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Acute hypoxia to endothelial cells induces activating transcription factor 3 (ATF3) expression that is mediated via nitric oxide

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Abstract

Endothelial cells (ECs) play an important role in hypoxia-induced vascular disorders. We investigated the acute hypoxia effect on endothelial expression of activating transcription factor 3 (ATF3), a stress-inducible transcription factor playing significant roles in cellular responses to stress. Bovine aortic ECs were subjected to acute hypoxia (1% O_2, pO_2 = 8 mmHg) and ATF3 expression was examined. ECs exposed to hypoxia transiently induced ATF3 expression. A transient increase in the activation of c-Jun-NH_2-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) in ECs was observed; however, only ECs pretreated with a specific inhibitor to JNK suppressed the hypoxia-induced ATF3 expression. ECs exposed to acute hypoxia transiently increased endothelial nitric oxide (eNOS) activity. Pre-treating ECs with a specific inhibitor to eNOS (L-NAME) or PI3-kinase significantly inhibited the hypoxia-induced JNK activation and ATF3 expression. ATF3 induction has been shown to inhibit matrix metalloproteinase-2 (MMP-2) expression. Consistently, ECs exposed to hypoxia attenuated the MMP-2 expression. This hypoxia-attenuated MMP-2 expression can be rescued by pre-treating ECs with an inhibitor of eNOS. These results suggest that the ATF3 induction by acute hypoxia is mediated by nitric oxide and the JNK pathway in ECs. Our findings provide a molecular basis for the mechanism in which ECs respond to acute hypoxia.

Keywords: Hypoxia; Activating transcription factor 3; Mitogen-activated protein kinase; Nitric oxide; Reactive oxygen species; Matrix metalloproteinase-2

1. Introduction

Hypoxia elicits a variety of functional responses in vascular endothelial cells (ECs) including cell proliferation, angiogenesis, and cell death [1]. Manipulation of these responses is important for biomedical aspects such as ischemia, stroke, and tumorigenesis [2]. Vascular ECs cope with hypoxia to maintain vascular homeostasis by expressing a number of genes, which are mediated by a variety of signaling cascades [3,4]. However, the detailed signaling mechanisms that regulate endothelial responses to hypoxia remain unclear. Understanding the oxygen-sensitive pathways in ECs might help to develop therapies for hypoxia-induced vascular diseases.

Activating transcription factor 3 (ATF3), a member of the ATF/cAMP-responsive element binding protein family, is a inducible transcription factor which can be activated by various stimuli, including ischemia/reperfusion [5], and cytokine [6]. Several studies demonstrated that ATF3 could function as a pro-apoptotic or anti-apoptotic regulator in cells [6,7]. ATF3 expression has been shown to alter a variety of cellular functions including cell proliferation, cell death, angiogenesis and

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metastasis [8,9]. Recent studies indicated that the expression of ATF3 was regulated by various stresses-mediated signaling or transcriptional factors, including nuclear factor-κB (NF-κB), and c-Jun-NH_2-terminal kinase (JNK) [10], and was independent of p53 and the hypoxia-inducible factor-1 signaling pathway in response to anoxia [11]. Studies including us have demonstrated that the induction of ATF3 is critical for endothelial migration [9,12]. Although there are considerable studies on the effect of various stimuli on ATF3 expression and cellular functions, the effect of acute hypoxia on ATF3 expression and the mechanism underlying this regulatory effect of acute hypoxia in ECs remain elusive.

Many studies have demonstrated that reactive oxygen species (ROS) are important mediators in hypoxia-induced intracellular signaling cascades [13,14]. Among those ROS, superoxide and hydrogen peroxide (H_2O_2) are two of the most important oxygen species in ECs. Superoxide is generated via different sources, including NADPH oxidase and mitochondria, and can be converted to hydrogen peroxide (H_2O_2) by the action of superoxide dismutase (SOD). Both superoxide and H_2O_2 can mediate a variety of downstream signaling cascades in ECs. In addition to ROS, ECs also constantly produce nitric oxide (NO). NO, a vasodilator, exerts an anti-atherogenic effect in the vasculature by inhibiting platelet aggregation and leukocyte adhesion to vessel wall [15]. Hypoxia has been shown to induce NO production [16]. We showed earlier that hypoxia induced the activation of ERK and resulted in an up-regulation of Egr-1 expression in ECs [4]. However, the detailed mechanisms are not clear. Our recent studies demonstrated that NO treatment to ECs inhibited the expression of matrix metalloproteinase-2 (MMP-2) via the induction of ATF3 [12]. When cells are under oxidative stress, NO may react with superoxide to form peroxynitrite, which can react with biomolecules that result in cell damage. Although it is known that ROS and NO are involved in the hypoxia-induced cellular injuries, the interplay between these reactive species and their contribution to the pathophysiological consequences in ECs under acute hypoxia remain unclear.

