Targeting histone acetylation in pulmonary hypertension and right ventricular hypertrophy

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Epigenetic mechanisms, including DNA methylation and histone post-translational modifications (PTMs), have been known to regulate chromatin structure and lineage-specific gene expression during cardiovascular development and disease. However, alterations in the landscape of histone PTMs and their contribution to the pathogenesis of incurable cardiovascular diseases such as pulmonary hypertension (PH) and associated right heart failure (RHF) remain largely unexplored. This review focusses on the studies in PH and RHF that investigated the gene families that write (histone acetyltransferases), read (bromodomain-containing proteins) or erase (histone deacetylases [HDACs] and sirtuins [SIRT]) acetyl moieties from the ε-amino group of lysine residues of histones and non-histone proteins. Analysis of cells and tissues isolated from the in vivo preclinical models of PH and human pulmonary arterial hypertension not only confirmed significant alterations in the expression levels of multiple HDACs, SIRT1, SIRT3 and BRD4 proteins but also demonstrated their strong association to proliferative, inflammatory and fibrotic phenotypes linked to the pathological vascular remodelling process. Due to the reversible nature of post-translational protein acetylation, the therapeutic efficacy of numerous small-molecule inhibitors (vorinostat, valproic acid, sodium butyrate, mocetinostat, entinostat, tubastatin A, apabetalone, JQ1 and resveratrol) have been evaluated in different preclinical models of PH and human pulmonary arterial hypertension not only confirmed significant alterations in the expression levels of multiple HDACs, SIRT1, SIRT3 and BRD4 proteins but also demonstrated their strong association to proliferative, inflammatory and fibrotic phenotypes linked to the pathological vascular remodelling process. Due to the reversible nature of post-translational protein acetylation, the therapeutic efficacy of numerous small-molecule inhibitors (vorinostat, valproic acid, sodium butyrate, mocetinostat, entinostat, tubastatin A, apabetalone, JQ1 and resveratrol) have been evaluated in different preclinical models of cardiovascular disease, which revealed the promising therapeutic benefits of targeting histone acetylation pathways in the attenuation of cardiac hypertrophy, fibrosis, left heart dysfunction, PH and RHF. This review also emphasizes the need for deeper molecular insights into the contribution of epigenetic changes to PH pathogenesis and therapeutic evaluation of isoform-specific modulation in ex vivo and in vivo models of PH and RHF.

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1 | INTRODUCTION

Pulmonary arterial hypertension (PAH) is a rapidly progressive vascular disease with multifactorial aetiology mediated by the interplay of a susceptible genetic background, epigenetic changes, and injurious events (Kim et al., 2015). Despite our increased understanding of the pathomechanisms of PAH and the recent therapeutic advances, PAH remains an incurable disease. Notably, preliminary findings support the hypothesis that there is a significant contribution of epigenetic mechanisms to PAH.

2 | EPIGENETIC MECHANISMS

An epigenetic trait is a stably inherited phenotype resulting from changes in a chromosome without alterations in the DNA sequence (Berger, Kouzarides, Shiekhattar, & Shilatifard, 2009). Several epigenetic mechanisms facilitate modification of chromatin structure, including (a) DNA methylation, (b) ATP-dependent chromatin remodelling, (c) histone post-translational modifications (PTMs), (d) non-coding RNAs, (e) replacement of canonical histones with histone variants and (f) (re)organization of the three-dimensional nuclear architecture (Bonisch & Hake, 2012). Regulation of the compaction or relaxation of chromatin at specific genes can lead to their repression or activation, respectively. Histone PTMs such as lysine acetylation, lysine or arginine methylation, serine/threonine phosphorylation and lysine ubiquitination on their N-terminal tails have been shown to modulate the chromatin structure by changing protein–DNA or protein–protein interactions (Lalonde, Cheng, & Cote, 2014).

3 | HISTONE ACETYLATION

Histone lysine acetylation plays a fundamental role in the epigenetic regulation of gene expression. Acetylated histones tend to be less compact and more accessible to RNA polymerase and the transcriptional machinery, thereby enabling transcription of nearby genes (Cohen, Poreba, Kamieniarz, & Schneider, 2011). Acetylated histones also serve as binding sites for bromodomain-containing proteins (BRDPs) to recruit transcriptional machinery and other chromatin-modifying elements (Bannister & Kouzarides, 2011). Conversely, histone deacetylation causes transcriptional repression via chromatin compaction. Acetylation of non-histone targets can lead to changes in enzyme activity and protein–protein interactions (Kim & Workman, 2010).

4 | REGULATORS OF HISTONE ACETYLATION

Histone PTMs can be deposited on or removed from chromatin by different enzyme families. These “writers” and “erasers” of histone acetylation include lysine acetyltransferases (HATs) and deacetylases (HDACs) gene families (Lalonde et al., 2014). The balance between the actions of these enzyme families serves as a critical regulatory mechanism for gene expression and governs numerous developmental processes and disease states (Lalonde et al., 2014). Notably, the recruitment of proteins to macromolecular complexes by acetylated lysine residues is mediated by BRDPs, which are the principal readers of ε-N-acetyl-lysine (Kac; Muller, Filipopapoulos, & Knapp, 2011). All three enzyme families are an integral component of gene regulatory complexes, which are well known to regulate transcription through modulation of chromatin modification states (Lalonde et al., 2014). Of the three enzyme families regulating histone acetylation, the HDAC family has been the most studied in the context of pulmonary hypertension (PH) and right ventricular hypertrophy (RVH; Figure 1, Table 1).

5 | DEACETYLASES

5.1 | Family and molecular functions

HDACs are a large family of enzymes that contain a highly conserved deacetylase domain and are responsible for the removal of acetyl groups and maintenance of the equilibrium of lysine acetylation in histones (Peserico & Simone, 2011). So far, 18 human HDACs have been identified and grouped into four classes according to functional and phylogenetic criteria. They are class I HDACs (HDAC1, 2, 3, and 8), class IIa HDACs (HDAC4, 5, 7 and 9), class IIb HDACs (HDAC6 and 10) and class IV HDAC (HDAC11) which are Zn2+-dependent, while the class III HDACs, also called sirtuins (SIRT1, 2, 3, 4, 5, 6, and 7), are NAD+-dependent enzymes (Haberland, Montgomery, & Olson, 2009). HDAC enzymes differ in structure, enzymatic function, subcellular localization and expression patterns. In addition to the nuclear roles of HDACs, HDAC isoforms also exhibit essential cytoplasmic functions by controlling the acetylation status and activity of numerous cytoplasmic proteins, including transcription factors (Glozak & Seto, 2007).

