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Review

Hypoxia and panvascular diseases: exploring the role of hypoxia-inducible factors in vascular smooth muscle cells under panvascular pathologies

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ABSTRACT

As an emerging discipline, panvascular diseases are a set of vascular diseases with atherosclerosis as the common pathogenic hallmark, which mostly affect vital organs like the heart, brain, kidney, and limbs. As the major responser to the most common stressor in the vasculature (hypoxia)—hypoxia-inducible factors (HIFs), and the primary regulator of pressure and oxygen delivery in the vasculature—vascular smooth muscle cells (VSMCs), their own multifaceted nature and their interactions with each other are fascinating. Abnormally active VSMCs (e.g., atherosclerosis, pulmonary hypertension) or abnormally dysfunctional VSMCs (e.g., aneurysms, vascular calcification) are associated with HIFs. These widespread systemic diseases also reflect the interdisciplinary nature of panvascular medicine. Moreover, given the comparable proliferative characteristics exhibited by VSMCs and cancer cells, and the delicate equilibrium between angiogenesis and cancer progression, there is a pressing need for more accurate modulation targets or combination approaches to bolster the effectiveness of HIF targeting therapies. Based on the aforementioned content, this review primarily focused on the significance of integrating the overall and local perspectives, as well as temporal and spatial balance, in the context of the HIF signaling pathway in VSMC-related panvascular diseases. Furthermore, the review discussed the implications of HIF-targeting drugs on panvascular disorders, while considering the trade-offs involved.

1. Introduction

Panvascular diseases, an emerging field in medicine, encompass a collection of vascular disorders that share a common etiology characterized by atherosclerosis [1–4]. These conditions primarily impact vital organs such as the heart, brain, kidneys, and extremities. The translation of panvascular medicine from theoretical concepts to clinical practice has gained momentum. Hypoxia, triggered by high altitude, strenuous exercise, and various pathological conditions, can induce oxygen deprivation in the body. Oxygen delivery to tissues and organs relies on hemoglobin in the bloodstream. Crucially, the tissues and organs implicated in panvascular diseases are particularly sensitive to fluctuations in oxygen levels and ischemia/hypoxia can potentiate disease progression. Hypoxia-inducible factors (HIFs), as major regulators of the hypoxic response in organisms, are multifaceted and spatiotemporally specific, and have cardiovascular correlations [5]. Consequently, a comprehensive examination of the interplay between hypoxia and panvascularity is imperative at this juncture.

HIFs play a crucial role in governing the cellular response to low oxygen levels (Fig. 1) [5,6]. HIF-1α was initially discovered as the first isoform of the HIF through affinity purification with oligonucleotides from the erythropoietin (*EPO*) gene locus [7]. HIF-1α is a highly conserved protein that serves as the primary regulator of cellular and organismal homeostasis in response to hypoxia. It is present in almost all aerobic organisms and is estimated to govern the regulation of more than 2% of human genes, specifically

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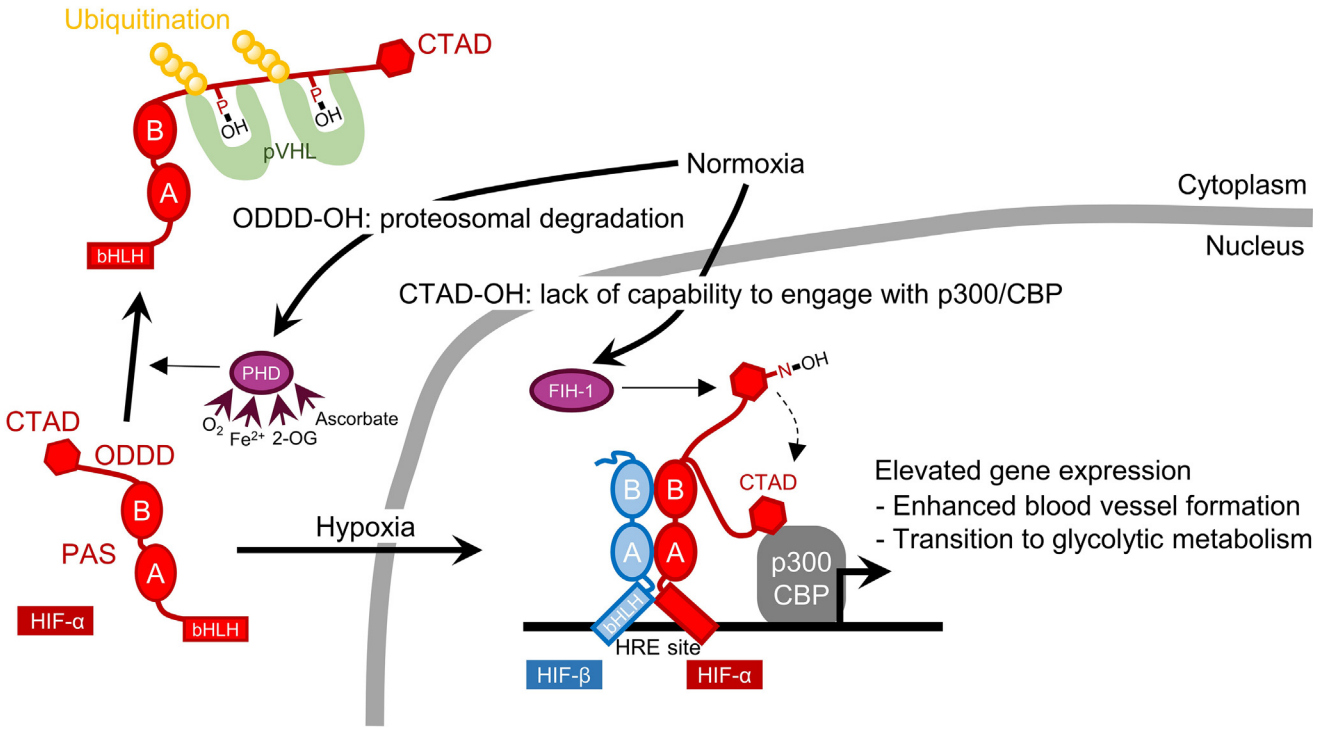

Fig. 1. Classical HIF pathway signaling. In normoxia, hydroxylation of HIF-α's conserved proline motifs in ODDD by PHDs leads to its degradation via the E3 ubiquitin ligase complex and 26S proteasomal pathway, preventing it from forming a heterodimer with HIF-β for transcriptional function. FIH-1 is an asparagine hydroxylase that blocks the interaction between HIF-α and co-transcription factor p300/CBP by hydroxylating N803 of HIF-α, providing an alternative way to inhibit HIF-α transcription. PHD and FIH, besides oxygen molecules, also require Fe^{2+}, ascorbate, and α-ketoglutaric acid (2-OG, an intermediate of the TCA cycle). In the context of hypoxia, PHD-mediated hydroxylation of HIF-α fails, leading to the rapid buildup of HIF-α. HIF-α subsequently combines with HIF-β to form a heterodimer that translocates into the nucleus. In order to bind to E-box-like HREs and initiate downstream adaptive responses to hypoxia, heterodimers form complexes with co-transcription factor p300/CBP. The GC-rich start-up region of HREs generally contains a 5′-(A/G)CGTG-3′ sequence. bHLH: basic-helix-loop-helix; CBP: cyclic adenosine monophosphate response element-binding protein (CREB) binding protein; CTAD: COOH-terminal transactivation domain; CTAD-OH: hydroxylation of CTAD; FIH-1: factor inhibiting HIF-1; HIF: hypoxia-inducible factor; HRE: hypoxia-response element; ODDD: O_2-dependent degradation domain; ODDD-OH: hydroxylation of ODDD; p300: 300-kilodalton coactivator protein; PAS: Per-Arnt-Sim; PHD: prolyl hydroxylase; TCA cycle: tricarboxylic acid cycle; VHL: von Hippel–Lindau disease tumor suppressor protein.

involved in maintaining oxygen homeostasis in diverse cellular contexts. HIF-2α and HIF-3α were lately detected through homology searches or by screening for interaction partners with HIF-1β. HIF-3α is the isoform that is more distantly related and, under certain splicing arrangements, produces a polypeptide that counteracts gene expression modulated by hypoxia response elements (HREs). However, both HIF-1α and HIF-2α are closely related, and activate gene transcription that is dependent on HREs [8]. The shift from oxidative phosphorylation to oxygen-independent glycolysis is the earliest cellular response to hypoxia, and it has been observed in organisms like *Caenorhabditis elegans* (*C. elegans*). Although HIF-1α is conserved across species, ranging from *C. elegans* to humans, the presence of HIF-2α is limited to more complex vertebrates like chickens, quails, and mammals [9]. In advanced organisms with specialized oxygen delivery systems, both HIF-1α and HIF-2α play crucial roles in facilitating an adaptive oxygen response mechanism [10]. Particularly, HIF-1 and HIF-2 play interconnected and sequentially complementary roles in the process of angiogenesis. In general, HIF-1 assumes dominance during vasculogenesis under conditions of profound hypoxia, while HIF-2 primarily orchestrates the concluding phases of vascular remodeling and stabilization under the hypoxic environment. These later stages involve crucial events such as the recruitment of vascular pericytes and vascular smooth muscle cells (VSMCs) [5].

VSMCs exhibit a complete and specialized phenotype in healthy blood vessels, while still possessing a high degree of plasticity, manifesting two distinct phenotypes: the contractile (differentiated) phenotype and the synthetic (dedifferentiated) phenotype. Dedifferentiated VSMCs exhibit decreased myofilament density and reduced expression of contractile proteins, while displaying elevated expression of extracellular matrix (ECM) components and ECM-remodeling enzymes [11]. VSMCs are inherently porous and elastic, and their phenotype that promotes ECM secretion can coordinately regulate biomechanical response properties such as vascular elasticity, tone, and compliance [12]. Dedifferentiated VSMCs regulate their proliferation, migration, and inflammatory capacity, crucial for arterial remodeling (e.g., atherosclerosis) by multiple mechanisms (e.g., enhanced secretory organelles and increased pro-inflammatory cytokine expression) [11,12]. Furthermore, HIFs are also been implicated in the pathogenesis of VSMCs in panvascular diseases (Fig. 2) [13]. Abnormally active VSMCs featuring phenotypes of proliferation and migration, as seen in atherosclerosis and pulmonary hypertension, are associated with HIFs [14,15]. HIFs in VSMCs of pulmonary hypertension may further display abnormal electrophysiological effects. On the other hand, abnormally dysfunctional VSMCs, such as those seen in aneurysms and vascular calcification (VC), also have an association with HIFs [16,17]. VC is currently a major therapeutic challenge in coronary artery disease, particularly in chronic total occlusion lesions. The pathological process is closely associated with VSMCs apoptosis and marks a critical transition toward functional failure of VSMCs. Thus, the search for HIF targets against panvascular diseases involved in VSMCs has significant implications.

The multifaceted nature of VSMCs and their intricate interactions with HIFs present a captivating area of study, particularly concerning the search for HIF targets against panvascular diseases in VSMCs. The interdisciplinary nature of panvascular medicine emphasizes the necessity of adopting a comprehensive approach

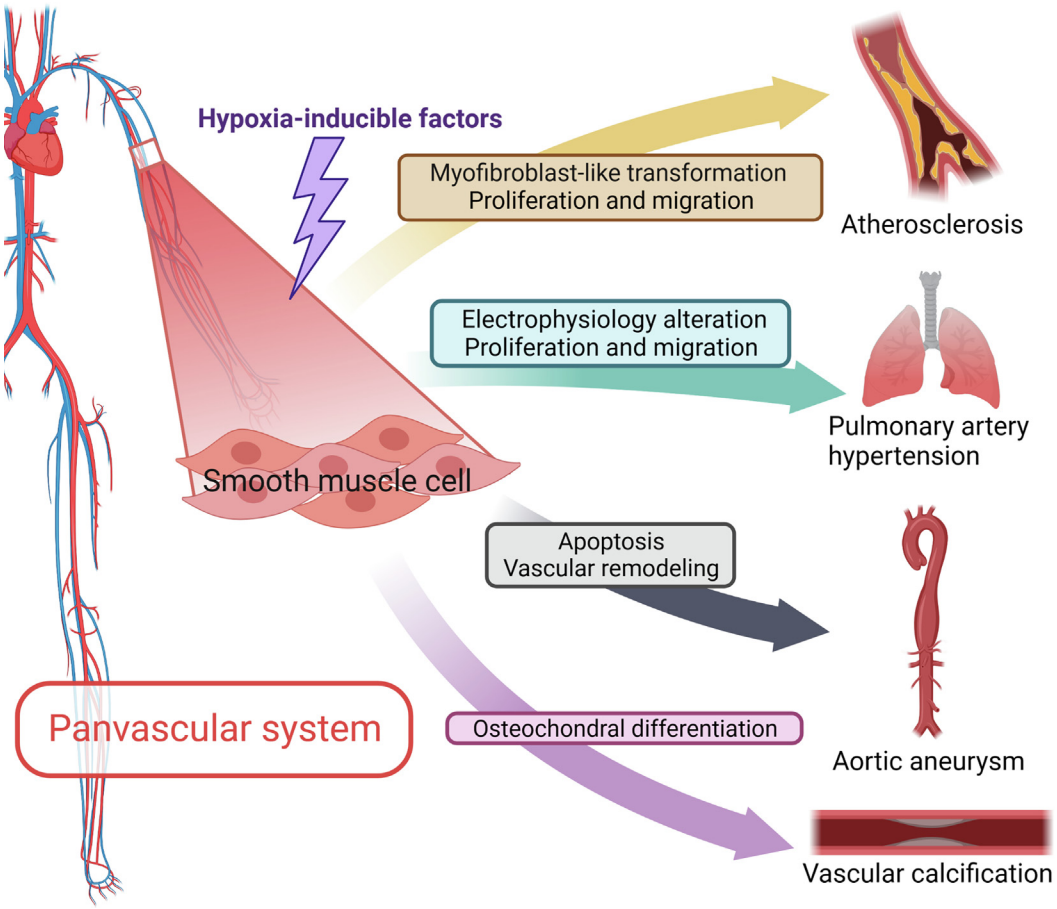

Fig. 2. Phenotypic shift of VSMCs in response to hypoxia condition of panvascular system. (Created with BioRender.com).

that considers the whole and local aspects, as well as temporal and spatial dimensions, when investigating these targets. This review aims to compare the similarity between VSMCs and cancer cells of plasticity and clonality, summarize the role of HIFs in panvascular diseases (atherosclerosis, pulmonary arterial hypertension (PAH), aortic aneurysm (AA), and VC), and discuss the challenges and opportunities for HIFs-targeted candidates/drugs for panvascular diseases.

