Epigenetic Regulation of Endothelial Dysfunction and Inflammation in Pulmonary Arterial Hypertension

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Abstract: Once perceived as a disorder treated by vasodilation, pulmonary artery hypertension (PAH) has emerged as a pulmonary vascular disease with severe endothelial cell dysfunction. In the absence of a cure, many studies seek to understand the detailed mechanisms of EC regulation to potentially create more therapeutic options for PAH. Endothelial dysfunction is characterized by complex phenotypic changes including unchecked proliferation, apoptosis-resistance, enhanced inflammatory signaling and metabolic reprogramming. Recent studies have highlighted the role of epigenetic modifications leading to pro-inflammatory response pathways, endothelial dysfunction, and the progression of PAH. This review summarizes the existing literature on epigenetic mechanisms such as DNA methylation, histone modifications, and non-coding RNAs, which can lead to aberrant endothelial function. Our goal is to develop a conceptual framework for immune dysregulation and epigenetic changes in endothelial cells in the context of PAH. These studies as well as others may lead to advances in therapeutics to treat this devastating disease.

Keywords: pulmonary arterial hypertension; endothelial cell; endothelial dysfunction; inflammation; epigenetic regulation

1. Introduction

Pulmonary Arterial Hypertension (PAH) is defined by abnormally high blood pressure, increased pulmonary vascular resistance, and pulmonary arteriopathy. This life-threatening disease can result in progressive symptoms such as shortness of breath, right heart failure, and death. Although the exact cause remains unknown, many studies seek to improve the poor prognosis and limited therapeutic options of the disease. One of the major limitations of currently approved PAH treatments is that these therapies mainly target the vasotonus which is not sufficient in mitigating the occlusive arteriopathy in PAH. Endothelial cells (ECs) have become a focal point in PAH pathophysiology due to their critical roles in lung vascular function [1]. The disruption of EC function can occur by cardiovascular risk factors which are known to promote oxidative stress and other metabolic changes [2–4]. Endothelial dysfunction manifests with features, such as a cancer cell-like phenotype including unchecked proliferation, apoptosis-resistance, increased pro-inflammatory signaling, and metabolic reprogramming towards glycolysis [5,6]. These unfavorable changes in ECs are often compounded with other irregularities such as altered hemostasis and thrombosis [7,8]. Injured ECs also undergo endothelial-to-mesenchymal transition (EnMT) which severely impacts their functional characteristics [9]. EnMT ECs show a pro-inflammatory phenotype and altered blood barrier function [9]. A more comprehensive understanding of the mechanisms leading to endothelial dysfunction will aid in designing specific approaches to treat PAH as well as other pulmonary diseases [10].

The observation that inflammation is present in the pulmonary arteries of PAH patients is one of the oldest histopathological findings in PAH [11]. Over the past decades, multiple investigations have yielded important results on how inflammation and dysregulation...
of the immune system contribute to pulmonary artery remodeling and PAH [11–13]. Yet, immune dysregulation can also exert fundamental effects on endothelial function and contribute to PAH pathophysiology on multiple levels [14]. One less well investigated area is how inflammation and epigenetic changes affect EC function. Here, we are summarizing existing literature to develop a conceptual framework for the regulation of endothelial function via immune dysregulation and epigenetic changes in PAH.

2. Endothelial Dysfunction

The endothelium serves many homeostatic functions in the blood vessels including regulation of leukocyte trafficking, gas exchange, and regulation of vascular smooth muscle cell (SMC) function [15–17]. It is therefore not surprising that changes in endothelial function have a fundamental effect on many key elements of vascular and immunological biology. Because of the integral role of EC dysfunction in PAH, a variety of scientific and clinical tools as well as assays have been developed to measure endothelial function. In vitro, these assays include the ones that measure the proliferative potential, including clonogenic expansion potential endothelial cell colony forming cell (ECFC) assay and various proliferation assays including the 5-bromodeoxyuridine incorporation assay [18,19]. Additional assays provide an analysis of the migratory and proliferative capacity, including gap closure assays (also known as “wound repair” assays) and angiogenesis assays. Multiple forms of angiogenesis assays have been developed, starting from simple 2-dimensional (2D) network/tube formation assays on a layer of extracellular matrix (ECM) [20]. These assays have been further developed to provide a 3-dimensional, more realistic environment for tube formation, including a 3D sandwich assay or a spheroid sprouting assay [18].

