Abstract

Pulmonary Arterial Hypertension (PAH) is a devastating disease in which mean survival from diagnosis is only 2.8 years and for which, despite efforts, there is currently no effective treatment. Although the mechanism of PAH is largely unknown, inflammation appears to play a role in the vascular remodeling that is typical of this disease. Recent findings indicate that microparticles isolated from the blood of PAH rat models induce increased intracellular adhesion molecule (ICAM-1) expression in pulmonary endothelial cells when compared to treatment with microparticles from control animals. It has also been found that the protein alpha-pix, a guanine exchange factor responsible for downstream ICAM-1 expression, is found in the microparticles of PAH patients at concentrations seven times that of microparticles isolated from control patients- causing speculation that this protein may be the source of increased ICAM-1 expression in the PAH microparticle-treated endothelial cells. Before it can be determined whether the microparticles are delivering or stimulating increased alpha-pix expression, we first had to determine the constitutive expression of alpha-pix in pulmonary endothelial cells. We used several methods to confirm protein expression in both pulmonary microvascular endothelial cells (PMVECs) and pulmonary artery endothelial cells (PAECs) including western blotting, immunocytochemistry, and flow cytometry. These methods proved that there was alpha-pix expression in both PMVECs and PAECs, but that expression was considerably higher in PAECs (25% vs 2%) and that localization of the protein was perinuclear. These results suggest that alpha-pix is expressed in both endothelial cell types but more abundantly and with clear nuclear localization in the pulmonary artery endothelial cells. These findings may contribute to our knowledge of the function of alpha-pix in PAH and help us determine the endothelial response to circulating microparticles in the setting of pulmonary arterial hypertension.

Methods

- Rat pulmonary artery and pulmonary microvascular endothelial cells were grown to confluence.
- Total protein was isolated from both cell types and Western analysis for alpha-Pix was performed (positive control HeLA cell extract)
- Cells were also grown on glass to confluence and using immunocytochemistry analyzed for alpha-Pix intracellularly
- Cells were also grown to confluence then trypsinized for analysis by flow cytometry for alpha-Pix
- Primary antibody for all experiments Santa Cruz sc-90127.

Results

Western analysis suggests alpha-Pix is expressed in both PAECs and PMVECs.

Flow cytometry: revealed that approximately 25% of the PAEC population expressed alpha-pix, whereas only 2% of MVECs express the protein.

Conclusions

- Alpha-pix is expressed in both pulmonary artery and pulmonary microvascular endothelial cells, however the abundance of expression and localization are significantly different.
- These findings may contribute to the function of alpha-pix and be determinate of the endothelial response to circulating microparticles in the setting of PAH.

Objective

Determine constitutive expression of alpha-pix in pulmonary endothelial cells.

Future Directions

Investigate whether isolated circulating microparticles from PAH rats deliver or stimulate alpha-PIX activity in pulmonary artery endothelial cells.

Literature Cited


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