5.2 | Class I histone deacetylases: Expression and preclinical studies

Although in vivo studies on the pulmonary vascular cell-specific roles of class I HDAC isoforms are lacking, the contribution of aberrant HDAC activity to the pathogenesis of PH and RVH is mainly based on the promising therapeutic benefits observed upon the application of small-molecule HDAC inhibitors in different animal models of PH (Chelladurai, Seeger, & Pullamsetti, 2016). Table 1 summarizes all research findings that reported the aberrant expression and activity of HDACs and the therapeutic effects of non-selective HDAC inhibitors targeting multiple HDAC isoforms (pan-HDAC). In PH, Li et al. (2011) first reported the association between the persistent pro-inflammatory phenotype exhibited by activated pulmonary adventitial fibroblasts isolated from a bovine model of hypoxia-induced PH and the abnormal activity of class I HDACs. Elevated class I HDAC
PH is a complex disease with multifactorial aetiology. Besides genetic predisposition, vascular injury caused by hypertensive stimuli such as shear stress, autoimmunity, hypoxia, infection, drugs and toxins may disrupt the cellular homeostasis. The persistence of injurious events alters the existing epigenetic state of the healthy pulmonary vascular cells and re-establishes an aberrant epigenetic signature that favours the acquisition of altered cellular phenotypes that aggravate the vascular remodelling process. Vascular cells isolated from PAH pulmonary vasculature exhibit stable pro-proliferative, pro-migratory, anti-apoptotic, pro-inflammatory and pro-fibrotic vascular cell phenotypes, which correlate with the observed changes in the transcriptional levels of genes associated with respective phenotypes. Histone acetylation plays a fundamental role in the epigenetic regulation of gene expression. Besides the recent focus on the BRD4 that recognizes and binds acetylated histones, the most investigated epigenetic regulators in the context of PH were the enzyme isoforms from HDAC and SIRT families. Mechanistically, aberrant expression of HDACs or HATS in vascular cells may cause an imbalance in acetylome (hypoacetylation or hyperacetylation) of histone tails and cellular proteins that consequently modulate DNA accessibility and transcriptional state (activation or repression) of critical PAH-associated genes that aggravate the vascular remodelling process in PAH. Ac, acetylation; BET, bromodomain and extra-terminal domain family; BRD, bromodomain; HATS, histone acetyltransferases; HDACs, histone deacetylases; POL2, RNA polymerase II; SIRT, sirtuins.
| Species          | Family                | Rodent model/cells       | Summary                                                                 | PH and RVH | Inhibitors         | Year/reference |
|------------------|-----------------------|--------------------------|--------------------------------------------------------------------------|------------|--------------------|----------------|
| Human            | HDACs                 | Hypoxia, PASMCs          | HDAC activity is required for hypoxic repression of BMP signalling.      |            |                    | WU X et al., Circ Res. 2006 |
| Bovine           | Class I, class II HDACs | Hypoxia, adventitial fibroblasts | Increase in HDAC1, HDAC2, HDAC3 expression and activity contributes to a pro-inflammatory phenotype of PH fibroblasts, which can be attenuated by HDAC inhibition. |            | SAHA, apicidin     | Li M et al., J Immunol. 2011 |
| Rat              |                       | Hypoxia, PASMCs          | TGF-β-mediated PPAR-γ suppression is mediated via increased binding of Smad2/3/4 and HDAC1 proteins to PPAR-γ. |            |                    | Gong K et al., AJPCLCM. 2011 |
| Rat              | Class I, class II HDACs | PAB                      | Suppression of HDACs worsens right ventricular dysfunction after PAB in rats. | Vascular remodelling, RVH | TSA, VPA        | Bogaard HJ et al., AJRCCM. 2011 |
| Human/bovine/rat | Class I, class II HDACs | IPAH, rat hypoxia bovine AF, R-cells | Increased expression of HDAC1, HDAC4, HDAC5 in IPAH lung homogenates, hypoxia-induced PH lungs and RV. HDAC inhibition attenuated hypoxia-induced PH. | Vascular remodelling, RVH | SAHA, VPA        | Zhao L et al., Circulation. 2012 |
| Rat              | Class I HDACs         | Hypoxia, RV, PASMCs      | Selective class I HDAC inhibitors suppress hypoxia-induced PH and RV.     | Vascular remodelling, RVH | MGCD0103, MS-275 | Cavasin MA et al., Circ Res. 2012 |
| Ovine/sheep      | Class I, class II HDACs | Newborn PASMCs           | Inhibition of class I and II HDACs by apicidin and HDACi VIII suppressed proliferation and migration of PASMCs. |            | Apicidin, HDACi VIII | Yang Q et al., Cell Prolif. 2013 |
| Rat              | Class I, class II HDACs | PDGF-PASMCs              | Sodium butyrate inhibits PDGF-induced proliferation and migration in PASMCs. | NaBU       |                    | Cantoni S et al., 2013 |
| Rat              |                       | PVECs                    | Maternal nutrient restriction increased the histone acetylation and HIF-1α binding in ET-1 gene promoter of PVEC. |            |                    | Xu et al., Respir Res. 2013 |
| Bovine           | Class I, class II HDACs | Hypoxia, adventitial fibroblasts | Pan-HDAC inhibitors rescued the hypoxia-induced down-regulation of miR-124 expression. |            | SAHA, apicidin     | Wang D et al., Circ Res. 2014 |
| Rat              | Class I, class II HDACs | PDGF, PASMCs             | Class I HDAC (MC1855), but not class II HDAC (MC1575) inhibitors counteract PDGF-induced proliferation. | MC1855, MC1575 |                    | Galetti M et al., Bio Pharmaco. 2014 |
| Species | Family | Rodent model/cells | Summary | PH and RVH | Inhibitors | Year/reference |
|---------|--------|--------------------|---------|------------|------------|----------------|
| Human   | Class IIa HDACs | IPAH PAECs, Sugen5416 + hypoxia, MCT | Increased nuclear accumulation of Class IIa HDACs, HDAC4 and HDAC5 impaired MEF2 activity in PAH PAECs. | Vascular remodelling, RVH | MC1568 | Kim J et al., Circulation. 2015 |
| Rat     | HDACs   | MCT                | Increased expression of HDAC3, HDAC4, HDAC5, and HDAC7 in PAs from MCT-treated rats reduced transcription of NOX and ROS. | VPA | Chen F et al., FRBM 2016 |
| Human rat | HDAC6 | PAH PASMCs, Sugen5416 + hypoxia, MCT | Hdac6-deficient mice were partially protected against chronic hypoxia-induced PH. Pharmacological inhibition of HDAC6 improved established PAH in two experimental models. | Vascular remodelling, RVH | Tubastatin A | Boucherat O et al., Sci Rep. 2017 |
| Rat     | Class I HDACs | PAB, MCT           | Sodium valproate significantly reduced RVH induced by either PAB or MCT injection. | RVH | VPA | Cho YK et al., Circ J. 2010 |
| Rat     | Class I, class II HDACs | PAB, MCT, Sugen5416 + hypoxia | HDAC activity levels are reduced in the lungs of rat with experimentally induced hypertension, whereas activity levels are increased in the hypertrophic hearts. HDAC inhibitor TSA had no effect on pulmonary vascular remodelling in the Sugen5416 + hypoxia model. | Vascular remodelling, RVH | TSA | De Raaf MA et al., Pulm. Circ. 2013 |
| Rat     | Class I HDACs | MCT + chronic hypoxia | Daily administration of valproic acid therapy prevented and partially reversed the development of severe PH in rats, and decreased inflammation and proliferation in remodelled pulmonary arteries. | Vascular remodelling, RVH | VPA | Lan B et al., PLoS One. 2015 |
| Human   | HDACs, HATs | PMVECs, human PAH | Lung tissue from IPAH patients showed decreased nuclear HDAC and increased nuclear HAT activity. | JQ1 | Mumby S et al., Respirology. 2017 |
| Rat     | BET    | Sugen5416 + hypoxia | BRD4 levels were dramatically elevated in hypertrophic RVs from Sugen5416 + hypoxia rats. | JQ1 | Stratton SM et al., BioCellBiol 2015 |
| Human rat | BET | IPAH, PASMCs, Sugen5416-hypoxia | BRD4 is up-regulated in lungs, distal PAs, and PASMCs of patients with PAH compared with controls. | Vascular remodelling, RVH | JQ1 | Meloche J et al., Circ Res. 2015 |

