Histone deacetylase inhibition with trichostatin A does not reverse severe angioproliferative pulmonary hypertension in rats (2013 Grover Conference series)

Michiel Alexander De Raaf,¹ Aysar Al Hussaini,² Jose Gomez-Arroyo,² Donatas Kraskaukas,² Daniela Farkas,² Chris Happé,¹ Norbert F. Voelkel,² Harm Jan Bogaard¹

¹Department of Pulmonology, Pulmonary Arterial Hypertension Knowledge Centre, VU University Medical Center, Amsterdam, The Netherlands; ²Pulmonary and Critical Care Medicine Division, Virginia Commonwealth University, Richmond, Virginia, USA

Abstract: Pulmonary arterial hypertension (PAH) is a rapidly progressive and devastating disease characterized by remodeling of lung vessels, increased pulmonary vascular resistance, and eventually right ventricular hypertrophy and failure. Because histone deacetylase (HDAC) inhibitors are agents hampering tumor growth and cardiac hypertrophy, they have been attributed a therapeutic potential for patients with PAH. Outcomes of studies evaluating the use of HDAC inhibitors in models of PAH and right ventricular pressure overload have been equivocal, however. Here we describe the levels of HDAC activity in the lungs and hearts of rats with pulmonary hypertension and right heart hypertrophy or failure, experimentally induced by monocrotaline (MCT), the combined exposure to the VEGF-R inhibitor SU5416 and hypoxia (SuHx), and pulmonary artery banding (PAB). We show that HDAC activity levels are reduced in the lungs of rat with experimentally induced hypertension, whereas activity levels are increased in the hypertrophic hearts. In contrast to what was previously found in the MCT model, the HDAC inhibitor trichostatin A had no effect on pulmonary vascular remodeling in the SuHx model. When our results and those in the published literature are taken together, it is suggested that the effects of HDAC inhibitors in humans with PAH and associated RV failure are, at best, unpredictable. Significant progress can perhaps be made by using more specific HDAC inhibitors, but before clinical tests in human PAH can be undertaken, careful preclinical studies are required to determine potential cardiotoxicity.

Keywords: experimental pulmonary hypertension, HDAC inhibitors, HDAC activity.

Pulm Circ 2014;4(2):237-243. DOI: 10.1086/675986.

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a rapidly progressive and devastating disease, characterized by obstructive remodeling of lung vessels. If left untreated, and often despite treatment, the increased pulmonary vascular resistance eventually results in right ventricular (RV) dysfunction, heart failure, and death.¹ After linking biological hallmarks of cancer^{2,3} with some of the abnormalities found in the PAH lung, the hypothetical etiological focus of PAH shifted from the concept of vasoconstriction to concepts of quasimalignancy.⁴ Endothelial cell proliferation is currently given a more prominent role in the pathogenesis of the disease, and the view on vasodilators has changed, as these treatments are incapable of curing

PAH.⁴⁻⁶ Newer treatments are being developed which aim to reduce pulmonary vascular resistance by reversing the quasimalignant behavior of cells in the pulmonary vascular wall of PAH patients.⁷ Unfortunately, antiproliferative treatment of PAH with the tyrosine kinase inhibitor imatinib was associated with adverse events in clinical trials (particularly subdural hematoma), despite very promising initial preclinical and clinical studies.^{8,9}

As the insight developed that epigenetic modifications play a major role in the pathogenesis and behavior of several tumors,^{10,11} histone deacetylases (HDAC) inhibitors were shown to have therapeutic effects in malignant disease, including promotion of growth arrest, cell differen-

Address correspondence to Dr. Harm Jan Bogaard, VU University Medical Center, Department of Pulmonology, Pulmonary Arterial Hypertension Knowledge Centre, De Boelelaan 1117, 1081 HZ, Amsterdam, Netherlands. E-mail: hj.bogaard@vumc.nl

Submitted September 12, 2013; Accepted January 9, 2014; Electronically published May 21, 2014.