In the present study, we investigated the effect of acute hypoxia on the expression of ATF3 in ECs and the potential signaling mechanisms involved in this hypoxia-mediated ATF3 expression. We demonstrated that acute hypoxia induced a transient increase in ATF3 protein expression in ECs. This hypoxia-induced ATF3 expression appears to be mediated via NO and JNK pathway, and is independent of the endogenous H_2O_2 and superoxide. In addition, ECs exposed to hypoxia attenuated their MMP-2 gene expression. This hypoxia-induced attenuation of MMP-2 expression was rescued by pre-treating ECs with an eNOS inhibitor. Our findings provide a molecular basis for the mechanism by which acute hypoxia plays a role in modulating signaling and gene expression in ECs.

2. Materials and methods

2.1. Antibodies and reagents

Rabbit polyclonal antibody against ATF3 (C-19), mouse monoclonal antibodies (mAbs) against JNK, phospho-JNK, SOD, and catalase were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse mAbs against p38 mitogen-activated protein kinase (MAPK), phospho-p38 MAPK, eNOS, and phospho-eNOS (Ser-1177) were purchased from BD Bioscience Pharmingen (San Diego, CA). Anti-tubulin antibody was obtained from NeoMarker (Fremont, CA). N^G-Nitro-L-arginine Methyl Ester (L-NAME), PD98059, SB203580, and SP600125 were purchased from Calbiochem (San Diego, CA). All other chemicals of reagent grade were obtained from Sigma (St Louis, MO).

2.2. Cell culture

Bovine aortic ECs were obtained commercially (Clonetics, Palo Alto, CA) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Hyclone) as described elsewhere [4]. ECs were seeded on 60 mm Petri dishes until ∼90% confluence, and the culture medium was then changed to DMEM containing 0.5% FBS prior to experiments.

2.3. Hypoxia apparatus

Hypoxic conditions ($pO_2 = 8$ mmHg) were achieved by adding medium pre-equilibrated with nitrogen gas to cells prior to the incubation in a Plexiglas chamber purged with water-saturated nitrogen gas to a $pO_2 = 8$ mmHg by a oxygen controller (PROOX model 110, BioSpherix, Ltd., Redfield, NY). The partial pressure of oxygen (pO_2) of culture medium under hypoxia was monitored using ISO_2 dissolved oxygen meter (World Precision Instruments, Inc., Sarasota, FL). The measurement indicated that a steady-state $pO_2 = 8$ mmHg in the culture medium was maintained during experiments.

2.4. RNA isolation and real-time quantitative PCR

Total cellular RNA was extracted from ECs (∼1.5×10^6 cells) using TRI Reagent (Molecular Research Center, Cincinnati, OH) according to manufacturer's instruction. One microgram of total RNA was reverse-transcribed by M-MuLV reverse transcriptase (Finnzyme, Finland) in a total volume of 20 μl. The reverse transcriptase products were amplified with DyNAmo HS SYBR Green qPCR Kit (Finnzyme, Finland) in the reaction mix containing DyNAmo SYBR Green master mix and primers. Primers were designed for detection of bovine MMP-2 gene expression (forward: 5′-CCTAAACACCTTCTACGGCT-3′; reverse: 5′-TGTGATCTGGTTCTTGTCCC-3′). The bovine GAPDH gene expression was used as internal controls

(forward: 5′-ATGACCACTGTCCACGCCAT-3′; reverse: 5′-GCCTGCTTC ACCACCTTCTT-3′).

2.5. Western blot analysis

ECs were washed in PBS and lysed with RIPA buffer containing Nonidet P-40 (1%), SDS (0.5%) and protease inhibitor cocktail. Cells were disrupted by intermittent sonication. After centrifugation, the protein level of supernatant was measured using BSA as standard. Cell lysates were then subjected to SDS-PAGE followed by Western blotting. Antigen–antibody complexes were detected by horseradish peroxidase-labeled rabbit anti-mouse or goat anti-rabbit IgG with an ECL detection system (Pierce, Rockford, IL).

