Biochemical and Biophysical Research Communications 440 (2013) 707–713

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Activation of PRMT1 and PRMT5 mediates hypoxia- and ischemia-induced apoptosis in human lung epithelial cells and the lung of miniature pigs: The role of p38 and JNK mitogen-activated protein kinases

Seul Ki Lim [a,1], Yong Wun Jeong [b,1], Dong Il Kim [a], Min Jung Park [a], Joo Hee Choi [a], Se Un Kim [a], Seong Soo Kang [a], Ho Jae Han [b], Soo Hyun Park [a,*]

[a] College of Veterinary Medicine, Chonnam National University, Gwangju 500-757, South Korea
[b] College of Veterinary Medicine, Seoul National University, Seoul, South Korea

ARTICLE INFO

Article history:
Received 25 September 2013
Available online 9 October 2013

Keywords:
Hypoxia
Lung epithelial cells
PRMT1, 5
Apoptosis
Mitogen activated protein kinase

ABSTRACT

Severe hypoxic and ischemic injury leads to primary graft dysfunction after lung transplantation. Arginine methylation, which is responsible for the regulation of a variety of biological functions, is mediated by protein arginine methylation transferases (PRMTs). This study examined the role of hypoxia in PRMT activation in A549 human lung epithelial cells, as well as the role of ischemia in PRMT activation in the lung of miniature pigs. In A459 cells, hypoxia increased the expression of PRMT1 and PRMT5, and overexpression of PRMT1 and PRMT5 induced apoptosis. The transfection of PRMT1 and PRMT5 small interfering RNA (siRNA) prevented hypoxia-inducible factor (HIF)-1α expression and apoptosis in A549 cells. Hypoxia-induced expression of PRMT1 and PRMT5 was blocked by p38 and JNK mitogen-activated protein kinase (MAPK) inhibitors, but not by an inhibitor of extracellular signal-regulated kinases (ERK) 1/2. In the lungs of miniature pigs, ischemia stimulated PRMT1 and PRMT5 expression and induced phosphorylation of p38 MAPK (p-p38), phosphorylation of JNK (p-JNK), and apoptotic molecules. These results demonstrate that PRMT1 and PRMT5 are involved in hypoxia and ischemia-induced apoptosis via p-p38 MAPK and p-JNK in *in vitro* and *in vivo* models.

1. Introduction

Prolonged hypoxia induces ischemic damage in lung tissue [1], and severe ischemic injury leads to primary graft dysfunction after lung transplantation [2]. The lung alveolar epithelium is critical for normal lung function, as it plays a central role in gas exchange and fluid transport and is very sensitive to ischemia [3]. Thus, *in vitro* studies often induce hypoxia in cultured human lung epithelial cells to study ischemia-related cellular and molecular changes [4].

Arginine methylation, which is responsible for the regulation of a variety of biological functions, is mediated by protein arginine methylation transferases (PRMTs) [5]. Type I PRMTs, including PRMT 1, 3, and 4, catalyze conversion of arginine to a monomethylarginine (MMA) intermediate, which is subsequently converted to asymmetric dimethylarginine (ADMA). Type II PRMTs, such as PRMT5, catalyze the formation of symmetric dimethylarginine (SDMA) [6]. However, the role of PRMTs in hypoxia and ischemia has not been clearly elucidated.

Diverse signaling molecules may be involved in the pathogenesis of lung diseases. Mitogen-activated protein kinases (MAPKs) are serine/threonine protein kinases that regulate proliferation, differentiation, cellular stress responses, and apoptosis [7]. Activation of MAPKs, including extracellular signal-regulated kinases 1 and 2 (ERK 1/2), p38 MAPK, and the stress activated protein kinase (SAPK)/c-Jun NH2-terminal kinase (JNK), has been implicated in the pathogenesis and pathophysiology of lung injury [8], and hypoxia-inducible factor (HIF)-1α is involved in airway obstruction.

Apoptosis is a complex process that can be initiated by an intrinsic mitochondria-dependent pathway and an extrinsic receptor-induced pathway. Bcl-2 and Bax proteins are intracellular membrane-bound proteins that have antagonistic effects; Bcl-2 extends cellular survival, and Bax promotes cell death following an apoptotic stimulus. Caspase-3 is a critical executioner of apoptosis, and an increase in caspase-3 cleavage is associated with lung injury [9]. Poly ADP ribose polymerase (PARP) is also involved in apoptosis during lung damage [10]. The role of apoptosis in lung

* Corresponding author. Address: Bio-therapy Human Resources Center, Department of Veterinary Physiology, College of Veterinary Medicine, Chonnam National University, Gwangju 500-757, South Korea. Fax: +82 62 530 2809.
E-mail address: parksh@chonnam.ac.kr (S.H. Park).
[1] The first two authors contributed equally to this work.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.09.136

ischemia was suggested by Zhang et al., who reported that lung ischemia–reperfusion injury was mediated by autophagy [11].

Pigs are a suitable animal model to study lung injury because of easier animal husbandry and organs with comparatively similar anatomical and physiological features to humans [12–14]. The elucidation of hypoxic and ischemic pathophysiology in human lung epithelial cells and the lungs of miniature pigs would be clinically useful for applications such as transplantation and xenotransplantation. Therefore, this study examined the role of hypoxia in PRMT activation in A549 human lung epithelial cells, as well as the role of ischemia in PRMT activation in the lung of miniature pigs, to address the hypothesis that PRMTs interact with MAPKs to mediate hypoxia-induced apoptosis during lung injury.

