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RESEARCH ARTICLE

Exposure to the Interior Environment of Water-Damaged Buildings Can Activate HIF 1A, Induce Proliferative Physiology and Impair Mitochondrial Metabolism

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ABSTRACT:
Hypoxia-inducible factor 1A (HIF 1A) is an oxygen-sensing nuclear transcription factor that regulates oxygen homeostasis in many illnesses ranging from, but not limited to, cancer, heart failure, premature infants to viral infections. We report here the measurement of HIF 1A using transcriptomics as a biomarker in Chronic Inflammatory Response Syndrome (CIRS), a systemic inflammatory and metabolic illness characterized by a multisystem, multi-symptom illness acquired following exposure to the interior environment of water-damaged buildings (WDB).
Pulmonary artery hypertension (PAH) is a well-established disease and is also associated with CIRS, but treatment is problematic, depending on its physiologic basis.
Both CIRS and PAH share a common pathogenesis of proliferative physiology. In the case of CIRS, genomic overexpression of the HIF 1A pathway represents a particularly concerning finding in our study population. Its re-regulation offers a salutary outcome, with the benefit of reducing the phenotypic expression represented by resolving PAH. By first reviewing the diverse pathophysiology of PAH, we present data that provide a basis for the demonstrated efficacy of our treatment protocol, which was used sequentially in CIRS patients to reduce HIF 1A by resolving aberrant mitochondrial transcriptomics associated with proliferative physiology and molecular hypometabolism. These data suggest a basis for a novel approach to treatment of PAH.
We demonstrate reduction of HIF 1A by a CIRS-treatment protocol with VIP therapy in the context of proper biotoxin treatment. Current literature shows that reduction of HIF 1A is crucial in PAH to avoid the significant morbidity and mortality associated with this condition.

Acronyms

CIRS Chronic Inflammatory Response Syndrome
GENIE Genomic Expression: Inflammation Explained
HIF 1A Hypoxia Inducible Factor-alpha
MHM Molecular hypometabolism
PAH Pulmonary artery hypertension
PASMCs Pulmonary artery smooth muscle cells Proliferative physiology Aerobic glycolysis
TGFBR Transforming growth factor beta receptors 1, 2 and 3.
VIP Vasoactive intestinal polypeptide
WDB Water-damaged buildings

Introduction:

Pulmonary arterial hypertension (PAH) is a group of syndromes characterized by irreversible vascular remodeling and persistent elevation of pulmonary vascular resistance and pressure, leading to right heart failure and even death. Current therapeutic strategies focus on pulmonary arterial vessel dilation. Unfortunately, the interventional management of vascular remodeling is not well-delineated. Hypoxia plays a significant role in the pathogenesis of PAH, and numerous studies have shown the relationship between PAH and the hypoxia-inducible factors family, especially HIF 1A[1,26,27,28].

PAH has a prevalence of 20 cases/1,000,000 population, and the incidence in women is four times higher than in men[2]. Epidemiological data of this disease differ in the literature; values of 15-26 cases per million inhabitants per year worldwide, affecting women with a female-to-male ratio of 1.7:1 and the onset of symptoms after the second or third decade of life[3].

Clinically, PAH is categorized into five groups: (i) pulmonary arterial hypertension; (ii) PAH associated with left heart disease; (iii) PAH associated with lung disease and/or hypoxia; (iv) PAH associated with pulmonary artery obstructions; and (v) PAH with unclear or multifactorial mechanisms. The diagnostic criteria for PAH were updated as mean pulmonary artery pressure (mPAP) >20 mmHg at rest during right heart catheterization according to the 2022 European Society of Cardiology/European Respiratory Society PAH guidelines[1,4].

The primary pathophysiology of pulmonary vascular remodeling involves (i) intimal endothelial cell proliferation; (ii) apoptotic resistance; (iii) medial pulmonary artery smooth muscle cell (PASMCs) hypertrophy and proliferation; (iv) adventitial fibroblast proliferation and activation; with (v) excessive extracellular matrix deposition; and (vi) interstitial or perivascular inflammatory infiltration. PAH patients have a low 5-year survival rate and high mortality, about 57%, due to the complexity of etiologies and limited interventions for irreversible pulmonary vascular remodeling[1,30].

In the treatment of pulmonary hypertension, currently available targeted therapeutic agents act on diastolic/contraction balance in the pulmonary arteries. These include soluble guanylate cyclase. (SGC) stimulants, antagonists of endothelin receptors and inhibitors of phosphodiesterase (PDE5). Despite improving the quality of life in patients with pulmonary hypertension, these drugs do not reverse or delay pulmonary vascular remodeling, leading to the progression of the disease[6]. Supplemental oxygen, however, decreases pulmonary vascular resistance and improves the quality of life in these patients[7].

The combined adverse effects of hypoxia and proliferative physiology have been recognized in cases of PAH. Pulmonary hypertension, marked by elevated levels of activation of hypoxia-inducible factor 1 alpha (HIF 1A), is characterized by a profound pulmonary artery remodeling that includes significant proliferative and inflammatory changes of the pulmonary artery adventitia[8]. PAH develops from a multistep process beginning with gene expression changes that directly change cell metabolism, inflammation and proliferation[31,32].

