

An AMPK paradox in pulmonary arterial hypertension

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ABSTRACT

Adenosine monophosphate-activated protein kinase (AMPK) is a heterotrimeric serine-threonine kinase important as a metabolic sensor for intracellular ATP levels and plays a key role in regulating cell survival and proliferation, particularly when cells are exposed to hypoxia. AMPK is critical for lung function, and abnormal AMPK signaling participates in many lung diseases. Recent studies suggest that both inhibition and activation of AMPK are preventive for the development of pulmonary arterial hypertension (PAH). However, the molecular mechanisms by which inhibition or activation of AMPK affects pulmonary hypertension (PH) appear to be distinct. Inhibition of AMPK by compound C blocks hypoxia-induced autophagy and induces apoptosis in pulmonary artery smooth muscle cells, leading to prevention of PAH; activation of AMPK by metformin attenuates the PH phenotype induced by hypoxia by regulating endothelial cell function. These seemingly opposing data on the function of AMPK in PH can be partly explained by off-target and compartment-specific effects of AMPK inhibitors and activators and the differentiated expression of AMPK in various cell types and subcellular locations. To elucidate the specific roles of AMPK in the pathogenesis of PAH, it is important to study the role of AMPK in a tissue specific manner combining genetic and biochemical approaches.

Keywords: AMPK; Pulmonary Hypertension; Pulmonary Artery Smooth Muscle Cells; Endothelial Cells

1. INTRODUCTION

Pulmonary arterial hypertension (PAH) is a disease characterized by increased pulmonary arterial pressure that leads to right ventricular failure, and ultimately, death

[1,2]. Active vasoconstriction as well as vascular remodeling leads to manifestation of the disease. Pulmonary artery remodeling includes survival and proliferation of pulmonary artery smooth muscle cells (PASMC), which cause the development and progression of high pulmonary vascular tone observed in PAH patients [3]. Hypoxia-induced pulmonary hypertension (PH) in several animal models is a well-established and commonly employed method to investigate the pathogenesis of PAH. Hypoxia is known to induce right ventricular hypertrophy (expressed as a ratio of right ventricular weight to left ventricular plus ventricular septum weight, RV/(LV + S), increased pulmonary arterial pressure, and pulmonary arterial wall remodeling [4]. Although major advancements have been made in the last two decades in the understanding of the development and treatment of PAH, the exact mechanisms in the pathogenesis are still not clear and there is currently no cure for this disease.

Adenosine monophosphate-activated protein kinase (AMPK) is a heterotrimeric serine-threonine kinase important as a metabolic sensor for intracellular ATP levels [5]. AMPK is composed of three subunits, the catalytic α subunit and the regulatory β and γ subunits. Each of the subunits can be found in multiple isoforms (α 1, α 2, β 1, β 2, γ 1, γ 2, γ 3), giving a total of 12 combinations having different tissue distribution and subcellular localization patterns [5,6]. AMPK is allosterically activated by the binding of two AMP molecules to the γ subunit, allowing the phosphorylation of α Thr 172 in the catalytic domain via an upstream kinase, thus increasing kinase activity, and inhibiting AMPK dephosphorylation [5]. AMPK is sensitive to stress and low energy states when the AMP/ATP ratio increases, such as in hypoxia. AMPK is also considered a “master switch” in regulating cell survival and proliferation. For example, AMPK activity is increased in rapidly proliferating cells like cardiac fibroblasts and cancer cells [7,8]. In addition, cell cycle regulation, decision to enter autophagy, apoptosis, and other cell fate decisions are regulated by AMPK (**Figure 1**) [9].

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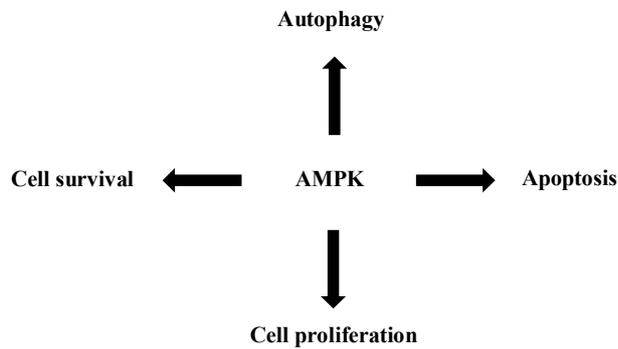


Figure 1. Schematic diagram of the roles of AMPK in the cellular response to hypoxia.

