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Review

Molecular targets of the Warburg effect and inflammatory cytokines in the pathogenesis of pulmonary artery hypertension

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ABSTRACT

Pulmonary arterial hypertension (PAH) is a progressive vascular disease characterized by increased pulmonary arterial pressure and vasoconstriction and structural remolding of pulmonary arterioles. Recent clinical and experimental studies have discovered the relationship between metabolic alterations and the pathogenesis of PAH. The primary metabolic alteration, previously demonstrated in various cancers, is a gradual change in energy generated from complete aerobic cellular respiration to from solely "aerobic glycolysis," termed the "Warburg effect." Understanding the Warburg effect of metabolic dysregulation and its interaction with inflammatory mechanisms in the pathogenesis of PAH has provided a valuable explanation of this disease and has guided formulation of new clinical treatments at the molecular level.

Contents

Abbreviations: DCA, dichloroacetate; FAO, fatty acid oxidation; GO, glucose oxidation; HIF-1, hypoxia-inducible factor 1; Ca_L channels, L-type calcium channels; NFATs, nuclear factor of activated T cells; NO, nitric oxide; MCT-PAH, monocrotaline-induced PAH; PAH, pulmonary arterial hypertension; PASMCs, pulmonary artery smooth muscle cells; PDK-1, pyruvate dehydrogenase kinase-1; PTPs, protein tyrosine phosphatases; PDGFRs, platelet-derived growth factor receptors; PDH, pyruvate dehydrogenase; PVR, pulmonary vascular resistance; ROS, reactive oxygen species; RV, right ventricular; Kv channels, voltage-gated potassium channels.

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1. Introduction

Pulmonary arterial hypertension (PAH) is a condition characterized by increased pulmonary arterial pressure and vascular remodeling of the small pulmonary arteries [1]. This vascular remodeling occurs via proliferation of vascular smooth muscle and endothelial cells and adventitial inflammation, often leading to fibrosis of the right ventricle [2,3]. The prevalence and geographic distribution of PAH vary depending on the type and etiology of the disease. Although worldwide prevalence remains unknown, previous studies estimate the incidence of PAH varies from 5 to 52 cases per million persons [4,5]. The prevalence of PAH associated with scleroderma is 30 to 286 cases per million [6]. In spite of outstanding progress in research and development of diagnostics and therapeutics in the last thirty years, the disease remains relatively incurable and the entire prognostication remains ill-defined. PAH is a fatal and progressive disease without treatment. Survival in untreated patients with PAH is estimated to be 2.8 years, with a 5-year survival estimated at only 34% [7]. However, with the development of therapies for PAH, its morbidity has decreased significantly as one year of treatment increases 5-year survival rates to 91–97% [8]. Patients with scleroderma-associated PAH have poorer survival rates [9].

Etiologies of PAH are numerous. PAH occurs as an idiopathic disease (formerly referred to as "primary pulmonary arterial hypertension"), as a heritable disease (heritable pulmonary arterial hypertension (HPAH)), and as a component of other disease states. PAH can also be associated with connective tissue diseases (CTDs), human immunodeficiency virus infection, congenital heart disease (CHDs) or toxins/drugs [10–12].

The pathogenesis of PAH is intricate. There are hypotheses that suggest elevated pulmonary artery pressure occurs when there is loss of balance between the endogenous vasodilators, such as prostacyclin and nitric oxide (NO), and the endogenous vasoconstrictors, such as thromboxane and endothelin [13,14]. Investigators are currently discovering that the disease may in fact be caused by a proliferative vascular remodeling process within the pulmonary artery smooth muscle cells (PASMCs) involving oxygen-sensitive voltage-gated potassium channels (Kv channels) and L-type calcium channels (Ca_L channels) [15–17].

2. Molecular pathogenesis of PAH

Hypoxia is frequently present in the pulmonary vasculature in patients with PAH, which induces the expression of hypoxia-inducible factor 1 (HIF-1). HIF-1 is a heterodimeric transcription factor and considered the master transcriptional regulator of cellular and developmental response to hypoxia [18,19]. HIF-1 is composed of an oxygen-regulated HIF-1α subunit and a constitutively expressed HIF-1β subunit [20]. The alpha subunit is normally prolyl hydroxylated and recognized by Von Hippo Lindau tumor suppressor protein (VHL) for ubiquitination and rapid degradation. However, in hypoxia, prolyl hydroxylation is inhibited, leading to the accumulation of HIF-1α. Accumulated HIF-1α freely translocates to the nucleus and binds to hypoxia response elements (HRE) causing upregulation of pyruvate dehydrogenase kinase-1 (PDK-1) and downregulation of protein tyrosine phosphatases (PTPs). Normally, PTPs antagonize platelet-derived growth factor receptors (PDGFRs); however, with downregulation of PTPs, PASMCs proliferate due to increased PDGFR activity [21]. In addition, the upregulation of PDK-1 induces metabolic dysregulation. PDK-1 inhibits pyruvate dehydrogenase (PDH), causing cessation of oxidative phosphorylation in the mitochondria. This causes the PASMCs to shift their energy generation from cellular respiration with oxidative phosphorylation to solely relying on glycolysis with the conversion of glucose to pyruvate and then lactate, as discussed below in the Warburg effect.

