

Review Article

Phenotypical Changes of the Pulmonary Artery Endothelial Cells in Fetal Sheep Model of Persistent Pulmonary Hypertension of the Newborn

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- Apoptosis
- Autophagy
- AMP activated protein kinase (AMPK)

Abstract

Persistent pulmonary artery hypertension of the newborn is a common lung disorder in neonates affecting one in every 500 live births with high mortality and morbidity rates. Intrauterine ductus arteriosus constriction in fetal sheep is the most commonly used animal model to study this disease entity. In vitro studies using pulmonary artery endothelial cells obtained from this animal model have provided us a lot of important information to understand this disease. Evidence suggests that there is a phenotypical change with pulmonary artery endothelial cells under pulmonary hypertension with increased formation of reactive oxygen species that lead to changes in several signaling pathways. Research using pulmonary artery endothelial cells from this animal model can help us not only to understanding the Pathophysiology of the persistent pulmonary artery hypertension of the newborn but also finding new targets that we can study to treat the disease in human neonates.

INTRODUCTION

Persistent pulmonary hypertension of the newborn is a disorder with failure to decrease pulmonary vascular resistance after birth. Persistent pulmonary hypertension of the newborn happens in one out of 500 live births with a mortality rate 10-50%. Right after birth the pulmonary vascular resistance decreases drastically within a few minutes when oxygen enters into the fluid-filled alveoli. Failure to achieve this transition impairs adequate blood flow into the lungs to be oxygenated. There are several mechanisms that can impede this transition such as decreased blood vessel count in the lungs or inadequate production of vasorelaxant by the endothelial cells. The most important vasorelaxants to the pulmonary vasculature are nitric oxide [1] and prostacyclin [2]. Endothelial cells also produce vasoconstricting substance such as endothelin-1. It is believed that the surge of ATP formation by the pulmonary vasculature right after birth is the agonist that prompts the release of nitric oxide and prostacyclin from the pulmonary vasculature [1]. There is evidence to suggest that the productions of nitric oxide and prostacyclin are impaired in persistent pulmonary hypertension of the newborn whereas formation of endothelin-1 is increased. Several animal models have been used to study persistent pulmonary hypertension of the newborn and readers

are recommended to read recent reviews [1]. In this short review we only focus on pulmonary artery endothelial cells as the in vitro studies.

Intrauterine ductus arteriosus constriction of fetal sheep as the animal model

Intrauterine ductus arteriosus constriction of fetal sheep at about 80-85% gestation (term=140-145 days) is a commonly used animal model to study persistent pulmonary hypertension of the newborn [3]. After the ductal constriction for 8 days most fetuses will establish severe persistent pulmonary hypertension of the newborn and cannot survive without for more than several hours even with mechanical ventilator support. This animal model has helped us to understand the Pathophysiology of persistent pulmonary hypertension of the newborn and efficacy of using inhalational nitric oxide and prostacyclin analogues in this disease. It was thought the ductal constriction mimics the prenatal exposure to non-steroid anti-inflammatory drugs but studies have shown the physiological changes are more extensive than just increased shear-stress to the pulmonary vasculature.

Increased formation of reactive oxygen species

The most important picture observed from the fetal sheep

model of persistent pulmonary hypertension of the newborn is the increased formation of reactive oxygen species in the pulmonary vasculature [4]. Both pulmonary artery endothelial cells [5] and smooth muscle cells from this model show increased reactive oxygen species formation [6]. The increased reactive oxygen species formation impairs the blood vessel formation (angiogenesis) in lungs that contributes to high pulmonary vascular resistance [5]. Poor alveolar formation is also observed in this model (Figure 1). One interesting finding is the pulmonary artery endothelial cells maintain the high reactive oxygen species formation even after multiple passages in vitro indicating a phenotypical change. In vitro studies show antioxidants are capable to improve pulmonary artery endothelial function with persistent pulmonary hypertension of the newborn [5].

