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Hypoxia reduces endothelial Ang1-induced Tie2 activity in a Tie1-dependent manner

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ABSTRACT

Despite the altered expression of Tie receptors and angiopoietin ligands during hypoxic conditions, the effect of hypoxia on Tie-mediated endothelial responses has not been elucidated. In this study, we found that hypoxia increased Tie receptor expression but attenuated angiopoietin-1 (Ang1)-induced Tie2 activity, including Tie2 phosphorylation, Tie2 downstream signaling activation, and endothelial cell tube formation. However, Ang1 binding to endothelial cells was increased during hypoxic conditions. We demonstrated that Tie1 suppression restored the Tie2 activity and that Tie1-mediated Tie2 suppression was independent of tyrosine phosphatase activity. These results suggest that under hypoxic conditions, Tie1 is critical for reducing Ang1-induced Tie2 activity and angiogenesis.

1. Introduction

Tie1 and Tie2 are receptor tyrosine kinases that are predominantly expressed in the vascular endothelium. Tie2 is well recognized as a receptor for angiopoietin (Ang) ligands [1]. Among the various Ang ligands, Ang1 is the best characterized Tie2 receptor agonist, whereas Ang2 has been reported to act as a context-dependent agonist or antagonist for Tie2 [2]. Tie2 stimulation is known to play a crucial role not only in the maturation and stabilization of blood vessels by activating phosphatidylinositol 3-kinase and subsequently the cell survival kinase Akt [3,4] but also the prevention of vascular leakage by potentiating endothelial barrier function and anti-inflammatory effects [5,6].

In contrast to the beneficial effect of Ang1/Tie2 signals on vascular remodeling and integrity, relatively little is known about Tie1 receptor function, its downstream signaling pathway, and even the ligands for the Tie1 receptor. One study, in which Tie1 was genetically ablated in mice, reveals that the Tie1 receptor plays a role not in early angiogenic processes but in the maintenance of micro-vessel integrity [7]. Ang1 was proposed to activate Tie1 directly [8] or indirectly via Tie2 activation [9]. A growing body of evidence suggests that Tie1 may interact with Tie2 and limit Tie2 responsiveness to Ang1 and the cellular function of Tie2 [9,10]. In this regard, the preference of agonistic Ang1 to induce Tie2 homo-multimer formation, whereas antagonistic Ang2 induces Tie1/Tie2 hetero-multimer formation [11], suggests that the inhibitory function of Tie1 on Tie2 activity is associated with impairment of ligand binding to the Tie2 receptor. However, it is not clear how Tie1 contributes to Ang1-induced Tie2 responses.

Hypoxia plays a critical role in angiogenesis by producing a variety of mediators, including growth factors. The vascular response to hypoxia includes an autocrine component of cell survival and vessel permeability and a paracrine component of smooth muscle cell and pericyte vasoactive action [12]. In terms of hypoxic regulation of Ang/Tie system, hypoxia increased Ang2 expression, which is dependent on hypoxia-inducible factor (HIF) via hypoxia-response element (HRE) [13,14]. Hypoxia also increased Tie1 expression in endothelial cells [15], whereas Ang1 and Tie2 expression during hypoxia remains controversial and differs *in vitro* and *in vivo* [16]. Despite the known alterations of expression of the Ang/Tie system, the effect of hypoxia on Ang/Tie-mediated signaling and angiogenic responses in endothelial cells has not been well elucidated. Thus, the objective of our study was to examine whether hypoxia affects Tie receptor expression, Ang1-induced signaling, and angiogenic responses in endothelial cells.

Abbreviations: Ang1, angiopoietin-1; DFX, desferrioxamine; HIF, hypoxia-inducible factor; HUVEC, human umbilical vein endothelial cell; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase; VEGF, vascular endothelial growth factor; VE-PTP, vascular endothelial protein tyrosine phosphatase.

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2. Materials and methods

2.1. Reagents

Recombinant human Ang1 and Ang2 proteins were purchased from R&D Systems (Minneapolis, MN). The following antibodies were used: anti-phosphotyrosine (Millipore, MA, USA), anti-Tie1 (Santa Cruz Biotechnology, CA, USA), anti-Tie2 (Millipore), anti-phospho-Erk1/2, anti-Erk1/2, anti-phospho-Akt, anti-Akt, anti-β-tubulin (Cell Signaling Technology, MA). Peroxidase-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology. Cell culture media, fetal bovine serum, and other chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Cell culture

Human umbilical vein endothelial cells (HUVECs) were purchased and cultured in EGM-2 basal medium containing growth factors supplied by the manufacturer (Lonza, Rockland, ME, USA). Cells were grown in a humidified 5% CO_2 atmosphere in an incubator, with oxygen tension at either 140 mmHg (20% O_2, v/v, normoxic conditions) or 7 mmHg (1% O_2, v/v, hypoxic conditions).

2.3. Western blot analysis

The cells were lysed in a buffer containing 20 mM Tris (pH 7.5), 150 mM NaCl, 1% Triton X-100, and a protease inhibitor cocktail. Proteins from cell lysates were separated on 7–10% SDS–PAGE gels and were transferred to nitrocellulose membranes. The membranes were incubated with the appropriate primary antibodies. Horseradish peroxidase-conjugated species-specific IgGs were used as secondary antibodies. An Enhanced Chemiluminescence Detection kit (Pierce, IL, USA) was used to detect and visualize the bands.

2.4. ShRNA knockdown of Tie receptors

Lentiviral particles encoding Tie1, Tie2, and control shRNA were purchased from Sigma–Aldrich. HUVECs were infected with the lentiviral particles according to the manufacturer's protocol (Sigma–Aldrich). After 7 days, the expression of Tie1 and Tie2 was monitored by Western blot analysis.