2. Proliferative properties of VSMCs: metabolic similarities with cancer cells

Modulating cellular metabolism is a prevailing characteristic during periods of oxygen deficiency [18]. Oxygen-starved cells elevate glucose uptake and glycolytic flux while decreasing mitochondrial metabolism. This promotes the availability of lipids to support critical processes such as the production of organelle and plasma membranes [6]. Hypoxic mechanisms impacting metabolic pathways are significant for cancer. Disorganized blood vessels, inadequate oxygen distribution, and limited nutrients in solid tumors create stressful environments, which cancer cells utilize to survive and proliferate, fueling tumor progression. Similarly, the plasticity and clonality of VSMCs exhibit a cancer-like phenotype switch under hypoxia, which is targeted in current therapies for some panvascular diseases.

Glucose metabolism is a significant contributor to vascular reactivity, with VSMCs exhibiting high levels of glucose utilization and lactate generation, even in normal and well-oxygenated surroundings [19,20]. VSMCs derive around 30% of their adenosine triphosphate (ATP) supply from aerobic glycolysis, and approximately 90% of the flux in glycolysis leads to the production of lactate [21]. In pulmonary hypertension, pulmonary arterial VSMCs (PASMCs) exhibit a phenotype that promotes proliferation and resists apoptosis, which is characterized by altered glycolysis metabolism and HIF-1α activation. Furthermore, HIF-1 activation under normal oxygen exacerbates the altered behavior of PASMCs in PAH [22]. Non-oxygen-dependent HIF-1 activation in PAH includes: (1) reduction of superoxide dismutase 2 (SOD2), the up-regulation of growth factors/receptor tyrosine kinases, and the activation of protein kinase B (AKT), extracellular signal-regulated kinase (ERK), and mammalian target of rapamycin (mTOR) that promote proliferation and survival; and (2) the deletion of growth inhibitory factors (such as phosphatase and tensin homolog (PTEN), p53 and Hippo) that increases hydrogen peroxide (H_2O_2) and subsequently activates HIFs [22]. Previously identified as a surface marker on rapidly proliferating cells [23], CD146 recently has been found to act as a co-receptor of vascular endothelial growth factor receptor 2 (VEGFR2) and platelet-derived growth factor receptor-β (PDGFR-β) and interplay with HIF-1α to facilitate PASMCs switch from contractile phenotype to proliferative, exacerbating pulmonary hypertension [24,25]. CD146 was detected in most cell types, comprised of vascular endothelial cells, vascular pericytes and smooth muscle cells, epithelial cells, fibroblasts, mesenchymal stem cells, and lymphocytes, except red blood cells [23]. The expression of CD146 is usually increased in rapidly proliferating cells, such as cells at developmental stages or cancer cells [23]. Ascites-derived stromal

cells Hospicells, which can promote tumorigenicity and angiogenesis, can express CD146 as a surface marker [26]. As a common receptor of PDGFR-β and VEGFR2, or an independent receptor of Wnt1, Wnt5a, VEGF-C, FGF4, netrin-1, and other growth factors, high expression of CD146 has been shown to enhance angiogenesis signaling and promote cellular multiplication, specification, and survival [24]. CD146-HIF-1α cross-regulation is a key factor in vascular remodeling and disease progression of PAH. CD146 has been demonstrated to be significantly increased in PASMCs of PAH and proportional to the severity of the disease. The expression of CD146 and the transcription mechanism of HIF-1α are mutually enhanced, which makes PASMCs lose contractile phenotype and become more synthetic phenotype. The destruction of either side can alleviate the established pulmonary hypertension and enhance cardiac function [25]. Besides the distribution around the blood vessels, $CD146^+$ cells express α-smooth muscle actin (α-SMA) and lack endothelial cell markers CD31 and CD34, and are considered to be perivascular cells (PVCs) [27]. PVCs and their "progeny" mesenchymal stromal cells (MSCs) regulate the development of tissue-specific vascular systems and the maturation and maintenance of endothelial "barrier integrity" and have high therapeutic potential for ischemic diseases [28,29]. Neural/glial antigen 2 $(NG2)^+PDGFR\beta^+CD146^+CD34^-CD31^-CD45^-$ PVCs can be isolated from cardiac PVCs. These cardiac PVCs can increase transmembrane resistance and reduce endothelial cell permeability. This kind of PVC group expresses contraction protein, which is stimulated by adrenaline signals, and is characterized by stereotypical contraction and relaxation. Under hypoxic conditions, the cardiac PVCs activate the HIF-1α pathway along with more resilience to hypoxic stress and the increased secretion of angiogenesis factors. Hyperphysiological low-density lipoproteins (LDLs) reduce PVCs proliferation and induce lipid droplet aggregation [29].

Collectively, the reprogramming of intracellular metabolism in response to oxygen deprivation plays a critical role in cancer and panvascular diseases such as pulmonary hypertension. The promotion of glycolytic flux and decreased mitochondrial metabolism are essential mechanisms for survival and proliferation in cancer cells and VSMCs under hypoxic conditions. In pulmonary hypertension, altered glucose metabolism and HIF-1α activation under normal oxygen further contribute to disease progression with a phenotype switch from contractile to proliferative in PASMCs. The identification of CD146 as a co-receptor of VEGFR2 and PDGFR-β and its interaction with HIF-1α emphasizes its promising role as a therapeutic target in the context of pulmonary hypertension.

3. HIFs in panvascular diseases

3.1. Atherosclerosis

Atherosclerosis, the development of fibrofatty lesions within the arterial wall, predominantly leads to various panvascular events such as coronary heart disease, critical limb ischemia, and the formation of emboli (ultimately resulting in strokes and myocardial infarctions) [30]. VSMC is a major contributor to plaque development at all stages with great plasticity and special clonality [11]. During the early stages of atherosclerosis, VSMCs lose their contractile phenotype and exhibit proliferation and migration, accompanied by a myofibroblast-like transformation (also known as "smooth muscle cell phenotypic switching"), which makes cells capable of becoming foam cells and secreting inflammatory factors. Later, VSMCs exhibit a fibroblast-like phenotype (also known as "reverse differentiation") in a self-repair mechanism but still retain some macrophage-like characteristics, such as the ability to phagocytose.

3.1.1. VSMC HIFs in lipid metabolism of atherosclerosis

Observations suggest that VSMCs in mice initially form the fibrous cap, when switching to a synthetic phenotype in the lesion core. This process involves transforming contractile proteins into ECM components and conducting a different lipid metabolism [11,31]. Hypoxia increases the uptake of LDL and palmitate in VSMCs [32]. LDL carries cholesterol and excess LDL in blood can cause atherosclerosis. Palmitate is a saturated fatty acid found in food that increases LDL levels. Cholesterol is important for cell function but excess in blood contributes to atherosclerosis. HIF-1α targets and increases the 3-hydroxy-3-methylglutaryl-coen zyme A (HMG-CoA) reductase gene to contribute the cholesterol synthesis (Fig. 3) [33]. Under hypoxic conditions, cells with reduced HIF-1 levels have been observed to display lower levels of HMG-CoA reductase mRNA, decreased triglyceride levels, and a higher efflux of cholesterol, resulting in a reduction of total cholesterol content [34]. The reduced ATP-binding cassette transporter (ABCA)-mediated cholesterol efflux observed in hypoxia may be due to subcellular redistribution and decreased protein levels of ABCA1, which could be regulated by HIF-1 via disturbance of vesicular trafficking, ABCA1 recycling, or protein misfolding [35]. Additionally, it has been demonstrated that loading VSMCs with cholesterol can cause them to undergo transdifferentiation into cells resembling macrophages [36]. HIF-1β may have a notable impact on the expression of ABCA1 in human macrophages [37]. Furthermore, VSMC-derived foam cells constitute the predominant population of foam cells observed in apolipoprotein E (ApoE)-deficient mice, with reduced levels of the cholesterol efflux transporter ABCA1 [38–40]. Furthermore, changes in shear stress can activate HIF-1α in VSMCs during atherosclerosis, linking intravascular pressure, vascular inflammation, and lipid deposition [41].

3.1.2. HIFs' effect on proliferation or migration of VSMCs in atherosclerosis

The clonal expansion of VSMCs in atherosclerosis shows similarity with smooth muscle cell tumors [42]. While hypoxia alone, in the absence of ligand binding, does not induce the proliferation and migration of VSMCs, co-stimulation with growth factors is essential for facilitating the hypoxia-mediated transition from the contractile to the synthetic phenotype [43], which enables VSMCs to proliferate and migrate to the intima of the artery. Hypoxic conditions enhance the proliferative response by several distinct receptor tyrosine kinase (RTK) activating growth factors (e.g., fibroblast growth factor-2 (FGF-2), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF)). These growth factors can induce the expression of HIF-1α even in normoxia [44,45]. PDGF is released by activated platelets and macrophages in the arterial wall. The PDGF family may contribute to the synthetic and clonal features for the switching VSMCs (Fig. 3). Notably, in mice, most medial cells of vessel wall have limited contribution to atherosclerotic plaque formation, and the VSMC migration independent of proliferation also plays a restricted role. However, phenotypically unique VSMC-derived cells within plaques originating from a common "ancestor" possess the potential of both proliferation and migration [11]. In the initial stage of fibrous cap formation, VSMCs exhibit a high proliferation rate and express smooth muscle myosin heavy chain (SMMHC), α-SMA, and PDGF receptor-β (PDGFRβ) [31]. Under hypoxic conditions, phosphatidyl-inositol-3-kinase (PI3K) and phospholipase Cγ (PLCγ) can mediate the proliferation and migration of primed $PDGFR\beta^+$ VSMC progenitors. Chronic hypoxia further leads to the accumulation of HIF-1 in human naive VSMCs, resulting in a decrease in the antagonist of PDGFR tyrosine phosphatase (PTP) and an increase in PDGFR phosphorylation, which contributes to VSMCs' proliferation and migration [43].