It is well established that the pathogenesis of PAH includes (i) pro-inflammatory activation, (ii) increased proliferation and (iii) apoptosis resistance, all occurring in changes in proliferative physiology, especially seen with glucose metabolism leading to aerobic glycolysis, the Warburg effect[42]. Historically assigned to cancer cells, this metabolic adaptation has recently been reported in PAH[9,10,11,12,13]. These changes have been reported to occur in smooth muscle cells, endothelial cells and fibroblasts. Immune changes include the activation of macrophages toward a pro-inflammatory effect through secretion of chemokines, cytokines and glycolytic metabolites, particularly pyruvate and lactate[8].

The hypoxia-inducible factor family is a group of transcription factors involved in the intracellular response sensing oxygen concentration, strongly associated with organism growth and development and disease pathogenesis. Microenvironmental hypoxia is commonly observed in various systemic inflammatory diseases, such as atherosclerosis, diabetes mellitus, inflammatory bowel disease, cancer, CIRS and pulmonary hypertension. Chronic

hypoxia signaling plays a significant role in the progression of pulmonary vascular remodeling, leading to irreversible PH and right heart failure. The hyperplastic proliferation of PASMCs is also attributed to the pathogenesis of pulmonary vascular remodeling. Different studies showed that HIF 1A is overactivated in PASMCs in PAH[1]. Adventitial fibroblast activation and transition to myofibroblasts, the primary origin of collagen and extracellular matrix synthesis, are believed to promote vasculopathy in PAH[1].

The current standard management of PH focuses on regenerating normal vasomotor function instead of preventing vascular remodeling, which causes high mortality and low quality of life. A new interventional strategy reversing pulmonary vascular remodeling is imperative for treating PAH[1]. The features of pulmonary vascular remodeling include thickening of the media, hyperproliferation of vascular cells, enhanced muscularity, increased migration and increased inflammatory cell recruitment[4]. Exposure of alveoli to regional hypoxia constricts the pulmonary vasculature to compensate for the diminished tissue perfusion and maintain adequate arterial oxygenation.

Chronic hypoxia leads to pulmonary artery remodeling driven by PASMC proliferation increases wall thickness, which causes an increase in flow resistance. This phenomenon leads to pressure overload of the right heart ventricle, potentially causing failure. The remodeling process is initiated by oxygen sensors in vascular cells that detect a decrease in partial pressure of oxygen (PO2) and activate the signaling system that leads to acute constriction of pulmonary arteries. Over time, given the persistence of hypoxia, the acute phase is marked by remodeling of the vascular wall marked by capillary lumen narrowing[14,33].

The role of reactive oxygen species (ROS) in developing PAH is strongly suggested by studies showing profound alteration in the mitochondrial structure and function (see mitochondria below). During hypoxia, mitochondria from vascular cells release superoxide (O2-) from complex III to the intermembrane space, which is converted to hydrogen peroxide (H202) by superoxide dismutase. H202 is delivered into the cytoplasm, activating smooth muscle contraction and remodeling[14,34.]

HIF 1A

Oxygen is essential for mammalian life, and cells are well-designed to alter gene expression profiles in response to changes in PO2. Hypoxia activates cellular sensing mechanisms to restore oxygen to the hypoxic regions and maintain cell viability. Previous studies from human and animal models point to the family of HIF 1A as essential regulators in pulmonary vascular responses to acute and chronic hypoxia[15,16].

Elevated HIF 1A, a marker for PAH, promotes the progress of vascular remodeling by regulating PASMC proliferation and migration[17]. HIF 1A, which is highly regulated by the intracellular oxygen concentration, is critical in regulating PASMC phenotypes in hypoxia. In response to hypoxia, the transcription factor HIF 1A is rapidly stabilized and translocated to the nucleus, where it binds to the hypoxia-responsive element[17].

Research shows that HIF-1A participates in hypoxia-induced PASMC proliferation and migration. Also, HIF 1A protein expression increases after 4 hours of hypoxia; its level declines slightly at 8-48 h but remains higher than in normoxia. The mRNA level of HIF 1A is significantly up-regulated at 12 h-48 h[17].

Acute hypoxia leads to an increase in intracellular calcium, reversible upon reoxygenation. In contrast, chronic hypoxia causes a sustained increase in Ca2+, which remains elevated after returning to normoxia. This effect is mediated by calcium channels, which are activated by the depletion of intracellular calcium stores during chronic hypoxia. These channels comprise transient receptor potential proteins, which HIF 1A controls[14].

The adaptive mechanism of vascular cells to chronic hypoxia is orchestrated via the activity of HIF 1A. HIF-mediated signaling is now regarded as the cornerstone of oxygen homeostasis that controls multiple hypoxia-responsive genes on the transcriptional level[14] This cellular response to hypoxia activates signaling pathways leading to cell survival, proliferation and metabolic modulations such as repression of mitochondria respiration[18,19]. These HIF-mediated signals augment angiogenesis and erythropoiesis on the tissue or organism level. EPO and VEGF are the primary mediators of the later effects[14,34].

Additionally, hypoxia exposure reduced the expression of the voltage-gated potassium channels, causing a reduction in potassium current subsequent membrane depolarization, voltage-gated calcium channel activation and increased intracellular Ca2+. Further evidence of the impact of HIF 1A activation in controlling both vascular tone and pulmonary vascular remodeling comes from studies showing that in isolated PASMCs with HIF 1A

deficiency, there was no decrease in the potassium current following exposure to chronic hypoxia[4].

Chronic hypoxia exposure causes inflammation as an early consequence and is an essential component in developing pulmonary hypertension. The targets of HIF activation include pro-inflammatory mediators, IL-6, NF-kB and VEGF in PAH[4].