(Continues)
catalytic activity also correlated with an increased abundance of HDAC1, HDAC2 and HDAC3 protein levels in PH fibroblasts (Li et al., 2011). Further, six HDACs (1, 2, 3, 4, 5, and 7) were screened in human idiopathic PAH (IPAH) lung homogenates and elevated expression of HDAC1 and HDAC5 was reported (Zhao et al., 2012b).

A large number of preclinical studies using different HDAC inhibitors have shown their promising antitumour responses in vivo, of which few of them have been approved by U.S. FDA for the treatment of human lymphoma (Segre & Chiocca, 2011; Suraweera, O’Byrne, & Richard, 2018). These compounds are generally well tolerated, with the most notable adverse effects being thrombocytopenia, nausea and fatigue (Tan, Cang, Ma, Petrillo, & Liu, 2010). In addition to their anticancer actions, several studies have reported beneficial effects of HDAC inhibitors in preclinical models of left ventricular (LV) dysfunction by attenuating pathological cardiac hypertrophy, inflammation, fibrosis and restenosis thereby improving ventricular function (Cavasin, Stenmark, & McKinsey, 2015).

Based on these promising findings, the therapeutic potential of commercially available broad-spectrum HDAC inhibitors, valproic acid (VPA, inhibits class I HDACs) and SAHA (vorinostat, inhibits class I and II HDACs), was evaluated in multiple rodent models of PH. Both SAHA and VPA mitigated the development of PH and reduced established hypoxia-induced PH in the rat model. Similarly, both drugs inhibited the hyperproliferative phenotype and inflammatory gene expression in fibroblasts, fibrocytes from PH bovine vessels and PDGF-stimulated human vascular smooth muscle cells (Zhao et al., 2012b). These studies established that HDAC activity inhibition effectively reverses PH in chronic hypoxia-induced PH via anti-proliferative and anti-inflammatory effects. Furthermore, different class I-selective HDAC inhibitors like MGCD0103 (mocetinostat) or MS-275 (entinostat) also significantly reduced pulmonary artery (PA) systolic pressure and pulse pressure, which suggests that these compounds increased arterial compliance in lungs of hypoxic rats (Cavasin et al., 2012a). Right ventricular (RV) function was maintained in MGCD0103-treated animals. Likewise, Cho et al. (2010) evaluated the effects of VPA, a class I HDAC inhibitor, on RVH induced by either pulmonary artery banding (PAB) or monocrotoline (MCT) injection in rats. VPA attenuated RVH and hypertrophy-associated protein markers and also improved RV systolic function in animals subjected to MCT or PAB. These findings also confirm that class-selective HDAC inhibitors are well tolerated in PH conditions.

### 5.3 | Class II and IV histone deacetylases: Expression and preclinical studies

The class Ila HDACs (subtypes 4, 5, 7, and 9) are characterized by tissue-specific expression and stimulus-dependent nucleo-cytoplasmic shuttling (Witt, Deubzer, Milde, & Oehme, 2009). Class II HDACs are expressed at the highest levels in the heart, brain and skeletal muscle. HDAC4 acts as a repressor of chondrocyte hypertrophy by interacting with transcription factor myocyte enhancer factor 2C (MEF2C). Mice lacking HDAC5 develop profoundly enlarged hearts in response to...
pressure overload resulting from aortic constriction or constitutive activation of cardiac stress signals. HDAC9 knockout mice show cardiac hypertrophy that is further exacerbated in the HDAC9 and HDAC5 double knockout animals. HDAC7 has a specific role in the clonal expansion of T cells by suppressing Nur77-dependent apoptosis and in vascular integrity through suppression of MMP10 (Witt et al., 2009). HDAC6 is the only deacetylase known to act on tubulin. Recently, Boucherat et al. (2017) reported up-regulation of HDAC6 protein levels in lungs, distal PAs and isolated PA smooth muscle cells (PASMCs), PA endothelial cells (PAECs) from PAH patients as well as in the RV of rats exposed to SU5416-hypoxia and MCT. Exogenous expression of HDAC10 in cervical cancer cells significantly inhibited cell motility and invasiveness in vitro and metastasis in vivo, by transcriptional repression of MMP2 and MMP9 genes (Song, Zhu, Wu, & Kang, 2013). HDAC11 expression is enriched in the brain, heart, muscle, kidney and testis (Gao, Cueto, Asselbergs, & Atadja, 2002), but little is known about its function.

Specifically, MEF2 is a transcription factor family that plays a prominent role in cardiovascular development and differentiation, and is constitutively associated with class IIa HDACs, which maintain MEF2 in a transcriptionally inactive state (Desjardins & Naya, 2016). In the context of PH, Kim et al. (2015) demonstrated that impaired MEF2 transcriptional activity found in PAH PAECs was mediated by excess nuclear accumulation of HDAC4 and HDAC5. Selective, pharmacological inhibition of class IIa HDACs using MC1568 restored MEF2 activity in IPAH PAECs, as demonstrated by increased expression of its transcriptional targets, decreased cell migration and proliferation, and rescue of experimental PH models (MCT and SU5416 + hypoxia). Importantly, class IIa HDAC inhibition did not promote RV fibrosis or coronary artery endothelial cell apoptosis (Kim, Hwangbo, et al., 2015). However, a recent study (Lemon et al., 2015) reported that the commercially available compound MC1568 (used in the above study by Kim, Hwangbo, et al.) failed to inhibit class IIa HDAC catalytic activity in vitro. Expression patterns of class II HDAC isoforms were proposed to be tissue specific, and therefore, thorough in vivo evaluation of their gene-specific functional roles in development and disease of the cardiovascular system is warranted.