One important determinant of endothelial function plays a prominent role in studying the microvascular capillary endothelium of the lung, particularly in the context of acute lung injury [21]. Lung microvascular ECs have a crucial role in regulating fluid and protein homeostasis of the blood during passage of the lung capillary network [22]. Common during acute lung injury, dysfunctional capillary ECs can allow massive fluid and protein extravasation with detrimental consequences for gas exchange [21]. This dysfunctional aspect of the endothelium is commonly studied by barrier function assays [22]. Some of these assays use the fact that an intact endothelial barrier has a higher electrical impedance than leaky, dysfunctional endothelium, whereas other assays make use of the impaired barrier for plasma proteins, such as albumin, in dysfunctional ECs [23,24].

A very important aspect of dysfunctional ECs is the impaired interaction with other mural/vascular cells, particularly vascular smooth muscle cells (SMCs) [25]. ECs produce a variety of mediators that limit vasoconstriction and SMC growth, including nitric oxide, prostacyclin, and bone morphogenetic protein ligands [26–28]. Dysfunctional endothelial to SMC crosstalk is derived from the lack or impaired signaling of these EC-derived mediators. The production of mediators that promote vasoconstriction and SMC growth such as endothelin-1 or transforming growth factor-β [29–31]. Together, vasodilators and vasoconstrictors function in counterpart of each other to regulate vascular integrity and function. Many potential mechanisms have been investigated over the years that have the potential to initiate and drive endothelial dysfunction in PAH, including genetics and inflammation, but the role of epigenetic regulation of endothelial function has only recently been studied in more detail.

3. Epigenetic Regulation of Endothelial Function

Epigenetic regulation links changes in chromatin structure to activation or repression of signaling pathways that alter EC function [32]. Vascular ECs require specific genes in the chromatin to be activated or silenced to maintain homeostasis. These modifications are reversible and impact endothelial gene expression rather than the DNA sequence itself. Increasing evidence has shown that endothelial activation is regulated by epigenetic mechanisms such as DNA methylation, histone modifications, and non-coding RNAs [33–35]. Defects in these pathways can lead to increased expression of pro-inflammatory cytokines
such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), nuclear factor kappa B (NF-κB), type I interferons (IFN), and interferon regulatory factors (IRFs), including IRF1 and IRF3. Understanding the wide range of epigenetic mechanisms may help identify potential biomarkers and therapeutic targets for PAH [36,37]. Methods such as chromatin immunoprecipitation (Ch-IP) and RNA-sequencing (RNA-seq) have shed light on pathways that alter endothelial function. Ch-IP assays are primarily used to identify specific genes associated with histone modifying marks whereas RNA-seq can quantitate the expression of spliced variants and non-coding RNAs [38]. Other approaches may include whole-genome reporters to depict a large set of genes regulated by epigenetic change [39].

DNA methylation is a covalent modification that adds a methyl group to the 5-position of cytosine in a sequence, thus yielding a 5-methylcytosine [40]. These modified groups are located within CpG dinucleotides and serve in either recruiting transcriptional repressors, i.e., methyl-CpG binding proteins, or inhibiting the transcription factors at the promoter region, i.e., CCCTC-binding factors (CTCF) [41]. DNA methylation reactions are catalyzed by two types of DNA methyltransferases (DNMTs), DNMT1 and DNMT3A/DNMT3B [42-44]. DNMT1 is responsible for maintaining the methylation in the newly synthesized strand and binds to hemi-methylated cytosines [44]. In contrast, DNMT3A and DNMT3B function in de novo methylation which does not require hemi-methylation [43,45]. Previous studies highlight the significance of DNA methylation in EC function as well as endothelium-specific expression of endothelial NO synthase (eNOS) via VEGF signaling pathways [33,46]. As the name indicates, the eNOS enzyme serves a critical role in producing nitric oxide in the vascular endothelium which enhances vasodilation and maintains endothelial homeostasis [47]. Furthermore, DNA methylation of the eNOS proximal promoter negatively impacts functional activity as well as vascular tone and angiogenesis. These studies are the first to show regulatory roles for DNA methylation in the constitutively expressed eNOS gene in the vascular endothelium [33]. In addition, DNA methylation activates or inhibits inflammatory pathways associated with PAH [48-50].