2. Materials and methods

2.1. Materials

Dulbecco's Modified Eagle's Medium (DMEM) and Ham's nutrient mixture F-12 (F-12) were purchased from Life Technologies (Gibco BRL, Grand Island, NY, USA). Fetal bovine serum (FBS) was purchased from HyClone (Logan, Utah, USA). Formaldehyde (FA; 36.5–38% in water, FW 30.03) was obtained from Sigma–Aldrich (St. Louis, MO, USA). Bcl-2, β-actin, and HIF-1α antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Caspase 3, CHOP, phospho-p38, total-p38, phospho-p44/42, total-p44/42, and PARP antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Bax antibody was purchased from Chemicon International (Billerica, MA, USA). GFP antibody was purchased from Abcam Ltd. (Cambridge, UK). PRMT1 and PRMT5 antibody were supplied by Dr. Mark T. Bedford. Anti-rabbit IgG, anti-mouse IgG, and anti-goat IgG were purchased from Cell Signaling Technology (Danvers, MA, USA). SB 203580, SP 600125, and PD 98059 were purchased from Enzo Life Sciences (AG, Switzerland). M-PER Mammalian protein extraction reagent was purchased from Thermo Scientific (Pierce, Fockford, IL, USA). All reagents were of the highest purity commercially available.

2.2. Cell cultures

The lung epithelial cell line A549 was obtained from the American Type Culture Collection (ATCC, Rockville, MD). The culture medium for A549 cells was DMEM/Ham's F-12 (1:1) supplemented with 10% FBS. Stock cultures of A549 cells were subcultured once a week (split ratio 1:4). A549 cells were grown to confluence in 60 mm dishes in DMEM/Ham's F-12 (DMEM, Gibco; F-12 Nutrient Mixture, Gibco; obtained without glucose and then supplemented by adding glucose to the appropriate concentrations) with 15 mM HEPES buffer, 10% FBS, 5.5 mM glucose, 0.35% additional sodium bicarbonate, 2.5 mM L-glutamine, and 1% penicillin/streptomycin

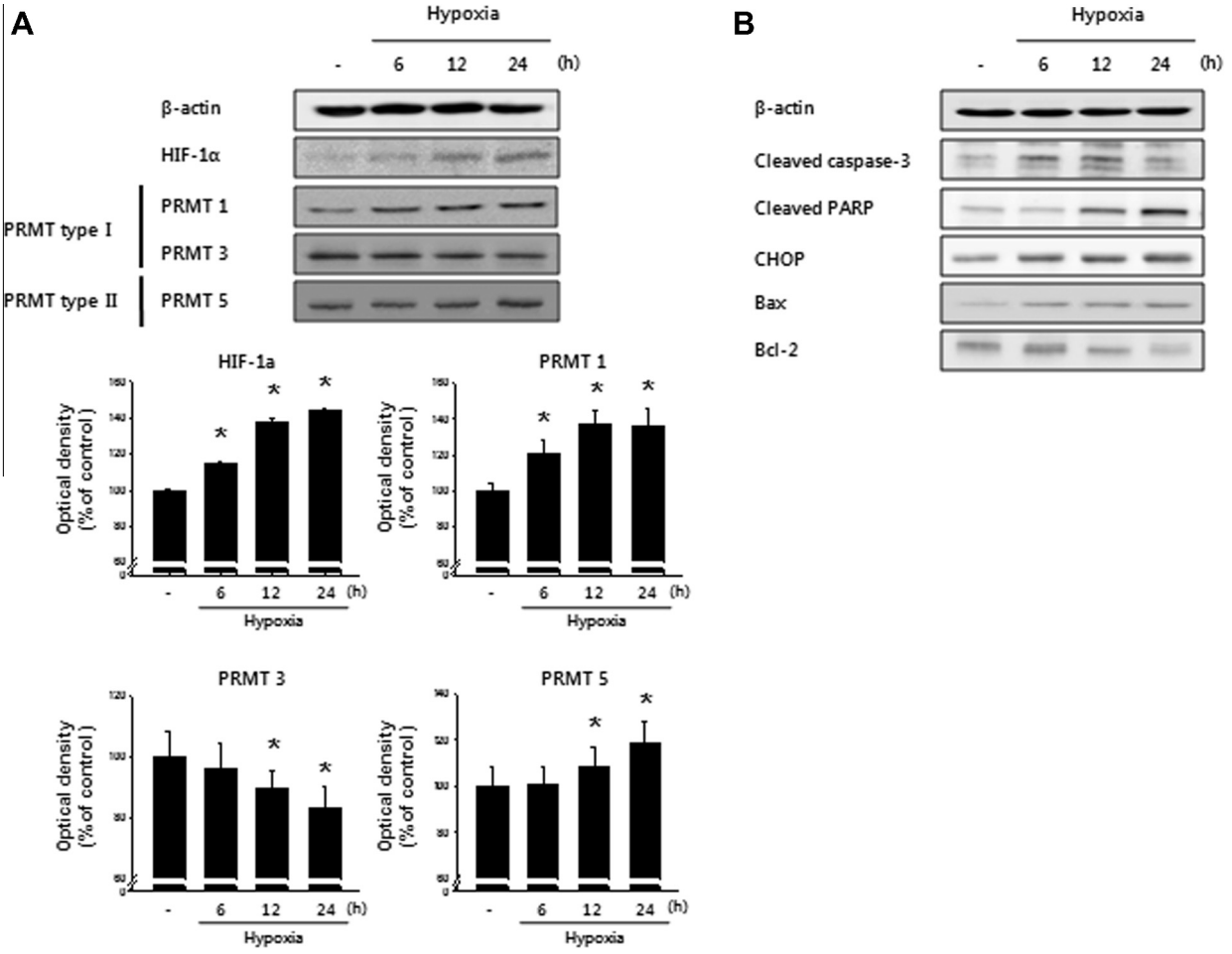

Fig. 1. Effect of hypoxia on the expression level of PRMT isoforms and apoptosis regulatory proteins in A549 cells. (A) The cells were exposed to hypoxia for 0–24 h. And then, PRMT1, PRMT3, and PRMT5 were detected by Western blotting as described in Section 2. (B) Cleaved caspase-3, cleaved PARP, CHOP, Bax, and Bcl-2 were detected by Western blotting under the same experimental conditions. The lower panels represent the mean ± S.E. of three independent experiments for each condition as determined from densitometry relative to β-actin. $^{*}p < 0.05$ vs. control.

at 37 °C. The cells were maintained at 37 °C in 5% CO_2 in a humidified cell culture incubator. The media was changed every other day. Passaged cells were plated to yield near-confluent cultures at the end of the experiments.