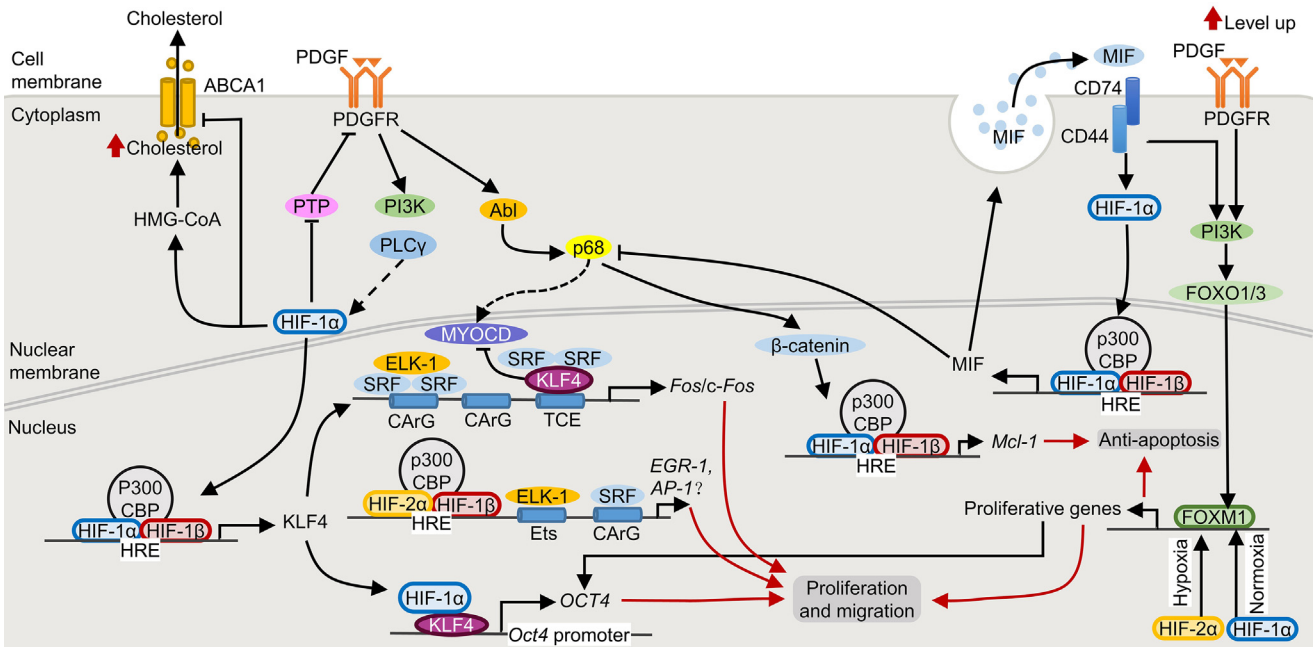

Fig. 3. VSMC HIFs in atherosclerosis. MYOCD and ELK-1 compete for binding to SRF, regulating VSMC phenotypic switching. KLF4 suppresses contraction-associated genes by binding to G/C repressor elements (the TCE element) and inhibiting SRF binding to CArG boxes. KLF4 recruits histone deacetylases to modify chromatin structure, restricting transcription factor access. The SRE contains both the TCF binding element (Ets box) and the SRF binding element (CArG box). ELK-1 binds to the Ets box, cooperating with HIF-2α to activate target genes, promoting endothelial cell and VSMC phenotypic changes. The SRE activation promotes actin cytoskeleton and hypertrophy responses, supporting VSMC proliferation and inhibiting apoptosis. Additionally, HIF-1α targets MIF, which acts on the CD74/CD44 receptor complex to induce VSMC proliferation, migration, and differentiation. MIF inhibits p68, reducing SRF expression, while MYOCD-related transcription factor A (MRTF-A), a vital co-activator of SRF, remains unaffected. The p68 overexpression counteracts MIF's inhibition of SRF recruitment. PDGF-BB activates c-Abl/p68, facilitating β-catenin nuclear translocation and HIF-1α-induced Mcl-1 upregulation against apoptosis. Furthermore, HIF-1 regulates the fundamental levels of FOXM1, while HIF-2 mediates hypoxia-induced FOXM1. In atherosclerosis, HIF-1α and KLF4 bind to the hydroxymethylated *Oct4* promoter, reactivating *Oct4* for atheroprotection by enhancing proliferation, migration, and stabilizing the fibrous cap. ABCA: ATP-binding cassette transporter; AP-1: activator protein-1; CBP: cyclic adenosine monophosphate response element-binding protein (CREB) binding protein; EGR-1: early growth response-1; ELK-1: E-26-like protein 1; FOXM1: forkhead box M1; FOXO: forkhead box O; HIF: hypoxia-inducible factor; HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A; KLF4: Kruppel-like factor 4; Mcl-1: myeloid cell leukemia-1; MIF: macrophage migration inhibitory factor; MYOCD: myocardin; OCT4: octamer 4; PDGF: platelet-derived growth factor; PDGFR: PDGF receptor; PI3K: phosphoinositide 3-kinase; PLC: phospholipase C; PTP: tyrosine phosphatase; SRE: serum response element; SRF: serum response factor; TCF: ternary complex factor; VSMC: vascular smooth muscle cells.

The phenotypic switch triggered by PDGF occurs through its influence on the transcription factors E-26-like protein 1 (ELK-1) and Kruppel-like factor 4 (KLF4) [46]. Pre-existing progenitor cells expressing both PDGFRβ and VSMC markers at the border between muscular and non-muscular arteries migrate and undergo clonal expansion in response to hypoxia. The migration process is facilitated by KLF4, while clonal expansion is mediated by HIF-1α [47]. These two processes also could increase excessive pulmonary vascular muscularization contributing to pulmonary hypertension advancement. The differential hypoxic response patterns of the lung in comparison with atherosclerosis may be a contributing factor to the disparate progression of proliferation and migration of VSMCs in pulmonary hypertension. HIF-1α and HIF-2α typically regulate acute and chronic hypoxia in various organs, respectively [5]. Unlike other organs, the lung can induce HIF-2α production even in mild oxygen deprivation, while HIF-1α is not present [10]. HIF-2α may play a larger role in starting PAH, whereas HIF-1α may be more involved in its progression and perpetuation [48]. Furthermore, KLF4 governs VSMCs phenotype switch as a downstream target of HIF-1α, with two HIF-1α binding elements located in the upstream regulatory zone of the *Klf4* locus (−150 to −163 and −3922 to −3932, respectively) (Fig. 3) [49,50]. KLF4 inhibits the level of contraction-related genes in various manners, involving binding to G/C repressor elements (the TCE element) and preventing serum response factor (SRF) from binding to CRMP-5-associated GTPase (CArG) boxes [11,51]. Furthermore, the remained binding of ELK-1 facilitates the expression of immediate early genes (IEGs) associated with growth, such as *Fos/c-Fos* genes. Conversely, the normal binding of myocardin (MYOCD) to SRF triggers the activation of contractile genes specific to VSMCs [52,53]. Furthermore, the serum response element (SRE) comprises the TCF binding element (Ets box) and the SRF binding element (CArG box) [54]. By binding to Ets box, ELK-1 has been demonstrated to specifically collaborate with HIF-2α to activate specific target genes [55], promoting pro-proliferation and anti-apoptosis role in several cell types (e.g., endothelial cells [56], MCF7 breast cancer cells [57], and cardiomyocytes [58]), which may also indicate its similar role in VSMCs [59,60]. Additionally, the decreased MYOCD and increased KLF4 expression consequently lead to the VSMC phenotype transitioning into macrophage-like cells [49]. VSMC-specific deletion of *Klf4* could decrease VSMC transitioning into macrophage-marker-positive cells ($CD68^+$) and lead to a significant enhancement in the thickness and α-SMA^+ cell population in the fibrous cap [49]. Macrophages also produce PDGF to induct the proliferation and migration of VSMCs [61].

3.1.3. HIFs' effect on the crosstalk between VSMC and vascular endothelial cells in atherosclerosis

Macrophage migration inhibitory factor (MIF), a multi-effect inflammatory cytokine, is expressed and secreted by endothelial cells under atherogenic stimulations, showing a cytokine-like effect and promoting leukocyte recruitment in atherosclerosis [62]. In hypoxic conditions, MIF has been demonstrated to be regulated by HIF-1α and secreted in two phases [63]. The first phase peaked at 60 min and was suppressed by glyburide, demonstrating that it was released by a nonclassical pathway from previously

formed MIF reserves. The second phase of MIF secretion peaked at about 8 h, probably due to HIF-1α-mediated *de novo* synthesis. The chemotaxis of endothelial progenitor cells (EPCs) was promoted by MIF in a dose-dependent approach (peak: 10 ng/mL MIF). Notably, the migration of EPCs induced by supernatant of hypoxic human umbilical vein endothelial cells (HUVECs) was completely prevented by anti-CXCR4 or anti-MIF antibodies. Under moderate hypoxia (3% O_2), the proliferation and migration of VSMCs increased, which was blocked by MIF-small hairpin RNA (shRNA) [64], and MIF dose-dependently triggered VSMC migration related to increased MMP-2 [65]. Moreover, during the second phase of synthesis, the MIF (HIF-1α-driven *de novo* synthesis) derived from EPCs plays a crucial role in the directed differentiation of VSMCs in further stages of angiogenesis, enveloping tubular endothelial cells to promote the formation of structurally intact, stable, and mature neovessels[66]. Furthermore, VSMCs themselves can also secrete MIF [65], and MIF secretion shows a notable increase in dedifferentiated VSMCs treated with PDGF-BB, which would be reversed by anti-MIF treatment [65]. Furthermore, in MIF-challenged vascular tissue and VSMCs, the p68 protein was observed to be downregulated by regulating the MAPK pathway [65]. The p68 protein, an RNA helicase belonging to the DEAD box family, is known for its ability to reorganize RNA structures and regulate RNA metabolism [67]. It has been demonstrated to function as a co-activator for multiple transcription factors involved in various cellular processes, including cell cycle control and tumorigenesis [68]. MIF incubation and p68 small interfering RNA (siRNA) application could inhibit microRNA (miR)-21, suppressing the differentiation of contractile VSMCs [65]. The p68 may serve as a molecular partner of SRF, and the inhibition of p68 by MIF may subsequently impact the molecular function of SRF, resulting in VSMC dedifferentiation (Fig. 3) [65].

Although both PDGF and MIF could promote dedifferentiation and proliferation of VSMCs through SRF/CArG, they could also stimulate the expression of forkhead box M1 (FOXM1) in VSMCs to achieve the same purpose independent of binding and altering the function of SRF (Fig. 3) [69,70]. FOXM1 appears to be of great importance in regulating G2-M phase transcription of mammalian cells [71], while FOXM1 also controls hypoxia-induced PASMC proliferation [72]. Tamoxifen-inducible VSMC-specific *Foxm1* knockout mice experiments have shown that increased FOXM1 expression could causally drive VSMC proliferation and pulmonary vascular remodeling, while not necessary for endothelial proliferation [69]. The PI3K/FOXO signaling is probably the shared mechanism responsible for the increased expression of FOXM1 [73]. Moreover, the *FOXM1* promoter harbors HIF response elements [72], and FOXM1 is regulated by HIF activation both *in vitro* and *in vivo* [74]. In PASMCs, HIF-1 regulates the fundamental levels of FOXM1, while HIF-2 is accountable for the hypoxia-induced FOXM1 [72]. HIF-2α can induce FOXM1 expression in hypoxic human PASMCs promoting pro-proliferation and anti-apoptosis [72], and activating the downstream gene target *Oct4* for idiopathic PAH (Fig. 3) [75]. However, in $ApoE^{-/-}$ mice, the reactivation of gene *Oct4* relies on the HIF-1α and KLF4 binding to the hydroxymethylated *Oct4* promoter under hypoxia resulting in atheroprotection by promoting proliferation and migration and further stabilizing the fibrous cap with thick fibrous cap, small necrotic core area, and minimal intraplaque hemorrhage [76].

Collectively, HIFs act as a critical role in the advancement of atherosclerosis by regulating the phenotype switch of VSMCs and promoting their proliferation and migration as hypoxia responses. HIF-1α and HIF-2α have different roles in the pathogenesis of atherosclerosis and pulmonary hypertension. HIF-1α targets and increases the HMG-CoA reductase gene to contribute to cholesterol synthesis, while HIF-2α may play a larger role in the development of pulmonary hypertension. The clonal expansion of VSMCs in atherosclerosis is driven by growth factors such as PDGF that act on PDGFR-β, which is expressed by VSMC progenitors. The phenotypic switch of VSMCs induced by PDGF is mediated by transcription factors ELK-1 and KLF4, with HIF-1α and KLF4 regulating VSMCs' proliferation and migration. The accumulation of HIF-1α in VSMCs under chronic hypoxia contributes to their proliferation and migration by decreasing the antagonist of PDGFR PTP, and increasing PDGFR phosphorylation. HIF-1α is also involved in regulating ABCA1-mediated cholesterol efflux in VSMCs. Overall, HIF is a critical mediator of VSMC phenotype switch, proliferation, and migration in response to hypoxia and is vital for atherosclerotic pathogenesis.

3.2. PAH

In the World Health Organization classification, Group III of pulmonary hypertension is associated with hypoxic lung disorders. And lower oxygen saturation in the pulmonary hypertension group is associated with the "external environment" of the patient's underlying conditions (such as chronic obstructive pulmonary disease (COPD)) or hypoxia in underlying conditions [77]. PAH is characterized by sustained pulmonary vasoconstriction, proliferative and occlusive vascular remodeling based on medial thickening (increased numbers and enlarged volumes of VSMCs), intimal dysregulation (endothelial cell dysfunction and apoptosis), perivascular inflammation, in situ thrombosis, and ultimately increased vascular stiffness, especially at distal pulmonary artery [78–81]. Recent studies have demonstrated that hypoxia can trigger pulmonary artery constriction, even in the absence of endothelial denudation [82]. Hypoxic pulmonary vasoconstriction occurs in response to an acute deficiency of lung oxygenation, aiming to optimize the exchange of gases. By contrast, chronic hypoxia initiates pathological vascular remodeling, leading to pulmonary hypertension, while ischemia can induce vascular injury, ultimately resulting in lung edema [83]. Furthermore, the pathological phenotype of VSMCs may play a role in vascular stiffening, regardless of endothelial dysfunction [84]. Overall, unfitted arterial constriction exclusively, conducted by VSMCs and its phenotype switch, introduces ventilation/perfusion mismatching and further exacerbates systemic oxygen delivery, as well as the PAH advancement [85,86].