Another layer of chromatin remodeling is the post-translation modification (PTM) of histones which have been well studied in vascular biology [51]. Gene expression begins with tightly packaged DNA, or chromosomes, that surround histone proteins to form an octamer complex known as a nucleosome [52]. Nucleosomes consist of two histone copies of histones H2A, H2B, H3, and H4 that is further compacted by linker DNA [53]. PTMs modulate the structural changes within the nucleosome and produce histone variants that regulate the transcription of genes. The most notable histone modifications are methylation and acetylation which both regulate inflammation as well as EC function [34]. ECs have distinct modifications of histones at the core promoter, specifically, enriched di- and tri-methylation of histone H3 lysine 4 (H3K4) and acetylation of histone H3 lysine 9 (H3K9), and histone H4 lysine 12 (H4K12) [34]. Histone methyltransferases (HMTs) are enzymes that regulate transcription by transferring one, two, or three methyl groups to the histones [54]. Some of the most prominent HMTs involved in endothelial gene expression are EZH2 and ASH2 [55]. Histone modifications such as H3K27me3 are associated with gene silencing, or inactivation, and contribute to altered endothelial gene expression [56,57]. Another histone modification, histone acetylation, activates gene transcription by adding negatively charged acetyl groups to DNA, ultimately relaxing the chromatin [58]. Acetylation of histones is performed by histone acetyltransferase (HATs) enzymes that transfer acetyl groups to conserved lysine residues and are linked to transcriptional activation [59]. Specific HATs, such as histone acetyltransferase 7 (KAT7) modulates endothelial function by activating the transcription of vascular endothelial growth factors (VEGFs) [60]. It was shown that KAT7 is necessary for VEGFR-2 transcription and blood vessel formation in zebrafish embryos [60].

Despite the lack of studies on RNA-based mechanisms, non-coding RNAs are recognized for their regulation of post-transcriptional activities in ECs. While non-coding RNAs are not epigenetic per se, their expression is frequently regulated by epigenetic modification of their respective gene promoter [61-65]. Non-coding RNAs such as microRNA
(miRNA) and long coding RNA (lncRNA) can dysregulate EC function by permitting smooth muscle cell proliferation and EnMT [66,67]. Long non-coding RNAs are defined as being larger than 200 nucleotides whereas the average size of miRNAs is approximately 20–21 nucleotides in length [68,69]. Microarray analysis has helped construct a spheroid model of miRNA, including miR-424-5p and miR-29a-3p that promote endothelial migration and repress genes in the VEGF pathway [70]. These studies have also validated the regulatory roles of miRNAs in the Mitogen-Associated Protein Kinase (MAPK) pathway which mediates VEGF-A-induced cell proliferation [70]. Other studies have shown the regulation of vascular remodeling and angiogenesis through the combined functions of novel miRNAs such as miR-181a-5p and miR-324-5p [71]. The investigation of long non-coding RNAs is also an emerging field in endothelial dysfunction and vascular biology [72]. Past studies have used chromosome- and genome-wide screening of cDNA libraries to show high expression of lncRNAs in the human genome [73]. Among the novel lncRNAs expressed in ECs are metastasis associated lung adenocarcinoma transcript 1 (MALAT1), a regulator of glucose-induced production by the inflammatory cytokines IL-6 and TNF-α [74]. Studies show that MALAT1 is increased in hypoxia condition and the knockdown of the lncRNA decreases proliferation and regulates endothelial functions, such as angiogenesis [75]. Other lncRNAs such as antisense non-coding RNA in the INK4 locus (ANRIL) and non-coding RNA activated by DNA damage (NORAD) also causes endothelial dysfunction by impeding apoptosis and increasing proliferation [76,77].