2.3. Hypoxic treatment of A549 cells

A549 cells were washed twice with phosphate-buffered saline (PBS) and the medium was exchanged with fresh DMEM supplemented with 5% FBS. Experiments were performed in a modular incubator chamber (Billups-Rothenberg, San Diego, CA, USA) at 37 °C for designed time under normoxic (92.3% air and 5.5% CO_2) or hypoxic (2.2% O_2, 5.5% CO_2, and 92.3% N_2) conditions at a flow rate of 20 L/min. The chamber was purged with gas, sealed, and placed in a conventional incubator at 37 °C.

2.4. Animals

The studies were performed using healthy Yucatan miniature pigs, all of which were purchased from PWG Genetics (Korea). Prior to their purchase, the pigs were physically examined and confirmed to be healthy. The pigs were housed indoors in individual cages, fed dry pig food freely and provided water.

2.5. Lung ischemia induction and surgical preparation

Twelve miniature pigs were used in this study. The bodyweight was 37–45 kg (mean 41 kg) and their ages 6–8 months. All animals received humane care in compliance with the 'Guide for the care and use of Laboratory Animals' published by National Institute of Health. After fasting for 12 h, the pigs were i.m. injected with atropine (atropine sulfate, Tai yu, Taiwan), followed by tiletamine and zolazepam 1:1 mixture (Zoletil, Virbac, France) 0.55–0.80 mg/kg i.m. injection to achieve the initial stage of anesthesia. Gas anesthesia with isoflurane 2.5–4.5% was used for induction, followed by 1–3% in maintenance after completion of intubation through the tracheotomy. The animals were placed in supine position, intubated through cervical tracheostomy, and mechanically ventilated with a tidal volume of 20 mL/kg at a rate of 15–20 per min using an ADS 1000 ventilator (Engler Engineering, Hialeah, Fla). An arterial line and a Swan-Ganz catheter were inserted through the left carotid artery and jugular vein. Then the miniature pig was placed on right decubitus position for surgical procedure. Heart rate, arterial, pulmonary artery and central venous pressures, and arterial oxygen saturation were monitored continuously. Dynamic lung compliance and pulmonary vascular resistance (PVR) (Sulla 808, Lubek, Germany), arterial blood gas analysis (ABL3 Radiometer, Copenhagen, Denmark) were measured at baseline and every 30 min thereafter until the termination of the experiment.

Three Miniature pigs underwent a left thoracotomy at the sixth intercostals space without ischemia of the lung and lung tissues in the left lower lobe were dissected and harvested. In each group, three miniature pigs underwent a left thoracotomy and a 30 min, 2 h, and 4 h ischemia of the left lung by means of clamping the ipsilateral pulmonary arterial trunk and the bronchoesophageal artery arising from the descending thoracic aorta.

2.6. Isolation of protein from tissue

Pig lung sections from normoxia and ischemia-treatment pig was isolated, as previously described and were re-suspended in tissue lysis buffer [10 mM Tris, 2 mM EDTA, 150 mM NaCl, 1% (v/v) Triton X-100 and 1.5% (v/v) NP40] with protease inhibitors added. Use the homogenizer to disperse tissues into the buffer. Use for about 5 s and then place the samples on ice again to keep it from getting warm. Wash the homogenizer between samples with PBS. When samples are in solution, centrifuge at 17,000 rpm for 15 min at 4 °C. The supernatant was saved as the whole tissue extraction.

