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Original Article

Pre-B-cell colony enhancing factor regulates the alveolar epithelial sodium-water transport system through the ERK and AKT pathways

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Abstract: The sodium-water transport system is crucial for alveolar fluid clearance. The pulmonary edema caused by extracorporeal circulation is mainly due to increased alveolar capillary permeability and reduced fluid clearance. We previously demonstrated that pre-B-cell colony enhancing factor (PBEF) increases alveolar capillary permeability and inhibits the sodium-water transport system. However, the specific mechanism by which PBEF inhibits the sodium-water transport system is unclear. In this study, we used HPAEpiC (alveolar type II epithelial cells) to construct an anoxia-reoxygenation model and simulate the extracorporeal circulation microenvironment. The impact of PBEF on the expression of genes and proteins implicated in sodium transport and its effect on the activation status of the ERK, P38, and AKT signaling pathways were explored in HPAEpiC by real-time fluorescent PCR and western blotting. Specific inhibitors were employed to verify the role of the three signaling pathways in the regulation of the sodium-water transport system. PBEF was substantially non-toxic to alveolar epithelial cells, inhibited the expression of ENaC, NKA, and AQP1, and affected the ERK, P38, and AKT signaling pathways. ERK pathway inhibitors attenuated PBEF-induced downregulation of EnaC, NKA, and AQP1, and increased NKA activity. P38 pathway inhibitors only attenuated PBEF-induced suppression of NKA expression. AKT pathway inhibitors potentiated the inhibitory effects of PBEF, reducing EnaC, AQP1, and NKA expression, as well as NKA activity. In conclusion, PBEF inhibited the sodium-water transport system by activation of ERK and suppression of AKT signaling.

Keywords: PBEF, HPAEpiC, sodium-water transport system, ERK, AKT

Introduction

Cardiopulmonary bypass surgery (CPB) is a necessary procedure in open-heart surgery and is widely used in clinical practice, owing to continuous technological advancements. Pulmonary edema following CPB is an important, frequently lethal complication [1]. The main cause of pulmonary edema is an increase in alveolar capillary permeability and a decrease in fluid clearance from the alveoli [2]. Recent evidence suggests that the sodium-water transport system of alveolar epithelial cells is the main driving force for alveolar fluid clearance [3]. We previously found that PBEF negatively regulates this transport system [4]. However, the specific underlying mechanism is unclear.

PBEF was first cloned from a human peripheral blood lymphocyte cDNA gene library in 1994 [5]. It was initially found to be a growth factor promoting early B cell differentiation. Subsequent studies have found that functional PBEF, also named visfatin or NAMPT (nicotinamide phosphoribosyltransferase), is expressed in visceral fat cells [6]. PBEF plays a crucial role in the occurrence and development of acute lung injury [7]. Currently, three main functional roles are attributed to PBEF: as a growth factor, a cytokine, and a nicotinamide phosphoribosyltransferase [8]. In recent years, most studies have focused on PBEF role as a cytokine. PBEF plays an important role in various acute and chronic inflammatory reactions. PBEF upregulation significantly increases the expression of inflammatory factors including IL-1, TNF-α, IL-8, and IL-16 [9, 10]. These inflammatory factors may regulate the permeability of vascular endothelial cells, increase fluid exudation in the alveolar space [11], and inhibit the sodium-water transport system, thus reducing fluid clearance

from the cavity and aggravating pulmonary edema [12].

The alveolar epithelium is composed of type I (ATI) and type II cells (ATII). The number of ATI cells is relatively small, and is approximately half that of ATII cells. ATII cells are small in size, exhibit proliferative ability and differentiation potential, synthesize and secrete pulmonary surfactant (SP) and immunologically active proteins, and regulate the level of alveolar fluid. On the other hand, type I alveolar cells have no proliferative capacity, and are induced to differentiate by type II alveolar cells after injury [13]. The sodium-water transport system of lung epithelial cells is mainly composed of a sodium channel (ENaC), a sodium potassium ATPase (NKA), and a water channel (AQP) [14]. The sodium channel is a transmembrane protein formed by a combination of three homologous subunits, α-, β-, and γ-EnaC [15]. The sodium channels on the alveolar side transport sodium into the cells, and then sodium ions are pumped into the interstitial cells by the sodium-potassium ATPases located in the basal domain, and returned through the capillaries into the circulation. Water is co-transported with sodium [16]. During active sodium transport, 90% of the sodium ions are transported into the cells via ENaC. Therefore, ENaC can be considered as a major factor in the sodium-water transport system [17]. Several studies have shown that inflammatory factors, such as TNF-a and IL-1β, inhibit ENaC expression in lung epithelial cells by acting on the ERK1/2 signaling pathway [18], and PBEF increases the expression of these inflammatory factors in acute lung injury during cardiopulmonary bypass [19]. The study of Tilg and Moschen [20], as well as other reports, have shown that PBEF can participate in the pro-inflammatory response through the insulin signaling pathway. Moreover, another study has confirmed that insulin regulates ENaC-mediated alveolar fluid clearance in acute lung injury through the PI3K/Akt pathway [21]. Furthermore, PBEF induces insulin resistance in the A549 lung epithelial cells PBEF inhibits insulin/AKT signaling by promoting NAMPT-dependent IR translocation into the non-raft domains of A549 epithelial cells [22].