2.5. Immunoprecipitation

Cell lysates were incubated with anti-phosphotyrosine antibody for 1 h at 4 °C. Protein A/G beads (Thermo Scientific, MA, USA) were added for collecting protein complexes for another 4 h at 4 °C. After washing, the precipitated proteins were separated on 7% SDS–PAGE gels and analyzed by Western blot analysis using anti-Tie1 and anti-Tie2 antibodies.

2.6. Flow cytometry analysis

The cells were incubated with His-tagged Ang1 (500 ng/mL × 10^5 cells) or bovine serum albumin (500 ng/mL × 10^5 cells) for 1 h at 4 °C. After washing, the cells were incubated with an anti-His antibody or a mouse IgG for 1 h at 4 °C, followed by the incubation with FITC-conjugated secondary antibodies. Fluorescence was detected using a FACScan flow cytometer with CELLQuest software (Becton Dickinson, UK).

2.7. Tube formation assay

A 50 μL suspension of HUVECs (5×10^4 cells per well) was added to 24-well plates coated with growth factor-reduced Matrigel™ (BD biosciences) and was incubated in a 5% CO_2 atmosphere for 16 h at 37 °C. The plate was examined under a light microscope, and the phase contrast image was captured by a digital camera. Vessel length and tube area were quantitated using ImageJ software (NIH, Bethesda, MD, USA).

2.8. Statistical analysis

The data are presented as the mean ± SEM. The statistical analyses were performed using Prism 5.0 software (GraphPad Software, Inc., CA, USA), and Student's *t*-test was used to compare the differences between the groups, with $P < 0.05$ considered to be statistically significant.

3. Results

3.1. Tie1 impairs Ang1-induced Tie2 activity during hypoxia

To examine the effect of hypoxia on the expression of Tie1 and Tie2, HUVECs were subjected to 1% O_2 for up to 24 h. Hypoxia significantly increased both Tie1 and Tie2 protein levels in a time dependent manner, beginning after 12 h of hypoxia (Fig. 1A). Although Tie receptor expression was increased after hypoxia, the basal activation of Erk1/2 and Akt was not changed (Fig. 1A).

To determine the consequences of increased Tie receptor expression, we examined the ability of Ang1 stimulation to activate and phosphorylate Tie1 and Tie2 receptors under hypoxic conditions. HUVECs were exposed to hypoxic or normoxic conditions for 12 h followed by the addition of Ang1 (300 ng/mL) for 10 min. Under the normoxic conditions, Ang1 induced both Tie1 and Tie2 phosphorylation (Fig. 1B). In contrast, Ang1-induced Tie2 phosphorylation was considerably lower after hypoxic conditions compared with normoxic conditions (Fig. 1B). To exclude the possibility that reoxygenation during Ang1 treatment affects Tie receptor activities, we examined the same phenomenon in the presence of the hypoxia mimetic iron chelator desferrioxamine (DFX). We confirmed that both Tie1 and Tie2 were activated after 10 min of Ang1 treatment (Fig. 1C). These results indicated that hypoxia increased both Tie1 and Tie2 expression but attenuated Ang1-induced Tie2 phosphorylation.

Because the activation of mitogen-activated protein kinase (Erk1/2) and phosphatidylinositol 3-kinase (PI3K) by Ang1 is a well-recognized pathway in endothelial cells [17], we examined whether Ang1-induced Erk1/2 and Akt signaling were also modified under hypoxic conditions. When HUVECs were exposed to hypoxic conditions, Ang1-induced Erk1/2 and Akt phosphorylation were decreased compared with those values in normoxic conditions (Fig. 1D).

We next examined whether the decreased activation of cellular signaling pathways during hypoxic conditions was associated with the decreased binding of Ang1 to the Tie2 receptor in endothelial cells. Unexpectedly, the binding of Ang1 to HUVECs increased under hypoxic conditions compared with the binding in normoxic conditions (Fig. 1E), indicating that the diminished activation of cellular signaling pathways by Ang1 after hypoxia was not related to Ang1 binding to the Tie2 receptor.

3.2. Suppression of Tie1 restores Ang1-induced Tie2 activity during hypoxia

Based on these findings, we hypothesized that Tie2 and its downstream cellular signaling activities were suppressed by hypoxia and that some negative regulator reduced agonist-induced Tie2 activity. Because Tie1 has the ability to inhibit Ang1/Tie2 signaling [9], we examined whether Tie1 was associated with the hypoxic suppression of Ang1/Tie2 signaling. Using HUVECs transfected with a lentiviral shRNA construct to knockdown Tie1, we