Inhibition of mitochondrial oxidative phosphorylation decreases generation of reactive oxygen species (ROS). Decreased ROS leads to inhibition of Kv channels, resulting in intracellular potassium accumulation. High intracellular potassium levels directly inhibit caspases and apoptosis as well as cause cellular depolarization of PASMCs. This depolarization activates Ca_L channels, which allow influx of calcium [22,23]. Cellular calcium sensitization mediated by rho kinase augments the effect of increased intracellular calcium on the PASMCs [16,24]. Rising intracellular calcium levels activate calcineurin, which dephosphorylates nuclear factor of activated T cells (NFATs).

NFATs are transcription factors in many cell types including PASMCs; however, as their name implies, their role was first described in lymphoid cells [25]. Specifically in PASMCs, NFATs promote proliferation and inhibits apoptosis as well as regulates transcription of glycolytic enzymes [26]. Dephosphorylated NFATs are active and able to translocate to the nucleus and inhibit KCNA5, a gene encoding Kv, further inhibiting efflux of potassium and creating a positive feedback loop for the activation of NFATs [26]. Activated NFATs also increase levels of mitochondrial BCL-2, leading to efflux of hydrogen ions and hyperpolarization of the mitochondria, and causing inhibition of the release of pro-apoptotic molecules [22] (Fig. 2 & Fig. 3).

These molecular pathways leading to PAH have been demonstrated in studies with 4-aminopyridine (4-AP), nifedipine, verapamil, and BAY K8644. 4-AP is a Kv channel blocker that was shown to cause pulmonary vasoconstriction in a rodent model [27]. The role of Ca_L channels in the pathogenesis of PAH was illustrated with the Ca_L channel blockers nifedipine and verapamil, as both of these medications were shown to inhibit pulmonary vasoconstriction [24]. Illustrating the same mechanism, BAY K8644 is a calcium channel agonist that was shown to worsen hypoxic vasoconstriction in PASMCs [28].

3. Warburg effect and inflammation

Research has revealed multiple similarities between PAH and cancer. PAH exhibits high expression of cellular growth factors, dysregulated angiogenesis and enhancement of antiapoptotic activity [29,30]. Discerning a link between cancer and PAH has recently revealed a common metabolic pathway named the "Warburg effect," which describes the cessation of mitochondrial oxidative phosphorylation and a dependence on only glycolysis, with conversion of glucose to pyruvate and subsequently lactate, for energy. In 1956 Otto Warburg discovered the Warburg effect while researching the pathogenesis of cancer and also called this effect "aerobic glycolysis" as the cancer cells he studied behaved metabolically as hypoxic cells, even when exposed to ample oxygen [31]. The Warburg effect was identified as central to malignant transformation in a number of tumor types and was characterized by the production of lactate to form an acidic environment, optimal for the proliferation of cancer cells [32] (Fig. 1). More recently, the Warburg effect was discovered to also be responsible for the mitochondrial changes in PAH. Mitochondria are the main site of ATP production through oxidative phosphorylation. In the pulmonary vasculature, mitochondria induce physiological intracellular signaling pathways through generation of reactive oxygen species and play a significant role as oxygen sensors in hypoxic pulmonary vasoconstriction. Changes in pulmonary artery mitochondria occur in PAH, specifically a chronic transition in energy production from mitochondrial oxidative phosphorylation to aerobic glycolysis. This occurs through the molecular pathways described above with HIF-1α causing upregulation of PDK-1. In summary, these studies emphasize a potential link between altered glucose metabolism and the pathogenesis of PAH, similar to that seen by Otto Warburg in the pathogenesis of cancers.

Recently, a study by Sutendra et al. demonstrated mechanistic connections between changes in glucose metabolism and inflammatory cytokines in the pathogenesis of PAH [33]. PASMCs in PAH have a glycolytic phenotype, characterized by increased glycolysis and inhibition of glucose oxidation (GO, also called 'oxidative phosphorylation'), previously described as the Warburg effect [34–37]. There is now emerging evidence that a glycolytic environment boosts a state of apoptotic resistance in both cancer and PAH [34,