© 2014 by the Pulmonary Vascular Research Institute. All rights reserved. 2045-8932/2014/0402-0011. \$15.00.

tiation, and apoptosis.¹² Although additional studies are necessary, HDAC inhibitors were well tolerated by patients with urological cancers and were shown to have the potential to arrest tumor growth.¹⁰ At the same time, it was discovered that class IIa HDACs are involved in cardiac hypertrophy and that HDAC inhibitors could regulate hypertrophic responses.¹³

The combination of antimalignant effects and suppressive effects on cardiac hypertrophy makes HDAC inhibitors attractive candidates for the treatment of PAH. Beneficial responses were observed in rats with monocrotaline (MCT)- and chronic hypoxia-induced pulmonary hypertension.^{14,15} Unlike human PAH, these experimental models do not exhibit endothelial hyperproliferation, however, which makes their use in the clinical situation unpredictable. Moreover, we have shown that HDAC inhibition induces a functional deterioration in the pressure-overloaded right ventricle (RV), which was associated with the development of fibrosis and capillary rarefaction.¹⁶ Lungs and heart have different adaptation strategies and their own specific roles in the pathophysiology and outcome of PAH. Although hampering remodeling in the lungs might be a beneficial effects of HDAC inhibitors, they may also worsen the adaptive processes in the heart.¹⁴⁻¹⁷

To contribute to a better assessment of the therapeutic potential of HDAC inhibitor in PAH, we determined HDAC activities in lung and right ventricular tissue derived from 2 models of experimental PH and 1 model of isolated RV pressure overload. In addition, to determine the effect of HDAC inhibition on lung endothelial hyperproliferation, the key hallmark of human PAH, we assessed the therapeutic efficacy of trichostatin A in the Sugen hypoxia (SuHx) model. This model is based on the combined exposure of rats to the VEGF-R inhibitor SU5416 and hypoxia and shows a striking hemodynamic and histological resemblance to human severe PAH.

METHODS

Animal models

Male Sprague Dawley rats, weighing 180–200 g at the start of the experiments, were exposed to MCT or Sugen plus 10% hypoxia, as described previously.^{18,19} MCT and SuHx rats were terminated after 28 days. To induce a fixed RV pressure overload insensitive to drug treatment, another group of rats underwent pulmonary artery banding (PAB), as described elsewhere.^{18,20} Control animals were housed under similar conditions as the other groups and sacrificed after 4 weeks. Lungs and hearts were collected and immediately placed in liquid nitrogen to be stored at -80°C . The study was approved by the local ani-

mal welfare committee (VU Fys 11-11, Fys 11-16, and Fys 12-08).

HDAC activity

HDAC activity was determined by using the Fluor-de-Lys HDAC kit (Enzo Life Sciences, Farmingdale, NY), according to their instruction manual and recommendations.

Echocardiography, hemodynamics, Fulton index, and histology

Transthoracic echocardiography (Vevo770 imaging system, VisualSonics, Toronto, Ontario, Canada) allowed assessment of triscupid annular plane systolic excursion (TAPSE) and right ventricular inner diameter (RVID/d). Hemodynamics were measured invasively using a 4.5-mm Millar conductance catheter, which was inserted into the right ventricular outflow tract, to measure right ventricular systolic pressure (RVSP). After hemodynamic assessments, hearts were excised, and after removal of atria and the great arteries, the RV was separated from the residual left ventricle plus septum (LV + S). RV weight and LV + S weight were both determined, and the Fulton index ($\text{FI} = \text{RV}/\text{LV} + \text{S}$) was calculated. Lung tissue was fixated in formalin and processed, and slides were stained with hematoxylin and eosin.

TSA treatment study in SuHx

Severe angioproliferative pulmonary hypertension was induced in 2 groups of 4 male Sprague Dawley rats by their combined exposure to a subcutaneous injection of SU5416 (20 mg/kg) suspended in CMC and subsequent housing in 10% oxygen, for the duration of 4 weeks.^{20,21} In one of the SuHx groups, TSA was administered (450 mg/kg) for 5 times a week intraperitoneally starting on the day of Sugen administration and lasting for 4 weeks; the other SuHx group received vehicle only. On the last day of hypoxia (study day 28), RV function was evaluated by transthoracic echocardiogram, hemodynamics, and FI. A naive control group without induction of SuHx or TSA treatment, consisting of 4 animals, was included.

Statistical analyses

After confirmation that data were normally distributed, variables were compared between groups using two-way ANOVA with Bonferroni post hoc tests.

RESULTS

In comparison with normal rats, lung HDAC activity was significantly lower in the two models of experimental PH

Figure 1. Histone deacetylase (HDAC) activity was decreased in lung tissue (A) of monocrotaline (MCT)- and Sugen hypoxia (SuHx)-exposed rats. Lung tissue after pulmonary artery banding (PAB) was not affected. HDAC activity was increased in the pressure-overloaded right ventricle of all experimental models used (B). Comparisons with controls are denoted by $*$ ($P < .05$), $**$ ($P < .01$), and $***$ ($P < .001$).