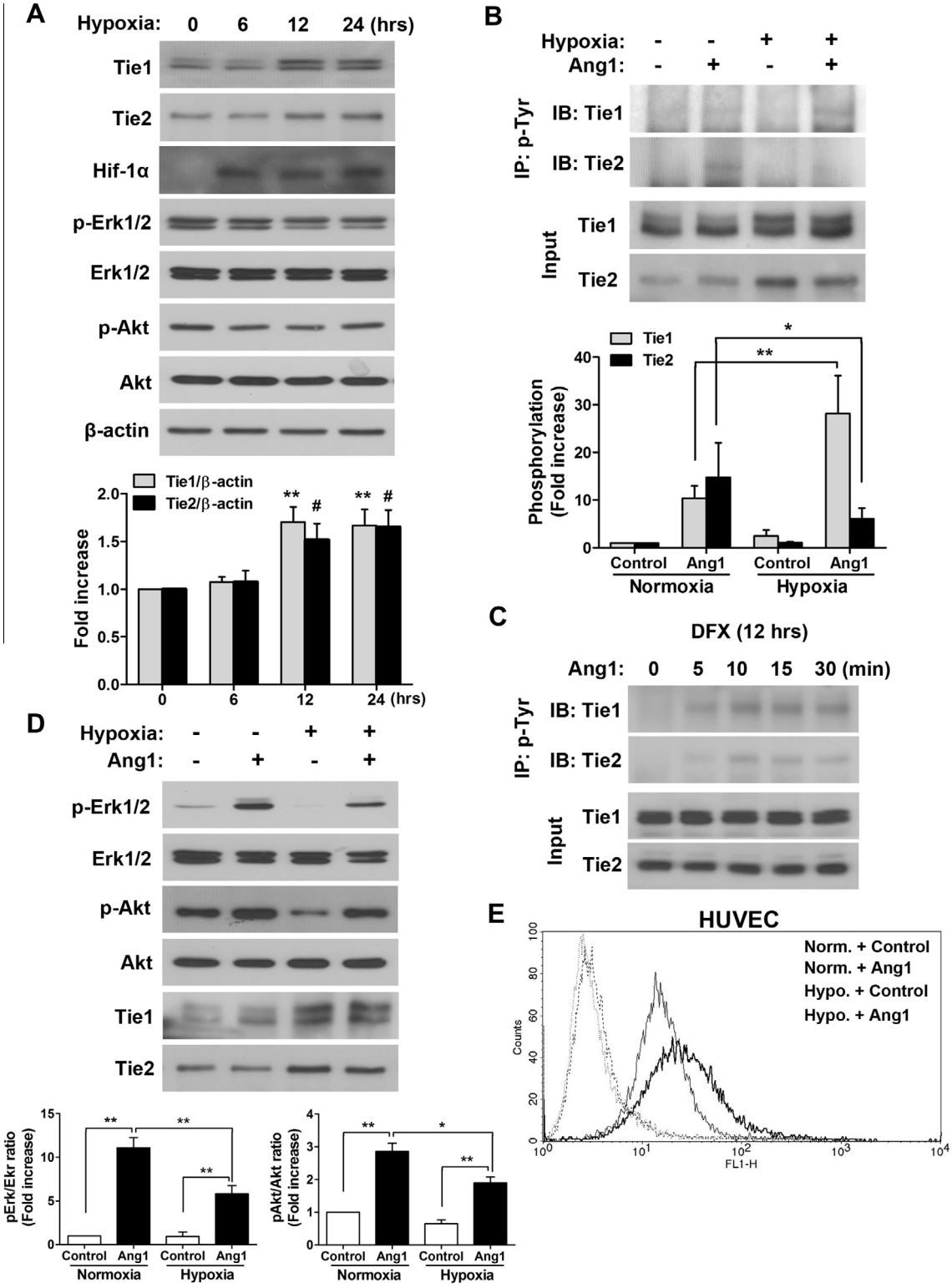

Fig. 1. Effects of hypoxia on Tie1 and Tie2 receptor expression and their responsiveness to Ang1. (A) HUVECs were incubated under hypoxic conditions (1% O_2) for 24 h. Cells were lysed at the indicated times, and the expression of the indicated proteins was examined by Western blot analysis. Results are given as fold increases versus 0 h values and plotted as mean ± SEM. ** and # denote $P < 0.01$ and $P < 0.05$ versus 0 h values, respectively ($n = 4$). (B) After 12 h of incubation under normoxic and hypoxic conditions, HUVECs were treated with Ang1 for 10 min. Cell lysates were subjected to immunoprecipitation with an anti-phosphotyrosine antibody. Tie receptor phosphorylation was detected with anti-Tie1 and Tie2 antibodies. Input indicates the Tie1 and Tie2 protein levels in the lysates used for immunoprecipitation. Results are given as fold increases versus control values under normoxia and plotted as mean ± SEM. * and ** denote $P < 0.05$ and $P < 0.01$ between the indicated groups, respectively ($n = 4$). (C) HUVECs were treated with DFX for 12 h and followed by Ang1 treatment for the indicated times. Cell lysates were subjected to immunoprecipitation with anti-phosphotyrosine antibody and blots were detected with anti-Tie1 and Tie2 antibodies. (D) After 12 h of normoxia or hypoxia, HUVECs were treated with Ang1 for 10 min and lysed. The indicated signaling proteins were detected by Western blot analysis. Results are given as fold increases versus control values under normoxia and plotted as mean ± SEM. * and ** denote $P < 0.05$ and $P < 0.01$ between the indicated groups, respectively ($n = 4$). (E) After 12 h of normoxia (Norm.) or hypoxia (Hypo.), Ang1 binding to HUVECs was determined by Ang1-stained flow cytometry. FL1-H indicates FITC fluorescence intensity, and counts indicate the number of cells. Data are representative of three independent experiments.

found that Ang1 induced an increase in phosphorylated Tie2 and its downstream signaling pathways (Erk1/2 and Akt) after hypoxia when compared with phosphorylation levels after normoxia (Fig. 2A). We also examined whether increased Tie1 phosphorylation after hypoxia solely produced the aforementioned cellular signaling. Utilizing a lentiviral shRNA knockdown construct targeting Tie2 in HUVECs, we observed that Ang1-induced phosphorylation of Erk1/2 and Akt was completely abolished under both normoxic and hypoxic conditions (Fig. 2B). Together, the results suggested that Ang1 activated cellular signaling predominantly in a Tie2-dependent manner but not in a Tie1-dependent manner.

