Biochemical and Biophysical Research Communications 453 (2014) 600–605

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Down-regulation of hypoxia-inducible factor-1 alpha and vascular endothelial growth factor by HEXIM1 attenuates myocardial angiogenesis in hypoxic mice

Noritada Yoshikawa [a,*,1], Noriaki Shimizu [a,1], Hidenori Ojima [b], Hiroshi Kobayashi [a], Osamu Hosono [a], Hirotoshi Tanaka [a,c]

[a] Department of Rheumatology and Allergy, IMSUT Hospital, Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan
[b] Department of Pathology, Keio University School of Medicine, 35, Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
[c] Division of Rheumatology, Center for Antibody and Vaccine, IMSUT Hospital, Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

ARTICLE INFO

Article history:
Received 28 September 2014
Available online 6 October 2014

Keywords:
Pulmonary hypertension
Cardiac hypertrophy
Angiogenesis
HEXIM1
HIF-1α
VEGF

ABSTRACT

Pulmonary hypertension (PH) sustains elevation of pulmonary vascular resistance and ultimately leads to right ventricular (RV) hypertrophy and failure and death. Recently, proangiogenic factors hypoxia-inducible factor-1 alpha (HIF-1α) and vascular endothelial growth factor (VEGF) have been known to promote left ventricular myocardial angiogenesis and lead to cardiac hypertrophy, and this would be involved in RV hypertrophy of PH patients. Previously, we revealed that overexpression of HEXIM1 prevents endothelin-1-induced cardiomyocyte hypertrophy and hypertrophic genes expression, and that cardiomyocyte-specific HEXIM1 transgenic mice ameliorates RV hypertrophy in hypoxia-induced PH model. Given these results, here we analyzed the effect of HEXIM1 on the expression of HIF-1α and VEGF and on myocardial angiogenesis of RV in PH. We revealed that overexpression of HEXIM1 prevented hypoxia-induced expression of HIF-1α protein and its target genes including VEGF in the cultured cardiac myocytes and fibroblasts, and that cardiomyocyte-specific HEXIM1 transgenic mice repressed RV myocardial angiogenesis in hypoxia-induced PH model. Thus, we conclude that HEXIM1 could prevent RV hypertrophy, at least in part, via suppression of myocardial angiogenesis through down-regulation of HIF-1α and VEGF in the myocardium under hypoxic condition.

1. Introduction

Pulmonary hypertension (PH) sustains elevation of pulmonary vascular resistance and ultimately leads to right ventricular (RV) hypertrophy and failure and death. Endothelin-receptor antagonists, phosphodiesterase type 5 inhibitors, and prostacyclin and its analog have been approved for the treatment of PH, however, these treatments are neither universally available nor always effective, thus, development of novel therapeutic strategies are anticipated [1]. Although the initial insult in PH involves the pulmonary vasculature, survival of patients with PH is closely related to RV function. However, it remains unknown how the RV adapts to the increased afterload and RV dysfunction occurs [2].

Disease-related cardiac remodeling is a complex process involving cardiac myocyte growth and death, vascular rarefaction, and fibrosis. In the left ventricle (LV), hypertrophic responses increase oxygen demand, promote myocardial angiogenesis, and sustain the increase in myocardial mass to dissolve the hypoxic situation and to maintain cardiac contractile function. The myocardium secretes various physiological substances including angiogenic growth factors and these responses induce crosstalk between cardiac myocytes and microvasculature and coordinate myocardial angiogenesis [3–5]. In the hypertrophied heart, myocardial angiogenesis is maintained by vascular endothelial growth factor (VEGF), which is mainly induced by hypoxia-inducible factor-1 alpha (HIF-1α) in relatively hypoxic conditions. In the advanced hypoxic condition of the failing heart, HIF-1α is inhibited by p53 accumulation in the myocardium, resulting in the suppression of myocardial angiogenesis and cardiac dysfunction [5–7]. However, the role of HIF-1α and VEGF on myocardial angiogenesis and remodeling in the RV has not been well defined.

* Corresponding author. Fax: +81 3 6409 2098.
E-mail address: n-yoshi@ims.u-tokyo.ac.jp (N. Yoshikawa).
[1] These co-authors are equally contributed this work.

http://dx.doi.org/10.1016/j.bbrc.2014.09.135
0006-291X/

HEXIM1 was initially identified as the protein that inhibits proliferation of vascular smooth muscle cells and has been shown to be involved in cancers, AIDS, inflammation, skeletal muscle regeneration, hormonal actions, and cardiac hypertrophy. One of the major biological functions of HEXIM1 is suppression of positive transcription elongation factor b (P-TEFb), which is a protein complex composed of cyclin-dependent kinase 9 (CDK9) and cyclin T1 (CycT1), and plays a key role in regulation of RNA polymerase II-dependent transcription elongation [8–12]. HEXIM1 knockout mice exhibited LV hypertrophy during the late stages of fetal development, whereas heart-specific activation of P-TEFb provoked LV hypertrophy in mice [13,14]. The genetic reduction of HEXIM1 in the background of elevated levels of CycT1 derepresses P-TEFb activity, emphasizing the importance of the role of HEXIM1 in the mechanism governing cardiac hypertrophy [15]. Recently, we also revealed that HEXIM1 suppressed endothelin-1 (ET-1)-induced myocyte growth and RV hypertrophy in hypoxia-induced PH model mice [16]. Together, HEXIM1 might play a critical role in the cardiac development and attenuation of the hypertrophic stimuli in the myocardium.