(Fig. 1). All experimental models showed an increased HDAC activity in cardiac tissues in comparison with control rats.

RVSP and FI were significantly increased in SuHx rats and unaffected by TSA administration. Echocardiography showed a decreased TAPSE and increased RVID/d in SuHx rats, and, again, TSA treatment did not change these parameters (Fig. 2). Likewise, lung histology findings for

SuHx rats were unaltered after TSA treatment, with significant presence of occlusive vascular lesions and media hypertrophy (Fig. 3).

DISCUSSION

This study shows that HDAC activity is decreased in the lungs of rats with experimentally induced PH, whereas HDAC activity is increased in the pressure-overloaded RV.

Figure 2. Echocardiographic assessment and Fulton index of control rats and rats exposed to Sugen hypoxia (SuHx), with or without treatment with trichostatin A (TSA). Tricuspid annular plane systolic excursion (TAPSE), right ventricular inner diameter (RVID/d), and right ventricular systolic pressure (RVSP) were altered because of the induction of experimental pulmonary hypertension by SuHx, but no differences were seen between the SuHx group and SuHx group with TSA treatment. The Fulton index showed similar results. Comparisons with controls are denoted by $*$ ($P < .05$) and $**$ ($P < .01$).

Figure 3. Pulmonary vessel remodeling was seen in rats exposed to Sugen and hypoxia (SuHx) and consisted of medial hypertrophy and intima hyperplastic lesions in small arteries (indicated by arrowheads and arrows). Trichostatin A (TSA) treatment in SuHx rats did not affect pulmonary vessel remodeling (as shown in the column labeled SuHx + TSA). Hematoxylin and eosin staining; top two rows = original magnification $\times 40$; bottom row = original magnification $\times 200$.

Furthermore, we show that the pan-HDAC inhibitor TSA has no therapeutic or beneficial effects in SuHx-induced PH, because it neither affected pulmonary vascular remodeling nor improved cardiac function.

Gene expression is regulated by folding and unfolding of the DNA by histones. Unfolding is controlled by histone acetyltransferases (HATs) and facilitates expression of specific genes.²² Hampering of expression is caused by folding, which is regulated by histone deacetylases (HDACs).²² In various cell types, HDAC inhibitors exert therapeutic effects by direct suppression of overexpressed genes or, through the release from effects of suppressor genes, by increasing transcriptional activity of silenced genes. Additional effects of HDAC inhibitors are related to acetylation of other substrates than histones, including proteins and enzymes.^{12,23,24} Via these mechanisms, HDAC inhibitors affect proliferation, apoptosis, and differentiation as well as cardiac hypertrophy. This combination of effects makes HDAC inhibitors seemingly attractive candidate drugs for the treatment of PAH.

To study the preclinical efficacy of HDAC inhibitors in PH, several experimental models are available.^{25,26} PH was reversed by the HDAC inhibitors valproic acid (VPA) and suberoylanilide hydroxamic acid (SAHA) in two of

these models, namely the chronic hypoxia and MCT model (Fig. 4).^{14,25,27,28} However, given the fact that HDAC activity was low in all PH models we tested, even without treatment, it seems unlikely that the therapeutic effects of SAHA and VPA were mediated by a further reduction in HDAC activity levels. The treatment effects of HDAC inhibitors in chronically hypoxic and MCT rats may have resulted from the deacetylation of nonhistone protein substrates, including those involved in hypoxic angiogenesis via hypoxia inducible factor 1 alpha (HIF-1 α)-related signaling pathways.¹² Lung HIF-1 α protein expression is increased in MCT and chronically hypoxic rats and is thought to play a role in the vascular remodeling in these experimental models.²⁹ It has been suggested that TSA reduces the gene expression DNA-binding activity of HIF-1 α in a nonhistone related fashion, which would lead to a reduction in VEGF expression and inhibition of hypoxic angiogenesis.³⁰ The reduced overall HDAC activity we observed in the SuHx and MCT lung seems at odds with the previously reported increased protein expression of several specific HDACs in the lungs of MCT and chronically