The specific mechanism by which PBEF regulates pulmonary edema and lung injury is currently unclear. Therefore, the purpose of this study was to establish a hypoxia-reoxygenation model to simulate the postoperative status of CPB, and the effect of PBEF on the sodium-water transport system of alveolar epithelial cells, as well as the underlying mechanism. The elucidation of molecular events critical for CPB-associated postoperative pulmonary edema may allow for the development of new strategies for disease prevention and treatment. This will help reduce the incidence of pulmonary dysfunction following CPB, and improve patient quality of life.

Materials and methods

Cell culture

HPAEpiC were purchased from ATCC (Manassas, VA, USA). Cells were cultured in α-MEM medium containing 10% FBS (Gibco, South America). Passages were performed at a cell density of approximately 80%.

Establishment of anoxic reoxygenation model

The Anaeropack package (MGC, Tokyo, Japan) was used to generate an oxygen-deficient environment. The original medium was removed and replaced with a low-sugar medium, and then the cells were placed in an anoxic culture box. After 12 h, normal serum containing FBS was supplied and cells were placed in an incubator in the presence of normal oxygen conditions.

Western blot

Cells were lysed with RIPA buffer (Beyotime Biotechnology, China) for 30 min and oscillated on ice. Lysates were then centrifuged at 12000 × g for 15 min at 4°C. The supernatant was extracted, added with 1/4 volumes of 5 × SDS, and heated at 100°C for 10 min, and 20 ml of sample were separated in a 10% SDS-PAGE gel. Next, proteins were transferred to a PVDF membrane at 300 mA for 70 min. The membrane was blocked with 5% skim milk for 1 h, incubated with the appropriate primary antibody (see below) at 4°C overnight, washed three times (10 min each) with TBST, incubated with the secondary antibody (CST, US) for 1 h at 25°C, and washed as above. Finally, the membrane was incubated for 1 min in ECL developer (Thermo, Berlin, Germany) and exposed with a TANON imager (Shanghai, China). The

Table 1. Primers used in this study

Gene and primer direction	Sequence
Human β-actin	
Forward	CATGTACGTTGCTATCCAGGC
Reverse	CTCCTTAATGTCACGCACGAT
Human ENaC	
Forward	CTTCGAGTTCTTCTGCAACAAC
Reverse	GTAGCTGAAGTACTCTCCGAAA
Human NKA	
Forward	ACAGACTTGAGCCGGGGATTA
Reverse	TCCATTCAGGAGTAGTGGGAG
Human SP	
Forward	TGTGTGCGAAGTGAAGGACG
Reverse	CTTTGAGACCATCTCTCCCGT

Note: ENaC, Epithelial sodium channel; NKA, Na^+-K^+-ATPase; SP, Surfactant protein.

primary antibodies used were ENaC, AQP1, β-actin (1:1000, Abcam, Cambridge, UK), AKT, ERK, P38 (1:1000, CST, US).

PCR

Total RNA was extracted according to the kit instructions (QIAGEN, Hilden, Germany) and the RNA concentration measured with NanoDrop (Thermo, Berlin, Germany). Each sample was diluted so as to obtain equal RNA concentrations. Two microliters of reverse transcriptase were added to 8 ml of RNA for reverse transcription. The reaction was carried out at 37°C for 15 min and then at 85°C for 5 s. Real-time quantitative PCR was then performed using TAKARA rapid PCR reagent (Shanghai, China). The expression of three water transport-related genes, i.e., ENaC-α, Na^+-K^+-ATPase, and AQP1, was measured by the Bio-Rad Quantitative Real-time PCR System (Hercules, CA, USA), and β-actin was used as an internal reference gene. The PCR primers used are listed in **Table 1** (Sangon Biotech, Shanghai, China).

CCK8 cell proliferation assay

The cells were digested, centrifuged, resuspended, and seeded in 96-well plates at 3,000 cells per well. After cell attachment, the appropriate concentration of PBEF was added, and incubations were carried out for 1, 3, or 5 days. One tenth of the CCK8 solution (Dojindo, Kumamoto, Japan) was added to medium containing 10% FBS, and incubated at 37°C for 2 h, followed by measurement of the absorbance at 450 nm.

Measurement of apoptosis by flow cytometry

Cells were seeded at 10^5 per well in a 6-well plate. After the cells attached to the bottom, different concentrations of PBEF were added. After 24 h of culture, cells were collected by trypsinization according to the instructions of the Annexin V-FITC Apoptosis Detection Kit (Beyotime, Shanghai, China). The binding solution, annexin V-FITC, and PI were sequentially added to approximately 100,000 cells, and a 20-min incubation was performed at room temperature in the dark. DxFLEX (Beckman coulter, Jiangsu, China) was used for detection. Finally, FlowJo software (US) was employed for data analysis and visualization.