2.6. Statistical analysis

Results are expressed as means ± S.E.M. Statistical analysis was performed by using an independent Student's *t*-test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Hypoxia induces ATF3 protein expression in ECs

To examine the effect of hypoxia on ATF3 expression in ECs, ECs were incubated in a hypoxic condition (1% O_2, $pO_2 = 8$ mmHg) for 0.5–12 h and the ATF3 protein expression was analyzed by Western blotting. As shown in Fig. 1, the induction of ATF3 was observed within 2 h and reached to its peak 4 h after the onset of hypoxia. The up-regulation of ATF3 was slightly decreased within 12 h after hypoxic treatment. These results indicate that acute hypoxia induces a transient increase in the ATF3 expression in ECs.

3.2. Hypoxia-induced ATF3 expression is mediated by the JNK pathway

The MAPK signaling pathways [i.e., ERK, JNK, and p38 MAPK] have been shown to regulate gene expression and cellular function in response to various stimuli [17]. We have previously demonstrated that hypoxia induced the activation of ERK and resulted in an up-regulation of Egr-1 in ECs [4]. In the present study, we further explored whether the acute hypoxia induced activations of JNK and p38 MAPK in ECs. As shown in Fig. 2A, ECs exposed to acute hypoxic induced a transient increase in the phosphorylation of JNK and p38 MAPK in ECs. The peak induction of JNK or p38 activity was observed 15 min after the onset of hypoxia. We further examined whether these hypoxia-induced JNK and p38 MAPK phosphorylation were involved in the hypoxia-induced ATF3 expression. ECs were pre-treated with a specific inhibitor either to MEK (PD98059), p38 MAPK (SB203580), or JNK (SP600125) for 1 h, and then subjected to hypoxia for 4 h with identical culture medium containing inhibitor. As shown in

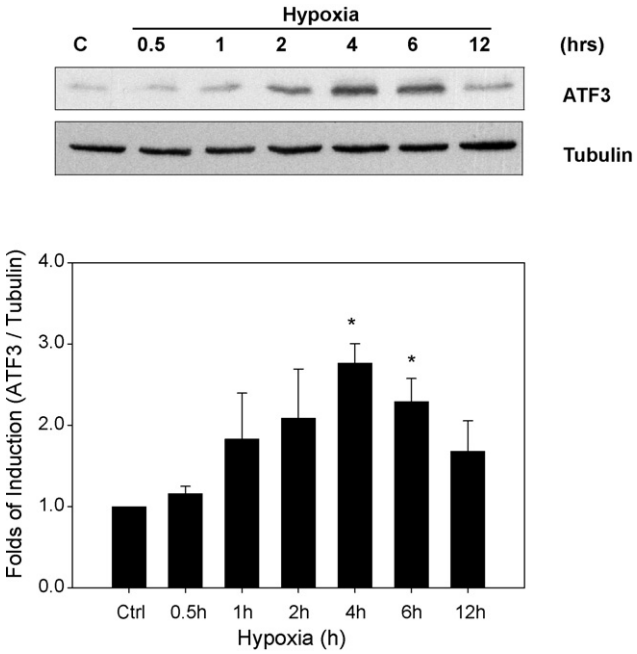

Fig. 1. Hypoxia induces ATF3 protein expression in endothelial cell. ECs were subjected to normoxia or hypoxia for various time intervals and total cell lysates were immunoblotted with anti-ATF3 antibody. Tubulin was shown for equal amounts of proteins loading in each lane. Data was presented as mean ± S.E. from three independent experiments. * $p < 0.01$ *vs.* normoxia controls.

Fig. 2B, the hypoxia-induced ATF3 expression in ECs was significantly inhibited by the JNK inhibitor (SP600125) but only partially suppressed by the p38 inhibitor (SB203580). In contrast, ECs pretreated with ERK inhibitor (PD98059) had no inhibitory effect on hypoxia-induced ATF3 expression in ECs. These results suggest that the hypoxia-induced ATF3 expression is mediated mainly by the JNK pathway in ECs.

3.3. NO is involved in hypoxia-induced ATF3 expression in ECs

Our previous study has shown that NO-induced MMP-2 down-regulation was mediated by the induction of ATF3 [12]. We examined the role of NO in mediating hypoxia-induced ATF3 expression in ECs. ECs subjected to acute hypoxia up for 1 h, and the phosphorylation of eNOS in hypoxia-treated ECs was examined. As shown in Fig. 3A, the phosphorylation of eNOS was induced by 15 min under hypoxic condition. This increased eNOS phosphorylation returned to the basal level 60 min after hypoxic treatment. When ECs were pretreated with a specific inhibitor to eNOS (i.e., L-NAME) or phosphatidylinositol 3-kinase (PI3-K; i.e., LY294002), the hypoxia-induced ATF3 expression was significantly suppressed (Fig. 3B). Furthermore, hypoxia-induced JNK phosphorylation was significantly down-regulated by pretreatment of ECs with L-NAME or LY294002 (Fig. 4A and B). These results suggest that the PI3K-eNOS pathway may be involved in hypoxia-induced ATF3 expression in ECs,