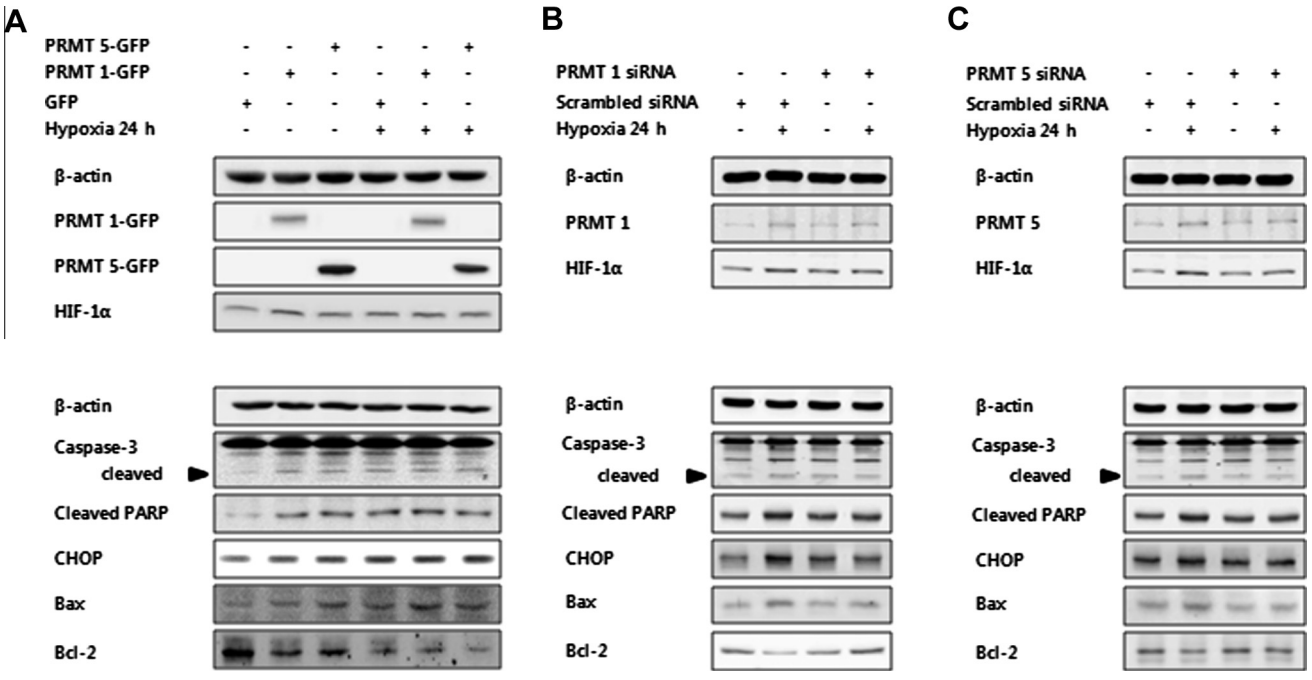

Fig. 2. Effects of PRMTs overexpression and siRNAs on hypoxia-induced stimulation of HIF-1α and apoptosis. (A) The transfection of PRMT1-GFP or PRMT5-GFP plasmid DNA influenced the expression of HIF-1α and apoptosis regulatory molecules. Overexpression of PRMT1 or PRMT5 increased the expression of the pro-apoptotic proteins, cleaved caspase-3, cleaved PARP, CHOP, and Bax and decreased expression of the anti-apoptotic protein, Bcl-2. (B and C) The knock down of PRMT1 and PRMT5 expression using PRMT1 or PRMT5 siRNA prevents the increased expression of HIF-1α and pro-apoptotic regulatory molecules. Each example shown is a representative of four experiments.

2.7. Western blotting

The resuspended cells in M-PER lysis buffer were lysed mechanically on ice by vortex. The protein level was quantified using the Bradford procedure. The cell homogenates were separated by 8–13% SDS–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to an enhanced nitrocellulose membrane. The blots were then washed with TBST (10 mM Tris–HCl, pH 7.6, 150 mM NaCl, 0.05% Tween-20), blocked with 5% skim milk powder in TBST for 1 h and incubated for 15 h at 4 °C with the primary antibody at the dilutions recommended by the supplier. The membrane was then washed with TBST, and the secondary antibodies conjugated to horseradish peroxidase were incubated for 1 h at room temperature. The bands were visualized using Amersham ECL™ Western Blotting Detection Reagents (GE Healthcare, UK) on LAS (Las 4000 mini, GE, USA).

2.8. Plasmid & transfection

PRMT1-GFP and PRMT5-GFP plasmid DNA were supplied by Dr. Mark T. Bedford. The A549 cell cultures were established and stabilized 24 h before they were transfected with the PRMT1-GFP, PRMT5-GFP or GFP-empty vector (E.V.) constructs. The culture medium was exchanged, and PRMT1-GFP, PRMT5-GFP or GFP-E.V. plasmid (3 μg) was transfected into the A549 cells using Gene-Expresso Max transfection reagent (Excellgen, Rockville, MD, USA). After 24 h, the transfected cells were incubated under hypoxic conditions for 24 h.

2.9. PRMT1 and PRMT 5 small interfering RNA (siRNA) transfection

PRMT1 and PRMT5 small interfering RNA (siRNA) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). A549 cells