The dominant phenotypes of pulmonary arterial SMCs (PASMCs) alter between proliferation, inflammation, and ECM production, which exhibits great similarity with VSMCs in atherosclerosis [87]. Distinct from atherosclerosis, a unique landscape of VSMCs in PAH focuses on electrophysiologic maladaptation and contraction, followed by mural hypertrophy. In the presence of alveolar hypoxia, a mitochondrial sensor undergoes dynamic alterations in reactive oxygen species (ROS) and redox couples within PASMCs. This process inhibits potassium channels, leading to the depolarization of PASMCs. It also activates calcium channels and elevates cytosolic calcium levels, ultimately resulting in vasoconstriction [85]. Furthermore, reduced expression of the voltage-gated potassium channels (Kv), which is dependent on HIFs [88–90], also contributes to remodeling by reducing apoptosis [91]. The decrease of apoptotic volume is an early sign of cell apoptosis, and this process is partly due to the enhancement of disordered potassium ion outflow through various potassium ion channels. The increase in the outflow of potassium ions from the potassium ion channel on the plasma membrane leads to the shrinkage of apoptotic cells [92]. Conversely, down-regulated potassium channel and inhibited potassium channel function are related to the alleviation of apoptosis in rat and human PASMCs (Fig. 4) [92]. Furthermore, a long-lived, apoptosis-resistant phenotype of PASMCs, derived from neoplasia-like metabolic transformation (e.g., Warburg effect), may impair the normal response of pulmonary vasoconstriction [85,87].

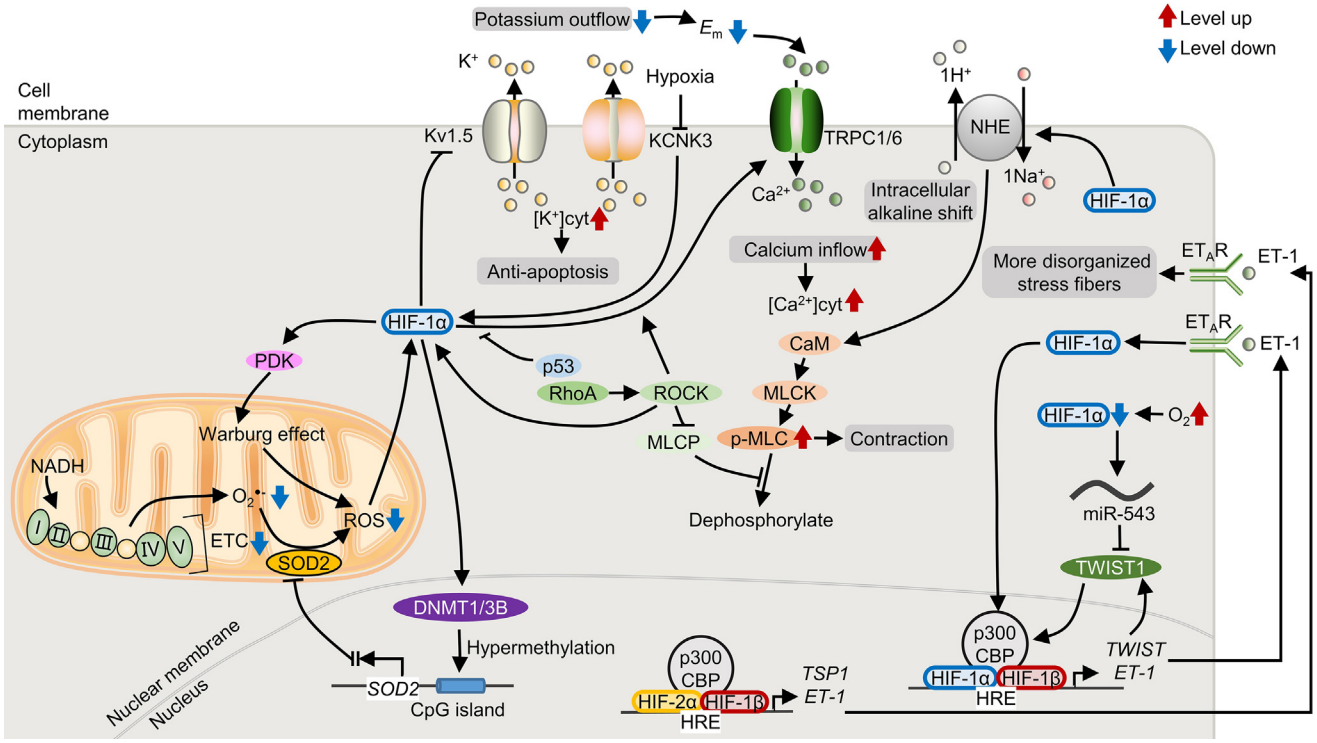

Fig. 4. VSMC HIFs in PAH. The plasticity of VSMCs shows great similarity in atherosclerosis and in PAH, where the proliferation and migration of VSMCs are important. However, PAH also focuses on the electronic activity of VSMCs. In the presence of alveolar hypoxia, a mitochondrial sensor modulates the levels of ROS and redox couples within PASMCs. This hinders potassium channels, depolarizes PASMCs, stimulates calcium channels, and stimulates cytosolic calcium, resulting in vasoconstriction. Further, maintaining a high concentration of cytoplasmic potassium ions ($[K^+]cyt$) is crucial not only for preserving ion homeostasis and regulating cell volume, but also for preventing caspases activation within the cytoplasm. The mitochondrial ETC generates ROS, with mitochondrial SOD2 converting superoxide anions produced at complexes I and III into hydrogen peroxide (H_2O_2). Increased expression of DNMT1 and DNMT3B hypermethylates critical CpG islands in the *SOD2* gene promoter, leading to downregulation. This impairs H_2O_2-mediated redox signaling, activates HIF-1α, and promotes cell proliferation and apoptosis resistance. HIF-1α activation then suppresses the level of oxygen-sensitive, voltage-gated potassium channels, where HIF-1α binds to a putative HRE at position − 1208 to 1203 within the 5′-untranslated region of Kv1.5. Meanwhile, Hypoxia suppresses KCNK3, causing membrane depolarization and calcium entry. Furthermore, hypoxic HIF-1α-induced TRPC1/6 increased resting $[Ca^{2+}]cyt$ leading to PASMCs contraction. Intracellular Ca^{2+} binds to CaM, forming a complex that activates cytosolic MLCK. Activated MLCK phosphorylates MLC, activating ATPase, which hydrolyzes ATP and releases energy. This energy causes myofilament sliding and PASMC contraction. Inactivation of MLCP, a ROCK target, through RhoA/ROCK signaling pathway phosphorylation leads to the accumulation of p-MLC, enhancing actin-myosin coupling and PASMC contraction. Also, The RhoA/ROCK pathway exacerbates pulmonary hypertension through HIF-1α-dependent functional TRPCs. Furthermore, overexpression of p53 in normal PASMCs suppresses store-operated Ca^{2+} entry induced by depleting stored Ca^{2+} in the sarcoplasmic reticulum. CaM: calmodulin; CBP: cyclic adenosine monophosphate response element-binding protein (CREB) binding protein; DNMT: DNA methyltransferase; *E*m: membrane potential; ET-1: endothelin 1; ET_AR: endothelin receptor type A; ETC: electron transport chain; HIF: hypoxia-inducible factor; KCNK3: potassium two pore domain channel subfamily K member 3; miR: microRNA; MLCK: myosin light chain kinase; MLCP: myosin light chain phosphatase; NADH: nicotinamide adenine dinucleotide; NHE: Na^+/H^+ exchanger; PAH: pulmonary arterial hypertension; PASMCs: pulmonary arterial vascular smooth muscle cells; PDK: pyruvate dehydrogenase kinase; p-MLC: phosphorylated myosin light chain; RhoA: Ras homolog family member A; ROCK: Rho-associated coiled-coil kinase; ROS: reactive oxygen species; SMMHC: smooth muscle myosin heavy chain; SOD2: superoxide dismutase 2; TRPC: canonical transient receptor potential channel; TSP1: thrombospondin-1; TWIST: twist family BHLH transcription factor; VSMC: vascular smooth muscle cells.

3.2.1. HIFs-associated potassium ion channels in PAH

PASMCs, in contrast to the majority of cultured primary cells, exhibit elevated levels of HIF-1 under both normoxia and hypoxia [93]. HIF-1α shows increased expression in cultured PASMCs derived from Fawn hooded rats (FHRs, a strain that spontaneously develops PAH) [88], PAH patients [94], and CH rats [95]. Both genetically inherited PAH (e.g., FHR) and idiopathic human PAH show early mitochondrial dysfunction in their pathogenesis, leading to disruptions in the mitochondria-ROS-HIF-Kv pathway (Fig. 4) [96–98]. This pathway is disrupted due to a chromosome 1 abnormality in FHRs, while idiopathic human PAH exhibits comparable irregularities [88]. The oxygen sensor function of mitochondria is a result of the physiological flow of electrons [85], where H_2O_2 functions as a diffusible redox signaling molecule. Moreover, the phenomenon of hypoxic suppression of normoxic ROS generation is exclusive to pulmonary arteries [88,99–107], and manifests within a brief period of moderate hypoxia, preceding the initiation of hypoxic pulmonary vasoconstriction. In the majority of systemic arteries, however, the production of ROS is comparatively lower than in the pulmonary arteries and remains unaffected by moderate hypoxia [108,109]. The distinctive capacity of PASMC mitochondria to swiftly modify the generation of diffusible ROS is what enables them to regulate Kv channel function in PAH [99,101], where PASMCs exhibit a sparse network of hyperpolarized mitochondria characterized by decreased level of electron transport chain (ETC) units and SOD2. Depressed mitochondrial ROS production creates a pseudohypoxic state and causes normoxic stimulation of HIF-1α (Fig. 4) [96,98]. This is accompanied by the downregulation of HIF-destabilizing enzyme proline dehydroxylase-1 (HPH-1), acting as a redox-sensitive prolyl hydroxylase (PHD) which destabilizes HIF-1α and leads to its degradation as well as depresses HIF-3α [96,110,111]. HIF-1α activation then suppresses the level of oxygen-sensitive, voltage-gated potassium channels (e.g., Kv1.5, Kv2.1, and Kv1.2) [81,96,106]. Furthermore, HIF-1α activation initiates the Warburg-like effect via upregulating PDK and further reducing ROS [98,112], causing increased PASMC proliferation and vasoconstriction. Lung-specific elevations in the expression of DNMT1 and DNMT3B lead to an epigenetic mechanism where methylation within the gene's promoter and enhancer regions decreases the expression of SOD2 [113]. The

subsequent reduction in H_2O_2 production initiates HIF-1α activation (Fig. 4) [98]. The reason behind the specific dysregulation of DNA methyltransferases in the lung of FHRs, while the systemic vasculature does not exhibit the same selective upregulation, remains uncertain [98]. Furthermore, the voltage-independent potassium channel, potassium two pore domain channel subfamily K member 3 (KCNK3) (also known as TASK1, and $K_{2P}3.1$) is one of the K_{2p} channels, which is a designated leak or background channel contributing to the resting potential of PASMCs, and is recently discovered to exacerbate PAH [81,114]. KCNK3 is suppressed under hypoxia, resulting in membrane depolarization and subsequent calcium influx [115]. Genetic *KCNK3* deficiency in rats through the CRISPR/Cas9 method led to increased susceptibility to pulmonary hypertension due to age-dependent pulmonary vascular remodeling [116]. Knocking out *KCNK3* significantly increases HIF-1α expression, promoting cell proliferation without altering HIF-1α mRNA expression (Fig. 4) [116].