Recently, HEXIM1 has been shown as an inhibitor of tumor angiogenesis via down-regulation of HIF-1α protein expression and inhibition of HIF-1α-mediated transcription [17,18]. The mechanistic basis for regulation of HIF-1α by HEXIM1 has been considered that HEXIM1 down-regulates HIF-1α protein stability [19]. On the other hand, HEXIM1 re-expression in the heart results in the induction of angiogenesis that allows physiological hypertrophy via activation of several transcription factors including HIF-1α [20]. The roles of HEXIM1 on HIF-1α and angiogenesis in the heart, thus, remain controversial. Given this, we studied the effect of HEXIM1 on the expression of HIF-1α and VEGF and myocardial angiogenesis using cultured cardiac myocytes and fibroblasts, and cardiomyocyte-specific HEXIM1 transgenic mice under hypoxic condition.

2. Materials and methods

2.1. Ethics statement

All animal experimental procedures and protocols were approved by the Animal Experiment Committee of Institute of Medical Science, The University of Tokyo and conducted according to the institutional ethical guidelines for animal experiments.

2.2. Animals

C57BL/6J mice were obtained from CLEA Japan (Tokyo, Japan). The heterozygous mice expressing Cre recombinase driven by the alpha-MHC promoter (alphaMHC-Cre) were kindly provided from Dr. Kinya Otsu (Department of Internal Medicine and Therapeutics, Osaka University Graduate School of Medicine, Osaka, Japan). To create cardiomyocyte-specific HEXIM1 transgenic mice (HEX-Tg), heterozygous mice, which encode FLAG-His-tagged human HEXIM1 preceded by a floxed stuffer sequence, were mated with alpha-MHC-Cre mice as described previously [16].

2.3. Reagents and antibodies

ET-1 (E7764) and anti-actin antibodies (A2103) were purchased from Sigma–Aldrich (St. Louis, MO). Anti-rat and human HIF-1α antibodies were purchased from Novus Biologicals (NB 100-105, Littleton, CO) and BD Biosciences (610958, San Jose, CA), respectively. Anti-PECAM-1 antibodies (M-20) were obtained from Santa Cruz Biotechnologies (Santa Cruz, CA). Anti-human and rat HEXIM1 antibodies were generated as previously described [16]. Other reagents were obtained from Nacalai Tesque (Kyoto, Japan) unless otherwise specified.

2.4. Cell culture

HeLa cells were obtained from the RIKEN Cell Bank (Tsukuba, Japan) and primary cultures of neonatal rat cardiomyocytes and cardiac fibroblasts were prepared as described previously [21]. Cardiac and HeLa cells were grown in medium 199/DMEM (Invitrogen, Carlsbad, CA) and DMEM (Sigma–Aldrich), respectively, supplemented with 10% fetal calf serum and antibiotics in a humidified atmosphere at 37 °C with 5% CO_2. The culture media was replaced to phenol red and serum-free medium Opti-MEM I (Invitrogen) and adenovirus infection was performed for 24 h before culture under hypoxic condition and with various treatments as described previously [16]. Hypoxia was achieved by using an AnaeroPack System anaerobic jar (Mitsubishi Gas Chemical, Japan) and a methylene blue indicator to monitor oxygen depletion as described previously [22].

2.5. Recombinant adenoviruses

Recombinant adenoviruses encoding FLAG- and 6xhistidine (FLAG-His)-tagged human HEXIM1 (AdCALNL/FHhHEXIM1) preceded by a floxed stuffer sequence were generated by using Adenovirus Cre/loxP-regulated Expression Vector Set (TaKaRa, Otsu, Japan) as manufacturer's instructions and previously described [10]. Recombinant adenoviruses encoding Cre-recombinase (AxCANCre) and beta-galactosidase (AxCALNLZ, used as irrelevant adenovirus) were purchased from TaKaRa. These adenoviruses were prepared and purified as described previously [10].

2.6. Western blotting

Whole cell extracts were prepared in RIPA buffer (50 mmol/L Tris–HCl (pH 7.6), 150 mmol/L NaCl, 1% Nonidet-P40, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with 1 mmol/L DTT, 100 nmol/L MG132, protease inhibitor cocktail, and phosphatase inhibitor cocktail as described previously [23]. They were boiled in SDS sample buffer, resolved by SDS–PAGE, and electrically transferred to a PVDF membrane (Millipore, Bedford, MA). Subsequently, Western blotting was performed with appropriate primary antibodies diluted at 1:1000 and horseradish peroxidase-conjugated secondary antibodies (Amersham Biosciences, Buckinghamshire, UK) diluted at 1:2000. Antibody-protein complexes were visualized using the enhanced chemiluminescence method according to the manufacturer's protocol (Amersham Biosciences). Signal intensities of the bands were quantified by using the analysis software Image J from National Institutes of Health as described previously [16].