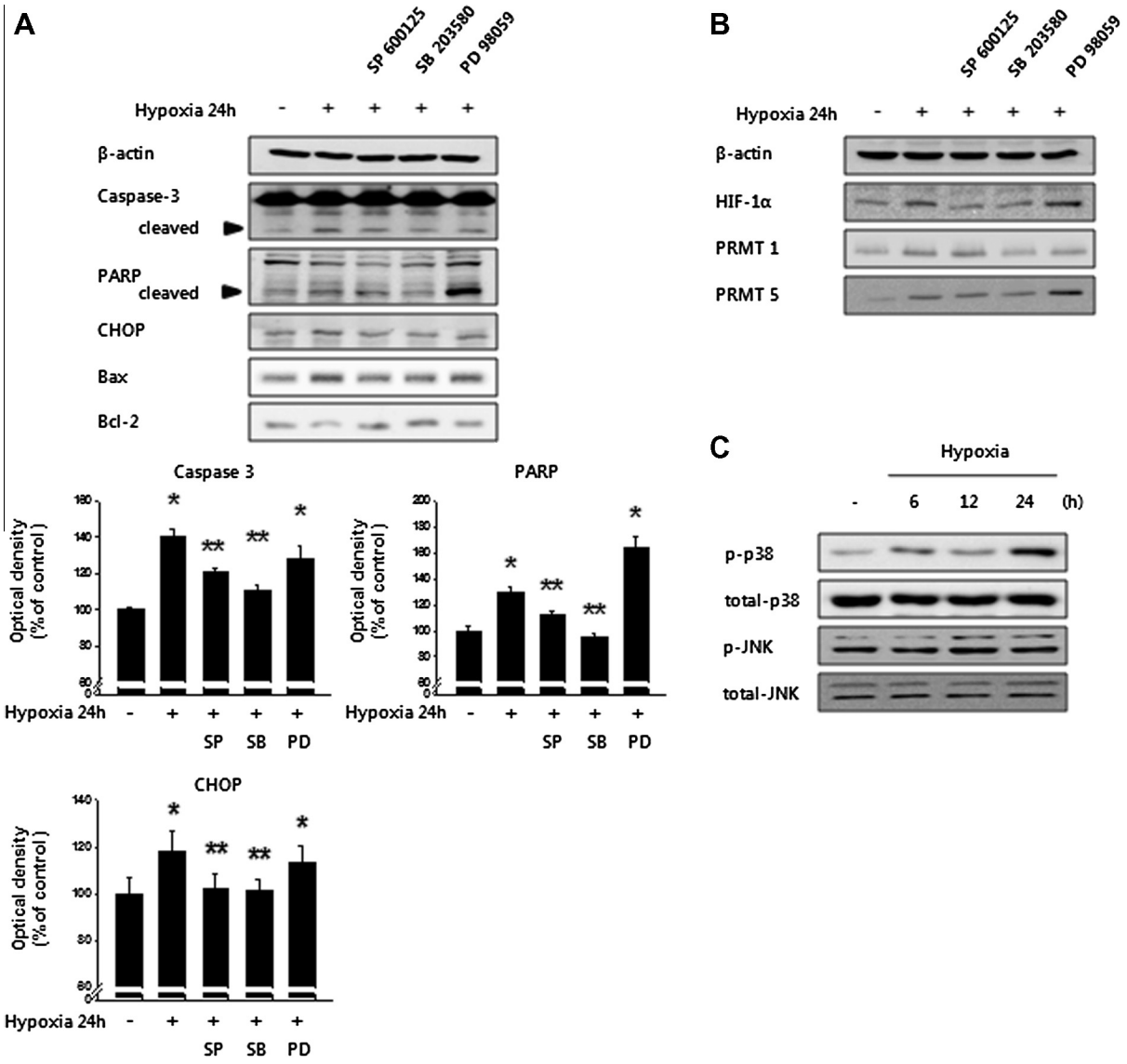

Fig. 3. Effect of hypoxia-induced p38 and JNK activation on expression of apoptosis regulatory proteins and PRMTs (PRMT1 and PRMT5). (A) The cells were pretreated with SP 600125 (JNK MAPKs inhibitor, 10^{-6} M), SB 203580 (p38 MAPK inhibitor, 10^{-6} M), or PD 98059 (p44/42 MAPKs inhibitor, 10^{-6} M) for 30 min prior to 24 h of hypoxia exposure and the total proteins were then subjected to SDS–PAGE and blotted with the cleaved caspase-3, cleaved PARP, CHOP, Bax, and Bcl-2 antibodies. The lower panels represent the mean ± S.E. of three independent experiments for each condition as determined from densitometry relative to β-actin. $^{*}p < 0.05$ vs. control. $^{**}p < 0.05$ vs. hypoxia alone. (B) Also, the total proteins were then subjected to SDS–PAGE and blotted with the HIF-1α, PRMT1, and PRMT5. (c) The cells were exposed to hypoxia for 0–24 h. And then, phospho-p38 and phospho-JNK were detected by Western blotting. Each example shown is a representative of four experiments.

were grown to 80% con confluence and transfection of PRMT1 siRNA, PRMT5 siRNA or scrambled siRNA was performed using Lipofectamine™ RNAiMAX Reagent (Invitrogen™, Carlsbad, CA, USA) according to the manufacturer's instructions. After 24 h, the transfected cells were incubated under hypoxic conditions for 24 h.

2.10. Statistical analysis

The results were expressed as the mean ± standard error (S.E.). All the experiments were analyzed by analysis of variance (ANOVA). In some experiments, a comparison of the treatment means was made with the control using the Bonnferroni–Dunn test. A p-value <0.05 was considered significant.

3. Results

3.1. Effect of hypoxia on protein arginine methyltransferases (PRMT) and apoptosis-related proteins

To examine the time-dependent effect of hypoxia on expression of PRMT isoforms, A549 cells were exposed to hypoxic conditions for 0–24 h. Fig. 1A confirms the expression of HIF-1α, which supports the induction of hypoxic conditions in these cells. We subsequently observed that protein expression of the PRMT type I isoform, PRMT1, and type II isoform, PRMT5, were increased in a time-dependent manner (>6 h) in response to hypoxic conditions, whereas the expression of PRMT3 decreased, and PRMT4 was not detected (Fig. 1A). Furthermore, we determined that the maximum expression of pro-apoptotic proteins such as cleaved caspase-3, cleared PARP, CHOP, and Bax occurred after 12–24 h of chronic hypoxic exposure, and the anti-apoptotic protein Bcl-2 decreased during the same time period (Fig. 1B).

3.2. Effects of PRMT1 and PRMT5 overexpression and small interfering RNA (siRNA) on hypoxia-induced apoptotic death

To determine whether overexpression of PRMT1 and PRMT5 influenced cell death, the green fluorescent protein (GFP)-tagged empty vector (GFP-EV), PRMT1-GFP, and PRMT5-GFP were overexpressed in A549 cells. As shown in Fig. 2A, the expression of transfected PRMT1-GFP and PRMT5-GFP was confirmed by Western blotting with a GFP-specific antibody and PRMT1-GFP- and