PAH is a condition that is characterized by pulmonary artery pressure above 25 mmHg. In treating PAH, the pulmonary vascular system is regulated to ensure a diastolic and contraction balance; this treatment does not prevent or reverse pulmonary vascular remodeling and still causes pulmonary hypertension to progress. According to Warburg, the link between metabolism and proliferation in PAH is like that of cancer, with a typical aerobic glycolytic phenotype. By activating HIF 1A, aerobic glycolysis is enhanced, and cell proliferation is triggered[6].

Mitochondria, Warburg Physiology and HIF 1A:

Research has shifted from mitochondrial metabolic plasticity to altered intracellular energy metabolism in pulmonary arteries. As part of PAH, cellular metabolism is abnormal, including aerobic glycolysis, fatty acid, and glutamine metabolism. A metabolic reprogramming mechanism may be involved in PAH pulmonary vascular remodeling[6,35].

PASMCs and extracellular vesicles isolated from PAH patients maintain a longer-lasting anti-apoptotic phenotype. The connection between PAH metabolism and proliferation is like that in cancer. Anti-proliferative or anti-cancer agents may play a role in PAH treatment[6].

The metabolism of PAH is accomplished by many cellular and molecular mechanisms with mitochondria at their cores. Evidence suggests metabolic dysfunction may be associated with PAH expression and susceptibility changes. Warburg's principle of dysregulated whole-body metabolism can now be included in the metabolic theory of PAH. Right ventricular metabolism may also be present in skeletal muscle tissue, indicating that PAH development could result from systemic effects[6,36].

The dysregulation of mitochondrial metabolism occurs in the early and late stages of PAH[6]. Abnormal immunometabolism suggests a functional role of perivascular inflammation in pulmonary vascular remodeling. There is a correlation between clinical outcomes and elevated levels of cytokines, chemokines and inflammatory mediators in PAH patients. In PAH lung biopsies, macrophages, mast cells and T lymphocytes are detected near remodeled pulmonary vasculature[6,37].

In PAH, altered intracellular glucose transport may contribute to glucose intolerance. The presence of A1C hemoglobin in PAH suggests that glucose intracellular influx and insulin resistance are present when chronic hyperglycemia is present. Insulin resistance was found to accompany proliferative physiology in CIRS patients[20].

HIF 1A activation may result from abnormalities in mitochondrial metabolism in PAH. The abnormalities reduce hydrogen peroxide production and eliminate inhibition of HIF 1A activation. DNA methyltransferase in the lung activates epigenetic silencing of superoxide dismutase (SOD2), interfering with gene transcription and reducing hydrogen peroxide levels in the blood, activating HIF 1A in normal PASMC[6].

In PAH, HIF 1A activation results in mitochondrial fission in human and animal PASMCs, suggesting its association with mitochondrial plasticity. In addition, it regulates mitochondrial dynamics, which decreases mitochondria and reduces NO utilization in PAH cells. In the mitochondrial respiratory chain, HIF 1A promotes the expression of cytochrome oxidase subunits 4.2[21,22,23].

McElroy[19] states, "Vascular obstruction increases the afterload faced by the right ventricle (RV), leading to RV failure. The proliferative, obstructive vasculopathy of PAH shares several mitochondrial abnormalities with cancer, notably a shift to aerobic glycolysis and mitochondrial fragmentation". The resulting metabolic shift to aerobic glycolysis reflects the inhibition of pyruvate dehydrogenase by pyruvate dehydrogenase kinase. In addition, altered mitochondrial dynamics result in mitochondrial fragmentation. The molecular basis of this structural change includes upregulation and activation of fission mediators, notably dynamin-related protein one and downregulation of fusion mediators, especially mitofusin-2. These pathogenic mitochondrial abnormalities offer new therapeutic targets. Inhibition of mitotic fission or enhancement of fusion in PASMCs slows cell proliferation, causes cell cycle arrest and induces apoptosis[2].

It is recognized that fixed mechanical vascular obstruction with loss of cross-sectional area, rather than vasospasm, is the predominant cause of increased PAH in most patients. Only 12.6% of patients respond to potent vasodilators, like inhaled nitric oxide, with a >20% mean pulmonary pressure falling to a value <40 mmHg while maintaining or

increasing cardiac output. Current PAH therapies are primarily vasodilators and do not directly address the vascular obstruction or cancer-like phenotype of vascular cells in PAH[2].

The mitochondria in PASMC of resistance-level arteries (<200 um) serve as vascular oxygen sensors, responding to local decreases in alveolar oxygen tension, affecting the lung's autoregulatory mechanism to match perfusion to ventilation. A modest decline in airway oxygen leads to localized vasoconstriction, which shunts perfusion to better-ventilated lobes within the lung. The mitochondria in PAH, PASMC have impaired metabolism due to transcriptionally-mediated inhibition of mitochondrial PDH and are fragmented due to an imbalance of mitochondrial fission versus fusion[19].

Functioning mitochondria are mandatory for biosynthetic and bioenergetic pathways controlled by the Krebs cycle and mitochondrial membrane potential. Mitochondria are vital to cellular oxygen-sensing and adaptation to hypoxia[4]. In addition, the impairment of the mitochondria ROS-HIF pathway leading to glycolysis and impaired energy metabolism was implicated in PAH[4].

