and investigated the possible mechanism involved. SAP was induced in Sprague-Dawley rats by retrograde infusion of 5% sodium taurocholate (1 ml/kg), after which, rats were divided into various groups and were administered emodin, FK866 [a competitive inhibitor of pre-B-cell colony-enhancing factor (PBEF)] or dexamethasone (DEX). DEX was used as a positive control. Subsequently, PBEF expression was detected in polymorphonuclear neutrophils (PMNs) isolated from rat peripheral blood by reverse transcription-quantitative polymerase chain reaction and western blotting. In addition, histological alterations, apoptosis in lung/pancreatic tissues, apoptosis of peripheral blood PMNs and alterations in the expression of apoptosis-associated proteins were examined by hematoxylin and eosin staining, terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling assay, Annexin V/propidium iodide (PI) assay and western blotting, respectively. Serum amylase activity and wet/dry (W/D) weight ratios were also measured. An *in vitro* study was also conducted, in which PMNs were obtained from normal Sprague-Dawley rats and were incubated with emodin, FK866 or DEX in the presence of lipopolysaccharide (LPS). Apoptosis of PMNs and the expression levels of apoptosis-associated proteins were examined in cultured PMNs *in vitro* by Annexin V/PI assay and western blotting, respectively. The results demonstrated that emodin, FK866 and DEX significantly downregulated PBEF expression in

peripheral blood PMNs. In addition, emodin, FK866 and DEX reduced serum amylase activity, decreased lung and pancreas W/D weight ratios, alleviated lung and pancreatic injuries, and promoted PMN apoptosis by regulating the expression of apoptosis-associated proteins: Fas, Fas ligand, B-cell lymphoma (Bcl)-2-associated X protein, cleaved caspase-3 and Bcl-extra-large. In addition, the *in vitro* study demonstrated that emodin, FK866 and DEX significantly reversed the LPS-induced decrease of apoptosis in PMNs by regulating the expression of apoptosis-associated proteins. In conclusion, the present study demonstrated that emodin may protect against SAP-associated ALI by decreasing PBEF expression, and promoting PMN apoptosis via the mitochondrial and death receptor apoptotic pathways.

## Introduction

Severe acute pancreatitis (SAP) is an acute abdominal disorder with a mortality rate of 9-24%, which can increase to 47-69% in SAP patients with multiple organ dysfunction syndromes (1). In addition, SAP-associated mortality has not exhibited a marked decrease in recent years (2). The pathogenesis of SAP remains unclear and the generation of novel therapeutic strategies is required for the treatment of patients with SAP (3,4). Acute lung injury (ALI) is a common complication of SAP, which can develop into acute respiratory distress syndrome (ARDS) (5); ARDS is the leading cause of mortality in patients with SAP (6). Excessive infiltration of polymorphonuclear neutrophils (PMNs) into the lungs is the a critical event in the progression of ALI (7). Neutrophils serve an important role in the inflammatory response (8), and it has previously been reported that depletion or inhibition of neutrophils may protect against tissue injury in pancreatitis (9).

*Rheum palmatum* is a traditional Chinese herb that is widely used for the treatment of numerous diseases in China, including acute pancreatitis (10,11). Emodin is a natural active component of *Rheum palmatum* and other Chinese herbs, including *Polygonum cuspidatum*, *Polygonum multiflorum* and *Cassia obtusifolia* (12,13). It has previously been reported that emodin possesses anti-inflammatory, antiatherogenic and

---

*Correspondence to:* Dr Hailong Chen, Department of General Surgery, The First Affiliated Hospital of Dalian Medical University, 222 Zhongshan Road, Dalian, Liaoning 116011, P.R. China  
E-mail: hailongchen2006@126.com

**Key words:** severe acute pancreatitis, emodin, pre-B-cell colony-enhancing factor, polymorphonuclear neutrophil, FK866, apoptotic pathway

antitumor activities (14). Emodin has also been widely used in animal models as a potent agent for the treatment of SAP (15). Yao *et al.* (16) demonstrated that emodin protects rats against SAP by inhibiting nuclear factor- $\kappa$ B activity, inflammation and oxidative stress. Furthermore, Ni *et al.* (17) reported that emodin enhances peritoneal macrophage phagocytosis and elevates intercellular adhesion molecule-3 expression in a SAP/systemic inflammatory response syndrome rat model.

Pre-B-cell colony-enhancing factor (PBEF), which is also known as visfatin or nicotinamide phosphoribosyl transferase, is an extracellular cytokine-like molecule (18). It inhibits neutrophil apoptosis, serves an important role in inflammation and primes neutrophil respiratory burst (18,19). PBEF is elevated in ventilator-induced lung injury and exacerbates ALI via the induction of neutrophil infiltration, alveolar permeability and oxidative stress (20,21).

The present study aimed to investigate whether emodin exerted its therapeutic effects by influencing PBEF expression and PMN apoptosis in SAP-associated ALI *in vivo* and *in vitro*.

## Materials and methods

**Animals.** Male Sprague-Dawley (SD) rats (n=30; age, 8 weeks; weight, 230-270 g) were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Rats were housed at a constant room temperature and humidity (21±2°C; 45-55%) under a 12-h light/dark cycle. The rats had *ad libitum* access to food and water. Animal experiments were approved by the Animal Care and Use Committee of Dalian Medical University (Dalian, China) and were performed according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (22).

**Induction of SAP and experimental grouping.** The rats were randomly assigned to the following five groups (n=6 rats/group): Control group, SAP group, emodin group, FK866 group and dexamethasone (DEX) group. To induce SAP, the rats were anesthetized with chloral hydrate (10%, 3.5 ml/kg bodyweight) and sodium taurocholate solution (5%, 1 ml/kg bodyweight) was retrogradely injected into the biliary-pancreatic duct. The control rats were anesthetized in the same manner however, without the sodium taurocholate solution injection. A total of 3 h after SAP induction, rats in the SAP, emodin, FK866 and DEX groups were intraperitoneally injected with a single dose of dimethyl sulfoxide, emodin (10 mg/kg bodyweight; both Aladdin, Shanghai, China), FK866 (10 mg/kg bodyweight; MedChem Express, Monmouth Junction, NJ, USA) or DEX (1 mg/kg bodyweight; Aladdin), respectively. After 24 h, peripheral blood was obtained, and lung and pancreatic tissues were excised. The rats were anesthetized with 10% chloral hydrate, and then sacrificed by exsanguination prior to tissue excision. Half of the tissues were immediately subjected to edema examination and the remaining tissues were fixed at room temperature for 48 h in 10% formaldehyde for hematoxylin and eosin (HE), and TUNEL staining.