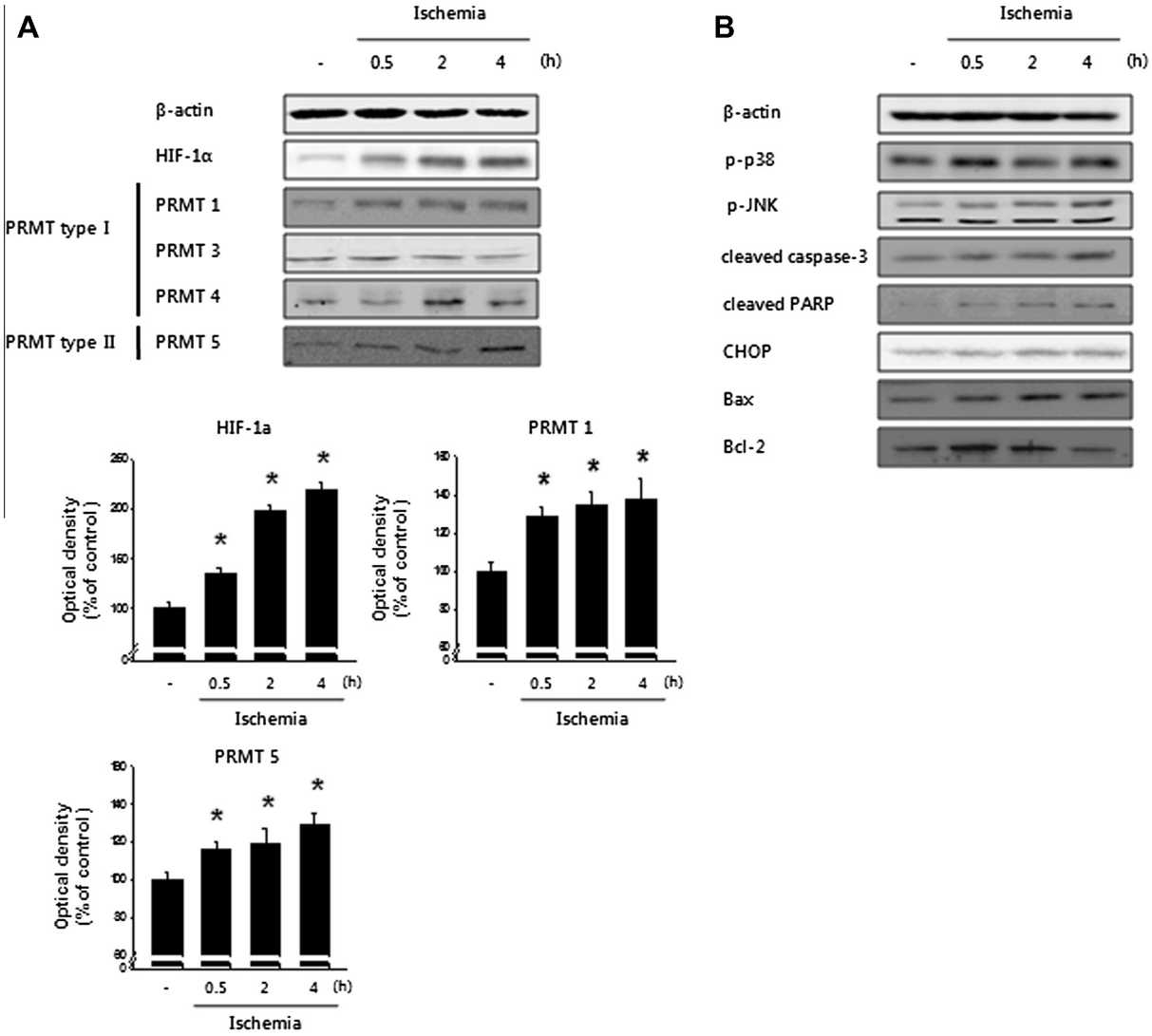

Fig. 4. Expression of PRMT isoforms and apoptosis regulatory proteins in lung ischemia induced pig model. (A) The ischemia-induced lung tissue total lysates were subjected to SDS–PAGE and blotted with specific antibodies. PRMT1 and PRMT5 were detected by Western blotting as described in Section 2. (B) Cleaved caspase-3, cleaved PARP, CHOP, Bax, and Bcl-2 were detected by Western blotting under the same experimental conditions. The lower panels represent the mean ± S.E. of three independent experiments for each condition as determined from densitometry relative to β-actin. *p < 0.05 vs. control.

PRMT5-GFP-induced HIF-1α protein expression. Overexpression of PRMT1 and PRMT5 in A549 cells increased pro-apoptotic molecules such as cleaved caspase-3, cleaved PARP, CHOP, and Bax and decreased the anti-apoptotic molecule Bcl-2. However, overexpression of PRMT1 and PRMT5 did not synergistically affect hypoxia-induced cell apoptosis.

We hypothesized that PRMT1 and PRMT5 knockdown would reverse the effects of hypoxia on HIF-1α and apoptosis regulatory proteins. We confirmed that transfection of PRMT1 or PRMT5 siRNA, but not scrambled siRNA, decreased PRMT1 and PRMT5 expression by Western blotting with isoform-specific antibodies (Fig. 2B and C). Furthermore, as expected, the expression of PRMT1 (B) or PRMT5 (C) siRNA decreased expression of hypoxia-induced HIF-1α, and the apoptosis regulatory proteins cleaved caspase-3, cleaved PARP, CHOP, and Bax, whereas expression of the anti-apoptotic molecule Bcl-2 was increased. These results suggest that PRMT1 or PRMT5 plays a major role in hypoxia-induced cell death in lung epithelial cells.

3.3. Effect of MAPKs signaling on expression of hypoxia-induced apoptosis regulatory proteins and PRMT1 and PRMT5

The relationship between MAPKs (JNK, p38, and ERK 1/2), apoptosis regulatory proteins, and PRMTs was examined in cells treated with 10^{-6} M MAPK inhibitor for 30 min prior to a 24 h hypoxia exposure. As shown in Fig. 3A and B, the expression levels of hypoxia-induced cell apoptosis regulatory proteins (A), HIF-1α, and PRMTs (PRMT1 and PRMT5) (B) were inhibited by the JNK inhibitor SP 600125 or the p38 MAPK inhibitor SB 203580, but not by the ERK 1/2 inhibitor PD 98059. Additionally, hypoxia increased the phosphorylation of p38 MAPK and JNK MAPKs in a time-dependent manner (Fig. 3C). These results suggest that p38 and JNK MAPK signaling regulate the expression levels of hypoxia-induced apoptosis regulatory proteins, HIF-1α, PRMT1, and PRMT5.