Cell detection by immunofluorescence

Immunofluorescence was performed to ensure that type II alveolar epithelial cells did not differentiate into type I alveolar epithelial cells and verify cell identity. An antibody against the type II alveolar epithelial cell protein marker, SP (1:500; Abcam, Cambridge, UK), was used for staining. In brief, 5,000 cells per well were seeded in 24-well plates. Cells were fixed with 4% paraformaldehyde for 30 min, and then permeabilized with 0.5% Triton-100 (Sigma-Aldrich, Shanghai, China) for 30 min. Then, blocking was performed with 10% goat serum for 1 h, followed by overnight incubation with the primary antibody at 4°C. Then, cells were washed three times for 10 min with PBST, incubated for 1 h at room temperature with the secondary antibody, and washed as above. Finally, a 10-min incubation with DAPI was performed, followed by additional washing with PBST.

ATPase activity assay

The ATPase activity assay was performed using the Solarbio Na^+K^+-ATPase activity assay kit (Beijing, China) according to the kit instructions.

Mechanism of PBEF-mediated regulation of the alveolar epithelial sodium-water transport system

To determine whether PBEF was involved in the regulation of sodium-water transport system, after oxygen depletion and reoxygenation, 50

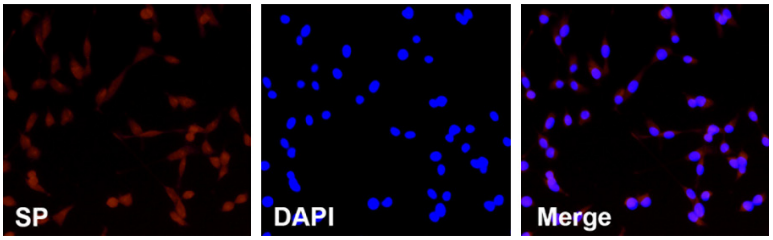

Figure 1. Detection of type II alveolar epithelial cell-specific marker SP using a fluorescently labelled antibody.

ng of PBEF were added to the HPAEpiC cell culture medium. Activation of the EKR, P38, and AKT pathways was evaluated by western blot. PD98059 (ERK inhibitor), SB203580 (P38 inhibitor) and LY294002 (AKT inhibitor) were also added to the medium to verify the influence of the respective pathways. The specific inhibitors were from Selleck (Houston, TX, USA).

Statistical analysis

All data were analyzed with Graphpad Prism 7 (GraphPad Software, Inc., La Jolla, CA, USA) and expressed as means ± standard error (SEM). Statistical comparisons between control and experimental groups were assessed by using one-way analysis of variance. *P* values < 0.05 were considered statistically significant.

Results

Characterization of alveolar type II epithelial cells

Immunofluorescence analysis of alveolar type II epithelial cells showed that the cells were round or fusiform, with large nuclei, and positive for the alveolar type II epithelial cell marker protein, SP (surface active substance binding protein; **Figure 1**). On the basis of these characteristics, we concluded that the cultured cells were the desired alveolar type II epithelial cells, and did not transform into alveolar type I epithelial cells.

PBEF cytotoxicity test

We tested the effect of different PBEF concentrations (10, 50, 100, 200, and 500 ng/ml) on cell proliferation and apoptosis. PBEF did not exhibit significant effects on either cell proliferation or apoptosis (**Figure 2**), indicating the absence of obvious cytotoxicity in this range of concentrations.

Effects of PBEF on the expression of proteins implicated in sodium transport

At 10 ng/ml, PBEF inhibited ENaC protein expression and AKT phosphorylation, and promoted P38 phosphorylation, whereas at concentrations of 50 ng/ml or higher it inhibited AQP1 protein expression and promoted ERK phosphorylation (**Figure 3**). The results were statistically significant ($P < 0.05$), and indicated that PBEF downregulated the expression of ENaC and AQP1, activated the ERK and P38 signaling pathways, and suppressed the AKT pathway.

Notably, the PBEF-induced decrease in the level of ENaC and AQP1 proteins was reversed by cell co-treatment with the ERK inhibitor (**Figure 4A** and **4B**), whereas it was exacerbated by the AKT inhibitor. Moreover, the P38 inhibitor had little effect on ENaC and AQP1 expression. Each inhibitor suppressed the corresponding signaling pathway ($P < 0.05$; **Figure 4C** and **4D**).