**Isolation of PMNs.** A total of 24 h after treatment, the rats in each group were sacrificed and peripheral blood was obtained. PMNs were isolated from the peripheral blood using

Histopaque-1083 (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) according to the manufacturer's protocol and were immediately used for flow cytometry experiments.

**Reverse transcription-quantitative polymerase chain reaction (RT-qPCR).** RNA was extracted from PMNs using the RNAPure Total RNA Rapid Extraction kit (BioTeke Corporation, Beijing, China) according to the manufacturer's protocol. RNA was then reverse-transcribed into cDNA with the following reaction system: 1  $\mu$ g RNA, 1.2  $\mu$ l RT primer (Tiangen Biotech Co., Ltd., Beijing, China), 0.75  $\mu$ l dNTP (BioTeke Corporation), 4  $\mu$ l 5X buffer, 0.25  $\mu$ l RNasin, 1  $\mu$ l super M-MLV reverse transcriptase (BioTeke Corporation) and sufficient ddH<sub>2</sub>O to produce a final reaction volume of 20  $\mu$ l. The following temperature protocol was applied for the RT reaction: 25°C for 10 min, 42°C for 50 min and 80°C for 5 min. qPCR analysis was performed using the Exicycler™ 96 Real-Time qPCR system (Bioneer Corporation, Daejeon, Korea) and the following reaction system: 1  $\mu$ l cDNA, 0.5  $\mu$ l forward primer, 0.5  $\mu$ l reverse primer (Sangon Biotech Co., Ltd., Shanghai, China), 10  $\mu$ l SYBR GREEN MasterMix (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) and sufficient ddH<sub>2</sub>O to produce a final reaction volume of 20  $\mu$ l. The thermocycling conditions were as follows: 95°C for 10 min, followed by 40 cycles at 95°C for 10 sec, 60°C for 20 sec and 72°C for 30 sec. The primer sequences were as follows: PBEF, forward 5'-GATGCCAAATCCCGAAGG-3', reverse 5'-CCC ACTCCAGCAGTTCATT-3'; and  $\beta$ -actin, forward 5'-GGA GATTACTGCCCTGGCTCCTAGC-3' and reverse 5'-GGC CGGACTCATCGTACTCCTGCTT-3'. Relative expression was determined using the 2<sup>- $\Delta\Delta$ C<sub>q</sub></sup> method (23).

**Western blotting.** The PMNs were lysed in kit lysis buffer (Whole Cell Lysis Assay kit; Wanleibio, Shenyang, China) and centrifuged at 10,005 x g for 10 min at 4°C. Total protein concentration was determined using a bicinchoninic acid kit (Wanleibio). Equal amounts of protein (40  $\mu$ g) were separated by 10 or 13% SDS-PAGE and were transferred onto polyvinylidene fluoride membranes (EMD Millipore, Billerica, MA, USA). Subsequently, the membranes were blocked with nonfat milk, followed by incubation with primary antibodies against PBEF (cat. no. ab45890; 1:500; Abcam, Cambridge, UK), B-cell lymphoma (Bcl)-extra-large (xL; cat. no. BA3368; 1:400; Wuhan Boster Biological Technology Ltd., Wuhan, China), Bcl-2-associated X protein (Bax; cat. no. BA0315; 1:400, Wuhan Boster Biological Technology Ltd.), cleaved caspase-3 (cat. no. ab2302; 1:1,000; Abcam), Fas (cat. no. ab82419; 1:1,000; Abcam) and Fas ligand (L; cat. no. ab15285; 1:1,000; Abcam) overnight at 4°C. Membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies (cat. nos. WLA023 and WLA024; Wanleibio) at 37°C for 45 min. The bands were visualized by enhanced chemiluminescence (Wanleibio) and the blots were semi-quantified using Gel-Pro Analyzer (version 4.0; Media Cybernetics, Inc., Rockville, MD, USA) (24).

**HE staining.** The lung and pancreatic tissues that were fixed in 10% formaldehyde at room temperature for 48 h were embedded in paraffin and cut into 5- $\mu$ m sections. Subsequently, the sections were deparaffinized and rehydrated prior to

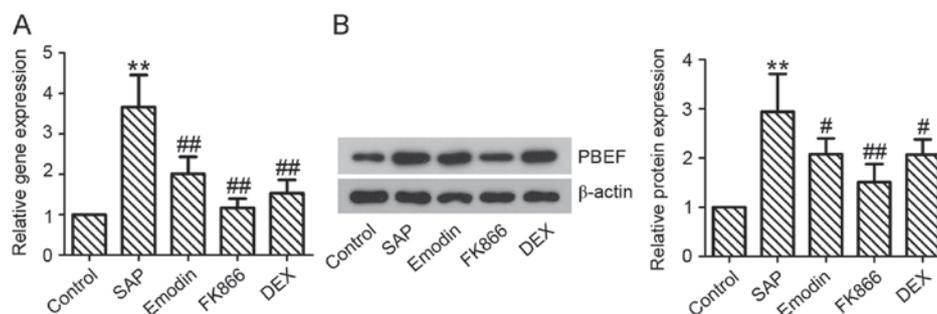


Figure 1. Effects of emodin, FK866 and DEX on PBEF expression in PMNs isolated from rat peripheral blood. PMNs were harvested from rat peripheral blood following various treatments. (A) The mRNA expression levels of PBEF were detected by reverse transcription-quantitative polymerase chain reaction. (B) Protein expression levels of PBEF were detected by western blotting. Data are presented as the mean  $\pm$  standard deviation. \*\* $P < 0.01$  compared with the control group; # $P < 0.05$ , ## $P < 0.01$  compared with the SAP group. DEX, dexamethasone; PBEF, pre-B-cell colony-enhancing factor; PMN, polymorphonuclear neutrophils; SAP, severe acute pancreatitis.