We next determined whether Tie1 was involved in Ang1 binding to HUVECs. Employing the same shRNAs to knockdown Tie1 in HUVECs, we found that the binding of Ang1 to HUVECs under hypoxic conditions was similar to the binding under normoxic conditions (Fig. 2C). These results suggested that Tie1 did not affect the Ang1 binding to HUVECs but modified Ang1/Tie2 activity.

3.3. Suppression of Tie1 restores Ang1-induced tube formation under hypoxic conditions

We next employed an *in vitro* model of endothelial tube formation to address the biological significance of our biochemical observations. When HUVECs were exposed to hypoxic conditions, Ang1-induced tube formation in HUVECs was suppressed compared with tube formation under normoxic conditions (Fig. 3). In contrast, upon knockdown of Tie1 with lentiviral shRNA particles, the hypoxic suppression of Ang1-induced tube formation in HUVECs was abolished. Image analyses showed that both the tube length and tube area were significantly reduced in HUVECs under hypoxic conditions but restored or increased after Tie1 knockdown (Fig. 3B and C).

3.4. Tie1-mediated suppression of Ang1/Tie2 signaling is not dependent on phosphatase activity

We next wondered how Tie2 activity was inhibited by Tie1. Because Tie2 activity can be negatively modulated by tyrosine phosphatases [18,19], we investigated the possibility of the involvement of tyrosine phosphatases in Tie1-induced suppression of Ang1/Tie2 signaling. When HUVECs were treated with the non-selective tyrosine phosphatase inhibitor pervanadate, Erk1/2 and Akt phosphorylation were induced without ligand stimulation. The levels of phosphorylation were comparable to the phosphorylation levels after Ang1 treatment. Under hypoxic conditions, pervanadate-induced Erk1/2 and Akt phosphorylation were

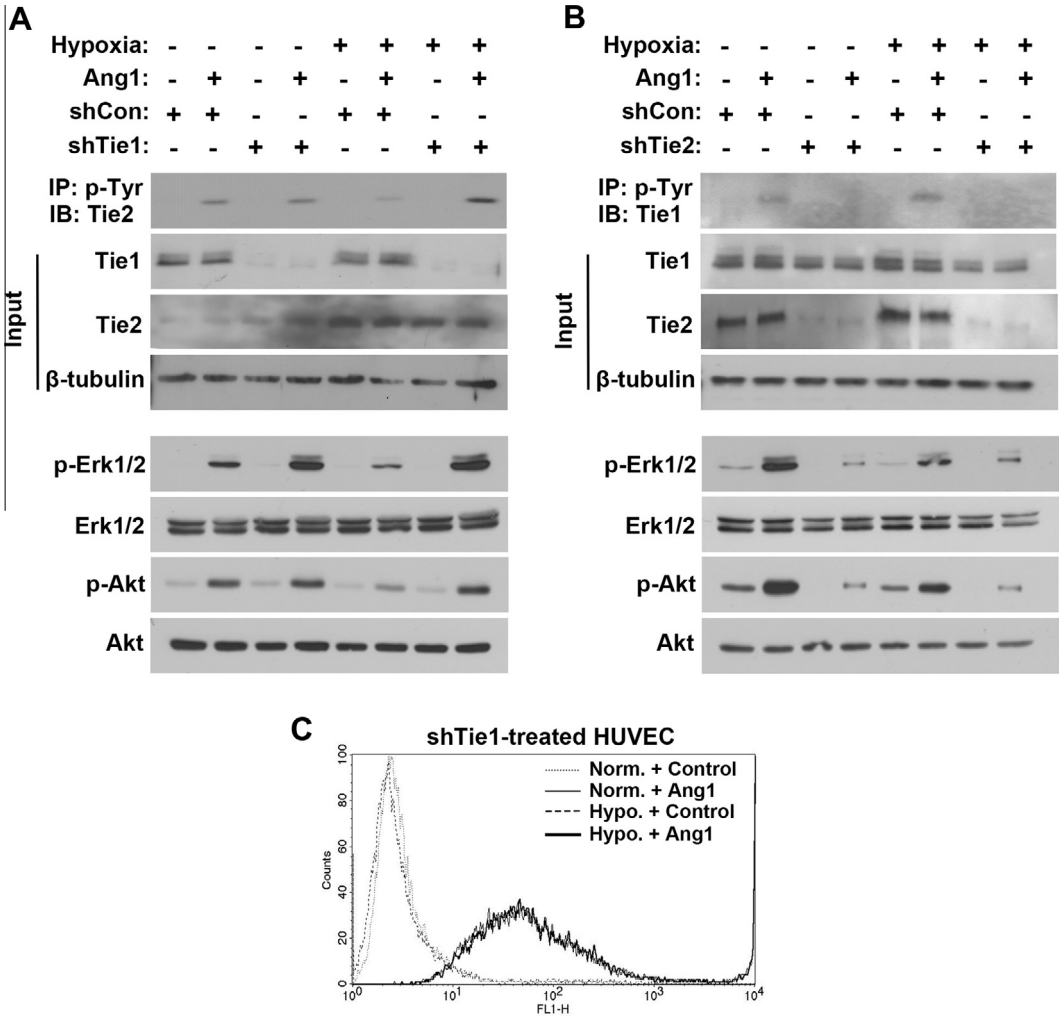

Fig. 2. Effect of Tie1 knockdown on Ang1-induced Tie2 activity. (A) ShRNA constructs targeting Tie1 (shTie1) or a control shRNA (shCon) were transfected into HUVECs, and the cells were incubated for 12 h of normoxia or hypoxia and followed by a 10 min Ang1 treatment. Cell lysates were subjected to immunoprecipitation with an anti-phosphotyrosine antibody. Tie2 phosphorylation was detected with an anti-Tie2 antibody. The indicated signaling proteins were also detected in the cell lysates. (B) An shRNA knockdown of Tie2 (shTie2) was transfected into HUVECs, and cells were analyzed to determine Tie1 activity and the effect of Tie2-knockdown on signaling pathways. (C) ShTie1-transfected HUVECs were incubated under normoxic (Norm.) and hypoxic (Hypo.) conditions. Ang1 binding to HUVECs was determined by Ang1-stained flow cytometry. FL1-H indicates FITC fluorescence intensity, and counts indicate the number of cells. Data are representative of three independent experiments.