PBEF effects on the transcription of genes implicated in sodium transport

The effect of PBEF on sodium-water transport-related genes was confirmed by PCR and the involved signaling pathways were evaluated by using specific inhibitors. In the model group (hypoxia reoxygenation), ENaC gene expression decreased and was further suppressed by treatment with PBEF, whereas cell exposure to the ERK inhibitor (PD98059) reversed PBEF effect. On the other hand, the AKT inhibitor, LY294002, exacerbated PBEF-induced suppression of ENaC gene expression. The P38 inhibitor (SB203580) showed no significant effects, consistently with the results of western blot (**Figure 5A**). Moreover, PBEF reduced NKA expression, whereas both the ERK inhibitor and the P38 inhibitor partially reversed this effect. On the other hand, the AKT inhibitor further reduced NKA expression (**Figure 5B**). PBEF also decreased SP expression, and the ERK inhibitor substantially prevented this effect. The P38 inhibitor slightly suppressed PBEF-induced SP downregulation. AKT blockade slightly aggravated the inhibitory effects of PBEF. The results were statistically significant ($P < 0.05$; **Figure 5C**). In conclusion, PBEF reduced the expres-

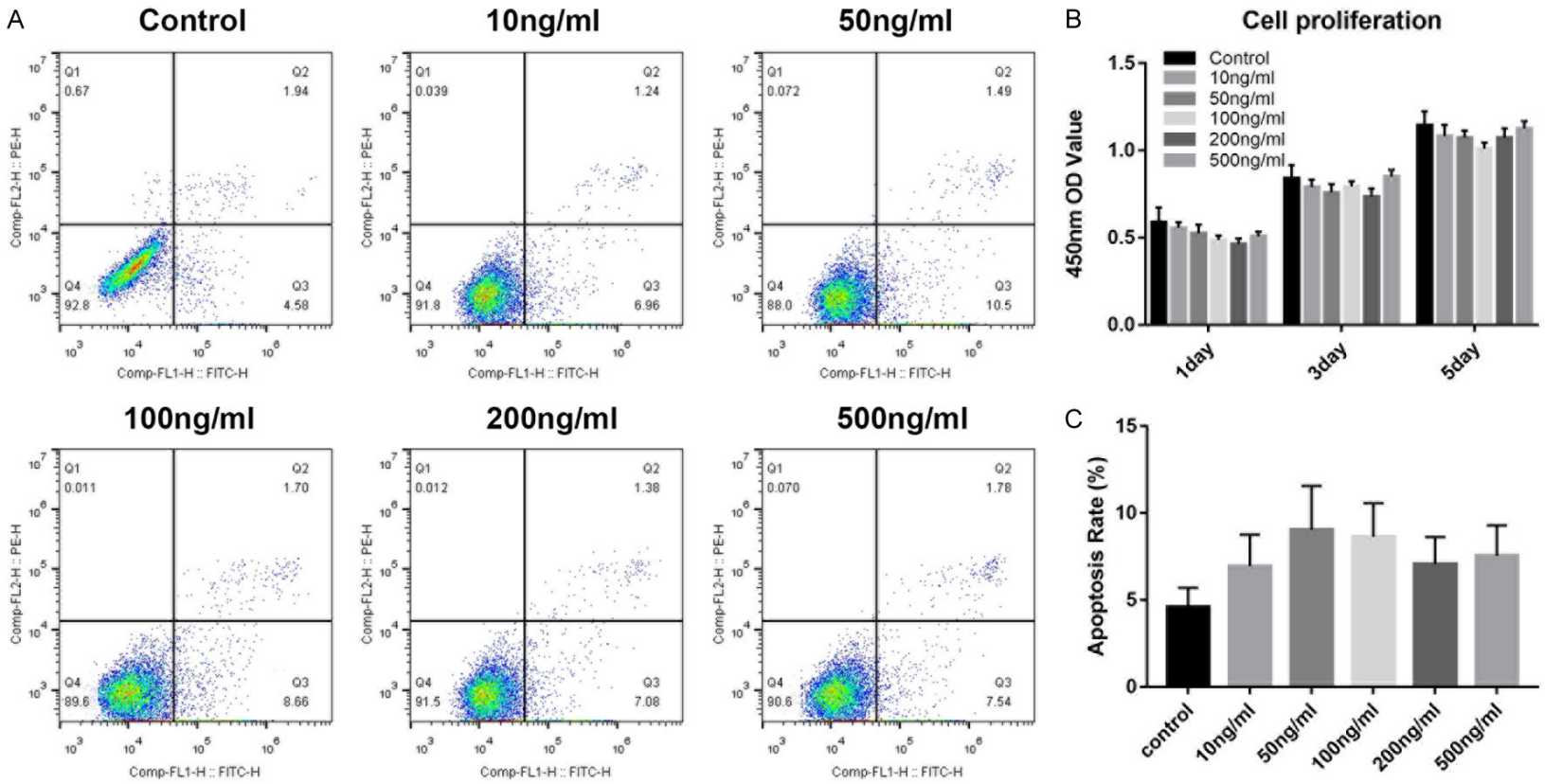

Figure 2. PBEF cytotoxicity assay. (A) Apoptotic rate of alveolar type II epithelial cells measured by flow cytometry. HPAEpiC were treated with 10, 50, 100, 200 and 500 ng/ml PBEF for 24 h. There was no treatment as a control. The number of each quadrant is expressed as a percentage. The data represent one of three independent experiments. (B) CCK8 assay for detection of cell proliferation. HPAEpiC were also stimulated by adding different concentrations of PBEF, and the value of CCK8 was measured after 1, 3, and 5 days of culture. Normally cultured HPAEpiC served as a control group. Data shown are means ± SD (n = 3). (C) Analysis of the apoptosis data shown in (A). Results are presented as mean ± SD (n = 3).