3.4. The expression of PRMT isoforms and apoptotic molecules in an in vivo model of lung ischemia

We investigated the regulation of PRMT isoforms and apoptotic molecules in miniature pig lungs exposed to ischemic conditions for 0–4 h. Western blotting analysis confirmed the expression of HIF-1α in this *in vivo* model of lung ischemia and revealed increased expression of PRMT1 and PRMT5 isoforms and decreased expression of PRMT3 in ischemic lung extracts (Fig. 4A), which correlates with our *in vitro* results. Moreover, we confirmed that ischemia was induced a time-dependent increase in p38 and JNK MAPK phosphorylation (Fig. 4B). We further determined that a 30 min ischemic exposure increased pro-apoptotic proteins such as cleaved caspase-3, cleaved PARP, and CHOP, whereas the anti-apoptotic protein Bcl-2 was decreased after 2 h of ischemic exposure (Fig. 4B).

4. Discussion

The results of this study demonstrate that hypoxia induces apoptosis by stimulating expression of PRMT1 and PRMT5 in lung epithelial cells. Recently, Betz et al. reported that ADMA and SDMA levels in serum are increased during acute ischemic kidney injury [15]. However, they observed that tissue-specific expression of PRMT and dimethylarginine dimethylaminohydrolases, the ADMA and SDMA synthesizing and metabolizing enzymes, were not altered. Yildirim et al. have provided the only report to date describing the involvement of PRMT in hypoxia, determining that chronic hypoxia increased PRMT2 expression in mouse lung [16].

Although it is known whether hypoxia induces apoptosis in chronic lung diseases [17], the involvement of PRMTs in hypoxia-induced apoptosis in the lung has not been examined. Our study provides new evidence on the association of hypoxia-induced increase in PRMT1 and PRMT5 expression in human lung epithelial cells with apoptosis. We demonstrated that hypoxia induced an increase in pro-apoptotic molecules such as Bax, CHOP, PARP, and caspase-3 and decreased the anti-apoptotic molecule Bcl-2. Furthermore, overexpression of PRMT1 and PRMT5 induced an increase in pro-apoptotic molecules, with a concomitant decrease in an anti-apoptotic molecule. Additionally, siRNA transfection of PRMT1 and PRMT5 blocked hypoxia-induced apoptosis. It is important to note that we did not examine the role of PRMT2 in hypoxia, as it is not clear that PRMT2 expression mediates hypoxia-induced apoptosis in lung epithelial cells and we did not detect expression of PRMT4 under normoxic or hypoxic conditions in human lung epithelial cells. Our *in vivo* results demonstrated that ischemia stimulates expression of PRMT1 and PRMT5 in the miniature pig, although PRMT4 expression was also increased, which remains to be studied in detail.

Diverse signaling molecules are involved in hypoxic lung injury. Taylor et al. provided evidence that p38 and JNK MAPK activation were involved in apoptosis of A549 cells, although they did not examine the role of hypoxia [18]. Tan et al. demonstrated that ischemia reperfusion (IR) caused lipid peroxidation, inflammation, and apoptosis, which required MAPK-dependent activation of NF-κB in rat pulmonary microvascular endothelial cells [8]. Lu et al. similarly showed that p38 and JNK MAPK activation mediated cigarette smoke-induced lung endothelial apoptosis [19]. This is the first study to clarify the interaction between PRMTs and MAPKs, and our results suggest that p38 and JNK MAPK, but not ERK 1/2, are involved in hypoxia-induced apoptosis mediated by PRMT1 and PRMT5 activation. Jiang et al. reported that inhibition of HIF-1α ameliorates lung injury, suggesting a role of HIF-1α in hypoxia [20]. In this study, hypoxia increased the expression of HIF-1α, and we determined that PRMT1 and PRMT5 are upstream molecules mediating the induction of HIF-1α in hypoxia. Therefore, we surmise that the MAPK–PRMT–HIF-1α signaling cascade is an important event in hypoxia-induced apoptosis in lung epithelial cells. We also confirmed that these signaling events are reproducible in an ischemic miniature pig lung, which may be clinically relevant to ischemic injury following lung transplantation.

In conclusion, this study extends our understanding of the roles of PRMTs and MAPKs in lung epithelial cells during hypoxia and suggests that inhibition of PRMT1, PRMT5, or p38 and JNK MAPK activation may provide possible therapeutic targets for the treatment of hypoxia and ischemia in the lung using a miniature pig lung model. Taken together, these findings identify a new pathway linking hypoxia to p38 and JNK MAPK activation, which subsequently induces the expression of PRMT1 and PRMT5, to promote apoptosis in lung epithelial cells via HIF-1α expression.

Acknowledgment

This work was supported by a grant from the Next-Generation BioGreen 21 Program (No.PJ009090), Rural Development Administration, Republic of Korea. This work was also supported by a research grant from the Korean Science & Engineering Foundation (2011-0016649). It would be grateful you consider our request when you make the gallery proof.

References

[1] Y. Wu, J. Lv, D. Feng, F. Jiang, X. Fan, Z. Zhang, R. Yin, L. Xu, Restoration of alveolar type II cell function contributes to simvastatin-induced attenuation of lung ischemia-reperfusion injury, Int. J. Mol. Med. 30 (2012) 1294–1306.