Hypoxia inhibits the opening of voltage-gated potassium channels, contributing to PASMC depolarization. The expression of these channels is also decreased in PASMC subjected to chronic hypoxia. In addition to increased calcium, chronic hypoxia also increases intracellular pH, an effect due to HIF 1A-dependent expression of the sodium-hydrogen exchanger. The role of HIF 1A in the pathogenesis of pulmonary hypertension is not restricted to hypoxia-induced pulmonary hypertension. The spontaneous development of PAH is associated with (i) increased HIF 1A expression, (ii) HIF-dependent reductions in potassium currents, (iii) increased PDK1 expression, and (iv) a switch from oxidative to glycolytic metabolism in pulmonary artery smooth muscle cells[14].

Pathogenesis:

The cell-proliferation/inflammation interaction is critical to the pathogenesis of PAH. Enhancing aerobic glycolysis attenuated pulmonary hypertension and vascular remodeling and reduced expression of the glucose transporters (Glut1 and 4) typically up-regulated in cells exhibiting enhanced glycolysis. Collectively, these observations support a metabolic hypothesis for the pathogenesis of pulmonary hypertension, whereby a rearrangement of the mitochondrial and cytoplasmic metabolism might explain the molecular and functional abnormalities seen in pulmonary hypertension, including excessive proliferation, apoptosis resistance and inflammatory activation[8].

Evidence supports the role of the Warburg effect in immune activation, particularly in macrophages and dendritic cells. Initial reports have demonstrated that Toll-like receptor-activated dendritic cells undergo a metabolic adaptation from oxidative phosphorylation to aerobic glycolysis, and this metabolic switch is critical for dendritic cell's maturation and function. These cells express HIF 1A and show increased aerobic glycolysis. Moreover, increased aerobic glycolysis produces increased amounts of lactate. Pyruvate is excreted as lactate and entered the citric acid cycle. In this regard, lactate is a potent activator of macrophages toward the expression of HIF 1A[8].

Hypoxia-induced pulmonary hypertension is associated with diminished endothelial nitric oxide (eNOS) production and increased superoxide (O2) production through eNOS uncoupling and defective mitochondrial respiration. This drives the activation of the transcription factor HIF 1A with dysregulation of pulmonary vasculature. Therapeutics aimed at increasing NO and targeting HIF 1A are now being considered[14].

TGF Beta, Fibrosis and PAH:

Pathological studies indicate that the cell types found in the intraluminal occlusions contain mesenchymal cells and smooth muscle cells, while cell markers of endothelial cells are not prominent. Endothelial-to-mesenchymal transition results in the transformation of endothelial cells to mesenchymal cells and in PASMCs.TGF-B1 induces EMT[38]. Transforming growth factor β is a fibrotic factor that induces EMT in many cell types[7].

Since the HIF signaling pathway is essential for developing pulmonary hypertension in chronic hypoxia, research suggests HIF signaling in vascular endothelium regulates the development of pulmonary fibrosis[39]. Endothelial HIF-deficient mice were protected against the development of PAH, including right ventricle and pulmonary vessel remodeling. Similarly, endothelial HIF-deficient mice were protected from PAH after a 4-week exposure to normobaric hypoxia. "Studies of pulmonary vascular endothelial cells isolated from the HIF-targeted mice revealed that endothelial HIF signaling (i) increases endothelial cell expression of connective tissue growth factor; (ii) enhances vascular permeability; and (iii) promotes PASMC proliferation, all of which have potential to impact the development of PAH." These studies demonstrate that vascular endothelial cell HIF

signaling is necessary for developing hypoxia and pulmonary fibrosis-associated PAH. PAH is associated[16] with decreased exercise capacity, independence, quality of life, and the 6-minute walk distance[7].

"In a recent study, PAH in the chronic hypoxia model was shown to be mediated by HIF activation specifically within vascular smooth muscle cells of the lung. A proposed mechanism for this finding is that HIF stabilization in SMCs leads to decreased potassium channel expression, resulting in increased cytosolic calcium concentration and tonic vasoconstriction with attendant expected changes in vascular remodeling leading to PAH[7]."

Methods:

We used a retrospective study design using a deidentified database of transcriptomics findings that report gene activity results compared to controls from a library of 210 genes in 1822 patients. We found 81 new cases of elevated HIF 1A, as shown by *z* scores > 1.29 in cases compared to controls in 1822 CIRS cases, suggesting that CIRS carries an incidence of PAH that is over 2,000 times higher than expected from the literature. We followed a standard, published assessment and treatment protocol to stratify cases by stage of therapy[5.] Additional information from a literature review to follow below supported that observation.

We extracted cases that showed positivity of gene activation of HIF 1A, see Table 1. We used the same protocol for reporting gene activation as reported previously[20]. We further defined the data extraction to compare cases of molecular hypometabolism (MHM) by four stages, representing cases 1) before treatment, 2) after the first eleven steps of a published treatment protocol[5], 3) during therapy with vasoactive intestinal polypeptide (VIP), and 4) after all treatment was completed, respectively.

We stratified data by MHM to determine the effect of the treatment protocol on HIF 1A. We further analyzed the impact of MHM on transforming growth factor beta receptors (TGFBR) 1, 2, and 3. Finally, we looked for confounding factors for HIF 1A, namely the nuclear transcription factor FOX3, stratified by MHM as before, in Table 2. Using these correlations, we could assess the potential for association with findings reported for HIF 1A with our control cohort and results from the literature.