Because both ECs and smooth muscle cells (SMCs) from PAH patients maintain features of dysfunction in vitro, it has been widely assumed that epigenetic modifications occur in multiple cell types of PAH patients which also contributes to pathogenesis and disease progression [78–81]. These studies are not limited to human patients as others have used newborn rat models to investigate the epigenetic regulation of eNOS expression [82]. Up to date, more epigenetic changes have been identified in pulmonary artery SMCs than in ECs [51,62,83–87]. Some studies suggest a potential role for SMC contact and endothelial metabolism in chromatin remodeling leading to EC regeneration, however, this level of gene regulation remains unclear [88]. Epigenetic modifications also modulate EnMT, lung vascular and right ventricular remodeling, as well as novel transcriptional targets for idiopathic PAH [89–91]. Important epigenetic mechanisms that have been identified in PAH include promoter DNA hypermethylation in pulmonary ECs from PAH patients that particularly affected the expression of genes within the lipid metabolism [62]. Further, in a hypoxic fetal lamb model of pulmonary hypertension (PH), expression of eNOS was decreased, and this reduction was associated with epigenetic changes, including promoter hypermethylation and histone acetylation and trimethylation [87]. A novel mechanism of endothelial dysfunction in PAH was based on reduced expression of Bola Family Member 3 (BOLA3) and its transcription was inhibited via histone deacetylation [92]. Extensive remodeling of H3K27AC may leads to aberrant gene-regulatory networks in PAECs in the context of PAH [93]. A combination of altered transcription factor activity and remodeling of active endothelial enhancers was found using prediction analysis in PAH ECs [93]. We have summarized the important studies regarding epigenetic regulation of endothelial function and PAH in Table 1.

Besides epigenetic changes, inflammation has a key role in the development and progression of PAH and to understand the role of inflammation in epigenetic regulation, it is imperative to immerse further into the detailed molecular mechanisms that drive inflammation and immune dysregulation [11,13].
Table 1. Previous literature of putative epigenetic pathways and regulatory factors associated with PAH and endothelial dysfunction.

| Source                                      | Epigenetic Regulators | Primary Model | Description                                      |
|----------------------------------------------|-----------------------|---------------|--------------------------------------------------|
| DNA methylation                              |                       |               |                                                  |
| Chan et al. (2004) [33]                      | multiple              | mice          | eNOS expression                                  |
| Fish et al. (2005) [34]                      | multiple              | in vitro      |                                                  |
| Quentmeier et al. (2012) [46]                | multiple              | in vitro      | angiogenesis, proliferation, EnMT, inflammation  |
| Hautefort et al. (2017) [62]                 | multiple              | in vitro      | proliferation, EnMT, inflammation, angiogenesis  |
| Wang et al. (2018) [49]                      | N/A                   | patients      | vascular remodeling                              |
| Ke et al. (2018) [87]                        | Specificity Protein 1 | in vitro/lamb | eNOS expression, vascular remodeling             |
| Yan et al. (2020) [45]                       | DNMT3B                | rat           | eNOS expression, mitochondrial metabolism        |
| Joshi et al. (2020) [48]                     | multiple              | mice          |                                                  |
| Histone modifications                        |                       |               |                                                  |
| Maleszewska et al. (2016) [55]               | EZH2                  | in vitro      | EC expression                                    |
| Hulshoff et al. (2018) [89]                  | multiple              | in vitro      |                                                  |
| Li et al. (2018) [94]                        | JARID1B               | in vitro      |                                                  |
| Yan et al. (2018) [60]                       | KAT7                  | zebrafish     | angiogenesis                                     |
| Yu et al. (2019) [92]                        | BOLA3                 | in vitro      | EC metabolism                                    |
| Non-coding RNAs                              |                       |               |                                                  |
| Michalik et al. (2014) [75]                  | MALAT1                | in vitro      | proliferation                                    |
| Simion et al. (2019) [72]                    | multiple              | in vitro      | angiogenesis                                     |
| Puthanveetil et al. (2015) [74]              | MALAT1                | in vitro/mice | inflammation                                     |
| Rosano et al. (2020) [70]                    | miR-424-5p, miR-29a-3p| in vitro      | EC expression                                    |
| Sindi et al. (2020) [71]                     | miR-181a-5p, miR-324-5p| in vitro      | proliferation, angiogenesis, EC dysfunction      |
| Liu et al. (2020) [76]                       | ANRIL                 | patients      |                                                  |
| Bian et al. (2021) [77]                      | NORAD                 | in vitro      | proliferation, EnMT                             |