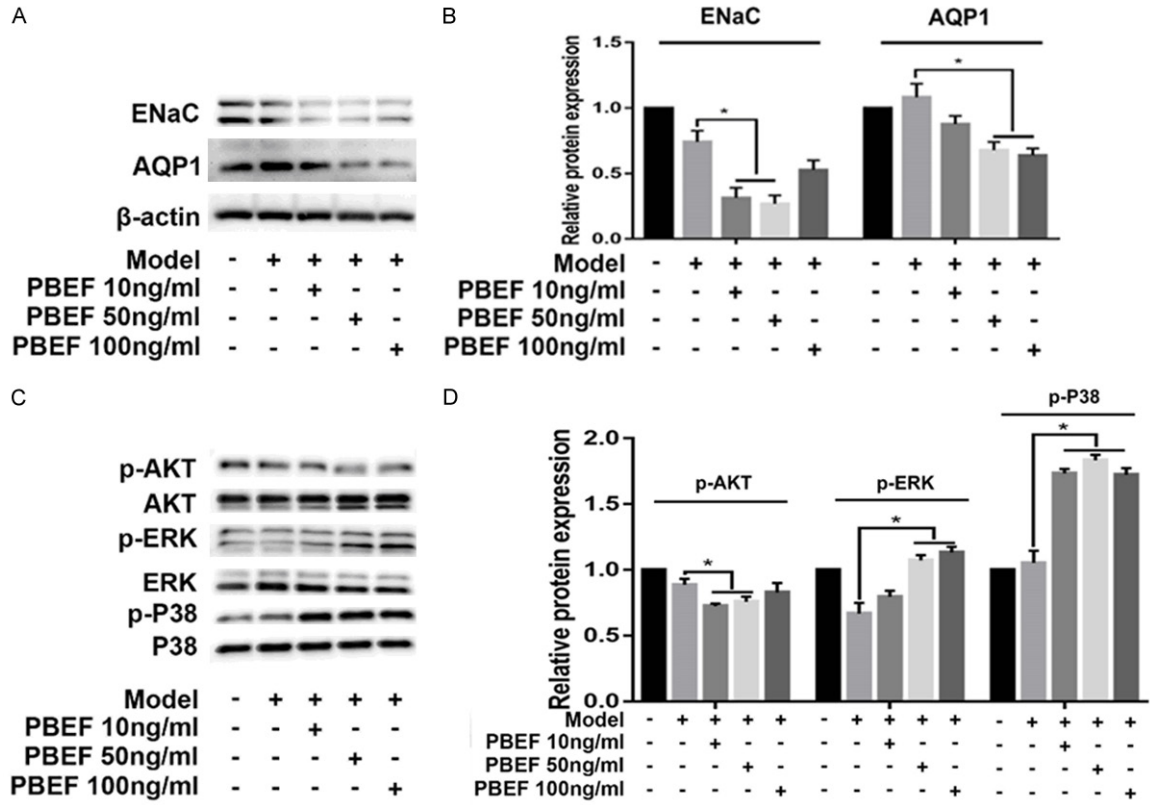

Figure 3. Effect of PBEF on protein expression. (A) Protein bands of ENaC and AQP1. HPAEpiC were subjected to hypoxia-reoxygenation and stimulated with 10, 50 and 100 ng/ml PBEF for 24 h. Untreated HPAEpiC served as a control group. (B) Histograms obtained from data shown in (A). β-actin was used for normalization. Data shown are means ± SD (n = 3). *, $P < 0.05$. (C) Electrophoretic bands of the signaling proteins AKT, ERK, and P38. HPAEpiC was hypoxic reoxygenated and stimulated with 10, 50 and 100 ng/ml of PBEF for 15 min. Untreated HPAEpiC served as a control group. (D) Histograms obtained from data shown in (C). For each signaling protein, the extent of phosphorylation was calculated by dividing the phosphorylated protein by the total protein, and then normalizing to the control cells. Data shown are means ± SD (n = 3). *, $P < 0.05$.

sion of the sodium ion channel and the sodium potassium pump in the sodium-water transport system, and downregulated surface-active substance in alveolar type II epithelial cells. Furthermore, whereas the ERK inhibitor attenuated these effects, the P38 inhibitor only attenuated PBEF effects on sodium potassium pump and surfactant. Finally, similarly to PBEF, the AKT inhibitor downregulated the expression of sodium ion channel, sodium potassium pump, and surface-active substance.

PBEF effects on Na^+-K^+-ATPase activity

Hypoxia-reoxygenation reduced the activity of NKA, which was also significantly inhibited by treatment with different concentrations of PBEF (10 ng/ml, 50 ng/ml, 100 ng/ml). The ERK and AKT inhibitors affected NKA activity ($P < 0.05$), while the P38 inhibitor did not exert significant effects (**Figure 6**). These findings indicated that PBEF inhibited NKA activity and that this effect was mediated by the ERK and AKT pathways.