Results:

There were 81 total HIF cases identified in our cohort out of 1822: 62 in Stage 1 (7.5 %), 14 in Stage 2 (3.4 %), 4 in Stage 3 (3.4 %) and 1 in Stage 4 (1.6 %), showing a 79 % reduction overall in the incidence of HIF 1A in cases due to the CIRS treatment protocol analyzing by stage. Isolating the HIF 1A data (Table 2), the % reduction of treated HIF cases is 98.3%.

TGFBR 1 also showed a response to treatment based on the stages of MHM. There were 136 cases of TGFBR in Stage 1, 50 in Stage 2, 17 in Stage 3 and 5 in Stage 4. Comparable results were obtained for TGFBR 2 and TGFBR 3.

FOX03 numbers were not significantly different when sorted by MHM by percentage, but we see a treatment effect when sorted by HIF.

Table 1: HIF sorted by molecular hypometabolism

MHM N=	STAGE	HIF 1A	TGFBR 1	TGFBR 2	TGFBR 3	FOX03
829	1	62= 7.5 %	136=16.4	113=13.3%	79=9.5	85=10.2%
409	2	14= 3.4 %	50=12.2	45=11.0%	38=9.3%	40=9.8%
116	3	4= 3.4%	17=14.6	13=11.2%	12=10.3%	10=8.6%
60	4	1=1.6%	3=5.0%	2= 3.3%	1=1.7%	6=10 %

HIF 1A falls with consecutive interventions.

Table 2: HIF sorted by stage compared to total HIF

HIF A	STAGE 1-4	TGFBR 1	FOXO3
81	62=76.5 %.	26= 32,1%	10=12.3%
81	14=17.3%	5=6.2%	5=6.2%
81	4= 4.9%	2=2.5%	2=2.5%
81	1= 1.2%	0=0	0=0

If we accept the incidence of PAH as 1 in 50,000[2,3], with HIF 1A ubiquitous in PAH, CIRS cases are enriched by over 2000-fold in the incidence of HIF 1A.

Discussion:

Our research indicates a leading role that HIF 1A pathophysiology plays in CIRS. It may stand at a nexus between pulmonary arterial hypertension

(PAH) and chronic inflammatory response syndrome (CIRS), offering a novel therapeutic target based on transcriptomic biomarkers. This data will enhance the depth of the analysis between these related conditions, highlight the significance of the findings, and offer future research directions.

ELABORATION ON THE METABOLIC AND INFLAMMATORY BASIS FOR PAH

Our study underscores the intricate link between metabolic dysregulation, particularly aerobic glycolysis (the Warburg effect), and a known pathogenetic cause of PAH mediated by expression of HIF1A. This link highlights a novel aspect of PAH akin to cancer biology and opens avenues for metabolic-targeted therapies. Discussing how the dysregulation of HIF 1A contributes to a shift towards a glycolytic metabolic profile in the pulmonary vasculature and linking this to potential therapeutic interventions could provide a comprehensive overview of the pathophysiological underpinnings of PAH and its similarities with cancer metabolism.

The integration of HIF 1A measurement into routine clinical assessments represents a promising advancement in managing pulmonary arterial hypertension (PAH), given the frequency of CIRS found in the general population. The results presented in the study provide compelling evidence that HIF 1A not only plays a crucial role in the pathophysiology of these diseases but also can serve as a valuable biomarker for their detection, monitoring, and treatment stratification. This development holds significant potential for enhancing patient care through more personalized and precise medical interventions.

THE POTENTIAL OF HIF 1A AS A BIOMARKER FOR PAH

The study reveals that HIF 1A can be measured non-invasively and may serve as a clinical biomarker. This is pivotal, and expanding on the mechanism of how HIF 1A levels correlate with disease severity, response to treatment, and prognosis will further strengthen the argument for its use in clinical settings in the CIRS population and have direct implications for managing PAH.

EARLY DETECTION

Early detection of PAH remains a clinical challenge due to its non-specific symptoms and the complexity of its diagnosis, which traditionally relies on invasive procedures like right heart catheterization. The study's findings suggest that measuring HIF 1A expression could serve as a non-invasive, easily accessible biomarker for early detection of PAH, especially in populations at risk or presenting with subtle symptoms. Incorporating HIF 1A measurement into routine screenings could enable clinicians to identify PAH earlier, potentially before significant vascular remodeling. This early detection is critical for improving patient outcomes, as it allows for initiating treatment at a stage when it is likely to be more effective in halting or slowing disease progression.

MONITORING DISEASE PROGRESSION AND TREATMENT RESPONSE

We feel there is a role for HIF 1A as a central regulator in the hypoxic response pathway, directly implicating it in the pathogenesis of both PAH and CIRS. Furthermore, it should raise suspicion that CIRS patients are at direct risk for PAH. Our study demonstrates significant improvement in HIF 1A levels with CIRS treatment, suggesting its utility in monitoring disease progression and response to therapy while improving PAH as a subgroup in this broader population. Regular assessment of HIF 1A could provide clinicians with real-time insights into the efficacy of prescribed treatments, enabling timely adjustments to therapeutic strategies. Finally, this responsive approach to treatment may have implications in management of PAH related and unrelated to CIRS, optimizing patient outcomes through personalized medicine.