2.7. Quantitative RT-PCR (qRT-PCR) analysis

Total RNA was extracted from cell pellets using Sepasol-RNA I Super G (Nacalai Tesque) and subjected to reverse-transcription with oligo-dT primers using SuperScript™III First-Strand Synthesis System for RT-PCR (Invitrogen). PCR was performed with the LightCycler TaqMan Master, Universal ProbeLibrary Set, and LightCycler® ST300 systems (Roche, Indianapolis, Ind) according to the manufacturer's instructions as described previously [23]. Expression levels of mRNA were calculated on the basis of standard curves generated for each gene. The sequences of the primers used in this study are shown below:

Vegfa: 5′-aaaaacgaaagcgcaagaaa-3′ and 5′-tttctccgctctgaacaagg-3′.

Gapdh: 5′-agccacatcgctcagaca-3′ and 5′-gcccaatacgaccaaatcc-3′.
Ldha: 5′-gatctcgcgcacgctact-3′and 5′-cacaatcagctggtccttgag-3′.

2.8. Chronic hypoxia model of PH

Adult male wild-type (C57BL/6J) and HEX-Tg mice were randomized to the normoxia or hypoxia group. In hypoxia group, the mice were placed in an airtight chamber with access to food and water ad libitum, and exposed to 10% O_2 using a hypoxic air generator (TEIJIN, Tokyo, Japan) as described previously [16].

2.9. Histopathological analysis

Formalin-fixed tissues from each animal were cut in paraffin sections (4 μm thick) and mounted onto slides, and Hematoxylin-Eosin and Azan staining were performed with heart sections as described previously [24]. Immunohistochemistry (IHC) was performed using a polymer-based method (Envision+Dual Link System-HRP; Dako, Glostrup, Denmark). Formalin-fixed, paraffin-embedded serial tissue sections (4 μm) were placed on silane-coated slides. Sections cut through the maximum heart diameter were selected for IHC evaluation. The sections were deparaffinized and rehydrated in xylene and diluted ethanol (50–100%), and submerged for 20 min in 0.3% hydrogen peroxide with absolute methanol to block endogenous peroxidase activity. For antigen retrieval, the sections were autoclaved in 10 mmol/L citrate buffer (pH 6.0) at 121 °C for 10 min. After protein blocking, the sections were incubated with primary antibodies against PECAM-1 (diluted at 1:50) at room temperature for 1 h, followed by incubation with Envision+Dual Link reagent at room temperature for 30 min, and visualized using 3,3′-diaminobenzidine tetrahydrochloride as a chromogen. Finally, the sections were counterstained with hematoxylin. Sections were gently rinsed in phosphate-buffered saline between the incubation steps. Three image fields (approximately 230 μm × 170 μm/field) of each section were observed using fluorescence microscope Biozero BZ-8000 system (Keyence, Osaka, Japan). The degree of fibrosis in the images was estimated by BZ-Analyzer (Keyence). The number of vessels and myocytes was counted by two independent operators, and the mean of these values was determined. The number of capillaries per myocyte was then calculated as the ratio of the number of capillaries per square millimeter to the number of myocytes per square millimeter as described elsewhere [25].

2.10. Statistical analysis

Data were analyzed with Student's *t* test for unpaired data. *P* values below 0.05 were considered statistically significant. Graphs represent means ± SD.

3. Results

3.1. Overexpression of HEXIM1 decreases protein expression levels of HIF-1α in HeLa cells and cultured cardiac myocytes under hypoxic condition

At first, we examined the effects of overexpression of HEXIM1 using adenovirus system on the protein expression levels of HIF-1α in HeLa cells (Fig. 1A). The protein expression levels of HIF-1α were significantly increased under hypoxic condition compared with normoxic condition. Infection of adenoviruses encoding HEXIM1 prevented hypoxia-induced expression of HIF-1α protein (Fig. 1A). Next, we did similar set of experiments in primary cultured neonatal rat cardiac myocytes (Fig. 1B). We showed that hypoxia significantly increased protein expression levels of HIF-1α and that overexpression of HEXIM1 again repressed hypoxia-induced expression of HIF-1α protein (Fig. 1B).