In the lung, both isoforms of the catalytic α subunit are expressed [5,10,11]. Accumulating evidence suggests that AMPK is critical for lung function, and abnormal AMPK signaling participates in lung disease [10,12-16]. For example, in alveolar epithelial cells, AMPK is critical for the regulation of sodium transport [12,13,16]. Recent reports also suggest that acute or moderate hypoxia can activate AMPK through Ca^{2+} /calmodulin-dependent protein kinase kinase- β (CaMKK β) independent of the AMP/ATP ratio [13-15]. In pulmonary endothelial cells, AMPK promotes endothelial barrier function [10]. PASMC express both α isoforms, of which the $\alpha 1$ isoform contributes up to 80% of the total AMPK activity [5]. However, the role of AMPK in lung, particularly in PAH, appears to be inconclusive: We have shown that AMPK inhibition is beneficial for the treatment of PH [17] whereas others suggest that AMPK activators such as metformin and AICAR are protective against experimental PH [18]

(<http://licensing.inserm.fr/fiche.php?artid=179>). In this review, we will present an overview on the current literature on the role of AMPK in PAH, analyze the causes of discrepancy, and discuss the future directions to elucidate the role of AMPK in the lung.

2. INHIBITION OF AMPK PREVENTS AND REVERSES HYPOXIA-INDUCED PH

Recently, we have demonstrated the physiological significance of AMPK in PAH [17]. In human pulmonary artery smooth muscle cells (HPASMC) isolated from PAH patients, levels of AMPK phosphorylated at α Thr 172 (pAMPK) are elevated compared to normal HPASMC while total AMPK levels remained the same. In a hypoxia-induced PH mouse model, pAMPK in the lung tissue of mice exposed to hypoxia for three weeks is also increased, and elevation of pAMPK occurs in mouse PASMC. These results suggest that AMPK is hyperphosphorylated in PASMC of PAH.

We also report that AMPK is necessary for PASMC

survival in hypoxia [17]. HPASMC treated with compound C, an AMPK inhibitor, exhibit decreased viability in hypoxia compared to untreated HPASMC, while inhibition of AMPK in normoxia using compound C has no effect on viability. As PASMC express both $\alpha 1$ and $\alpha 2$ isoforms of AMPK, the two different isoforms of the catalytic subunit are found to regulate separate functions that prevent cell death in hypoxia. Specifically, AMPK $\alpha 2$ promotes HPASMC survival by increasing expression of pro-survival proteins such as MCL-1. On the other hand, AMPK $\alpha 1$ functions through regulating autophagy. The inhibition of AMPK $\alpha 1$ prevents autophagy and, thus, causes cell death independent of apoptosis (**Figure 2**).

Furthermore, using an *in vivo* mouse model, we show that inhibition of AMPK by compound C is able to prevent the development of hypoxia-induced PH when compound C is administered before a three-week hypoxia exposure. When mice are treated with compound C prior to hypoxia exposure, they have significantly reduced RV/(LV + S) ratio, right ventricular systolic pressure (RVSP), and vascular remodeling (arterial wall thickness). Compound C can also partially reverse hypoxia-induced PH when it is administered after the onset of hypertension. In this model, mice are exposed to hypoxia for two weeks to induce hypertension and are then treated with compound C. RV/(LV + S) ratio and vessel wall thickness are significantly reduced, but RVSP was not affected. In both models, a marker of hypoxia, HCT, is unaffected by compound C treatment, suggesting that it is unlikely that compound C affects hypoxia-induced PH though an off-target effect on HIF. These results indicate that an inhibitor of AMPK may be a novel therapeutic approach for the treatment of PAH.

3. ACTIVATION OF AMPK PLAYS A PROTECTIVE ROLE AGAINST PH

Metformin, a commonly used drug for treating type 2 diabetes mellitus, has recently been shown to treat PH in animal models. Metformin improves hyperglycemia by increasing peripheral sensitivity to insulin, reducing gas-

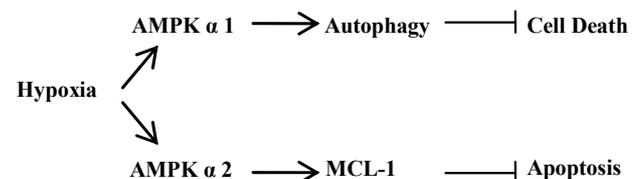


Figure 2. The mechanism by which activation of APMK promotes PASMC survival during hypoxia. Activation of AMPK $\alpha 1$ and $\alpha 2$ during hypoxia plays a differentiated role in the survival of PASMC: Activation of AMPK α_1 induces hypoxia-induced autophagy, preventing PASMC death; activation of AMPK α_2 maintains the expression levels of MCL-1 during hypoxia, preventing cell apoptosis.

trointestinal absorption of glucose, and inhibiting glucose production by the liver [19,20]. In addition, metformin has been shown to improve cardiovascular function [21]. Studies have demonstrated that metformin carries out a large part of these functions through activation of AMPK [18,22].