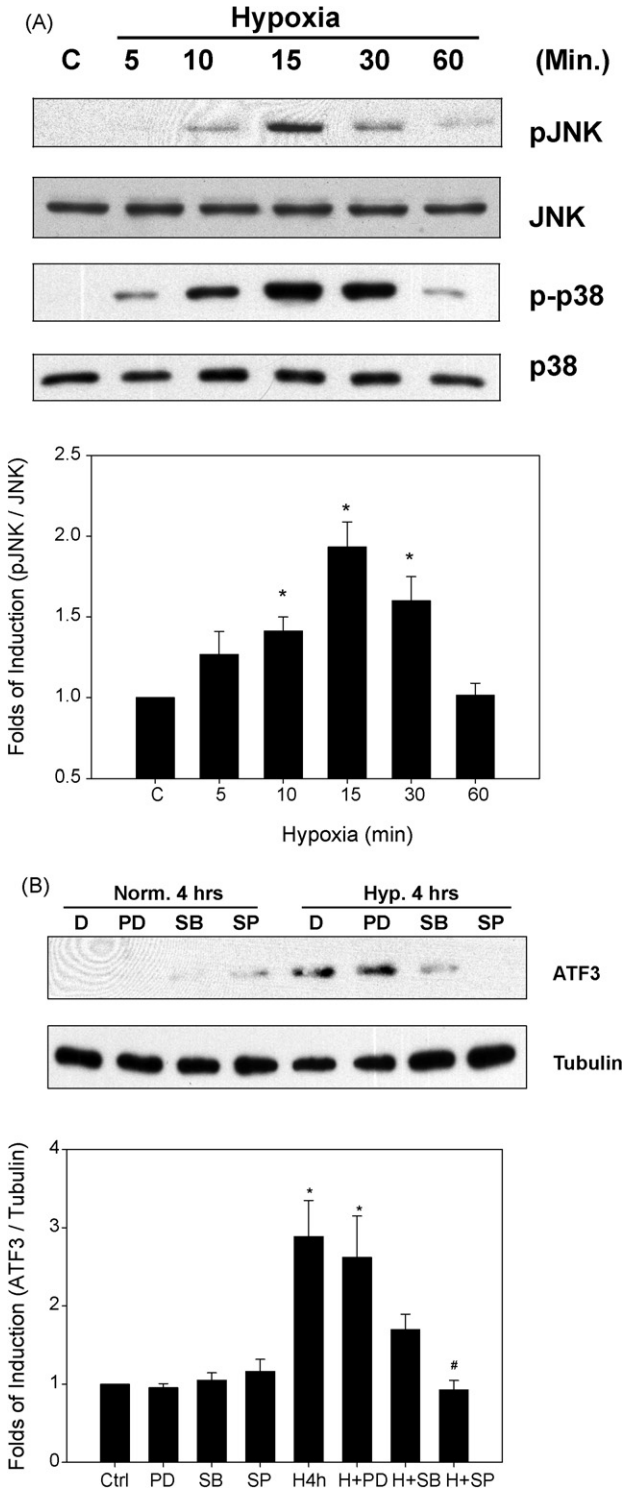

Fig. 2. JNK/SAPK mediates hypoxia-induced ATF3 expression in ECs. (A) ECs were subjected to normoxia or hypoxia for 5–60 min and cell lysates were collected for Western analysis using antibody for phospho-JNK. Hypoxic induction of p38 phosphorylation was analyzed by Western blot using antibodies to phospho-p38 and p38. Antibodies to p38 and JNK were shown for equal amounts of proteins loading in each lane. Densitometric analysis of phospho-JNK was performed. (B) ECs were pretreated with a MEK inhibitor (PD98059, PD), a p38 kinase inhibitor (SB203580, SB) or a JNK inhibitor (SP600125, SP) for 1 h followed by normoxia or hypoxia for 4 h. ECs were harvested and analyzed by Western blotting using anti-ATF3 antibody and the result was normalized to tubulin. Data was presented as mean ± S.E. from three independent experiments. *, $p < 0.05$ *vs.* normoxia controls. #, $p < 0.05$ *vs.* hypoxia.

probably through the JNK signaling pathway. NO appears to be a key molecule in mediating the JNK pathway, and subsequently modulates the ATF3 induction by hypoxia.