4. PAMPs and DAMPs

ECs serve as the primary defensive response against microbial infection and tissue injury in the vascular system. An important function of ECs is to recognize stress-associated signals that activate an inflammatory response leading to defects in endothelial structure and function. Pathogen-associated molecular pattern (PAMPs) and damage-associated molecular patterns (DAMPs) are two classes of ligands that are detected by host cells through a family of pattern recognition receptors (PRRs) [95]. PAMPs are exogenous molecules produced by microorganisms which includes lipoteichoic acids (LTAs), lipopolysaccharides (LPSs), peptidoglycan, flagellin, lipoproteins, and double stranded (ds) RNA [96]. In contrast, DAMPs are endogenous molecules secreted from damaged or dying cells that are recognized by the same PRRs as PAMPs and thereby activate similar pathways as PAMPs [97]. Notable DAMPs are DNA released from replication stress, heat shock proteins, S100 proteins, and high mobility group box 1 (HMGB-1). Also known as signal 0s, PAMPs and DAMPs bind to highly conserved regions on PRRs and stimulate intracellular signaling [98]. This interaction stimulates a signaling cascade responsible for upregulating the expression of type I IFNs, pro-inflammatory cytokines, chemokines, and other mediators that can promote endothelial dysfunction in the pulmonary circulation [11,12,15]. As a result, ECs undergo apoptosis or the more specialized pyro-apoptosis, and exhibit impaired barrier function, causing decreased blood flow [67,76,99–104].

Past research has focused on how PAMPs and DAMPs activate specific PRR-mediated inflammatory response pathways amplifying cardiovascular disease [97,105]. PAMP/DAMPs also induce inflammatory pathways in response to severe acute respiratory syndrome (SARS)-coronavirus (CoV) and Middle East respiratory syndrome CoV (MERS) infections [103,106]. RNA sequencing of virus-infected ECs yields upregulation of innate immune regulator genes (IGs) in response to PAMPS/DAMPS such as lipopolysaccharide (LPS) and oxidized low density lipoproteins (oxLDL) [103]. IG induction result in transcriptomic changes leading to thrombosis specifically in microvascular ECs [103].

There are several families of PRRs that recognize PAMPs and DAMPs including toll-like receptors (TLRs), C-type lectin receptors (CLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), NOD-like receptors (NLRs), and leucin rich repeats-containing receptors (LRRs) [98,107–109]. TLRs are highly conserved type I membrane glycoproteins that were first discovered in *Drosophila melanogaster* [110,111]. Humans and mice have 10
and 12 known TLRs, respectively [105,109]. TLRs either exist as preformed dimers or form homo- or heterodimers following binding of PAMPs and DAMPs, subsequently leading to the activation of signaling pathways mediated by NF-κB, IRFs, and MAP kinases [112]. As a result, TLR stimulation causes EC activation, cell proliferation, inflammation, and ultimately PAH [111]. TLR activation and inflammation can lead to the development of PAH through several mechanisms. One of which involves the increased expression of endothelin-1 (ET-1), an endogenous vasoconstrictor released from the vascular smooth muscle of pulmonary arteries [113,114].