5.4 The mixed outcome of HDAC inhibition in RV

Contrary to the previous findings of beneficial effects of HDAC inhibition using VPA on PAB- or MCT-induced cardiac hypertrophy (Cho et al., 2010) and trichostatin A (TSA) on transverse aortic constriction model-induced cardiac hypertrophy (Kong et al., 2006), Bogaard et al. (2011) reported deleterious effects of TSA and VPA on the RV using PAB model, which cast doubt on the utility of this class of compounds for patients with RV failure caused by PH. In contrast to the data obtained with the transverse aortic constriction model, TSA treatment did not decrease the degree of RV hypertrophy but worsened RV function, as revealed by reduced cardiac output and increased RV dilatation (Bogaard et al., 2011). TSA treatment in the setting of PAB was also associated with exaggerated RV fibrosis, increased numbers of apoptotic cells in the RV, and induced capillary rarefaction in the RV, but not in control rats (Bogaard et al., 2011). However, the authors also reported that VPA did not increase fibrosis or reduce capillary density. A second study by the same group (in 2014) employed three different rodent models (MCT, SU5416 + hypoxia and PAB) to evaluate the effect of pan-HDAC inhibitor TSA on experimentally induced PH and RVH. No beneficial effects of TSA were observed in SU5416 + hypoxia-induced PH because it neither affected pulmonary vascular remodelling nor improved cardiac function. However, in all three animal models of PH and RV dysfunction, they confirmed that HDAC activity was significantly increased in the hypertrophied RV tissue but decreased in the lung homogenates (De Raaf et al., 2014).

5.5 Histone deacetylase inhibitors: Mechanism of action in PH

Regarding the cellular responses and mechanisms associated with HDAC inhibition, Li et al. (2011) demonstrated that catalytic inhibition of class I HDACs using apicidin significantly suppressed the production of pro-inflammatory mediators by PH fibroblasts and reduced the capacity of PH fibroblasts to induce monocyte migration and activation. Moreover, this study strengthened the hypothesis that the persistent pro-inflammatory phenotype of PH fibroblasts was due to epigenetic alterations, which can be reversed upon HDAC inhibition. On similar lines, Galletti et al. highlighted that only class I-selective inhibitor (MC1855) dose- and time-dependently inhibited PDGF-induced proliferation and migration in PASMCs isolated from MCT-PAH rats, while class II inhibition with MC1575 was ineffective. Similar to class I inhibitor MC1855, RNA interference experiments of class I HDACs revealed that only silencing of HDAC1 and HDAC2 significantly reduced proliferation and migration. However, only HDAC1 siRNA reduced PDGF-induced CyclinD1 protein expression significantly, highlighting the prominent role of HDAC1 in PASMC proliferation induced by PDGF (Galletti et al., 2014). However, pan-HDAC inhibitors like sodium butyrate also decreased the expression of cell cycle regulators (PCNA, c-Myc and cyclin D1 and p21). PDGFR-β and endothelin-1 receptors ETα and ETβ and pulmonary artery smooth muscle cells (PASMC) migration (Cantoni et al., 2013).

With regard to the epigenetic mechanisms associated with mitochondrial metabolism and glyoxylate homeostasis in PH, HDAC1 was recently reported to repress transcription of an iron–sulfur biogenesis protein known as BoiA Family Member 3 (BOLA3) via enrichment of
HDAC1 binding and deacetylation of lysine 9 of histone H3 (H3K9ac) at the BOLA3 promoter (Yu et al., 2019). The transcriptional repression of BOLA3 via HDAC1 was hypoxia-inducible factor-2α dependent, which consequently induced alterations in mitochondrial electron transport, glycolysis, fatty acid oxidation and repressed lipoate biosynthesis, thus inhibited the glycine cleavage system, thereby increased glycine accumulation, and promoted endothelial proliferation (Yu et al., 2019).

Recent studies have also explored the crosstalk between HDACs and miRNAs and the molecular basis that facilitates the adventitial fibroblasts to exhibit constitutively activated phenotypes, in the context of hypoxic PH. Wang et al. (2014) investigated whether small-molecule pan- and class-selective HDAC inhibitors can restore the down-regulated miR-124 expression levels in PH fibroblasts isolated from calves and humans with severe PH. They demonstrated that class I HDAC inhibition with apicidin not only led to a significant increase in miR-124 expression and concomitantly decreased polypyrimidine tract binding protein (PTBP1) expression but also attenuated the pro-inflammatory phenotype of constitutively activated PH fibroblasts (Wang et al., 2014). A recent study identified a novel regulatory axis involving the role of HDAC4 in PDGF-BB-induced PASMC proliferation and migration (Li et al., 2018). It is well established that PDGF-BB is a potent mitogen found at high levels in lung tissues of patients with PAH and induces PASMCs to switch from a contractile phenotype to a proliferative and migratory phenotype. PDGF-BB treatment elevated the expression and activity of HDAC4, while HDAC4 reduction inhibited PDGF-BB-induced PASMC proliferation and migration (Li et al., 2018). However, the pro-proliferative effects of PDGF-BB were antagonized by overexpression of miR-1281, which significantly inhibited PASMC proliferation and migration, at least in part through direct reduction of HDAC4 protein levels. Although this study confirmed the pro-proliferative roles of HDAC4 in the context of PDGF-induced functional alterations in PASMCs, how the
deacetylase activity of HDAC4 contributes to the downstream regulation of histone and non-histone targets is still to be explored.

NADPH oxidases (Nox) are significant sources of ROS, but the mechanisms regulating changes in Nox expression in disease states remain poorly understood. Multiple HDAC isoforms (HDAC 3, 4, 5, and 7) are up-regulated in isolated pulmonary arteries, and HDAC inhibitors attenuate Nox expression in isolated pulmonary arteries and reduce indices of MCT rat model of PAH (Chen et al., 2016). In immune cells, multiple HDAC inhibitors (scriptaid, SAHA, TSA and VPA) robustly decreased Nox1, Nox2, Nox4 and Nox5 mRNA and protein expression in a dose-dependent manner concomitant with reduced superoxide production. This study confirms that HDAC inhibitors can potently suppress Nox gene expression, overproduction of ROS, and reduced indices of PAH, including RVH and PA stiffening (Chen et al., 2016).

SODs constitute significant antioxidant responses against ROS-mediated injury, and oxidant/antioxidant imbalance may promote abnormal vascular responses. Recent studies have emphasized that elevated ROS and decreased SOD activity are associated with PH (Villegas et al., 2013). Previously, epigenetic silencing of the mitochondrial isoform of SOD2 via selective hypermethylolation of CpG islands was confirmed to be involved in the down-regulation in PASMCs from PAH patients and fawn-hooded rats with PAH. Similarly, extracellular SOD3 that is reported to be significantly down-regulated in multiple animal models of lung or vascular injury is also associated with an increase in disease severity (Nozik-Grayck et al., 2016). Importantly, the treatment with 5-aza-2’-deoxycytidine failed to increase PASMC SOD3 mRNA, suggesting that DNA methylation was not responsible for reduced SOD3 expression in PAH. Interestingly, treatment with class I-selective HDAC inhibitor MGCD0103 or HDAC3 siRNA restored SOD3 mRNA levels in IPAH PASMCs. These data indicate that histone deacetylation, specifically via HDAC3, contributes to the impaired SOD3 expression and enhanced cell proliferation in IPAH PASMC, which can be countered using selective HDAC inhibitors that restore SOD3 expression in PAH (Nozik-Grayck et al., 2016). Similarly, exposure of human PAECs to HDAC inhibitors (scriptaid and TSA) for 24 hr induced expression of SOD3 up to 10-fold, whereas expression of the pro-oxidant gene NADPH oxidase 4 (NOX4) was decreased by more than 95% (Zelko & Folz, 2015). These findings altogether confirm that targeting the acetylation pathway with HDAC inhibitors is a viable approach to abrogate disease phenotypes associated with the pathological vascular remodelling process.