[2] P.D. Weyker, C.A. Webb, D. Kiamanesh, B.C. Flynn, Lung ischemia reperfusion injury: a bench-to-bedside review, Semin. Cardiothorac. Vasc. Anesth. 17 (2013) 28–43.
[3] L.A. Dada, E. Novoa, E. Lecuona, H. Sun, J.I. Sznajder, Role of the small GTPase RhoA in the hypoxia-induced decrease of plasma membrane Na, K-ATPase in A549 cells, J. Cell. Sci. 120 (2007) 2214–2222.
[4] A.K. Sharma, L.G. Fernandez, A.S. Awad, I.L. Kron, V.E. Laubach, Proinflammatory response of alveolar epithelial cells is enhanced by alveolar macrophage-produced TNF-alpha during pulmonary ischemia-reperfusion injury, Am. J. Physiol. Lung Cell Mol. Physiol. 293 (2007) 105–113.
[5] D. Zakrzewicz, O. Eickelberg, From arginine methylation to ADMA: a novel mechanism with therapeutic potential in chronic lung diseases, BMC Pulm. Med. 9 (2009) 5.
[6] Y. Yang, M.T. Bedford, Protein arginine methyltransferases and cancer, Nat. Rev. Cancer 13 (2013) 37–50.
[7] P.A. Klekotka, S.A. Santoro, H. Wang, M.M. Zutter, Specific residues within the alpha 2 integrin subunit cytoplasmic domain regulate migration and cell cycle progression via distinct MAPK pathways, J. Biol. Chem. 276 (2001) 32353–32361.
[8] J. Tan, D. Liu, X. Lv, L. Wang, C. Zhao, Y. Che, Q. Xie, X. Cui, MAPK mediates inflammatory response and cell death in rat pulmonary microvascular endothelial cells in an ischemia-reperfusion model of lung transplantation, J. Heart Lung Transplant. 32 (2013) 823–831.
[9] M. Korfei, C. Ruppert, P. Mahavadi, I. Henneke, P. Markart, M. Koch, G. Lang, L. Fink, R.M. Bohle, W. Seeger, T.E. Weaver, A. Guenther, Epithelial endoplasmic reticulum stress and apoptosis in sporadic idiopathic pulmonary fibrosis, Am. J. Respir. Crit. Care Med. 178 (2008) 838–846.
[10] K.Y. Kwon, J.H. Jang, W.I. Choi, S. Ramachandran, C.H. Cho, P.T. Cagle, Expression of apoptotic nuclei by ultrastructural terminal deoxyribonucleotidyl transferase mediated dUTP nick end labeling and detection of FasL, caspases and PARP protein molecules in cadmium induced acute alveolar cell injury, Toxicology 218 (2006) 197–204.
[11] J. Zhang, J.S. Wang, Z.K. Zheng, J. Tang, K. Fan, H. Guo, J.J. Wang, Participation of autophagy in lung ischemia-reperfusion injury *in vivo*, J. Surg. Res. 182 (2003) 79–87.
[12] H.C. Hughes, Swine in cardiovascular research, Lab. Anim. Sci. 36 (1986) 348–350.
[13] M.A. Pereira-Sampaio, L.A. Favorito, F.J. Sampaio, Pig kidney: anatomical relationships between the intrarenal arteries and the kidney collecting system. Applied study for urological research and surgical training, J. Urol. 172 (2004) 2077–2081.
[14] P. Vodicka, K. Smetana Jr, B. Dvoránková, T. Emerick, Y.Z. Xu, J. Ourednik, V. Ourednik, J. Motlík, The miniature pig as an animal model in biomedical research, Ann. N.Y. Acad. Sci. 1049 (2005) 161–171.
[15] B. Betz, K. Möller-Ehrlich, T. Kress, J. Kniepert, E. Schwedhelm, R.H. Böger, C. Wanner, C. Sauvant, R. Schneider, Increased symmetrical dimethylarginine in ischemic acute kidney injury as a causative factor of renal L-arginine deficiency, Trans. Res. 162 (2013) 67–76.
[16] A.O. Yildirim, P. Bulau, D. Zakrzewicz, K.E. Kitowska, N. Weissmann, F. Grimminger, R.E. Morty, O. Eickelberg, Increased protein arginine methylation in chronic hypoxia: role of protein arginine methyltransferases, Am. J. Respir. Cell Mol. Biol. 35 (2006) 436–443.
[17] T. Weng, H. Karmouty-Quintana, L.J. Garcia-Morales, J.G. Molina, M. Pedroza, R.R. Bunge, B.A. Bruckner, M. Loebe, H. Seethamraju, M.R. Blackburn, Hypoxia-induced deoxycytidine kinase expression contributes to apoptosis in chronic lung disease, FASEB J. 27 (2013) 2013–2026.
[18] C.A. Taylor, Q. Zheng, Z. Liu, J.E. Thompson, Role of p38 and JNK MAPK signaling pathways and tumor suppressor p53 on induction of apoptosis in response to Ad-eIF5A1 in A549 lung cancer cells, Mol. Cancer 12 (2013) 35.
[19] Q. Lu, P. Sakhatskyy, J. Newton, P. Shamirian, V. Hsiao, S. Curren, G.A. Gabino Miranda, M. Pedroza, M.R. Blackburn, S. Rounds, Sustained adenosine exposure causes lung endothelial apoptosis: a possible contributor to cigarette smoke-induced endothelial apoptosis and lung injury, Am. J. Physiol. Lung Cell Mol. Physiol. 304 (2013) 361–370.
[20] H. Jiang, Y. Huang, H. Xu, R. Hu, Q.F. Li, Inhibition of hypoxia inducible factor-1α ameliorates lung injury induced by trauma and hemorrhagic shock in rats, Acta Pharmacol. Sin. 33 (2012) 635–643.