Excessive Nuclear Matrix Anchoring Hampers Endothelial Shear Adaptation and Promotes Shear-Mediated Cell Damage in Pulmonary Arterial Hypertension

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Background: The hallmark of pulmonary arterial hypertension (PAH) is extensive lung vascular remodeling following from a combination of predisposition and acute activation. We have previously shown that the pulmonary endothelium (HPEC) of PAH patients is prone to shear-induced cell damage due to dysfunctional morphological shear adaptation. Based on our preliminary results, we hypothesize that increased nuclear matrix anchoring results in a reduced ability of HPEC to adjust to shear stress, rendering the cells in a continuous state of injury and repair that promotes pathological vascular remodeling.

Methods: Human lung samples (n=5 control; n=8 PAH) were obtained from lung transplantations and autopsies (METC: 2012/306) and submitted to global proteomic analysis. Commercial control HPEC were subjected to 24 hours of physiological fluid shear stress (2.5 dyn/cm^2), followed by 96 hours of supra-physiological shear stress (HSS, 15 dyn/cm^2) with 2 µM cell-permeable fluoromethyl ketone-derivatized peptides acting as irreversible and non-cytotoxic caspase inhibitors.

Results:
The PAH lung proteome exhibits significantly increased levels of proteins associated with the nucleus, with 19 proteins in the nuclear domain identified as being significantly overexpressed in PAH samples. Using gene ontology enrichment analysis on the ranked full-data set, significant enrichment was found in “nuclear matrix anchoring at nuclear membrane” (p=4.23E-5), “integral component of nuclear inner membrane” (p=2.87E-7), and “lamin binding” (p=7.05E-6). Partial least squares-discriminant analysis was applied to then cluster the control *vs*. PAH samples. From this, several “VIP” proteins were identified that drive cluster separation, with multiple LINC complex (Linker of Nucleoskeleton and Cytoskeleton) and inner nuclear membrane proteins in the top 15, including SUN1/2, SYNE1, and EMD. However, the upregulated inner nuclear membrane/LINC complex signature observed in the proteomics was not significantly changed on transcript level, suggesting post-translational modification. We have previously shown that caspase-mediated protein cleavage - one form of post-translational modification targeting the nuclear envelope - plays a role in the shear phenotype of PAH HPEC. Therefore, we probed caspase group inhibition (initiator, executioner, inflammatory) in HSS conditions, revealing that exclusively executioner caspases modulate morphological shear adaptation.

Conclusions:
Our multiomic analysis reveals a post-translational upregulated nuclear matrix-binding signature in PAH lung samples, with an emphasis on the LINC complex. Furthermore, we demonstrate the necessity of executioner caspase activity to permit shear adaptation of HPEC in HSS conditions. Therefore, we postulate that reduced caspase cleavage promotes overaccumulation of the LINC complex proteins in PAH HPEC inhibiting morphological shear adjustment, and consequently promoting shear-induced cell injury.