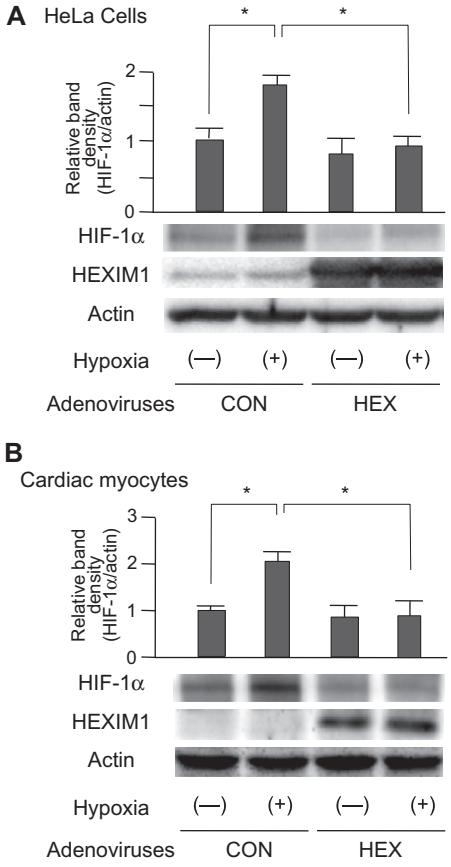

Fig. 1. Overexpression of HEXIM1 decreases protein expression levels of HIF-1α in HeLa cells and cultured cardiac myocytes. HeLa cells (A) and cultured cardiac myocytes (B) were infected with irrelevant AxCALNLZ (CON) or recombinant adenoviruses, which express FLAG-tagged human HEXIM1 (HEX) in the co-presence of Cre recombinase, along with Cre recombinase-expressing recombinant adenoviruses for 24 h. The cells were further cultured under normoxic or hypoxic condition for 24 h. Protein expression levels of HIF-1α, HEXIM1, and actin were analyzed by Western blotting and representative images from 5 independent experiments are shown. The band densities of HIF-1α detected by Western blotting were quantified and normalized to those of actin. Relative band densities compared to the values obtained from control cells (AxCALNLZ-infected and cultured under normoxic condition) are presented (means ± SD, $n = 5$). $^*P < 0.05$.

3.2. Overexpression of HEXIM1 prevents hypoxia- and ET-1-induced mRNA expression of HIF-1 target genes in cardiac myocytes and fibroblasts

Activation of HIF-1α induces expression of its target genes, including VEGF (*Vegfa*), glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*), and lactate dehydrogenase A (*Ldha*). Given this, we tested the effect of exogenously expressed HEXIM1 on those HIF-1α target gene expression. Hypoxia-triggered enhancement of mRNA expression was significantly repressed by overexpression of HEXIM1 in *Vegfa*, *Gapdh*, and *Ldha* in cardiac myocytes. HEXIM1 also repressed ET-1-induced expression of *Vegfa* mRNA (Fig. 2A). Moreover, in cultured cardiac fibroblasts, HEXIM1 also repressed hypoxia-induced gene expression of *Vegfa* (Fig. 2B).

3.3. HEXIM1 exerts antiangiogenic effects on hypertrophied right ventricle in hypoxia-induced PH mice

Myocardial capillary proliferation is believed to be involved in cardiac hypertrophy (See Section 1). Given that HEXIM1 sup-

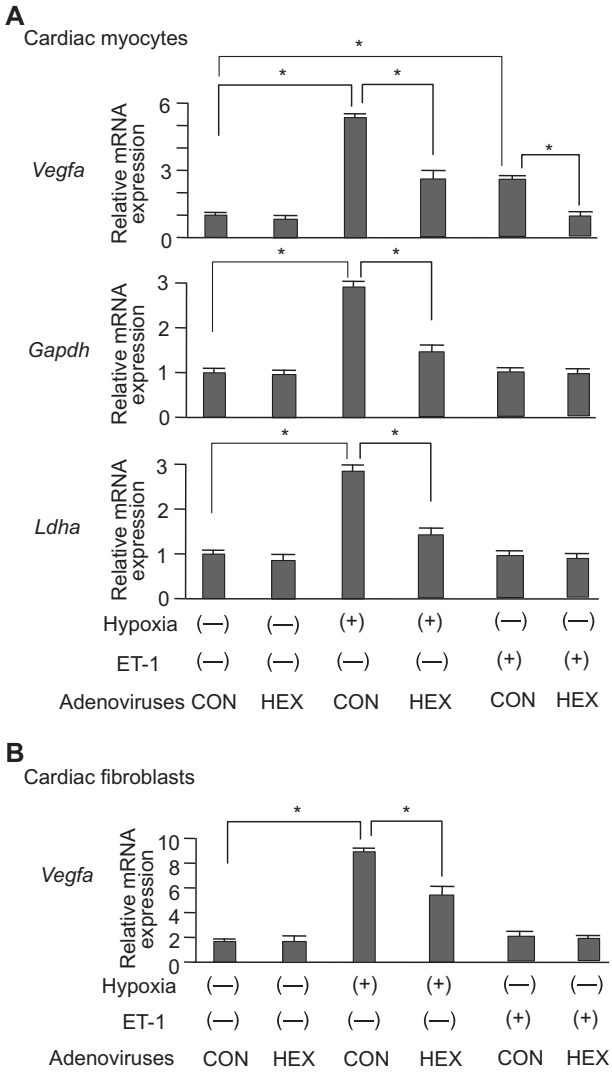

Fig. 2. Overexpression of HEXIM1 prevents hypoxia and ET-1-induced mRNA expression of vascular endothelial growth factor in cardiac myocytes and fibroblasts. Cardiac myocytes (A) or fibroblasts (B) were infected with irrelevant AxCALNLZ (CON) or recombinant adenoviruses, which express FLAG-tagged human HEXIM1 (HEX) in the co-presence of Cre recombinase, along with Cre recombinase-expressing recombinant adenoviruses for 24 h. The cells were treated with vehicle or 100 nmol/L ET-1 and further cultured under normoxic or hypoxic condition for 24 h. Total RNA was extracted from the cells and expression levels of indicated mRNA were assessed in qRT-PCR analysis. Results are shown as relative mRNA expression levels in the control cells (AxCALNLZ-infected, vehicle-treated, and cultured under normoxic condition). Error bars represent SD ($n = 5$). $^{*}P < 0.05$.