3.2.2. HIFs-associated calcium channels in PAH

Another significant factor triggering pulmonary vasoconstriction is the increase of cytoplasmic calcium ions ($[Ca^{2+}]$cyt). Initially, it was believed that the downregulation of voltage-gated potassium channels and membrane depolarization was responsible for the increase of $[Ca^{2+}]$cyt, leading to the assumption that voltage-gated Ca^{2+} channels were responsible [117–119]. However, it is now known that under anoxic conditions, sustained Ca^{2+} influx is mainly regulated by the upregulation of nonselective store-operated Ca^{2+} channels (SOCCs), which consist of canonical transient receptor potential channels (TRPC) [120–122]. HIF-1 may function as a downregulator of TRPC expression in nonhypoxic settings and as an upregulator in hypoxic environments (Fig. 4) [123]. Hypoxic HIF-1α-induced TRPC1/6 increases resting $[Ca^{2+}]$cyt leading to PASMCs contraction, and vice versa [123]. The Ras homolog family member A (RhoA)/Rho-associated coiled-coil kinase (ROCK) signaling pathway exacerbates PAH through HIF-1α-dependent functional TRPCs (Fig. 4), and HIF-1 inhibition through RhoA/ROCK inhibitors significantly reduces Ca^{2+} influx (induced by receptor- or store-operated Ca^{2+} channels) and subsequent PASMC contraction [124]. In murine PAH models, the decrease of p53 in PASMCs was associated with an increase in HIF-1α, while inhibition of p53 in normal PASMCs amplified store-operated Ca^{2+} entry triggered by passive depletion of sarcoplasmic reticulum-stored Ca^{2+} (Fig. 4) [125]. Furthermore, changes in intracellular pH are tightly connected to $[Ca^{2+}]$cyt signaling and alterations in artery tone [126]. Acidosis generally results in muscle relaxation and an alkaline shift in intracellular pH, and activation of sodium-hydrogen exchanger (NHE) is necessary for PASMC proliferation stimulated by growth factors [127,128]. The hypoxia-introduced HIF-1-dependent NHE1 expels H^+ in exchange for Na^+ and controls intracellular alkalinization [129]. To be noted, elevated $[Ca^{2+}]$cyt not only modulates vasoconstriction but also promotes PASMC growth and proliferation, which synergistically leads to hypertension and concentric remodeling of pulmonary vessels [81,123].

3.2.3. The relationship between HIF-1 and HIF-2 in PAH

Furthermore, the interactive and differential roles of HIF-1 and HIF-2 in PAH are worth exploring. Endothelin 1 (ET-1) serves as a potent vasoconstrictor, highly expressed in pulmonary arterial endothelial cells (PAECs), promoting PAH [130]. ET-1 also promotes the division of PASMCs and fibroblasts, facilitates the production of ECM, and affects pulmonary vascular remodeling [130]. Nevertheless, the VSMC phenotype switch has been raising the controversial role of HIF-1α in PASMCs of PAH interconnected with ET-1. Several studies have indicated that both overexpression and deletion of HIF-1α in human PASMCs can lead to the production and secretion of ET-1 [131–134]. Meanwhile, the specific knockout of the *Hif1a* gene in mouse PASMCs can either protect [135] or aggravate PAH [136]. It has been postulated that there may be two distinct phenotypes of PAH, characterized by increased or decreased HIF-1α in PASMCs, but both phenotypes can lead to ET-1 production and vasoconstriction [22]. ET-1 can be regulated by the upstream HIF-1 signaling and can inhibit Kv (Fig. 4) [90,137–139]. A rise in ET-1 results in an escalation in HIF-1 expression via either an augmentation in synthesis, a reduction in hydroxylation [140], or a decline in proteasomal degradation [141]. Conversely, HIF-1α deficiency increases ET-1 expression by increasing miR-543 expression and mediating Twist family BHLH transcription factor (TWIST) inhibition [131]. TWIST is a direct transcriptional subject of HIF-1 [142]. Furthermore, studies have proposed a potential feedforward approach in the development of chronic hypoxia-induced PAH whereby increased HIF-2α expression in PAECs during chronic hypoxia may result in elevated production of ET-1, enhanced HIF-1α in PASMCs, thereby leading to the upregulation of HIF target genes [143]. However, it is not clear whether the ET-1 derived from PASMCs is associated with ET-1 produced by PAECs, and the correlation between PASMCs and PAECs in regulating ET-1 remains unclear [22].

Furthermore, HIF-2α in PAECs shows a predominant role in PAH [79,144,145], while HIF-1α is primarily expressed in PASMCs, HIF-2α is predominantly found in PAECs, and HIF-3α is predominantly expressed in pulmonary fibroblasts [133]. However, the role of HIF-2α in PASMC still cannot be underestimated. The hypoxic and pseudohypoxic states in various groups of PAH can differ in intensity and duration. And the intricate interplay between HIF-1 and HIF-2 is essential for driving the pathological processes responsible for pulmonary vascular and right ventricular remodeling. HIF-2α mutations have been demonstrated to enhance PAH in both mouse models and human patients [146,147], and HIF-2α might have a significant impact on the onset of PAH, while HIF-1α may predominantly contribute to the advancement and persistence of the condition [15]. The overall HIF-1α deficiency in mice could not prevent hypoxic-induced PAH in the fifth week. Furthermore, although the overall deficiency of HIF-2α in mice could not survive during prolonged hypoxia, the conditional absence of HIF-2α or the application of HIF-2α antisense oligonucleotide in mice for 4–5 weeks of hypoxia reduced the degree of vascular muscularization, pulmonary arterial pressure, and right ventricular hypertrophy [148]. Stabilizing HIF-1α may be intentional for the later stages of PAH. Upregulation of PHD with its growing activity downregulated HIF-1α in PASMCs from patients of severe PAH, who finally received lung transplantation [133]. By contrast, the well-preserved HIF-1 level of PASMCs could constrain myosin light chain phosphorylation (p-MLC) and ET-1 generation, thus keeping the normal pulmonary circulation in a low tone [131]. Although the efficacy of HIF-1 inhibitors for PAH has been broadly explored and proofed, such as R59949, 2-methoxyestradiol (2-ME2), topotecan, anti-CD146 monoclonal antibody AA98, CAPE, celastramycin, YC-1, and apigenin [15], HIF-2 inhibitors open a novel potent approach. A HIF-2 inhibitor, small molecule PT2567, significantly alleviated the early events (monocyte recruitment and vascular cell proliferation) of mice exposed to hypoxia for 4 days, and also alleviated the vascularization, the accumulation of tenascin C (TNC) and the progress of PAH of mice exposed to hypoxia for 5 weeks [148]. Furthermore, iron deficiency can slow the breakdown of HIFs by disturbing PHD's function and iron-deficient people may face a heightened vulnerability to PAH when exposed temporarily or constantly to oxygen deprivation caused by lung diseases or high-altitude residence. A clinical trial (NCT01847352) observed that exaggerated hypoxic PAH, caused by clinical iron deficiency, could be reversed by subsequent iron administration [149]. Additionally, the translation of HIF-2α (in-

stead of HIF-1α) is associated with iron metabolism because of an iron-responsive element within its 5′-untranslated region [150,151].

Furthermore, the *Hif2a* gene gain-of-function (GOF) mutation alone is expected to be sufficient to increase PASMC stiffness via the *EDN1* (encoding ET-1), independent of endothelial dysfunction (Fig. 4) [84]. Humans with GOF mutations in HIF-2α also exhibit enhanced hypoxic pulmonary vasoconstriction [152]. Heterozygous HIF-2α GOF mutations have been linked to polycythemia, thrombosis, and vascular complications, contributing to morbidity and mortality in PAH patients [84]. Cellular stiffness is primarily determined by augmented F-actin stress fibers, smooth muscle myosin heavy chain (SMMHC), and elevated acetylated α-tubulin. The PASMCs of *Hif2a* heterozygous mice showed increased disarray of stress fibers compared to their wild-type counterparts [84]. Furthermore, thrombospondin 1 (TSP1)—upregulated in lungs in PAH patients [150,151,153], and in mice of hypoxia-mediated PAH [153,154]—is another factor triggered by HIF-2α via HIF-2α triggered HREs near the *Tsp1* promoter (Fig. 4) [155]. Under hypoxia, elevated levels of TSP1 promote the migration of fibroblasts and PASMCs, while simultaneously compromising the integrity of the endothelial barrier [155]. Overactive TSP1 signaling partially restricts vasodilation by reducing nitric oxide production and signaling [156,157]. Furthermore, the expression of the Kv1.5 channel was maintained, while the protein levels of endothelin receptors were reduced in the lungs of *Tsp1*$^{-/-}$ mice when compared to wild-type mice [155,156]. As ET-1 has been shown to hinder Kv1.5 channels [90], the influence of TSP1 on Kv channel-driven contraction in hypoxic PASMCs might be mediated by ET-1. Additionally, the hypoxia resistance observed in *Tsp1*$^{-/-}$ PASMCs might be attributed to its impact on other signaling molecules, such as ROS-mediated vasoconstriction [155]. However, it remains to be explored the role of TSP1 on ROS production in the pulmonary vasculature.

Collectively, the dominant phenotype of PASMCs in PAH alters between proliferation, inflammation, and ECM production. PASMCs respond to alveolar hypoxia by dynamically changing ROS and redox couples, which leads to changes in electrophysiological status and corresponding vasoconstriction. Mitochondrial dysfunction and disrupted ROS-HIF-Kv pathway are crucial for the pathogenesis of PAH. HIF-1 is upregulated in PASMCs under both hypoxic and nonhypoxic conditions and inhibits the level of oxygen-sensitive voltage-gated potassium channels, resulting in enhanced PASMC proliferation and vasoconstriction. The malfunction of DNA methyltransferases in the lung also contributes to reduced H_2O_2 production and HIF-1α activation. Furthermore, HIF-2α may be an important promoting factor in the early stage of the disease, and has a transitional interaction with HIF-1α.

3.3. AA

Aortic dissection is a severe vascular condition characterized by inflammation, limited treatment options, and high mortality rates. It involves the infiltration of inflammatory cells, angiogenesis within the aortic wall, and the breakage of elastic fibers [158,159]. During the development of AA, VSMCs exhibit plasticity and undergo phenotypic changes in response to environmental stresses, transitioning from contractile cells to cells involved in matrix remodeling, accompanied by changes in contractile protein expression and upregulation of matrix metalloproteinases (MMPs) and other mediators of inflammation and apoptosis [160,161]. Genes highly expressed in the VSMCs and fibroblasts of the aneurysmal wall, are positively associated with abdominal AA enlargement, and they are also related to angiogenesis and vascular remodeling and functionally converge with HIF-1α pathway-upregulated genes exclusively found in abdominal AA rupture [162]. HIF-1α overexpression has been observed in the rupture edge of AA of human tissues, localized primarily in VSMCs and adventitia of aneurysmal tissues [163,164]. HIF-1α inhibitors (e.g., digoxin and 2-ME2) have been used to inhibit the progression of abdominal AA and alleviate multiple related conditions [17]. However, maintaining a certain level of HIF-1 is necessary for the repair and stabilization of AA progression, which is what this review focuses on.

3.3.1. Effect of HIF-1 stabilization on AA

Studies have demonstrated that VSMC-specific deficiency of HIF-1α suppresses the expression of fibrosis-related genes (e.g., *collagen I* gene) induced by angiotensin II (Ang II) in the aorta [165]. VSMC-specific HIF-1α depletion does not affect the activity of MMP-2, relating to the degradation of elastic fibers, but suppresses the function of lysyl oxidase (LOX) and the level of tropoelastin mRNA, thereby reducing the formation of elastic fibers and worsening AA in the Ang II- and β-aminopropionitrile (BAPN)-treated aorta [164,166]. Furthermore, excessive suppression of the transcription of HIF-1 downstream genes in the nucleus with the accumulation of HIF-1α subunits in the cytoplasm can lead to the progression of AA. In AA, H19 long non-coding RNA (lncRNA) recruits Sp-1 to the HIF-1α promoter region and inhibits the nuclear translocation of HIF-1α, which then inhibits MDM2-mediated p53 reduction and increases VSMCs' apoptosis (Fig. 5) [167]. Furthermore, BRG1, a central catalytic subunit of various enzymatic complexes involved in chromatin modification, can utilize ATP hydrolysis-derived energy to alter the chromatin structure of targeted promoters. Overexpression of BRG1 in VSMCs of human aortic could enhance apoptosis and inhibit proliferation to exacerbate thoracic AA. The expression of HIF-1α-antisense RNA1 (HIF1A-AS1) has been found significantly downregulated in BRG1 knockdown VSMCs, while upregulated in BRG1 overexpressing VSMCs. Upregulated by BRG1, HIF1A-AS1 then contributes to the apoptosis of VSMCs while inhibiting proliferation [168]. Moreover, histone acetyltransferases (HATs) and ATP-driven remodeling enzymes, such as BRG1, typically work in conjunction and collaborate to regulate gene transcription. BRG-1 can be recruited to the enhancer through an "enhancersome" composed of HIF-1, p300, and other related coactivators. This complex directly controls the transcription of the *EPO* gene [169]. Although BRG-1 is not necessary for the hypoxic induction of the VEGF gene, it is still recruited to the VEGF promoter during hypoxia treatment (Fig. 5) [169]. It is possible that HIF-1 routinely recruits these coactivators regardless of their necessity. However, it still needs to be clarified whether HIF-1 directly interacts with BRG-1 in any way. Intriguingly, the stabilization of HIFs has been used to reduce the bad outcomes of surgery for aneurysms. Open thoracoabdominal AA repair has long been associated with significant perioperative complications and risk of death, mainly due to distal aortic ischemia leading to conditions like renal failure [170]. A phase 2 study of daprodustat, a PHD inhibitor, to restrict ischemic events in patients undergoing thoracoabdominal AA has been initiated (NCT01920594). Additionally, the markers of hypoxia, specifically the protein level of HIF and SIRT1, remain unchanged in thoracoabdominal AA, compared to the control aorta [171], which implicates the highly possibility of spatiotemporal fluctuation of HIFs.