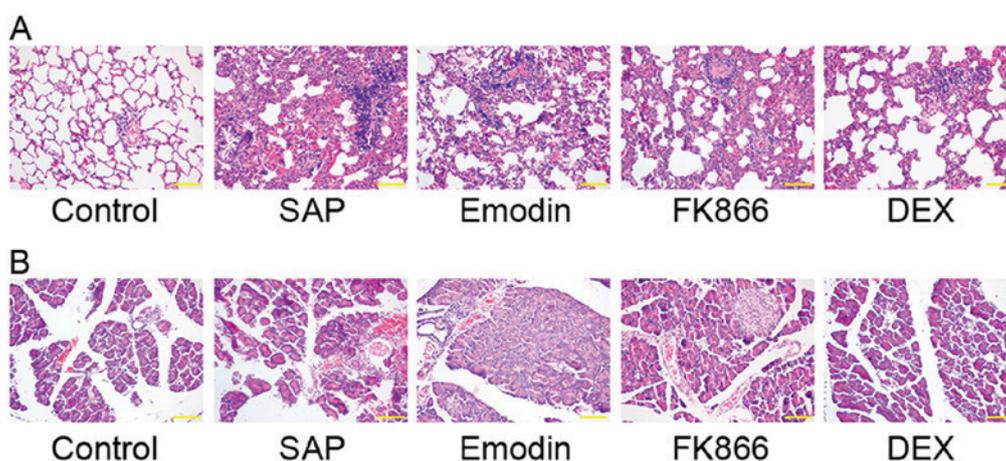


Figure 2. Representative HE-stained sections of lung and pancreatic tissues. (A) Lung and (B) pancreatic tissues were excised from rats in each group following various treatments and histological alterations were evaluated by HE staining. Scale bar, 100  $\mu$ m. DEX, dexamethasone; HE, hematoxylin and eosin; SAP, severe acute pancreatitis.

staining with HE at 37°C (hematoxylin for 5 min and eosin for 3 min). The stained sections were examined, and images were captured under a fluorescence microscope (Olympus Corporation, Tokyo, Japan).

**Determination of serum amylase activity.** Blood was harvested and serum was separated by centrifugation at room temperature and 1,111 x g for 10 min. Subsequently, serum was diluted at a ratio of 1:10 in normal saline and incubated with pre-heated substrate buffer containing soluble starch (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) at 37°C for 7.5 min, followed by incubation with 5 mM iodine solution (Sangon Biotech). Absorbance was measured at 660 nm.

**Edema.** At 24 h post-treatment, lung and pancreatic samples were immediately excised and weighed. The samples were then dried for 72 h, until the weight remained unchanged, and were then weighed again. The wet/dry (W/D) weight ratio was subsequently calculated.

**Terminal deoxynucleotidyl-transferase (TdT)-mediated dUTP nick end labeling (TUNEL) assay.** DNA fragmentation was measured by TUNEL assay using an *In Situ* Cell Death

Detection kit (Roche Diagnostics, Basel, Switzerland) (25). Briefly, 5- $\mu$ m tissue sections were deparaffinized and rehydrated. Endogenous peroxidase activity was blocked with 3% H<sub>2</sub>O<sub>2</sub>. The sections were washed with PBS, followed by incubation with TdT reaction mixture at 37°C for 1 h. Subsequently, the sections were incubated with converter-POD for 30 min and then with DAB substrate (Beijing Solarbio Science & Technology). Images of the stained cells were captured under a fluorescence microscope (Olympus Corporation).

**Cell culture and treatment.** PMNs were isolated from the peripheral blood of rats in the control group (n=6) and were cultured *in vitro* in Dulbecco's modified Eagle's medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (HyClone; GE Healthcare Life Sciences, Logan, UT, USA) at 37°C in a 5% CO<sub>2</sub> incubator. PMNs were then treated with emodin (30  $\mu$ g/ml), FK866 (10 nM) or DEX (100 nM) for 16 h in the presence of lipopolysaccharide (LPS; 50 ng/ml; Aladdin). Untreated PMNs were used as controls.

**Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) assay.** Cell apoptosis was analyzed using an

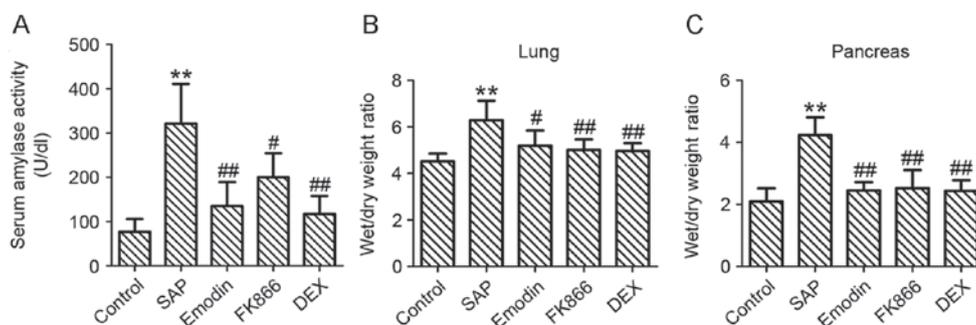


Figure 3. Effects of emodin, FK866 and DEX on serum amylase activity and W/D weight ratio. (A) Serum amylase activity was measured; values are expressed as U/dl. (B) Lung and (C) pancreatic W/D weight ratios. Data are presented as the mean  $\pm$  standard deviation. \*\* $P < 0.01$  compared with the control group; # $P < 0.05$ , ## $P < 0.01$  compared with the SAP group. DEX, dexamethasone; SAP, severe acute pancreatitis; W/D, wet/dry.

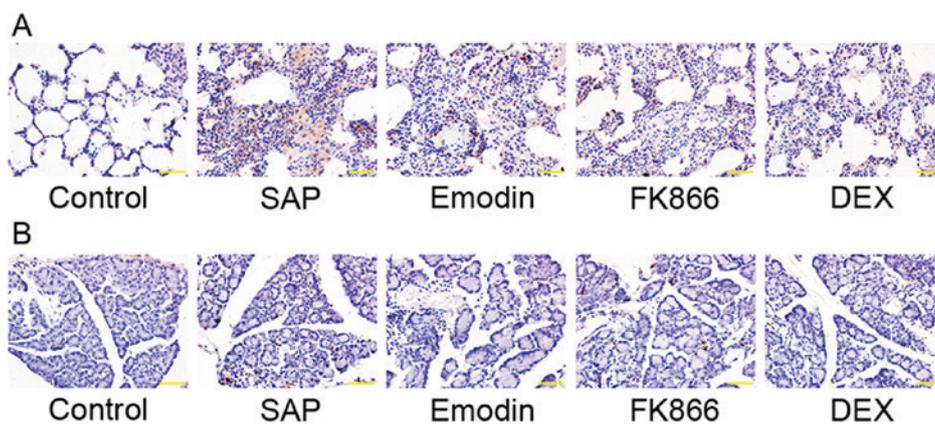


Figure 4. Representative TUNEL-stained sections of lung and pancreatic tissues. (A) Lung and (B) pancreatic sections were subjected to TUNEL staining, in order to detect apoptotic cells. Representative images are shown. Scale bar, 50  $\mu$ m. DEX, dexamethasone; SAP, severe acute pancreatitis; TUNEL, terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling.