Figure 4. The physiological response on histone deacetylase (HDAC) activity (blue) decreases in the lung (left panel), whereas cardiac HDAC activity (right panel) increases (blue) upon different experimental PH exposures. Treatment of HDAC inhibitors (HDACi) was beneficial in hypoxia (and monocrotaline; green), was neither worsening nor beneficial in sugen hypoxia (orange), and was detrimental for pulmonary artery banding in the heart (red). Bogaard = Bogaard et al.,¹⁸ Cavasin = Cavasin et al.,¹⁵ PAB = pulmonary artery banding; SuHx = Sugen and hypoxia; Zhao = Zhao et al.¹⁴

hypoxic rats.¹⁴ Unfortunately, HDAC activity levels have never been determined in human PAH lung tissue. A discrepancy between human HDAC protein expression and activity could point to dysfunction of specific HDAC enzymes, which were not studied at this time. A limitation of the current study is that, using an activity kit, it remains unknown which specific HDACs are responsible for overall HDAC activity.

We chose to test TSA in the SuHx model, because this model features intima remodeling in the arterioles and precapillaries and thereby gives new insights into the pathobiology and treatment of PAH.^{21,25,31} A therapeutic effect of TSA was not observed in this model (Figs. 2, 3). Because the TSA dose level and dosing strategy used in this study caused detrimental cardiac effects in PA-banded rats,¹⁶ we cannot attribute the absence of a treatment effect in SuHx rats to ineffective HDAC inhibition. This discrepancy with the therapeutic effects of HDAC inhibitors in other PH models is perhaps explained by the fact that the HIF-VEGF signaling pathway in this model is inhibited by SU5416, thereby rendering the model insensitive to further inhibition of HIF-1 α related pathways.²¹

Several investigators have shown that left ventricular hypertrophy, induced by stimuli varying from pressure overload to overexpression of prohypertrophic genes and continuous agonist infusion, is reduced or prevented by HDAC inhibitors.^{13,14,32-34} In preclinical studies, it is clear that HDAC inhibitors are actively lessening myocardial hypertrophy.^{14,15} HDAC inhibitors were almost exclusively tested in preventive treatment strategies. Studying the effects of HDAC inhibitors on RV function and hypertrophy in MCT or chronically hypoxic rats is problematic, because the treatment simultaneously changes RV afterload. To assess the therapeutic effect of HDACs on cardiac hypertrophy per se, PA-banding has been shown to be an important tool,³⁵ because this model only induces RV hypertrophy, without affecting the pulmonary vasculature.^{18,20} In pulmonary artery-banded or aortic-banded animals, HDAC inhibitors have resulted in controversial outcomes (Fig. 4).^{16,17,28} We showed that the adaptive cardiac hypertrophy, as induced by PAB, requires upregulation of HDAC activity and that TSA or VPA treatment leads to a deterioration of cardiac function.¹⁶

When the RV is pushed toward hypertrophy, major shifts in fetal gene expression are taking place to remodel the ventricle into a high-pressure pump.^{20,34,36,37} This compensatory gene response needs HDAC activity; blocking these fetal genes indirectly by HDAC inhibitors prevents cardiac hypertrophy.^{38,39} In line with this hypothesis, we

found an increased HDAC activity in RV tissue. Furthermore, Sano et al.⁴⁰ observed a bimodal pattern in gene expression during the hypertrophic response, with an initial increase in HIF-1 α expression early in the hypertrophic process, followed by a later decrease in HIF-1 α expression due to p53 accumulation when hypertrophy is established. The bimodal pattern has also been confirmed in MCT-induced RV hypertrophy.⁴¹ The shift in expression during established hypertrophy hampers angiogenesis and the fetal remodeling program. Growth and maintenance of the capillary network are critical for the adaptation of the RV to pressure overload,¹⁸ and the inhibition of angiogenic pathways seems critical for the induction of RV failure by HDAC inhibitors.¹⁶

To date, preclinical work in models of pulmonary hypertension and RV pressure overload has relied on the exclusive use of rather unselective HDAC inhibitors. Significant progress can perhaps be made by using more specific isoform-selective HDAC inhibitors, which are currently under development and have been used with success in left heart disease animal models.^{13,42} When isoform-specific HDAC inhibitors do not provoke failure of the pressure overloaded RV, they would be worth considering for the treatment of PAH.