Agard *et al.* have demonstrated that metformin exhibits a protective effect against hypoxia-induced PH in a rat model [18]. Rats exposed to hypoxia while being treated with metformin showed near normal levels of pulmonary arterial pressure, RV wall thickness, and RV/(LV + S) ratio, with the effect being dependent on drug dose. In addition, metformin treatment significantly reduced pulmonary artery remodeling in the lungs of hypoxic rats. As expected, there was an increase in the phosphorylation of acetyl CoA carboxylase, a direct target of AMPK, in the pulmonary arteries of metformin-treated hypoxic rats, demonstrating an increase in AMPK activity. This study also demonstrates attenuation of hypoxic pulmonary vasoconstriction due to improved endothelial function and decreased RhoA/Rho kinase activity after treatment with metformin, which is in agreement with previous studies on metformin and vascular tone [22-24]. Furthermore, treatment with metformin on cultured rat PASMCM inhibited proliferation. Consistently, AICAR, another AMPK activator, has also been shown to be protective against experimental PH [18] (<http://licensing.inserm.fr/fiche.php?artid=179>). Thus, these studies suggest that an AMPK activator may be used as therapeutic agent for the treatment of PAH.

4. DISCUSSION

In this review, we have discussed seemingly opposing data on the function of AMPK in PAH and hypoxia models of PH. On the one hand, we have suggested that inhibition of AMPK activity prevents and reverses hypoxia-induced PH by inducing PASMCM death; on the other hand, Agard *et al.* have suggested that activation of AMPK protects against hypoxia-induced PH, presumably by regulating endothelial cell function [18]. However, these results need to be viewed with caution due to non-specific effects of these drugs. This inconsistency can therefore be explained partly by off-target and compartment-specific effects of AMPK inhibitors and activators [25-27] and partly by the differentiated expression of AMPK in various cell types and subcellular locations [5,10,28].

Indeed, the role of AMPK in cell survival, for example, appears to be cell-specific. Some studies show that AMPK is activated in rapidly proliferating cells [7,8] and that inhibition of AMPK induces growth arrest and reduces viability [8,29]. Others report that activation of AMPK inhibits growth and/or survival of cells, particu-

larly in systemic vascular smooth muscle cells [30-32]. Our study demonstrates that AMPK $\alpha 1$ plays a role in regulating autophagy while AMPK $\alpha 2$ upregulates the pro-survival protein MCL-1, inhibiting apoptosis. Both isoforms are necessary for PASMCM survival in hypoxia; however, suppression of either or both isoforms does not induce cell death under normoxic conditions. Krymskaya and colleagues show that hypoxic activation of AMPK does not contribute to hypoxia-induced proliferation of PASMCM [33], supporting our finding that the role of AMPK in hypoxia-induced PH is mediated by its regulation of PASMCM survival. In addition, AMPK is known to be required for hypoxia-mediated vasoconstriction [5,34,35], a feature of PAH. Thus, these studies suggest that activation of AMPK contributes to the pathogenesis of PAH.

In the study by Agard *et al.*, however, indirect AMPK activation by the drug metformin seems to attenuate, and almost eliminate, PAH phenotype induced by hypoxia. An increase in the phosphorylation of endothelial NOS (eNOS) as a marker of endothelial function and a decrease in phosphorylated MYPT as a marker of RhoA/Rho kinase activity were observed. Improvement in endothelial function is likely mediated by AMPK activity as several previous studies have shown AMPK activity to lead directly to phosphorylation of eNOS [22,24]. It is worth pointing out that the inhibition of PASMCM proliferation with metformin treatment is possibly due only in part to AMPK activity. Previous studies on metformin's inhibitory effects on cancer cell proliferation were shown to be only partly dependent upon AMPK [36,37]. Therefore, the protective effects of metformin from hypoxia-induced PH may not be completely attributed to increased AMPK activity.

In conclusion, recent studies have shown what seems like opposite functions for AMPK in PAH. However, in reconciliation, the molecular pathways and the type of cells in which AMPK functions are different. In one set of studies, inhibition of AMPK leads to elevated PASMCM cell death, thus preventing PH; in another set of studies, AMPK activation increases eNOS function to attenuate PH. Given the fact that these studies are carried out with chemical AMPK inhibitors and activators, these results need to be interpreted with caution as long-term success in the treatment of PAH with these agents being uncertain due to their non-specific effects [27]. Thus, to elucidate the specific roles of AMPK in pathogenesis of PAH, it is important to study the role of AMPK in a tissue-specific manner combining genetic and biochemical approaches.

5. ACKNOWLEDGEMENTS

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