Apoptosis Detection kit (Wanleibio). PMNs were harvested after the indicated treatments, and were resuspended in binding buffer. Subsequently, Annexin V-FITC and PI were added to stain the cells. Following a 15-min incubation at room temperature in the dark, the cells were analyzed by flow cytometry (Accuri™ C6; BD Biosciences, San Jose, CA, USA) and the system software (BD Accuri C6 Software; BD Biosciences, USA).

**Statistical analysis.** Data are presented as the mean  $\pm$  standard deviation of three independent repeated experiments. GraphPad Prism 5 software (GraphPad Software Inc., La Jolla, CA, USA) was used to analyze statistical differences. The results were analyzed using one-way analysis of variance with Bonferroni post hoc test for multiple comparisons.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Emodin decreases PBEF expression in PMNs.** Following treatment with the indicated reagents; PMNs were isolated from the peripheral blood of rats in each group. Subsequently, PBEF expression was detected in PMNs using RT-qPCR and western blotting. The results indicated that the mRNA (Fig. 1A) and protein expression levels of PBEF (Fig. 1B) were significantly

higher in the SAP group compared with in the control group. Conversely, PBEF expression in the emodin, FK866 and DEX groups was markedly decreased compared with in the SAP group.

**Emodin, FK866 and DEX alleviate SAP-induced histopathological alterations.** The results of histological examination with HE staining indicated that the lung and pancreatic tissue structures of the control rats were normal. Following the induction of SAP, a thickened alveolar septum, edema, hemorrhage and infiltration of inflammatory cells were all observed in the lung tissues (Fig. 2A). In the SAP group, HE staining of the pancreatic tissues identified pancreatic edema, hemorrhage, acinar cell necrosis and inflammatory cell infiltration (Fig. 2B). Conversely, these histopathological alterations were alleviated in SAP rats treated with emodin, FK866 and DEX.

**Emodin, FK866 and DEX reduce serum amylase activity.**

The severity of SAP-associated ALI was confirmed by measuring serum amylase activity. As presented in Fig. 3A, serum amylase activity was significantly increased in rats in the SAP group compared with in the control group. However, following treatment with emodin, FK866 and DEX, SAP rats exhibited reduced serum amylase activity compared with in the untreated SAP rats.

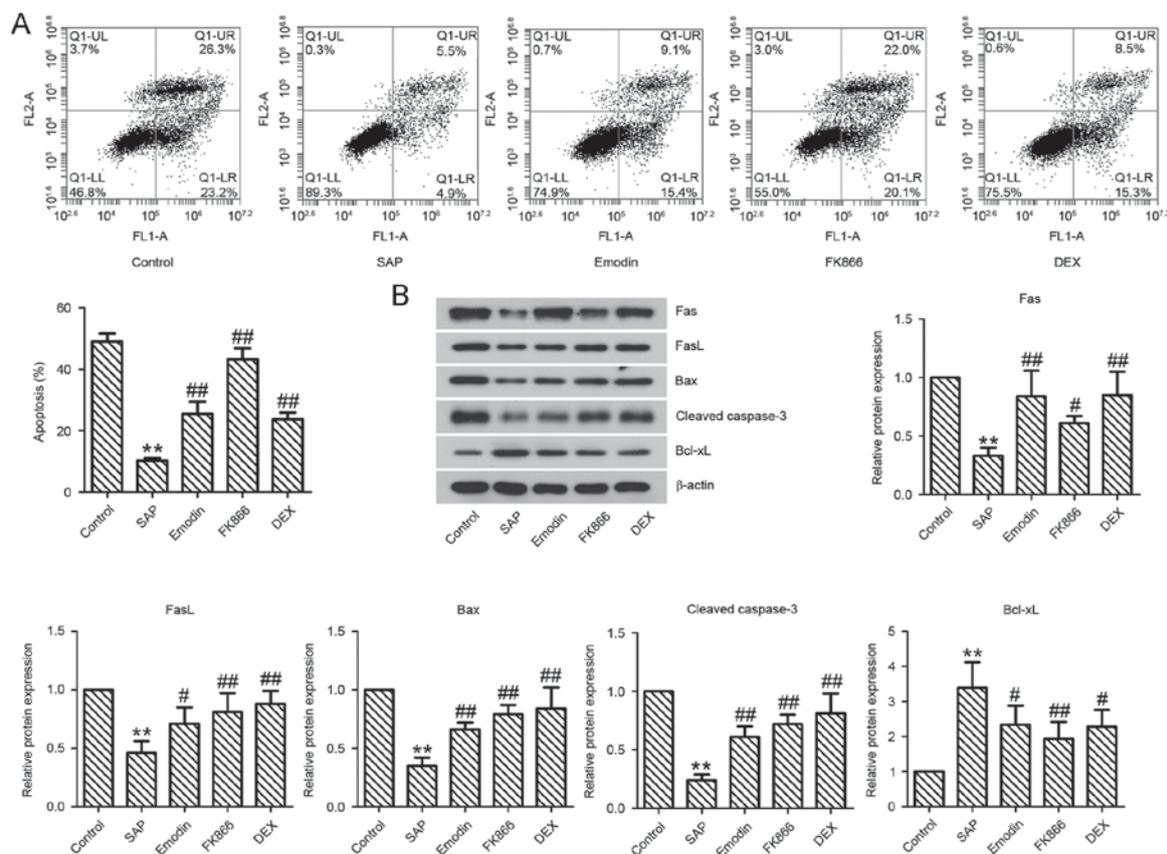


Figure 5. Effects of emodin, FK866 and DEX on PMN apoptosis in SAP rats. (A) PMN apoptosis was examined by Annexin V/propidium iodide staining and flow cytometry. (B) Protein expression levels of Fas, FasL, Bax, cleaved caspase-3 and Bcl-xL were detected by western blotting.  $\beta$ -actin served as an internal control. Data are presented as the mean  $\pm$  standard deviation. \*\* $P$ <0.01 compared with the control group; # $P$ <0.05, ## $P$ <0.01 compared with the SAP group. Bax, B-cell lymphoma 2-associated X protein; Bcl-xL, B-cell lymphoma-extra-large; DEX, dexamethasone; FasL, Fas ligand; PMN, polymorphonuclear neutrophils; SAP, severe acute pancreatitis.