Essential differences are found in HDAC activity between heart and lung. Because lung HDAC activity is decreased in several experimental PH models, remodeling of the pulmonary vascular vessel seems not to require a continued increase in HDAC activity. In SuHx rats, HDAC inhibition by TSA did not lead to a reduction in pressure overload. RV HDAC activity was increased in several models of RV hypertrophy, which suggests that HDAC activity plays an important role in the compensation to pressure overload. The clinical implication of our findings is that the effects of HDAC inhibitors in humans with PAH are unpredictable and may include RV functional deterioration, especially when a reduction in pulmonary vascular resistance is not feasible.

ACKNOWLEDGMENTS

We would like to thank Silvia Rain, of the VU University Medical Center, for her supportive hands-on assistance regarding protein analyses.

Source of Support: We acknowledge support from the Netherlands CardioVascular Research Initiative: the Dutch Heart Foundation, Dutch Federation of University Medical Centers, the Netherlands Organization for Health Research and Development, and the Royal Netherlands Academy of Sciences.

Conflict of Interest: None declared.

REFERENCES

1. Simonneau G, Robbins IM, Beghetti M, et al. Updated clinical classification of pulmonary hypertension. *J Am Coll Cardiol* 2009;54(1 suppl):S43–S54.
2. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100(1):57–70.
3. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011;144(5):646–674.
4. Rai PR, Cool CD, King JAC, et al. The cancer paradigm of severe pulmonary arterial hypertension. *Am J Crit Care Med* 2008;178(6):558–564.
5. Stacher E, Graham BB, Hunt JM, et al. Modern age pathology of pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2012;186(3):261–272.
6. Morrell NW, Adnot S, Archer SL, et al. Cellular and molecular basis of pulmonary arterial hypertension. *J Am Coll Cardiol* 2009;54(1 suppl):S20–S31.
7. Humbert M, Sitbon O, Simonneau G. Treatment of pulmonary arterial hypertension. *N Engl J Med* 2004;351(14):1425–1436.
8. Hoeper MM, Barst RJ, Bourge RC, et al. Imatinib mesylate as add-on therapy for pulmonary arterial hypertension: results of the randomized IMPRES study. *Circulation* 2013;127(10):1128–1138.
9. Ghofrani HA, Morrell NW, Hoeper MM, et al. Imatinib in pulmonary arterial hypertension patients with inadequate response to established therapy. *Am J Respir Crit Care Med* 2010;182(9):1171–1177.
10. Sharma NL, Groselj B, Hamdy FC, Kiltie AE. The emerging role of histone deacetylase (HDAC) inhibitors in urological cancers. *BJU Int* 2013;111(4):537–542.
11. Gallinari P, Di Marco S, Jones P, Pallaoro M, Steinkühler C. HDACs, histone deacetylation and gene transcription: from molecular biology to cancer therapeutics. *Cell Res* 2007;17(3):195–211.
12. Ververis K, Hiong A, Karagiannis TC, Licciardi PV. Histone deacetylase inhibitors (HDACIs): multitargeted anticancer agents. *Biologics* 2013;7:47–60.
13. McKinsey TA. Therapeutic potential for HDAC inhibitors in the heart. *Annu Rev Pharmacol Toxicol* 2012;52:303–319.
14. Zhao L, Chen C-N, Hajji N, et al. Histone deacetylation inhibition in pulmonary hypertension: therapeutic potential of valproic acid and suberoylanilide hydroxamic acid. *Circulation* 2012;126(4):455–467.
15. Cavaasin MA, Demos-Davies K, Horn TR, et al. Selective class I histone deacetylase inhibition suppresses hypoxia-induced cardiopulmonary remodeling through an antiproliferative mechanism. *Circ Res* 2012;110(5):739–748.
16. Bogaard HJ, Mizuno S, Hussaini AAA, et al. Suppression of histone deacetylases worsens right ventricular dysfunction after pulmonary artery banding in rats. *Am J Respir Crit Care Med* 2011;183(10):1402–1410.
17. Kong Y, Tannous P, Lu G, et al. Suppression of class I and II histone deacetylases blunts pressure-overload cardiac hypertrophy. *Circulation* 2006;113(22):2579–2588.
18. Bogaard HJ, Natarajan R, Henderson SC, et al. Chronic pulmonary artery pressure elevation is insufficient to explain right heart failure. *Circulation* 2009;120(20):1951–1960.