STRATIFICATION OF PATIENTS FOR TAILORED THERAPEUTIC INTERVENTIONS

The variability in response to PAH treatments among patients highlights the need for personalized therapeutic strategies based on individual disease characteristics and mechanisms. The study's findings suggest that CIRS patients with elevated HIF 1A levels could serve as a stratification tool, identifying patient subsets more likely to benefit from specific interventions, especially those targeting hypoxia-related pathways due to exposure to a WDB. This stratification could extend to CIRS treatment, where interventions that effectively reduce HIF 1A levels could be preferentially used in patients showing elevated HIF 1A as part of their disease profile.

BROADER IMPLICATIONS FOR TRANSCRIPTOMIC BIOMARKERS IN CLINICAL PRACTICE

Our study positions transcriptomics at the forefront of precision medicine in complex diseases like PAH and CIRS, highlighting the importance of genotyping patients with different phenotypic expressions. Transcriptomic biomarkers have already revolutionized the diagnosis, monitoring, and treatment of such diseases and could underscore the broader significance of our findings. Integrating transcriptomic biomarkers into managing chronic, complex diseases represents a transformative approach to medical care,

heralding a shift towards more personalized, targeted, and effective treatments. The study's findings on the role of HIF 1A in CIRS exemplify the potential of this approach in pulmonary arterial hypertension (PAH). By leveraging the insights gained from transcriptomics, healthcare providers can achieve a more nuanced understanding of disease mechanisms, patient variability, and the impact of therapeutic interventions at the molecular level. This depth of insight is poised to fundamentally change how chronic, complex diseases are managed, offering several key advancements:

PERSONALIZED MEDICINE

The core of this paradigm shift is the move towards personalized medicine, where treatments are tailored to the individual characteristics of each patient, including their genetic makeup, disease phenotype, and response to previous treatments. Transcriptomic biomarkers like HIF 1A enable the identification of molecular signatures that predict disease progression, treatment response, and risk of complications more accurately than traditional clinical markers. This precision allows for the customization of therapy to the patient's specific disease profile, maximizing efficacy and minimizing the risk of adverse effects.

TARGETED THERAPIES

Understanding the molecular underpinnings of diseases enables the development and application of targeted therapies that directly modulate specific disease pathways. The study's insights into the role of HIF 1A in CIRS suggest new therapeutic targets within the hypoxia response pathway with direct implications for PAH. By focusing on these targets, treatments can be designed to interrupt the disease process at a fundamental level, offering the potential for more effective interventions compared to conventional, non-specific therapies.

DYNAMIC DISEASE MONITORING

Transcriptomic biomarkers enable dynamic monitoring of disease progression and response to treatment in real time. By tracking changes in gene expression patterns, clinicians can adjust therapeutic strategies in response to the patient's current disease state, ensuring that treatments remain effective over time and adapt to changes in the disease's evolution.

ENHANCED DRUG DEVELOPMENT

The detailed molecular insights transcriptomics provides can streamline drug development, focusing efforts on pathways that yield therapeutic benefits. This specificity can reduce the time and cost of bringing new treatments to market, accelerating the availability of effective therapies for complex diseases.

Novel Treatment: Resveratrol

As we consider the benefits of the use of transcriptomics in new therapies, the case of resveratrol in PAH is a cogent example. Resveratrol, a plant-derived polyphenolic compound and phytoestrogen, has multiple protective effects, enhancing anti-inflammatory and anti-oxidative cellular stress response. Recent anecdotal reports have also suggested that resveratrol has anti-proliferative effects. The present study[25] showed that resveratrol treatment alleviated right ventricular systolic pressure and pulmonary arterial remodeling induced by hypoxia. Resveratrol has also been reported to have inhibited the proliferation of PASMCs and HIF 1A expression and decreased reactive oxygen species induced by hypoxia in PAMSCs[25].

The inflammatory compounds TNF, IL6 and IL1B have been reported to be suppressed by resveratrol treatment. Resveratrol also inhibited the expression of HIF 1A by suppressing the intracellular signaling pathways MAP/ERK1 and P13K. The suppression of VEGF, a downstream gene of HIF 1A, verified those results[25,40,41].

Resveratrol may prevent PAH through its anti-proliferative, anti-inflammatory and antioxidant effects. A recent study showed that NO and ROS can also activate HIF 1A production besides hypoxia. ROS and HIF-1 were both shown to decrease significantly with resveratrol administration[24].

Resveratrol could attenuate the development of PAH by (i) reduction of vascular remodeling, (ii) inhibition of proliferation of the intima and media, (iii) inhibition of endothelial dysfunction, (iv) decreased vasoconstriction, (v) and activation of inflammatory processes[3].

Resveratrol costs pennies, and has been in use for years. Given its impact on PAH, a transcriptomic study on use of resveratrol to lower HIF 1A and correct PAH in CIRS could show safety and efficacy rapidly.

Conclusion:

The advancements brought about by integrating transcriptomic biomarkers into clinical practice represent a paradigm shift in managing chronic, complex diseases. This shift towards personalized, targeted, and effective treatments promises improved patient outcomes and heralds a new era

in medical science. Moving beyond one-size-fits-all approaches and embracing the complexity of individual disease processes can offer patients more hope for effective treatment and a better quality of life. The journey from broad-spectrum interventions to precise molecular medicine, exemplified by the study on HIF 1A, is a testament to the power of modern biomedical research and its potential to redefine health care for complex diseases.