3.3.2. Effect of HIF-1 and TGF-β on AA

This spatiotemporal specificity of HIF is also associated with the protective effect of transforming growth factor-β (TGF-β) on AA. The relationship between TGF-β and AA is complex. While some studies suggest that TGF-β may promote AA formation and development by modulating cell proliferation, the synthesis of ECM, and inflammation [172–174], other research indicates that TGF-β acts as a protective role in preventing AA formation and progression

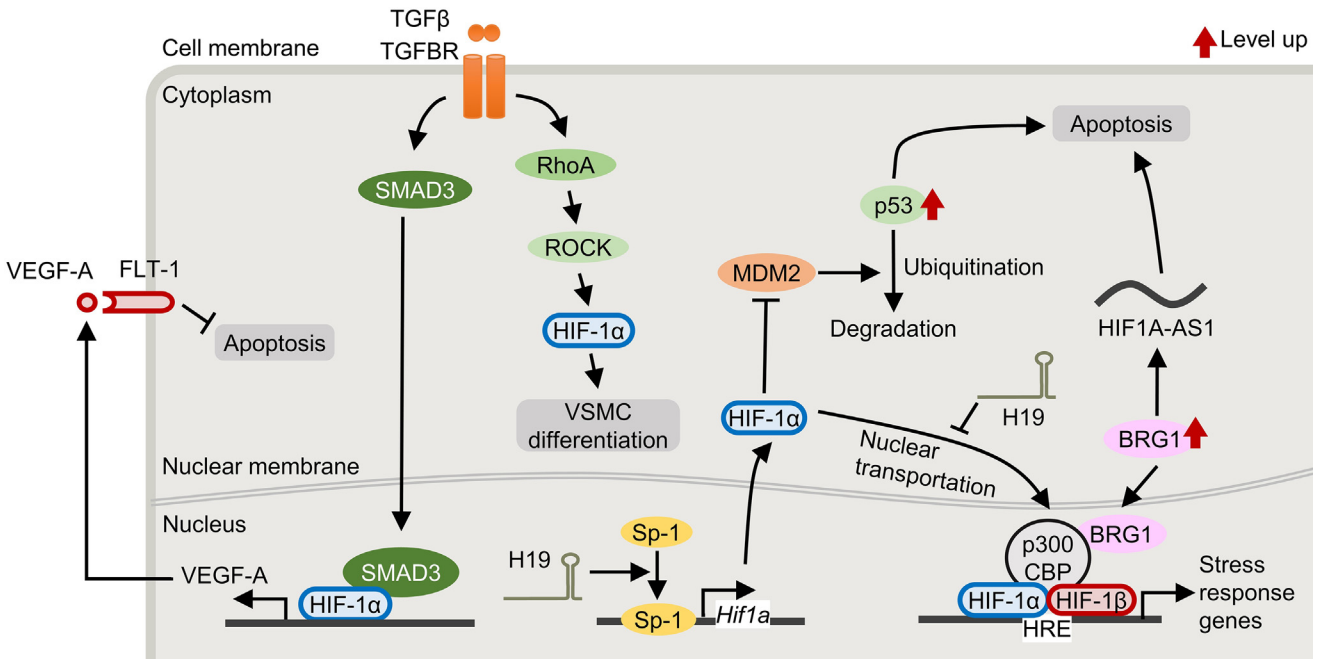

Fig. 5. VSMC HIFs in AA. Excessive aggregation of HIF-1α in the cytoplasm without entering the nucleus leads to AA progression. In aortic dissection, in the cytoplasm, lncRNA H19 recruits the transcription factor Sp-1 to the promoter region of HIF-1α. This prevents the MDM2-mediated decrease of p53 and increases the apoptosis of VSMCs. Further, H19 inhibits the HIF-1α nuclear transportation, which further stabilizes p53. Of note, H19-dependent apoptosis in VSMCs does not depend on miR-675, which exists in the first exon of the *H19* gene. However, the proper activation of target genes after HIF-1α enters the nucleus facilitates the stabilization of AA. AA: aortic aneurysm; BRG1: Brahma/SWI2-related gene 1; CBP: cyclic adenosine monophosphate response element-binding protein (CREB) binding protein; FLT-1: fms-related receptor tyrosine kinase 1; HIF: hypoxia-inducible factor; HIF1A-AS1: HIF-1α-antisense RNA1; lncRNA: long non-coding RNA; MDM2: mouse double minute 2 homolog; miR: microRNA; RhoA: Ras homolog family member A; ROCK: Rho-associated coiled-coil kinase; SMAD3: SMAD family member 3; Sp-1: specific protein 1; TGF-β: transforming growth factor-β; TGFBR: transforming growth factor-β receptor 1; VSMC: vascular smooth muscle cell.

by regulating the balance between cell proliferation and apoptosis, as well as ECM remodeling and repair [159,175,176]. Our discussion will focus primarily on the protective effects of TGF-β in the development of AA. While TGF-β exhibits a proapoptotic impact on VSMCs in culture when SMAD3 levels are at their basal state, it undergoes a transformation into an inhibitory factor for VSMC apoptosis and a promoter of cell survival in the context of arterial injury and elevated SMAD3 levels, thereby contributing to the progression of intimal hyperplasia [177]. Or rather, the presence of increased SMAD3 levels transforms TGF-β from an inhibitory factor to a promoter of VSMC proliferation (Fig. 5) [178]. SMAD3 is upregulated in human restenotic disease, in conjunction with cell proliferation, whereas it remains unaltered in primary atherosclerotic lesions [179]. Moreover, the activation of VSMCs by TGF-β resulted in the assembly of a SMAD3-HIF-1α complex, which subsequently triggered the activation of the VEGF-A promoter and its transcription following angioplasty [177]. The secreted VEGF-A acts through an autocrine pathway to bind and activates the FLT-1 receptor, a VEGF receptor, on VSMCs, resulting in a decrease in apoptosis [177]. Meanwhile, after angioplasty, TGF-β enhances VSMC proliferation for the repair responses of the arterial wall [180,181]. Additionally, the non-canonical RhoA/ROCK pathway mediated by HIF-1α acts as the primary downstream mediator of TGF-β to regulate the hypoxic effect on VSMC differentiation of epicardium-derived cells (Fig. 5) [182].

In general, HIF-1α and TGF-β, which are traditionally considered to be the factors that promote the progression of AA in VSMCs, also have the effect of stabilizing and preventing the progression of AA after considering the spatiotemporal specificity of HIFs. This reminds researchers to consider the integrity of the disease and the course of the disease more when taking medication, and to make a more precise grasp of the timing of administration.

3.4. VC

VC is an advanced pathological process in atherosclerosis that resembles osteogenesis and occurs in the intima or media layer of blood vessels [183]. It is strongly linked to elevated cardiovascular morbidity and mortality in patients with end-stage renal disease or diabetes mellitus [184,185]. Importantly, this association exists independently of traditional cardiovascular risk factors [186]. Previously, VC was regarded as a passive degenerative process characterized by the accumulation of calcium and phosphate, but now it's widely accepted that it's a regulated process with similarities to the embryonic bone formation [187]. During the early phase of bone formation, HIF-1 is necessary for mesenchymal cell condensation, chondrocyte proliferation, as well as the synthesis of the cartilaginous ECM. Then, HIF-1 promotes continued proliferation, while HIF-2 is necessary for hypertrophic differentiation in cells within the hypertrophic zone. The vascular invasion of the hypertrophic zone is subsequently initiated, necessitating the involvement of both HIF-1 and HIF-2. Ultimately, the degradation of cartilage and its subsequent substitution with the bone are both facilitated by HIF-2 [188].

3.4.1. Effect of HIF-1α and RUNX2 on VC

Osteochondrogenic differentiation of VSMCs, through apoptosis and osteochondrogenic conversion, is the main cellular mechanism of VC [11,189]. Hypoxia induces an osteochondrogenic differentiation program in VSMCs, characterized by increased expression of bone-specific proteins and related osteochondrogenic transcription factors. Consequently, this process ultimately results in enhanced ECM calcification *in vitro* [189]. Furthermore, intraplaque neovascularization, which is a significant indicator of plaque

hypoxia, frequently associates with intimal spotty calcification of atherosclerosis, which is a characteristic feature of the disease [190,191]. Studies have shown that HIF-1α coexists with neoangiogenesis and calcification regions in stenotic valves [192]. Moreover, patients with COPD exhibits accelerated coronary artery calcification, which strongly correlated with reduced arterial blood oxygen levels [193,194]. Furthermore, plasma HIF-1α levels have been found to be a significant and independent predictor of coronary arterial calcification in individuals with type 2 diabetes mellitus [195]. A study has determined that the majority of osteochondrogenic precursor runt-related transcription factor 2 $(RUNX2)^+$ cells and chondrocyte-like cells (positive for type II collagen) within atherosclerotic plaques in mice originate from VSMCs [196]. RUNX2 is a key modulator of osteogenic differentiation and elevated RUNX2 level could improve transdifferentiation of PASMCs towards osteoblast-like cells, accelerating mineralization of the vascular wall. A positive feedback loop involving HIF-1α and RUNX2 has been indicated (Fig. 6) [197,198]. Additionally, RUNX2-related PAH often co-occurs with VC, and up-regulated RUNX2 may facilitate calcium-mediated biomineralization in distal arteries of PAH patients [28]. Moreover, proteomic analysis on human lungs indicates that calcification-related proteins are prominently overexpressed in PAH patients [199]. PAH-PASMCs demonstrate reduced miR-204 expression, resulting in subsequent RUNX2 upregulation. Sustained expression of RUNX2, in turn, enhances HIF-1α activation, leading to its nuclear localization. This axis disrupts the balance between proliferation and apoptosis in PAH-PASMCs, promoting excessive proliferation and inhibiting apoptosis. These actors also enhance VC by inducing the upregulation of osteocalcin (OCN), osterix, and alkaline phosphatase (ALP) in PAH-PASMCs [198].

3.4.2. Effect of HIF-1α and ROS on VC

Vascular cells expressing osteogenic markers were recognized as the origins of excessive ROS in the vicinity of calcifying regions, which exacerbate the pathological VC [200,201]. Unfettered ROS production under hypoxia is enhanced for stabilizing HIF-1α and promoting osteochondrogenic response in VSMCs [189,200]. The stabilization of HIF-1α and the induction of calcification under hypoxic conditions can be effectively suppressed by inhibiting ROS production using N-acetyl cysteine and the mitochondrial complex I inhibitor, rotenone [189]. HIF-1α not only targets GLUT1 and VEGF-A, but also induces the marker expression of osteochondral differentiation, such as RUNX2, Sry-related HMG box-9 (SOX9), ALP, and OCN, and consequently promotes ECM calcification of VSMCs in the time-dependent manner (Fig. 6) [189,197]. Furthermore, HIF-1α has been observed as an important factor in AGE-induced [202] and phosphate-induced [197] VSMC calcification (Fig. 6). Advanced glycation end products (AGEs) contribute to the osteoblastic phenotype transition from VSMCs to VC by several mechanisms [203], and the receptor for advanced glycation end products (RAGE) has also been demonstrated to enhance the expression of RUNX2 in systemic blood vessels [204]. A reciprocal interaction among mitochondrial ROS, HIF-1α, and PDK4 might be implicated in the process of calcified VSMCs induced by AGEs. AGEs promote VSMC calcification by activating the HIF-1α/PDK4 signaling pathway, while also impairing normal glucose metabolism. AGEs increased the expression of HIF-1α and PDK4 in a dose-dependent way and reached the maximum stimulation at 24 h. Stable HIF-1α or HIF-1α nuclear translocation increases PDK4 expression [202]. PDK4 facilitates VC by SMAD1/5/8 phosphorylation [205]. Furthermore, AGEs have been demonstrated to enhance oxidative stress in VSMC calcification [206], and PDK4 activation also contributes to mitochondrial dysfunction along with excessive mitochondrial ROS [205,207]. Additionally, hypoxia collaborates with increased levels of inorganic phosphate (Pi) to amplify the process of VSMC osteogenic transdifferentiation, leading to subsequent VC [197]. Elevated Pi could quickly trigger HIF-1 activation, even under normal oxygen conditions. Hypoxia and HIF-1 activators then upregulate bone-related markers in conditions of high phosphate levels ($HiPO_4$) [197], mirroring the effects of hypoxia on bone formation [208]. $HiPO_4$ stabilizes HIF-1α by inhibiting VHL binding and potentially HIF-1α hydroxylation [197]. And mitochondrial ROS production in VSMCs plays a crucial role in stabilizing HIF-1α during conditions of high $HiPO_4$ levels [197].