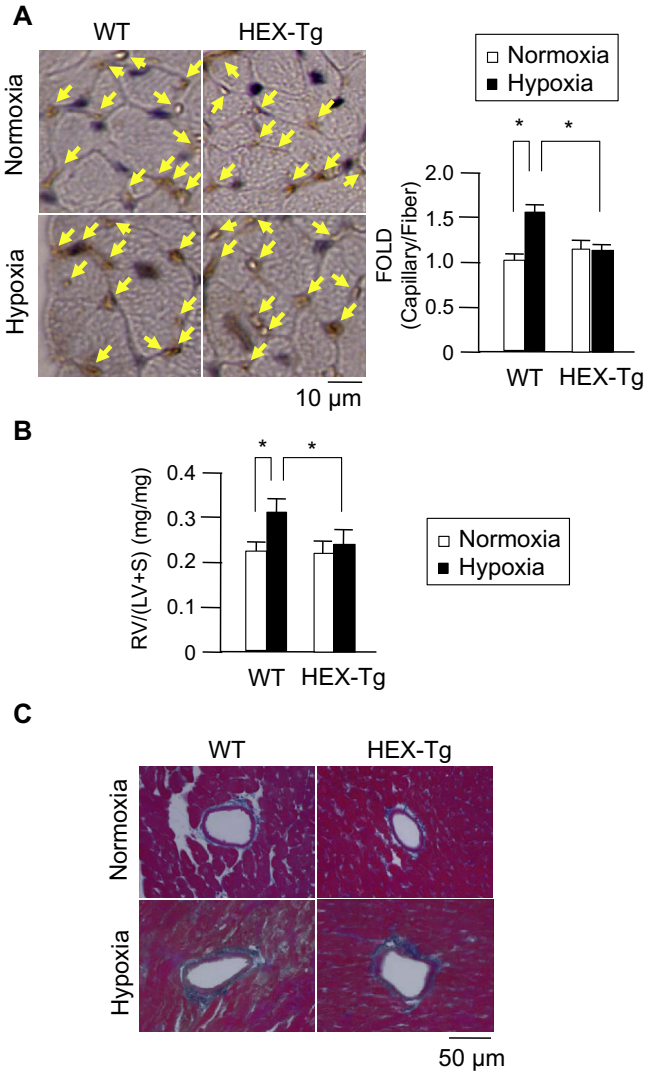

Fig. 3. HEXIM1 exerts antiangiogenic effects on hypertrophied right ventricle in hypoxia-induced PH mice. Wild-type (WT) and cardiomyocyte-specific HEXIM1 transgenic mice (HEX-Tg) were placed in normoxic or hypoxic conditions for 10 weeks and the effect of HEXIM1 on the development of RV myocardial capillary angiogenesis and remodeling in mice was analyzed. (A) Immunohistopathological analysis using anti-PECAM-1 antibodies was performed to demonstrate the presence of endothelial cells, the sections were counterstained with hematoxylin, and representative images of RV sections are shown. For each condition, the number of capillaries (yellow arrows) per myocytes (Capillary/Fiber) was calculated, and results are expressed as fold to those of WT mice placed in normoxic condition. (B) Assessments of the RV weight to LV + septum weight ratio (RV/LV + S) are shown. Results are expressed as means ± SD ($n = 10$). $^{*}P < 0.05$. (C) Azan staining was performed and representative images of RV sections in each mouse are shown.

presses proangiogenic substances including HIF-1α and VEGF *in vitro* (Figs. 1 and 2) and RV hypertrophy *in vivo* [16], we measured the capillary density with immunohistochemistry using anti-PECAM-1 antibodies in the RV walls of wild-type (WT) and cardiomyocytes-specific HEXIM1 transgenic (HEX-Tg) mice after exposure to chronic hypoxia. As shown in Fig. 3A, the capillary density was significantly increased in WT mice, but not in HEX-Tg mice in hypoxic condition. In this setting, we again confirmed that the RV weight to LV and septum weight ratio was increased by chronic hypoxia in WT mice but not in HEX-Tg mice, suggesting that the degree of RV hypertrophy was less marked in HEX-Tg mice compared with WT mice (Fig. 3B) [16]. On the other hand, chronic hypoxia appeared to induce mild fibrosis at perivascular space in RV wall in both mice at almost similar level (Fig. 3C). We, therefore, conclude that HEXIM1 prevents RV hypertrophy, at least in part, via suppression of myocardial angiogenesis through preventing HIF-1α protein accumulation and VEGF gene expression under hypoxic condition.