Mechanisms of increased reactive oxygen species formation

NADPH oxidase, especially isoform II, has been shown to contribute to the increased reactive oxygen species formation in both cell types [5,6] but other reactive oxygen species forming enzymes/systems have also been shown in pulmonary artery endothelial cells. Endothelial nitric oxide synthase is the enzyme that produces nitric oxide to relax pulmonary arteries but this enzyme requires multiple co-factors including tetrahydrobiopterin. Without tetrahydrobiopterin the endothelial nitric oxide synthase will be uncoupled [7] and forms superoxide instead of nitric oxide as the end product. The superoxide formation will decrease the availability of nitric oxide to the pulmonary artery smooth muscle cells with pulmonary vasoconstriction. GTP cyclohydroplase-1 is the rate limit-limiting enzyme for tetrahydrobiopterin formation. The GTP cyclohydroplase-1 level decreases in pulmonary artery endothelial cells with persistent pulmonary hypertension that contributes to the endothelial nitric oxide synthase uncoupling [8].

Impaired mitochondrial function

Mitochondria are the power plants for cells through oxidative phosphorylation. As the by-product of oxidative phosphorylation superoxide will be formed. At least one percent

of oxygen consumed by cell will be converted into superoxide. Although pulmonary artery endothelial cells have low number of mitochondria the organelle remains one of the sources for reactive oxygen species. Mitochondrial bioenergetics is impaired in pulmonary artery endothelial cells with persistent pulmonary hypertension of the newborn.

Mitochondrial manganese superoxide dismutase is the first defense mechanism inside of mitochondria to protect against reactive oxygen species induced injury. The uncoupled endothelial nitric oxide synthase in persistent pulmonary hypertension of the newborn impairs manganese superoxide dismutase transport into mitochondria after its synthesis in cytosol [9] and also decreases manganese superoxide dismutase activity by post-translational nitration [10]. Impaired manganese superoxide dismutase activity leads to decreased ATP formation in pulmonary artery endothelial cells [9].

Decreased AMP-activated protein kinase activity

Decreased ATP formation should activate AMP-activated protein kinase but such mechanism is not achieved in persistent pulmonary hypertension of the newborn pulmonary artery endothelial cells [11]. This unexpected finding indicates there is a Phenotypical change of persistent pulmonary hypertension of the newborn pulmonary artery endothelial cells. Through peroxisome proliferator-activated receptor gamma co activator 1AMP-activated protein kinase promotes mitochondrial biogenesis. The impaired AMP-activated protein kinase signaling contributes to decreased angiogenesis and mitochondrial function, and increased reactive oxygen species formation in persistent pulmonary hypertension of the newborn pulmonary artery endothelial cells. In vitro metformin treatment can activate the AMP-activated kinase in pulmonary artery endothelial cells with persistent pulmonary hypertension of the newborn [11] with increased GTP-chclohydrolase-1 levels and improved endothelial nitric oxide synthase activity.

Endoplasmic reticulum stress and Nogo-B receptor

The functional changes in persistent pulmonary hypertension of the newborn pulmonary artery endothelial cells also involve the endoplasmic reticulum. The increased reactive oxygen

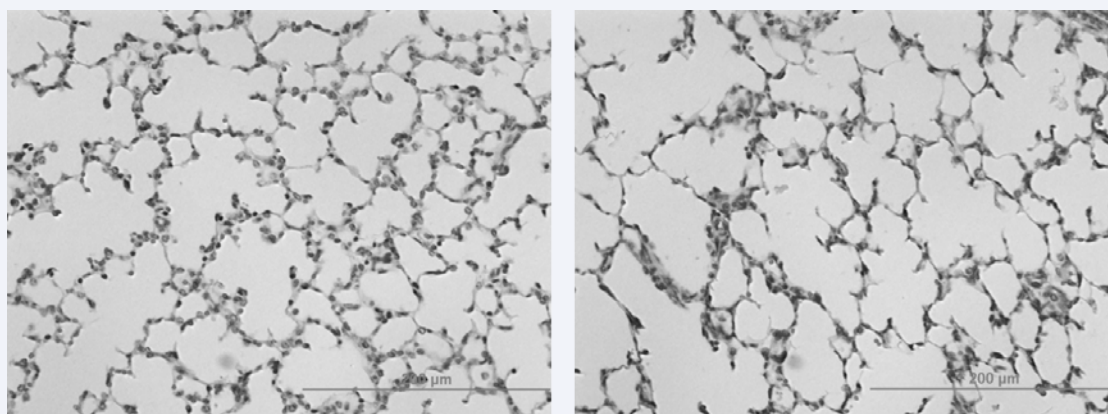


Figure 1 Intrauterine ductus arteriosus constriction of fetal sheep produce picture of persistent pulmonary hypertension of the newborn and poor lung development mimicking human disease. Control (left) sheep has better alveolar formation than the co-twin (right) sheep with pulmonary hypertension.