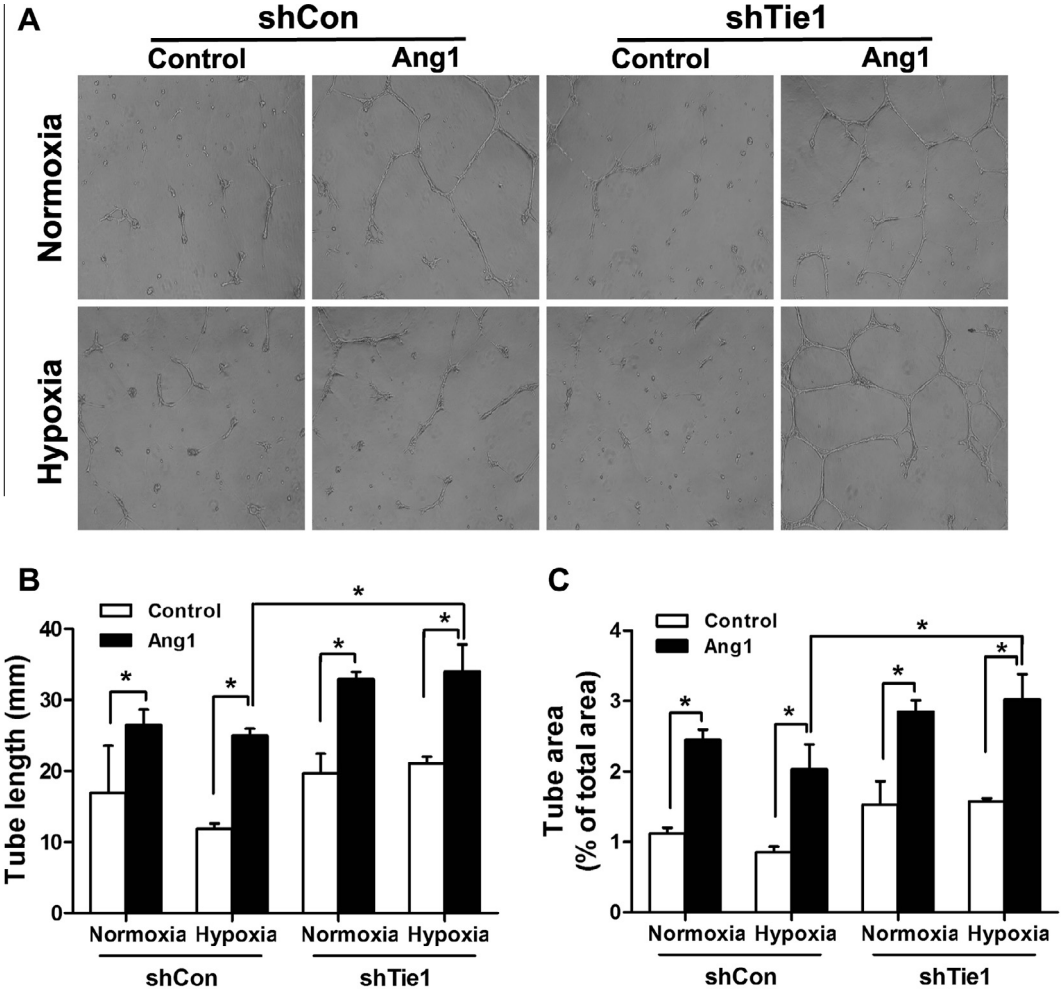

Fig. 3. Effect of Tie1 knockdown on Ang1-induced endothelial tube formation. (A) HUVECs were transfected with a control shRNA (shCon) or an shRNA knockdown of Tie1 (shTie1) and were cultured on Matrigel™-coated plates for 16 h of normoxia or hypoxia in the presence or absence of Ang1. The images of tube formation were captured at 40× magnification under a microscope. (B, C) A quantitative analysis of tube lengths (mm) and tube area (% of total area) in (A) was performed. * denotes $P < 0.05$ between the indicated groups, respectively ($n = 4$).

diminished to similar levels as with Ang1 treatment under hypoxic conditions (Fig. 4A). In addition, the potentiating effect of Tie1 knockdown on Erk1/2 and Akt phosphorylation under hypoxic conditions was synergistically enhanced by the phosphatase inhibitor (Fig. 4B). These results suggested that Tie1-mediated inhibition of Ang1/Tie2 signaling was not associated with tyrosine phosphatase activity.

4. Discussion

In the present study, we found that hypoxia suppressed Ang1-induced signaling pathways and responses in HUVECs through the increase of Tie1 expression independent of tyrosine phosphatase activity, even though hypoxia increased Tie2 receptor expression and Ang1 binding on HUVECs. These results suggested that Tie1 was critical for attenuating Ang1-induced Tie2 activity and angiogenesis under hypoxic conditions (Fig. 4C).