4. Discussion

Previous reports suggest that hypoxia-driven cardiac factors might independently participate in RV remodeling rather than pulmonary vascular constriction or remodeling in hypoxia-induced PH model [25–30]. Given that hypoxia induces HIF-1 activity and VEGF expression, we, in the present study, focused on the effects of HEXIM1 on those hypoxia-induced factors in the myocardium.

Here we showed that HEXIM1 suppressed myocardial protein expression of HIF-1α and mRNA induction of its target genes under hypoxic condition (Figs. 1 and 2). There have been pros and cons in the relationship between HEXIM1 and HIF-1α. In line with our results, the levels of HIF-1α protein was reported to be increased in HEXIM1+/− heterozygous hearts subjected ischaemic stress and that down-regulation of HIF-1α protein by HEXIM1 allows not only for inhibition of VEGF-regulated angiogenesis, but also for inhibition of migration and invasion of breast cancer cells [18,31]. Recently, HEXIM1 was shown to down-regulate HIF-1α protein stability by direct interaction with HIF-1α and up-regulation of hydroxylation of HIF-1α, resulting in the induction of the interaction of HIF-1α with pVHL (von Hippel–Lindau protein) and ubiquitination of HIF-1α [19]. On the other hand, HEXIM1 was suggested to interact with HIF-1α and increase HIF-1α protein expression in H9C2 cells [20]. HEXIM1, thus, could contribute to variable modulation of HIF-1α and adaptive myocardial remodeling via multiple mechanisms.

Angiogenesis-induced myocardial hypertrophy has been known that, in the pressure-overloaded heart, the myocardium becomes ischemic, and the DNA-binding activity of HIF-1α significantly increases and HIF-1α is stabilized in hypoxic conditions to transactivate various genes encoding hypoxia- and angiogenesis-associated proteins, such as VEGF, and this would be involved in the development of RV hypertrophy [2,5,32]. In this line, here we showed that not only the myocardial capillary density in RV wall but also RV mass is significantly increased under chronic hypoxia in wild-type mice, and we revealed that HEXIM1 transgenic mice ameliorates these cardiac responses (Fig. 3A and B). Moreover, we previously showed that HEXIM1 suppressed ET-1-induced hypertrophic cellular growth in cardiac myocytes and RV remodeling in hypoxia-induced PH model mice [16]. Together, HEXIM1 might efficiently control angiogenesis-induced myocardial hypertrophy via modulation of angiogenic factors including ET-1, HIF-1α, and VEGF in PH. Since direct interruption of cardiac remodeling, i.e., LV hypertrophy, has been suggested to be beneficial to decrease the risk of heart failure [33], we may propose that those negative effects of HEXIM1 on HIF-1α and VEGF might intervene excessive adaptive response of RV and delay consecutive progression to RV failure in PH. Our present study prompts us to work with cardiomyocyte-specific HIF-1α-knockout mice for verification of the role of HEXIM1 in prevention of RV remodeling in PH model for future publication. On the other hand, it is shown that prolonged pathological hypertrophy reveals down-modulation of HIF-1α and VEGF despite persistent myocardial hypoxia [5]. Moreover, HEXIM1 re-expression is shown to induce angiogenesis and hypertrophy via activation of several transcription factors including HIF-1α, c-Myc, GATA4, and PPAR-α [20]. We, therefore, conclude that pathological milieu to cardiac remodeling is multiple, but that HEXIM1 might be beneficial at a certain stage of RV remodeling in PH.

Acknowledgments

We thank Dr. Kinya Otsu and Dr. Motoaki Sano for providing alphaMHC-Cre and cardiomyocyte-specific HEXIM1 transgenic mice, respectively. We also thank all members of Tanaka Laboratory for helpful comments and clerical works.

This work was supported by Grants-in-Aid for Scientific Research (B) to HT (24390236), for Scientific Research (C) to NY (25461469) and to NS (26461375), and for Research Activity Start-up to HK (25893051), and by grants from SENSHIN Medical Research Foundation and Takeda Science Foundation to NY.