3.4. Reactive oxygen species are not involved in hypoxia-induced ATF3 protein expression in EC

We have demonstrated that the PI3K/eNOS and JNK pathways are involved in hypoxia-induced ATF3 expression in ECs, and that NO plays an important role in the hypoxia-induced ATF3 expression. Since there have been reports showing that hypoxia can induce generation of ROS, including superoxide [14], which can instantly react with NO to form peroxinitrite and subsequently contribute to the induction of JNK and ATF3, we investigated whether superoxide plays a role in modulating hypoxia-induced ATF3 expression in ECs. Adenovirus carrying the Cu/Zn SOD was infected to ECs to remove the superoxide, if produced, during hypoxic condition. The SOD protein expression was increased in the adenoviral-infected ECs. However, the increased SOD expression in these ECs had no effect on hypoxia-induced ATF3 expression. To investigate whether H_2O_2 is involved in hypoxia-induced ATF3 expression in ECs, ECs were infected with adenovirus carrying catalase and then exposed to the hypoxic condition for 4 h. The catalase protein expression was significantly increased in those adenoviral-infected ECs. However, this increased expression of catalase in ECs did not alter the hypoxia-induced ATF3 expression (Supplementary data). ECs pretreated with catalase exogenously which was shown previously to reduce the intracellular ROS levels [18] also did not affect the ATF3 expression by hypoxia (Supplementary data). These results suggest that the ROS is not involved in hypoxia-induced ATF3 expression in ECs.

3.5. ECs exposed to hypoxia attenuated MMP-2 gene expression via NO

Our previous report demonstrated that the NO-induced MMP-2 down-regulation was mediated by the ATF3-dependent pathway [12], we postulated that hypoxic condition might modulate the expression of MMP-2 in ECs. We examined the MMP-2 mRNA expression in ECs exposed to hypoxia and the role of NO in this MMP-2 expression. As shown in Fig. 5A, hypoxia to ECs greatly suppressed the MMP-2 mRNA level at 8 h. Pre-treatment of ECs with an eNOS inhibitor, L-NAME, recovered this hypoxia-induced attenuation of MMP-2 mRNA levels (Fig. 5B). These results suggest that MMP-2 expression in ECs is down-regulated by hypoxia and NO is involved in this suppression of MMP-2 by hypoxia.