A classical response of cells to hypoxia is a delay of proliferation or growth arrest. To combat these phenomena, cells or tissues need to trigger a series of events to induce angiogenesis to supply oxygen. Thus, endothelial cells need to proliferate to form blood vessels under hypoxic conditions. Two possible mechanisms enable endothelial cells to survive or proliferate during hypoxic conditions to promote angiogenesis. The first is the hypoxic induction of growth factors by the HIF-1, which stimulates endothelial proliferation [20]. Vascular endothelial growth factor (VEGF) is the canonical angiogenic growth factor, which is strongly up-regulated by hypoxia and induces endothelial proliferation [21]. During hypoxia, VEGF also induces proper vascular development requiring the coordination with the expression of angiopoietin and Tie2 [22]. In the context of hypoxia, increased expression of Tie1 [15] and Tie2 in endothelial cells [23] and Ang1 in pericytes [24] contributes to growth factor-induced angiogenesis. However, it is not clear that the increased expression of Tie2 under hypoxic conditions reflects whether Tie2 activity is also increased. Here, we found that Tie2 expression was consistently increased and that Ang1 binding to HUVECs was also increased under hypoxic conditions (Fig. 1C). Nonetheless, hypoxia blunted the Ang1-induced Tie2 phosphorylation, Tie2 downstream signaling pathways, and endothelial tube formation (Figs. 1 and 3), suggesting that although hypoxia initiated angiogenesis, proper angiogenesis could not be fully supported due to decreased Ang1/Tie2 activity.

Along with VEGF induction, the second possible mechanism could be a direct induction of endothelial proliferation by hypoxia itself [25]. Whether hypoxia alone induces endothelial proliferation remains controversial. It has been reported that hypoxic stimulation of endothelial proliferation was not dependent on VEGF

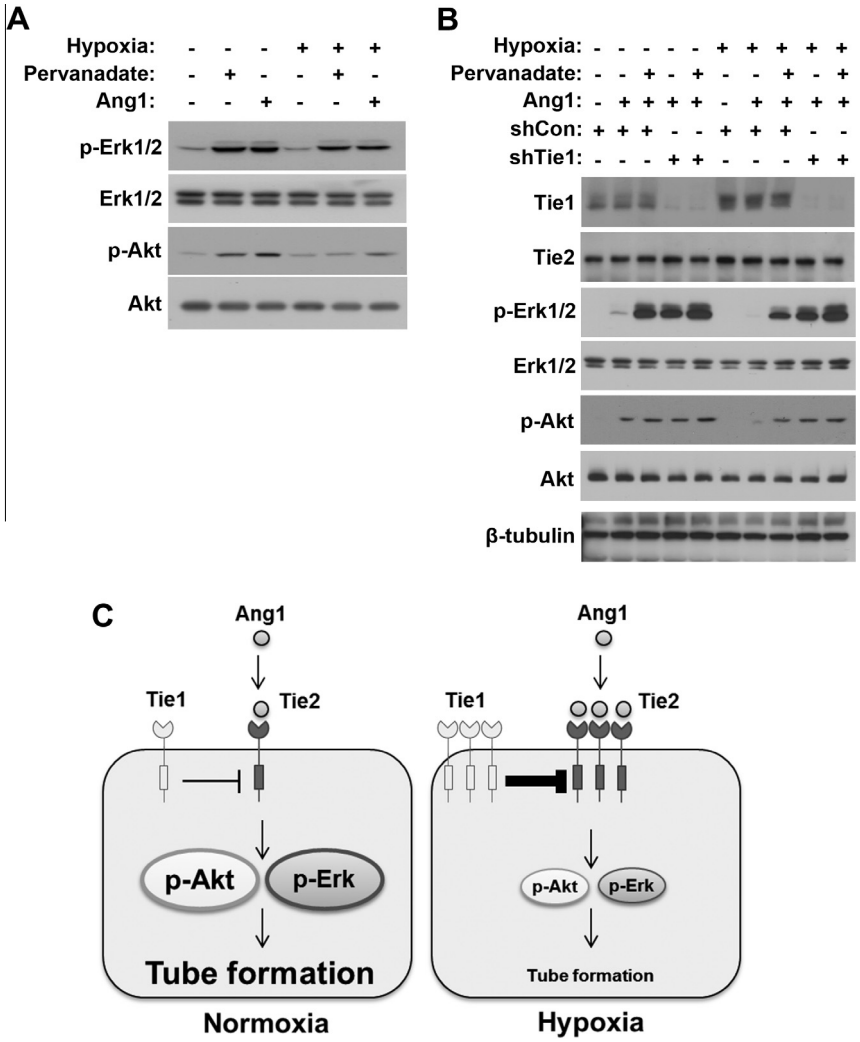

Fig. 4. Effect of tyrosine phosphatase inhibition on Tie1-mediated suppression of Ang1/Tie2 signaling. (A) After 12 h of normoxia or hypoxia, HUVECs were treated with Ang1 or pervanadate (1 mM) for 10 min. The indicated signaling proteins were detected by Western blot analysis of cell lysates. (B) A control shRNA (shCon) and an shRNA targeting Tie1 (shTie1) were transfected into HUVECS, and transfected cells were incubated for 12 h of normoxia or hypoxia and followed by a 10 min treatment of Ang1 or pervanadate. The indicated signaling proteins were detected by Western blot analysis of cell lysates. Data are representative of three independent experiments. (C) Schematic diagram of the proposed role of Tie1 in Ang1-induced Tie2 activity during hypoxia. Ang1 normally binds to and activate Tie2 downstream signaling including Akt and Erk1/2 phosphorylation. During hypoxic condition, Ang1-induced tube formation is suppressed compared with normoxic conditions. The hypoxic induction of Tie1 is critical to suppress the Ang1-induced Akt and Erk1/2 signaling and tube formation.