References

[1] R.M. Tuder, S.L. Archer, P. Dorfmüller, S.C. Erzurum, C. Guignabert, E. Michelakis, M. Rabinovitch, R. Schermuly, K.R. Stenmark, N.W. Morrell, Relevant issues in the pathology and pathobiology of pulmonary hypertension, J. Am. Coll. Cardiol. 62 (2013) D4–12.
[2] A. Vonk-Noordegraaf, F. Haddad, K.M. Chin, P.R. Forfia, S.M. Kawut, J. Lumens, R. Naeije, J. Newman, R.J. Oudiz, S. Provencher, A. Torbicki, N.F. Voelkel, P.M. Hassoun, Right heart adaptation to pulmonary arterial hypertension: physiology and pathobiology, J. Am. Coll. Cardiol. 62 (2013) D22–33.
[3] J.S. Burchfield, M. Xie, J.A. Hill, Pathological ventricular remodeling: mechanisms: part 1 of 2, Circulation 128 (2013) 388–400.
[4] M. Xie, J.S. Burchfield, J.A. Hill, Pathological ventricular remodeling: therapies: part 2 of 2, Circulation 128 (2013) 1021–1030.
[5] T. Oka, H. Akazawa, A.T. Naito, I. Komuro, Angiogenesis and cardiac hypertrophy: maintenance of cardiac function and causative roles in heart failure, Circ. Res. 114 (2014) 565–571.
[6] G.L. Semenza, Oxygen sensing, hypoxia-inducible factors, and disease pathophysiology, Annu. Rev. Pathol. 9 (2014) 47–71.
[7] Z. Taimeh, J. Loughran, E.J. Birks, R. Bolli, Vascular endothelial growth factor in heart failure, Nat. Rev. Cardiol. 10 (2013) 519–530.
[8] B.M. Peterlin, D.H. Price, Controlling the elongation phase of transcription with P-TEFb, Mol. Cell 23 (2006) 297–305.
[9] P. Hong, K. Chen, B. Huang, M. Liu, M. Cui, I. Rozenberg, B. Chaqour, X. Pan, E.R. Barton, X.C. Jiang, M.A. Siddiqui, HEXIM1 controls satellite cell expansion after injury to regulate skeletal muscle regeneration, J. Clin. Invest. 122 (2012) 3873–3887.
[10] N. Shimizu, R. Ouchida, N. Yoshikawa, T. Hisada, H. Watanabe, K. Okamoto, M. Kusuhara, H. Handa, C. Morimoto, H. Tanaka, HEXIM1 forms a transcriptionally abortive complex with glucocorticoid receptor without involving 7SK RNA and positive transcription elongation factor b, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 8555–8560.
[11] A. Dey, S.H. Chao, D.P. Lane, HEXIM1 and the control of transcription elongation: from cancer and inflammation to AIDS and cardiac hypertrophy, Cell Cycle 6 (2007) 1856–1863.
[12] E.J. Mascareno, I. Belashov, M.A. Siddiqui, F. Liu, M. Dhar-Mascareno, Hexim-1 modulates androgen receptor and the TGF-β signaling during the progression of prostate cancer, Prostate 72 (2012) 1035–1044.
[13] F. Huang, M. Wagner, M.A. Siddiqui, Ablation of the CLP-1 gene leads to down-regulation of the HAND1 gene and abnormality of the left ventricle of the heart and fetal death, Mech. Dev. 121 (2004) 559–572.
[14] M. Sano, M. Abdellatif, H. Oh, M. Xie, L. Bagella, A. Giordano, L.H. Michael, F.J. DeMayo, M.D. Schneider, Activation and function of cyclin T-Cdk9 (positive transcription elongation factor-b) in cardiac muscle-cell hypertrophy, Nat. Med. 8 (2002) 1310–1317.
[15] J. Espinoza-Derout, M. Wagner, L. Salciccioli, J.M. Lazar, S. Bhaduri, E. Mascareno, B. Chaqour, M.A. Siddiqui, Positive transcription elongation factor b activity in compensatory myocardial hypertrophy is regulated by cardiac lineage protein-1, Circ. Res. 104 (2009) 1347–1354.
[16] N. Yoshikawa, N. Shimizu, T. Maruyama, M. Sano, T. Matsuhashi, K. Fukuda, M. Kataoka, T. Satoh, H. Ojima, T. Sawai, C. Morimoto, A. Kuribara, O. Hosono, H. Tanaka, Cardiomyocyte-specific overexpression of HEXIM1 prevents right ventricular hypertrophy in hypoxia-induced pulmonary hypertension in mice, PLoS One 7 (2012) e52522.
[17] N. Ogba, Y.Q. Doughman, L.J. Chaplin, Y. Hu, M. Gargesha, M. Watanabe, M.M. Montano, HEXIM1 modulates vascular endothelial growth factor expression and function in breast epithelial cells and mammary gland, Oncogene 29 (2010) 3639–3649.
[18] W. Ketchart, K.M. Smith, T. Krupka, B.M. Wittmann, Y. Hu, P.A. Rayman, Y.Q. Doughman, J.M. Albert, X. Bai, J.H. Finke, Y. Xu, A.A. Exner, M.M. Montano, Inhibition of metastasis by HEXIM1 through effects on cell invasion and angiogenesis, Oncogene 32 (2013) 3829–3839.
[19] I.J. Yeh, N. Ogba, H. Bensigner, S.M. Welford, M.M. Montano, HEXIM1 down-regulates hypoxia-inducible factor-1α protein stability, Biochem. J. 456 (2013) 195–204.
[20] M.M. Montano, C.L. Desjardins, Y.Q. Doughman, Y.H. Hsieh, Y. Hu, H.M. Bensinger, C. Wang, J.E. Stelzer, T.E. Dick, B.D. Hoit, M.P. Chandler, X. Yu, M. Watanabe, Inducible re-expression of HEXIM1 causes physiological cardiac hypertrophy in the adult mouse, Cardiovasc. Res. 99 (2013) 74–82.
[21] N. Yoshikawa, M. Nagasaki, M. Sano, S. Tokudome, K. Ueno, N. Shimizu, S. Imoto, S. Miyano, M. Suematsu, K. Fukuda, C. Morimoto, H. Tanaka, Ligand-based gene expression profiling reveals novel roles of glucocorticoid receptor in cardiac metabolism, Am. J. Physiol. Endocrinol. Metab. 296 (2009) E1363–1373.
[22] S. Tokudome, M. Sano, K. Shinmura, T. Matsuhashi, S. Morizane, H. Moriyama, K. Tamaki, K. Hayashida, H. Nakanishi, N. Yoshikawa, N. Shimizu, J. Endo, T. Katayama, M. Murata, S. Yuasa, R. Kaneda, K. Tomita, N. Eguchi, Y. Urade, K. Asano, Y. Utsunomiya, T. Suzuki, R. Taguchi, H. Tanaka, K. Fukuda, Glucocorticoid protects rodent hearts from ischemia/reperfusion injury by activating lipocalin-type prostaglandin D synthase-derived PGD2 biosynthesis, J. Clin. Invest. 119 (2009) 1477–1488.
[23] N. Shimizu, N. Yoshikawa, N. Ito, T. Maruyama, Y. Suzuki, S. Takeda, J. Nakae, Y. Tagata, S. Nishitani, K. Takehana, M. Sano, K. Fukuda, M. Suematsu, C.