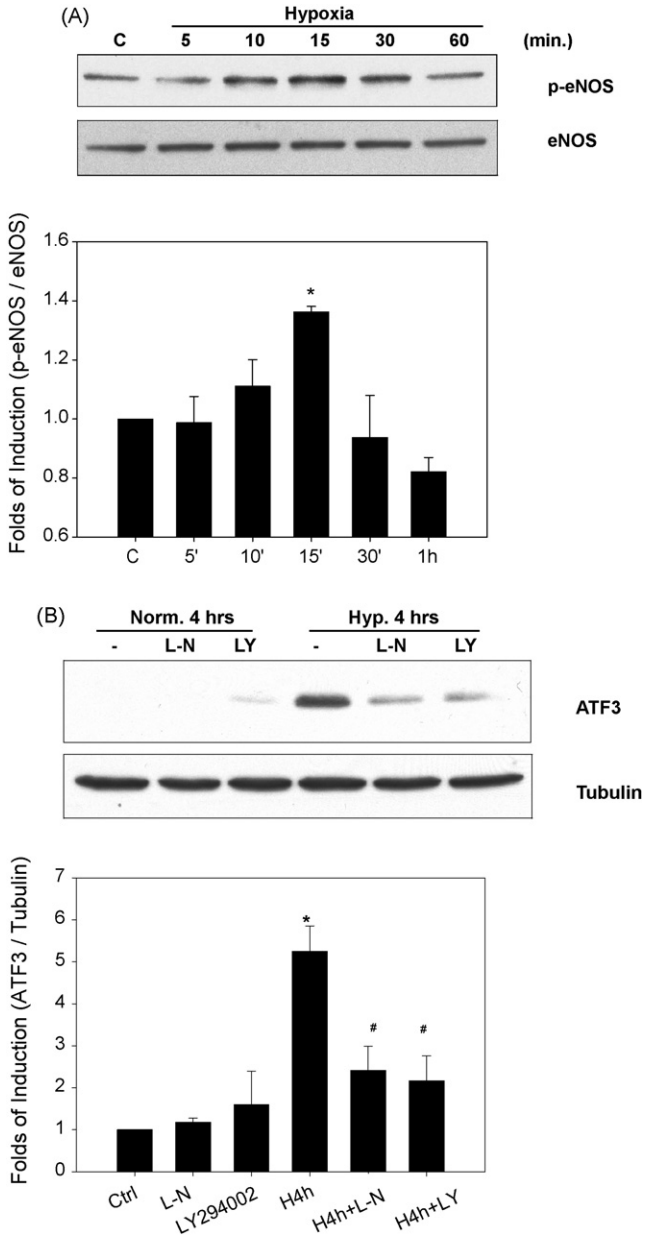

Fig. 3. NO is involved in hypoxia-induced ATF3 expression. (A) ECs were subjected to normoxia (C) or hypoxia for 5–60 min and total cell lysates were collected and analyzed by immunoblotting assay with antibody to phospho-eNOS (Ser-1177). Densitometric analysis was performed by normalization of phospho-eNOS with eNOS. (B) ECs were pretreated with an inhibitor to eNOS (L-NAME, 500 μM) or PI3-Kinase (LY294002, 5 μM) for 1 h followed by hypoxia for 4 h. Cells lysates were harvested and analyzed by Western blot. Tubulin indicates an equal loading of proteins in each lane. Data was presented as mean ± S.E. from three independent experiments. *, $p < 0.01$ *vs.* normoxia controls. #, $p < 0.01$ *vs.* hypoxia.

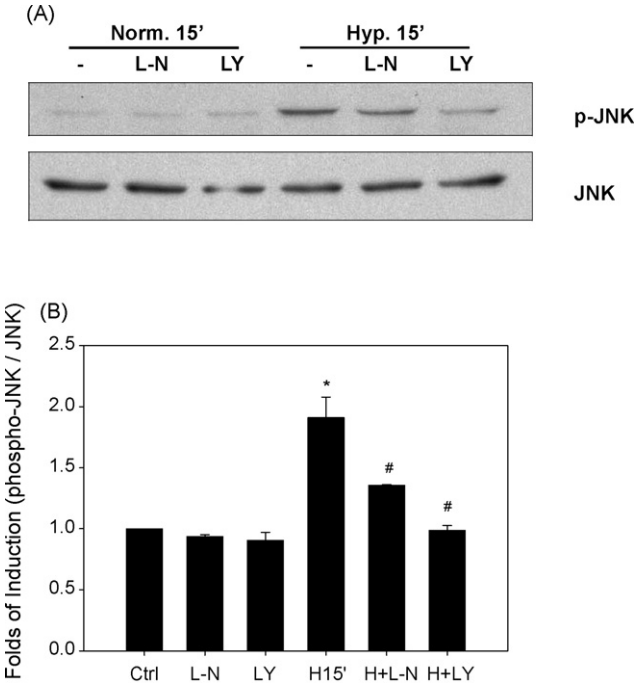

Fig. 4. Hypoxic induction of JNK phosphorylation is NO- and PI3-kinase-dependent. (A) ECs were pretreated with or without L-NAME (100 μM) or LY294002 (5 μM) for 1 h and then subjected to normoxia or hypoxia for 15 min. Total cell lysates were analyzed by Western blotting using phospho-JNK or JNK antibody. (B) Densitometric analysis of NO- and PI3 kinase-mediated JNK activation (p-JNK) was performed. Data was presented as mean ± S.E. from three independent experiments. *, $p < 0.05$ *vs.* normoxia controls. #, $p < 0.05$ *vs.* hypoxia.

4. Discussion

The present study was aimed to elucidate the initial response mechanism of ECs to acute hypoxia. Our results indicate that ECs exposed to hypoxia induces a transient activation of eNOS and the increase of NO appears to be involved in the ATF3 expression and consequently results in a suppression of MMP-2 levels in ECs. Several lines of evidence indicated that hypoxia induced ATF3 expression in ECs and that this hypoxia-induced ATF3 expression was mediated by NO and JNK signaling pathways. First, ECs exposed to hypoxia induced ATF3 protein expression in a transient manner. Second, JNK and p38 MAPK activities in ECs were transiently induced by hypoxia; however, only the inhibitor for JNK completely abolished the hypoxia-induced ATF3 expression. Third, hypoxia induced a transient eNOS activation in ECs. Pre-treatment of ECs with the NOS inhibitor, i.e., L-NAME significantly inhibited the hypoxia-induced JNK phosphorylation and ATF3 expression. In addition, the specific inhibitor of PI3K (i.e., LY294002), which was shown to be the upstream signals for eNOS [19], had inhibitory effects on hypoxia-induced JNK phosphorylation and ATF3 expression. These results suggest that the PI3K-eNOS-JNK pathway is involved in hypoxia-induced ATF3 expression in ECs. Fourth, reactive oxygen species (ROS) including superoxide and H_2O_2 might not be involved in hypoxia-induced ATF3 expression, as indicated by a lack of inhibition by treating ECs with SOD or catalase. Finally, ECs exposed to hypoxia significantly inhibited their MMP-2 expression, which was shown previously [12] to be modulated by ATF3 induced by NO. Consistently, pre-treatment of ECs with an eNOS inhibitor recovered the MMP-2 expression suppressed