*Emodin, FK866 and DEX attenuate pulmonary and pancreatic edema.* The degrees of pulmonary and pancreatic edema were examined by measuring water content. The results demonstrated that the W/D weight ratios of the lung (Fig. 3B) and pancreatic tissues (Fig. 3C) in the SAP group were higher than those in the control group. However, the W/D weight ratios were markedly decreased following treatment with emodin, FK866 and DEX compared with in the SAP group.

*Emodin, FK866 and DEX inhibit cell apoptosis in the lung and pancreatic tissues.* The present study detected cell apoptosis in the lung and pancreatic tissues using TUNEL staining. The results indicated that the number of apoptotic cells in the lung (Fig. 4A) and pancreatic tissue sections (Fig. 4B) were markedly increased following the induction of SAP compared with in the control group. However, treatment with emodin, FK866 and DEX markedly decreased the number of apoptotic cells in the lung and pancreatic tissues compared with in the SAP group.

*Emodin, FK866 and DEX promote PMN apoptosis in vivo.* The present study also examined PMN apoptosis using flow cytometry. The results demonstrated that rats in the SAP group ( $10.28 \pm 0.77\%$ ) exhibited reduced apoptosis of PMNs compared with in the control rats ( $49.01 \pm 2.62\%$ ) (Fig. 5A). However, emodin ( $25.48 \pm 4.02\%$ ), FK866 ( $43.2 \pm 3.65\%$ ) and

DEX treatment ( $23.72 \pm 2.13\%$ ) significantly increased cell apoptosis of PMNs, compared with in the SAP group.

The protein expression levels of cleaved caspase-3, Bax, Bcl-xL, Fas and FasL were measured by western blotting. The results demonstrated that the expression levels of Bax, cleaved caspase-3, Fas and FasL in the SAP group were significantly decreased compared with in the control group, whereas Bcl-xL expression was increased in the SAP group (Fig. 5B). Following treatment of the SAP rats with emodin, FK866 and DEX, the expression levels of Bax, cleaved caspase-3, Fas and FasL were markedly increased, whereas Bcl-xL expression was decreased compared with the untreated SAP rats.

*Emodin, FK866 and DEX induce apoptosis of LPS-treated PMNs.* Flow cytometric analysis indicated that LPS significantly inhibited PMN apoptosis compared with the control group (Fig. 6A). Compared with the LPS group, incubation with emodin, FK866 and DEX significantly induced PMN apoptosis and attenuated the anti-apoptotic effects of LPS on PMNs.

The expression levels of apoptosis-associated proteins were detected using western blotting. The results demonstrated that, following LPS stimulation, the expression levels of Bax, cleaved caspase-3, Fas and FasL were markedly decreased, whereas Bcl-xL was increased (Fig. 6B). However, treatment