[26,27]. Moreover, a later study showed that hypoxia-induced endothelial proliferation was related to the mammalian target of rapamycin (mTOR) signaling pathway [28]. Conversely, other studies have suggested that persistent hypoxia alters the endothelial cell cycle to induce cell growth arrest and apoptosis [29,30]. In our experimental settings, we found that hypoxia alone induced neither Erk1/2 and Akt activation nor endothelial tube formation (Figs. 1A and 3). Moreover, when we examined cell cycle after hypoxia by staining DNA content, we found that hypoxia reduced the percentage of endothelial cells entering S phase (Supplement Fig. 1), suggesting that hypoxia led to endothelial cell cycle arrest. Therefore, growth factors induced by hypoxia may be critical for stimulating endothelial proliferation and angiogenesis during hypoxic conditions.

The mechanism responsible for the inhibition of Ang1/Tie2 activity under hypoxic conditions was associated with the increased expression of Tie1 (Fig. 2). Similar to Tie2 expression, Tie1 expression was also up-regulated under hypoxic conditions [15]. In addition, Tie1 down-regulation in endothelial cells is known to potentiate Ang1/Tie2 signaling pathways [9]. The inhibitory role of Tie1 in Tie2 signaling is in part associated with Tie1 phosphorylation, which is mediated by Ang1-induced Tie2 phosphorylation [8,9] (Fig. 2). A more detailed mechanism of how Tie1 activation inhibits Tie2 activity remains unknown. There are two possible explanations for a detailed mechanism. One possible explanation is that Tie1 interferes with Ang1 binding to Tie2, which decreases Tie2 activity. Another possible explanation is that Tie1 activation affects Tie2 downstream signaling pathways. Our results showing unchanged Ang1 binding to HUVECs in hypoxic conditions after Tie1 depletion ruled out the former possibility (Fig. 2C). As for the latter possibility, we hypothesized that Tie1-mediated Tie2 inhibition was associated with negative regulators of Tie2 activity. Tie2 is known to interact with tyrosine phosphatases, such as the vascular endothelial protein tyrosine phosphatase (VE-PTP) [18] and the tyrosine phosphatase SHP-2 [19], and both phosphatases are negative regulators of Tie2 phosphorylation. We found that treatment with a tyrosine phosphatase inhibitor, pervanadate, increased Tie2 phosphorylation (Fig. 4A). Moreover, we also found that Ang1-induced Tie2 activity was further potentiated after Tie1 suppression in the presence of pervanadate

(Fig. 4B), suggesting that Tie1-mediated Tie2 inhibition was independent of phosphatase activity. Therefore, how activated Tie1 inhibits Tie2 activity remains to be explored.

In summary, our results demonstrate that hypoxia increases Tie1 and Tie2 receptor expression, but reduces Ang1-induced Tie2 activation and tube formation. We also demonstrate that the induction of Tie1 expression impairs Ang1/Tie2 activity under hypoxic conditions in a tyrosine phosphatase-independent manner. Therefore, targeting Tie1 in hypoxic conditions could contribute to the control of vascular cell proliferation and angiogenesis in physiologic and pathologic environments.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.06.018.