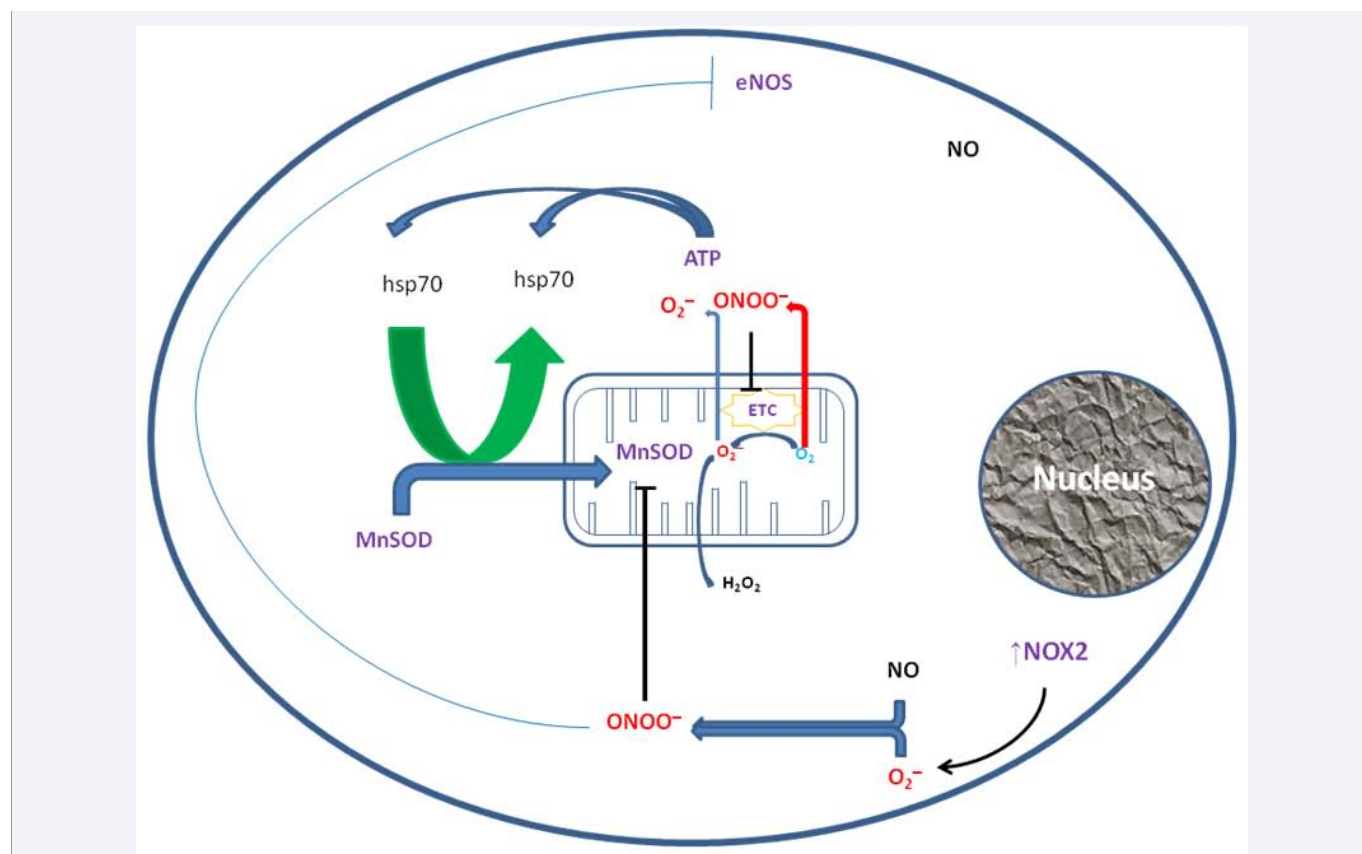


Figure 2 Summary of the phenotypic changes in pulmonary artery endothelial cells with persistent pulmonary hypertension of the newborn.