The subcellular localization of TLRs is tightly controlled at regions such as the endosomes and lysosomes [18,115]. TLRs located at the cell surface, such as TLR1, 2, 4, 5, 6, and 11, bind lipids, lipoproteins, and other membrane molecules [96,106,110,116]. In contrast, the TLRs 3, 7, 8, and 9 are located at membranes of intracellular vesicles, such as endosomes [96,106,110,113,116]. TLR2 and TLR4 signaling is triggered by PAMP molecules such as LPS and oxidized phospholipids. TLR3 is a nucleotide-sensor of dsRNA, which is a result of viral pathogens, and other self-RNAs including mRNA [112,116]. Further, TLR3 affects multiple fundamental aspects of cellular function, including autophagy and apoptosis [117,118]. In the systemic and the pulmonary circulation, TLR3 has a protective function, and our group has shown that silencing of TLR3 expression in pulmonary artery ECs promotes apoptosis, whereas a TLR3 agonist reduced PH in rats [119,120]. However, other reports also demonstrate that severe systemic arterial injury predisposes to increased vascular injury following activation of TLR3 [121]. In models of PH, various TLR4 ligands, including high mobility group box 1 promote PH in experimental models and genetic knockout of TLR4 prevents PH in mice [122–129]. Our group has shown that endothelial TLR3 deficiency contributes to PAH via increased endothelial apoptosis [120]. In addition, treatment with a TLR3 agonist reverses severe PH in a rodent model [120]. To further understand the effects of PRR signaling, a closer look needs to be taken into central pro-inflammatory pathways, which are frequently activated downstream of PRRs.

5. Central Pathways Driving Inflammation

Endothelial dysfunction is driven by pro-inflammatory mediators that act downstream of PRR signaling pathways [1,101]. These pathways specifically induce genes encoding cytokines, chemokines, IRF transcription factors, adhesion molecules, and regulators that recruit leukocytes to sites of damage and infection. Upon recognizing PAMPs and DAMPs, PRRs, such as TLRs, induce an intracellular signaling cascade defined by several phosphorylation events as PTMs [106,110,116]. The combined efforts of pro-inflammatory factors affect inflammation, coagulation, endothelial barrier permeability, and gas exchange between the blood and lung tissue [11]. Although inflammatory response factors help protect against additional damage to the endothelium, these pathways lead to pathogenesis of PAH [11].

TLR signaling, for example, primarily induces a type I IFN response leading to the transcription of specific cytokines, such as IL-6, CXC chemokine ligand 8 (CXCL8), and CC chemokine ligand 2 (CCL2) as well as other stimulatory molecules [96,106,110,116]. TLR activation can also lead to activation of ECs which is controlled by gene expression [130]. These cytokines also help promote angiogenesis, recruit leukocytes, and upregulate leukocyte adhesion molecules in ECs [131–133]. Vascular ECs have contrasting classic- and trans-signaling of IL-6 [134]. Demethylation in the promoter activates transcription of the IL-6 gene in ECs [135], a mechanism that could also play a role in PAH. The following paragraphs discuss potential disease specific mechanisms in a selected set of pathways.

Nuclear factor-κB (NF-κB) is a central switchboard of inflammation in most cell types of the body [136]. Multiple inflammatory signals, including downstream of TNF-α or TLRs, induce NF-κB activation. The canonical NF-κB pathway requires nuclear translocation of a p65/p50 dimer to the nucleus, leading to activation of gene transcription [136]. Activation of this pathway is a key player in vascular and cardiac remodeling, including in PAH and experimental models of severe PH, as shown by our group [94,137–143]. Our group has
also shown that inhibition of NF-κB reduces occlusive pulmonary arteriopathy in severe PH in rats [141]. Epigenetic modifications, including histone deacetylation, miRNAs or demethylation, can alter NF-κB activity and hence may contribute to the amplification of NF-κB signaling in pulmonary arteries from PAH patients [144–149].