6 SIRTUINS

6.1 Family and molecular functions

In mammals, seven sirtuin isoforms (SIRT1–SIRT7) have been classified as NAD+-dependent class III HDACs with different tissue distributions, subcellular localizations, diversity in substrate affinities, and PTMs of histone and non-histone targets (Sebastian, Satterstrom, Haigis, & Mostoslavsky, 2012). SIRT1 and SIRT2 were localized in nucleus and cytoplasm, SIRT3 in nucleus, cytoplasm and mitochondria (Scher, Vaquero, & Reinberg, 2007; Sundareshan, Samant, Pillai, Rajamohan, & Gupta, 2008), SIRT4 and SIRT5 in mitochondria, while SIRT6 and SIRT7 were prominently found in nucleus (Poulose & Raju, 2015). Sirtuin isoforms possess NAD+-dependent deacetylase, and ADP-ribosyltransferase activities, which are required for diverse cellular processes like proliferation, differentiation, apoptosis, senescence, mitochondrial biogenesis, metabolism, cellular stress response, insulin secretion, aging and inflammation (Poulose & Raju, 2015).

6.2 Expression and preclinical studies

The most extensively studied member among the sirtuin family is nicotinamide adenine dinucleotide (NAD+)-dependent SIRT1 deacetylase, whose activity is regulated in a context-dependent manner by cellular stress, redox state, transcription factors, and PTMs (Yu & Auwerx, 2010). Although neither the expression levels or activity status of SIRT1 nor the lysine acetylation state in human PAH setting have been explored in detail, a few studies have reported a potential protective role for SIRT1 in rodent models of PH and RVH (Figure 2, Table 2).

A recent study demonstrated that inactivation of Sirt1 aggravates chronic hypoxia-induced PA muscularization and cardiac remodelling in vivo (Zurlo et al., 2018). Sirt1 global knockout mice displayed a more intense vascular remodelling and RVH upon exposure to chronic hypoxia. Aggravated vascular remodelling exhibited by Sirt1 ablation was associated with a significant increase in the percentage of fully muscular pulmonary arteries and α-smooth muscle actin expression, paralleled by a decreased percentage of non-muscular arteries. However, at the molecular level, the authors observed no changes in both mRNA and protein levels of SIRT1 in human PAH PASMCs compared to control PASMCs (Zurlo et al., 2018). Even though the catalytic activity of SIRT1 was not quantified in PAH or hypoxia, there is an imbalance in the acetylation/deacetylation state of the non-histone targets of SIRT1 in PH, wherein the acetylated forms of PGC-1α, histone H1, and FOXO1 were observed in PAH PASMCs. Pharmacological activation of SIRT1 using Stac-3 reduced not only PDGF-induced proliferation in PASMCs from PAH patients but also reduced mitochondrial fragmentation and increased mitochondrial biogenesis (Zurlo et al., 2018). Multiple observations confirm the proposition that SIRT1 is a negative regulator of PASMC proliferation and promotes mitochondrial biogenesis, and SIRT1 inactivation may be strongly associated with the pathogenesis of PH. Although the study used global Sirt1 knockout mice, investigating vascular cell-specific roles of Sirt1 may yield more in-depth insights into the complex regulatory mechanisms associated with Sirt1 activity regulation.

Along the same lines, short-term calorie restriction (CR) ameliorated MCT-induced mean pulmonary arterial pressure and reduced vascular remodelling and RVH that was accompanied by a significant increase in Sirt1 expression (Ding et al., 2015). Remarkably, Sirt1 overexpression in the rat model of MCT-induced PAH and hypoxia-induced PH is sufficient to mimic the reduction in mean
pulmonary arterial pressure (mPAP) that was achieved merely with short-term CR (Ding et al., 2015). These studies demonstrated the biological role of Sirt1 in the protective effects of CR against the development of MCT- or hypoxia-induced vascular remodelling in PH and RVH.

It is well established that alterations in metabolic state and impaired mitochondrial function play critical roles in the pathogenesis of PAH (Marshall, Bazan, Zhang, Fares, & Lee, 2018). SIRT3 is predominately a mitochondrial NAD-dependent deacetylase. Although Sirt3-deficient mice appeared to have normal activity, they showed

| Species        | Family | Rodent model/cells | Summary                                                                 | PH and RVH | Treatment | Year/reference               |
|----------------|--------|--------------------|-------------------------------------------------------------------------|------------|-----------|------------------------------|
| Rat            | SIRT   | MCT                | Resveratrol inhibits RVH induced by MCT in rats.                        | RVH        | Resveratrol | Yang DL et al., Clin Exp Pharmacol Physiol 2010 |
| Rat            | SIRT   | MCT                | Resveratol treatment attenuates pulmonary vascular remodelling and prevents the development of PAH. | Vascular remodelling, RVH | Resveratrol | Shi W et al., Life Sci. 2018   |
| Human, mouse   | SIRT1  | PASMCs, hypoxia    | Sirtuin 1 down-regulation aggravates chronic hypoxia-induced vascular remodelling. SIRT1 activation could be considered as an effective tool to inhibit PASMC proliferation. | Vascular remodelling, RVH | Stac-3       | Zurlo G et al., J Hypertension 2008 |
| Rat            | SIRT   | MCT                | Oral resveratrol attenuated established MCT-induced PH indices, including RVSP, RVH, and medial thickening of intrapulmonary arteries. | Vascular remodelling, RVH | Resveratrol | Paffett ML et al., Vascul Pharmacol. 2012 |
| Human/bovine/rat| SIRT  | MCT                | Resveratrol treatment attenuated RVSP and pulmonary arterial remodelling. | Vascular remodelling, RVH | Resveratrol | Csiszar A et al., Hypertension 2009 |
| Rat            | SIRT   | MCT                | Resveratrol does not inhibit the abnormal remodelling of the RV induced by MCT but attenuates the development of medial hypertrophy in the pulmonary trunk. | Vascular remodelling, RVH | Resveratrol | Wilson DN et al., Pathophysiology. 2016 |
| Rat            | SIRT1  | MCT, hypoxia       | Increasing SIRT1 expression is sufficient to attenuate both MCT-induced and hypoxia-induced PH. | Vascular remodelling, RVH |                      | Ding M et al., J Cardiovasc Pharmacol. 2015 |
| Human, mice    | SIRT3  | PAH PASMCs         | SIRT3 is down-regulated, and its normalization with adenovirus gene therapy reverses PAH in rats in vivo. Presence of the SIRT3 loss-of-function SNP rs11246020 is associated with clinical IPAH. | Vascular remodelling, RVH |                      | Paulin R et al., Cell Metab. 2014 |
| Human, mice    | SIRT3  | Angiotensin II     | Diminished Sirt3 expression and redox inactivation of Sirt3 lead to SOD2 inactivation and contribute to the pathogenesis of hypertension. Hypertension was markedly increased in Sirt3-knockout mice in response to low dose of angiotensin II. |                      |                      | Dikalova AE et al., Circulation research 2017 |

