



Embracing the Potential of
**YOUNG
INVESTIGATORS**

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Endothelial to mesenchymal transition in pulmonary hypertension: Forming a new identity

INTRODUCTION:

The actin cytoskeleton is a key component of endothelial structure and function, and alterations may effect monolayer integrity. The transformation of endothelial cells to mesenchymal cells, and ultimately proliferation increasing vessel stiffness and creating obliterative lesions, is a hallmark of pulmonary hypertension. Cytoskeletal influence on this process is unknown.

BACKGROUND:

Investigations into the pathogenesis of pulmonary hypertension (PH) have defined multiple pathways including inflammation, fibrosis, smooth muscle cell proliferation, and endothelial cell (EC) injury and dysfunction. EC and monolayer functions are abnormal in PH with altered apoptosis, increased cellular migration, and augmented vascular stiffness. A prominent component of this EC dysfunction is the formation of plexiform lesions. The origin is thought to be EC in nature with the transformed cells showing phenotypic variations consistent with hyperproliferation, resistance to apoptosis, and a mesenchymal-like phenotype. Pathways underlying endothelial to mesenchymal transition (EndMT) are not well understood, but an early step in transformed EC migration is the loss of vascular endothelial cardherin (VE-cad), a key component of EC adherens junctions. VE-cad, through β -catenin, is linked to the actin cytoskeleton and alterations in the cytoskeletal arrangement can change the organization of the adherens junction. Formins are integral in actin cytoskeleton polymerization and maintenance. Two in particular, the mammalian Diaphanous-related formin 1 (mDia1) and formin-like protein 3 (FMNL3) accelerate actin nucleation and bundles filaments and localizes to EC junctions, respectively. Decreased or absent formin activity is associated with increased cell migration and invasiveness. Isolated rat pulmonary microvascular endothelial cells from the Sugen-Hypoxia model show evidence of EndMT with co-expression of the endothelial markers von Willebrand Factor and Griffonia simplicifolia II with the smooth muscle cell markers smooth muscle α -actin and myosin heavy chain. Additionally, these cells show an altered spindle like form and exhibit enhanced migration, proliferation, and altered endothelial nitric oxide synthase levels.

HYPOTHESIS AND OBJECTIVES:

We hypothesize that cytoskeletal-organizing formins have a critical functional impact on pulmonary vascular endothelium that is lost in PH, and our objectives are to determine the impact of formins on endothelial form and function in human cells, the Sugen-Hypoxia animal model, and in patients affected with PH.

SPECIFIC AIM 1:

Determine the role of formin mDia1 in pulmonary endothelial adherens junction formation via epifluorescence and super resolution microscopy.

SPECIFIC AIM 2:

Analyze the effect of formin mDia1 inhibition on endothelial monolayer barrier function.

SPECIFIC AIM 3:

Characterize the role of formins mDia1 and FMNL3 in endothelial to mesenchymal transition in the microvasculopathy of pulmonary hypertension in the Sugen-Hypoxia animal model and in patients with PH.