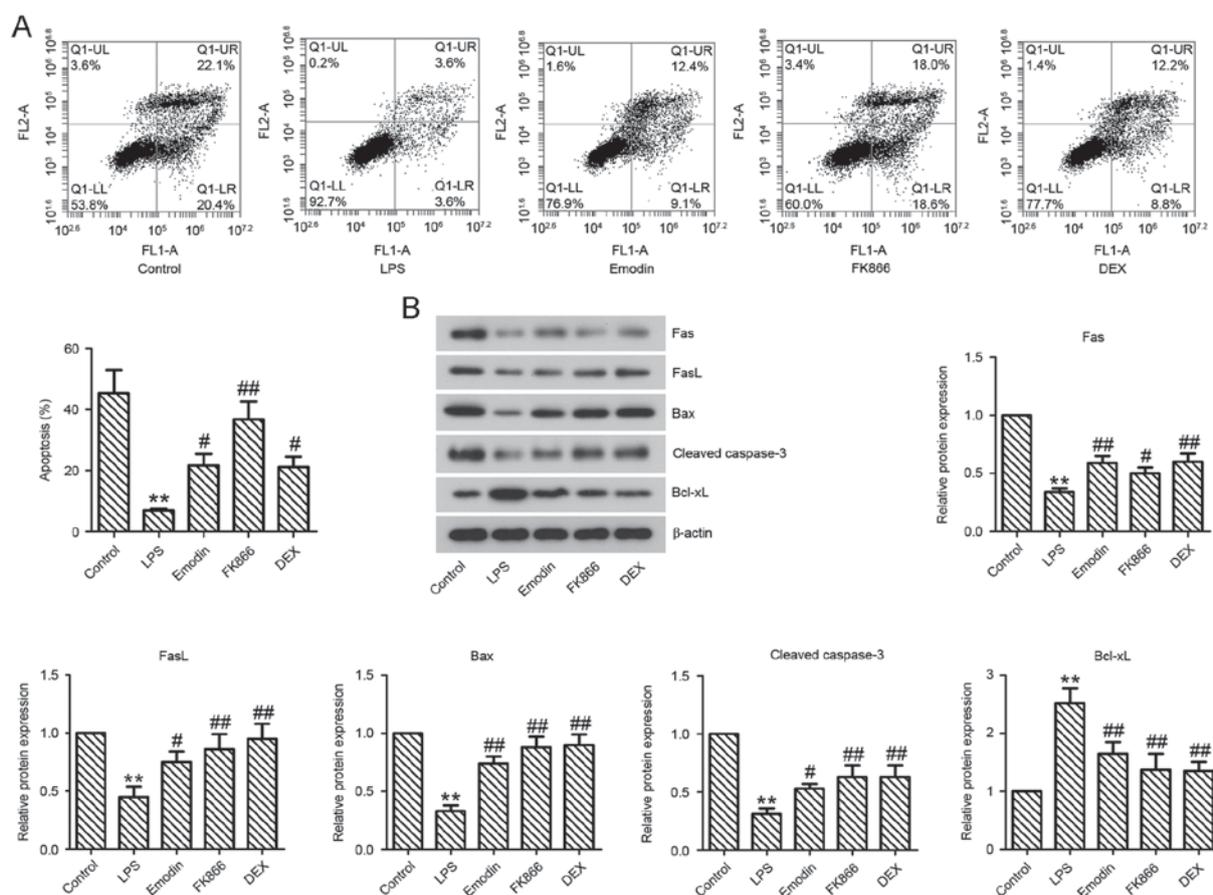


Figure 6. Effects of emodin, FK866 and DEX on apoptosis of LPS-stimulated PMNs *in vitro*. PMNs were isolated from normal rats and cultured *in vitro*. Subsequently, PMNs were stimulated with LPS and incubated with emodin, FK866 and DEX for 16 h. (A) Flow cytometric analysis of PMN apoptosis, using Annexin V/propidium iodide staining. (B) Protein expression levels of Fas, FasL, Bax, cleaved caspase-3 and Bcl-xL were examined by western blotting. Data are presented as the mean  $\pm$  standard deviation. \*\* $P < 0.01$  compared with the control group; # $P < 0.05$ , ## $P < 0.01$  compared with the SAP group. Bax, B-cell lymphoma 2-associated X protein; Bcl-xL, B-cell lymphoma-extra-large; DEX, dexamethasone; FasL, Fas ligand; LPS, lipopolysaccharide; PMN, polymorphonuclear neutrophil.

with emodin, FK866 and DEX markedly elevated Bax, cleaved caspase-3, Fas and FasL expression in LPS-stimulated PMNs, and reduced Bcl-xL expression.

## Discussion

DEX is a glucocorticoid that is widely used in the treatment of SAP (26,27). Therefore, in the present study, DEX was used as a positive control to investigate the therapeutic effects of emodin on SAP-associated ALI, and the mechanism involved *in vivo* and *in vitro*.

PBEF is a proinflammatory cytokine that is associated with numerous diseases, including ALI, Leber congenital amaurosis and rheumatoid arthritis (RA) (28). It is a highly conserved protein with a molecular weight of 52 kDa (29). It has previously been reported that PBEF expression is increased in the synovial fluid and serum of patients with RA (30). In addition, PBEF may delay neutrophil apoptosis in experimental and clinical sepsis (31). In the present study, a rat model of SAP was successfully established using sodium taurocholate solution. The expression levels of PBEF were detected in PMNs isolated from rat peripheral blood by RT-qPCR and western blotting. The results demonstrated that treatment with emodin and FK866 markedly reduced

the SAP-induced elevation of PBEF expression in PMNs compared with in the untreated SAP rats. Therefore, it may be hypothesized that PBEF is involved in the protective effects of emodin on SAP-associated ALI.

To further confirm this hypothesis, histopathological alterations, serum amylase activity and edema were analyzed in SAP-associated ALI. FK866 is a competitive inhibitor of PBEF, which is widely used (32). Matsuda *et al* (33) demonstrated that FK866 protects against ALI in C57BL/6J mice subjected to intestinal ischemia and reperfusion injury. Emodin has previously been reported to be effective in SAP treatment (34); however, the underlying mechanism remains unclear. The present study indicated that emodin and FK866 significantly ameliorated histopathological alterations induced by SAP-associated ALI in the lung and pancreatic tissues, as determined by HE staining. Serum amylase is commonly used as a biochemical marker of SAP (35); therefore, serum samples were obtained and serum amylase activity was measured. The results suggested that emodin and FK866 reduced SAP-enhanced serum amylase activity. In addition, W/D weight ratio can be used to evaluate tissue edema, including in the lung and pancreas (36,37). Alterations in the lung and pancreas W/D weight ratios were significantly decreased following treatment with emodin and FK866 in the present

study. These results suggested that emodin may alleviate histopathological alterations and edema via downregulation of PBEF.

Takeyama (38) reported that inhibition of apoptosis attenuates multi-organ injuries in SAP. In the present study, a TUNEL assay indicated that emodin and FK866 markedly inhibited SAP-induced apoptosis in the lung and pancreatic tissues, thus suggesting that emodin may reduce the lung and pancreatic cell apoptotic rate by downregulating PBEF. Bcl-2 family members control the mitochondrial pathway in cancer and other diseases (39), and can be divided into three sub-families, including proapoptotic proteins (Bcl-2 homologous antagonist/killer and Bax), anti-apoptotic proteins (Bcl-xL, Bcl-2 and induced myeloid leukemia cell differentiation protein) and BH3-only proteins (Bcl-2-associated death promoter, BH3 interacting-domain death agonist and Bcl-2-like protein 11) (40). In the present study, treatment with emodin and FK866 significantly induced PMN apoptosis, alongside increases in the expression levels of Bax and cleaved caspase-3, and a decrease in Bcl-xL expression, in PMNs. Fas is a member of the tumor necrosis factor (TNF) family and FasL belongs to the TNF receptor family (41). It is well known that activation of Fas/FasL signaling leads to cell apoptosis (42). Initially, the interaction of Fas with FasL activates caspase-8 via the Fas-associated death domain protein, which then activates downstream caspases-3, 6 and 7 (41,43). The present study demonstrated that the protein expression levels of Fas and FasL were downregulated in PMNs from the SAP group. Following treatment with emodin and FK866, Fas and FasL levels were upregulated. These results suggested that emodin may promote PMN apoptosis by decreasing PBEF expression via the mitochondrial and the death receptor apoptotic pathways.

A previous study reported that delayed apoptosis of PMNs was observed in ALI lung tissues, which contributed to ALI development (44). LPS, which is an important risk factor of ALI, is a component of the outer membrane of Gram-negative bacteria, which can induce inflammation (45). The present study performed several *in vitro* studies to verify the results obtained from the animal studies. Briefly, PMNs were isolated from normal Sprague-Dawley rats and were cultured *in vitro*. PMNs were then exposed to LPS, to simulate ALI, and were treated with emodin and FK866. The results demonstrated that emodin and FK866 ameliorated the LPS-induced inhibition of PMN apoptosis, which was associated with upregulation of Bax, cleaved caspase-3, Fas and FasL, and downregulation of Bcl-xL. The *in vitro* and *in vivo* experiments conducted in the present study confirmed that PBEF and the mitochondrial apoptotic/death receptor pathway were involved in emodin-mediated PMN apoptosis.

In conclusion, emodin and FK866 significantly decreased PBEF expression in rat peripheral blood PMNs. The results of the present study demonstrated that emodin and DEX exert similar therapeutic effects on SAP-associated ALI. The protective mechanism underlying the effects of emodin on SAP-associated ALI may be associated with the downregulation of PBEF and the promotion of PMN apoptosis via activation of mitochondrial and death receptor apoptotic pathways. Further studies are required to verify these conclusions and elucidate the underlying mechanism.