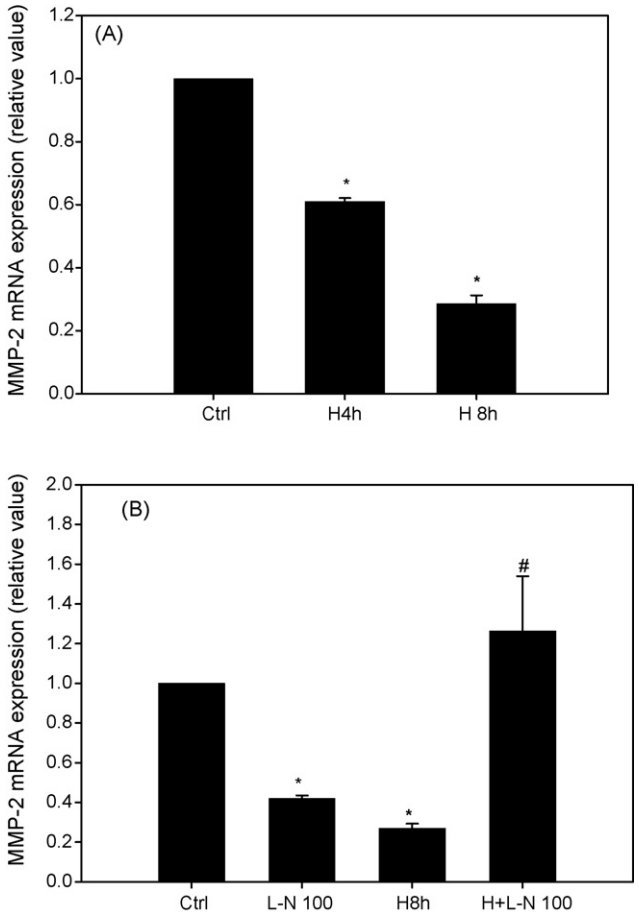

Fig. 5. Hypoxic-suppressed MMP-2 expression is NO-dependent. (A) ECs were subjected to normoxia or hypoxia for 4 and 8 h and total RNA was extracted. MMP-2 mRNA level was analyzed by real-time quantitative PCR as described in Section 2. Data was presented as mean ± S.E. from three independent experiments. *, $p < 0.005$ *vs.* normoxia controls. (B) ECs were pretreated with L-NAME (100 μM) for 1 h followed by hypoxia for 8 h. RT-PCR was performed to analyze MMP-2 mRNA levels. * indicates $p < 0.05$ *vs.* normoxia controls. #, $p < 0.05$ *vs.* hypoxia groups (H8 h).

by hypoxia. Our findings provide a molecular basis for mechanisms by which acute hypoxia regulates signaling and gene expression in ECs.

The molecular response of cells to hypoxia is a complex and poorly understood process. ATF3, a member of the ATF/cAMP response element-binding proteins (CREB) family of transcriptional factors, has been shown to be induced in various tissue by a variety of stresses including ischemia/reperfusion [5], anoxia in cancer cells [11] and prolonged hypoxia in neuroblastoma [20]. Similarly, anoxia-induced ATF4 in HeLa cells was shown to be transiently expressed [21]. Although different cells were used in these studies, the sensing of oxygen appears to be a widespread phenomenon. However, in contrast to the prolonged ATF3 expression under anoxia and prolonged hypoxia, our results of ATF3 expression in ECs was transiently up-regulated by acute milder hypoxia (1% oxygen) that reaches maximal level 4 h after the onset of hypoxia. These results imply that signaling mechanisms may be different between sensing acute hypoxia versus prolonged hypoxia. Hypoxia was shown to activate a number of intracellular signaling pathways in ECs. Our previous study [4] and others [22] demonstrated that hypoxia induced phosphorylation of ERK, which subsequently modulated the *Egr-1* and *c-fos* expressions. In this context, we have shown that all MAPKs, including ERK, JNK, and p38 MAPK, can be activated by hypoxia in ECs, with similar temporal patterns of inductions (reaching maximal levels within 15 min of hypoxia). JNK pathway was shown to be activated by hypoxia in ECs [23] and in neuronal nuclei [24]. Similarly, low oxygen in solid tumor microenvironments also transiently activated JNKs pathway [25]. This JNK activation was shown to be a critical regulator of ATF3 expression under various stimuli including anoxia [9–11]. ATF3 mRNA under anoxia was demonstrated to be more stable than that under normoxic condition. The relationship between JNK and ATF3 expression, however, is not clear in ECs exposed to hypoxia. By using specific inhibitors of ERK (PD98059), JNK (SP600125), and p38 MAPK (SB203580), our present study clearly demonstrates that JNK, but not ERK or p38 MAPK, is involved in regulating ATF3 expression in ECs under acute hypoxia. These results support the notion that JNK is a major stress-activated protein kinase that is involved in regulating endothelial responses to acute hypoxia. Our study also indicate that acute hypoxia-induced NO, rather than superoxide and H_2O_2 contributes to the JNK activation in ECs.