Discussion

Acute lung injury is one of the possible complications of cardiopulmonary bypass, and seriously endangers the life of patients. Decreased body temperature during extracorporeal circulation, blood cell damage, ischemia, endotoxemia, and surgical trauma can lead to systemic inflammatory response syndrome [23]. Acute lung injury following cardiopulmonary bypass is characterized by increased alveolar capillary permeability, endothelial cell injury, lung tissue and alveolar neutrophil infiltration, congestion, and edema, followed by a series of physiological changes [24]. Pulmonary edema is an im-

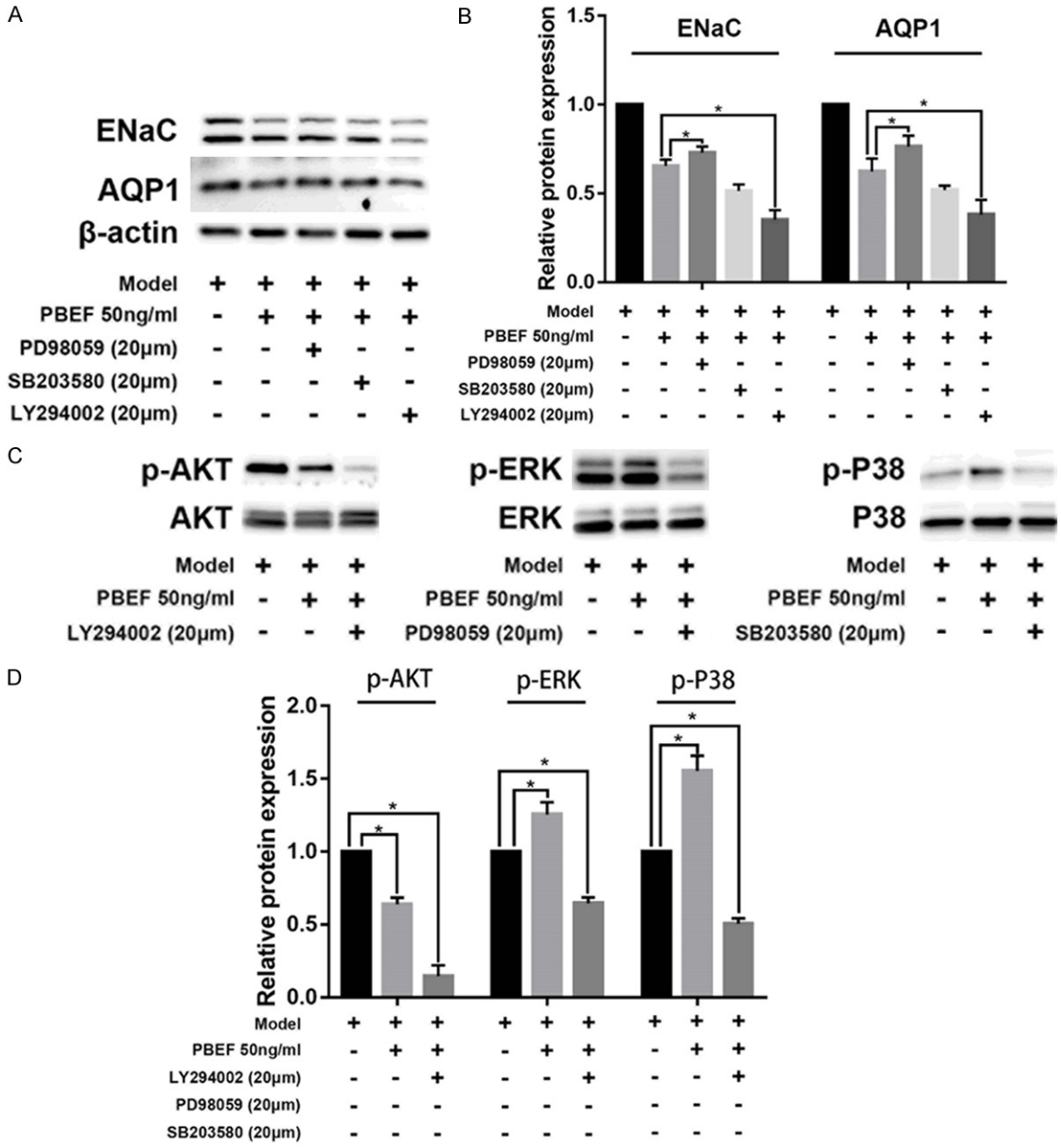

Figure 4. PBEF signaling pathway. (A) ENaC and AQP1 protein bands. HPAEpiC were subjected to hypoxia-reoxygenation in the presence of PD98059, SB203580, and LY294002 inhibitors for 12 h, and then stimulated with 50 ng/ml PBEF for 24 h. Hypoxic-reoxygenated HPAEpiC were used as controls. (B) Histograms obtained from data in (A). β-actin was used for normalization and then standardized to control cells. Data are means ± SD (n = 3). *, $P < 0.05$. (C) Protein bands corresponding to the signaling proteins AKT, ERK, and P38. HPAEpiC were exposed to hypoxia-reoxygenation in the presence of PD98059, SB203580 and LY294002 inhibitors for 12 hours, and then incubated with 50 ng/ml PBEF for 15 min. Hypoxic-reoxygenated HPAEpiC were used as a control group. (D) Histograms obtained from data in (C). For each signaling protein, the proportion of phospho-protein was calculated by dividing the phosphorylated protein by the total protein, and then normalized to the control group. Data shown are means ± SD (n = 3). *, $P < 0.05$.