Abbreviations: ET, endothelin; FHR-PH, fawn-hooded rats; HIF, hypoxia-inducible factor; IPAH, idiopathic pulmonary arterial hypertension; MCT, monocrotaline; MEF2, myocyte enhancer factor 2; NOX, NADPH oxidases; PAB, pulmonary artery banding; PAECs, pulmonary artery endothelial cells; PAs, pulmonary arteries; PASMCs, pulmonary artery smooth muscle cells; PMVEC, pulmonary microvascular endothelial cells; RV, right ventricle; RVH, RV hypertrophy; RVSP, RV systolic pressure; SIRT, sirtuins; SNP, single nucleotide polymorphism; SU5416 + hypoxia-induced PH (SU5416: VEGF receptor [VEGFR-2] inhibitor); TSA, trichostatin A.
signs of cardiac hypertrophy, interstitial fibrosis and an increased cross-sectional area of cardiomyocytes compared with wild-type controls. In the context of PH pathogenesis, Sirt3 was significantly down-regulated in MCT-induced PAH and human PAH tissues. Both Sirt3 and PGC-1α expressions were also decreased in hypertrophic RV induced by MCT, but not in the corresponding LV. Notably, Sirt3-deficient mice developed spontaneous PAH in a gene dose-dependent manner in vivo and displayed increased muscularization and the medial wall thickness of resistance PAs compared to the wild-type controls at baseline. The disease phenotype associated with Sirt3 deficiency was reversed by Sirt3 overexpression in MCT-injected rats. At the cellular level, Sirt3-deficient PASMCs exhibited suppressed mitochondrial oxidative phosphorylation and had increased levels of mitochondrial protein lysine acetylation compared to wild type (Paulin et al., 2014). Furthermore, the authors confirmed that suppression of SIRT3 activity in IPAH can be either due to the down-regulation of the expressed protein or due to the presence of a loss-of-function single nucleotide polymorphism in the SIRT3 gene (rs11246020) within the conserved catalytic deacetylase domain or due to the occurrence of both in a PAH patient (Paulin et al., 2014). These findings support the metabolic basis of PAH and confirm the central role of the SIRT family of deacetylases both during the physiological maintenance of metabolic and mitochondrial homeostasis and during the pathogenesis of PH, thereby rapidly emerging as potential therapeutic targets.

On the contrary to cardioprotective role of Sirt3 described by Paulin et al., observed in the transgenic mice from 129/Sv strain, Waypa et al. (2013) revealed that their Sirt3-deficient mice from C57/BL6 background did not exhibit any differences from their wild-type littermates in PA wall remodelling, and RVH, when exposed to chronic hypoxia. The disparity in the outcome between the two studies carried out by Waypa et al. and Paulin et al. led to the supposition that Sirt3 responses may be cell type specific or restricted to specific genetic backgrounds, which has to be taken into account and correlated with the status of expression pattern in cells and tissues from human PH setting, in all the studies to be carried out in future.

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a natural phytoalexin, which has high antioxidant properties and also found to be useful in the treatment of numerous diseases because of its cardioprotective, neuroprotective, anti-diabetic, anti-inflammatory and anti-carcinogenic effects and also claimed to possess vasoprotective properties (Carrizzo et al., 2013). Over the last decade, several studies have demonstrated that resveratrol can attenuate myocardial ischaemic reperfusion injury (Hung et al., 2002), atherosclerosis (Tomayko, Cachia, Chung, & Wilund, 2014), arterial wall inflammation (Mattison et al., 2014) and cardiac hypertrophy associated with spontaneously hypertensive rats (Thandapilly et al., 2010). With regard to the effects of resveratrol in the pathophysiology of RV, resveratrol treatments ameliorated established RV systolic pressure, RVH (Yang et al., 2010) and pulmonary arterial remodelling in chronic hypoxia-induced PAH (Chen et al., 2014) and MCT-induced PAH (Csiszar et al., 2009; Paffett, Lucas, & Campen, 2012), in part by exerting anti-proliferative, anti-inflammatory and antioxidant effects in PASMCs. Although the specificity of resveratrol in the direct activation of SIRT1 is debated, the studies evaluating the therapeutic benefits of resveratrol have highlighted the potential protective role of resveratrol in cardiovascular diseases.

7 | ACETYLTRANSFERASES

7.1 | Family and molecular functions

Acetylation of cellular proteins is carried out by histone acetyltransferases (HATs), which catalyse the transfer of an acetyl group from acetyl-CoA to the lysine ε-amino groups on the N-terminal tails of histones (Bannister & Kouzarides, 2011). HATs exist as components of multi-subunit protein complexes that determine their binding preferences and catalytic activity (Carrozza, Utley, Workman, & Cote, 2003). About 30 different HATs were identified and grouped into five different families. These include CREBBP/EP300, GCN5-related N-acetyltransferase (GNAT: HAT1, GCN5, PCAF, ELP3), MYST (TIP60, MOZ, MORF, MOF, HBO1), basal transcriptional factor-related HATs (TAF1 and TIFIIIC90), and nuclear receptor co-activators (P600, SRC1, CLOCK and AIB1/ACTR/SCR3; Carrozza et al., 2003). Both EP300 and CREBBP are transcriptional co-activators able to acetylate histones and non-histone proteins (Lau et al., 2000). Acetylation of non-histone proteins has been demonstrated to modulate protein functions by altering their stability, cellular localization and protein–nucleotide/protein–protein interactions. For example, post-translational acetylation regulates the activity of key DNA-binding transcription factors and coregulators, such as TP53, RELA, NFKB1, STAT3, MYB, hypoxia-inducible factor-1α, FOXO1, E2F1, MYOD1, MEF2D, BCL6, KLF5, PGC1α, GATA factors, CREBBP, EP300 and nuclear receptors, to name a few (Singh et al., 2010). Remarkably, most of these DNA-associated factors have been demonstrated to play pivotal roles in the pathogenesis of PH and RVH (Pullamsetti, Perros, Chelladurai, Yuan, & Stenmark, 2016).

7.2 | Expression and preclinical studies

Dysregulated HAT activity has been largely linked to cancer formation and progression (Avvakumov & Cote, 2007). Although in-depth studies dissecting the contribution of HAT isoforms in vascular remodelling associated with PH are lacking, few studies provide insights into the role of HATs in development and disease in cardiovascular development and disease setting. The Ep300/Crebbp family has mainly been studied and found to play critical roles in the physiological and pathological growth of cardiac myocytes. With regard to heart development, Ep300 knockout mice displayed defects like pericardial effusion, weaker heart contractions, reduced trabeculation, proliferation and reduced expression of cardiac muscle structural proteins such as β-myosin heavy chain and α-actinin (Yao et al., 1998). A knock-in approach was further used to demonstrate the significance of the HAT domain of Ep300 in heart development and coronary vascularization (Shikama et al., 2003). Mice overexpressing Ep300 in the
heart exhibit increased mortality and marked eccentric dilatation and systolic dysfunction of the LV. Cardiomyocyte-specific overexpression further established the crucial role of Ep300 in cardiac hypertrophy and heart failure in vivo (Miyamoto et al., 2006; Yanazume et al., 2003). In a murine model of myocardial infarction, cardiac overexpression of Ep300 promoted LV remodelling after myocardial infarction in adult mice in vivo (Miyamoto et al., 2006). Acetylation mediated by Ep300 increased DNA-binding activity of hypertrophy-responsive transcription factor Gata4, which preceded the development of LV dilatation and dysfunction (Yanazume et al., 2003).