Chronic kidney disease (CKD) patients with anemia, residing at high altitudes, or receiving treatment with PHD inhibitors are more prone to VC. HIF-1 is an early event associated with CKD, serving as a prospective marker and potential target for combating VC in CKD-related conditions [197]. FG-4592 (also referred to as roxadustat), a PHD inhibitor (PHI)/HIF-1 activator, is a promising candidate for treating anemia in CKD, and could recreate a procalcifying environment where Pi-induced calcification of VSMCs is markedly exacerbated [197]. However, FG4592 alone does not accelerate VC at the normal Pi level. High Pi and PHI FG4592 together increase calcification-related gene levels in VSMCs, indicating that CKD patients with elevated Pi levels may be more susceptible to VC during PHI FG4592 therapy. PHI FG4592 enhances VC by facilitating Pi absorption and promoting calcium deposition within VSMCs. This process initiates phenotypic reprogramming of VSMCs, leading to the induction of PDK4 expression and modulation of the phosphorylation level of RUNX2. Furthermore, zinc serum levels have been observed to be inhibited in CKD, and preserved zinc exhibits a dose-dependent inhibition on the Pi-induced mineralization and PHI-enhanced procalcification of VSMCs [209]. Zinc suppresses the osteochondrogenic phenotypic transition of VSMCs by reducing Pi uptake, downregulating the expression of osteochondrogenic genes (e.g., BMP-2, Sp7, and Msx-2), and averting the loss of VSMC-specific markers (e.g., SM22α, ACTA-2, and MYH11) (Fig. 6) [209]. Furthermore, zinc supplementation *in vitro* may induce ROS scavenger proteins, ameliorate ROS and oxidative stress, and thus reduce VC [210]. Metallothioneins are highly induced by elevated zinc and cadmium concentrations [211], exhibiting strong antioxidant properties [212]. Therefore, close monitoring of zinc plasma levels is advisable, and appropriate supplementation may be considered. Similar to the findings on CKD, individuals who underwent carotid endarterectomy also exhibit decreased levels of zinc in their plasma [209]. The data indicate that a low plasma zinc concentration may be a potential risk factor associated with HIF-1α in various vascular diseases. Collectively, the metabolism of glucose and inorganic minerals in the body can be involved with the ROS-HIF axis in VSMCs and integrated into the HIF-1/RUNX2 complex, and there is also a positive feedback loop between HIF-1 and RUNX2. These contribute to the calcification of VSMCs.

4. Challenges and opportunities in clinical use

4.1. The balance between anemia/ischemic treatments and cancer promotion

Besides gene-transfer approaches to raise HIF-1α levels, orally bioavailable prolyl hydroxylases (PHD) inhibitors to stabilize HIF under normoxia leads the fashion. Both CKDs and cancers can cause anemia. Erythropoiesis-stimulating agents (ESAs), like recombinant human EPO (rhEPO) and darbepoetin alfa (Aranesp), have been used as the standard treatment for anemia with CKDs, however, rises the risks of cardiovascular adverse events [213,214]. A considerable number of patients dose-dependently exhibits progressive deterioration of blood pressure after the use of ESAs, resulting from excessive hemoglobin, increased hemat-

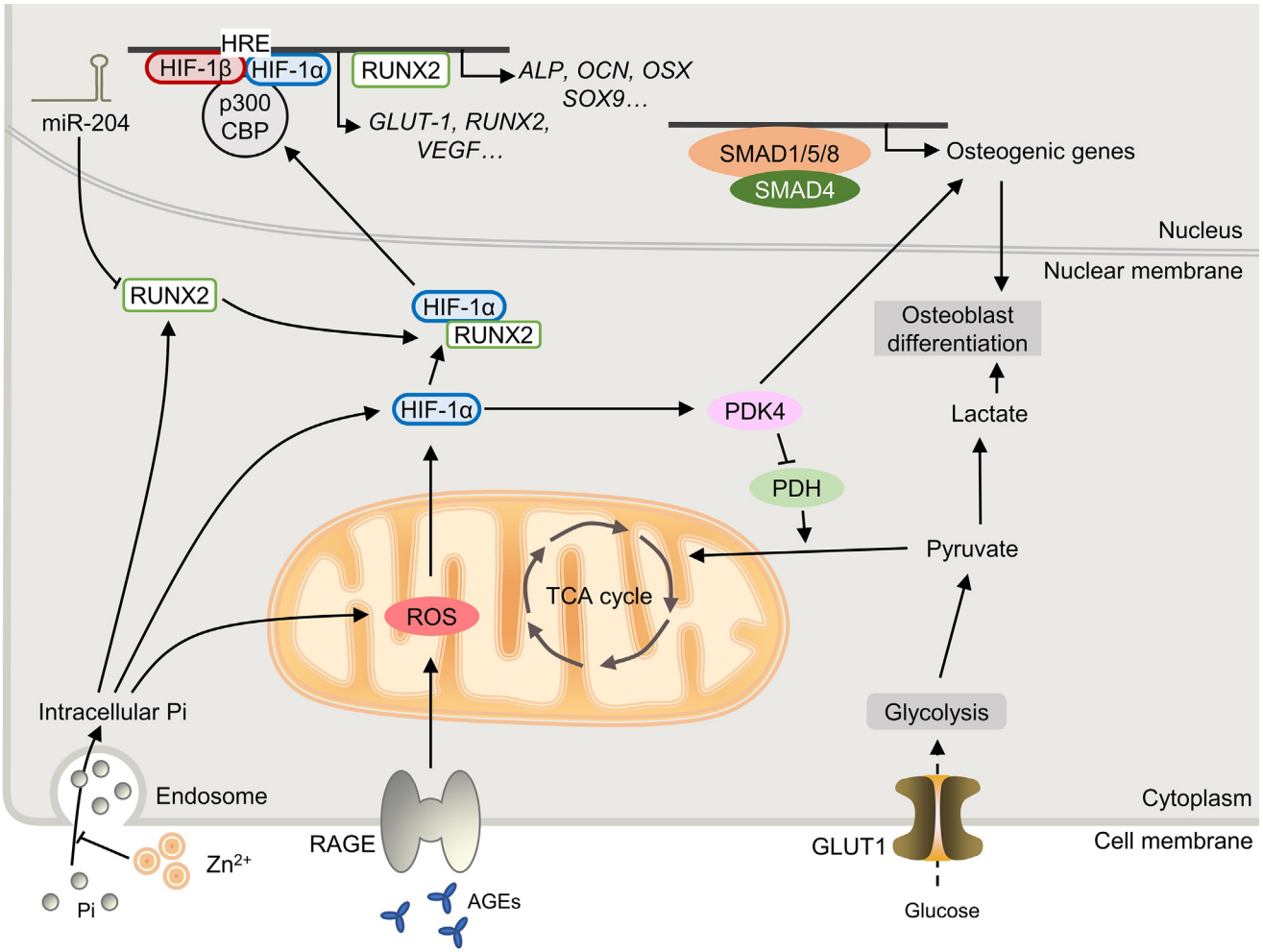

Fig. 6. VSMC HIFs in vascular calcification apoptosis and fibrosis seem to be two major ends of VSMCs during the pathological process. HIF-1α coexists with neoangiogenesis and regions of calcification. AGE: advanced glycation end products; ALP: alkaline phosphatase; CBP: cyclic adenosine monophosphate response element-binding protein (CREB) binding protein; ECM: extracellular matrix; GLUT1: glucose transporter 1; HIF: hypoxia-inducible factor; miR: microRNA; OCN: osteocalcin; OSX: osterix; PDH: pyruvate dehydrogenase; PDK: pyruvate dehydrogenase kinase; Pi: inorganic phosphate; RAGE: receptor for AGE; ROS: reactive oxygen species; RUNX2: runt-related transcription factor 2; SMAD1/4/5/8: SMAD family member 1/4/5/8; SOX9: Sry-related HMG box-9; TCA cycle: tricarboxylic acid cycle; VEGF: vascular endothelial growth factor; VSMC: vascular smooth muscle cell.

ocrit, elevated blood viscosity, and ultimately raised vascular resistance [214,215]. Furthermore, rhEPO may excessively elevate EPO-derived pro-angiogenesis *in vivo* and increase the risk of cancer occurrence and tumor progression [216,217].

Recently, the exploration of PHD inhibition as one of the alternative treatment methods for CKD anemia has become popular [218–228]. Several "-dustats" are PHD inhibitors consisting of a 2-oxoglutarate (2-OG) scaffold (Table 1). These compounds inhibit the interaction of 2-OG with PHD, leading to the stabilization of the PHD-inhibitor complex in its closed conformation. As a result, they effectively block HIFs' degradation. PHD inhibitors enhance renal and hepatic erythropoiesis, while also facilitating optimal iron utilization. The peak concentration of EPO achieved with these compounds, when administered at a clinically effective dose (once daily or thrice a week), is significantly lower than that attained through the administration of recombinant erythropoietin. This characteristic enhances the safety profile of these compounds, making them a preferable therapeutic option. An additional overlooked benefit of PHD inhibitors is their ability to positively regulate iron homeostasis during anemic conditions. Elevated levels of hepcidin are linked to CKDs and other inflammatory disorders. PHD inhibitors can enhance iron availability in the bloodstream by inhibiting hepcidin production, potentially decreasing the requirement for iron supplementation [237]. Intriguingly, Chuvash polycythemia patients exhibit a clinical phenotype related to systemic HIFs' activation without increased malignant tumors [238]. And hemodialysis-dependent CKD patients, who receives proactive IV iron therapy, have not shown the results of more cardiovascular events in the high-dose iron group [239]. Furthermore, JTZ-951 is a new PHD inhibitor that can be taken orally and has a quick absorption rate and short duration of action. It can induce EPO in both normal and anemic conditions, without influencing other factors associated with HIF stabilization. Daily doses of JTZ-951 can stimulate erythropoiesis without disturbing endogenous EPO levels, but higher doses may induce the expression of other HIF-targeted proteins. JTZ-951 can also potentially induce VEGF at high doses without affecting its normal function, while the elevated plasma VEGF is unlikely to promote edema in retinopathy or affect tumor growth [240]. In addition, JTZ-951 has been demonstrated to suppress cardiac hypertrophy and improve myocardial fibrosis [241]. JTZ-951 is on the phase 3 clinical trial which is completed and waiting for results (NCT04027517). Other drugs such as DS-1093, JNJ-42905343, DDO-3055, and HEC53856 are also being developed for renal anemia. Still, efforts to pursue PHDs are ongoing in order to achieve the desired half-life while balancing potency and reducing side effects, considering the safety concerns

Table 1
Overview of "-dustats" for HIFs stabilization.

	Roxadustat (ASP1517, FG-4592) [218,219,229]	Daprodustat (GSK1278863) [220,221,230]	Vadadustat (AKB-6548, MT-6548) [222,223,231]	Molidustat (BAY 85–3934) [224,232]	Desidustat (ZYAN1) [225–227,233]	Enarodustat (JTZ-951) [234–236]
Specificity for PHD	PHD1–3	PHD1 and PHD3 are favored	PHD3	PHD1–3, particularly PHD3	PHD1–3	PHD1–3
Effects on EPO elevation	Improved the release of EPO (maximum serum levels of EPO after administering 1 mg/kg roxadustat were 115 mU/mL, which were significantly lower compared to the levels achieved through intravenous injection of recombinant erythropoietin)	A dose-dependent increase of EPO and hemoglobin	Elevated the peak serum concentration of EPO and raised hemoglobin in a dose-dependent manner	A dose-dependent increase of EPO	A trend of dose–response of EPO	Increased plasma EPO levels and dose-dependently increased hemoglobin levels (once-daily administration of short-lived JTZ-951 successfully induces erythropoiesis without impacting endogenous EPO levels)
Effects on hepcidin suppression	Suppression	N/A	Suppression (also in ferritin, with increased iron-binding capacity)	N/A	Suppression	Suppression
Primary safety concerns on abnormal VEGF promotion	Activation (despite inducing erythropoiesis, it does not facilitate oncogenesis, tumor advancement, or metastasis in a VEGF-sensitive model)	No change (with serum levels of EPO exceeding 500 mU/mL in the patients treated with a 10 mg dosage)	No change (also no change in blood pressure, total cholesterol, or C-reactive protein)	N/A	N/A	Activation (JTZ-951 at a high dose: elevated plasma VEGF, no change in retinal VEGF mRNA, no effect on tumor growth)
Primary safety concerns on adverse events on cardiovascular system	N/A	Affecting cardiac repolarization: decreases in the ΔQTc	A temporary decline of mean arterial blood pressure and a slight, temporary rise in serum uric acid levels	N/A	N/A	N/A
Other safety concerns	N/A	Higher incidences of gastrointestinal adverse events	N/A	N/A	N/A	N/A
Clinical status (target diseases)	First approval in China in December 2018 (dialysis- and non-dialysis-dependent CKD anemia)	First approval in Japan in June 2020 (renal anemia)	First approval in Japan in June 2020 (dialysis- and non-dialysis-dependent CKD anemia)	First approval in Japan in January 2021 (renal anemia)	First approval in India in March 2022 (dialysis- and non-dialysis-dependent CKD anemia)	First approval in Japan in September 2020 (CKD anemia)

The retrieval time is up to May 2023. N/A: not available.