This research demonstrates the significant role HIF 1A plays in CIRS. It is found in up to 7.5% of CIRS patients, and its significant reduction is responsive to a staged treatment approach. HIF 1A is also elevated in PAH and serves as a primary indicator of pathogenesis, disease progression and therapeutic target in this difficult-to-treat population. Future research should focus on the incidence of PAH in CIRS and its response to CIRS treatment as a subpopulation.

Additionally, while Vasoactive Intestinal Peptide (VIP) therapy has been used to treat PAH directly, are outcomes improved when patients demonstrate co-morbidities of CIRS and PAH that undergo a staged treatment approach that concludes with VIP compared to VIP therapy alone? Is a staged treatment approach to PAH/CIRS population better than standard PAH therapy alone? If our model, based in transcriptomics, demonstrates improved PAH outcomes in a CIRS population compared to standard of care, this would be a breakthrough in managing a subgroup of PAH.

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References:

1. Wang N, Hua J, An J, et al. Updated perspective of EPAS1 and the role in pulmonary hypertension. *Frontiers* 2023; Doi: 10.3389.
2. Ryan J, Dasgupta A, Huston J, et al. Mitochondrial dynamics in pulmonary arterial hypertension. *J Mol Med* 2015; 93: 229-242.
3. Ferreira A, Serejo J, Durans R, et.al. Dose-related effects of resveratrol in different models of pulmonary arterial hypertension: A systematic review. *Curr Cardio Rev* 2020; 16: 231-240.
4. Zeidan E, Akbar Hossain M, El-Daly M, et al. Mitochondrial regulation of the hypoxia-induced factor in the development of pulmonary hypertension. *J. Clin Med* 2022; 11: 5219.
5. Shoemaker R, House D, Ryan J. Vasoactive intestinal polypeptide (VIP) corrects chronic inflammatory response syndrome (CIRS) acquired following exposure to water-damaged buildings. *Health* 2013; *5*(3): 396-401.
6. Liu X, Zhang L, Zhang W. Metabolic reprogramming: A novel metabolic model for pulmonary hypertension. *Review* 2022; 10.3389/fcvm
7. Bryant A, Carrick R, McConaha M, et al. Endothelial HIF regulates pulmonary fibrosis-associated pulmonary hypertension. *Am J Physiol Lung Cell Mol Physiol* 2016; 310: L249-L262.
8. Stenmark K, Tuder R, Kasmi K. Metabolic reprogramming and inflammation act in concert to control vascular remodeling in hypoxic pulmonary hypertension. *J Appl Physiol* 1985; 119: 1164-1
9. Cuttica MJ, Kalhan R, Shlobin OA, Ahmad S, Gladwin M, Machado RF, Barnett SD, Nathan SD. Categorization and impact of pulmonary hypertension in patients with advanced COPD. *Respir Med* 2010; 104: 1877-1882.
10. Fijalkowska I, Xu W, Comhair SA, Janocha AJ, Mavrakis LA, Krishnamachary B, Zhen L, Mao T, Richter A, Erzurum SC, Tuder RM. Hypoxia inducible-factor alpha regulates the metabolic shift of pulmonary hypertensive endothelial cells. *Am J Pathol* 2010; 176: 1130-1138.
11. Paulin R, Michelakis ED. The metabolic theory of pulmonary arterial hypertension. *Circ Res* 2014; 115: 148-164.
12. Tuder RM, Archer SL, Dorfmuller P, Erzurum SC, Guignabert C, Michelakis E, Rabinovitch M, Schermuly R, Stenmark KR, Morrell NW. Relevant issues in the pathology and pathobiology of pulmonary hypertension. *J Am Coll Cardiol* 2013; 62: D4-D12.
13. Zhao L, Ashek A, Wang L, fang W, Dabral S, Dubois O, Cupitt J, Pullamsetti SS, Cotroneo E, Jones H, Tomasi G, Nguyen QD, Aboagye EO, El-Bahrawy MA, Barnes G, Howard LS, Gibbs JS. Gsell W, He JG, Gsell W. He JG, Wilkins MR. Heterogeneity in the lung (18) FDG uptake in pulmonary hypertension: the potential of dynamic (18) FDG positron emission tomography with kinetic analysis as a bridging biomarker for pulmonary vascular remodeling targeted treatments 2013; 128: 1214-1224.
14. Jaitovich A, Jourd'heuil D. A brief overview of nitric oxide and reactive oxygen species signaling in hypoxia-induced pulmonary hypertension. *Adv Exp Med Biol* 2017; 967: 71-81
15. Labrousse-Aria D, Castillo-Gonzalez C, Rogers N, et al. HIF-2a-mediated induction of pulmonary thrombospondin-1 contributes to hypoxia-driven vascular remodelling and vasoconstriction. *Cardiovascular Research* 2016; 109: 115-130
16. Lei W, Shui X, Li G, et al. Expression and analysis of the HIF-1 pathway in humans with pulmonary arterial hypertension lungs. *Mol Med Rep* 2016; 14: 4383-4390.
17. Han X, Zhang W, Wang Q et al. HIF 1A promotes the proliferation and migration of pulmonary arterial smooth muscle cells via activation of Cx43 *Cell Moll Med*. 2022; 25: 10663-10673.
18. Marshall J, Bazan L, Zhang Y, Fares W, Lee J. Mitochondrial dysfunction and pulmonary hypertension: Cause, effect or both? *Am J Physio Cell Molec Phys*, 2018; 314: L782?
19. McElroy G, Chandel N. Mitochondria control acute and chronic responses to hypoxia. *Exp Cell Res*.2017; 356:217.
20. Shoemaker, R. Metabolism, molecular hypometabolism and inflammation: Complications of proliferative physiology include metabolic acidosis, pulmonary hypertension, T reg cell deficiency, insulin resistance and neuronal injury. *Trends Diabetes Metab 2021; Doi: 10.15761/TDM.1000118*
21. Kracun D, Klop M, Knirsch A, et al. NADPH oxidase and HIF1 promote cardiac dysfunction and pulmonary hypertension in response to glucocorticoid excess. *Redox Biology* 2020; 34: 101536
22. Archer SL, Gomberg-Maitland M, Maitland ML, Rich S, Garcia JGN, Weir EK. Mitochondrial metabolism, redox signaling and fusion: a mitochondrial ROS-HIF-1-alpha-Kv15 O2 sensing pathway at the intersection of pulmonary hypertension and cancer. *Am J*