species formation can lead to disturbed protein folding and stress to the endoplasmic reticulum. The sustained endoplasmic reticulum stress can further increase reactive oxygen species formation. One of the endoplasmic reticulum proteins, Nogo-B, and its receptor, Nogo-B receptor, has been studied in persistent pulmonary hypertension of the newborn. In adult patient with idiopathic pulmonary hypertension the serum levels of Nogo-B are increased and are believed to aggravate endoplasmic reticulum stress and mitochondrial dysfunction [12]. Nogo-B receptor on the other hand has been shown to mediate angiogenesis via PKB/Akt pathway [13]. The Nogo-B receptor levels decrease in both pulmonary artery endothelial cells and pulmonary artery smooth muscle cells and manipulating Nogo-B receptor level in pulmonary artery endothelial cells affect the angiogenesis *in vitro*. The exact mechanism by which Nogo-B receptor modulates angiogenesis remains to be explored but GTP cyclohydrolase-1 and mitochondrial biogenesis seems to be involved [14].

Increased autophagy and apoptosis

Pulmonary artery endothelial cells from adult patients with idiopathic pulmonary hypertension are apoptosis-resistant but in persistent pulmonary hypertension of the newborn the pulmonary artery endothelial cells are pro-apoptotic [5]. The increased reactive oxygen species formation is believed to be an important contributing factor to the apoptosis. Apoptosis, necrosis and autophagy are three major types of cell death mechanisms. Autophagy is an adaptive mechanism for cells that facing extreme stressful environment. Autophagy can either anti-death or

pro-death depends upon the context. In persistent pulmonary hypertension of the newborn pulmonary artery endothelial cells there is an increased autophagy [15]. Contrary to the autophagy in aortic endothelial cell which is needed in angiogenesis [16] the autophagy in persistent pulmonary hypertension of the newborn pulmonary artery endothelial cells is pro-apoptotic. The increased reactive oxygen species formation from NADPH oxidase-2 in persistent pulmonary hypertension of the newborn pulmonary artery endothelial cells seems to enhance autophagy and contributes to the impaired angiogenesis [15].

Impaired prostanoid synthesis

Another signaling pathway that control angiogenesis in the developing lungs is the prostacyclin. Not only the levels of prostacyclin synthase decreased in persistent pulmonary hypertension of the newborn pulmonary artery endothelial cells the uncoupled endothelial nitric oxide synthase in persistent pulmonary hypertension of the newborn pulmonary artery endothelial cells also lead to prostacyclin synthase nitration [17]. The decreased prostacyclin synthase activity explains the decreased prostacyclin production with the precursor shunts to thromboxane A2 formation that either constricts pulmonary arteries or impairs angiogenesis.

CONCLUSION

In conclusion, pulmonary artery endothelial cells in persistent pulmonary hypertension of the newborn show a phenotype with (1) increased reactive oxygen species formation; (2) increased

autophagy and apoptosis; (3) impaired prostacyclin and nitric oxide production; (4) impaired AMP-activated protein kinase signaling pathway; (5) decreased Nogo-B receptor expressions; (6) increased endoplasmic reticulum stress; and (7) decreased GTP cyclohydrolase-1 levels and tetrahydrobiopterin formation (Figure 2). All these phenotypical changes lead to impaired angiogenesis which contributes to the increased pulmonary vascular resistance in persistent pulmonary hypertension of the newborn. The increased reactive oxygen species formation also promotes pulmonary artery smooth muscle cells proliferation that is the other path gnomic finding in persistent pulmonary hypertension of the newborn. Understanding of these newly described phenomena can help us to develop new therapeutic strategies in managing persistent pulmonary hypertension of the newborn.

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