Similar to NF-κB, IRF3 and IRF7 are key transcription factors downstream of many PRRs, including TLR3 and TLR4 [150–153]. Phosphorylation and nuclear translocation of IRF3 and IRF7 promote transcription of type I IFNs IFN-α and IFN-β, which triggers a succinct signaling cascade with strong antiviral activity [150–155]. However, the role of type I IFNs is controversial in PAH. On one side, patients with significant pre-existing morbidity, i.e., Multiple Sclerosis, can develop a reversible form of PH under IFN therapy [156–158]. A potential adverse role for type I IFN has been suggested by the protective effect of type I IFN receptor knockout for chronic hypoxic PH in mice [12]. However, type I IFN treatment decreased severe PH in rats, suggesting a context- and disease phenotype-specific role for interferons in lung vascular remodeling [159]. Some of the differences could be explained by epigenetic modification of IRF3 and IRF7 signaling molecules, such as their methylation status, which can alter the extent of downstream signaling [160,161]. Yet, more work is necessary to understand this interaction in ECs.

Hypoxia is a well-known trigger for lung vascular remodeling and endothelial dysfunction [162–167]. Hypoxia signaling is mediated via the hypoxia-inducible factors (HIFs), which are stabilized in the cell following reduced oxygen levels and induce gene transcription by activation of hypoxia-responsive elements (HRE) in the promoter of target genes [168]. Three isoforms of HIF have been shown in humans, HIF-1α, HIF-2α, and HIF-3α that show a vast variety in cell-specific functions [169]. Under normal conditions HIF-1α, and HIF-2α are rapidly degraded, however, during hypoxia the degradation of HIF-1α and HIF-2α is inhibited, hence contributing to the development of PAH [170,171]. HIF-1α is considered the master regulator of homostatic response to hypoxia by promoting the transcription of genes involved in angiogenesis, vasculogenesis, apoptosis, and energy metabolism [172–174]. Important targets of HIF-1α are vascular endothelial growth factors (VEGFs) which bind to and stimulate pro-angiogenic receptors as well as promote vascular permeability [175,176]. In contrast, HIF-2α has a distinct role in promoting EnMT, which contributes to pulmonary vascular remodeling [171]. In addition, HIF1α expression is rapidly increased following induced hypoxia whereas HIF-2α is known to gradually accumulate later in time [170]. There is a variety of ways by which HIF transcription factors affect inflammation and epigenetics, including in ECs. One example is activation of NF-κB signaling by hypoxia via the prolyl hydroxylases that also regulate HIF activity and degradation [169]. Hypoxia and HIFs have been shown to induce significant functional changes in ECs in PH, including metabolic reprogramming in ECs, dysfunction of endothelial colony-forming cells, impaired angiogenesis, and altered estrogen metabolism via HIF-1α [177–180]. More recently, HIF-2α has been identified as another major driver of lung vascular remodeling and PH, and HIF-2α drives EnMT, the process by which ECs change phenotype and function to a myofibroblast/SMC-like cell [171,181–183]. EnMT promotes lung vascular remodeling and contributes to the dysfunctional endothelial phenotype in PAH [9,184,185]. Interestingly, EnMT is regulated via epigenetic modification of multiple potential target genes [62,93]. In addition, hypoxia and HIFs can induce various epigenetic changes, including histone acetylation and methylation [61,64,94,186,187].

6. Conclusions

Epigenetic modifications and inflammation have significant effects on the function of the pulmonary artery endothelium, and thereby contribute to the pathogenesis of PAH. While epigenetics and inflammation also contribute to PAH by affecting cellular signaling in other important cell types and organs, including pulmonary artery SMCs, pulmonary artery adventitia fibroblasts and the right ventricle [61,85,188,189]. Although the complete mechanisms of epigenetic regulation of endothelial dysfunction remain unclear, here we summarize the current literature on putative modifications and pathways leading to
inflammation, lung vascular remodeling and PAH. The development of novel therapies targeting these changes will open new possibilities to treat this life-threatening disease.

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