With regard to the therapeutic evaluation, only limited numbers of HAT inhibitors have been investigated in cardiovascular diseases. Although an increased level of Ep300 is documented in multiple fibrotic tissues (Rai et al., 2017), only a few studies have evaluated pharmacological modulation of Ep300 in fibrosis-associated organ dysfunction (Ghosh, 2014; Rai et al., 2017). For instance, the acetyltransferase activity of Ep300 was shown to enhance Smad-dependent Tgf-β stimulation of collagen gene expression in fibroblasts (Ghosh, Yuan, Mori, & Varga, 2000). In a murine model of hypertensive cardiac-renal fibrosis, Ep300 inhibitor (L002) suppresses pro-fibrotic processes and associated cell proliferation, migration, myofibroblast differentiation and collagen synthesis. Systemic administration of L002 in angiotensin II infused mice reduced angiotensin II-induced perivascular and interstitial collagen deposition and hypertension-associated pathological hypertrophy, cardiac fibrosis and renal fibrosis. However, these anti-hypertrophic and anti-fibrotic benefits of L002 were independent of vasodilatory effects (Rai et al., 2017). Some natural products such as anacardic acid, garcinol and curcumin have been reported as potent Ep300 and PCAF inhibitors, and γ-butyrolactone (MB-3) and a series of isothiazolones have been revealed as inhibitors of both Ep300 and PCAF HAT activities (Mai et al., 2006). Considering the extent of biological roles of HAT enzymes in cardiovascular system development and disease, both mechanistic and pharmacological studies targeting the acetyltransferases in the context of PH and RVH have to be evaluated in detail.

8 | BROMODOMAINS

8.1 | Family and molecular functions

The bromodomain (BRD) is a conserved structural module composed of several α-helices connected by two loops forming a hydrophobic cavity that selectively recognizes and binds acetyl-lysine residues present on histone and non-histone proteins (Filippakopoulos et al., 2012; Fujisawa & Filippakopoulos, 2017). Given that they act in concert with proteins responsible for writing (HATs) and erasing (HDACs) histone acetylation marks, BRDPs mainly serve as epigenetic “readers,” interpreting the histone acetylation landscape to influence gene transcription through multiple mechanisms. BRDPs (at least 46 identified to date) are phylogenetically split into multiple families (Filippakopoulos et al., 2012) and include (a) direct chromatin modifiers that possess in addition to their BRD domain an intrinsic lysine acetyltransferase (EP300/CREBBP, etc.) or lysine methyltransferase (KMT2A/ASH1L) activity, (b) chromatin remodelers, which contain a BRD and an ATPase domain (SMARCA2/SMARCA4), and (c) the “bromodomain and extra-terminal domain” (BET) family that is distinguished by the presence of two BRDs and a so-called extra-terminal domain. Composed of four members (BRD2, BRD3, BRD4 and the testis-specific isoform BRD7), BET proteins are chromatin-binding adaptors able to recruit various transcriptional regulatory complexes to modify the transcriptional programme. Besides stimulating cell cycle progression, BRD4 was documented to facilitate telomere elongation (Wang et al., 2017) as well as the DNA damage response by stimulating expression of DNA repair factors (Li et al., 2018; Zhang et al., 2018). Taken together, these experimental findings illustrate the multifaceted roles of BRDPs and their importance in regulating major biological processes.

8.2 | Expression and preclinical studies

Altered expression levels of BRDPs have been linked to various disease states (Sanchez, Meslamani, & Zhou, 2014). Among them, a particular attention has been paid to BET proteins, especially BRD4, essential for embryonic viability (Houzelstein et al., 2002) and found to be overexpressed in many types of cancers (Dawson et al., 2011; Delmore et al., 2011; Liao et al., 2016; Segura et al., 2013; Zhang et al., 2016). Small-molecule inhibitors have been developed, causing displacement of BET proteins from chromatin by competing with the acetyl-binding pockets present in the BRDs (Filippakopoulos et al., 2010; Nicodeme et al., 2010). Pharmacological inhibition of BETs repeatedly reduced cancer cell growth and tumour formation in multiple preclinical cancer models (Dawson et al., 2011; Delmore et al., 2011; Segura et al., 2013). Considering that PAH may be viewed as a chronic inflammatory, proliferative disease with a cancer-like nature (Boucherat et al., 2017; Pullamsetti, Savai, Seeger, & Goncharova, 2017), the possible implication of BET proteins, especially BRD4, in the process of pulmonary vascular remodelling was recently explored (Figure 2, Table 1).

Increased expression of BRD4 was found in RV, lungs, dissected PAs and isolated PASMCs from PAH patients compared to controls (Meloche et al., 2015). The authors demonstrated that reduced miR-204 expression accounts, at least in part, for the increased level of BRD4 and that inhibition of BRD4 by JQ1 or RNA interference strikingly diminishes expression of the oncogenic factors NFAT, BCL2, BIRC5 (Survivin), and FOXM1 leading to the normalization of the hyperproliferative and apoptosis-resistant phenotype of PAH-PASMCs (Bourgeois et al., 2018; Meloche et al., 2015; Van der Feen et al., 2019).

NF-κB represents a family of inducible transcription factors (NF-κB1, NF-κB2, RelA, RelB and c-Rel) that induces the expression of various pro-inflammatory genes, including those encoding cytokines and chemokines (Liu, Zhang, Joo, & Sun, 2017). Additionally, BRD4 has emerged as a critical regulatory factor of inflammatory and immune...
responses. Specifically, BRD4 interacts with acetylated RelA (a subunit of nuclear factor κ light chain enhancer of activated B cells, NF-κB) and enhances the transcriptional activity of the latter (Huang, Yang, Zhou, Ozato, & Chen, 2009; Nicodeme et al., 2010). Chromatin immunoprecipitation analyses showed that BRD2 and BRD4 physically associate with the promoters of the pro-inflammatory cytokines genes IL-6, TNFα and MCP-1 increasing their expression in activated macrophages (Belkina, Nikolajczyk, & Denis, 2013). Similarly, JQ1 was reported to inhibit serum-stimulated proliferation and migration of human pulmonary microvascular ECs as well as expression of pro-inflammatory cytokines (IL-6 and IL-8) by preventing the recruitment of RelA to these gene promoters (Mumby et al., 2017). Based on these data, the potential benefit of intratracheal nebulization of JQ1 and siBRD4 was explored in the SU5416/hypoxia rat model with established PH. Both approaches significantly reduced pulmonary vascular remodelling and improved pulmonary haemodynamic parameters and cardiac function (Meloche et al., 2015), underscoring the pivotal role of BET proteins, particularly BRD4, in the acquired abnormal phenotype of PA cells. In support of this, the pan-BET inhibitor I-BET151 also reduced RV hypertrophy and PH in rats exposed to chronic hypoxia combined with pulmonary inflammation (Chabert et al., 2018).