portant manifestation of acute lung injury. A previous study found that pulmonary edema is mainly determined by increased capillary permeability and involves the sodium-water transport system [25]. Enhanced capillary permeability leads to an increase in alveolar fluid, which is the basis for the formation of pulmonary edema, and alterations in the sodium-

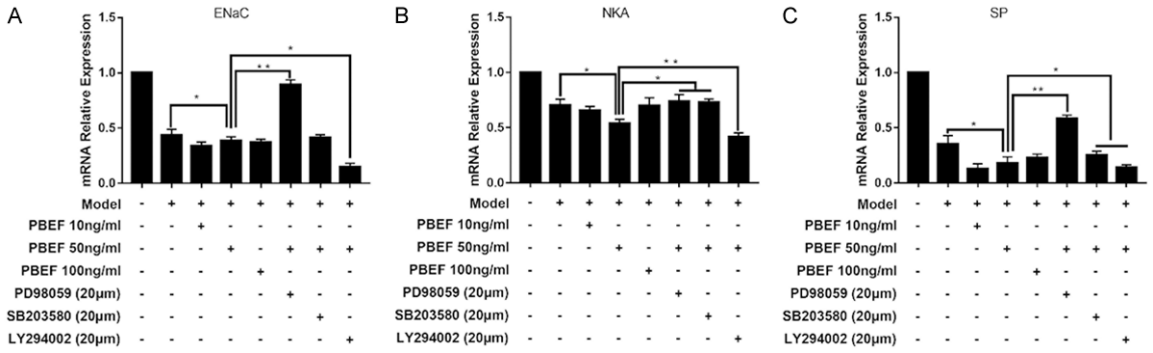

Figure 5. PCR detection. Different concentrations of PBEF and various specific inhibitors were used, depending on whether or not hypoxia-reoxygenation was applied, and PCR was performed 3 days after treatment. A. Expression of the ENaC gene. B. Expression of the NKA gene. C. Expression of the SP gene. Data shown are means ± SD (n = 3). * denotes significant difference vs cells treated with PBEF (50 ng/ml). *, $P < 0.05$.

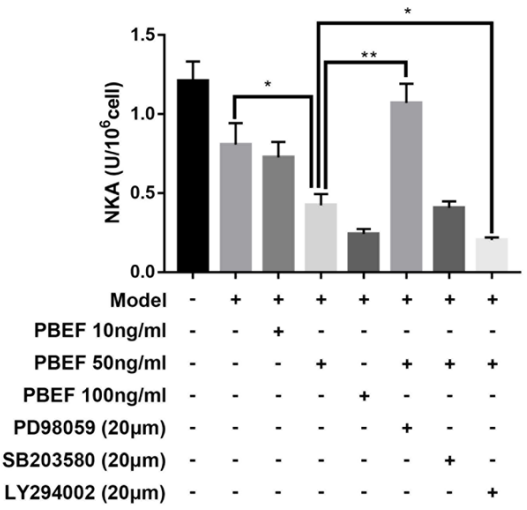

Figure 6. Na^+-K^+-ATPase activity after treatment of hypoxic-reoxygenated alveolar epithelial cells with different concentrations of PBEF and specific inhibitors. According to whether or not hypoxia-reoxygenation treatment was carried out, different concentrations of PBEF and specific inhibitors were added. Na^+-K^+-ATPase activity was measured after 12 h of treatment. Data shown are means ± SD (n = 3). *denotes significant difference vs cells treated with PBEF (50 ng/ml). *, $P < 0.05$. **, $P < 0.01$.

water transport system may affect the clearance of fluid from alveoli, further promoting pulmonary edema. Our previous studies showed that PBEF increases the permeability of pulmonary vascular endothelial cells and inhibits the sodium-water transport system of alveolar epithelial cells [4, 26]. However, the mechanism by which PBEF exerts this inhibition is still unclear. This study demonstrated that PBEF inhibited the sodium-water transport system of alveolar epithelial cells by activating the ERK signaling pathway and inhibiting the AKT signaling pathway.

Undesired effects of extracorporeal circulation may include body hypoxia and reperfusion injury. The hypoxia-reoxygenation model consists in the initial exposure of cells to anoxic conditions in the presence of low-glucose serum-free medium, followed by restoration of the aerobic state [27, 28]. The model effectively simulates the environmental changes experienced by cells during cardiopulmonary bypass. We previously used a hypoxia-reoxygenation model to study the sodium-water transport system of alveolar epithelial cells after cardiopulmonary bypass [4]. In this study, hypoxia-reoxygenation was used *in vitro* to analyze the changes in the cellular sodium-water transport system that may accompany cardiopulmonary bypass. In addition, the effectiveness of the hypoxia-reoxygenation model was further confirmed, providing a reference for future *in vitro* experiments focusing on extracorporeal circulation.