Table 2
Current available indirect HIF activators.

Mechanisms for indirect HIF activation	Characteristics of specific inhibitors	Agents	Major targets
PHD inhibition	Noncompetitive and nonselective iron chelators [244,245]	Deferoxamine	Chelating with Fe^{2+}, reducing the concentration of ferrous iron
		3,4-dihydroxybenzoic acid	
		1,10-phenanthroline	
	Competitive and selective 2-OG mimetics [246–250]	Dimethyloxalylglycine	Inhibiting the oxoglutarate-dependent dioxygenase that typically inactivates HIF-1α
		FG-2216 (IOX-3, YM-311, BIQ)	A coordination fragment of Fe^{2+}, forming ionic bonds with Arg383
		IOX-2	Competition with 2-OG and bidentate iron-chelation
		IOX-4	
		FG-4592, AKB-6548, BAY-85–3934, GSK-1278863, ZYAN1, JTZ-951(triazolopyridine derivatives), see Table 1	
		JNJ-42905343	
		DS-1093	
	Others [251–255]	MK-8617	Chelating Fe^{2+}; one of the methoxy-substituted aryl rings stacking above Arg322
		1,2,4-triazolo-[1,5-a]pyridine	Coordinating in an undisclosed monodentate interaction with the Fe^{2+} ion at the active site; accepting a hydrogen bond from the Asn315 side chain residue of
		Spirohydantoins (1,3,8-triazaspiro[4.5]decane-2,4-diones)	Unknown
		TP0463518 (2-[[1-[[6-(4-chlorophenoxy) pyridin-3-yl]methyl]-4-hydroxy-6-oxo-2,3-dihydropyridine-5-carbonyl]amino]acetic acid)	Unknown
		[3-hydroxy-5-(1H-1,2,3-triazol-4-yl)picolinoyl] glycines	Unknown
FIH inhibition	Nonselective inhibitors: Fe^{2+} chelators [256,257]	Pyridines	Chelating Fe^{2+}
		Catechols	
		hydroxypyrones/hydroxypyridines	
	Selective inhibitors: N-oxalyl amino acids with hydrophobic side chain [258]	N-oxalyl-*D*-phenylalanine	Associated with the structure of N-oxalylglycine, a derivative of 2-OG
		Dimethyl-N-oxalyl-*D*-phenyl-alanine	
VHL inhibition	Inhibitors of VHL: HIF-1α protein–protein interaction [259–261]	VH298	Disrupting complex of VHL:ElonginB:ElonginC
		CM11	Inducing target protein degradation by exploiting E3 ligase activity

of prolonged HIF stabilization [242]. Overall, the "-dustats" is a novel type of drug that can inhibit PHD, thus activating the HIF-EPO pathway, and its effect on increasing EPO in the body is gentle. In existing research, the drug "-dustats" has been found to improve iron metabolism while treating anemia, and it generally does not exhibit significant cardiovascular side effects or promote cancer occurrence.

4.2. More precise modulating targets or combination strategies for enhancing HIF targeting therapies

Although PHD inhibitors are increasingly used to regulate HIFs, more precise regulation is being advocated due to possible off-target effects [243]. Considering the limited knowledge of the worthwhile direct target of HIFs, indirect HIF activation strategies are still the hot spot in the field (Table 2). Compared with PHD2 inhibitors, there has been relatively less research conducted on the activation of HIF using PHD1 inhibitors. A series of 1,2,4-triazolo[1,5-a]pyridines were recently identified as inhibitors of the PHD1 enzyme [252]. These PHD1 inhibitors have been found to exhibit a previously unidentified monodentate mode of chelation with Fe^{2+} ions, involving the triazole N1 atom. The benzonitrile group of the inhibitors then interacts and binds to the hydrogen of the Asn315 residue. Compared with other documented PHD inhibitors, this type of PHD1 inhibitors demonstrates remarkable bioactivity and exhibits favorable oral exposure in both the central nervous system and peripheral circulation. These characteristics position it as a highly promising and potent candidate for *in vivo* applications [262]. However, researchers have discovered that hypoxia can influence PHD1 expression through a non-HRE-mediated mechanism, while two distinct PHD1 isoforms have been detected in the nucleus, each exhibiting unique roles in cellular proliferation [263]. Additionally, PHDs do not necessarily prevent unwanted angiogenesis and glycolysis in normoxic cells. By contrast, the specific inhibition of factor inhibiting HIF (FIH) does not give rise to these issues as FIH solely hydroxylates HIF-1α that is stabilized under hypoxic conditions. FIH is an additional 2-OG-dependent non-heme iron enzyme that performs an asparaginyl hydroxylation, which subsequently impedes the interplay between HIF-1α and p300 [264], and FIH has demonstrated greater sensitivity to HIF-1α compared to PHDs under hypoxic conditions [265]. N-Oxalyl amino acids with hydrophobic side chains selectively and competitively inhibit FIH, as determined by the crystal structure of the FIH-Fe^{2+}-2-OG complex. This inhibition is closely linked to the structure of N-oxalylglycine, which is an analog of 2-OG [258]. Additional research regarding the impact of FIH on oxidative metabolism is imperative.

Furthermore, as such mentioned method falls under indirect regulation of HIFs, the direct regulation of HIFs molecules is currently the focus of research. However, HIF is a DNA-binding transcription factor that lacks a recognized ligand-binding domain, unlike steroid hormone receptors [266]. Due to the structural similarity between HIF-1/2α isomers, the regulation of specific isomers is currently a fortress to be overcome. Intriguingly, a 290-A cavity within the HIF-2α PAS-B domain has been detected with the capacity to hold small molecules and plays a key role in allosteric inhibition and hydrogen bond formation [267]. The PAS domain of HIF-2α consists of two subdomains, PAS-A and PAS-B,

which are located at the N- and C-termini of HIF-2α, respectively. The PAS-B subdomain plays a critical role in maintaining the stability and functionality of HIF-2α, as it contains the binding site of aryl hydrocarbon receptor nuclear translocator (ARNT), also known as HIF-1β, and enables HIF-2α to form an active transcription complex with ARNT and induce HIF-2α-mediated gene expression. By contrast, the function of the PAS-A subdomain is not well understood, but it is believed to be involved in regulating the activity and stability of HIF-2α. Antagonists to the HIF-2α PAS-B (e.g., MK-6482 and PT2385) cavity disrupt its heterodimerization with HIF-1β and are effective inhibitors in treating renal cell carcinoma [268–270]. No adverse effect on the cardiovascular system has been so far reported on PT2385 [271].

However, due to the potentially highly variable pharmacokinetics of PT2385, some patients may be under-exposed, resulting in poor efficacy [271]. Consequently, a highly potent and selective second-generation inhibitor of HIF-2α, MK-6482 (also known as belzutifan, or PT2977) has been developed [272]. MK-6482 is similar to PT2385, but with a structural modification—the geminal difluoro group present in the parental compound is substituted with a *cis*-vicinal difluoro group—that improves its pharmacokinetic profile, resulting in greater potency, reduced affinity for serum proteins, and less susceptibility to glucuronidation [273]. The variation in PT2385 exposure is attributed to the secondary metabolic pathway wherein the hydroxyl group is exposed to glucuronidation by UDP-glucuronosyltransferases. However, these HIF-α inhibitors are still expected to overcome drug-resistant variations. G323E of HIF-2α and F446L of HIF-1β reject MK-6482 and enhance the HIF-2 complex, respectively [269,274,275], while p53 mutations have been associated with resistance to HIF-2α blockade [269,275]. Furthermore, another two HIF-2 agonists, M1001 and M1002, also bind to the HIF-2α PAS-B domain [267]. After binding to the pocket, Tyr281 undergoes internal pressure, causing it to move towards the subunit binding surface and establish hydrogen bonds with Tyr456 of HIF-1β. This alteration improves the stability of the HIF-2 complex, leading to an augmentation in its activity. Studies have shown that the impact of M1002 on HIF-2α target genes is limited. However, when combined with a PHD inhibitor, which enhances the levels of HIF-2α, M1002 can substantially increase intracellular EPO levels [267]. In the future, it will be necessary to conduct comparable studies on other mammalian bHLH-PAS family members (e.g., HIF-2α PAS-B domain) to determine whether they can be activated or inhibited using direct-binding chemical ligands. In sum, more precise and targeted HIF pathway-activating drugs require either more specific indirect activation of HIF (e.g., inhibitors targeting specific PHD1-3 or FIH) or more effective direct activation targeting the specific HIF isoforms. Additionally, the issue of drug resistance also needs to be addressed.

5. Perspectives and conclusion

Three key implications for advancing the translation of VSMC HIFs-associated therapeutic strategies in panvascular medicine when considered collectively:

(1) Commonality and specificity of HIFs in panvascular diseases: HIFs are involved in the balanced regulation of proliferation/apoptosis and proliferation/migration of VSMCs, transforming VSMCs phenotype from contractile to synthetic, promoting the development of atherosclerosis and PAH. However, in PAH, HIF-2 appears to be the initiating factor of the disease where the interaction between HIFs and the electrophysiological characteristics in VSMCs is a key feature. In addition, moderate activation of HIFs in VSMCs contributes to the repair and stabilization responses of AAs. In the transition of VSMCs to a calcified phenotype, HIFs integrate ROS-glycolysis metabolism and Pi-related mineral metabolism. Furthermore, given the role of HIFs in remote ischemic preconditioning (RIPC) [5], it remains worthwhile to explore whether HIFs play an interactor between organs/systems in panvascular disease. Moreover, considering that short-term hypertrophic preconditioning can effectively alleviate ischemia–reperfusion injury, reduce infarct size, and attenuate cardiomyocyte apoptosis, it remains to be investigated whether HIF-1α undergoes pre-accumulation in this process, partially simulating the effects of RIPC [276]. Thus, how to induce HIF-1α towards a proliferative and hypertrophic phenotype to counteract fibrosis, inflammation, and cell apoptosis holds great promise as a valuable therapeutic intervention in the future.

(2) Holistic consideration of HIFs-related applications: in the clinical translational use of HIFs, it is imperative to consider not only the intrinsic properties of HIFs (e.g., HIFs' switch) and their spatiotemporal specificity with respect to organs and diseases, but also the impact of various internal and external environmental factors on the overall function of HIFs within the organism. A significant internal environmental factor is gender-related disparities, where circulating sex hormones are interconnected with HIF-1α. A higher baseline of HIF-1α in human PASMCs of females may increase susceptibility to pulmonary hypertension [277]. Estrogen metabolite 2-methoxyestradiol (2-ME2) has the characteristics of anti-proliferation and is also an inhibitor of HIF-1α. 2-ME2 promotes apoptosis and leads to significant disruption of the microtubule network. 2-ME2 reduces the proliferation of human PASMCs and the baseline expression of HIF-1α [278]. Furthermore, in the natural world, the habitat of an organism is arguably its most critical external environment. In high altitudes, HIF-2α shoulders the role of the short-term adaptive responser, instead of HIF-1α as generally considered. However, HIF-2α can also result in excessive production of red blood cells, causing chronic mountain sickness, which can ultimately lead to death and impair reproductive capabilities. Several mutations linked to hypertension and stroke are known to elevate HIF-2α expression, exhibiting symptoms akin to mountain sickness, even at low altitudes. Furthermore, populations residing in high-altitude regions undergo natural selection on HIF-2α, favoring mutations that mitigate the adverse effects of excessive red blood cell production, thereby reducing fitness costs [279].

(3) Precisely targeted drugs for HIFs-associated pathways: candidates for HIFs with preferable pharmacological and pharmacokinetic profiles should possess the following characteristics: oral formulations, higher target affinity, appropriate blood drug concentration and half-life, avoidance of excessive dosage load and prolonged use. In addition, it is important to avoid the use of drugs that excessively alter the endogenous hormone and endocrine environment.

Collectively, the application of HIFs-associated therapies from bench side to bedside requires doctors to focus more on individual differences (e.g., residence, gender, and disease) in diagnosis and treatment to discover root problems, researchers to investigate and explicit the distinct roles and interactions of HIFs in different organs/systems or the distinct stage of diseases, and pharmaceutical experts or engineers to strive for the industrialized production of targeted drugs with robust pharmacodynamics and pharmacokinetics. Therefore, the substantial translation and implementation of HIFs-related treatments based on the principle of "from doctors,

by engineers/researchers, for patients" can be achieved in the pan-vascular medicine.

Author contributions

Yiqing Hu, Yongchao Zhao, and Peng Li participated in the literature search, outline design, and manuscript writing. Junbo Ge introduced the central concept of this review and elaborated on the overall framework of the review. Hao Lu, Hua Li, and Junbo Ge provided guidance and supervision throughout the writing process, reviewed and revised the manuscript, and provided critical input and expertise.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary materials

Supplementary materials to this review can be found online at https://doi.org/10.1016/j.scib.2023.07.032.

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