## Acknowledgements

The present study was supported by grants from the National Natural Science Foundation of China (grant nos. 81173452 and 81573751).

## References

- Nieminen A, Maksimow M, Mentula P, Kyhälä L, Kylänpää L, Puolakkainen P, Kempainen E, Repo H and Salmi M: Circulating cytokines in predicting development of severe acute pancreatitis. *Crit Care* 18: R104, 2014.
- Li G, Wu X, Yang L, He Y, Liu Y, Jin X and Yuan H: TLR4-mediated NF- $\kappa$ B signaling pathway mediates HMGB1-induced pancreatic injury in mice with severe acute pancreatitis. *Int J Mol Med* 37: 99-107, 2016.
- Liang X, Zhang B, Chen Q, Zhang J, Lei B, Li B, Wei Y, Zhai R, Liang Z, He S and Tang B: The mechanism underlying alpinetin-mediated alleviation of pancreatitis-associated lung injury through upregulating aquaporin-1. *Drug Des Devel Ther* 10: 841-850, 2016.
- Zhang ZW, Zhang QY, Zhou MT, Liu NX, Chen TK, Zhu YF and Wu L: Antioxidant inhibits HMGB1 expression and reduces pancreas injury in rats with severe acute pancreatitis. *Dig Dis Sci* 55: 2529-2536, 2010.
- Wang G, Zhang J, Xu C, Han X, Gao Y and Chen H: Inhibition of SOCs attenuates acute lung injury induced by severe acute pancreatitis in Rats and PMVECs injury induced by lipopolysaccharide. *Inflammation* 39: 1049-1058, 2016.
- Guo H, Suo DW, Zhu HP, Sun XM and Chen J: Early blood purification therapy of severe acute pancreatitis complicated by acute lung injury. *Eur Rev Med Pharmacol Sci* 20: 873-878, 2016.
- Yuan Q, Jiang YW, Ma TT, Fang QH and Pan L: Attenuating effect of Ginsenoside Rb1 on LPS-induced lung injury in rats. *J Inflamm (Lond)* 11: 40, 2014.
- Xu LT, Xu HL and Fu MS: Association between glucose-regulated protein and neutrophil apoptosis in severe acute pancreatitis. *Int J Clin Exp Pathol* 8: 9300-9306, 2015.
- Merza M, Wetterholm E, Zhang S, Regner S and Thorlacius H: Inhibition of geranylgeranyltransferase attenuates neutrophil accumulation and tissue injury in severe acute pancreatitis. *J Leukoc Biol* 94: 493-502, 2013.
- Yao P, Cui M, Li Y, Deng Y and Wu H: Effects of rhubarb on intestinal flora and toll-like receptors of intestinal mucosa in rats with severe acute pancreatitis. *Pancreas* 44: 799-804, 2015.
- Zhao YQ, Liu XH, Ito T and Qian JM: Protective effects of rhubarb on experimental severe acute pancreatitis. *World J Gastroenterol* 10: 1005-1009, 2004.
- Pang X, Liu J, Li Y, Zhao J and Zhang X: Emodin inhibits homocysteine-induced C-Reactive protein generation in vascular smooth muscle cells by regulating PPAR $\gamma$  expression and ROS-ERK1/2/p38 signal pathway. *PLoS One* 10: e0131295, 2015.
- Tu C, Gao D, Li XF, Li CY, Li RS, Zhao YL, Li N, Jia GL, Pang JY, Cui HR, *et al*: Inflammatory stress potentiates emodin-induced liver injury in rats. *Front Pharmacol* 6: 233, 2015.
- Jia X, Yu F, Wang J, Iwanowycz S, Saaoud F, Wang Y, Hu J, Wang Q and Fan D: Emodin suppresses pulmonary metastasis of breast cancer accompanied with decreased macrophage recruitment and M2 polarization in the lungs. *Breast Cancer Res Treat* 148: 291-302, 2014.
- Wang G, Sun B, Gao Y, Meng QH and Jiang HC: The effect of emodin-assisted early enteral nutrition on severe acute pancreatitis and secondary hepatic injury. *Mediators Inflamm* 2007: 29638, 2007.
- Yao WY, Zhou YF, Qian AH, Zhang YP, Qiao MM, Zhai ZK, Yuan YZ and Yang SL: Emodin has a protective effect in cases of severe acute pancreatitis via inhibition of nuclear factor- $\kappa$ B activation resulting in antioxidation. *Mol Med Rep* 11: 1416-1420, 2015.
- Ni Q, Zhang W, Sun K, Yin C, An J and Shang D: In vitro effects of emodin on peritoneal macrophage intercellular adhesion molecule-3 in a rat model of severe acute pancreatitis/systemic inflammatory response syndrome. *Biomed Rep* 2: 63-68, 2014.
- Peng Q, Jia SH, Parodo J, Ai Y and Marshall JC: Pre-B cell colony enhancing factor induces Nampt-dependent translocation of the insulin receptor out of lipid microdomains in A549 lung epithelial cells. *Am J Physiol Endocrinol Metab* 308: E324-E333, 2015.