In a translational perspective, a preclinical multicentre study was recently conducted to evaluate the therapeutic potential of the clinically available BET inhibitor RVX-208 (also called apabetalone, Picaud et al., 2013) in PAH (Van der Feen et al., 2019). The authors showed that RVX-208 exerts beneficial effects in complementary animal models of PAH and can be combined safely with current PAH therapies. As improved RV functions may be due to reduced PA pressure and subsequent decreases in RV afterload, effects of RVX-208 in the face of PAH-independent RV-pressure overload generated by PA banding were assessed. In this model, RVX-208 augmented cardiac output and RV stroke volume with no signs of adverse remodelling indicating a satisfactory safety profile or even therapeutic benefit for PAH patients with RV dysfunction (Van der Feen et al., 2019). Although substantial differences exist between the right and left ventricles including embryological origin, chamber geometry and response to therapies, the beneficial effects of BET inhibition on the RV are consistent with studies reporting that treatment with JQ1 preserves LV function following transverse aortic constriction (Anand et al., 2013; Duan et al., 2017). JQ1 also prevented the main pathological hallmarks of heart failure (i.e. cardiomyocyte hypertrophy, apoptosis, fibrosis and capillary rarefaction) associated with this model. Recent work further demonstrated that increased levels of circulating pro-inflammatory cytokines in PAH patients stimulate expression of BRD4 in coronary arteries that, in turn, favour their remodelling along with maintaining or even intensifying a high inflammatory status (Meloche et al., 2017). Collectively, these compelling data pinpoint BRD4 as a critical determinant of pulmonary vascular remodelling in PAH and underlying co-morbidities. Nevertheless, because BRD4 dysregulation is functionally integrated with multiple other epigenetic events, the precise mechanisms by which up-regulation of BRD4 promotes pathological remodelling in PAH remain to be elucidated.

9 | THERAPEUTIC IMPLICATIONS OF TARGETING EPIGENETIC ENZYMES

An extensive literature now supports the fact that epigenetic alterations are indeed associated with PH pathogenesis (Cheng, Wang, & Du, 2019; Hulshoff, Xu, Krenning, & Zeisberg, 2018; Olschewski et al., 2018). Given the reversible nature of these epigenetic modifications, considerable efforts have been made to therapeutically target the enzymes responsible for the observed alterations in the epigenome during disease. Moreover, new compounds targeting epigenetic enzymes are being developed with enhanced specificity, selectivity and potency as well as improved pharmacokinetic properties (Dhanak & Jackson, 2014). The risk of side effects due in part to an incomplete understanding of the mechanisms of action has fed scepticism about the use of drugs targeting epigenetic factors. Notwithstanding, clinical trials using histone acetylation modifiers as a single agent or in combination with standard therapy have been conducted over the last 15 years to primarily treat cancer, with many demonstrating clinical benefit with tolerable side effects (Suraweera et al., 2018). Since multiple epigenetic enzymes have been dysregulated in PH, the epigenomic landscape of cells from pulmonary vasculature or circulation can be first profiled to document alterations in DNA methylation, post-translational histone modifications (PTMs), and RNA-based mechanisms, which can be further tested for their potential as a clinical biomarker in the diagnosis and prognosis of PAH.

Available PAH therapies improve the functional capacity of PAH patients but do not cure the disease. Current efforts are now under way to target multiple mechanisms that drive excessive proliferation and resistance to apoptosis of resident vascular cells. Owing to their ability to affect the transcriptional expression of numerous genes simultaneously (along with impacting protein stability and enzymatic activity), drugs targeting either lysine acetylation modifiers or readers hold the potential to deliver a combinatorial attack on multiple pathogenic pathways. As mentioned in the above sections, numerous preclinical studies have been undertaken in PAH with a particular focus on HDAC and BET inhibitors. Although pan-HDAC inhibition using TSA was associated with development of emphysema (Mizuno et al., 2011) as well as adverse remodelling and dysfunction of the pressure-overloaded RV (Bogaard et al., 2011), the positive results obtained with two class I HDAC inhibitors (VPA and MGCD0103; Cavasin et al., 2012b; Zhao et al., 2012a) advocate that member-selective HDAC inhibitors could eventually expand the limited armamentarium against PAH.

Besides, the enthusiastic preclinical findings using BET inhibitors have strengthened the initiation of a clinical trial designed to investigate the safety, tolerability and effectiveness of RVX-208 in PAH patients (NCT03655704), offering a glimmer of hope for a way to stop the progression of the disease. Unfortunately, most drugs fail to reproduce the promise shown in preclinical studies. Along with the implementation of the standard and methodological rigour in PAH preclinical studies (Provencher et al., 2018) and the development of predictive biomarkers to identify poor- and good-responder patients,
a deeper mechanistic understanding of the altered epigenetic landscape is warranted to reduce the gap between preclinical animal studies and clinical trials.

Lessons gleaned from cancer biology can be harnessed for optimizing epigenetic therapy in PAH. It is important to note that the pathogenesis of PAH and RVH involves the complex interaction between resident vascular cells (e.g., PAECs, PASMCs, and PAAFs), infiltrating immune cell types, vasoconstrictive, pro-proliferative, pro-inflammatory and pro-fibrotic mediators. Therefore, treatment with a single epigenetic drug may not completely reverse the complex disease process of PAH but may require a combination of drugs to promote reverse remodelling of severe human PAH. For instance, it has been demonstrated that BET inhibitors synergize with HDAC or PARP inhibitors to combat the growth of cancer cells (Karakashev et al., 2017; Zhao, Okhovat, Hong, Kim, & Wood, 2019). Given that PARP1 inhibitor shows beneficial effects in different animal models mimicking PAH (Melch et al., 2014), it can be envisaged that this drug combination maximizes the desired intracellular effects. Besides, small chimeric molecules have been recently designed to target BET proteins for degradation providing an alternative approach to inhibit BET functions (Choi et al., 2019). This strategy allows us to circumvent a possible compensatory accumulation of BET protein in response to their inhibition and to abolish the kinase- and HAT-mediated functions of BRD4 (potentially involved in PAH development and progression) in addition to the interaction between the BRD and the acetyl group.

Another line of active research involves strategies to improve drug delivery for specifically targeting diseased cells while minimizing the potential unwanted toxicity within healthy tissues. Notably, the majority of HDAC inhibitors currently evaluated in PAH non-selectively modulate the activities of HDAC isoforms and also exert off-target effects, which may limit the clinical application. Moreover, the mixed results of HDAC inhibition on RV function highlight the need for identification of specific HDAC isoforms dysregulated in human PAH. The expression pattern of individual enzyme isoforms in different tissues and vascular cells of the cardiopulmonary system in rodent and human PAH should be profiled in order to circumvent the harmful side effects of broad-spectrum epigenetic modulation.

9.1 Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/2020 (Alexander et al., 2019; Alexander, Keely, et al., 2019).

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