Physiol Heart Circ Physiol (2008) 294: H570-8.Doi: 10.1152/ajpheart.01324.2007.

23. Tuder RM, Davis LA, Graham BB. Targeting energetic metabolism: a new frontier in the pathogenesis and treatment of pulmonary hypertension. *Am J Resp Crit Care Med* (2012) 18.260-6. Doi: 10.1164/rccm.201108-1563PP.
24. Dasgupta A, Wu D, Tian L, et al. Mitochondria in the pulmonary vasculature in health and disease: oxygen—sensing, metabolism and dynamics. *Compr Physiol* 2020; 10: 713-765.
25. Xu D, Li Y, Zhang Bo, et al. Resveratrol alleviates hypoxic pulmonary hypertension via anti-inflammation and antioxidant pathways in rats. *Int. J Med. Sci*. 2016; 13: 942-954
26. Slingo M. Oxygen-sensing pathways and pulmonary circulation. *J Physiol* 2023; Doi: 10.1113/JP284591.
27. Young J, Williams D, Thompson R. Thin air, thick vessels: Historical and current perspectives on hypoxic pulmonary hypertension. *Frontiers in Medicine* 2019; 6: Doi: 10.3389
28. Shimoda L, Laurie S. HIF and pulmonary vascular responses to hypoxia. J Appl Physiol 1985; 116: 867-874.
29. Tuder R, Archer S, Dorfmuller P, et al. Relevant issues in the pathology and pathobiology of pulmonary hypertension. *J Am Coll Cardiol* 2013; 62: D4-D12.
30. Dunham K, Wu D, Sykes E, et al. Hypoxic pulmonary vasoconstriction from molecular mechanisms to medicine. *Chest* 2017; 151: 181-192.
31. Yu B, Wang X, Xie F, et.al. The role of hypoxia-inducible factors in cardiovascular diseases. *Pharmacol Ther* 2022; Doi: 10.1016
32. Shen Y, Gonchoarova E, et al. Twisted HIF: revisiting smooth muscle HIF-1a signaling in pulmonary hypertension. *AM J Physio Lung Cell Mol Physio*. 315: L387-389
33. Pullamsetti S, Mamazhakypov A, Weissman N, et al. Hypoxia-inducible factor signaling in pulmonary hypertension. *J Clin Invest* 2020; 130: 5638-5651.
34. Liu J, Wang W, Wang L, et al. IL-33 initiates vascular remodeling in hypoxic pulmonary hypertension by up-regulating HIF-1a and VEGF expression in vascular endothelial cells. *EBioMedicine* 2018; 33: 196-210.
35. Archer S, Gomberg-Maitland M, Maitland M, et al. Mitochondrial metabolism, redox signaling, and fusion: a mitochondria-ROS-HIF-1alpha-Kv1.5 o2-sensing pathway at the intersection of pulmonary hypertension and cancer. *Am J Physiol Heart Circ Physiol* 2008; 2194: H570-8.
36. Tian L, Wu D, Dasgupta A, et al. Epigenetic metabolic reprogramming of right ventricular fibroblasts in pulmonary arterial hypertension: A pyruvate dehydrogenase kinase-dependent shift in mitochondrial metabolism promotes right ventricular fibrosis. *Circ Res* 2020; 126: 1723-1745.
37. Mathew R. Inflammation and pulmonary hypertension. *Cardiol Rev* 2010; 18: 67-72.
38. D'Alessandro A, Kasmi K, Plecita-Hiavata L, et al. Hallmarks of pulmonary hypertension: Mesenchymal and inflammatory cell metabolic reprogramming. *Antioxidants & Redox Signaling* 2018; 28: Doi: 10.1089/ars.2017.7217
39. Luo Y, Teng X, Zhang L, et al. CD146-HIF-1a hypoxic reprogramming drives vascular remodeling and pulmonary arterial hypertension. *Nature Communication* 2019; 10: 3551.
40. Christou H, Khalil R. Mechanisms of pulmonary vascular dysfunction in pulmonary hypertension and implications for novel therapies. *Am J Physiol Heart Circ Physiol* 2022; 322: H702-H724.
41. Yu Z, Xiao J, Chen X, et al. Bioactivities and mechanisms of natural medicines in the management of pulmonary arterial hypertension. *Chinese Medicine* 2022; 17: 13
42. Xu W, Erzurum S. Endothelia cell energy metabolism, proliferation, and apoptosis in pulmonary hypertension. *Compr Physiol* 2011; 1: 357-272