19. Yang W, Zeng Y, Li B, Zhou J, Gong Y, Xu J and Dong X: Pre-B-cell colony enhancing factor (PBEF) increases endothelial permeability in hypoxia/re-oxygenation model. *Int J Clin Exp Med* 8: 8842-8847, 2015.
20. Jones HD, Yoo J, Crother TR, Kyme P, Ben-Shlomo A, Khalafi R, Tseng CW, Parks WC, Arditi M, Liu GY and Shimada K: Nicotinamide exacerbates hypoxemia in ventilator-induced lung injury independent of neutrophil infiltration. *PLoS One* 10: e0123460, 2015.
21. Hong SB, Huang Y, Moreno-Vinasco L, Sammani S, Moitra J, Barnard JW, Ma SF, Mirzapioazova T, Evenoski C, Reeves RR, *et al*: Essential role of pre-B-cell colony enhancing factor in ventilator-induced lung injury. *Am J Respir Crit Care Med* 178: 605-617, 2008.
22. Institute of laboratory animal resources: Guide for the care and use of laboratory animals. National institutes of health publication no: 85-23, 1996 (revised 1996). *Nation Acad Press*, Washington, D.C Available from [http://www.nap.edu/openbook.php?record\\_id=5140](http://www.nap.edu/openbook.php?record_id=5140).
23. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *methods* 25: 402-408, 2001.
24. Wang LN, Wang Y, Lu Y, Yin ZF, Zhang YH, Aslanidi GV, Srivastava A, Ling CQ and Ling C: Pristimerin enhances recombinant adeno-associated virus vector-mediated transgene expression in human cell lines in vitro and murine hepatocytes in vivo. *J Integr Med* 12: 20-34, 2014.
25. Kabeer FA, Sreedevi GB, Nair MS, Rajalekshmi DS, Gopalakrishnan LP, Kunjuraman S and Prathapan R: Antineoplastic effects of deoxyelephantopin, a sesquiterpene lactone from *Elephantopus scaber*, on lung adenocarcinoma (A549) cells. *J Integr Med* 11: 269-277, 2013.
26. Xiping Z, Jun F, Jie Z, Bingyan Y, Jing M, Wei Z, Jing Y, Penghui J, Wenqin Y, Ninnin Z and Jiao H: Influence of dexamethasone on the expression levels of P-selectin protein in multiple organs of rats with severe acute pancreatitis. *Inflamm Res* 59: 31-39, 2010.
27. Kandil E, Lin YY, Bluth MH, Zhang H, Levi G and Zenilman ME: Dexamethasone mediates protection against acute pancreatitis via upregulation of pancreatitis-associated proteins. *World J Gastroenterol* 12: 6806-6811, 2006.
28. Kaja S, Shah AA, Haji SA, Patel KB, Naumchuk Y, Zabaneh A, Gerdes BC, Kunjukunju N, Sabates NR, Cassell MA, *et al*: Namp1/PBEF/visfatin serum levels: A new biomarker for retinal blood vessel occlusions. *Clin Ophthalmol* 9: 611-618, 2015.
29. Lee K, Huh JW, Lim CM, Koh Y and Hong SB: Clinical role of serum pre-B cell colony-enhancing factor in ventilated patients with sepsis and acute respiratory distress syndrome. *Scand J Infect Dis* 45: 760-765, 2013.
30. Brentano F, Schorr O, Ospelt C, Stanczyk J, Gay RE, Gay S and Kyburz D: Pre-B cell colony-enhancing factor/visfatin, a new marker of inflammation in rheumatoid arthritis with proinflammatory and matrix-degrading activities. *Arthritis Rheum* 56: 2829-2839, 2007.
31. Jia SH, Li Y, Parodo J, Kapus A, Fan L, Rotstein OD and Marshall JC: Pre-B cell colony-enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis. *J Clin Invest* 113: 1318-1327, 2004.
32. Song TY, Yeh SL, Hu ML, Chen MY and Yang NC: A Namp1 inhibitor FK866 mimics vitamin B3 deficiency by causing senescence of human fibroblastic Hs68 cells via attenuation of NAD(+)-SIRT1 signaling. *Biogerontology* 16: 789-800, 2015.
33. Matsuda A, Yang WL, Jacob A, Aziz M, Matsuo S, Matsutani T, Uchida E and Wang P: FK866, a visfatin inhibitor, protects against acute lung injury after intestinal ischemia-reperfusion in mice via NF- $\kappa$ B pathway. *Ann Surg* 259: 1007-1017, 2014.
34. Xu J, Huang B, Wang Y, Tong C, Xie P, Fan R and Gao Z: Emodin ameliorates acute lung injury induced by severe acute pancreatitis through the up-regulated expressions of AQP1 and AQP5 in lung. *Clin Exp Pharmacol Physiol* 43: 1071-1079, 2016.
35. Deng YY, Shamoan M, He Y, Bhatia M and Sun J: Cathelicidin-related antimicrobial peptide modulates the severity of acute pancreatitis in mice. *Mol Med Rep* 13: 3881-3885, 2016.
36. Tao W, Su Q, Wang H, Guo S, Chen Y, Duan J and Wang S: Platycodin D attenuates acute lung injury by suppressing apoptosis and inflammation in vivo and in vitro. *Int Immunopharmacol* 27: 138-147, 2015.
37. Luan ZG, Zhang J, Yin XH, Ma XC and Guo RX: Ethyl pyruvate significantly inhibits tumour necrosis factor- $\alpha$ , interleukin-1 $\beta$  and high mobility group box 1 releasing and attenuates sodium taurocholate-induced severe acute pancreatitis associated with acute lung injury. *Clin Exp Immunol* 172: 417-426, 2013.
38. Takeyama Y: Significance of apoptotic cell death in systemic complications with severe acute pancreatitis. *J Gastroenterol* 40: 1-10, 2005.
39. Hockings C, Anwari K, Ninnis RL, Brouwer J, O'Hely M, Evangelista M, Hinds MG, Czabotar PE, Lee EF, Fairlie WD, *et al*: Bid chimeras indicate that most BH3-only proteins can directly activate Bak and Bax, and show no preference for Bak versus Bax. *Cell Death Dis* 6: e1735, 2015.
40. Zhou Z, Lu X, Wang J, Xiao J, Liu J and Xing F: microRNA let-7c is essential for the anisomycin-elicited apoptosis in Jurkat T cells by linking JNK1/2 to AP-1/STAT1/STAT3 signaling. *Sci Rep* 6: 24434, 2016.
41. Bayram S, Kizilay G and Topcu-Tarlacalisir Y: Evaluation of the Fas/FasL signaling pathway in diabetic rat testis. *Biotech Histochem* 91: 204-211, 2016.
42. Yuan HJ, Han X, He N, Wang GL, Gong S, Lin J, Gao M and Tan JH: Glucocorticoids impair oocyte developmental potential by triggering apoptosis of ovarian cells via activating the Fas system. *Sci Rep* 6: 24036, 2016.
43. Yin XH, Yan JZ, Yang G, Chen L, Xu XF, Hong XP, Wu SL, Hou XY and Zhang G: PDZ1 inhibitor peptide protects neurons against ischemia via inhibiting GluK2-PSD-95-module-mediated Fas signaling pathway. *Brain Res* 1637: 64-70, 2016.
44. Feng Y, Yang Q, Xu J, Qian G and Liu Y: Effects of HMGB1 on PMN apoptosis during LPS-induced acute lung injury. *Exp Mol Pathol* 85: 214-222, 2008.
45. Xu C, Chen G, Yang W, Xu Y, Xu Y, Huang X, Liu J, Feng Y, Xu Y and Liu B: Hyaluronan ameliorates LPS-induced acute lung injury in mice via Toll-like receptor (TLR) 4-dependent signaling pathways. *Int Immunopharmacol* 28: 1050-1058, 2015.