PBEF stimulates the expression of inflammatory factors, acts as a rate-limiting enzyme for the synthesis of nicotinamide adenine dinucleotide, and has an islet-like function in combination with the insulin receptor [28]. PBEF is highly expressed in lung tissue after cardiopulmonary bypass and is a marker of CBP-related acute lung injury [29]. We used different concentrations of PBEF to stimulate hypoxia-reoxygenation of alveolar type II epithelial cells and evaluate cell proliferation and apoptosis under such conditions. No significant PBEF cytotoxic-

ity was detected in alveolar type II epithelial cells. A previous study demonstrated that PBEF overexpression promotes early apoptosis in human pulmonary microvascular endothelial cells [30]. This inconsistency with our results is probably due to the use of different cell lines and PBEF concentrations.

Surfactant binding protein (SP) has the function to reduce the surface tension of the alveoli, stabilizing alveolar size and protecting alveolar epithelial cells from inflammatory damage [31]. Decreased SP expression makes lung epithelial cells more susceptible to changes in the surrounding microenvironment. Notably, real-time quantitative PCR analysis demonstrated that PBEF inhibited SP gene expression, which may have an effect on the sodium-water transport system of alveolar epithelial cells.

ENaC is the major pathway and a rate-limiting factor in the highly selective transfer of Na^+ from the apical to the basal side of epithelial cells [32-34]. We demonstrated an inhibitory effect of PBEF on ENaC expression in alveolar II epithelial cells, at both the gene and protein levels. Zhao *et al*. have shown that knocking out IL-17 not only reduces the inflammation of lung tissue after acute lung injury, but also reduces ENaC protein loss and promotes alveolar fluid clearance [35]. Moreover, Roux and collaborators demonstrated that IL-1β reduces the expression of ENaC in the alveolar epithelium via the P38 signaling pathway [36]. Both IL-17 and IL-1β are inflammatory factors, and PBEF promotes inflammation. Thus, PBEF may inhibit ENaC through inflammation. Looney reported that reduced expression of the α subunit of Na, K-ATPase is associated with decreased maximum lung clearance [37]. Overexpression of Na, K-ATPase in the lung improves lung clearance and prevents pulmonary edema. We found that PBEF inhibited Na, K-ATPase activity and reduced its transcription. Among the twelve identified aquaporins (AQP1-12), alveolar type II epithelial cells mainly express AQP1. In a rat model of lung injury, administration of LPS reduced the expression of AQP1 and AQP5 and induced pulmonary edema [38]. Moreover, pulmonary edema was aggravated when AQP1 and AQP5 were blocked [39]. These findings indicate a role of AQP in edema prevention and protection from lung injury. Our experiments demonstrated that PBEF inhibited the expression of AQP1 in a concentration-dependent manner. In summary, our data indicated that PBEF exerted an inhibitory effect on the sodium-water transport system (ENaC, NKA, AQP) of alveolar type II epithelial cells, and suggested that changes in the interactions between these components may aggravate edema and reduce alveolar fluid clearance.

The specific mechanism by which PBEF regulates the sodium-water transport system in alveolar epithelial cells is unclear. We previously showed that PBEF increases the permeability of pulmonary vascular epithelial cells through the MAPK signaling pathway [19]. Our study also demonstrated that PBEF can activate the ERK and P38 pathways and inhibit the phosphorylation of AKT. Fan and coworkers demonstrated that the pro-inflammatory effect of PBEF depends on the PBEF-MAPK (P38, ERK)-NF-κB pathway [40]. PBEF was found to increase fibroblast motility by activating the P38 signaling pathway [41]. Another study explored the relationship between PBEF and insulin signaling, and found that the addition of PBEF to insulin-treated cells resulted in decreased AKT phosphorylation [42]. PBEF inhibits insulin signaling by promoting insulin receptor exclusion from raft domains, thereby inactivating its entry into A549 epithelial cells. These studies are consistent with our results. By using pathway-specific inhibitors, we observed that ERK inhibition attenuated the PBEF-induced downregulation of ENAC, NAK, and AQP, whereas P38 blockade only alleviated the PBEF-induced suppression of NKA activity, and AKT inhibition exacerbated the PBEF-induced inhibition of ENaC, NAK, and AQP. Similarly, previous studies showed that the activation of the ERK pathway inhibits ENaC, NAK, and AQP [37, 43, 44]. Moreover, it has been demonstrated that activation of the AKT pathway promotes the expression of ENaC, NAK, and AQP [45-47]. Regarding the P38 signaling pathway, we found that PBEF activated the P38 pathway, and that changes in the sodium-water transport system were not dependent on the P38 pathway. The functional significance of the PBEF-induced activation of the P38 signaling pathway needs to be further investigated.

In conclusion, PBEF regulated the alveolar epithelial sodium-water transport system through the ERK and AKT pathways. This study provides

a new potential molecular target for the prevention and treatment of pulmonary edema after cardiopulmonary bypass.

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Disclosure of conflict of interest

None.

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