19. Bogaard HJ, Natarajan R, Mizuno S, et al. Adrenergic receptor blockade reverses right heart remodeling and dysfunction in pulmonary hypertensive rats. *Am J Respir Crit Care Med* 2010;182(5):652–660.
20. Bogaard HJ, Abe K, Vonk Noordegraaf A, Voelkel NF. The right ventricle under pressure: cellular and molecular mechanisms of right-heart failure in pulmonary hypertension. *Chest* 2009;135(3):794–804.
21. Taraseviciene-Stewart L, Kasahara Y, Alger L, et al. Inhibition of the VEGF receptor 2 combined with chronic hypoxia causes cell death-dependent pulmonary endothelial cell proliferation and severe pulmonary hypertension. *FASEB J* 2001;15(2):427–438.
22. Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. *Cell* 2007;128(4):669–681.
23. Ocker M. Deacetylase inhibitors: focus on non-histone targets and effects. *World J Biol Chem* 2010;1(5):55–61.
24. Dobaczewski M, Bujak M, Li N, et al. Smad3 signaling critically regulates fibroblast phenotype and function in healing myocardial infarction. *Circ Res* 2010;107(3):418–428.
25. Nicolls MR, Mizuno S, Taraseviciene-Stewart L, et al. New models of pulmonary hypertension based on VEGF receptor blockade-induced endothelial cell apoptosis. *Pulm Circ* 2012;2(4):434–442.
26. Ryan J, Bloch K, Archer SL. Rodent models of pulmonary hypertension: harmonisation with the world health organisation's categorisation of human PH. *Int J Clin Pract Suppl* 2011;(172):15–34.
27. Stenmark KR, Meyrick B, Galie N, Mooi WJ, McMurtry IF. Animal models of pulmonary arterial hypertension: the hope for etiological discovery and pharmacological cure. *Am J Physiol Lung Cell Mol Physiol* 2009;297(6):L1013–L1032.
28. Cho YK, Eom GH, Kee HJ, et al. Sodium valproate, a histone deacetylase inhibitor, but not captopril, prevents right ventricular hypertrophy in rats. *Circ J* 2010;74(4):760–770.
29. Lai Y-L, Law TC. Chronic hypoxia- and monocrotaline-induced elevation of hypoxia-inducible factor-1 alpha levels and pulmonary hypertension. *J Biomed Sci* 2004;11(3):315–321.
30. Kim MS, Kwon HJ, Lee YM, et al. Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. *Nat Med* 2001;7(4):437–443.
31. De Raaf MA, Schalij I, Gomez-Arroyo J, et al. SuHx rat model: partly reversible pulmonary hypertension and progressive intima obstruction. *Eur Respir J* 2014 (forthcoming).
32. Kee HJ, Sohn IS, Nam KI, et al. Inhibition of histone deacetylation blocks cardiac hypertrophy induced by angiotensin II infusion and aortic banding. *Circulation* 2006;113(1):51–59.
33. Kook H, Lepore JJ, Gitler AD, et al. Cardiac hypertrophy and histone deacetylase-dependent transcriptional repression mediated by the atypical homeodomain protein Hop. *J Clin Invest* 2003;112(6):863–871.
34. Kee HJ, Kook H. Roles and targets of class I and IIa histone deacetylases in cardiac hypertrophy. *J Biomed Biotechnol* 2011. doi:10.1155/2011/928326.
35. Voelkel NF. Pulmonary hypertension: the present and future. Shelton, CT: People's Medical, 2011.

36. Rajabi M, Kassiotis C, Razeghi P, Taegtmeyer H. Return to the fetal gene program protects the stressed heart: a strong hypothesis. *Heart Fail Rev* 2007;12(3–4):331–343.
37. Peacock AJ, Naeije R, Rubin LJ. *Pulmonary circulation*. 3rd ed. Boca Raton, FL: CRC, 2011.
38. Kee HJ, Kook H. Krüppel-like factor 4 mediates histone deacetylase inhibitor-induced prevention of cardiac hypertrophy. *J Mol Cell Cardiol* 2009;47(6):770–780.
39. Trivedi CM, Luo Y, Yin Z, et al. Hdac2 regulates the cardiac hypertrophic response by modulating Gsk3 beta activity. *Nat Med* 2007;13(3):324–331.
40. Sano M, Minamino T, Toko H, et al. p53-induced inhibition of Hif-1 causes cardiac dysfunction during pressure overload. *Nature* 2007;446(7134):444–448.
41. Sutendra G, Dromparis P, Paulin R, et al. 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* 2013;91(11):1315–1327.
42. McKinsey TA. Isoform-selective HDAC inhibitors: closing in on translational medicine for the heart. *J Mol Cell Cardiol* 2011;51(4):491–496.