Morimoto, H. Tanaka, Crosstalk between glucocorticoid receptor and nutritional sensor mTOR in skeletal muscle, Cell Metab. 13 (2011) 170–182.
[24] J. Endo, M. Sano, J. Fujita, K. Hayashida, S. Yuasa, N. Aoyama, Y. Takehara, O. Kato, S. Makino, S. Ogawa, K. Fukuda, Bone marrow derived cells are involved in the pathogenesis of cardiac hypertrophy in response to pressure overload, Circulation 116 (2007) 1176–1184.
[25] C. Partovian, S. Adnot, S. Eddahibi, E. Teiger, M. Levame, P. Dreyfus, B. Raffestin, C. Frelin, Heart and lung VEGF mRNA expression in rats with monocrotaline- or hypoxia-induced pulmonary hypertension, Am. J. Physiol. 275 (1998) H1948–1956.
[26] M.K. Ball, G.B. Waypa, P.T. Mungai, J.M. Nielsen, L. Czech, V.J. Dudley, L. Beussink, R.W. Dettman, S.K. Berkelhamer, R.H. Steinhorn, S.J. Shah, P.T. Schumacker, Regulation of hypoxia-induced pulmonary hypertension by vascular smooth muscle hypoxia-inducible factor-1α, Am. J. Respir. Crit. Care Med. 189 (2014) 314–324.
[27] Y. Huang, R.P. Hickey, J.L. Yeh, D. Liu, A. Dadak, L.H. Young, R.S. Johnson, F.J. Giordano, Cardiac myocyte-specific HIF-1alpha deletion alters vascularization, energy availability, calcium flux, and contractility in the normoxic heart, FASEB J. 18 (2004) 1138–1140.
[28] J. Krishnan, M. Suter, R. Windak, T. Krebs, A. Felley, C. Montessuit, M. Tokarska-Schlattner, E. Aasum, A. Bogdanova, E. Perriard, J.C. Perriard, T. Larsen, T. Pedrazzini, W. Krek, Activation of a HIF1alpha-PPARgamma axis underlies the integration of glycolytic and lipid anabolic pathways in pathologic cardiac hypertrophy, Cell Metab. 9 (2009) 512–524.
[29] M. Hölscher, K. Schäfer, S. Krull, K. Farhat, A. Hesse, M. Silter, Y. Lin, B.J. Pichler, P. Thistlethwaite, A. El-Armouche, L.S. Maier, D.M. Katschinski, A. Zieseniss, Unfavourable consequences of chronic cardiac HIF-1α stabilization, Cardiovasc. Res. 94 (2012) 77–86.
[30] J. Wu, P. Chen, Y. Li, C. Ardell, T. Der, R. Shohet, M. Chen, G.L. Wright, HIF-1α in heart: protective mechanisms, Am. J. Physiol. Heart Circ. Physiol. 305 (2013) H821–828.
[31] E. Mascareno, I. Manukyan, D.K. Das, M.A. Siddiqui, Down-regulation of cardiac lineage protein (CLP-1) expression in CLP-1 +/− mice affords, J. Cell Mol. Med. 13 (2009) 2744–2753.
[32] G. Sutendra, P. Dromparis, R. Paulin, S. Zervopoulos, A. Haromy, J. Nagendran, E.D. Michelakis, A metabolic remodeling in right ventricular hypertrophy is associated with decreased angiogenesis and a transition from a compensated to a decompensated state in pulmonary hypertension, J. Mol. Med. (Berl) 91 (2013) 1315–1327.
[33] G. Esposito, A. Rapacciuolo, S.V. Naga Prasad, H. Takaoka, S.A. Thomas, W.J. Koch, H.A. Rockman, Genetic alterations that inhibit in vivo pressure-overload hypertrophy prevent cardiac dysfunction despite increased wall stress, Circulation 105 (2002) 85–92.