References

[1] N.W. Gale, G.D. Yancopoulos, Growth factors acting via endothelial cell-specific receptor tyrosine kinases: VEGFs, angiopoietins, and ephrins in vascular development, Genes Dev. 13 (1999) 1055–1066.
[2] H.G. Augustin, G.Y. Koh, G. Thurston, K. Alitalo, Control of vascular morphogenesis and homeostasis through the angiopoietin–Tie system, Nat. Rev. Mol. Cell Biol. 10 (2009) 165–177.
[3] I. Kim, H.G. Kim, J.N. So, J.H. Kim, H.J. Kwak, G.Y. Koh, Angiopoietin-1 regulates endothelial cell survival through the phosphatidylinositol 3′-Kinase/Akt signal transduction pathway, Circ. Res. 86 (2000) 24–29.
[4] C.D. Kontos, T.P. Stauffer, W.P. Yang, J.D. York, L. Huang, M.A. Blanar, T. Meyer, K.G. Peters, Tyrosine 1101 of Tie2 is the major site of association of p85 and is required for activation of phosphatidylinositol 3-kinase and Akt, Mol. Cell. Biol. 18 (1998) 4131–4140.
[5] J. Gavard, V. Patel, J.S. Gutkind, Angiopoietin-1 prevents VEGF-induced endothelial permeability by sequestering Src through mDia, Dev. Cell 14 (2008) 25–36.
[6] J.R. Gamble, J. Drew, L. Trezise, A. Underwood, M. Parsons, L. Kasminkas, J. Rudge, G. Yancopoulos, M.A. Vadas, Angiopoietin-1 is an antipermeability and anti-inflammatory agent in vitro and targets cell junctions, Circ. Res. 87 (2000) 603–607.
[7] T.N. Sato, Y. Tozawa, U. Deutsch, K. Wolburg-Buchholz, Y. Fujiwara, M. Gendron-Maguire, T. Gridley, H. Wolburg, W. Risau, Y. Qin, Distinct roles of the receptor tyrosine kinases Tie-1 and Tie-2 in blood vessel formation, Nature 376 (1995) 70–74.
[8] P. Saharinen, K. Kerkela, N. Ekman, M. Marron, N. Brindle, G.M. Lee, H. Augustin, G.Y. Koh, K. Alitalo, Multiple angiopoietin recombinant proteins activate the Tie1 receptor tyrosine kinase and promote its interaction with Tie2, J. Cell Biol. 169 (2005) 239–243.
[9] H.T. Yuan, S. Venkatesha, B. Chan, U. Deutsch, T. Mammoto, V.P. Sukhatme, A.S. Woolf, S.A. Karumanchi, Activation of the orphan endothelial receptor Tie1 modifies Tie2-mediated intracellular signaling and cell survival, FASEB J. 21 (2007) 3171–3183.
[10] M.B. Marron, H. Singh, T.A. Tahir, J. Kavumkal, H.Z. Kim, G.Y. Koh, N.P. Brindle, Regulated proteolytic processing of Tie1 modulates ligand responsiveness of the receptor-tyrosine kinase Tie2, J. Biol. Chem. 282 (2007) 30509–30517.
[11] T.C. Seegar, B. Eller, D. Tzvetkova-Robev, M.V. Kolev, S.C. Henderson, D.B. Nikolov, W.A. Barton, Tie1–Tie2 interactions mediate functional differences between angiopoietin ligands, Mol. Cell 37 (2010) 643–655.
[12] D.V. Faller, Endothelial cell responses to hypoxic stress, Clin. Exp. Pharmacol. Physiol. 26 (1999) 74–84.
[13] P. Pichiule, J.C. Chavez, J.C. LaManna, Hypoxic regulation of angiopoietin-2 expression in endothelial cells, J. Biol. Chem. 279 (2004) 12171–12180.
[14] M.P. Simon, R. Tournaire, J. Pouyssegur, The angiopoietin-2 gene of endothelial cells is up-regulated in hypoxia by a HIF binding site located in its first intron and by the central factors GATA-2 and Ets-1, J. Cell. Physiol. 217 (2008) 809–818.
[15] M.J. McCarthy, M. Crowther, P.R. Bell, N.P. Brindle, The endothelial receptor tyrosine kinase tie-1 is upregulated by hypoxia and vascular endothelial growth factor, FEBS Lett. 423 (1998) 334–338.
[16] H.H. Marti, Angiogenesis-a self-adapting principle in hypoxia, EXS (2005) 163–180.
[17] R. Harfouche, J.P. Gratton, G.D. Yancopoulos, M. Noseda, A. Karsan, S.N. Hussain, Angiopoietin-1 activates both anti-and proapoptotic mitogen-activated protein kinases, FASEB J. 17 (2003) 1523–1525.
[18] G. Fachinger, U. Deutsch, W. Risau, Functional interaction of vascular endothelial-protein-tyrosine phosphatase with the angiopoietin receptor Tie-2, Oncogene 18 (1999) 5948–5953.
[19] L. Huang, C.W. Turck, P. Rao, K.G. Peters, GRB2 and SH-PTP2: potentially important endothelial signaling molecules downstream of the TEK/TIE2 receptor tyrosine kinase, Oncogene 11 (1995) 2097–2103.
[20] D.J. Manalo, A. Rowan, T. Lavoie, L. Natarajan, B.D. Kelly, S.Q. Ye, J.G. Garcia, G.L. Semenza, Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1, Blood 105 (2005) 659–669.
[21] A.K. Olsson, A. Dimberg, J. Kreuger, L. Claesson-Welsh, VEGF receptor signalling-in control of vascular function, Nat. Rev. Mol. Cell Biol. 7 (2006) 359–371.
[22] J. Holash, S.J. Wiegand, G.D. Yancopoulos, New model of tumor angiogenesis: dynamic balance between vessel regression and growth mediated by angiopoietins and VEGF, Oncogene 18 (1999) 5356–5362.
[23] C. Willam, P. Koehne, J.S. Jurgensen, M. Grafe, K.D. Wagner, S. Bachmann, U. Frei, K.U. Eckardt, Tie2 receptor expression is stimulated by hypoxia and proinflammatory cytokines in human endothelial cells, Circ. Res. 87 (2000) 370–377.
[24] Y.S. Park, N.H. Kim, I. Jo, Hypoxia and vascular endothelial growth factor acutely up-regulate angiopoietin-1 and Tie2 mRNA in bovine retinal pericytes, Microvasc. Res. 65 (2003) 125–131.
[25] R. Humar, F.N. Kiefer, H. Berns, T.J. Resink, E.J. Battegay, Hypoxia enhances vascular cell proliferation and angiogenesis in vitro via rapamycin (mTOR)-dependent signaling, FASEB J. 16 (2002) 771–780.
[26] M. Schafer, C. Schafer, N. Ewald, H.M. Piper, T. Noll, Role of redox signaling in the autonomous proliferative response of endothelial cells to hypoxia, Circ. Res. 92 (2003) 1010–1015.
[27] M. Schafer, N. Ewald, C. Schafer, A. Stapler, H.M. Piper, T. Noll, Signaling of hypoxia-induced autonomous proliferation of endothelial cells, FASEB J. 17 (2003) 449–451.
[28] W. Li, M. Petrimpol, K.D. Molle, M.N. Hall, E.J. Battegay, R. Humar, Hypoxia-induced endothelial proliferation requires both mTORC1 and mTORC2, Circ. Res. 100 (2007) 79–87.
[29] C. Li, R. Issa, P. Kumar, I.N. Hampson, J.M. Lopez-Novoa, C. Bernabeu, S. Kumar, CD105 prevents apoptosis in hypoxic endothelial cells, J. Cell Sci. 116 (2003) 2677–2685.
[30] T. Iida, S. Mine, H. Fujimoto, K. Suzuki, Y. Minami, Y. Tanaka, Hypoxia-inducible factor-1alpha induces cell cycle arrest of endothelial cells, Genes Cells 7 (2002) 143–149.