NO derived from eNOS has been shown to play an important role in modulating endothelial functions including pro-apoptotic and anti-apoptotic effects. These effects of NO on cells depend on the quantity of NO and the expression of NO synthase isoform in the relevant tissues [26]. Recent data indicate that NO is an important mediator in ischemia-reperfusion injury [27]. The increased NO production was found in ischemia-reperfusion injury, and this injury was significantly reduced by treating with NO inhibitor [28]. However, NO has been found to have protective effect during ischemia/reperfusion-induced injury. The effect of NO on ECs during acute hypoxia remains poorly understood. In the present study, we have shown that NO contributes to the ATF3 expression during acute hypoxia in ECs. First, the eNOS activity was transiently activated in ECs during acute hypoxia. Second, a NO synthase inhibitor, L-NAME, significantly inhibited the hypoxia-induced ATF3 expression in ECs. Moreover, a specific inhibitor (LY294002) for the upstream signals of eNOS, i.e., PI3K/Akt [19] had similar inhibitory effects on hypoxia-induced ATF3 expression. These results provided an evidence for the role of NO in the ATF3 expression in hypoxic ECs. In contrast to the role of NO in this acute hypoxia, ROS have been suggested to contribute to gene regulation in prolonged hypoxia and anoxia-induced ATF3 expression [29]. However, present study indicates that intracellular ROS level is not involved in the acute milder hypoxia-induced ATF3 expression, as demonstrated by a lack of inhibition in ATF3 expression after infecting ECs

with adenovirus carrying SOD and catalase or treating ECs with catalase exogenously. Our results thus suggest that an acute hypoxia to ECs initiates a transient eNOS activation followed by a NO-mediated JNK activation that results in a transient ATF3 expression. These sensing mechanisms of ECs to acute hypoxia may be different from those non-acute hypoxia treated cells in which ROS are involved in a prolonged ATF3 expression. In the present study, our results clearly show that PI3K-eNOS-JNK mediates acute hypoxia-induced ATF3 expression in ECs.

The present study has demonstrated that hypoxia down-regulates the expression of MMP-2, a proteinase that functions to degrade the extracellular matrix components surrounding ECs during many physiological and pathophysiological processes including angiogenesis and tumor metastasis. We have demonstrated that NO inhibits the MMP-2 expression in ECs via the induction of ATF3 [12]. Thus, the reduction in MMP-2 expression by hypoxia may be attributable to the hypoxia-induced NO and ATF3 expression in ECs. ECs treating with L-NAME recovered the MMP-2 expression that was suppressed by hypoxia further suggests the role of NO in the inhibitory effect of hypoxia on MMP-2 expression. Previous study demonstrated that ATF3 repressed MMP-2 expression by decreasing the trans-acting activity of P53. Over-expression of ATF3 also suppressed mRNA level and protein expression of MMP-2 [30]. Thus, the transient expression of ATF3 may result in a transient suppression of MMP-2. However, future work is needed to determine whether MMP-2 is a target protein of ATF3 in ECs under acute hypoxia. Nevertheless, our results implicate that acute hypoxia plays a significant role in modulating gene expression and function in ECs by regulating intracellular signaling, including MAPKs and NO, and transcription factors, such as ATF3.

In summary, the present study indicates that acute hypoxia induces the ATF3 protein expression in ECs via the JNK pathway, which is mediated by NO. The consequence of these acute hypoxia-mediated signaling and ATF3 expression may result in the reduction of MMP-2 expression in ECs. Our findings support the importance of PI3K-eNOS-JNK pathway in the regulation of endothelial responses to acute hypoxia.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2008.02.014.

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